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Herb–drug interaction prediction based on the high specific inhibition of andrographolide derivatives towards UDP-glucuronosyltransferase (UGT) 2B7

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Abstract

Herb–drug interaction strongly limits the clinical application of herbs and drugs, and the inhibition of herbal components towards important drug-metabolizing enzymes (DMEs) has been regarded as one of the most important reasons. The present study aims to investigate the inhibition potential of andrographolide derivatives towards one of the most important phase II DMEs UDP-glucuronosyltransferases (UGTs). Recombinant UGT isoforms (except UGT1A4)-catalyzed 4-methylumbelliferone (4-MU) glucuronidation reaction and UGT1A4-catalyzed trifluoperazine

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Conflict of interest statement

The authors have declared that there are no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.taap.2014.02.021>.

(TFP) glucuronidation were employed to firstly screen the andrographolide derivatives' inhibition potential. High specific inhibition of andrographolide derivatives towards UGT2B7 was observed. The inhibition type and parameters (K_i) were determined for the compounds exhibiting strong inhibition capability towards UGT2B7, and human liver microsome (HLMs)-catalyzed zidovudine (AZT) glucuronidation probe reaction was used to furtherly confirm the inhibition behavior. In combination of inhibition parameters (K_i) and *in vivo* concentration of andrographolide and dehydroandrographolide, the potential *in vivo* inhibition magnitude was predicted. Additionally, both the *in vitro* inhibition data and computational modeling results provide important information for the modification of andrographolide derivatives as selective inhibitors of UGT2B7. Taken together, data obtained from the present study indicated the potential herb–drug interaction between *Andrographis paniculata* and the drugs mainly undergoing UGT2B7-catalyzed metabolic elimination, and the andrographolide derivatives as potential candidates for the selective inhibitors of UGT2B7.

Keywords

Andrographolide derivatives; Herb–drug interaction (HDI); UDP-glucuronosyltransferases (UGTs); Adverse effects

Introduction

With the extensive utilization of herbs in the modern world, herb–drug interaction (HDI) has become a serious problem challenging the clinical application of drugs and herbs (Fang et al., 2011; Hu et al., 2012). *Andrographis paniculata* (*A. paniculata*), known as “kalmegh” or “chuanxinlian”, has been demonstrated to exert a variety of biochemical and pharmacological activities, including the activities towards common cold, diarrhea, and infectious disease-induced fever (Lin et al., 2009; Saxena et al., 2010). The clinical safety of *A. paniculata* has been frequently speculated, and many evidences have indicated the possibility of *A. paniculata*'s influence towards the pharmacokinetic behavior of drugs. For example, *in vivo* administration of *A. paniculata* extract and its major medicinally active component andrographolide significantly decreased the activity of cytochrome P450 (CYP) 2C11 (Pekthong et al., 2009). The experiment performed by Pan et al. demonstrated the *in vitro* modulatory effects of *A. paniculata* towards CYP2C19 (Pan et al., 2011). Additionally, *A. paniculata* extract and andrographolide showed strong influence towards the pharmacokinetic behavior of theophylline which has been regarded to be a typical substrate of CYP1A2 (Chien et al., 2010). Diterpenoid ligands isolated from *A. paniculata* can induce the expression of CYP1A1 through aryl hydrocarbon receptor (Chatuphonprasert et al., 2011). All the above data indicated the risk of HDI related with the utilization of *A. paniculata*.

UDP-glucuronosyltransferase (UGT)-catalyzed glucuronidation reaction has been considered to be one of the most important elimination pathways for clinical drugs (King et al., 2000). More than 35% phase II drug metabolism reaction was catalyzed by UGT isoforms. Besides the important role in drug metabolism, UGTs also play a key role in metabolism of endogenous substances such as bilirubin, bile acids and estrogen (Zucker et

al., 2001). Many severe drug–drug interaction (DDI), herb–drug interaction (HDI), and toxicity mechanisms can be explained using the inhibition of UGTs. For example, the induced unconjugated bilirubin in serum after administration of HIV therapeutic drug indinavir can be explained with the inhibition of UGT1A1-mediated bilirubin glucuronidation (Zucker et al., 2001). Sorafenib-induced elevation of serum bilirubin can also be explained through inhibition of UGT1A1 activity (Meza-Junco et al., 2009). Many herbal components have been indicated to exhibit inhibition potential towards UGT isoforms (Shi et al., 2012; Xing and Che, 2012). Additionally, elevated concentrations of some endogenous substances can also affect the metabolic activity of UGT isoforms, which might partially contribute the pathogenesis of some diseases (Fang et al., 2013a).

