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Inhibition potential of UDP-glucuronosyltransferases (UGTs) 1A isoforms by the analogue of resveratrol, bakuchiol

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Bakuchiol is a promising anti-tumor candidate with resveratrol-like structure. The present study aims to evaluate the inhibition potential of bakuchiol towards UDP-glucuronosyltransferases (UGT) 1A isoforms. An *in vitro* incubation system using 4-methylumbelliferone (4-MU) glucuronidation was used to evaluate the inhibition capability of bakuchiol towards UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9 and 1A10. The glucuronidation of trifluoperazine (TFP) was employed as the probe reaction to determine bakuchiol's inhibition towards UGT1A4. At 1 μM and 10 μM of bakuchiol, no or weak inhibition was observed for all the tested UGT1A isoforms. At 100 μM of bakuchiol, the activity of UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9 and 1A10 was inhibited by -46.2%, 74.7%, 17.8%, 98.7%, 70.4%, 99.2%, 75.8%, and 93.3%, respectively. Further inhibition kinetic behaviour was determined for UGT1A6, 1A8, and 1A10. Both Dixon plot and Lineweaver-Burk plot showed the noncompetitive inhibition of bakuchiol towards all these three UGT isoforms. The inhibition kinetic parameters (K_i) were calculated to be 5.3, 1.8, and 92.6 μM for UGT1A6, 1A8, and 1A10, respectively. In combination with the *in vivo* exposure of bakuchiol, the high possibility of *in vivo* inhibition of UGT1A6 and 1A8 was predicted. However, relatively low possibility of *in vivo* inhibition towards UGT1A10 was predicted due to lower *in vivo* concentration of bakuchiol than its inhibition parameter (K_i). All these information will be helpful for the R&D of bakuchiol as a promising anti-tumor drug.

1. Introduction

Resveratrol (3,5,4-trihydroxy-*trans*-stilbene, Fig. 1A), is an important herbal component found in grapes, berries, and peanuts, and has been regarded as a promising agent for cancer prevention (Athar et al. 2007; Dong 2003). To date, the core structure of resveratol has been drawing much attention of researchers, and it is expected to discover new promising anti-tumor agents with resveratrol-like structure.

Bakuchiol (Fig. 1B) is a prenylated phenolic monoterpene extracted from the seeds of *Psoralea corylifolia* L. (Leguminosae), and has been demonstrated to exhibit various biochemical and pharmacological activities, including antimicrobacterial activity and inhibition of iNOS expression (Park et al. 2005; Sun et al. 1998). Bakuchiol shares the 4-hydroxystyryl moiety with resresatrol and has been reported to have anti-cancer activity (Chen et al. 2010).

UDP-Glucuronosyltransferases (UGTs) are the most important enzymes catalyzing the conjugation of xenobiotics and endogenous substances, which accounts for >35% of all phase II drug metabolism (Song et al. 2013). Compounds with a phenol hydroxyl group are the preferred substrates of UGT isoforms, and reveratrol has been demonstrated to be a good substrate of UGTs. The involved UGT isoforms comprise UGT1A1, 1A7, 1A9 and 1A10 (Iwuchukwu et al. 2008). All these information indicate the possibility for an interaction between compounds with resveratrol-like structure and the UGT1A isoforms.

The present study aims to evaluate the inhibition potential of bakuchiol towards important UGT1A isoforms in the liver and intestine. As previously reported (Fang et al. 2013; Lu et al. 2013), recombinant UGTs-catalyzed 4-methylumbelliferone (4-MU) glucuronidation was employed as the probe reaction. However, due to the lack of catalytic activity of UGT1A4 towards 4-MU glucuronidation, the recombinant UGT1A4-catalyzed trifluoperazine glucuronidation was adopted to evaluate the inhibition of bakuchiol towards UGT1A4.

