Properties of Drugs

What makes a chemical compound acting as pharmaceutically active agent ?

- high affinity towards the target:

High binding constant (the drug should bind to the enzyme in concentrations as low as micro to nano molar)

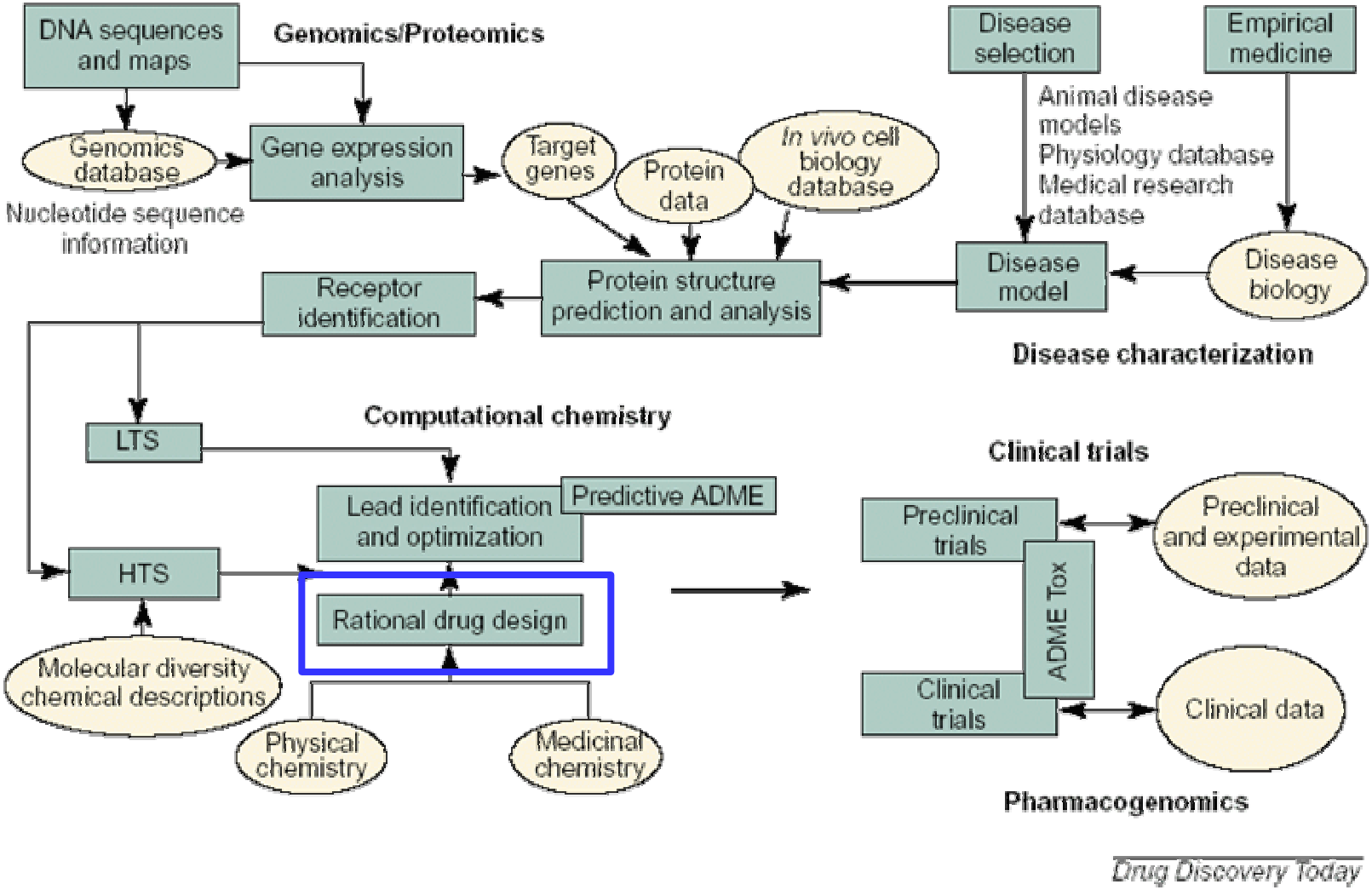
- selectivity with respect to the target:

The drugs should bind preferably to the target and not to other enzymes

- high bioavailability und low toxicity:

Sufficient concentration in the body and a broad therapeutic range (dosage) along a minimum of adverse side effects

Flow of information in a drug discovery pipeline



Rational drug design

Basic principles:

- Improving the affinity
- Improving the selectivity } specificity
- Improving the bioavailability } allows lower dosage
- Reducing toxicity and adverse side effects

Frequently only possible by testing on animals and clinical trials

What are rational strategies? → create and test similar compounds

- systematic modification of the lead structure
- High Throughput Screening
- Combinatorial Synthesis
- bioisosteric exchange → lecture 4

Lit: H.Gohlke & G.Klebe, *Angew.Chem.* **114** (2002) 2764.

Improving Specificity (I)

How to increase the affinity of a molecule to its receptor ?

binding constant K_i (association of the complex)

$$K_i = \frac{[\text{ligand}]\cdot[\text{enzyme}]}{[\text{ligand-enzyme complex}]} \cdot \frac{[\text{mol/l}]\ [\text{mol/l}]}{[\text{mol/l}]}$$

dimension: K_i [mol/l = molar] ; e.g. $K_i = 10^{-9}$ M = 1 nM

The binding constant is associated with the change in free energy upon binding: $-RT \ln K = \Delta G = \Delta H - T\Delta S$

suitable values of K_i are in the range of 10^{-6} to 10^{-12} *M*
(micro to pico molar range).

this confers to values for ΔG of –4 to –17 kcal / mol

Improving Specificity (II)

The binding constant K_i can be determined experimentally by microcalorimetric measurements, such as isothermal titration calorimetry (ITC).

More often IC_{50} values are reported, which can be determined more easily.

IC_{50} : added amount or concentration of the ligand that produces an effect of 50%. e.g. reduces the enzymatic activity by 50%.

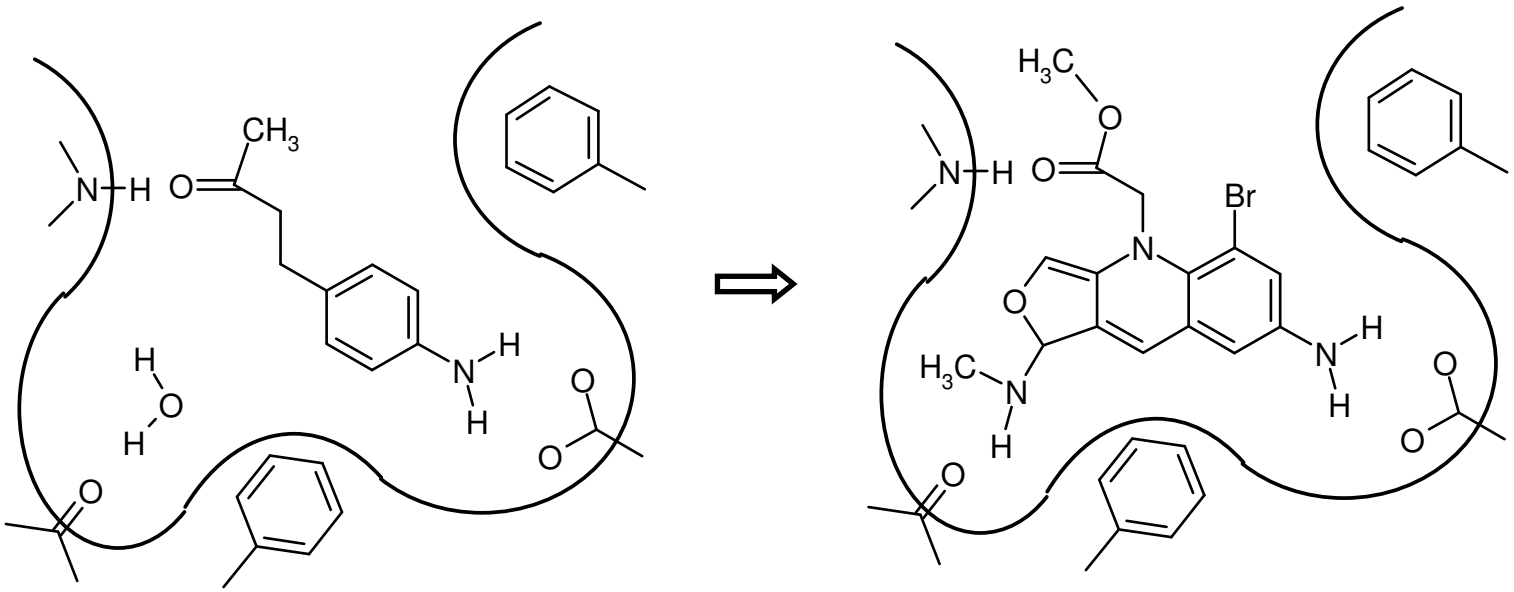
Testing of the enzymatic assay with different concentrations of the ligand and interpolation to 50%.

