Interrogating the Druggable Proteome:

Target-Fishing and Drug Design

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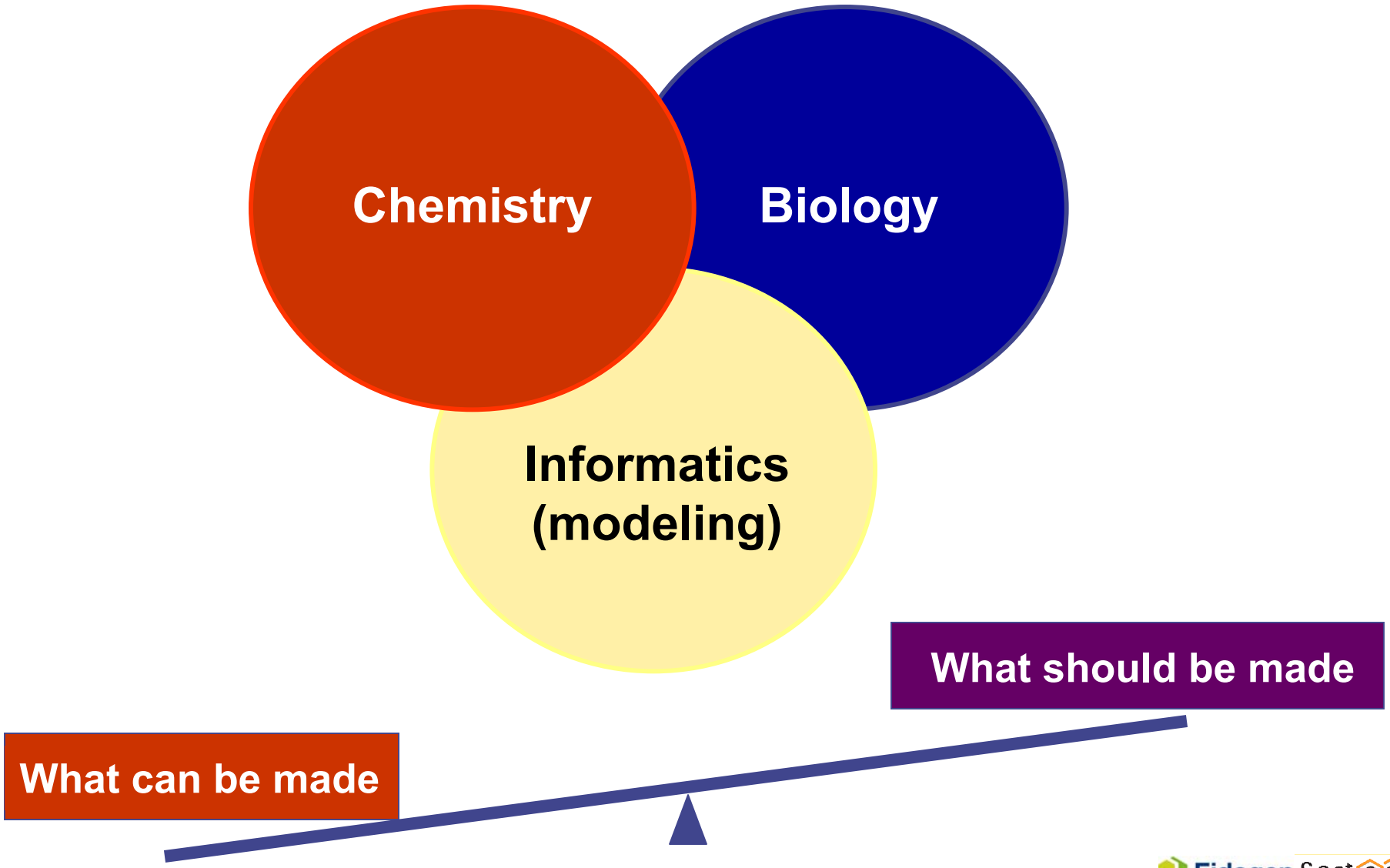
Discovery results from efficient collaboration

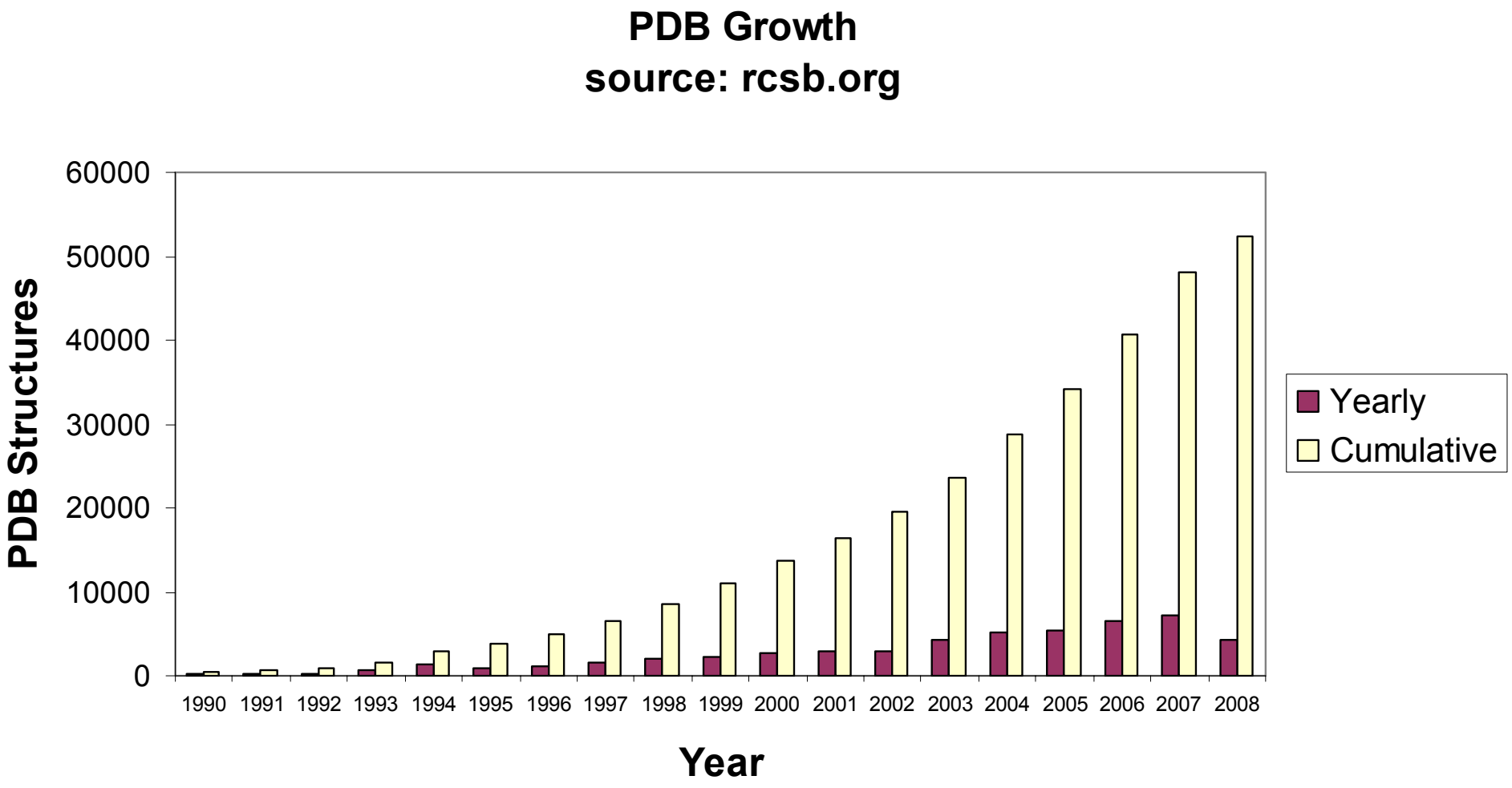


Pharma companies are playing "on the same end of the field"

- Number druggable targets: ~2000-3000
  - Commercially validated targets: ~200
- Same synthetic methods used through industry
  - Methods are published, “same” training
- Commercially available starting materials ~500-750K
  - “Clean”/Suitable ~ 250-300K

Protein structure growth is accelerating

> 50K Structures/co-complexes (Aug-2008)
> 600 deposits per month → **>150/week!**



Drugs developed using SBDD

Inhibitor/Drug	Disease	Company(s)	Protein targeted	Enzyme Family
STI-571/Gleevec	Chronic Myeloid Leukemia	Novartis	c-Abl kinase	Tyrosine kinase
Fluoroquinolone/Ciprofloxacin	Bacterial infection	Bayer	Gyrase	ATP Hydrolase
Saquinavir/Invirase, Ritonavir/Norvir, Indinavir/ Crixivan, Nelfinavir/Viracept, Amprenavir/Agenerase, Fosamprenavir/Lexiva,	AIDS	Roche, Abbott, Agouron, Merck, Vertex	HIV-1 Protease	Aspartylprotease
Trusopt	Glaucoma	Merck	Carbonic Anhydrase	Lyase
Thymitaq	Cancer	Agouron	Thymidylate synthase	Methyl transferase
Celecoxib/Celebrex, Rofecoxib/Vioxx	Inflammation, rheumatoid arthritis	Searle, Merck	Cox-2	Oxidoreductase
AG3340/Prinomastat	Cancer	Agouron	Matrix metalloprotease	Metalloprotease
Oseltamivir phosphate/Tamiflu, Zanamivir/Relenza	Influenza	Roche	Neuraminidase	Glycosidase

Source: http://www.active-sight.com/science/sbdd.html

About Eidogen-Sertanty

- Knowledge-Driven Discovery Solutions Provider
  - Formed in March 2005 when Sertanty (Libraria→Sertanty 2003) acquired Eidogen (Bionomix 2000)
  - >$20M Invested in Technology Development
  - 12 FTE's
  - Worldwide Customerbase
  - Cash-Positive

- Chemogenomic Databases & Analysis Software
  - *TIP*™ - Structural Informatics Platform
  - *KKB*™ - Kinase SAR and Chemistry Knowledgebase
  - CHIP™ - Chemical Intelligence Platform

- DirectDesign™ Discovery Collaborations
  - In Silico Target Screening ("Target Fishing" and Repurposing)
  - Target and compound prioritization services
  - Fast Follower Design: Novel, Patentable Leads

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Eidogen-Sertanty Investors

Tavistock Life Sciences

HighBAR Ventures (Bill Joy, Andreas Bechtolsheim, Roy Thiele-Sardiña)

Technofyn (Alejandro Zaffaroni)

Band of Angels

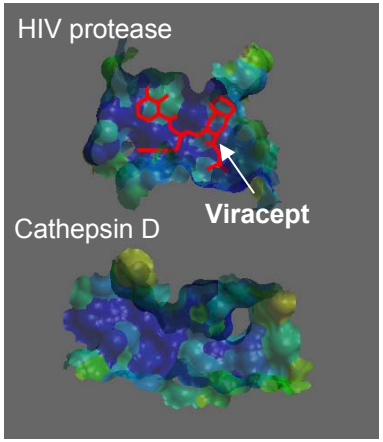
The Athenaeum Fund

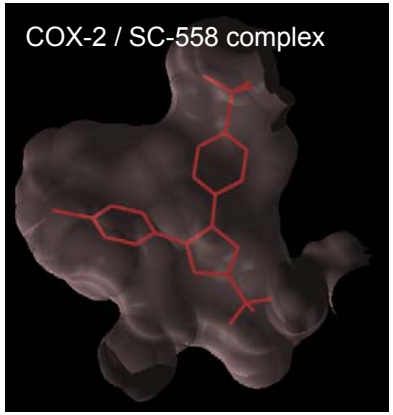
Bringing Proteomic riches to non-specialists

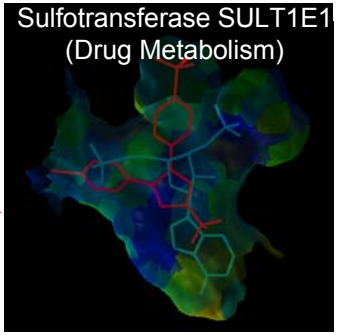
Automated Modeling and Proteomic Structural Mining

- ➢Sequence-to-Structure Calculation Cascade
- ➢Search-by: KeyWord, Sequence, Ligand, Protein Structure, Receptor-Sites, etc.
- ➢Exploit Structural fold and receptor-site conservation

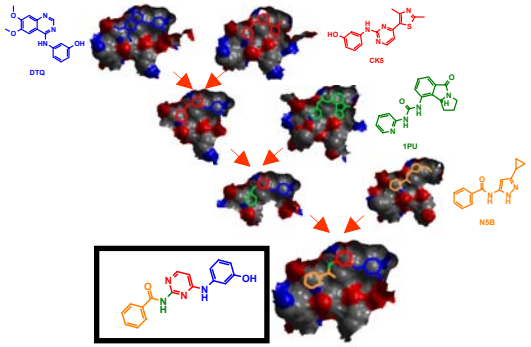
→Off-Target Identification (opportunities v. liabilities)







→Borrowing Matter Ideas from co-complexes and other drugs



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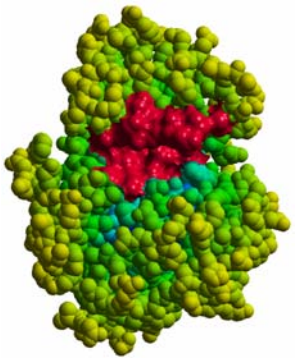
Using both target and compound similarity

