Advanced Medicinal Chemistry

Lecture 3:

Molecular Interactions and Drug Potency

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AstraZeneca R&D Charnwood

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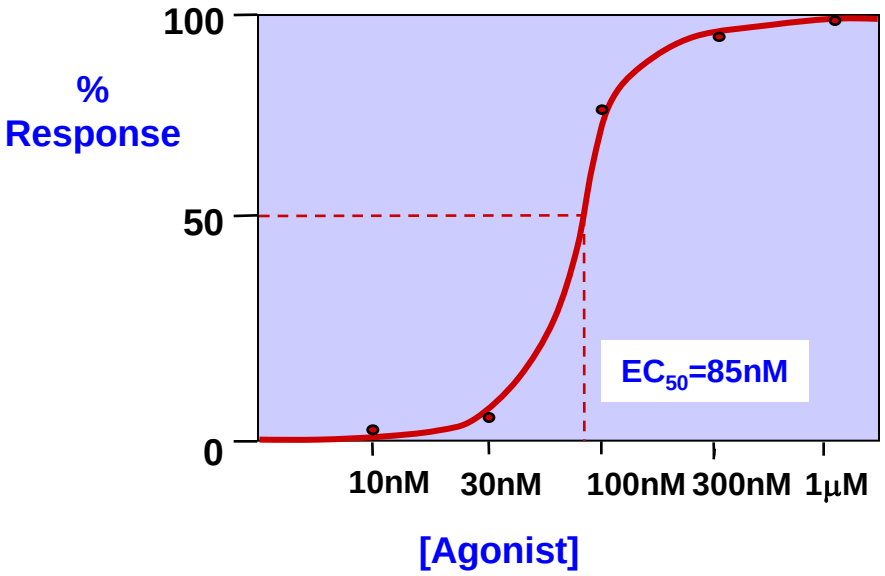
Dose-Response Curves



Enzyme Inhibitors (competitive):

Measure inhibition at differing concentrations of 'drug'.

IC_{50} - The inhibitor concentration that causes a 50% reduction in intrinsic enzyme activity

$pIC_{50} = -\log_{10}(IC_{50})$

IC_{50} 1μM = pIC_{50} 6.0
IC_{50} 1nM = pIC_{50} 9.0

Agonists: Measure % Response vs Agonist concentration
EC_{50} - The agonist concentration that causes 50% of the maximum response. $pEC_{50} = -\log_{10}(EC_{50})$

Antagonists: Situation more complex. Antagonists displace the agonist dose-response curve rightwards – most accurate measure of potency (pA_2) requires measurement of agonist binding at multiple concentrations of antagonist

For a drug, typically target affinity values of $pIC_{50} \geq 8$ (<10 nM concentration)

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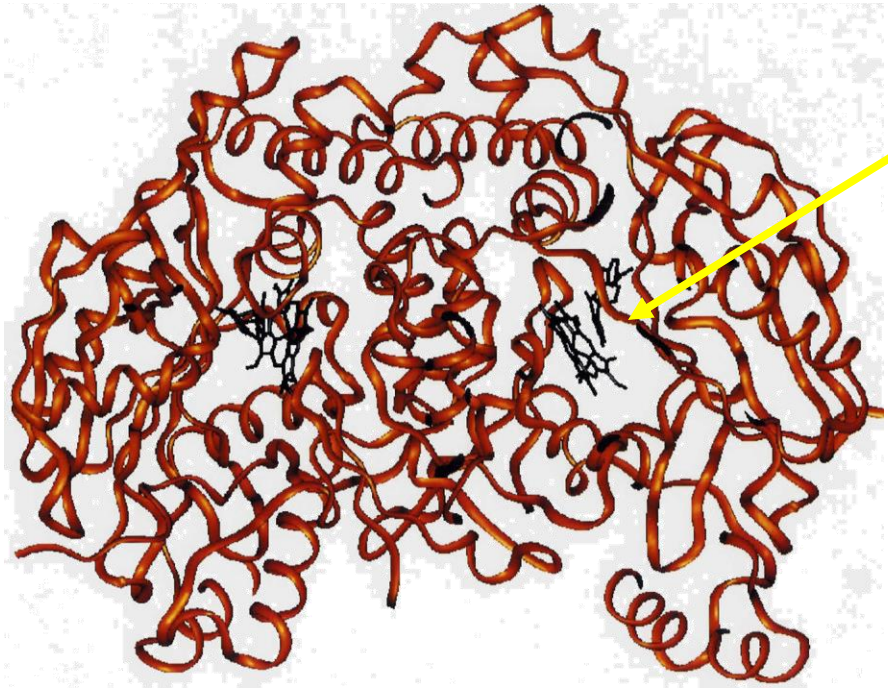
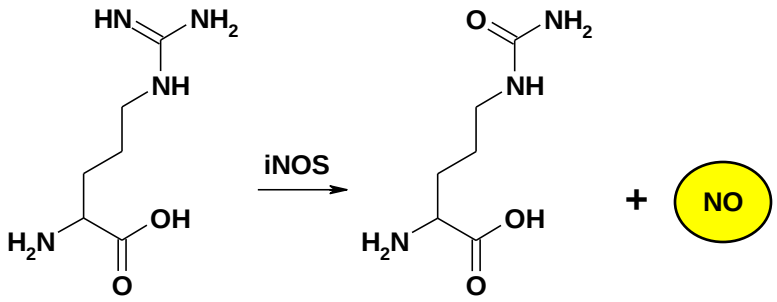
iNOS - An AZ Charnwood Discovery Project