2. Investigations and results

As shown in Fig. 2, three concentrations of bakuchiol (1,10,100 μM) were used for screening of the inhibitory potential of bakuchiol towards the activity of UGT1A isoforms. At 1 μM and 10 μM of bakuchiol, bakuchiol exhibited weak or no inhibition towards all the tested UGT1A isoforms. At 100 μM of bakuchiol, the activity of UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9 and 1A10 was inhibited by -46.2%, 74.7%, 17.8%, 98.7%, 70.4%, 99.2%, 75.8%, and 93.3%, respectively. Furthermore, the inhibition type and kinetic parameters (K_i) were determined for the UGT1A isoforms which activity were inhibited by more

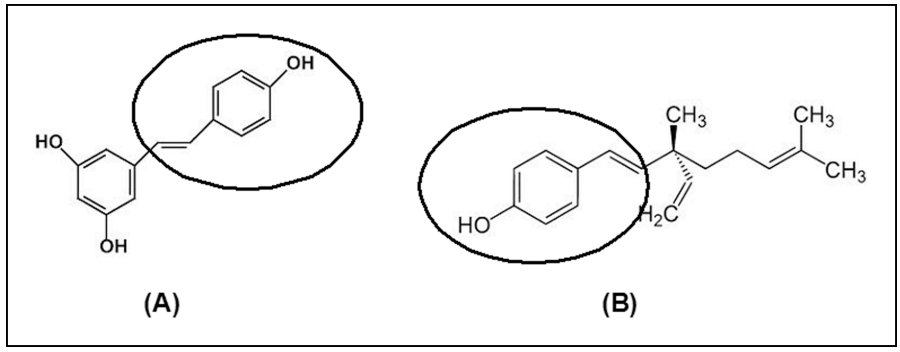

Fig. 1: Structures of reveratrol (A) and bakuchiol (B).

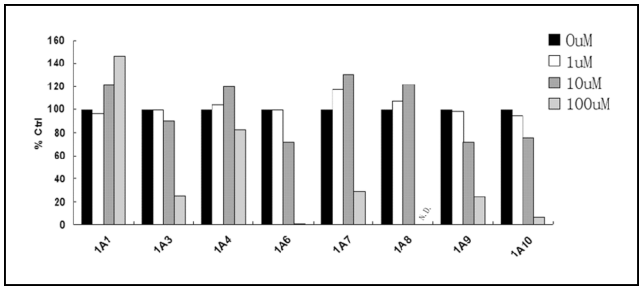

Fig. 2: Initial screening of the inhibition of bakuchiol (100 μM) towards UGT1A isoforms. The experiment was performed in duplicate.

than 80%. Dose-dependent inhibitory behaviour can be observed for the inhibition of bakuchiol towards UGT1A6, 1A8, and 1A10 (Fig. 3A, 4A & 5A). Dixon plot and Lineweaver-Burk plot are common methods to determine the inhibition kinetic type, and the second plot with the slopes from the Lineweaver-Burk plots *versus* the concentrations of bakuchiol. For the inhibition of bakuchiol towards UGT1A6, 1A8 and 1A10, the intersection point was located in the horizontal axis in both the Dixon plot (Fig. 3B, 4B & 5B) and the Lineweaver-Burk plot (Fig. 3C, 4C & 5C), indicating noncompetitive inhibition of bakuchiol towards all these three UGT1A isoforms. The second plot with the slopes from the Lineweaver-Burk plot *versus* the concentrations of bakuchiol was employed to calculate the inhibition kintic parameters (K_i), and the K_i values were determined to be 5.3, 1.8, and 92.6 μM for UGT1A6, 1A8, and 1A10, respectively (Fig. 3D, 4D & 5D).

