Combinatorial and Fragment-based Ligand Design

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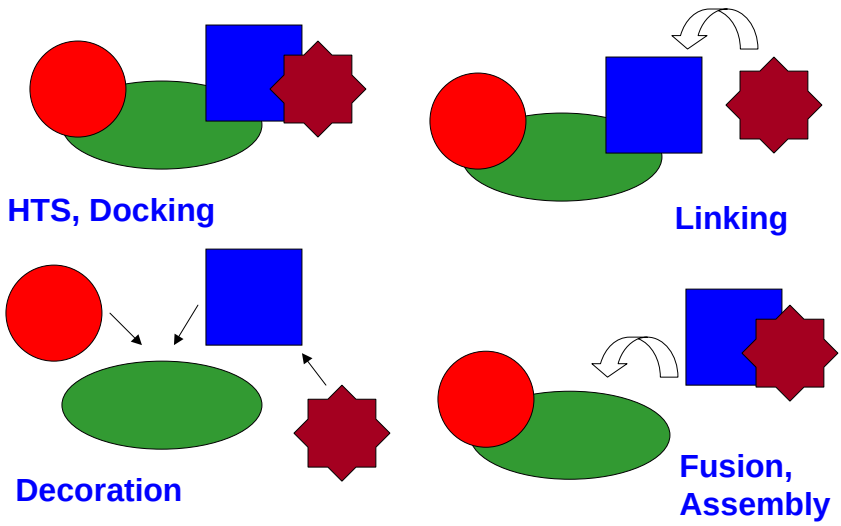
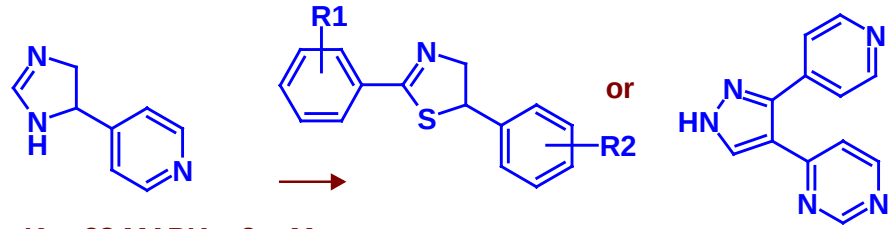
„Classical“ and „Combinatorial“ Drug Design

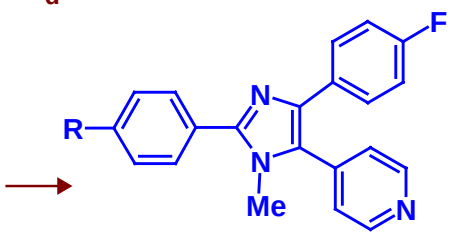


SHAPES Strategy: NMR Screening of Ligands



K_d p38 MAPK = 2 mM

K_d p38 MAPK = 200-300 µM

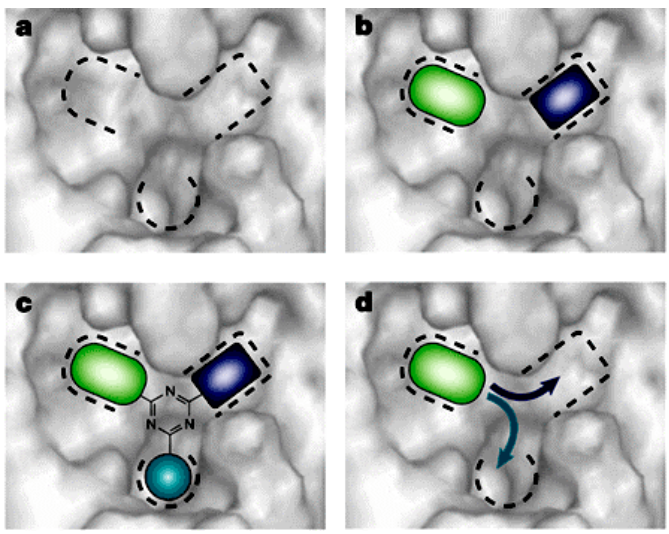


R = COOH or S(=O)Me

K_d p38 MAPK = 10-200 nM

J. Fejzo et al., Chem. Biol. 6, 755-769 (1999)

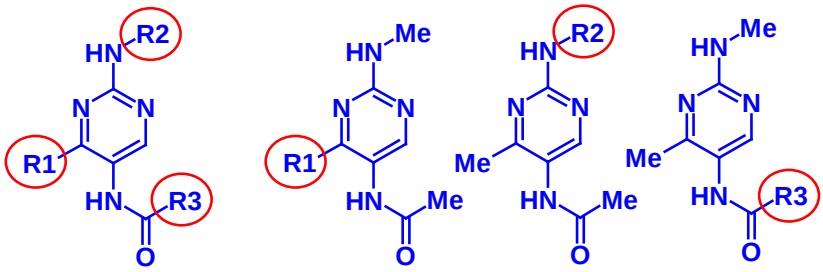
Fragment-Based Approach (Astex)



a) complex binding site
b) fragments in different pockets
c) assembly of different fragments
d) growing out from a fragment

T. L. Blundell et al., Nature Rev. Drug Discov. 1, 45-54 (2002)

Fragment-Based Approach With Central Scaffold



A library with

100 x R1,
100 x R2, and
100 x R3 yields

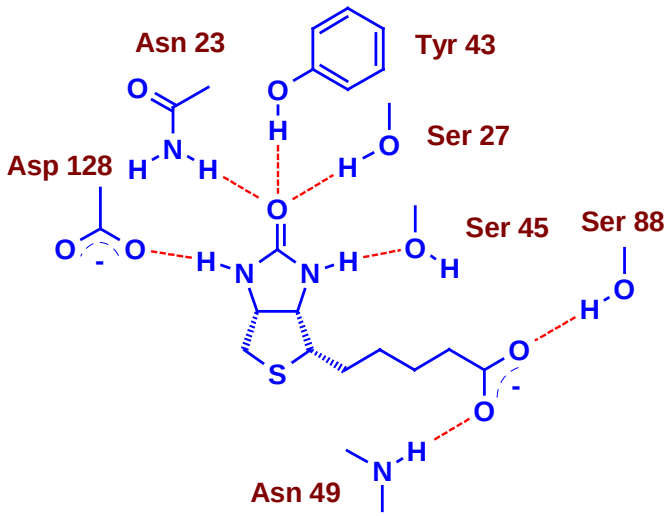
1 mio compounds

100 + 100 + 100 variations yield a library of only 300 compounds

R. Carr and M. Hann, Modern Drug Discov., April 2002, 45-48

Hugo Kubinyi, www.kubinyi.de

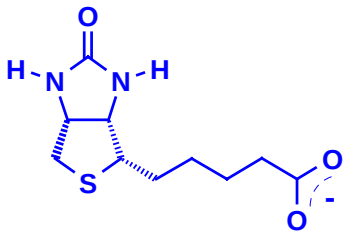
Enthalpic and Entropic Contributions to Binding: 3D Structure of the Biotin-Streptavidin Complex



