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Novel polyisoprenylated benzophenone derivatives from *Garcinia paucinervis*

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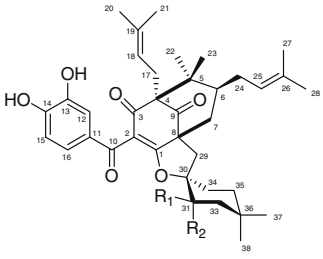
ABSTRACT

Four novel polyisoprenylated benzophenone derivatives, paucinones A–D (**1**–**4**), were isolated from the leaves of the plant *Garcinia paucinervis*. Paucinones A–C (**1**–**3**) contained an unexpected cyclohexane-spiro-tetrahydrofuran moiety. A 1-methylene-3,3-dimethylcyclohexane group never reported before was found in the structure of paucinone D (**4**). The structures of these compounds were elucidated with spectroscopic evidences. The relative stereochemistries of **1**–**4** were determined by NOESY correlations. These compounds showed significant cytotoxicities against HeLa cells.

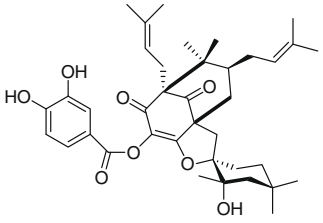
1. Introduction

The genus *Garcinia* (Guttiferae family) is known to be a rich source of polyisoprenylated benzophenones and xanthones in which some have shown various biological activities including antibacterial,[1] antifungal,[2] anti-inflammatory,[3] antioxidant,[4] apoptosis-inducing,[5] and cytotoxic effects.[6] With our continuing efforts for finding new bioactive compounds in this genus, four novel polyisoprenylated benzophenone derivatives, paucinones A–D (**1**–**4**), were isolated from *Garcinia paucinervis*. In this Letter, we describe the isolation and structure elucidation of **1**–**4**, and their cytotoxicities against Hela cells.

An acetone extract prepared from the leaves of *G. paucinervis* (2.8 kg) was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2-soluble portion (182 g) was decolorized by MCI. The 90% methanol portion (57 g) was chromatographed on a silica gel column eluting with hexane/acetone (1:0, 4:1, 2:1, 1:1, and 0:1) to afford five fractions, I–V. Fraction II-1 (7 g) was then separated on reversed phase column (RP-18) eluting with MeOH/H_2O (80%-100%) to give 17 fractions. Fraction II-1-10 was separated over Sephadex LH-20 gel eluting with MeOH and then subjected to semi-preparative HPLC (MeOH/H_2O, 80:20) to yield paucinones A (**1**, 2.1 mg), B (**2**, 2.0 mg), C (**3**, 3.4 mg), and D (**4**, 2.1 mg).



	R_1	R_2
1	Me-32	OH
2	OH	Me-32



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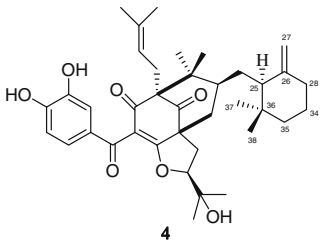

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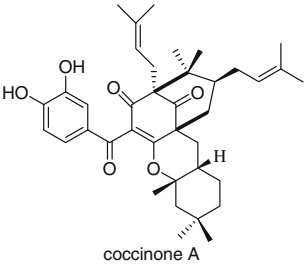

coccinone A

2. Results and discussion

Compounds **1**–**4** shared several common spectral characteristics. The UV spectra showed absorption bands consistent with

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those of aromatic rings and conjugated carbonyl groups. The IR spectra exhibited bands for hydroxyl groups, conjugated carbonyl groups, and aromatic rings.

Compound **1** was obtained as a white powder. Its molecular formula was determined to be $C_{38}H_{50}O_7$ by HRESIMS at *m*/*z* 619.3624 $[M+H]^+$, suggesting the existence of 14 degrees of unsaturation. The ^1H and ^{13}C NMR data of **1** (Table 1) showed the presence of nine methyls, seven methylenes, six methines (five olefinic), and sixteen quaternary carbons (seven olefinic, one oxygenated, and three carbonylic). In addition, the IR spectrum showed the presence of hydroxyl groups (3435 cm^{-1}), carbonyl groups (1733 cm^{-1}), and aromatic rings (1606 cm^{-1}). Analyzing 2D NMR spectra using HMQC and HMBC techniques enabled the assignment of ^1H and ^{13}C NMR signals. The foregoing data indicated that **1** was a benzophenone derivative that contained four isoprene units.

