Four New 1,4-Benzoquinone Derivatives and One New Coumarin Isolated from *Ardisia gigantifolia*

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Four new 1,4-benzoquinone derivatives, belamcandaquinones J (**1**), K (**2**), L (**3**), and M (**4**), and one new coumarin, **5**, were isolated from the rhizome of *Ardisia gigantifolia*. Their structures were established by means of spectroscopic methods, and their cytotoxicity was tested *in vitro* against the cell lines PC-3, EMT6, A549, Hela, RM-1, and SGC7901. In comparison with cisplatin, compound **5** showed strong cytotoxicity with an IC_{50} value of < 30 μM against the cell lines PC-3 and A549. Compounds **1**–**4** showed no cytotoxicity against all cell lines.

Introduction. – *Ardisia* species have been recorded as sources of food and folk medicine for a long time and are known for their richness in biologically active agents, such as saponins, coumarins, resorcinols, and quinones [1]. Various bioactivities including antitumor, anti-inflammation, antivirus, anti-HIV, and anti-oxidative properties have been reported for compounds isolated from this genus [1]. In our previous chemical investigation on *Ardisia gigantifolia* STAPF (Myrsinaceae), four new 1,4-benzoquinone derivatives, belamcandaquinones F–I, and one new resorcinol derivative were isolated [2]. Belamcandaquinones have a unique structure with an alkenyl- or alkyl-substituted 1,4-benzoquinone ring and an alkenyl- or alkyl-substituted benzene ring linked together by a C–C bond. Such derivatives, belamcandaquinones A–E, have been also isolated from *Belamcanda chinensis* and *A. punctata* [3][4]. Isolation of belamcandaquinones from *A. gigantifolia* promoted our interest to further investigate other minor components. In this study, we describe the isolation, structure determination, and cytotoxicity test of another four minor belamcandaquinones and of one new coumarin derivative from *A. gigantifolia*. Their structures were elucidated on the basis of spectroscopic data, including extensive 1D- and 2D-NMR data analyses. All compounds were tested against cell lines PC-3, EMT6, A549, Hela, RM-1, and SGC7901 for cytotoxicity *in vitro*.

Results and Discussion. – The dried MeOH extract of *A. gigantifolia* was dissolved in H_2O and partitioned with AcOEt. The AcOEt portion was subjected to silica gel, *LH-20*, and *ODS* chromatography. Compounds **1**–**5**[1]) (*Fig. 1*) were finally purified by reversed-phase HPLC.

Belamcandaquinones J (**1**) and K (**2**) were obtained as red oils with the identical molecular formula $C_{45}H_{70}O_5$ as determined by HR-TOF-MS. The UV and IR absorption spectra of **1** and **2** were similar to each other and indicative of the presence of a 1,4-benzoquinone and a phenol moiety. The ^{1}H-NMR spectrum of **1** showed signals for two alkenyl side chains at δ(H) 1.99–2.01 (*m*, CH_2(13,13′,16,16′)), 2.19–2.21 (*m*, 1 H–C(7)), 2.23–2.25 (*m*, CH_2(7′)), 2.34–2.36 (*m*, 1 H–C(7)), 5.35–5.37 (*m*, H–C(14,14′,15,15′)), 1.20–1.44 (*m*), and 0.91 (*t*, J = 7.0 Hz, Me(21′,23)), one aromatic Me group at δ(H) 1.98 (*s*, Me(24)), as well as two aromatic H-atoms at δ(H) 6.18 (br. *s*, H–C(5′)) and 6.35 (br. *s*, H–C(3′)). Analysis of the ^{13}C-NMR spectrum of **1** (*Table 1*) indicated the presence of three Me groups at δ(C) 7.9 (C(24)) and 14.0 (C(23,21′)), CH_2 groups at δ(C) 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 the substitution pattern of two rings and indicated that the 1,4-benzoquinone ring and the benzene ring in **1** are linked together by a C(4)–C(1′) bond, with two alkenyl chains attached at C(3) and C(2′), respectively (*Fig. 2*) [2–4]. The length of the two alkenyl side chains was deduced from ESI-MS and NMR data and comparison with belamcandaquinone F (**6**) [2]. In the ESI-MS (negative-ion mode) of **1**, two fragment-ion peaks corresponding to the 1,4-benzoquinone unit (*A*) and the benzene unit (*B*) were observed at *m*/*z* 372 and 317, which indicated the length of the alkenyl chain to be C_{17} and C_{15}, respectively. In our previous report, the C=C bond position in **6** was confirmed by NMR and ESI-MS data of an oxidized product of **6** obtained by *m*-CPBA (= 3-chloroperbenzoic acid) treatment [2]. From the viewpoint of biosynthesis and NMR data comparison, the position of the C=C bond in **1** was inferred to be identical to that of **6**. The configuration of each C=C bond in **1** was assigned as (*Z*) on the basis of the diagnostic chemical-shift values of the allylic C-atom signals observed around δ(C) 27 [5]. We previously reported the isolation of (8*Z*)-heptadec-8-enyl- and (8*Z*)-pentadec-8-enylresorcinol derivatives from this plant, which further support the location and configuration of the C=C bonds [2]. In circular dichroism (CD) experiments, both **1** and belamcandaquinone C [4] showed a positive *Cotton* effect near 287 nm. Thus, the configuration of **1** was deduced to be the same as that of the known belamcandaquinone C. The H- and C-atom signal assignments were achieved by careful elucidation of ^{1}H,^{1}H-COSY, HSQC, and HMBC spectra.

