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Tacrolimus strongly inhibits multiple human UDP-glucuronosyltransferase (UGT) isoforms

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The objective of the present study is to clearly evaluate the inhibitory effects of tacrolimus (tacro) on important UGT isoforms in human liver, including determination of inhibition kinetic type and calculation of inhibition kinetic parameters. An *in vitro* incubation system was used to investigate the inhibitory effect of tacro on UGT isoforms. The recombinant UGT isoforms were used as enzyme source, and a nonspecific substrate 4-methylumbelliferone (4-MU) was utilized as substrate. Among the tested UGT isoforms, UGT1A1, UGT1A3, UGT2B7 and UGT2B15 were strongly inhibited by tacro in a concentration-dependent manner. Dixon and Lineweaver-Burk plots showed that the inhibition of UGT1A1, UGT1A3, and UGT2B7 was all best fit to competitive inhibition type, and the inhibition of UGT2B15 was best fit to noncompetitive type. The inhibition kinetic parameters (K_i) were determined to be 4.7, 1.3, 1.9, and 4.3 μM for UGT1A1, UGT1A3, UGT2B7, and UGT2B15, respectively. Inhibition of these important UGT isoforms in human liver might be an important reason for clinically frequent drug-drug interaction between tacro and other drugs.

1. Introduction

UDP-glucuronosyltransferases (UGTs) are membrane-bound drug metabolizing enzymes which can glucuronidate various endogenous and exogenous substances. Glucuronidation has been regarded to be a powerful detoxification pathway (Kiang et al. 2005). However, UGTs also play an important role in the formation of bioactive and toxic compounds. The pharmacokinetic behaviour of drugs could be altered by inhibition of UGT isoforms, and many drugs and herbal components have been reported to inhibit the activity of UGTs to potentially induce the drug-drug interaction or herb-drug interaction (Huang et al. 2010, 2011; Dong et al. 2012). For example, plasma concentration of 3'-azido-3'-deoxythymidine (AZT) was elevated with concomitant administration of valproic and fluconazole (Lertora et al. 1994; Sahai et al. 1994). The experiment performed by Knights et al. (2010) demonstrated that the increased plasma concentrations of aldosterone in patients treated with spironolactone might be due to the inhibition of UGT2B7 by spironolactone and canrenone.

Tacrolimus (Tacro) is an immunosuppressant drug widely used to optimize the clinical outcomes in post-transplantation. The mechanism may be the suppression of lymphocyte proliferation and interleukin synthesis (Halloran 2004). The metabolic behaviour of tacro by drug-metabolizing enzymes has been investigated. Tacro can be metabolized by cytochrome P450 (CYP3A4 and CYP3A5) and UGT enzymes (UGT1A4) (Wallemacq et al. 2009; Laverdiere et al. 2011). High incidence of drug-drug interactions (DDI) has been reported between tacro and other drugs (Hosohata et al. 2008), and the reason of which might be explained by the inhibition or induction of cytochrome P450 (CYP) and P-glycoprotein (ABCB1) efflux transporter. However, there is some evidence suggesting that tacro might be a potential inhibitor of UGT isoforms (Hara et al. 2007).

Comprehensive understanding of inhibitory effects of tacro on important UGT isoforms in human liver is helpful for a deeper understanding of DDI between tacro and other drugs. To date, only limited data are available with regards to the inhibitory effects of tacro on UGT isoforms. Therefore, the aim of the present study was to investigate the inhibition effects of tacro on important UGT isoforms in human liver. The recombinant UGT isoforms were used as enzyme sources, and a nonspecific substrate 4-methylumbelliferone (4-MU) was utilized as substrate.

