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Synthesis of New 4-(*tert*-Octyl)phenol Derivatives and Their Anticancer Activity against Human Prostate and Lung Cancer Cell Lines

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4-(*tert*-Octyl)phenol derivatives bearing the D-mannitol substructure (**6a**, **6b**, **7**) were prepared from D-mannitol and evaluated their anticancer activity against human lung (A549) and prostate (Lncap, Du145, PC3) cancer cell lines. Among derivatives tested, the bis(*tert*-octyl)phenoxy compound **7** exhibited strongest proliferation inhibitory activities against human cancer cell lines tested, especially high sensitivity to human Du145 prostate cancer cells (IC_{50} = 7.3 μM).

Key Words : *Cordyceps* militaris, Militarin, 4-(*tert*-Octyl)phenol derivatives, D-Mannitol substructure, Anticancer activity

Introduction

Cordyceps militaris (*C. militaris*), a caterpillar-grown traditional insect-flower (medicinal mushroom), is a fungus that parasitizes Lepidoptera Larvae and is known to possess diverse biological activities in the human body including immune, respiratory, circulatory and glandular systems. *C. militaris* has been used in traditional chinese medicine to treat numerous illnesses, promote longevity, relieve exhaustion and increase athletic prowess. Precedent literatures showed various biological activities such as antitumor and antidiabetic,[1] antiangiogenesis,[2] antimutagenic[3] and hypoglycemic effect.[4] It was also reported that butanol fraction of *C. militaris* produces a number of bioactive constituents and one of the major components was identified as cordycepin which markedly inhibited the phosporylation of Akt and MAP kinase in LPS-activated macrophage, suppressed tumor necrosis factor (TNF-α) expression.[5] Cordycepin was reported to possess various biological properties in precedent literatures.[6-12] Bioactivity-guided further fractionation of butanol fraction of *C. militaris* yielded a novel anticancer substance with polyethylene glycol moiety in its structure named by us as militarin (Figure 1). An efficient synthetic method of militarin was developed by us to prepare multigram scale sample for bioassay in diverse therapeutic areas.[13] Also a number of militarin derivatives bearing different numbers of ethylene glycol unit (Figure 1) were synthesized. It was proved from bioassay that 4-(*tert*-octyl)phenol derivatives with 1 and 4 ethylene glycol unit exhibited strong anticancer and antiinflammatory activities.[14]

Structure-activity relationship study (SARs) of bioactive natural products is a general and powerful procedure, and one of very promising approach to discover new lead compounds as proved in many precedent drug discovery. Recently, several polysaccharide components have been isolated from *C. militaris* and suggested as anticancer therapeutic agents in preliminary *in vitro* and animal studies.[15-17] Militarin derivatives functionalized with a carbohydrate group (D-mannitol) as the congener of the ethylene glycol substructure of militarin were designed (Figure 1) since the introduction of a carbohydrate moiety allowed the generation of glycosides linked through an ethylene ether bridge; this is a very promising approach for the development of novel drugs with improved cellular membrane permeability and stability as therapeutic reagents.

Experimental

General. All chemicals were obtained from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agent under nitrogen gas. All solvents used for

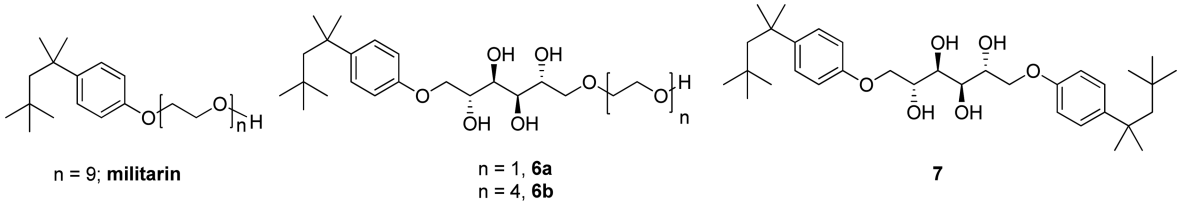


Figure 1. Structures of militarin and new target compounds (**6a**, **6b**, **7**).

chromatography were purchased and directly applied without further purification. ^{1}H-NMR spectra were recorded on a Varian Gemini 3000 instrument (300 MHz) and a Bruker DPX 400 (400 MHz) spectrometers. Chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Peak splitting patterns are abbreviated as m (multiplet), s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), t (triplet), dd (doublet of doublets) and ddd (doublet of double doublet). ^{13}C-NMR spectra were recorded on a Bruker DPX 400 (100 MHz) spectrometer, fully decoupled and chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Melting points were recorded on a Fisher-Johns microscopic scale melting point apparatus. Mass spectra were recorded on a Autospec M363 and MALDI-TOM MS (Voyager DE STR). Analytical thin-layer chromatography (TLC) was performed using commercial glass plate with silica gel $60F_{254}$ purchased from Merck. Chromatographic purification was carried out by flash chromatography using Kieselgel 60 (230-400 mesh, Merck).

