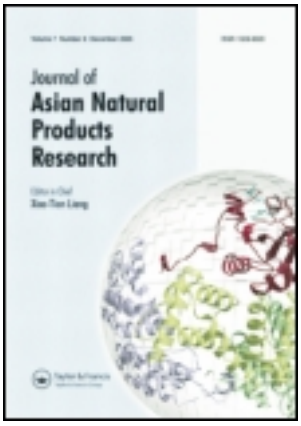

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Synthesis and biological evaluation of bilobol and adipostatin A

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Synthesis and biological evaluation of bilobol and adipostatin A

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This paper is dedicated to Prof. Koji Nakanishi on the occasion of his 85th birthday

Concise total synthesis of bilobol 5-pentadecenylresorcinol (**1**), isolated from *Gingko biloba* fruits, has been achieved in 10 steps with 51% overall yield from 3,5-dihydroxybenzoic acid (**3**). Adipostatin A (**2**), isolated from the fruits as well as from *Streptomyces cyaneus* 2299-SV1, has also been synthesized in two steps from methylated bilobol (**10**). The structure–activity relationship study of synthetic products was described by means of cytotoxic assay against human KB carcinoma cell lines.

Keywords: bilobol; adipostatin A; total synthesis; cytotoxicity; structure–activity relationship

1. Introduction

More than 100 species of 5-alk(en)ylresorcinols have been isolated from higher plant families, algae, mosses, fungi, and several bacterial families as secondary metabolites [1]. The natural products comprise homologous 1,3-dihydroxybenzenes with 5-alk(en)yl side chains from C_5 to C_{29}, structurally related monoenes, dienes, and trienes in *Z* configurations. The side chain on resorcinols in most cases is odd numbered, which is significant with regard to their biosynthetic pathway.

Alk(en)ylresorcinols have been recognized to exert numerous biological qualities such as DNA cleavaging [2,3], tumor promoting activity [4], cytotoxicity toward HaCaT cell lines [5], KB cell lines [6], antibacterial activity [7], inhibitory activity for tyrosinase [8], and inhibition to HIV-1 protease [9]. As a remarkable molecule among the resorcinol family, bilobol 5-pentadecenylresorcinol (**1**, Figure 1), isolated from some plants such as *Gingko biloba* fruits [10,11], is well known. It was found that an increase in the number of double bonds in the side chain of **1** enhanced the biological activities, such as cytotoxicity, antibacterial activity, and inhibitory activity for tyrosinase [6–8]. However, the distinction of alkane, alkene, and alkyne moieties in the side chain for the biological activity of resorcinols has been unknown.

Interest in such attractive biological aspects and in the understanding of the structure–activity relationship of 5-alk(en)ylresorcinols prompted us to conduct a study of efficient synthesis and biological assay. Among the numerous papers of synthetic study of 5-alk(en)ylresorcinols [3,6,12–16], one of the previous synthesis of **1** was reported by Hecht and co-workers [3] with 27% overall yield in

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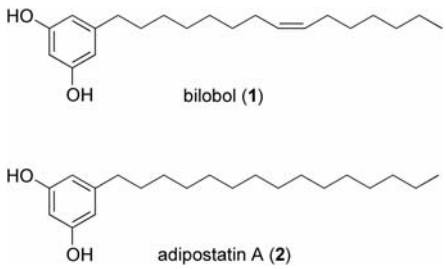


Figure 1. Structures of bilobol (**1**) and adipostatin A (**2**).

