Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 8751–8755

Novel sesquiterpenoids from the Formosan soft coral *Paralemnalia thyrsoides*

Ho-Cheng Huang,[a,b] Zhi-Hong Wen,[a] Chih-Hua Chao,[a] Atallah F. Ahmed,[a,c] Michael Y. Chiang,[d] Yao-Haur Kuo,[e] Chi-Hsin Hsu[a] and Jyh-Horng Sheu[a,*]

[a]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[b]*Department of Chemical and Materials Engineering, Cheng Shiu University, Kaohsiung 833, Taiwan*
[c]*Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt*
[d]*Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[e]*National Research Institute of Chinese Medicine, Taipei 112, Taiwan*

Received 5 September 2006; revised 29 September 2006; accepted 2 October 2006

Abstract—Three sesquiterpenoids with unprecedented skeletons, paralemnanone (**1**), isoparalemnanone (**2**), and paralemnanol (**3**), were isolated from the Formosan soft coral *Paralemnalia thyrsoides*. Their structures were elucidated by extensive spectroscopic analysis, and the structure of **1** was further confirmed by X-ray crystallographic analysis. The absolute stereochemistries of **1**–**3** were established by application of the Mosher's method on **2**.

Soft corals of the genus *Paralemnalia*[1] and *Lemnalia*[2] have been found to be a rich source of sesquiterpenoids (neolemnane and nardosinane carbon skeleton) and norsesquiterpenoids. Our previous study on the secondary metabolites of a Taiwanese soft coral *Paralemnalia thyrsoides* has resulted in the isolation of three terpenoids, paralemnolins A–C.[3] Continuing investigation on the chemical constituents of this soft coral has led to the isolation of three novel sesquiterpenoids: paralemnone (**1**), isoparalemnone (**2**), and paralemnol (**3**). The structures of sesquiterpenoids **1**–**3** were elucidated by spectroscopic analysis and the absolute stereochemistries were established by application of modified Mosher's Method on **2**.[4] The inhibition of these metabolites toward the pro-inflammatory proteins (iNOS and COX-2) expression was also investigated.

The soft coral *P. thyrsoides* (1.8 kg) was collected by hand using scuba at Green Island, Taiwan in July, 2004. The EtOH extract (67.3 g) of the frozen organism was partitioned between EtOAc and H_2O. The EtOAc-soluble portion (33.0 g) was subjected to column chromatography over silica gel using *n*-hexane–EtOAc mixtures of increasing polarity. A fraction eluted *n*-hexane–EtOAc (3:1) was further purified by reverse phase HPLC (Purospher RP-18e, 5 μm, 10 × 250 mm), using acetonitrile–H_2O (2:1) to afford **3** (2.9 mg). Another fraction eluted with *n*-hexane–EtOAc (2:1) was chromatographed by reverse phase HPLC using acetonitrile–H_2O (2:1), and followed by normal phase HPLC (Lichrosorb Si 60, 7 μm, 25 × 250 mm), eluting with *n*-hexane–EtOAc (5:1), to yield **1** (3.1 mg) and **2** (12.6 mg).

Paralemnone (**1**) was obtained as colorless prisms, mp 106–108 °C, $[\alpha]_D^{25}$ −41 (*c* 1.24, $CHCl_3$). The molecular formula of **1**, $C_{15}H_{22}O_2$, was established by HRESIMS (*m*/*z* calcd 257.1517; found 257.1517, $[M+Na]^+$), indicating five degrees of unsaturation. Its IR absorptions (ν_{max} 3447 and 1741 cm^{-1}) revealed the existence of hydroxy and carbonyl functionalities. The ^{13}C NMR (Table 1) and DEPT spectra indicated the presence of 15 carbon signals, including three methyls, three methylenes, six methines, and three quaternary carbons. The ^{13}C and ^1H NMR spectra revealed the presence of one hydroxyl-containing methine [δ_H 4.03 (t, $J = 7.5$ Hz), δ_C 71.7 (CH)], one ketone (δ_C 218.5) and one trisubstituted double bond [δ_H 5.60 br t, δ_C 125.4 (CH), 136.9 (qC)]. The above finding suggests **1** to be a tricyclic

