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Biotransformation of baicalin to baicalein significantly strengthens the inhibition potential towards UDP-glucuronosyltransferases (UGTs) isoforms

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The aim of the present study was to investigate the influence of biotransformation of baicalin into baicalein towards the inhibition potential towards one of the most important drug-metabolizing enzymes (DMEs) UDP-glucuronosyltransferases (UGTs). *in vitro* incubation method using recombinant UGTs-catalyzed 4-methylumbelliferone (4-MU) glucuronidation was used to evaluate the inhibition towards important UGT isoforms in the liver, including UGT1A1, 1A3, 1A6, 1A9, and 2B7. At the same concentration (100 μM), baicalein showed stronger inhibition potential than baicalin towards all the tested UGT isoforms. Data fitting using Dixon plot and Lineweaver-Burk plot was carried out to determine the inhibition type, and the second plot with the slopes from Lineweaver-Burk plot towards baicalein's concentrations was used to calculate the inhibition kinetic parameters (K_i). Competitive inhibition type was observed for UGT1A1, 1A6, 1A9 and 2B7, and noncompetitive inhibition was detected for UGT1A3. The inhibition kinetic parameters (K_i) were calculated to be 1.2, 5.1, 15.3, 26.3, and 48.9 μM for UGT1A1, 1A3, 1A6, 1A9, and 2B7, respectively. All these information reminds us of the necessary monitoring when oral administration of baicalin or baicalin-containing herbs.

1. Introduction

Flavonoids, a vast group of heterogenous polyphenols, have been widely found in a variety of fruits, vegetables, tea and wine, and demonstrated to exhibit multiple biochemical and pharmacological activities, including anti-tumor activity (Yao et al. 2004). Some flavonoids have been developed as efficient drugs. For example, silymarin, the active complex containing three isomer flavonolignans (silybin, silychristin, silydianin), has been regarded as an efficient treatment of cirrhosis, chronic hepatitis, and non-alcoholic/alcoholic liver diseases (Ferenci 2012). Baicalin (the chemical name: 5,6-dihydroxy-7-O-glucuronide) is a flavonoid isolated from *Scutellariae radix*, and has been demonstrated to exhibit various biochemical and pharmacological activities, including anti-inflammatory, anti-tumor, anti-allergic, and anti-oxidation effects (Cao et al. 2011; Huang et al. 2005; Li-Weber 2009; Li et al. 2010). Baicalein, the hydrolysis product of baicalin, has been regarded as the main active iffectively of baicalin in the serum (Li et al. 2011).

The interaction between the active components of herbs and drug-metabolizing enzymes (DMEs) might influence the pharmacokinetic behaviour of co-administered drugs (Zhang *et al.* 2012). The biotransformation process of compounds in the intestine can often complicate the inhibition situation of compounds towards DMEs. For example, the deglycosylation of liquiritin into liquiritigenin can significantly strengthen the inhibition potential towards one of the important DME UDP-glucuronosyltransferases (UGTs) (Guo et al. 2012). The experiment carried out by Liu et al. (2006) showed that ginsenosides' degradation products exhibited stronger inhibition towards cytochrome P450 enzymes. The aim of the present study was to compare the inhibition potential between baicalin and baicalein towards important UGT isoforms located in the liver, trying to indicate the influence of biotransformation of baicalin into baicalein towards the inhibition potential towards UGTs.

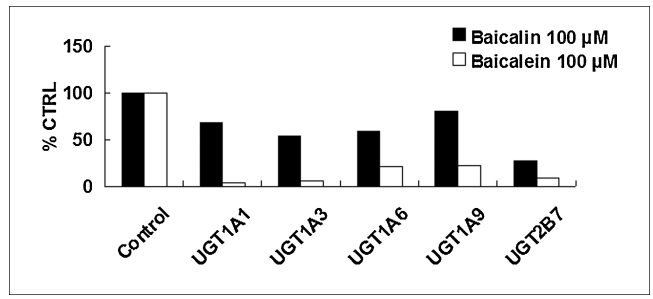


Fig. 1: Screening the inhibition of UGT isoforms by baicalin and baicalein. Recombinant UGT isoforms-catalyzed 4-MU glucuronidation was used, and 100 μM of baicalin and baicalein was utilized.