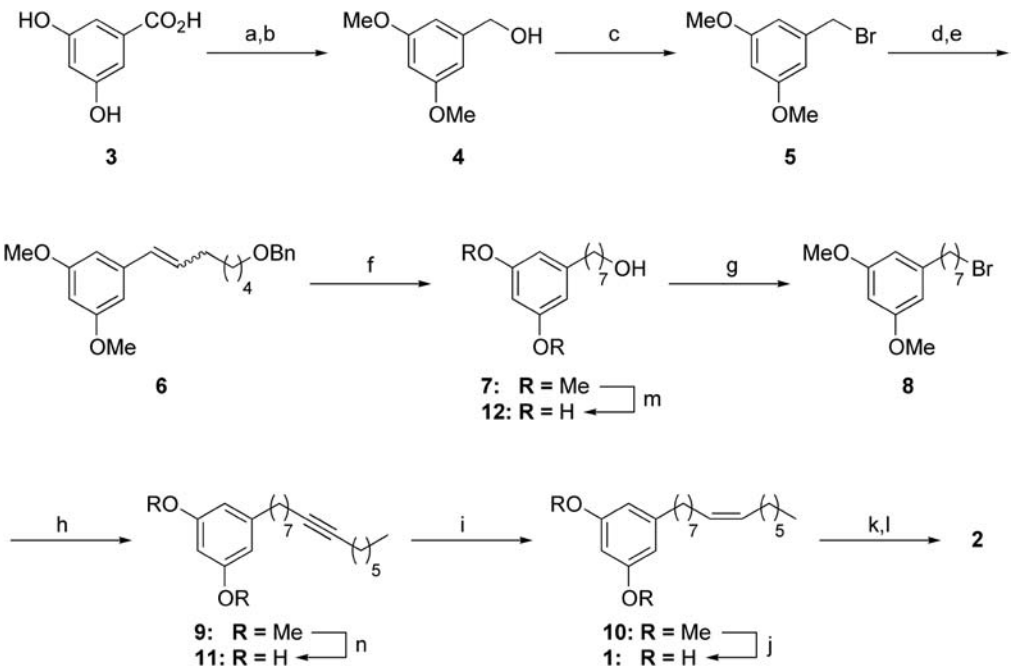
eight steps from 3,5-dimethoxybenzaldehyde. In this paper, synthesis and KB cell cytotoxicity assay of resorcinols are described. Target molecules include unnatural analogs of bilobol **1** as well as 5-pentadecylresorcinol adipostatin A (**2**, Figure 1), which is isolated not only from *Ginkgo* fruits but also from *Streptomyces cyaneus* 2299-SV1 [17].

2. Results and discussion

As shown in Scheme 1, synthesis of **1** and **2** in this research involved Appel reaction, Wittig reaction, alkyne coupling reaction, and Lindlar reduction, beginning with 3,5-dihydroxybenzoic acid (**3**). Treatment of **3** with potassium carbonate followed by dimethyl sulfate under reflux condition for 4 h gave a trimethyl product in 91% yield [18], and reduction of methyl ester with lithium aluminum hydride (LAH) gave alcohol **4** in 92% yield [19]. Alcohol **4** was then converted to bromide **5** with Appel reaction in 98% yield by using triphenylphosphine and carbon tetrabromide. The obtained **5** was transformed into the corresponding phosphonium salt, followed by deprotonation with *n*-BuLi for Wittig reaction. 1.1 equivalent of the resulting benzyl phosphorus ylide was reacted with 6-benzyloxy-1-hexanal, which was led from 1,6-hexanediol [20]. The Wittig reaction gave **6** in 87% yield in two steps. The ratio of *E*/*Z* configuration of olefin **6** was determined to be 3/2 by ^{1}H NMR analysis. Hydrogenation of **6** gave alcohol **7** by H_2 with Pd/C in 97% yield, followed by Appel reaction to give bromide **8** in 98% yield. Coupling with lithium acetylide of 1-octyne gave alkyne **9** in the presence of tetrabutylammonium iodide (TBAI) [21]. Alkyne **9** was quantitatively hydrogenated to give *Z*-selective alkene **10** by Lindlar catalyst with a drop of quinoline. Finally, removal of methyl groups of **10** gave desired bilobol **1** in 93% yield using methylmagnesium iodide at 170°C [22]. Synthetic **1** was identical in all respects (^{1}H, ^{13}C NMR, MS) with authentic data [3]. Thus, total synthesis of bilobol **1** was achieved with overall 51% yield in 10 steps.