Active Site,
Haem & Inhibitor

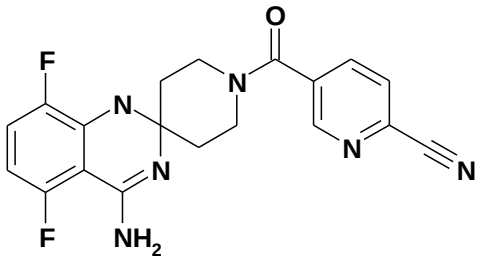


Nitric Oxide Synthases – catalyse production of NO from arginine in the body – implicated in inflammatory conditions e.g. rheumatoid arthritis

AZ10896372

pIC_{50} 7.5

A potent, selective iNOS inhibitor



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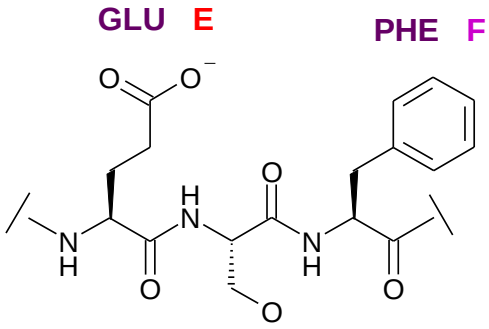
How Do Drugs Bind to Enzymes & Receptors?

Drugs bind to particular sites on enzymes and receptors. In the case of an enzyme, this will often be the active site. Receptors have binding pockets formed between transmembrane helixes where drugs usually bind (not always the agonist's binding site).

These sites are comprised of a variety of amino acid residues which give rise to a specific 3-D shape and molecular features:

- **Charges:** CO_2^- , NH_3^+, =NH-$^+$
- **Polar groups:** OH, C=O, CONH
- **Hydrophobic groups:** Ph, Alkyl, SMe

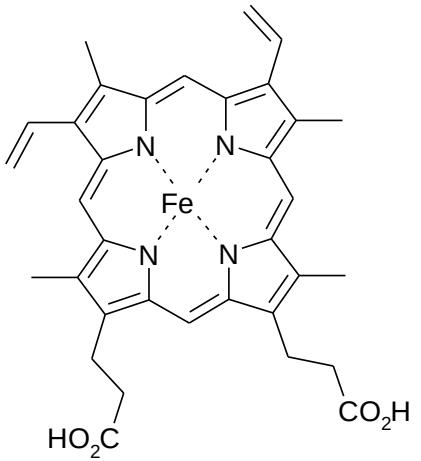


In enzymes, reaction centres are also present:

- **Asp-His-Ser in esterases**
- **SH in some proteases**
- **Metal ions (CYP-450, iNOS).**

Small molecules bind to these pockets by a combination of:

- **Shape complementarity**
- **Energetically favourable interactions**



Haem group – iNOS, CYP-450

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Shape Complementarity

iNOS Enzyme Inhibitor

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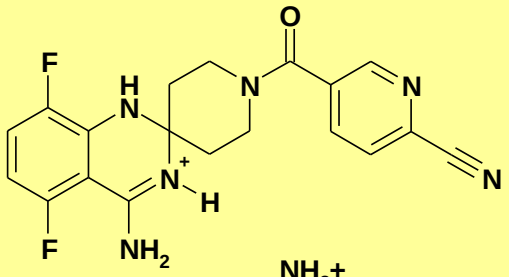
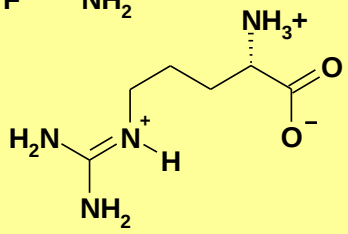
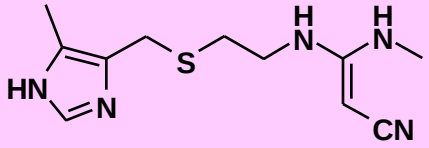


