Hit Search for ATPase Inhibitors of the GyrB-Subunit

Moritz Münsterberg, Iris Fransson, and Thomas Lemcke
University of Hamburg, Institute of Pharmacy, Bundesstr. 45, 20146 Hamburg, Germany

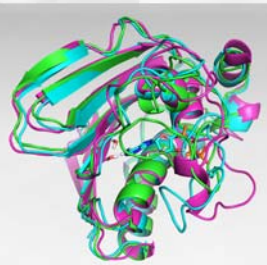
Introduction

Bacteria are a growing thread for mankind. The development of new drugs is essential for the future treatment of bacterial infection [1]. Nonetheless, the development of new and superior antibacterial is neglected [2]. Gyrase is a well-known target for the treatment of bacterial infections. It is an ATP-dependent type II topoisomerase, which consists of four subunits (two gyrA, two gyrB). Fluoroquinolones are clinical used inhibitors of the gyrase-DNA-complex. Besides this mechanism of action there are known inhibitors of the ATPase in gyrB, e. g. the aminocoumarines like novobiocin. Currently, no drugs, which act as gyrB ATPase inhibitors, are used in clinical therapy [3].

We have performed a structural based hit search for new inhibitors of the ATPase gyrB-subdomain: target optimization, library generation, virtual screening, post-processing, acquisition, biological testing and evaluation.

Target Optimization

Three x-ray crystal structures of the Protein Data Bank [4] were chosen, which cover different conformations of the binding site. The chosen complexes contain ANP (PDB-code: 1EI1) [5], novobiocin (1AJ6) [6] and a pyrazolylthiazol derivative (3G7E) [7]. The structures were prepared with the Protein Preparation Wizard of Schrödinger and mutation and missing loops were fixed [8]. We identified two important water molecules in the crystal structure: One water molecule bound to Asp73 (*E. coli* numbering), which should be conserved in every structure and a second water molecule, which might be conserved in 3G7E. We decided to use four structures: 1EI1, 1AJ6 and 3G7E with one water molecule and another 3G7E structure with two water molecules.



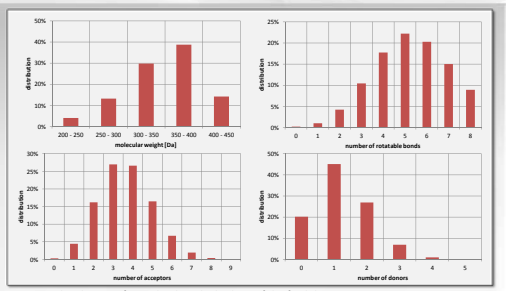
ATPase Domain of GyrB: 1EI1 with ANP (cyan), 1AJ6 (magenta), 3G7E (green) (created with PyMol [9])

Library Generation

We started with the "Clean-Drug-Like" ZINC-subset [10] (11 million compounds) and optimized the filter criteria with Mona [11] to the following rules:

- 200 – 450 Da molecular weight
- ≤ 8 rotatable bonds
- ≥ 1ring system
- 0 chiral centers; 0 E/Z-isomers

The final dataset was reduced to 3.8 million compounds.

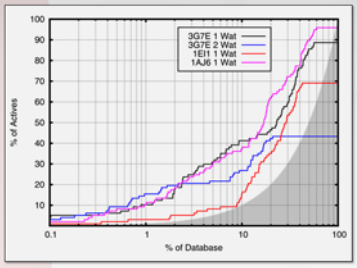


Property distribution of a randomly picked subset of the final dataset

$11 \cdot 10^6$ $3.8 \cdot 10^6$ $360{,}0$ 20

Virtual Screening

The virtual screening was performed with the recently developed high-throughput virtual screening tool TrixX [12]. The dataset was reduced in two docking steps with increasing accuracy to 360,000 compounds.

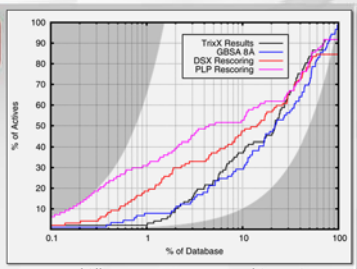


Enrichment of final screening

Post-Processing

We optimized the enrichment with a manual generated DUD-E dataset [13]:

1. rescoring of all poses with PLP-Score [14]
2. leader clustering of all poses with Daylight-Fingerprints (tanimoto coefficient 0.2) [15]
3. redocking with Glide SP-Method [16] of the top 1000 cluster leader
4. visual inspection of poses



Comparison of different optimization processes of the test dataset

Acquisition: 20 Compounds

Conclusion

We performed a complete, rational based hit search for new inhibitors of the ATPase domain of the gyrase.

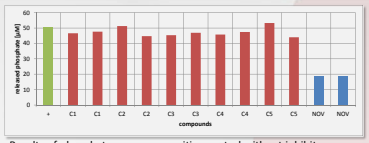
However, none of our so far purchased compounds showed significant inhibition at 100 µM.

A possible reasons may be the very small number of tested compounds. Additionally, the binding modes of the chosen compounds have to be reviewed critically.

To fully elaborate the hits, higher concentrations of the purchased compounds will be tested.

Results

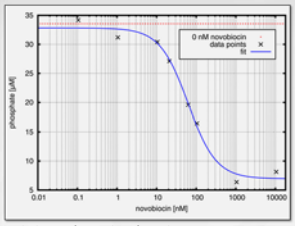
None of the purchased compounds showed significant inhibition at an 100 µM level. Additional hit compounds will be selected for further biological evaluation and repetitive assays are still necessary to consolidate the results.



Results of phosphate assay – +: positive control without inhibitor; C1-5: tested compounds; NOV: novobiocin

Biological Testing

Free inorganic phosphate was determined with an improved phosphomolybdate malachite green complex method [17-18]. For conformation of the hits we utilized a supercoiling assay as orthogonal test system.



Calculation of IC_{50}-value of Novobiocin: 68 ± 9 nM (literature: 0.08 µM [19])

supercoiling assay – S: substrate (relaxed DNA plasmid); +: positive control without inhibitor; C1-3: tested compounds; NOV: novobiocin

References

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