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Polycyclic polyprenylated acylphloroglucinol derivatives from *Hypericum scabrum*

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ABSTRACT

Three new polycyclic polyprenylated acylphloroglucinol derivatives (PPAPs), hyperibrins E–G (**1**–**3**), along with seven known compounds were identified from the air-dried aerial parts of Hypericum scabrum. Their structures were determined by NMR spectroscopic methods, both experimental and calculated electronic circular dichroism (ECD) spectra and comparison with known compounds. Compound **1** was derived from an analogue of compound **2** by cyclization, while a *retro*-Claisen reaction transformed another analogue of compound **2** into compound **3**. Compounds **3**, **4**, **5**, and **10** showed obvious hepatoprotective activities against paracetamol-induced HepG2 cell damage.

Polycyclic polyprenylated acylphloroglucinols (PPAPs), a family of natural products with intriguing chemical structures and appealing biological activities, such as anti-HIV, antimicrobial, antioxidant, antidepressant, and tumor inhibitory,[1] are mainly obtained from the plants of the genus *Hypericum* (Guttiferae) and have been used for centuries as folk medicine for the treatment of burns, bruises, swelling, inflammation, anxiety, and bacterial and viral infections.[2] Consequently, PPAPs have attracted scientific interest because of their novel structures and significant bioactivities.

Hypericum scabrum exhibits various activities and has been used as an herbal treatment of hepatitis.[2e] In our current study of *Hypericum scabrum*, three new PPAPs, hyperibrins E–G (**1**–**3**), along with seven known compounds (**4**–**10**) were obtained.[3] These diverse carbon skeletons were all derived from less complex monocyclic polyprenylated acylphloroglucinols (MPAPs) by different cycloadditions or cleavage reactions, such as aldol condensations, Diels-Alder additions, and *retro*-Claisen reactions.[4] 1 D and 2 D NMR experimental methods coupled with comparisons with known compounds were used to verify the structures. The absolute configurations were determined by the electronic circular dichroism (ECD) exciton chirality method. Additionally, compounds **3**, **4**, **5**, and **10** showed obvious hepatoprotective activities against paracetamol-induced HepG2 cell damage at 10 μM.

Hyperibrin E[5] (**1**) was isolated as a colorless oil {$[\alpha]_D^{20}$ +47.27 (c 0.03, CH_3OH)}. The HRESIMS ion at *m*/*z* 503.2777 $[M+H]^+$ (calcd for $C_{32}H_{39}O_5$, 503.2797) indicated that its molecular formula was $C_{32}H_{38}O_5$. The ultra violet (UV) spectrum of **1** showed a maximum absorption at 209 nm (log ε 4.24) and 247 nm (log ε 4.14), indicating the presence of a benzoyl group. The infrared (IR) spectrum showed absorption bands for carbonyl groups (1743 and 1701 cm^{-1}). The ^1H NMR spectrum revealed the presence of a mono-substituted benzene moiety [δ_H 7.46 (1 H, t, J = 7.6 Hz), 7.30 (2H, t, J = 7.6 Hz), and 7.19 (2H, d, J = 7.6 Hz)], two vinyl protons [δ_H 5.17 (1 H, t, J = 7.2 Hz) and 4.89 (1H, t, J = 6.4 Hz)], two methine protons [δ_H 3.96 (1H, d, J = 2.4 Hz) and 1.90 (1 H, m)], and seven methyl groups (δ_H 2.22, 1.69, 1.66 × 2, 1.61, 1.25, and 1.17). The ^{13}C NMR spectra of compound **1** displayed signals for 28 carbons and indicated the main signals of a benzene ring (δ_C 134.2, 132.9, 129.2 × 2, and 128.1 × 2), five carbonyl carbons (δ_C 205.6, 203.1, 202.2, 200.8, and 194.0), four olefinic carbons (δ_C 135.2, 134.6, 119.1, and 118.4) (Table 1). Based on these observations, **1** was preliminarily inferred to be a PPAP containing a trioxygenated adamantyl ketone skeleton.

