Introduction to ADMET: Solving Problems Chemically

Wednesday 20 October 2010

SCI HQ, London, UK

SCI
where science meets business

Organised by SCI's Young Chemists' Panel of SCI's Fine Chemicals Group

Your Speakers

Peter C. Astles

Peter completed his Chemistry degree and Ph.D. at the University of Oxford. He then spent two years of postdoctoral research with Prof. Leo Paquette at the Ohio State University, USA prior to joining Rhone-Poulenc Rorer, now Sanofi Aventis, in 1992. Peter gained experience of medicinal chemistry working on cardiovascular and asthma/ inflammation projects, becoming a section leader in 1997. In 2000, Peter moved to Eli Lilly based at Windlesham in the UK where he is a Medicinal Chemistry Group Leader and project leader in the CNS therapeutic area.

Darren McKerrecher

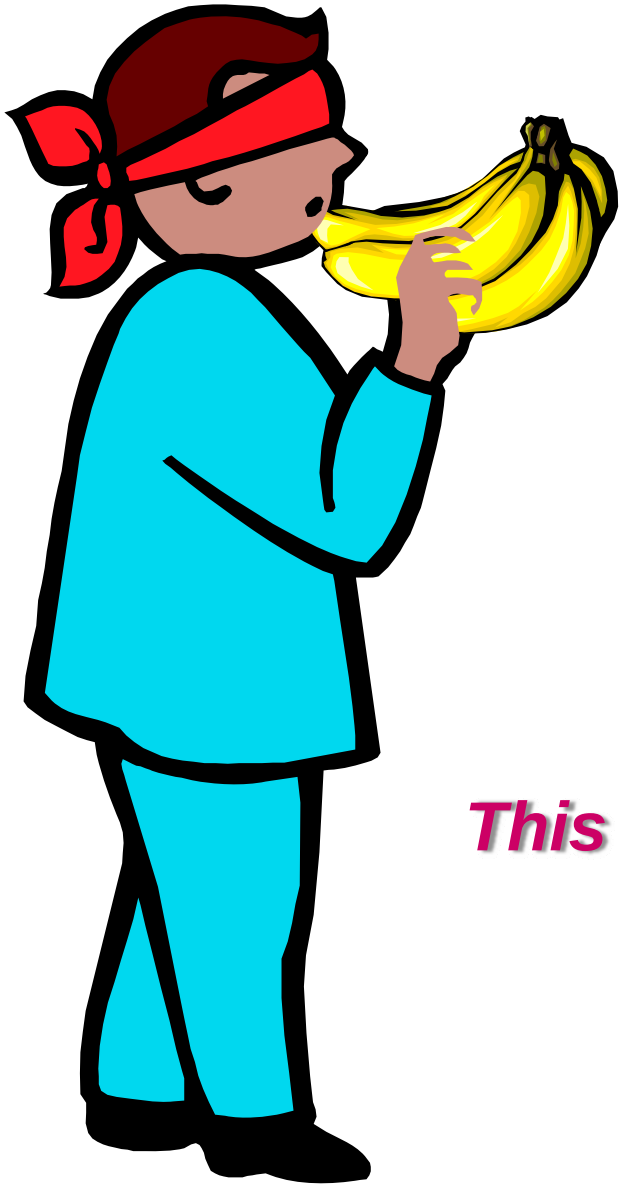
Darren obtained his Chemistry degree at Edinburgh, and completed his D.Phil with Richard Taylor at York. He joined Zeneca, now AstraZeneca, at Alderley Park in 1997. He has been involved in a number of projects with ADMET challenges, in disease areas as diverse as cancer, diabetes, obesity, asthma and COPD.
In 2008, Darren returned to Alderley Park after a 2-year secondment in Lund (Sweden), and is now Associate Director of Medicinal Chemistry and Project Leader in the Diabetes & Obesity research area.

Dr Ted (AH) Parton

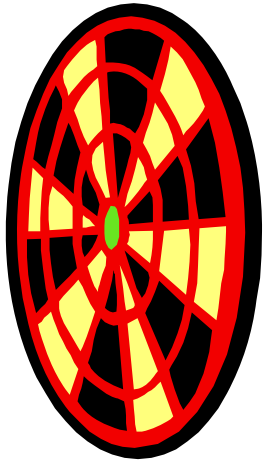
Ted studied Chemistry at Cambridge and after two years in industry began research into insect pheromones at the University of Southampton. After three years as Experimental Officer in mass spectrometry at the University of York, he began his career in pharmaceutical development in 1985 at Upjohn Laboratories in Crawley. In 1993, he moved to Celltech in Slough, acquired by UCB in 2004, where he is a Director in Research DMPK. His current interests include mathematical modelling of PK/PD interactions.

icinal Chemistr
n exercise in precis

Did we pay sufficient attention to the requirements of the dart?

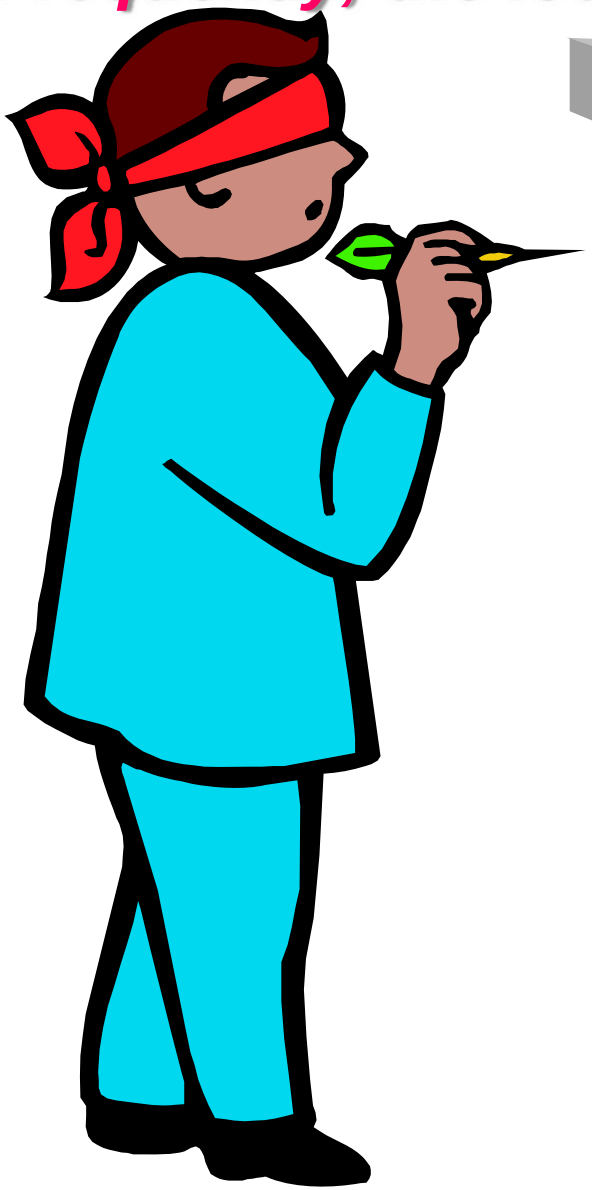


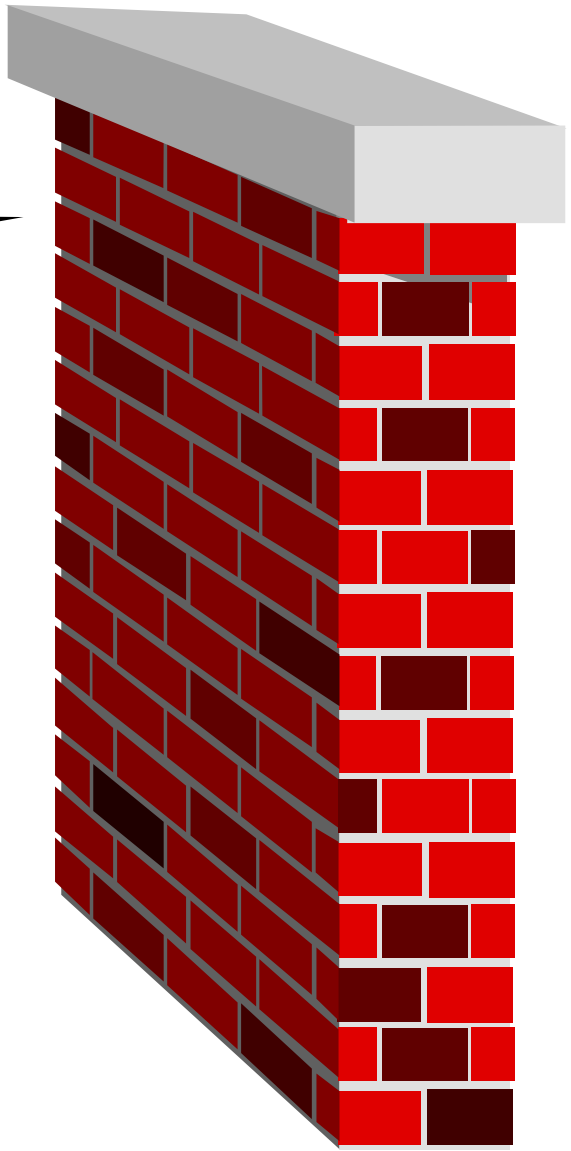
Sometimes the compound was unlikely to become a drug:

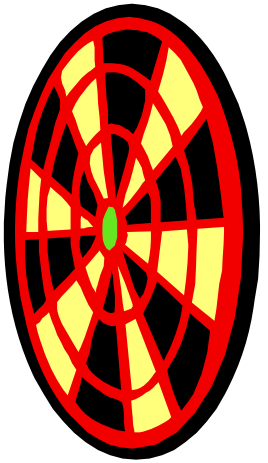


This was not good Medicinal Chemistry

Frequently, the location of the target adds problems:





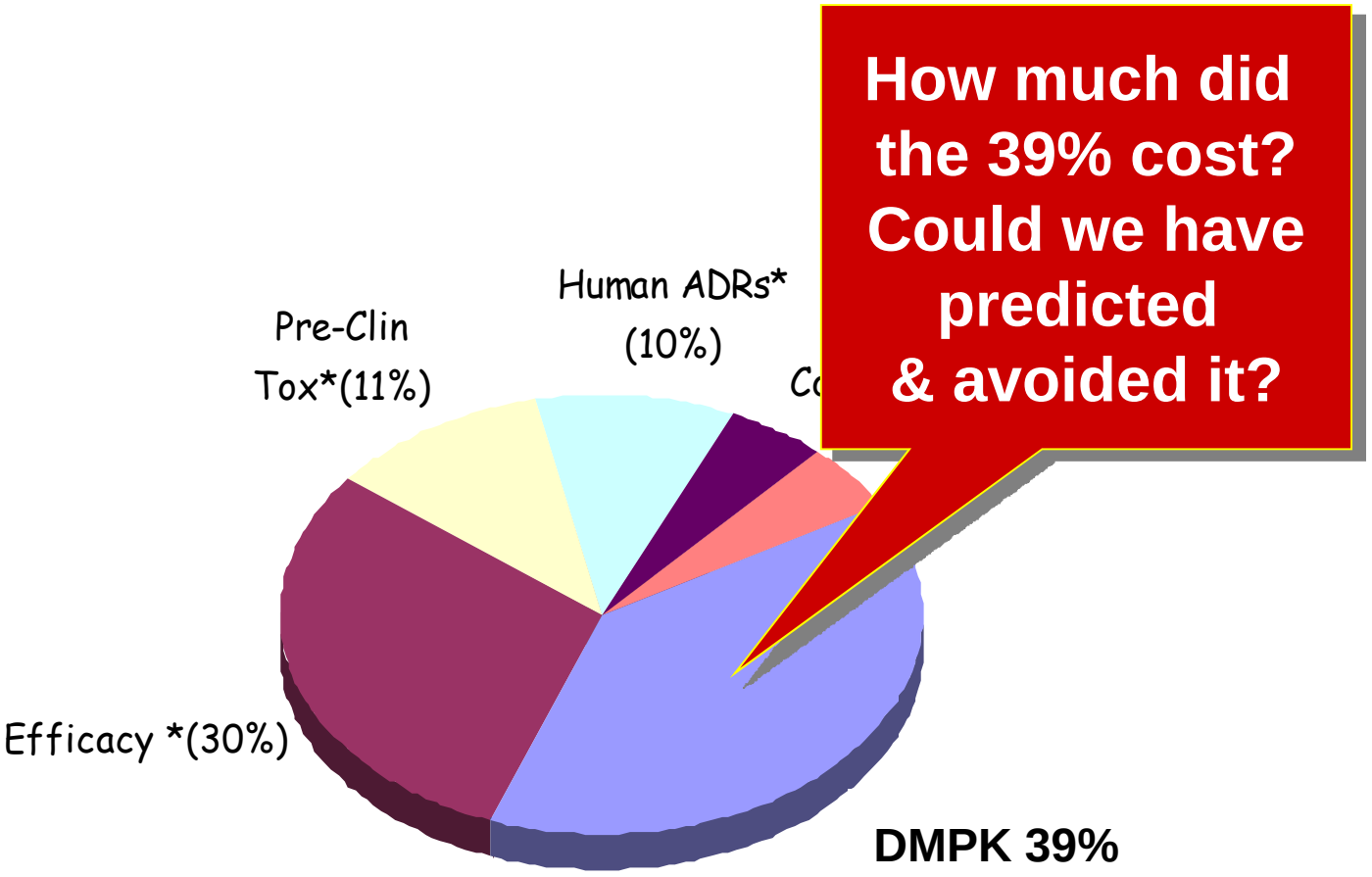


This requires a very powerful dart

Pharmacokinetics, Physical & Pharmaceutical Properties in Medicinal Chemistry

Potential drugs... or merely good ligands?

DMPK & Compound Attrition



Kennedy,T. DDT (1997). 2: 436-444

DMPK & Compound Attrition

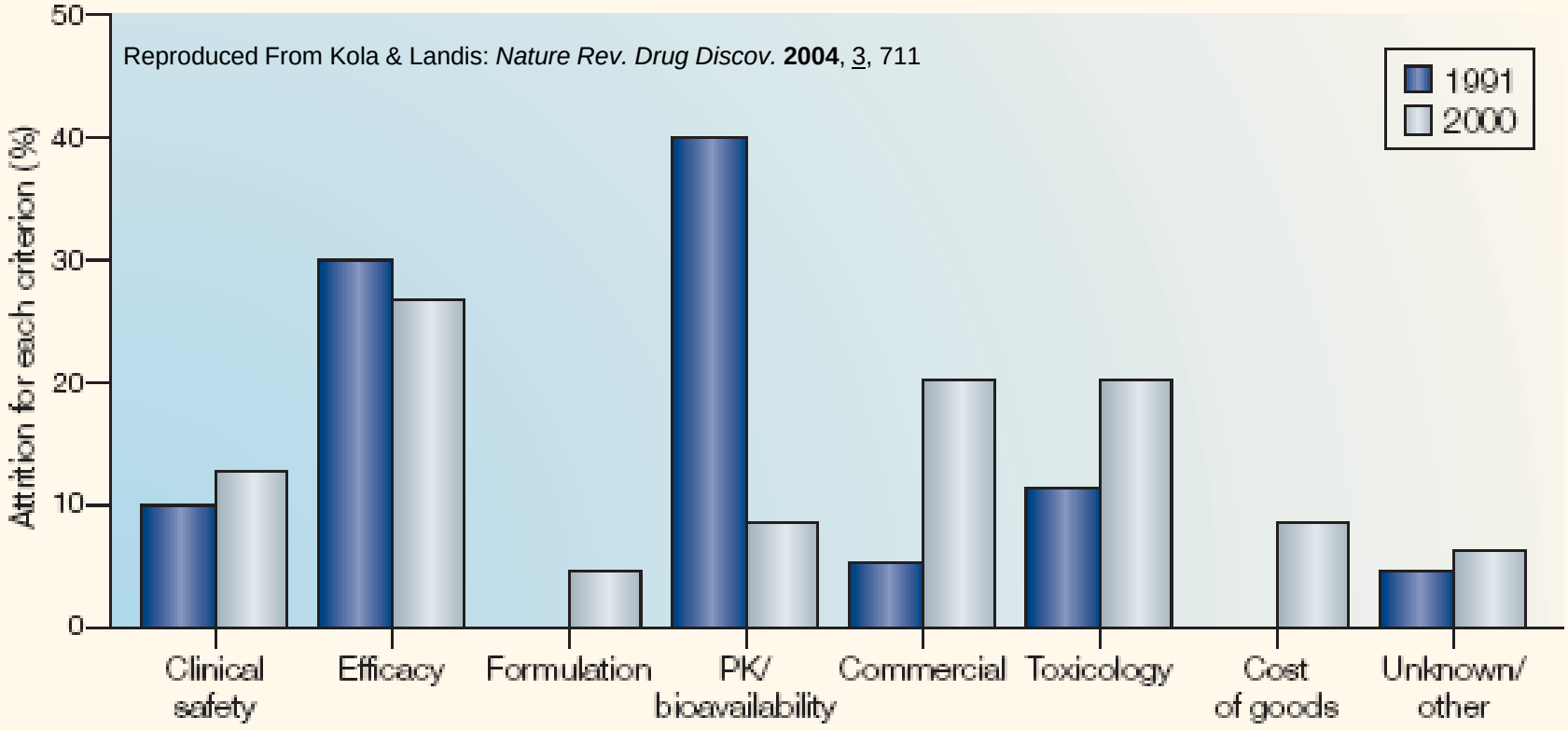


Figure 3 | **Reasons for attrition (1991–2000).** PK, pharmacokinetics.

Apparent improvement in DMPK-attributed attrition, but still significant
(& perhaps underestimated – Efficacy? Formulation? Cost of Goods? Toxicology?)

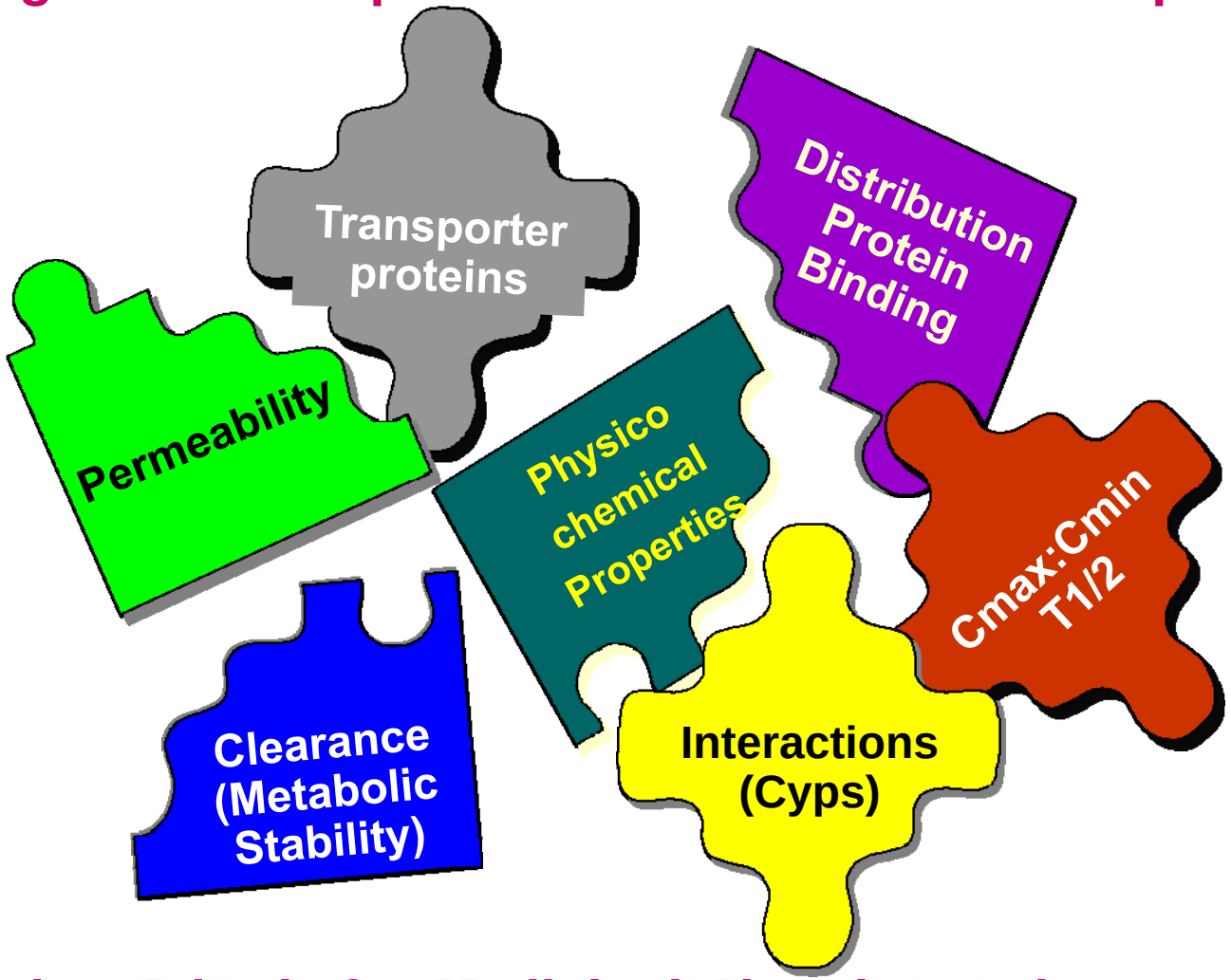
DMPK & Compound Attrition

Poor oral exposure
Sub-optimal duration
Polymorphic metabolism
Active/toxic Metabolites
Cyp inhibition & induction
Poor margins

Standard reasons for failure over 20 yrs

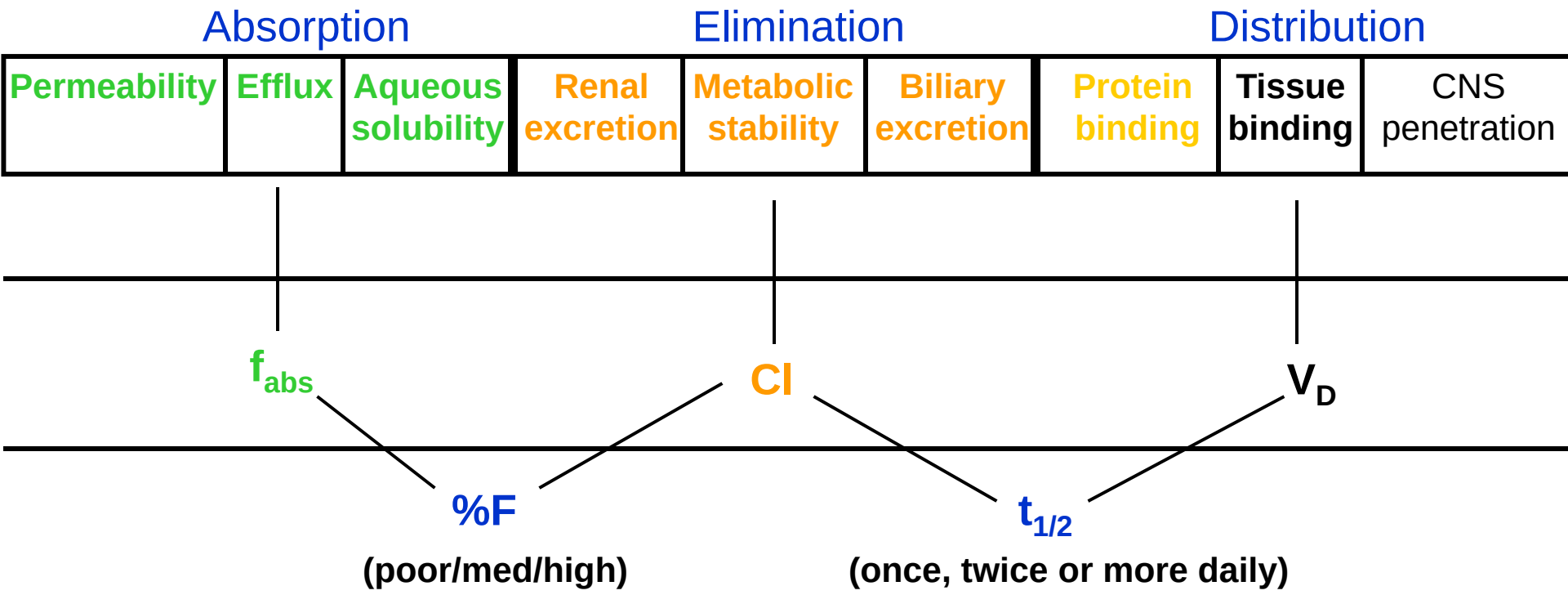
DMPK & Candidate Drugs

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

ADME Overview

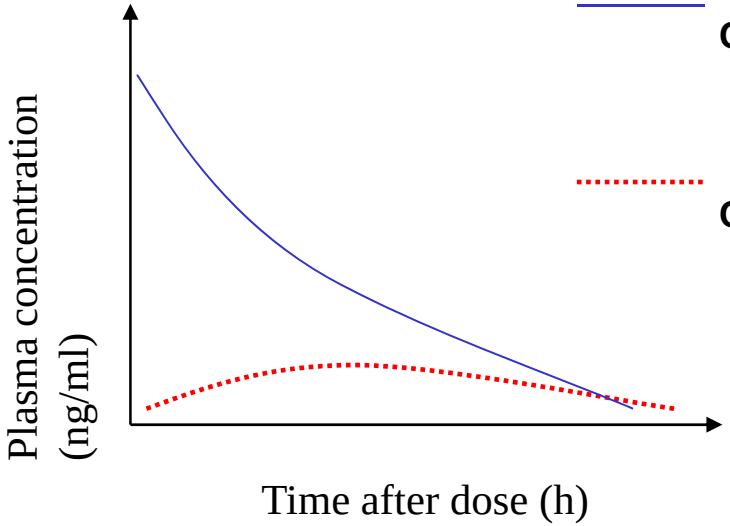


And once you've cracked all that, compounds can still be toxic!

Absorption

Absorption from an oral dose

How do you know you have a problem?



Compound A i.v. bolus injection 1 mg/kg

- **Good plasma exposure (area under curve AUC)**
- **Metabolic or other plasma clearance appears low**

Compound A oral dose (p.o.) 10 mg/kg

- **Low plasma exposure (AUC)**
- **Unchanged parent drug may appear in faeces**

Oral Bioavailability (F)

= fraction of the dose which makes it to the systemic circulation

(Combination of absorption & clearance)

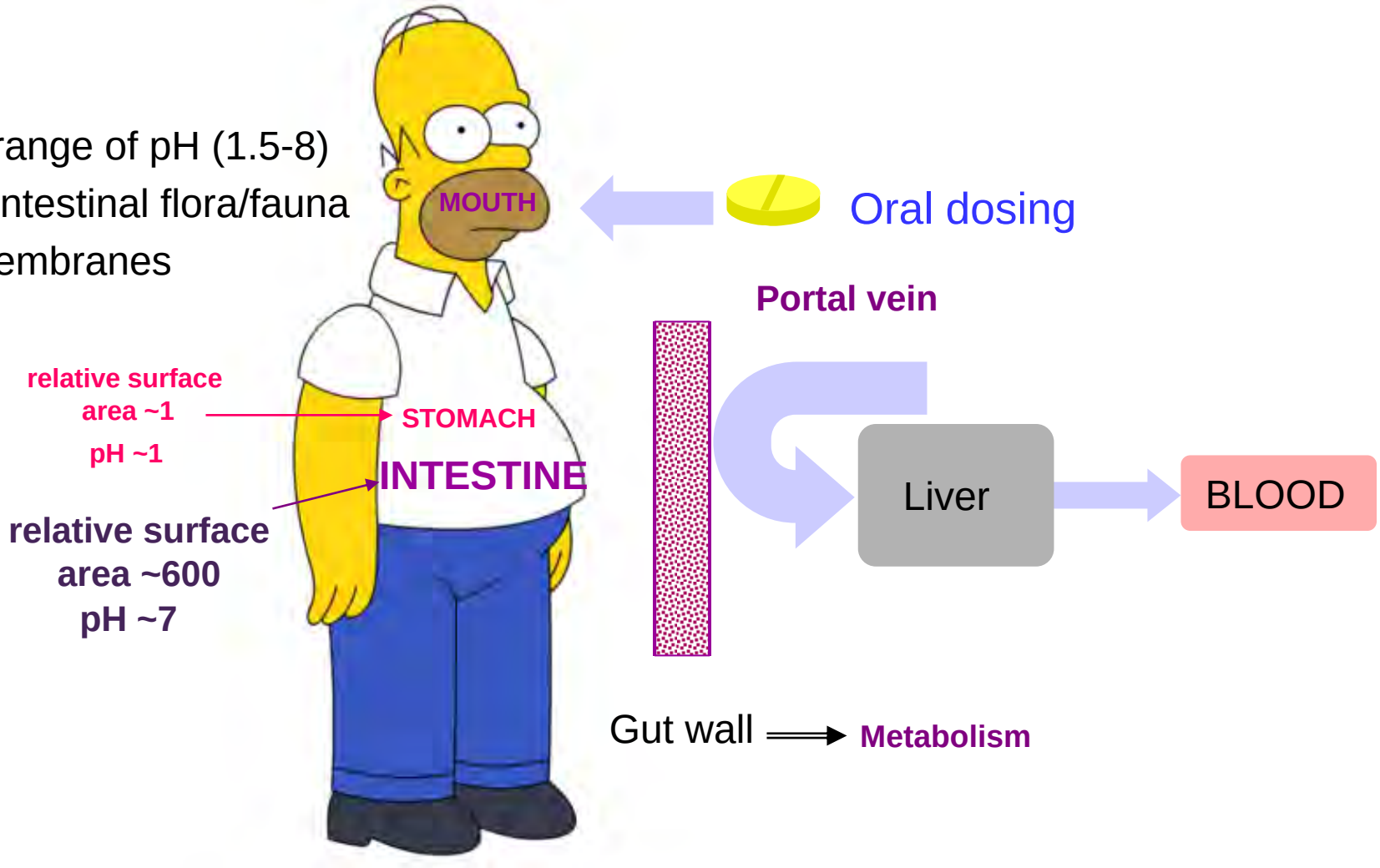
$$F\% = \frac{\text{AUC po / dose}}{\text{AUC iv / dose}} \times 100$$

Compound A has low oral bioavailability

Absorption

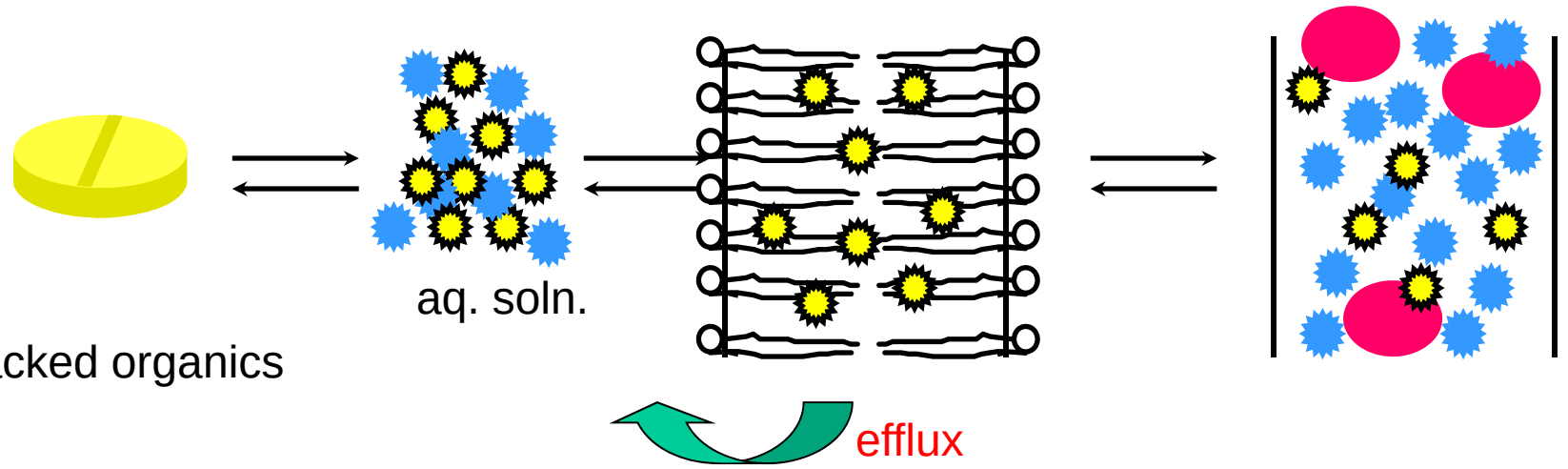
The process by which a drug moves from its site of administration to the systemic circulation

- dissolve
- survive range of pH (1.5-8)
- survive intestinal flora/fauna
- cross membranes



Adapted from a slide by Rhona Cox, AZ Charnwood

Absorption – sources of the problem



Dissolving in stomach/intestine
Stable pH 1-7

Crossing membranes (permeability)

- Solubility
- Instability
- Permeability
- Efflux

Absorption - Solubility

Solubility can be measured in a number of
different media: eg, water, (simulated gastric fluid) and
pH values: pH 7.4 (blood), pH 6.5 (small intestine – major site of absorption)

Typical assays for measuring solubility/ dissolution rate:

- "Traditional" solubility / dissolution measurements
  - **Thermodynamic** (equilibrium) measurements
  - values will depend on the crystalline form of the compound
  - caution with amorphous solids!
  - lower throughput

- High throughput turbidometric measurements
  - **Kinetic** measurement from DMSO solutions
  - for newly synthesised compounds
  - quick indication of low solubility

- Calculation/ Prediction from molecular structure
  - in house and commercial programs available

Caution! Need to be aware of differences between thermodynamic and kinetic solubility

Solubility Guidelines

Water solubility values (pH 6.5) and impact on absorption

mg/ml		µM*
> 1	Ideal, absorption not limited by dissolution rate	>2000
0.1 – 1	Acceptable, absorption unlikely to be limited by dissolution rate Formulation could be important	200 – 2000
0.01 – 0.1	Dissolution rate is likely to limit absorption Could be a big problem for high dose drugs Formulation & salt selection critical, may add to development time	20 – 200
< 0.01	Dose size, dissolution rate and formulation critical	< 20

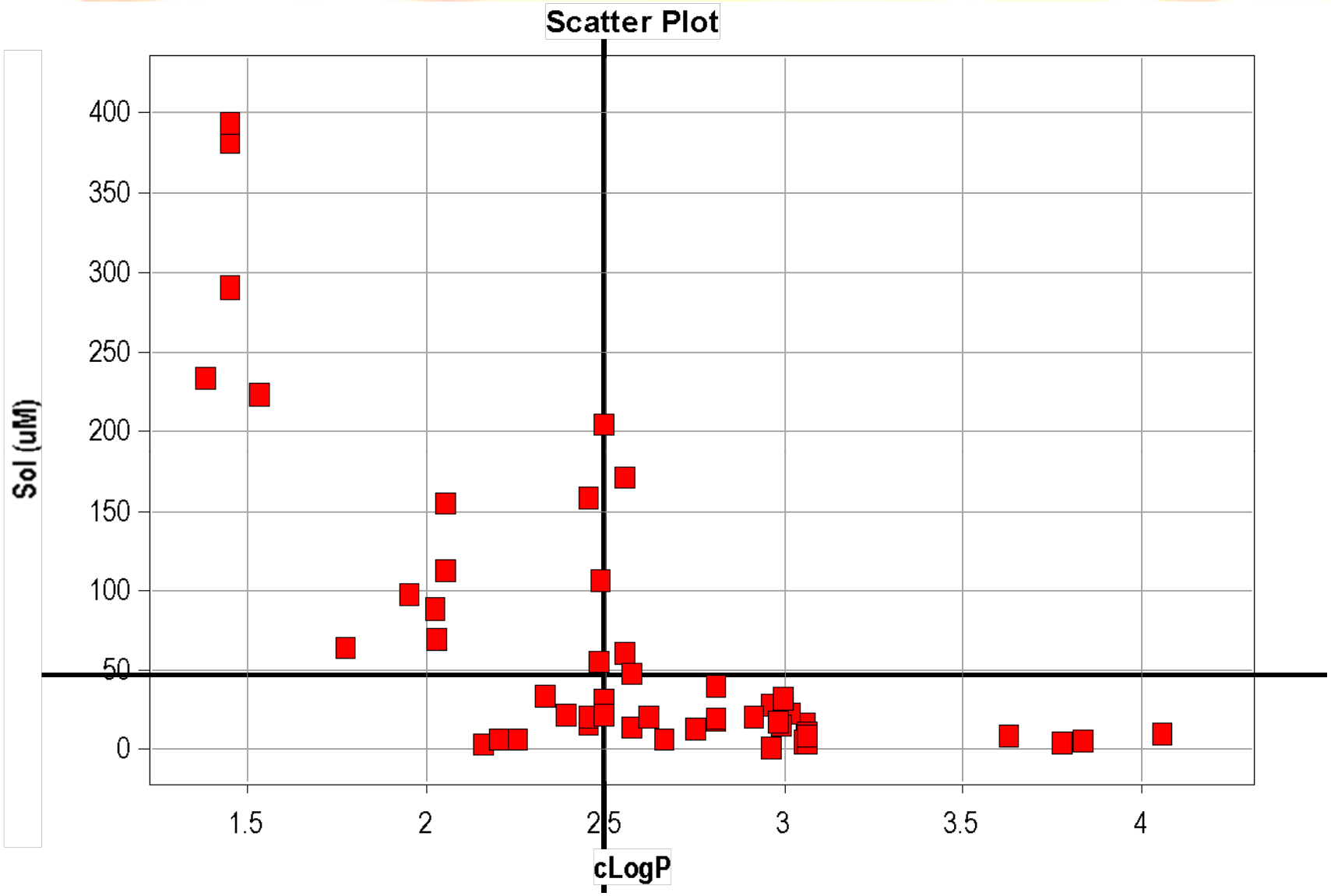
*for typical MWt = 500 compound

Solubility is physical chemistry

What factors govern solubility?

"Brick Dust or Greaseballs"*:*
J. Med. Chem. **2007,** *50,* 5858-5862

Solubility vs ClogP



Series needs clogP <2.5 for solubility >50µM (~0.025mg/ml)

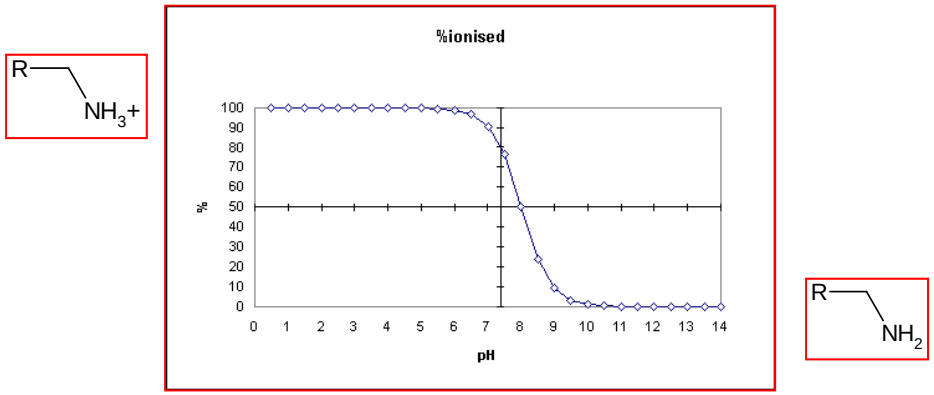
LogP and logD

LogP is pH independent

LogD is pH dependent

LogP only takes into account the concentration of ***neutral*** species in the organic and aqueous layers.

LogD is the log distribution coefficient at a particular pH ***and will vary according to the ability of the molecule to ionise.***



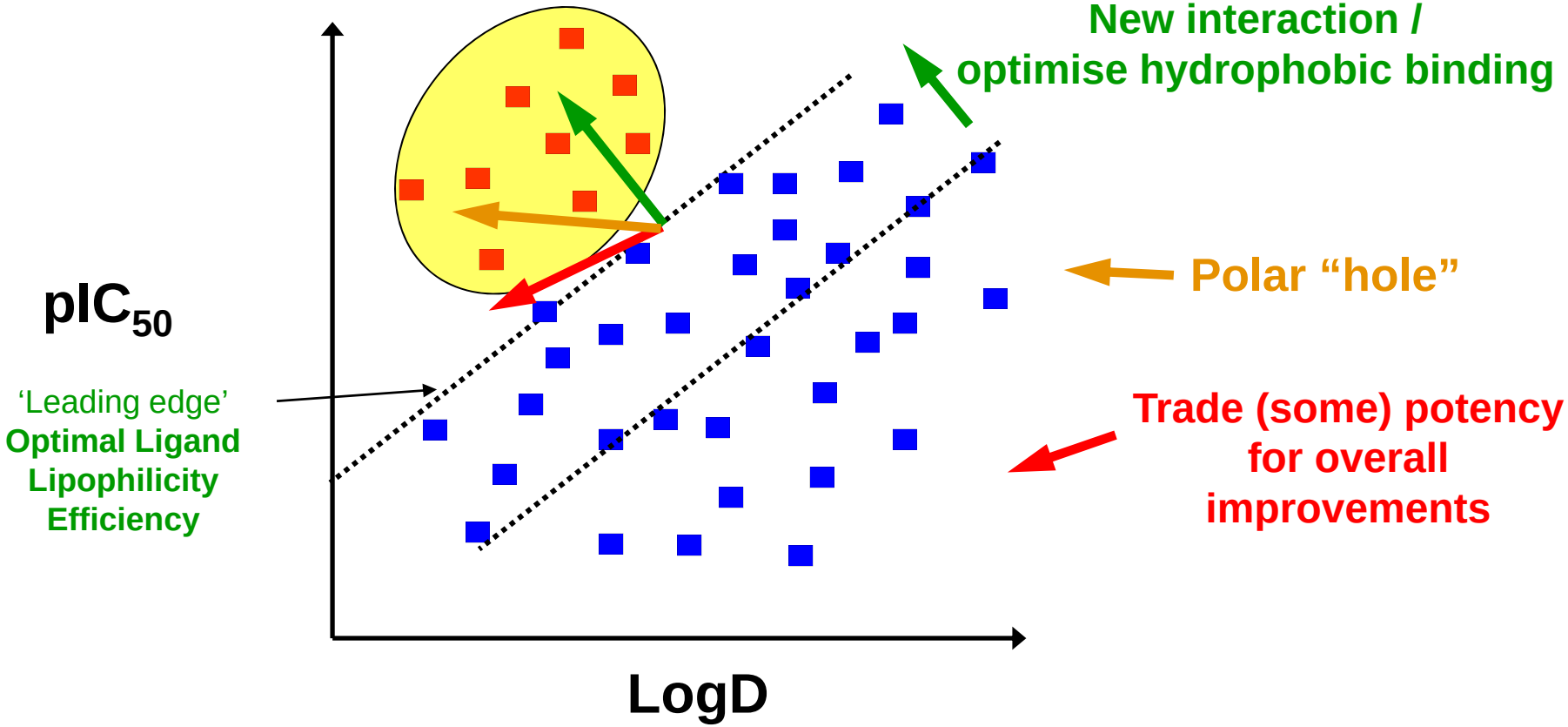
LogD at pH 7.4 is often quoted to give an indication of the ‘true’ lipophilicity of a drug at the pH of blood plasma (pH 7.4) – can be calculated from LogP values.

