Optimization of UGT Induction and Inhibition Assays

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1 BACKGROUND

Uridine 5'-diphosphoglucuronosyltransferases (UGTs) are a superfamily of enzymes that can detoxify a large variety of xenobiotics and endogenous compounds by catalyzing the conjugation of the substrates with uridine 5-phosphoglucuronic acid (UDPGA) to convert them into more polar glucuronides. Glucuronidation is the major Phase II drug metabolizing pathway and a significant number of drugs are known to be metabolized by UGTs, either directly or following Phase I metabolism. Induction and inhibition of UGTs by xenobiotics may alter the pharmacokinetics of drugs that are eliminated primarily via glucuronidation. It is therefore very important to evaluate the potential of UGT induction and inhibition for new chemical entities (NCEs) during drug development. In human liver, seven major UGT enzymes (1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15) are present and assays to determine the induction and inhibition of these enzymes have been developed.

2 ASSAY DEVELOPMENT - INHIBITION

Based on literature research, SN-38 (1A1), CDCA (1A3), trifluoperazine (TFP; 1A4), serotonine (SER; 1A6), propofol (PRO; 1A9), zidovudine (ZVD; 2B7), oxazepam (OXA; 2B15) were selected as substrates. Analytical methods (UPLC-PDA-MS) were developed and implemented for each substrate and the corresponding glucuronide metabolite and incubations with human recombinant UGT isoenzymes were performed to evaluate the selectivities of the selected substrates.

Sub-strate	Metabo-lite	Relative Metabolite Formation (Highest Peak set at 100%)						
		UGT 1A1	UGT 1A3	UGT 1A4	UGT 1A6	UGT 1A9	UGT 2B7	UGT 2B15
SN-38	SN-38-G	**100**	3	0	1	14	0	0
CDCA	CDCA-G	2	**100**	0	0	1	5	0
TFP	TFP-G	0	0	**100**	0	0	0	0
SER	SER-G	0	0	0	**100**	2	0	0
PRO	PRO-G	0	0	0	0	**100**	0	0
ZVD	ZVD-G	0	0	0	0	0	**100**	0
OXA	R-OXA-G	0	3	0	0	**100**	94	6

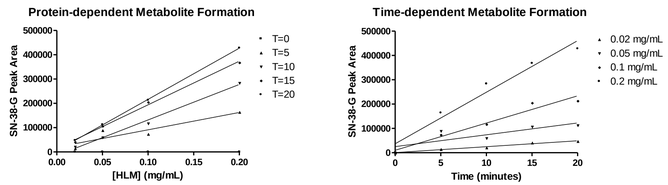


Figure 1 Representative Protein- and Time-dependency Results
Left: Protein-dependency and Right: Time-dependency of the HLM-mediated formation of SN-38-glucuronide after incubating 5 µM of SN-38 at 37ºC at various HLM concentrations using different incubation times.

- Specificity for the intended UGT was confirmed for each substrate.
- For each of the substrates, HLM-mediated metabolite formation was protein- and time-dependent up to the highest HLM concentration tested and the longest incubation time used.

3 INHIBITOR SELECTIVITY - INHIBITION

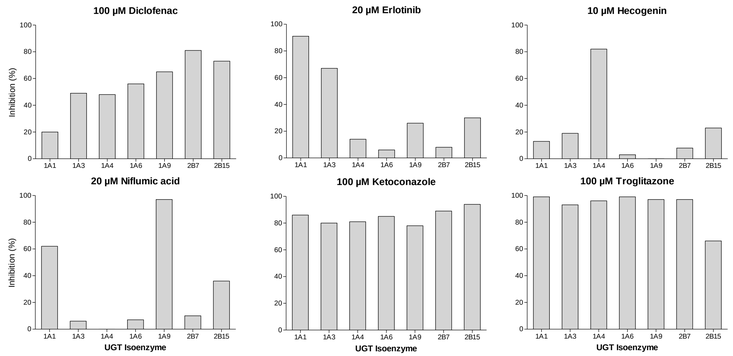


Figure 2 Results of the Inhibitor Screening Experiments
Six UGT inhibitors were tested for their ability to inhibit HLM-mediated formation of UGT-specific substrate reactions (UGT1A1: SN-38; UGT1A3: CDCA; UGT1A4: trifluoperazine; UGT1A6: serotonine; UGT1A9: Propofol; UGT2B7: zidovudine; UGT2B15: oxazepam). Incubations were performed in duplicate using optimized reaction conditions.

4 IC_{50} DETERMINATIONS - INHIBITION

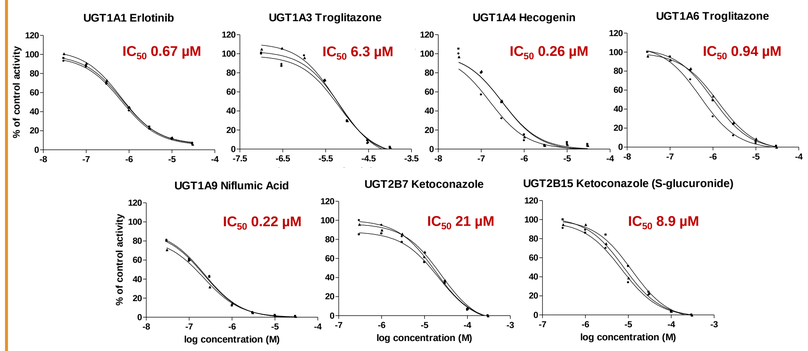


Figure 3 Results of the IC_{50} Experiments
Selected UGT inhibitors were tested for their ability to inhibit HLM-mediated formation of UGT-specific substrate reactions (UGT1A1: SN-38; UGT1A3: CDCA; UGT1A4: trifluoperazine; UGT1A6: serotonine; UGT1A9: Propofol; UGT2B7: zidovudine; UGT2B15: oxazepam). Incubations were performed in triplicate using optimized reaction conditions.

