Electronic Supplementary Information

Chemical inhibitors and stable knock-down of efflux transport leads to reduced glucuronidation of wushanicaritin in UGT1A1-overexpressing HeLa cells: The role of breast cancer resistance protein (BCRP) and multidrug resistance-associated proteins (MRPs) in the excretion of glucuronides

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Table List and Figure Caption

Table S1 Relative protein expression of transporters (normalized to the levels of GAPDH) in HeLa1A1 and engineered HeLa1A1 cells based on western blotting.

Figure S1 Chemical structures and MS/MS spectra of wushanicaritin (a), WICT-3-G (b) and WICT-7-G (c). The sample was generated after incubation of HeLa1A1 cell lysate with wushanicaritin (1 μM) for 60 min. WICT-3-G, wushanicaritin-3-*O*-glucuronide; WICT-7-G, wushanicaritin-7-*O*-glucuronide.

Figure S2 Effects of dipyridamole (5 and 20 μM) on the glucuronidation disposition upon incubation of HeLa1A1 cells with wushanicaritin. The concentration of wushanicaritin was 1 μM. (a) Effects of dipyridamole on the excretion rates of WICT-3-G; (b) Effects of dipyridamole on the excretion rates of WICT-7-G; (c) Effects of dipyridamole on the intracellular levels of WICT-3-G and WICT-7-G; (d) Effects of dipyridamole on the efflux clearances ($CL_{ef,app}$) of WICT-3-G and WICT-7-G. WICT-3-G, wushanicaritin-3-*O*-glucuronide; WICT-7-G, wushanicaritin-7-*O*-glucuronide. * compared with the parameters of dipyridamole (5 μM) treated HeLa1A1 cells, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; $^{\#}$ compared with the parameters of dipyridamole (20 μM) treated HeLa1A1 cells, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$. All experiments were performed in triplicate.

Figure S3 Effects of LTC4 (0.1 and 0.4 μM) on the glucuronidation disposition upon incubation of HeLa1A1 cells with wushanicaritin. The concentration of wushanicaritin was 1 μM. (a) Effects of LTC4 on the excretion rates of WICT-3-G; (b) Effects of LTC4 on the excretion rates of WICT-7-G; (c) Effects of LTC4 on the intracellular levels of WICT-3-G and WICT-7-G; (d) Effects of LTC4 on the efflux

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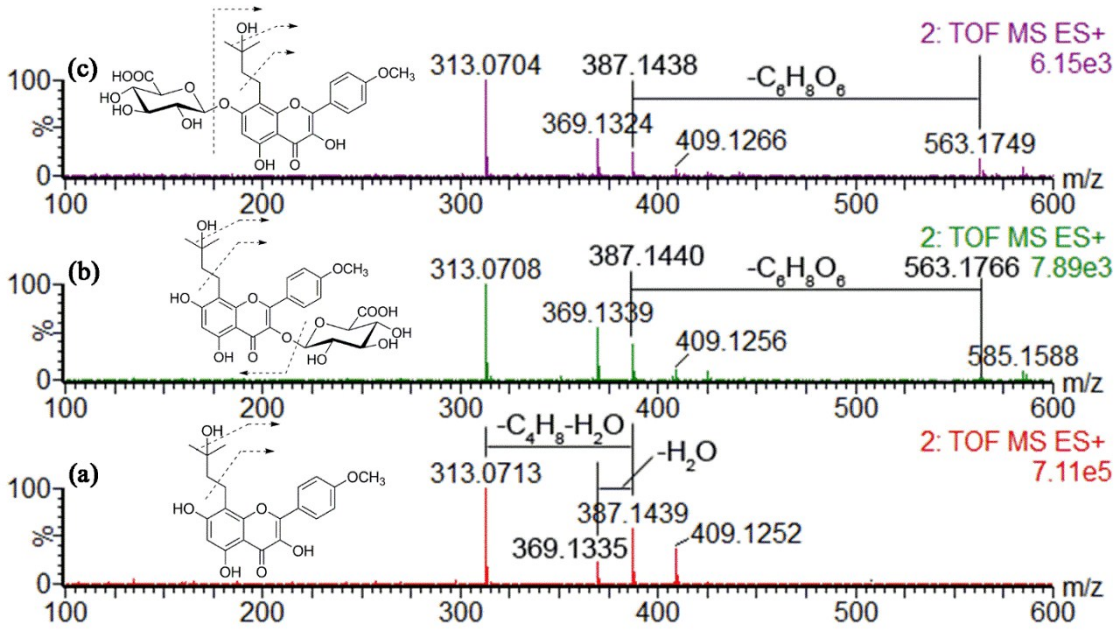
Figure S4 Determination of the mRNA levels of transporters in various types of cells. Comparisons of the mRNA expression of (a) BCRP after stable transfection of BCRP-shRNA to HeLa1A1 cells; (b) of MRP1 after stable transfection of MRP1-shRNA to HeLa1A1 cells; (c) of MRP3 after stable transfection of MRP3-shRNA to HeLa1A1 cells and (d) of MRP4 after stable transfection of MRP4-shRNA to HeLa1A1 cells. (* compared with the parameters of control (scramble) cells, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). All experiments were performed in triplicate.

