Modulation of NAD(P)H:Quinone Oxidoreductase (NQO1) Activity Mediated by 5-Arylamino-2-methyl-4,7-dioxobenzothiazoles and their Cytotoxic Potential

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Synthesized 5-arylamino-2-methyl-4,7-dioxobenzothiazoles **3a-3o** were evaluated for modulation of 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 4,7-dioxobenzothiazoles affected the reduction potential by NQO1 activity and showed a potent cytotoxic activity against human cancer cell lines. The tested compounds **3a, 3b, 3g, 3h, 3n** and **3o** were considered as more potent cytotoxic agents, and comparable modulators of NQO1 activity.

INTRODUCTION

Quinones were frequently studied with their cytotoxic activities against cancer cells. Many quinones showed that their bioreduction seems to be required for antitumor activity (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 a cytosolic enzyme, an obligate two-electron reductase that is characterized by its capacity for using either NADH or NADPH as reducing cofactors (Ernster, 1967). NQO1 is considered as a bifunctional enzyme which can protect the cell from a broad range of chemically reactive metabolites (a detoxification enzyme) through reducing 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 and other bioreductive antitumor compounds. 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
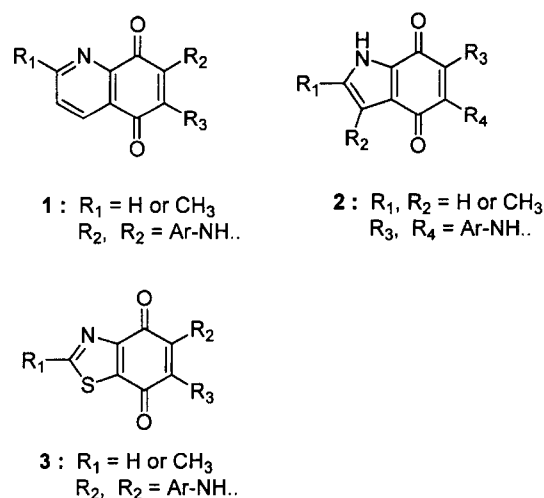

1 : R₁ = H or CH₃
    R₂, R₂ = Ar-NH..

2 : R₁, R₂ = H or CH₃
    R₃, R₄ = Ar-NH..

3 : R₁ = H or CH₃
    R₂, R₂ = Ar-NH..

Fig. 1. Quinones and 4,7-dioxobenzothiazoles derivatives

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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 dioxobenzothiazole derivatives 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). Thus, in the present study, we further extended to evaluate whether 5-arylamino-2-methyl-4,7-dioxobenzothiazole derivatives **3a-3o** can modulate the potential of NQO1 activity, and additionally we also evaluated these compounds for the cytotoxicities on human solid tumor cell lines (Scheme 1 and Table I). A variety of quinones with different substituents could exhibit the antitumor activities with different pattern and improve sometimes the activities. In the previous report, the presence of substituents such as alkyl, and phenyl-

3a-3j: R$_1$, R$_2$ =H, F, Cl,...
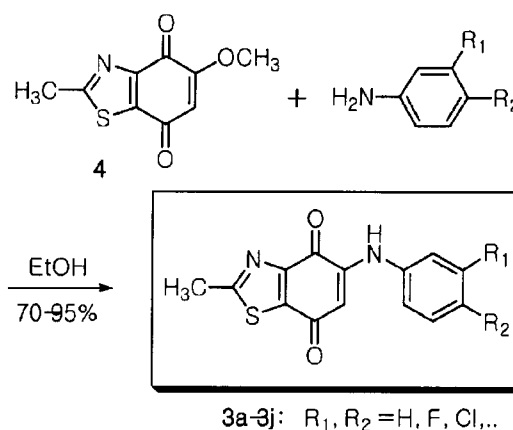
Scheme 1. Synthesis of 5-arylamino-2-methyl-4,7-dioxobenzothiazoles