Unlike most adamantly-type PPAPs, compound **1** contained an acetonyl group {CH_3-CO-CH_2-, [δ_H 2.22 (27-CH_3, s), 3.15 (25-H_a, dd, J = 18.0, 3.2 Hz), and 2.26 (25-H_b, m); δ_C 205.6, 41.9, and 30.5]} and was only discovered in oblongifolin J and oblongifolin K.[6] Additionally, comparing the molecular formula and NMR data of **1** to those of the known compounds oblongifolin J and oblongifolin K revealed that they had the same skeleton, and compound **1**

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Table 1
^{1}H NMR and ^{13}C NMR spectroscopic data for compounds **1**–**3**[a] (δ in ppm, J in Hz).

No.	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	68.8		70.7		203.1	
2	202.2		171.9		66.4	4.49 s
3	78.4		118.5		44.4	
4	200.8		190.3		46.0	1.73 m
5	76.9		65.3		36.8	2.54 m; 1.41 m
6	53.8		41.8	2.00 dd (13.2, 3.8); 1.43 m	61.6	
7	45.0	1.90 m	43.1	1.65 m	196.9	
8	41.4	2.24 m; 2.54 m	47.1		138.9	
9	203.1		206.9		127.9	7.89 d (7.2)
10	194.0		192.7		128.5	7.43 t (7.2)
11	134.2		137.1		132.9	7.53 t (7.2)
12	129.2	7.19 d (7.6)	128.2	7.58 d (7.6)	128.5	7.43 t (7.2)
13	128.1	7.30 t (7.6)	128.5	7.35 t (7.6)	127.9	7.89 d (7.2)
14	132.9	7.46 t (7.6)	132.9	7.50 t (7.6)	26.7	1.08 s
15	128.1	7.30 t (7.6)	128.5	7.35 t (7.6)	16.1	1.15 s
16	129.2	7.19 d (7.6)	128.2	7.58 d (7.6)	27.4	2.24 m; 1.72 m
17	23.2	2.45 m	26.5	2.96 d (10.0)	122.7	5.17 t (6.4)
18	119.1	4.88 t (6.4)	93.5	4.65 t (10.0)	133.0	
19	134.6		70.3		25.9	1.76 s
20	26.0	1.69 s	23.7	0.90 s	18.0	1.62 s
21	18.1	1.66 s	26.5	0.90 s	33.3	2.54 m; 2.32 m
22	23.8	1.17 s	29.3	2.59 dd (10.4, 7.0) 2.49 dd (10.4, 7.0)	118.1	5.06 t (7.6)
23	22.6	1.25 s	119.5	5.06 t (7.4)	135.3	
24	48.0	3.96 d (2.4)	134.7		26.0	1.68 s
25	41.9	3.15 dd (18.0, 3.2); 2.26 m	26.1	1.68 s	17.8	1.57 s
26	205.6		18.2	1.70 s	172.4	
27	30.5	2.22 s	27.7	2.13 m; 1.70 m	52.4	3.80 s
28	27.4	2.51 m	122.3	4.96 t (6.8)		
29	118.4	5.17 t (7.2)	133.5			
30	135.2		25.9	1.68 s		
31	26.0	1.61 s	18.0	1.57 s		
32	18.1	1.66 s	15.7	1.24 s		
33			24.1	1.34 s		

[a] ^{1}H NMR spectra measured at 400 MHz, ^{13}C NMR spectra measured at 125 MHz; obtained in $CDCl_3$. Assignments supported by the 2D NMR spectra.

and oblongifolin J were determined to be C-24 epimers. This was confirmed by key HMBC and NOESY correlations, as shown in Figs. 2 and 3. Key HMBC correlations were observed as follows: H-17/C-5, C-9, and C-18; H-28/C-2, C-8, C-9, and C-29; H-24/C-25, C-3, and C-2; and H-23/C-5 and C-7.

The relative configurations at the chiral centers C-1, C-3, C-5, and C-7 were evident for the adamantyl core.[6,7] Furthermore, in the NOESY spectrum of **1**, we did not find the correlations of H-8 with H-23 and H-25 with H-22 that oblongifolin J indicated, but proton signals were observed that indicated that H-25a (3.14, m)

