Approaches of
Classical Medicinal Chemistry

Optimizing Drug Binding Affinity: (Semi) Empirical Studies

Drug Design:
Optimizing Binding Interactions

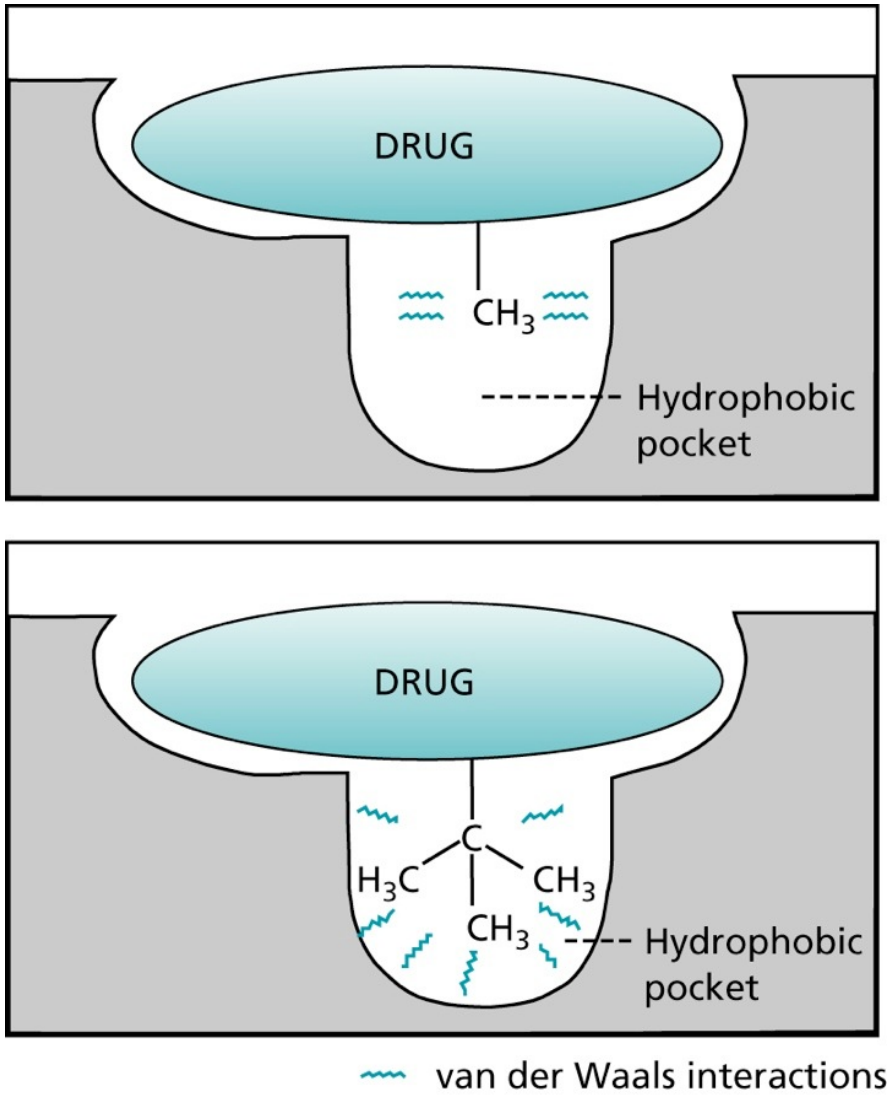
- increase activity and reduce dose levels
- increase selectivity and reduce side effects

Strategies:
Vary alkyl substituents
Vary aryl substituents
Extension
Chain extensions / contractions
Ring expansions / contractions
Ring variation
Isosteres
Simplification
Rigidification

Vary Alkyl Substituents

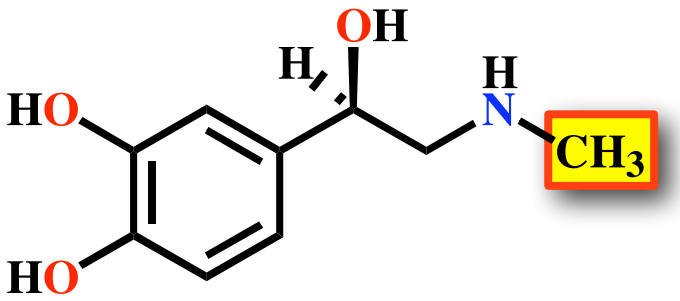
Rationale :

- Alkyl group in lead compound may interact with hydrophobic region in binding site
- Vary length and bulk of group to optimize interaction

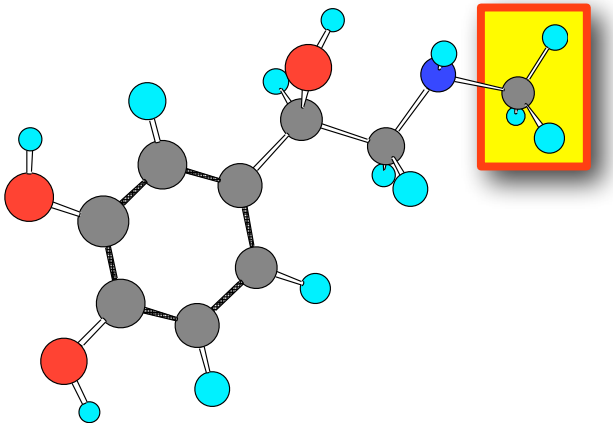


Vary Alkyl Substituents: Increasing selectivity

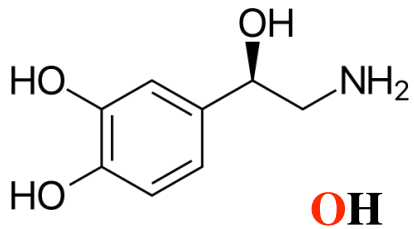
Adrenaline



$\beta_2 > \beta_1$

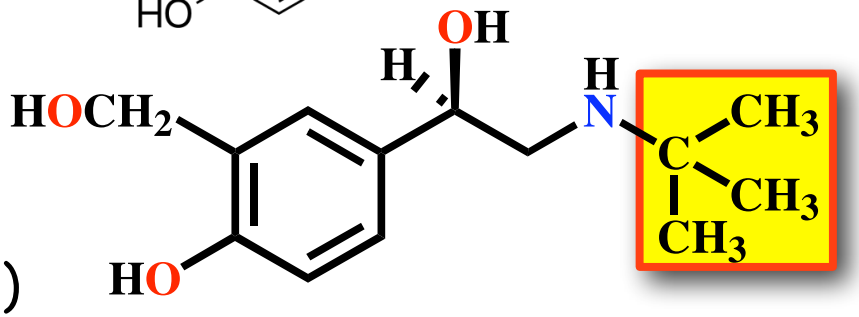


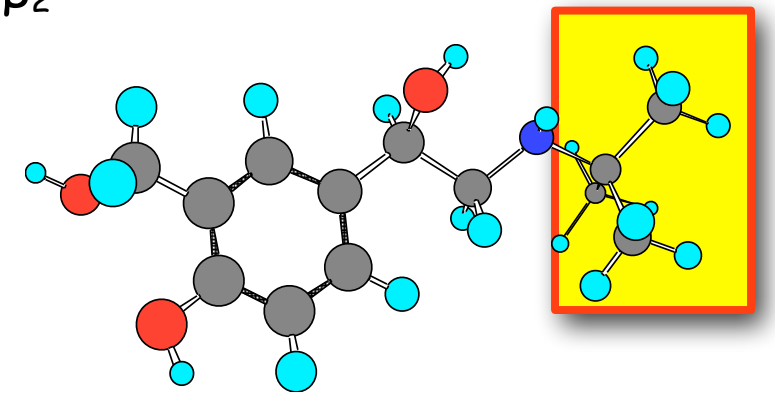
Noradrenaline



$\beta_1 > \beta_2$

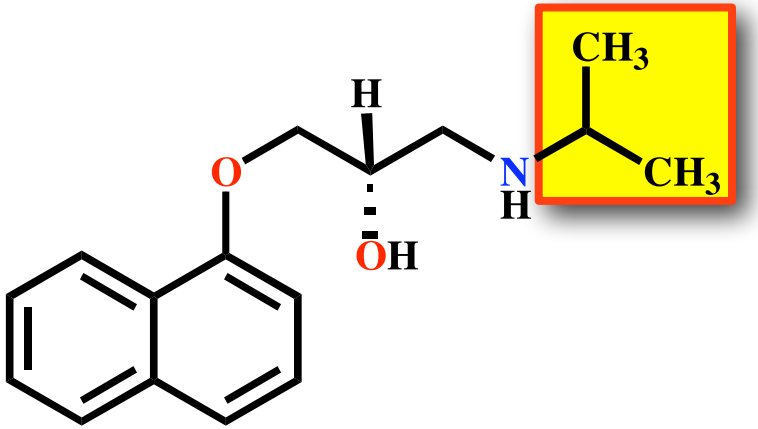
Salbutamol
(Ventolin)
(Anti-asthmatic)

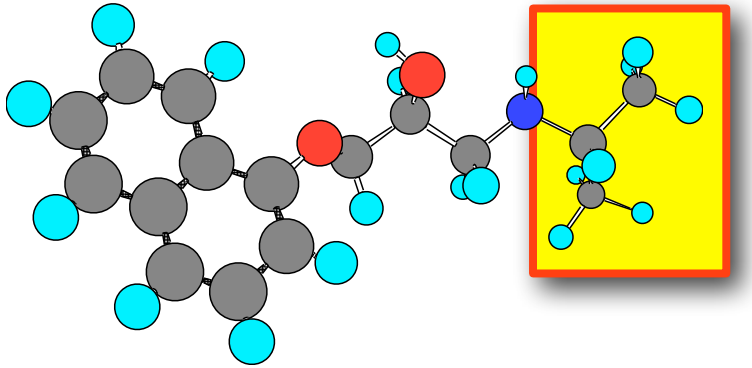




selective β_2-receptor agonist

Propranolol
(β-Blocker)

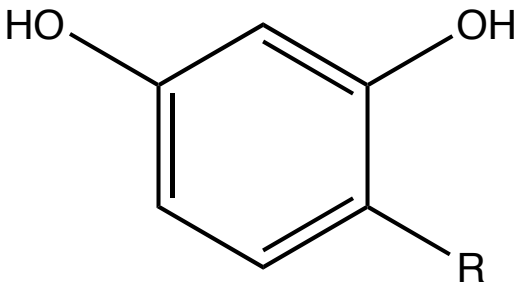




blocks the action of epinephrine and norepinephrine
on both β_1- and β_2-adrenergic receptors

Modulating lipophilicity

antimicrobial activity



R=	Activity
propyl	1.0
butyl	4.2
pentyl	6.6
hexyl	10.2
heptyl	6.0
octyl	0.0

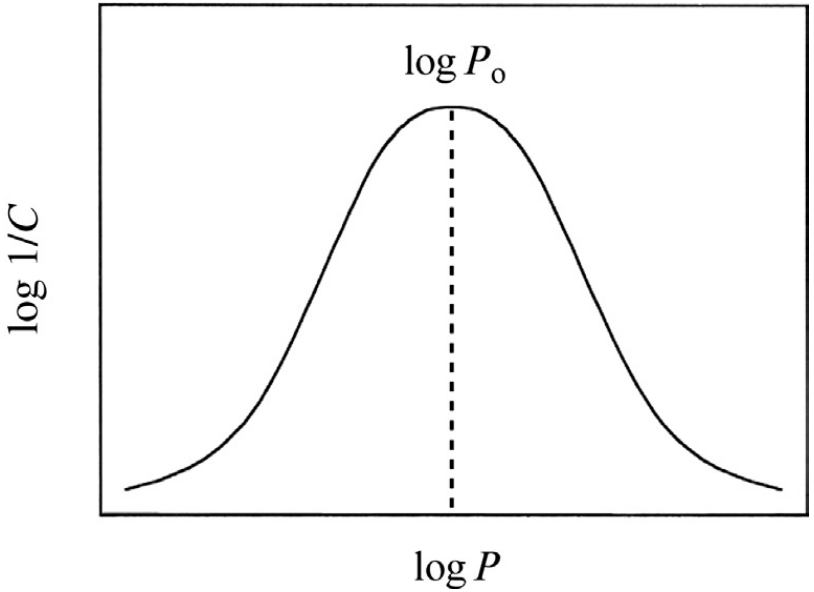
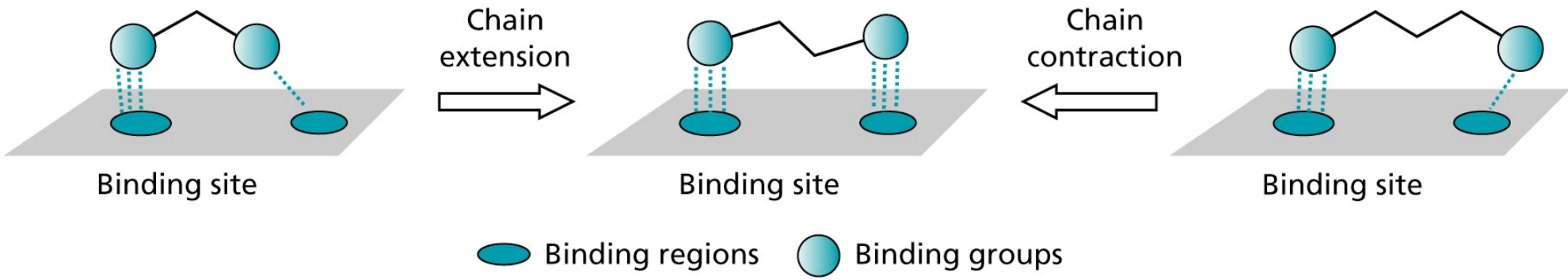
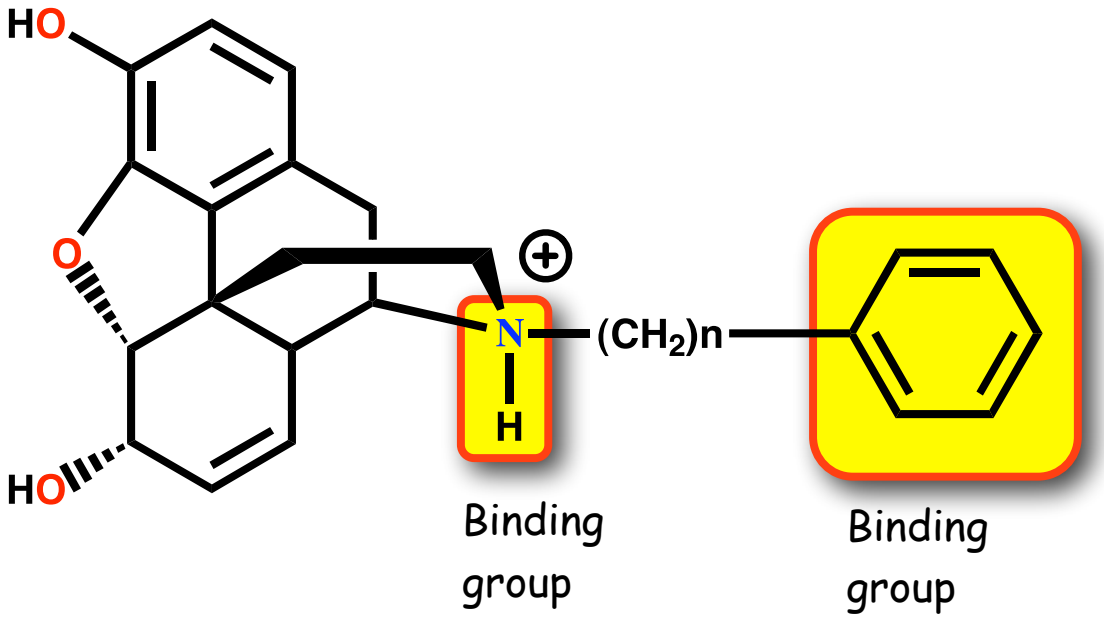


Fig. from Silvermann, pg.75

Chain Extension / Contraction



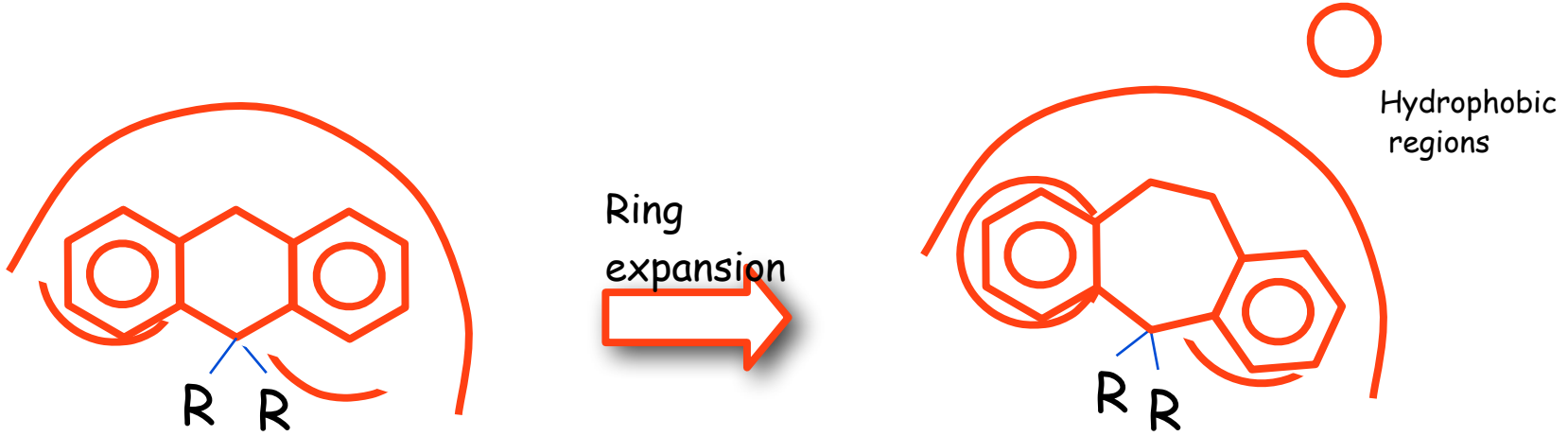
Example : N-Phenethylmorphine

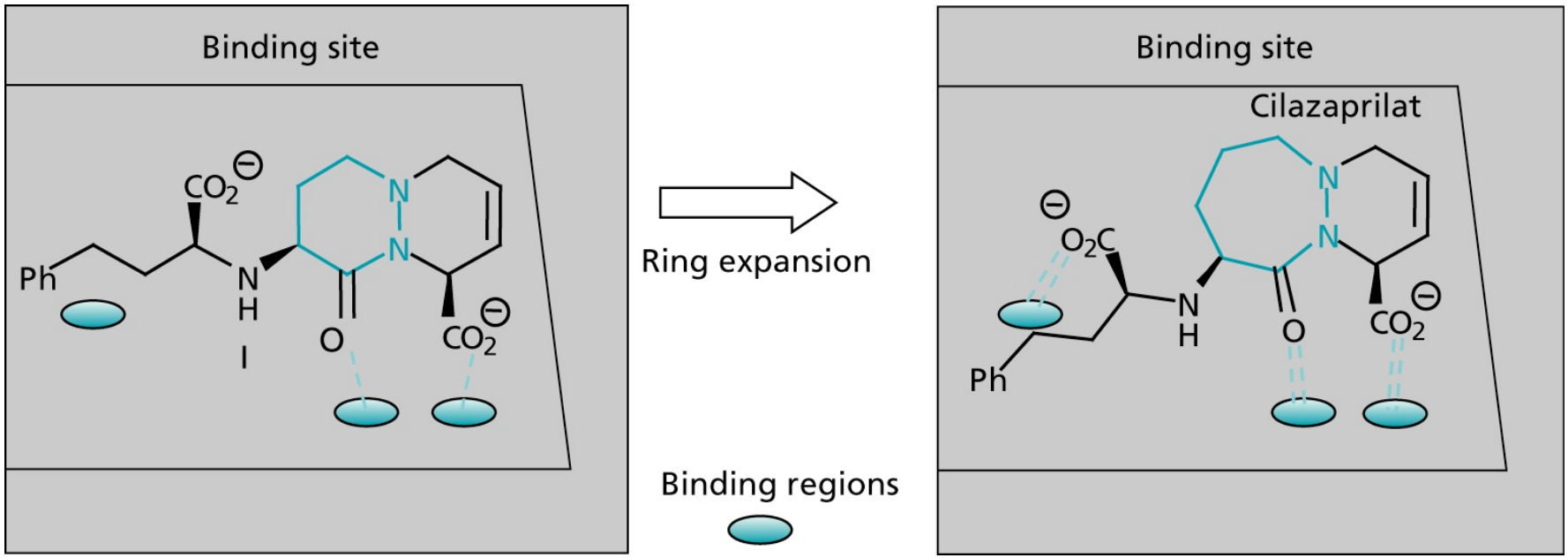


Optimum chain length = 2

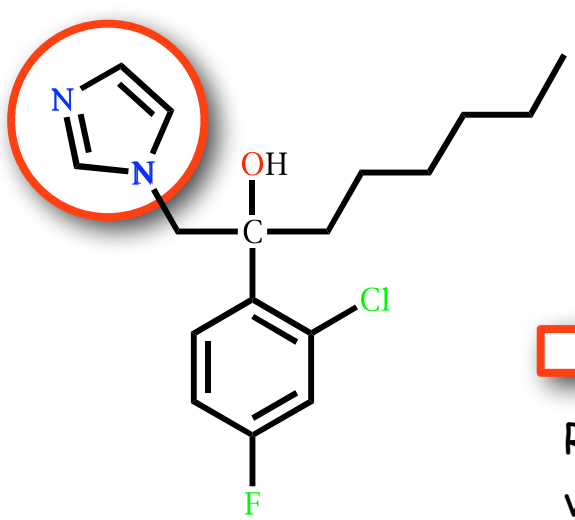
Ring Expansion / Contraction

Rationale : To improve overlap of binding groups with their binding regions





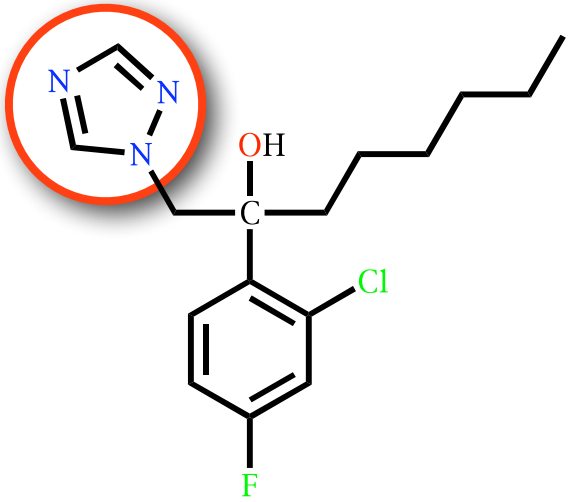
Ring Variations



Structure I

Antifungal agent

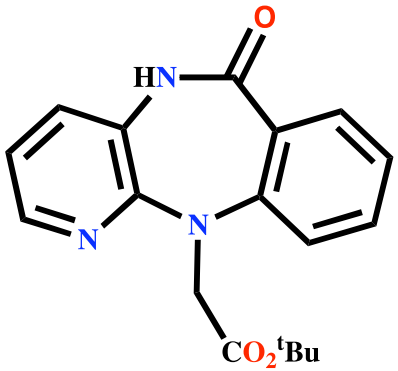
Ring variation



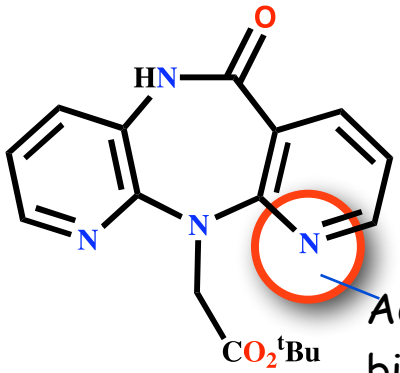
UK-46245

Improved selectivity vs. fungal enzyme

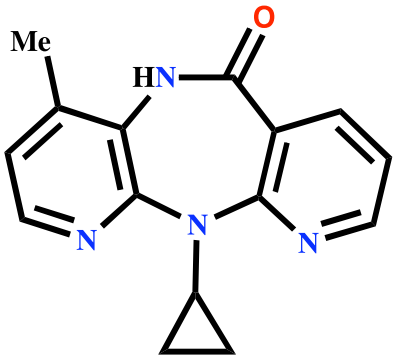
Nevirapine (antiviral agent)



Lead compound

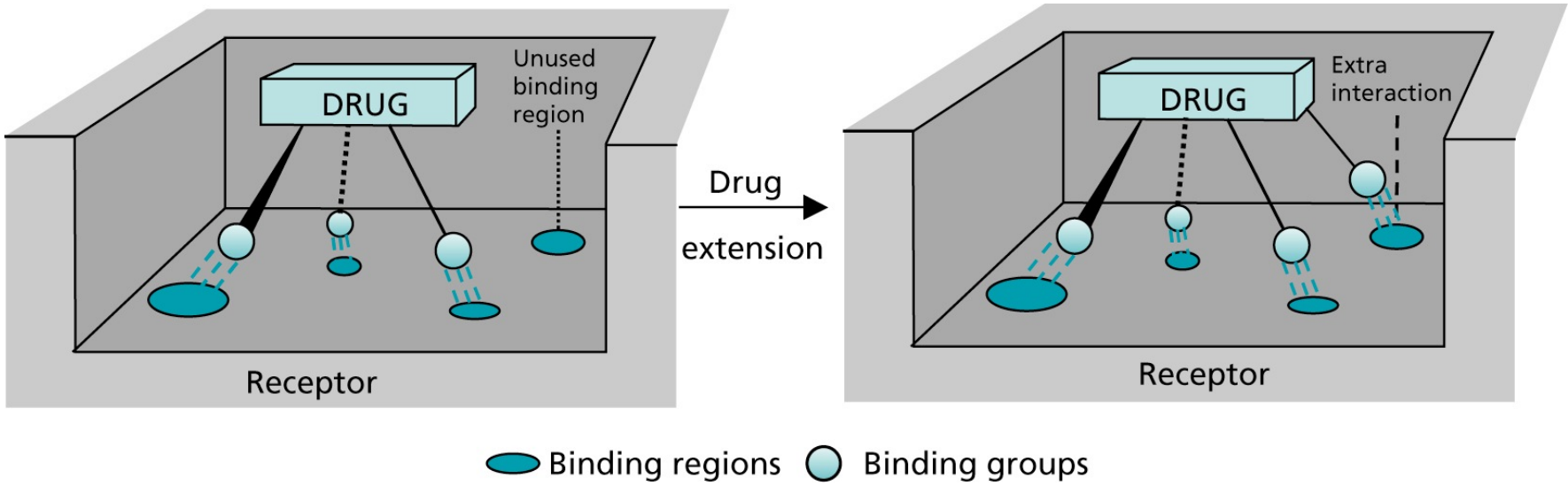


Additional binding group

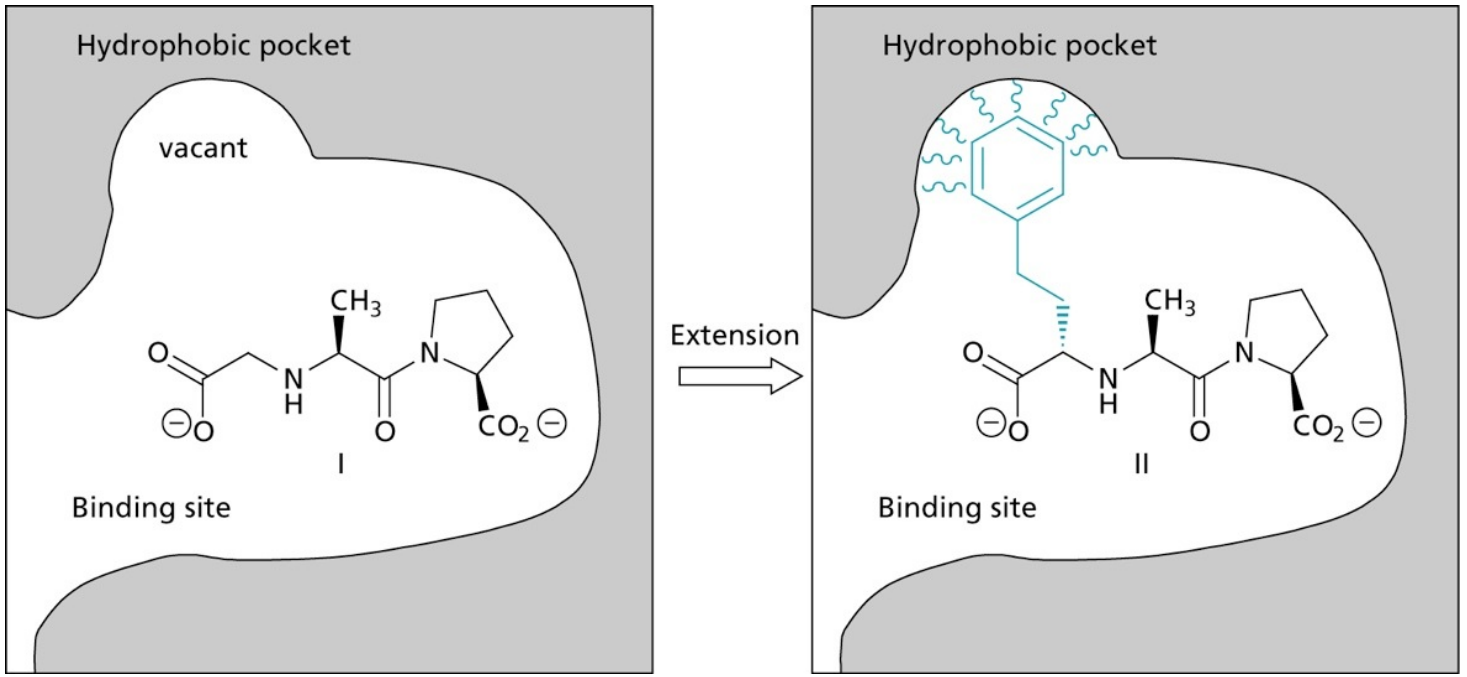


Nevirapine

Extension - Extra Functional Groups



ACE Inhibitors

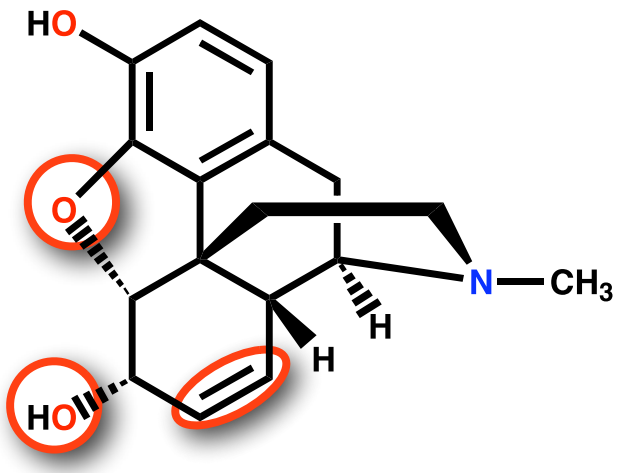


Simplification

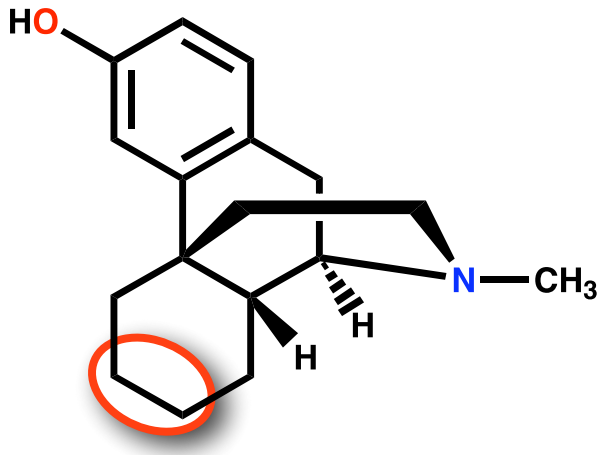
Rationale :

- Lead compounds from natural sources are often complex and difficult to synthesize
- Simplifying the molecule makes synthesis of analogues easier, quicker and cheaper
- Simpler structures may fit binding site easier and increase activity
- Simpler structures may be more selective and less toxic if excess functional groups removed

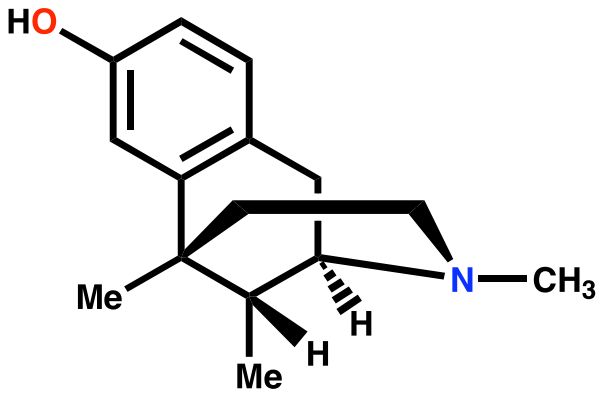
Remove excess rings



Morphine

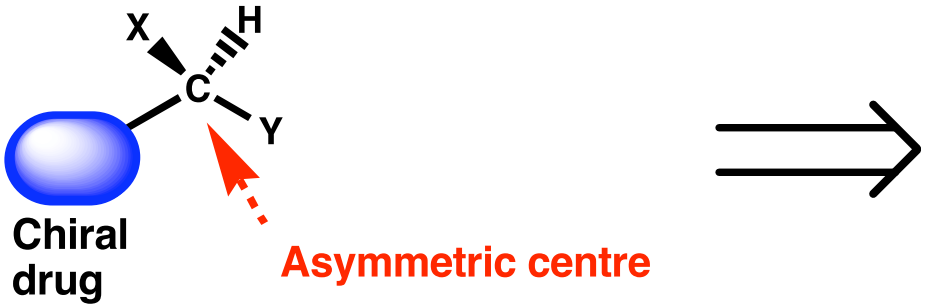


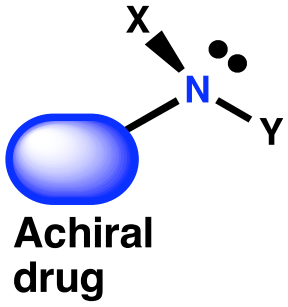
Levorphanol

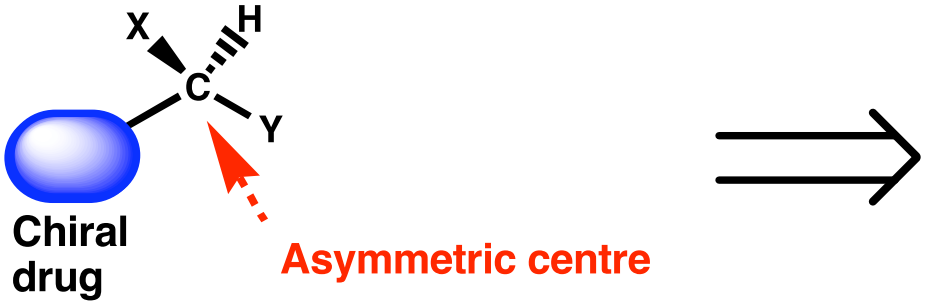


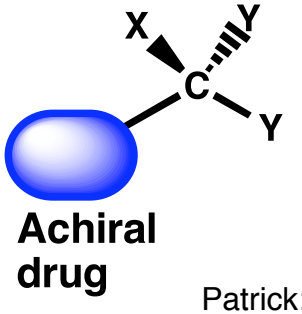
Metazocine

Remove asymmetric centres

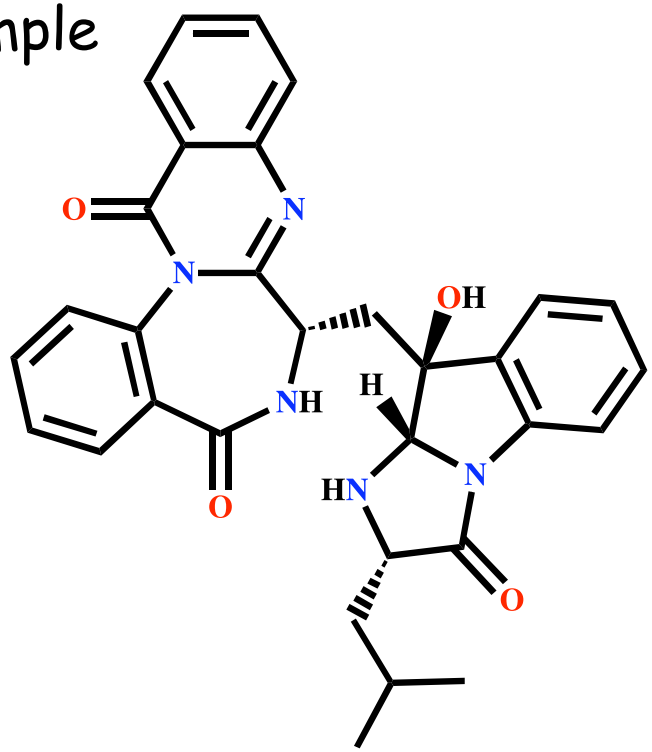


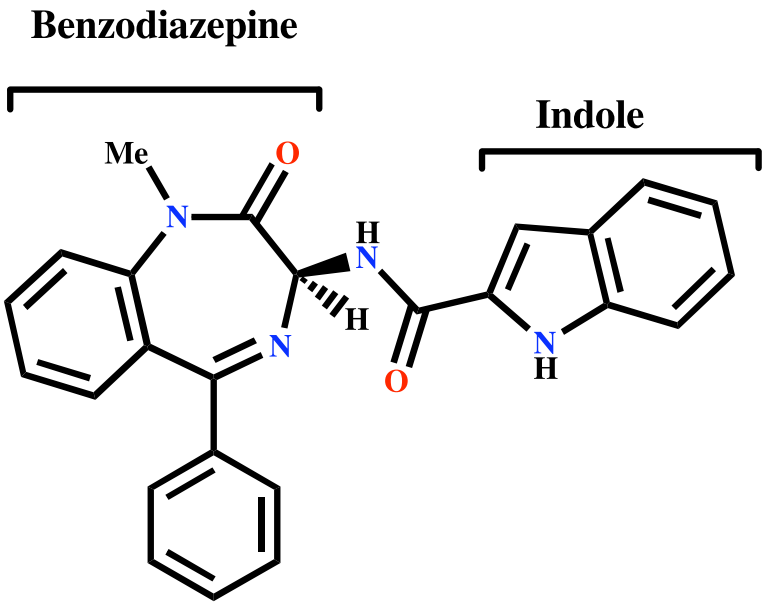






Example





Asperlicin - CCK antagonist
Possible lead for treating panic attacks

Devazepide
Excess rings removed

Disadvantages:

- Oversimplification may result in decreased activity and selectivity
- Simpler molecules have more conformations
- More likely to interact with more than one target binding site.

