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### Coumarins from *Daphne feddei* and their potential anti-inflammatory activities

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## Coumarins from *Daphne feddei* and their potential anti-inflammatory activities

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Chemical examination of the methanolic extract from the stem bark of *Daphne feddei* led to the isolation of three new dicoumarin glucosides (**1–3**), and eight known coumarins, dicoumarins and dicoumarin glucosides. Their structures were elucidated by extensive analysis of spectral data and comparison with the literature values. All compounds were tested for inhibitory activity against lipopolysaccharide-induced NO production in RAW 264.7 macrophages, and compounds **4** and **5** showed potent inhibitory activity with IC<sub>50</sub> values of 0.161 and 0.127 μM, respectively.

**Keywords:** *Daphne feddei*; coumarin; RAW 264.7 macrophages; nitric oxide

### 1. Introduction

Coumarins are secondary metabolites widely distributed in the plant kingdom, and especially abundant in the families of Umbelliferae, Fabaceae, Asteraceae, and Thymelaeaceae [1]. In the past years, research efforts on the coumarins focused on isolation and identification of new compounds, biosynthetic patterns, and their potential biological actions. Modern pharmaceutical studies have proved that coumarins and their derivatives have a wide range of bioactivities [2–4], which is consistent with the fact that plants rich in coumarins have long been used as medicinal herbs in different cultures. Recently, the search for lead compounds in drug discovery from medicinal plants has led to a resurgence of interest in coumarins.

*Daphne feddei* Levl., a common ever-green shrub in Southwest China, is a rich resource of coumarins from Thymelaeaceae, and its stem bark is used in folk medicine for the treatment of injuries such as falls and bruises [5]. A detailed investigation on the title plant resulted in the isolation of three new dicoumarin glucosides (**1–3**; Figure 1), together with eight known coumarins: 7-hydroxycoumarin (**4**) [6], daphnetin (**5**) [7], skimmin (**6**) [8], daphnin (**7**) [9], daphnetin-8-*O*-β-glucopyranoside (**8**) [9], giraloid A (**9**) [10], 7,7'-dihydroxy-6,8'-bicycoumarin (bicycoumarin) (**10**) [11], and daphgilin-7-*O*-β-glucopyranoside (daphnolin) (**11**) [12]. This paper describes the structural elucidation of compounds **1–3**, and the inhibitory activity of the isolated compounds against NO production induced by lipopolysaccharide (LPS) in macrophages.

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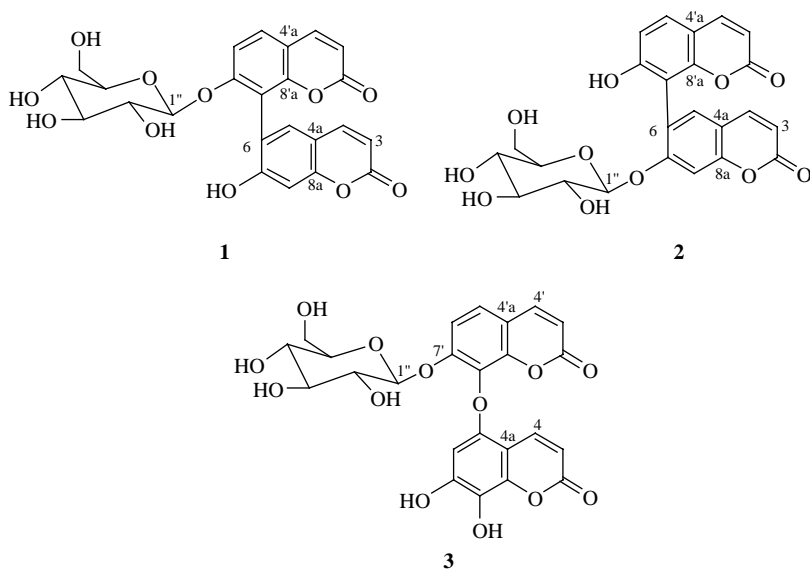


Figure 1. Chemical structures of compounds **1–3**.