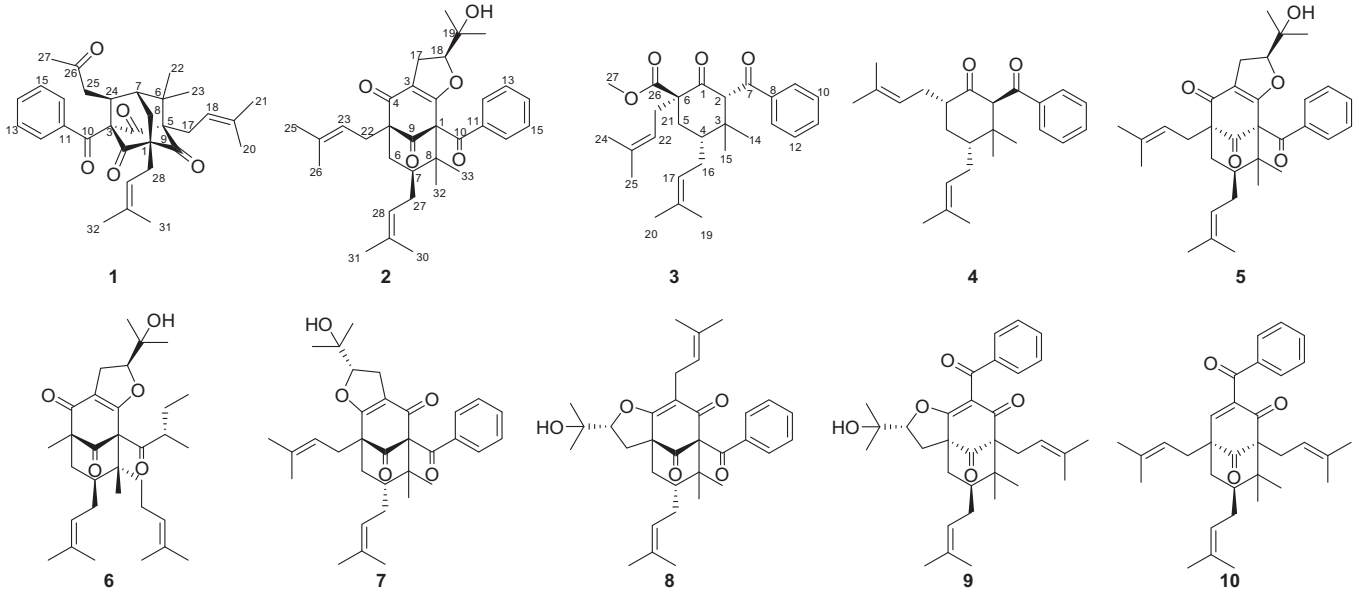


Figure 1. Structures of compounds **1**–**10**.

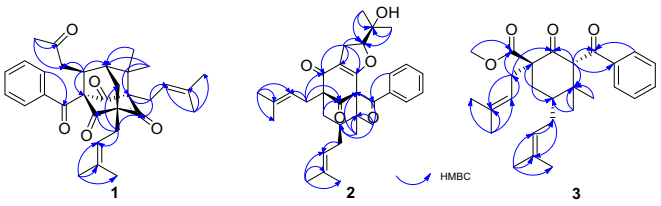


Figure 2. Selected key HMBC correlations for **1**–**3**.

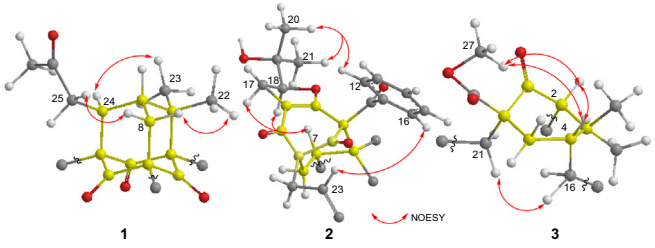


Figure 3. Selected key NOESY correlations for **1**–**3**.

was related to H-8b (2.24, m), H-8a (2.54, m) was related to H-22 (1.17, s), and H-23 (1.25, s) was related to H-24 (3.96, d) (Fig. 2). Thus, the spectrum indicated an α-H of C-24 and confirmed hyperibrin E to be the C-24 epimer of oblongifolin J, as shown in Fig. 1.

