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2- or 6-(1-Azidoalkyl)-5,8-Dimethoxy-1,4-Naphthoquinone: Synthesis, Evaluation of Cytotoxic Activity, Antitumor Activity and Inhibitory Effect on DNA Topoisomerase-I

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6-(1-azidoalkyl)-DMNQ derivatives compared to 2-(1-azidoalkyl)-DMNQ isomers, exhibited higher cytotoxic activity against L1210 mouse leukemia cells and stronger inhibition of DNA topoisomerase-I (TOPO-I), suggesting involvement of steric hindrance. However, similar antitumor activity against mice bearing S-180 cell was shown by 2- and 6-(1-azidoalkyl)-DMNQ derivatives.

Key words: Azidoalkylated naphthoquinones, DNA topoisomerase-I inhibition, Antitumor activity, Structure-activity relationship

INTRODUCTION

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). Steric modification of alkyl chains play an important role in expressing bioactivity. 6-(1-Hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone (DMNQ, 5,8-dimethoxy-1,4-naphthoquinone) derivative, possessing an unmodified quinonoid moiety, displayed stronger antiproliferative activity than sterically hindered 2-(1-hydroxyalkyl)-DMNQ derivative (Ahn *et al.*; 1995, Song *et al.*, 1999, in press). Another important factor may be the inherent electrophilicity in the quinonoid system. 6-Acyl-DMNQ derivatives exhibited more potent antitumor activity than the corresponding 6-(1-hydro-xyalkyl)-DMNQ derivatives (Song *et al.*, 1999). In another study, some cytotoxic naphthazarin derivatives were shown to exert an inhibitory effect on DNA topoisomerase-I. This suggests mode of cytotoxic effect of the DMNQ derivatives is through inhibition of the TOPO-I enzyme. (Ahn *et al.*, 1995).

Azido group possesses both positive mesomeric and electron-withdrawing properties (Biffin *et al.*, 1971). Electron-withdrawing effect of the azido group is relatively strong, being between that of bromo group and that of iodo group. It was expected that introduction of azido group at the side chain substituted at the 2 or 6 position of DMNQ, electrophilicity of the napthoquinone ring might be enhanced. On the other hand, the mesomeric effect was expected to be be negligible because the azido group would be attached to a sp^3 carbon atom of alkyl side chain. Furthermore, presence of the azido group in molecules could improve water solubility of these com-pounds through polarisation.

Based on this rationale, in the present study azido group was introduced at C-1 of 2-alkylated DMNQ and cytotoxic activity, antitumor activity and DNA topoisomerase-I (TOPO-I) inhibition of 2- or 6-(1-azidoalkyl)-DMN Q derivatives were investigated.

MATERIALS AND METHODS

Chemical reagents were obtained from Aldrich Chemical Company. Solvents were of reagent grade and used without further purification. L1210 and A549 cells were obtained from Korea Research Institute for Chemical Technology. RPMI 1640, fetal bovine serum and other reagents used for cell culture were purchased from Gibco (GIBCO Co. USA). Proton NMR spectra were recorded on a JEOL 90 MHz spectrometer using tetramethylsilane as internal standard. Analytical thin layer chromatography was performed on plastic sheet (0.2 mm) coated with silica gel 60 F254 (E. Merk). Silica gel 60 (70-230mesh, E. Merk) was used for column chromatography.

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Synthesis of compounds

1'-Hydroxyl group of 2-(1-hydroxyalkyl)-1,4,5,8-tetramethoxynaphthalene (TMN,1,4,5,8-tetramethoxynaphthalene) derivatives (Baik *et al.*, 1997; Terada *et al.*, 1987) was replaced with azido group using Mitsunobu reaction (Mitsunobu, 1981) to produce 2-(1-azidoalkyl)-TMN derivatives (I~XIII). These compounds were then oxidatively demethyated with cerium ammonium nitrate (CAN) to yield 2-and 6-(1-azidoalkyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives in a 69-80% yield (Fig. 1).

General Synthesis of 2-(1-azidoalkyl)-TMN derivatives

2-(1-Hydroxyalkyl)-1,4,5,8-tetramethoxynaphthalene (TMN, 1,4,5,8-tetramethoxynaphthalene (5.4 mM), triphenylphosphine (5.8 mM), diethylazodicarboxylate (5.8 mM) were dissolved in tetrahydrofuran (50 ml) under nitrogen gas, and diphenylphosphorylazide (5.8 mM) dissolved in tetrahydrofuran (15 ml) was added, dropwise, to this solution during 10 min. The reaction mixture was stirred for 24 h at room temperature. The reaction mixture was evaporated to dried mass, which was chromatographed on a silica gel column to give yellow oils. The purity was checked on a silica gel plate in a developing system of hexane/ethyl acetate (3 : 2).

