Supporting Information

Paper Title: Mutual Regioselective Inhibition of Human UGT1A1-Mediated Glucuronidation of Four Flavonoids

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Table S1 Apparent Enzyme Kinetic Parameters of Glucuronidation of 3HF,7HF and 4′HF in the Presence of 3,7,4'THF Fitted to Different Inhibition Kinetics Model (*n*=3, $\bar{x}$ ±SD)

Glucuronides \ Inhibition model		Competitive Inhibition	Noncompetitive Inhibition	Uncompetitive Inhibition	Mixed-type Inhibition
3HF-*O*-G	K_m (μM)	1.382±0.240	2.273±0.287	2.857±0.475	1.675±0.286
	V_{max}(nmol/mg/min)	1.909±0.083	2.189±0.099	2.328±0.155	2.015±0.097
	K_i (μM)	0.267±0.045	1.312±0.124	0.876±0.116	0.509±0.151
	α	-	-	-	5.034±1.080
	R^2	0.943	0.946	0.921	0.953
	AIC	1.759	-0.980	21.808	-7.600
7HF-*O*-G	K_m (μM)	1.628±0.212	2.472±0.185	3.166±0.333	2.212±0.233
	V_{max}(nmol/mg/min)	1.302±0.045	1.480±0.041	1.599±0.070	1.430±0.049
	K_i (μM)	0.456±0.061	1.936±0.123	1.236±0.115	1.286±0.329
	α	-	-	-	1.903±0.799
	R^2	0.959	0.976	0.961	0.977
	AIC	-72.612	-105.595	-75.240	-106.328
4′HF-*O*-G	K_m (μM)	1.821±0.230	2.810±0.243	3.616±0.448	2.289±0.269
	V_{max}(nmol/mg/min)	0.664±0.024	0.757±0.025	0.821±0.044	0.711±0.027
	K_i (μM)	0.410±0.051	1.706±0.120	1.072±0.115	0.871±0.205
	α	-	-	-	3.147±1.056
	R^2	0.965	0.972	0.952	0.975
	AIC	-163.055	-176.976	-145.000	-182.221

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Table S2 Apparent Enzyme Kinetic Parameters of Glucuronidation of 3,7,4'THF (3,7,4'THF-3-*O*-G and 3,7,4'THF-7-*O*-G) in the Presence of 3HF, 7HF and 4′HF Using Different Inhibition Kinetics Model, respectively. (*n*=3, $\bar{x}$ ±SD)

Metabolites	Inhibitors	Inhibition model	Competitive Inhibition	Noncompetitive Inhibition	Uncompetitive Inhibition	Mixed-type Inhibition
3,7,4'THF-3-O-G	3HF	K_m (μM)	0.113±0.037	0.257±0.038	0.290±0.048	0.200±0.045
		V_{max}(nmol/mg/min)	0.541±0.021	0.635±0.024	0.644±0.029	0.609±0.026
		K_i (μM)	1.333±0.297	11.509±1.570	9.626±1.491	4.142±1.243
		α	--	--	--	3.966±1.760
		R^2	0.735	0.796	0.768	0.809
		AIC	-69.843	-85.703	-77.813	-87.505
	7HF	K_m (μM)	0.078±0.041	0.262±0.041	0.296±0.056	0.194±0.045
		V_{max}(nmol/mg/min)	0.558±0.027	0.686±0.028	0.697±0.035	0.653±0.029
		K_i (μM)	0.351±0.185	4.947±0.541	3.998±0.517	1.766±0.720
		α	--	--	--	3.955±1.273
		R^2	0.766	0.861	0.829	0.873
		AIC	-52.258	-83.323	-71.038	-86.884
	4′HF	K_m (μM)	0.089±0.054	0.246±0.055	0.284±0.070	0.214±0.069
		V_{max}(nmol/mg/min)	0.577±0.036	0.707±0.040	0.722±0.047	0.690±0.046
		K_i (μM)	0.660±0.419	6.696±1.117	5.437±0.992	3.664±0.768
		α	--	--	--	2.168±1.161
		R^2	0.601	0.716	0.697	0.719
		AIC	-11.833	-32.092	-28.240	-30.763
3,7,4'THF-7-O-G	3HF	K_m (μM)	0.146±0.036	0.247±0.035	0.274±0.042	0.213±0.045
		V_{max}(nmol/mg/min)	0.612±0.021	0.687±0.025	0.697±0.029	0.670±0.029
		K_i (μM)	3.363±1.189	20.769±3.681	17.517±3.379	9.043±2.715
		α	--	--	--	3.051±1.229
		R^2	0.703	0.745	0.729	0.751
		AIC	-63.476	-72.745	-68.942	-72.122
	7HF	K_m (μM)	0.078±0.040	0.256±0.041	0.288±0.055	0.186±0.046
		V_{max}(nmol/mg/min)	0.619±0.030	0.759±0.032	0.771±0.039	0.721±0.033
		K_i (μM)	0.392±0.203	5.377±0.617	4.362±0.585	1.809±0.762
		α	--	--	--	4.331±1.600
		R^2	0.763	0.845	0.814	0.859
		AIC	-40.305	-65.960	-54.749	-69.591
	4′HF	K_m (μM)	0.120±0.057	0.255±0.058	0.293±0.072	0.231±0.076
		V_{max}(nmol/mg/min)	0.651±0.039	0.775±0.046	0.791±0.053	0.761±0.054
		K_i (μM)	1.434±0.796	10.190±2.057	8.301±1.803	6.223±1.738
		α	--	--	--	1.886±0.588
		R^2	0.552	0.641	0.626	0.642
		AIC	3.050	-8.466	-7.695	-10.151

