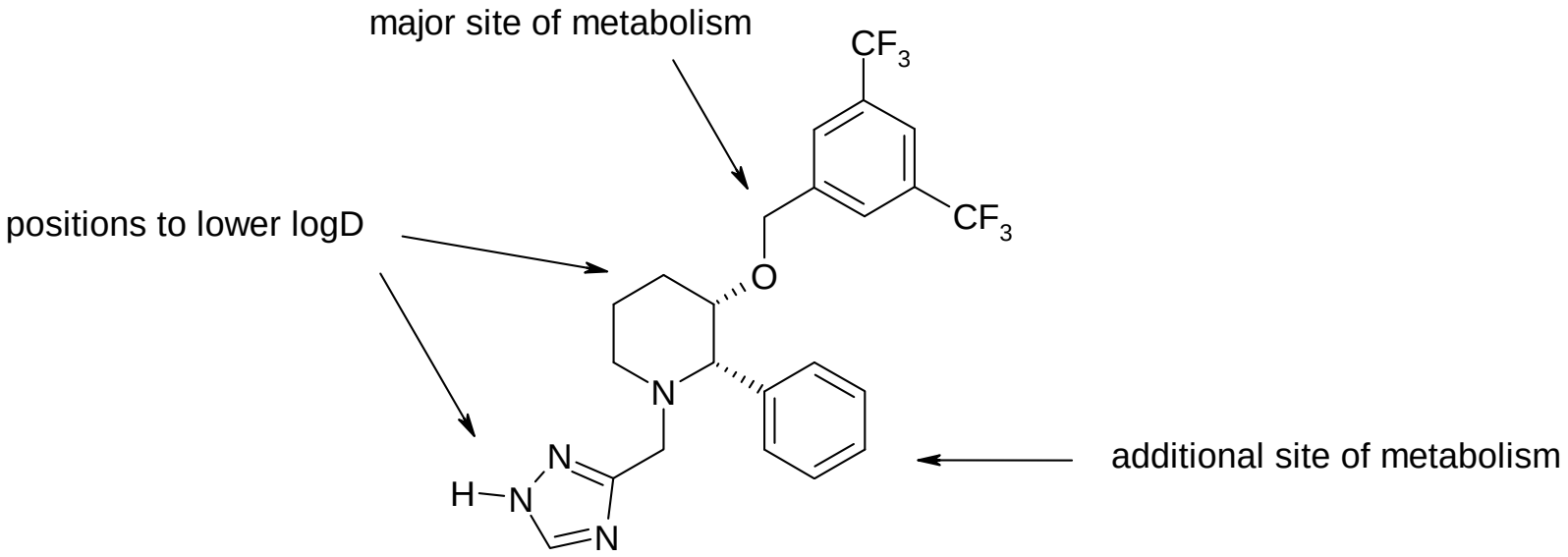
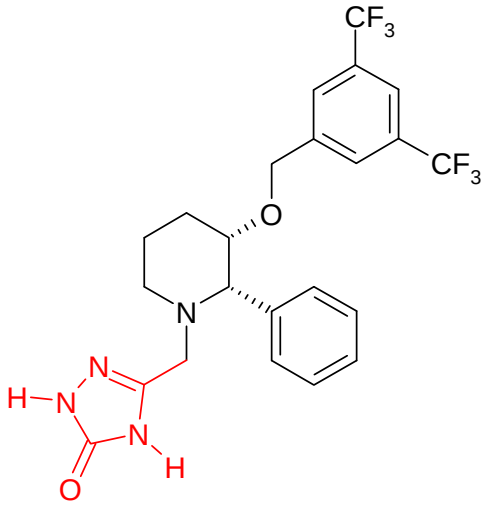
Brainteaser – NK-1 receptor antagonists

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

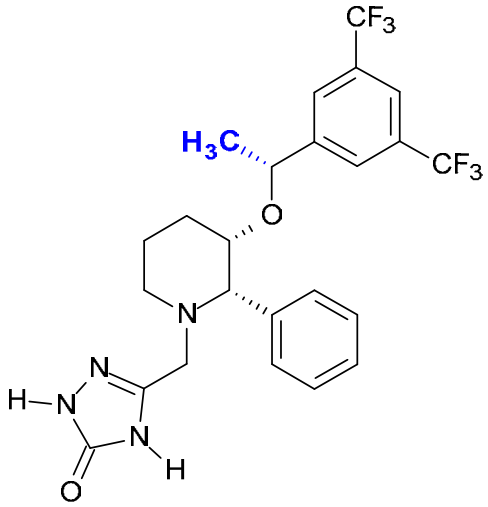


A Solution…..

cLogD = 3.9

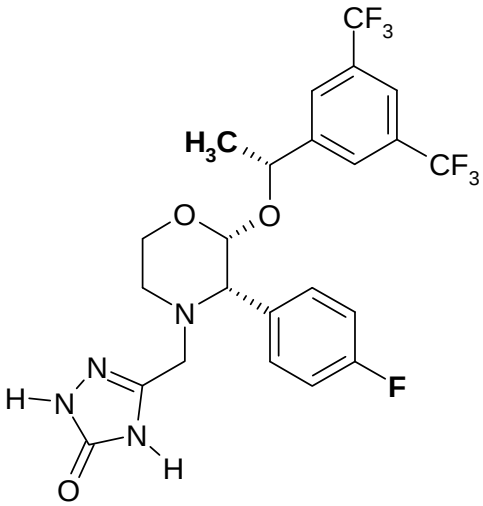


NK-1 IC_{50} = 0.1 nM



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

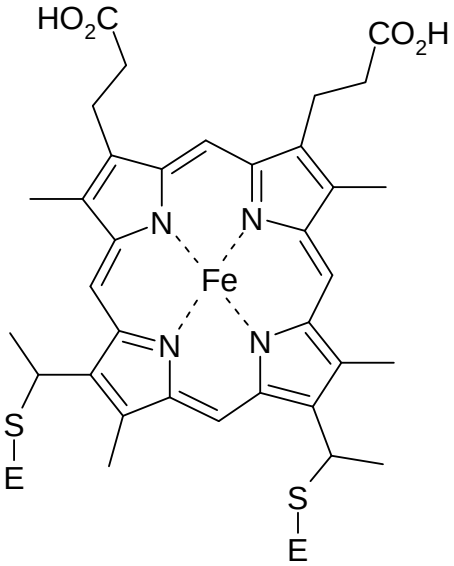
- Clearance
  - Decrease MW
  - Increase logD
  - Decrease logD / electron density
  - Decrease logD / planarity
  - Increase logD / rigidity