Results were as follows:

2-(1-Azidomethyl)-1,4,5,8-tetramethoxynaphthalene(I):

Yield : 78%, Rf : 0.35 (hexane : ethyl acetate=3 : 2). 1H-NMR (CDCl3) : δ 6.98(s, 1H), 6.83(s, 2H), 4.58(s, 2H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1610, 1060.

2-(1-Azidoethyl)-1,4,5,8-tetramethoxynaphthalene(II):

Yield : 70 %, Rf : 0.38(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.95(s, 1H), 6.81(s, 2H), 5.13(q, J =7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77 (s, 3H), 1.54(d, J=7.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1600, 1060.

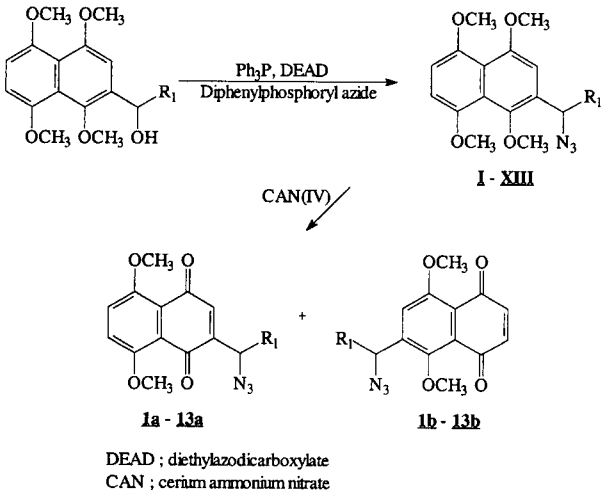


Fig. 1. Synthetic pathways of 2- and 6-(1-azidoalkyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives

2-(1-Azidopropyl)-1,4,5,8-tetramethoxynaphthalene(III):

Yield : 70%, Rf : 0.60 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.99(s, 1H), 6.83(s, 2H), 5.12(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77 (s, 3H), 1.611.47(m, 2H), 0.89(t, J=6.10Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2940, 2100, 1610, 1070.

2-(1-Azidobutyl)-1,4,5,8-tetramethoxynaphthalene(IV):

Yield : 71 %, Rf : 0.62(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.01(s, 1H), 6.84(s, 2H), 5.09(t, J=7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.70~1.52(m, 4H), 0.87(t, J=6.10Hz, 3H). IR_{max} cm^{-1} (neat) : 2930, 2100, 1600, 1060.

2-(1-Azidopentyl)-1,4,5,8-tetramethoxynaphthalene(V):

Yield : 68%, Rf : 0.64 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.98(s, 1H), 6.83(s, 2H), 5.14(t, J =7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.75~1.10(m, 6H), 0.87(t, J=6.0Hz, 3H). IR $\nu_{max}cm^{-1}$ (neat) : 2950, 2100, 1610, 1060.

2-(1-Azidohexyl)-1,4,5,8-tetramethoxynaphthalene(VI):

Yield : 65%, Rf : 0.65(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.01(s, 1H), 6.85(s, 2H), 5.12(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77 (s, 3H), 1.82~1.10(m, 8H), 0.87(t, J=6.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2940, 2100, 1610, 1060.

2-(1-Azidoheptyl)-1,4,5,8-tetramethoxynaphthalene(VII):

Yield : 73%, Rf : 0.68 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.95(s, 1H), 6.83(s, 2H), 5.12(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.82~0.82(m, 13H). IR ν_{max} cm^{-1} (neat) : 2940, 2100, 1610, 1060.

2-(1-Azidooctyl)-1,4,5,8-tetramethoxynaphthalene(VIII):

Yield : 70%, Rf : 0.71(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.92(s, 1H), 6.83(s, 2H), 5.13(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77 (s, 3H), 1.85~0.84(m, 15H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1610, 1060.

2-(1-Azidononyl)-1,4,5,8-tetramethoxynaphthalene(IX):

Yield : 76%, Rf : 0.76(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.96(s, 1H), 6.82(s, 2H), 5.13(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.91~0.83(m, 17H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1610, 1060.

2-(1-Azidodecyl)-1,4,5,8-tetramethoxynaphthalene(X):

Yield: 71 %, Rf : 0.78(hexane : ethyl acetate=3 : 2).

^{1}H-NMR ($CDCl_3$) : δ 6.95(s, 1H), 6.84(s, 2H), 5.13(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77 (s, 3H), 1.85~0.84(m, 19H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1610, 1060.

2-(1-Azidoundecyl)-1,4,5,8-tetramethoxynaphthalene(XI):
Yield : 69%, Rf : 0.81 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.94(s, 1H), 6.83(s, 2H), 5.13(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.89~0.84(m, 21H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1610, 1060.

