Arch Pharm Res Vol 27, No 9, 893-900, 2004

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis and Evaluation of Antitumor Activity of 2- and 6-[(1,3-Benzothiazol-2-yl)aminomethyl]-5,8-dimethoxy-1,4-naphthoquinone Derivatives

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(Received July 22, 2004)

2- or 6-Substituted BZT-N derivatives were synthesized, and their cytotoxic activity against cancer L1210 and SNU-1 cells was examined. The antitumor action was also assessed in mice bearing S-180 cells in peritoneal cavity. In a comparison, it was found that 6-substituted BZT-N derivatives exhibited higher potencies in both bioactivities than 2-substituted BZT-N derivatives against L1210 cells in *in vitro* and S-180 *in vitro* tests exception of compound **36**. Interestingly, it was observed that 2-substituted compound **36,** which has methyl group at R_1 position, exhibited a better antitumor activity than 6-substituted compounds against L1210 and SNU-1 *in vitro*. The ED_{50} value of 2-substituted compound **36** against L1210 was found to be comparable to the ED_{50} value of adriamycin and was even better against the solid cancer cell line SNU-1. It was also observed that 2-substituted compound **36** showed better antitumor activity in mice bearing S-180 cells in the peritoneal cavity. The T/C (%) value of 2-substituted compound **36** was similar to that of adriamycin. Quantitative structure-activity relationship (QSAR) tests reveal that the experimental ED_{50} values against SNU-1 closely correlate with both the calculated HOMO energies (E_{HOMO}) and the measured ^{1}H-NMR chemical shift of 3-H (δ_H). The results suggests that a compound having higher E_{HOMO} and δ_H values usually should have a lower ED_{50} (SNU-1) value.

Key words: Naphthoquinone, Cytotoxicity, Antitumor activity

INTRODUCTION

Quinones are widely distributed in nature and many clinically important antitumor drugs containing quinone nucleus, such as anthracyclines, mitoxantrones and saintopin, show an excellent anticancer activity. These anticancer agents are effective inhibitors of DNA topoisomerase, and it is generally known that the cytotoxicity of quinone analogues results from the inhibition of DNA topoisomerase-II (Foye, 1995; Liu, *et al.*, 1984; Leopold, *et al.*, 1984; Scheithauer, *et al.*, 1986). The quinone analogues can also induce the formation of semiquinone radical, which can transfer an electron to oxygen to produce superoxide. This process is catalyzed by flavoenzymes such as NADPH-cytochrome-*P*-450 reductase. Both 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-dioxy-1,4-naphthoquinone derivatives exhibited good antitumor activity (Baik *et al.*, 1997). In view of these facts, previously we had synthesized some 1,4-naphthoquinone derivatives with a propenoate substituent functional group and reported their cytotoxicity and antitumor activity (Cho, *et al.*, 1998). In this study, we report the synthesis of 2- and 6-[(substituted 1,3-benzothiazol-2-yl)aminomethyl]-5,8-dimethoxy-1,4-naphthoquinones (BZT-N, 1,3-benzothiazol-2-yl)aminomethyl]-5,8-dimethoxy-1,4-naphthoquinone) and their cytotoxic activity against cancer L1210 and SNU-1

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cells. As evidenced in earlier reports, the introduction of electron withdrawing group such as electronegative fluorine (Cho *et al.*, 1998) and acetoxy or oxo group (You *et al.*, 1998a) on the side chain of naphthoquinone increased the cytotoxicity. These previous works indicate that the electron density in the quinoid ring may be important in cytotoxicity and antitumor activity. In order to find a relationship between cytotoxicity and electron density of quinoid ring, quantitative structure-activity relationship (QSAR) analysis was performed. We also assessed the antitumor action of 2- and 6-substituded BZT-N derivatives in mice bearing S-180 cells in peritoneal cavity.

MATERIALS AND METHODS

Materials

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 as KBr pellet. 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°C and 12 h cycle of light/dark. Feed and water were freely accessible to the mice. L1210, SNU-1 and S-180 cells were a gift from Dr. B. Z. Ahn, College of Pharmacy, Chungnam National University (Korea).

In vitro cytotoxicity (MTT assay)

SNU-1 and Vero target cells were prepared from confluent cultures by trypsinization (trypsin-EDTA, GIBCO Cat. No. 25300-054). Target cells were suspended at 2×10^5 cells/mL in complete medium (10% fetal bovine serum) containing each concentrations of synthesized naphthoquinone derivatives, vigorously vortexed and then 100 μL aliquots were dispensed into 96-well flat-bottomed microtiter plates using a multichannel pipette. Plates were then incubated at 37°C for 72 h in 5% CO_2 incubator. MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was dissolved in PBS at 5 mg/mL and filtered to remove a small amount of insoluble residue present in some batches of 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 0.01 N HCl solution containing 10% sodium dodecyl sulfate were added to solubilize the MTT formazan. Plates were gently shaken until all crystals were dissolved, and the absorbance at 540 nm was determined with a Microplate Reader (SPECTRA MAX 340). All results were corrected for background absorbance detected in wells without added MTT. Preliminary experiments showed a linear relationship between the cell numbers and the absorbance at 540 nm when cells are in the range of 4×10^2 to 4×10^5 per well 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 to male ICR mice. 24 h after the transplantation, the mice were divided so that each group contains 8 mice. The test compounds were administered into the intraperitoneal cavity of the mouse daily for 5 days. The rate of growth inhibition (T/C, %) was calculated by following equation;

$$T/C(\%)=\frac{\text{Average survival period in the test group}}{\text{Average survival period in the control group}}\times100$$

Computational methods

Geometries of molecules **32** to **41** were first fully optimized by performing PM3 calculations. The optimized geometries were used to carry out *ab initio* molecular orbital calculations by using Hartree-Fock (HF) theory with 6-31G* basis set (Hehre *et al.*, 1987). All of these electronic structure calculations were performed by using Gaussian 03 program (Frisch *et al.*, 2003) on a 34-processors IBM Linux computer cluster. The electronic wave functions calculated at the HF/6-31G* level were used to derive possibly useful electronic structure-based molecular descriptors, such as molecular dipole moments, net atomic charges, and energies of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). These molecular descriptors were used to explore their possible correlation relationship with the measured anticancer activity data.

