Four New Sesquiterpenoids from the Roots of *Incarvillea arguta* and Their Inhibitory Activities against Lipopolysaccharide-Induced Nitric Oxide Production

Jian-Jun Fu,[a,b] Jiang-Jiang Qin,[a] Qi Zeng,[a] Ying Huang,[a] Hui Zi Jin,*[,a] and Wei-Dong Zhang*[,a,c]

[a] *School of Pharmacy, Shanghai Jiao Tong University; Shanghai 200240, P.R. China:* [b] *College of Pharmacy, Soochow University; Suzhou 215123, P.R. China: and* [c] *School of Pharmacy, Second Military Medical University; Shanghai 200433, P.R. China.*

Received May 15, 2010; accepted June 14, 2010; published online June 17, 2010

Four new sesquiterpenoids, argutosines A—D (1—4), together with two known ones (5, 6), were isolated from the roots of *Incarvillea arguta*. Their chemical structures were elucidated on the basis of MS, NMR spectroscopic analysis. All the sesquiterpenoids were tested for inhibitory activities against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages. Argutosines A, B and C (1—3) showed potent inhibitory activities with IC_{50} values of 2.05, 0.55, and 9.87 μM, respectively.

Key words *Incarvillea arguta*; argutosine; sesquiterpenoid; nitric oxide production

The genus *Incarvillea* is notable for being a temperate and herbaceous genus of the primarily tropical and woody family Bignoniaceae. It is composed of 16 species, which mainly occurred in the Himalayas and southwest China.[1,2] As a folk herbal medicine, *Incarvillea arguta* has been widely used for treating hepatitis and diarrhea among the Yi nationality in Yunnan province of China.[3] Previous chemical investigation of *I. arguta* has led to the isolation of monoterpene alkaloids, ceramides, triterpenes, and flavones.[4—7] Recently, we have reported the isolation and structure elucidation of two monoterpene alkaloid dimers and two biphenyls from the roots of *I. arguta*.[8,9] Further investigation of this plant led us to isolate four new sesquiterpenoids, argutosines A—D (**1**—**4**) (Fig. 1), together with two known compounds, oxysolavetivone (**5**) and 1,10-didehydrolubimin (**6**). This paper describes the isolation and structure elucidation, as well as the inhibitory activities of all six compounds against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages.

Results and Discussion

Argutosine A (**1**) was obtained as yellow oil. The molecular formula was determined as $C_{15}H_{22}O_2$ by positive high-resolution (HR)-electrospray ionization (ESI)-MS at *m*/*z* 257.1529 $[M+Na]^+$ (Calcd for $C_{15}H_{22}O_2Na$, 257.1517) in conjunction with the NMR spectroscopic data (Table 1), which required five degrees of unsaturation. The ^{1}H-NMR spectrum showed signals of six methyl protons at [δ_H 0.93 (3H, s, H_3-15); 0.94 (3H, d, J=6.5 Hz, H_3-14)], two oxygenated methylene protons at [δ_H 4.16 (2H, s, H_2-12)], and three olefinic protons at [δ_H 5.14 (1H, s, H-13a); 4.96 (1H, s, H-13b); 6.48 (1H, t, J=3.5 Hz, H-1)]. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra established that **1** possessed two methyls (δ_C 15.8, 20.3), six methylenes (δ_C 109.5, 65.0, 45.8, 42.1, 26.1, 25.7), three methines (δ_C 133.9, 39.3, 33.5), one α,β-unsaturation carbonyl carbon (δ_C 202.7; IR 1683 cm^{-1}), and three quaternary carbons (δ_C 151.7, 144.6, 38.3), that is, a total of 21 protons attached to 15 carbons. One more proton was inferred from IR spectra showing that a 3425 cm^{-1} band was attributed to a hydroxyl group. The ^{1}H- and ^{13}C-NMR spectroscopic data of **1** showed features similar to those of an eremophilane-type sesquiterpenoid, dehydroflourensic acid (NMR data in ref. 10), but with a new primary alcohol group [δ_C 65.0; δ_H 4.16 (2H, s, H_2-12)], instead of the aldehyde group present in dehydroflourensic acid. The planar structure of **1** was further supported by heteronuclear single quantum coherence (HSQC), heteronuclear multiple-bond correlation (HMBC) and ^{1}H–^{1}H correlation spectroscopy (COSY) experiments in Fig. 2. The long-range correlations between H_2-12 and C-7/C-11/C-13, H-13a/H-13b and C-7/C-11, H_3-14 and C-3/C-4, H_3-15 and C-4/C-5/C-6, were observed in the HMBC spectrum. In the ^{1}H–^{1}H COSY experiment, a spin-system of $CHCH_2CH_2CHCH_3$ (C1/C2/C3/C4/C14) was deduced by the correlations starting from the olefinic methine

