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Article

Novel Polyprenylated Phloroglucinols from *Hypericum sampsonii*

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Abstract: *Hypericum sampsonii* Hance (Clusiaceae) is a folk medicine used in Taiwan to treat blood stasis, relieve swelling, and as an anti-hepatitis drug. Two new polyprenylated phloroglucinol derivatives, hypersampsone R (**1**) and hypersampsone S (**2**), and a known prenylated benzophenone, hyperibone K (**3**) were isolated from the aerial parts of *H. sampsonii*. Their structures were determined by extensive 1D and 2D NMR, and MS spectral analyses.

Keywords: *Hypericum sampsonii*; Guttiferae; polyprenylated phloroglucinol

1. Introduction

Hypericum sampsonii Hance (Guttiferae) is a folk herbal medicine used in Taiwan for treating blood stasis, to relieve swelling, and as an anti-hepatitic drug [1]. Due to not only the biological activities, but also the structural diversity, the chemical constituents of *Hypericum* species have attracted much attention, and different kinds of compounds such as xanthones [2–5], benzophenones [5,6], bisanthraquinones [6,7], and polyprenylated phloroglucinols [8–16] have been isolated. A continuing chemical investigation on the secondary metabolites of this plant resulted in the isolation of a new

ring-opened polyprenylated benzophenone, hypersampsone R (**1**) and a new polyprenylated phloroglucinol, hypersampsone S (**2**) (Figure 1), as well as a known polyprenylated benzophenone, hyperibone K (**3**) (Figure 2). This paper describes the structural elucidation of compounds **1** and **2**.

Figure 1. The chemical structures of new compounds **1** and **2** isolated from *H. sampsonii*.

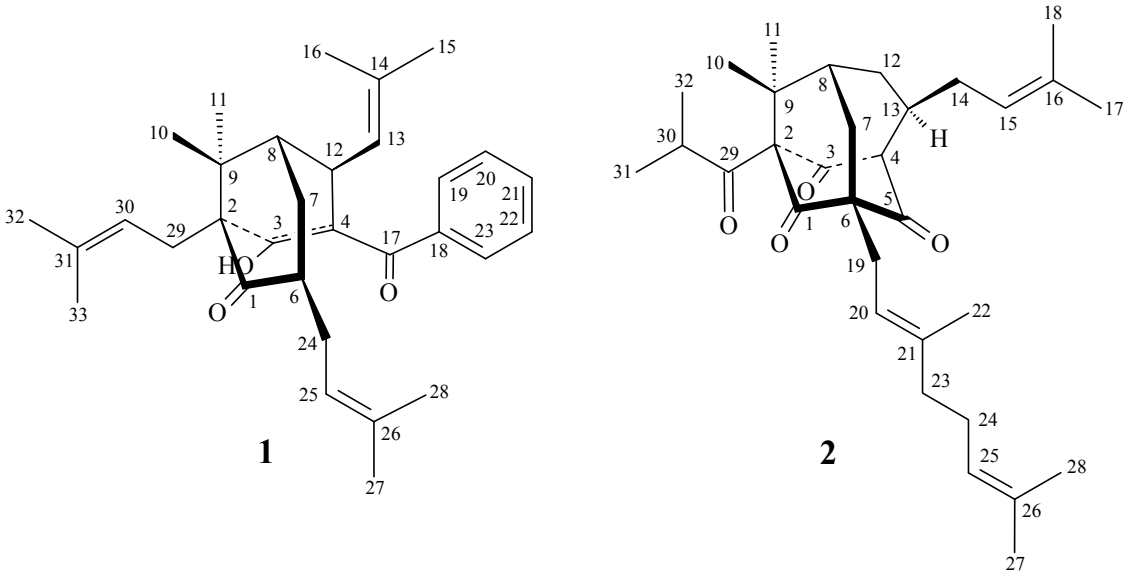
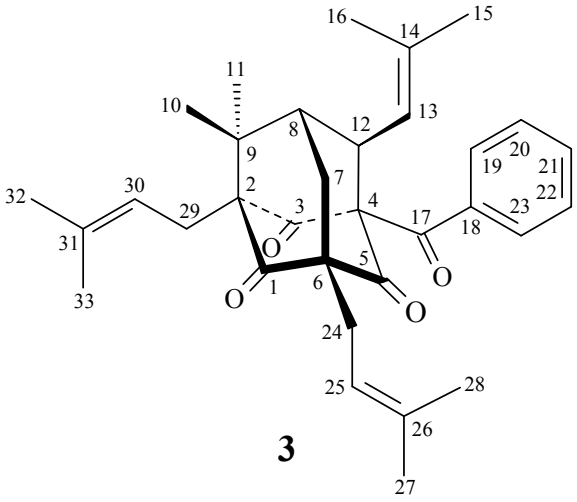


