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Synthesis, Cytotoxicity and Topoisomerase II Inhibitory Activity of Benzonaphthofurandiones

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Benzonaphthofurandiones containing four coplanar fused aromatic rings were synthesized and evaluated for their cytotoxicity against five human cancer cell lines, and their inhibitory activity on topoisomerases. These benzonaphthofurandiones were prepared by condensation of 2,3-dichloronaphthoquinone and three aromatic diols with base catalysts in alcohol. The synthesized compounds were *o*-alkylated with six dialkylaminoalkyl halides. The hydroxy derivatives (**8a**-**8g**) exhibited relatively potent cytotoxicity among the prepared compounds. These compounds were evaluated as excellent inhibitors against topoisomerase II (topo II). Especially, the hydroxy analogue with branched methyl side chain (**8e**) showed high cytotoxicity against cancer cell lines and good inhibitory activity on topo II.

Key Words : Benzonaphthofurandiones, Cytotoxicity, Topoisomerase II

Introduction

The benzonaphthofurandiones are an important class of compounds belong to quinone derivatives having a variety of biological activities. Coenzyme Q (**1**) and Vitamin K (**2**), are also benzoquinone and a naphthoquinone derivatives, respectively (Figure 1)[1]. These two classes of compounds are acting as an electron carrier in the biological system, and the cytotoxicity of quinones is correlated to their chemical actions. They can accept one electron to form the semiquinone radical, followed by accepting one more electron to give the hydroquinone.[2] Mitomycin C (**3**), a bioreductive alkylating agent, expresses antitumor activity by the reductive redox-cycling hydroquinone.[3] Another representative quinone derivative with antitumor activity, Doxorubicin (**4**), acts as an intercalater. Although it shows anticancer properties through intercalation-induced DNA binding, the functional group of Doxorubicin, available to generate hydrogen bond with DNA, plays an important role for the compound to anchor strongly to DNA double helix as well.[4] They cause an extensive increase in DNA strand breaks, which stimulate various cellular processes including apoptosis that finally lead to cell death. There is a coincidence that the antitumor activity of anthracyclines mainly results from an inhibition of topoisomerase II (topo II).[5,6] Topoisomerases are essential enzymes regulating DNA topology through single- (topo I) or double-strand breaks (topo II) during normal cellular growth. They are potentially involved in DNA metabolism including replication, transcription and chromosome conformational changes.[7] Therefore, they have been identified as clinically important targets for cancer chemotherapy and their inhibitors are principal factors in many therapeutic systems. Topo II forms a covalent linkage to both strands of the DNA helix and catalyzed a transient DNA double strand break, allowing for the passing of the intact double helix and religation of the cleaved DNA.[5,6] This delicate and susceptible process makes topo II an attractive target for anticancer drugs. [8] In our laboratory, the study for development of various heterocyclic quinones as an anticancer agent with topo II inhibitory activity has been continued.[9-11] However, the intrinsic cytotoxicity related to these benzonaphthofurandiones[12,13] prompted us to pursue the preparation and cytotoxicity study on the more promising anlogues towards the five human tumor cell lines.

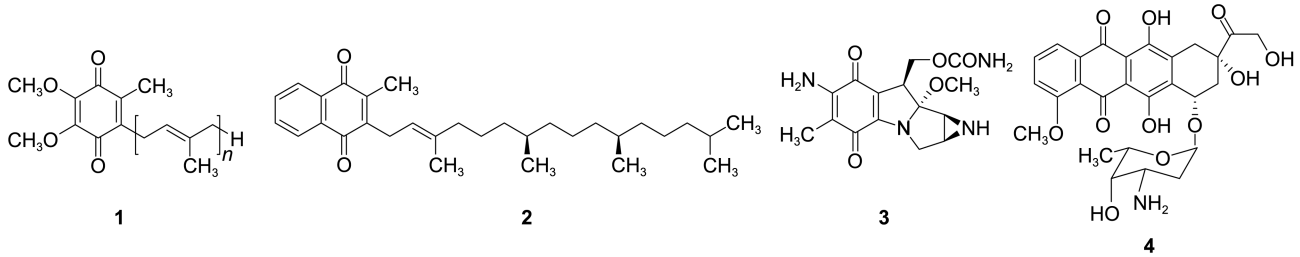

Figure 1. Natural quinones.