2. Investigations and results

As shown in Fig. 1, the residual activity of 4-MU glucuronidation was $7.8 \pm 0.2\%$ (UGT1A1), $15.9 \pm 1.3\%$ (UGT1A3), $67.9 \pm 4.6\%$ (UGT1A6), $138.4 \pm 2.8\%$ (UGT1A9), $56.2 \pm 4.6\%$ (UGT2B4), $4.9 \pm 0.1\%$ (UGT2B7), and $2.5 \pm 0.0\%$ (UGT2B15) of the control activity at 100 μM of tacro, respectively. Because the activities of UGT1A1, UGT1A3, UGT2B7 and UGT2B15 were inhibited by more than 50% at 100 μM of tacro, their half inhibition concentration (IC_{50}) values were determined. The IC_{50} values were calculated to be 3.2 ± 0.3, 0.4 ± 0.1, 23.5 ± 0.7, 16.5 ± 0.7 μM for UGT1A1, UGT1A3, UGT2B7 and UGT2B15, respectively. Dixon and Lineweaver-Burk plots showed that the

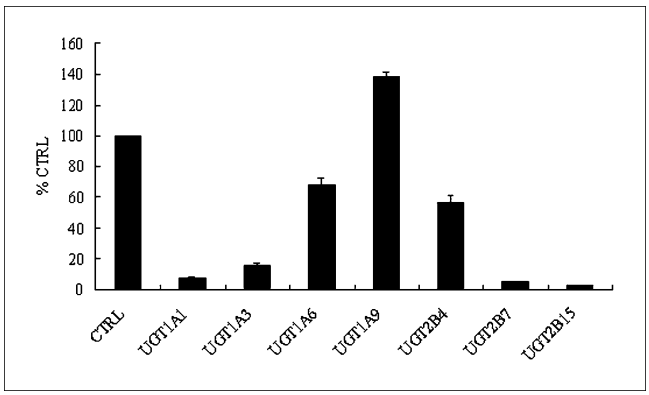

Fig. 1: Inhibitory effects of tacro on important UGT isoforms in human liver. Recombinant UGT isoforms were used as enzyme sources. 4-MU was utilized as probe substrate

inhibition of UGT1A1, UGT1A3, and UGT2B7 was all best fit to competitive inhibition type, and the inhibition of UGT2B15 was best fit to noncompetitive type. The inhibition kinetic parameters were determined to be 4.7, 1.3, 1.9, and 4.3 μM for UGT1A1, UGT1A3, UGT2B7, and UGT2B15, respectively.

3. Discussion

Drug-metabolizing enzymes (DMEs)-mediated biotransformation plays a key role in determining the optimal window between the drug safety parameters and its therapeutic potential (Lin and Lu 1997). DDI induced by inhibition of DMEs is the major reason for drugs being withdrawn from the market and clinical adverse drug reactions (Michalets 1998). Compared with CYP-mediated DDIs (Sun et al. 2010a, b), little attention has been paid to UGT-mediated DDIs (Tanaka 1998). Therefore, a clarification of inhibitory potential of clinical drugs on UGT isoforms is necessary for a deeper understanding of the mechanism of clinical DDIs.

In the present study, tacro demonstrated to exert a strong inhibitory potential towards UGT1A1, UGT1A3, UGT2B7 and UGT2B15. UGT1A1 is an important UGT isoform involved in the metabolism of many clinically used drugs, and the inhibition of UGT1A1 often leads to severe adverse effects for some drugs with narrow therapeutic windows, such as etoposide and and irinotecan (Kawato et al. 1991; Wen et al. 2007). Additionally, the hyperbilirubinemia caused by atazanavir and indinavir can be attributed to reduced glucuronidation of bilirubin induced by UGT1A1 inhibition (Zhang et al. 2005). UGT1A3 is also an important UGT isoform involved in the metabolism of many xenobiotics, including UGT2B7 has been considered as the most important UGT isoform because approximately 35% of the top 200 drugs prescribed in the United States in 2002. UGT2B15, a large group of human UGT isoforms, is involved in the inactivation of steroid hormones (mainly androgens), and also plays a key role in the metabolism of many drugs (Green et al. 1994). The inhibition of these UGT isoforms by tacro should be paid much attention.

