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Synthesis and Evaluation of Antitumor Activity of Novel 1,4-Naphthoquinone Derivatives (IV)

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1,4-Naphthoquinones are widely distributed in nature and many clinically important antitumor drugs containing a quinone moiety, such as anthracyclines, mitoxantrones and saintopin, show excellent anticancer activity. In this study, 2- or 6-substituted 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) and 5,8-dihydroxy-1,4-naphthoquinone (DHNQ) derivatives were synthesized, and their cytotoxic activity against L1210 and P388 cancer cells was examined. Their antitumor activity was also assessed in mice bearing S-180 cells in the peritoneal cavity. In comparison with the DMNQ derivatives, the DHNQ derivatives exhibited more potent bioactivities than the DMNQ derivatives against both L1210 and P388 cells *in vitro* and S-180 cells *in vivo*. The ED_{50} values of the DHNQ derivatives against P388 cells were in the range of 0.18-1.81 μg/mL whereas those of the DMNQ derivatives were in the range of 0.26-40.41 μg/mL. The T/C (%) values of the DHNQ derivatives, **8**, **17**, **18**, **19**, and **20**, were found to be comparable to or even better than that of adriamycin. It was also observed that the 2-substituted derivatives (**8**, **19**, **20**) showed better antitumor activity than the 6-substituted derivatives (**7**, **17**, **18**) in the mice bearing S-180 cells in the peritoneal cavity.

Key words: Naphthoquinone, Cytotoxicity, Antitumor activity

INTRODUCTION

1,4-Naphthoquinones are widely distributed in nature and there are many clinically important antitumor drugs containing a quinone nucleus, such as anthracyclines, mitoxantrones and saintopin, that show excellent anticancer activity. These anticancer agents are effective inhibitors of DNA topoisomerase, and it is generally accepted that the cytotoxicity of quinone analogues results from the inhibition of DNA topoisomerase-II (Foye, 1995; Leopold, *et al*., 1984; Scheithauer *et al*., 1986). Quinone analogues can also induce the formation of semiquinone radicals, which can transfer an electron to oxygen to produce superoxide. This process is catalyzed by flavoenzymes such as NADPH-cytochrome-P-450 reductase. Both the superoxide and semiquinone radical anions of naphthoquinone analogues can generate the hydroxyl radical, which is known to cause DNA strand breaks (Lown *et al*., 1977; Tewey *et al*., 1984; Hertzberg *et al*., 1984; Silverman *et al*., 1992). In addition, a number of 1,4-naphthoquinone derivatives have been found to possess powerful pharmacological effects and are also associated with marked antimicrobial and antitumor activities (Aviado *et al*., 1969; Skelton *et al*., 1971; Kelker *et al*., 1986). Previously, it was found that 2- or 6-(1-hydroxyalkyl)-5,8-dihydroxy-1,4-naphthoquinone derivatives exhibited good antitumor activity (Baik *et al*., 1997). In view of these facts, we previously synthesized various 1,4-naphthoquinone derivatives and reported their cytotoxicity and antitumor activity (Cho *et al*., 1998; Chung *et al*., 2004). Of particular interest was the discovery that the 2-substituted compound, 2-[(4-methyl-1,3-benzothiazol-2-yl)aminomethy]-5,8-dimethoxy-1,4-naphthoquinone, exhibited better antitumor activity than the 6-substituted DMNQ derivatives (Chung

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et al., 2004). It has also been reported that compounds having higher ^{1}H-NMR chemical shifts of 3-H (δ_H) usually have a lower ED_{50} value (Chung *et al.*, 2004).

In this study, 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) derivatives and 5,8-dihydroxy-1,4-naphthoquinone (DHNQ) derivatives were synthesized, and their cytotoxicity against L1210 and P388 cancer cells was examined. Their antitumor activity was also assessed in mice bearing S-180 cells in the peritoneal cavity.

MATERIALS AND METHODS

Materials

All chemical reagents were obtained from Aldrich Chemical Company. Solvents were of reagent grade and used without further purification. Melting points were determined on an Electrothermal capillary melting point apparatus and were uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 200 (200 MHz) and Bruker DPX-300 (300 MHz) spectrometer using tetramethylsilane as the internal standard. Infrared (IR) spectra were obtained on a Shimadzu IR-470 spectrometer using the KBr pellet method. Male ICR mice were purchased from Daehan Laboratory Animal Co. (Korea) and used when they weighed from 20 to 23 g. The mice were acclimated for at least four days to the animal facilities, which were maintained at 23 ± 1 with a 12 h light/dark cycle. Feed and water were freely accessible to the mice. L1210, P388, HL-60, A549, SNU-1 and normal Vero cells were a gift from Dr. B. Z. Ahn, College of Pharmacy, Chungnam National University (Korea).

