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Effects of 6-Arylamino-5,8-quinolinediones and 6-Chloro-7-arylamino-5,8-isoquinolinediones on NAD(P)H: Quinone Oxidoreductase (NQO1) Activity and Their Cytotoxic Potential

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Synthesized 6-arylamino-5,8-quinolinediones **4a-4j** and 6-chloro-7-arylamino-5,8-isoquinolinediones **5a-5g** were evaluated for effects on NAD(P)H: quinone oxidoreductase (NQO1) activity with the cytosolic fractions derived from cultured human lung cancer cells and their cytotoxicity in cultured several human solid cancer cell lines. The 5,8-quinolinediones **4** and 5,8-isoquinolinediones **5** affected the reduction potential by NQO1 activity and showed a potent cytotoxic activity against human cancer cell lines. The tested compounds **4a, 5c, 5f,** and **5g** were considered as more potent cytotoxic agents. The compounds **4d, 5b, 5c, 5e** and **5g** were comparable modulators of NQO1 activity.

Key words: 6-Arylamino-5,8-quinolinediones, 6-Chloro-7-arylamino-5,8-isoquinolinediones, NAD(P)H: Quinone oxidoreductase (NQO1), Cytotoxicity

INTRODUCTION

Cytotoxic mechanism of quinones against cancer cells is highly related to bioreduction process for activation of the quinones (Shaikh *et al.*, 1986). In the bioreduction, a plausible enzyme is NAD(P)H: quinone oxidoreductase (NQO1, DT-Diaphorase) that are overexpressed in cancer cells (Beall *et al.*, 1995). NQO1 is an cytosolic two-electron reductase that is characterized by its capacity for using either NADH or NADPH as reducing cofactors (Ernster, 1967). NQO1 reduces quinones to hydroquinones bypassing the potentially toxic semiquinone radical intermediates and can also function as an activating enzyme, specifically for the reductive activation of antitumor quinones. NQO1 has been found to be highly overexpressed in tumor of the colon, breast and lung relative to normal tissue, indicating that bioreductive quinones which are activated by NQO1 may be selectively toxic to those tumors (Beall *et al.*, 1995). Further it showed that the best quinone substrates for NQO1 were also the most toxic to the high NQO1 cell line when compared to the NQO1-deficient cell line (Beall *et al.*, 1996). As exemplified, quinoid compounds such as 5,8-quinolinediones **1** or novel indolequinones **2** (Fig. 1) are an excellent substrate for NQO1, and they are selectively toxic to colon and lung cancer cell lines with elevated NQO1 (Beall *et al.*, 1995; 1996; Fryatt *et al.*, 1999).

In our continuous searching for antitumor agents, we previously showed that some quinolinediones and 4,7-dioxobenzothiazoles **3** as bioisotere of the quinones **1** and **2** exhibited potent cytotoxic activities against several cancer cell lines (Ryu *et al.*, 1999; Ryu *et al.*, 2000). On the line of this study, we further extended to evaluate whether 6-arylamino-5,8-quinolinediones **4** and 6-chloro-7-arylamino-5,8-isoquinolinediones **5** can modulate the potential of NQO1 activity, and additionally we also evaluated these compounds for the cytotoxicities on human solid tumor cell lines (Fig. 1). A variety of quinones with different substituents could exhibit the antitumor activities with different pattern and sometimes improve the activities. In the previous report, the presence of substituents such as halo, alkyl, and phenylamino groups of quinones could improve their antitumor activity (Rao *et*

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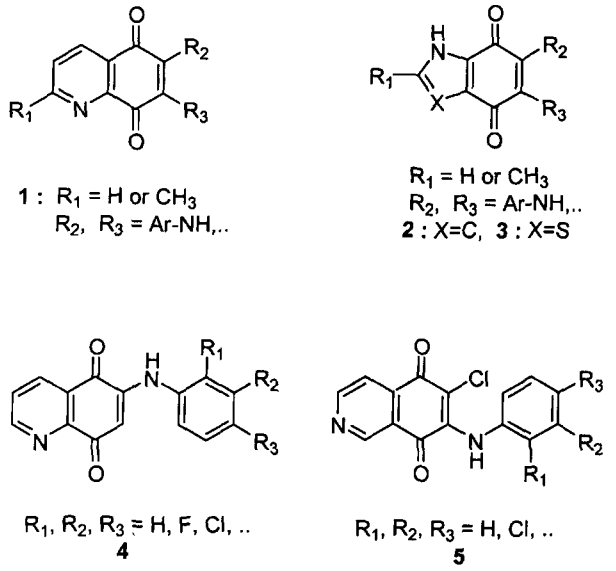


