Supplemental data for

Drug Metabolism and Disposition

# In vitro assay of six UGT isoforms in human liver microsomes, using cocktails of probe substrates and LC-MS/MS

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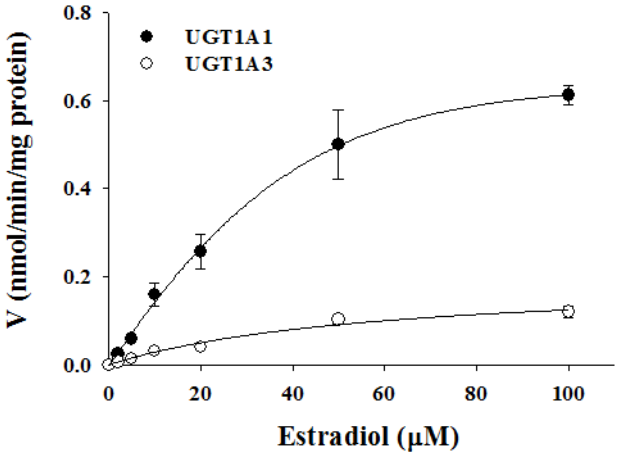
**Supplemental Table 1.** Published $K_m$ values for six UGT substrates.

| UGT isoform | Substrates | Reported $K_m$ (μM) | | References |
|---|---|---|---|---|
| | | Recombinant | HLMs | |
| UGT1A1 | β-estradiol | 22 | 20 - 30 | (Soars et al., 2003, Alkharfy and Frye, 2002) |
| UGT1A3 | Chenodeoxycholic acid | 18.6 - 130 | 10.6 - 372 | (Trottier et al., 2006, Gagez et al., 2012, Matern.S. et al., 1984) |
| UGT1A4 | Trifluoperazine | 23 - 39 | 61 - 106 | (Uchaipichat et al., 2006, Gagez et al., 2012) |
| UGT1A6 | 4-Hydroxyindole | 178.7 | 30 - 63.5 | (Manevski et al., 2010), in house |
| UGT1A9 | Propofol | 45 - 59.8 | 64 - 280 | (Liang et al., 2011, Fujiwara et al., 2007, Soars et al., 2001, Soars et al., 2003) |
| UGT2B7 | Naloxone | 40 – 60 | 423 - 870 | (Coffman et al., 1998, Donato et al., 2010, Soars et al., 2001) |

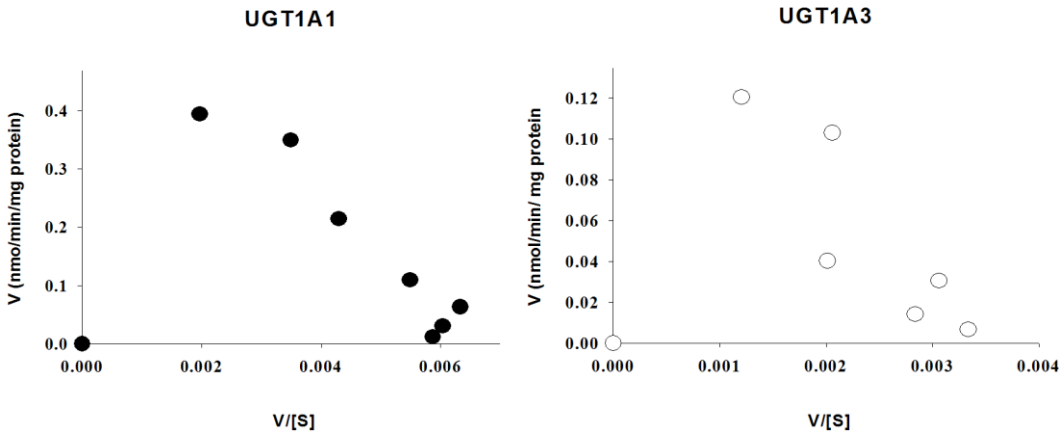
**Supplemental Table 2.** Kinetic parameters for the glucuronide conjugates mediated by human cDNA-expressed UGT isoforms.