Ligand Lipophilicity Efficiency in Optimisation

$pIC_{50} - logD$

Leeson & Springthorpe, *Nat. Rev. Drug Disc.* **2007**, *6*, 881

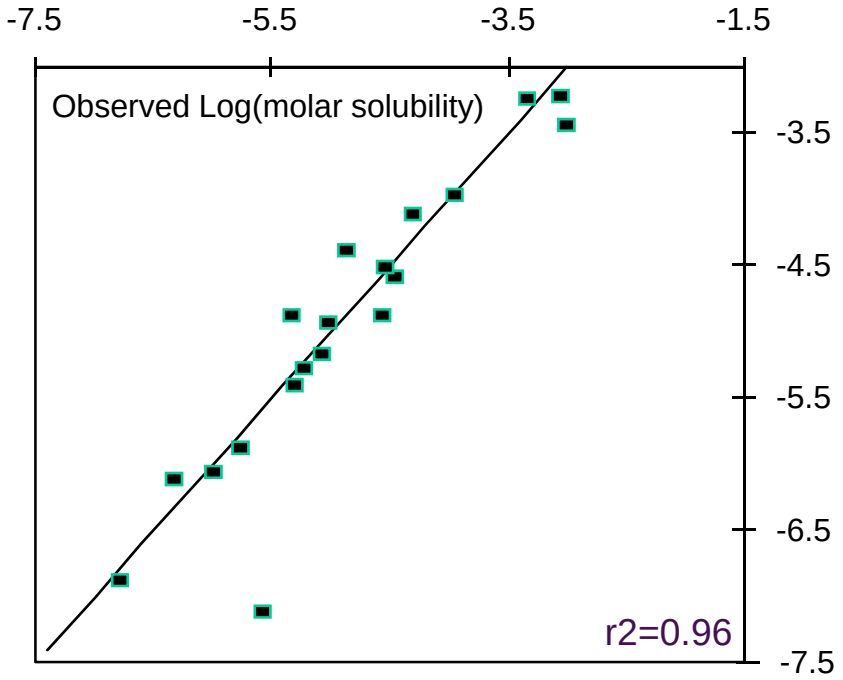
- **In optimising leads, seek potency increases *without* increasing lipophilicity**
- **Control *ligand lipophilicity efficiency* -** How efficient is every lipophilic portion of the compound?
- **Target: LLE>5.0 when IC_{50} < 10nM, logD ≤ 3**



….& promiscuity, hERG, phospholipidosis, PPB, P450, insolubility, metabolic clearance, exposure (Vd)…*etc*

Predicted vs Observed Aqueous Solubility

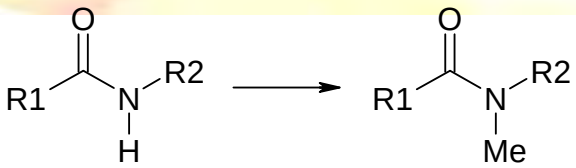
Series of Lipoxygenase Inhibitors:



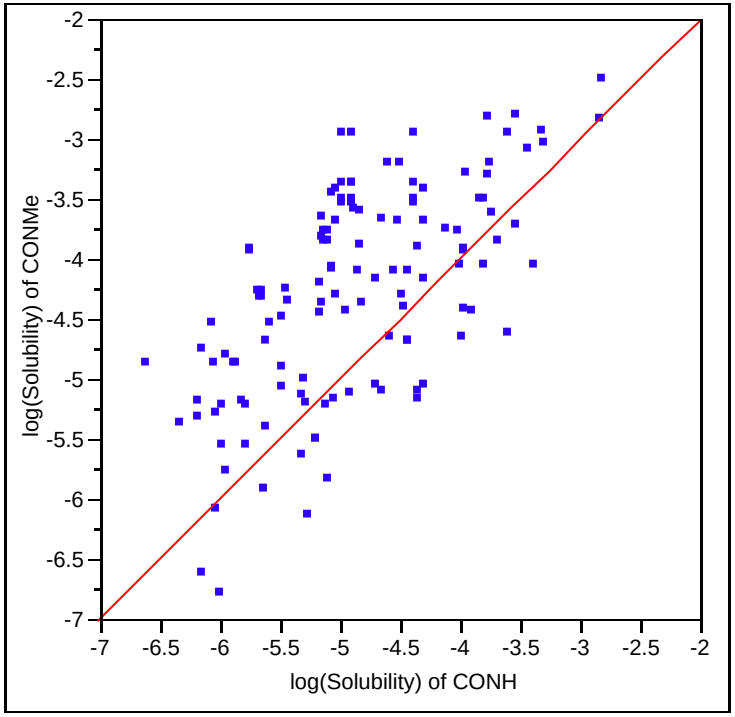
$$\log S = -1.16 \times \log P - 0.018 \times Mpt + 0.93$$

- Mpt reflects energy required to break crystal lattice
- LogP reflects energy required for solute to enter aqueous phase
- Lowering melting point and logP increases solubility

Example: Methylation of amides

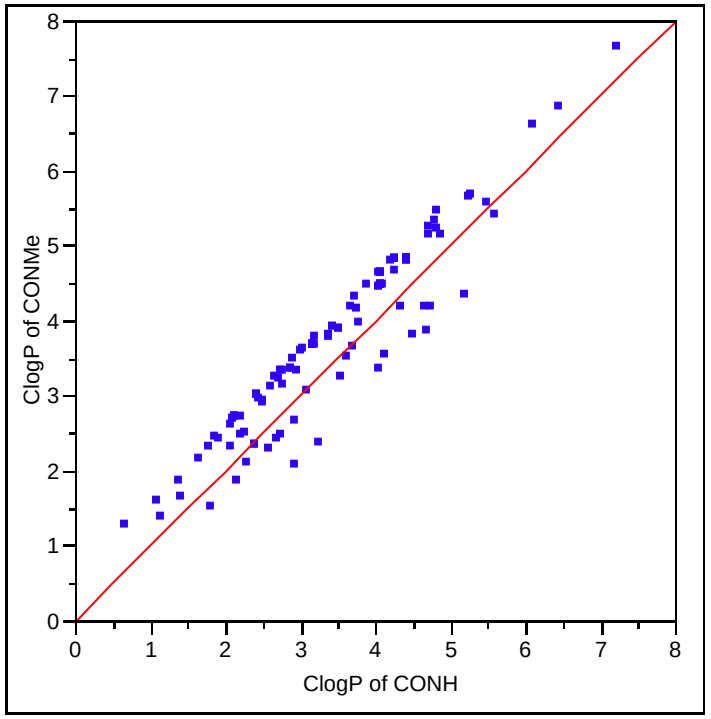


Survey of whole company database of solubility



Mean change = +0.61
For 77% of cases, CONMe is more soluble than CONH

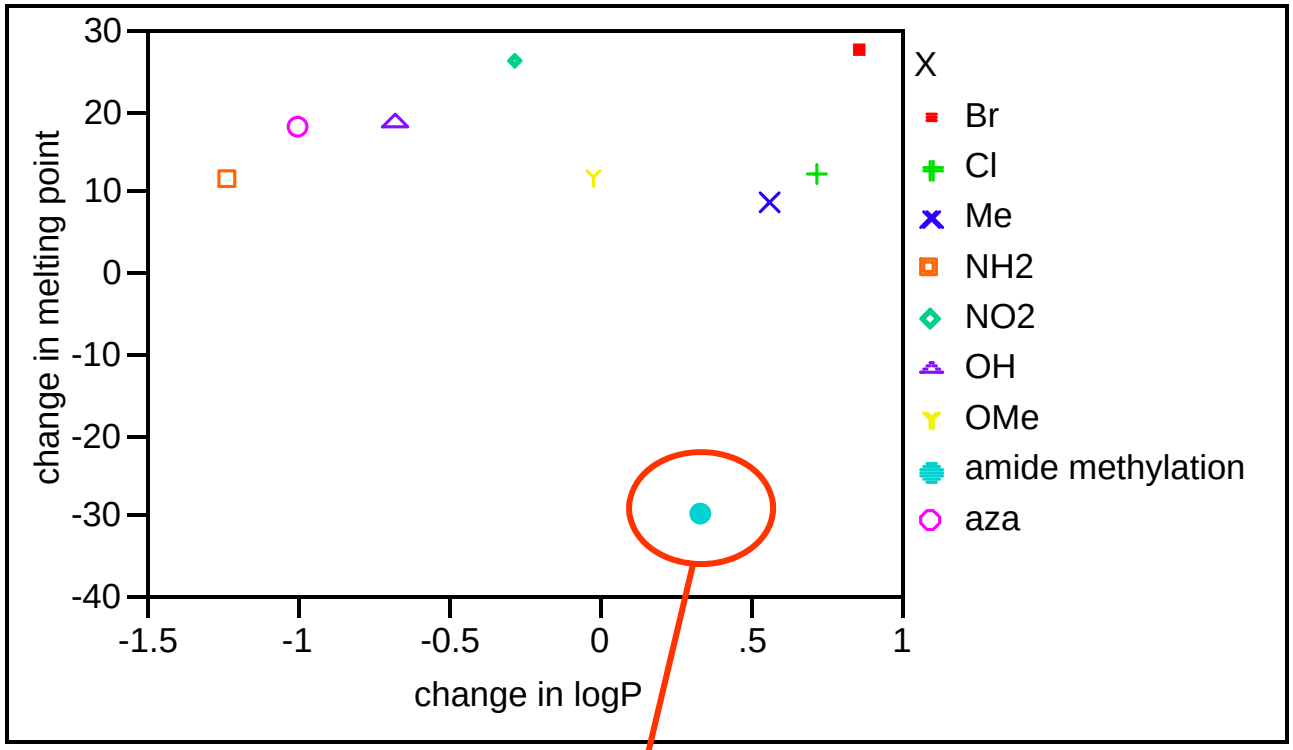
Not lipophilicity driven



Mean change = +0.34
For 82% of cases, CONMe is more lipophilic than CONH

Thanks to: Andrew Leach, AstraZeneca Alderley Park

The solid state & melting points



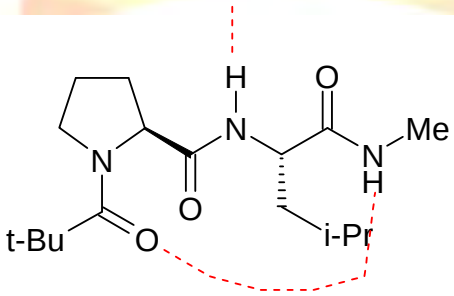
Average change in logP and MPt for matched pairs (data from Beilstein)

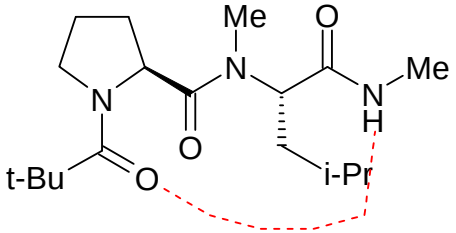
CONMe has higher average logP **lower average melting point** than CONH

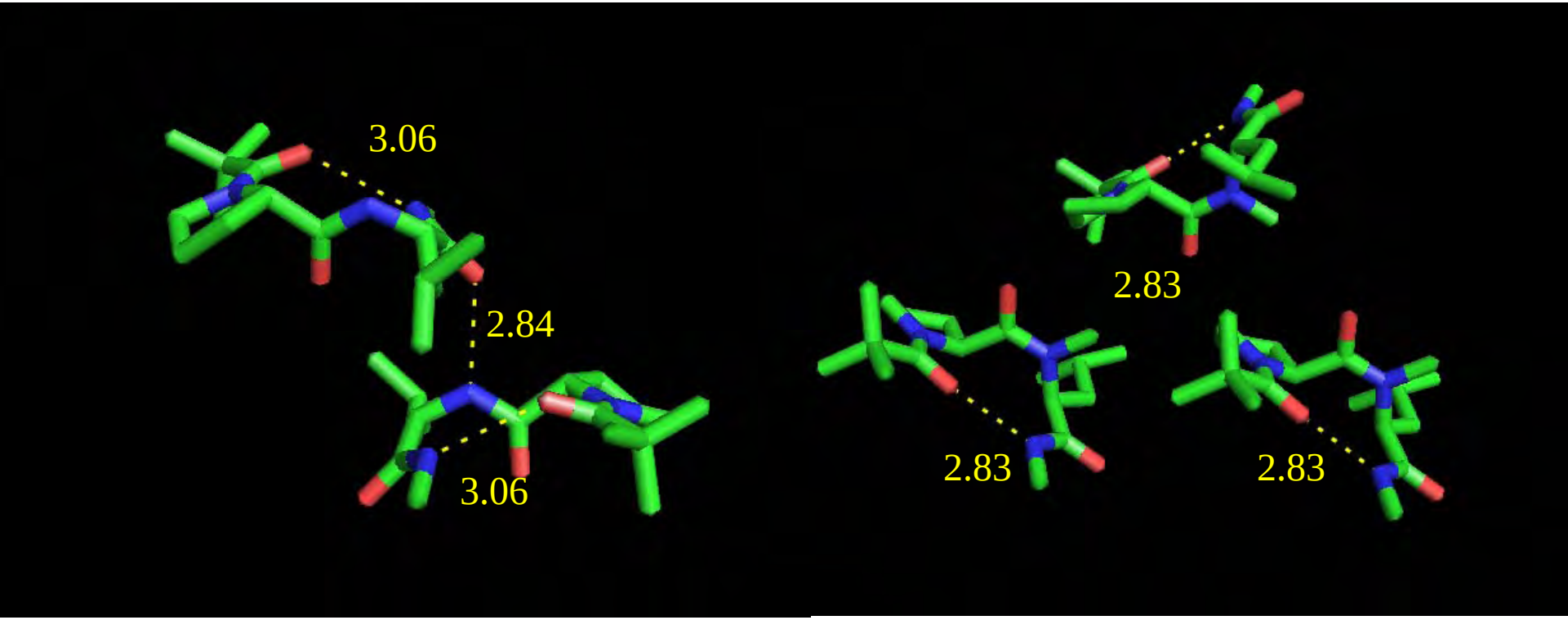
Thanks to: Andrew Leach, AstraZeneca Alderley Park

Journal of Medicinal Chemistry (2006), 49(23), 6672-6682

The solid state & melting points



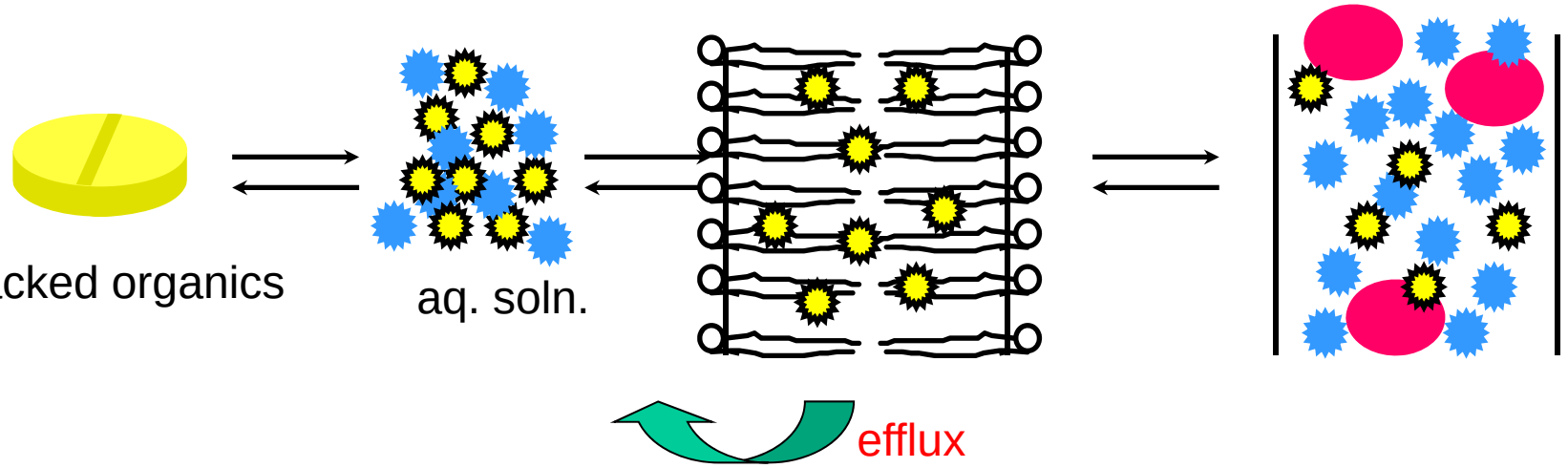




Introduction of CONMe eliminates intermolecular H-bonding:
lowers lattice energy, lowers melting point & increases solubility

Thanks to: Andrew Leach, AstraZeneca Alderley Park

Absorption – sources of the problem



Dissolving in stomach/intestine
Stable pH 1-7

Crossing membranes (permeability)

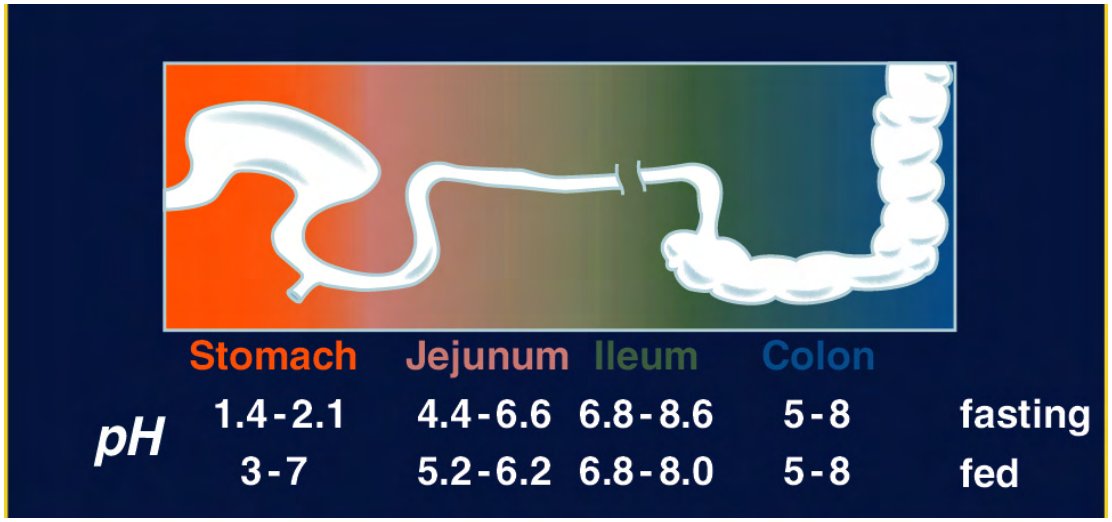
- Solubility
- Instability
- Permeability
- Efflux

→ Measure stability in GI fluids/range of pHs

Absorption: pH ranges and GI stability

Compounds administered orally will encounter:

- A pH range from 1 to 8 in the GI tract
- Digestive and bacterial enzymes



Compounds may be unstable to acid pH range (1-3)

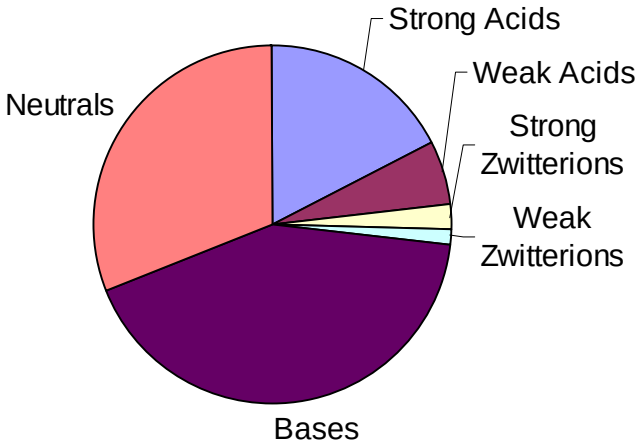
- measure stability over time as a measure of pH

Compounds may be unstable to lipases, peptidases, esterases etc

- use gastric fluid ex vivo or purified enzymes

Why is pKa important ?

Many marketed drugs are acids or bases



Acids, bases and neutrals have very different ADMET properties:

- Adding ionizable groups can enhance solubility (pH dependent)

but...

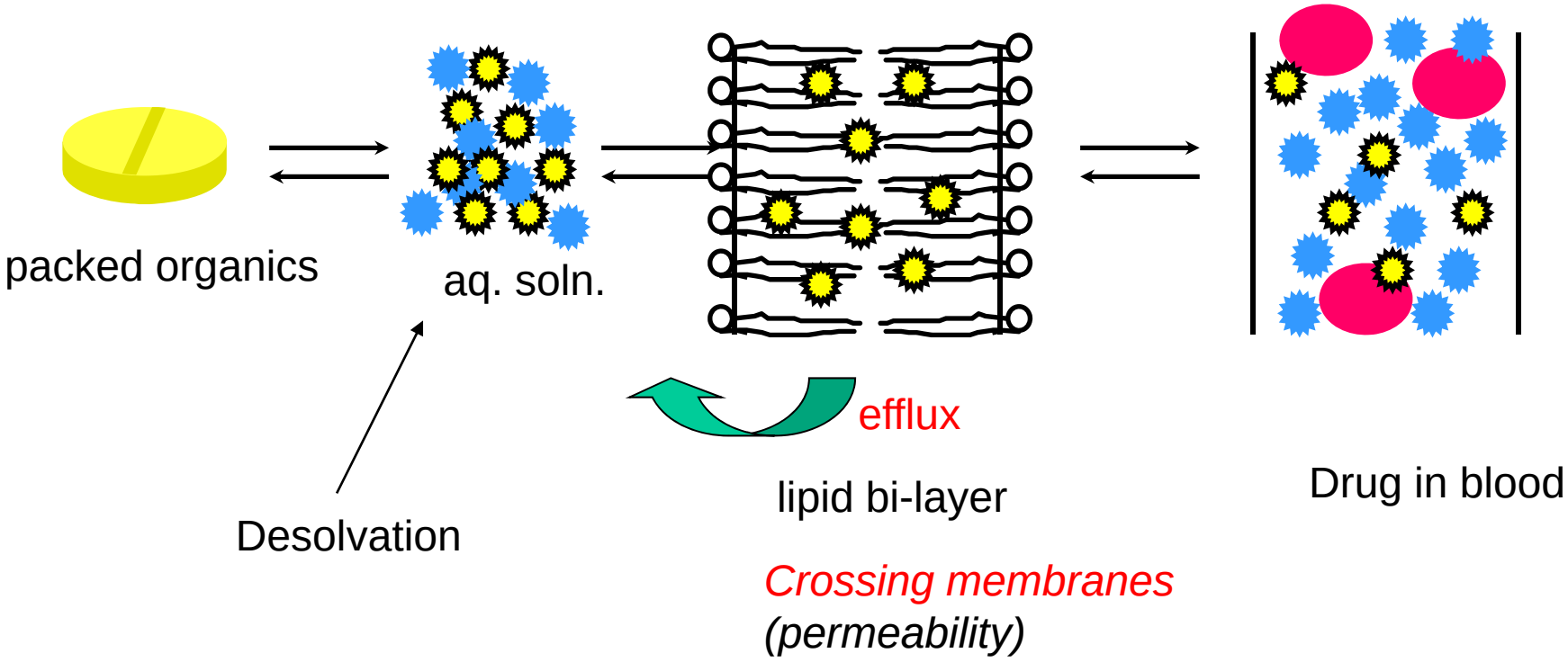
- Ionized species pass through lipid membranes at a much lower rate than neutrals

pKa can be calculated, measured ... and modified by the chemist!

The proportions of charged and uncharged forms depends on the **pH** and **pKa**:

$$\% \text{ ionized} = \frac{100}{1 + \text{antilog}(pH - pKa)}$$

Absorption – sources of the problem



Crossing membranes
(permeability)

- Solubility
- Instability
- Permeability
- Efflux

Cell membrane permeability assays

Caco-2 cell system

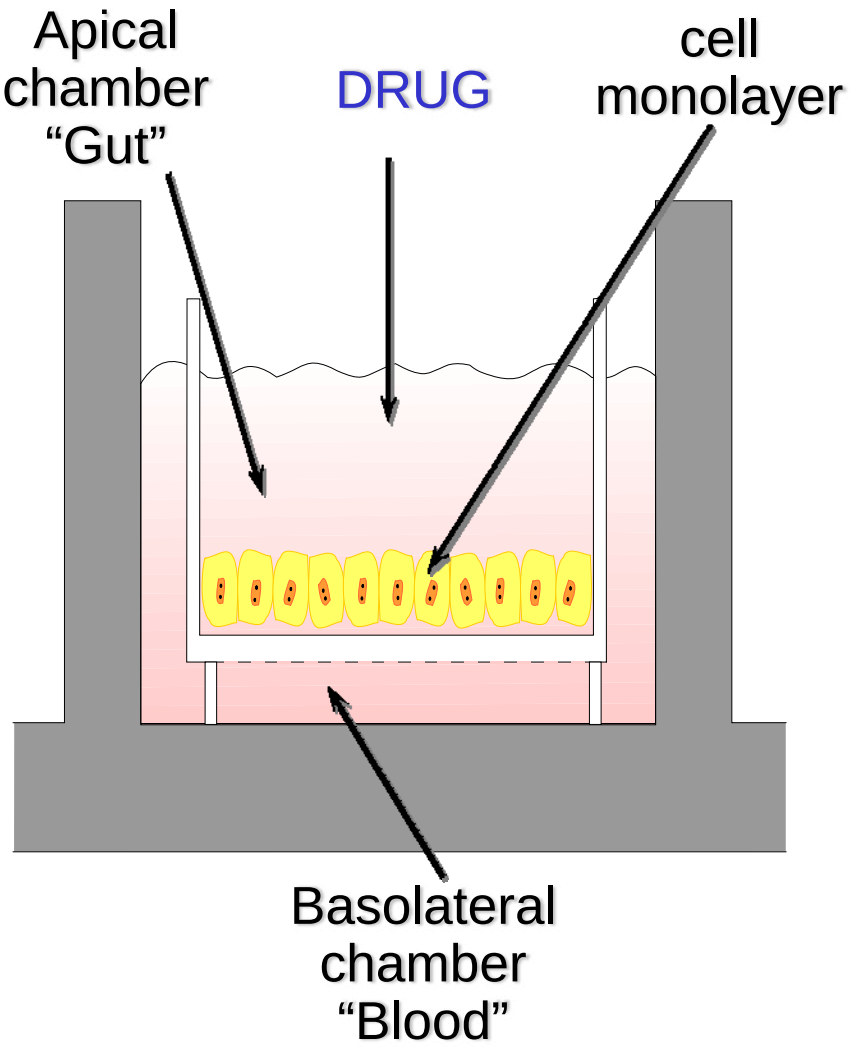
- High throughput method, widely used

- Monolayer of a human intestinal cell line (Caco-2) is grown on a filter support

- Transport is typically measured in Apical ('A') to Basolateral ('B') direction

-Best measure of passive transcellular transport

- Many reports of good correlations between Caco-2 cell permeability & in vivo absorption



Other cell lines available
(eg. *Madin-Darby Canine Kidney (**MDCK**)* epithelial cells)

Journal of Pharmaceutical Sciences 1999, 88, 28-33

Permeability is also physical chemistry

What factors govern permeability?

Lipinski Rule of 5

• Poor permeability is more likely when:

- Mol Weight > 500
- LogP > 5
- > 5 H- bond donors (eg OH, NH)
- The sum of N and O atoms > 10

Adv. Drug Delivery Rev. 1997, 23, 4-25
J. Pharm. Toxicol. Methods 2000, 44 235-249

• Since Lipinski's data set relates to marketed drugs, and
• Lead optimisation often involves increasing complexity,
• The concepts of 'lead-like' parameters and 'ligand efficiency' have arisen:

"Astex Rule of 3" for optimal lead compounds:

- Mol Weight < 300
- LogP < 3
- No. donors < 3
- No. acceptors < 3

See: Congreve et al: *J. Med. Chem.*, **2008**, 51, 3661 (excellent recent review) & *DDT,* **2003**, 8, 876 (Rule of 3)
Teague et al: *Angewandte Chemie, International Edition* **1999**, 38, 3743 (lead-like)

Molecular Size & Lipophilicity

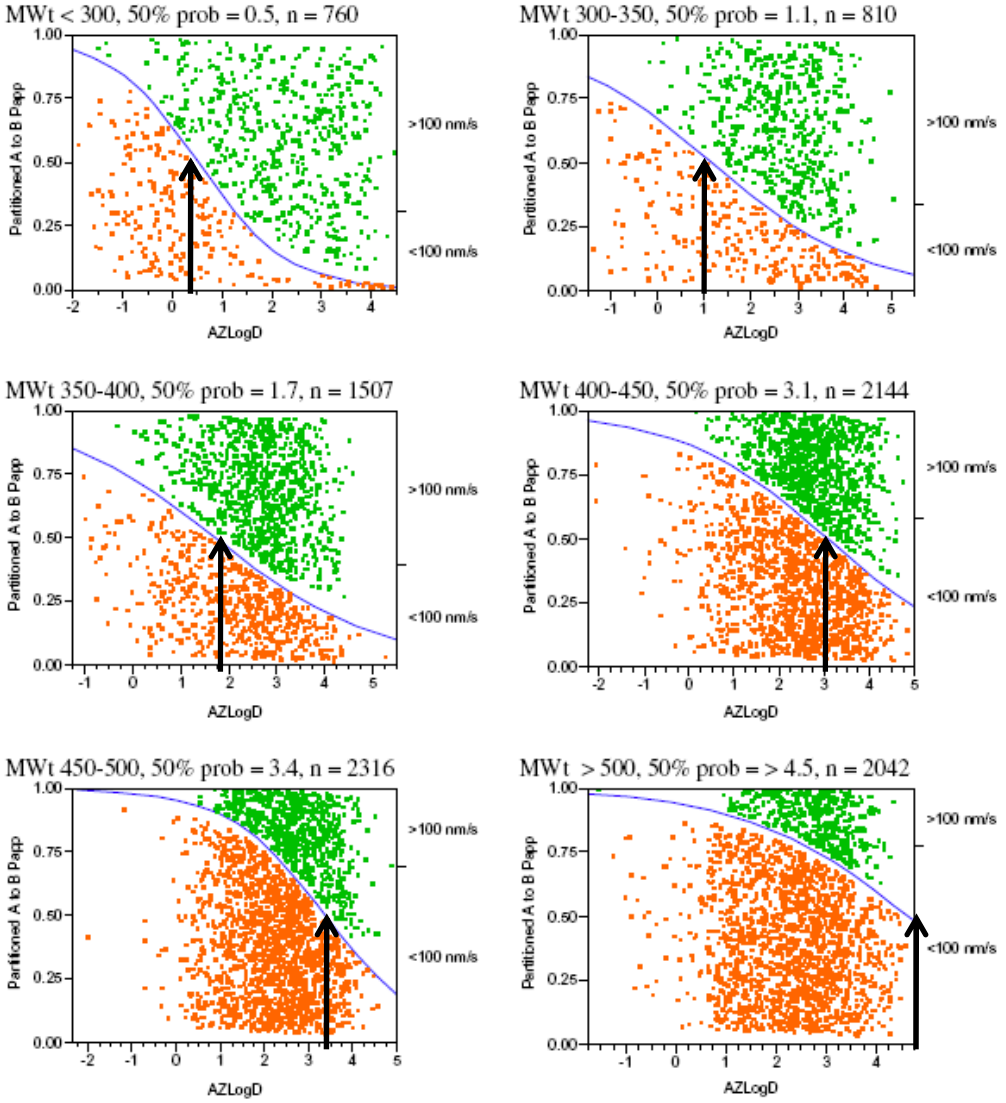


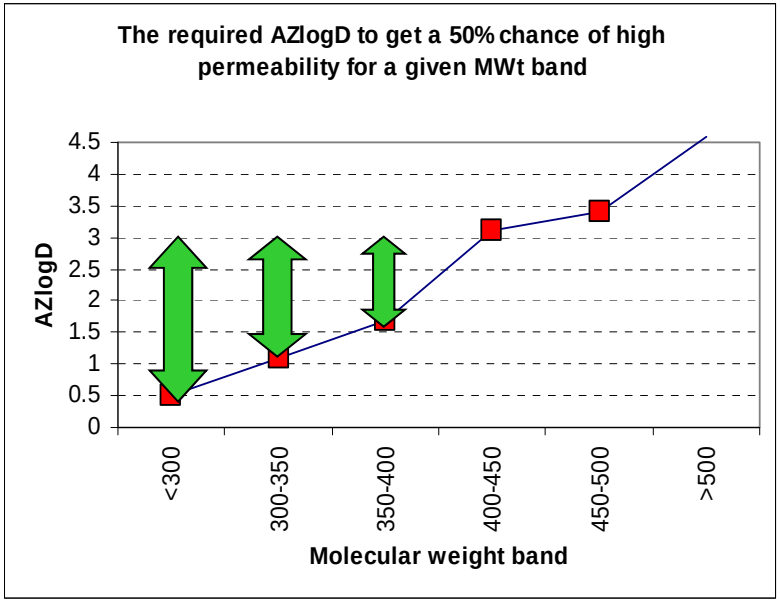
Figure 5. Logistic plots versus *AZlogD* for individual molecular weight bands.
Arrows depict logD where 50% of compounds show 'good' permeability

Generally, good permeability can be achieved by:

- low molecular weight
- high lipophilicity

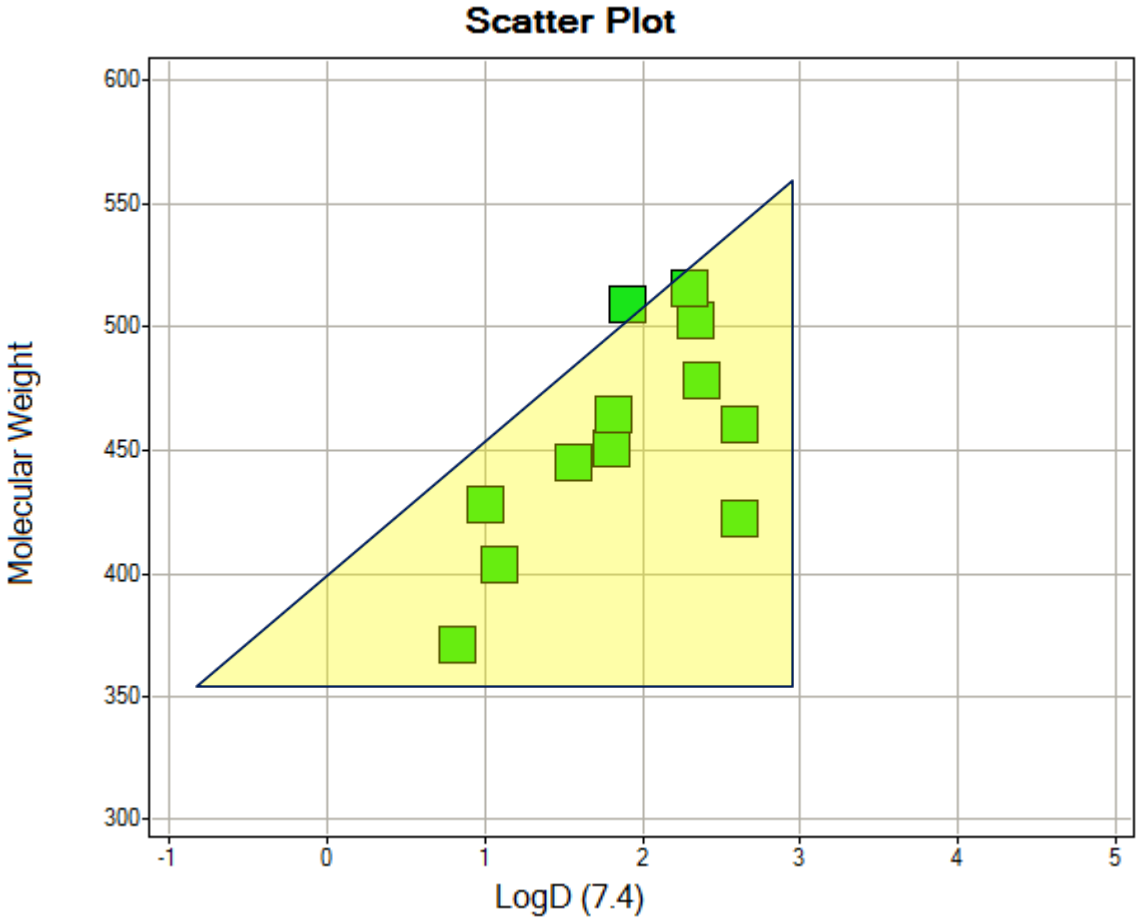
Since we don't want to increase lipophilicity too much, need to keep an eye of molecular weight...

As MWt increases, observe a diminishing logD window to maintain good permability



Waring, M. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2844
See also: Johnson, T.; Dress, K.R.; Edwards, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5560

Optimal Window & Development Compounds



- Development compounds often lie within optimal window – ‘Golden Triangle’
- More polar compounds allowed by lower MWt
- Does this lead to increased chance of success?

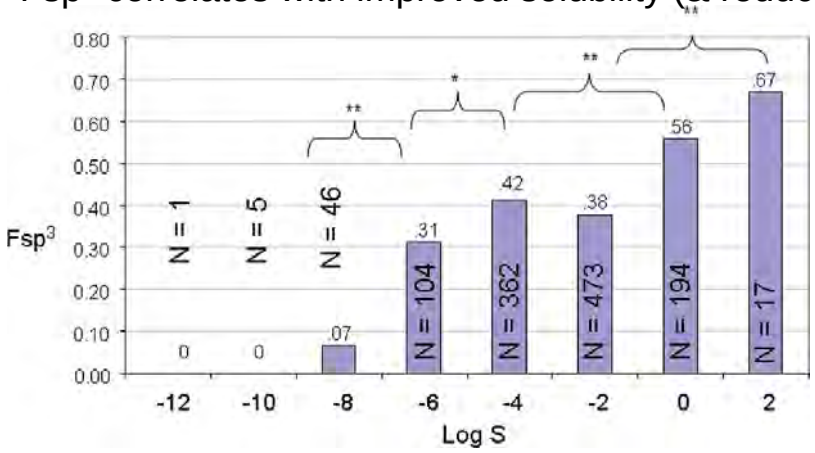
See also: Johnson, T.; Dress, K.R.; Edwards, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5560

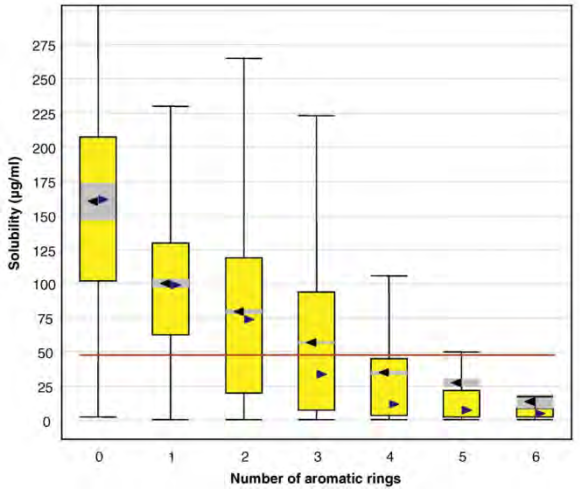
Impact of Molecular Shape / Complexity

- Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success (Lovering, Wyeth)

J. Med. Chem. 2009, 52, 6752–6756

- A simple & interpretable measure of the complexity of molecules is carbon bond saturation, as defined by Fraction sp^3 (Fsp^3) where: $Fsp^3 = (\text{number of } sp^3 \text{ hybridized carbons}/\text{total carbon count})$
- Significant enrichment of increased saturation as compounds progress through clinical testing:
- Fsp^3 correlates with improved solubility (& reduced Mpt):





- The impact of aromatic ring count on compound developability – are too many aromatic rings a liability in drug design? (Ritchie & Macdonald, GSK)

Drug Discov. Today 2009, 14, 1011-1020

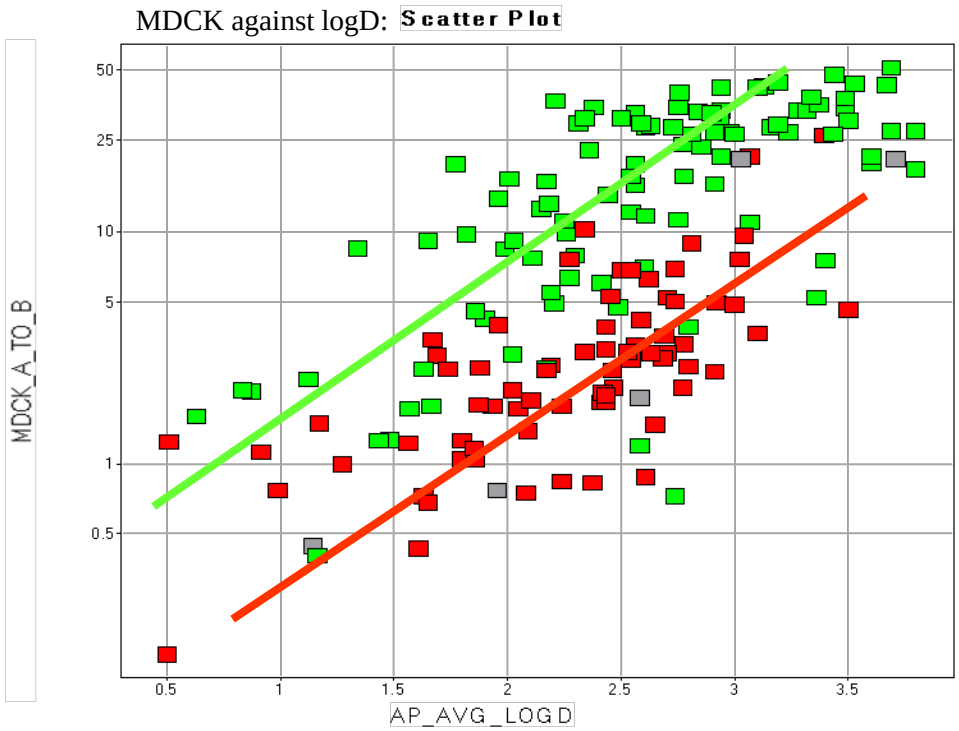
  - As aromatic ring count increases:
    - Lipophilicity increases
    - Solubility decreases (even when clogP remains constant)
    - Protein binding, Cyp inhibition & hERG liability increase (later...)
  - >3 Ar rings correlates with poorer compound developability & increased risk of attrition in development

- Molecular flexibility (# of rotatable bonds) has also been shown to correlate with oral bioavailability (*Veber, GSK)*

J. Med. Chem. 2002, 45, 2615

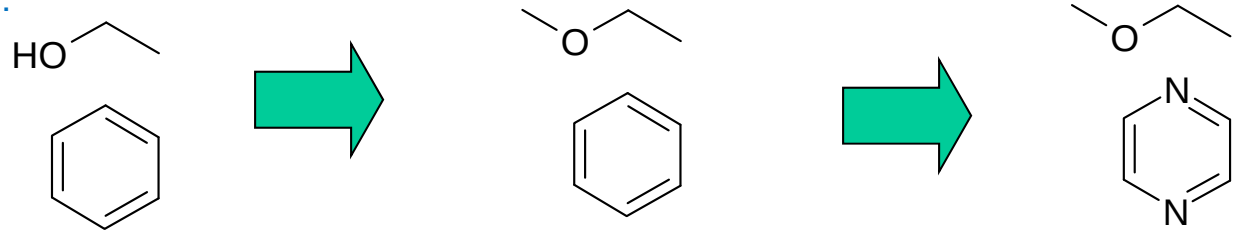
H-bonding & Permeability

Minimising number of H-bond donors is a good strategy to improve permeability:



1 donor green,
2 donors red,
>2 grey

Structural changes:

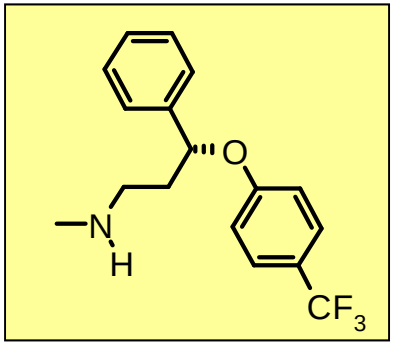


Removal of donor improves permeability but increases logD outside target range

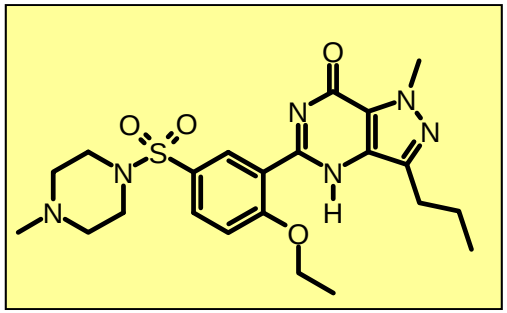
logD increase can be offset by introducing heteroatoms

Polar Surface Area (PSA)

The Polar Surface Area (PSA) of a molecule is defined as the area of a molecule's van der Waal's surface that arises from O or N atoms, or hydrogen atoms attached to O or N atoms.

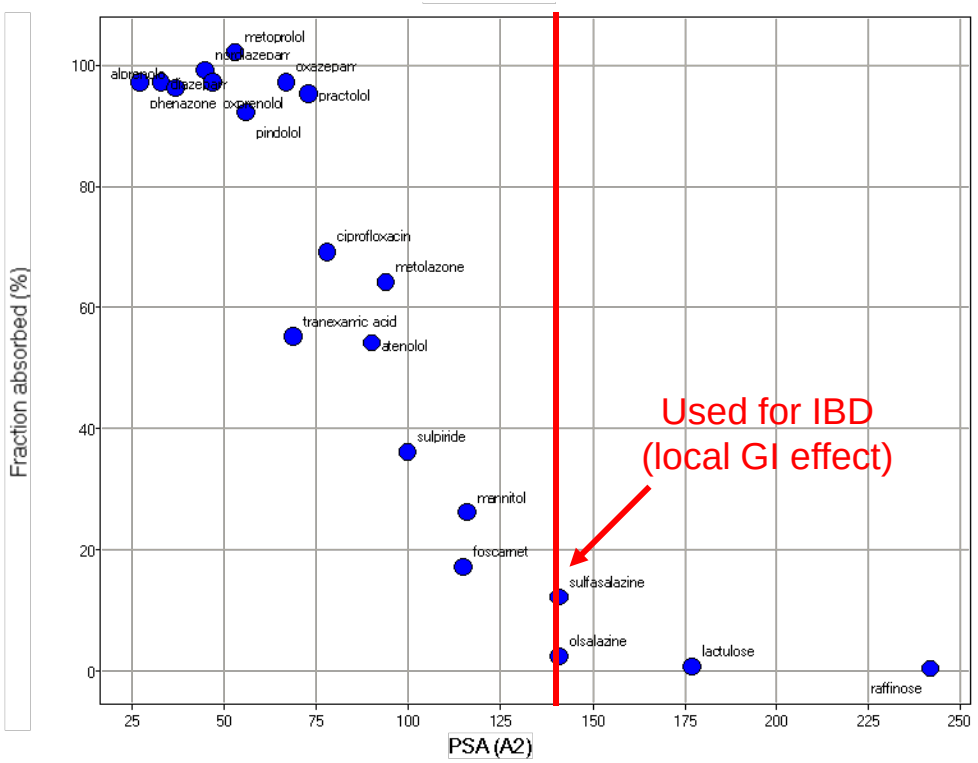


Fluoxetine 23A^2



Sildenafil 109A^2

Human intestinal permeability v PSA



Veber reported that best probability of good oral bioavailability if PSA < 140 A^2

J. Med. Chem. 2002, 45, 2615

Maximum Absorbable Dose (MAD)

MAD (mg) = S x Ka x SIWV x SITT

Pharmaceutical Research, Vol. 13,1996,1795-1798

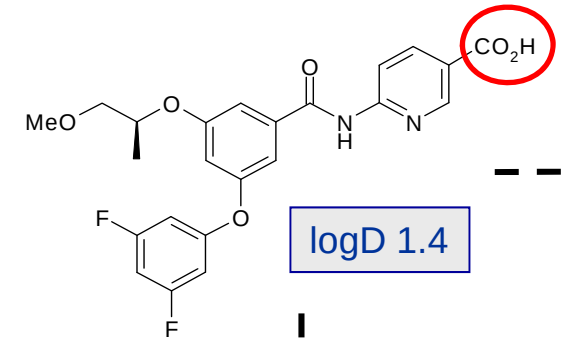
S = solubility (mg/ml) at pH 6.5
Ka = intestinal absorption rate constant (min^{-1})
(derived from rat intestinal perfusion expt - similar to man)
SIWV = small intestine water volume ~ 250 ml for man
SITT = small intestine transit time ~ 270 min (4.5h) for man

MAD = quantity absorbed if the small intestine were saturated with drug for 4.5h (eg, dose 10g/kg to saturate small intestine, how much of the dose will be absorbed)

Impact of MAD:
Take two compounds with projected human dose of 70 mg

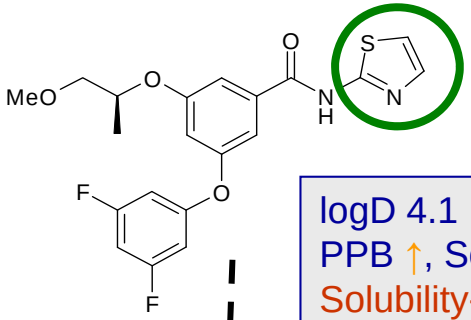
Compound	Ka	Solubility	MAD
Cmpd A	0.001 min^{-1}	5 mg/ml	337 mg
Cmpd B	0.03 min^{-1}	0.001 mg/ml	2 mg

Balancing Solubility & Permeability



logD 1.4

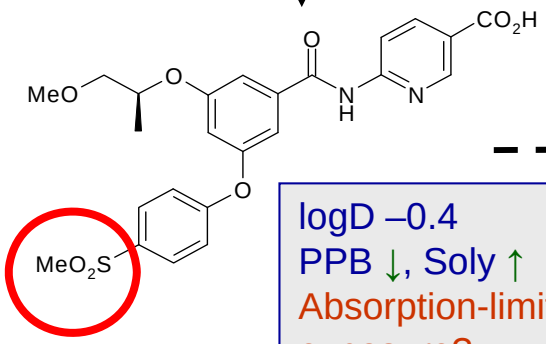
Want to remove acid functionality



logD 4.1
PPB ↑, Soly ↓
Solubility-limited exposure?

