

## Dimeric 1,4-benzoquinone derivatives and a resorcinol derivative from *Ardisia gigantifolia*

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### ARTICLE INFO

#### Article history:

Received 17 October 2008

Received in revised form 5 February 2009

Available online 4 May 2009

#### Keywords:

*Ardisia gigantifolia*

Myrsinaceae

1,4-Benzoquinone derivative

Resorcinol derivative

Cytotoxicity

### ABSTRACT

Naturally occurring dimeric 1,4-benzoquinone derivatives, belamcandaquinones F, G, H, and I, as well as one resorcinol derivative and four known compounds, were isolated from rhizomes of *Ardisia gigantifolia*. Their structures were established by means of spectroscopic analyses. All compounds were tested against cell lines PC-3, EMT6, A549, Hela, RM-1, and SGC7901 for cytotoxicity *in vitro*. In comparison with cisplatin, compounds **5** and **6** showed a strong cytotoxicity with IC<sub>50</sub> values less than 30 μM for most cell lines tested.

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## 1. Introduction

The *Ardisia* is the largest genus in the family of the Myrsinaceae, consisting of approximately 500 species of evergreen shrubs and trees throughout the subtropical and tropical regions of the world (Chen and Pipoly, 1996). *Ardisia* species have been used as sources of both food and folk medicine. Diverse types of compounds have been isolated from this genus, such as saponins, coumarins, and quinones (Kobayashi and De Mejía, 2005). In addition, very interesting bioactivities, such as anti-tumor, anti-inflammation, anti-virus, anti-HIV, and anti-oxidation properties have been described for compounds isolated from this genus (Kobayashi and De Mejía, 2005). A literature survey showed that *A. punctata* (Li et al., 2006) contained dimeric 1,4-benzoquinone derivatives that are rarely found in nature, which prompted us to undertake a phytochemical investigation on *Ardisia gigantifolia* whose clinical usage and appearance are similar with those of *A. punctata* as herbal medicine in China (Dai et al., 1996). As a result, four dimeric 1,4-benzoquinone derivatives, belamcandaquinones F (**1**), G (**2**), and H (**3**), I (**4**), and one new resorcinol derivative (**5**), together with four

known resorcinol derivatives (**6–9**) were isolated. This paper deals with their structural determination on the basis of spectroscopic analysis, including extensive 1D and 2D NMR spectroscopic data. All compounds were tested against cell lines PC-3, EMT6, A549, Hela, RM-1, and SGC7901 for cytotoxicity *in vitro*.

## 2. Results and discussion

The dried methanol extract of *A. gigantifolia* was dissolved in water and partitioned with ethyl acetate. The ethyl acetate portion was subjected to silica gel, LH-20, and ODS chromatographic steps. Compounds **1–9** were finally purified by reversed phase HPLC.

Belamcandaquinones F (**1**) and G (**2**) were obtained as red oils with identical molecular formulae of C<sub>48</sub>H<sub>76</sub>O<sub>5</sub> as determined by HRTOFMS. The UV and IR absorption spectra of **1** and **2** were similar to each other, and were indicative of the presence of a 1,4-benzoquinone moiety and a phenol group. The <sup>1</sup>H NMR spectrum of **1** showed signals for two alkenyl side chains [δ 2.00 (8H, *m*), 2.18 (1H, *m*), 2.24 (2H, *m*), 2.33 (1H, *m*), 5.36 (4H, *m*), 1.20–1.44 (*m*) and 0.91 (6H, *t*, *J* = 7.0 Hz)], two aromatic methyl groups (δ 2.00 and 2.03), as well as one aromatic proton [δ 6.32 (1H, *s*)]. Analysis of the <sup>13</sup>C NMR spectrum indicated the presence of two methyl groups (δ 7.9, 8.1), a group of methylenes (δ 22.7–33.2), and 12 signals belonging to a 1,4-benzoquinone ring and a benzene ring. The HMBC experiment together with literature comparison confirmed

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the substitution pattern on the two rings, and indicated that the 1,4-benzoquinone and the benzene rings in **1** were linked together by a C4 and C1' bond, with two alkenyl chains attached at C3 and C2', respectively (Fig. 2) (Fukuyama et al., 1993a; Li et al., 2006). The remaining problems to be solved were the length of the two alkenyl side chains and the position of the double bonds. In the negative ESI-MS spectrum of **1**, two fragment ion peaks corresponding to a *p*-benzoquinone unit (A) and a benzene unit (B) were observed at  $m/z$  372 and 359, which indicated the length of two alkenyl chains to be C<sub>17</sub>. The double bond on the C<sub>17</sub> side chain was oxidized with *m*-chloroperoxybenzoic acid to afford **10**, whose structure was definitively assigned by NMR (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HSQC, HMBC) and ESI-MS data analyses. Compound **10** was a symmetrical dimer of 1,4-benzoquinone. In the negative ESI-MS of **10**, the presence of a fragment ion peak at  $m/z$  389, at half the molecular weight (778), further confirmed the length of the side chains; detection of characteristic fragment ion peaks at  $m/z$  764, 750, 736, 722, 708, 694, 680, 666, 624 [ $M - \text{CH}_2 \times 8 - \text{C}_2\text{H}_2\text{O}$ ], 610 [ $M - \text{CH}_2 \times 8 - \text{C}_2\text{H}_2\text{O} - \text{CH}_2$ ], 454 [ $M - (\text{CH}_2 \times 8 - \text{C}_2\text{H}_2\text{O}) \times 2$ ], and 426 [ $M - (\text{CH}_2 \times 8 - \text{C}_2\text{H}_2\text{O} - \text{CH}_2) \times 2$ ] established the location of the double bond to be at C-14 and 14' in **1** (Fig. 3). The configuration of each double bond in **1** was assigned as *Z* on the basis of the diag-