| Substrate | UGT isoforms | Kinetic Parameters | | | |
|---|---|---|---|---|---|
| | | $K_m$ (μM) | $V_{max}$ (nmol/min/mg protein) | $CL_{int}$ ($V_{max}/K_m$) (μl/min/mg protein) | Hill coefficient (n) |
| Estradiol | UGT1A1 | 30.9 | 0.752 | 24.3 | 1.3 |
| | UGT1A3 | 58.5 | 0.198 | 3.4 | |
| Chenodeoxycholic acid | UGT1A1 | 55.8 | 1.075 | 19.3 | 1.2 |
| | UGT1A3 | 24.4 | 8.653 | 354.8 | |
| 4-Hydroxyindole | UGT1A6 | 135.5 | 9.848 | 72.7 | |
| | UGT1A9 | 375.4 | 1.544 | 4.1 | 1.5 |

**(A)**

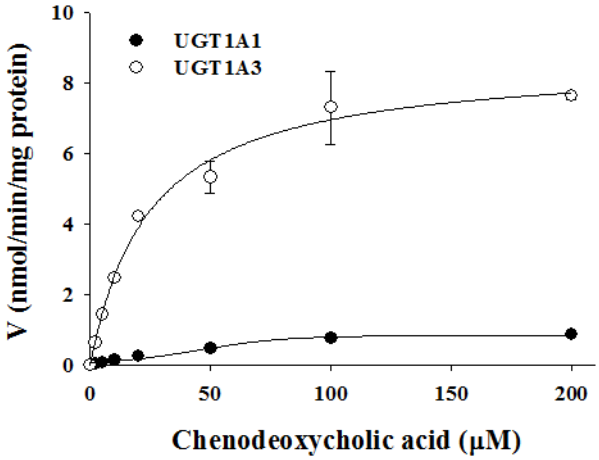



**(B)**

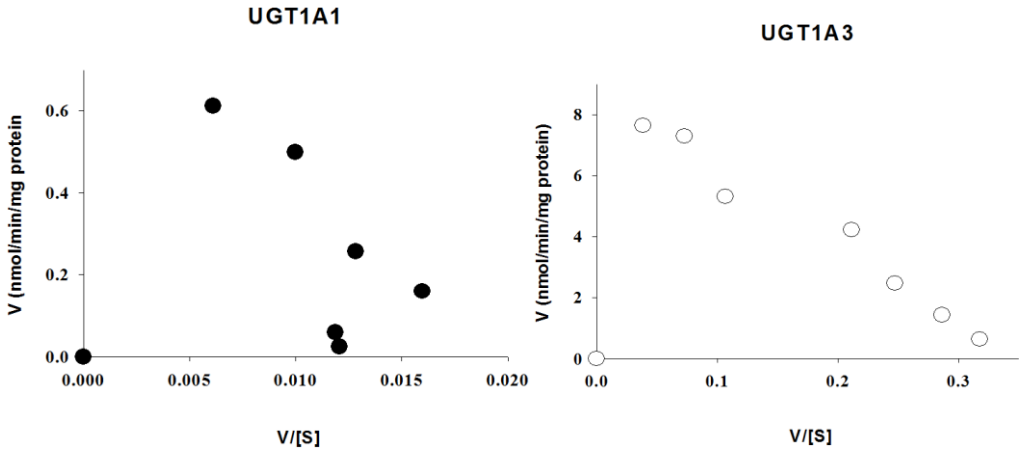



**Supplemental Figure 1**. Kinetics for the formation of estradiol 3-glucuronide from estradiol in human cDNA-expressed UGT1A1 and 1A3 (A) and the respective corresponding Eadie-Hofstee plots (B). An increasing concentration of estradiol was incubated with each recombinant UGT and UDPGA at 37$^{o}$C for 30 min. The kinetic data were fitted by Michaelis-Menten equation for UGT1A3 and a Hill equation for UGT1A1. Each data point represents the mean ± SD of triplicate determinations.

