



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 PolyUGTs® enzymes, which facilitates quick access to metabolites for structure elucidation by NMR, quantitation and biological profiling. MetID case studies conducted in hepatocytes with the CYP2C8 probe tool compound repaglinide and the PROTAC tool molecule MZ1 are used to illustrate the process. Biocatalysis provides a rapid route to accessing key metabolites for activity testing and evaluation of DMPK properties, or for use in analytical method development or as reference standards. This unified approach enhances confidence in metabolite characterization and supports informed decision-making in drug development.

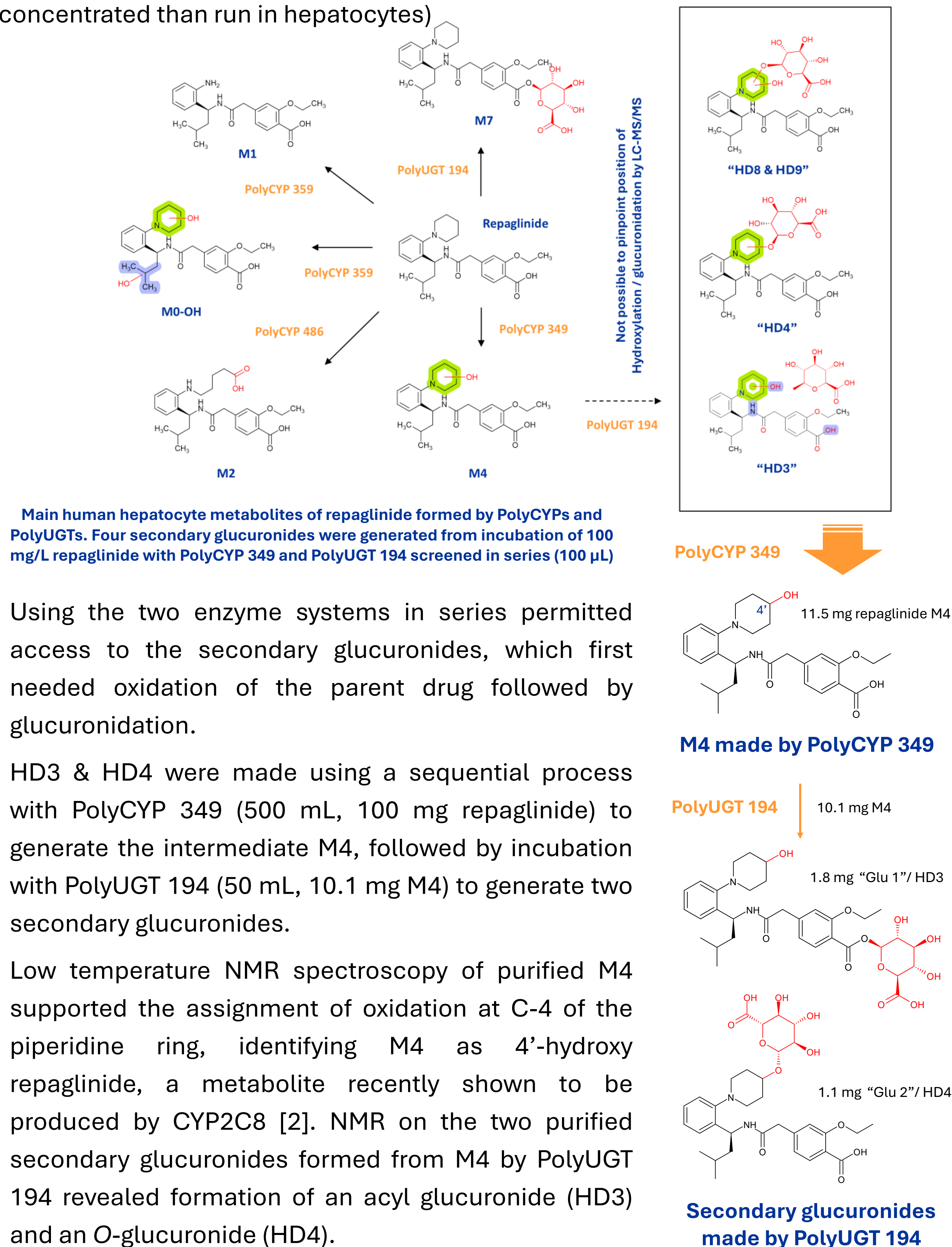
MetID of repaglinide

- LC-MS/MS profiling of 5 μ M repaglinide in human and rat hepatocytes (2 h) was performed in positive and negative ionization modes.
- 13 metabolites were detected in human hepatocytes above 1% MS peak area, including the major named circulating CYP derived metabolites M1 and M2, together with the acyl glucuronide M7 [1].
- Reported enzymes involved in humans are CYP3A4, CYP2C8 and UGT1A1/1A3.
- Biocatalysis screening using recombinant PolyCYPs and PolyUGTs enzyme systems reproduced the key human metabolites at good conversion yields.

M1 (HD10)	M0-OH (HD1)	M2 (HD14)	M4 (HD7)	M7 (HD6)	% conversions
2%	2%	11%	6%	5%	Human hepatocytes
83%	8%	38%	42%	21%	PolyCYPs/PolyUGTs

PolyCYP 359 PolyCYP 359 PolyCYP 486 PolyCYP 349 PolyUGT 194 Best isoform

- PolyCYPs & PolyUGTs were incubated with 225 μ M repaglinide (x45 more concentrated than run in hepatocytes)



Take-home message

Sequential use of PolyCYPs and PolyUGTs enabled generation of previously uncharacterized secondary glucuronides of a drug compound for definitive structure elucidation by NMR spectroscopy.

