Eur. J. Med. Chem. 35 (2000) 291–298

S022352340000129X/FLA

Original article

Naphthazarin derivatives (IV): synthesis, inhibition of DNA topoisomerase I and cytotoxicity of 2- or 6-acyl-5,8-dimethoxy-1,4-naphthoquinones

Gyu-Yong Song[a], Yong Kim[a], Xiang-Guo Zheng[a], Young-Jae You[a], Hoon Cho[b], Jin-Ho Chung[c], Dai-Eun Sok[a], Byung-Zun Ahn[a]*

[a]College of Pharmacy, Chungnam National University, Taejon 305-764, Korea
[b]Kuhnil Pharmaceutical Co. Ltd., Chunan 333-810, Korea
[c]College of Pharmacy, Seoul National University, Seoul 151-742, Korea

Received 17 May 1999; revised 6 September 1999; accepted 7 September 1999

Abstract – Some 2- or 6-acyl-5,8-dimethoxy-1,4-naphthoquinone (DMNQ) derivatives were synthesized and evaluated for inhibition of DNA topoisomerase I and cytotoxicity against L1210 cells. Compared with 2-acyl-DMNQ derivatives, 6-acyl-DMNQ compounds, bearing a higher electrophilic quinone moiety, showed a higher potency in the inhibition of DNA topoisomerase I and the cytotoxicity, implying the possible participation of electrophilic arylation in their bioactivities. Time and temperature dependence of the enzyme inhibition suggests that the arylation occurs irreversibly. Among the 6-acyl-DMNQ derivatives, the ones possessing an acyl group of an intermediate size (C_5–C_9) showed higher potency in their bioactivities than other derivatives. Furthermore, for the effective inhibition of DNA topoisomerase I, the size of acyl moiety of 6-acylated derivatives seems to be limited to < 12 carbon atoms. © 2000 Éditions scientifiques et médicales Elsevier SAS

naphthazarin / DNA topoisomerase I inhibition / cytotoxicity / structure–activity relationship

1. Introduction

In previous studies [1, 2] it had been reported that 6-(1-hydroxyalkyl)-5,8-dimethoxy-1,4-naphthoquinone (DMNQ) derivatives showed a stronger potency in the inhibition of TOPO I and cytotoxicity compared with 2-(1-hydroxyalkyl)-DMNQ derivatives. This result led to the suggestion that the steric hindrance of side chain was a cause for the lowered bioactivities of 2-substituted DMNQ derivatives. Therefore, it was supposed that the higher bioactivities of 6-substituted DMNQ derivatives were ascribed to the higher electrophilicity of the quinonoid moiety, sterically less hindered, of 6-substituted DMNQ derivatives.

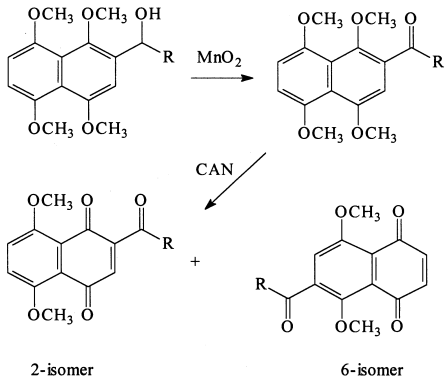
In the present study we synthesized a series of 2- or 6-acylated DMNQ derivatives in order to enhance the electrophilicity of the quinone moiety of DMNQ derivatives, and determined the inhibitory effects on TOPO I and cytotoxicity against L1210 cells. In addition, the oxygen consumption of those compounds as an indicator for redox cycling and the bioactivities were correlated to establish a structural importance.

2. Result and discussion

2.1. Chemistry

For the preparation of 2- or 6-acyl-DMNQ derivatives, 2-(1-hydroxyalkyl)-TMN derivatives as starting materials were oxidized with MnO_2 to give 2-acyl-TMN derivatives. Next, oxidative demethylation of 2-acyl-TMN derivatives with cerium (IV) ammonium nitrate (CAN) produced two isomeric mixtures, 2- and 6-acyl-DMNQ derivatives, in an average ratio of 1/10 (*figure 1*). Preferential formation of 6-substituted DMNQ could be explained by the mechanism of the CAN oxidation [3]; first, the oxidation of 2-substituted-TMN derivatives leads to a formation of the radical cation intermediate induced by

*Correspondence and reprints: ahnbj@hanbat.chungnam.ac.kr
Abbreviations: DMNQ: 5,8-dimethoxy-1,4-naphthoquinone; TMN: 1,4,5,8-tetramethoxynaphthalene; TOPO I: DNA topoisomerase I; CAN: cerium (IV) ammonium nitrate



* CAN : cerium(IV) ammonium nitrate
R ; H, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl and dodecyl

Figure 1. Synthesis of 2- or 6-acyl-5,8-dimethoxy-1,4-naphthoquinone derivatives.

one-electron oxidation. Then, the radical cation intermediate is more stabilized in the electron-rich ring B of naphthalene than in the ring A with electron-withdrawing acyl groups, resulting in the facilitated oxidation of the ring B.

