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Sesquiterpenoids from the Formosan Soft Coral *Lemnalia flava*

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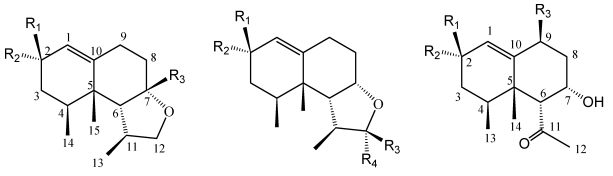
Four new nardosinane-type sesquiterpenoids, flavalins E—H (1—4) and two new nornardosinane-type norsesquiterpenoids, flavalins I (5) and J (6), along with five known compounds (7—11) have been isolated from a Formosan soft coral *Lemnalia flava*. The structures of these compounds were elucidated on the basis of their spectroscopic data. Moreover, the absolute configuration of 10 was further determined by Mosher's method.

Key words sesquiterpenoid; soft coral; *Lemnalia flava*

Our chemical studies on the Formosan soft corals have resulted in the discovery of series of bioactive natural products, including sesquiterpnoids,[1—3] diterpenoids,[4—8] steroids[9—11] and others.[12] Previous chemical investigations on soft corals of the genus *Lemnalia* have led to the isolation and identification of varieties of sesquiterpenoids. Some of these were found to possess several kinds of biological activities such as cytotoxic,[13,14] antimicrobial,[15] and anti-inflammatory properties.[16] In a previous paper, we reported the discovery of four new sesquiterpenoids with nardosinane-type skeleton from a Formosan soft coral *Lemnalia flava*.[17] During our continuing studies on the chemical constituents from the same collection of *L. flava*, we further yielded four new nardosinane-type sesquiterpenoids, flavalins E—H (**1**—**4**), and two new nornardosinane-type norsesquiterpenoids, flavalins I (**5**) and J (**6**), along with five known compounds, 2-oxolemnacarnol (**7**),[18] lemnacarnol (**8**),[18,19] armatin F (**9**),[20] (2*R*)-2-hydroxylemnal-1(10)-en-12-one (**10**)[21] and laevinol B (**11**).[14] The structures of the new compounds were established by extensive spectroscopic analysis, including careful examination of 2D-NMR correlations, and the absolute configuration of **10** was further determined using Mosher's method.[22] The cytotoxicity of compounds **1**—**11** against several cancer cell lines, including 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 studied.

The high resolution (HR) electrospray ionization-mass spectrum (ESI-MS) (*m*/*z* 287.1621 $[M+Na]^+$) of flavalin E (**1**) established the molecular formula $C_{16}H_{24}O_3$, appropriate for five of unsaturation, and the IR spectrum revealed the presence of carbonyl (1710 cm^{-1}) group. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) (Table 1) spectroscopic data showed signals of four methyls (including one methoxyl), four sp^3 methylenes (including one oxymethylene), three sp^3 methines, one sp^2 methines, two sp^3 (including one dioxymethine) and two sp^2 quaternary carbons (including one carbonyl). The ^{13}C data of **1**, measured in $CDCl_3$ (Table 1), showed a α,β-unsaturated keto group resonating at δ 198.4. The ^1H data (Table 2) of **1** showed signals of two secondary methyls (δ 0.98, d, J=6.0 Hz; δ 1.07, d, J=6.0 Hz) and a tertiary methyl (δ 1.23, s). The above data accounted for one of the five degrees of unsaturation, indicating a tricyclic structure of **1**. In the ^1H–^1H correlation spectroscopy (COSY) spectrum, it was possible to identify three different structural units, which were assembled with the assistance of an heteronuclear multiple bond correlation (HMBC) experiment (Fig. 1). Key HMBC correlations of H_3-14 to C-3, C-4 and C-5; H_3-15 to



