EXPANDING CHEMICAL SPACE VIA MICROBIAL LATE STAGE FUNCTIONALIZATION

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Abstract:

Introducing chemical diversity into a drug candidate late in the optimisation process has several applications including exploration of SAR (structure-activity relationships). Biocatalysis can provide access to chemical space in a complementary manner to chemical synthesis, thereby broadening coverage of the SAR map to better understand how small changes in the molecular structure affect biological potency. In this late stage functionalization project undertaken by Hypha and AstraZeneca, biotransformation of a small quantity of a drug lead was explored using a subset of microbes from Hypha's oxidative strain panel, resulting in the identification of eight active oxidised derivatives. Sufficient purified material was generated for structure elucidation by 2D NMR and subsequent pIC50 determination *via* application of qNMR.

Process summary

Total of 6mg drug dosed over 12 microbial strains (subset of Hypha's panel)

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Small scale isolation to generate fractions

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ID via LC-MS/MS and NMR spectroscopy

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Concentration by quantitative NMR

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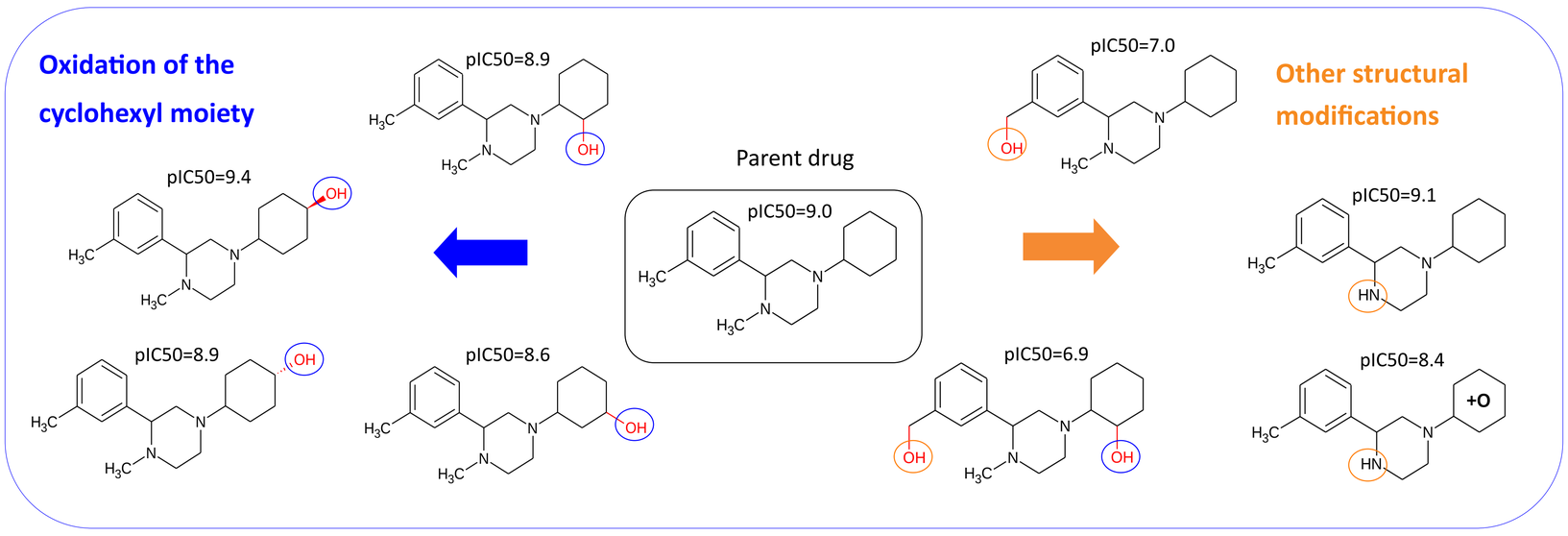
Activity testing to generate pIC_{50} values

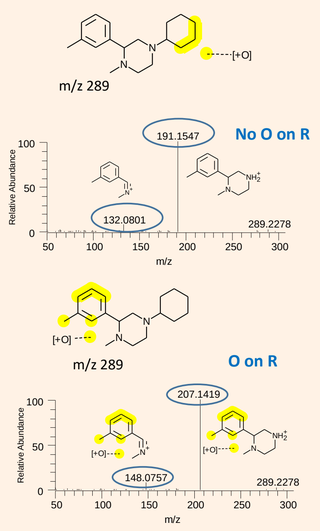


pIC_{50} values for derivatives produced by microbial biotransformation of a drug lead, including oxidation of the cyclohexyl moiety, demethylation and other hydroxylation reactions.