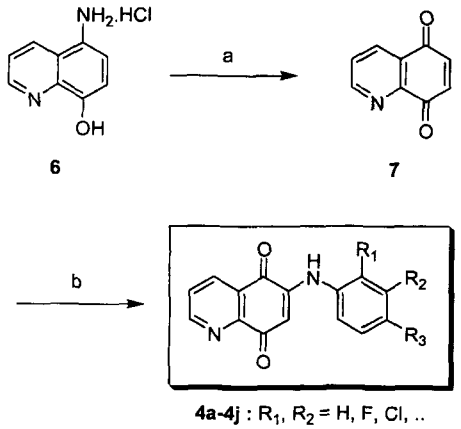
Fig. 1. Structures of 5,8-quinolinediones and 5,8-isoquinolinediones

al., 1991 and 1996, Ryu *et al.*, 1999). Based on these considerations, the 6-arylamino-5,8-quinolinediones, **4a-4j** and 6-chloro-7-arylamino-5,8-isoquinolinediones, **5a-5g** with various substituents were synthesized and evaluated for the metabolism by the NQO1 and their cytotoxicities. (Scheme 1, Scheme 2, Table I).

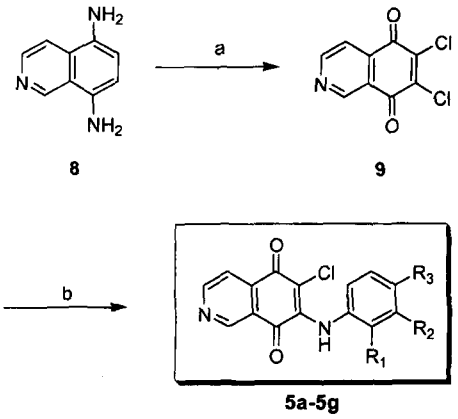
MATERIALS AND METHODS

All melting points were measured in open capillary tubes with Thomas Hoover Capillary Apparatus model and were uncorrected. The TLC was performed on precoated silica gel (60G 254, Merck) using $CHCl_3$ for solvent. The compounds were detected under UV light (254 nm) or by heating at 110°C after spraying 30% H_2SO_4-vanillin solution. Column chromatography was performed on silica gel G60 (70-230 mesh, ASTM, Merck). The IR spectra were obtained from Perkin-Elmer 1420r IR spectrometer with KBr pellets. ^1H NMR spectra were recorded on Brucker DPX 250 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents, and chemical shifts are given in ppm with TMS as a standard. Mass spectra were obtained on JMS AX 505 WA spectrometer (electronic impact at 70 eV). Elemental analyses were performed by CE instruments EA1110 with sulfanilamide as a standard material. 8-Hydroxyquinoline, cerium (III) chloride heptahydrate, arylamines, $CDCl_3$, DMSO-d_6 and other reagents were obtained from Aldrich Chemical Co.

MEME medium was obtained from Gibco-BRL. 2,6-Dichlorophenol-indophenol (DCPIP), dicoumarol, and NADH were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were at least of analytical grade.



Scheme 1. Synthesis of 6-aryamino-5,8-quinolinediones



Scheme 2. Synthesis of 6-chloro-7-aryamino-5,8-isoquinolinediones

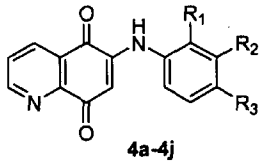
Preparation of the 5,8-quinolinediones, 4a-4j and 5,8-isoquinolinediones, 5a-5g

The 6-arylamino-5,8-quinolinediones, **4a-4j** and 6-chloro-7-arylamino-5,8-isoquinolinediones, **5a-5g** were synthesized according to the previously reported method (Ryu *et al.*, 1999a, 1999b). The quinones, **4a-4j**, and **5a-5g** (Table I), whose data were not reported elsewhere, were newly synthesized for their cytotoxicity and modulation of NQO1 activity.

