Short Communication

Chirality Influence of Zaltoprofen Towards UDP-Glucuronosyltransferases (UGTs) Inhibition Potential

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ABSTRACT Zaltoprofen (ZLT) is a nonsteroidal antiinflammation drug, and has been clinically employed to treat rheumatoid arthritis, osteoarthritis, and other chronic inflammatory pain conditions. The present study aims to investigate the chirality influence of zaltoprofen towards the inhibition potential towards UDP-glucuronosyltransferases (UGTs) isoforms. In vitro a recombinant UGT isoforms-catalyzed 4-methylumbelliferone (4-MU) glucuronidation incubation system was employed to investigate the inhibition of (R)-zaltoprofen and (S)-zaltoprofen towards UGT isoforms. The inhibition difference capability was observed for the inhibition of (R)-zaltoprofen and (S)-zaltoprofen towards UGT1A8 and UGT2B7, but not for other tested UGT isoforms. (R)-zaltoprofen exhibited noncompetitive inhibition towards UGT1A8 and competitive inhibition towards UGT2B7. The inhibition kinetic parameters were calculated to be 35.3 μM and 19.2 μM for UGT1A8 and UGT2B7. (R)-zaltoprofen and (S)-zaltoprofen exhibited a different inhibition type towards UGT1A7. Based on the reported maximum plasma concentration of (R)-zaltoprofen in vivo, a high drug–drug interaction between (R)-zaltoprofen and the drugs mainly undergoing UGT1A7, UGT1A8, and UGT2B7-catalyzed glucuronidation was indicated. *Chirality 27:359–363, 2015.* © 2015 Wiley Periodicals, Inc.

KEY WORDS: zaltoprofen; chirality; UDP-glucuronosyltransferases (UGTs); inhibition potential

INTRODUCTION

Chirality describes a molecule with a nonsuperimposeable mirror image of itself, and the molecules with chirality are called enantiomers.[1] The enantiomers always exhibit different, even contrasting, properties for pharmacodynamic and pharmacokinetic behaviors. For the anticoagulant drug warfarin, (S)-warfarin exhibits a stronger therapeutic role than (R)-warfarin.[2] Additionally, different binding of (R)-warfarin and (S)-warfarin with human albumin has been reported.[3] Tramadol and its active O-demethylated metabolite exhibited stereoselective pharmacokinetics in rats.[4]

UDP-glucuronosyltransferases (UGTs), the important phase II drug-metabolizing enzyme, plays an important role in the metabolic elimination of many important clinical drugs. For example, UGT1A9-catalyzed glucuronidation reaction is the major elimination pathway for propofol.[5] UGT2B7 can catalyze the glucuronidation elimination reaction of anti-HIV drugs, including efavirenz and zidovudine.[6] UGTs also play an important role in the metabolism of some endogenous substances, including bile acids, estrogen, and bilirubin.[7,8] The inhibitory capability of various xenobiotics and endogenous substances towards the activity of UGT isoforms has been reported. For example, lipid components showed strong inhibition towards UGT isoforms, including UGT1A4, 1A6, 1A8, 1A9, and 2B7.[9] Indinavir inhibited UGT1A1-catalyzed glucuronidation of bilirubin, and resulted in the elevation of unconjugated bilirubin in serum.[10]

Zaltoprofen (ZLT) is a nonsteroidal antiinflammation drug, and has been clinically employed to treat rheumatoid arthritis, osteoarthritis, and other chronic inflammatory pain conditions.[11] The influence of the chirality of ZLT towards the drug efficiency has been reported. The results reported by Cho et al. have shown that the enantiomers of ZLT exerted different antiinflammation and analgesic effects.[12]

Given that our previous study found the enantioselective inhibition of carprofen towards UGT isoforms,[13] the present study aimed to investigate the chirality influence of zaltoprofen towards UDP-glucuronosyltransferases (UGTs) inhibition potential.