2. Investigations and results

As shown in Fig. 1, 100 μM of baicalin and baicalein was utilized to initially screen the inhibition potential towards UGT isoforms, and the results showed that baicalein exerted stronger

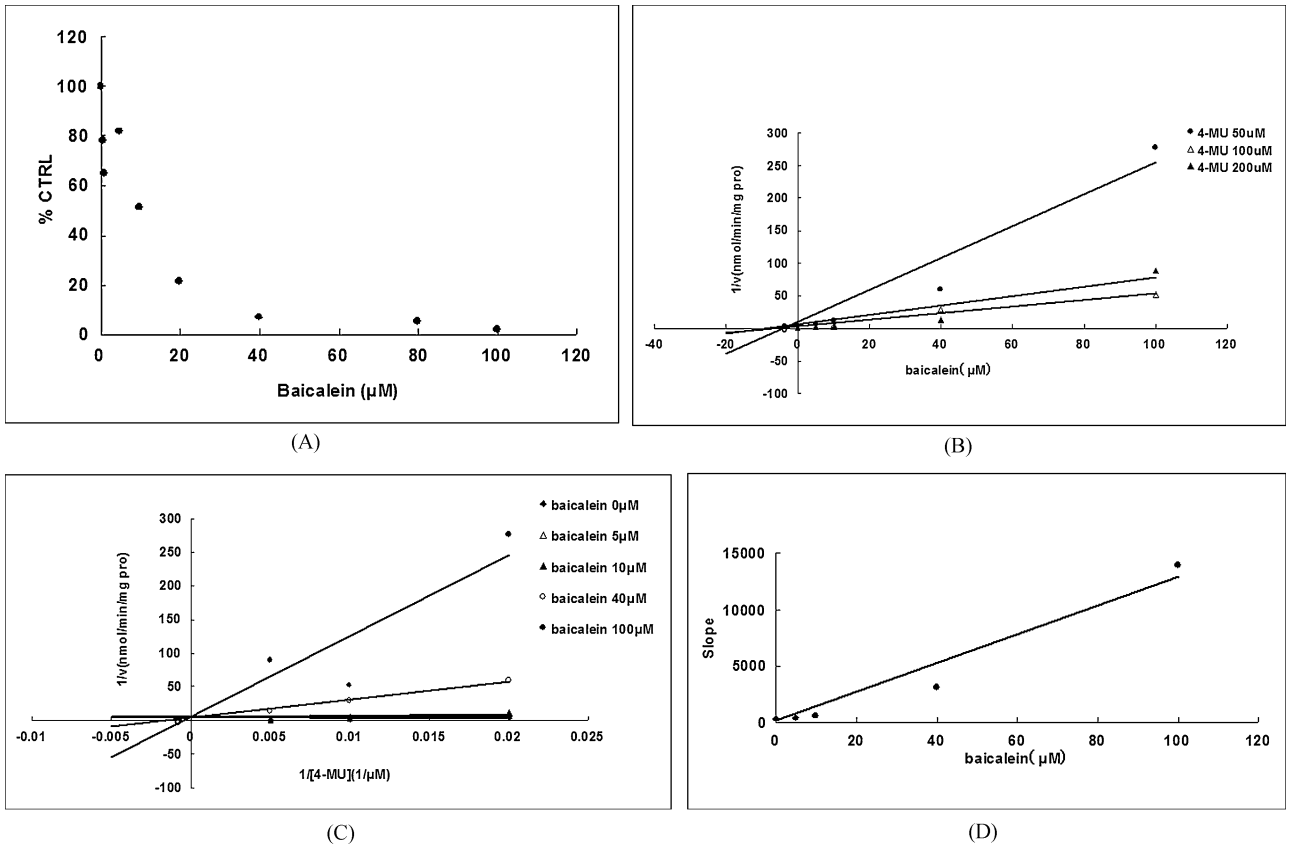


Fig. 2: Baicalein exerts competitive inhibition towards UGT1A1-catalyzed 4-MU glucuronidation. (A) Dose-dependent inhibition of baicalein towards UGT1A1-catalyzed 4-MU glucuronidation. (B) Dixon plot of baicalein's inhibition towards UGT1A1-catalyzed 4-MU glucuronidation. (C) Lineweaver-Burk plot of baicalein's inhibition towards UGT1A1-catalyzed 4-MU glucuronidation. (D) Second plot to calculate the inhibition kinetic parameters.