MetID of PROTAC tool molecule MZ1

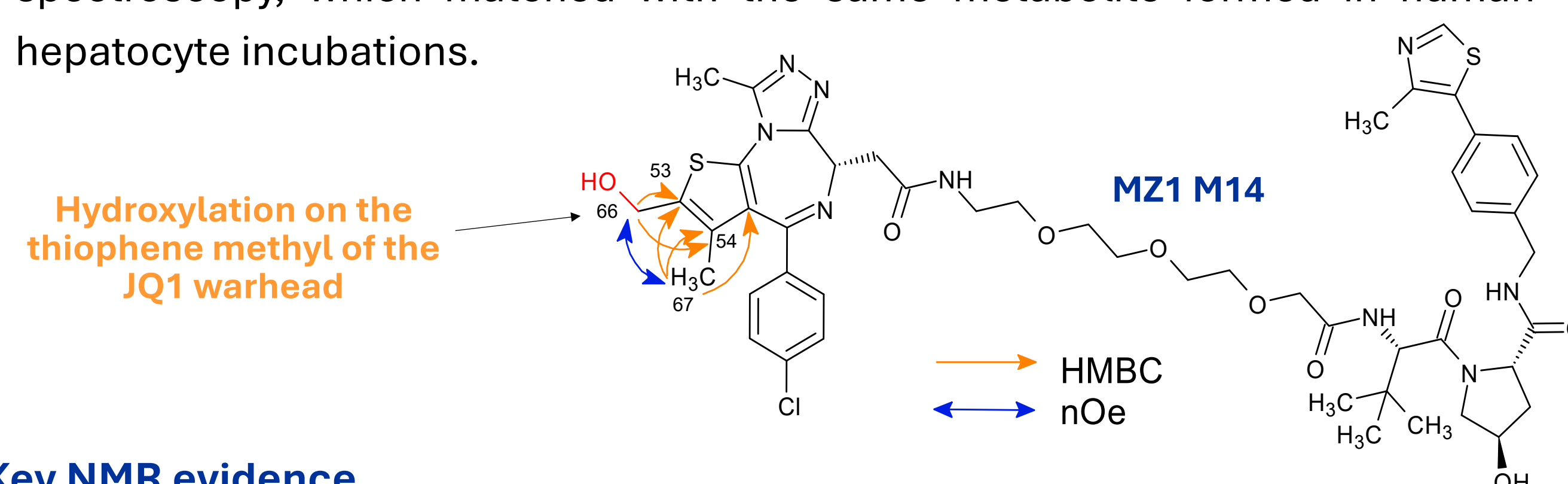
- LC-MS/MS (+ve mode) profiling of the PROTAC MZ1 (5 μ M) across multiple species (human, monkey, dog, and rat) hepatocytes (2 h) identified 18 metabolites, with 16 observed in human hepatocytes.
- Metabolic pathways were dominated by PEG linker cleavage, oxidative transformations, N-dealkylation, and amide hydrolysis, with no human-specific metabolites detected.

- Of the three hydroxylated metabolites detected, two were hydroxylated in the VHL ligand (M17 and M18) and one was hydroxylated in the fused three-ring region of the JQ1 warhead (M14).

- Screening with a panel of PolyCYPs enzymes, human recombinant CYPs and microbial strains generated an array of oxidised metabolites, including high conversion to M14 by PolyCYP 196. The same hydroxylation was undertaken by human recombinant CYP2C8.

- The PolyCYP 196 reaction was scaled up using 40 mg MZ1 in a 200 mL reaction (200 mg/L substrate dose) to provide material for identification of the position of hydroxylation in M14.

- An in-extract yield of 16% was obtained and 2.58 mg M14 purified for NMR spectroscopy, which matched with the same metabolite formed in human hepatocyte incubations.



Key NMR evidence

- NOESY: New methylene group at C-66 shows correlations to the C-67 methyl group.
 - HMBC: New methylene group shows correlations to the thiophene ring carbons C-53 & C-54.
- Confirms selective hydroxylation at the C-66 methyl group.
- Hydroxylation at this position has also been confirmed as the major CYP3A4 metabolic route of the JQ1 compound alone. The metabolite was made in two steps by chemical catalysis using a tetrabutylammonium decatungstate (TBADT) photocatalyst to form the aldehyde followed by reduction with NaBH_4 to generate the alcohol [3].

Take-home message

A biocatalytic route using PolyCYPs enzymes provided a one-step method for specific hydroxylation at a metabolically vulnerable position in the warhead region of a complex PROTAC molecule.

References:

- 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%.
- Sharma et al., 2025. Human cytochrome P4502C8 metabolizes repaglinide to 4'-hydroxyrepaglinide, not 3'-hydroxyrepaglinide. Drug Metab Dispos. 53(11):100177. doi: 10.1016/j.dmd.2025.100177.
- Holmes et al., 2024. Chemical catalysis guides structural identification for the major in vivo metabolite of the BET inhibitor JQ1. ACS Med Chem Lett. 15(1):107-115. doi: 10.1021/acsmchemlett.3c00464.