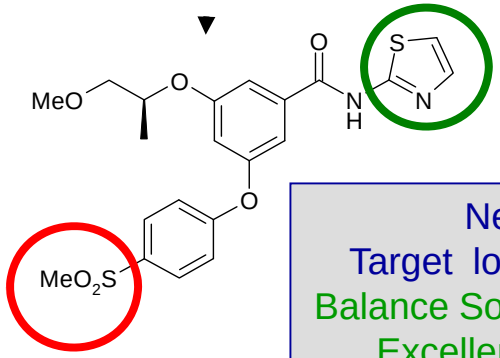
Sought to increase solubility

Combine



logD –0.4
PPB ↓, Soly ↑
Absorption-limited exposure?

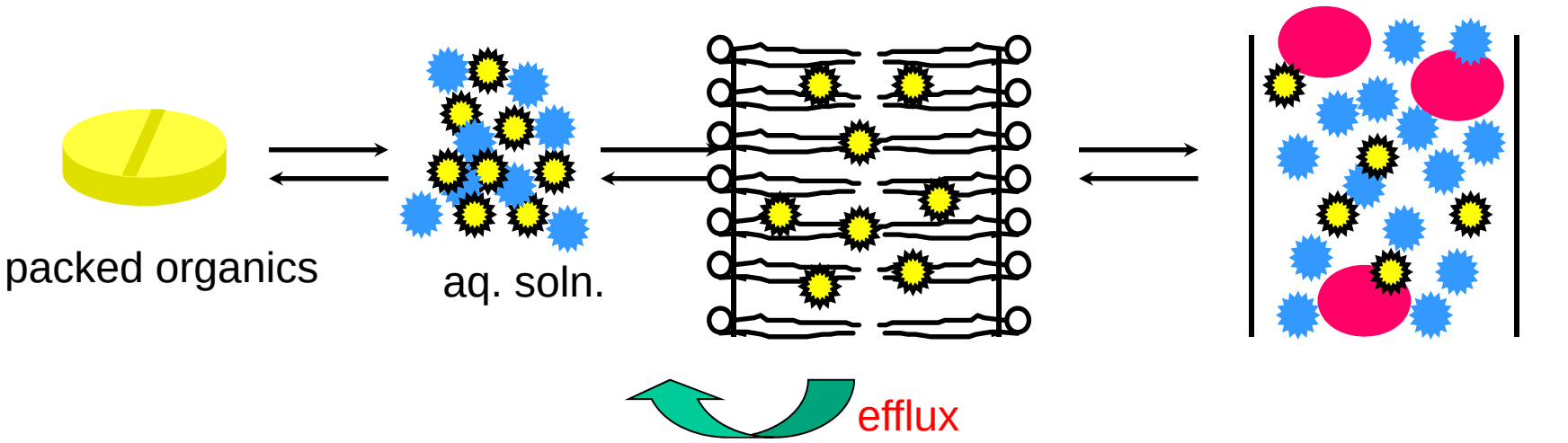
Combine



Neutrals
Target logD range 2-3
Balance Soly & Absorption
Excellent exposure

Example of need to balance permeability & solubility to optimise in vivo exposure

Absorption – sources of the problem



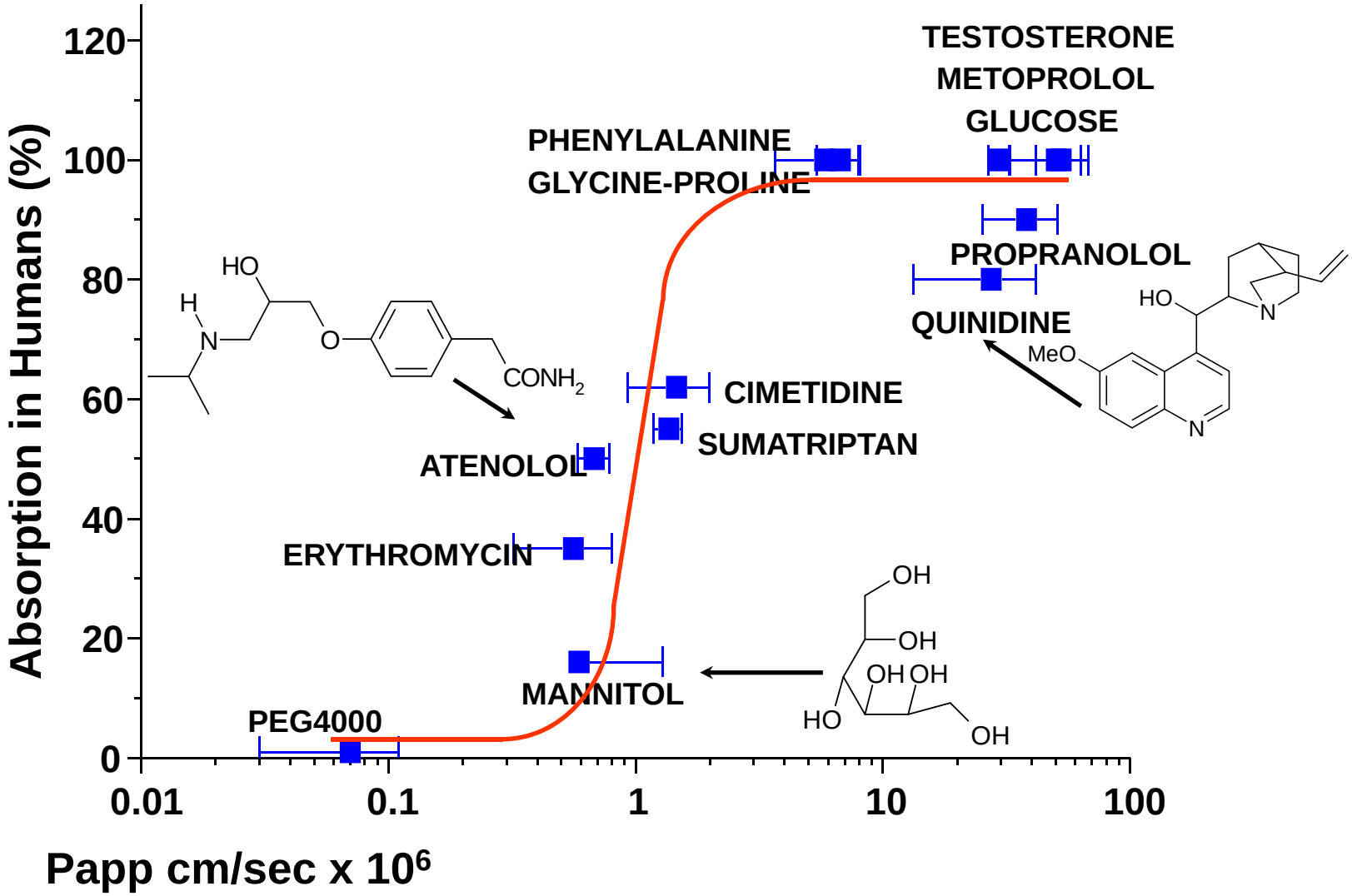
Dissolving in stomach/intestine
Stable pH 1-7

Crossing membranes (permeability)

- Solubility
- Instability
- Permeability
- Efflux

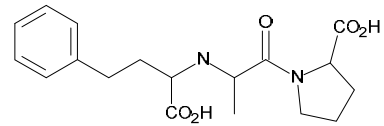
Active Transport

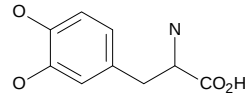
Caco-2 Model of Absorption

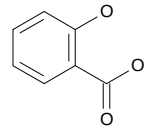


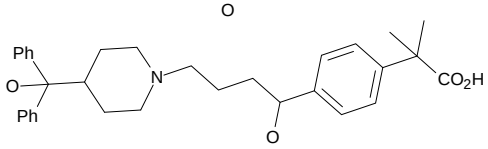
Uptake Transporters

- **Uptake transporters enhance the absorption of drug molecules from the intestine (*Current Drug Metabolism 2004, 5, 109-124*)**
- **They may also enhance the distribution of drugs into certain organs such as the brain and into heptatocytes to enable metabolic or biliary clearance**
- **In contrast to passive diffusion, active transport can be saturated**
  - **Finite number of transporter protein molecules on cell**
- **Examples of uptake transporters and their substrates**
  - **Oligopeptide transporters PEPT1, PEPT2 - enalapril**
  - **Large neutral amino acid transporter (LAT1) - L-dopa**
  - **Monocarboxylic acid transporter (MCT1) – salicylic acid**
  - **Organic anion transporters (OATs) - Fexofenadine**





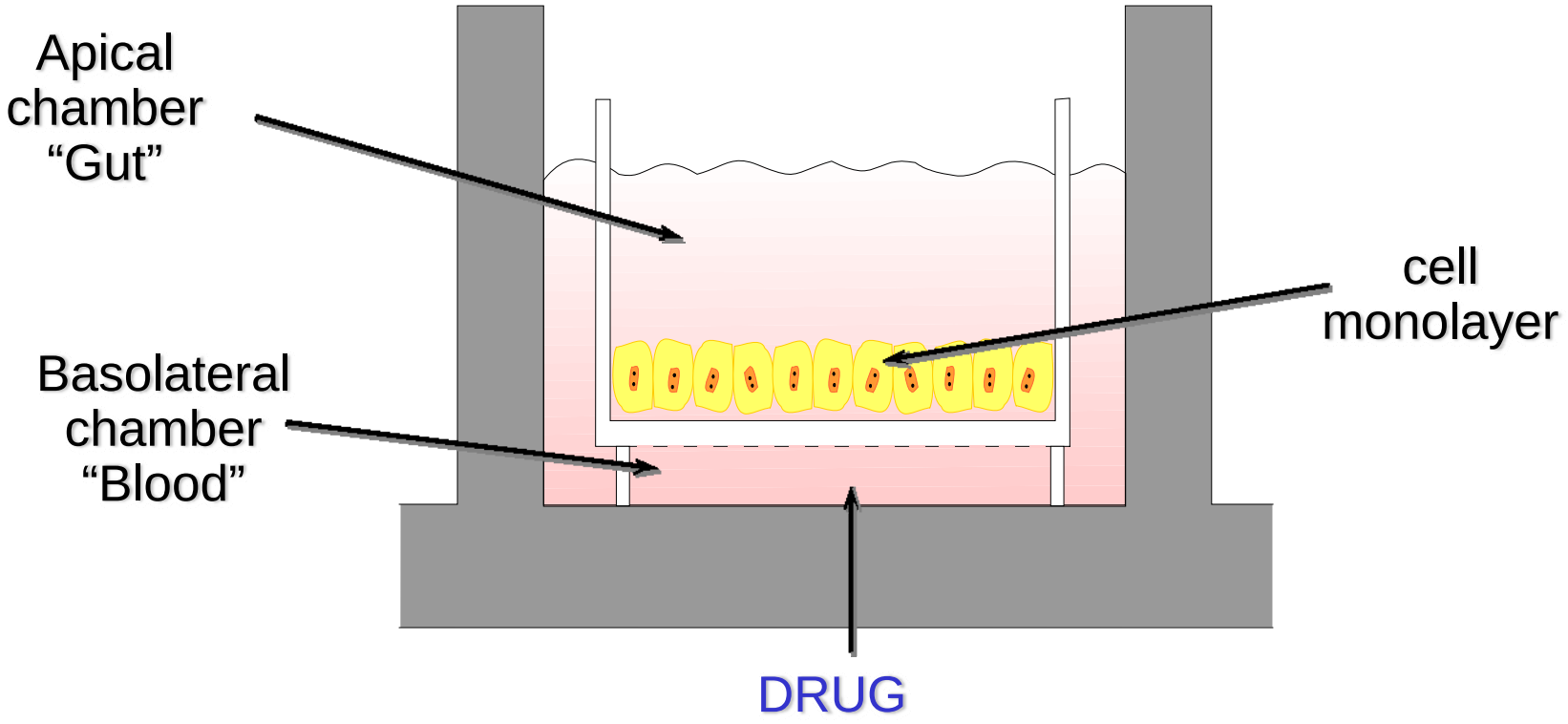




Efflux (P-glycoprotein, P-gp, MDR-1)

- Efflux transporters on the intestinal lumen (apical) oppose the absorption of certain drug molecules
- Mainly a function of a transporter in the cell membrane called P-glycoprotein. Abundant in “protective cells – BBB, intestine, liver, kidney
- Some compounds are a substrate for P-gp
  - Enter the cell by passive diffusion, some of the compound is transported back into the intestinal lumen.
  - No clear SAR but common features emerging
- Some compounds inhibit P-gp
  - An inhibitor (eg verapamil) will increase the absorption of P-gp substrates
- Other efflux transporters exist eg BCRP, MRP2 which effect drug disposition

Caco-2 cells - Transport Experiment (efflux measurement)

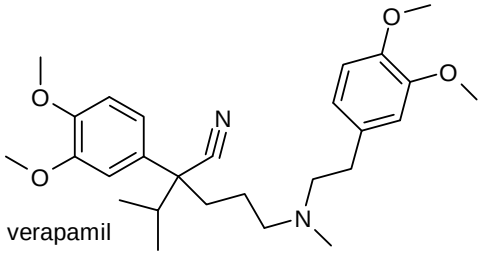


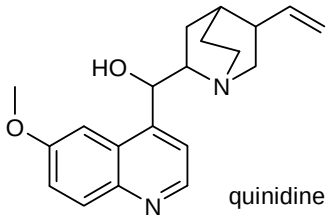
If Papp B-A > A-B then efflux may be operating

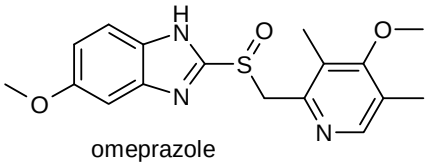
General Characteristics of P-glycoprotein Substrates

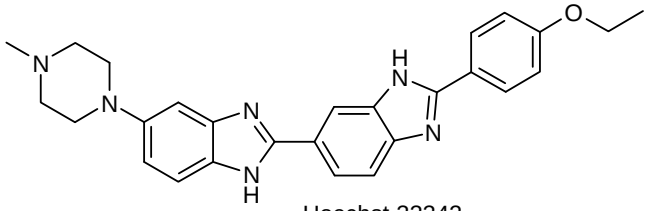
- Lipophilic often with multiple aromatic rings
- High Mol Wt (>400) (increased probability for points of interaction)
- Ampiphilic often with weak cationic group present
- Electronegative groups contributing dipole moment
- 1-3 H-bond acceptors (N, 0) and/or 1-2 H-bond donors (NH, OH)
  - Alkoxy and Carbonyl are frequent functionalities

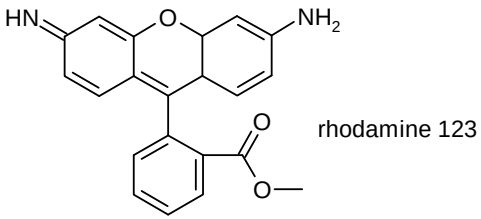
- **As membrane passive diffusion increases, P-gp pump efficiency decreases**
- Review – T.J. Raub, Molecular Pharmaceutics, 2006, 3(1), 3-25.





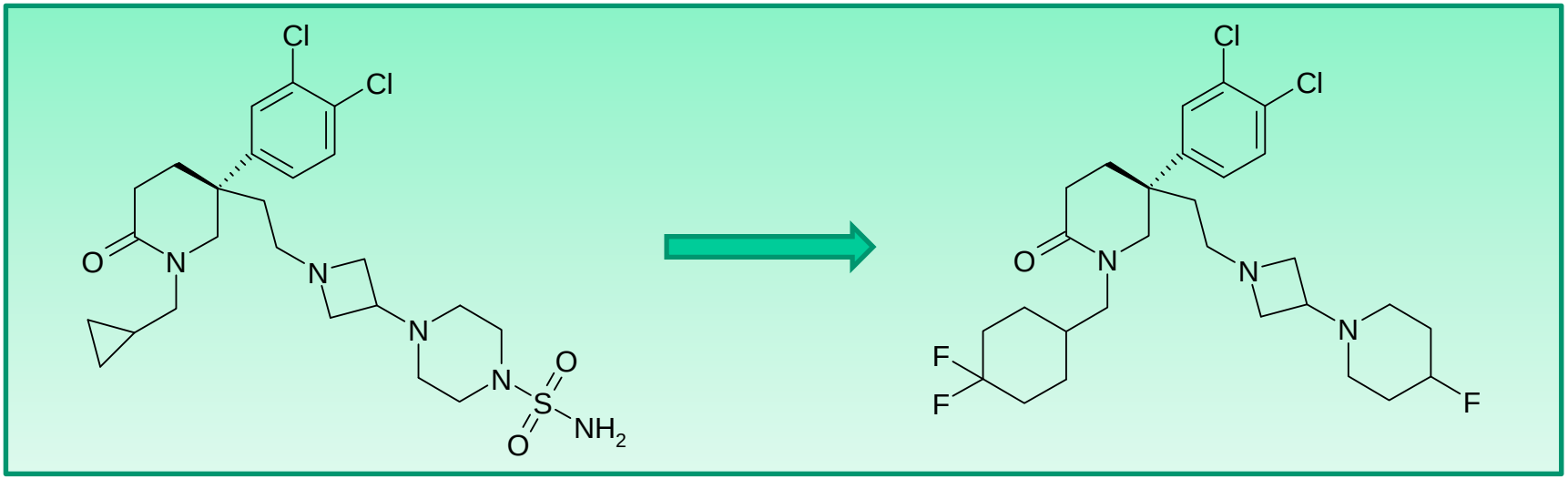






Pfizer NK2 Antagonists

Journal of Medicinal Chemistry (2002),45(24),5365-5377.



UK-224,671
NK2 pIC_{50} = 8.4

clogP = 2.2
Mol weight = 545
PSA = 98 A^2, HBD = 2

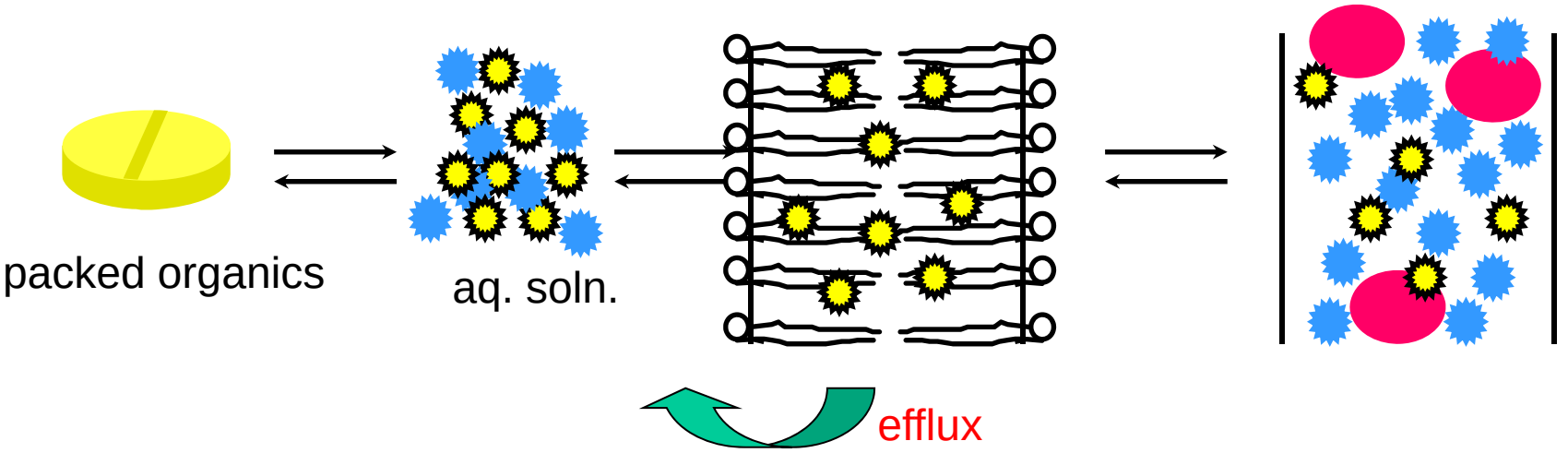
Caco-2 %/h A-B/B-A = 1/18
Rat %F < 20
P-gp KO mice > 20%

UK-290,795
NK2 pIC_{50} = 9.4

clogP = 4.1
Mol weight = 561
PSA = 27 A^2, HBD = 0

Caco-2 %/h A-B/B-A = >35/>35
Rat %F > 80

Absorption – sources of the problem

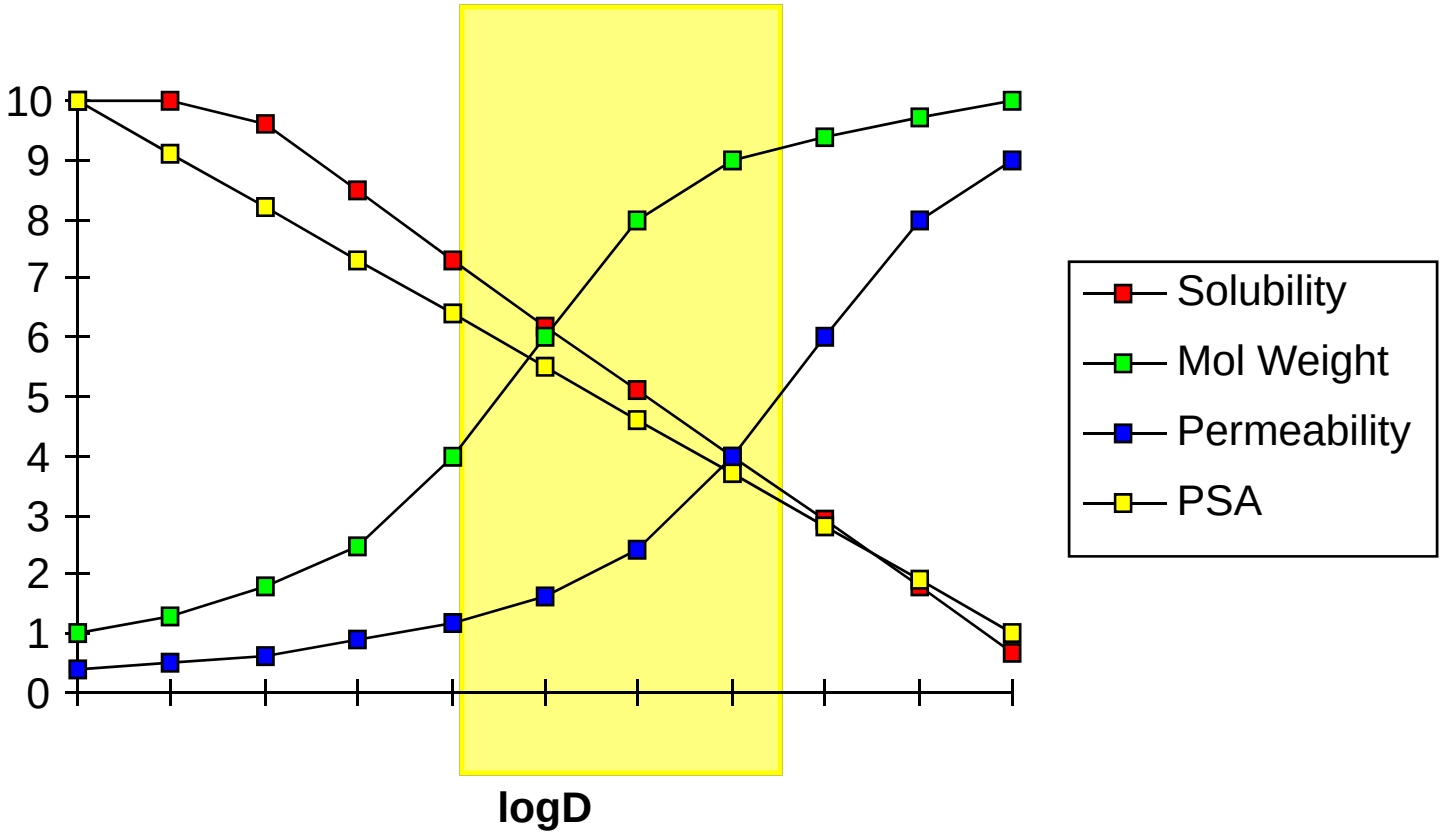


Dissolving in stomach/intestine
Stable pH 1-7

Crossing membranes (permeability)

- Solubility
- Instability
- Permeability
- Efflux

logD vs physicochemical parameters



Over-simplification and series-dependent, but can be a useful working guide to chemistry

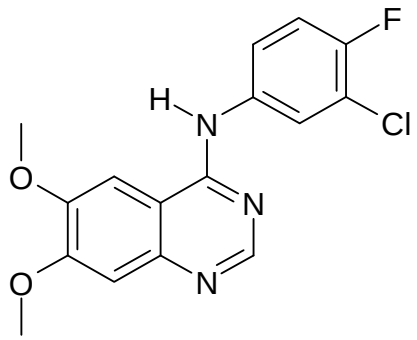
eg see Smith et al, *Med. Research Rev*. 1996, 16, 243-266

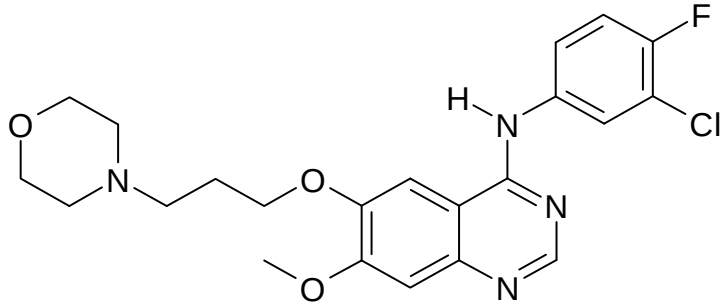
In Summary..what *you* can do:

- Poor absorption may be due to :
- Poor solubility
  - Reduce lipophilicity/ add polar/ ionizable groups
  - Reduce melting point (by reducing symmetry, planarity)
- Poor permeability
  - Increase lipophilicity
  - Decrease polar surface area/H-bonding
  - Decrease mol weight
- Efflux
  - Increase passive permeability to reduce impact of efflux

Worked examples…

Solubility of Iressa





EGF - RTK IC_{50} 0.009 μM

Stim. Cell Growth IC_{50} 0.08 μM

Solubility at pH 7 7.2 μM

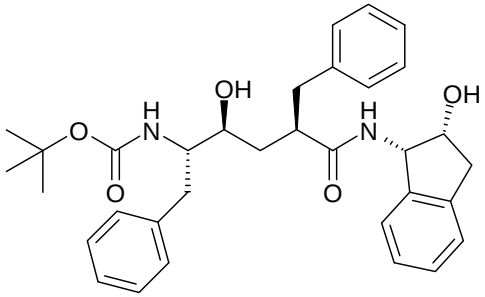
Solubility at pH 7 (phosphate) 3.7 μM

Solubility at pH 3 (phosphate) 2.2 mM

Solubility at pH 1 (HCl) 48 mM

Solubility and oral absorption

HIV protease inhibitors (J. Med. Chem. 1994, 37, 3443-3451)

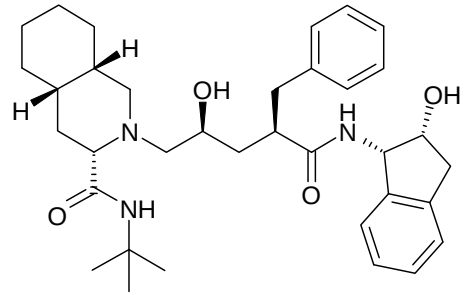


I

IC_{50} = 0.3nM

No oral bioavailability in dog

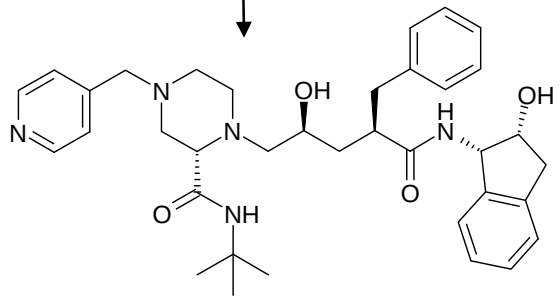
Solubility (pH 7.4) < 0.001 mg/ml



II

IC_{50} = 7.8 nM

15% oral bioavailability in dog



III

IC_{50} = 0.3 nM

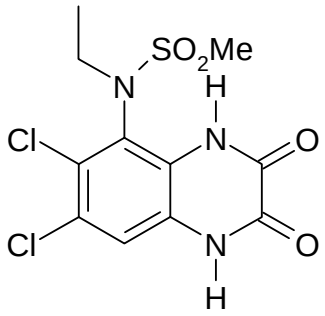
Solubility (pH 7.4) = 0.07 mg/ml

70% oral bioavailability in dog

Indinavir – marketed for HIV infection

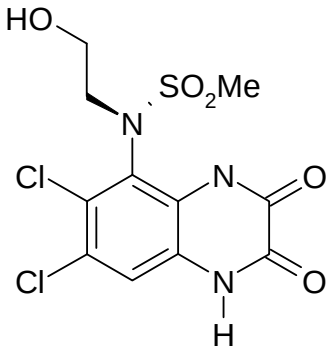
- **Incorporation of solubilising groups (weakly basic amine, pyridine) increases oral absorption**

Pfizer Glycine Antagonists



Potency 20nM
LogD 0.7
pKa 7.6
Solubility <1mg/ml

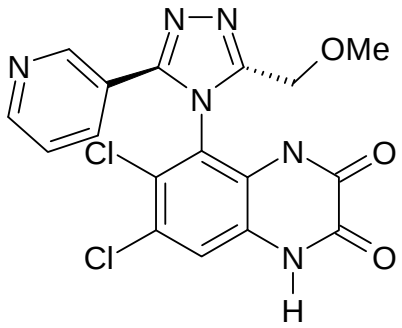
Increase solubility by reducing lipophilicity



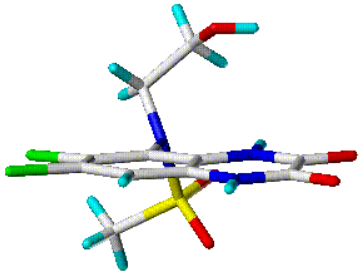
Potency 3nM

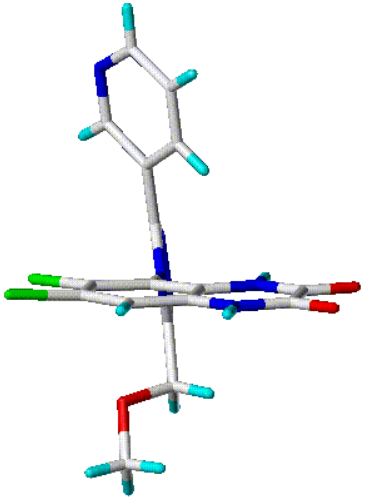
Solubility 5-30mg/ml

Increase solubility by decreasing lattice energy



Potency 2.6nM
LogD -0.4
pKa 6.7
Solubility >30mg/ml

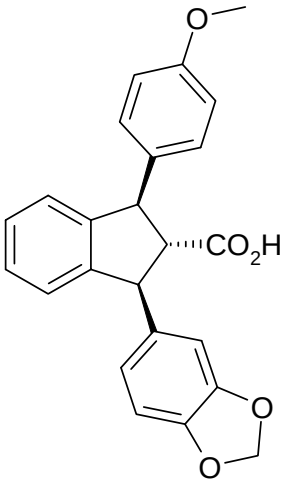




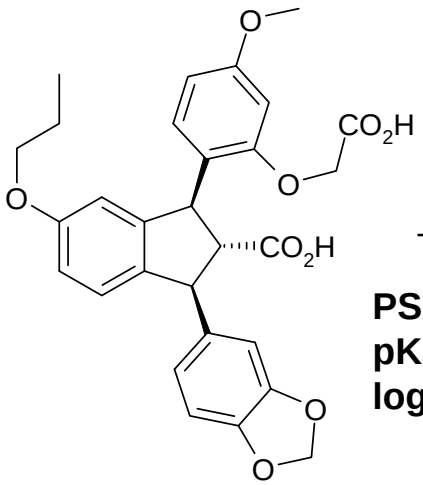
(thanks to Alan Stobie)

Intestinal permeability and oral absorption

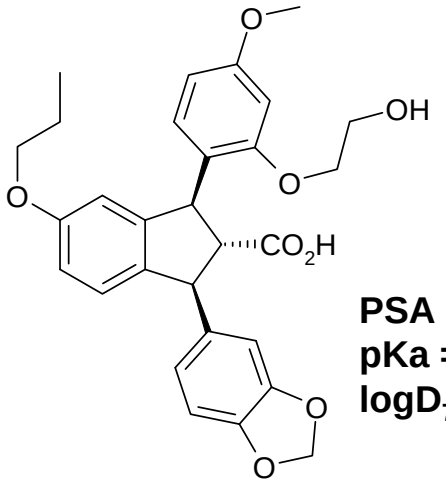
Endothelin (ET) A receptor antagonists (J. Med. Chem. 1994, 37, 1553-1557)



PSA = 75
pKa = 4.1
logD$_{7.4}$ = 2.2



PSA = 141
pKa = 3.1, 4.1
logD$_{7.4}$ = 0.4



PSA = 130
pKa = 4.1
logD$_{7.4}$ = 1.8

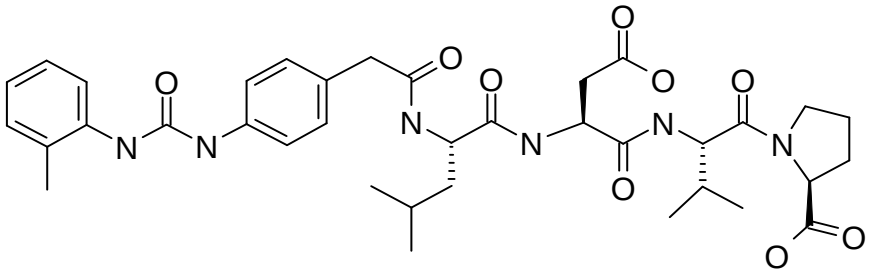
Lead
Ki ET$_A$ = 43 nM
Caco-2 cell permeability
Papp = 0.17 cm/hr

SB 209670
Ki ET$_A$ = 0.4 nM
Papp = 0.0075 cm/hr
< 5% bioavailable (rat)

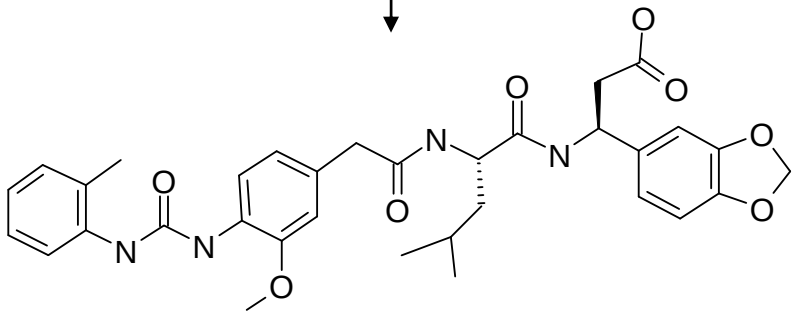
SB 217242
Ki ET$_A$ = 1.1 nM
Papp = 0.2 cm/hr
66% bioavailable

- **Caco-2 cell assay used to identify issue with SB 209670 – low intestinal permeability and rapidly identify non acidic sides chains with improved permeability**

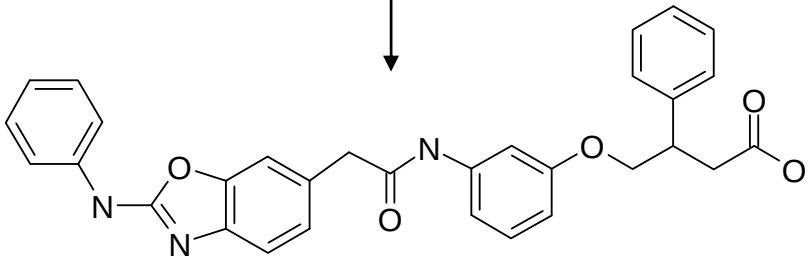
VLA4 Antagonists



Potent VLA4 antagonist
clogP 3.6
MW 708
PSA 222
Administered topically



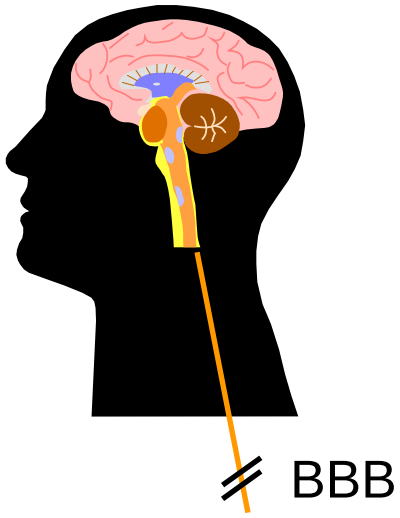
clogP 3.9
MW 618
PSA 174
Caco Papp <1



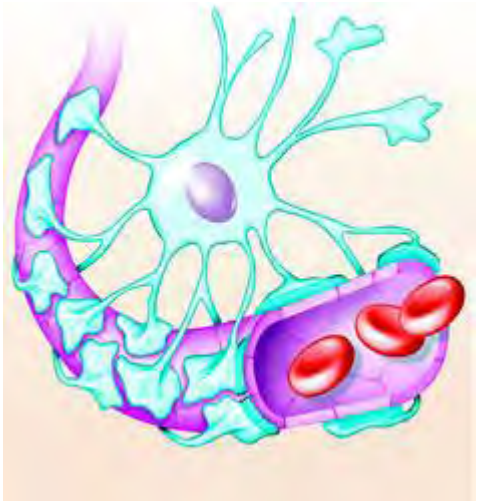
clogP 5.9
MW 521
PSA 120
Caco Papp 4-8
Rat Bioavailability 44%

Distribution

And you thought getting
from the gut to the blood
was a challenge…
think some more…



Distribution to Site of Action
Blood Brain Barrier and CNS Penetration



What is the BBB?

- Blood Brain Barrier is the interface between blood vessels and brain cells
- Protective lipid membrane with tight cellular junctions
- Polar, hydrophilic molecules are prevented from entering CNS
  - Active transport does operate eg for peptides, amino acids, glucose, fatty acids
- Efflux pumps (eg P-gp) acts to keep “foreign” drug molecules out of CNS
- BBB has some metabolic capacity
- Main route of CNS drug penetration is by passive diffusion

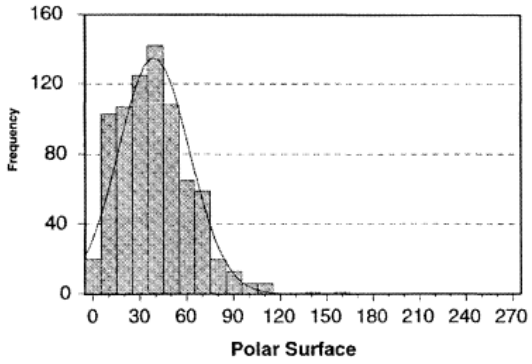
Blood Brain Barrier Penetration
Features of CNS drugs

- **Mol Weight < 400**

- **logP/ logD 2 – 4 (optimum ~ 2)**

Strong correlation of logD and passive permeability to BBB penetration

- **PSA < 60-90 Å2**



PSA range for 776 oral CNS drugs that reached phase 2 efficacy studies

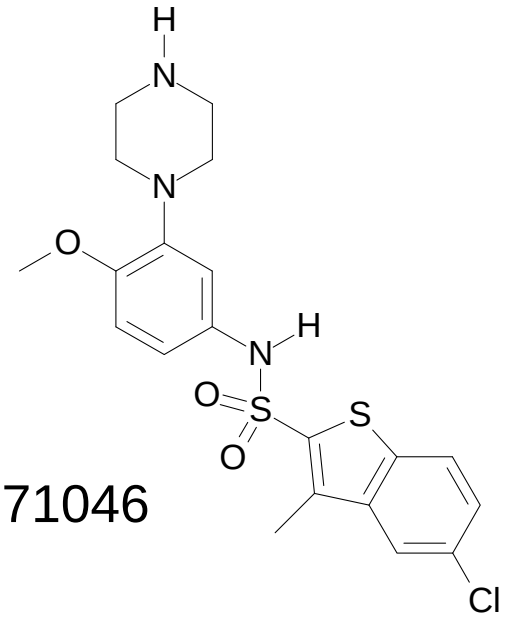
- **pKa - optimum pKa range is 7.5 – 10.5**

- **H-bond donors 0 - 1**

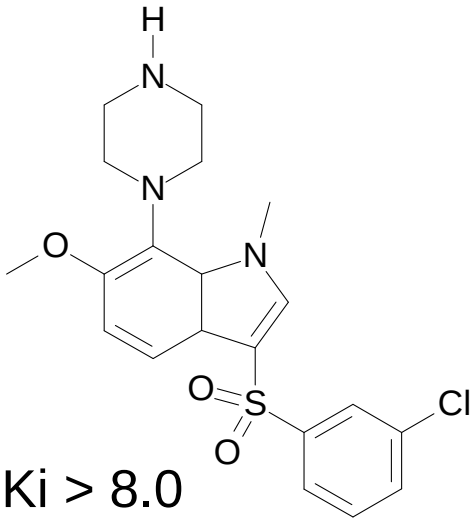
- **Few CNS drugs are P-gp substrates - harder to achieve saturating concentrations in plasma.**

Journal of Medicinal Chemistry, 2006, 49, 26, 7559

5-HT6 Antagonists



SB-271046

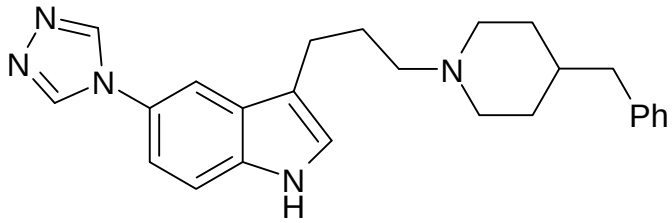


5-HT6 pKi > 8.0

MW	**452**	**390**
clogP	**4.1**	**3.0**
clogD	**3.6**	**1.4**
PSA (A^2)	**71**	**54**
HBD	**2**	**1**
Brain / Plasma	**0.05**	**2.6**

Brain Teaser – 5-HT$_{1D}$ receptor agonists

(J. Med. Chem. 1999, 42 2087 – 2104)



1

Compound	5-HT1D Ki	pKa	cLogD	Concentration in rat plasma HPV sampling 0.5h after 3 mg/kg p.o.
1	0.3 nM	9.7	2.5	25 ng/ ml

Compound 1 is a potent 5-HT$_{1D}$ agonist but is poorly absorbed orally

Basic Nitrogen is important to activity

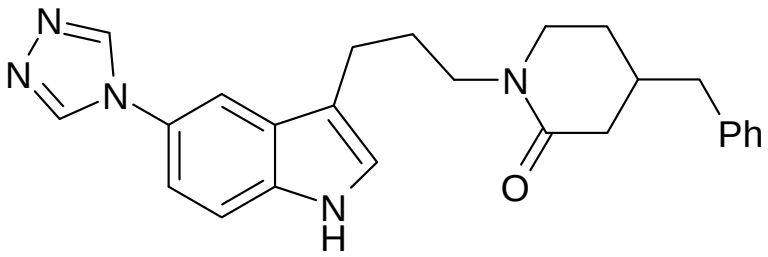
What is a possible barrier to absorption?

What strategies would you use to attempt to improve oral absorption?

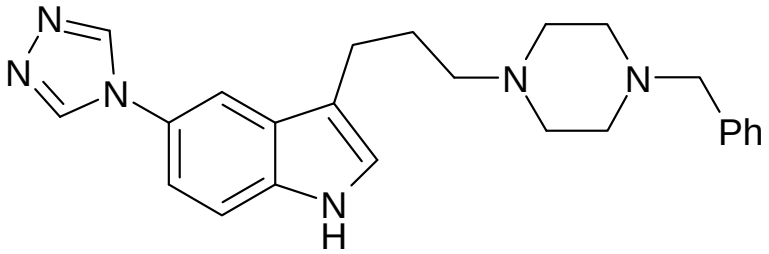
HPV = hepatic portal vein

Coffee Break

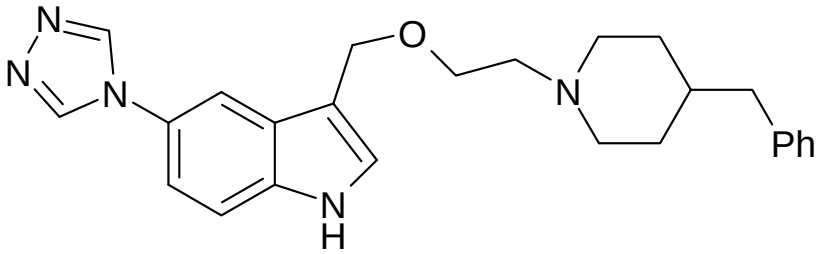
Which one would you make……?



A



B

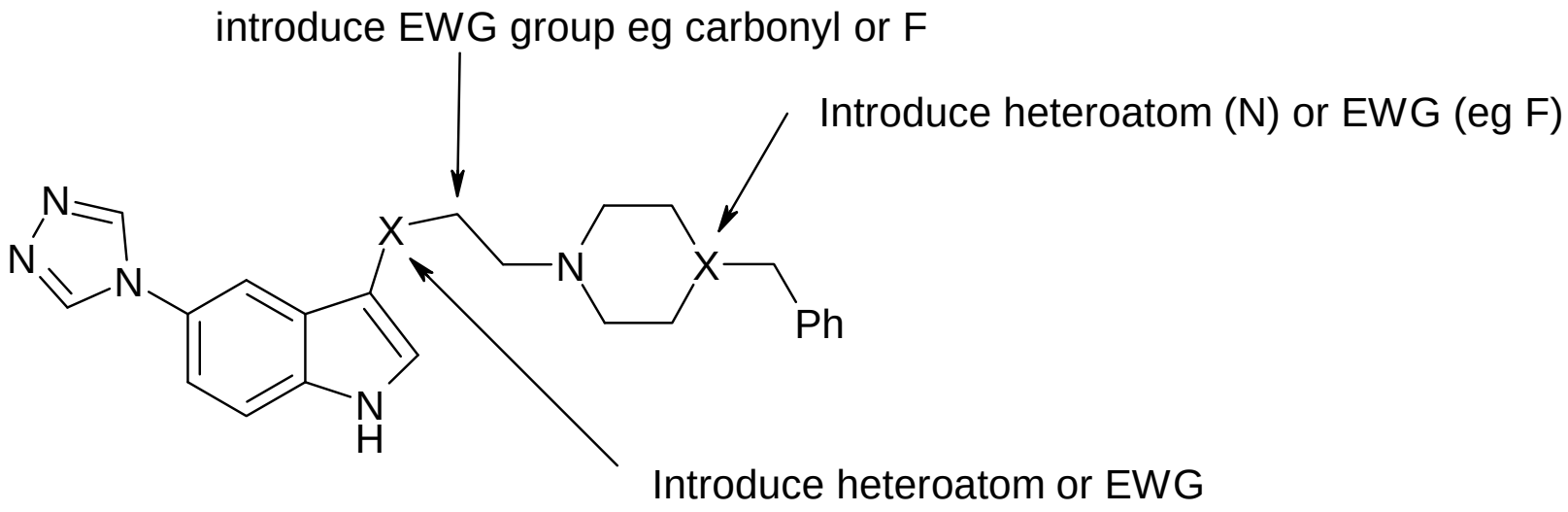


C

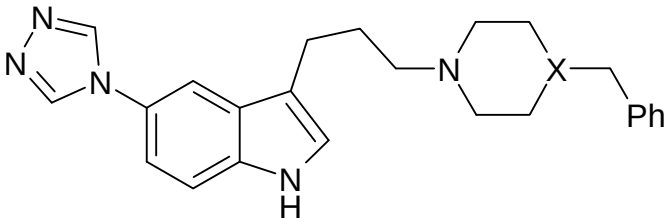
Hypothesis: Lower pKa of basic N to influence absorption

Effect of lowering pKa is to increase logD and decrease % of ionized compound at gastric pH - both will favour membrane permeability

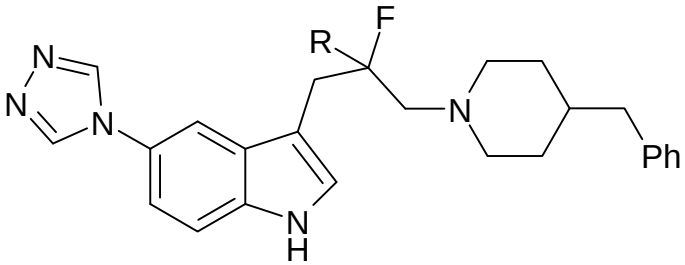
Strategy: Introduce heteroatoms, EWG's β or γ to nitrogen



What was tried…….