The possible structure of **1** was by a detailed comparison of its NMR data with those of the known coccinone A,[7] which suggested the same core structure of both compounds. However, different carbon and proton chemical shifts for C-29, C-30, and C-31 indicated that the structure of **1** differed from that of coccinone A with respect to the side chain attached at C-1 and C-8. In the HMBC spectrum of **1**, the correlations of the proton signals at δ_H 2.64 and 2.03 (H_2-29) with the carbon signals at δ_C 98.0 (C-30) and 72.9 (C-31), and with the carbon signals at δ_C 176.3 (C-1), 39.4 (C-7), 59.9 (C-8), and 206.7 (C-9) suggested that an oxygen bridge was formed between C-30 and C-1. The connection between the cyclohexane ring and the tetrahydrofuran ring through C-30 was evidenced by the HMBC correlations of H_2-33 (δ_H 1.28–1.30) with C-30 (δ_C 98.0, s), C-31 (δ_C 72.9, s), C-34 (δ_C 34.8, t), C-35 (δ_C 35.3, t), and C-36 (δ_C 31.1, s), of H_2-34 (δ_H 1.94, 1.23) with C-29 (δ_C 36.3, t), C-30, C-31, C-35, and C-36, and of H_2-35 (δ_H 0.86, 0.68) with C-30, C-33 (δ_C 48.4, t), C-34, and C-36 (Fig. 1). The above deduction revealed that compound **1** contained a cyclohexane-spiro-tetrahydrofuran group. The HMBC correlation of H_3-32 (δ_H 1.12) with C-30, C-31, and C-33, together with the molecular formula $C_{38}H_{50}O_7$ indicated the presence of a methyl group and a hydroxyl group at C-31. The presence of a gem-dimethyl group at C-36 was deduced by the HMBC correlations of H_3-37 (δ_H 0.63) and H_3-38 (δ_H

Table 1
^1H and ^{13}C NMR data for paucinones A–D (**1**–**4**) in CD_3OD[a]

