One-Stop Metabolite Biosynthesis and Structural Characterization

The disproportionate drug metabolites (>10% of total drug-related substances, Safety Testing of Drug Metabolites Guidance for Industry, Revision 2 (2020, FDA)), unique metabolites, and metabolites with potential pharmacological activity or toxicity play a critical role in the investigational new drug (IND) application and approval for marketing. It is crucial to obtain these metabolite standards in early-stage R&D studies and preclinical filing for pharmacological activity or toxicity evaluation.

The precise structures of most metabolites are often challenging to predict based solely on high-resolution mass spectrometry (HRMS) data during *in vitro* or *in vivo* metabolite identification (MetID). This challenge leads to hurdles in achieving targeted synthesis of the desired metabolites using traditional chemical methods. Consequently, the chemical synthesis process becomes time-consuming, labor-intensive, and intricate, with a potential for mismatches. In addition, other metabolites are difficult to synthesize by chemical method due to the stability, configuration or long synthetic route issues, etc. Metabolite biosynthesis, which is performed by using biotransformation system of *in vitro* incubation and *in vivo* animal administration, is a good choice to solve these problems. Utilizing preparative liquid chromatography (LC), high-resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR) identification technology, the WuXi AppTec DMPK Department has established a biosynthesis platform for targeted metabolites. This platform can efficiently synthesize metabolites at milligram (mg) level and accurately identify their precise structures.

Biosynthesis Platforms

- Recombinase (CYPs, UGTs, AO, FMO, GSTs, etc.)
- Hepatocytes and their subfractions (microsomes, S9, cytoplasm, etc.)
- Matrices of animals (urine, feces, bile, plasma, etc.)
- Preparative LC, HRMS, and NMR

Customized Delivery

- Precise metabolite structure (based on HRMS and NMR)
- Metabolite standards (powder or solution)
- Metabolite standards (powder or solution) + precise metabolite structure (based on HRMS and NMR)
- Standard COA including HPLC-UV chromatograms, ^{1}H-NMR data, etc.

Our Strengths

Extensive experience in the identification of metabolites of large and small molecules in *in vivo* and *in vitro*, which allows for the efficient identification of target metabolite(s) and suitable biosynthesis systems.

Comprehensive *in vivo* and *in vitro* systems support diverse types (e.g., phase I and phase II metabolites) of metabolites bio-synthesis.

Characterized enzyme-oriented biosynthesis with high yield and short turnaround time (~ 4 weeks), without the need for further matching with MetID results.

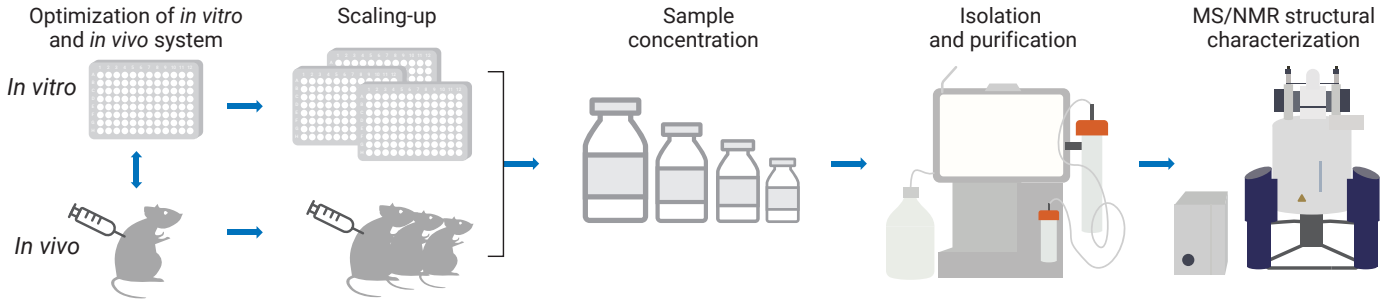
Expertise in biosynthesizing challenging metabolites with low relative abundance or complex chromatographic separation, and optimizing incubation systems.

Efficient and convenient one-stop metabolite synthesis, isolation, and structural characterization.