The present study aims to investigate the inhibition of UGTs by the ingredients isolated from *A. paniculata*, including andrographolide, neoandrographolide, dehydroandrographolide, deoxyandrographolide, 3-oxo-dehydroandrographolide, 9 β -hydroxy dehydroandrographolide, 3-oxo-deoxyandrographolide, 9 β -hydroxy deoxyandrographolide, 3-oxo-9 β -hydroxy deoxyandrographolide, and 3,17,19-trihydroxy-7,11,13-ent-labdatrien-15,16-olide. The influence of structures towards UGT inhibition activity was discussed, and the prediction of *in vivo* herb–drug interaction was also performed. The present study indicated the possible herb–drug interaction prediction based on the high specific inhibition of andrographolide derivatives towards UDP-glucuronosyltransferase (UGT) 2B7.

Materials and methods

Chemicals.

4-Methylumbelliferone (4-MU), 4-methylumbelliferone- β -D-glucuronide (4-MUG), Tris–HCl, 7-hydroxycoumarin, 3'-azido-3'-deoxythymidine (AZT) and uridine-5'-diphosphoglucuronic acid (UDPGA) (trisodium salt) were obtained from Sigma-Aldrich (St. Louis, MO). Andrographolide, neoandrographolide, dehydroandrographolide, deoxyandrographolide, 3-oxo-dehydroandrographolide, 9 β -hydroxy dehydroandrographolide, 3-oxo-deoxyandrographolide, 9 β -hydroxy deoxyandrographolide, 3-oxo-9 β -hydroxy deoxyandrographolide, and 3,17,19-trihydroxy-7,11,13-ent-labdatrien-15,16-olide were isolated from *A. paniculata* in our lab, and the purity was demonstrated to be above 95%. 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). Pooled human liver microsomes (HLMs, 20 mg/mL) were purchased from a commercial source (BD Gentest, Woburn, MA). All other reagents were of HPLC grade or of the highest grade commercially available.

Initial screening of inhibition potential of andrographolide derivatives towards a variety of UGT isoforms.

Evaluation of inhibition capability of andrographolide derivatives towards various UGT isoforms was performed as previously described (Fang et al., 2013b). The inhibition potential of these compounds towards all the tested UGT isoforms except UGT1A4 was

investigated using recombinant UGT isoform-catalyzed 4-MU glucuronidation reaction. In brief, the incubation system (volume = 200 μ L) contained recombinant UGT isoforms, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl (pH = 7.4), and 4-MU in the absence and presence of andrographolide derivatives at various concentrations. The used incubation time and protein concentration were determined to ensure the reaction velocity within the linear range, and listed in Supplemental Table 1. The used 4-MU concentrations were equal or close to their K_m or S_{50} values. After 5-min preincubation at 37 $^{\circ}C$, the reaction was initiated through adding UDPGA. 100 μ L of ice-cold acetonitrile with 100 μ M of 7-hydroxycoumarin as internal standard was used to terminate the reaction. After centrifugation at 20,000 $\times g$ for 10 min, the aliquots (20 μ L) of the supernatants were transferred to an auto-injector vial for HPLC analysis. The HPLC system (Shimadzu, Kyoto, Japan) contained a SCL-10A system controller, two LC-10AT 140 pumps, a SIL-10A auto injector, and a SPD-10AVP UV detector. Chromatographic separation was carried out using a C18 column (4.6 $\times$ 200 mm, 5 μ m, 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.

Previous literatures have showed low catalytic activity of UGT1A4 towards the glucuronidation of 4-MU (Uchaipichat et al., 2004). Therefore, recombinant UGT1A4-catalyzed trifluoperazine (TFP) glucuronidation was used to phenotype the activity of UGT1A4 as previously described (Uchaipichat et al., 2006). The corresponding incubation time and the used concentrations of TFP were described in the previous literatures (Uchaipichat et al., 2006).

Computational modeling.