Keywords: Paralemnone; Isoparalemnone; Paralemnol; *Paralemnalia thyrsoides*; Soft coral.
* Corresponding author. Tel.: +886 7 5252000x5030; fax: +886 7 5255020; e-mail: sheu@mail.nsysu.edu.tw

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.10.002

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**3**

C/H	1		2		3	
	^{13}C[a]	^{1}H[b]	^{13}C[a]	^{1}H[b]	^{13}C[a]	^{1}H[b]
1	125.4 d[c]	5.60 br t (2.4)[d]	126.0 d[c]	5.71 t (2.4)[d]	121.2 d[c]	5.41 t (2.4)[d]
2	25.7 t	2.03 m	25.7 t	2.03 m	25.6 t	1.96 m
3	25.8 t	1.44 m	25.6 t	1.45 m	26.7 t	1.38 m
4	34.2 d	1.70 m	33.9 d	1.74 m	34.0 d	1.42 m
5	48.6 s		48.3 s		37.6 s	
6	59.1 d	1.79 brs	61.1 d	1.85 m	39.9 d	2.70 dt (11.1, 7.8)
7	218.5 s		216.5 s		43.8 d	
8	56.2 d	2.28 dt (2.4, 3.6)	52.8 d	2.45 m	26.1 t	α 1.84 m β 1.35 m
9	38.4 t	α 2.35 m β 2.83 m	35.2 t	α 2.62 m β 2.73 m	30.0 t	1.93 2.12
10	136.9 s		137.9 s		142.6 s	
11	35.0 d	2.38 m	41.4 d	1.89 m	36.2 t	1.76 m
12	71.7 d	4.03 d (7.5)	75.1 d	3.69 t (4.8)	75.0 s	
13	14.8 q	1.02 d (7.5)	20.9 q	1.06 d (7.2)	24.0 q	1.20 s
14	15.3 q	0.83 d (6.6)	15.3 q	0.82 d (6.9)	15.7 q	0.81 d (6.3)
15	17.8 q	0.90 s	18.3 q	0.91 s	20.3 q	0.94 s

[a] Spectra recorded at 75 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 300 MHz in $CDCl_3$ at 25 °C.
[c] Multiplicity is deduced by HSQC and DEPT spectra and indicated by the usual symbol.
[d] J value (in Hz) in parentheses.

sesquiterpenoid with a ketone and a secondary hydroxy group. The gross structure of **1** was determined by 2D NMR spectroscopic analysis. The ^{1}H–^{1}H COSY spectrum revealed three spin systems (**a**–**c**) as shown in Figure 2. The molecular framework of **1** was further established by an HMBC experiment, in which the planar structure was connected through HMBC correlations from H_3-14 to C-3, C-4, and C-5, H_3-15 to C-4, C-5, C-6 and C-10, H_3-13 to C-6, C-11 and C-12, H-6 to C-7 and C-8, H_2-9 to C-1, C-5, C-7, C-8, C-10 and C-12, H-11 to C-7, and H-12 to C-6 and C-7, as presented in Figure 2.

The relative stereochemistry of **1** was established from the NOE correlations observed in a NOESY experiment (Fig. 3). Assuming the β-orientation of H_3-15, H_3-15 was found to show NOE correlations with H_3-14, H_β-9 (δ_H 2.83, m) and H-6, and H-6 in turn showed an NOE response with H_3-13, but not with H-11, suggesting the β-orientations of H_3-13, H_3-14, and H-6. The β-orientation of H-8 was determined by an NOE correlation between H-8 and H_β-9. Furthermore, H-12 showed NOE correlations with H-11 and H_α-9, but not with H_3-13 and H-8, suggesting the β position of 12-OH. To confirm the structure of **1**, a single-crystal X-ray diffraction experiment was undertaken (Fig. 4).[5] Thus, the structure of **1** was fully established and the molecular skeleton was found to be unprecedented.

