www.hyphadiscovery.com

HYPHA DISCOVERY

Metabolites and Molecules for Tomorrow's Drugs

Stable-labelled metabolites

Synthesis of deuterated metabolites

Hypha is experienced in making labelled phase I and conjugated metabolites for clients. We can produce both stable-labelled (^{2}H or ^{13}C) and radiolabelled (^{3}H or ^{14}C) metabolites of drugs and agrochemicals using the techniques in our one-stop metabolite shop, including microbial biotransformation, recombinant enzymes and chemical synthesis. This is achieved through application of late-stage methods using the labelled parent compound. Most commonly requested are stable-labelled metabolites, in particular deuterated metabolites for use as internal standards.

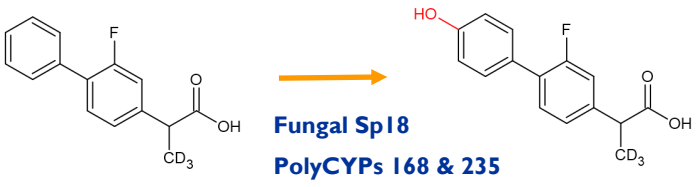
Deuterated metabolites

Both oxidised and conjugated metabolites can be produced using either biotransformation or late-stage chemical synthesis from the deuterated parent compound. The route and rate of metabolism of deuterated compounds can be different to the unlabelled material.

Flurbiprofen case study

The non-steroidal anti-inflammatory flurbiprofen is subject to both CYP and UGT mediated metabolism. In humans CYP2C9 is responsible for the major metabolite, 4'-hydroxyflurbiprofen.

Screening of flurbiprofen against a panel of PolyCYPs® enzymes and microbes resulted in several products. A fungal biotransformation produced the major metabolite 4'-hydroxyflurbiprofen with almost complete conversion. The correct product was confirmed by NMR spectroscopy of the purified metabolite.



Flurbiprofen-*d3* 4'-Hydroxyflurbiprofen-*d3*

Metabolism of flurbiprofen-*d3* also results in hydroxylation at the same position by the same enzymes and microbes, albeit the fungal biotransformation proceeding more slowly than observed with the non-deuterated drug. It is expected that biotransformation processes may be affected by deuteration at sites of metabolism in compounds.

Client projects

Hydroxylated *O*-sulfate: Sometimes both biotransformation and chemical synthesis routes are utilised in order to make a metabolite. This tandem approach is especially useful for multi-step metabolites. In one example, 100s of milligrams of both unlabelled and deuterated analogues of an *O*-sulfated metabolite were produced using these methods in series.
The parent drug was subject to multiple routes of metabolism with the major route involving oxidation followed by sulfation. Microbial biotransformation was used to make the correct isomer of the aryl hydroxylated intermediate. Following purification of the intermediate metabolite, chemical synthesis was used to make 430 mg of an *O*-sulfated metabolite observed in human liver microsomes at a purity of >99% by LC-UV-ELSD.

Acyl glucuronide: In another project involving only late-stage chemical synthesis, 51 mg of a deuterated acyl glucuronide at >99% purity by LC-UV-ELSD was made from 100 mg of the *d6*-labelled parent drug using a two-step process. The process was optimised using unlabelled parent before transferring the method to the deuterated analogue.

***N*-glucuronide:** This project necessitated transitioning from a human liver S9 biotransformation to a more scalable late-stage chemical synthesis method. Following a quick optimisation, gram amounts of a *d7 N*-glucuronide was supplied to the client at >99% purity by LC-UV-ELSD and 97% purity by qNMR analysis.

Contact us to discuss your metabolite project

enquiries@hyphadiscovery.com