Figure 2. The chemical structure of known compound **3** isolated from *H. sampsonii*.



2. Results and Discussion

The ethanol extract of the air-dried aerial parts of *H. sampsonii* was successively partitioned with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) to give EtOAc, *n*-BuOH and H_2O fractions. The EtOAc soluble partition enriched in polyprenylated phloroglucinols was subjected to silica gel and RP-18 column chromatography in combination with preparative silica-gel HPLC to yield two new compounds **1** and **2**, together with a known compound **3**.

Hypersamsone R (**1**) was isolated as an optically active ($[\alpha]_D^{25} = +160°$), colorless amorphous powder. The molecular formula was established as $C_{32}H_{42}O_3$ on the basis of HR-EI-MS (found *m/z* 474.3128,

calcd. for $C_{32}H_{42}O_3$ 474.3134) with twelve indices of hydrogen deficiency (IHD). The IR spectrum displayed absorptions of hydroxyl (3443 cm^{-1}) and carbonyl groups (1709 and 1677 cm^{-1}). The ^{1}H-NMR spectrum of **1** showed three olefinic protons [δ_H 4.63 (1H, d, J = 7.8 Hz), 4.98 (1H, t, J = 6.5 Hz), and 5.15 (br s)], eight methyls [δ_H 0.92, 1.18, 1.24, 1.26, 1.54, 1.56, 1.65, and 1.66 (each 3H, s)], a benzoyl [δ_H 7.36 (3H, m) and 7.39 (2H, m)], and a conjugated hydroxyl group [δ_H 16.25 (s)]. The ^{13}C-NMR, DEPT and HMQC spectra indicated the presence of 32 carbons, including two carbonyls (δ_C 195.2 and 209.4), eight quaternary carbons with one conjugated oxygenated quaternary carbon (δ_C 185.5), five fully substituted aromatic and olefinic quarternary carbons (δ_C 111.4, 130.3, 132.7, 133.2, and 139.3) and two quaternary carbons (δ_C 42.4 and 65.4), eight double-bond methine carbons [δ_C 121.3, 122.9, 125.7, 126.7, 126.7, 128.0, 128.0 and 130.1], three methine carbons [δ_C 35.4, 45.3, 45.5], three methylene carbons [δ_C 26.3, 28.3, 29.9], and eight methyl carbons [δ_C 17.3, 17.9, 17.9, 25.1, 25.1, 25.7, 26.0, 26.0]. The ^{1}H-^{1}H COSY indicated the correlations of H-12 (δ_H 4.00) and H-13 (δ_H 4.63) and H-8 (δ_H 1.50); H-6 (δ_H 2.50) and H-7 (δ_H 1.44, 2.06) and H-24 (δ_H 2.32). Comparison of the ^{1}H- and ^{13}C-NMR data (Table 1) of **1** with those of hyperibone K (**3**) [17] suggested that their structures were closely related, except that the C3,C4-double bond, 3-hydroxy group, and the lack of carbonyl group between C-4 and C-6 of **1** replaced the C3,C4-single bond and 3-oxo group of hyperibone K (**3**) [17]. This was supported by HMBC correlations (Figure 3) between OH-3 (δ_H 16.25) and C-2 (δ_C 65.4), C-3 (δ_C 185.5), and C-4 (δ_C 111.4); between H-12 (δ_H 4.00) and C-3 (δ_C 185.5), C-4 (δ_C 111.4), C-7 (δ_C 29.9), C-8 (δ_C 45.3), C-13 (δ_C 125.7) and C-14 (δ_C 132.7). The NOESY cross-peaks (Figure 3) between H-7/Me(10), H-7/H-13, H-7/H-24, H$_\alpha$-12/H-19, H$_\alpha$-12/Me(11), and H-30/Me(11) suggested that H-12, Me(11), 2-isoprenyl, and the 4-benzoyl groups are α-oriented, and 6-isoprenyl, Me(10), and C-12 2-methylprop-1-enyl groups are β-oriented. The full assignment of ^{1}H- and ^{13}C-NMR resonances was supported by ^{1}H-^{1}H COSY, DEPT, HMQC, NOESY (Figure 3), and HMBC (Figure 3) spectral analyses. According to the above data, hypersamsone R was identified as structure **1**.