In vitro cytotoxicity (MTT assay)

The target cancer cells were suspended at 2×10^5 cells/mL in medium (10% fetal bovine serum) containing various concentrations of the synthesized naphthoquinone derivatives, vigorously vortexed and then 100 μL aliquots were dispensed into 96-well flat-bottomed microtiter plates using a multichannel pipette. The plates were then incubated at 37°C for 72 h in a 5% CO_2 incubator. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] was dissolved in PBS at 5 mg/mL and filtered to sterilize and remove the small amount of insoluble residue present in some batches of the MTT. An aliquot of 10 μL of MTT stock solution was added to each well using a multichannel pipette and the plate was incubated at 37°C for 4 h. To each well, 150 μL of 0.01N HCl solution containing 10% sodium dodecyl sulfate were added to solubilize the MTT formazan. The plates were gently shaken until all of the formazan crystals were dissolved, and the absorbance at 540 nm was determined with a Microplate Reader (SPECTRA MAX 340). All of the results were corrected for background absorbance detected in wells without added MTT. The preliminary experiments showed a linear relationship between the cell numbers and the absorbance at 540 nm, when cell numbers in the range of 4×10^2 to 4×10^5 per well were examined.

In vivo antitumor activity in ICR mice bearing S-180 cells

The test samples dissolved in saline including 2% DMSO and 4% Tween 80 were stored at 4°C. S-180 cells (0.1 mL per mouse) suspended in saline (1×10^7 cells/mL) were inoculated intraperitoneally into male ICR mice. 24 h after the transplantation, the mice were divided into groups containing 8 mice each. The test compounds were administered into the intraperitoneal cavity of the mice daily for 5 days. The rate of growth inhibition (T/C, %) was calculated by the following equation;

$$T/C(\%) = \frac{\text{Average survival period in the test group}}{\text{Average survival period in the control group}} \times 100$$

General procedure for the synthesis of compounds 2-12

Compound 2

To a 100 mL round bottom flask fitted with a Dean-Stark trap and a condenser were added benzene (20 mL), 2-formyl-1,4,5,8-tetramethoxynaphthalene (5 g, 18.13 mmol), 4-nitro-3-(trifluoromethyl)aniline (3.75 g, 18.1 mmol), triethylamine (2.51 mL, 18.1 mmol), and acetic acid (300 μL, 14 mmol), the mixture was refluxed for 20 h and then the water was removed by azeotropic distillation. After cooling to room temperature, the reaction mixture was washed successively with 5% HCl, saturated $NaHCO_3$, 5% acetic acid and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl acetate and ether to afford 6.27 g (74.6%) of compound **2** as an orange solid. Yield 74.6%; m.p. 181-182°C; IR (KBr, cm^{-1}) 2940, 2840, 1605, 1580, 1526, 1377, 1355, 1265, 1142, 1070; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.98 (1H, s), 8.03 (1H, d, *J*=5.39 Hz), 7.64 (1H, d, *J*=2.11 Hz), 7.53 (1H, s), 7.45 (1H, dd, *J*=8.58 and 2.11 Hz), 7.03 (1H, d, *J*=8.73 Hz), 6.94 (1H, d, *J*=8.73 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.93 (3H, s), 3.87 (3H, s).

Compound 3

A solution of compound **2** (5 g, 10.8 mmol) in 150 mL of ethanol was stirred with 5% Pd/C (500 mg) under hydrogen (5 atm) at room temperature overnight. The reaction mixture was filtered and the solvent was evaporated off. The separation of compound **3** by column chromatography on silica gel (ethyl acetate/hexane) afforded compound **3** (4.7 g, 79.2%) as a white solid.

Yield 79.2%; m.p. 128-129°C; IR (KBr, cm^{-1}) 3390, 2950, 1600, 1510, 1360, 1260, 1065; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.94 (1H, s), 6.84 (1H, dd, *J*=6.81 and 8.65 Hz), 6.70-6.62 (3H, m), 5.70 (1H, t, *J*=6.14 Hz), 4.60 (2H, d, *J*=6.81 Hz), 4.29 (2H, d, *J*=6.14 Hz), 3.80 (3H, s), 3.71 (3H, s), 3.68 (3H, s), 3.64 (3H, s).