inhibition towards all the tested UGT isoforms, including UGT1A1, 1A3, 1A6, 1A9, and 2B7. Furthermore, the inhibition kinetics of baicalein towards the tested UGT isoforms were determined, and the inhibition parameters (Ki) were calculated. For UGT1A1, 1A6, 1A9 and 2B7, the intersection point was located in the second quadrant in the Dixon plot (Fig. 2B, Fig. 4B, Fig. 5B & Fig. 6B), and located in the verticle axis in Lineweaver-Burk plot (Fig. 2C, Fig. 4C, Fig. 5C & Fig. 6C), indicating the competitive inhibition of baicalein towards these UGT isoforms. For UGT1A3, the intersection point was located in the horizontal axis in both Dixon plot (Fig. 3B) and Lineweaver-Burk plot (Fig. 3C), indicating the noncompetitive inhibition of baicalein towards UGT1A3. The inhibition kinetic parameters (K_i) were calculated to be 1.2, 5.1, 15.3, 26.3, and 48.9 μM for UGT1A1, 1A3, 1A6, 1A9, and 2B7, respectively (Fig. 2D-6D).

3. Discussion

The UGT enzymes located in the liver play a key role in the metabolism of various xenobiotics and endogenous substances (Malik and Black 2012). Therefore, although orally administrated herbal components might not inhibit UGT isoforms in the liver, hydrolysis products of the herbal ingredients taken into the plasma might exert strong inhibition potential towards UGT isoforms. In the present study, the influence of this kind of biotransformation towards UGT inhibition potential was demonstrated for baicalin and baicalein.

A competitive inhibition type was detected for the inhibition of baicalein towards UGT1A1, 1A6, 1A9 and 2B7, and a noncompetitive inhibition type was observed for baicalein's inhibition towards UGT1A3. All these UGT isoforms have been demonstrated to be involved in the metabolic elimination of important clinical drugs and endogenous substances. For example, UGT1A1 is the key enzyme for conjugation elimination of bilirubin which is a nonpolar metabolite formed in the catabolism of hemoglobin, and the limited activity of UGT1A1 will result in unconjugated hyperbilirubinemia, and then induce Crigler–Najjar syndrome type 1 (CN1) and 2 (CN2) and Gilbert's syndrome (GS) (D'Silva et al. 2013). Additionally, UGT1A1 has been demonstrated to mainly contribute to the metabolism of estrogen, and decreased activity of UGT1A1 might increase the exposure dose of estrogen, possibly resulting in the high risk of carcinogenesis (Deming et al. 2008). The altered activity of UGT1A3 was well correlated with the changed lipid-lowering effect (Cho et al. 2012). The altered activity of UGT1A6 might be related with the high risk of cancer (Kua et al. 2012). UGT1A9 mainly participates in the metabolism of propofol, which is a short-acting, intravenously administered hypnotic agent with narrow therapeutic window (Kansaku et al. 2011). UGT2B7 is arguably the most important drug metabolizing UGT isoform in human and could catalyze the glucuronidation of many drugs including nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids (Miners et al. 2010). In conclusion, the biotransformation process of baicalin into baicalein can significantly increase the inhibition potential towards important UGT isoforms in the liver, including UGT1A1, 1A3, 1A6, 1A9, and 2B7. All these information reminds us of the necessary monitoring after oral administration of baicalin or baicalin-containing herbs.

4. Experimental

4.1. Chemicals

Baicalin (≥99%), baicalein (≥98%), 4-methylumbelliferone (4-MU), 4-methylumbelliferone-β-d-glucuronide (4-MUG), Tris-HCl, 7-hydroxycoumarin and uridine 5'-diphosphoglucuronic acid (UDPGA)

