



8-9' linked neolignans with cytotoxicity from *Alpinia conchigera*

Jun-Ju Xu^{a,b}, Guang-Zhi Zeng^c, Sheng-Chao Yang^a, Yong Shen^{a,*}, Ning-Hua Tan^{c,*}

^a Yunnan Research Center on Good Agricultural Practice for Dominant Chinese Medicinal Materials, Yunnan Agricultural University, Kunming 650201, P. R. China

^b College of Tobacco Science, Yunnan Agricultural University, Kunming 650201, P. R. China

^c State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P. R. China

ARTICLE INFO

Article history:

Received 20 June 2013

Accepted in revised form 12 August 2013

Available online 23 August 2013

Keywords:

Zingiberaceae

Alpinia conchigera

Neolignans

Conchigeranals A–E

Cytotoxicity

ABSTRACT

Five new 8-9' linked neolignans conchigeranals A–E (**1–5**), together with three known compounds galanganal (**6**), galanganols A (**7**) and B (**8**), were isolated from the whole plant of *Alpinia conchigera*. Their structures were established by spectroscopic analysis, including 2D-NMR spectroscopic techniques. Cytotoxicities of compounds **1–8** were tested against two cancer cell lines A549 and Hela. Results showed that **4**, **5**, **7** and **8** exhibited cytotoxicity against A549 with the IC₅₀ values of 12.36, 9.72, 10.26, 13.05 µg/ml, respectively, and **1–8** against Hela with the IC₅₀ values from 1.53 to 5.29 µg/ml.

© 2013 Published by Elsevier B.V.

1. Introduction

The genus *Alpinia* belongs to the family of Zingiberaceae, and comprises approximately 250 species throughout the world, 46 of which grow in China [1,2]. Some plants from this genus have been used as a folk medicine for the treatment of various diseases in China and phytochemical investigations of this genus revealed the presence of diarylheptanoids [3–5], sesquiterpenes [6–8], diterpenes [9,10], and phenolics [11–13]. However, seldom neolignans were isolated from this genus [12,13]. The plant of *Alpinia conchigera* is traditionally used for treating gastrointestinal disorders, indigestion and snakebite [14]. Previous studies of this plant have reported the isolation of diarylheptanoids, flavonoids and phenylpropanoids [15,16]. In our study, five rare 8-9' linked neolignans conchigeranals A–E (**1–5**), together with three known compounds galanganal (**6**), galanganols A (**7**) and B (**8**) [13], were isolated from the whole plant of *Alpinia conchigera*. Moreover, the cytotoxicities of compounds **1–8** were measured in vitro against three cancer cell lines, A549, Hela and SMMC-7721 according to the method

[17,18]. In this paper, we report the isolation, identification and structure elucidation of these compounds, along with their cytotoxic results.

2. Experimental

2.1. General

Column chromatography (CC) was performed on silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Ltd. Co., China), silica gel H (60 µm, Qingdao Marine Chemical Ltd. Co., China) and Lichroprep RP-18 gel (40–63 µm, Merck, Germany); Semiprep. reverse-phase (RP) HPLC was subjected to an Agilent 1100 liquid chromatograph with a Zorbax SB-C₁₈ column; MCI was performed on CHP-20P (75–150 µm, Mitsubishi Chemical Co. Japan); NMR spectra were recorded on a Bruker AVANCE III-600 instrument with chemical shifts given in ppm (δ) using TMS as an internal standard; IR Spectra (KBr pellets) were obtained on a Bio-Rad FTS-135 spectrometer; UV spectra were recorded on a Shimadzu 210A double-beam spectrophotometer with λ_{max} (log ε) in cm^{−1}; Optical rotations were recorded on a Jasco DIP-370 digital polarimeter; EI and HR-EI-MS were recorded on a Waters Autospec Premier P776.

* Corresponding authors. Tel./fax: +86 871 65227213.

E-mail addresses: pharmsy@sina.com (Y. Shen), nhtan@mail.kib.ac.cn (N.-H. Tan).

Table 1¹H NMR data of **1–5** at 600 MHz in MeOD; δ in ppm, *J* in Hz.