2-(1-Azidotridecyl)-1,4,5,8-tetramethoxynaphthalene(XII):
Yield: 71%, Rf : 0.87 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.97(s, 1H), 6.83(s, 2H), 5.13(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.85~0.84(m, 25H). IR ν_{max} cm^{-1} (neat) : 2930, 2100, 1610, 1070.

2-(1-Azido-4-methylpentyl)-1,4,5,8-tetramethoxynaphthalene(XIII):
Yield: 69%, Rf : 0.63(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 6.98(s, 1H), 6.83(s, 2H), 5.14(t, J= 7.0Hz, 1H), 3.94(s, 3H), 3.91(s, 3H), 3.87(s, 3H), 3.77(s, 3H), 1.78~1.10 (m, 5H), 0.87(d, J=6.0Hz, 6H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1610, 1060.

General Synthesis of 2-or 6-(1-azidoalkyl)-5,8-dimethoxy-1,4-naphthoquinones(1a~13a, 1b~13b)
2-(1-Azidoalkyl)-TMN (3.33 mM) was dissolved in acetonitrile (30 ml) and cooled to 5°C. Cerium ammonium nitrate (CAN) (10.98 mM) in water (15 ml) was added to the solution during 10 min, and stirred for 20 min further. The reaction mixture was diluted with water (50 ml) and extracted with dichloromethane (100 ml) twice. Dichloromethane solution was dehydrated with anhydrous sodium sulfate (25 g). The dried solution was evaporated to a dry mass, which was chromatographed on a silica gel coulmn to give red to red brown quinone isomers. (**1a~13a, 1b~13b**) Purity was checked on a silica gel plate in developing system of hexane/ethyl acetate (3 : 2).

Following results were observed:
2-(1-Azidomethyl)-5,8-dimethoxy-1,4-naphthoquinone(1a):
Yield : 44%, m.p. : 98.7~99.3°C. Rf : 0.10 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.33(s, 2H), 6.83(s, 1H), 4.36(s, 2H), 3.97(s, 6H). IR ν_{max} cm^{-1} (KBr) : 2950, 2100, 1660, 1600, 1060. Anal.($C_{13}H_{11}N_3O_4$) calcd.: C, 57.14; H, 4.06; N, 15.38; found : C, 57.31; H, 4.12; N, 15.30.

6-(1-Azidomethyl)-5,8-dimethoxy-1,4-naphthoquinone (1b) :
Yield : 36%. m.p. : 78.2~79.4°C. Rf : 0.31(hexane : ethyl acetate=3 : 2). 1H-NMR (CDCl3) : δ 7.36(s, 1H), 6.76(s, 2H), 4.55(s, 2H), 3.96(s, 6H). IR ν_{max} cm^{-1} (KBr) : 2950, 2100, 1660, 1590, 1060. Anal. ($C_{13}H_{11}N_3O_4$) calcd.: C, 57.14; H, 4.06; N, 15.38; found : C, 57.29; H, 4.11; N, 15.31.

2-(1-Azdoethyl)-5,8-dimethoxy-1,4-naphthoquinone(2a):
Yield : 47%, Rf : 0.11(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.31(s, 2H), 6.81(s, 1H), 4.85(q, J= 7.0Hz, 1H), 3.91(s, 6H), 1.51(d, J=7.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{14}H_{13}N_3O_4$) calcd.: C, 58.53; H, 4.56; N, 14.63; found: C, 58.41; H, 4.48; N, 14.59.

6-(1-Azidoethyl)-5,8-dimethoxy-1,4-naphthoquinone(2b) :
yield : 32%, Rf : 0.36(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.39(s, 1H), 6.80(s, 2H), 5.01(q, J=7.1Hz, 1H), 4.01(s, 3H), 3.86(s, 3H), 1.54(d, J=7.1Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{14}H_{13}N_3O_4$) calcd.: C, 58.53; H, 4.56; N, 14.63; found : C, 58.67; H, 4.49; N, 14.57.

2-(1-Azidopropyl)-5,8-dimethoxy-1,4-naphthoquinone(3a) :
yield : 49 %, Rf : 0.12(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.29(s, 2H), 6.74(s, 1H), 4.76(t, J=7.0Hz, 1H), 3.92(s, 6H), 1.711.60(m, 2H), 0.97(t, J=6.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2940, 2100, 1660, 1600, 1060. Anal. ($C_{15}H_{15}N_3O_4$) calcd.: C, 59.79; H, 5.02; N, 13.95; found : C, 59.87; H, 5.11; N, 13.79.