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Inhibitory activity against topo I and II have been evaluated in this study too.

Experimental Section

Synthesis. All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Büchi). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity Inova 400 MHz NMR spectrometer and Bruker Avance 400 MHz NMR spectrometer using tetramethylsilane as an internal standard. Samples were dissolved in $CDCl_3$ or DMSO-d_6. Mass spectra were obtained on the Mass spectrometer JMS-700 (Jeol, Japan) at the Korea Basic Science Institute (Daegu). Most of the reagents were purchased from Sigma-Aldrich Chemical Company.

General procedure for the preparation of benzonaphthofurandiones followed the method of previous paper.[12] To a stirred ethanolic sodium ethoxide solution or solution of methanol containing KOH was added portionwise at less than 20 °C of powdered 2,3-dichloronaphthoquinone. After stirring for about 10 minutes, the alcoholic solutions of phloroglucinol or resorcinol or 4-chlororesorcinol were added dropwise with continuous stirring, respectively. The mixture was stirred at room temperature overnight. The reaction mixture was acidified with HCl at 0 °C. The resulting solid was collected by filtration, washed successively with water, methanol, diethyl ether and dried *in vacuo* at 60 °C. These were recrystallized from dimethylformamide (DMF).

1,3-Dihydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (7a). Dark brown solid (28%); mp > 310 °C; ^{1}H NMR (DMSO-d_6) δ 10.46 (s, 1H), 9.69 (s, 1H), 8.15 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 9.2 Hz, 1H), 7.86-7.96 (m, 2H), 6.69 (s, 1H), 6.40 (s, 1H); HR-FABMS Calcd for $C_{16}H_8O_5$ $(M+H)^+$: 281.0450, Found: 281.0448.

3-Hydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (7b). Deep yellow solid (24%); mp > 310 °C; ^{1}H NMR (DMSO-d_6) δ 10.53 (s, 1H), 8.10-8.13 (m, 2H), 8.00 (d, J = 8.8 Hz, 1H), 7.88-7.90 (m, 1H), 6.69 (s, 1H), 7.08 (d, J = 8.8 Hz, 1H); HR-FABMS Calcd for $C_{16}H_8O_5$ $(M+H)^+$: 265.0501, Found: 265.0505.

2-Chloro-3-hydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (7c). Brick red solid (31%); mp 309-310 °C; ^{1}H NMR (DMSO-d_6) δ 11.37 (s, 1H), 8.11-8.13 (m, 2H), 8.08 (s, 1H), 7.89-7.92 (m, 2H), 7.41 (s, 1H); HR-FABMS Calcd for $C_{16}H_7ClO_4$ $(M+H)^+$: 299.0111, Found: 299.0112.

General Procedure for the Preparation of (dialkylamino)alkoxy-benzo[*b*]naphtha [2,3-*d*]furan-6,11-diones. To a suspension of 0.15 mmol of benzo[*b*]naphtho[2,3-*d*]furan-6,11-diones in 10 mL of $CHCl_3$, a solution of 1.20 mmol K_2CO_3 and 0.01 mmol benzyltriethylammonium chloride in 2 mL of H_2O was added followed by 0.3 mmol of (dialkylamino)alkyl chloride hydrochloride in 2 mL of H_2O. The mixture was stirred at rt for 1h and refluxed overnight with vigorous stirring. It was cooled and the organic layer was separated. The aqueous portion was extracted with $CHCl_3$ (2 × 10 mL). The combined organic phase was washed with brine and water and dried (anhydrous Na_2SO_4). The solids were acquired by evaporation of the solvent, which gave pure products upon recrystallization (from $CHCl_3$-MeOH) or flash column chromatography ($CHCl_3$/MeOH=2/1).