Position	1		2		3		4	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		176.3		177.6		167.8		177.7
2		120.0		118.8		121.3		119.0
3		196.6		196.9		193.6		197.1
4		69.2		69.2		69.8		69.2
5		47.2		47.1		46.9		47.0
6	1.54 (1H, m)	47.6	1.53 (1H, m)	47.6	1.65 (1H, m)	47.0	1.97 (1H, m)	48.6
7	eq 2.55 (1H, m)	39.4	2.44 (1H, m)	40.5	2.58 (1H, m)	38.6	2.10 (2H, m)	42.9
	ax 2.08 (1H, m)		2.17 (1H, m)		2.07 (1H, m)			
8		59.9		60.2		59.9		61.7
9		206.7		206.3		205.5		208.5
10		192.8		193.2		164.8		192.9
11		130.8		130.8		129.0		130.7
12	7.22 (1H, d, 2.1)	116.2	7.28 (1H, d, 2.1)	116.0	7.49 (1H, m)	124.4	7.33 (1H, d, 2.0)	116.6
13		146.8		146.8		146.3		146.7
14		152.7		152.7		152.4		152.9
15	6.70 (1H, d, 8.2)	115.6	6.67 (1H, d, 8.2)	115.4	6.83 (1H, d, 8.8)	116.0	6.73 (1H, d, 8.2)	115.5
16	6.98 (1H, dd, 8.2, 2.1)	124.4	6.94 (1H, dd, 8.2, 2.1)	125.1	7.46 (1H, m)	118.0	7.14 (1H, dd, 8.2, 2.0)	125.4
17	2.64 (1H, m)	26.8	2.48 (1H, m)	26.7	2.57 (1H, m)	26.8	2.26 (1H, m)	27.5
	2.47 (1H, dd, 13.7, 5.8)		2.65 (1H, m)		2.52 (1H, dd, 14.3, 5.8)		2.59 (1H, dd, 13.8, 4.4)	
18	4.90 (1H, m)	120.9	4.91 (1H, m)	121.0	4.76 (1H, m)	121.3	5.05 (1H, m)	126.1
19		135.7		135.6		134.0		134.0
20	1.57 (3H, s)	26.5	1.62 (3H, s)	26.6	1.59 (3H, s)	26.1	1.66 (3H, s)	26.0
21	1.56 (3H, s)	18.2	1.58 (3H, s)	18.2	1.62 (3H, s)	18.2	1.57 (3H, s)	18.4
22	1.03 (3H, s)	27.1	1.04 (3H, s)	27.2	1.04 (3H, s)	27.1	1.10 (3H, s)	27.5
23	1.19 (3H, s)	22.7	1.17 (3H, s)	22.7	1.16 (3H, s)	22.6	1.23 (3H, s)	24.1
24	2.69 (1H, m)	30.4	2.72 (1H, m)	30.2	2.10 (1H, m)	30.4	1.38 (2H, m)	31.1
	2.13 (1H, m)		2.07 (1H, m)		1.28 (1H, m)			
25	4.92 (1H, m)	126.0	4.93 (1H, m)	126.0	4.94 (1H, m)	125.6	1.65 (1H, m)	49.2
26		134.1		134.3		133.9		151.6
27	1.67 (3H, s)	25.8	1.70 (3H, s)	26.1	1.69 (3H, s)	26.0	4.56 (1H, m)	108.5
							4.47 (1H, br s)	
28	1.65 (3H, s)	18.6	1.70 (3H, s)	18.6	1.69 (3H, s)	18.3	1.97 (2H, m)	35.5
29	2.64 (1H, ABd, 14.0)	36.3	3.27 (1H, ABd, 14.2)	36.2	2.63 (1H, ABd, 14.0)	37.0	2.24 (2H, m)	39.9
	2.03 (1H, ABd, 14.0)		1.45 (1H, ABd, 14.2)		2.05 (1H, ABd, 14.0)			
30		98.0		97.4		98.3	4.60 (1H, m)	94.0
31		72.9		74.1		73.0		71.8
32	1.12 (3H, s)	26.0	0.76 (3H, s)	25.0	1.20 (3H, s)	25.8	1.07 (3H, s)	25.6
33	1.29 (2H, m)	48.4	1.20 (1H, m)	48.4	1.45 (2H, m)	50.0	1.02 (3H, s)	25.4
			1.07 (1H, m)					
34	ax 1.94 (1H, td, 13.1, 3.7)	34.8	2.23 (1H, m)	33.2	1.95 (1H, td, 13.6, 3.7)	34.3	2.50 (1H, m)	31.0
	eq 1.23 (1H, dt, 14.2, 3.8)		1.75 (1H, dt, 14.4, 3.5)		1.38 (1H, m)		2.42 (1H, m)	
35	ax 0.86 (1H, dt, 13.1, 2.8)	35.3	1.48 (1H, m)	35.8	1.34 (1H, m)	35.8	1.46 (1H, m)	38.2
	eq 0.68 (1H, dd, 13.1, 2.8)		1.26 (1H, m)		1.11 (1H, m)		1.21 (1H, m)	
36		31.1		31.1		31.2		33.4
37	0.63 (3H, s)	33.1	0.76 (3H, s)	33.8	0.72 (3H, s)	33.1	0.83 (3H, s)	29.0
38	0.98 (3H, s)	28.1	1.05 (3H, s)	27.2	1.04 (3H, s)	28.3	0.84 (3H, s)	28.6

[a] Data were recorded with a Bruker DRX-400 MHz spectrometer, chemical shifts (δ) were expressed in ppm, *J* in Hz; assignments were confirmed by ^1H–^1H COSY, HMQC, and HMBC.

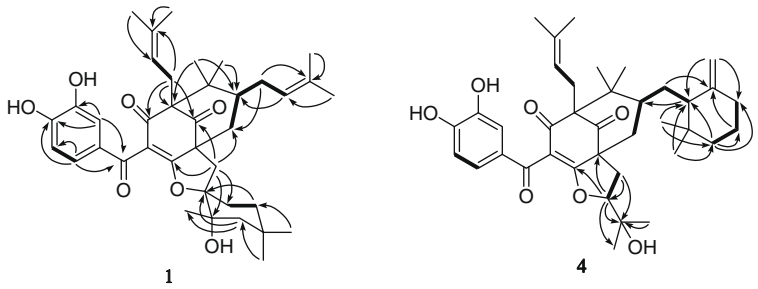

Figure 1. Selected HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of **1** and **4**.

