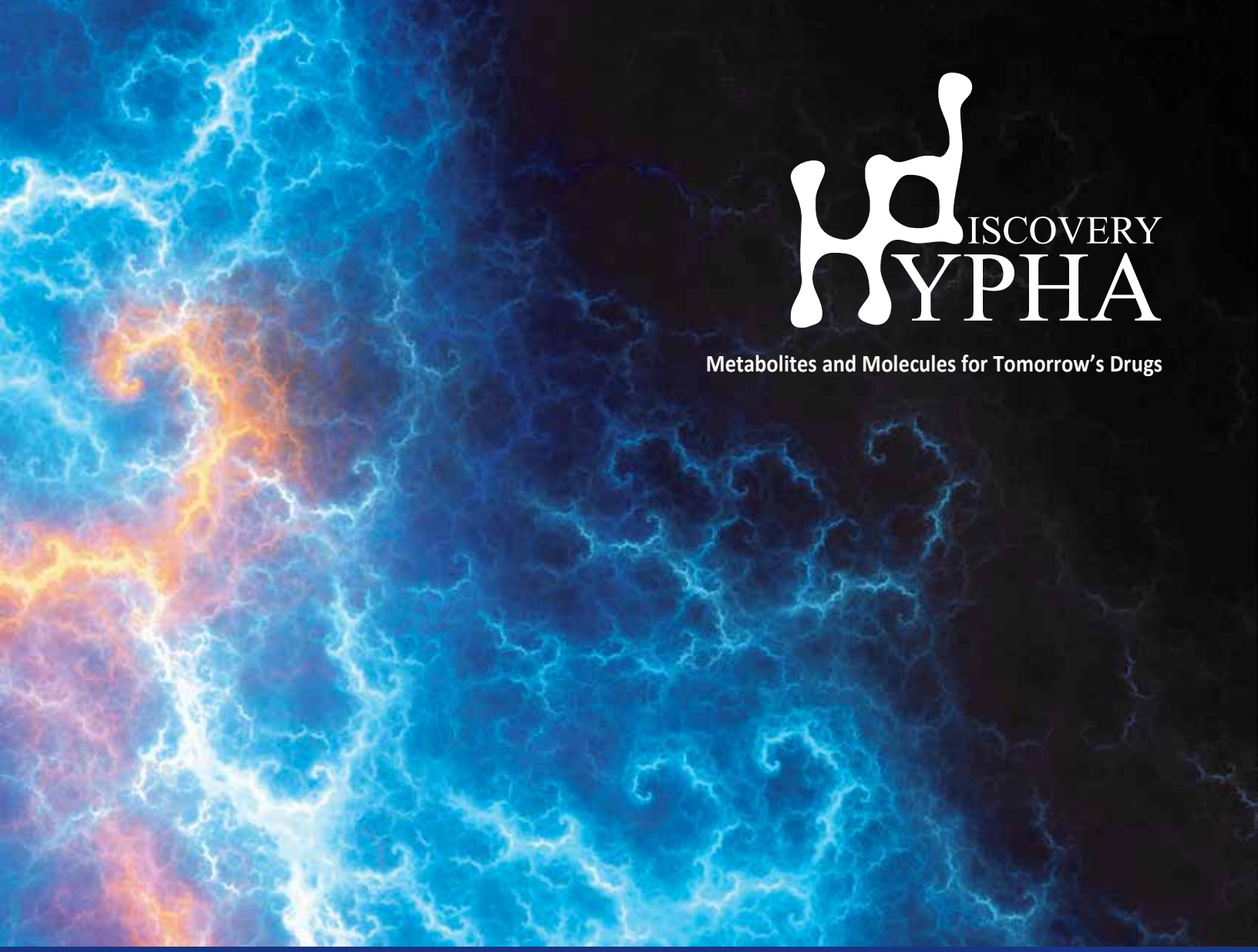


HYPHA DISCOVERY

Metabolites and Molecules for Tomorrow's Drugs

Drug metabolites and C-H activated derivatives

Phase I CYP and non-CYP metabolites

Phase II conjugated metabolites

Isotopically labelled metabolites

Lead diversification and late stage functionalization

Polar / C-H activated derivatives

www.hyphadiscovery.co.uk

Contents

Contact us for more information

Hypha Discovery Ltd
Russell Building
Brunel Science Park
Uxbridge UB8 3PQ
UK

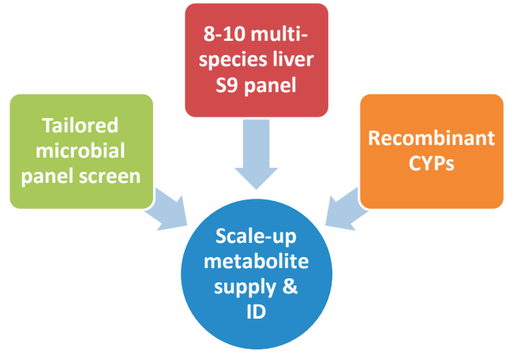
www.hyphadiscovery.co.uk
Phone: +44 (0)1895 814585
Email: mail@hyphadiscovery.co.uk

Accessing pharmaceutical and agrochemical metabolites

Hypha is a trusted supplier of difficult-to-synthesize metabolites to support development programs in eight of the ten largest pharma and four of the six largest agrochemical companies worldwide, as well as numerous other companies and institutes in these and other sectors.

We mimic human and other mammalian phase I and phase II metabolism using our proven microbial and mammalian S9 panels to produce metabolites for MetID, for use as quantitation standards or for DMPK / ADME / TOX studies. Metabolites arising from a variety of reactions are accessible, including both phase I CYP and non-CYP derived mechanisms. We also produce phase II conjugates such as *N*-, *O*- and acyl glucuronides, other glycosides, *N*-acetates and sulfates.

Microbes are able to produce most human drug metabolites due to evolutionarily-related enzyme systems. Critically, scalability of microbial biocatalytic systems enables cost-effective production up to multiple gram amounts.



Hypha's biotransformation platform is also effective for the production of metabolites from agrochemicals such as environmental fate (eFate) derivatives. Our processes have been specially adapted to accept and metabolize pesticides which often have poor aqueous solubility.

We provide metabolites to clients for a variety of applications:

- **DMPK/Tox studies**
- **Structure identification / confirmation**
- **Authentic standards for quantification assays**