Table S1 Relative protein expression of transporters (normalized to the levels of GAPDH) in HeLa1A1 and engineered HeLa1A1 cells based on western blotting.

Protein	control	scramble	BCRP-shRNA	MRP1-shRNA	MRP3-shRNA	MRP4-shRNA
BCRP	0.98±0.07	1.01±0.06	0.21±0.03***	0.99±0.05	0.97±0.08	1.02±0.05
MRP1	1.06±0.08	1.10±0.08	1.03±0.10	0.25±0.07***	1.05±0.10	0.99±0.08
MRP3	1.10±0.06	1.20±0.09	1.12±0.11	1.09±0.13	0.32±0.06***	1.14±0.06
MRP4	0.99±0.04	1.03±0.08	0.97±0.07	1.00±0.06	0.99±0.10	0.55±0.04***
UGT1A1	0.75±0.06	0.78±0.04	0.80±0.09	0.77±0.08	0.81±0.10	0.74±0.09
GAPDH	1	1	1	1	1	1

* Statistically significant compared with the scramble cells (*** $p < 0.001$).



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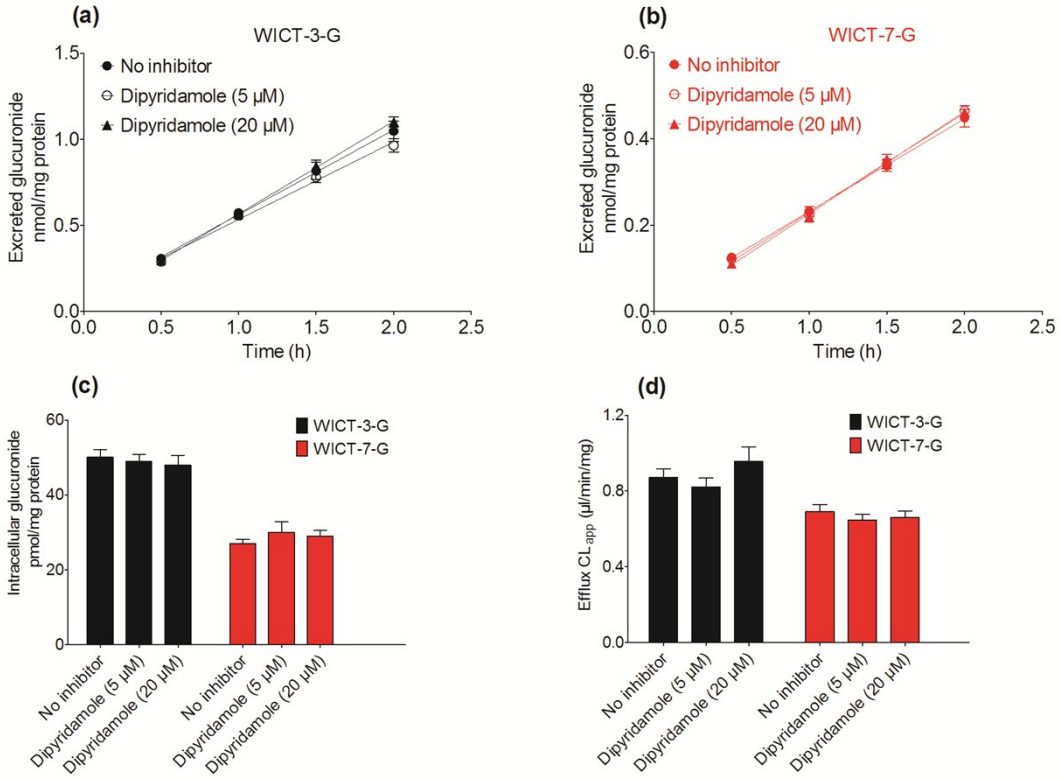