Given that the 3D structure of UGT2B7 with substrate binding domain has yet been crystallized, a homology model was constructed with FUGUE and ORCHESTRAR package within SYBYL 7.3 package (Smith et al., 2007). Protein sequence of UGT2B7 was obtained from NCBI database coded as NP_001065 which was further submitted to FUGUE for the identification of its homologues. The crystal structures of macrolide glycosyltransferases (PDB code: 2IYF) and UDP-glucosyltransferase GtfB (PDB code: 1IIR) were selected as templates for the relatively higher identities. With the establishment of the backbone of the structurally conserved regions (SCR) based on template, the structurally variable regions were further constructed with Loop Search. After the sidechains of UGT2B7 model were added, the model was optimized with staged energy minimization.

The molecular docking study was performed using the Surflex-Dock of the SYBYL procedure to evaluate the interaction modes and binding affinities of andrographolide derivatives. Surflex-Dock uses an empirical scoring function and a patented search engine to dock ligands into a protein's binding site. The modeled UGT2B7 protein was used as receptor without any constraint during the calculations. The active pocket for inhibitor binding was generated beside UDP binding site in an automatic mode with the setup of float radius as zero. And the inhibitors were docked into the active site to give a sight into the interaction mode and each ligand was generated with 20 conformations.

Andrographolide derivatives' inhibition towards human liver microsome (HLM)-catalyzed zidovudine (AZT) glucuronidation.

For AZT glucuronidation, the typical incubation system (total volume = 200 μ L) contained 0.5 mg/mL HLMs, 5 mM UDPGA, 5 mM $MgCl_2$, 50 mM Tris-HCl (pH = 7.4), 50 μ g/mg protein alamethicin, and AZT (the concentration is corresponding to the K_m value). The incubation time was 30 min. After centrifugation at 20,000 $\times g$ for 10 min, aliquots of the supernatants were analyzed on UFLC system (Shimadzu, Kyoto, Japan) coupled with ABI 4000 MS instrument. Shim-pack XR-ODSII column (2.0 mm $\times$ 75 mm, 2.2 μ m) was used with flow rate 0.4 mL/min. The mobile phase consisted of acetonitrile (A) and H_2O containing 0.2% (v/v) formic acid (B). The following gradient condition was used: 0–3 min, 88% B; 3–5 min, 5% B; 5–8 min, 88% B. The MS conditions were as follows: CRU Gas, 20.0 psi; IonSpray Voltage (IS), –4500 V; TEM, 550 $^{\circ}C$; GS1, 55 psi; GS2, 55 psi. Negative mode was used, and the ions 266 and 442 ($[M-H]^-$) were used to monitor AZT and its glucuronide.

Inhibition kinetic evaluation and in vitro–in vivo extrapolation (IVIVE).

To determine the inhibition kinetic behavior (including inhibition type and parameters), the reaction velocity was determined at various concentrations of andrographolide derivatives and probe substrates. Dixon and Lineweaver–Burk plots were employed to determine the inhibition kinetic type, and the second plot with the slopes from Lineweaver–Burk plot towards the concentrations of andrographolide derivatives was utilized to determine the inhibition kinetic parameters (K_i). *In vitro*–*in vivo* extrapolation (IVIVE) was carried out using the following equation.

$$AUC_i/AUC = 1 + [I]_{in\ vivo}/K_i$$

The terms are defined as follows: AUC_i/AUC is the predicted ratio of *in vivo* exposure of xenobiotics or endogenous substances with and without co-administration of andrographolide derivatives. K_i is the reversible inhibition constant, and $[I]_{in\ vivo}$ is the *in vivo* concentration of andrographolide derivatives.

Results

Andrographolide derivatives exert structure-dependent specific inhibition towards UGT2B7

100 μ M of andrographolide derivatives was used to perform the initial inhibition screening towards various UGT isoforms, and the preliminary results were given in Supplemental Table 2. All the andrographolide derivatives showed little influence towards UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, and 2B4, with the residual activity more than 20%. Andrographolide derivatives showed different inhibition capabilities towards UGT2B7 in the structure-dependent behavior. 100 μ M of andrographolide, deoxyandrographolide, dehydroandrographolide, neoandrographolide, 3-oxo-dehydroandrographolide, 9 β -hydroxy dehydroandrographolide, 3-oxo-deoxyandrographolide, 9 β -hydroxy deoxyandrographolide, 3-oxo-9 β -hydroxy deoxyandrographolide, and 3,17,19-trihydroxy-7,11,13-ent-

