JOURNAL OF NATURAL PRODUCTS

ARTICLE

pubs.acs.org/jnp

Nardosinane Sesquiterpenoids from the Formosan Soft Coral *Lemnalia flava*

Yi Lu,[†] Po-Ju Li,[†] Wen-Yu Hung,[†] Jui-Hsin Su,[‡] Zhi-Hong Wen,[†] Chi-Hsin Hsu,[†] Chang-Feng Dai,[§] Michael Y. Chiang,[⊥] and Jyh-Horng Sheu*[,†,∥]

[†]Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[‡]Taiwan Coral Research Center, National Museum of Marine Biology & Aquarium, Checheng, Pingtung 944, Taiwan, Republic of China

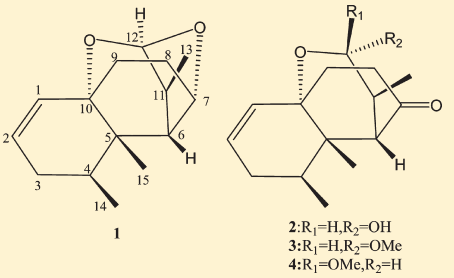
[§]Institute of Oceanography, National Taiwan University, Taipei 112, Taiwan, Republic of China

[⊥]Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

[∥]Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan, Republic of China

Supporting Information

ABSTRACT: Four new nardosinane-type sesquiterpenoids, flavalins A−D (**1**−**4**), have been isolated from the Formosan soft coral *Lemnalia flava*. The structures of the new metabolites were determined by extensive spectroscopic analysis, and the structure of **2** was confirmed by X-ray diffraction analysis. A plausible biosynthetic pathway to **1** and **2** is proposed. Compound **1** was found to display dose-dependent inhibition of iNOS protein expression, and **1** and **2** were shown to possess significant neuroprotective activity.



Recently we have discovered a series of bioactive marine natural products including steroids,[1] cembranoids,[2] and sesquiterpenoids[3] from soft corals inhabiting waters around Taiwan. Soft corals of the genera *Lemnalia* and *Paralemnalia* also have been proven to be rich sources of ylangene,[4−6] capnellane,[6] aristolane,[7−9] nardosinane,[6−11] nornardosinane,[6,7,9,10,12] calamenane,[11] guaiane,[7,13] africanane,[7,9,10] eremophilane,[6,7,12,13] neolemnane,[7,12,14] and germacrane[7,9,13] type sesquiterpenes, and some metabolites of these types were shown to possess biological activities such as cytotoxic[10,12] and anti-inflammatory[15] properties. These observations led us to carry out an investigation on the chemical constituents of the Formosan soft coral *Lemnalia flava* (May 1898) for the discovery of bioactive compounds. In this study, four new nardosinane sesquiterpenoids, flavalins A−D (**1**−**4**), were discovered. The structures of **1**−**4** were established by extensive spectroscopic analysis, including examination of 2D NMR (^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY) correlations, and the structure of **2** was unambiguously proven by X-ray diffraction analysis. The cytotoxicity of compounds **1**−**4** against several cancer cell lines was studied, and none of them were found to be cytotoxic. However, the ability of **1**−**4** to inhibit the up-regulation of pro-inflammatory iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) proteins in LPS (lipopolysaccharide)-stimulated RAW264.7 macrophage cells showed that **1** could reduce the levels of iNOS and COX-2 in a dose-dependent manner. The neuroprotective effect of **1**−**4** against the damage of 6-OHDA toward SH-SY5Y cells was also measured. The cytotoxicity of 6-OHDA on SH-SY5Y cells was significantly reduced by pretreatments of **1** and **2** at various concentrations.

Flavalin A (**1**) was obtained as an optically active pale oil. The HRESIMS of **1** exhibited a pseudomolecular ion peak at m/z 257.1519 $[M + Na]^+$, consistent with the molecular formula $C_{15}H_{22}O_2$, which required five degrees of unsaturation. The IR spectrum of **1** showed an absorption at 1658 cm^{-1}, indicating the presence of the olefinic group. The ^{1}H NMR data of **1**, measured in $CDCl_3$ (Table 1), showed signals of a *cis* 1,2-disubstituted double bond (δ 5.70, ddd, J = 10.0, 5.5, 2.0 Hz; δ 5.43, d, J = 10.0 Hz). Also, the signals of two secondary methyls (δ 0.90, d, J = 7.0 Hz; δ 0.84, d, J = 7.0 Hz) and a tertiary methyl (δ 0.84, s) were observed, suggesting the terpenoidal character of this metabolite. The ^{13}C NMR data of **1** (Table 1) showed the presence of 15 carbons, which were assigned by the assistance of a DEPT spectrum to three methyls, three methylenes, five sp^3 methines (including a monooxygenated carbon and an acetal carbon, resonating at δ_C 78.3 and 107.9, respectively), two sp^2 methines of an olefin, and two quaternary carbons (including an oxygenated carbon appearing at δ_C 77.1). The above data accounted for one of the five degrees of unsaturation, indicating a tetracyclic structure for **1**. From the COSY spectrum measured in $CDCl_3$, it was possible to establish three proton sequences from H-1 to H$_3$-14, H-6 to H$_2$-9, and H-11 to H$_3$-13 (Figure 1). The HMBC experiment showed the following correlations: H-2 to C-10; H-4

