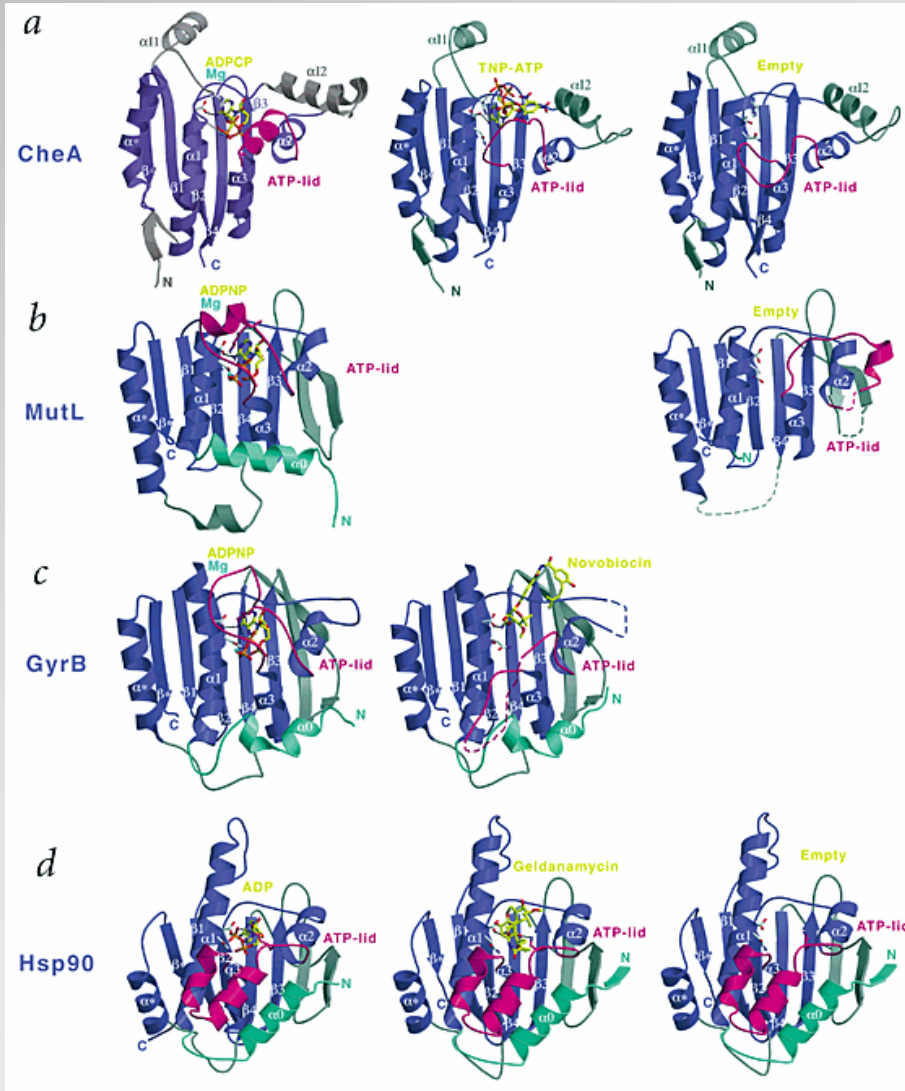


***Drug Discovery Targeting the GHKL Superfamily
- HSP90, DNA Gyrase, Histidine Kinases***

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The GHKL Superfamily Proteins



GHKL:

G – DNA Gyrase

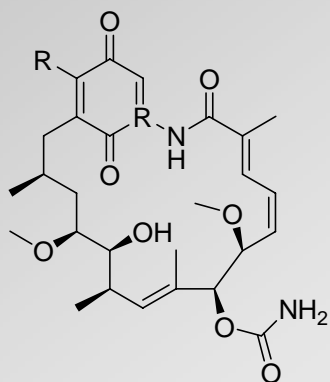
H – HSF90

K – Histidine Kinases

L – DNA Mismatch Repair Enzyme MutL

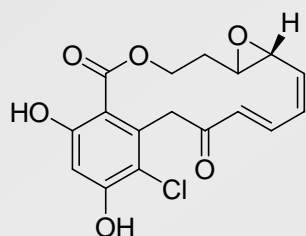
This structural superfamily has as its defining feature a novel ATP binding domain, whose adenine binding pocket is both conserved within the family and distinct from those of human protein kinases. The superfamily has as members a number of established and emerging drug targets, including bacterial histidine kinases, bacterial Gyrase B, and human HSP-90.

