

DeNovo Design as a tool for medicinal chemists: minimal requirements for a renewed consideration

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Talk

- Why is DeNovo Design a so unpopular approach within drug discovery?
- Novobench project to address unmet needs in DeNovo design
- Focus on:
 - Fragment spaces (source of candidate molecules)
 - Druggability (“on the fly” filtering of candidate molecules)
 - “Med. Chem.” controls
 - Synthetic Feasibility control
- Retrospective Validation and Results
- Examples



DeNovo – too early, too technical or already successful but hidden?

- Old themes in DeNovo applications
 - Progressive cavity filling driven by scoring functions
 - Druggability & synthetic feasibility of results
 - Diversity with respect to known ligands
 - Slow surfacing of promising retrospective validation
 - Lack of published prospective validation with wide applicability
 - **Inherent usage as idea- more than compound- generator**
- Why publish? Patent!



Novobench – Rethinking DeNovo with medchem focus

- Project granted by BMBF (grant #313342A) in Germany
- *Technical partners* (design & programming): Molecular Network (MoINet), BioSolveIT (BIT)
- *Pharma partners* (specifications & testing) Nycomed GmbH, Lilly Forschung GmbH and 4SC AG
- *Deliverables*: new modular software addressing DeNovo design for drug discovery



Novobench – Rethinking DeNovo with medchem focus

- Delivered so far

- **CoLibri** (Lemmen, Christian; Claussen, Holger; Gastreich, Marcus; Paern, Juri; Degen, Jorg; Rarey, Matthias. *Fragment -based DeNovo design*. Abstracts of Papers, 233rd ACS National Meeting, Chicago, IL, United States, 2007)
- **FlexNovo** (Degen, Joerg; Rarey, Matthias. *FlexNovo : structure-based searching in large fragment spaces*. ChemMedChem (2006), 1(8), 854-868)
- **Sylvia/Syntree** (Boda, Krisztina; Seidel, Thomas; Gasteiger, Johann. *Structure and reaction based evaluation of synthetic accessibility*. Journal of Computer-Aided Molecular Design (2007), 21(6), 311-325)
- **FragView/FragEnum** (Paern, Juri; Degen, Joerg; Rarey, Matthias. *Exploring fragment spaces under multiple physicochemical constraints*. Journal of Computer-Aided Molecular Design (2007), 21(6), 327-340)



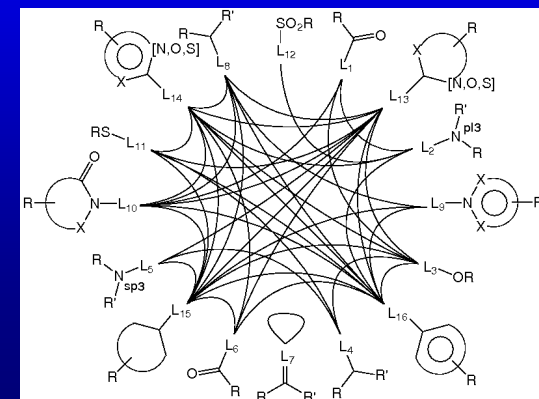
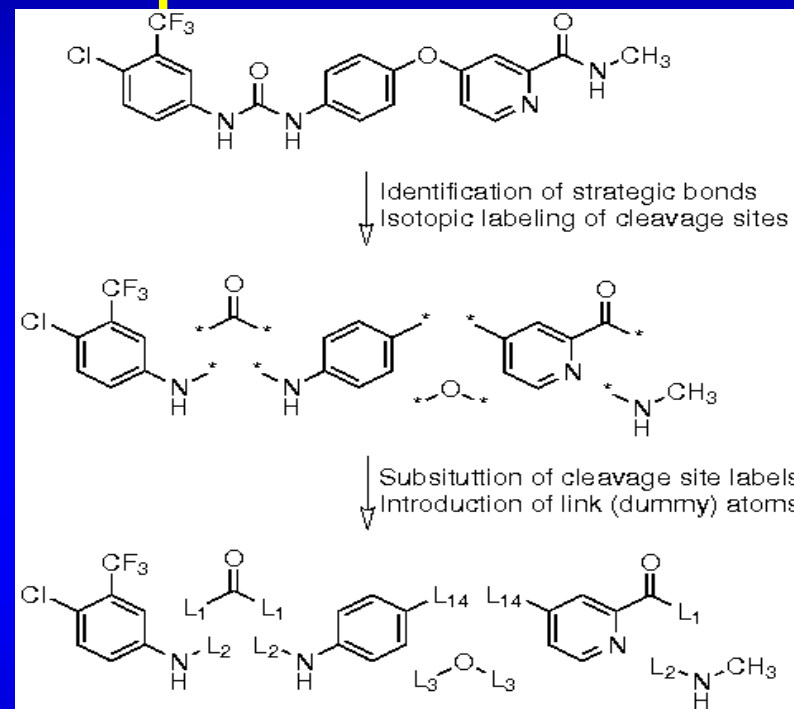
FlexNovo – Design of approach