Improving Specificity (III)

How to increase the affinity of a ligand to its receptor ?

Energy of binding ΔH must become more negative

The energetic interactions between ligand and receptor have to become more favorable



Improving Specificity (IV)

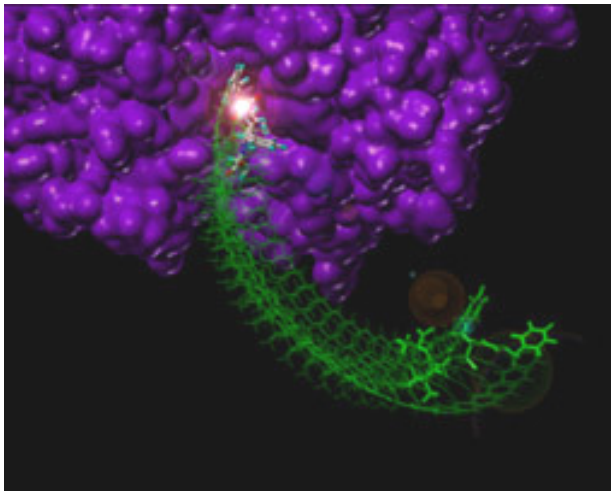
The energy terms can be calculated according to force fields

$$E = E_{stretch} + E_{bend} + E_{tors} + E_{vdW} + E_{ES}$$

$$= \sum_{bonds\,(ij)} \frac{k^{(ij)}}{2}\left(r_{ij} - r_0^{(ij)}\right)^2 + \sum_{angles\,(ijk)} \frac{k^{(ijk)}}{2}\left(\phi_{ij} - \phi_0^{(ijk)}\right)^2$$

$$+ \sum_{torsions\,(ijkl)} \frac{k^{(ijkl)}}{2}\left(1 + \cos(n^{(ijkl)}\tau - \tau_0^{(ijkl)}\right)^2$$

$$+ \sum_{pairs\,(ij)} \left(\frac{A_{(ij)}}{r_{ij}^{12}} - \frac{B_{(ij)}}{r_{ij}^{6}} \right) + \frac{1}{4\pi\varepsilon\varepsilon_0} \sum_{pairs\,(ij)} \frac{q_i q_j}{r_{ij}}$$



Most docking program apply this concept.

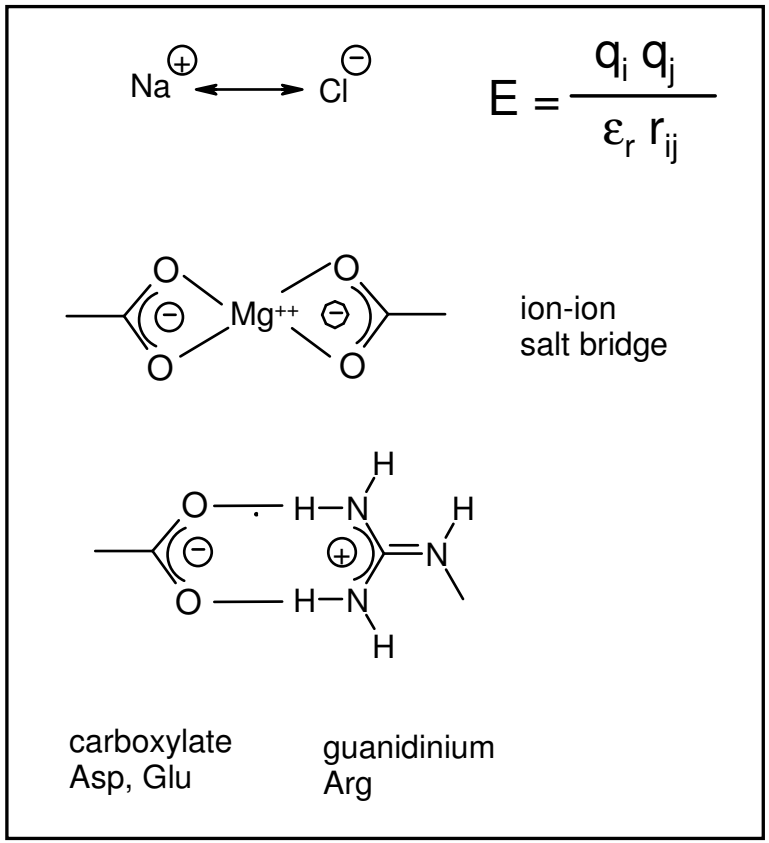
Furthermore, a high resolution X-ray structure or an appropriate homology model of the target are necessary.

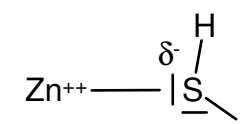
Enzyme-Ligand Interactions (I)

Which do exist ?	range in energy upto:
electrostatic interactions: salt bridges	≈250 kcal/mol
coordinative binding of metals (complexes)	≈200 kcal/mol
hydrogen bonds: also to charged groups	1-8 kcal/mol (neutral)
van der Waals interactions	0.5 kcal/mol (per atom pair)

Enzyme-Ligand Interactions (II)

Strong and medium electrostatic interactions (static)