General procedure for the synthesis of compounds (2-11)

Into a 100 mL round bottom flask fitted with a Dean-Stark trap and a condenser were added bezene (20 mL), 2-formyl-1,4,5,8-tetramethoxynaphthalene (2 g, 7.25 mmol), 2-aminobenzothiazole (1.21 g, 7.25 mmol), triethylamine (1.03 mL, 7.25 mmol) and acetic acid (300 μL, 5.25 mmol) and the mixture was refluxed for 20 h and 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 2.7 g (91%) of compound **2** as an orange solid.

Compound 2. Yield 91%; mp 161-162°C; IR (KBr, cm^{-1}) 2925, 2830, 1578, 1371, 1259, 1080; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.48 (1H, s), 8.02 (1H, d, *J*=8.1 Hz), 7.85 (1H, d, *J*=7.9 Hz), 7.68 (1H, s), 7.47 (1H, m), 7.38 (1H, m), 7.04 (1H, d, *J*=8.7 Hz), 6.94 (1H, d, *J*=8.7 Hz), 4.06 (3H, s), 4.01 (3H, s), 3.94 (3H, s), 3.93 (3H, s).

Compound 3. Yield 90%; mp 161-163°C; IR (KBr, cm^{-1}) 2945, 1560, 1440, 1372, 1267, 1250, 1070; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.44 (1H, s), 7.94 (1H, m), 7.65 (1H, s), 7.53 (1H, dd, *J*=8.2 and 2.6 Hz), 7.21 (1H, td, *J*=9.0 and 2.6 Hz), 7.04 (1H, d, *J*=8.8 Hz), 6.94 (1H, d, *J*=8.8 Hz), 4.05 (3H, s), 4.00 (3H, s), 3.93 (3H, s), 3.92 (3H, s).

Compound 4. Yield 69%; mp 169-176°C; IR (KBr, cm^{-1}) 2945, 2840, 1575, 1512, 1445, 1372, 1255, 1172, 1082, 1070; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 9.43 (1H, s), 7.73 (1H, dd, *J*=8.0 and 1.0 Hz), 7.66 (1H, s), 7.50 (1H, dd, *J*=7.8 and 1.0 Hz), 7.04 (1H, t, *J*=7.9 Hz), 7.04 (1H, d, *J*=8.7 Hz), 6.94 (1H, d, *J*=8.7 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.93 (3H, s), 3.92 (3H, s).

Compound 5. Yield 92%; mp 184-185°C; IR (KBr, cm^{-1}) 2940, 2850, 1582, 1560, 1515, 1372, 1265, 1080, 1070; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.45 (1H, s), 7.90 (1H, d, *J*=8.8 Hz), 7.81 (1H, d, *J*=2.0 Hz), 7.63 (1H, s), 7.43 (1H, dd, *J*=*J*=8.6 and 2.0 Hz), 7.04 (1H, d, *J*=8.7 Hz), 6.94 (1H, d, *J*=8.7 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.92 (3H, s), 3.91 (3H, s).

Compound 6. Yield 92%; mp 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 7. Yield 86%; mp 161-162°C; IR (KBr, cm^{-1}) 2925, 2820, 1606, 1580, 1559, 1512, 1372, 1264, 1152, 1080; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.44 (1H, s), 7.89 (1H, d, *J*=8.4 Hz), 7.67 (1H, s), 7.63 (1H, s), 7.29 (1H, d, *J*=8.4 Hz), 7.03 (1H, d, *J*=8.8 Hz), 6.93 (1H, d, *J*=8.8 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.91 (3H, s), 2.50 (3H, s).

Compound 8. Yield 82%; mp 162-169°C; IR (KBr, cm^{-1}) 2940, 1585, 1512, 1450, 1370, 1259, 1080; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.42 (1H, s), 7.77 (1H, s), 7.67 (1H, s), 7.57 (1H, s), 7.01 (1H, d, *J*=8.8 Hz), 6.91 (1H, d, *J*=8.8 Hz), 4.04 (3H, s), 3.99 (3H, s), 3.92 (3H, s), 3.90 (3H, s), 2.39 (3H, s), 2.38 (3H, s).

Compound 9. Yield 82%; mp 173-176°C; IR (KBr, cm^{-1}) 1600, 1460, 1370, 1260, 1225, 1212, 1080, 1065; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.40 (1H, s), 7.89 (1H, d, *J*=8.8 Hz), 7.66 (1H, s), 7.31 (1H, d, *J*=2.6 Hz), 7.07 (1H, dd, *J*=8.8 and 2.6 Hz), 7.02 (1H, d, *J*=8.8 Hz), 6.93 (1H, d, *J*=8.8 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.92 (3H, s), 3.91 (3H, s), 3.90 (3H, s).