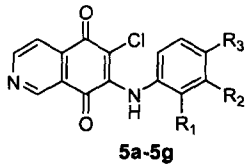
Synthesis of 5,8-quinolinedione (7)

5-Amino-8-hydroxyquinolineHCl (**6**) was prepared according to the previously reported method (Ryu *et al*, 1994). To a solution of compound **6** (1.96 g, 10 mmol) in 400 mL of acetone was added a solution of potassium nitrosodisulfonate (5 g, 18.65 mmol) in NaH_2PO_4 buffer (0.3 M, 800 mL). The mixture was stirred at room temperature for 1 h and was extracted twice with CH_2Cl_2. The extract was evaporated and crystallized from CH_2Cl_2. 5,8-Quinolinedione (**7**) was obtained (1.34 g, 84%).

Table I. Modulation of NQO1 by quinones and their cytotoxicities



4a-4j



5a-5g

Compound	R_1	R_2	R_3	NQO1 activity (% of control)[a]	Cytotoxicity[b] IC_{50} (μg/mL)		
					A549[c]	SK-OV-3	SK-MEL-2
4a	H	H	OCF_3	23.0	0.85	0.50	0.36
4b	H	H	CF_3	12.5	1.74	0.49	1.60
4c	H	H	Ethyl	27.0	0.62	0.92	2.84
4d	H	H	n-Butyl	33.4	4.29	1.62	3.61
4e	H	H	NO_2	21.9	1.39	0.38	1.66
4f	H	CH_3	Br	12.6	3.28	1.16	4.14
4g	H	H	CN	28.4	3.71	3.67	4.94
4h	H	H	Cl	31.9	0.17	1.15	1.09
4i	H	H	OH	31.2	0.79	1.33	3.31
4j	H	H	$COCH_3$	22.2	0.56	1.77	3.18
5a	F	F	F	30.9	1.10	3.92	1.07
5b	H	CH_3	Br	38.0	7.41	6.94	1.44
5c	H	H	CH_2H_2OH	36.8	0.61	0.78	0.07
5d	H	H	$COCH_3$	29.7	1.11	1.22	0.29
5e	H	H	OCF_3	34.2	1.30	4.87	1.04
5f	H	$COCH_3$	H	18.7	0.34	0.33	0.07
5g	H	H	$COOCH_2CH_3$	50.2	0.68	0.45	0.29
Cisplatin				NT[d]	0.51	0.88	0.85
Streptonigrin				37.2	0.33	0.28	0.02

[a]NQO1 activity: NQO1 activity was determined with reduction potential of DCPIP with the cytosolic fractions from human lung cancer cells; control activity was expressed as 621.8 ± 45.7 μmole/min/mg protein and relative activity with sample treatment was evaluated as mentioned in Materials and Methods.
[b]Cytotoxicity evaluation: SRB assay according to the NCI (National Cancer Institute) protocols
[c]Human cancer cell lines: Human solid tumor cell lines: A 549, SK-OV-3 and Sk-MEL-2 from National Cancer Institute (NCI) in USA.
[d]NT: not tested

General procedure for synthesis of the 6-arylamino-5,8-quinolinediones 4a-4j

A solution of 5,8-quinolinedione (**7**, 0.159 g, 1 mmol) and $CeCl_3 \cdot 7H_2O$ (0.373 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylamine (1.1 mmol) in 5 mL of 95% EtOH with stirring at room temperature for 2 h and then refluxed for 4-5 h. After the mixture was kept overnight in the refrigerator or poured into 15 mL of ice water, the precipitate was collected by filtration. Crystallization from aq. EtOH afforded the 6-arylamino-5,8-quinolinediones (**4a-4j**). Some compounds were purified by column chromatography using $CHCl_3$ as eluents. (Table I).

