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Identification and evaluation of apoptotic compounds from *Garcinia oligantha*

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ABSTRACT

Four new compounds, oligantins A–D (**1**–**4**), and one known caged xanthone gaudichaudione H (**5**) were isolated from the stems of *Garcinia oligantha*. The structures of the new compounds were elucidated by spectroscopic evidences. All of the five compounds were evaluated for their apoptosis-inducing effects using HeLa-C3 cells which have been genetically engineered to produce a fluorescent biosensor capable of detecting caspase-3 activation. All of them induced cell apoptosis at 10 μM or lower concentrations. The apoptotic activity of oligantins A, B and gaudichaudione H were further confirmed by detecting the cleavage of PARP, which is the substrate of activated caspase-3, in these compounds-treated cells using the method of Western blot. Moreover, the values of IC_{50} were measured for all five compounds on HeLa cells using the MTT assay. Among them, gaudichaudione H had the lowest IC_{50} value of 0.90 μM, while the other four new compounds had IC_{50} values of 1.58, 1.52, 4.15, and 7.82 μM, respectively. These results show that gaudichaudione H has the strongest apoptosis-inducing effect and cell growth inhibition effect among these xanthones and it may have the potential to be developed into a new anticancer agent.

The genus *Garcinia* (Guttiferae family) is known to be a rich source of polyisoprenylated benzophenones and xanthones. Up to now, series of active components extracted from this genus displayed clear apoptosis-inducing effect against different cancer cells such as HeLa.[1–3]

Apoptosis is a programmed cell death that leads to the removal of unwanted and abnormal cells.[4] Preferential and efficient inductions of apoptosis in tumor cells are regarded as one of the most effective anti-cancer therapies. Activation of caspase-3 is the most critical event signifying the occurrence of apoptosis which forms the basis for measuring the apoptotic extent.[5] Compounds isolated from natural sources leading to caspase-3 activation may serve as potential anticancer drug candidates. Previously, we reported to identify a series of benzophenones and xanthones isolated from the *Garcinia* genus as apoptosis inducers of HeLa cells.[1–3]

With our continuing efforts for finding more bioactive compounds from *Garcinia* species, we explored the chemical constituents of *Garcinia oligantha* (Guttiferae), a Shrub 1–3 m tall, which is distributed in 200–1200 m dense forests in Hainan and Guangdong province of China.[6] In the folkloric medicine, this plant is known to have clinical efficacies of anti-inflammation, analgesic cool the internal heat and detoxify the body. It has been used to treat toothache, sore mouth, and scald. This plant is also used as folkloric medicine in Laos.[7] Here we describe the isolation and structure elucidation of four new prenylated xanthones, oligantins A–D (**1**–**4**), and a known caged xanthone gaudichaudione H[8] from the acetone extract of the dried stems of *G. oligantha*. All compounds were also assayed for apoptotic activity using engineered Hela-C3 cells that can detect caspase-3 activation in live cells.[9,10]

An acetone extract prepared from the stems of *G. oligantha* was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was subjected repeatedly to column chromatography over silica gel, Sephadex LH-20, and RP-18 and to HPLC to afford four new compounds, oligantins A–D (**1**–**4**), together with one known caged xanthone, namely gaudichaudione H[8] (**5**).

Compound **1** was obtained as a yellow amorphous powder. Its molecular formula was determined to be $C_{28}H_{30}O_7$ by HRESIMS at *m/z* 479.1982 $[M+H]^+$, suggesting the existence of 14 degrees of unsaturation. The ^1H and ^{13}C NMR data of **1** (Table 1) showed the presence of six methyls, two methylenes, five olefinic methines,

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Table 1
^{1}H and ^{13}C NMR Data of compounds 1–4[a]