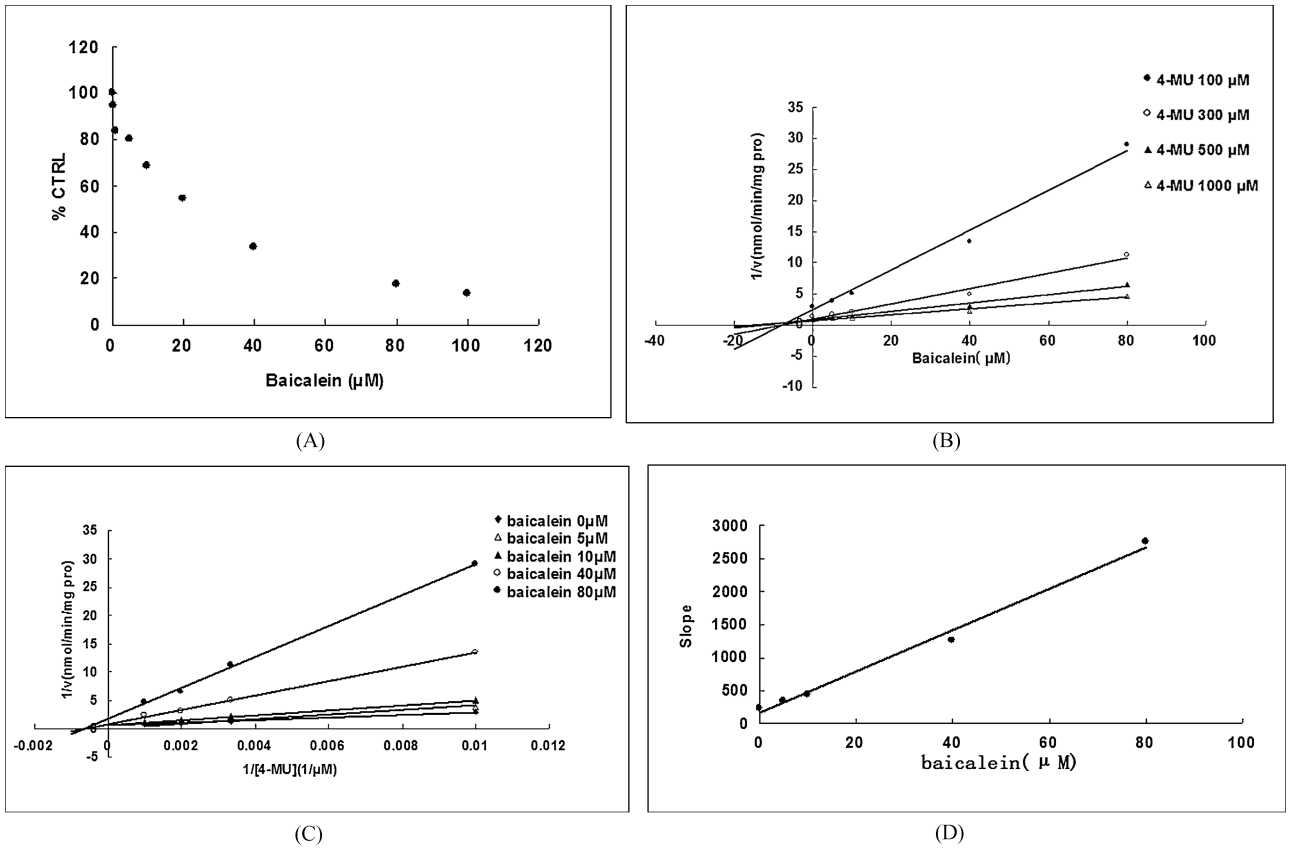


Fig. 3: Baicalein exerts noncompetitive inhibition towards UGT1A3-catalyzed 4-MU glucuronidation. (A) Dose-dependent inhibition of baicalein towards UGT1A3-catalyzed 4-MU glucuronidation. (B) Dixon plot of baicalein's inhibition towards UGT1A3-catalyzed 4-MU glucuronidation. (C) Lineweaver-Burk plot of baicalein's inhibition towards UGT1A3-catalyzed 4-MU glucuronidation. (D) Second plot to calculate the inhibition kinetic parameters.