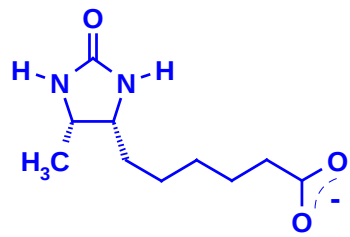
„Entropic Stabilisation“ of a Ligand-Receptor Complex

Binding Constants of Biotin and Analogs

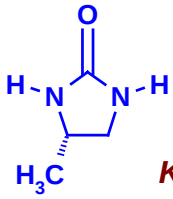
(N. M. Green, Adv. Protein Chem. 29, 85-133 (1975))



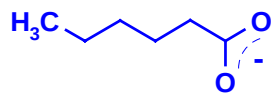
Biotin, K_i = 1,3 x 10^{-15} M



Desthiobiotin, K_i = 5 x 10^{-13} M

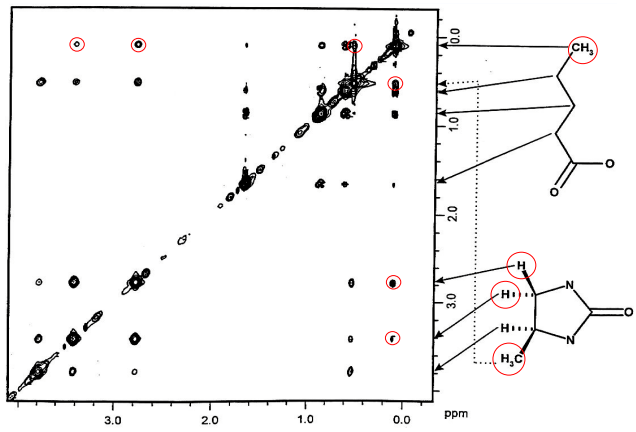


K_i = 3,4 x 10^{-5} M



K_i = 3 x 10^{-3} M

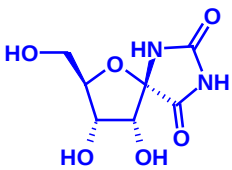
„Re-discovering“ Biotin by NMR Fragment-Based Screening



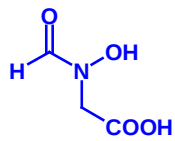
NOESY experiment

streptavidin plus two biotin fragments;
intermolecular NOEs indicate the „correct“ linkage of the fragments
(A. Kline et al., The NMR Newsletter 472, 13 (1997))

Design of a Bisubstrate Inhibitor of Adenylosuccinate Synthase (AdSS)



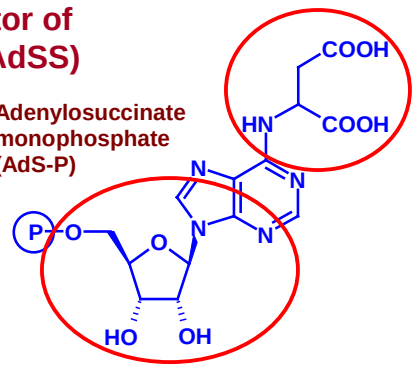
Hydantocidin

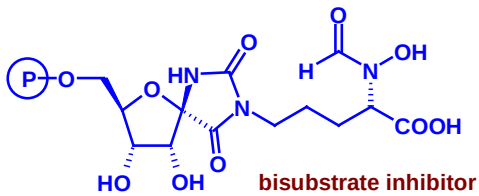


Hadacidin

are natural product inhibitors of AdSS

Adenylosuccinate monophosphate (AdS-P)





bisubstrate inhibitor

S. Hanessian et al., Angew. Chem. Int. Ed. 38, 3159-3162 (1999)

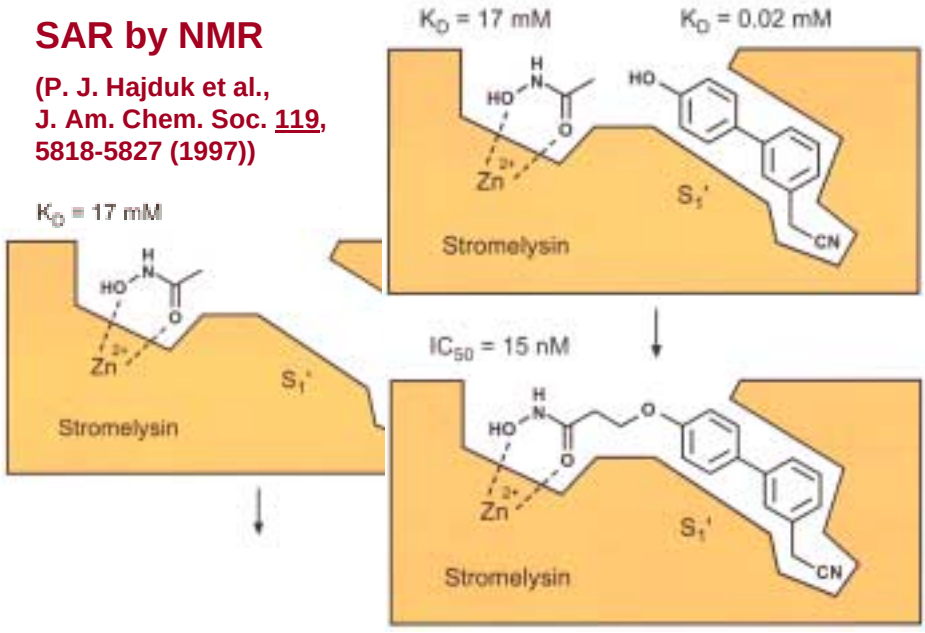
	IC_{50} in µMol, AdSS from E. coli	wheat
hyd-P	0.675	1.35
hadacidin	3.5	12
bisubstrate inhibitor	0.043	0.20

Michelangelo, The Creation of Adam

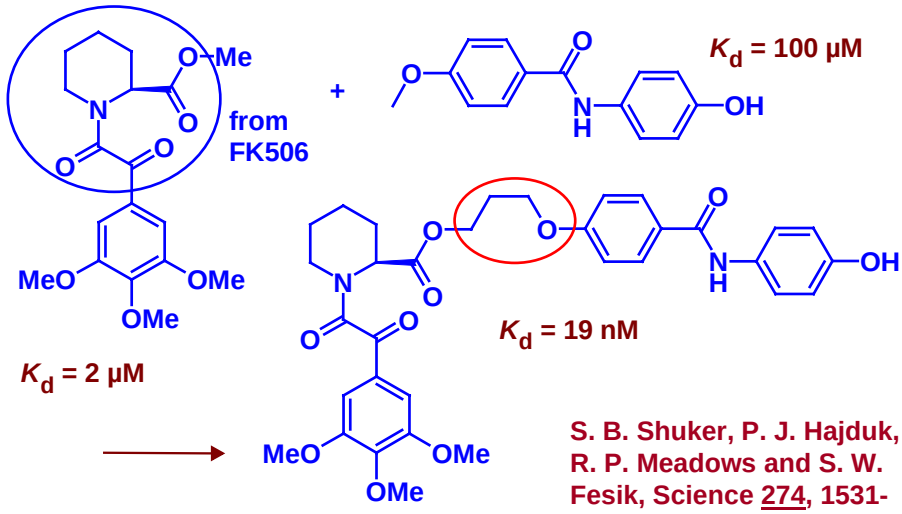
(fresco, detail from the Sistine Chapel ceiling, Vaticane)

