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HYPHA DISCOVERY

Metabolites and molecules for tomorrow's drugs

Sulfated drug metabolites

Scaled up supply of *O*- and *N*- sulfate conjugates

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

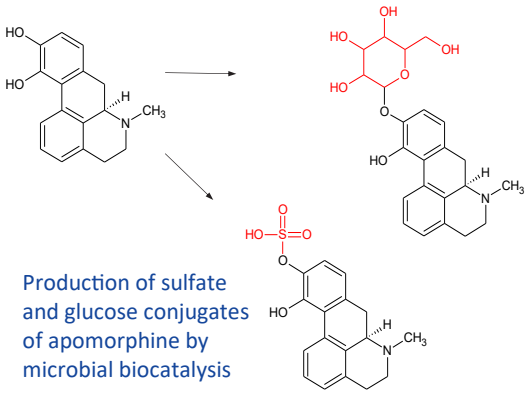
- For DMPK / ADME / TOX
- For Met ID
- As standards for quantitation
- For bioactivity testing
- For stability studies

Proven Reactions

Methyl hydroxylation
Methylene hydroxylation
Methine hydroxylation
Aromatic hydroxylation
***N*-oxidation**
***N*-demethylation**
***O*-demethylation**
Carbonyl reduction
Heterocycle oxidation (AO)
Aromatic *O*-glucuronidation
Aromatic *N*-glucuronidation
Non-aromatic *O*-glucuronidation
Non-aromatic *N*-glucuronidation
Acyl-glucuronidation
***N*-sulfation**
***O*-sulfation**
Glycosidation
Thiol conjugation (GSH/NAC)
Sequential reactions e.g. hydroxylation & glucuronidation
***N*-acetylation**
Transamination

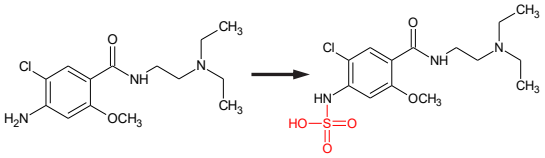
Phase II conjugated metabolites such as sulfates can be screened for and scaled-up using either microbial biocatalysis and/or liver S9 fractions. Metabolites can be supplied at microgram to multi mg scale, and at gram scale *via* the microbial route.

Sulfated metabolites of several drugs have been produced by microbes in Hypha's panel, such as *O*-sulfated metabolites of apomorphine and genistein, Scale-up of the apomorphine biotransformation generated 3.2 mg of the sulfate conjugate for definitive MetID. A glucose conjugate was produced in parallel, demonstrating broader conjugative capabilities of microbial biotransformation.



Production of sulfate and glucose conjugates of apomorphine by microbial biocatalysis

To demonstrate *N*-sulfation, the dopamine receptor antagonist metoclopramide was screened against Hypha's microbial and S9 panels. Metoclopramide has significant individual variation in metabolism, featuring multiple metabolites generated *via* both oxidative and conjugative pathways. Argikar *et al.* reported ten principal metabolites comprising five oxidation products, four glucuronides and one sulfate conjugate. Sulfate conjugation occurs at the primary amine *in vivo* with marked variability in levels due to polymorphism of human sulfotransferases.



The sulfated metabolite of metoclopramide was produced by two fungal species but not detected in *in vitro* liver S9 incubations, despite being a known circulating metabolite. Glucuronides were produced by both microbial and S9 systems, with several microbes also generating oxidative metabolites and other sugar conjugates.

Case Study

A recent project for a large pharma client involved supply of two sulfated metabolites in mg amounts. Following a dual system screening approach, more than 50mg of an O-sulfate was produced from porcine S9 fractions and more than 10mg of a N-sulfate via a microbial biocatalytic route. Employing the best yielding path for creating each sulfated metabolite meant that requirements were successfully met for supply of both metabolites at high purity.

Argikar *et al.*, 2010. Identification of novel metoclopramide metabolites in humans; *in vitro* and *in vivo* studies. DMD 38(8), 1295-1307.

For more information about our services, contact us at
mail@hyphadiscovery.co.uk

We work with 8 out of 10 of the top pharma companies and 4 out of 6 of the top agrochemical companies worldwide. Some of our clients include:

DuPont, Gilead, Lundbeck, LEO, Selcia, Glactone Pharma, Phenex Pharmaceuticals AG, Incyte, syngenta, ucb, Scynexis, ferrer

About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company providing solutions to pharmaceutical and agrochemical R&D partners worldwide through the production of mammalian and microbial metabolites, as well as specialising in microbially-derived chemicals.