Received: August 4, 2010
Published: January 4, 2011

dx.doi.org/10.1021/np100541a | *J. Nat. Prod.* 2011, 74, 169–174

Table 1. ^{1}C NMR and ^{13}C NMR Data for Compounds 1−4

	1		2		3		4	
position	δ_C[a]	δ_H[b]	δ_C[c]	δ_H[d]	δ_C[a]	δ_H[b]	δ_C[a]	δ_H[b]
1	130.2, CH[e]	5.43, d (10.0)[f]	129.0, CH	5.50, brd (10.0)	129.2, CH	5.50, brd (10.0)	130.1, CH	5.60, brd (9.5)
2	128.2, CH	5.70, ddd (10.0, 5.5, 2.0)	130.2, CH	5.81, ddd (10.0, 4.8, 2.4)	130.0, CH	5.78, ddd (10.0, 5.0, 2.5)	129.1, CH	5.80, ddd (9.5, 5.0, 2.0)
3	31.5, CH_2	1.97, m	31.6, CH_2	2.17, m	31.6, CH_2	2.19, m	31.6, CH_2	2.14, dddd (18.5, 6.5, 5.0, 2.0)
		1.79, ddt (18.0, 11.5, 2.0)		1.77, ddt (18.8, 12.0, 2.4)		1.77, ddt (19.0,12.0, 2.5)		1.79, ddt (18.5, 12.0, 2.0)
4	30.7, CH	2.36, ddq (11.5, 7.0, 7.0)	27.5, CH	2.78, ddq (12.0, 6.8, 6.8)	27.4, CH	2.77, ddq (12.0, 7.0, 7.0)	27.6, CH	2.58, ddq (12.0, 6.5, 6.5)
5	38.0, C		39.0, C		39.0, C		38.4, C	
6	46.2, CH	1.94, d (5.0)	57.6, CH	2.49, d (5.2)	57.6, CH	2.47, d (5.0)	54.2, CH	2.29, d (5.0)
7	78.3, CH	4.57, t (5.0)	211.8, C		212.2, C		214.5, C	
8	25.3, CH_2	2.14, ddd (14.0, 7.5, 2.5)	38.3, CH_2	2.64, dd (19.2, 10.0)	38.5, CH_2	2.63, dd (19.0, 9.5)	35.0, CH_2	3.08, ddd (19.0, 10.5, 7.5)
		1.97, m		2.32, dd (19.2, 9.6)		2.36, dt (19.0, 10.0)		2.32, m
9	30.9, CH_2	2.01, m	31.5, CH_2	2.10, m	31.5, CH_2	2.19, m	30.6, CH_2	2.05, ddd (15.0, 10.5, 1.0)
		1.67, dd, (14.0, 8.5)		2.05, m		2.08, m		1.93, ddd (15.0, 10.0, 7.5)
10	77.1, C		75.1, C		74.4, C		72.3, C	
11	38.8, CH	2.55, q (7.0)	33.9, CH	1.99, m	31.9, CH	2.04, m	31.3, CH	2.32, m
12	107.9, CH	4.97, s	96.8, CH	5.05, d (9.2)	104.0, CH	4.58, d (9.5)	99.8, CH	4.66, d (5.0)
13	15.5, CH_3	0.90, d (7.0)	14.8, CH_3	0.92, d (6.8)	13.1, CH_3	0.86, d (7.0)	14.1, CH_3	0.90, d (7.5)
14	13.0, CH_3	0.84, d (7.0)	13.1, CH_3	0.80, d (6.8)	14.7, CH_3	0.79, d (7.0)	13.1, CH_3	0.79, d (6.5)
15	14.3, CH_3	0.84, s	14.9, CH_3	0.75, s	14.9, CH_3	0.72, s	15.0, CH_3	0.73, s
12-OMe					57.0, CH_3	3.51, s	55.2, CH_3	3.39, s

[a] Spectrum recorded at 125 MHz in $CDCl_3$. [b] 500 MHz in $CDCl_3$. [c] 100 MHz in $CDCl_3$. [d] 400 MHz in $CDCl_3$. [e] Attached protons were deduced by DEPT experiments. [f] J values (in Hz) in parentheses.