Known Inhibitors Against GHKL Members

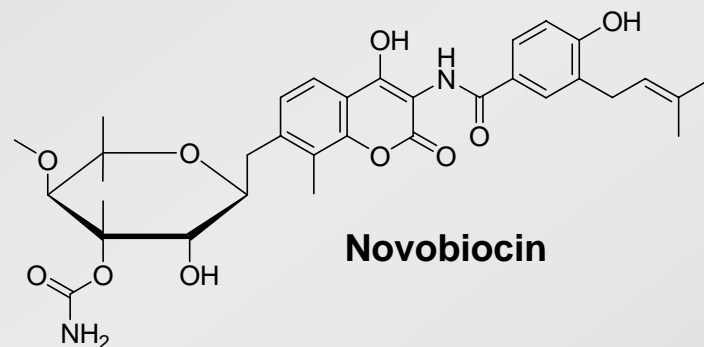


**Geldanamycin
17-AAG**

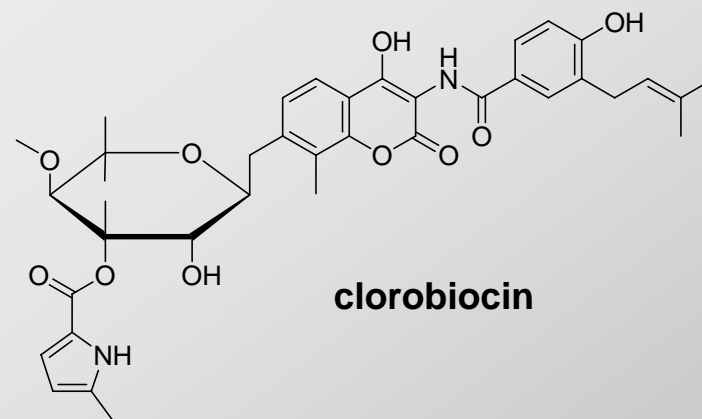
R =
-OCH₃
-NHCH₂CH=CH₂



Radicicol



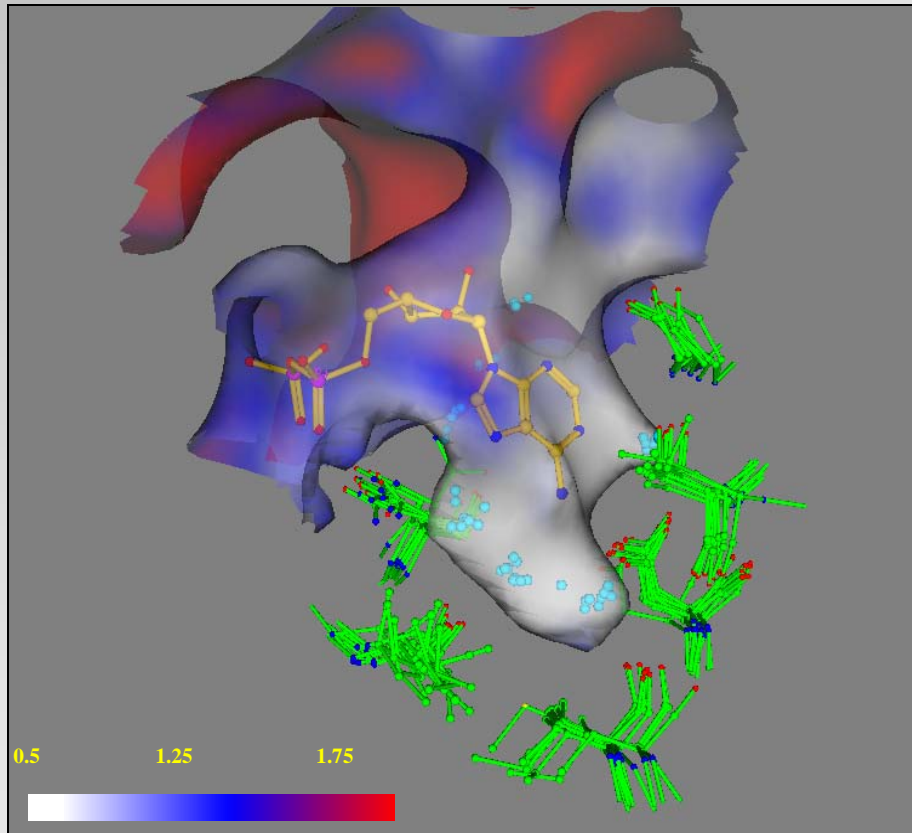
Novobiocin



clorobiocin

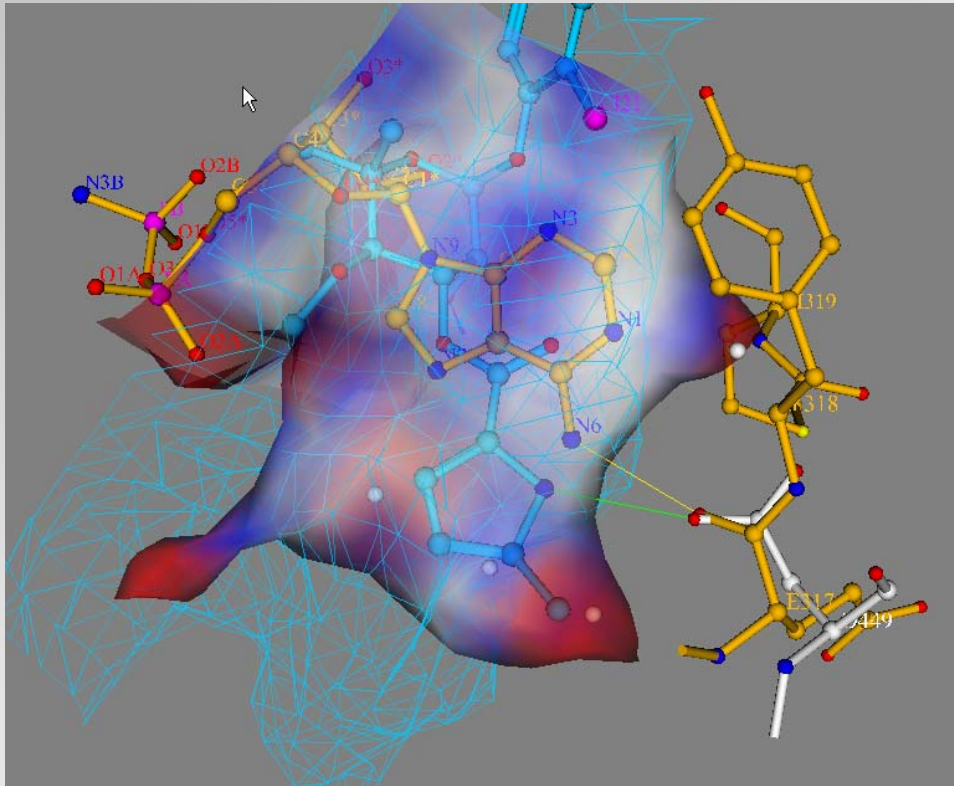
Coumarins

The GHKL Adenine Binding Pocket



1. The six key residues (green) line the adenine pocket, and are well conserved both in sequences and in 3D structures;
2. These residues coordinate the structurally conserved waters in the superfamily (blue spheres).
3. Surface of a representative member is shown, color-coded by the averaged distance difference for each surface point.

