Development of *in vitro* Methods to Assess Inhibition Potential in Nine Recombinant UGT Isozymes

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Introduction

UDP-glucuronosyltransferases (UGTs) are major contributors to drug metabolism, ranking second in importance only to cytochrome P450 enzymes. The implementation of the newly published ICH M12 Harmonized Guideline for Drug Interaction Studies has emphasized the importance of evaluating investigational new drugs (INDs) as potential UGT inhibitors during drug development, as it recommends assessing up to eleven UGT enzymes when direct glucuronidation is a major route of drug elimination. The objective of this study is to use guidance-recommended probe substrates [1] (17β-estradiol, telmisartan, trifluoperazine, 5-hydroxytryptophol, propofol, zidovudine, cotinine, oxazepam, and testosterone) to establish optimal conditions for inhibition potential assessment of nine UGT enzymes (UGT 1A1, 1A3, 1A4, 1A6, 1A9, 2B7, 2B10, 2B15, and 2B17) in recombinant human isozymes.

Methods

- Linear conditions were established for each of the nine probe substrates in the respective recombinant human UGT (rhUGT) enzymes up to 0.4 mg/mL.
- To characterize enzyme kinetics parameters, probe substrates at various concentrations were incubated with rhUGT enzymes under determined linear conditions.
- A probe substrate concentration at or below K_m (or K_{half}), along with a protein concentration and incubation time that maintained initial rate conditions were chosen for the UGT Inhibition incubation conditions.
- The IC_{50} dose-response curves and IC_{50} values were determined for recommended UGT inhibitors [1] or zafirlukast (a pan UGT inhibitor).

Results and Discussion

- The UGT inhibition assay conditions were optimized to use 0.1 mg/mL of rhUGT for all isozymes with varying incubation times up to 30 minutes.
- The enzyme kinetics model and the IC_{50} of each inhibitor are presented in Table 1.

Table 1. UGT probe substrate kinetics model and UGT inhibitor IC_{50}

UGT Enzyme	Substrate	Kinetics Model[a]	Inhibitor	IC_{50} (μM)
UGT1A1	17β-Estradiol	AS	Nilotinib	0.145
UGT1A3	Telmisartan	SI	Zafirlukast	3.83
UGT1A4	Trifluoperazine	AS	Hecogenin	0.466
UGT1A6	5-Hydroxytryptophol	SI	Zafirlukast	0.694
UGT1A9	Propofol	SI	Niflumic acid	0.031
UGT2B7	Zidovudine	MM	16β-Phenyllongifolol	0.062
UGT2B10	Cotinine	MM	Desloratadine	6.62
UGT2B15	Oxazepam	SI	Zafirlukast	9.73
UGT2B17	Testosterone	MM	Imatinib	0.029

[a] AS, MM, and SI refer to allosteric sigmoidal, Michaelis-Menten, and substrate inhibition.

