LATE STAGE FUNCTIONALISATION OF DRUG CANDIDATES USING POLYCYPS® ENZYMES

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Abstract:

Introducing oxygen into a drug candidate late in the optimisation process has several applications including exploration of SAR (structure-activity relationships) and the ability to access derivatives that may possess superior properties such as improved metabolic stability and LLE (ligand-lipophilicity efficiency) . Biocatalysis can provide access to chemical space in a complementary manner to chemical synthesis and provide a "one-experiment" solution to accessing multiple derivatives in parallel. This poster illustrates the application of a new biocatalysis kit, PolyCYPs®, to enable parallel synthesis of hydroxylated derivatives of drugs. The PolyCYPs platform is comprised of a set of cloned cytochrome P450 enzymes and redox partners derived from some of the most talented bacteria in Hypha's biotransformation panel. Enzymes in the kit catalyse oxidation reactions of a wide variety of substrate types to generate multiple derivatives in one pot, including hydroxylated and dealkylated derivatives useful for diversification and late stage functionalization of drug leads. This case study compares the hydroxylated derivatives produced from a drug candidate under development by AstraZeneca using a whole cell microbial approach with those obtained employing selected PolyCYPs® enzyme reagents, as part of a study exploring the effect of different polar chemical space on potency.

Background

- As part of a previous project characterising derivatives generated from one of AstraZeneca's drug leads by whole cell biotransformation using microbes in Hypha's biocatalysis panel, eight regio- and stereoisomers were isolated. Analogues were characterised by 2D NMR and derived from oxidation of the cyclohexane moiety, together with desmethyl and benzylic hydroxylated derivatives, and combinations thereof.
- In this current study, we investigated the extent of derivatisation possible using the same drug lead screened against a selected number of Hypha's new PolyCYPs kit enzymes.

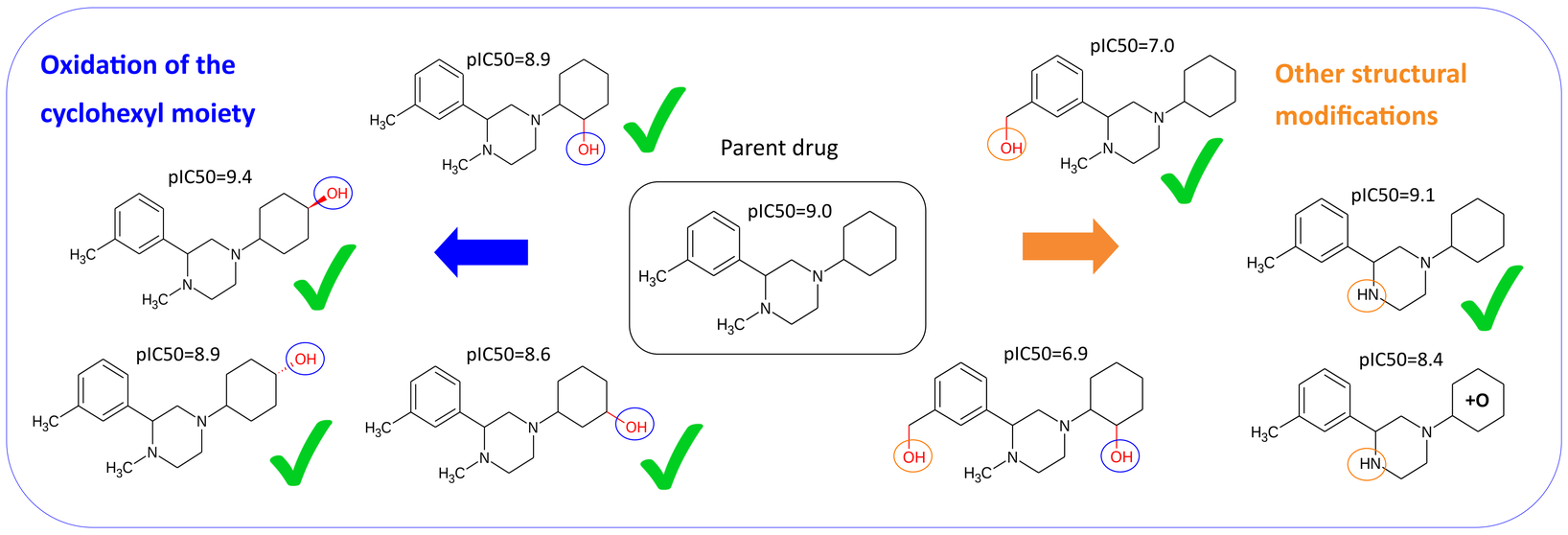
Reagents included in the PolyCYPs kits:

PolyCYPs enzymes (**blue lid) -** PolyCYPs tested in this experiment were 6, 14, 152, 166, 168

Co-factors (**green lid)** - NADP+, G6P, G6PDH

Formulant **(red lid) -** not needed for this substrate

pIC_{50} values for oxidised derivatives produced from microbial biocatalysis of AstraZeneca's drug lead (mock structures given due to confidentiality). The addition of a green tick on the structures illustrates all those produced by a PolyCYPs enzyme.



Process summary for PolyCYPs screen

Five lyophilised PolyCYPs enzymes reconstituted in water according to PolyCYPs kit instructions

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Drug dosed in DMSO at 0.125mg/ml (0.4% final DMSO concentration)

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Lyophilised co-factors reconstituted in water and added to the PolyCYPs and substrate to initiate the reaction

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Incubated overnight (18 hrs) with gentle shaking at 27°C

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Reaction stopped with equal volume of acetonitrile

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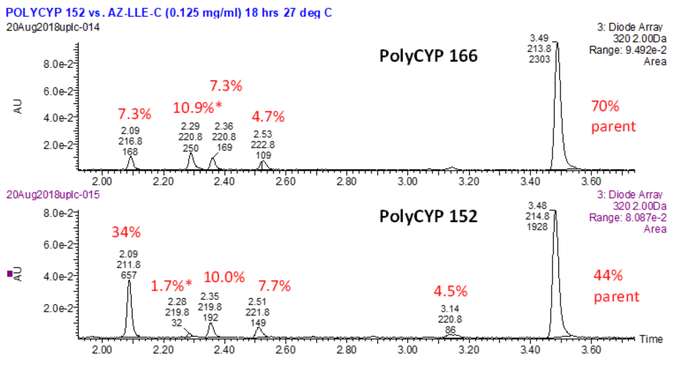
LC-MS analysis and comparison to reference samples of derivatives previously isolated from whole cell biotransformation of the parent drug



Estimated % conversion to various monohydroxylated derivatives of the parent drug based on UV absorbance

Results

- All five PolyCYPs enzymes used generated some hydroxylated derivatives of the parent drug (mock structures shown due to confidentiality) although PolyCYPs 152 and 166 generated the best array for this compound.
- PolyCYP 152 gave a 56% conversion to produce all 5 monohydroxylated derivatives observed in the whole cell biotransformation whereas PolyCYP 6 gave a 30% conversion to 4 of these monohydroxylated derivatives.
- Minor amounts of a dihydroxylated product was produced by PolyCYPs 6, 14 and 166, although this derivative did not appear to match with the dihydroxylated product identified originally. Minor amounts of a demethylated derivative was produced by PolyCYPs 152 and 166 which matched with the *N*-demethylated derivative originally seen.

Conclusions / Discussion

- Biocatalytic approaches, such as that provided by PolyCYPs kits, are an effective way to access metabolites and hydroxylated derivatives of lead compounds in parallel for late stage functionalisation. These reactions can readily be scaled-up to provide more material for evaluation and comparison with properties of the parent drug compound.
- There is increasing focus on exploiting properties of hydroxylated metabolites for lead optimisation as highlighted in a 2018 paper by Bostrom *et al.* who state that, "Hydroxylation is a key tactic to consider as part of a late stage functionalisation strategy where small changes can result in improved activity, selectivity, solubility and lipophilicity." PolyCYPs enzymes address this need by hydroxylating susceptible lead compounds in parallel *via* an easy-to-use kit.
- In addition, biocatalysis can provide a route to obtaining novel analogues with improved metabolic stability with improved renal clearance, exemplified in a paper by Stepan *et al.* exemplifying Pfizer's routine late stage oxidation approach.

References

Bostrom *et al*., 2018. Nature Reviews Drug Discovery 17, 709-727.

Stepan *et al*., 2018. ACS Med. Chem. Lett. 9, 68-72.

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About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company helping partners in pharmaceutical and agrochemical R&D worldwide succeed through the production of human and other mammalian metabolites, as well as specialising in lead-diversification and production of microbially-derived chemicals.