Rigidification

Rationale :

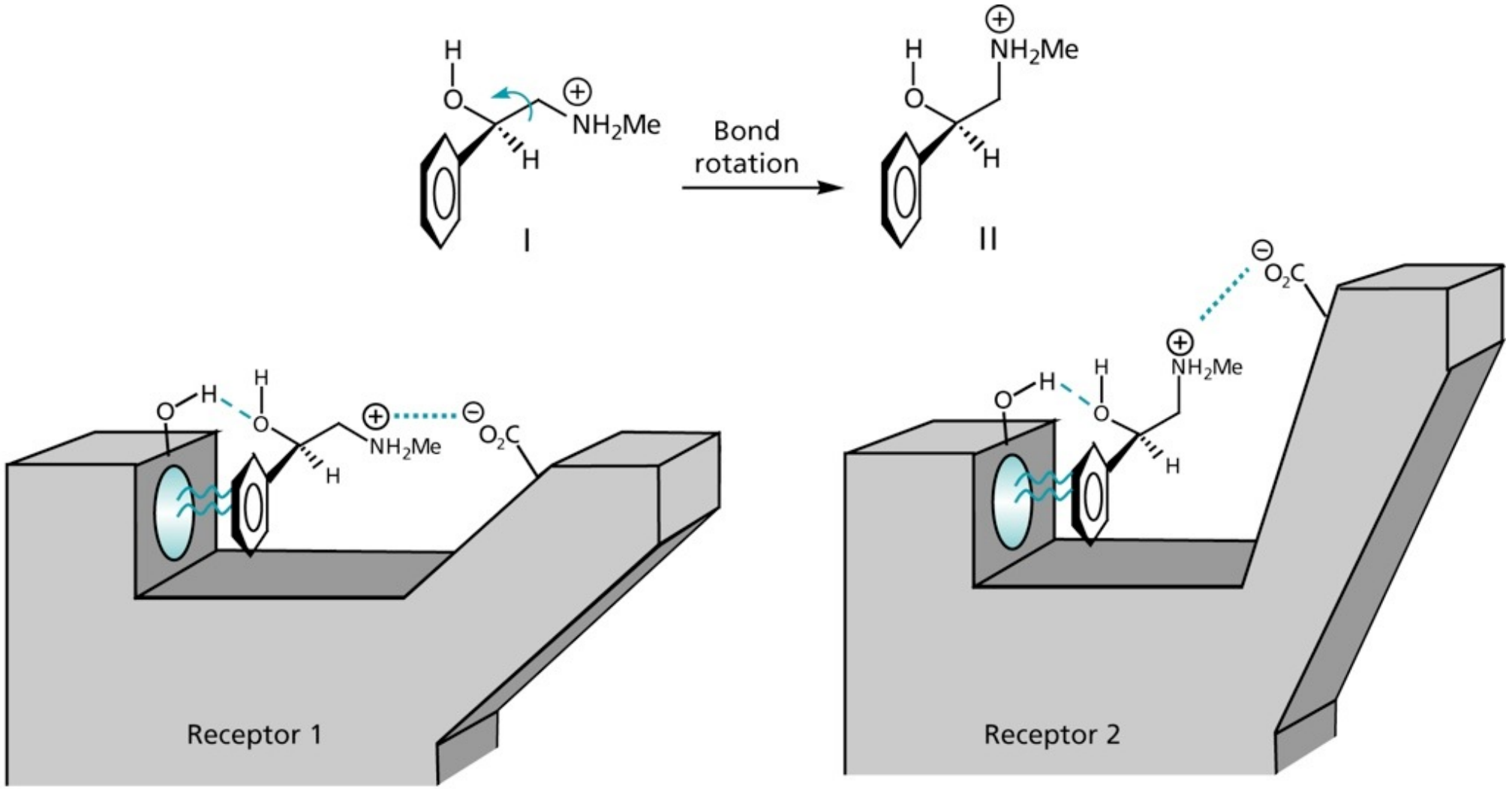
- Endogenous lead compounds often simple and flexible (e.g. adrenaline)
- Fit several targets due to different active conformations (e.g. adrenergic receptor types and subtypes)

- Rigidify molecule to limit conformations - conformational restraint
- Increases activity (more chance of desired active conformation)
- Increases selectivity (less chance of undesired active conformations)

Disadvantage:

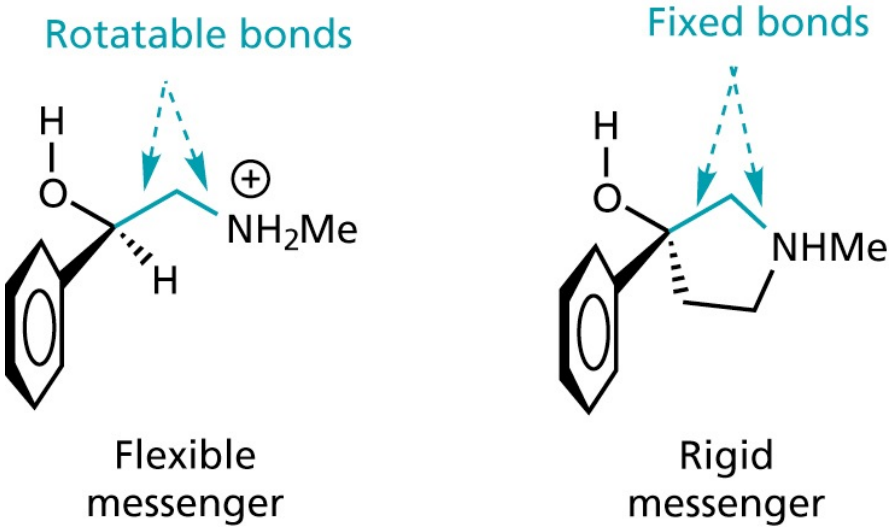
Molecule more complex and may be more difficult to synthesize

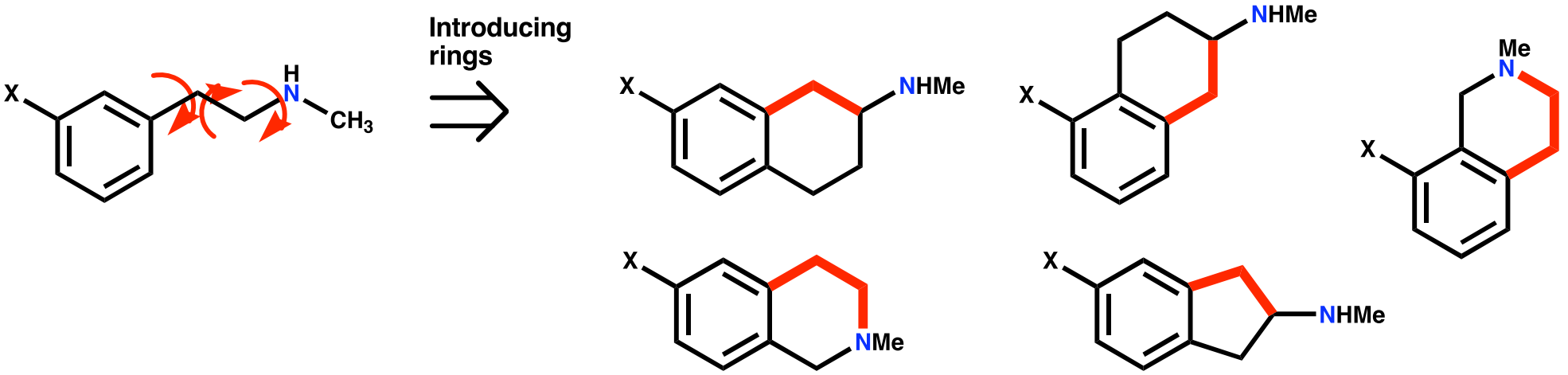
Rigidification



Methods - Introduce rings

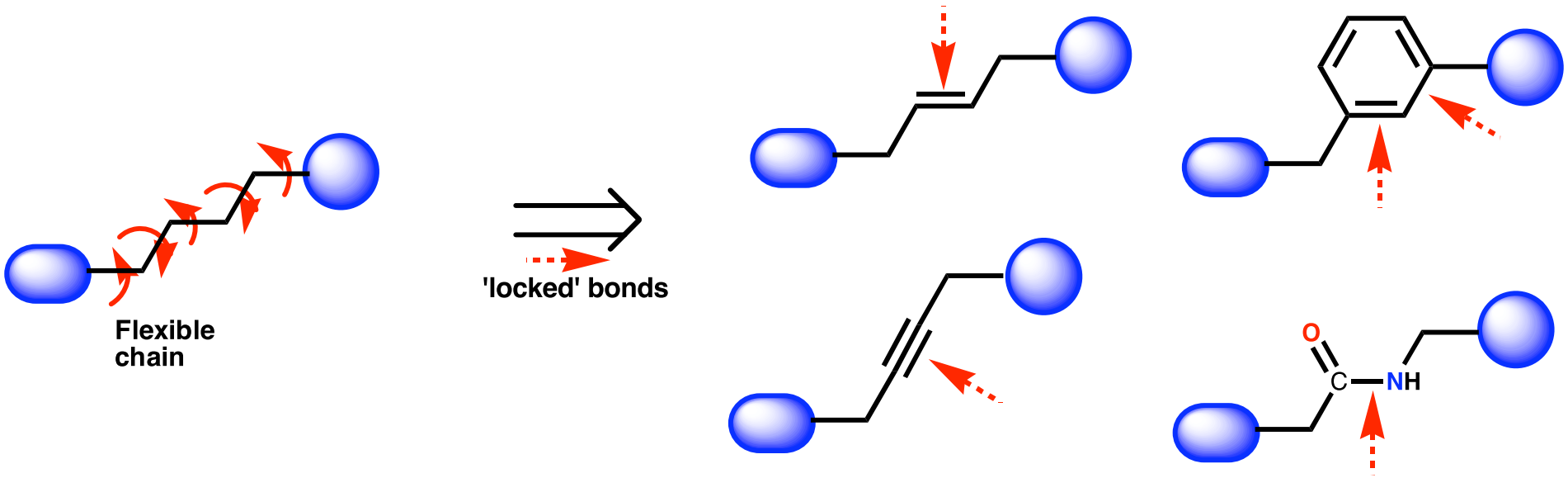
Bonds within ring systems are locked and cannot rotate freely



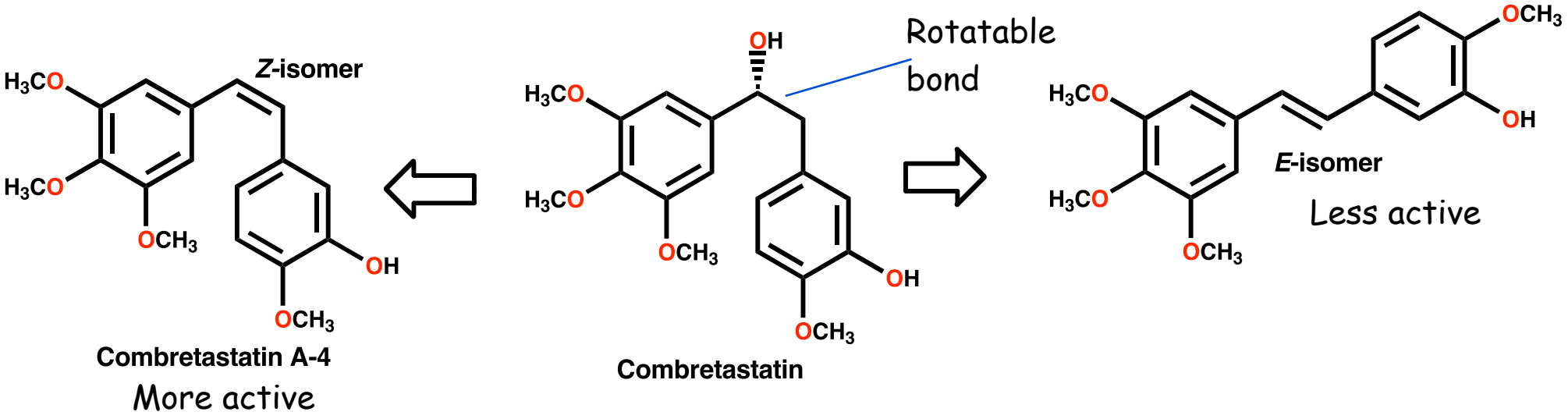


Test rigid structures to see which ones have retained active conformation

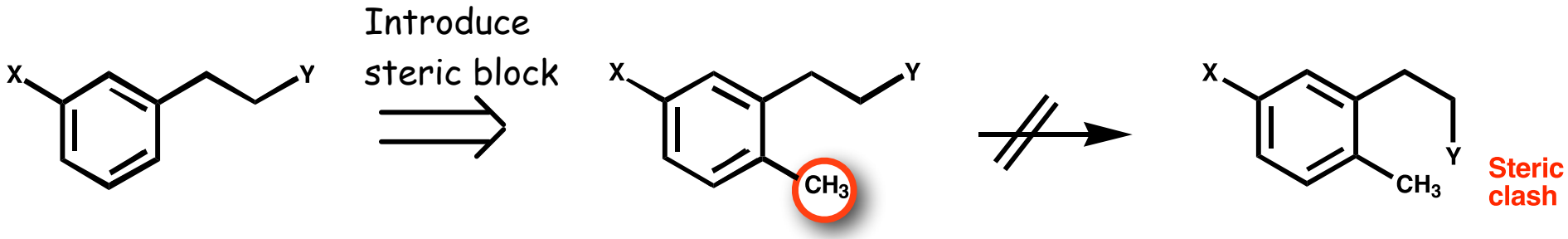
Methods - Introduce rigid functional groups



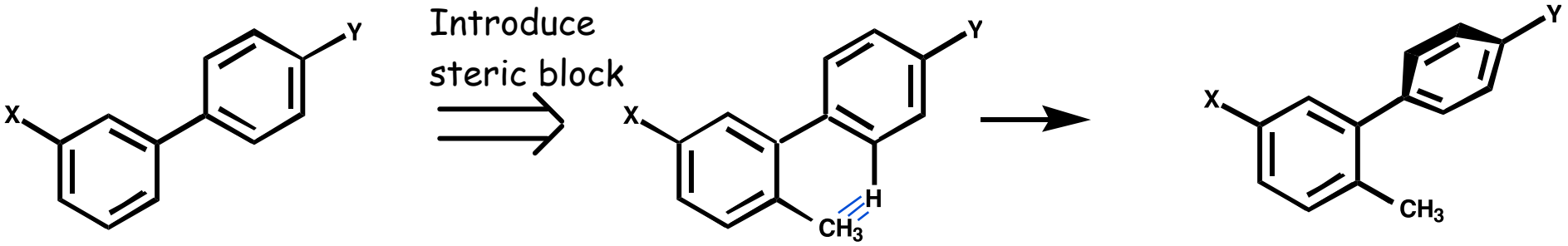
Combretastatin (anticancer agent)



Methods - Steric Blockers



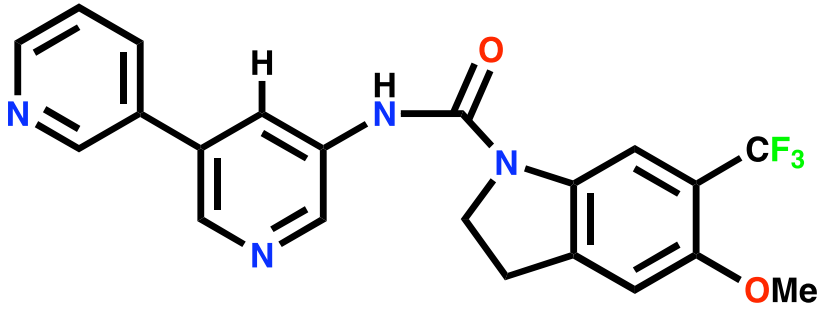
Flexible side chain



Coplanarity allowed

Orthogonal rings preferred

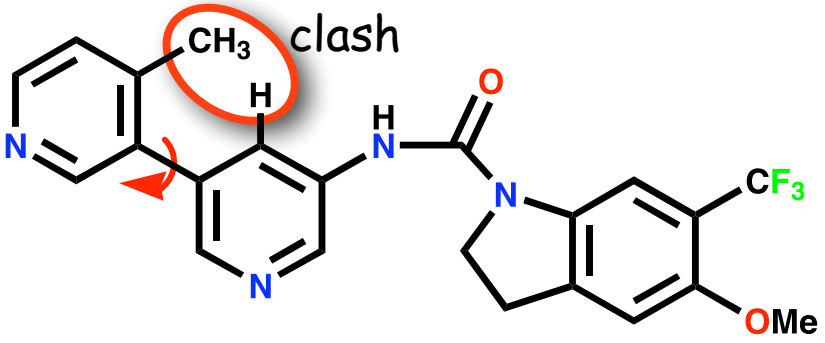
Steric Blockers

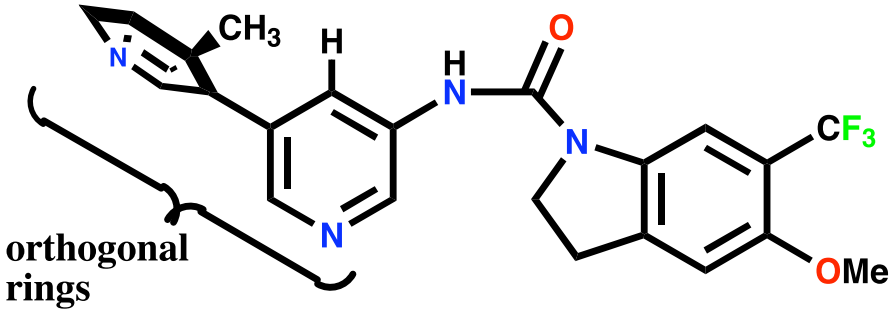


Serotonin antagonist

Introduce methyl group

Steric clash





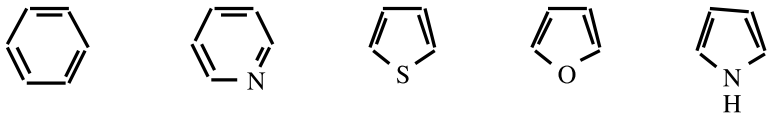
Increase in activity
Active conformation retained

Isosterism in Drugs

- Often used by pharmaceutical companies
- Isosteric replacement may result in drugs having similar, opposite or different biological activity: there are no general rules which will predict if activity will increase or decrease
- Isosteric replacement of the bridge connecting groups necessary for activity and is itself not essential, may result a graduation of similar activity
- When the isosteric replacement involves a part of the molecule essential for an interaction with a receptor, loss of activity or antagonism may result
- Commonly encountered isosteres include the following:

  –O– –S– –NH– $-CH_2-$ –CH=CH–

- Another common set of isosteres are the aromatic rings; phenyl, pyridyl, thiophene, furan, and pyrrole;



Lima and Barreiro (2005). Bioisosterism: a useful strategy for molecular modification and drug design. Curr. Med. Chem. 2005. 12, 23-49.

Isosteres and Bio-isosteres

Rationale (Isosteres) :

- Replace a functional group with a group of same valency (isostere) e.g. OH replaced by SH, NH_2, CH_3 or O replaced by S, NH, CH_2
- Leads to more controlled changes in steric/electronic properties
- May affect binding and / or stability

Rationale (Bio-isosteres) :

Replace a functional group with another group which retains the same biological activity
Not necessarily the same valency

Why making isosteres/bioisosteres?

a drug candidate is already available but..

- improve potency of the drug
- enhance selectivity
- change lipophilicity/hydrophilicity
- reduce metabolism/ increase metabolism
- eliminate or modify toxic metabolites
- create new intellectual properties

Classical Isotere Groups and Atoms

1. Univalent atoms and groups

   a. CH_3 NH_2 OH F Cl
   b. Cl PH_2 SH
   c. Br *i*-Pr
   d. I *t*-Bu

2. Bivalent atoms and groups

   a. $-CH_2-$ $-NH-$ $-O-$ $-S-$ $-Se-$
   b. $-COCH_2R$ $-CONHR$ $-CO_2R$ $-COSR$

3. Trivalent atoms and groups

   a. $-CH=$ $-N=$
   b. $-P=$ $-As=$

4. Tetravalent atoms

   a. $-\overset{|}{\underset{|}{C}}-$ $-\overset{|}{\underset{|}{Si}}-$
   b. $=C=$ $=\overset{+}{N}=$ $=\overset{+}{P}=$

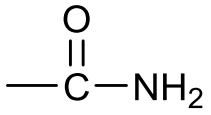
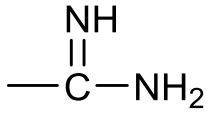
5. Ring equivalents

   a. $-CH=CH-$ $-S-$ (e.g., benzene, thiophene)
   b. $-CH=$ $-N=$ (e.g., benzene, pyridine)
   c. $-O-$ $-S-$ $-CH_2-$ $-NH-$ (e.g., tetrahydrofuran, tetrahydrothiophene, cyclopentane, pyrrolidine)

from: Organic Chemistry of Drug Design and Drug Action, Silverman & Holladay

Non Classical Isosteres

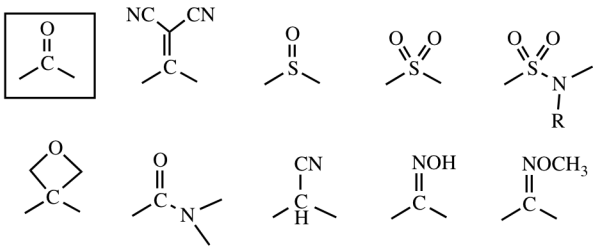
As time progressed the definition of isosterism was broadened to include functional groups, such as O and NH (amide and amidine), cyclic/acyclic etc.

	
Amide	Amidine

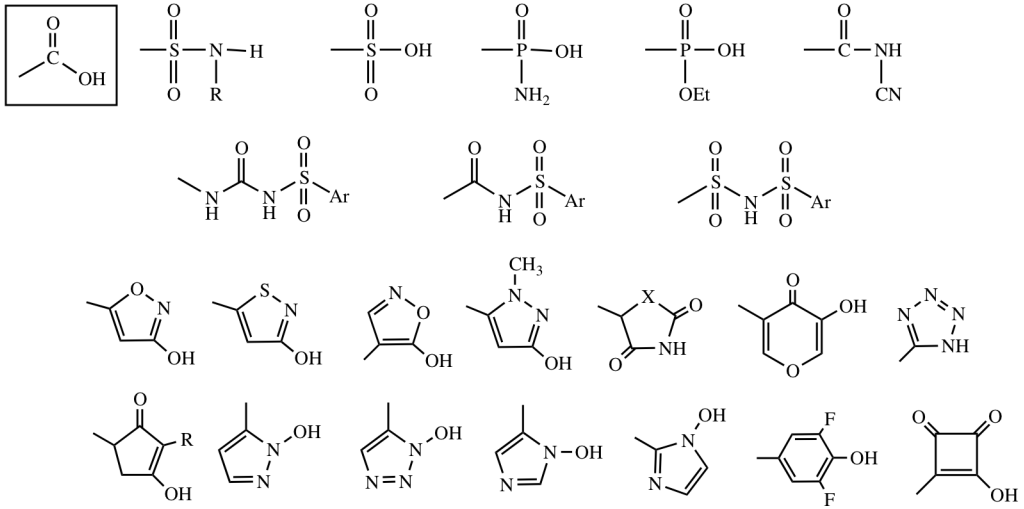
- NCI do not have the same number of atoms and do not fit the steric and electronic rules
- Friedman (1951) introduced the term Bioisostere to fit the broadest definition for isosteres: This definition includes all isosteres that produce similar biological activity

Non-Classical Isosteres

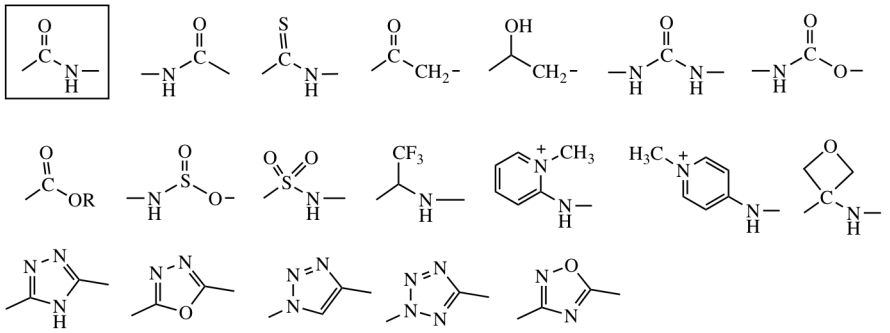
1. **Carbonyl group**



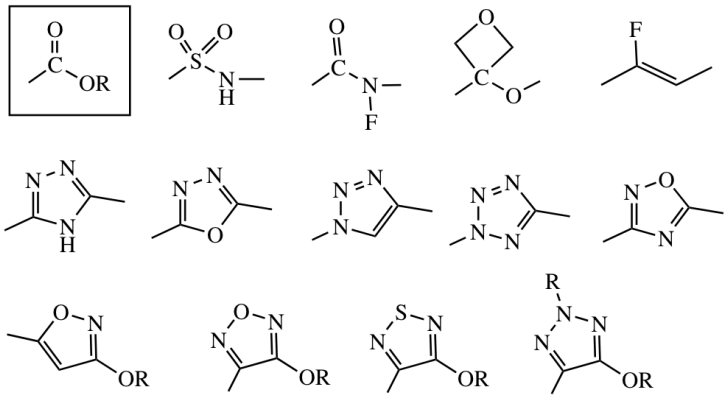
2. **Carboxylic acid group**



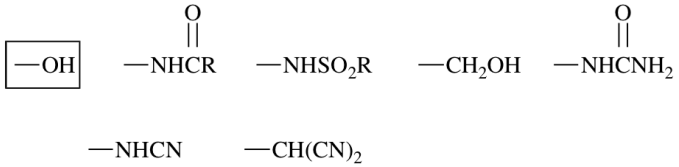
3. **Amide group**



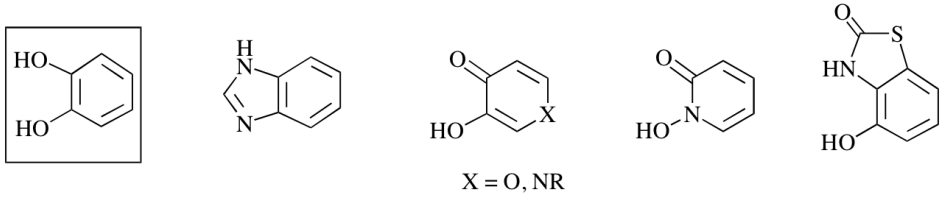
4. **Ester group**



5. **Hydroxyl group**

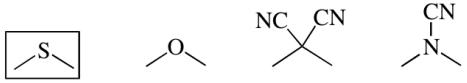


6. **Catechol**

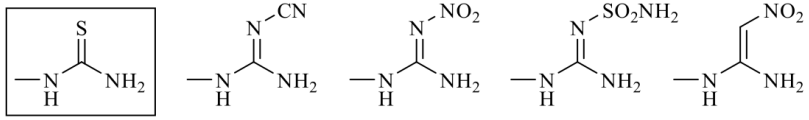


7. **Halogen**

8. **Thioether**

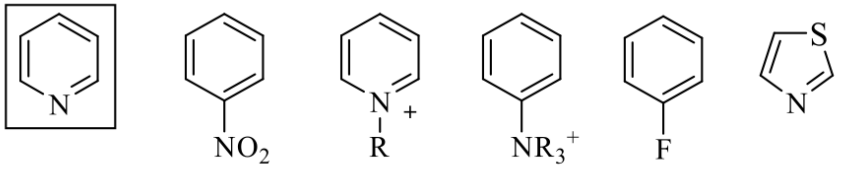


9. **Thiourea**

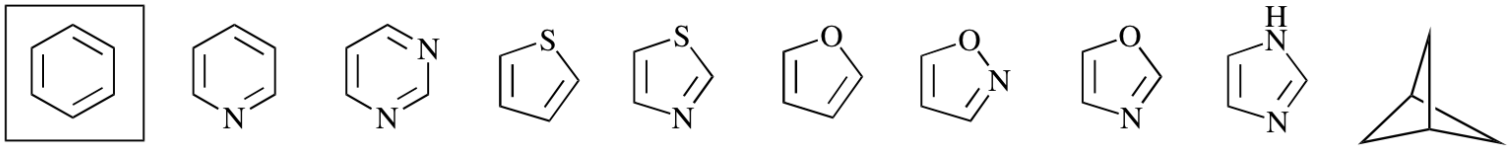


10. **Azomethine**

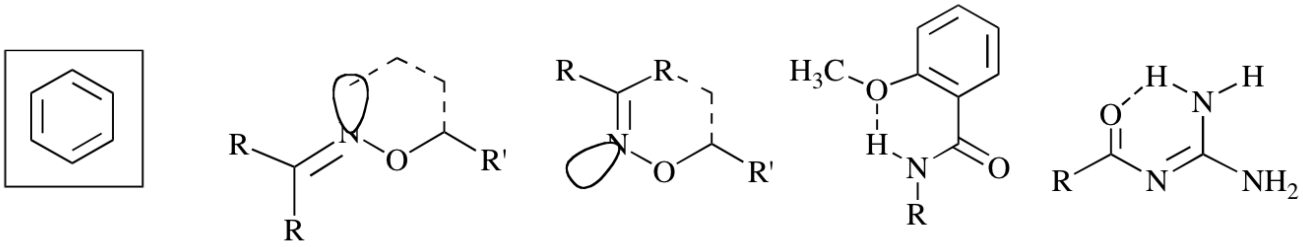
11. **Pyridine**



12. **Benzene**



13. **Ring equivalents**



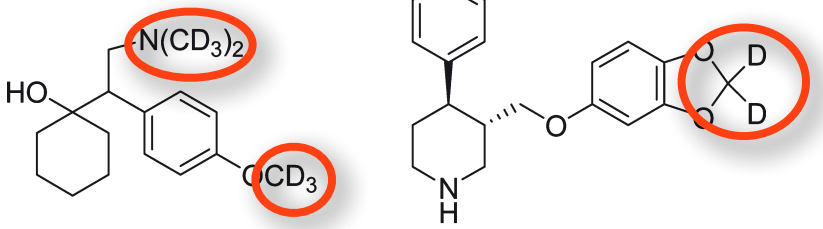
14. **Spacer group**

15. **Hydrogen**

F D

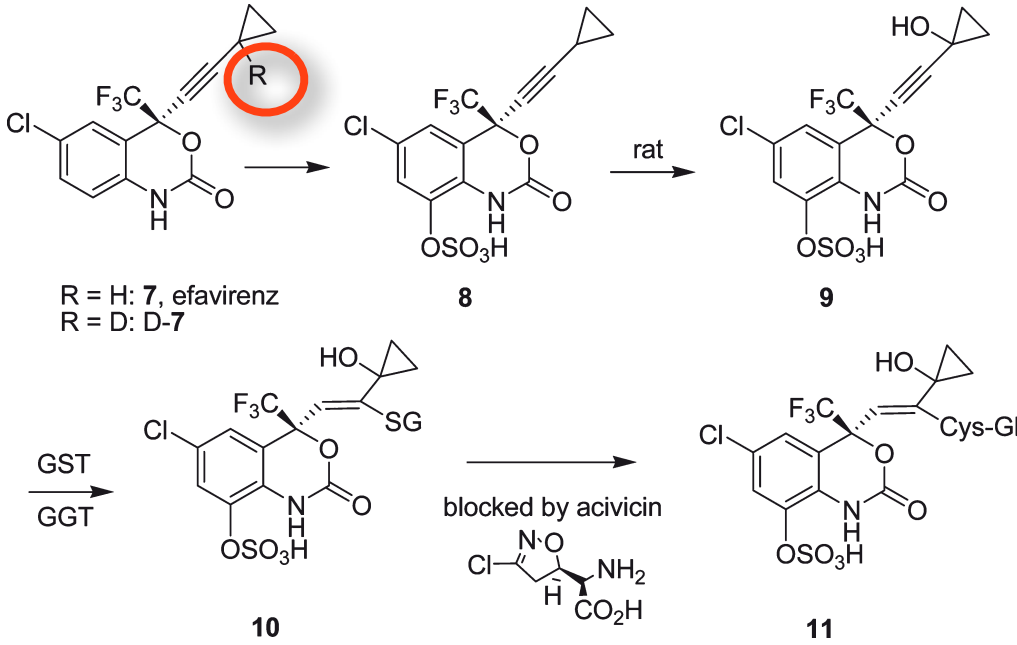
Replacement of H by ^2H

- least disturbing replacement
- D is slightly less hydrophobic than H
- the molar volume is slightly smaller
- C-D bonds are shorter
- D can be introduced to increase metabolic stability



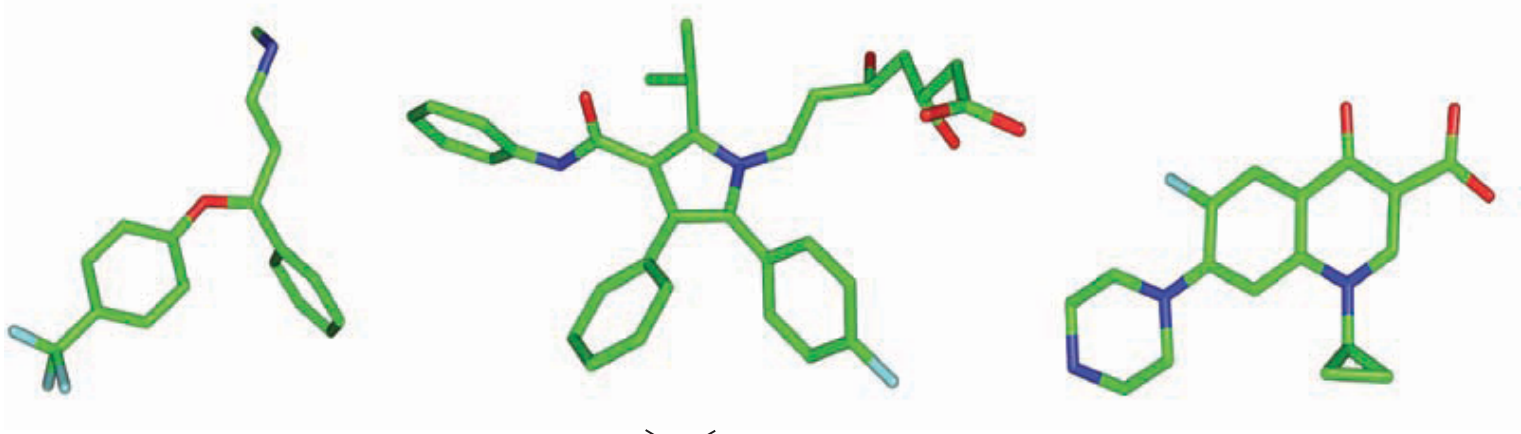
1, SD-254

2, CTP-347



increased metabolic stability

Halogens (Fluorine) in Pharmaceutical Products



Prozac
anti-depressant

Lipitor
cholesterol-lowering

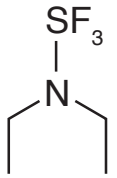
Ciprobay
antibiotic

20% of all drugs are fluorinated compounds!

