Rational Drug Design lecture 12

Łukasz Berlicki

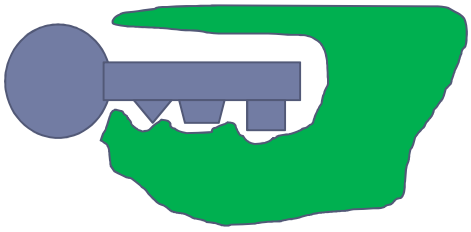
Computer-aided design

- **Ligand-based design** – QSAR and 3D-QSAR

- **Structure-based design** – design based on the structure of molecular target

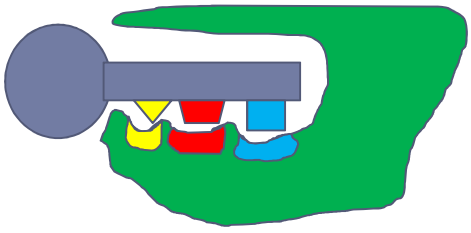
Key - Lock

- The shape of the ligand must match the molecular target like the key to the lock - **steric effects**.



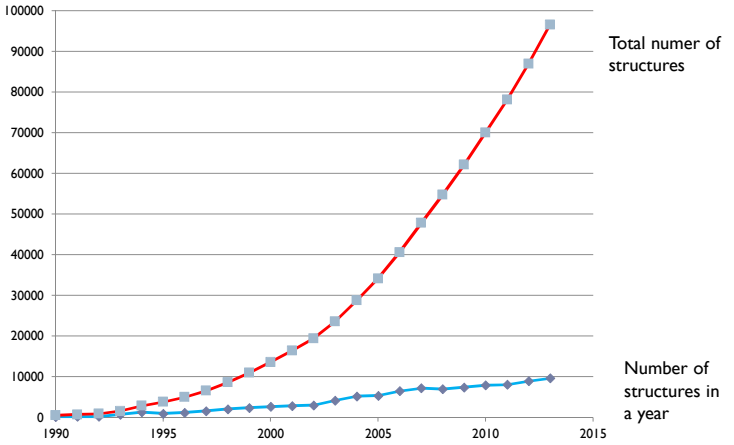
Key - Lock

- The shape of the ligand must match the molecular target like the key to the lock - **steric effects**.
- Ligand must form a network of interactions with the molecular target - **electron effects**.



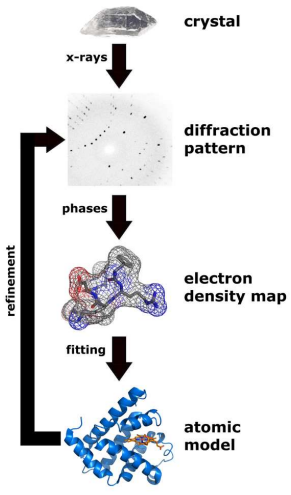
Structures of macromolecules

- **Protein Data Bank** (rcsb.org) – a database that collects macromolecular structures



Protein crystallography

- Protein preparation and purification
- Crystallization
- Collection of diffraction data
- Obtaining an electron density map
- Model matching

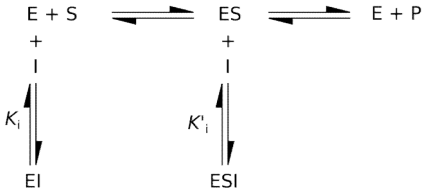


Inhibition Constant

- The inhibition constant is a measure proportional to the binding energy of the ligand to the protein

$$\Delta G = \Delta H - T\Delta S = RT \ln K_i$$

- ΔG – Gibbs energy
- ΔH – entalphy
- ΔS – entropy

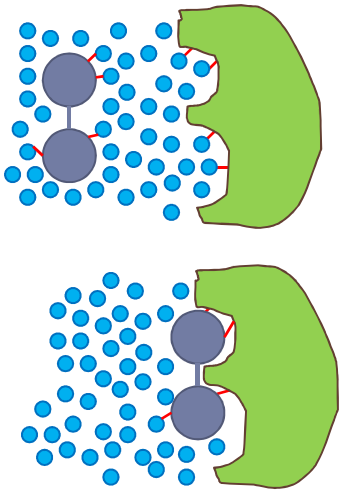


- K_i in range 10^{-6} - 10^{-12} M, what is related to

-4 - -17 kcal/mol

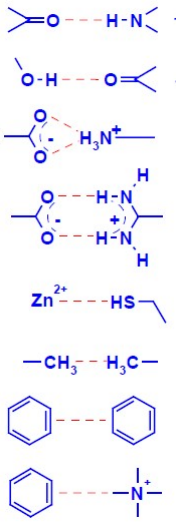
Ligand binding

- An important element of the inhibitor - enzyme balance is a solvent (water), which also interacts with the ligand.
- Ligand-protein interactions reduce enthalpy of the system.
- All broken ligand-water and water-protein interactions should be reconstituted in the ligand-protein complex.
- Binding the ligand through the protein results in a change in entropy.