1 : X = CH
2 : X = CF

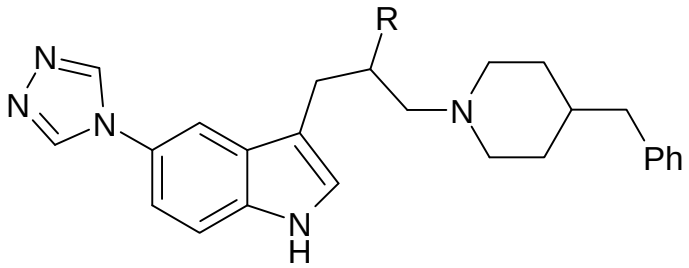


3 : R = H
4: R = F

Compound	5-HT1D Ki	pKa	cLogD	Concentration in rat plasma HPV sampling 0.5h after 3 mg/kg p.o.
1	0.3 nM	9.7	2.5	25 ng/ ml
2	0.9 nM	8.8	3.5	570 ng /ml
3	0.9 nM	8.7	3.5	781 ng/ ml
4	78 nM	6.7	4.7	ND

- **Lowering pKa improves permeability and oral absorption**
- **Fluorine atoms have minimal steric influence on structure**
- **NB: fluoropiperidines are possibly toxic but any heteroatom β or γ to a nitrogen will lower pKa**

But…….



1 : R = H
3 : R = F

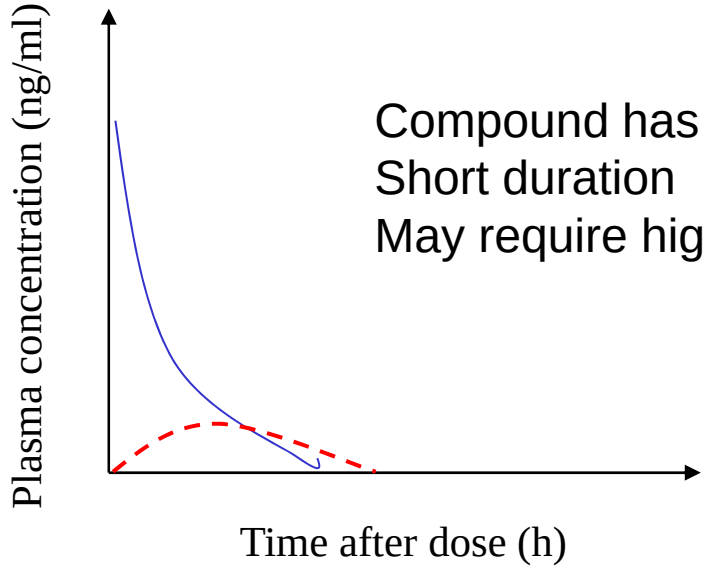
				Concentration in rat plasma 0.5h after 3 mg/kg p.o.	
Compound	**5-HT1D Ki**	**pKa**	**cLogD**	**HPV sampling**	**systemic (cardiac) sampling**
1	**0.3 nM**	**9.7**	**2.5**	**25 ng/ ml**	**< 2 ng/ ml**
3	**0.9 nM**	**8.7**	**3.5**	**781 ng/ ml**	**196 ng/ ml**

Increase in lipophilicity leads to extensive first pass metabolism
- lower than expected systemic exposure……

Metabolism

Metabolism and Clearance

How do you know you have a problem?



Compound has a short $T_{1/2}$ (half life)
Short duration
May require high or multiple dosing to see a biological effect

Compound A iv bolus injection 1 mg/kg

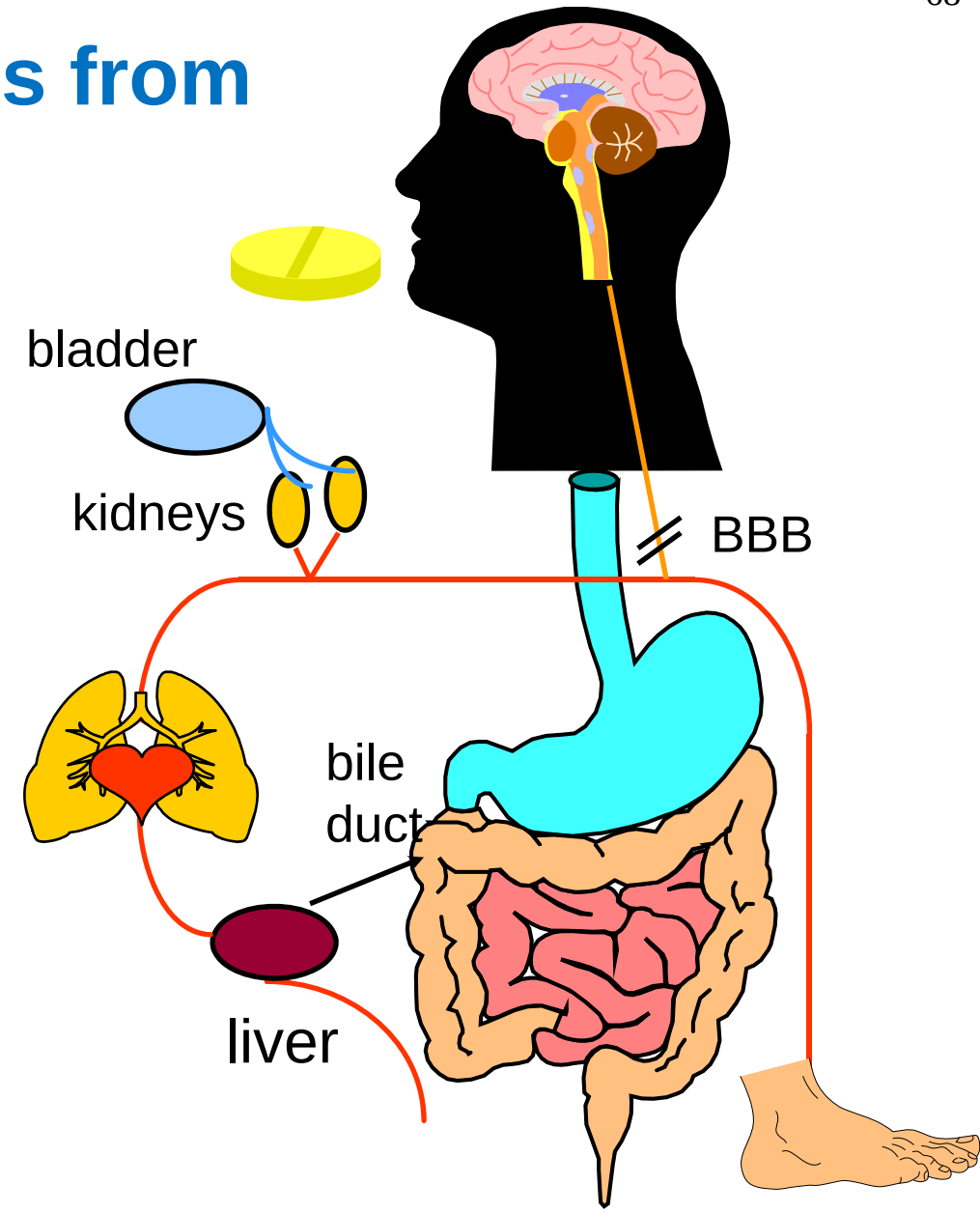
- **Low plasma exposure (area under curve AUC)**
- **Drug disappears rapidly from plasma**

Compound A oral dose (po) 10 mg/kg – jugular vein detection

- **Low plasma exposure (AUC)**
- **Extensive first pass metabolism**

“Clearance” of drugs from plasma

- Successfully entered plasma
- survive plasma contents (hydrolysis etc)
- survive metabolism in liver (oxid. and conj.)
- avoid active transport to bile
- avoid excretion by kidneys

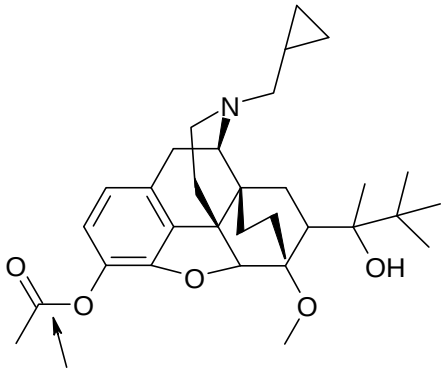


Common sources of the problem

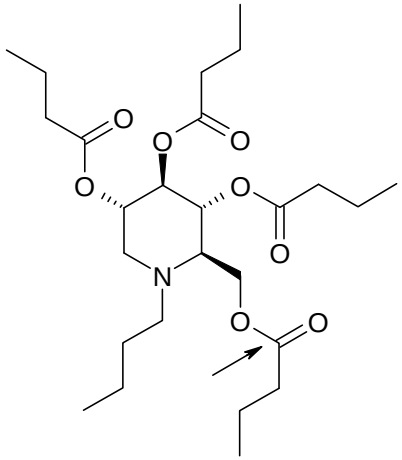
- Plasma instability
- Biliary elimination of compound unchanged
- Metabolism by the liver
- Renal elimination of compound unchanged

Plasma Instability

Enzymatically mediated, usually hydrolases and peptidases
Therefore compounds containing esters and some electrophilic amides can be a concern
Rates of hydrolysis usually (but not always) faster in rodents than man



Isocarbacyclin



glycovir

Human blood T1/2	17min	45min

Rates are hard to predict but are sensitive to electrophilicity, sterics and lipophilicity
J Med Chem, 1999, 42, 5161

Clearance by the Liver

Plumbing and liver physiology

Clearance - an important concept

GI Tract & Liver

Plumbing and liver physiology

Clearance - an important concept

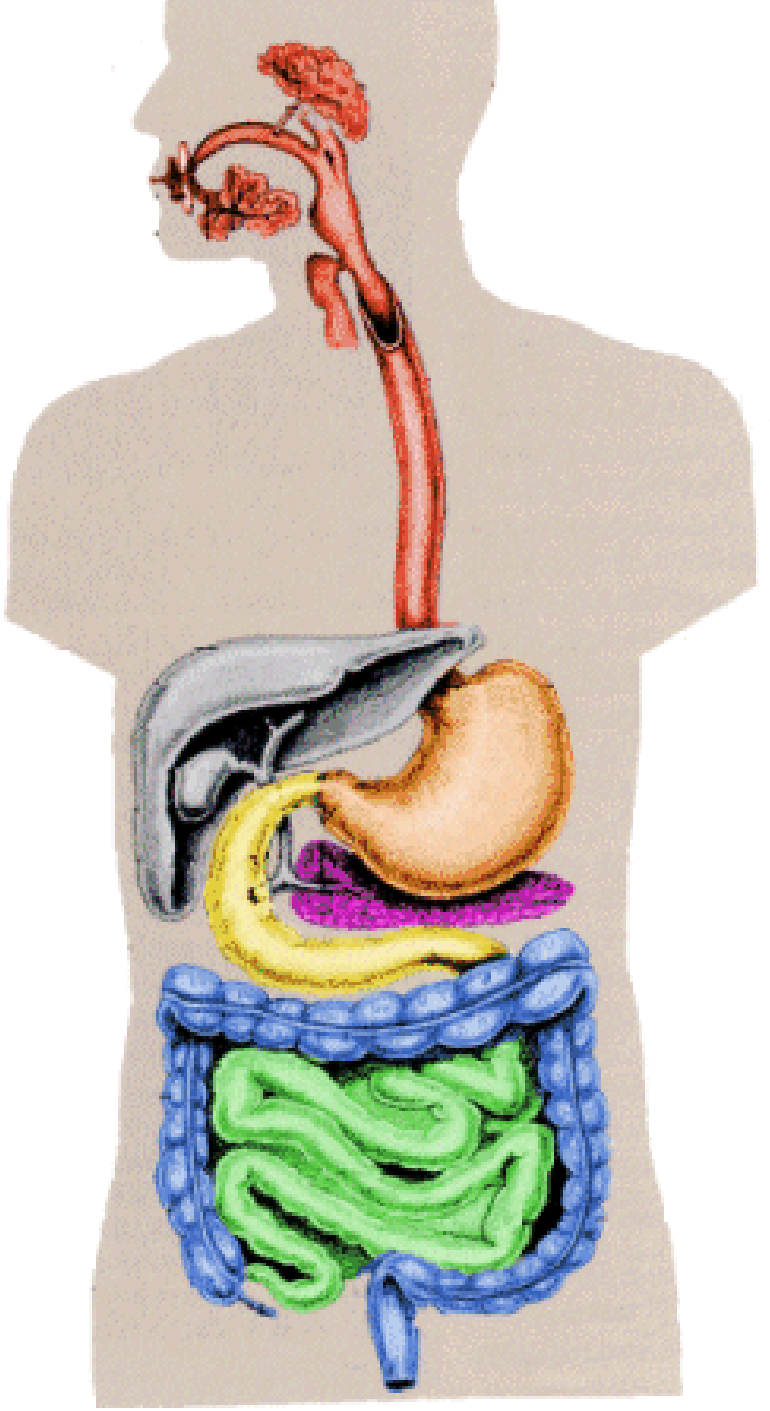
Double whammy – first pass and every pass

Clearance also affects bioavailability (F) because of pass extraction

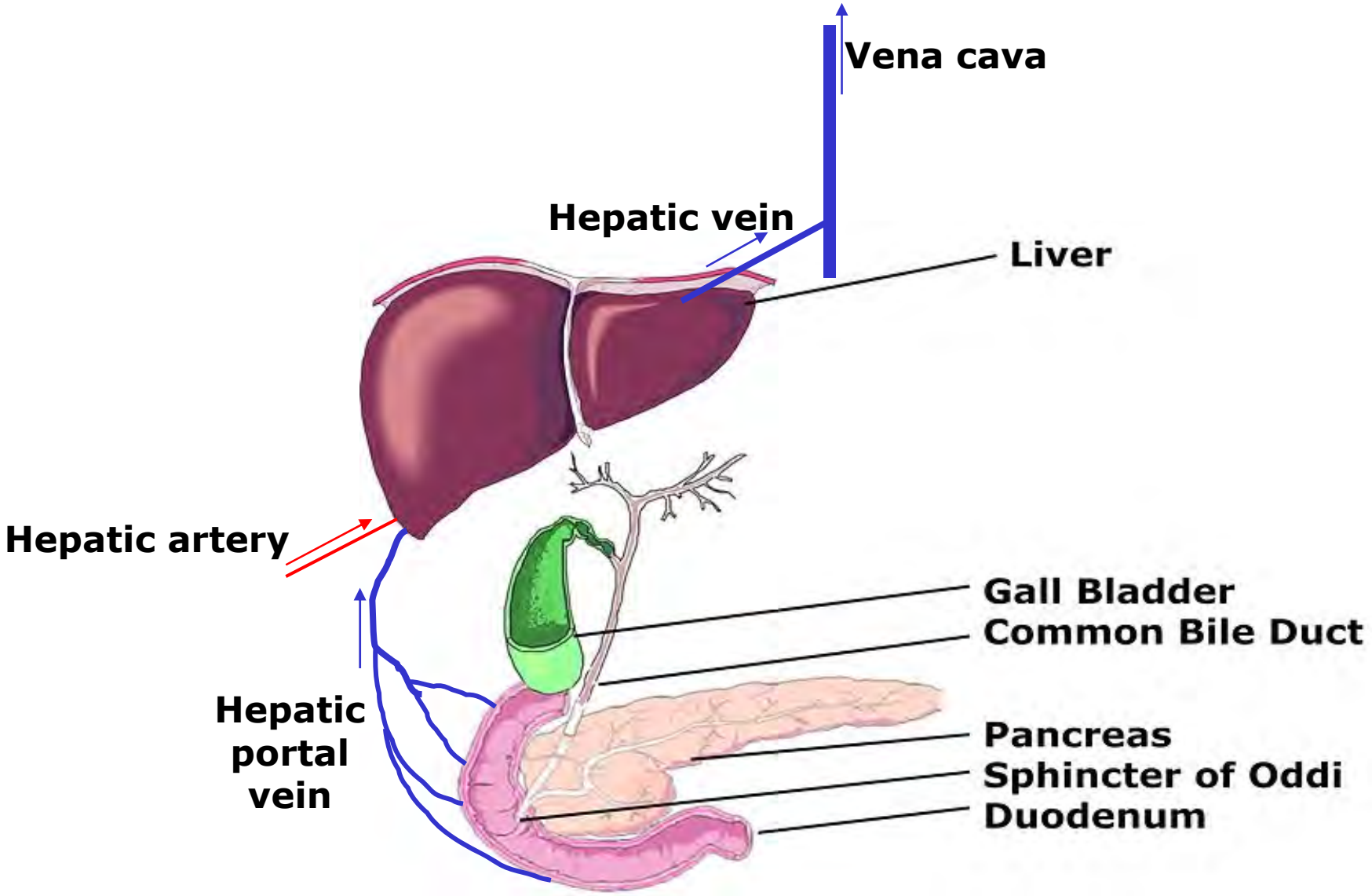
$F = F_{abs} * F_{gut} * F_{hep}$ where

F_{abs} = fraction absorbed
F_{gut} = fraction which survives metabolism in the intes
F_{hep} = fraction which survives extraction (metabolism the liver

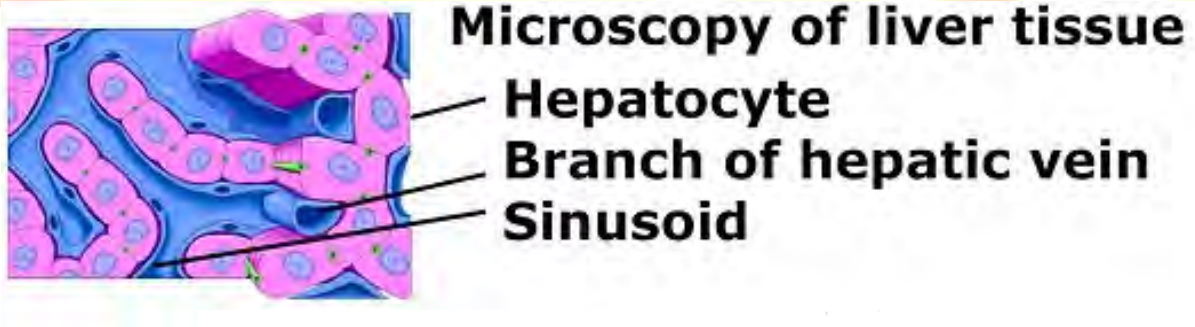
- Video explanation of anatomy of liver
- http://www.nottingham.ac.uk/nursing/sonet/rlos/bioproc/liv eranatomy/index.html
- Dr Viv Rolfe, Uni Nottingham



Liver & its Connections



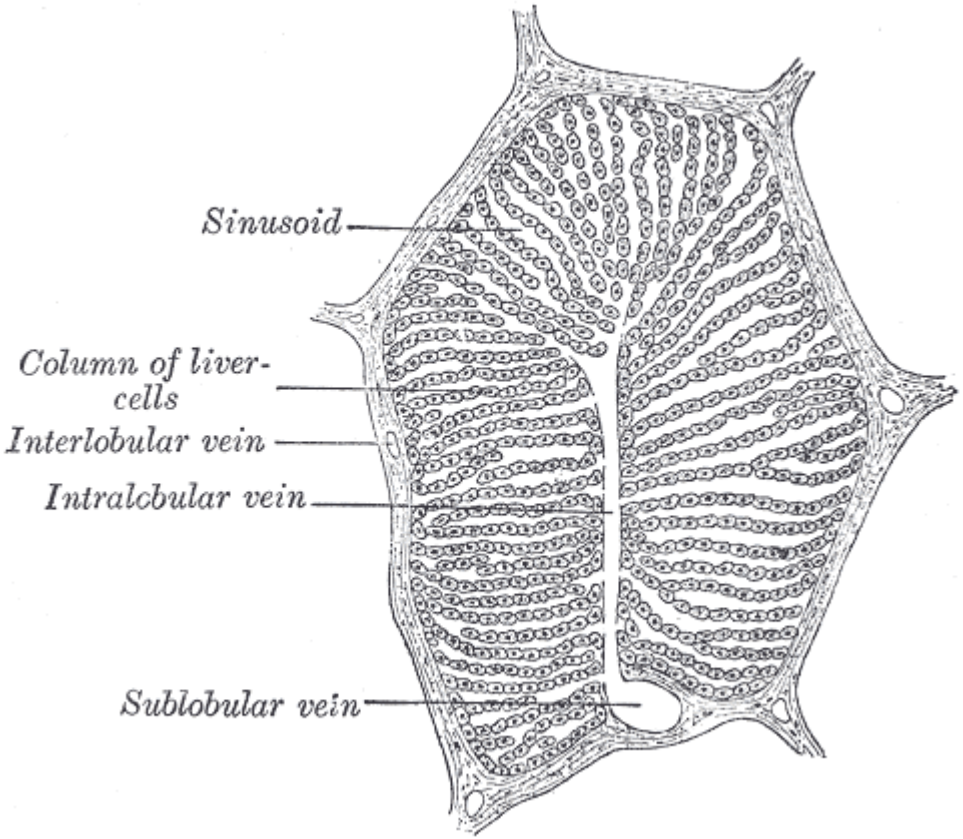
Liver Lobule



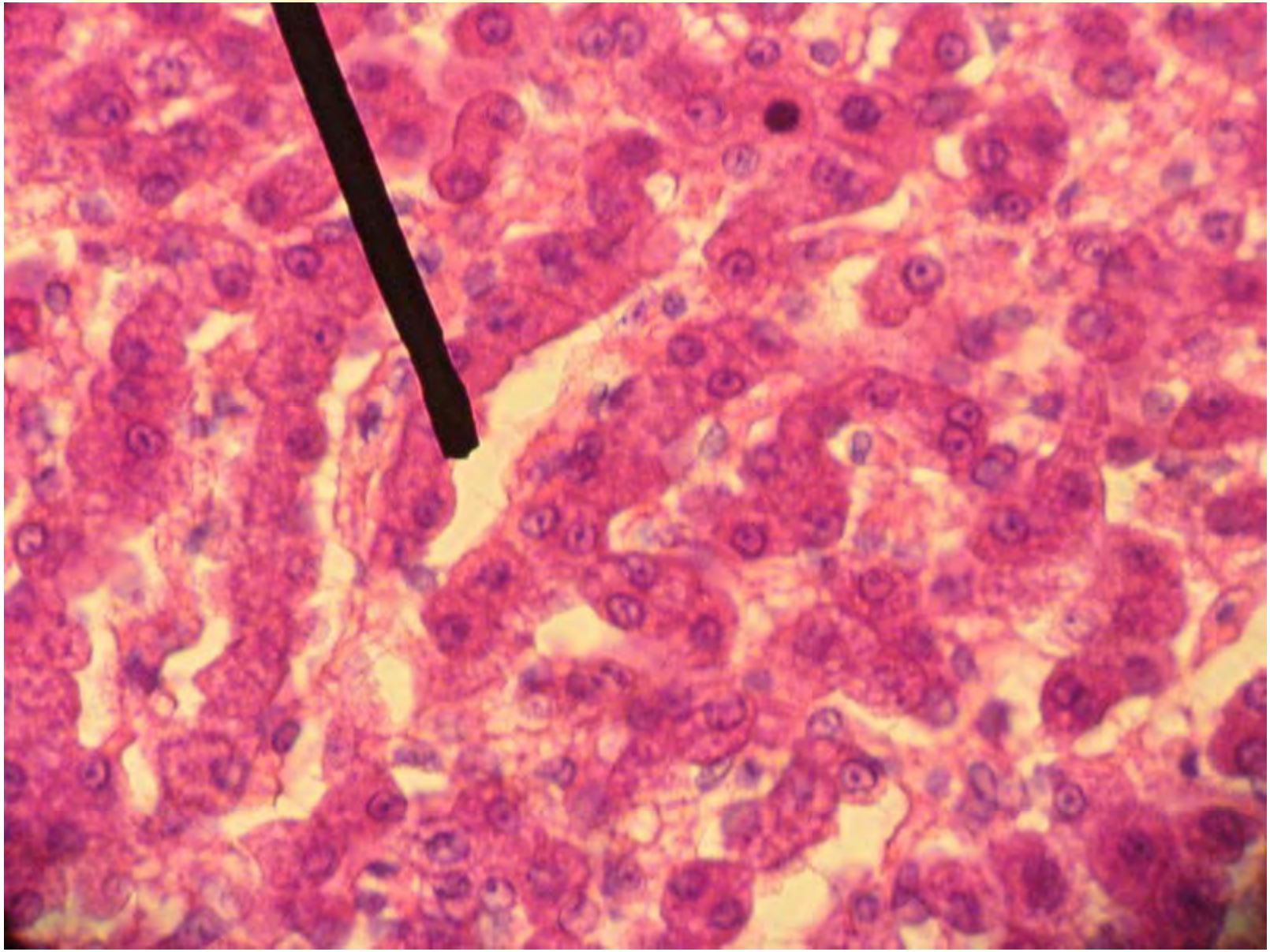
Blood from the hepatic portal vein and the hepatic artery flow through sinusoids towards centre.

Bile flows in the opposite direction.

Blood leaves through central vein & returns to heart.



Liver Histology



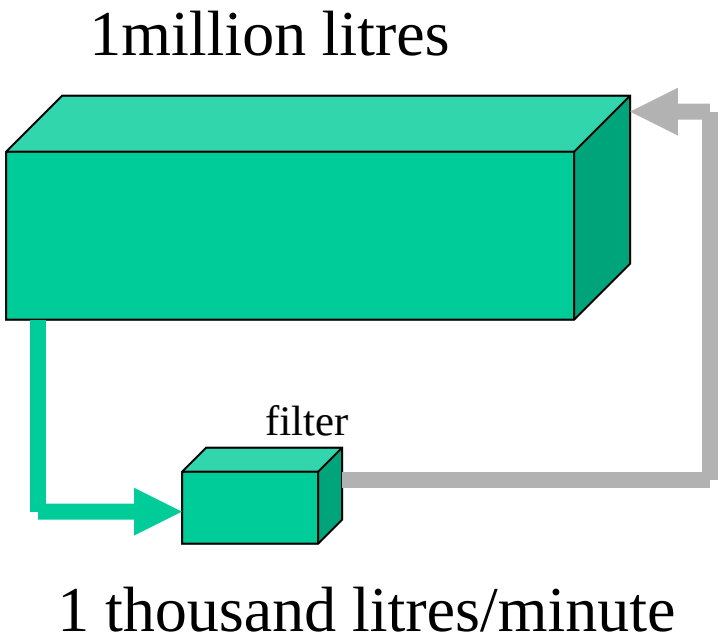
Plasma clearance – an analogy

Imagine a swimming pool.

Drop green ink into it and mix it.

A pump sends water through a filter.

The filter destroys the ink and returns clean water to the pool.



The flow rate is the CLEARANCE.

The half-life = $\log_e(2)$ x volume / clearance =
0.693x1000000/1000 = **693 minutes**!!!

Plasma clearance – an analogy

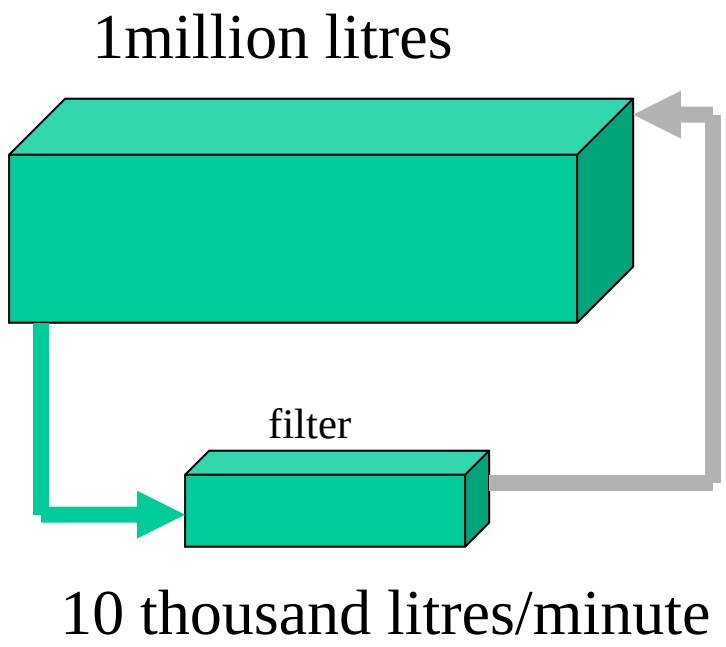
So fit a bigger pump and filter!

Drop green ink into it and mix it again.

The filter destroys the ink and returns clean water to the pool.

The flow rate is the CLEARANCE.

The half-life = $\log_e(2)$ x volume / clearance =
0.693x1000000/10000 = **69.3 minutes**



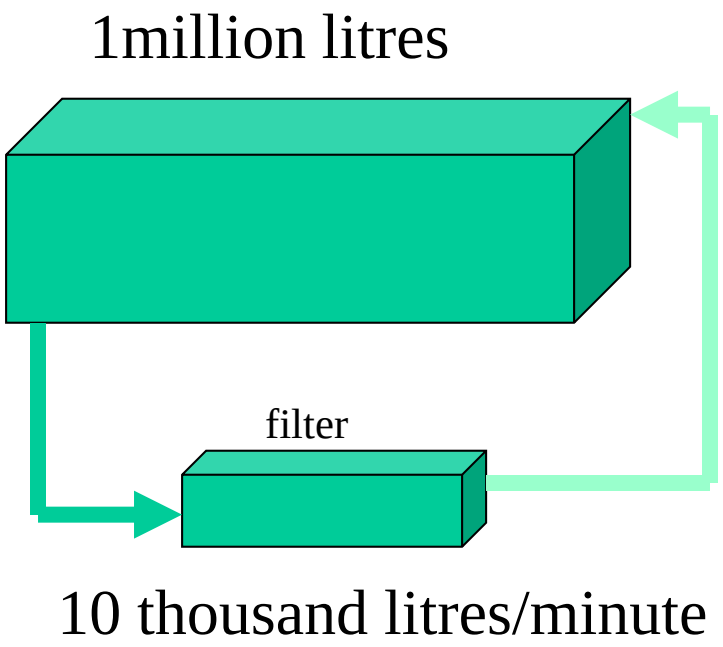
Plasma clearance – an analogy

Suppose the filter is only 50% efficient (extraction ratio = 0.5).

Now the CLEARANCE is 5000 litres/minute

The half-life is doubled to:

0.693x1000000/5000 = **138.6 minutes**



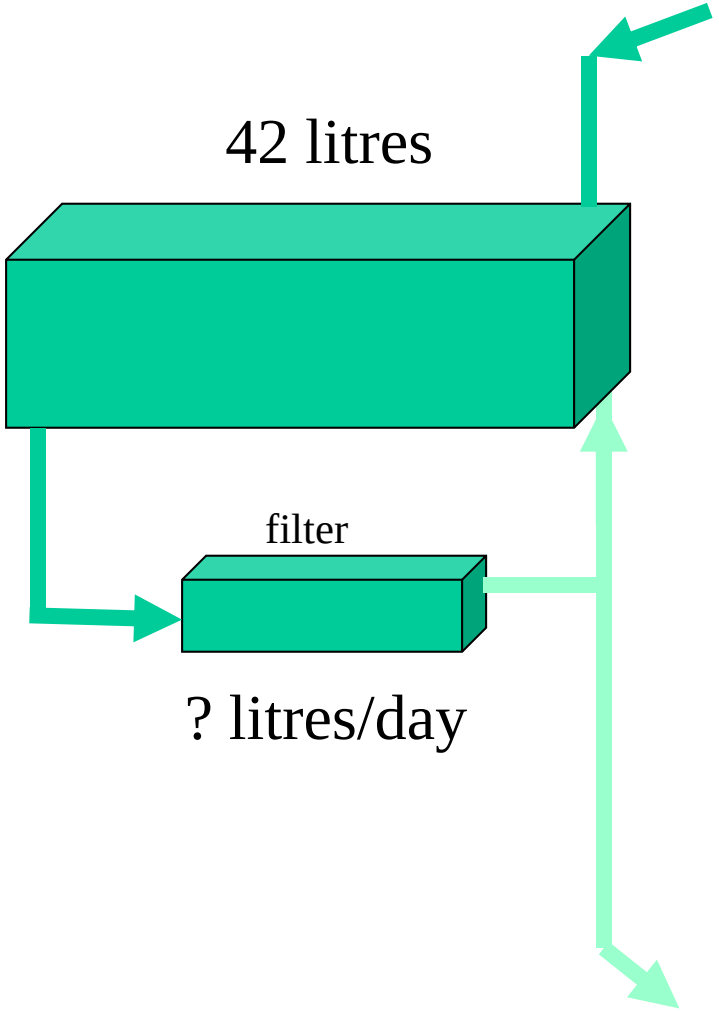
Clearance – worked example

Total body water is 42L (70kg person)

The half-life of water is 8 days

What is the clearance of water?

CL = $\log_e(2)$ x volume / half-life



Clearance – worked example

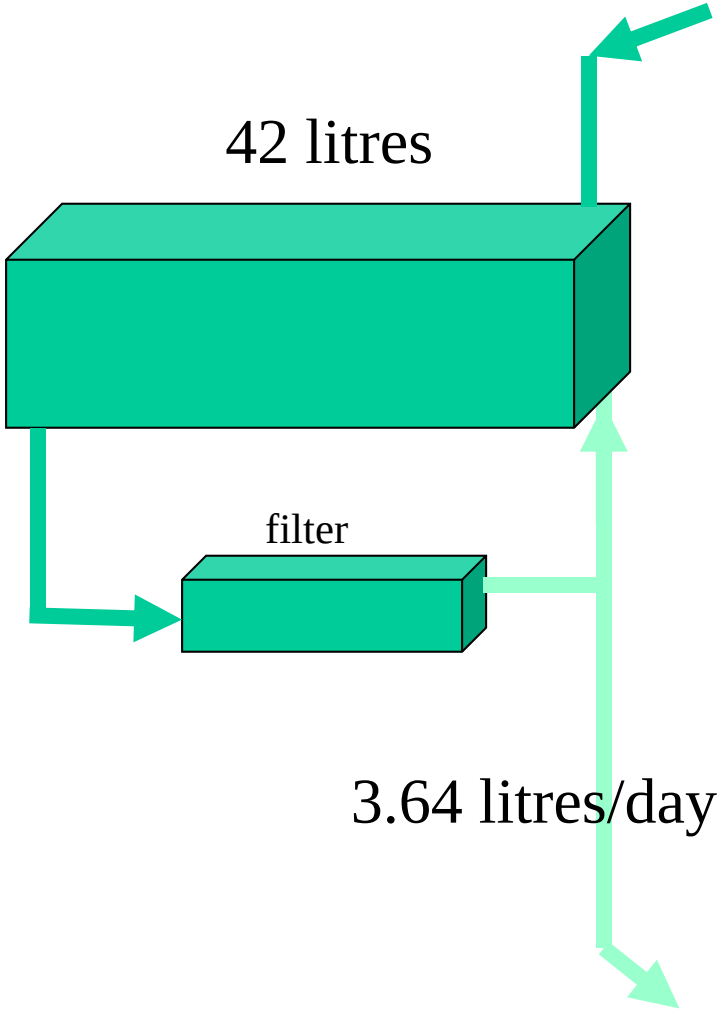
Total body water is 42L (70kg person)

The half-life of water is 8 days

What is the clearance of water?

CL = $\log_e$ (2) x volume / half-life

= 0.693 x 42 / 8 = **3.64L/day**



- *Is this reasonable?*
- *Where does it come from?*
- *Where does it go?*

Clearance – worked example

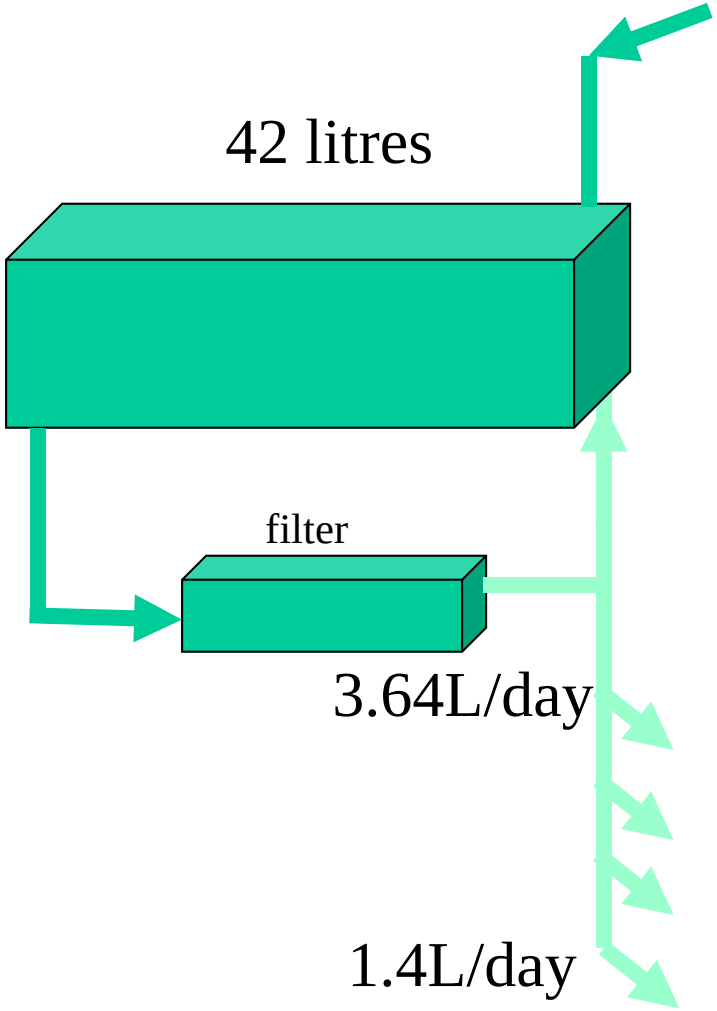
Total body water is 42L (70kg person)

The half-life of water is 8 days

What is the clearance of water?

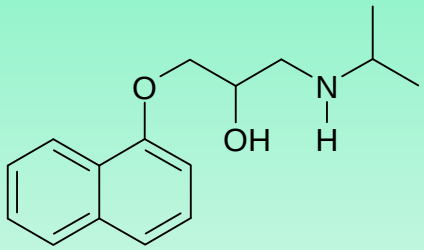
CL = 3.64L/day = 2.5mL/min

Urine flow = 1.4L/day = 1mL/min

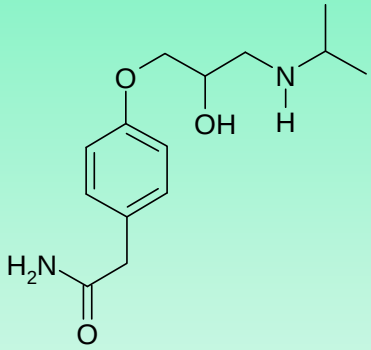


Renal Clearance

- Typically only relevant for low lipophilicity compounds e.g. log D $\leq$0
- Therefore not very common!



Propanolol (logD = 1.5)
99% of clearance by metabolism



Atenolol (clogP = -1.9)
> 90% excreted unchanged in urine

Physicochemical Determinants of Human Renal Clearance. Journal of Medicinal Chemistry (2009), 52(15), 4844-4852.

Biliary Elimination of Compound

Once believed to be solely a function of molecular weight
(MW >500 for human)

However, now more widely regarded as an “active transport” problem

Can affect acids, bases and polar neutrals; bile is alkaline and this can “attract” acid drugs

Concentration gradient from bile to plasma can be 10000 to 1 for low permeability drugs

Difficulty - need to surgically cannulate rats and look for drug in bile fluid
- bile is not the easiest matrix to analyse

Biliary Clearance

We think we are starting to understand what controls it……

Most drugs are sufficiently lipophilic for membrane permeability and oral absorption
Compounds which are less lipophilic tend to experience active transport.
Probably active transport is the norm, but permeable compounds can leak out again

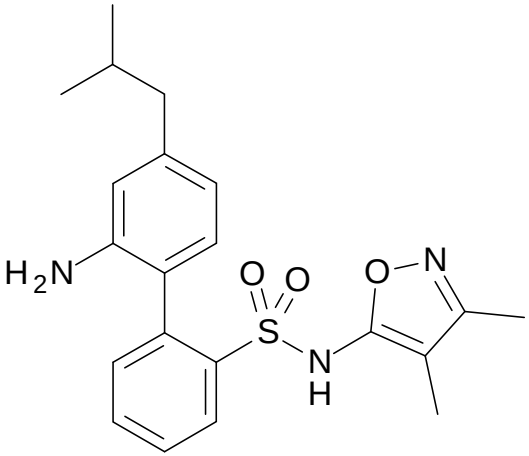
So increase in PSA →
decrease in permeability →
increased likelihood of biliary clearance

Details of specific transporters are hard to get and harder to interpret.
We have seen compounds which are
>99% plasma bound
E_h ~ 1 (mostly biliary)
bile / plasma ratio ~ 1000:1 (bile unbound plasma ~ 100,000:1!)

Biliary clearance often leads to a high concentration inside hepatocytes, blocking transport of bile acids or other toxins → hepatotoxicity

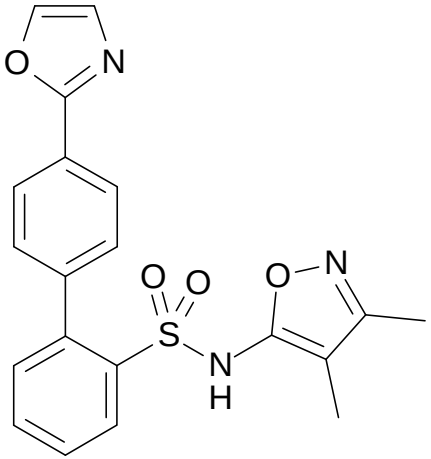
Efflux in Caco-2 assay or increasing PSA increase the risk of hepatic uptake

Example of biliary clearance: BMS ET_A antagonists



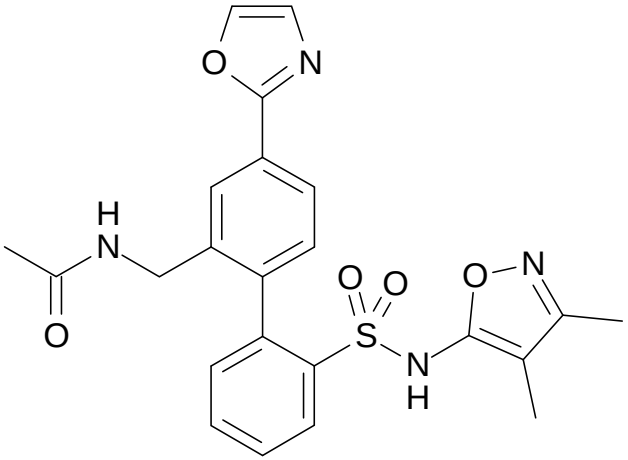
BMS-187308

moderately fast
in vitro & in vivo



BMS-193884

slow in vitro
slow in vivo

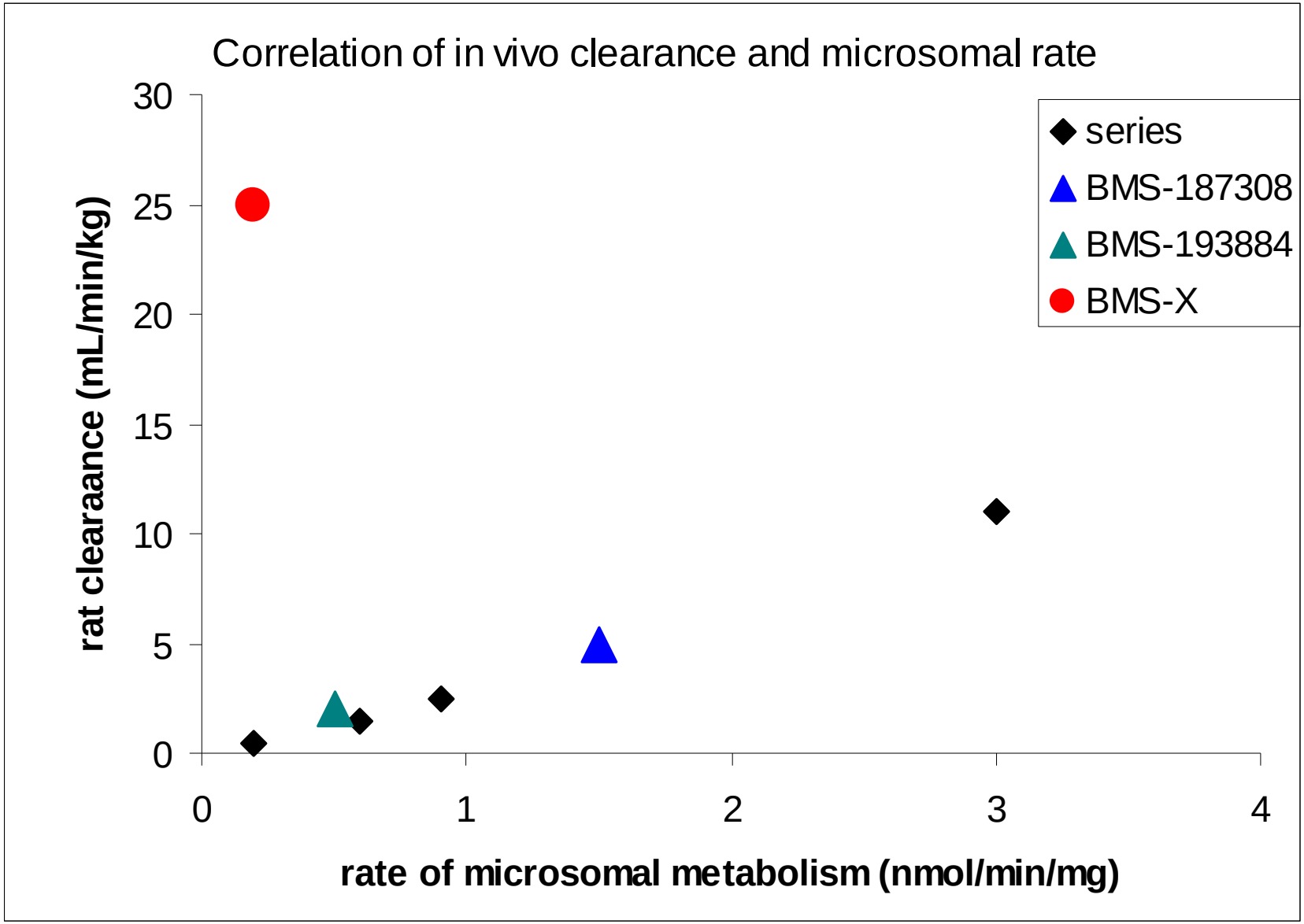


BMS-X

very slow in vitro
very fast in vivo -
biliary clearance!