Specificity Against Human Ser, Tyr Kinases



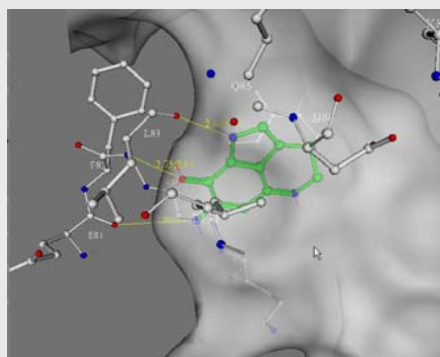
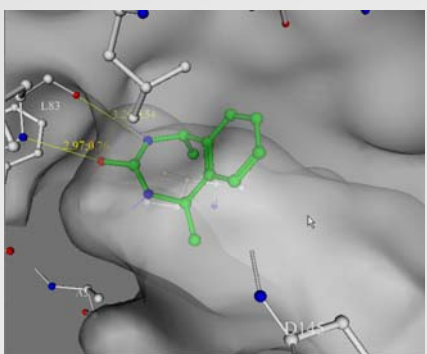
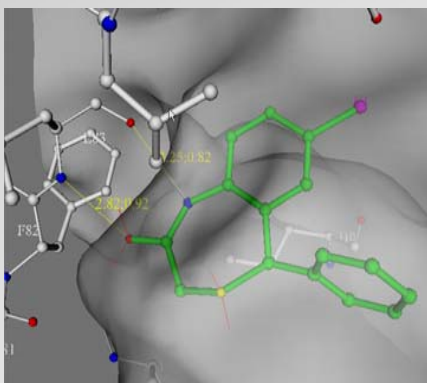
1. The conserved adenine-binding pocket within GHKL superfamily (CheA, solid) shows distinct features from those of serine/threonine and tyrosine kinases (LCK, net) and offers opportunities to achieve specificity.
2. Clorobiocin (blue) binds to GyrB with an affinity at least 10000 times higher than that of ATP.
3. Clorobiocin hits a "specificity" pocket (red).

Fragment-Based Drug Discovery

We have constructed a Lipinsky “Rule of 3” lead-like library of low-molecular-weight compounds that can be docked and subjected to footprint analysis to identify novel scaffolds for development into leads and libraries.

Rule of 3 library:

MW < 300; HBd ≤ 3; HBa ≤ 3; RotB ≤ 5; LogP ≤ 3;	60,006
MW < 300; HBd ≤ 3; HBa ≤ 3; RotB ≤ 3; LogP ≤ 3; Ring# = 1,2	30,901
MW < 250; HBd ≤ 3; HBa ≤ 3; RotB ≤ 3; LogP ≤ 3; Ring# = 1,2	23,179