labdatrien-15,16-olide inhibited the activity of 2B7 by 94%, 95%, 80%, 42%, 90%, 73%, 94%, 56%, 56%, and 39%, respectively. The deoxygenation conversion of andrographolide into deoxyandrographolide slightly affected the inhibition capability towards UGT2B7. However, the dehydrated product of andrographolide dehydroandrographolide showed much lower inhibition activity towards UGT2B7. Through comparing the structures of deoxyandrographolide, 3-oxo-deoxyandrographolide, and 9 β -hydroxy deoxyandrographolide, the introduction of 3-oxo group slightly influence the inhibition ability, and 9 β -hydroxy group significantly weakened the inhibition potential towards UGT2B7 (Fig. 1). The similar situation was also detected for dehydroandrographolide, 3-oxo-dehydroandrographolide, and 9 β -hydroxy dehydroandrographolide.

Molecular docking was carried out using a homology model of the protein to explore the interaction between the andrographolide derivatives and UGT2B7. Because the present crystallized structure is a UDP binding domain, it is a great task to recognize the ligand binding domain. In an automatic mode, the active pocket was generated for ligand (either substrate or inhibitor) binding in adjacent to the UDP binding site. The active site is mainly composed by Gln399, Phe396, Gly376, Asp398, Ser173, His35, Ser34, Ala31, Trp29, Asp151, Ala152 and Val313, just as shown in Supplemental Fig. 1, where the UDP is expressed as a stick-ball style. The inhibitors andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, and 9 β -hydroxy deoxyandrographolide were then docked into the active pocket respectively. Assuming these analogs bind with the enzyme in similar conformation, we select reasonable bioactive mode from the top five conformations ranked by Hammet score. For andrographolide and 9 β -hydroxy deoxyandrographolide, ranks 3 and 4 were selected respectively, and deoxyandrographolide, dehydroandrographolide, and 3-oxo-deoxyandrographolide all select the top 1 conformations. These inhibitors can well match with the cavity and amino acids like Ser173, Asp398, and Phe396 tend to form hydrogen bonds with the hydroxyls or carbonyls of the inhibitor, as shown in Fig. 2A, where andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, and 9 β -hydroxy deoxyandrographolide were colored by atom type, orange, violet, purple and yellow respectively. These inhibitors tend to adopt conformations with the fused ring inside the pocket to interact with Asp398, Ser173, and Ser34 while the single five member ring at the entrance to interact with Phe 396. To view the interaction mode more clearly, these inhibitors were classified into two groups individually, *e.g.* andrographolide, deoxyandrographolide, and dehydroandrographolide, and deoxyandrographolide, 3-oxo-deoxyandrographolide, and 9 β -hydroxy deoxyandrographolide, which can be viewed in Figs. 2B and C. We have also employed the Chemscore to evaluate the binding affinities. For the group of andrographolide, deoxyandrographolide, and dehydroandrographolide, their Chemscore values were -19.3782, -18.5522 and -23.2971, which is in accordance the experimental result as the inhibition activity is andrographolide > deoxyandrographolide > dehydroandrographolide. While for the group deoxyandrographolide, 3-oxo-deoxyandrographolide, and 9 β -hydroxy deoxyandrographolide, values of Chemscore were -18.5522, -16.2158 and -16.8693, respectively. When comparing with the experimental potency, we found that the binding affinities of deoxyandrographolide and 3-oxo-deoxyandrographolide were equivalent accordant with the *in vitro* assays, whereas 9 β -

hydroxy deoxyandrographolide exhibits similar Chemscore value but a much lower inhibition activity, we suppose that the disagreement between molecular modeling and *in vitro* results for 9 β -hydroxy deoxyandrographolide may be due to a different interaction mechanism.

