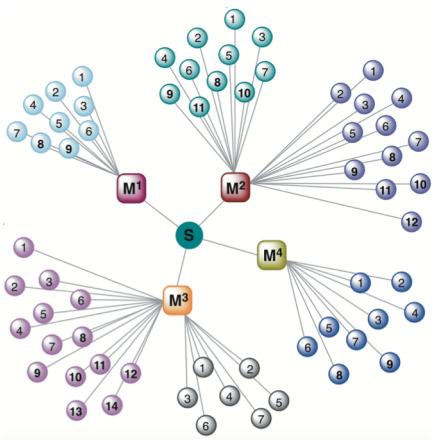
ISCFPOCMiB035 BB/L013711/1 BB/SCA/MetalsinBiology/17
1 November 2017 – 28 February 2018
Funded by a Metals in Biology BBSRC NIBB Seeding Catalyst grant
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

Adding value to biocatalytic hydroxylation products for synthesis and drug discovery

"We were pleased to see such encouraging results from the project, both in terms of the whole-cell bioconversions and the subsequent derivatisation of the metabolites." Jason King, Oxford Biotrans

Jeremy Robertson and Luet Wong, University of Oxford; Jason King, Oxford Biotrans



Engineered $P450_{BM3}$-mediated hydroxylation of fragment-sized unfunctionalised substrates (S) provides a series of metabolites (M) that may then be elaborated into a diversified set of new fragment molecules.

RESULTS: Following preliminary optimisation studies, engineered *E. coli* cells expressing cytochrome P450 mutants from *Bacillus megaterium* ($P450_{BM3}$) were used to produce two hydroxylated derivatives (termed **A** and **B**) of a nitrogen heterocyclic fragment molecule (*N*-Boc-8-azaspiro[4,5]decane). For **A**, 91% conversion of the starting material was achieved after 43 h; for **B**, 85% conversion was achieved after just 3 h and the reaction was complete within 20 h; the cells were still active and were re-used in an identical reaction. Both reactions scaled well to 340 mg of substrate. These proof-of-concept reactions provided synthetically useful outcomes, with reactions processing 1.70–3.4 g of substrate per litre of culture. The second phase of the project scoped a proposed method for elaborating the hydroxylated fragments into a variety of derivatives, based on tethered C–H insertion reactions. Two Rh(II)-catalysed variants were studied: (1) Du Bois' conditions for nitrenoid insertion; (2) Doyle–Lee carbenoid insertion. These reactions generated several derivatives that included three C–H amination products, currently assigned as two stereoisomers of the five-membered cyclic sulfamate (β-insertion) and the product of γ-insertion, a six-membered bridged cyclic sulfamate. This work provided insight into the likely outcomes of C–H insertion reactions in conformationally-biased molecules.

INITIAL AIMS: We intend to use iron-containing enzymes to produce compounds of value to the pharmaceutical industry. Drug discovery campaigns based on fragment-based screening combine the features of a number of 'fragment' compounds that are weakly-active at the desired target to identify promising 'lead' compounds. The highest chance of identifying leads that develop into successful drugs arises when the initial fragment collection is structurally diverse. Therefore, we aim to diversify compound collections in a two-stage process that mimics the biosynthesis of known medicines such as the anti-cancer drug paclitaxel (Taxol). Stage one employs engineered *E. coli* cells to enzymatically introduce a 'handle' (a hydroxyl group) onto a nitrogen heterocyclic fragment. In stage two, the chemical properties of this handle will be exploited to introduce features that promote favourable interactions with drug targets.

- Collaboration to continue to identify parameters that lead to shorter reaction times and higher conversions and substrate concentrations, and to improve product isolation
- Literature survey undertaken to identify alternatives to the Rh(II)-catalysed reactions
- Manuscript published: Syntrivanis et al. (2018) EUR J ORG CHEM.: https://onlinelibrary.wiley.com/doi/full/10.1002/ejoc.201801206