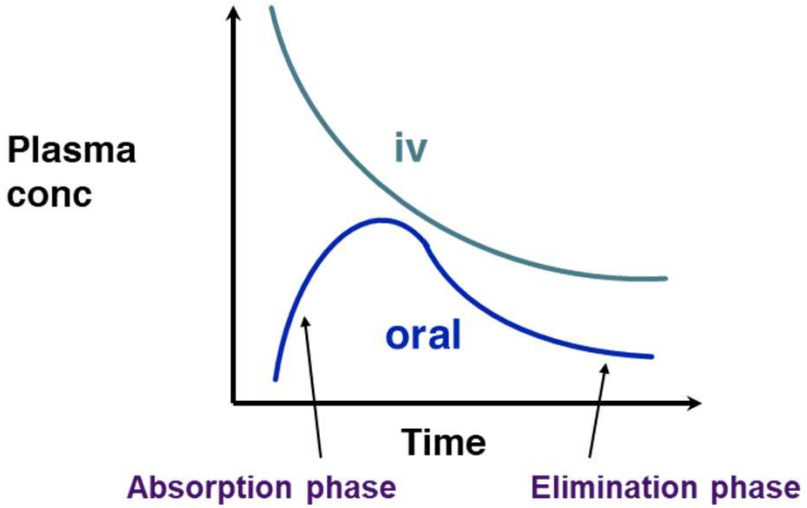
Oral Dosing - Bioavailability



Upon oral dosing of a drug, there is an initial increase in the systemic concentration of the drug, as it is absorbed from the gut.

As absorption is completed and the compound is eliminated from the body, the concentration of drug decreases over time.

Oral Bioavailability (F%) is defined as:

The fraction of the dose which makes it to the systemic circulation (i.e. survives 1st pass metabolism).

$$F = \frac{\textbf{AUC after an oral dose}}{\textbf{AUC after an equivalent iv dose}}$$

Limiting factors include: Chemical instability, eg acid sensitive compound in the stomach; Incomplete absorption - solubility, formulation; Gut wall metabolism - labile functional groups

How does pH vary in the body?

Fluid	pH
Aqueous humour	7.2
Blood	7.4
Colon	5-8
Duodenum (fasting)	4.4-6.6
Duodenum (fed)	5.2-6.2
Saliva	6.4
Small intestine	6.5
Stomach (fasting)	1.4-2.1
Stomach (fed)	3-7
Sweat	5.4
Urine	5.5-7.0

So the same compound will be ionised to different extents in different parts of the body.

This means that, for example, basic compounds will not be so well absorbed in the stomach than acidic compounds since it is generally the unionised form of the drug which diffuses into the blood stream.

Ionisation constants

- The equilibrium between un-ionised and ionised forms is defined by the acidity constant K_a or $pK_a = -\log_{10} K_a$
- For an acid:

$$HA \overset{K_a}{\rightleftharpoons} H^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[AH]} \qquad \% \text{ ionised} = \frac{100}{1 + 10^{(pK_a - pH)}}$$

- For a base:

$$BH^+ \overset{K_a}{\rightleftharpoons} H^+ + B$$

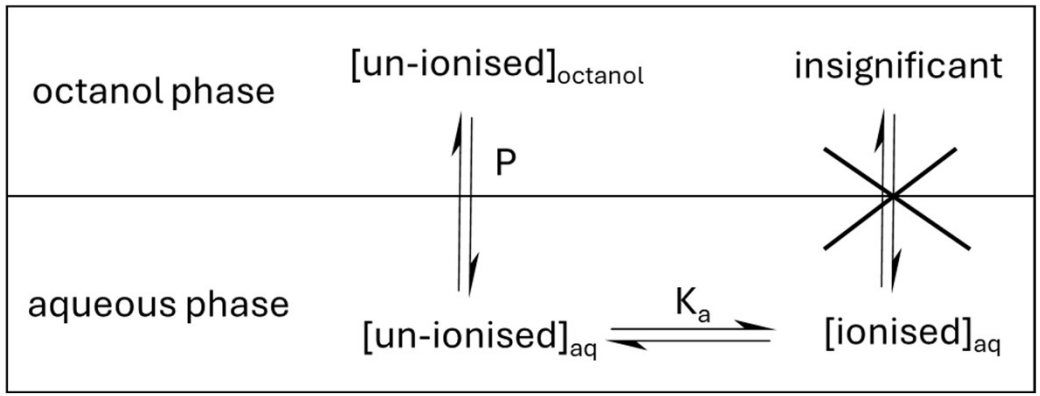
$$K_a = \frac{[H^+][B]}{[BH^+]} \qquad \% \text{ ionised} = \frac{100}{1 + 10^{(pH - pK_a)}}$$

When an acid or base is 50% ionised:

$$pH = pK_a$$

Distribution coefficients

If a compound can ionise then the observed partitioning between water and octanol will be pH dependent.



