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6-(1-Alkenoyloxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone Derivatives: Synthesis and Evaluation of Antitumor Activity

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Thirty six 5,8-dimethoxy-1,4-naphthoquinone derivatives, which bear unsaturated alkyl side chain with ester bond, were synthesized and tested cytotoxic activity on L1210 cells and antitumor activity against ICR mice bearing S-180 cells. It could be recognized that the cytotoxicities of naphthoquinones with R_1 being methyl and propyl (**IV1~24**) were not enhanced by replacing the alkanoyls with alkenoyls. In contrast, the introduction of the alkenoyl moieties on the compounds with R_1=hexyl (**IV25~36**) resulted in the enhancement of their cytotoxicities. Replacement of alkanoyl group with an alkenoyl group generally increased the T/C value of the mice bearing S-180 cells.

Key word : 6-Substituted 5,8-dimethoxy-1,4-naphthoquinone, Esters of alkenoic acids, Antitumor activity

INTRODUCTION

Shikonin containing 5,8-dihydroxy-1,4-naphthoquinone (naphthazarin) and some acylated derivatives were isolated from *Boraginaceae* plants (Brockmann, 1936; Morimoto *et al.*, 1965). It was reported that they showed relatively good cytotoxic activity against L1210 and antitumor activity in ICR mice bearing S-180 cells (Sankawa *et al.*, 1981). Ahn and coworkers (Kim *et al.*, 1990) found that acetylshikonin showed a higher T/C value on ICR mice bearing S-180 fluid tumor than shikonin. The cytotoxicity-enhancing effect of acetyl group in shikonin prompted us to synthesize various acyl derivatives of shikonin and some synthesized 2-(1-hydroxyalkyl)-5,8-dihydroxy(or dimethoxy)-1,4-naphthoquinone derivatives, and to evaluate their antitumor effect and inhibitory effect on DNA topisomerase-I, to show a general potentiation of the activities (Ahn *et al.*, 1995; Ahn, 1996; Ahn *et al.*, 1993). Meanwhile, we have recently found that 6-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone showed stronger antitumor activities than 2-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone. The decreased activity of 2-substituted 5,8-dimethoxy-1,4-aphthoquinone derivatives was explained by ensuing from the steric hindrance of the substituent at C-2 (Ahn *et al.*, 1993; You *et al.*, 1998). From these previous results, it may be deduced that the side chain modification of these 1,4-naphthoquinone analogs considerably vary their antitumor activities. Therefore we further investigated side chain variation of these analogs with alkenoyl motif for finding the more desirable compounds. Thus 6-(1-alkenoyloxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives were synthesized and evaluated against L1210 cells *in vitro* and mice bearing S-180 cells *in vivo*.

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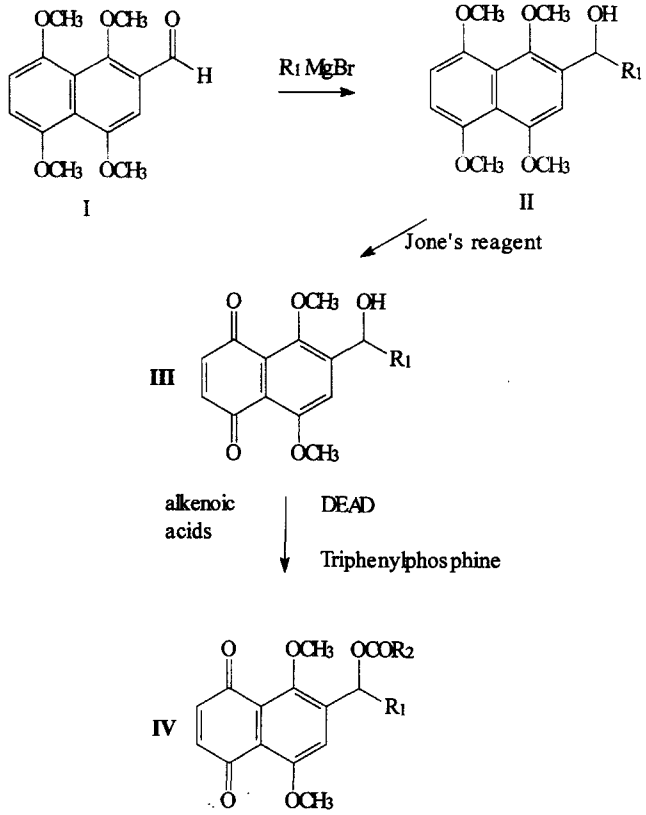
MATERIALS AND METHODS

Chemical reagents were obtained from Aldrich Chemical Company. Solvents were of reagent grade and used without further purification. L1210 cells were obtained from Korea Institute for Chemical Technology. RPMI 1640, Fetal bovine serum and other reagents used for cell culture were purchased from Gibco Co. Proton NMR spectra were recorded on a JEOL 90 MHz spectrometer using tetramethylsilane as an 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 (70~230 mesh, E. Merk) was used for column chromatography.