Compound 10. Yield 87%; mp 173-176°C; IR (KBr, cm^{-1}) 2940, 1595, 1512, 1443, 1371, 1255, 1220, 1080, 1067; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.39 (1H, s), 7.88 (1H, d, *J*=8.8 Hz), 7.66 (1H, s), 7.29 (1H, d, *J*=2.4 Hz), 7.07 (1H, dd, *J*=8.8 and 2.4 Hz), 7.02 (1H, d, *J*=8.8 Hz), 6.92 (1H, d, *J*=8.8 Hz), 4.11 (2H, q, *J*=7.0 Hz), 4.04 (3H, s), 4.00 (3H, s), 3.92 (3H, s), 3.91 (3H, s), 1.47 (3H, t, *J*=7.0 Hz).

Compound 11. Yield 92%; mp 240-241°C; IR (KBr, cm^{-1}) 2930, 1600, 1580, 1558, 1510, 1460, 1428, 1370, 1330, 1265; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.53 (1H, s), 8.76 (1H, d, *J*=2.4 Hz), 8.34 (1H, dd, *J*=9.0 and 2.4 Hz), 8.05 (1H, d, *J*=9.0 Hz), 7.60 (1H, s), 7.06 (1H, d, *J*=8.8 Hz), 6.95 (1H, d, *J*=8.8 Hz), 4.05 (3H, s), 4.01 (3H, s), 3.93 (6H, s).

General procedure for the synthesis of compounds (12-21)

To a stirred solution of compound **2** (2 g, 4.9 mmol) in 30 mL of tetrahydrofuran at room temperature was slowly added $LiAlH_4$ (196 mg, 4.9 mmol) over for 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 1.75 g (87%) of compound **12** as an orange solid.

Compound 12. Yield 87%; mp 160°C; IR (KBr, cm^{-1}) 3345, 2940, 2845, 1598, 1549, 1443, 1369, 1257, 1072; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.55 (1H, d, *J*=7.8 Hz), 7.46 (1H, d, *J*=8.2 Hz), 7.23 (1H, t, *J*=8.0 Hz), 7.09 (1H, t, *J*=7.4 Hz), 6.96 (1H, s), 6.83 (2H, s), 4.83 (2H, s), 3.96 (3H, s), 3.88 (3H, s), 3.82 (6H, s).

Compound 13. Yield 88%; mp 165-166°C; IR (KBr, cm^{-1}) 3325, 2925, 1600, 1458, 1380, 1073; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.40 (1H, m), 7.25 (1H, dd, *J*=8.2 and 2.6 Hz), 6.94 (1H, td, *J*=9.0 and 2.6 Hz), 6.93 (1H, s), 6.84 (2H, s), 6.26 (1H, s), 4.79 (2H, s), 3.96 (3H, s), 3.89 (3H, s), 3.86 (3H, s), 3.81 (3H, s).

Compound 14. Yield 84%; mp 87-88°C; IR (KBr, cm^{-1}) 3325, 2930, 1595, 1555, 1450, 1415, 1365, 1255, 1075; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.42 (1H, dd, *J*=7.8 and 0.6 Hz), 7.29 (1H, dd, *J*=8.0 and 0.6 Hz), 7.18 (1H, s), 6.96 (1H, t, *J*=8.2 Hz), 6.96 (1H, s), 6.83 (2H, s), 4.82 (2H, s), 3.95 (3H, s), 3.87 (3H, s), 3.81 (3H, s), 3.79 (3H, s).

Compound 15. Yield 94%; mp 162-163°C; IR (KBr, cm^{-1}) 3345, 2925, 1604, 1442, 1379, 1266, 1252, 1072; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.52 (1H, d, *J*=2.0 Hz), 7.40 (1H, d, *J*=8.4 Hz), 7.21 (1H, dd, *J*=8.6 and 2.0 Hz), 6.92 (1H, s), 6.85 (2H, s), 6.19 (1H, s), 4.80 (2H, s), 3.97 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.82 (3H, s).

Compound 16. Yield 97%; mp 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*=8.81 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 17. Yield 93%; mp 162-163°C; IR (KBr, cm^{-1}) 3325, 2940, 1600, 1570, 1520, 1460, 1372, 1265, 1079; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.40 (1H, d, *J*=8.4 Hz), 7.36 (1H, d, *J*=1.8 Hz), 7.06 (1H, dd, *J*=8.4 and 1.8 Hz), 6.95 (1H, s), 6.84 (2H, s), 6.26 (1H, s), 4.81 (2H, s), 3.96 (3H, s), 3.88 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 2.37 (3H, s).

Compound 18. Yield 97%; mp 173-174°C; IR (KBr, cm^{-1}) 3350, 2940, 1596, 1560, 1475, 1377, 1265, 1075; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.36 (1H, t, *J*=5.5 Hz), 7.45 (1H, s), 7.26 (1H, s), 7.09 (1H, s), 6.98 (1H, d, *J*=8.7 Hz), 6.91 (1H, d, *J*=8.7 Hz), 4.75 (2H, d, *J*=5.5 Hz), 3.90 (3H, s), 3.80 (6H, s), 3.76 (3H, s), 2.27 (3H, s), 2.26 (3H, s).