SAR by NMR

(P. J. Hajduk et al.,
J. Am. Chem. Soc. 119,
5818-5827 (1997))



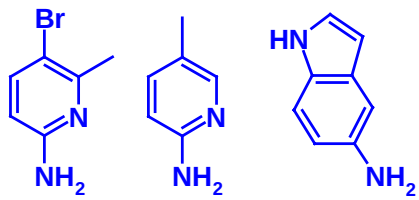
SAR by NMR: FKBP Ligands



S. B. Shuker, P. J. Hajduk, R. P. Meadows and S. W. Fesik, Science 274, 1531-1534 (1996).

CrystaLEAD - Crystallographic Screening

Crystallographic hits (Urokinase inhibitors)

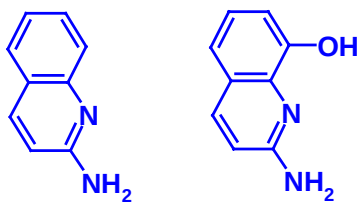


K_i > 500 µM

K_i = 200 µM

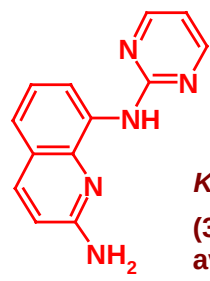
V. L. Nienaber et al., Nat. Biotechnol. **18**, 1105-1108 (2000)

optimized structure:



K_i = 71 µM

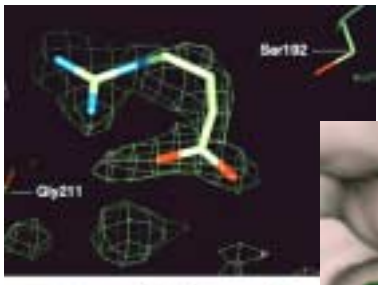
K_i = 56 µM

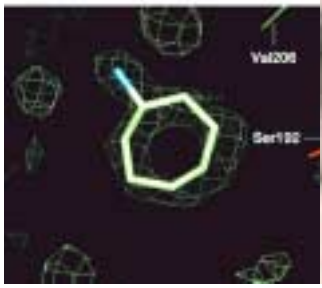


K_i = 370 nM

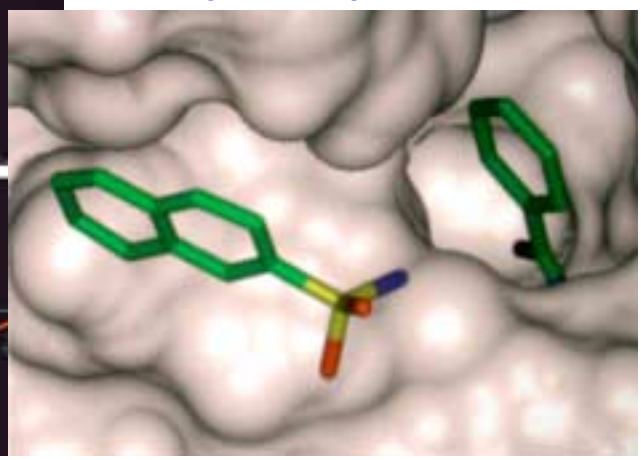
(38% bio-availability in the rat)

HTX in Drug Design: The Astex Pyramid Approach

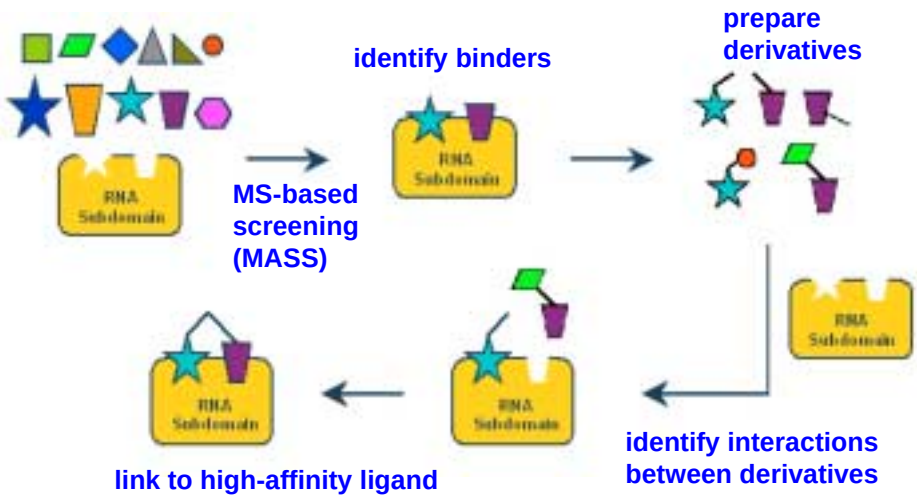




Virtual screening (AutoDock, up to 10^{14} compounds), soaking (AutoSolve software) and linking of building block libraries

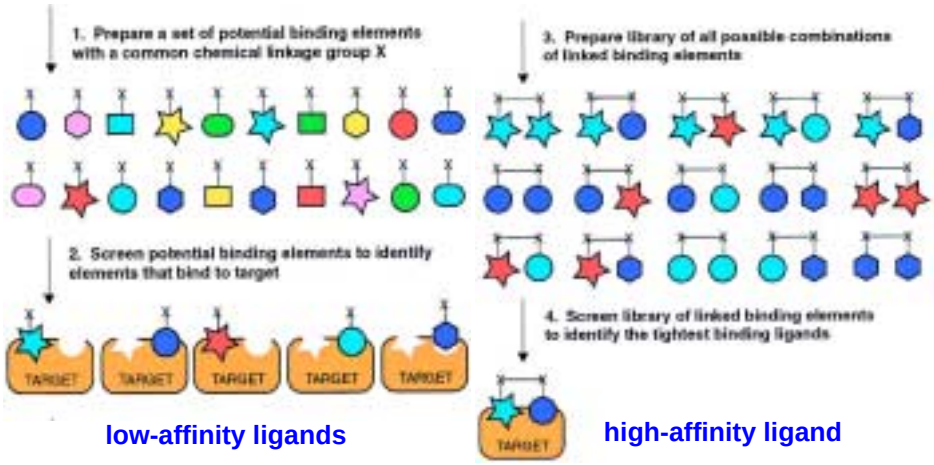


The SAR by MS Approach for RNA Targets



© Ibis Pharmaceuticals, www.ibisrna.com/darpa_ii/chemistry.html
E. E. Swayze et al., J. Med. Chem. 45, 3816-3819 (2002)