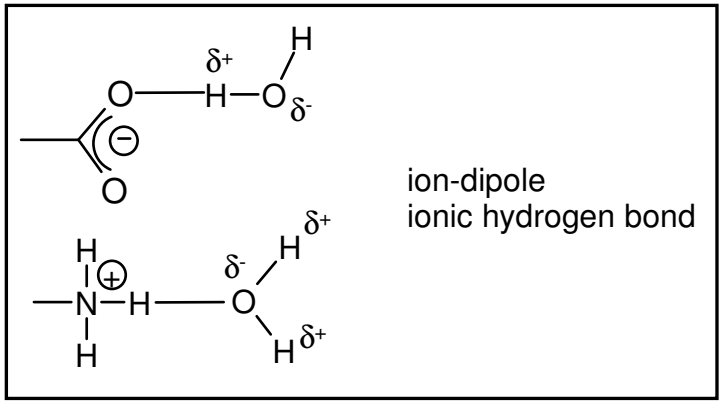


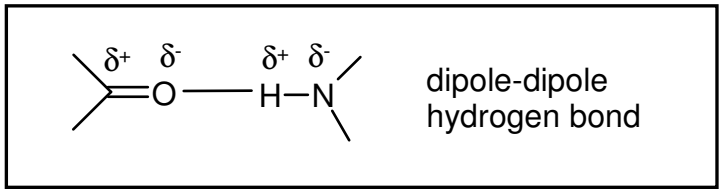


ion-dipole interaction

coordinative binding

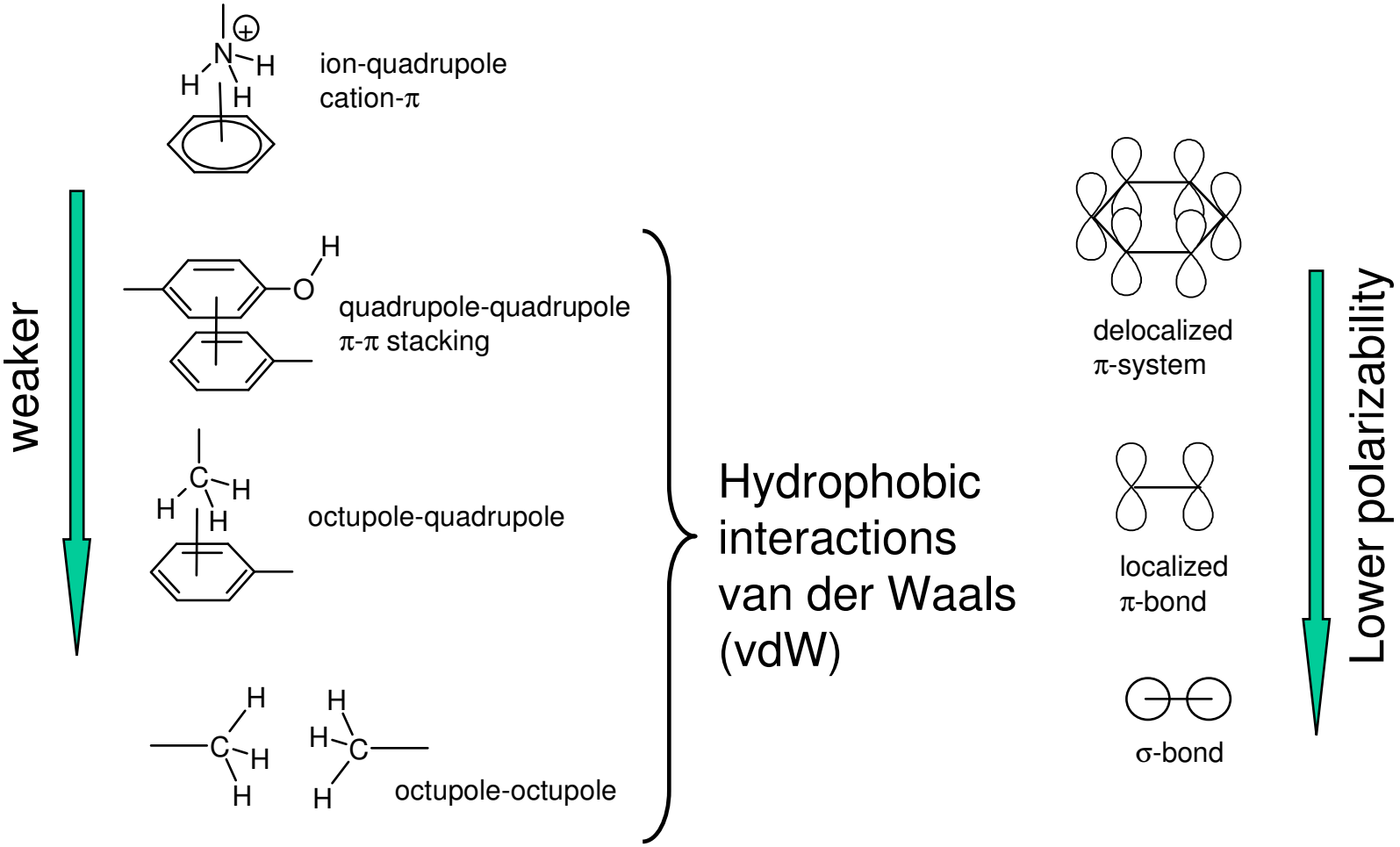
MO interaction
almost covalent binding





Enzyme-Ligand Interactions (III)

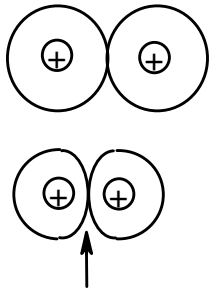
weak electrostatic interactions (induced, dynamic)



Enzyme-Ligand Interactions (IV)

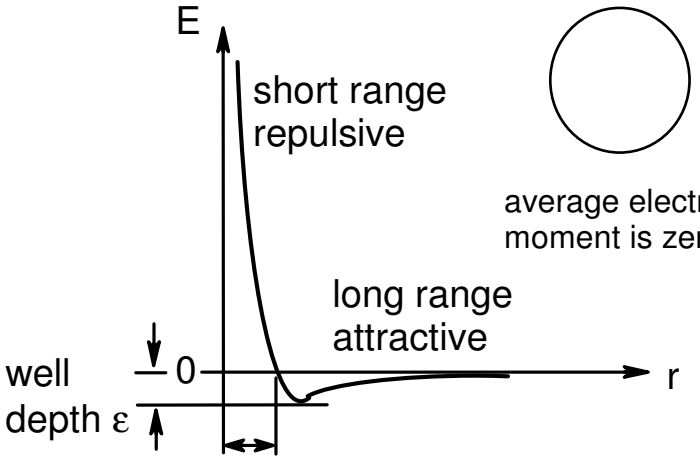
Dispersive interactions: London forces and van der Waals

The attractive force is due to instantaneous dipols which arise from fluctuations in the electron clouds. These induce mutual dipole moments.

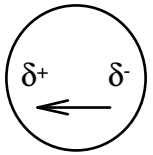


decreased electron density
repulsion of atomic cores

$$E = \frac{q_i \, q_j}{\varepsilon_r \, r_{ij}}$$



average electric moment is zero



dynamic behaviour of the electrons causes instantanous dipole moment

$$E_{London} = \frac{-C_6}{r^6} + \ldots$$

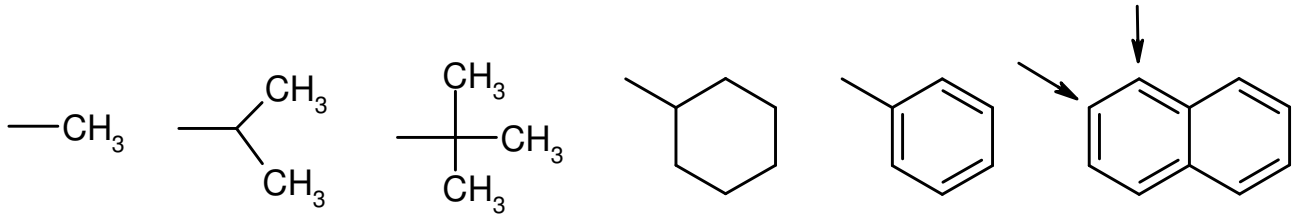
Lennard-Jones potential

$$E_{vdW} = 4\varepsilon\left(\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6}\right)$$

Enzyme-Ligand Interactions (V)

Hydrophobic Interactions are characterized by the absence of polar hydrogens and low differences in electronegativity between the atoms.

Examples of non-polar groups:



methyl
Me

isopropyl
ipr

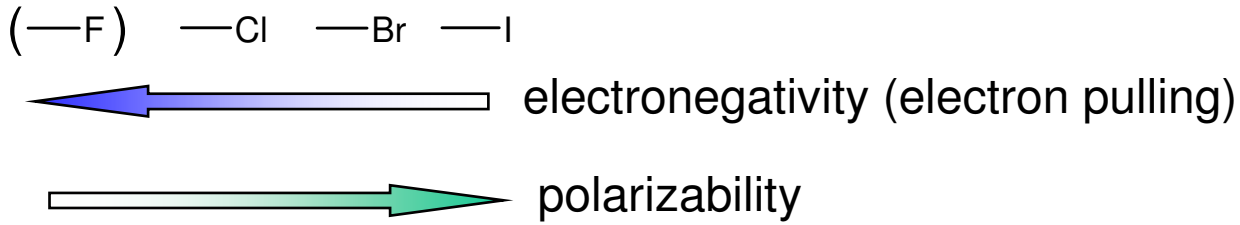
tert-butyl
t-butyl

cyclohexyl

phenyl

naphthyl

Examples of non-polar substituents:



Electronegativity (EN)

The EN is a measure of the ability of an atom (or group) to attract electrons in the context of a chemical bond.

Concepts and definitions (not comprehensive !)

R.S. Mulliken: $$EN = \frac{E_{Ionization} + E_{ElectronAffinity}}{2}$$

L. Pauling: using the bond dissociation energies D of diatomic molecules containing the elements A and B

$$D_{AB} - \sqrt{D_{AA} - D_{BB}} = 96.48 \frac{kJ}{mol} \cdot (EN_A - EN_B)^2$$

Element	H	C	N	O	F	Cl	Br	I	Si	P	S
Mulliken	2.2	2.5	2.9	3.5	3.9	3.3	2.7	2.2	1.7	2.1	2.4
Pauling	2.2	2.5	3.0	3.4	4.0	3.2	3.0	2.7	1.9	2.2	2.6

Improving Specifity (V)

Favorable intermolecular interactions lower the energy:
Many side chains of amino acids can change their protonation state, depending on the local environment and pH ! (which ones?)