nostic chemical shift values of the allylic carbon signals observed around 27 ppm (De Haan and Van de Ven, 1973). The stereochemistry of **1** was deduced to be the same as that of known belamcand-aquinone C (**11**) determined by X-ray diffraction (Fig. 1) (Li et al., 2006). In circular dichroism (CD) experiments, both **1** and **11** showed a positive cotton effect near 287 nm. The proton and carbon signal assignments were achieved by careful elucidation of <sup>1</sup>H-<sup>1</sup>H COSY, HSQC, and HMBC spectra (Table 1).

The <sup>1</sup>H- and <sup>13</sup>C NMR spectrum of **2** were similar to those of **1**, having proton signals for two alkenyl side chains, one aromatic methyl group ( $\delta$  2.13), one methoxyl group ( $\delta$  3.87) and two aromatic protons [ $\delta$  6.03 (1H, s), 6.39 (1H, s)], as well as 12 carbon resonances belonging to 1,4-benzoquinone ring and benzene rings. Detailed analysis of the 2D NMR spectra established the structure and helped assign the proton and carbon signals (Table 1). The length of the two alkenyl chains was determined to be C<sub>17</sub> from the two fragment ion peaks at  $m/z$  386 and 345 corresponding to a *p*-benzoquinone unit and a benzene unit in the negative ESI-MS spectrum of **2**. The configuration of each double bond in **2** was assigned as *Z* from the allylic carbon signals observed at 27.2 ppm (De Haan and Van de Ven, 1973). Due to the small amount obtained, **2** was not submitted to oxidation. The position