3. Discussion

UGT1A6 is one of the most important UGT1A isoforms involved in the glucuronidation of clinical drugs, toxins, and endogenous substrates, including acetaminophen, benzopyrene, and serotonin (Navarro et al. 2011; Court 2005). Additionally, UGT1A6 has been reported to exhibit high capability towards the detoxification of carcinogenic arylamines and aryl hydro-

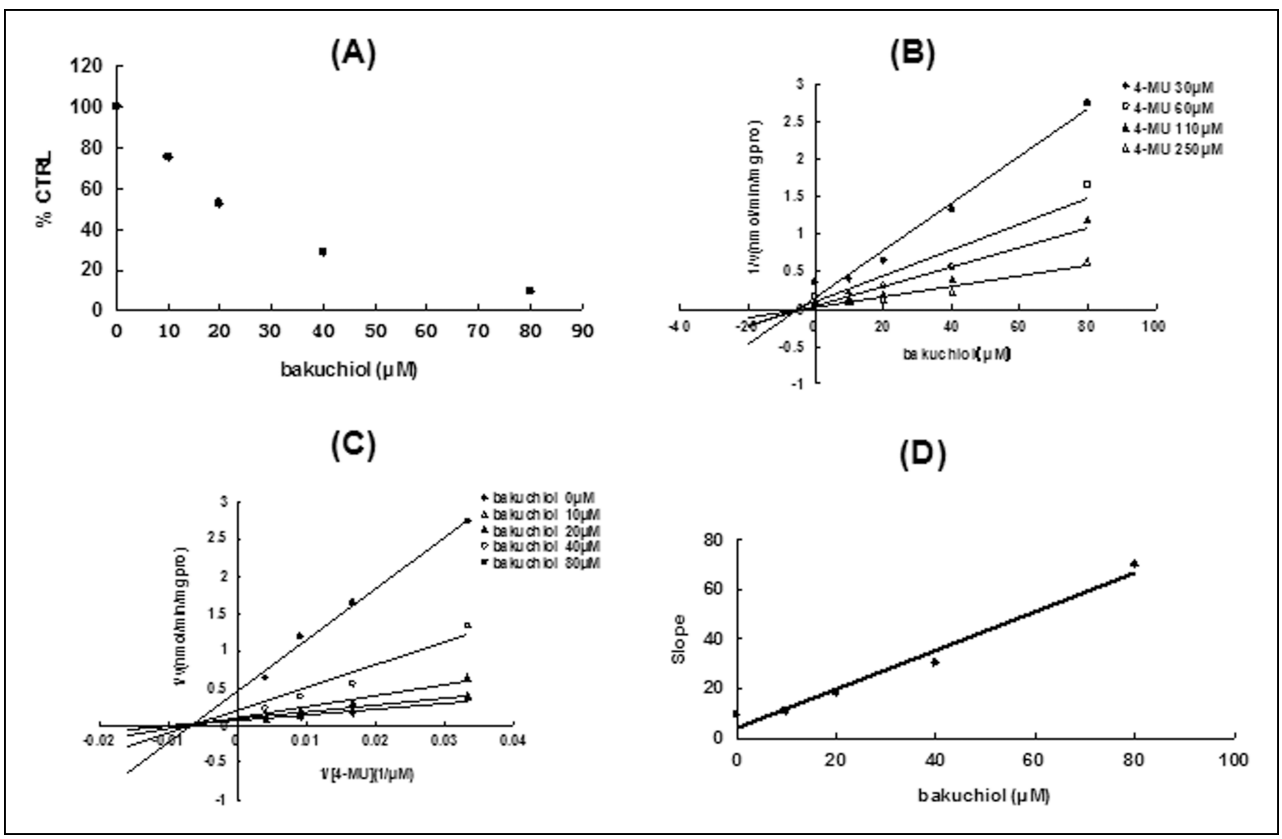

Fig. 3: Inhibition kinetic analysis of bakuchiol towards UGT1A6-catalyzed 4-MU glucuronidation. (A) Bakuchiol showed dose-dependent inhibition towards UGT1A6; (B) Dixon plot of bakuchiol inhibition towards UGT1A6-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of bakuchiol inhibition towards UGT1A6-catalyzed 4-MU glucuronidation; (D) Second plot using slope (obtained from Lineweaver-Burk plot) vs. the concentration of bakuchiol.