6-(1-Azidopropyl)-5,8-dimethoxy-1,4-naphthoquinon(3b):
yield : 30%, Rf : 0.37(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 1H), 6.80(s, 2H), 4.96(t, J=7.0Hz, 1H), 4.01(s, 3H), 3.85(s, 3H), 1.741.62(m, 2H), 1.00(t, J=6.5Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{15}H_{15}N_3O_4$) C, 59.79; H, 5.02; N, 13.95; found :C, 59.88; H, 5.09; N, 13.78.

2-(1-Azidobutyl)-5,8-dimethoxy-1,4-naphthoquinone(4a):
yield : 41%, Rf : 0.13(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 4.78(t, J=7.0Hz, 1H), 3.96(s, 6H), 1.82~1.25 (m, 4H), 0.87(t, J=6.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{16}H_{17}N_3O_4$) C, 60.94; H, 5.43; N, 13.33; found : C, 60.82; H, 5.49; N, 13.25.

6-(1-Azidobutyl)-5,8-dimethoxy-1,4-naphthoquinone(4b):
yield: 28%. Rf : 0.38(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.35(s, 1H), 6.80(s, 2H), 5.01(t, J=7.0Hz, 1H), 3.96(s, 3H), 3.86(s, 3H), 1.71~0.82(m, 7H). IR ν_{max} cm^{-1}(neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{16}H_{17}N_3O_4$) C, 60.94; H, 5.43; N, 13.33; found : C, 60.99; H, 5.48; N, 13.21.

2-(1-Azidopentyl)-5,8-dimethoxy-1,4-naphthoquinone (5a):
yield : 41 %. Rf : 0.15(hexane : ethyl acetate=3 : 2). ^{1}H-

NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.80(s, 1H), 4.79(t, J=7.0Hz, 1H), 3.97(s, 6H), 1.70~1.10(m, 6H), 0.90(t, J=6.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{17}H_{19}N_3O_4$) C, 62.00; H, 5.81; N, 12.76; found : C, 61.73; H, 5.73; N, 12.60.

6-(1-Azidopentyl)-5,8-dimethoxy-1,4-naphthoquinone(5b): yield : 31%. Rf : 0.62 (hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.29(s, 1H), 6.80(s, 2H), 5.01(t, J=7.1Hz, 1H), 3.85(s, 3H), 3.86(s, 3H), 1.75~0.85(m, 9H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1660, 1600, 1060. Anal. ($C_{17}H_{19}N_3O_4$) C, 62.00; H, 5.81; N, 12.76; found : C, 61.85; H, 5.93; N, 12.62.

2-(1-Azidohexyl)-5,8-dimethoxy-1,4-naphthoquinone(6a): yield : 45%. Rf : 0.17(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 4.76(t, J=7.0Hz, 1H), 4.00(s, 6H), 1.91~1.18(m, 8H), 0.82(t, J=6.0Hz, 3H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{18}H_{21}N_3O_4$) C, 62.96; H, 6.16; N, 12.24; found : C, 63.11; H, 6.25; N, 12.15.

6-(1-Azidohexyl)-5,8-dimethoxy-1,4-naphthoquinone(6b): yield : 32%. Rf : 0.63(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 1H), 6.80(s, 2H), 5.00(t, J=7.0Hz, 1H), 3.97(s, 3H), 3.91(s, 3H), 1.81~0.85(m, 11H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{18}H_{21}N_3O_4$) C, 62.96; H, 6.16; N, 12.24; found : C, 63.12; H, 6.27; N, 12.12.

2-(1-Azidoheptyl)-5,8-dimethoxy-1,4-naphthoquinone (7a): yield : 41%. Rf : 0.18(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 4.78(t, J=7.0Hz, 1H), 3.96(s, 6H), 1.90~0.85(m, 13H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{19}H_{23}N_3O_4$) C, 63.85; H, 6.49; N, 11.76; found : C, 64.02; H, 6.37; N, 11.59.

6-(1-Azidoheptyl)-5,8-dimethoxy-1,4-naphthoquinone(7b): yield : 30 %. Rf : 0.64(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.35(s, 1H), 6.80(s, 2H), 5.01(t, J=7.0Hz, 1H), 3.96(s, 3H), 3.86(s, 3H), 1.79~0.83(m, 13H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{19}H_{23}N_3O_4$) C, 63.85; H, 6.49; N, 11.76; found : C, 63.98; H, 6.54; N, 11.60.

2-(1-Azidooctyl)-5,8-dimethoxy-1,4-naphthoquinone(8a): yield : 39%. Rf : 0.20(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 4.76(t, J= 7.0Hz, 1H), 3.96(s, 6H), 1.90~0.83(m, 15H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{20}H_{25}N_3O_4$) C, 64.67; H, 6.78; N, 11.31; found : C, 64.77; H, 6.74; N, 11.26.