Allied chemical synthesis team to provide APIs for formulation, PK study, pharmacodynamics study, safety testing, and IND-enabling study through chemical scale-up.

Flow Chart of Metabolite Biosynthesis

The metabolite biosynthesis process mainly includes optimization and scale-up of the experiment system (screening of animal species, matrix types, and assay conditions), isolation and purification of the target metabolites, and structural identification by HRMS and NMR.



Case Study of Metabolite Biosynthesis

This case is the biosynthesis of the oxidation metabolite of carbazeran. This metabolite was observed to be abundant (~60% relative abundance) in human hepatocytes (Figure 1A). After comparing the metabolite profiles of carbazeran in liver microsomes, S9, and hepatocytes, it was hypothesized that the oxidation metabolite was a product of the AO enzyme. Considering the accessibility of the bio-matrix and cost, the monkey cytosol enriched in AO enzyme was chosen for further optimization of incubation conditions (Figure 1B). Eventually more than 95% of carbazeran was metabolized to oxidative metabolite. After isolation and purification using preparative LC, this metabolite was characterized as 4-oxo-carbazeran by MS/MS (Figure 2 A) and NMR (Figure 2 B).

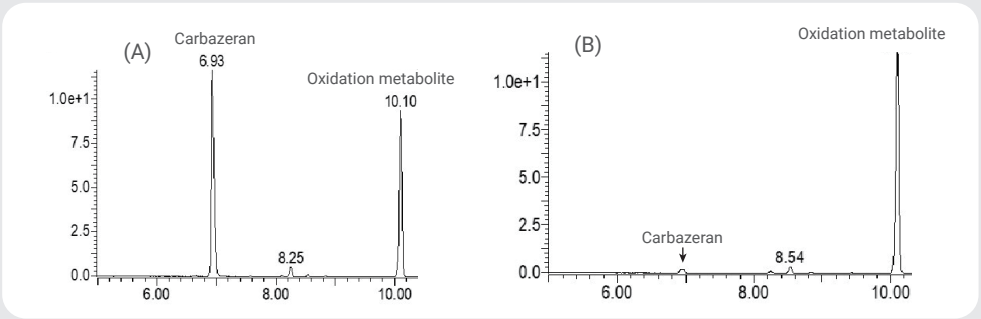


Figure 1. LC-UV Chromatograms of carbazeran in human hepatocytes after incubation 2 h (A) and in monkey cytosol after incubations 4 h (B)

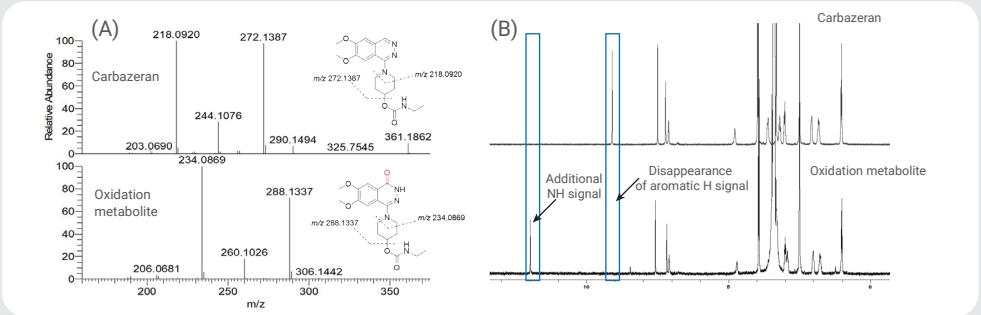


Figure 2. MS/MS (A) and NMR (B) spectra of carbazeran and its oxidation metabolite

Metabolite Profiling and Identification Services

We provide comprehensive metabolite profiling and structural characterization services, covering lead compound optimization from drug discovery to clinical development, including radiolabeled metabolite profiling and identification. In addition, our extensive experience in metabolite biosynthesis provides our clients with more convenient and efficient synthesis methods to support drug discovery and safety assessment. We also offer customized services based on the client's research needs. Our global research centers and departments collaborate to ensure a timely delivery of high-quality reports that meet the requirements of regulatory authorities (NMPA, FDA, EMA, etc.).

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