# Accessing Difficult-to-Synthesise Metabolites in Drug Development Programmes

Scientists may benefit from exploring the available options to make metabolites, thus meeting the criteria set in regulatory guidelines

Julia Shanu-Wilson
at Hypha Discovery

Access to major and/or disproportionate metabolites as part of drug development programmes is critical to ensure adherence to regulatory guidelines issued by the EMA and FDA, as well as to comply with internal safety standards within pharmaceutical companies themselves (1-2).

Identification and characterisation of metabolites ensures not only rigorous safety evaluation of significant metabolites, but can also expand patent coverage and reveal superior potency, reduced side effects, or improved physical properties compared to the parent drug.

Multiple options are available for making metabolites through both chemical and biological routes, as discussed by scientists at Abbvie (3) and summarised in Figure 1.

Chemical synthesis is often attempted first, either via direct modification of the drug candidate or via modified intermediates in the synthetic pathway. However, alternative methods are required if poor reaction yields, severe reaction conditions, or unwanted by-products are encountered. Equally, constraints on medicinal chemistry resources can mean that investing significant time in chemical synthesis of metabolites may not be the best route, particularly in cases where the structure of the metabolite is not known and where multiple possibilities need to be made.

Access to metabolites through direct biological methods that involve incubation of the drug with matrices, such as liver fractions, recombinant enzyme kits, microbial cultures, or even direct purification of metabolites from clinical materials, are proven solutions. For difficult-to-synthesise drug metabolites with the possibility of several regio- or stereoisomers, biological approaches are the logical choice to minimise costly, and sometimes, time-consuming, speculative synthesis of all possible structures. Sometimes, a suite of metabolites is accessed using a combination of both chemical and biological routes, as described by scientists at Janssen, Merck and Co, and Hypha Discovery (4).

Both Phase 1 and Phase 2 primary single-step and secondary human metabolites can be readily made via surrogate biological routes, including the generation of aromatic and aliphatic hydroxylated metabolites and conjugates such as glucuronides and sulphates. Additionally, metabolites made by gut microbiota can also be accessed.