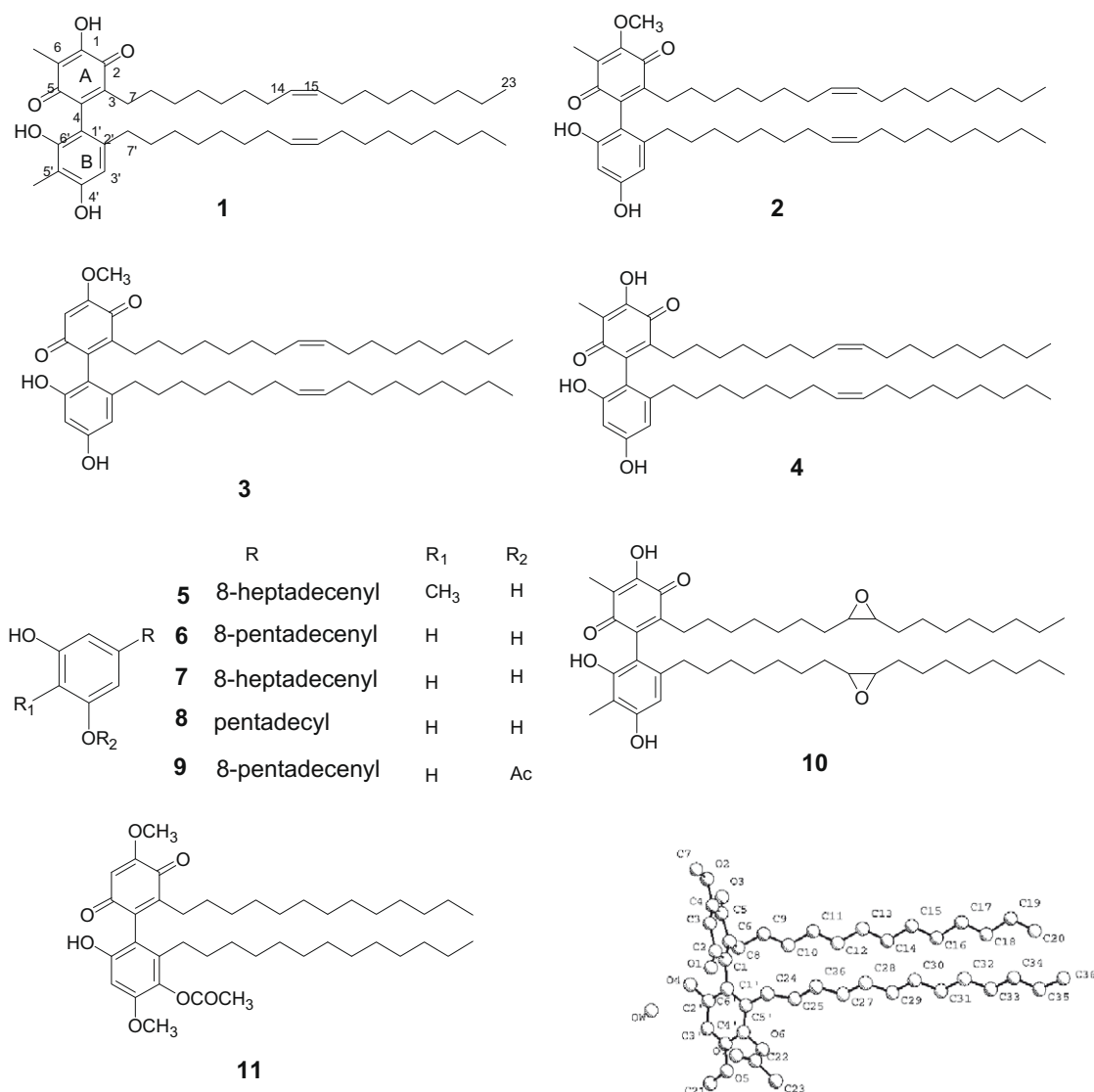


Fig. 1. Structures of compounds **1–11** and perspective view of **11** obtained by X-ray diffraction study.

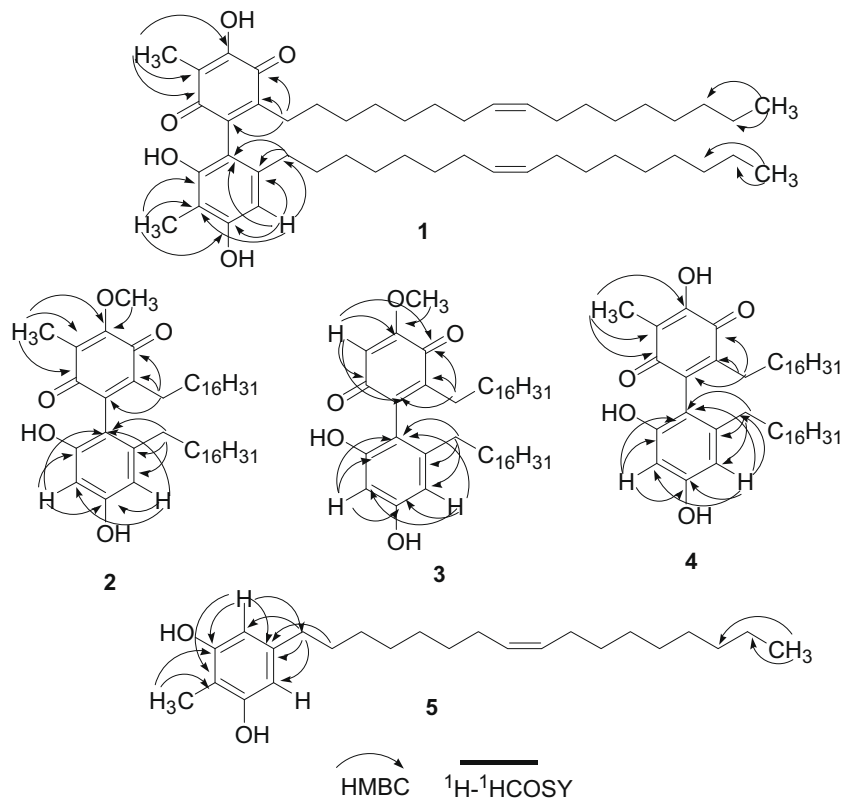


Fig. 2. HMBC and  $^1\text{H}$ - $^1\text{H}$  COSY correlations for compounds 1–5.

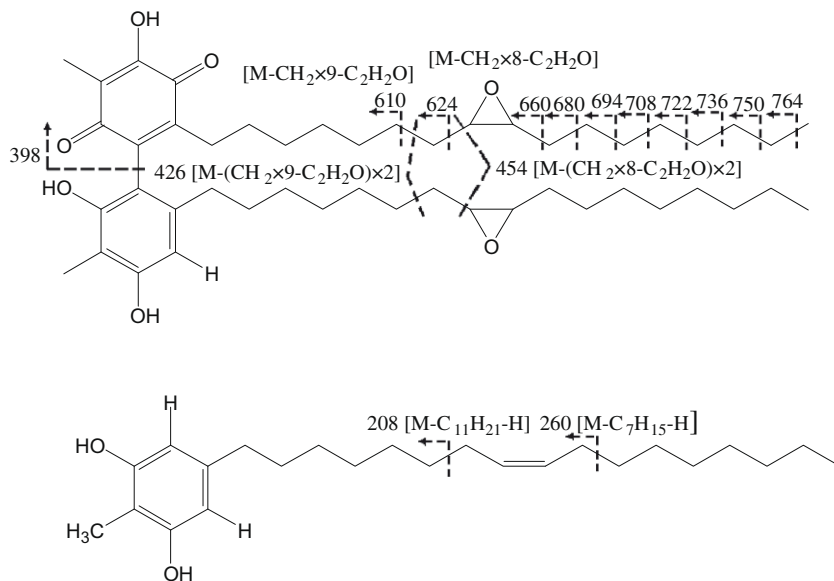


Fig. 3. Major fragment ions observed in negative ESI-MS spectrum of **10** and EI-MS spectrum of **5**.

of the double bond was inferred identical to that of **1** by NMR spectroscopic data comparisons. The stereochemistry of **2** was determined to be the same as that of **1** from the positive cotton effect at 287 nm.

