

Synthesis and *In Vitro* Cytotoxicity of 2-Alkylaminosubstituted Quinoline Derivatives

Heesoon Lee¹, Jeeman Lee¹, Seung-Soo Hong¹, Sung-Il Yang², Sang-Hun Jung³, Yurngdong Jahng⁴, and Jungsook Cho⁵

¹College of Pharmacy, Chungbuk National University, Cheongju, 361-763, Korea, ²College of Medicine, Kunkuk University, Chungju, 380-701, Korea, ³College of Pharmacy, Chungnam National University, Taejon 305-764, Korea, ⁴College of Pharmacy, Yeoungnam University, Gyongsan, 712-749, Korea, and ⁵College of Medicine, Dongguk University, Gyeongju, 780-714, Korea

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Eight 2-alkylaminosubstituted 5,8-dimethoxy-4-methylquinolines and nine 2-alkylaminosubstituted or 2,6-disubstituted 4-methylquinoline-5,8-diones were synthesized and evaluated *in vitro* cytotoxicity against four human cancer cell lines (HOP62, SK-OV-3, HCT15 and SF295).

Key words: Antitumor agents, Cytotoxic activity, Doxorubicin, TAS-103

INTRODUCTION

Quinoline derivatives have been extensively studied as potential antitumor agents, since they are capable of binding to DNA (Peacocke *et al.*, 1973). A great number of quinolines has been synthesized and assessed as antimalarial, anti-allergic, anti-inflammatory, fungicidal, and antiviral agents (Alvarez-Ibarra *et al.*, 1997). Among them, 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno[2,1-c]quinolin-7-one dihydrochloride (TAS-103) (Fig. 1) was a novel lead structure which has potential for the treatment of resistant tumors. TAS-103 was developed to target both topoisomerase (Topo) I and Topo II (Minderman *et al.*, 2000; Aoyagi *et al.*, 1999).

In this report, we describe the synthesis and *in vitro* cytotoxic activities of 2-alkylaminosubstituted quinoline derivatives (Fig. 1). These compounds were designed as truncated analogues of TAS-103. It would seem to be interesting to prepare the compounds with simplified structure of TAS-103 to probe the structure-activity relationship.

MATERIALS AND METHODS

Melting points were recorded on a Electrothermal IA9100 digital melting point apparatus and are uncorrected. IR

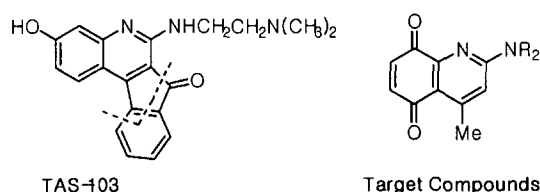


Fig. 1. Structures of TAS-103 and target compounds

spectra were determined with a Jasco FT/IR-300E spectrophotometer and reported in cm^{-1} . ¹H-NMR spectra were recorded on Bruker DPS300 NMR spectrometer using TMS as an internal standard and chemical shifts are reported as δ ppm units. Thin-layer chromatography was performed on E. Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray of concentrated sulfuric acid followed by heating. Column chromatography was carried out on silica gel 60 (230-400mesh ASTM). Commercially available reagents and solvents were used without additional purification unless otherwise stated. RPMI1640 media was obtained from Gibco BRL. Dimethylsulfoxide (DMSO) and other chemicals were purchased from Sigma.