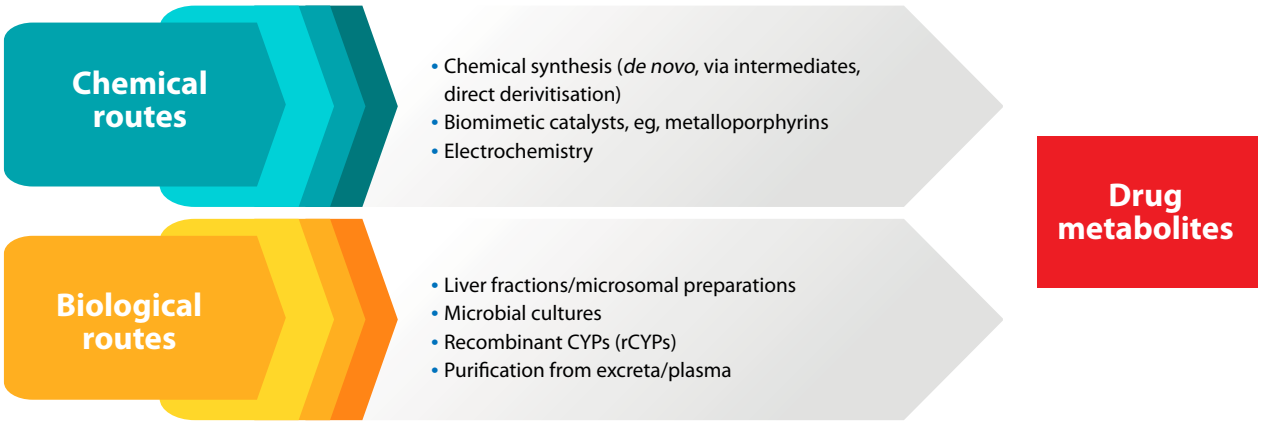


Figure 1: Commonly used routes to access human and other mammalian metabolites

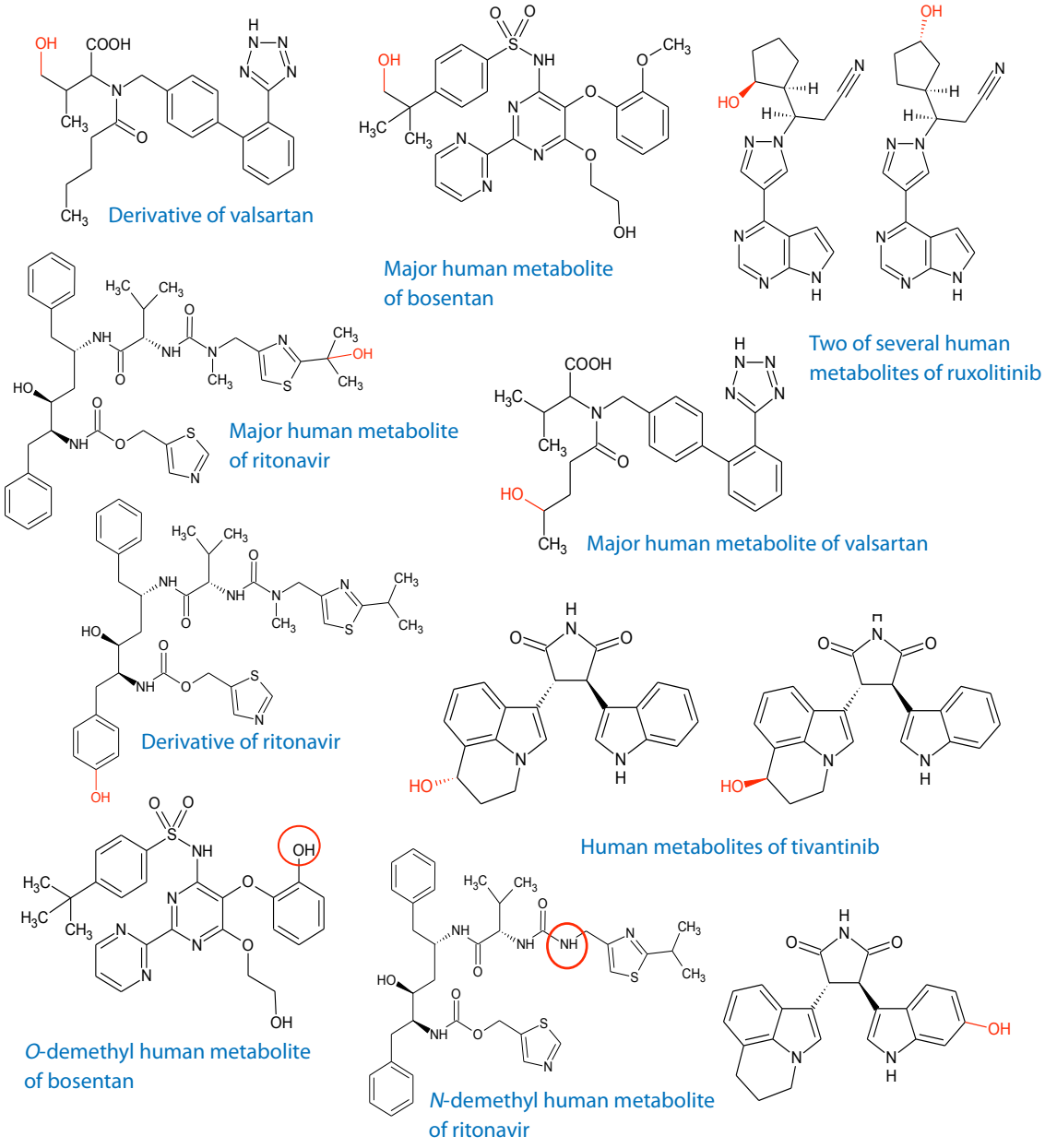


Figure 2: Human CYP-derived metabolites of a variety of drugs hydroxylated at different positions by PolyCYPs® enzymes

Examples of different metabolite types accessed through biological means are discussed in the following series of case studies.