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MATERIALS AND METHODS

Chemicals

4-Methylumbelliferone (4-MU), 4-methylumbelliferone-β-D-glucuronide (4-MUG), Tris–HCl, 7-hydroxycoumarin, and uridine-5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human UGT isoforms (UGT1A1, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7) expressed in baculovirus-infected insect cells were obtained from BD Gentest (Woburn, MA). (R)-zaltoprofen and (S)- zaltoprofen were made according to the previous literature. The purities for both enantiomers were more than 98.0%. All other reagents were of high-performance liquid chromatography (HPLC) grade or of the highest grade commercially available.

Initial Screening of Zaltoprofen Enantiomers' Inhibition Towards Recombinant UGTs-Catalyzed 4-MU Glucuronidation

4-MU, a nonspecific probe substrate for all the UGT isoforms, was employed to investigate the inhibition of zaltoprofen enantiomers towards the activity of UGT isoforms. The incubation and analytical methods have been previously described.[9] In brief, the typical incubation system (total volume = 200 μL) contained recombinant UGTs, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris–HCl (pH = 7.4), and 4-MU in the absence or presence of different concentrations of (R)-zaltoprofen or (S)-zaltoprofen. The compounds were dissolved in DMSO and the final concentration of DMSO was below 0.5% (v/v). The incubation time and protein concentration have been demonstrated to ensure the linear glucuronidation reaction. The concentration of 4-MU used was equal to known K_m or S_{50} values reported in the previous literatures.[9] After a 5-min preincubation, UDPGA was added to initiate the glucuronidation reaction, and after the incubation the reaction was terminated with 100 μL acetonitrile with 7-hydroxycoumarin (100 μM) as internal standard. Centrifuged at 22,000*g* for 10 min, the aliquots (10 μL) of supernatant were transferred to an autoinjector vial for HPLC analysis. The analysis instrument (Shimadzu, Kyoto, Japan) contained an SCL-10A system controller, two LC-10AT pumps, an SIL-10A autoinjector, and an 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 H_2O 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 $r^2 > 0.99$. The limits of detection and quantification were determined at signal-to-noise ratios of 3 and 10, respectively. The accuracy and precision of the back-calculated values for each concentration were less than 5%.

Inhibition Kinetic Determination

Multiple concentrations of 4-MU and (R)-zaltoprofen or (S)-zaltoprofen were used to determine the reaction velocity. A Dixon plot and Lineweaver–Burk plot were used to fit the data as previously described. The inhibition kinetic type was evaluated through determining the intersection point in the Dixon and Lineweaver–Burk plots. The second plot of slopes from Lineweaver–Burk plot vs. the concentrations of (R)-zaltoprofen or (S)-zaltoprofen was utilized to calculate the K_i value.

RESULTS

Comparison of Inhibition Capability of Zaltoprofen Enantiomers Towards ugt Isoforms

The inhibition potential of (R)-zaltoprofen and (S)-zaltoprofen is given in Figure 1. Among the tested UGT isoforms, both (R)-zaltoprofen and (S)-zaltoprofen exhibited similar inhibition potential towards UGT1A1, 1A3, 1A6, 1A7, 1A9, 1A10, and 2B4. (R)-zaltoprofen exhibited stronger inhibition capability towards UGT1A8 and UGT2B7 than (S)-zaltoprofen ($P < 0.01$, Figure 1).

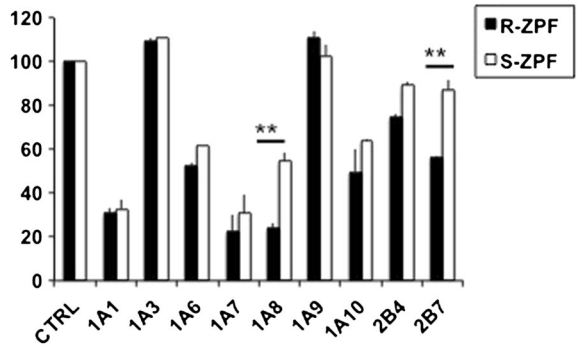

Fig. 1. Comparison of the inhibition capability of zaltoprofen enantiomers towards UGT isoforms. In this screening process, 100 μM of (R)-zaltoprofen and (S)-zaltoprofen were used. Data are given as mean plus SD ($n = 3$).

