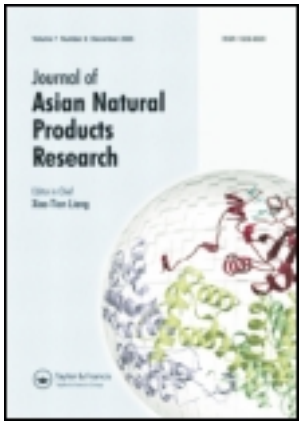

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Three unusual sesquineolignans from *Alpinia conchigera*

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Three unusual sesquineolignans conchignans A (**1**), B (**2**), and C (**3**), together with two known compounds vanillin (**4**) and phloroglucinol (**5**), were isolated from the whole plants of *Alpinia conchigera*. Their structures were established by spectroscopic analysis, including 2D NMR spectroscopic techniques.

Keywords: Zingiberaceae; *Alpinia conchigera*; sesquineolignans; conchignans A–C

1. Introduction

The plants of *Alpinia* genus, belonging to the family of Zingibereae, are mainly distributed in subtropical parts of many countries including south of China [1,2]. Chemical investigations on the plants from this genus led to the isolation of active diarylheptanoids, sesquiterpenes, diterpenes, and phenolics [3–9]. The plant of *Alpinia conchigera* is traditionally used as a Chinese herbal medicine to treat gastrointestinal disorders, indigestion, and snakebite [10]. Previous research on this plant revealed several diarylheptanoids, flavonoids, and phenylpropanoids [11,12]. In our study, three new sesquineolignans conchignans A (**1**), B (**2**), and C (**3**) were isolated from the whole plants of *Alpinia conchigera*, along with two known compounds, namely vanillin (**4**) and phloroglucinol (**5**) [13,14]. Among them, compounds **1**–**3** bearing a tetrahydropyrane ring and containing three C_6–C_3 units that are not linked by a β—β' bond are rare [6,15,16]. Their inhibitory activity against nitric oxide (NO) production in lipopolysaccharide and interferon-γ-induced RAW 264.7 murine macrophages at 25 μM and cytotoxicities against human-tumor A549, SMMC-7721, and HL-60 cell lines at 40 μM were, respectively, tested according to the methods described in Refs. [17,18]. However, none of them showed bioactivity. This paper mainly deals with the isolation and structure elucidation of new compounds **1**–**3** (Figure 1).

2. Results and discussion

Compound (**1**) possessed the molecular formula $C_{28}H_{30}O_5$ as inferred from HR-ESI-MS data at *m/z* 469.2000 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra showed signals assignable to three pairs of *para*-substituted aromatic proton signals {[δ 7.26 (2H, d, $J = 8.5$ Hz, H-2, 6), 6.81 (2H, d, $J = 8.5$ Hz, H-3, 5)], [δ 7.10 (2H, dd, $J = 8.4$, 1.5 Hz, H-2′, 6′), 6.68 (2H, d, $J = 8.4$ Hz, H-3′, 5′)] and [δ 7.10 (2H, dd, $J = 8.4$, 1.5 Hz, H-2″, 6″), 6.74 (2H, d, $J = 8.4$ Hz, H-3″, 5″)]}, a pair of *trans*-olefinic proton signals [δ 6.15 (d, $J = 15.8$ Hz, H-7′), 5.83 (*m*, H-8′)], four methine signals including two oxygenated

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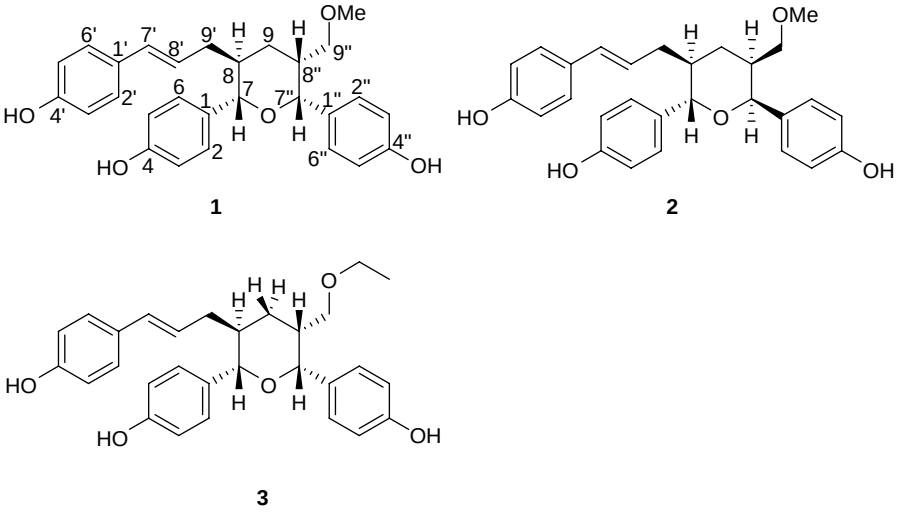