Direct hydrogenation of bilobol **1** by Pd/C did not undergo toward natural adipostatin A **2**. Then, hydrogenation of methylated bilobol **10** was quantitatively succeeded, and following deprotection of methyl groups by methylmagnesium iodide without solvents at 170°C in 95% yield gave the desired **2**. Thus, total synthesis of **2** was also achieved as well. Meanwhile, for the structure–activity relationship study of the resorcinols, removal of methyl groups of synthetic intermediates **7** and **9** was carried out under the same conditions, respectively. Synthetic unnatural analogs **12** and **11** with free phenols were thereby obtained (Scheme 1).

Five synthetic samples **1**, **2**, and **10**–**12** were tested for the biological activity by inhibiting proliferation on human carcinoma KB cell lines utilized by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) method (Table 1). Both **10**, which is not a free phenol group, and **12**, which has a shorter alkyl chain with free terminal alcohol, were inactive. Interestingly, compound **11**, which has an alkyne moiety in the side chain, showed cytotoxic activities with IC_{50} value of 14.0 μM, whereas bilobol **1** and adipostatin A **2** exerted IC_{50} values of 14.3 and 10.6 μM, respectively. Other biological tests such as anti-obesity and



Scheme 1. Synthesis of bilobol **1** and adipostatin A **2**. Reagents and conditions: (a) $(MeO)_2SO_2$, K_2CO_3, acetone, reflux, 4 h, 91%; (b) LAH, THF, rt, 2 h, 92%; (c) PPh_3, CBr_4, CH_2Cl_2, −78 to −50°C, 80 min, 98%; (d) PPh_3, benzene, reflux, 5 h; (e) *n*-BuLi, THF, 0°C, then, $BnO(CH_2)_5CHO$, THF, rt, 3 h, 87% (two steps); (f) H_2, 10% (w/w) Pd/C, THF, 50°C, 50 h, 97%; (g) PPh_3, CBr_4, CH_2Cl_2, −78 to −40°C, 2 h, 98%; (h) 1-octyne, *n*-BuLi, THF, −78°C to rt, TBAI, reflux, 30 h, 81%; (i) H_2, $Pd/CaCO_3$ with Pd, quinoline, cyclohexane, 4 h, quant; (j) MeMgI, 170°C, 1 h, 93%; (k) H_2, 10% (w/w) Pd/C, THF, 50°C, 18 h, quant; (l) MeMgI, 170°C, 1 h, 95%; (m) MeMgI, 170°C, 1 h, 89%; (n) MeMgI, 170°C, 1 h, 80%.

Table 1. Cytotoxicity against KB cell lines.

Synthetic products	IC_{50} (μM)
Bilobol **1**	14.3
Adipostatin A **2**	10.6
Alkyne-type bilobol **11**	14.0
Methylated bilobol **10**	>100
5-(7-Hydroxyheptyl)resorcinol **12**	>100

diabetes using peroxisome proliferator-activated receptor's cell-based assays showed no activity (data not shown).

In summary, we have achieved the total synthesis of bilobol **1** with overall 51% yield in 10 steps. Meanwhile natural adipostatin A **2** and the related unnatural 5-resorcinol derivatives were successfully synthesized. Three properties upon the cytotoxicity assay against KB cell lines were found by means of the structure activity–relationship study. First, the compound which has short carbon chain length with terminal hydroxyl groups is not cytotoxic. Second, it is important to have free phenol groups to exert cytotoxicity. Third, alkane, alkene, and alkyne moieties in C_{15} chains at 5-position of resorcinols do not affect IC_{50} values.