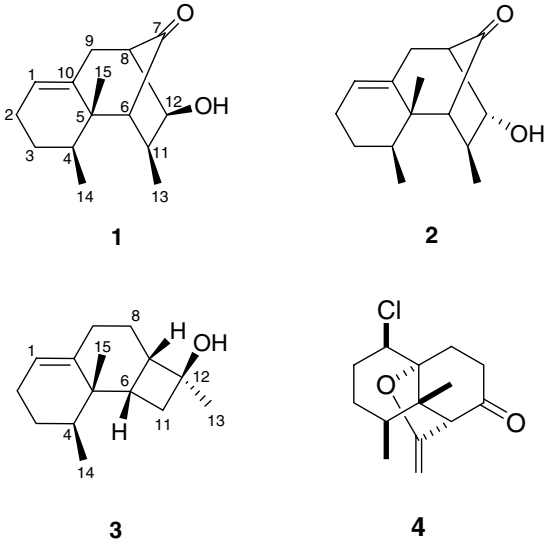


Figure 1. Structures of metabolites **1**–**4**.

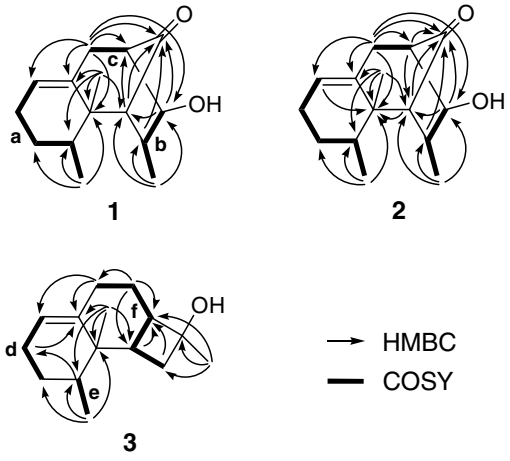


Figure 2. Selective ^{1}H–^{1}H COSY and HMBC correlations of **1**–**3**.

Isoparalemnone (**2**) was obtained as a white powder, mp 45–46 °C, $[\alpha]_D^{25}$ +14 (*c* 1.3, $CHCl_3$). On the basis of its HRESIMS (*m*/*z* 257.1518, $[M+Na]^+$) and NMR spectral data, the molecular formula of **2** was established as $C_{15}H_{22}O_2$. The ^{1}H NMR, ^{13}C NMR (Table 1) and IR (ν_{max} 3447 and 1745 cm^{-1}) spectra were found to be quite similar to those of **1**, suggesting that **2** could

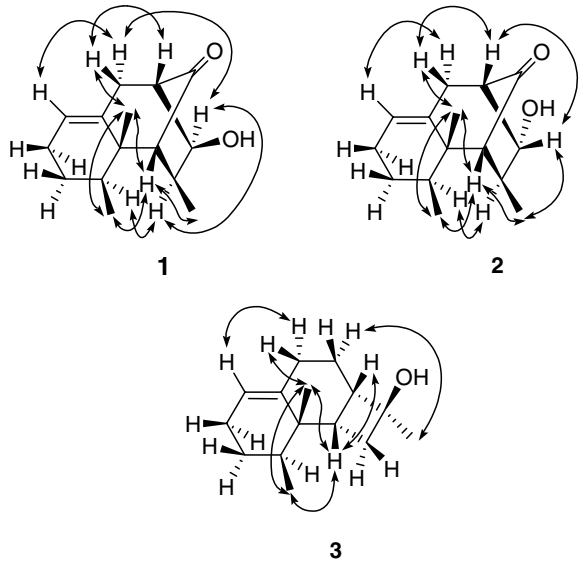


Figure 3. Selective NOESY correlations of **1**–**3**.