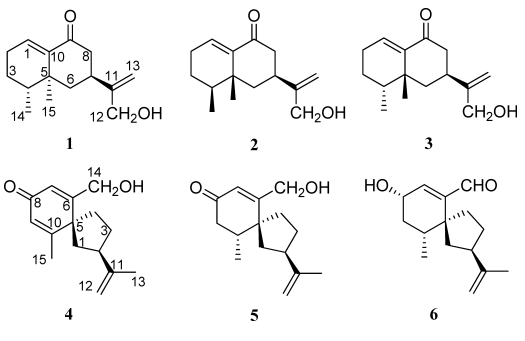

Fig. 1. Chemical Structures of Compounds **1**—**6**

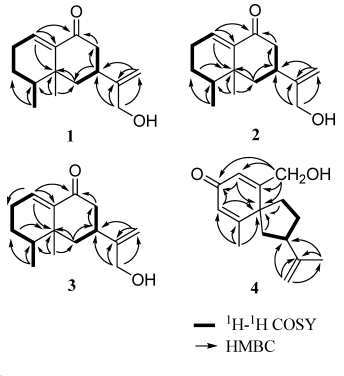

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC Correlations for Compounds **1**—**4**

* To whom correspondence should be addressed. e-mail: kimhz@sjtu.edu.cn; wdzhangy@hotmail.com

Table 1. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) Data for **1**—**3** ($CDCl_3$; δ in ppm; J in Hz)

No.	**1**		**2**		**3**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	133.9 d	6.48 (t, 3.5)	135.6 d	6.58 (t, 3.5)	135.5 d	6.61 (t, 4.0)
2	25.7 t	2.19 (m) (a)	25.5 t	2.21 (m) (a)	22.7 t	2.25 (m) (a)
		2.22 (m) (b)		2.25 (m) (b)		2.19 (m) (b)
3	26.1 t	1.50 (m) (a)	26.5 t	1.51 (m) (a)	25.2 t	1.92 (m) (a)
		1.55 (m) (b)		1.55 (m) (b)		1.39 (m) (b)
4	39.3 d	1.57 (m)	38.6 d	1.62 (m)	38.9 d	1.68 (m)
5	38.3 s		35.9 s		35.9 s	
6	42.1 t	2.08 (tt, 13.0, 3.0) (a)	41.9 t	2.05 (m) (a)	41.3 t	2.11 (m) (a)
		1.40 (t, 13.0) (b)		1.46 (m) (b)		1.36 (m) (b)
7	33.5 d	2.75 (tt, 13.5, 4.0)	34.7 d	2.44 (m)	35.6 d	2.36 (m)
8	45.8 t	2.65 (ddd, 16.5, 4.5, 2.5) (a)	43.4 t	2.48 (m) (a)	44.1 t	2.48 (m) (a)
		2.26 (dd, 16.5, 13.5) (b)		2.40 (m) (b)		2.38 (m) (b)
9	202.7 s		203.7 s		203.3 s	
10	144.6 s		144.2 s		142.0 s	
11	151.7 s		151.4 s		151.3 s	
12	65.0 t	4.16 (s)	64.9 t	4.14 (s)	65.1 t	4.15 (s)
13	109.5 t	5.14 (s) (a)	109.7 t	5.12 (s) (a)	109.5 t	5.11 (s) (a)
		4.96 (s) (b)		4.92 (s) (b)		4.91 (s) (b)
14	15.8 q	0.94 (d, 6.5)	15.9 q	0.95 (d, 6.7)	15.0 q	0.97 (d, 6.5)
15	20.3 q	0.93 (s)	24.9 q	0.97 (s)	33.2 q	1.17 (s)