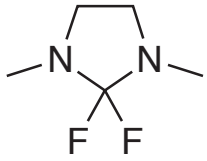
Müller et al, Science **317**, 2007, 1881-86

Fluorination Reagents

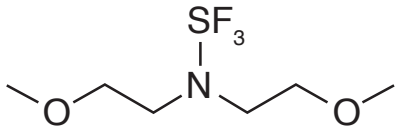
nucleophilic



DAST

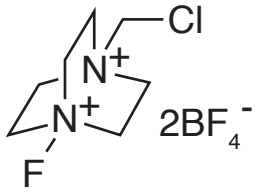


DFI

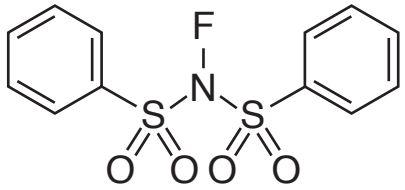


Deoxofluor

electrophilic

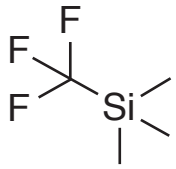


Selectfluor

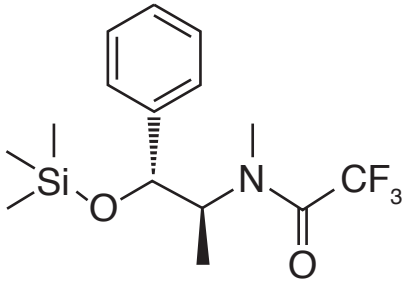


NFSI

reagents to introduce CF_3



Ruppert-Prakash

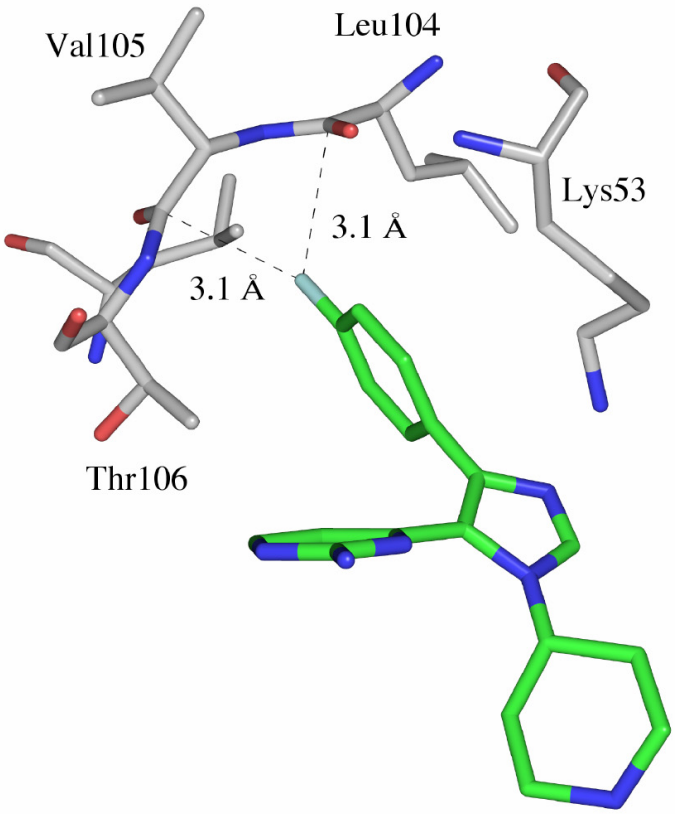


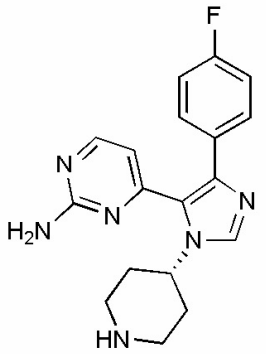
Trifluoroacetamide

Properties of Fluor in Organic Molecules

- Fluor enhances metabolic stability by lowering the susceptibility of nearby moieties to cytochrome P450 enzymatic oxidation.
- F-C bonds are extremely stable, as well as adjacent C-C bonds
- Fluor can serve as **bioisotere** of a C-O bond, C=CHF for the peptide bond, the C-CF3 fragment for a C=O group, C-F for C-OH and C-OMe.
- Substitution of H by F may completely alter conformational preferences
- The basicity of adjacent N centers can be lowered, so that compounds tend to be more neutral and hence bioavailability is increased
- Replacing H by F in general increases lipophilicity of the compound

Van der Waals radius	Fluorine	1.35 Å 1.47 Å
	Hydrogen	1.20 Å
	Oxygen	1.40 Å 1.52 Å
Bond length	C–F	1.35 Å 1.41 Å
	C–H	1.09 Å
	C–O	1.43 Å
Total exensions	C–F	2.82 Å
	C=O	2.72
Van der Waals volume	CF3	39.8 Å3
	CH3	21.6 Å3
	((CH3)2CH	56.2 Å3
	CH3CH2	38.9 Å3



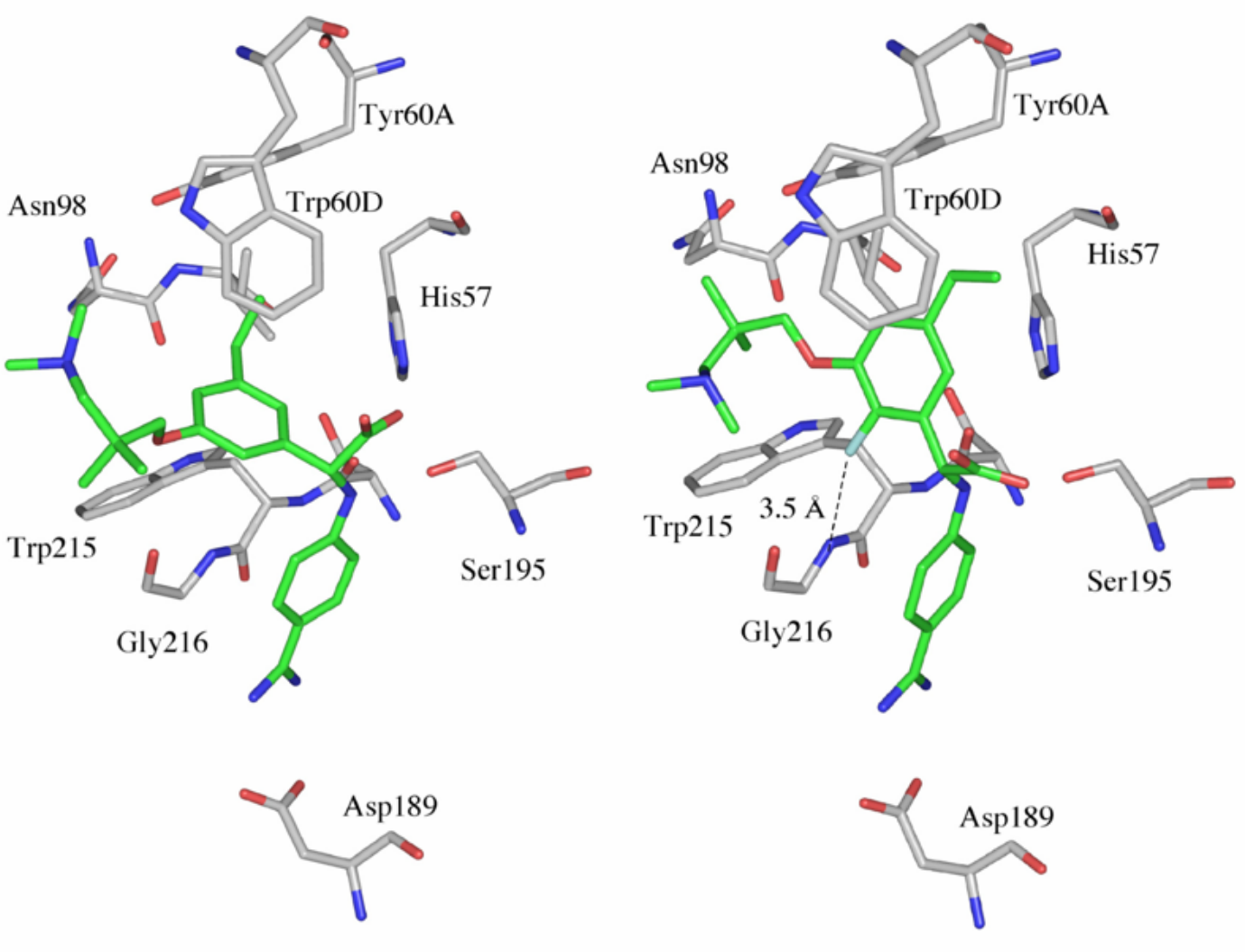


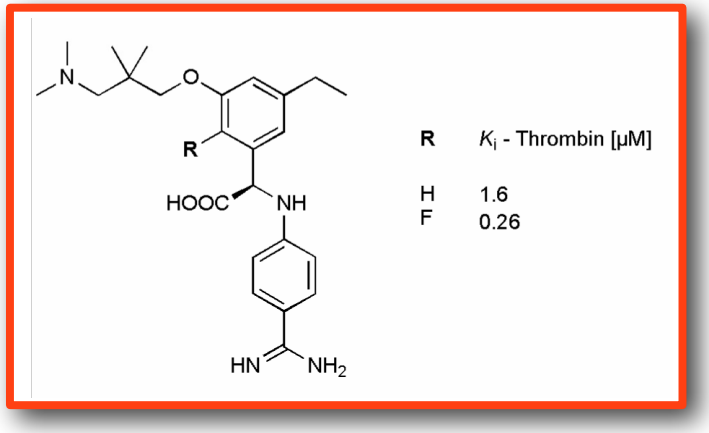
IC_{50} = 0.019 µM

Müller et al, Science **317**, 2007, 1881-86

- Fluor is not a good H-bond acceptor, because F is not polarizable
- However, the C-F dipole undergoes multipolar interactions
- C-F bonds pointing into polar environments reduce binding affinity
- C-F bonds avoid point to C=O groups
- C-F groups interact with C=O group in an orthogonal fashion
- C-F groups interact with guanidinium groups of Arg residues

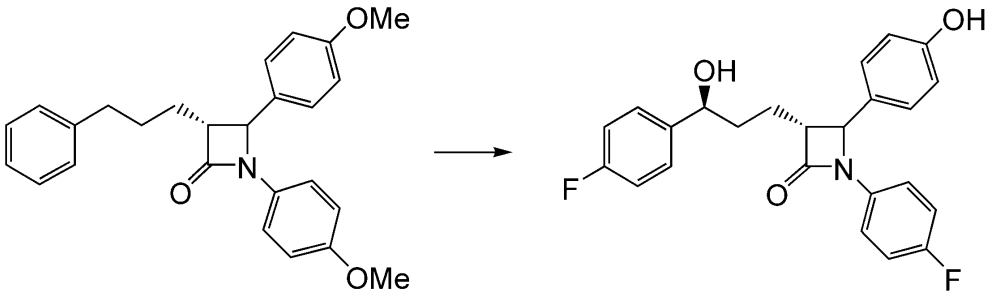
Introduction of Fluor may dramatically alter the binding mode





Müller et al, Science **317**, 2007, 1881-86

Metabolic stabilization by introducing F

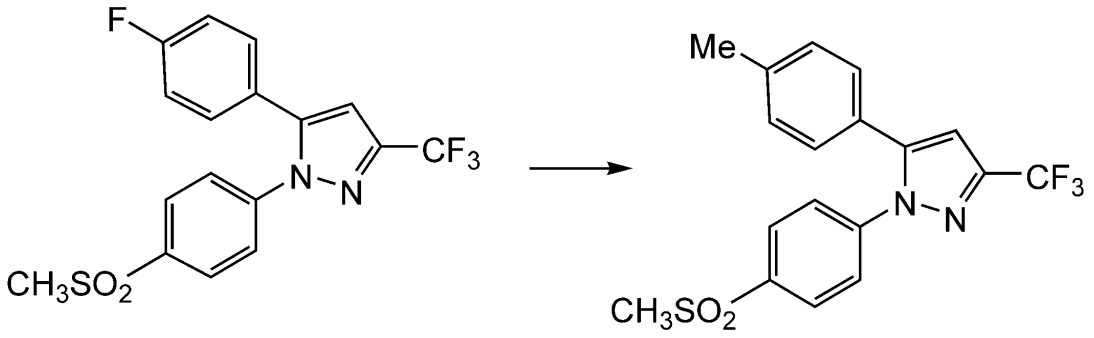


SCH 48461
ED_{50} (hamster) = 2.2 mg kg^{-1}

Ezetimib (SCH 58235,
oral cholesterol absorption inhibitor)
ED_{50} (hamster) = 0.04 mg kg^{-1}

Scheme 1. Development of Ezetimibe (SCH58235) by optimization of the lead SCH48461.[12,13] As part of the optimization, two metabolically labile sites are blocked by fluorine substituents.

Metabolic destablization by removing F



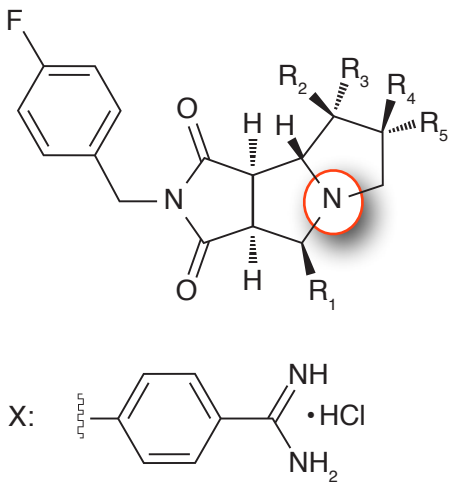
Early COX II inhibitor
$t_{1/2}$ (rat) up to 220 h

Celecoxib
$t_{1/2}$ (rat) = 3.5 h

Böhm, *ChemBioChem* 2004, **5**, 637

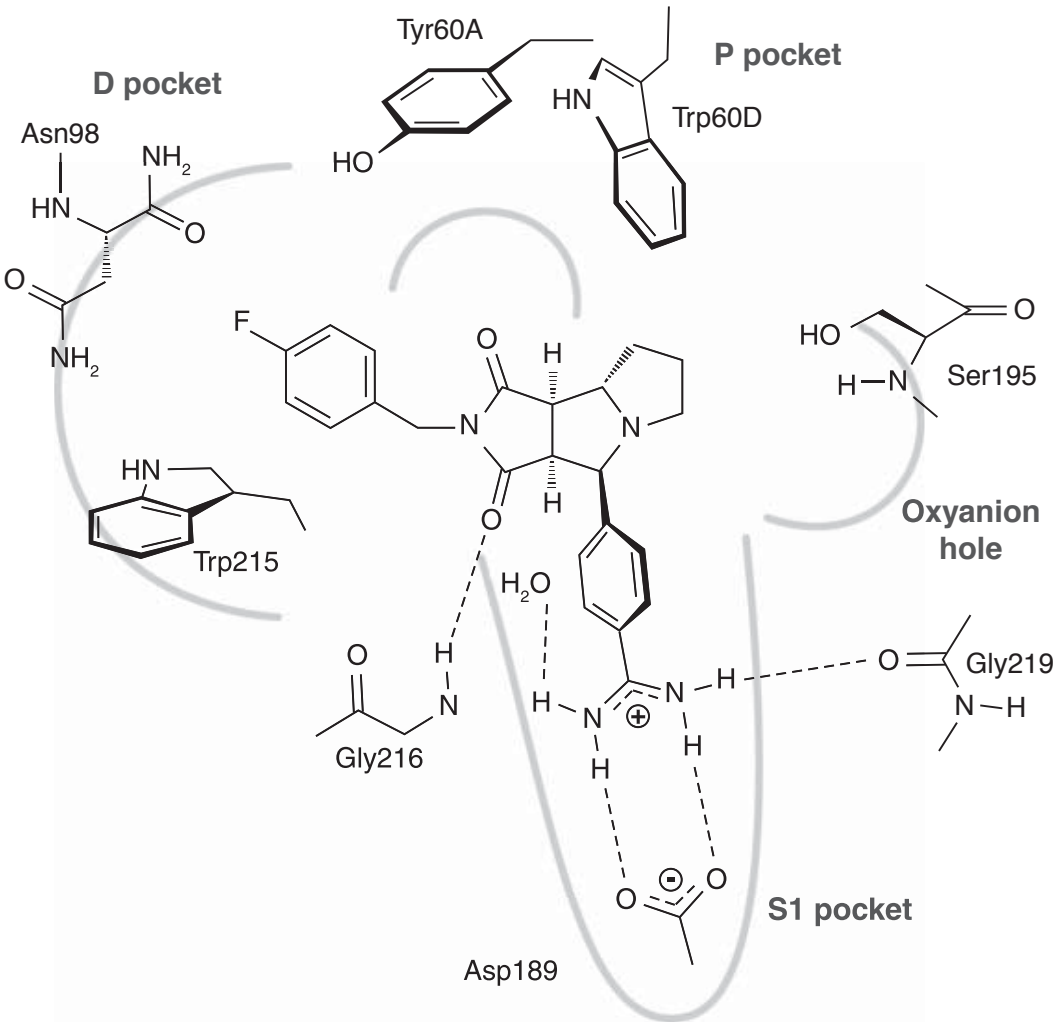
Fluor substitution alters the basicity of adjacent N centers

A



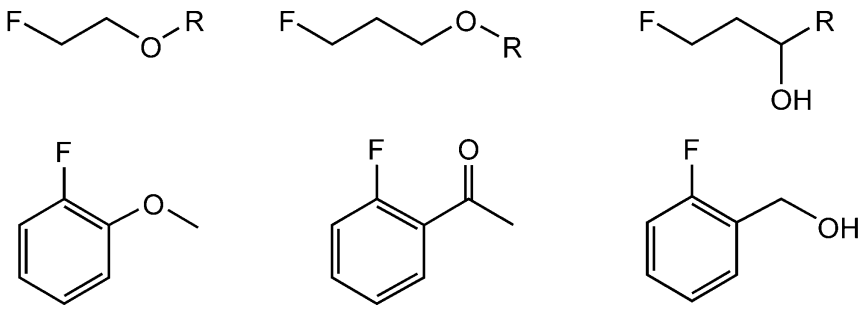
Compound	R^1	R^2	R^3	R^4	R^5	pK_a
(±)-**1**	H	H	H	H	H	7.0
(±)-**2**	X	H	H	H	H	4.5
(+)-**3**	X	F	H	H	H	3.4
(+)-**4**	X	H	F	H	H	3.3
(+)-**5**	X	H	H	F	H	3.3
(+)-**6**	X	H	H	H	F	3.3
(+)-**7**	X	OH	H	H	H	4.1
(+)-**8**	X	OMe	H	H	H	3.7
(+)-**9**	X	F	F	H	H	<2
(±)-**10**	X	H	H	F	F	<2

B



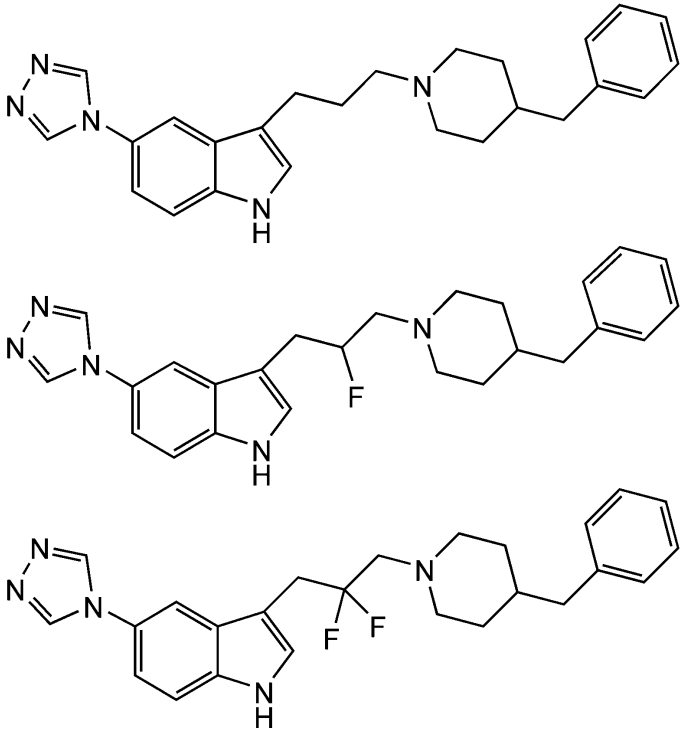
Müller et al, Science **317**, 2007, 1881-86

Improving water-solubility



Scheme 3. Chemical substructures observed in compounds for which a fluorine substituent decreases log D.

Improving bioavailability by improving membrane passage



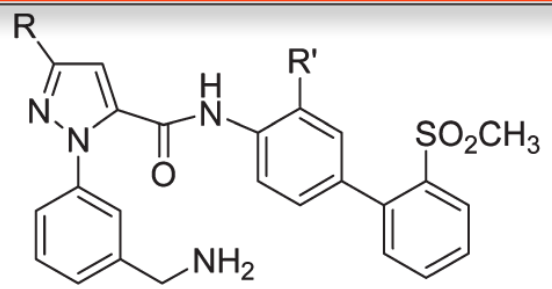
IC_{50} = 0.3 nM
EC_{50} = 0.6 nM
pK_a = 9.7
very low bioavailability

IC_{50} = 0.9 nM
EC_{50} = 0.9 nM
pK_a = 8.7
medium bioavailability
(14% in rat)

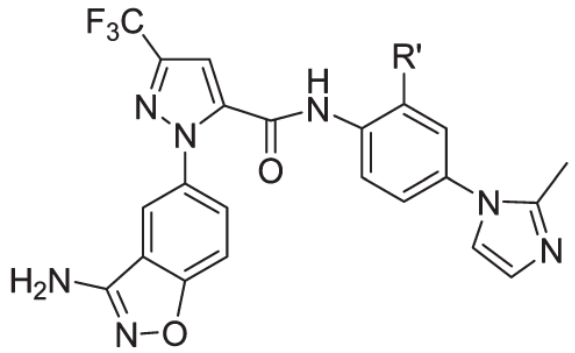
IC_{50} = 78 nM
pK_a = 6.7

Böhm et al, ChemBioChem 2004, 5, 637 ± 643

Imcreasing membrane permeability



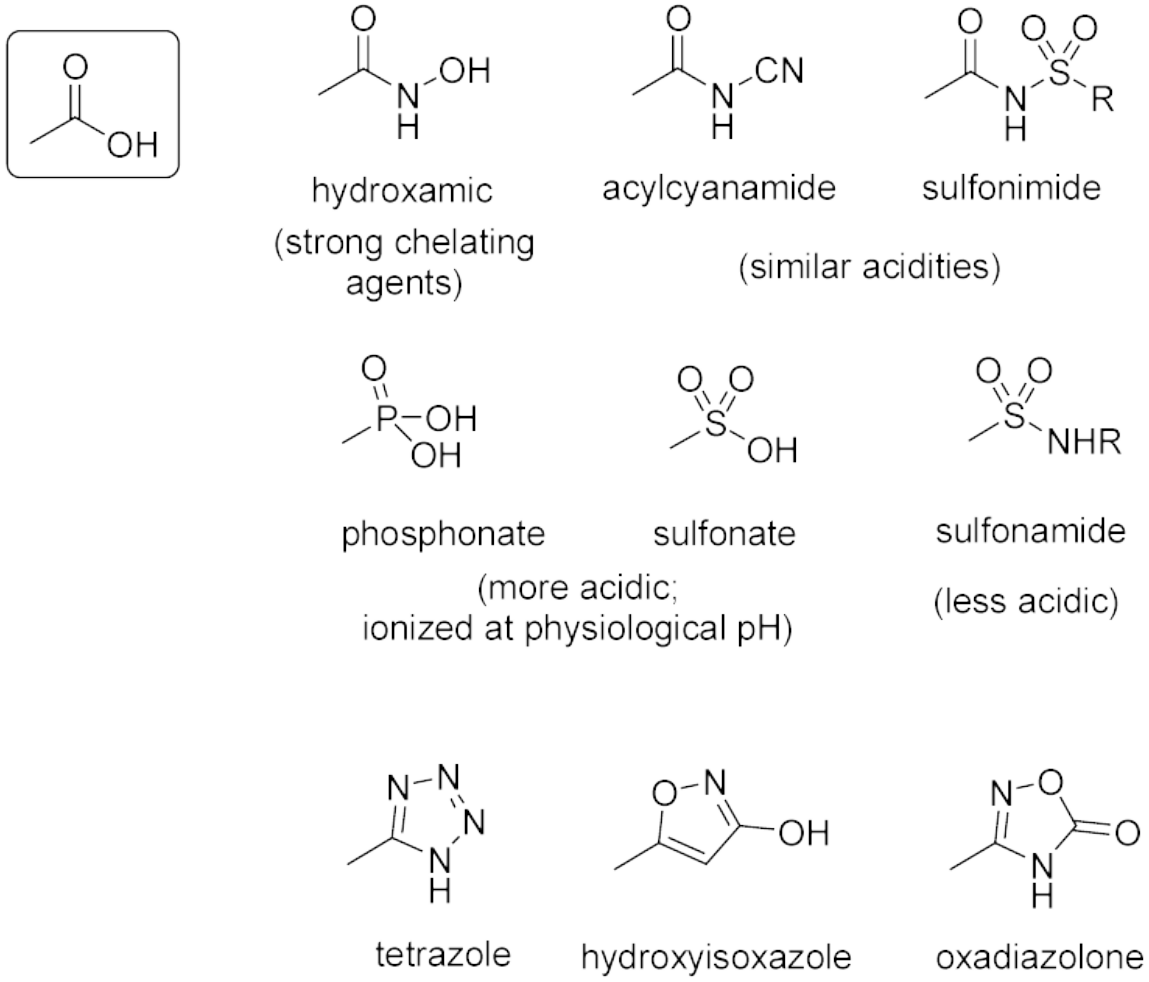
R	R'	Caco-2 permeability
CH_3	H	1.20 x 10^{-6} cm/s
CH_3	F	3.14 x 10^{-6} cm/s
CF_3	H	3.38 x 10^{-6} cm/s
CF_3	F	4.86 x 10^{-6} cm/s



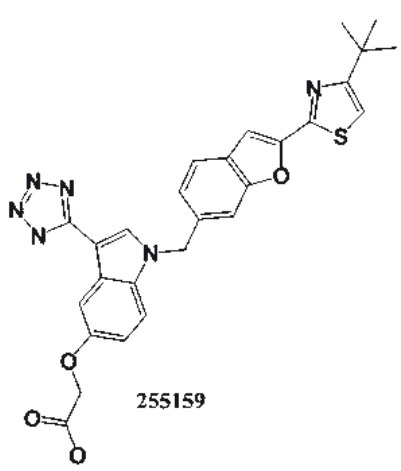
R	Caco-2 permeability
CN	<0.1 x 10^{-6} cm/s
H	0.82 x 10^{-6} cm/s
F	7.41 x 10^{-6} cm/s

Improving membrane permeability

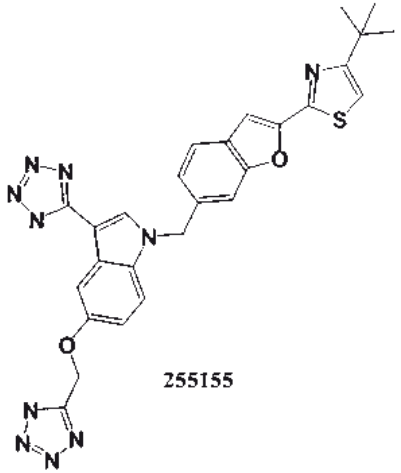
COOH replacements



Carboxyl group may be replaced in order to alter acidity, or modify lipophilicity without affecting pKa