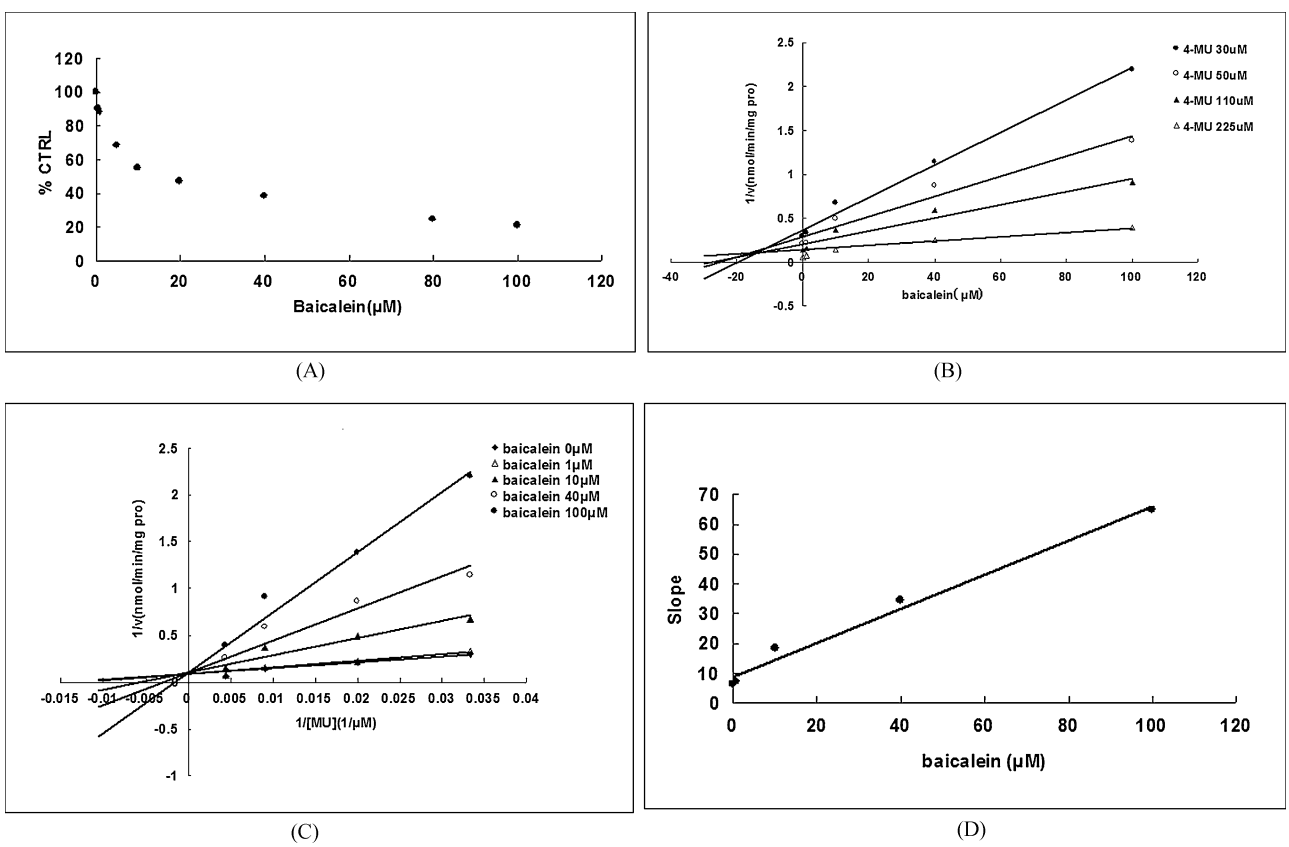
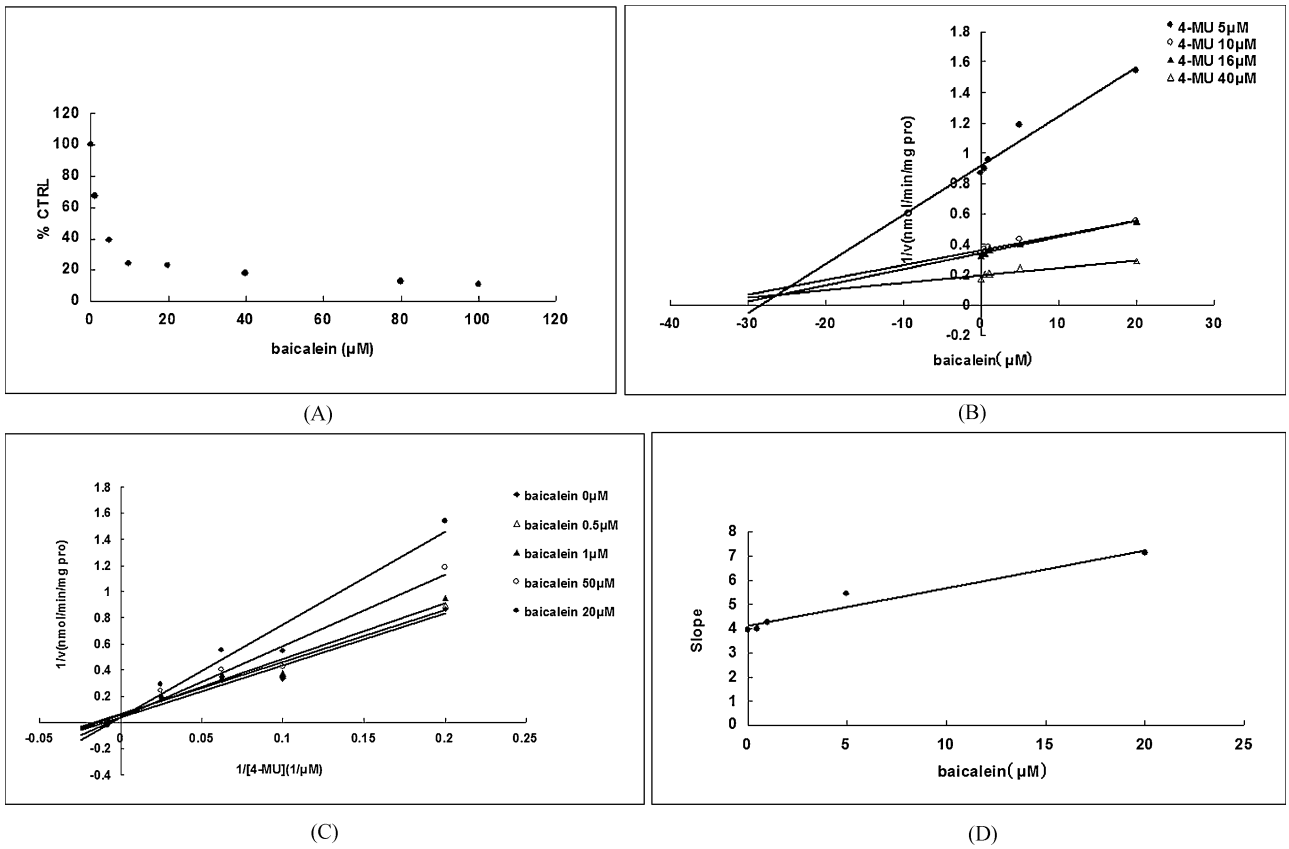