3-[2-(Dimethylamino)ethoxy]-1-hydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (8a).[13] Black solid (19%); mp 220-221 °C; ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1H), 8.21-8.27 (m, 2H), 7.76-7.85 (m, 2H), 6.72 (s, 1H), 6.58 (s, 1H), 4.13 (t, J = 5.8 Hz, 2H), 2.77 (t, J = 5.6 Hz, 2H), 2.36 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.15, 173.61 (2C, carbonyl), 163.88, 158.74, 153.03, 151.35 (4C, aromatic-O-), 135.01, 133.85, 132.81, 131.89, 127.35, 127.18, 126.12, 106.35, 100.18, 89.65 (10C, aromatic), 66.95 (1C, O-C-), 58.05 (1C, -C-N), 45.94 (2C, N-CH_3 x 2); HR-FABMS Calcd for $C_{20}H_{17}NO_5$ $(M+H)^+$: 352.1185, Found: 352.1185.

3-[2-(Dimethylamino)propoxy]-1-hydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione(8b).[13] Black solid (22%); mp 209-210 °C; ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1H), 8.21-8.27 (m, 2H), 7.76-7.85 (m, 2H), 6.70 (s, 1H), 6.55 (s, 1H), 4.10 (t, J = 6.4 Hz, 2H), 2.51 (t, J = 7.4 Hz, 2H), 2.30 (s, 6H), 1.97-2.07 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.21, 173.64 (2C, carbonyl), 164.02, 158.83, 153.07, 150.06 (4C, aromatic-O-), 135.08, 133.85, 132.84, 131.91, 127.37, 127.20, 126.18, 125.10, 100.15, 89.52 (10C, aromatic), 56.13 (1C, -C-N), 47.45 (2C, N-CH_3 x 2), 22.70 (1C, -CH_2-); HR-FABMS Calcd for $C_{21}H_{19}NO_5$ $(M+H)^+$: 366.1341, Found: 366.1338.

3-[2-(Diethylamino)ethoxy]-1-hydroxybenzo[*b*]naphtho-[2,3-*d*]furan-6,11-dione (8c).[13] Black solid solid (58%); mp 204-205 °C; ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1H), 8.21-8.26 (m, 2H), 7.76-7.85 (m, 2H), 6.71 (s, 1H), 6.55 (s, 1H), 4.12 (t, J = 6.0 Hz, 2H), 2.92 (t, J = 6.2 Hz, 2H), 2.67 (q, J = 7.2 Hz, 4H), 1.09 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.32, 173.75 (2C, carbonyl), 164.15, 158.96, 153.19, 151.49 (4C, aromatic-O-), 135.24, 134.01, 133.01, 132.08, 127.53, 127.36, 126.30, 106.47, 100.44, 89.71 (10C, aromatic), 67.83 (1C, O-C-), 51.74 (1C, -C-N), 48.14 (2C, N-CH_2- x 2). 12.10 (2C, -CH_3 x2); HR-FABMS Calcd for $C_{22}H_{21}NO_5$ $(M+H)^+$: 380.1498, Found: 380.1494.

3-[2-[Bis(1-methylethyl)amino]ethoxy]-1-hydroxybenzo-[*b*]naphtho[2,3-*d*]furan-6,11-dione (8d).[13] Brick red solid (78%); mp 197-198 °C; ^{1}H NMR ($CDCl_3$) δ 9.59 (s, 1H), 8.18-8.26 (m, 2H), 7.73-7.83 (m, 2H), 6.73 (s, 1H), 6.50 (s, 1H), 4.73 (t, J = 6.0 Hz, 2H), 3.72-3.80 (m, 2H), 3.39 (q, J = 5.6 Hz, 2H), 1.57 (d, J = 6.4 Hz, 6H), 1.48 (d, J = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.22, 173.61 (2C, carbonyl), 164.14, 158.87, 153.02, 151.29 (4C, aromatic-O-), 135.06, 133.82, 132.86, 131.92, 127.36, 127.18, 126.20, 106.20, 100.25, 89.50 (10C, aromatic), 70.18 (1C, O-C-), 49.69 (2C, N-CH- x 2), 44.20 (1C, -CH_2-N), 20.90 (4C, -CH_3 x4); HR-FABMS Calcd for $C_{24}H_{25}NO_5$ $(M+H)^+$: 408.1811, Found: 408.1811.