2.2. Inhibition of DNA topoisomerase I and cytotoxicity of naphthoquinone derivatives

As demonstrated in *table I*, the acylation of the DMNQ derivatives resulted in an increase of the cytotoxicity against L1210 cells and the inhibition of TOPO I. 6-Acyl-DMNQ derivatives were more active than 2-acylated ones. As shown in a previous study [1], the steric hindrance of the alkyl side chain may be responsible for the differences in bioactivities of 2-acyl-DMNQ and 6-acyl-DMNQ derivatives. Noteworthy, 6-acyl-DMNQ derivatives exhibited approximately 1.3-fold higher inhibitory effect on TOPO I and 2.3-fold greater cytotoxicity against L1210 cells than the 6-(1-hydroxyalkyl)-DMNQ derivatives [2]. The higher bioactivities of 6-acyl-DMNQ derivatives could be due to enhancement of electrophilicity through the presence of a carbonyl group in the side chain of 6-acylated DMNQ derivatives. The importance of electrophilicity in the cytotoxicity of DMNQ derivatives was discussed in a previous observation [1, 2], that the rate of GSH conjugate formation as an indicator of arylation capability was correlated with cytotoxicity of substituted DMNQ derivatives. Taken together, it is suggested that the steric effect and the electrophilicity may be involved in the inhibition of the enzyme and the cytotoxicity. Furthermore, there was a similarity in size dependence as well as position dependence of alkyl chain for the inhibition of TOPO I and the cytotoxicity; 6-substituted isomers showed more potent bioactivities than 2-substituted isomers, and derivatives bearing an acyl chain of an intermediate size ($C_5 \sim C_8$) were more potent than those with longer ($C_9 \sim C_{13}$) or shorter ($C_1 \sim C_4$). The greatest bioactivity was observed with 6-acyl-DMNQ derivatives bearing a hexanoyl or heptanoyl moiety, which showed IC_{50} values of 16–18 μM and an ED_{50} value of 0.11 μM. Meanwhile, such a size dependence was not expressed for 2-acyl-DMNQ derivatives.

Table I. Relationship between cytotoxicity and DNA topoisomerase I inhibition of 2- and 6-acyl-5,8-dimethoxy-1,4-naphthoquinones.

R	IC_{50} (μM) onTOPO I[a]		ED_{50} (μM) in L1210[b]	
	2-Isomer	6-Isomer	2-Isomer	6-Isomer
Formyl	156 ± 5.67	38 ± 8.56	0.41 ± 0.06	0.20 ± 0.07
Acetyl	163 ± 6.87	40 ± 9.43	0.42 ± 0.13	0.24 ± 0.10
Propanoyl	165 ± 5.62	38 ± 5.78	0.56 ± 0.27	0.19 ± 0.04
Butanoyl	174 ± 7.32	29 ± 8.01	0.46 ± 0.16	0.16 ± 0.01
Pentanoyl	169 ± 8.82	21 ± 6.32	0.49 ± 0.17	0.17 ± 0.05
Hexanoyl	171 ± 5.87	18 ± 5.78	0.50 ± 0.13	0.11 ± 0.01
Heptanoyl	181 ± 7.43	16 ± 3.56	0.53 ± 0.16	0.11 ± 0.01
Octanoyl	189 ± 8.99	19 ± 9.43	0.78 ± 0.18	0.16 ± 0.05
Nonanoyl	> 200	32 ± 7.34	1.15 ± 0.33	0.24 ± 0.05
Decanoyl	> 200	59 ± 6.34	1.58 ± 0.24	0.29 ± 0.06
Undecanoyl	> 200	81 ± 10.02	2.19 ± 0.53	0.40 ± 0.07
Tridecanoyl	> 200	> 200	2.45 ± 0.50	0.66 ± 0.23
Camptothecin	6 ± 0.98			

[a]IC_{50} values and [b]ED_{50} values were measured as described in the Experimental section (mean ± SD)

In a further study, where TOPO I was pre-incubated with 6-hexanoyl-DMNQ for various times at different temperatures and then the remaining activity of TOPO I was determined, it was found that the activity of TOPO I dramatically decreased in a time-dependent manner and the inactivation degree was greater at higher temperatures, exhibiting a pattern typical of an irreversible inhibition (*figure 2*). These results indicate that TOPO I is one of targets for the electrophilic arylation by 6-substituted DMNQ derivatives as suggested from the role of the electrophilicity of DMNQ derivatives [1, 2], although it is not excluded that there may be nucleophile acceptors more susceptible to DMNQ derivatives than TOPO I. After further study, 6-acyl-DMNQ derivatives were found to be more potent in the inhibition of TOPO I than 6-(1-hydroxyalkyl)-DMNQ derivatives with a quinone moiety with less electrophilicity [1]. As for the size dependence of the alkyl group in the inhibition of TOPO I, there seems to exist a boundary size for 6-acyl-DMNQ derivatives. As shown in *table I*, 6-undecanoyl-DMNQ showed a considerable inhibitory effect (IC_{50}, 81 ± 10.02 μM) on DNA topoisomerase I, while 6-tridecanoyl-DMNQ, a two carbon higher homologue, was inactive (IC_{50} > 200 μM). Thus, the active site of TOPO I seems to express a limitation in accommodating the alkyl group.