2-Chloro-4-methyl-5,8-dihydro-5,8-quinolinedione (5)

A stirred solution of 2-chloro-5,8-dimethoxy-4-methylquinoline (4) (6 g, 25.2 mmol) in acetonitrile (400 mL) was treated with a solution of ceric ammonium nitrate (41.65 g, 75.7 mmol) in water (100 mL) over 15 min. The

Correspondence to: Heesoon Lee, College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea
E-mail: medchem@trut.chungbuk.ac.kr

reaction mixture was stirred at room temperature for an additional 30 min, during which time a transient blue-black color was observed. The mixture was stirred for 30 min at room temperature, the yellow precipitate was collected by filtration, washed with water (50 mL), and dried thoroughly *in vacuo* to give a yellow powder. The filtrate was extracted with chloroform (3 × 100 mL). The organic layer was washed with brine (100 mL) and dried (magnesium sulfate). The solvent was removed *in vacuo* to give a crude product. Crystallization of the combined crude product from ethyl acetate afforded **5** (5.03 g, 95 %) as yellow needles: mp 145–146°C (lit. 145–146°C); IR (KBr) 3070, 1683, 1659 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 2.78 (s, 3H), 6.94 (d, *J*=10Hz, 1H), 7.07 (d, *J*=10Hz, 1H), 7.47 (s, 1H).

General procedure for 2,6-disubstituted 4-methyl-5,8-quinolinediones

2-Chloro-4-methyl-5,8-Dihydro-5,8-quinoline-dione (**5**) (150 mg, 0.725 mmol) was treated with corresponding amine (3.625 mmol) in DMF (10 mL) for 24 h under nitrogen atmosphere. The solvent was removed *in vacuo* and the resulting residue was purified by flash column chromatography (3% methanol in dichloromethane).

4-Methyl-2,6-di-pyrrolidin-1-yl-5,8-quinolinedione (**6a**)

The product was obtained in 44% yield as a dark brown powder; mp 163–165°C; IR (KBr) 2924, 1668, 1647, 1603 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 2.03 (m, 8H), 2.64 (s, 3H), 3.65 (m, 8H), 5.74 (s, 1H), 6.24 (s, 1H)

4-Methyl-2,6-di-morpholin-4-yl-5,8-quinolinedione (**6b**)

The product was obtained in 53% yield as a dark brown powder; mp 210–212°C; IR (KBr) 2965, 1651, 1632 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 2.62 (s, 3H), 3.47 (t, *J*=4.8Hz, 4H), 3.83 (m, 6H), 5.97 (s, 1H), 6.52 (s, 1H).

General procedure for 2-substituted 5,8-dimethoxy-4-methylquinolines

2-Chloro-5,8-dimethoxy-4-methylquinoline (**4**) (1.26 mmol) was treated with corresponding amine (12.6 mmol) in pyridine (10 mL). The reaction mixture was stirred at reflux for 24 h under nitrogen atmosphere. The solvent was removed *in vacuo* and the resulting residue was purified by flash column chromatography (5% methanol in dichloromethane).

5,8-Dimethoxy-4-methyl-2-pyrrolidin-1-ylquinoline (**7a**)

The product was obtained in 68% yield as a cream colored powder; mp 118–119°C; IR (KBr) 2925, 1604 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 2.02 (t, *J*=6.4Hz, 4H), 2.76 (s, 3H), 3.65 (m, 4H), 3.84 (s, 3H), 3.97 (s, 3H), 6.46 (d, *J*=8.5Hz, 1H), 6.48 (s, 1H), 6.84 (d, *J*=8.5Hz, 1H).

5,8-Dimethoxy-4-methyl-2-piperidin-1-ylquinoline (**7b**)

The product was obtained in 35% yield as a cream colored powder; mp 243–245°C; IR (KBr) 2948, 2805, 1604, 1585, 1459 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 2.04 (m, 6H), 2.72 (s, 3H), 3.49 (m, 4H), 3.90 (s, 3H), 3.99 (s, 3H), 6.62 (d, *J*=8.5Hz, 1H), 6.92 (s, 1H), 6.97 (d, *J*=8.5Hz, 1H).

5,8-Dimethoxy-4-methyl-2-(4-methylpiperazino)quinoline (**7c**)

The product was obtained in 73% yield as a yellow powder; mp 187–189°C; IR (KBr) 2995, 1601, 1572 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 2.37 (s, 3H), 2.56 (m, 4H), 2.77 (s, 3H), 3.75 (m, 4H), 3.85 (s, 3H), 3.95 (s, 3H), 6.49 (d, *J*=8.5Hz, 1H), 6.78 (s, 1H), 6.81 (d, *J*=8.5Hz, 1H).

