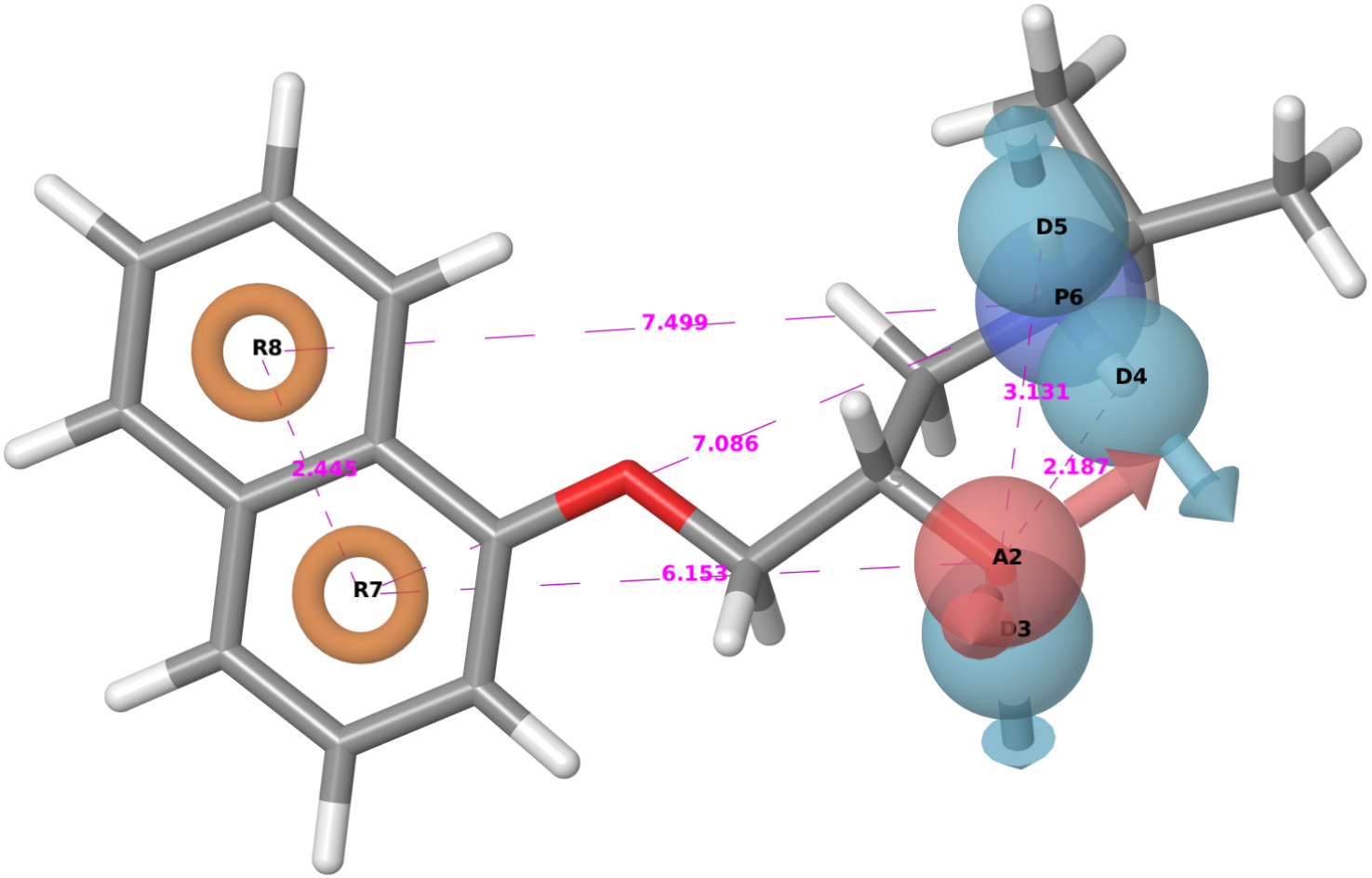
Ligand-Based Design



What is Ligand-Based Design?

Traditional approach in the Computer-Aided Drug Design applied especially in the era before protein crystallography (→ structure based design), **more than 50%** of current FDA-approved drugs were optimized by (some technique related to) LBD*

Applicability domain

- 3D structure of the receptor is unknown (e.g. membrane anchored proteins, receptors or ion channels composed of multiple subunits, problematic expression and purification)
- known hit(s) from screening of compounds from natural extracts or synthetic libraries

Examples of drugs designed by LBD:

- antidepressants and most of psychopharmaca (G-protein coupled receptors, ion channels)
- ACE inhibitors (membrane-anchored enzyme) → case study
- local anesthetics (ion-channel)

*Shim J., MacKerell A.D., Jr.: Computational ligand-based rational design: role of conformational sampling and force fields in model development. *Med. Chem. Commun.* (2011) 2, 356.

Theory behind LBD

Ligand-receptor complementarity

arrangement of functional groups of affine ligands is complementary to the arrangement of the functional groups in receptor (*lock & key, induced fit, conformational selection, population shift*)

Internal strain

biologically active molecules (ligands) bind with their macromolecular counterpart (receptor) in a conformation energetically not too far from the global minimum, i.e. in a conformation with low internal strain

Pharmacophore

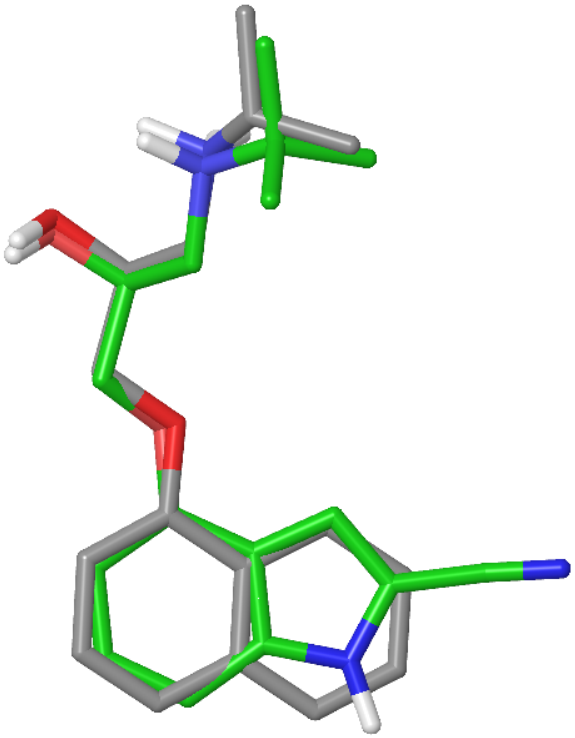
based on a superposition (alignment) of the low energy conformers (identified in a conformational search) of a single or several compounds a common pharmacophore can be derived

Similarity

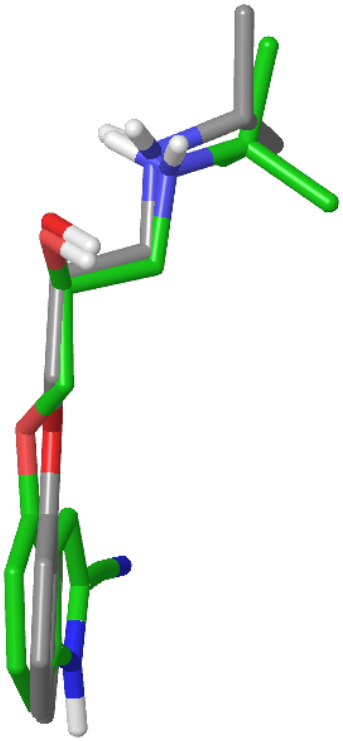
ligands with a similar structure bind to receptor in a similar mode (no multiple binding modes)

Internal strain – a real-world example

Superposition of the bioactive conformation of Cyanopindolol (green carbon atoms, PDB ID: 2VT4) at β1-receptor and low-energy conformer of Propranolol (grey carbon atoms) identified in conformational search using the OPLS2005 force-field in water; ΔE vs. glob.min. = 0.89 kcal/mol



TOP VIEW



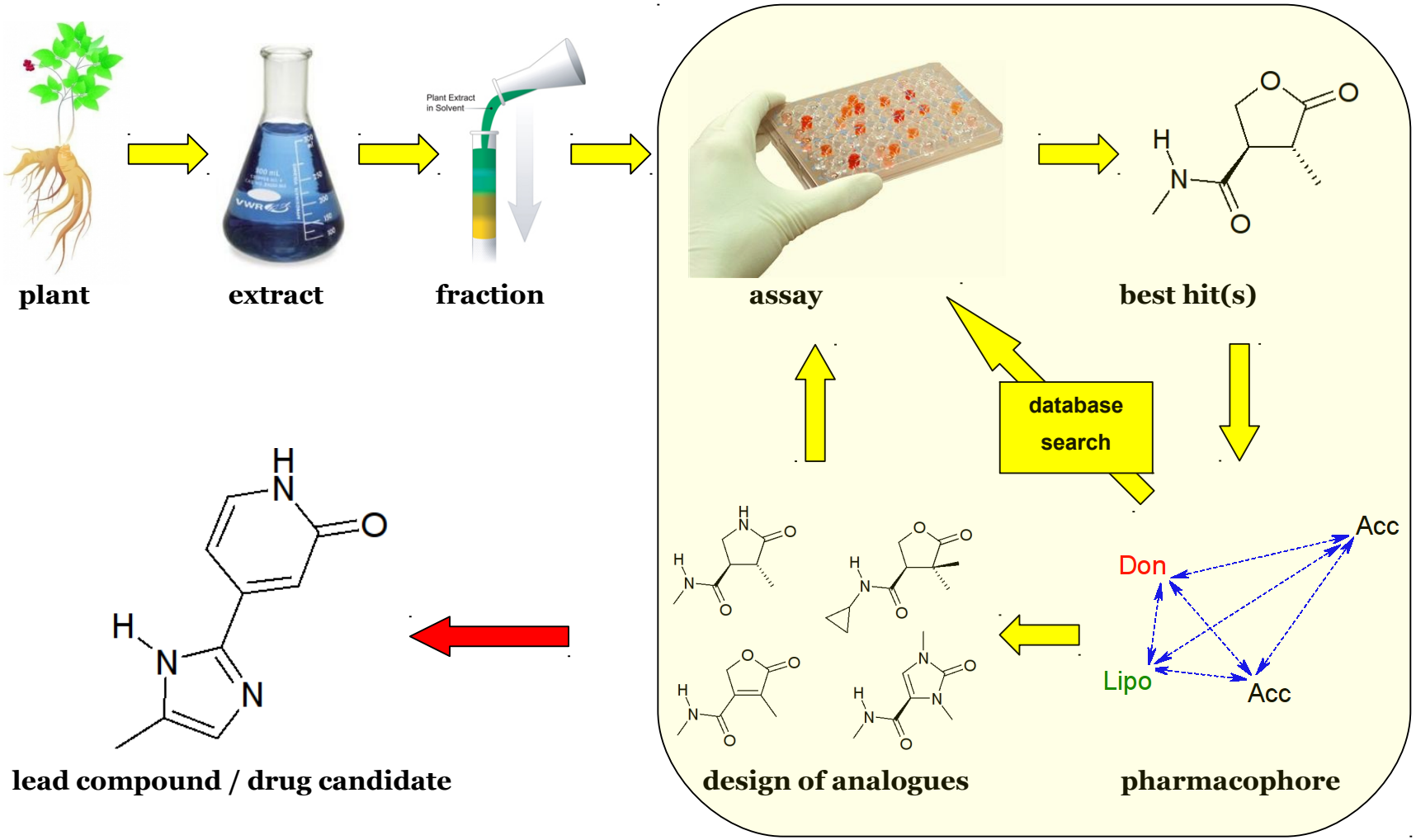
SIDE VIEW

What do we need for a successful LBD?

