Title:

Diversification of Mitragynine Using Biocatalytic Approaches for Improved Therapeutics

Abstract:

Opioid abuse and overdoses rank among the leading causes of unintentional drug overdose fatalities in the United States. Therefore, identifying a novel mu opioid receptor (MOR) agonist molecular scaffold that possesses robust analgesic effects with minimal respiratory suppression, and abuse potential is an urgent challenge. We aim to diversify mitragynine, a plant-derived alkaloid natural product with intriguing analgesic properties toward the goal of a safer alternative to current opioid MOR agonists. We have pursued the creation of new analogs of mitragynine through late-stage hydroxylation and halogenation, which enables further interrogation of chemical space through additional functionalization. The incorporation of biocatalysis into synthetic chemistry approaches is increasing largely due to growing number of protein engineering approaches to tune their selectivity, efficiency and stability. However, current knowledge of biocatalyst driven late-stage C-H functionalization is limited to a narrow substrate and enzyme scope. In this work, we have employed two complementary strategies to identify and optimize biocatalysts capable of site-selectively modifying mitragynine. First, we are utilizing directed evolution on the 4V flavin dependent halogenase to enhance chlorination of mitragynine at variant positions on the indole ring. Directed evolution is being conducted to generate a diverse library of enzyme variants with improved activity and selectivity. Secondly, we are employing density functional theory (DFT), inverse docking, and molecular dynamics (MD) simulations as a workflow to identify novel cytochrome P450s capable of late-stage C-H functionalization. Finally, the bioactivity of our analogs at the MOR will be assessed through M-SPOTIT and PathHunter β-galactosidase enzyme-complementation assays, providing detailed insights into their signaling and potential side effects. Through this comprehensive approach we strive to develop novel, improved mitragynine derivatives with improved analgesic properties.