Table S3. IC_{50} Values of 3,7,4'THF as the Inhibitor of Glucuronidation of 3HF,7HF and 4′HF

Substrate Conc. (μM) / Substrate name	0.625	2.5	5	10
3HF	1.07±0.08	1.10±0.03	1.22±0.01	1.55±0.06
7HF	1.80±0.07	1.81±0.06	2.24±0.07	2.40±0.06
4′HF	1.69±0.09	1.57±0.08	1.95±0.10	2.03±0.06

Concentrations of the inhibitor 3,7,4'THF were 0, 0.313, 0.625, 1.25, 5 μM.

Table S4 IC_{50} Values of MHF-mediated Inhibition of Glucuronidation of 3,7,4'THF

Substrate Glucuronides/Inhibitor \ 3,7,4'THF (μM)		0.313	0.625	1.25	5
3,7,4'THF-3-*O*-G	3HF	6.74±1.53	8.14±0.35	10.98±1.09	13.68±0.95
	7HF	3.61±0.41	4.85±0.33	4.64±0.20	7.27±0.14
	4'HF	6.70±0.99	5.66±0.30	6.59±0.70	9.34±0.59
3,7,4'THF-7-*O*-G	3HF	10.30±1.05	18.13±2.05	15.29±1.83	24.49±2.68
	7HF	3.96±0.22	5.21±0.66	4.77±0.08	8.93±0.37
	4'HF	11.30±0.55	8.21±0.48	8.25±0.60	13.94±0.36

Concentrations of the inhibitor 3HF,7HF and 4′HF were 0, 0.625, 2.5, 5, 10 μM, respectively.