In LBD the ligands are the only source of data and many molecular descriptors are calculated in order to rationalize scoring and selection (p*K*a, polar surface area, molecular weight, etc.)

- a **greater number** of chemical compounds (usually in later stages of development)
- **diverse scaffolds** help to restrict conformational space
- some **rigid** or at least **conformationally limited** compounds
- active as well as **inactive** molecules, large **range of activities**
- some **directional properties** (e.g. H-bond extension vectors, lone pair vectors, ring planes)
- know **protonation state** at the site of action
- advanced molecular modeling software to perform **conformational search**, **superimpose** conformers (alignment), build **pharmacophore** and calculate **score**

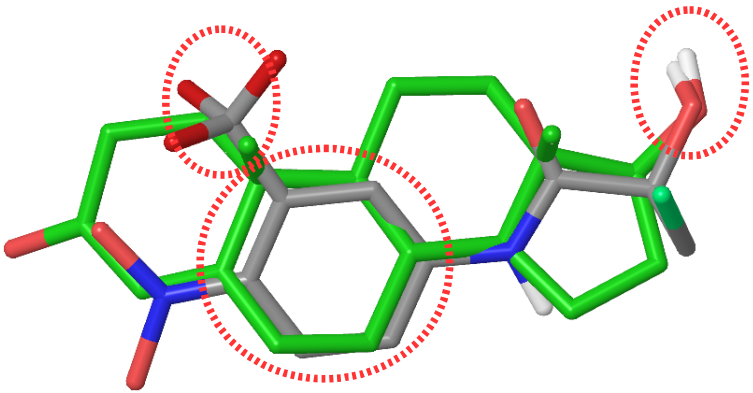
LBD - Schematic overview



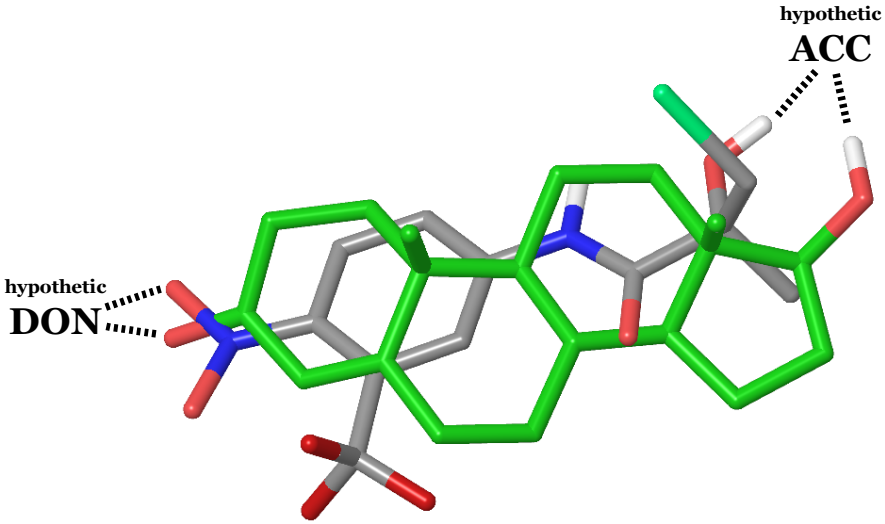
Alignment

Good Alignment → Good Pharmacophore

Molecular alignment – finding the best overlap between multiple molecules (conformers)
atom-based, property-based (e.g. electrostatic potential, field based), hypothetic partner-based...



Functional group-based alignment



Hypothetic partner-based alignment

Alignment

Good Alignment → Good Pharmacophore

Advanced mathematical algorithms

Flexible alignment – simultaneous minimization of strain and fit to a specified template or pharma-cophore; Weigted alignment – pairs have unequal importance (weight)

The most simple measure of goodness of fit is the ***Root Mean Square Deviation* (*RMSD*)**:

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\delta_i^2}$$

, where *N* is number of pairs, δ is distance between two points

Interaction energy-based scoring methods → QSAR models (receptor surrogate), Comparative Molecular Field Analysis (CoMFA), Comparative Molecular Similarity Indices Analysis (CoMSIA)

Special scoring methods → Hologram QSAR (fragments), GRIND (does not need alignment, grid-based), VolSurf (3D voxels – shape, electro, volume → compressed to 2D descriptors)…

Pharmacophore

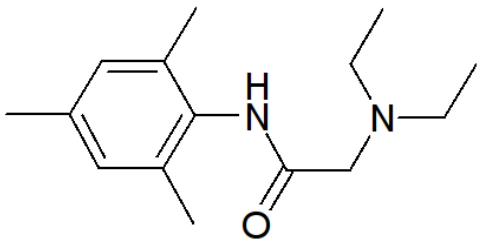
IUPAC definition: "The ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interaction with a specific biological target structure and to trigger (or block) its biological response"

The most active (sometimes the most rigid compound) is usually taken as a **template** from which initial pharmacophore is derived. In the course of the study the pharmacophore is further refined.

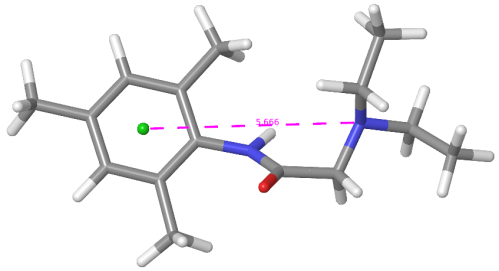
Various levels of abstraction for representing molecules

Descriptional → *arom. ring linked by a heteroatom and two carbon atoms to a tertiary amine…*

1-dimensional → e.g. SMILES code: *CCN(CC)CC(=O)Nc1ccccc1*



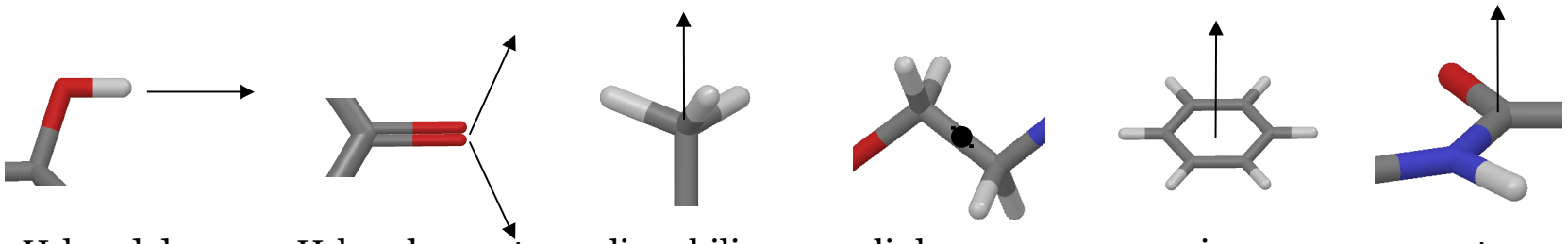
2-dimensional → e.g. 2D formula



3-dimensional → e.g. 3D conformation

Pharmacophore features

Typical molecular features (functional groups) recognized by modeling software



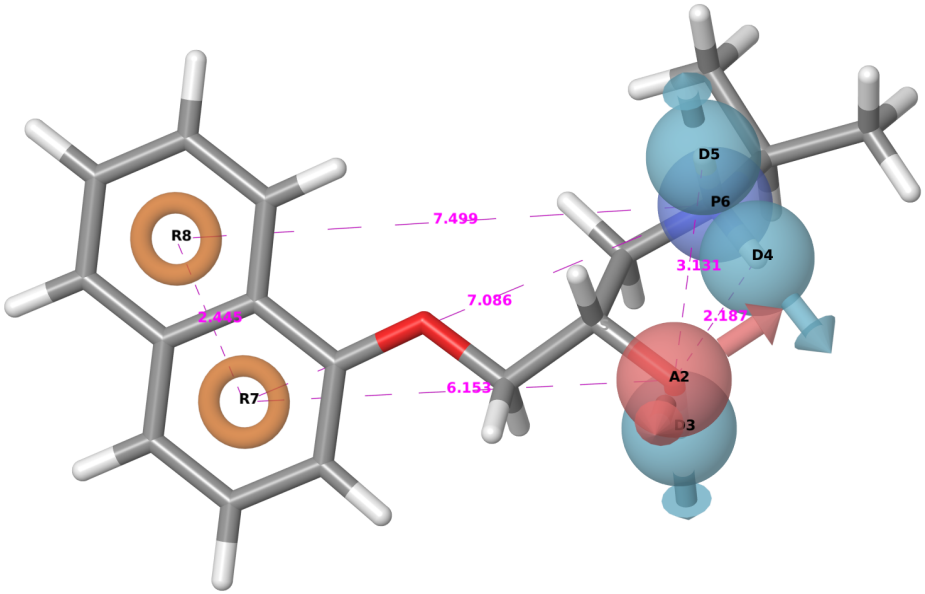
H-bond donor | H-bond acceptor | lipophilic | linker | ring | π-system

Extended pharmacophore representatio

- Information about forbidden zones
- Directional properties like:

extension vectors, ring normals, -systems

- Angles, dihedral angles between properties
- Others, depending on which properties are supported by the database to be searched



Database Searching – Novel Scaffolds

The better the search query, the better the results :)

Similarity searches using

- Pharmacophore - certain form of pharmacophore is used to identify similar molecules
- Fingerprint – combination of various properties (descriptors)

Most popular freely accessible databases

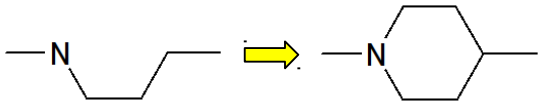
- PubChem - http://pubchem.ncbi.nlm.nih.gov/
- ZINC Database - http://zinc.docking.org/
- ChEMBL - https://www.ebi.ac.uk/chembldb/
- eMolecules - http://www.emolecules.com/
- Relibase (search within data stored at the Protein Data Bank) - http://relibase.ccdc.cam.ac.uk/