WG Humphreys *et al*, Xenobiotica, 33 (11), 1109-23, 2003

Example of biliary clearance



Metabolism and Clearance

Most drugs are sufficiently lipophilic for membrane permeability and oral absorption
Metabolism in the liver is therefore the major route of clearance.

Where metabolism in the liver is the principal method of elimination then

$$\text{Clearance } (CL_H) = Q_H E \text{ ml/min/kg}$$

Q_H is the blood flow through the liver
E is the liver extraction ratio = $(C_A - C_V)/ C_A$

C_A = Concentration of drug entering liver. C_V = Concentration of drug leaving liver

Metabolism and Clearance

What are high and low clearance values?

Clearance (CL_H) = Q_H * E
For drugs where hepatic elimination is high then E $\rightarrow$ 1 and CL ~ Q_H
Clearance is high and approaches hepatic drug flow

	Rat	Dog	Man
Hepatic blood flow (ml/min/kg)	90	40	21
High clearance; E > 0.7 (ml/min/kg)	>63	>28	>15
Low clearance; E < 0.3 (ml/min/kg)	<30	<12	<7

Clearance is measured after an iv dose of compound (all the dose is “absorbed”)

Clearance also affects bioavailability (F) because of first pass extraction

$F = F_{abs} * F_{gut} * F_{hep}$ where

F_{abs} = fraction absorbed
F_{gut} = fraction which survives metabolism in the intestine
F_{hep} = fraction which survives extraction (metabolism) by the liver

(21mL/min/kg = 2100L/day!!!)

Metabolism

Why are drugs metabolised?

- **High molecular weight or high lipophilicity**
  - metabolism makes compounds more polar and more water soluble
  - trend for metabolism to increase with lipophilicity

- **Reactive/ labile groups eg:**
  - benzylic or allylic positions,
  - electron rich aromatic rings
  - N-methyl or O-methyl groups, Sulphur atoms
  - acidic OH or NH groups

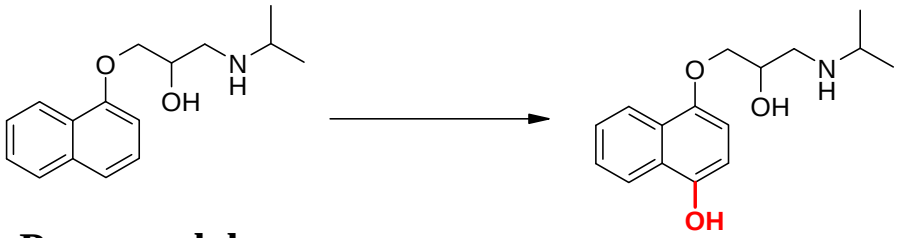
- **High affinity for metabolising enzyme**
  - Good fit into active site, specific interactions

Phase I Metabolism

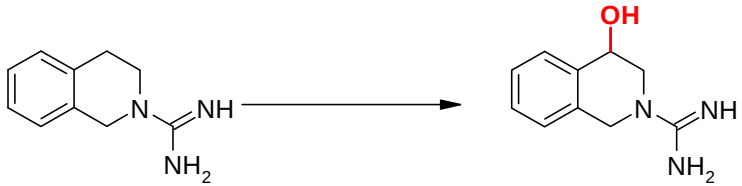
- Principally by:-
- (i) Oxidation
  - Aliphatic or aromatic hydroxylation
  - N-, or S-oxidation - cycling
  - N-, O-, S-dealkylation
- (ii) Reduction
  - Nitro reduction to hyroxylamine/ amine
  - Carbonyl reduction to alcohol - cycling
- (iii) Hydrolysis
  - Ester or amide to acid and alcohol or amine
  - Hydrazides to acid and substituted hydrazine

Examples of Phase I Metabolism

- Oxidation

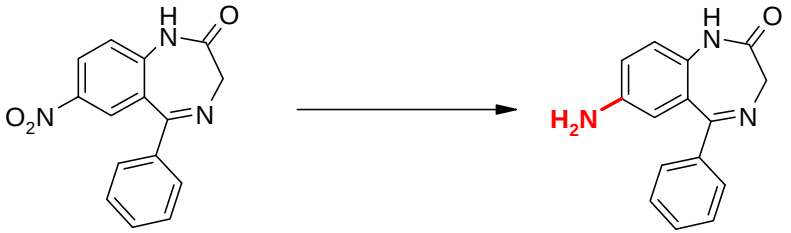


Propranolol (β-blocker)



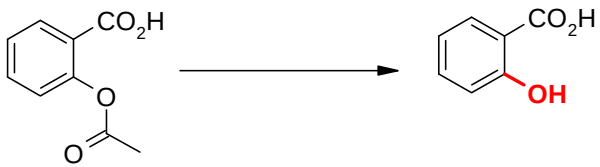
Debrisoquine (anti-hypertensive)

- Reduction



Nitrazepam (hypnotic)

- Hydrolysis



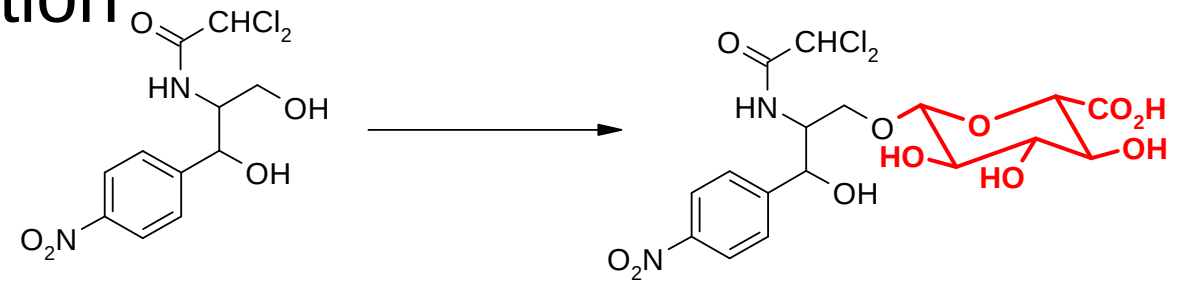
Aspirin (Analgesic)

Phase II Metabolism

- Principally by:-
- (i) Glucuronidation
  - Carboxylic acid, alcohol, phenol, amine
- (ii) Sulphation
  - Alcohol, phenol, amine
- (iii) Acetylation
  - Amines
- (iv) Amino acids
  - Carboxylic acids
- (v) Glutathione conjugation (gly-cys-glu)
  - Halo-cpds, epoxides, arene oxides, quinone-imine

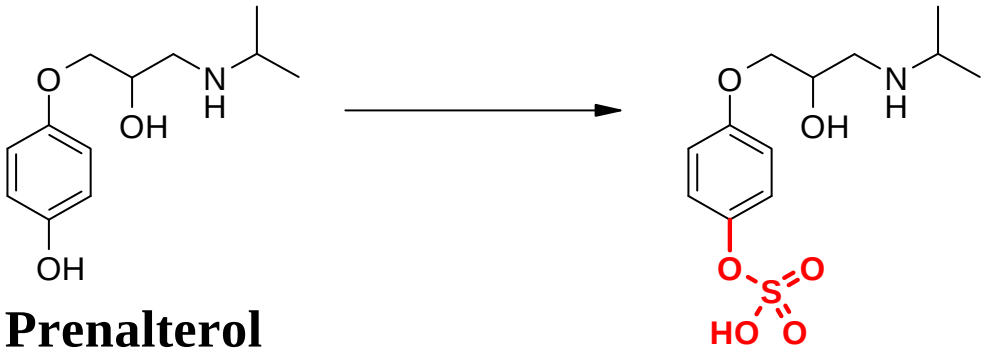
Examples of Phase II Metabolism

- Glucuronidation



Chloramphenicol (antibiotic)

- Sulphation



Prenalterol (β-blocker)

In vitro measurement of metabolism

- **Microsomes (species)**
  - **A subcellular fraction obtained by centrifugation of liver cells. Mainly composed of the endoplasmic reticulum**
  - ➢ **Perform Phase I reactions only**
- **Hepatocytes (species)**
  - **Isolated whole liver cells. (must be used fresh)**
  - **Harder to get hold of human hepatocytes**
  - ➢ **Capable of performing both Phase I and II reactions**

➢ **Purified metabolising enzymes can be prepared**

➢ **Rates of metabolism are generated**

➢ **Metabolite identification may be possible**

➢ **Extrapolation from *in vitro* to *in vivo* is possible (with caution!)**

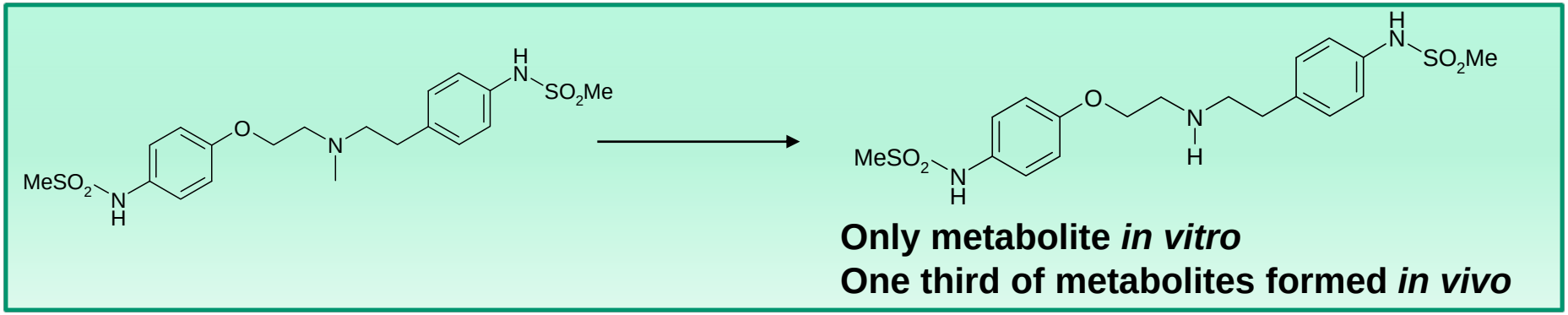
Metabolism

Identification of metabolites

Knowing the exact structure of major metabolite(s) is a powerful aid to the medicinal chemist

- metabolism can be blocked/ suppressed
- potential toxicity can be predicted
- predict if the same metabolites formed in human as rat/ dog

- Advances in LCMS, MS/MS and NMR have allowed minute quantities of metabolites to be identified
- *In vitro* liver preparations (microsomes, hepatocytes)
- *Ex vivo* analysis of plasma or tissue samples
- Analysis of urine/ faeces
- ***In vitro* and *in vivo* metabolite profiles may be different eg: dofetolide**



Only metabolite *in vitro*
One third of metabolites formed *in vivo*

What can *you* do?

The medicinal chemist can reduce metabolic clearance by altering chemical structure

- knowing what structural features or properties favour metabolism
- knowing/ predicting the structure of metabolites

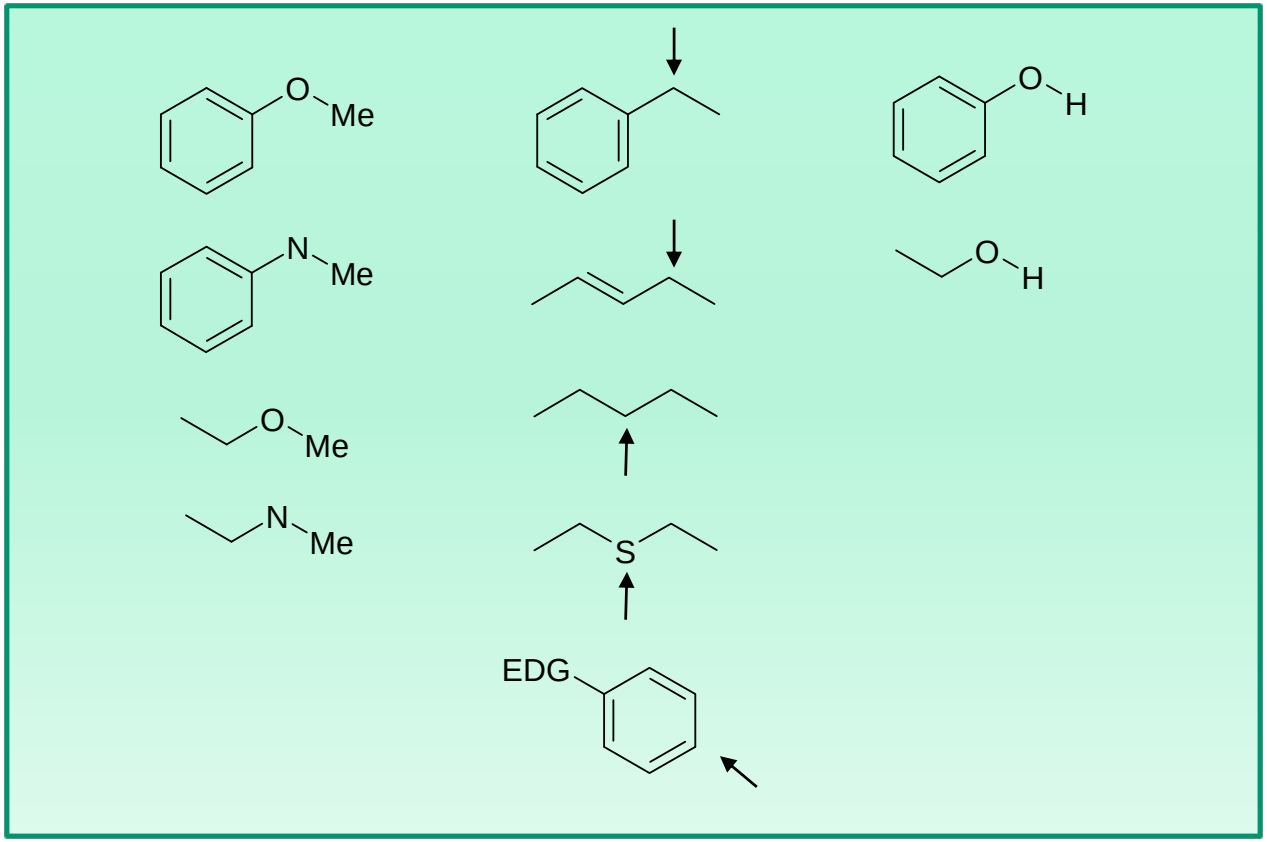
- **Lower the overall lipophilicity of a compound**
  - introduce polar atoms/ groups, basic or acidic groups
  - remove/ modify highly lipophilic regions (polyalkyl chains, unsubstituted aryl rings)

- **Block / sterically hinder sites of metabolism**

- **Remove reactive/ labile sites or replace with bioisoteres**

- **Make aryl rings more electron deficient**

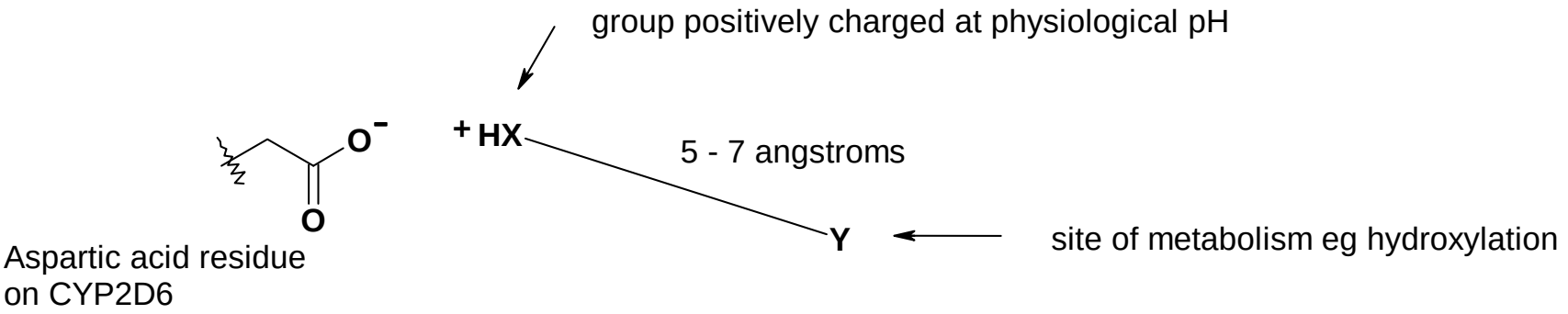
Summary of common metabolic soft spots

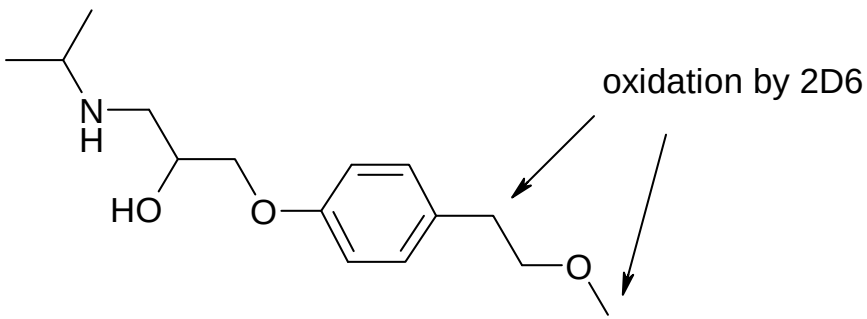


Block sterically (adjacent substituent or bigger than methyl)
or electronically (reduce/remove electron density) with halogens,
heteroatoms, EWGs

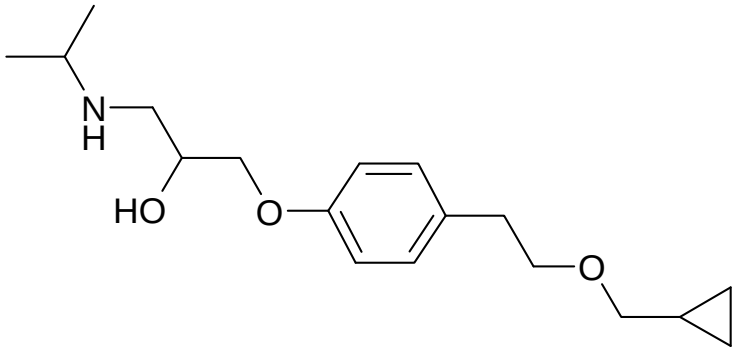
Metabolism by CYP2D6

Model of CYP2D6 and substrate





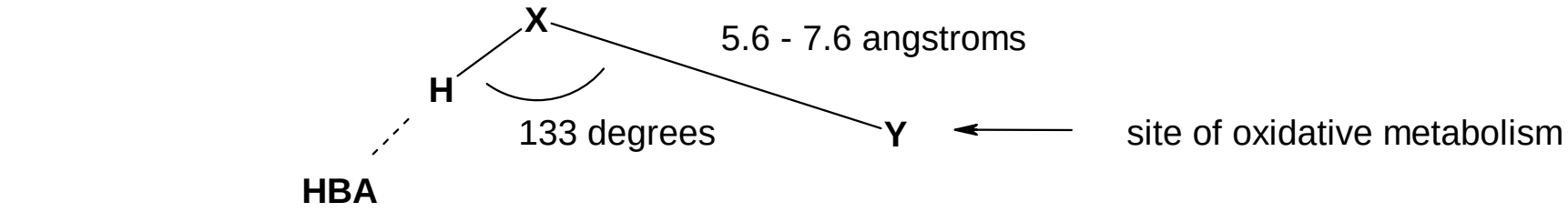
Metoprolol
38% oral bioavailability
Clearance 15 ml/min/kg

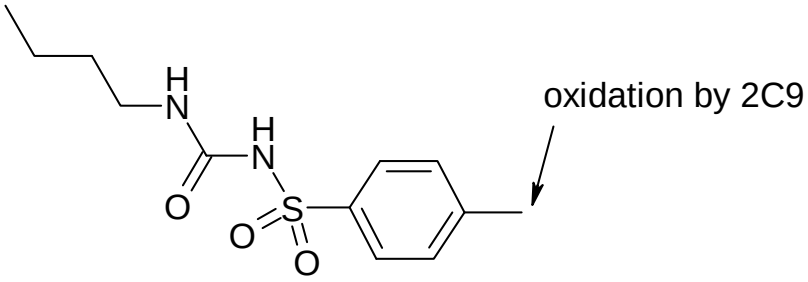


Betaxolol
89% oral bioavailability
Clearance 4.7 ml/min/kg

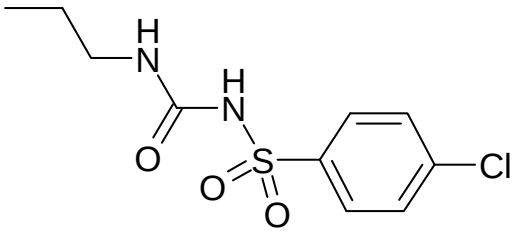
Metabolism by CYP2C9

Model of CYP2C9 and substrate





Tolbutamide
Half life = 5 hours

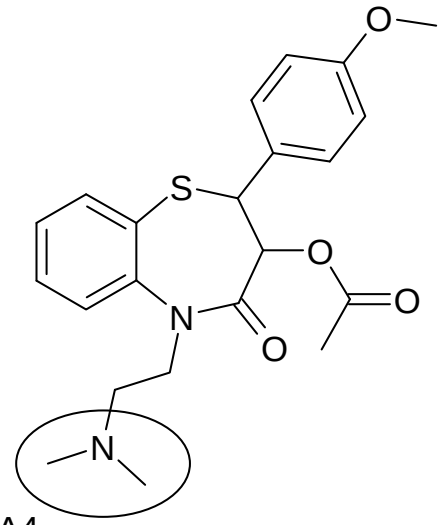


Chlorpropamide
Half life = 35 hours

Metabolism by CYP3A4

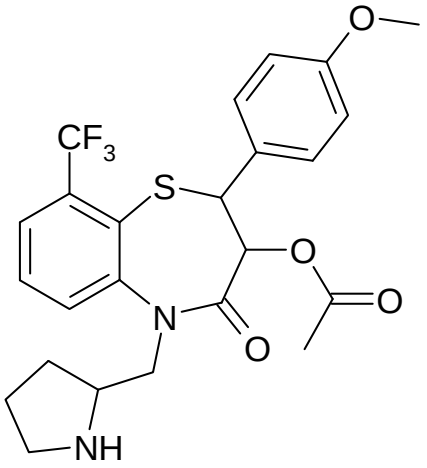
Substrates of CYP3A4 – lipophilic neutral or basic compounds

Sites of metabolism – allylic positions, nitrogen atoms (eg N-dealkylations)



N-dealkylation by CYP3A4

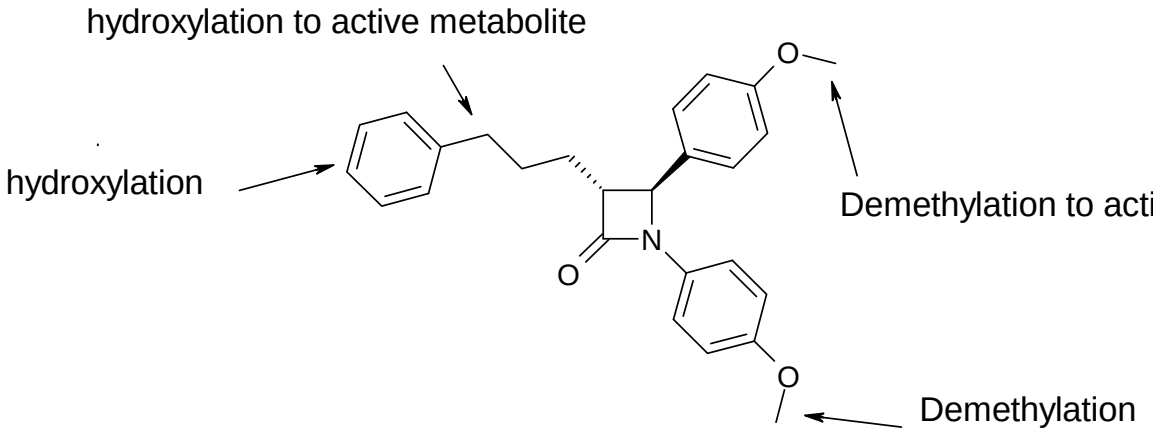
Diltiazem



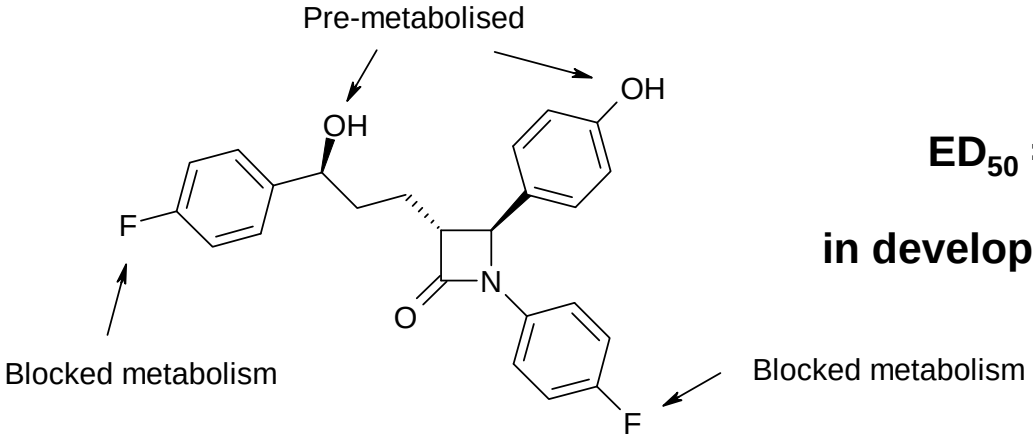
Reduced metabolism by 3A4

Use of metabolite identification to drive medicinal chemistry

Cholesterol absorption inhibitors (J. Med. Chem. 2004, 47, 1-9)



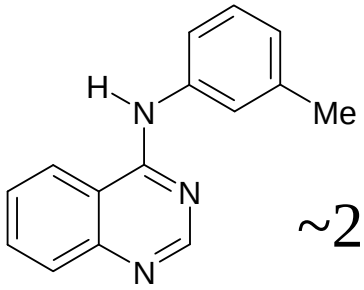
ED_{50} = 0.2 mg/Kg p.o. (Monkey)



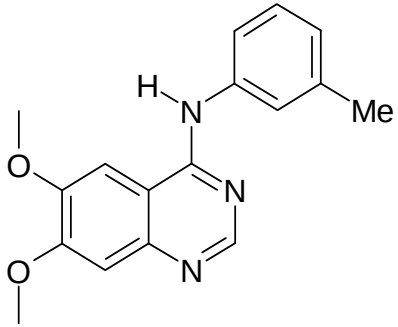
ED_{50} = 0.0005 mg/Kg p.o. (Monkey)
Ezetimibe
in development as cholesterol lowering agent

- Metabolites identified and synthesised
- Tested to identify active and inactive metabolites
- Sites of deactiviating metabolism blocked, sites of productive metabolism incorporated

Discovery of Iressa…

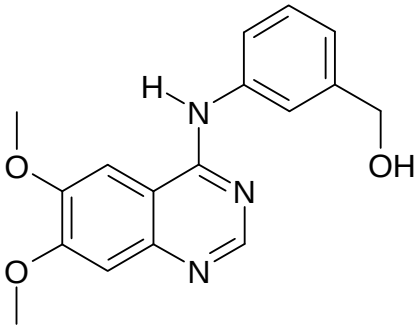


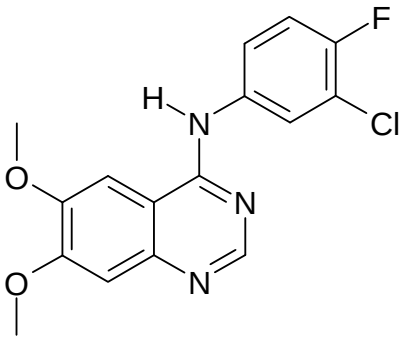
~200nM lead

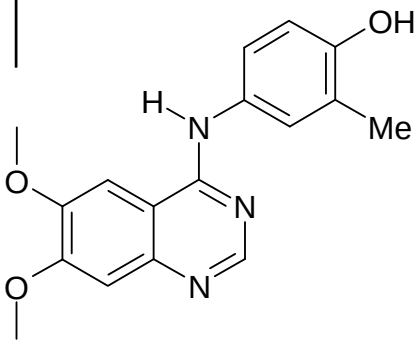


~5nM

Metabolised in vivo to

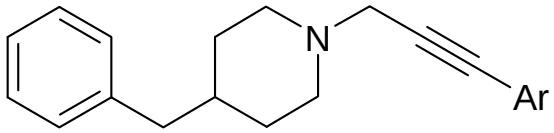


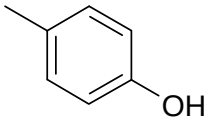
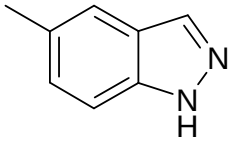
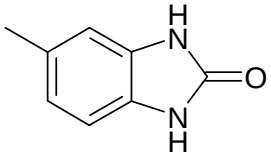




Blocking Phase II conjugation processes

- Exploration of phenol bioisosteres in a series of NMDA (NR1A/2B) receptor antagonists
- Phenol has low oral exposure and no oral activity due to extensive glucuronide formation
- Correctly placed phenol bioisostere is resistant to glucuronidation

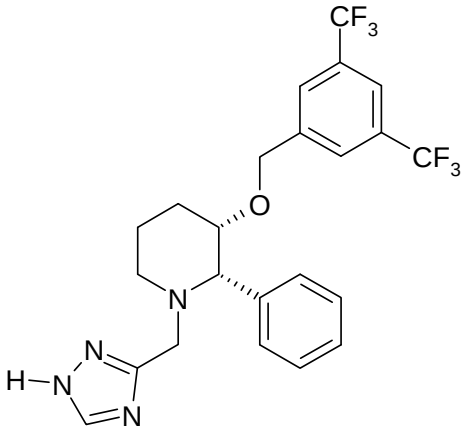


Ar	NR1A/2B IC_{50} nM	In vivo activity
	100	Inactive po
	38	NT
	5.0	active @ 10 mg/kg po

Brainteaser – NK-1 receptor antagonists

(J. Med. Chem. 1996, 39, 2907-2914 and J. Med. Chem. 1998, 41, 4607-4614)

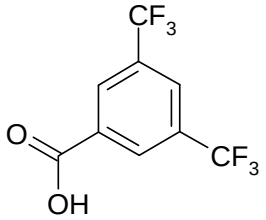
How would you attempt to increase the duration of action of this lead compound?



CLUE: cLogD = 5.2

NK-1 IC_{50} = 0.18 nM
Biological effect at 8 hours (guinea pig): 55% inhibition @ 1mg/kg po
24 hours: 0%

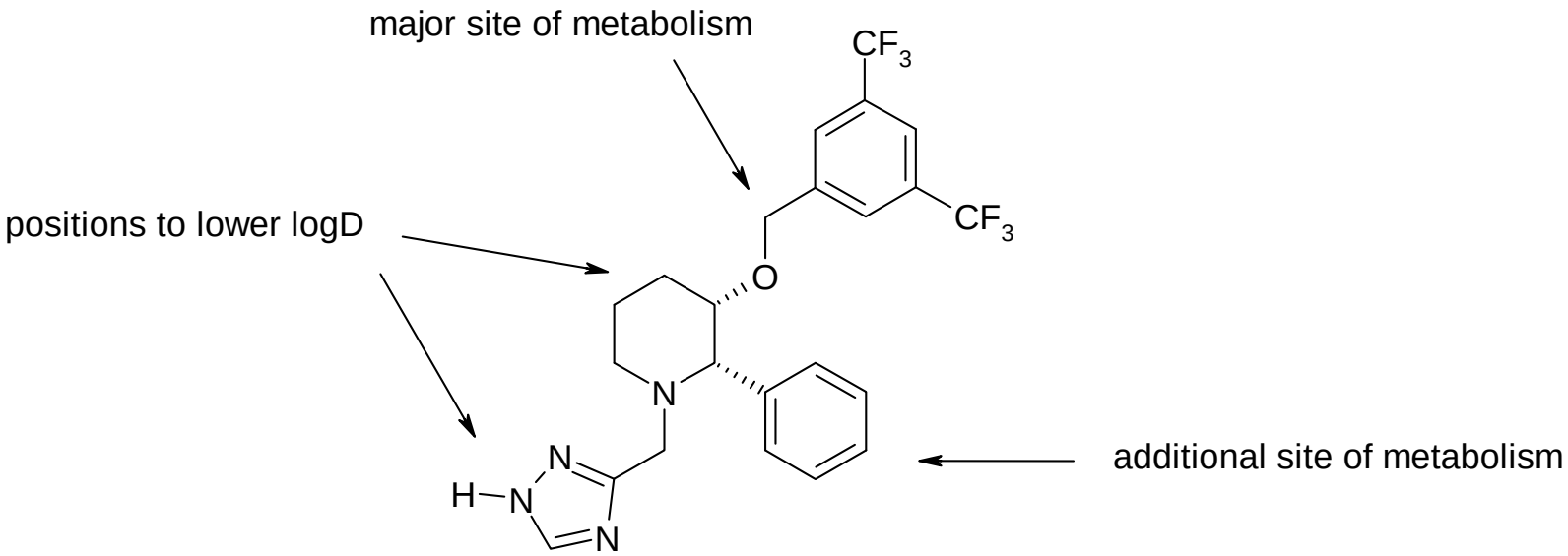
CLUE: A major metabolite was identified as:



Lunch

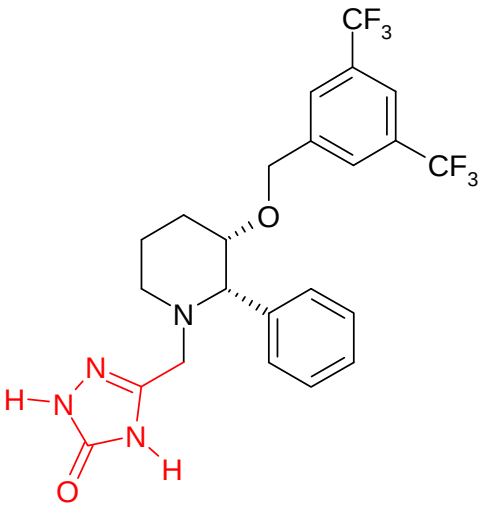
Brainteaser – NK-1 receptor antagonists

Strategies: **Lower overall lipophilicity of compound**
- find areas of the molecule where logD can be lowered
Identify and block sites of metabolism

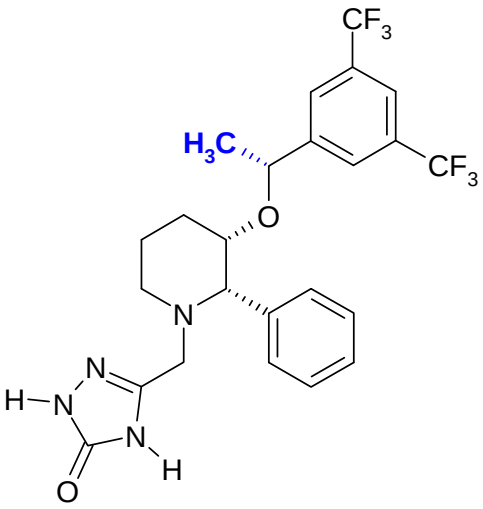


A Solution…..

cLogD = 3.9

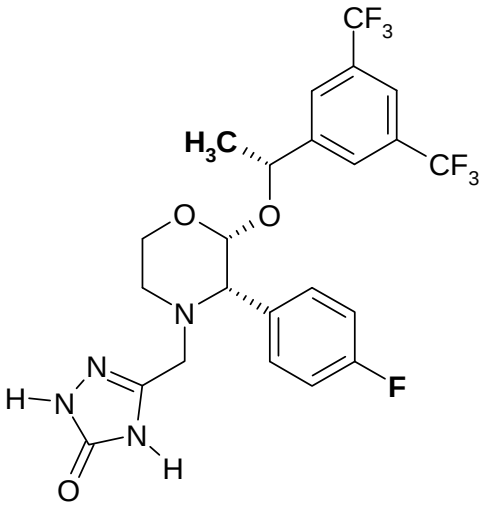


NK-1 IC_{50} = 0.1 nM



NK-1 IC_{50} = 0.16 nM
Effect at 8 hours: 97%
24 hours: 66%

cLogD = 4.1



NK-1 IC_{50} = 0.09 nM
Effect at 8 hours: 100%
24 hours: ID_{50} = 0.55 mg/kg p.o.

MK-869 for emesis

Before Lunch….a re-cap

- Absorption
  - Solubility
  - GI Instability
  - Permeability
  - Efflux
- Clearance
  - Plasma instability
  - Biliary elimination
  - Renal elimination
  - Liver metabolism

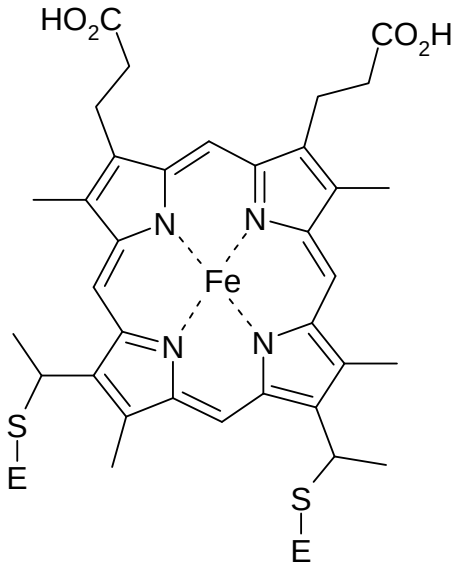
- Clearance
  - Decrease MW
  - Increase logD
  - Decrease logD / electron density
  - Decrease logD / planarity
  - Increase logD / rigidity

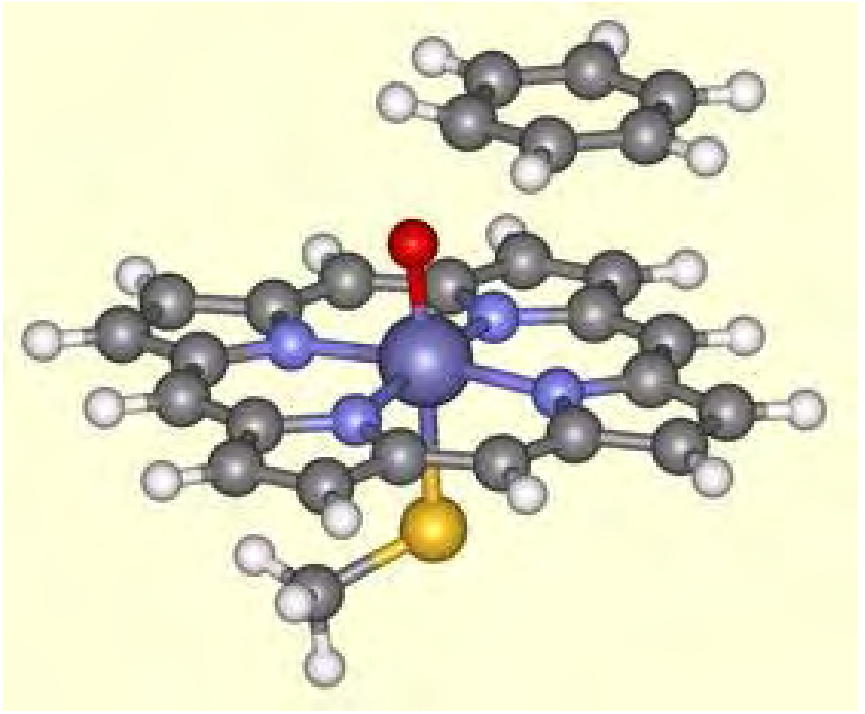
Now…

- Clearance continued
  - What enzymes are involved in PhI metabolism
  - Drug:Drug Interactions
  - Clearance and link to duration of action
    - Volume of distribution, half-life, PPB

Ph I - Cytochrome P450 Enzymes

- Carry out Phase I oxidations in liver cells (also present in the intestine)
- Membrane-bound Haem-containing proteins coordinating $Fe^{II/III}$ at the active site
- Found embedded in the endoplasmic reticulum (a cellular transport system composed of a honeycomb of membrane pervading the entire cytoplasm)
- Account for the biotransformation of approx. 60% of commonly prescribed drugs
- Cofactors: NADPH and molecular oxygen





Cytochrome P450 (CYP, P450)

- ~ 1000 isoforms known, > 100 in man!
- 74 families, 17 in man
- Many are responsible for metabolism of endogenous agents – eg steroids
- Some have multiple alleles (polymorphism) eg CYP2D6
- Some are not expressed in liver, but in lung, nasal mucosa, kidney, white blood cells
- CYP2D6 also found in brain
- CYP3A4 also found in intestine
- Some isoforms are inducible – 3A4, 2C9, 2C19, 2E1, 1A1, 1A2, 2B6
- Some are not – 2D6

CYP substrate specificity

- 1A2 - flat aromatic molecules & halo benzenes – caffeine, haloperidol + erythromycin
- 2B6 – cyclophosphamide
- 2C9 - S-warfarin, phenytoin, diclofenac & other NSAIDs, tolbutamide, losartan
- 2C19 – diazepam, tricyclic antidepressants, dextromethorphan, omeprazole
- 2D6 – debrisoquine, beta blockers, antipsychotics, dextromethorphan, SSRIs, TCAs, tolteridine, etc
- 2E1 – paracetamol, ethanol, tolbutamide, isoflurane
- 3A4 – terfenadine (hERG!), Ca blockers, midazolam, CsA, TCAs, opiates, steroids, many others

Cytochrome P450 Alleles

- Some have multiple alleles (polymorphism) eg CYP2D6
  - CYP2D6*1 (wt) + > 25 others (*5 = deleted)
  - Most are “poor” metabolisers compared to wt, but depends on substrate
  - Some people have multiple copies of CYP2D6 to be ultra-rapid metabolisers
    - Can lead to lack of effect or toxicity from metabolites
  - Homozygous CYP2D6*4 associated with red/blonde hair and melanoma
- CYP2C9 – 3 alleles – poor metabolisers may suffer phenytoin toxicity
- CYP2C19 – 8 alleles
- CYP3A4 – NO ALLELES!
  - but wide range of expression and activity, easily induced

CYP2D6 Polymorphism

Predicted effects of CYP2D6 alleles and multiple copies:

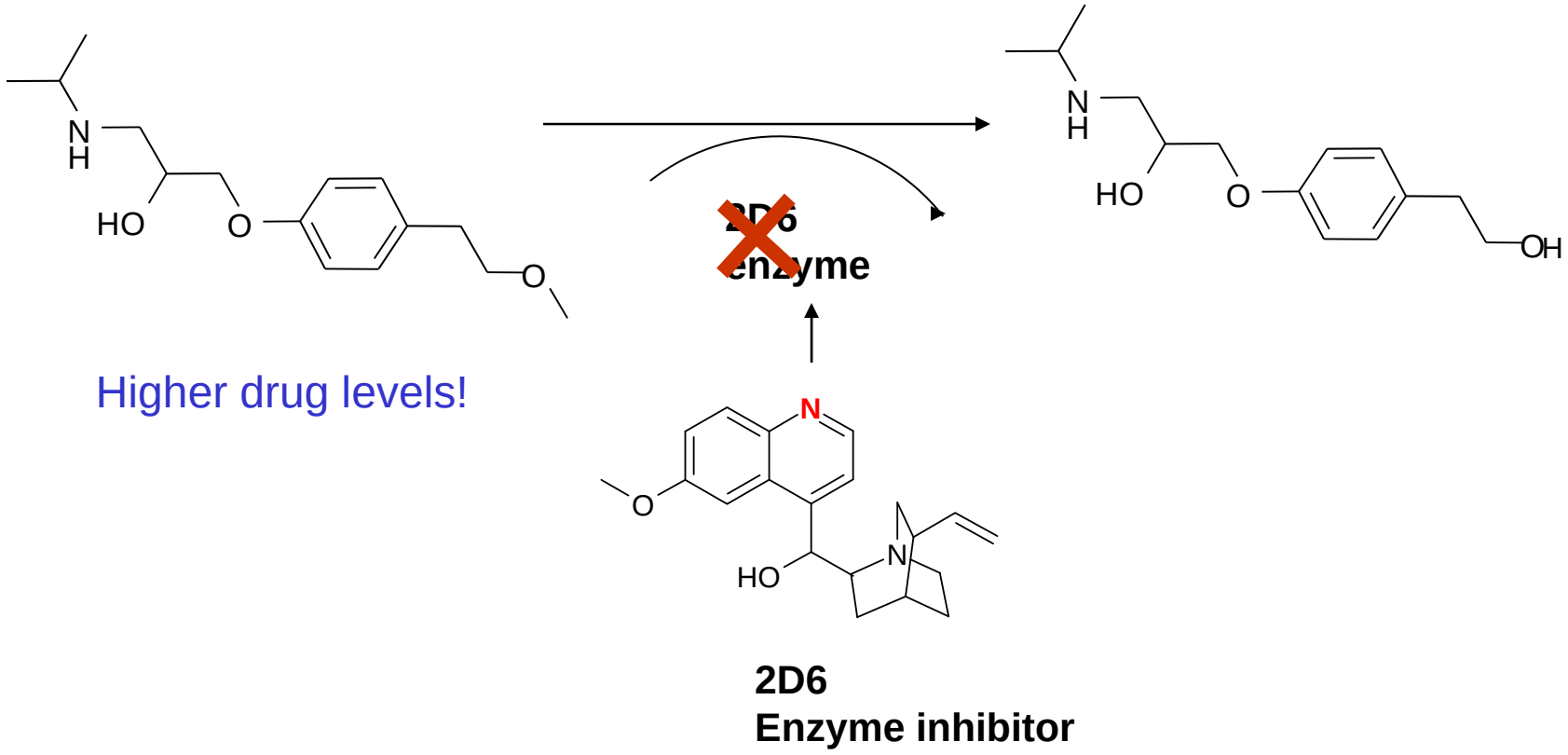
Allele	1	2	3	4	5	6	7	8	9	10	11	14A	14B	15	17	19	20	25	26	29	30	31	35	36	40	41	1XN	2XN	4XN	10XN	17XN	35XN	41XN
1	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E
2		E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	U	U	E	E	E	U	E
3			P	P	P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
4				P	P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
5					P	P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
6						P	P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
7							P	P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
8								P	I	I	P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
9									I	I	I	I	N	I	I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
10										I	I	I	N	I	I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
11											P	P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
14A												P	N	P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
14B													N	N	N	N	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
15														P	I	P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
17															I	I	I	N	N	I	N	N	E	I	I	I	E	E	I	I	I	E	I
19																P	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
20																	P	N	N	I	N	N	E	I	P	I	E	E	P	I	I	E	I
25																		N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
26																			N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
29																				I	N	N	E	I	I	I	E	E	I	I	I	E	I
30																					N	N	E	N	N	N	N	N	N	N	N	N	N
31																						N	E	N	N	N	N	N	N	N	N	N	N
35																							E	E	E	E	U	U	E	E	E	U	E
36																								I	I	I	E	E	I	I	I	E	I
40																									P	I	E	E	P	I	I	E	I
41																										I	E	E	I	I	I	E	I

E = effective P = poor I = intermediate N = unknown U = ultra-rapid

CYP Advice

- Avoid metabolism by sole isoform – bigger risk of clinically significant drug-drug interactions (DDIs)
- Avoid predominant metabolism by CYP2D6 – too many poor metabolisers
  - In silico screening for easily oxidised position 5 or 7 Å from basic nitrogen
- Or CYP3A4 – very wide range of activity in population
- CYP oxidation requires two properties:
  - 1 binding to protein
  - 2 oxidisable position
  - If you prevent oxidation by blocking without lowering affinity, you will turn a good substrate into a good inhibitor! Some blocking groups increase lipophilicity, increase binding, increase inhibition
- Avoid notorious problem groups – eg 4-pyridyl-, 4-imidazolyl-
- Use suitable (PBPK) software – Simcyp includes variability in populations and extrapolates from in vitro data to predict PK and drug-drug interactions

Drug:Drug Interactions – the basic concept



Higher drug levels!