## Case Study 1

This case study concerns use of recombinant enzymes for scalable synthesis of cytochrome (CYP)-derived drug metabolites.

Recombinant enzymes, derived from either human or microbial origins, are capable of synthesising human and other mammalian metabolites. Scalable systems provided by microbial-derived recombinant enzyme kits enable synthesis of CYP-derived human metabolites for definitive metabolite identification and pharmacological testing, as illustrated in Figure 2. They can also be used to explore new chemical space of lead compounds by late-stage oxidation, producing multiple derivatives through oxidation at susceptible aliphatic and aromatic positions.

## Case Study 2

Case study 2 assesses accessing human metabolites of drug subjects to metabolic shunting.

As part of a medicinal chemistry strategy to reduce major metabolism by single polymorphic CYPs, there has been an increase in metabolism of new drug candidates through non-CYP pathways, mediated by other key drug metabolising enzymes such as aldehyde oxidase (AO) (5).

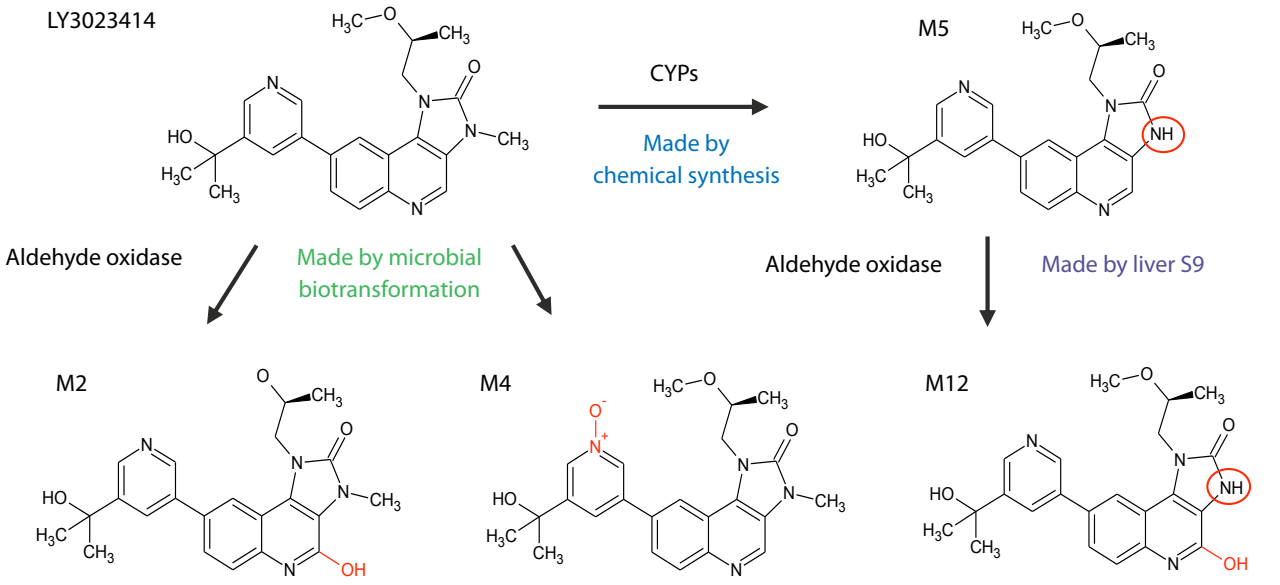


Figure 3: Provision of human CYP and non-CYP metabolites M2, M4, and M12 at multi mg scale utilising both microbial biotransformation and liver S9 incubations. Metabolites M2 and M4 were obtained via microbial biosynthesis, and metabolite M12 was obtained from incubation with liver S9. All three metabolites were prepared and isolated at Hypha Discovery, and parent compound and metabolites were identified by accurate mass LC-MS and NMR at Eli Lilly

Furthermore, mixed AO/CYP drug candidates may be subject to metabolic shunting, an important consideration during toxicology and drug-drug interaction (DDI) assessment of these compounds (6). Thus, access and evaluation of metabolites is important to consider for drugs subject to mixed metabolism.