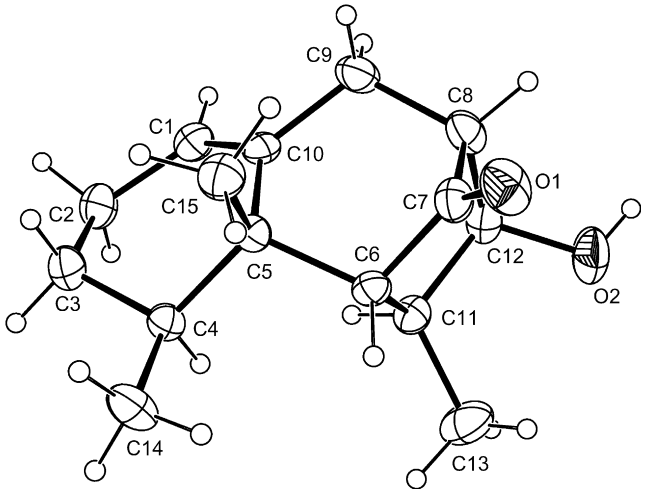


Figure 4. X-ray ORTEP diagram of **1**.

be an isomer of **1**. By the assistance of 2D NMR spectra (^{1}H–^{1}H COSY, HMQC, and HMBC), **2** was shown to possess the same molecular framework as that of **1** (Fig. 2). However, the significant differences in chemical shifts for C-8 ($\Delta\delta_C$ −3.4 ppm), C-11 ($\Delta\delta_C$ +6.4 ppm), C-12 ($\Delta\delta_C$ +3.4 ppm), and C-13 ($\Delta\delta_C$ +6.1 ppm) relative to those of **1** (Table 1) revealed that **2** might be the C-12 epimer of **1**. In the NOESY spectrum of **2** (Fig. 3), H-12 showed significant NOE interactions with both H-8 and H_3-13, but not with H-11, revealing the β-orientation of H-12. Further analysis on the other NOE interactions revealed that **2** possessed the same relative configurations at C-4, C-5, C-6, C-8, and C-11 as those of **1** (Fig. 3). Therefore, **2** was demonstrated to be a 12-epimer of **1** as shown in formula **2**.

The absolute stereochemistry of isoparalemnone (**2**) was further determined by application of the Mosher's method.[4] Comparison of ^{1}H NMR chemical shifts between the (*R*)- and (*S*)-MTPA esters of compound **2** (see Fig. 5) led to the assignment of *R*-configuration at

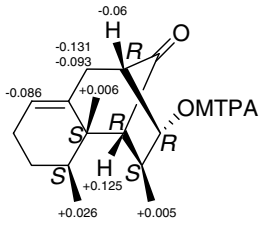


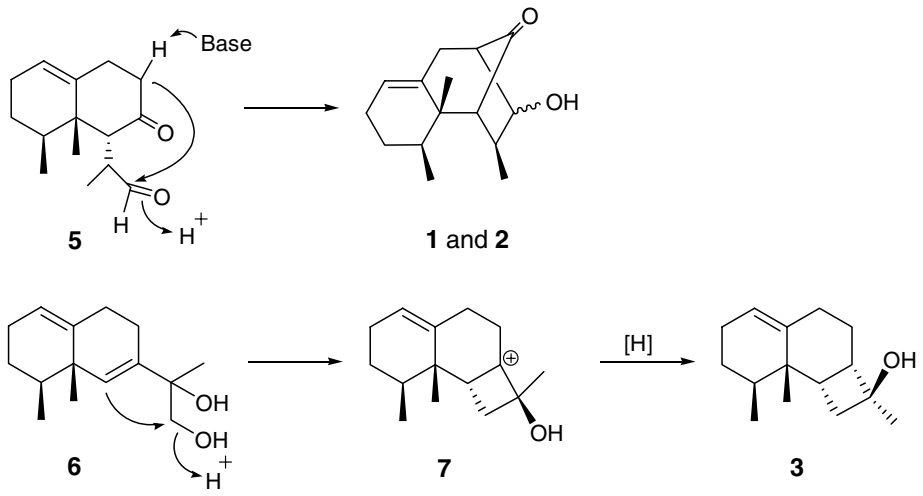
Figure 5. ^{1}H NMR chemical shift differences ($\delta_S - \delta_R$) in ppm for the MTPA esters of **2**.

C-12. On the basis of the above results and because a related metabolite **4**, which was isolated previously from the same organism has been found to possess the absolute structure as shown in Figure 1 by a single-crystal X-ray diffraction analysis,[3] the absolute structure of **2** was fully established and was found to possess the (4*S*,5*S*,6*R*,8*R*,11*S*,12*R*)-stereochemistry as shown in formula **2**. From biogenic consideration, the absolute stereochemistry of **1** was thus established as 4*S*,5*S*,6*R*, 8*R*,11*S*,12*S*.