- Engine and Score used: FlexX
- Approach:
 1. in a fixed protein cavity, to flexibly position and score fragments taken from user-prepared space,
 2. sort them and grow them with a k-greedy algorithm under several user-defined controls.
 3. iterate 2. until molecular growth ends because of lack of possible attaching points or limitations from user-defined controls
- Examples of controls implemented
 - Rot_bonds, TPSA, Lipinski's rules
 - Geometrical constraints (e.g. important H-bonds)
 - Synthetic feasibility score (Chem.complexity, presence of similar reagents and of precise retrosynthetic paths)
 - Multiple occurrences of identical fragments



FlexNovo– Fragment Space @ basis

- The optimization of lists of docked fragments (and of growing combinations thereof) is an overwhelming task due to its size
- Can we make it manageable through fragment space definition?
- Fragment space is defined and practically generated by:
 - shredding a molecule(s) at all recognized scissile bonds at once (no overlapping fragments)
 - storing not only the fragments but also the nature of linker atom





FlexNovo– Fragment Space @ basis

- The fragment space used in a DeNovo experiment depends greatly on what we want to achieve:
 - Target-specific run may require fragments from known ligands
 - Combinatorial chemistry-inspired run may require list of commercial reagents
 - What makes a fragment a good fragment ?
- Generally, a good collection of fragments might be that smallest one, which allows to rebuild the highest number of known and stable molecules (<http://www.zbh.uni-hamburg.de/BRICS/>)
- In a retrospective validation study we needed to start from several fragment spaces obtained from shredding known ligands



FlexNovo – Validation problems

- Prospective Validation : how ?
- Published prospective validations in DeNovo design are few or with low efficacy designed molecules
- No valid effective and general solutions found yet
- Reasons for this silence
 - Publication restrictions in drug discovery business
 - Lack of multidisciplinary teams within academic groups
 - Why not to patent first?
 - Overhyped technology => SBDD made automatic ???!
 - Risky business for major software vendors



FlexNovo – Retrospective Validation

- Here we propose a minimal requirement set in a DeNovo validation experiment:
 1. be able to reconstruct the reference ligand from its own fragment space
 2. find it within the first five best ranked
 3. find it within a fixed RMSD (same binding mode)
 4. find it within a reasonable complexity
 5. show that results with lower similarity* vs. reference ligands, could be useful in idea generation process

* in this study FTree similarity was used



FlexNovo – Validation set

Protein cavities and fragment spaces used in 3 growing cycles*				
Family	Protein	No. of Ligands with respective cavities	No. of unique fragments	Enumerated results within filtering limits
Kinases	CDK2	99	137	NA (>1Mio.)
	CHEK1	17	24	640
	Rock-1	4	7	19
	JNK3	7	21	7771
PDE	PDE4b	9	15	34
	PDE5a	6	14	42
NHR	ESR1	24	32	604
Protease	BACE	18	49	32585*

NA=not assessed - * = 5 cycles allowed



FlexNovo – Controls set

Max values of control variables allowed for final solutions

Family	Protein	MW	Hacc	Hdon	cLogP	NRings	RotBonds
Kinases	CDK2	550	8	5	6	5	8
	CHEK1	550	8	5	6	5	8
	Rock-1	550	8	5	6	5	8
	JNK3	550	8	5	6	5	8
PDE	PDE4b	550	8	4	6	5	8
	PDE5a	550	8	4	6	5	8
NHR	ESR1	550	8	4	6	5	11
Protease	BACE	850	10	10	6	10	9



