ONE-STOP METABOLITE HUB

MetID Services

Multi species *in vitro* panels

LC-MS/MS metabolite profiling

Metabolite synthesis and identification

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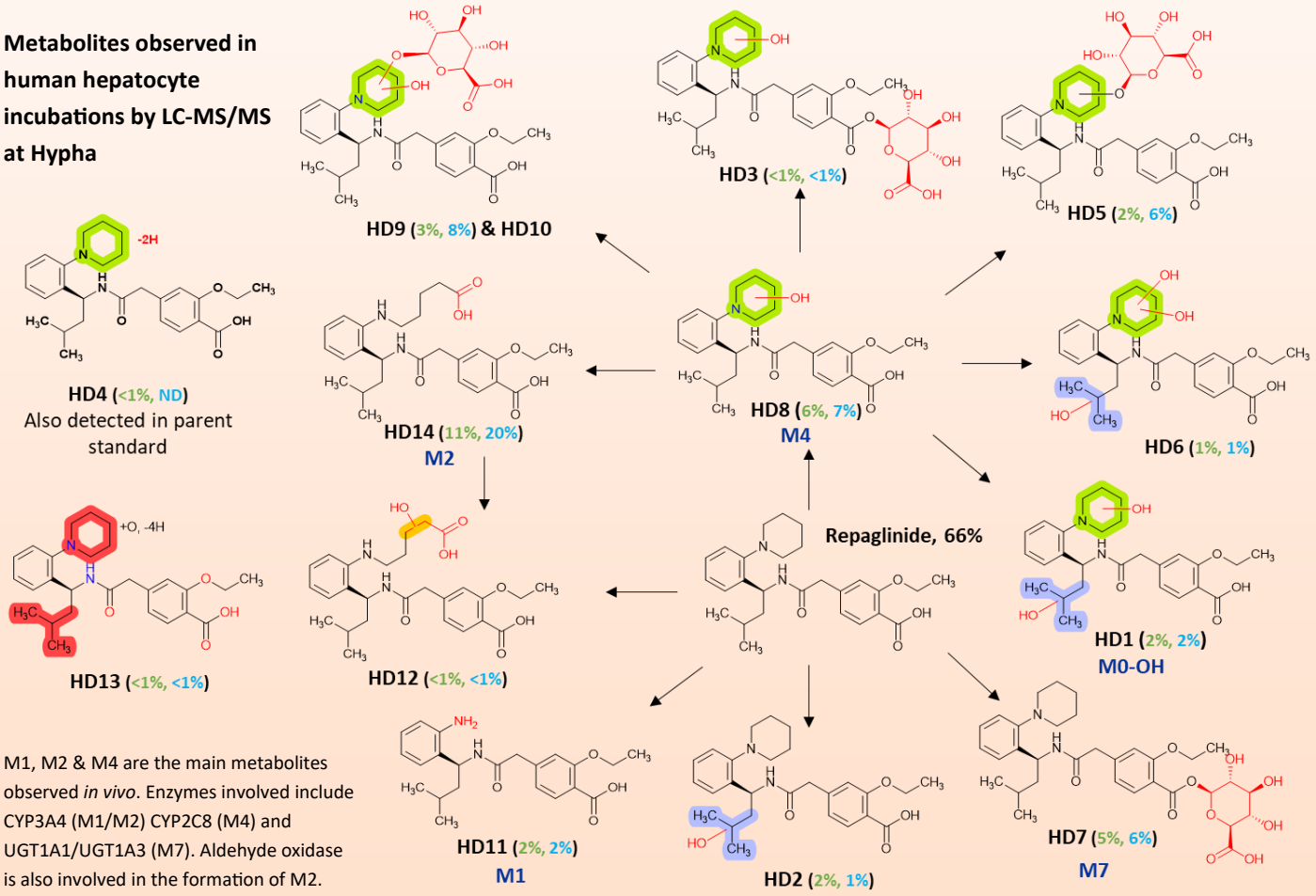
MetID Services: Part of Hypha's One-Stop Metabolite Hub

Hypha offers a range of MetID packages tailored to client requirements. We can generate metabolites using a variety of multi species *in vitro* systems such as hepatocytes and microsomes, including use of methods suitable for slowly metabolised compounds. We utilise a Thermo Scientific Vanquish Horizon UHPLC and Orbitrap Exploris™ 120 LC-MS/MS system, which enables the detection and identification of a wide spectrum of both expected and unexpected metabolites, including those present at low abundance. Its sub-ppm mass accuracy and high resolution allow for differentiation of isobaric compounds, providing high confidence in metabolite identification.

Case study – MetID of repaglinide

Metabolism of repaglinide (5 µM) was studied in human and rat cryopreserved hepatocytes (1 million cells/mL) at 37°C for 120 mins, with subsequent analysis by LC-MS/MS in positive and negative ionisation modes. In human hepatocytes, 14 metabolites were detected. The 3 main *in vitro* metabolites reported by Gan *et al.* (M2, M4 and the glucuronide M7) corresponded to the top 3 metabolites (HD14, HD8, HD7) observed in this study. Ten other metabolites were annotated, arising from oxidation of the aliphatic side chain, oxidative dehydrogenation, further oxidations and secondary glucuronidation. Only 6 of the human metabolites were detected in rat hepatocyte incubations (data not shown), including M2 and M7 but not M4. The de-ethylated metabolite M5 was only seen in rat hepatocytes.

