STRUCTURE BASED DESIGN OF NEW THIENO[2,3-*B*]PYRIDINES AS SELECTIVE PLASMODIAL GLYCOGEN SYNTHASE KINASE-3 (*Pf*GSK-3) INHIBITORS USING MOLECULAR DYNAMICS SIMULATIONS AND MM/GBSA-CALCULATIONS

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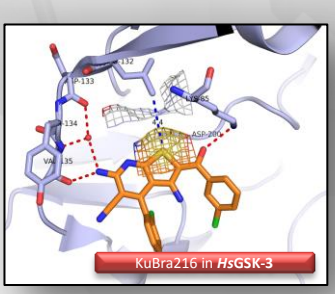
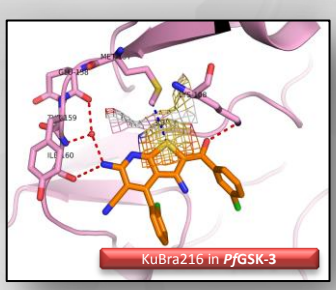
Introduction

Malaria is one of the most widespread infectious diseases besides HIV and Tuberculosis: In 2012 207 million infected patients have been registered of which 627.000 died [1]. The most serious form of malaria, *Malaria tropica*, is caused by *Plasmodium falciparum*. There are effective malaria therapeutics on the market, however rapid emergence of resistance and high costs necessitate the development of new drugs [2]. In a screening campaign a thieno[2,3-*b*]pyridine derivative has been identified as a potential lead structure to inhibit the plasmodial Glycogen Synthase Kinase 3 (*Pf*GSK-3) [3] and a small library of derivatives has been synthesized [3]. A homology model of the *Pf*GSK-3 [4] was used for Docking- and 3D-QSAR-studies [3] as well as molecular dynamics simulations and MM/GBSA calculations of the respective protein-ligand-complexes considering pre-optimized calculation parameters [5] to clarify the binding mode [3]. As a result the GBSA energies could be correlated to the biologically measured IC_{50} values of the individual compounds for complexes with binding mode A [6].
This was used as a starting point for further structure based design modifications. Following a thorough binding site/mode analysis, we generated 42 new thieno[2,3-*b*]pyridine derivatives and prioritized them via MD simulations and MM/GBSA calculations to identify promising synthesis candidates. Finally some of the top-ranked ligands were successfully synthesized and will be tested for antimalarial activity against *Pf*GSK-3.

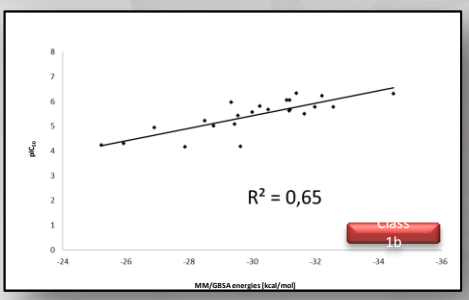
The binding mode A



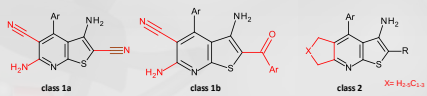
Figures show the best scoring docking poses in the *Hs*GSK-3 (left) and the *Pf*GSK-3 (right) for KuBra216 (most active & selective inhibitor). Both complexes show similar hydrogen bonding contacts to the Hinge region, the water and to this important lysine deep in the binding site. There are two key points to explain the selectivity. The first is with the gatekeeper sidechain. While in *Hs*GSK-3 it is a relative small leucine, it is a more bulky methionine in *Pf*GSK-3. Due to the size of the gatekeepers side chain and the way the ligand is fixed in the binding site only the methionine with a distance of 3.3 Å towards the sulfur of the ligand has the ability to assume favorable Van-der-Waals interactions [3].
The second key point is the position of the halogen substituent on the aryl ring. It points downwards to a shallow groove at the bottom of the binding site. This is the region were a positive molecular interaction field for Iodine was identified in *Pf*GSK-3 and not in the human structure [4].



Scoring



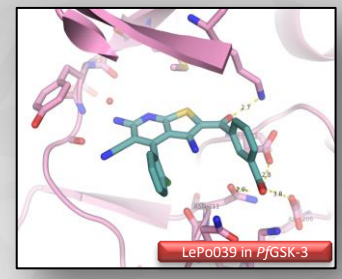
The binding mode A poses of 34 thieno[2,3-*b*]pyridines (dividable into three classes, see figure below) were used for the ongoing procedure. 1 ns MD simulations with periodic boundary conditions were carried out as production runs on each system in an NPT-ensemble (300 K, 1 atm). The MM/GBSA energies were calculated using *AMBER11* [7]. For GBSA calculations only MD simulations were selected that were consistent with pre-defined stability-criteria (e.g. H-bonds, rmsd etc).
The GBSA energies could be correlated to the biologically measured IC_{50} values of the individual compounds (Results for selective class 1b shown see figure).

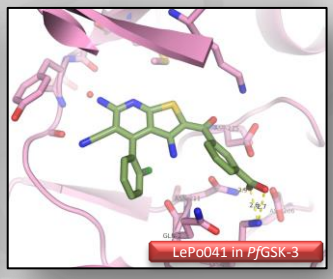


Structure-based design

We focused the design process on the aroyl substitution of the class 1b compounds due to potential new interaction points we found by a binding site analysis. Mode A poses of 42 different new and 3 known thieno[2,3-*b*]pyridines for control purpose were ranked via MD simulations and MM/GBSA calculations using previously described parameters. MM/GBSA energies of the top-ranked new thieno[2,3-*b*]pyridines see table below. We successfully synthesized two derivatives which were interesting for several reasons. (see box below and figures).

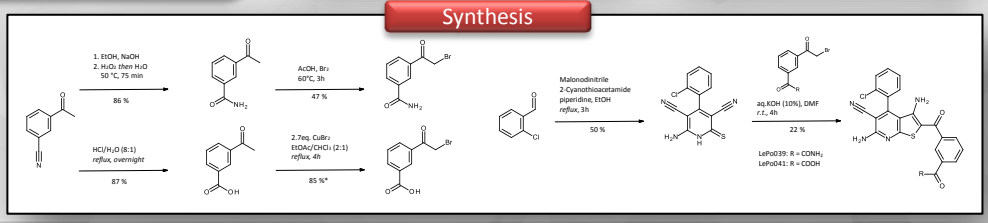
- MM/GBSA energies promising compared to KuBra216
- Carboxylate and amide functionality add new hydrogen bond
  -> INCREASE IN ACTIVITY
- New interactions work as anchor groups to stabilize pose and enhance gatekeeper-interaction
  -> INCREASE IN SELECTIVITY





Name	Aroyl-substitution	MM/GBSA [kcal/mol]
LePo041	*m*-carboxylate	-41.0836
LePo039	*m*-amide	-37.4584
LePo042	*m*-sulfonate	-37.3445
LePo034	*p*-nitro	-35.0632
KuBra216	*m*-chloro	-31.4006
LePo033	*m*-nitro	-31.2565

Synthesis



Conclusion & Outlook

- Design process resulted in at least five new thieno[2,3-*b*]pyridines with increased MM/GBSA energies compared to KuBra216 by adding new interactions
- Increased activity & selectivity proposed due to added interactions
- Two top-ranked derivatives were successfully synthesized
- Biological testing is yet to be done
- Further design & synthesis of top-ranked ligands

References

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