3-[2-(Dimethylamino)isopropoxy]-1-hydroxybenzo[*b*]-naphtho[2,3-*d*]furan-6,11-dione (8e). Brown solid (16%); mp 135-136 °C; ^{1}H NMR ($CDCl_3$) δ 9.52 (s, 1H), 8.21-8.27 (m, 2H), 7.76-7.85 (m, 2H), 6.75 (s, 1H), 6.56 (s, 1H), 4.54-4.66 (m, 1H), 2.67-2.79 (m, 2H), 2.34 (s, 6H), 1.37(d, J = 6.4

Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.19, 173.62 (2C, carbonyl), 163.02, 158.87, 153.13, 151.36 (4C, aromatic-O-), 135.06, 133.83, 132.86, 131.92, 127.36, 127.19, 126.17, 106.24, 101.08, 90.81 (10C, aromatic), 73.34 (1C, O-C-), 64.53 (1C, -CH_2-N), 46.27 (2C, N-CH_3 x 2) 18.33 (1C, -CH_3); HR-FABMS Calcd for $C_{21}H_{19}NO_5$ $(M+H)^+$: 366.1341, Found: 366.1340.

3-[2-(Dimethylamino)propoxy]-1-hydroxybenzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (8f). Dark brown solid (12%); mp 196-197 °C; ^{1}H NMR ($CDCl_3$) δ 9.55 (s, 1H), 8.21-8.27 (m, 2H), 7.76-7.85 (m, 2H), 6.72 (s, 1H), 6.57 (s, 1H), 4.08-4.15 (m, 1H), 3.87-3.98 (m, 1H), 2.97-3.10 (m, 1H), 2.39 (s, 6H), 1.18 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.15, 173.46 (2C, carbonyl), 161.80, 157.85, 152.63, 151.78 (4C, aromatic-O-), 135.14, 134.23, 132.32, 131.90, 126.77, 126.55, 125.41, 106.40, 99.88, 89.95 (10C, aromatic), 67.38 (1C, O-C-), 59.11 (1C, -CH_2-N), 13.91 (1C, -CH_3); HR-FABMS Calcd for $C_{21}H_{19}NO_5$ $(M+H)^+$: 366.1341, Found: 366.1364.

3-[2-(Morpholino)ethoxy]-1-hydroxybenzo[*b*]naphtho-[2,3-*d*]furan-6,11-dione (8g). Brick brown solid (18%); mp 218-219 °C; ^{1}H NMR ($CDCl_3$) δ 9.56 (s, 1H), 8.22-8.27 (m, 2H), 7.77-7.86 (m, 2H), 6.71 (s, 1H), 6.56 (s, 1H), 4.18(t, *J* = 5.6 Hz, 2H), 3.75 (t, *J* = 4.8 Hz, 4H), 2.85 (t, *J* = 5.2 Hz, 2H), 2.60 (t, *J* = 4.4 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.16, 173.62 (2C, carbonyl), 163.70, 158.73, 153.10, 151.40 (4C, aromatic-O-), 135.09, 133.88, 132.81, 131.89, 127.38, 127.21, 100.22, 98.80, 89.60 (10C, aromatic), 66.94 (two overlapping signals), 66.79 (3C, O-C-), 57.39, 54.15 (two overlapping signals) (3C, -CH_2-N); HR-FABMS Calcd for $C_{22}H_{19}NO_6$ $(M+H)^+$: 394.1291, Found: 394.1292.

3-[2-(Dimethylamino)ethoxy]benzo[*b*]naphtho[2,3-*d*]-furan-6,11-dione (8h). Yellow solid (64%); mp 239-240 °C; ^{1}H NMR ($CDCl_3$) δ 8.20-8.25 (m, 2H), 8.16 (d, *J* = 7.6 Hz, 1H), 7.74-7.79 (m, 2H), 7.17 (s, 1H), 7.15 (d, *J* = 7.2 Hz, 1H), 4.18 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 5.2 Hz, 2H), 2.39 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.06, 174.90 (2C, carbonyl), 161.41, 158.17, 153.04 (3C, aromatic-O-), 134.91, 133.45, 132.93, 126.90, 124.44, 124.20, 117.29, 115.91, 114.79, 99.13, 98.08 (11C, aromatic), 58.13 (1C, -C-N), 46.19 (2C, N-CH_3 x 2); HR-FABMS Calcd for $C_{20}H_{17}NO_4$ $(M+H)^+$: 336.1236, Found: 336.1236.

