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Abstract: Scalable methods are needed for producing synthetically intractable major human metabolites of drugs in sufficient quantity for further biological testing, and for use as standards in bioanalytical studies. Ideally the method used should eliminate use of animal liver fractions on a larger scale but yet retain the ability to produce the target human metabolite(s).¹ This poster discusses the application of PolyCYPs and PolyUGT enzymes in making clinically relevant metabolites of drugs.

PolyCYPs are promiscuous cytochrome P450 enzymes that can produce oxidized metabolites of common human CYPs involved in drug metabolism. PolyUGTs mimic human UGTs in making *O*-, acyl and some *N*-glucuronides of drugs. Both enzyme types have been cloned from actinomycete bacteria and expressed in *E. coli* together with the necessary co-factors. PolyUGTs were further purified by affinity chromatography. Whole cell biotransformation methods using the Streptomyces clones containing the enzyme, or the source strain itself, enabled mg to gram quantities of human metabolites of JNJ-61393215 and camonsertib to be produced.

Production of a major hydroxylated metabolite of JNJ-61393215

PolyCYPs® were used to generate sufficient material for structure identification, and for scaling up the production of M54, a major circulating human hydroxylated metabolite of the deuterated orexin-1 receptor antagonist JNJ-61393215.

- Screening JNJ-61393215 with a PolyCYPs kit revealed PolyCYP isoform 152 to be the most proficient at producing the human CYP3A4-derived metabolite M54, with a conversion of 52% at a substrate concentration of 100 µg/ml.
- A subsequent small scale up using PolyCYP 152 scale-up vials provided sufficient quantities of M54 for structure elucidation by NMR spectroscopy, revealing stereoselective hydroxylation on the deuterated 2-aza-[2.2.1]-bicycle core structure.



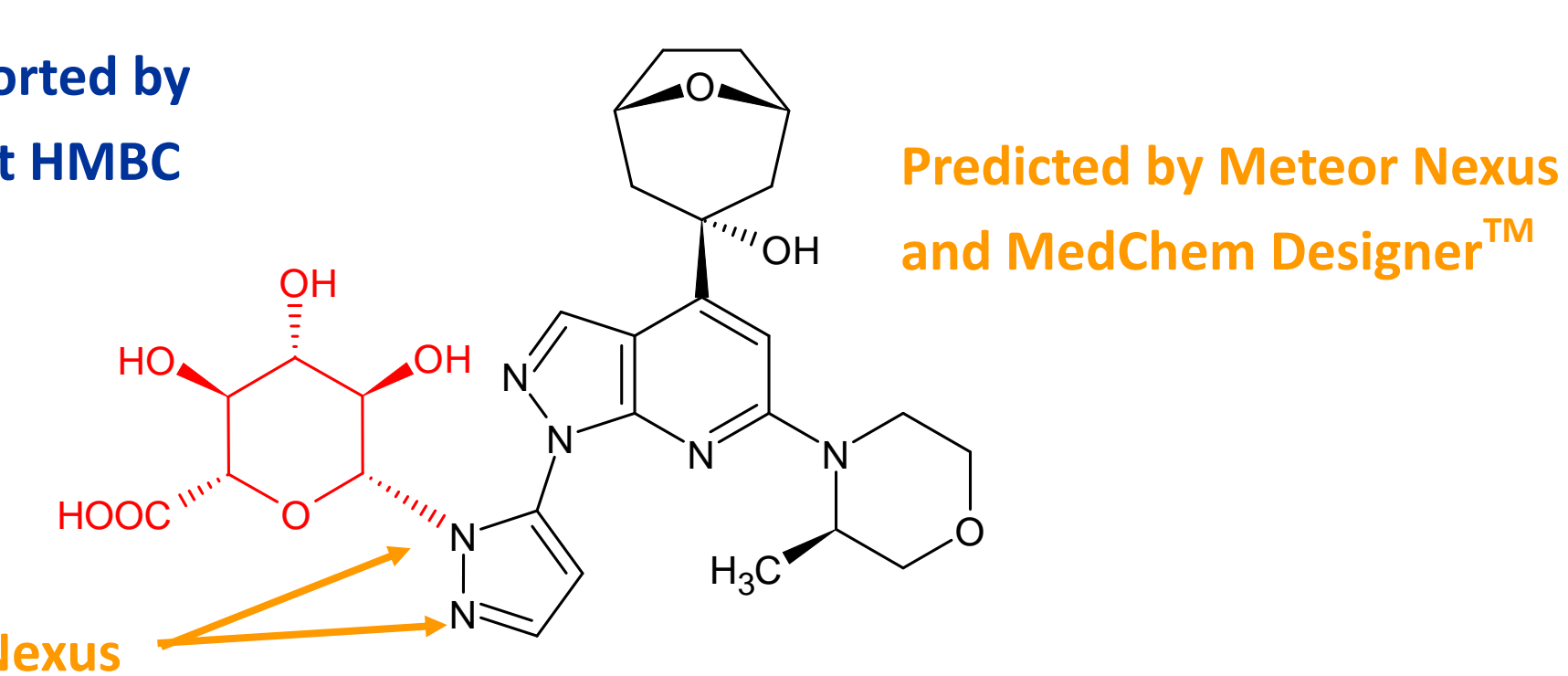
- The PolyCYPs reaction was replicated in a live whole cell biotransformation with a Streptomyces strain from which PolyCYP 152 was cloned at near full conversion to M54 using the same substrate concentration.
- Increasing the substrate concentration to 500 mg/L allowed a reduction in volume of the biotransformation reaction whilst still permitting a high conversion to M54.
- 7.5 grams of >99% pure M54 was isolated from a 50L biotransformation (5 x 10L batches) using 25g substrate at an overall 30% isolated yield.
- This biotransformation would have been challenging and resource-intensive to try and achieve via chemical synthesis due to the nature of the ring system in which the biotransformation occurred.

