



ONE-STOP METABOLITE HUB
MetID Services

Multi species *in vitro* panels

LC-MS/MS metabolite profiling

Metabolite synthesis and identification by NMR

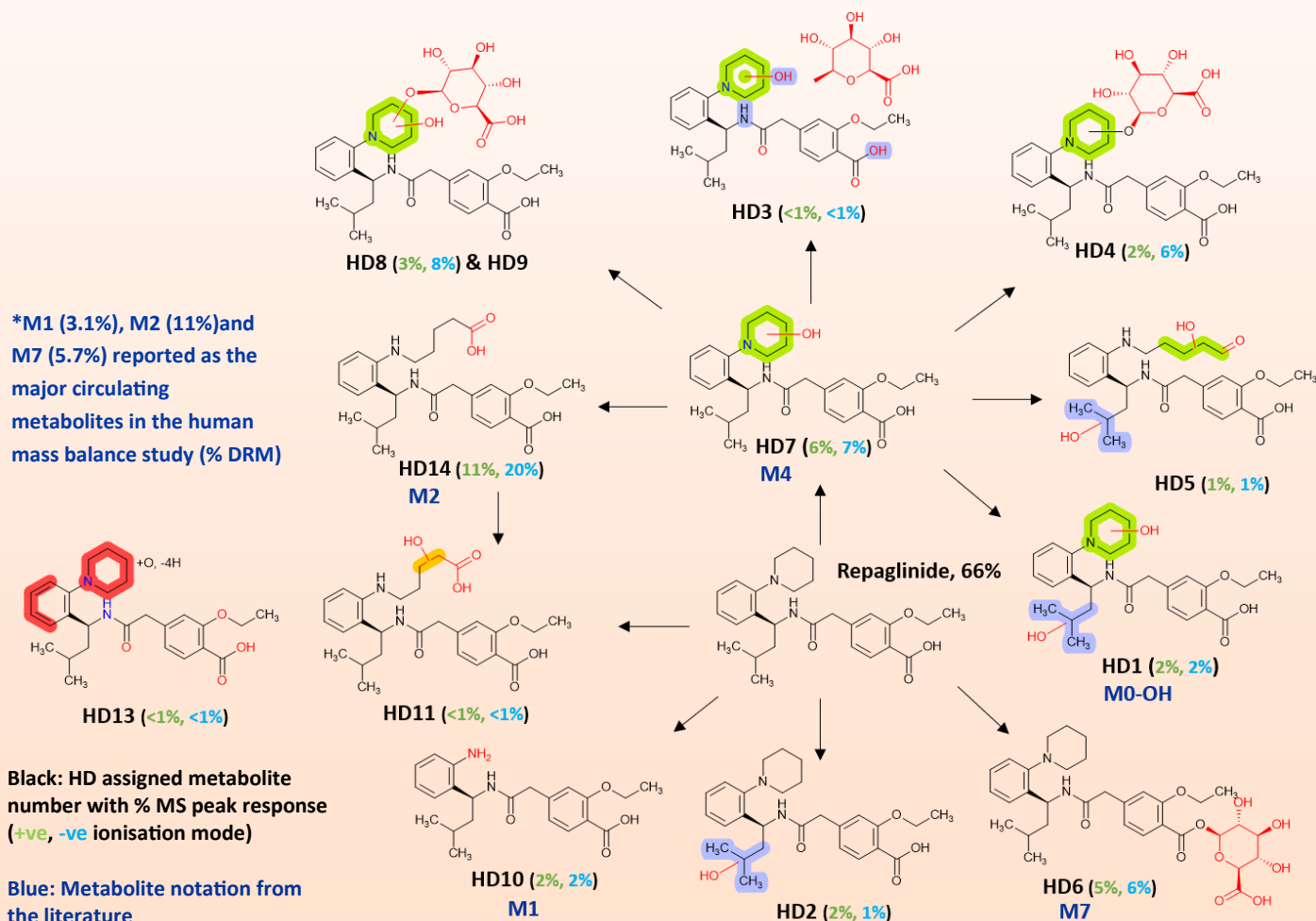
www.hyphadiscovery.com

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

Hypha offers a range of MetID packages tailored to client requirements - see our table of service tiers on the back page. 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, 13 metabolites were detected, including the 3 major metabolites reported in the literature. 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.