0.98) with C-33, C-35, and C-36. Finally, the key information was again provided by the correlations between protons H_2-29 and the two sets of carbons indicating the connection on one hand with carbons at C-1, C-7, C-8, and C-9 of the benzophenone moiety and on the other hand with C-30, C-31, and C-34 of the cyclohexane ring.

The relative configuration of **1** was revealed by an NOE experiment. The NOESY correlation of H-6/H_3-22 suggested the existence of equatorial prenyl group at C-6. (Fig. 2) This was confirmed by the ^{13}C NMR chemical shift of Me-22, which was due to the γ-gauche interaction shielding of the axial methyl by the C-6 substituent when this group is equatorial.[5]

The axial or equatorial positions of the cyclohexanic protons were assigned by taking into account the coupling constant values and the NOESY correlations. (Fig. 2) The NOESY correlations of H-35ax (δ_H 0.86) with H_3-37 (δ_H 0.63) and of H-34ax (δ_H 1.94) with H_3-38 (δ_H 0.98) suggested the axial position of both hydrogens. Conversely, protons at δ_H 1.23 and 0.68 were in equatorial position (H-34 eq and H-35 eq). These assignments were confirmed by the coupling constant ^{3}J = 13.1 Hz between H-34ax and H-35ax, the characteristic of an anticoplanar stereochemistry. Finally, the strong NOESY correlation of H_3-32 (δ_H 1.12) with H-29b (δ_H 2.03) and H-7 eq (δ_H 2.53–2.56) led to the determination of the equatorial position of Me-32 and conversely the axial position of hydroxyl group. From these spectroscopic data, the structure of compound **1** is determined as shown.

Paucinone B (**2**) was isolated as a white amorphous solid. The molecular formula of **2** was determined to be $C_{38}H_{50}O_7$ by HRESIMS at *m*/*z* 619.3624 $[M+H]^+$, which was the same as that of **1**. Comparison of the NMR data between **2** and **1** indicated that they are isomers (Table 1). The only structural difference between **2** and **1** was found to be the opposite configuration of Me-32. This was deduced from the chemical shifts and the NOESY correlations. The ^{13}C NMR chemical shift of C-31 was at δ_C 74.1 in **2**, while the signal of C-31 with an equatorial position was located at δ_C 72.9 in **1**. There were no correlations of H_3-32 with H-7 eq and very weak correlation of H-32 with H-29b in the NOESY spectrum of **2**. These observations led to the determination of the axial Me-32 and conversely the equatorial hydroxyl group. Comparing to compound **1**, the above deduction was confirmed by the upfielded chemical shifts of H-29b (δ_H 1.45), H_3-32 (δ_H 0.76), and H_2-33 (δ_H 1.20, 1.07), and downfielded chemical shifts of H-29a (δ_H 3.27), H_2-34 (δ_H 2.23, 1.75), H_2-35 (δ_H 1.48, 1.26), H_3-37 (δ_H 0.76), and H_3-38 (δ_H 1.05) due to gauche effect. Therefore, the structure of **2** was elucidated as an isomer of **1** as shown.

Paucinone C (**3**) was obtained as a white amorphous solid. The HRESIMS showed an ion peak at *m*/*z* 635.3580 $[M+H]^+$, giving the molecular formula $C_{38}H_{50}O_8$. The NMR data of **3** were similar to those of **1** and **2** indicating that the three compounds have the same carbon skeleton. In contrast, the only difference between them was that there was one more oxygen atom in **3** than in **1** and **2**. The NMR spectra of **3** showed difference at C-10 when compared with those of **1** and **2**. The chemical shift of C-10 was upfielded to δ_C 164.8 instead of δ_C 192.8 and 193.2 in **1** and **2**, respectively. Within the given molecular formula $C_{38}H_{50}O_8$, an ester group was found to be located between C-2 and C-11, which has never been reported among publicly disclosed benzophenone analogs. This was confirmed by strong IR absorption at 1741 cm^{-1}, 1292 cm^{-1}, and 1100 cm^{-1}. According to the observed ROESY correlations and comparisons of ^{1}H and ^{13}C NMR data with those of **1** and **2** (Table 1), the relative configuration of **3** was deduced to be the same as that of **1** with equatorial Me-32 at C-31. Consequently, the structure of compound **3** was deduced as shown.