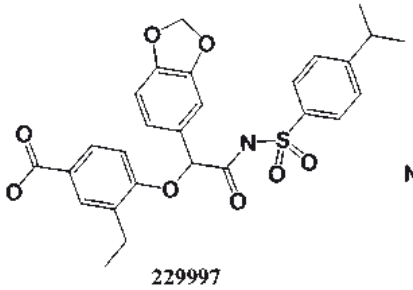


255159

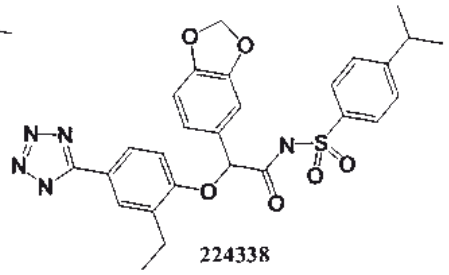


255155

T27210 Leukotriene antagonist

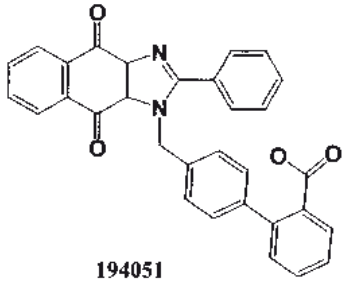


229997

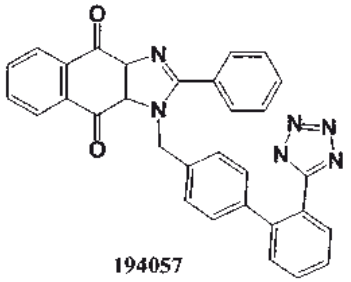


224338

T31655 Endothelin ETA antagonist

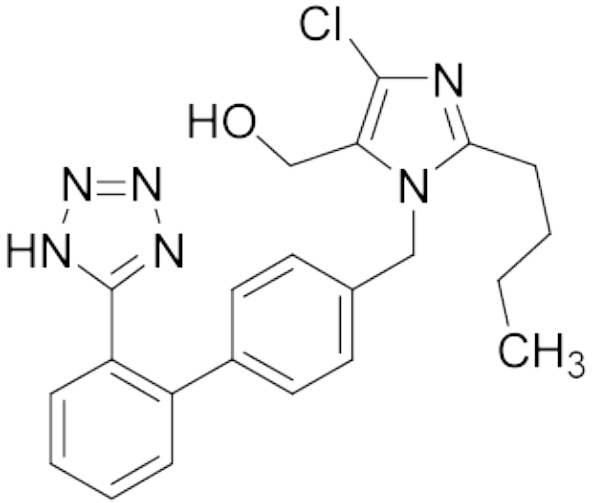


194051



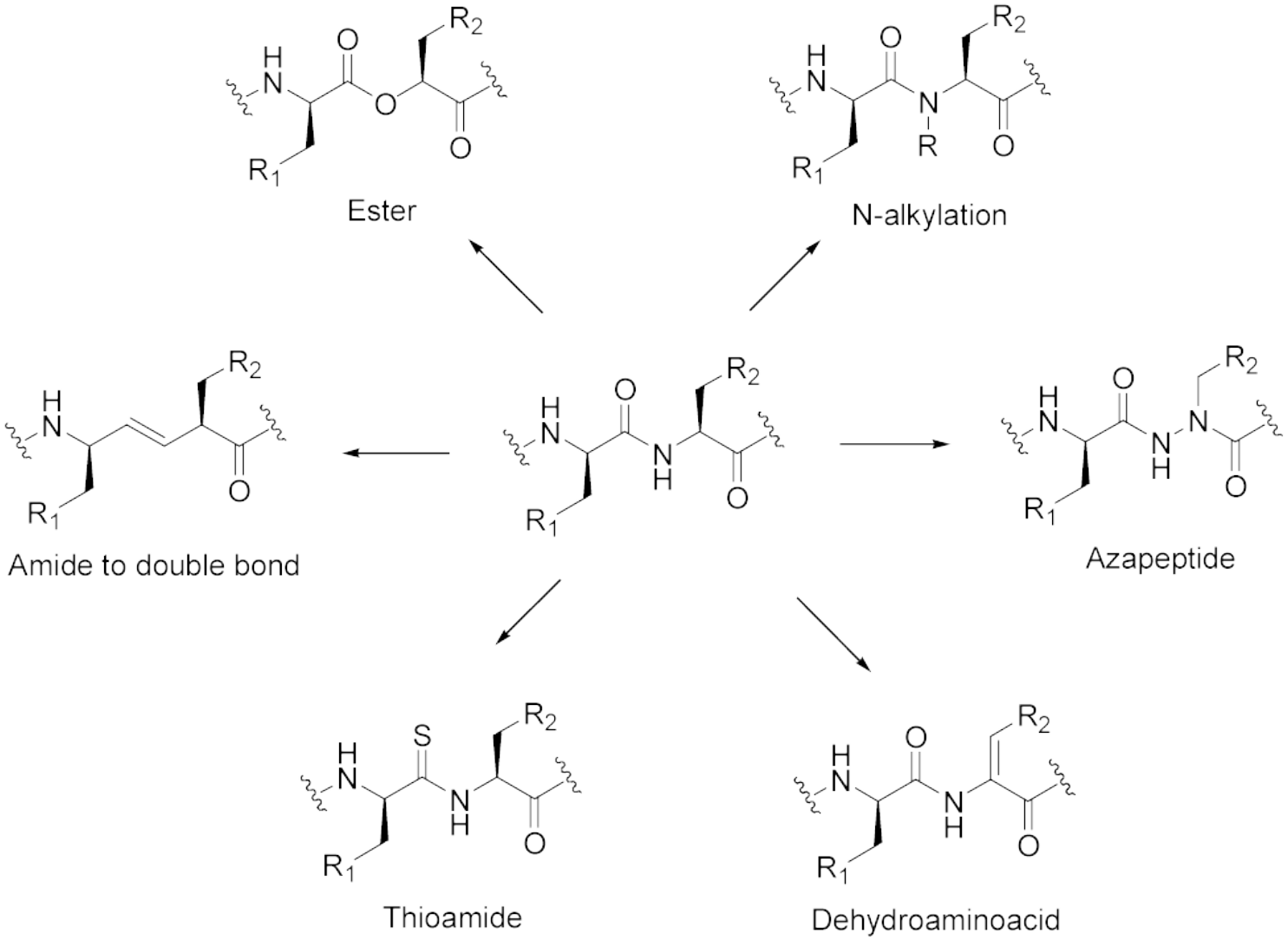
194057

T31432 Angiotensin-II AT1 antagonist



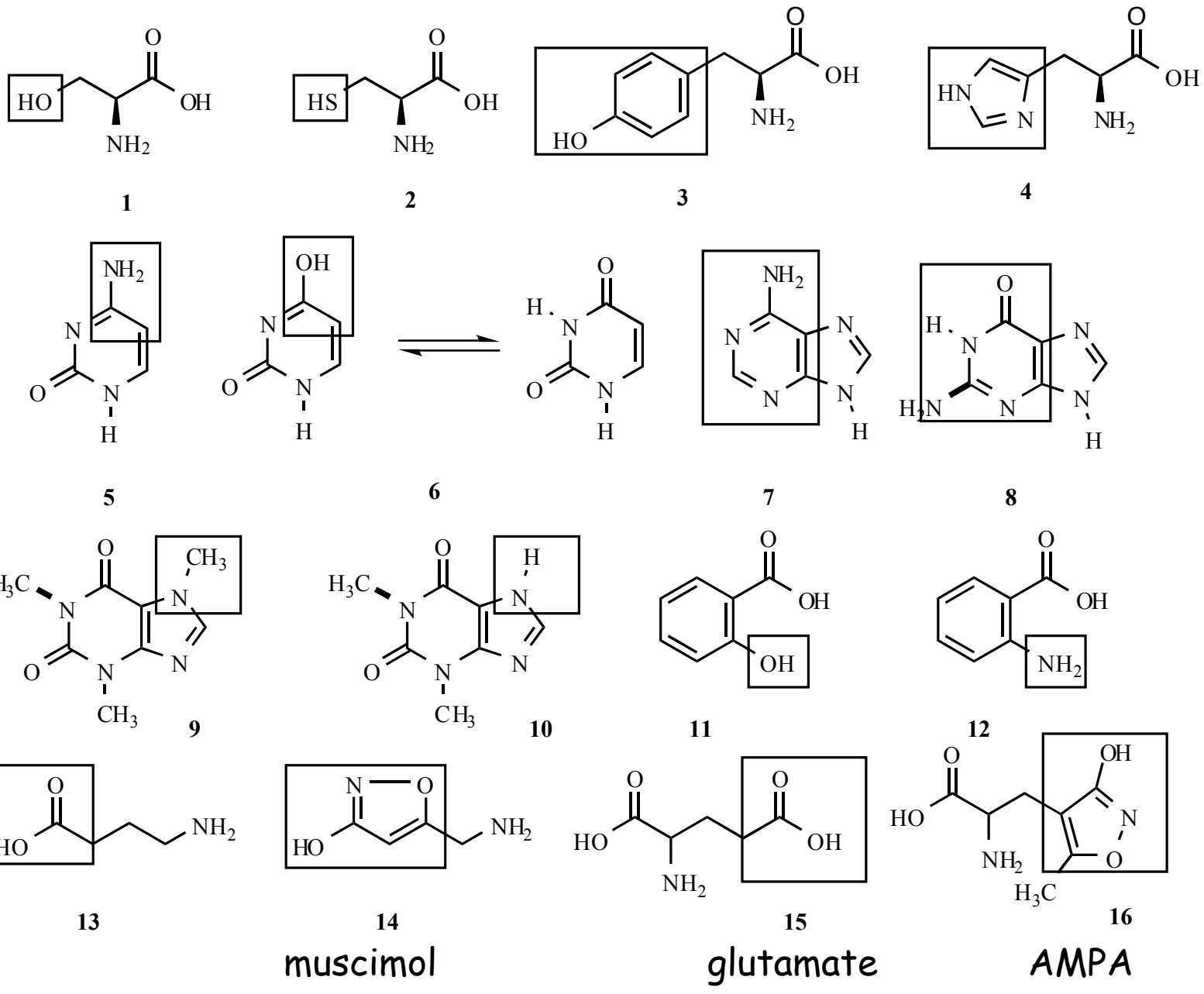
Losartan
(antihypypertensive)

Bioisosteres of the peptide bond



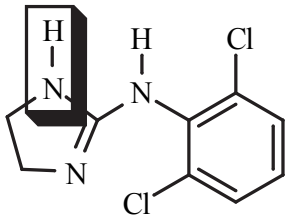
Naturally occuring bioisoteres

Current Medicinal Chemistry, 2005, Vol. 12, No. 1 **25**

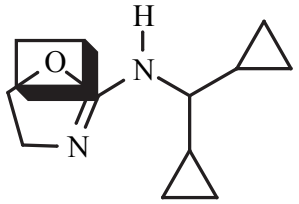


Examples

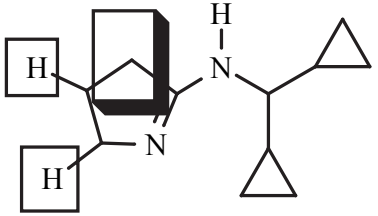
Development of anti-hypertensive drugs analogous to Clonidine (23)



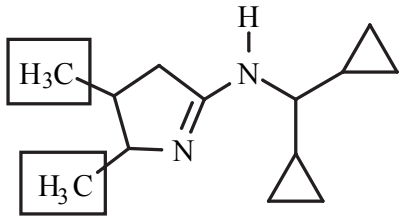
23



24



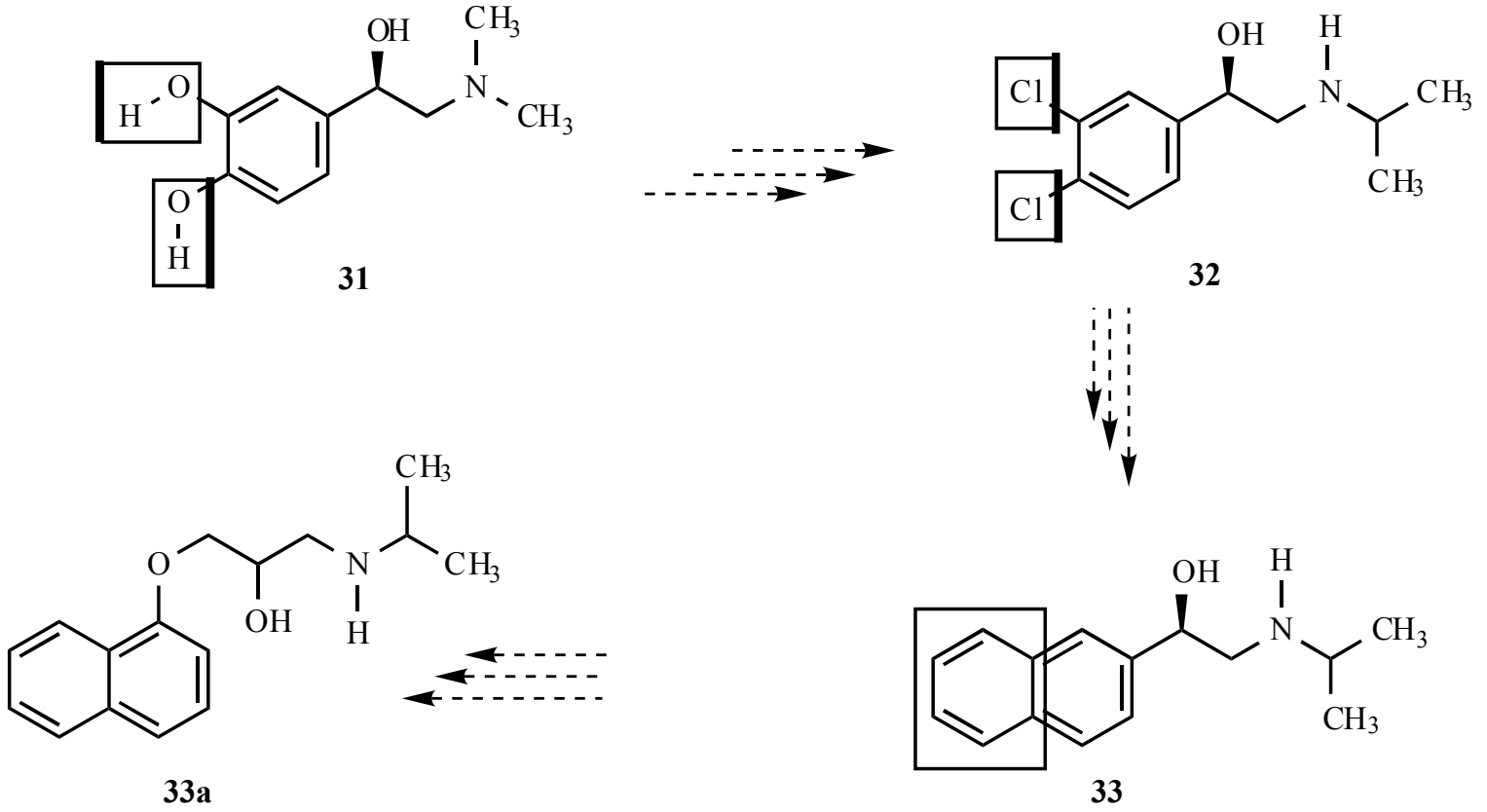
25



cis/trans

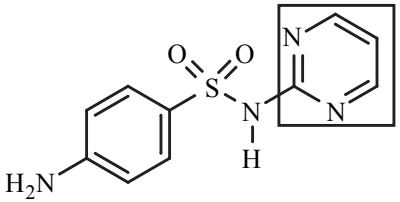
26

Conversion of isoproterenol (31), that is **susceptible to metabolic degradation**, into dichloroisoproterenol (32). Form 32 **pronethalol** (33) was discovered, a selective antagonist for adrenergic receptors, and a precursor of **propranolol** (33a)

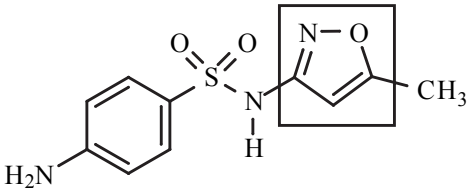


Ring-Bioisosteres

ANTIBACTERIAL

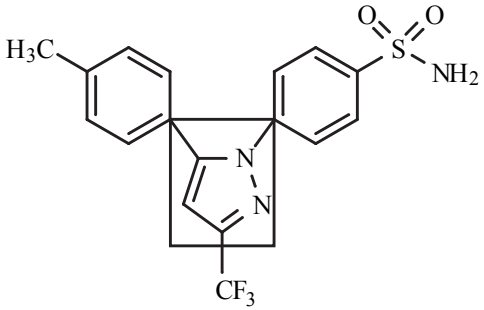


sulfadiazine, 52

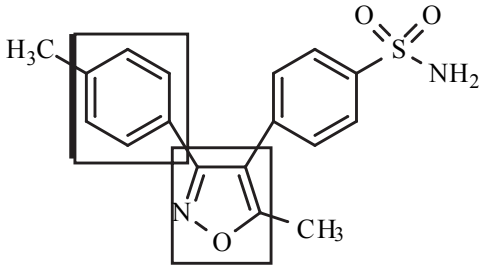


sulfamethoxazole, 53

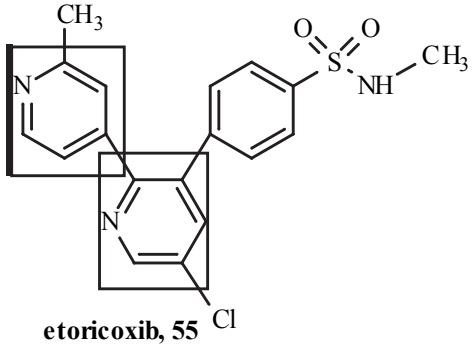
ANTI-INFLAMATORY



celecoxib, 30

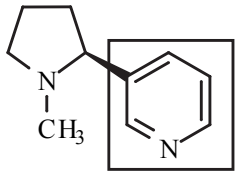


valdecoxib, 54

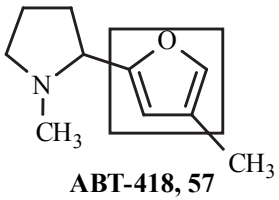


etoricoxib, 55

ANALGESIC



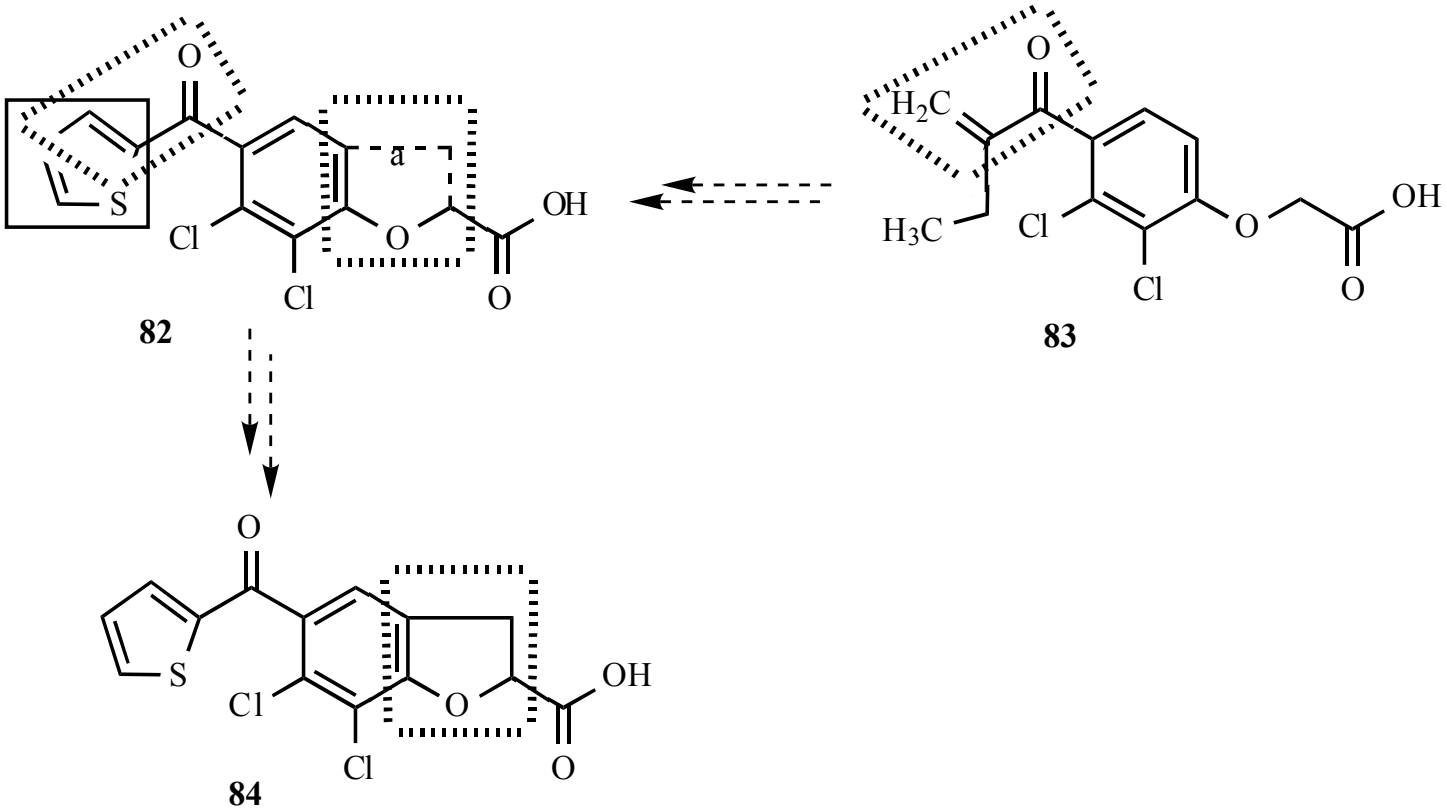
nicotine, 56



ABT-418, 57

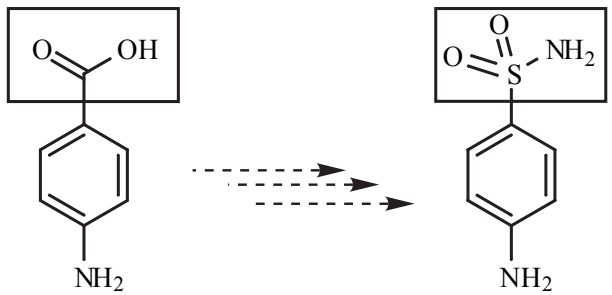
Non-classical Bioisosteres

Cyclic vs. Non-Cyclic



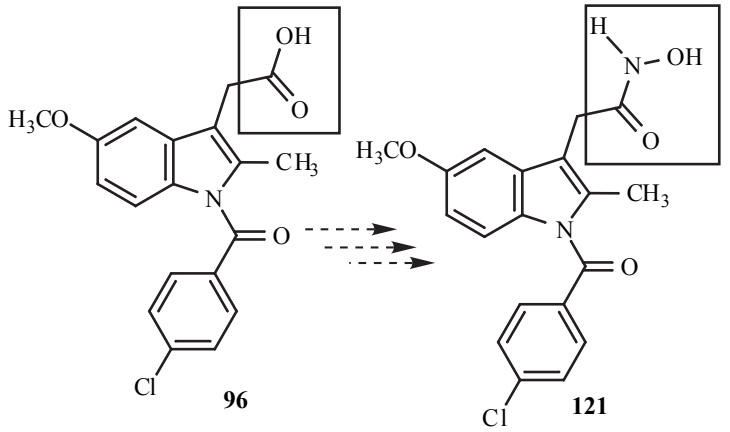
Non-classical Bioisosteres

Functional group replacements



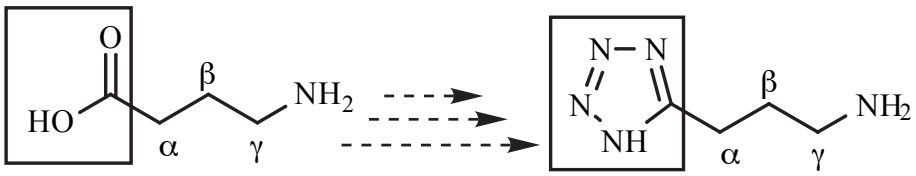
116 **117**

sulfanilamide
(active metabolite of Prontosil)
(used for chemotherapy)



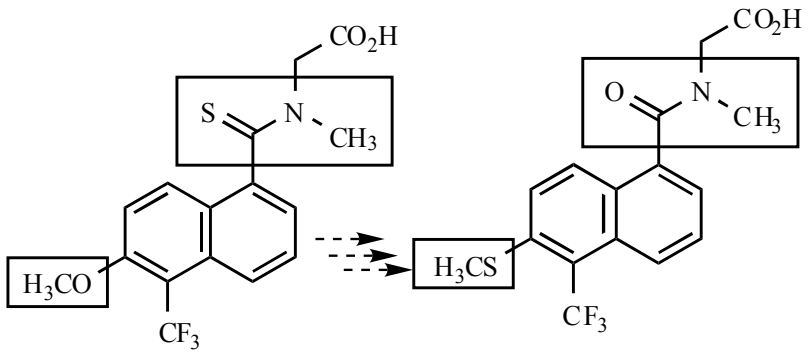
96 **121**

metabolically stable



GABA, 13 **113**

ligand to GABA receptor
passes the BBB



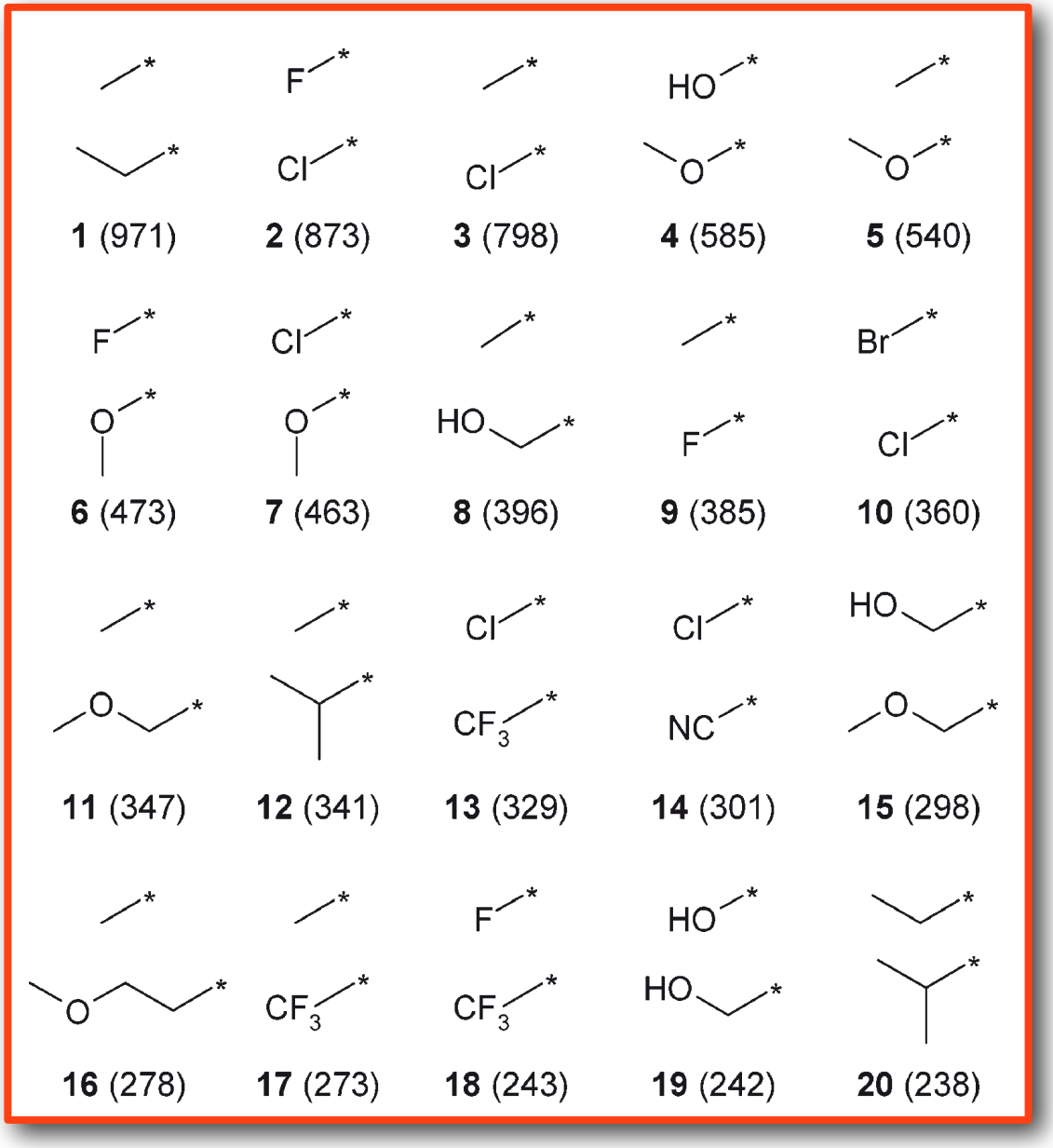
122 **123**

potent aldolase inhibitor

Making Analogues

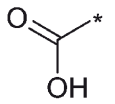
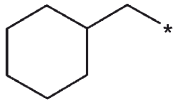
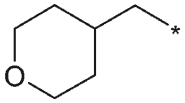
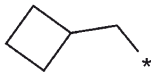
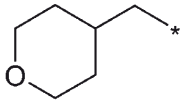
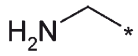
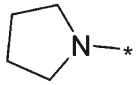
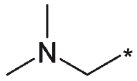
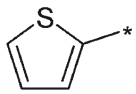
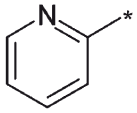
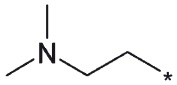
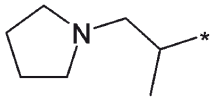
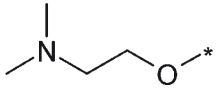
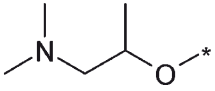
- Modifications may disrupt binding by electronic / steric effects
- Easiest analogues to make are those made from lead compound
- Possible modifications may depend on other groups present
- Some analogues may have to be made by a full synthesis(e.g. replacing an aromatic ring with a cyclohexane ring)
- Allows identification of important groups involved in binding
- Allows identification of the pharmacophore

Common sidechain replacements



Haubertin, J. Chem. Inf. Model. **47** (2007), 1294-1302

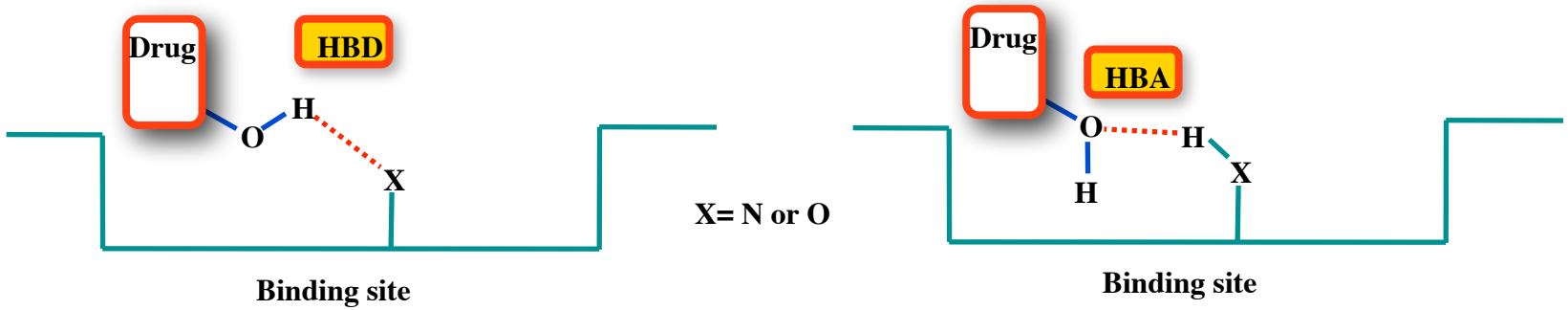
Sidechain modifications resulting in increased solubility

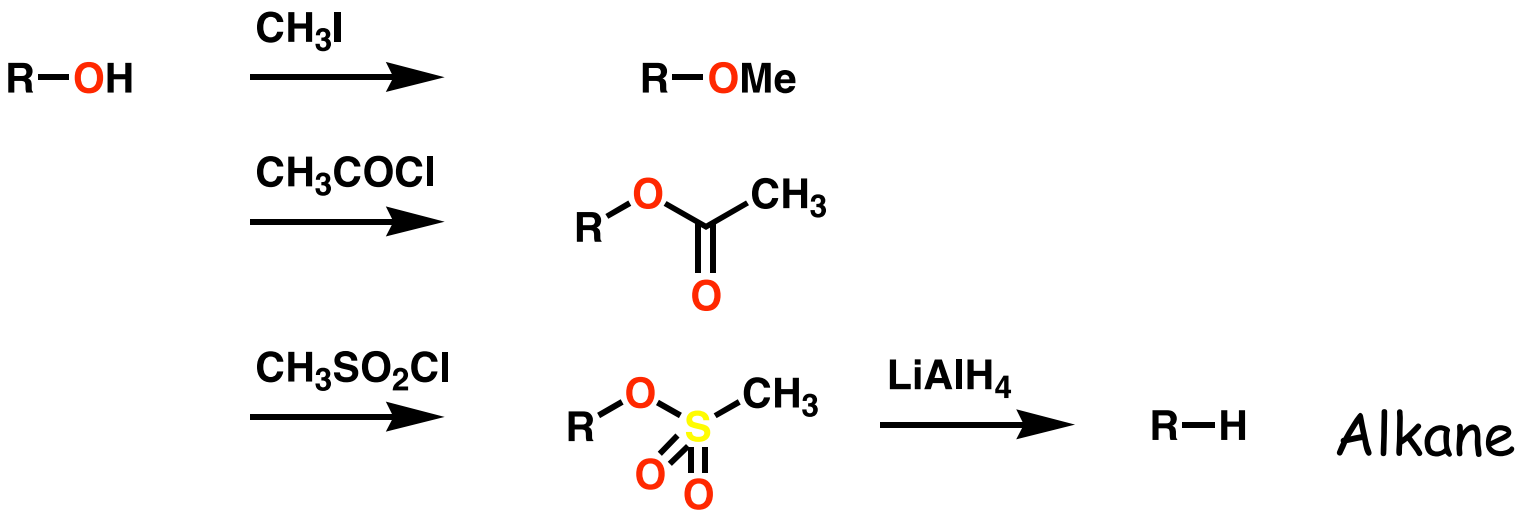
ij	Sch. i	Sch. j	Δ Log (Solubility / M)
1			c 2.32 +/- 1.11 (16) P > 0 : 0.998
2			N 2.16 +/- 0.82 (12) P > 0 : 0.999
3			N 1.66 +/- 0.58 (16) P > 0 : 0.999
4			c 1.65 +/- 0.73 (21) P > 0 : 0.999
5			C 1.46 +/- 0.86 (62) P > 0 : 0.991
6			C 1.46 +/- 0.86 (77) P > 0 : 0.991
7			C 1.28 +/- 0.85 (42) P > 0 : 0.984
8			O 1.06 +/- 0.33 (12) P > 0 : 0.999
9			c 1.02 +/- 0.57 (26) P > 0 : 1