(2*R*,3*R*,4*R*,5*R*)-1,2,3,4,5,6-Hexakis(benzyloxy)hexane (1). D-Mannnitol (5.0 g, 26.90 mmol) was added to a solution of powdered KOH (22.64 g, 403.47 mmol) and tetrabutylammonium bromide (TBAB, 0.87 g, 2.69 mmol) in 25 mL DMSO. The mixture was stirred for 1 h at room temperature. The suspension was cooled in an ice bath, and benzyl chloride (BnCl, 25 mL, 215.18 mmol) was immediately added dropwise. The reaction was allowed to come slowly to room temperature by allowing the ice to melt. After stirring for 24 h, the reaction was diluted with 60 mL of cold water and then extracted with ethyl acetate (3 × 100 mL). The combined organic phases were washed with water (100 mL) and sat brine (100 mL), dried ($MgSO_4$) and concentrated to give a cream-colored solid. Purification by column chromatography (hexane) afforded the title product **1** (18.26 g, 94%) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.27-7.31 (m, 30H, 6xPh), 4.41-4.70 (m, 12H, 6x-C<u>H</u>$_2$-Ph), 4.02-4.03 (dd, 2H, H3, H4), 3.85-3.88 (m, 4H, H1a, H6a, H2, H5), 3.68-3.74 (dd, 2H, *J* = 11.1 Hz, 5.8 Hz, H1b, H6b).

(2*R*,3*R*,4*R*,5*R*)-1,6-Dimesyl-2,3,4,5-tetrakis(benzyloxy)-hexane (2). To $ZnCl_2$ (11.36 g, 83.34 mmol) was added 1:5 HOAc–Ac_2O (65 mL). The mixture was cooled down to 0 °C, and then a solution of compound **1** (12.04 g, 16.67 mmol) in 1:5 HOAc–Ac_2O (65 mL) was added dropwise. The reaction mixture was stirred at room temperature for 3 hours, and then 300 mL of ice water was added. The resulting organic layer was washed with water (3 × 200 mL) and brine (100 mL), dried ($MgSO_4$) and filtered to gave 1,6-diacetate compound as a light-yellow oil. K_2CO_3 (11.5 g, 83.35 mmol) was added to a solution of crude l,6-diacetate compound in 80 mL MeOH. The reaction mixture was stirred at room temperature for 3 h, and filtered, concentrated to provided the corresponding alcohol as colorless oil. The reaction mixture of the crude alcohol (1.62 g, 2.99 mmol), methanesulfonyl chloride (0.50 mL, 6.27 mmol) and DMAP (0.0 g, 0.24 mmol) in pyridine (15 mL) was stirred at 0 °C and the mixture was allowed to warm to room temperature. The reaction mixture was quenched with saturated NH_4Cl aqueous solution, extracted with CH_2Cl_2 and 3% HCl. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure to obtain the title product **2** (1.80 g, 86%) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.28-7.35 (m, 20H, 4xPh), 4.56-4.74 (m, 8H, *J* = 11.4 Hz, 4x-C<u>H</u>$_2$-Ph), 4.35-4.43 (m, 4H, H1a, H1b, H6a, H6b), 3.85 (m, 4H, H2, H3, H4, H5), 2.84 (s, 6H, 2xOMs).