Hyperibrin F[8] (**2**) had a molecular formula of $C_{33}H_{42}O_5$, as determined by HRESIMS (*m*/*z* 519.3088 $[M+H]^+$, calcd for 519.3094), which is in agreement with its ^{13}C NMR data. Eight methyl groups (δ_H 0.90 × 2, 1.24, 1.34, 1.57, 1.68 × 2, and 1.70), two olefinic protons of isoprenyl groups [δ_H 4.96 (1H, t, J = 6.8 Hz), 5.06 (1H, t, J = 7.4 Hz)], a mono-substituted benzene ring [δ_H 7.58 (2 H, d, J = 7.6 Hz), 7.50 (1H, t, J = 7.6 Hz), and 7.35 (2 H, t, J = 7.6 Hz)] were observed in the ^1H NMR spectrum. The ^{13}C NMR spectrum contained 33 carbon signals, including three carbonyl carbons (δ_C 206.9, 192.7, and 190.3). The relevance between the ^1H NMR and ^{13}C NMR signals was confirmed by analyzing the HSQC and HMBC spectrum shown in Fig. 2. These data indicated the similarities between **2** and hyperibrin C,[9] which was previously isolated in our labs. The chemical shifts of C-2∼3 and C-17∼19 differed from δ_C of compound **2**: 171.9, 118.5, 26.5, 93.5, and 70.3 to δ_C of hyperibrin C: 172.9, 118.2, 26.2, 93.4, and 71.3. Except for the differences listed above, most of the correlations of the proton signals with the carbon signals in the HMBC spectra were all the same, suggesting that compound **2** and hyperibrin C have the same planar structures. In the NOESY spectrum of **2**, H-12/16 (7.58, d) were related to H-20/21 (0.9, s) and H-23 (5.06, t), indicating that the isoprenyl groups at C-5 and benzoyl group at C-1 were on the same side of the molecule. Additionally, the proton signal at H-18 (4.65, t) was only related to H-17 (2.96, d), H-20/21 (0.90, s), H-33 (1.34, s), and H-7 (1.65, m) (Fig. 3). The signal of C-7 (43.1) and difference in chemical shifts of the two H-6 atoms (2.00 and 1.43; $\Delta\delta$ = 0.57) were in agreement with the rule that when H-7 is α-oriented, the chemical shift of C-7 is δ_C 41–44, and the difference in chemical shifts of H-6β and H-6α was always 0.3–1.2 ppm, regardless of the NMR solvent used.[4b,10] Finally, the structure of hyperibrin F (**2**) was confirmed to be the C-18 epimer of hyperibrin C, as shown in Fig. 1.

Hyperibrin G[11] (**3**) was also isolated as a colorless oil, and its molecular formula was determined to be $C_{27}H_{36}O_4$ according to the HRESIMS ion peak at *m*/*z* 447.2494 $[M+Na]^+$ (calcd. for $C_{27}H_{36}NaO_4$, 447.2511), which indicated 10 indices of hydrogen deficiency. The presence of carbonyl and benzoyl groups was speculated by strong IR absorptions (ν_{max} 1745 and 1719 cm^{-1}) and (ν_{max} 1687, 1598, 1581, and 1447 cm^{-1}). The ^1H NMR signals at δ_H 7.53 (1 H, t, J = 7.2 Hz), 7.43 (2H, t, J = 7.2 Hz) and 7.89 (2H, d, J = 7.2 Hz) and ^{13}C NMR resonances at δ_C 138.9, 132.9, 127.9 × 2, and 128.5 × 2 also indicated the presence of a mono-substituted benzene moiety. The ^1H NMR spectrum of **3** also showed two olefinic protons [δ_H 5.17 (1 H, t, J = 6.4 Hz), 5.06 (1 H, t, J = 7.6 Hz)] and seven methyl groups (δ_H 1.08, 1.15, 1.57, 1.62, 1.68, 1.76, and 3.80), including one methoxy group that is infrequently observed in PPAPs. The ^{13}C NMR of **3** indicated 27 carbon signals, and three keto carbonyl groups at δ_C 203.1, 196.9, and 172.5 were observed. The details of the ^1H and ^{13}C NMR spectra are listed in Table 1. Correlation signals from the HSQC and HMBC spectra (Fig. 2) confirmed and clarified the planar structure. Key correlations from H-2 to C-1, C-3, C-7, and C-8; H-16 to C-3, C-4, C-5, C-17, and C-19; H-21 to C-1, C-6, C-22, C-23, and C-26; and H-27 to C-26 were observed. The spatial configuration was determined by the NOESY spectrum of **3** in which the correlations (Fig. 3) H-2 (4.49, s) with H-4 (1.73, m), H-2 (4.49, s) with H-27 (3.80, s), H-4 (1.73, m) with H-27 (3.80, s), and H-16b (1.72, m) with H-21a (2.54, m) were observed and confirmed the structure of compound **3**, as shown in Fig. 1.

