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## Isolation and identification of urinary metabolites of tectoridin in rats

Xue Bai<sup>a</sup>, Jia-Lin Qu<sup>a</sup>, Jia Liu<sup>a</sup>, Jia-Hong Sun<sup>b</sup> and Dan Yuan<sup>a\*</sup>

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Tectoridin is an active isoflavone from the rhizome of *Belamcanda chinensis* and flower of *Pueraria thomsonii* Benth, possessing an anti-inflammation action and the protective effect against ethanol-induced intoxication and hepatic injury. The metabolism of tectoridin was investigated in rats. Nine metabolites were isolated by using repeated chromatographic methods and identified by spectroscopic methods including UV, IR, mass spectrometry, and NMR experiments. A new compound was determined as tectorigenin-7-*O*- $\beta$ -D-glucuronide (M-1), together with eight known compounds identified as irisolidone-7-*O*- $\beta$ -D-glucuronide (M-2), tectorigenin-7-*O*-sulfate (M-3), tectorigenin-4'-*O*-sulfate (M-4), tectorigenin (M-5), irisolidone (M-6), isotectorigenin (M-7), genistein (M-8), and daidzein (M-9), respectively. The metabolic pathway of tectoridin was proposed, which is important to understand its metabolic fate and disposition in humans.

**Keywords:** tectoridin; metabolism; glucuronidation; rat; urine

### 1. Introduction

Tectoridin is the main active isoflavone of *Belamcanda chinensis*, a Chinese traditional medicine for the treatment of asthma and tonsillitis. The flower of *Pueraria thomsonii* Benth, which has been used to relieve some symptoms such as drunkenness, headache, and red, also contains high content (1–3%) of tectoridin [1]. Pharmacologically, tectoridin shows a wide spectrum of bioactivities such as anti-inflammatory, antioxidative, hepatoprotective and cytotoxic activities against tumor cells, etc [2–10].

Tectoridin was reported to be transformed into tectorigenin by human intestinal bacteria [11,12]. Chen *et al.* [13] reported the identification of urinary and fecal metabolites of tectoridin *in vivo* and *in vitro* after oral administration of

tectoridin to rats by liquid chromatography-tandem mass spectrometry, which included tectorigenin, hydrogenated tectorigenin, mono-hydroxylated tectorigenin, di-hydroxylated tectorigenin, glucuronide-conjugated tectorigenin, and sulfate-conjugated tectorigenin in urine and tectorigenin, di-hydroxylated tectorigenin, and sulfate-conjugated tectorigenin in feces. Among these metabolites, however, the conjugated tectorigenin could not be structurally confirmed due to the failure in determination of the conjugated position by LC-MS<sup>n</sup> technology. It has been reported that glucuronidation of the 3'- or 4'-hydroxyl group in the B-ring of flavonoids decreased the antioxidant activity, whereas glucuronidation in the A-ring affected antioxidant activity to a lesser extent [14]. The above results

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indicated that identification of the structures of metabolites is important for further pharmacological research. Thus, the isolation of metabolites and their structural confirmation on the basis of UV, IR, NMR, and MS data are valuable as well.

In this study, we conducted the systematic isolation of urinary metabolites in rat given tectoridin orally, and determined their structures by means of chemical and spectroscopic experiments. On the basis of the metabolite profile, the possible metabolic pathway of tectoridin was proposed.

## 2. Results

Representative HPLC profiles showing the rat urinary metabolites are shown in Figure 1. Tectoridin and its metabolites were selectively detected at 265 nm due to their characteristic benzoyl group. Six major metabolites M-1–M-5 and M-7 were clearly observed in the urine of rats. By means of repeated chromatographic methods on the columns of silica gel, Sephadex LH-20, or reverse phase ODS, nine metabolites were isolated from the rat urinary sample including one new compound, a glucuronide conjugate M-1