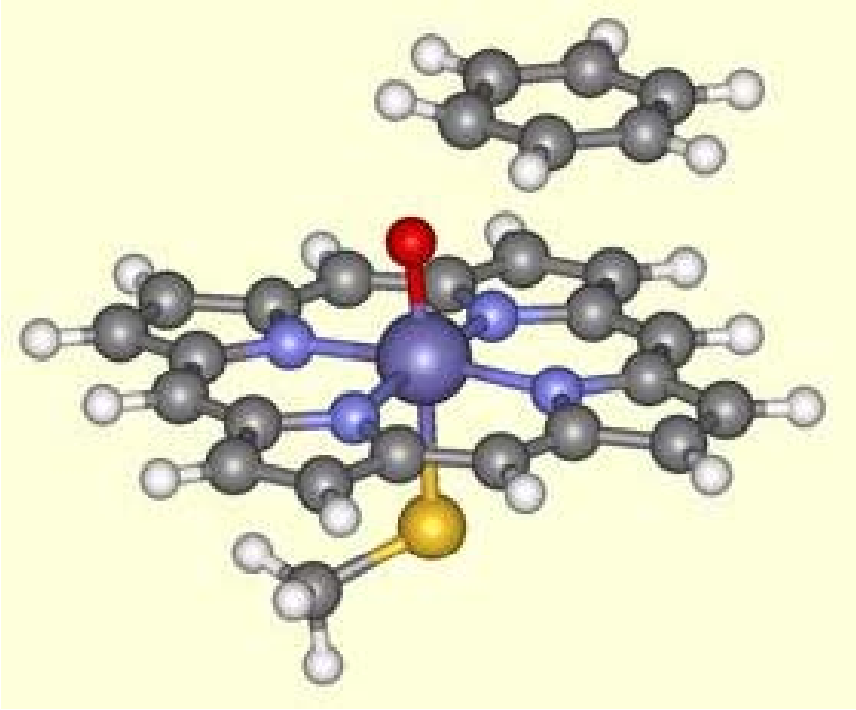
Now…

- Clearance continued
  - Which 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; easily induced by smoking, broccoli
- 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; important polymorphism
- 2E1 – paracetamol, ethanol, tolbutamide, isoflurane
- 3A4 – terfenadine (hERG!), Ca blockers, midazolam, CsA, TCAs, opiates, steroids, many others; very wide range of activity and easily induced and inhibited

CYP inhibition (competitive)

- Every substrate of a enzyme must also be an inhibitor of that enzyme
- To be a substrate of a CYP, a compound must first bind to the protein before it can be oxidised
- This is why higher logP often leads to faster metabolism, by increasing the affinity for the protein
- Sometimes, if you block all the sites of oxidation, the new compound binds very well to the CYP protein, but cannot be easily oxidised, making it a potent inhibitor
- CYP inhibition is a growing problem in drug discovery and development because there are so many other drugs around that there are many possible drug-drug interactions – DDIs
- Investigations into possible DDIs can delay approval of a drug by years