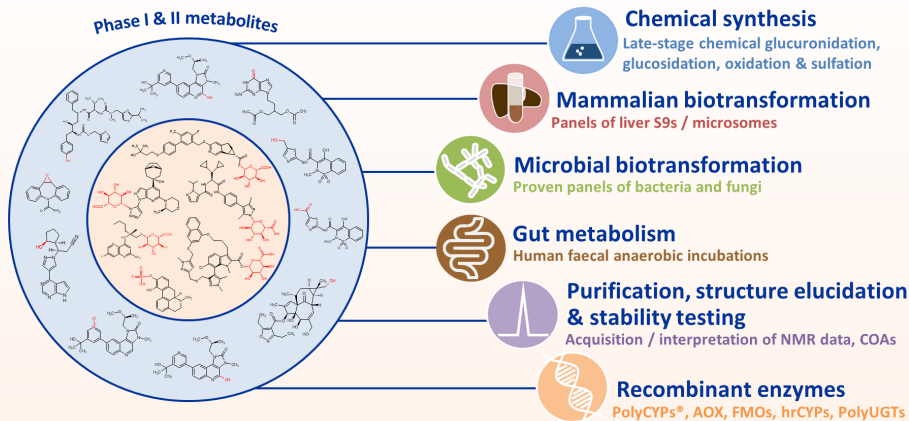


Synthesis by PolyCYPs and PolyUGT enzymes (% conversions are calculated UV data with a substrate dose at 100 μg/ml)

13 metabolites were produced by PolyCYPs including major metabolites M1 (83%, PC359), M0-OH (8%, PC359), M2 (39%, PC486) and M4 (42%, PC349). M7 was produced by multiple PolyUGTs e.g. PolyUGT194 (21%). Oxidised / secondary glucuronides could be made using a combination of PolyCYPs and PolyUGTs.

*FDA Prescribing Information for repaglinide: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/020741s0441bl.pdf

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



Routes to accessing metabolites

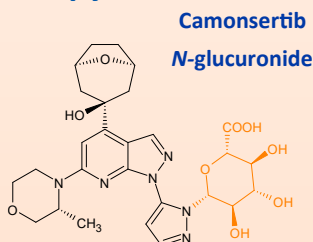
Users of Hypha's MetID services benefit from Hypha's world-leading metabolite synthesis service using our proven scalable methods. In addition to providing the LC-MS/MS MetID data, we can make metabolites for definitive identification by NMR spectroscopy, for further testing, or for use as reference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

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 small molecules 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 (human & rat)
- Coculture systems (human hepatocytes and stromal cells) for slowly metabolised compounds
- Multiple scalable chemical synthesis and biotransformation methods for accessing Phase I, Phase II and mixed phase / multi-step metabolites
- Metabolite purification and Certificates of Analysis
- Definitive structure elucidation by cryoprobe NMR spectroscopy
- qNMR for bioanalysis standards
- Stable-labelled metabolites
- Stability testing (informal)



For more information or to discuss a project email us at:
enquiries@hyphadiscovery.com

MetID Service Tiers

| Tier | Package | Best suited for | Typical deliveries |
|----------------|---|---|---|
| Tier 1 | Rapid Discovery MetID | Early discovery screen; quick view of major metabolites | Parent + approx. 3-5 most abundant metabolites; concise report |
| Tier 2 | Enhanced Discovery MetID | Broader discovery-level profile across selected systems/species | Main metabolites, e.g. top 10 or agreed threshold; relative abundance table |
| Tier 3A | Comprehensive In Vitro MetID | More complete profile for lead optimisation / candidate selection | Main metabolites plus relevant minor metabolites listed separately |
| Tier 3B | Comprehensive + MS/MS Interpretation | When stronger structural support is required for key metabolites | Tabular profile plus EICs, spectra and key fragment interpretation |
| Tier 4 | In Vivo Sample MetID | Submitted plasma, urine or other biological samples | Bespoke in vivo profile; AUC-pooled plasma where appropriate |

Contact us

For more information or to discuss a project email us at enquiries@hyphadiscovery.com

