Summary and perspectives

MCR chemistry represents a specific yet minor branch of organic chemistry. MCRs have been described in documents almost as early as organic chemistry. Nevertheless, MCRs have been overlooked for so long. Only in recent decades, the advantages embraced by MCRs, e.g., structural complexity, skeletal diversity, and green experimental environment have been gradually recognized in academia and industry. Especially in the pharmaceutical industry, alongside an increasing number of preclinical and clinical drug candidates incorporating MCRs, MCRs have attracted intensive focus, they are gaining their place as a powerful engineering tool to produce complex, diverse structures, large chemical space and rapidly generate structure-activity relationships.

This thesis has discussed four aspects of MCRs that could potentially find their application in drug discovery.

In chapter 1, we describe the automated acoustic dispensing-enabled (ADE) nanoscale synthesis of 16 different MCR scaffolds in parallel, resulting in 1536 reactions. HT analysis of this large number of reactions provides insight into the scope and limitations of compatible building blocks and subsequently can be instrumental for reaction optimization. Furthermore, this rapid library synthesis can be used to generate diverse libraries for identifying biologically active compounds. Our chemoinformatic analysis of the 16 scaffolds supports the high diversity and drug-likeliness of our in situ synthesized chemical space. More importantly, this automated nanomole synthesis manifests its sustainability for minimizing building block consumption in HT screening. (figure 1)

Figure 1. 1536 reactions based on 16 different chemistries for the parallel assembly of a highly diverse library of small molecules. The generic scaffolds are shown in the green boxes left and right of the 1536 well plate. The MS-analysis is plotted on the 1536-well plate based on a three-colour code (blue = no product formation, yellow = medium product formation, green = major product formation).

In chapter 2, we give an overview of the molecular biology of SARS-CoV-2. Four viral proteins (3C-like protease, Papain-like protease, Spike glycoprotein, RNA-dependent RNA polymerase) and one host protein (AP2-associated protein kinase 1) are discussed as potential targets to fight SARS-CoV-2 in the context of drug repurposing. Many different drug modalities are thinkable to fight SARS-CoV-2, such as vaccines, antibodies, small molecules, biologics, modified cells, peptides, proteins, oligonucleotides or natural medicines. It should be noted that, in a HT screening campaign, there are several Ugi-4CR products found to be noncovalent SARS-CoV 3CLpro inhibitors of moderate MW with good enzyme
and antiviral inhibitory activity which may serve as a promising starting point for further drug development. (figure 2)

![Chemical structures](image)

**Figure 2.** Synthesis and some molecular, DMPK and pharmacology data of the probes ML188 and ML300.

In chapter 3, we have developed a mild method to conveniently synthesize in a single step 2,3-disubstituted imidazo[1,2-a]pyrazin-8-amines as adenine mimetics through the GBB-3CR, 22 reactions were performed and achieved products in overall good to excellent yields. Compared to previous methods, our method is much superior in terms of the number of steps and generality. The mild reaction conditions allow a large variety of substituents, including functional groups. This reaction scaffold can be potentially investigated to discover novel adenine mimetics with biological activity. (figure 3)

![Chemical structures](image)

**Figure 3.** Facile GBB-3CR method to synthesize adenine mimetics.

**Chapter 4** focuses on cleavable Ugi-tetrazole reactions, aiming to achieve the 5-substituted 1H-tetrazole scaffold as an important bioisostere of the carboxylic acid. We first successfully designed and synthesized a novel 4-hydroxy-3,5-di-tert butyl benzyl isocyanide. This specific isocyanide shows, in general, excellent reactivity in the Ugi-tetrazole reactions by a selection of different amine and oxo components as building blocks. More importantly, as expected, the 4-Hydroxy-3,5-di-tert butyl benzyl moiety can be readily cleaved under basic condition to achieve our targeted 5-substituted 1H-tetrazoles. (figure 4)
Figure 4. The employment of our designed 4-hydroxyl-3,5-di-tert butyl benzyl isocyanide in Ugi-tetrazole reactions followed by deprotection under basic condition with LiOH.

Perspectives

This thesis has investigated and discussed four different topics, each of which has directly or indirectly manifested its potential in promoting the application of MCRs in drug design. In chapter 1, HT synthesis of 16 different MCR scaffolds was performed in a highly automated and miniaturized fashion. This work opens a promising avenue to screen more isocyanide-based MCR (IMCR) scaffolds with more structural diversity to identify distinguished bioactive MCR compounds. Possibly more significantly, the true advantage of our work has been revealed: it is expected to achieve a more sustainable ‘design-make-test-analyse’ cycle in the future as the time could be largely reduced by highly automated screening and the throughput will also substantially increase by employing miniaturized chemistry. In chapter 2, we describe specifically several Ugi-4CR compounds as potential noncovalent inhibitors against 3CL protease of SARS-CoV-2. Since Ugi-4CR as a classic IMCR scaffold is easy to handle experimentally and can rapidly generate structure-activity relationship, HT screening campaign, e.g., applying ADE technology discussed in chapter 1, could be undertaken in the lead discovery stage to select optimized inhibitors against 3CL protease among a diversity of Ugi-4CRs with better ADME (absorption, distribution, metabolism, excretion) properties. Chapter 3 and Chapter 4 center on the synthesis of 2 MCR scaffolds, GBB and Ugi-tetrazole reactions, respectively, to obtain 2,3-di-substituted imidazo[1,2-a]pyrazin-8-amines as adenine mimetics and 5-substituted 1H-tetrazole scaffold as an important bioisostere of the carboxylic acid. Owing to the structural complexity and diversity of MCR chemistry, it is unquestionable that more MCRs can be utilized or further modified to explore their unique bioactivities against different therapeutic targets. Last but not least, all above discussed areas related to MCRs, can be smartly exploited or integrated, which could promisingly lead to the rise of MCR-specific drug candidates in both academia and industry.