H	1	2	3	4	5
2	6.90 (s)	7.16 (d, 8.4)	7.13 (d, 8.4)	7.12 (d, 8.3)	7.10 (d, 8.5)
3		6.81 (d, 8.4)	6.80 (d, 8.4)	6.77 (d, 8.3)	6.73 (d, 8.5)
5	6.81 (d, 8.0)	6.81 (d, 8.4)	6.80 (d, 8.4)	6.77 (d, 8.3)	6.73 (d, 8.5)
6	6.78 (d, 8.0)	7.16 (d, 8.4)	7.13 (d, 8.4)	7.12 (d, 8.3)	7.10 (d, 8.5)
7	4.20 (d, 10.0)	4.19 (d, 10.0)	4.07 (d, 7.2)	4.17 (d, 6.8)	4.10 (d, 7.8)
8	2.83 (m)	2.81 (m)	2.12 (m)	1.86 (m)	1.88 (m)
9			4.26 (dd, 6.0, 10.8), 4.19 (dd, 3.6, 10.8)	3.48 (dd, 5.8, 11.5), 3.30 (m)	3.75 (dd, 4.9, 10.8), 3.61 (dd, 4.7, 10.8)
2'	7.08 (d, 8.5)	7.08 (d, 8.5)	7.13 (d, 8.4)	7.16 (d, 8.4)	7.10 (d, 8.5)
3'	6.66 (d, 8.5)	6.66 (d, 8.5)	6.68 (d, 8.4)	6.68 (d, 8.4)	6.65 (d, 8.5)
5'	6.66 (d, 8.5)	6.66 (d, 8.5)	6.68 (d, 8.4)	6.68 (d, 8.4)	6.65 (d, 8.5)
6'	7.08 (d, 8.5)	7.08 (d, 8.5)	7.13 (d, 8.4)	7.16 (d, 8.4)	7.10 (d, 8.5)
7'	6.14 (d, 15.7)	6.13 (d, 15.7)	6.16 (d, 15.6)	6.29 (d, 15.8)	6.15 (d, 15.7)
8'	5.76 (m)	5.75 (m)	5.87 (m)	6.01 (m)	5.86 (m)
9'	2.17 (m), 1.96 (m)	2.18 (m), 1.94 (m)	2.10 (m), 2.01 (m)	2.41 (m), 2.30 (m)	2.01 (m)
3-OCH ₃	3.86 (s)				
7-OCH ₃	3.10 (s)	3.07 (s)	3.14 (s)	3.19 (s)	3.09 (s)
9-OCH ₃	3.67 (s)	3.67 (s)			
9-OCOCH ₃			2.03 (s)		

2.2. Plant materials

The whole plant of *Alpinia conchigera* was collected from Mengla county of Xishuangbanna, Yunnan province, People's Republic of China in October, 2010, and identified by Doc. *Tao Su* of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, where a voucher number (HITBC048450) was deposited.

2.3. Extraction and isolation

The air-dried powdered whole plant (12 kg) was extracted with methanol under reflux for 8 h (3 × 30 L). The resulting residue was partitioned between AcOEt and H₂O, and then *n*-BuOH and H₂O. Column chromatography with the AcOEt extract (180 g) was performed on silica gel, eluting with petroleum ether/Me₂CO (9:1 to 1:1, V/V) to yield nine fractions (Fr.) 1–9. Fr. 4 (12 g) was subjected to CC (RP-18, MeOH/H₂O 15:85–1:0, V/V) to afford 4 subfractions (Fr.) 4.1–4.4. Fr. 4.1 was subjected to CC (silica gel, CHCl₃/AcOEt 8:2, V/V) and further purified by CC (MCI) and HPLC (MeOH/H₂O 4:6, V/V) to yield **1** (5 mg), **2** (3 mg), **3** (5 mg) and **6** (10 mg). Fr. 4.3 was subjected to CC (silica gel, CHCl₃/AcOEt 8:2, V/V) and petroleum ether/Me₂CO 75:25, V/V) and further purified by HPLC (MeOH/H₂O 4:6, V/V) to yield **4** (3 mg), **5** (6 mg). Fr. 4.4 was subjected to CC (silica gel, petroleum ether/Me₂CO 8:2 and petroleum ether/AcOEt 6:4, V/V) and further purified by HPLC (CH₃CN/H₂O 4:6, V/V) to yield **7** (6 mg), **8** (4 mg).