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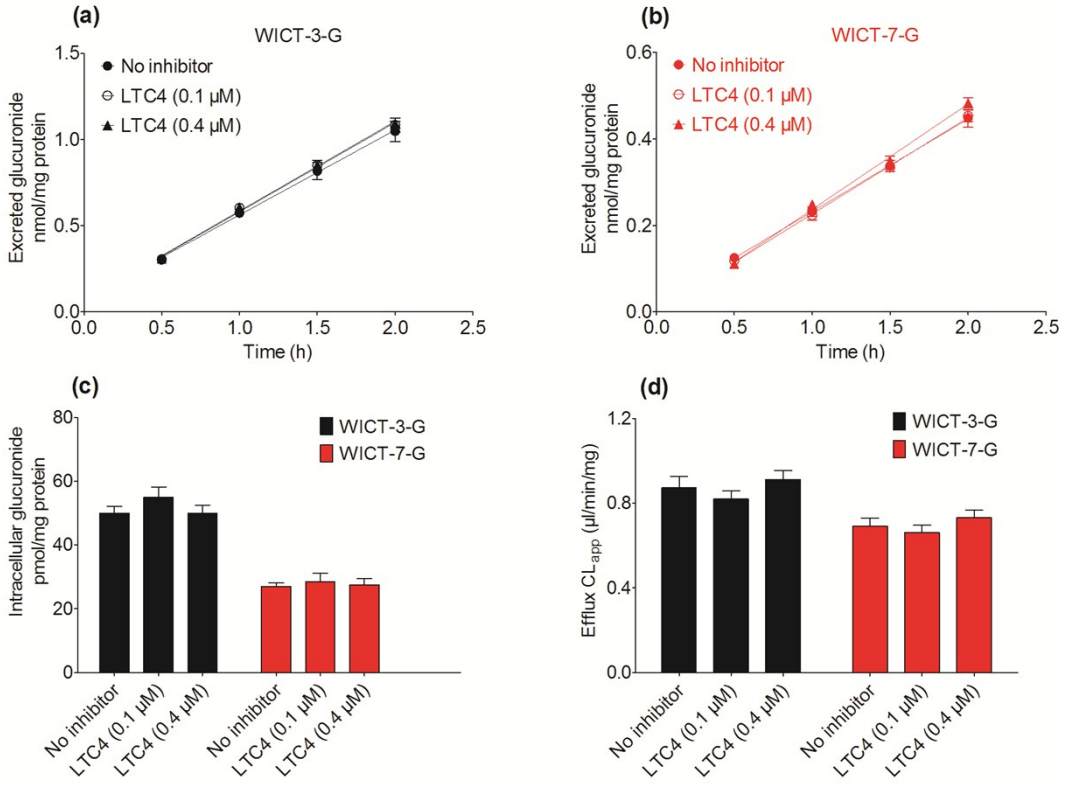

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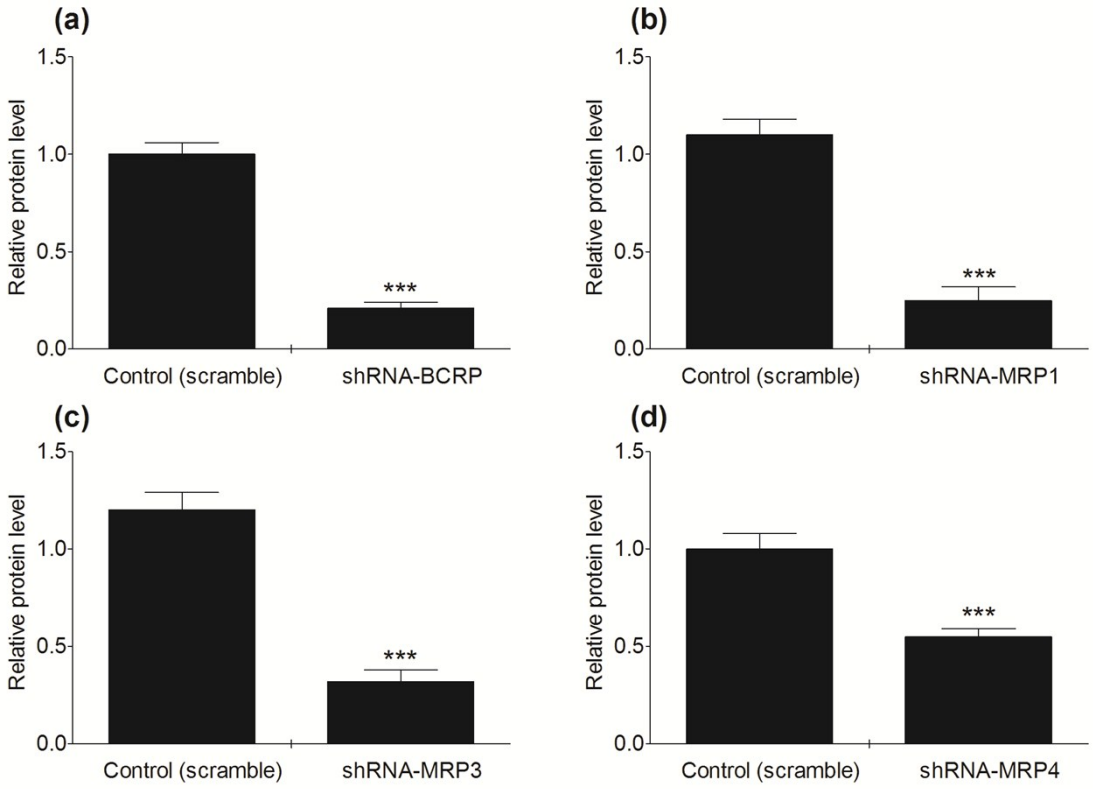

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