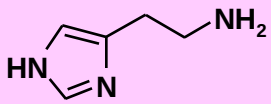
Arginine



H_2 Receptor Antagonist



Cimetidine



Histamine

The drug must fit into the Binding Site and shape complementarity is an important feature of a drug molecule. Competitive enzyme inhibitors often bear a resemblance to the substrate, as they bind to the same Active Site. This is also true for some receptor antagonists, but not all.

The strength of an interaction depends on the complementarity of the physico-chemical properties of atoms that bind, i.e. protein surface and ligand structure.

The 'Binding Sites' are not totally rigid. The side chains of the amino acids that make up the pocket have some mobility. A variety of related structures can thus be accommodated by movements that change the shape of the active site. This is known as the 'Induced Fit Hypothesis'.

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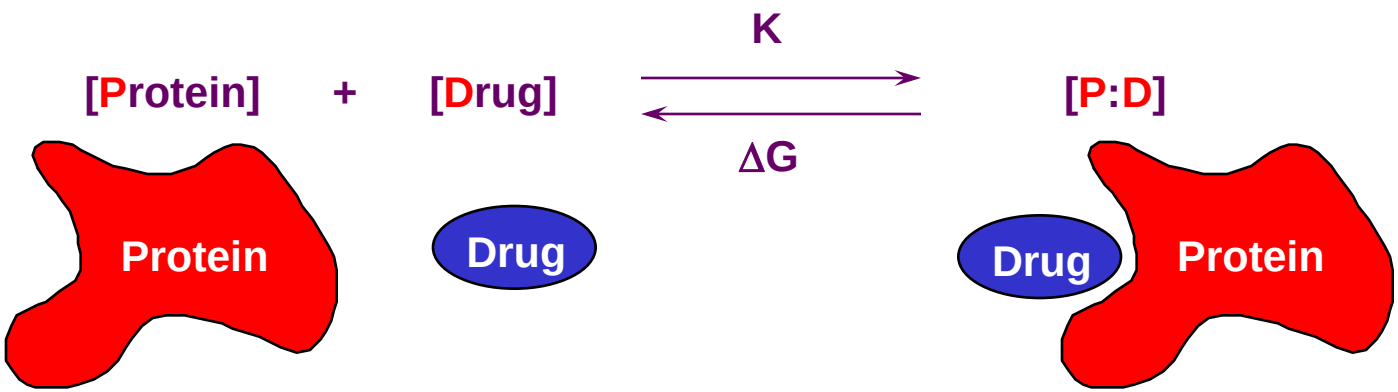
Drug-Protein Binding Energies

For a binding Equilibrium between a Protein & a Drug



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

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

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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.

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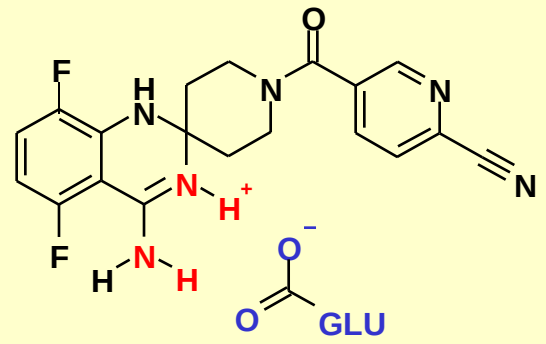
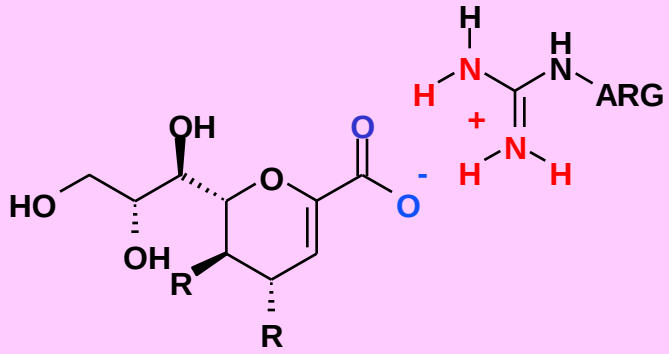
Electrostatic Interactions

- 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.