Metabolites observed in human hepatocyte incubations by LC-MS/MS at Hypha

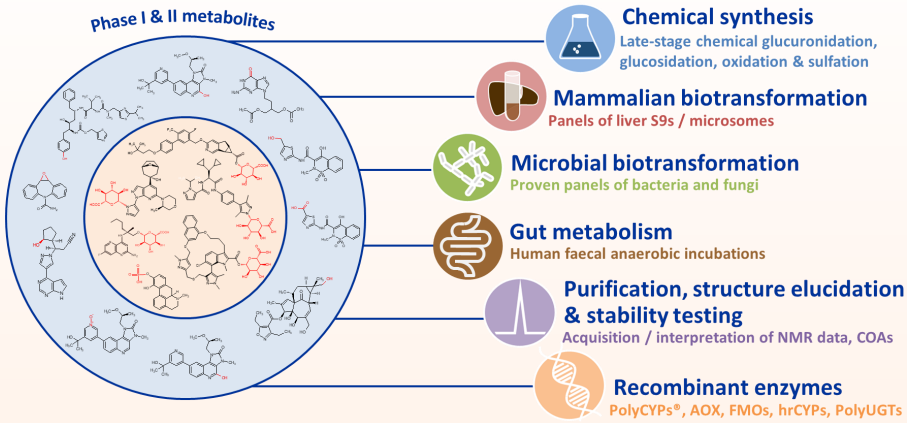


M1, M2 & M4 are the main metabolites observed *in vivo*. Enzymes involved include CYP3A4 (M1/M2) CYP2C8 (M4) and UGT1A1/UGT1A3 (M7). Aldehyde oxidase is also involved in the formation of M2.

Black: HD assigned metabolite number with % MS peak response (+ve, -ve ionisation mode)
Blue: Metabolite numbers reported in the literature

Refs: Gan *et al.* Br. J. Clin. Pharmacol. 2010; 70(6): 870-880. Säll *et al.*, Drug Metab. Dispos. 2012; 40(7): 1279-1289.

Hypha's One-Stop Metabolite Shop - multiple techniques under one roof



Routes to accessing metabolites

Users of Hypha's MetID services benefit from Hypha's world-leading metabolite synthesis service using our proven scalable methods. In addition to providing the LC-MS/MS MetID data, we can make metabolites for definitive identification by NMR spectroscopy, for further testing, or for use as reference standards.

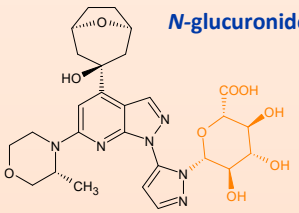
Techniques encompass the use of scalable surrogate biotransformation systems such as microbes and recombinant enzymes as well as proprietary late-stage chemical methods. In addition, gut metabolites can be accessed using our human and rat faecal incubation system.

Structure elucidation by NMR spectroscopy

For definitive MetID we elucidate structures using NMR spectroscopy. Typically only tens of micrograms are needed for acquiring full data sets using a 700 MHz machine in conjunction with a 1.7mm micro-cryoprobe. We also routinely use qNMR to assess the purity of the metabolites we produce.

Camonsertib

***N*-glucuronide**



The position of glucuronidation of camonsertib was not possible to assign using LC-MS/MS or prediction software. Microbial biotransformation was used to scale-up the UGT1A4-derived metabolite to provide material for structure elucidation by NMR spectroscopy, stability studies, and for use as a standard for bioanalysis.

MetID Services

Hypha's expertise spans a full range of services for confident MetID. From identifying metabolic soft spots of discovery stage compounds to conducting more in depth biotransformation studies for lead compounds and beyond, our capabilities support critical stages of drug development.

- **Multi species *in vitro* panels**
- **Hepatocytes, microsomes, S9 etc.**
- **Biofluids and tissues from *in vivo* studies, e.g. plasma, urine etc.**
- **Metabolite profiling and ID by LC-MS/MS**
- **Anaerobic faecal incubation system**
- **HμREL® coculture system (human hepatocytes and stromal cells) for slowly metabolised compounds**
- **Multiple scalable biotransformation and chemical synthesis methods for accessing Phase I, Phase II and mixed phase metabolites**
- **Metabolite purification and Certificates of Analysis**
- **Definitive structure elucidation by cryoprobe NMR spectroscopy**
- **qNMR for bioanalysis standards**
- **Unlabelled, stable-labelled and radiolabelled metabolites**
- **Stability testing**

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

enquiries@hyphadiscovery.com

HYPHA DISCOVERY

Metabolite Experts

Recent papers

- Song F, Chen J, Dallas S, Lam D, Lim HK, Zhou R, Kokubun T, Phipps R, Steele J, Salter R. Biosynthesis and structure assignment of a hydroxylated metabolite of the orexin-1 receptor antagonist JNJ-61393215. Bioorganic & Medicinal Chemistry, **2025,** 121: 118130.
- Papp R, Trimble L, Fretland AJ, Manohar R, Phipps R, Kvaerno L, Perryman AL, Reynolds G and Black WC. *N*-Glucuronide Metabolite of Camonsertib. Drug Metabolism and Disposition , **2024**, 52(5): 368-376.
- 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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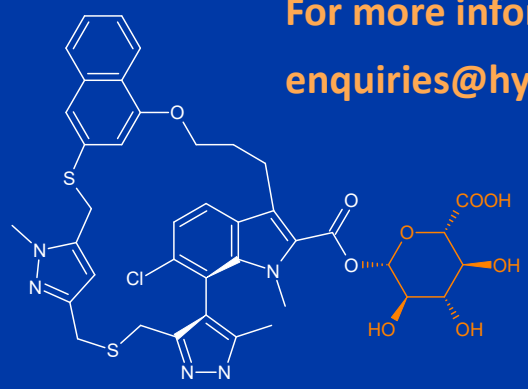

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