6-[*N*-(4-trifluoromethoxyphenyl)amino]-5,8-quinolinedione (4a)

Yield: 79%; m.p.146-148°C; color: dark brown cake; IR (KBr, cm^{-1}): 3300 (w, NH), 3070 (w, Ar), 1670 (s, C=O), 1600~1400 (v), 1400(m), 1290, 1090, 1000, 740 (s); ^{1}H-NMR (DMSO-d_6): δ 9.5 (s,1H,NH), 9.1 (dd,1H,Ha), 8.5 (dd,1H,Hc), 7.9 (dd,1H,Hb), 7.6 &7.5 (4H, *J*=9Hz, benzene ring), 6.3 (s, 1H, Hd); MS (m/z): 69, 77, 95, 200, 228, 249, 277, 334(M^+).

6-[*N*-(4-trifluoromethylphenyl)amino]-5,8-quinolinedione (4b)

Yield: 86%; m.p. 264-265°C; color: reddish crystalline; IR (KBr, cm^{-1}): 3200 (m, NH), 3090 (w, Ar), 1670 (s C=O), 1620~1520, 1410, 1320, 1300, 1000, 790; ^{1}H-NMR (DMSO-d_6): δ 9.6 (s, 1H, NH), 9.1 (dd, 1H, Ha) 8.5 (dd,1H,Hc), 7.9 (dd,1H,Hb), 7.8 & 7.7 (4H,*J*=8Hz benzene ring), 6.5 (s, 1H, Hd); MS (m/z): 77, 145, 184 212, 249, 261, 289, 318 (M^+).

6-[*N*-(4-ethylphenyl)amino]-5,8-quinolinedione (4c)

Yield: 89%; m.p. 239-240°C; color: red-brownish leaflet; IR (KBr, cm^{-1}): 3300 (m, NH), 3070, 2950, 1680 (s, C=O), 16001500, 1300, 1000, 820; ^{1}H-NMR (DMSO-d_6): δ 9.4 (s,1H,NH), 9.1 (dd,1H,Ha), 8.5 (dd,1H,Hc), 7.9 (dd,1H,Hb), 7.4 (s,4H, benzene ring), 6.25 (s, 1H, Hd), 2.7 (q, *J*=7.5Hz, 2H, -$\underline{CH_2}$-), 1.3 (t, *J*=7.5gz, 3H, -$\underline{CH_3}$); MS (m/z): 76, 77, 103, 116, 172, 249, 263, 278 (M^+).

6-[*N*-(4-n-butylphenyl)amino]-5,8-quinolinedione (4d)

Yield: 91%; m.p.125-127°C; color: dark reddish leaflet; IR (KBr, cm^{-1}): 3200 (m, NH), 3060, 2920, 1670 (s, C=O), 1620~1520, 1400, 1300, 1100, 980, 790; ^{1}H-NMR (DMSO-d_6): δ 9.3 (s,1H,NH), 9.1 (dd,1H,Ha), 8.5 (dd,1H,Hc), 7.9 (dd,1H,Hb), 7.4 (s, 4H, benzene ring), 6.8 (s,1H,Hd), 2.7 (t, *J*=7.5gz, 2H, Ar-$\underline{CH_2}$-), 1.7 (m, *J*=7.5Hz, 2H, CH_2-$\underline{CH_2}$-CH2), 1.4 (m, *J*=7.5Hz, 2H,-$\underline{CH_2}$-CH_3), 1.0 (t, *J*=7.5Hz, 3H, -$\underline{CH_3}$); MS (m/z): 77, 116, 192, 205, 249, 263, 306 (M^+).

6-[*N*-(4-nitrophenyl)amino]-5,8-quinolinedione (4e)

Yield: 73%; m.p. 290-293°C; color: red-brownish amorphous powder; IR (KBr, cm^{-1}): 3200 (s,NH), 3060, 1660 (s, C=O), 16201520, 1400, 1330, 1100, 1000, 830, 780; ^{1}H-NMR (DMSO-d_6): δ 9.6 (s, 1H, NH), 9.1 (dd, 1H, Ha), 8.5 (dd,1H,Hc), 7.9 (dd,1H,Hb), 8.3 &.7 (4H, *J*= 9Hz, benzene ring), 6.7 (s,1H,Hd); MS (m/z): 63, 76, 77, 90, 102, 143, 189, 192, 220, 295 (M^+).

