Integrated Metabolite Identification and Biocatalytic Synthesis: Case Studies with Repaglinide and PROTAC MZ-1

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Abstract: **Metabolite identification (MetID) is essential for understanding the metabolic fate of drugs in discovery and development. This poster illustrates an integrated workflow combining LC–MS/MS-based metabolite profiling and scalable biocatalysis with PolyCYPs® and PolyUGT enzymes, which facilitates rapid access to metabolites for structure elucidation by NMR, quantitation and biological profiling. MetID case studies conducted in hepatocytes with repaglinide and the PROTAC tool molecule MZ-1 are used to illustrate the process.**

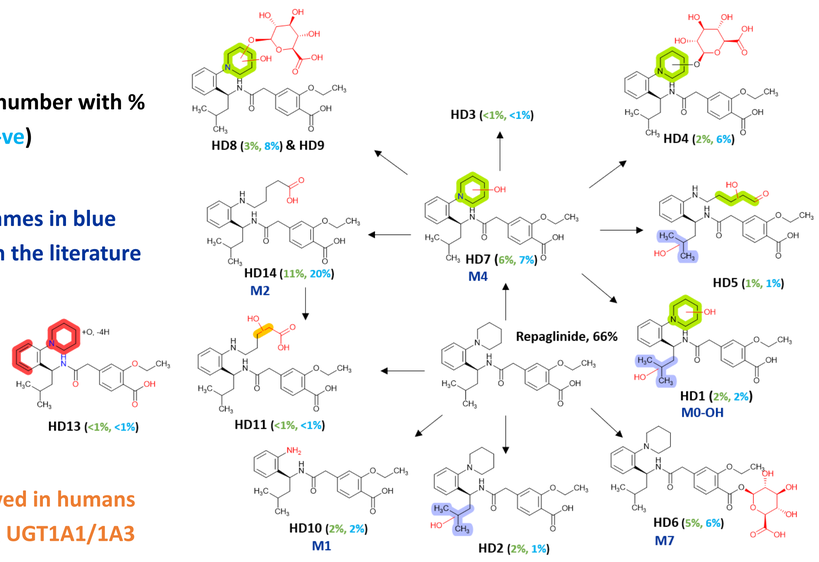
MetID of Repaglinide

- LC-MS/MS profiling of 5µM repaglinide in human and rat heptatocytes (2h) was performed in positive and negative ionisation modes.
- 13 metabolites were detected in human hepatocytes above 1%, including the major circulating CYP derived metabolites M1 and M2, together with the acyl glucuronide M7*.

HD assigned metabolite number with % MS peak response (+ve, -ve)

Metabolite numbers / names in blue refer to those reported in the literature

Reported enzymes involved in humans include CYP3A4, CYP2C8, UGT1A1/1A3

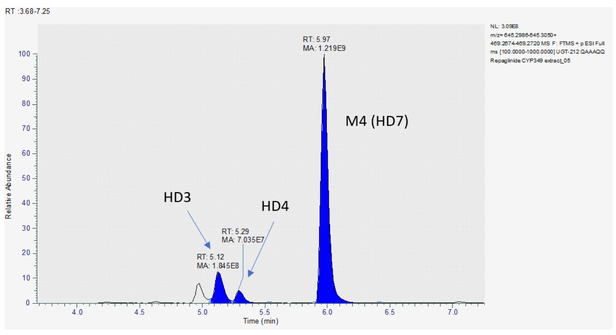


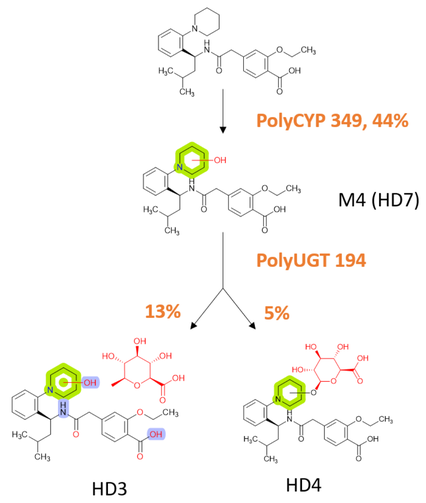
- Biocatalysis screening using recombinant PolyCYPs and PolyUGTs enzyme systems reproduced the key human metabolites at good conversion yields, supporting scalable production.

M1 (HD10)	M0-OH (HD1)	M2 (HD14)	M4 (HD7)	M7 (HD6)	
2%	2%	11%	6%	5%	% conversions
83%	8%	38%	42%	21%	Human hepatocytes
PolyCYP 359	PolyCYP 359	PolyCYP 486	PolyCYP 349	PolyUGT 194	PolyCYPs/PolyUGTs
					Best isoform

PolyCYPs / PolyUGTs screened at 225 µM repaglinide (x45 more concentrated than run in hepatocytes)

- Using the two enzyme systems in tandem resulted in the generation of indirect glucuronides. Indirect glucuronides need oxidation of the parent drug first followed by glucuronidation.





