Effects of Mace and Nutmeg on Human Cytochrome P450 3A4 and 2C9 Activity

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Pharmacokinetic or pharmacodynamic interactions between herbal medicines or food constituents and drugs have been studied as crucial factors determining therapeutic efficacy and outcome. Most of these interactions are attributed to inhibition or induction of activity of cytochrome P450 (CYP) metabolic enzymes. Inhibition or induction of CYP enzymes by beverages, including grapefruit, pomegranate, or cranberry juice, has been well documented. Because spices are a common daily dietary component, other studies have reported inhibition of CYP activity by spices or their constituents/derivatives. However, a systematic evaluation of various spices has not been performed. In this study, we investigated effects of 55 spices on CYP3A4 and CYP2C9 activity. Cinnamon, black or white pepper, ginger, mace, and nutmeg significantly inhibited CYP3A4 or CYP2C9 activity. Furthermore, bioassay-guided fractionation of mace (Myristica fragrans) led to isolation and structural characterization of a new furan derivative (1) along with other 16 known compounds, including an acylphenol, neolignans, and phenylpropanoids. Among these isolates, (1S,2R)-1-acetoxy-2-(4-allyl-2,6-dimethoxyphenoxoxy)-1-(3,4-dimethoxyphenyl)propane (9) exhibited the most potent CYP2C9 inhibitory activity with an IC50 value comparable to that of sulfaphenazole, a CYP2C9 inhibitor. Compound 9 competitively inhibited CYP2C9-mediated 4′-hydroxylation of diclofenac. The inhibitory constant (Kd) of 9 was determined to be 0.037 μM. Compound 9 was found to be ca. 14-fold more potent than was sulfaphenazole.

Key words  spice; cytochrome P450; drug interaction; lignan

Several studies have investigated potential interactions between herbal medicines or food constituents and clinical drugs.1) One of the most important metabolic enzymes affected by drugs is cytochrome P450 (CYP), which may be inhibited or induced by these interactions. For example, concomitant intake of the dihydropyridine-type calcium-channel blockers with grapefruit juice leads to elevated drug levels in blood as a result of suppression of CYP3A4 activity in the intestinal epithelium.2) Recently, effects on efflux and influx transporters such as P-glycoprotein and organic anion-transporting polypeptides, respectively, have also been shown to mediate these interactions.3,4) However, CYPs are involved in ca. 96% of metabolic drug interactions.5)

Various CYP isoforms, including CYP1A2 (13%), CYP2A6 (4%), CYP2C (20%), CYP2D6 (2%), CYP2E1 (7%), and CYP3A (30%) are expressed in the human liver.6) Among the CYP isoforms, CYP3A4, which is abundantly present in liver microsomes and the intestinal epithelium, is involved in the metabolism of more than 50% of clinical drugs, including calcium-channel blockers, midazolam, and cyclosporin. The second predominant CYP isoform, CYP2C9, is mostly expressed in the liver and is known to metabolize drugs such as warfarin and phenytoin. Inhibition of CYP enzymes by common beverages, including citrus, pomegranate, or cranberry juice, has been investigated and well documented. Similarly, there are some reports about inhibitory effects of spices or their constituents/derivatives on the activity of CYPs, because spices are very common in our daily diet. For example, black-pepper extracts or alkaloids derived from white pepper have been shown to inhibit CYP3A4 activity.7,8) However, a systematic evaluation of a wide variety of spices has not been performed. While searching for CYP inhibitors derived from food or other supplement sources, we recently reported the inhibitory effects of 60 dietary polyphenols on CYP3A4 and CYP2C9 activities, indicating that some flavonoids can potently inhibit these CYPs in vitro.9) In this study, we assayed inhibitory effects of 55 common spices on CYP3A4 and CYP2C9 activities in vitro. Among other spices, the bioassay-guided fractionation of mace (Myristica fragrans) with potent inhibitory activities on CYPs, led to isolation and characterization of 17 phytochemicals including a new compound.