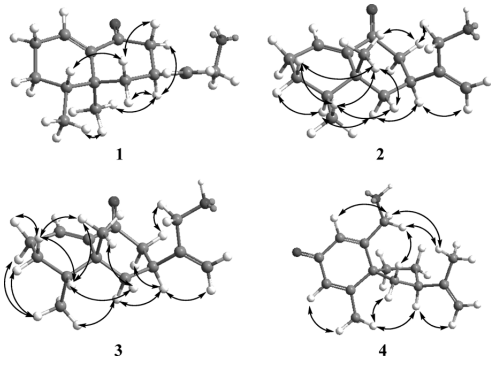

Fig. 3. Key NOESY Correlations for Compounds **1**—**4**

at δ_H 6.48 (H-1). Cross-peaks between H-7 and H-8, and H-7 and H-9 were also observed in the ^{1}H–^{1}H COSY spectrum. Thus, **1** was characterized as 9-oxoeremophila-1(10),11(13)-diene-12-ol.

The relative configuration of **1** was confirmed by careful analysis of nuclear Overhauser effect spectroscopy (NOESY) data. Biogenetically, the relative configuration of H-7 in eremophilane-type sesquiterpenoids was α-orientation. The strong NOESY correlations of H-7/H-6a, H-7/H-8a, H-7/H_3-15, and H_3-14/H_3-15 suggested that H-6a, H-8a, H_3-14, H_3-15 and H-7 were at the same orientation. The strong NOESY correlations of H-8b/H-6b, and H-6b/H-4 revealed that H-8b, H-6b and H-4 were β-oriented. The absence of any NOESY correlations of H-8b/H_3-15, and H-6b/H_3-15 supported these assignments. An energy-minimized structure of **1** by MM2 molecular modeling with the selected NOESY correlations was shown in Fig. 3. On the basis of above evidence, the structure of **1** was elucidated as (4α-methyl,5α-methyl, 7αH)-9-oxoeremophila-1(10),11(13)-diene-12-ol, and named argutosine A.

Argutosine B (**2**) was obtained as yellow oil. The molecular formula was determined as $C_{15}H_{22}O_2$ by positive HR-ESI-MS at m/z 257.1524 $[M+Na]^+$ (Calcd for $C_{15}H_{22}O_2Na$, 257.1517) in conjunction with the NMR spectroscopic data (Table 1), which required five degrees of unsaturation. The ^{1}H-, ^{13}C-NMR, and DEPT spectra implied that **2** share the same planar structure with **1**, which was confirmed by detailed analysis of HMBC and ^{1}H–^{1}H COSY experiments (Fig. 2). The relative configuration of **2** was determined as 4β-methyl, 5β-methyl, 7αH by NOESY correlations (Fig. 3), which was different from those of **1**. Thus, the structure of **2** was elucidated as (4β-methyl,5β-methyl,7αH)-9-oxoeremophila-1(10),11(13)-diene-12-ol, and named argutosine B.

Argutosine C (**3**) was obtained as yellow oil. The molecular formula was determined as $C_{15}H_{22}O_2$ by positive HR-ESI-MS at m/z 257.1519 $[M+Na]^+$ (Calcd for $C_{15}H_{22}O_2Na$, 257.1517) in conjunction with the NMR spectroscopic data (Table 1), which required five degrees of unsaturation. The ^{1}H-, ^{13}C-NMR and DEPT spectra implied that **3** share the same planar structure with **1** and **2**, which was confirmed by detailed analysis of HMBC and ^{1}H–^{1}H COSY experiments (Fig. 2). The difference between the three isomers was the relative configuration. The relative configuration of **3** was determined as 4α-methyl, 5β-methyl, 7αH by NOESY correlations (Fig. 3). Thus, the structure of **3** was elucidated as (4α-methyl,5β-methyl,7αH)-9-oxoeremophila-1(10),11(13)-diene-12-ol, and named argutosine C.