Haubertin, J. Chem. Inf. Model. **47** (2007), 1294-1302

SAR on Alcohols

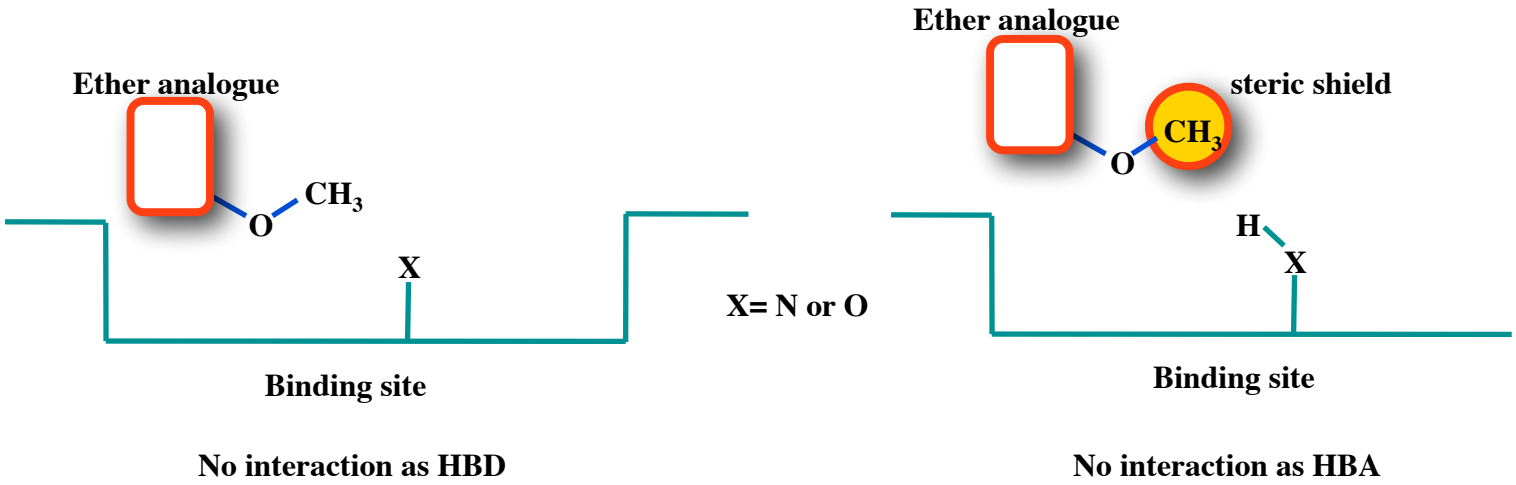
Possible binding interactions





SAR on Alcohols

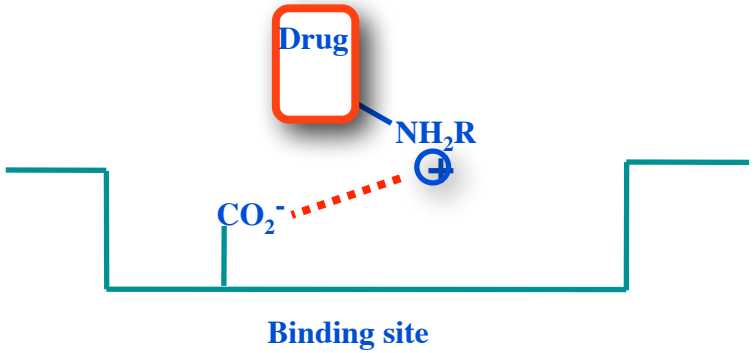
Possible effect of analogues on binding
(e.g. ether)



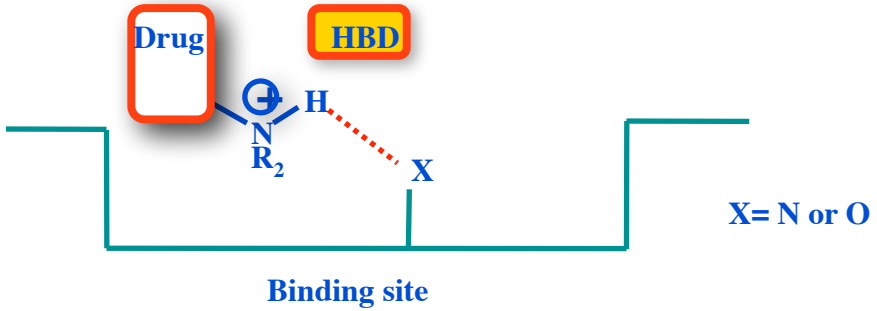
SAR on 1°, 2° & 3° Amines (RNH_2, RNHR, R_3N)

Possible binding interactions if amine is ionized

Ionic



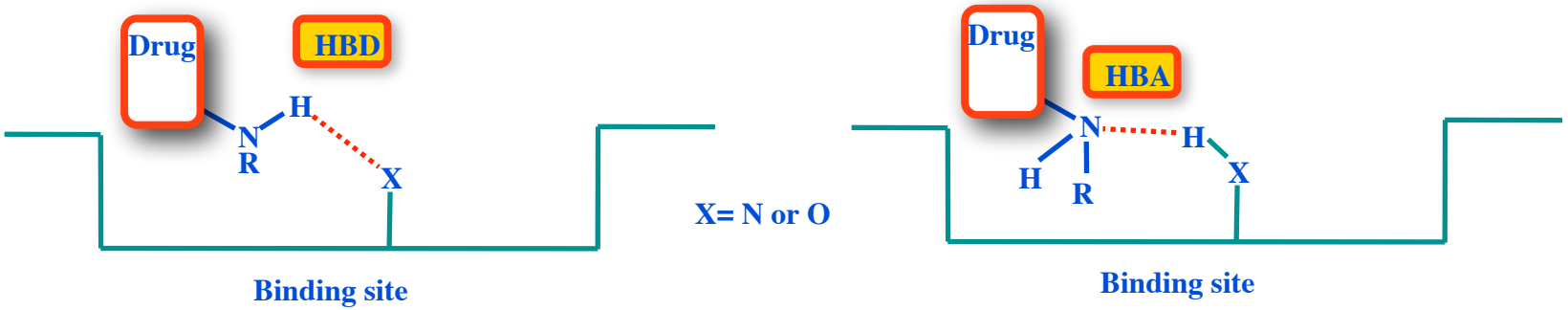
H-Bonding



SAR on 1°, 2° & 3° Amines (RNH_2, RNHR, R_3N)

Possible binding interactions for free base

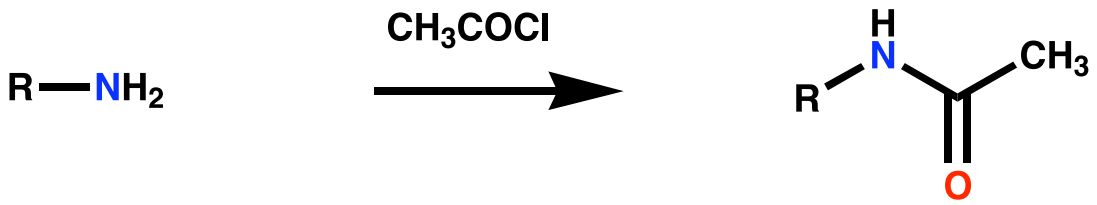
H-Bonding



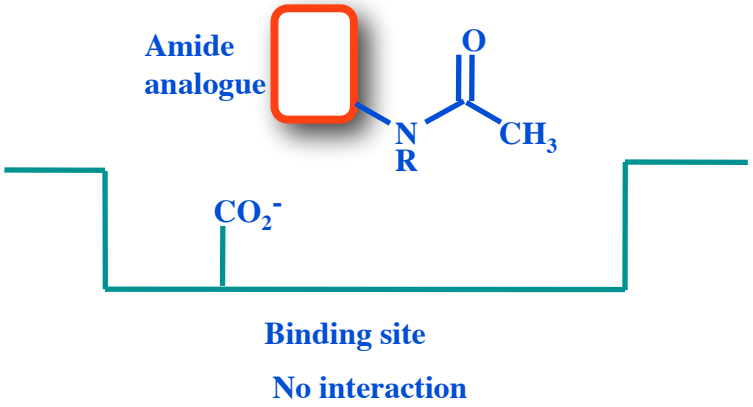
Note:
3° Amines are only able to act as HBA's - no hydrogen available to act as HBD

SAR on 1°, 2° & 3° Amines (RNH_2, RNHR, R_3N)

Analogues of
1° & 2° amines



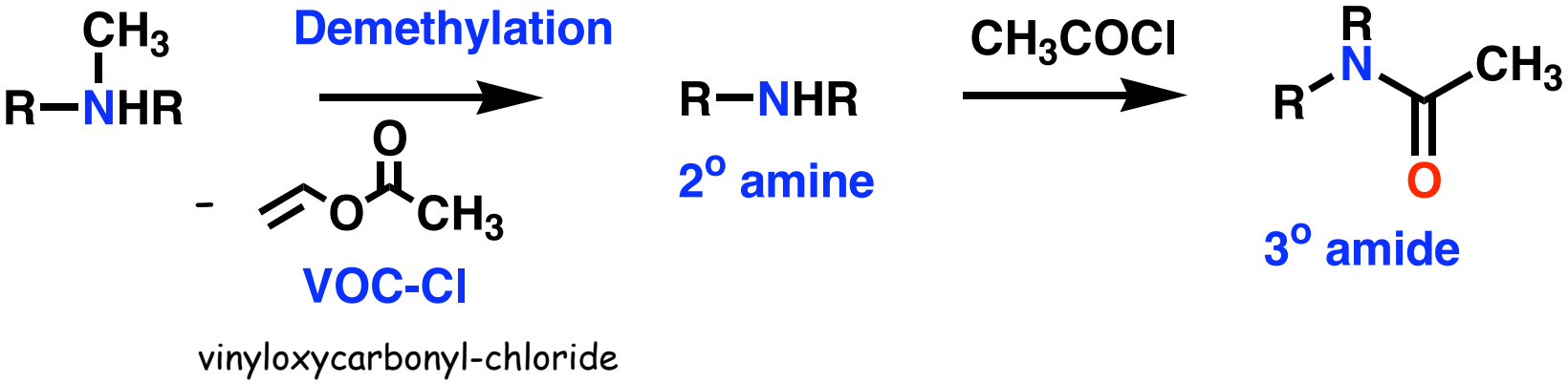
Effect on binding



- 1° and 2° amines are converted to 2° and 3° amides respectively
- Amides cannot ionize and so ionic bonding is not possible
- An amide N is a poor HBA and so this eliminates HBA interactions
- Steric effect of acyl group is likely to hinder NH acting as a HBD (2o amide)

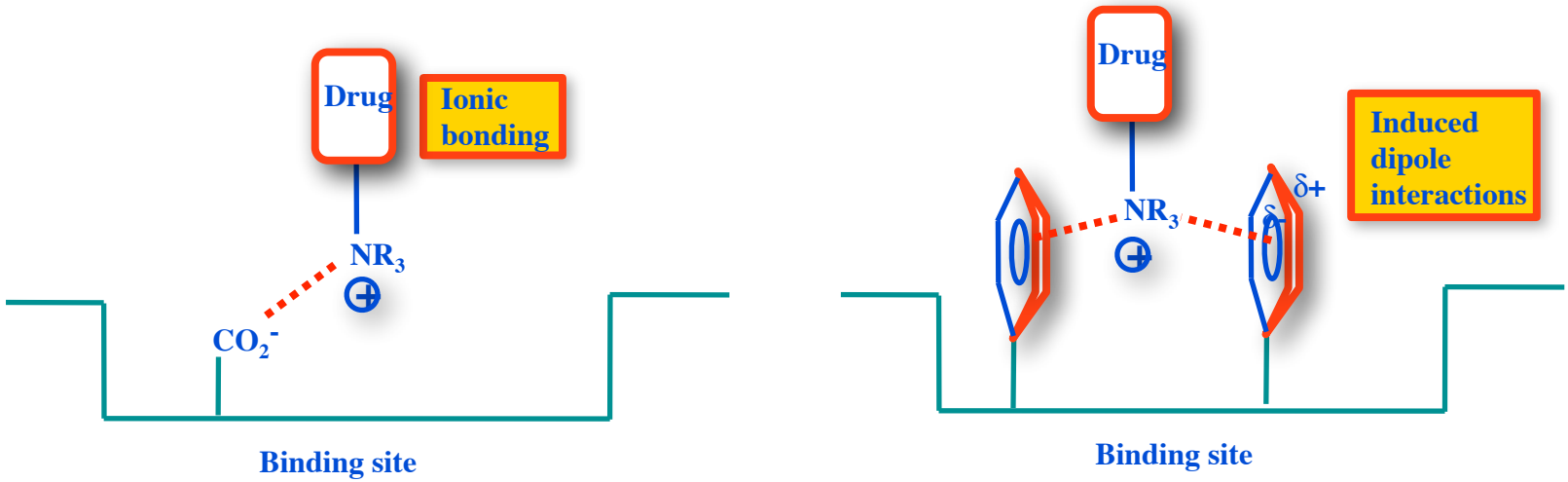
SAR on 1°, 2° & 3° Amines (RNH_2, RNHR, R_3N)

Analogues of 3° amines containing a methyl substituent



SAR on Quaternary Ammonium Salts (R_4N^+)

Possible binding interactions

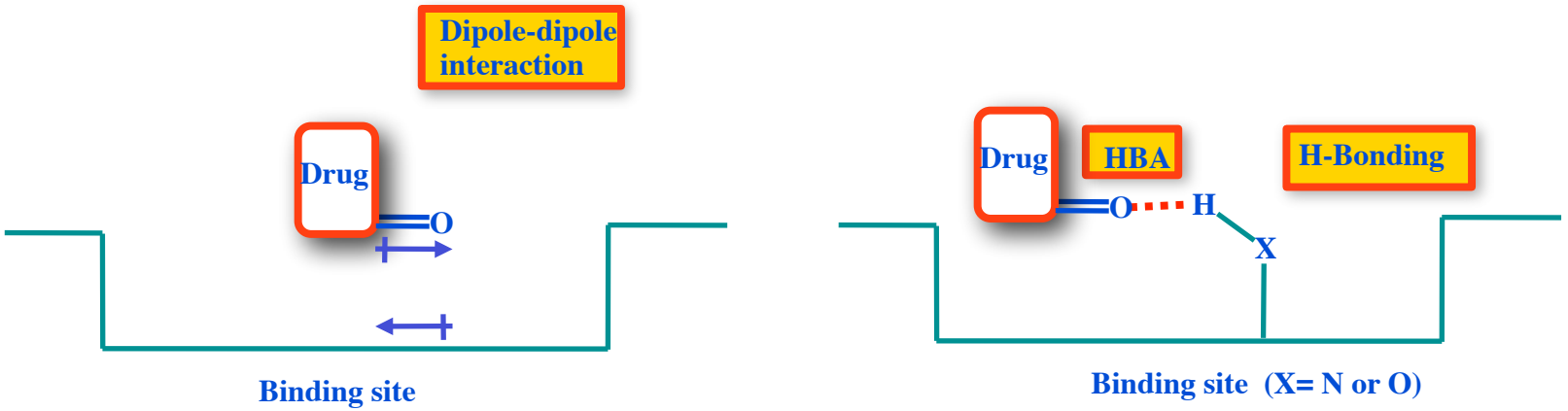


Analogues

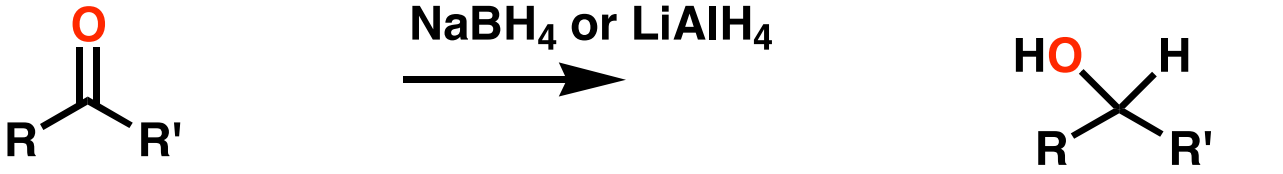
Full synthesis of 1°-3° amines and amides

SAR on Aldehydes and Ketones

Possible binding interactions



Analogues



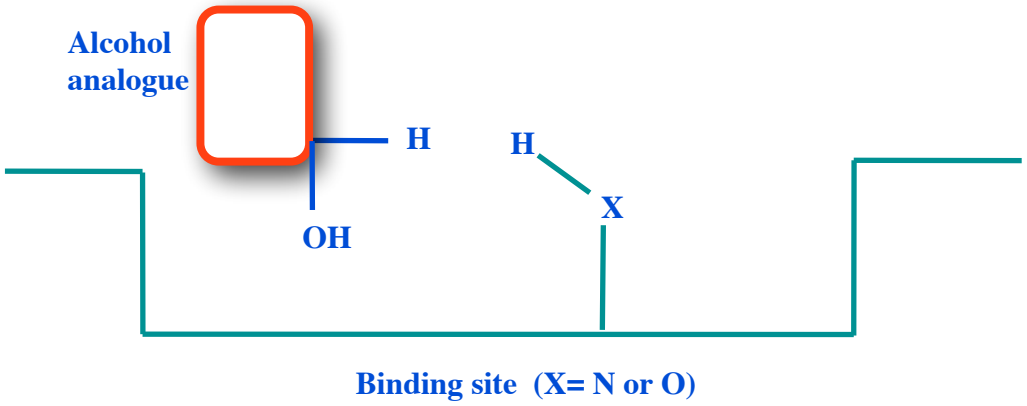
Ketone
Planar sp^2 carbon centre

2^o Alcohol
Tetrahedral sp^3 carbon centre

SAR on Aldehydes and Ketones

Effect on binding

Change in stereochemistry (planar to tetrahedral)
May move oxygen out of range



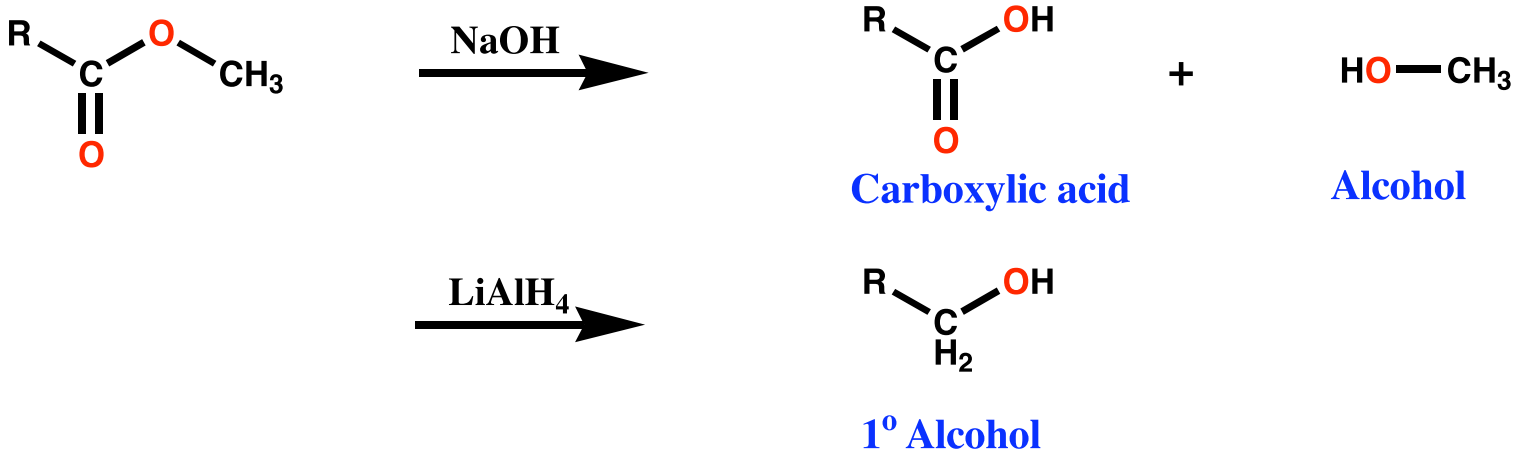
If still active, further reactions can be carried out on alcohol to establish importance of oxygen

SAR on Esters

Possible binding interactions

H-bonding as HBA by either oxygen

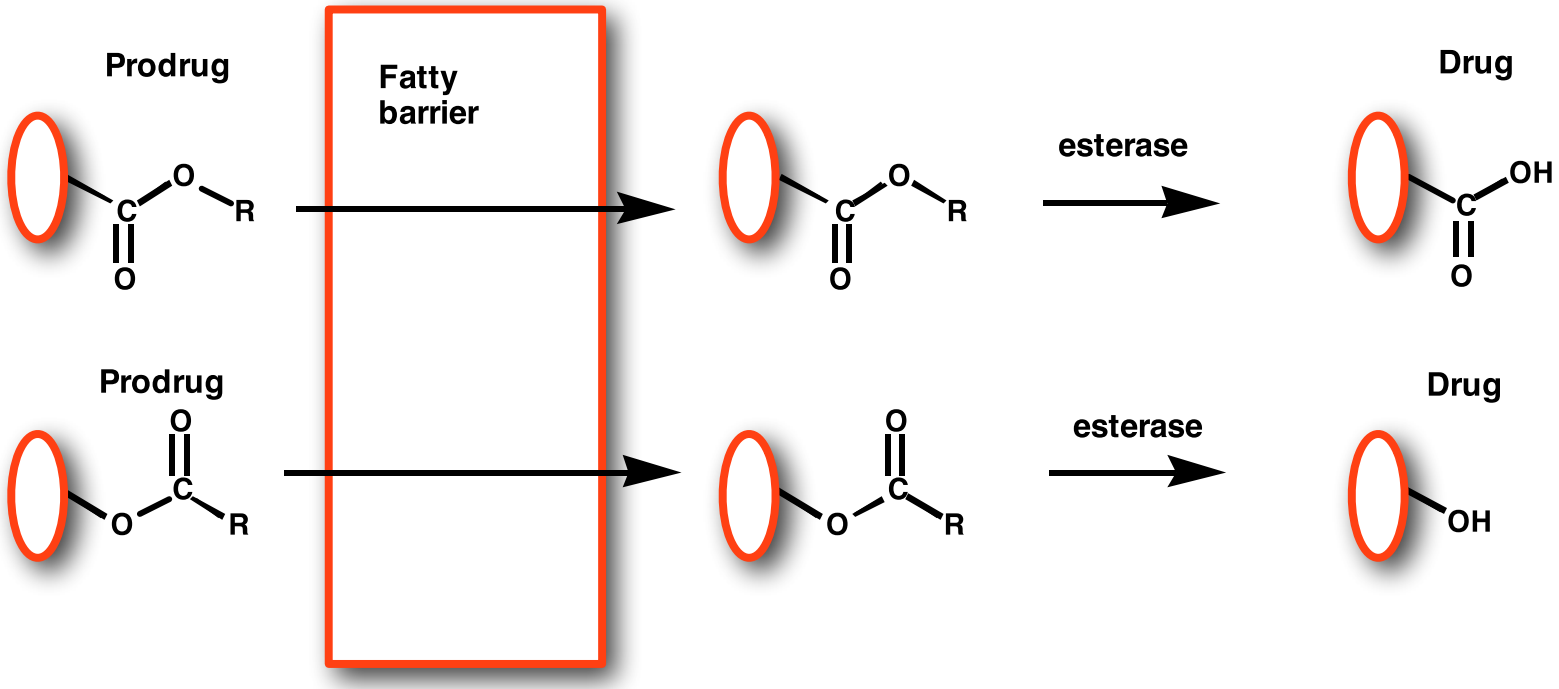
Analogues



- Hydrolysis splits molecule and may lead to a loss of activity due to loss of other functional groups - only suitable for simple esters.
- Hydrolysis leads to a dramatic increase in polarity which may influence ability of analogue to reach target if in-vivo tests are used
- Reduction to alcohol removes carbonyl group and can establish importance of the carbonyl oxygen, but reaction can be difficult to do if other labile functional groups are present

SAR on Esters

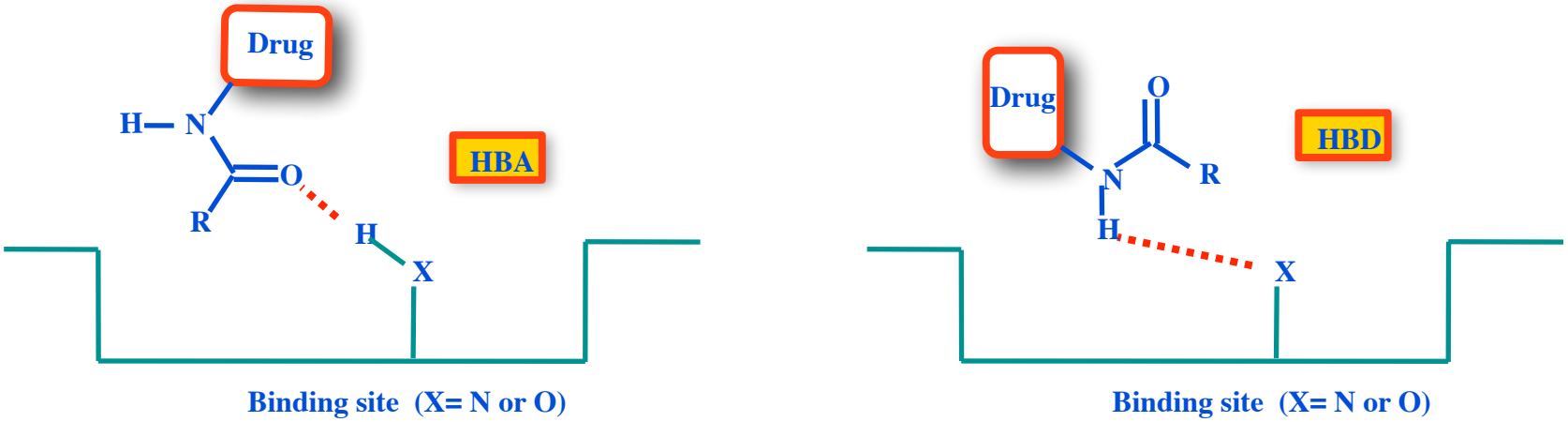
Esters are usually hydrolyzed by esterases in the blood
Esters are more likely to be important for pharmacokinetic reasons i.e. acting as prodrugs



Ester masking polar groups allowing passage through fatty cell membranes

SAR on Amides

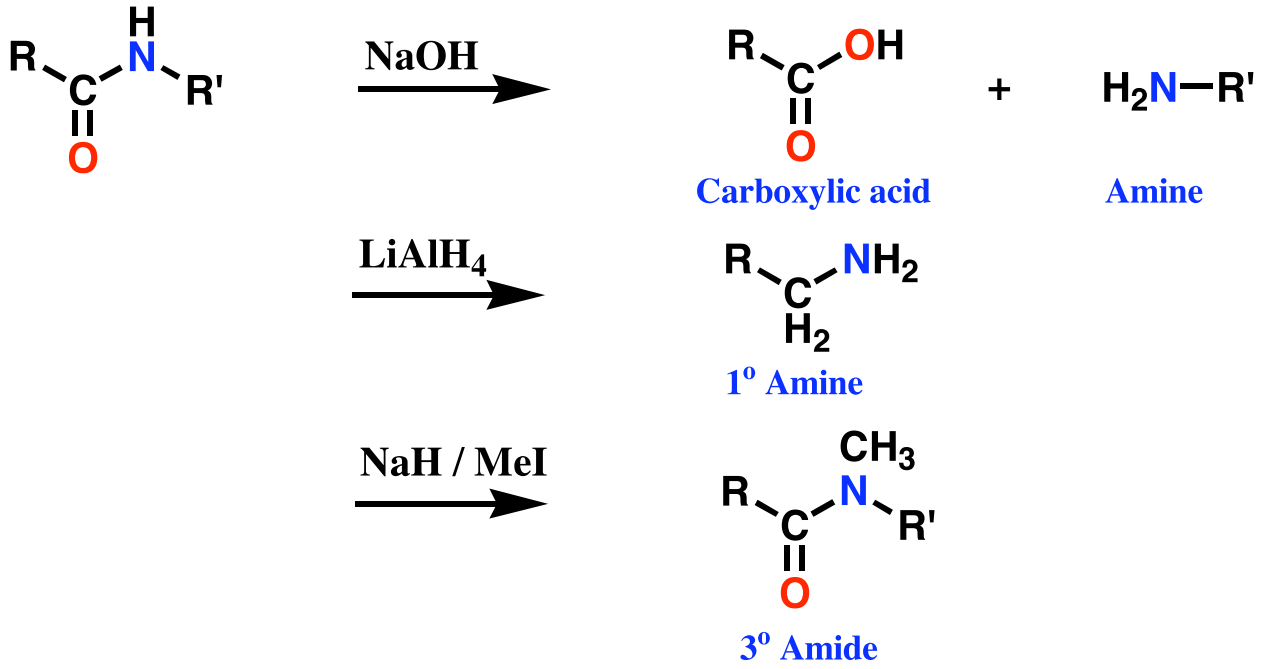
Possible binding interactions



- The nitrogen of an amide cannot act as a HBA - lone pair interacts with neighboring carbonyl group
- Tertiary amides unable to act as HBD's

SAR on Amides

Analogues

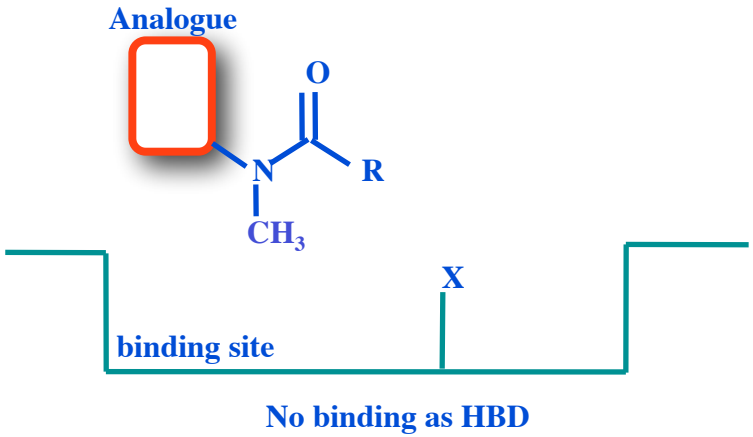


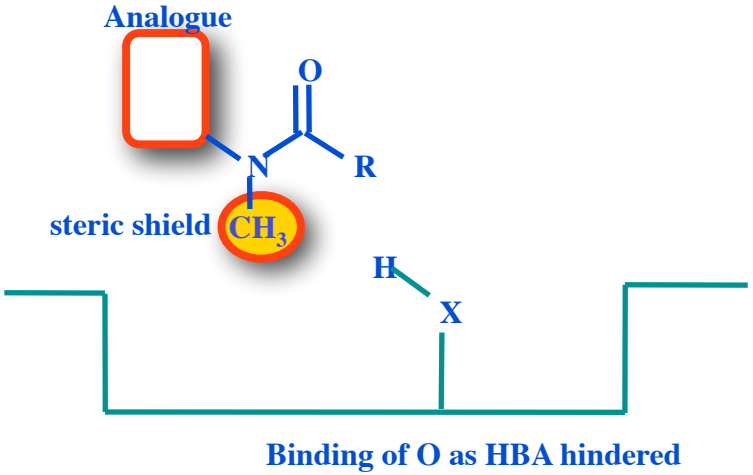
- Hydrolysis splits molecule and may lead to loss of activity due to loss of other functional groups - only suitable for simple amides.
- Hydrolysis leads to dramatic increase in polarity which may affect ability of analogue to reach target if in-vivo tests are done
- Reduction to amine removes carbonyl group and can establish importance of the carbonyl oxygen, but reaction may be difficult to do if other labile groups are present

SAR on Amides

Analogues

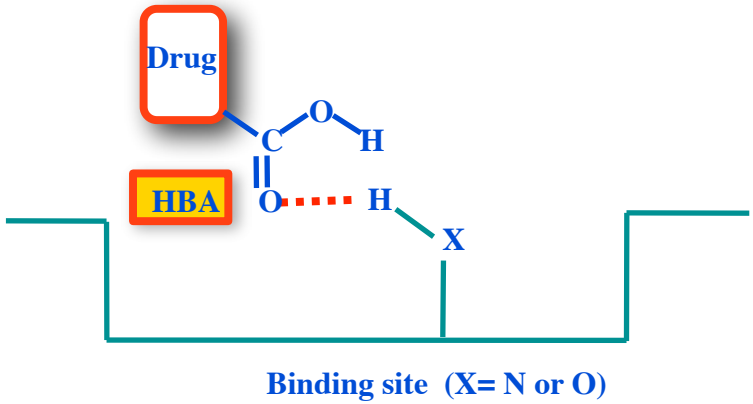
N-methylation prevents HBD interaction and may introduce a steric effect that prevents an HBA interaction

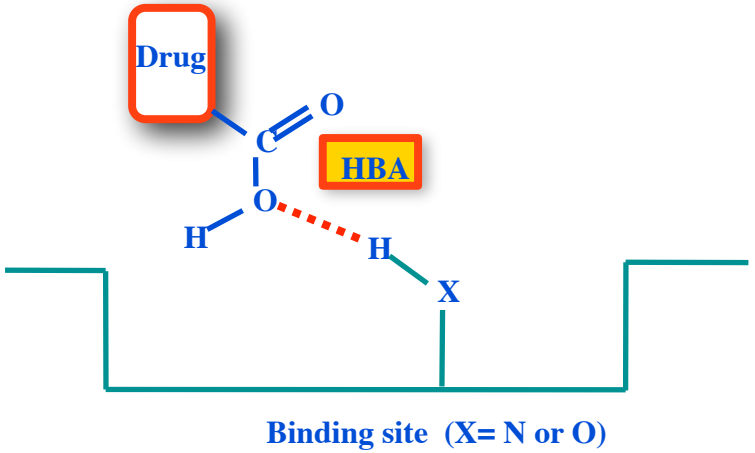


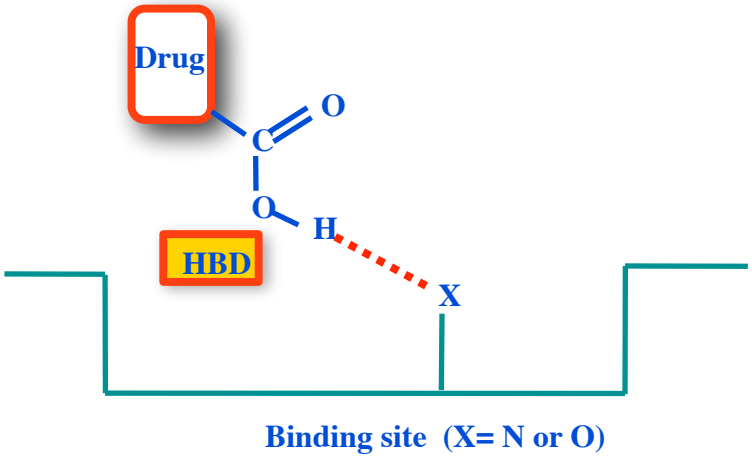


SAR on Carboxylic Acids

Possible binding interactions as free acid

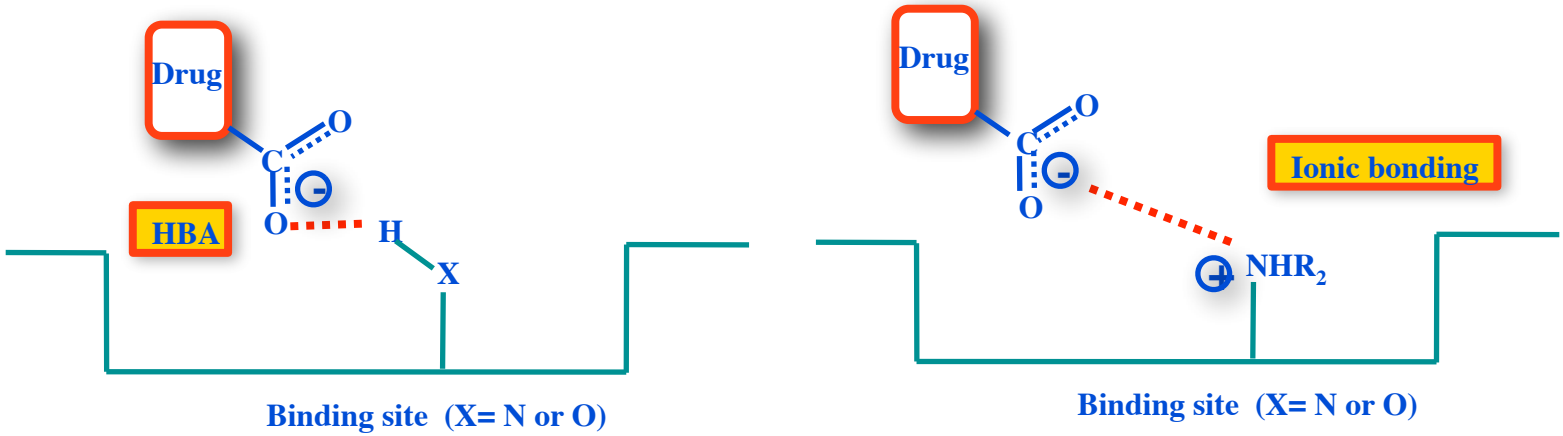






SAR on Carboxylic Acids

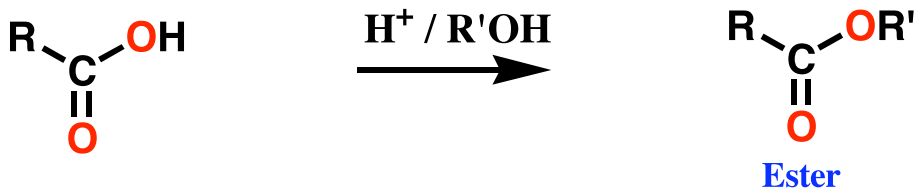
Possible binding interactions as carboxylate ion