## 2. Results and discussion

Compound **1** was obtained as a white powder. The molecular formula  $C_{24}H_{20}O_{11}$  was determined by HR-ESI-MS. The  $^{13}C$  NMR (Table 1) and DEPT spectra of **1** showed 24 carbon resonances ascribed to a methylene, 13 methines, and 10 quaternary C atoms. In the  $^1H$  NMR spectrum, two doublets with an AB coupling pattern ( $\delta = 6.38$ , d,  $J = 9.6$  Hz, H-3;  $\delta = 8.03$ , d,  $J = 9.6$  Hz, H-4), along with two singlets ( $\delta = 7.58$ , H-5;  $\delta = 7.31$ , H-8), were indicative of a 6,7-disubstituted coumarin moiety. Two pairs of doublets with an AB coupling pattern ( $\delta = 6.15$ , d,  $J = 9.6$  Hz, H-3';  $\delta = 7.92$ , d,  $J = 9.6$  Hz, H-4';  $\delta = 7.54$ , d,  $J = 8.4$  Hz, H-5';  $\delta = 6.94$ , d,  $J = 8.4$  Hz, H-6') indicated the existence of a 7,8-disubstituted coumarin moiety. The  $^{13}C$  NMR spectrum suggested that **1** contained a glucose unit ( $\delta$  100.3, 77.1, 76.6, 73.0, 69.6, and 60.7). The anomeric H-1'' of the glucose moiety was determined to be  $\beta$ -oriented on the basis of the coupling constant for H-1'' ( $\delta = 4.99$ , d,  $J = 7.2$  Hz). The HMBC correlation of

H-1''/C-7' ( $\delta = 158.3$ ) suggested that the sugar moiety was attached to C-7' (Figure 2). In fact, compared with the known bicoumol [11], the NMR spectral data of **1** showed a great similarity to the known compound except for the additional signals due to a  $\beta$ -glucosyl group. Thus, compound **1** was deduced as 7-hydroxy-7'-( $\beta$ -glucopyranosyloxy)-(6,8'-bi-2H-1-benzopyran)-2,2'-dione, and named bicoumol-7'-O- $\beta$ -glucopyranoside.

Compound **2** was obtained as a white powder. The molecular formula  $C_{24}H_{20}O_{11}$  was established by HR-ESI-MS, which is the same as that of **1**. Its  $^1H$  and  $^{13}C$  NMR spectral data almost resembled those of **1**, suggesting that it is an isomer of **1**. The HMBC correlation of H-1''/C-7 ( $\delta = 158.8$ ) revealed that the sugar moiety was attached to C-7, rather than C-7' (Figure 2). Thus, compound **2** was deduced as 7-( $\beta$ -glucopyranosyloxy)-7'-hydroxy-(6,8'-bi-2H-1-benzopyran)-2,2'-dione, and named bicoumol-7-O- $\beta$ -glucopyranoside. Compounds **1** and **2** changed into each other in the MeOH–H<sub>2</sub>O solution within 24 h (Figure 3), and acid hydrolysis of them afforded glucose and corresponding

Table 1.  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectral data of compounds **1–3** ( $\delta$  in ppm,  $J$  in Hz).

	<b>1</b> <sup>a</sup>		<b>2</b> <sup>a</sup>		<b>3</b> <sup>b</sup>	
	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$
2	160.2		160.2		161.1	
3	113.1	6.38 (d, 9.6)	113.2	6.38 (d, 9.6)	113.8	6.36 (d, 9.6)
4	144.1	8.03 (d, 9.6)	144.0	8.03 (d, 9.6)	139.3	8.04 (d, 9.6)
4a	111.5		111.4		108.1	
5	131.2	7.58 (s)	131.4	7.60 (s)	145.5	
6	119.0		119.6		104.4	7.42 (s)
7	158.5		158.8		150.6	
8	102.9	7.31 (s)	102.6	7.30 (s)	133.6	
8a	153.1		152.1		145.2	
2'	160.4		160.4		161.1	
3'	112.3	6.15 (d, 9.6)	112.3	6.21 (d, 9.6)	115.2	6.43 (d, 9.6)
4'	144.2	7.92 (d, 9.6)	144.8	8.00 (d, 9.6)	144.9	7.71 (d, 9.6)
4'a	111.3		111.3		116.8	
5'	128.5	7.54 (d, 8.4)	128.8	7.58 (d, 8.4)	115.6	6.96 (d, 8.4)
6'	112.9	6.94 (d, 8.4)	112.8	6.98 (d, 8.4)	118.5	7.03 (d, 8.4)
7'	158.3		158.0		149.0	
8'	111.2		111.1		138.0	
8'a	154.7		154.7		145.5	
1''	100.3	4.99 (d, 7.2)	100.1	5.09 (d, 7.8)	103.2	5.82 (d, 6.6)
2''	73.0	2.92 (m)	73.1	2.93 (m)	75.1	4.28–4.40 (m)
3''	76.6	3.23 (m)	76.3	3.23 (m)	79.5	3.99 (m)
4''	69.6	3.05 (m)	69.4	3.07 (m)	71.4	4.28–4.40 (m)
5''	77.1	3.39 (m)	77.0	3.39 (m)	78.8	4.28–4.40 (m)
6''	60.7	3.39 (m), 3.74 (m)	60.5	3.43 (m), 3.79 (m)	62.5	4.28–4.40 (m)