1:R_1,R_3=O, R_3=OMe
2:R_1=OH, R_2=H, R_3=OH
7:R_1,R_2=O, R_3=OH
8:R_1=H, R_2=OH, R_3=OH

3:R_1,R_2=O, R_3=H, R_4=OMe
4:R_1=H, R_2=OAc, R_3=OMe, R_4=H
9:R_1,R_2=O, R_3=OMe, R_4=H
10:R_1=OH, R_2=H, R_3,R_4=O

5:R_1,R_2=O, R_3=H
6:R_1,R_2=O, R_3=OH
11:R_1=OH, R_2=H, R_3=H

Chart 1

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**—**6**

	1[a)]	**2**[a)]	**3**[a)]	**4**[b)]	**5**[a)]	**6**[a)]
1	126.5,CH[c)]	127.3, CH	126.8, CH	119.1, CH	125.6, CH	128.0, CH
2	198.4, qC	67.6, CH	198.5, qC	67.2, CH	198.0, qC	199.0, qC
3	41.7, CH_2	36.9, CH_2	41.8, CH_2	34.8, CH_2	41.1, CH_2	41.3, CH_2
4	35.7, CH	33.7, CH	35.3, CH	26.8, CH	35.1, CH	35.3, CH
5	41.9, qC	40.6, qC	41.6, qC	40.8, qC	42.6, qC	41.9, qC
6	58.4, CH	59.1, CH	53.2, CH	54.9, CH	60.3, CH	60.4, CH
7	110.5, qC	107.3, qC	77.6, CH	78.5, CH	68.5, CH	65.2, CH
8	28.6, CH_2	33.4, CH_2	28.3, CH_2	31.7, CH_2	29.0, CH_2	35.5, CH_2
9	28.0, CH_2	27.2, CH_2	29.2, CH_2	27.8, CH_2	30.4, CH_2	73.6, CH
10	169.6, qC	142.2, qC	168.3, qC	151.6, qC	166.5, qC	165.5, qC
11	38.7, CH	36.7, CH	42.4, CH	40.6, CH	211.1, qC	211.3, qC
12	72.6, CH_2	72.1, CH_2	114.0, CH	108.8, CH	35.6, CH_3	35.6, CH_3
13	17.1, CH_3	19.0, CH_3	19.7, CH_3	13.5, CH_3	15.2, CH_3	14.9, CH_3
14	15.9, CH_3	16.2, CH_3	15.7, CH_3	18.0, CH_3	17.8, CH_3	19.1, CH_3
15	19.1, CH_3	21.2, CH_3	19.4, CH_3	19.2, CH_3		
2-OAc				21.5, CH_3		
				170.9, qC		
7-OMe	48.9, CH_3					
12-OMe			55.8, CH_3	54.8, CH_3		

a) Spectrum recorded at 125 MHz in $CDCl_3$. *b*) 100 MHz in $CDCl_3$. *c*) Attached protons deduced by DEPT experiment.

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Table 2. ^{1}H-NMR Spectral Data for Compounds **1**—**6**

	1[a)]	**2**[a)]	**3**[a)]	**4**[b)]	**5**[a)]	**6**[a)]
1	5.92 s	5.51 s	5.90 br s	5.42 d (4.0)	5.87 d (1.5)	5.95 s
2		4.24 dd (8.0, 6.5)		5.06 dd (4.8, 4.8)		
3	2.28 m	1.80 m	2.26 m	1.59 m	2.19 m	2.27 m
	2.34 m	1.43 ddd (12.0, 12.0, 6.5)	2.24 dt (17.0, 6.0)	1.78 td (15.2, 4.8)		2.24 m
4	2.33 m	1.84 m	2.28 m	2.00 m	2.19 m	2.23 m
6	1.99 d (10.0)[c)]	1.78 d (10.0)	2.23 dd (10.0, 7.5)	1.97 m	3.52 d (5.0)	3.53 d (5.5)
7			4.49 dd (13.5, 7.0)	3.73 ddd (11.2, 9.6, 7.2)	4.37 dt (12.0, 5.0)	4.74 dt (12.0, 5.0)
8	2.07 ddd (13.5, 5.0, 3.0)	1.95 m	2.01 tdd (13.5, 7.0, 6.0)	2.13 m	1.99 qd (12.0, 6.0)	2.14 dd (12.0, 4.5)
	1.95 td (13.5, 6.0)	1.78 m	1.91 m	1.57 m	1.87 m	2.05 dd (13.5, 5.0)
9	2.57 m	2.45 m	2.66 dt (17.0, 6.0)	2.54 q (10.4)	2.57 dd (6.0, 2.5)	4.59 dd (4.5, 2.0)
	2.54 m	2.22 m	2.43 dddd (17.0, 9.5, 5.5, 1.5)	1.99 m	2.54 td (6.0, 2.0)	
11	1.84 ddq (11.0, 8.0, 6.0)	1.93 m	1.92 m	2.19 m		
12	3.90 dd (8.0, 8.0)	3.88 t (8.5)	4.57 d (3.5)	4.74 d (4.8)	2.27 s	2.27 s
	3.41 dd (11.0, 8.0)	3.48 t (9.0)				
13	1.07 d (6.0)	1.10 d (6.5)	1.21 d (6.5)	1.17 d (6.8)	1.01 d (6.0)	1.00 d (6.5)
14	0.98 d (6.0)	0.90 d (6.5)	0.99 d (5.5)	1.01 d (6.8)	1.15 s	1.37 s
15	1.23 s	1.18 s	1.15 s	1.02 s		
2-OAc				2.03 s		
7-OMe	3.32 s					
12-OMe			3.36 s	3.34 s		

