Solid-Phase Biocatalysis for Pharmaceutical Lead Optimization

by

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ABSTRACT

The focus of this thesis work is on the development of a new technology to generate and screen small molecule-based therapeutics for drug discovery. Solid-phase biocatalysis, as demonstrated here, is a powerful tool that can be used to generate novel biologically active small molecules in a combinatorial or iterative fashion. Using principles of microarray technology and biocatalysis, we have employed several enzymes to modify substrates attached to glass slides and then detected the products in a spatially addressable microarray format. To further develop the enabling tools for solid-phase biocatalysis, we have also employed non-planar supports to compliment the glass slide screens and to provide characterization of the molecules that are identified using high-throughput screens. In addition to generating novel molecules using solid-phase biocatalysis, we have also used soybean peroxidase enzyme, in solution, to generate a library of oligophenols as potential inhibitors of NADPH oxidase. This enzyme has been implicated in inflammatory cascades as a result of oxidative stress in the body.

Our work supports the first demonstration of using enzymes to modify substrates that are attached to glass slides. This approach would potentially enable rapid and efficient lead optimization, hence overcoming a critical bottleneck in the drug discovery process. Lead optimization involves modification of pharmaceutical leads (e.g., "hits" from high-throughput screening) with some demonstrated level of biological activity to generate analogs with more suitable pharmacokinetic properties for ultimate use as a drug. A major goal of this thesis work was to develop the enabling tools needed to exploit the exquisite selectivity and unique reactivity of enzymes to perform high-throughput biocatalytic derivatization of solid-supported small-molecule compounds.

The microarray platform can be ultimately used to attach any lead onto a glass slide and employ enzymes to rapidly generate several analogues on a single glass slide, whereby the binding of the analogues can be determined when probed with their target proteins. This methodology will thus provide novel capabilities for discovering new biologically active molecules and can serve as a general platform for the enzymatic lead optimization of lead compounds in an easy and efficient manner.