AZ-10896372 iNOS Inhibitor



Neuraminidase Inhibitor (Antiviral GSK)



- 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

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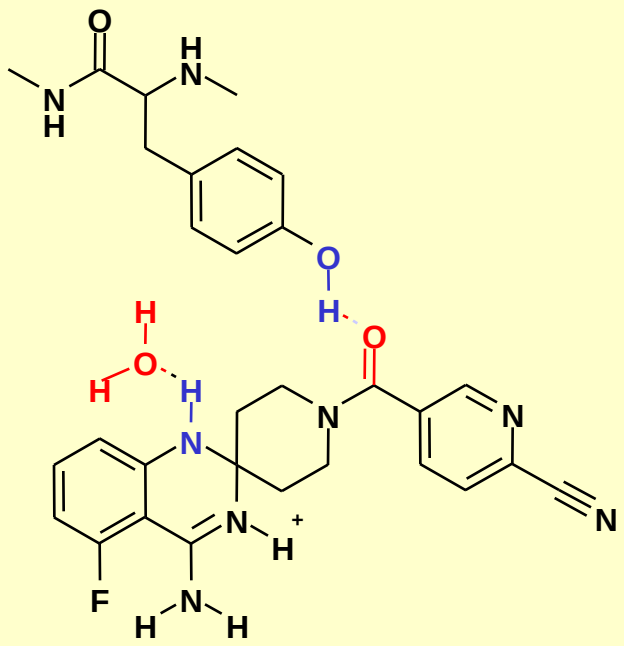
Hydrogen Bonding Interactions

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

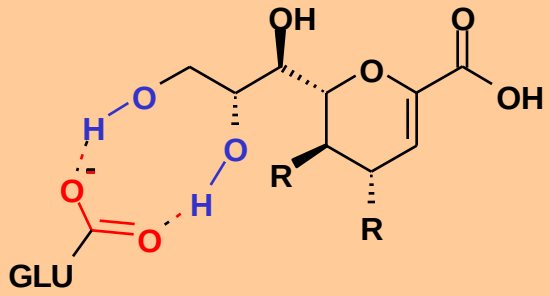
The Donor provides the H, while the Acceptor provides an electron pair

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

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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.**

- **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**

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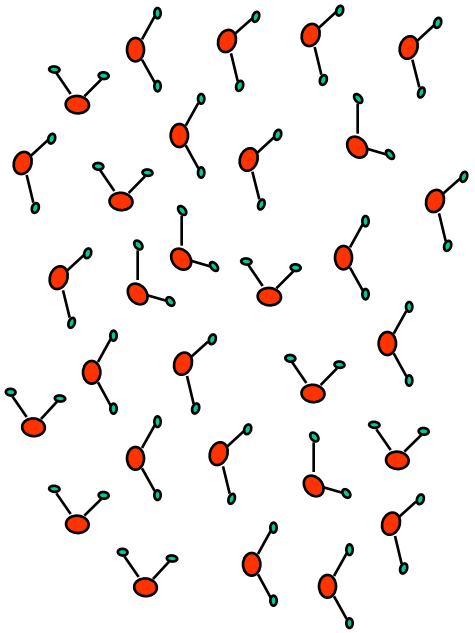
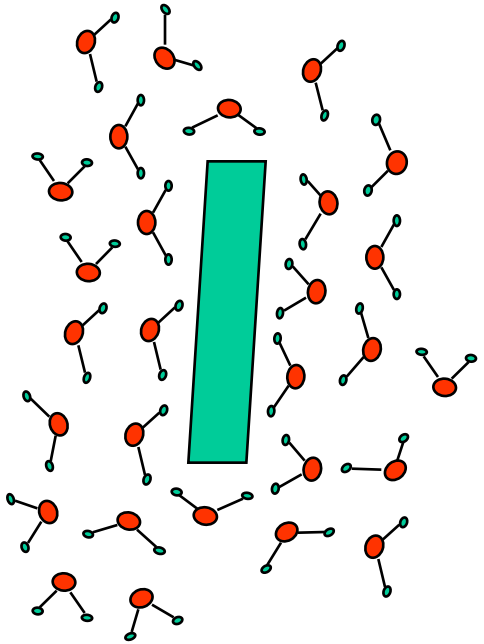
Hydrophobic Bonding : Δ Entropy



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.