Synthesis of compounds

The synthetic pathways are shown in Scheme 1. Jone's oxidation of 6-(1-hydroxyalkyl)-1,4,5,8-tetramethoxynaphthalene (**II-series**) (Baik *et al.*, 1997; Terada *et al.*, 1987) produced 6-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives (**III-series**). Compounds III were subsequently acylated with various alkenoic acids using Mitsunobu reaction to produce 6-(1-alkenoyl-



Scheme 1. Synthesis of 6-(1-alkenoyloxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives.

oxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives (**IV-series**).

General synthesis of 6-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone, III series

Chromium trioxide (17.6 mmole) was dissolved in 250 ml water followed by addition of 651 μl concentrated. sulfuric acid (a Jone's reagent). 17.5 mmole of 2-(1-hydroxyalkyl)-1,4,5,8-tetramethoxynaphthalene was dissolved in 200 ml acetone, and this solution was added dropwise to Jone's reagent cooled in the ice bath and stirred for 30 min at room temperature. The reaction mixture was extracted three times with dichloromethane. The organic phase was washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated to a brown red mass. This was purified by silica gel column chromatography using ethyl acetate/hexane (1 : 3) to afford compounds **III** series.

6-(1-Hydroxyethyl)-5,8-dimethoxy-1,4-naphthoquinone III-1: yield=75%, Rf=0.53 (hexane:ethyl acetate =3 : 1), ^{1}H-NMR (ppm): 7.55 (s, 1H), 6.78 (s, 2H), 5.31 (q, J=18.8 Hz, 1H), 3.99 (s, 3H), 3.84 (s, 3H), 2.31 (br, s, 1H), 1.53 (d, J=6.6 Hz,2H), IR (cm^{-1}): 3475, 2950, 1650, 1460.

6-(1-Hydroxybutyl)-5,8-dimethoxy-1,4-naphthoquinone III-2: yield=70%, Rf=0.15 (hexane:ethyl acetate =3 : 1), ^{1}H-NMR (ppm): 7.54 (s, 1H), 6.75 (s, 2H), 5.15 (m, 1H), 3.97 (s, 3H), 3.80 (s, 3H), 3.19 (d, J=3.45 Hz, 1H), 1.74~1.26 (m, 4H), 0.96 (t, J=13.6 Hz, 3H), IR (cm^{-1}): 3470, 2950, 1645, 1450.

6-(1-Hydroxyheptyl)-5,8-dimethoxy-1,4-naphthoquinone III-3: yield=74%, Rf=0.25 (hexane:ethyl acetate =3 : 1), ^{1}H-NMR (ppm): 7.51 (s, 1H), 6.78 (s, 2H), 5.12 (m, 1H), 3.99 (s, 3H), 3.84 (s, 3H), 2.19 (d, J=3.45 Hz, 1H), 1.60~1.18 (m, 10H), 0.91 (t, J=5.00 Hz, 3H), IR (cm^{-1}): 3470, 2950, 1645, 1450.

General synthesis of 6-(1-alkenoyloxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone, IV series

1.23 mmole 6-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone (1.23 mmole) was dissolved in 20 ml tetrahydrofuran, and 85 mmole alkenoic acid, 1.85 mmole triphenylphophine and 1.85 mmole diethylazodicarboxylate were added to the substrate solution. The mixture was stirred for 2 h at room temperature. The reaction mixture was extracted with dichloromethane. The organic phase was washed with brine and dried over anhydrous sodium sulfate. The organic phase was evaporated give a red crude mass, which was purified by the same method as above.

6-(1-Butanoyloxyethyl)-5,8-dimethoxy-1,4-naphthoquinone IV-1: yield=48%, Rf=0.38 (hexane:ethyl acetate= 3 : 1), ^{1}H-NMR (ppm): 7.32 (s, 1H), 6.78 (s, 2H), 6.20 (q, J=18.2 Hz, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.38 (t, J=14,7 Hz, 2H), 1.81~1.48 (m, 5H), 0.97 (t, J=12.2 Hz, 3H) , IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(*trans*-But-2-enoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-2: yield=64%, Rf=0.16 (hexane: ethyl acetate=3 : 1), ^{1}H-NMR (ppm): 7.31 (s, 1H), 6.9~7.2 (m, 1H), 6.77 (s, 2H), 6.24 (q, J=6.44 Hz, 1H), 5.75~6.05 (m, 1H), 5.91 (d, J=15.7 Hz, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 1.40 (d, J=6.62 Hz, 3H), 1.90 (d, J=7.07 Hz, 3H), IR (cm^{-1}): 2920, 1710, 1650, 1240, 1070.