For an acidic compound: $HA_{aq} \rightleftharpoons H^+_{aq} + A^-_{aq}$

$$D = \frac{[HA]_{octanol}}{[HA]_{aq} + [A^-]_{aq}}$$

For a basic compound: $BH^+_{aq} \rightleftharpoons H^+_{aq} + B_{aq}$

$$D = \frac{[B]_{octanol}}{[BH^+]_{aq} + [B]_{aq}}$$

Distribution coefficient D (usually expressed as logD) is the effective lipophilicity of a compound at a given pH, and is a function of both the lipophilicity of the un-ionised compound and the degree of ionisation.

Lipinski's 'Rule of 5'

1. Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
2. Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
3. A molecular mass less than 500 daltons
4. An octanol-water partition coefficient log *P* not greater than 5

All the above numbers are multiples of five and that is the origin of the name

An additional rule was proposed by **Veber**

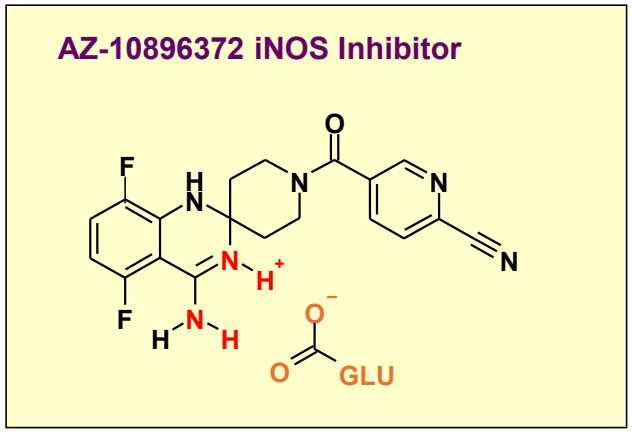
➢ < 10 rotatable bonds

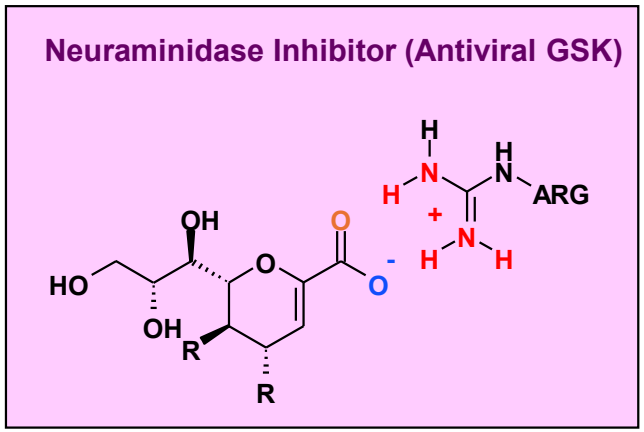
Otherwise absorption and bioavailability are likely to be poor.

NB - This is for oral drugs only.

Electrostatic Interactions

- These result from the attraction between molecules bearing opposite electronic charges.
- Strong ionic interactions can contribute very strongly to binding.
- Proteins contain both CO_2^- and NH_3^+ residues and these may be present at the binding site to interact with oppositely charged groups on the drug.





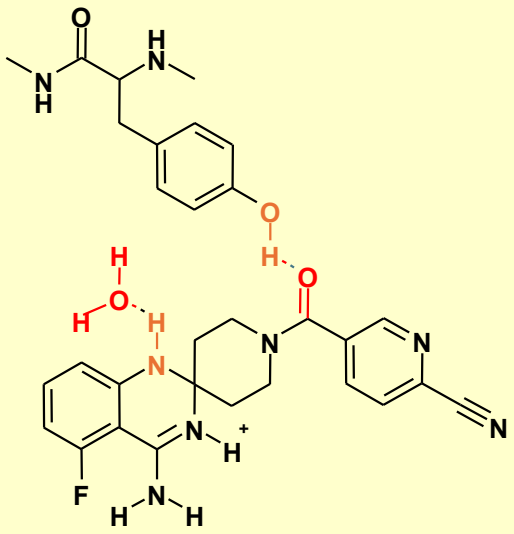
- The energies involved in a 'salt bridge' can be in the order of >30 kJ/mol
- This can lead to increase in observed binding of $>10^6$ fold