<sup>a</sup> Measured in DMSO-*d*<sub>6</sub>.<sup>b</sup> Measured in pyridine-*d*<sub>5</sub>.

aglycones, of which NMR spectral data were in accordance with the respective data reported before [11]. These pieces of evidence further confirmed their structures.

Compound **3** was obtained as a white powder. Its HR-ESI-MS gave a pseudomolecular ion peak at  $m/z$  539.0836  $[\text{M} + \text{Na}]^+$ , corresponding to the molecular formula of  $\text{C}_{24}\text{H}_{20}\text{O}_{13}$ . Its  $^1\text{H}$  NMR spectrum displayed signals of two pairs of doublets with an AB coupling pattern ( $\delta = 6.43$ , d,  $J = 9.6$  Hz, H-3';  $\delta = 7.71$ , d,  $J = 9.6$  Hz, H-4';  $\delta = 6.96$ , d,  $J = 8.4$  Hz, H-5';  $\delta = 7.03$ , d,  $J = 8.4$  Hz, H-6'), indicating the existence of a 7,8-dioxygenated coumarin moiety, and signals of two doublets with an AB coupling pattern ( $\delta = 6.36$ , d,  $J = 9.6$  Hz, H-3 and  $\delta = 8.04$ , d,  $J = 9.6$  Hz, H-4) and a singlet ( $\delta = 7.42$ , H-6), indicative of a trioxxygenated coumarin moiety. The  $^{13}\text{C}$  NMR and

DEPT spectra revealed 24 carbon resonances of a methylene, 12 methines, and 11 quaternary C atoms including those for a glucose unit ( $\delta = 103.2$ , 79.5, 78.8, 75.1, 71.4, and 62.5). The anomeric H-1'' of the glucose moiety was determined to be  $\beta$ -oriented on the basis of its coupling constant ( $\delta = 5.82$ , d,  $J = 6.6$  Hz). The HMBC correlation of H-1''/C-7' ( $\delta = 149.0$ ) further suggested that the sugar moiety was attached to C-7' (Figure 2). In fact, the NMR spectra of **3** were very similar to those of daphgilin [13] except for the additional signals due to a  $\beta$ -glucose group. Thus, compound **3** was deduced as 7'-( $\beta$ -glucopyranosyloxy)-5-[(2-oxo-7,8-dihydroxy-2H-1-benzopyran-8-yl)oxy]-2H-1-benzopyran-2-one, and named daphgilin-7'- $O$ - $\beta$ -glucopyranoside. Similar to compounds **1** and **2**, compounds **3** and daphgilin-7'- $O$ - $\beta$ -glucopyranoside