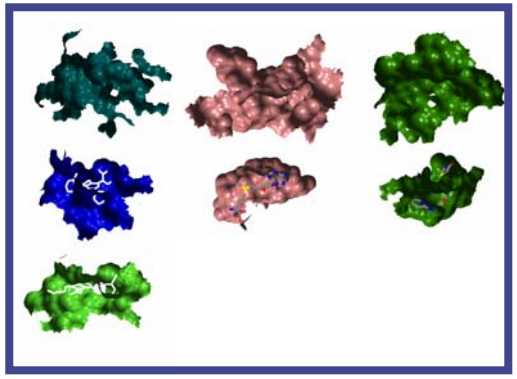


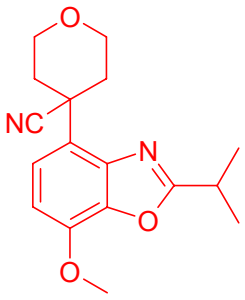
SiteSorter™

Similar Sites In The Human Proteome

Same Family

Different Target Family

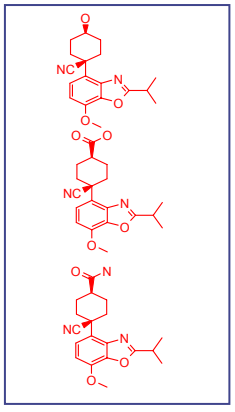




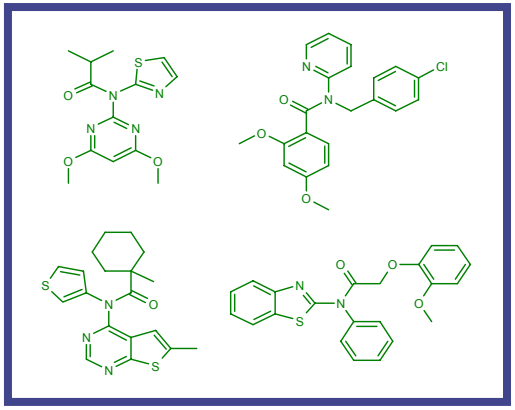
PharmSim™

Similar Drugs & Available Compounds

2D Similar



Pharmacophore Similarity



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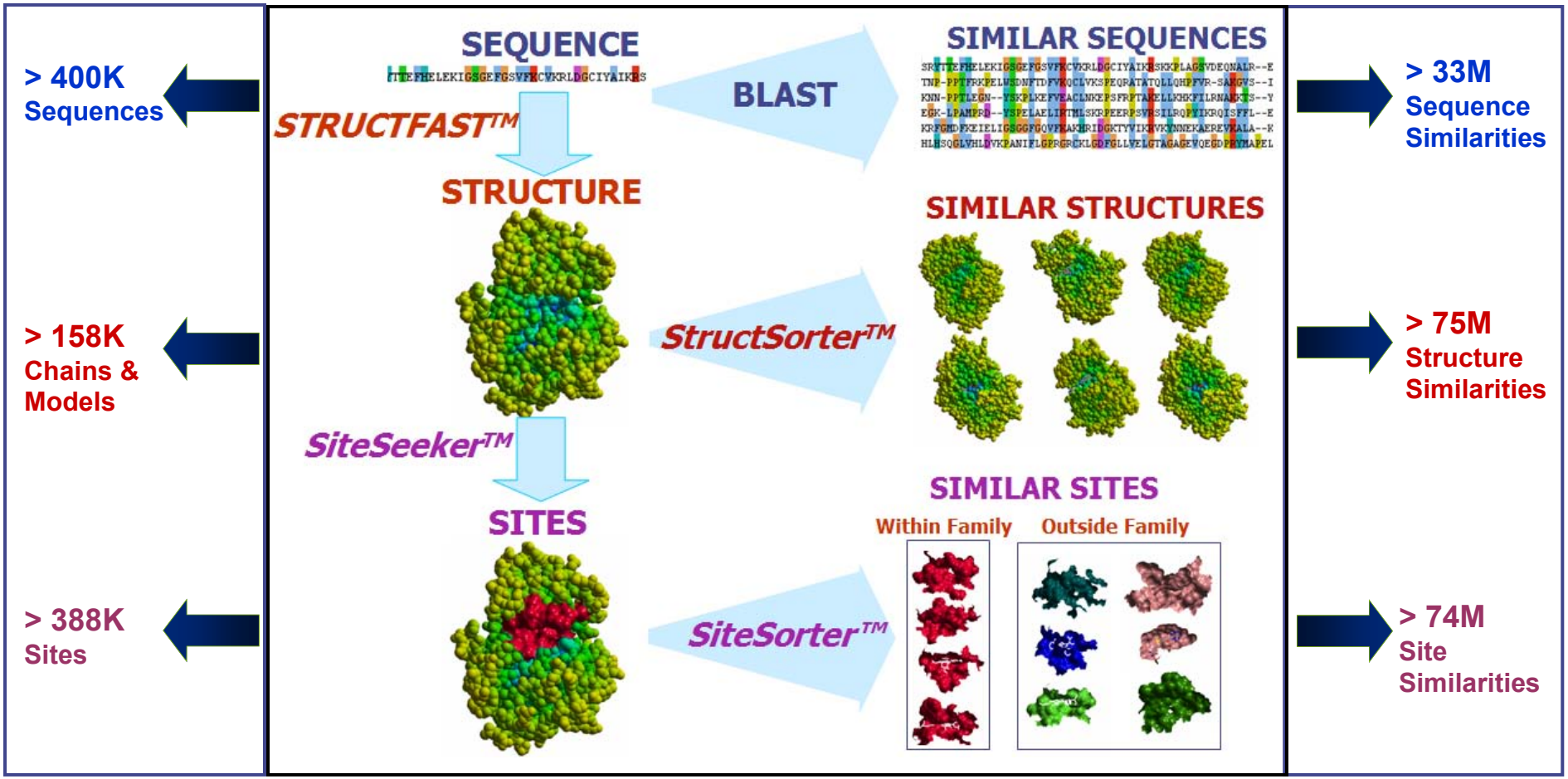
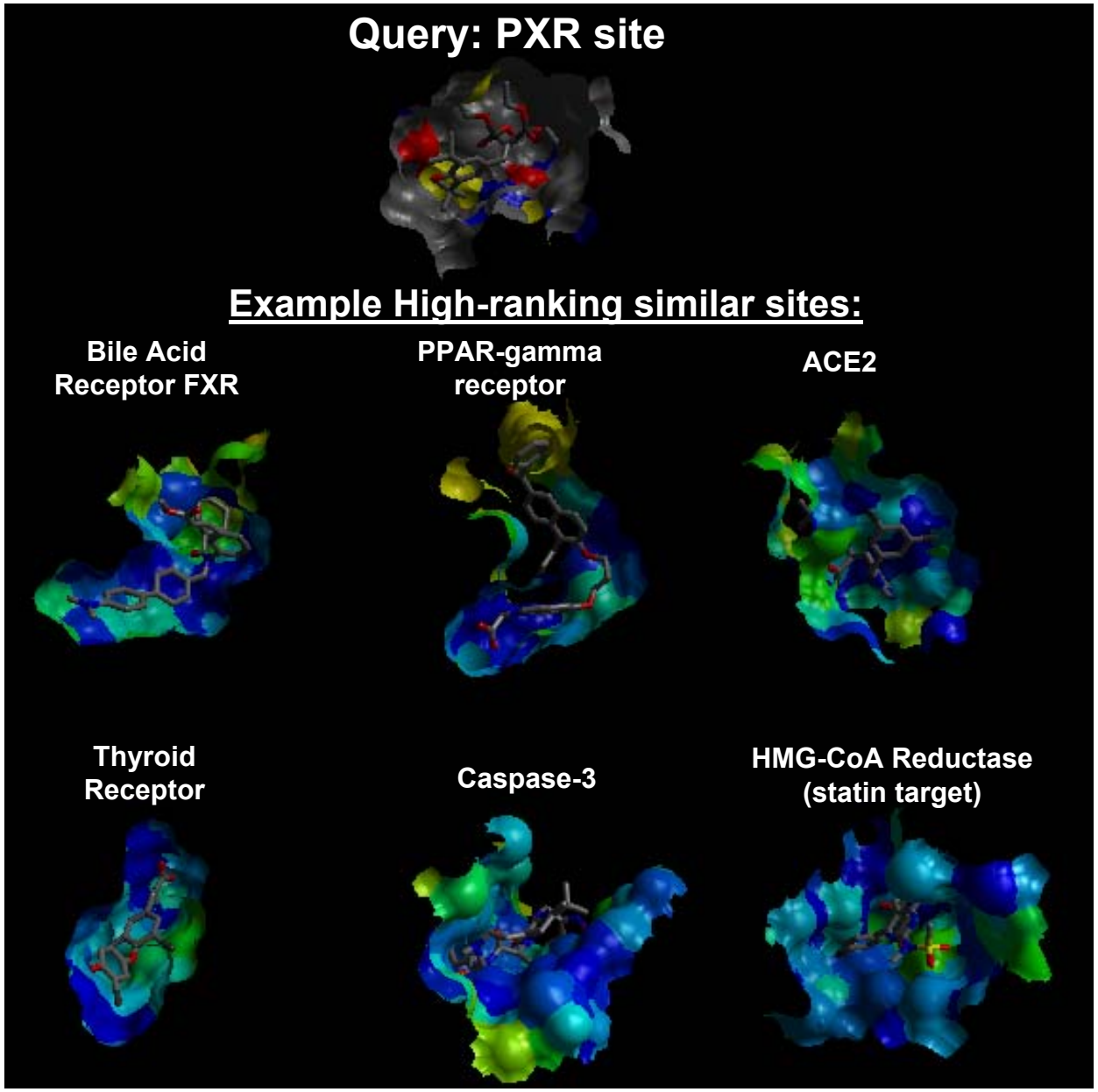
TIP algorithm engine



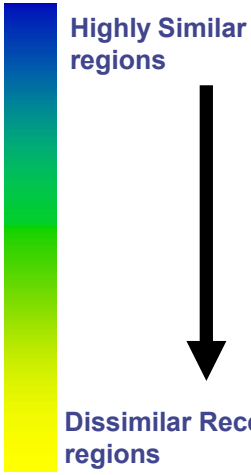
Reference: Interrogating the druggable genome with structural informatics, MolecularDiversity (2006)

PXR – Promiscuous ligand-binding site



Pregnane X-receptor – PXR ("sensor)" →CYP3A4 ("executioner")
PXR Binds > 50% drugs
Including some bile acids, statins, herbal components, a selection of HIV protease inhibitors, calcium channel modulators, numerous steroids, plasticizers and monomers, organochlorine pesticides, a peroxisome proliferator-activated receptor-ãantagonist, xenobiotics and endobiotics…