In conclusion, the experimental results in the present study demonstrated that tacro exerts inhibitory effect towards multiple important UGT isoforms in the human liver. Given that many factors can affect the extrapolation of *in vivo* situation using these *in vitro* inhibition kinetic parameters, these *in vitro* parameters should be considered with great caution.

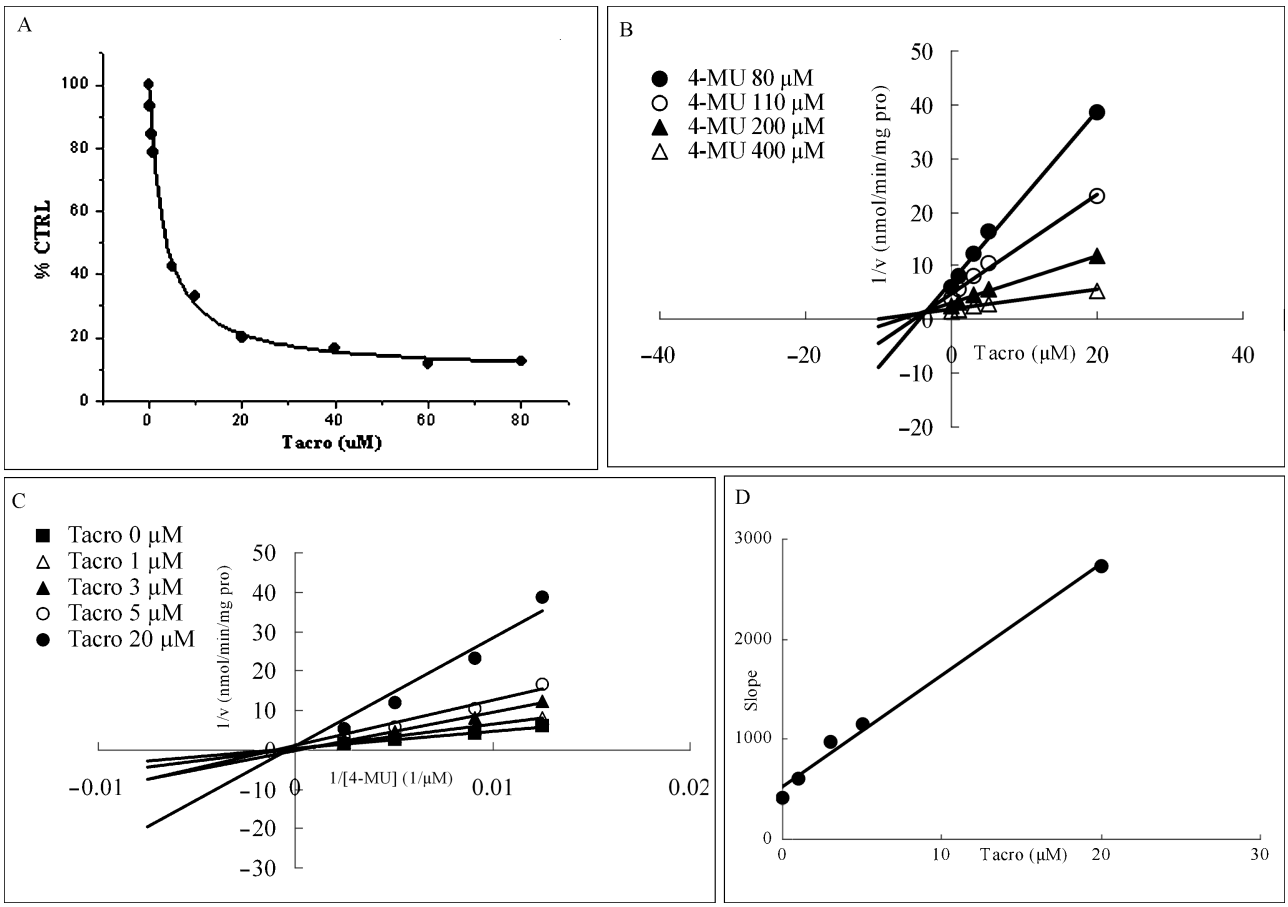

Fig. 2: Reversible inhibition of UGT1A1 by tacro. A. Determination of IC50 value of UGT1A1. B. Dixon plot of inhibitory effect of tacro on UGT1A1-mediated 4-MU glucuronidation reaction. C. Lineweaver-Burk plot inhibitory effect of tacro on UGT1A1-mediated 4-MU glucuronidation reaction. D. Second plot of slope from Lineweaver-Burk plot vs. tacro concentration