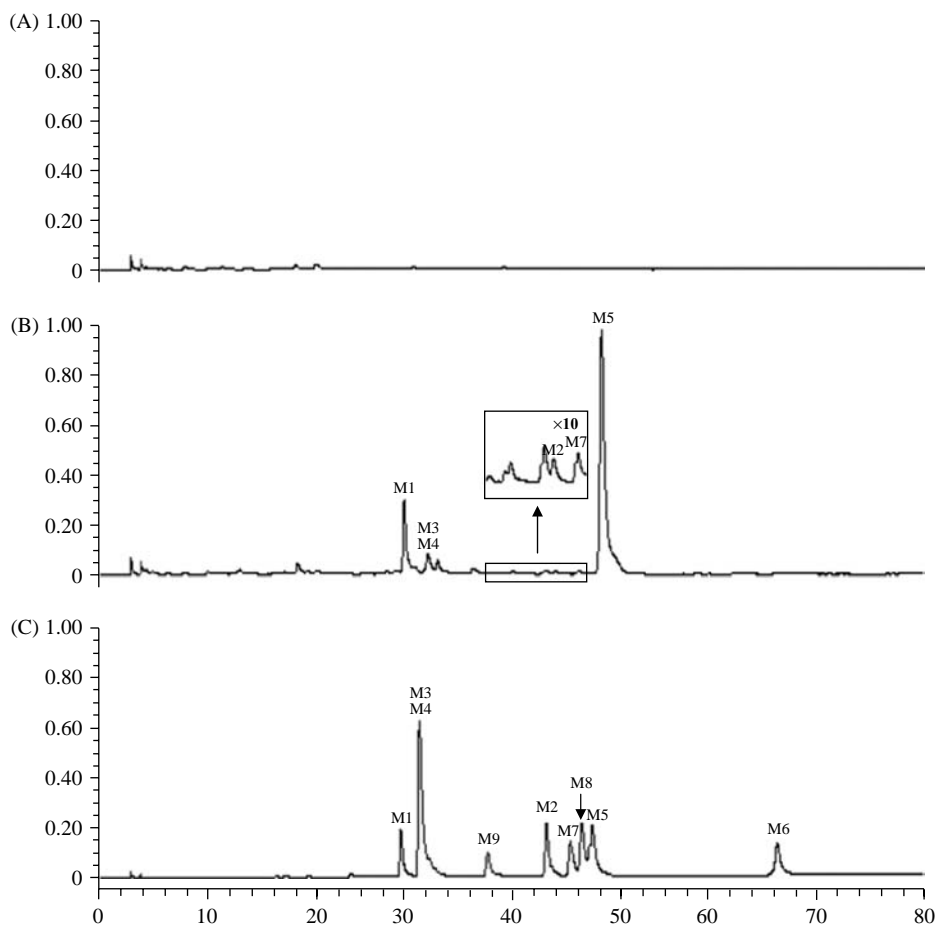


Figure 1. (A) HPLC-UV chromatograms of blank urine, (B) a rat urine sample during 0 to 24 h after oral administration of tectoridin at a dose of 100 mg/kg, and (C) metabolite standards.

(7.5 mg), together with eight known isoflavones, M-2 (1.9 mg), M-3 (6.5 mg), M-4 (2.8 mg), M-5 (13 mg), M-6 (7.4 mg), M-7 (1.1 mg), M-8 (1.1 mg), and M-9 (1.1 mg).

The maximal absorption at 263–265 nm in UV spectrum and the absorption bands at 1648–1659  $\text{cm}^{-1}$  due to conjugated carbonyl and at 1456–1622  $\text{cm}^{-1}$  due to aromatic functions in IR spectrum indicated that M-1–M-9 had an isoflavone skeleton. The structures of M-1–M-9, as shown in Figure 2, were elucidated using UV, IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, and MS techniques.