6-(1-Azidooctyl)-5,8-dimethoxy-1,4-naphthoquinone(8b): yield : 27%. Rf : 0.65(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.35(s, 1H), 6.80(s, 2H), 4.99(t, J= 7.1Hz, 1H), 3.96(s, 3H), 3.86(s, 3H), 1.82~0.83(m, 15 H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{20}H_{25}N_3O_4$) C, 64.67; H, 6.78; N, 11.31; found : C, 64.60; H, 6.69; N, 11.27.

2-(1-Azidononyl)-5,8-dimethoxy-1,4-naphthoquinone (9a): yield : 46%. Rf : 0.21(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 4.80(t, J= 7.0Hz, 1H), 3.96(s, 6H), 1.92~0.81(m, 17H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{21}H_{27}N_3O_4$) C, 65.44; H, 7.06; N, 10.90; found: C, 65.59; H, 7.01; N, 10.81.

6-(1-Azidononyl)-5,8-dimethoxy-1,4-naphthoquinone(9b): yield : 31%. Rf : 0.68(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.35(s, 1H), 6.80(s, 2H), 5.02(t, J= 7.0Hz, 1H), 3.96(s, 3H), 3.86(s, 3H), 1.81~0.85(m, 17H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{21}H_{27}N_3O_4$) C, 65.44; H, 7.06; N, 10.90; found:C, 65.37; H, 7.00; N, 10.80.

2-(1-Azidodecyl)-5,8-dimethoxy-1,4-naphthoquinone(10a): yield : 43%. Rf : 0.23(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 4.75(t, J= 7.0Hz, 1H), 3.96(s, 6H), 1.91~0.82(m, 19H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{22}H_{29}N_3O_4$) C, 66.14; H, 7.32; N, 10.52; found : C, 66.07; H, 7.29; N, 10.47.

6-(1-Azidodecyl)-5,8-dimethoxy-1,4-naphthoquinone(10b): yield : 29%, Rf : 0.71(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.35(s, 1H), 6.80(s, 2H), 5.01(t, J= 7.0Hz, 1H), 3.96(s, 3H), 3.86(s, 3H), 1.73~0.82(m, 19 H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{22}H_{29}N_3O_4$) C, 66.14; H, 7.32; N, 10.52; found : C, 66.21; H, 7.25; N, 10.45.

2-(1-Azidoundecyl)-5,8-dimethoxy-1,4-naphthoquinone (11a): yield : 42%. Rf : 0.25(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.34(s, 2H), 6.78(s, 1H), 5.02(t, J=7.0Hz, 1H), 3.96(s, 6H), 1.91~0.82(m, 21H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{23}H_{31}N_3O_4$) C, 66.81; H, 7.56; N, 10.16; found : C, 66.95; H, 7.48; N, 9.97.

6-(1-Azidoundecyl)-5,8-dimethoxy-1,4-naphthoquinone (11b): yield : 31%, Rf : 0.76(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.35(s, 1H), 6.80(s, 2H), 5.02(t, J=7.0Hz, 1H), 3.96(s, 3H), 3.86(s, 3H), 1.91~0.82(m, 21H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{23}H_{31}N_3O_4$)

C, 66.81; H, 7.56; N, 10.16; found : C, 66.93; H, 7.51; N, 10.01.

2-(1-Azidotridecyl)-5,8-dimethoxy-1,4-naphthoquinone (12a):

yield : 36%. Rf : 0.31(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.33(s, 2H), 6.79(s, 1H), 4.76(t, J=7.0Hz, 1H), 3.97(s, 6H), 1.90~0.81(m, 25H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{25}H_{35}N_3O_4$) C, 68.00; H, 7.99; N, 9.52; found : C, 68.19; H, 7.84; N, 9.41.

6-(1-Azidotridecyl)-5,8-dimethoxy-1,4-naphthoquinone (12b):

yield : 22 %. Rf : 0.80(hexane : ethyl acetate=3 : 2). ^{1}H-NMR ($CDCl_3$) : δ 7.33(s, 1H), 6.78(s, 2H), 4.99(t, J=7.0Hz, 1H), 3.99(s, 3H), 3.84(s, 3H), 1.82~0.83(m, 25H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{25}H_{35}N_3O_4$) C, 68.00; H, 7.99; N, 9.52; found : C, 67.88; H, 7.87; N, 9.40.

2-(1-Azido-4-methylpentyl)-5,8-dimethoxy-1,4-naphthoquinone(13a):

yield : 41%. m.p. : 75.2~77.2. Rf : 0.17(hexane : ethyl acetate=3 : 2). ^{1}H-NMR($CDCl_3$): δ 7.33(s, 2H), 6.79(s, 1H), 4.75(t, J=7.0Hz, 1H), 3.96(s, 6H), 1.60~1.10(m, 5H), 0.88 (d, J=6.12Hz, 6H). IR ν_{max} cm^{-1} (KBr): 2950, 2100, 1650, 1600, 1060. Anal. ($C_{18}H_{21}N_3O_4$) C, 62.96; H, 6.16; N, 12.24; found : C, 62.83; H, 6.07; N, 12.15.