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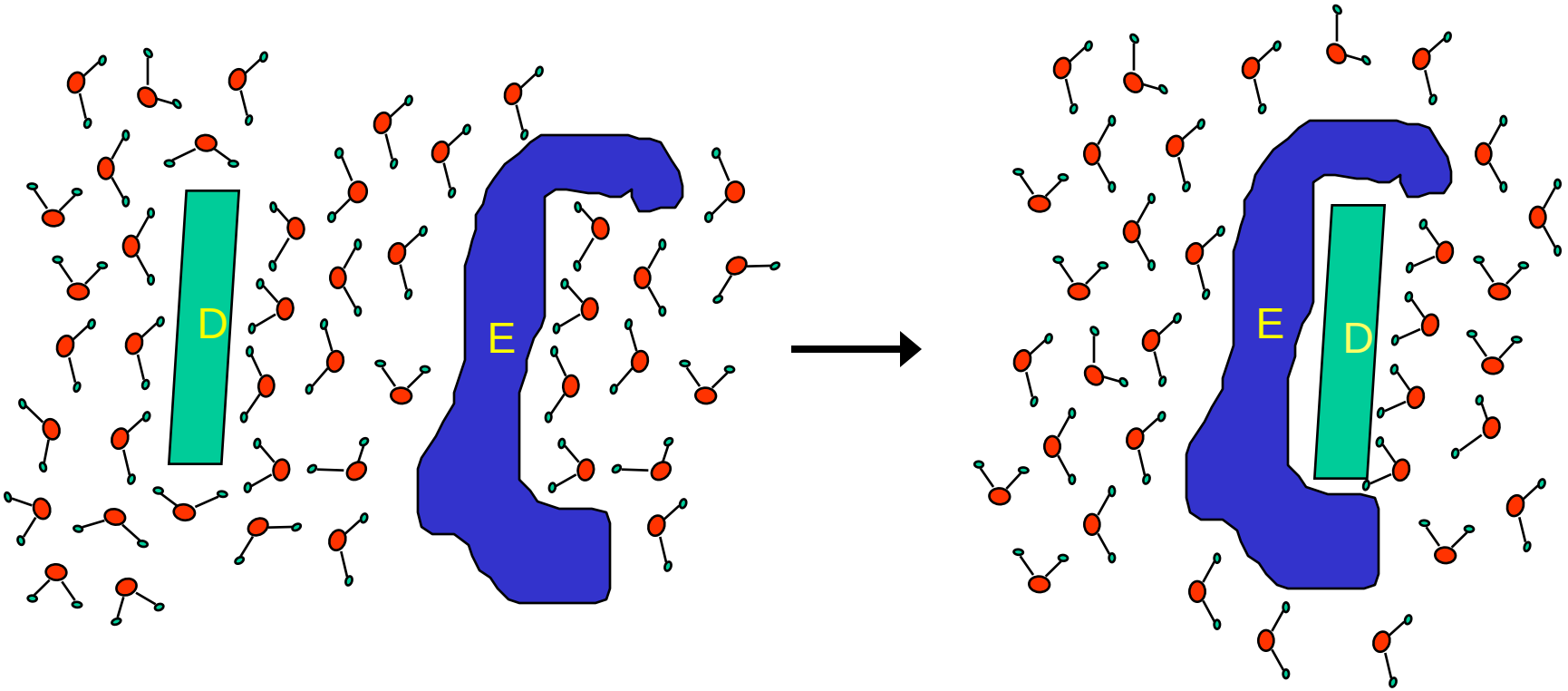
Hydrophobic Bonding : Δ Entropy



- **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)**

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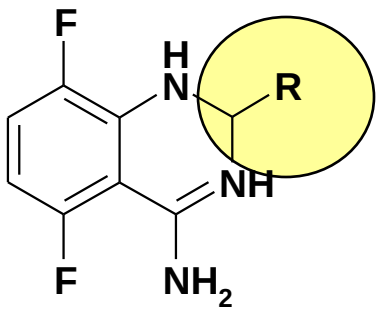
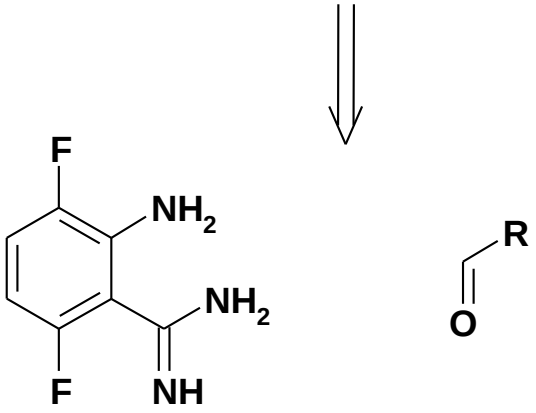
Probing Hydrophobicity in Drug Discovery



New iNOS lead identified: R =Me, small lipophilic substituent iNOS pIC_{50} 7.8

Aim: Probe lipophilic pocket – what else could we put there?

