Acceleration of Hit-to-Lead Drug Development by Nanoliter-scale Screening of Mutasynthetic BamA Inhibitor Library

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The increasing number of multi-drug resistant pathogens calls for new chemical scaffolds with novel molecular targets to serve as lead structures. For antibiotics, natural product - inspired molecules represent a most promising resource, but optimization by derivatization is often impeded by their relative inaccessibility to medicinal chemistry programs. We addressed this limitation with a mutasynthetic library of bicyclic heptapeptides based on the natural BamA inhibitor darobactin. Substrate flexibility of the ring closing enzyme DarE and structure-activity-information obtained from previously investigated analogues directed the master library design. Using a microfluidics approach, single clone-cells were encapsulated into alginate beads to express the ~16k different peptides. Simultaneously, the method allowed us to screen all expressed derivates against a fluorescent sensor strain in a high-throughput manner. Loss of fluorescence sorting led to the identification of HIT compounds. The most intriguing new peptides were purified and compared to known darobactins by MIC determination against a broad panel of Gram-negative pathogens. From all tested heptapeptides, darobactin B remained the most promising candidate and was chosen for further characterization. Time-kill curve and early ADMET experiments were carried out. The *in vivo* efficacy was confirmed in pneumonia mouse models. The bacterial load of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was significantly reduced by intraperitoneal, as well as intratracheal administration. These positive results push darobactin B further as lead compound towards drug development. Our study showcases the potential of mutasynthetic libraries to accelerate identification of functional peptides for drug lead discovery.