Hydroxylated and *N*-methylated metabolites *via* microbial biocatalysis

A case study using the experimental anti-cancer drug tivantinib

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
O-dealkylation
Carbonyl reduction
Heterocycle oxidation (AO)
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)
Sequential reactions e.g. hydroxylation & glucuronidation
N-acetylation
Transamination

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The experimental anti-cancer drug tivantinib, is a MET tyrosine kinase inhibitor which exerts a cytotoxic effect through interfering with tubulin polymerization independently of MET inhibition[1]. The drug is extensively metabolized in humans, in which the primary CYP isoforms involved are CYP2C19 and CYP3A4/5. Of the oxidation products, M4, M5, M7, M8 and M9 are observed in humans with M4 and M5 being major metabolites over the 10% AUC threshold[2], implicating these metabolites under the FDA MIST guideline.

Tivantinib was screened against a subset of Hypha's microbial panel - all of these strains produced M4, M5, M7 and M9 to varying extents, with 2 strains also producing a novel microbe-specific *N*-methyl metabolite.

One actinomycete strain was selected for scale-up to purify and characterize metabolites by NMR spectroscopy as shown in Figure 1 below. One of the hydroxylated metabolites could only be putatively assigned as M9, since previous researchers did not have sufficient material for definitive Met ID. Although the microbe-specific mono *N*-methyl metabolite is novel, synthetic *N*,*N*-dimethyl analogues are known, as *N*-alkylation was a strategy used to make derivatives in two patent applications.[3]

As well as giving scalable access to human metabolites, microbial biocatalysis also provides a route to obtaining known and novel derivatives, useful for lead diversification and late stage functionalization.

[1]Munoz, 2017. Non-kinase targets of protein kinase inhibitors. Nature Reviews Drug Discovery (2017). doi:10.1038/nrd.2016.266

[2]Nishiya *et al.*, 2016. Stereoselective hydroxylation by CYP2C19 and oxidation by ADH4 in the *in vitro* metabolism of tivantinib. Xenobiotica 46 (11), 967-976

[3]Chan *et al.*, 2010. From PCT Int. Appl. WO 2010093789 A2 20100819 and Li *et al.*, 2006, From PCT Int. Appl. WO 2006086484 A1 20060817

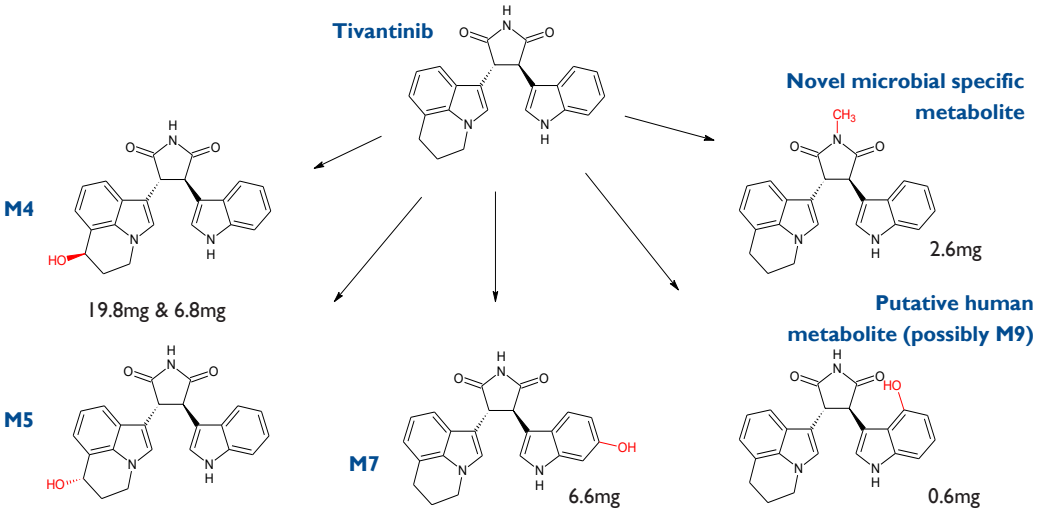

Figure 1: Human hydroxylated metabolites purified from a 200mg incubation of tivantinib with one of Hypha's microbial strains, together with a novel microbial-specific *N*-methylated metabolite.

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 work with 8 out of 10 of the top pharma companies and 4 out of 6 of the top agrochemical companies worldwide.