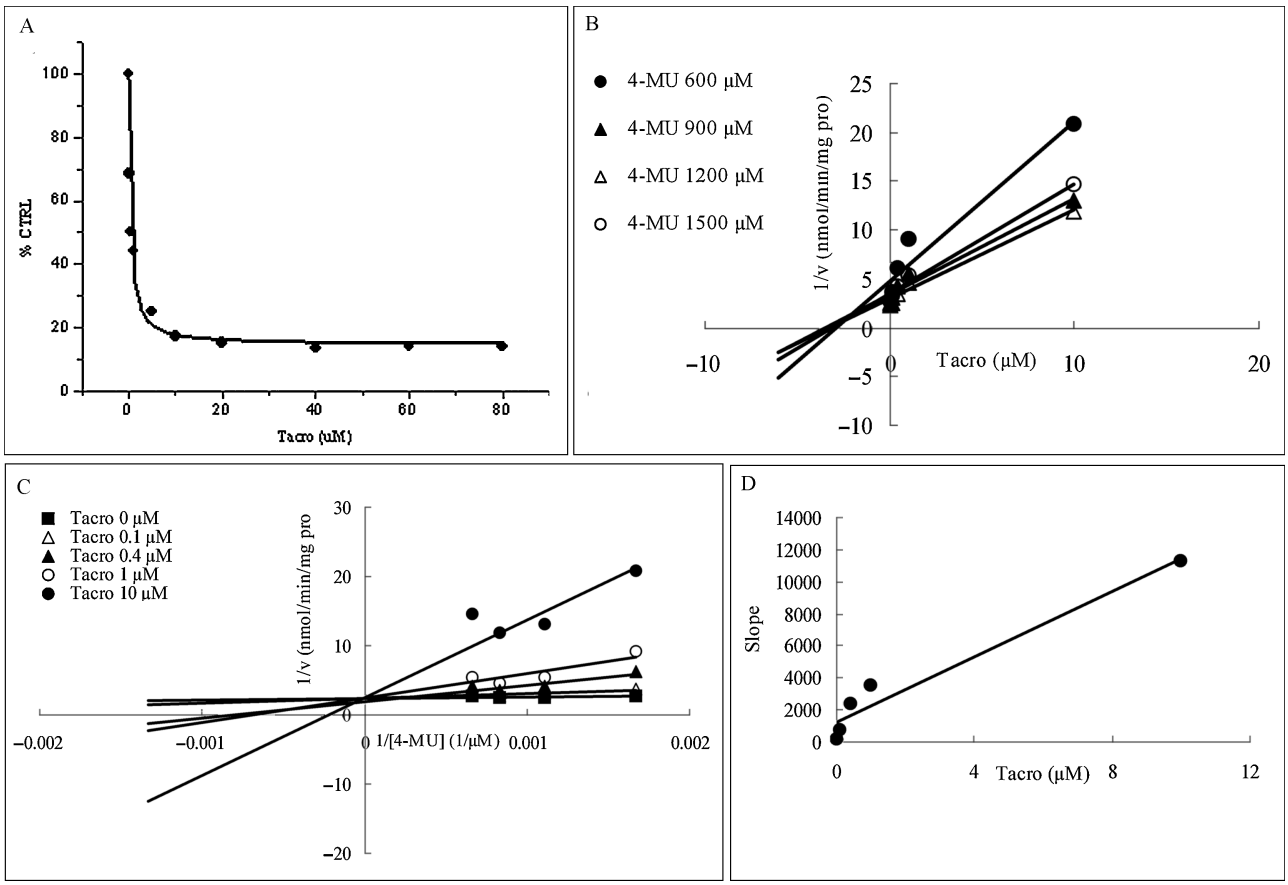

Fig. 3: Reversible inhibition of UGT1A3 by tacro A. Determination of IC50 value of UGT1A3. B. Dixon plot of inhibitory effect of tacro on UGT1A3-mediated 4-MU glucuronidation reaction. C. Lineweaver-Burk plot inhibitory effect of tacro on UGT1A3-mediated 4-MU glucuronidation reaction. D. Second plot of slope from Lineweaver-Burk plot vs. tacro concentration