Paucinone D (**4**), obtained as a white amorphous solid, gave the molecular formula $C_{38}H_{50}O_7$, as revealed by its HRESIMS at *m*/*z* 619.3625 $[M+H]^+$. The ^{1}H and ^{13}C NMR data for **4** were similar to those of **1** with differences in substituents at C-30 and C-24. The COSY and HMBC spectra suggested the presence of a 1-methy-

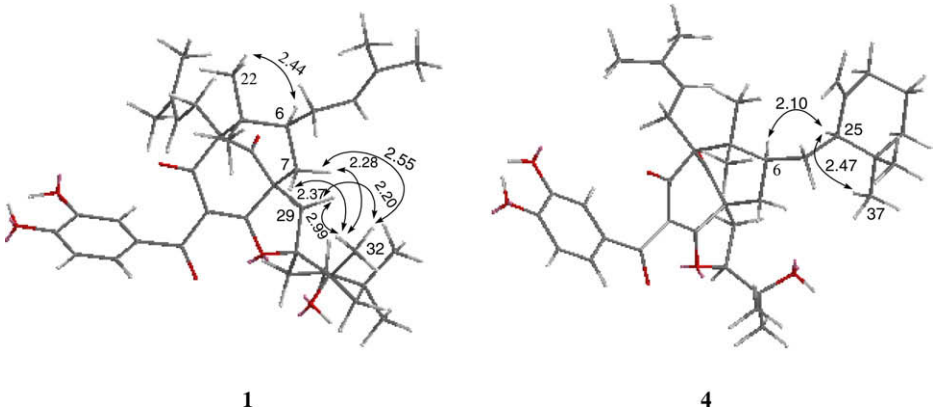

Figure 2. Key NOESY correlations and relative configurations assigned for **1**, **4**, and their corresponding interatomic distance (Å).

lene-3,3-dimethylcyclohexane at C-24 (Fig. 1). In the HMBC spectrum, the presence of a gem-dimethyl group was deduced from the correlations of the methyl protons H_3-37 (δ_H 0.83) and H_3-38 (δ_H 0.84) with the quaternary carbon C-36 (δ_C 33.4), the methylene carbon C-35 (δ_C 38.2), and the methine C-25 (δ_C 49.2). Correlations were also observed from C-28 (δ_C 35.5) and C-25 to a characteristic exocyclic methylene protons H_2-27 (δ_H 4.47 and 4.56–4.60), from C-25, C-26 (δ_C 151.6), and C-36 to the methylene protons H_2-24 (δ_H 1.36–1.40), and from C-26, C-28, C-35, and C-36 to H_2-34 (δ_H 2.40–2.44 and 2.48–2.53), which confirmed the presence of 1-methylene-3,3-dimethylcyclohexane moiety located at C-24. HMBC correlations of H_2-29 with C-1, C-8, C-9, C-30, and C-31 suggested the existence of an oxygen bridge between C-30 and C-1. The HMBC correlations of H-30 with C-31, C-32, and C-33 (Fig. 1) and of H_3-32 and H_3-33 with C-30 and C-31 suggested that there is an iso-propyl group at C-30. The carbon signal of C-31 (δ_C 71.8, s) together with the molecular formula $C_{38}H_{50}O_7$ indicated the presence of a hydroxyl group at C-31.

In the NOESY spectrum, the correlation of H-25 (δ_H 1.65) with H-6 (δ_H 1.98) and H_3-37 (δ_H 0.83) suggested an α-orientation for H-25. The absence of correlation between H-30 and H_2-7 indicated the α-orientation of H-30. (Fig. 2) These data together with other results from 2D NMR analysis confirmed the structure of compound **4**.

The cyclohexane-spiro-tetrahydrofuran moiety of compounds **1**–**3** and the 1-methylene-3,3-dimethylcyclohexane moiety of compound **4** shed new insights into structural diversity of benzophenone analog libraries. The possible biosynthetic pathways of these four new benzophenones are given in Figures 3 and 4.