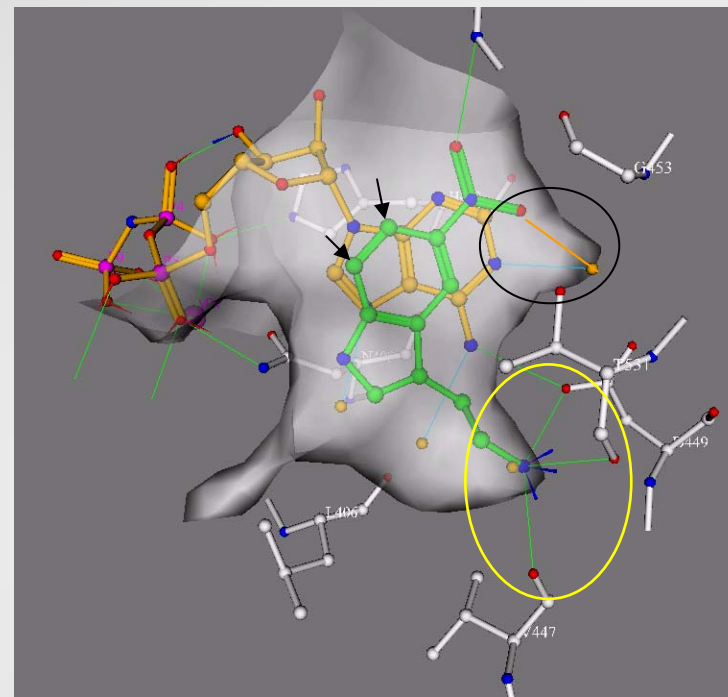
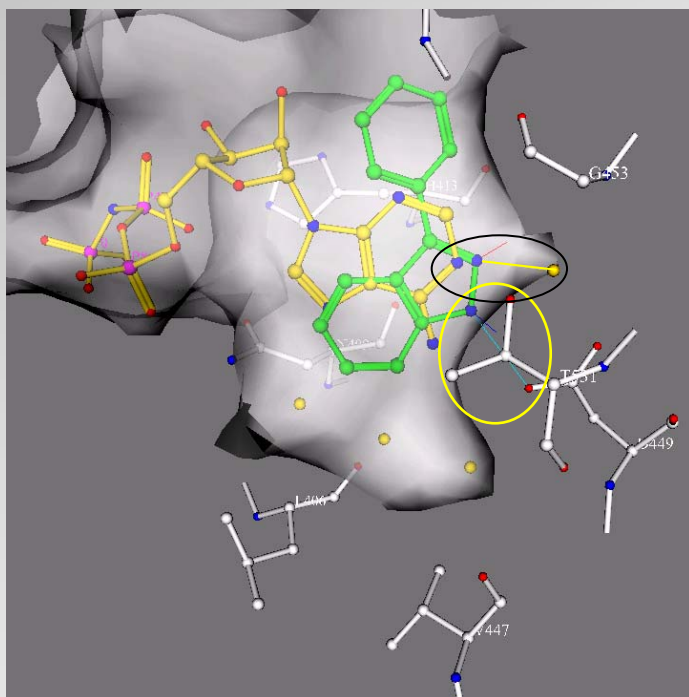


Scaffold candidates are selected as:

- appropriate to combichem
- novel to the literature

Teague, SJ. *The Design of Leadlike Combinatorial Libraries. Angew. Chem. Int. Ed.* 1999, 38, 3743

The Fragment-based Approach

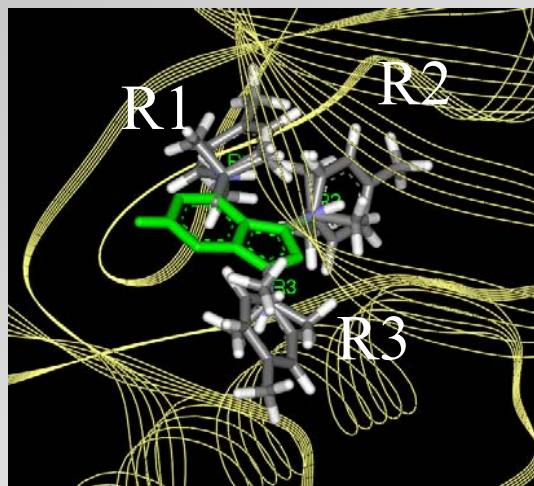


1. One of the known compounds (green, left figure) from *Boehm et al., 2000* fits the pocket of CheA well. The co-crystallized ANP (yellow) was shown as reference.
2. One example molecule (green, right figure) from our fragment-based library was shown to fit into the pocket and maintain the key interactions with CheA (right figure);
3. R-groups attached at black arrow positions can probe the portions of the active site more distal to the adenine binding site, to achieve enhanced affinities and specificities.

Boehm H-J et al., Novel Inhibitors of DNA Gyrase: 3D Structure Based Biased Needle Screening, Hit Validation by Biophysical Methods, and 3D Guided Optimization. A Promising Alternative to Random Screening. J. Med. Chem. 2000, 43, 2664-2674.