Figure 3. Key NOESY (**a**) and HMBC (**b**) correlations of **1**.

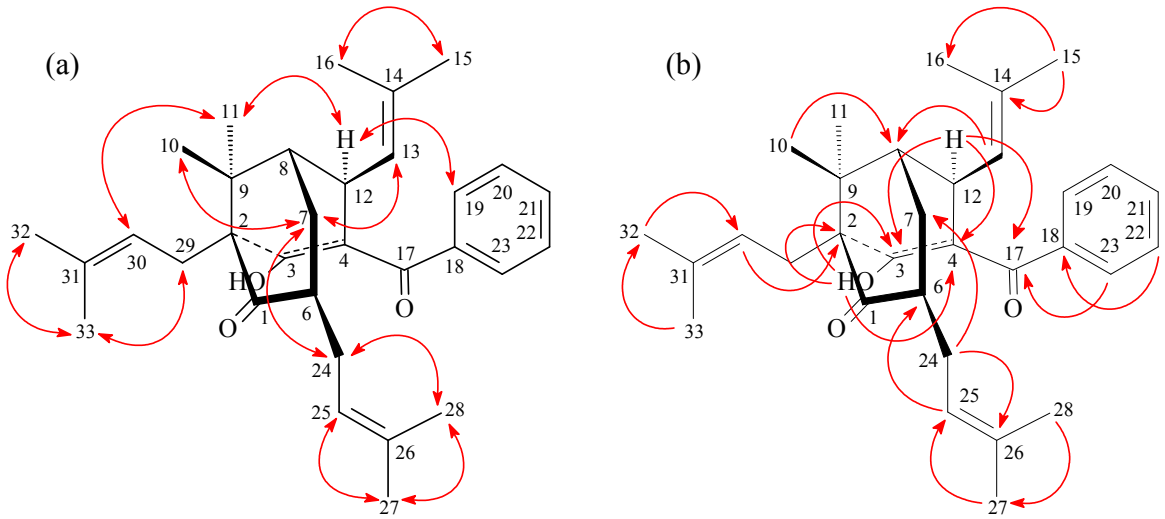


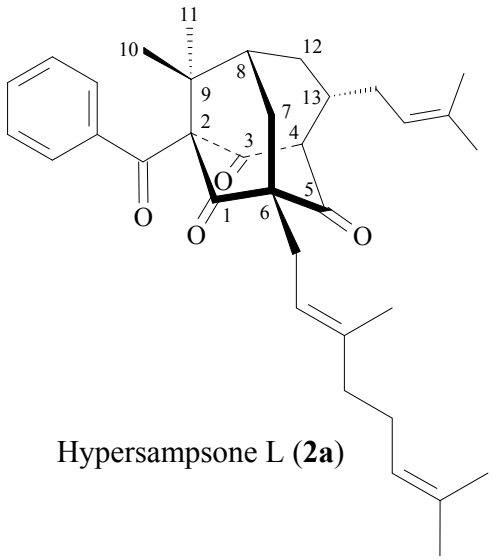
Table 1. ^{1}H- and ^{13}C-NMR data of **1** and **2**.