a) Spectrum recorded at 500 MHz in $CDCl_3$. *b*) 400 MHz in $CDCl_3$. *c*) *J* values in Hz in parentheses.

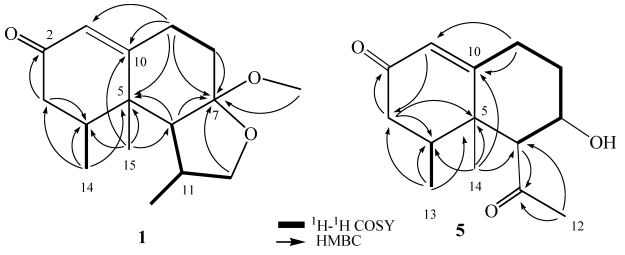


Fig. 1. Key ^{1}H–^{1}H COSY and HMBC Correlations for **1** and **5**

C-4, C-5, C-6 and C-10; H_2-12 to C-7, H_2-3 to C-2 and C-4; H-6 to C-5 and C-7; H_2-8 and C-7; and H_2-9 to C-1, C-5, and C-10, allow the establishment of the carbon skeleton. On the basis of the above analysis, the planar structure of **1** was established unambiguously. Furthermore, the relative structure of **1** was elucidated by the analysis of nuclear Overhauser effect (NOE) correlations, as shown in Fig. 2. It was found that H_3-15 (δ 1.23, s) showed NOE interactions with H-6 (δ 1.99, d, J=10.0 Hz), H_3-14 (δ 0.98, d, J=6.0 Hz) and a proton at C-8 (δ 2.07, ddd, J=13.5, 5.0, 3.0 Hz); therefore, assuming a β-orientation of H_3-15, H_3-14 and H-6 should also be positioned on the β-face. NOE correlations of the methoxyl and both protons at C-8 were observed, indicating a β-orientation of methoxyl at C-7. Moreover, the structure was also confirmed by comparison of the similar ^{1}H- and ^{13}C-NMR data of **1** with those of known compound **7**,[18)] with the difference that **1** contains one methoxyl relative to the hydroxyl of **7**. On the basis of the above findings and other detailed NOE correlations (Fig. 2), the relative structure of **1** was determined.

Flavalin F (**2**) was obtained as a white solid. A molecular formula $C_{15}H_{24}O_3$ for **2** was suggested by ESI-MS, ^{13}C- and ^{1}H-NMR spectral data (Tables 1, 2), and the HR-ESI-MS of