3-[2-(Dimethylamino)propoxy]benzo[*b*]naphtho[2,3-*d*]-furan-6,11-dione (8i). Yellow solid (28%); mp 225-226 °C; ^{1}H NMR ($CDCl_3$) δ 8.20-8.25 (m, 2H), 8.15 (d, *J* = 8.8 Hz, 1H), 7.74-7.79 (m, 2H), 7.15 (s, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 4.13 (t, *J* = 6.2 Hz, 2H), 2.50 (t, *J* = 7.4 Hz, 2H), 2.29 (s, 6H), 1.99-2.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.30, 174.93 (2C, carbonyl), 161.49, 158, 153, (3C, aromatic-O-), 133.89, 133.85, 132.57, 126.81, 126.70, 124.22, 116.66, 115.91, 96.79 (11C, aromatic), 66.95 (1C, O-C-), 56.20 (1C, -C-N), 45.52 (2C, N-CH_3 x 2), 27.35 (1C, -CH_2-); HR-FABMS Calcd for $C_{21}H_{19}NO_4$ $(M+H)^+$: 350.1392, Found: 350.1392.

3-[2-(Morpholino)ethoxy]benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (8j). Yellow solid (37%); mp 226-227 °C; ^{1}H NMR ($CDCl_3$) δ 8.20-8.26 (m, 2H), 8.17 (d, *J* = 9.2 Hz, 1H), 7.76-7.79 (m, 2H), 7.15 (s, 1H), 7.13 (s, 1H), 4.21(t, *J* = 5.6 Hz, 2H), 3.76 (t, *J* = 4.6 Hz, 4H), 2.87 (t, *J* = 5.6 Hz, 2H), 2.61 (t, *J* = 4.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.71, 174.89 (2C, carbonyl), 160.96, 158.01, 152.97 (3C, aromatic-O-), 133.97, 133.90, 133.21, 132.50, 126.84, 126.73, 124.65, 124.36, 116.63, 96.91 (11C, aromatic), 69.82, 66.77 (two overlapping signals) (3C, O-C-), 57.36, 54.01 (two overlapping signals) (3C, -CH_2-N); HR-FABMS Calcd for $C_{22}H_{19}NO_5$ $(M+H)^+$: 378.1341, Found: 378.1337.

2-Chloro-3-[2-(dimethylamino)ethoxy]benzo[*b*]naphtho-[2,3-*d*]furan-6,11-dione (8k). Yellow solid (15%); mp 226-227 °C; ^{1}H NMR ($CDCl_3$) δ 9.74 (s, 1H), 8.35 (s, 1H), 8.22-8.27 (m, 2H), 7.78-7.82 (m, 2H), 4.74 (t, *J* = 4.4 Hz, 2H), 3.57-3.61 (m, 2H), 2.17 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.29, 174.74 (2C, carbonyl), 156.27, 156.22, 153.29 (3C, aromatic-O-), 134.11, 134.00, 133.10, 132.42, 126.92, 126.83, 124.16, 124.04, 123.38, 115.92, 96.79 (11C, aromatic), 68.60 (1C, O-C-), 57.69 (1C, -C-N), 46.27 (2C, N-CH_3 x 2); HR-FABMS Calcd for $C_{20}H_{16}ClNO_4$ $(M+H)^+$: 370.0846, Found: 370.0848.

2-Chloro-3-[2-(dimethylamino)propoxy]benzo[*b*]naphtho-[2,3-*d*]furan-6,11-dione (8l). Dark yellow solid (16%); mp 216-217 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 8.21-8.26 (m, 2H), 7.77-7.80 (m, 2H), 7.22 (s, 1H), 4.22 (t, *J* = 6.2 Hz, 2H), 2.55-2.76 (m, 2H), 2.37 (s, 6H), 1.96-2.06 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.37, 174.72 (2C, carbonyl), 162.06, 156.35, 151.20 (3C, aromatic-O-), 133.99, 133.09, 132.43, 126.91, 124.05, 123.35, 118.00, 96.77 (**11C**, aromatic), 67.76 (1C, O-C-), 55.92, 45.29 (two overlapping signals) (3C, -CH_2-N), 29.71 (1C, -C-); HR-FABMS Calcd for $C_{21}H_{18}ClNO_4$ $(M+H)^+$: 384.1003, Found: 384.1004.