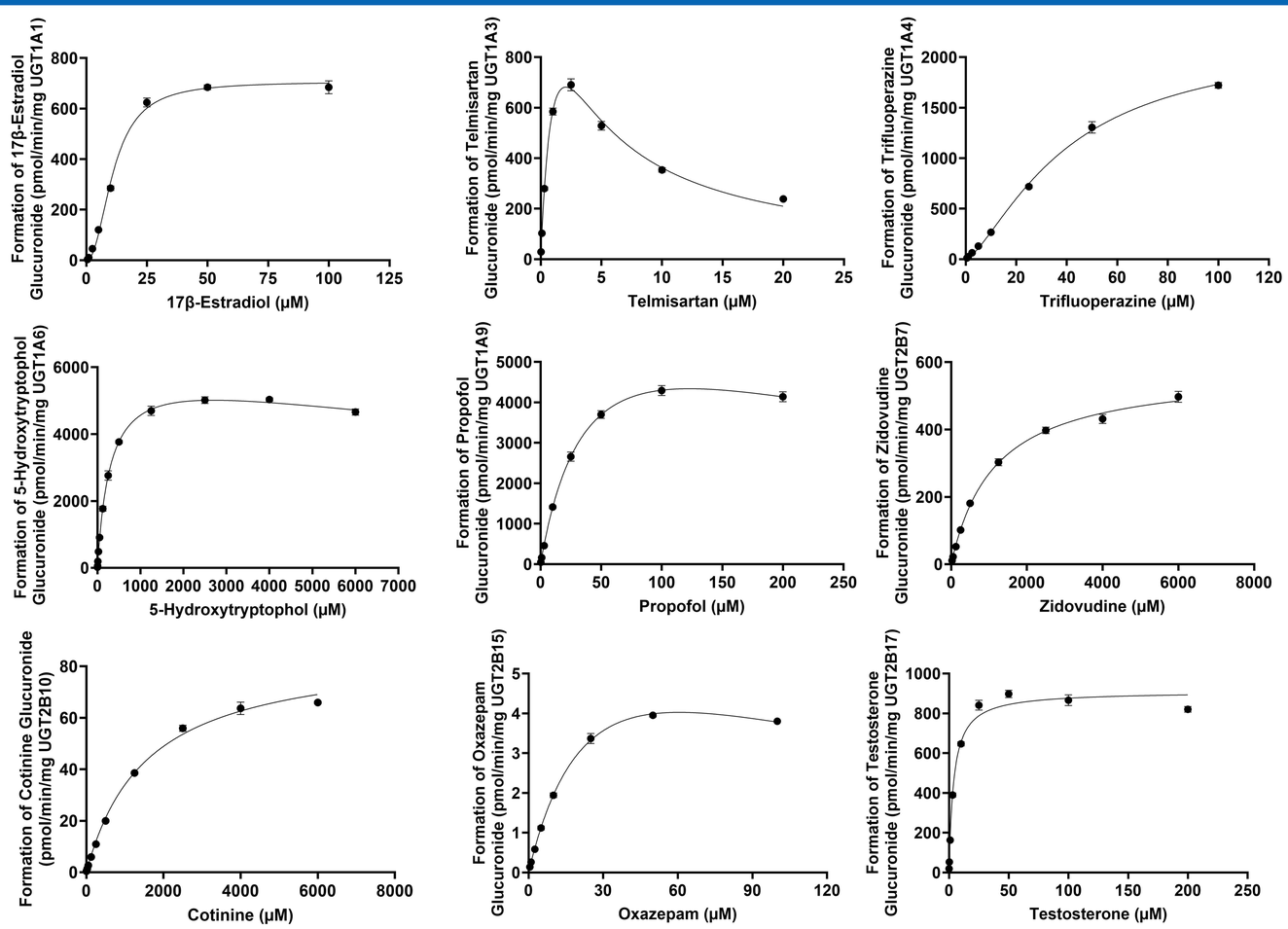

Figure 1. Enzyme kinetics of probe substrate metabolism in rhUGT enzymes.

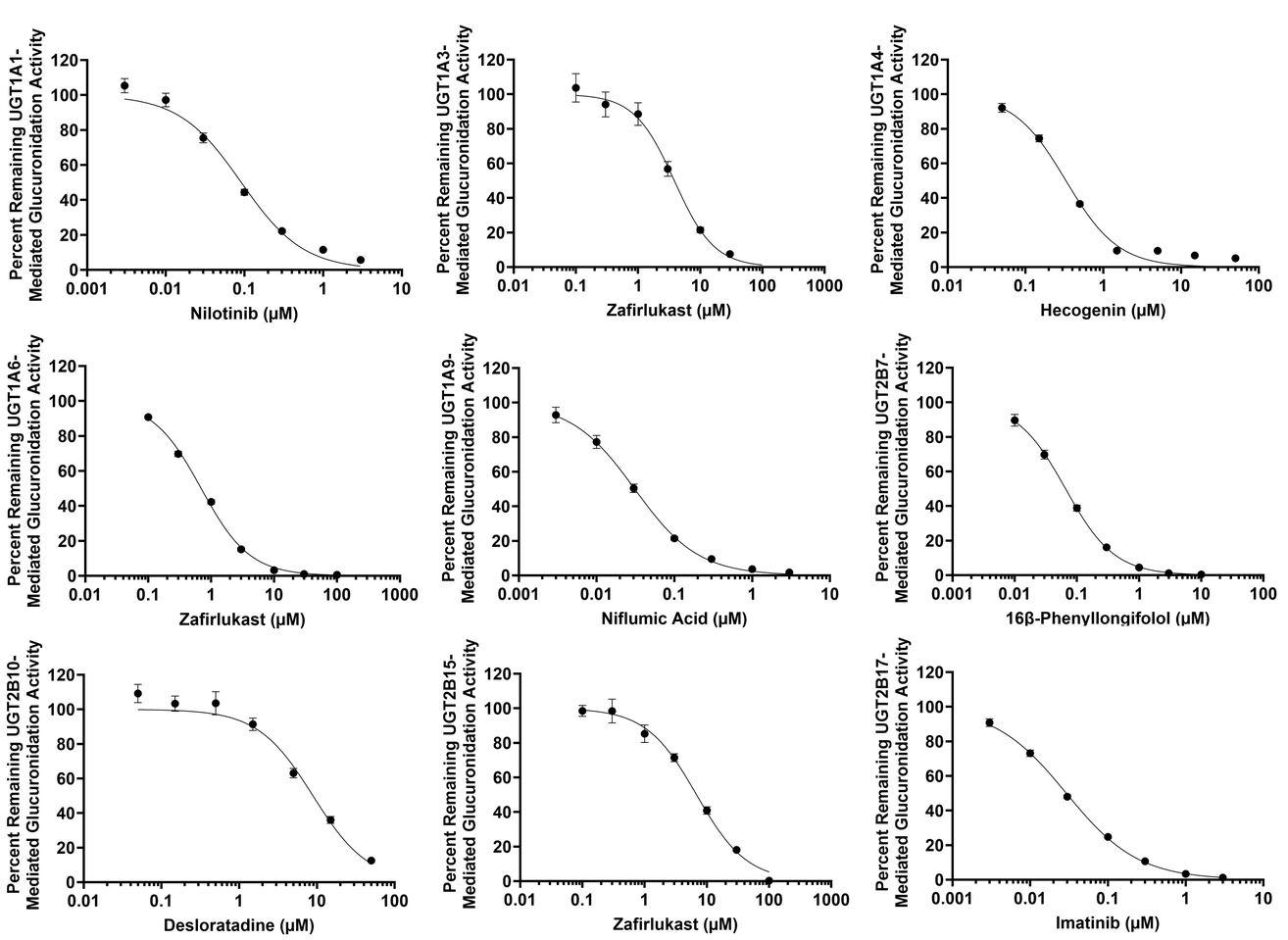

Figure 2. Inhibition of rhUGT-mediated metabolism of probe substrates by UGT inhibitors

Conclusion

The results of this study provided optimized assay conditions aligned with current guidance recommendations for evaluating the UGT inhibitory potential of drug candidates.

Reference

[1] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), M12 Drug Interaction Studies, Guidance for Industry, August 2024.

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