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Inhibition of human CYP3A4 and CYP3A5 enzymes by gomisin C and gomisin G, two lignan analogs derived from *Schisandra chinensis*

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Abstract

Gomisin C (GC) and gomisin G (GG) are two lignan analogs isolated from the Traditional Chinese Medicine *Schisandra chinensis* which possesses multiple pharmacological activities. However, the potential herb-drug interactions (HDI) between these lignans and other drugs through inhibiting human cytochrome P450 3A4 (CYP3A4) and CYP3A5 remains unclear. In the present study, the inhibitory action of GC and GG on CYP3A4 and CYP3A5 were investigated. The results demonstrated that both GC and GG strongly inhibited CYP3A-mediated midazolam 1'-hydroxylation, nifedipine oxidation and testosterone 6 β -hydroxylation. Notably, the inhibitory intensity of GC towards CYP3A4 was stronger than CYP3A5 when using midazolam and nifedipine as substrates. While inhibition of GC towards CYP3A5 was weaker than CYP3A4 when using testosterone as substrate. In contrast, GG showed a stronger inhibitory activity on CYP3A5 than CYP3A4 without substrate-dependent behavior. In addition, docking simulations indicated that the π - π interaction between CYP3A4 and GC, and hydrogen-bond interaction between CYP3A5 and GG might result in their different inhibitory actions. Furthermore, the AUC of drugs metabolized by CYP3A was estimated to increase by 8%–321% and 2%–3190% in the

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Conflict of interest

The authors have declared that there is no conflict of interest.

presence of GC and GG, respectively. These findings strongly suggested that GC and GG showed high HDI potentials, and the position of methylenedioxy group determined their different inhibitory effect towards CYP3A4 and CYP3A5, which are of significance for the application of *Schisandra chinensis*-containing herbs.

Keywords

Gomisin C; Gomisin G; CYP3A4; CYP3A5; Inhibition; Herb-drug interactions

1. Introduction

The concomitant use of herbal medicines with synthetic drugs has been extensively investigated in order to achieve the goal of reducing toxicity and enhancing efficacy. Nevertheless, the combined use of herbs and drugs may produce herb-drug interactions (HDI) resulting in serious adverse events [1]. Cytochrome P450 (CYP) 3A is among the most important human CYPs due to its high relative abundance in the liver and broad substrate specificity [2]. CYP3A4 is generally thought to be the predominant form expressed in the human liver and intestine, whereas CYP3A5, which is polymorphically expressed, might contribute as much as 50% of hepatic CYP3A metabolism in a third of Caucasians and in one half of African-Americans [3]. Since CYP3A5 and CYP3A4 are 83% homologous in amino acid sequence, it is believed that the substrate specificity of CYP3A5 is similar to that of CYP3A4. However, certain differences have been demonstrated both in catalytic and inhibitory activities [4]. The differences in contribution of CYP3A4 and CYP3A5 to drug metabolism have been documented [4]. For example, the catalytic activity of CYP3A4 is generally higher than that of CYP3A5 for most of drugs. Nevertheless, the exception is that CYP3A5 contributes more than CYP3A4 to the metabolism of several commonly-used drugs with narrow therapeutic indexes. For example, the intrinsic clearance of the anticancer drug vincristine and the immunosuppressant tacrolimus metabolized by CYP3A5 are higher than those of CYP3A4 [5,6]. As a result, the difference in the inhibitory actions to the CYP3A4 and CYP3A5 will lead to different drug interaction potentials, and this risk could be further amplified in vivo. However, there are few reports that compared in detail the herbal inhibition potentials towards metabolic reactions mediated by CYP3A4 and CYP3A5. Therefore, the illumination of comparable inhibition by herbal ingredients towards CYP3A4 and CYP3A5 will help to understand and predict adverse HDIs.