Compound 19. Yield 94%; mp 166-167°C; IR (KBr, cm^{-1}) 3320, 2950, 1606, 1575, 1552, 1464, 1375, 1253, 1210, 1079; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 7.38 (1H, d, *J*=8.8 Hz), 7.09 (1H, d, *J*=2.6 Hz), 6.95 (1H, s), 6.83 (2H, s), 6.83 (1H, dd, *J*=8.8 and 2.6 Hz), 6.35 (1H, s), 4.79 (2H, s), 3.95 (3H, s), 3.88 (3H, s), 3.84 (3H, s), 3.81 (3H, s), 3.79 (3H, s).

Compound 20. Yield 87%; mp 158-161°C; IR (KBr, cm^{-1}) 3330, 2920, 1600, 1545, 1456, 1365, 1255, 1220, 1072; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.24 (1H, t, *J*=5.6 Hz), 7.30 (2H, m), 7.05 (1H, s), 6.85 (2H, m), 4.70 (2H, d, *J*=5.6 Hz), 3.98 (2H, q, *J*=6.9 Hz), 3.89 (3H, s), 3.76 (6H, s), 3.71 (3H, s), 1.31 (3H, t, *J*=6.9 Hz).

Compound 21. Yield 97%; mp 222-223°C; IR (KBr, cm^{-1}) 3360, 2950, 1625, 1605, 1560, 1445, 1430, 1367, 1330, 1295; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.41 (1H, d, *J*=2.4 Hz), 8.04 (1H, dd, *J*=9.0 and 2.4 Hz), 7.32 (1H, d, *J*=9.0 Hz), 6.84 (3H, s), 4.81 (2H, s), 3.95 (3H, s), 3.88 (3H, s), 3.84 (3H, s), 3.79 (3H, s).

General procedure for the synthesis of compounds (22-41)

Oxidation with chromium (VI) oxide

To a stirred solution of compound **12** (800 mg, 1.95 mmol) in acetone (20 mL) at room temperature were added H_2SO_4 (164 μL, 2.93 mmol), 4 mL of water and CrO_3 (203 mg, 1.95 mmol). The mixture was then stirred at room temperature for 1 h. Separation of the compounds **22** and **32** by column chromatography on silica gel (ethyl acetate : hexane = 3 : 7) afforded compound **22** (593.2 mg, 80%) as an orange solid and compound **32** (66 mg, 9%) as a reddish brown solid.

Oxidation with ammonium cerium (IV) nitrate

To a stirred solution of compound **12** (800 mg, 1.95 mmol) in acetonitrile (15 mL) at room temperature were added a solution of ammonium cerium (IV) nitrate (2.72 g, 4.87 mmol) in 3.5 mL of water. The mixture was then stirred at room temperature for 1 h. Separation of the compounds **22** and **32** by column chromatography on silica gel (ethyl acetate : hexane = 3 : 7) afforded compound **22** (156 mg, 20%) as a yellow solid and compound **32** (533 mg, 72%) as a reddish brown solid.

Compound 22. Yield 80%; mp 154-160°C; IR (KBr, cm^{-1}) 3395, 2945, 1652, 1545, 1452, 1250, 1220, 1050; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.58 (1H, s), 7.69 (1H, d, *J*=8.0 Hz), 7.63 (1H, s), 7.40 (1H, d, *J*=7.6 Hz), 7.23 (1H, t, *J*=7.2 Hz), 7.04 (1H, t, *J*=6.6 Hz), 6.83 (2H, s), 4.73 (2H, s), 3.84 (3H, s), 3.81 (3H, s).

Compound 23. Yield 80%; mp 134-135°C; IR (KBr, cm^{-1}) 3320, 2930, 1658, 1552, 1532, 1458, 1255, 1055, 850; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.53 (1H, t, *J*=5.6 Hz), 7.60 ((2H, m), 7.38 (1H, m), 7.03 (1H, td, *J*=9.2 and 2.6 Hz), 6.80 (2H, s), 4.67 (2H, d, *J*=5.6 Hz), 3.80 (3H, s), 3.76 (3H, s).

Compound 24. Yield 93%; mp 165-167°C; IR (KBr, cm^{-1}) 3350, 2930, 1652, 1590, 1555, 1537, 1250, 1110, 1050; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.89 (1H, t, *J*=5.8 Hz), 7.69 (1H, s), 7.64 (1H, d, *J*=7.8 Hz), 7.28 (1H, d, *J*=7.8 Hz), 6.99 (1H, t, *J*=8.0 Hz), 6.79 (2H, s), 4.70 (2H, d, *J*=5.8 Hz), 3.86 (3H, s), 3.82 (3H, s).

Compound 25. Yield 86%; mp 175-176°C; IR (KBr, cm^{-1}) 3360, 2945, 1655, 1545, 1445, 1255, 1212, 1065, 1015, 855; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.65 (1H, t, *J*=6.0 Hz), 7.80 (1H, d, *J*=2.0 Hz), 7.58 (1H, s), 7.35 (1H, d, *J*=8.6 Hz), 7.21 (1H, dd, *J*=8.6 and 2.0 Hz), 6.80 (2H, s), 4.68 (2H, s), 3.80 (3H, s), 3.76 (3H, s).

Compound 26. Yield 90%; mp 133-134°C; IR (KBr, cm^{-1}) 3330, 2950, 1650, 1598, 1525, 1465, 1395, 1252, 1515, 1052; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.54 (1H, s), 7.40 (1H, d, *J*=7.8 Hz), 7.11 (1H, d, *J*=7.3 Hz), 7.00 (1H, t, *J*=7.6 Hz), 6.75 (2H, s), 6.14 (1H, broad), 4.77 (2H, s), 3.92 (3H, s), 3.90 (3H, s), 2.56 (3H, s).