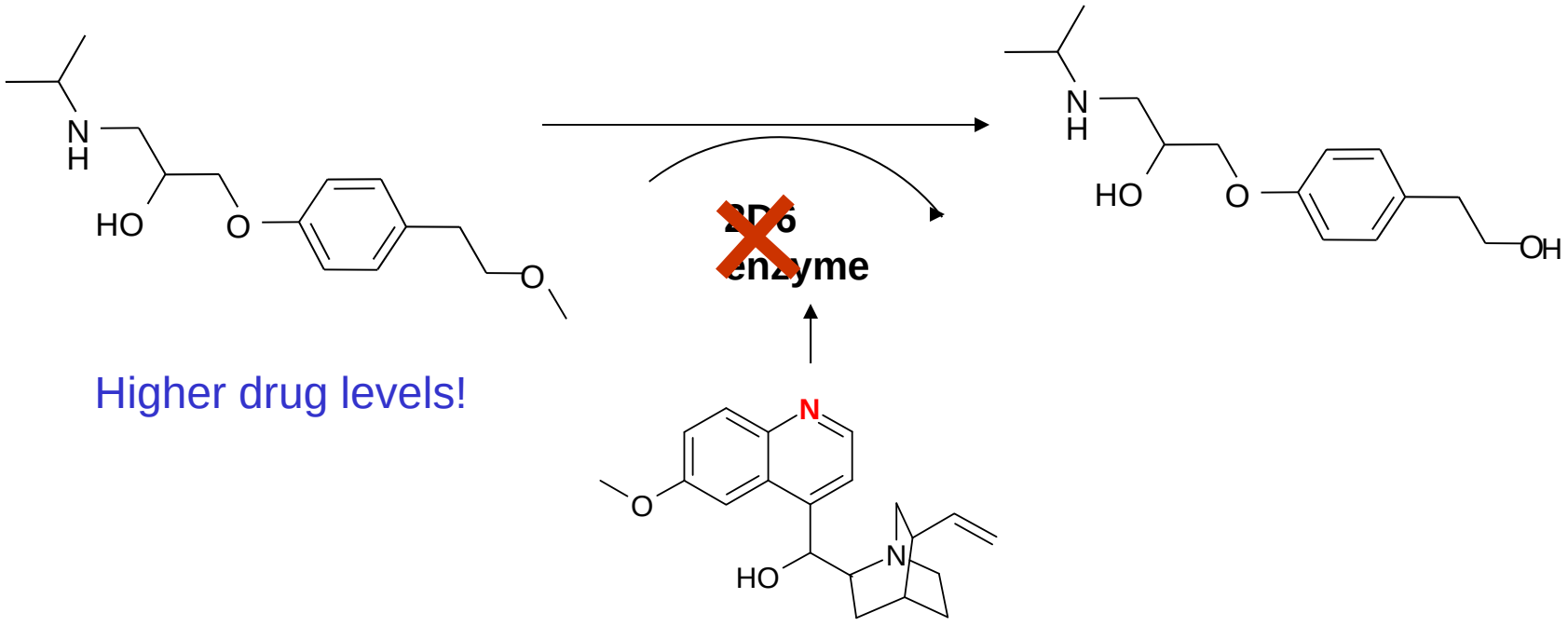
CYP induction and TDI

- Some compounds bind to nuclear transcription factors which promote the expression of certain CYPs, leading to increased expression and activity
- This is another cause of DDI.
- Another type of DDI can be caused by time-dependent inhibition (TDI) which is often caused by the formation of a reactive metabolite which permanently inactivates an enzyme
- DDIs are usually dependent on the concentration of drug in the liver. Thus, DDIs can limit the dose and exposure of a new drug and indirectly be the cause of insufficient efficacy at an acceptable dose size.
- Therefore, it is best to eliminate as many as possible causes of DDIs in Lead Optimisation

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



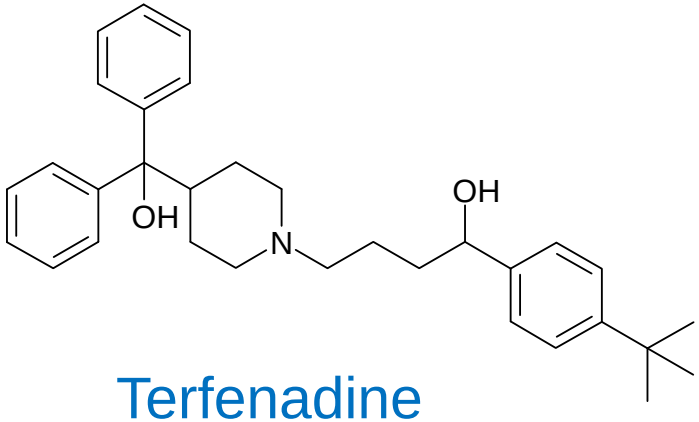
Cytochrome P450s
Drug-Drug Interactions

- 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

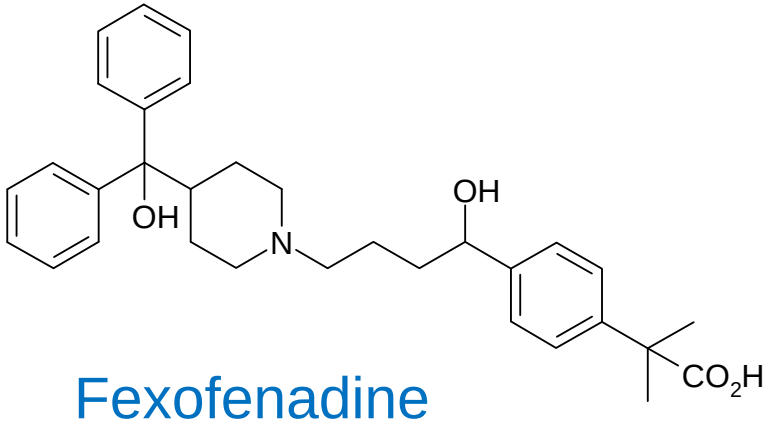
- A clear understanding of CYP interactions is important for all new drugs (inhibition can be measured *in vitro*)

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
  - Terfenadine also blocks cardiac K-channel (hERG)
  - Potential for increased amount of terfenadine in the body leading to cardiac toxicity