Site Similarity Coloring



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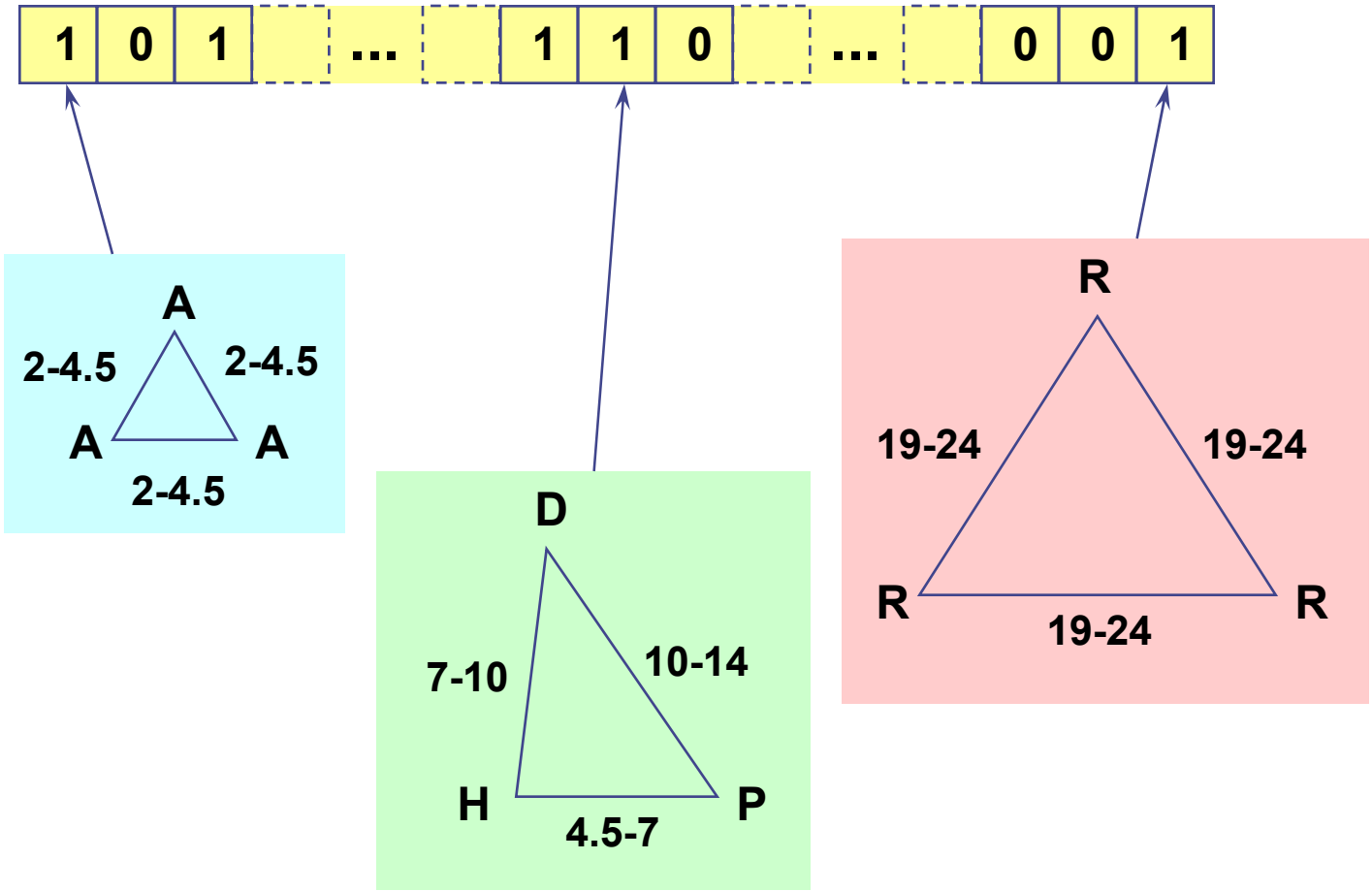
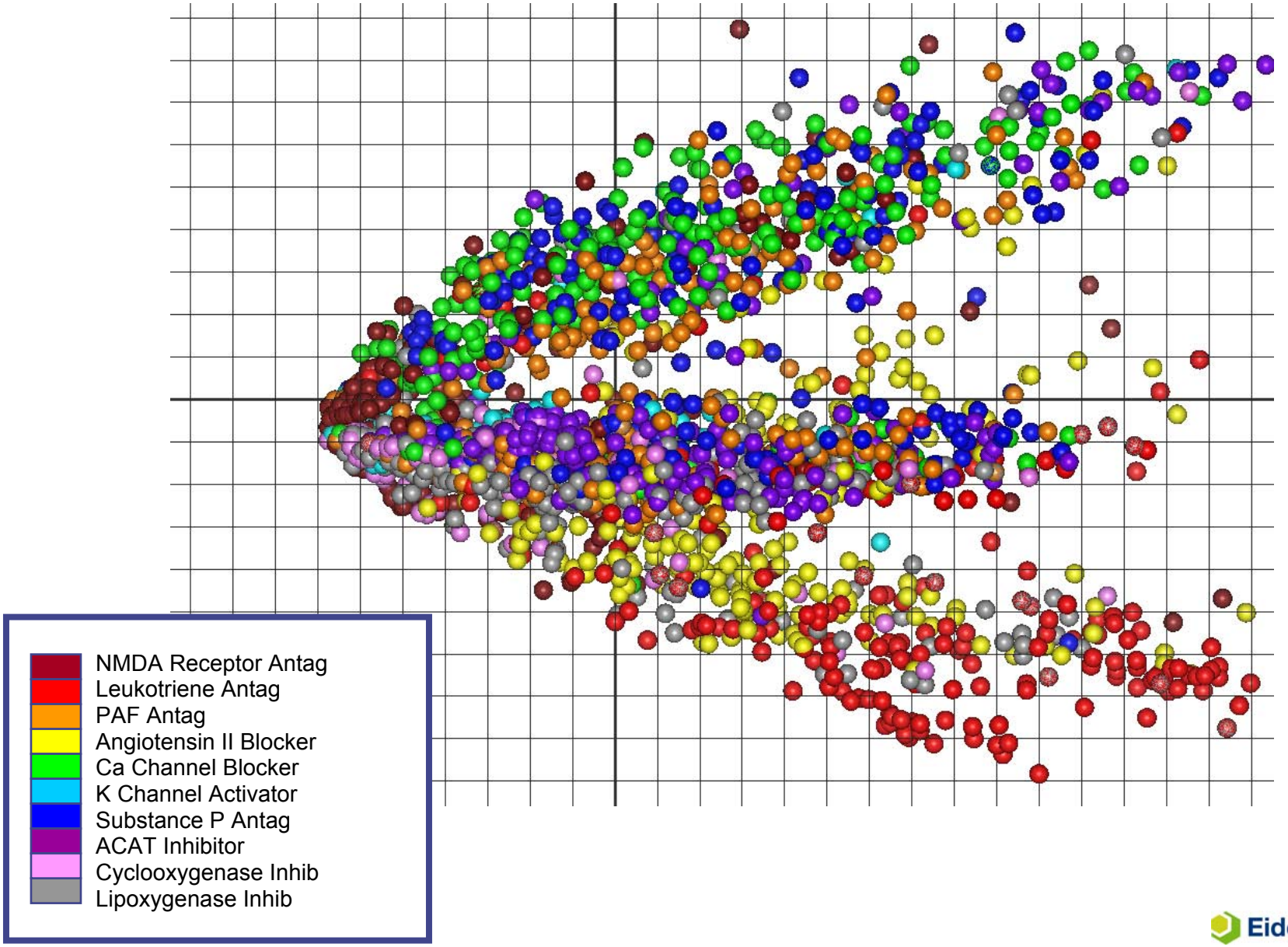
Ligand pharmacophoric potential



Journal of Chemical Information and Computer Sciences, 1999, 39 (3) : 569-74

Ligands grouped by pharmacophoric potential



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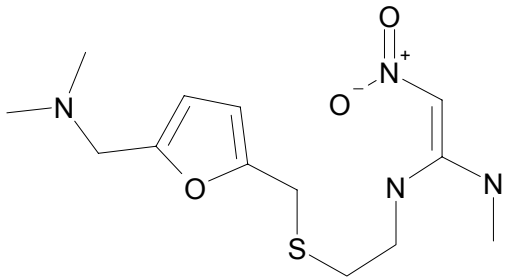
Journal of Chemical Information and Computer Sciences, 2000, 40 : 117-125

Drug similarity example - Non-obvious “Me-Too’s”

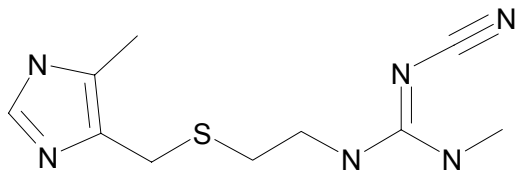
RANITIDINE (Zantac)



Target: Histamine H2-antagonist

MWT: 314.4; LogP: 0.27; pKa [2.30, 8.20]
Oral Avail.: 52% (±11)
Urinary Excretion: 69% (±6)
Plasma Bound: 15% (±3)
Clearance: 730 mL/min (±80)
Half-Life: 2.1 hr (±0.2)
Effective Conc.: 100 ng/mL

CIMETIDINE (Tagamet)



Target: Histamine H2-antagonist

MWT: 252.3; LogP: 0.40; pKa: [6.80]
Oral Avail.: 62% (±6)
Urinary Excretion: 62% (±20)
Plasma Bound: 19%
Clearance: 540 mL/min (±130)
Half-Life: 1.9 hr (±0.3)
Effective Conc.: 800 ng/mL

Eidogen-Sertanty Pharmacophoric Similarity Score, PharmSim= 0.88/1.00

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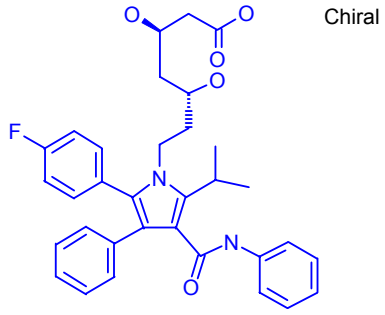
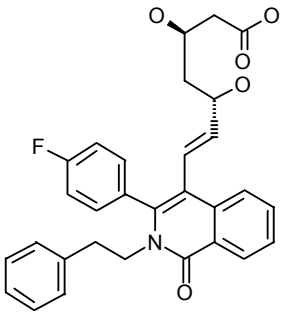
Drug similarity example (cont)

ATORVASTATIN (Lipitor)



Target: HMG-CoA Reductase
2007 Sales >$12.8B (worldwide)

172577 – Takeda



Target: HMG-CoA Reductase

Eidogen-Sertanty Pharmacophic Similarity Score, PharmSim= 0.79/1.00

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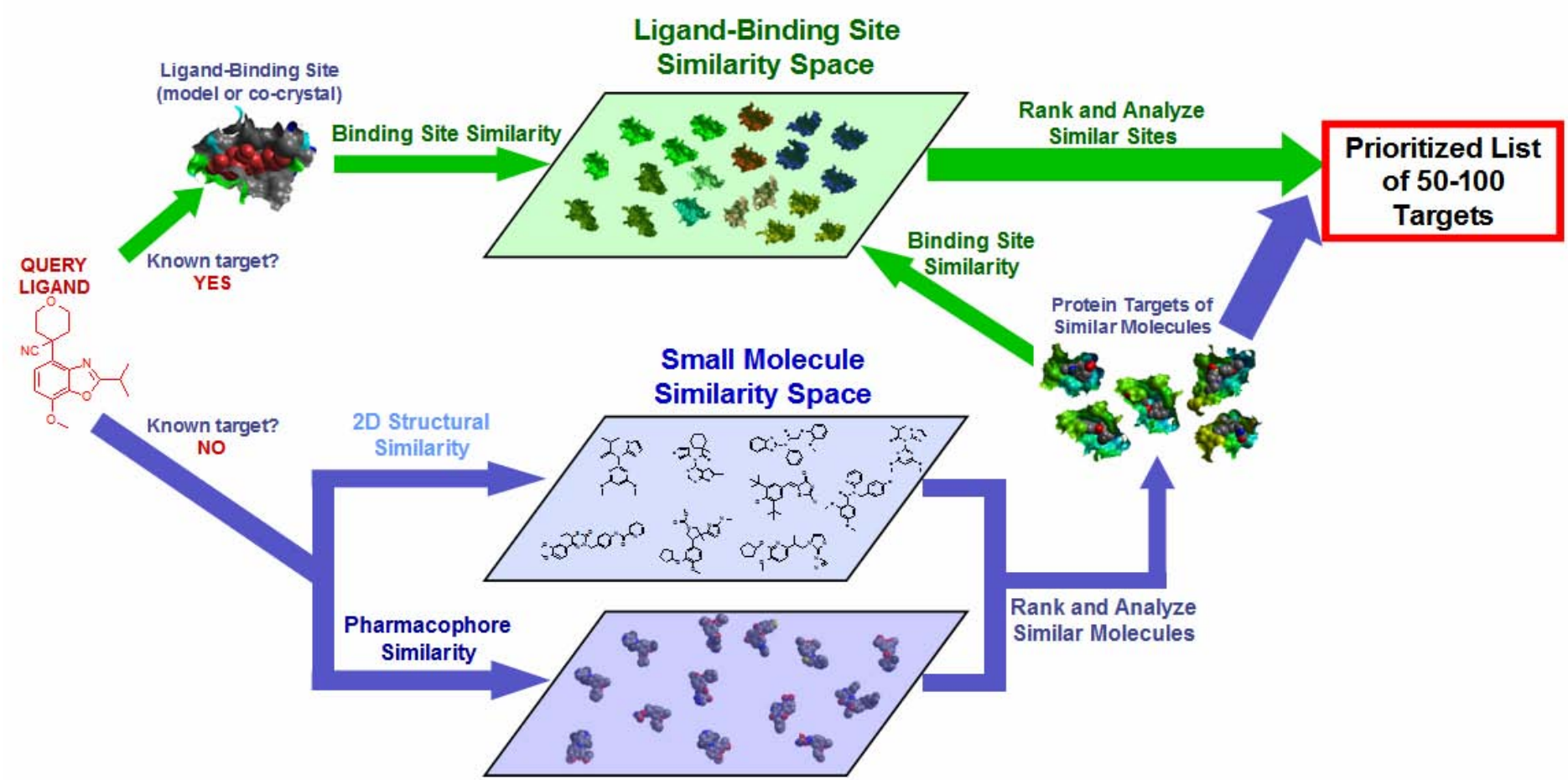
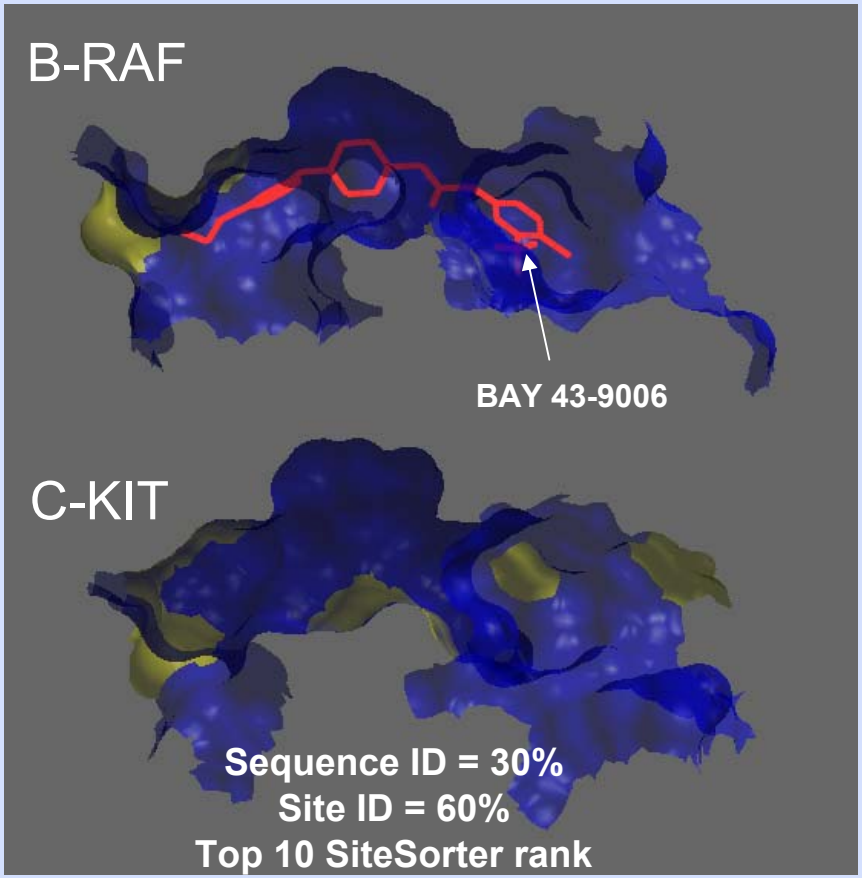
In silico target screening ("Target Fishing")