Compound 27. Yield 72%; mp 159-160°C; IR (KBr, cm^{-1}) 3350, 2950, 1660, 1220, 1465, 1392, 1330, 1250, 1050, 850; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.43 (1H, t, *J*=5.6 Hz), 7.58 (1H, s), 7.45 (1H, d, *J*=1.2 Hz), 7.25 (1H, d, *J*=8.0 Hz), 7.00 (1H, dd, *J*=8.0 and 1.2 Hz), 6.79 (2H, s), 4.67 (2H, d, *J*=5.6 Hz), 3.79 (3H, s), 3.76 (3H, s), 2.76 (3H, s).

Compound 28. Yield 86%; mp 135-137°C; IR (KBr, cm^{-1}) 3350, 2950, 1650, 1558, 1538, 1456, 1250, 1221, 1055, 852; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.40 (1H, t, *J*=5.6 Hz), 7.60 (1H, s), 7.41 (1H, s), 7.20 (1H, s), 6.82 (2H, s), 4.68 (2H, d, *J*=5.6 Hz), 3.81 (3H, s), 3.79 (3H, s), 2.21 (6H, s).

Compound 29. Yield 82%; mp 131-132°C; IR (KBr, cm^{-1}) 3340, 2940, 1652, 1532, 1465, 1259, 1221, 1050; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.32 (1H, t, *J*=5.4 Hz), 7.58 (1H, s), 7.27 (2H, m), 6.80 (3H, m), 4.65 (2H, s), 3.80 (3H, s), 3.76 (3H, s), 3.70 (3H, s).

Compound 30. Yield 87%; mp 166-168°C; IR (KBr, cm^{-1}) 3320, 2970, 1650, 1605, 1575, 1545, 1459, 1392, 1251, 1229; ^{1}H0NMR (200 MHz, $CDCl_3$) δ 8.33 (1H, s), 7.61 (1H, s), 7.28 (2H, m), 6.81 (3H, m), 4.68 (2H, s), 3.96 (2H, q, *J*=7.0 Hz), 3.83 (3H, s), 3.79 (3H, s), 1.30 (3H, t, *J*=7.0 Hz).

Compound 31. Yield 75%; mp 209-210°C; IR (KBr, cm^{-1}) 3345, 2940, 1655, 1553, 1495, 1450, 1390, 1340, 1300, 1250; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.22 (1H, s), 8.72 (1H, s), 8.11 (1H, d, *J*=8.8 Hz), 7.60 (1H, s), 7.48 (1H, d, *J*=8.8 Hz), 6.81 (2H, s), 4.76 (2H, s), 3.82 (3H, s), 3.76 (3H, s).

Compound 32. Yield 72%; mp 95-97°C; IR (KBr, cm^{-1}) 3320, 2945, 1645, 1525, 1442, 1405, 1272, 1225, 1050; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.40 (1H, t, *J*=5.7 Hz), 7.69 (1H, d, *J*=7.3 Hz), 7.53 (2H, s), 7.39 (1H, d, *J*=8.0 Hz), 7.21 (1H, t, *J*=8.0 Hz), 7.04 (1H, t, *J*=7.9 Hz), 6.58 (1H, s), 4.46 (2H, d, *J*=5.7 Hz), 3.87 (3H, s), 3.82 (3H, s).

Compound 33. Yield 72%; mp 181-182°C; IR (KBr, cm^{-1}) 3350, 2920, 1645, 1555, 1455, 1280, 1255, 1210, 1050, 965; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.37 (1H, t, *J*=5.6 Hz), 7.55 (2H, m), 7.33 (1H, m), 7.03 (1H, td, *J*=9.2 and 2.6 Hz), 6.53 (1H, s), 4.40 (2H, s), 3.84 (3H, s), 3.80 (3H, s).

Compound 34. Yield 68%; mp 165-167°C; IR (KBr, cm^{-1}) 3300, 2940, 1642, 1558, 1410, 1272, 1215, 1052, 958; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.68 (1H, t, *J*=5.8 Hz), 7.68 (1H, d, *J*=7.7 Hz), 7.57 (2H, s), 7.31 (1H, d, *J*=7.9 Hz), 7.03 (1H, t, *J*=7.9 Hz), 6.65 (1H, s), 4.48 (2H, s), 3.88 (3H, s), 3.85 (3H, s).

Compound 35. Yield 69%; mp 185-186°C; IR (KBr, cm^{-1}) 3350, 1630, 1545, 1435, 1280, 1255, 1210, 1050, 965; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.50 (1H, t, *J*=5.4 Hz), 7.80 (1H, d, *J*=2.0 Hz), 7.52 (2H, s), 7.34 (1H, d, *J*=8.6 Hz), 7.19 (1H, dd, *J*=8.6 and 2.0 Hz), 6.54 (1H, s), 4.42 (2H, s), 3.84 (3H, s), 3.80 (3H, s).

Compound 36. Yield 82%; mp 151-153°C; IR (KBr, cm^{-1}) 3300, 2940, 1645, 1580, 1559, 1532, 1470, 1405, 1270, 1210; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.39 (1H, d, *J*=7.8 Hz), 7.30 (2H, s), 7.09 (1H, d, *J*=7.3 Hz), 6.98 (1H, t, *J*=7.7 Hz), 6.87 (1H, s), 5.87 (1H, broad), 4.55 (2H, s), 3.97 (3H, s), 3.93 (3H, s), 2.53 (3H, s).