N-(5,8-Dimethoxy-4-methyl-2-quinolyl)-*N*-propylamine (**7d**)

The product was obtained in 81% yield as a cream colored powder; mp 102–103°C; IR (KBr) 3322, 3007, 2926, 1612, 1566 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 1.02 (t, *J*=7.3Hz, 3H), 1.69 (m, 2H), 2.77 (s, 3H), 3.33 (m, 2H), 3.85 (s, 3H), 3.97 (s, 3H), 6.47 (s, 1H), 6.50 (d, *J*=8.5Hz, 1H), 6.86 (d, *J*=8.5Hz, 1H), 8.61 (brs, 1H).

*N*1-(5,8-Dimethoxy-4-methyl-2-quinolyl)-*N*2,*N*2-dimethyl-1,2-ethanediamine (**7e**)

The product was obtained in 99% yield as a cream colored powder; mp 225–227°C; IR (KBr) 3226, 2934, 1617, 1552 cm⁻¹; ¹H-NMR (DMSO-*d*₆/TMS) δ 2.66 (s, 3H), 2.90 (s, 6H), 3.28 (m, 2H), 3.56 (m, 2H), 3.82 (s, 3H), 3.91 (s, 3H), 6.66 (s, 1H), 6.70 (d, *J*=8.6Hz, 1H), 7.08 (d, *J*=8.6Hz, 1H), 7.89 (brs, 1H).

*N*1-(5,8-Dimethoxy-4-methyl-2-quinolyl)-*N*3,*N*3-dimethyl-1,3-propanediamine (**7f**)

The product was obtained in 94% yield as a cream colored powder; mp 110–112°C; IR (KBr) 3274, 2931, 1620, 1563 cm⁻¹; ¹H-NMR (CDCl₃/TMS) 1.83 (m, 2H), δ 2.26 (s, 6H), 2.43 (t, *J*=7.5Hz, 2H), 2.75 (s, 3H), 3.49 (t, *J*=6.3Hz, 2H), 3.84 (s, 3H), 3.96 (s, 3H), 5.43 (brs, 1H), 6.48 (m, 2H), 6.84 (d, *J*=8.5Hz, 1H).

*N*1-(5,8-Dimethoxy-4-methyl-2-quinolyl)-*N*2,*N*2-diethyl-1,2-ethanediamine (**7g**)

The product was obtained in 88% yield as an orange powder; mp 185–187°C; IR (KBr) 3397, 2973, 1616, 1558 cm⁻¹; ¹H-NMR (CDCl₃/TMS) δ 1.14 (t, *J*=7.1Hz, 6H), 2.73 (s, 3H), 2.85 (q, *J*=7.1Hz, 4H), 2.98 (t, *J*=5.2Hz, 2H), 3.66 (t, *J*=5.2Hz, 2H), 3.84 (s, 3H), 3.95 (s, 3H), 6.52 (d, *J*=8.7Hz, 1H), 6.59 (s, 1H), 6.88 (d, *J*=8.7 Hz, 1H), 8.11 (brs, 1H).

General procedure for 2-substituted 4-methyl-5,8-quinolinediones.

A stirred solution of 2-substituted 5,8-dimethoxy-4-

methylquinoline (**7**) (0.37 mmol) in acetonitrile (3 mL) was treated with a solution of ceric ammonium nitrate (1.47 mmol) in water (1 mL). The reaction mixture was stirred at room temperature for 5 h. The solvent was removed *in vacuo* and the resulting residue was purified by flash column chromatography (5% methanol in dichloromethane).