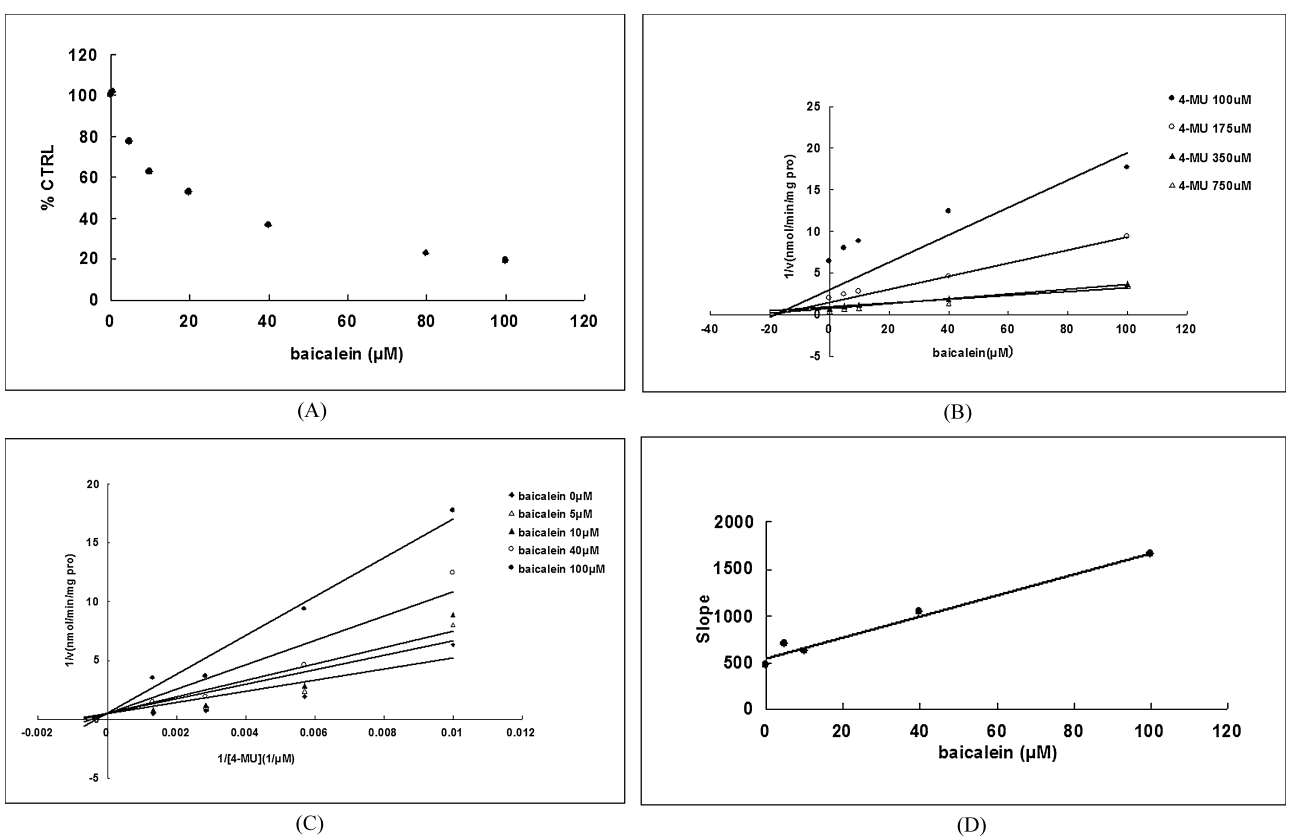
Fig. 4: Baicalein exerts competitive inhibition towards UGT1A6-catalyzed 4-MU glucuronidation. (A) Dose-dependent inhibition of baicalein towards UGT1A6-catalyzed 4-MU glucuronidation. (B) Dixon plot of baicalein's inhibition towards UGT1A6-catalyzed 4-MU glucuronidation. (C) Lineweaver-Burk plot of baicalein's inhibition towards UGT1A6-catalyzed 4-MU glucuronidation. (D) Second plot to calculate the inhibition kinetic parameters.