2-Chloro-3-[2-(morpholino)ethoxy]benzo[*b*]naphtho[2,3-*d*]furan-6,11-dione (8m). Pale brown solid (14%); mp 236-237 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (s, 1H), 8.21-8.26 (m, 2H), 7.77-7.80 (m, 2H), 7.21 (s, 1H), 4.27 (t, *J* = 5.4 Hz, 2H), 3.75 (t, *J* = 4.4 Hz, 4H), 2.94 (t, *J* = 5.0 Hz, 2H), 2.67 (t, *J* = 4.4 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.46, 174.94 (2C, carbonyl), 161.10, 156.37, 153.55 (3C, aromatic-O-), 134.34, 134.23, 133.29, 132.61, 127.14, 127.05, 124.41, 123.54, 116.23, 97.04 (11C, aromatic), 68.61, 67.19 (two overlapping signals) (3C, O-C-), 57.28, 54.52 (two overlapping signals) (3C, -CH_2-N); HR-FABMS Calcd for $C_{22}H_{18}O_5$ $(M+H)^+$: 412.0952, Found: 412.0949.

***In vitro* Antitumor Activity Evaluation by SRB Assay.** The *in vitro* cytotoxic activities were evaluated by SRB method. The human tumor cell lines; lung (A549), stomach (SNU-638), colon (HCT116) and fibro sarcoma (HT1080) (5×10^4 cells/mL) were treated with different concentrations of the test agents for 3 days. After treatment, cells were fixed with TCA and cell viability was determined with sulforhodamine B (SRB) protein staining method. The result was expressed as a percentage, relative to solvent-treated control incubations, and the IC_{50} values were calculated using non-linear regression analysis (percent survival *versus* concentration). Myeloid leukemic (HL-60) cell was cultured according to the supplier's instructions. Cells were seeded in 96-well plates at a density of $2\sim4 \times 10^4$ cells per a well and

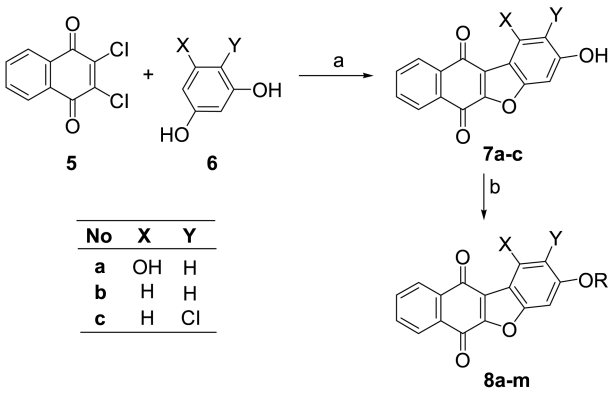
incubated for overnight in 0.1 mL of media supplied with 10% Fetal Bovine Serum (Hyclone, USA) in 5% CO_2 incubator at 37 °C. On day 2, culture medium in each well was exchanged with 0.1 mL aliquots of medium containing graded concentrations of compounds. On day 4, each well was added with 5 μL of the cell counting kit-8 solution (Dojindo, Japan) then incubated for additional 4 h under the same condition. The absorbance of each well was determined by an Automatic Elisa Reader System (Bio-Rad 3550) with a 450 nm wavelength. For determination of the IC_{50} values, the absorbance readings at 450 nm were fitted to the four-parameter logistic equation. The compounds of doxorubicin, etoposide, and camptothecin were purchased from Sigma and used as positive controls.[14,15]