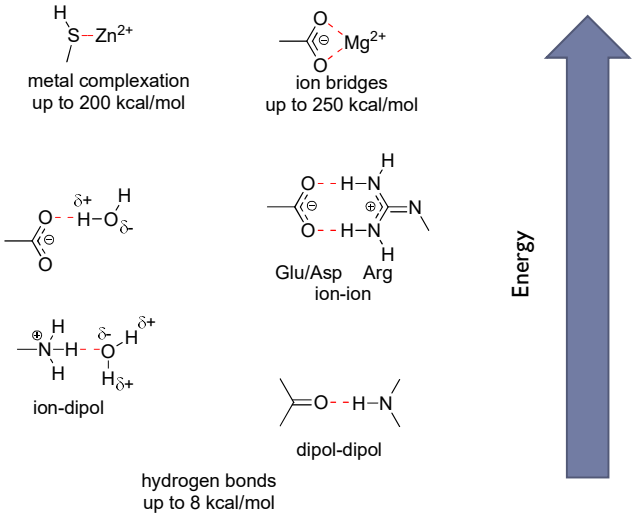
Non-covalent interactions

- Hydrogen bonds
- Ionic bonds
- Complexing of metal ions
- Hydrophobic interactions
- Interactions of π-cation



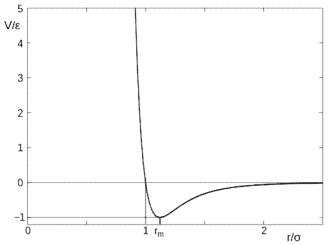
Electrostatic bonds - strong and medium

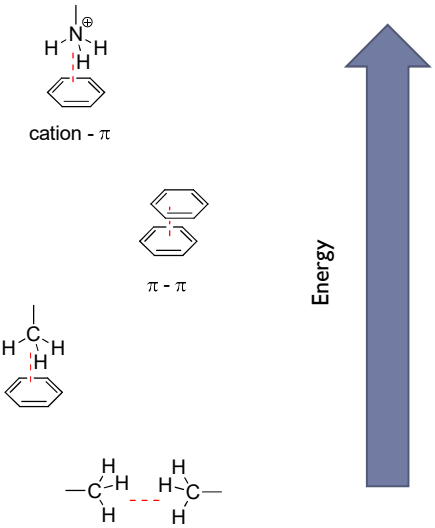
$$E = \frac{q_1 \; q_2}{\varepsilon_r \; r}$$



Hydrophobic interactions, van der Waalsa

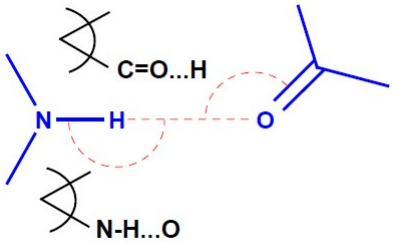
- Hydrophobic interactions are described by Lennard-Jones potential
- $V_{LJ} = 4\varepsilon\left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right]$





Hydrogen bonds

- Hydrogen bonding is one of the most important ligand-protein interactions.
- Hydrogen bonding energy strongly depends on its geometry (distances and angles).
- The binding energy is inversely proportional to its length