Argutosine D (**4**) was obtained as yellow oil. The molecular formula was determined as $C_{15}H_{20}O_2$ by HR-ESI-MS at m/z 255.1347 $[M+Na]^+$ (Calcd for $C_{15}H_{20}O_2Na$, 255.1361) in conjunction with the NMR spectroscopic data (Table 2), which required six degrees of unsaturation. The ^{1}H-NMR spectrum showed signals of six methyl protons at [δ_H 2.08 (3H, s, H_3-15); 1.78 (3H, s, H_3-13)], two oxygenated methylene protons at [δ_H 4.47 (1H, d, J=15.9 Hz, H-14a); 4.39 (1H, d, J=15.9 Hz, H-14b)], and four olefinic protons at [δ_H 4.78 (2H, s, H-12); 6.05 (1H, s, H-9); 6.47 (1H, s, H-7)]. The ^{13}C-NMR and DEPT spectra established that **4** possessed two methyls (δ_C 20.5, 21.3), five methylenes (δ_C 109.8, 61.7,

Table 2. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) Data for **4** ($CDCl_3$; δ in ppm; J in Hz)

No.	**4**	
	δ_C	δ_H
1	41.4 t	1.90 (d, 7.9)
2	49.1 d	2.84 (m)
3	33.5 t	2.12 (m)
		1.74 (m)
4	36.5 t	2.02 (m)
		1.87 (m)
5	51.6 s	
6	167.5 s	
7	122.4 d	6.47 (s)
8	186.7 s	
9	126.1 d	6.05 (s)
10	164.6 s	
11	145.9 s	
12	109.8 t	4.78 (d, 3.7)
13	21.3 q	1.78 (s)
14	61.7 t	4.47 (d, 15.9)
		4.39 (d, 15.9)
15	20.5 q	2.08 (s)

41.4, 36.5, 33.5), three methines (δ_C 126.1, 122.4, 49.1), one carbonyl carbon (δ_C 186.7), and four quaternary carbons (δ_C 167.5, 164.6, 145.9, 61.6), that is, a total of 19 protons attached to 15 carbons. One more proton was inferred from IR spectra showing that a 3381 cm^{-1} band was attributed to hydroxyl group. The NMR spectral data were found to be very similar to those of oxysolavetivone (**5**), except for the presence of the olefinic signals [C-9 (δ_C 126.1), δ_H 6.05 (1H, s, H-9); C-10 (δ_C 164.6)] in **4**, instead of the saturated alkyl signals [C-9 (δ_C 43.0), δ_H 2.70 (1H, dd, J=17.0, 4.6 Hz, H-9a), 2.24 (1H, dd, J=17.0, 4.6 Hz, H-9b); C-10 (δ_C 39.2), δ_H 2.15 (1H, m, H-10)] in **5**. The planar structure of **4** was further confirmed by HSQC, HMBC and ^{1}H–^{1}H COSY experiments in Fig. 2. The relative configuration of **4** was confirmed by careful analysis of NOESY data. C-3 and C-4 of the rigid spiro[4,5]deca skeleton were determined to be α-orientation, when C-1 was randomly assigned to be β-orientation. The strong NOESY correlations of Me-13 to H-14a and H-14b, H-2 to Me-15 and H-12 suggested that Me-13 would be close to H_2-14, and H_2-12 would be close to Me-15, giving a relative configuration of **4** as shown in Fig. 3. The specific rotation of **4** was positive. Thus, the structure of **4** was elucidated to be (+)-2-isopropenyl-6-hydroxymethyl-10-methylspiro[4,5]deca-6,9-dien-8-one with as yet unknown absolute configuration, and named argutosine D.