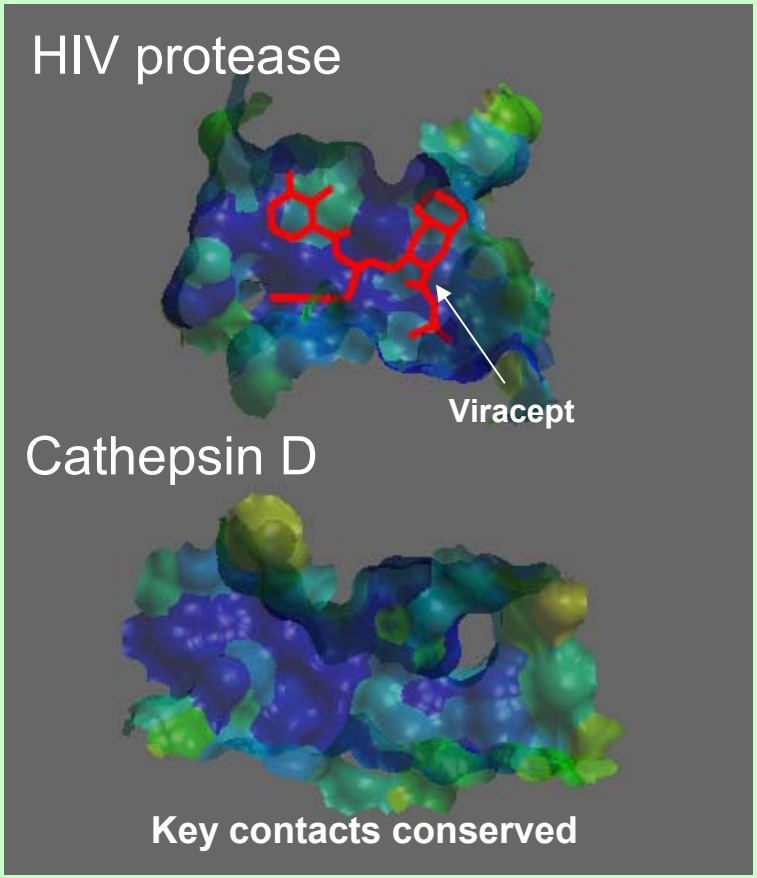
Off-target opportunities

Intra-Family Opportunities



B-RAF inhibitor BAY 43-9006 also inhibits C-KIT

Inter-family Opportunities

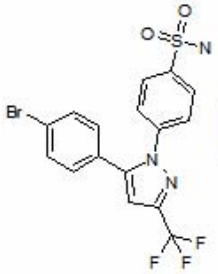


Cathepsin D is inhibited by HIV protease inhibitors

Identifying “off-target” opportunities and liabilities

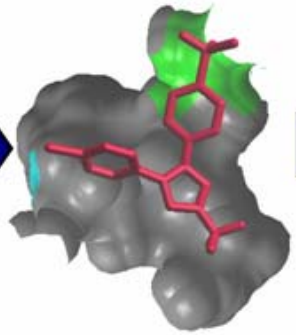
Example: Prioritizing potential “off-targets” for COX-2 inhibitors

Ligand



Celebrex analog SC-558
(Pfizer – Celebrex 2004 Sales $3.3 B,
black-box warning in 2005)
{Bextra pulled from market in 2005]

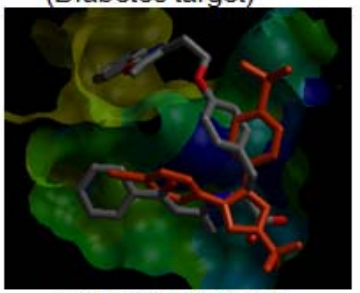
Determine Ligand-Binding Site



Search Database and Rank Similar Binding Sites

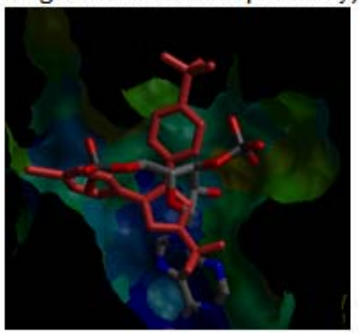
Prioritize “off-targets” based on Site-Ligand Contact analysis and/or biological relevance

Rank #1: PPAR-Gamma
(Diabetes target)



- Confirmed “Off-Target” for COX-2 inhibitors

Rank #10: Estrogen Sulfotransferase
(Estrogen Metabolism pathway)



- Possible “Off-Target” for COX-2 inhibitors

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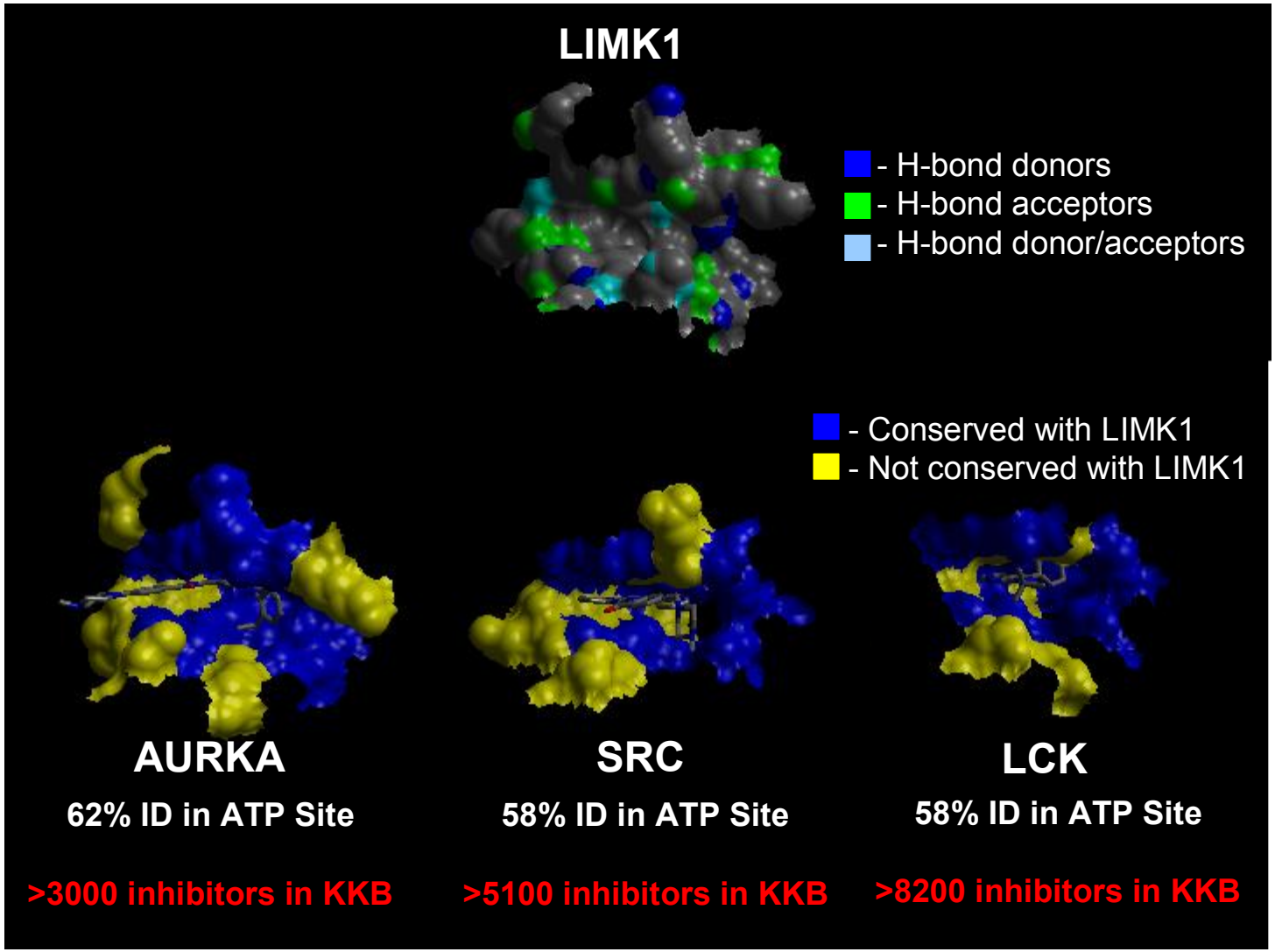
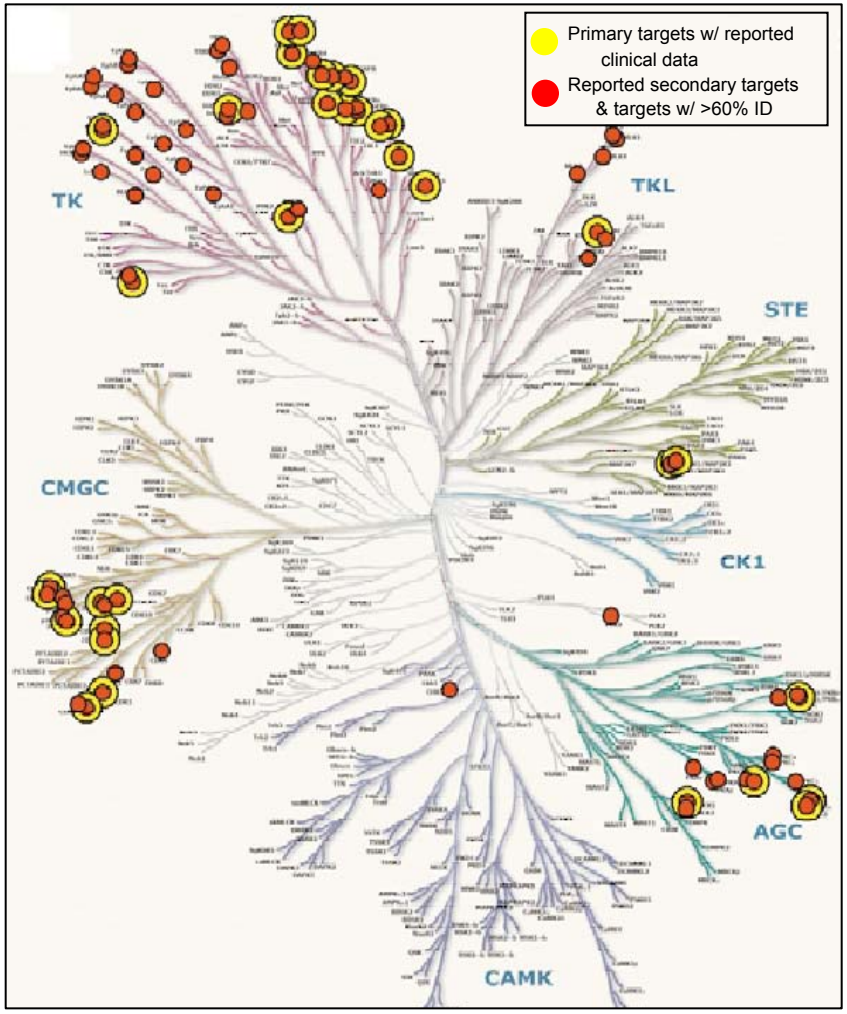
LIMK1 – ATP binding site comparison



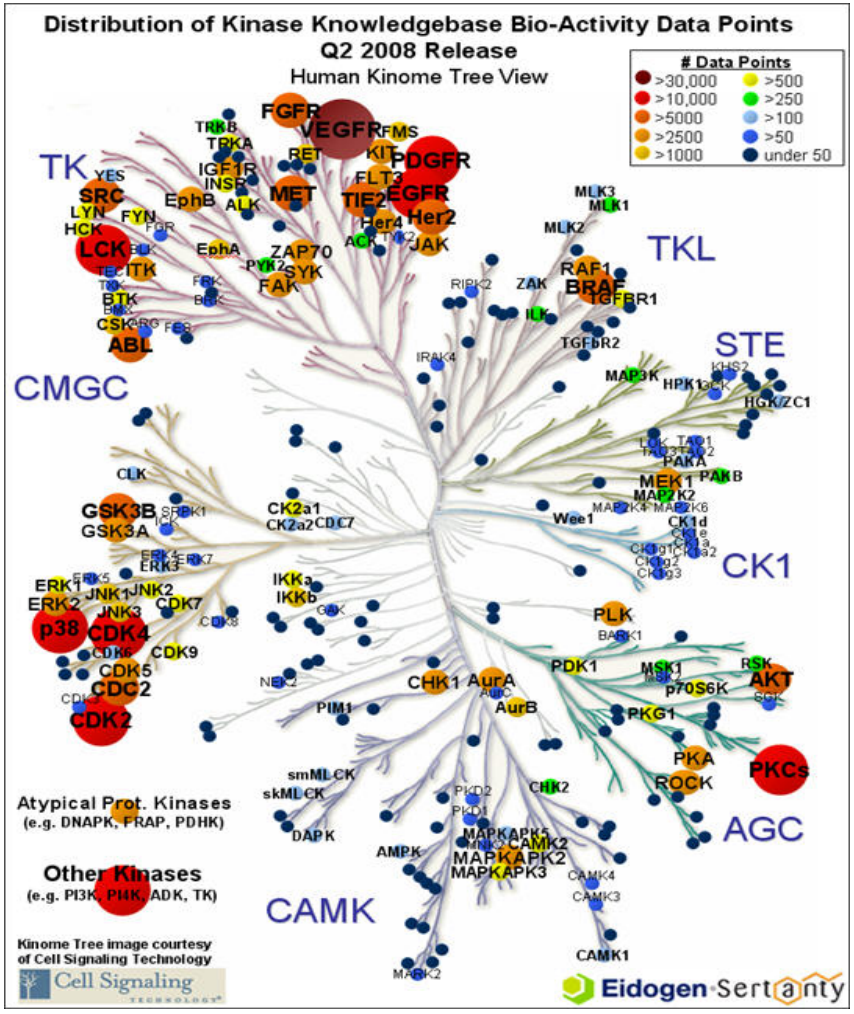
The ATP site of LIMK1 shares a high level of homology with several well-studied kinases

Kinase SAR Knowledgebase – Hot Targets

Kinase Targets of Clinical Interest
from Vieth *et al*. *Drug Disc. Today* **10**, 839 (2005).