The ^{1}H- and ^{13}C-NMR spectra of **2** were similar to those of **1**, showing H-atom signals for two alkenyl side chains, one MeO group at δ(H) 3.86 (Me(24)) and three aromatic H-atoms at δ(H) 6.02 (H–C(6)), 6.20 (H–C(5′)), and 6.35 (H–C(3′)), and 12 C-atom resonances belonging to a 1,4-benzoquinone ring and a benzene ring. Detailed analysis of 2D-NMR spectra established the structure and helped to assign the H- and C-atom signals (*Table 1*). The length of the two alkenyl chains was determined to be C_{17} and C_{15} from two fragment-ion peaks at *m*/*z* 372 and 317, corresponding to a 1,4-benzoquinone unit and a benzene unit in the negative-ion mode

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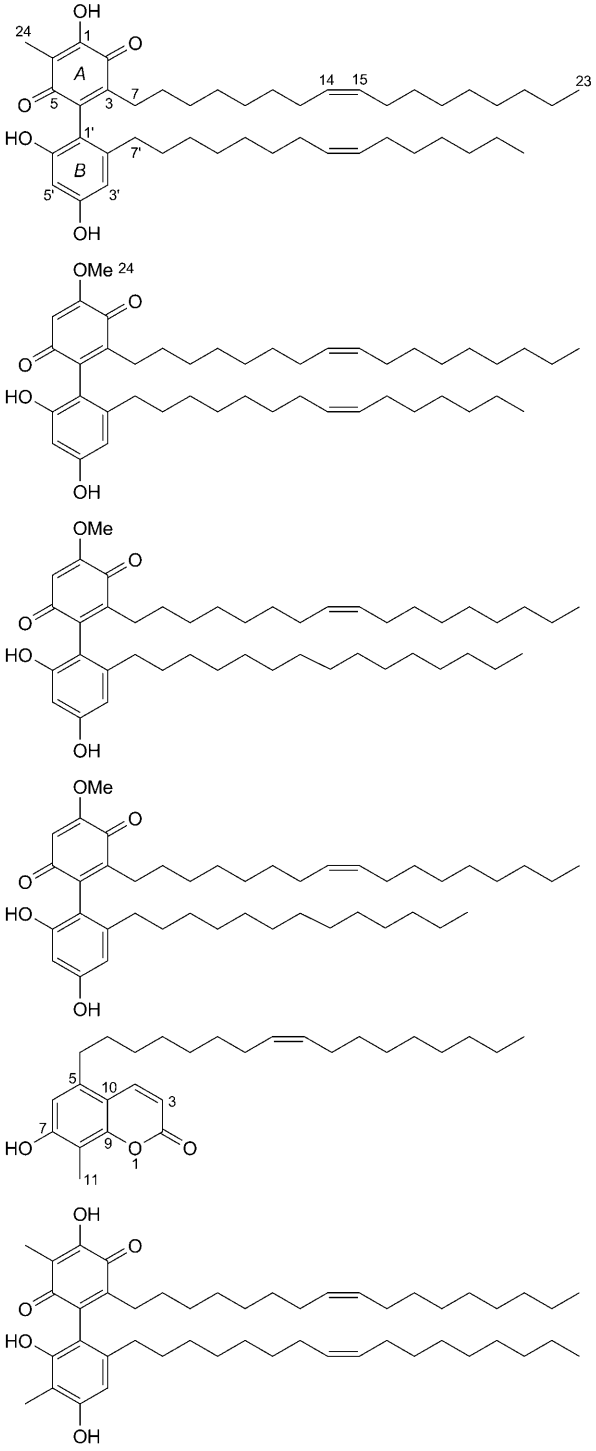
[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