FlexNovo – Results analysis I

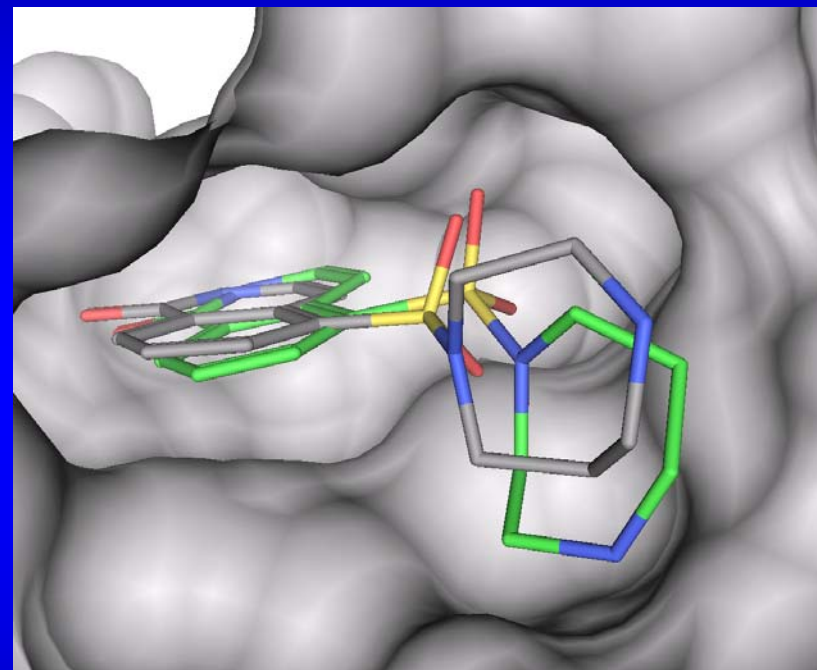
Analysis of solutions according to FTree similarity to reference ligands

Family	Protein	Total no. of solutions produced	No. of solutions identical* to reference ligand	Highest rank of Identical-to-ref. ligand solutions	RMDS (Å)
Kinases	CDK2	5702	6	4th	2.172
	CHEK1	1224	4	21st	6.922
	Rock-1	32	5	1st	1.614
PDE	JNK3	136	0	-	
	PDE4b	148	11	2nd	2.118
	PDE5a	33	0	-	
NHR	ESR1	232	3	3rd	6.285
Protease	BACE	1531	0	-	

* FTree similarity = 1.0 does not necessarily imply identity, but visual inspection assured it

FlexNovo – Results analysis II

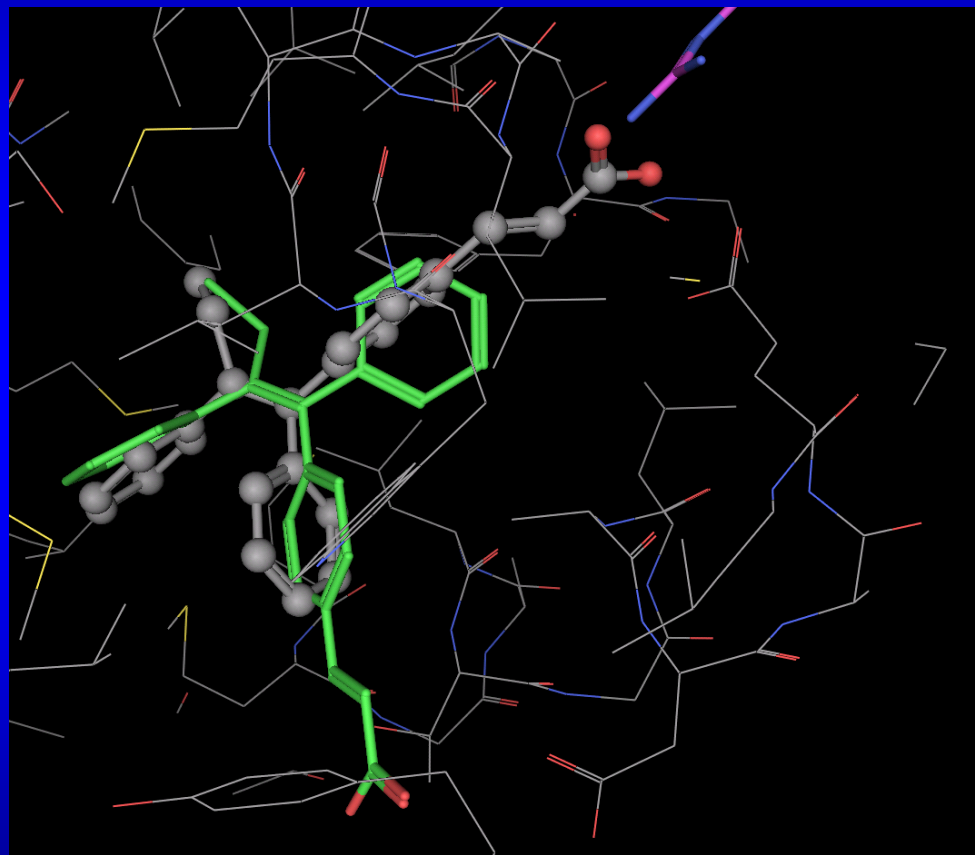
- Kinases example Rock1
- Successful rebuilding of ligand in 2ETK (green = xray ligand)
- RMSD= 1.614
- Ranked 1st





