ACCESSING MAMMALIAN DRUG METABOLITES USING POLYCYPS® ENZYMES

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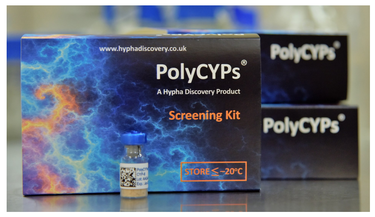
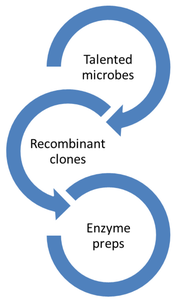


Abstract: This poster illustrates the application of a new biocatalysis kit, PolyCYPs®, to enable scalable synthesis of CYP-derived metabolites of drugs. The PolyCYPs platform is comprised of a set of recombinant cytochrome P450 enzymes and redox partners cloned from some of the talented actinomycetes in Hypha's biotransformation panel. Enzymes in the kit catalyze the oxidation of a wide variety of substrate types to generate multiple mammalian and microbial-derived CYP metabolites. The poster features application of selected PolyCYPs isoforms to produce oxidized human metabolites of drugs. Further, the utility of PolyCYPs enzymes for introducing oxygen into a drug candidate as part of a late stage functionalization program is illustrated, in which derivatives can be generated in parallel which may possess superior properties such as improved metabolic stability and LLE (lipophilic ligand efficiency), and for exploration of structure-activity relationships.

PolyCYPs enzymes are proprietary recombinant microbial CYPs expressed in *E.coli* together with ferredoxin / ferredoxin reductase redox partners. They are derived from a subset of Hypha's actinomycetes talented at oxidizing drugs.



Lyophilized and vacuum-sealed reagents included in PolyCYPs screening kits:

PolyCYPs enzymes (**blue lid) -** PolyCYPs include 6, 152, 166, 168, 194, 196, 217, 235

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

Formulant **(red lid) -** for highly lipophilic substrates

Control compound (silver lid) - bosentan

Process summary for PolyCYPs screen

Lyophilised PolyCYPs enzymes reconstituted in water according to PolyCYPs kit instructions

↓

Drugs dosed in DMSO at 0.1 mg/ml in 0.4% final DMSO concentration (up to 2% possible)

↓

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

↓

Shaken overnight (16-20 hrs) in 24 square well plate at 27°C

↓

Reaction stopped with equal volume of acetonitrile, centrifuged and supernatant analysed by LC-MS

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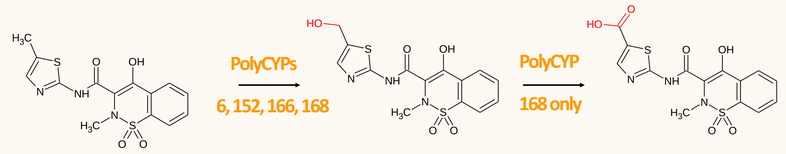
Generation of human CYP-derived metabolites

Results

Step 1: Multiple drugs (20) screened using 8 PolyCYPs enzymes

> 10% conversion of 18 drugs to at least one metabolite



Meloxicam

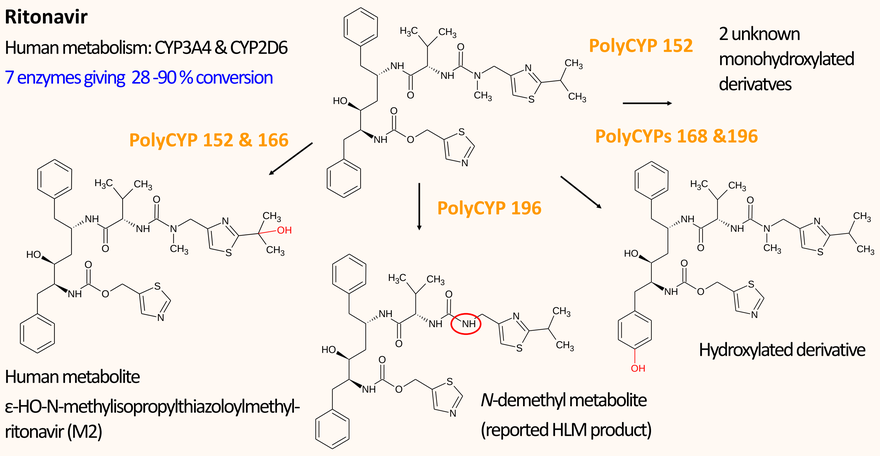
Human metabolism: CYP2C9 (CYP3A4)

5'-hydroxymethyl meloxicam (M5)
Major human *in vivo* metabolite
Max 66% conversion

5'-carboxy meloxicam (M5)
Major human *in vivo* secondary metabolite (low turnover in HLMs)
7.4% conversion