1[1])

2[1])

3

4

5[1])

6

Fig. 1. *Compounds* **1**–**5** *isolated from* A. gigantifolia*, and the known* [2] *belamcandaquinone F* (**6**)

Table 1. *^{13}C-NMR Data for Compounds* **1**–**4**[1]) *in $CDCl_3$* (500 MHz)

	1	**2**		**3**		**4**
C(1)	151.2	158.8	C(1)	158.9	C(1)	158.7
C(2)	183.5	182.2	C(2)	182.2	C(2)	182.2
C(3)	144.1	146.8	C(3)	146.9	C(3)	146.9
C(4)	142.6	140.8	C(4)	140.9	C(4)	140.9
C(5)	187.4	187.2	C(5)	187.7	C(5)	187.7
C(6)	117.3	107.5	C(6)	107.4	C(6)	107.4
C(7)	27.2	27.2	C(7)	27.2	C(7)	27.2
C(8)–C(12)	27.8–30.1	27.9–30.1	C(8)–C(12)	27.6–30.1	C(8)–C(12)	27.6–30.1
C(13)	27.2	27.2	C(13)	27.2	C(13)	27.2
C(14)	129.9	129.9	C(14)	129.9	C(14)	129.9
C(15)	129.9	129.9	C(15)	129.9	C(15)	129.9
C(16)	27.2	27.2	C(16)	27.2	C(16)	27.2
C(17)–C(20)	27.8–30.1	27.9–30.1	C(17)–C(20)	27.6–30.1	C(17)–C(20)	27.6–30.1
C(21)	31.9	31.9	C(21)	31.9	C(21)	31.9
C(22)	22.7	22.7	C(22)	22.7	C(22)	22.7
C(23)	14.0	14.0	C(23)	13.9	C(23)	13.9
C(24)	7.9	56.3	C(24)	56.2	C(24)	56.2
C(1′)	112.9	112.2	C(1′)	112.4	C(1′)	112.4
C(2′)	143.4	143.0	C(2′)	143.2	C(2′)	143.2
C(3′)	108.4	108.2	C(3′)	108.3	C(3′)	108.3
C(4′)	156.7	156.6	C(4′)	156.7	C(4′)	156.7
C(5′)	100.9	101.0	C(5′)	100.9	C(5′)	100.9
C(6′)	153.5	153.8	C(6′)	153.7	C(6′)	153.5
C(7′)	33.4	33.5	C(7′)	33.4	C(7′)	33.4
C(8′)–C(12′)	27.8–30.1	27.9–30.1	C(8′)–C(18′)	27.6–30.1	C(8′)–C(16′)	27.6–30.1
C(13′)	26.9	26.9	C(19′)	31.9	C(17′)	31.9
C(14′)	129.9	129.9	C(20′)	22.7	C(18′)	22.7
C(15′)	129.9	129.9	C(21′)	13.9	C(19′)	13.9
C(16′)	26.9	26.9				
C(17′)–C(18′)	27.8–30.1	27.9–30.1				
C(19′)	31.8	31.8				
C(20′)	22.3	22.3				
C(21′)	14.0	14.0				

ESI-MS of **2**. The configuration of each C=C bond in **2** was assigned as (*Z*) from the allylic C-atom signals observed at δ(C) 27.2 [5]. The position of the C=C bond was inferred to be identical to that of **1** by NMR-data comparison. The configuration of **2** was determined to be the same as that of **1** from the positive *Cotton* effect at 287 nm.