The inhibition kinetics of andrographolide derivatives towards UGT2B7

The inhibition kinetic evaluation was performed for the inhibition of UGT2B7 by andrographolide (Fig. 3), deoxyandrographolide (Fig. 4), dehydroandrographolide (Fig. 5), 3-oxo-deoxyandrographolide (Fig. 6), and 3-oxo-dehydroandrographolide (Fig. 7). The dose-dependent inhibition of andrographolide (Fig. 3A), deoxyandrographolide (Fig. 4A), dehydroandrographolide (Fig. 5A), 3-oxo-deoxyandrographolide (Fig. 6A), and 3-oxo-dehydroandrographolide (Fig. 7A) towards UGT2B7 was observed. Furthermore, both Dixon plot and Lineweaver–Burk plot were employed to determine the inhibition kinetic type. The location of intersection points in Dixon plot and Lineweaver–Burk plot can be used to determine the inhibition kinetic type. When the intersection point was located in the second quadrant and vertical axis in Dixon plot and Lineweaver–Burk plot respectively, the inhibition kinetic type belongs to be competitive inhibition. When the intersection point was located in the horizontal axis in both Dixon plot and Lineweaver–Burk plot, the inhibition type was best fit to noncompetitive inhibition type. Based on the corresponding interaction point information, the activity of UGT2B7 was competitively inhibited by deoxyandrographolide (Figs. 4B & C), dehydroandrographolide (Figs. 5B & C), 3-oxo-deoxyandrographolide (Figs. 6B & C), and 3-oxo-dehydroandrographolide (Figs. 7B & C), and noncompetitively inhibited by andrographolide (Figs. 3B & C). The inhibition kinetic parameters (K_i) were 5.7 μ M (Fig. 3D), 1.5 μ M (Fig. 4D), 6.9 μ M (Fig. 5D), 4.2 μ M (Fig. 6D), and 13.7 μ M (Fig. 7D) for the inhibition of andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, and 3-oxo-dehydroandrographolide towards the activity of UGT2B7. Furthermore, the inhibition behavior was demonstrated using human liver microsome (HLM)-catalyzed AZT glucuronidation reaction as probe reaction. The glucuronidation activity of AZT in HLMs was competitively inhibited by deoxyandrographolide (Supplemental Fig. 3), 3-oxo-deoxyandrographolide (Supplemental Fig. 5), and 3-oxo-dehydroandrographolide (Supplemental Fig. 6). Andrographolide (Supplemental Fig. 2) and dehydroandrographolide (Supplemental Fig. 4) noncompetitively inhibited the glucuronidation of AZT. The inhibition kinetic parameters (K_i) were calculated to be 6.1 μ M, 7.1 μ M, 90.4 μ M, 25.0 μ M, and 22.0 μ M for the inhibition of andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, and 3-oxo-dehydroandrographolide towards HLMs-catalyzed AZT glucuronidation.

In vitro–in vivo extrapolation (IVIVE) of UGT2B7 inhibition by andrographolide derivatives

The *in vitro*–*in vivo* extrapolation (IVIVE) was performed for the inhibition of UGT2B7 by andrographolide and dehydroandrographolide due to the availability of their *in vivo* concentrations. The maximum plasma concentration of andrographolide and dehydroandrographolide was reported to be 2.28 μ g/mL (6.5 μ M) and 1.33 μ g/mL (4.0 μ M) after oral administration of *A. paniculata* extract at a dose of 2 mL/kg in rabbits (Chen et al., 2007). In combination of *in vitro* inhibition kinetic parameters (K_i), the AUC was predicted to increase by 110% and 58% due to the inhibition of andrographolide and

dehydroandrographolide towards UGT2B7 activity. When using the inhibition kinetic parameters (K_i) obtained using HLM-catalyzed AZT glucuronidation probe reactions, the values of AZT AUC were predicted to increase by 107% and 4.4%, respectively.

Discussion

The excellent safety of herbs results in the prevalence of herbal utilization. However, more and more cases on herbal adverse effects remind us unavoidable adverse effects in clinical application of many herbs, which might result in severe results. For example, FDA banned the utilization of ephedra-containing herbal products due to its toxicity (Woolf et al., 2005). Besides the direct herbal toxicity towards target organs (*e.g.* cardiac, pulmonary, renal, hematologic, hepatic, endocrine and neurological toxicity), herb–drug interaction is another severe adverse effect influencing the application of herbs.