Compound 4

To a 250 mL round bottom flask fitted with a condenser were added toluene (100 mL), compound **3** (3 g, 6.9 mmol) and succinic anhydride (1.42 g, 13.8 mmol) and the mixture was refluxed for 2 h. The reaction mixture was extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from acetone to afford 3.28 g (88.9%) of compound **4** as a white solid. Yield 88.9%; m.p. 138-139°C; IR (KBr, cm^{-1}) 3380, 2930, 1730, 1600, 1510, 1360, 1255, 1070; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.10 (1H, s), 7.26 (1H, s), 7.08 (1H, d, *J*=4.31 Hz), 6.88 (2H, dd, *J*=10.38 and 8.73 Hz), 6.80 (1H,s), 6.74 (1H, d, *J*=8.73 Hz), 5.76 (1H, t, *J*=3.04 Hz), 4.96 (2H, s), 3.78 (3H, s), 3.77 (3H, s), 3.76 (3H, s), 3.34 (4H, m), 2.47 (2H, t, *J*=6.56 Hz), 2.27 (2H, t, *J*=6.56 Hz).

Compound 5

To a stirred solution of compound **4** (1 g, 1.9 mmol) in acetone (20 mL) at room temperature were added H_2SO_4 (156.8 μL, 2.8 mmol), 4 mL of water and CrO_3 (194.3 mg, 1.9 mmol). The mixture was then stirred at room temperature for 1 h. The separation of compound **5** by column chromatography on silica gel (ethyl acetate/hexane) afforded compound **5** (654.2 mg, 69.4%) as a yellow solid. Yield 69.4%; m.p. 196-197°C; IR (KBr, cm^{-1}) 3350, 1735, 1655, 1510, 1400, 1250, 1050; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.16 (1H, s), 7.37 (1H, s), 7.35 (1H, d, *J*=2.28 Hz), 7.23 (1H, dd, *J*=8.68 and 2.28 Hz), 6.83 (1H, s), 6.80 (2H, s), 5.82 (1H, t, *J*=2.07 Hz), 4.92 (2H, s), 3.88 (3H, s), 3.56 (3H, s), 3.36 (1H, s), 2.49 (2H, t, *J*=5.55 Hz), 2.31 (2H, t, *J*=5.55 Hz).

Compound 6

To a stirred solution of compound **4** (1 g, 1.9 mmol) in acetonitrile (15 mL) at room temperature were added a solution of ammonium cerium (IV) nitrate (2.96 g, 4.7 mmol) in 3.5 mL of water. The mixture was then stirred at room temperature for 1 h. The separation of compound **6** by column chromatography on silica gel (ethyl acetate/hexane) afforded compound **6** (645.7 mg, 68.4%) as a yellow solid. Yield 68.4%; m.p. 190°C; IR (KBr, cm^{-1}) 3375, 1720, 1650, 1505, 1435, 1210, 1055; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.13 (1H, s), 7.53 (2H, s), 7.35 (1H, d, *J*=2.02 Hz), 7.29 (1H, d, *J*=4.34 Hz), 6.83 (1H, d, *J*=4.34 Hz), 6.66 (1H, s), 5.82 (1H, d, *J*=2.02 Hz), 4.57 (2H, s), 3.84 (3H, s), 3.83 (3H, s), 3.60 (1H, t, *J*=6.09 Hz), 2.43 (2H, t, *J*=6.09 Hz), 2.29 (2H, t, *J*=5.93 Hz).

Compound 7

To a stirred solution of compound **6** (600 mg, 1.2 mmol) in 40 mL of dry methylene chloride at 0-5°C was slowly added $AlCl_3$ (1.61 g, 11.9 mmol). The mixture was then stirred for 2 h. To a stirred cold solution of 10% HCl (500 mL) were slowly added the reaction mixture and ethyl ether (500 mL). The mixture was then stirred overnight. The reaction mixture was extracted with ethyl ether and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl ether to afford 321.3 mg (56.7%) of compound **7** as a red brown solid. Yield 56.7%; m.p. 97-99°C; IR (KBr, cm^{-1}) 3390, 1730, 1610, 1510, 1450, 1200, 1105; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.44 (1H, s), 12.40 (1H, s), 12.14 (1H, s), 7.36 (1H, d, *J*=1.18 Hz), 7.35-7.18 (4H, m), 7.10 (1H, s), 6.78 (1H, d, *J*=4.30 Hz), 4.87 (2H, s), 4.33 (1H, s), 2.76 (2H, t, *J*=7.70 Hz), 2.48 (2H, t, *J*=6.65 Hz).