Conchigeranal A (**1**): colorless oil. $[\alpha]_D^{18.8} - 5.53^\circ$ (*c* = 0.26, MeOH). UV (MeOH): 263 (3.95), 203 (4.29). IR (KBr): 3431, 2924, 1632, 1514. ¹H- and ¹³C-NMR: see [Tables 1 and 2](#). HR-EI-MS *m/z*: 372.1570 ([M]⁺, C₂₁H₂₄O₆; calcd. 372.1573).

Conchigeranal B (**2**): colorless oil. $[\alpha]_D^{18.7} - 1.60^\circ$ (*c* = 0.58, MeOH). UV (MeOH): 262 (4.15), 204 (4.39). IR (KBr): 3424, 2925, 1713, 1613, 1442, 1216. ¹H and ¹³C NMR spectral data see [Tables 1 and 2](#). HR-EI-MS *m/z*: 342.1462 ([M]⁺, C₂₀H₂₂O₅; calcd. 342.1467).

Conchigeranal C (**3**): colorless oil. $[\alpha]_D^{20.7} - 9.67^\circ$ (*c* = 0.17, MeOH). UV (MeOH): 263 (4.13), 203 (3.76). IR (KBr): 3431, 2924, 1635, 581. ¹H and ¹³C NMR spectral data see [Tables 1 and 2](#). HR-EI-MS *m/z*: 356.1619 ([M]⁺, C₂₁H₂₄O₅; calcd. 356.1624).

Conchigeranal D (**4**): colorless oil. $[\alpha]_D^{19.1} - 6.07^\circ$ (*c* = 0.30, MeOH). UV (MeOH): 262 (4.11), 204 (4.36). IR (KBr): 3430, 2924, 1633, 1514, 1229. ¹H and ¹³C NMR spectral data see [Tables 1 and 2](#). HR-EI-MS *m/z*: 314.1520 ([M]⁺, C₁₉H₂₂O₄; calcd. 314.1518).

Conchigeranal E (**5**), colorless oil. $[\alpha]_D^{19.1} - 0.61^\circ$ (*c* = 0.11, MeOH). UV (MeOH): 262 (4.11), 203 (4.30). IR (KBr): 3431, 2925, 1629, 1514, 1234. ¹H and ¹³C NMR spectral data see [Tables 1 and 2](#). HR-EI-MS *m/z*: 314.1522 ([M]⁺, C₁₉H₂₂O₄; calcd. 314.1518).

Table 2¹³C NMR data of **1–5** at 150 MHz in MeOD; δ in ppm.

C	1	2	3	4	5
1	131.4 (s)	130.3 (s)	131.9 (s)	132.4 (s)	132.1 (s)
2	111.9 (d)	130.1 (d)	130.0 (d)	129.5 (d)	129.9 (d)
3	149.4 (s)	116.4 (d)	116.3 (d)	116.0 (d)	116.1 (d)
4	148.0 (s)	158.9 (s)	158.5 (s)	158.0 (s)	158.2 (s)
5	116.1 (d)	116.4 (d)	116.3 (d)	116.0 (d)	116.1 (d)
6	122.1 (d)	130.1 (d)	130.0 (d)	129.5 (d)	129.9 (d)
7	86.3 (d)	86.0 (d)	84.7 (d)	84.4 (d)	85.5 (d)
8	55.0 (d)	55.0 (d)	46.1 (d)	49.2 (d)	48.8 (d)
9	176.5 (t)	176.6 (t)	65.0 (t)	62.0 (t)	62.2 (t)
1'	130.5 (s)	130.5 (s)	130.8 (s)	130.9 (s)	130.8 (s)
2'	128.3 (d)	128.3 (d)	128.3 (d)	128.1 (d)	128.1 (d)
3'	116.3 (d)	116.2 (d)	116.3 (d)	116.2 (d)	116.2 (d)
4'	157.9 (s)	157.9 (s)	157.9 (s)	157.6 (s)	157.6 (s)
5'	116.3 (d)	116.2 (d)	116.3 (d)	116.2 (d)	116.2 (d)
6'	128.3 (d)	128.3 (d)	128.3 (d)	128.1 (d)	128.1 (d)
7'	133.0 (d)	132.9 (d)	133.0 (d)	132.5 (d)	132.5 (d)
8'	124.0 (d)	123.8 (d)	125.6 (d)	126.6 (d)	126.2 (d)
9'	33.9 (t)	34.0 (t)	32.8 (t)	30.9 (t)	32.1 (t)
3-OCH ₃	56.5 (q)				
7-OCH ₃	56.7 (q)	56.6 (q)	57.0 (q)	57.0 (q)	56.7 (q)
9-OCH ₃	52.1 (q)	52.1 (q)			
9-OCOCH ₃			173.3 (s)		
9-OCOCH ₃			21.0 (q)		