Non-free

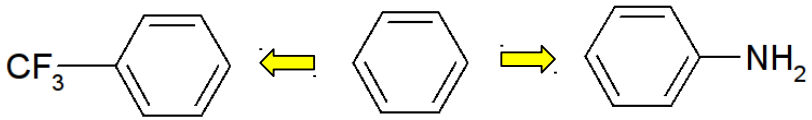
- Cambridge Crystallographic Data Bank (CSD) - www.ccdc.cam.ac.uk
- commercial libraries (pharma companies)

Ligand Optimization Techniques in LBD

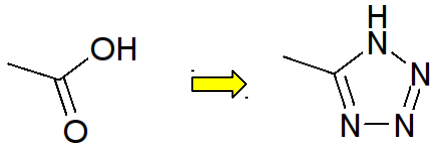
- rigidification



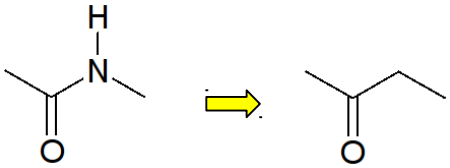
- optimizing electronic distribution



- isosteric & isoelectronic replacements



- replacing scissile bonds



- removing chirality (synthesis is usually easier without chiral centers), if it does not worsen selectivity
- exploring new existence of new pockets/interactions by extending ligand and substitutions

All of the above must be done while **monitoring or actively co-optimizing ADMET** properties and checking **compatibility** with the **pharmacophore**

Ligand Based Design - Conclusion

Advantages
- no need to know 3D structure of target
- can produce drug candidates comparable to structure-based design

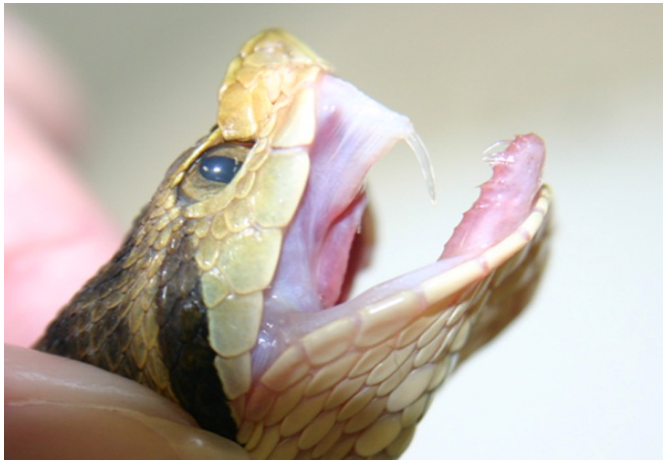
Disadvantages
- need of a higher number of synthesized and tested compounds (systematic structural changes)
- compared to structure-based design: the solvation pattern of the binding site is unknown → cannot improve binding by displacing water; interacting partners on target macromolecule not known → are assumed protonation states correct?

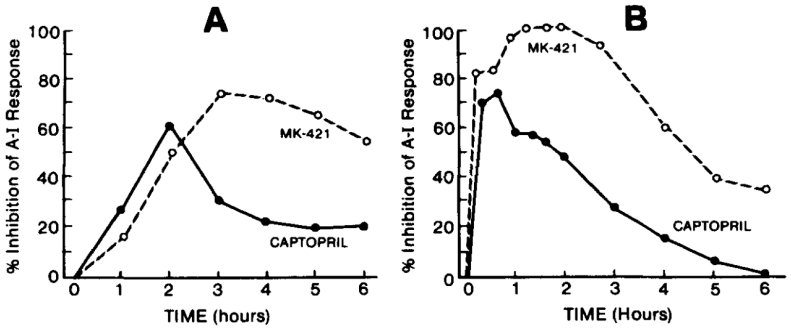
Prerequisites
- a classical target (constant, small or neglegible induced fit)
- good conformational search algorithm, force-field parameters, alignment protocol, scoring function

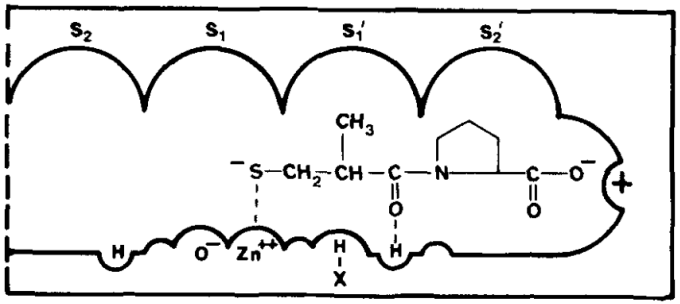
Might fail if
- big conformational changes at receptor site, depending on the ligand
- multiple binding modes
- internal strain needed for proper ligand accommodation higher than assumed