Combinatorial Libraries of Linked Low-Affinity Binders



D. J. Maly, I. C. Choong and J. A. Ellman, PNAS 97, 2419-2424 (2000)

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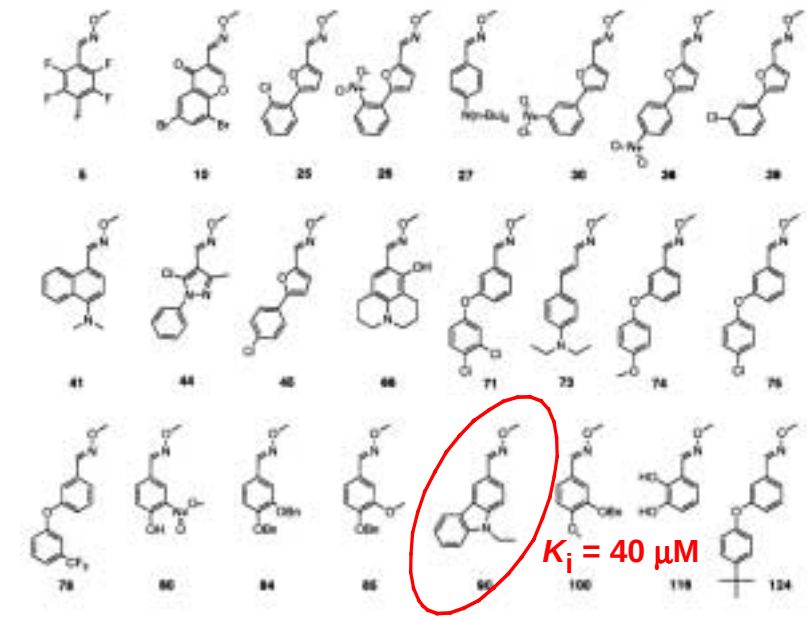

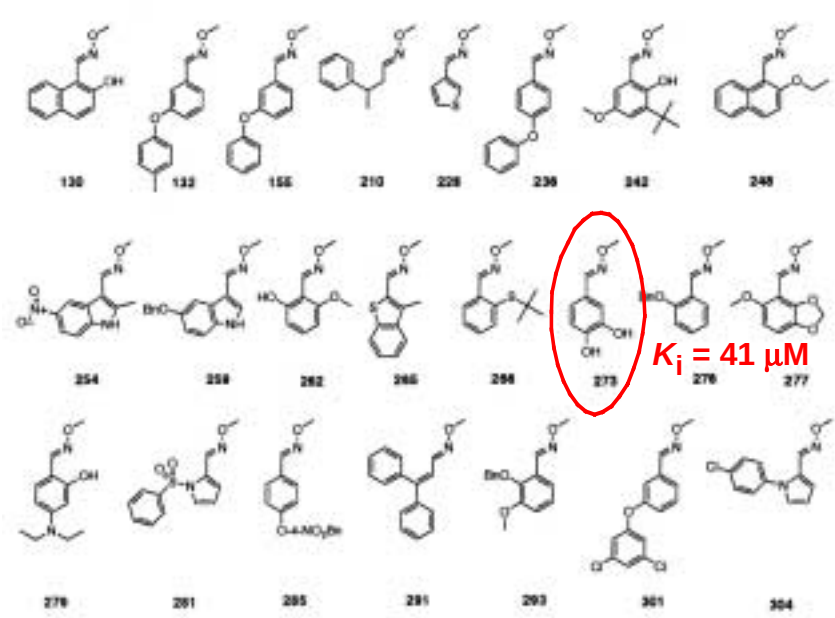

K_i = 41 µM

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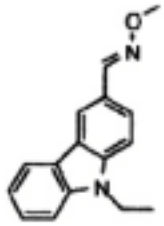
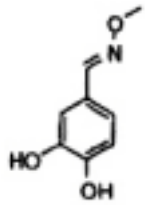
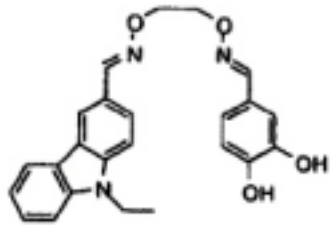
Fragment-Based Design: Protein Kinase Inhibition



[90]



[273]

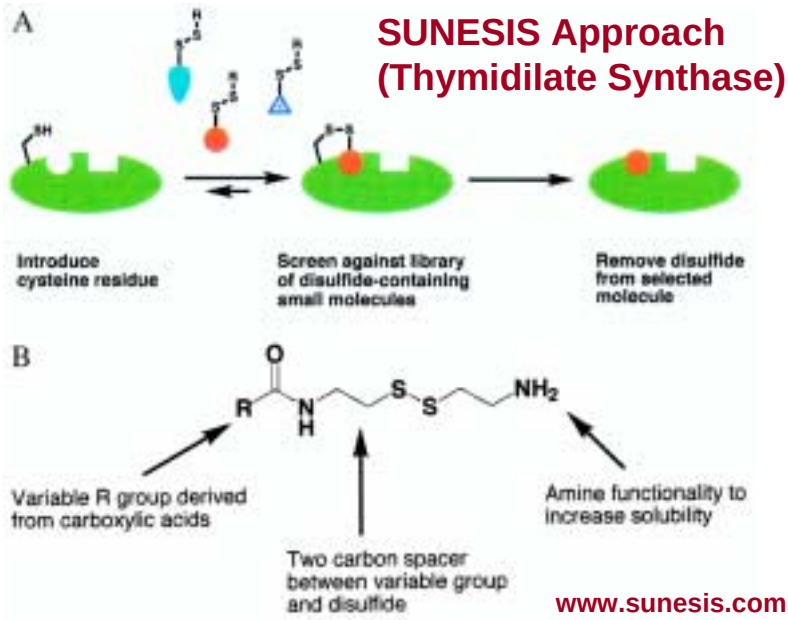


[90, 273, n=2]

Compound	IC_{50}, µM c-Src	Fyn	Lyn	Lck
[273]	41 ± 5	>1000	>1000	>1000
[90]	40 ± 16	64 ± 50	400 ± 170	>500
[90, 273, *n* = 2]	0.064 ± 0.038	5.0 ± 2.4	13 ± 3	>250

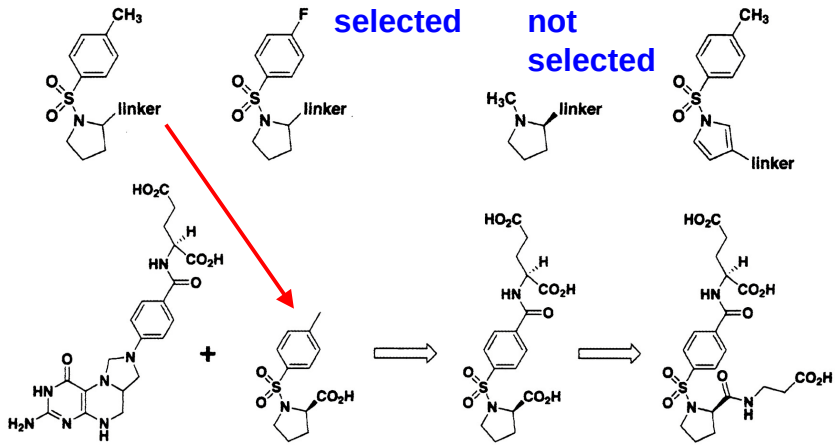
D. J. Maly, I. C. Choong and J. A. Ellman, PNAS 97, 2419-2424 (2000)