6-[1-(But-3-enoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-3: yield= 60%, Rf=0.17 (hexane: ethyl acetate=3 : 1), ^{1}H-NMR (ppm): 7.31 (s, 1H), 6.79 (s, 2H), 6.24 (q, J=6.62 Hz, 1H), 5.7~6.1 (m, 1H), 5.29 (q, J=1.52 Hz, 1H), 5.13 (q, J=1.52 Hz, 1H), 3.97 (s, 3H), 3.91 (s,3H), 3.17 (d, J=5.46 Hz, 2H), 1.54 (d, J= 6.62 Hz, 3H), IR (cm^{-1}): 2920, 1730, 1650, 1240, 1075, 850.

6-[1-(*trans*-2-Methylbut-2-enoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-4: yield=48%, Rf= 0.31 (hexane:ethyl acetate=3 : 1), ^{1}H-NMR (ppm): 7.33 (s, 1H), 6.9~7.1 (m, 1H), 6.78 (s, 2H), 6.25 (q, J=6.62 Hz, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 1.53~1.88 (m, 9H), IR (cm^{-1}): 2930, 1705, 1655, 1330, 1240, 1075, 850, 730.

6-[1-(3-Methyl-but-2-enoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-5: yield=52%, Rf=0.22 (hexane:

ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.33 (s, 1H), 6.78 (s, 2H), 6.22 (q, J=6.53, 1H), 5.78 (t, J=1.3, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 2.16 (d, J=1.16, 2H), 1.93 (d, J= 1.1, 3H), 1.59 (d, J=4.1, 3H), IR(cm^{-1}): 2900, 1710, 1650, 1220, 1140, 1075.

6-(1-Hexanoyloxyethyl)-5,8-dimethoxy-1,4-naphthoqu- inone IV-6: yield=49%, Rf=0.41 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.32 (s, 1H), 6.78 (s, 2H), 6.23 (q, J=18.0, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 2.39 (t, J=13.4 Hz, 2H), 1.66~1.42 (m, 9H), 0.88 (t, J= 12.2, 3H), IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(trans-Hex-2-enoyloxy)-ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-7: yield=55%, Rf=0.26 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.33 (s, 1H), 6.88~ 7.20 (m, 1H), 6.78 (s, 2H), 6.26 (q, J=3.31 Hz, 1H), 5.78~6.04 (m, 1H), 3.98 (s, 3H), 3.90 (s, 3H), 2.22 (q, J=7.42 Hz, 2H), 1.39~1.64 (m, 2H), 0.95 (t, 6.80 Hz, 3H), IR (cm^{-1}): 2930, 1710, 1650, 1240, 1070.

6-[1-(trans-Hex-3-enoyloxy)ethyl-5,8-dimethoxy-1,4-naphthoquinone IV-8: yield=64%, Rf=0.28 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.31 (s, 1H), 6.79 (s, 2H), 6.22 (q, J=6.62 Hz, 1H), 5.52~5.68 (m, 2H), 3.97 (s, 3H), 3.84 (s, 3H), 3.08~3.14 (m, 2H), 1.89~ 2.18 (m, 2H), 1.53 (d, J=6.62 Hz, 3H), 0.99 (t, J=7.51 Hz, 3H), IR (cm^{-1}): 2930, 1730, 1650, 1240, 1070.

6-[1-(2-Ethyl-hex-2-enoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-9: yield=66%, Rf=0.39 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.78 (s, 2H), 6.83 (t, J=7.87 Hz, 1H), 6.07~6.22 (m, 1H), 3.95 (s, 3H), 3.93 (s, 3H), 2.1~2.5 (m, 4H), 0.9~1.95 (m, 11H), IR (cm^{-1}): 2920, 1700, 1650, 1450, 1240, 1050.