No.	1[b]		2[b]		3[c]		4[c]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		55.0		55.1		58.6		57.2
2		210.0		210.0		207.4		205.0
3		139.3		83.8		85.1		82.6
4		157.9		189.6		187.9		188.8
5	6.18 (s)	100.9	6.18 (s)	100.9	6.37 (s)	100.0	6.31 (s)	100.8
6		160.8		160.8		160.8		161.9
7		102.4		102.4		105.1		101.2
8		156.5		156.5		153.4		151.7
9		181.0		180.6		179.9		181.5
4α		163.2		163.2		161.1		160.8
8α		106.1		106.1		105.6		106.3
9α		121.2		130.8		130.8		124.8
10α		152.0		152.1		153.1		150.7
11	6.85 (d, 10.0)	115.5	6.84 (d, 10.0)	115.6	3.60 (d, 7.1)	21.8	6.81 (d, 10.0)	114.4
12	5.70 (d, 10.0)	128.8	5.68 (d, 10.0)	128.7	5.34 (t, 7.1)	121.3	5.62 (d, 10.0)	127.6
13		79.5		79.4		135.5		78.8
14	1.46 (overlap)	28.5	1.45 (overlap)	28.5	1.77 (overlap)	17.9	1.46 (s)	28.4
15	1.46 (overlap)	28.5	1.45 (overlap)	28.4	1.86 (s)	30.8	1.45 (s)	28.4
16a	2.65–2.70 (m)	38.0	2.64–2.71 (m)	40.6	2.77–2.84 (m)	36.7	3.00–3.05 (m)	34.5
16b	3.37–3.42 (m)							
17	4.60–4.64 (overlap)	119.5	4.62–4.64 (overlap)	119.6	4.58–4.64 (overlap)	117.8	4.77–4.81 (overlap)	119.5
18		135.8		139.5		139.1		137.8
19	1.46 (overlap)	18.0	1.46 (overlap)	18.0	1.48 (overlap)	16.2	1.53 (s)	17.9
20	1.46 (overlap)	25.9	1.52 (s)	25.9	1.49 (s)	25.5	1.57 (s)	25.9
21a	2.65–2.70 (m)	38.0	3.38–3.45 (m)	40.6	3.40–3.47 (m)	39.7	3.25–3.31 (m)	36.9
21b	3.37–3.42 (m)							
22	4.60–4.64 (overlap)	119.5	4.62–4.64 (overlap)	119.6	4.58–4.64 (overlap)	117.9	4.77–4.81 (overlap)	118.3
23		135.8		135.8		135.2		136.1
24	1.46 (overlap)	18.0	1.41 (s)	17.6	1.47 (s)	17.5	1.49 (s)	17.9
25	1.46 (overlap)	24.2	1.46 (overlap)	24.2	1.58 (s)	25.7	1.56 (s)	25.8
26			1.75–1.79 (m)	27.6	2.19 (br)	26.7	2.89–2.95 (m)	45.4
27			4.82 (br)	123.7	4.88 (t, 6.5)	123.9		206.9
28				132.2		131.3	2.25 (s)	31.4
29			1.47 (overlap)	16.5	1.48 (overlap)	17.9		
30			1.47 (overlap)	25.8	1.77 (overlap)	25.8		
8-OH					12.97 (s)		12.60 (s)	

[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 ^{1}H–^{1}H COSY, HMQC and HMBC.
[b] Data were recorded in CD_3OD.
[c] Data were recorded in $CDCl_3$.

and fifteen quaternary carbons (eleven olefinic, one oxygenated, and two carbonylic), including those with two prenyl groups [δ_H 1.46 (12H, H_3-19 and H_3-20, H_3-24 and H_3-25), 2.65–2.70 (2H, H-16a and H-21a), 3.37–3.42 (2H, m, H-16b and H-21b), and 4.60–4.64 (2H, H-17 and H-22); δ_C 38.0 (C-16 and C-21), 119.5 (C-17 and C-22), 135.8 (C-18 and C-23), 18.0 (C-19 and C-24), 25.9 (C-20) and 24.2 (C-25)], and a dimethyl-2*H*-pyrano group [δ_H 6.85 (1H, d, 10.0, H-11), 5.70 (1H, d, 10.0, H-12), 1.46 (6H, H_3-14 and H_3-15); δ_C 115.5 (C-11), 128.8 (C-12), 79.5 (C-13), 28.5 (C-14 and C-15)]. In addition, the IR spectrum showed the presence of hydroxyl groups (3447 cm^{-1}), and aromatic rings (1617 cm^{-1}), and a xanthone carbonyl group (1653 cm^{-1}). The foregoing data indicated that **1** was a xanthone derivative.

