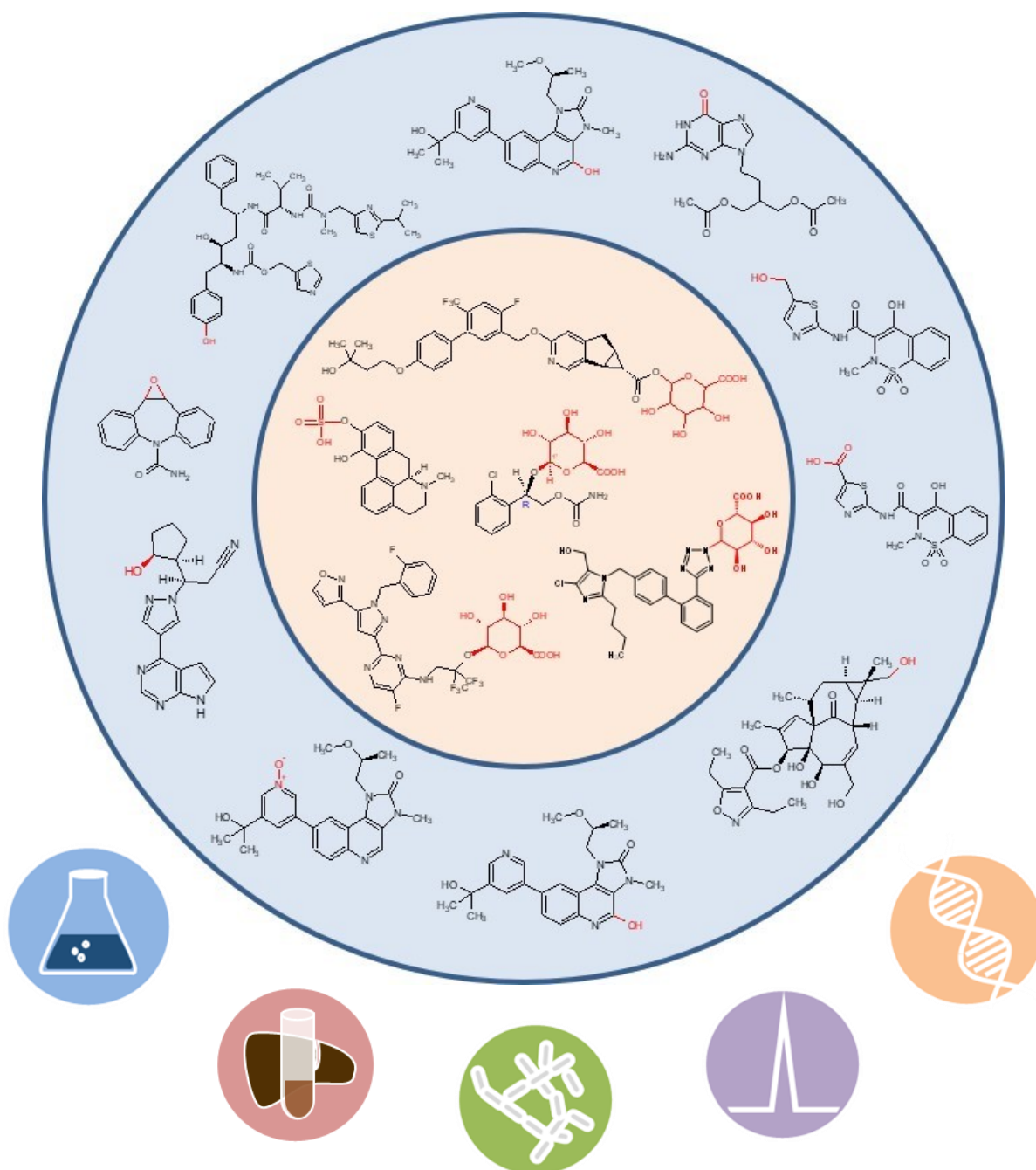


One-Stop Metabolite Shop



Synthesis, purification and characterization of phase 1 and 2
metabolites of drugs and agrochemicals