MATERIALS AND METHODS

Materials Human CYP3A4 (Cat. No. 456202) and CYP2C9 (Cat. No. 456258) microsomes expressed in Baculovirus-infected insect cells were purchased from BD Gentest (Woburn, MA, U.S.A.). Each microsome preparation contained only one P-450 enzyme, the P-450 reductase or cytochrome b5. 4′-Hydroxydiclofenac was obtained from BD Gentest. Diclofenac (DIC), testosterone (TST), magnesium chloride hexahydrate (MgCl2·6H2O), and ketoconazole (KET) were obtained from Wako Pure Chemicals Industries Ltd. (Osaka, Japan). Sulfaphenazole (SPZ), 6β-hydroxytestosterone, piperine, and myristicin were purchased from Sigma-Aldrich Inc. (St. Louis, MO, U.S.A.). Glucose-6-phosphate (G-6-P), β-nicotinamide adenine dinucleotide phosphate (reduced form, NADPH), and glucose-6-phosphate dehydrogenase (G-6-P-DH) were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents were of analytical grade and obtained commercially. The 55 powdered spices were provided by House Foods Corp. (Tokyo, Japan).

General Circular dichroism (CD) spectra were recorded using a Jasco J-720W spectrometer. 1H- and 13C-NMR spectra, including hetero-nuclear single quantum coherence (HSQC), hetero-nuclear multiple-bond connectivity
(HMBC), nuclear Overhauser enhancement and exchange spectroscopy (NOESY), and 1H–1H correlation spectroscopy (COSY)] were measured on a Varian Unity INOVA AS600NB instrument operated at 600 MHz for 1H and 150 MHz for 13C. The chemical shifts are given in δ (ppm) values relative to that of the solvent [acetone-d6 (Δδ 2.04, Δc 29.8), CD3OD (Δδ 3.35, Δδ 49.0)]. The high-resolution electrospray-ionization (HR-ESI)-MS data were acquired using a Bruker micrOTOF II spectrometer.

**Extraction and Isolation** The powdered mace or nutmeg (170/190 g) was macerated in MeOH (3×1 l) at room temperature. The concentrated MeOH extract (49.0/49.0 g) was suspended in H2O (1 l) and then partitioned with n-hexane (3×1 l), EtOAc (3×1 l), and n-ButOH (3×1 l), to afford n-hexane (33.8/19.8 g), EtOAc (13.0/4.5 g), n-ButOH (0.8/1.8 g), or H2O (2.1/2.7 g) extracts, respectively. A portion (0.5 g) of the EtOAc extract derived from mace was separated using a silica-gel column chromatography (11 cm i.d.×20 cm) using a n-hexane/EtOAc solvent system. The n-hexane/EtOAc (2:1) eluate was purified using a preparative TLC (Silica gel 60 F254, Merck) (toluene/acetone/ hexane/EtOAc (2:1) eluate was purified using a preparative HPLC [YMC-Pack ODS A-302 column, (4.6 mm i.d.×250 mm; YMC Co., Ltd.), 40 °C, 1.0 ml/min, 280 nm, H2O/CH3CN/H2O=45:50:5] yielding compounds 2 (1.2 mg), 3 (7.9 mg), 4 (2.9 mg), 5 (1.7 mg), 6 (4.7 mg), 7 (1.7 mg), and 9 (5.3 mg). The n-hexane/EtOAc (1:1) eluate was purified using a normal phase preparative HPLC [YMC-Pack Sil A-003 column, (4.6 mm i.d.×250 mm; YMC Co., Ltd.), room temperature, 1.5 ml/min, 280 nm, n-hexane/EtOAc/HCOOH=19:1:0.5] to afford compounds 8 (2.8 mg) and 10 (2.4 mg). A portion (2.5 g) of the n-hexane extract derived from mace was similarly separated using silica-gel column chromatography developed with n-hexane–EtOAc solvent. Compound 14 (37.1 mg) was derived from the n-hexane eluate. The eluate of n-hexane/EtOAc (19:1) furnished compound 11 (110 mg). The n-hexane/EtOAc (19:1) eluate was purified using a preparative TLC, normal- and reversed-phase HPLC, as mentioned above, to yield compounds 1 (2.4 mg), 12 (8.0 mg), 13 (2.5 mg), 16 (5.5 mg), and 17 (1.4 mg). The n-hexane/EtOAc (9:1) eluate was further separated using TLC, normal- and reversed-phase HPLC to afford compounds 8 (7.9 mg), 9 (3.8 mg), 10 (1.8 mg), and 15 (2.4 mg).