4-Methyl-2-pyrrolidin-1-yl-5,8-dihydro-5,8-quinolinedione (**8a**)

The product was obtained in 42% yield as a red-brown powder; mp 152~154°C; IR (KBr) 2924, 1647, 1591, cm^{-1} ; $^1\text{H-NMR}$ ($\text{DMSO-d}_6/\text{TMS}$) δ 1.99 (m, 4H), 2.50 (s, 3H), 3.55 (m, 4H), 7.34 (d, $J=4.5\text{Hz}$, 1H), 7.68 (m, 2H).

4-Methyl-2-(4-methylpiperazino)-5,8-dihydro-5,8-quinolinedione (**8c**)

The product was obtained in 42% yield as a dark brown powder; mp 178~180°C; IR (KBr) 2922, 2851, 1655, 1595 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3/TMS) δ 2.31 (s, 3H), 2.51 (m, 4H), 2.75 (s, 3H), 3.65 (m, 4H), 7.46 (s, 1H), 7.52 (d, $J=5.5\text{Hz}$, 1H), 7.69 (d, $J=5.5\text{Hz}$, 1H).

4-Methyl-2-(propylamino)-5,8-dihydro-5,8-quinolinedione (**8d**)

The product was obtained in 10% yield as a dark brown powder; mp 186~189°C; IR (KBr) 3400, 2924, 1653, 1594 cm^{-1} ; $^1\text{H-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{OD}/\text{TMS}$) δ 0.95 (t, $J=6.2\text{Hz}$, 3H), 2.12 (m, 2H), 2.68 (s, 3H), 3.39 (m, 2H), 6.67 (s, 1H), 6.89 (d, $J=5.4\text{Hz}$, 1H), 6.92 (d, $J=5.4\text{Hz}$, 1H).

2-[(2-Dimethylamino)ethyl]amino-4-methyl-5,8-dihydro-5,8-quinolinedione (**8e**)

The product was obtained in 18% yield as a dark brown powder; mp 210~212°C; IR (KBr) 3400, 2924, 1637, 1592 cm^{-1} ; $^1\text{H-NMR}$ ($\text{CDCl}_3\text{-CD}_3\text{OD}/\text{TMS}$) δ 2.69 (s, 3H), 3.23 (s, 6H), 4.03 (m, 4H), 6.68 (s, 1H), 6.89 (d, $J=8.6\text{Hz}$, 1H), 6.95 (d, $J=8.6\text{Hz}$, 1H), 7.60 (brs, 1H).

2-[(3-Dimethylamino)propyl]amino-4-methyl-5,8-dihydro-5,8-quinolinedione (**8f**)

The product was obtained in 21% yield as a dark brown powder; mp 193~196°C; IR (KBr) 3397, 2927, 1684, 1636, 1594 cm^{-1} ; $^1\text{H-NMR}$ ($\text{DMSO-d}_6/\text{TMS}$) δ 1.75 (m, 2H), 2.04 (s, 6H), 2.52 (m, 2H), 2.75 (s, 3H), 3.78 (t, $J=6.3\text{Hz}$, 2H), 7.39 (s, 1H), 7.42 (d, $J=8.6\text{Hz}$, 1H), 7.69 (d, $J=8.6\text{Hz}$, 1H), 8.11 (brs, 1H).

2-[(2-Diethylamino)ethyl]amino-4-methyl-5,8-dihydro-5,8-quinolinedione (**8g**)

The product was obtained in 8% yield as a dark brown powder; mp 182~185°C; IR (KBr) 3399, 2924, 1680, 1636, 1594 cm^{-1} ; $^1\text{H-NMR}$ (CDCl_3/TMS) δ 1.03 (m, 6H),

2.89 (s, 3H), 2.96 (m, 4H), 3.18 (m, 2H), 4.20 (t, $J=5.0\text{Hz}$, 2H), 7.34 (s, 1H), 7.52 (d, $J=8.7\text{Hz}$, 1H), 7.70 (d, $J=8.7\text{Hz}$, 1H), 8.02 (brs, 1H).