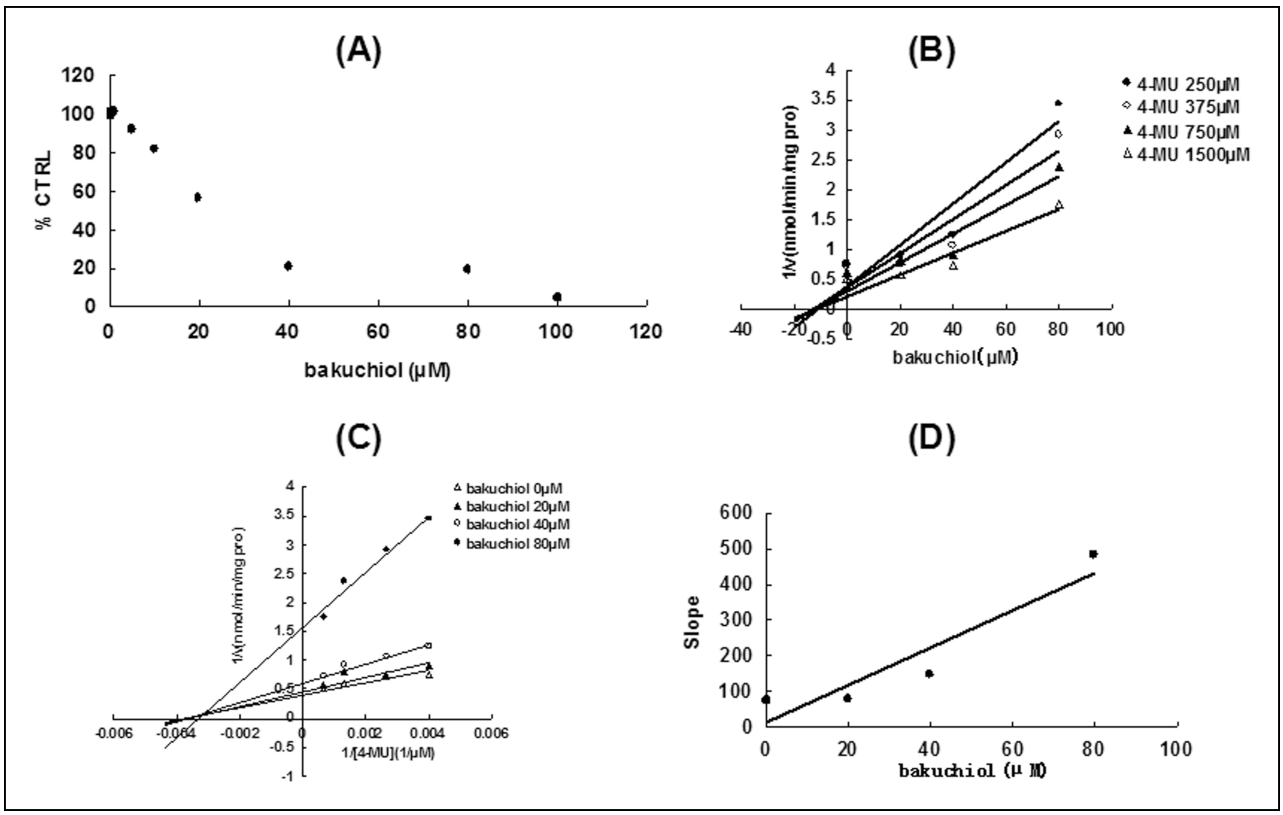

Fig. 4: Inhibition kinetic analysis of bakuchiol towards UGT1A8-catalyzed 4-MU glucuronidation. (A) Bakuchiol showed dose-dependent inhibition towards UGT1A8; (B) Dixon plot of bakuchiol inhibition towards UGT1A8-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of bakuchiol inhibition towards UGT1A8-catalyzed 4-MU glucuronidation; (D) Second plot using slope (obtained from Lineweaver-Burk plot) vs. the concentration of bakuchiol.