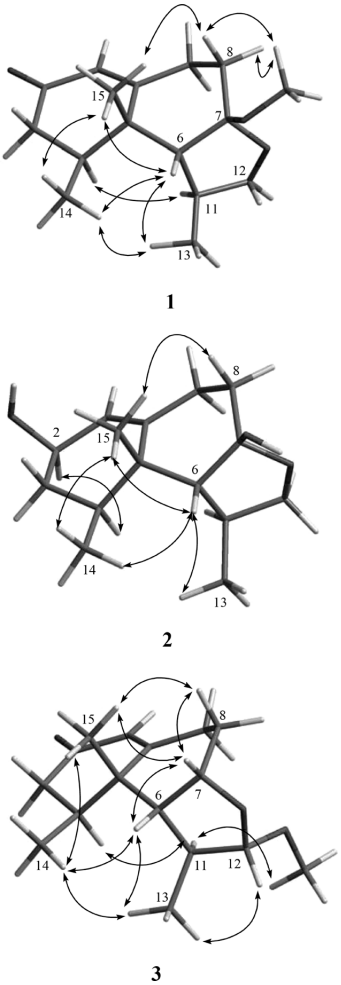


Fig. 2. Selective NOESY Correlations of **1**—**3**

the acetyl derivative of **2** (see Experimental). The IR spectrum of **2** showed the absorption of hydroxy group (3356 cm^{-1}). By comparison of the NMR spectroscopic data (Tables 1, 2) of **2** and **8**,[18,19] compound **2** was shown to possess the same planar structure as that of **8**. Furthermore, assuming the β-orientation of H_3-15, the key NOE correlations between H-2 (δ 4.24, dd, J=8.0, 6.5 Hz) and H-4 (δ 1.84, m), and H_3-14 (δ 0.90, d, J=6.5 Hz) and H_3-15 (δ 1.18, s) suggested the β-orientation of hydroxy group at C-2. From the above observations and further analysis of other NOE interactions (Fig. 2), **2** was revealed to be the C-2 epimer of **8**. Thus, the structure of **2** was established.

The HR-ESI-MS flavalin G (**3**) determined the molecular formula $C_{16}H_{24}O_3$ (m/z 287.1622 $[M+Na]^+$). By 2D-NMR spectroscopic data, including ^1H–^1H COSY, heteronuclear multiple quantum correlation (HMQC), and HMBC, compound **3** was shown to possess the same molecular framework as that of **9**.[20] Furthermore, it was found that the NMR data of **3** were very similar to those of **9**, suggesting that **3** might be an isomer of **9**. By nuclear Overhauser effect spectroscopy (NOESY) (Fig. 2), it was found that H_3-15 (δ 1.15, s) showed NOE interactions with H-7 (δ 4.49, dd, J=13.5, 7.0 Hz) and H_3-14 (δ 0.99, d, J=5.5 Hz); therefore, assuming the β-orientation of H_3-15, H-7 and H_3-14 should also be positioned on the β face. NOE correlations of H_3-13 with H-6, H-12 and H_3-14 exhibited the β-orientation of C-13 at C-11. On the other hand, H-11 showed NOE correlations with H-4 and methoxyl, indicating α-orientation of methoxyl at C-12. On the basis of the above findings, **3** was revealed to be the C-12 epimer of **9**.

The HR-ESI-MS of flavalin H (**4**) showed that it possesses the molecular formula $C_{18}H_{28}O_4$ (m/z 331.1881 $[M+Na]^+$). The IR spectrum of **4** showed the absorption of carbonyl group (1717 cm^{-1}). Comparison of the ^1H- and ^{13}C-NMR spectroscopic data (Tables 1, 2) of compounds **4** and **9**[20] showed that the structure of **4** should be very similar to that of **9**, with the exception of signals assigned to C-2, where a keto group in **9** was replaced by one acetoxymethine (δ_H 2.03, 3H, s, δ_C 170.9 and 21.5; δ_H 5.06, dd, J=4.8, 4.8 Hz, δ_C 67.2) in **4**. Furthermore, the key NOE correlation between H-11 (δ 2.19, m) and both H-4 and H-12 (δ 4.74, d, J=4.8 Hz) showed the β-orientation of H_3-13 and methoxyl at C-12, assuming the α-orientation of H-4. H-2 exhibited NOE correlations with both protons of H_2-3, but not H-4, reflecting the α-orientation of the acetoxy group at C-2. From the above observations and further analysis of other NOE interactions (Fig. 3), the structure of **4** was established.

The HR-ESI-MS of flavalin I (**5**) revealed that it possesses the molecular formula $C_{14}H_{20}O_3$ (m/z 259.1310 $[M+Na]^+$).