By analyzing 2D NMR spectra and a detailed comparison of its NMR data with those of the known allanxanthone C[11], the possible structure of **1** was established. The assignment of ^{1}H and ^{13}C signals confirmed that **1** was a xanthenedione with the same core structure as allanxanthone C.[11]

The two prenyl groups were located at (δ_C 55.0, C-1), respectively, according to the HMBC correlations between H_2-16 and H_2-21 with C-1. These resonances were typical of a gem bis (3-methylbut-2-enyl) group linked to a sp^3 carbon atom.[11] The additional carbonyl signal at δ_C 210.0 suggested a xanthenedione skeleton of this compound. Compared to allanxanthone C[11], this new compound did not exhibit signals of the third prenyl group. One of the C5 group was proved to be attached at C-7, forming a dimethyl-2*H*-pyrano-xanthone skeleton, by the HMBC correlations between δ_C 160.8 (C-6) with the chelated 8-OH and the cis-coupled H-11 and H-12, and those between H-12 with the oxygenated quaternary carbon C-13 and the tertiary methyl carbons C-14 and C-15. The remaining aromatic proton was indicated to be δ_H 6.18 (1H, H-5) by its HMBC correlations with C-6, C-7, C-8a, and C-10a. Considering the signal for δ_C 139.3 (C-3), 157.9 (C-4) and the molecular formula of **1**, two hydroxyl groups were located at C-3 and C-4, respectively.

To unequivocally corroborate the substitution pattern of **1**, the HMBC spectrum was also analyzed in full detail as shown in Figure 2. The complete HMBC analysis confirmed the assigned structure. From the ^{1}H and ^{13}C NMR spectroscopic data displayed in Table 1, the structure of compound **1** was determined and named as oliganthin A (Fig. 1).

Compound **2** was obtained as a yellow amorphous solid. The HRESIMS showed an ion peak at *m/z* 547.2678 $[M+H]^+$, giving the molecular formula of $C_{33}H_{38}O_7$. The NMR data of **2** were similar to those of **1** indicating that they have similar carbon skeleton. However, different carbon chemical shifts for C-3, and C-4 indicated that the structure of **2** is different from that of **1** with respect to the side chain attached at C-3 and C-4. In contrast, there was one more prenyl group in **2** than in **1** [δ_H 1.47 (6H, H_3-29 and H_3-30), 1.75–1.79 (2H, H-26), and 4.82 (1H, H-27); δ_C 27.6 (C-26), 123.7 (C-27), 132.2 (C-28), 16.5 (C-29), and 25.8 (C-30)]. The HMBC correlation between H_2-26 with C-3 and C-2 located the prenyl

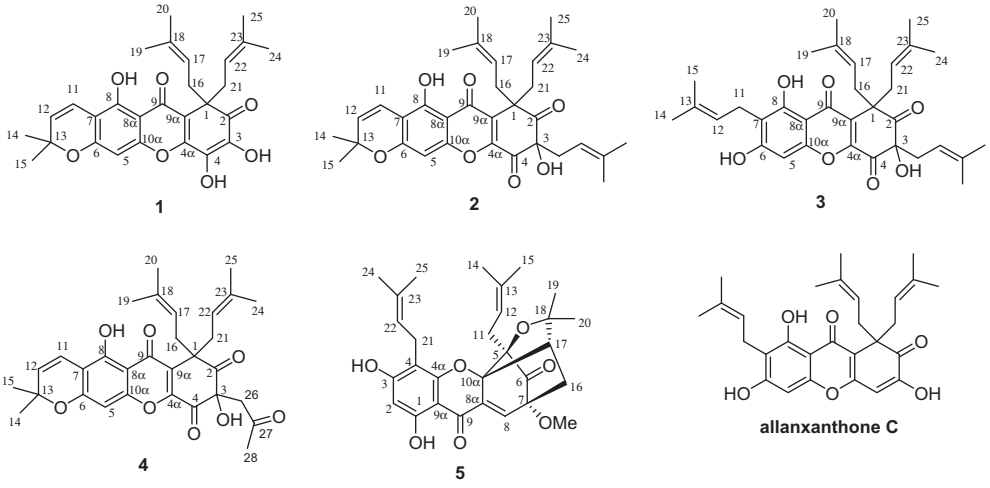

Figure 1. Structures of compounds **1**–**5**.