2D6
Enzyme inhibitor

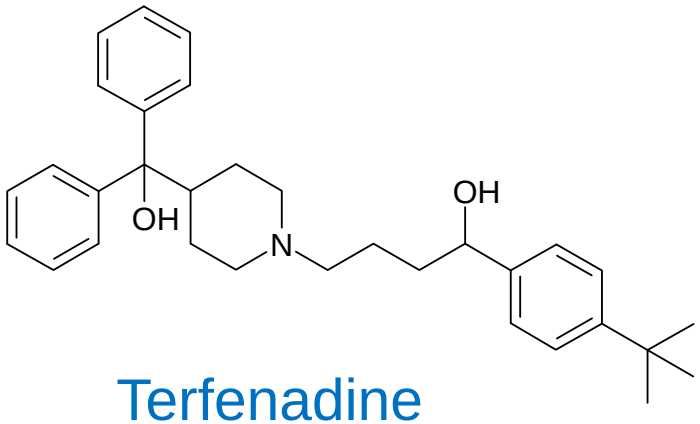
Cytochrome P450s
Drug-Drug Interactions

- Drugs may inhibit/promote P450 enzymes
  - Phenobarbitone induces (promotes) P450 enzymes
  - Cimetidine inhibits P450 enzymes
  - Both interact with the anti-coagulant warfarin
    - Phenobarbitone makes it less effective
    - Cimetidine slows the metabolism (potential safety issues)
  - Administration of a CYP3A4 inhibitor with cyclosporin (immunosuppresant) allows lower dose to be used

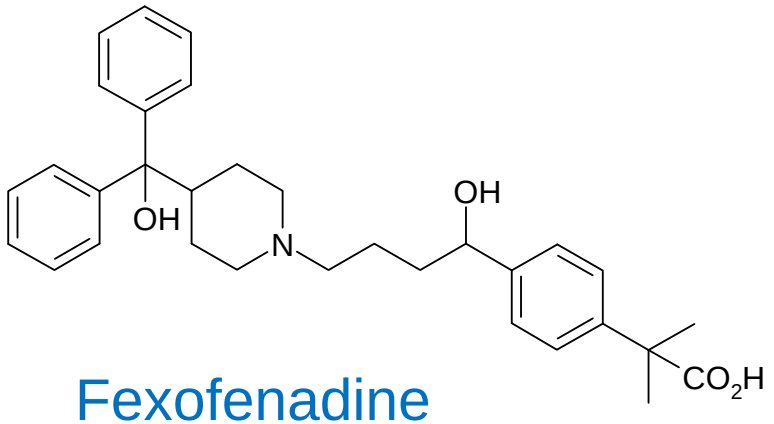
- A clear understanding of CYP interactions is important for all new drugs (inhibition can be measured *in vitro*)

Cytochrome P450s
Impact of food & smoking

- Some foods affect P450 activity
  - Brussel sprouts and smoking enhance P450 activity
  - Grapefruit juice inhibits activity
- Terfenadine (inactive) is metabolised to fexofenadine (active, antihistamine)
  - Metabolism is inhibited by grapefruit juice
  - Potential for increased amount of terfenadine in the body leading to cardiac toxicity



Terfenadine



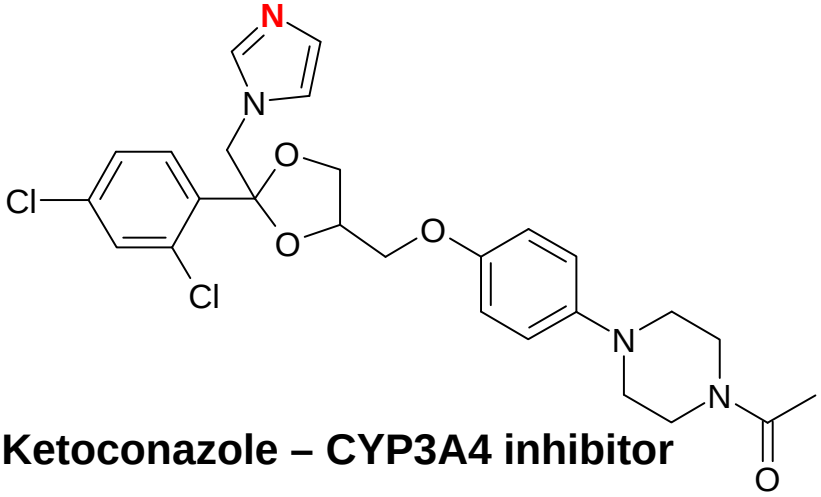
Fexofenadine

Inhibition of cytochrome P450's

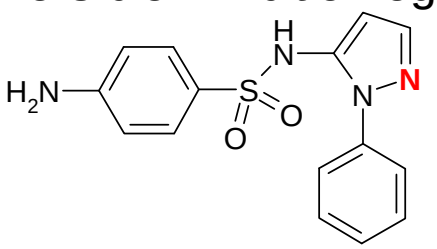
- Potency of inhibition has been correlated to lipophilicity of compounds
  - lowering logP is a good strategy for reducing CYP450 inhibition

- Reactive metabolites of compounds may covalently bind to P450
  - mechanism based inhibitors (usually irreversible)
  - N-methyl groups, alkenes, alkynes, furans, thiophenes, methylenedioxy groups

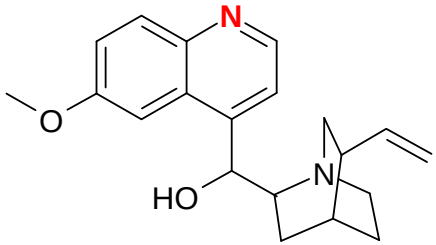
- Certain structural features may lead to reversible inhibition eg aza, diaza groups



Ketoconazole – CYP3A4 inhibitor



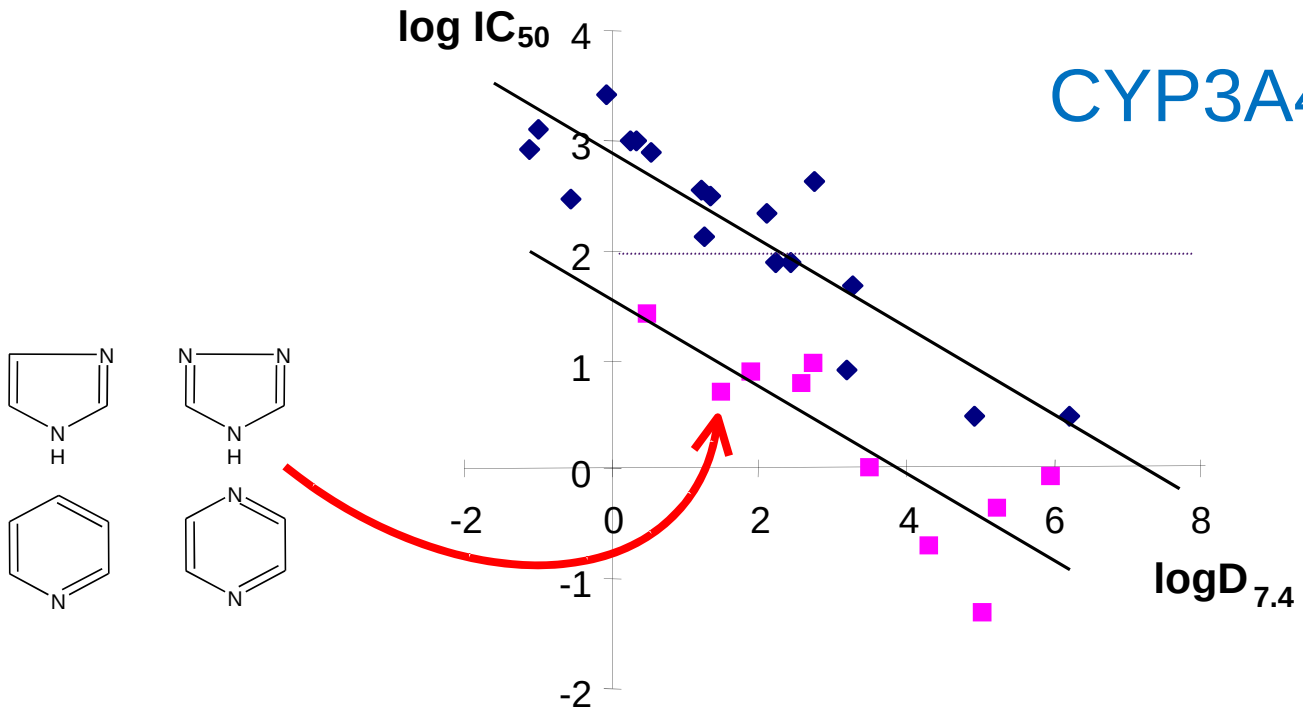
Sulphaphenazole
- CYP2C9 inhibitor



quinidine
- CYP2D6 inhibitor

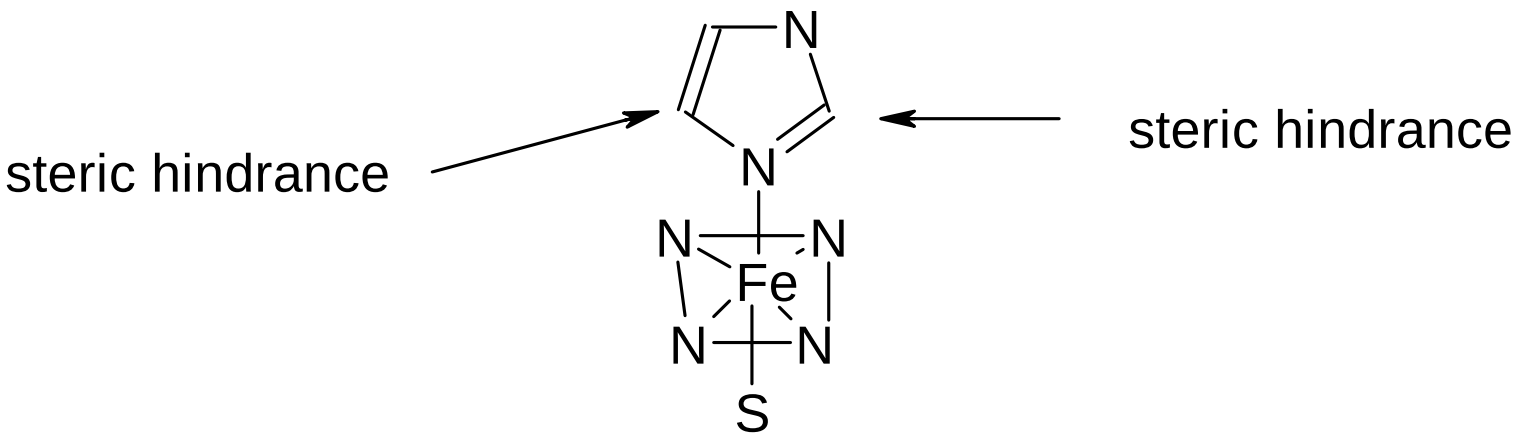
Drug Interactions

- Cyp 3A4 has logD dependence



- General $LogD_{7.4}$ trend (consistent with active site)
- Sterically uninhindered N-cont. heterocycles
- Applicable to Project Chemistry .

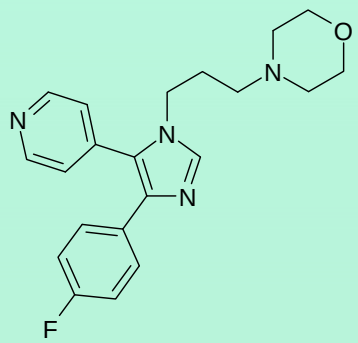
Inhibition of Cytochrome P450's

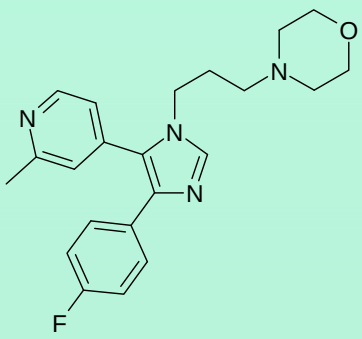


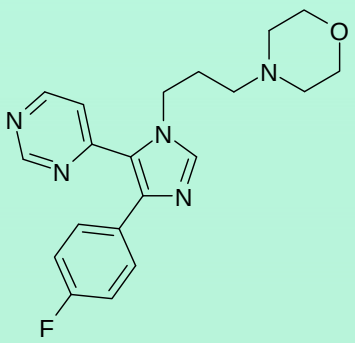
- **Nitrogen atom displaces water from haem complex**
- **Introduction of steric hindrance around N-atom (eg alkyl groups) may reduce interaction**
- **Look for isosteres of the *aza/* diaza groups and reduction of electron density**

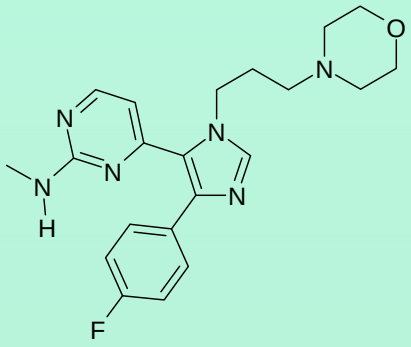
Example – p38 MAP kinase inhibitors

(Bio Med Chem Lett 1998, 8, 3111-3116)









p38 IC_{50} (μM)	**1.3**	**2.1**	**0.22**	**1.9**
CYP 2D6 inhibition % inhib @ 10 μM	**86%**	**51%**	**34%**	**11%**
cLogD (7.4)	**2.5**	**2.9**	**1.8**	**1.9**

Summary, what can you do about p450 inhibition?

- Reduce lipophilicity of molecules
- Increase steric hindrance around metal-binding heterocycles

And drink less grapefruit juice!

Distribution & Duration

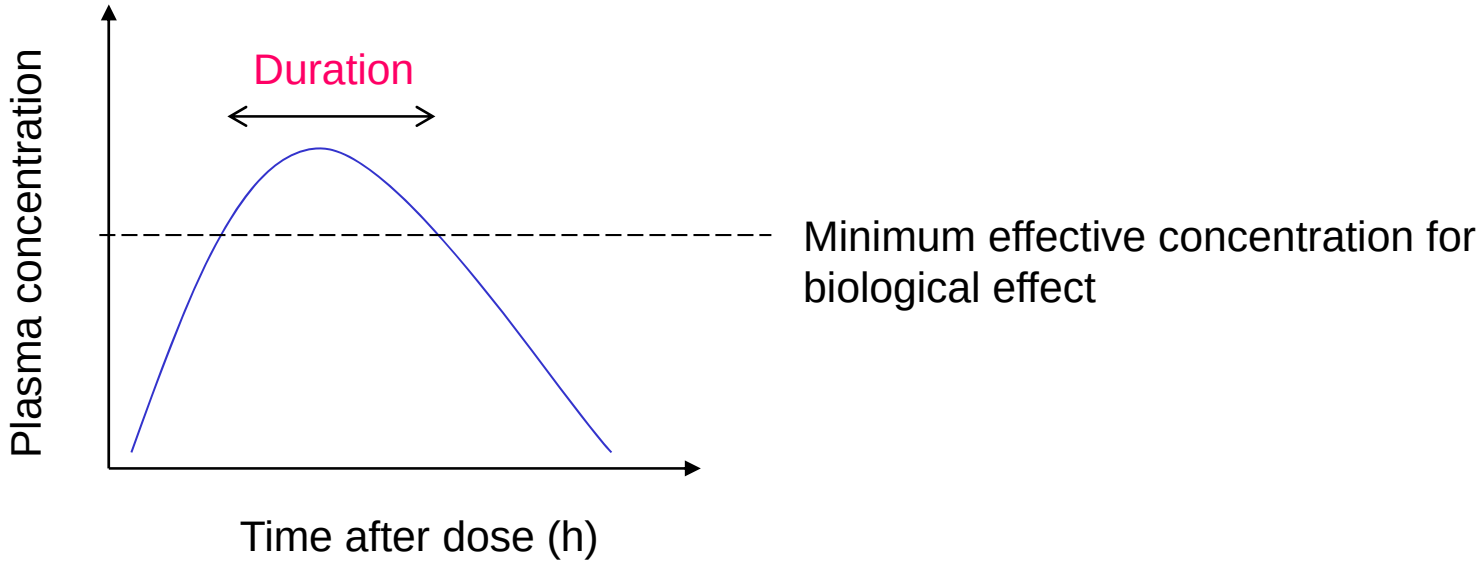
From clearance to duration of action…

What is “good” or “low” plasma exposure of a compound?

How much for how long?

Depends on:

- the affinity (potency) of the compound at the biological target
- what plasma concentration is required to give the desired biological effect
- how well the compound reaches the tissue or biological target from plasma



How to increase half life ($T_{1/2}$)

The elimination half life of a compound is determined by two factors
- Volume of distribution (theoretical volume into which a drug distributes)
- Clearance (the volume cleared of drug per unit time)

$$T_{1/2} = \frac{0.693\ V}{CL}$$

V = volume of distribution
CL = clearance

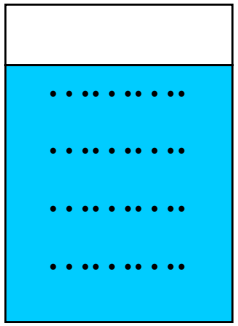
Half life in plasma can be increased by:
- increasing V, or
- decreasing CL

Volume of Distribution

- Not a real volume!
- A parameter relating the plasma drug conc to the total amount of drug in the body

Best way to understand this is an example:

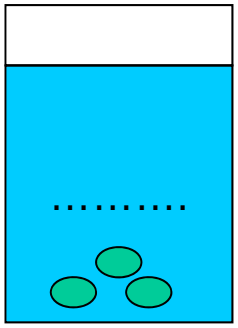
Addition of a cpd to water:



10 mg added to 1L of water

Concn. is 10 mg/L

Addition of a cpd + Charcoal:



Know that still 10 mg cpd in total

Now, concn. measured is 2 mg/L

To find the 10 mg total, the volume should be 5 L

The cpd appears more dilute than anticipated - as it has distributed to other compartments!

In real life, we know the total drug administered (i.v. dose), and measure plasma concn.

It follows that the major determinant of V_d is how well a drug partitions from plasma into other compartments - not charcoal (!), but into tissues such as liver, muscle, heart, fat

A drug that partitions well will have a high V_d as less will remain in the plasma
A drug that partitions poorly will have a low V_d as it will be retained in the plasma

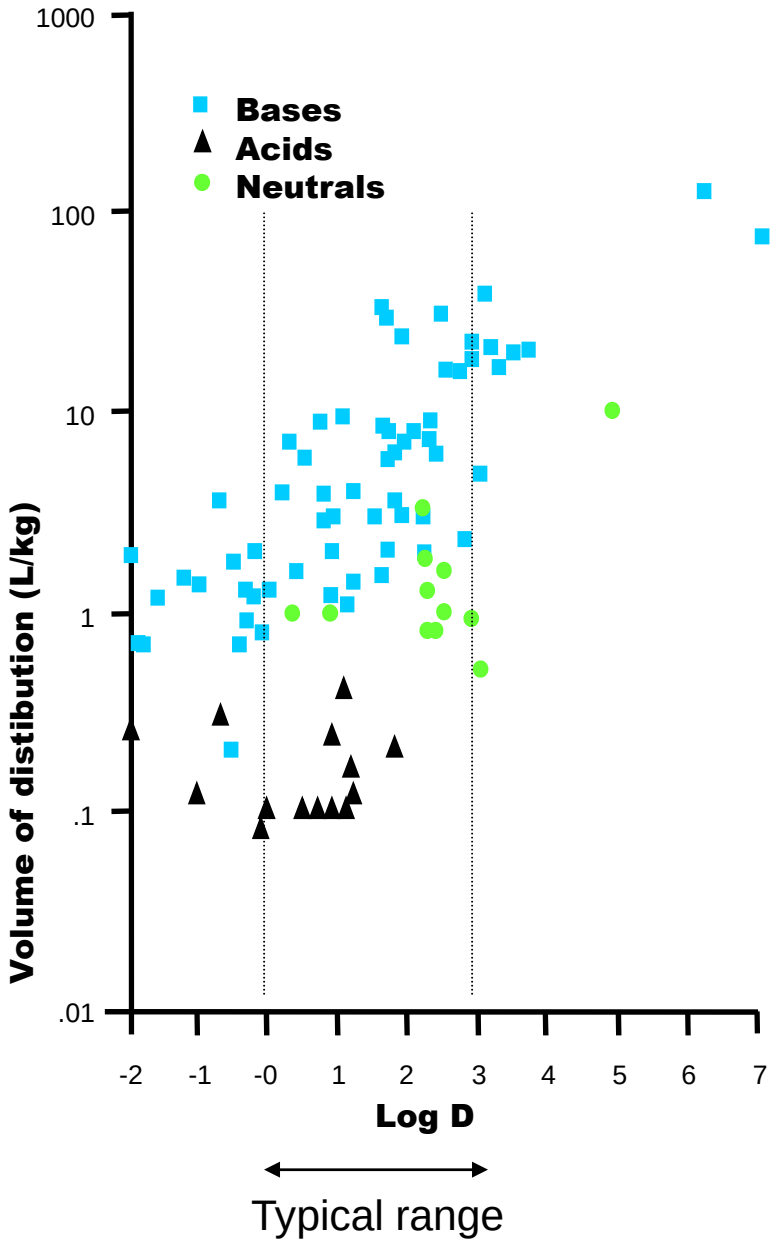
What factors govern volume of distribution?

Volume of distribution is also physical chemistry

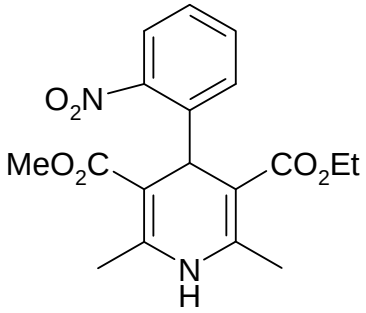
Influenced by:

- pKa (tissue pH ~6.5 is slightly lower than plasma ~7.4)
  - generally bases > neutrals > acids
- Lipophilicity (tissue is generally lipophilic)
  - increase logD, increase Vdss
- Plasma protein binding (unbound drug free to cross membranes)
  - increase PPB, decrease Vdss

Volume of Distribution correlates with LogD

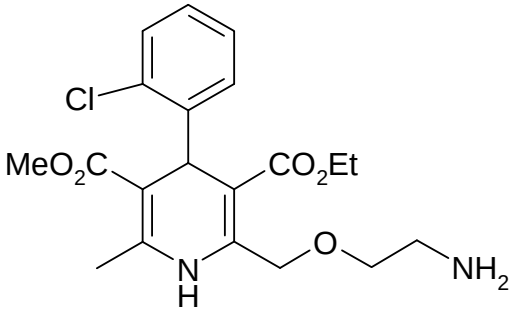


Volume of distribution can be modified



Nifedipine
CL = 8.4 ml/min/kg
V = 1.0 L/Kg
Half life = 1.9 h

Clearance is unchanged, but going from neutral (low Vdss) to base (high Vdss) increases half-life and duration of action

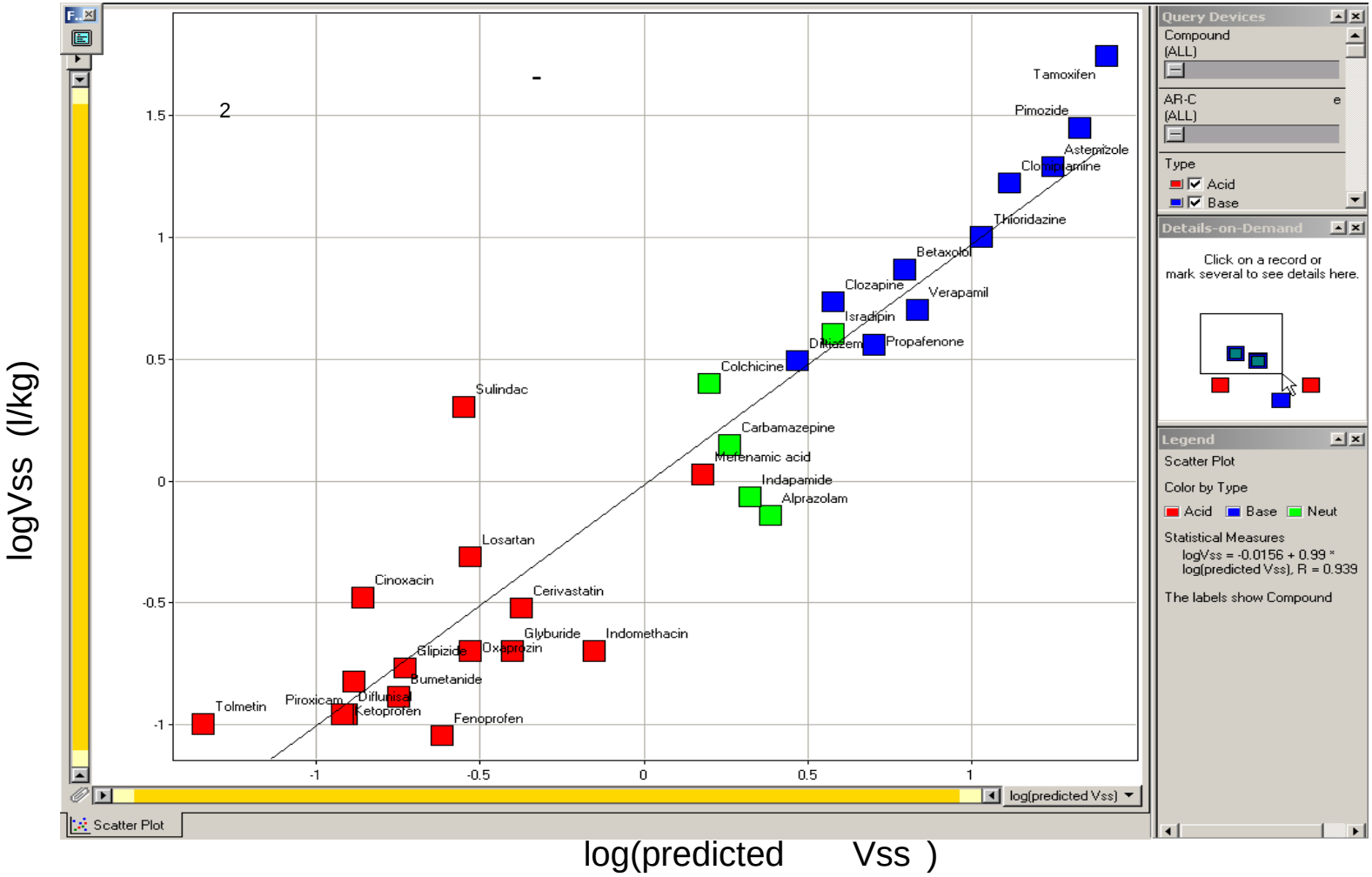


Amlodipine
CL = 7.0 ml/min/kg
V = 21.4 L/Kg
Half life = 33.8 h !!

Sales 2006: $5bn

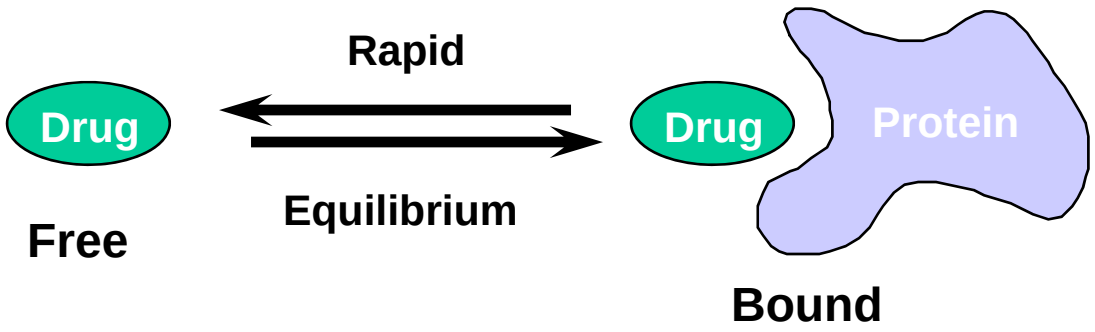
Volume of distribution can be predicted

Equations which combine lipophilicity, PPB and pKa give good predictions of Vdss. See ***J Med Chem* 2004, 47, 1242-1250**



Plasma Protein Binding

PPB has a big impact on Vdss:



- Compounds with high plasma binding are retained in plasma

  0-50% bound = negligible
  50-90% = moderate
  90-99% = high
  >99% = very high

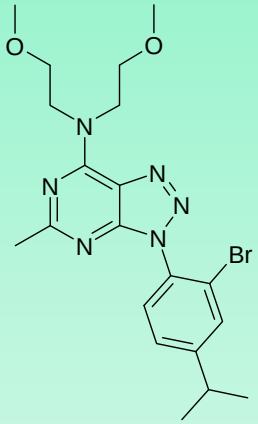
- Usually consider binding to albumin which is lipophilic & slightly basic, hence acids tend to have very high PPB, bases less so

- NB:- it is the %free or fraction unbound (fu) that matters
  The difference between 99.9% bound and 99.0% (10-fold)
  is greater than the difference between 90% and 50% (5-fold).

Plasma Protein Binding

- PPB also has a big impact on in vivo efficacy*
- Unbound / 'free' levels determine in-vivo efficacy
- If Protein Binding too High
  - High cell IC_{50}s & Lack of efficacy in-vivo:

*Assuming reversible binding with biological target: PPB not relevant for β-lactam antibiotics & some mechanism-based protease inhibitors



SC241

SC241- a CRF antagonist considered for clinical development

Ki = 4.7 nM, rat BA = 22%, T1/2 = 6h.

BUT – completely inactive in an anxiety behavioural assay (rat) @ 30 mg/kg po (1 h time point)

- **30 mg/kg - Plasma levels 400 nM @ 1 hour**
- **Brain/ plasma ratio of 0.5**
- **Predict whole brain levels to be 200 nM**

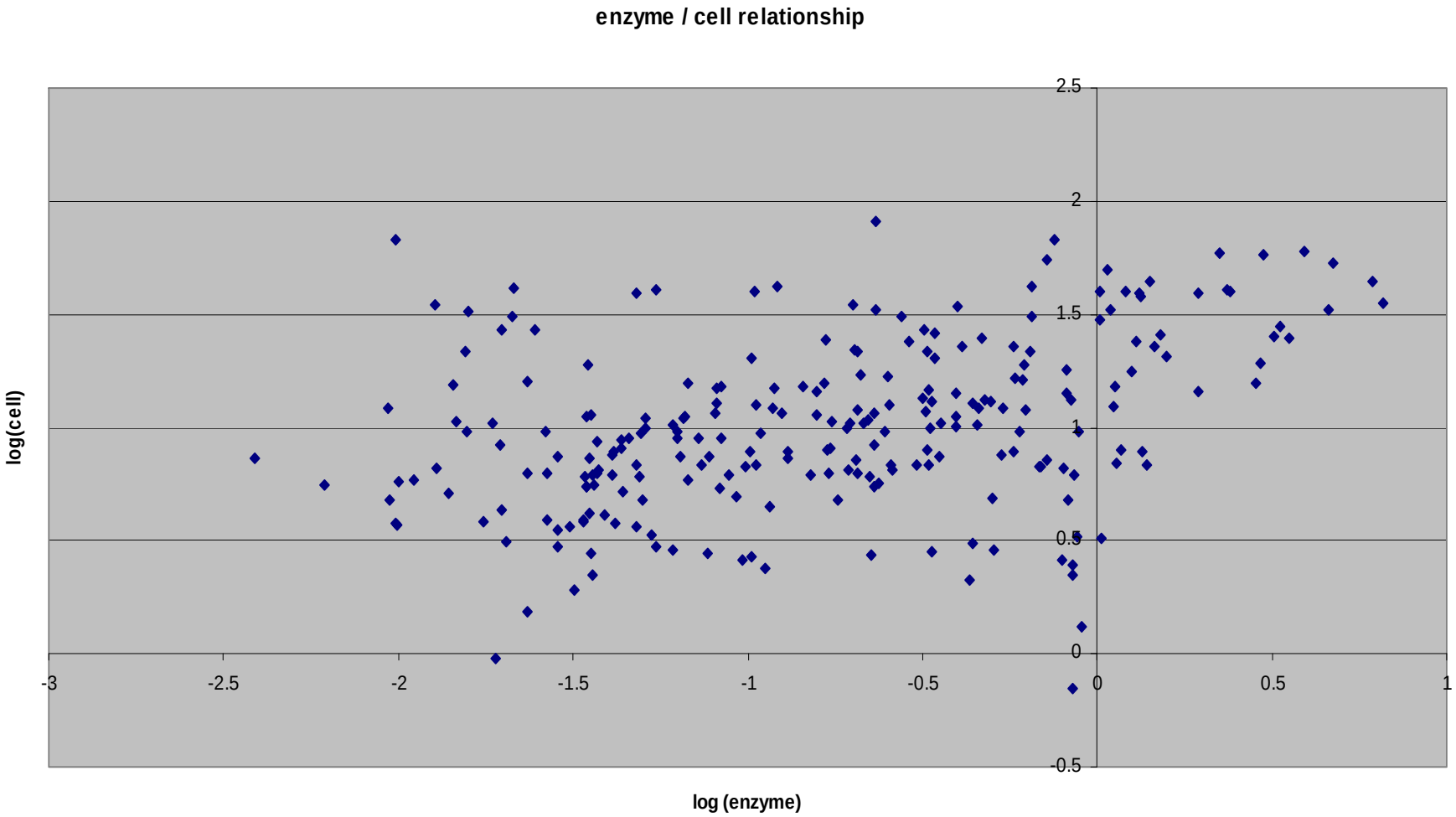
SC241 is highly PP bound – human plasma 99.88%, rat plasma 99.76%

- **Actual free CNS levels at 1 hour < 1 nM**

Efficacy failure due to insufficient free drug exposure

Danger of using whole brain levels

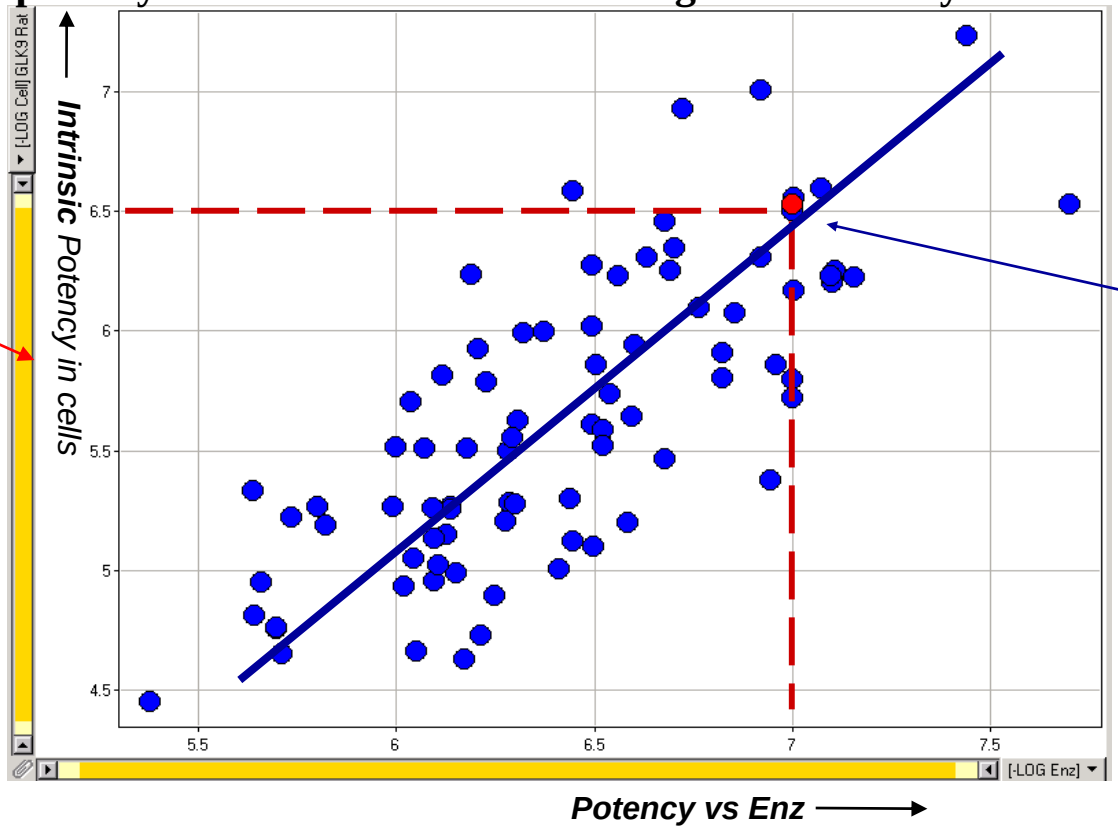
Project Enzyme – Cell Relationship



No clear correlation between enzyme & cell potency...

PPB Correction of Enzyme-Cell Correlation

Cell potency corrected to reflect free drug levels in assay



This axis includes PPB & cellular Potency parameters

Enzyme 100nM
0.15% free
Cell potency 3uM

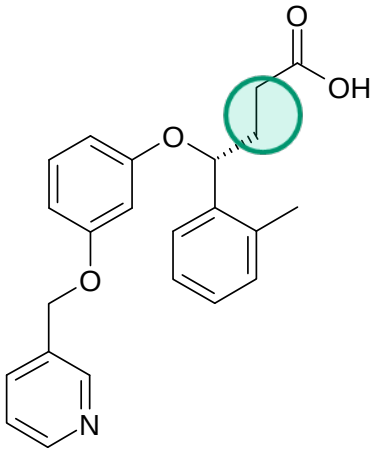
Factoring in protein binding shows
clearer correlation between enzyme & cell potency

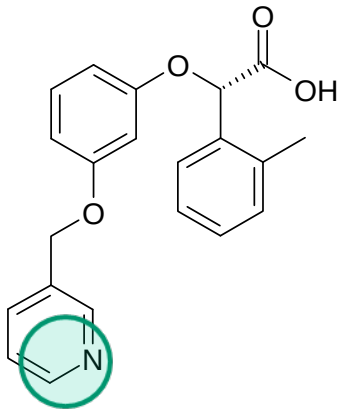
What you can do about PPB
– reduce lipophilicity!

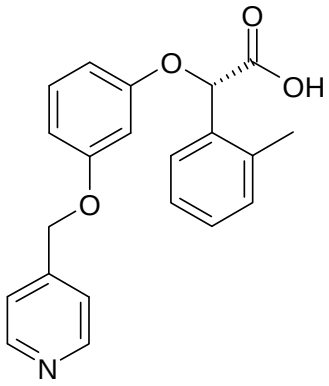
Example:

Reducing PPB in a series of acidic endothelin ET_A receptor antagonists

J. Med. Chem. 2000, 43, 900-910



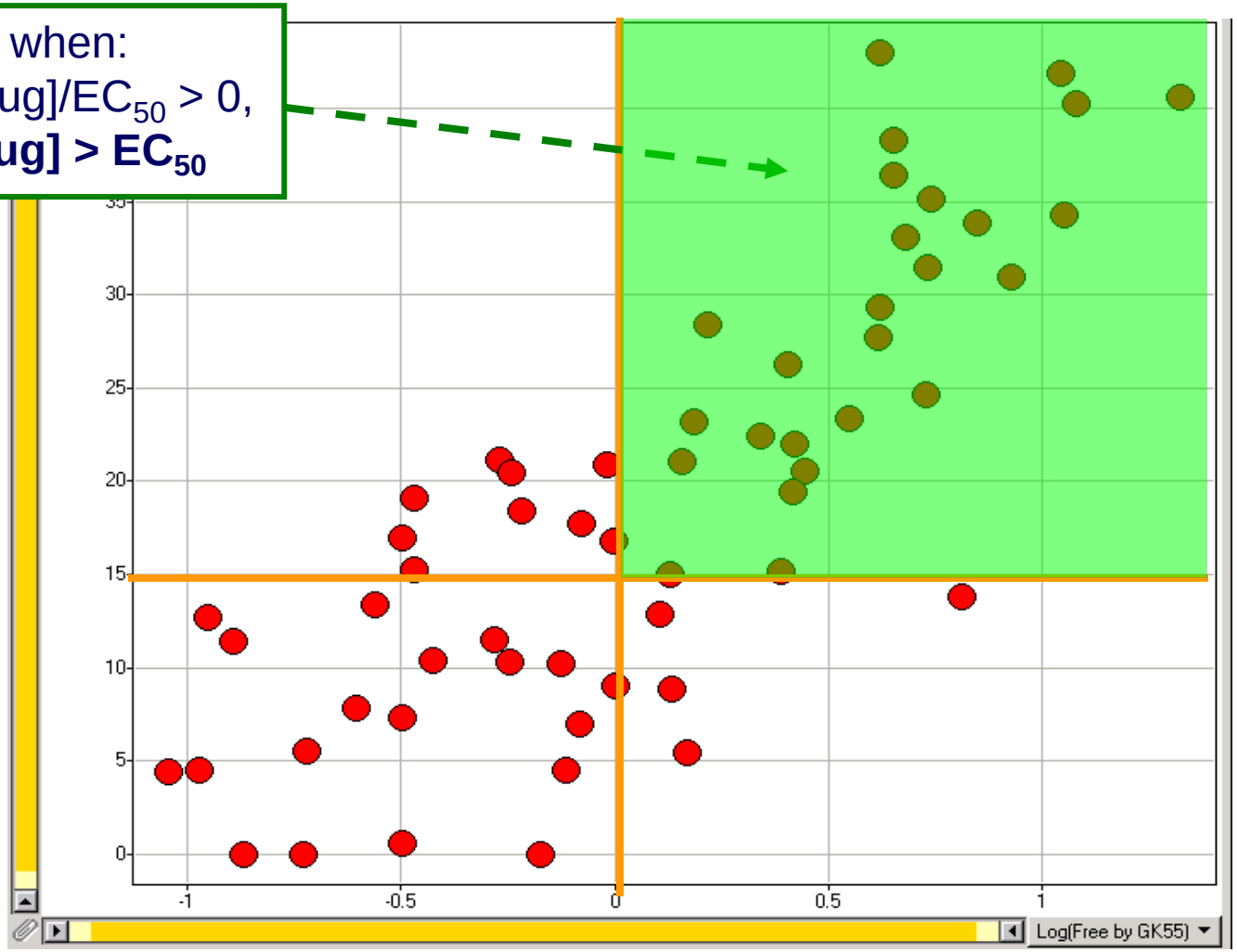




logD (7.4)	**1.6**	**-0.05**	**-0.04**
ET_A IC_{50}	**4.0 nM**	**70 nM**	**3.0 nM**
ET_A IC_{50} + 10% plasma	**95 nM**	**150 nM**	**9.0 nM**
Decrease in blood pressure (rat) @ 25 μmol/kg i.v. (shift in drc to ET-1)	**10X**		**90X**

PKPD Relationship

>15% effect when:
log [Free Drug]/EC_{50} > 0,
ie. **[Free Drug] > EC_{50}**



Log (Free Drug Level/Enz EC_{50})

% effect driven by ↑ Potency, ↑ Exposure & ↑ % Free

So now you can predict in vivo activity!

- Imagine you are in the project team using the model on the slide before.
- You have two compounds, but which is the best?

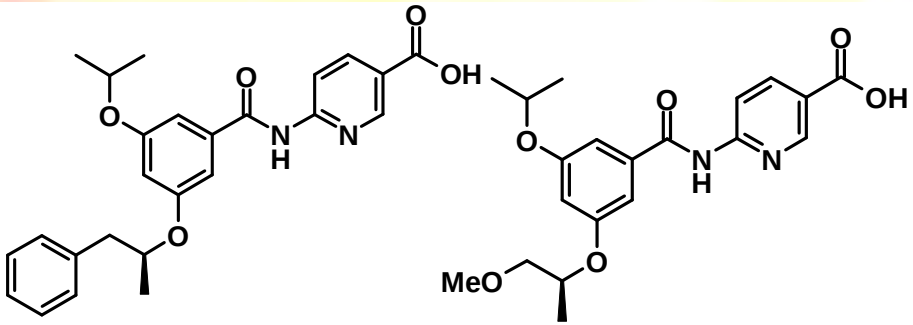
EC50	0.02	0.07
PPB	99.7%	98%
Oral Cmax	2.0uM	4.5uM
Predicted in vivo activity	?	?

