A model of in vitro UDP-glucuronosyltransferases inhibition by bile acids predicts possible metabolic disorders

Zhong-Ze Fang¹,²,³,#, Rong-Rong He⁴,#, Yun-Feng Cao², Naoki Tanaka³, Changtao Jiang³, Kristopher W. Krausz³, Yunpeng Qi³, Pei-Pei Dong⁵, Chun-Zhi Ai², Xiao-Yu Sun², Mo Hong², Guang-Bo Ge², Frank J. Gonzalez³, Xiao-Chi Ma⁶*, Hong-Zhi Sun¹,*

¹The First Affiliated Hospital of Liaoning Medical University, Jinzhou 121001, China
²Joint Center for Translational Medicine, Dalian Institute of Chemical Physics Chinese Academy of Sciences and The first Affiliated Hospital of Liaoning Medical University, No.457, Zhongshan Road, Dalian 116023, China
³Laboratory of Metabolism, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892, USA
⁴Pharmacy College, Jinan University, Guangzhou 510632, China
⁵Academy of Integrative Medicine, Dalian Medical University, Dalian 116044, China
⁶College of Pharmacy, Pharmacokinetic and Drug Transport Key Laboratory, Dalian Medical University, Dalian, China.

# These two authors equally contributed to this work.

Running Title: Inhibition of UGTs by bile acids

*Corresponding author:
Hong-Zhi Sun, The First Affiliated Hospital of Liaoning Medical University, Jinzhou 121001, China; E-mail: zzfang228@gmail.com

Xiao-Chi Ma, College of Pharmacy, Pharmacokinetic and Drug Transport Key Laboratory, Dalian Medical University, Dalian, China.

Abbreviations:

azidothymidine, AZT; bile acids, BAs; cholic acid, CA; sodium chenodeoxycholate, CDCA; Sodium deoxycholate, DCA; dehydrocholic acid, DHCA; glycocholic acid hydrate, GCA; hyodeoxycholic acid, HDCA; lithocholic acid, LCA;
4-methylumbelliferone, 4-MU; 4-methylumbelliferone-β-D-glucuronide, 4-MUG;
taurocholic acid sodium salt hydrate, TCA; taurochenodeoxycholate, TCDCA;
sodium tauroliothocholate, TLCA; ursodeoxycholic acid, UDCA; trifluoperazine, TFP; sodium tauroursodeoxycholate, TUDCA; sodium taurodeoxycholate hydrate, TDCA; UDP-glucuronosyltransferases, UGTs; uridine-5′-diphosphoglucuronic acid, UDPGA; pregnane X receptor, PXR; human liver microsomes, HLMs; vitamin D receptor, VDR; farnesoid X receptor, FXR; peroxisome proliferator-activated receptors, PPAR
Abstract

Increased levels of bile acids due to the various hepatic diseases could interfere with the metabolism of xenobiotics such as drugs, and endobiotics including steroid hormones. UDP-glucuronosyltransferases (UGTs) are involved in the conjugation and elimination of many xenobiotics and endogenous compounds. The present study sought to investigate the potential for inhibition of UGT enzymes by bile acids. The results showed that tauroolithocholic acid (TLCA) exhibited the strongest inhibition towards UGTs, followed by lithocholic acid (LCA). Bile acids structure-UGT inhibition relationships were examined and in vitro-in vivo extrapolation performed by using in vitro inhibition kinetic parameters (K_i) in combination with calculated in vivo levels of tauroolithocholic acid (TLCA). Substitution of a hydrogen with a hydroxyl group in the R1, R3, R4, R5 site of bile acids significantly weakens their inhibition ability towards most UGTs. The in vivo inhibition by tauroolithocholate (TLCA) towards UGT forms was determined with following orders of potency: UGT1A4 > UGT2B7 > UGT1A3 > UGT1A1 ~ UGT1A7 ~ UGT1A10 ~ UGT2B15.

In conclusion, these studies suggest that disrupted homeostasis of bile acids, notably tauroolithocholic acid, found in various diseases such as cholestasis, could lead to altered metabolism of xenobiotics and endobiotics through inhibition of UGT enzymes.