Cell culture

Four human cancer cell lines, HOP62, SK-OV-3, HCT15, and SF295 were used in this study. SK-OV-3 and HCT15 were obtained from national cancer institute, U.S.A. HOP62 and SF295 were purchased from American Type Culture Collection. These cells were maintained in Dulbecco's modified eagle media supplemented with 10% fetal calf serum at 37°C under a humidified atmosphere of 5% CO_2 .

In vitro Cytotoxicity assay

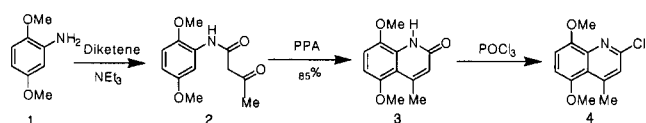
Cell numbers were measured indirectly by sulforhodamine B (SRB) method according to the NCI (USA)'s protocol (Skehan *et al.*, 1990). Briefly, cells were plated into 96 well plate at a density of 2×10^3 cells per well. Next day (day 0), compounds of interest dissolved in DMSO/media were added in quadruplicate. The final concentrations of each compound were 1 nM-10 μM and the final concentration of DMSO was <0.1%. 72 h later, cells were fixed with 10% trichloroacetic acid (TCA) for overnight at 4°C. The TCA-treated cells were extensively washed with distilled water and dried in the air. Then, SRB solution (0.4% in 1% acetic acid) was added to the well at room temperature for one hour. Bound dye was solubilized with 10 mM Tris after washing the wells with 1% acetic acid, and absorbances at 690 nm were measured using a microplate reader. The absorbance value of day 0 was subtracted from the absorbance values of day 3.

RESULTS AND DISCUSSION

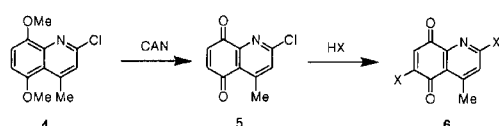
In order to synthesize quinoline derivatives bearing 2-alkylaminosubstituent, 2-chloro-4-methyl-5,8-dihydro-5,8-quinolinedione (**5**) was prepared according to the literature method (Kaslow *et al.*, 1946; Potts *et al.*, 1986). 5,8-Dimethoxy-4-methylquinoline (**4**) was prepared using Knorr cyclization starting from dimethoxyaniline (**1**) (Kaslow *et al.*, 1946) (Scheme 1).

Oxidative demethylation of **4** gave the compound **5** (Potts *et al.*, 1986). Treatment of **5** with pyrrolidine and morpholine in DMF afforded 2,6-disubstituted-4-methylquinoline derivatives **6a** (52%) and **6b** (44%) (Scheme 2).

In order to synthesize monosubstituted quinoline derivatives, 2-chloro-4-methyl-5,8-dimethoxyquinoline (**4**) was treated with various amines (pyrrolidine, morpholine, piperidine, *N*-methylpiperazine, propylamine, dimethylaminoethylamine, dimethylaminopropylamine, and diethylaminoethylamine) to give **7a-g** in 35 to 99% yield. These were then oxidized by ceric ammonium nitrate to give the



Scheme 1. Synthesis of 2-chloro-5,8-dimethoxy-4-methylquinoline



Compound No	X	Yield(%)
6a	-N(CH ₂ CH ₂) ₂	52
6b	-N(CH ₂ CH ₂) ₂ O	44

Scheme 2. Synthesis of 2,6-disubstituted 5,8-quinolinediones

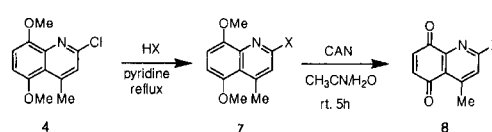
corresponding quinolinedione **8a**, and **8c-g** (Scheme 3).