ID by MS/NMR and concentration by quantitative NMR

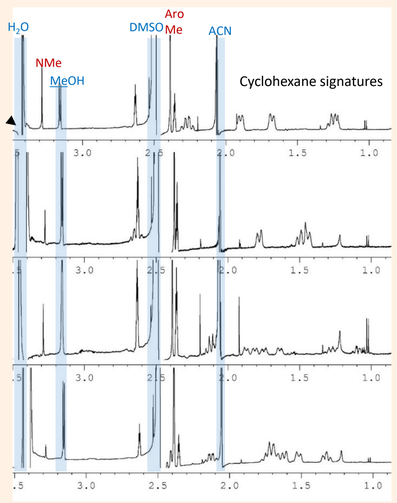
- A total turnover of 21% was observed for this drug compound providing 643 μg of purified fractions.
- Purified fractions used for NMR structure elucidation were reused to create assay-ready plates suitable for pIC_{50} determination using concentrations determined by qNMR. Concentrations determined ranged from 0.16 mM to 2.88 mM.

LC-MS/MS — **Functionalisation**

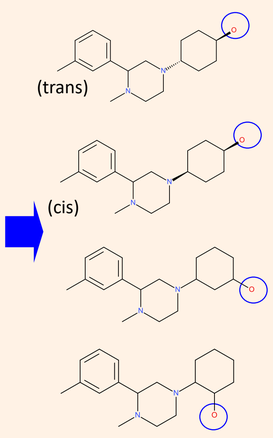


Oxidation on the cyclohexyl moiety

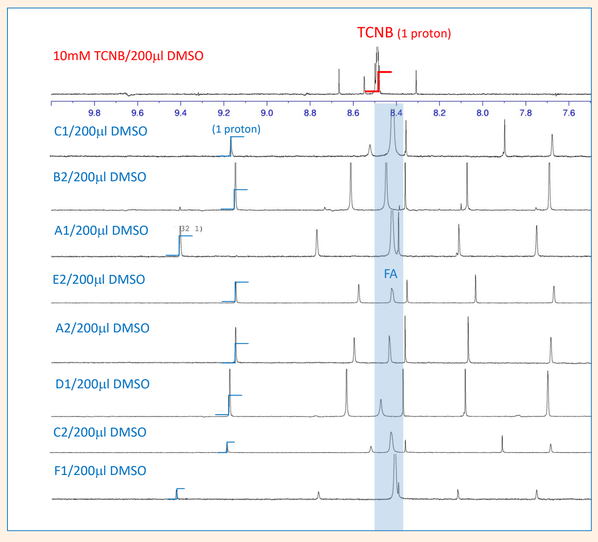
NMR spectroscopy — **Structural ID/regioisomery & concentration**



2-, 3- & 4-OH regioisomers



qNMR uses external unrelated standard (TCNB)



NMR parameters
3 mm tube
noesypr
ns=1024
rg=16

Aromatic region of NMR spectrum

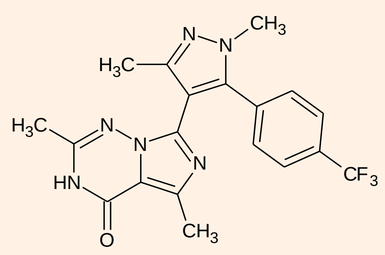
Absolute quantitation $c_{compound} \propto c_{TCNB} \frac{S_{compound}}{S_{TCNB}}$

compound	integral	concentration
TCNB	1000	**10mM**
A1	54.4	0.54mM
A2	66.96	0.67mM
B2	176.16	1.76mM
C1	30.52	0.31mM
C2	66.61	0.67mM
D1	287.61	2.88mM
E1	17.22	0.17mM
E2	114.95	1.15mM
F1	15.83	0.16mM

10 mM assay plate created for pIC_{50} determination

Outcome and discussion

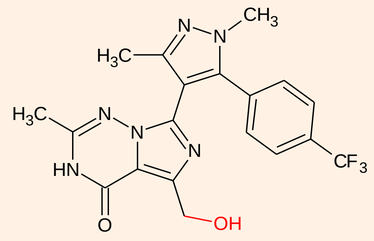
- Eight regio- and stereoisomers were isolated as a result of oxidation on the cyclohexane moiety, together with desmethyl and benzylic hydroxylated derivatives, and combinations thereof.
- Hydroxylated derivatives were obtained that overlapped with those produced synthetically, in addition to novel "trickier to synthesise" compounds where hydroxylation was achieved in two distinct areas of the molecule.
- The study was valuable in revealing that different polar chemical space could be accessed in parallel which did not compromise potency, as part of a wider SAR map.
- There is increasing focus on exploiting properties of hydroxylated metabolites for lead optimization including speedy generation of novel analogies with improved metabolic stability, exemplified by Pfizer's routine biocatalytic approach in which lead compounds are screened in microsomal systems. Hydroxylated derivatives are scaled up *via* microbial biotransformation and ultimately by chemical synthesis.



Lead compound

Liver microsomes

Microbial biotransformation produced 100s mg in 20% yield. Subsequently synthesised for *in vivo* studies



Clinical candidate
Reduced lipophilicity
Mixed metabolic profile
Increased renal clearance

- Hypha's microbial-based process, provides access to a late-stage hydroxylation platform delivering aliphatic and aromatic hydroxylation in one reaction, including regio– and stereoisomers.

About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company helping partners in pharmaceutical and agrochemical R&D worldwide succeed through the production of human and other mammalian metabolites, as well as specialising in lead-diversification and production of microbially-derived chemicals.