6-[*N*-(4-bromo-3-fluorophenyl)amino]-5,8-quinolinedione (4f)

Yield: 77%; m.p. 253-255°C; color: deep purple needle; IR (KBr, cm^{-1}): 3200 (m,NH), 3040, 1690 (s, C=O), 1630~1490°C, 1390, 1300, 1100, 1030, 990 ; ^{1}H-NMR (DMSO-d_6): δ 9.42 (s,1H,NH), 9.1 (dd,1H,Ha), 8.5 (dd,1H,Hc), 7.9 (dd,1H,Hb), 7.7~7.34 (m, 3H, benzene ring), 6.3 (s,1H,Hd), 2.3 (s,3H, CH_3); MS (m/z): 77, 90, 118, 207, 236, 238, 263, 342 (M^+), 344.

6-[*N*-(4-cyanophenyl)amino]-5,8-quinolinedione (4g)

Yield: 71%; m.p. 307-309°C; color: brilliant scalet needle; IR (KBr, cm^{-1}): 3200 (m, NH), 3060, 2220 (m, CN), 1680 (s, C=O), 1640~1530°C, 1400, 1300, 1100, 760; ^{1}H-NMR (DMSO-d_6): δ 9.7 (s,1H,NH), 9.1 (dd, 1H, Ha), 8.5 (dd, 1H, Hc), 7.9 (dd, 1H, Hb), 7.0 & 7.7 (4H, *J*=6.9Hz, benzene ring), 6.6 (s, 1H, Hd); MS (m/z): 76, 77, 102, 141, 169, 218, 246, 275 (M^+).

6-[*N*-(4-chlorophenyl)amino]-5,8-quinolinedione (4h)

Yield: 78%; m.p. 227-229°C; color: dark purple plate; IR (KBr, cm^{-1}): 3200 (m, NH), 3100, 1690 (s, C=O), 1640~1500, 1400, 1300, 1100, 820, 720; ^{1}H-NMR (DMSO-d_6): δ 9.4 (s, 1H, NH), 9.1 (dd, 1H, Ha), 8.5 (dd, 1H, Hc), 7.9 (dd,1H,Hb), 7.6 &7.5 (4H, *J*=9Hz, benzene ring), 6.3 (s, 1H, Hd); MS (m/z): 75, 77, 111, 150, 178, 180, 227, 228, 249, 284 (M^+).

6-[*N*-(4-hydroxyphenyl)amino]-5,8-quinolinedione (4i)

Yield: 93%; m.p. 256-258°C; color : dark purple flake; IR (KBr, cm^{-1}): 3340 (b, OH), 3320 (ww, NH), 3060, 1690 (s, C=O), 1620~1470, 1330, 1300, 1000, 800; ^{1}H-NMR (DMSO-d_6): δ 9.7 (s,1H,OH), 9.2 (s,1H,NH), 9.1 (dd, 1H, Ha), 8.5 (dd, 1H, Hc), 7.9(dd, 1H, Hb), 7.3 & 6.9 (4H, *J*=8.7Hz, benzene ring), 5.6(s, 1H, Hd); MS (m/z): 75, 95, 134, 162, 211, 239, 268 (M^+)

6-[*N*-(4-acetophenyl)amino]-5,8-quinolinedione (4j)

Yield: 72%; m.p. 245-247°C; color: bright purple platelet; IR (KBr, cm^{-1}): 3220 (m, NH), 3010 (w, Ar), 1680 (s, C=O), 1630~1500, 1330, 1270, 800; ^{1}H-NMR (DMSO -d_6): δ 9.7 (s, 1H, NH), 9.1 (dd, 1H, Ha), 8.5 (dd, 1H, Hc), 7.9 (dd, 1H, Hb), 8.1 & 7.6 (4H, benzene ring), 6.7 (s, 1H, Hd); MS (m/z): 65, 76, 77, 166, 186, 249, 277(M^+)