The evaluations of the biological activity for the compounds were performed *in vitro* following the protocols developed by the National Cancer Institute (Skehan *et al.*, 1990). The *in vitro* cytotoxic activities of the quinoline derivatives **4**, **5**, **6a-b**, **7a-g**, **8a**, and **8c-g** against human cancer cell lines originated from lung (HOP62), ovarian (SK-OV-3), colon (HCT-15), CNS carcinoma (SF295) along with comparative data for doxorubicin are listed in Table I.

Table I. *In Vitro* cytotoxic activity of quinoline derivatives

Compound No	X	IC ₅₀ (μM)			
		HOP62	SK-OV-3	HCT15	SF295
6a	-N(CH ₂ CH ₂) ₂	12	27	16	13
6b	-N(CH ₂ CH ₂) ₂ O	30	30	9	29
4	-Cl	33	35	30	40
7a	-N(CH ₂ CH ₂) ₂	23	27	22	24
7b	-N(CH ₂ CH ₂) ₂ CH ₂	>100	>100	90	55
7c	-N(CH ₂ CH ₂) ₂ NCH ₃	53	61	40	49
7d	-NHCH ₂ CH ₂ CH ₃	17	9.2	15	13
7e	-NHCH ₂ CH ₂ N(CH ₃) ₂	23	29	29	26
7f	-NHCH ₂ CH ₂ CH ₂ N(CH ₃) ₂	34	38	36	42
7g	-NHCH ₂ CH ₂ N(CH ₂ CH ₃) ₂	5.2	10	23	13
5	-Cl	14	15	3.1	16
8a	-N(CH ₂ CH ₂) ₂	69	>100	41	40
8c	-N(CH ₂ CH ₂) ₂ NCH ₃	29	>100	28	38
8d	-NHCH ₂ CH ₂ CH ₃	29	>100	31	37
8e	-NHCH ₂ CH ₂ N(CH ₃) ₂	14	25	5	24
8f	-NHCH ₂ CH ₂ CH ₂ N(CH ₃) ₂	16	21	7.9	27
8g	-NHCH ₂ CH ₂ N(CH ₂ CH ₃) ₂	3.9	21	4.1	13
Doxorubicin		0.11	0.068	0.036	0.09

^aIC₅₀=concentration of compound(μM) required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by the SRB assay. Each experiment was run at least three times, and the results are presented as an average value. ^bHuman cancer cell lines: HOP62(lung cancer cell), SK-OV-3(ovarian cancer cell), HCT-15(colon cancer cell), SF295 (CNS carcinoma cell).



Compound No	X	Yield(%)
7a	-N(CH ₂ CH ₂) ₂	68
7b	-N(CH ₂ CH ₂) ₂ CH ₂	35
7c	-N(CH ₂ CH ₂) ₂ NCH ₃	73
7d	-NHCH ₂ CH ₂ CH ₃	81
7e	-NHCH ₂ CH ₂ N(CH ₃) ₂	99
7f	-NHCH ₂ CH ₂ CH ₂ (CH ₃) ₂	94
7g	-NHCH ₂ CH ₂ N(CH ₂ CH ₃) ₂	88
8a	-N(CH ₂ CH ₂) ₂	42
8c	-N(CH ₂ CH ₂) ₂ NCH ₃	14
8d	-NHCH ₂ CH ₂ CH ₃	10
8e	-NHCH ₂ CH ₂ N(CH ₃) ₂	18
8f	-NHCH ₂ CH ₂ CH ₂ N(CH ₃) ₂	21
8g	-NHCH ₂ CH ₂ N(CH ₂ CH ₃) ₂	8

Scheme 3. Synthesis of 2-alkylamino-4-methylquinolines

The compounds were generally less potent than doxorubicin. The compounds **7g** and **8g** bearing diethylaminoethyl side chain were more potent than the others. However, there is no significant activity difference between quinolines **7a-g** and quinolindiones **8a-g**. This result

suggests that additional ring system may be necessary for good activity. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

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