DNA Relaxation Assay of Topoisomerase I. Experiments were performed as described previously.[15] The test compounds were dissolved in DMSO at 20 mM as stock solution. The activity of DNA topoisomerase I (topo I) was determined by assessing the relaxation of supercoiled DNA pBR322. The mixture of 100 ng of plasmid pBR322 DNA and 0.2 units of calf thymus DNA topo I (Fermentas, USA) was incubated without and with the prepared compounds at 37 °C for 30 minutes in the relaxation buffer (35 mM Tris-HCl; pH 8.0, 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin). The reaction in the final volume of 10 μL was terminated by adding 2.5 μL of the stop solution containing 10% SDS, 0.2% bromophenol blue, 0.2% xylene cyanol and 30% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

DNA Relaxation Assay of Topoisomerase II. DNA topoisomerase II (topo II) inhibitory activity of compounds was measured as follows.[15] The mixture of 100 ng of supercoiled pBR322 plasmid DNA and 1 units of human DNA topoisomerase IIα (Amersham, USA) was incubated without and with the prepared compounds in the assay buffer (10 mM Tris-HCl; pH 7.9) containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin) for 30 min at 30 °C. The reaction in a final volume of 20 μL was terminated by the addition of 3 μL of 7 mM EDTA. Reaction products are analyzed on a 1% agarose gel at 25 V for 4 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager™ (Alpha Innotech Corporation).

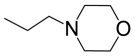
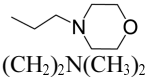
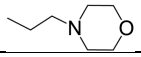
Results and Discussion

Chemistry. Condensation of 2,3-dichloronaphthoquinone (**5**) and **6** (Phloroglucinol **6a**, or resorcinol **6b**, or 4-chlororesorcinol **6c**) was achieved by modification of the reported reaction conditions.[12] Treatment of the synthesized benzonaphthofurandiones (**7a-7c**) with dialkylaminoalkyl chloride in base and phase transfer catalyst (PTC, benzyltriethylammonium chloride) gave the corresponding dialkylaminoalkoxy derivatives, as shown in Scheme 1. Compounds **7a-7c** were synthesized by base-catalyzed condensation of compound **5** with phenolic derivatives (**6a-6c**) in alcohols. The compounds were purified by recrystallization in dimethylformamide (DMF). The dialkylaminoalkoxy derivatives (**8a-8m**) were prepared by base-catalyzed condensation of prepared compounds **7a-7c** with dialkylaminoalkyl chlorides. The products were purified by recrystallization in co-solvent of chloroform and methanol or flash column chromatography (chloroform/methanol=4/1) with 12-84% yields. Introduction of (dimethylamino)isopropyl group to compounds **7a** gave two isomers for each compound (**8e** and **8f**, respectively). Two isomers were isolated by column chromatography and rearrangement of methyl group was identified on the ^1H NMR spectra.



No	X	Y
a	OH	H
b	H	H
c	H	Cl

a. Na in EtOH or KOH in MeOH
b. RCl·HCl, K_2CO_3, benzyltriethylammonium chloride in $CHCl_3$ and H_2O

No	X	Y	R
8a	OH	H	$(CH_2)_2N(CH_3)_2$
8b	OH	H	$(CH_2)_3N(CH_3)_2$
8c	OH	H	$(CH_2)_2N(C_2H_5)_2$
8d	OH	H	$(CH_2)_2N\{CH(CH_3)_2\}_2$
8e	OH	H	$CH(CH_3)CH_2N(CH_3)_2$
8f	OH	H	$CH_2CH(CH_3)N(CH_3)_2$
8g	OH	H	
8h	H	H	$(CH_2)_2N(CH_3)_2$
8i	H	H	$(CH_2)_3N(CH_3)_2$
8j	H	H	
8k	H	Cl	$(CH_2)_2N(CH_3)_2$
8l	H	Cl	$(CH_2)_3N(CH_3)_2$
8m	H	Cl	

Scheme 1. Synthesis of benzonaphthofurandiones.