6-(1-Heptanoyloxyethyl)-5,8-dimethoxy-1,4-naphthoquinone IV-10: yield=45%, Rf=0.45 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.32 (s, 1H), 6.79 (s, 2H), 6.23 (q, J=18.1 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 2.39 (t, J=13.9 Hz, 2H), 1.66~1.31 (m, 11H), 0.88 (t, J=12.0 Hz, 3H), IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(Hept-6-enoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-11: yield=56%, Rf=0.26 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.33 (s, 1H), 6.79 (s, 2H), 6.24 (q, J=6.62 Hz, 1H), 5.5~5.9 (m, 1H), 4.9~ 5.1 (m, 1H), 4.90 (d, J=1.34 Hz, 1H), 3.98 (s, 3H), 3.92 (s, 3H), 2.41 (t, J=6.62 Hz, 2H), 2.09 (q, J=7.07 Hz, 2H), 1.35~1.8 (m, 7H), IR (cm^{-1}): 2930, 1730, 1655, 1460, 1240, 1075.

6-[1-(Hepta-2,6-dienoyloxy)ethyl]-5,8-dimethoxy-1,4-naphthoquinone IV-12: yield=87%, Rf=0.22 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.25 (s, 1H), 6.8~ 7.1 (m, 1H), 6.71 (s, 2H), 6.24 (q, J=6.62 Hz, 1H), 5.95 (s, 1H), 5.55~5.75 (m, 1H), 5.09 (d, J=5.28 Hz, 1H), 4.85~4.95 (m, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 2.2~2.4 (m, 4H), 1.56 (d, J=6.62 Hz, 3H), IR (cm^{-1}): 2920, 1720, 1655, 1240, 1075.

6-(1-Butanoyloxypropyl)-5,8-dimethoxy-1,4-naphthoquinone IV-13: yield=48%, Rf=0.38 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.78 (s, 2H), 6.07 (t, J=14.3 Hz, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 2.39 (t, J=13.8 Hz, 2H), 2.04~1.26 (m, 7H), 0.96 (t, J=14.5 Hz, 3H), IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(*trans*-But-2-enoyloxy)butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-14: yield=57%, TLC (hexane: EA=3:1): Rf=0.24, ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.9~ 7.2 (m, 1H), 6.78 (s, 2H), 6.09 (t, J=6.35 Hz, 1H), 5.8~ 6.05 (m, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 1.7~2.0 (m, 5H), 1.2~1.4 (m, 2H), 1.94 (t, J=7.16 Hz, 3H), IR (cm^{-1}): 2940, 1710, 1650, 1240, 1050.

6-[1-(But-3-enoyloxy)butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-15: yield=65%, Rf=0.25 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.80 (d, J=1.34 Hz, 1H), 6.17 (t, J=6.62 Hz, 1H), 5.75~6.05 (m, 1H), 5.25~5.35 (m, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.24 (t, J=3.58, 1H), 3.17 (t, J=1.16 Hz, 1H), 0.9~1.9 (m, 7H), IR (cm^{-1}): 2940, 1730, 1650, 1240, 1045.

6-[1-(*trans*-2-Methylbut-2-enoyloxy)butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-16: yield=49%, Rf =0.27 (hexane:ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.25 (s, 1H), 6.9~7.1 (m, 1H), 6.77 (s, 2H), 6.15 (t, J=6.62 Hz, 1H), 3.94 (s, 3H), 3.95 (s, 3H), 0.8~1.9 (m, 13H), IR (cm^{-1}): 2930, 1705, 1655, 1330, 1240, 1075, 850, 730.

6-[1-(3-Methylbut-2-enoyloxy)butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-17: yield=51%, Rf=0.29 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.78 (s, 2H), 6.17 (t, J=6.62 Hz, 1H), 5.7~5.8 (m, 1H), 3.90 (s, 6H), 0.8~2.2 (m, 13H), IR (cm^{-1}): 2940, 1720, 1650, 1230, 1135.

6-[1-(Hex-2-enoyloxy)butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-18: yield=49%, Rf=0.27 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.32 (s, 1H), 6.78 (s, 2H), 6.23 (q, J=18.0 Hz, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 2.39 (t, J=13.4 Hz, 2H), 1.66~1.42 (m, 9H), 0.88 (t, J=12.2 Hz, 3H), IR (cm^{-1}): 2950, 1720, 1650, 1450, 1240, 1045.

6-[1-(*trans*-Hex-2-enoyloxy)-butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-19: yield=63%, Rf=0.34 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.87~ 7.12 (m, 1H), 6.77 (s, 2H), 6.08 (t, J=6.35 Hz, 1H), 5.90 (d, J=14.3 Hz, 1H) 3.93 (s, 6H), 2.05~2.35 (m, 2H), 0.8~1.9 (m, 12H), IR (cm^{-1}): 2930, 1710, 1650, 1240, 1050.