Evaluation of the modulatory effects of herbal major ingredients towards drug-metabolizing enzymes (DMEs) will be helpful for indicating potential herb–drug interaction (HDI). The present study investigated the inhibitory potential of *A. paniculata* main ingredients andrographolide derivatives towards UGT isoforms to predict the possible herb–drug interaction between *A. paniculata* and clinical drugs. High specific inhibition of these compounds towards UGT2B7 was observed, providing the risk indication for co-administration utilization of *A. paniculata* and the drugs mainly undergoing UGT2B7-catalyzed metabolic elimination, including mycophenolic acid (Frymoyer et al., 2013) and 3'-azido-3'-deoxythymidine (zidovudine, AZT) (Uchaipichat et al., 2008). The present study also adopted two incubation systems to confirm the inhibition of andrographolide derivatives towards UGT2B7. Andrographolide, deoxyandrographolide, 3-oxo-deoxyandrographolide, and 3-oxo-dehydroandrographolide exhibited the same inhibition type towards recombinant UGT2B7-catalyzed 4-MU glucuronidation, and HLM-catalyzed AZT glucuronidation. For dehydroandrographolide, the competitive inhibition and noncompetitive inhibition types were observed for the recombinant UGT2B7-catalyzed 4-MU glucuronidation and HLMs-catalyzed AZT glucuronidation, respectively. This enzyme sources and substrate-dependent inhibition behaviors have been observed in our previous studies (Dong et al., 2012). Whether these *in vitro* inhibition behaviors will finally result in *in vivo* herb–drug interaction can be not only affected by *in vitro* inhibition kinetic parameter (K_i) but also influenced by their *in vivo* concentrations. Therefore, we estimated the *in vivo* inhibition potential of andrographolide and dehydroandrographolide due to the availability of their *in vivo* concentrations. The possible *in vivo* herb–drug interaction was indicated to exist given the increased fold of AUC (110% and 58% for the inhibitions of andrographolide and dehydroandrographolide towards UGT2B7-catalyzed 4-MU glucuronidation, and 107% and 4.4% for the inhibitions of andrographolide and dehydroandrographolide towards HLM-catalyzed AZT glucuronidation). The influence of *A. paniculata* exposure in human remains unclear due to the limited reports for the *in vivo* concentration of the ingredients from *A. paniculata* after the exposure of *A. paniculata*.

When the *in vivo* pharmacokinetics of the *A. paniculata* ingredients will become more clear in the future, the *in vivo* herb–drug interaction between *A. paniculata* and clinical drugs will be better predicted using recent data.

Searching the high specific inhibitors of UGT2B7 is an important task to phenotype the UGT isoforms involved in the metabolism of drugs. The experiment performed by Bichlmaier et al. developed epimeric tricyclic sesquiterpenoid alcohols as the potent inhibitors for UGT2B7, including globulol, epiglobulol, cedrol, epicedrol, longifolol, and isolongifolol (Bichlmaier et al., 2007a). A set of 48 derivatives of the tricyclic sesquiterpenol alcohol isolongifolol was synthesized to obtain one highly selective inhibitor for UGT2B7 tricyclic secondary alcohol 26b (true selectivity > 1000) (Bichlmaier et al., 2007b). Recent study indicated that andrographolide derivatives were a class of good lead compounds that can be developed as the selective inhibitors of UGT2B7. Additionally, some structural modification information has been indicated. For example, the dehydration reaction between 13 and 14 sites in andrographolide will significantly weaken the inhibitory capability, explained by weakened ability to bind to the activity cavity as indicated by computational modeling. Additionally, the introduction of hydroxyl group in 9 sites also significantly weakened the inhibitory potential towards UGT2B7 activity.

In conclusion, the complete inhibition profile of andrographolide derivatives towards UGT isoforms was given, and high specific inhibition of andrographolide derivatives towards UGT2B7 was demonstrated in the present study, indicating the possible herb–drug interaction between *A. paniculata* and clinical drugs mainly undergoing UGT2B7-catalyzed metabolic elimination. Additionally, some structures–UGT2B7 inhibition relationship was observed in the present study, providing helpful information to synthesize the strong selective inhibitors of UGT2B7.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CYP	cytochrome P450
DDI	drug–drug interaction
HDI	herb– drug interaction
HLMs	human liver microsomes
4-MU	4-methylumbelliferone
4-MUG	4-methylumbelliferone-β-D-glucuronide
TFP	trifluoperazine
UGTs	UDP-glucuronosyltransferases

UDPGA uridine-5'-diphosphoglucuronic acid

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Fig. 1.