(2*R*,3*R*,4*R*,5*R*)-6-Mesyl-1-(4-(*tert*-octyl)phenoxy)-2,3,4,5-tetrakis(benzyloxy)hexane (3). Compound **2** (4.63 g, 6.63 mmol) and potassium iodide (0.11 g, 0.66 mmol) were dissolved in CH_3CN (33 mL), and stirred for 5 min. A mixture of 4-(*tert*-octyl)phenol (1.69 g, 7.96 mmol) and K_2CO_3 (2.75 g, 15.9 mmol) dissolved in acetonitrile (33 mL) was added. The reaction mixture was refluxed for 24 h. The white solid in the mixture was removed by filtration and the solvent was removed *in vacuo* to give yellow oil. Purification of crude product by chromatography (silica, 10:1 to 5:1 hexane-acetone) provided the title product **3** (2.3 g, 39%) and **4** (0.99 g, 15%) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.23-7.36 (m, 22H, Ph), 6.81-6.83 (d, 2H, *J* = 7.7 Hz, Ph), 4.57-4.76 (m, 8H, 4x-C<u>H</u>$_2$-Ph), 4.35-4.51 (m, 4H, H1a, H1b, H6a, H6$_b$), 4.12-4.15 (br, 1H, H5), 4.00 (br, 2H, H3, H4), 3.89 (br, 1H, H2), 2.80 (s, 3H, OMs), 1.71 (s, 2H, -CH_2-), 1.35 (s, 6H, 2xMe), 0.72 (s, 9H, 3xMe).

(2*R*,3*R*,4*R*,5*R*)-1,6-Bis(4-(*tert*-octyl)phenoxy)-2,3,4,5-tetrakis(benzyloxy) hexane (4). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.22-7.31 (m, 24H, Ph), 6.81-6.84 (d, 4H, *J* = 8.8 Hz, Ph), 4.60-4.76 (m, 6H, *J* = 11.7 Hz, 11.2 Hz, 3x-C<u>H</u>$_2$-Ph) 4.89-4.53 (d, 2H, *J* = 11.7 Hz, -C<u>H</u>$_2$-Ph), 4.37-4.42 (m, 2H, H1a, H6a), 4.12-4.18 (m, 4H, *J* = 10.6 Hz, 5.2 Hz, H1b H6b, H2, H5), 4.05 (br, 2H, H3, H4), 1.71 (s, 4H, 2x-CH_2-), 1.35 (s, 12H, 4xMe), 0.72 (s, 18H, 6xMe).

(2*R*,3*R*,4*R*,5*R*)-2,3,4,5-Tetrakis(benzyloxy)-6-(2-hydroxy-ethoxy)-1-(4-(*tert*-octyl)phenoxy)hexane (5a). A solution of the ethylene glycol (0.19 mL, 3.41 mmol) in anhydrous THF (9 mL) was treated with NaH (0.50 g; 11.37 mmol, 55% in mineral oil) and stirred for 1 h at room temperature. The reaction mixture was cooled to 0 °C, and the compound **3** (2.30 g; 2.84 mmol) in dry THF (9 mL) was added dropwise over 30 min at 0-5 °C. After completed addition, the mixture was stirred at room temperature for 10 h and filtered. The filtrate was washed with THF and concentrated by using rotary evaporation, redissolved in chloroform (100 mL), washed with brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a pale yellow oil, which was subjected to flash chromatography on silica with up to 5% gradient elution of methanol in dichloromethane to give **5a** (0.70 g; 32%) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.13-7.33 (m, 22H, Ph), 6.70-6.73 (d, 2H, *J* = 8.8 Hz, Ph), 4.62-4.73 (m, 6H, 3x-C<u>H</u>$_2$-Ph), 4.44-4.50 (m, 2H, *J* = 11.7 Hz, -C<u>H</u>$_2$-Ph), 4.25-4.37 (m, 2H, H1a, H1b), 3.77-4.01 (m, 5H, H6a, H2, H3, H4, H5), 3.66-3.76 (m, 3H, H6b, C<u>H</u>$_2$), 3.54-3.61 (m, 2H, C<u>H</u>$_2$), 1.71 (s, 2H, -CH_2-octyl), 1.34 (s, 6H, 2xMe), 0.70 (s, 9H, 3xMe).

