Fragment-based Design - A Promising Strategy

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Fragment-based Design - Not Just Another Hype !

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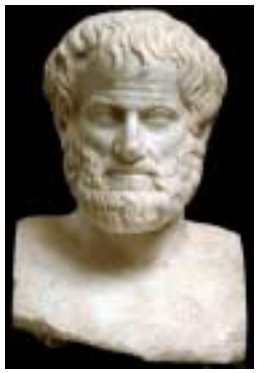

"The whole is more than the sum of its parts"

Aristotle, Metaphysica

The Fundamental Axiom of Fragment-based Design

if C = A + B then A + B = C

binding site:	+	+	-	+	-	-	+	-	+	+	+
complex ligand	-	-	+	-	+	+	-	+	-	-	-
small ligand		-	+	-			-	+	-		

M. M. Hann et al., J. Chem. Inf. Comput. Sci. 41, 856-864 (2001)

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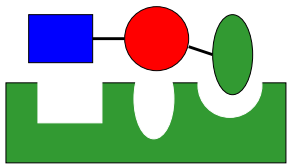
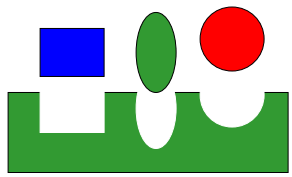
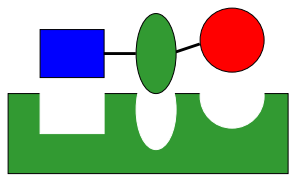
To Fit or Not to Fit a Binding Site



a common situation in screening or docking: the ligand does not fit the binding site

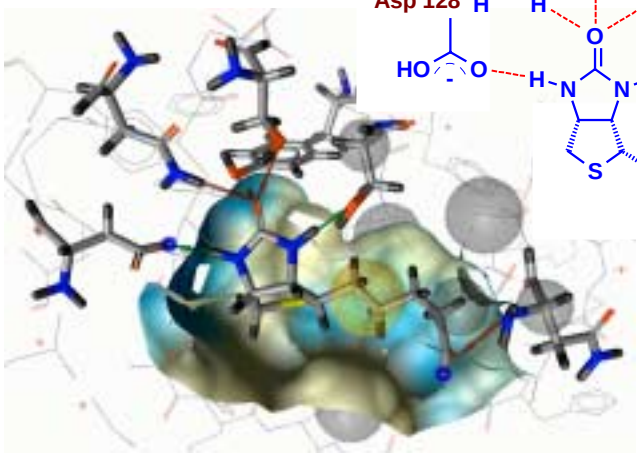


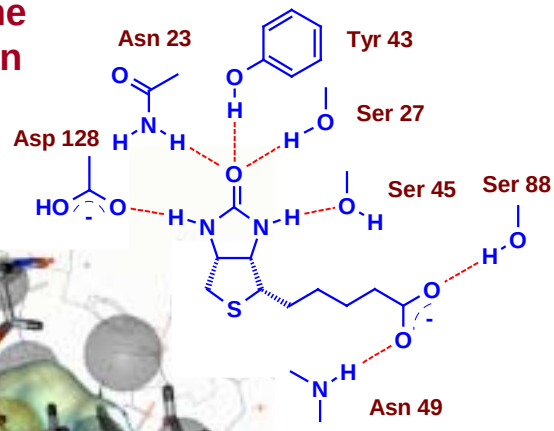
fragments fit the pockets of the binding site



ligand fits the binding site

3D Structure of the Biotin Streptavidin Complex

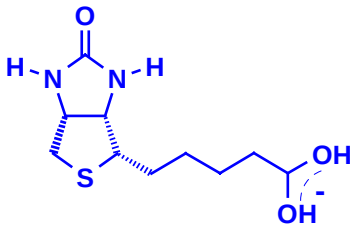




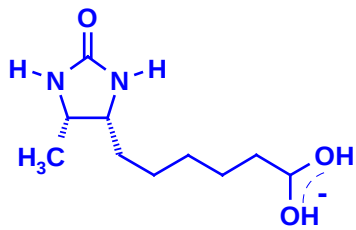
2rtf (1.47Å)