Figure 1. Structures of compounds **1–3**.

ones [δ 4.12 (d, $J = 10.0$ Hz, H-7), 4.73 (1H, d, $J = 2.8$ Hz, H-7″)], three methylene groups, a methoxy group [δ 3.18 (s)], six quaternary carbon signals including three oxygenated ones resonating at δ 157.6 (s, C-4′), 158.2 (s, C-4), and 157.2 (s, C-4″). As shown in Figure 2, the ^{1}H–^{1}H COSY correlations indicated the presence of partial structures drawn in bold lines. Particularly, the unusual linkages between C-8 with C-9′ and C-8″ with C-9 were confirmed by the key ^{1}H–^{1}H COSY correlations (Figure 2) from H-8 to H-9′, and H-8″ to H-9, respectively. In addition, HMBC experiment (Figure 2) showed the key correlations of H-7 with C-1, H-2 and H-3 with C-4, H-8′ with C-1′, H-2′ and H-3′ with C-4′, H-7″ with C-1′, H-2″ and H-3″ with C-4″, H-7 with C-7″. Moreover, the presence of a novel tetrahydropyrane ring was determined by the correlation of H-7 with C-7″. Therefore, the constitution of **1** was determined to be a novel sesquineolignan [6]. The correlations between H_α-8 and H-6, H-9″; H_β-7 and H-9′, H-8″, H-7″ in the NOESY spectrum, together with the coupling constants of 10.0 Hz between H_β-7 and H_α-8, and of 2.8 Hz between H_β-7″ and H_β-8″ [6], determined the stereostructure of the tetrahydropyrane unit of **1** (Figure 3).

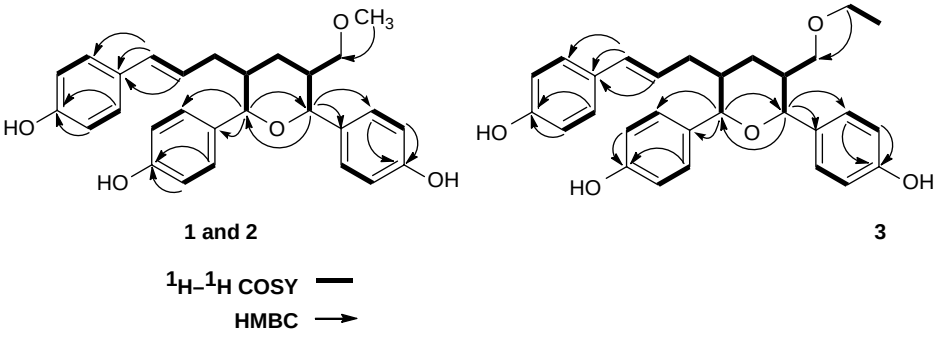

Figure 2. ^{1}H–^{1}H COSY and key HMBC correlations for compounds **1–3**.