(3*R*,4*R*,5*R*,6*R*)-4,5,6-Tris(benzyloxy)-1-phenyl-3-((4-(2,4,4-trimethylpentan-2-yl)phenoxy)methyl)-2,8,11,14,17-pentaoxanonadecan-19-ol (5b). A solution of the tetraethylene glycol (0.13 mL, 0.65 mmol) in anhydrous THF (3 mL) was treated with NaH (0.09 g; 1.95 mmol, 55% in mineral oil) at room temperature, stirred for 1 h, and cooled to 0 °C, and the compound **3** (0.53 g; 0.65 mmol) in dry THF (4 mL) was added dropwise over 30 min at 0-5 °C. After complete addition, the mixture was stirred at room temperature for 1 day and filtered. The filtrate was washed with THF and concentrated by using rotary evaporation, redissolved in chloroform (100 mL), washed with brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a pale yellow oil, which was subjected to flash chromatography on silica with up to 5% gradient elution of methanol in dichloromethane to give **5b** (0.14 g; 23%) as colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.23-7.33 (m, 22H, Ph), 6.78-6.81 (d, 2H, *J* = 8.8 Hz, Ph), 4.59-4.75 (m, 6H, 3x-C$\underline{H}_2$-Ph), 4.46-4.53 (m, 2H, *J* = 11.7 Hz, -C$\underline{H}_2$-Ph), 4.33-4.37 (m, 1H, *J* = 10.4 Hz, 2.6 Hz, H18a), 4.08-4.16 (m, 2H, *J* = 10.4 Hz, 5.5 Hz, 4.9 Hz, 4.0Hz, H18b, H17), 3.87-4.03 (m, 4H, H13a, H14, H15, H16, H17), 3.66-3.76 (m, 3H, H13b, -OC$\underline{H}_2$-CH_2OH), 3.54-3.61 (m, 14H, 3x-OC$\underline{H}_2$-C$\underline{H}_2$O-, C$\underline{H}_2$OH), 1.71 (s, 2H, -CH_2-octyl), 1.34 (s, 6H, 2xMe), 0.72 (s, 9H, 3xMe).

(2*R*,3*R*,4*R*,5*R*)-1-(2-Hydroxyethoxy)-6-(4-(2,4,4-trimethylpentan-2-yl)phenoxy)hexane-2,3,4,5-tetraol (6a). To a solution containing compound **5a** (0.21 g, 0.27 mmol) and HCOOH (0.3 mL) in dry MeOH (3 mL) under an argon atmosphere, Pd 10 wt % on activated carbon (0.5 g) was added and the mixture was stirred for overnight at reflux. After removal of the catalyst by filtration, the organic layer was evaporated. Purification by flash chromatography (100:1-10:1 $CHCl_3$:MeOH) to get product **6a** (0.05 g, 47%) as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.23-7.25 (d, 2H, *J* = 8.8 Hz, Ph), 6.80-6.82 (d, 2H, *J* = 8.8 Hz, Ph), 4.08-4.16 (m, 2H, *J* = 10.1 Hz, 5.7 Hz, 4.3 Hz, H6a, H6b), 3.89-3.93 (m, 1H, H5), 3.61-3.85 (m, 9H, H1a H1b, H2, H3, H4, -OC$\underline{H}_2$-C$\underline{H}_2$OH), 1.69 (s, 2H, -CH_2-octyl), 1.31 (s, 6H, 2xMe), 0.70 (s, 9H, 3xMe). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.0 (C-1-Ph), 142.8 (C-4-Ph), 127.1 (C-3-Ph, C-5-Ph), 113.7 (C-2-Ph, C-6-Ph), 72.6 (C-3, C-4), 72.2 (-C$\underline{H}_2$-CH_2OH), 71.3 (C-2, C-5), 67.6 (C-1), 64.2 (C-6), 61.4 (CH_2OH), 56.9 (-C$\underline{H}_2$-octyl), 38.0 (C-Me$_2$), 32.3 (C-Me$_3$), 31.8 (Me$_3$), 31.7 (Me$_2$). Calc'd exact mass for $C_{22}H_{35}Na_3O_7$: 480.2076. HRMS (MALDI-TOF): *m/z* $[M + 3Na]^+$ 480.0655.