Inhibition Kinetic Determination for Zaltoprofen Enantiomers Towards Representative ugt Isoforms

Due to the stronger inhibition of (R)-zaltoprofen towards UGT1A8 and UGT2B7 than (S)-zaltoprofen, the inhibition kinetic behavior of (R)-zaltoprofen towards UGT1A8 and UGT2B7 was determined. Additionally, UGT1A7 was selected as an example of UGT isoforms not differently inhibited by zaltoprofen, and the inhibition kinetic behavior of both zaltoprofen enantiomers towards UGT1A7 was determined. As shown in Figure 2A,B, (R)-zaltoprofen exhibited competitive inhibition towards UGT1A7, as indicated by the location of the intersection point in the second quadrant in the Dixon plot and in the vertical axis in the Lineweaver–Burk plot. For (S)-zaltoprofen, the intersection point was located in the horizontal axis in both Dixon (Figure 3A) and Lineweaver–Burk (Figure 3B) plots. The second plots (Figure 2C,C) were used to calculate the inhibition kinetic parameters (K_i) to be 25.1 and 35.0 μM μM for (R)-zaltoprofen and (S)-zaltoprofen, respectively. As shown in Figure 4, (R)-zaltoprofen noncompetitively inhibited the activity of UGT1A8, and the K_i value was determined to be 35.3 μM. For the inhibition of (R)-zaltoprofen towards UGT2B7, the inhibition kinetic type was competitive, and the inhibition kinetic parameter (Ki) was calculated to be 19.2 μM (Figure 5).

DISCUSSION

Drug–drug interactions remain the limiting factor for the clinical application of drugs, and the inhibition of drug-metabolizing enzymes is a key factor for drug–drug interaction.[14] Drug-metabolizing enzymes contain phase I (e.g., cytochrome P450 (CYP), esterase, etc.) and phase II (e.g., UDP-glucuronosyltransferases (UGTs), etc.) enzymes. The influence of drug chirality on the inhibition potential towards CYP isoforms has been reported. For example, the experiment performed by Dilmaghanian et al. showed the enantioselectivity of inhibition of cytochrome P450 3A4 (CYP3A4) by ketoconazole.[15] The stereoselective interaction between tetrahydropalmatine enantiomers and CYP enzymes in human liver microsomes has been reported.[16]

The present study demonstrated the stereoselective inhibition of zaltoprofen towards the activity of UGT1A8 and UGT2B7. UGT1A8 is an important intestinal UGT isoform, and is involved in the metabolism of some clinical drugs. For example, UGT1A8 has been reported to exhibit catalytic activity towards raloxifene, and the UGT1A8 genotype plays an important role in the overall response to raloxifene.[17] Additionally, the activity of UGT1A8 has high correlation with the