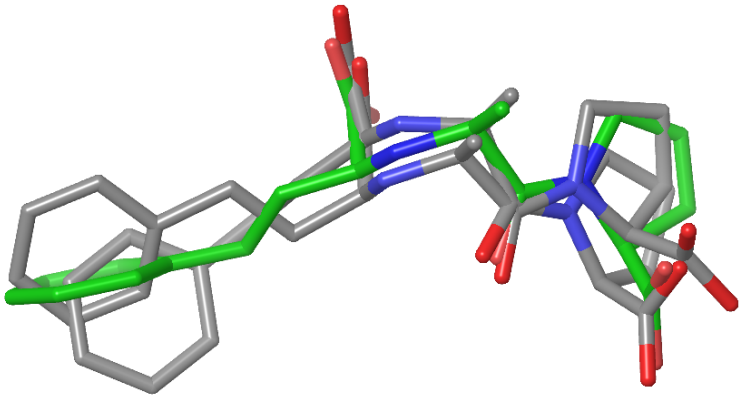
Ligand-Based Design – Case Study

Angiotensin-Converting Enzyme Inhibitors

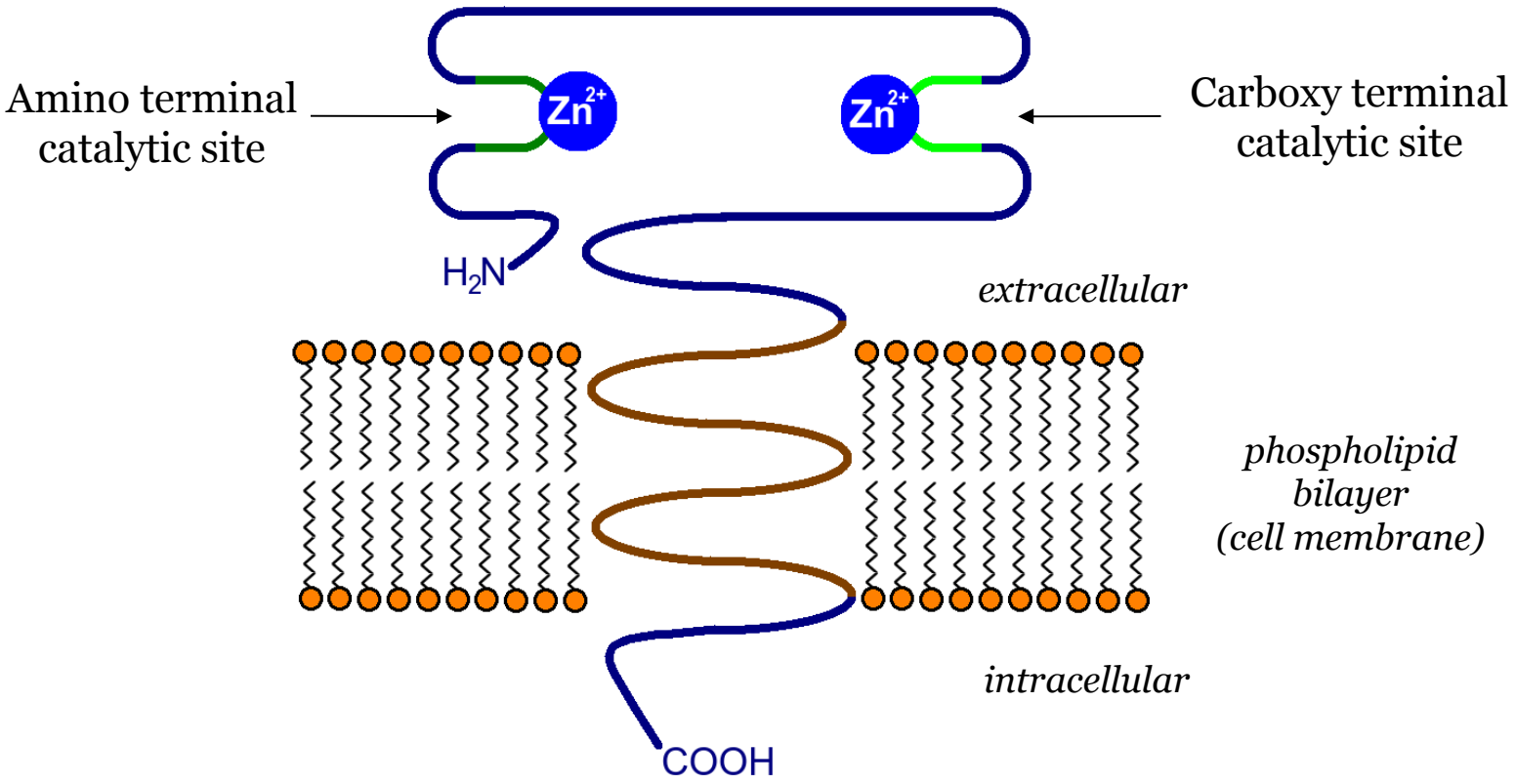






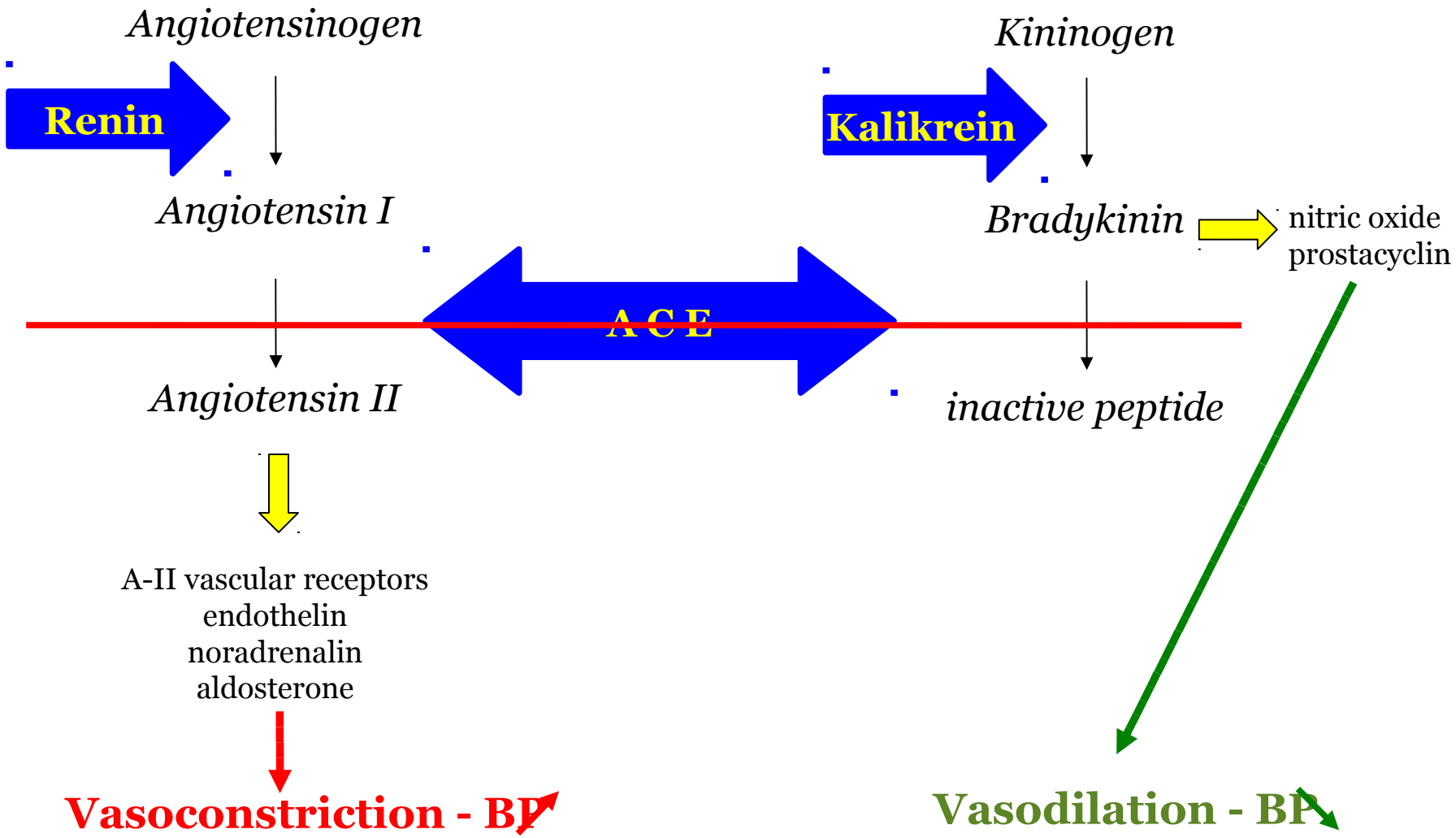


Ligand-Based Design – Case Study

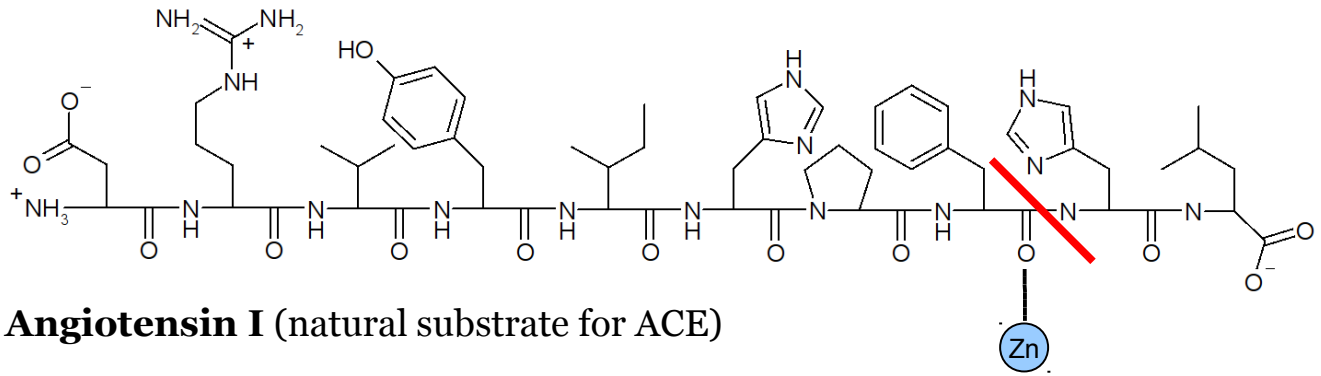


Structure of ACE C-domain was elucidated as late as in 2003.

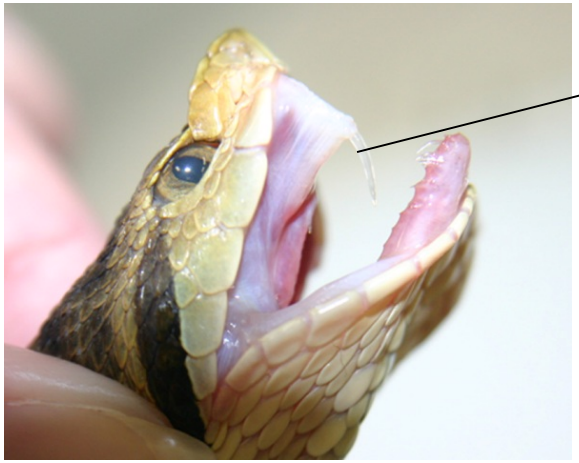
ACE in the Renin-Angiotensin-Aldosterone System



Discovery of Teprotide



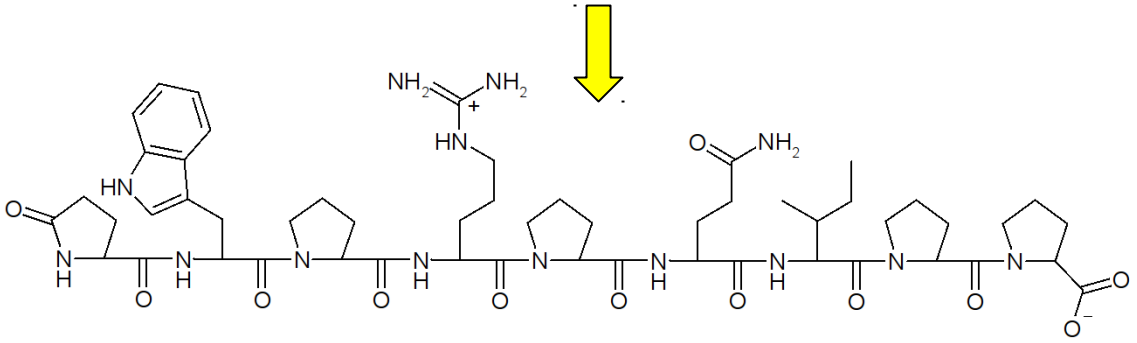
Angiotensin I (natural substrate for ACE)



Bothrops jararaca

Snake venom (bradykinin "potentiating" effect)

S. H. Ferreira, et al., Biochemistry, 9, 2583 (1970).



Teprotide (active compound) – the first peptidic ACE inhibitor

M. A. Ondetti, et al. , Biochemistry, 10, 4033 (1971).

Ligand Based Design of ACE inhibitors

based on

Angiotensin-Converting Enzyme Inhibitors: Medicinal Chemistry and Biological Actions

Medicinal Research Reviews, Vol. 2, No. 1, 1-41 (1982)

+

Recent Developments in the Design of Angiotensin-Converting Enzyme Inhibitors

Medicinal Research Reviews, Vol. 5, No. 4, 483-531 (1985)

+ additional literature > 1985

Mapping binding site using peptides

S_1 Subsite

Hip–X–His–Leu

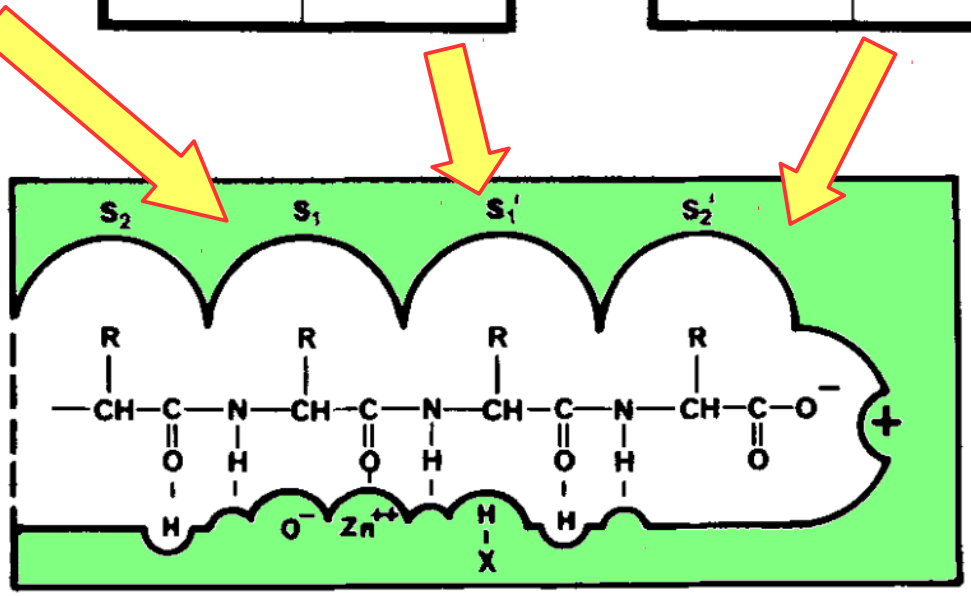
X	K_i
Arg	0.4
Phe	0.8
Pro	1.2
Ala	1.3
Ile	1.6
Ser	1.8
Glu	4.5
D-Phe	1.5

S_1' Subsite

Hip–X–Leu		X–Gly	
X	K_i	X	I_{50}
Pro	0.14	Val	1100
Arg	0.77	Arg	1200
His	*	Ala	2500
Ala	1.8	Lys	3200
Phe	1.9	Phe	3700
Glu	4.0	His	6300
		Gly	7200
		Leu	8800
		Glu	10000
		Pro	17000

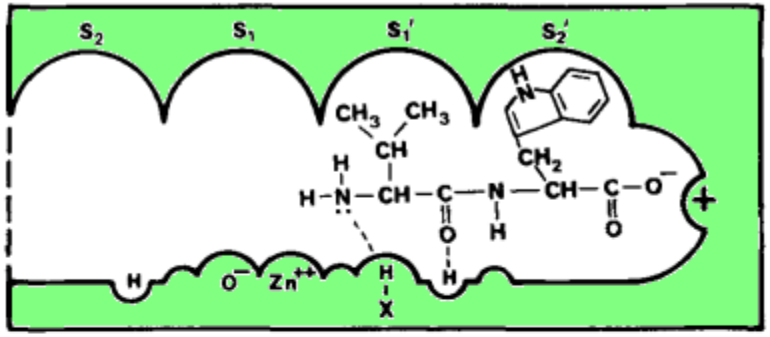
S_2' Subsite

Hip–His–X		Gly–X		$HSCH_2CH_2COX$	
X	K_i	X	I_{50}	X	I_{50}
Arg	0.08	Trp	30	Trp	0.07
Pro	0.64	Pro	210	Pro	0.20
Leu	*	Phe	450	Phe	0.43
Ala	3.2	Ala	2000	Ala	0.85
Phe	3.7	Leu	2500	Leu	1.6
Glu	>50.	Arg	3200	Arg	1.7
		Lys	5400	Lys	2.4
		Glu	5400	Gly	3.2
		Gly	7200	Asp	68.
		Asp	9200	D-Pro	1800.