The absolute configurations of hyperibrins E–G (**1**–**3**) were elucidated from their experimental and calculated ECD spectra. The ECD spectra of three pairs of enantiomers, (1*R*,3*R*,5*S*,7*S*,27*R*)-1a, (1*S*,3*S*,5*R*,7*R*,27*S*)-1b; (1*S*,5*R*,7*S*,18*S*)-2a, (1*R*,5*S*,7*R*,18*R*)-2b and (2*S*,4*R*,6*R*)-3a, (2*R*,4*S*,6*S*)-3b were calculated based on the known relative configurations of compounds **1**–**3**. Thus, the overall patterns of the calculated ECD spectra of 1b, 2a, and 3a were in good agreement with the experimental data of **1**–**3** (Fig. 4). Therefore, the absolute configurations of hyperibrins E–G (**1**–**3**) were defined as shown in Fig. 1.

The seven known compounds were identified by comparison of their NMR and MS data with the literature values as (2*R*,4*R*,6*S*)-2-benzoyl-3,3-dimethyl-4,6-bis(3-methylbut-2-en-1-yl)cyclohexan-1-one (**4**),[12] sampsonione N (**5**),[13] hypermongone B (**6**),[2d]

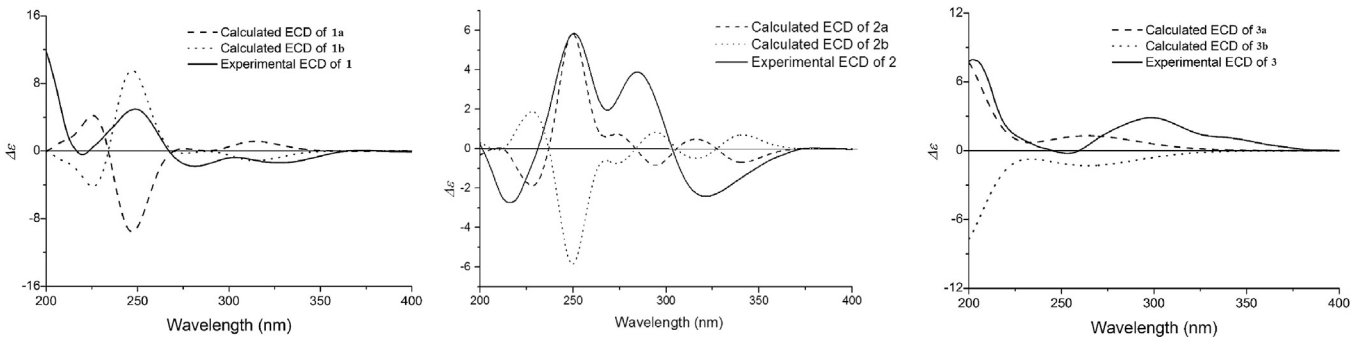
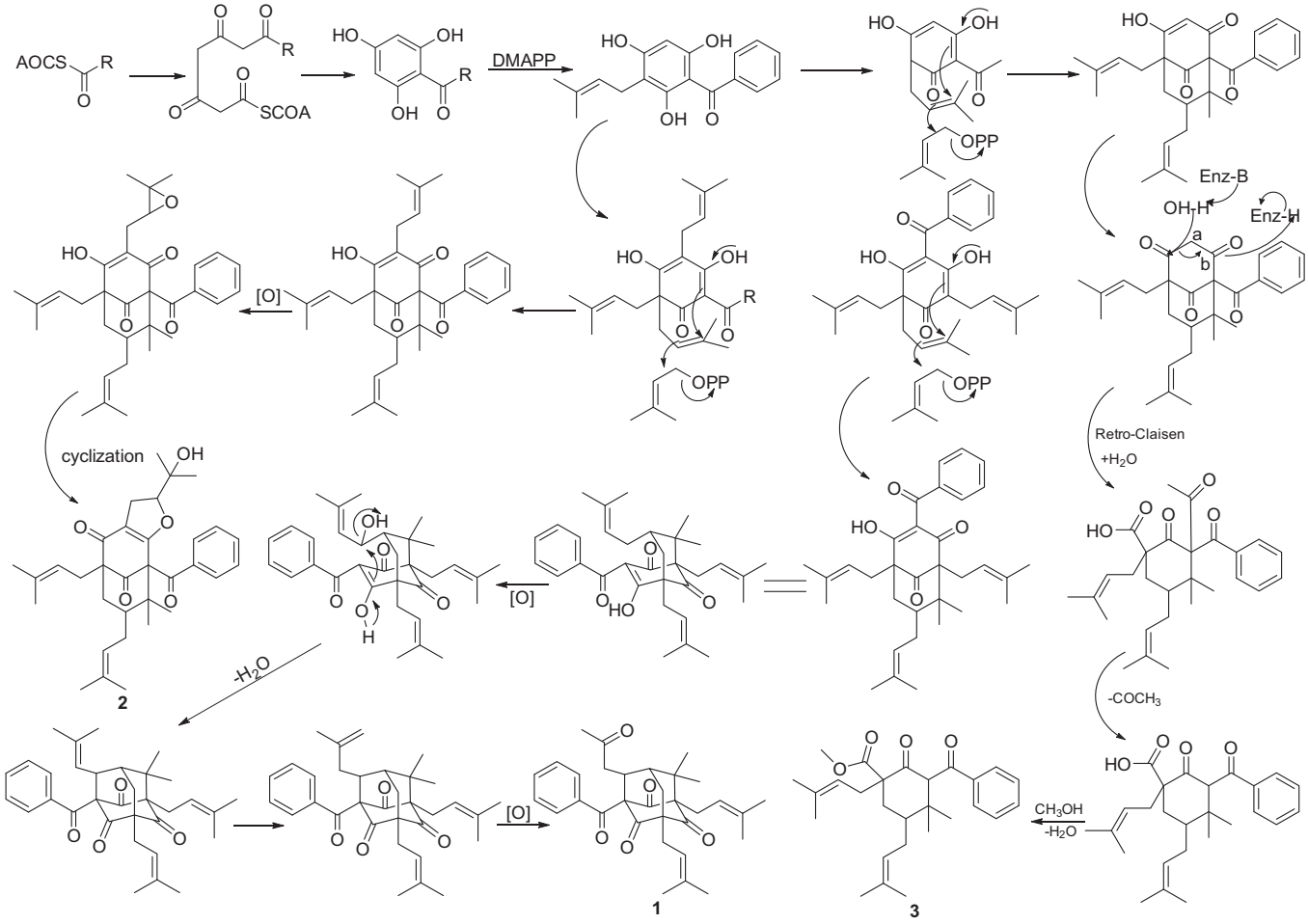