Overall there seems to be a parallel relationship between the inhibition of TOPO I and the cytotoxicity of 6-substituted DMNQ analogues. From these data it is implied that the inhibition of topoisomerase I could be one of mechanisms for the cytotoxicity. However, a great difference between IC_{50} values and ED_{50} values suggests that other mechanisms in addition to TOPO I inhibition need to be considered to explain the cytotoxicity of the substituted DMNQ derivatives. Since it is well known that one of the mechanisms for the cytotoxicity of naphthoquinone is the redox cycling [4, 5], the oxygen consumption by 2- or 6-acyl-DMNQ derivatives was measured to examine the participation of the redox cycling in the cytotoxicity. As shown in *table II*, there was no remarkable difference in O_2 consumption between 2-and 6-substituted derivatives, implying that both isomers may be subjected to the redox cycling to a similar extent. From this observation the cytotoxicity of 2-substituted DMNQ derivatives, much smaller in arylating capacity, is supposed to be mainly due to the redox cycling. Meanwhile, the higher cytotoxcity of 6-acyl-DMNQ analogues, possessing a sterically non-hindered quinoid moiety as a Michael acceptor, seems to be ascribed to both of the electrophilic arylation and the redox cycling.

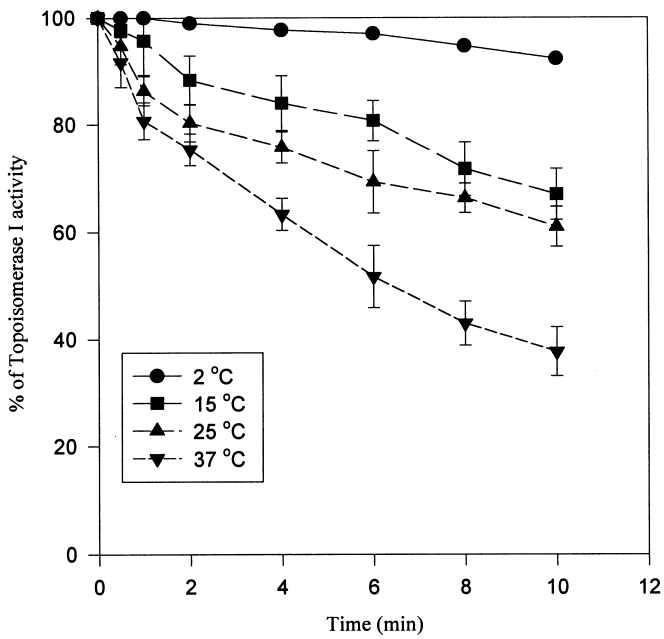


Figure 2. Changes in DNA topoisomerase I activity after pre-incubation with 6-hexanoyl-5,8-dimethoxy-1,4-naphthoquinone. DNA topoisomerase I (1 unit) was incubated with 6-hexanoyl-DMNQ before adding 0.5 μg of pBR322 DNA in 20 μL of relaxation buffer for various reaction times (2, 4, 6, 8 or 10 min) at each of the following temperatures: 2, 15, 25, or 37 °C.

Table II. Oxygen consumption and cytotoxicity of 2- or 6-acyl-5,8-dimethoxy-1,4-naphthoquinones (refer to structure of *table I*).

	R		
2-Isomer	6-Isomer	O_2 consumption (μL/min)	ED_{50} (μM)[a]; mean ± SE
Butanoyl		0.200	0.46 ± 0.16
Hexanoyl		0.167	0.50 ± 0.13
	Propanoyl	0.251	0.19 ± 0.04
	Butanoyl	0.251	0.16 ± 0.01
	Pentanoyl	0.247	0.17 ± 0.05
	Hexanoyl	0.238	0.11 ± 0.01
	Heptanoyl	0.226	0.11 ± 0.01
	Octanoyl	0.206	0.16 ± 0.05
	Nonanoyl	0.100	0.24 ± 0.05
	Decanoyl	0.126	0.29 ± 0.06
	Undecanoyl	0.095	0.40 ± 0.07
	Tridecanoyl	0.113	0.66 ± 0.23

[a]ED_{50} values were measured as described in the Experimental section (mean ± SD).

3. Experimental protocols

Melting points were determined on an electrothermal melting point apparatus and were uncorrected. The IR spectra were recorded on a Jasco Report-100 FT IR spectrometer, and only the principal bands were described. The ^{1}H-NMR spectra were recorded on either a JEOL EX-90 (90 MHz) or a Bruker ARX (300 MHz) NMR spectrometer, and proton chemical shifts are relative to tetramethylsilane as an internal standard in $CDCl_3$ or in DMSO-d_6. High resolution mass spectra were obtained on VG70-VSEQ (VG analytical) under a standard condition. All fractions from column chromatography (EM Kiesegel 60, 230–400 mesh) were monitored by thin layer chromatography (silica gel 60 GF-254, Merck, 230–400 mesh ASTM). All reagents, commercially available, were used without further purification unless otherwise stated.

3.1. Synthesis

3.1.1. General procedure for synthesis of 2-acyl-1,4,5,8-tetramethoxynaphthalenes

To a solution of 2-(1-hydroxyalkyl)-1,4,5,8-tetramethoxynaphthalene [6, 7] (1,4,5,8-tetramethoxynaphthalene, TMN, 20 mmol) in dry benzene (70 mL) was added activated manganese (IV) oxide (8.69 g). The mixture was stirred under reflux for 10 h, and then filtered and concentrated in vacuo. The crude product was recrystallized from methanol.

3.1.1.1. 2-Formyl-1,4,5,8-tetramethoxynaphthalene **1**

This compound was synthesized as described previously [7].