- hydrogen bonds 1-8 kcal mol^{-1} (average ≈3 kcal mol^{-1})

electrostatic interactions

- salt bridges up to 250 kcal mol^{-1}

coordinative binding of metals (complexes)

- van der Waals max. 0.5 kcal mol^{-1} per atom pair

burying of hydrophobic fragments

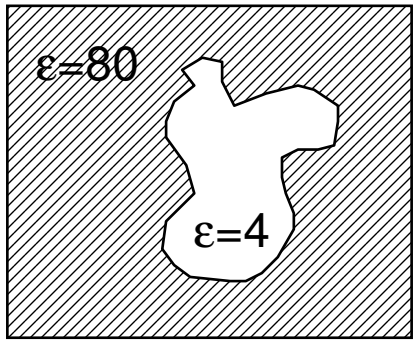
Sign of interaction energies : positiv = repulsive; negative = attractive

Improving Specificity (VI)

enzyme-ligand interactions that are energetically unfavorable upon binding:

Burying of polar or charged fragments (amino acid side chains) up to 7 kcal mol^{-1}. Reason:

Transition from a medium of high dielectric constant (physiological solution ≈78) into an environment of much lower ε
(hydrophobic pocket ε ≈ 2-20)



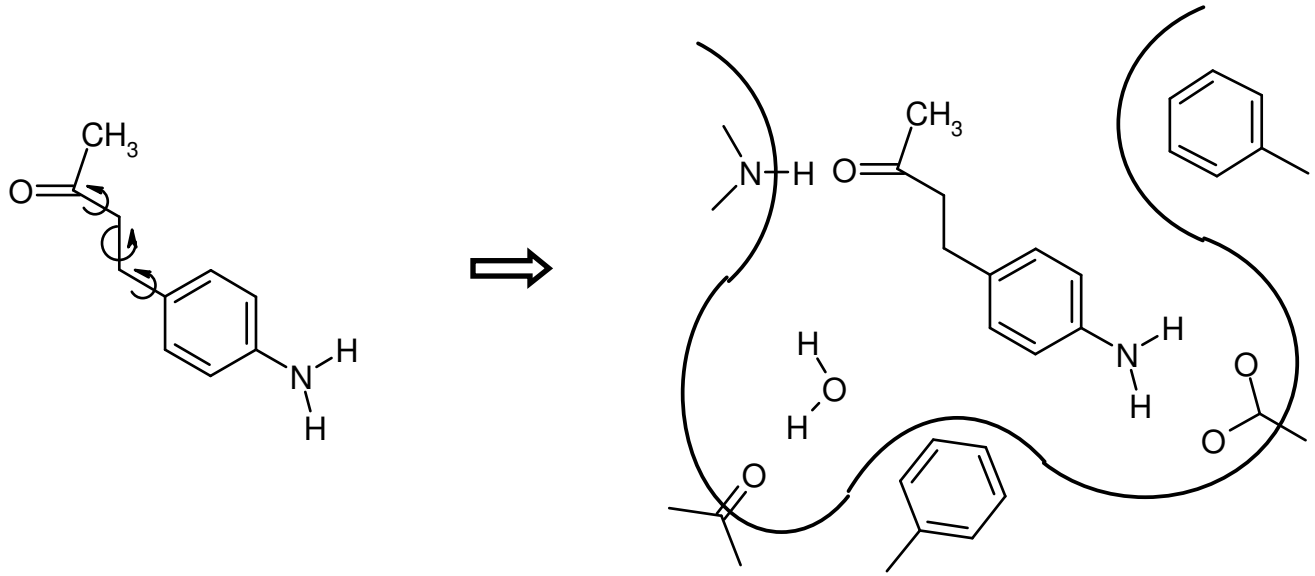
Desolvation:
displacement of water molecules involved in hydrogen-bonds from the binding pocket. Breaking of H-bonds and formation of an empty cavity which allows the ligand to enter.

Improving Specificity (VII)

Entropically (ΔS) unfavorable during binding are :

- Loss of all translational degrees of freedom (x,y,z direction)
- Loss of rotational degrees of freedom

about 1 kcal mol^{-1} per rotatable bond (single bonds) between two non-hydrogen atoms



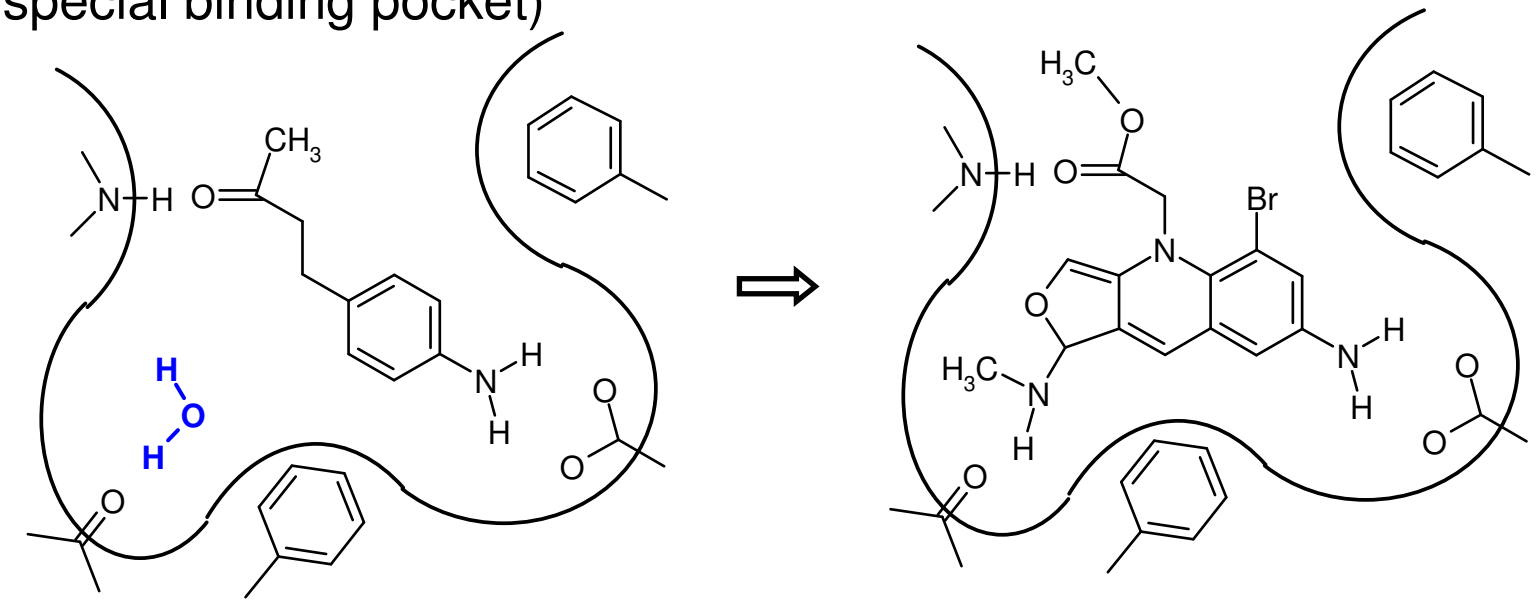
Improving Specificity (VIII)

Entropic (ΔS) considerations:

Displaced water molecules can form usually more hydrogen bonds (with other waters) outside the binding pocket. Likewise the dynamic exchange of H-bonds is simplified in bulk solution.

Thus: The ligand should fit more precisely and thoroughly into the binding pocket.

Simultaneously, the selectivity is improved (ligand fits only in one special binding pocket)



Improving Specificity (IX)

Experience in *rational drug design* shows:

- binding pockets are predominately hydrophobic, so are the ligands
- hydrogen-bonds are important for selectivity
- energy - entropy compensation:

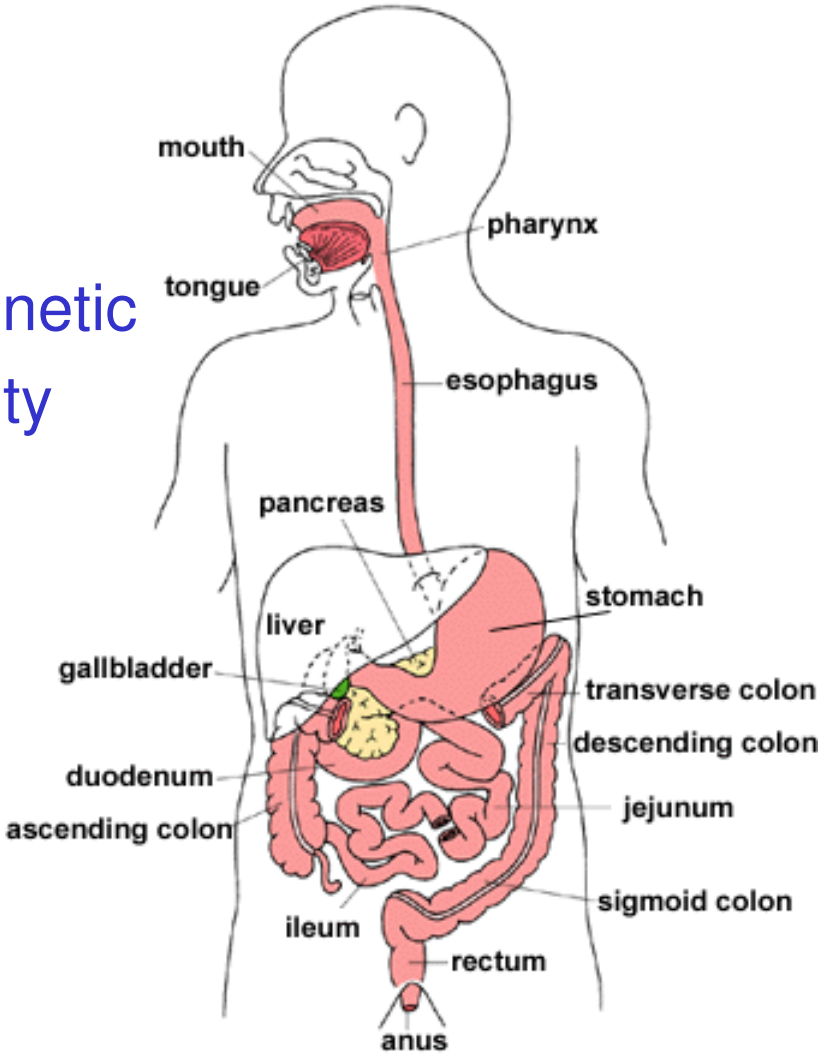
Adding one OH-group to the ligand in order to form an additional H-bond in the binding pocket will lead to displacement of a water molecule, but this water will be solvated in the surrounding bulk water. Thus no additional H-bonding energy is gained.

