USE OF MICROBIAL BIOTRANSFORMATION TO PRODUCE SCALABLE QUANTITIES OF A GLUCURONIDATED METABOLITE OF THE CLINICAL STAGE SOLUBLE GUANYLATE CYCLASE STIMULATOR PRALICIGUAT

Jonathan Steele[1], Alicja Borowik[1], Liam Evans[1], Deepa Karunakaran[1], Ravi Manohar[1], Richard Phipps[1], Frank Scheffler[1], Julia Shanu-Wilson[1], Ali Banjamali[2], Andrew Carvalho[3]

[1]Hypha Discovery, 957 Buckingham Avenue, Slough, SL1 4NL, UK. [2]Ironwood Pharmaceuticals, Cambridge, MA 02141, US. [3]Cyclerion Therapeutics, Cambridge, MA 02141, US

Abstract: In recent years, FDA guidance has advised initiating human metabolite profiling earlier in drug development, emphasizing the importance of metabolite identification and quantification to evaluate a drug metabolite's safety and pharmacological activity. Praliciguat (IW-1973) is a soluble guanylate cyclase (sGC) stimulator in Phase 2 clinical trials for diabetic nephropathy and heart failure with preserved ejection fraction (HFpEF). During studies on metabolism of praliciguat in preclinical species and in human hepatocytes, a prominent direct *O*-glucuronide metabolite was detected. Initial attempts by Cyclerion to isolate the metabolite in rat bile, *via* administration of praliciguat to bile duct-cannulated rats, lacked scalability and resulted in low yields. Access to larger quantities of this glucuronide metabolite for structure elucidation and pharmacological activity was achieved through microbial biotransformation of the parent compound. The purified glucuronide metabolite produced by the actinomycete strain was used as a quantitative analytical standard for clinical sample analysis and for other pharmacological and DMPK studies.

Background

Praliciguat is a member of a new pyrazole-pyrimidine series of sGC stimulators and is extensively metabolized in rats *via* hydroxylation, *N*-dealkylation, glucuronidation, sulfation, and glutathione conjugation. The most abundant metabolites recovered in bile were praliciguat-glucuronide and hydroxy-praliciguat-glucuronide [1].

Milligram quantities of praliciguat-glucuronide were required as a standard for clinical sample analysis and other studies, however, these could not be obtained via purification of the metabolite from rat bile. Instead, microbial biotransformation was explored as a scalable option for producing the clinically relevant glucuronide.

Process summary

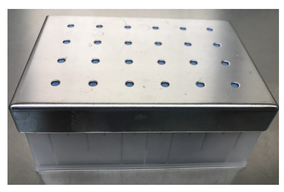
Initial screening of praliciguat against a glucuronidating panel of 23 filamentous actinomycetes and fungi identified two strains capable of producing a glucuronide whilst two other strains produced oxidized metabolites. One actinomycete strain produced the *O*-glucuronide in excellent yield without onward metabolism. Following confirmation of the correct metabolite, this producing organism was scaled up to produce 66 mg of the *O*-glucuronide at > 99.5% purity from a 4L fermentation. A further 6L scale-up was subsequently performed to provide an additional 118 mg of the *O*-glucuronide.

Outcome

- Screening of a panel of microbes revealed one actinomycete (Sp45) capable of producing the target glucuronide with no onward metabolism.
- Scale-up of Sp45 resulted in a near 100% conversion of praliciguat, with a single glucuronide as the dominant metabolite.
- The glucuronide was purified to >99% purity by two orthogonal prep HPLC rounds resulting in 66 mg of praliciguat-glucuronide. The high level of purity was determined by inspection of the ELSD and ^{1}H NMR spectrum.
- The structure was elucidated by NMR spectroscopy and the position of glucuronidation confirmed as 1-*O*-beta.
- A request for 100 mg of further material was fulfilled by a repeat scale-up biotransformation to provide 118 mg of pure praliciguat-glucuronide.