Compound 8

Compound **8** was prepared using the same procedure as that described for compound **7**. Yield 58.2%; m.p. 79-80°C; IR (KBr, cm^{-1}) 3400, 2940, 1720, 1605, 1510, 1450, 1110; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.42 (1H, s), 12.41 (1H, s), 12.15 (1H, s), 7.33 (1H, d, *J*=2.27 Hz), 7.24-7.16 (4H, m), 7.07 (1H, s), 6.76 (1H, d, *J*=8.60 Hz), 4.84 (2H, s), 4.31 (1H, s), 2.74 (2H, t, *J*=5.69 Hz), 2.45 (2H, t, *J*=6.62 Hz).

Compound 9

To a 200 mL round bottom flask fitted with a Dean-Stark trap and a condenser were added benzene (50 mL), 2-formyl-1,4,5,8-tetramethoxynaphthalene (5 g, 18.1 mmol), 2-amino-4-methylbenzothiazole (3.04 g, 18.1 mmol), triethylamine (2.53 mL, 18.1 mmol) and acetic acid (800 μL, 14 mmol), the mixture was refluxed for 20 h and then the water was removed by azeotropic distillation. After cooling to room temperature, the reaction mixture was washed successively with 5% HCl, saturated $NaHCO_3$, 5% acetic acid and water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl acetate and hexane to afford 6.7 g (87.5%) of compound **9** as an orange solid. Yield 87.5%; m.p. 125-126°C; IR (KBr, cm^{-1}) 2930, 1590, 1512, 1490, 1450, 1370, 1252, 1170, 1152, 1070; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.45 (1H, s), 7.68 (1H, s), 7.65 (1H, m), 7.27 (2H, m), 7.02 (1H, d, *J*=8.7 Hz), 6.92 (1H, d, *J*=8.7 Hz), 4.05 (3H, s), 3.99 (3H, s), 3.92 (3H, s), 3.91 (3H, s), 2.78 (3H, s).

Compound 10

To a stirred solution of compound **9** (6.5 g, 15.4 mmol) in 50 mL of tetrahydrofuran at room temperature was slowly added $LiAlH_4$ (614.6 mg, 15.4 mmol) over a period of 10 min. The mixture was then stirred at room temperature for 30 min. The reaction mixture was extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl ether to afford 6.46 g (98.9%) of compound **10** as a white solid. Yield 98.9%; m.p. 69-71°C; IR (KBr, cm^{-1}) 3350, 2930, 1595, 1522, 1450, 1365, 1252, 1210, 1072; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.41 (1H, d, *J*=7.7 Hz), 7.10 (1H, d, *J*=7.4 Hz), 6.98 (2H, m), 6.88 (1H, d, *J*=8.8 Hz), 6.81 (1H, d, *J*=8.8 Hz), 6.02 (1H, s), 4.80 (2H, s), 3.96 (3H, s), 3.89 (6H, s), 3.83 (3H, s), 2.59 (3H, s).

Compound 11

To a stirred solution of compound **10** (6 g, 14.1 mmol) in 120 mL of dry tetrahydrofuran at 0-5°C was slowly added NaH (802.3 mg, 18.4 mmol). The mixture was then stirred for 10 min at 0-5°C. To the reaction mixture was slowly added a solution of iodomethane (2.66 mL, 42.4 mmol) in 10 mL of tetrahydrofuran. The mixture was then stirred for 3 h. The reaction mixture was extracted with methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from acetonitrile to afford 5.56 g (89.7%) of compound **11** as a white solid. Yield 89.7%; m.p. 114-115°C; IR (KBr, cm^{-1}) 2930, 1595, 1550, 1370, 1260, 1070; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.45 (1H, d, *J*=7.87 Hz), 7.13 (1H, d, *J*=3.63 Hz), 6.98 (1H, t, *J*=7.62 Hz), 6.94 (1H, s), 6.85 (2H, s), 5.03 (2H, s), 3.97 (3H, s), 3.89 (3H, s), 3.81 (6H, s), 3.14 (3H, s), 2.60 (3H, s).

Compound 12

To a 250 mL round bottom flask fitted with a condenser were added toluene (60 mL), compound **10** (3 g, 7.1 mmol) and succinic anhydride (2.92 g, 28.3 mmol) and the mixture was refluxed for 5 h. After cooling to room temperature, the precipitates were collected. The resulting residue was recrystallized from ethyl ether to afford 4.26 g (96.7%) of compound **12** as a white solid. Yield 96.7%; m.p. 210-211°C; IR (KBr, cm^{-1}) 3440, 2920, 1700, 1595, 1495, 1405, 1365, 1255, 1205, 1072; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.20 (1H, s), 7.79 (1H, t, *J*=4.69 Hz), 7.22 (1H, s), 7.20 (1H, s), 6.96 (1H, d, *J*=8.7 Hz), 6.87 (1H, d, *J*=8.7 Hz), 6.60 (1H, s), 5.76 (1H, s), 3.88 (3H, s), 3.82 (3H, s), 3.74 (3H, s), 3.62 (3H, s), 2.99 (2H, t, *J*=4.95 Hz), 2.61 (2H, t, *J*=6.45 Hz), 2.43 (3H, s).