The Fragment-based Approach

– Library Construction

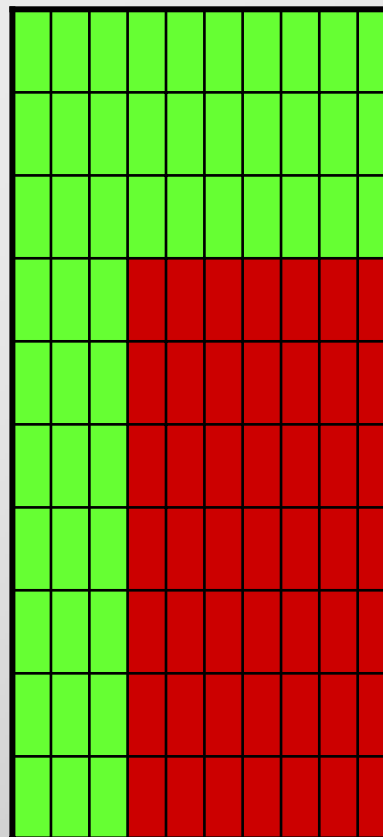


Structure-based R group selection:

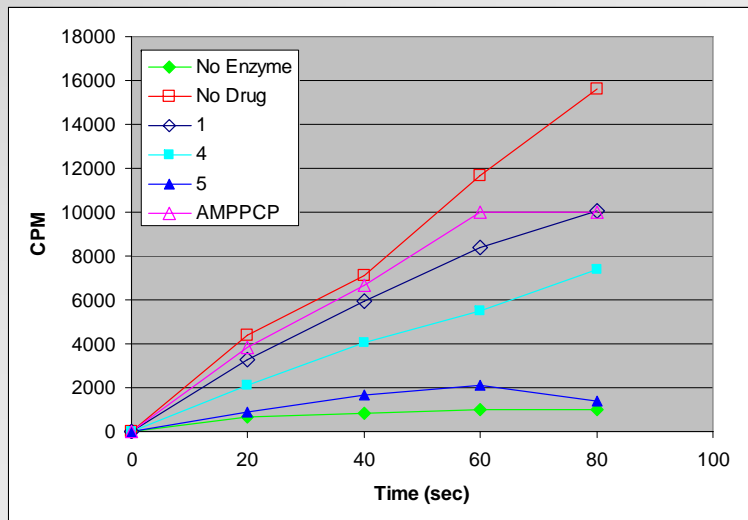
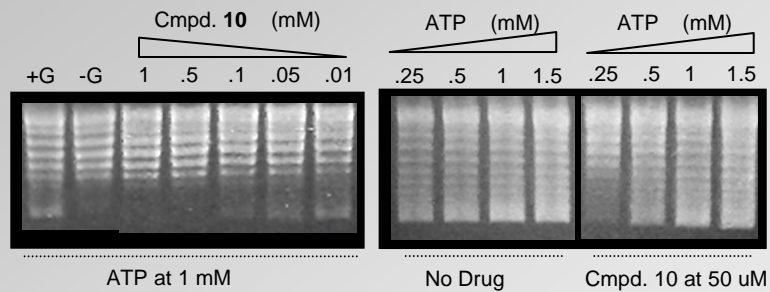
1. Scaffold held fixed;
2. R groups are “sprouted” one at a time and scored;
3. Selected high scoring R-groups are retained to comprise the library;
4. Entire library is re-docked, re-scored, refined.

Results:

1. A 100 X 100 X 100 (1 million) library turns into a 10 X 10 X 10 (1000) library;
2. There is still significant redundancy in a 10 X 10 X 10 library in terms of SAR exploration, *if the R groups are relatively independent of each other. On this account, only 216 compounds need to be synthesized.*