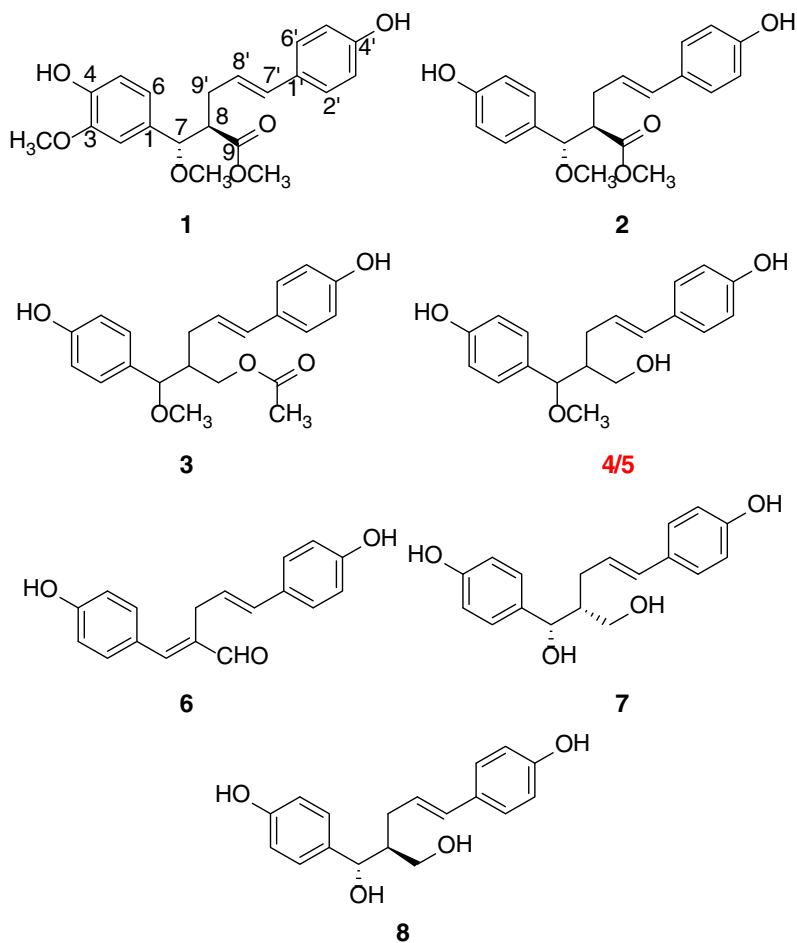


Fig. 1. Structures of compounds 1–8 isolated from *Alpinia conchigera*.