Eidogen-Sertanty KKB
SAR Data Point Distribution



>362,000 SAR data points curated from
>4,270 journal articles and patents
>130 Bayesian QSAR Models

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Kinase Knowledgebase (KKB)

Kinase inhibitor structures and SAR data mined from

Kinase Validation Set

Three sizable datasets freely available to the research community

http://www.eidogen-sertanty.com/kinasednld.php

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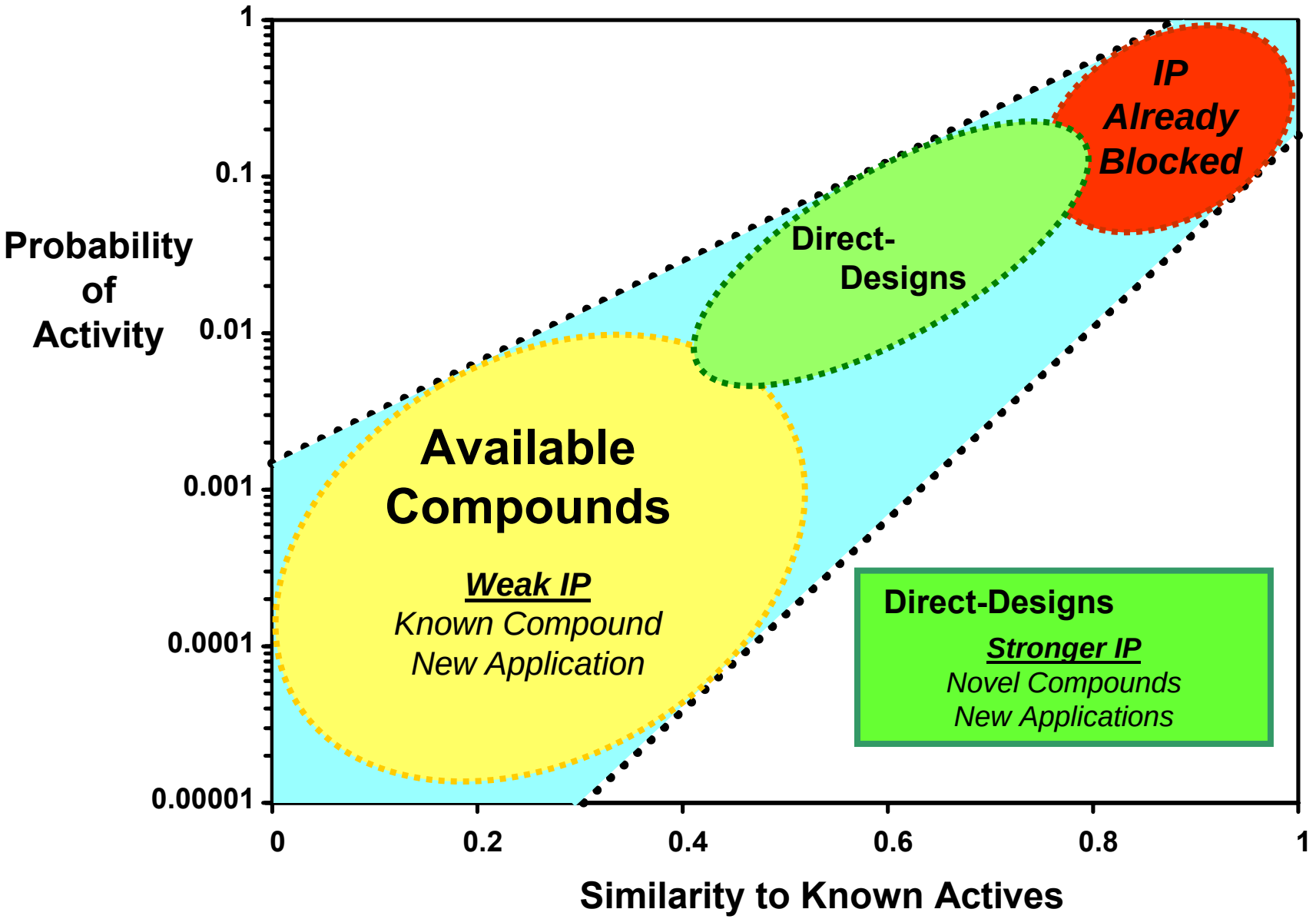
Fast follower design - Novel active chemotypes in 5-50 molecules



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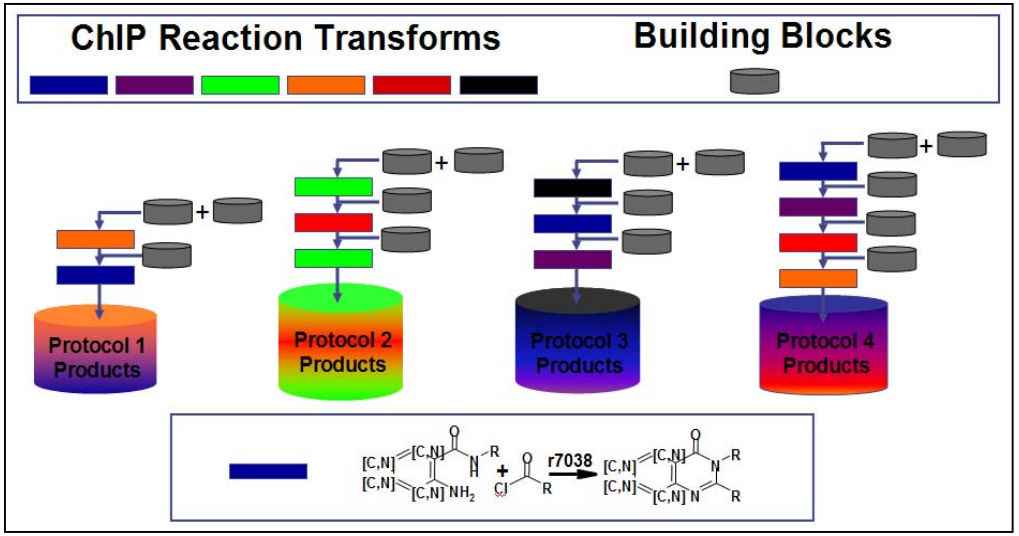
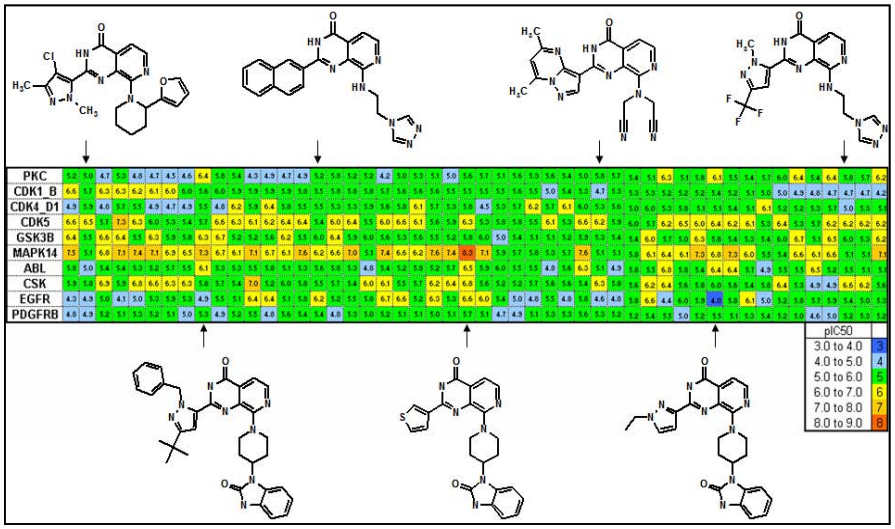
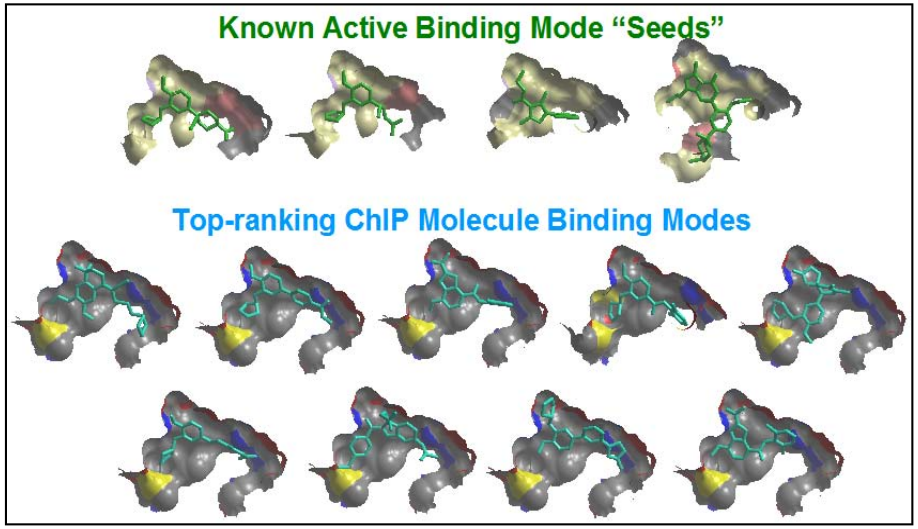
Synthetically accessible molecule design



ChIP™





Schurer et al. J. Chem. Inf. Model. 45(2), 239-248, 2005.

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Success requires efficient “cause-effect” feedback

What can be made?

That which is synthetically “feasible:”

- In-house expertise
- Starting material availability
- Cost/time

What should be made?

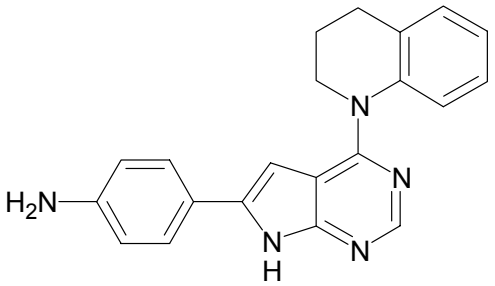
That which is medicinally relevant, efficacious, and safe:

- Potency
- Selectivity
- Bioavailability
- Low toxicity
- Cost effective

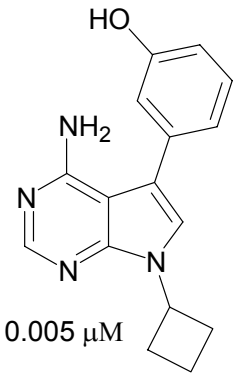
Direct Design Case 1: Using SAR information

ABL Kinase Project

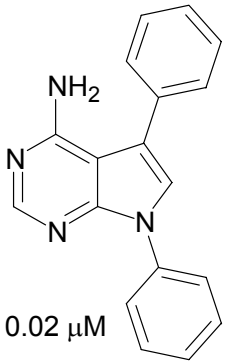
Previously published ABL inhibitors



0.002 μM (Traxler et. al. Novartis)