Production of a major *N*-glucuronide of camonsertib

A major *N*-glucuronide of the ATR kinase inhibitor camonsertib (RP3500) was first observed in human hepatocyte incubations. Material was subsequently needed to determine the structure, evaluate its stability as part of bioanalytical method development, and for use as a standard for estimating its concentration in Phase I clinical samples.

- Camonsertib is metabolized by Phase I mechanisms and to a single UGT1A4-derived glucuronide, the position of attachment of which was challenging to pinpoint by LC-MS/MS and prediction software.
- Late stage chemical synthesis generated 2 *N*-linked glucuronides at the pyrazole but at low yield, whereas microbial biotransformation generated the specific *N*-glucuronide matching the human hepatocyte product.
- The *N*-glucuronide was scaled-up using Sp288 and 31.7 mg was purified from a 900 ml biotransformation with a 255 mg parent dose (12.4% isolated yield).
- NMR spectroscopy revealed the specific point of attachment of the glucuronic acid through NOE data and the gradient HMBC experiment.³

Point of attachment supported by NOE data and the gradient HMBC experiment.³



- Subsequent to this work, camonsertib was screened against Hypha's PolyUGTs. The *N*-glucuronide was specifically produced by PolyUGT 179 at a conversion of 12.4%. The source strain of PolyUGT 179 is the same microbe (Sp. 288) that was originally used to scale-up the *N*-glucuronide.
- Use of a PolyUGTs scale-up kit would have provided a quick way to generate sufficient material for structure elucidation by cryoprobe NMR spectroscopy, with a whole cell biotransformation subsequently required for generation of more metabolite for analytical studies.

Conclusions

- Application of PolyCYPs and PolyUGT enzymes provided a scalable route to access human CYP- and UGT-derived synthetically challenging human metabolites of JNJ-61393215 and camonsertib, respectively.
- The biocatalytic platforms bridge the gap between early MetID needs and large-scale metabolite supply, reducing reliance on animal-derived systems and circumventing the need for complex chemical synthesis.

References

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About Hypha: Hypha Discovery is a specialist CRO supporting pharmaceutical and agrochemical companies worldwide through MetID, metabolite synthesis and identification. We are experts in the scalable production, purification and definitive identification of drug metabolites, and also possess a wealth of experience in the production, purification and structure elucidation of natural products.