General procedure for synthesis of 6-chloro-7-arylamino-5,8-isoquinolinediones, 5a-5g

6,7-Dichloro-5,8-isoquinolinedione (**9**) was prepared from 5,8-diaminoisoquinoline (**8**) according to the previously reported method (Ryu et al, 1999). A mixture of compound **9** (0.227 g, 1 mmol) and appropriate arylamine (1.1 mmol) in 95% EtOH (20 mL) was refluxed for 4-10 h. After the mixture was kept overnight in the refrigerator or was poured into 15 mL ice water, the precipitate was collected by filtration. The precipitate was filtered and recrystallized from 95% EtOH or MeOH. And the recrystallized **5a-5h** were filtered, washed with cold EtOH and dried (Table I).

6-Chloro-7-[*N*-(2,3,4-trifluorophenyl)amino]-5,8-isoquinolinedione (5a)

Yield: 62%; color: bright brown powder; m.p. 254-256°C; IR (KBr, cm^{-1}) : 3250 (NH), 1660 (s, C=O), 1580, 1520, 1330, 1200, 1140, 1000, 860, 830; ^{1}H-NMR (DMSO-d_6): δ 8.0-7.9 (2H, m, benzene ring), 8.0-9.2 (3H, m), 9.3 (1H, s, NH); MS (m/e) : 106, 220, 275, 303, 338 (M^+)

6-Chloro-7-[*N*-(4-bromo-3-methylphenyl)amino]-5,8-isoquinolinedione (5b)

Yield: 67%; color: dark brown powder; m.p. 240-241°C; IR (KBr, cm^{-1}) : 3310 (NH), 1655 (s, C=O), 1610, 1580, 1535, 1330, 1310, 1250, 1190, 1140, 1040, 900, 840, 700; ^{1}H-NMR (DMSO-d_6): δ 2.4 (3H, s, $ArCH_3$), 7.2-7.6 (3H, m, benzene ring), 8.4-9.2 (3H, m), 9.3 (1H, s, NH); MS (m/e) : 89, 90, 148, 261, 262, 297, 340, 341, 376, 378 (M^+)

6-Chloro-7-[*N*-(4-hydroxyethylphenyl)amino]-5,8-isoquinolinedione (5c)

Yield: 72%; color: dark violet plate; m.p. 207-208°C; IR (KBr, cm^{-1}) : 3260 (NH), 1640 (s, C=O), 1620, 1580, 1520, 1430, 1320, 1190, 920, 700; ^{1}H-NMR (DMSO-d_6): δ 2.8-2.8 (4H, t, CH_2CH_2, J=7.5Hz), 4.8 (1H, s, OH), 7.1-7.3 (4H, m, benzene ring), 8.0-9.2 (3H, m), 9.3 (1H, s, NH); MS (m/e) : 89, 90, 261, 297, 328 (M^+).

6-Chloro-7-[*N*-(4-acetophenyl)amino]-5,8-isoquinolinedione (5d)

Yield: 65%; color: black powder; m.p. 175-177°C; IR (KBr, cm^{-1}): 3250 (NH), 1660 (s, C=O), 1610, 1580, 1540, 1330, 1290, 1200, 850, 700; ^{1}H-NMR (DMSO-d_6): δ 2.6 (3H, s, $COCH_3$), 6.7-7.4 (2H, m, benzene ring), 8.0-9.2 (3H, m), 9.3 (1H, s, NH); MS (m/e) : 120, 138, 248, 311, 328 (M^+).

6-Chloro-7-[*N*-(4-trifluoromethoxyphenyl)amino]-5,8-isoquinolinedione (5e)

Yield: 87%; color: red powder; m.p. 210-212°C; IR (KBr, cm^{-1}) : 3220 (NH), 1640 (s, C=O), 1590, 1520, 1410, 1340, 1320, 1210, 1160, 1000, 910, 850; ^{1}H-NMR (DMSO-d_6): δ 7.3-7.5 (4H, m, benzene ring), 7.8-9.2 (3H, m), 9.3 (1H, s, NH); MS (m/e) : 95, 247, 283, 299, 333, 368 (M^+).