Metabolite M-1 (tectoridin-7-*O*- $\beta$ -D-glucuronide) was isolated as a yellowish amorphous powder. The molecular formula was determined to be  $\text{C}_{22}\text{H}_{19}\text{O}_{12}$  from the quasi-molecular ion peak at  $m/z$  475.0877  $[\text{M} - \text{H}]^-$  in the ESI-TOF-MS. The  $[\text{M} - \text{H}]^-$  ion at  $m/z$  475 and an important fragment ion at  $m/z$  299 originating from the eliminating 176 mass units (glucuronic acid) from  $[\text{M} - \text{H}]^-$  ion indicated that M-1 should be a glucuronide conjugate. M-1 was hydrolyzed with  $\beta$ -glucuronidase to give the aglycone tectorigenin, the identity of which was confirmed through co-chromatography with tectorigenin standard by HPLC coupled with ultraviolet photodiode array detection based on the same retention time and UV spectral values. In the  $^1\text{H}$

NMR spectrum, the characteristic isoflavone signal for H-2 was observed at  $\delta$  8.43 (1H, s). A singlet at  $\delta$  6.89 due to an aromatic proton suggested that three substituents existed in A-ring. The protons due to an AA'BB' aromatic system appeared at  $\delta$  7.39 (d,  $J = 8.4$  Hz, H-2', 6') and 6.81 (d,  $J = 8.4$  Hz, H-3', 5'), indicating that B-ring was hydroxylated at C-4'. Also a singlet at  $\delta$  3.76 due to a methoxyl group was observed. The resonances for carbons and protons of the aglycone moiety had a close resemblance to those of the known tectorigenin, and they are assigned according to the literature values of the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra for tectorigenin [1,15]. The signals of an anomeric proton ( $\delta$  5.07, 1H, d,  $J = 7.8$  Hz) and a carboxylic group at C-6'' at  $\delta_c$  171.2 indicated the presence of a  $\beta$ -D-glucuronic acid moiety [16]. The  $\beta$ -D-glucuronic acid moiety could be attached to C-7 position according to the upfield shift of C-7 ( $-1.0$  ppm) and the downfield shifts of C-6 ( $+1.0$  ppm), C-8 ( $+0.2$  ppm), and C-10 ( $+1.6$  ppm) relative to the corresponding signals of tectorigenin [1,16,17]. The full assignments of carbon and proton signals are summarized in Table 1. Thus, M-1 was determined to be tectorigenin-7-*O*- $\beta$ -D-glucuronide.

Other metabolites were identified as irisolidone-7-*O*- $\beta$ -D-glucuronide (M-2),

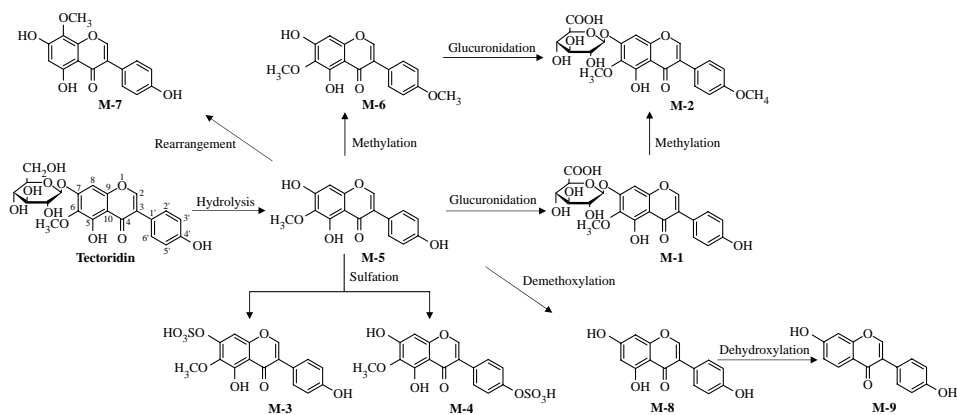


Figure 2. Proposed metabolic pathways of tectoridin.

Table 1.  $^1\text{H}$  and  $^{13}\text{C}$  NMR of tectoridin metabolite M-1.

