BIOTRANS
LA ROCHELLE 2023

N°1747 / PC
TOPIC(s) : Enzyme engineering & Discovery / (Chemo)enzymatic strategies

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

AUTHORS

Diana-Alexandra AMARIEI / HEINRICH-HEINE UNIVERSITY, DÜSSELDORF, WILHELM-JOHNEN-STRASSE, JÜLICH
Julia TENHAEF / IBG-1, FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Tobias ROSCH / IBG-1, FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Mona HAASE / HEINRICH-HEINE UNIVERSITY, DÜSSELDORF, WILHELM-JOHNEN-STRASSE, JÜLICH
Pascal SCHNEIDER / HEINRICH-HEINE UNIVERSITY, DÜSSELDORF, WILHELM-JOHNEN-STRASSE, JÜLICH
Stephan NOACK / IBG-1, FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH
Jörg PIETRUSZKA / HEINRICH-HEINE UNIVERSITY, DÜSSELDORF, IBG-1, FORSCHUNGSZENTRUM JÜLICH, WILHELM-JOHNEN-STRASSE, JÜLICH

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

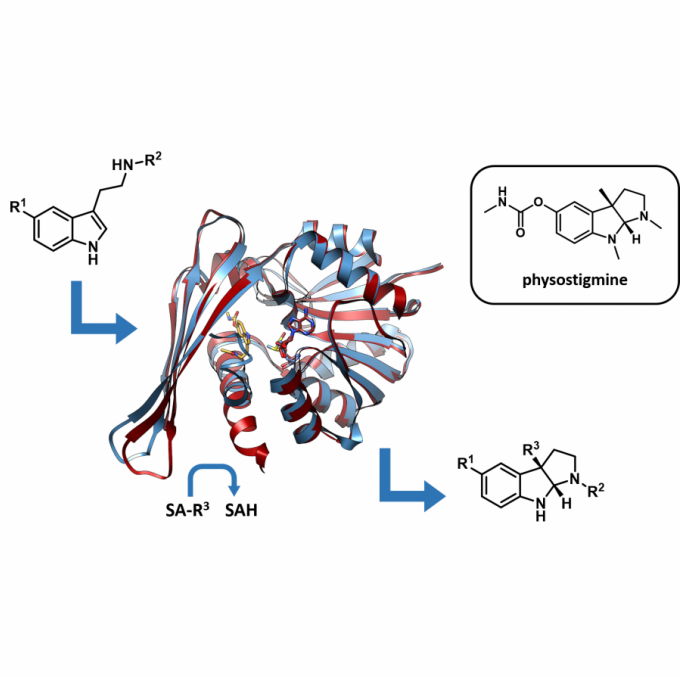

FIGURE 1

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 possition (ex. R3=Me for methylation)

FIGURE 2

KEYWORDS

methyltransferase | enzyme engineering | indole methylation | library screening

BIBLIOGRAPHY

[1] de Sa Alves, F. R.; Barreiro, E. J.; Manssour Fraga, C. A. Mini Rev. Med. Chem. 2009, 9, 782-793.

[2] Liu, J.; Tailun, Ng.; Rui, Z.; Ad, O.; Zhang, W. Angew. Chem. Int. Ed. 2014, 53, 136-139.

[3] Amariei, D. A.; Pozhydaieva, N.; David, B.; Schneider, P.; Classen, T.; Gohlke, H.; Weiergräber, O. H; Pietruszka, J. ACS Catal. 2022, 12(22), 14130-14139.