Belamcandaquinone H (**3**) and belamcandaquinone I (**4**) were isolated as red oils. They possessed the same molecular formula of  $\text{C}_{47}\text{H}_{74}\text{O}_5$  as determined by HRTOFMS. The UV and IR absorption spectra of **3** and **4** were similar to those of **1** and **2**, supporting the presence of a 1,4-benzoquinone moiety and a phenol group. The  $^1\text{H}$

NMR spectrum of **3** showed signals for two alkenyl side chains, one methoxyl group ( $\delta$  3.85), and three aromatic protons [ $\delta$  5.99 (1H, s), 6.16 (1H, s), 6.26 (1H, s)]. The  $^{13}\text{C}$  NMR spectrum of **3** suggested the presence of one methoxyl group ( $\delta$  56.3), a group of methylenes, and 12 signals belonging to a 1,4-benzoquinone ring and a benzene ring. In the  $^1\text{H}$  NMR spectrum of **4**, signals for two alkenyl side chains, one aromatic methyl group 1.97 (3H, s), and two aromatic protons [ $\delta$  6.10 (1H, s) and 6.25 (1H, s)] were observed. The  $^{13}\text{C}$  NMR spectrum of **4** showed resonances for one methyl group ( $\delta$

**Table 1**  
<sup>13</sup>C NMR spectroscopic data for compounds **1–4** and **10** in CDCl<sub>3</sub>.

Position	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>10</b>
1	151.3	158.7	159.1	151.7	151.2
2	183.6	182.2	182.2	183.4	182.7
3	144.2	146.9	146.9	144.3	142.4
4	143.1	141.0	141.1	142.8	139.5
5	187.8	186.9	188.4	188.8	185.7
6	117.4	107.5	107.3	117.1	117.4
7	27.2	27.2	27.2	27.2	27.2
8	27.6–30.1	27.8–30.1	27.6–30.1	27.8–30.1	26.6
9–12	27.6–30.1	27.8–30.1	27.6–30.1	27.8–30.1	27.9–30.1
13	27.2	27.2	27.2	27.2	27.8
14	130.0	129.9	129.9	129.9	57.2
15	130.0	129.9	129.9	129.9	57.2
16	27.2	27.2	27.2	27.2	27.8
17–20	27.6–30.1	27.8–30.1	27.6–30.1	27.8–30.1	27.9–30.1
21	31.9	31.9	31.9	31.9	31.8
22	22.7	22.7	22.7	22.7	22.6
23	14.0	14.0	14.0	14.0	14.0
1-OCH <sub>3</sub>		56.2	56.3		
6-Me	7.9			7.9	8.1
1'	108.1	112.7	112.2	112.3	
2'	139.1	139.4	143.0	143.0	
3'	108.3	108.2	108.2	108.2	
4'	154.8	154.6	156.6	156.6	
5'	112.7	107.8	101.0	101.0	
6'	151.3	151.3	153.8	153.8	
7'	33.2	33.2	33.5	33.5	
8'–12'	27.6–30.1	27.8–30.1	27.6–30.1	27.8–30.1	
13'	27.2	27.2	27.2	27.2	
14'	130.0	129.9	129.9	129.9	
15'	130.0	129.9	129.9	129.9	
16'	27.2	27.2	27.2	27.2	
17'–20'	27.6–30.1	27.8–30.1	27.6–30.1	27.8–30.1	
21'	31.9	31.9	31.9	31.9	
22'	22.7	22.7	22.7	22.7	
23'	14.0	14.0	14.0	14.0	
5'-Me	8.1				

8.1), a group of methylenes, and signals due to a 1,4-benzoquinone ring and a benzene ring. The structure determination and resonance assignment for **3** and **4** were achieved by analyses of the 2D NMR experiments. The length of two alkenyl chains in both **3** and **4** were determined to be C<sub>17</sub> from two fragment ion peaks at *m/z* 372 and 345 corresponding to a *p*-benzoquinone unit and a benzene unit in their negative ESI–MS spectrum. The configuration and position of each double bond in both **3** and **4** were identical with that of **1** by NMR spectroscopic data comparison. The stereochemistry of both **3** and **4** were determined to be same as that of **1** by CD experiment.