Compound 37. Yield 71%; mp 173-174°C; IR (KBr, cm^{-1}) 3340, 2900, 1650, 1470, 1279, 1258, 1210, 1050, 965, 810; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.28 (1H, t, *J*=5.6 Hz), 7.56 (2H, s), 7.47 (1H, s), 7.25 (1H, d, *J*=8.0 Hz), 7.01 (1H, d, *J*=8.0 Hz), 6.54 (1H, s), 4.44 (2H, s), 3.85 (3H, s), 3.81 (3H, s), 2.89 (3H, s).

Compound 38. Yield 73%; mp 129-130°C; IR (KBr, cm^{-1}) 3340, 2950, 1640, 1559, 1455, 1272, 1252, 1211, 1050, 960; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.23 (1H, s), 7.52 (2H, s), 7.40 (1H, s), 7.18 (1H, s), 6.55 (1H, s), 4.41 (2H, s), 3.86 (3H, s), 3.82 (3H, s), 2.19 (6H, s).

Compound 39. Yield 56%; mp 114-116°C; IR (KBr, cm^{-1}) 3350, 2950, 1645, 1545, 1469, 1252, 1212, 1058, 961; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.16 (1H, t, *J*=6.0 Hz), 7.53 (2H, s), 7.31 (1H, d, *J*=2.0 Hz), 7.26 (1H, d, *J*=8.8 Hz), 6.78 (1H, dd, *J*=8.8 and 2.0 Hz), 6.53 (1H, s), 4.38 (2H, s), 3.84 (3H, s), 3.70 3H, s).

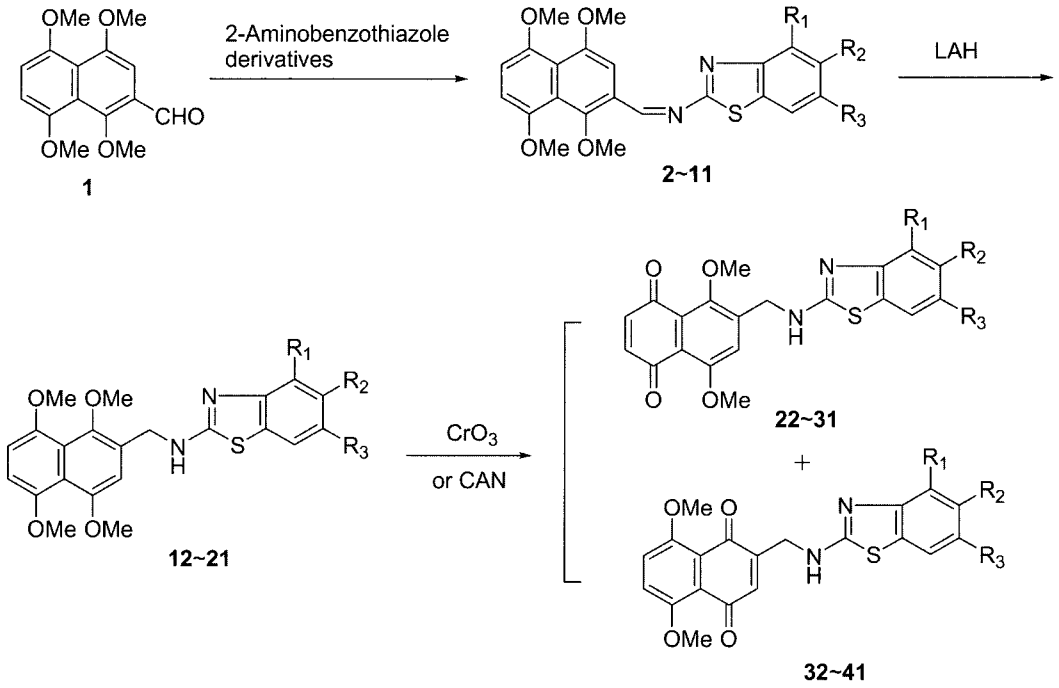
Compound 40. Yield 52%; mp 117-119°C; IR (KBr, cm^{-1}) 3330, 2920, 1647, 1600, 1579, 1550, 1460, 1400, 1270, 1250; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 8.17, (1H, t, *J*=5.6 Hz), 7.52 (2H, s), 7.38 (2H, m), 6.79 (1H, dd, *J*=8.6 and 2.4 Hz), 6.56 (1H, s), 4.41 (2H, s), 3.97 (2H, q, *J*=7.0 Hz), 3.86 (3H, s), 3.82 (3H, s), 1.30 (3H, t, *J*=7.0 Hz).

Compound 41. Yield 50%; mp 210°C; IR (KBr, cm^{-1}) 3300, 1640, 1545, 1500, 1439, 1408, 1330, 1300, 1210, 1120; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 9.06 (1H, t, *J*=5.6 Hz), 8.72 (1H, d, *J*=2.2 Hz), 8.08 (1H, dd, *J*=9.0 and 2.2 Hz), 7.54 (2H, s), 7.46 (1H, d, *J*=9.0 Hz), 6.57 (1H, s), 4.50 (2H, s), 3.85 (3H, s), 3.81 (3H, s).