- **Target /off-target activity testing**
- **Enzyme kinetic and intrinsic clearance assays**
- **As handles for introduction of fluorine into metabolic hotspots for enhancing metabolic stability**
- **Lead diversification**
- **Late stage functionalization**
- **Improved potency / solubility / bioavailability**

Key features

- Phase I CYP and non-CYP phase I metabolites.
- Phase II metabolites, including *N*-, *O*- & acyl glucuronides, sulfates and other conjugates.
- Multiple metabolites captured in a single screen.
- No requirement to reveal structural information; the entire process can be conducted blind.
- Metabolites purified to >90%.
- Optional structure elucidation.
- Scalable to multi-gram amounts.
- Formulation know-how for poorly-soluble compounds.
- Simple fee-for-service structure, with no downstream terms or royalties.

Industry-tested biocatalysis capability

The effectiveness of our microbial panels has been confirmed in an independent evaluation presented at the 20th North American ISSX meeting in 2015[1]. Hypha's microbial metabolite synthesis platform produced 83% of the major *in vivo* human metabolites from 16 commercial drugs, while human liver microsomes and hepatocytes were only able to generate 40% of these. Based upon these data, combining our capabilities in microbial and liver biotransformations, Hypha is able to provide **over 90% coverage of target metabolites.**

[1]Katyayan, Alberts and Cassidy, Evaluation of metabolite synthesis for sixteen commercial drugs using the Hypha Discovery system. Poster, ISSX, Orlando, FL , 2015.

Case study: metabolite scale-up

Hypha supplies phase I and II metabolites to clients worldwide. We work flexibly with all sizes of organizations to supply metabolites from microgram to gram scale.

Client metabolite projects typically start with a screen using microbes, and optionally mammalian S9 panels, to determine the most cost effective route to generate scaled-up amounts of metabolites.

Once a route to synthesis is identified, this can be readily scaled-up to supply up to mutigram amounts of metabolites.