| Position | $\delta_{\text{C}}^{\text{a}}$ | $\delta_{\text{H}}^{\text{a}}$ | Position | $\delta_{\text{C}}^{\text{a}}$ | $\delta_{\text{H}}^{\text{a}}$ (J in Hz) |
|----------|--------------------------------|--------------------------------|----------|--------------------------------|--|
| 2        | 154.8                          | 8.43, s                        | 2',6'    | 130.2                          | 7.39, d (8.4)                            |
| 3        | 122.1                          |                                | 3',5'    | 115.2                          | 6.81, d (8.4)                            |
| 4        | 180.9                          |                                | 4'       | 157.5                          |  |
| 5        | 152.9                          |                                | 1''      | 100.1                          | 5.07, d (7.8)                            |
| 6        | 132.6                          |                                | 2''      | 73.1                           |  |
| 7        | 156.8                          |                                | 3''      | 76.8                           |  |
| 8        | 94.2                           | 6.89, s                        | 4''      | 71.9                           |  |
| 9        | 152.2                          |                                | 5''      | 73.9                           |  |
| 10       | 106.5                          |                                | 6''      | 171.2                          |  |
| 1'       | 121.1                          |                                | 6-OMe    | 60.3                           | 3.76, s                                  |

Notes: <sup>a</sup>All spectra were recorded on a Bruker ARX-600 spectrometer, in DMSO-*d*<sub>6</sub>. 1''–6'' refer to the numbering of glucuronic acid moiety.

tectorigenin-7-*O*-sulfate (M-3), tectorigenin-4'-*O*-sulfate (M-4), tectorigenin (M-5), irisolidone (M-6), isotectorigenin (M-7), genistein (M-8), and daidzein (M-9), respectively, by comparing their UV, NMR, and MS data with the reported values [15–22]. M-2, M-3, and M-6–M-9 were first isolated as the metabolites of tectoridin.

### 3. Discussion

In this study, nine metabolites were isolated from the urine of rats given tectoridin orally, and structurally confirmed on the basis of UV, IR, NMR, and MS data. According to the metabolite profile, the possible metabolic pathways of tectoridin in rats are proposed as shown in Figure 2.

Two phase I metabolites (M-5 and M-7) and four phase II metabolites (M-1, M-2, M-3/M-4) were clearly identified in the HPLC-UV profile of rat urine (Figure 1). Tectorigenin (M-5), a major metabolite, is believed to be formed through the microbial hydrolysis of tectoridin in the gastrointestinal tract, which was consistent with the previous literature [13]. Moreover, M-5 is also a reactive metabolite that can be successively converted to a glucuronide conjugate (M-1) via further biotransformation at C-7 position catalyzed by UDP-glucuronosyltransferases. And the sulfate conjugation at the C-7 and C-4' positions of M-5 (Figure 2) was catalyzed by

sulfotransferase, forming M-3 and M-4. Structural identification of tectorigenin-4'-*O*- $\beta$ -D-glucuronide and M-4 was studied on the basis of LC-MS<sup>n</sup> data by Chen *et al.* [13]. However, LC-MS<sup>n</sup> experiments could not provide full evidence for the determination of the glucuronidation or sulfation positions because the 4'-OH, 5-OH, and 7-OH positions are all possible conjugated sites for tectorigenin. In this study, tectorigenin-7-*O*- $\beta$ -D-glucuronide was isolated and structurally confirmed using UV, NMR, and MS, rather than tectorigenin-4'-*O*- $\beta$ -D-glucuronide. Thus, the isolation of metabolite and the structural confirmation are valuable for some polyhydroxy compounds.

M-7, the isomeric metabolite of M-5, was also clearly detected in the HPLC profiles of the rat urinary metabolites. A similar pathway related to methoxyl group migration was found in the urinary metabolites of kakkalide in our previous study, which produced two unusual metabolites 5,7-dihydroxy-8,4-dimethoxyisoflavone and isotectorigenin formed by the rearrangement of irisolidone and tectorigenin [18]. Furthermore, we found that M-6 is another minor metabolite derived from the *O*-methylation of M-5 at C-4' position catalyzed by methyltransferase [23], which has not been reported in the previous study on the fecal and urinary metabolites of tectoridin in rat [13].

It might subsequently undergo glucuronide-conjugation at C-7 position, forming M-2 (Figure 2). M-2 might also be formed by M-1 via the methylation of 4'-hydroxyl group. In addition, we also isolated two minor phase I metabolites (M-8 and M-9).