SUNESIS Approach (Thymidilate Synthase)



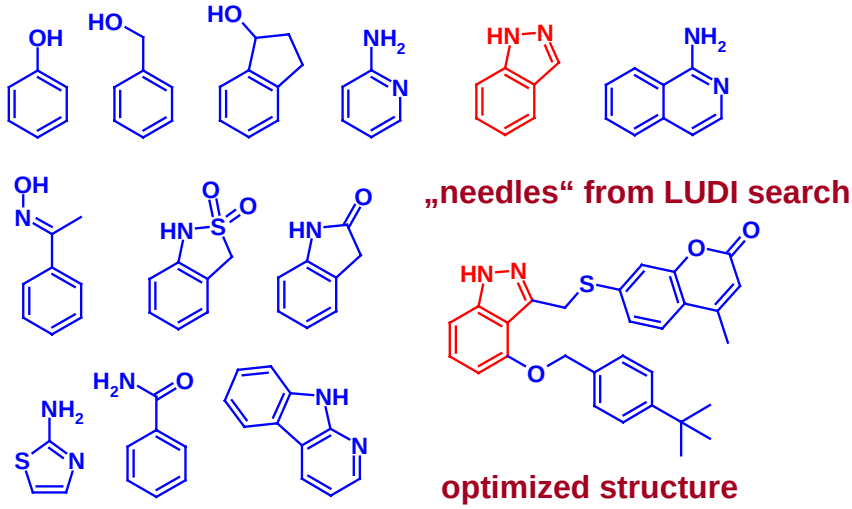
www.sunesis.com

Design of a Submicromolar TS Inhibitor



D. A. Erlanson et al., PNAS 97, 9367-9372 (2000)

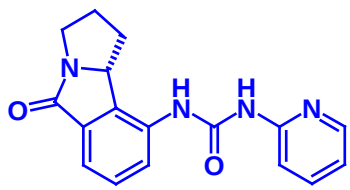
„Needle Screening“: Gyrase Inhibitors



H.-J. Boehm et al., J. Med. Chem. 43, 2664-2674 (2000)

Cdk4 Inhibitors: Virtual Screening and Library Design

1. Construction of a Cdk4 homology model (starting from the 3D structure of activated Cdk2)
2. de novo design of ligands in the binding site (programs LEGEND and SEEDS)
3. 382 hits acquired and tested
4. design of a diarylurea library
5. further chemical modifications

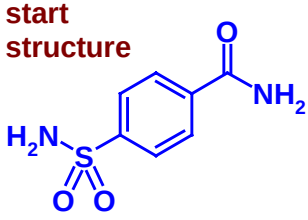


Cdk4 inhibitor
IC_{50} = 42 nM

T. Honma et al., J. Med. Chem. 44, 4615-4627 (2001)

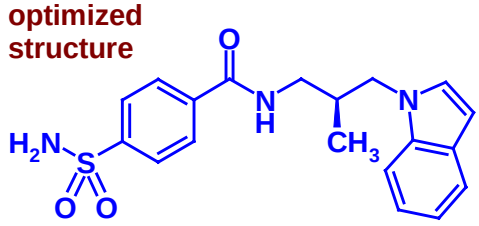
Combinatorial Design of Carbonic Anhydrase Inhibitors

start structure



K_d = 120 nM

optimized structure



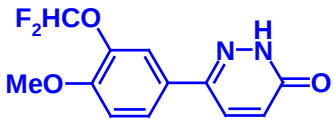
R enantiomer, K_d = 30 pM

(*S* enantiomer: K_d = 230 pM)

Program CombiSMoG, „best“ N-substituents from 100,000 candidates (20 scored by knowledge-based potentials)

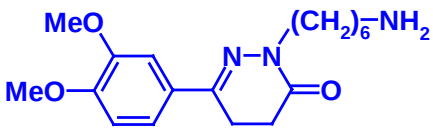
B. A. Grzybowski et al., Acc. Chem. Res. 35, 261-269 (2002);
B. A. Grzybowski et al., Proc. Natl. Acad. Sci. USA 99, 1270-1273 (2002)

Scaffold-Linker-Functional Group Approach



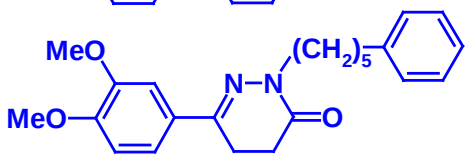
Zardaverine
IC_{50} PDE4 = 800 nM

Design of a structure-based 320-member virtual library with four different scaffolds or ring connections, five linkers and 16 different functional groups; best docking results with FlexX