dx.doi.org/10.1021/np100541a |J. Nat. Prod. 2011, 74, 169–174

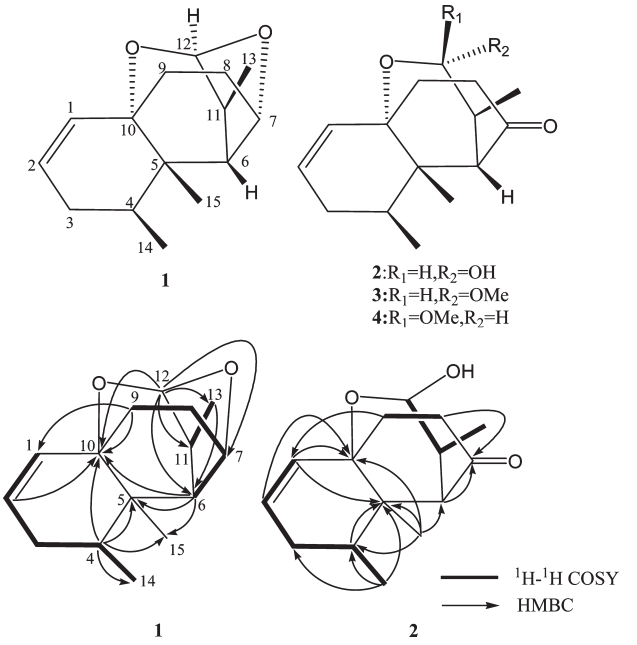


Figure 1. ^{1}H−^{1}H COSY and HMBC correlations for **1** and **2**.

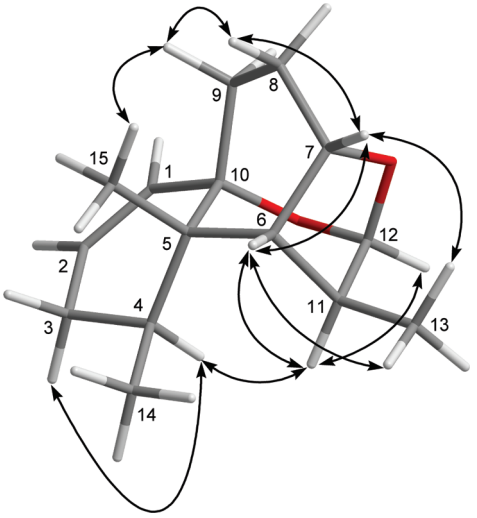


Figure 2. Key NOESY correlations of **1**.

to C-5, C-10, C-14, and C-15; H-6 to C-5, C-10, C-13, and C-15; H-7 to C-5; H-9 to C-1 and C-10; H-12 to C-6, C-7, C-10, and C-11. Thus, the ring juncture C-15 methyl should be located at C-5, one of the two ring-fused carbons of two six-membered carbocyclic rings. Also, one oxygen atom should be inserted between C-10 and C-12, and another between C-7 and C-12, to form a 2,7-dioxabicyclo[3.2.1]octane ring. This is further fused to the cyclohexane ring at C-5−C-7 and C-10, which fulfills the formation of a dioxaprotoadamantane ring by considering the molecular formula, the chemical shift values of the acetal methine 12-CH (δ_H 4.97 and δ_C 107.9), and the above HMBC correlations. Therefore, the planar structure of **1**, possessing a tetracyclic oxa-cage ring system, was established.

The relative configuration of **1** was elucidated by the analysis of NOE correlations, as shown in Figure 2. It was found that H-6 (δ 1.94) showed strong NOE interactions with H-7 (δ 4.57) and H_3-13 (δ 0.90) and a weak correlation with H-11 (δ 2.55), and

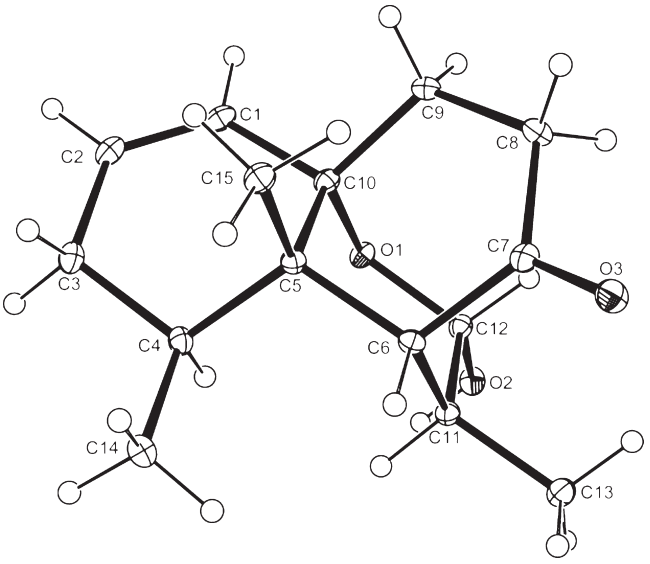


Figure 3. X-ray crystal structure of **2**.