Binding Constants of Biotin and Analogs

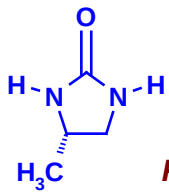
(N. M. Green, Adv. Protein Chem. 29, 85-133 (1975))



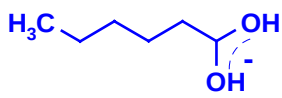
Biotin, K_i = 1.3 fM



Desthiobiotin, K_i = 0.5 pM

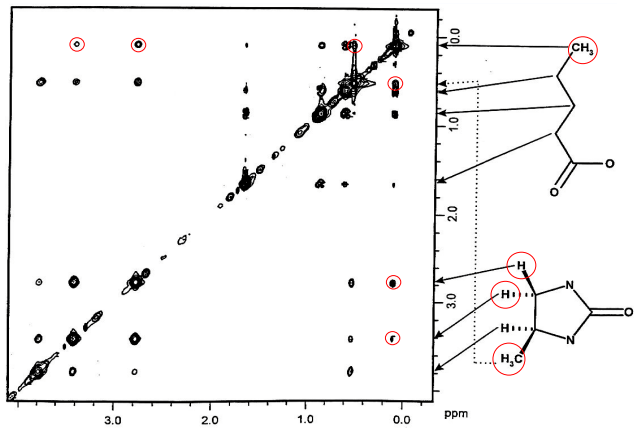


K_i = 34 µM



K_i = 3 mM

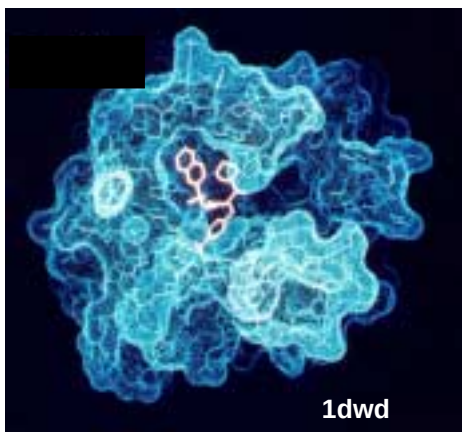
„Re-discovering“ Biotin by NMR Fragment-Based Screening

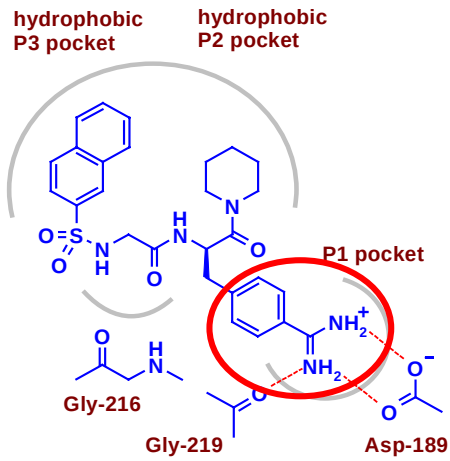


NOESY experiment

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

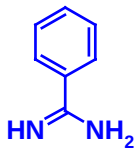
NAPAP in Thrombin / Schematic Binding Mode





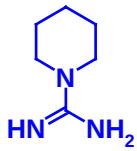
„Needle Screening“: Thrombin Inhibitors

needle screening of 200 small molecules with low affinity for thrombin vs. trypsin selectivity



K_i (thrombin) = 300 µM
K_i (trypsin) = 31 µM

benzamidine binds specifically to trypsin, whereas N-amidino-piperidine has a slightly higher specificity for thrombin