HYPHA'S ONE-STOP METABOLITE SHOP

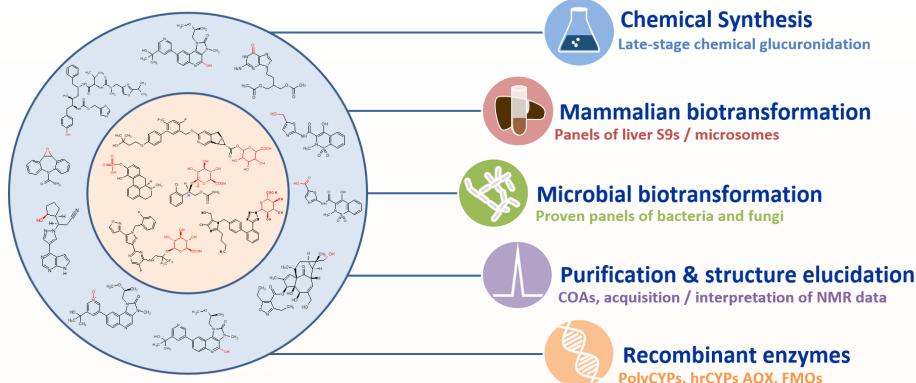
Hypha's One-Stop Metabolite Shop enables synthesis, purification and characterization of all the main types of mammalian phase 1 and 2 metabolites.

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, sulfates and other conjugates
- Multiple metabolites and multi-step metabolites
- Purification of metabolites
- Structure elucidation by NMR Spectroscopy
- Provision of Certificates of Analysis
- 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:

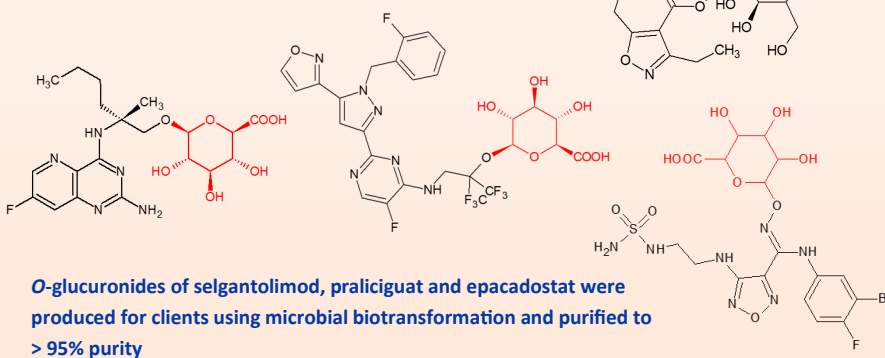
enquiries@hyphadiscovery.com



Microbial biotransformation

Hypha's microbes mimic human and other mammalian CYP and non-CYP phase 1 metabolic reactions, including aromatic and aliphatic hydroxylation, as well as being effective for conjugative reactions. 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 Met1D and various *in vitro* assays.



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



Mammalian biotransformation

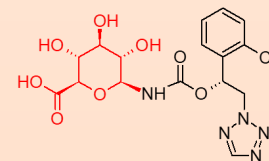
We use multiple species of S9s and microsomes from liver and other tissues to make metabolites that are more difficult-to-synthesize using other routes.

1g of the major *N*-glucuronide of cenobamate was synthesized by late-stage chemical glucuronidation



Late-stage chemical synthesis

We have developed late-stage chemical methods for synthesis of all types of glucuronides, and of sulfated metabolites. Reactions are fully scalable to supply gram amounts, and are a proven and cost-effective way to access conjugated metabolites synthesized using these methods.





Recent 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."



Recombinant enzymes

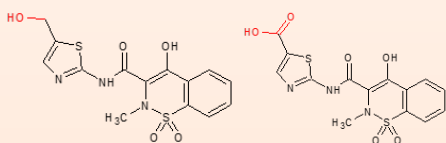
A number of recombinant enzymes are used to produce phase 1 metabolites. These include PolyCYPs® enzymes which have been mined from some of our talented actinomycete bacteria, providing a diverse set of CYPs effective for producing human and other mammalian CYP-mediated metabolites. We also have a panel 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 enzymes, 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.



PolyCYPs+ kits

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



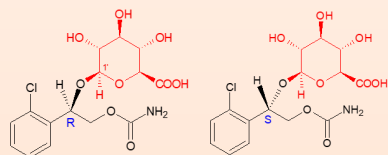
Metabolites of meloxicam produced by PolyCYPs enzymes, mimicking those produced by CYP2C9/CYP3A4 in humans



Purification and structure elucidation

In addition to making metabolites, we also can purify them from biological matrices such as urine.

Hypha uses modern NMR instruments and state-of-the-art probe technology for 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. Interpretation of the data can be performed by clients or by our in-house experts.



R and S epimers of carisbamate glucuronides which were purified from urine, and structures assigned from interpretation of NMR spectra



The Metabolite Experts

Further reading

Recent Hypha Publications

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.

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.

Contact us

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