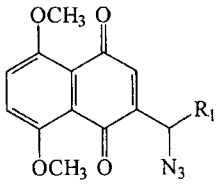
6-(1-Azido-4-methylpentyl)-5,8-dimethoxy-1,4-naphthoquinone(13b):

yield : 29%. Rf : 0.61(hexane : ethyl acetate=3 : 2). ^{1}H-NMR($CDCl_3$) : δ 7.36(s, 1H), 6.80(s, 2H), 4.99(t, J= 7.0Hz, 1H), 4.01(s, 3H), 3.86(s, 3H), 1.63~1.10(m, 5H), 0.90(d, J=6.13Hz, 6H). IR ν_{max} cm^{-1} (neat) : 2950, 2100, 1650, 1600, 1060. Anal. ($C_{18}H_{21}N_3O_4$) C, 62.96; H, 6.16; N, 12.24; found : C, 62.99; H, 6.11; N, 12.15.

***In vitro* cytotoxicity test**

Cytotoxic activity was measured against L1210 cells using the reported method (Thayer *et al.*, 1971). RPMI 1640 supplemented with 10% fetal bovine serum (FBS) was used in proliferation of L1210 cells. Cell numbers were counted using a haemacytometer, and ED_{50} value was defined as the concentration of drug to produce a 50% reduction in viability relative to control in three independent experiments. A549 cells in exponential growth phase were trypsinized, dispersed in single cell suspen-sion and 180 μl dispensed into 96-well plates. Cells (3×10^4) were allowed to attach and grow overnight. 20 μl of medium containing test sample were

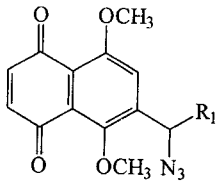
Table I. Cytotoxic activity and antitumor activity of 2-(1-azidoalkyl)-5,8-dimethoxy-1,4-naphthoquinones, and their inhibitory effect on DNA topoisomerse-I



No.of compd.	R_1	ED_{50} (μM, mean±S.D.)		T/C (%)	Survivor (50days)[a]	IC_{50} (μM) (mean±S.E.)
		L1210	A549			
1a	H	0.92±0.43	42.3±7.58	149	0/8	>200
2a	Methyl	0.73±0.45	>50	152	0/8	>200
3a	Ethyl	1.06±0.76	>50	164	0/8	>200
4a	Propyl	1.11±0.38	>50	171	0/8	>200
5a	Butyl	0.94±0.73	47.4±10.7	182	1/8	>200
6a	Pentyl	1.11±0.43	39.4±5.10	188	1/8	>200
7a	Hexyl	1.20±0.59	>50	199	1/8	>200
8a	Heptyl	1.13±0.62	43.7±7.38	209	2/8	>200
9a	Octyl	1.51±0.96	47.1±9.64	221	2/8	>200
10a	Nonyl	1.78±1.23	>50	201	2/8	>200
11a	Decyl	2.23±1.40	>50	194	1/8	>200
12a	Dodecyl	2.53±2.11	>50	178	1/8	>200
13a	*i*-pentyl	1.02±0.61	39.3±4.31	192	1/8	>200

Dosage for animal test; 15 μmole/kg/day for naphthoquinones and 1.1 μmole for adriamycin, intraperitoneally injected for 7 consecutive days. [a] x/8; number of mice which survived for more than 50 days from 8 test mice

Table II. Cytotoxic activity and antitumor activity of 6-(1-azidoalkyl)-5,8-dimethoxy-1,4-naphthoquinones, and their inhibitory effect on DNA topoisomerase-I



No.of compd.	R_1	ED_{50} (μM, mean±S.D.)		T/C (%)	Survivor (50days)[a]	IC_{50}(μM) (mean±S.E.)
		L1210	A549			
1a	H	0.77±0.55	>50	151	0/8	126±4.7
2b	Methyl	0.66±0.38	44.18±10.7	159	0/8	136±5.34
3b	Ethyl	0.80±0.47	47.67±5.81	173	1/8	149±2.32
4b	Propyl	0.86±0.51	>50	176	1/8	137±3.56
5b	Butyl	0.76±0.46	39.30±8.33	193	1/8	129±3.42
6b	Pentyl	0.76±0.44	45.45±10.9	191	1/8	139±6.40
7b	Hexyl	0.92±0.36	>50	205	2/8	147±3.21
8b	Heptyl	0.86±0.40	35.26±4.82	211	2/8	193±5.39
9b	Octyl	1.06±0.44	45.51±10.3	216	2/8	187±5.81
10b	Nonyl	1.13±0.63	>50	214	1/8	>200
11b	Decyl	1.26±0.92	>50	196	1/8	>200
12b	Dodecyl	1.47±0.70	>50	181	1/8	>200
13b	i-pentyl	0.76±0.35	34.17±6.38	196	1/8	131±4.24
ADR		0.016±0.016	1.01±0.27	240	4/8	
CPT						6±0.98