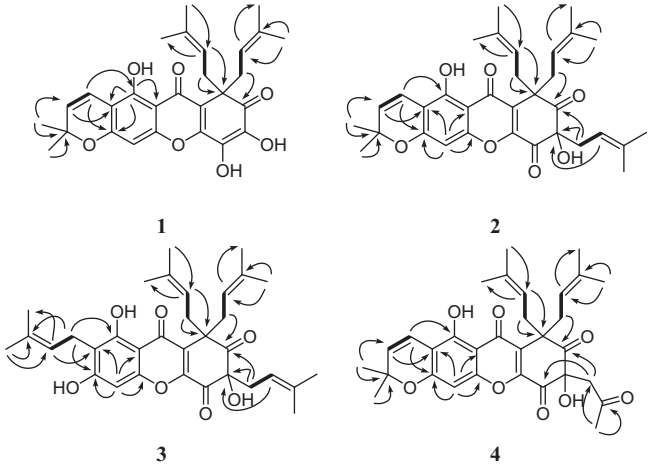

Figure 2. Selected HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of **1**–**4**.

group at C-3. The chemical shift of C-3 was up fielded to δ_C 83.8 instead of δ_C 139.3 in **1**. Considering the molecular formula of $C_{33}H_{38}O_7$ and the signal of δ_C 83.8 (C-3), 188.8 (C-4), a hydroxyl group and a carbonyl group were located at C-3 and C-4, respectively, which is different from that of compound **1**. Consequently, the structure of compound **2** was established as shown in Figure 1 and named as oliganthin B.

Compound **3** was obtained as a yellow amorphous solid. It was assigned the molecular formula of $C_{33}H_{40}O_7$ by the HRESIMS at *m*/*z* 549.2813 $[M+H]^+$. By contrast, the NMR data of **3** were quite similar to those of **2**, except for the substituent at C-7. The NMR data indicated that a prenyl group [δ_H 1.77 (3H, H_3-14), 1.86 (3H, H_3-15), 3.60 (2H, d, 7.1, H-11), and 5.34 (1H, t, 7.1, H-12); δ_C 21.8 (C-11), 121.3 (C-12), 135.5 (C-13), 17.9 (C-14), and 30.8 (C-15)] at C-7 in **3** instead of dimethyl-pyrano-xanthone structure in **2**. The HMBC correlation of H-11 with C-6, C-7, C-8 and that of H-12 with C-7, together with the molecular formula of $C_{33}H_{40}O_7$ further confirmed the above deduction. As a result, the structure of compound **3** was established as shown in Figure 1 and named as oliganthin C.

The HRESIMS of oliganthin D (**4**) showed an ion peak at *m*/*z* 535.2125 $[M+H]^+$, giving the molecular formula $C_{31}H_{34}O_8$. The UV spectra showed absorption bands consistent with those of aromatic rings and conjugated carbonyl groups. The IR spectra exhibited bands for hydroxyl groups and xanthone carbonyl group. Furthermore, the NMR data of **4** were similar to those of **2** and **3** indicating that they were based on same carbon skeleton, and showed difference at C-3 on comparing with those of **2**. The HMBC correlations of δ_H 2.77–2.82 (m) (H-26) with δ_C 212.2 (C-2), 75.9 (C-3), 208.4 (C-27), 31.9 (C-28), and of δ_H 2.26 (s) (H-28) with the carbon signals at δ_C 42.8 (C-26), 208.4 (C-27) indicated an acetonyl at C-3. The ^{13}C data of **4** showed C-3 as oxygenated quaternary carbon, indicating a hydroxyl group at C-3. Considering the molecular formula $C_{31}H_{34}O_8$ and the signal of δ_C 188.8 (C-4), a carbonyl group were located at C-4, which is the same as that of compound **2**. Consequently, the structure of compound **4** was established as shown.