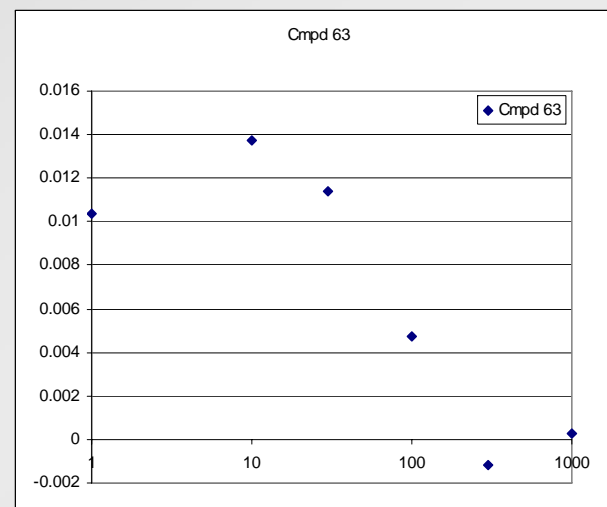
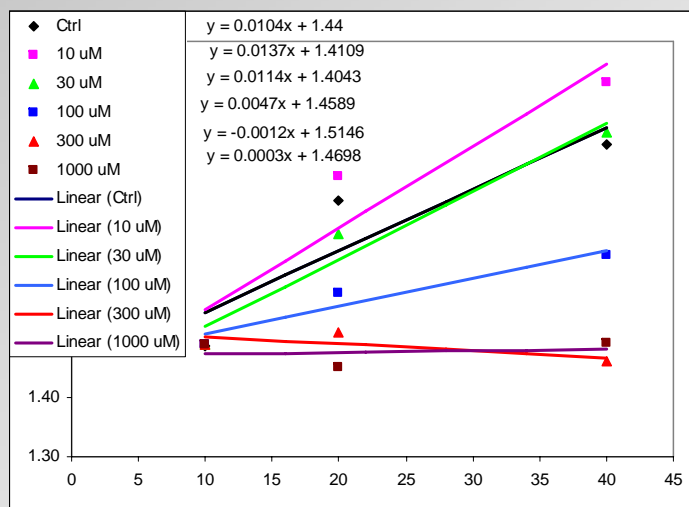


Work is Ongoing With Promising Results



1. Out of the first tested 50 compounds in a GyrB supercoiling assay, 12 showed inhibition at 1 mM concentration, with 3 of them completely inhibited the supercoiling of the DNA at 100 uM (ATP binds at ~500 uM).
2. 5 compounds showed a similar transition pattern as AMPPCP – a non-hydrolyzable ATP analog, suggesting a binding mode that is competitive with ATP.
3. 8 out of 12 showed inhibition comparable or better than AMPPCP in a secondary CheA phosphorylation assay. 4 of them belong to the 5 ATP competitive hits from the primary assay.