3. Experimental

3.1 General experimental procedures

Melting point was measured by an AS one ATM-01 apparatus. Infrared (IR) spectra were measured with a JASCO FT-IR 4100 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a JEOL Lambda 300 spectrometer in deuterium solvents using $CHCl_3$ or MeOH as an internal standard. EI-MS spectra were recorded on a Shimadzu GCMS QP-5050 instrument. ESI-MS spectra were recorded on a JEOL JMS-T100LC instrument or on a Thermo Exactive spectrometer. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), benzene, and diethyl ether (Et_2O) were dried over molecular sieves. Other solvents were used without further purification.

3.2 Synthetic procedures

3.2.1 1,3-Dimethoxy-5-hydroxymethylbenzene (4)

To a solution of 3,5-dihydroxybenzoic acid **3** (3.08 g, 20.0 mmol) and K_2CO_3 (11.1 g, 80.0 mmol) in acetone (30.8 ml), dimethyl sulfate (6.64 ml, 70.0 mmol) was added at room temperature. The reaction mixture was vigorously stirred at reflux for 4 h, and filtered. The filter solid was rinsed with acetone, and most of acetone was removed. The residue was diluted with saturated aqueous $NaHCO_3$ (15 ml), stirred for 5 min, and extracted with EtOAc (3 × 20 ml). The organic layer was washed with aqueous 5% HCl, then with saturated aqueous $NaHCO_3$, and dried over Na_2SO_4. Concentration and flash column chromatography (hexane:EtOAc = 8:1) gave 3,5-dimethoxybenzoic acid methyl ester (3.57 g, 18.2 mmol, 91%): white powder; $C_{10}H_{12}O_4$, mp 42–44°C; IR (KBr): ν_{max} 3017, 2959, 1721, 1599, 1458, 1427, 1301, 1244, 1208, 1158, 1102, 1052, 930, 886, 846, 762, 675, 627, 549, 442 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.19 (2H, d, J = 2.4 Hz, Ar), 6.65 (1H, t, J = 2.4 Hz, Ar), 3.91 (3H, s, COOMe), 3.83 (6H, s, OMe); EI-MS m/z 196 $[M]^+$.

To a solution of LAH (1.03 g, 27.2 mmol) in THF (22 ml), 3,5-dimethoxybenzoic acid methyl ester (3.56 g, 18.2 mmol) in dry THF (50 ml) was slowly added at 0°C for 10 min. After being stirred at room temperature for 2 h, the mixture was cooled to 0°C, and the reaction quenched with H_2O (40 ml). The mixture was acidified to pH 4–5 with aqueous 2 N HCl, and then filtered through Celite. The filtrate was extracted with EtOAc (4 × 50 ml). The organic layer was washed with brine and dried over Na_2SO_4. Concentration and flash column chromatography (hexane:EtOAc = 4:1) gave product **4** (2.80 g, 16.6 mmol, 92%): white powder; $C_9H_{12}O_3$, mp 48–50°C; IR (KBr) ν_{max}: 3398, 3008, 2930, 1600, 1458, 1363, 1295, 1207, 1065, 1011, 914, 864, 828,

702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.53 (2H, d, $J = 2.4$ Hz, Ar), 6.39 (1H, t, $J = 2.4$ Hz, Ar), 4.64 (2H, s, CH_2), 3.80 (6H, s, OMe); EI-MS *m/z* 168 $[M]^+$.

3.2.2 5-Bromomethyl-1,3-dimethoxybenzene (*5*)

