"Exploration of *Stig* Cyclases as Biocatalytic Tools for Unnatural Indole Alkaloid Production"

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The lack of development of new antibiotics has pushed drug discovery methods back towards natural products to help combat the threat of multi-drug resistant bacteria. Hapalindoles and related compounds (ambiguines, fischerindoles, welwitindolinones) are a diverse class of indole alkaloid natural products that were originally isolated from the cyanobacteria species, *Hapalosiphon fontinalis*. These complex metabolites possess a large variety of biological activities including antibacterial, antimycotic and anticancer. Their large number of regio- and stereo-diverse core ring systems and late-stage modifications has motivated several synthetic routes. Only recently has the biosynthetic pathway for hapalindole-type metabolites been elucidated. In order to assemble the core ring system, *L*-tryptophan is converted into the *cis*-indole isonitrile subunit by a three enzyme cascade before being prenylated with geranyl pyrophosphate at the C-3 position. A new class of cyclase (Stig) catalyzes a three step process including a Cope rearrangement, 6-*exo*-trig cyclization and electrophilic aromatic substitution followed by diverse late-stage tailoring steps catalyzed by a range of enzymes that mediate C-H functionalization (Figure **1**). The presence of Ca^{2+} with distinct combinations of Stig cyclase(s) promotes the assembly of variant hapalindole and fischerindole type core molecules. This project focuses on exploring the nature of selectivity of the Fam prenyltransferases and Stig cyclases in order to diversify this class of indole alkaloids via cascade biocatalysis. To date, three Stig cyclases have been examined with 14 unique *cis*-indole isonitrile derivatives to produce 11 distinct hapalindole and 8 fischerindole derivatives. This work showcases the potential of using enzymes to produce complex, unnatural indole alkaloid molecules for further medicinal screening against human microbial pathogens.

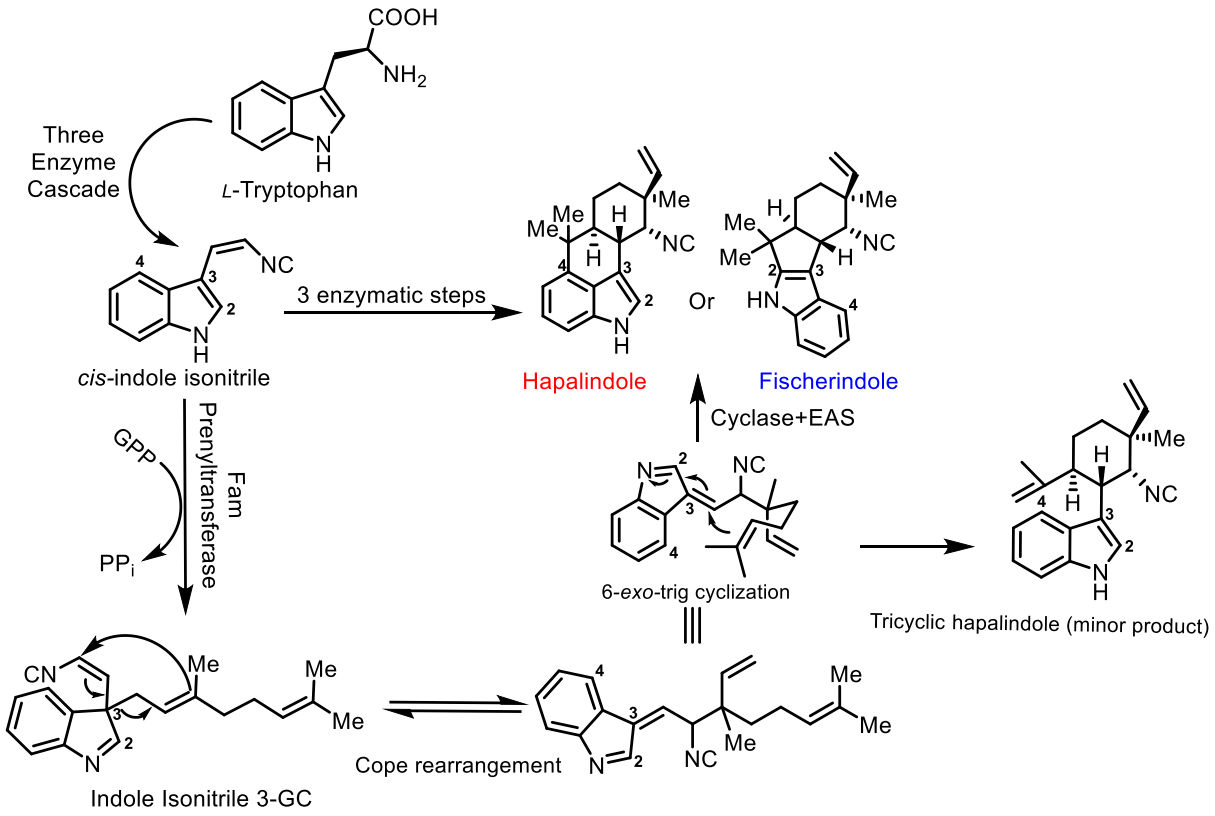

Figure 1: Biosynthesis of hapalindole-type molecules. *L*-tryptophan is converted into *cis*-indole isonitrile by a three enzyme cascade, which is geranylated at the indole C-3 position to produce the indole isonitrile 3-GC intermediate. In the presence of a dimerized cyclase combination, this intermediate undergoes a Cope rearrangement, 6-*exo*-trig cyclization and a terminal electrophilic aromatic substitution (EAS) at either the indole C-4 or C-2 position to produce the core hapalindole or fischerindole molecule, respectively.