Kakkalide, the predominant isoflavone of *P. lobata*, has a similar structure to tectoridin. We isolated 13 metabolites from the urine of rats after orally given kakkalide [18]. This study showed that most of the metabolites of tectoridin were similar to those of kakkalide, which should be ascribed to their common key metabolites, tectorigenin and irisolidone, *in vivo*.

## 4. Materials and methods

### 4.1 Materials and chemicals

Tectoridin and tectorigenin were isolated from the flowers of *P. thomsonii*, and irisolidone from the flowers of *P. lobata*, following the previously reported methods [24,25]. The identity of these compounds was confirmed by melting point, UV, IR,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, and MS. The purity of tectoridin evaluated with HPLC-UV was above 98%, and that of others was more than 95%.  $\beta$ -glucuronidase was purchased from Sigma (St Louis, MO, USA), macroporous resin D101 from Fushun Xintai Fine Chemical Factory (Fushun, China), Sephadex LH-20 from GE Healthcare (Uppsala, Sweden), ODS from YMC Co., Ltd (Kyoto, Japan), silica gel for column chromatography (CC) from Qingdao Ocean Chemical Co. (Qingdao, China), and MDS-5 RP (200-300 mesh) from Beijing Medicine Technology Center (Beijing, China). Other chemical reagents were of analysis or HPLC grades. Double distilled water was used in this study.

### 4.2 Animals

Male Wistar rats ( $220 \pm 20$  g body weight) were purchased from the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The experimental protocol was approved by the

Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University. They were kept in a breeding room to be acclimated for 4 days before use. Normal foods were provided to the rats before experiments, so was normal water at all times.

### 4.3 Urine collection

Male Wistar rats were fasted 12 h before experiments. Tectoridin (2.5 g) was given orally to 28 rats at a dose of 100 mg/kg and reiteratively in an interval of 7 d (2 d for administration and 5 d for recovery) for the collection of urinary samples. The urine samples were collected from 0 to 36 h. During the collection, water and sugar were available freely. Phosphoric acid was added to urinary samples to adjust pH to 5.0, and they were placed in the refrigerator at  $-10^\circ\text{C}$  subsequently.

### 4.4 Isolation of metabolites

The cumulative urinary samples (approximately 1.8 l in total) were thawed at room temperature, and successively passed through a macroporous absorption resin D101 column eluting with a gradient of EtOH-H<sub>2</sub>O (H<sub>2</sub>O, 30% EtOH, 70% EtOH, and 95% EtOH elutions) to yield four major fractions.

The 30% EtOH fraction was further separated through an ODS open column eluting with a gradient of MeOH-H<sub>2</sub>O (10:90-100:0). The fraction eluted with MeOH-H<sub>2</sub>O (30:70) was further subjected to a Sephadex LH-20 column eluting with MeOH-H<sub>2</sub>O (50:50) to yield M-1 and M-2, so was the fraction eluted with MeOH-H<sub>2</sub>O (40:60) to a Sephadex LH-20 column eluting with MeOH-H<sub>2</sub>O (50:50) to yield M-3 and M-4.

The 70% EtOH fraction was further separated through an MDS column eluting with a gradient of MeOH-H<sub>2</sub>O (10:90-100:0). The fraction eluted with MeOH-H<sub>2</sub>O (60:40) was further

separated by silica gel CC eluting with a  $\text{CHCl}_3$ -MeOH gradient solvent system. The fraction eluted with  $\text{CHCl}_3$ -MeOH (50:1) was further subjected to a Sephadex LH-20 column eluting with  $\text{CHCl}_3$ -MeOH (1:1) to give M-8, so was the fraction eluted with  $\text{CHCl}_3$ -MeOH (30:1) to a Sephadex LH-20 column eluting with  $\text{CHCl}_3$ -MeOH (1:1) to give M-9. The fraction eluted with MeOH-H<sub>2</sub>O (70:30) was further separated by silica gel CC eluting with a  $\text{CHCl}_3$ -MeOH gradient solvent system. M-5 was given from the fraction eluted with  $\text{CHCl}_3$ -MeOH (100:1). The fraction eluted with  $\text{CHCl}_3$ -MeOH (80:1) was further subjected to a Sephadex LH-20 column eluting with  $\text{CHCl}_3$ -MeOH (1:1) to give M-7.