The molecular formula of belamcandaquinones L (**3**) and M (**4**) were determined by HR-TOF-MS to be $C_{45}H_{72}O_5$ and $C_{43}H_{68}O_5$, respectively. The ^{1}H- and ^{13}C-NMR spectra of **3** and **4** were quite similar to each other, showing H-atom signals for one alkenyl and one alkyl side chain, one MeO group at δ(H) 3.86 (Me(24)) and three aromatic H-atoms at δ(H) 6.00 (**3**, H–C(6)), 6.02 (**4**, H–C(6)), 6.18 (**3**, H–C(5′)), 6.25 (**4**, H–C(5′)), 6.32 (**3**, H–C(3′)), and 6.38 (**4**, H–C(3′)), and 12 C-atom resonances belonging to a 1,4-benzoquinone ring and a benzene ring. In comparison with NMR data of **2**, compounds **3** and **4** were proposed to have the same 1,4-

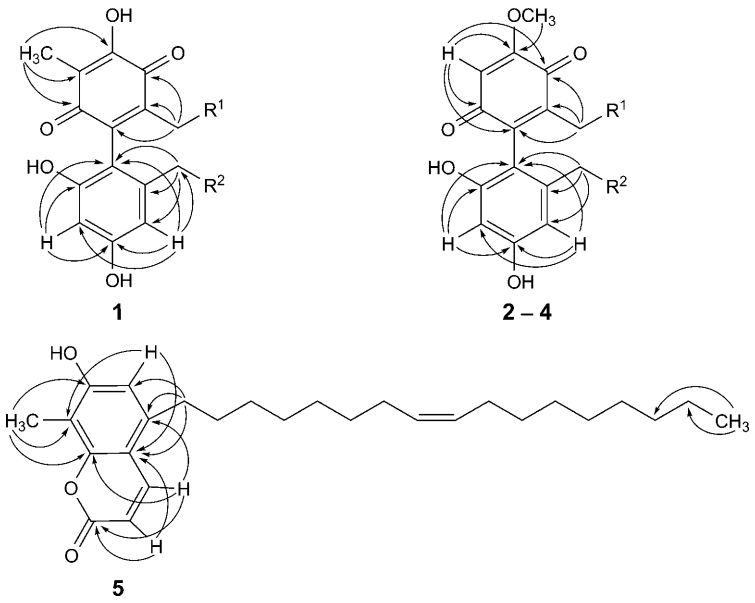


Fig. 2. *HMBCs* (H → C) *of compounds* **1**–**5**

benzoquinone unit as that of **2**. The molecular-mass difference between **3** and **4** was calculated for a C_2H_4 fragment, indicating a pentadecyl group and a tridecyl group attached to the benzene unit, respectively. HMBC (*Fig. 2*) and HSQC spectra confirmed the substitution pattern of the 1,4-benzoquinone and benzene units, and assigned the H- and C-atom signals (*Table 1*). Analysis of the ESI-MS (negative-ion mode) confirmed the length of both the alkenyl and alkyl chain to be C_{17} and C_{15} for **3** from two fragment-ion peaks at *m*/*z* 372 and 319, corresponding to the 1,4-benzoquinone unit and the benzene unit, respectively. The length of both the alkenyl and alkyl chain in **4** was determinded to be C_{17} and C_{13} in the same way from two fragment-ion peaks at *m*/*z* 372 and 291. The configuration of the C=C bond in **3** and **4** was assigned as (*Z*) from the allylic C-atom signals observed at δ(C) 27.2 [5]. The position of the C=C bond was inferred to be identical to that of **2** by NMR-data comparison. The configuration of **3** and **4** was determined to be the same as that of **1** from the positive *Cotton* effect at 287 nm.