Therewith, all possibilities of *ligand design* by *docking* are exploited.

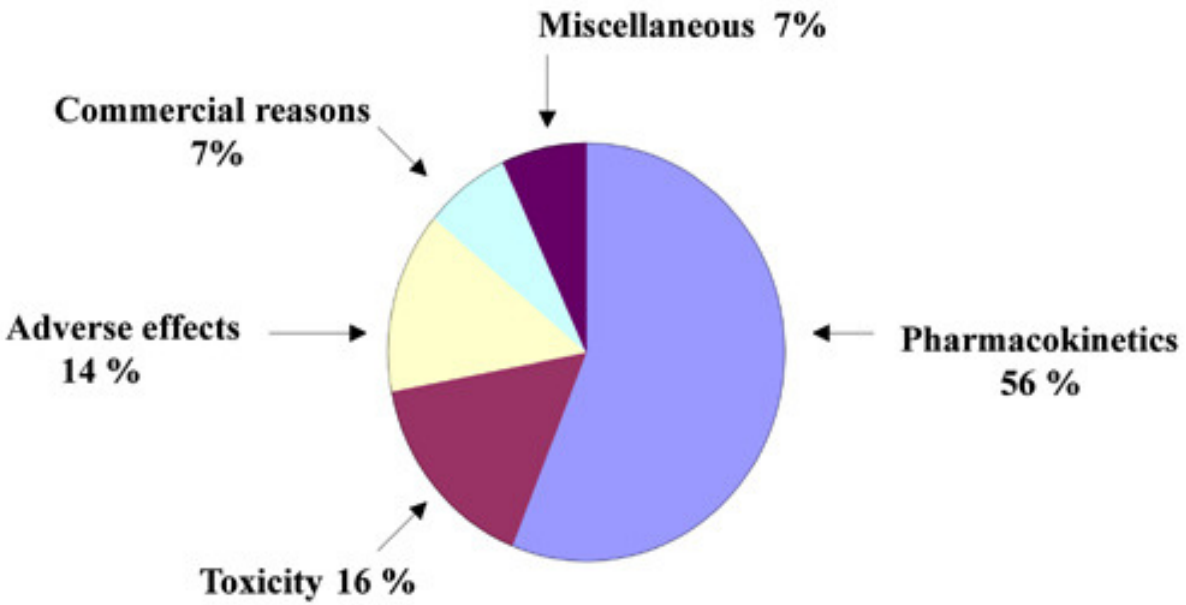
Bioavailablity & ADME prediction

Absorption
Distribution
Metabolism
Elimination

} Pharmacokinetic Bioavailability

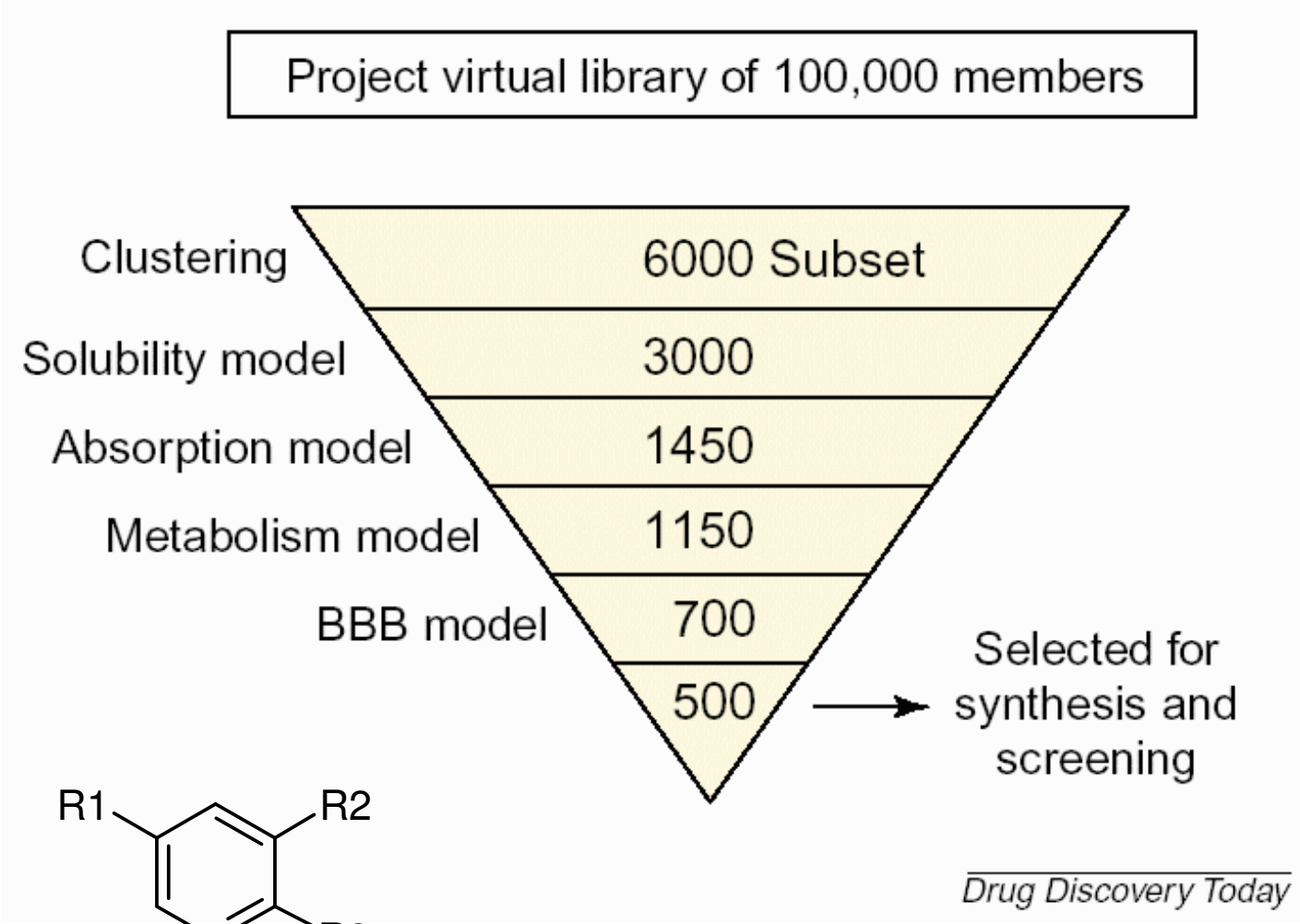


Why is AMDE prediction so important ?



Reasons that lead to the failure of a potential drug

In silico ADME filter



More about ADME-models in lecture 7

Which physico-chemical properties are recommended for drugs ?

Solubility and absorption: A hardly soluble compound is hardly transfered into the systemic blood flow.