FlexNovo – Results analysis III

- NHR example ESR1
- Partially successful rebuilding of GW5638 in 1R5K (green = xray ligand)
- RMSD=6.285
- Ranked 3rd
- Here scoring function privileged false interaction to Arg101. Again a constraint to precise cavity region would have been decisive to drive the first fragment pose to the correct flipped binding mode

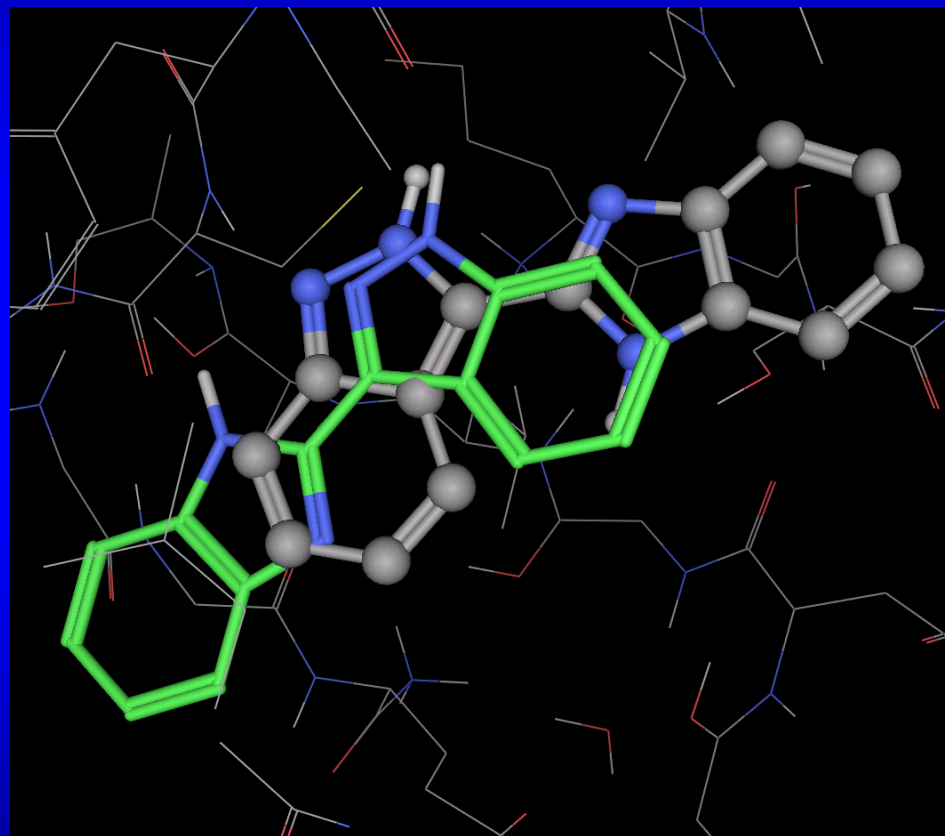




FlexNovo – Results analysis IV

- Kinases example CHEK1
- Unsuccessful rebuilding of ligand in 2HXL (green = xray ligand)
- RMSD=6.922
- Ranked 21st