Compound BP_0063 in ATPase Assay of Gyrase B



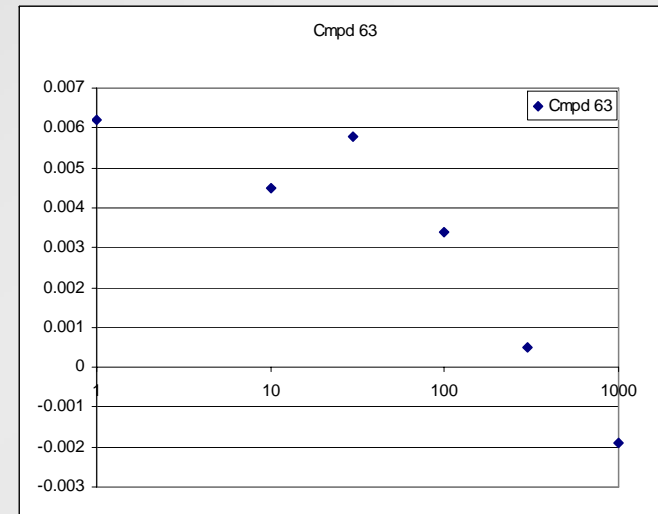
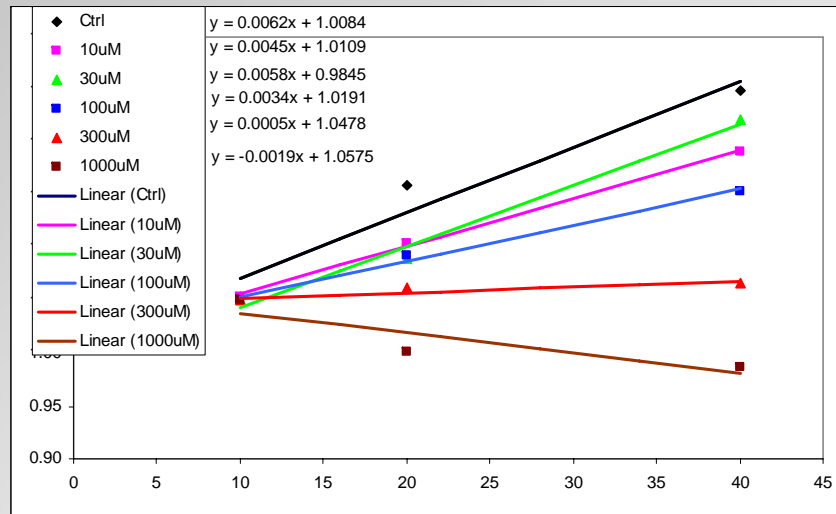
1. About 100 compounds were selected and subjected to an ATPase assay of Gyrase B in the second round. The active hits from a single concentration screening were further subjected to an IC50 measurement of Gyrase B;
2. Results for one of the representatives were shown above. The ATP hydrolysis rates were measured in the presence of Compound BP_0063 in concentrations of 10, 30, 100, 300 and 1000 uM.
3. The increasing inhibition was observed with the increasing concentrations of the compound, with an IC50 of ~50uM. MW of the compound is 286. CLogP is ~3.

Compound Screening in ATPase Assay of Gyrase B

Classes	# of Compounds Screened	# of Hits from GyrB Screening	# of Hits Confirmed by GyrB IC50 Measurements
0	6	1	1
1	10	2	2
2	9	3	2
3	6	3	1
4	5	1	0
5	7	4	3
6	21	9	5
7	12	5	2
8	17	6	2
Total	93	34	18

1. 34 out of 93 (36.6%) screened compounds for the second round showed inhibition against Gyrase B at 1 mM;
2. 18 out of 93 (19.4%) screened compounds were confirmed in the IC50 measurements against Gyrase B;
3. The best compounds showed IC50's around 50 – 100 μ M. The molecular weights are between 180 to 300, and the CLogP's are around 3 or lower.

Compound BP_0063 in ATPase Assay of HSP90



1. All 18 confirmed hit molecules in the Gyrase B assays are being subjected to IC50 measurements based on an ATPase assay of HSP90.
2. Results for one of the representatives were shown above. The ATP hydrolysis rates were measured in the presence of Compound BP_0063 in concentrations of 10, 30, 100, 300 and 1000 uM.
3. The increasing inhibition was observed with the increasing concentrations of the compound, with an IC50 of ~100-200 uM. MW of the compound is 286. CLogP is ~3.

Summary and Ongoing Plans

1. Structures of the ATP binding pockets of the GHKL super family have been carefully analyzed, using all the available crystal structures in the family plus the known inhibitor information of the family;
2. A hypothesis of the ATP binding pocket was constructed based on the above information, including the key site interactions between the potential inhibitors and the family proteins;
3. A rule-of-three lead-like compound library was built and screened *in silico* to identify potential inhibitors;
4. 93 compounds, a small and highly enriched but versatile selected set, include 9 classes of molecules based on their key structural elements that fit to our hypothesis;
5. After two rounds of ATPase assays against Gyrase B, 18 out of 93 compounds showed active IC₅₀'s, with the best ones around 50 – 100 μ M;
6. Ongoing ATPase assays against HSP90 demonstrated that compounds from the 18 hits against Gyrase B also inhibit ATP hydrolysis of HSP90, suggesting they hitting the common ATP binding pocket;
7. Library designs are underway based on the best hit molecules served as the scaffolds from the above assays. The extended library molecules will further increase the affinities and fine tune specificity.