The best system for making the indirect glucuronides (HD3 & HD4) of repaglinide is a sequential process with PolyCYP 349 followed by incubation of the crude extract with PolyUGT 194.

*M1, M2 and the acyl glucuronide M7 are reported at major circulating metabolites in humans (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/020741s044lbl.pdf).
% DRM in plasma (human mass balance study): M1 3.1%, M2 11%, M7 5.7%. All others < 2%.

About Hypha: **Hypha Discovery is a specialist CRO supporting pharmaceutical and agrochemical companies worldwide through LS-MS/MS based MetID, scalable metabolite synthesis and structure elucidation by NMR spectroscopy.**

MetID of PROTAC tool molecule MZ1

CYP3A4-mediated oxidation
Fused 3-ring core
Thiophene ring

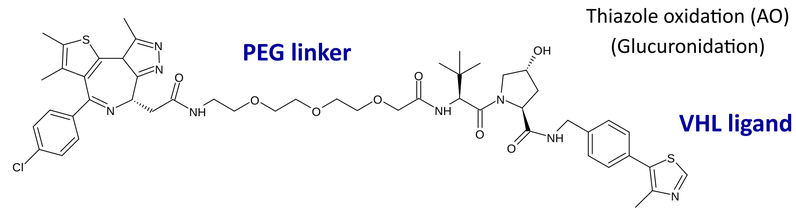
Oxidative *O*-dealkylation
Hydroxylation

CYP3A4 & AO mediated oxidation
Tert butyl group
Hydroxyproline ring
Thiazole oxidation (AO)
(Glucuronidation)

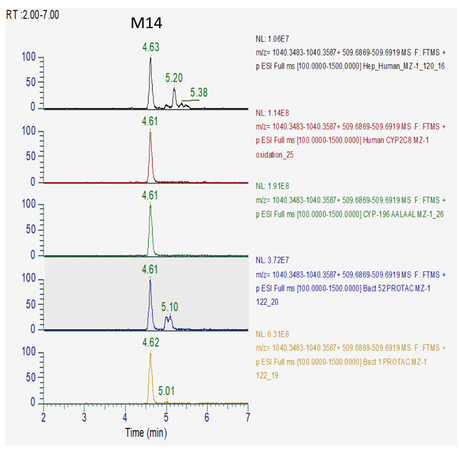
JQ1 warhead

PEG linker

VHL ligand



- LC–MS/MS (+ve mode) profiling of MZ1 across multiple species (human, monkey, dog, and rat) hepatocytes (2h) identified 18 metabolites, with 16 observed in human hepatocytes.
- Metabolic pathways were dominated by PEG linker cleavage, oxidative transformations, *N*-dealkylation, and amide hydrolysis, with no human-specific metabolites detected.
- 3 hydroxylated metabolites were detected; two with hydroxylation in the VHL ligand (M17 & M18) and one with hydroxylation in the fused three-ring core of the JQ1 warhead (M14).
- Screening with a PolyCYPs+ kit and a panel of human recombinant CYPs and microbial strains generated an array of oxidised metabolites, including high-conversion to M14 by PolyCYP196. The same hydroxylation was undertaken by CYP2C8 and by PolyCYP196.



Human hepatocytes

hrCYP2C8 (M14 also produced by CYP2C9)

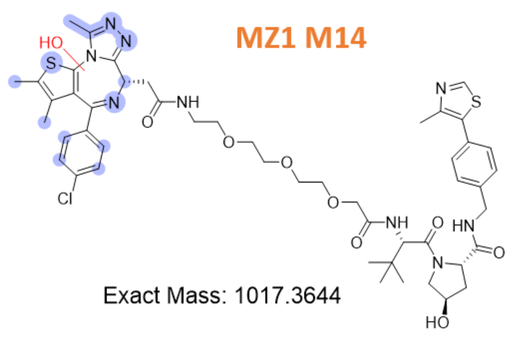
PolyCYP 196

Microbial Sp52

Microbial Sp1

64% conversion by MS response by PolyCYP 196 in the screen at 100 mg/L MZ1 dose

PolyCYP 196 scale-up



- Scale-up of the PolyCYP196 reaction is in progress to generate material for purification and structure elucidation by NMR spectroscopy to pinpoint the position of hydroxylation in the warhead moiety.

Conclusions

- These case studies demonstrate the effectiveness of integrating metabolite profiling with biocatalytic enzymatic platforms to enable rapid identification, scalable production, and structural confirmation of drug metabolites.
- Biocatalysis provides a rapid route to accessing key metabolites for activity testing and evaluation of DMPK properties. This unified approach enhances confidence in metabolite characterisation and supports informed decision-making in drug development.

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