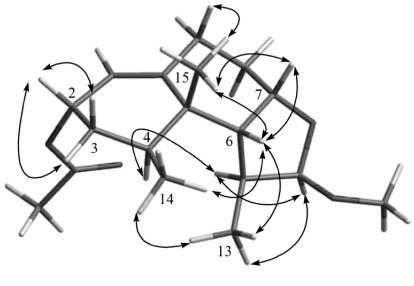


Fig. 3. Selective NOESY Correlations of **4**

The IR spectrum of **5** showed the absorption of carbonyl groups (1716, 1669 cm^{-1}) and hydroxy groups (3333 cm^{-1}). Comparison of the ^1H- and ^{13}C-NMR spectroscopic data of **5** (Tables 1, 2) with those of **11**[13] showed that the structure of **5** should be very close to that of **11**, with the exception of signals assigned to C-2, where a hydroxymethine in **11** was replaced by a keto group (δ_C 198.0) in **5**. This was further supported by the planar structure established by 2D-NMR analysis of **5** (Fig. 1). Furthermore, H_3-14 (δ_H 1.15, s) exhibited NOE correlations with H_3-13 (δ_H 1.01, d, J=6.0 Hz), H-6 (δ_H 3.52, d, J=5.0 Hz) and H-7 (δ_H 4.37, dt, J=12.0, 5.0 Hz) showed the α-orientation of both acetyl group at C-6 and hydroxy group at C-7. Thus, the structure of **5** was established.

A molecular formula for $C_{14}H_{20}O_4$ for flavalin J (**6**) was suggested by HR-ESI-MS. The IR spectrum of **6** showed the absorptions of carbonyl groups (1719, 1657 cm^{-1}) and hydroxy group (3393 cm^{-1}). The NMR spectroscopic data of **6** were found to be very similar to those of **5** (Tables 1, 2) with the exception of signals assigned to C-9, where a methylene in **5** was replaced by an oxymethine (δ_H 4.59, dd, J=4.5, 2.0 Hz; δ_C 73.6) in **6**. The NOESY spectrum of **6** (Fig. 4) showed correlations of H-9 with H-1 (δ 5.95, s) and both protons at C-8 (δ_H 2.14, d, J=12.0, 4.5 Hz and 2.05, dd, J=13.5, 5.0 Hz), indicating the β-orientation of the C-9 hydroxy group. Further analysis of NOE correlations revealed that **6** possessed the same configurations at C-4, C-5, C-6, and C-7, as those in compound **5**. On the basis of the above findings, the structure of **6** was established.

The absolute configuration of **10** was also determined by the use of Mosher's method.[22] The (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of **10** (**10a**, **b**, respectively) were prepared using the corresponding *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenyl-

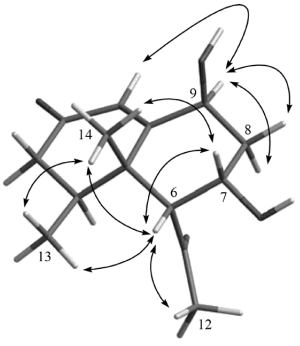
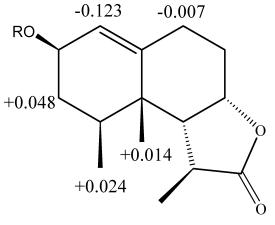


Fig. 4. Selective NOESY Correlations of **6**



10a: R = (*S*)-MTPA
10b: R = (*R*)-MTPA

Fig. 5. ^1H-NMR Chemical Shift Differences $\Delta\delta$ (δ_S–δ_R) in ppm for the MTPA Esters of **10**

acetyl chlorides, respectively. The determination of the chemical shift differences (δ_S–δ_R) for the protons neighboring C-2 led to the assignment of the *R* configuration at C-2 in **10** (Fig. 5). Thus, the absolute configuration of **10** has been determined.

The presence of both the C-12 methoxy epimers (**3**, **9**) and the use of MeOH in the purification suggest that methoxy-containing compounds **1**, **3**, **4** and **9** might be artifacts. Treatment of **7** with MeOH and silica gel for 10 d, however, led to the quantitative recovery of **7** without the formation of **1**. Based on the shared biosynthetic pathway, compounds **1**—**9** and **11** are assumed to have the same absolute configurations as **10** at C-4 and C-5, as these compounds were isolated from the same organism.