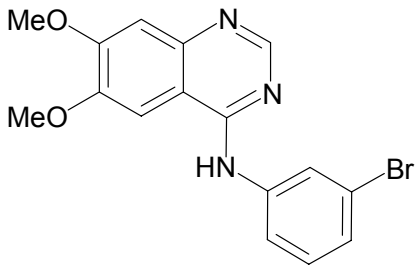


0.005 μM

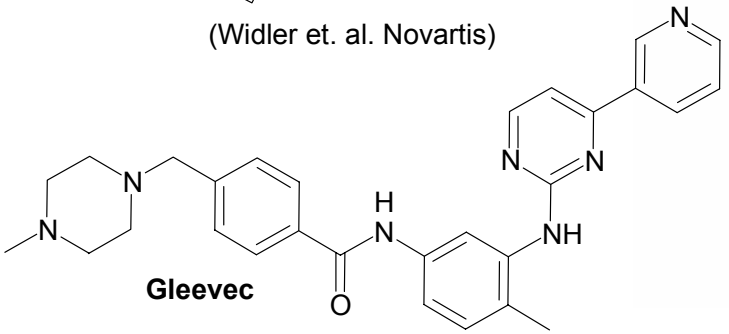


0.02 μM

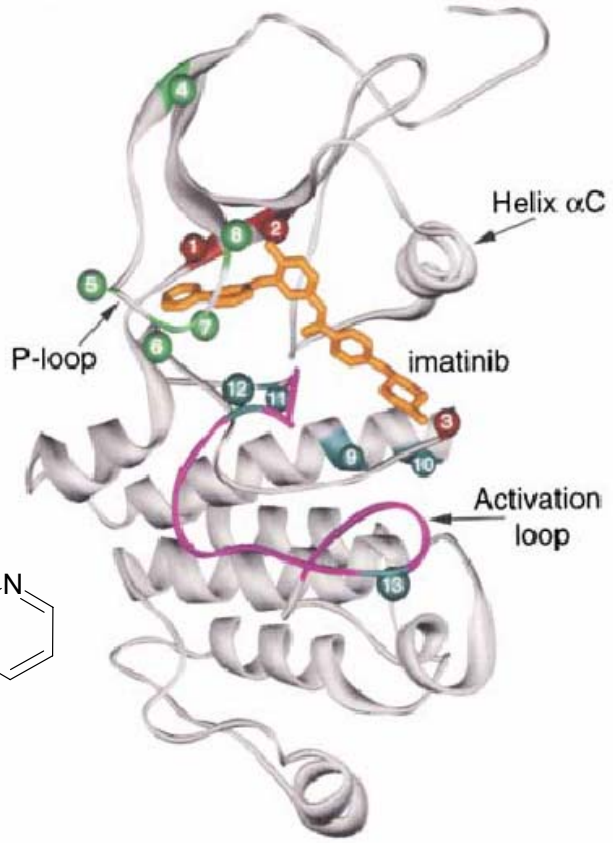
(Widler et. al. Novartis)



0.03 μM (Bridges et. al. Parke Davis)



0.038 μM (Zimmermann et. al. Novartis)



Sawyers, C. L. et. al. *Cancer Cell* **2002**, *2*, 117

Data Capture – Model Development

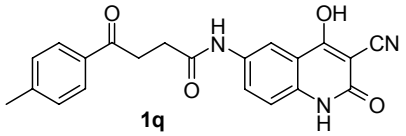
- Capture known ABL-kinase SAR data
  - 27 journal articles / patents
  - 413 small molecules
- Build a pharmacophoric eScreen™ model
  - Grouping of comparable IC50 SAR data based on assay type, binding site, assay conditions
  - 3-point pharmacophore fingerprints

Virtual Library Analysis – Synthesis

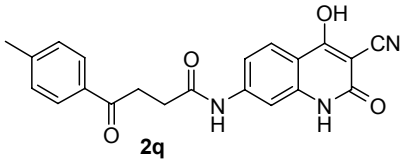
- Generation of 60,000 member virtual Library using 15,000 commercially available carboxylic acids
- Identification of best building blocks by pharmacophoric-based eScreen™ and eADME prioritization
- Synthesis of 80 compounds of this scaffold

Assay Results

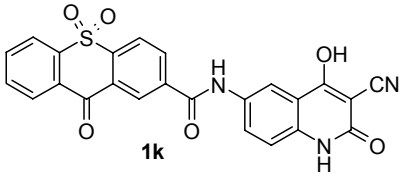
Measured IC50 (predicted IC50)



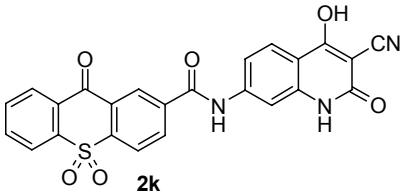
4 (9) μM



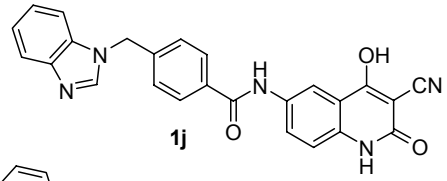
3 (4) μM



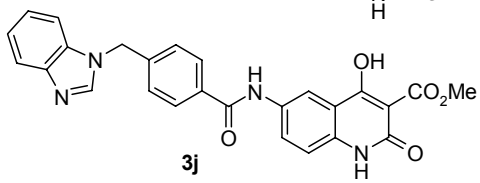
5 (7) μM



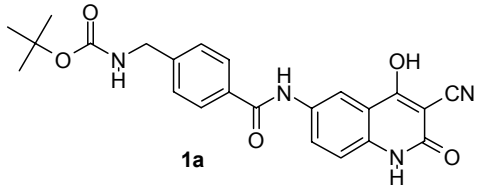
5 (5) μM



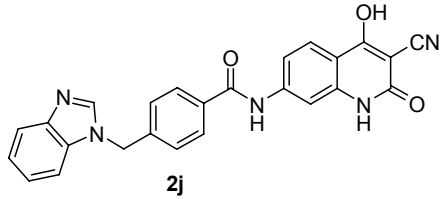
8 (8) μM



10 (21) μM

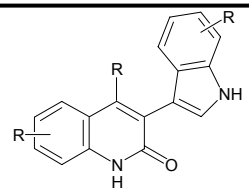


10 (9) μM



7 (6) μM

8 Compounds with IC50 ≤ 10 μM (initial synthesis)



JP2001-089471, Japan Tobacco, Inc.

(claimed as PDGFR inhibitors)

Eidogen-Sertanty

ABL Project Summary

4 months

Data Capture

Predictive model building

Data analysis and scaffold extraction

Scaffold hopping

Design proliferation,
Prioritization, and in silico screening

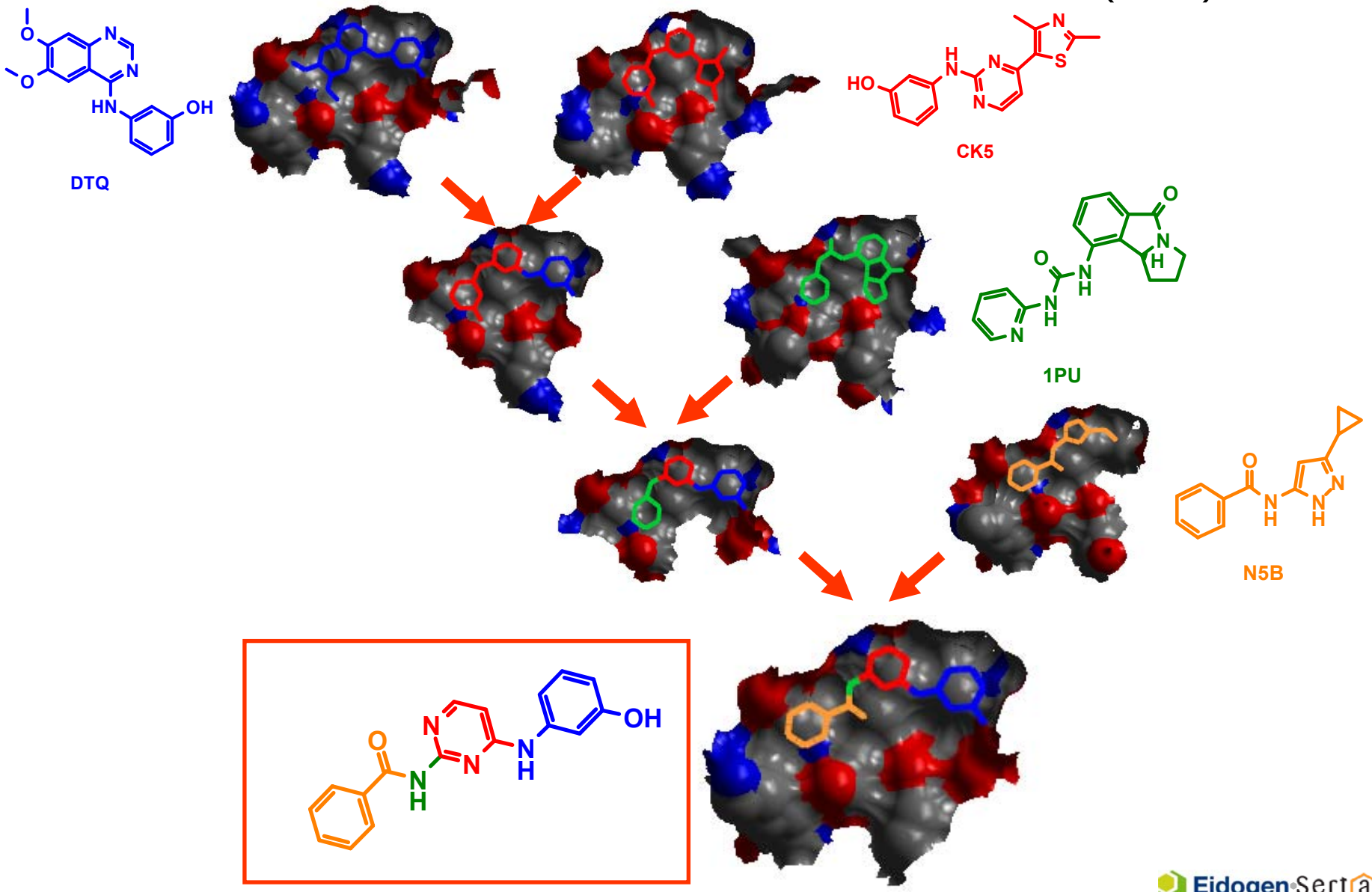
Synthesis of compounds

→ **Bio assay**

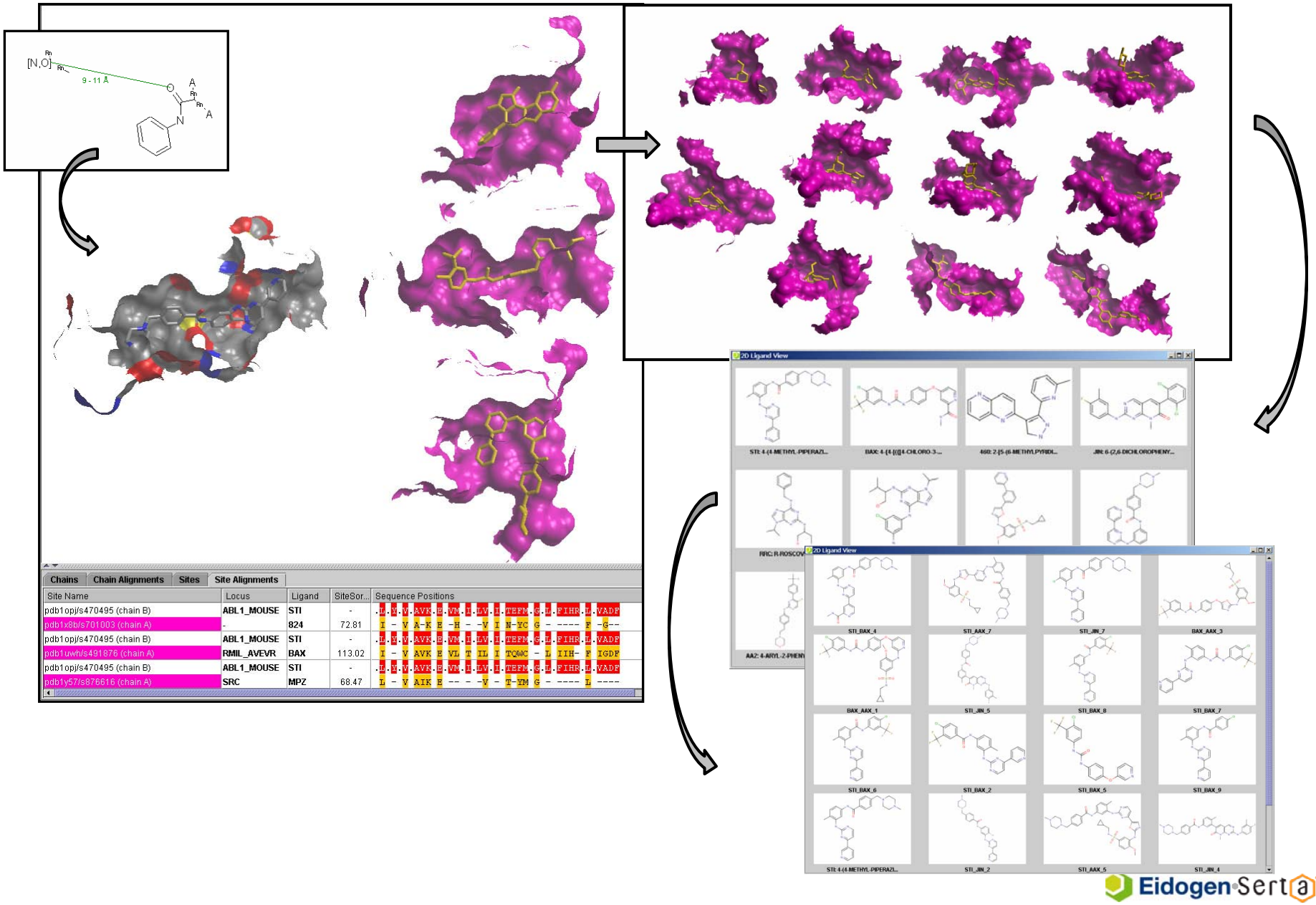
Direct Design Case 2: Using co-complex information

Lead Discovery: Knowledge-Based Design

Similar to Vertex's BREED: J. Med. Chem. **47**, 2768 (2004).