Position	δ_H			
	1 [a]		**2** [a]	
	^{1}H	**^{13}C**	**^{1}H**	**^{13}C**
1		209.4 s		202.2 s
2		65.4 s		88.8 s
3		185.5 s		205.4 s
4		111.4 s	2.97 m	50.0 d
5				204.7 s
6	2.50 m	45.5 d		69.2 s
7	1.44 m	29.9 t	1.87 d (10.5)	36.2 t
	2.06 m		2.50 m	
8	1.50 m	45.3 d	1.75 m	41.4 d
9		42.4 s		50.0 s
10	1.26 s	26.0 q	1.20 s	24.8 q
11	0.92 s	25.1 q	1.18 s	24.2 q
12	4.00 dd (7.8, 7.0)	35.4 d	1.84 d (7.8)	35.4 d
			2.74 m	
13	4.63 d (7.8)	125.7 d	2.81 m	58.1 d
14		132.7 s	2.06 m	31.1 t
			2.30 m	
15	1.24 s	25.1 q	4.97 t (6.5)	119.9 d
16	1.18 s	17.3 q		135.8 s
17		195.2 s	1.67 s	25.8 q
18		139.3 s	1.56 s	17.9 q
19	7.36 m	128.0 d	2.50 m	32.3 t
			2.66 dd (11.5, 7.0)	
20	7.39 m	126.7 d	4.99 t (7.0)	119.3 d
21	7.36 m	130.1 d		139.6 s
22	7.39 m	126.7 d	1.61 s	16.4 q
23	7.36 m	128.0 d	2.01 m	40.1 t
24	1.88 m	28.3 t	2.03 m	26.6 t
	2.32 m			
25	4.98 t (6.5)	121.3 d	5.00 t (6.5)	124.1 d
26		133.2 s		131.5 s
27	1.65 s	26.0 q	1.64 s	25.7 q
28	1.54 s	17.9 q	1.56 s	17.6 q
29	2.57 dd (13.0, 7.0)	26.3 t		211.5 s
	2.89 br d (13.0)			
30	5.15 br s	122.9 d	2.45 hepta (7.0)	42.8 d
31		130.3 s	1.19 d (7.0)	21.0 q
32	1.66 s	25.7 q	1.21 d (7.0)	21.0 q
33	1.56 s	17.9 q		
OH	16.25 s			

[a] Recorded in $CDCl_3$ at 500 MHz (^{1}H) and 125 MHz (^{13}C). Values in ppm (δ). *J* (in Hz) in parentheses.

Hypersamsone S (2) was obtained as a colorless amorphous powder. The molecular formula was determined to be $C_{32}H_{46}O_4$ on the basis of HR-EI-MS (found *m/z* 494.3396, calcd. for $C_{32}H_{46}O_4$ 494.3394) with nine IHD. The ^{13}C-NMR, DEPT and HMQC spectra indicated ten quaternary carbons [including four carbonyl (δ_C 202.2, 204.7, 205.4, and 211.5), three olefinic quaternary (δ_C 131.5, 135.8, and 139.6), and three other quaternary carbons (δ_C 50.0, 69.2, and 88.8)], seven tertiary carbons [including three olefinic (δ_C 119.3, 119.9, and 124.1) and four other tertiary carbons (δ_C 41.4, 42.8, 50.0, and 58.1)], and six methylene carbons (δ_C 26.6, 31.1, 32.3, 35.4, 36.2, and 40.1), and nine methyl carbons (δ_C 16.4, 17.6, 17.9, 21.0, 21.0, 24.2, 24.8, 25.7, and 25.8). Comparison of the ^{1}H- and ^{13}C-NMR data (Table 1) of 2 with those of hypersampsone L (2a) [18] suggested that their structures were closely related, except that 2-isobutyryl and 13β-isoprenyl groups of 2 replaced 2-benzoyl and 13α-isoprenyl groups of hypersampsone L (**2a**, Figure 4) [18]. This was supported by HMBC correlations (Figure 5) between H-15 (δ_H 4.97) and C-13 (δ_C 58.1); between H-20 (δ_H 4.99) and C-6 (δ_C 69.2); and between H-30 (δ_H 2.45) and C-2 (δ_C 88.8). The NOESY cross-peaks (Figure 5) between H_α-4/ H_α-13, H_α-13/Me(11), H-32/Me(11), H-7/Me(10), H-7/H-14, H-7/H-19, suggested that H_α-4, H_α-13, Me(11), and the 2-isobutyryl groups are α-oriented, and 6-geranyl, Me(10), and 13-isoprenyl groups are β-oriented. On the basis of the evidence above, the structure of hypersamsone S was elucidated as 2, which was further substantiated through 2D-experiments, including HMQC, ^{1}H-^{1}H COSY, HMBC (Figure 5), and NOESY (Figure 5) spectra.