Hydrogen Bonding Interactions

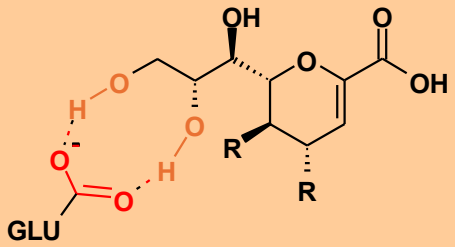
A hydrogen bond results when a hydrogen is shared between two electronegative atoms

The Donor provides the H, while the Acceptor provides an electron pair

D-X-H….Y-A **e.g. R-O-H…..O=C**



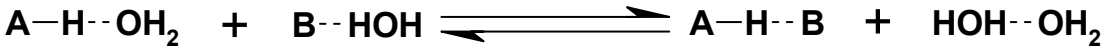
AZ10896372 - iNOS complex
Amide to Tyrosine H-Bond



Neuraminidase Inhibitor
Charge re-inforced H-Bond

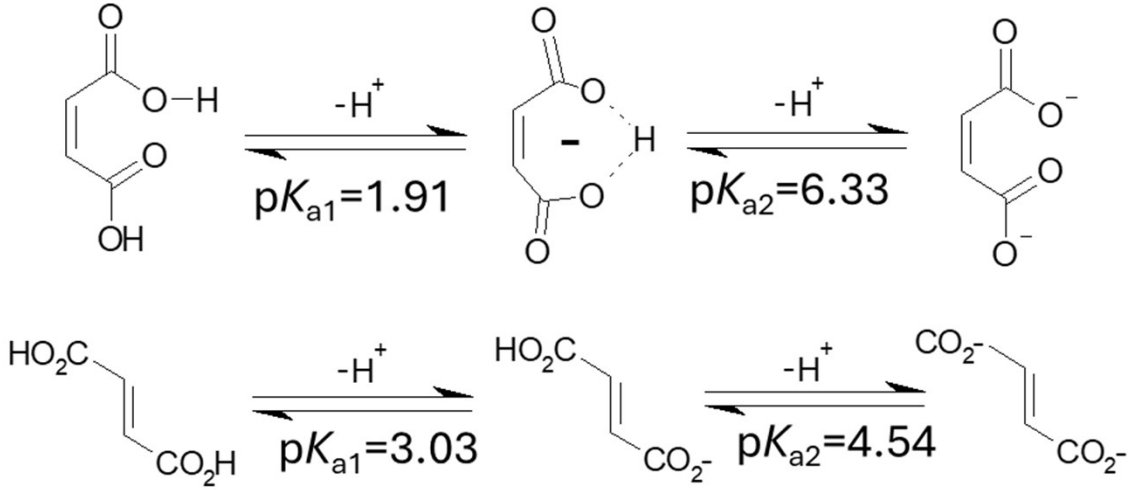
Hydrogen bonding

- **Intermolecular** hydrogen bonds are virtually non-existent between small molecules in water. To form a hydrogen bond between a donor and acceptor group, both the donor and the acceptor must first break their hydrogen bonds to surrounding water molecules



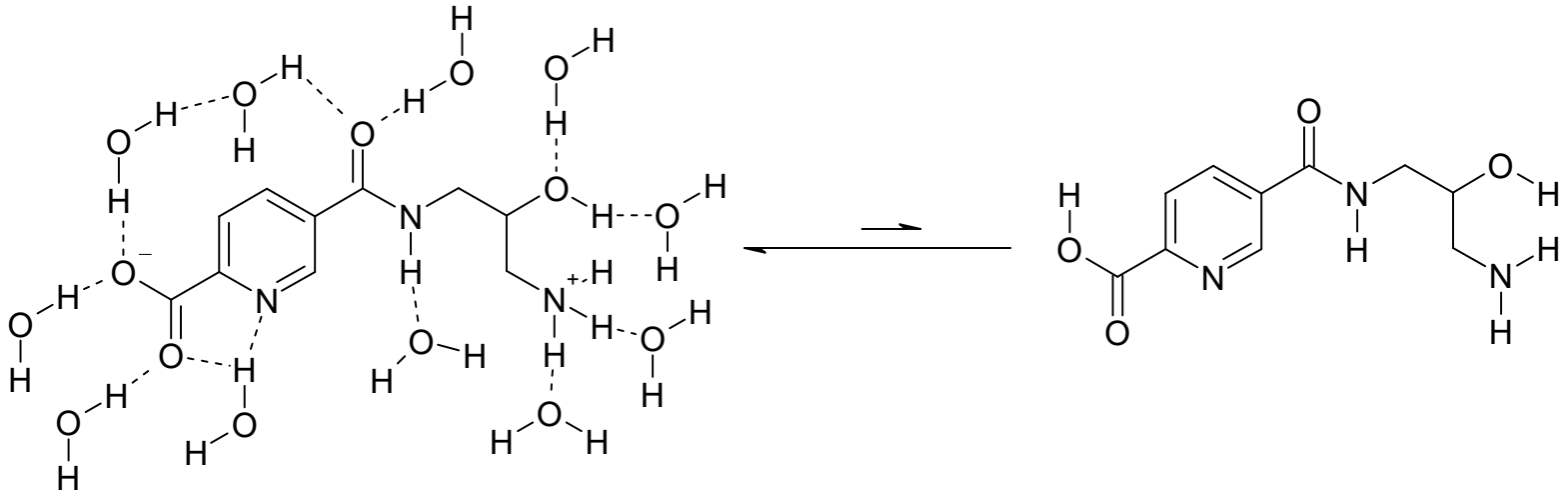
- The position of this equilibrium depends on the relative energies of the species on either side, and not just the energy of the donor-acceptor complex