To a solution of **4** (1.91 g, 11.3 mmol) in dry CH_2Cl_2 (46 ml), triphenylphosphine (5.95 g, 22.6 mmol) was added at room temperature. After being stirred for 10 min, carbon tetrabromide (7.52 g, 22.6 mmol) was added at $-78°C$, and was stirred at $-50°C$ for 80 min. The reaction mixture was treated with hexane–EtOAc mixture (20 ml) and passed through flash short column chromatography (EtOAc 300 ml). Concentration and flash column chromatography (hexane:EtOAc = 20:1) gave product **5** (2.57 g, 11.1 mmol, 98%): white powder; $C_9H_{11}BrO_3$, mp 73–75°C; IR (KBr): ν_{max} 3060, 3003, 2936, 2837, 1721, 1595, 1477, 1331, 1167, 1069, 941, 853, 822, 697, 646, 608, 526, 475 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.54 (2H, d, $J = 2.4$ Hz, Ar), 6.40–6.39 (1H, t, $J = 2.4$ Hz, Ar), 4.42 (2H, s, CH_2Br), 3.80 (6H, s, OMe); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1, 139.9, 107.1, 100.8, 55.5, 33.8; EI-MS *m/z* 230 $[M]^+$.

3.2.3 1,3-Dimethoxy-5-(7′-benzyloxy-1-heptenyl)benzene (*6*)

A mixture of **5** (1.16 g, 5.0 mmol) and triphenylphosphine (1.31 g, 5.0 mmol) in distilled benzene (10 ml) was stirred under reflux for 5 h, and on concentration gave phosphonium salt as product. A solution of the obtained crude salt in dry THF (25 ml) at 0°C was added dropwise to 2.6 M *n*-BuLi in hexane (1.93 ml, 5.02 mmol). After being stirred for 15 min at 0°C, 6-benzyloxy-1-hexanal (0.93 ml, 4.5 mmol) was added. After stirring for 3 h at room temperature, the reaction was quenched with ice water (15 ml), and extracted with Et_2O (3 × 30 ml). The organic layer was washed with brine and dried over Na_2SO_4. On concentration and flash column chromatography (hexane:EtOAc = 8:1) it gave product **6** (1.42 g, 4.17 mmol, 87%): colorless oil; IR (neat): ν_{max} 2934, 2855, 1592, 1456, 1425, 1360, 1204, 1154, 1065, 966, 833, 737, 698 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34–7.29 (5H, m, Ar), 6.50–6.42 (2H, m, Bn), 6.35 (2H, d, $J = 2.1$ Hz, Ar), 6.33 (1H, t, $J = 2.1$ Hz, Ar), 4.50–4.49 (2H, m, OCH_2Ar), 3.79 (6H, s, OMe), 3.50–3.43 (2H, dd, $J = 6.6$, 6.6 Hz, CH_2OBn), 2.35–2.17 (2H, m, $CHCH_2$), 1.67–1.41 (6H, m, CH_2); HR-ESI-MS: *m/z* 363.1915 $[M + Na]^+$ (calcd for $C_{22}H_{28}O_3Na$, 363.1931).

3.2.4 1,3-Dimethoxy-5-(7′-hydroxyheptyl)benzene (*7*)

A mixture of **6** (216 mg, 0.63 mmol) and 10% Pd/C (51.4 mg) in dry THF (5 ml) was stirred under H_2 for 50 h. The reaction mixture was passed through short flash column chromatography (Et_2O, 200 ml). On concentration and flash column chromatography (hexane:EtOAc = 3:1) it gave product **7** (156 mg, 0.62 mmol, 97%): colorless oil; data, see Reference [3].

3.2.5 5-(7′-Bromoheptyl)-1,3-dimethoxybenzene (*8*), 1,3-dimethoxy-5-(8′-pentadecyn-1-yl)benzene (*9*), and 1,3-dimethoxy-5-[8′(Z)-pentadecen-1-yl]benzene (*10*)

See Reference [3].