6-[1-(*trnas*-Hex-3-enoyloxy)-butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-20: yield=54%, Rf=0.34 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.24 (s, 1H), 6.78 (s, 2H), 6.12 (t, J=5.90 Hz, 1H), 5.51~5.67 (m, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.10 (d, J=5.64 Hz, 2H), 0.8~2.2 (m, 12H), IR (cm^{-1}): 2950, 1730, 1650, 1240, 1050.

6-[1-(2-Ethyl-hex-2-enoyloxy)-butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-21: yield=56%, Rf=0.41 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.83 (t, J=7.33 Hz, 1H), 6.78 (s, 2H), 6.17 (t, J=5.81 Hz, 1H),

3.95 (s, 3H), 3.93 (s, 3H), 2.1~2.5 (m, 4H), 0.9~1.9 (m, 15H), IR (cm^{-1}): 2950, 1710, 1650, 1220, 1050.

6-[1-Heptanoyloxy)-butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-22: yield=43%, Rf=0.28 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.32 (s, 1H), 6.79 (s, 2H), 6.23 (q, J=18.1 Hz, 1H), 3.98 (s, 3H), 3.91 (s, 3H), 2.39 (t, J=13.9 Hz, 2H), 1.66~1.31 (m, 11H), 0.88 (t, J=12.0 Hz, 3H), IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(Hept-6-enoyloxy)-butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-23: yield=72%, Rf=0.35 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.24 (s, 1H), 6.77 (s, 2H), 6.12 (t, J=6.35 Hz, 1H), 5.55~5.95 (m, 1H), 4.85~5.15 (m, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 2.40 (t, J=6.35 Hz, 2H), 2.07 (q, J=6.62 Hz, 2H), 1.2~1.9 (m, 8H), 0.93 (t, J=7.25 Hz, 3H), IR (cm^{-1}): 2920, 1730, 1650, 1240, 1050.

6-[1-(Hepta-2,6-dienoyloxy)-butyl]-5,8-dimethoxy-1,4-naphthoquinone IV-24: yield=59%, Rf=0.35 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.25 (s, 1H), 6.9~7.15 (m, 1H), 6.77 (s, 2H), 6.15 (t, J=5.99 Hz, 1H), 6.0 (s, 1H), 5.6~5.9 (m, 1H), 5.13 (d, J=11.2 Hz, 1H), 4.95 (s, 1H), 3.93 (s, 3H), 3.92 (s, 3H), 2.2~2.4 (m, 4H), 1.25~1.9 (m, 4H), 0.93 (t, J=6.80 Hz, 3H), IR (cm^{-1}): 2930, 1710, 1650, 1240, 1050.

6-[1-Butanoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-25: yield=48%, Rf=0.55 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.79 (s, 2H), 6.13 (t, J=12.7 Hz, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 2.39 (t, J=14.9 Hz, 2H), 1.88~1.17 (m, 12H), 0.97 (t, J=12.2 Hz, 6h), IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(*trans*-But-2-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-26: yield=68%, Rf=0.36 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.24 (s, 1H), 6.9~7.2 (m, 1H), 6.80 (s, 2H), 6.15 (t, J=6.26 Hz, 1H), 5.95 (m, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 0.8~2.1 (m, 16H), IR (cm^{-1}): 2920, 1720, 1650, 1240, 1050.

6-[1-(But-3-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-27: yield=71%, Rf=0.38 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.80 (s, 2H), 6.13 (t, J=6.26 Hz, 1H), 5.75~6.05 (m, 1H), 5.31 (m, 1H), 5.25 (m, 1H), 3.97 (s, 3H), 3.94 (s, 3H), 3.23 (t, J=1.07 Hz, 1H), 3.16 (t, J=1.25 Hz, 1H), 0.8~1.9 (m, 13H), IR (cm^{-1}): 2920, 1740, 1655, 1240, 1050.

6-[1-(*trans*-2-Methyl-but-2-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-28: yield=47%, Rf=0.48 (hexane:ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.8~7.0 (m, 1H), 6.78 (s, 2H), 6.12 (t, J=6.62 Hz, 1H), 3.94 (s, 6H), 0.8~2.0 (m, 19H), IR (cm^{-1}): 2920, 1710, 1655, 1245, 1050.

6-[1-(3-Methyl-but-2-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-29: yield=52%, Rf =0.32 (hexane:ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.78 (s, 2H), 6.15 (t, J=6.26 Hz, 1H), 5.85 (s, 1H), 3.95(s, 6H), 2.16 (d, J=1.07 Hz, 3H), 0.8~2.0 (m, 16H), IR (cm^{-1}): 2910, 1710, 1650, 1220, 1130.