How would we make it?



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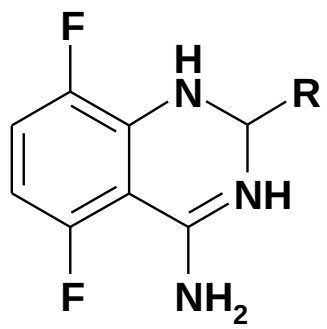
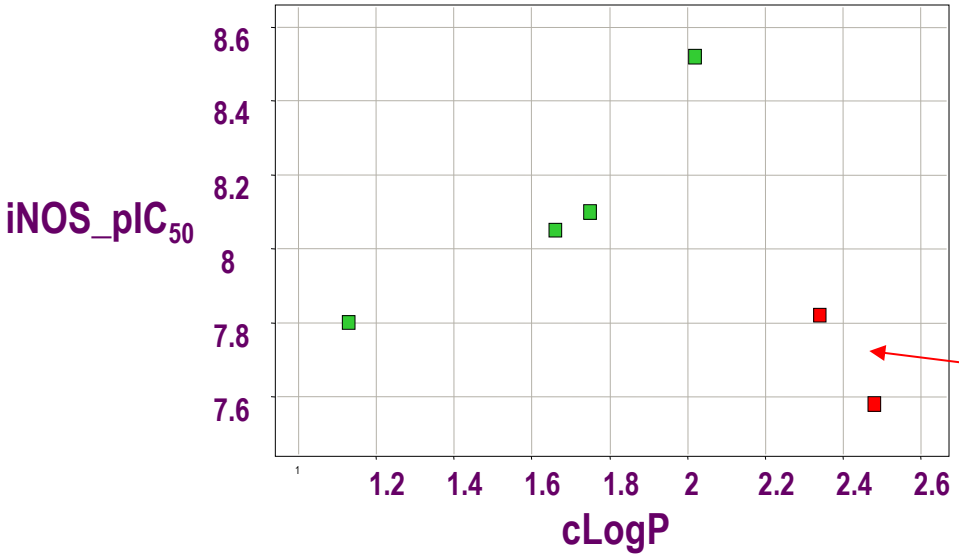
Effect of Hydrophobicity on Activity

Binding into Lipophilic pocket of iNOS



R	cLogP	IC_{50} μM
Me	1.13	0.016
Et	1.66	0.009
CF_3	1.75	0.008
Thiophene	2.02	0.003
Phenyl	2.34	0.015
2-Me-Thiophene	2.48	0.026



Too big to fit in pocket optimally
(Shape complementarity)

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Bioisosteres

Isostere:

Similarities in physicochemical props. of atoms/groups/molecules with similar electronic structures (no. and arrangement of electrons in outermost shell). Often observed with groups in the same periodic table column (Cl → Br, C → Si).

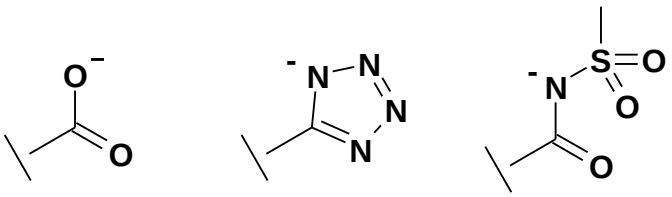
Grimm – Hydride Displacement Law (1925) - Replacement of chemical groups by shifting one column to the right & adding H.

C	N	O	F	Ne	Na^+
	CH	NH	OH	FH	
		CH_2	NH_2	OH_2	FH_2^+
			CH_3	NH_3	NH_4+

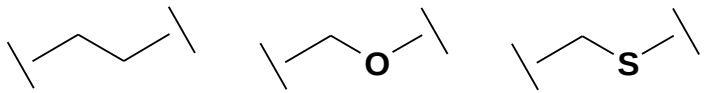
Bioisostere:

Simplest definition - any group replacement which improves the molecule in some way
Two different interchangeable functionalities which retain biological activity.