The Traditional Chinese Medicine *Schisandra chinensis* has been widely used due to its multiple pharmacological activities, which results in a high frequency of the combination with other drugs. Gomisin C (GC) and gomisin G (GG) are two lignan analogs isolated from *Schisandra chinensis*, in which the content of GC and GG could reach up to 0.79 mg/g and 0.69 mg/g respectively [7]. Notably, GG exhibited the most anti-HIV pharmacological activity with an EC₅₀ value of 0.006 µg/ml among all the natural dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* [8]. In contrast, GC exhibited observable antitussive activity with an IC₅₀ value of 21.5 µg/ml via partly by the suppression of NADPH oxidase [9]. Interestingly, GC and GG have the same molecular weight and their chemical structures are very similar. They possess a bulky benzoyl at the same position,

while the methylenedioxy group locates at different positions (Fig. 1). More importantly, as previously reported, the dibenzocyclooctadiene lignans with six methoxyl groups on two benzene rings, are the most potent CYP3A substrates [10,11]. Whereas, the lignans are prone to be the potent CYP3A inhibitors when the methoxyl groups on phenyl are replaced with methylenedioxy ring [12], the potential HDI risks between these lignans and other drugs through inhibiting CYP3A4 and CYP3A5, remains unclear. Therefore, in the present study, the differential inhibitory effects of GC and GG on the human CYP3A enzymes and its potential HDI risk in the clinic were investigated.

2. Materials and methods

2.1. Chemicals

Gomisin C (GC) and Gomisin G (GG) (purity N 98%) were purchased from Sichuan WeiKeqi Biological Technology Co., Ltd. (Chengdu, China). testosterone (TST), 6 β -hydroxytestosterone (6 β -HTS), nifedipine (NIF), oxidized nifedipine (OX-NIF), midazolam (MDZ), 1'-hydroxymidazolam (1'-OH-MDZ) glucose-6-phosphate dehydrogenase, NADP⁺, and D-glucose-6-phosphate were purchased from Sigma-Aldrich (St. Louis, Miss., USA). All other reagents were of the highest purity commercially available or HPLC grade. cDNA-expressed recombinant human cytochrome P450 (rhCYP) isoforms, including CYP3A4 and CYP3A5, derived from baculovirus-infected insect cells co-expressing NADPH-CYP reductase and cytochrome *b5* were purchased from Cypex (Dundee, UK), and stored at -80 °C until use.

2.2. CYP3A probe substrate reactions

Three well-characterized CYP3A probe substrates, TST, MDZ and NIF with distinct binding domains were used this study [13]. testosterone 6 β -hydroxylation, midazolam-1'-hydroxylation, and nifedipine oxidization were used as selective probe reactions for CYP3A as previously reported [14].

2.3. Inhibition of CYP3A activities by GC and GG

All incubations were performed in duplicate, and the mean values were used for analysis. The basic incubation mixture consisted of 100 mM potassium phosphate buffer (pH 7.4), NADPH-generating system (10 mM NADP⁺, 10 mM glucose-6-phosphate, 1 unit/ml of glucose-6-phosphate dehydrogenase, and 4 mM MgCl₂), appropriate concentration of rhCYP3A4 or rhCYP3A5 enzyme, respective probe substrate and inhibitor in a final volume of 200 μ L. The substrates were used at concentrations approximately equal to their respective *K_m* values: 5 μ M midazolam, 20 μ M nifedipine, and 50 μ M testosterone. After a 5-min preincubation period at 37 °C, the reactions were initiated by addition of an NADP⁺ generating system and incubated for 15 min at 37 °C in a shaking metal bath. Preliminary experiments were conducted to make sure all experiments were performed within the linear range of incubation time and protein concentration. All substrates, GC and GG were dissolved in acetonitrile or methanol. The final concentration of organic solvent in the incubation media was 1% (v/v). The reaction was terminated by adding ice cold 200 μ L acetonitrile and the reactions kept on ice. The reaction mixture was centrifuged at 20,000g

for 20 min during -4°C ; An aliquot of supernatant was transferred to a 0.4 ml auto-injector vial for UFLC analysis.