Table I. Modulation of NQO1 by 4,7-dioxobenzothiazoles and their cytotoxicities
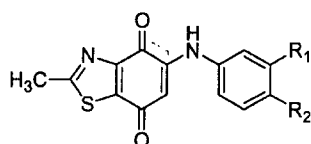
Compound	R$_1$	R$_2$	NQO1 activity (% of control)[a]	Cytotoxicity[b] IC$_{50}$ (μg/mL)				
				A549[c]	SK-OV-3	SK-MEL-2	XF498	HCT15
3a	H	H	47.1	0.35	0.27	0.16	0.33	0.30
3b	H	F	35.5	0.38	0.30	0.29	0.33	0.32
3c	H	Cl	17.5	>20	1.48	1.12	8.47	1.44
3d	H	Br	27.9	>20	1.11	0.37	18.44	1.24
3e	H	I	35.6	1.26	0.32	0.32	1.10	0.32
3f	H	OH	25.7	0.40	0.16	0.22	0.33	0.23
3g	H	CH$_3$	32.1	0.37	0.28	0.26	0.33	0.27
3h	H	OCH$_3$	37.9	0.37	0.29	0.22	0.32	0.31
3i	H	OCH$_2$CH$_3$	29.0	0.42	0.24	0.25	0.28	0.32
3j	H	CF$_3$	39.4	0.52	0.32	0.32	1.31	0.61
3k	H	OCF$_3$	34.3	>20	16.99	1.83	>20	4.87
3l	H	n-C$_6$H$_{13}$	47.7	16.16	1.74	1.00	18.66	1.90
3m	Cl	H	27.1	>20	1.46	0.46	>20	1.22
3n	Br	H	43.2	0.34	0.31	0.28	0.34	0.26
3o	CH$_3$	CH$_3$	42.6	0.40	0.22	0.27	0.36	0.27
Cisplatin			NT[d]	0.51	0.88	0.85	0.43	0.53
Streptonigrin			37.2	0.33	0.28	0.02	0.31	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 mmole/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, SK-MEL-2, HCT-15 and XF 498 from National Cancer Institute (NCI) in USA.
d) NT: not tested

amino groups of quinones could improve their antitumor activity (Rao et al, 1991 and 1996, Ryu et al, 1999). Based on these considerations, the synthesized 5-aylamino-2-methyl-4,7-dioxobenzothiazoles (3a-3o) with various substituents were evaluated for the metabolism by the NQO1 and their cytotoxicities. (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 CHCl₃ for solvent. The compounds were detected under UV light (254 nm) or by heating at 110°C after spraying 30% H₂SO₄-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 CDCl₃ 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. 5-Methoxy-2-methylbenzothiazole was obtained from TCI Co. CDCl₃, DMSO-d_6 and other reagents were purchased 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.

Preparation of the 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 3

The 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 3a -3i were prepared according to the previously reported method (Ryu et al, 2000). 4,7-Dioxobenzothiazoles 3j-3o (Table I), whose data were not reported elsewhere, were newly synthesized for their cytotoxicity and modulation of NQO1 activity.

General procedure for synthesis of 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 3j-3o

5-Methoxy-2-methyl-4,7-dioxobenzothiazole (4) was prepared according to the previously reported method (Ryu et al., 2000). A solution of compound 4 (0.209 g, 1 mmol) in 20 mL of 95% EtOH was added to a solution of the arylamine (1.1 mmol) in 10 mL of 95% EtOH and then refluxed for 4-5 h. After the reaction mixture was kept overnight, the precipitate was collected by the filtration. The crude product was purified by silica gel column chromatography with CHCl₃ or crystallized from 95% EtOH (Scheme 1 and Table I). Crystallization from aq.

EtOH afforded 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 3j-3o.