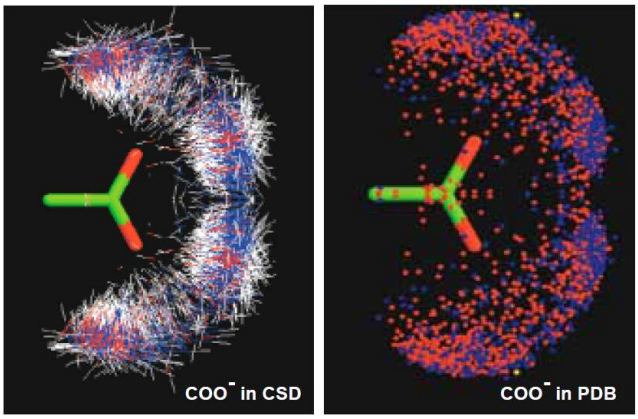


Distance N-H…O	**2.8-3.2 Å**
Angle N-H…O	**150-180°**
Angle C=O…H	**100-180°**

movie

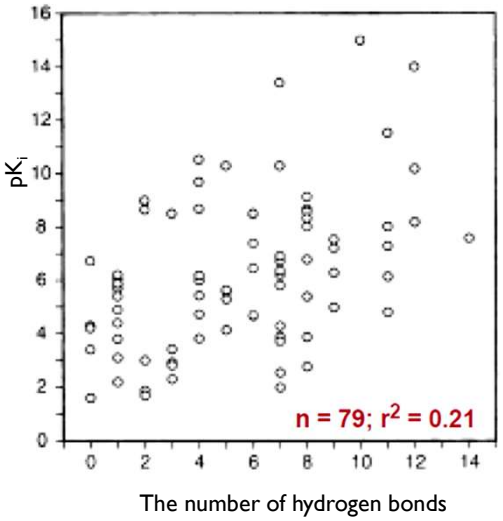
Hydrogen bonds

- Arranging the hydrogen bond donors around the carboxyl group indicates preferences in its geometry.



Hydrogen bonds

- Hydrogen bonds are a key element of the ligand-protein interactions and strongly influence the inhibition constant

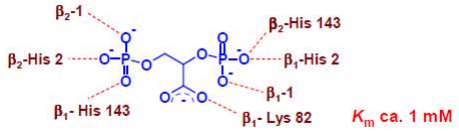


Hydrogen bonds

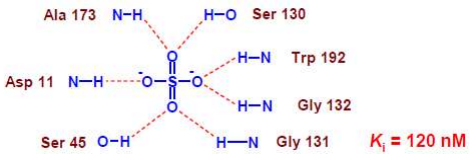
- Neutral Hydrogen bonds: 2-6 kJ / mol, increase ligand binding 2-15 fold
- Ionic hydrogen bonds: up to 20 kJ / mol, increase ligand binding to 3,000 times

Hydrogen bonds - examples

- Binding of 2,3-phosphoglyceric acid to hemoglobin

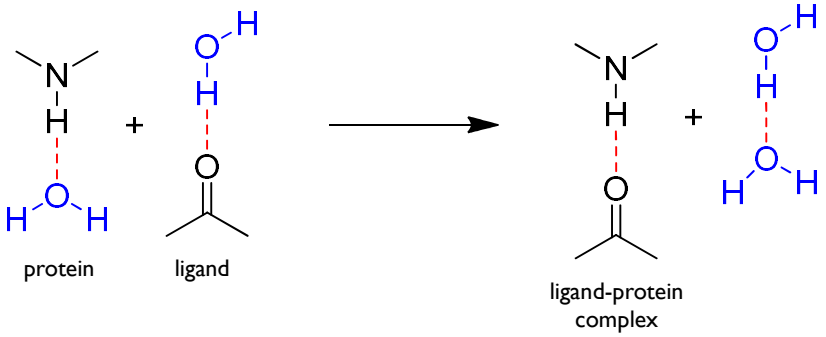


- Binding of sulfate to SBP (*sulfate binding protein*)



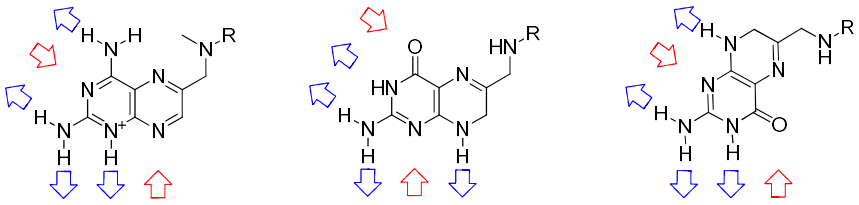
Hydrogen bonds

- All donors and acceptors in the aqueous solution form hydrogen bonds with the solvent