2.4. Analytical instruments and methods

The UFLC system (Shimadzu, Kyoto, Japan) was equipped with a SCL-20A system controller, two LC-20AD pumps, a SIL-20AC auto sampler, a DGU-20AC vacuum degasser, a CTO-20AC column oven and a SPD-20AVP UV detector. A SHIMADZU VP-ODS ($5\text{ }\mu\text{m}$, $150\text{ mm} \times 2.1\text{ mm}$) analytical column was used to separate the probe substrates and their metabolites. As for MDZ, TST and NIF, the mobile phase consisted of methanol (A pump) and water (B pump) with the following gradient profile: 0–9 min, 50%–5% B; 9–12 min, 5% B; 12–16.5 min, balanced to 50% B. The flow rate was 0.4 ml/min and the column temperature was kept at 40°C . The respective metabolites of midazolam, testosterone and nifedipine were detected at the wavelengths of 254 nm, 245 nm and 237 nm, respectively. Data processing was performed using the LC-MS Solution version 3.41 software. MDZ, TST, NIF and their respective metabolites were quantified by the standard curve of authentic standards, which was linear from 0.1 to $30\text{ }\mu\text{M}$, with correlation coefficient of >0.999 . The quantitative method displayed good sensitivity.

2.5. Data analysis

For CYPs whose activities were inhibited by N50%, a second study was carried out to obtain the 50% inhibition concentration (IC_{50}). Inhibitory potencies (IC_{50} values) of GC and GG were determined using CYP assays in the presence and absence of GC and GG (final concentrations of 1–200 μM with an acetonitrile concentration 0.1% v/v) using pooled HLMs. midazolam 1'-hydroxylation, testosterone 6β -hydroxylation and nifedipine dehydrogenation activities were determined as probe activities for CYP3A4 and CYP3A5 using in vitro rhCYP incubation systems and UFLC.

2.6. Computational simulations

To illustrate the molecular mechanism of the selective catalysis of CYP3A5 for SE, docking simulation was employed and performed using the knowledge-based homology modeling package from Advanced Protein Modeling (APM) that was distributed within SYBYL (X-1.1). The homology model of CYP3A5 was constructed based on the template structure of CYP3A4 (PDB ID: 3TJS) as previously described [15]. With the established 3D-structure of CYP3A5 as well as the crystal structure of CYP3A4, the bioactive binding conformations of GC and GG were respectively generated using Surflex-Dock and were evaluated by an empirical function ChemScore, one of the most suitable scoring functions for P450s. The docking results were further visualized using PyMOL Molecular Graphics System, version 0.99 (DeLano Scientific LLC). A 2D molecular descriptor (Discovery Studio Visualizer software, version 3.5) was employed to show the interacting amino acid residue plot of ligands located within the active site of CYP3A4 or CYP3A5.

2.7. Calculation of AUC ratio

The magnitudes of inhibitory interactions were estimated as the ratio of the area under the plasma concentration-time curve (AUC) in the presence and absence of GC and GG. This

ratio was predicted by using the formula (Eq. (1)) for intravenous administration of a high hepatic clearance drug [16].

$$\text{AUC ratio} = \frac{1}{f_{\text{hep}} \cdot \left(\frac{1/E_h}{(1/E_h - 1) \cdot (1 + I/K_i) + 1} \right) + (1 - f_{\text{hep}})} \quad (1)$$

where f_{hep} is the percentage of hepatic clearance mediated by an enzyme in total clearance; E_h is the hepatic extraction ratio.

3. Results

3.1. Inhibition of recombinant human CYP3A4 and CYP3A5 activities by GC and GG

The inhibitory activities of GC and GG towards CYP3A4 and CYP3A5 were compared using rhCYP3A4 and rhCYP3A5 enzymes, respectively. Both GC and GG significantly inhibited CYP3A-mediated midazolam 1'-hydroxylation, nifedipine oxidation and testosterone 6 β -hydroxylation, with IC_{50} values of 0.01–2.5 μ M (Table 1). However, the inhibitory behaviors of GC and GG towards CYP3A4 and CYP3A5 were comparable. The inhibitory intensity of GC towards CYP3A4 was slightly stronger than towards CYP3A5 when using midazolam and nifedipine as the probe substrates; Whereas the inhibition of GC towards CYP3A4 was weaker than CYP3A5 when using testosterone as the substrate, with less than a 2-fold shift of IC_{50} (Fig. 2). In contrast, GG showed a stronger inhibitory activity on CYP3A5 than CYP3A4 without substrate-dependent behavior, with 4.3- to 9.4-fold variation of IC_{50} (Fig. 3). These findings demonstrated that the inhibitory intensity of GC was almost equal to CYP3A4 and CYP3A5, while GG can more strongly inhibit the activity of CYP3A5 than CYP3A4.