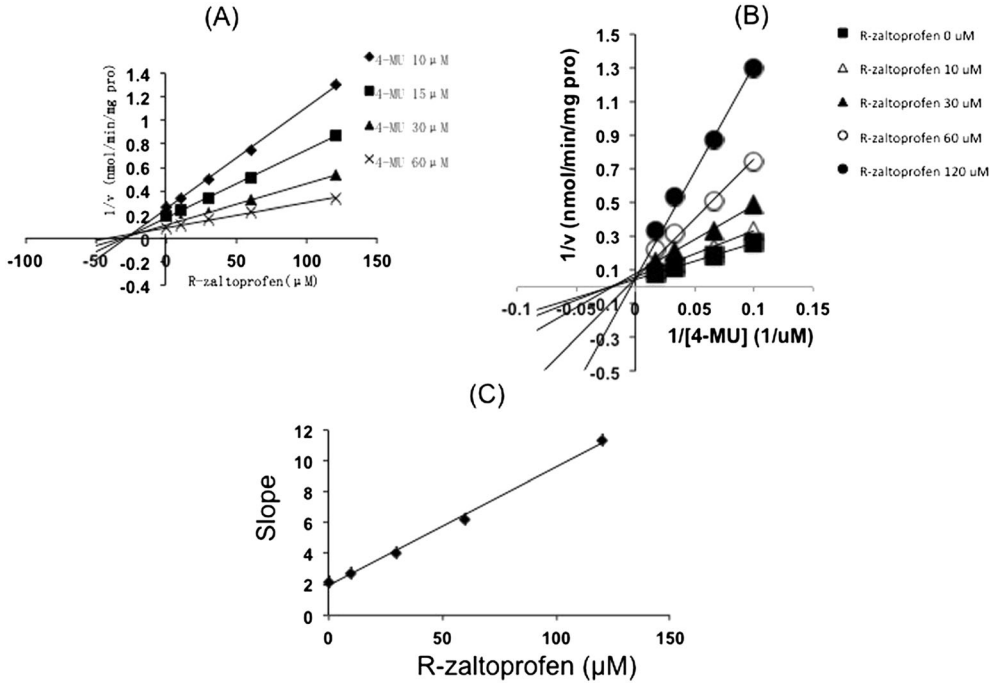

Fig. 2. Inhibition kinetics of (R)-zaltoprofen towards UGT1A7-catalyzed glucuronidation of 4-MU. (*A*) Dixon plot of (R)-zaltoprofen's inhibition towards UGT1A7-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (B) Lineweaver–Burk plot of (R)-zaltoprofen's inhibition towards UGT1A7-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (C) The second plot of (R)-zaltoprofen's inhibition towards UGT1A7-catalyzed glucuronidation of 4-MU.

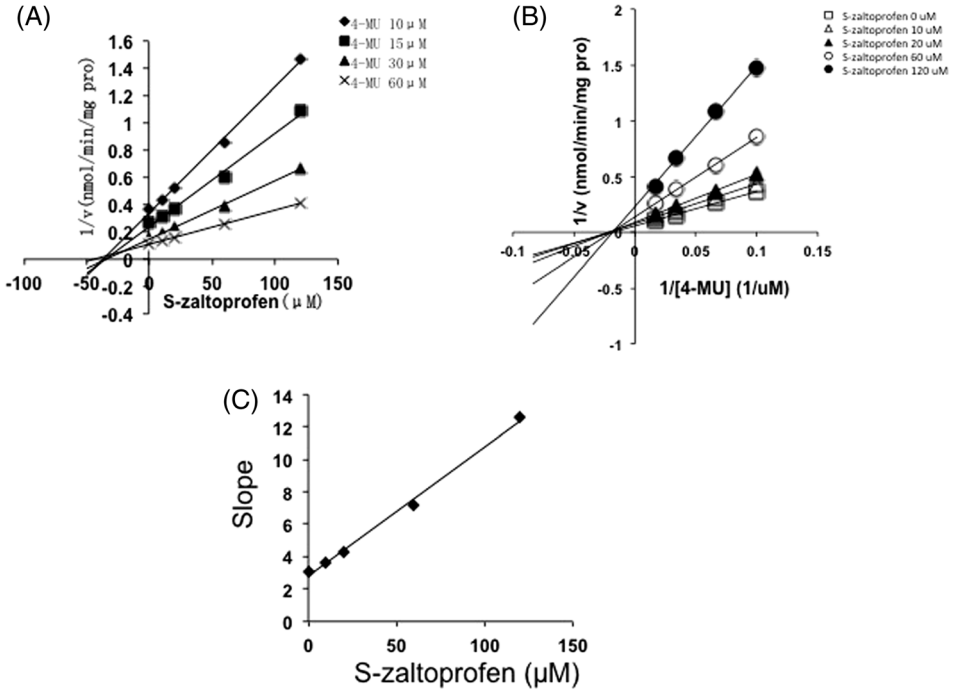

Fig. 3. Inhibition kinetics of (S)-zaltoprofen towards UGT1A7-catalyzed glucuronidation of 4-MU. (A) Dixon plot of (S)-zaltoprofen's inhibition towards UGT1A7-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (B) Lineweaver–Burk plot of (S)-zaltoprofen's inhibition towards UGT1A7-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (C) The second plot of (S)-zaltoprofen's inhibition towards UGT1A7-catalyzed glucuronidation of 4-MU.

occurrence of cancers.[18] UGT2B7 is regarded as one of the most important UGT isoforms involved in drug metabolism, such as the glucuronidation process of zidovudine.[19] Therefore, compared with (S)-zaltoprofen, (R)-zaltoprofen exhibited higher risk when coadministered with the drugs mainly undergoing UGT1A8 and UGT2B7-catalyzed metabolism.