C. Lipinski‘s rule of five: Orally administered substances

Molecular weight < 500

logP < 5

H-bond donors (N-H, O-H) < 5

H-bond acceptors (N, O) < 10

Less than 8 rotatable bonds

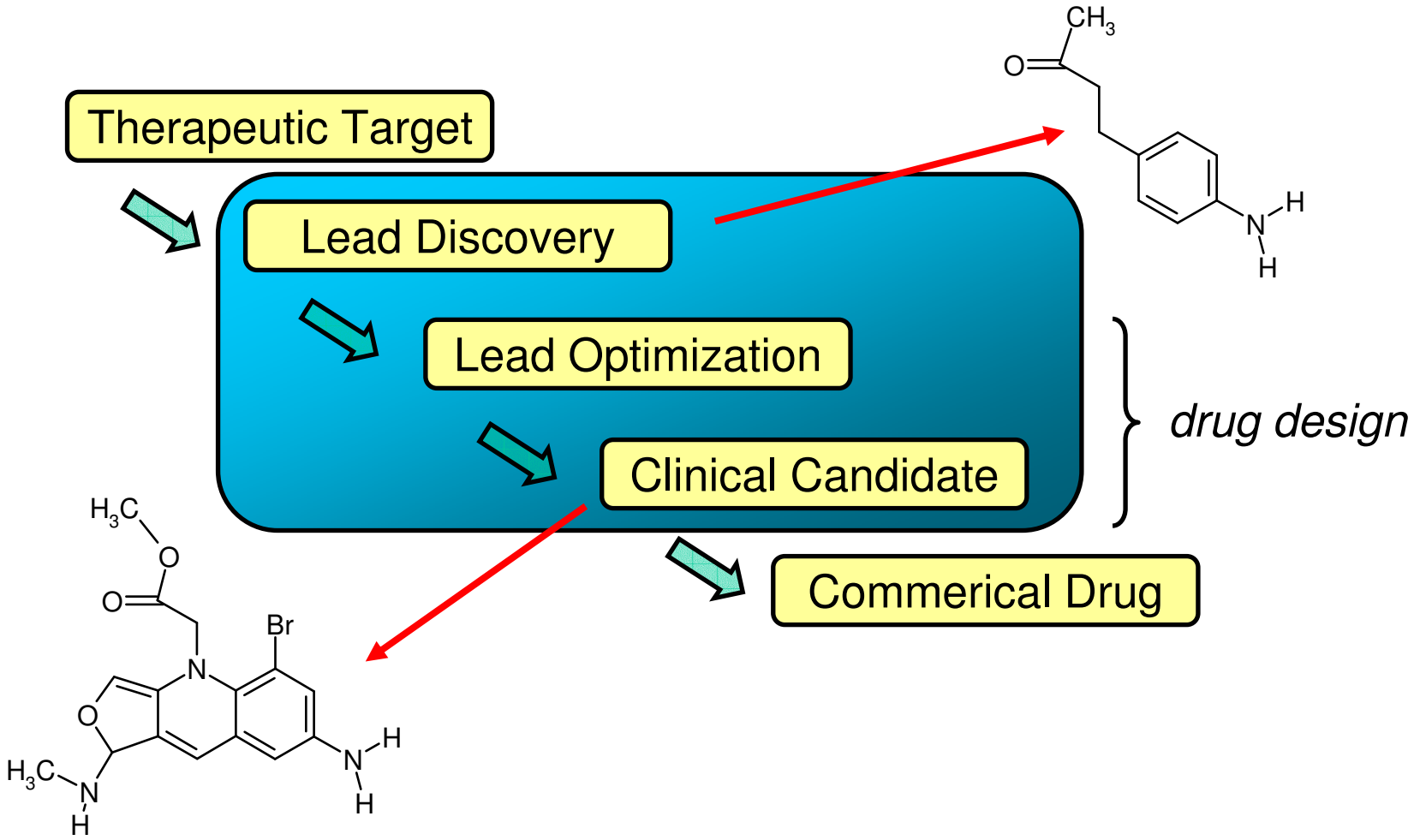
polar surface area < 140 Å^2

} Influence on the membrane passage

} not part of his original rules

→ *drug-like* compounds

From the lead compound to the drug (I)

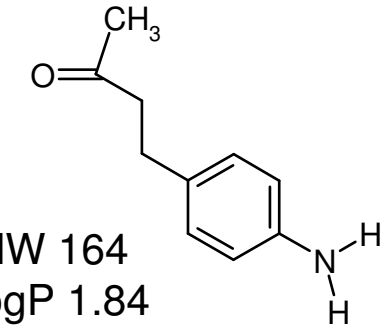


From the lead compound to the drug (II)

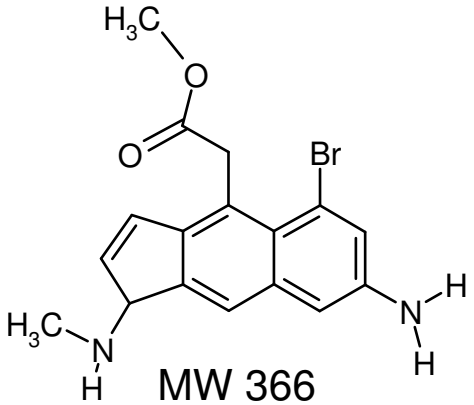
During the optimization from the lead compound to the clinical candidate, molecules are usually becoming larger and more lipophilic (binding pocket is filled better).

Thus, following properties are desirable for *lead-like* compounds:

- molecular weight < 250
- low lipophily (logP<3) for oral administration
- enough possibilities for side chains
- sufficient affinity and selectivity



MW 164
logP 1.84



MW 366
logP 2.58

More about substance libraries in lecture 4

What makes a compound *drug-like* ?

„typical“ pharmaceutic compounds show following properties:

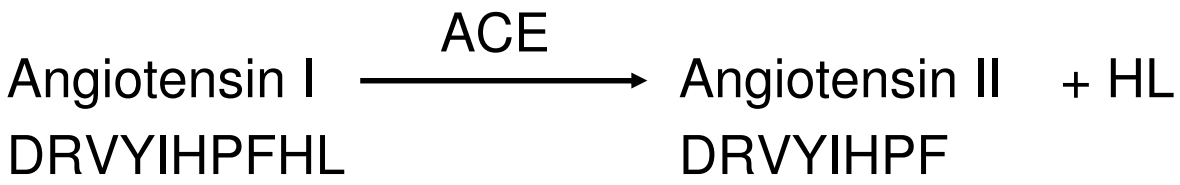
- Molecular weight in the range of 160 < MW < 480
- Number of atoms between 20 and 70
- lipophily in the range of –0.4 < logP < +5.6
- Molar refractivity in the range of 40 < MR < 130
- few H-bond donors (< 5)
- few H-bond acceptors (< 10)
- At least one OH-group (exception: CNS-active substances)

Lit: A.K.Ghose et al. *J.Comb.Chem.* **1** (1999) 55.

More about *in silico* drug/non-drug prediction in lecture 12

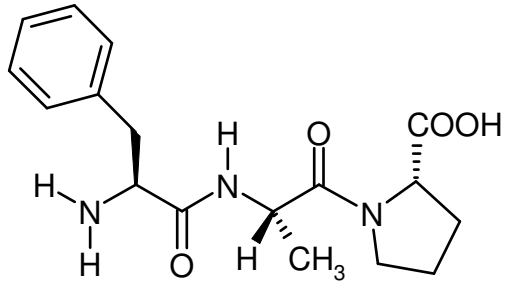
From the lead compound to the drug (III)

Example: Inhibitors of the Angiotensin Converting Enzyme



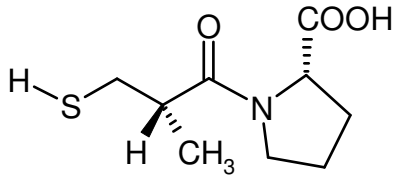
Lead compound: Phe-Ala-Pro

K_i in μM range



Captopril (1977) X-Ala-Pro

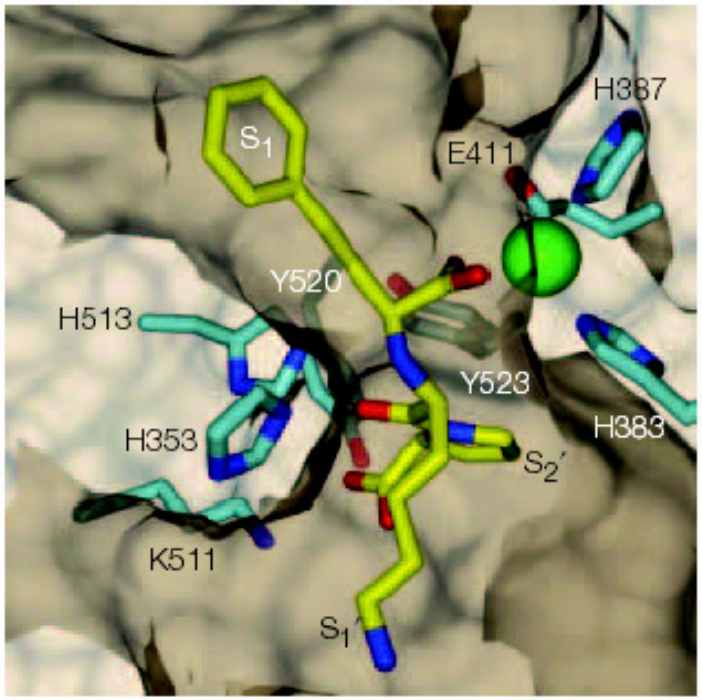
IC_{50} = 23 nM; K_i = 1.7 nM



From the lead compound to the drug (IV)

The somatic ACE (sACE) is a membrane bound protein.
The X-Ray structure of the N-terminal domain (2C6F.pdb) is known since 2006.