The 95% EtOH fraction was subjected to a Sephadex LH-20 column eluting with  $\text{CHCl}_3$ -MeOH (3:1) to give M-6.

#### 4.4.1 Tectoridin-7-O- $\beta$ -D-glucuronide (M-1)

A yellow amorphous powder; UV (MeOH)  $\lambda_{\text{max}}$ : 266 nm; IR (KBr)  $\nu_{\text{max}}$  ( $\text{cm}^{-1}$ ): 3387, 1650, 1613, 1515, 1459; <sup>1</sup>H and <sup>13</sup>C NMR spectral data, see Table 1; HR-ESI-TOF-MS:  $m/z$  475.0877 [ $\text{M} - \text{H}$ ]<sup>-</sup> (calcd for C<sub>22</sub>H<sub>19</sub>O<sub>12</sub>, 475.0871).

#### 4.5 Enzymatic hydrolysis of metabolite M-1

M-1 (0.1 mg) was incubated with  $\beta$ -glucuronidase (20  $\mu\text{l}$ , 2000 units, type B-1) in 0.05 M ammonium dihydrogen phosphate buffer (1.0 ml, pH 5.0) for 2 h at 37°C. The reaction mixture was extracted with ethyl acetate, and the organic layer was evaporated to dryness *in vacuo* to give the aglycone powder, which was dissolved in 0.2 ml methanol for HPLC-UV analysis.

#### 4.6 Spectroscopic methods

ESI-single quadrupole MS (Shimadzu QP8000 $\alpha$ , Shimadzu Co. Ltd, Kyoto,

Japan) was used in the beginning to scan each fraction for novel metabolites. The mass spectrometer was operated under the following conditions: direct-infusion of sample, ESI in positive and negative mode, an electrospray voltage of 4.0 KV, a mass scan range at  $m/z$  50–800, a heating capillary temperature at 250°C, and dry air at a flow rate of 4.5 l/min.

TOF-MS (Bruker MicroTOF-Q 125, Bruker, Newark, DE, USA) was used to acquire both the exact molecular weight and the product ion spectra of any novel compound detected by the single quadrupole mass spectrometer. Parameters for analysis were set using full scan negative ion mode with spectra acquired over a mass range from  $m/z$  50 to 1000. The ESI source was set to the following conditions: drying gas (N<sub>2</sub>); flow rate, 4.0 l/min; drying gas temperature, 190°C; nebulizer, 0.4 bar; capillary voltage, 3.2 KV.

NMR spectra were measured on a Bruker ARX-600 spectrometer, and chemical shifts are given in ppm downfield relative to TMS. All compounds were dissolved in DMSO-*d*<sub>6</sub>. UV spectra were obtained using a Shimadzu UV-2201 spectrophotometer. IR spectra were obtained on a Bruker IFS-55 infrared spectrometer.

#### 4.7 HPLC condition

Waters HPLC system (Waters Co., Milford, MA, USA) consisting of model 510 pump, automated gradient controller, model 2996 photodiode array detector, and Millennium32 PDA software was used. HPLC analyses were carried out at 35°C on a Kromasil C18 column (4.6  $\times$  250 mm, 5  $\mu\text{m}$ , Tianjin Scientific Instruments Co. Ltd, Tianjin, China). The on-line UV spectra were recorded in the range of 200–400 nm. The injection volume was 20  $\mu\text{l}$ . The mobile phase consisted of a gradient system of solution A, water containing 0.05% TFA, and solution B, acetonitrile containing 0.05%

TFA at a flow rate of 0.8 ml/min. The gradient program was as follows: linear gradient from solution A-B (90:10, v/v) to solution A-B (60: 40, v/v) in 48 min, followed by linear gradient to solution A-B (30:70, v/v) in 32 min.