Kinetic studies were investigated for the inhibition of (R)-zaltoprofen towards UGT1A8 and UGT2B7. It was found

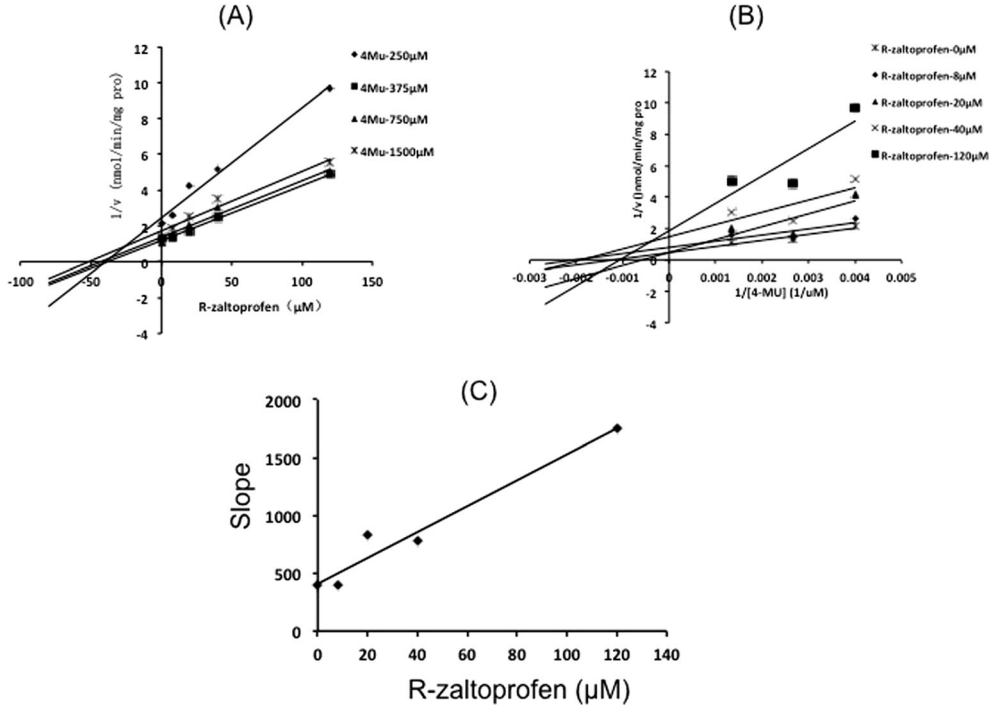

Fig. 4. Inhibition kinetics of (R)-zaltoprofen towards UGT1A8-catalyzed glucuronidation of 4-MU. (A) Dixon plot of (R)-zaltoprofen's inhibition towards UGT1A8-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (B) Lineweaver–Burk plot of (R)-zaltoprofen's inhibition towards UGT1A8-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (C) The second plot of (R)-zaltoprofen's inhibition towards UGT1A8-catalyzed glucuronidation of 4-MU.