In work presented by scientists at Eli Lilly, both AO and CYP enzymes were found to be responsible for the metabolic clearance of LY3023414, with non-CYP enzymes mediating approximately half of the clearance (7). No metabolism was observed when tested against human recombinant CYPs, however, a microbial biotransformation panel was able to generate a number of metabolites, including milligrams of an AO-mediated hydroxylated metabolite (M2) and an *N*-oxide (M4). Additionally, use of a liver S9 fraction enabled production of a further CYP/AO metabolite (M12) from a synthetic intermediate (M5). Therefore, use of a combined biotransformation and synthesis approach enables access to metabolites created through different pathways, as illustrated in Figure 3.

## Case Study 3

This case study is based on accessing disproportionate human metabolites for toxicology studies.

In cases where major drug metabolites are formed at disproportionately higher levels in humans than found in the species used in preclinical studies, safety regulations require the pharmacological and toxicological testing of the metabolite, dictating the need for production and identification of the disproportionate metabolite.

In this case study, a disproportionate mono-hydroxylated metabolite (M27) of ingenol disoxate, originally under development by Leo Pharma, was formed in humans (8).

Metabolite identification by liquid chromatography-mass spectrometry (LC-MS) only narrows the point of hydroxylation in a metabolite to a specific moiety of the parent drug and not the specific location of attachment. Definitive identification of the position of hydroxylation in the metabolite M27 was achieved via microbial biotransformation and milligram quantities purified for bioanalysis requirements after structure elucidation by nuclear magnetic resonance (NMR). A subsequent request by the FDA to undertake *in vitro* studies to assess the drug interaction potential of the metabolite was facilitated by scaling up the reaction. In total, over half a gram of this chemically intractable metabolite was made using microbial biotransformation.

## Case Study 4

Case study 4 covers purification of metabolites from *excreta* for use as analytical standards.

If clearance mechanisms of the test drug results in sufficient quantities of the major metabolites in biological material such as faeces or urine, purification and subsequent identification of metabolites from such matrices is possible. One such project undertaken resulted in tens of milligrams of the R (95mg) and S (23mg) *O*-glucuronides of carisbamate, a neuromodulator developed by SK Life Science, which was purified to >95% purity from 150ml of rabbit urine using a three-step purification method. Confirmation of the structures was obtained by NMR