Coffee Break

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



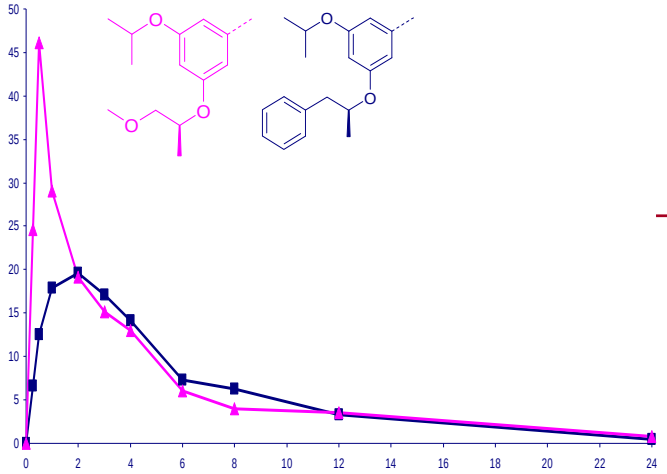
	GKA 31	GKA 30
Enzyme EC_{50} (μM)	0.02	0.61
% free (Rat)	0.23	5.34
Solubility (μM)	8	3140
Cl (ml/min/kg)	3.3	2.3
Unbound Clearance	20	45
F (%)	100	85

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

How to rank compounds?

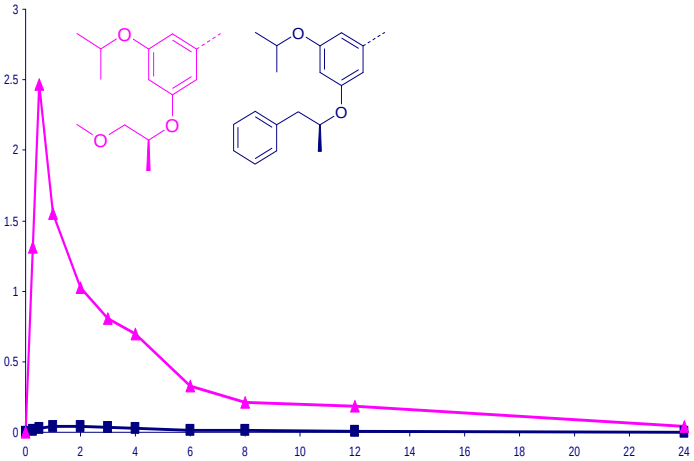
Best cpds will have best coverage above PKPD free drug multiple

Total drug conc (dose normalised)

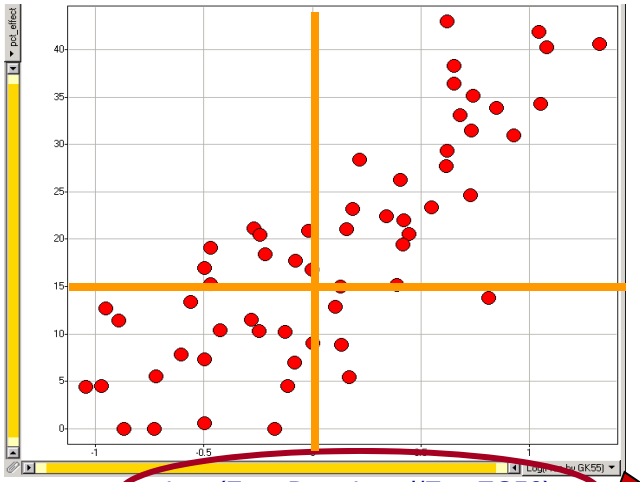


Correct for free drug

Free drug conc

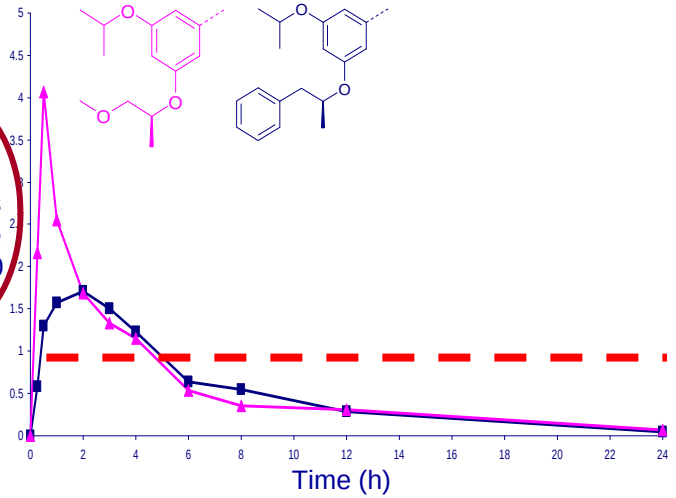


Correct for potency

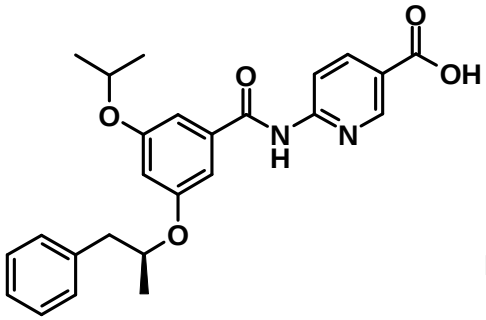


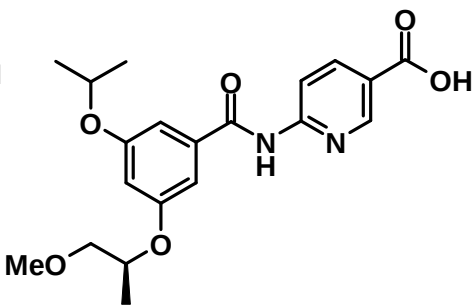
Same axis as PK:PD

Free drug conc as multiple of EC50

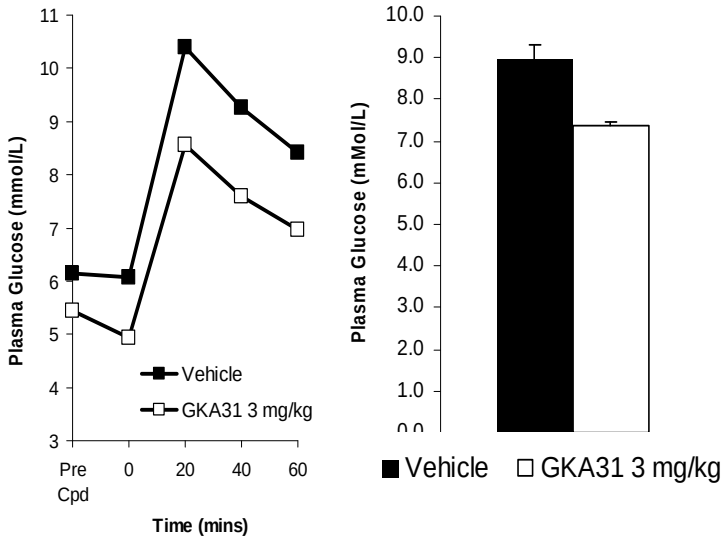


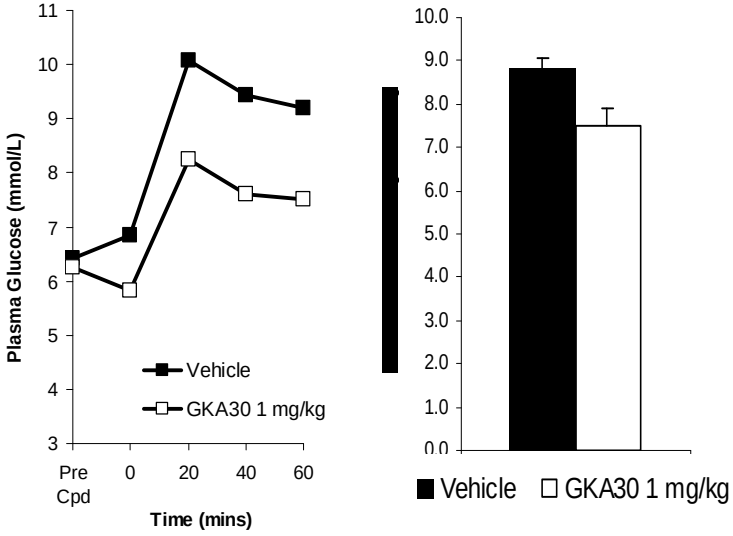
In vivo efficacy data





	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

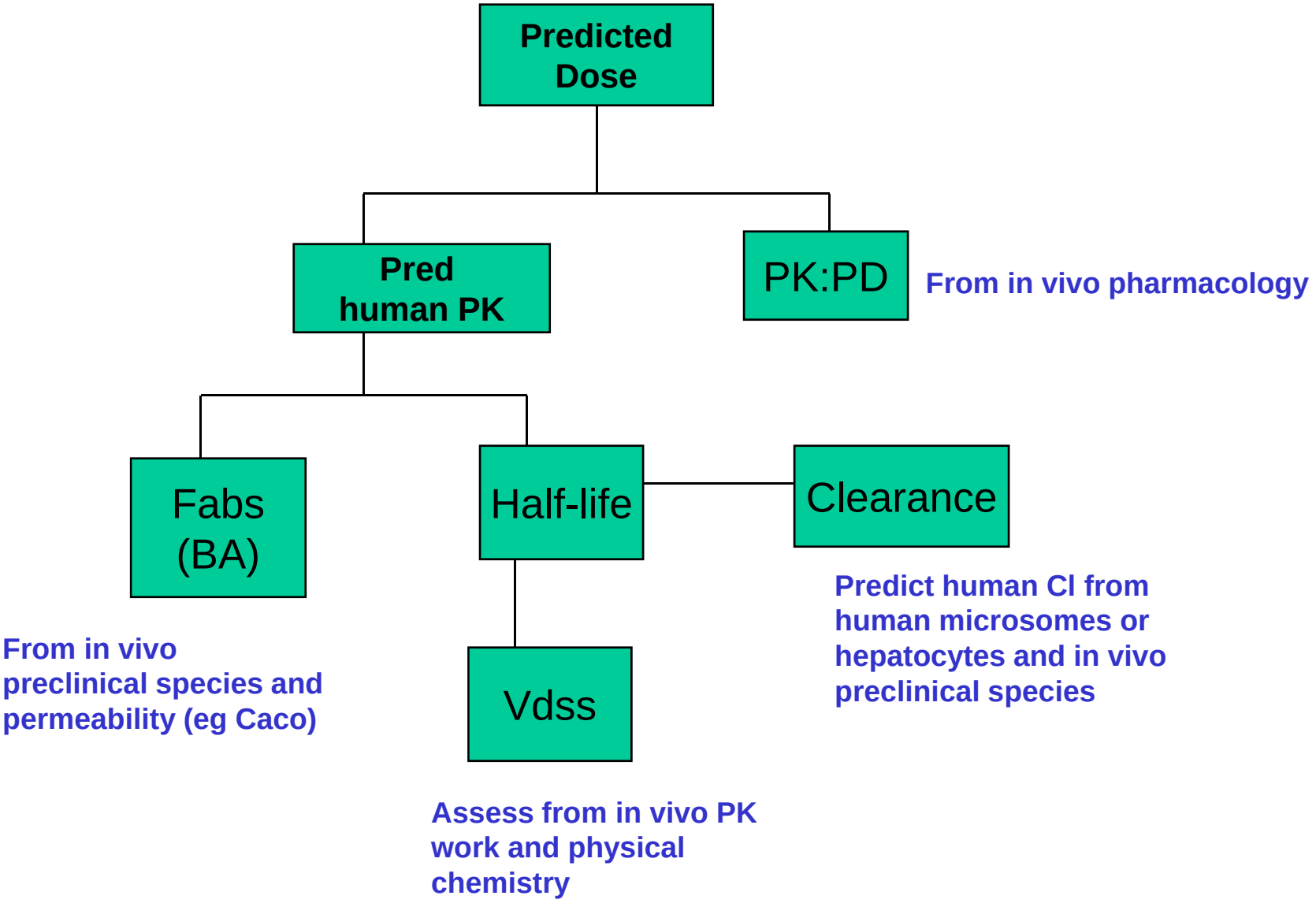




Biorg. Med. Chem. Lett., **2006**, 16, 2705

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!

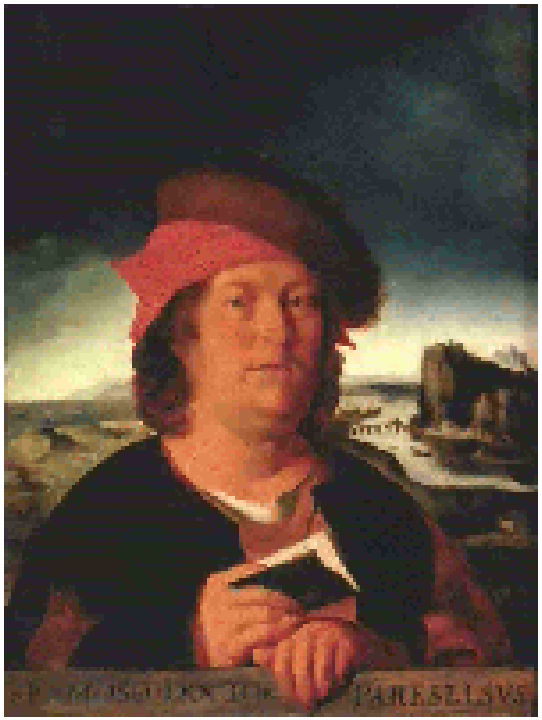
The Role of Toxicology

- **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
    - 2g/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)

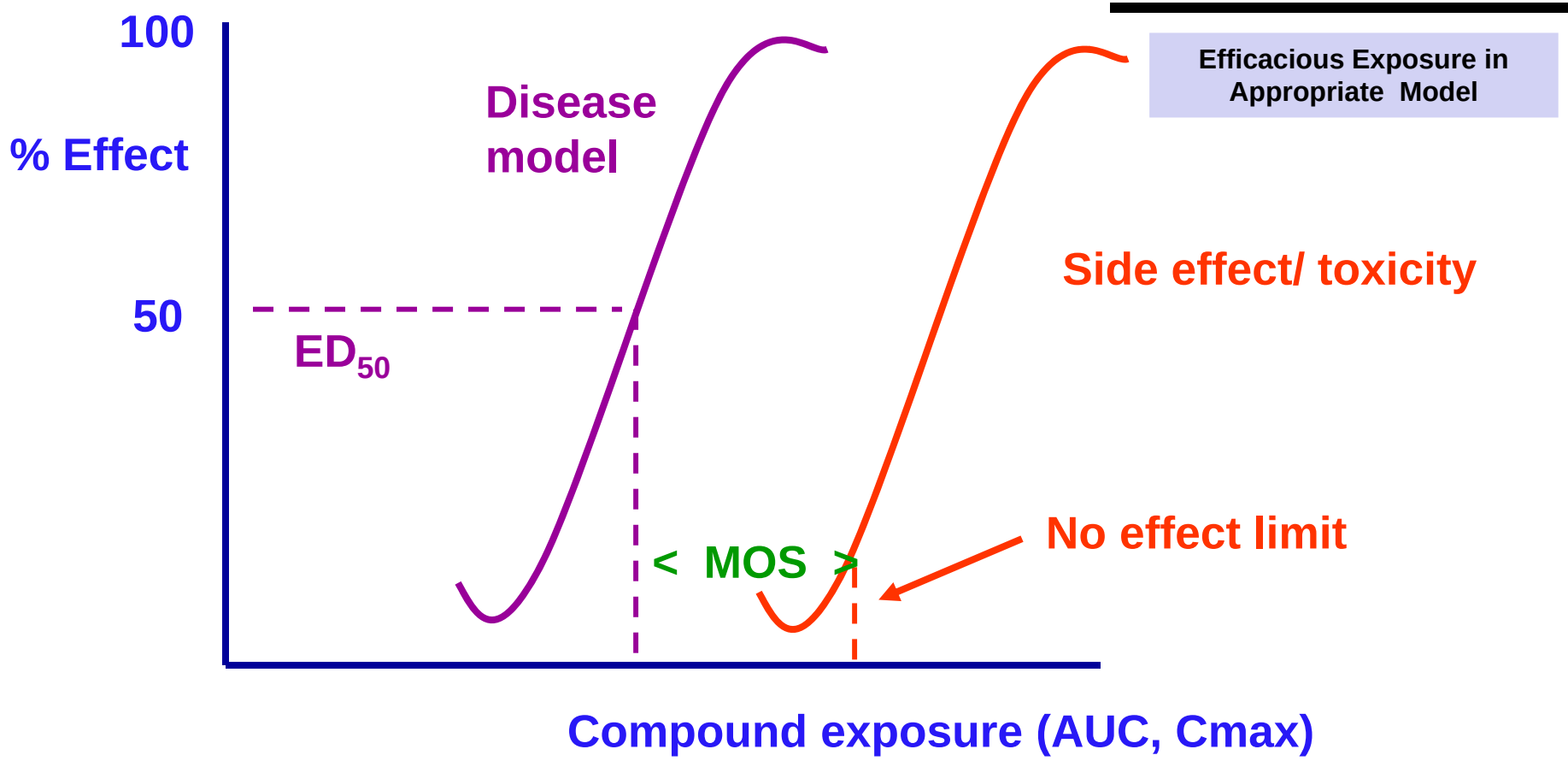
"All substances are [toxic];

There is none which is not [toxic].

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

Margin of Safety

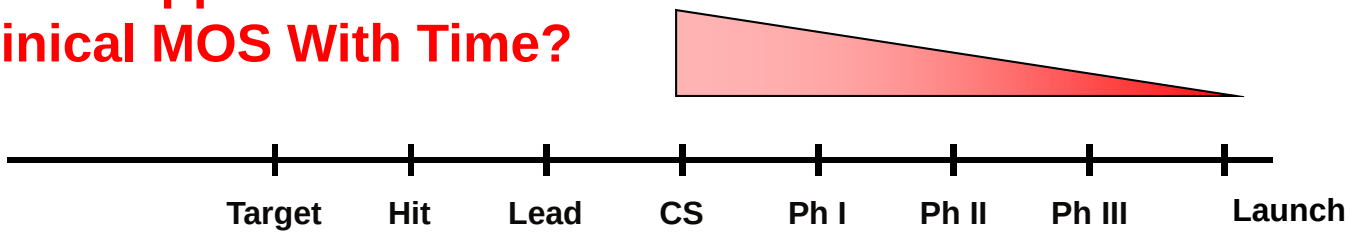
Highest Exposure Associated with No Toxicity (No Effect Limit, NOEL) or "Manageable" Toxicity (No Adverse Effect Limit, NOAEL)



- Based on exposure, not dose!

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?

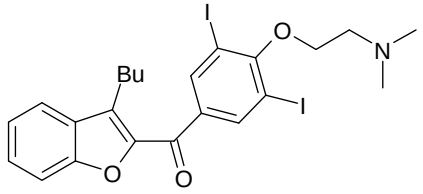
1. Lack of Monitorability
2. Lack of Reversibility
3. Uncertainty Regarding the Translation to Man

Common Toxicities

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

Common Toxicities

- Genetic toxicity/ Mutagenicity
  - Mini-Ames and in vitro micronucleus tests
- 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?

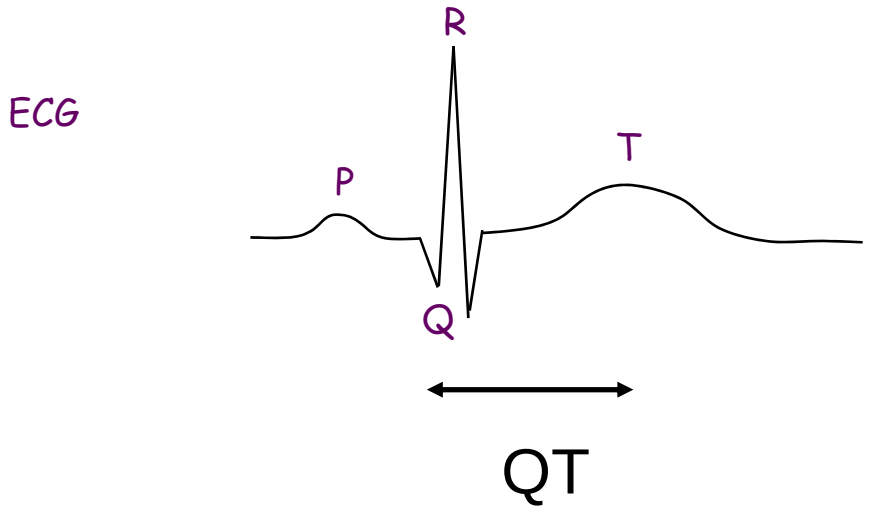
- Ideally, want efficacious compounds with no side effects
- More often…
- Observe side effects in one or more species
- Mechanism related
  - Exaggerated pharmacology (hypoglycaemia when taking glucose lowering agents or positional hypotension when taking blood-pressure lowering agents)
  - Undesirable 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!

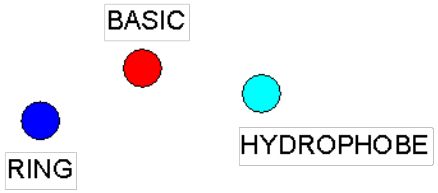
hERG - Background

- 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

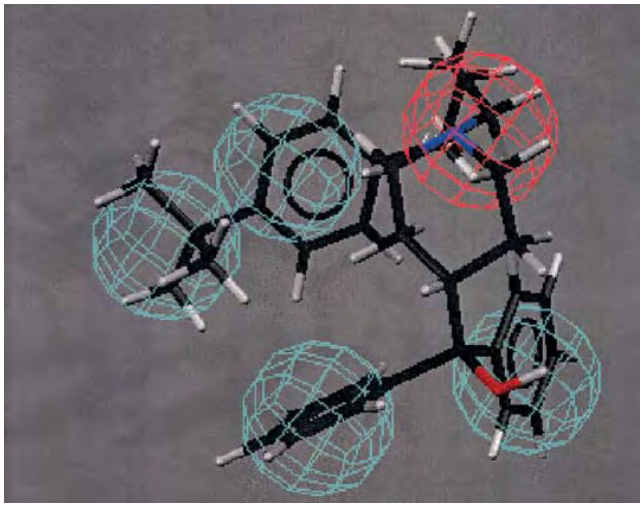


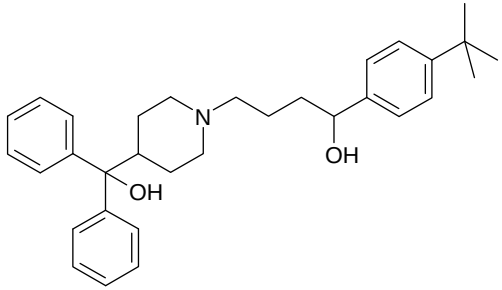
hERG – What can chemists do?

- 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 recent review of assays and strategies for reducing hERG activity.



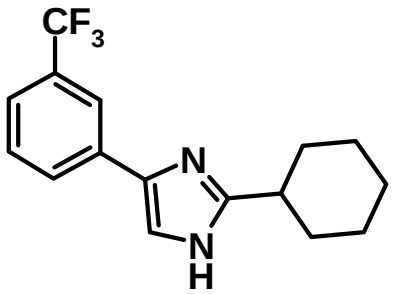


Terfenadine fitted to a QSAR derived Herg Pharmacophore
Hydrophobic regions in cyan
Positive ionizable regions in red

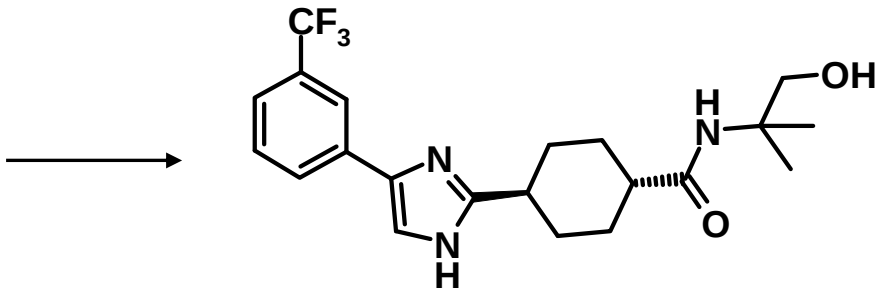
Reducing Activity at hERG

Neurogen: Neuropeptide Y-Y5 antagonists

- Lower lipophilicity-adding hydrophilic groups

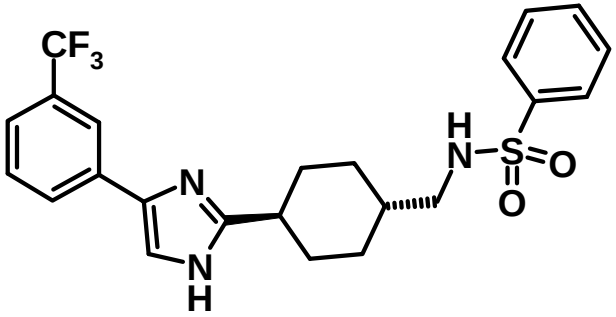


hERG 60% @ 3μM
logP = 3.34

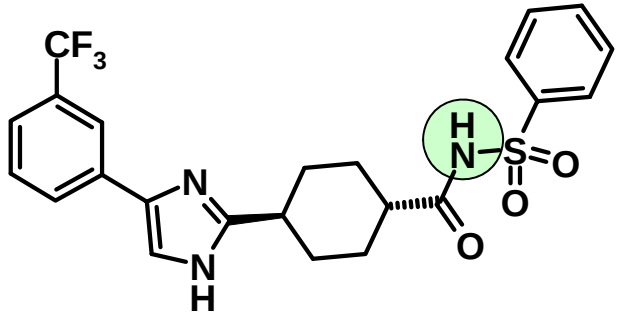


hERG 6% @ 3μM
logP = 2.3

- Acidic



hERG 87% @ 300nM



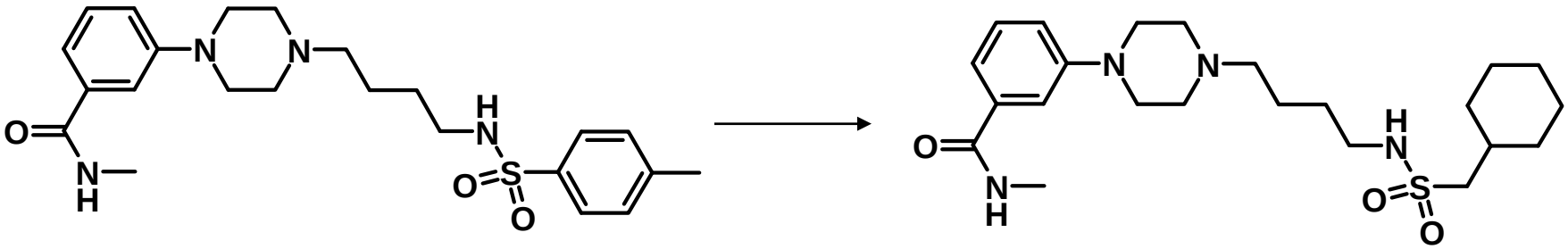
hERG 7% @ 3μM

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

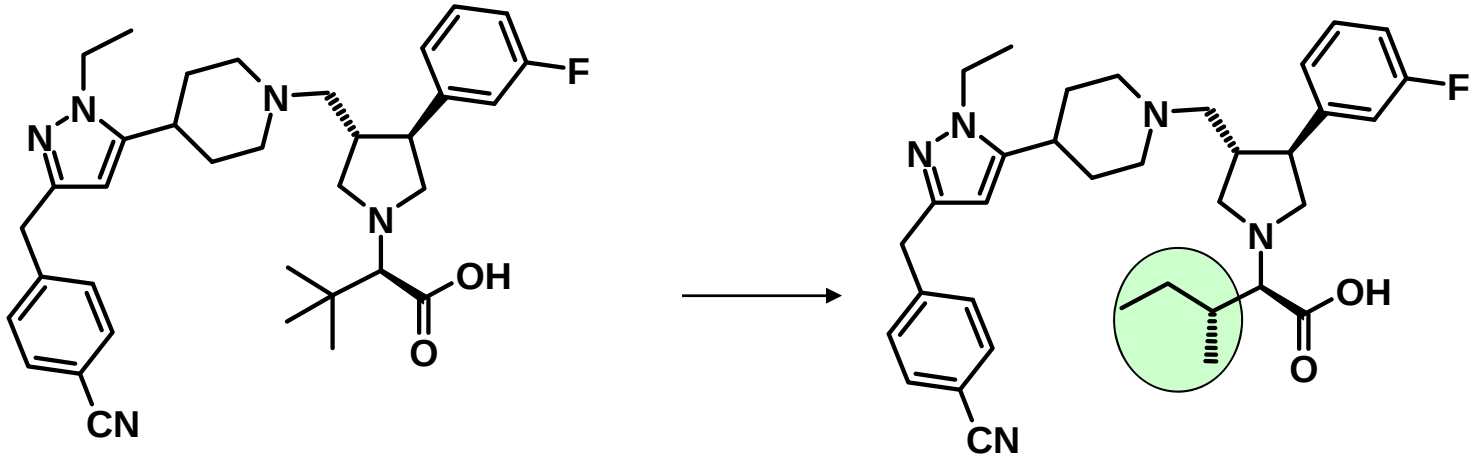
Insilico based methods as primary tool
-Model 3D hERG channel

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

Reducing Activity at hERG

Merck: CCR5 Antagonists HIV

- Subtle structural changes



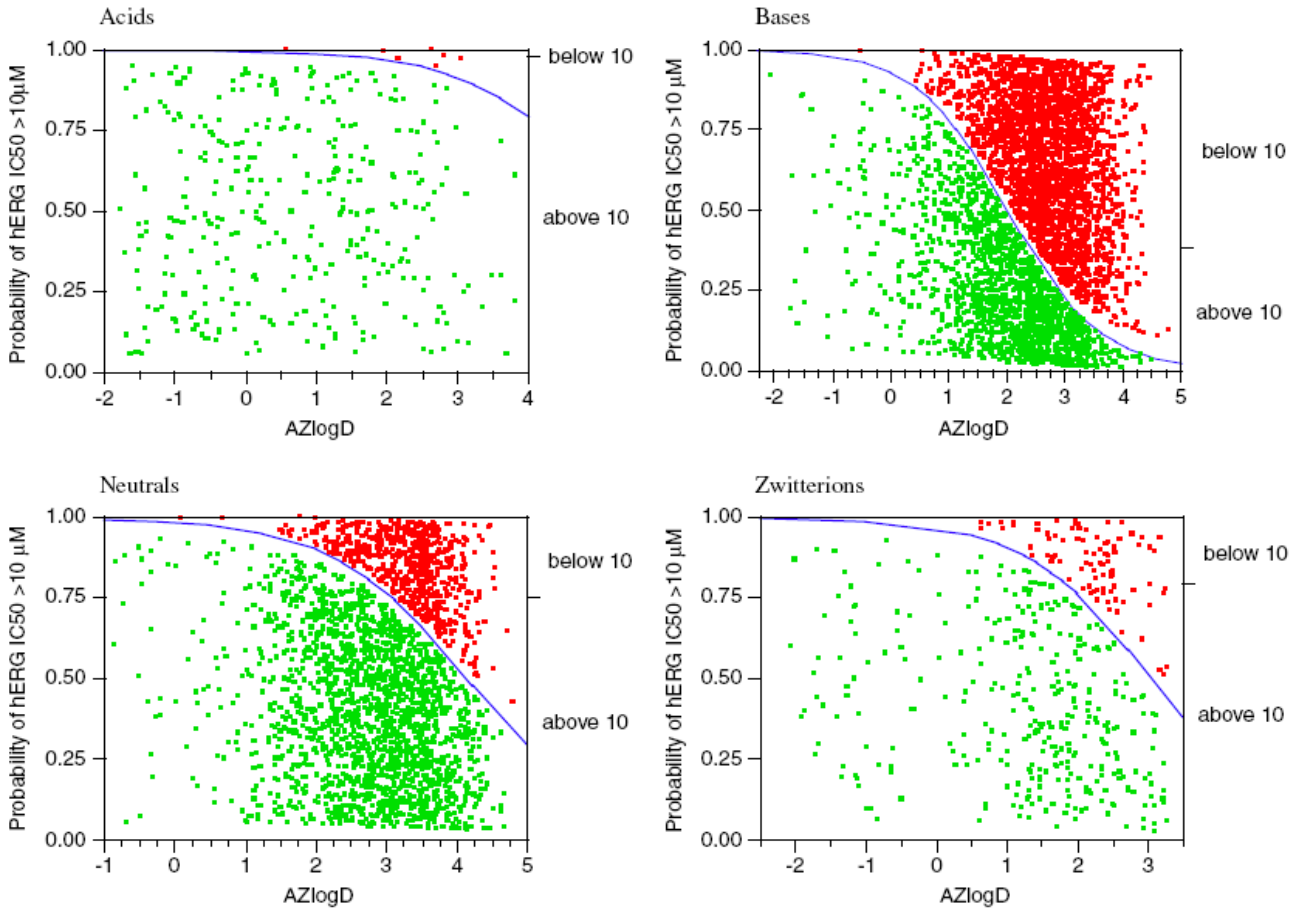
hERG IC_{50} = 1µM

hERG IC_{50} = >10µM

ACD Log P,D, pKa are same

Bio Med Chem Letts; 14, 2004, 947-952

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.

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

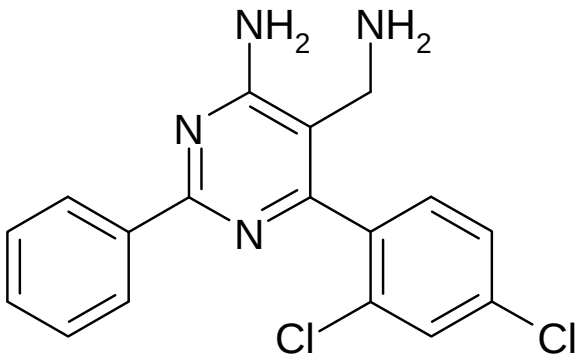
	Acids	Bases	Neutrals	Zwitterions
logD	>4	1.4	3.3	2.3
clogP	>9	1.9	4.0	4.4

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

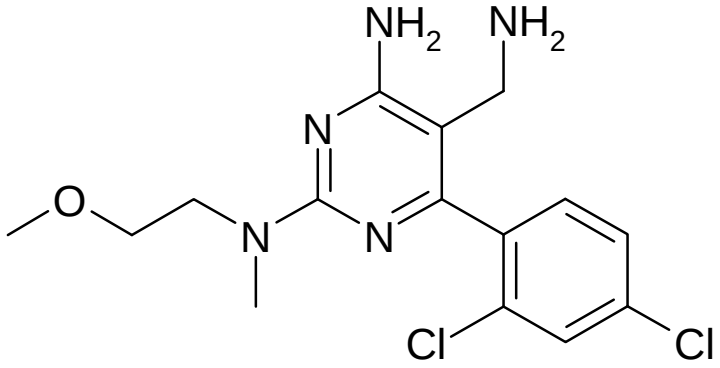
Phospholipidosis – What can chemists do?

- Reduce lipophilic/ amphiphilic nature of compound
- Reduce or remove 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

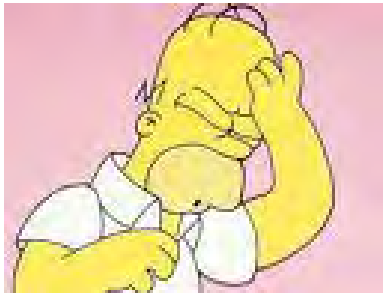


DPP-IV IC_{50} = 10 nM
$logD_{7.4}$ = 3.0, pKa = 7.8
Phospholipodosis in fibroblasts
CYP 3A4 IC_{50} = 5.4 μM



DPP-IV IC_{50} = 9 nM
$logD_{7.4}$ = 1.6
No Phospholipodosis
CYP 3A4 IC_{50} = 30 μM

Review - Drug-Induced Phospholipidosis: Are There Functional Consequences? Mark J Reasor and Sam Kacew, Exp Biol Med, 226(9), 825-830, 2001.



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)

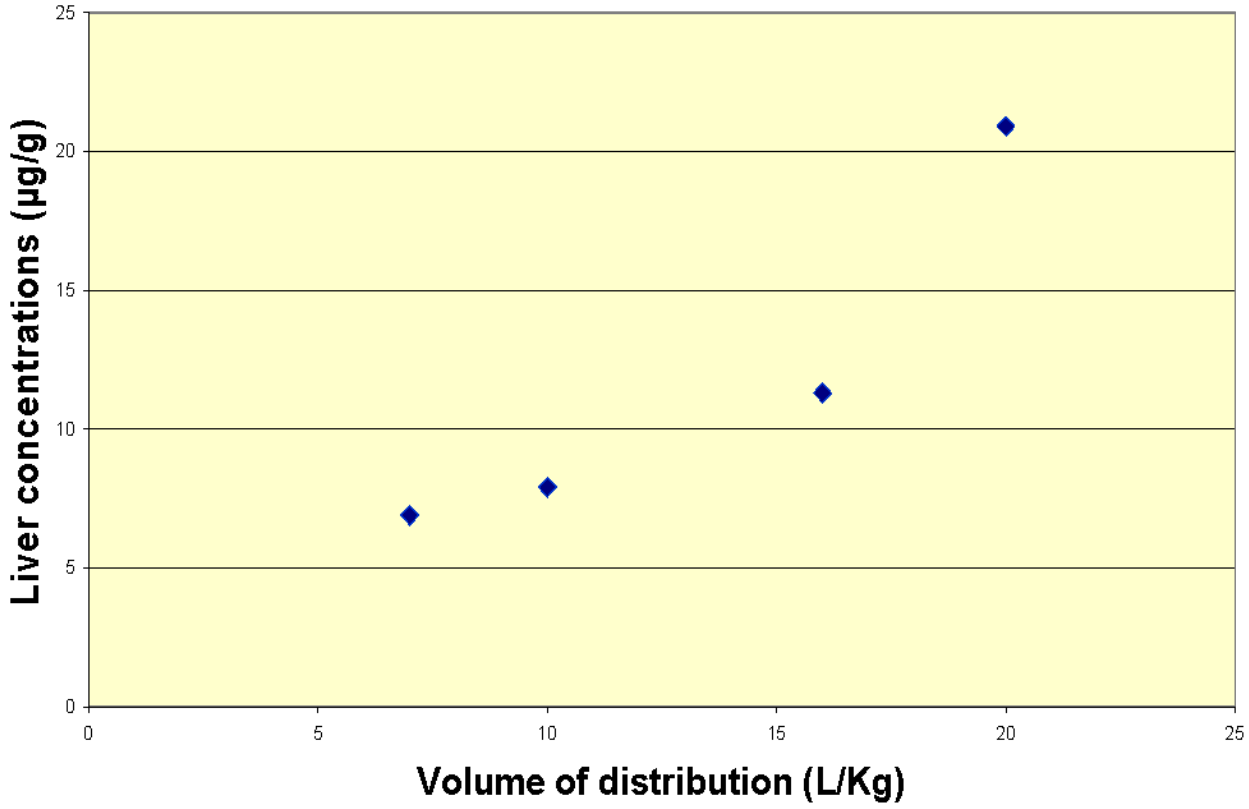
Drug levels in liver were determined….

Liver/plasma concentration ratios of lead compounds at end of 7 day toxicity studies

	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

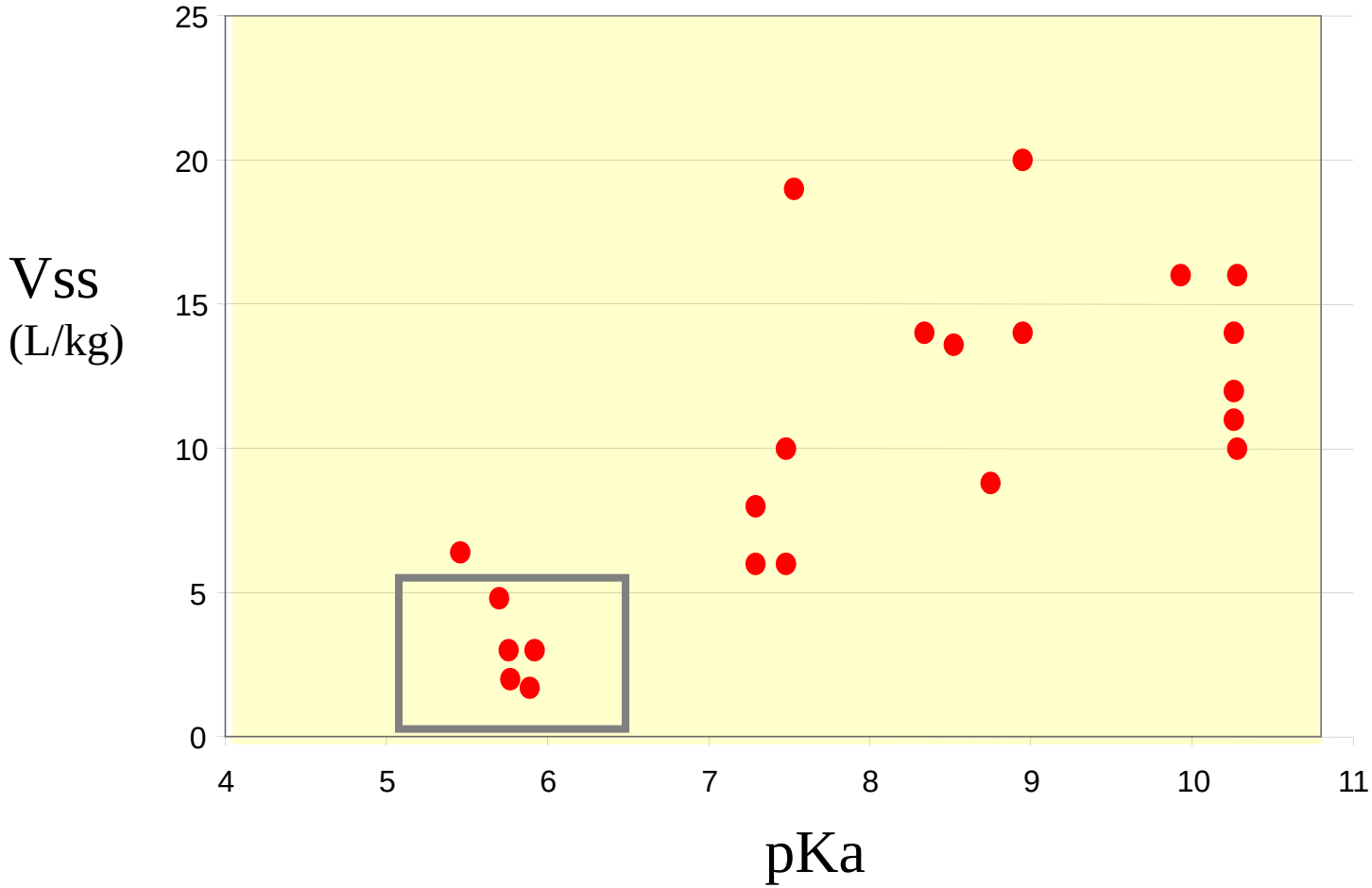
Volume of Distribution

- 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!

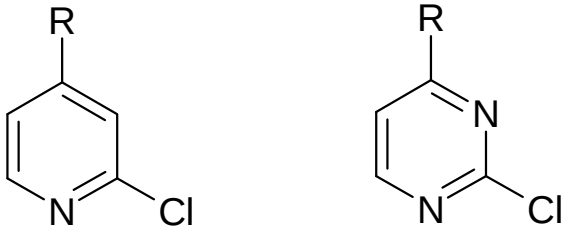
- Low pKa compounds identified and tested
- Low liver/plasma ratios (1-5) in acute low dose studies
- Best compounds gave no hepatotox signs in preliminary tox studies at any dose.
- Low toxicity for candidated compound was confirmed in 28 day studies in rat and dog.
- Improved brain penetration
- Compound has recently entered phase 1 studies

Reactive molecules and metabolites

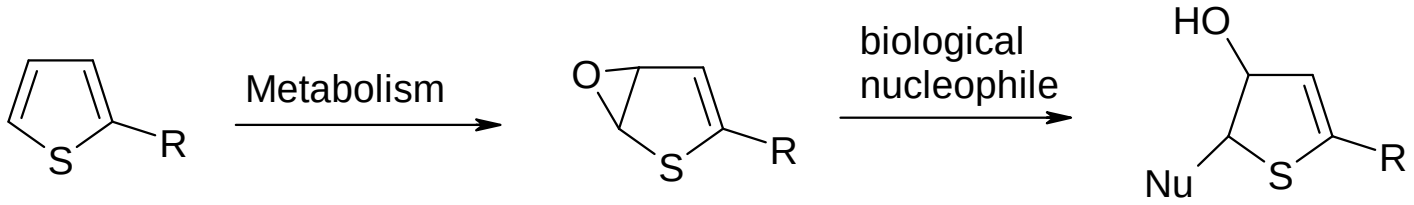
- The body is full of mild nucleophiles (proteins, peptides, glutathione etc)
- Reaction between small molecules and proteins or peptides can give rise to foreign adducts
- These adducts can cause immunological responses or further organ toxicities
- This kind of toxicology is often spotted late – very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

What can chemists do?