Germinal ACE (tACE) which is soluble shows a high sequence similarity and was used in modified form for crystallization with known inhibitors. Furthermore, structure-based design of new inhibitors is possible as the shape of the binding pocket around the catalytic zinc-ion is known.

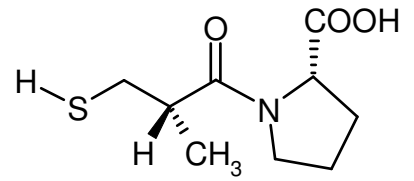


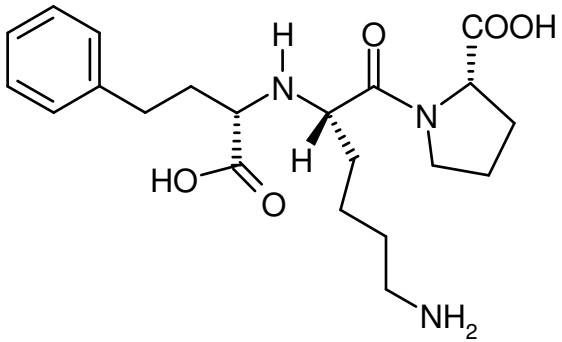
Lit: K.R.Acharya *Nature Rev. Drug Discov.* **2** (2003) 891.

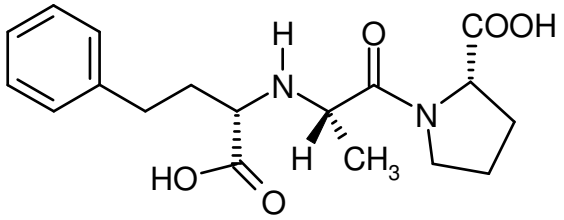
From the lead compounds to the drug (V)

Available X-Ray structures of tACE

	inhibitor (patent as of year)
1UZF.pdb	Captopril (1977)
1O86.pdb	Lisinopril (1980)
1UZE.pdb	Enalapril (1980)

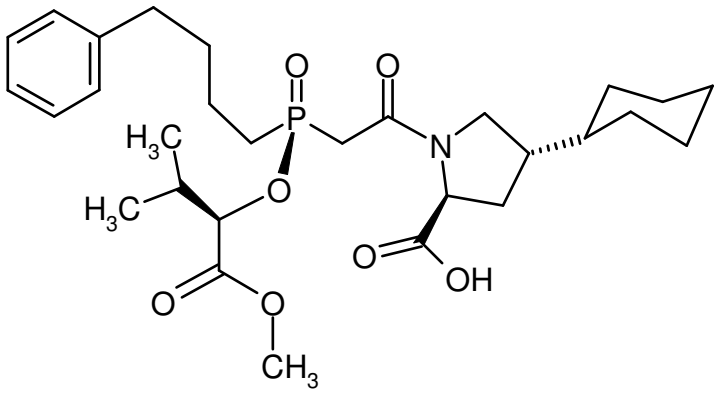


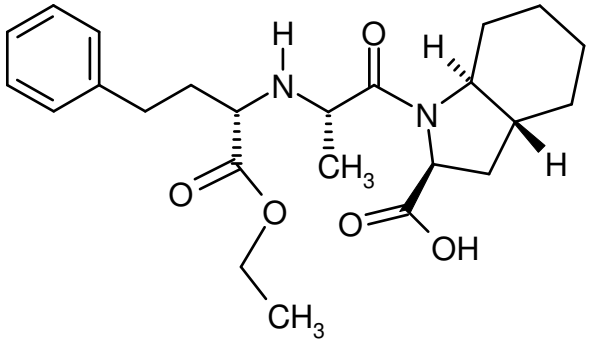




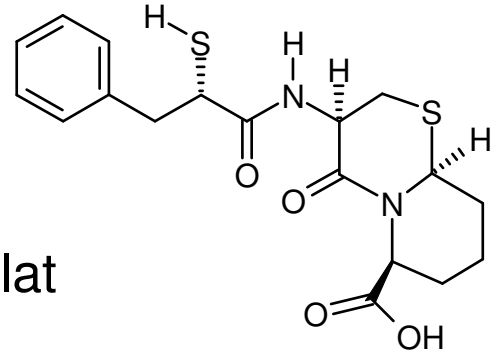
From the lead compound to the drug (VI)

Trandolapril (1980)





Fosinopril (1982)



Omapatrilat

From the lead compound to the drug (VII)

Another possibility to obtain information about the structure is to crystallize homolog enzymes from model organisms followed by homology modelling.

In the case of human tACE (E.C. 3.4.15.1) an ortholog protein of *Drosophila melanogaster* (ANCE) is present, from which another X-Ray structure is available.

In vivo screening of inhibitors is possible with according animal models that possess orthologue enzymes (mouse, rat). For hypertension the rat is establish as animal model.

Lit: K.R.Acharya *Nature Rev. Drug Discov.* **2** (2003) 891.

2nd assignment

Scope:

Ligand-enzyme interactions

Considered systems:

Comparison of lisinopril and captopril bound to tACE

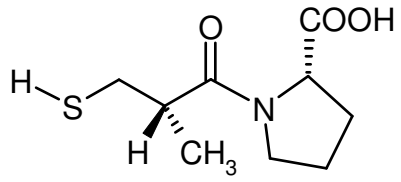
biotin – streptavidin complex

Searching Compound Databases

Problem: How to encode structural information of chemical compounds alphanumerically ?

Solution 1: Not at all. Drawn structure is used directly as query, e.g. in in CAS-online (SciFinder) database.

Assignment of a so-called CAS-registry number



Captopril [62571-86-2]

Solution 2: as so-called SMILES or SMARTS

SMILES (Daylight Chemical Infomation Systems Inc.)

SMILES and SMARTS

Simplified **M**olecular **I**nput **L**ine **E**ntry **S**pecification

Depiction of molecular 2D-structures (configuration) in 1D-form as an alphanumerical string

CC	$H_3C\text{-}CH_3$
C=C	$H_2C{=}CH_2$
C#C	$HC{\equiv}CH$
CCO	$H_3C\text{-}CH_2OH$

rules:

1) Atoms are given by their element names

C B N O P S Cl Br I H organic subset

others: [Si] [Fe] [Co]

Hydrogens are added automatically: C becomes CH_4

SMILES tutorial see http://www.daylight.com/

D. Weininger *J. Chem. Inf. Comput. Sci.* **28** (1988) 31.

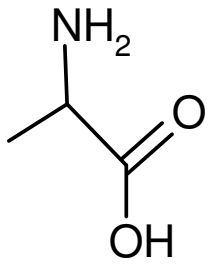
SMILES (II)

2) atoms and bonds

CC	single bonds are not needed to be specified
C=C	bouble bonds
C#C	triple bonds
c:c	aromatic bond between aromatic carbons (no need to specify)
C@C	any kind of bond in any ring
C~C	any kind of bond (single, double, ring, etc.)

SMILES (III)

3) Parenthesis denote branching



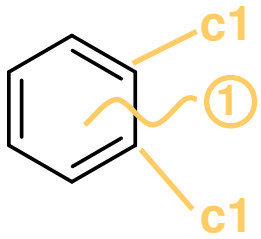
CC(N)C(=O)O

hint: Determine the longest possible chain in the molecule, first

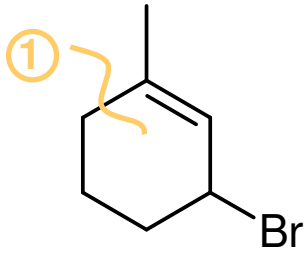
SMILES (IV)

4) Cyclic compounds: Cutting through a bond yield a chain

Also find the longest chain, first.