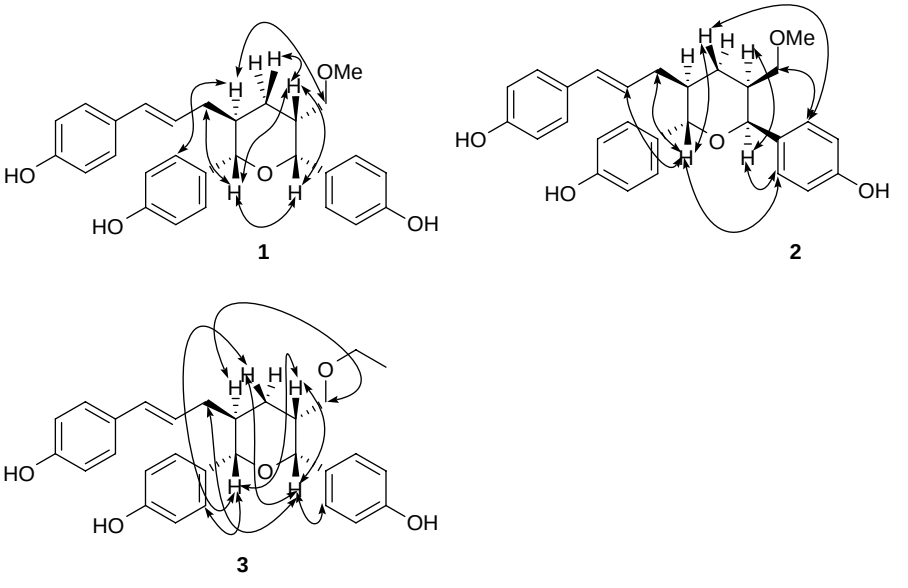

Figure 3. Key NOESY correlations for compounds **1–3**.

Thus, the relative configuration of **1** was determined as shown.

Compound **2** was confirmed as a sesquineolignan with the same planar structure as **1**, by comparison of their NMR data, which was further confirmed by HSQC, ^{1}H–^{1}H COSY, and HMBC correlations (Figure 2). Moreover, NOESY correlations between H_β-7 and H-8′, H-9′, H_β-9, and H-6″; H-9″ and H-2″ revealed that H-8, H-7″, and H-8″ were all α-oriented (Figure 3), which were further confirmed by the coupling constants of 9.7 Hz between H_β-7 and H_α-8, and of 5.8 Hz between H_α-7″ and H_α-8″ (Table 1).

A detailed comparison of the NMR spectroscopic data of **3** with those of **1** indicated that they were both analog compounds. The most prominent difference between them was that the methyl group was replaced by ethyl in **3**, which was confirmed by HMBC correlation of methylene protons [δ 3.33 (q, $J = 7.0$ Hz)] with C-9″ and ^{1}H–^{1}H COSY correlation of these protons with methyl ones at δ 1.05 (t, 7.0) in **3** (Figure 2). Thus, they were also sesquineolignans with the same relative configuration, which was supported by HSQC, ^{1}H–^{1}H COSY, HMBC, and ROESY experiments (Figures 2 and 3), and the coupling constants of H-7/H-8 and H-7″/H-8″ (Table 1).

3. Experimental

3.1 General experimental procedures

NMR spectra were measured on a Bruker AVANCE III-600 instrument (Bruker BioSpin International AG, Karlsruhe, Germany) with TMS as internal standard, δ in ppm, J in Hz. IR spectra were obtained on a Bio-Rad FTS-135 spectrometer (Bio-Rad Laboratories, Inc., Richmond, CA, USA). UV spectra were recorded on a Shimadzu 210A double-beam spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Optical rotations were recorded on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). EI and HR-EI-MS were measured on Waters Autospec Premier P776 (Water Corporation, Billerica, MA, USA). ESI and HR-ESI-MS were recorded on a API Qstar Pulsar instrument (Applied Biosystems/MDS Sciex, Ontario, Vaughan, Canada). Column chromatog-

Table 1. ^{1}H NMR spectral data of compounds **1**–**3** (600 MHz, δ in ppm and J values in Hz).

