ONE-STOP METABOLITE SHOP

Synthesis, purification and characterisation of phase 1 and 2 metabolites of drugs

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HYPHA'S ONE-STOP METABOLITE SHOP

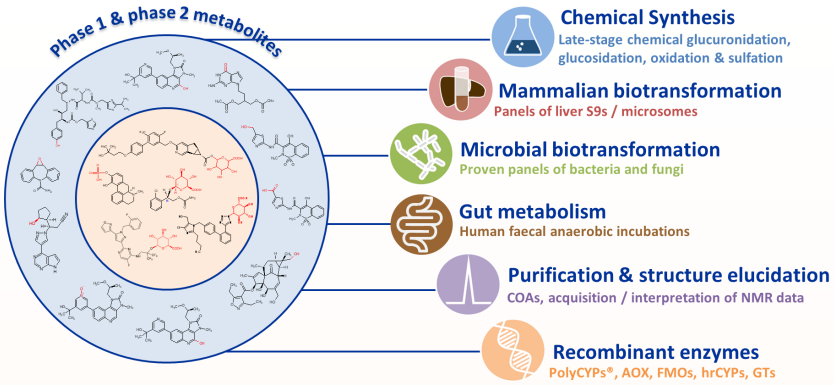
Hypha's One-Stop Metabolite Shop enables synthesis, purification and characterisation of all the main types of human and other mammalian phase 1 and 2 metabolites.

We use chemical synthesis, microbial biotransformation, mammalian tissue fractions (multiple species of S9s and microsomes) plus recombinant enzymes such as PolyCYPs, and human recombinant CYPs, AOX1 and FMOs 1 to 5.

- Phase 1 CYP and non-CYP metabolites
- Phase 2 metabolites, including *O*-, acyl, *N*- & *N*-carbamoyl glucuronides, glucosides, sulfates and other conjugates
- Multiple metabolites and multi-step metabolites
- Purification of metabolites
- Structure elucidation by cryoprobe NMR spectroscopy
- Provision of Certificates of Analysis including qNMR
- Scalable to multi-gram amounts
- Formulation know-how for poorly-soluble compounds
- Cold, stable-labelled and radiolabelled metabolites

For more information or to discuss a project email us at:

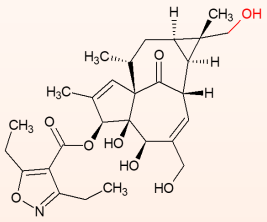
enquiries@hyphadiscovery.com

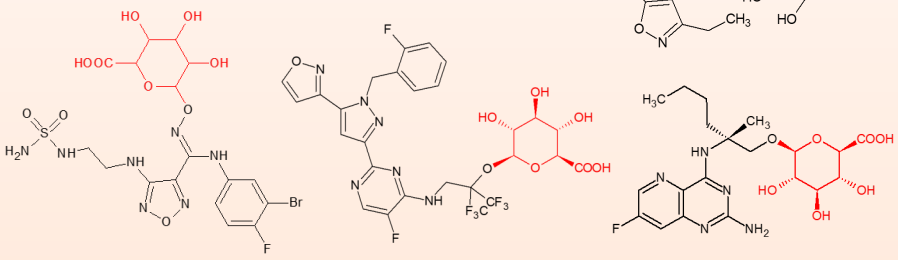


Microbial biotransformation

Hypha's microbes mimic human and other mammalian CYP and non-CYP phase 1 metabolic reactions, as well as being effective for making phase 2 conjugates. Using this approach, it is also possible to obtain metabolites formed from multiple sequential reactions in a single incubation, e.g. hydroxylation and subsequent glucuronidation.

Hundreds of milligrams of M27, the major disproportionate human metabolite of ingenol disoxate, was purified from scale-up of one of Hypha's microbes for MetID and various *in vitro* assays.



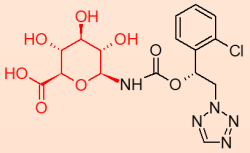


***O*-glucuronides of selgantolimod, praliciguat and epacadostat were produced for clients using microbial biotransformation and purified to > 95% purity**

Mammalian biotransformation

We use multiple species of S9s and microsomes from liver and other tissues to make metabolites that are more difficult-to-synthesise using other routes.