Hydrogen bond - example

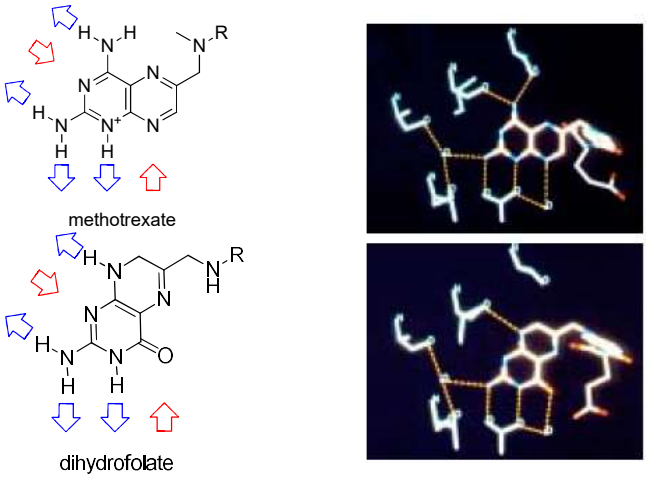
- Binding of ligands to dihydrofolate reductase



- The hydrogen bonding scheme indicates that dihydrofolate binds to the enzyme in a different conformation to methotrexate.

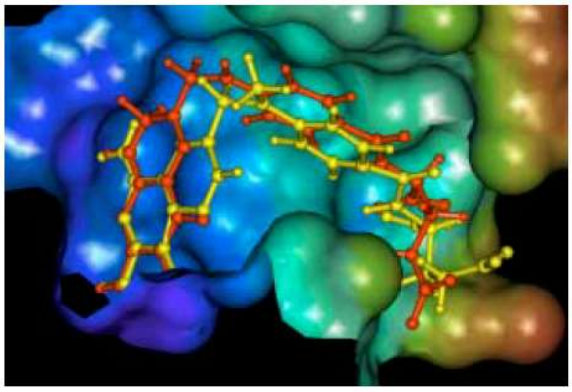
Hydrogen bond - example

- Binding of ligands to dihydrofolate reductase



Hydrogen bond - example

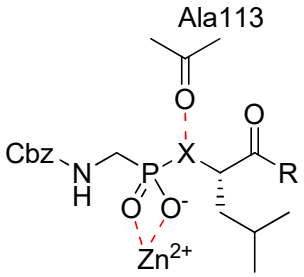
- Binding of ligands to dihydrofolate reductase