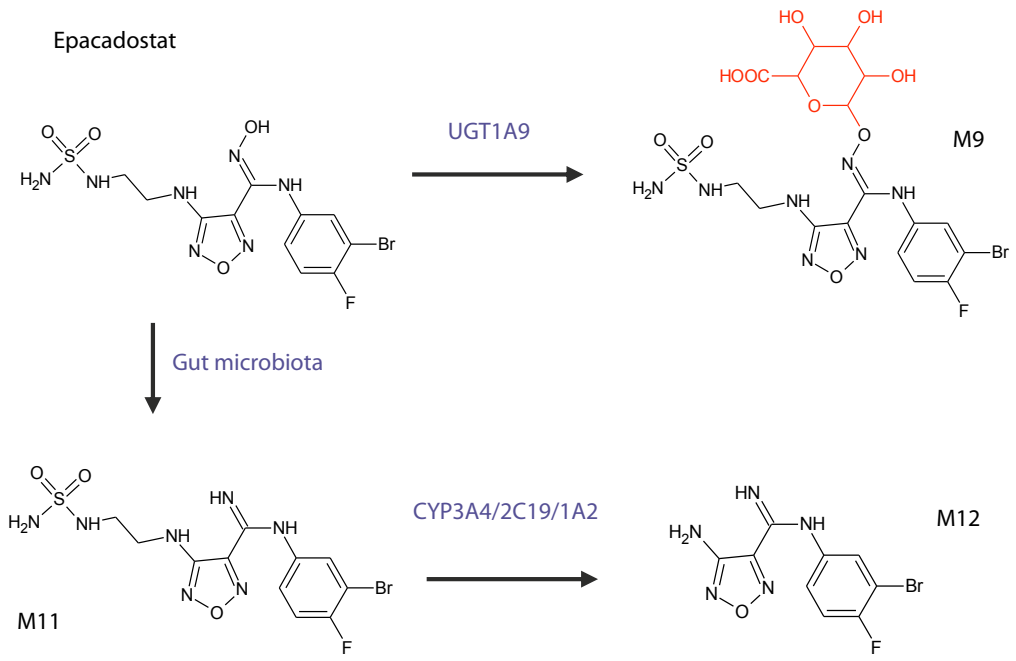


Figure 4: Major metabolites of epacadostat produced by microbial biotransformation via mixed metabolic pathways

**"Despite the clinical need for accessing these metabolites, *N*-glucuronides are sometimes difficult to produce in scalable amounts by either chemical or biological routes"**


spectroscopy, enabling the use of the purified material as analytical standards for bioanalysis.

**Case Study 5**

This case study focusses on metabolites produced by gut microbiota.

There is increasing focus on the effect of gut microbiota on drugs, which can have consequences for efficacy and toxicity (9). Most commonly, the gut microbiota undertake reductive reactions as exemplified in the case study illustrated in Figure 4, where a major metabolite of Incyte's investigational drug epacadostat was found to be derived from metabolism of the drug in the gut (10). This gut metabolite (M11) is then absorbed and further metabolised by CYP enzymes to form the secondary metabolite (M12).

Microbial biotransformation was able to produce both the gut and secondary metabolite, as well as an additional major metabolite, the glucuronide M9. The ability of this method to access metabolites derived from mixed metabolic pathways

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showcases the versatility of specific actinomycetes and fungi to produce metabolites formed by varied, and sometimes, sequential, pathways.

### Case Study 6

Case study 6 reviews the increase in formation of *N*-glucuronides of drug candidates.

*N*-glucuronides are a class of metabolites increasingly observed as a significant route of drug elimination and can exhibit transporter-mediated pharmacologic activity and drug-drug interactions, thereby necessitating their evaluation (11). Despite the clinical need for accessing these metabolites, *N*-glucuronides are sometimes difficult to produce in scalable amounts by either chemical or biological routes. Therefore, various strategies to improve synthesis of these metabolites are required.

Glucuronides are produced by uridine 5'-diphospho-glucuronosyltransferases (UGTs), which are expressed by many organisms – from microbes to humans – to facilitate elimination of xenobiotics. Many subtypes catalyse the glucuronidation of different compound types, including UGTs which specifically form *N*-glucuronides. Recombinant human UGT products currently available, eg, Corning's Supersomes, target preclinical reaction phenotyping and inhibition studies, still require addition of expensive uridine diphosphate glucuronic acid cofactor, and are only suitable for small-scale production. One project at Hypha Discovery, funded by Innovate UK and in collaboration with Professor John Ward at University College London, UK, will aim to address this clinical need through provision of engineered UGT-based biocatalytic solutions to provide a mechanism for readily obtaining larger scale quantities of *N*-glucuronide metabolites for testing (12).

These case studies described provide just a snapshot of the many challenges and solutions for the provision of metabolites for drug development programmes through biological routes. Both chemical and biological solutions will continue to evolve to meet the challenges of ensuring that sufficient quantities of drug metabolites are accessible to meet all regulatory guidelines, enabling the provision of higher quality medicines to patients.

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## About the author


Julia Shanu-Wilson has over 25 years' experience in the biotechnology and drug discovery industries. Following a BSc and PhD in microbial biochemistry at Imperial College London, UK, Julia worked in the biotech and pharma industries holding research leadership positions with Xenova, Cubist Pharmaceuticals, and TMO. She joined Hypha Discovery in 2013 and is on the management team, leading scientific communications for the company.
Email: julia.shanuwilson@hyphadiscovery.co.uk