3.1.1.2. 2-Acetyl-1,4,5,8-tetramethoxynaphthalene **2**

Yield: 88%, yellow solid (Rf = 0.34 on silica gel in 40% ethyl acetate in n-hexane), m.p. 68–68.5 °C, ^{1}H-NMR ($CDCl_3$): δ 7.09 (s, 1H, H-3), 6.92 (s, 2H, H-6,7), 3.98 (s, 3H, H-OCH_3), 3.96 (s, 3H, H–OCH_3), 3.90 (s, 3H, H–OCH_3), 3.79 (s, 3H, H–OCH_3), 2.77 (s, 3H, H–$COCH_3$), IR ν_{max} cm^{-1} (KBr): 2 950, 1 665, 1 600, 1 050.

3.1.1.3. 2-Propanoyl-1,4,5,8-tetramethoxynaphthalene **3**

Yield: 90%, yellow solid (Rf = 0.39 on silica gel in 40% ethyl acetate in n-hexane), m.p. 68.3–69.5 °C, ^{1}H-NMR ($CDCl_3$): δ 6.98 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.97 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.90 (s, 3H, H–OCH_3), 3.76 (s, 3H), 3.10 (q, *J* = 7.29 Hz, 2H, H-2′), 1.12 (t, *J* = 7.29 Hz, 3H, H–CH_3), IR ν_{max} cm^{-1} (KBr): 2 925, 1 665, 1 600, 1 060.

3.1.1.4. 2-Butanoyl-1,4,5,8-tetramethoxynaphthalene **4**

Yield: 89%, yellow solid (Rf = 0.43 on silica gel in 40% ethyl acetate in n-hexane), m.p. 82.5–83.7 °C, ^{1}H-NMR ($CDCl_3$): δ 6.96 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.97 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.90 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.10 (t, *J* = 7.29 Hz, 2H, H-2′), 1.70≈1.60 (m, 2H, H-3′), 0.98 (t, *J* = 7.11 Hz, 3H, H–CH_3), IR ν_{max} cm^{-1} (KBr): 2 950, 1 670, 1 580, 1 060.

3.1.1.5. 2-Pentanoyl-1,4,5,8-tetramethoxynaphthalene **5**

Yield: 87%, yellow oil (Rf = 0.49 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.96 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.97 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.90 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.13 (t, *J* = 7.29 Hz, 2H, H-2′), 1.71≈1.26 (m, 4H, H-3′,4′), 0.93 (t, *J* = 6.39 Hz, 3H, H–CH_3), IR ν_{max} cm^{-1} (neat): 2 925, 1 660, 1 600, 1 060.

3.1.1.6. 2-Hexanoyl-1,4,5,8-tetramethoxynaphthalene **6**

Yield: 85%, yellow oil (Rf = 0.56 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.97 (s, 1H, H-3), 6.90 (s, 2H, H-6,7), 3.95 (s, 6H, H-2×OCH_3), 3.89 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.12 (t, *J* = 7.29 Hz, 2H, H-2′), 1.76≈1.17 (m, 6H, H-3′≈5′), 0.90 (t, *J* = 6.21 Hz, 3H, H–CH_3), IR ν_{max} cm^{-1} (neat): 2 925, 1 665, 1 600, 1 050.

3.1.1.7. 2-Heptanoyl-1,4,5,8-tetramethoxynaphthalene **7**

Yield: 88%, yellow oil (Rf = 0.61 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.96 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.96 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.89 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.12 (t, *J* = 7.29 Hz, 2H, H-2′), 1.62≈0.83 (m, 11H, H-3′≈6′ and CH_3), IR ν_{max} cm^{-1} (neat): 2 925, 1 665, 1 600, 1 050.

3.1.1.8. 2-Octanoyl-1,4,5,8-tetramethoxynaphthalene **8**

Yield: 82%, yellow oil (Rf = 0.64 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.95 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.96 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.89 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.11 (t, *J* = 7.29 Hz, 2H, H-2′), 1.73≈0.87 (m, 13H, H-3′≈7′ and CH_3), IR ν_{max} cm^{-1} (neat): 2 925, 1 665, 1 600, 1 060.

3.1.1.9. 2-Nonanoyl-1,4,5,8-tetramethoxynaphthalene **9**

Yield: 80%, yellow oil (Rf = 0.68 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.98 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.97 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.90 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.12 (t, *J* = 7.29 Hz, 2H, H-2′), 1.75≈0.82

(m, 15H, H-3′≈8′ and CH_3) IR ν_{max} cm^{-1} (neat): 2 925, 1 665, 1 600, 1 050.

3.1.1.10. 2-Decanoyl-1,4,5,8-tetramethoxynaphthalene **10**

Yield: 78%, yellow oil (Rf = 0.72 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.94 (s, 1H, H-3), 6.90 (s, 2H, H-6,7), 3.96 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.89 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.12 (t, *J* = 7.29 Hz, 2H, H-2′), 1.75≈0.81 (m, 17H, H-3′≈9′ and CH_3), IR ν_{max} cm^{-1} (neat): 2 925, 1 665, 1 600, 1 060.