6-Chloro-7-[*N*-(4-ethylcarboxy)amino]-5,8-isoquinolinedione (5f)

Yield: 64%; color: red plate; m.p. 201-203°C; IR (KBr, cm^{-1}): 3220 (NH), 1640 (s, C=O), 1580, 1550, 1300, 1280, 1000, 850, 770; ^{1}H-NMR (DMSO-d_6): δ 1.4-1.5 (3 H, t, CH_3, J=7.3Hz), 4.5 (2H, s, CH_2), 7.4-7.5 (3H, m, benzene ring), 8.0-9.2 (3H, m), 9.3 (1H, s, NH); MS (m/e): 138, 248, 249, 283, 311, 328, 356 (M^+).

6-Chloro-7-[*N*-(3-acetophenyl)amino]-5,8-isoquinolinedione (5g)

Yield: 69%; clolor: dark red brown powder; m.p. 250-251°C; IR (KBr, cm^{-1}) : 3180 (NH), 1635 (s, C=O), 1590, 1530, 1480, 1310, 1375, 1180, 1010, 920, 790, 680; ^{1}H-NMR (DMSO-d_6): δ 2.6 (3H, s, $COCH_3$), 7.5-7.6 (4H, m, benzene ring), 7.8-9.2 (3H, m), 9.3 (1H, s, NH); MS(m/e) : 138, 248, 249, 291, 311, 326 (M^+).

Cytotoxicity assay

Cytotoxic potential was determined according to the NCI protocols (Lee *et al.*, 1998; Skehan *et al.*, 1990). The following human solid tumor cell lines were used: A 549 (non-small cell lung cancer), SK-OV-3 (ovarian cancer), SK-MEL-2 (melanoma), HCT-15 (colon cancer) and XF 498 (CNS cancer). The cells were grown at 37°C in MEME media (Gibco-BRL, USA) supplemented with 10% fetal bovine serum (FBS) and separated using PBS containing 0.25% trypsin and 3 mM EDTA. 5×10^3 - 2×10^4 cells were added to each well of 96 well plate and incubated at 37°C for 24 h. Each compounds **4a-4j** and **5a-5h** was dissolved in DMSO and diluted with the above medium at various concentrations with the range of 0.01-30 μg/mL. The DMSO concentration was set to be below 0.5%. After removing the well medium by aspiration, a portion 200 μL of the solution was added to the above well plates, which were placed in 5% CO_2 incubator for 48 h. The protein contents were determined according to SRB assay method (Skehan *et al.*, 1990). The results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using non-linear regression analyses (percent survival versus concentration).

NAD(P)H:quinone oxidoreductase (NQO1) assay

NQO1 activity was determined according to modified method previously reported (Beall *et al.*, 1995; 1996). Cells (A549, human non-small lung cancer cells) were grown to 75-80% confluence, tripsynized, washed in PBS, and resuspended in 25 mM Tris-HCl solution containing 250 mM sucrose, pH 7.4. Cell suspensions were sonicated on ice. The cell preparations were centrifuged at 100,000 × g for 1 h at 4°C, and the cytosolic supernatants were retained and stored at 70°C before use. Protein was determined by the method of Bradford (Bradford, 1976). NQO1 activity was determined spectrophotometrically by monitoring DCPIP reduction at 595 nm (Ernster, 1967; Beall *et al.*, 1995; Phillips, 1999). In brief, the reactions contained 0.2 mM DCPIP (2,6-dichlorophenolindophenol), cytosolic fractions for NQO1 sources (10 μL, 0.0013 mg/mL) and 0.1 mM sample in a final volume of 200 μL, 25 mM Tris-HCl (pH 7.4) containing bovine serum albumin (BSA) (0.7 mg/mL). All reactions were carried out at room temperature and initiated by the addition of cofactor, 1 mM NADH. Rates of reduction were calculated from the linear portion of the progress curves over time period of 5 min incubation. Results were expressed as the reduction of DCPIP using a molar extinction coefficient of 21 $mM^{-1}cm^{-1}$ [μmole/min/mg protein]. Relative activity with sample treatment was compared to the control activity (% of control).