Two known compounds oxysolavetivone (**5**)[11] and 1,10-didehydrolubimin (**6**)[12] were identified by comparison of their spectroscopic data (ESI-MS, IR, ^{1}H-NMR, ^{13}C-NMR) with reported values.

All of these isolated sesquiterpenoids (**1**—**6**) were evaluated for inhibitory activities against lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages under the concentration range from 1 to 50 μM. Aminoguanidine (AG) was used as a positive control. Among the compounds tested, argutosines A—C (**1**—**3**) showed potent inhibitory activities with the IC_{50} values of 2.05, 0.55, and 9.87 μM, respectively (Table 3). These results

Table 3. Inhibitory Activities of Compounds Isolated from *I. arguta* against LPS-Induced NO Production in RAW264.7 Macrophages and Their Cytotoxicities (n=4, mean±S.D.)

Groups	IC_{50} [a] (μM)	IC_{50} [b] (μM)
AG[c]	0.06	NT[d]
1	2.05±0.12	18.09±1.20
2	0.55±0.04	12.68±0.90
3	9.87±0.22	>50
4	>50	>50
5	>50	>50
6	>50	>50

a) Inhibitory activity of compounds **1**—**6** against LPS-Induced NO production in RAW264.7 macrophages. *b*) Cytotoxicity of compounds **1**—**6** in RAW264.7 macrophages. *c*) Positive control; AG: Aminoguanidine. *d*) Not tested.

were in accordance with the results of the effects of eremophilane-type sesquiterpenoids on the NO produciton in previous study.[13] Cell viability, as measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, also showed that these three compounds had no significant cytotoxicity to the RAW264.7 cells at their effective concentrations for the inhibition of NO production.

Experimental

General Experimental Procedures NMR spectra were determined on Bruker Avance-500 NMR, for ^{1}H-NMR at 500 MHz and ^{13}C-NMR at 125 MHz. ESI-MS spectra were recorded on Varian MAT-212 mass spectrometer. The time-of flight (TOF)-ESI spectra were carried out on a Q-Tof micro YA019 mass spectrometer. IR spectra were recorded with a Bruker FTIR Vector 22 spectrometer. Optical rotations were obtained with a Perkin-Elmer 341 polarimeter. UV spectra were recorded with a Shimadzu UV-2550 spectrophotometer. TLC analysis was run on $HSGF_{254}$ silica gel plates (10—40 μm, Yantai, China). Column chromatography was performed on silica gel (100—200, 200—300 mesh, Yantai, China), and silica gel H (10—40 μm, Qingdao, China). HPLC was performed using a system composed of a SHIMADZU LC-6AD pump, a SHIMADZU UV–VIS detector SPD-20A, a SHIMADZU 7725 injection port, and a preparative column (PRC-ODS, 20 mm i.d.×250 mm, 15 mm, Japan). A preparative column (Shimadzu PRC-ODS EV0233) was used for preparative HPLC (Shimadzu LC-6AD).

Plant Material The roots of *I. arguta* were collected from Anning, Yunnan Province, P. R. China, in May 2006 and identified by Prof. Bao-kang Huang, School of Pharmacy, Second Military Medical University. The voucher specimens (LTM20060514) were deposited with the Herbarium of the School of Pharmacy, Shanghai Jiao Tong University, Shanghai, P. R. China.