Figure 4. Experimental and calculated ECD spectra of **1**–**3**.



Scheme 1. Plausible biogenetic pathway for **1**–**3**.

hyperibone A (**7**),[14] hyperibone G (**8**),[14] sampsonione P (**9**),[13] 7-epi-clusianone (**10**).[14]

Generally, most of the PPAPs isolated from *Hypericum* formed a unique family of structurally related caged metabolites that were most likely biosynthesized from the biogenetically acceptable 2,4,6-trihydroxybenzophenon via a series of C-alkylations with dimethylallyl diphosphate (DMAPP).[4d,15,16] Hyperibrin G (**3**) was biogenetically derived by the cleavage of the C-a and C-b of a bicyclic polyprenylated acylphloroglucinol (BPAP) derivative through a *retro*-Claisen reaction and subsequent esterification. Compound **2** contained a dihydrofuran ring that was formed by the cyclization of a 3-methylbut-2-enyl side chain with an enolic hydroxy group. The plausible biosynthetic pathway for new compounds **1**–**3** is proposed as shown in Scheme 1.

Compounds **1**–**10** were evaluated for their hepatoprotective activities against paracetamol-induced HepG2 cell damage, and bicyclol, a hepatoprotective activity drug, was used as the positive control.[17] As shown in Table 2, compounds **3**, **4**, **5**, and **10** exhibited obvious hepatoprotective activities at 10 μM.