Position	**1**[a]	**2**[a]	**2**[b]	**3**[a]
2	7.26 (d, $J = 8.5$)	7.12 (d, $J = 8.0$)	7.47 (d, $J = 8.5$)	7.26 (d, $J = 8.5$)
3	6.81 (d, $J = 8.5$)	6.74 (d, $J = 8.0$)	7.22 (overlapped)	6.81 (d, $J = 8.5$)
5	6.81 (d, $J = 8.5$)	6.74 (d, $J = 8.0$)	7.22 (overlapped)	6.81 (d, $J = 8.5$)
6	7.26 (d, $J = 8.5$)	7.12 (d, $J = 8.0$)	7.47 (d, $J = 8.5$)	7.26 (d, $J = 8.5$)
7	4.12 (d, $J = 10.0$)	4.36 (d, $J = 9.8$)	4.76 (d, $J = 9.7$)	4.14 (d, $J = 10.1$)
8	1.94 (m)	2.00 (m)	2.21 (m)	1.96 (m)
9	2.35 (m, H_α) 1.62 (m, H_β)	1.99 (m, H_α), 1.72 (m, H_β)	2.25 (m, H_α), 1.94 (m, H_β)	2.37 (m, H_α), 1.61 (m, H_β)
2′	7.10 (dd, $J = 8.4, 1.5$)	7.14 (d, $J = 8.4$)	7.48 (d, $J = 8.5$)	7.11 (dd, $J = 8.7, 2.3$)
3′	6.68 (d, $J = 8.4$)	6.70 (d, $J = 8.4$)	7.23 (overlapped)	6.67 (d, $J = 8.7$)
5′	6.68 (d, $J = 8.4$)	6.70 (d, $J = 8.4$)	7.23 (overlapped)	6.67 (d, $J = 8.7$)
6′	7.10 (dd, $J = 8.4, 1.5$)	7.14 (d, $J = 8.4$)	7.48 (d, $J = 8.5$)	7.11 (dd, $J = 8.7, 2.3$)
7′	6.15 (d, $J = 15.8$)	6.19 (d, $J = 15.6$)	6.48 (d, $J = 15.7$)	6.16 (d, $J = 15.7$)
8′	5.83 (m)	5.89 (m)	6.20 (m)	5.83 (m)
9′	2.00 (m) 1.80 (m)	2.01 (m), 1.86 (m)	2.35 (m), 2.09 (m)	1.99 (m), 1.80 (m)
2″	7.10 (dd, $J = 8.4, 1.5$)	7.33 (d, $J = 8.4$)	7.71 (*d*, $J = 8.5$)	7.11 (dd, $J = 8.7, 2.3$)
3″	6.74 (d, $J = 8.4$)	6.76 (d, $J = 8.4$)	7.27 (d, $J = 8.5$)	6.71 (d, $J = 8.7$)
5″	6.74 (d, $J = 8.4$)	6.76 (d, $J = 8.4$)	7.27 (d, $J = 8.5$)	6.71 (d, $J = 8.7$)
6″	7.10 (dd, $J = 8.4, 1.5$)	7.33 (d, $J = 8.4$)	7.71 (d, $J = 8.5$)	7.11 (dd, $J = 8.7, 2.3$)
7″	4.73 (d, $J = 2.8$)	4.93 (overlapped)	5.39 (d, $J = 5.8$)	4.75 (d, $J = 2.5$)
8″	2.20 (m)	2.60 (m)	2.88 (m)	2.23 (m)
9″	3.64 (t, $J = 10.1$), 3.08 (dd, $J = 10.1, 4.3$)	3.26 (m), 3.18 (m)	3.36 (d, $J = 7.2$)	3.66 (t, $J = 10.0$), 3.16 (dd, $J = 10.0, 4.3$)
9″-OCH_3	3.18 (s)	3.18 (s)	3.18 (s)	
9″-OCH_2CH_3				3.33 (q, $J = 7.0$)
9″-OCH_2CH_3				1.05 (t, $J = 7.0$)

[a] The spectra were recorded in CD_3OD at 600 MHz.
[b] The spectra were recorded in C_5D_5N at 600 MHz.

raphy (CC) was carried out on silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Ltd. Co., Qingdao, China), silica gel H (60 μm, Qingdao Marine Chemical Ltd. Co.), and Lichroprep RP-18 gel (40–63 μM, Merck, Darmstadt, Germany). Semiprep. reverse-phase (RP) HPLC was subjected to an Agilent 1100 liquid chromatograph, with a Zorbax SB-C_{18} column. MCI was done on CHP-20P (75–150 μm, Mitsubishi Chemical Co., Tokyo, Japan).

3.2 Plant material

The whole plants of *Alpinia conchigera* were collected from Mengla county of Xishuangbanna, Yunnan province, China, and identified by Dr. Tao Su of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, where a voucher number (HITBC048450) has been deposited.

3.3 Extraction and isolation

The air-dried powdered whole plants (12 kg) were extracted with methanol under reflux for 8 h (3 × 30 liters). The resulted residue was partitioned between EtOAc and H_2O, and then between *n*-BuOH and H_2O. CC of the EtOAc extract (180 g) was carried out on silica gel, eluting with petroleum ether–acetone (9:1–1:1) to yield nine fractions (Frs 1–9). Fr. 3 (9 g) was subjected to CC (RP-18, MeOH–H_2O; 15:85–1:0) to afford five subfractions (Fr. 3.1–Fr. 3.5). Fr. 3.1 (1.5 g) was subjected to CC (silica gel, $CHCl_3$–EtOAc; 9:1) to give compounds **4** (6 mg) and **5** (8 mg). Fr. 3.2 (2 g) was subjected to CC (silica gel, $CHCl_3$–EtOAc; 9:1) and further purified by CC (MCI) and HPLC (MeOH–H_2O 4:6; wavelength, 265 nm; flow rate, 2.0 ml/min) to yield compounds **1** (4 mg, Rt = 35 min), **2** (3 mg, Rt = 36 min), and **3** (4 mg, Rt = 39 min).