- **Intramolecular** hydrogen bonds are more readily formed in water - they are entropically more favourable.



Hydrogen bonding and bioavailability

Remember! Most oral drugs are absorbed through the gut wall by transcellular absorption.



- De-solvation and formation of a neutral molecule is unfavourable if the compound forms many hydrogen or ionic bonds with water.
- So, as a good rule of thumb, you don't want too many hydrogen bond donors or acceptors, otherwise the drug won't get from the gut into the blood.
- There are some exceptions to this – sugars, for example, but these have special transport mechanisms.

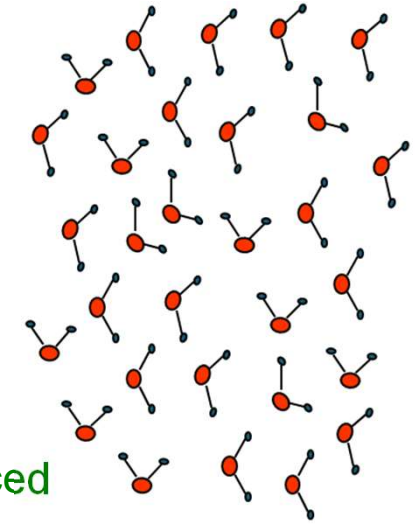
Hydrophobic Interactions

- Drugs, in general, are hydrophobic molecules
- The 'Binding Sites' of proteins are also hydrophobic in character
- Thus a mutual attraction can result (like attracts like).

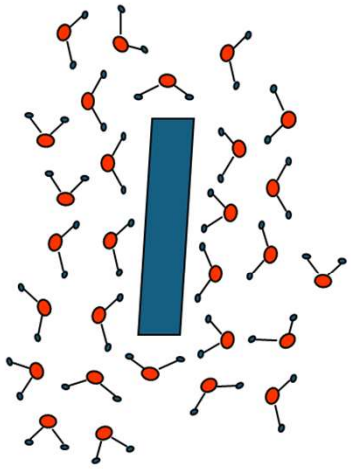
- What drives this attraction?
- Enthalpy gains may result from van der Waals bonding:
  - Between Alkyl, Aryl, Halogen groups
  - π–π Stacking is an important type of this
- Entropy gains are achieved when water molecules are displaced from 'active site', and return to a more random (high S) state.

NOTE:

- *Each -(CH_2)- group can contribute >1 kJ/mol towards binding*
- *Each -Ph ring can contribute >2 kJ/mol towards binding*
- *These effects are additive and hence Hydrophobic Bonding can make a very high contribution to binding*