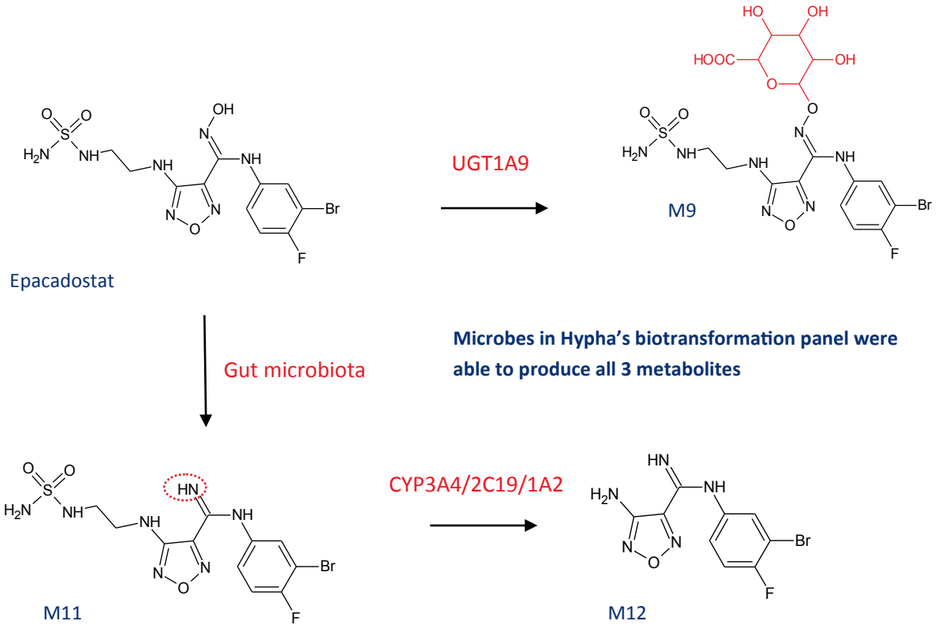
Client needs for scaled-up metabolite supply vary and we regularly produce amounts from a few mgs up to multigram scale.

One such project for Incyte in the US, involved the generation of diverse metabolites of epacadostat (EPA). EPA is a first-in-class IND targeting the enzyme indoleamine-2,3-dioxygenase 1 which is implicated in tumor escape from immune surveillance.

Three major metabolites are formed from EPA; M9 - a glucuronide formed by the action of UGT1A9, M11 - a *N*-dehydroxylated metabolite formed by gut microbiota, and M12 - a secondary metabolite formed from M11 by the action of CYP enzymes[2].

Several microbial species were capable of producing all 3 metabolites. The client requested supply of 25mg each of M9 and M11, for which 2 different species were used for scale-up resulting in 112mg of the glucuronide and 69mg of the phase I gut metabolite supplied at 95% purity.

[2]Roles of UGT, P450 and Gut Microbiota in the Metabolism of Epacadostat in Humans. Boer *et al.*, Drug Metab Dispos. 2016 Oct, 44(10): 1668-74.



Microbes in Hypha's biotransformation panel were able to produce all 3 metabolites

Scalable solutions for your metabolite needs

Met ID service

Hypha offers the option of using recombinant enzymes and multiple species liver preparations as well as microbial routes to produce metabolites. Together, it has been shown that these methods are able to produce 90% of human drug metabolites in an independent evaluation.

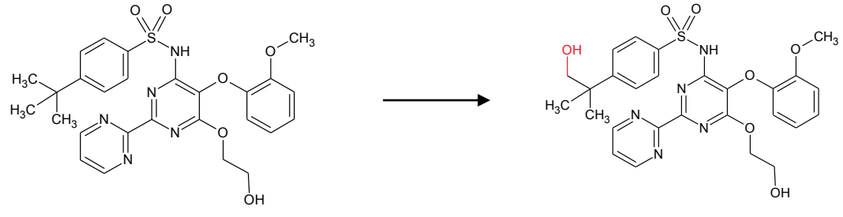
The low amounts required for metabolite identification using modern NMR instruments and state-of-the-art probe technology, means metabolite synthesis using mammalian liver preparations is a pragmatic solution for some applications. Only sub-milligram amounts of metabolites need to be generated using this flexible and adaptable process without requiring any optimisation steps.

Our parent-to-product process can generate 50-500 µgs of metabolite at >90% purity. This process saves time compared to a standard metabolite production process and is designed to make use of the latest NMR technology for rapid and unambiguous structural identification. Hypha has access to a 700MHz NMR spectrometer equipped with a 1.7mm Micro-cryoprobe, which means only low µg amounts of metabolites are needed to acquire data sets for full structural elucidation. Interpretation can be performed by clients or by our in-house experts.

"We commissioned Hypha to produce 50-200 µg of an oxidative metabolite where structural details were ambiguous. They provided 1mg of highly pure metabolite enabling full structure elucidation."

