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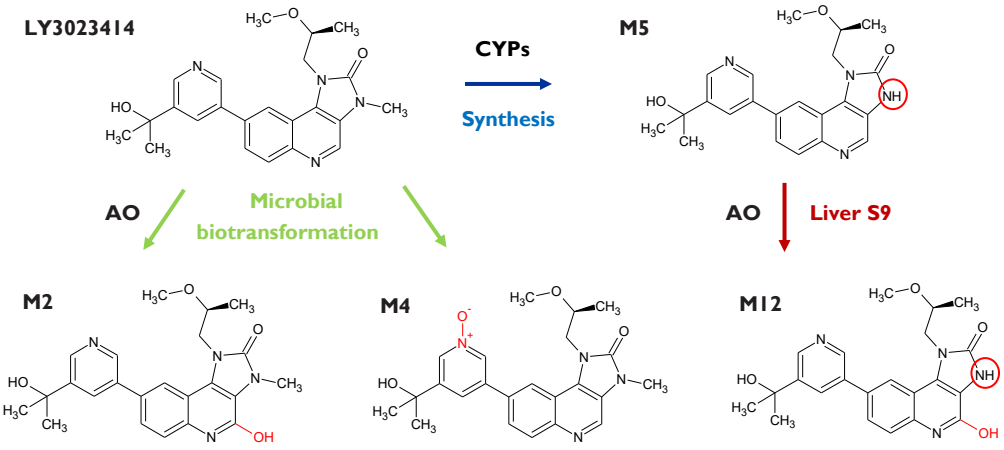
HYPHA DISCOVERY

Metabolites and molecules for tomorrow's drugs

Identifying and scaling up AO metabolites

Provision of multiple human metabolites via biotransformation



Provision of human CYP and non-CYP metabolites M2, M4 and M12 at multi mg scale utilising both microbial biotransformation and liver S9 incubations.

There has been a notable increase in metabolism of new drug candidates through non-CYP phase I pathways such as those mediated *via* aldehyde oxidase (AO).[1] Further, mixed AO/P450 substrates may be subject to metabolic shunting, an important consideration during toxicology and DDI assessment of these drugs.[2] Access to metabolites may thus be important to consider for drugs with mixed metabolism.

Zhou *et al.* presented a poster at the 2018 ISSX meeting in Montreal on "Elimination of [^{14}C]-LY3023414 by Aldehyde Oxidase and CYP Enzymes in Humans Following Oral Administration." Both AO and CYP enzymes were responsible for the metabolic clearance of LY3023414 with the non-CYP enzymes mediating approximately half of the clearance of the drug. The predominant metabolic clearance pathways were aromatic hydroxylation of the quinoline moiety (M2), *N*-demethylation (M5) and quinoline oxidation with *N*-demethylation (M12).

No metabolism was observed when tested vs 5 human recombinant CYPs, however screening of LY3023414 against a subset of Hypha's microbial biotransforming strains generated a number of metabolites. The best strain was scaled-up to 6L to access target metabolites M2 and M4. Subsequent incubation of the synthesised intermediate M5 vs Cyno S9 enabled production of a further target metabolite M12. Metabolites were purified to > 95% purity by Hypha and the structures confirmed by LC-MS and NMR. The **AO mediated hydroxylated metabolite** (M2, 20.1mg) and an **N-oxide** (M4, 66.3mg) were made *via* microbial biosynthesis and a **CYP/AO mediated metabolite** (M12, 18.4mg) was generated through liver S9 incubations.

Use of combined biotransformation approaches can therefore enable access to metabolites created through different pathways, including AO and CYP mediated metabolites.

[1]Rashidi & Soltani, 2017. Expert Opin. Drug Discov.12 (3), 305-316.

[2]Crouch *et al.*, 2016. Drug Metab Dispos 44, 1296-1303.

We can produce and scale-up mammalian phase I and II metabolites using microbial catalysts, mammalian tissue fractions and recombinant enzymes:

- **For DMPK / ADME / TOX**
- **For Met ID**
- **As standards for quantitation**
- **For bioactivity testing**
- **For stability studies**

Proven Reactions

Methyl hydroxylation
Methylene hydroxylation
Methine hydroxylation
Aromatic hydroxylation
***N*-oxidation**
***N*-methylation**
***N*-dealkylation**
***N*-acetylation**
***O*-dealkylation**
Carbonyl reduction
Heterocycle oxidation via aldehyde oxidase
Aromatic *O*-glucuronidation
Aromatic *N*-glucuronidation
Non-aromatic *O*-glucuronidation
Non-aromatic *N*-glucuronidation
Acyl-glucuronidation
Other glycosidations (AgChem)
***N*-sulfation**
***O*-sulfation**
Thiol conjugation (GSH/NAC)
Transamination
Amino acid conjugations
Sequential reactions e.g. hydroxylation & glucuronidation

For more information contact us at mail@hyphadiscovery.com

About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company providing solutions to pharmaceutical and agrochemical R&D partners through the production of mammalian and microbial metabolites, as well as specialising in microbially-derived chemicals. We have an extensive client base and work with many of the top pharma and agrochemical companies worldwide.