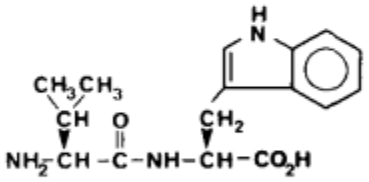


Dipeptide Inhibitors without Zn ligand

(Combining the best fragments from the peptide scan)



I_{50} μM



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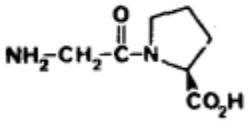
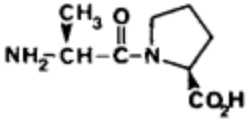
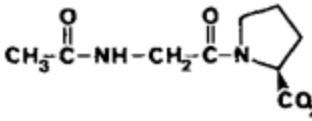


450.



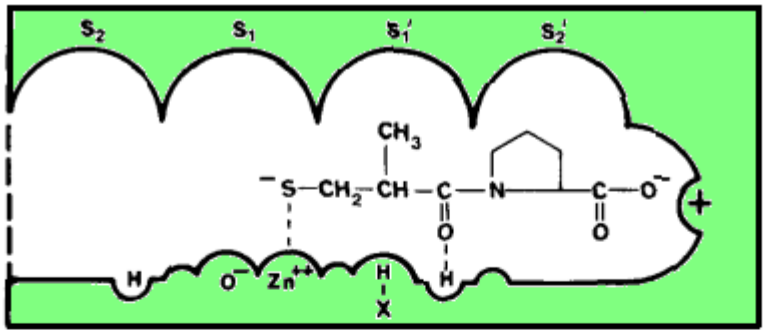
230.

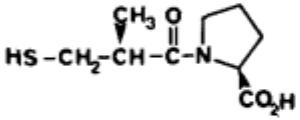
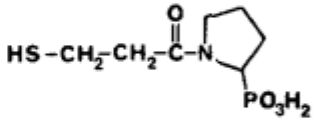
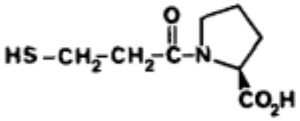
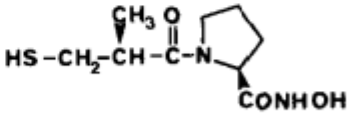
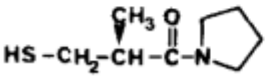
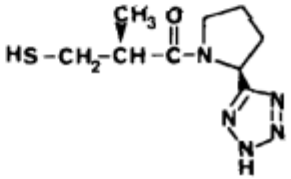


27,000.

Dipeptide Inhibitors with Zn ligand

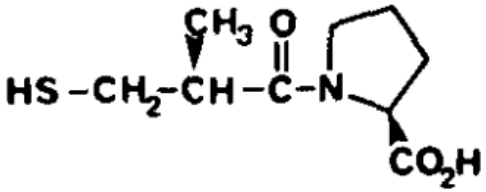
(Inspecting role of the terminal carboxyl by isosteric replacements)



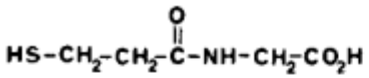
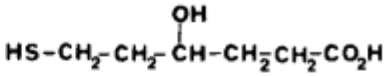
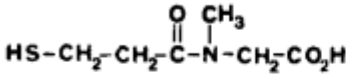
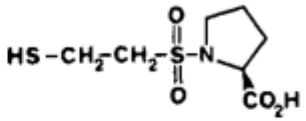
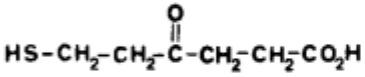
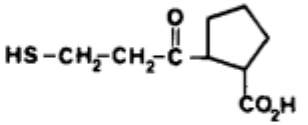
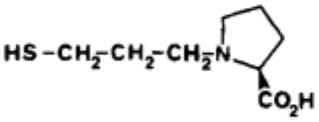
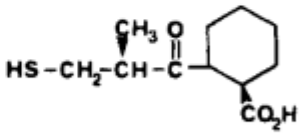
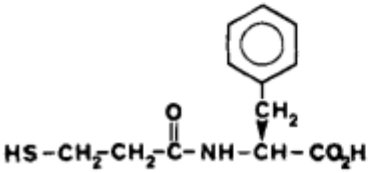
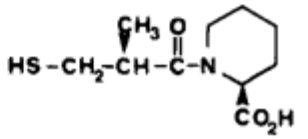
	I_{50} μM
	0.023
	1.7
	0.20
	0.022
	240.
	0.26

Dipeptide Inhibitors with Zn ligand

(Searching for the key features – ring size, flexibility, carbonyl)

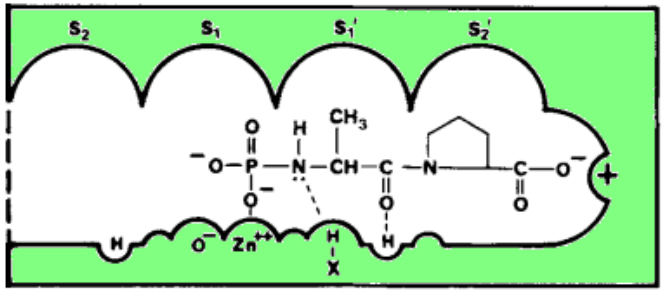


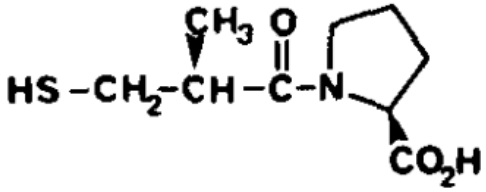
IC_{50} = 0.023µM

	I_{50} µM		
	2.8		1200.
	3.0		11.
	11.		0.74
	240.		2.4
	0.85		0.13

Dipeptide Inhibitors with Zn ligand

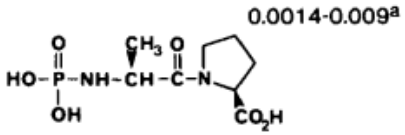
Phosphoric acid-based better than thiols?

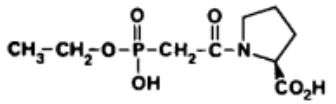


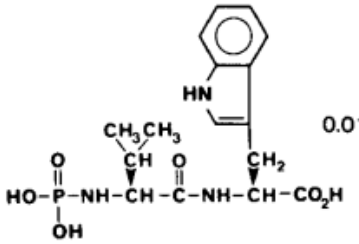


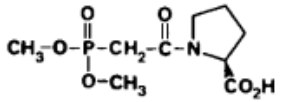
IC50 = 0.023µM

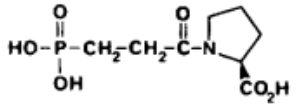
I_{50} µM

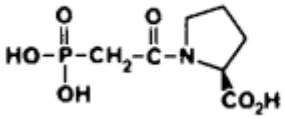


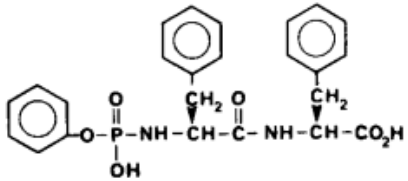


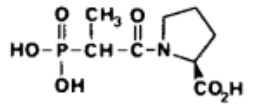




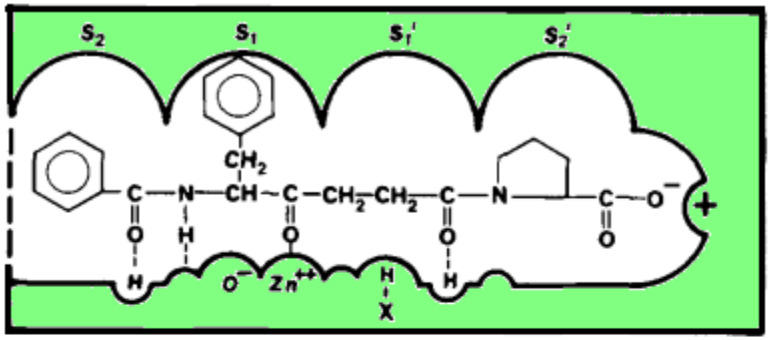




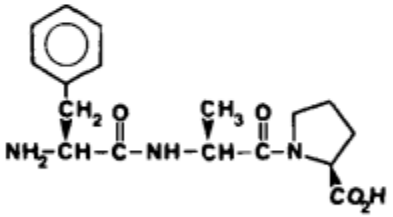




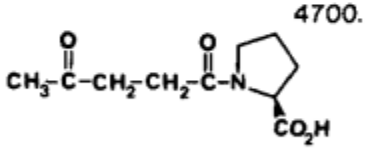
Tripeptide Inhibitors



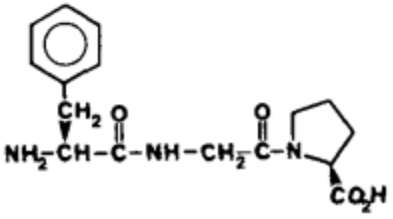
I_{50} μM



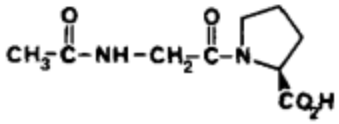
1.4[a]
4.2[b]



4700.