Supplementary key words bile acids; cholestasis; endobiotics; UDP-glucuronosyltransferases (UGTs); structure-UGT inhibition relationship; metabolic disorders; xenobiotics.
Introduction:

Bile acids (BA), the major end-products of cholesterol metabolism, play a key role in the solubilization, absorption, and transportation of dietary lipids in the intestine (1). In humans, the most abundant BAs contain cholic acid, chenodeoxycholic acid, deoxycholic acid, lithocholic acid, and ursodeoxycholic acid (2). In the livers of mice and rats, chenodeoxycholic acid can be further converted into α- and β-muricholic acid (MCA) (3). Under normal conditions, BAs exist at low concentrations in the peripheral circulation. Various hepatic and intestinal diseases can significantly affect the total BA levels and disrupt the BA homeostasis. For example, cholestasis can disturb the bile secretory process, leading to the accumulation of toxic bile acids in the liver (4).

Human UDP-glucuronosyltransferases (UGTs), endogenous membrane proteins located in endoplasmic reticulum, can conjugate various endogenous and exogenous compounds (5). Bile acids are substrates of UGTs, and UGT-catalyzed glucuronidation of bile acids facilitate their hydrophilicity, increase their elimination from the liver and decrease their potential for hepatic toxicity (6-8). For example, UGT1A3 can catalyze C24-glucuronidation of chenodeoxycholic acid, lithocholic acid, and hyodeoxycholic acid through forming acyl glucuronides (9). UGT2B4 is involved in the 6α-glucuronidation of BAs such as hyodeoxycholic acid (10). UGT2B7 is the UGT isoform involved in the 3α- and 6α-glucuronidation of primary, secondary and hydroxylated BA (9).
As substrates for UGT enzymes, bile acids might also exhibit inhibitory effects towards various UGT forms. Previous studies revealed that some bile acids components exhibit inhibitory effects towards 4-methylumbelliferone (4-MU) glucuronidation, including lithocholic acid, dehydrocholic acid, and chenodeoxycholate (11). However, the complete inhibition profile of bile acids towards different UGT forms remains unclear. Additionally, the bile acid structures-UGT inhibition relationships are important to determine what structural basis of bile acids affects their inhibition potentials towards different UGT. Therefore, the present study evaluated the inhibition capability of various bile acids towards UGT forms in liver and intestine. As previously described (12), recombinant UGT isoforms-catalyzed 4-MU glucuronidation reactions were used to evaluate the inhibition potential of bile acids towards UGTs except for UGT1A4, and where trifluoperazine (TFP) glucuronidation was performed as the standard reaction to evaluate the inhibition potential of bile acids towards UGT1A4 activity.

MATERIALS AND METHODS

Materials

4-Methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin, azidothymidine (AZT, purity ≥ 98%), trifluoperazine (TFP, purity ≥ 99%), estradiol (purity ≥ 98%) and uridine-5′-diphosphoglucuronic acid trisodium salt (UDPGA) were purchased from Sigma-Aldrich (St Louis, MO). Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6,
UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15) expressed in baculovirus-infected insect cells were obtained from BD Gentest Corp. (Woburn, MA, USA). Hyodeoxycholic acid (HDCA), lithocholic acid (LCA), sodium taurochenodeoxycholate (TCDDA), taurocholic acid sodium salt hydrate (TCA), sodium chenodeoxycholate (CDCA), sodium tauroolithocholate (TLCA), ursodeoxycholic acid (UDCA), cholic acid (CA), dehydrocholic acid (DHCA), sodium deoxycholate (DCA), sodium tauroursodeoxycholate (TUDCA), sodium taurodeoxycholate hydrate (TDCA), and glycocholic acid hydrate (GCA) were obtained from Sigma-Aldrich (St Louis, MO). The purity of these compounds were above 95%. All other reagents were of HPLC grade or of the highest grade commercially available.