3.3.1 Conchignan A (*1*)

Colorless gum. $[\alpha]_D^{20.7}$ −13.67 ($c = 0.18$, MeOH). UV (MeOH) λ_{max} (log ε): 262 (4.13), 201 (4.41). IR (KBr): ν_{max} 3419, 2922, 1613, 1514, 1446, 1232, 1082, 1051 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-ESI-MS: *m/z* 469.2000 $[M + Na]^+$ (calcd for $C_{28}H_{30}NaO_5$, 469.1990).

3.3.2 Conchignan B (*2*)

Colorless gum. $[\alpha]_D^{20.7}$ −4.90 ($c = 0.51$, MeOH). UV (MeOH) λ_{max} (log ε): 263 (4.18), 226 (4.31), 201 (4.55) nm. IR (KBr): ν_{max} 3424, 2924, 1613, 1514, 1450, 1384, 1086, 1033 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-EI-MS *m/z*: 446.2082 $[M]^+$ (calcd for $C_{28}H_{30}O_5$, 446.2093).

3.3.3 Conchignan C (*3*)

Colorless gum. $[\alpha]_D^{17.3}$ −12.38 (*c* 0.15, MeOH). UV (MeOH) λ_{max} (log ε): 262 (4.11), 201 (4.43) nm. IR (KBr): ν_{max} 3422, 2921, 1613, 1514, 1446, 1384, 1236, 1170, 1088, 835 cm^{-1}. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-ESI-MS *m/z*: 483.2142 $[M + Na]^+$ (calcd for $C_{29}H_{32}NaO_5$, 483.2147).

3.4 Cell lines and assay

Three cancer cell lines, SMMC-7721 (human hepatocellular carcinoma), A549 (human non-small cell lung carcinoma), and Hela (human cervical carcinoma), were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) under a humidified atmosphere of 5% CO_2 at 37°C. Cytotoxicities of compounds **1–3** were measured by the sulforhodamine B method [17,18]. The murine monocytic macrophage cell lines RAW 264.7 were cultured in RPMI 1640 medium (Hyclone, Logan, UT, USA) with 10% FBS under a humidified atmosphere

Table 2. ^{13}C NMR spectral data of compounds **1**–**3** (150 MHz).

Position	**1**[a]	**2**[a]	**2**[b]	**3**[a]
1	134.0 (s)	133.3 (s)	133.6 (s)	134.1 (s)
2	129.9 (d)	130.2 (d)	130.2 (d)	130.1 (d)
3	116.0 (d)	116.1 (d)	117.0 (d)	116.1 (d)
4	158.2 (s)	158.1 (s)	159.1 (s)	158.2 (s)
5	116.0 (d)	116.1 (d)	117.0 (d)	116.1 (d)
6	129.9 (d)	130.2 (d)	130.2 (d)	130.1 (d)
7	87.5 (d)	78.6 (d)	78.2 (d)	87.7 (d)
8	38.1 (d)	42.9 (d)	42.8 (d)	38.4 (d)
9	32.7 (t)	29.7 (t)	29.8 (t)	33.0 (t)
1′	130.8 (s)	130.8 (s)	130.1 (s)	130.8 (s)
2′	128.2 (d)	128.2 (d)	128.5 (d)	128.3 (d)
3′	116.2 (d)	116.3 (d)	116.5 (d)	116.3 (d)
4′	157.6 (s)	158.4 (s)	158.9 (s)	157.8 (s)
5′	116.2 (d)	116.3 (d)	116.5 (d)	116.3 (d)
6′	128.2 (d)	128.2 (d)	128.5 (d)	128.3 (d)
7′	132.3 (d)	132.3 (d)	132.4 (d)	132.5 (d)
8′	125.7 (d)	125.5 (d)	125.8 (d)	125.9 (d)
9′	36.7 (t)	36.9 (t)	37.0 (t)	36.7 (t)
1″	133.3 (s)	131.1 (s)	131.2 (s)	133.5 (s)
2″	127.4 (d)	132.2 (d)	132.1 (d)	127.5 (d)
3″	115.7 (d)	115.9 (d)	116.5 (d)	115.8 (d)
4″	157.2 (s)	157.7 (s)	158.9 (s)	157.3 (s)
5″	115.7 (d)	115.9 (d)	116.5 (d)	115.8 (d)
6″	127.4 (d)	132.2 (d)	132.1 (d)	127.5 (d)
7″	81.4 (d)	78.2 (d)	77.2 (d)	81.5 (d)
8″	40.7 (d)	41.3 (d)	41.0 (d)	40.9 (d)
9″	71.1 (t)	75.9 (t)	75.6 (t)	69.0 (t)
9″-OCH_3	59.0 (q)	59.0 (q)	59.1 (q)	
9″-OCH_2CH_3				67.4 (t)
9″-OCH_2CH_3				15.6 (*q*)