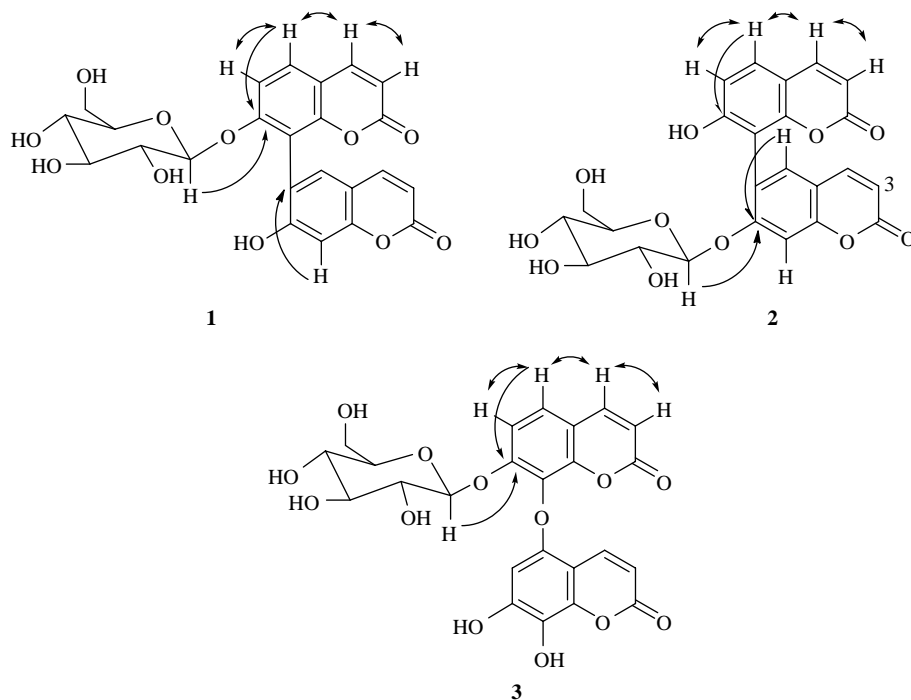


Figure 2. Key HMBC ( $H \rightarrow C$ ) and NOESY ( $H \leftrightarrow H$ ) correlations of compounds **1**–**3**.

(**11**) changed into each other in MeOH–H<sub>2</sub>O solution slowly within a month.

All 11 isolates were tested for inhibitory activity against LPS-induced NO production in RAW 264.7 macrophages. Compounds **4** and **5** showed inhibitory activity against the production

of NO with IC<sub>50</sub> values of 0.161 and 0.127  $\mu$ M, respectively.

Since nitric oxide (NO) plays an important role in the inflammatory process [14], inhibitors of NO release may be considered as potential therapeutic agents in inflammatory diseases [15]. Our

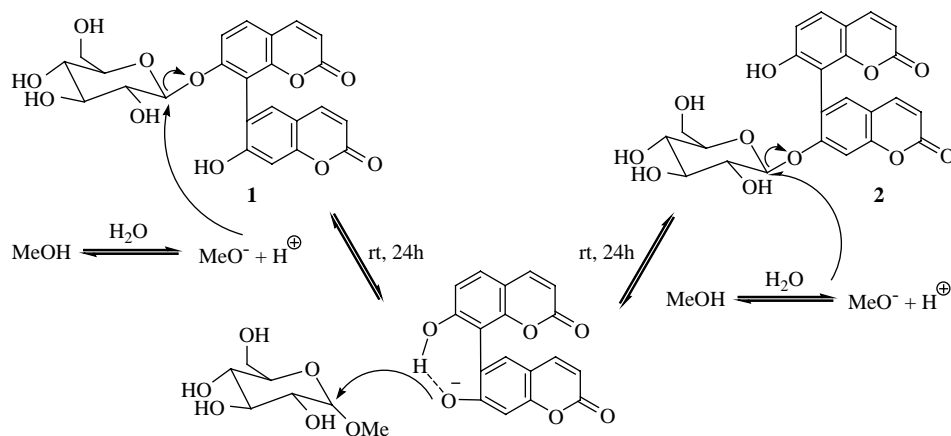


Figure 3. Presumed mechanism of compounds **1** and **2**. (There are two S<sub>N</sub>2 conversions, and absorption configuration of sugar is maintained.)

investigation showed that compounds **4** and **5** may represent potential nitric oxide synthase inhibitors.

### 3. Experimental

#### 3.1 General experimental procedures

Optical rotations were acquired with a Perkin-Elmer 341 polarimeter (Perkin-Elmer, Beaconsfield, UK), whereas UV spectra were obtained by using a Shimadzu UV-2550 UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded on a Bruker Vector 22 spectrometer (Bruker Optik GmbH, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded on a Bruker Avance 400 NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with TMS as an internal standard. HR-ESI-MS were measured using a Q-TOF micro mass spectrometer (Waters, Millford, MA, USA). Materials for column chromatography were silica gel (100–200 mesh; Huiyou Silica Gel Development Co. Ltd, Yantai, China), silica gel H (10–40  $\mu\text{m}$ ; Yantai), Sephadex LH-20 (40–70  $\mu\text{m}$ ; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and YMC-gel ODS-A (50  $\mu\text{m}$ ; YMC, Allentown, PA, USA). Preparative TLC (0.4–0.5 mm) was conducted with glass precoated silica gel GF<sub>254</sub> plates (Yantai). Aminoguanidine, LPS, sulphanilamide, and naphthylethylenediamine were purchased from Sigma-Aldrich (St Louis, MO, USA). RAW 264.7 murine macrophages were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and maintained in media recommended by the suppliers, supplemented with 10% fetal bovine serum (Gibco, Paisley, UK), penicillin (100 U ml<sup>-1</sup>), and streptomycin (100 mg ml<sup>-1</sup>) in a humidified 5% CO<sub>2</sub> atmosphere at 37°C.