Initial screening of bile acids' inhibition towards the activity of recombinant UGTs

The inhibition capability of bile acids towards all the UGT forms, except UGT1A4, was evaluated using recombinant UGT-catalyzed 4-MU glucuronidation as the probe reaction, as previously described (12,13). The incubation system in a total volume of 200 μL, contained recombinant UGTs, 5 mM UDPGA, 5 mM MgCl₂, 50 mM Tris-HCl (pH=7.4), and 4-MU in the absence or presence of different concentrations of various bile acids. The used incubation time and protein concentration were previously determined to ensure the reaction rate within the linear range. The 4-MU concentration was equal to known Kₘ or S₅₀ values for each UGT form. The concentrations of 4-MU and recombinant UGTs, and incubation time were given in Supplemental Table 1. The incubation reaction was initiated through addition of
UDPGA to the mixture after a 5 min pre-incubation at 37 °C. The reactions were quenched by adding 100 µL acetonitrile with 7-hydroxycoumarin (100 µM) as internal standard. The mixture was centrifuged at 20000 × g for 10 min, and an aliquot of supernatant was transferred to an auto-injector vial for HPLC analysis. The HPLC system (Shimadzu, Kyoto, Japan) contained a SCL-10A system controller, two LC-10AT pumps, a SIL-10A auto injector, a SPD-10AVP UV detector.

Chromatographic separation was carried out using a C18 column (4.6 × 200 mm, 5 µm, Kromasil) at a flow rate of 1 mL/min and UV detector at 316 nm. The mobile phase consisted of acetonitrile (A) and H2O containing 0.5% (v/v) formic acid (B). The following gradient condition was used: 0–15 min, 95–40% B; 15–20 min, 10% B; 20–30 min, 95% B. The calculation curve was generated by peak area ratio (4-MUG/internal standard) over the concentration range of 4-MUG 0.1-100 mM. The curve was linear over this concentration range, with an r² value > 0.99. The limits of detection and quantification were determined at signal-to-noise ratios of 3 and 10, respectively. The accuracy and precision for each concentration were more than 95%.

Due to the low catalytic activity of UGT1A4 towards 4-MU glucuronidation, the UGT1A4-catalyzed trifluoperazine (TFP) glucuronidation was performed to evaluate the inhibition potential of bile acids towards UGT1A4 activity. TFP (40 μM, near its Kₘ value), was incubated with recombinant UGT1A4 (0.1 mg/mL) at 37°C for 20 min in the absence or presence of bile acids (14).
Taurolithocholate (TLCA)’s inhibition towards hepatocyte UGTs-catalyzed 4-MU glucuronidation

Primary hepatocytes were isolated from C57BL/6NCr mice and cultured as previously described (15). Fifty μM of 4-MU and 50 nM of TLCA were added in the medium. After a 1h incubation at 37°C, the medium and cells were isolated. Methanol v/v=1:1, and 5 μM chlorpropamide as an internal standard, were added to the medium, and 1 mL methanol with 5 μM chlorpropamide as an internal standard, added to extract the compounds in the cells. After centrifugation at 20000 × g for 10min, the aliquot of supernatant was determined to detect the formation of 4-MUG.

Bile acids' inhibition towards human liver microsomes (HLMs)-catalyzed zidovudine (AZT) and estradiol glucuronidation

Twenty five donor pooled human liver microsomes (HLMs) were purchased from Research Institute for Liver Diseases (RILD, Shang Hai, China). For AZT glucuronidation, the typical incubation system (total volume=200 μL) contained 0.5 mg/ml HLMs, 5 mM UDPGA, 5 mM MgCl₂, 50 mM Tris-HCl (pH=7.4), 50 μg/mg protein alamethicin, and AZT (the concentration is corresponding to the Kₘ value). The incubation time was 30 min. After centrifugation at 20,000×g for 10 min, aliquots of the supernatants were analyzed by HPLC (Shimadzu, Kyoto, Japan), equipped with a SCL-10A system controller, two LC-10AT pumps, a SIL-10A auto sampler, and a SPD-10AVP UV detector. A C-18 column (250mm×4.6 mm I.D., 5 μm, Kromasil) was used to separate AZT and its glucuronide. The mobile phase was acetonitrile (A)
and 0.2% formic acid (B) at a flow rate of 1.0 ml/min, with an isocratic: 0-25 min 90% B. The detector wavelength was set at 260 nm. Since there was no standard for the AZT glucuronide, a standard curve of AZT was used to quantify glucuronide formation.