- For each UGT isoform, a specific substrate has been identified.
- For each UGT isoform/substrate/metabolite combination, analytical methods have been implemented and validated (data not shown).
- For each UGT isoform/substrate/metabolite combination, inhibitors have been identified that can be used as positive controls for UGT inhibition studies. Selected inhibitors can also be used for UGT characterization studies.

5 ASSAY IMPLEMENTATION - INDUCTION

To implement induction assays, experiments were performed with cryopreserved plateable rat hepatocytes to investigate the time-dependent glucuronide formation for each of the selected probe substrates and the general Cytochrome P450 (CYP) substrates phenacetin, bupropion and midazolam. In addition, it was evaluated if a cocktail approach could be used to monitor several substrates simultaneously.

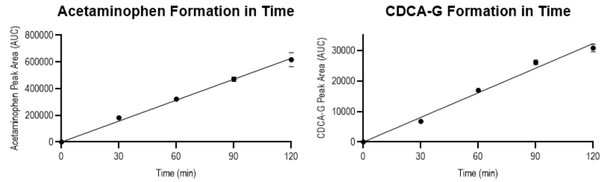
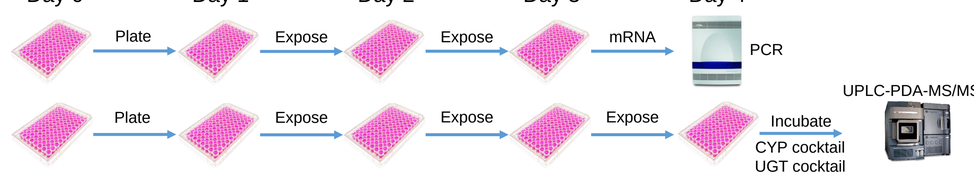


Figure 4 Time-dependent Metabolite Formation
Time-dependency of the formation of acetaminophen and CDCA-glucuronide in cryopreserved plateable male rat hepatocytes after incubation at 37ºC.

The optimized reaction conditions were used to investigate the possible induction of CYP and UGT activities in male rat (donor pool) and male human (one individual donor) hepatocytes by a selection of known CYP inducers using both enzyme activity and mRNA analysis as endpoints using the approach depicted below:



Inducer	Target Gene																			
	CYP 1A2	CYP 2B2	CYP 3A*	UGT 1A1	UGT 1A5	UGT 1A6	UGT 1A7	UGT 2B1	UGT 2B3	UGT 2B12	CYP 1A2	CYP 2B6	CYP 3A4	UGT 1A1	UGT 1A3	UGT 1A4	UGT 1A6	UGT 1A9	UGT 2B7	UGT 2B15
	Male Rat										Male Human									
3-MC	**3.84**	0.60	1.01	0.59	0.58	**4.38**	**16.2**	0.69	0.87	0.78	**28.3**	0.76	0.29	1.29	0.64	0.56	0.44	0.26	0.77	0.44
PB	1.76	**32.2**	**43.8**	1.07	1.17	1.41	1.48	**16.7**	1.99	**2.10**	1.90	**4.01**	**13.7**	1.40	1.12	1.98	0.75	1.16	0.45	0.47
RIF	0.99	1.33	0.98	0.79	0.85	0.82	0.78	0.99	0.91	0.84	0.79	**2.58**	**11.6**	1.17	1.25	1.37	0.62	0.60	0.45	0.48
DEX	1.47	0.74	**435**	1.29	1.08	0.78	0.55	**49.4**	**2.53**	**2.66**	1.31	1.51	**3.21**	1.16	1.03	1.03	0.74	0.95	0.53	0.42
OME	Not performed										**16.1**	1.91	**5.77**	1.92	1.20	1.75	0.77	1.00	0.26	0.28
PCN	0.92	1.25	**160**	1.07	1.44	0.98	1.12	**25.2**	**2.41**	**2.82**	0.84	0.99	**3.82**	1.17	1.05	0.98	1.19	**2.12**	1.08	0.84

Inducer	Metabolite													
	Acetaminophen		OH-bupropion		OH-midazolam		SN38-G		CDCA-G		Trifluoperazine-G		Propofol-G	
	Rat	Human	Rat	Human	Rat	Human	Rat	Human	Rat	Human	Rat	Human	Rat	Human
3-MC	**8.30**	**2.93**	0.81	0.80	0.91	0.83	**2.10**	1.03	0.94	1.14	ND	0.75	**2.18**	0.72
PB	**1.77**	0.72	0.98	**3.38**	1.09	**2.63**	1.35	1.17	1.57	1.49	ND	1.20	1.18	0.96
RIF	1.10	1.24	0.91	**2.42**	0.99	**2.24**	1.05	1.10	1.26	1.46	ND	1.12	1.10	0.87
DEX	1.33	1.30	1.09	1.14	**2.40**	**1.71**	1.19	0.94	**1.76**	1.42	ND	0.87	**1.51**	0.86
OME	NP	**3.37**	NP	1.22	NP	1.12	NP	1.22	NP	**1.57**	NP	1.05	NP	0.84
PCN	1.05	1.16	0.52	0.87	1.15	1.22	1.16	0.87	**1.76**	1.22	ND	0.87	1.23	0.83

- mRNA analysis was successfully used to measure the induction of 3 CYP and 7 UGT genes in both rat and human hepatocytes.
- The cocktail setup was successfully used to measure CYP and UGT enzyme activities in both rat and human hepatocytes.
- A strategy was successfully implemented to monitor CYP and UGT induction and which may help to determine if specific metabolism-related effects observed in animal models are human relevant and this includes induction of hepatic glucuronidation