#### 3.2 Plant Material

The plant material was collected in July 2006 in Kunming City, Yunnan Province,

China, and identified as *D. feddei* Levl. by Prof. Li-Shan Xie of the Kunming Institute of Botany. A voucher specimen (No. 200607-12) has been deposited in the Herbarium of the School of Pharmacy, Second Military Medical University.

#### 3.3 Extraction and isolation

The air-dried and powdered stem bark of *D. feddei* (6.5 kg) was percolated with methanol for 4 h at room temperature for three times. The solvent was evaporated under reduced pressure. Then, the extract was suspended in H<sub>2</sub>O and partitioned with petroleum ether, EtOAc, and BuOH, successively. The EtOAc extract (400 g) was subjected to column chromatography on silica gel (200–300 mesh, 1000 g), eluted successively with gradient CHCl<sub>3</sub>–MeOH mixtures of increasing polarity and separated into 18 fractions (F<sub>1</sub>–F<sub>18</sub>). F<sub>5</sub> (35 g) was chromatographed on silica gel with CHCl<sub>3</sub>–MeOH (100:0, 100:1) to give compounds **4** (200 mg) and **5** (10 g). F<sub>11</sub> (3.5 g) was chromatographed on silica gel with CHCl<sub>3</sub>–MeOH (50:1, 25:1) followed by Sephadex LH-20 with MeOH to give compounds **6** (30 mg), **7** (10 mg), **8** (200 mg), and **9** (400 mg). F<sub>15</sub> (3.5 g) was chromatographed on silica gel with CHCl<sub>3</sub>–MeOH (10:1) and separated into five sub-fractions (F<sub>15-1</sub>–F<sub>15-5</sub>). F<sub>15-2</sub> was re-purified by preparative thin layer chromatography (PTLC), eluting with CHCl<sub>3</sub>–MeOH (20:1) to afford compounds **1** (10 mg) and **2** (20 mg). F<sub>15-4</sub> was further purified by column chromatography over octadecylsilan (MeOH–H<sub>2</sub>O 30:70, 40:60), affording compounds **3** (400 mg), **10** (150 mg), and **11** (50 mg).

##### 3.3.1 Bicoumol-7'-O- $\beta$ -glucopyranoside (**1**)

A white powder (MeOH); TLC (silica gel, CHCl<sub>3</sub>–MeOH, 10:1):  $R_f = 0.62$ ;  $[\alpha]_D^{18}$ : +9 ( $c$  0.17, MeOH); UV (MeOH):  $\lambda_{\text{max}}$  (log  $\epsilon$ ) 317 (3.29); IR (KBr):  $\nu_{\text{max}}$

3401, 2917, 1708, 1602, 1560, 1498, 1381, 1231, 1037, 829, and 618  $\text{cm}^{-1}$ ; For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data, see Table 1; HR-ESI-MS:  $m/z$  507.0906  $[\text{M} + \text{Na}]^+$  (calculated for  $\text{C}_{24}\text{H}_{20}\text{O}_{11}\text{Na}$ , 507.0903).

### 3.3.2 *Bicoumol-7'-O- $\beta$ -glucopyranoside* (2)

A white powder (MeOH); TLC (silica gel,  $\text{CHCl}_3$ -MeOH, 10:1):  $R_f = 0.50$ ;  $[\alpha]_D^{17}$ : +5 ( $c$  0.14, MeOH); UV (MeOH):  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 317 (3.31); IR (KBr):  $\nu_{\text{max}}$  3411, 2920, 1707, 1602, 1498, 1383, 1312, 1231, 1163, 1078, 901, 829, and 618  $\text{cm}^{-1}$ ; For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data, see Table 1; HR-ESI-MS:  $m/z$  507.0909  $[\text{M} + \text{Na}]^+$  (calculated for  $\text{C}_{24}\text{H}_{20}\text{O}_{11}\text{Na}$ , 507.0903).

### 3.3.3 *Daphgilin-7'-O- $\beta$ -glucopyranoside* (3)

A white powder (MeOH); TLC (silica gel,  $\text{CHCl}_3$ -MeOH, 5:1):  $R_f = 0.46$ ;  $[\alpha]_D^{18}$ : -75 ( $c$  0.10, MeOH); UV (MeOH):  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) 259 (3.42), 313 (3.47); IR (KBr):  $\nu_{\text{max}}$  3421, 2924, 1707, 1619, 1575, 1491, 1384, 1339, 1267, 1166, 1077, 826, and 609  $\text{cm}^{-1}$ ; For  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data, see Table 1; HR-ESI-MS:  $m/z$  539.0836  $[\text{M} + \text{Na}]^+$  (calculated for  $\text{C}_{24}\text{H}_{20}\text{O}_{13}\text{Na}$ , 539.0802).