RESULTS AND DISCUSSION

Chemistry

A method for the synthesis of the 6-arylamino-5,8-quinolinediones **4a-4j** (Table I) is shown in Scheme 1. 5-Amino-8-hydroxyquinoline (**6**) was prepared according to the known method (Ryu *et al.*, 1994). Oxidation of the compound **6** with Fremys salt (potassium nitrosodisulfonate) gave 5,8-quinolinediones (**7**) in about 84% yield. 6-Arylamino-5,8-quinolinediones **4a-4j** were synthesized by regioselective nucleophilic substitution of 5,8-quinolinedione (**7**) with appropriate arylamines in the presence

of Ce(III). Most of these substitutions were produced as expected, and had overall high yields of 71-93%.

A convenient method for the synthesis of the 6-chloro-7-arylamino-5,8-isoquinolinediones **5a-5g** (Table I) is shown in Scheme 2. 5,8-Diaminoisoquinoline (**8**) was prepared according to the previously reported method (Ryu *et al.*, 1999a). The 6,7-dichloro-5,8-isoquinolinedione (**9**) was synthesized by oxidizing the compound **8** with the $KClO_3$/HCl variation in 76% yield. The 5,8-isoquinolinediones **5a-5g** were synthesized by nucleophilic substitution of the compound **9** with appropriate arylamines. Most of these nucleophilic substitutions went as expected and had overall high yields of 62-87%.

Cytotoxic potential against human cancer cells

The *in vitro* cytotoxic potential of compounds **4a-4j** and **5a-5h** against human cancer cells was determined by the sulforhodamine B (SRB) assay according to the NCI protocols (Skehan *et al.*, 1990). The following human solid tumor cell lines were used: A549, SK-OV-3 and SK-MEL-2. The IC_{50} values of compounds **4a-4j** and **5a-5g** were compared with those of cisplatin and quinoid antibiotic streptonigrin as reference agents. As indicated in Table I, many quinones showed generally potent cytotoxic activities against all cancer cell lines tested. Indeed, activities of the compounds **4a, 5c, 5f** and **5g** were superior with the range of IC_{50} values of 0.07-0.78 μg/mL, or comparable to those of cisplatin against all cell lines. These results were highly consistent with the previous reports tested with other cancer cell lines (Ryu *et al.*, 1999). Unexpected, substitutions of CN, Br, Cl, CH_3 in R_2 or R_3 position of the quinones **4** and **5** were decreased the cytotoxic activities.

The structure-activity relationship may not exist within substituents (R_1, R_2 and R_3) of 6/7-arylamino moiety of compounds **4** and **5**.

Modulation of NQO1 activity

The compounds **4a-4j** and **5a-5g** were evaluated for the effects on NQO1 activity using the cytosolic fractions of human lung cancer cells A549. Bioreductive antitumor agents were considered as potential substrates for NQO1, and thus activated by the enzyme for their cytotoxic activity. In the present study, we used the cytosolic fractions derived from human lung cancer cells (A549) as the enzyme sources for NQO1, and tested the reduction potential using DCPIP as a substrate. The cell preparations showed the potent DCPIP reduction activity, and also showed dicoumarol-sensitive manner. As a positive control, streptonigrin was used and showed approximately 37% of control of NQO1 activity at the test concentration of 100 μM. In this condition, the compound **4a-4j** and **5a-5g** were in the range of approximately 12.5 ~50.2% of control of NQO1 activity. Among them, compounds **4d, 5b, 5c, 5e** and **5g** are comparable with streptonigrin as shown in the Table I. This suggests that the synthesized 6-arylamino-5,8-quinolinediones **4** and 6-chloro-7-arylamino-5,8-isoquinolinediones **5** will be good substrates for NQO1, and thus possibly be activated for their cytotoxic potential. Further, the results might encourage the synthesis of new 5,8-quinolinediones and 5,8-isoquinolinediones analogs for improving NQO1 modulators and potent cytotoxic agents.

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