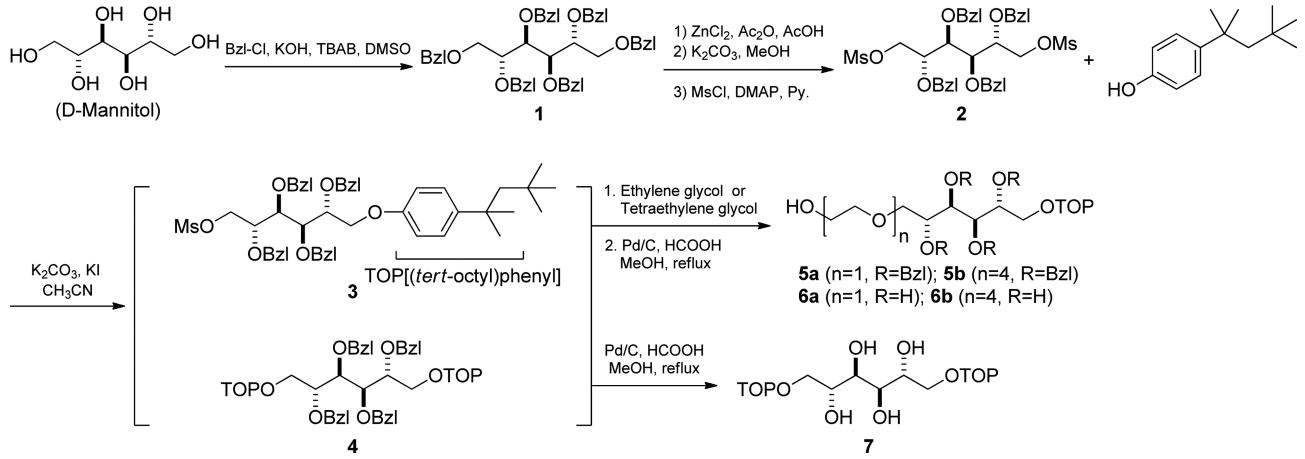
(14*R*,15*R*,16*R*,17*R*)-18-(4-(2,4,4-Trimethylpentan-2-yl)-phenoxy)-3,6,9,12-tetraoxaoctadecane-1,14,15,16,17-pentaol (6b). Following the procedure applied for synthesis of **6a**, but replacing compound **5b** (0.14 g, 0.15 mmol) for compound **5a**, the title product **6b** was obtained as colorless oil. Yield 55%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.25-7.27 (d, 2H, *J* = 8.8 Hz, Ph), 6.85-6.87 (d, 2H, *J* = 8.8 Hz, Ph), 4.25-4.28 (m, 1H, H18a), 4.07-4.14 (m, 2H, H18b, H17), 3.89-3.98 (m, 3H, H14, H13a H13b), 3.57~3.79 (m, 18H, H15, H16, 4x-OC$\underline{H}_2$-C$\underline{H}_2$O-), 1.90 (s, 1H, OH), 1.70 (s, 2H, -CH_2-), 1.33 (s, 6H, 2xMe), 0.71 (s, 9H, 3xMe). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.3 (C-1-Ph), 142.6 (C-4-Ph), 127.1 (C-3-Ph, C-5-Ph), 113.8 (C-2-Ph, C-6-Ph), 72.9 (C-15), 72.7 (C-16), 71.0 (C-11), 70.6 (C-14), 70.4 (C-17), 70.3 (C-4, C-5, C-7, C-8, C-10, C-11), 70.2 (C-2) 69.9 (C-13), 69.8 (C-18), 61.3 (CH_2OH), 57.0 (-C$\underline{H}_2$-octyl), 38.0 (C-Me$_2$), 32.3 (C-Me$_3$), 31.8 (Me$_3$), 31.7 (Me$_2$). Calc'd exact mass for $C_{28}H_{49}NaO_{10}$: 568.3223. HRMS (MALDI-TOF): *m/z* $[M + Na]^+$ 568.3385.

(2*R*,3*R*,4*R*,5*R*)-1,6-Bis(4-(2,4,4-trimethylpentan-2-yl)-phenoxy)hexane-2,3,4,5-tetraol (7). Following the procedure applied for synthesis of **6a**, the title product **7** was obtained as colorless oil. Yield 50%; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.27-7.29 (d, 4H, *J* = 8.8 Hz, Ph), 6.84-6.86 (d, 4H, *J* = 8.8 Hz, Ph), 4.23-4.26 (dd, 2H, *J* = 8.8 Hz, 3.1 Hz, H1a, H6a), 4.11-4.19 (m, 4H, *J* = 8.8 Hz, 6.2 Hz, H1b, H6b, H2, H5), 4.04-4.07 (t, 2H, *J* = 5.8 Hz, H3, H4), 3.01-3.02 (d, 2H, 2xOH), 2.83 (s, 2H, 2xOH), 1.71 (s, 4H, 2x-CH_2-), 1.34 (s, 12H, 4xMe), 0.71 (s, 18H, 6xMe). ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.9 (C-1-Ph), 143.1 (C-4-Ph), 127.2 (C3-Ph, C-5-Ph), 113.8 (C-2-Ph, C-6-Ph), 71.3 (C-3, C-4), 70.6 (C-2, C-5), 69.2 (C-1, C-6), 56.9 (-C$\underline{H}_2$-octyl), 38.00 (C-Me$_2$), 32.3 (C-Me$_3$), 31.8 (Me$_3$), 31.7 (Me$_2$). Calc'd exact mass for $C_{33}H_{51}NaO_6$: 566.3583. HRMS (MALDI-TOF): *m/z* $[M + Na]^+$ 566.3637.