Dosage for animal test; 15 μmole/kg/day for naphthoquinones and 1.1 μmole for adriamycin(ADR), intraperitoneally injected for 7 consecutive days. [a] x/8; number of mice which survived for more than 50 days from 8 test mice. CPT; camptothecin

added and incubated for further 48 h. Cytotoxic activity was measured by SRB method (Skehan *et al.*, 1990) and the IC_{50} value was calculated using Probits method. Absorbance was read with microtiter plate reader at 520 nm. IC_{50} value was the concentration of drug required to reduced absorbance to 50% of vehicle-treated controls.

In vivo antitumor activity in ICR mice bearing Sarcoma 180 cells

The following procedure was followed by protocol (NCI, 1972). The test sample dissolved in a predetermined amount of 50% PEG200 and were stored at 4°C. Sarcoma 180 cells (0.1 ml per mouse) suspended in saline (1×10^7 cells/ml) were inoculated intraperitoneally to male ICR mice (NCI, 1972). Twenty four hours after transplantation, mice were divided so that each group containsed 8 mice. The sample was administered into the rodents intraperitoneal cavity daily for 7 days. Survival rate (T/C, %) was calculated using the following equation;

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

DNA Topoisomerase-activity was determined as follows (Liu *et al*, 1981, 1983).

Enzymatic activity was analyzed by DNA unwinding assay. Calf thymus DNA topoisomerase I (1 unit) was incubated with 0.5 μg of *E-coli* pBR322 (TAKARA Co. Ltd.) DNA, in the presence or absence of test compounds, in 20 mL of 5 mM Tris-HCl (pH 8.0) containing 72 mM KCl, 5 mM $MgCl_2$, 5.0 mM dithiothreitol, 5 mM spermidine and 0.01% bovine serum albumin for 30 min at 37°C. The reaction was terminated by addition of 5 μl of a stop solution consisting of 2% glycerol, 2% sodium dodecyl sulfate (SDS) and 0.05% bromophenol blue. Electrophoresis was carried out over 1% agarose gel plates, equilibrated with TBE buffer (50 mM Tris base, 50 mM boric acid and 2.5 mM EDTA). The gel was stained with 0.5 μg/ml ethidium bromide solution after electrophoresis. IC_{50} was the concentration which caused 50% inhibition of relaxation of supercoiled pBR322 DNA under test conditions. In each test, camptothecin was used as positive control. The value expressed was the mean (±SE) of triplicate experiments.

RESULTS AND DISCUSSION

Chemistry

Hydroxyl group of 2-(1-hydroxyalkyl)-TMN (TMN, 1,4, 5, 8-tetramethoxynaphthalene) was replaced with azido group using the Mitsunobu method resulting in formation of 2-(1-azidoalkyl)-TMN deivatives. These were oxidatively demthylated with CAN to produce 2-(1-azidoalkyl)-and 6-(1-azidoalkyl)-DMNQ derivatives (DMNQ, 5,8-di-methoxy-1,4-naphtho-quinone). The ratio of 2-(1-azidoalkyl)-to 6-(1-azidoalkyl)-isomers was 3:2 on average. The preferential formation of 2-isomers was due to the stabi-lization of methoxy radical cation at C-1 and C-4 observed during the oxidation process of 2-(1-azidoalkyl)-TMN derivatives by a field effect of azido group.

In vitro Cytotoxicity and in vivo antitumor activity

The bioactivities of 2- and 6-(1-azidoalkyl)-DMNQ derivatives were evaluated against L1210 cells *in vitro* (Thayer *et al.*, 1971), and results demonstrated in Tables I and II. It was found that 6-(1-azidoalkyl)-DMNQ derivatives (ED_{50}, 0.66~1.47 μM) on the whole showed a higher cytotoxic activity against L1210 cells than 2-(1-azidoalkyl)-DMNQs (ED_{50}, 0.73~2.53 μM). As evidenced in earlier reports (You *et al.*, 1998; Song *et al.*, 1999, in press), steric hindrance of C-2 substituent of the DMNQ derivatives may explain the lower cytotoxic activity of 2-substituted derivatives.

