Routes for Synthesis of Metabolites of Ruxolitinib and Epacadostat

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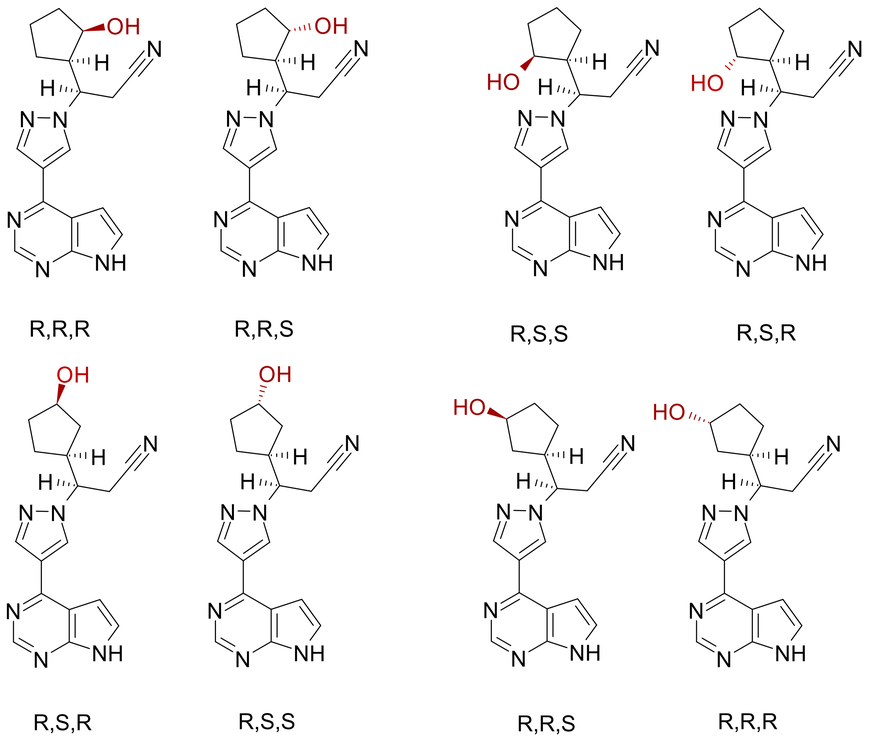
Abstract: Significant metabolites of small molecule drugs sometimes need to be accessed at scale for further testing. Hypha have developed multiple techniques as part of their one-stop metabolite shop to tackle synthesis of these using both biotransformation techniques or late-stage chemical synthesis.

In this poster we describe the routes taken to make metabolites of Incyte's drugs, ruxolitinib and epacadostat, where the majority of metabolites were not accessible through facile chemical synthesis. Instead, microbial biotransformation was used to make the phase 1 and phase 2 metabolites needed.

Phase 1 metabolites of ruxolitinib

The JAK inhibitor ruxolitinib is largely metabolized by CYP3A4, to multiple oxidized metabolites. The major human circulating metabolites in human are M18 formed by 2-hydroxylation of the cyclopentyl ring, and two other hydroxylated stereoisomers formed by 3-hydroxylation. Active metabolites contribute 18% of the overall pharmacodynamics of ruxolitinib. A method was needed for accessing all the possible stereoisomers of hydroxylated metabolites of ruxolitinib.



