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Enzyme engineering approaches on a stereospecific indole C-methyltransferase – challenges, new functions and solutions for practical applications

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PURPOSE OF THE ABSTRACT

The indole and pyrroloindoline structural motifs are common among bioactive natural product alkaloids with various biological activities.[1] AChE inhibitor physostigmine was the inspiration for the development of a stereoselective enzymatic methylation platform for these scaffolds. A key step in the biosynthesis of physostigmine is the enzymatic C-methylation of an indole derivative, which drives the subsequent intramolecular cyclization, forming a stereogenic centre.[2] By using a chemo-enzymatic approach, we were able to produce physostigmine analogs in an enantiopure fashion. However, the limits of the enzyme are preventing application on a broader scope.[3] Site-specific and saturation mutagenesis approaches allowed the modification of the catalytic site, with implications on the product size and chemistry. Our mechanistic study, as well as an analysis of known methyltransferases containing a Rossmann-fold motif are a source of inspiration for targeted modifications, while directed evolution techniques were able to fill in the gaps towards a substrate promiscuous – but stereoselective engineered catalyst. Further enzymatic alkylations of the indole scaffold were also enabled by this approach, using SAM analogs. In order to support the screening effort, we developed an automated process for the cultivation, expression and activity testing of the resulting mutant libraries. Our results lead us to an enhanced biocatalytic approach for the production of physostigmine analogs.

FIGURES

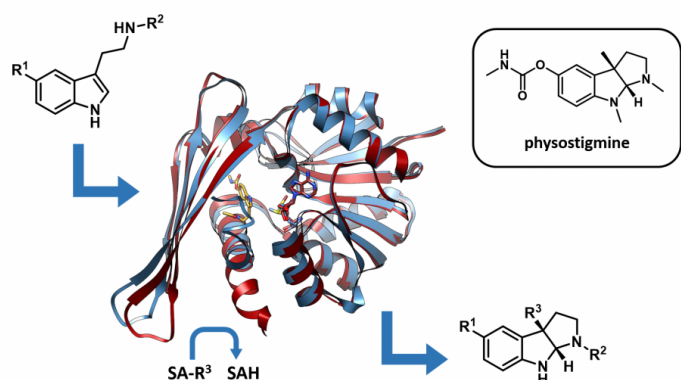


FIGURE 1

General process catalyzed by the engineered methyltransferase

3 points of interest were selected for the structural diversification of the products. SAM analogs were used for alkylation in the 3 position (ex. $R^3=\text{Me}$ for methylation)

FIGURE 2

KEYWORDS

methyltransferase | enzyme engineering | indole methylation | library screening

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