RESULTS AND DISCUSSION

2-Formyltetramethoxynaphthalene (1) was prepared from 1,5-dihydroxynaphthalene through 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 substituted anilines at pH 5 and the resulting imine compounds were reduced to amine compounds **12-21** using LAH in good yields. We could obtain 6-substituted naphthoquinone derivatives **22-31** with a yield of 73-94% by oxidation with chromium (VI) oxide and 2-substituted naphthoquinone derivatives **32-41** with a yield of 51-82% by oxidation with cerium (IV) ammonium nitrate (CAN) from corresponding 2-substituted-1,4,5,8-tetramethoxynaphthalenes **12-21** (Scheme 1).

The cytotoxicity of naphthoquinone derivatives was measured against cancer cells L1210 and SNU-1 cells using the MTT colorimetric method (Carmichael, *et al.*, 1987). ED_{50} value (μg/mL) was defined as the concentration of compound to produce a 50% reduction in viability relative to the control in three independent experiments. The results were shown in Table I and II. It could be recognized that the solid cancer cell line SNU-1 were more resistant to the synthesized naphthoquinone derivatives, compared to the L1210 cells (Lymphocytic leukemia). The ED_{50} values of compounds **22-31** were 0.19 μg/mL - 0.56 μg/mL and those of compounds **32-41** were 0.09 μg/mL - 5.13 μg/mL against L1210. In a comparison, it was found that 6-substituted BZT-N derivatives exhibited higher antitumor activity than 2-substituted BZT-N derivatives in mice bearing S-180 cells in peritoneal cavity (Table I and II). However, interestingly, it was observed that 2-substituted compound **36,** which has methyl group at R_1 position, exhibited a better inhibitory activity than 6-substituted compounds against L1210 and SNU-1 *in vitro*. The ED_{50}



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

Table I. Cytotoxic activity and antitumor activity of 6-[(substituted 1,3-benzothiazol-2-yl)aminomethyl]-5,8-dimethoxy-1,4-naphthoquinones

Compound	R_1	R_2	R_3	ED_{50} (μg/mL)		T/C (%)
				L1210	SNU-1	
22	H	H	H	0.22	1.00	205
23	H	H	F	0.37	3.52	185
24	Cl	H	H	0.29	1.06	189
25	H	H	Cl	0.56	4.91	182
26	CH_3	H	H	0.19	1.43	172
27	H	H	CH_3	0.24	1.75	196
28	H	CH_3	CH_3	0.51	2.34	221
29	H	H	OCH_3	0.28	1.72	192
30	H	H	OCH_2CH_3	0.28	2.85	189
31	H	H	NO_2	0.53	2.50	177
Adriamycin				0.07	2.01	234

Table II. Cytotoxic activity and antitumor activity of 2-[(substituted 1,3-benzothiazol-2-yl)aminomethyl]-5,8-dimethoxy-1,4-naphthoquinones

Compound	R_1	R_2	R_3	ED_{50} (μg/mL)		T/C (%)
				L1210	SNU-1	
32	H	H	H	1.78	1.26	108
33	H	H	F	0.45	1.56	101
34	Cl	H	H	1.71	1.75	109
35	H	H	Cl	1.89	1.31	107
36	CH_3	H	H	0.09	0.65	241
37	H	H	CH_3	0.69	0.95	110
38	H	CH_3	CH_3	1.11	1.16	105
39	H	H	OCH_3	0.12	1.60	105
40	H	H	OCH_2CH_3	5.13	1.12	103
41	H	H	NO_2	0.91	2.06	104
Adriamycin				0.07	2.01	234

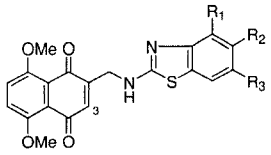
value of 2-substituted compound **36** against L1210 was found to be comparable to the ED_{50} value of adriamycin and was even better against the solid cancer cell line SNU-1. It was also observed that 2-substituted compound **36** showed better antitumor activity in mice bearing S-180 cells in the peritoneal cavity. The T/C (%) value of 2-substituted compound **36** was similar to that of adriamycin. In earlier works, we observed that the cytotoxic activity was dependent upon the location of the substituent groups. In the case of naphthoquinone derivatives with a propenoate substituent functional group at C6 or C2 position, we had shown that 6-substituted derivatives were more effective than the 2-substituted derivatives and the introduction of electronegative fluorine on the benzoyl group increased the cytotoxicity (Cho *et al.*, 1998). This result was in accord with the works reported by other researchers and it was said that the C2 or C3 of 6-substituted compounds would be better Michael acceptors than the C3 of 2-substituted compounds and attacked more easily by nucleophiles such as amine or thiol functional groups in the cell (You, *et al.*, 1998a, 1998b; Song, *et al.*, 2001). As evidenced in earlier reports (You *et al.*, 1998a; Chae,

et al., 1999), steric hindrance of C-2 substituent of the naphthoquinone derivatives may explain the lower cytotoxic activity of 2-substituted derivatives. Also, electron density in the quinoid ring may be important. Previously, it had been reported that an electron withdrawing group such as acetoxy or oxo group at C1 in side chain of naphthoquinone analogues enhanced cytotoxic activity (You *et al.*, 1998a). Table III indicates the relationship between cytotoxic activity and ^{1}H-NMR chemical shifts of 2-substituted BZT-N derivatives. Compound **36**, which possess a higher electrophilicity of C3 position, was found to be good cytotoxic activity than others. The relatively high cytotoxic activity of 2-substituted compound **36**, despite steric hindrance, could be explained by the assumption that steric effect of side chain at C2 position could be compensated by the electrophilicity of the C3 position. It was also observed that 6-substituted BZT-N derivatives showed better antitumor activity than 2-substituted BZT-N derivatives in mice bearing S-180 cells in the peritoneal cavity (Table I and II). However, compound **36** also exhibited a higher antitumor activity than 6-substituded BZT-N derivatives.