Hydrogen bond

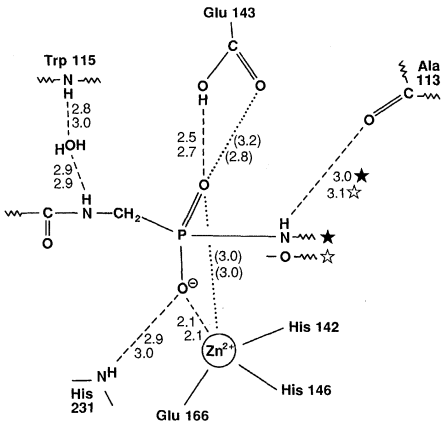
- Phosphonic thermolysin inhibitors

R	-NH-	-O-	$-CH_2-$
-OH	0.76	660	1.4
-Gly-OH	0.27	230	0.3
-Leu-OH	0.01	9	0.01



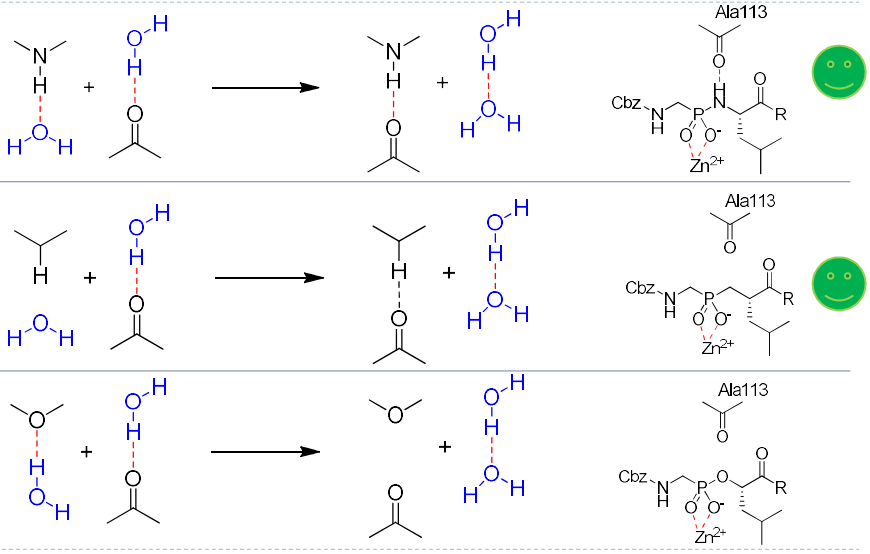
Hydrogen bond

- Phosphonic thermolysin inhibitors



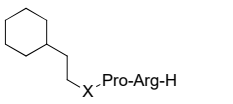
Struktura krystaliczna

Hydrogen bond

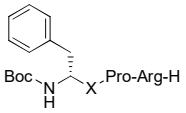


Hydrogen bond

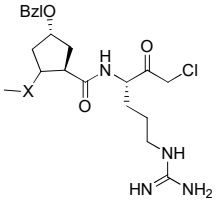
- Examples of binding differences for C=O and CH_2



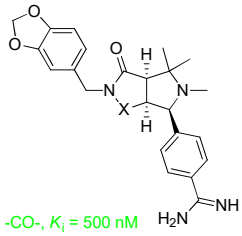
X = -CO-, IC_{50} = 150 nM
X = -CH_2-, IC_{50} = 56 000 nM



X = -CO-, IC_{50} = 28 ng/ml
X = -CH_2-, IC_{50} = 52 000 ng/ml



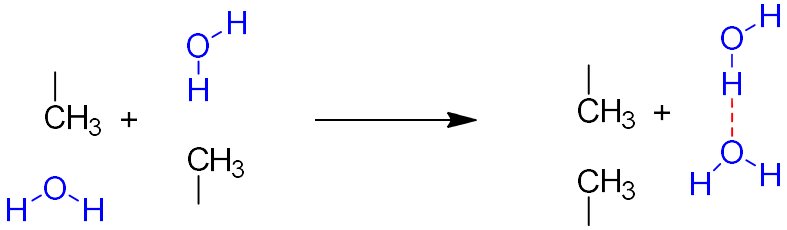
X = -CO-, IC_{50} = 0.9 nM
X = -CH_2-, IC_{50} = 8 600 nM



X = -CO-, K_i = 500 nM
X = -CH_2-, K_i = 2 000 nM

Lipophilicity

- The formation of hydrogen bonds in the solvent is an important element of the hydrophobic effect that enhances hydrophobic interactions (van der Waals, π–π, cation-π)



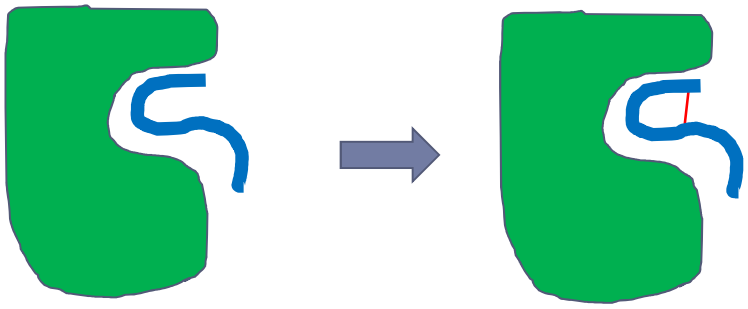
Entropy

- Ligand binding causes a decrease in its entropy due to:
  - Decreasing the freedom of translational movements (x, y, z)
  - Reduce the freedom of rotation (around individual bonds). Each single bond (between non-hydrogen atoms) in the ligand reduces binding energy by about 1.0 kcal / mol

- It is advantageous to design compounds with low conformational freedom (rigid).

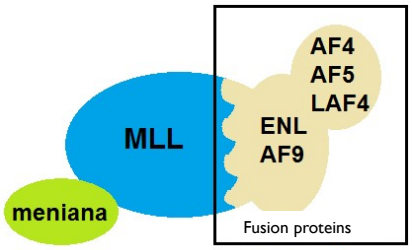
Reducing the conformational freedom

- If the conformation of the ligand in the active site is known, it is preferred to design compounds with a stiffened structure and an analogous conformation.