Process for microbial biotransformation of praliciguat

SCREEN



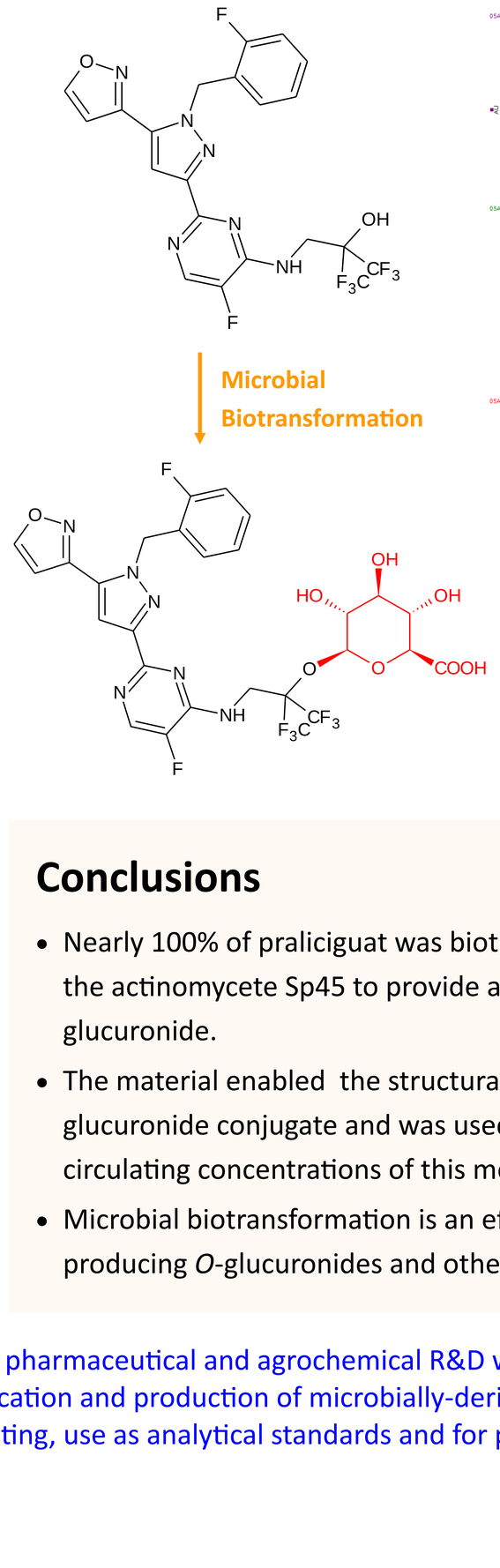
- 23 microbe panel screened in a shaken 24 well block vs praliciguat dosed at 100 mg/L in the presence of a cyclodextrin formulant.
- Samples time-coursed and analysed for the presence of glucuronides by LC-MS.
- Hits matched against a clinical sample by LC-MS/MS to identify the best biotransforming microbe.

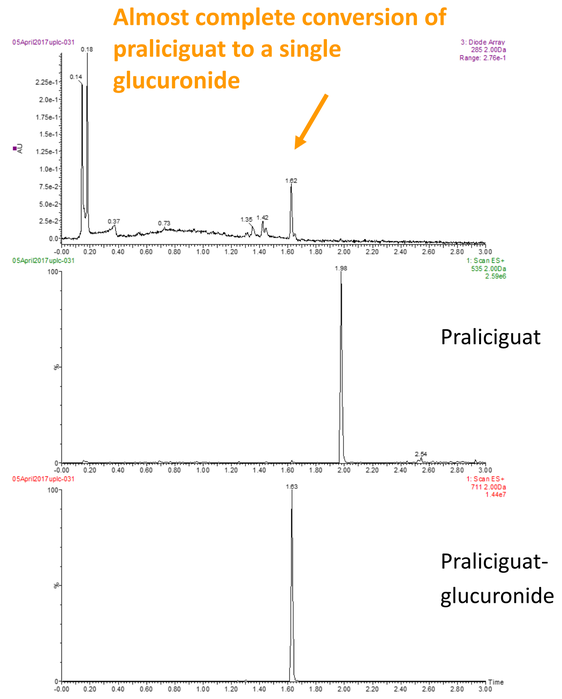
SCALE-UP

- Sp45 scaled up to 4L (80 flasks of 50 ml M3G medium) with dosing at 100 mg/L after 24h pre-growth at 27°C & 200 rpm.
- Flasks time coursed to monitor production and harvested 24h post-dosing time.

PURIFICATION

- Broth bound to HP20 resin, washed, eluted and fractionated on prep HPLC using two orthogonal reverse phase separation modes.
- Purity confirmation by ELSD and ^{1}H NMR spectroscopy.





UV_{285nm}, positive $EIC_{535m/z}$ and positive $EIC_{711m/z}$ chromatograms of Sp45 24 hours after dosing with praliciguat. Praliciguat (1.98 min) had been almost completely converted to the *O*-glucuronide (1.63 mins).

Conclusions

- Nearly 100% of praliciguat was biotransformed to a single *O*-glucuronide by the actinomycete Sp45 to provide a total of 184 mg of purified praliciguat-glucuronide.
- The material enabled the structural elucidation of the position of the glucuronide conjugate and was used for determining the identification and circulating concentrations of this metabolite in clinical samples.
- Microbial biotransformation is an efficient and scalable method for producing *O*-glucuronides and other metabolites of drug candidates.

Scan code for a pdf of the poster

Reference

[1] Ali Banijamali, Andrew Carvalho, James Wakefield, Maria Ribadeneira, Timothy Barden, Daniel Zimmer, Jaime Masferrer, Albert Profy, Mark Currie, Todd Milne, Peter Germano. 2019. Quantitative mass balance, tissue distribution, pharmacokinetics and biotransformation pathway of praliciguat, a clinical-stage sgc stimulator, after oral administration in rats. Drug Metabolism and Pharmacokinetics 34(1), Supplement: S56-S57.

About Hypha Discovery

Hypha Discovery Ltd is a UK-based microbial biotechnology company helping partners in pharmaceutical and agrochemical R&D worldwide succeed through the production of human and other mammalian metabolites, as well as specialising in lead-diversification and production of microbially-derived chemicals. Clients routinely access our biocatalysis technology to generate phase I and II metabolites for MetID, stability testing, use as analytical standards and for producing larger amounts for pharmacological testing.