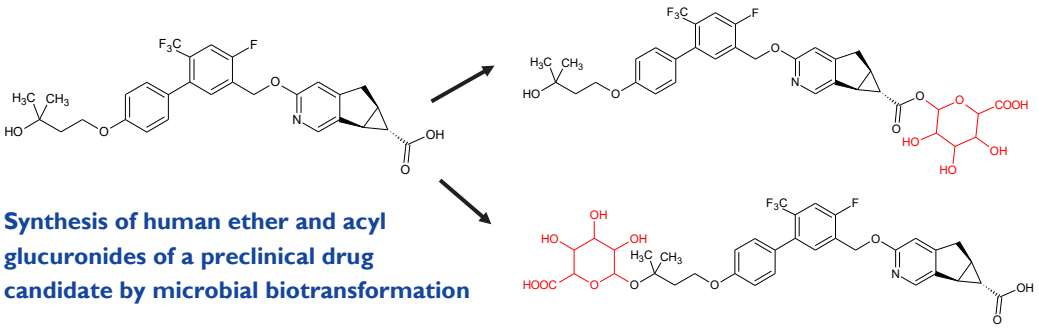
Glucuronides of carboxylic acid-containing drugs

Provision of human glucuronide conjugates *via* biotransformation



Synthesis of human ether and acyl glucuronides of a preclinical drug candidate by microbial biotransformation

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

The FDA's 2016 MIST guidance states that phase II conjugates are generally pharmacologically inactive, however where a potentially toxic conjugate, such as an acyl glucuronide is formed, additional safety assessments may be needed. Idiosyncratic drug toxicity of carboxylic acid-containing drugs can be caused by the formation of reactive acyl glucuronides,[1] which have the ability to directly acylate proteins and undergo intramolecular rearrangement producing reactive aldehydes leading to protein glycation.

Further, there is evidence to suggest that on-target pharmacological studies of acyl glucuronides of drugs are also warranted.[2] This is particularly relevant where acyl glucuronidation constitutes the primary clearance mechanism, or where the pharmacological target is in the extracellular matrix and does not require penetration by the acyl glucuronide conjugate.

Glucuronides can also be responsible for clinically relevant DDIs, such as those attributed to the acyl glucuronides of clopidogrel[3] and gemfibrozil[4], which selectively inhibit CYP2C8. As humans readily oxidise acidic drugs, there is also a potential complication arising from the presence of acyl glucuronides of oxidative metabolites of the drug, and which may later alter conjugate reactivity if oxidation occurs on a moiety nearby.[4] Further issues can arise due to β-glucuronidase-mediated hydrolysis of the patent drug, the propensity for which differs due to marked species differences in expression of β-glucuronidases.[5]

In the client project illustrated above, quantities of both the acyl and ether glucuronides were needed to study pathways responsible for the drug candidate's clearance. A subset of Hypha's microbes were able to produce both glucuronides in good yields. Due to chemical intractability of the ether glucuronide, a streptomycete strain was scaled up to provide the metabolite, which was identical to that formed by incubation of the parent compound with recombinant human UGT1A4.[6]

In a more recent project for another client, Hypha produced and purified 20 mg of an acyl glucuronide for further studies. The acyl glucuronide was produced by 4 microbes in Hypha's glucuronidation panel screen; subsequently a *Streptomyces* mutant was scaled up to provide sufficient material for purification.

[1]Lassila *et al.*, 2015. Chem Res Toxicol 28(2):2292-2303
[2]Ryder *et al.*, 2018. J Med Chem 61(16):7273-7288
[3]Tornio *et al.*, 2014. Clin Pharmacol Ther 96(4):498-507
[4]Ogilivie *et al.*, 2006. Drug Metab Dispos 34(1):191-197
[5]Smith *et al.*, 2018. Drug Metab Dispos 46(6):980-912
[6]Salter *et al.*, 2018. Xenobiotica 21:1-10

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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.