3.1.1.11. 2-Undecanoyl-1,4,5,8-tetramethoxynaphthalene **11**

Yield: 72%, yellow oil (Rf = 0.76 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.96 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.97 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.90 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.12 (t, *J* = 7.29 Hz, 2H, H-2′), 1.76≈0.82 (m, 19H, H-3′≈10′ and CH_3), IR ν_{max} cm^{-1} (neat): 2 925, 1 665, 1 600, 1 360.

3.1.1.12. 2-Tridecanoyl-1,4,5,8-tetramethoxynaphthalene **12**

Yield: 68%, yellow oil (Rf = 0.81 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 6.96 (s, 1H, H-3), 6.91 (s, 2H, H-6,7), 3.97 (s, 3H, H–OCH_3), 3.95 (s, 3H, H–OCH_3), 3.89 (s, 3H, H–OCH_3), 3.76 (s, 3H, H–OCH_3), 3.11 (t, *J* = 7.29 Hz, 2H, H-2′), 1.72≈0.83 (m, 23H, H-3′≈12′ and CH_3), IR ν_{max} cm^{-1} (neat): 2 900, 1 690, 1 590, 1 060.

3.1.2. General procedure for synthesis of 2- or 6-acyl-5,8-dimethoxy-1,4-naphthoquinones

To a solution of cerium ammonium nitrate (12.5 mmol) in water (30 mL) was added dropwise a solution of 2-acyl-TMN derivatives (5 mmol) in acetonitrile (80 mL) at room temperature. After 30 min the reaction mixture was poured into ice water (200 mL) and then extracted twice with dichloromethane (200 mL). The combined organic phase was dried over anhydrous magnesium sulfate, and then concentrated in vacuo. The crude product was chromatographed on a silica gel column with ethyl acetate/hexane (3:7) to give 2- and 6-acyl-5,8-dimethoxy-1,4-naphthoquinones (5,8-dimethoxy-1,4-naphthoquinone, DMNQ).

3.1.2.1. 2-Formyl-5,8-dimethoxy-1,4-naphthoquinone **13a**

Yield: 1.8%, red–brown solid (Rf = 0.09 on silica gel in 40% ethyl acetate in n-hexane), m.p. 102–103 °C, ^{1}H-NMR ($CDCl_3$): δ 10.50 (s,1H), 7.75 (s, 2H), 6.89 (s, 1H), 4.00 (s, 6H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{13}H_{10}O_5$: C, 63.42; H, 4.09. Found: C, 63.51; H, 4.14.

3.1.2.2. 6-Formyl-5,8-dimethoxy-1,4-naphthoquinone **13b**

Yield: 77%, yellow solid (Rf = 0.24 on 40% ethyl acetate in n-hexane), m.p. 173–174 °C, ^{1}H-NMR ($CDCl_3$): δ 10.51 (s, 1H), 7.77 (s, 1H), 6.87 (s, 2H), 4.03 (s, 3H), 4.00 (s, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{13}H_{10}O_5$: C, 63.42; H, 4.09. Found: C, 63.42; H, 4.14.

3.1.2.3. 2-Acetyl-5,8-dimethoxy-1,4-naphthoquinone **14a**

Yield: 2.6%, red–brown oil (Rf = 0.24 on silica gel in 40% ethyl acetate in n-hexane), ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 2H), 6.98 (s, 1H), 3.97 (s, 6H), 2.60 (s, 3H), IR ν_{max} cm^{-1} (neat): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65. Found: C, 64.52; H, 4.59.

3.1.2.4. 6-Acetyl-5,8-dimethoxy-1,4-naphthoquinone **14b**

Yield: 73%, yellow solid (Rf = 0.28 on silica gel in 40% ethyl acetate in n-hexane), m.p. 159–160 °C, ^{1}H-NMR ($CDCl_3$): δ 7.49 (s, 1H), 6.84 (s, 2H), 4.00 (s, 3H), 3.86 (s, 3H), 2.69 (s, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{14}H_{12}O_5$: C, 64.61; H, 4.65. Found: C, 64.74; H, 4.72.

3.1.2.5. 2-Propanoyl-5,8-dimethoxy-1,4-naphthoquinone **15a**

Yield: 3.6%, red–brown solid (Rf = 0.10 on silica gel in 40% ethyl acetate in n-hexane), m.p. 134–135 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 2H), 6.89 (s, 1H), 3.99 (s, 6H), 3.01 (q, *J* = 7.29 Hz, 2H), 1.21 (t, *J* = 7.29 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{15}H_{14}O_5$: C, 65.59; H, 5.14. Found: C, 65.54; H, 5.16.

3.1.2.6. 6-Propanoyl-5,8-dimethoxy-1,4-naphthoquinone **15b**

Yield: 74%, yellow solid (Rf = 0.36 on silica gel in 40% ethyl acetate in n-hexane), m.p. 148.3–149.6 °C, ^{1}H-NMR ($CDCl_3$): δ 7.37 (s, 1H), 6.83 (s, 2H), 3.99 (s, 3H), 3.83 (s, 3H), 3.01 (q, *J* = 7.29 Hz, 2H), 1.21 (t, *J* = 7.29 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 680, 1 655, 1 620, 1 050. Anal. calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.14. Found: C, 65.61; H, 5.12.