Compound **5** was obtained as white lamellar crystals. Positive ESI–MS gave an [M+H]<sup>+</sup> ion peak at *m/z* 361 and a negative ESI–MS had an [M–H]<sup>–</sup> at *m/z* 359. Analysis of the HRTOFMS together with NMR spectra indicated a molecular formula of C<sub>24</sub>H<sub>40</sub>O<sub>2</sub>. The <sup>1</sup>H NMR spectrum of **5** contained signals corresponding to two equivalent aromatic protons at δ 6.26 (2H, *s*), one aromatic methyl group at δ 2.13 (3H, *s*), and resonances due to a long alkenyl side chain at δ 0.91 (3H, *t*, *J* = 7.0 Hz), 1.20–1.40 (20H), 1.57 (2H, *m*), 2.03 (4H, *m*), 2.48 (2H, *t*, *J* = 8.0 Hz), and 5.38 (2H, *m*). The <sup>13</sup>C NMR spectrum of **5** showed signals for a tetra-substituted benzene at δ 107.3, 107.8 (2×C), 142.2, and 154.5 (2×C), and resonances for an alkenyl chain. Based on the analyses above, compound **5** was predicted to be a benzene derivative tetra-substituted by two hydroxyl groups, a methyl group, and an alkenyl chain. The substitution pattern was verified by HMBC experiment as shown in Fig. 2. The length of the alkenyl chain was deduced to be C<sub>17</sub> by subtraction of the benzene part (C<sub>7</sub>H<sub>34</sub>O<sub>2</sub>) from the molecular formula. The configuration of each double bond in **5** was assigned as *Z* on the basis of the diagnostic chemical shift values of the allylic carbon signals observed around 27 ppm. The position of the double bond was

determined by observation of fragment ion peaks at *m/z* 208 [M–C<sub>11</sub>H<sub>21</sub>–H] and 260 [M–C<sub>7</sub>H<sub>15</sub>–H] from the allylic cleavages of the side chain in the EIMS spectrum (Fig. 3). Assignment of the <sup>1</sup>H- and <sup>13</sup>C NMR signals was achieved by combination of data from the <sup>1</sup>H–<sup>1</sup>H COSY, HMQC, and HMBC experiments (Table 2). Thus, structure **5** was proposed to be 2-methyl-5-(8*Z*-heptadecenyl)resorcinol.

Four known resorcinol derivatives (**6–9**), but new to the species, were also isolated with their structures identified by <sup>1</sup>H- and <sup>13</sup>C NMR and MS analyses, and comparisons with literature data (Barbero et al., 1989; Suzuki et al., 1996).

To date, only cyclooxygenase inhibitory activity was reported for belamcandaquinone A (Fukuyama et al., 1993a). In this study, we evaluated the cytotoxicity of compounds **1–10**. Their cytotoxicity towards cell lines PC-3, EMT6, A549, HeLa, RM-1, SGC7901 was determined by MTT method. Cisplatin, a clinical anti-cancer drug with strong cytotoxic activity to most tumor cell lines *in vitro*, was used as a positive control in this experiment. In comparison with the IC<sub>50</sub> of cisplatin, compounds with IC<sub>50</sub> less than 30 μM were considered to be strongly cytotoxic; compounds with IC<sub>50</sub> in the range of 30–50 μM were considered to be moderately cytotoxic; compounds with IC<sub>50</sub> in the range of 50–100 μM were considered to be weakly cytotoxic; compounds with IC<sub>50</sub> over 100 μM were considered to be inactive. To most cell lines compounds **5** and **6** showed strong cytotoxicity; compounds **7–9** showed moderate cytotoxicity; and compound **1** was weakly cytotoxic as resulted in Table 3.

### 3. Concluding remarks

Four new dimeric 1,4-benzoquinone derivatives, belamcandaquinones F, G, H, and I, as well as one new resorcinol derivative (**5**), along with four known resorcinol derivatives (**6–9**), were isolated from rhizomes of *A. gigantifolia*, and the structures of the new compounds were determined by NMR data spectroscopic analyses. Belamcandaquinones or, however, distributed to a very limited extent in higher plants. To date, only five belamcandaquinones, A–E, have been reported from *Belamcanda chinensis* plants (Fukuyama et al., 1993a) and *A. punctata* plants (Li et al., 2006). This is also the first report of belamcandaquinones with C<sub>17</sub> long alkenyl side chains. The isolation of belamcandaquinones with C<sub>17</sub> long alkenyl side chains from *A. gigantifolia* in present study can serve as chemotaxonomic marker for *A. gigantifolia*, and some may be bioactive.

**Table 2**  
<sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data for compound **5** in CDCl<sub>3</sub>.

Position	<b>5</b>	
	δ <sub>H</sub> (m, J in Hz)	δ <sub>C</sub>
1		154.5
2		107.3
3		154.5
4	6.26 ( <i>s</i> )	107.8
5		142.2
6	6.26 ( <i>s</i> )	107.8
1'	2.48 ( <i>t</i> , <i>J</i> = 8.0 Hz)	35.5
2'	1.57 ( <i>m</i> )	31.8
3'–6'	1.20–1.40 ( <i>m</i> )	29.0–29.8
7'	2.03 ( <i>m</i> )	27.2
8'	5.38 ( <i>m</i> )	129.9
9'	5.38 ( <i>m</i> )	129.9
10'	2.03 ( <i>m</i> )	27.2
11'–14'	1.20–1.40 ( <i>m</i> )	29.0–29.8
15'	1.28–1.32 ( <i>m</i> )	31.2
16'	1.28–1.32 ( <i>m</i> )	22.7
17'	0.91 ( <i>t</i> , <i>J</i> = 7.0 Hz)	14.1
2-CH <sub>3</sub>	2.13 ( <i>s</i> )	7.9