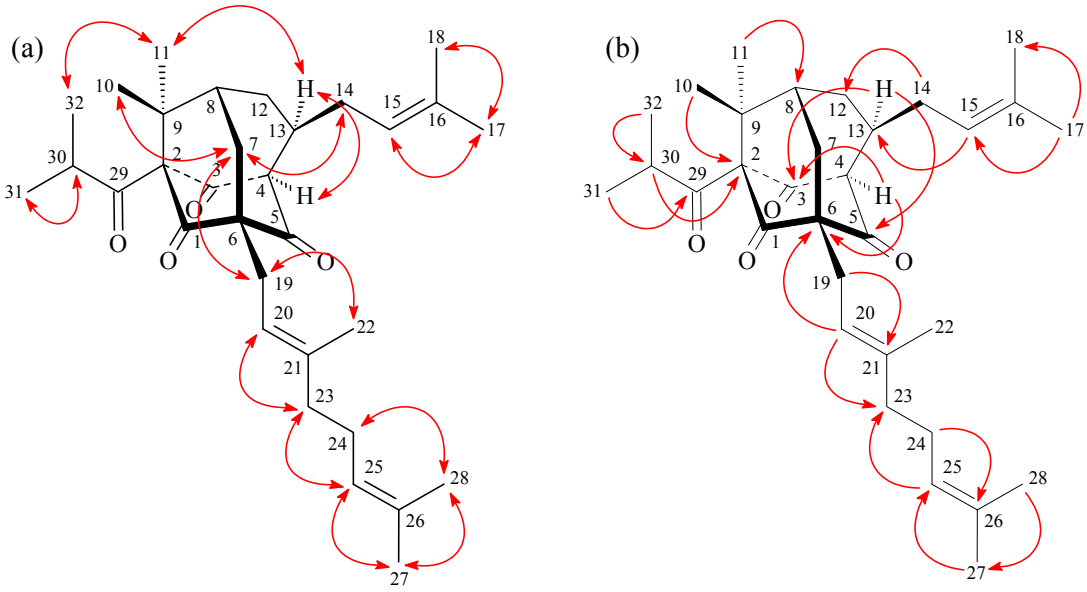
Figure 4. The chemical structure of hypersampsone L (**2a**).



Hypersampsone L (**2a**)

The known isolate, hyperibone K (**3**) was readily identified by a comparison of physical and spectroscopic data (IR, ^{1}H-NMR, $[\alpha]_D$, and MS) with the literature values [17].

Figure 5. Key NOESY (**a**) and HMBC (**b**) correlations of **2**.



3. Experimental Section

3.1. General Experimental Procedures

Optical rotations were measured using a Jasco P-2000 polarimeter in $CHCl_3$. Infrared (IR) spectra (KBr) were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer. Nuclear magnetic resonance (NMR) spectra, including correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), heteronuclear multiple-bond correlation (HMBC), and heteronuclear multiple quantum coherence (HMQC) experiments, were acquired using a Varian Inova 500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using $CDCl_3$ as solvent. Chemical shifts were referenced to the residual solvent peaks (δ_H 7.24 and δ_C 77.0). Mass spectra (EIMS and HREIMS) were recorded on a Finnigan LCQ and JEOL Finnigan MAT 95S Mass Spectrometer, respectively. Column chromatography was performed using silica gel (70-230 mesh, Merck, Darmstadt, Germany) and Sephadex^{TM} LH-20 (Amersham Biosciences, Uppsala, Sweden). Preparative HPLC was conducted using a L-2130 pump (Hitachi, Tokyo, Japan) anda LiChrosorb Si-60 column (Merck).

3.2. Plant Material

The aerial parts of *Hypericum sampsonii* Hance were collected from Chia-Yi county in June 2007. The plant was identified by Mr. Jun-Chih Ou, former associate research fellow of National Research Institute of Chinese Medicine, and compared with a voucher specimen which was deposited in the Herbarium of the Institute of Ecology and Evolutionary Biology, National Taiwan University, Taipei, Taiwan (No.077152).