Compound **5** was obtained as a yellow oil. HR-TOF-MS (negative-ion mode) revealed its molecular formula to be $C_{27}H_{40}O_3$. The UV spectrum (MeOH) displayed strong absorption bands at 342 and 260 nm that were characteristic of a coumarin skeleton. The IR spectrum showed the presence of aromatic rings (1616, 1590, and 1450 cm^{-1}) and of a lactone (1720 cm^{-1}). The ^{1}H-NMR spectrum exhibited three aromatic H-atom signals at δ(H) 6.27 (*d*, *J* = 10.0 Hz, H–C(3)), 6.65 (H–C(6)), and 7.86 (*d*, *J* = 10.0 Hz, H–C(4)), one Me signal at δ(H) 2.32 (Me(11)), and signals for an alkenyl chain at δ(H) 0.91 (*t*, *J* = 7.0 Hz, Me(17′)), 1.27–1.29 (*m*, CH_2(16′)), 1.30–1.32 (*m*, CH_2(15′)), 1.59–1.61 (*m*, CH_2(2′)), 1.28–1.35 (*m*, CH_2(3′,6′,11′,14′)), 2.03–2.05 (*m*, CH_2(7′,10′)), 2.76 (*t*, *J* = 8.0 Hz, CH_2(1′)), and 5.36–5.38 (*m*, H–C(8′,9′)) (*Table 2*).

In the HMBC spectrum (*Fig. 2*), the observed correlations H–C(3)/δ(C) 161.5 (C(2)) and 111.1 (C(10)), H–C(4)/δ(C) 161.5 (C(2)), 139.3 (C(5)), and 154.4 (C(9)), H–C(6)/δ(C) 111.1 (C(10)) and 156.8 (C(7)), Me–C(11)/δ(C) 109.5 (C(8)), 154.4 (C(9)), and 156.8 (C(7)), H–C(1′)/δ(C) 111.1 (C(10)), 112.7 (C(6)), and 139.3 (C(5)) established a coumarin skeleton and confirmed the substitution pattern of the aromatic ring. The length of the alkenyl chain was deduced to be C_{17} by subtracting the coumarin part ($C_{11}H_7O_3$) from the molecular formula. The configuration of the C=C bond in **5** was assigned as (*Z*) on the basis of the diagnostic chemical-shift values of the allylic C-atom signal observed around δ(C) 27. The position of the C=C bond was determined by observation of fragment-ion peaks at *m*/*z* 259 ($[M - C_{11}H_{21}]^+$) and 313 ($[M - C_7H_{15}]^+$) from the allylic cleavages of the side chain in the EI-MS (*Fig. 3*). Thus, structure **5** was proposed to be 5-[(8*Z*)-heptadec-8-en-1-yl]-7-hydroxy-8-methyl-2*H*-1-benzopyran-2-one.

Table 2. *^{1}H- and ^{13}C-NMR Data for Compound* **5**[1]) *in $CDCl_3$* (500 MHz). δ in ppm, *J* in Hz.

	δ(C)	δ(C)
C(2)		161.5
H–C(3)	6.27 (*d*, *J* = 10.0)	111.9
H–C(4)	7.86 (*d*, *J* = 10.0)	140.6
C(5)		139.3
H–C(6)	6.65 (*s*)	112.7
C(7)		156.8
C(8)		109.5
C(9)		154.4
C(10)		111.1
Me(11)	2.32 (*s*)	7.9
CH_2(1′)	2.76 (*t*, *J* = 8.0)	31.9
H–CH_2(2′)	1.59–1.61 (*m*)	31.5
CH_2(3′) to CH_2(6′)	1.28–1.35 (*m*)	29.2–29.7
CH_2(7′)	2.03–2.05 (*m*)	27.2
H–C(8′)	5.36–5.38 (*m*)	130.0
H–C(9′)	5.36–5.38 (*m*)	129.8
CH_2(10′)	2.03–2.05 (*m*)	27.2
CH_2(11′) to CH_2(14′)	1.28–1.35 (*m*)	29.2–29.7
CH_2(15′)	1.30–1.32 (*m*)	31.9
H–CH_2(16′)	1.27–1.29 (*m*)	22.7
Me(17′)	0.91 (*t*, *J* = 7.0)	14.1