**Table 3**  
Cytotoxicity of compounds **1**, **5**–**9**<sup>a</sup>.

Compounds	Cancer cell lines (IC <sub>50</sub> , μm)					
	PC-3	EMT6	A549	Hela	RM-1	SGC7901
<b>1</b>	65.3 ± 4.2	83.9 ± 4.6	44.7 ± 3.2	73.7 ± 5.1	70.2 ± 4.9	>100
<b>5</b>	27.5 ± 3.1	22.5 ± 3.6	19.3 ± 2.1	33.5 ± 3.4	22.5 ± 3.0	15.5 ± 1.8
<b>6</b>	16.8 ± 1.6	35.4 ± 3.1	12.7 ± 1.4	19.4 ± 2.0	18.6 ± 1.6	19.1 ± 1.5
<b>7</b>	27.5 ± 2.2	22.5 ± 2.3	19.3 ± 1.7	33.5 ± 3.1	22.5 ± 2.4	15.5 ± 1.7
<b>8</b>	33.9 ± 2.8	71.1 ± 4.6	31.8 ± 3.0	39.8 ± 3.8	43.9 ± 4.1	68.3 ± 5.8
<b>9</b>	50.8 ± 4.8	81.4 ± 7.2	33.1 ± 4.1	49.1 ± 3.9	47.8 ± 4.5	97.1 ± 7.5
Cisplatin	12.6 ± 1.3	16.9 ± 1.7	18.7 ± 1.8	14.3 ± 1.3	13.8 ± 1.4	11.6 ± 1.3

<sup>a</sup> Compounds **2**–**4**, **10** were inactive against all cell lines tested (IC<sub>50</sub> > 100 μm).

## 4. Experimental

### 4.1. General

Optical rotations were measured using a P-1020 digital polarimeter (JASCO). UV spectra were obtained with a Shimadzu UV2401PC UV–vis recording spectrophotometer in MeOH. IR spectra were recorded on Shimadzu FT/IR-8400 spectrometer. EI–MS data was obtained on a TRAC spectrometer. ESI–MS was conducted using a Brüker esquire 2000 mass spectrometer. HRTOFMS spectra were measured on a Bruck microTOF-Q instrument. <sup>1</sup>H- and <sup>13</sup>C NMR, along with 2D NMR spectra were obtained on a Bruker AV-500 (500 MHz for <sup>1</sup>H, 125 MHz for <sup>13</sup>C) NMR spectrometer, using TMS as an internal standard. Chemical shifts were expressed in δ (ppm) and coupling constants (*J*) were reported in Hertz (Hz). TLC was carried out on Silica gel 60F<sub>254</sub> and spots were visualized by spraying with 10% H<sub>2</sub>SO<sub>4</sub> and heating. LH-20 (Amersham Biosciences) and ODS (Lobar, 40–63 μm, Merck) were used for column chromatography (CC). Preparative HPLC was performed using an ODS column (C-18, 250 × 20 mm, Shimadzu Pak; detector: UV).

### 4.2. Plant material

Rhizomes of *A. gigantifolia* were collected in the Guangxi province, China, in October 2006 and identified by Dr. Changli Liu, School of Traditional Chinese Medicine, Capital University of Medical Sciences. A voucher specimen (LHW-2005-1201) was deposited at the School of Chemical Biology and Pharmaceutical Sciences, Capital University of Medical Sciences, China.

### 4.3. Extraction and isolation

The rhizomes of *A. gigantifolia* (2.5 kg) were ground and macerated with MeOH (10 l × 3, for a week) at room temperature. The combined methanol extracts (220 g) were dried, then dissolved in H<sub>2</sub>O (1 l) and partitioned with EtOAc (1 l × 3) to yield an EtOAc extract (100 g). A portion of the EtOAc extract (40 g) was subjected to silica gel CC (hexane/EtOAc, 1:0–0:1, v/v) to afford 23 subfractions (AG1–AG23). Four main fractions consisting of AG7, AG9, AG11, and AG13 were selected for further separation based on TLC comparisons (hexane/EtOAc 80:20; silica gel). Fraction AG7 (hexane/EtOAc 9:1, 2 g) was subjected to Sephadex LH-20 CC (eluted with MeOH) to afford 8 subfractions (AG7-1–AG7-8). Compound **5** (100.6 mg) was crystallized from subfraction AG7-4 in MeOH. Compounds **6** (15.2 mg), **7** (12.6 mg), and **9** (12.4 mg) were obtained from subfraction AG7-5 by reversed phase HPLC (MeOH–H<sub>2</sub>O, 95:5, v/v). Fraction AG9 (hexane/EtOAc, 9:1, 3 g) was applied to a Sephadex LH-20 column (eluted with MeOH) to yield compound **8** (1.5 g). Fraction AG11 (hexane/EtOAc, 85:15, 0.8 g) was subjected to ODS CC (MeOH/H<sub>2</sub>O, 4:1–1:0, v/v) to give five subfractions (AG11-1–AG11-5). Belamcandaquinone F (**1**, 40.6 mg) and G (**2**, 5.8 mg) were purified by reversed HPLC (MeOH–H<sub>2</sub>O, 9:1, v/v)