Evaluation of Anticancer Activity against Human Lung (A549) and Prostate (Lncap, Du145, PC3) Cancer Cell Lines: Militarin derivatives (**6a**, **6b**, **7**) were dissolved in DMSO and diluted with media. The solutions were added to cancer cell lines at the concentration of 0-200 ug/mL each. For the growth inhibition assay, non adherent (10,000 cells) or adherent (10,000 cells) cancer cell were cultured in the 96-well culture plate in the presence or absence of the extracts or fractions of *C. militaris*. Cell growth inhibition was observed by MTT colorimetric method. Briefly, 20 uL of 5 ug/mL MTT was added to each 96 well. After 30 minutes incubation, 100 uL of the culture medium was replaced with 100 uL of isopropanol for the extraction of dye. All crystals were dissolved by repeated pipeting of the medium and measured by ELISA reader (570 nm). The anticancer activities of compounds **6a**, **6b** and **7** were determined using the MTT assay after the incubation of A549 cells in the presence of the militarin derivatives (0, 10, 30, 50 and 100 μM) for 24 h. Also anticancer activities of compound **7** against three human prostate cancer cell lines (DU145, LNCap and PC3) and a human lung adenocarcinoma (A549) were evaluated. The cells were treated with compound **7** (0, 2.5, 5, 7.5 and 10 μM) for 24 h and determined using the MTT assay.

Results and Discussion

Chemistry. Militarin derivatives bearing the D-mannitol substructure (**6a**, **6b**, **7**) were prepared following the synthetic pathway as shown in Scheme 1. The target compound **6a** and **6b** were generated from commercially available D-mannitol, 4-(*tert*-octyl)phenol, ethylene glycol and tetraethylene glycol. The compound **7** was prepared from the compound **4**, which was obtained as a by-product from coupling reaction between the dimesylate intermediate (**2**)



Scheme 1. Synthesis of 4-(*tert*-octyl)phenol derivatives with D-mannitol substructure.

and 4-(*tert*-octyl)phenol. D-Mannitol was converted to the hexabenzylated compound **1** by reaction with BnCl, KOH and tetra-butylammonium bromide (TBAB) in DMSO at room temperature.[18] Selective debenzylation–acetylation of compound **1** using $ZnCl_2$–Ac_2O–HOAc, then deacetylation with K_2CO_3 in MeOH at room temperature gave alcohol intermediate,[18] which was converted to dimesylate compound **2** by reaction with MsCl and DMAP in pyridine at room temperature.[12] Compound **2** was converted to mono- and di-substituted phenyl ether compound **3** and **4** by reaction with 4-(*tert*-octyl)phenol, K_2CO_3 and KI in CH_3CN at reflux.[13] Compound **3** was converted to the compound **5a** (or **5b**) by reaction with ethylene glycol (or tetraethylene glycol) and NaH in dry THF at room temperature.[19] Hydrogenolysis of compounds **5a**, **5b** and **4** gave carbohydrate bearing militarin derivatives **6a**, **6b** and **7**, respectively.