H-7 showed a NOE interaction with H_3-13, but not with H-11; therefore, assuming the β-orientation of H-6, the locations of H-7 and H_3-13 on the β-face and H-11 on the α-face were determined. Furthermore, H-11 exhibited NOE correlations with H-4 (δ 2.36) and H-12 (δ 4.97), revealing the α-orientation of both H-4 and H-12 and also the β-orientation of H_3-14 at C-4. Moreover, significant NOE interactions were observed between H-7 and H-8β (δ 1.97), H-8β and H-9β (δ 1.67), and H-9β and a methyl at δ 0.84. Models indicate that although H_3-15 and H_3-14 have almost identical chemical shifts (δ 0.84), H_3-14 is not in close spatial proximity to H-9β, so the correlation is to H_3-15. Overall, these interactions indicated the β-orientations of both 15-methyl and 9-methylene at C-5 and C-10, respectively, as seen in Figure 2. On the basis of the above findings, the relative configuration of flavalin A was established as shown in formula **1**.

The HRESIMS peak for **2** (m/z 273.1469 [M + Na]$^+$) established the molecular formula $C_{15}H_{22}O_3$, which indicated five degrees of unsaturation, and the IR spectrum revealed the presence of carbonyl (1706 cm^{-1}) and hydroxy (3318 cm^{-1}) groups. Comparison of the ^{1}H and ^{13}C NMR data (Table 1) of compounds **1** and **2** showed that the structure of **2** should be very close to that of **1**, with the exception of the signals assigned to C-7, where an oxymethine in **1** (δ_H 4.57, t, J = 5.0 Hz, δ_C 78.3 CH) was replaced by a keto group (δ_C 211.8, C) in **2**. In the ^{1}H−^{1}H COSY spectrum, it was possible to identify three different structural units, which were assembled with the assistance of an HMBC experiment. Key HMBC correlations of 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-1 to C-5 and C-10; H-6 to C-5 and C-7; H-8 to C-7; and H-9 to C-1 permitted the establishment of the carbon skeleton (Figure 1). Furthermore, the detailed structure and relative configuration of **2** could be established unambiguously from single-crystal X-ray diffraction analysis (Figure 3).

Compound **3** possesses the molecular formula $C_{16}H_{24}O_3$, as revealed by HRESIMS (m/z 287.1622 [M + Na]$^+$). Furthermore, the ^{1}H and ^{13}C NMR data of **3** were very similar to those of **2**, with the difference that **3** contains an additional methyl relative to **2**. The observations of the chemical shift of H-12 in **3** (δ 4.58), which was shifted downfield in **2** (δ 5.05), and key HMBC correlation of the methoxy protons to C-12 suggested that **3** is

dx.doi.org/10.1021/np100541a |J. Nat. Prod. 2011, 74, 169–174

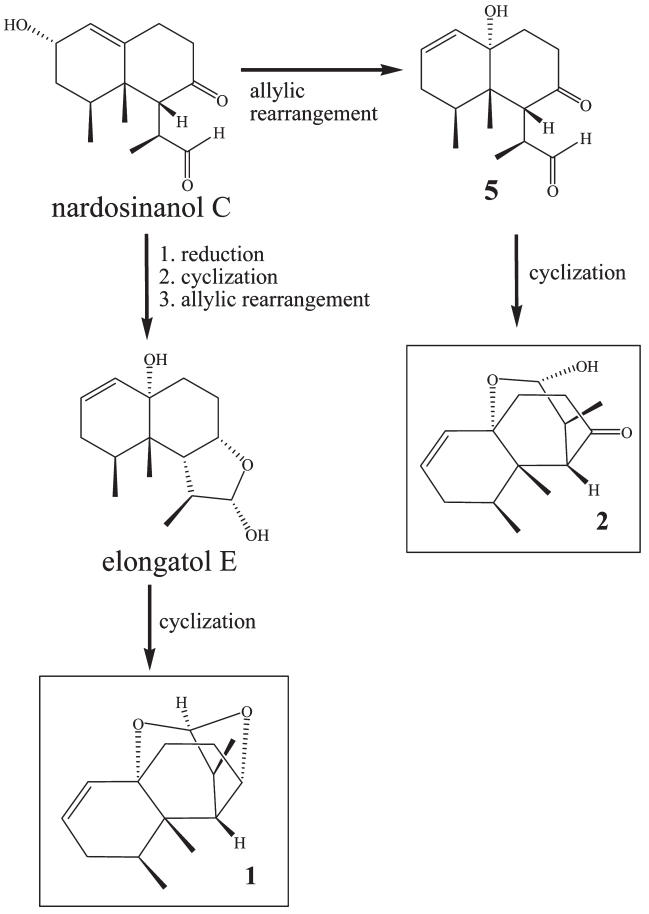


Figure 4. Proposed biosynthetic pathway to **1** and **2**.