Manfred Birkel, Head of DMPK, Phenex Pharmaceuticals AG, Germany

For applications where higher amounts of material are required, the microbial route provides a cost effective way of generating **mgs to grams** of metabolites for further study.



2D COSY & HMQC correlations on 4.5 µg material were consistent with the expected *t*-butyl hydroxylation of bosentan.

Delivering a first class service to our clients worldwide

Production of phase I metabolites

Hypha's microbes mimic human and other mammalian CYP and non-CYP phase I metabolic reactions, including aromatic and aliphatic hydroxylation, as well as being effective for conjugative reactions. Using this method, it is possible to obtain metabolites formed from multiple sequential reactions in a single incubation, e.g hydroxylation and subsequent glucuronidation.

Our microbial metabolite synthesis method is scalable to enable production of up to gram amounts of pure metabolites without the co-factor costs associated with liver fraction incubations.

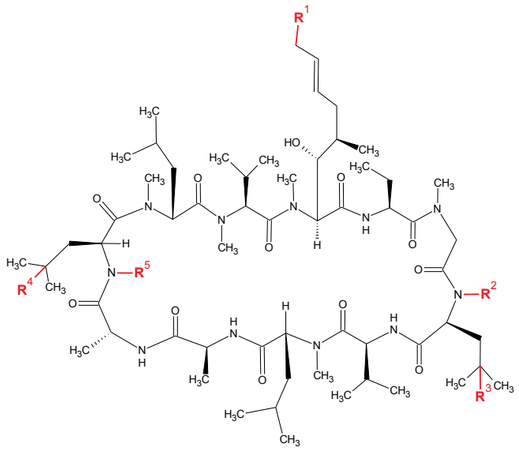
CYP reactions

CYP mediated reactions include methine, methylene and methyl hydroxylations as well as aromatic hydroxylations, dihydrodiol formation and creation of dealkylated metabolites.

Compound	R^1	R^2	R^3	R^4	R^5
Cyclosporin A	H	CH_3	H	H	CH_3
AM1	**OH**	CH_3	H	H	CH_3
AM19	**OH**	CH_3	H	**OH**	CH_3
AM19N	**OH**	CH_3	H	H	**H**
AM4	H	CH_3	**OH**	H	CH_3
AM4N	H	**H**	H	H	CH_3
AM49	H	CH_3	**OH**	**OH**	CH_3
AM4N9	H	**H**	H	**OH**	CH_3
AM9	H	CH_3	H	**OH**	CH_3
AM9N	H	CH_3	H	H	**H**

Phase I metabolite synthesis - proven reactions

- Aliphatic and aromatic hydroxylation (single & multiple)
- Heteroatom oxidation (*N* & *S* oxides)
- *N*- & *O*- dealkylation
- Dihydrodiols from phenyls
- Alcohol oxidation/carbonyl reduction
- Others incl. epoxidation, dehydrogenation, dehydration, hydrogenation, methylation, deacetylation/*N*-acetylation

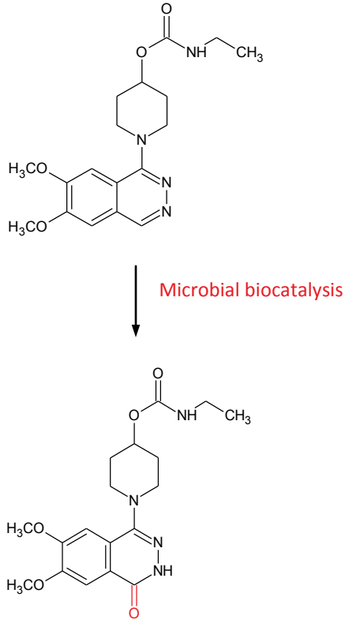


Derivatives of cyclosporine A obtained include the major human metabolites and more minor human and microbial –specific metabolites.

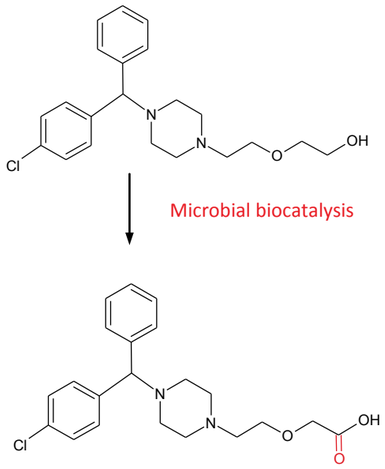
Non-CYP phase I reactions

A consequence of the development of drugs that are less susceptible to some mechanisms of CYP metabolism, has been the increase in drugs that undergo metabolism *via* alternative routes. Non-CYP phase I mechanisms involve monoamine oxidases, flavin-containing monooxygenases, xanthine oxidases, carboxylesterases and alcohol / aldehyde dehydrogenases.