(A) (B)

(C) (D)

Fig. 5: Baicalein exerts competitive inhibition towards UGT1A9-catalyzed 4-MU glucuronidation. (A) Dose-dependent inhibition of baicalein towards UGT1A9-catalyzed 4-MU glucuronidation. (B) Dixon plot of baicalein's inhibition towards UGT1A9-catalyzed 4-MU glucuronidation. (C) Lineweaver-Burk plot of baicalein's inhibition towards UGT1A9-catalyzed 4-MU glucuronidation. (D) Second plot to calculate the inhibition kinetic parameters.



(A) (B)

(C) (D)

Fig. 6: Baicalein exerts competitive inhibition towards UGT2B7-catalyzed 4-MU glucuronidation. (A) Dose-dependent inhibition of baicalein towards UGT2B7-catalyzed 4-MU glucuronidation. (B) Dixon plot of baicalein's inhibition towards UGT2B7-catalyzed 4-MU glucuronidation. (C) Lineweaver-Burk plot of baicalein's inhibition towards UGT2B7-catalyzed 4-MU glucuronidation. (D) Second plot to calculate the inhibition kinetic parameters.

(trisodium salt) were purchased from Sigma-Aldrich (St. Louis, MO). Recombinant human UGT isoforms (UGT1A1, 1A3, 1A6, 1A9 and 2B7) were were obtained form BD Gentest Corp. (Woburn, MA, USA), and all these recombinant UGTs were expressed in baculovirus-infected insect cells. All other reagents were of HPLC grade or of the highest grade commercially available.

4.2. Inhibition potential of baicalin and baicalein towards recombinant UGTs-catalyzed 4-MU glucuronidation reaction

The 4-MU glucuronidation reaction was utilized as the probe reaction to evaluate the inhibition towards all the tested UGT isoforms as previously described (Gao et al. 2012). The incubation system (total volume = 200 μM) consisted of different concentrations of recombinant UGT isoforms (0.25, 0.05, 0.025, 0.05, and 0.05 mg/ml for UGT1A1, 1A3, 1A6, 1A9 and 2B7), 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl buffer (pH = 7.4), and various concentrations of 4-MU and baicalin/baicalein. The concentrations of 4-MU, incubation time, and analysis method were previously described (Zhao et al. 2012; Liu et al. 2012).

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