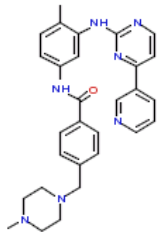
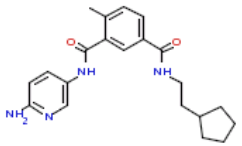
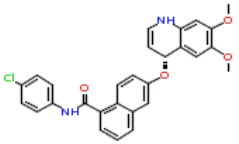
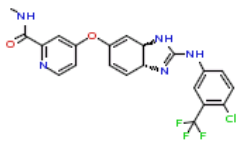
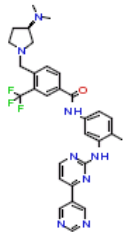
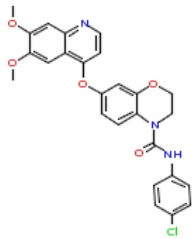
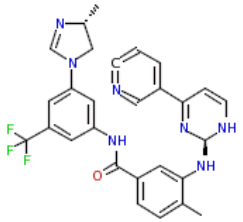
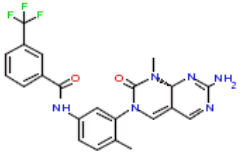
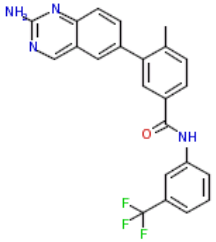
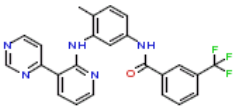
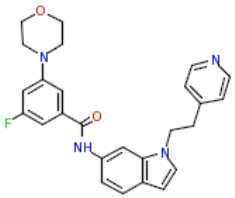
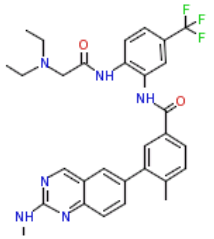
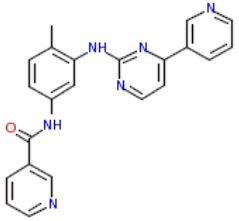
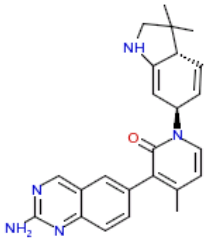
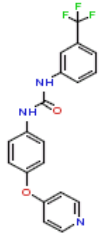
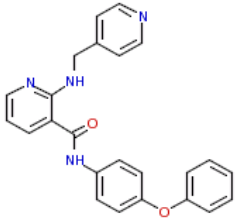
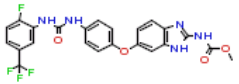
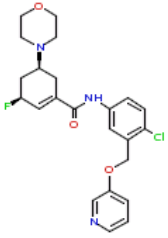
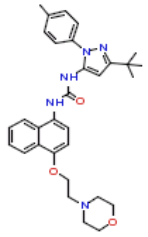
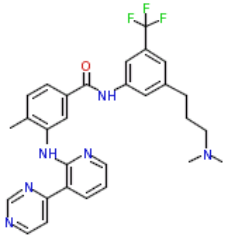


From ligand query to sites to new ligand ideas



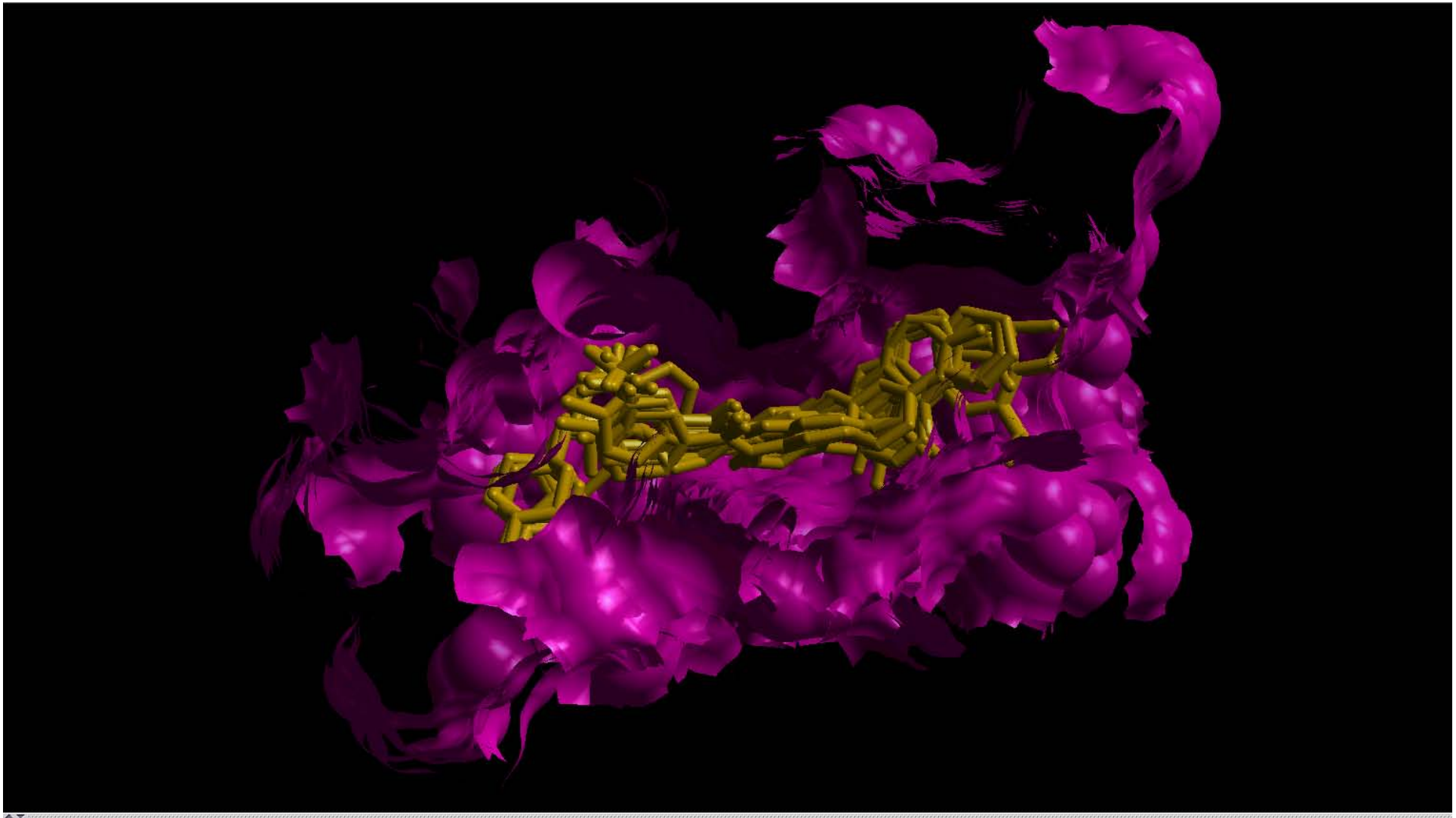
Eidogen-Sertanty

Example Ligands Extracted from Similar Sites

 STI	 C92	 900	 276	 406
 2RL	 NIL	 7MP	 242	 MUH
 WBT	 1N8	 PRC	 C19	 KIN
 608	 GIG	 L11	 B96	 RAJ

Eidogen-Sertanty

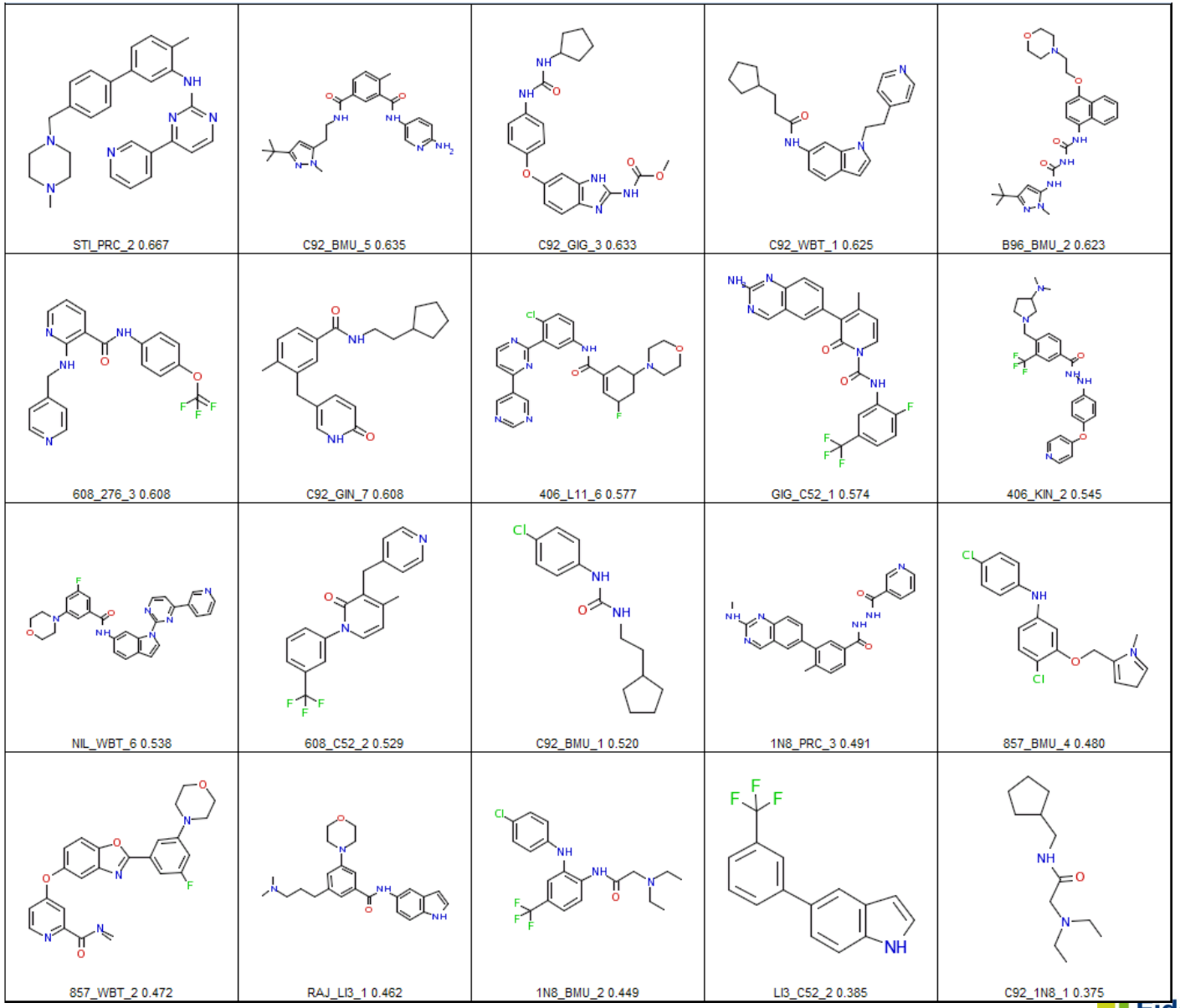
LigandCross – Mixing Ligand Features from Aligned Sites



Chains | Chain Alignments | Sites | Site Alignments

Site Name	Locus	Ligand	%Conf	Sequence Positions
pdb2pl0/s1309707 (chain A)	LCK	STI	100	.L.V.AVK.E.LM.L.LV.I.TEYM.GS.I.YIHR.L.IADF
pdb2of/s916648 (chain B)	LCK	242	100	.L.V.AVK.E.LM.L.LV.I.TEYM.G.I.Y.H.L.IADF.I
pdb2rl5/s1396160 (chain A)	-	2RL	100	.LG.V.AVK.L.E.IL.I.VV.V.TEFCKFGN.L.CIH.L.ICDF
pdb2e2b1/s1284639 (chain B)	ABL	406	100	.L.Y.V.A.K.E.VM.I.LV.I.TEFMT.G.L.FIHRD.L.VADF