Micro-organisms in Hypha's biotransformation panel are able to undertake non-CYP phase I reactions, offering a viable solution to scaling up the production of metabolites formed by these mechanisms.



Oxidation of carbazeran by aldehyde oxidase to form the human phthalazinone metabolite



Formation of the active metabolite cetirizine from hydroxyzine by the action of alcohol dehydrogenase. Alongside cetirizine, many other metabolites were produced *via* reactions including dealkylations, conjugations and other oxidations.

"When the need to generate, and characterize, oxidative metabolites of a complex natural product emerged, Scynexis recognized that direct synthesis may be difficult and instead turned to Hypha Discovery to explore biosynthetic opportunities. The Hypha team was extremely professional in their approach to the problem and rapidly identified organisms that produced the desired metabolites. The group then went on to scale-up the biosynthesis such that full structural characterization was achieved. Scynexis appreciates the problem-solving abilities of the Hypha team and will certainly turn to their expertise for further projects when needed."

David Angulo, Chief Medical Officer, Scynexis Inc, NJ, USA

Production of conjugated metabolites

We solve the challenges in synthesizing glucuronides and other phase II conjugates using talented microbes, which are adept at these biotransformations.

Our microbes perform reactions to provide up to gram amounts of *N*-, *O*- and acyl glucuronides and other conjugated metabolites for a range of applications:

- Drug-drug interaction studies, e.g. investigation of interaction with drug transporters.
- Assessment of ring-migration kinetics to check for formation of reactive acyl glucuronides.
- Standards to validate stability studies, e.g. quantifying reversion to the aglycone during bioanalytical processing.
- Pure analytical references.
- DMPK studies.

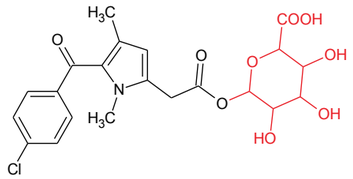
"We contacted Hypha Discovery to generate specific phase I and phase II metabolite standards in sufficient quantities and purity to allow structural confirmation and quantitation. Hypha exceeded expectations, providing 60mg of a phase I metabolite and over 100mg of a phase II metabolite at high purity. Hypha's team was a pleasure to work with and communicative and responsive throughout the process. We will undoubtedly be working with Hypha Discovery in the future."

Jason Boer, Senior Principal Investigator, Incyte Corporation, USA

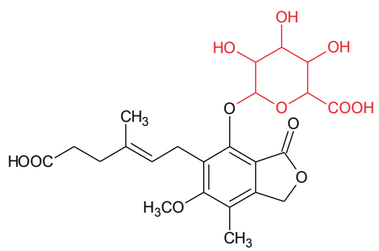
Phase II metabolite synthesis - proven reactions

- *N*-glucuronidation
- *O*-glucuronidation
- Acyl-glucuronidation
- Sulfation
- Glycosylations
- *N*-acetylations

Zomepirac acyl glucuronide formation led to the withdrawal of the drug due to irreversible protein binding. Hypha provides custom-made acyl glucuronides to enable toxicity studies and for use as analytical standards.