- Charged oxygen atoms are strong HBA's
- Group could interact both as an ion and as a HBA at the same time

SAR on Carboxylic Acids

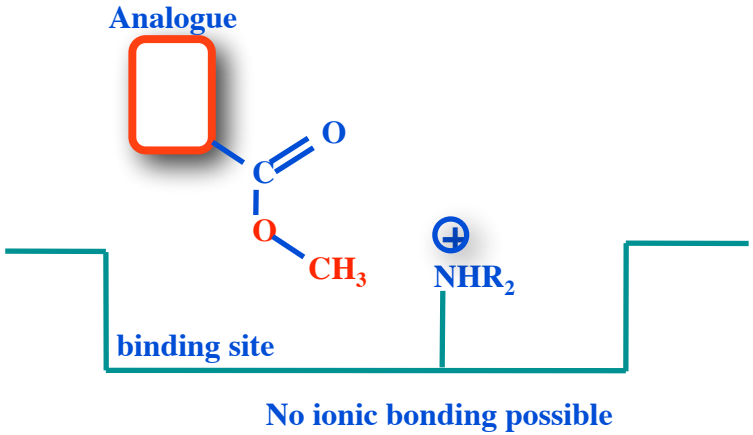
Possible analogues

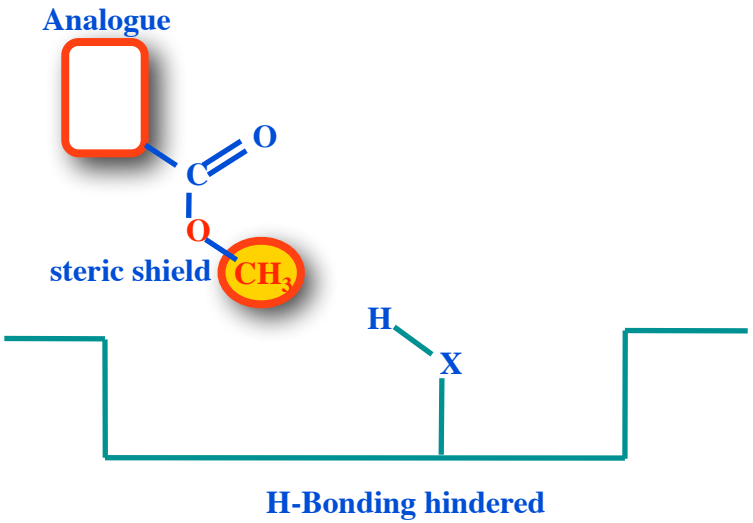


Possible effects

Reduction removes carbonyl oxygen as potential HBA and prevents ionization

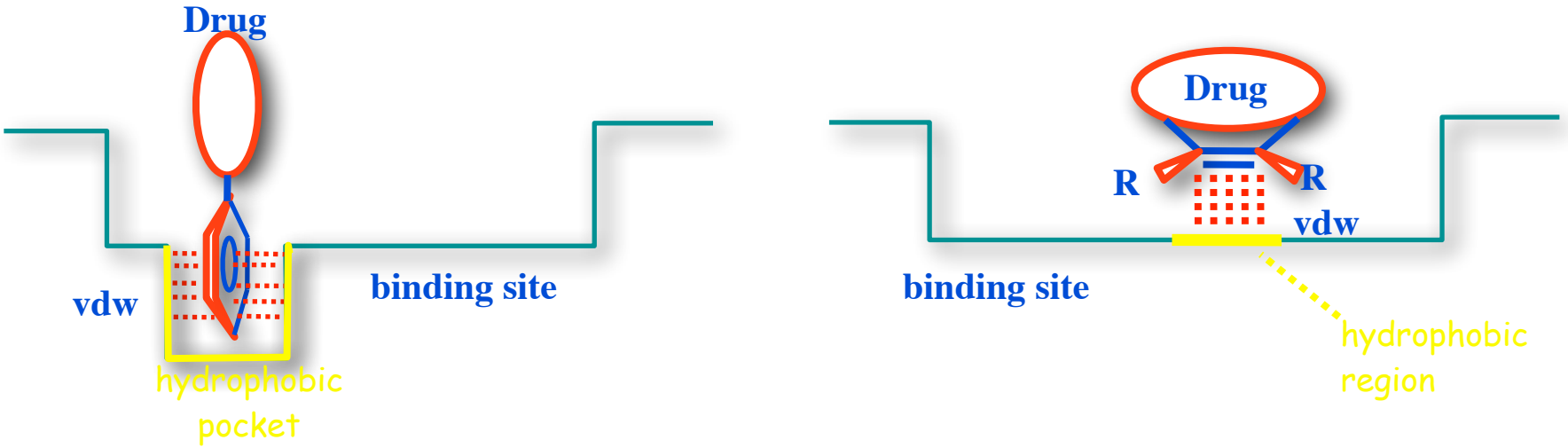
Esterification prevents ionization, HBD interactions and may hinder HBA by a steric effect



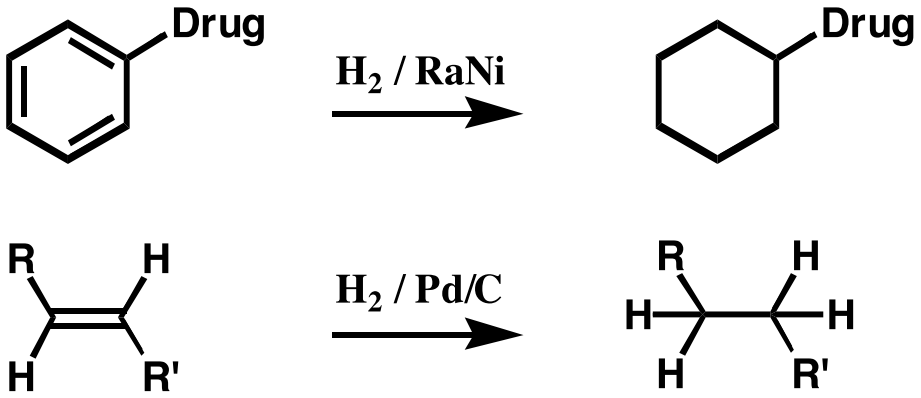


SAR on Aromatic Rings and Alkenes

Possible binding interactions

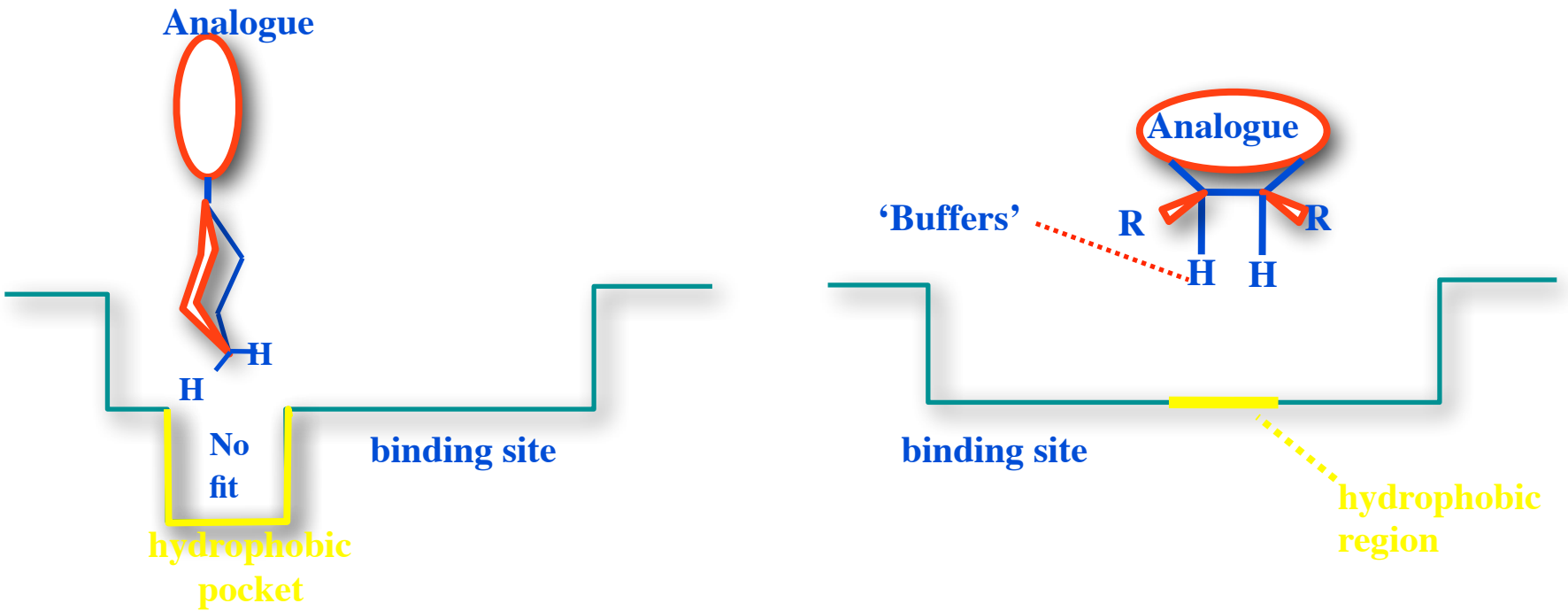


Possible analogues



SAR on Aromatic Rings and Alkenes

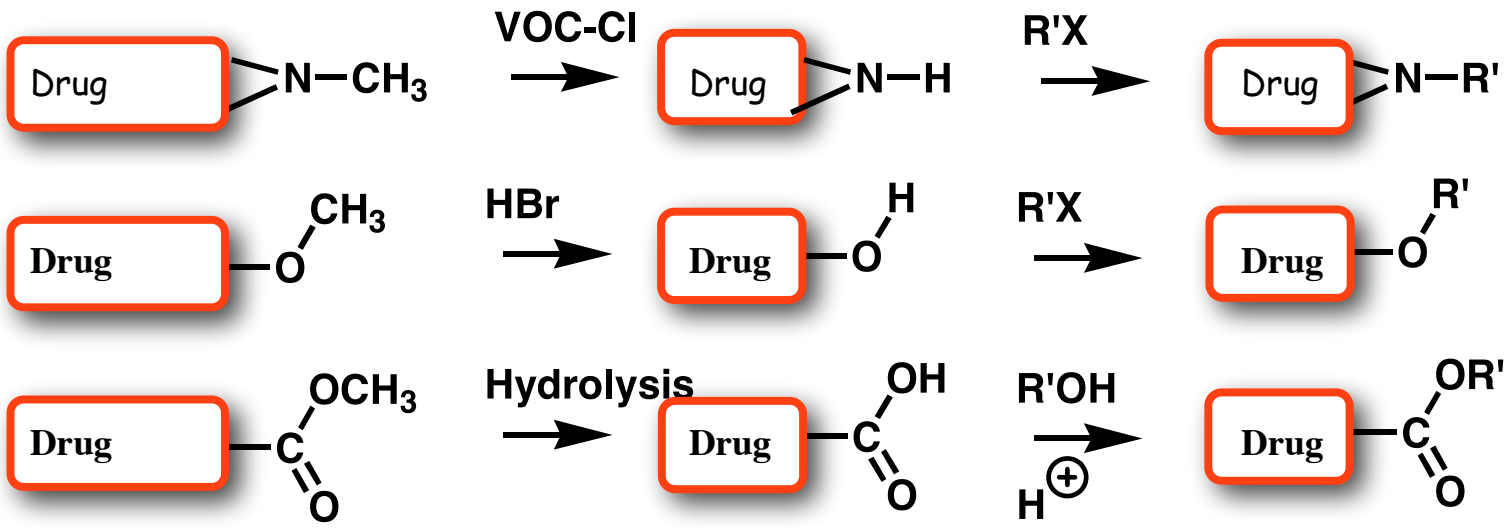
Possible effects on binding



SAR of Alkyl Groups

Analogues

Easiest alkyl groups to vary are substituents on heteroatoms
Vary length and bulk of alkyl group to test space available



Quantitative Structure-Activity Relationships (QSAR)

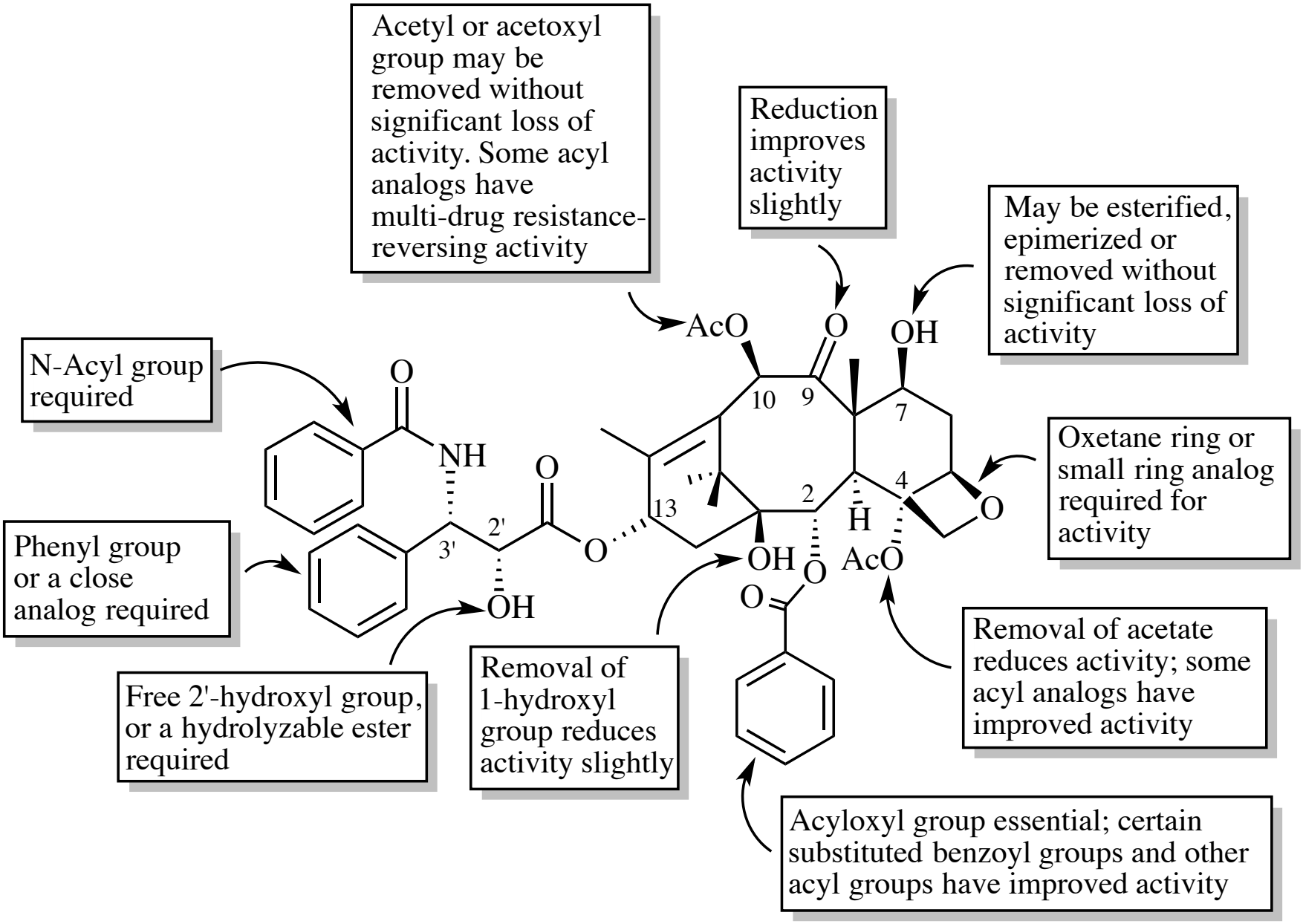
- Attempts to identify and quantitate physicochemical properties of a drug in relation to its biological activity or binding
- Studies hydrophobic, electronic, and steric properties-either whole molecule or pieces

the aim is to set up an equation that quantifies the relationship & allows one to predict (to some extent) biological activity

- Alter, remove or mask a functional group
- Test the analogue for activity
- Conclusions depend on the method of testing in vitro - tests for binding interactions with target in vivo - tests for target binding interactions and/or pharmacokinetics
- If in-vitro activity drops, it implies group is important for binding
- If in-vivo activity unaffected, it implies group is not important

Ligand binding affinities should cover three to four orders of magnitude to allow for statistically plausible model development and testing

SAR of Taxol



from: Organic Chemistry of Drug Design and Drug Action, Silverman & Holladay

Hansch Equation

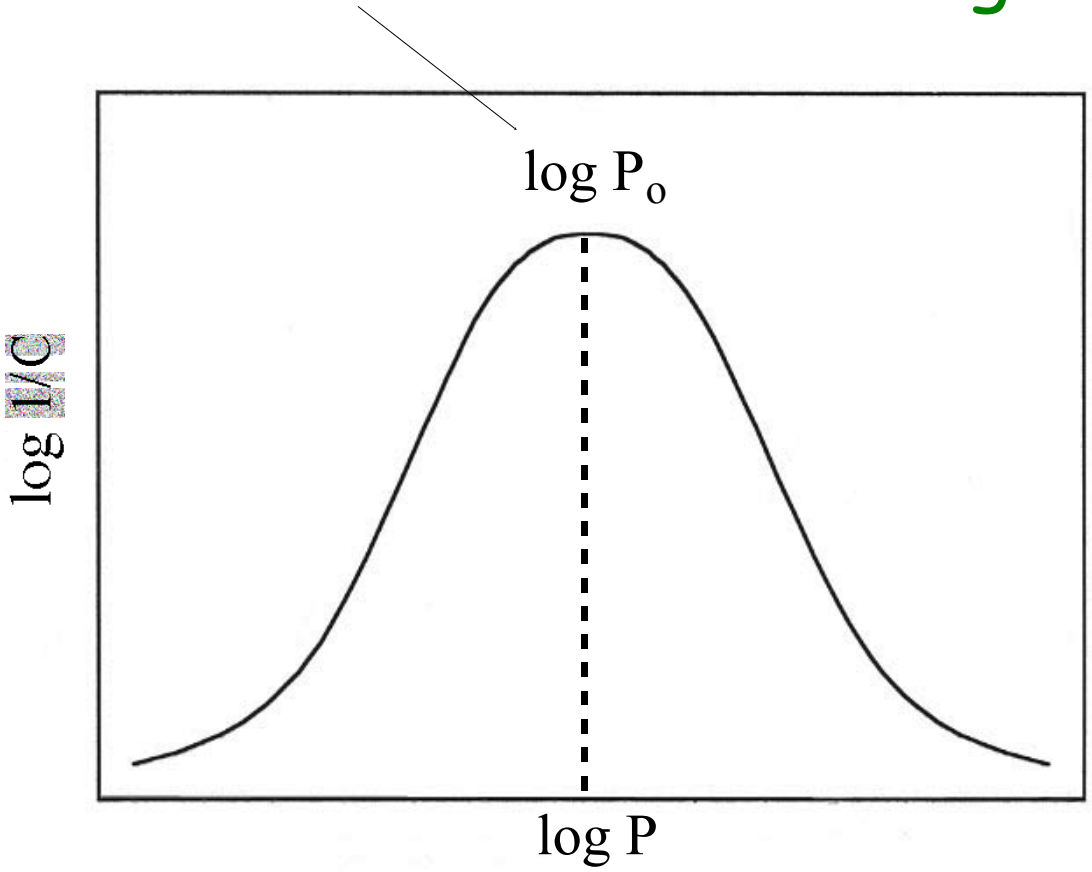
- A QSAR equation relating various physicochemical properties to the biological activity of a series of compounds
- Usually includes log P, electronic(σ, Hammett) and steric (*Es, Taft*) factors: positive p means hydrophobic, negative σ means e-donating
- Start with simple equations and elaborate as more structures are synthesized
- Typical equation for a wide range of log P is parabolic

$$\text{Log}\left(1/C\right) = -k_1(\log P)^2 + k_2 \log P + k_3\sigma + k_4 Es + k_5$$

(C is the concentration to achieve a biological effect)

Parabolic Relationship Between Potency (log 1/C) and $(\log P)^2$

optimum partition coefficient for biological activity



log P_0 should be ≥ 2 to penentrate the CNS (blood-brain barrier)

Hydrophobicity: π vs P

- **P** measures drug's **overall hydrophobicity** & measures drug's transportability
- **π** measures the hydrophobicity of a **specific region** on the drug-hydrophobic bonding to a receptor

substituent hydrophobicity constant, π

- Possible to calculate the substituent hydrophobicity constant (π)
- A measure of how hydrophobic the substituent is relative to H

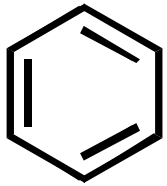
- Measure P experimentally for a standard compound with and without a substituent (X). Use this equation:

$$\pi_x = \log P_x - \log P_H = \log (P_x/P_H)$$

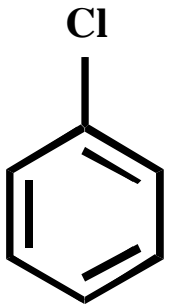
- positive π = substituent more hydrophobic than H
  negative π = less hydrophobic than H

Hydrophobicity of Substituents
- the substituent hydrophobicity constant (π)

- *A* measure of a substituent's hydrophobicity relative to hydrogen
- Tabulated values exist for aliphatic and aromatic substituents
- Measured experimentally by comparison of log P values with log P of parent structure

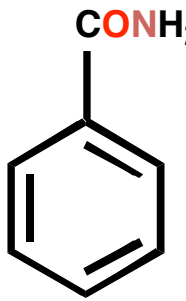


Benzene
(Log *P* = 2.13)



Chlorobenzene
(Log *P* = 2.84)

π_{Cl} **= 0.71**



Benzamide
(Log *P* = 0.64)

π_{CONH_2} **= -1.49**

- Positive values imply substituents are more hydrophobic than H
- Negative values imply substituents are less hydrophobic than H

π values for various substituents on aromatic rings

CH_3	t-Bu	OH	$CONH_2$	CF_3	Cl	Br	F
0.52	1.68	-0.67	-1.49	1.16	0.71	0.86	0.14

Theoretical Log P for chlorobenzene

= log P for benzene + π for Cl

= 2.13 + 0.71 = 2.84

Theoretical Log P for meta-chlorobenzamide

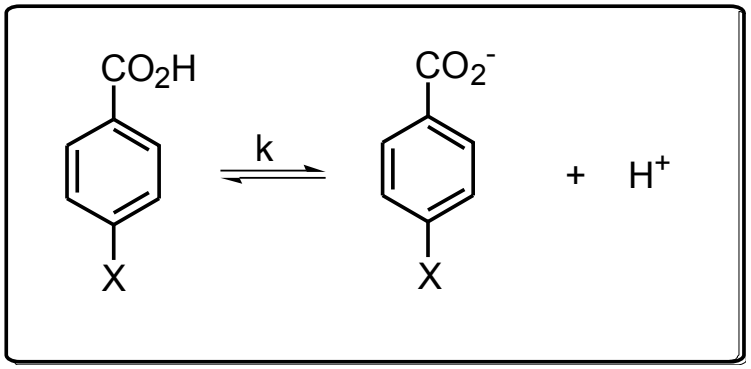
= log P for benzene + π for Cl + π for $CONH_2$

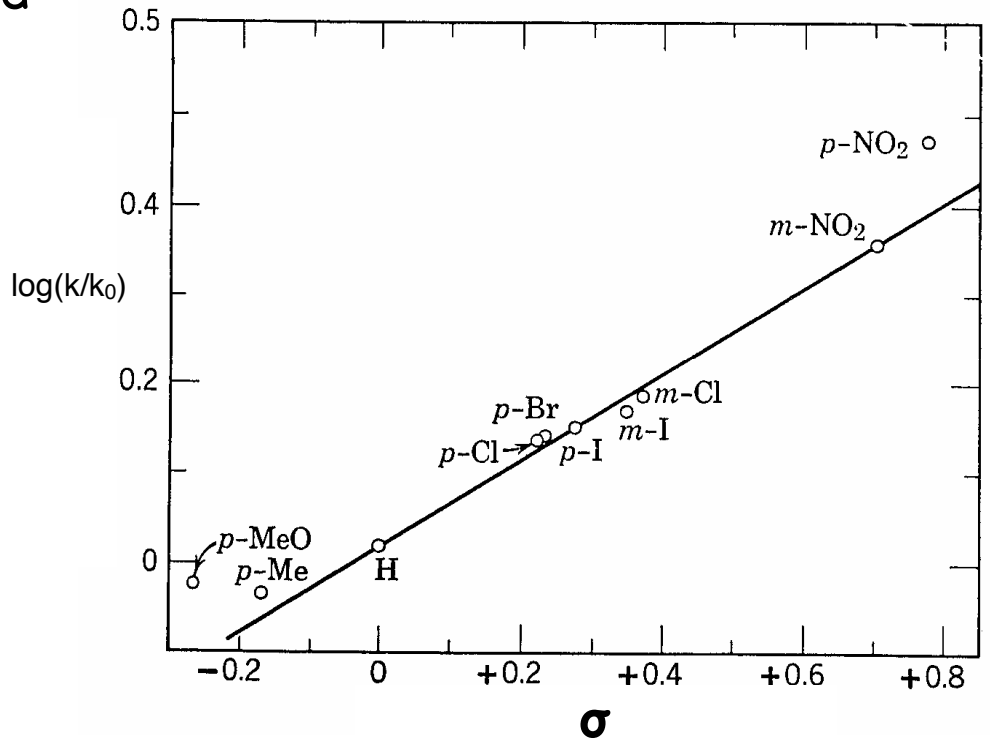
= 2.13 + 0.71 - 1.49 = 1.35

Electronic Effects: The Hammett Constant σ

Hammett constant (1940) σ

Measure e-withdrawing or e-donating effects and compare it to benzoic acid to see how its ionization state is affected





$$\sigma = \log [K_X/K_H]$$

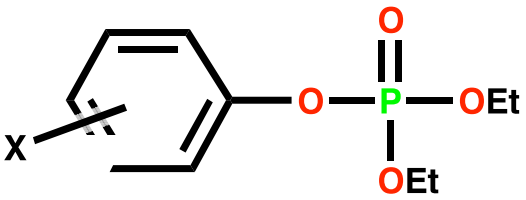
(X = substituent on benzene ring)

electron-withdrawing groups:

equilibrium shifts right & $K_x > K_{benzoic}$

since $\sigma_x = \log K_x - \log K_{benzoic}$, then σ will be **positive**

$\sigma_x = \log (K_x/K_{benzoic})$



$$\log\left(1/C\right) = 2.282\,\sigma - 0.348$$

Diethylphenylphosphates
(Insecticides)

Conclusion: e-withdrawing substituents increase activity

σ value depends on inductive and resonance effects

σ value depends on whether the substituent is meta or para

ortho values are invalid due to steric factors

Steric Effects

- much harder to quantitate

Examples are:

- **Taft's steric factor** (Es) (~1956), an experimental value based on rate constants

- **Molar refractivity** (MR)--measure of the volume occupied by an atom or group-equation includes the MW, density, and the index of refraction-

- **Verloop steric parameter**-computer program uses bond angles, van der Waals radii, bond lengths

Steric Factors

Taft's Steric Factor (E_s)

- Measured by comparing the rates of hydrolysis of substituted aliphatic esters against a standard ester under acidic conditions

$$E_s = \log k_x - \log k_o$$

k_x represents the rate of hydrolysis of a substituted ester

k_o represents the rate of hydrolysis of the parent ester

- Limited to substituents which interact sterically with the tetrahedral transition state for the reaction
- Cannot be used for substituents which interact with the transition state by resonance or hydrogen bonding
- May undervalue the steric effect of groups in an intermolecular process (i.e. a drug binding to a receptor)

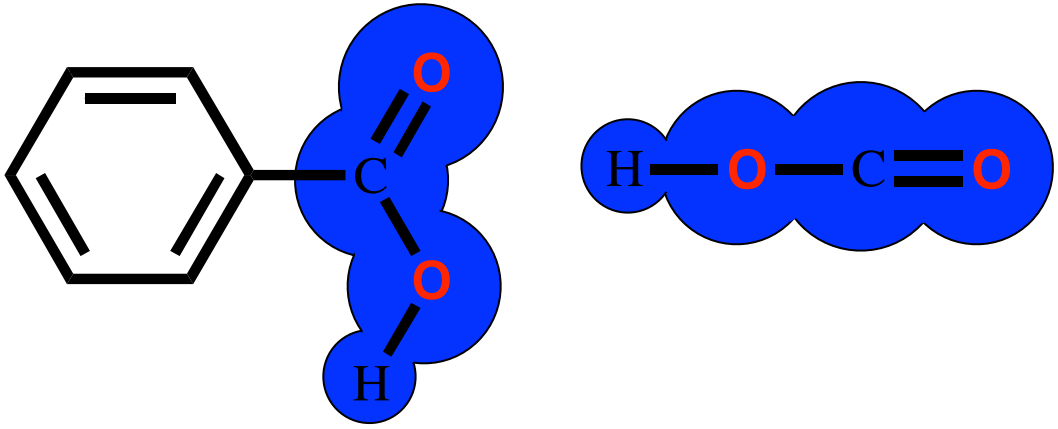
Steric Factors

Molar Refractivity (MR) - a measure of a substituent's volume and its polarizability

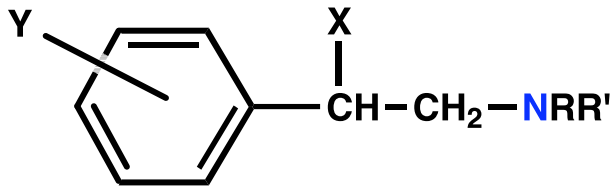
$$MR = \underbrace{\frac{(n^2 - 1)}{(n^2 - 2)}}_{\text{Correction factor for polarisation (n=index of refraction)}} \times \underbrace{\frac{\text{mol. wt.}}{\text{density}}}_{\text{Defines volume}}$$

Verloop Steric Parameter

- calculated
- gives dimensions of a substituent
- can be used for any substituent



Example: Adrenergic blocking activity of β-halo-β-arylamines

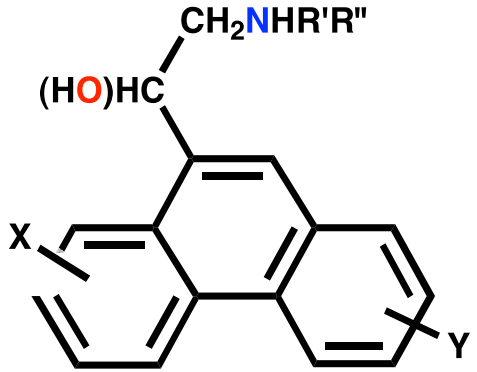


$$\text{Log}\left(\frac{1}{C}\right) = 1.22\ \pi - 1.59\ \sigma + 7.89$$

Conclusions:

- Activity increases if π is +ve (i.e. hydrophobic substituents, π is the hydrophobicity constant)
- Activity increases if σ is negative (i.e. e-donating substituents, σ is the Hammett constant)

Example: Antimalarial activity of phenanthrene aminocarbinols



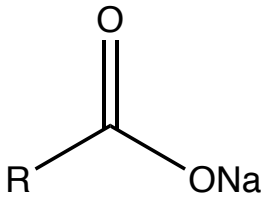
$$\mathrm{Log}\left(\frac{1}{C}\right) = -0.015\,(\log P)^2 + 0.14 \log P + 0.27\,\Sigma\pi_X + 0.40\,\Sigma\pi_Y + 0.65\,\Sigma\sigma_X + 0.88\,\Sigma\sigma_Y + 2.34$$

Conclusions:

- Activity increases slightly as log P (hydrophobicity) decreases
- Parabolic equation implies an optimum log P^o value for activity
- Activity increases for hydrophobic substituents (esp. ring Y)
- Activity increases for e-withdrawing substituents (esp. ring Y)

Case Study: Antifungal activity of aliphatic carboxylic salts

$$\log(1/C) = -1.54 \log P - 0.64 (\log P)^2 + 2.15$$



entry	r	LogP	$(LogP)^2$	Exptl. Log 1/C	Calc. Log 1/C
1	Butyl	-2.7	7.29	1.7	1.64
2	Pentyl	-2.2	4.84	2.4	2.44
3	Hexyl	-1.7	2.89	3.0	2.92
4	Heptyl	-1.2	1.44	3.0	3.08
5	Octyl	-0.7	0.49	3.0	2.91
6	Nonyl	-0.2	0.04	2.4	2.43