To determine the cytotoxicities of these four compounds, we measured their IC_{50} on HeLa cells. As the results show in Table 2, the **1**, **2**, and **4** have strong HeLa cell growth-inhibiting effects for which IC_{50} values are below or near 10 μM.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a JASCO DIP-1000 polarimeter. Ultraviolet absorption spectra were recorded using a Perkin–Elmer Lambda L14 spectrometer. A Perkin-Elmer spectrum 100 FT-IR spectrometer was used for scanning IR spectroscopy with KBr pellets. The 1D and 2D NMR spectra were recorded on a Bruker AV-400 spectrometer with TMS as an internal standard. Chemical shifts (δ) were expressed in parts per million with reference to the solvent signals. HRESIMS were obtained using a nanoLC–MS/MS system, with a nanoAcquity ultra performance liquid chromatography (UPLC) module and a quadrupole time-of-flight (Q-TOF) spectrometer equipped with a nanoelectrospray ion source (Waters, Milford, MA) and supported by a lock-mass apparatus to perform a real-time calibration correction. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China), Sephadex LH-20 gel (Pharmacia), and reversed phase C18 silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF_{254} were used. An Agilent 1100 series equipped with an Alltima C18 column (4.6 × 250 mm) was used for HPLC analysis, and semi-preparative and preparative Alltima C18 columns or Zorbax SB-C18 columns (9.4 × 250 mm and 22 × 250 mm) were used in sample preparation. Spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

3.1.1. Plant material

The leaves of *G. paucinervis* were collected in October 2008 from Xishuangbanna Prefecture of Yunnan Province, China. The plant was identified by Pan-Yu Ren. A voucher specimen (CMED-047404) has been deposited at Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine.

3.1.2. Paucinone A (1)

White amorphous powder; $[\alpha]_D^{23}$ −6.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 273 (2.30), 234 (2.32), 203 (2.50) nm; IR (KBr) ν_{max} 3435, 2924, 1733, 1606, 1442, 1384, 1293, 1202, 1109, 1064, 997, 957 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive HRESIMS *m/z* 619.3624 $[M+H]^+$ (calcd 619.3635 for $C_{38}H_{51}O_7$).

3.1.3. Paucinone B (2)

White amorphous solid; $[\alpha]_D^{25}$ +58.7 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 275 (2.33), 234 (2.32) nm; IR (KBr) ν_{max} 3436, 2925, 1735, 1608, 1519, 1442, 1372, 1294, 1194, 1108, 990, 959 cm^{-1};

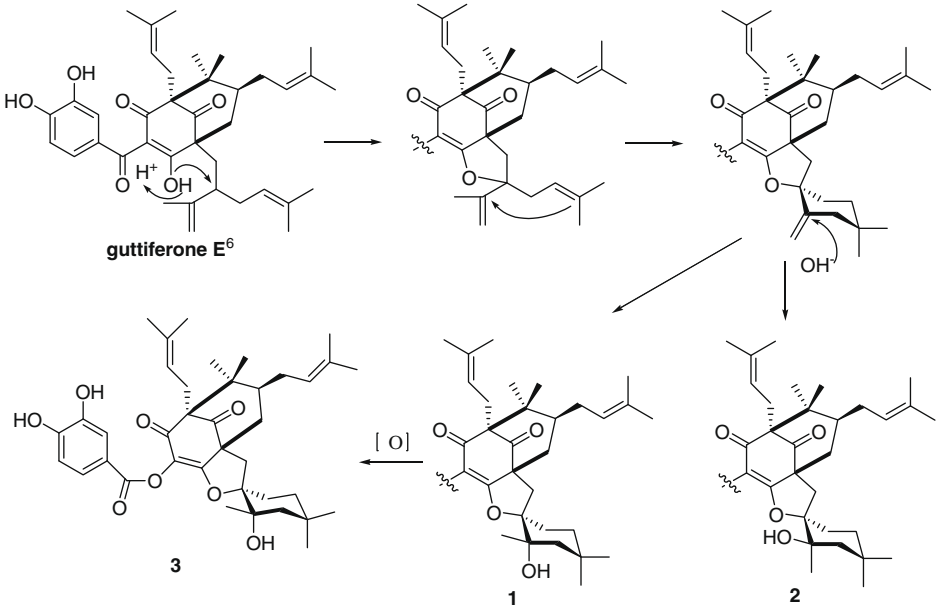

Figure 3. Possible biosynthetic pathway of paucinones A–C (**1**–**3**).