General procedure for the synthesis of compounds 13-16

Oxidation with chromium (VI) oxide

To a stirred solution of compound **11** (1 g, 2.3 mmol) in acetone (20 mL) at room temperature were added H_2SO_4 (164 μL, 2.9 mmol), 4 mL of water and CrO_3 (240 mg, 2.3 mmol). The mixture was then stirred at room temperature for 1 h. The separation of compounds **13** and **15** by column chromatography on silica gel (ethyl acetate : hexane = 3 : 7) afforded compound **13** (598.9 mg, 64.3%) as an orange solid and compound **15** (116.4 mg, 12.5%) as a red brown solid.

Oxidation with ammonium cerium (IV) nitrate

To a stirred solution of compound **11** (1 g, 2.20 mmol) in acetonitrile (15 mL) at room temperature was added a solution of ammonium cerium (IV) nitrate (3.29 g, 5.7 mmol) in 3.5 mL of water. The mixture was then stirred at room temperature for 1 h. The separation of compounds **13** and **15** by column chromatography on silica gel (ethyl acetate : hexane = 3 : 7) afforded compound **13** (122.9 mg, 13.2%) as an orange solid and compound **15** (600.7 mg, 64.5%) as a red brown solid, respectively.

Compound 13

Yield 64.3%; m.p. 69-71°C; IR (KBr, cm^{-1}) 2930, 1655, 1545, 1400, 1260, 1050; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.49 (1H, s), 7.46 (1H, d, *J*=7.83 Hz), 7.13 (1H, d, *J*=7.30 Hz), 7.00 (1H, t, *J*=7.61 Hz), 6.80 (2H, s), 4.97 (2H, s), 3.90 (3H, s), 3.86 (3H, s), 3.22 (3H, s), 2.56 (3H, s).

Compound 14

Yield 20%; m.p. 140-141°C; IR (KBr, cm^{-1}) 3450, 2930, 1725, 1655, 1495, 1395, 1250, 1210, 1050; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.31 (1H, s), 7.79 (1H, t, *J*=4.38 Hz), 7.22 (1H, s), 7.20 (1H, s), 7.17 (1H, s), 6.86 (1H, d, *J*=10.25 Hz), 6.81 (1H, d, *J*=10.25 Hz), 5.70 (2H, s), 3.91 (3H, s), 3.74 (3H, s), 2.99 (2H, t, *J*=4.93 Hz), 2.66 (2H, t, *J*=6.57 Hz), 2.41 (3H, s).

Compound 15

Yield 64.5%; m.p. 85-87°C; IR (KBr, cm^{-1}) 2940, 1650, 1540, 1400, 1280, 1205, 1060; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.43 (1H, d, *J*=3.89 Hz), 7.32 (2H, s), 7.10 (1H, d, *J*=3.68 Hz), 6.97 (1H, t, *J*=10.20 Hz), 6.75 (1H, s), 4.71 (2H, s), 3.99 (3H, S), 3.94 (3H, s), 3.26 (3H, s), 2.53 (3H, s).

Compound 16

Yield 58.6%; m.p. 141-142°C; IR (KBr, cm^{-1}) 3440, 2950, 1726, 1649, 1505, 1405, 1277, 1210, 1059; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.24 (1H, s), 7.77 (1H, t, *J*=4.76 Hz), 7.57 (1H, s), 7.56 (2H, s), 7.23 (1H, d, *J*=2.61 Hz), 7.21 (1H, d,

J=4.91 Hz), 6.34 (1H, s), 5.43 (2H, s), 3.90 (3H, s), 3.82 (3H, s), 2.99 (2H, t, *J*=4.82 Hz), 2.69 (2H, t, *J*=7.48 Hz), 2.41 (3H, s).

General procedure for the synthesis of compounds 17-20

To a stirred solution of compound **13** (500 mg, 1.22 mmol) in 40 mL of dry methylene chloride at 0-5°C was slowly added $AlCl_3$ (1.66 g, 12.24 mmol). The mixture was then stirred for 2 h. To a stirred cold solution of 10% HCl (500 mL) were slowly added the reaction mixture and ethyl ether (500 mL). The mixture was then stirred overnight. The reaction mixture was extracted with ethyl ether and washed with water. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The resulting residue was recrystallized from ethyl ether to afford 220 mg (46.9%) of compound **17** as a red brown solid.