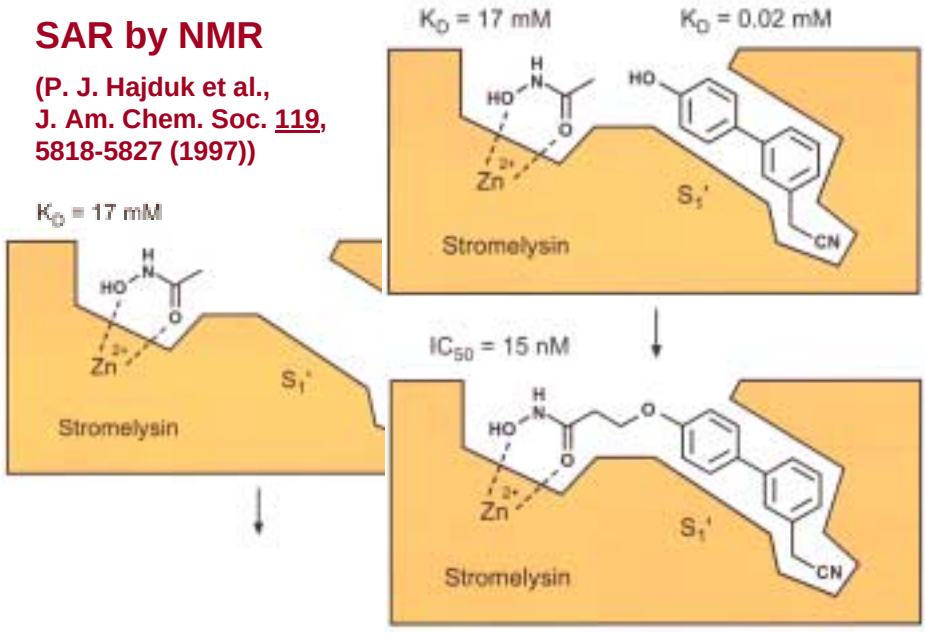
K_i (thrombin) = 150 µM
K_i (trypsin) = 360 µM

Thrombin 189-200: DACEGDSGGPFV
Trypsin 189-200: DSCQGDSGGPVV

K. Hilpert et al., J. Med. Chem. 37, 3889-3901 (1994)

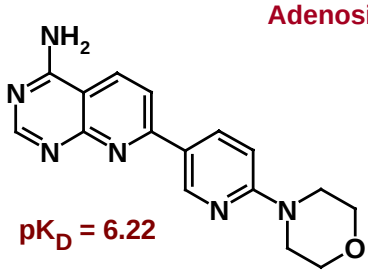
SAR by NMR

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

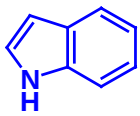


SAR by NMR: Other Results

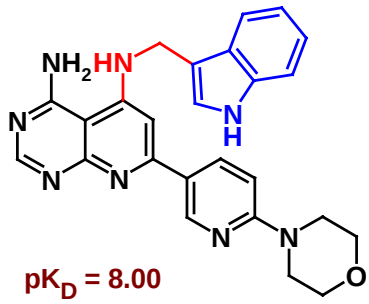
Adenosine Kinase



$pK_D = 6.22$



$pK_D = 2.52$



$pK_D = 8.00$

P. J. Hajduk, J. R. Huth and C. Sun, in W. Jahnke and D. A. Erlanson, Eds., Fragment-based Approaches in Drug Discovery, Wiley-VCH, 2006, pp. 181-192

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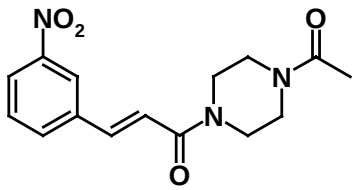
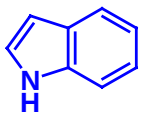
SAR by NMR: Other Results