Late-stage chemical synthesis



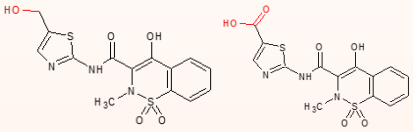
Effective late-stage chemical methods for synthesis of all types of glucuronides, glucosides and sulfated metabolites have been developed in house. Reactions are fully scalable to supply gram amounts, and are a proven and cost-effective way to access conjugated metabolites.

Oxidised metabolites and API degradation products may also be accessed through a broad range of chemical oxidation conditions, effective for oxidising α-amine, α-carbonyl, benzylic, aliphatic, *N*-alkyl and *N*-heterocycles.

Recombinant enzymes

We have a number of recombinant enzymes for making phase 1 metabolites. Our PolyCYPs® enzymes have been mined from talented actinomycete bacteria, providing a diverse set of CYPs effective for producing human and other mammalian CYP-mediated metabolites. We also have a panel of human recombinant CYPs available.

PolyCYPs+ kits contain 20 enzymes effective for producing a wide range of phase 1 metabolites. In addition to 18 PolyCYPs isoforms, the kit also contains human aldehyde oxidase (AOX1) and the main human hepatic flavin-containing monooxygenase (FMO3), with the other human FMO isoforms also available at Hypha.



Metabolites of meloxicam produced by PolyCYPs enzymes, mimicking those produced by CYP2C9/CYP3A4 in humans

Reactions are scalable either by resupply of lyophilised enzymes for mg scale production in-house, or larger scale production up to gram scale at Hypha, with optional purification and structure elucidation.

Gut metabolites

Human faecal extracts from mixed sex sources are used to make metabolites made by gut bacteria under anaerobic conditions. The technique is suitable for generation of µg to mg amounts needed for MetID and biological testing.

Purification and structure elucidation

In addition to synthesising metabolites, we can also purify them direct from biological matrices such as plasma, urine and faeces.

Hypha provides rapid and unambiguous structural identification through access to a 700MHz NMR spectrometer equipped with a 1.7mm micro-cryoprobe. This means only small amounts of metabolites are needed to acquire data sets for full structural elucidation. Our scientists are experts in data interpretation.

Stable-labelled and radiolabelled metabolites

Both stable-labelled (^{2}H or ^{13}C) and radiolabelled (^{3}H or ^{14}C) metabolites can be produced using scalable biotransformation and chemical synthesis techniques.

Client feedback

Director of Chemistry, US Pharma

"Hypha Discovery did a fantastic job synthesizing N– and O– glucuronides of our clinical stage drug substance. The project updates were detailed, our questions were answered in a timely manner, and the overall timeline was maintained. Hypha was highly recommended to us and I would not hesitate to recommend them to a colleague."

Director of DMPK, US Pharma

"Hypha Discovery has been a valuable metabolite ID partner. They *have provided answers to some of our most challenging metabolism and metabolite ID problems. We really appreciate the breadth of expertise available at Hypha Discovery and will definitely reach out for future work."*

Senior VP, US pharma

"Hypha Discovery was a huge help to our drug development timeline, when an ADME study revealed significant metabolites that were challenging to synthesize chemically. Hypha was able to rapidly reproduce the metabolites to confirm chemical structure, and then scale up to support nonclinical testing and bioanalytical method development, with far greater speed than chemical synthesis could achieve. The Hypha people were very pleasant to work with and the material they produced was of very high quality, which rounded out an overall great experience. I would recommend them without reservation."

HYPHA DISCOVERY

Metabolite Experts

Further reading

Selected Hypha publications

Shanu-Wilson, J., Evans, L., Wrigley, S., Steele, J., Atherton, J., Boer, J., **2020.** Biotransformation: Impact and Application of Metabolism in Drug Discovery. ACS Medicinal Chemistry Letters, 11: 2087-2107.

Evans, L., Phipps, R., Shanu-Wilson, J., Steele, J., Wrigley, S., **2020.** Chapter 4 Metabolite generation and characterization by NMR. In: Identification and quantification of drugs, metabolites, drug metabolizing enzymes and transporters. Second edition. Eds Shuguang Ma and Swapan Chowdhury. Elsevier Science. ISBN: 9780128200186.

Salter, R., Beshore, D.C., Colletti, S.L., Evans, L., Gong, Y., Helmy, R., Liu, Y., Maciolek, C.M., Martin, G., Pajkovic, N., Phipps, R., Small, J., Steele, J., de Vries, R., Williams, H., Martin, I.J., **2018.** Microbial biotransformation – an important tool for the study of drug metabolism. Xenobiotica, 49:8, 877-886.

Contact us

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