Estradiol (10 μM) was incubated with HLMs for 20 min, with the final protein concentrations of 0.25 mg/ml. Estradiol glucuronidation samples were analyzed on the UFLC system. A Shim-pack XR-ODS (50.0 mm×2.0 mm I.D., 2.2 μm, Shimadzu) analytical column with an ODS guard column (5 mm × 2.0 mm I.D., 2.2 μm, Shimadzu) was used and kept at 40 °C. The mobile phase consisted of acetonitrile (A) and 0.2% formic acid (B) at a flow rate of 0.3 ml/min, with a gradient: 0-9 min, 90% B-30% B; 9-12.5 min, 5% B; 12.5-16min, balance to 90% B. The detector wavelength was set at 250 nm. A standard curve of the estradiol-3-O-glucuronide was used to quantify glucuronide formation.

In vitro-in vivo extrapolation (IVIVE)

The reaction velocity was determined at different concentrations of substrates and inhibitors. The durations in the inhibition kinetic study were the same with the initial screening study. Dixon and Lineweaver-Burk plots were used for determination of inhibition kinetic type. The second plot of slopes from the Lineweaver-Burk plot vs inhibitor concentrations was used to calculate the K_i value. The in vivo inhibition magnitude is affected by both in vitro inhibition kinetic parameters (K_i) and in vivo
concentrations of inhibitors. Therefore, the following equation was employed to predict *in vivo* situation.

\[
\frac{\text{AUC}_i}{\text{AUC}} = 1 + \frac{[I]_{\text{in vivo}}}{K_i}
\]

The terms are defined as follows: \(\frac{\text{AUC}_i}{\text{AUC}}\) is the predicted ratio of *in vivo* exposure of xenobiotics or endogenous compounds in control and disease situations. \(K_i\) is the reversible inhibition constant, \([I]_{\text{in vivo}}\) is the *in vivo* concentration of bile acids.

Results

*Structure-inhibition relationship of bile acids towards UGT isoforms*

The structures of tested bile acids were listed in Fig. 1, and the inhibitory capabilities of these bile acids towards different UGT forms given in Table 1. Among the tested UGT enzymes, the activity of UGT1A6, UGT1A8 and UGT1A9 were not affected by various bile acids. Among the tested bile acids, at 100 \(\mu\)M, TCDCA, TCA, CDCA, UDCA, CA, DHCA, DCA, TUDCA, TDCA, and GCA exhibited no or weak inhibition towards all the tested UGT forms, with inhibition magnitudes less than 90%. HDCA exerted 90.5% inhibition towards UGT2B7-catalyzed 4-MU glucuronidation, and low inhibition towards other UGTs. LCA exhibited the strongest inhibition (more than 90%) towards UGT1A4 and UGT2B15. TLCA showed strong inhibition towards most of UGT forms, including UGT1A1 (94.6%), 1A3 (96.6%), 1A4 (100%), 1A7 (90.9%), 2B7 (98.3%), and 2B15 (94.7%).
The structure-UGTs inhibition relationship can be observed using the combination of the bile acids' structures (Fig. 1) and the UGT inhibition results (Table 1). The substitution of hydrogen with hydroxyl group at the R₁, R₃, R₄, R₅ sites significantly weaken the inhibition towards most of UGT forms. In comparison with the inhibition magnitude of LCA and TLCA towards UGT2B7, a conclusion can be drawn that the taurine conjugation of LCA can strengthen the inhibition potential towards UGT2B7.

To demonstrate the physiologically significance of these observations, TLCA (50 nM) was added in the medium to investigate whether it can inhibit hepatocyte UGTs-catalyzed 4-MU glucuronidation activity. The results showed significant inhibition of TLCA towards hepatocyte UGT-catalyzed 4-MU glucuronidation (Supplemental Fig. 1).