Biological Evaluation. The anticancer activities of compounds **6a**, **6b** and **7** were determined using the MTT assay[14] after the incubation of A549 cells in the presence of the militarin derivatives (0, 10, 30, 50 and 100 μM) for 24 h. The treatment of compound **6a** or **6b** resulted in a slight decrease in cell viability at 100 μM, whereas treatment of compound **7** significantly suppressed A549 cell viability (36.9%) at 10 μM (Figure 2(a)) without cytotoxicity (data not shown). Thus, the compound **7**, which has additional 4-(*tert*-octyl)phenol in its structure, showed better anticancer activity than that of compound **6a** or **6b** (Figure 2(a)). We next examined whether compound **7** affects proliferation of three human prostate cancer cell lines (DU145, LNCap and PC3) and a human lung adenocarcinoma (A549). The cells were treated with compound **7** (0, 2.5, 5, 7.5 and 10 μM) for 24 h and determined using the MTT assay. As shown in Figure 2(b), the compound **7** treated in A549, DU145, LNCap and PC3 decreased cell viability in a concentration-dependent manner after 24 h incubation. From the more precise comparison from MTT analysis from Figure 2(b), compound **7** showed an anticancer effect towards A549, Du145, LNCap and PC3 cells with an IC_{50} value of 8.5, 7.3, 12.1 and 9.3 μM, respectively. Human Du145 prostate cancer cells showed the highest sensitivity to the anti-cancer

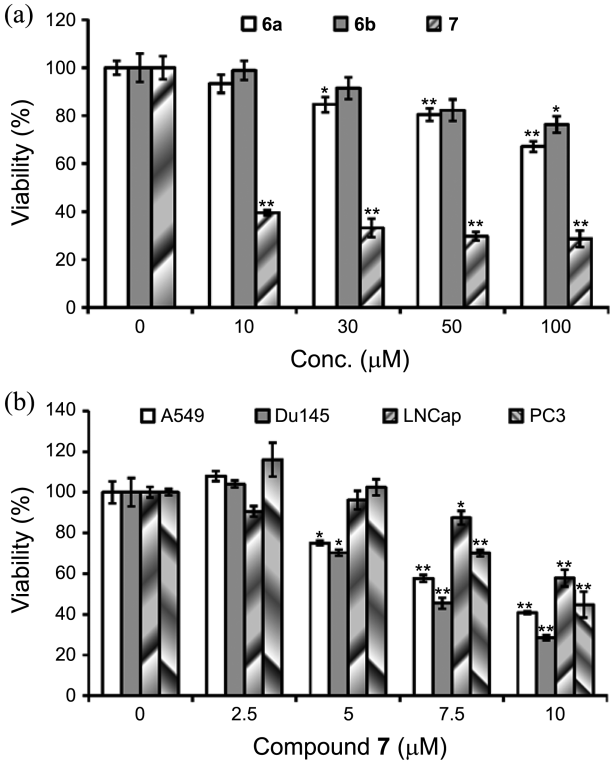


Figure 2. Cell viability as determined by the MTT assay. (a) A549 cells were treated for 24 h with three militarin derivatives (Compound **6a**, **6b** and **7**). (b) A549, Du145, LNCap and PC3 cells were treated for 24 h with compound **7** at the indicated doses. Data are presented as the mean ± SEM of three independent experiments. *; $p < 0.05$ *vs.* control, **; $p < 0.01$ *vs*. control.

effect on compound **7** among the 4 cancer cell lines tested as shown in Figure 2(b). Our previous results also indicated similar trends that the 4-(*tert*-octyl)phenol derivatives bearing different numbers of ethylene glycol substructure exhibited generally reduced anticancer activities against HeLa, B16F19 and HT-29 cancer cell lines than those of the parent compound (militarin).[14] Based on the previous and present results, we found that increased number (length) of ethylene glycol unit generally resulted in decreased anticancer activity.

Conclusion

In summary, three militarin derivatives bearing a carbohydrate (D-mannitol) as the congener of the polyethylene glycol substructure of militarin were successfully synthesized to decipher the effects of structural alterations on the anticancer activity. Anticancer activities of the synthesized derivatives (**6a**, **6b** and **7**) were evaluated against human lung (A549) and prostate (Lncap, Du145, PC3) cancer cell lines. Among tested derivatives, the compound with bis-4-*tert*-octylphenoxy group (**7**) exhibited strong proliferation inhibitory activities against tested human cancer cell lines, especially high sensitivity to human Du145 prostate cancer cells (IC_{50} = 7.3 μM). The proliferation inhibitory activity of compound **7** against Du145 prostate cancer cells was slightly potent than that of militarin (IC_{50} = 9.4 μM). On the other hand, derivatives bearing a carbohydrate (D-mannitol) and ethylene glycol substructures (**6a** and **6b**) exhibited less potent anticancer activities than those of compound **7**. Our present results may imply that the polyethylene glycol substructure is not essential for the anticancer activity and could be replaced by other congeners. Further SARs results related with militarin derivatives bearing diverse carbohydrates will be reported in due course.

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