New applications of dynamic combinatorial chemistry to medicinal chemistry

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Summary & Perspectives
7.1 Context and scope of this thesis
Applying dynamic combinatorial chemistry (DCC) to medicinal chemistry projects can be a helpful strategy for finding starting points in the drug-discovery process. Especially, when information of the target is known, structure-based or fragment-based drug design approaches combined with DCC could lead to potent compounds. As a first aim of this thesis, we have applied DCC on a number of biologically relevant targets, Chapters 3 and 4.

As relevant drug target, 14-3-3 proteins play a role in several diseases and many biological processes. Proteins of this family engage in protein-protein interactions (PPIs), and can do so with numerous different binding partners. By forming these PPIs, these proteins regulate their binding partner's activity. The activity can be both up- or down-regulated.

Another family of relevant targets are glucansucrases, which are important enzymes in the initiation and development of cariogenic dental biofilms, commonly known as dental plaque. Inhibiting these enzymes might therefore prevent dental caries.

The second aim of this thesis was to extend the list of reversible reactions applicable in a tDCC setting. We have shown in Chapters 5 and 6 that nitrones and thiazolidines could be applied in DCC under physiological conditions. Based on this finding, we ran ptDCC experiments using these reversible linkages with endothiapepsin as a target protein. An important aspect in DCC is the stability of compounds, as noted for the nitrones and thiazolidines. Dynamic combinatorial libraries (DCLs) of these types of linkages should therefore be carefully monitored. Another important aspect in DCC is the analysis of the DCLs, which is best performed by determining the surface area under each peak in the UV chromatogram of the LC-MS. For the libraries in Chapters 5 and 6, analysis via UV was not possible, as most products were not UV-active under the screened wavelengths. We therefore analysed these libraries using the total ion counts of each product. To avoid possible problems of ionisation efficiency and the injection volume, which can deviate per run, we have taken the relative ion counts. This gives the ratio of products as a percentage, which allows the comparison of the libraries even when factors like injection volume or the ionisation might not be identical per run. One could consider adding an internal standard, which allows for a quantitative analysis.

In the last two chapters, endothiapepsin was used for protein-templated DCC (ptDCC). Endothiapepsin belongs to the family of the aspartic proteases. Aspartic proteases are involved in numerous biological processes and diseases, for example the maturation of the HIV virus particle.
Throughout this thesis, we focus on applying DCC to various projects. The main achievements are: 1) the description of the in-house protocol of DCC, in which aspects like solubility of building blocks and products, protein stability and more need to be taken into account, 2) the application of acylhydrazone-based DCC to two attractive targets, a (PPI)-target and a glucansucrase, 3) the identification of small-molecules, which stabilise PPIs of 14-3-3/synaptopodin, 4) expanding the reaction toolbox of ptDCC by two additional reactions: nitrone and thiazolidine formation.

Here, we summarise the main results reported in this thesis and we give an outlook on DCC for medicinal chemistry projects.

7.2 Summary
In Chapter 1, provides a clear and concise description of a comprehensive DCC protocol. This work focused on important aspects concerning the practical handling in ptDCC. The best biological and chemical conditions should be harmoniously combined for successful application in ptDCC. Moreover, analytical techniques used to determine which compounds are favoured were briefly discussed.

In Chapter 2, we present an overview of modulators of 14-3-3 proteins. We critically analysed the known modulators in terms of their structures and molecular recognition by 14-3-3 proteins. We proposed small modifications of certain modulators, based on reported cocrystal structures.

In Chapter 3, we describe the identification of modulators of 14-3-3 PPIs via DCC. We targeted a PPI-complex of 14-3-3(ζ) with synaptopodin, a 21 amino acid peptide chain. Hit compounds of the DCC experiments were analysed for their biochemical activities via surface plasmon resonance (SPR). These compounds show a stabilising effect on the PPI-complex. Co-crystallisation studies are ongoing to confirm the binding mode, setting the stage for SBDD to optimise the affinity.

In Chapter 4, we applied acylhydrazone-based DCC on a glucansucrase. Glucansucrases play an important role in cariogenic dental biofilms, which are causative for dental caries. Glucansucrase inhibitors should therefore prevent caries and may be added as additives in toothpaste. We described the syntheses, DCC experiments as well as biochemical characterisation of the hit compounds.

In Chapter 5, we describe the first application of nitrone-based ptDCC. We optimised conditions for ptDCC, keeping in mind both the target protein endothiasepsin as well as the applicability of the nitrone reaction. Besides the application to ptDCC, we determined the optimal pH window of this `new`
reaction. Synthesis of hit compounds from the DCC experiments enabled evaluation of their biochemical activity in a fluorescence-based activity assay. Preliminary results on the cytotoxicity of the nitrene linkage showed no cytotoxicity on the liver cell line, HepG2.

In Chapter 6, we portray another new reaction in ptDCC, using thiazolidines. Thiazolidines can be found in numerous natural compounds and are therefore very attractive scaffolds from a biochemical point of view. We described a number of DCC experiments, illustrating that this reaction can also be applied in medicinal chemistry projects. Expansion of the reaction scope from cysteine derivatives towards aromatic aminothiol building blocks resulted in the oxidation of the products. This oxidation makes the reaction irreversible, and therefore thiazolidine DCC is limited for now to cysteine derivatives.

7.3 Perspectives
As can be seen from this thesis, dynamic combinatorial chemistry can be applied to a variety of different targets. It opens up the possibility of finding starting points in medicinal-chemistry projects. It can be expected that the number of successful DCC projects will increase over time, as more and more research groups are showing interest in this technique. The applicability of DCC in a high-throughput manner is still limited but this might change in the near future, if the analysis via LC-MS can be automated. This would open-up the possibility of running library sizes over 100 compounds, especially when extracted ion counts of each individual product will be used instead of analysis via UV.

DCC will likely become also more important in optimisation rounds. Hit compounds of previous studies, obtained via DCC or via other hit-discovery strategies, can be valuable starting points. Molecules can for example also be grown by applying DCC. Drug discovery requires multiparameter optimisation, focusing on more than just affinity. Therefore, DCC will most likely stay most useful in the early phases of the drug-discovery process.

Since the chemical linkage formed in the reversible reactions used in DCC (for example: imine, hydrazone, thiazolidine) is part of the drug-molecule, it could also be involved in binding to the protein. Depending on the type of target, it would be helpful to have a variety of reversible reaction at hand. This would require a constant search for new reactions and the corresponding scaffolds which can be applied in a tdDCC setting.

All in all, we have given examples of the broad utility of DCC, showing why this technique is evolving over time!