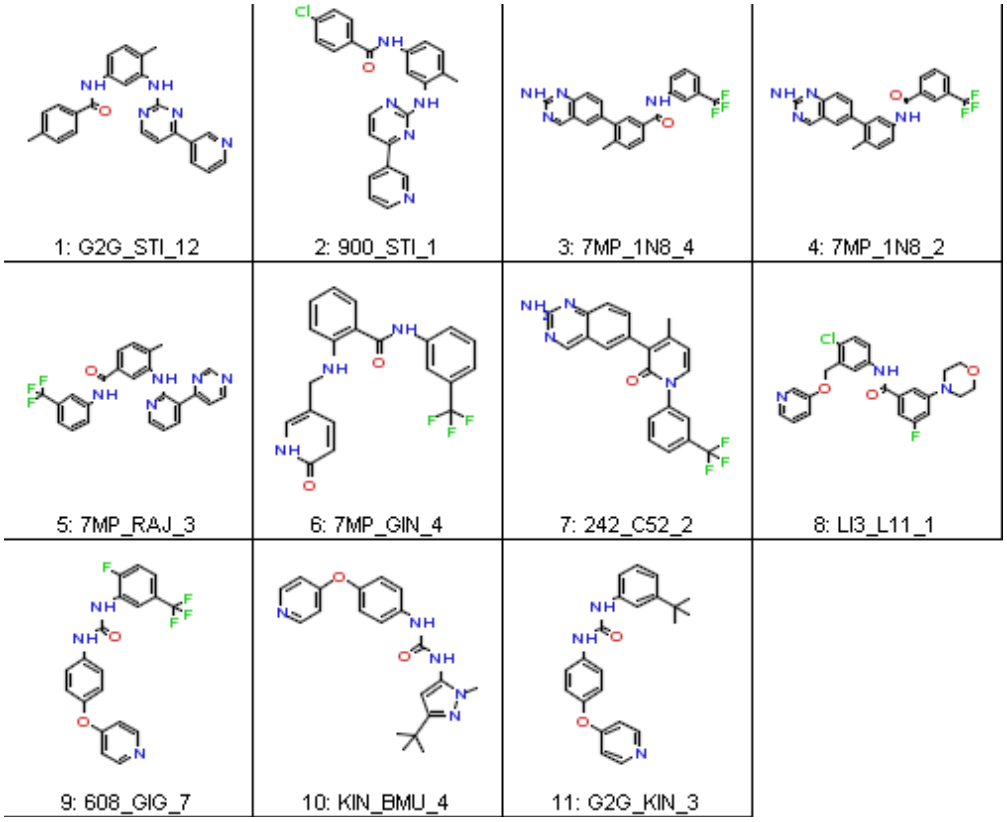
Example LigandCross Results



Eidogen-Sertanty

LigandCross Ligands with Reported Biological Activity

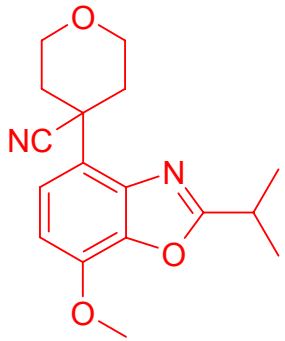
	Kinase Knowledgebase (pIC50)										Bayesian Model Predictions (PP)									
LC-ID	ABL	PDGFR	PDGFRB	JAK3	KDR	LCK	MAPK14	TEK	KIT	RAF1	ABL	PDGFR	PDGFRB	JAK3	KDR	LCK	MAPK14	TEK	KIT	RAF1
G2G_STI_12	6.7	8	8								0.40	0.90	0.76	0.81	0.59	0.15	0.89	0.45	0.70	0.37
900_STI_1	6.1	8	8								0.38	0.91	0.76	0.72	0.55	0.16	0.88	0.42	0.71	0.55
7MP_1N8_4				7.8	9	9.5	8.7				0.36	0.49	0.34	0.32	0.94	1.00	0.95	0.67	0.86	0.39
7MP_1N8_2				6.8	8.3	9.5	9				0.37	0.46	0.31	0.44	0.92	1.00	0.92	0.69	0.84	0.45
7MP_RAJ_3					8.4			8.4			0.35	0.73	0.50	0.49	0.92	0.81	0.86	0.94	0.74	0.37
7MP_GIN_4					7.6						0.16	0.50	0.40	0.82	0.95	0.67	0.70	0.41	0.76	0.51
242_C52_2									7.9		0.30	0.28	0.29	0.74	0.80	0.66	0.74	0.31	1.00	0.43
LI3_L11_1							7.2				0.31	0.73	0.55	0.84	0.74	0.69	0.62	0.36	0.76	0.85
608_GIG_7										6.1	0.28	0.61	0.57	0.69	0.93	0.50	0.60	0.68	0.85	0.50
KIN_BMU_4										6.1	0.31	0.43	0.45	0.78	0.75	0.57	0.77	0.33	0.81	0.25
G2G_KIN_3										6.1	0.25	0.51	0.52	0.75	0.89	0.59	0.64	0.43	0.84	0.43



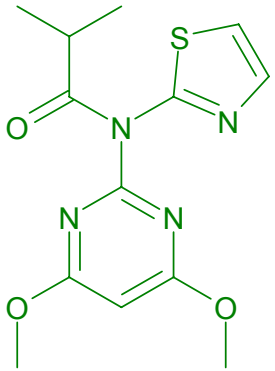
Eidogen-Sertanty

Direct Design Case 3: Using Active Molecule(s)

ChIP-ing Towards Me-Too's

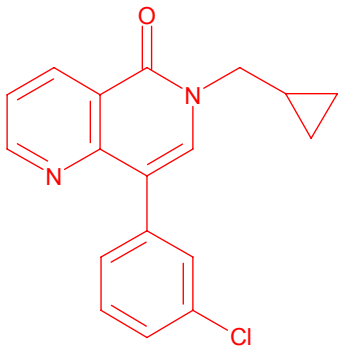


ChIP'd Me2

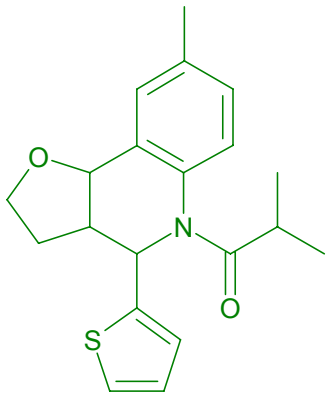


PharmSim: 0.93
MDLSim: 33.1/100.0

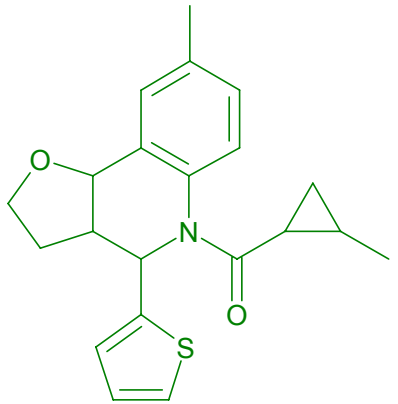
Known PDE-IV Inhibitors



ChIP'd Me2

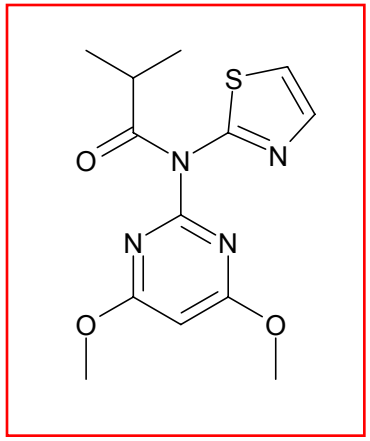


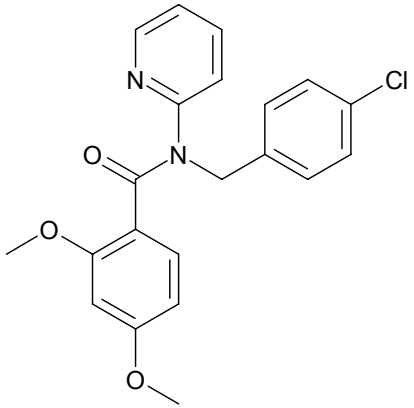
PharmSim: 0.92
MDLSim 44.4/100.0

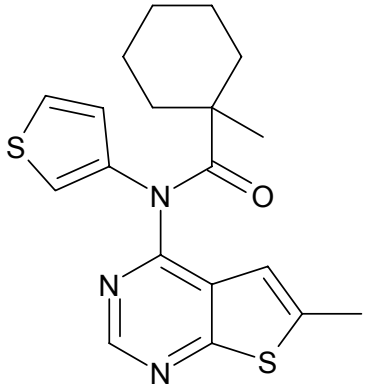


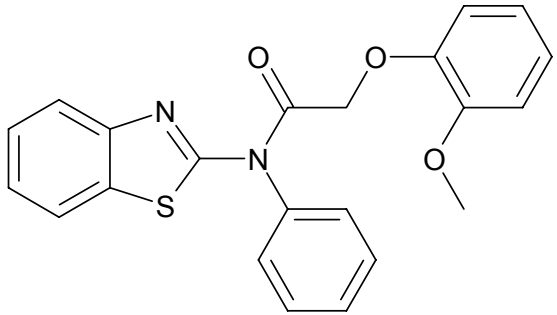
PharmSim: 0.92
MDLSim 50.6/100.0

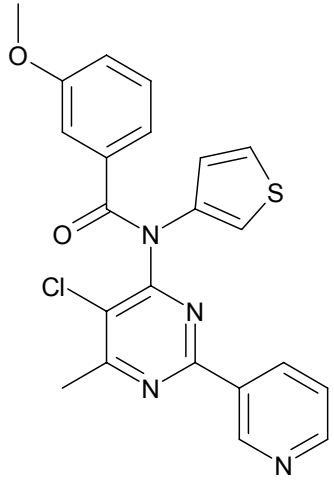
Other ChIP Generated PDE-IV “Me-Too’s”

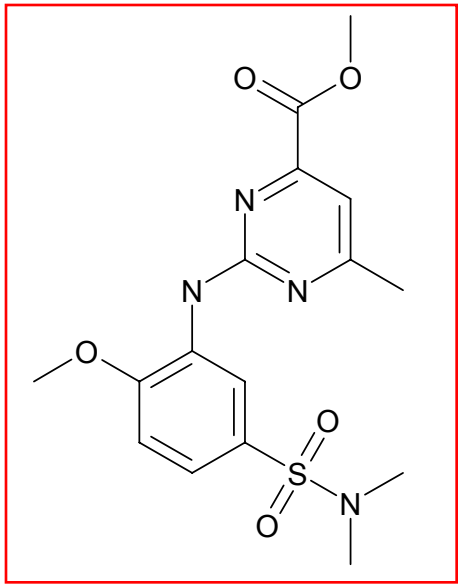


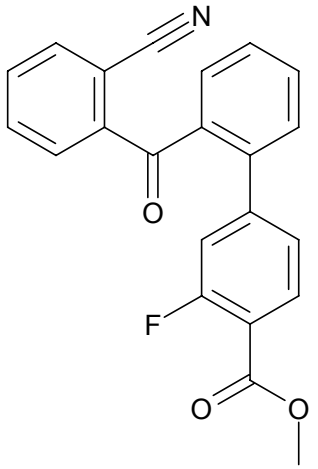


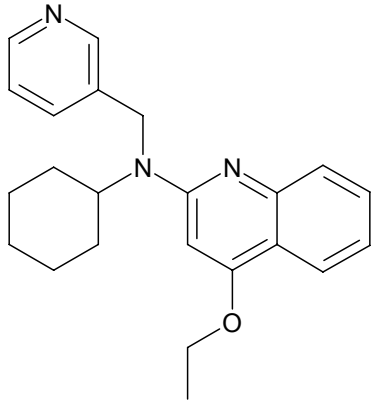






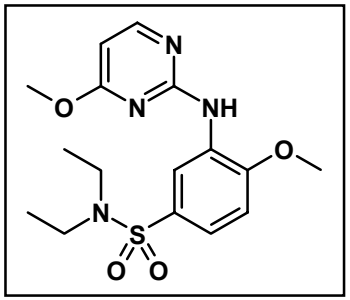




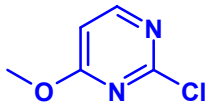


Eidogen-Sertanty

Example ChIP Generated Synthetic Road-Maps



PFP Sim 0.8623



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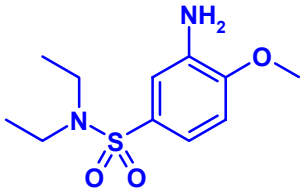

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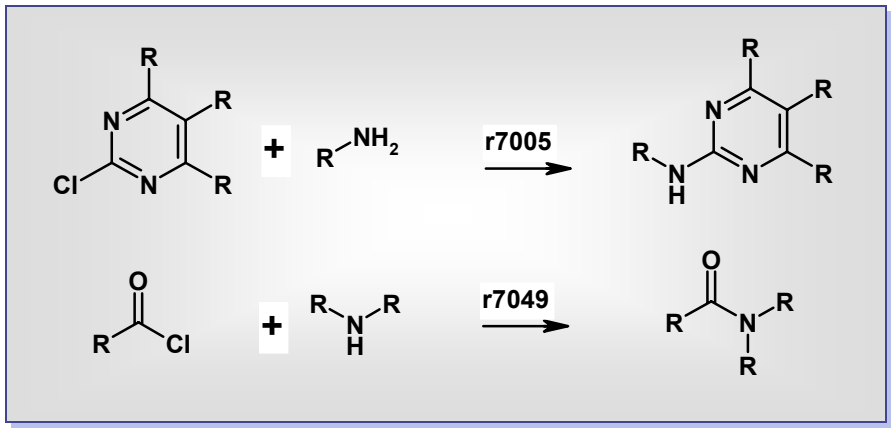

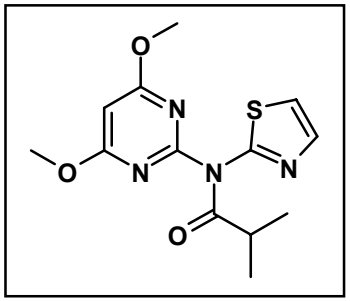

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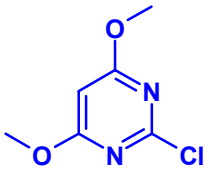

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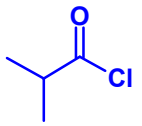

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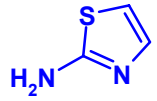

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Conclusions

- Significant receptor-site similarities exist within and across target families

- The structurally resolved and modelable proteome is a very rich source for new matter ideas

- Biologically active molecules can be used to generate other very novel, bioactive molecules

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