3.2. Molecular docking to understand the selective inhibition of lignans

GC and GG were docked into the binding pocket of CYP3A4 and the corresponding homology model of CYP3A5, respectively. The interaction of GC and CYP3A4 and CYP3A5 was further analyzed by amino acid residues plotting. The optimal conformational matching was given based on each of the highest ChemScore value. The π – π interaction observably occurred between the heme of CYP3A4 and the benzoyl group of GC, while this interaction was not found between GC and CYP3A5 (Fig. 4). Furthermore, the docking simulation studies indicated that two hydrogen-bonds interactions were observed between Arg105 of CYP3A5 and the two oxygen atoms of methoxyl at position 12 and position 13 of GG, respectively (Fig. 5), whereas these interactions were not found between GG and CYP3A4. These findings might explain, at least in part, the different inhibitory actions of GC and GG towards CYP3A4 and CYP3A5.

3.3. Quantitative prediction of HDI risk (AUC ratio)

The magnitudes of the potential HDI risk of GC or GG with CYP3A4 and CYP3A5 were evaluated by estimating the ratio of AUC in the presence and absence of the inhibitor (AUC ratio). The AUC ratios were calculated by the hepatic extraction ratio (E_h) and percentage of

hepatic clearance mediated by CYP3A in total clearance (f_{hep}) for substrate drugs, IC_{50} values and the predicted maximum concentration of inhibitors in human plasma (C_{max}). The predicted C_{max} of GC and GG ranged from 0.305–2.11 μM and 0.387–3.54 μM , respectively (Table 2), the f_{hep} values are set to an estimated average value of 1.0, and the E_{h} of drugs metabolized by CYP3A varies between 0.1 and 0.9. Then, the plasma exposure (AUC) of drugs predominantly metabolized by CYP3A4 was estimated to increase by 22%–321% and 2%–332% in the presence of GC and GG, respectively. In contrast, the AUC of drugs that primarily eliminated by CYP3A5 was predicted to elevate by 8%–233% and 7%–3190% in the presence of GC and GG, respectively (Table 3).

4. Discussion

HDI mediated by CYP3A enzymes has received the most widespread attention due to the important role of CYP3A4 in the metabolism of >50% of clinically-used drugs. However, comparative investigations on the herbal inhibition potentials towards CYP3A4 and CYP3A5 are limited. Here, the differential inhibitory behaviors of the herbal ingredients GC and GG towards CYP3A4 and CYP3A5 were investigated.

CYP3A4 can mediate adverse drug interactions due to its broad substrate specificity. Despite the fact that CYP3A4 and CYP3A5 are 83% homologous in amino acid sequence and have overlapping substrate specificity, differences both in catalytic properties and inhibitory activities have been demonstrated. As previously reported, the intrinsic clearance of anticancer drugs mediated by CYP3A5 are comparable with or greater than those mediated by CYP3A4. For example, the intrinsic clearance of vincristine dehydrogenation by CYP3A5 were >10 times those for CYP3A4 [6]. In contrast, most of the intrinsic clearance for dehydrogenation of many of the dihydropyridine calcium antagonists, such as benidipine, felodipine, and nifedipine, and steroid hydroxylation, such as testosterone 6 β -hydroxylation and estradiol 2- or 4-hydroxylation, mediated by CYP3A5, are lower than those mediated by CYP3A4 [19,20]. Therefore, the determining the comparable inhibition by GC and GG, two active constituents of *Schisandra chinensis*, towards CYP3A4 and CYP3A5 will help in predicting adverse HDIs.