Ritonavir

Human metabolism: CYP3A4 & CYP2D6
7 enzymes giving 28 -90 % conversion



Human metabolite
ε-HO-N-methylisopropylthiazoloylmethyl-ritonavir (M2)

N-demethyl metabolite
(reported HLM product)

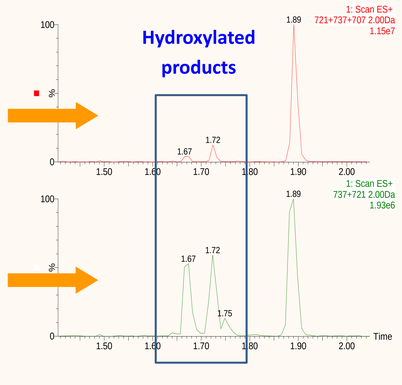
Step 2: Scale-up of reaction of interest

Multiple scale-up vials, or fresh enzyme prep, can be used to generate multi mg amounts of metabolites. For larger quantities, scale-up is achieved using a recombinant *Streptomyces* clone expressing the PolyCYP undertaking the biotransformation of interest, or the originating wild type strain.

Ritonavir scale-up

Hydroxylated derivatives of ritonavir produced in an unoptimized screening reaction with PolyCYP 168

Hydroxylated derivatives or ritonavir produced in a bioreactor by a *Streptomyces* clone containing PolyCYP 168



Conclusions

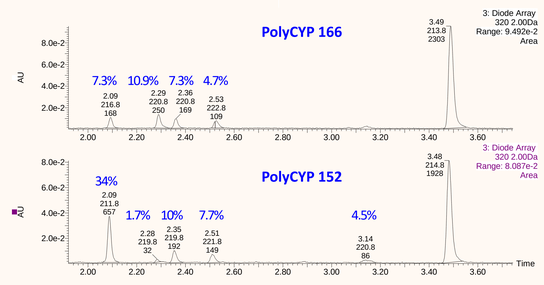
- PolyCYPs enzymes can be used to generate mg quantities of human metabolites and other hydroxylated derivatives of drugs for MetID & biological evaluation.
- Recombinant streptomycete clones expressing PolyCYP enzymes enable large scale production of synthetically challenging metabolites / derivatives.

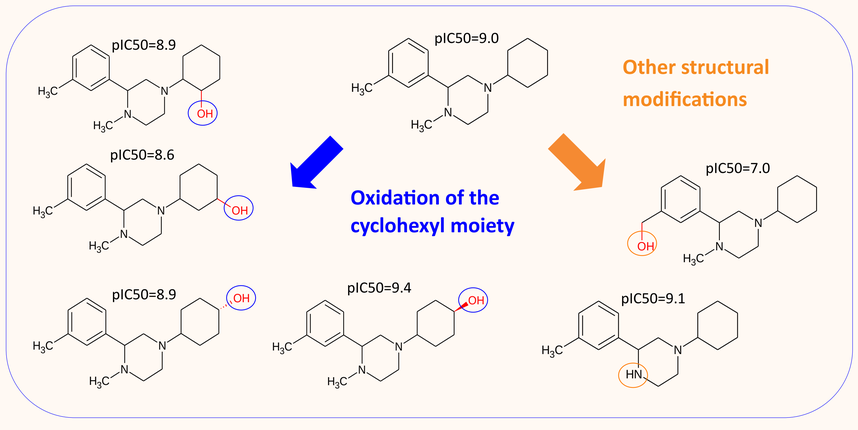
Late stage oxidation of drug compounds

- Previously we generated several derivatives of one of AstraZeneca's drug leads by microbial whole cell biotransformation.
- Analogues were characterised/quantified by 2D NMR/qNMR, and found to be derived from oxidation of the cyclohexane moiety, together with desmethyl and benzylic hydroxylated derivatives.
- Subsequently we investigated the ability of 5 PolyCYPs enzymes (6, 14, 152, 166, 168) to generate the same oxidized derivatives.

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 parent compound.
- PolyCYP 166 gave a 30% conversion to 4 monohydroxylated derivatives.
- PolyCYP 152 gave a 58% conversion to produce 5 derivatives that matched with all monohydroxylated compounds previously observed.





With kind permission from AstraZeneca, mock structures given due to confidentiality

Conclusions

- This study exploring polar SAR, indicated that polarity could be incorporated in the cyclohexyl ring and by *N*-demethylation without reducing potency. However benzylic hydroxylation had a negative effect on potency.
- PolyCYPs enzymes are an effective way to rapidly access metabolites and hydroxylated derivatives of lead compounds in parallel for late stage oxidation. These reactions can be scaled-up to provide more material for evaluation and comparison with properties of the parent drug compound.

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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. Clients routinely access our biocatalysis technology to generate phase I and II metabolites for MetID, stability testing, use as analytical standards and for producing larger amounts for pharmacological testing.