The n-hexane nutmeg extract was separated using silica-gel column chromatography using n-hexane/EtOAc. The EtOAc eluate was further separated using a C18 column (Strata, 1000 mg/6 ml, Phenomenex, Inc.) developed with aqueous MeOH. The compounds eluted with 100% MeOH were purified using reversed-phase HPLC to yield compound 1 (2.5 mg).

**Compound 1**: A white powder, 1H-NMR (600 MHz, acetone-d6) δ: 7.31 (1H, d, J=15.6 Hz, H-2), 6.12 (1H, s, H-3), 6.05 (1H, d, J=15.6 Hz, H-3), 2.63 (2H, t, J=7.8 Hz, H-1’), 2.12 (3H, s, 3’-CH3), 1.66 (2H, m, H-2’), 1.30—1.40 (4H, m, H-3’—4’), and 0.89 (3H, m, H-5’); 13C-NMR (150 MHz, acetone-d6) δ: 168.0 (C-1), 159.8 (C-5’), 146.2 (C-2’), 129.9 (C-2), 128.2 (C-3’), 112.7 (C-3), 111.6 (C-4’), 32.5 (C-3’), 28.1 (C-1’), 28.1 (C-4’), 21.9 (C-4”), 14.1 (C-5’), and 10.1 (3’-CH3). HR-ESI-MS m/z 221.1175 [M+H]+ (Calcd for C10H19O3·H, 221.1183).

CD (MeOH; [θ]) nm; compound 4: 244 (−12800), 280 (−2430), compound 5: 235 (−5180), 269 (+3450), compound 6: 238 (−1820), 273 (+2980), compound 8: 239 (−6860), 283 (+13500), compound 9: 244 (−12000), 280 (−2300), compound 10: 243 (−7080), 271 (+14300).

**Inhibition Assays of Cytochrome P450** 6β-Hydroxylation of TST by CYP3A4 and 4’-hydroxylation of DIC by CYP2C9 were assayed as indicators of an inhibitory activity. The inhibitory assays were carried out as previously described. 9 A mixture of G-6-P (330 μg/ml, final concentration), β-NADPH (330 μg/ml), MgCl2·6H2O (200 μg/ml), G-6-P-DH (0.4 U/ml in 5 mM sodium citrate), and each substrate (25 μM of TST or 5 μM of DIC) in 0.1 M Tris–HCl buffer (pH 7.6) was added to a tested sample dissolved in dimethylsulfoxide. These substrate concentrations were chosen to approximate the Michaelis constant (Km) for each CYP enzyme. The Km values for CYP3A4 (17.4 μM) and CYP2C9 (2.4 μM) were calculated using the Michaelis–Menten equation. After 5-min preincubation at 37 °C, the reaction mixture was incubated at 37 °C for 1 h followed by addition of the respective enzymes (10 pmol/ml). The reaction was terminated by addition of an ice-cold solution of 0.2 ml CH3CN and CH3COOH (96:4).

**Analytical Method** After 4-min centrifugation at 13400 g, the supernatant (20 μl) was analyzed using the following reversed-phase HPLC system. The HPLC analysis was performed as described previously. 9 The limits of detection (LOD) (signal to noise=3) and quantification (LOQ) (signal to noise=10) for 4’-hydroxydiclofenac were 0.1 and 0.8 μM, respectively. The LOD and LOQ for 6β-hydroxysterone were 0.04 and 0.5 μM, respectively. The range of coefficient of variation (CV) and accuracy for each concentration (n=3) for 4’-hydroxydiclofenac were calculated to be 0.08—7.7% and -6.4—0.3%, respectively. The CV and accuracy for 6β-hydroxysterone were calculated to be 0.1—4.2% and -12.0—0.4%. The calibration curves were linear and had mean correlation coefficients of r=0.999.

**Enzyme-Inhibition Kinetics** To determine the mode of inhibition and inhibitory constants (Ki), the final concentration of DIC was 2, 5, 10, or 20 μM. Incubation was stopped at 0, 5, 10, or 15 min. The concentration of compound 9 was set around its IC50 value. The mode of inhibition and Ki values were determined using duplicate measurements.

**Data Analysis** Concentrations of metabolites formed by CYP reactions were determined by calculating the corresponding peak areas in HPLC analysis. IC50 values were calculated graphically using the linear extrapolation of enzyme activity versus inhibitor concentration. Type of inhibition and Ki values were evaluated using Sigma Plot 11.0, Enzyme Kinetic Module 1.3 (SPSS Inc., Chicago, IL, U.S.A.).