Compound 17

Yield 92.6%; m.p. 135-136°C; IR (KBr, cm^{-1}) 2910, 1610, 1545, 1455, 1405, 1200, 1110; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.57 (1H, s), 12.41 (1H, s), 7.45 (1H, d, *J*=7.85 Hz), 7.20 (2H, s), 7.16-7.08 (2H, m), 6.99 (1H, *t*, *J*=7.66 Hz), 4.82 (2H, s), 3.28 (3H, s), 2.54 (3H, s).

Compound 18

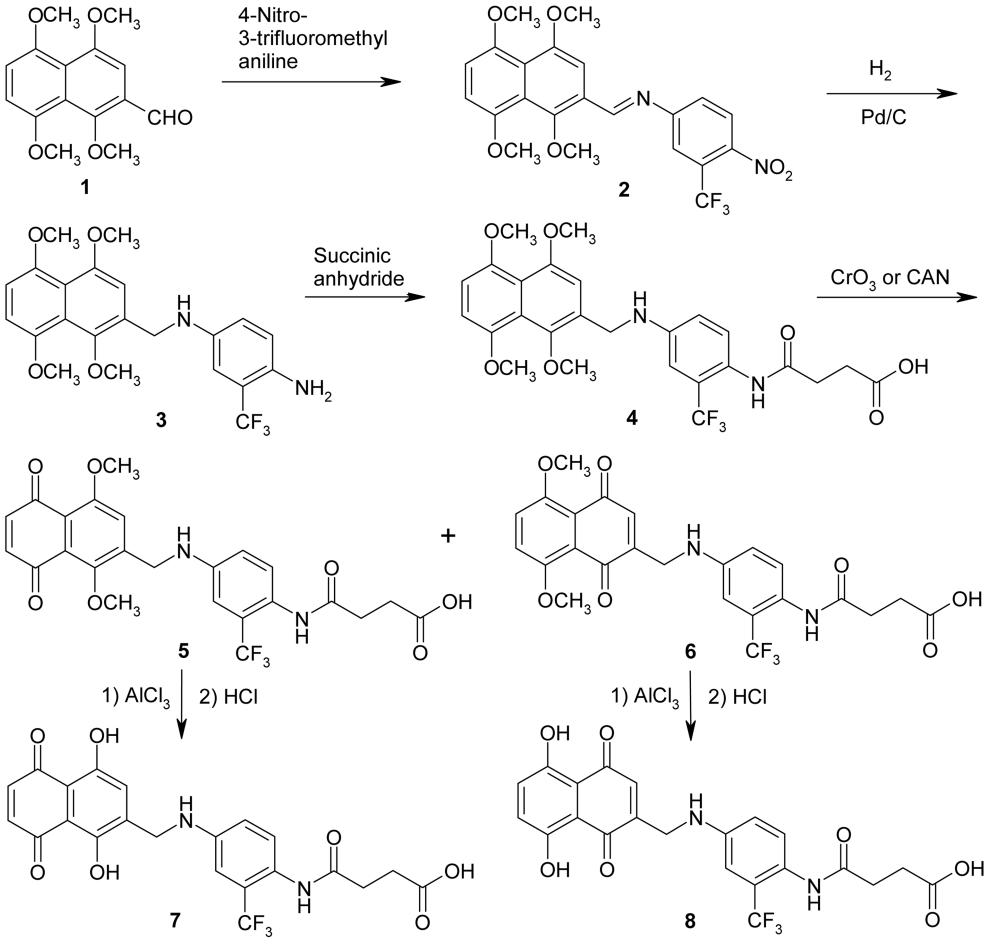
Yield 46.9%; m.p. 115-116°C; IR (KBr, cm^{-1}) 3450, 2930, 1675, 1610, 1500, 1450, 1405, 1265, 1205; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.35 (1H, s), 12.31 (1H, s), 12.17 (1H, s), 7.78 (1H, t, *J*=4.53 Hz), 7.37 (1H, s), 7.14 (3H, m), 5.64 (1H, d, *J*=1.01 Hz), 5.53 (2H, s), 3.01 (2H, t, *J*=6.52 Hz), 2.65 (2H, t, *J*=2.15 Hz), 2.42 (3H, s)

Compound 19

Yield 79.6%; m.p. 130-132°C; IR (KBr, cm^{-1}) 2910, 1605, 1540, 1450, 1340, 1200, 1110; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 12.56 (1H, s), 12.40 (1H, s), 7.48 (1H, d, *J*=7.60 Hz), 7.19 (2H, s), 7.13-7.09 (2H, m), 6.99 (1H, t, *J*=7.60 Hz), 4.82 (2H, s), 3.28 (3H, s), 2.55 (3H, s).