3.1.2.7. 2-Butanoyl-5,8-dimethoxy-1,4-naphthoquinone **16a**

Yield: 2.5%, red–brown solid (Rf = 0.12 on silica gel in 40% ethyl acetate in n-hexane), m.p. 97.4–98.2 °C,

^{1}H-NMR ($CDCl_3$): δ 7.32 (s, 2H), 6.83 (s, 1H), 3.90 (s, 6H), 3.01 (t, *J* = 7.21 Hz, 2H), 1.70–1.60 (m, 2H), 0.99 (t, *J* = 6.30 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 65.54; H, 5.52.

3.1.2.8. 6-Butanoyl-5,8-dimethoxy-1,4-naphthoquinone **16b**

Yield: 73%, yellow solid (Rf = 0.40 on silica gel in 40% ethyl acetate in n-hexane), m.p. 70.3–71.2 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 1H), 6.83 (s, 2H), 3.90 (s, 3H), 3.84 (s, 3H), 3.01 (t, *J* = 7.21 Hz, 2H), 1.70–1.60 (m, 2H), 0.99 (t, *J* = 6.30 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.60; H, 5.52.

3.1.2.9. 2-Pentanoyl-5,8-dimethoxy-1,4-naphthoquinone **17a**

Yield: 2.8%, red–brown solid (Rf = 0.14 on silica gel in 40% ethyl acetate in n-hexane), m.p. 101.2–102.5 °C, ^{1}H-NMR ($CDCl_3$): δ 7.33 (s, 2H), 6.93 (s, 1H), 3.97 (s, 6H), 2.92 (t, *J* = 7.21 Hz, 2H), 1.71–1.26 (m, 4H), 0.93 (t, *J* = 6.39 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.42; H, 5.92.

3.1.2.10. 6-Pentanoyl-5,8-dimethoxy-1,4-naphthoquinone **17b**

Yield: 75%, yellow solid (Rf = 0.42 on silica gel in 40% ethyl acetate in n-hexane), m.p. 62.1–63.2 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 1H), 6.91 (s, 2H), 3.97 (s, 3H), 3.90 (s, 3H), 3.03 (t, *J* = 7.21 Hz, 2H), 1.71–1.26 (m, 4H), 0.93 (t, *J* = 6.39 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{17}H_{18}O_5$: C, 67.54; H, 6.00. Found: C, 67.61; H, 6.07.

3.1.2.11. 2-Hexanoyl-5,8-dimethoxy-1,4-naphthoquinone **18a**

Yield: 3.0%, red–brown solid (Rf = 0.17, eluent: 40% ethyl acetate in n-hexane), m.p. 138.9–140.3 °C, ^{1}H-NMR ($CDCl_3$): δ 7.32 (s, 2H), 6.90 (s, 1H), 3.95 (s, 6H), 3.02 (t, *J* = 7.21 Hz, 2H), 1.76–1.17 (m, 6H), 0.90 (t, *J* = 6.21 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.45; H, 6.31.

3.1.2.12. 6-Hexanoyl-5,8-dimethoxy-1,4-naphthoquinone **18b**

Yield: 77%, yellow solid (Rf = 0.44 on silica gel in 40% ethyl acetate in n-hexane), m.p. 70.3–71.2 °C, ^{1}H-NMR ($CDCl_3$): δ 7.34 (s, 1H), 6.84 (s, 2H), 3.95 (s, 3H), 3.82 (s, 3H), 3.10 (t, *J* = 7.21 Hz, 2H), 1.76–1.17 (m, 6H), 0.90 (t, *J* = 6.21 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{18}H_{20}O_5$: C, 68.34; H, 6.37. Found: C, 68.45; H, 6.44.

3.1.2.13. 2-Heptanoyl-5,8-dimethoxy-1,4-naphthoquinone **19a**

Yield: 2.3%, red–brown solid (Rf = 0.19 on silica gel in 40% ethyl acetate in n-hexane), m.p. 76.4–77.9 °C, ^{1}H-NMR ($CDCl_3$): δ 7.31 (s, 2H), 6.92 (s, 1H), 3.92 (s, 6H), 2.99 (t, *J* = 7.29 Hz, 2H), 1.82–1.07 (m, 8H), 0.88 (t, *J* = 6.21 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 680, 1 655, 1 620, 1 050. Anal. calcd. for $C_{19}H_{22}O_5$: C, 69.08; H, 6.71. Found: C, 69.16; H, 6.65.

3.1.2.14. 6-Heptanoyl-5,8-dimethoxy-1,4-naphthoquinone **19b**

Yield: 69%, yellow solid (Rf = 0.46 on silica gel in 40% ethyl acetate in n-hexane), m.p. 71.5–72.8 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 1H), 6.83 (s, 2H), 3.94 (s, 3H), 3.83 (s, 3H), 2.99 (t, *J* = 7.29 Hz, 2H), 1.82–1.07 (m, 8H), 0.88 (t, *J* = 6.21 Hz, 3H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{19}H_{22}O_5$: C, 69.08; H, 6.71. Found: C, 69.16; H, 6.86.

3.1.2.15. 2-Octanoyl-5,8-dimethoxy-1,4-naphthoquinone **20a**

Yield: 2.6%, red–brown solid (Rf = 0.22 on silica gel in 40% ethyl acetate in n-hexane), m.p. 93.2–94.6 °C, ^{1}H-NMR ($CDCl_3$): δ 7.31 (s, 2H), 6.94 (s, 1H), 3.95 (s, 6H), 2.98 (t, *J* = 7.11 Hz, 2H), 1.89–0.81 (m, 13H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.84; H, 6.98.