**RESULTS AND DISCUSSION**

**Inhibitory Effects of 55 Spices on CYP3A4 or CYP2C9 Activity** Inhibitory effects of 55 common spices on CYP3A4 or CYP2C9 activity are shown in Fig. 1. The inhibitory effects on CYP3A4 or CYP2C9 activity were based on TST 6β-hydroxylation and DIC 4’-hydroxylation reactions. Some 36 spices exhibited more than 50% inhibition of CYP3A4 or CYP2C9 activity. Among these spices, black pepper, Chinese cinnamon, Sri Lankan cinnamon, ginger,
Japanese pepper, mace, nutmeg, sage, Sichuan pepper, turmeric, and white-pepper extracts significantly suppressed the TST or DIC hydroxylation reactions at 100 mg/ml. The IC\textsubscript{50} values of these 11 spices are summarized in Table 1.

Black pepper and ginger selectively inhibited CYP3A4 activity with IC\textsubscript{50} values of 4.1 and 5.1 mg/ml, respectively. The inhibitory effects of pepper (\textit{Piper nigrum} L.) extract and its constituents on CYP3A4 have been reported previously.\textsuperscript{7) The IC\textsubscript{50} value for black-pepper extract on CYP3A4-mediated erythromycin metabolism was reported to be 29 mg/ml. Another report demonstrated that alkaloids isolated from white pepper exhibited remarkable inhibition of CYP3A4-mediated nifedipine oxidation with IC\textsubscript{50} values of 0.18—0.79 \(\mu\)M, whereas the main alkaloid piperine showed a moderate inhibition with an IC\textsubscript{50} value of 1100 M.\textsuperscript{8) In this study, piperine at 10 \(\mu\)M revealed 66% and 2.2% of inhibition on CYP3A4-mediated TST and CYP2C9-mediated DIC hydroxylation, respectively (data not shown). These findings suggest that inhibitory effects of alkaloids and piperine derived from black/white pepper on CYP3A4 activity are stronger than those on CYP2C9 activity.

On the other hand, mace and nutmeg derived from \textit{Myristica fragrans} showed potent inhibition on CYP3A4 and CYP2C9 enzymes with IC\textsubscript{50} values of 1.1—4.2 \(\mu\)g/ml. The content of a major constituent of mace and nutmeg—myristicin (11)—was 1.3% or 2.7%, respectively.\textsuperscript{10) It was also reported that myristicin is sensitive to oxidation by human liver CYP3A4 and 1A2,\textsuperscript{11) indicating that the competitive inhibition may occur in CYP3A4-mediated metabolism. In the present study 10 \(\mu\)M myristicin inhibited CYP3A4 and CYP2C9 activities by 40% and 2%, respectively. These results disagree with the data shown in Table 1; if myristicin contributes to CYP inhibition. Further, although safrole (14) in mace and nutmeg is known to undergo 1'-hydroxylation by CYP2C9 and CYP2E1,\textsuperscript{12) safrole did not inhibit CYP2C9 activity in this study. Thus, we investigated and characterized CYP inhibitors derived from mace and nutmeg by a bioassay-guided fractionation assay \textit{in vitro}.

\section*{Assay-Guided Fractionation of Mace}

Total methanol extracts of mace/nutmeg were extracted successively in \textit{n}-hexane, EtOAc, and \textit{n}-BuOH to give the respective extracts, of which inhibitory activities on CYP3A4 and CYP2C9 are summarized in Table 2. \textit{n}-Hexane or EtOAc extract derived from mace or nutmeg exhibited a more potent inhibition than other extracts. The EtOAc extracts at 5 mg/ml completely inhibited the CYP2C9-mediated hydroxylation reaction. The composition of each extract derived from mace was almost similar to that from nutmeg as analyzed by HPLC and TLC, suggesting that the same constituents may cause CYP inhibition. We further separated and purified the active extracts from mace to characterize CYP inhibitors. The \textit{n}-hexane and EtOAc extracts were subjected to column chromatography over a silica gel developed with \textit{n}-hexane—EtOAc solvent system. The eluates were further purified by a preparative TLC and/or HPLC to furnish a new compound (1) together with 16 compounds, 1-(2,6-dihydroxyphenyl)-9,3,4-dihydroxyphenylnonan-1-one (malabaricone C) (2),\textsuperscript{13) 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)propan-1-ol (3),\textsuperscript{14) 1-acetoxy-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propan-1-ol (4),\textsuperscript{14) 7,8-dihydro-7-(4-hydroxy-3,5-dimethoxyphenyl)-3'-methoxy-8-methyl-1'-trans-propenylbenzofuran (5),\textsuperscript{15) 7,8-dihydro-7-(4-hydroxy-3-methoxyphenyl)-3'-methoxy-8-methyl-1'-trans-propenylbenzofuran (6),\textsuperscript{15) 5-hydroxyeugenol (7),\textsuperscript{16) 7,8-dihydroxy-1,2,3,4-tetrahydroxybenzofuran (8),\textsuperscript{16) 7,8-dihydroxy-1,2,3,4-tetrahydroxybenzofuran (9),\textsuperscript{16) and 5-hydroxy-1,2,3,4-tetrahydroxybenzofuran (10).\textsuperscript{16) The final concentration of each spice extract was 100 \(\mu\)g/ml (\(n=2\).)