6-[1-(Hexanoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-30: yield=49%, Rf=0.19 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.78 (s, 2H), 6.12 (t, J=12.8 Hz, 1H), 3.93 (s, 3H), 3.97 (s, 3H), 2.39 (t, J=13.4 Hz, 2H), 1.72~1.31 (m, 16H), 0.89 (t, J=12.2 Hz, 6H), IR (cm^{-1}): 2910, 1710, 1650, 1220, 1130.

6-[1-(*trans*-Hex-2-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-31: yield=62%, Rf=0.31 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.28 (s, 1H), 6.9~7.2 (m, 1H), 6.79 (s, 2H), 6.16 (t, J=6.53 Hz, 1H), 5.93 (d, J=15.6 Hz, 1H) 3.96 (s, 6H), 2.15~2.35 (m, 2H), 0.8~1.9 (m, 18H), IR (cm^{-1}): 2910, 1710, 1650, 1225, 1050.

6-[1-(*trans*-Hex-3-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-32: yield=52%, Rf=0.31 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.28 (s, 1H), 6.79 (s, 2H), 6.11 (t, J=6.26 Hz, 1H), 5.5~5.7 (m, 2H), 3.96 (s, 3H), 3.93 (s, 3H), 0.9~2.2 (m, 18H), IR (cm^{-1}): 2930, 1730, 1650, 1230, 1050.

6-[1-(2-Ethyl-hex-2-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-33: yield=63%, Rf=0.38 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.25 (s, 1H), 6.82 (t, J=7.51 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 2.1~2.5 (m, 4H), 0.8~1.9 (m, 15H), IR (cm^{-1}): 2950, 1710, 1650, 1240, 1070.

6-[1-(Heptanoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-34: yield=32%, Rf=0.24 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.79 (s, 2H), 6.13 (t, J=13.1 Hz, 1H), 3.91 (s, 3H), 3.88 (s, 3H), 2.42 (t, J=13.9 Hz, 2H), 1.72~1.21 (m, 18H), 0.95 (t, J=12.0 Hz, 6H), IR (cm^{-1}): 2950, 1730, 1660, 1460.

6-[1-(Hept-6-enoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-35: yield=66%, Rf=0.34 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.26 (s, 1H), 6.80 (s, 1H), 6.78 (s, 1H), 6.12 (t, J=6.26 Hz, 1H), 5.6~6.0 (m, 1H), 4.9~5.15 (m, 2H), 3.97 (s, 3H), 3.94 (s, 3H), 2.42 (t, J=6.35 Hz, 2H), 0.9~2.25 (m, 19H), IR (cm^{-1}): 2910, 1730, 1650, 1240, 1050.

6-[1-(Hepta-2,6-dienoyloxy)-heptyl]-5,8-dimethoxy-1,4-naphthoquinone IV-36: yield=56%, Rf=0.44 (hexane: ethyl acetate=3:1), ^{1}H-NMR (ppm): 7.27 (s, 1H), 6.85~7.15 (m, 1H), 6.78 (s, 2H), 6.14 (t, J=6.26 Hz, 1H), 5.6~6.03 (m, 2H), 4.9~5.2 (m, 2H), 3.95 (s, 3H), 3.94 (s, 3H), 2.25~2.4 (m, 4H), 0.9~1.9 (m, 15H), IR (cm^{-1}): 2920, 1720, 1650, 1240, 1050.

Measurement of cytotoxicity

Cytotoxicity was measured against L1210 cells using the reported method (Thayer *et al.*, 1971). RPMI 1640 supplemented with Fetal bovine serum in 10% was used for the proliferation of L1210 cells. Cell numbers were counted using a haemacytometer, and ED50 value was defined as the concentration of drug to produce a 50% reduction in viability relative to the control in

three independent experiments.

Antitumor activity in ICR mice bearing Sarcoma 180 cells

The following procedure was due to the protocol of National Cancer Institute USA, 1972. The test sample dissolved in a predetermined amount of 50% PEG200 were stored 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 (National Cancer Institute USA, 1972). 24 Hrs after the transplantation, mice were divided so that each group contains 8 mice. The sample was administered into the intraperitoneal cavity of the mouse daily for 7 days. The survival rate (T/C, %) was calculated by following equation;

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

RESULTS AND DISCUSSION

Chemistry

Acylation of 6-(hydroxyalkyl)-5,8-dimethoxy-1,4-naph-

Table 1. Cytotoxicity and antitumor activity of 6-(1-alkenoyloxyethyl)-5,8-dimethoxy-1,4-naphthoquinone derivatives against L1210 cells and ICR mice bearing S-180 cells