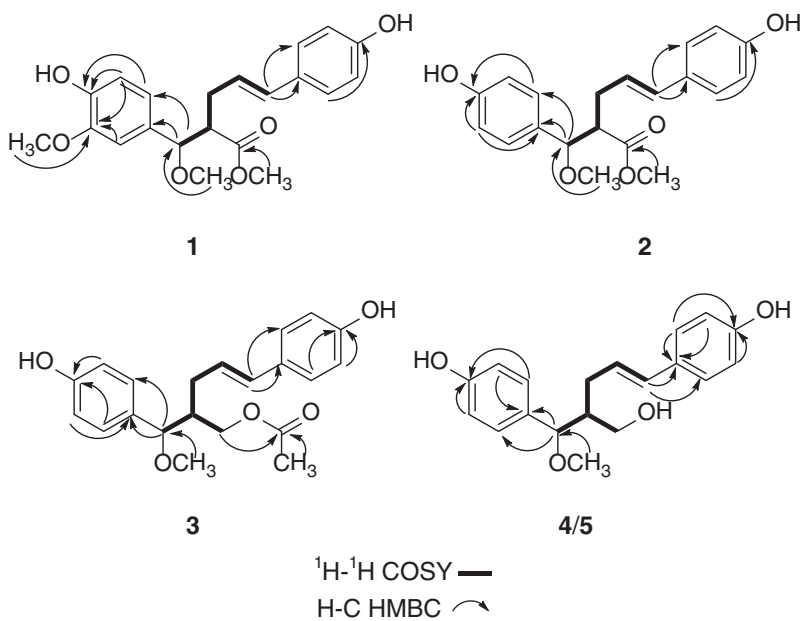


Fig. 2. ¹H–¹H COSY and key HMBC correlations for compounds 1–5.

2.4. Cytotoxicity assay in vitro (SRB method)

Cytotoxicities of compounds **1–8** against two cancer cell lines, A549 (human non-small cell lung carcinoma) and Hela (human cervical carcinoma) were measured by the SRB method. Briefly, cells were plated in 96-well culture plates. After 24 h the cells were treated with serial dilutions of compounds **1–8** with the maximum concentration of 50 µg/ml. Each compound was initially dissolved in DMSO and further diluted in medium to produce different concentrations. After 48 h cells were fixed by the addition of 25 µl of ice-cold 50% TCA (trichloroacetic acid) and incubated at 4 °C for 1 h. After being washed with distilled water and air-dried, the plate was stained for 15 min with 100 µl of 0.4% SRB (sulforhodamine B, Sigma) in 1% glacial acetic acid. The plates were washed with 1% acetic acid and air-dried overnight. For reading the plate, the bound dye (SRB) was solubilized with 100 µl of 10 mM Tris base solution. And the absorbance was measured at 560 nm on a Molecular Devices SpectraMax 340 Microplate spectrophotometer (MWG-Biotech, Inc., Sunnyvale, USA). Cell survival was measured as the percentage absorbance compared to the untreated control. Taxol was used as a positive control.

3. Results and discussion

Compound **1** was obtained as a colorless oil, and HR-EI-MS data gave a molecular ion peak at m/z 372.1570 (calcd for 372.1573), corresponding to a molecular formula of $C_{21}H_{24}O_6$. The 1H NMR spectrum showed a *para*-substituted aromatic ring at δ_H 7.08 (d, $J = 8.5$ Hz) and 6.66 (d, $J = 8.5$ Hz), a 1,3,4-trisubstituted phenyl group at δ_H 6.90 (s), 6.81 (d, $J = 8.0$ Hz) and 6.78 (d, $J = 8.0$ Hz), three methoxyl singlets at δ_H 3.86 (s), 3.10 (s) and 3.67 (s), a methylene, four methines including an oxymethine proton at δ_H 4.20 (d, $J = 10.0$ Hz), and a trans-olefin pair at δ_H 6.14 (d, $J = 15.7$ Hz) and 5.76 (m). The ^{13}C -NMR data additionally revealed the presence of six quaternary carbons including an ester carbonyl group at δ_C 176.5 (s) and five aromatic carbons.