N-substituted dihydro-pyridazinone analogs

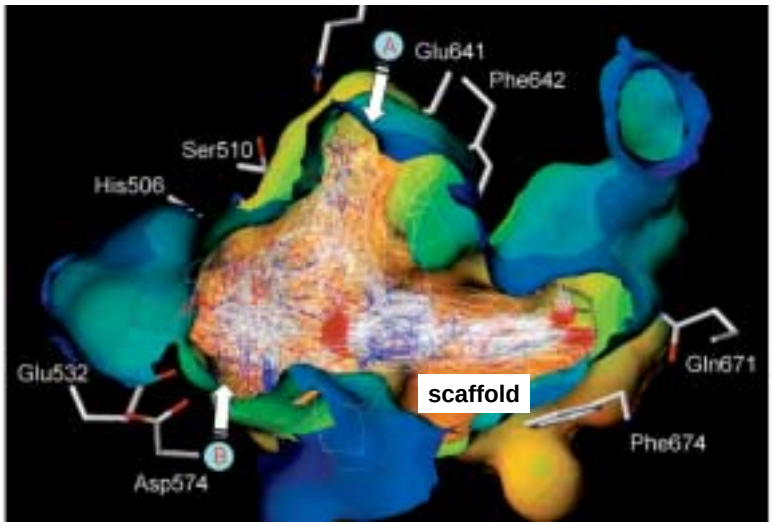
IC_{50} PDE4 = 20 nM



IC_{50} PDE4 = 0.9 nM

M. Krier et al., J. Med. Chem. 48, 3816-3822 (2005)

Scaffold-Linker-Functional Group Approach



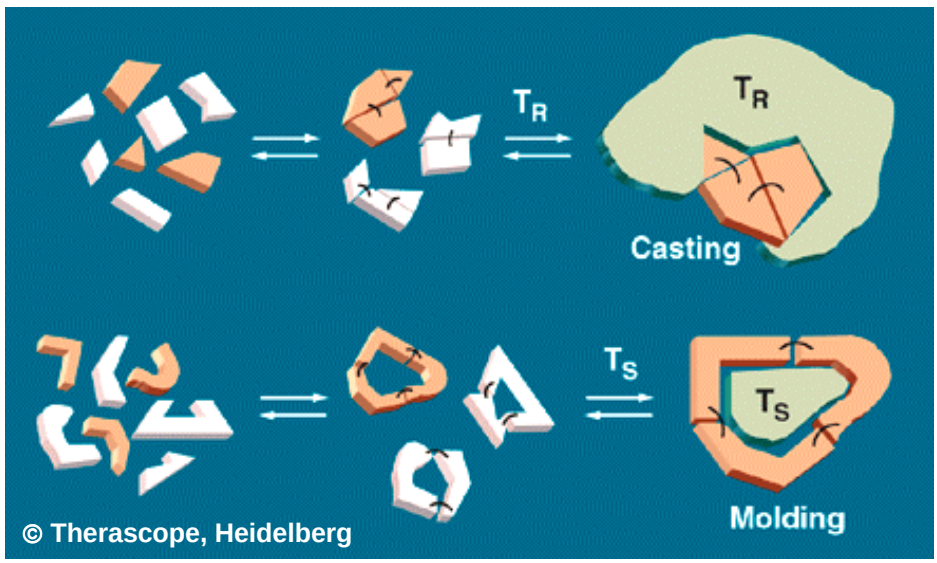
Docking of a 320-member library into PDE4 pocket

subsite A favors a phenyl ring

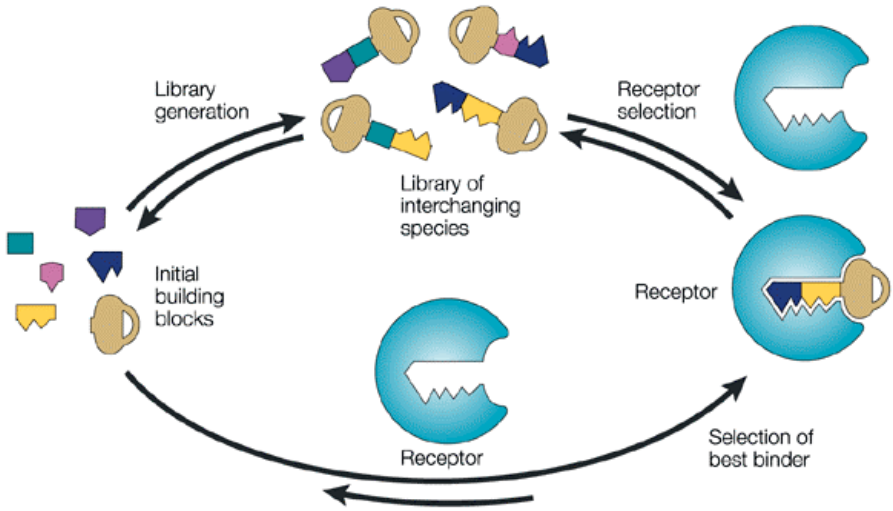
subsite B favors a basic group (amine)

M. Krier et al., J. Med. Chem. 48, 3816-3822 (2005)

Dynamic Self-Assembly of Ligands and Receptors

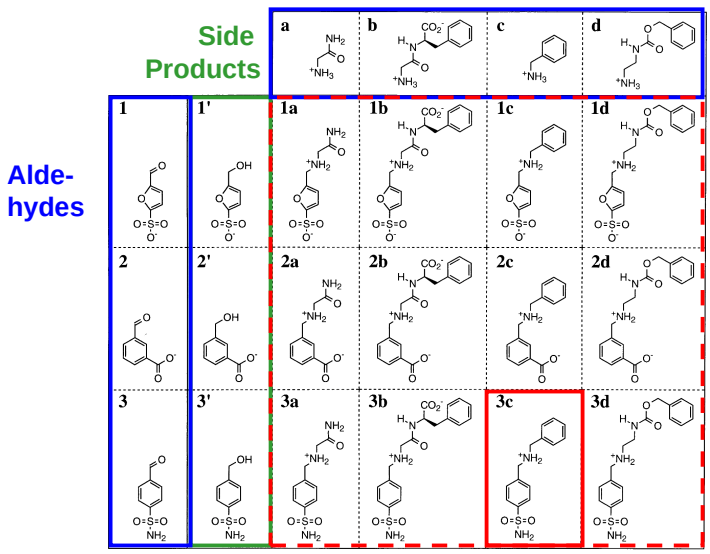


Dynamic Ligand Assembly in a Binding Site



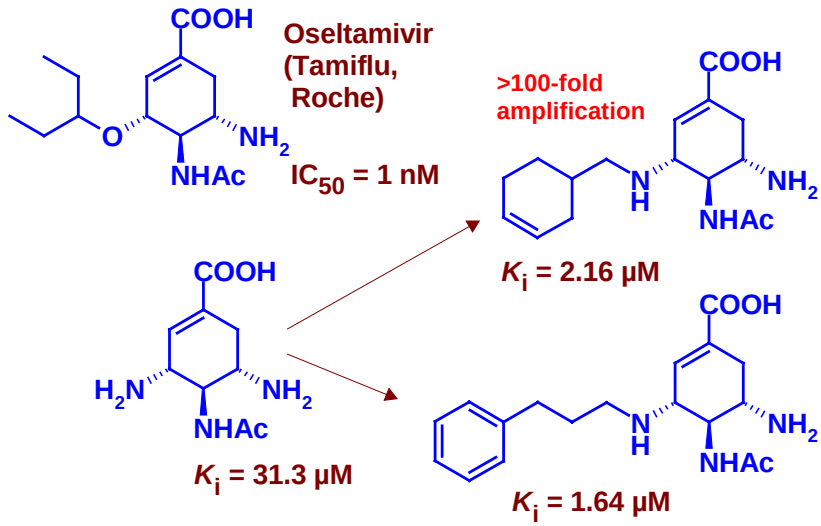
O. Ramström and J. M. Lehn, Nature Rev. Drug Discov. 1, 26-36 (2002)

Ligand Assembly in Carbonic Anhydrase



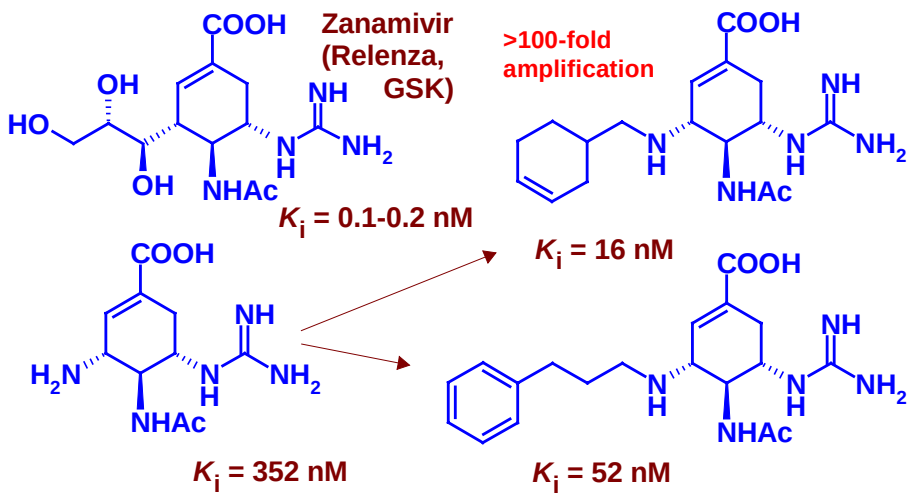
I. Huc and J.-M. Lehn, Proc. Natl. Acad. Sci. USA 94, 2106-2110 (1997)