from subfraction AG11-4. Fraction AG13 (hexane/EtOAc, 8:2, 0.5 g) was subjected to CC on ODS (MeOH/H<sub>2</sub>O, 4:1–1:0, v/v) to give seven subfractions (AG13-1–AG13-7). Belamcandaquinone H (**3**, 8.1 mg) and I (**4**, 7.5 mg) were purified from fraction AG13-5 by reversed phase HPLC (MeOH–H<sub>2</sub>O, 9:1, v/v).

#### 4.3.1. Belamcandaquinone F (**1**)

Red oil; [ $\alpha_D^{25}$ ] + 6.2 (c 1.0, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 280 (4.25); CD (MeOH) [ $\theta$ ]<sub>287</sub> + 2.6 × 10<sup>4</sup>; IR (KBr, disc)  $\nu_{\max}$  cm<sup>-1</sup>: 3414, 2925, 2854, 1670, 1640, 1457, 1390, 1315, 1090, 723; positive ESI–MS: *m/z* 755 [M+Na]<sup>+</sup>; negative ESI–MS: *m/z* 731 [M–H]<sup>-</sup>, 372, 359; Negative HRTOFMS: *m/z* [M–H]<sup>-</sup> 731.5596 (calcd for C<sub>48</sub>H<sub>75</sub>O<sub>5</sub>, 731.5614); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.91 (6H, *t*, *J* = 7.0 Hz, H-23, H-23'), 1.20–1.44 (44H, *m*), 2.00 (3H, *s*, Me-6), 2.00 (8H, *m*, H-13, H-16, H-13', H-16'), 2.03 (3H, *s*, Me-5'), 2.18 (1H, *m*, H-7), 2.24 (2H, *m*, H-7'), 2.33 (1H, *m*, H-7), 5.36 (4H, *m*, H-14, H-15, H-14', H-15'), 6.32 (1H, *s*, H-3'); for <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1.

#### 4.3.2. Belamcandaquinone G (**2**)

Red oil; [ $\alpha_D^{25}$ ] + 4.6 (c 0.50, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 282 (4.25); CD (MeOH) [ $\theta$ ]<sub>287</sub> + 2.3 × 10<sup>4</sup>; IR (KBr, disc)  $\nu_{\max}$  cm<sup>-1</sup>: 3320, 2920, 2854, 1680, 1642, 1600, 1457, 1380, 1227, 1060, 722; positive ESI–MS: *m/z* 755 [M+Na]<sup>+</sup>; negative ESI–MS: *m/z* 731 [M–H]<sup>-</sup>, 386, 345. Negative HRTOFMS: *m/z* [M–H]<sup>-</sup> 731.5601 (calcd for C<sub>48</sub>H<sub>75</sub>O<sub>5</sub>, 731.5614); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.91 (6H, *t*, *J* = 7.0 Hz, H-23, H-23'), 1.18–1.44 (44H, *m*), 2.00 (8H, *m*, H-13, H-16, H-13', H-16'), 2.13 (3H, *s*, Me-5'), 2.20 (1H, *m*, H-7), 2.24 (2H, *m*, H-7'), 2.36 (1H, *m*, H-7), 3.87 (3H, *s*, 1-OMe), 5.36 (4H, *m*, H-14, H-15, H-14', H-15'), 6.03 (1H, *s*, H-6), 6.39 (1H, *s*, H-3'); for <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1.

#### 4.3.3. Belamcandaquinone H (**3**)

Red oil; [ $\alpha_D^{25}$ ] + 5.8 (c 0.50, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 280 (3.85); CD (MeOH) [ $\theta$ ]<sub>286</sub> + 2.2 × 10<sup>4</sup>; IR (KBr, disc)  $\nu_{\max}$  cm<sup>-1</sup>: 3286, 2925, 2854, 1678, 1640, 1600, 1457, 1338, 1227, 1148, 1049, 848, 722; positive ESI–MS: *m/z* 741 [M+Na]<sup>+</sup>; negative ESI–MS: *m/z* 717 [M–H]<sup>-</sup>, 372, 345. Negative HRTOFMS: *m/z* [M–H]<sup>-</sup> 717.5432 (calcd for C<sub>47</sub>H<sub>73</sub>O<sub>5</sub>, 731.5458); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 0.91 (6H, *t*, *J* = 7.0 Hz, H-23, H-23'), 1.20–1.44 (44H, *m*), 2.00 (8H, *m*, H-13, H-16, H-13', H-16'), 2.20 (1H, *m*, H-7), 2.24 (2H, *m*, H-7'), 2.35 (1H, *m*, H-7), 3.85 (3H, *s*, 1-OMe), 5.36 (4H, *m*, H-14, H-15, H-14', H-15'), 5.99 (1H, *s*, H-6), 6.16 (1H, *s*, H-5'), 6.26 (1H, *s*, H-3'); for <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) spectroscopic data, see Table 1.