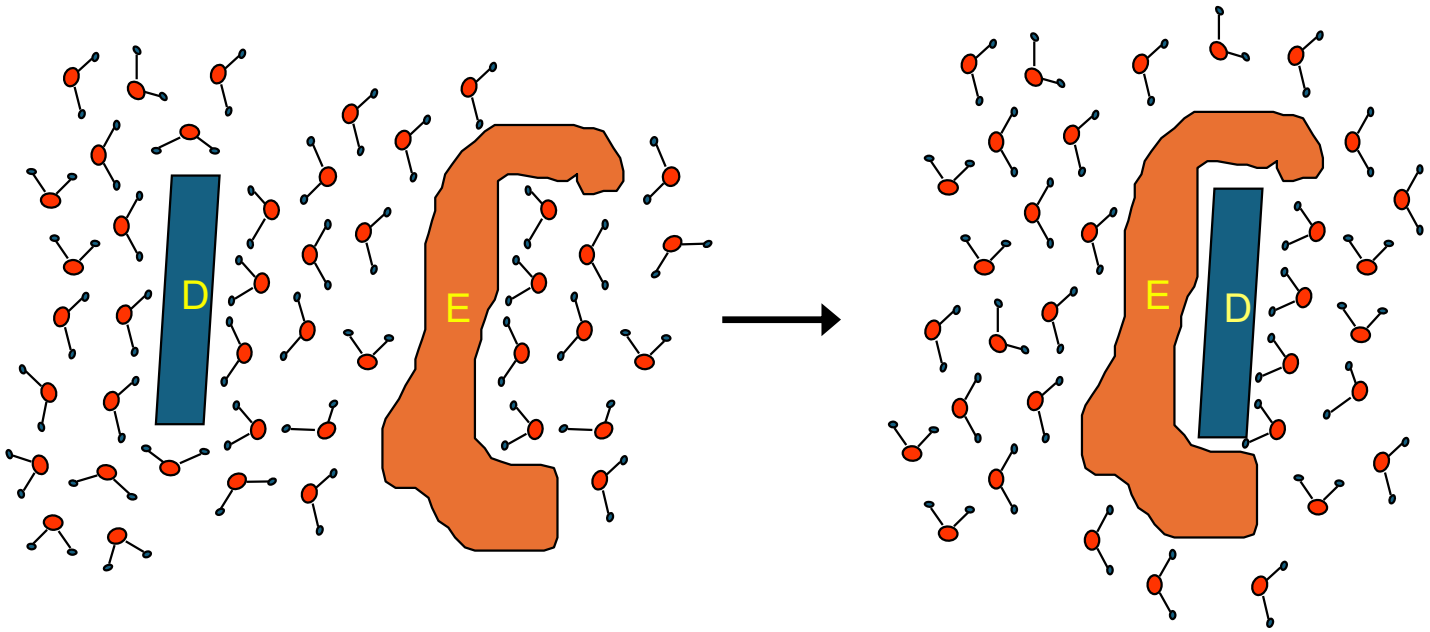


Water molecules are in a highly disordered state. Each molecule maximises H-Bonds to other molecules of water.



When a hydrophobic drug is placed into water, the structure of the water around the drug is more ordered. This allows the H_2O-H_2O H-bonds to be maintained. This leads to lower entropy and is not favoured.

Hydrophobic Bonding : Δ Entropy



- Hydrophobic interaction between protein and drug is favoured by entropy gains:
  - Bulk water returns to less ordered state
  - Water molecules may be expelled from being bound in active site.
  - In addition enthalpy gains due to new bonds may also be favourable (e.g. van der Waals interactions)

Lipophilicity

Lipophilicity ('fat-liking') is the most important physical property of a drug in relation to its absorption, distribution, potency, and elimination.

Lipophilicity is often an important factor in all of the following, which include both biological and physicochemical properties:

- Solubility
- Absorption
- Plasma protein binding
- Metabolic clearance
- Volume of distribution
- Enzyme / receptor binding
- Biliary and renal clearance
- CNS penetration
- Storage in tissues
- Bioavailability
- Toxicity

Partition coefficients

Partition coefficient P (usually expressed as $\log_{10}P$ or logP) is defined as:

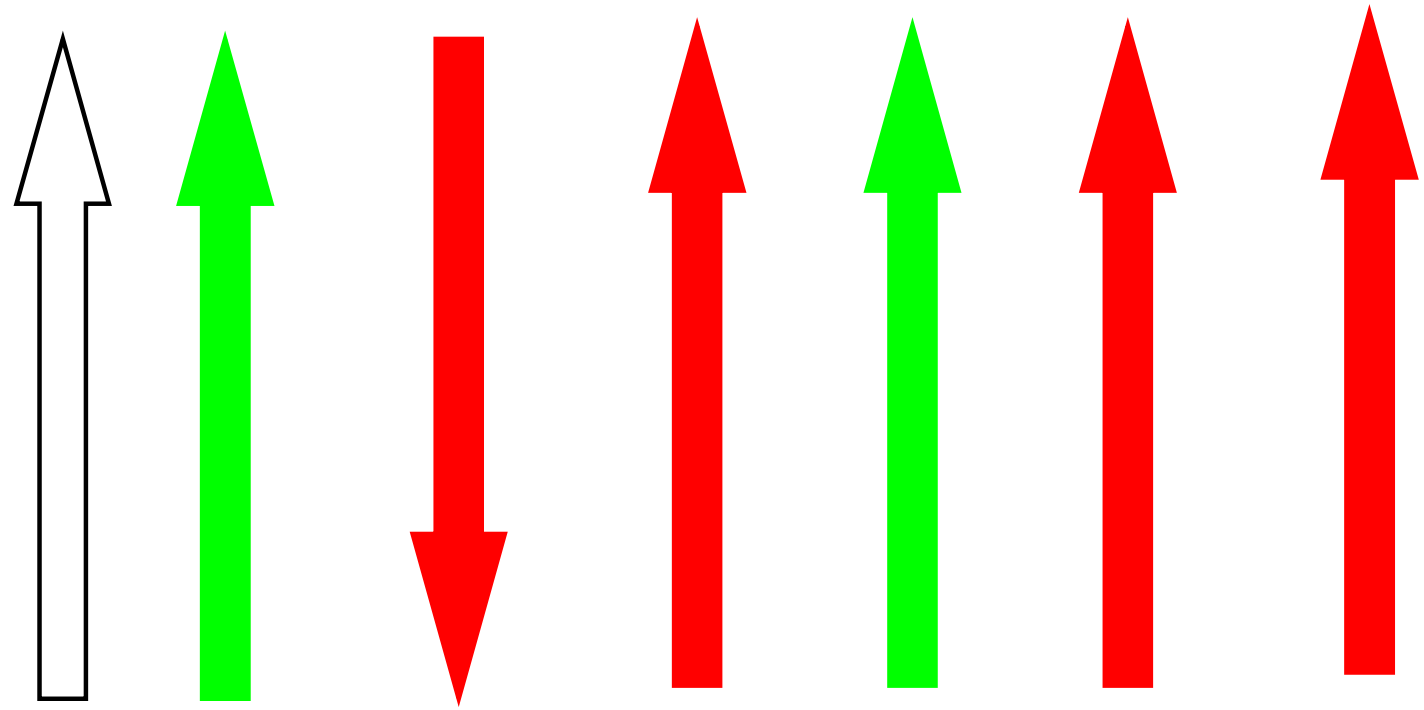
$$P = \frac{[X]_{octanol}}{[X]_{aqueous}}$$

P is a measure of the relative affinity of a molecule for the lipid and aqueous phases in the absence of ionisation.

1-Octanol is the most frequently used lipid phase in pharmaceutical research. This is because:

- It has a polar and non polar region (like a membrane phospholipid)
- $P_{o/w}$ is fairly easy to measure
- $P_{o/w}$ often correlates well with many biological properties
- It can be predicted fairly accurately using computational models

What else does logP affect?



logP

Binding to enzyme / receptor

Aqueous solubility

Binding to P_{450} metabolising enzymes

Absorption through membrane

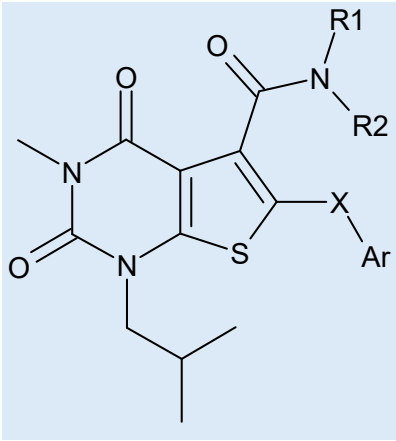
Binding to blood / tissue proteins – less drug free to act

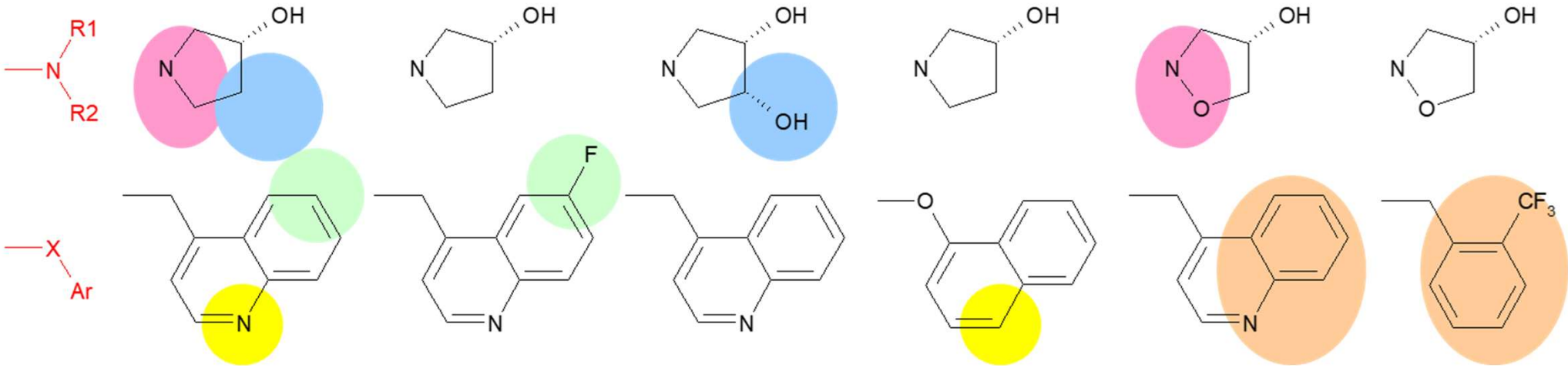
Binding to hERG heart ion channel - cardiotoxicity risk

So log P needs to be *optimised*

How can lipophilicity be altered?