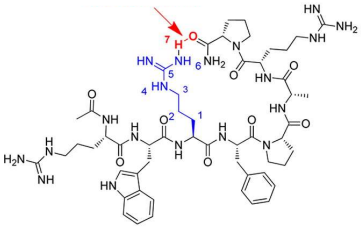
Menin-MLL

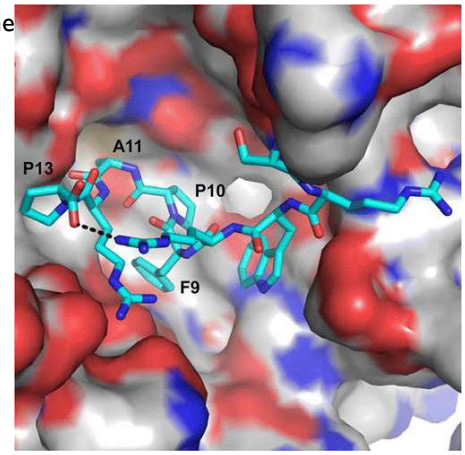
- Menin is a tumor suppressor protein
- Menin interacts specifically with the MLL protein
- Blocking the effects of menin-MLL reduces the oncogenic properties of the fusion proteins interacting with MLL
- Inhibitors of menin-MLL interaction are potential medicines for acute leukemia.



Menin-MLL

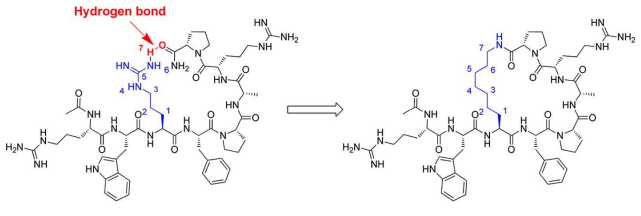
- The crystal structure of the MLL protein fragment with menin shows the active conformation of this peptide.
- The C-terminal residue interacts with the arginine side chain





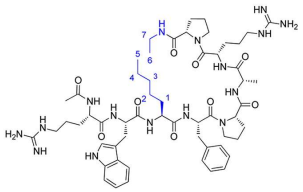
Menin-MLL

- Stiffening of the peptide by cyclization



K_i = 71 nM

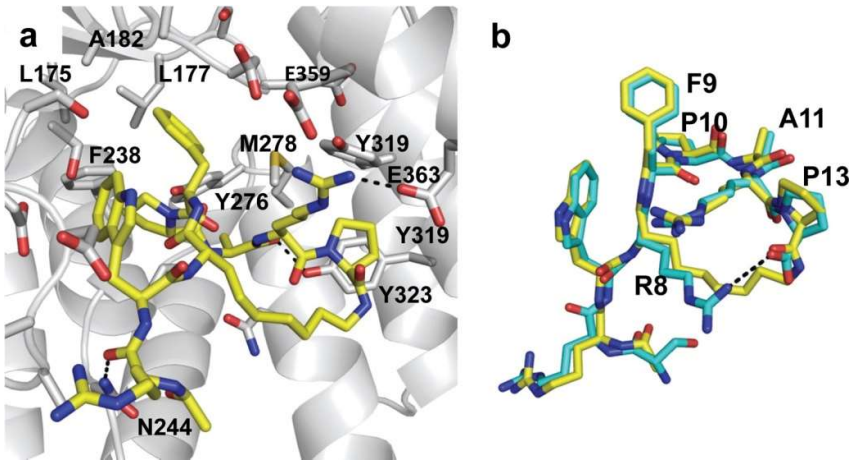
K_i = 24 nM



K_i = 2170 nM

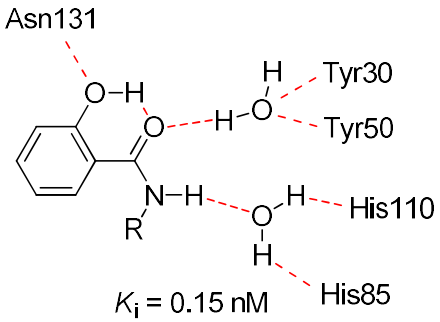
Menin-MLL

- Stiffening of the peptide by cyclization



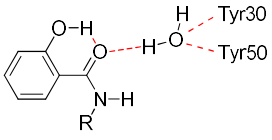
Water molecule in the active site

- Introduction to the compound of the functional group, which replaces the water molecule in the active site, results in a beneficial **enthalpy effect** (increased interaction energy) and **entropy**.

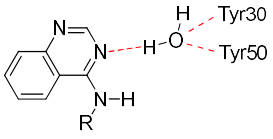


Water molecule in the active site

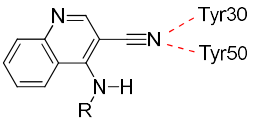
- If a newly introduced functional group reproduces the effects of the replaced water molecule, we will obtain a compound with higher activity.