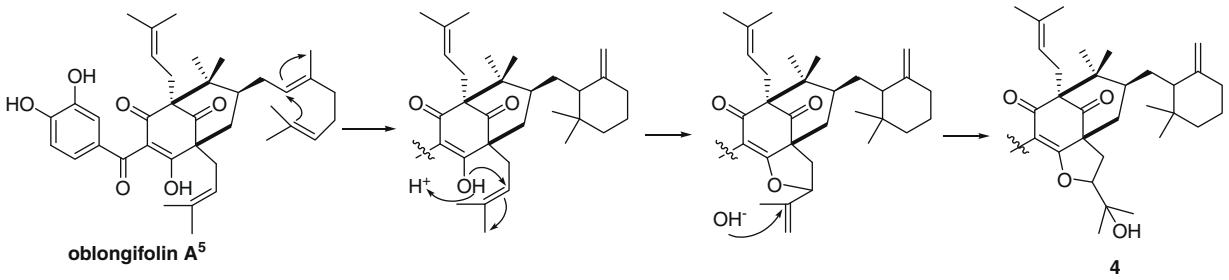

Figure 4. Possible biosynthetic pathway of paucinone D (**4**).

Table 2
IC_{50} values of HeLa cells treated with compounds **1**–**4** for 72 h

Compound	IC_{50} (μM)
1	10 ± 0.5
2	8.2 ± 0.8
3	24.3 ± 0.6
4	5.8 ± 0.6

^1H and ^{13}C NMR data, Table 1; positive HRESIMS *m/z* 619.3624 $[M+H]^+$ (calcd 619.3635 for $C_{38}H_{51}O_7$).

3.1.4. Paucinone C (3)

White amorphous solid; $[\alpha]_D^{24}$ +19.2 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 268 (2.41), 222 (2.41) nm; IR (KBr) ν_{max} 3435, 2926, 1741, 1631, 1444, 1367, 1292, 1200, 1100, 1066, 1025 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive HRESIMS *m/z* 635.3580 $[M+H]^+$ (calcd 635.3584 for $C_{38}H_{51}O_8$).

3.1.5. Paucinone D (4)

White amorphous solid; $[\alpha]_D^{27}$ +41.6 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 274 (2.40), 251 (2.22) nm; IR (KBr) ν_{max} 3435, 2924, 1732, 1630, 1443, 1375, 1291, 1199, 1114, 979 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; positive HRESIMS *m/z* 619.3625 $[M+H]^+$ (calcd 619.3635 for $C_{38}H_{51}O_7$).

3.2. Bioassay

Firstly, 2500 HeLa cells suspended in 100 μL minimum essential medium were seeded, respectively, in each well of a 96-well plate. After 24 h incubation, a fresh medium that contained various concentrations of each compound was added into the 96-well plate to replace the old medium. All the testing compounds were dissolved in DMSO to make stock solutions. The stock concentration was at least 1000 times higher than the working concentration. The concentrations applied were ranged from 100 μM to 1.5625 μM, which was achieved by doing twofold dilutions for 6 times. The OD values of the control group at 0 h and 72 h together with the compound-treated groups at 72 h were subjected to the methylthiazol tetrazolium (MTT) assays and their optical density (OD) values were measured using a plate reader. We measure the IC_{50} of four new compounds using MTT assay where IC_{50} is the concentration of a compound inhibiting 50% of the cell growth. MTT powder was dissolved in PBS at a concentration of 5 mg/mL. For MTT assay, 10 μL of MTT solution was added into each well of a 96-well plate. After 2 h incubation at 37 °C, 100 μL of 10% SDS solution with 0.01 M HCl was added to dissolve the purple crystals. After 24 h incubation, the optical density (OD) readings at 595 nm were measured using a plate reader.

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Supplementary data

Supplementary data (^1H and ^{13}C NMR spectra of paucinones A–D (**1**–**4**)) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.02.147.

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