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### References

- [1] G.F. Zhang, Master's dissertation, Shenyang Pharmaceutical University, 2008, p. 39.
- [2] J.S. Park, M.S. Woo, D.H. Kim, J.W. Hyun, W.K. Kim, J.C. Lee, and H.S. Kim, *J. Pharmacol. Exp. Ther.* **320**, 1237 (2007).
- [3] H.I. Moon, J. Lee, O.P. Zee, and J.H. Chung, *Biol. Pharm. Bull.* **28**, 1123 (2005).
- [4] K.A. Kang, K.H. Lee, S. Chae, R. Zhang, M.S. Jung, S.Y. Kim, H.S. Kim, D.H. Kim, and J.W. Hyun, *Eur. J. Pharmacol.* **519**, 16 (2005).
- [5] H.U. Lee, E.A. Bae, and D.H. Kim, *J. Pharmacol. Sci.* **97**, 541 (2005).
- [6] H.U. Lee, E.A. Bae, and D.H. Kim, *Biol. Pharm. Bull.* **28**, 531 (2005).
- [7] S.H. Jung, Y.S. Lee, S.S. Lim, S. Lee, K.H. Shin, and Y.S. Kim, *Arch. Pharm. Res.* **27**, 184 (2004).
- [8] H.W. Lee, M.K. Choo, E.A. Bae, and D.H. Kim, *Liver Int.* **23**, 221 (2003).
- [9] R. Fang, P.J. Houghton, and P.J. Hylands, *J. Ethnopharmacol.* **118**, 257 (2008).
- [10] K.T. Lee, I.C. Sohn, Y.K. Kim, J.H. Choi, J.W. Choi, H.J. Park, Y. Itoh, and K.I. Miyamoto, *Biol. Pharm. Bull.* **24**, 1117 (2001).
- [11] E.A. Bae, M.J. Han, and K.T. Lee, *Biol. Pharm. Bull.* **22**, 1314 (1999).
- [12] J.E. Shin, E.A. Bae, Y.C. Lee, J.Y. Ma, and D.H. Kim, *Biol. Pharm. Bull.* **29**, 1202 (2006).
- [13] Y. Chen, W. Song, Z.H. Peng, B.Y. Ge, and F.M. Han, *J. Pharm. Pharmacol.* **60**, 709 (2008).
- [14] J.E. Brown, H. Khodr, R.C. Hider, and C.A. Rice-Evans, *Biochem. J.* **330**, 1173 (1998).
- [15] J.T. Yin, Y. Zhong, J.Y. Sun, L. Liu, and B. Wang, *Chin. Tradit. Herb. Drugs* **37**, 350 (2006).
- [16] T. Yasuda, Y. Kano, K. Saito, and K. Ohsawa, *Biol. Pharm. Bull.* **17**, 1369 (1994).
- [17] S.W. Kang, M.C. Kim, C.Y. Kim, S.H. Jung, and B.H. Um, *Chem. Pharm. Bull.* **56**, 1452 (2008).
- [18] X. Bai, Y.Y. Xie, J. Liu, J.L. Qu, Y. Kano, and D. Yuan, *Drug Metab. Dispos.* **38**, 281 (2010).
- [19] A. Bashir, M. Hamburger, J.D. Msonthi, and K. Hostettmann, *Phytochemistry* **31**, 309 (1991).
- [20] M. Moriyasu, Y. Igi, M. Ichimaru, K. Iwasa, J. Kobayakawa, F. Sato-Nishimori, Y. Matsukawa, and C. Nagase, *J. Nat. Med.* **61**, 329 (2007).
- [21] A.C. Talukdar, N. Jain, S. De, and H.G. Krishnamurty, *Phytochemistry* **53**, 155 (2000).
- [22] T. Yasuda and K. Ohsawa, *Biol. Pharm. Bull.* **21**, 953 (1998).
- [23] M.R. Meyer, F.T. Peters, and H.H. Maurer, *Biochem. Pharmacol.* **77**, 1725 (2009).
- [24] X. Chang, Y. Yuan, Y.Y. Xie, J.L. Hu, and D. Yuan, *Chin. J. Med. Chem.* **19**, 284 (2009).
- [25] D. Yuan, Y.Y. Xie, X. Bai, X. Wu, J.Y. Yang, and C.F. Wu, *J. Asian Nat. Prod. Res.* **11**, 471 (2009).