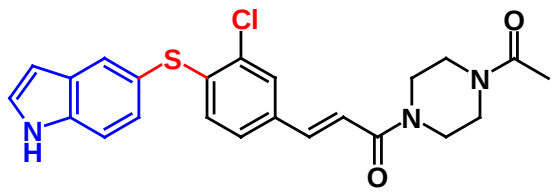
Leucocyte-Function-Associated Antigen



$pK_D = 4.10$



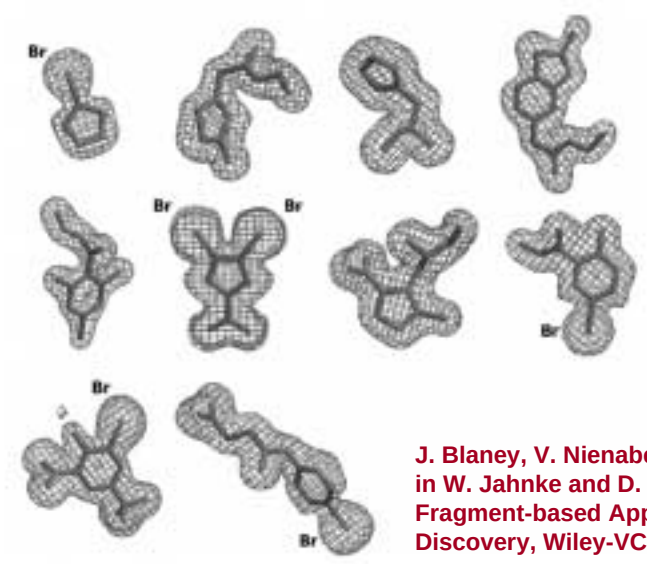
$pK_D = 3.52$



$pK_D = 7.70$

P. J. Hajduk, J. R. Huth and C. Sun, in W. Jahnke and D. A. Erlanson, Eds., Fragment-based Approaches in Drug Discovery, Wiley-VCH, 2006, pp. 181-192

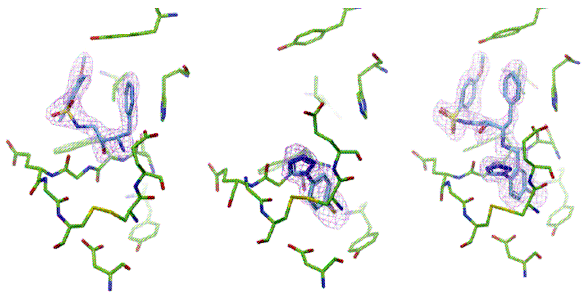
Shape-Diverse Crystallographic Screening at SGX

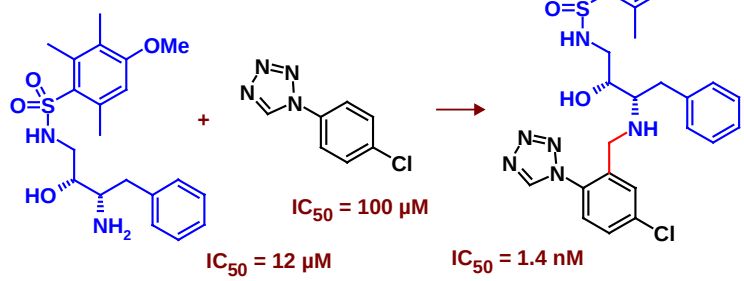


fitting fragments are identified by their difference electron density

J. Blaney, V. Nienaber and S.K. Burley, in W. Jahnke and D. A. Erlanson, Eds., Fragment-based Approaches in Drug Discovery, Wiley-VCH, 2006, pp. 215-248

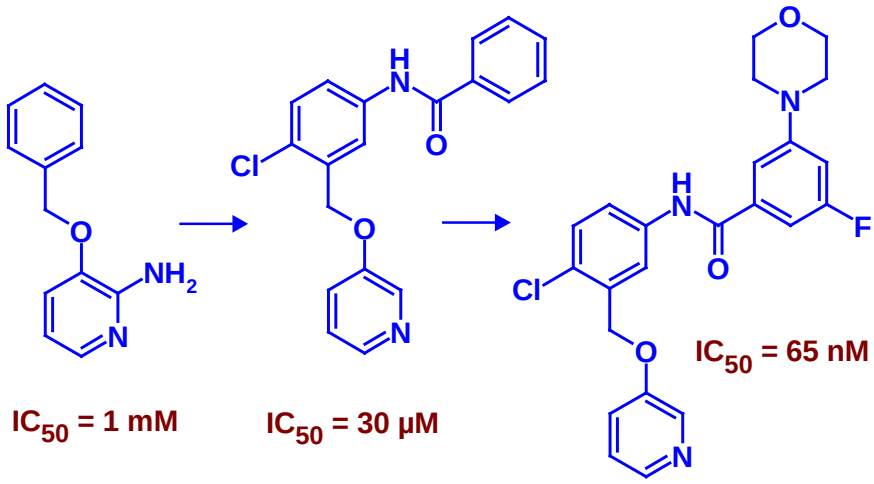
Fragment-based Design of a Thrombin Inhibitor