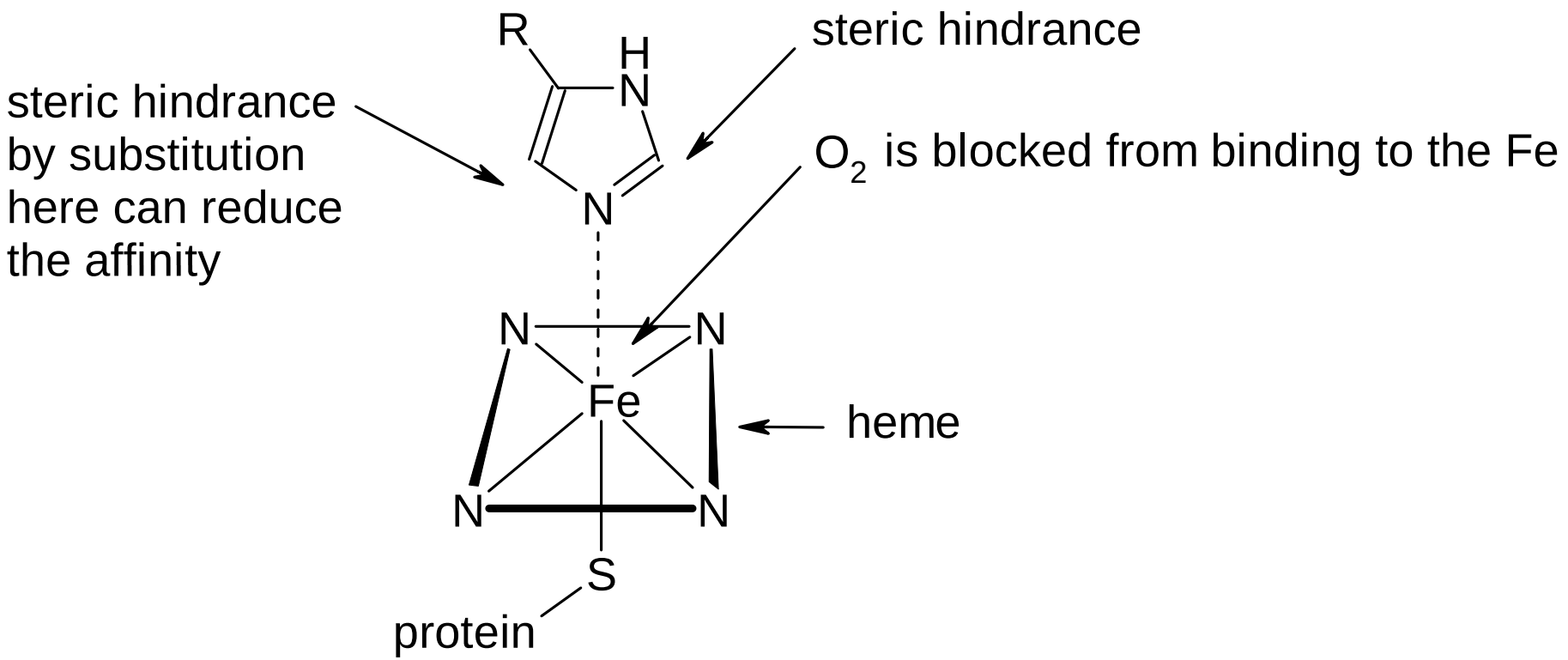


Terfenadine



Fexofenadine

Inhibition of Cytochrome P450's



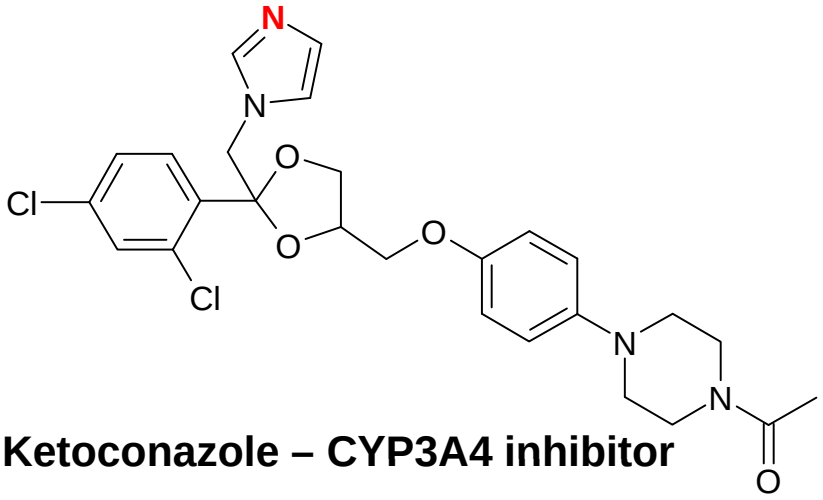
- **Nitrogen atom displaces water from heme complex**
- **Introduction of steric hindrance around N-atom (eg alkyl groups) may reduce interaction**
- **Look for isosteres of the aza/ diaza groups and reduction of electron density**

Inhibition of cytochrome P450's

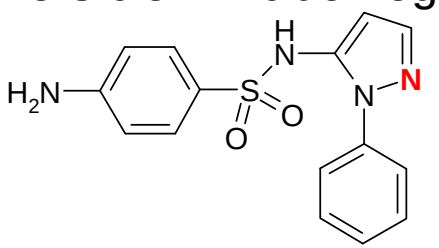
- Potency of inhibition has been correlated to lipophilicity of compounds
  - lowering logP is a good strategy for reducing CYP450 inhibition

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

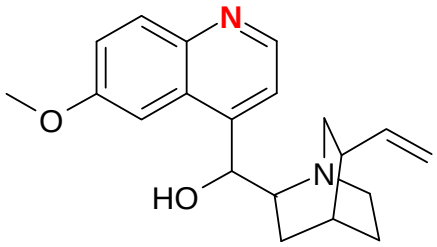
- Certain structural features may lead to reversible inhibition eg aza, diaza groups