Cpd	R1	R2	ED50 (μg/ml)	T/C (%)	Dose (μmole/kg/d)
IV 1	Methyl	***Butanoyl***	***0.07***	***124***	17
IV 2	Methyl	*trans*-2-butenoyl	0.08	206	14
IV 3	Methyl	3-butenoyl	0.11	210	14
IV 4	Methyl	*trans*-2-methyl-2-butenoyl	0.10	172	14
IV 5	Methyl	3-methyl-2-butenoyl	0.13	144	14
IV 6	Methyl	***Hexanoyl***	***0.08***	***195***	19
IV 7	Methyl	*trans*-2-hexenoyl	0.09	193	14
IV 8	Methyl	*trans*-3-hexenoyl	0.11	202	14
IV 9	Methyl	2-ethyl-2-hexenoyl	0.20	140	14
IV 10	Methyl	***Heptanoyl***	***0.17***	***130***	16
IV 11	Methyl	6-heptenoyl	0.10	267	14
IV 12	Methyl	2,6-heptadienoyl	0.08	249	14
IV 13	Propyl	***Butanoyl***	***0.08***	***130***	17
IV 14	Propyl	*trans*-2-butenoyl	0.08	183	14
IV 15	Propyl	3-butenoyl	0.11	199	14
IV 16	Propyl	*trans*-2-methyl-2-butenoyl	0.08	219	14
IV 17	Propyl	3-methyl-2-butenoyl	0.10	245	14
IV 18	Propyl	***Hexanoyl***	***0.08***	***143***	17
IV 19	Propyl	*trans*-2-hexenoyl	0.12	210	14
IV 20	Propyl	*trans*-3-hexenoyl	0.08	NT	NT
IV 21	Propyl	2-ethyl-2-hexenoyl	0.11	193	14
IV 22	Propyl	***Heptanoyl***	***0.16***	***NT***	NT
IV 23	Propyl	6-heptenoyl	0.12	213	14
IV 24	Propyl	2,6-heptadienoyl	0.07	248	14
IV 25	Hexyl	***Butanoyl***	***0.21***	***165***	15
IV 26	Hexyl	*trans*-2-butenoyl	0.15	171	14
IV 27	Hexyl	3-butenoyl	0.15	141	14
IV 28	Hexyl	*trans*-2-methyl-2-butenoyl	0.16	171	14
IV 29	Hexyl	3-methyl-2-butenoyl	0.16	177	14
IV 30	Hexyl	***Hexanoyl***	***0.18***	***165***	16
IV 31	Hexyl	*trans*-2-hexenoyl	0.14	183	14
IV 32	Hexyl	*trans*-3-hexenoyl	0.18	154	14
IV 33	Hexyl	2-ethyl-2-hexenoyl	0.17	227	14
IV 34	Hexyl	***Heptanoyl***	***0.17***	***NT***	NT
IV 35	Hexyl	6-heptenoyl	0.20	224	14
IV 36	Hexyl	2,6-heptadienoyl	0.13	186	14

thoquinone (5,8-dimethoxy-1,4-naphthoquinone; DMNQ) with DCC/DMAP as catalysts has brought a negligible yield of 6-(1-alkenoyloxyalkyl)-DMNQ derivatives. It is general that DCC reacts with organic acid to result in strong electrophilic intermediate to which a alcoholic hydroxyl group adds readily. However, in the case of an α,β-unsaturated alkenoic acid-DCC adduct, the alcoholic hydroxyl group could add both of carbonyl and β-carbon atom to lower the yield. Therefore, for acylation of 6-(hydroxyalkyl)-DMNQ with α,β-unsaturated carboxylic acids, application of the Mitsunobu' method (Leo A. Paquette *et al.*, 1995) was expected to be more beneficial for the synthesis of 6-(1-alkenoyloxyalkyl)-DMNQ derivatives. Triphenylphosphine in Mitsunobu's condition activates the alcoholic hydroxyl group to be a strong alkylating agent which in turn reacts with α,β-unsaturated alkenoylate ion (O. Mitsunobu, *et al.*, 1971, G. Grynkiewicz *et al.*, 1976).

Cytotoxicity and antitumor activities

The cytotoxic activities of the alkenoyl compounds, most of them being *trans*-form, were compared with those of the alkanoyl compounds, and the results were shown in Table I. It could be recognized that the cytotoxicity of the naphthoquinones with R_1 being methyl and propyl (**IV1~24**) was not enhanced by replacing the alkanoyls with alkenoyls. In contrast, the compounds with R_1=hexyl (**IV25~36**) tended to increase the cytotoxicity through the introduction of the alkenoyl groups.