A. Ciulli and C. Abell, Curr. Opin. Biotechnol. 18, 489-496 (2007)

Fragment-based Design of a p38α Inhibitor



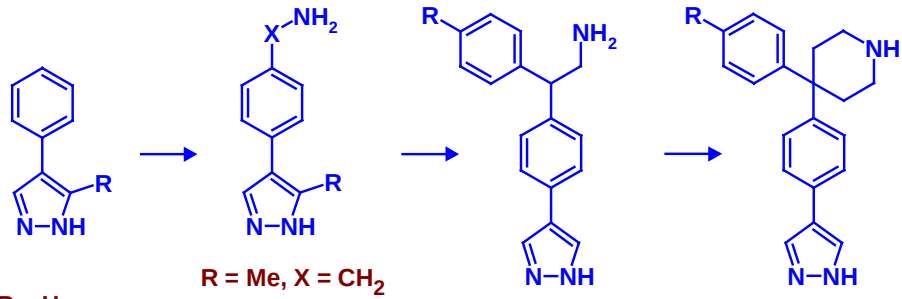
IC_{50} = 1 mM

IC_{50} = 30 µM

IC_{50} = 65 nM

A. L. Gill et al., J. Med. Chem. 48, 414-426 (2005)

Fragment-based Design of a PKB Inhibitor



R = H
IC_{50} = 135 µM

R = Me
IC_{50} = 80 µM

R = Me, X = CH_2
IC_{50} = 12 µM

R = Me, X = $(CH_2)_2$
IC_{50} = 5.2 µM

R = H, X = $(CH_2)_3$
IC50 = 3.0 µM

R = H
IC_{50} = 0.51 µM

R = Cl
IC_{50} = 31 nM

R = H
IC_{50} = 0.20 µM

R = Cl
IC_{50} = 18 nM

G. Saxty et al., J.Med. Chem. 50, 2293-2296 (2007)

Table 2. Clinical and Preclinical Candidates Derived from Fragments

compd	company	target	progress
LY-517717[a]	Lilly/Protherics	FXa	phase 2
PLX-204[b]	Plexxikon	PPAR agonist	phase 2
ABT-263[c]	Abbott	Bcl-X_L	phase 1/2a
AT9283[b]	Astex	Aurora	phase 1/2a
ABT-518[d]	Abbott	MMP-2 and 9	phase 1
AT7519[b]	Astex	CDKs	phase 1
PLX-4032[b]	Plexxikon	B-Raf^{V600E}	phase 1
SGX523[b]	SGX Pharmaceuticals	MET	phase 1
SNS-314[b]	Sunesis	Aurora	phase 1
NVP-AUY922[e]	Vernalis/Novartis	HSP90	phase 1
AT9311/LCQ195[b]	Astex/Novartis	CDKs	preclinical
AT13148[b]	Astex	PKB/Akt	preclinical
AT13387[b]	Astex	HSP90	preclinical
PLX-4720[f]	Plexxikon	B-Raf^{V600E}	preclinical
RO6266[g]	Roche	P38	preclinical
SGX393[b]	SGX Pharmaceuticals	BCR-Abl^{T315I}	preclinical

M. Congreve et al., J. Med. Chem. 51, 3661-3680 (2008)

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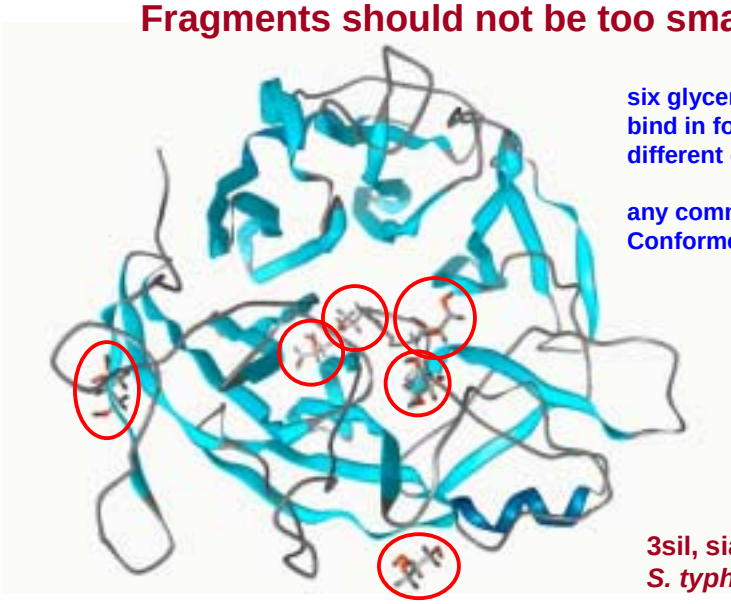
Fragments should not be too small

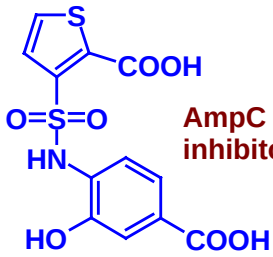


six glycerol molecules bind in four completely different conformations

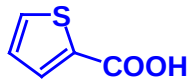
any comments from Conformetrix Ltd ?