3.1.2.16. 6-Octanoyl-5,8-dimethoxy-1,4-naphthoquinone **20b**

Yield: 72%, yellow solid (Rf = 0.50 on silica gel in 40% ethyl acetate in n-hexane). m.p. 78.8–79.7 °C, ^{1}H-NMR ($CDCl_3$): δ 7.34 (s, 1H), 6.83 (s, 2H), 3.97 (s, 3H), 3.82 (s, 3H), 2.99 (t, *J* = 7.11 Hz, 2H), 1.85–0.81 (m, 13H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.83; H, 7.07.

3.1.2.17. 2-Nonanoyl-5,8-dimethoxy-1,4-naphthoquinone **21a**

Yield: 3.0%, red–brown solid (Rf = 0.25 on silica gel in 40% ethyl acetate in n-hexane), m.p. 83.2–84.6 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 2H), 6.91 (s, 1H), 3.99 (s, 6H), 2.99 (t, *J* = 7.11 Hz, 2H), 1.87–0.82 (m, 15H), IR ν_{max} cm^{-1} (KBr): 2 960, 1 680, 1 655, 1 620, 1 050. Anal. calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.31; H, 7.25.

3.1.2.18. 6-Nonanoyl-5,8-dimethoxy-1,4-naphthoquinone **21b**

Yield: 71%, yellow solid (Rf = 0.54 on silica gel in 40% ethyl acetate in n-hexane). m.p. 83.7–84.9 °C, ^{1}H-NMR ($CDCl_3$): δ 7.36 (s, 1H), 6.83 (s, 2H), 3.95 (s, 3H), 3.84 (s, 3H), 2.99 (t, *J* = 7.11 Hz, 2H), 1.89–0.83 (m, 15H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 680, 1 655, 1 620, 1 050. Anal. calcd. for $C_{21}H_{26}O_5$: C, 70.37; H, 7.31. Found: C, 70.45; H, 7.40.

3.1.2.19. 2-Decanoyl-5,8-dimethoxy-1,4-naphthoquinone **22a**

Yield: 3.6%, red–brown solid (Rf = 0.28 on silica gel in 40% ethyl acetate in n-hexane), m.p. 87.8–89.5 °C, ^{1}H-NMR ($CDCl_3$): δ 7.33 (s, 2H), 6.92 (s, 1H), 3.94 (s, 6H), 3.01 (t, *J* = 7.11 Hz, 2H), 1.85–0.87 (m, 17H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{22}H_{28}O_5$: C, 70.95; H, 7.58. Found: C, 70.84; H, 7.63.

3.1.2.20. 6-Decanoyl-5,8-dimethoxy-1,4-naphthoquinone **22b**

Yield: 70%, yellow solid (Rf = 0.56 on silica gel in 40% ethyl acetate in n-hexane), m.p. 83.7–84.9 °C, ^{1}H-NMR ($CDCl_3$): δ 7.36 (s, 1H), 6.83 (s, 2H), 3.95 (s, 3H), 3.84 (s, 3H), 2.99 (t, *J* = 7.11 Hz, 2H), 1.89–0.83 (m, 17H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{22}H_{28}O_5$: C, 70.95; H, 7.58. Found: C, 70.82; H, 7.62.

3.1.2.21. 2-Undecanoyl-5,8-dimethoxy-1,4-naphthoquinone **23a**

Yield: 3.0%, red–brown solid (Rf = 0.30 on silica gel in 40% ethyl acetate in n-hexane), m.p. 94.3–95.1 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 2H), 6.90 (s, 1H), 3.98 (s, 6H), 2.99 (t, *J* = 7.11 Hz, 2H), 1.91–0.82 (m, 19H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.59; H, 7.91.

3.1.2.22. 6-Undecanoyl-5,8-dimethoxy-1,4-naphthoquinone **23b**

Yield: 71%, yellow solid (Rf = 0.59 on silica gel in 40% ethyl acetate in n-hexane), m.p. 89.7–90.4 °C, ^{1}H-NMR ($CDCl_3$): δ 7.35 (s, 1H), 6.85 (s, 2H), 3.95 (s, 3H), 3.84 (s, 3H), 2.98 (t, *J* = 7.11 Hz, 2H), 1.91–0.83 (m, 19H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.57; H, 7.91.

3.1.2.23. 2-Tridecanoyl-5,8-dimethoxy-1,4-naphthoquinone **24a**

Yield: 2.3%, red–brown solid (Rf = 0.35 on silica gel in 40% ethyl acetate in n-hexane), m.p. 67.8–68.4 °C, ^{1}H-NMR ($CDCl_3$): δ 7.31 (s, 2H), 6.92 (s, 1H), 3.95 (s, 6H), 2.99 (t, *J* = 7.11 Hz, 2H), 1.89–0.83 (m, 23H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.37; H, 8.32.

3.1.2.24. 6-Tridecanoyl-5,8-dimethoxy-1,4-naphthoquinone **24b**

Yield: 67%, yellow solid (Rf = 0.64 on silica gel in 40% ethyl acetate in n-hexane), m.p. 92.3–93 °C, ^{1}H-NMR ($CDCl_3$): δ 7.36 (s, 1H), 6.82 (s, 2H), 3.99 (s, 3H), 3.82 (s, 3H), 3.02 (t, *J* = 7.11 Hz, 2H), 1.91–0.81 (t, 23H), IR ν_{max} cm^{-1} (KBr): 2 950, 1 685, 1 655, 1 620, 1 050. Anal. calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.51; H, 8.32.