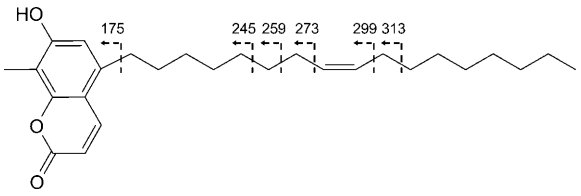


Fig. 3. *Major fragment ions observed in the EI-MS of compound* **5**

The cytotoxicity of each compound against the cell lines PC-3, EMT6, A549, Hela, RM-1, and SGC7901 was determined by the MTT method. Cisplatin was used as a

positive control with IC_{50} of 12.6 ± 1.3, 16.9 ± 1.7, 18.7 ± 1.8, 14.3 ± 1.3, 13.8 ± 1.4, and 11.6 ± 1.3 μM for the tested cell lines. Compound **5** showed strong cytotoxicity against the cell lines PC-3 (IC_{50} 14.4 ± 1.8 μM) and A549 (IC_{50} 20.6 ± 2.1 μM), moderate cytotoxicity against the cell lines EMT6 (IC_{50} 66.6 ± 3.8 μM), Hela (IC_{50} 32.8 ± 2.8 μM), and RM-1 (IC_{50} 36.2 ± 2.3 μM), and no cytotoxicity against SGC7901 with an IC_{50} value larger than 100 μM. Belamcandaquinones J–M (**1**–**4**) showed no cytotoxicity against all cell lines. It is worth noting that belamcandaquinones F–I similarly did not show cytotoxicity in our previous study [2].

This work was co-sponsored by the 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*), *Beijing Scientific Research Foundation for the Returned Overseas Chinese Scholars*, and partially supported by a *National Key Basic Research Project of China* (2009CB522300).

Experimental Part

General. TLC: silica gel *60F*$_{254}$ (SiO_2); visualization by spraying with 10% H_2SO_4 soln. and heating. Column chromatography (CC): *LH-20* (*Amersham Biosciences*) and *ODS* (*Lobar*, 300 × 20 mm, 40–63 μm; *Merck*). Prep. HPLC: *Agilent-1200* HPLC system (UV detector: 254 nm); *ODS* column (*YMC-Pack ODS-A*, 250 × 10 mm, 10 μm); t_R in min. Optical rotations: *Jasco-P-1020* digital polarimeter. Circular dichroism (CD) Spectra: *Jasco J-810* circular dichroism chiroptical spectrometer; λ ([θ]) in nm. UV Spectra: *Shimadzu-UV2401PC* UV/VIS recording spectrophotometer; in MeOH; λ_{max} (log ε) in nm. IR Spectra: *Shimadzu FT/IR-8400* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-, and 2D-NMR: *Bruker-AV-500* (500 MHz for ^1H, 125 MHz for ^{13}C) NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *Shimadzu-QP-5050* GC/MS spectrometer; in *m*/*z* (rel. %). ESI-MS: *Bruker Esquire-2000* mass spectrometer. HR-TOF-MS: *Bruker microTOF-Q* instrument.

Plant Material. The rhizome of *Ardisia gigantifolia* STAPF was collected in Guangxi Province, P. R. 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 with the School of Chemical Biology and Pharmaceutical Sciences, Capital University of Medical Sciences, P. R. China.

Extraction and Isolation. The rhizomes of *A. gigantifolia* (2.5 kg) were ground and macerated with MeOH at r.t. The dried MeOH extract (220 g) was dissolved in H_2O and partitioned with AcOEt to yield an AcOEt extract (100 g). A part of the AcOEt extract (40 g) was subjected to CC (SiO_2; hexane/AcOEt 1:0 → 0:1): *Fractions AG1–AG23*. Separation of the four main fractions of *AG7*, *AG9*, *AG11*, and *AG13* resulted in the isolation of belamcandaquinones F–I, *2-methyl-5-[(8Z)-heptadec-8-en-1-yl]resorcinol*, and four known resorcinol (= benzene-1,3-diol) derivatives [2]. TLC Comparison revealed that *Frs. AG6*, *AG14*, and *AG15* also contained compounds with similar structural characteristics. *Fr. AG6* (eluted with hexane/AcOEt 9:1; 0.2 g) was subjected to CC (*Sephadex LH-20*; MeOH): *Fr. AG6-1–AG6-5*. *Fr. AG6-3* containing one major spot was further purified by a reversed-phase HPLC (MeOH/H_2O 95:5): **5** (2.1 mg; t_R 20.2). *Fr. AG14* (eluted with hexane/AcOEt 8:2; 0.2 g) was subjected to CC (*ODS*; MeOH/H_2O 4:1 → 1:0): *Fr. AG14-1–AG14-5*. The main subfraction, *Fr. AG11-4*, was further purified by a reversed-phase HPLC (MeOH/H_2O 9:1): *belcamcandaquinone J* (**1**; 5.6 mg; t_R 14.2). *Fr. AG15* (eluted with hexane/AcOEt 8:2; 0.4 g) was first separated by CC (*ODS*; MeOH/H_2O 4:1 → 1:0): *Fr. AG15-1–AG15-6*. *Belcamcandaquinones K* (**2**; 3.1 mg; t_R 30.8), *L* (**3**; 4.5 mg; t_R 29.1), and *M* (**4**; 3.5 mg; t_R 20.1) were isolated from *Fr. AG15-3* by a reversed-phase HPLC (MeOH/H_2O 9:1).