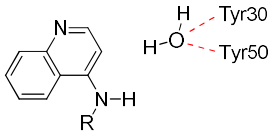
K_i = 0.15 nM



K_i = 0.14 nM



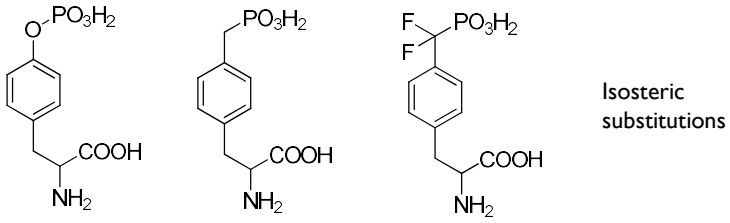
K_i = 0.0066 nM



K_i = 200 nM

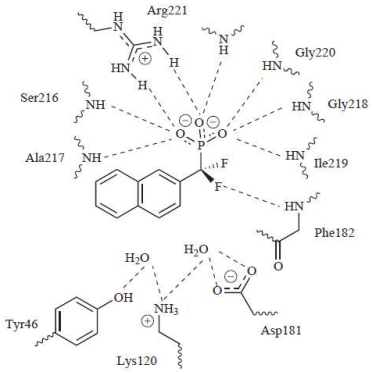
PTP (*protein tyrosine phosphatase*)

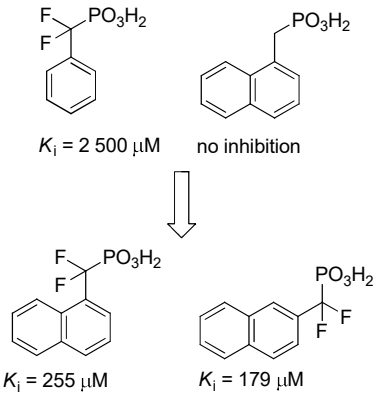
- Protein tyrosine phosphatase (PTP) - an enzyme that controls the signaling by dephosphorylation of protein tyrosyl residues.
- PTP 1B inhibitors can be effective drugs of type 2 diabetes and obesity.



PTP (*protein tyrosine phosphatase*)

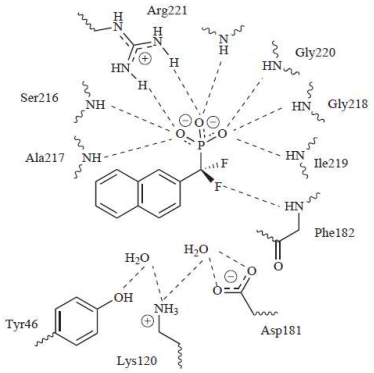
- The phosphonic group is bound by 8 hydrogen bonds
- Hydrogen bonding also forms one of the fluorine atoms

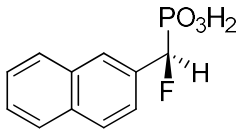




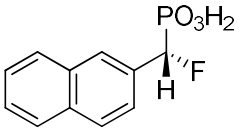
PTP (*protein tyrosine phosphatase*)

- The phosphonic group is bound by 8 hydrogen bonds
- Hydrogen bonding also forms one of the fluorine atoms





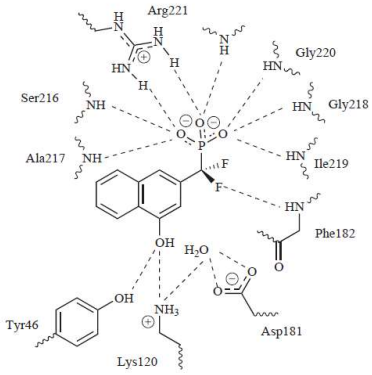
IC_{50} = 675 μM

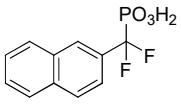


IC_{50} = 7 500 μM

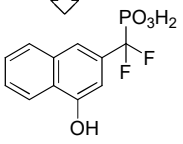
PTP (*protein tyrosine phosphatase*)

- Replacing one molecule of water bound in the active site by the -OH group of the inhibitor increases its activity.