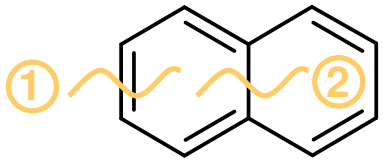
c1ccccc1



CC1=CC(Br)CCC1

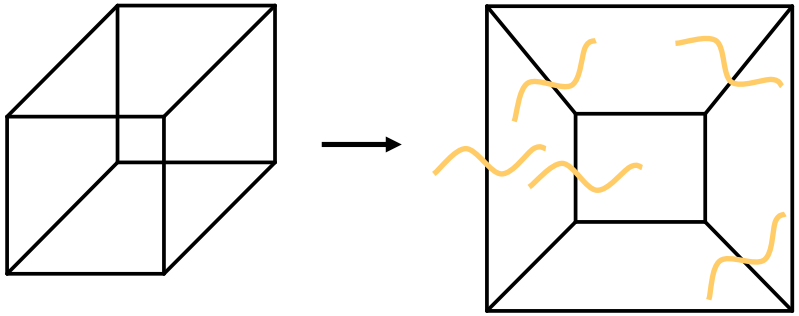
SMILES (V)

polycyclic compounds



c1cc2ccccc2cc1

There can be more than one ring closures at one atom:

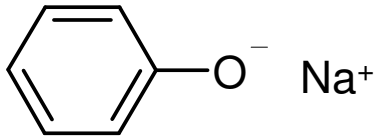


c12c3c4c1c5c4c3c25

Numbers larger than 9 are denoted by a preceeding % : c%11

SMILES (VI)

5) non-covalently bonded fragments are separated by a **.**



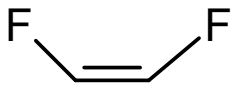
[Na+].[O-]c1ccccc1

6) isotopes

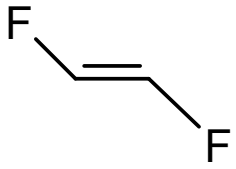
^{13}C	[13C]	
$^{13}CH_4$	[13CH4]	specify the hydrogens !
D_2O	[2H]O[2H]	

SMILES (VII)

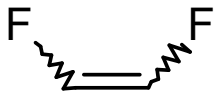
7) Configuration at double bonds



F/C=C\F above, above



F/C=C/F below, below

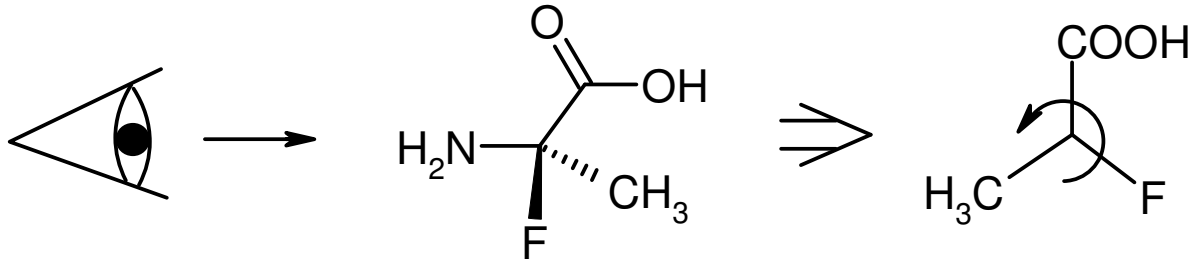


FC=CF unspecified

matches both possibilities

SMILES (VIII)

8) chirality



N[C@] (C)(F)C(=O)O

@ anti-clockwise sequence of substituents

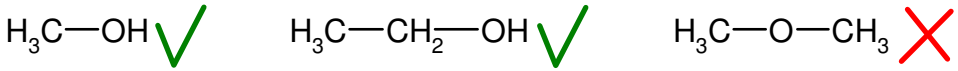
@@ clockwise sequence of substituents (anti-anti-clockwise)

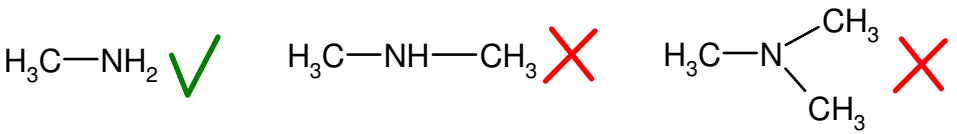
Caution: Not conform with the R/S nomenclature at stereo centers.

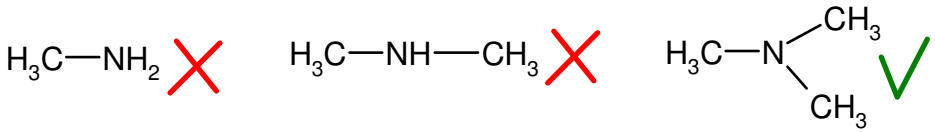
SMILES (IX)

9) Explicit hydrogen atoms

Since hydrogens are added automatically, they only have to be specified if a certain number of hydrogens is required:

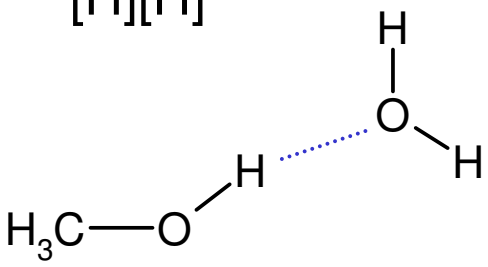
C[OH1] 

C[NH2] 

C[NH0] 

H^+ [H+] proton

H_2 [H][H]



CO[H][OH2] hydrogen bond

SMARTS (I)

Description of possible substructures

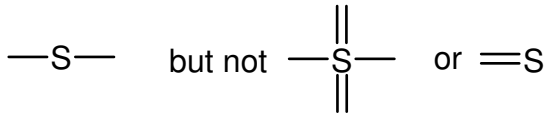
SMARTS are a superset of SMILES with molecular patterns.
A pattern ist grouped by [ ]

example:

[F,Cl,Br,I] one atom being either F or Cl or Br or I

1) atoms

c	aromatic carbon
a	aromatic atom (C, N, O, S,...)
A	aliphatic atom (= not aromatic)
*	any atom (including no atom)
[#16]	element no.16 (any kind of sulfur)
[r*n*]	atom in a *n*-membered ring
[SX2]	sulfur with two substituents
[Fe]	iron atom of arbitary charge



SMARTS (II)

2) logical (boolean) operators

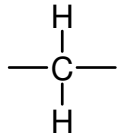
A,B	A or B
A&B	A and B (high priority)
A;B	A and B (low priority)
!A	not A

examples:

[F,Cl,Br,I] F or Cl or Br or I

[!C;R] non-aliphatic carbon and in a ring (c, N, O,...)

[CH2] aliphatic carbon with 2 Hs (methylene group)



[c,n&H1] aromatic carbon or aromatic NH [A or (B and C)]

[c,n;H1] aromatic C or N, and exactly one H [(A or B) and C]

[#7;r5] any nitrogen in a 5-membered ring

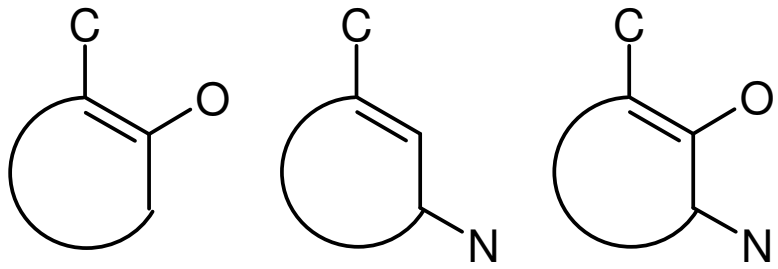
SMARTS (III)

3) configuration of substituents. Examples:

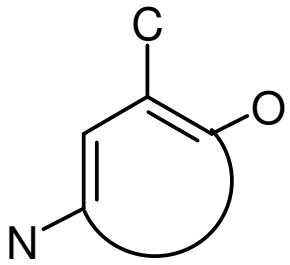
[CaaO] C ortho to O

[CaaaN] C meta to N

[Caa(O)aN]

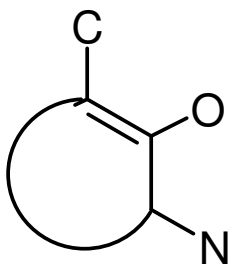


[Ca(aO)aaN]

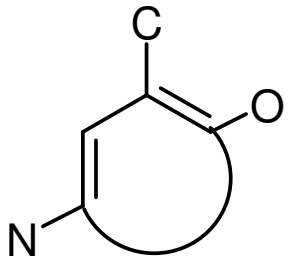


The environment of an atom is specified as follows:

C[$(aaO);$(aaaN)]



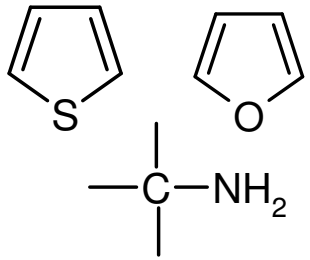
as well as



SMARTS (IV)

typical datebase queries

[s,o]1cccc1	thiophenes and furanes
[CX4][NH2]	primary aliphatic amine
[C1OC1]	epoxides
C(=O)[OH,O-,O-.+]	carbonic acid, carboxylate, or with cation
C(=O)[NH1]	peptide linkage
=[OH]	acids and enoles
F.F.F.F.F	a total of 5 fluorine atoms in the molecule (does not (yet) work with Open Babel)



further examples: E.J. Martin *J. Comb. Chem.* **1** (1999) 32.

Converting different formats of molecule files with Open Babel:
http://openbabel.sourceforge.net