SYNTHESIS OF *N*-GLUCURONIDES OF PYRAZOLE MOIETIES IN DRUGS ARISING FROM UGT-1A4 MEDIATED GLUCURONIDATION IN HUMANS

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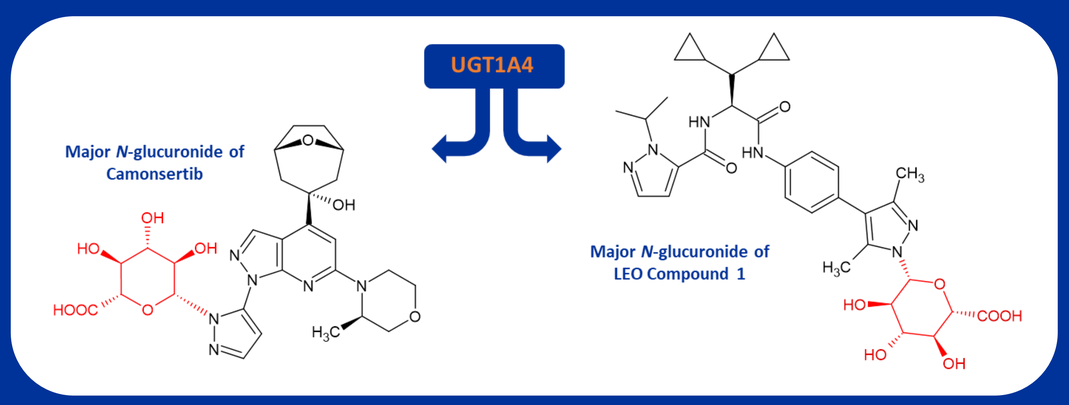


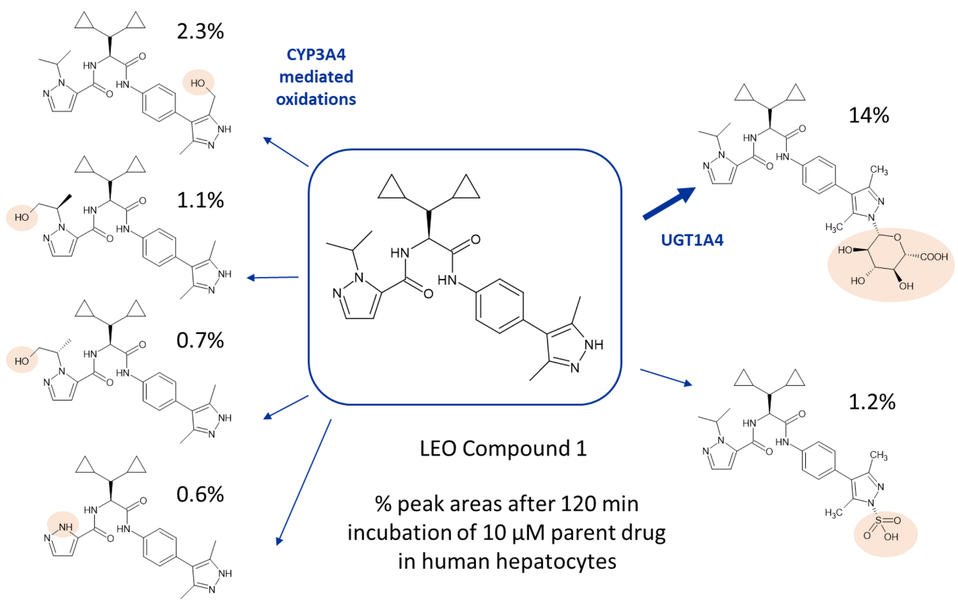
Abstract: Glucuronidation is the most common phase II reaction observed in the metabolism of drugs in humans, involving conjugation of small molecules to glucuronic acid by UDP-glucuronosyltransferases (UGTs). *N*-glucuronides can be formed as a result of aliphatic and aromatic conjugations with pyrazole, pyridine, pyridazine, pyrrolidine, pyrimidine, imidazole, triazole, and tetrazole moieties all being susceptible ring structures. This poster illustrates the synthesis of two human *N*-glucuronides of pyrazole-containing drugs in clinical development, using both biotransformation and late-stage chemical glucuronidation techniques.

Case study 1

Liver S9 biotransformation to scalable late-stage chemical synthesis of a major human *N*-glucuronide metabolite of LEO Compound 1

LEO compound 1: an oral IL-17A protein-protein interaction modulator under development for the treatment of psoriasis and other inflammatory disorders [1].

- Metabolised through multiple Phase I and Phase II routes, including various CYP3A4 mediated hydroxylations and *N*-dealkylation, as well as *N*-sulfation and *N*-glucuronidation.
- Conjugation reactions occur in the pyrazole moiety with an *N*-glucuronide being a major metabolite in humans, observed at 14% in human hepatocytes in the MetID study.
- UGT1A4 responsible for *N*-glucuronidation of LEO compound 1
- Only small amounts of the *N*-glucuronide observed in other species.