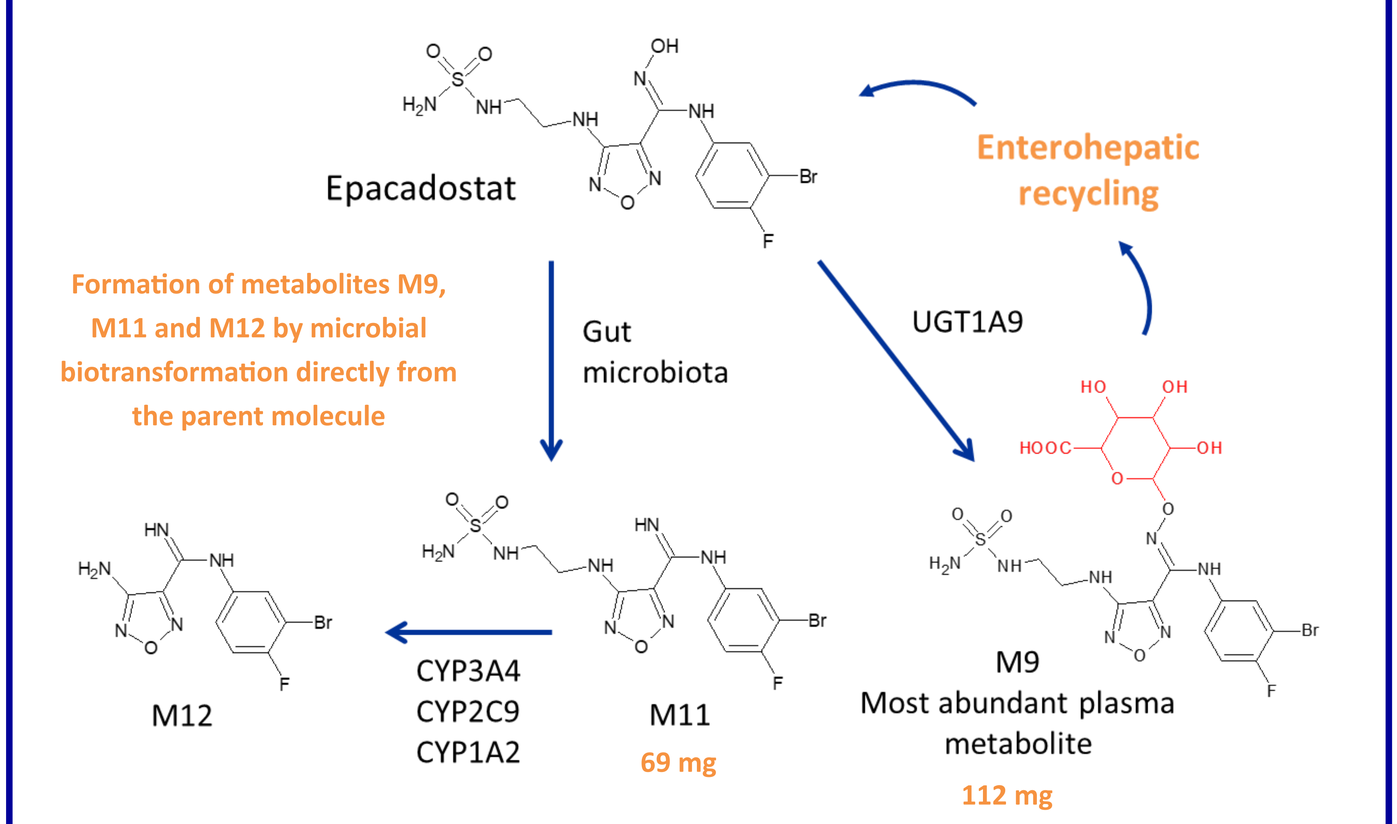
Up to 120 mgs of 2- and 3-cyclopentyl hydroxylated and keto metabolites

Metabolites of epacadostat produced via mixed pathways

Metabolism of epacadostat results in the formation of 3 major circulating metabolites in humans from both primary and secondary pathways. Glucuronidation is the major metabolic pathway, resulting in M9 which is subject to enterohepatic recycling and thus influencing the PK of epacadostat.

Additionally an amidine M11 is formed by gut microbiota, which is absorbed and modified by CYP enzymes to the *N*-dealkylated metabolite M12.

Pure standards of each metabolite were needed for clinical DDI studies



Process

- Ruxolitinib was screened against a panel of oxidising microbes at 100 µg/ml and samples analyzed by LC-MS.
- 15 strains were found to produce at least 1 metabolite.
- 1 actinomycete strain was identified that could produce all of the 2- and 3-cyclopentyl hydroxylated metabolites.
- Further oxidized keto derivatives were also observed.
- The best strain was scaled up at 10L to provide material for purification and structure confirmation. Production of the target metabolites was monitored in real time by LC-MS.
- Up to 120 mgs of all oxidized metabolites were isolated and identified at Incyte.

Process

- Epacadostat was dosed at 100 µg/ml to a panel of microbes known to mimic both phase 1 and phase 2 pathways and samples analyzed by LC-MS
- 5 strains were found to produce M9, 14 able to produce M11 and 8 able to generate M12
- The best 2 actinomycete strains for production of M9 and M11 were scaled-up at 3L. Production of the target metabolites was monitored in real time by LC-MS.
- 112 mg of the glucuronide (M9) and 69 mg of the gut metabolite (M11) were purified and supplied at 95% purity to Incyte.
- The NMR spectra of M9 suggested the presence of rotamers and this metabolite was thus supplied at >95% purity of mixed rotational isomers.
- M12 was produced by the microbes but was synthesized by Incyte in parallel and thus not targeted for scaled-up production and purification.

Outcome

Microbial biotransformation was successful in producing all diastereoisomers of the 2– and 3– hydroxylated metabolites of ruxolitinib, in addition to the ketone derivatives. Several of these corresponded with those shown to be circulating in humans, including active metabolites.

Outcome

Microbial biotransformation was used to make quantities of M9 and M11 for studies at Incyte investigating interactions with major drug transporters. M9 was shown to be a substrate for multiple uptake and efflux transporters but was not an inhibitor of P-gp or BRCP, and only a weak inhibitor of OAT3 and OATP1B1. Studies showed a low risk of clinical DDIs.

References

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About Hypha: Hypha Discovery is a specialist CRO with expertise in the scalable synthesis, purification and identification of drug metabolites. Using our biocatalytic and organic chemistry capabilities, we also make oxidized derivatives of lead compounds and API degradation products. Hypha also has a wealth of experience in the production, purification and structure elucidation of natural products. We work with pharmaceutical and agrochemical companies globally.