3.2.6 1,3-Dihydroxy-5-[8′(Z)-pentadecenyl]benzene (*1*)

To a solution of **10** (79.9 mg, 0.23 mmol) in dry Et_2O (1 ml), 0.94 M of MeMgI (4.9 ml, 0.46 mmol) in Et_2O was added at 0°C. After the mixture was heated to 100°C *in vacuo*, the residue was heated at 170°C for 1 h under N_2. The cooled reaction mixture was quenched with saturated aqueous NH_4Cl (10 ml), and

extracted with EtOAc (4 × 20 ml). The organic layer was washed with brine, and dried over Na_2SO_4. On concentration and flash column chromatography (hexane:EtOAc = 5:1) it gave product **1** (68.0 mg, 0.21 mmol, 93%): yellow oil; IR (neat) ν_{max}: 3356, 2925, 2854, 1599, 1466, 1339, 1154, 995, 837, 694 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.24 (2H, d, $J = 1.8$ Hz, Ar), 6.17 (1H, t, $J = 1.8$ Hz, Ar), 5.38–5.32 (2H, m, C*H*═C*H*), 4.63 (2H, s, OH), 2.48 (2H, t, $J = 7.8$ Hz, ArCH_2), 2.02–2.00 (4H, m, CH_2-CH═CHCH_2), 1.57 (2H, m, ArCH$_2$CH_2), 1.30–1.24 (16H, m, CH_2), 0.88 (3H, t, $J = 6.5$ Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.3, 145.9, 129.7, 129.6, 107.8, 99.8, 35.5, 31.5, 30.8, 29.5, 29.1, 29.0, 28.9, 28.7, 27.0, 26.9, 22.4, 13.9; HR-ESI-MS: *m/z* 363.1915 $[M + Na]^+$ (calcd for $C_{21}H_{34}O_2$, 363.1931), 318.2538 $[M]^+$ (calcd for $C_{21}H_{34}O_2$, 318.2559).

3.2.7 1,3-Dihydroxy-5-pentadecylbenzene

A mixture of 1,3-dimethoxy-5-[8′(*Z*)-pentadecen-1-yl]benzene **10** (0.21 g, 0.61 mmol) and 10% Pd/C (50 mg) in 5 ml of dry THF was stirred under H_2 for 18 h. The reaction mixture was passed through a short column of silica gel and eluted with EtOAc (150 ml). On concentration it gave 1,3-dimethoxy-5-pentadecylbenzene (215 mg, 0.67 mmol) in quant.: white powder; mp 49–51°C; IR (KBr) ν_{max}: 2917, 2849, 1599, 1346, 1293, 1206, 1151, 1056, 842, 821, 696 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.34 (2H, s, Ar), 6.30 (1H, s, Ar), 3.78 (6H, s, OMe), 2.54 (2H, t, $J = 7.8$ Hz, ArCH_2), 1.60–1.55 (2H, m, ArCH$_2$CH_2), 1.25 (24H, m, CH_2), 0.88 (3H, t, $J = 6.0$ Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$), δ 160.9, 145.7, 106.8, 106.7, 97.8, 55.4, 36.6, 32.2, 31.6, 30.0, 29.9, 29.8, 29.7, 29.6, 22.9, 14.4.

To a solution of 1,3-dimethoxy-5-pentadecylbenzene (0.123 g, 0.35 mmol) in dry Et_2O (1.5 ml) and dry THF (0.1 ml), 0.94 M of MeMgI (7.5 ml, 7.04 mmol) in Et_2O was added at 0°C. After the mixture was heated to 100°C *in vacuo*, the residue was then heated at 170°C for 1 h under N_2. The cooled reaction mixture was quenched with saturated aqueous NH_4Cl (10 ml), and extracted with EtOAc (4 × 20 ml). The organic layer was washed with brine and dried over Na_2SO_4. Flash column chromatography (hexane:EtOAc = 4:1) gave product **2** (107 mg, 0.34 mmol, 95%): white powder; mp 85–87°C; IR (KBr): ν_{max} 3323, 2916, 2847, 1607, 1512, 1469, 1331, 1200, 1146, 991, 830, 721, 697, 569 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 6.12 (2H, d, $J = 2.4$ Hz, Ar), 6.07 (1H, t, $J = 2.4$ Hz, Ar), 2.46–2.40 (2H, m, ArCH_2), 1.55 (2H, m, ArCH$_2$CH_2), 1.28 (24H, m, CH_2), 0.91–0.87 (3H, m, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 159.2, 159.1, 146.3, 108.0, 107.9, 101.0, 100.9, 37.1, 37.0, 33.2, 33.1, 32.5, 30.9, 30.9, 30.8, 30.6, 30.6, 30.5, 30.5, 30.4, 23.2, 23.7, 14.7, 14.5; HR-EI-MS: *m/z* 320.2713 $[M]^+$ (calcd for $C_{21}H_{36}O_2$, 320.2715).