Screening

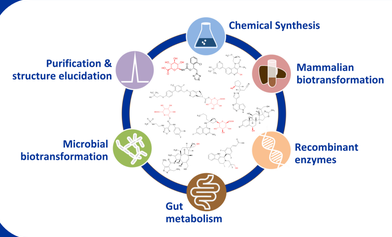
- Due to the low aqueous solubility of LEO compound 1, a phosphorylated prodrug [2] was screened against 23 microbial strains and liver S9 fractions of 15 species.
- High turnover was observed for the prodrug with conversions of 79% in human liver S9 and 7% in microbial species Sp.45, which were shown to match to the target by LC-MS using two different column chemistries with acidic and alkaline pH and comparison of fragmentation patterns.

Scale-up

- The S9 route was initially scaled up due to limited availability of the prodrug substrate, and the higher turnover in this system. A conversion around 60-75% was observed over multiple batches yielding a total of 76.4mg of the unlabelled glucuronide at an isolated yield of 33-37%. The method was also used to make 12.0mg of the deuterated metabolite at a similar yield.
- For production of larger amounts, a late stage chemical synthesis method was subsequently developed. Initially this resulted in only very low conversion, however various modifications in reaction temperature, solvent and reagent stoichiometry allowed the identification of conditions that provided a higher and cleaner conversion, increasing isolated yield from 1.1% to 8.8%.
- Scale up of the optimum chemical synthesis method generated 4.3g of the unlabelled glucuronide at 7% isolated yield with 97% purity by qNMR analysis. The same method was also used to make 213.7mg of the deuterated metabolite.
- Synthesis of the *N*-glucuronide allowed investigations to be conducted, showing excretion of the metabolite in bile, its hydrolysis and the subsequent reabsorption of parent. This was not predicted to lead to significant enterohepatic recirculation.

Case study 2

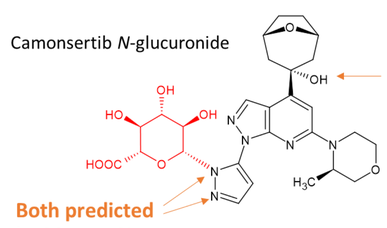
Use of microbial biotransformation to access a human *N*-glucuronide of camonsertib



Screening against multiple biocatalytic and late-stage chemical synthesis conditions

Scale-up of best condition, purification and structure elucidation by NMR spectroscopy

Camonsertib: a novel ATR kinase inhibitor in clinical development for advanced cancers targeting sensitising mutations [3].



- Challenging to pinpoint attachment by LC-MS/MS
- Resolved by NMR on material synthesized using microbial biotransformation
- Point of attachment supported by NOE data and a gradient HMBC experiment

Screening

- Camonsertib (RP3500) was screened against 36 microbial strains and liver S9 fractions of 12 species. Eight microbes produced 5 oxidised metabolites. Two bacteria produced 2 glucuronides, the major one of which matched that observed in human hepatocyte incubations. In liver S9 biotransformations, the glucuronide was best produced in human and NZ rabbit.

Scale up

- Bacterial species 288 was scaled up to 900ml to generate a total of 31.7mg of the *N*-glucuronide at > 95% purity by LC-UV-ELSD from a 255mg dose of the parent.
- To aid delivery of the parent drug to the biotransformation, a relatively high % of a formulant was used, enabling a x5 increase in loading of the parent drug.
- As well as confirmation of structure and accurate quantitation, the pure metabolite was used for short term stability studies (max 6 hrs) in human whole blood and plasma, and deemed stable over the evaluation period [3].
- Despite low turnover, studies with recombinant UGTs suggested camonsertib is a substrate for UGT1A4, with minor contributions from four other UGTs.

Impact of UGT1A4-mediated glucuronidation

Interspecies variability in *N*-glucuronidation is relatively high and is compounded by much higher rates of *N*-glucuronidation in humans. This is largely due to UGT1A4 and UGT2B10. This species difference can lead to the observation of disproportionate *N*-glucuronides during studies in humans. Here, late-stage chemical glucuronidation and microbial biotransformation provided scalable quantities of UGT-1A4 mediated pyrazole-linked *N*-glucuronides of two clinical stage drugs for definitive structure elucidation by NMR and testing of the metabolites.

References

[1] Discovery of an oral, rule-of-5 compliant, IL-17A protein-protein interaction modulator (PPIm) for the treatment of psoriasis and other inflammatory diseases. Mark Andrews. Presentation at the 3rd RSC Anglo-Nordic Medicinal Chemistry Symposium, 13th-16th June 2023.

[2] Amino-acid anilides as small molecule modulators of IL-17. Dack *et al.* (2020). WO2020127685.

[3] Identification and biosynthesis of an *N*-glucuronide metabolite of camonsertib. Papp *et al.* Drug Metabolism and Disposition 2024, 52 (5), 368-376; DOI: https://doi.org/10.1124/dmd.123.001611.

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