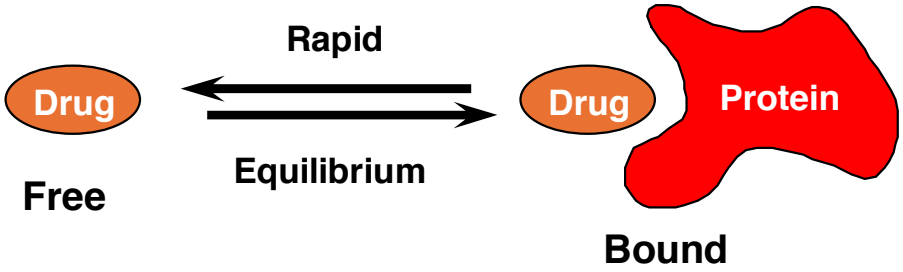
e.g. Monocarboxylate transporter 1 blockers





logD	1.7	2.0	1.2	2.9	2.2	3.2

Plasma Protein Binding (PPB)



Drugs can bind to macromolecules in the blood – known as plasma protein binding (PPB)

Only unbound compound is available for distribution into tissues

Acids bind to basic binding sites on albumin, bases bind to alpha-1 acid glycoprotein

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

For bases and neutrals, PPB is proportional to logD.

Acidic drugs tend to have higher PPB than neutral/basic drugs.

Drug-Protein Binding Energies

For a binding Equilibrium between a Protein & a Drug

$$[\text{Protein}] + [\text{Drug}] \underset{\Delta G}{\overset{K}{\rightleftharpoons}} [\text{P:D}]$$

$$K = \frac{[\text{P:D}]}{[\text{P}] \times [\text{D}]}$$

Gibbs Free Energy Changes

$\Delta G = -RT\ln K$ and $\Delta G = \Delta H - T\Delta S$

Both Enthalpy (ΔH) and Entropy (ΔS) changes affect binding strength

Drug-Protein Interactions

Bond	Example	kJ/mol
Van der Waal	Xe…Xe, alkyl groups	2
Hydrophobic	Ph…Ph (π-stacking)	5
Dipole - Dipole	C=O…HN-R (δ+/δ-)...(δ+/δ-)	5
Hydrogen	H_2O…H_2O (X-H) …(Y-R)	35
Ion - Dipole	F^-…H_2O (+/-ve)…(δ+/δ-)	170
Ion - Ion	H^+…Cl^- (+ve)…(-ve)	450
Covalent	C-O	350

NB. When a drug moves from the aqueous medium into the 'Binding Site' it has to break H-Bonds with water, de-solvate etc. These processes require energy, so the net energy available for binding is only a fraction of the above bond energies.