All five compounds isolated from *G. oligantha* were evaluated for their apoptosis-inducing effects using genetically engineered HeLa-C3 cells that can detect caspase-3 activation in live cells based on the principle of fluorescence resonance energy transfer (FRET).[9,10]

As shown in Table 2, compounds were tested at the concentrations of 1, 2.5, 5 and 10 μM. Among the five tested compounds, compound **1** and **5** were found to have the strongest apoptotic effect since they could reduce the YFP/CFP emission ratio below 3 within 72 h at the concentration of 2.5 μM. Other three compounds also exhibited some apoptosis-inducing effects. The effective concentration of compounds **2**, **3** and **4** are 5, 10 and 10 μM, respectively.

Western blot was used to confirm the apoptosis-inducing effects of three compounds **1**, **2** and **5** as they displayed good caspase-3 activation ability in HeLa cells containing the biosensor. As shown in Figure 3, after 24 h treatment of compounds **1** and **5** at 5 μM as well as **2** at 10 μM to HeLa cells, there is a clear cleavage of PARP, which is the endogenous substrate protein of caspase-3. Moreover, after 48 h treatment, almost all the PARP protein was

Table 2
Apoptosis-inducing effect of five compounds at 72 h in HeLa-C3 cells

Compound	Apoptotic effect at			
	10 μM	5 μM	2.5 μM	1 μM
1	+	+	+	–
2	+	+	–	–
3	+	–	–	–
4	+	–	–	–
5	+	+	+	–

"+" means the YFP/CFP emission ratio of compound treated HeLa-C3 cells reached to 3 or was below 3 at 72 h. "–" means the YFP/CFP emission ratio of compound treated HeLa-C3 cells was above 3 at 72 h.

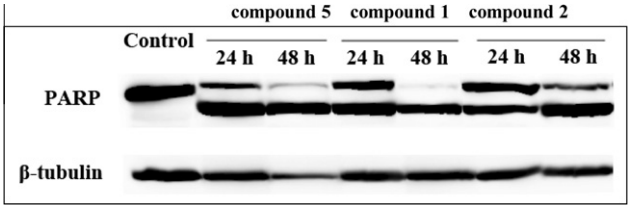

Figure 3. PARP cleavages were observed after compounds **1**, **2** and **5** treatment. HeLa cells were treated with 5 μM of compounds **1** or **5** and 10 μM of compound **2** for 24 and 48 h, respectively. β-Tubulin was probed as a loading control. HeLa cells treated with culture medium containing 0.1% DMSO were used as a negative control.

Table 3
IC_{50} values of five compounds at 72 h in HeLa cells

Compound	IC_{50} (μM)
1	1.58 ± 0.09
2	1.52 ± 0.12
3	4.15 ± 0.12
4	7.82 ± 0.06
5	0.90 ± 0.03

cleaved in cells treated with compound **1** or **5**. And for compound **2** treated cells, much more PARP was cleaved after 48 h treatment. Taken together, these results demonstrated that caspase-3 was indeed activated during the treatment of compounds **1**, **2** and **5** in HeLa cells.

In order to compare the cytotoxicity of these four apoptotic compounds, we measured their IC_{50} values in HeLa cells using MTT assay. As the results shown in Table 3, compound **5** has the lowest IC_{50} value of 0.90 μM, while the other four new compounds had IC_{50} values of 1.58, 1.52, 4.15 and 7.82 μM, respectively. The IC_{50} value of compound **5** is below 1 μM, which means that this compound has the strongest inhibition effect against HeLa cell growth than the other four compounds. Interestingly, although the effective apoptotic concentration of compound **2** is twofold higher than that of compound **1**, they have the similar IC_{50}, which is about 1.5 μM, indicating these compounds may use different mechanisms to induce cell apoptosis and inhibit cell proliferation.

In summary, all five compounds isolated from *G. oligantha* could induce HeLa-C3 cell apoptosis at 10 μM or lower concentration. Specifically, compounds **1** and **5** demonstrated stronger apoptosis-inducing effects than other three compounds. Moreover, these compounds also exhibited strong growth inhibition effect against human cervical cancer HeLa cells. Among them, the IC_{50} value of compound **5** is the lowest, suggesting its potential to be developed into a new anticancer agent.

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Supplementary data

Supplementary data (complete experimental details and characterization data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.068.

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