\begin{table}[h]
\centering
\caption{The IC\textsubscript{50} (\(\mu\)g/ml) of 11 Spices on CYP3A4 or CYP2C9 Activity}
\begin{tabular}{|c|c|c|}
\hline
Spices & CYP3A4 & CYP2C9 \\
\hline
Black pepper & 4.1 & 12.1 \\
Cinnamon (Chinese) & 24.0 & 12.4 \\
Cinnamon (Sri Lanka) & 30.8 & 15.2 \\
Ginger & 5.1 & 10.0 \\
Japanese pepper & — & 36.0 \\
Mace & 3.9 & 1.1 \\
Nutmeg & 4.2 & 2.6 \\
Sage & — & 29.0 \\
Sichuan pepper & — & 3.2 \\
Turmeric & 17.0 & 14.8 \\
White pepper & 1.0 & 3.2 \\
\hline
\end{tabular}
\end{table}

Each value shows the average of 3 measurements. a) Not tested.
dro-7-(5-methoxy-3,4-methylenedioxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran (8),19 1-acetoxy-2-(4-allyl-2,6-dimethoxyphenox)-1-(3,4-dimethoxyphenyl)propane (9),20 7,8-dihydro-7-(3,4-methylenedioxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran (10),15 myristicin (11),15 methyleugenol (12),21 2,3-dimethoxy-5-(1-propenyl)-phenol (13),22 safrole (14),23 methoxyeugenol (15),24 elemicin (16),25 and eugenol (17)26 (Fig. 2). These known compounds were identified by comparison of their physicochemical data with those reported in the literature. Absolute structures of neolignans 3–6 and 8–10 among these known compounds still remain unclear. The absolute configuration of these neolignans was supported as (7S)-8,8-dihydro-7-(3,4-methylenedioxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran, respectively, based on the rule reported by Konya et al.29 Compound 3 was characterized as a racemic mixture because of lack of a Cotton effect in the CD spectrum. The absolute configuration of dihydrobenzo[b]furan-type neolignans was determined based on the aromatic quadruplet and the P/M helicity rules.30–32 Compounds 5, 6, 8, and 10 showed all negative and positive signs at 1Ls band (around 230 nm) and 1Ls band (around 280 nm), respectively, establishing the 2S, 3S configurations for all compounds. Compounds 5, 6, 8, and 10 were thus characterized as (7S,8S)-7,8-dihydro-7-(4-hydroxy-3,5-dimethoxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran, (7S,8S)-7,8-dihydro-7-(4-hydroxy-3-methoxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran, (7S,8S)-7,8-dihydro-7-(5-methoxy-3,4-methylenedioxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran, and (7S,8S)-7,8-dihydro-7-(3,4-methylenedioxyphenyl)-3’-methoxy-8-methyl-1’-trans-propenylbenzofuran, respectively.