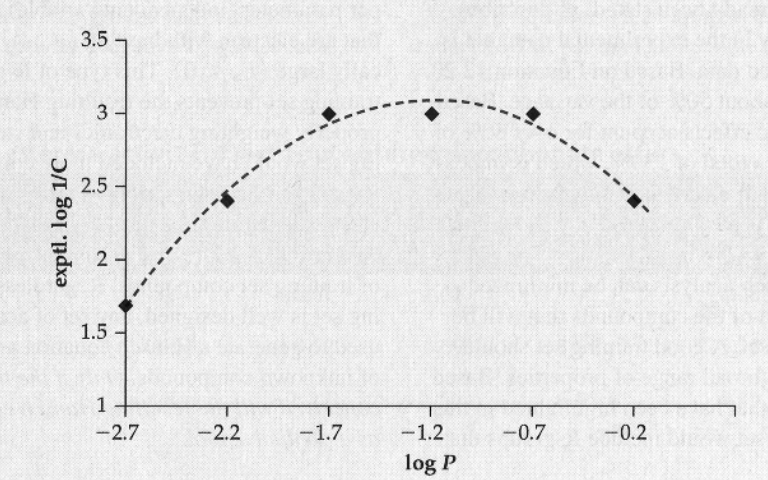


FIGURE 12.3 Biological activity versus log *P* for aliphatic carboxylic acids

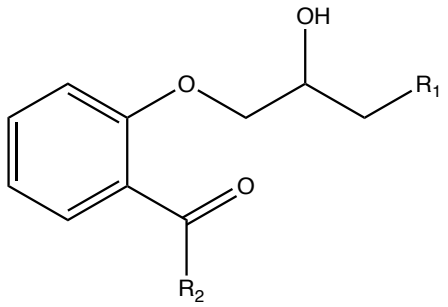
Orig.: Hansch et al., J. Med. Chem 1971,14,653

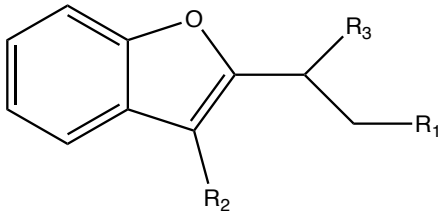
Case Study: Reduction of multi drug resistance exhibited by tumor cells

$$\log(1/C) = 0.86 \log P - 1.16 I_{BF} - 3.33$$

o-alkoxyphenone

$I_{BF} = 0$





benzofuran

$I_{BF} = 1$

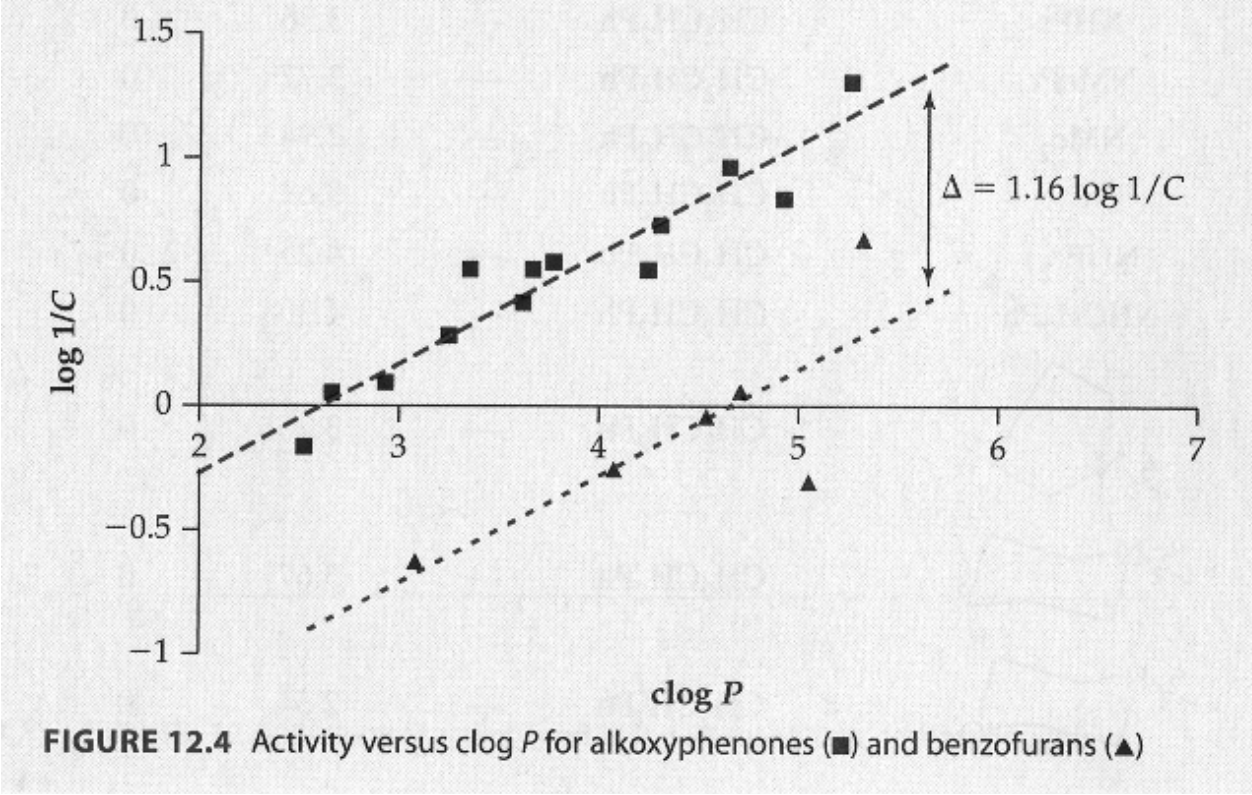
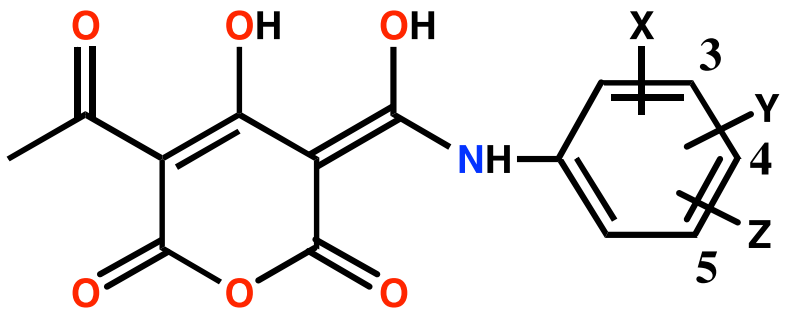


FIGURE 12.4 Activity versus clog *P* for alkoxyphenones (■) and benzofurans (▲)

Case Study: Using the predictive power to develop drugs

QSAR analysis of pyranenamines (Anti-allergy compounds)



Stage 1 19 structures were synthesised to study π and σ

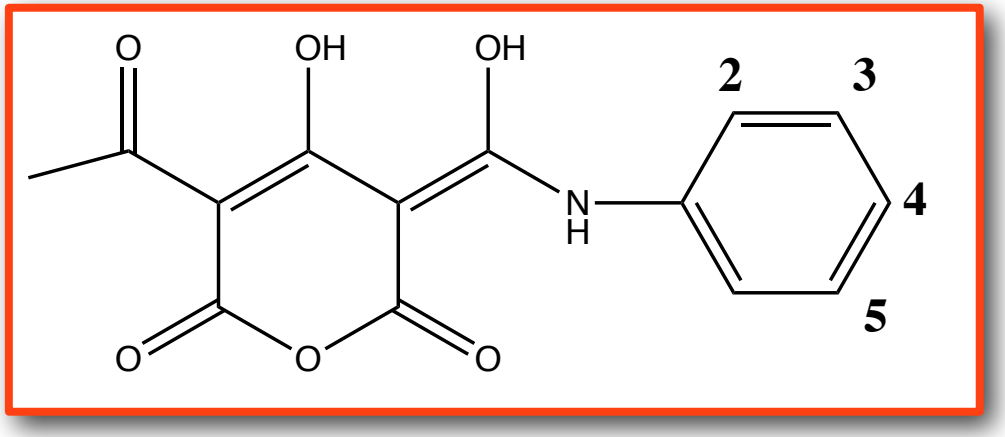
$$\text{Log}\left(\frac{1}{C}\right) = -\ 0.14\ \Sigma\pi - 1.35(\Sigma\sigma)^2 - 0.72$$

$\Sigma\pi$ and $\Sigma\sigma$ = total values for π and σ for all substituents

Conclusions:

- Activity drops as π increases
- Hydrophobic substituents are bad for activity - unusual
- Any value of σ results in a drop in activity
- Substituents should not be e-donating or e-withdrawing (activity falls if σ is +ve or -ve)

Stage 2 61 structures were synthesised, concentrating on hydrophilic substituents to test the first equation



Anomalies

a) 3-NHCOMe, 3-NHCOEt, 3-NHCOPr.
Activity should drop as alkyl group becomes bigger and more hydrophobic, but the activity is similar for all three substituents

Possible steric factor at work. Increasing the size of R may be good for activity and balances out the detrimental effect of increasing hydrophobicity

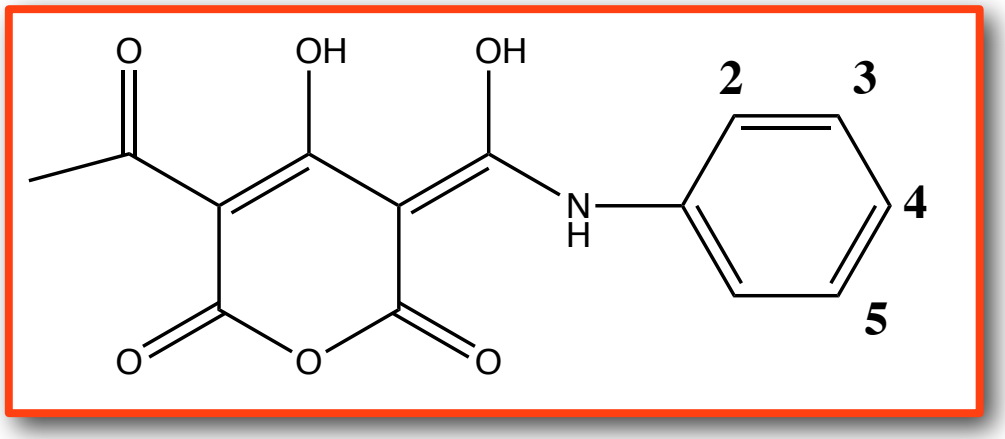
b) OH, SH, NH_2 and NHCOR at position 5 : Activity is greater than expected

Possibly involved in H-bonding

c) $NHSO_2R$: Activity is worse than expected

Exception to H-bonding theory - perhaps bad for steric or electronic reasons

Theories



d) 3,5-$(CF_3)_2$ and 3,5-$(NHMe)_2$: Activity is greater than expected

The only disubstituted structures where a substituent at position 5 was electron withdrawing

e) 4-Acyloxy : Activity is 5 x greater than expected

Presumably acts as a prodrug allowing easier crossing of cell membranes.
The group is hydrolysed once across the membrane.

Stage 3 Alter the QSAR equation to take account of new results

$$\text{Log}\left(\frac{1}{C}\right) = -0.30\ \Sigma\pi - 1.35(\Sigma\sigma)^2 + 2.0\sigma(F5) + 0.39\ (345 - \text{HBD}) - 0.63\ (\text{NHSO}_2) + 0.78(M\text{-}V) + 0.72\ (4\text{-OCO}) - 0.75$$

Conclusions

(F-5)	Electron-withdrawing group at position 5 increases activity (based on only 2 compounds though)
(3,4,5-HBD)	HBD at positions 3, 4,or 5 is good for activity Term = 1 if a HBD group is at any of these positions Term = 2 if HBD groups are at two of these positions Term = 0 if no HBD group is present at these positions Each HBD group increases activity by 0.39
($NHSO_2$)	Equals 1 if $NHSO_2$ is present (bad for activity by -0.63). Equals zero if group is absent.
(M-V)	Volume of any meta substituent. Large substituents at meta position increase activity
4-O-CO	Equals 1 if acyloxy group is present (activity increases by 0.72). Equals 0 if group absent

Stage 3 Alter the QSAR equation to take account of new results

$$\mathrm{Log}\left(\frac{1}{C}\right) = -0.30\ \Sigma\pi - 1.35(\Sigma\sigma)^2 + 2.0(F-5) + 0.39\ (345 - \mathrm{HBD}) - 0.63\ (\mathrm{NHSO_2})$$
$$+ 0.78(M-V) + 0.72(4 - \mathrm{OCO}) - 0.75$$

Stage 4

37 Structures were synthesised to test steric and F-5 parameters, as well as the effects of hydrophilic, H-bonding groups

Anomalies

Two H-bonding groups are bad if they are ortho to each other

Explanation

Possibly groups at the ortho position bond with each other rather than with the receptor - an intramolecular interaction

Stage 5 Revise Equation

$$\mathrm{Log}\left(1/C\right) = -0.034(\Sigma\pi)^2 - 0.33\,\Sigma\pi + 4.3(F\text{-}5) + 1.3\,(R\text{-}5) - 1.7(\Sigma\sigma)^2 + 0.73\,(345 - \mathrm{HBD})$$
$$- 0.86\,(\mathrm{HB} - \mathrm{INTRA}) - 0.69\,(\mathrm{NHSO_2}) + 0.72\,(4 - \mathrm{OCO}) - 0.59$$

NOTES

a) Increasing the hydrophilicity of substituents allows the identification of an optimum value for π ($\Sigma\pi$ = -5). The equation is now parabolic (-0.034 $(\Sigma\pi)^2$)

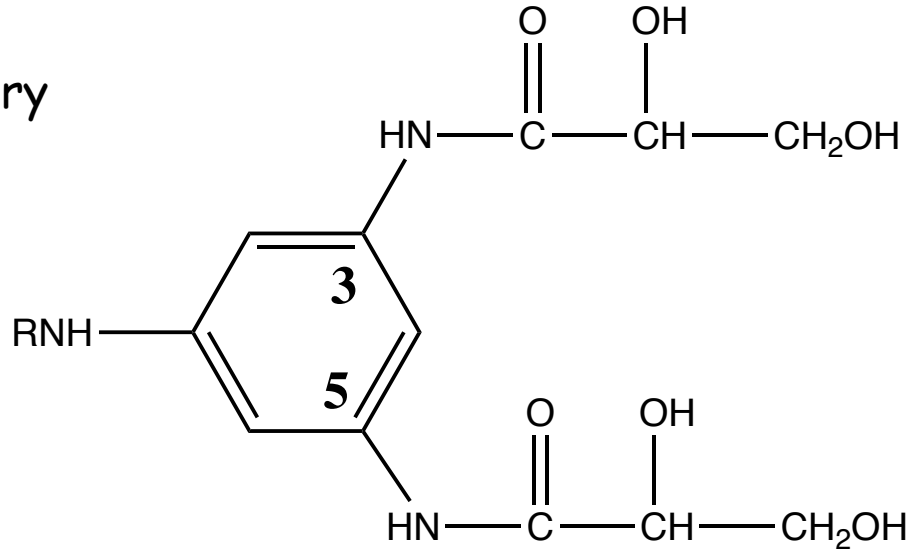
b) The optimum value of $\Sigma\pi$ is very low and implies a hydrophilic binding site

c) R-5 implies that resonance effects are important at position 5

d) HB-INTRA equals 1 for H-bonding groups ortho to each other
equals 0 if H-bonding groups are not ortho to each other

e) The steric parameter is no longer significant and is not present

Stage 6 Optimum Structure and binding theory



NOTES on the optimum structure

- It has unusual NHCOCH(OH)CH_2OH groups at positions 3 and 5

- It is 1000 times more active than the lead compound

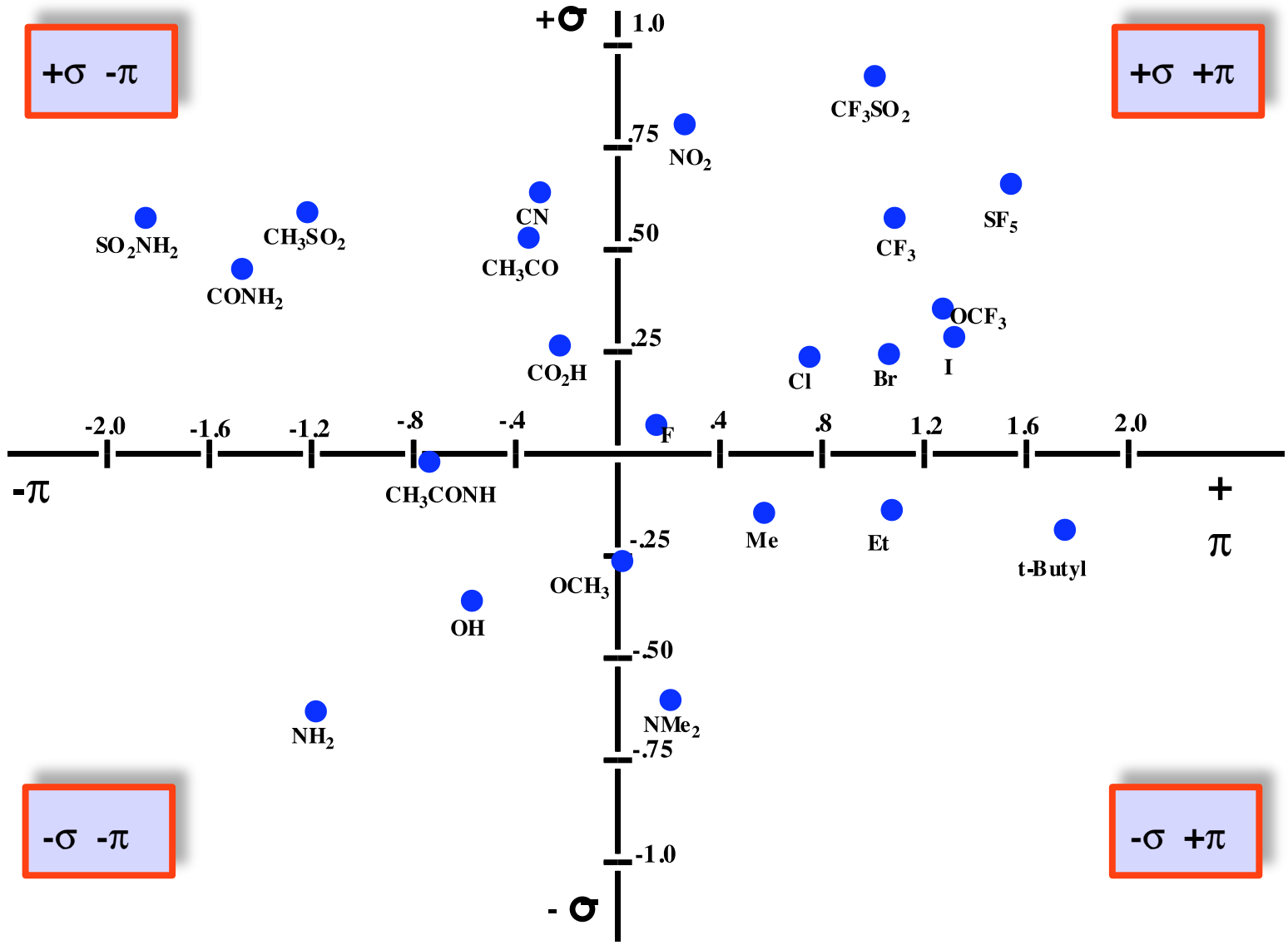
- The substituents at positions 3 and 5
  - are highly polar,
  - are capable of hydrogen bonding,
  - are at the meta positions and are not ortho to each other
  - allow a favourable F-5 parameter for the substituent at position 5

- The structure has a negligible $(\Sigma\sigma)^2$ value

Craig Plot

Craig plot shows values for 2 different physicochemical properties for various substituents; for **para-aromatic substituents**

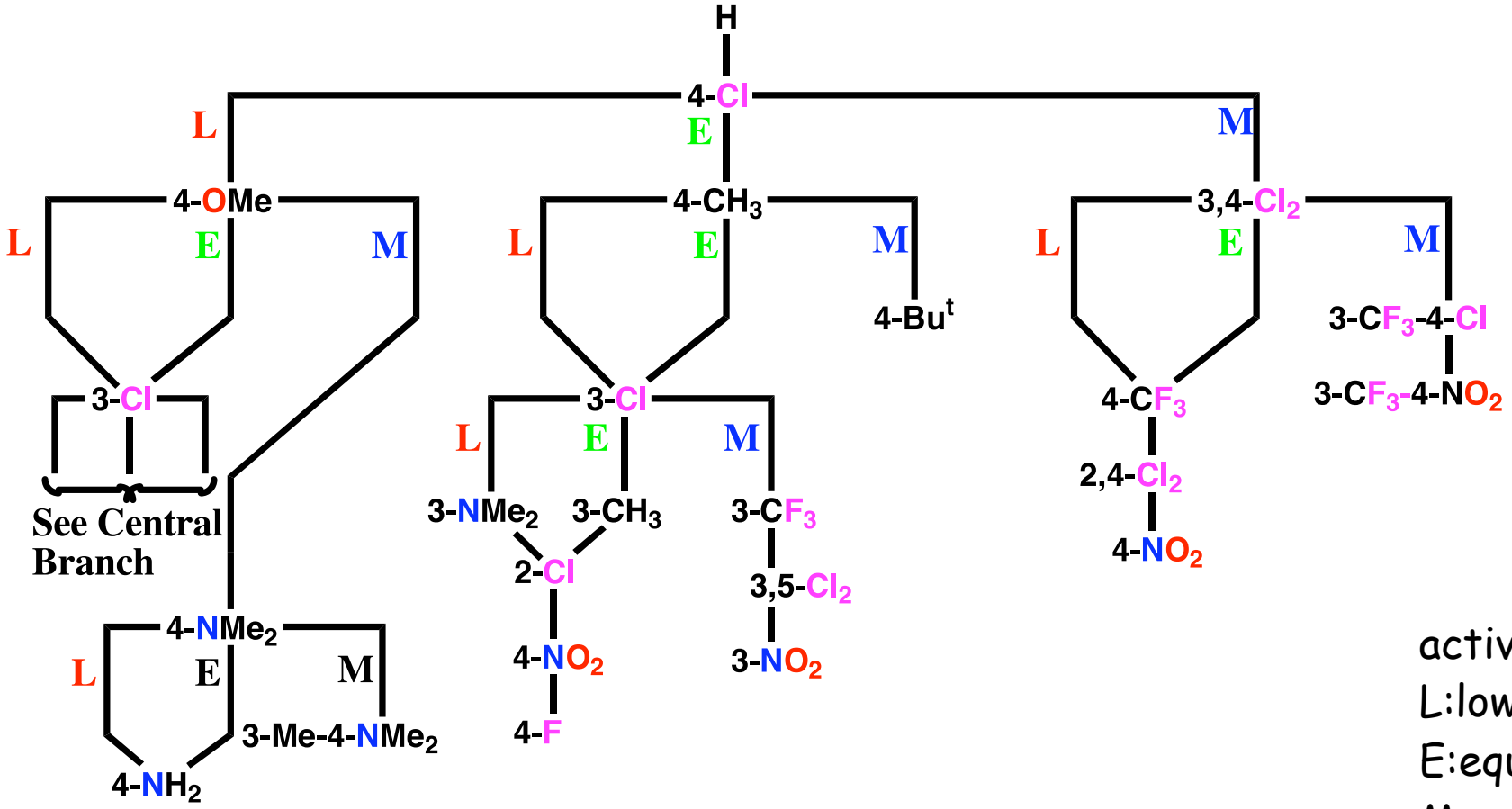
- Allows an easy identification of suitable substituents for a QSAR analysis which includes both relevant properties
- Choose a substituent from each quadrant to ensure orthogonality
- Choose substituents with a range of values for each property



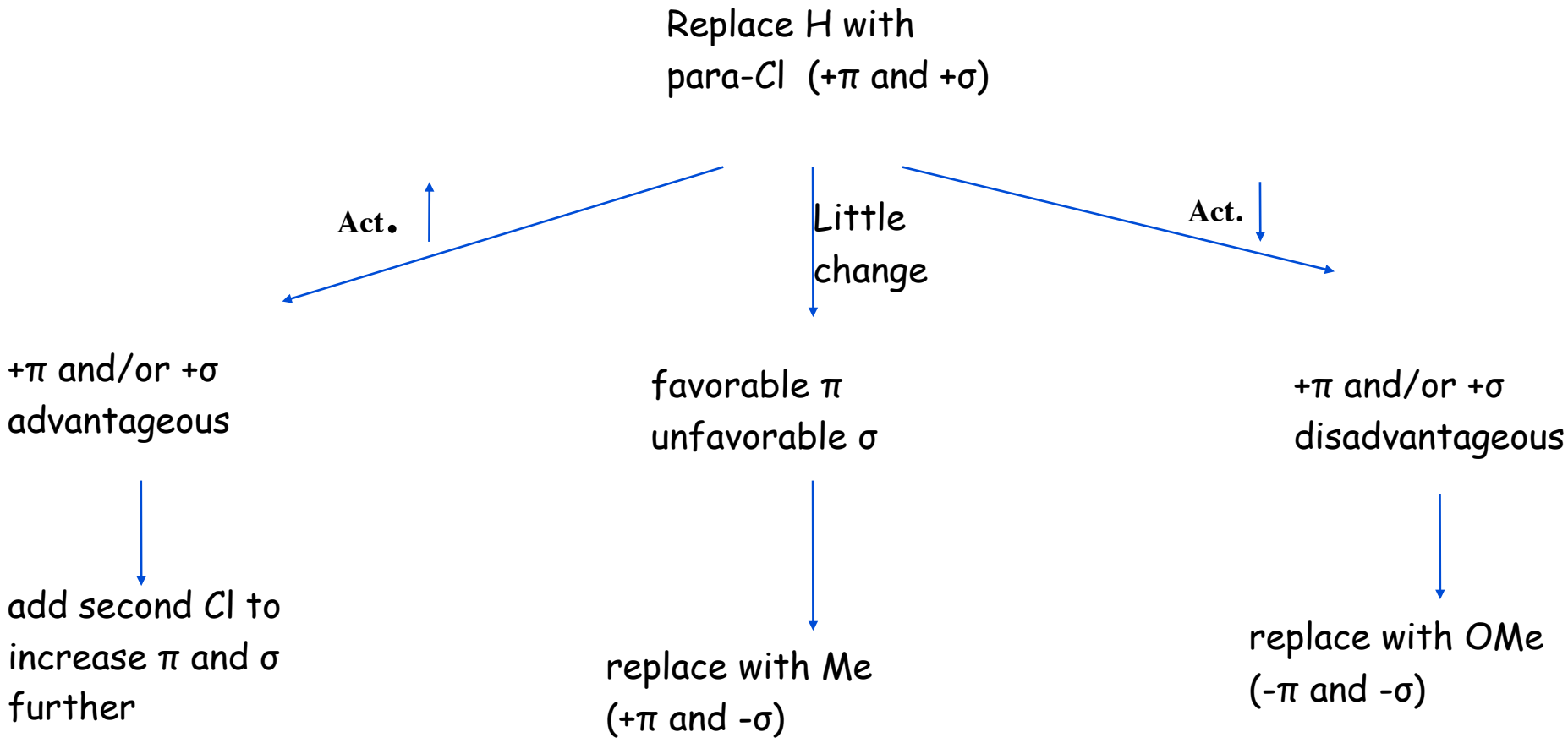
Topliss Scheme

Used to decide which substituents to use if optimizing compounds one by one (where synthesis is complex and slow)

Example: Aromatic substituents



activities:
L:lower
E:equal
M:more



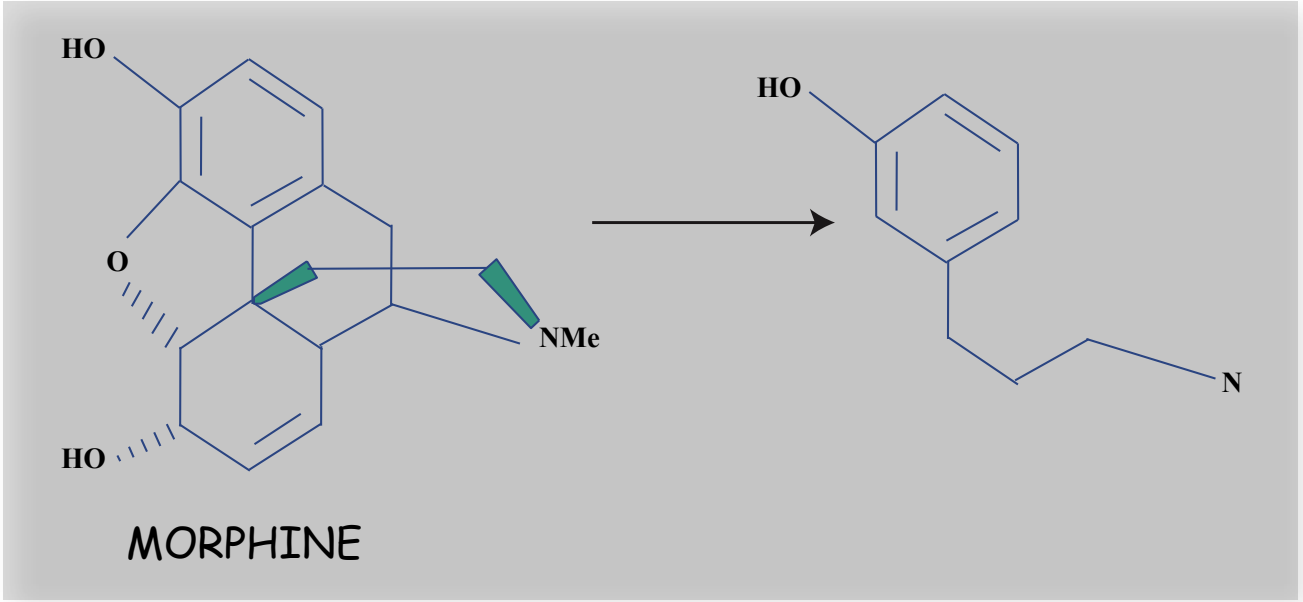
Further changes suggested based on arguments of π, σ and steric strain

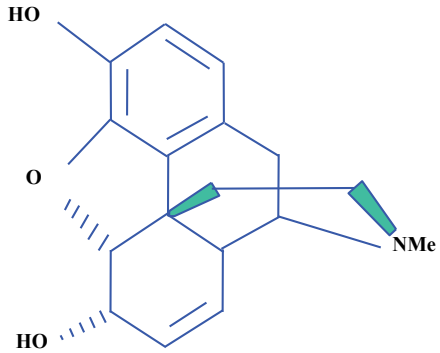
Pharmacophores

- Defines the important groups involved in binding
- Defines the relative positions of the binding groups
- Need to know Active Conformation
- Important to Drug Design
- Important to Drug Discovery