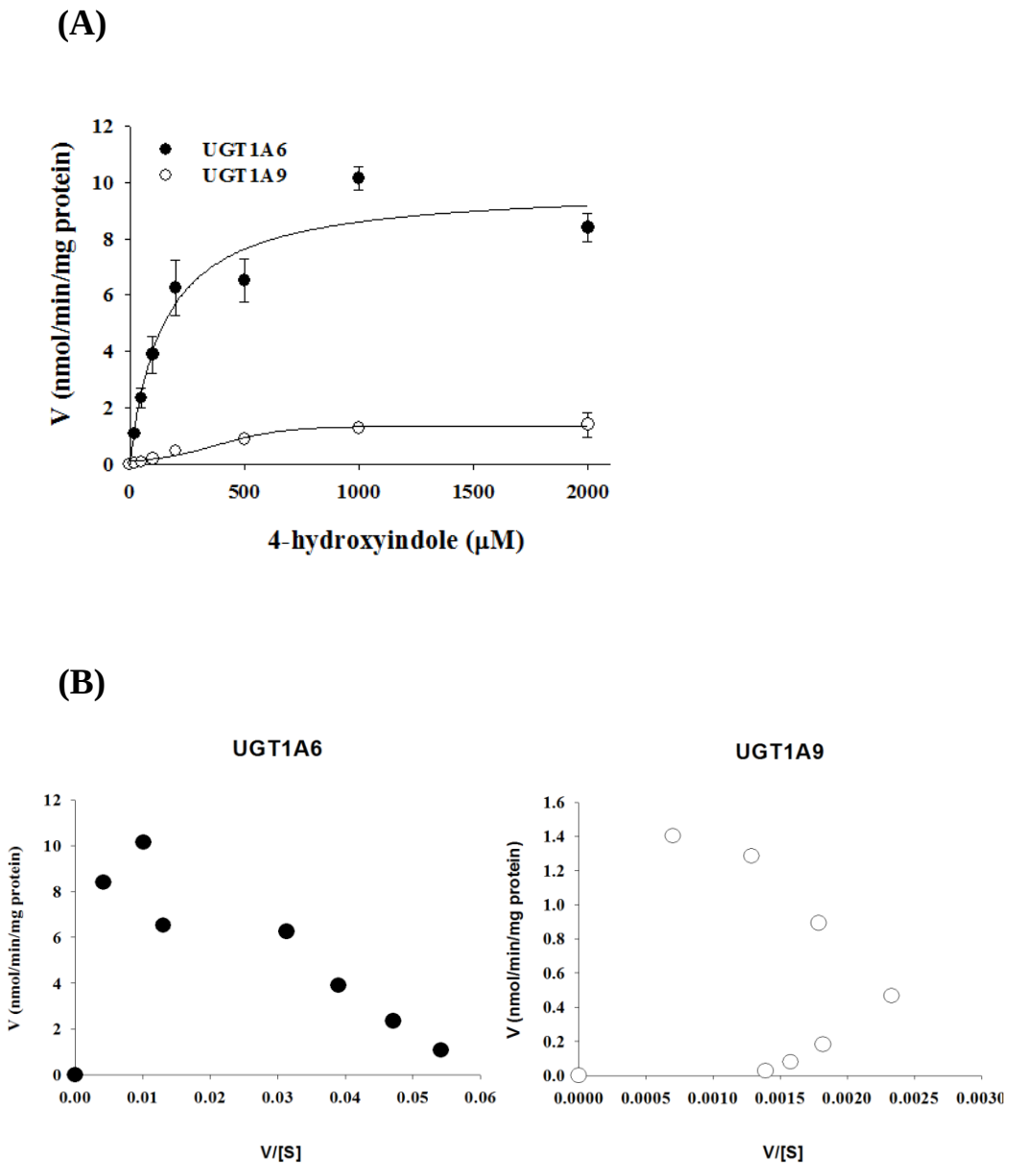
**(A)**



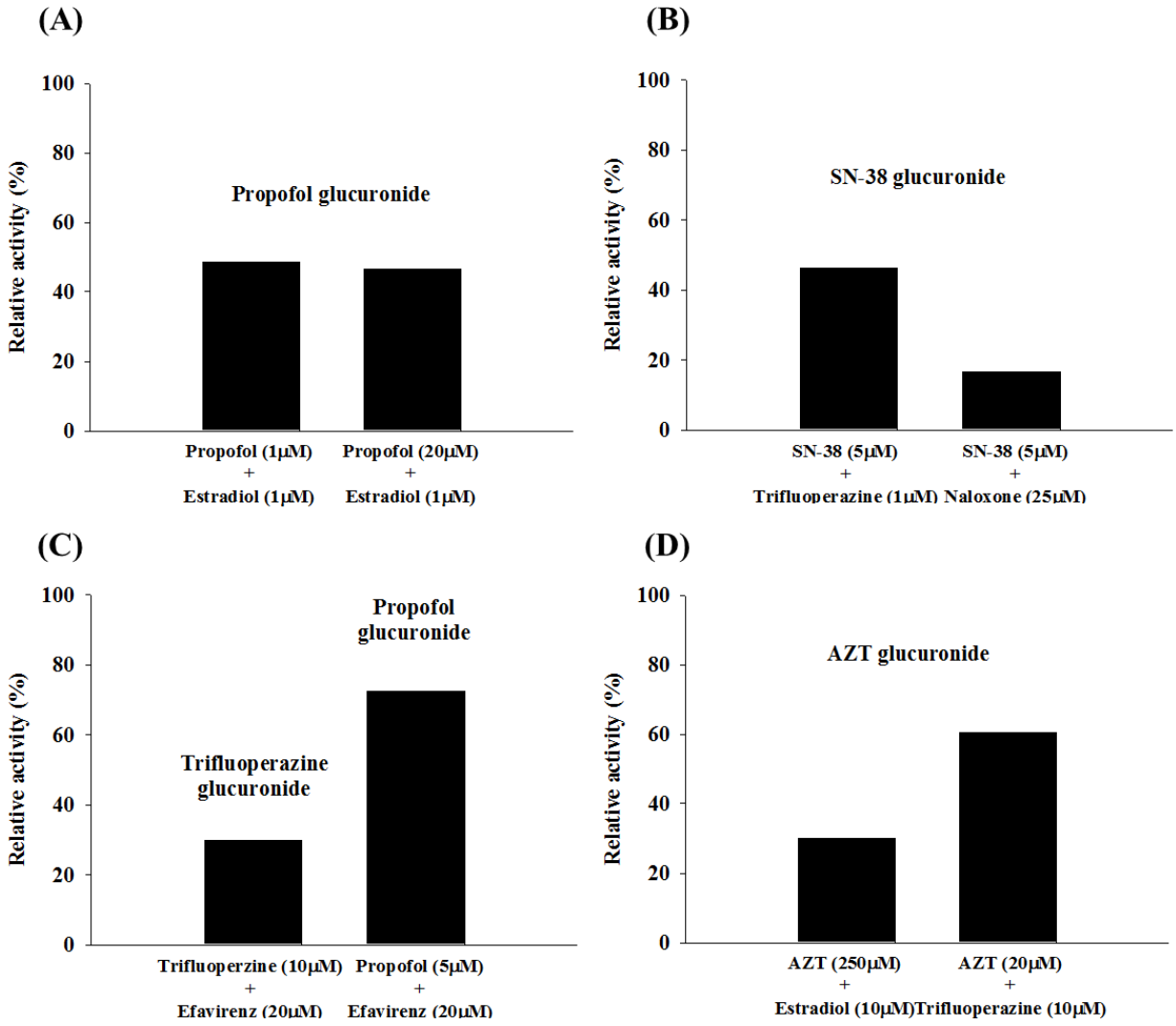

**(B)**



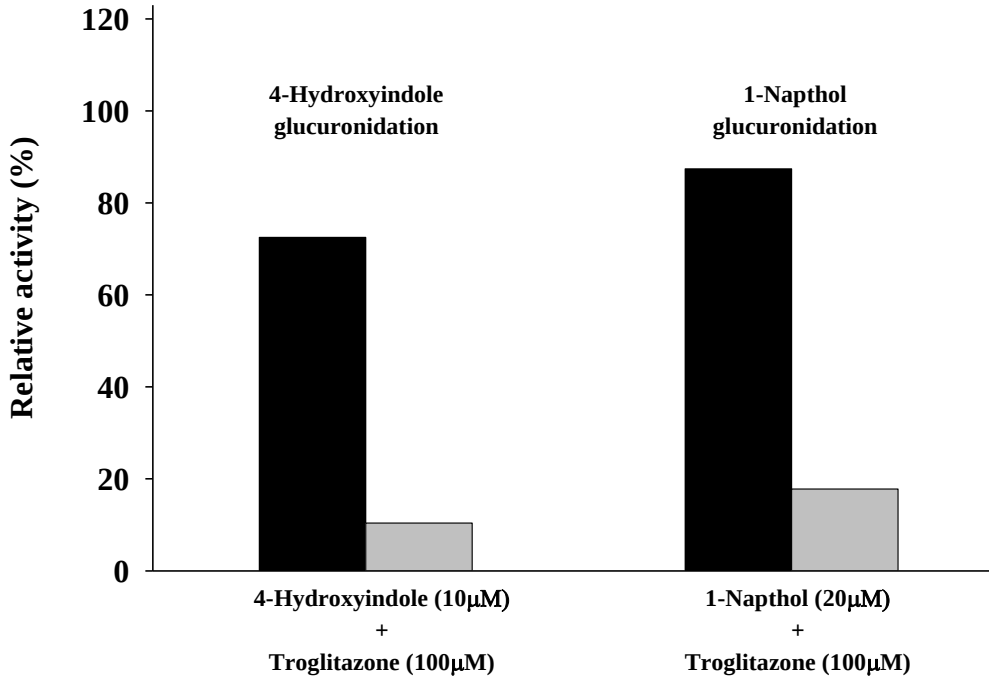

**Supplemental Figure 2**. Kinetics for the formation of chenodeoxycholic acid glucuronide from chenodeoxycholic acid in human cDNA-expressed UGT1A1 and 1A3 (A) and the respective corresponding Eadie-Hofstee plots (B). Increasing concentrations of chenodeoxycholic acid was incubated with each recombinant UGT and UDPGA at 37$^{o}$C for 30 min. The kinetic data were fitted by Michaelis-Menten equation for UGT1A3 and a Hill equation for UGT1A1. Each data point represents the mean ± SD of triplicate determinations.




**Supplemental Figure 3**. Kinetics for the formation of 4-hydroxyindole glucuronide from 4-hydroxyindole in human cDNA-expressed UGT1A6 and 1A9 (A) and the respective corresponding Eadie-Hofstee plots (B). Increasing concentrations of 4-hydroxyindole was incubated with each recombinant UGT and UDPGA at 37$^{o}$C for 30 min. The kinetic data were fitted by Michaelis-Menten equation for UGT1A6 and a Hill equation for UGT1A9. Each data point represents the mean ± SD of triplicate determinations.




**Supplemental Figure 4**. Inhibition of UGT isoform selective activities by various substrates in human liver microsomes: propofol glucuronidation by estradiol (A), SN-38 glucuronidation by trifluoperazine and naloxone (B), trifluoperazine and propofol glucuronidation by efavirenz (C), and zidovudine (AZT) glucuronidation by estradiol and trifluoperazine (D). Each data point represents the mean of duplicate determinations.




**Supplemental Figure 5**. Inhibition of UGT1A6-mediated 4-hydroxyindole and 1-napthol glucuronidation by troglitazone in human liver microsomes (black) and recombinant UGT1A6 (grey). Each data point represents the mean of duplicate determinations.

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