**Different inhibition type and kinetic parameters of TLCA towards UGT forms**

Due to the potent inhibition of TLCA towards many UGT forms, the inhibition kinetic type and parameters (Kᵢ) were further investigated. Both Dixon and Lineweaver-Burk plots were employed to evaluate the inhibition type, and the second plot using the slopes obtained from Lineweaver-Burk plot versus the concentrations of TLCA were used to calculate the inhibition kinetic parameters(Kᵢ). If the intersection point was located in the vertical axis and the second quadrant in the Lineweaver-Burk plot and Dixon plot respectively, the inhibition type is classified as competitive inhibition. If the intersection point was located in horizontal axis in both Dixon and Lineweaver-Burk plots, the inhibition type is noncompetitive inhibition. The results
showed that TLCA competitively inhibited UGT1A1 (Fig. 2B & C), UGT1A3 (Fig. 3B & C), UGT1A4 (Fig. 4B & C), UGT2B7 (Fig. 7B & C), and UGT2B15 (Fig. 8B & C), and noncompetitively inhibited UGT1A7 (Fig 5B & C) and UGT1A10 (Fig. 6B & C). The inhibition kinetic parameters (K_i) were calculated to be 2.4, 0.3, 0.03, 16.0, 12.9, 0.1, and 3.3 μM for the activity of UGT1A1 (Fig. 2D), UGT1A3 (Fig. 3D), UGT1A4 (Fig. 4D), UGT1A7 (Fig. 5D), UGT1A10 (Fig. 6D), UGT2B7 (Fig. 7D) and UGT2B15 (Fig. 8D), respectively.

Two typical substrates AZT and estradiol were then selected as representative of xenobiotic and endogenous substrates to clarify the influence of TLCA towards the glucuronidation of these two compounds. The concentration-dependent inhibition of TLCA towards the glucuronidation of AZT and estradiol was also demonstrated (Supplemental Fig. 2A and Fig. 3A). Furthermore, the competitive inhibition of TLCA towards the glucuronidation of AZT (Supplemental Fig. 2B & 2C) and estradiol (Supplemental Fig. 3B & 3C) were demonstrated. The inhibition kinetic parameters (K_i) were calculated to be 0.3 and 2.2 μM for inhibition of TLCA towards AZT and estradiol glucuronidation (Supplemental Fig. 2D & 3D).

**Prediction of in vivo metabolic disorders in humans**

Due to the strongest inhibition potential of TLCA towards different UGT enzymes, changes in in vivo metabolism could be predicted based on the concentration of TLCA. The concentration of TLCA in serum obtained from mice with intrahepatic cholestasis of pregnancy (ICP) was reported to be 0.3 μM (16). Using this concentration in
combination with the $K_i$ values for the inhibition of TLCA towards 4-MU glucuronidation, the $AUC_i/AUC$ value was calculated to be 1.1, 2, 11, 1.02, 1.02, 4, and 1.1 for UGT1A1, 1A3, 1A4, 1A7, 1A10, 2B7 and 2B15, respectively. In humans, the TLCA concentration was reported to never exceed 25nM (17). Using this maximum value, the $AUC_i/AUC$ value was calculated to be 1.01, 1.08, 1.83, 1.002, 1.002, 1.25, and 1.008 for UGT1A1, 1A3, 1A4, 1A7, 1A10, 2B7 and 2B15, respectively.

DISCUSSION

The inhibitory capability of bile acids towards UGTs is difficult to study using conventional animal models due to the complexity of factors influencing activity in vivo. For example, the role of bile acid in the activation of nuclear receptors might interfere with the evaluation of bile acid inhibition towards UGT enzymes. Pregnane X receptor (PXR) was found to be activated by LCA (18, 19). LCA can also activate the vitamin D receptor (VDR) (20). CDCA, CA and their conjugate GCA, GCDC were as agonist for the farnesoid X receptor (FXR) (21-23). Tauro-β-muricholic acid in mice was reported to be a naturally occurring farnesoid X receptor (FXR) antagonist (24). All affected nuclear receptors regulate the expression and activity of different genes encoding UGT enzymes. For example, bile acids can induce the activity of UGT2B4 via activation of FXR (25). The UGT2B4 promoter also contains a PPARα response element, and can be activated by PPARα agonist fenofibrate (26). The activity of UGT2B7 can be inhibited by hydrophobic bile acids via a negative FXR response element located in the UGT2B7 promoter (27). FXR activation can
also regulate the expression of UGT1A3 (28). To avoid these complex factors, a relatively simple in vitro UGT enzyme incubation system was utilized in the present study.

