Fragment-based Discovery Aiming at a Novel Modulation of Malate Dehydrogenase and Beyond

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Chapter 6

Summary and Future Perspectives

The validation of a target represents the first step in the early-stage drug discovery pipeline. In the specific case of antimalarial research, the protein interference assay represents a solid approach to the validation of an entire metabolic pathway, as exemplified by the study cases of *Pf*AspAt/ *Pf*MDH [1] and *Pf*ATC [2]. The primary results of this thesis provide further experimental evidence of an allosteric modulation of a class of enzymes at the oligomeric interface in a fragment-based fashion. This result represents a possible strategy for the development of initial molecular candidates against malaria. Accordingly, **Chapter 2** illustrates the crystallographic structure of *Pf*MDH bound to 4-(3,4-difluorophenyl) thiazol-2-amine. Additionally, the low sequence identity of the aminoacids located at the oligomeric surfaces suggests that subunits of *Pf*MDH assembles themselves in a distinctive manner compared to other causative agents of diseases like *Leishmania Major* or *Brucella Abortus*. These data suggest that the same approach might be reproduced in these microorganisms. 4-(3,4-difluorophenyl) thiazol-2-amine results from a high-throughput screening of 1500 fragments by STD – NMR, followed by an orthogonal validation by microscale thermophoresis and differential scanning fluorimetry. With a measured K_D of 99.0 ± 1.7 μM and a ΔT_m of -2 °C, the molecule binds and weakly destabilized *Pf*MDH. Nevertheless, a significant inhibition of the malate oxidation has been observed in the activity assay. The discovery of a distal bonding site from the orthostatic pocket represents a significant step forward towards the *in vitro* modulation of this class of enzymes and a unique opportunity to develop the compound into more potent leads.

Alternatively, *Pf*AspAt and *Pf*MDH offer a valuable application for macrocycles that specifically bind their oligomeric interfaces as both proteins have large interface loop structures meditating protein-protein interactions [3]. With more than 100 macrocycles in clinical market or in drug discovery pipelines (**Figure 2**), these molecules describe a mature field of chemistry [4] with surprisingly underexplored applications only in oncology and antibiotics [4]. In **Chapter 3** the software Moloc has been benchmarked. Without producing thousands of conformational ensembles or random ring splitting into two half-loops, this software has processed three-quarters of a database of 208 molecules with high accuracy of to the ring atoms. The time taken to complete a job in a laptop is acceptable (median: 39 minutes). Moreover, the structural diversity of the conformational ensembles is statistically similar to three commercial software tested, namely Prime, MacroModel and Molecular Dynamics. Our contribution in improving the conformational analysis of macrocycles is exemplified by a script for the automatic calculation of accuracy of the macrocycle structural components (ring or whole molecule), diversity (torsional fingerprints and radius of gyration),

number of conformation and the relative energy after minimization. However, macrocycles with branched side chains still represent still a limitation for Moloc because the algorithm deprecates the atomic coordinates in beta position from the ring network. We are planning to implement a second module considering the flexibility of complex side chains in the current version of Moloc in order to improve the all-atom accuracy.

In **Chapter 4** we shifted our focus on a solid methodology for the synthesis of highly substituted acrylamides as a covalent warhead. In this chapter, we designed an MCR route where reactions were performed on a 0.5 mmol scale in 96-well plates, on a nano scale in an automated fashion in 386-well plates yielding 1.536 reactions and also one example has been formed on a 10g scale. Thus, scalability has been established over 6 orders of magnitude. Finally, in order to underscore the usefulness and applicability of our libraries we have screened for covalent inhibitors against the enzyme protein tyrosine phosphatase 1B. Several compounds have been found to inhibit the enzymatic activity of PTP1B, a major negative regulator of both insulin and leptin signaling and approved drug target in the treatment of obesity and type 2 diabetes [5,6]. The reproducibility of two of the most active compounds has been assessed by a colorimetric assay. Mass spectrometry experiments, followed by trypsin digestion, have confirmed the presence of a covalent bond with the solvent exposed cysteines, including the catalytic Cys215 and the allosteric Cys121 [7]. Conformational modifications of WPD-loop, a highly conserved loop among phosphatase and P-loop that stabilize the in-transition state of the substrate have been elucidate by post-docking minimization. These results represent an interesting addition to the current state of the art since some of the most potent and selective inhibitors of PTP1B contain a negative charge on the phosphate, sulfate, carboxylate or selenate moieties [8-11], thus limiting the passive membrane permeability.

The last part the thesis focuses on a study case of nitrile containing compounds. **Chapter 5** fits into the context of the recent COVID-19 outbreak, showing a virtual screening protocol for gliptins repurposing. Gliptins are a class of oral hypoglycemics that inhibit the enzyme dipeptidyl peptidase-4 (DPP-4), a serine protease that rapidly inactivates incretin hormones in plasma. They are widely used as drugs to treat diabetes mellitus type 2. The first compound – sitagliptin – received market approval in 2006. In this chapter the rational inhibition of 3C-like proteinase (3CLpro) and Papain-like proteinase (PLpro) is presented using two software and the most relevant conformational changes are disclosed by post minimization. We have demonstrated that the docking scores of Vildagliptin, Anagliptin and Denagliptin

were preserved and in some cases even enhanced, suggesting that the binding of these gliptins could induce and adjust the fit which potentiates the binding and the stabilization of these compounds into the catalytic site of 3CLpro. Therefore, these gliptins might have a direct impact on the protease 3CLpro function, and they could consequently impact the replication cycle of SARS-CoV-2.

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