A new compound (1) was isolated as a white powder. The HR-ESI-MS of compound 1 had a molecular ion peak at m/z 221.1175 [M–H]−, consistent with the molecular formula C11H10O3. The 1H-NMR and 1H–1H COSY spectra of compound 1 indicated the presence of trans olefinic protons [δH 7.31 (1H, d, J=15.6 Hz, H-2), 6.05 (1H, d, J=15.6 Hz, H-3)], an aromatic proton [δH 6.12 (1H, s, H-4’)], 2 methyl groups [δH 2.12 (3H, s, CH3-3’), 0.89 (3H, s, H-5’)], and 4 methylene groups [δH 2.63 (2H, t, J=7.8 Hz, H-1’), 1.66 (2H, m, H-2’), 1.30–1.40 (4H, m, J=7.8 Hz, H-3’,4’)]. The resonances of a carbonyl carbon (δ 168.0) besides 6 sp2 (δ 159.8, 146.2, 129.9, 128.2, 112.7, 111.6) and 6 sp3 (δ 132.5, 28.5, 28.1, 22.9, 14.1, 10.1) carbons were observed in the 13C-NMR spectrum of compound 1. These spectral features indicated that compound 1 is a furan derivative having methyl, pentyl, and arylid acid moieties. The key HMBC correlations indicated a methyl group at C-3’ position and a pentyl group at C-5’ position on furan ring of compound 1 (Fig. 3). Based on these findings, the structure of compound 1 was elucidated as 3’-methyl-5’-pentyl-furlylacr acid. Compound 1 was also isolated from n-hexane extract of nutmeg.

**Inhibitory Activity of the Isolated Compounds on CYP3A4 and CYP2C9**

The isolated compounds 1–17 derived from mace were tested for their inhibitory effects on 6β-hydroxylation of TST by CYP3A4 and 4’-hydroxylation of DIC by CYP2C9. The IC50 values (μM) of the tested compounds are summarized in Table 3. The mace components except for phenylpropanoids inhibited the activities of CYP3A4 and CYP2C9 with an IC50 values less than 11.2 μM. Among these inhibitors, compound 10 belonging to dihydrobenzo[b]furan-type neolignan, specifically inhibited CYP3A4 with an IC50 of 1.5 μM. 8-O-4’-Neolignan compound 9 markedly inhibited CYP2C9 activity with an IC50 of 0.19 μM, the potency of which was comparable to that of the positive control SPZ.

**Kinetic Studies of Inhibition of CYP2C9 by Compound 9**

Lineweaver–Burk plot analysis of compound 9, which inhibited CYP2C9 activity remarkably, was performed to determine its mode of inhibition (Fig. 4). Compound 9 displayed a competitive inhibitory effect on CYP2C9-mediated DIC 4’-hydroxylation with Ki=0.037 μM. The Ki value of compound 9 was 14-fold lower than that of SPZ (0.5 μM),33 suggesting that compound 9 is a more significant CYP2C9 inhibitor than SPZ.

**CONCLUSION**

In this study, we investigated the inhibitory effects of 55 spices on CYP3A4 or CYP2C9 activity. Mace, nutmeg, black...
pepper, Chinese cinnamon, Sri Lankan cinnamon, ginger, Japanese pepper, sage, Sichuan pepper, turmeric, and white pepper inhibited CYP3A4 and CYP2C9 activities relatively strongly. The bioassay-guided fractionation of mace gave the (1S,2R)-1-acetoxy-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)propane (9) as a potent inhibitor of CYP2C9-mediated DIC 4′-hydroxylation. Kinetic studies of compound 9 indicated that the $K_i$ value of compound 9 was 14-fold lower than that of SPZ.

A sesame lignan, sesamin, was shown to cause elevation of tissue tocopherol levels in rats by inhibiting CYP3A-dependent tocopherol catabolism. Schisandra lignan extracts were shown to induce CYP3A activity strongly in rats. A neolignan kadsurenolone isolated from the stems of Piper kadsura revealed a specific antagonistic activity on platelet-activating factor. However, there was no significant interaction between kadsurenolone and cyclosporin A when kadsurenolone and cyclosporin A were co-administered in rats. Although there are several reports studying pharmacokinetic interactions between lignans and some components or drugs, our results demonstrated for the first time that 8-O-4′-neolignan, compound 9, exhibited a potent inhibitory effect on CYP2C9 activity. As the daily intake of myristicin, a major component of mace, has been estimated to be a few mg in humans, neolignans showing CYP inhibition in the present study may also be estimated similarly to the daily consumption levels of myristicin. Further, in vivo bioavailability studies on these CYP inhibitors are required to investigate mechanisms of potential interactions between mace and drugs. Our findings provide primary data for future in vivo and clinical studies on risk prediction related to interactions between drugs and foodstuff.

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