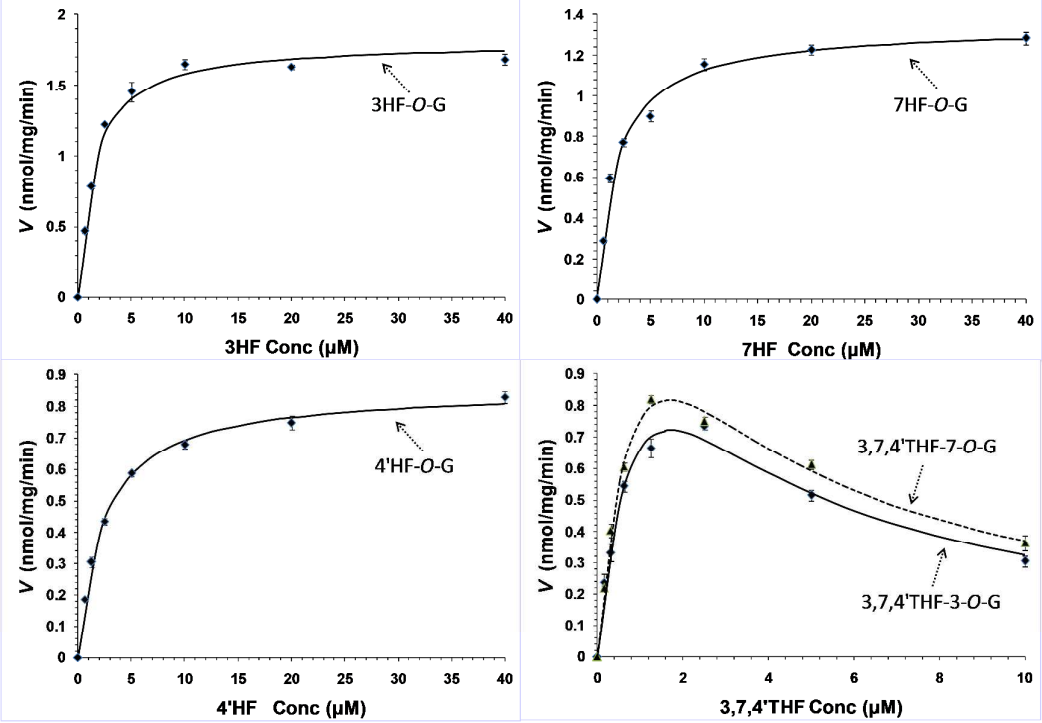

Figure S1. Kinetics profiles of UGT1A1-mediated glucuronidation of four flavonoids (3HF,7HF, 4′HF and 3,7,4' THF). Diamonds (triangle) and smooth lines denote observed and predicted glucuronidation rates of flavonoids, respectively. Solid and dashed lines denote formation rates of 3,7,4'THF-3-*O*-G and 3,7,4' THF-7-*O*-G, respectively. Predicted glucuronidation rates of 3HF, 7HF, 4′HF were from Michaelis-Menten models. Predicted glucuronidation rates of 3,7,4'THF were from substrate inhibition models. Each data point represents the average of three replicates. Experimental details are presented under *Materials and Methods*. For kinetic parameters, please see Table 2.

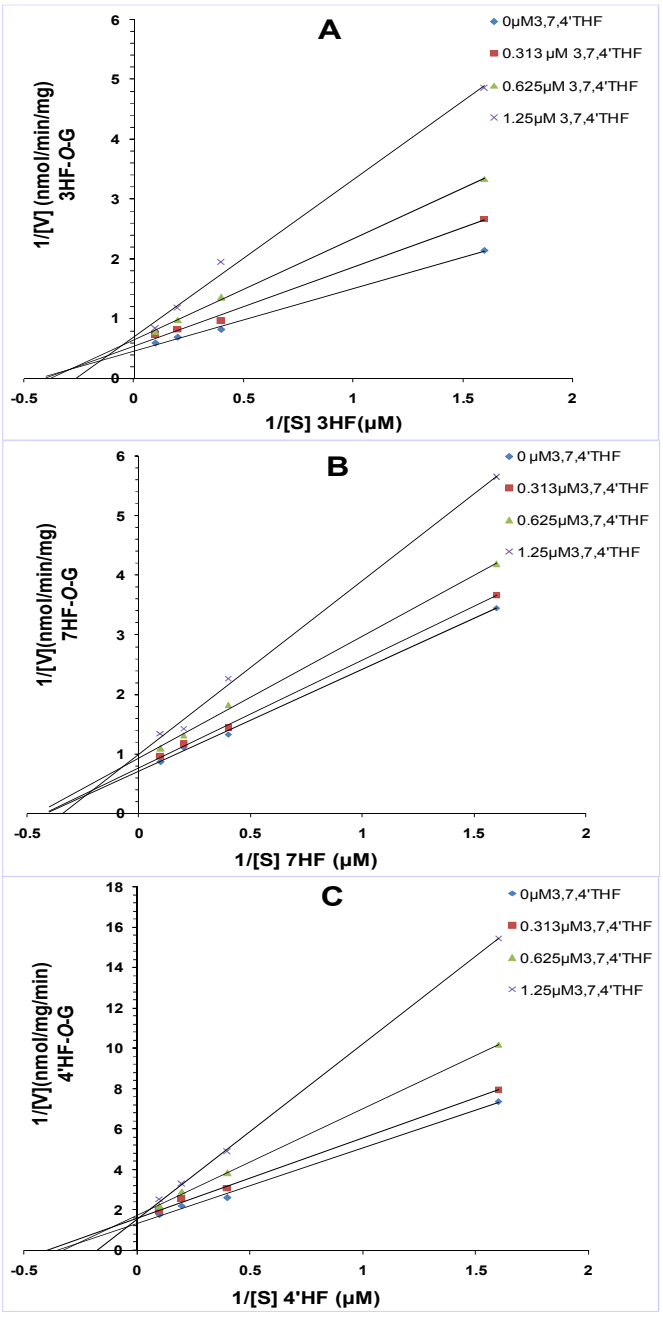

Figure S2. Lineweaver-Burk (double-reciprocal) plots (1/*V* versus 1/[*S*]) of UGT1A1-mediated glucuronidation of 3HF (A, 3HF-*O*-G), 7HF (B, 7HF-*O*-G) and 4′HF (C, 4′HF-*O*-G) in the presence of different concentrations of 3,7,4′THF, respectively. Concentrations of the substrate 3HF, 7HF, 4′HF were 0.625, 2.5, 5 and 10 μM, respectively. Concentrations of the inhibitor 3,7,4'THF were 0, 0.313, 0.625 and 1.25 μM. This is a corresponding ampliative Lineweaver-Burk plots at the lower inhibitor concentrations from Figure 3. Each plot represents the mean of duplicate measurements.