Ligand Assembly in Neuraminidase, I



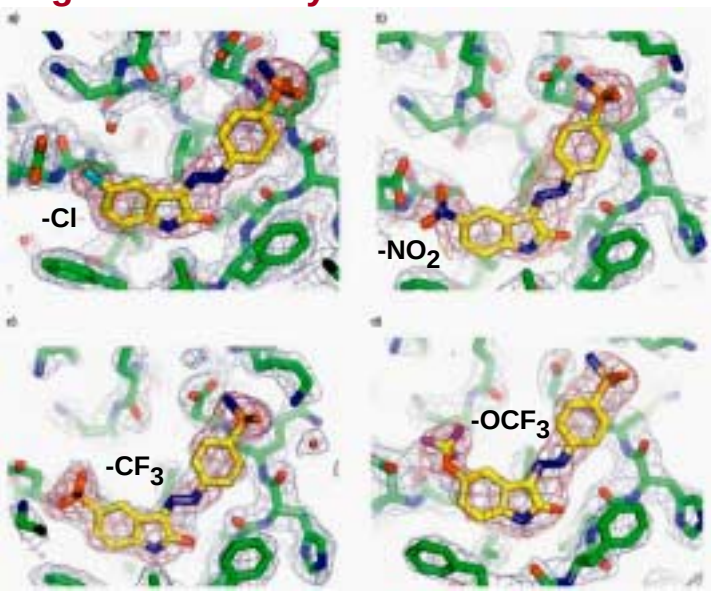
M. Hochgürtel et al., Proc. Nat. Acad. Sci. USA 99, 3382-3387 (2002)

Ligand Assembly in Neuraminidase, II



M. Hochgürtel et al., Proc. Nat. Acad. Sci. USA 99, 3382-3387 (2002)

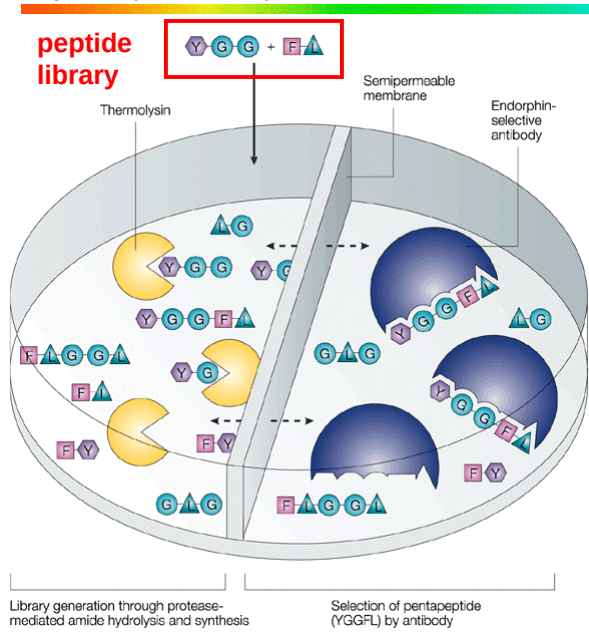
Ligand Assembly in Cdk 2



Four soaking experiments of different isatins and arylhydrazines in the presence of Cdk2.

all sulfonamido-arylhydrazones IC_{50} = 30 nM

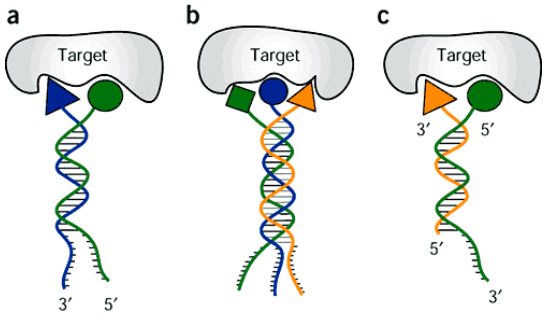
M. S. Congreve et al., Angew. Chem. Int. Ed. 42, 4479-4482 (2003)



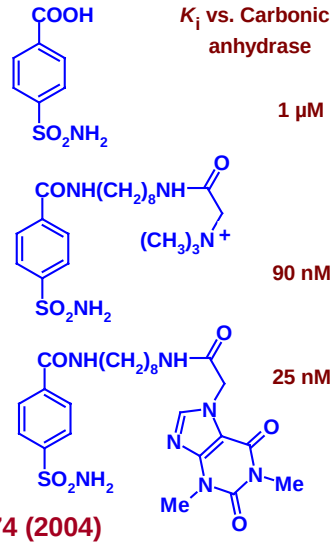
Dynamic Selection of Peptides by Antibodies

O. Ramström and J. M. Lehn, Nature Rev. Drug Discov. 1, 26-36 (2002)

Dynamic Self-Assembly of DNA-Coded Fragments



a) DNA duplex, pairing DNA sequence and two coding DNA sequences
b) DNA triplex, same as a)
c) DNA duplex with lead fragment (orange, without coding sequence)



K_i vs. Carbonic anhydrase

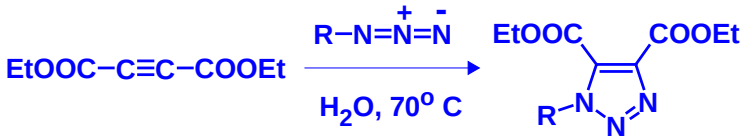
1 µM

90 nM

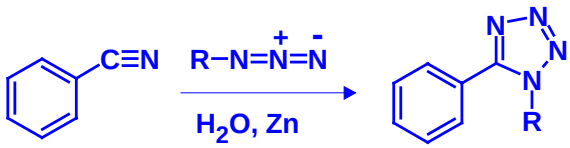
25 nM

S. Melkko et al., Nat. Biotechnol. 22, 568-574 (2004)

„Click Chemistry“ (K. Barry Sharpless)



triazoles

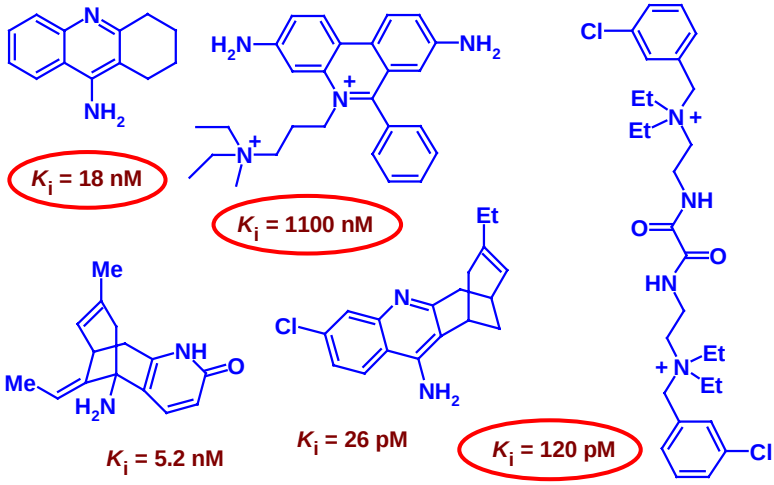


tetrazoles

The reaction of azides with acetylenes to triazoles is significantly accelerated in the binding site of acetylcholinesterase.