3.2.8 1,3-Dihydroxy-5-(7′-hydroxyheptyl)-benzene (*12*)

Yield 89%: white powder; mp: 123–125°C; IR (KBr): ν_{max} 3420, 3256, 2939, 2855, 2536, 2422, 2277, 1595, 1464, 1336, 1161, 1065, 1036, 925, 856, 836, 698, 582, 523 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 6.11 (2H, d, $J = 2.4$ Hz, Ar), 6.07 (1H, t, $J = 2.4$ Hz, Ar), 3.52 (2H, t, $J = 6.6$ Hz, CH_2OH), 2.43 (2H, t, $J = 7.5$ Hz, ArCH_2), 1.60–1.51 (4H, m, CH_2), 1.34 (6H, m, CH_2); ^{13}C NMR (75 MHz, CD_3OD) δ 159.3, 146.3, 107.9, 101.0, 63.0, 37.0, 33.6, 32.4, 30.4, 30.3, 26.9; HR-EI-MS: *m/z* 224.1411 $[M]^+$ (calcd for $C_{13}H_{20}O_3$, 224.1412).

3.2.9 1,3-Dihydroxy-5-(8′-pentadecynyl)-benzene (*11*)

Yield 80%: colorless oil; IR (neat) ν_{max}: 3388, 2929, 2856, 1706, 1602, 1466, 1336, 1155, 998, 838, 695 cm^{-1}; ^1H NMR

(300 MHz, $CDCl_3$) δ 6.24 (2H, d, $J = 2.4$ Hz, Ar), 6.17 (1H, t, $J = 2.4$ Hz, Ar), 4.68 (2H, s, OH), 2.49 (2H, t, $J = 7.5$ Hz, ArCH_2), 2.16–2.11 (4H, m, $CH_2C \equiv CCH_2$), 1.60–1.28 (20H, m, CH_2), 0.89 (3H, t, $J = 6.6$ Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.7, 146.2, 108.2, 100.3, 96.6, 80.5, 80.4, 35.9, 31.5, 31.1, 29.3, 29.3, 29.1, 28.9, 28.7, 22.7, 18.9, 18.9, 14.2; HR-EI-MS: *m/z* 316.2386 $[M]^+$ (calcd for $C_{21}H_{32}O_2$, 316.2402).

3.3 *Cell lines and cytotoxicity test*

The human fibroblast carcinoma KB cells (purchased from ATCC) were grown in Roswell Park Memorial Institute (RPMI) 1640 media (Invitrogen, Carlsbad, CA, USA) containing 5% fetal bovine serum (HyClone Laboratories, Logan, UT, USA), and 0.1 mg/ml kanamycin (Invitrogen) at 37°C in 5% CO_2. All chemicals were dissolved in DMSO (Sigma, St Louis, MO, USA) and were stored at −70°C until use. Cytotoxicity was determined by the MTS assay (Promega, Madison, WI, USA) according to the manufacturer's instruction. Briefly, 2×10^4 cells/well of KB cells in a 96-well plate were treated with 0, 1, 3, 10, 30, and 100 μM compounds and incubated for 48 h. For determining cytotoxicity, MTS/phenazine methosulfate solution was added into each well of the 96-well plate containing cells, and was incubated at 37°C in 5% CO_2. After 2 h, the absorbance was read at 490 nm and their cytotoxicity was calculated by comparing with 0.1% DMSO-treated cells, the cytotoxicity of which being 100%.

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