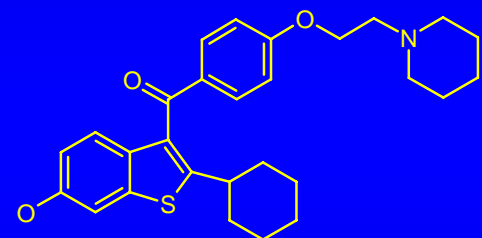
- Here a constraint to precise atom of hinge region would have been decisive to drive the correct flipped binding mode





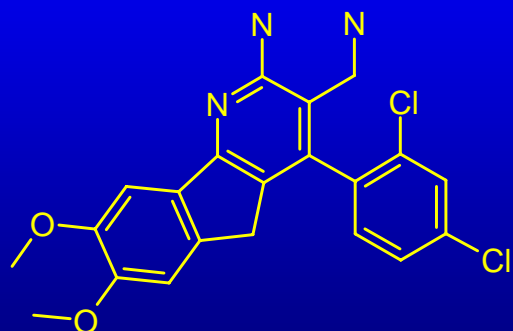
Arteminolide B (MW588)

Sylvia_score=9.9



ESR1 antagonist (MW468)

Sylvia_score=5.4



Roche DPPIV (MW416)

Sylvia_score=4.2

DeNovo – Synthetic feasibility

Sylvia's Score [Boda K. *et al.* 2007] for references and solutions

Family	Protein	Mean of reference ligands	Std. Dev.	Mean of candidate solutions	Std. Dev.
Kinases	CDK2	4.22	0.83	4.21	0.70
	CHEK1	4.42	0.50	4.24	0.75
	Rock-1	4.11	0.12	4.49	0.18
	JNK3	4.86	0.44	3.74	0.65
PDE	PDE4b	4.14	0.59	3.73	0.49
	PDE5a	5.23	0.86	3.88	0.49
NHR	ESR1	4.85	0.58	4.15	0.63
Protease	BACE	6.01	1.20	3.82	0.72



DeNovo – Exercise or value added?

- We checked the first 100 solutions looking for interesting candidates
- We were curious about:
 - Novelty of scaffolds
 - Geometries
- Scifinder searches for references



DeNovo – Speculations or value added?



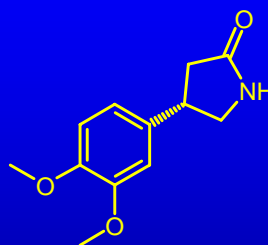
2is0 BACE_ligand



FlexNovo Candidate Rank=1
FTree Sim=0.935



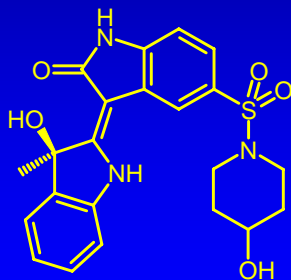
1xm6 PDE4b_ligand



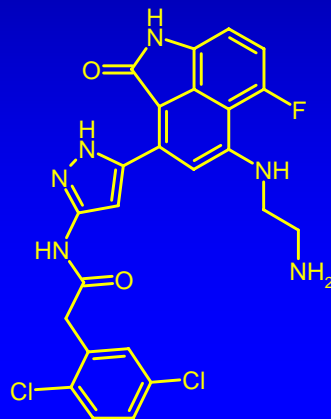
FlexNovo Candidate Rank=1
FTree Sim=0.908
RMSD 2.118 from 1ro6 (rolipram)



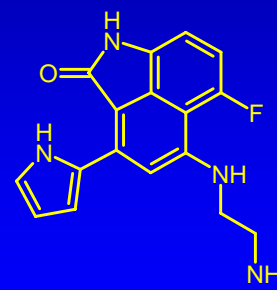
DeNovo – Speculations or value added?