Quantitative structure-activity relationship (QSAR) tests reveal that the experimental ED_{50} values against SNU-1 closely correlate with both the calculated HOMO energies (E_{HOMO}) and the measured ^{1}H-NMR chemical shift of 3-H (δ_H). By using the least-squares procedure, we obtain

$$ED_{50}(\text{SNU-1}) = -21.21 \cdot E_{HOMO} - 2.008 \cdot \delta_H + 8.355 \ (\mu g/mL) \quad (1)$$

Table III. Correlation of the ED_{50} values (in μg/mL) against SNU-1 with the HOMO energy (E_{HOMO}, in Hartree) and ^{1}H-NMR chemical shifts (δ_H, in ppm) of 2-[(substituted 1,3-benzothiazol-2-yl)aminomethyl]-5,8-dimethoxy-1,4-naphthoquinones



Compound	R_1	R_2	R_3	ED_{50}(SNU-1) Calc.	ED_{50}(SNU-1) Expt.	E_{HOMO}	δ_H
32	H	H	H	1.406	1.26	-0.295	6.58
33	H	H	F	1.528	1.56	-0.296	6.53
34	Cl	H	H	1.393	1.75	-0.301	6.65
35	H	H	Cl	1.508	1.31	-0.296	6.54
36	CH_3	H	H	0.739	0.65	-0.291	6.87
37	H	H	CH_3	1.296	0.95	-0.286	6.54
38	H	CH_3	CH_3	1.233	1.16	-0.284	6.55
39	H	H	OCH_3	1.188	1.60	-0.280	6.53
40	H	H	OCH_2CH_3	1.086	1.12	-0.278	6.56
41	H	H	NO_2	2.042	2.06	-0.324	6.57

with a correlation coefficient of 0.822 and root-mean-square (RMS) deviation of 0.22 μg/mL. In Equation (1), the descriptors E_{HOMO} and δ_H values are given in atomic unit (*i.e.* Hartree; 1 Hartree = 627.51 kcal/mol) and ppm, respectively. The results are summarized in Table III and depicted in Fig. 1. Depicted in Fig. 2 are the optimized geometries of molecules **36** and **41**, in which the net atomic charges were obtained from the natural population analysis on the molecular orbitals calculated at the HF/6-31G* level. Equation (1) suggests that a compound

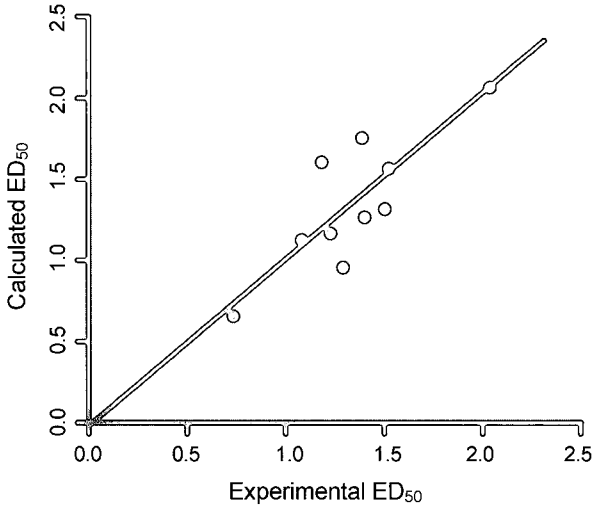


Fig. 1. Plot of the calculated ED_{50} values *versus* the experimental ED_{50} values (in μg/mL) against SNU-1

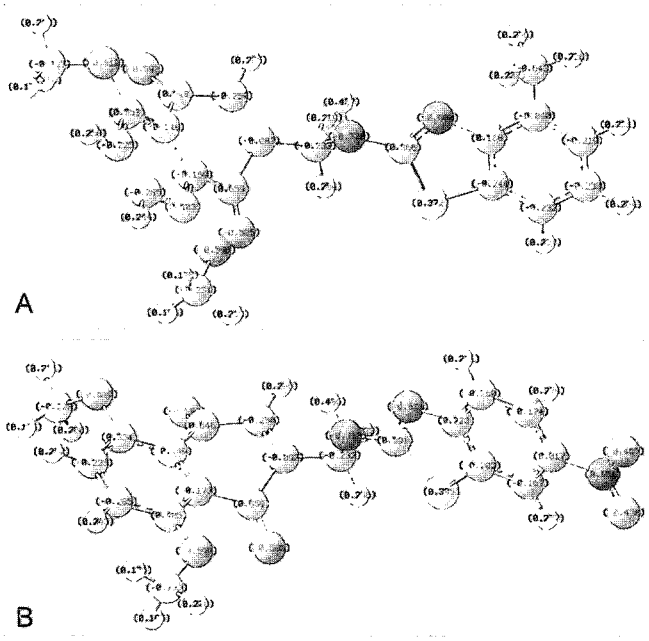


Fig. 2. Optimized geometries of molecules **36** and **41**, in which the net atomic charges were obtained from the natural population analysis on the molecular orbitals calculated at the HF/6-31G* level. A, compound **36**; B, compound **41**

having higher E_{HOMO} and δ_H values usually should have a lower ED_{50} (SNU-1) value.

ACKNOWLEDGEMENT

This work was supported by Chungbuk National University.

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