THE ONE STOP METABOLITE SHOP: EMPLOYING MULTIPLE TOOLS TO SOLVE CHALLENGING METABOLITE PROJECTS

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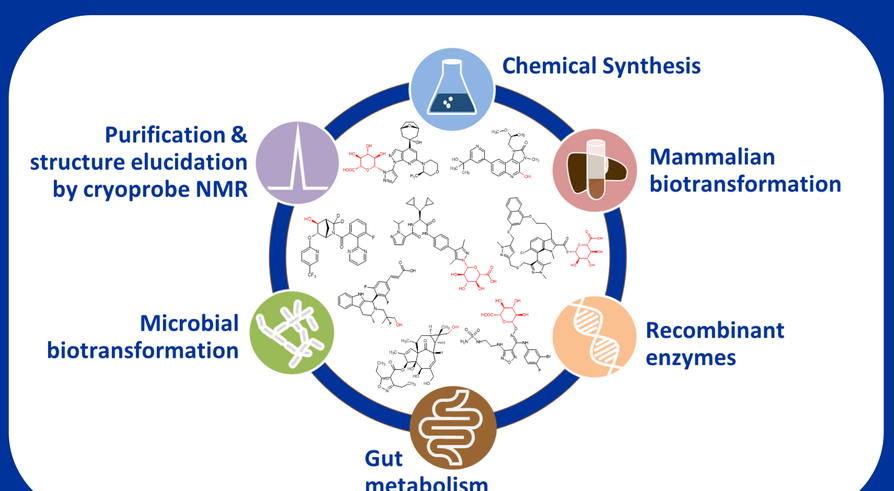


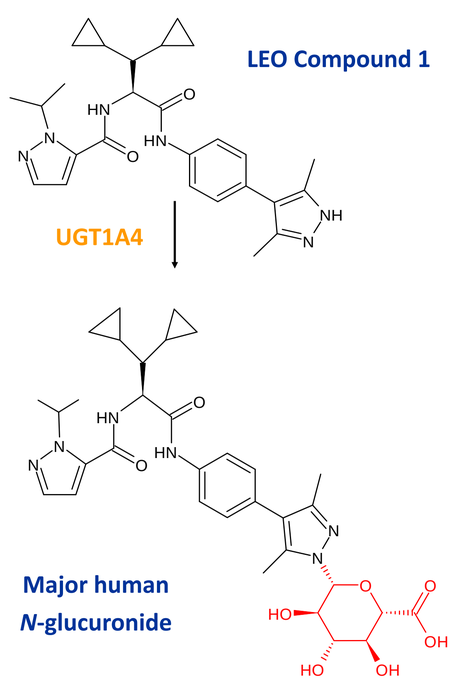
Abstract: Often several strategies are needed to access all key metabolites observed during drug development programs. Hypha has developed a "one-stop metabolite shop" scheme, which utilizes a combination of biological and chemical techniques in order to fulfil requirements to access any type of metabolites. The one-stop metabolite concept offers a parallel or sequential screening step to identify the most productive and cost-effective method to produce target metabolites. Depending on the type and quantity of metabolite required, a combination of chemical synthesis, mammalian S9, microbial biotransformation and recombinant phase I enzymes can be employed. Once the optimal production system is identified, the method can be scaled up to provide up to tens of grams of purified metabolites. The synergy of using the one-stop shop concept to access metabolites will be exemplified using recent client case studies.

Case study 1

S9 fraction biotransformation to scalable late-stage chemical synthesis of a major human *N*-glucuronide metabolite of LEO Compound 1

LEO compound 1 is an oral IL-17A protein-protein interaction modulator under development for the treatment of psoriasis and other inflammatory disorders [1].

- Metabolised through multiple Phase I and Phase II routes, including various CYP3A4 mediated hydroxylations and *N*-dealkylation, as well as *N*-sulfation and *N*-glucuronidation.
- Conjugation reactions occur in the pyrazole moiety with an *N*-glucuronide being a major metabolite in humans, observed at 14% in human hepatocytes in the MetID study.
- Only small amounts of the *N*-glucuronide are observed in other species.



Case study 2

Scale-up of gram amounts of a hydroxylated metabolite produced by PolyCYPs 152

PolyCYPs® screening kit

Scale-up vials | Bulk enzyme | Whole cell

PolyCYPs kit - screening and small scale-up (client lab)

- Hydroxylated target metabolite produced by an enzyme in a PolyCYPs screening kit at 54% conversion (done in client labs).

- Two scale-up vials of PolyCYP 152 used to generate and purify material for structure elucidation by NMR spectroscopy (2mg dosed).
- Structure not amenable to chemical synthesis.

Screening

- Due to the low aqueous solubility of LEO compound 1, the phosphorylated prodrug [2] was screened in 15 liver S9 species and 23 microbial strains.
- Higher turnover was observed for the prodrug with estimated yields of 79% in human liver S9 and 7% in microbial species Sp.45, which were shown to match by LC-MS using two different columns with acidic and alkaline pH and cone induced association to compare fragmentation patterns.

Scale-up

- The S9 route was initially scaled up due to limited availability of the prodrug substrate and the higher turnover. This yielded 76.4mg of the unlabelled glucuronide and 12.0mg of the deuterated version of the compound.
- For production of larger amounts a late stage chemical synthesis method was subsequently developed. Initially this resulted in only very low conversion, however various modifications in reaction temperature, solvent and reagent stoichiometry allowed the identification of conditions that provided a higher and cleaner conversion, increasing isolated yield from 1.1% to 8.9%.
- Scale up of the optimum method generated 4.3g of the unlabelled glucuronide at 7% isolated yield. The same method was also used to make 213.7mg of the deuterated metabolite.

Scale up (Hypha labs)

- Conversion to the metabolite confirmed with PolyCYP 152 (58%).
- Streptomyces clone and parent actinomycete tested in whole cell biotransformation (>95% conversion).
- Substrate dose escalation study (100mg - 1,000mg/L) performed with highest absolute conversion of 80% at 500mg/L parent dose.
- Purification of target from dose escalation studies yielded 128mg at >98% purity.
- Larger scale up biotransformation yielded 8g of the target hydroxylated metabolite at >98% purity for toxicity studies.

Reference

[1] Discovery of an oral, rule-of-5 compliant, IL-17A protein-protein interaction modulator (PPIm) for the treatment of psoriasis and other inflammatory diseases. Mark Andrews. Presentation at the 3rd RSC Anglo-Nordic Medicinal Chemistry Symposium, 13th-16th June 2023.

[2] K.N. Dack et al. (2020). Amino-acid anilides as small molecule modulators of IL-17. WO2020127685.

About Hypha: Hypha Discovery is a specialist CRO with expertise in the scalable synthesis, purification and identification of drug metabolites. Using our biocatalytic and organic chemistry capabilities, we also make oxidised derivatives of lead compounds and API degradation products. Hypha also has a wealth of experience in the production, purification and structure elucidation of natural products. We work with pharmaceutical and agrochemical companies globally.

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