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Expanding the toolbox of protein-templated reactions for early drug discovery

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Summary and Outlook

Despite recent developments in technology and techniques in today's drug discovery, accessing new bioactive compounds is still challenging. It is of utmost importance both for medicinal chemists as well as pharmaceutical companies to accelerate this lengthy trajectory. In this thesis, we aimed to establish novel strategies to facilitate faster and more efficient hit identification, the early stage of the drug-discovery process. In order to achieve this goal, we used target-guided synthesis (TGS) in which the biological target, in our case a protein, templates the formation of its own inhibitors from a pool of complementary building blocks. This efficient technique accelerates the drug-discovery process, namely hit-identification/optimization, enabling the selection of the best binders without prior synthesis, purification and biochemical evaluation of each individual compound.

In Chapter 1, we discuss the difficulties in today's drug discovery and how this trajectory could be improved and accelerated by using the TGS approach. Firstly, we give an overall introduction to the drug-discovery process and fragment-based drug design (FBDD). Secondly, we focus on target-guided synthesis in a broad perspective, subsequently kinetic-target guided synthesis (KTGS) in detail. KTGS is a type of TGS approach in which the assembly of the inhibitors takes place in an irreversible manner. This elegant approach has found numerous applications in drug discovery, especially in hit-identification. However, it is still premature in terms of the target scope and the number of compatible reactions. In this chapter, we present a detailed literature overview, the reaction and the target scope as well as some practical aspects in conducting KTGS experiments.

In Chapter 2, we demonstrate a strategic combination of two known techniques, protein-templated click chemistry (PTTC) and FBDD, to facilitate hit-identification. FBDD starts with the identification of the fragments, which are optimized in two ways: fragment growing and fragment linking. Fragment-growing is studied in more detail in the literature, whereas fragment-linking is rarely reported owing to the challenge in identifying a linker with optimal fit. Therefore, we aim to tackle this challenge as it is very attractive because of the potential for super-additivity. To overcome this hurdle, we used KTGS, namely PTCC, to link/optimize two fragments to afford inhibitors of endothiapepsin. We generated a library of azides and alkynes derived from fragments identified as inhibitors of endothiapepsin and selected the best combination by using the target itself in catalytic amount. We used the UPLC-TOF-SIM method to identify four of the 36 possible triazole combinations as the best binders. The hits identified have IC_{50} values of 43–121 μM. This method represents a successful example of fragment-linking facilitated by PTCC, affording the first triazole inhibitor of endothiapepsin. As it is a proof-of-principle work and the target is a model enzyme, we did not perform further rounds of optimization. A next

development of this method should be the use of real drug targets with iterative rounds of optimization.

In Chapter 3, we present a novel protein-templated reaction, the in situ Ugi-four component reaction (Ugi-4CR), to facilitate hit identification by opening up access to a larger part of chemical space. By using a protein-templated multicomponent reaction for the first time, we screened a library of complex molecules derived from two multicomponent reactions (Ugi-4CR and Passerini) by using a catalytic amount of protein in a very short time. This new protein-templated reaction goes beyond PTCC given that it enables simultaneous screening of four subpockets and provides access to the hits identified in one synthetic step. To demonstrate this concept, we generated a library of building blocks comprised of aldehydes, amines, carboxylic acids and isocyanides and used endothiapepsin in situ, enabling the selection of two hits with low micromolar activity (IC_{50}= 1.3 and 3.5 μM) in 18 h. Thanks to the use of the Ugi-4CR, the inhibitors identified can be optimized in a straightforward manner. As it is the first example in the field, the mechanism should be studied in detail and the target scope and the number of multicomponent reactions in the KTGS toolbox should be expanded.

In Chapter 4, we demonstrate the applicability of KTGS in targeting protein-protein interactions (PPIs) as well as flexible binding pockets by using a new reaction in KTGS. By using a protein-templated reductive amination, we screened a library of potential inhibitors of the p53-Mdm2 PPI and identified one hit showing a K_i value of 0.76 μM. Mdm2 has a flexible pocket, the Leu26 pocket, which is enlarged upon ligand binding, making it difficult to target by using structure-based drug design (SBBD) or other computational techniques such as virtual screening. Imine chemistry has found applications in the dynamic combinatorial chemistry (DCC) context and freezing the equilibrium was mostly achieved by in situ reduction with the risk of loss in affinity. In our work, we used reductive amination reaction in a KTGS setting by using a reducing agent from the beginning, making the reaction irreversible. The disadvantage of using this technique is the difficulty to compare activities of the amines after reduction since the selection takes place at the imine stage. Therefore, future focus in this reaction should be on the synthesis of the mimic of the imine scaffold formed in the protein-templated reaction.

In Chapter 5, we disclose our attempts to use an esterification reaction for the first time in KTGS. In this work, we used the model enzyme endothiapepsin and generated a library of building blocks bearing alcohol and acid/ester functional groups. Our first attempts afforded the templated formation of an ester inhibitor from a carboxylic acid and alcohol building blocks. Starting from the optimized conditions for the library reaction did not afford the assembly of any products under various conditions. There are many drawbacks in using esterification reactions in

this context. Firstly, hydrolysis of the esters may occur in the reaction buffer, which should be monitored carefully for the time duration needed by the templated-reaction. Another important point is the use of a protease as a model enzyme. As this family of enzymes is responsible for hydrolysis, another target family may be a better option for further optimization studies. In addition, fragment ligation screening methods could be more suitable method to identify the best binders.

In Chapter 6, we describe the design, synthesis and biochemical evaluation of bioisosteres of the acylhydazone inhibitor (IC_{50}=12 $\pm$ 0.4 µM), which was developed in our group previously and used as a parent inhibitor for chapters 3 and 5. We synthesized three new compounds and evaluated their inhibitory activity against endothiapepsin, showing that the best bioisostere inhibits endothiapepsin with an IC_{50} value of 27 $\pm$ 8 µM. We resolved the cocrystal structure of one bioisostere in complex with endothiapepsin. Therefore, we could demonstrate replacements for the sensitive and hydrolyzable acylhydrazone linker without a significant loss in activity, an important point for future drug development.

To conclude, KTGS is a very successful technique, which facilitates cheaper, faster and more efficient hit identification. The primary goal of the thesis is the expansion of the toolbox of protein-templated reactions in terms of reaction and target scope. In this thesis, we introduced three novel reactions, namely situ Ugi-4CR, reductive amination and esterification. In addition, we presented an efficient combination of techniques, fragment-linking and PTCC, which benefits from the advantages of both drug-discovery techniques and finally the design, synthesis and biochemical evaluation of stable bioisosteres of acylhydrazones as inhibitors of endothiapepsin. Morever, we used Mdmd2 for the first time as a target in KTGS, and demonstrated the benefit for exploring flexible binding pockets. We strongly believe that the methods that we developed could find many applications in hit-identification as well as in later stages, namely hit/lead optimization until the pre-clinical stage.