3.2. Measurement of the cytotoxicity against L1210 tumour cells

Cytotoxicity of compounds against L1210 cell lines was measured as described previously [8]. Fishers medium supplemented with 10% horse serum was used for the proliferation of L1210 cells. One day before the test, a cell suspension ($2 \approx 3 \times 10^5$ cells/mL) in a logarithmic phase (viability, > 95%) was prepared and incubated at 37 °C in an atmosphere of 5% CO_2. For the test, the cell suspension was adjusted to 5×10^4 cells/mL. A sample (0.1 mg/mL in dimethylsulfoxide) was diluted in 10-fold dilutions with fresh medium, 15, 30, 60 μL of the diluted sample was put in cell suspension (3 mL) and 5 wells were used for each concentration of the test sample. After 48 h incubation, viability was determined using a haemocytometer. ED_{50} values (μg/mL), calculated by an available computerized program, was defined as the concentration of drug to produce a 50% reduction in the viability relative to the control; the value was expressed as the average (± SD) of three independent measurements.

3.3. Determination of DNA topoisomerase I activity [9, 10]

The enzymatic activity was analysed by the DNA unwinding assay. Calf thymus DNA topoisomerase I (1 unit) was incubated with 0.5 μg of *Escherichia coli* pBR322 DNA (TAKARA Co. Ltd.), in the presence or absence of test compounds, in 20 μL 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 the 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 ethidium bromide solution (0.5 μg/mL) after electrophoresis. The IC_{50} value is expressed as the concentration of compound that caused 50% inhibition of relaxation of supercoiled pBR322 DNA under the conditions used. In each test, camptothecin was used as a positive control. Separately, DNA topoisomerase I (1 unit) was pre-incubated with 6-(1-oxohexyl)-DMNQ (8 μM) at various times (2, 4, 6, 8 or 10 min) at each temperature (2, 15, 25 or 37 °C). After designated time intervals at the respective temperature, 0.5 μg of pBR322 DNA in 20 μL of the incubation buffer was added to the above reaction mixture, which was incubated for 30 min at 37 °C. The value was expressed as the mean (± SE) of triplicate experiments (*figure 1*).

3.4. Oxygen consumption determination

To prepare liver microsomal fractions, mice were sacrificed by cervical dislocation. Livers were excised and homogenized at 4 °C in 4 volumes of 0.25 M sucrose containing 0.025 M KCl, 0.01 M $MgCl_2$ and 0.05 M Tris (pH 7.5) for 15 s using a Polytron homogenizer (Brinkmann, USA). The homogenate was then spun at 9 000 *g* for 15 min at 4 °C. After the centrifugation (100 000 *g*, 1 h), the final microsomal pellet was resuspended in the above buffer (approximately 10 mg protein/mL), and then rapidly frozen in liquid nitrogen and stored at –80 °C. The oxidative stress was measured by monitoring oxygen consumption essentially as described by Nakagawa and Moldeus [11]. The consumption of oxygen was measured with a Yellow Springs Instruments Model 5300 oxygen monitor equipped with a Clark-type electrode. The system was initialized with 0.05 M Tris buffer (pH 7.5) and was allowed to run for 10–15 min at 25 °C. After the baseline was established the microsome (0.25 mg/mL), NADPH (0.2 mM), and quinone sample (0.25 mM) were added to the incubation buffer. Oxygen consumption was measured at 5 min intervals and the initial linear portion of the curve was used to estimate the rate of oxygen consumption.

Acknowledgements

This study was financially supported by the Korea Science and Engineering Foundation (KOSEF) and Kuhnil Pharmaceutical Co. Ltd.

References

[1] You Y.J., Zheng X.G., Kim Y., Ahn B.Z., Arch. Pharm. Res. 21 (1998) 595–598.

[2] Song G.Y., Zheng X.G., Kim Y., You Y.J., Sok D.E., Ahn B.Z., Bioorg. Med. Chem. Lett. 9 (1999) 2407–2412.

[3] Tanoue A., Terada A., Bull. Chem. Soc. Jpn. 61 (1988) 2039–2045.

[4] Brunmark A., Cadenas E., Free Radic. Biol. Med. 7 (1988) 435–477.

[5] O'Brien P.J., Chem. Biol. Interact. 80 (1991) 1–41.

[6] Terada A., Tanoue Y., Hatada A., Sakamoto H., Bull. Chem. Soc. Jpn. 60 (1987) 203–213.

[7] Baik K.U., Song G.Y., Kim Y., Sok D.E., Ahn B.Z., Arch. Pharm. Med. Chem. 330 (1997) 377–382.

[8] Thayer A.S., Himnelfarb P., Watt G.L., Cancer Chemother. Rep. 2 (1971) 1–25.

[9] Liu L.F., Miller K.G., Proc. Natl. Acad. Sci. USA 78 (1981) 3487–3491.

[10] Liu L.F., Rowe T.C., Yang L., Tewey K.M., Chen G.L., J. Biol. Chem. 258 (1983) 15365–15370.

[11] Nakagawa Y., Moldeus P., Biochem. Pharmacol. 44 (1992) 1059–1065.