3.3. Extraction and Isolation

The dried aerial parts of *H. sampsonii* (12.0 kg) were extracted overnight with 95% ethanol (EtOH) at 60 °C three times (80 L each). The EtOH extracts were concentrated under reduced pressure, and the residue (215 g) was partitioned successively with ethyl acetate (EtOAc) and *n*-butanol (BuOH), respectively. The EtOAc fraction (103 g) was subjected to silica gel column chromatography (8 × 80 cm) and eluted with a EtOAc/hexane gradient. Fractions of 10%–15% EtOAc eluate were collected and rechromatographed over silica gel and RP-18 (MeOH) columns in combination with silica-gel preparative HPLC (15% or 20% EtOAc/Hex) to give hypersampsone R (**1**) (15 mg), hypersampsone S (**2**) (12 mg), and hyperibone K (**3**) (25 mg).

Hypersamsone R (**1**). Colorless amorphous powder. $[\alpha]_D^{25}$: +160 (*c* 0.1, $CHCl_3$). IR (KBr): υ_{max} = 3443, 2965, 2928, 1709, 1677, 1598, 1257, 1115, and 747 cm^{-1}. ^1H- and ^{13}C-NMR spectroscopic data, see Table 1. Key COSY correlations: H-6/H-7; H-6/H-24; H-7/H-8; H-8/H-12; H-12/H-13; H-24/H-25; H-29/H-30. Key NOESY correlations: H-7/H-10; H-7/H-13; H-7/H-24; H-11/H-12; H-11/H-30; H-12/H-19. Key HMBC correlations: H-6/C-1, -7, -24; H-10/C-2, -8, -9; H-12/C-3, -4, -7, -8, -13, -14, -17; H-13/C-15, -16; H-19/C-17, -18, -20, -21; H-24/C-6, -7, -25, -26; H-29/C-2, -3, -9, -30, -31; H-32 (H-33)/C-30, -31. EI-MS: *m*/*z* (%) = 474 $[M]^+$ (8), 446 (15), 128 (23), 105 (100). HR-EI-MS: *m*/*z* = 474.3128 $[M]^+$ (calcd for $C_{32}H_{42}O_3$: 474.3134).

Hypersamsone S (**2**). Colorless amorphous powder. $[\alpha]_D^{25}$: +33 (*c* 0.3, $CHCl_3$). IR (KBr): υ_{max} 2969, 2927, 1718, 1703, 1441, 1136, 1067, and 826 cm^{-1}. ^1H- and ^{13}C-NMR spectroscopic data, see Table 1. Key COSY correlations: H-8/H-7, -12; H-13/H-4, -12, -14; H-24/H-23, -25; H-30/H-31, -32. Key NOESY correlations: H-4/H-13; H-7/H-10, H-14, H-19; H-8/H-7; H-11/H-13, -32; H-15/H-14, -17, -18; H-25/H-23, H-24, -27, -28; H-30/H-31, -32. Key HMBC correlations: H-4/C-2, -3, -6, -12, -14; H-7/C-1, -5, -6, -8, -9, -12; H-12/C-4, -7, -8; H-13/C-4, -5, -12, -14; H-14/ C-4, -12, -13, -15, -16; H-15/C-13, -14, -16, -17, -18; H-17/C-15, -16, -18; H-19/C-1, -5, -6, -7, -20, -21; H-31/C-29, -30, -32. EI-MS: *m*/*z* (%) = 494 $[M]^+$ (10), 466 (16), 397 (25), 355 (35), 189 (38), 135 (45), 109 (52), 91 (55), 71 (100). HR-EI-MS: *m*/*z* = 494.3396 $[M]^+$ (calcd for $C_{32}H_{46}O_4$: 494.3394).

4. Conclusions

Three compounds, including two new compounds **1** and **2**, were isolated from the aerial parts of *H. sampsonii*. The structures of these compounds were established on the basis of spectroscopic data. The discovery of new compounds from the genus *Hypericum* may not only provide more spectroscopic data about these isolates, but may also contribute to enhancing our understanding of the taxonomy and evolution of the genus *Hypericum*.

Acknowledgments

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Author Contributions

Yun-Lian Lin designed the whole experiment and contributed to manuscript preparation; Jih-Jung Chen analyzed the data and wrote the manuscript; Hong-Jhang Chen contributed to the data collection.

Conflicts of Interest

The authors declare no conflict of interest.

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Sample Availability: Samples of the all compounds are available from the authors.