Ketoconazole – CYP3A4 inhibitor



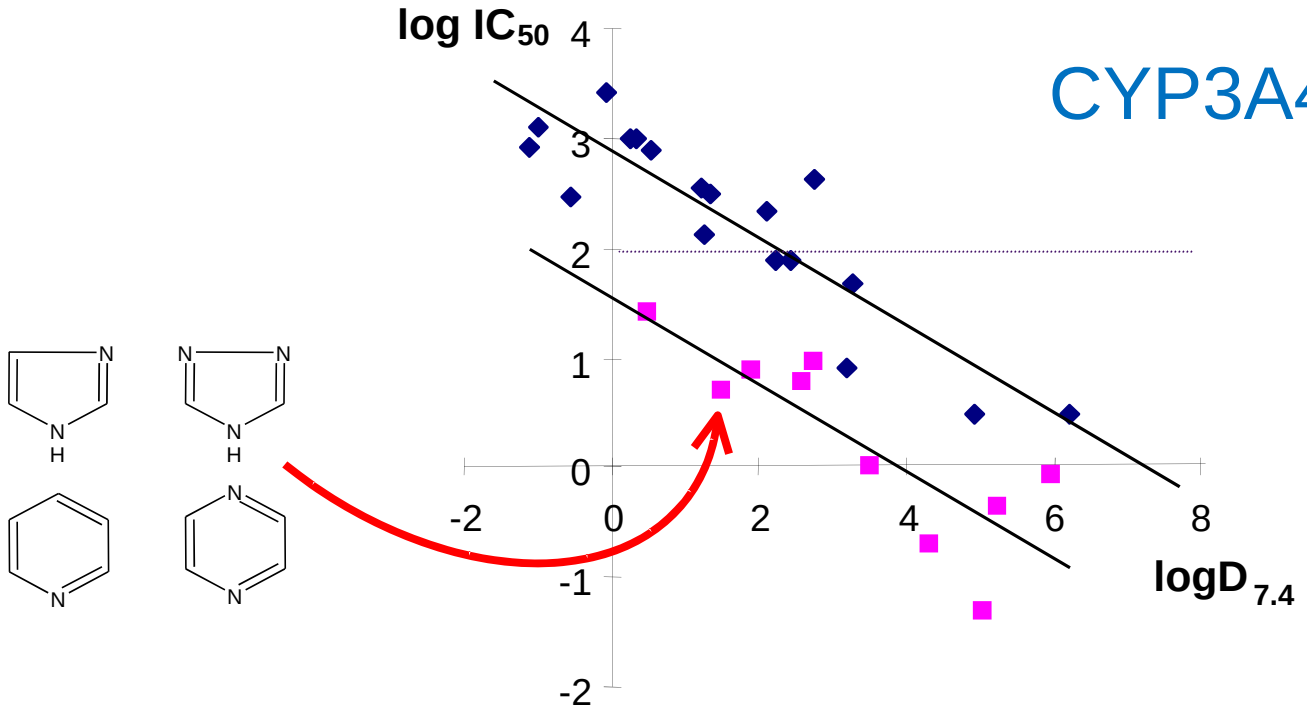
Sulphaphenazole
- CYP2C9 inhibitor



quinidine
- CYP2D6 inhibitor

Drug Interactions

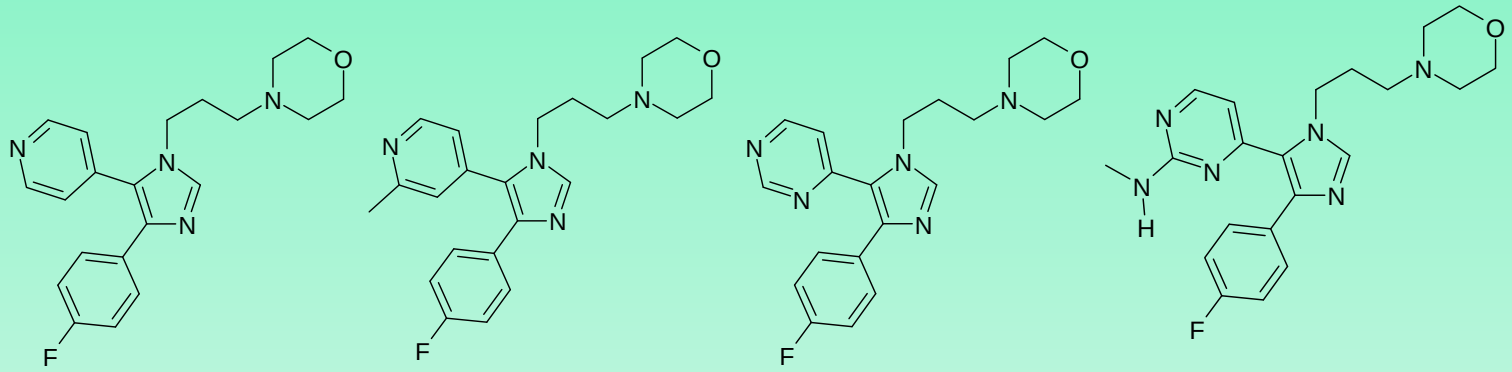
- Cyp 3A4 has logD dependence



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

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!
(but eating grapefruit is ok!)