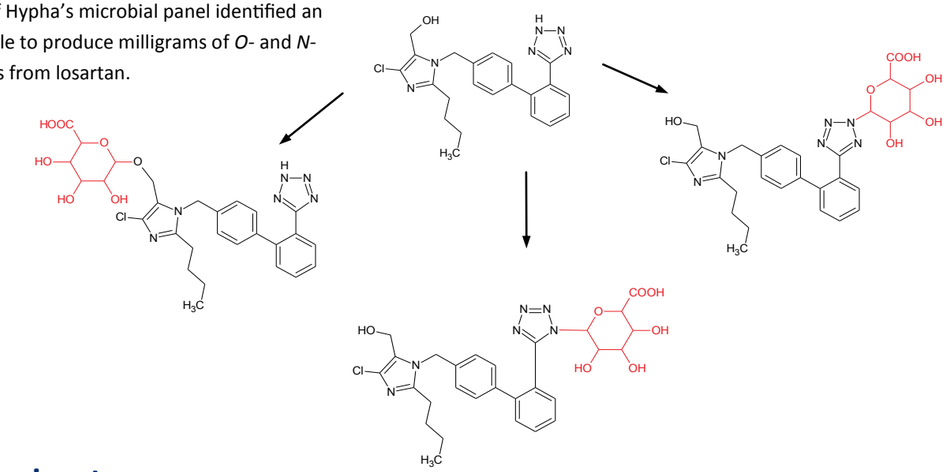


Mycophenolic acid is metabolized mainly to the *O*-glucuronide MPAG, which is known to inhibit hOAT3 transporters. An early warning of such liabilities is made possible by Hypha's glucuronide production service.



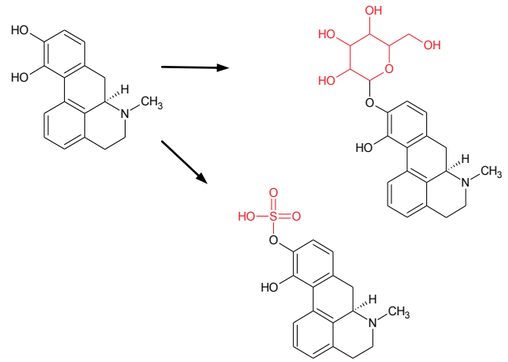
Glucuronide conjugate case studies

Screening of Hypha's microbial panel identified an organism able to produce milligrams of *O*- and *N*-glucuronides from losartan.



Other conjugates

Other conjugated metabolites such as sulfates and glycosylated products can also be formed using microbial biotransformation, as illustrated by the sulfate and glucose conjugates formed from apomorphine.



Use of liver preparations

We use liver preparations to augment our capabilities to produce both phase I and II metabolites, and where clients have specifically requested scale-up and purification of metabolites formed in liver incubations, including GSH conjugates.

Isotopically labelled metabolites

Through our partnership with the UK radiolabelling company Selcia, [^{13}C], [^{14}C], [^{2}H], [^{3}H] and [^{15}N]-labelled metabolites can be accessed to support regulatory, development or research projects in the pharma and crop protection industries. Hypha establishes optimized processes using unlabelled or stable labelled parent substrates, which can then be transferred to Selcia's state-of-the-art radiochemistry labs for the production of radiolabelled metabolites.

Lead diversification and late stage functionalization

A wide variety of compounds are metabolized by our talented microbes to generate oxidized derivatives that can solve issues such as excessive lipophilicity, or to provide chemical handles for introducing further structural changes. The resulting polar analogues can have significantly improved biological activities and may not have been accessible *via* synthetic routes.

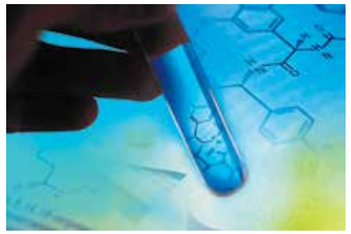
The process is complementary to medicinal chemistry and can be exploited to diversify hit or lead compounds by generating oxidised molecules with greater activity, better selectivity or an improved DMPK profile compared to the parent molecule. It can also be used to help widen and protect IP and inform SAR. The technology is flexible and can be applied at the lead optimisation stage or to generate hydroxylated analogues of hit compounds. It consists of an initial screen available at various scales with the provision of enriched derivatives for testing in assays, and subsequent scale-up of selected compounds.

Improving polarity / solubility

If you are looking to create more polar metabolites to improve solubility, we can apply our proven lipophilic rescue service to achieve improvement in LLE /LipE. The technology has the added benefit that multiple analogues can be produced in a single process. Production of selected derivatives can then be scaled-up.

Create handles for synthesis

Our process can create handles for further structural changes such as fluorination used for metabolism blocking or PET ligand formation.



Key Features

- Provides a method for generating multiple unique hydroxylated derivatives that are challenging to create synthetically.
- Scalable to multiple gram amounts.
- Applicable to broad structural types and complex molecules, including natural products.
- Metabolites are produced on a simple fee-for-service basis, i.e. there are no downstream terms.
- No requirement to reveal structural information; the entire process can be conducted “blind”.
- Flexible options – the process can be tailored to client needs.

“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 boosted the potency of a kinase inhibitor 20-fold, such that the LLE was increased by an extraordinary 2.6 units.”