the 12-*O*-methyl derivative of **2**. Furthermore, the relative structure of **3** was elucidated by the analysis of NOE correlations. It was found that NOE correlations of H-4 with H-11 and of H-12 with H_3-13 and a proton of C-8 but not with H-11 reflected the β-orientation of H_3-13 and H-12. On the basis of the above findings, the relative configuration of **3** was established as shown in formula **3**.

Compound **4** possesses the same molecular formula as that of **3**, as revealed from HRESIMS (m/z 287.1624 $[M+Na]^+$), indicating **4** to be an isomer of **3**. By comparison of the ^1H and ^{13}C NMR data (Table 1) and 2D NMR spectroscopic data of compounds **3** and **4**, including $^1H-^1H$ COSY, HMQC, and HMBC, compound **4** was shown to possess the same planar structure as that of **3**. Furthermore, H-12 showed NOE correlation with H-11, indicating the β-orientation of the methoxy group. On the basis of the above findings, **4** was revealed to be the C-12 epimer of **3**. Thus, the structure of **4** was established.

Plausible biosynthetic pathways to **1** and **2** from the possible precursor nardosinanol C are proposed as shown in Figure 4. A biosynthetic pathway to nardosinanol C from 1(10)-aristolene was described previously.[10] The allylic rearrangement of nardosinanol C to its isomer **5** and the ensuing cyclization to form the tetrahydropyran ring could furnish **2**. Also, the reduction of nardosinanol C produces elongatol A,[16] which could be rearranged to elongatol E.[16] Further cyclization from the C-10 hydroxy group to C-12 of elongatol E could afford **1**. The presence of both epimers of the C-12 methoxy compounds (**3** and **4**) and the use of MeOH in the purification protocol suggest that **3** and **4** might be artifacts. Treatment of **2** with MeOH and silica gel for two days, however, did not lead to the formation of either **3** or **4**.

No cytotoxicity of compounds **1**−**4** against a limited panel of human breast carcinoma (MCF-7), human colon carcinoma (WiDr), human laryngeal carcinoma (HEp 2), human medulloblastoma (Daoy), T-cell acute lymphoblastic leukemia (CCRF-CEM), colon adenocarcinoma (DLD-1), human promyelocytic leukemia (HL-60), and murine leukemia (P388D1) cell lines was found. The *in vitro* anti-inflammatory effects inhibiting LPS-induced up-regulation of pro-inflammatory proteins iNOS and COX-2 in RAW264.7 macrophage cells of compounds **1**−**4** were also tested by immunoblot analysis, and only flavalin A (**1**) was shown to significantly reduce the accumulation of iNOS and COX-2 relative to the control cells stimulated with LPS only. It was found that at concentrations of 5.0, 7.5, 10.0, and 12.5 μg/mL, **1** could reduce the levels of iNOS and COX-2 in LPS-induced macrophage cells relative to the control cells stimulated with LPS only (Figure 5). Thus, **1** was found to exhibit concentration-dependent inhibition of LPS-induced iNOS and COX-2 protein expression, and the ED_{50} values toward both proteins were 4.8 ± 0.3 μg/mL (20.5 ± 1.3 μM) and 6.2 ± 0.6 μg/mL (26.5 ± 2.6 μM), respectively. Furthermore, in a neuroprotective assay using 6-OHDA (6-hydroxydopamine)-induced neurotoxicity in neuroblastoma SH-SY5Y cells, a human dopaminergic neuron often used for the study of Parkinson's disease,[17] the neuroprotective effects of **1**−**4** against the damage of 6-OHDA were measured.[18] It was observed that the cytotoxicity of 6-OHDA (20 μM) on SH-SY5Y cells was significantly reduced by pretreatments of **1** and **2** at various concentrations. The relative neuroprotective activities of **1** and **2** at 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1}, 1, and 10 μM were 16.9 ± 1.6, 5.84 ± 1.61, 30.8 ± 3.3, 25.3 ± 3.0, 35.1 ± 3.5, and 20.9 ± 2.8%, and 29.6 ± 2.4, 32.6 ± 2.8, 30.2 ± 3.4, 24.3 ± 2.4, 21.3 ± 2.9, and 30.1 ± 5.0%, respectively. Both compounds **3** and **4** gave less than 20% protection at concentrations of 10^{-4} to 10 μM.