GC and GG have the same molecular weight and their chemical structures are very similar. However, the pharmacological activities of GC and GG are different. GC shows an observable antitussive activity, while GG possesses a distinct anti-HIV pharmacological activity [8,9]. More interestingly, these two lignan analogs have distinct activity on CYP3A4 and CYP3A5. It is notable that GC possesses a methylenedioxy group located on the phenyl ring distant from the benzoyl group at position 6. In contrast, the structure of GG comprises a methylenedioxy group located on the phenyl ring close to the benzoyl group at position 6. The presence of the methylenedioxy group was reported to be a prerequisite for a dibenzocyclooctadiene-based lignan to display inhibitory effects on CYP3A4 activity. Also, the presence of a lipophilic and bulky functional group of benzoyl at position 6 for GC and GG might increase the inhibitory intensity [12]. The results of this study demonstrated that both GC and GG strongly inhibited CYP3A-mediated metabolite formation, with low IC_{50} values (0.01–2.5 μM), in agreement with a previous report [12]. However, it is worth noting that the different position of methylenedioxy group in lignans may alter their comparable

inhibitory preferences towards CYP3A4 and CYP3A5. When the methylenedioxy group in GC is at position 12 and 13, distant from the benzoyl group at position 6, the inhibitory intensity towards CYP3A4 was a stronger than towards CYP3A5 by using midazolam and nifedipine, but not testosterone as the probe substrates. In contrast, when the methylenedioxy group in GG is at position 2 and 3, close to the benzoyl group at position 6, the inhibitory intensity towards CYP3A5 was stronger than towards CYP3A4 by using midazolam, nifedipine, and testosterone as probe substrates. As a result, the inhibitory intensity of GC was almost equal to CYP3A4 and CYP3A5, while GG can more strongly inhibit the CYP3A5 activity compared to CYP3A4 activity. These findings suggest that subtle structural differences in lignans might amplify their inhibitory behaviors towards CYP3A4 and CYP3A5, which would further result in differential clinical HDI potentials.

Notably, an in silico docking method is considered to be an effective method to elucidate the interactions between inhibitors and drug-metabolizing enzymes [21], such as hydrogen bonding and π - π interaction, both of which could help the formation of optimal combination model between ligand and a CYP enzyme [22,23]. In this study, the π - π interaction was observed between the heme of CYP3A4 and the benzoyl group of GC, while this interaction was not found between GC and CYP3A5. This finding might explain why the inhibitory intensity of GC was almost equal to both CYP3A4 and CYP3A5. More interestingly, docking simulation studies indicated that two hydrogen-bonds interactions were observed between Arg105 of CYP3A5 and the two oxygen atoms of methoxyl at position 12 and 13 of GG, whereas these interactions were not found between GG and CYP3A4. These results might explain why GG can more strongly inhibit the activity of CYP3A5 than that of CYP3A4. The docking results agreed with the inhibitory activities of GC and GG towards CYP3A4 and CYP3A5 that observed in this study. Taken together, these findings suggested that the potential HDI risk induced by GC for CYP3A4 was slightly higher than that for CYP3A5, whereas the potential HDI risk induced by GG for CYP3A5 was obviously higher than that for CYP3A4.

Furthermore, the clinical HDI risk induced by GC or GG was predicted based on each of the inhibitory intensity and the inhibitor concentration in plasma. The magnitude of AUC variation for most drugs that predominantly metabolized by CYP3A4 was estimated to increase by 22%–321% in the presence of GC, similar to the magnitude of 2%–332% in the presence of GG. More importantly, the magnitude of AUC variation for drugs that are primarily eliminated by CYP3A5, such as vincristine and tacrolimus [5,6], was predicted to increase by 8%–233% in the presence of GC, while this magnitude would be elevated by 7%–3190% in the presence of GG. These findings strongly hinted that GC and GG showed high HDI potentials via CYP3A4 and CYP3A5, in particular, that caution should be taken when drugs with narrow therapeutic indices, such as the vincristine and tacrolimus, are co-administrated with GG-containing herbs.

5. Conclusion

GC with the methylenedioxy group at position 12 and 13, its inhibitory intensity towards CYP3A4 was almost equal to that towards CYP3A5. In contrast, GG with the methylenedioxy group at position 2 and 3, showed a stronger inhibitory activity on CYP3A5

than CYP3A4. The π – π interaction between CYP3A4 and GC, and hydrogen-bond interaction between CYP3A5 and GG might result in their different inhibitory actions. These findings suggest that GC and GG showed high HDI potentials, and the position of methylenedioxy group determined their different inhibitory effect towards CYP3A4 and CYP3A5, which are of significance for the application of *Schisandra chinensis* containing herbs.