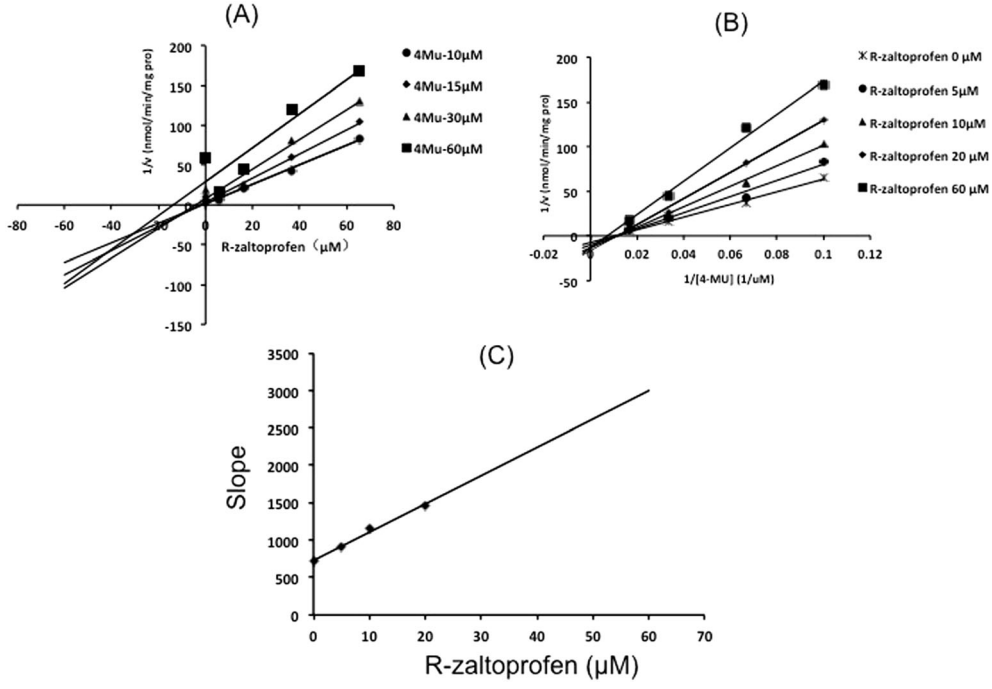

Fig. 5. Inhibition kinetics of (R)-zaltoprofen towards UGT2B7-catalyzed glucuronidation of 4-MU. (A) Dixon plot of (R)-zaltoprofen's inhibition towards UGT2B7-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (B) Lineweaver–Burk plot of (R)-zaltoprofen's inhibition towards UGT2B7-catalyzed glucuronidation of 4-MU. Each data point represents the mean value of duplicate experiments. (C) The second plot of (R)-zaltoprofen's inhibition towards UGT2B7-catalyzed glucuronidation of 4-MU.

that (R)-zaltoprofen exhibited noncompetitive inhibition towards UGT1A8 and competitive inhibition towards UGT2B7. The inhibition kinetic parameters were calculated to be 35.3 μM and 19.2 μM for UGT1A8 and UGT2B7, respectively. It should be noted that the inhibition kinetic type of zaltoprofen enantiomers towards UGT1A7 (competitive inhibition of

(R)-zaltoprofen towards UGT1A7, and noncompetitive inhibition of (S)-zaltoprofen towards UGT1A7) was detected, although there was no significant difference for the inhibitory capability towards UGT1A7. This indicates that the increased concentration of substrates can eliminate the inhibition of (R)-zaltoprofen towards UGT1A7, but not the inhibition of (S)-zaltoprofen towards UGT1A7. The maximum plasma concentration of (R)-zaltoprofen was reported to be 33.8 μg/mL (116.7 μM) after oral administration of 50 mg/kg zaltoprofen.[20] The ratio for the concentration of inhibitors versus the inhibition kinetic parameters ([I]/Ki) can reflect the in vivo inhibition magnitube ([I]/Ki<0.1, low possibility; 0.1<[I]/Ki<1, medium possibility; [I]/Ki>1, high possibility). Based on this value, the [I]/Ki values were calculated to be 4.6, 3.3, 3.3, and 6.1 for the inhibition of (R)-zaltoprofen towards UGT1A7, (S)-zaltoprofen towards UGT1A7, (R)-zaltoprofen towards UGT1A8, and (R)-zaltoprofen towards UGT2B7.

In conclusion, the chirality influence of zaltoprofen towards UGT1A8 and UGT2B7 was demonstrated, and a potential drug–drug interaction might occur between zaltoprofen and drugs undergoing multiple UGT isoforms-catalyzed metabolism, including UGT1A7, UGT1A8, and UGT2B7. This work was supported by the National Natural Science Foundation of China (No. 81102460, 81202586, 81202587, 81202588).

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