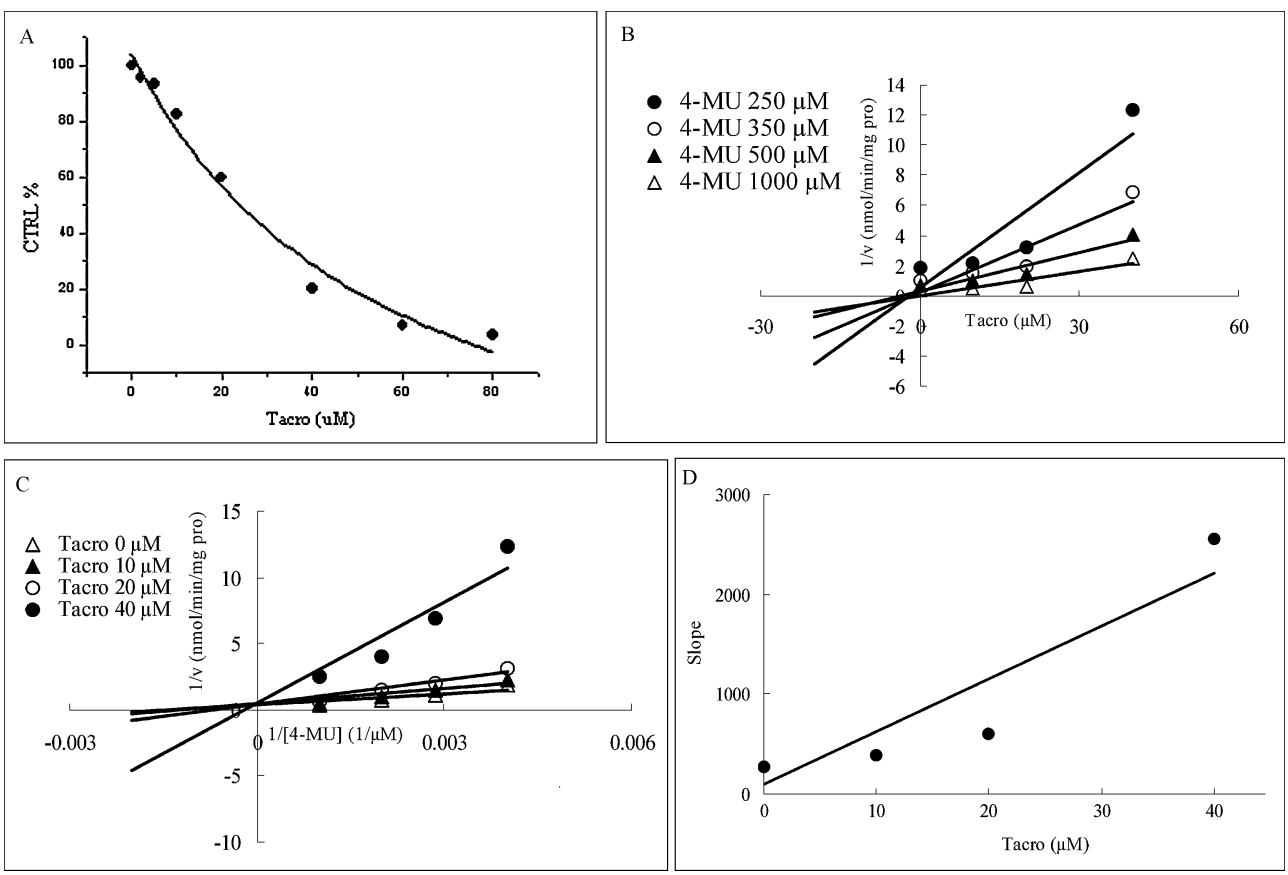

Fig. 4: Reversible inhibition of UGT2B7 by tacro. A. Determination of IC50 value of UGT2B7. B. Dixon plot of inhibitory effect of tacro on UGT2B7-mediated 4-MU glucuronidation reaction. C. Lineweaver-Burk plot inhibitory effect of tacro on UGT2B7-mediated 4-MU glucuronidation reaction. D. Second plot of slope from Lineweaver-Burk plot vs. tacro concentration