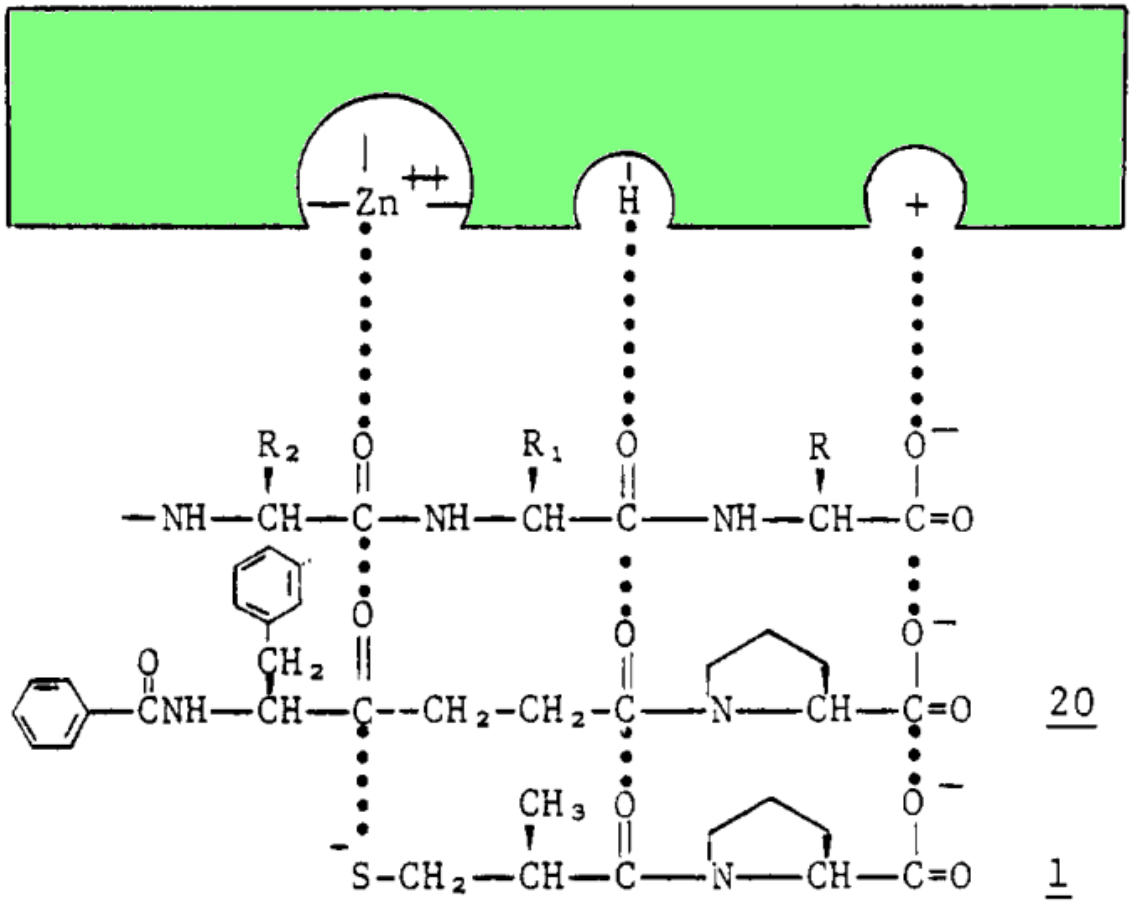


20.



27000.

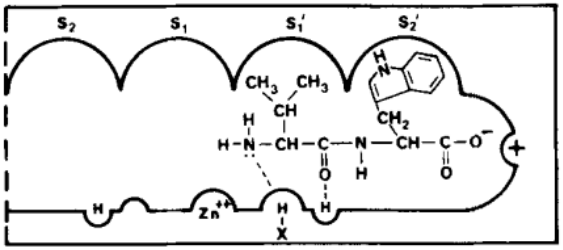
Ketomethylene Tripeptide Analogs



J. Med. Chem. 1980, 23, 1392-1398

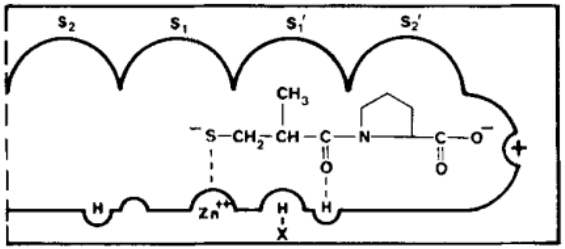
Overview

Dipeptide



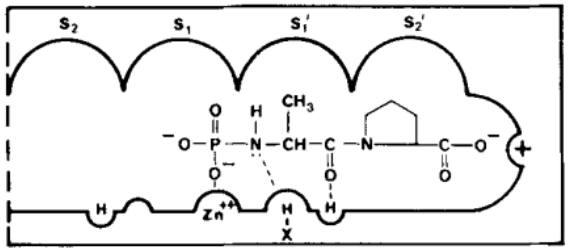
$I_{50} \cong 10^{-6}$

Mercaptoacyl amino acid



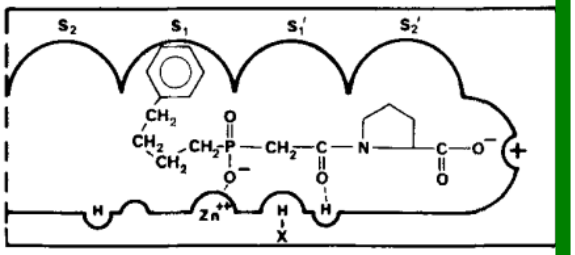
$I_{50} \cong 10^{-8}$

Phosphoryl dipeptide



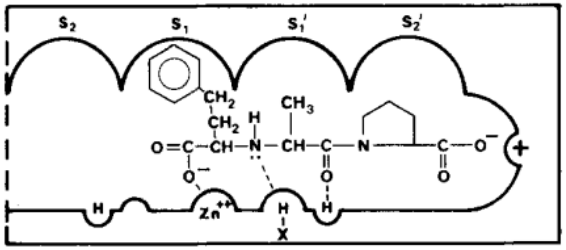
$I_{50} \cong 10^{-8}$

Phosphinic acid



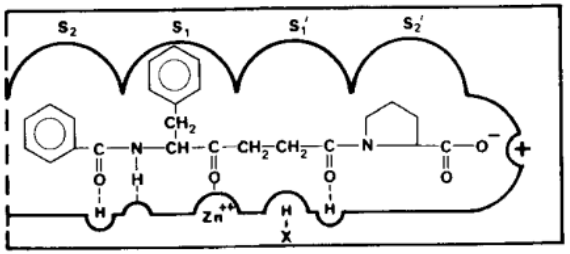
$I_{50} \cong 10^{-7}$

Carboxyalkyl dipeptide



$I_{50} \cong 10^{-9}$

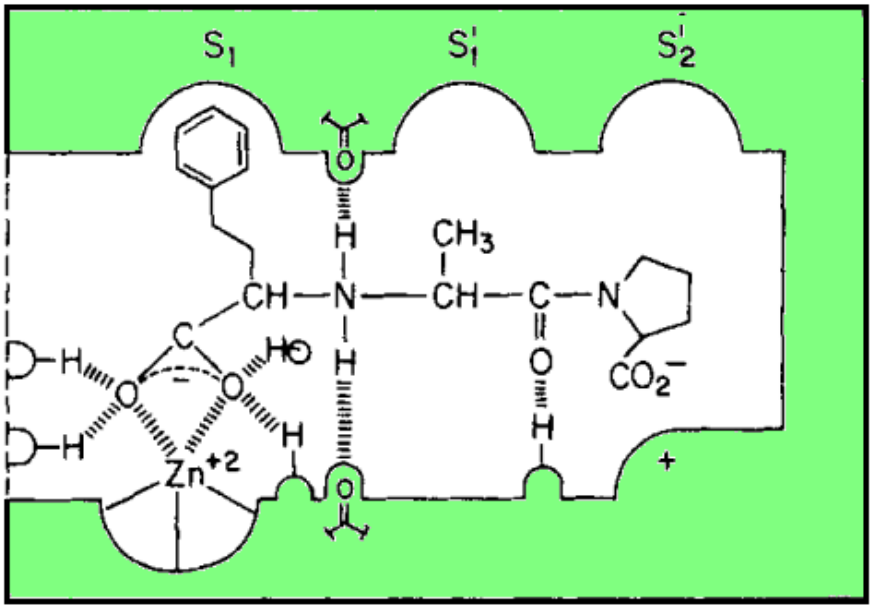
Tripeptide ketone

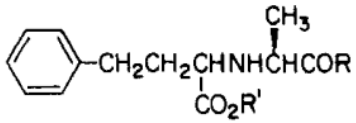


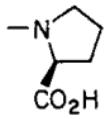
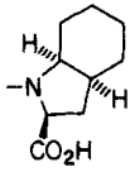
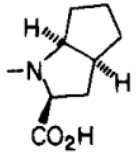
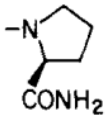
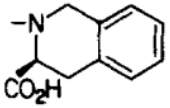
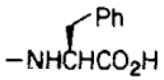
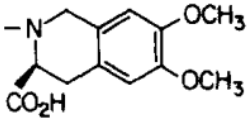
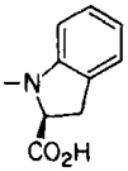
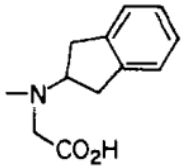
$I_{50} \cong 10^{-8}$

Carboxy alkyl dipeptides

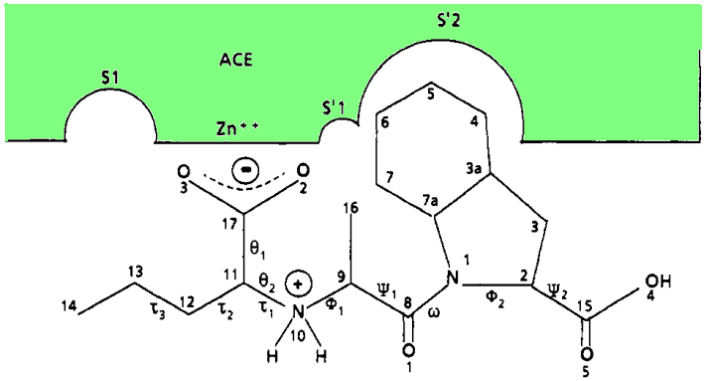
(Searching for the best C-terminal scaffold @ S'_2)