The detailed metabolic inhibition profile of bile acids towards important UGT isoforms was clarified in the present study, and TLCA was demonstrated to be the strongest inhibitor towards most of UGT isoforms, followed by LCA, which was consistent with the reports in which LCA and TLCA were reported to be able to significantly induce the liver damage (29, 30). LCA can be metabolized through UGT isoforms-catalyzed glucuronidation elimination, so, the wide inhibition of LCA towards UGT isoforms was observed in the present study. TLCA has similar structure with LCA, and can enter the activity cavity. However, TLCA can not be metabolized through glucuronidation by our present study (data not shown). Therefore, TLCA exhibited stronger inhibition than LCA. CA and GCA at 100 μM, were found to increase the activity of UGT1A3 by 35% and 45%, respectively. Given that induced expression of UGT1A3 through nuclear receptors does not occur in the present in vitro system, the allosteric activation of CA and GCA towards UGT1A3 might be the potential reason. However, the detailed mechanism needs further investigated.

According to the predicted values of AUCi/AUC, TLCA exhibited the strongest inhibition towards UGT1A4-catalyzed metabolic reaction. UGT1A4 was widely accepted to be the main UGT enzyme involved in the N-glucuronidation of primary, secondary, and tertiary amines located in the compounds. Xenobiotics catalyzed by UGT1A4 include nicotine, cotinine, and nitrosamines (31). The strongest inhibition of
UGT1A4 by bile acids might significantly affect the metabolism of these compounds. TLCA also exerted strong inhibition potential towards UGT2B7 and UGT1A3. These two UGT forms were also involved in the metabolism of bile acids, thus inhibition of these two UGT forms by bile acids could in turn affect bile acid metabolism. Additionally, these two UGT forms can also participate in the metabolism of many drugs, including AZT. Therefore, this substrate was selected to evaluate whether its glucuronidation could be affected by TLCA. Indeed, TLCA competitively inhibited HLM-mediated glucuronidation of AZT with a potent inhibition potential of $K_i = 0.3 \mu$M. In addition to the UGT1A4 isoform, bile acids can inhibit other UGTs including UGT1A1, UGT1A7, UGT 1A10, and UGT2B15. UGT1A1 plays a key role in the metabolism of two important endogenous compounds, bilirubin and estradiol. The inhibition of TLCA towards the glucuronidation of estradiol was also demonstrated in the present study. The accumulation of bile acids was reported potentiate colon carcinogenesis (32). Low activity of UGT1A7 and UGT1A10 was also strongly correlated with the risk of colon cancer (33, 34). Inhibition of UGT1A7 and 1A10 by TLCA could provide a possible link to the correlate of increased bile acids and high risk of colon cancer. In the present study, two concentrations of TLCA were used to predict in vivo situation due to the relatively few reports on the serum concentrations of TLCA. Information on the serum concentrations of TLCA in various diseases, might reveal a better understanding of disease risk.

In conclusion, the present study detailed inhibition profiles of bile acids towards UGT enzymes, and the bile acid structure-UGT inhibition relationships. In vivo risk was
predicted based on the inhibition of TLCA towards UGTs due to the strongest inhibition of TLCA towards UGT different forms. However, the influence of other bile acids towards UGTs should not be neglected. It should also be noted that the present results should be consider in the context of the complex environment in vivo and in cells, such as the influence of bile acids on nuclear receptors as summarized in Fig. 9. The disrupted homeostasis of bile acids due to diseases (e.g., cholestasis) could inhibit UGT-catalyzed metabolism resulting in altered metabolism of xenobiotics and endogenous compounds.