Table 2
Hepatoprotective effects of compounds **1**–**10** (10 μM) against paracetamol-induced HepG2 cell.[a]

Compound	Cell viability (% of normal)	Inhibition (% of control)
Normal	100 ± 8.17	
Control	42.72 ± 5.88	
Bicyclol	54.80 ± 1.99[b]	28.28
1	48.66 ± 3.44[c]	13.90
2	51.05 ± 1.25[b]	19.52
3	56.53 ± 4.74[b]	32.33
4	61.96 ± 1.83[b]	45.04
5	59.97 ± 1.07[b]	40.38
6	49.14 ± 4.43[c]	15.03
7	53.20 ± 6.65[c]	24.54
8	38.58 ± 3.05[c]	-9.68
9	48.45 ± 4.96[c]	13.41
10	58.62 ± 3.28[b]	37.22

[a] Results are expressed as the means ± SD (n = 3; for normal and control, n = 6); bicyclol was used as positive control (10 μM).
[b] $p < 0.01$.
[c] $p < 0.5$.

Acknowledgments

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A. Supplementary data

Supplementary data (the original ESIMS, UV, IR, 1D NMR, 2D NMR, and ECD spectra for all new compounds) associated with this

article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.09.001.

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3. *Extraction and isolation procedure of compounds **1**–**10***: The air-dried aerial parts of Hypericum scabrum (15.0 kg) were extracted with 95% EtOH (75.0 L) 3 times, and the extracts were partitioned with petroleum ether and H_2O. The resulting petroleum ether extracts (169.2 g) were subjected to silica gel column chromatography to obtain nine fractions (Fr. A–I), Fr. G (14.0 g) was further purified by an ODS RP-18 column using MeOH-H_2O (7:3 to 1:0, v/v) as the eluent to provide five fractions (Ga-Ge). Fr. Ga (6.311 g) was loaded onto a silica gel column and was eluted with petroleum ether-EtOAc (10:1, v/v) to give seven fractions that were further purified by preparative HPLC (MeOH-H_2O) to give **1** (4.5 mg), **3** (10.9 mg), and **4** (11.2 mg). Fr. H (38.8 g) was separated on a silica gel column using ether-EtOAc (50:1–0:1, v/v) as the elution solvent to give Fr. Ha to Fr. He and Fr. Hc was further purified by an ODS RP-C18 column using MeOH-H_2O (7:3 to 1:0, v/v) to obtain six fractions (Hc1-Hc6). Fr. Hc2 (3.795 g) was loaded onto two silica gel columns (CHCl3:acetone and petroleum ether-EtOAc) and finally afforded **2** (199.6 mg), **5** (10 mg), and **9** (40 mg) by preparative HPLC. Fr. Hc3 (3.627 g) was further purified by silica gel column chromatography and preparative HPLC to afford **7** (12 mg), **8** (8 mg), and **10** (13 mg).
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8. *Hyperibrin F(**2**)*: colorless oil; $[\alpha]_D^{20}$ −50.8 (c 0.07, MeOH); UV (MeOH) λ_{max} ($\log\varepsilon$) 209 (4.26), 249 (4.15), 281 (4.05) nm; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 215 (−2.74), 259 (5.85), 284 (3.89), 321(−2.40) nm; IR (KBr) ν_{max} 3573, 2974, 2917, 1722, 1700, 1629, 1446, 1395 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 519.3088 $[M+H]^+$ (calculated for $C_{33}H_{43}O_5$, 519.3094).
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17. The hepatoprotective effects of compounds **1**–**3** were determined by a (MTT) colorimetric assay in HepG2 cells. Each cell suspension of 2×10^4 cells in 200 μL of RPMI 1640 containing fetal calf serum (10%), penicillin (100 U/mL), and streptomycin (100 μg/mL) was placed in a 96-well microplate and pre-cultured for 24 h at 37 °C under a 5% CO_2 atmosphere. Fresh medium (100 μL) containing bicyclol and test samples was added, and the cells were cultured for 1 h. The cultured cells were exposed to 8 mM paracetamol for 24 h. Then, 100 μL of 0.5 mg/mL MTT was added to each well after the withdrawal of the culture medium and incubated for an additional 4 h. The resulting formazan was dissolved in 150 μL of DMSO after aspiration of the culture medium. The optical density (OD) of the formazan solution was measured on a microplate reader at 570 nm. Inhibition (%) was obtained by the following formula: Inhibition (%) = [(OD(sample) − OD(control))/(OD(normal) − OD(control))] × 100.