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Fig. 1.
Chemical structure of GC and GG.

Fig. 2. The selective inhibitory effects of GC on the metabolism of midazolam (a), nifedipine (b) and testosterone (c) in rhCYP3A4 and rhCYP3A5 systems. Data are shown as mean $\pm$ S.D. from three experiments carried out in duplicate.

Fig. 3.

The selective inhibitory effects of GG on the metabolism of midazolam (a), nifedipine (b) and testosterone (c) in rhCYP3A4 and rhCYP3A5 systems. Data are shown as mean $\pm$ S.D. from three experiments carried out in duplicate.

Fig. 4.
Docking simulation and interacting amino acid residues plot of GC located within the active site of CYP3A4 (a) and CYP3A5 (b).

Fig. 5.
Docking simulation and interacting amino acid residues plot of GG located within the active site of CYP3A4 (a) and CYP3A5 (b).

Table 1

Inhibition of CYP3A-catalyzed reactions by GC and GG in human recombinant CYP3A4 and CYP3A5.

Inhibitor	Substrate	IC ₅₀		
		CYP3A4 (μM)	CYP3A5 (μM)	Ratio (3A4/3A5)
GC	Midazolam ^a	0.059 ± 0.03	0.088 ± 0.04	0.67
	Nifedipine ^b	0.14 ± 0.07	0.37 ± 0.02	0.38
	Testosterone ^c	0.13 ± 0.04	0.085 ± 0.04	1.6
GG	Midazolam ^a	0.19 ± 0.09	0.034 ± 0.001	5.7
	Nifedipine ^b	25 ± 1	0.59 ± 0.4	4.3
	Testosterone ^c	0.096 ± 0.02	0.010 ± 0.004	9.4

^aMidazolam-1'-hydroxylation, nifedipine dehydrogenation and testosterone 6β-hydroxylation are involved as the probe reaction, respectively.^bMidazolam-1'-hydroxylation, nifedipine dehydrogenation and testosterone 6β-hydroxylation are involved as the probe reaction, respectively.^cMidazolam-1'-hydroxylation, nifedipine dehydrogenation and testosterone 6β-hydroxylation are involved as the probe reaction, respectively.

Table 2

Prediction of the maximum exposure ($C_{\max}$) levels of GC and GG in vivo.

Inhibitor	MW.	Referential fold*	Intake (mg)*	$C_{\max}$ (μM)*
GC	536.57	0.08–0.52	0.85–5.9	0.305–2.11
GG	536.57	0.10–0.88	1.1–9.9	0.387–3.54

* The referential content fold of GC and GG towards deoxyschizandrin is based on the literature [7]. The standard amount of deoxyschizandrin in WuZhi capsule is 11.25 mg as a quality control [17], then we calculated the intake of GC and GG. Furthermore, the volume of human blood is about 5.2 L [18].

Table 3

Prediction of the potential HDI risks in vivo based on the AUC ratios.

Substrates	E_h^a	f_{hep}^b	inhibitor	$IC_{50}(\mu M)^c$	C_{max}/IC_{50}	AUC ratio ^d	AUC increased (%)
CYP3A4-predominant metabolism	0.1–0.9	1.0	GC	0.059–0.14	2.18–35.7	1.22–33.1	22%–321%
			GG	0.096–2.5	0.155–36.9	1.02–34.2	2%–332%
CYP3A5-predominant metabolism			GC	0.085–0.37	0.824–24.8	1.08–23.3	8%–223%
			GG	0.010–0.59	0.656–354	1.07–320	7%–3190%

^a E_h values of the CYP3A substrate drugs were varied between 0.1 and 0.9.

^b f_{hep} was set to 1.0, based on the assumption that the drugs involved are entirely eliminated through the liver.

^c The ranges of IC_{50} of inhibitors were derived from Table 1.

^d Prediction methods are described in the Materials and methods.