Our present investigation demonstrated that the Formosan soft coral *Lemnalia flava* is a good source of bioactive substances. It is worthwhile to mention that **1**, possessing a novel tetracyclic oxacage ring structure, showed significant *in vitro* anti-inflammatory and neuroprotective activities. Compound **2** also exhibited significant neuroprotective activity. Ylangene-type sesquiterpenoids[4] and decalin-type diterpene glycosides,[19] which have been discovered from previous chemical investigations of this marine organism, were not isolated in the present study.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Fisher-Johns melting point apparatus. Optical rotations were measured on a JASCO P-1020 polarimeter. IR spectra were recorded on a JASCO FT/IR-4100 infrared spectrophotometer. The NMR spectra were recorded on a Varian 400MR FT-NMR (or Varian Unity INOVA500 FT-NMR) instrument at 400 MHz (or 500 MHz) for ^1H and 100 MHz (or 125 MHz) for ^{13}C in $CDCl_3$. LRMS and HRMS spectra were obtained by ESI on a Bruker APEX II mass spectrometer. Silica gel (Merck, 230−400 mesh) was used for column chromatography. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography was performed on a Hitachi L-7100 HPLC apparatus with a Merck Hibar Si-60 column (250 × 21 mm, 7 μm) and on a Hitachi L-2455 HPLC apparatus with a Supelco C18 column (250 × 21.2 mm, 5 μm).

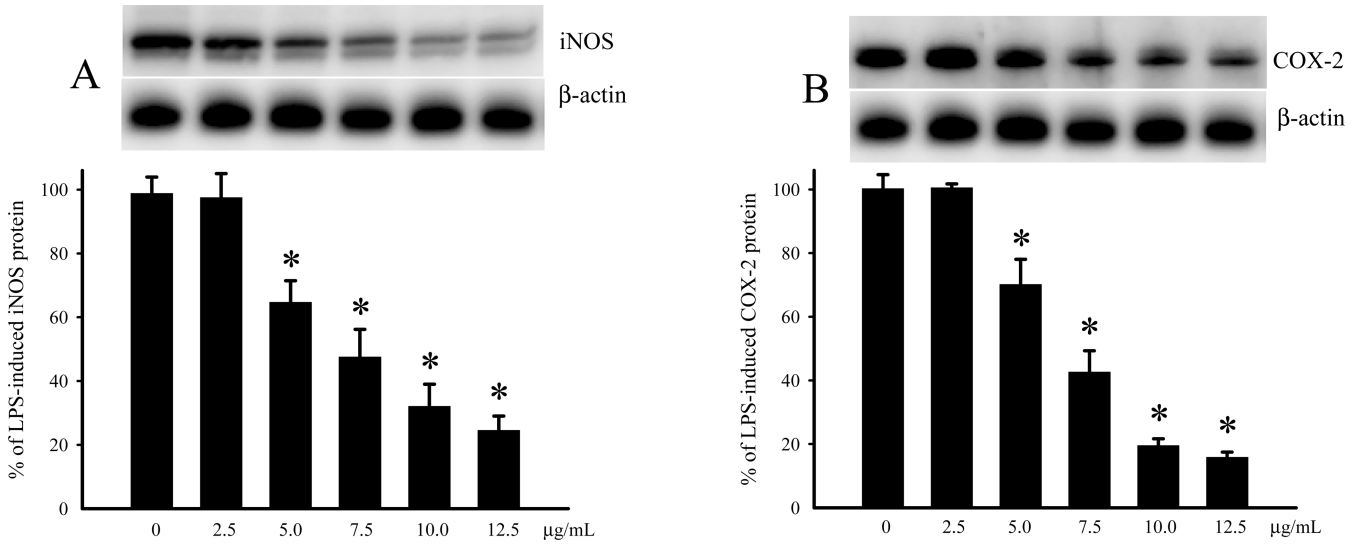


Figure 5. Concentration-dependent inhibition of LPS-induced iNOS and COX-2 expression by flavalin A (**1**) in RAW264.7 macrophage cells. (A) Relative density of iNOS immunoblot; (B) relative density of COX-2 immunoblot. The relative intensity of the LPS-stimulated group was taken to be 100%. Band intensities were quantified by densitometry and were indicated as a percentage change relative to that of the LPS-stimulated group. Western blotting with β-actin was performed to verify that equivalent amounts of protein were loaded in each lane. The experiment was repeated four times. *$P < 0.05$ indicates significant inhibition by flavalin A.

Animal Materials. *Lemnalia flava* was collected by hand using scuba off the coast of Green Island, Taiwan, in September 2007, at a depth of 10−15 m, and stored in a freezer until extraction. A voucher sample was deposited at the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. GI20070910-4).