3sil, sialidase from *S. typhimurium*

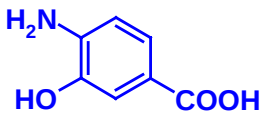
Different Binding Modes of Fragments



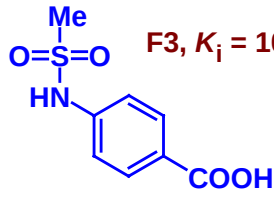
AmpC ß-lactamase inhibitor L1, K_i = 1 µM



F1, K_i = 40 mM



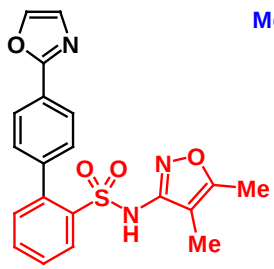
F2, K_i = 19 mM



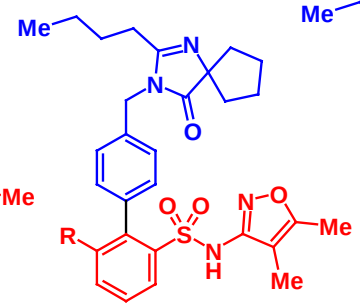
F3, K_i = 10 mM

K. Babaoglu and B. K. Shoichet, Nature Chem. Biol. 2, 720-722 (2006)

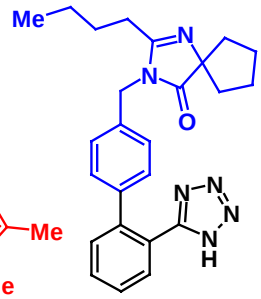
Design of a Dual AT_1 and ET_A Antagonist



AT_1 K_i > 10,000 nM
ET_A K_i = 1.4 nM



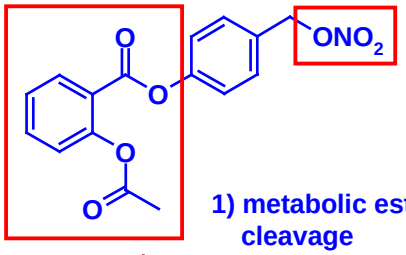
R = CH_2OEt
AT_1 K_i = 0.8 nM
ET_A K_i = 9.3 nM



AT_1 K_i > 0.8 nM
ET_A K_i >10,000 nM

N. Murugesan et al., J. Med. Chem. 45, 3829-3835 (2002);
N. Murugesan et al., J. Med. Chem. 48, 171-179 (2005)

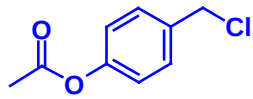
Failure of the Fragment-based Approach: A „Hybrid Drug“ is indeed a Prodrug

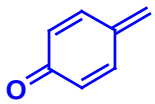


“NO-ASA”, strongly inhibits colon cancer growth *in vitro* and *in vivo* (J. L. Williams et al., BBRC 313, 784-788 (2004)); however, cGMP levels are not increased.

1) metabolic ester cleavage
2) 1,6-elimination of NO_3^-

the linker is a prodrug of the active agent:

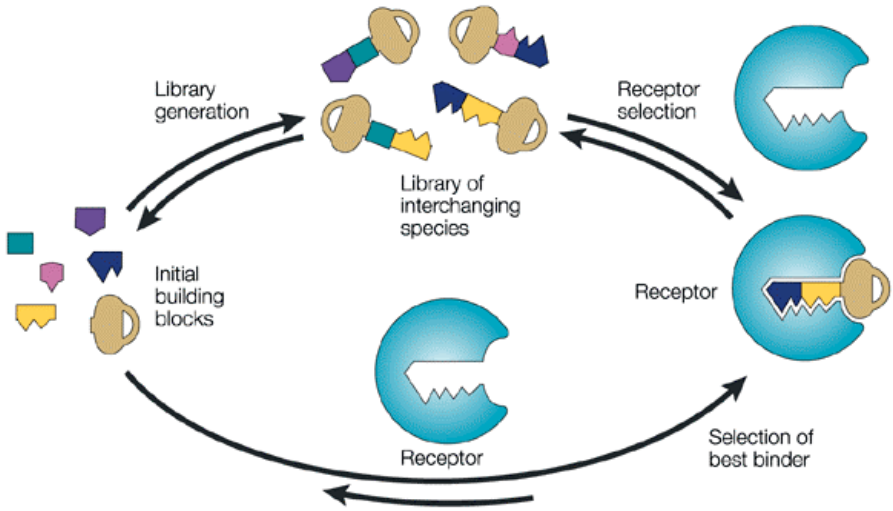




highly toxic, reacts with nucleophiles, e.g. GSH

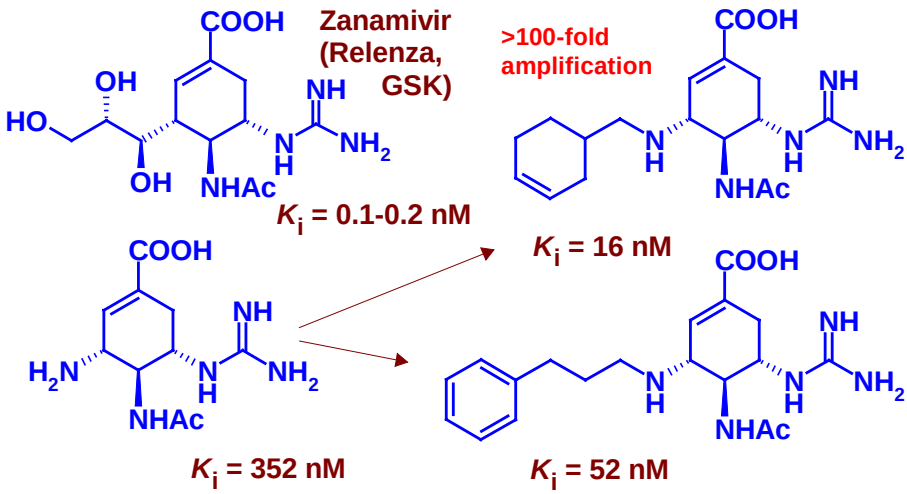
about 10 times more active against colon cancer cells (N. Hulsman et al., J. Med. Chem. 50, 2424-2431 (2007); M. Wijtmans, personal communication

Dynamic Ligand Assembly in a Binding Site



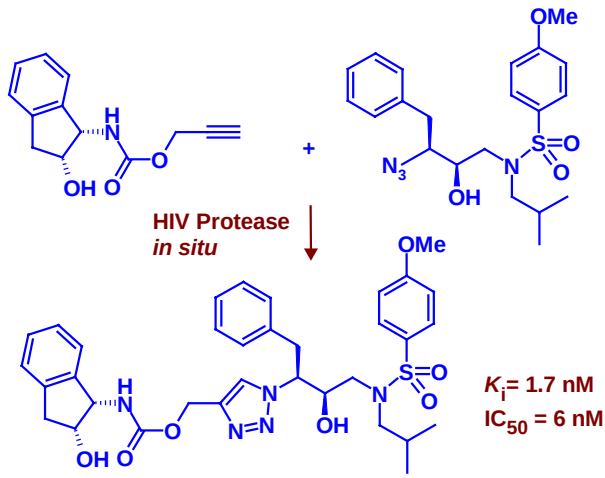
O. Ramström and J. M. Lehn, Nature Rev. Drug Discov. 1, 26-36 (2002)

Ligand Assembly in Neuraminidase, II



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

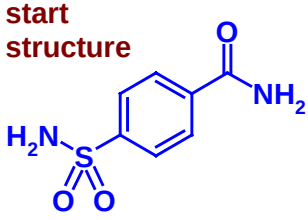
in situ Click Chemistry: HIV Protease Inhibitors