[a] The spectra were recorded in CD_3OD at 150 MHz.
[b] The spectra were recorded in C_5D_5N at 150 MHz.

of 5% CO_2 at 37°C. Inhibitory activity of NO production of compounds **1–3** was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [17].

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References

[1] Delectis Florae Reipularis Agendae Academiae Sinicae Edita, *Flora Reipublicae Popularis Sinicae* (Science Press, Beijing, 1981), Vol. 16, p. 148.
[2] Insitute Botanicum Kunmingense Academiae Sinicae Edita, *Flora Yunnancia* (Science Press, Beijing, 1997), Vol. 8, p. 96.
[3] Y. Tezuka, M.B. Gewali, M.S. Ali, A.H. Banskota, and S. Kadota, *J. Nat. Prod.* **64**, 208 (2001).
[4] L.Y. Kong, M.J. Qin, and M. Niwa, *Planta Med.* **68**, 813 (2002).
[5] L.K. Sy and G.D. Brown, *Phytochemistry* **45**, 537 (1997).
[6] T. Morikawa, S. Ando, H. Matsuda, S. Kataoka, O. Muraoka, and M. Yoshikawa, *Chem. Pharm. Bull.* **53**, 625 (2005).

[7] T.N. Ly, M.S. Yamada, K. Kato, and R.J. Yamauchi, *J. Agric. Food Chem.* **51**, 4924 (2003).
[8] J.J. Xu, C.J. Ji, Y.M. Zhang, J. Su, Y. Li, and N.H. Tan, *Bioorg. Med. Chem. Lett.* **22**, 1660 (2012).
[9] J.J. Xu, N.H. Tan, Y.S. Chen, X.L. Pan, G.Z. Zeng, H.J. Han, C.J. Ji, and M.J. Zhu, *Helv. Chim. Acta* **92**, 1621 (2009).
[10] Jiangsu New Medical College, *Dictionary of Chinese Herb Medicine* (Shanghai Scientific and Technologic Press, Shanghai, 1997), (pp. 538).
[11] K. Awang, M.N. Azmi, L.I.L. Aun, A.N. Aziz, H. Ibrahim, and N.H. Nagoor, *Molecules* **15**, 8048 (2010).
[12] S. Athamaprasangsa, U. Buntrarongroj, P. Dampawan, N. Ongkavoranan, V. Rukachaisirikul, S. Sethjinda, M. Sornnarintra, P. Sriwub, and W.C. Taylor, *Phytochemistry* **37**, 871 (1994).
[13] F.J. Hanke and I. Kubo, *J. Nat. Prod.* **52**, 1237 (1989).
[14] J.H. Lee, J.C. Park, and J.S. Choi, *Arch. Pharmacol. Res.* **19**, 223 (1996).
[15] B.N. Su and Y. Takaishi, *Chem. Lett.* ***12***, 1315 (1999).
[16] B.N. Su, Y. Takaishi, and T. Kusumi, *Tetrahedron* **55**, 14571 (1999).
[17] J.T. Fan, J. Su, Y.M. Peng, Y. Li, J. Li, Y.B. Zhou, G.Z. Zeng, H. Yan, and N.H. Tan, *Bioorg. Med. Chem.* **18**, 8226 (2010).
[18] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, and M.R. Boyd, *J. Natl. Cancer* **82**, 1107 (1990).