### 3.3.4 *Acid hydrolysis of compounds 1-3*

Each compound (5 mg) was heated in methanol (10 ml) and 1 M HCl (1 ml) under reflux for 4 h. The solution was concentrated under reduced pressure and diluted with  $\text{H}_2\text{O}$ . Then the residue was extracted with EtOAc (20 ml, three times) and the extract concentrated to afford a white powder. The NMR spectral data of the powder are in good agreement with those of the corresponding aglycone, respectively, and the  $R_f$  value of the monosaccharides from compounds 1-3 is in accordance with D-glucose.

### 3.3.5 *Assay for inhibition ability against LPS-induced NO production*

RAW 264.7 macrophages were seeded in 24-well plates ( $10^5$  cells per well). The cells were pretreated with compounds at different concentrations, aminoguanidine or vehicle solution for 20 min, and then, incubated with LPS ( $1 \mu\text{g ml}^{-1}$ ) for 24 h. The amount of NO was assessed by determining the nitrite concentration in the cultured RAW 264.7 macrophage supernatants with Griess reagent. One hundred microliters of supernatants were incubated, in sequence, with 50  $\mu\text{l}$  1% sulphanilamide and 50  $\mu\text{l}$  0.1% naphthylethylenediamine in 2.5% of phosphoric acid solution. The absorbances at 570 nm were read using an absorbance microplate reader Elx800 (BioTek Instruments, Inc., Winooski, VT, USA) [16].

### Acknowledgements

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### References

- [1] J.R. Hoult and M. Paya, *Gen. Pharmacol.* **27**, 713 (1996).
- [2] M.E. Riveiro, N. Kimpe, A. Moglioni, R. Vázquez, F. Monczor, C. Shayo, and C. Davio, *Curr. Med. Chem.* **17**, 1325 (2010).
- [3] D. Egan, R. Kennedy, E. Moran, D. Cox, E. Prosser, and R.D. Thornes, *Drug Metab. Rev.* **22**, 503 (1990).
- [4] A. Lacy and R. Kennedy, *Curr. Pharm. Des.* **10**, 3797 (2004).
- [5] C.Z. Gu, *Flora Reipublicae Popularis Sinicae*, (Beijing, 1999, Science Press), Vol. 52, p. 378.
- [6] F. Yin and F.C. Lou, *Chin. Pharm. J.* **39**, 20 (2004).
- [7] L.G. Zhuang, O. Seligmann, K. Jurcic, and H. Wagner, *Planta Med.* **45**, 172 (1982).



- [8] Y.L. Ren, J.S. Yang, and J.M. Chen, *Chin. Chem. Lett.* **36**, 732 (2001).
- [9] S. Mitsuhiro and H. Masao, *Phytochemistry* **8**, 1211 (1969).
- [10] S.H. Li, L.J. Wu, H.Y. Gao, Y.H. Chen, and Y. Li, *J. Asian Nat. Prod. Res.* **7**, 839 (2005).
- [11] B.N. Su, E.J. Park, Z.H. Mbwambo, B.D. Santarsiero, A.D. Mesecar, H.S. Fong, J.M. Pezzuto, and A. Douglas, *J. Nat. Prod.* **65**, 1278 (2002).
- [12] W.X. Sun, Q. Zhang, and J.Q. Jiang, *Chin. Chem. Lett.* **17**, 1054 (2006).
- [13] Q. Zhang and J.Q. Jiang, *Chin. J. Med.* **5**, 251 (2007).
- [14] J.B. Calixto, M.F. Otuki, and A.R. Santos, *Planta Med.* **69**, 973 (2003).
- [15] H.J. Lee, N.Y. Kim, M.K. Jang, H.J. Son, K.M. Kim, D.H. Sohn, S.H. Lee, and J.H. Ryu, *Planta Med.* **65**, 104 (1999).
- [16] S.M. Li, X.W. Yang, Y.H. Sheng, L. Feng, Y.H. Wang, H.W. Zheng, X.W. Liu, J.M. Tian, Y.N. Shi, C.L. Long, and W.D. Zhang, *Phytochemistry* **69**, 2200 (2008).