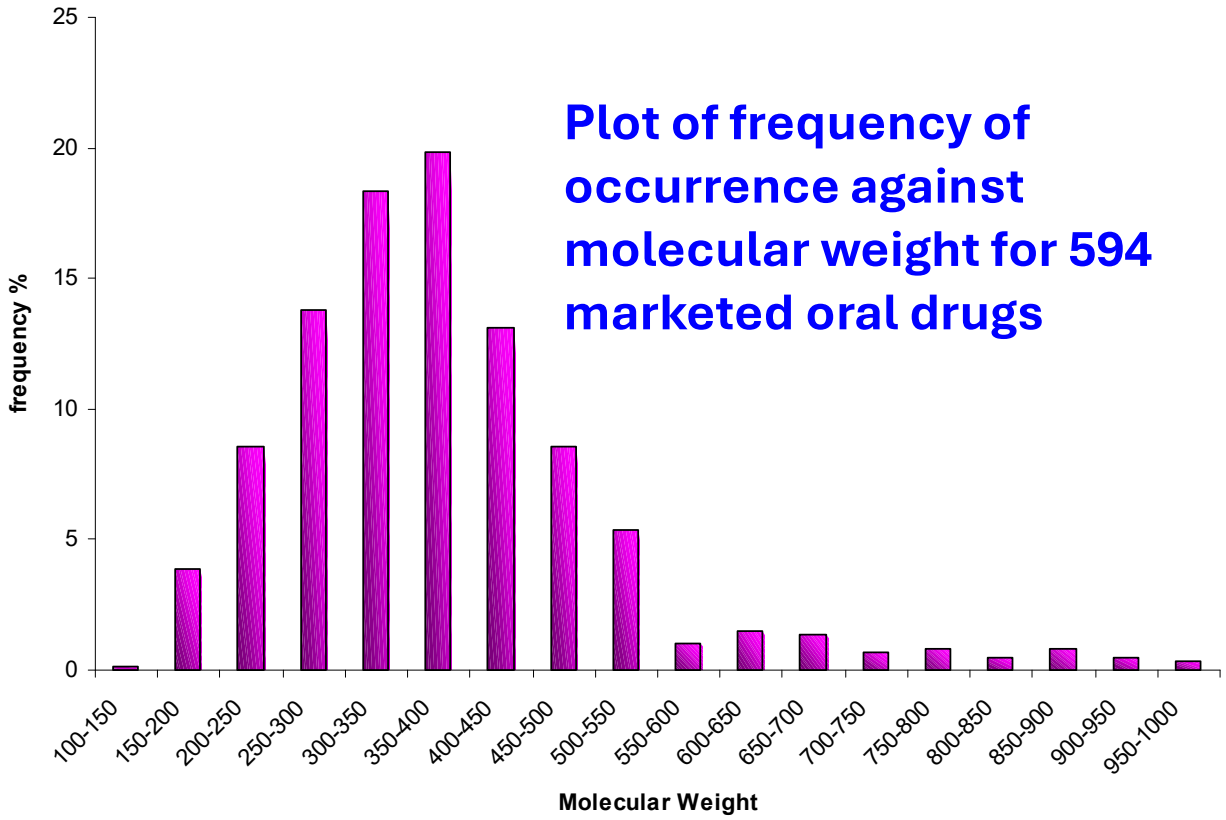
Molecular size

Molecular size is one of the most important factors affecting biological activity, but it's also one of the most difficult to measure.

There are various ways of investigating the molecular size, including measurement of:

- Molecular weight (most important
- Electron density
- Polar surface area
- Van der Waals surface
- Molar refractivity

Molecular weight

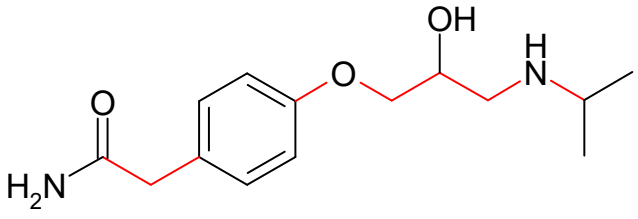


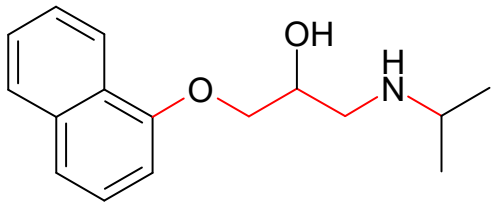
Plot of frequency of occurrence against molecular weight for 594 marketed oral drugs

Most oral drugs have molecular weight < 500

Number of rotatable bonds

A rotatable bond is defined as any single non-ring bond, attached to a non-terminal, non-hydrogen atom. Amide C-N bonds are not counted because of their high barrier to rotation.



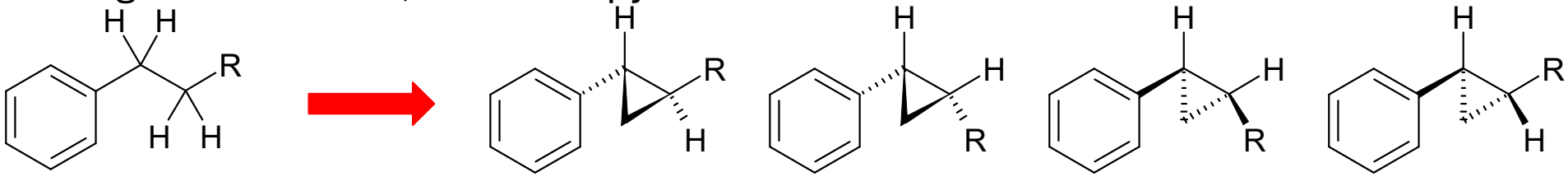


	No. of rotatable bonds	Bioavailability
Atenolol	8	50%
Propranolol	6	90%

The number of rotatable bonds influences, in particular, bioavailability and binding potency. Why should this be so?

Number of rotatable bonds

Remember δG = δH – TδS ! A molecule will have to adopt a fixed conformation to bind, and to pass through a membrane. This involves a loss in entropy, so if the molecule is more rigid to start with, less entropy is lost. But beware!



Any, or none, of these could be the active conformation!