From the 1H - 1H COSY spectrum, the protons resonating at δ_H 4.20 (1H, d, $J = 10.0$ Hz, H-7), 2.83 (1H, m, H-8), 2.17 (1H, m, H-9a), 1.96 (1H, m, H-9b), 5.76 (1H, m, H-8'), (1H, d, $J = 15.7$ Hz, H-7'), were assigned to a moiety CH(7)/CH(8)/CH₂(9')/CH(8')/CH(7') (Fig. 2). The location of three methoxyl groups on C-3, C-7 and C-9' was confirmed via HMBC correlations between the methoxyl protons at δ_H 3.86 (s), 3.10 (s), 3.67 (s) with carbons at δ_C 149.4 (s, C-3), 86.3 (d, C-7) and 176.5 (s, C-9), respectively (Fig. 2). Thus, the planar structure of compound **1** was determined as a neolignan as shown in Fig. 1. In addition, the larger coupling constant of 10.0 Hz between H-7 and H-8, compared with that of a known analogues galanganol B (**8**), suggested that the relative configuration of 7-8 positions was a 7,8-*threo*-form [13].

The molecular formula $C_{20}H_{22}O_5$ of compound **2** was determined on the basis of HR-EI-MS data at m/z 342.1462 (Calcd for 342.1467). The 1H NMR and ^{13}C -NMR spectrum of **2** were similar to that of compound **1**, except that H-3 [δ_H 6.81 (1H, d, $J = 8.4$ Hz)] of **2** replaced the 3-methoxy group [δ_H 3.86 (1H, s)] of **1**. This was supported by HMBC correlations between H-3 with C-1 (δ_C 130.3) and C-4 (δ_C 158.9). The relative stereostructure between H-7 and H-8 was also elucidated to be a 7,8-*threo*-form by the larger coupling constant $J_{7,8} = 10.0$ Hz

[13]. Therefore, the structure of compound **2** was elucidated as shown in Fig. 1. The full assignment of **2** was confirmed by HSQC, 1H - 1H COSY and HMBC.

The 1H NMR spectrum of **3** showed the resonances of two sets of aromatic proton signals assignable to two *para*-substituted aromatic rings, four methines including an oxygenated one and two olefinic ones attributing to an (*E*)-double bond, two methenes, a methoxy group, and an acetoxy group, which was similar to those of compound **2**. In the COSY spectrum, a spin system corresponding to CH(7), CH₂(9)/CH(8)/CH₂(9')/CH(8')/CH(7') was observed (Fig. 2). The acetoxy group was positioned on C-9 by HMBC correlations between the methyl proton at δ_H 2.03 (s) and H-9 with a carbonyl signal at δ_C 173.3 (Fig. 2). However, the stereostructure of **3** can't be determined.

A detailed comparison of the 1H and ^{13}C NMR data of compounds **4** and **5** with those of compound **3** suggested that both of them were neolignans with same planar structure and their structures were closely related to compound **3**, except that the 9-hydroxy of **4** and **5** replaced the 9-acetoxy group of **3**. This was further confirmed by 1H - 1H COSY, HMBC experiments (Fig. 2). These two neolignans with two chiral carbons suggested that the stereostructures of the 7-8 positions in them were a 7,8-*threo*-form or a 7,8-*erythro*-form [13]. However, the relative stereostructures of them can't be determined.

Cytotoxicities of compounds **1–8** were tested against three cancer cell lines A549 and Hela (Table 3). As a result, compounds **1–8** showed significant cytotoxicity against the tested cell lines with values of IC₅₀ values from 1.53 to 5.29 µg/ml. Compounds **4**, **5**, **7** and **8** exhibited cytotoxicity against A549 with the IC₅₀ values of 12.36, 9.72, 10.26, 13.05 µg/ml, respectively.

Conflict of interest

There is no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (31000159 and 31360080). The authors are grateful to the staff of analytical group at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for the spectral data.

Table 3

Cytotoxicity of compounds **1–8** against two human tumor cell lines (IC₅₀, µg/ml).

Compounds	Cell lines	
	A549	Hela
1	ND ^a	3.33
2	ND	1.53
3	ND	3.33
4	12.36	1.89
5	9.72	5.29
6	ND	3.19
7	10.26	2.59
8	13.05	3.73
Taxol ^b	0.02	0.01

^a ND: no detected (>25 µg/ml).

^b Positive control.