Compound 20

Yield 69.7%; m.p. 114-115°C; IR (KBr, cm^{-1}) 3440, 2910, 1675, 1605, 1500, 1450, 1405, 1265, 1205; ^{1}H-NMR (300

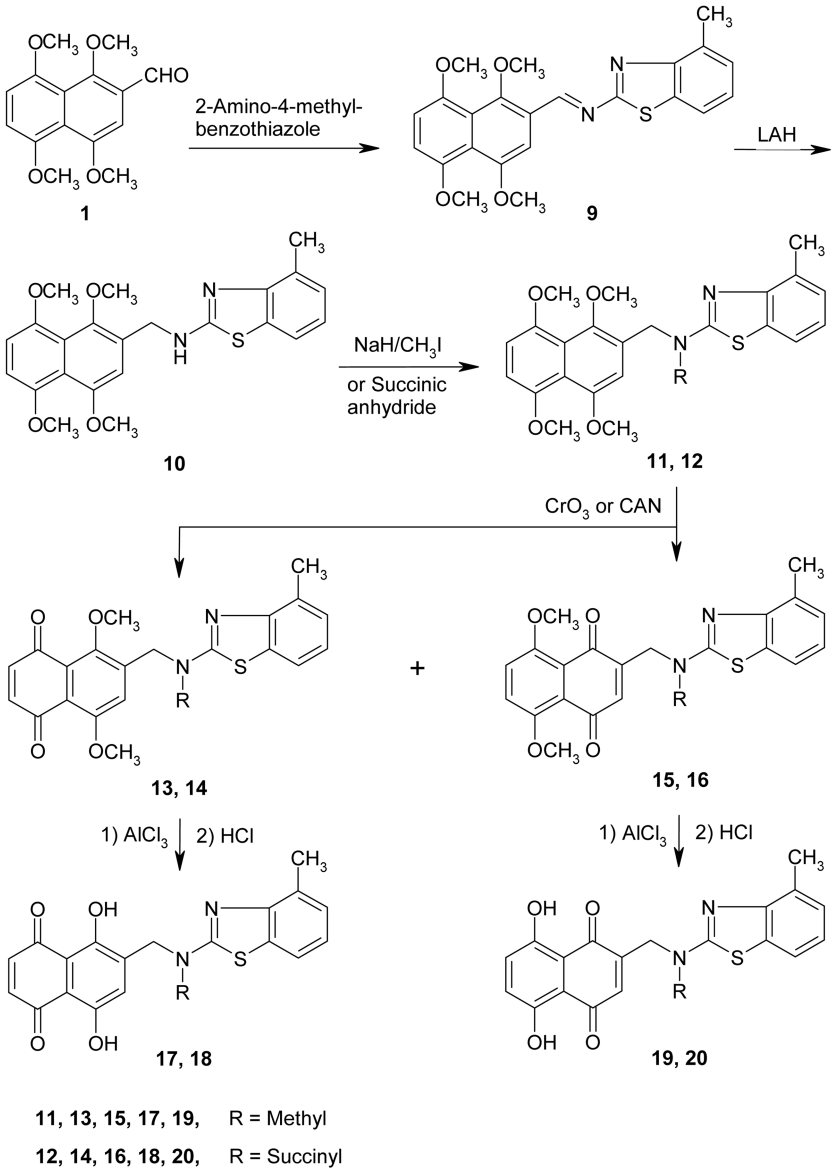


Scheme 1. Synthetic pathways of 2 and 6-anilinomethyl-5,8-dihydroxy-1,4-naphthoquinones. CAN, cerium (IV) ammonium nitrate

MHz, $CDCl_3$) δ 12.35 (1H, s), 12.24 (1H, s), 12.17 (1H, s), 7.80 (1H, t, *J*=8.81 Hz), 7.37 (1H, s), 7.22 (3H, m), 5.60 (1H, d, *J*=10.47 Hz), 5.53 (2H, s), 3.03 (2H, t, *J*=5.72 Hz), 2.61 (2H, t, *J*=5.95 Hz), 2.51 (3H, s).