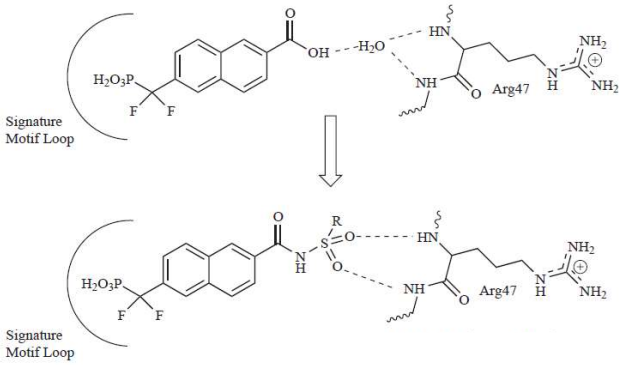
K_i = 179 μM



K_i = 93 μM

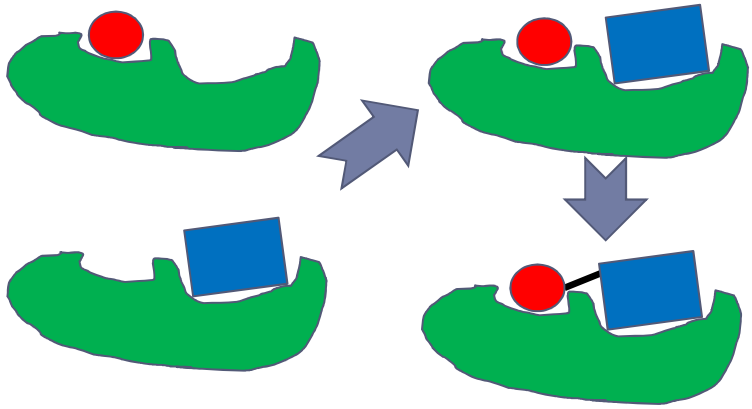
PTP (*protein tyrosine phosphatase*)

- Replacing the water molecule



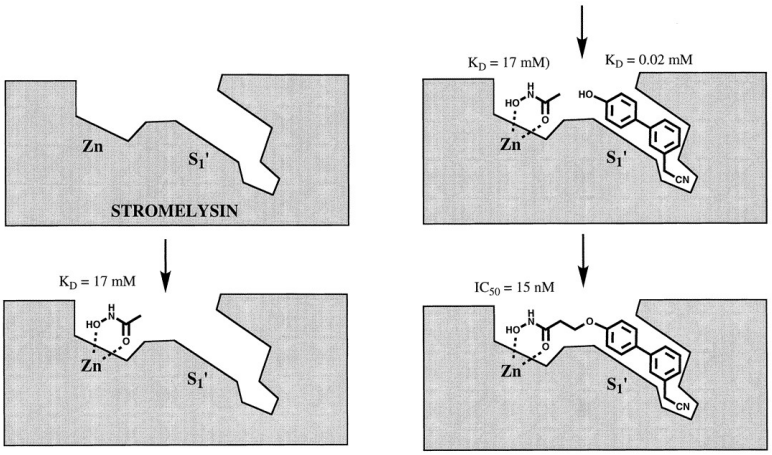
Fragment-based drug design

- *Fragment-based drug design* – joining fragments that interact with different parts of the active site leads to compounds with high activity.



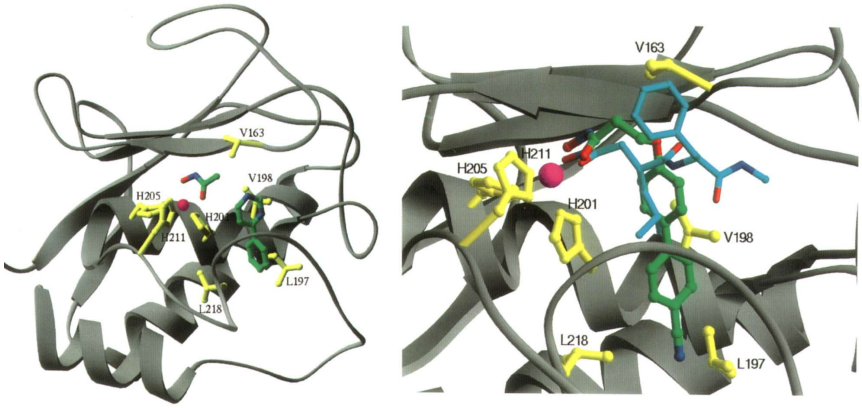
Stromelysin inhibitors

- Stromelysin is a metal-dependent protease with a Zn2 + ion in the active site



Stromelysin inhibitors

- The structure of the complex with 2 fragments (on the left) and a new inhibitor (on the right)



Summary

- Knowledge of the molecular target structure significantly increases the efficiency of ligand design.
- Structural and electronic matching is necessary.
- The creation of a specific set of protein-inhibitor interactions gives a chance to obtain a highly active and specific inhibitor.