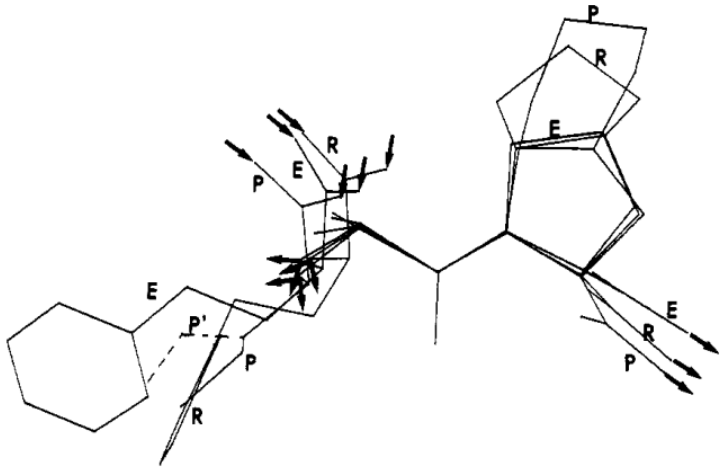


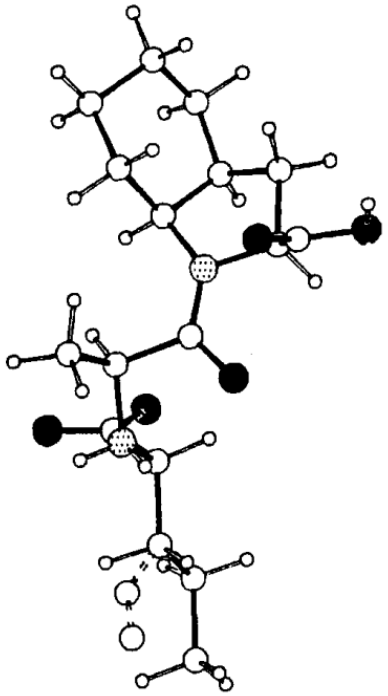


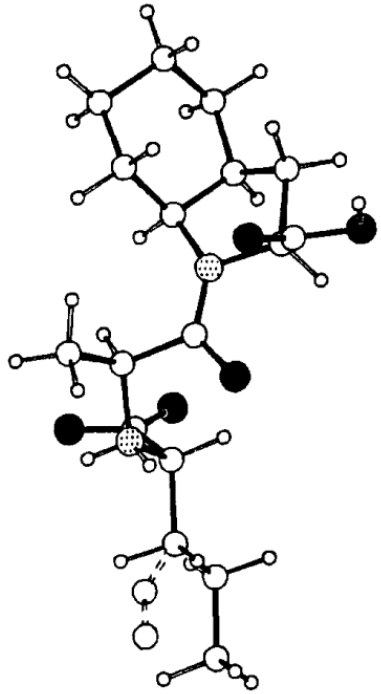
R	I_{50} (nM)	R	I_{50} (nM)
	1.2		2.2
—OH	>16700[b]		4.2[d]
	>167[b]		2.8
	86[b,c]		2.6
	3.0		40

1991
Perindoprilat – moving from 2D to 3D pharmacophore





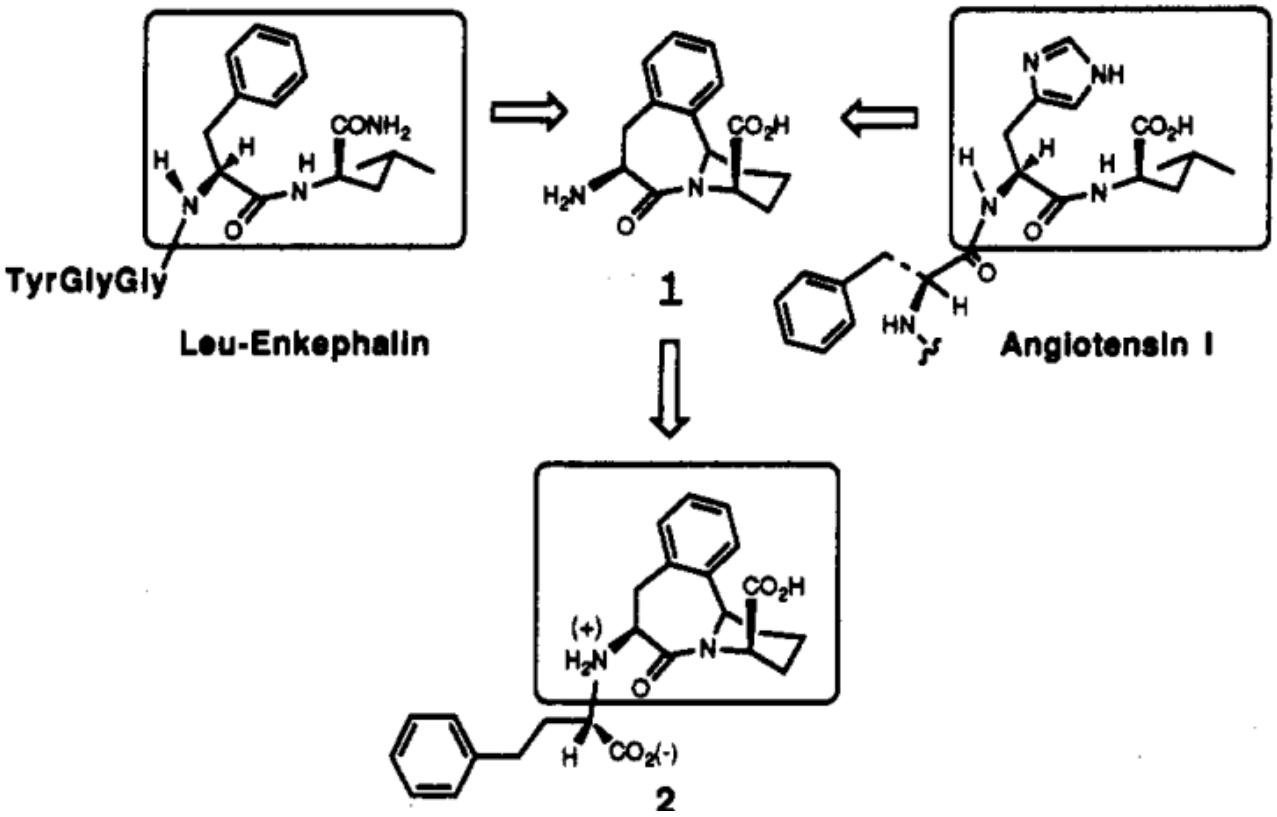




J. Med. Chem. 1991, 34(2), 663

1993

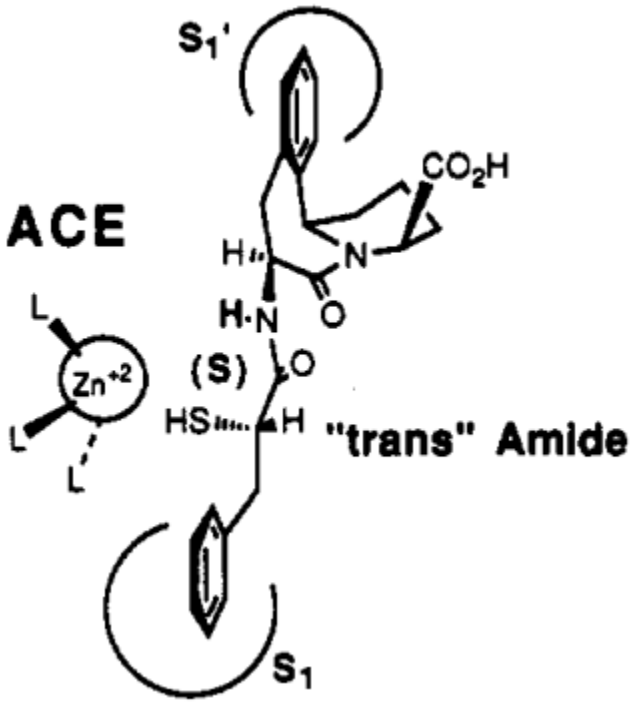
Conformationally Restricted Phe-Leu Dipeptide Mimetic