Conflict of interest statement

The authors have declared no conflict of interest.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 81202586) and the National Cancer Institute, Center for Cancer Research, Intramural Research Program.

References:


Table 1 Initial screening of bile acids (100 μM) towards various important UGT forms. The values shown are the residual activity, which was calculated using the following equation: % residual activity=(the activity at 100 μM bile acids/the control activity at 0 μM bile acids) *100%.

*, p<0.05, **, p<0.01, ***, p<0.001, compared with control activity.

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* p < 0.05  ** p < 0.01  *** p < 0.001
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Figure Legends

Fig. 1 The structures of bile acids used in this study.

Fig. 2 Evaluation of TLCA's inhibition towards recombinant UGT1A1-catalyzed 4-MU glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT1A1-catalyzed 4-MU glucuronidation; (B) Dixon plot of TLCA's inhibition towards recombinant UGT1A1-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT1A1-catalyzed 4-MU glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT1A1-catalyzed 4-MU glucuronidation. The data point represents the mean of the duplicate experiments.

Fig. 3 Evaluation of TLCA's inhibition towards recombinant UGT1A3-catalyzed 4-MU glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT1A3-catalyzed 4-MU glucuronidation; (B) Dixon plot of TLCA's inhibition towards recombinant UGT1A3-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT1A3-catalyzed 4-MU glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT1A3-catalyzed 4-MU glucuronidation. The data point represents the mean of the duplicate experiments.

Fig. 4 Evaluation of TLCA's inhibition towards recombinant UGT1A4-catalyzed TFP glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT1A4-catalyzed TFP glucuronidation; (B) Dixon plot of
TLCA's inhibition towards recombinant UGT1A4-catalyzed TFP glucuronidation;

(C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT1A4-catalyzed TFP glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT1A4-catalyzed TFP glucuronidation. The data point represents the mean of the duplicate experiments.

Fig. 5 Evaluation of TLCA’s inhibition towards recombinant UGT1A7-catalyzed 4-MU glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT1A7-catalyzed 4-MU glucuronidation; (B) Dixon plot of TLCA's inhibition towards recombinant UGT1A7-catalyzed 4-MU glucuronidation;

(C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT1A7-catalyzed 4-MU glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT1A7-catalyzed 4-MU glucuronidation. The data point represents the mean of the duplicate experiments.

Fig. 6 Evaluation of TLCA’s inhibition towards recombinant UGT1A10-catalyzed 4-MU glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT1A10-catalyzed 4-MU glucuronidation; (B) Dixon plot of TLCA's inhibition towards recombinant UGT1A10-catalyzed 4-MU glucuronidation;

(C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT1A10-catalyzed 4-MU glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT1A10-catalyzed 4-MU glucuronidation. The data point represents the mean of the duplicate experiments.
Fig. 7 Evaluation of TLCA's inhibition towards recombinant UGT2B7-catalyzed 4-MU glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT2B7-catalyzed 4-MU glucuronidation; (B) Dixon plot of TLCA's inhibition towards recombinant UGT2B7-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT2B7-catalyzed 4-MU glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT2B7-catalyzed 4-MU glucuronidation. The data point represents the mean of the duplicate experiments.

Fig. 8 Evaluation of TLCA's inhibition towards recombinant UGT2B15-catalyzed 4-MU glucuronidation. (A) TLCA exhibited concentration-dependent inhibition towards recombinant UGT2B15-catalyzed 4-MU glucuronidation; (B) Dixon plot of TLCA's inhibition towards recombinant UGT2B15-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of TLCA's inhibition towards recombinant UGT2B15-catalyzed 4-MU glucuronidation; (D) Second plot of TLCA's inhibition towards recombinant UGT2B15-catalyzed 4-MU glucuronidation. The data point represents the mean of the duplicate experiments.

Fig. 9 Brief summary of the influence of elevated bile acids towards hepatic metabolism of xenobiotics and endobiotics
### Fig. 1

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Fig. 7

(A) 

(B) 

(C) 

(D)