Combinatorial Solid-Phase Synthesis of Proteolysis Targeting Chimeras for High-Throughput Cell Screening on Droplet Microarray

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The pace of technological advancement nowadays is mostly limited by economical factors. Thus, much effort is put into reduction of both time and cost of processes in all branches of the chemical industry. The main strategies to achieve this are miniaturization and parallelization. For this, a platform was designed that fulfills both these criteria, the droplet microarray (DMA). The DMA is an array of hydrophilic spots on a superhydrophobic background, which allows handling of sub microliter volumes for chemical and biological experiments. In this work, a combinatorial solid-phase synthesis strategy for proteolysis targeting chimeras (PROTACs) through multi component reaction on DMA was designed that allows synthesis, purification and biological screening on the same platform for the identification of potential drug candidates. PROTACs are a novel type of therapeutic to treat protein associated diseases, with their two main advantage being a catalytic mode of action, reducing the necessary concentration of drug drastically, and their ability to target proteins not targetable by inhibitors.

As protein of interests in this study BRD4 and MEK1/2, both important in pathways involved in cancer proliferation were chosen. The binding motifs for these were derivatives of prominent inhibitors (+)JQ-1 and PD0325901. They are combined with CRBN-ligand Thalidomide through various linker systems of different length and branching, utilizing the

surface amino-groups of the DMA as anchor point. The different PROTACs that were synthesized could be released on demand through UV irradiation. The formation and purity of the products is verified via chromatography and mass-spectrometry, the ability to initiate protein degradation is studied in viability and proliferation assays on cancer cells, with the focus being the linkers´ influence on the degradation potency of PROTACs bearing the same terminal binding motifs.