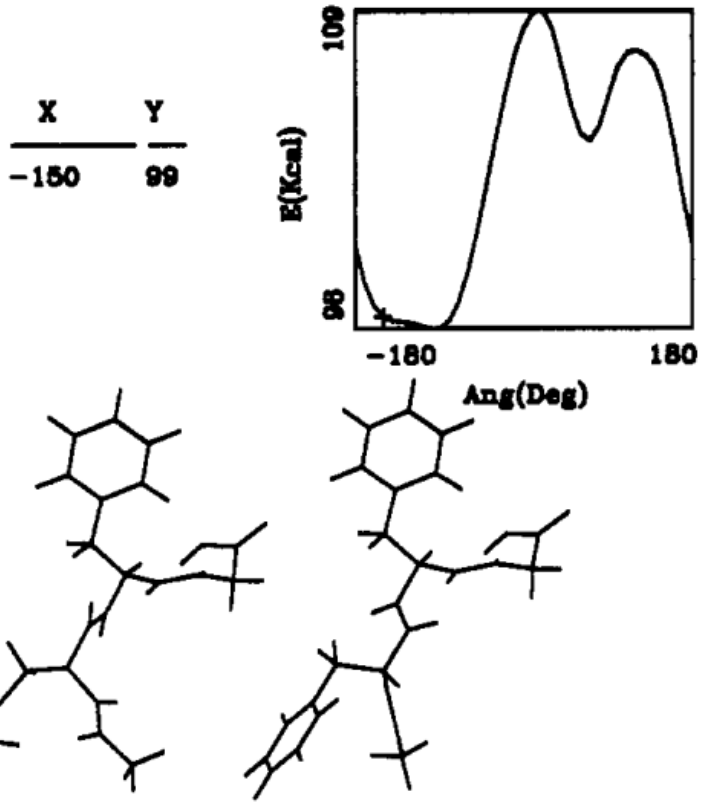


J. Med. Chem. 1993, 36(16), 2420

1993

Conformationally Restricted Phe-Leu Dipeptide Mimetic

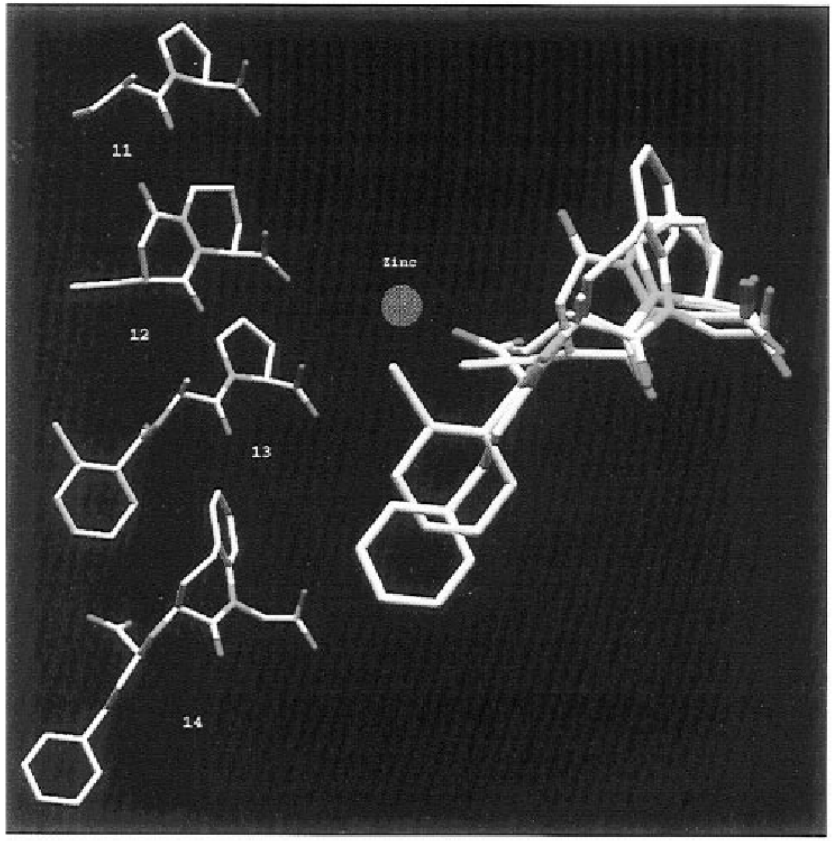


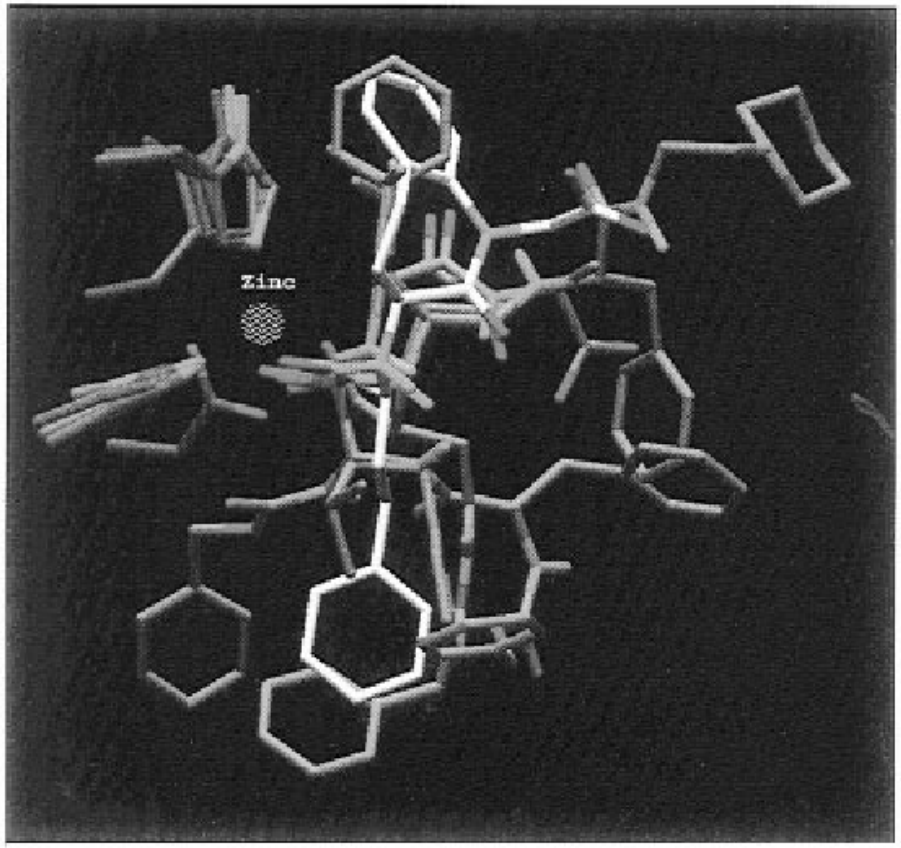


J. Med. Chem. 1993, 36(16), 2420

1996

Dual NEP/ACE Inhibitors

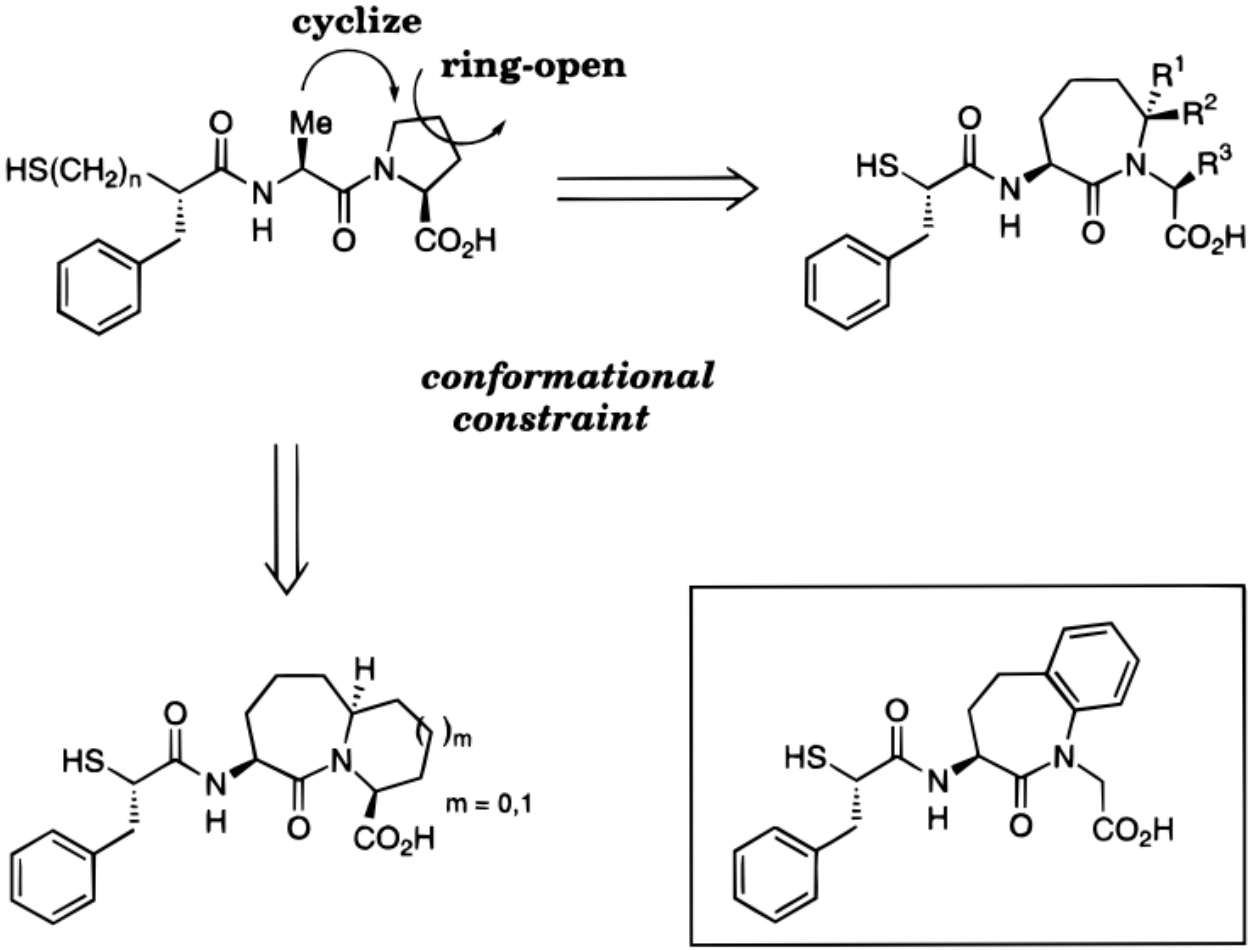




J. Am. Chem. Soc. 1996, 118(35), 8237

1999

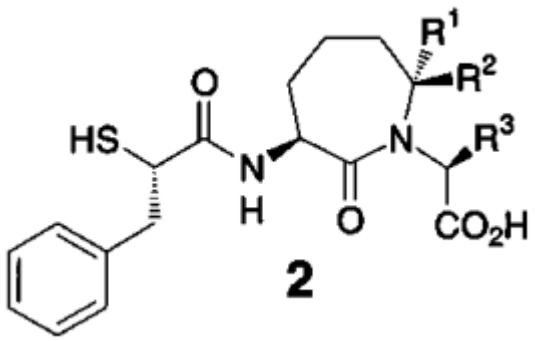
Cyclic Analogues



J. Med. Chem. 1996, 39, 494-502

1999

Cyclic Analogues – a classical "med-chem" approach



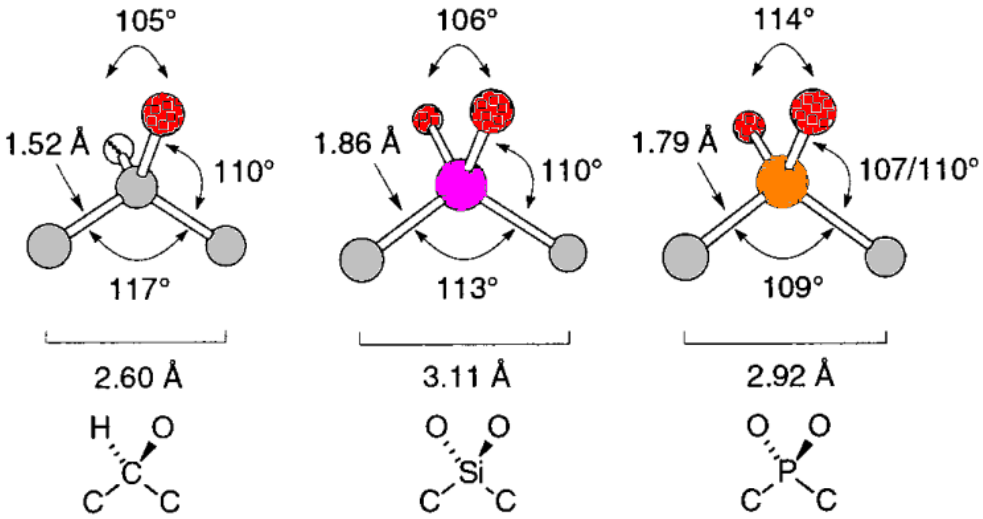
2

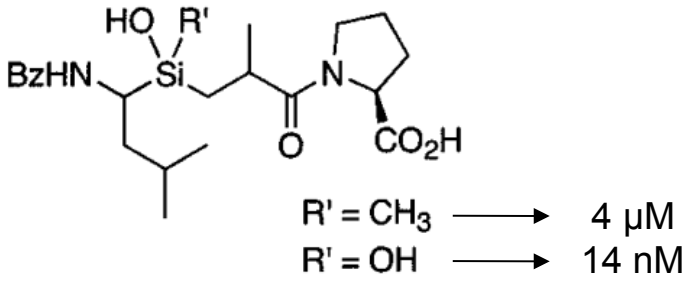
R^1	R^2	R^3	(nM) ACE[c]
H	H	H	22
H	H	methyl	26
H	H	benzyl	479
H	H	isopropyl	907
methyl	H	H	8
H	methyl	H	16
propyl	H	H	11
H	propyl	H	14
allyl	H	H	7
hydroxyethyl	H	H	25
methylcyclopropyl	H	H	23
isobutyl	H	H	10
cyclopentyl	H	H	17
propyl	H	methyl	5
propyl	H	ethyl	25

J. Med. Chem. 1996, 39, 494-502

2002

Mimicking transition state → silicon-based ACE inhibitors





R' = CH_3 ⟶ 4 μM

R' = OH ⟶ 14 nM

J. Am. Chem. Soc. 2002, 124, 7363

Conclusion

- Peptide scan followed by pharmacophore determination
- Large number of compounds synthesized
- Gradually improving and extending the pharmacophore helped in increasing the affinity of the lead compound
- Various additional techniques were employed
- Drug (candidates) with excellent affinity and properties could be designed