Distribution & Duration

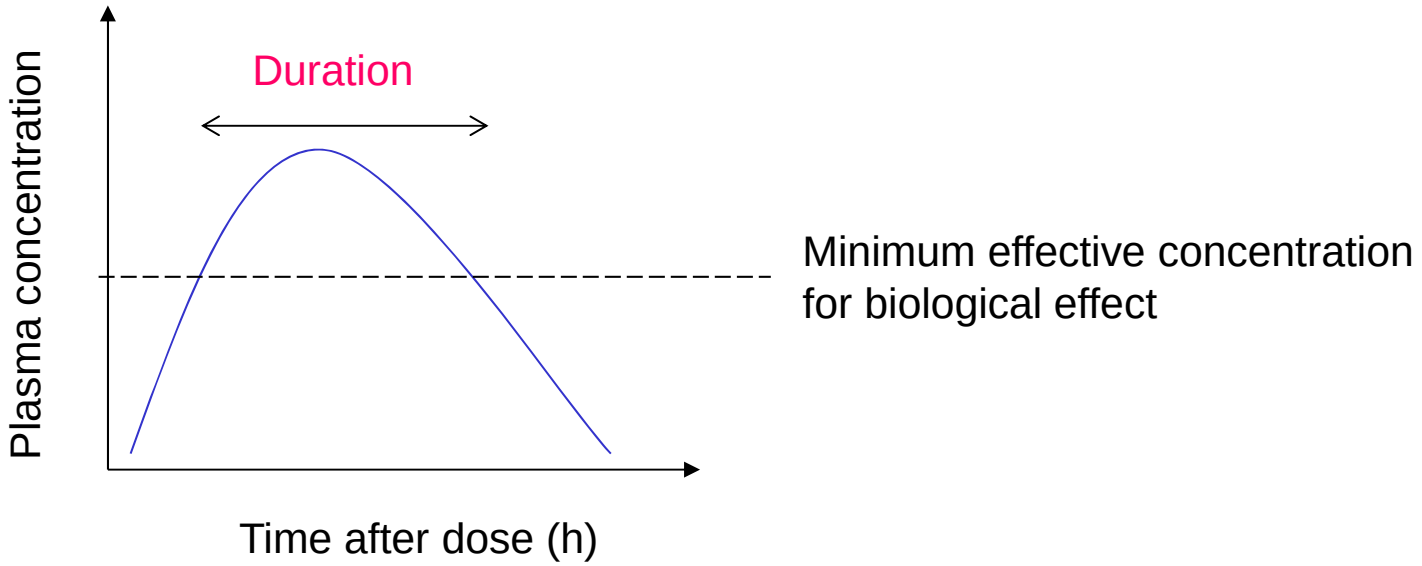
From clearance to duration of action…

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

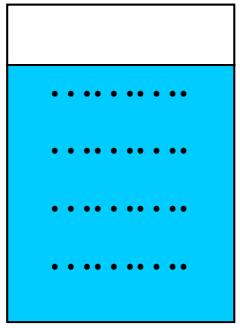
Half life in plasma can be increased by:
- increasing V, or
- decreasing CL

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:

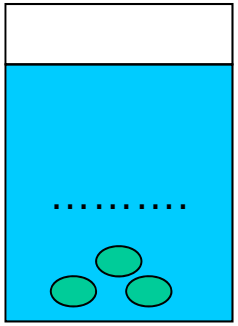
Addition of a cpd to water:



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

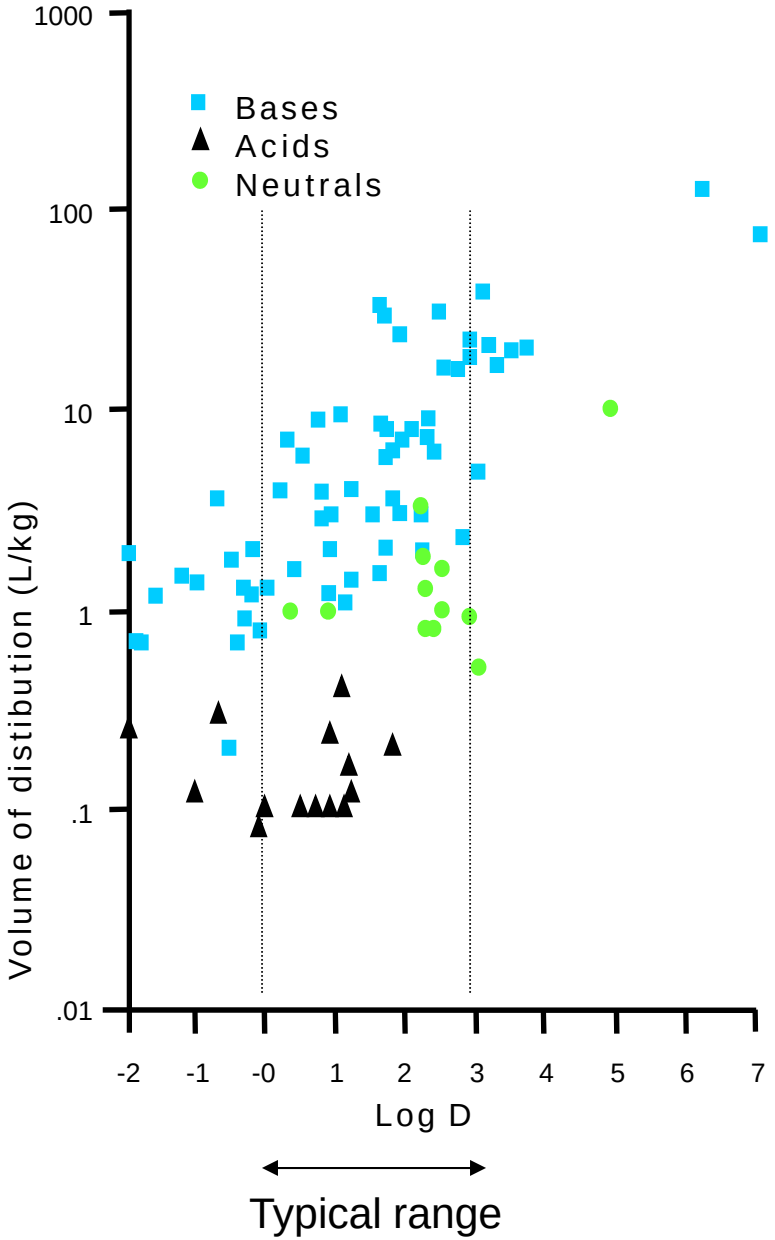
What factors govern volume of distribution?

Volume of distribution is also physical chemistry

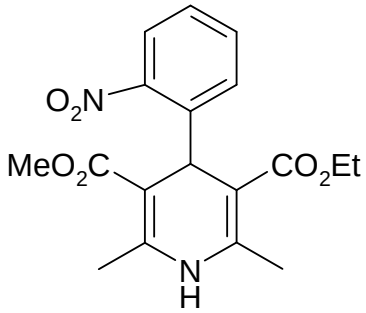
Influenced by:

- 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

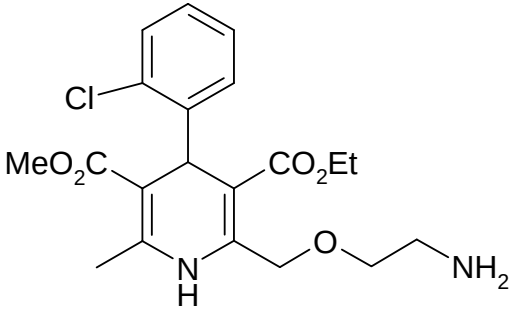


Volume of distribution can be modified



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

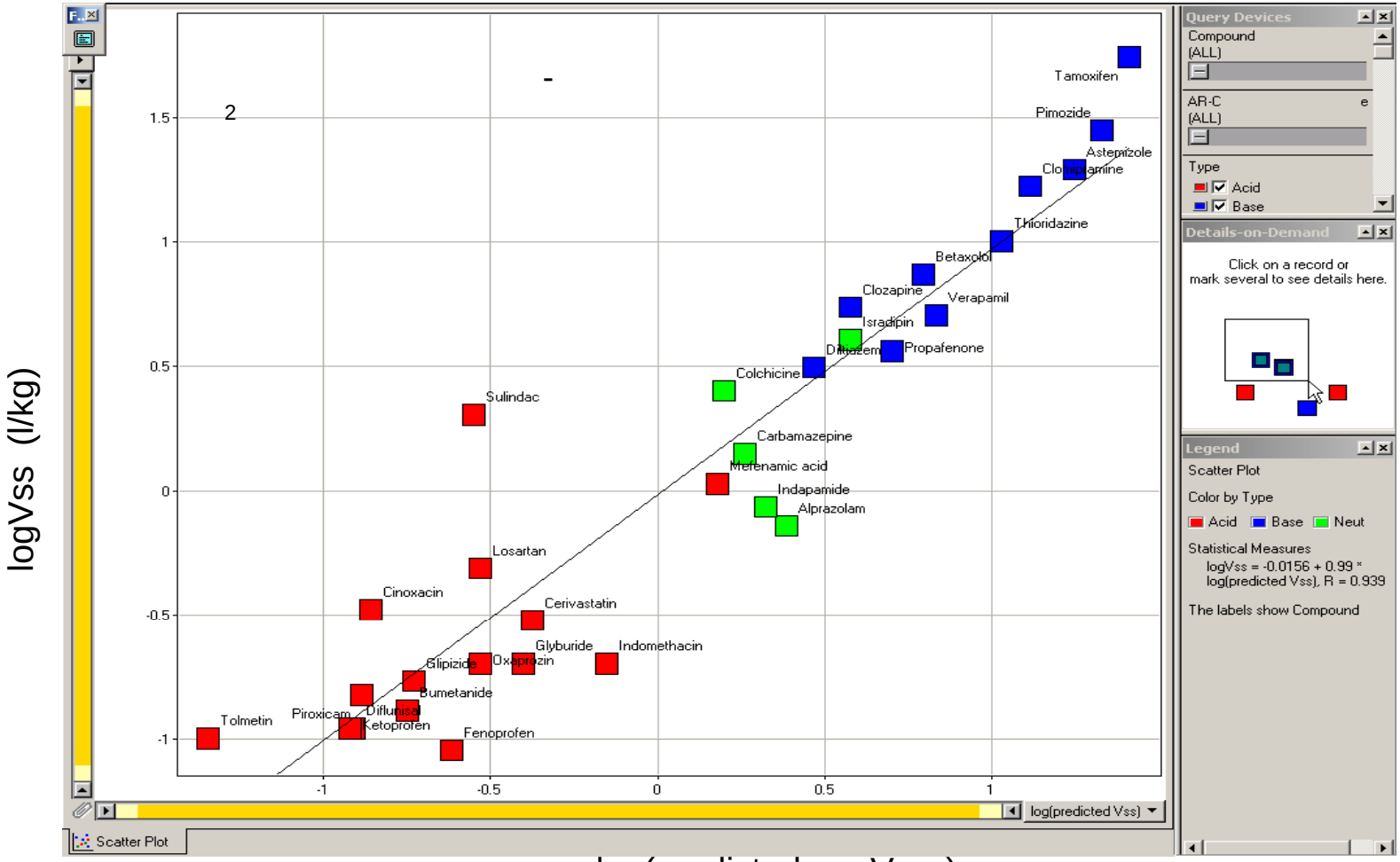


Amlodipine
CL = 7.0 ml/min/kg
V = 21.4 L/Kg
Half life = 33.8 h !!

Sales 2006: $5bn

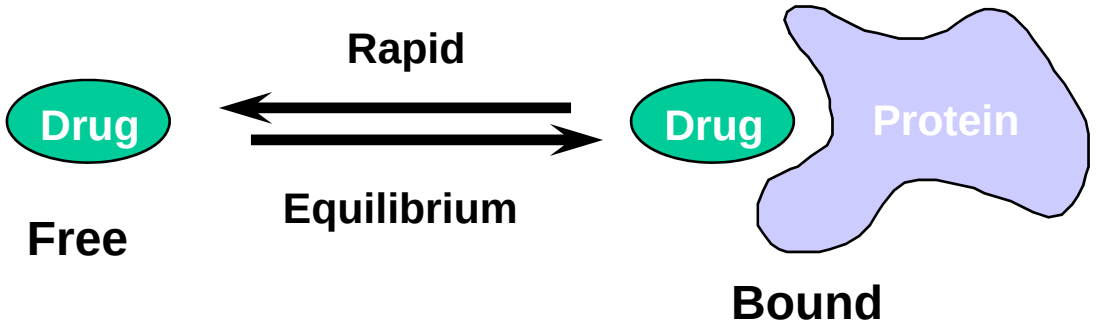
Volume of distribution can be predicted

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	Low lipophilicity
50-90%	= moderate	
90-99%	= high	
>99%	= very high	High lipophilicity

- 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).