The cytotoxicity of compounds **1**—**11** was tested against the proliferation of a limited panel of cancer cell lines, including MCF-7, WiDr, HEp 2, Daoy, CCRF-CEM, DLD-1, HL-60 and P388D1 carcinoma cells. The results showed that all of the compounds were not cytotoxic toward the above cancer cells (IC_{50}'s $>20\ \mu$g/ml).

Experimental

Melting points were determined using a Fisher–Johns melting point apparatus. Optical rotations were measured on a JASCO P-1020 polarimeter. Ultraviolet spectra were recorded on a JASCO V-650 spectrophotometer. 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 ^{1}H and 100 MHz (or 125 MHz) for ^{13}C in $CDCl_3$. Low resolution (LR)-MS and HR-MS 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).

Animal Material *L. flava* (specimen no. GI20070910-4) 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.

Extraction and Isolation The frozen bodies of *L. flava* (0.8 kg, wet weight) were 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 reverse-phase HPLC using MeOH–H_2O (2.4 : 1) to afford **4** (2.2 mg). Fraction 14, eluting with *n*-hexane–EtOAc (10 : 1), was further purified over silica gel using *n*-hexane–EtOAc (2 : 1) to afford eight subfractions (B1—B11). Subfraction B9 was separated by reverse-phase HPLC using MeOH–H_2O (3 : 1) to afford **1** (6.5 mg), **3** (4.3 mg) and **9** (12.8 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 eleven subfractions (C1—C11). Subfraction C3 was separated by reverse-phase HPLC using MeOH–H_2O (1 : 1.4) to afford **7** (16.5 mg) and **10** (12.5 mg). Subfraction C4 was separated by reverse-phase HPLC using MeOH–H_2O (1 : 1.5) to afford **2** (1.7 mg), **8** (6.3 mg) and **11** (3.8 mg). Fraction 16, eluting with *n*-hexane–EtOAc (5 : 1), and was further purified over silica gel using CH_2Cl_2–acetone (9 : 1) to afford seven subfractions (D1—D7). Subfraction D2 was separated by reverse-phase HPLC using MeOH–H_2O (1 : 2.5) to afford **5** (2.0 mg). Fraction 20, eluting with *n*-hexane–EtOAc (1 : 1), and was further purified over silica gel using *n*-hexane–acetone (10 : 3) to afford seven subfractions (E1—E7). Subfraction E5 was separated by reverse-phase HPLC using MeOH–H_2O (1 : 2.1) to afford **6** (1.3 mg).

Flavalin E (**1**): Colorless oil; $[\alpha]_D^{23}$ −154 (c=0.18, $CHCl_3$); IR (neat) ν_{max} 2970, 2938, 2884, 1654, 1456, and 1273 cm^{-1}; UV (MeOH) λ_{max} 242 (log ε=3.7); ^{13}C- and ^{1}H-NMR data, see Tables 1 and 2; ESI-MS *m*/*z* 287 $[M+Na]^+$; HR-ESI-MS *m*/*z* 287.1621 $[M+Na]^+$ (Calcd for $C_{16}H_{24}O_3Na$, 287.1623).

Flavalin F (**2**): White solid; mp 124—126 °C; $[\alpha]_D^{23}$ −160 (c=0.17, $CHCl_3$); IR (neat) ν_{max} 3356, 2928, 2865, 2654, 1739, 1656, 1460, 1388, and 1262 cm^{-1}; ^{13}C- and ^{1}H-NMR data, see Tables 1 and 2; ESI-MS *m*/*z* 275 $[M+Na]^+$.

Flavalin G (**3**): White solid; mp 128—130 °C; $[\alpha]_D^{23}$ −310 (c=0.1, $CHCl_3$); IR (neat) ν_{max} 2968, 2920, 2850, 1709, 1670, 1456, 1373, and 1263 cm^{-1}; UV (MeOH) λ_{max} 239 (log ε=4.4); ^{13}C- and ^{1}H-NMR data, see Tables 1 and 2; ESI-MS *m*/*z* 287 $[M+Na]^+$; HR-ESI-MS *m*/*z* 287.1622 $[M+Na]^+$ (Calcd for $C_{16}H_{24}O_3Na$, 287.1623).