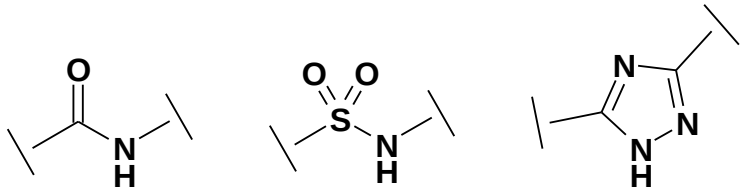
Bioisosteric replacements can offer improvements both in potency and other properties (e.g. metabolic stability, absorption)



Carboxylic acid & bioisosteres



-CH_2 & bioisosteres



amide & bioisosteres

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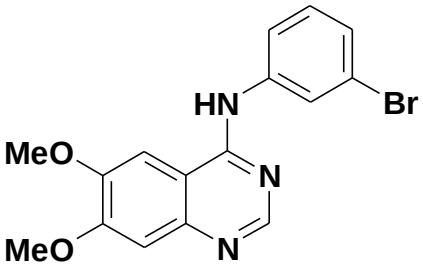
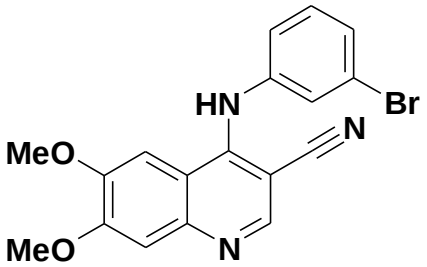
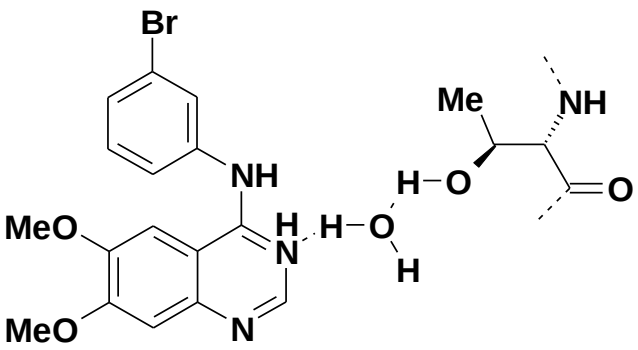
Invisible Bioisosteres

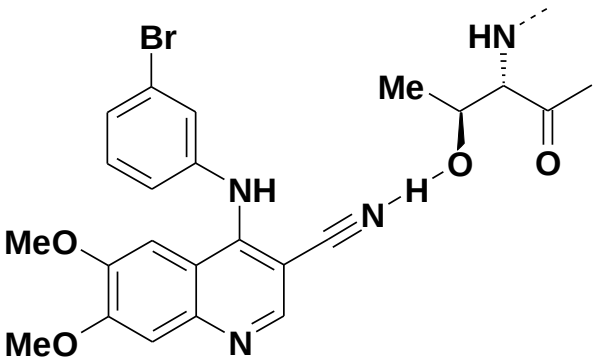


EGF-R 2.2 nM



EGF-R 7.5 nM





H-bonds can be directly to protein or via water molecules

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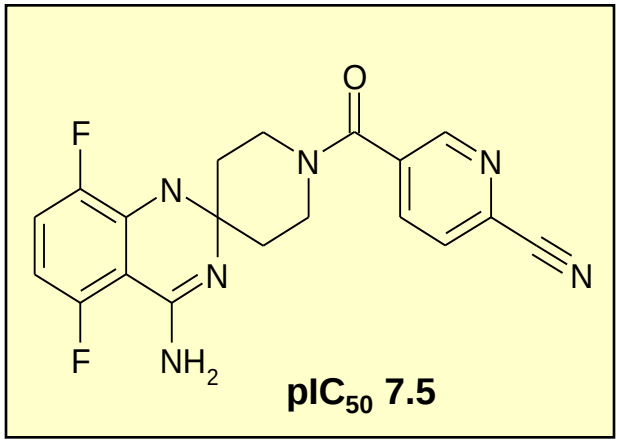
Optimising Potency



pIC_{50} 7.5

How might we improve potency further from this compound?

Develop understanding of which molecular features are important for activity – remove substituents.

Look at incorporating new groups for additional potency e.g. through lipophilic interactions, hydrogen bonds etc.

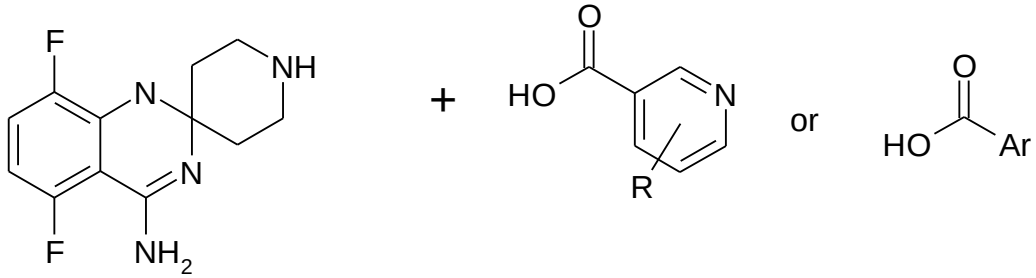
Functional group bioisosteres.