Extraction and Isolation The dried roots (24.9 kg) of *I. arguta* were chopped and extracted with 80% EtOH at room temperature. The extract was dissolved in water to form a suspension and acidified to pH=2 with 20% H_2SO_4 and filtered. The filtrate was basified to pH=10 with saturated $NaHCO_3$ aqueous solution and then extracted successively with $CHCl_3$. The $CHCl_3$ fraction (70 g) was chromatographed over a silica gel column with a gradient CH_2Cl_2–MeOH (100 : 1, 50 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1, 1 : 1, each 15 l) to give fourteen fractions 1—14. Fraction 6 (1.6 g) was separated on a silica gel column chromatography (CC) (CH_2Cl_2–MeOH, 50 : 1, 20 : 1, 10 : 1, 5 : 1, each 5 l) to afford six subfractions (Fr. 6.1—6.6). Subfraction 6.5 (600 mg) was subjected to a silica gel CC (CH_2Cl_2–MeOH, 20 : 1, 10 : 1, 5 : 1, each 3 l) to afford five subfractions (Fr. 6.5.1—6.5.5). Subfraction 6.5.3 (210 mg) was purified by preparative HPLC (60% aqueous MeOH, 8 ml/min) to yield compounds **1** (28.2 mg), **2** (13.5 mg), and **3** (5.2 mg). Fraction 7 (5.3 g) was separated on a silica gel CC (CH_2Cl_2–MeOH, 50 : 1, 20 : 1, 10 : 1, 5 : 1, 2 : 1, each 8 l) to afford five subfractions (Fr. 7.1—7.5). Subfraction 7.4 (900 mg) was subjected to a silica gel CC (CH_2Cl_2–MeOH, 20 : 1, 10 : 1, 5 : 1, each 3 l) to afford six subfractions (Fr. 7.4.1—7.4.6). Fr. 7.4.3 (120 mg) was further purified by preparative HPLC (50% aqueous MeOH, 8 ml/min) to yield compound **4** (12.0 mg). Fr. 7.4.2 (185 mg) was further purified by preparative HPLC (70% aqueous MeOH, 8 ml/min) to yield compounds **5** (43.0 mg) and **6** (20.1 mg).

Argutosine A (**1**): Yellow oil; $C_{15}H_{22}O_2$; $[\alpha]_D^{20}$ +54.0 (c=1.0, $CHCl_3$); HR-ESI-MS m/z: 257.1529 $[M+Na]^+$ (Calcd for $C_{15}H_{22}O_2Na$, 257.1517);

IR (KBr) ν_{max}: 3425, 2960, 2929, 2873, 1683, 1620, 1460, 1418, 1380, 1257, 1045, 902 cm^{-1}; UV (MeOH) λ_{max} nm: 241; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data see Table 1.

Argutosine B (**2**): Yellow oil; $C_{15}H_{22}O_2$; $[\alpha]_D^{20}$ −153.0 (c=1.0, $CHCl_3$); HR-ESI-MS m/z: 257.1524 $[M+Na]^+$ (Calcd for $C_{15}H_{22}O_2Na$, 257.1517); IR (KBr) ν_{max}: 3423, 2960, 2928, 2871, 1687, 1620, 1458, 1419, 1379, 1264, 1228, 1050, 902 cm^{-1}; UV (MeOH) λ_{max} nm: 241; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data see Table 1.

Argutosine C (**3**): Yellow oil; $C_{15}H_{22}O_2$; $[\alpha]_D^{20}$ −17.4 (c=0.3, $CHCl_3$); HR-ESI-MS m/z: 257.1519 $[M+Na]^+$ (Calcd for $C_{15}H_{22}O_2Na$, 257.1517); IR (KBr) ν_{max}: 3425, 2958, 2930, 2878, 1684, 1614, 1458, 1418, 1379, 1258, 1228, 1046, 902 cm^{-1}; UV (MeOH) λ_{max} nm: 242; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data see Table 1.

Argutosine D (**4**): Yellow oil; $C_{15}H_{20}O_2$; $[\alpha]_D^{20}$ +39.8 (c=0.6, MeOH); HR-ESI-MS m/z: 255.1347 $[M+Na]^+$ (Calcd for $C_{15}H_{20}O_2Na$, 255.1361); IR (KBr) ν_{max}: 3381, 2962, 2928, 2880, 1660, 1612, 1441, 1377, 1326, 1099, 1049, 891 cm^{-1}; UV (MeOH) λ_{max} nm: 247; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) data see Table 2.