References

- [1] Delectis Florae Reipularis Agendae Academiae Sinicae Edita. Flora Reipublicae Popularis Sinicae, 16. Beijing: Science Press; 1981. p. 67.
- [2] Insitute Botanicum Kunmingense Academiae Sinicae Edita. Flora Yunnancia, 8. Beijing: Science Press; 1997. p. 96.
- [3] Gewali MB, Tezuka Y, Banskota AH, Ali MS, Saiki I, Dong H, et al. Epicalyxin F and calyxin I: two novel antiproliferative diarylheptanoids from the seeds of *Alpinia blepharocalyx*. *Org Lett* 1999;1:1733–6.
- [4] Yang Y, Kinoshita K, Koyama K, Takahashi K, Tai T, Nunoura Y, et al. Two novel anti-emetic principles of *Alpinia katsumadai*. *J Nat Prod* 1999;62:1672–4.
- [5] Tezuka Y, Gewali MB, Ali MS, Banskota AH, Kadota S. Eleven novel diarylheptanoids and two unusual diarylheptanoid derivatives from the seeds of *Alpinia blepharocalyx*. *J Nat Prod* 2001;64:208–13.
- [6] Muraoka O, Fujimoto M, Tanabe G, Kubo M, Minematsu T, Matsuda H, et al. Absolute stereostructures of novel norcadinane- and trinoreudesmane-type sesquiterpenes with nitric oxide production inhibitory activity from *Alpinia oxyphylla*. *Bioorg Med Chem Lett* 2001;11:2217–20.
- [7] Morikawa T, Matsuda H, Toguchida I, Ueda K, Yoshikawa M. Absolute stereostructures of three new sesquiterpenes from the fruit of *Alpinia oxyphylla* with inhibitory effects on nitric oxide production and degranulation in RBL-2H3 cells. *J Nat Prod* 2002;65:1468–74.
- [8] Xu JJ, Ji CJ, Zhang YM, Su J, Li Y, Tan NH. Inhibitory activity of eudesmane sesquiterpenes from *Alpinia oxyphylla* on production of nitric oxide. *Bioorg Med Chem Lett* 2012;22:1660–3.
- [9] Kong LY, Qin MJ, Niwa M. New cytotoxic bis-labdanic diterpenoids from *Alpinia calcarata*. *Planta Med* 2002;68:813–7.
- [10] Sy LK, Brown GD. Labdane diterpenoids from *Alpinia chinensis*. *J Nat Prod* 1997;60:904–8.
- [11] Elzaawely AA, Xuan TD, Tawata S. Essential oils, kava pyrones and phenolic compounds from leaves and rhizomes of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. and their antioxidant activity. *Food Chem* 2007;103:486–94.
- [12] Ly TN, Yamada MS, Kato K, Yamauchi R. Isolation and characterization of some antioxidative compounds from the rhizomes of smaller galanga (*Alpinia officinarum* Hance). *J Agric Food Chem* 2003;51:4924–9.
- [13] Morikawa T, Ando S, Matsuda H, Kataoka S, Muraoka O, Yoshikawa M. Inhibitors of nitric oxide production from the rhizomes of *Alpinia galanga*: structures of new 8–9' linked neolignans and sesquieneolignan. *Chem Pharm Bull* 2005;53:625–30.
- [14] Jiangsu New Medical College. Dictionary of Chinese Herb Medicine. Shanghai: Shanghai Scientific and Technologic Press; 1997 538.
- [15] Awang K, Azmi MN, Aun LIL, Aziz AN, Ibrahim H, Nagoor NH. The apoptotic effect of 1'S-1'-acetoxychavicol acetate from *Alpinia conchigera* on human cancer cells. *Molecules* 2010;15:8048–59.
- [16] Athamaprasangsa S, Buntrarongroj U, Dampawan P, Ongkavoranan N, Rukachaisirikul V, Sethjinda S, et al. A 1,7-diarylheptanoid from *Alpinia conchiger*. *Phytochemistry* 1994;37:871–3.
- [17] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. *J Natl Cancer Inst* 1990;82:1107–12.
- [18] Xu JJ, Huang HQ, Zeng GZ, Tan NH. Cytotoxic sesquiterpenes and lignans from *Saussurea deltoidea*. *Fitoterapia* 2012;83:1125–30.