carbons (Schrenk et al. 1996). UGT1A8 and UGT1A10 are specific UGT1A isoforms in the GI tract. UGT1A8 has been found to highly express in esophagus, duodenum, jejunum, ileum, and colon, and the expression of UGT1A10 has been detected in esophagus, stomach, duodenum, jejunum, ileum, and colon (Gregory et al. 2004). UGT1A8 and UGT1A10 can

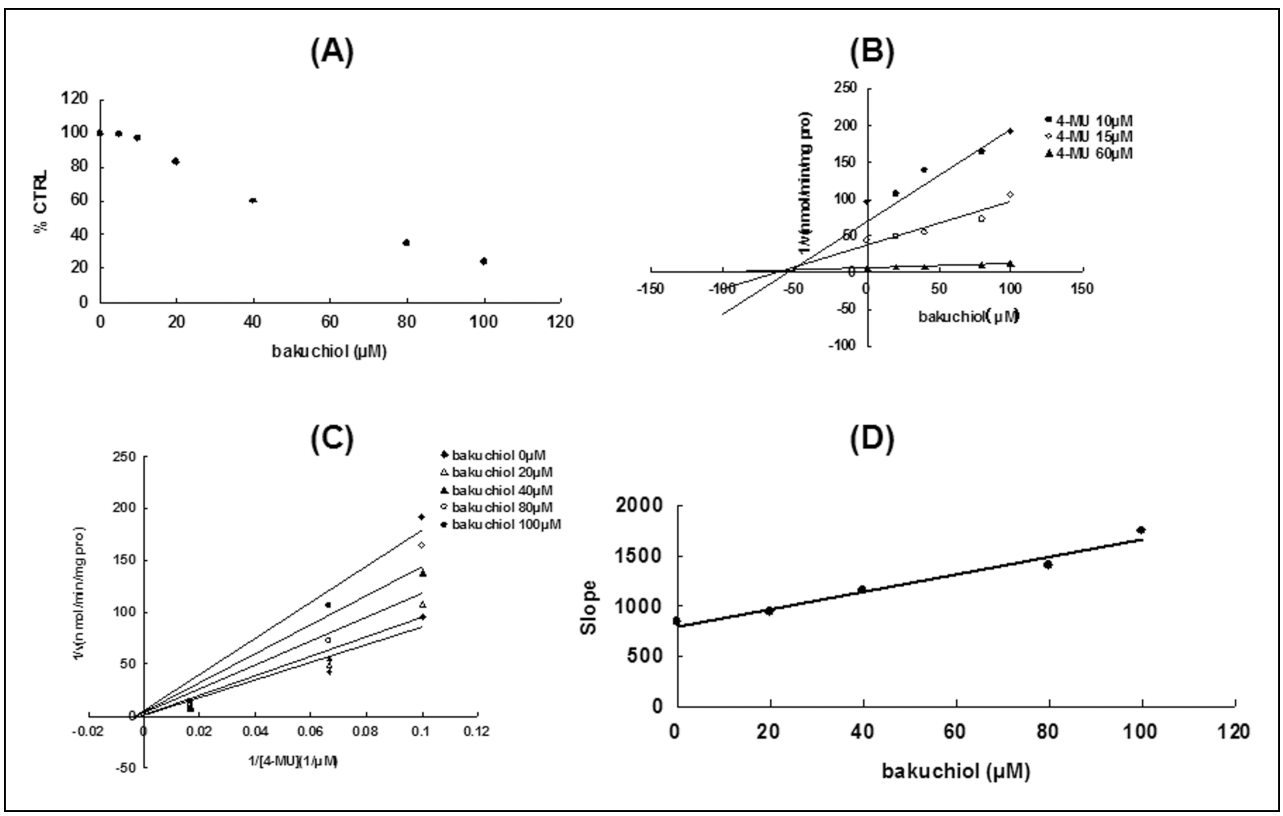

Fig. 5: Inhibition kinetic analysis of bakuchiol towards UGT1A10-catalyzed 4-MU glucuronidation. (A) Bakuchiol showed dose-dependent inhibition towards UGT1A10; (B) Dixon plot of bakuchiol inhibition towards UGT1A10-catalyzed 4-MU glucuronidation; (C) Lineweaver-Burk plot of bakuchiol inhibition towards UGT1A10-catalyzed 4-MU glucuronidation; (D) Second plot using slope (obtained from Lineweaver-Burk plot) vs. the concentration of bakuchiol.