2bhh CDK2_ligand



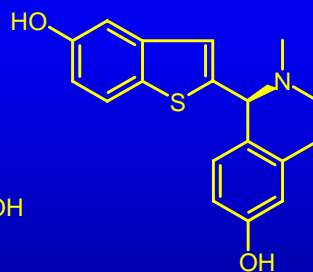
FlexNovo Candidate Rank=1
FTree Sim=0.922



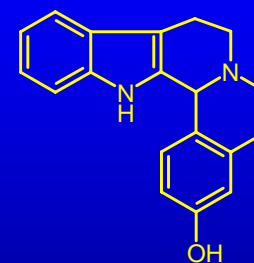
FlexNovo Candidate Rank=4
PDB 1p2a



2i0j ESR1_ligand



FlexNovo Candidate Rank=2
FTree Sim=0.906

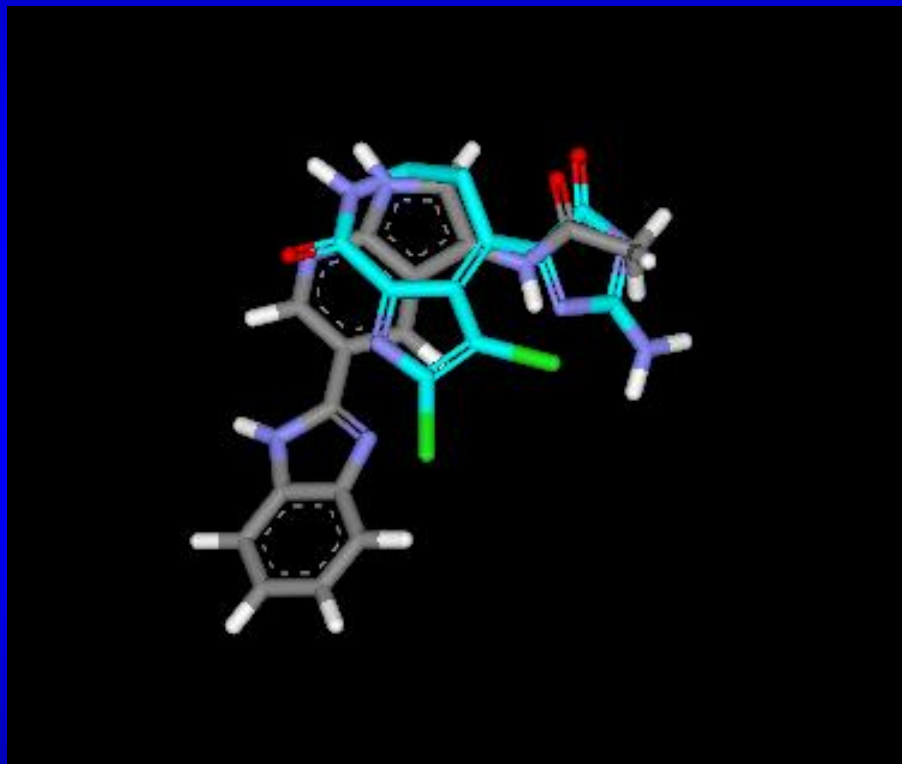


beta-carbolines and NHR
Pharmacol Biochem Behav.
1991 40(2):335



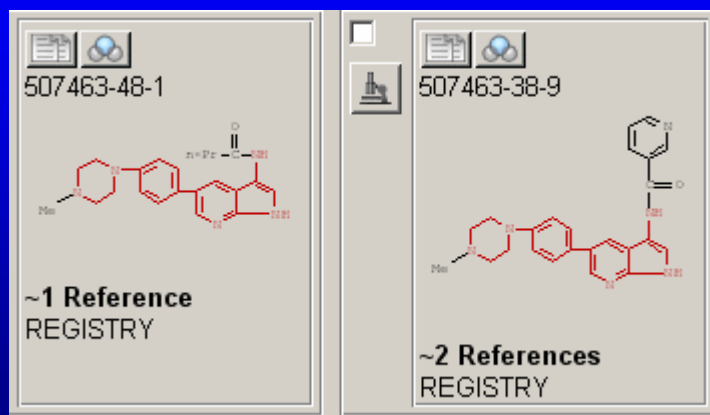
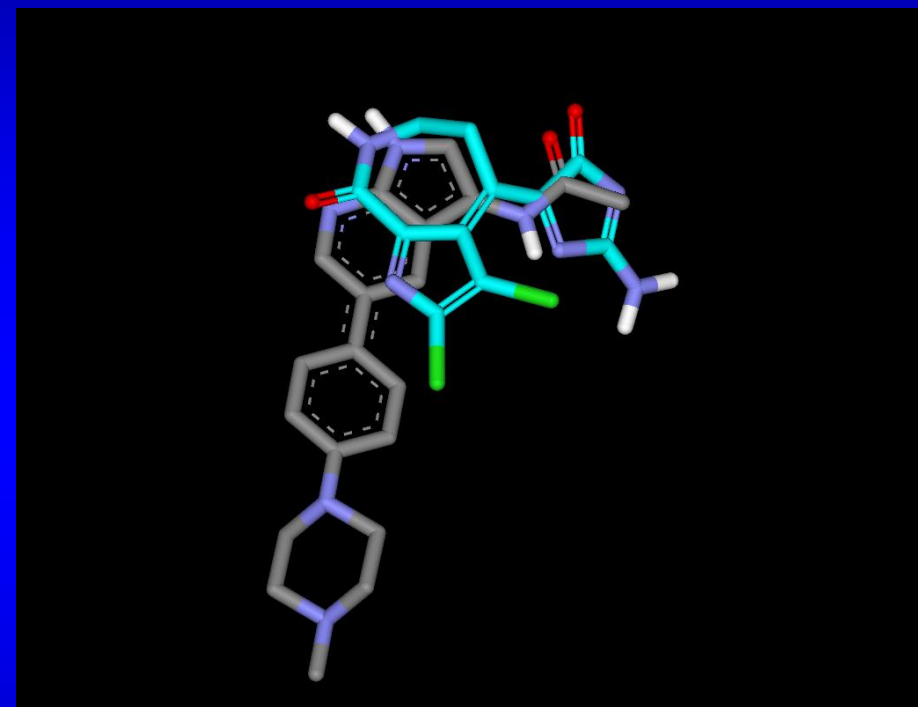
DeNovo – Beyond validation are ideas

- 1st ranked result from CHEK1 run (1zlt)
- Pyrrolopyridinyl-benzimidazole scaffold not found in Scifinder (July 08)



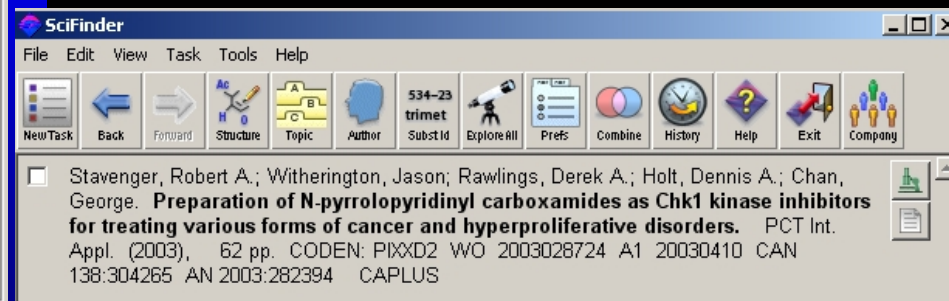
DeNovo – Beyond validation are ideas

- 8th ranked result from CHEK1 (1zlt)
- Pyrrolopyridinyl scaffold found in Scifinder



507463-48-1
~1 Reference
REGISTRY

507463-38-9
~2 References
REGISTRY



SciFinder
File Edit View Task Tools Help
NewTask Back Forward Structure Topic Author 534-23 trimet Subst Id Explore All Prefs Combine History Help Exit Company

Stavenger, Robert A.; Witherington, Jason; Rawlings, Derek A.; Holt, Dennis A.; Chan, George. **Preparation of N-pyrrolopyridinyl carboxamides as Chk1 kinase inhibitors for treating various forms of cancer and hyperproliferative disorders.** PCT Int. Appl. (2003), 62 pp. CODEN: PIXXD2 WO 2003028724 A1 20030410 CAN 138:304265 AN 2003:282394 CAPLUS



FlexNovo – Retrospective Validation

- **Summary**

1. be able to reconstruct what is known to bind (ref. Ligand) from its own fragment space
2. find it within the first five best ranked
3. find it within a fixed RMSD (same binding mode)
4. find it within a reasonable complexity
5. show that other results could be useful in idea generation process

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