Use available structural information – e.g. crystal structures of compound bound to enzyme.

Use of modelling to design/evaluate new targets.

Develop and test hypotheses.

Identify good disconnections/robust chemistry to allow rapid synthesis of multiple analogues – build up information.



N.B. Potency is one of many properties that needs to be optimised in drug discovery - need to consider absorption, metabolism, selectivity etc.

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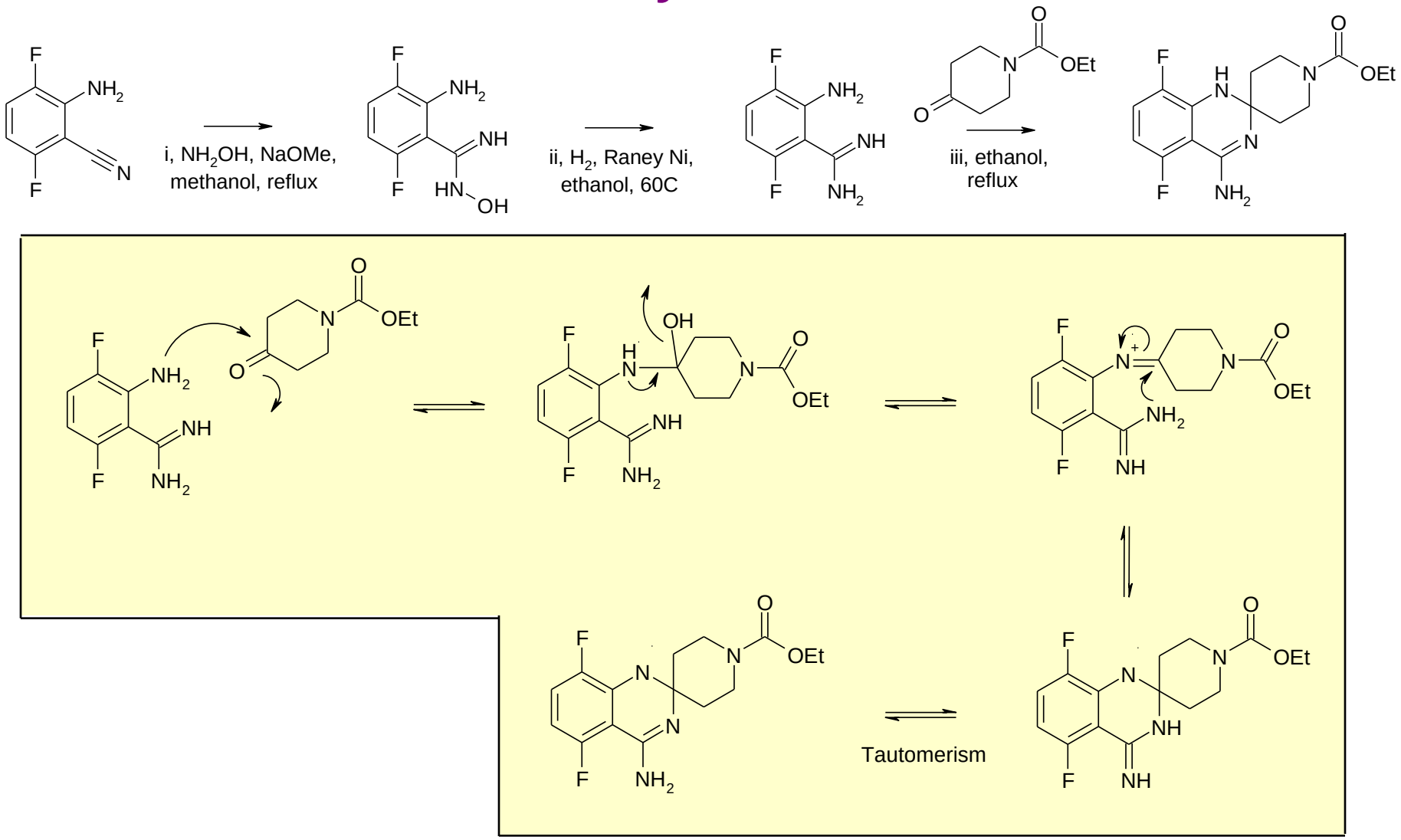
Forward Synthesis - 1



Forward Synthesis - 2