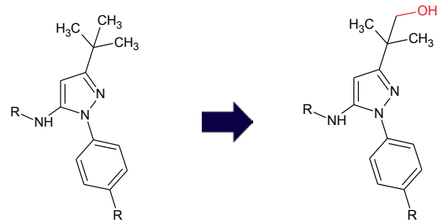
Will Watkins. Senior Director, Gilead Sciences, USA

Polar active derivatives of lead compounds

Lead diversification / late stage functionalization case studies

We have successfully generated polar active derivatives for clients, including Gilead for whom we produced a 20-fold more potent hydroxylated metabolite with significantly improved LLE.

More potent polar metabolite of a kinase inhibitor



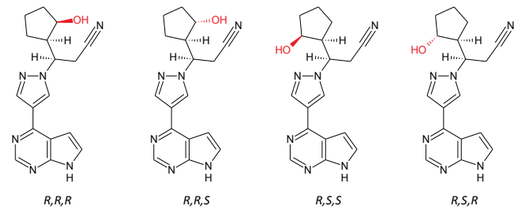
LogD	3.9 →	2.6
Kd (nM)	53 →	2.6
LLE	3.4 →	6.0

Biocatalytic C-H activation

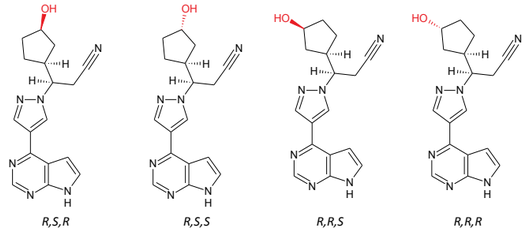
The activation of specific C-H bonds is one of the most challenging reactions in synthetic chemistry. However, nature is highly effective at selectively oxidizing C-H bonds enzymatically and Hypha’s microbial-based process provides access to a platform delivering such aliphatic and aromatic hydroxylations. Application of this technology to hit and lead compounds can result in the production of an array of analogues, including molecules with different biological activity and selectivity providing normally unattainable levels of SAR information. These products can include those observed in mammalian metabolism, or new derivatives specifically produced by microbes.

Application of biocatalytic C-H bond activation to the first-in-class JAK inhibitor ruxolitinib (trade names Jakafi and Jakavi) resulted in the production of an array of hydroxylated and further oxidized keto metabolites, many of which corresponded to circulating human metabolites. All possible oxidized isomers of the aliphatic cyclopentyl moiety were derived from a variety of microbial species which were scaled up for further characterization and testing.

Up to 120mg of all cyclopentyl hydroxylated derivatives of Incyte’s ruxolitinib were produced by microbial species in Hypha’s biotransformation panel. Further metabolized keto derivatives of metabolites were also produced.



2-hydroxylation of cyclopentyl moiety



3-hydroxylation of cyclopentyl moiety

Exploiting active metabolites for lead optimization

Many metabolites of drugs are wholly or in part responsible for the desired *in vivo* activity. This has been used to advantage in creating pro-drugs to optimize therapeutic effects, or alleviate issues in metabolism caused by polymorphic CYPs.

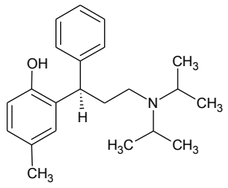
Rigorous studies of metabolism have also resulted in the development of improved drugs superseding the original, through harnessing knowledge of both active and inactive metabolites.

Application of Hypha's biotransformation panels allows exploration of the metabolism of lead compounds, which are readily scalable to generate sufficient amounts of derivatives for further investigation of activity, specificity and DMPK properties.

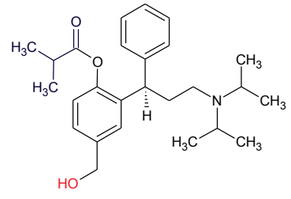
One powerful case study which highlights the importance of the utility of assessing metabolites is the development of the drug fesoterodine, which was subsequently developed as a prodrug of the active 5-hydroxymethyl metabolite of tolterodine.

The more polar pro-drug fesoterodine lacks the CNS penetration of the parent drug, and circumvents dosing issues associated with metabolism of the drug *via* the polymorphic CYP, CYP2D6.