RESULTS AND DISCUSSION

In the present paper, we compared the cytotoxic and antitumor activities of 5,8-dimethoxy-1,4-naphthoquinone (DMNQ) derivatives and 5,8-dihydroxy-1,4-naphthoquinone (DHNQ) derivatives. 2-Formyltetramethoxynaphthalene (**1**) was prepared from 1,5-dihydroxynaphthalene through the 4-step reactions of methylation (86%), bromination (85 %), methoxylation (61%), and formylation (96%) (Benthey *et al.*, 1907; Carter *et al.*, 1942). 2-formyltetramethoxynaphthalene was condensed with 4-nitro-3-(trifluoromethyl) aniline at pH 5 and the resulting imine compound (**2**) was reduced to 2-anilinonomethyl-1,4,5,8-tetramethoxynaphthalene (**3**) using LAH or $NaBH_4$ in good yields. Compound **4** was prepared from compound **3** and succinic anhydride with a yield of 88.9%. We were able to obtain the 6-substituted naphthoquinone derivatives **5** with a yield of 69.4% and the 2-substituted naphthoquinone derivatives **6** with a yield 68.4% by the oxidation of the corresponding 2-anilinomethyl-1,4,5,8-tetramethoxynaphthalenes with chromium (VI) oxide and cerium (IV) ammonium nitrate (CAN), respectively. Compounds **7** and **8** were prepared from compound **5** and **6**, respectively, by the demethylation



Scheme 2. Synthetic pathways of 2 and 6-[(1,3-benzothiazol-2-yl)aminomethyl]-5,8-dihydroxy-1,4-naphthoquinones. LAH, lithium aluminum hydride; CAN, cerium (IV) ammonium nitrate

reaction with $AlCl_3$ and HCl (Scheme 1). Compounds **13**-**20** were prepared from compound **4** through the reactions described above.

The cytotoxicity of the naphthoquinone derivatives was measured against the cancer cells, L1210 (Lymphocytic leukemia) and P388 (Lymphoid neoplasma) using the MTT colorimetric method (Carmichael *et al*., 1987). The ED_{50} value (μg/mL) was defined as the concentration of the compound required to produce a 50% reduction in viability relative to the control in three independent experiments.

The cytotoxicities of the DMNQ and DHNQ derivatives were examined and, interestingly, it was observed that the DHNQ derivatives exhibited higher cytotoxic activity than the DMNQ derivatives against L1210 and P388 cells *in vitro*. It was also observed that the DHNQ derivatives showed better antitumor activity than the DMNQ derivatives in mice bearing S-180 cells in the peritoneal cavity (Table I). The ED_{50} values of the DHNQ derivatives against P388 cells were in the range of 0.18-1.81 μg/mL, whereas those of the DMNQ derivatives were in the range of 0.36-40.41 μg/mL. The T/C (%) values of compounds **8**, **17**, and **19** were found to be comparable to that of adriamycin and those of compounds **18** and **20** were even better.

In earlier works, we observed that the cytotoxic activity was dependent upon the location of the substituent groups in the DMNQ. In the case of the naphthoquinone derivatives with a propenoate substituent functional group at the C6 or C2 position, we showed that the 6-substituted derivatives were more effective than the 2-substituted derivatives and that the introduction of electronegative fluorine into the benzoyl group increased the cytotoxicity (Cho *et al*,. 1998). This result was in accordance with the findings of other researchers and the C2 or C3 positions of the 6-substituted compounds would likely be better Michael acceptors than the C3 position of the 2-substituted compounds and would probably be attacked more easily by nucleophiles, such as those containing amine or thiol functional groups, in the cells (You *et al*., 1998a, 1998b; Song *et al*., 2001). The electron density in the quinoid ring may be important. Previously, it was reported that compounds having higher ^{1}H-NMR chemical shifts of 3-H (δ_H) usually have a lower ED_{50} value (Chung *et al*., 2004). Meanwhile, in the case of the DHNQ derivatives, interestingly, it was observed that the 2-substituted derivatives (**8**, **19**, and **20**) showed better antitumor activity than the 6-substituted derivatives (**7**, **17**, and **18**) in mice bearing S-180 cells in the peritoneal cavity (Table I). The reason why 2-substituted DHNQ derivatives are more effective than 6-substituteed DHNQ derivatives has not yet been elucidated and further investigations are currently under way.

Table I. Cytotoxicity and antitumor activity of DMNQ and DHNQ derivatives

No. of Compd.	ED_{50} (μg/mL)		T/C (%)
	L1210	P388	
5	5.87	31.18	108
6	8.92	40.41	101
7	1.52	1.81	199
8	1.57	1.64	240
13	0.80	0.36	229
14	1.49	3.61	138
15	1.21	0.56	214
16	4.76	4.19	105
17	0.72	0.29	228
18	0.34	0.22	321
19	0.45	0.26	257
20	0.34	0.18	331
Adriamycin	0.07	0.14	234

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