Also, electron density in the quinoid ring may be impotant. Previously, it had been reported that an electron-withdrawing group such as acetoxy group or oxo group at C-1 in side chain of naphthoquinone analogues enhanced cytotoxic activity (You *et al.*, 1998). Likewise, it is suggested that increase of electrophilicity in the quinoid moiety of azido DMNQ derivatives would make their electrophilic arylation or redox cycling more favorable, and consequently more cytotoxic. When we compared the cytotoxic activity of two representative quinonoid compounds: 2-(4-methylpentyl)-DMNQ (quinonoid carbonyl, 1650 cm^{-1}) and 2-(1-azido-4-methyl-pentyl)-DMNQ (quinonoid carbonyl, 1658 cm^{-1}), the latter (ED_{50}, 0.16 μM) was found to be more cytotoxic against L1210 cells than the former (ED_{50}, 0.93 μM). From these results, it is evident that introduction of azido moiety, an electron-withdrawing group, at C-1 of 2-alkylated DMNQ derivatives enhanced cytotoxic activity. Moreover, cytotoxic activity of 2-(1-azidoalkyl)-DMNQ derivatives against L 1210 cells seem to be dependent on the size of the alkyl group. The shorter the alkyl group, the stronger the cytotoxic activity; 2-(1-azidoethyl)-DMNQ (**2a**, R_1=methyl), possessing an ED_{50} value of 0.73 μM, expressed a stronger cytotoxic activity than 2-(1-azidoundecyl)-DMNQ (**11a**, R_1=decyl, ED_{50}; 2.23 μM).

6-(1-Azidoalkyl)-DMNQ derivative, which possess a more electrophilic quinoid moiety, was found to be slightly more cytotoxic than sterically hindered 2-isomers, (Table II). The relatively high cytotoxic activity of 2-(1-azidoalkyl)-DMNQ derivatives, despite steric hindrance, could be explained by the assumption that steric effect of azido alkyl group at C-2 could be compensated by the enhancing effect of azido group on electrophilicity of the neighboring quinonoid moiety.

In addition, branching of the alkyl chain, as shown with compounds **13a** and **13b** bearing isopentyl group did not change the cytotoxic activity significantly, compared to **6a** and **6b** with straight chains. When the cytotoxic activity of 2- and 6-substituted DMNQ derivatives against A549 cells was examined, both isomers were found to show a much lower cytotoxic activity against A549 cells than L1210 cells, indicating that A549 cells, solid tumor cell line, were more resistant to the azido DMNQ derivatives than L1210 cells.

Separately, antitumor action of these DMNQ derivatives was evaluated against S-180 cells in the peritoneal cavity of ICR mice (NCI, 1972). As shown in Table I and II, some of 2- and 6-(1-Azidoalkyl)-DMNQ derivatives showed T/C values higher than 150% in ICR mice bearing S-180 cells in intraperitoneal cavity at a dose of 15 μmol /kg/day.

Of noteworthy, these compounds expressed an optimal range of alkyl size in showing T/C values higher than 200%; viz. R_1, heptyl to nonyl derivatives **8a**, **9a**, **10a** and hexyl to nonyl derivatives **7b, 8b, 9b, 10b** Mice administered with these compounds exhibited a survival rate of 2/8, indicating that two of eight mice treated with each compound survived more than 50 days after transplantation of tumor cells. Overall, there was no remarkable difference in T/C value between 2- and 6-(1-azidoalkyl)-DMNQ derivatives.

Inhibition of DNA topoisomerase I

As demonstrated in Table I, 2-(1-azidoalkyl)-DMNQ derivatives showed no remarkable inhibiton (IC_{50}, >200 μM) of TOPO-I, while 6-isomers exhibited considerable inhibitory action (IC_{50}, 126~193 μM). The lower inhibitory effect of 2-(1-azidoalkyl)-DMNQ derivatives could be explained by the steric hindrance of azido alkyl group at C-2, as had been observed with 2-(1-hydroxyethyl)- or 2-(1-hydroxyiminoalkyl)-DMNQ derivatives. As for 6-(1-azidoalkyl)-DMNQ derivatives, inhibitory effect was found to be dependent on the size of alkyl group; compounds with alkyl groups of shorter lengths (**1b** to **9b**) exhibited stronger inhibitory activity than those with longer alkyl chains (**10b** to **12b**). In addition, branching of the alkyl group, as shown in compound **13b**, did not improve the activity further, compared with corresponding compound **6b** with a straight chain.

Though the inhibitory effect of 6-(1-azidoalkyl)-DMNQ derivatives on TOPO-I may not be related to their cytotoxic activity, the more electrophilic 6-substituted DMNQ derivatives showed higher potency in both inhibitory effect and cytotoxocity, compared to 2-substituted isomers. This suggests that inhibition of TOPO-I may be one of the cytotoxic mechanisms of action for cytotoxicity of (1-azidoalkyl)-DMNQ derivatives.

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