Extraction and Isolation. The frozen soft coral of *L. flava* (0.8 kg, wet wt) was minced and exhaustively extracted with EtOAc (1 L × 5). The EtOAc extract (16.5 g) was chromatographed over silica gel by column chromatography and eluted with EtOAc in *n*-hexane (0−100%, stepwise), then with acetone in EtOAc (50−100%, stepwise), to yield 26 fractions. Fraction 13, eluting with *n*-hexane−EtOAc (20:1), was further purified over silica gel using *n*-hexane−EtOAc (3:1) to afford six subfractions (A1−A6). Subfraction A3 was separated by reversed-phase HPLC using MeOH−H_2O (2.4:1) to afford **3** (6.4 mg) and **4** (6.5 mg). Fraction 15, eluting with *n*-hexane−EtOAc (10:1), was further purified over silica gel using CH_2Cl_2−EtOAc (10:1) to afford 11 subfractions (B1−B11). Subfraction B2 was separated by normal-phase HPLC with acetone−CH_2Cl_2−*n*-hexane (1:1:2) to afford **1** (20.1 mg), and subfraction B10 was purified by reversed-phase HPLC using MeOH−H_2O (1:1) to afford **2** (6.8 mg).

Flavalin A (***1***): yellow oil; $[\alpha]^{25}_D$ +8.5 (*c* 2.5, $CHCl_3$); IR (neat) ν_{max} 2959, 2930, 1658, 1380, and 1260 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m/z* 257 $[M + Na]^+$; HRESIMS *m/z* 257.1519 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_2Na$, 257.1517).

Flavalin B (***2***): white solid; mp 169 °C; $[\alpha]^{25}_D$ −33 (*c* 0.32, $CHCl_3$); IR (neat) ν_{max} 3318, 2978, 2960, 1706, 1653, 1375, and 1253 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m/z* 273 $[M + Na]^+$; HRESIMS *m/z* 273.1469 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_3Na$, 273.1467).

Flavalin C (***3***): white solid; mp 98 °C; $[\alpha]^{25}_D$ −121 (*c* 0.11, $CHCl_3$); IR (neat) ν_{max} 2953, 2880, 1706, 1654, 1453, 1392, and 1264 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m/z* 287 $[M + Na]^+$; HRESIMS *m/z* 287.1622 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3Na$, 287.1623).

Flavalin D (***4***): white solid; mp 112 °C; $[\alpha]^{25}_D$ −270 (*c* 0.06, $CHCl_3$); IR (neat) ν_{max} 2966, 2927, 1696, 1653, 1432, 1370, and 1273 cm^{-1}; ^{13}C and ^1H NMR data, see Table 1; ESIMS *m/z* 287 $[M + Na]^+$; HRESIMS *m/z* 287.1624 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_3$, 287.1623).

X-ray Crystallography Data for Flavalin B (2) (ref 20). *X-ray Diffraction Analysis of Flavalin B* (***2***). A suitable colorless crystal (0.5 × 0.4 × 0.3 mm^3) of **2** was grown by slow evaporation of the *n*-hexane−acetone (1:5) solution. Diffraction intensity data were acquired with a Rigaku AFC7S single-crystal X-ray diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Crystal data for **2**: $C_{15}H_{22}O_3$ (formula weight 250.33), approximate crystal size, 0.5 × 0.4 × 0.3 mm^3, monoclinic, space group, $P2_1$ (# 4), T = 150(2) K, a = 6.7198(2) Å, α = 90°, b = 12.2064(4) Å, β = 107.7460(10)°, c = 8.2198(2) Å, γ = 90°, V = 642.14(3) $Å^3$, D_c = 1.295 Mg/m^3, Z = 2, $F(000)$ = 272, $\mu_{(MoK\alpha)}$ = 0.088 mm^{-1}. A total of 4275 reflections were collected in the range 2.60° < θ < 25.05°, with 2100 independent reflections [R(int) = 0.0185], completeness to $\theta_{max.}$ was 99.7%; psi-scan absorption correction applied; full-matrix least-squares refinement on F^2, the numbers of data/restraints/parameters were 2100/1/168; goodness-of-fit on F^2 = 1.100; final R indices [$I > 2\sigma(I)$], R_1 = 0.0271, wR_2 =0.0739; R indices (all data), R_1 = 0.0273, wR_2 = 0.0740, largest difference peak and hole, 0.193 and −0.182 e/$Å^{-3}$.

