MICROBIAL HYDROXYLATION OF LEAD COMPOUNDS; A WAY OUT OF THE LIPOPHILIC CUL-DE-SAC

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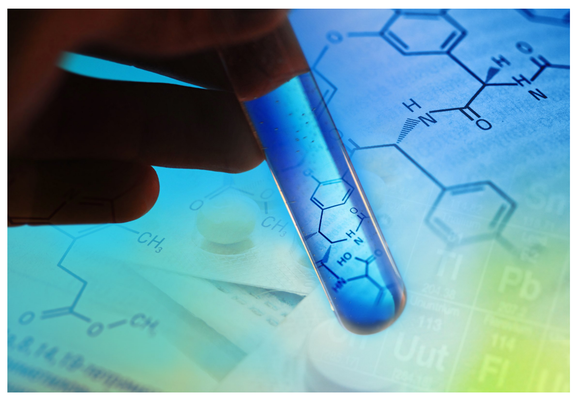
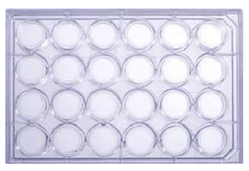


Summary: Microbial biotransformation offers an alternative and complementary approach to synthetic medicinal chemistry for the structural diversification of hit or lead compounds. Hypha Discovery has a scalable microbial process effective for activating C-H bonds to achieve aliphatic and aromatic hydroxylation. Applications include boosting ligand lipophilicity efficiency, improving selectivity, exploring SAR, creating handles for further derivatisation (e.g. fluorination for PET ligand formation or to block metabolism), or simply to highlight the conversion into active mammalian metabolites. This poster features a case study describing the biotransformation of a kinase inhibitor to explore lead diversification using Hypha's microbial panel. One of the derivatives possessed a reduced logD and an unpredicted 20-fold increase in potency, resulting in an increase in the LLE of 2.6 units. The process provides a scalable and reproducible way to access greater quantities of metabolites for further characterisation.

Process for capturing metabolites



Dose strain panel with parent hit or lead compound(s). This can be performed at various scales but in this case study a 24 well microbioreactor was used for the initial screen

Incubate, extract and analyse by LC-MS to look for hit reaction

Selected reactions combined and re-extracted

Pooled extracts fractionated by prepHPLC by time slicing over an extended gradient

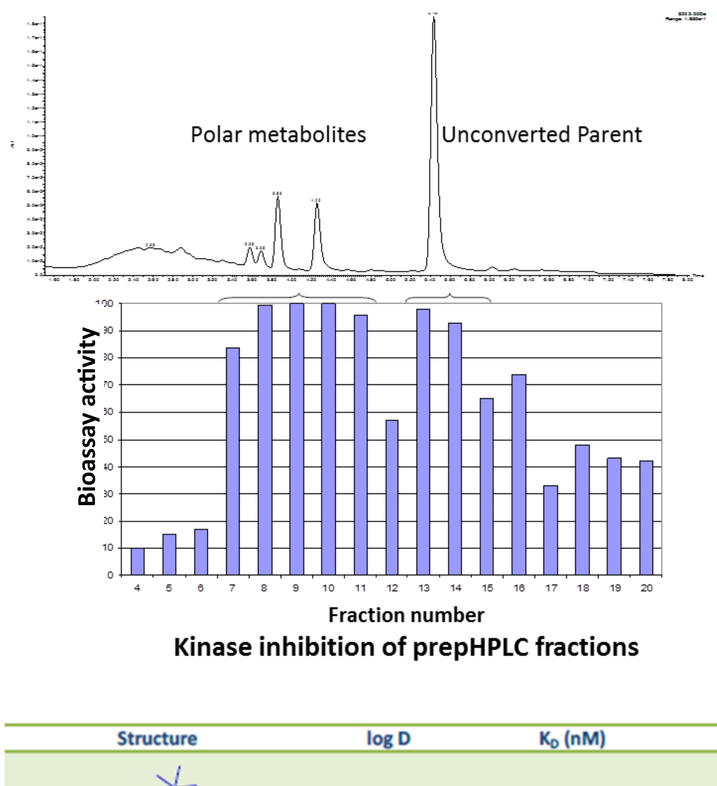
Test and control fraction returned for bioassay

Reactions possible

- Alkyl hydroxylation
- Aryl hydroxylation
- *N*-oxidation
- *N*- + *O*– dealkylation
- Hydrogenation / dehydrogenation
- *N*-, *O*– and acyl glucuronidation
- Other glycosylation / conjugation

Results

Assay of enriched fractions revealed several active polar metabolites. LC-MS was then used to prioritise compounds for scale-up.