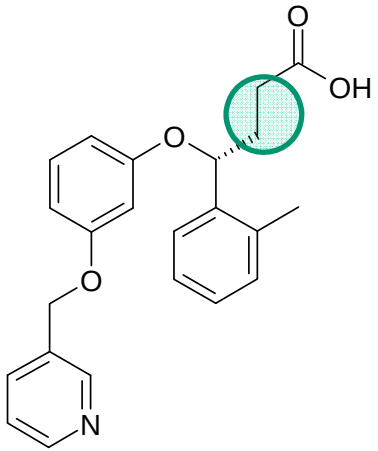
Impact of Protein Binding

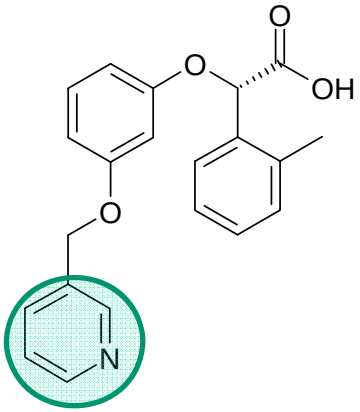
- PPB also has a big impact on in vivo efficacy
- Unbound / 'free' levels determine in-vivo efficacy
- Protein Binding too high can lead to lack of efficacy in cells, whole blood or in vivo:

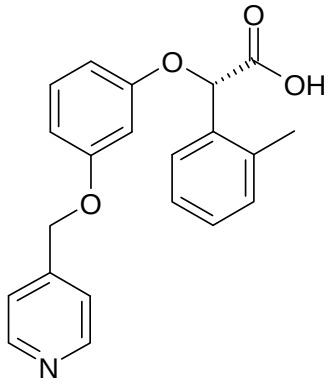
Example:

Reducing PPB in a series of acidic endothelin ET_A receptor antagonists

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



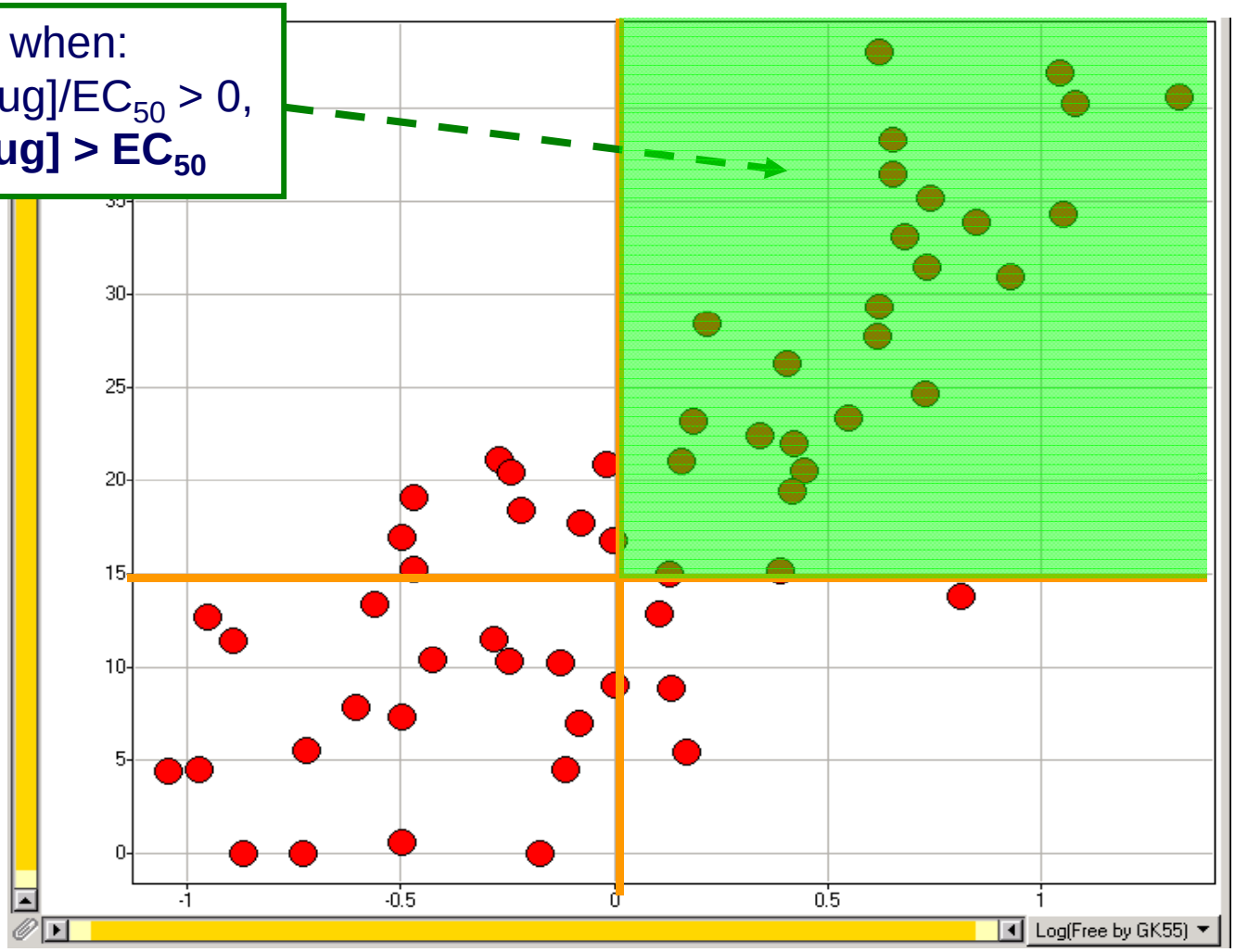




logD (7.4)	**1.6**	**-0.05**	**-0.04**
ET_A IC_{50}	**4.0 nM**	**70 nM**	**3.0 nM**
ET_A IC_{50} + 10% plasma	**95 nM**	**150 nM**	**9.0 nM**
Decrease in blood pressure (rat) @ 25 μmol/kg i.v. (shift in drc to ET-1)	**10X**		**90X**

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	?	?