***In Vitro* Anti-inflammatory Assay.** The macrophage (RAW264.7) cell line was purchased from ATCC. *In vitro* anti-inflammatory activities of compounds **1**−**4** were measured by examining the inhibition of lipopolysaccharide (LPS)-induced upregulation of iNOS (inducible nitric oxide synthetase) and COX-2 (cyclooxygenase-2) proteins in macrophage cells using western blotting analysis.[15]

Neuroprotective Assay. The neuroblastoma (SH-SY5Y) cell line was purchased from ATCC, and the neuroprotective effects of compounds **1**−**4** against the damage of 6-OHDA (6-hydroxydopamine) toward SH-SY5Y cells were measured by a method reported previously.[18]

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra of **1**−**4** and X-ray data are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 886-7-5252000, ext. 5030. Fax: 886-7-5255020. E-mail: sheu@mail.nsysu.edu.tw.

■ ACKNOWLEDGMENT

This work was supported by grants from the Ministry of Education (97C031702) and National Science Council of Taiwan (NSC 98-2113-M-110-002-MY3) awarded to J.-H.S.

■ REFERENCES

(1) Chao, C.-H.; Wen, Z.-H.; Chen, I.-M.; Su, J.-H.; Huang, H.-C.; Chiang, M. Y.; Sheu, J.-H. *Tetrahedron* **2008,** *64*, 3554–3560.

(2) Lu, Y.; Huang, C.-Y.; Lin, Y.-F.; Wen, Z.-H.; Su, J.-H.; Kuo, Y.-H.; Chiang, M. Y.; Sheu, J.-H. *J. Nat. Prod.* **2008,** *71*, 1754–1759.

(3) Sheu, J.-H.; Chao, C.-H.; Wang, G.-H.; Hung, K.-C.; Duh, C.-Y.; Chiang, M. Y.; Wu, Y.-C.; Wu, C.-C. *Tetrahedron Lett.* **2004,** *45*, 6413–6416.

(4) Duh, C.-Y.; El-Gamal, A. H. A.; Song, P.-Y.; Wang, S.-K.; Dai, C.-F. *J. Nat. Prod.* **2004,** *67*, 1650–1653.

(5) Kikuchi, H.; Tsukitani, Y.; Yamada, Y.; Iguchi, K.; Drexler, S. A.; Clardy, J. *Tetrahedron Lett.* **1982,** *23*, 1063–1066.

(6) Cheng, S.-Y.; Lin, E.-H.; Huang, J.-S.; Wen, Z.-H.; Duh, C.-Y. *Chem. Pharm. Bull.* **2010,** *58*, 381–385.

(7) Jurek, J.; Scheuer, P. J. *J. Nat. Prod.* **1993,** *56*, 508–513.

(8) Bowden, B. F.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980,** *33*, 681–684.

(9) Bowden, B. F.; Coll, J. C.; Mitchell, S. J.; Nemorin, J. L. E.; Sternhell, S. *Tetrahedron Lett.* **1980,** *21*, 3105–3108.

(10) Bishara, A.; Yeffet, D.; Sisso, M.; Shmul, G.; Schleyer, M.; Benayahu, Y.; Rudi, A.; Kashman, Y. *J. Nat. Prod.* **2008,** *71*, 375–380.

(11) Bowden, B. F.; Coll, J. C.; Engelhardt, L. M.; Tapiolas, D. M.; White, A. H. *Aust. J. Chem.* **1986,** *39*, 103–121.

(12) El-Gamal, A. A. H.; Chiu, E.-P.; Li, C.-H.; Cheng, S.-Y.; Dai, C.-F.; Duh, C.-Y. *J. Nat. Prod.* **2005,** *68*, 1749–1753.

(13) Izac, R. R.; Fenical, W.; Tagle, B.; Clardy, J. *Tetrahedron* **1981,** *37*, 2569–2573.

(14) Huang, H.-C.; Chao, C.-H.; Su, J.-H.; Hsu, C.-H.; Chen, S.-P.; Kuo, Y.-H.; Sheu, J.-H. *Chem. Pharm. Bull.* **2007,** *55*, 876–880.

(15) Jean, Y.-H.; Chen, W.-F.; Duh, C.-Y.; Huang, S.-Y.; Hsu, C.-H.; Lin, C.-S.; Sung, C.-S.; Chen, I.-M.; Wen, Z.-H. *Eur. J. Pharmacol.* **2008,** *578*, 323–331.

(16) Wang, S.-K.; Duh, C.-Y. *Chem. Pharm. Bull.* **2007,** *55*, 762–765.

(17) Blum, D.; Torch, S.; Lambeng, N.; Nissou, M.; Benabid, A. L.; Sadoul, R.; Verna, J. M. *Prog. Neurobiol.* **2001,** *65*, 135–172.

(18) Lee, K.-Y.; Sung, S.-H.; Kim, Y.-C. *J. Nat. Prod.* **2006,** *69*, 679–681.

(19) Rudi, A.; Levi, S.; Benayahu, Y.; Kashman, Y. *J. Nat. Prod.* **2002,** *65*, 1672–1674.

(20) Crystallographic data for compound **2** has been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 775719). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).