Regio-selective formation of *N*-oxide metabolites

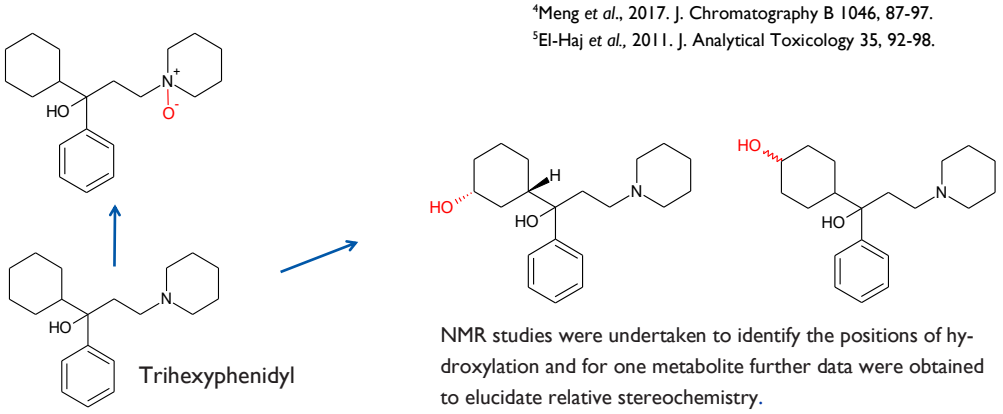
Phase I metabolism of tertiary nitrogen-containing drugs

Formation of *N*-oxide metabolites is one of the major pathways for metabolism of tertiary nitrogen-containing drugs. Some *N*-oxide metabolites have similar or greater pharmacological activity to the parent drug and thus require exposure assessment. They can also be unstable and can revert to the parent drug.[1] Conversion of an *N*-oxide metabolite back to the parent *in vivo* is a well-known phenomenon which may result in an altered tissue distribution of the metabolite and parent drug, such as that proposed for tamoxifen,[2] or cause adverse reactions as reported for soratenib.[3] In the lab, it is possible to reduce the potential for conversion of *N*-oxide metabolites to the parent through careful sample handling, as described for the clinically-significant metabolite loxapine *N*-oxide,[4]

Trihexyphenidyl (THP) is an anti-cholinergic agent used to treat Parkinson's disease and is also subject to drug abuse[5]. Major human metabolites of THP constitute hydroxycyclohexyl derivatives and an *N*-oxide, none of which have been structurally characterized in the literature. Through microbial biotransformation, we were able to prepare and isolate the *N*-oxide metabolite of THP together with two metabolites mono-hydroxylated in the cyclohexyl ring. Although the *N*-oxide of THP can be readily accessed synthetically, where there is potential for *N*-oxidation at multiple positions, a more selective approach is required.

N-oxides are formed through the action of cytochrome P450 and/or flavin-containing monooxygenase (FMO) enzymes. Microbial isoforms of these enzymes can selectively form the homologous human drug metabolites in quantities that allow full structural elucidation and further biological testing. For one client project Hypha was able to produce multi-gram amounts of the correct bis-*N*-oxide where multiple isomers were possible, as well as the correct human-relevant active mono-*N*-oxide in parallel, which in humans has been shown to be formed primarily *via* a FMO.

[1]Majunder, 2013. Regulated Bioassay of *N*-oxide Metabolites Using LC-MS, in Handbook of LC-MS Bioanalysis: Best practices, Experimental Protocols, and Regulations.
[2]Gjerde *et al.*, 2012. Breast Cancer Res. Treat. 134(2), 693-700.
[3]Gillani *et al.*, 2015. Chem. Res. Toxicol. 28(1), 92-102.
[4]Meng *et al.*, 2017. J. Chromatography B 1046, 87-97.
[5]El-Haj *et al.*, 2011. J. Analytical Toxicology 35, 92-98.



NMR studies were undertaken to identify the positions of hydroxylation and for one metabolite further data were obtained to elucidate relative stereochemistry.

Microbial biotransformation of trihexyphenidyl by two different microbes to yield trihexyphenidyl-*N*-oxide and metabolites mono hydroxylated in the cyclohexane ring

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

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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 the provision of microbially-derived chemicals. We work with many of the top pharma and agrochemical companies worldwide.