Flavalin H (**4**): Colorless oil; $[\alpha]^{23}{}_D$ −188 (c=0.22, $CHCl_3$); IR (neat) ν_{max} 2931, 2983, 1717, 1684, 1452, 1371 and 1220 cm^{-1}; ^{13}C- and ^{1}H-NMR data, see Tables 1 and 2; ESI-MS *m*/*z* 331 $[M+Na]^+$; HR-ESI-MS *m*/*z* 331.1881 $[M+Na]^+$ (Calcd for $C_{18}H_{28}O_4Na$, 331.1885).

Flavalin I (**5**): White solid; mp 213—215 °C; $[\alpha]_D^{23}$ −198 (c=0.2, $CHCl_3$); IR (neat) ν_{max} 3333, 2956, 2916, 2869, 1716, 1669, 1383, and 1261 cm^{-1}; UV (MeOH) λ_{max} 245 (log ε=3.9); ^{13}C- and ^{1}H-NMR data, see Tables 1 and 2; ESI-MS *m*/*z* 259 $[M+Na]^+$; HR-ESI-MS *m*/*z* 259.1311 $[M+Na]^+$ (Calcd for $C_{14}H_{20}O_3Na$, 259.1310).

Flavalin J (**6**): White solid; mp 160 °C; $[\alpha]_D^{23}$ −147 (c=0.13, $CHCl_3$); IR (neat) ν_{max} 3393, 2924, 2857, 1719, 1657, 1361 and 1288 cm^{-1}; ^{13}C- and ^{1}H-NMR data, see Tables 1 and 2; ESI-MS *m*/*z* 275 $[M+Na]^+$; HR-ESI-MS *m*/*z* 275.1262 $[M+Na]^+$ (Calcd for $C_{14}H_{20}O_4Na$, 275.1259).

Acetylation of 2 A solution of **2** (0.5 mg) in pyridine (0.1 ml) was mixed with Ac_2O (10 μl), and the mixture was stirred at room temperature for 12 h. After evaporation of excess reagent, the residue was subjected to column chromatograph over Si gel using *n*-hexane–acetone (3 : 1) to give flavalin H acetate (0.3 mg, 53%). Selective ^{1}H-NMR ($CDCl_3$, 400 MHz) data of flavalin H acetate: δ 5.44 (1H, s, H-1), 5.32 (1H, t, J=8.4 Hz, H-2), 2.44 (1H, m, H-9a), 2.44 (1H, m, H-9a), 3.88 (1H, t, J=8.0 Hz, H-12a), 3.48 (1H, t, J=8.4 Hz, H-12b), 1.09 (3H, d, J=6.0 Hz, H-13) 0.90 (3H, d, J=6.4 Hz, H-14), 1.19 (3H, s, H-15), 2.06 (3H, s, OAc). ESI-MS *m*/*z* 317 $[M+Na]^+$; HR-ESI-MS *m*/*z* 317.1728 $[M+Na]^+$ (Calcd for $C_{17}H_{26}O_4Na$, 317.1729).

Preparation of (*S*)- and (*R*)-MTPA Esters of 10 To a solution of **10** (1.0 mg) in pyridine (0.1 ml) was added (*R*)-MTPA chloride (10 μl), and the mixture was allowed to stand for 12 h at room temperature. After the evaporation of the solvent, the residue was subjected to short silica gel column chromatography using *n*-hexane–acetone (2 : 1) to yield the (*S*)-MTPA ester, **10a** (0.5 mg). The same procedure was applied to obtain the (*R*)-MTPA ester **10b** (0.5 mg) from the reaction of (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride with **1** in pyridine. Selective ^{1}H-NMR ($CDCl_3$, 400 MHz) data of **10a**: δ 5.428 (1H, s, H-1), 1.963 (H, m, H-3a), 2.138 (1H, m, H-9a), 0.966 (3H, d, J=6.8 Hz, H-14), 1.098 (3H, s, H-15); selective ^{1}H-NMR ($CDCl_3$, 400 MHz) data of **10b**: δ: 5.551 (1H, s, H-1), 1.915 (H, m, H-3a), 2.145 (1H, m, H-9a), 0.942 (3H, d, J=6.4 Hz, H-14), 1.084 (3H, s, H-15).

Cytotoxicity Testing Cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of compounds **1**—**11** were performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric method.[23,24]

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