Inhibitory Activities against LPS-induced NO Production RAW264.7 macrophages were seeded in 96-well plates (1×10^5 cells/well). The cells were co-incubated with drugs and LPS (1 μg/ml) for 24 h. The amount of NO was assessed by determined the nitrite concentration in the cultured RAW264.7 macrophage supernatants with Griess reagent. Aliqueots of supernatants (100 μl) were incubated, in sequence, with 50 μl 1% sulphanilamide and 50 μl 1% naphthylethylenediamine in 2.5% phosphoric acid solution. The absorbances at 570 nm were read using an Experimental Study of Enzyme-linked Immunosorbent Assay (ELISA) reader. Aminoguanidine was used as a positive control.[14)]

MTT Assay for Cytotoxicity MTT assay was selected as cytotoxicity screening method. Cytotoxicity assay for six compounds was performed *in vitro* against RAW264.7 macrophages. RAW264.7 macrophages were maintained in a water-saturated atmosphere of 5% CO_2 at 37 °C. Experiments were carried out according to the reported protocol. Aminoguanidine was used as a positive control.[14)]

Acknowledgements The work was supported by program for Changjiang Scholars and Innovative Research Team in University (PCSIRT), NCET Foundation, NSFC (30725045), National 863 Program (2006AA02Z338), "973" program of China (2007CB507400), China (2009ZX09311-001, 2008ZX09101-Z-029, and 2009ZX0910 3-375), National comprehensive technology platforms for innovative drug R&D, 2009ZX09301-007, Shanghai Leading Academic Discipline Project (B906) and in part by the Scientific Foundation of Shanghai China (08DZ1971302, 09dZ1972200).

References

1) Chen S. T., Guan K. Y., Zhou Z. K., Olmstead R., Cronk Q., *Am. J. Bot.*, **92**, 625—633 (2005).
2) Chen S. T., Guan K. Y., Zhou Z. K., Fujiki T., *Acta Bot. Yunnan*, **25**, 458—464 (2003).
3) Li G. D., *Chin. Herb. Med.*, **17**, 32—36 (1986).
4) Luo Y. G., Yi J. H., Li B. G., Zhang G. L., *Lipids*, **39**, 907—913 (2004).
5) Ji T. F., Feng X. Z., *Chin. Tradit. Herb Drugs*, **33**, 967—969 (2002).
6) Yu Z. W., Zhu H. Y., Yang X. S., Sun Q. Y., Hao X. J., *China J. Chin. Mater. Med.*, **30**, 1335—1338 (2005).
7) Zhou D. Y., Yang X. S., Yang B., Zhu H. Y., Hao X. J., *Nat. Prod. Res. Dev.*, **19**, 807—808 (2007).
8) Fu J. J., Jin H. Z., Shen Y. H., Zhang W. D., Xu W. Z., Zeng Q., Yan S. K., *Helv. Chim. Acta*, **90**, 2151—2155 (2007).
9) Fu J. J., Jin H. Z., Shen Y. H., Qin J. J., Wang Y., Huang Y., Zeng Q., Yan S. K., Zhang W. D., *Helv. Chim. Acta*, **92**, 491—494 (2009).
10) Mata R., Bye R., Linares E., Macias M., Rivero-Cruz I., Perez O., Timmermann B. N., *Phytochemistry*, **64**, 285—291 (2003).
11) Murai A., Yoshizawa Y., Katsui N., *Chem. Lett.*, **5**, 771—772 (1986).
12) Tan Q. G., Cai X. H., Feng T., Du Z. Z., Luo X. D., *Z. Naturforsch. B*, **64**, 439—442 (2009).
13) Hwang B. Y., Lee J. H., Koo T. H., Kim H. S., Hong Y. S., Ro J. S., Lee K. S., Lee J. J., *Planta Med.*, **68**, 101—105 (2002).
14) Denizot F., Lang R., *J. Immunol. Methods*, **89**, 271—277 (1986).