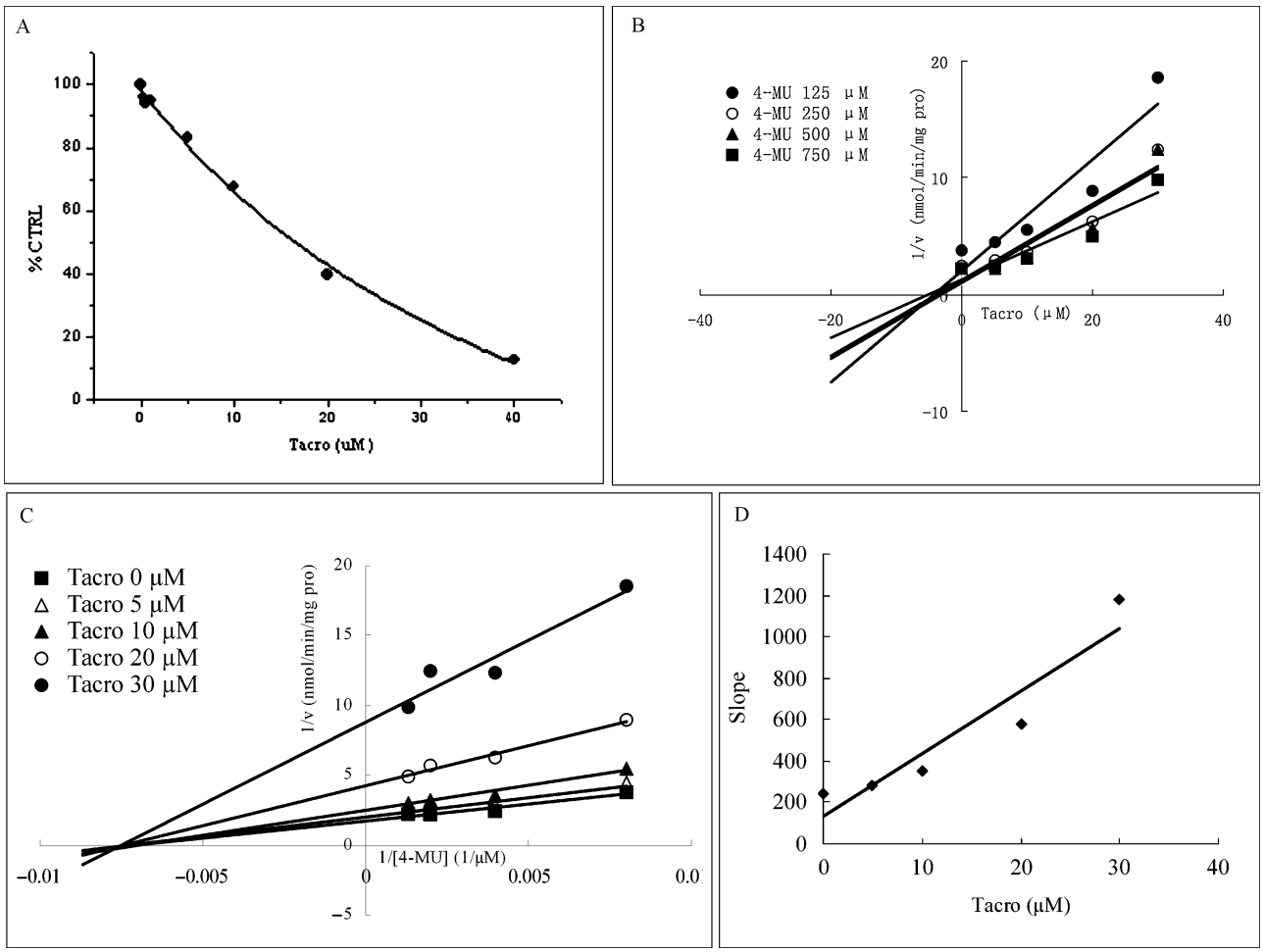

Fig. 5: Reversible inhibition of UGT2B15 by tacro. A. Determination of IC50 value of UGT2B15. B. Dixon plot of inhibitory effect of tacro on UGT2B15-mediated 4-MU glucuronidation reaction. C. Lineweaver-Burk plot inhibitory effect of tacro on UGT2B15-mediated 4-MU glucuronidation reaction. D. Second plot of slope from Lineweaver-Burk plot vs. tacro concentration

4. Experimental

4.1. Chemicals and reagents

Tacro (purity > 98%) was purchased from Aladdin Corp. (Shanghai, China). 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 supersomes (UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B4, UGT2B7, UGT2B15) expressed in baculovirus-infected insect cells were obtained form BD Gentest Corp. (Woburn, MA, USA). All other reagents were of HPLC grade or of the highest grade commercially available.

4.2. Inhibition of 4-MU glucuronidation assay

The probe substrate for all tested UGT isoforms is 4-MU which is a non-selective substrate of UGTs. Incubations with each UGT isoform were carried out as previously reported (Huang et al. 2010). The mixture (200 μl total volume) contained recombinant UGTs (final concentration: 0.25, 0.05, 0.025, 0.05, 0.5, 0.05, and 0.75 mg/ml for UGT1A1, UGT1A3, UGT1A6, UGT1A9, UGT2B4, UGT2B7, and UGT2B15, respectively), 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH 7.4), and 4-MU in the absence or presence of different concentrations of tacro. The