Pro-drug of the active metabolite



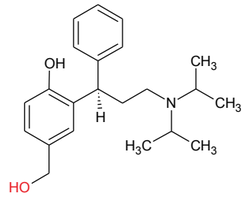
Tolterodine (Detrol®)



Fesoterodine (Toviaz®)

CYP2D6

Esterase

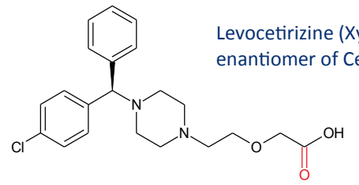


5-hydroxymethyltolterodine

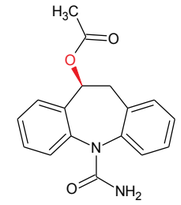
The active metabolite was produced by microbes in Hypha's microbial panels, alongside 3 other monohydroxylated metabolites.

More examples of metabolites as drugs

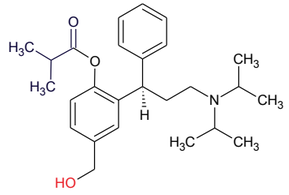
There are many cases where active metabolites have superior properties to the parent drug, some of which are developed as drugs. A selection are illustrated here.



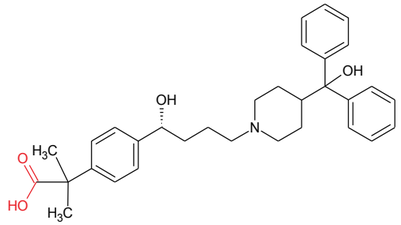
Levocetirizine (Xyzal®) - active *R* enantiomer of Cetirizine (Zyrtec®)



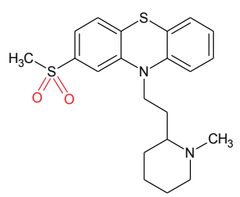
Eslicarbazepine (Aptiom®, Zebinix®)



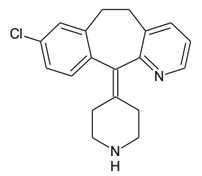
Fesoterodine (Toviaz®)



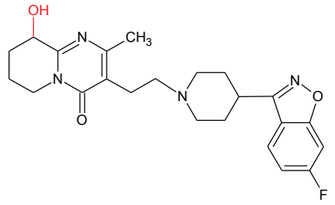
Fexofenadine (Telfast®, Allegra®)



Sulforidazine (Imagotan®, Psychoson®, Inofal®)



Desloratadine (Clarinex®, Aerius®, Neoclarityn®)



Paliperidone (Invega®)

References for the selection of active metabolites illustrated:

Levocetirizine. The latest treatment option for allergic rhinitis and chronic idiopathic urticaria. DuBuske, 2007. Allergy Asthma Proc. 28 (6), 724-734.

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The pharmacokinetics of Paliperidone versus Risperidone. Leon *et al.*, 2010. Psychosomatics 51(1), 80-88.

Meet the team

Our team members are always pleased to answer your questions and help determine if our solutions will meet your requirements.

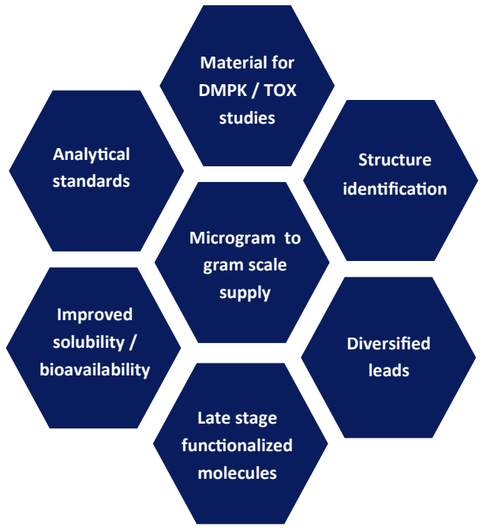
Hypha attends various US and European conferences throughout the year—check our website www.hyphadiscovery.co.uk to find out where we will be next. In addition to arranging WebEx calls, our business development team is also happy to meet with you at your organization - please let us know if you would appreciate a visit or tailored scientific seminar. We also host visits from clients and prospective clients who may be in the London Heathrow area.

Contact us for a no obligation discussion to find out more about the applications of our technology.

Phone: +44 (0)1895 814585

Email: mail@hyphadiscovery.co.uk

www.hyphadiscovery.co.uk



Contact us for more information

Hypha Discovery Ltd
Russell Building
Brunel Science Park
Uxbridge UB8 3PQ
UK

www.hyphadiscovery.co.uk
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Email: mail@hyphadiscovery.co.uk

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