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

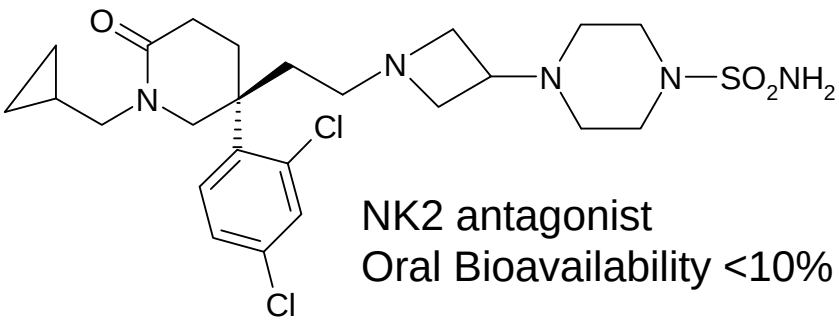


- And motifs/ groups which could give reactive metabolites
  - Eg thiophenes, furans



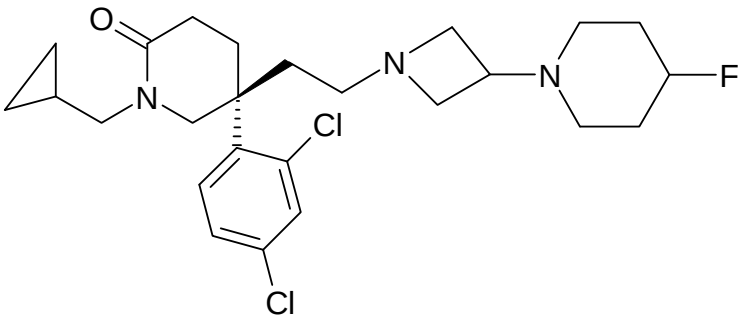
Reviews - A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups
A.S.Kalgutkar et al , Current Drug Metabolism, 2005, 6, 161-225.
- Biotransformation Reactions of Five-Membered Aromatic Heterocyclic Rings,
Chem. Res. Toxicol., 2002, 15, 269-299

Reactive metabolite example from Pfizer



NK2 antagonist
Oral Bioavailability <10%

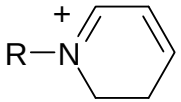
Absorption increased by raising logP



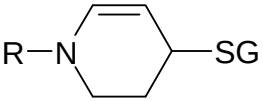
Compound stopped due to testicular toxicity

Oxidation

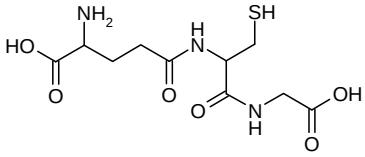
-F



GSH

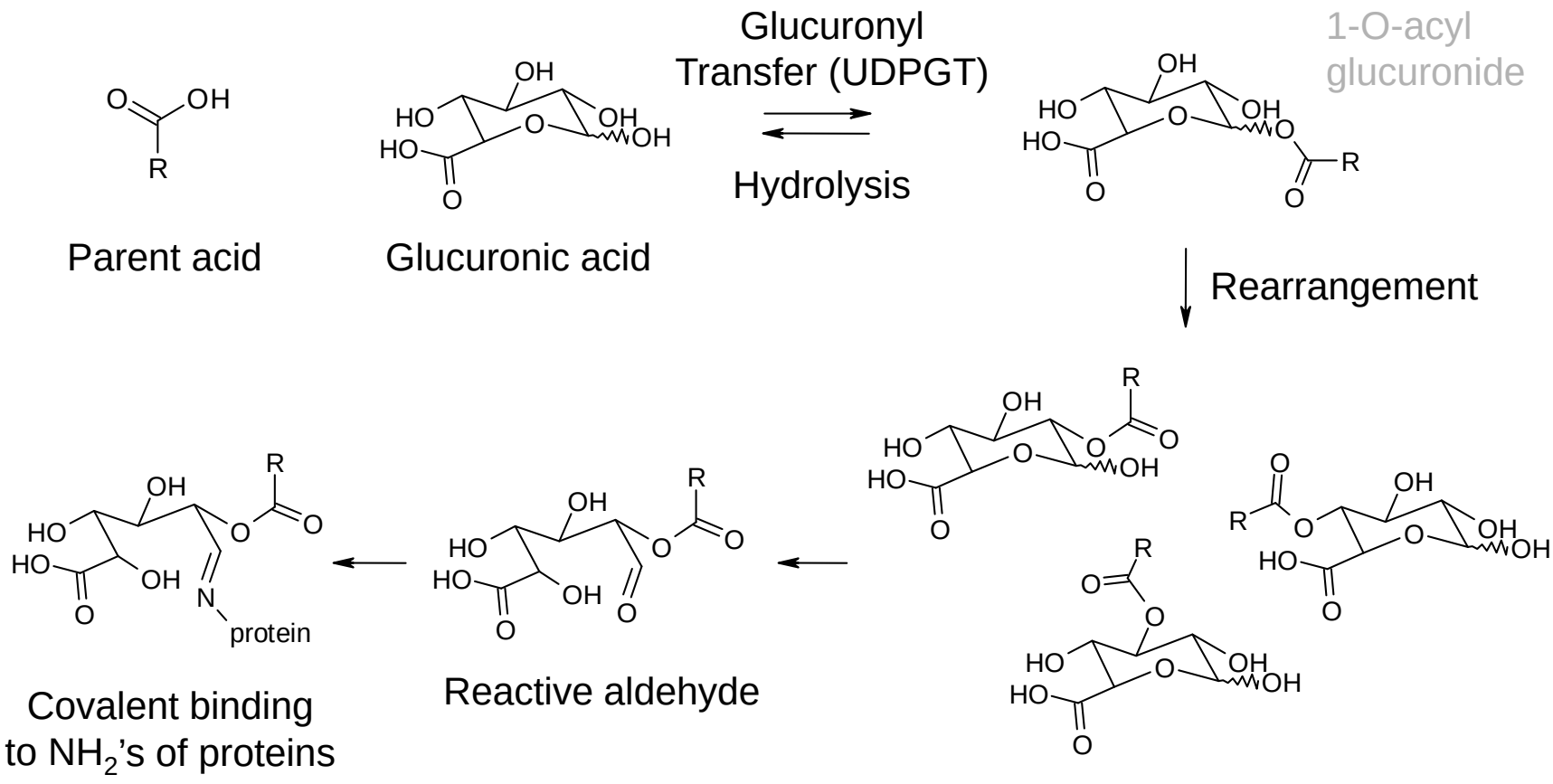


Glutathione
GSH =



Acyl glucuronides

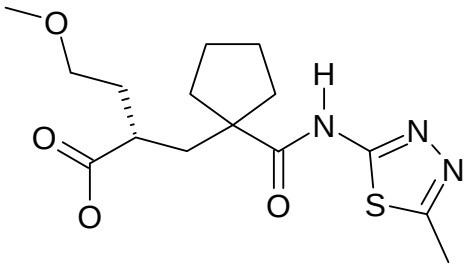
Acyl Migration and Covalent Binding



Immunogenic
Implicated in GI toxicity

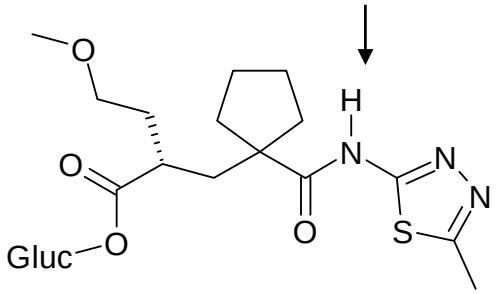
For a review Current Opinion in Drug Discovery & Development 2007 **10**(1):58-66

Reactive metabolite example from Pfizer

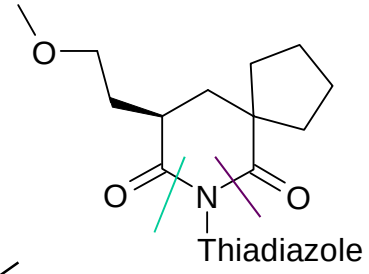


UK 414,495
Potent NEP inhibitor
Prototype candidate
stopped due to GI tox

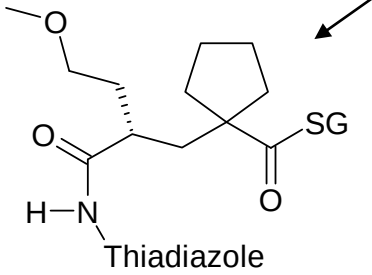
pKa 9.6



$t_{1/2}$ = 0.17h

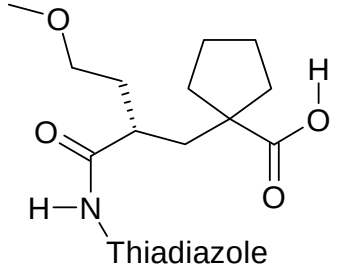


GSH



Toxic glutathione conjugate

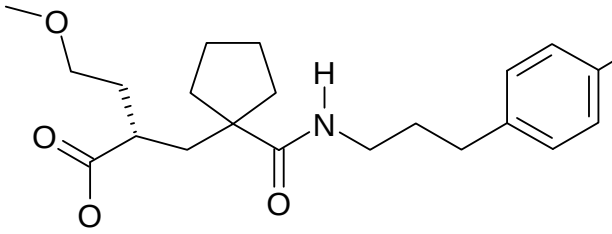
$t_{1/2}$ = 8.7h



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.



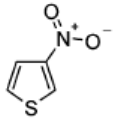
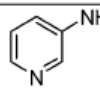
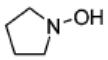
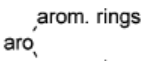
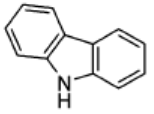
Calc pKa = 16

Thanks to David Pryde

Toxicophores for Mutagenicity

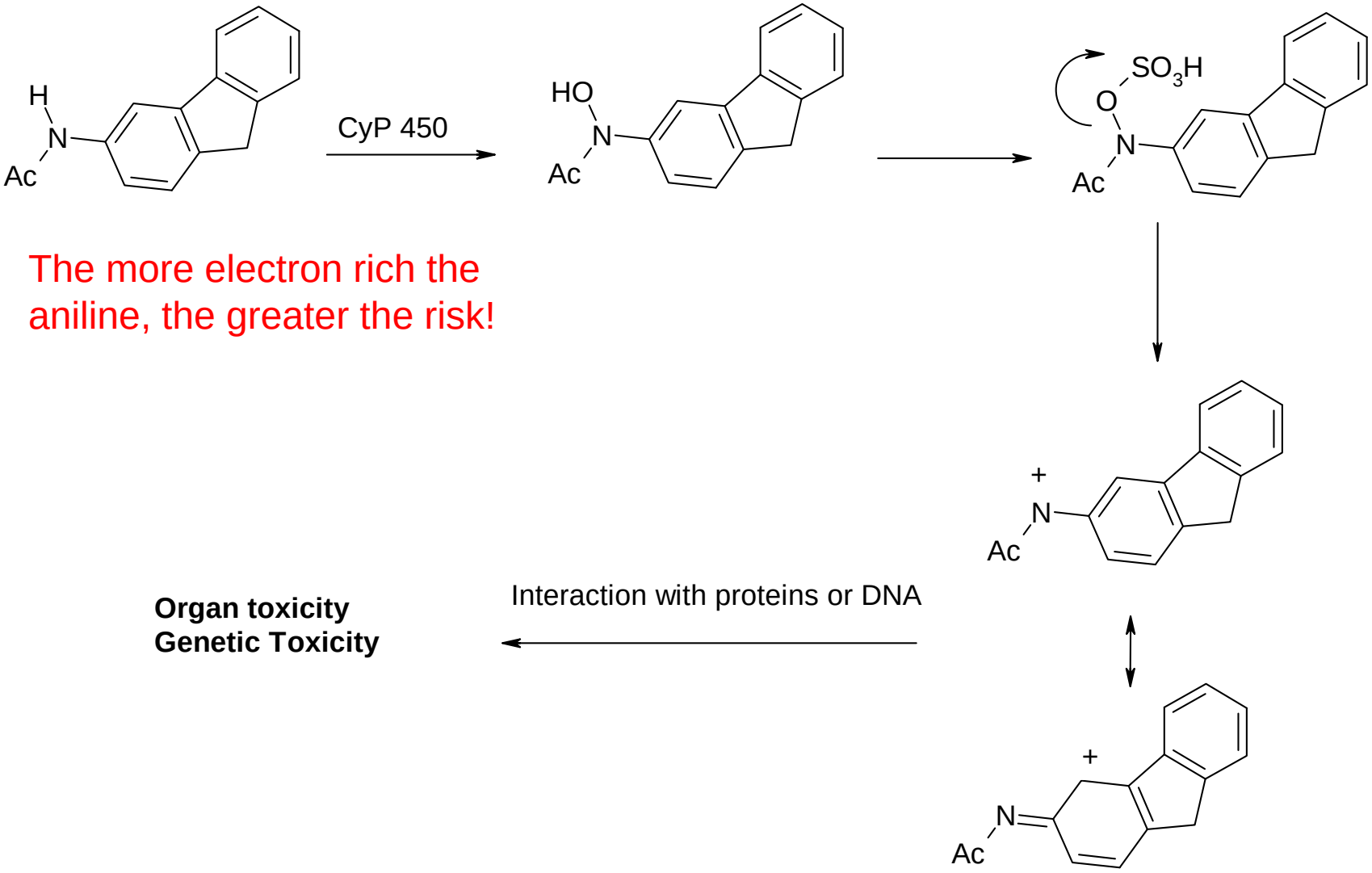
Structural alerts for DNA Reactivity

- 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		

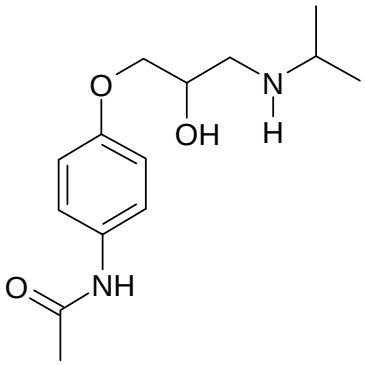
J. Med. Chem. **2005,** *48,* 312-320

Toxicity of anilines and derivatives

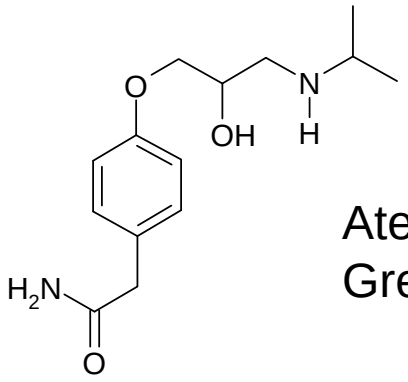


The more electron rich the aniline, the greater the risk!

Look for alternatives

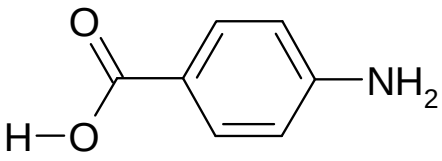


Practolol
Ocular toxicity

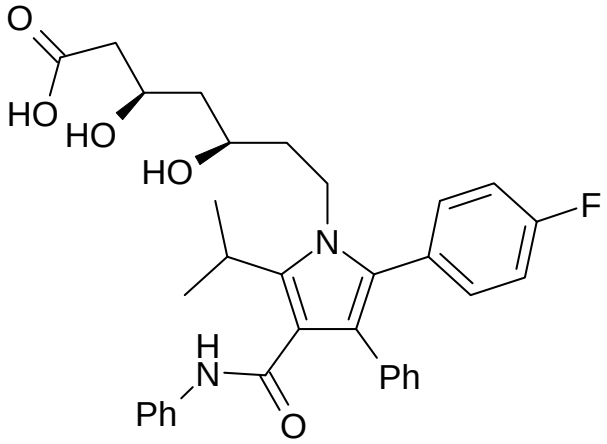


Atenolol
Greater Safety

OR….



Electron deficient anilines
eg PABA - Safe metabolite

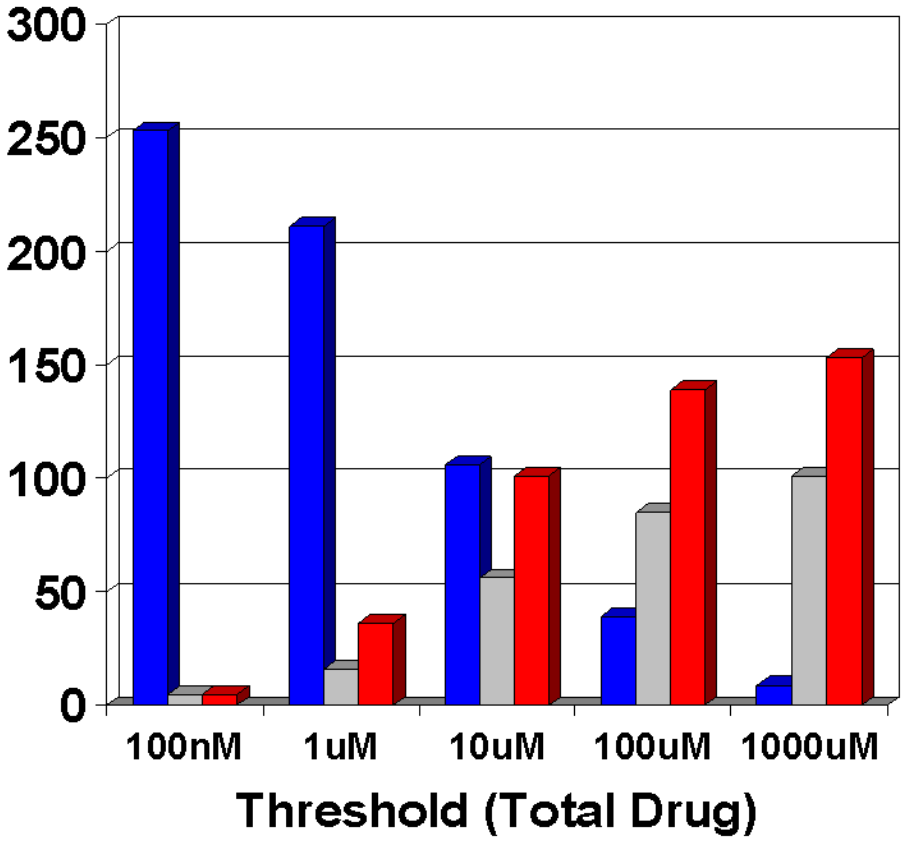


Atorvastatin
Anilide NH is hindered

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.

- 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

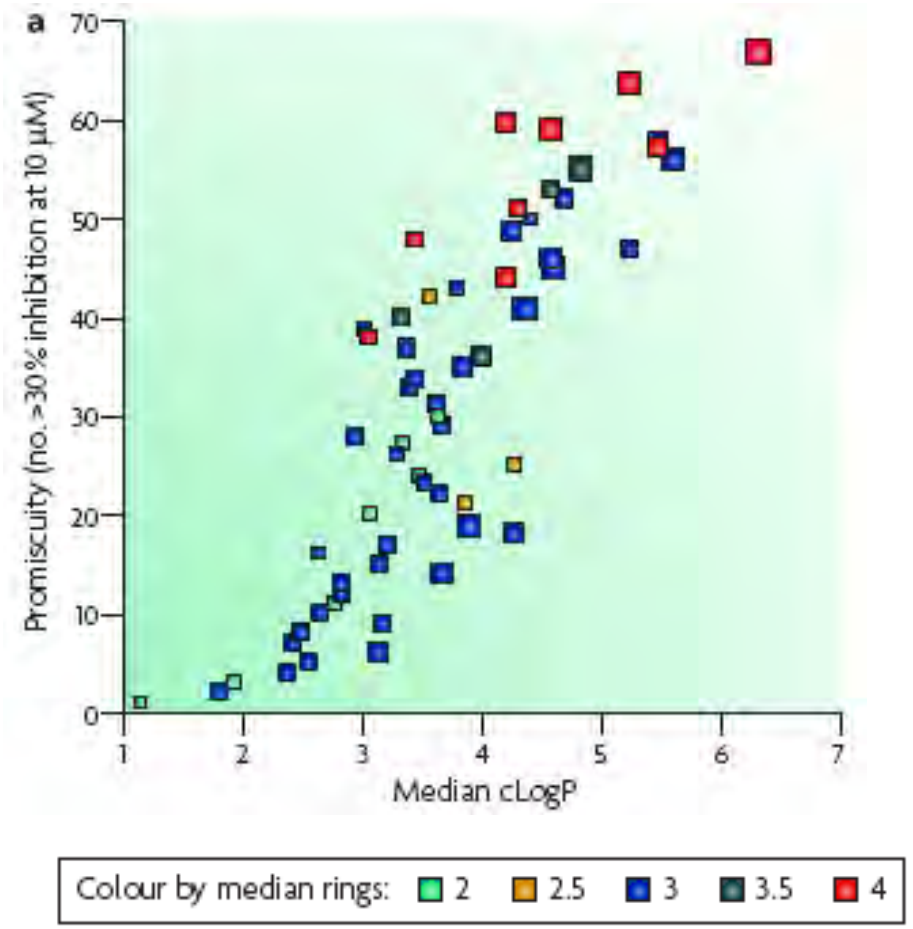
- Numbers in parentheses indicate number of outcomes in database
- Holds for both free-drug or total-drug thresholds

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

Lipophilicity and Promiscuity

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

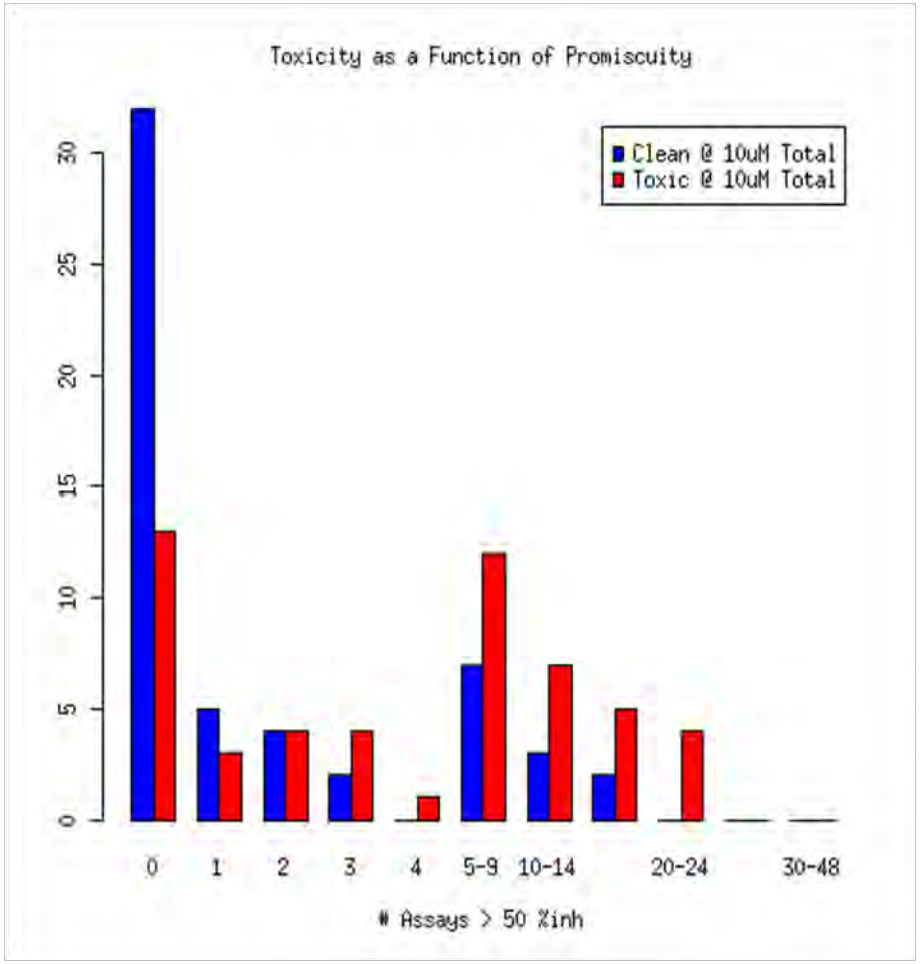
- Promiscuity = # Compounds with >30% inhibition at [10 µM]
- Greater propensity for off-target binding for compounds with cLogP≤3



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

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)

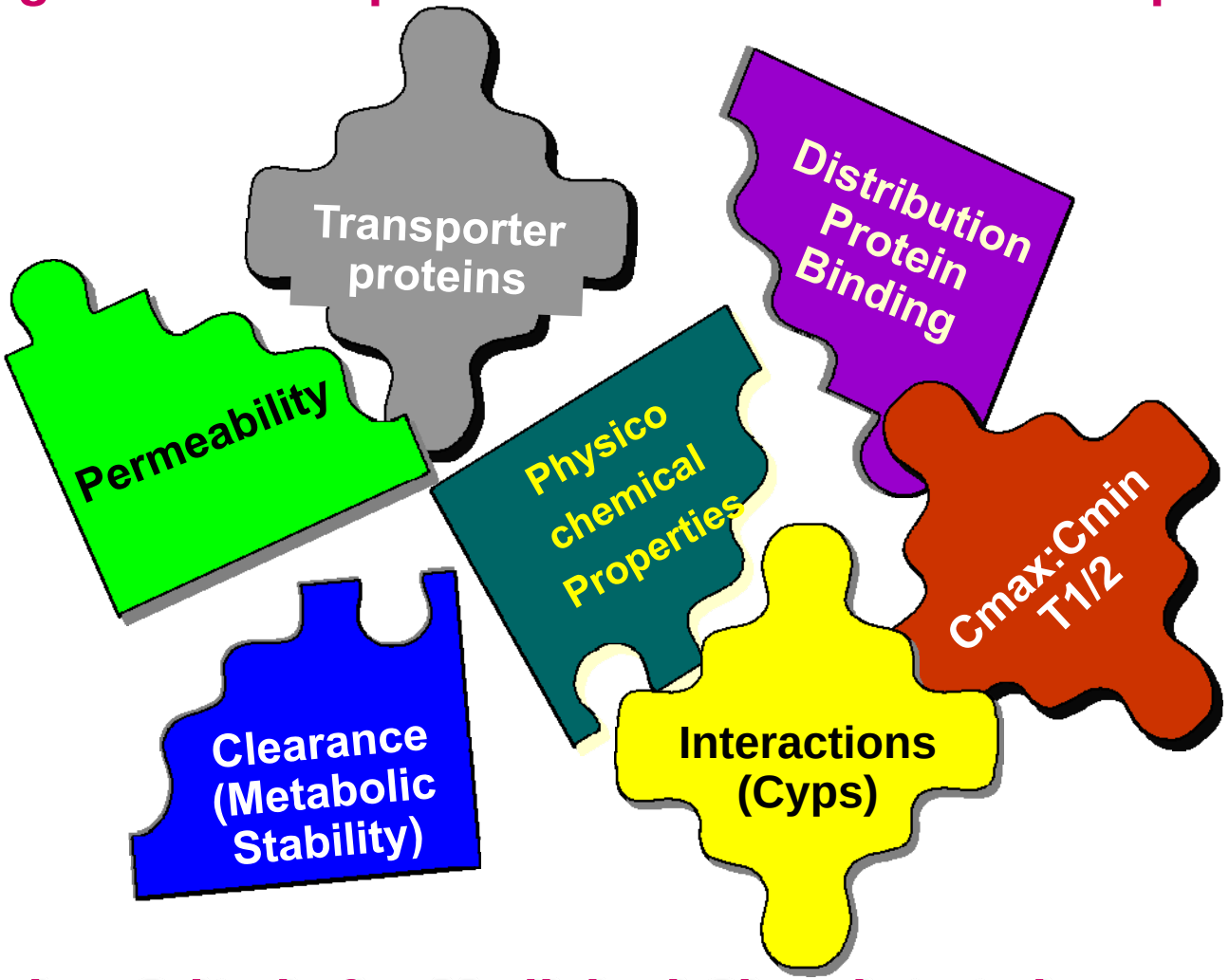
Summary – chemistry and toxicology

- 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 increases the risk of toxicity

Closing Remarks

DMPK & Candidate Drugs

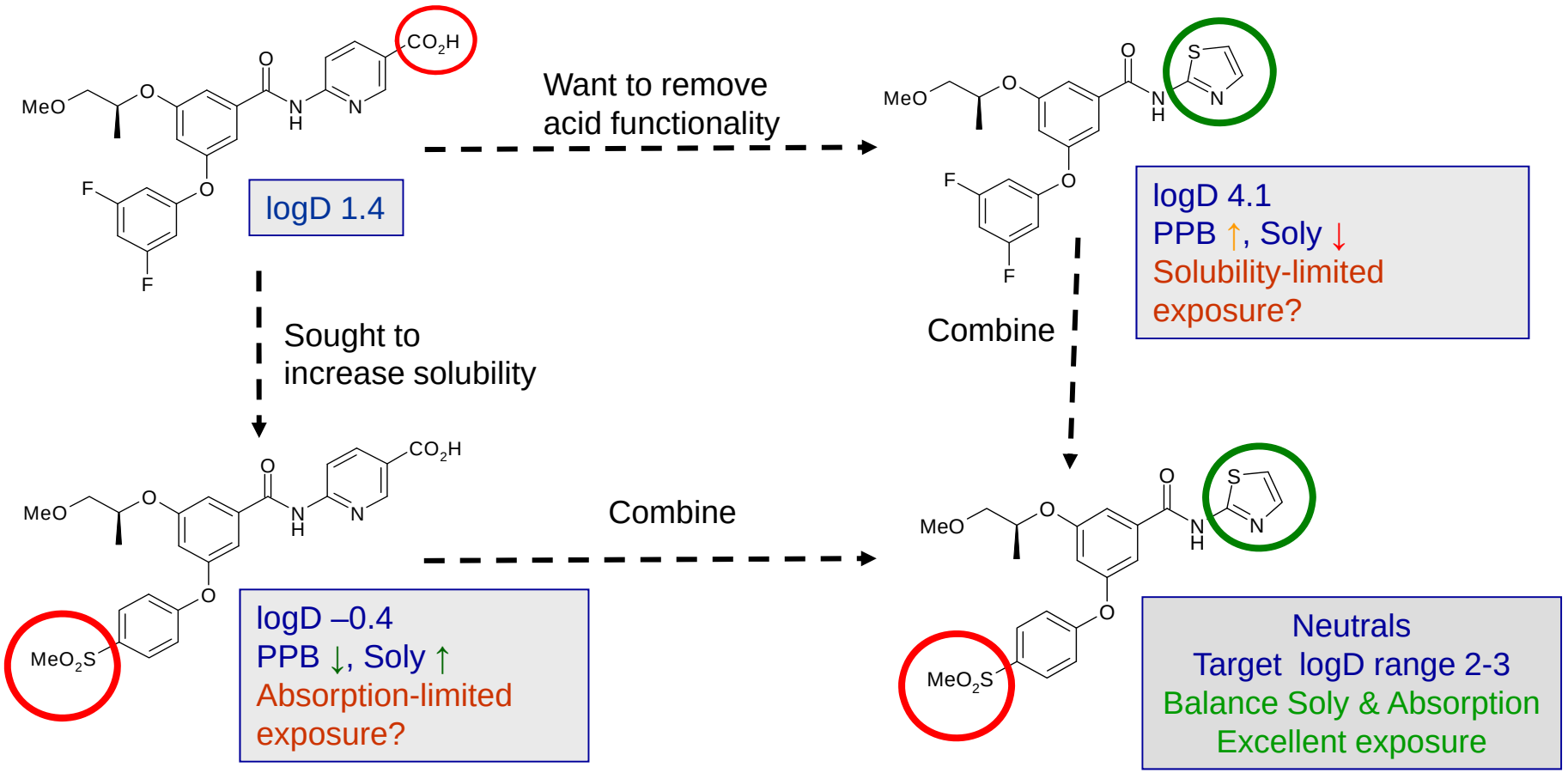
Candidate Drugs need good predicted human PK & minimal drug-drug interaction potential to have a chance of progress



Drug Design Criteria for Medicinal Chemists to be worried about

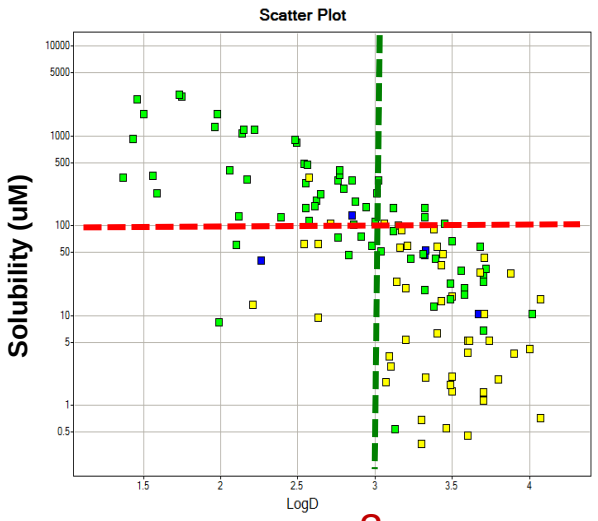
It's all about Balance

Example of need to balance permeability & solubility to optimise in vivo exposure

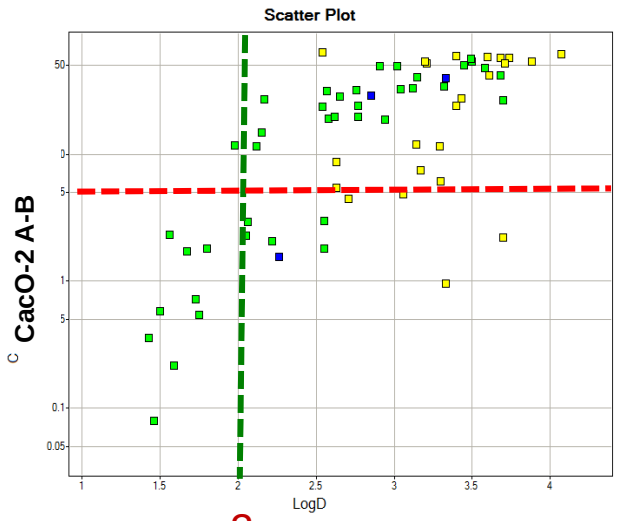


'Beware the yellow jersey':
For wider context, read the excellent article by DeWitte,
Curr. Drug Discov., **2002**, p.19-22

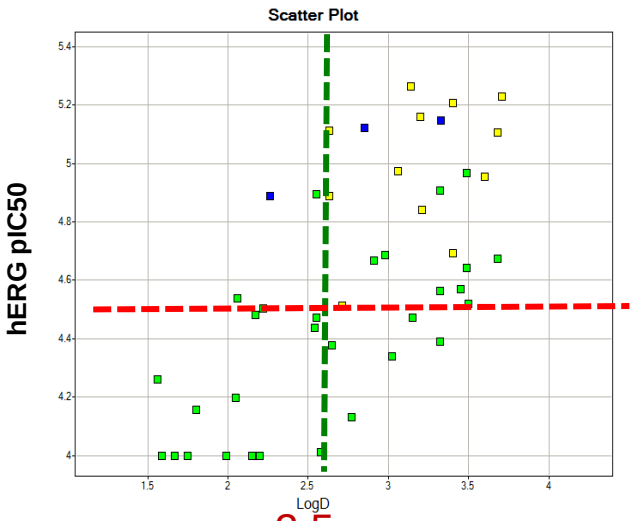
Lipophilicity Window



3



2



2.5

Blue thiazoles Yellow thiadiazoles Green pyrazoles

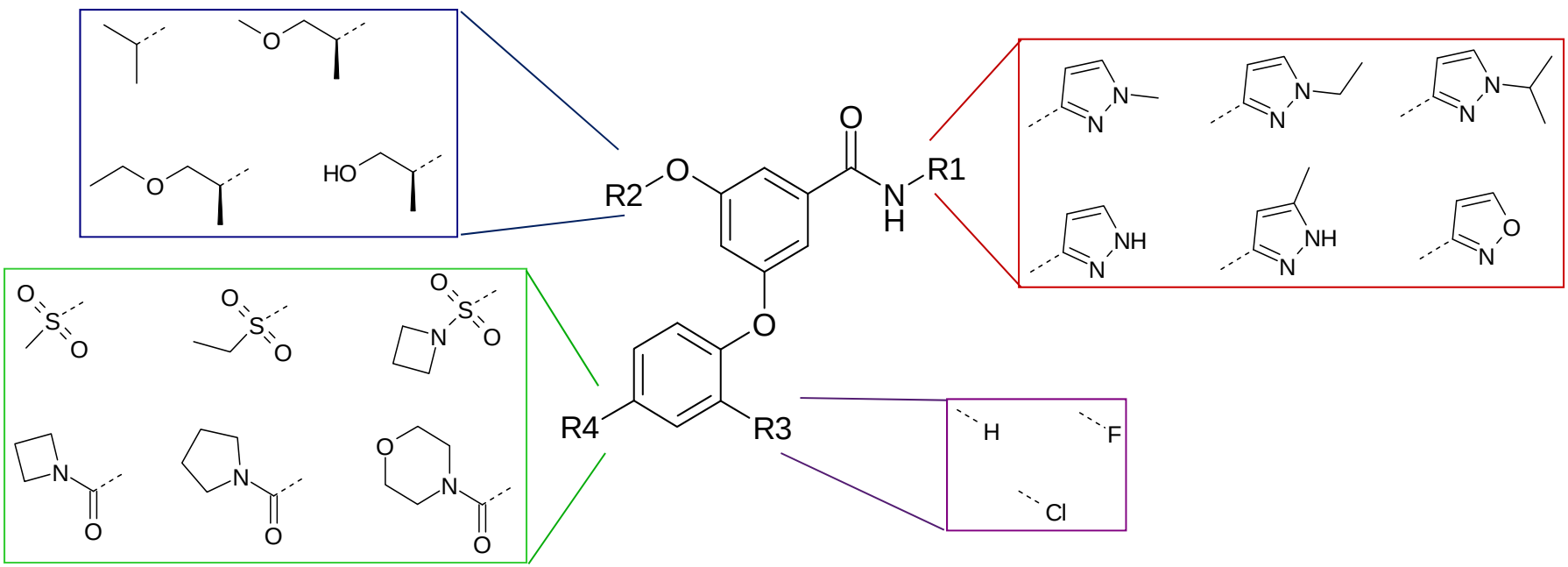
Define relationships, Set goals, Define Scope, Select Candidates

Parameters	**Target value**	**Log D range**
Solubility	>100 uM	< 3
Caco P_{app} (A to B)	>5*10^{-6} cm/s	> 2
hERG IC_{50}	>30 uM	< 2.5
Rat nAUC	>0.5	1 - 3

Overall ‘Sweet Spot’

LogD 2 – 2.5

Designing ‘Endgame’ Scope



- Inputs chosen to retain putative pharmacophore
- Create Virtual library - every permutation created and descriptors (eg, logD) calculated
- Synthesis targets refined & selected using calculated properties to target ‘sweet spot’
- Make, test & analyse...

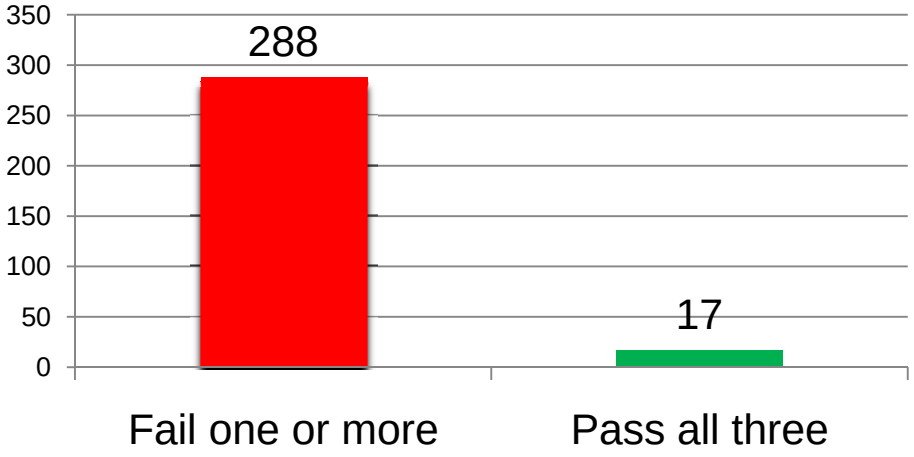
Assessment of Success

- Comparison of Endgame compounds vs. previous dataset

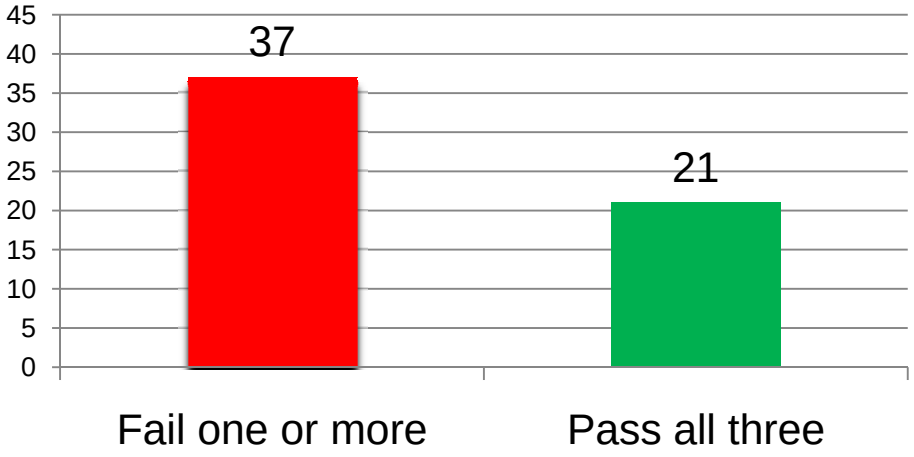
- Counts of compounds with:

rat nAUC	>0.5 mM.h
Solubility	>100 mM
hERG IC_{50}	>30 mM

Previous compounds



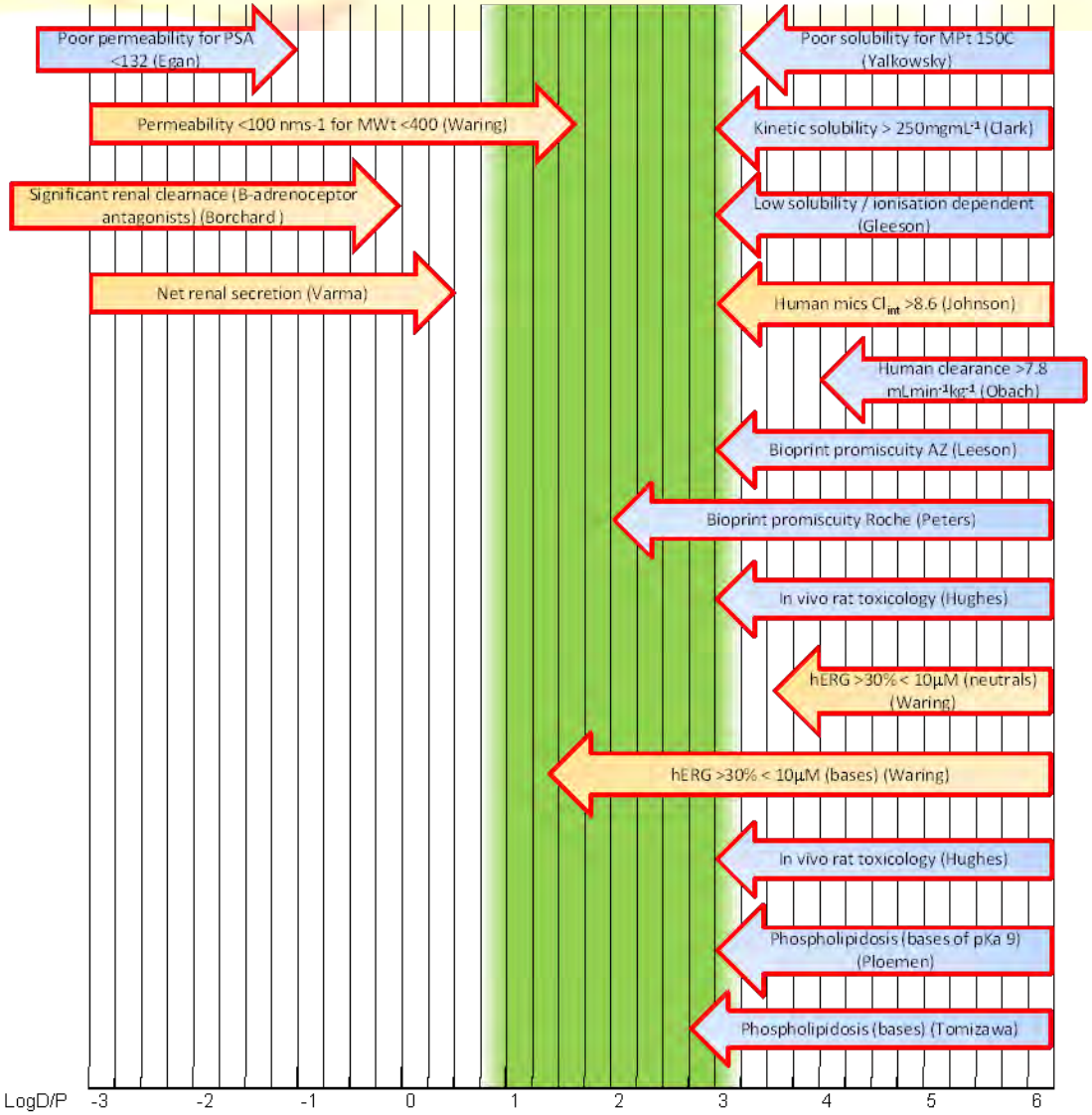
'Endgame' compounds



(including several clinical candidates)

- Significant increase in 'design hit rate' vs desired properties

‘Optimal’ Lipophilicity



logP blue
logD orange

Problems with high logD are widespread (and increasing)
Problems with low logD primarily permeability based

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

Bringing it all together...

Generation of a Set of Simple, Interpretable ADMET Rules of Thumb

M. Paul Gleeson*

J. Med. Chem. **2008**, *51*, 817–834

Table 3. Indication of How Changes in Key Molecular Properties will Affect a Range of ADMET Parameters[a]

neutral molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	average	lower
permeability*	higher	average/higher
bioavailability	average	lower
volume of Dist.**	average	average
plasma protein binding	average	higher
CNS penetration***	higher/average	average/lower
brain tissue binding	lower	higher
P-gp efflux	average	higher/average
in-vivo clearance	average	average
hERG Inhibition	lower	lower
P450 inhibition****	lower 2C9, 2C19, 2D6 & 3A4 inhibition	higher 2C9, 2C19 & 3A4 inhibition
P450 inhibition****	higher 1A2 inhibition	lower 1A2 inhibition
P450 inhibition****		average 2D6 inhibition

(a)

basic molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	higher/average	lower/average
permeability*	higher/average	average
bioavailability	average	lower
volume of Dist.**	higher/average	higher
plasma protein binding	lower	average
CNS penetration***	higher/average	average/lower
brain tissue binding	lower	higher
P-gp efflux	average	higher/average
in-vivo clearance	average	higher/average
hERG Inhibition	average/higher	higher
P450 inhibition****	lower 1A2, 2C9, & 2C19 inhibition	lower 1A2 inhibition
P450 inhibition****	average 2D6 & 3A4 inhibition	average 2C9, 2C19 inhibition
P450 inhibition****		higher 2D6 & 3A4 inhibition

(b)

ADMET rules of thumb II: A comparison of the effects of common substituents on a range of ADMET parameters

Paul Gleeson †,*, Gianpaolo Bravi †, Sandeep Modi ‡, Daniel Lowe §

Bioorganic & Medicinal Chemistry 17 (2009) 5906–5919

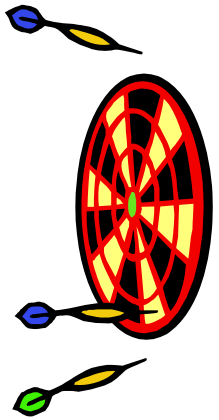
acidic molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	higher	average/higher
permeability*	lower	average/lower
bioavailability	average	average
volume of Dist.**	lower	lower
plasma protein binding	average/higher	higher
CNS penetration***	lower	lower
brain tissue binding	lower	higher
P-gp efflux	lower	lower
in-vivo clearance	lower/average	average
hERG Inhibition	lower	lower
P450 inhibition****	lower 1A2, 2C9, 2C19, 2D6 & 3A4 inhibition	lower 1A2, 2C19, 2D6 & 3A4 inhibition
P450 inhibition****		higher 2C9 inhibition

(c)

(d) zwitterionic molecules	MWT < 400 and clogP < 4	MWT > 400 and/or clogP > 4
solubility	higher	average/higher
permeability*	lower	lower/average
bioavailability	lower	lower
volume of Dist.**	lower	average/lower
plasma protein binding	average/lower	higher
CNS penetration***	average/lower	lower
brain tissue binding	lower	higher
P-gp efflux	average	average
in-vivo clearance	average	average
hERG Inhibition	lower	average/lower
P450 inhibition****	lower 1A2, 2C9, 2C19, 2D6 & 3A4 inhibition	lower 1A2, 2C19 & 3A4 inhibition
P450 inhibition****		average 2C9, 2D6 inhibition

(d)

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!