Cytotoxic Activity. The *in vitro* cytotoxic activities of the synthesized compounds were evaluated against five human tumor cell lines (lung; A549, stomach; SNU-638, colon; HCT116, fibro sarcoma; HT1080, myeloid leukemic; HL-60) by SRB assay method.[14] The inhibitory activities were

Table 1. Cytotoxicity of benzonaphthofurandiones against human cancer cells

Compound	IC_{50} (μM)				
	A549	SNU-638	HCT 116	HT 1080	HL-60
8a	0.044	0.069	0.0096	0.145	0.05
8b	0.28	0.61	0.29	0.72	0.04
8c	0.088	0.1	0.0091	0.017	0.18
8d	0.33	0.26	0.18	0.18	0.16
8e	0.07	0.15	0.17	0.30	0.02
8f	0.08	0.24	0.28	0.18	0.46
8g	1.54	4.39	1.88	1.26	2.00
8h	0.6	2.35	0.27	0.26	0.47
8i	1.04	1.4	1.25	0.38	0.81
8j	12.36	> 20	> 20	19.45	1.44
8k	1.74	2.57	0.93	1.27	0.44
8l	1.55	1.43	1.43	0.99	1.33
8m	13.11	> 20	17.1	11.88	0.75
Doxorubicin	0.062	0.16	0.064	0.018	0.04

A549; human lung tumor cell line, SNU-638; human stomach tumor cell line, HCT116; human colon tumor cell line, HT1080; human fibro sarcoma tumor cell line, HL-60; human myeloid leukemic tumor cell line

Table 2. Inhibitory activity of benzonaphthofurandiones on topoisomerases

Compound	Relaxation activity for Topo II (% Inhibition)	Relaxation activity for Topo I (% Inhibition)
8a	72	6
8b	**100**	22
8c	48	4
8d	39	5
8e	**100**	6
8f	**100**	8
8g	31	7
8h	68	5
8i	85	6
8j	25	5
8k	58	6
8l	74	30
8m	32	4
Etoposide (200 μM)	89	-
Camptothecin (100 μM)	-	83
Doxorubicin (5 μM)	78	-

The assays were done at a concentration of 5 μM of samples, respectively.

presented as micromolar concentrations of the compounds and compared with that of doxorubicin, clinically used agents for the treatment of solid tumors (Table 1). In general, benzonaphthofurandiones with hydroxyl group (**8a-8g**) exhibited more potent inhibitory activity against all tested cancer cell lines than the corresponding hydrogen or chlorine congeners.[12]

The cytotoxicty of compounds **8a-8d** against HL-60 was reported,[13] but these compounds were synthesized for a structure activity relationship on other cancer cell lines. The cytotoxicity of compounds **8a** and **8c** were similar or superior to that of doxorubicin. Compounds containing branched methyl side chain (**8e**, and **8f**) presented relatively high activity: the cytotoxiciy was higher than non-branched congener **8b**, but lower than non-branched congener **8a**. However, the introduction of chlorine at Y generally reduced the cytotoxicity compared with hydrogen congeners (**8h** *vs* **8k**, **8i** *vs* **8l**).

Molecules with bulky groups, such as bis(1-methylethyl)-amino group (**8d**) or morpholino group (**8g**, **8j** and **8m**), showed low cytotoxic activity in comparison with the other.

Inhibitory Activity for Topoisomerases. Topoisomerase relaxation assay was conducted using human topo I and topo II with the anticancer drugs such as camptothecin (100 μM as a topo I positive control), doxorubicin (5 μM as a topo II positive control) and etoposide (200 μM as a topo II positive control). The data were analyzed and calculated with LabWork 4.5 Software for the inhibition ratio.[15] The results of topoisomerase inhibition of the tested compounds are shown in Table 2. All the tested compounds showed clearly very low topo I inhibitory activity. At 5 μM concentration, most of compounds showed over 50% inhibitory activity against topo II. Especially, highly cytotoxic compounds **8b**, **8e**, and **8f** inhibited completely topo II at this concentration. The results of these tests suggest that some benzonaphthofurandiones are potent topo II inhibitors.

However, highly cytotoxic compounds **8c** and **8d** showed poor topo II inhibition, and there could be some other mechanisms for the cytotoxicity. Indeed, we recently reported the activity of **8e** was associated with the induction of cell cycle arrest in the G0/G1 phase and the **8e**-induced cell cycle arrest was well correlated with the suppression of CDK2, CDK4, cyclin D1, cyclin E, c-Myc, and pRb.[16]

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