2-{2,4-Dihydroxy-6-[(8Z)-pentadec-8-en-1-yl]phenyl}-3-[(8Z)-heptadec-8-en-1-yl]-5-hydroxy-6-methylcyclohexa-2,5-diene-1,4-dione (= *Belamcandaquinone J*; **1**): Red oil. $[\alpha]_D^{25} = +7.2$ ($c = 0.50$, $CHCl_3$). UV: 280 (3.95). CD (MeOH): 287 ($+2.3 \cdot 10^4$). IR: 3416, 2920, 2850, 1675, 1642, 1450, 1315, 1090, 722. ^1H-NMR[1]) (500 MHz, $CDCl_3$): 0.91 (*t*, *J* = 7.0, Me(23), Me(21′)); 1.20–1.44 (*m*, 40 H); 1.98 (*s*, Me(24)); 1.99–2.01 (*m*, CH_2(13), CH_2(16), CH_2(13′), CH_2(16′)); 2.19–2.21 (*m*, 1 H–C(7)); 2.23–2.25

(*m*, CH_2(7′)); 2.34–2.36 (*m*, 1 H–C(7)); 5.35–5.37 (*m*, H–C(14), H–C(15), H–C(14′), H–C(15′)); 6.18 (br. *s*, H–C(5′)); 6.35 (br. *s*, H–C(3′)). ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 1*. ESI-MS (pos.): 713 ($[M+Na]^+$). ESI-MS (neg.): 689 ($[M-H]^-$), 372, 317. HR-TOF-MS (neg.): 689.5090 ($[M-H]^-$, $C_{45}H_{69}O_5^-$; calc. 689.5145).

2-[2,4-Dihydroxy-6-[(8Z)-pentadec-8-en-1-yl]phenyl]-3-[(8Z)-heptadec-8-en-1-yl]-5-methoxycyclohexa-2,5-diene-1,4-dione (= *Belamcandaquinone K*; **2**): Red oil. $[\alpha]_D^{25} = +5.7$ ($c = 0.50$, $CHCl_3$). UV: 282 (4.20). CD (MeOH): 287 ($+2.6 \cdot 10^4$). IR: 3320, 2910, 2840, 1670, 1640, 1595, 1455, 1370, 1220, 1050, 720. ^{1}H-NMR[1]) (500 MHz, $CDCl_3$): 0.91 (*t*, *J* = 7.0, Me(23), Me(21′)); 1.20–1.44 (*m*, 40 H); 1.99–2.01 (*m*, CH_2(13), CH_2(16), CH_2(13′), CH_2(16′)); 2.19–2.21 (*m*, 1 H–C(7)); 2.24–2.26 (*m*, CH_2(7′)); 2.34–2.36 (*m*, H–C(7)); 3.86 (*s*, Me(24)); 5.35–5.37 (*m*, H–C(14), H–C(15), H–C(14′), H–C(15′)); 6.02 (*s*, H–C(6)); 6.20 (br. *s*, H–C(5′)); 6.35 (br. *s*, H–C(3′)). ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 1*. ESI-MS (pos.): 713 ($[M+Na]^+$). ESI-MS (neg.): 689 ($[M-H]^-$), 372, 317. HR-TOF-MS (neg.): 689.5120 ($[M-H]^-$, $C_{45}H_{69}O_5^-$; calc. 689.5145).