M. Whiting et al., Angew. Chem. Int. Ed. 45, 1435-1439 (2006); K. B. Sharpless and R. Manetsch, Expert Opin. Drug Discov. 1, 525-538 (2006)

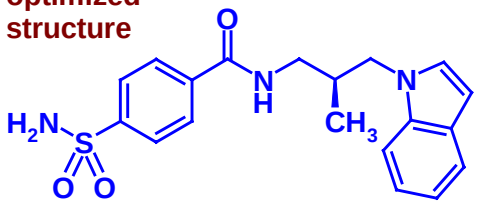
Combinatorial Design of Carbonic Anhydrase Inhibitors

start structure



K_d = 120 nM

optimized structure



R enantiomer, K_d = 30 pM

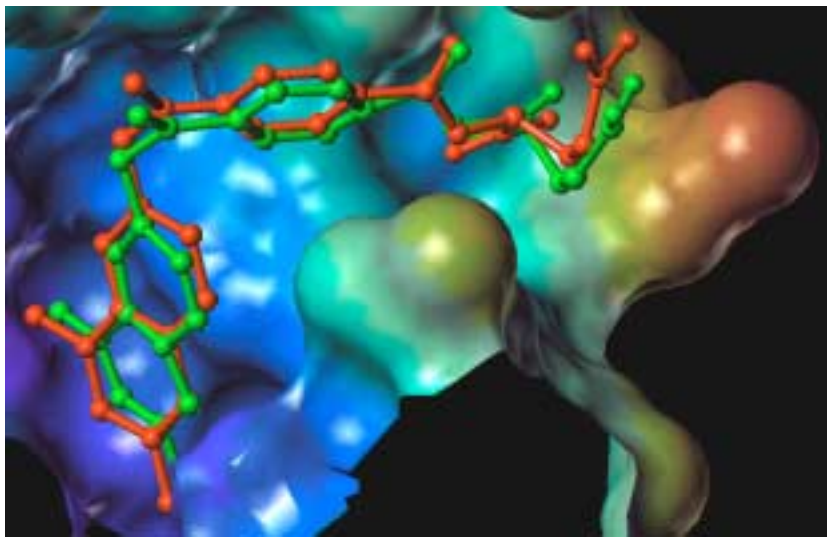
(*S* enantiomer: K_d = 230 pM)

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)

Hugo Kubinyi, www.kubinyi.de

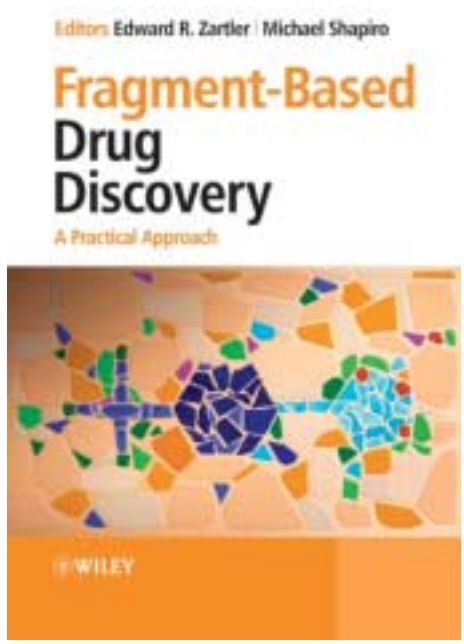
The Future: Combinatorial Drug Design



Advantages and Problems

+ many fragments are tested in short time, especially by NMR techniques
+ also low affinity ligands are discovered
+ hit rates are much higher than in HTS and VS
+ protein crystallography shows binding mode
+ all different pockets of a binding site are explored
+ scaffold hopping

- no binding site information from NMR experiments
- only relaxed protein conformation is explored
- construction of a ligand in a favorable conformation is difficult
- problems in lead structure optimization?



References

a) **Books and Reviews**

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D. A. Erlanson et al., Fragment-based drug discovery, J. Med. Chem. 47, 3462- 3482 (2004).

R. E. Hubbard et al., Curr. Opin. Drug Disc. Dev. 10, 289-297 (2007).

M. Congreve et al., J. Med. Chem. 51, 3661-3680 (2008).

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)

RECORE: P. Maass et al., J. Chem. Inf. Model. 47, 390-399 (2007)