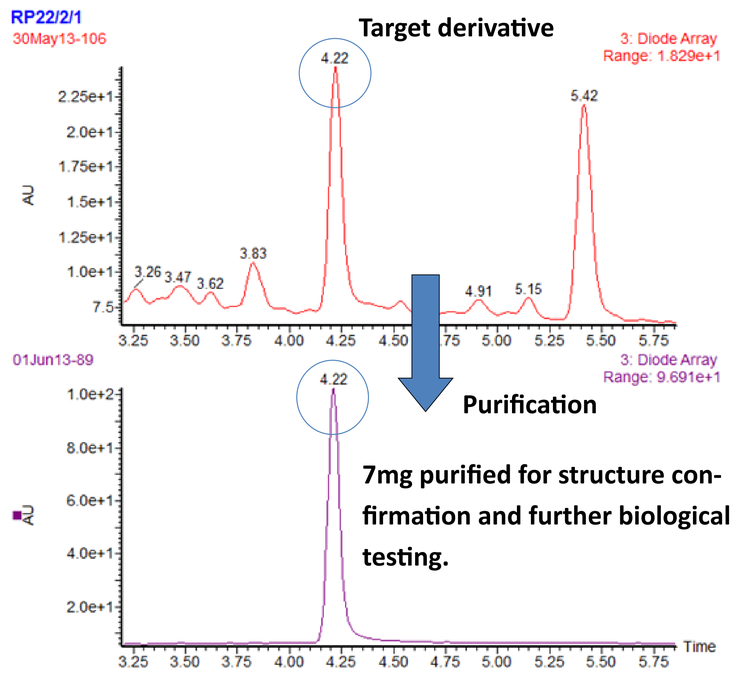


Kinase inhibition of prepHPLC fractions

Structure	log D	K_D (nM)	LLE
Parent	3.9	53	3.4
Metabolite	2.6	2.6	6.0

28mg parent fed for scale-up of a particular mono hydroxy derivative.

Scale-up



7mg purified for structure confirmation and further biological testing.

Outcome

- The derivative isolated was a hydroxylation of a *t*-butyl substituent.
- Application of the process resulted in an 20-fold boost in activity, improved solubility and achievement of the target LLE value.

Application

- The technology can be applied to compounds in the hit to lead stage of the discovery and optimisation pathway.
- The process can be conducted at various scales to produce quantities of derivatives tailored to the requirements of the project.
- We have screened multiple compounds for clients and successfully created polar derivatives of many (currently 86%).
- In addition to increased solubility, we have produced analogues that have improved potency and/or selectivity.
- Scale-up of derivatives is readily achieved by increased fermentation volume (96% reproducibility rate).

With thanks for Will Watkins, Senior Director at Gilead Sciences for permitting publication of this work and for the following testimonial:

"Upon hearing of Hypha's expertise in microbial transformation, we were intrigued to explore whether it might be useful for exploring the incorporation of polar groups into lead molecules in ways that were independent of synthetic considerations. We piloted two compounds from two separate projects with Hypha, choosing examples that we knew to have moderate microsomal stability.

We were very pleasantly surprised at the productive outcome where microbial incorporation of a hydroxyl group on a t-butyl substituent in one compound boosted the potency of a kinase inhibitor 20-fold, such that the LLE was increased by an extraordinary 2.6 units."

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 mammalian and microbial metabolites, as well as specialising in microbially-derived chemicals and provision of natural product libraries derived from higher fungi. In addition to our lead diversification capabilities, clients routinely use our biotransformation technology to gener-ate phase I & II metabolites for MetID, Stability testing, use as Analytical standards and for producing larger amounts for further DMPK testing.

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