Paralemnol (**3**) was obtained as a colorless oil, $[\alpha]_D^{25}$ −72 (*c* 1.24, $CHCl_3$). The molecular formula of **3** was determined as $C_{15}H_{24}O$ by HRESIMS (*m*/*z* calcd 243.1725; found 243.1727, $[M+Na]^+$), implying four degrees of unsaturation. The IR spectrum also suggested the presence of hydroxy group (ν_{max} 3327 cm^{-1}). The ^{13}C NMR (Table 1) and DEPT spectra showed the presence of 15 carbon signals, including three methyls, five methylenes, four methines, and three quaternary carbons. The ^{13}C and ^{1}H NMR spectra revealed the presence of one tertiary hydroxy group [δ_C 75.0 (qC)] and one trisubstituted double bond [δ_H 5.41 (t, $J = 2.4$ Hz); δ_C 121.2 (CH), 142.6 (qC)]. The above finding suggested **3** to be a tricyclic sesquiterpenoid with a tertiary hydroxy group.

The ^{1}H–^{1}H COSY spectrum of **3** revealed the presence of three spin systems (**d**–**f** in Fig. 2). The HMBC correlations (Fig. 2) from H_3-14 to C-3, C-4, and C-5, H_3-15 to C-4, C-5, C-6, and C-10, H_3-13 to C-7, C-11, and C-12, H_2-9 to C-1 and C-10 led to the establishment of the planar structure of **3**. The NOESY spectrum of **3** displayed correlations (Fig. 3) between the H_3-14 and H_3-15, H_3-14 and H-6, H_3-15 and H-6, and H-6 and H-7, suggesting that H-6, H-7, H_3-14, and H_3-15 should be positioned on the β-face. Furthermore, H_3-13 showed NOE interaction with one proton (δ_H 1.84, m) of H_2-8, but not with H-7, revealing that H_3-13 should be positioned on the α-face. Also **1**–**3** are biogenetically related metabolites (latter discussed, see Scheme 1) and should have the same absolute configurations at C-4 and C-5. Hence, **3** was suggested to possess the (4*S*,5*S*,6*S*,7*S*,12*S*)-stereochemistry.

The cytotoxicity of **1**–**3** toward Daoy (human medulloblastoma), HeLa (human cervical epitheloid carcinoma), Hepa59T/VGH (human liver carcinoma), and KB (human oral epidermoid carcinoma) was assayed.



Scheme 1. Proposed biosynthetic pathway for **1**–**3**.