2-(2,4-Dihydroxy-6-pentadecylphenyl)-3-[(8Z)-heptadec-8-en-1-yl]-5-methoxycyclohexa-2,5-diene-1,4-dione (= *Belamcandaquinone L*; **3**): Red oil. $[\alpha]_D^{25} = +6.5$ ($c = 0.50$, $CHCl_3$). UV: 280 (4.50). CD (MeOH): 286 ($+2.5 \cdot 10^4$). IR: 3310, 2925, 2854, 1670, 1640, 1600, 1450, 1400, 1190, 1049, 718. ^{1}H-NMR[1]) (500 MHz, $CDCl_3$): 0.91 (*t*, *J* = 7.0, Me(23), Me(21′)); 1.20–1.44 (*m*, 48 H); 1.99–2.01 (*m*, CH_2(13), CH_2(16)); 2.17–2.19 (*m*, 1 H–C(7)); 2.23–2.25 (*m*, CH_2(7′)); 2.34–2.36 (*m*, 1 H–C(7)); 3.86 (*s*, Me(24)); 5.33–5.35 (*m*, H–C(14), H–C(15)); 6.00 (*s*, H–C(6)); 6.18 (br. *s*, H–C(5′)); 6.32 (br. *s*, H–C(3′)). ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 1*. ESI-MS (pos.): 715 ($[M+Na]^+$). ESI-MS (neg.): 691 ($[M-H]^-$), 372, 319. HR-TOF-MS (neg.): 691.5273 ($[M-H]^-$, $C_{45}H_{71}O_5^-$; calc. 691.5301).

2-(2,4-Dihydroxy-6-tridecylphenyl)-3-[(8Z)-heptadec-8-en-1-yl]-5-methoxycyclohexa-2,5-diene-1,4-dione (= *Belamcandaquinone M*; **4**): Red oil. $[\alpha]_D^{25} = +4.9$ ($c = 0.20$, $CHCl_3$). UV: 280 (4.40). CD (MeOH): 286 ($+2.8 \cdot 10^4$). IR: 3330, 2915, 2845, 1670, 1640, 1590, 1440, 1380, 1220, 1060, 726. ^{1}H-NMR[1]) (500 MHz, $CDCl_3$): 0.91 (*t*, *J* = 7.0, Me(23), Me(19′)); 1.20–1.44 (*m*, 44 H); 1.99–2.01 (*m*, CH_2(13), CH_2(16)); 2.19–2.21 (*m*, 1 H–C(7)); 2.23–2.25 (*m*, CH_2(7′)); 2.34–2.36 (*m*, 1 H–C(7)); 3.86 (*s*, Me(24)); 5.35–5.37 (*m*, H–C(14), H–C(15)); 6.02 (*s*, H–C(6)); 6.25 (br. *s*, H–C(5′)); 6.38 (br. *s*, H–C(3′)). ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 1*. ESI-MS (pos.): 687 ($[M+Na]^+$). ESI-MS (neg.): 663 ($[M-H]^-$), 372, 291. HR-TOF-MS (neg.): 663.5036 ($[M-H]^-$, $C_{43}H_{67}O_5^-$; calc. 663.4988).

*5-[(8Z)-Heptadec-8-en-1-yl]-7-hydroxy-8-methyl-2*H*-1-benzopyran-2-one* (**5**): Yellow oil. UV: 220 (3.89), 245 (1.70), 260 (2.82), 342 (4.18). IR: 3450, 2925, 2830, 1720, 1650, 1616, 1590, 1450, 1280, 1070. ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): *Table 2*. ESI-MS (pos.): 435 ($[M+Na]^+$). ESI-MS (neg.): 411 ($[M-H]^-$). HR-TOF-MS (neg.): 411.2925 ($[M-H]^-$, $C_{27}H_{39}O_3^-$; calc. 411.2899).

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 compound treatment, cell viability was determined by measuring the metabolic conversion of MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) into purple formazan crystals by active cells [6]. The MTT-assay results were read with 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 with Microsoft Excel software.

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Received May 6, 2009