concentrations of 4-MU are as follows: 110 μM for UGT1A1, 1200 μM for UGT1A3, 110 μM for UGT1A6, 30 μM for UGT1A9, 1200 μM for UGT2B4, 350 μM for UGT2B7, and 250 μM for UGT2B15. Tacro was dissolved in DMSO and the final concentration of DMSO was 0.5% (v/v). After 5 min pre-incubation at 37 °C, the UDPGA was added in the mixture to initiate the reaction. Incubation time was 120 min for UGT1A1, UGT2B4, UGT2B7, and UGT2B15, 75 min for UGT1A3, 30 min for UGT1A6 and UGT1A9, respectively. The reactions were quenched by adding 100 μl acetonitrile with 7-hydroxycoumarin (100 μM) as internal standard. The mixture was centrifuged at 20,000 × 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 C_{18} 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.

4.3. Determination of half inhibition concentration (IC_{50})

For UGT isoforms that were inhibited by more than 50% at 100 μM of tacro, the half inhibition concentration values (IC_{50}) were determined as previously described (Fang et al. 2010, 2011).

4.4. Determination of inhibition kinetic parameters

Inhibition kinetic parameters (K_i) were determined utilizing various concentrations of 4-MU in the presence of different concentrations of tacro. Dixon and Lineweaver-Burk (L-B) plots were adapted to determine the inhibition type, and second plot of slopes from Lineweaver-Burk plot vs. tacro concentrations was utilized to calculate K_i value.

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