Replacement of alkanoyl group of IV with an alkenoyl group generally increased the T/C value of the mice bearing S-180 cells: the compounds **IV1** vs. **IV2, IV3, IV4, IV5; IV6** vs. **IV8; IV10** vs. **IV11, IV12** and so on. Within the compounds **IV2, IV3, IV4** and **IV5** (R1=methyl, R2=butenoyl), the T/C value was not dependent on the position of the double bond as **IV2** and **IV3** showed (T/C, 206 and 210%). Introduction of a methyl group to **IV2** and **IV3**, producing **IV4** and **IV53** (T/C, 172 and 144%), respectivlely, decreased the antitumor activity. This effect of alkenoyl group seemed to be reversed for the compound with R1 and R2 being methyl and butenoyl, and R1 and R2 being hexyl and butenoyl, respectively: **IV14** (R2=2-butenoyl, T/C, 183%) vs. **IV17** (R2=3-methyl-2-butenoyl, T/C, 245%); **IV26** (R1=hexyl, R2=2-butenoyl, T/C, 171%) vs. **IV29** (R=hexyl, R2=3-methyl-2-butenoyl, T/C, 177%). The enhancing effect of the butenoyl moiety on the T/C value was leveled out for the compounds with larger R1. Particulary introduction of 6-heptenoyl or 2,6-heptadienoyl group enhanced the antitumor effect considerably (**IV11, IV12, IV23, IV24**). Enhancing effect of alkenoyl groups on the antitumor activity may be based on enhancement of the affinity to cell membrane and the easiness of uptake into it. However, it was difficult to abstract the relationships between a larger size of side chains and the antitumor activity.

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REFERENCES CITED

Ahn, B. Z. and Sok, D. E.; Micheal Acceptors as a Tool for anticancer Drug Design, Current *Pharmaceutical Design.*, 2, 247-262 (1993).

Ahn, B. Z., Song, G. Y., Baik, K. U. and Sok, D. E.; Cytotoxicity of acylshikonin analogues against L1210 cells and their antitumor activity against sarcoma tumor, *Korean J. of Med. Chem.*, 6, 98-109 (1996) .

Ahn, B. Z., Baik, K. U., Kweon, G. R., Lim, K. and Hwang, B. D.; Acyshikonin Analogues: Synthesis and Inhibition of DNA Topoisomerase-I, *Journal of Medicinal Chemistry,* 38, 1044-1047, (1995).

Baik, K. U., Song, G. Y., Kim, Y., Sok, D. E. and Ahn, B. Z.; 2-Substituted Naphthazarins; Synthesis and Antitumor Activity, *Arch. Pharm. Med. Chem.* 330, 377-382 (1997).

Brockmann, H.; The constitutions of alkanin, shikonin and alkannan, *Liebigs Ann Chem* 521, 1-47 (1936).

G. Grynkiewicz *et al., Tetrahedron* 32, 2109 (1976).

Kim, H. and Ahn, B. Z.; Antitumor effect acetylshikonin and some synthesized naphthazarines on L1210 and S-180 systems, *Yakhak Hoeji,* 34, 262-266 (1990).

Leo, A. Paquette *et al.*; Encyclopedia of Reagents for Organic Synthesis, 8, 5379-5390, John Wiley & Sons, Inc., New York, (1995)

Morimoto, I., Kishi, T., Ikegami, S. and Hirata, Y.; Naphthoquinone derivatives from Lithospermum erythrorhizon., *Tetrahedron Lett* 3677-3680 (1965).

O. Mitsunobu, *et al., Bull. Chem. Soc. Jpn.* 44, 3427 (1971).

Sankawa, U., Otsuka, H., Kataoka, Y., Hoshi, Y. A. and Kuretani, K.; Antitumor activity and their derivatives, *Chem. Pharm. Bull.*, 29, 116-122 (1981).

Terada, Y. Tanoue, Hatada, A. and Sakamoto, H.; Synthesis of shikalkin and related compounds, *Bull. Chem. Soc. Jpn,* 60, 205-213, (1987)

Thayer, P. S., Himnelfarb, P. and Watt, G.L.; *Cancer Chemother. rep.*, 2, 1-25 (1971).

You, Y.-J. Zheng, X.-G. Kim, Y. and Ahn, B.-Z.; Naphthazarin derivatives: synthesis, cytotoxic mechanism and evalution of antitumor activity, *Arch. Pharm. Res.,* 21(5), 595-598 (1998).