ONE-STOP METABOLITE SYNTHESIS SERVICES AND KITS

Scalable Synthesis, Purification and Structure Elucidation of Drug Metabolites

www.hyphadiscovery.com

HYPHA'S ONE-STOP METABOLITE SERVICES

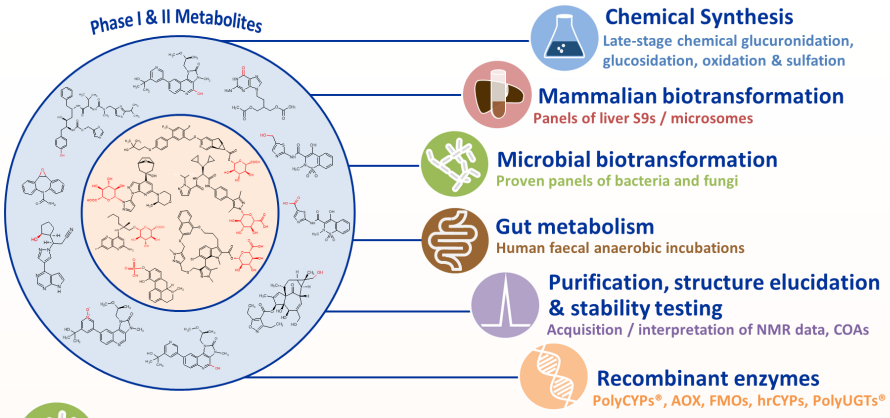
Hypha's One-Stop Metabolite Services enables synthesis, purification and characterisation of all the main types of human and other mammalian phase I and II metabolites.

We use chemical synthesis, microbial biotransformation, mammalian tissue fractions (multiple species of S9s and microsomes) plus proprietary recombinant enzymes such as PolyCYPs® and PolyUGTs®, and human recombinant CYPs, AOX and FMOs.

- **Phase I CYP and non-CYP metabolites**
- **Phase II metabolites, including *O*-, acyl, *N*- & *N*-carbamoyl glucuronides, glucosides, sulfates and other conjugates**
- **Gut metabolites using human faecal anaerobic incubations**
- **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**
- **Unlabelled, stable-labelled and radiolabelled metabolites**
- **Stability testing**

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

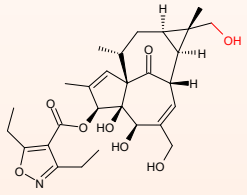
enquiries@hyphadiscovery.com

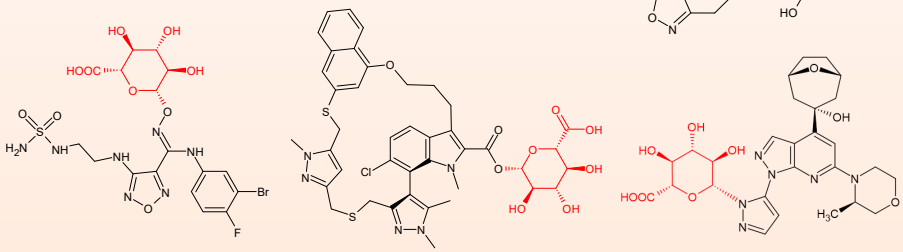


Microbial biotransformation

Hypha's microbes mimic human and other mammalian CYP and non-CYP phase I metabolic reactions, as well as being effective for making phase II 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 a scale-up of one of Hypha's microbes for MetID and various *in vitro*





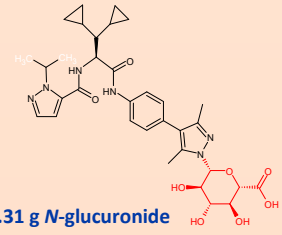
Glucuronides of epacadostat, AZD5991 and camonsertib were produced for clients using microbial biotransformation and purified to > 95% purity

Mammalian biotransformation

We use multiple species of S9 and microsomes from liver and other tissues to make metabolites that are more difficult to synthesise using other routes.

Late-stage chemical synthesis

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.



4.31 g *N*-glucuronide of LEO compound 1 synthesised

Oxidised metabolites and API degradation products may also be accessed using a range of chemical oxidation techniques, including electrochemistry and photochemistry.

Metabolite Kits

PolyCYPs+ and PolyUGTs Screening and Scale-up Kits

Ready-to-use kits for synthesis of oxidised metabolites and glucuronides.

- **Patented recombinant microbial enzymes in a kit format**
- **Derived from bacteria in Hypha's culture collection**
- **Produce a wide diversity of human CYP -derived metabolites & glucuronides**
- **Come with all reagents needed, including co-factors, a control compound, a 24-well reaction plate and seal**
- **Easy to use - reagents are provided as lyophilised powders - just add water and incubate**
- **Readily scalable, either in-house or outsource to Hypha**

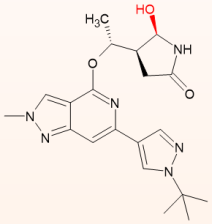
Recombinant enzymes

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

PolyCYPs+ kits contain 20 enzymes effective for making phase I metabolites including 18 PolyCYP isoforms, human aldehyde oxidase and the main human hepatic flavin-containing monooxygenase FMO3. Other FMOs are available at Hypha.

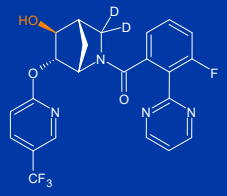
PolyUGTs kits contain 11 isoforms for making a variety of glucuronides. UDPGA co-factor is included.

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.



Major CYP3A4 metabolite of BI 894416 made in a client lab using PolyCYP 152

PolyCYPs: For CYP, AO & FMO3 metabolites



Gut metabolites

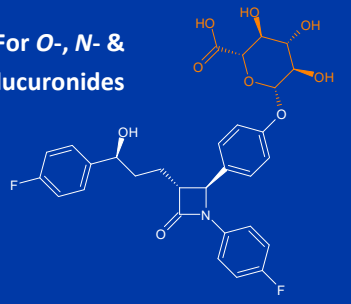
Human faecal extracts from mixed sex sources are used to make metabolites made by gut bacteria under anaerobic conditions. The technique is suitable for generation of µg to mg amounts needed for MetID and biological testing.

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 micrograms of metabolites are needed to acquire data sets for full structural elucidation. Our scientists are experts in data interpretation, and our reports are used in submission documents to regulatory authorities.

PolyUGTs: For *O*-, *N*- & acyl glucuronides



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.

Order kits via PO, VWR (Avantor) or Fisher Scientific

HYPHA DISCOVERY

Metabolite Experts

Further reading

Selected Hypha publications

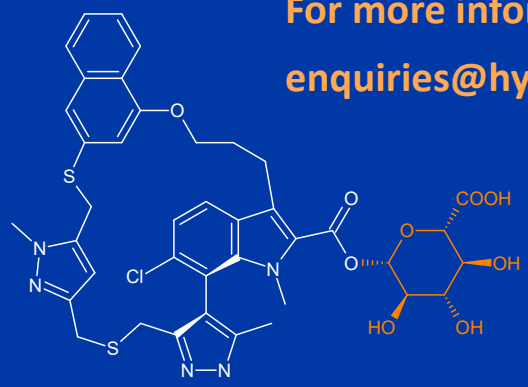
Shanu-Wilson J, Coe S, Evans L, Steele J, Wrigley S. Small molecule drug metabolite synthesis and identification: why, when and how? Drug Discovery Today. **2024**; 29(5):103943.

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

Contact us



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