The structures of andrographolide (A), deoxyandrographolide (B), dehydroandrographolide (C), neoandrographolide (D), 3-oxo-dehydroandrographolide (E), 9β-hydroxy dehydroandrographolide (F), 3-oxo-deoxyandrographolide (G), 9β-hydroxy deoxyandrographolide (H), 3-oxo-9β-hydroxy deoxyandrographolide (I), and 3,17,19-trihydroxy-7,11,13-ent-labdatrien-15,16-olide (J).

Fig. 2.

Computational modeling of andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, 9β-hydroxy deoxyandrographolide towards UGT2B7 active cavity. (A) The interaction binding mode of andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, 9β-hydroxy deoxyandrographolide with UGT2B7, where andrographolide, deoxyandrographolide, dehydroandrographolide, 3-oxo-deoxyandrographolide, 9β-hydroxy deoxyandrographolide were colored by atom type, orange, violet, purple and yellow. (B) The interaction binding mode of andrographolide, deoxyandrographolide, and dehydroandrographolide with UGT2B7, where andrographolide, deoxyandrographolide, and dehydroandrographolide were colored by atom type, orange, violet. (C) The interaction binding mode of deoxyandrographolide, 3-oxo-deoxyandrographolide, and 9β-hydroxy deoxyandrographolide with UGT2B7, where deoxyandrographolide, 3-oxo-deoxyandrographolide, and 9β-hydroxy deoxyandrographolide were colored by orange, purple and yellow.

Fig. 3.

The inhibition behavior of andrographolide towards the activity of UGT2B7. (A) Dose-dependent inhibition behavior of andrographolide towards the activity of UGT2B7; (B) Dixon plot of andrographolide's inhibition towards the activity of UGT2B7; (C) Lineweaver-Burk plot of andrographolide's inhibition towards the activity of UGT2B7; (D) determination of inhibition kinetic parameter (K_i) using the slopes from Lineweaver-Burk plot towards the concentration of andrographolide. The data points represent the mean value of duplicate experiments.

Fig. 4.

The inhibition behavior of deoxyandrographolide towards the activity of UGT2B7. (A) Dose-dependent inhibition behavior of deoxyandrographolide towards the activity of UGT2B7; (B) Dixon plot of deoxyandrographolide's inhibition towards the activity of UGT2B7; (C) Lineweaver-Burk plot of deoxyandrographolide's inhibition towards the activity of UGT2B7; (D) determination of inhibition kinetic parameter (K_i) using the slopes from Lineweaver-Burk plot towards the concentration of deoxyandrographolide. The data points represent the mean value of duplicate experiments.

Fig. 5.

The inhibition behavior of dehydroandrographolide towards the activity of UGT2B7. (A) Dose-dependent inhibition behavior of dehydroandrographolide towards the activity of UGT2B7; (B) Dixon plot of dehydroandrographolide's inhibition towards the activity of UGT2B7; (C) Lineweaver-Burk plot of dehydroandrographolide's inhibition towards the activity of UGT2B7; (D) Determination of inhibition kinetic parameter (K_i) using the slopes from Lineweaver-Burk plot towards the concentration of dehydroandrographolide. The data points represent the mean value of duplicate experiments.

Fig. 6.

The inhibition behavior of 3-oxo-deoxyandrographolide towards the activity of UGT2B7. (A) Dose-dependent inhibition behavior of 3-oxo-deoxyandrographolide towards the activity of UGT2B7; (B) Dixon plot of 3-oxo-deoxyandrographolide's inhibition towards the activity of UGT2B7; (C) Lineweaver-Burk plot of 3-oxo-deoxyandrographolide's inhibition towards the activity of UGT2B7; (D) Determination of inhibition kinetic parameter (K_i) using the slopes from Lineweaver-Burk plot towards the concentration of 3-oxo-deoxyandrographolide. The data points represent the mean value of duplicate experiments.

Fig. 7.

The inhibition behavior of 3-oxo-dehydroandrographolide towards the activity of UGT2B7.

(A) Dose-dependent inhibition behavior of 3-oxo-dehydroandrographolide towards the activity of UGT2B7; (B) Dixon plot of 3-oxo-dehydroandrographolide's inhibition towards the activity of UGT2B7; (C) Lineweaver-Burk plot of 3-oxo-dehydroandrographolide's inhibition towards the activity of UGT2B7; (D) determination of inhibition kinetic parameter (K_i) using the slopes from Lineweaver-Burk plot towards the concentration of 3-oxo-dehydroandrographolide. The data points represent the mean value of duplicate experiments.