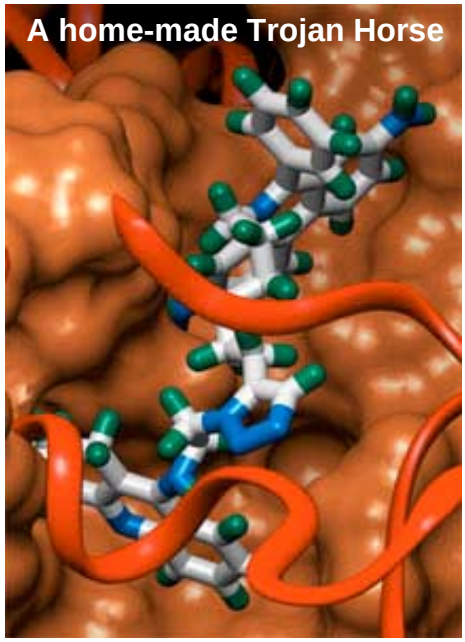
W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radic, P. R. Carlier, P. Taylor, M. G. Finn and K. B. Sharpless, Angew. Chem. Int. Ed. Engl. 41, 1053-1057 (2002).

Click Chemistry (B. Sharpless): AChE Inhibitors

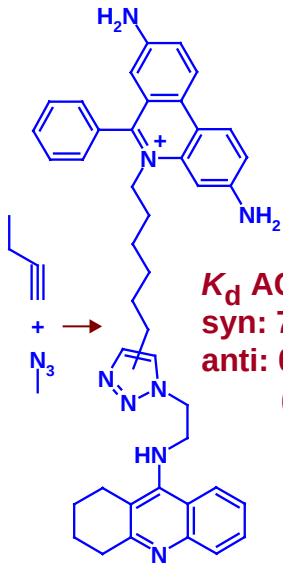


W. G. Lewis et al., Angew. Chem. 114, 1095-1099 (2002);
Angew. Chem. Int. Ed. Engl. 41, 1053-1057(2002).

A home-made Trojan Horse

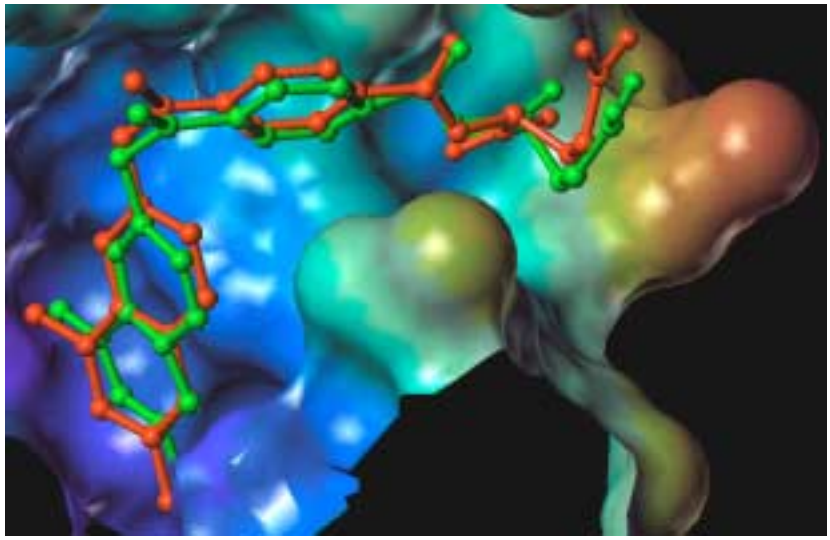


„Click Chemistry“

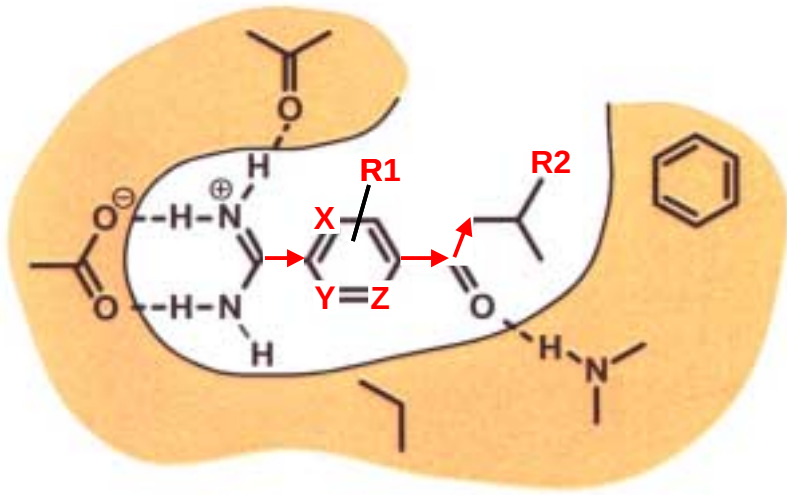


K_d AChE
syn: 77-410 fM
anti: 0.7-14 pM
(different species)

The Future: Combinatorial Drug Design



Combinatorial Ligand Construction



Further Progress in Computer-Aided Ligand Design

Ligand flexibility

Protein flexibility

Flexibility of the ligand-protein complex

Geometry and strength of hydrogen bonds

Solvation and desolvation effects (entropy)

Inserted (conserved) water molecules

Synthetic accessibility of ligands

Combinatorial docking of ligands

„Last Problem“ - The Scoring Function

Fragment-Based Drug Discovery

a) Reviews: D. A. Erlanson et al., Fragment-based drug discovery, J. Med. Chem. 47, 3462- 3482 (2004);
M. J. Hartshorn et al., J. Med. Chem. 48, 403-413 (2005)

b) Fragment-based de novo design:
SKELGEN: M. Stahl et al., JCAMD 16, 459-478 (2002)
COREGEN: A. M. Aronov and G. W. Bemis, Proteins 57, 36-50 (2004)

c) Fragment space for de novo design:
Modified RECAP procedure for Feature tree-based 2D design and 3D design by SkelGen
M. Stahl, WATOC January 2005.

d) SeeDs libraries for NMR screening, selected by kinase pharmacophore features, MWs about 160-240.
N. Baurin et al., JCICS 44, 2157-2166 (2004)