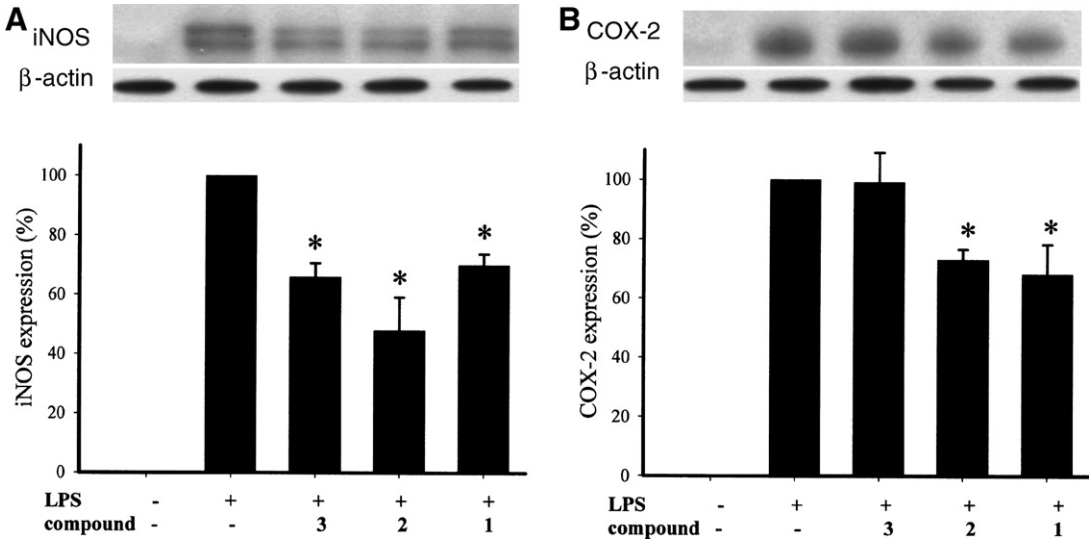


Figure 6. Effect of compounds **1**–**3** on the pro-inflammatory iNOS and COX-2 protein expression of RAW264.7 macrophage cells: (A) immunoblot of iNOS and β-actin; (B) immunoblot of COX-2 and β-actin.

It was found that all of the three metabolites were inactive (ED_{50}'s >20 μg/ml) toward the above cancer cell lines. We also investigated about the inhibition of these metabolites toward the LPS-induced pro-inflammatory proteins (iNOS and COX-2) expression. In this assay, stimulation of the RAW 264.7 cells with LPS resulted in accumulation of the pro-inflammatory iNOS and COX-2 proteins by immunoblot analysis.[6,7] Both **1** and **2** at a concentration of 10 μM could reduce the levels of the iNOS to 48.7 ± 11.2% and 70.6 ± 3.8%, respectively, and COX-2 to 73 ± 3.1% and 68.5 ± 10.1%, respectively, in comparison with those of the control cells stimulation with LPS (100% for both iNOS and COX-2). Metabolite **3** did not inhibit the COX-2 expression (99.7 ± 10.4%), but could reduce iNOS expression (66 ± 4.6%) by LPS treatment. These results can be seen in Figure 6.

A plausible biosynthetic pathway for **1**–**3** was proposed as illustrated in Scheme 1. Both **1** and **2** may be arisen from the intramolecular aldol condensation of an expected precursor **5**. Acid-catalyzed reaction of **6**[2c] as shown would lead to the formation of a four-membered ring cation **7**, which could be further reduced to metabolite **3**.

Acknowledgements

Financial support was provided by the Ministry of Education (C030313) and National Science Council of Taiwan (NSC94-2323-B-110-002) to J.-H.S.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.10.002.

References and notes

1. (a) Su, J. Y.; Zhong, Y. L.; Su, J. Y.; Zeng, L. M. *J. Nat. Prod.* **1993**, *56*, 288–291; (b) Izac, R. R.; Schneider, P.; Swain, M.; Fenical, W. *Tetrahedron Lett.* **1982**, *23*, 817–820; (c) Bowden, B. F.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 885–890.
2. (a) Daloze, D.; Braekman, J. C.; Georget, P.; Tursch, B. *Bull. Soc. Chim. Belg.* **1977**, *86*, 47–54; (b) Ahond, A.; Chiaroni, A.; Coll, J. C.; Fourneron, J. D.; Riche, C. *Bull. Soc. Chim. Belg.* **1979**, *88*, 313–324; (c) Izac, R. R.; Fenical, W.; Tagle, B.; Clardy, J. *Tetrahedron* **1981**, *37*, 2569–2573; (d) El-Gamal, A. A.; Chiu, E.-P.; Li, C.-H.; Cheng, S. Y.; Dai, C.-F.; Duh, C.-Y. *J. Nat. Prod.* **2005**, *68*, 1749–1753.
3. Huang, H.-C.; Chao, C.-H.; Liao, J.-H.; Chiang, M. Y.; Dai, C.-F.; Wu, Y.-C.; Sheu, J.-H. *Tetrahedron Lett.* **2005**, *46*, 7711–7714.
4. Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
5. Crystallography data of **1** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 611680. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].
6. Ho, F. M.; Lai, C. C.; Huang, L. J.; Kuo, T. C.; Chao, C. M.; Lin, W. W. *Br. J. Pharmacol.* **2004**, *141*, 1037–1047.
7. Park, E. K.; Shin, Y. W.; Lee, H. U.; Kim, S. S.; Lee, Y. C.; Lee, B. Y.; Kim, D. H. *Biol. Pharm. Bull.* **2005**, *28*, 652–656.