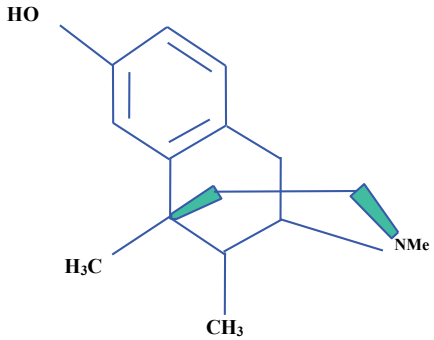
Structural (2D) Pharmacophore

Defines minimum skeleton connecting important binding groups

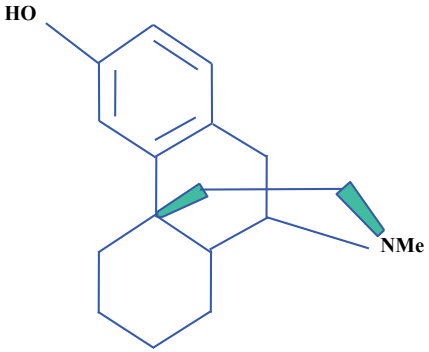




MORPHINE



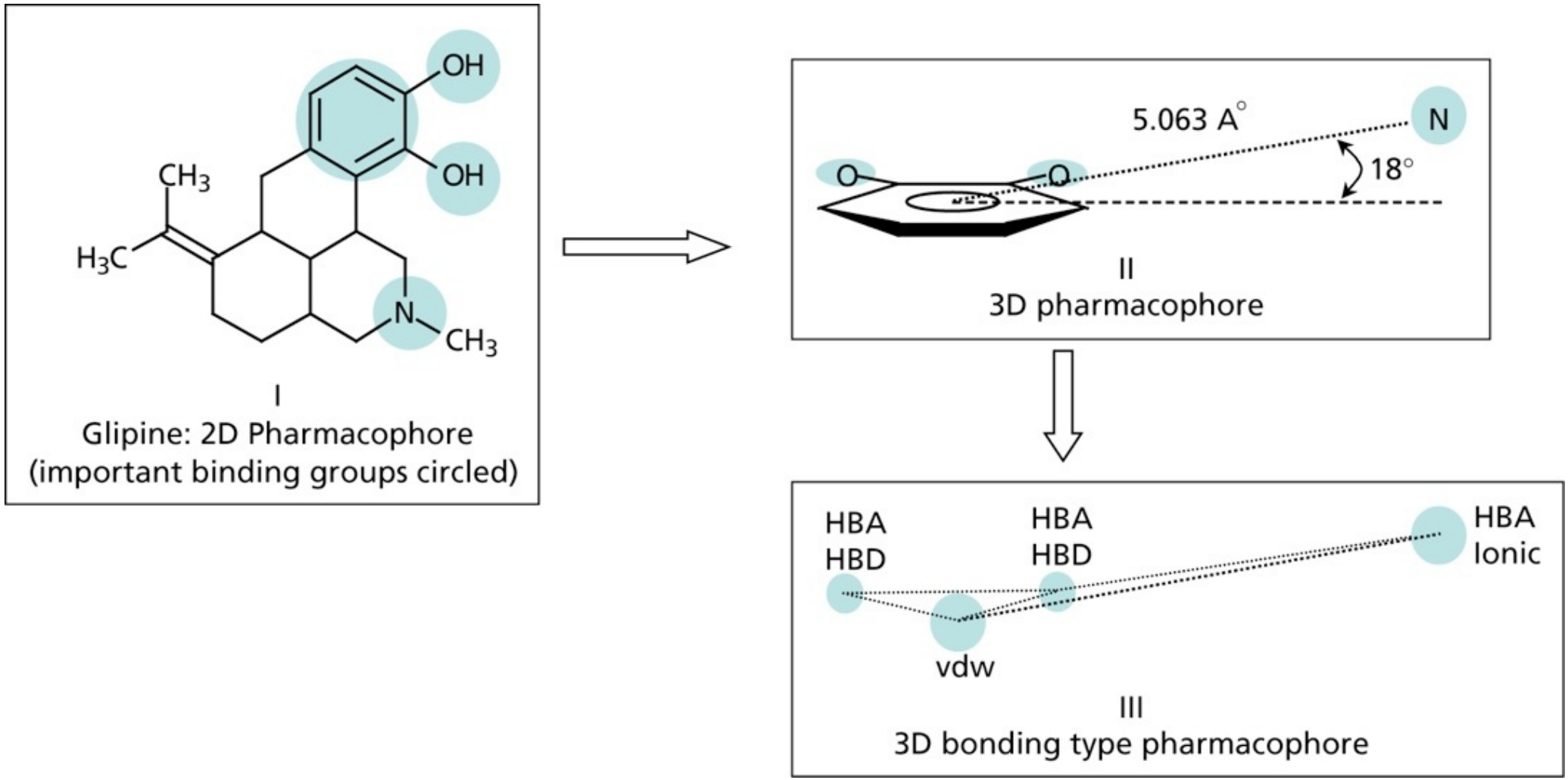
METAZOCINE

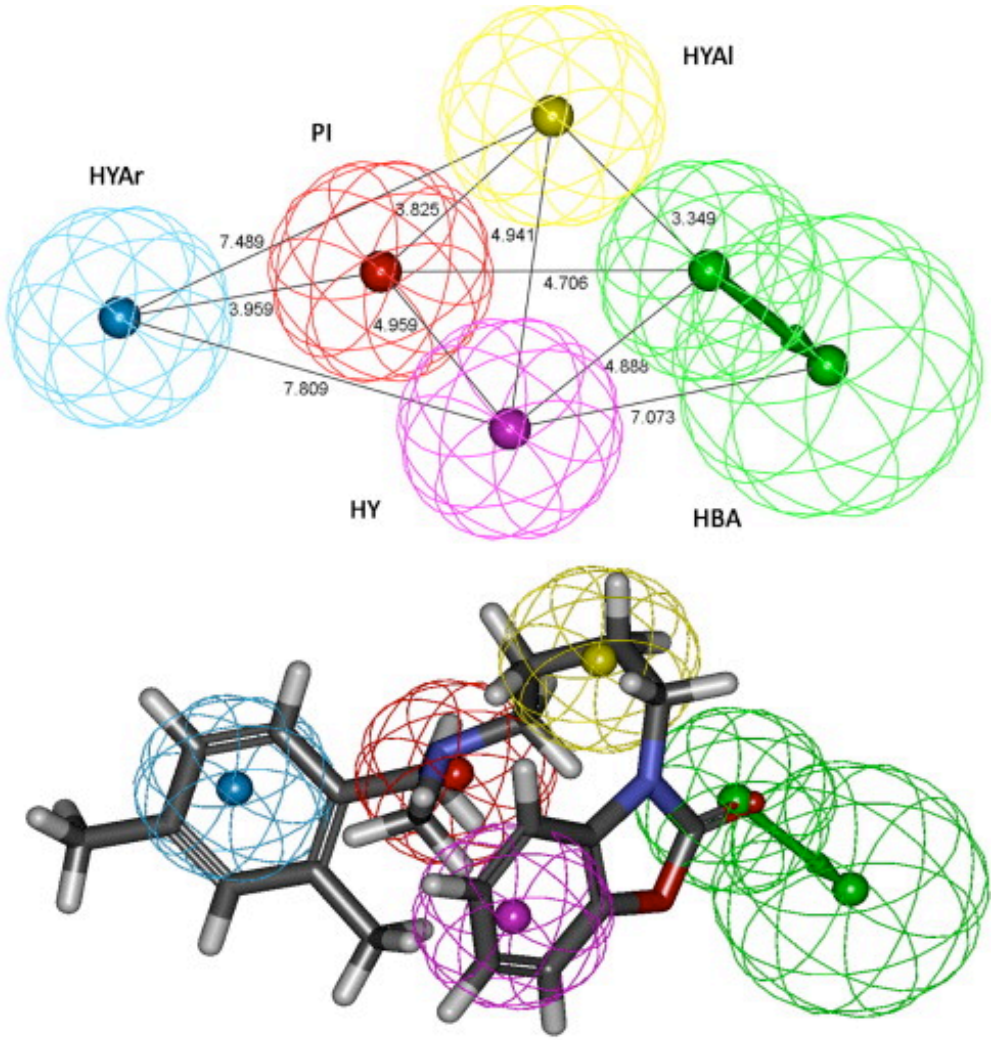


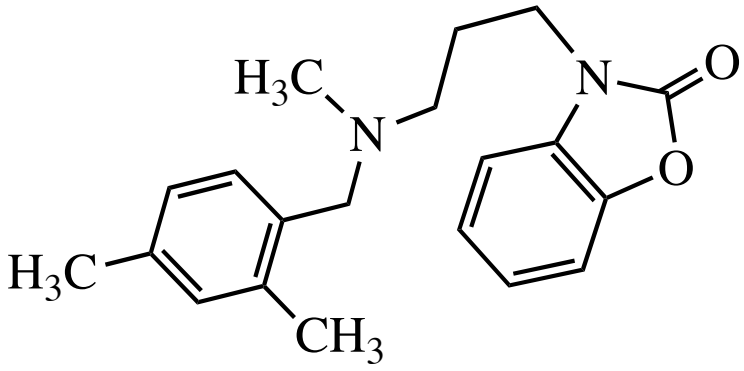
LEVORPHANOL

3D Pharmacophore

Defines relative positions in space of important binding groups

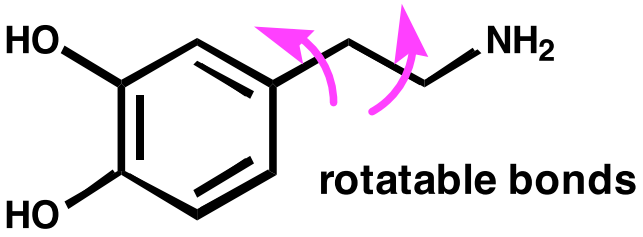



HYAl
PI
HYAr
3.825
7.489
4.941
3.349
4.706
3.959
4.959
4.888
7.809
7.073
HY
HBA

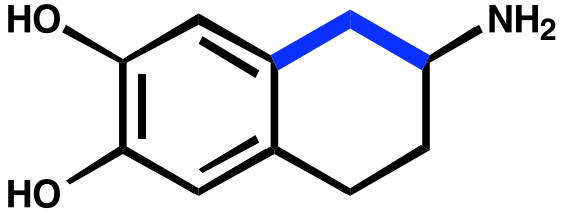

H3C
N
N
O
O
H3C
CH3

The Active Conformation

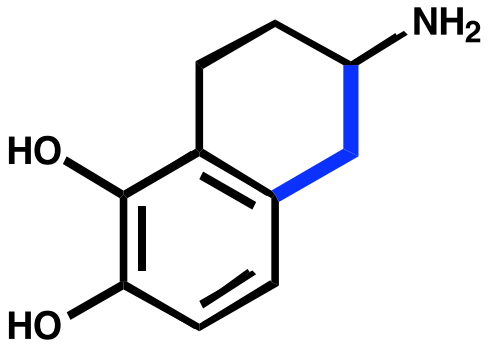
- Need to identify the active conformation in order to identify the 3D pharmacophore
- Conformational analysis - identifies possible conformations and their activities
- Conformational analysis is difficult for simple flexible molecules with large numbers of conformations
- Compare activity of rigid analogues



Dopamine



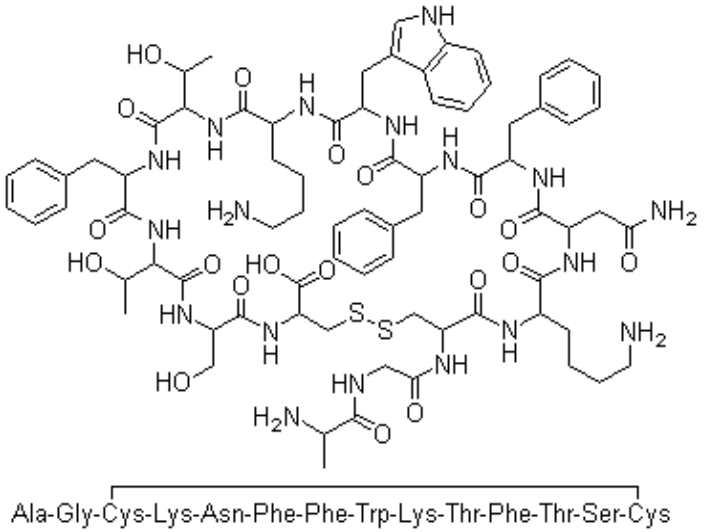
I

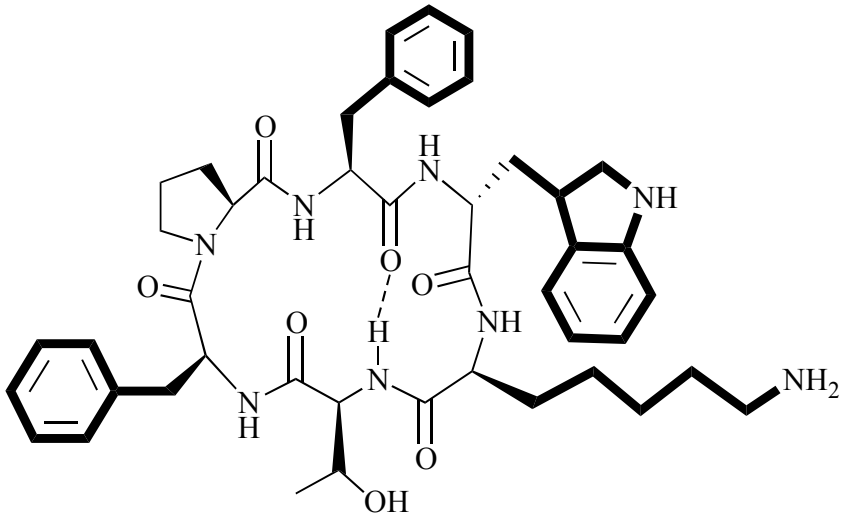


II

Locked bonds

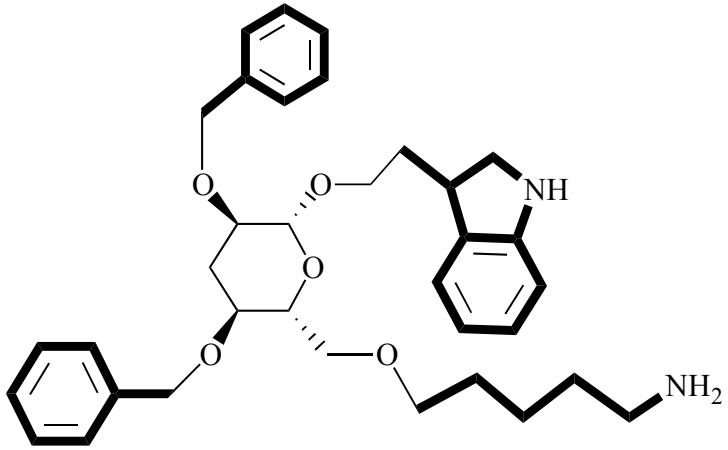
somatostatin





2.97

somatostatin agonist



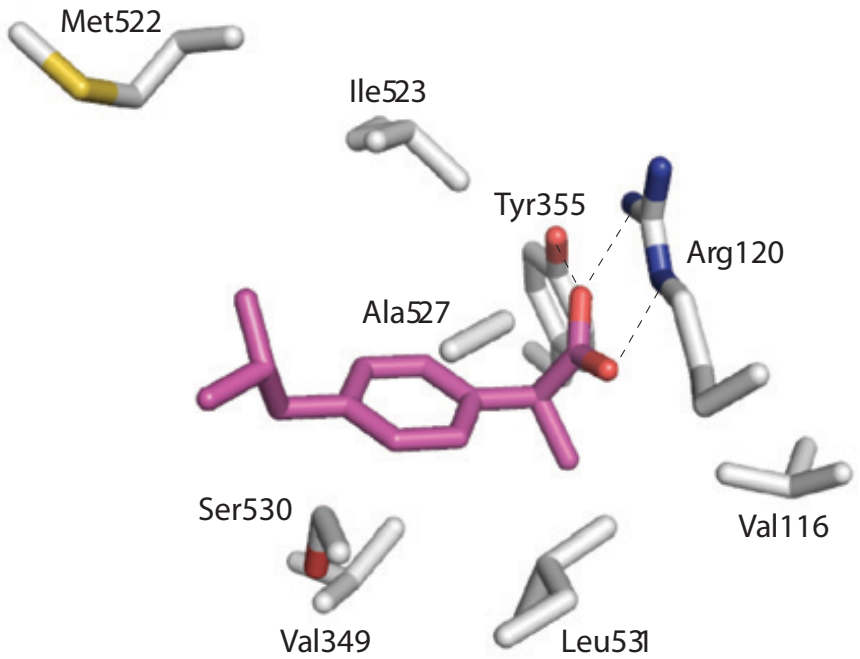
2.98

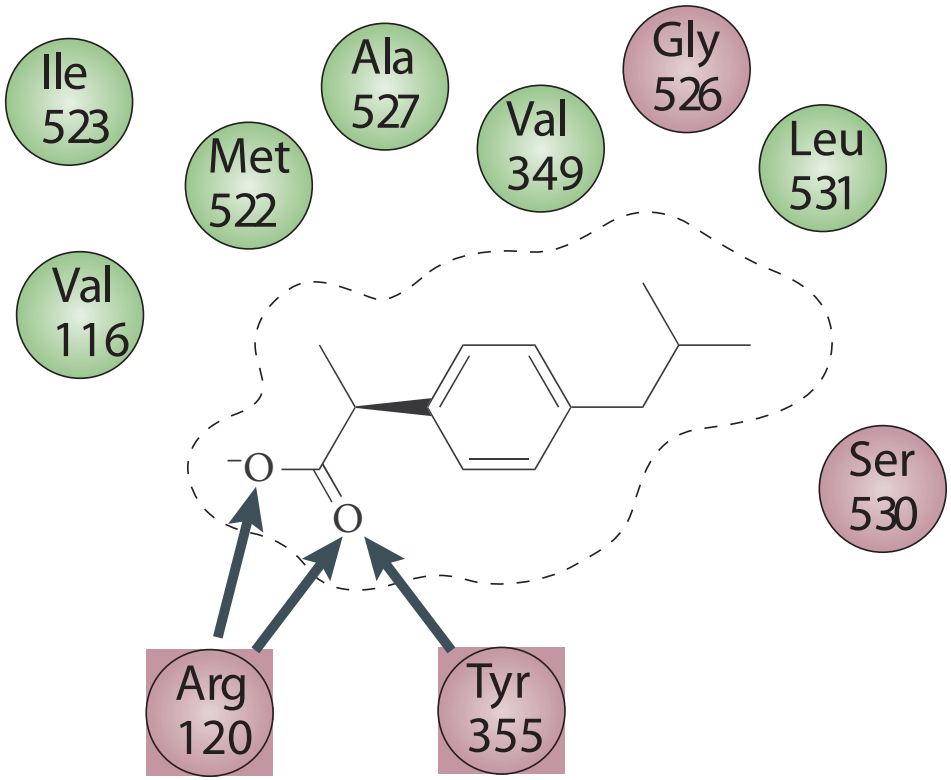
scaffold peptidomimetic of **2.97**

Somatostatin—a peptide hormone that affects neurotransmission and cell proliferation

Pseudoreceptor models

The idea of pseudoreceptor models is to construct a surrogate for the 3D target structure around a single ligand conformation. The models attempt to capture the shape of the binding site and its interaction points that need to be occupied for successful ligand binding





Definition of key interaction sites (anchor points) of the ligand-receptor complex
the core pseudoreceptor model is assembled around these hypotheses
model coordinates are optimized to gain more accurately calculated binding energies in validation studies

Pseudoreceptor applications in drug design

- exploration of key ligand–receptor interaction sites.
- evaluation of new candidate compounds.
- estimation of interaction energies.
- discerning the way in which a receptor protein sequence folds.
- energy minimization of ligands in the pseudoreceptor to indicate their active conformation.
- Direct medicinal chemists in the design of novel compounds.

3D-QSAR

- Physical properties are measured for the molecule as a whole
- Properties are calculated using computer software
- **No experimental constants or measurements are involved**
- Properties are known as '**Fields**'
- Steric field - defines the size and shape of the molecule
- Electrostatic field - defines electron rich/poor regions of molecule
- Hydrophobic properties are relatively unimportant

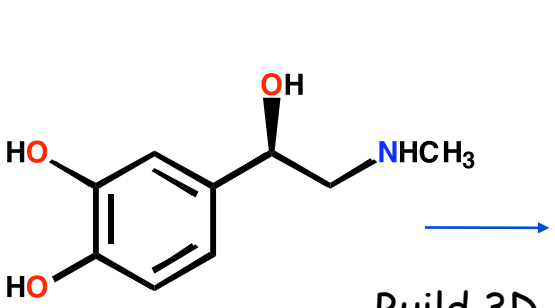
Advantages over QSAR

- No reliance on experimental values
- Can be applied to molecules with unusual substituents
- Not restricted to molecules of the same structural class
- Predictive capability

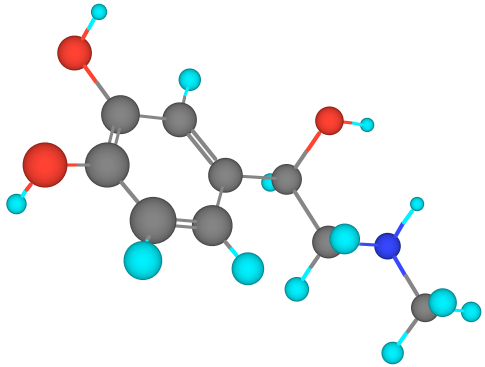
3D-QSAR

Method

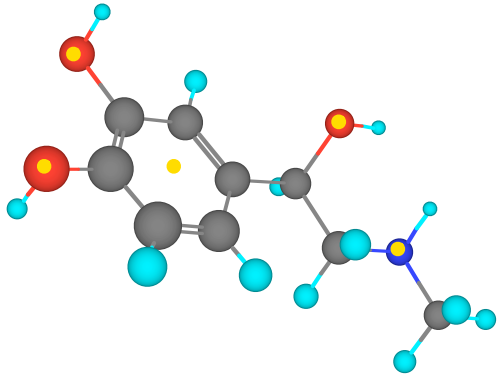
- **Comparative molecular field analysis** (CoMFA) - Tripos
- Build each molecule using modelling software
- Identify the active conformation for each molecule
- Identify the pharmacophore



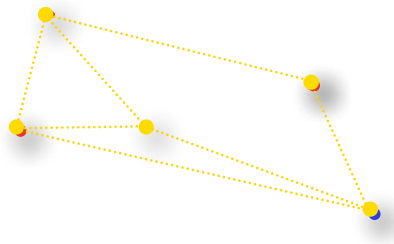
Build 3D model



Active conformation

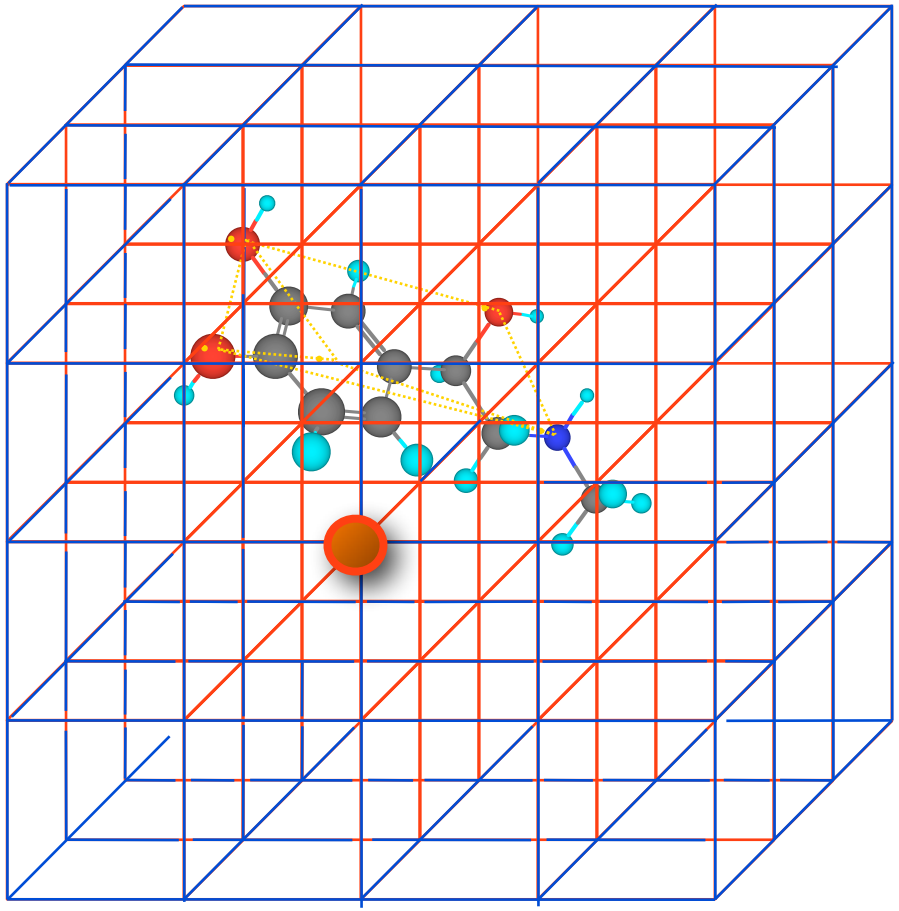


Define pharmacophore



3D-QSAR

- A probe atom is placed at each grid point in turn



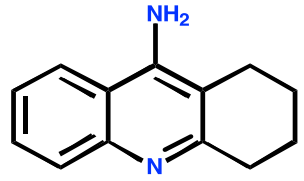
Probe atom

- Measure the steric or electrostatic interaction of the probe atom with the molecule at each grid point

3D-QSAR CASE STUDY

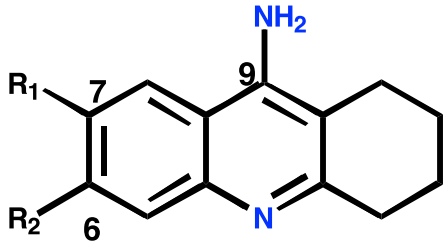
Tacrine

Anticholinesterase agonist used in the treatment of Alzheimer's disease



Conventional QSAR Study

12 analogues were synthesised to relate their activity with the hydrophobic, steric and electronic properties of substituents at positions 6 and 7



Substituents: CH_3, Cl, NO_2, OCH_3, NH_2, F

(Spread of values with no correlation)

$$\mathbf{Log\left(\frac{1}{C}\right) = pIC_{50} = -3.09\ MR\ (R^1) + 1.43\ \sigma(R^1,R^2) + 7.00}$$

Conclusions

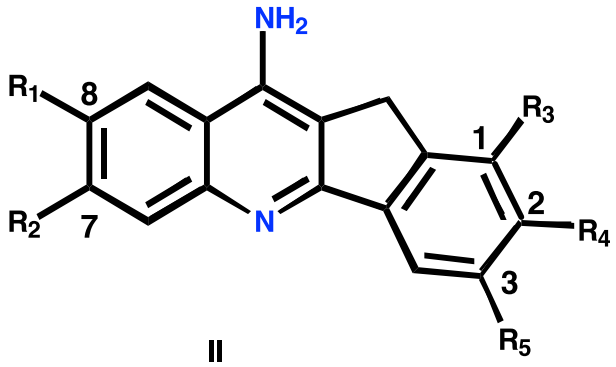
Large groups at position 7 are detrimental

Groups at positions 6 & 7 should be electron-withdrawing

No hydrophobic effect

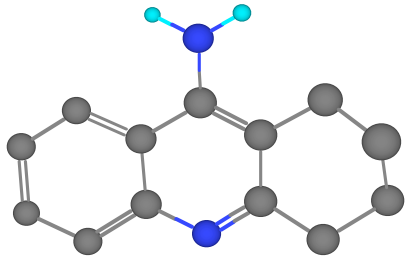
CoMFA Study

Analysis includes tetracyclic anticholinesterase inhibitors (II)

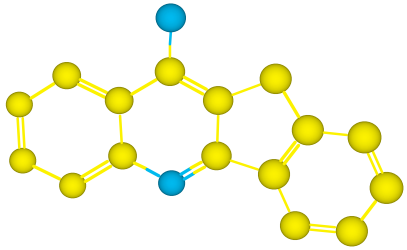


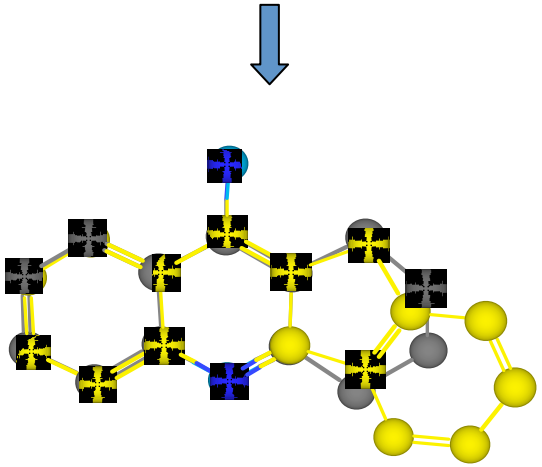
II

- Not possible to include above structures in a conventional QSAR analysis since they are a different structural class
- Molecules belonging to different structural classes must be aligned properly according to a shared pharmacophore



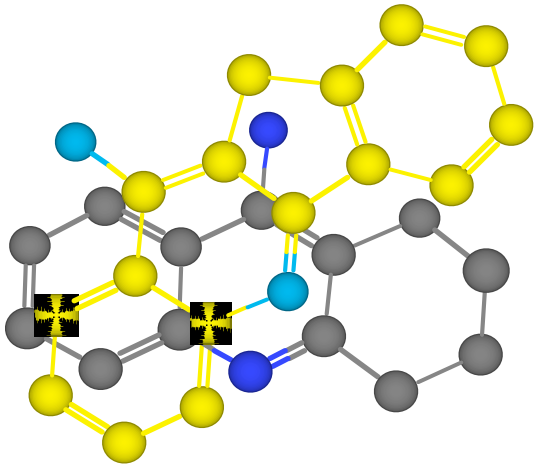
Overlay





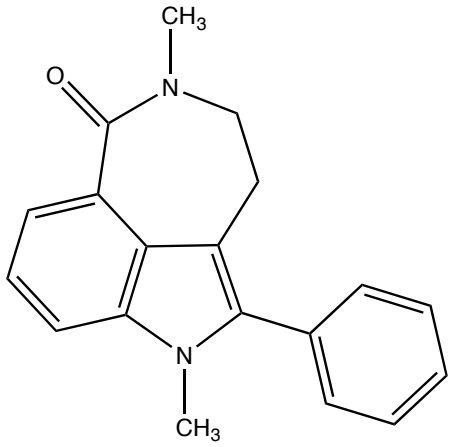
X-Ray Crystallography

- A tacrine / enzyme complex was crystallised and analysed
- Results revealed the mode of binding for tacrine
- Molecular modelling was used to modify tacrine to structure (II) while still bound to the binding site (in silico)
- The complex was minimized to find the most stable binding mode for structure II
- The binding mode for (II) proved to be different from tacrine

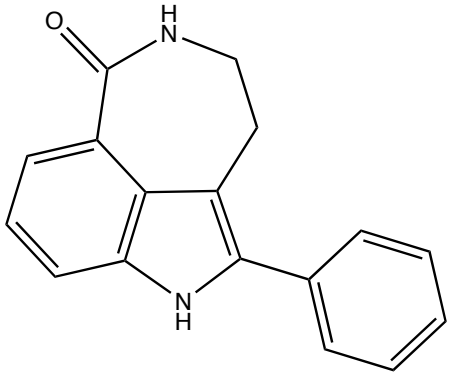


SAR Exercise

1

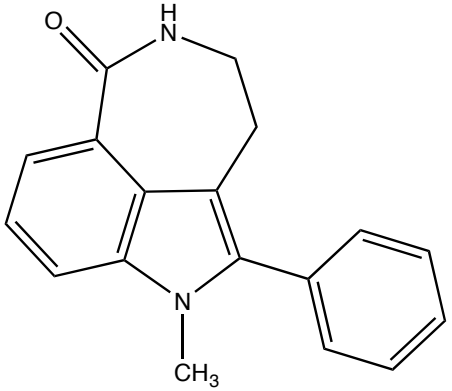


2



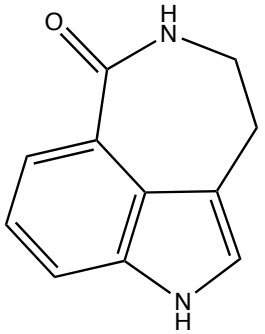
1000*1

3



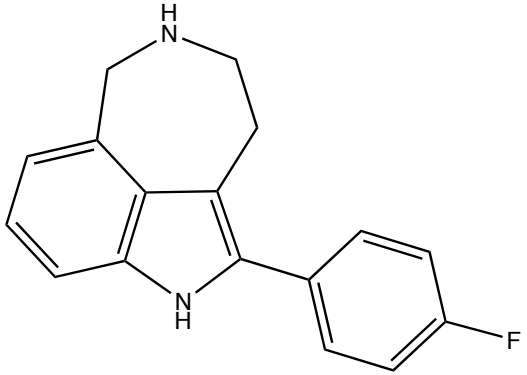
same as 2

4



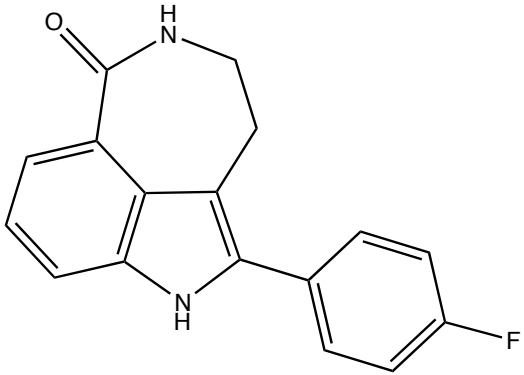
1/6 of 2

5



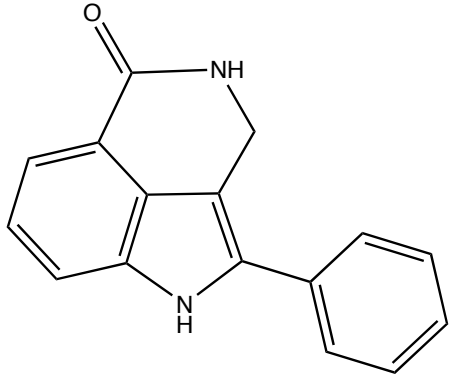
1/1000*2

6



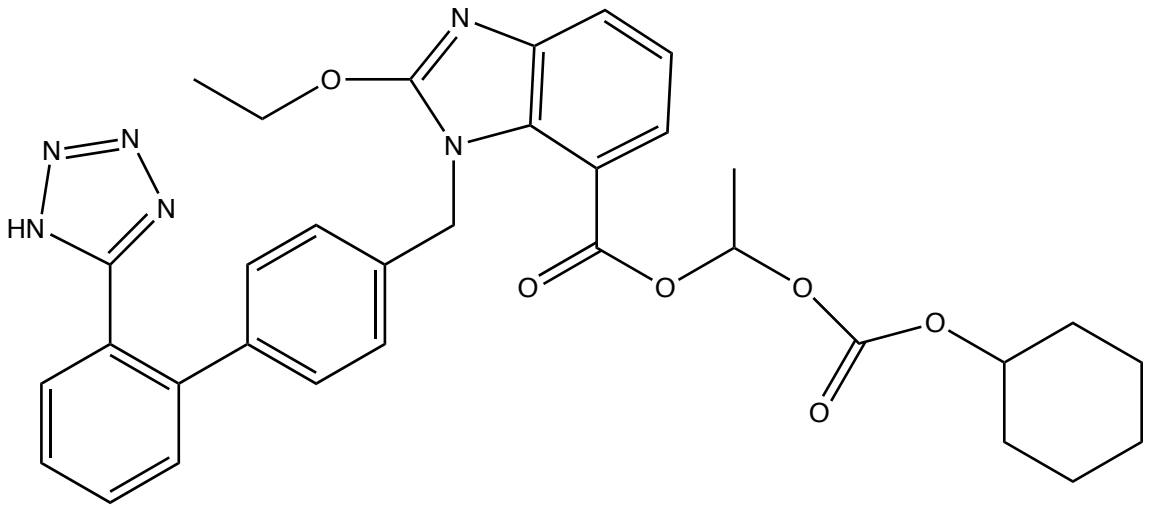
a little more than 2

7



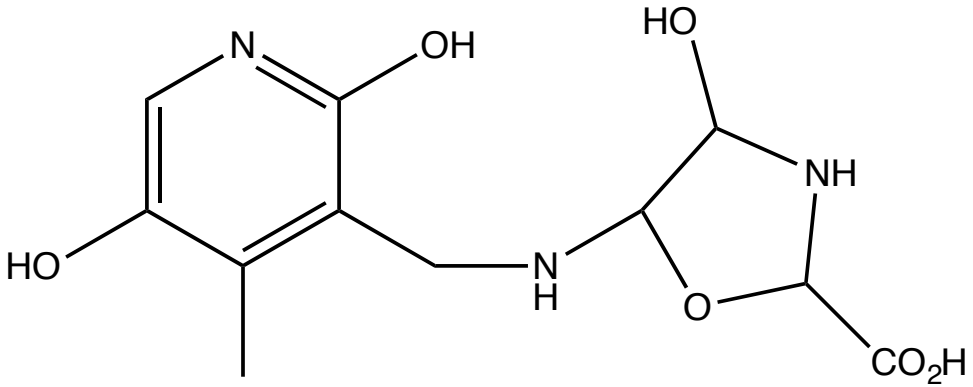
a little more than 2

This angiotensin antagonist was developed through four lead modifications. What was the potential original lead?



Candesartan cilexetil

what structural modifications can be made to improve membrane permeability?



MW=285
cLogP=-0.9
PSA=144

Benzoic acid has a pK_a of 4.2

Estimate the degree of ionisation in the fasted state for the stomach, duodenum and blood

Location	pH	[HA]/[A-]	Ionization
Stomach	1.5		
Duodenum	5.5		
Blood	7.4		