Directed evolution of terpene synthases to alter product specificity towards compounds with anxiolytic properties

Garrett Zinck[1] • Joe Chappell, PhD[1]

[1]*Pharmaceutical Sciences , University of Kentucky*

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Background: The anxiolytic activity of valerian, Valeriana officinalis, has been attributed to the sesquiterpenoids, a C15 family of terpenes naturally produced within valerian roots. Valerenic acid, a sesquiterpenoid found within Valerian roots, is a positive allosteric modulator of GABAA receptors – ligand-gated ion channels responsible for fast inhibitory neurotransmission which lead to decreases in motor activity and mitigation in feelings of anxiety. Through transcriptomic analysis and heterologous gene expression, we have identified valerenadiene synthase (VDS), the enzyme responsible for synthesizing valerena-1,10-diene (the non-acidified precursor of valerenic acid) from farnesyl diphosphate (FPP). Our goal was to selectively mutate key amino acids governing product specificity within VDS in order to generate constrained, rigid scaffolds of valerena-1,10-diene that would possess lower EC50 values and increase anxiolytic properties in vivo in comparison to valerenic acid.

Hypothesis: Constrained, rigid scaffolds of valerenic acid will bind with greater specificity to the β2,3 subunits of the GABAA resulting in a greater anxiolytic effect in comparison with valerenic acid.

Methods: We generated homology models of VDS from crystal structures of tobacco epi- aristolochene synthase (TEAS), a 40% identical sesquiterpene cyclase, and identified key amino acids interacting with FPP in order to change product specificity. We have selectively mutated these residues to see if we can generate some of the early intermediates along the catalytic cascade.

Results: Thus far, we have generated single-point mutants that either are catalytically inactive or produce low levels of valerena -1,10-diene.

Conclusions: Shifting from product specificity within VDS will more than likely require 1) multiple single point mutations 2) multiple complimentary single point mutations to generate the desired rigid analogs of valerena-1,10-diene.

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