#### 4.3.4. Belamcandaquinone I (**4**)

Red oil; [ $\alpha_D^{25}$ ] + 6.7 (c 0.50, CHCl<sub>3</sub>); UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 280 (4.15); CD (MeOH) [ $\theta$ ]<sub>286</sub> + 2.4 × 10<sup>4</sup>; IR (KBr, disc)  $\nu_{\max}$  cm<sup>-1</sup>: 3330, 2915, 2850, 1682, 1640, 1580, 1450, 1380, 1220, 1060, 734; positive ESI–MS: *m/z* 741 [M+Na]<sup>+</sup>; negative ESI–MS: *m/z* 717 [M–H]<sup>-</sup>. Negative HRTOFMS: *m/z* [M–H]<sup>-</sup> 717.5440 (calcd for

$C_{47}H_{73}O_5$ , 731.5458);  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  0.91 (6H, t,  $J = 7.0$  Hz, H-23, H-23'), 1.20–1.44 (44H, m), 1.97 (3H, s, 6- $CH_3$ ), 2.00 (8H, m, H-13, H-16, H-13', H-16'), 2.20 (1H, m, H-7), 2.24 (2H, m, H-7'), 2.35 (1H, m, H-7), 5.36 (4H, m, H-14, H-15, H-14', H-15'), 6.10 (1H, s, H-5'), 6.25 (1H, s, H-3'); for  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ) spectroscopic data, see Table 1.

#### 4.3.5. 2-methyl-5-(8Z-heptadecenyl) resorcinol (5)

White lamellar crystal; UV (MeOH)  $\lambda_{max}$  nm (log  $\epsilon$ ): 272 (3.84); IR (KBr, disc)  $\nu_{max}$   $cm^{-1}$ : 3364, 2921, 2850, 1633, 1586, 1521, 1465, 1426, 1166, 1070, 843, 720; positive ESI-MS:  $m/z$  361 [M+H] $^+$ ; negative ESI-MS:  $m/z$  259 [M-H] $^-$ . Negative HRTOFMS:  $m/z$  [M-H] $^-$  359.2925 (calcd for  $C_{24}H_{39}O_2$ , 359.2950); for  $^1H$  NMR (500 MHz,  $CDCl_3$ ) and  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ) spectroscopic data, see Table 2.

#### 4.4. Oxidation of double bond in 1 (Fukuyama et al., 1993b)

Belamcandaquinone F (1) (10 mg) was dissolved in  $CHCl_3$  (3 mL). *m*-Chloroperoxybenzoic acid (5 mg) was added to this solution, and then the reaction mixture was left to stand at room temperature for 3 h.  $CHCl_3$  (5 mL) was added to the mixture and then the organic layer was washed with saturated  $NaHCO_3$  solution and saturated  $NaCl$  solution. After being dried over anhydrous  $Na_2SO_4$ , the organic layer was evaporated in vacuo to give a residue which was purified by preparative TLC (hexane:ethyl acetate, 8:2) to afford compound 10 (6 mg): red oil; positive ESI-MS:  $m/z$  801 [M+Na] $^+$ ; negative ESI-MS:  $m/z$  777 [M-H] $^-$ , 764, 750, 736, 722, 708, 694, 680, 666, 624 (M- $CH_2 \times 8-C_2H_2O$ ), 610 (M- $CH_2 \times 8-C_2H_2O-CH_2$ ), 454 [M-( $CH_2 \times 8-C_2H_2O$ ) $\times 2$ ], 426 [M-( $CH_2 \times 8-C_2H_2O-CH_2$ ) $\times 2$ ], 389;  $^1H$  NMR (500 MHz,  $CDCl_3$ ):  $\delta$  0.91 (3H, t,  $J = 7.0$  Hz, H-23), 1.25–1.50 (26H, m), 1.98 (3H, s, Me-6), 2.18 (1H, m, H-7), 2.33 (1H, m, H-7), 2.92 (2H, m, H-14, 15); for  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ) spectroscopic data, see Table 1.

#### 4.5. Cytotoxicity bioassay

PC-3, EMT6, A549, Hela, RM-1, and SGC7901 cells were seeded in 96-well microtiter plates at 1200 cells/well. After 24 h, the compounds were added to the cells. After 48 h of drug treatment, cell viability was determined by measuring the metabolic conversion

of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) into purple formazan crystals by active cells (Carmichael et al., 1987). MTT assay results were read using a microplate reader (Bio-Rad) at 570 nm. All compounds were tested at five concentrations and were dissolved in 100% DMSO to give a final DMSO concentration of 0.1% in each well. Each concentration of the compounds was tested in three parallel wells.  $IC_{50}$  values were calculated using Microsoft Excel software.

#### Acknowledgements

This work was co-sponsored by Key Laboratory of Medicinal Chemical Resources and Molecular Engineering of Guangxi Normal University, Beijing Municipal Project for Developing Advanced Human Resources for Higher Education (BAHED), and Beijing Scientific Research Foundation for the Returned Overseas Chinese Scholars.

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