glucuronidate mycophenolic acid (Mackenzie 2000) and environmental pollutants such as benzo(a)pyrenes and metabolites, as well as N-hydroxy-PhIP (Mojarrabi and Mackenzie 1998; Nowell et al. 1999). Therefore, the inhibition effect of bakuchiol towards UGT1A6, 1A8 and 1A10 will not only affect the metabolic behaviour of some clinical drugs, but also induce some diseases, including cancers.

The *in vivo* maximum exposure of bakuchiol was reported to be 4090.6 ng/ml (16 μM) after an intravenous administration of bakuchiol (15 mg/kg) (Zhuang et al. 2013), which 3.0-fold and 8.9-fold as the K_i values of UGT1A6 and UGT1A8, indicating the high possibility of inhibition of UGT1A6 and UGT1A8-catalyzed metabolism of drugs or endogenous substrates. Due to the lower concentration *in vivo* than the K_i value of UGT1A10, a relatively low possibility occured for the *in vivo* inhibition of bakuchiol towards UGT1A10-catalyzed metabolism.

The results of the present study can also indicate possible herb-drug interactions between bakuchiol-containing herbs and drugs mainly undergoing UGT1A6, 1A8, and 1A10-catalyzed glucuronidation. However, many complex herbal factors might affect this process, including the various factors (e.g., soil, temperature, altitude, etc.) influencing the quantities of bakuchiol in herbs.

The inhibition of bakuchiol towards UGT1A isoforms was investigated in the present study, and a strong inhibition of bakuchiol towards UGT1A6, 1A8, and 1A10 was demonstrated. The inhibition kinetic type was determined, and the inhibition parameters (Ki) were calculated. In combination with *in vivo* concentration of bakuchiol, the *in vivo* inhibition magnitude was also predicted.

4. Experimental

4.1. Chemicals and reagents

Bakuchiol with a purity≥ 98% was obtained from Weikeqi Biotechnology Co.Ltd (Sichuan, 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 UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9 and 1A10 expressed in baculovirus 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. Screening of the inhibition potential of bakuchiol towards UGT1A isoforms

4-MU, the nonspecific probe substrate for UGT isoforms except UGT1A4, was employed to investigate the inhibition of UGT1A1, 1A3, 1A6, 1A7,1A8, 1A9 and 1A10 by bakuchiol. The mixture (200 μl total volume) contained recombinant UGTs, 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 bakuchiol. Bakuchiol was dissolved in methanol and the final concentration of methanol was 0.5% (v/v). After 5 min pre-incubation at 37 °C, the UDPGA was added to the mixture to initiate the reaction. The concentration of recombinant UGTs, 4-MU, incubation time, and analytical conditions were performed as previously described (Fang et al. 2013; He et al. 2013). Due to the lack of the catalytic activity towards the glucuronidation of 4-MU, the trifluoperazine (TFP) glucuronidation reaction was used to evaluate the inhibitory effect of thienorphine towards UGT1A4 as previously described (Uchaipichat et al. 2006; Dong et al. 2013).

4.3. Determination of inhibition kinetic type and parameters (K_i)

The reaction velocity was determined at different concentrations of probe substrates and inhibitors. Dixon and Lineweaver plots were adapted to determine the inhibition type, and the second plot of slopes from Lineweaver-Burk plot *vs*. bakuchiol concentrations was used to calculate K_i value.

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