5-(4-Trifluoromethylphenyl)amino-2-methyl-4,7-dioxobenzothiazole (3j)

Purple powder. mp: 237.6°C. IR (KBr): v 3290 (NH), 3050 (w, aromatic ring), 2362, 1685 (s, C=O), 1590-1470 (benzene ring), 1244 cm⁻¹ (s). ¹H NMR (DMSO.d_6): δ 9.6 (s, 1H, NH), 7.6-7.8 (m, 4H, benzene), 6.1 (s, 1H, H6), 2.8 (s, 3H, -CH₃). MS (m/z): 338 (M+), 269 (M⁺-69, -CF₃), 241 (C₁₃H₇NO₂S), 126, 77 (C₆H₅⁺). Anal. Calcd for C₁₅H₉F₃N₂O₂S (338.31): C, 53.25; H, 2.68; N, 8.28; S, 9.48. Found: C, 54.87; H, 3.5; N, 7.50; S, 10.30.

5-(4-Trifluoromethoxyphenyl)amino-2-methyl-4,7-dioxobenzothiazole (3k)

Dark violet powder. mp: 253.1°C. IR (KBr): 3270 (NH), 3050 (w, aromatic ring), 2356, 1695 (s, C=O), 1590-1470 (benzene ring), 1270 (s, C-O-C), 1230 cm⁻¹ (w, CF₃). ¹H NMR (DMSO.d_6):δ 9.5 (s, 1H, NH), 7.4-7.5 (m, 4H, benzene), 5.9 (s, 1H, H6), 2.8 (s, 3H,-CH₃). MS (m/z): 354 (M⁺), 285 (M⁺-69, -CF₃), 269 (M⁺-85, -OCF₃), 241 (C₁₃H₇NO₂S), 126, 77 (C₆H₅⁺). Anal. Calcd for C₁₅H₉F₃N₂O₃S (354.30): C, 50.85; H, 2.56; N, 7.91; S, 9.05. Found: C, 50.84; H, 2.17; N, 7.11; S, 8.00.

5-(4-Hexylphenyl)amino-2-methyl-4,7-dioxobenzothiazole (3l)

Bright purple powder. mp: 140.1°C. IR (KBr): 3270 (NH), 3050 (w, aromatic ring), 2356, 1695 (s, C=O), 1590-1470 cm⁻¹ (benzene ring). ¹H NMR (DMSO.d_6):δ 9.4 (s, 1H, NH), 7.3 (m, 4H, benzene), 5.9 (s, 1H, H6), 2.8 (s, 3H, -CH₃), 2.6 (s, 2H, -C₆H₁₃), 1.2-1.6 (m, 8H, -C₆H₁₃), 0.9 (m, 3H, -C₆H₁₃). MS (m/z): 354 (M⁺), 283 (M⁺-71, -C₅H₁₁), 269 (M⁺-85, -C₆H₁₃), 241 (C₁₃H₇NO₂S), 126, 77 (C₆H₅⁺). Anal. Calcd for C₂₀H₂₂N₂O₂S (354.47): C, 67.77; H, 6.26; N, 7.90; S, 9.05. Found: C, 66.34; H, 6.13; N, 7.16; S, 9.62.

5-(3-Chlorophenyl)amino-2-methyl-4,7-dioxobenzothiazole (3m)

Dark purple powder. mp: 237.6°C. IR (KBr): v 3250 (NH), 3070 (w, aromatic ring), 2360, 1690 (s, C=O), 1590-1490 cm⁻¹ (benzene ring). ¹H NMR (DMSO.d_6): δ 9.4 (s, 1H, NH), 7.2-7.5 (m, 4H, benzene), 5.9 (s, 1H, H6), 2.8 (s, 3H, -CH₃). MS (m/z): 304 (M⁺), 269 (M⁺-35, -Cl), 126, 77 (C₆H₅⁺). Anal. Calcd for C₁₄H₉ClN₂O₂S (304.75): C, 55.18; H, 2.98; N, 9.19; S, 10.52. Found: C, 56.68; H, 1.85; N, 8.64; S, 10.26.

5-(3-Bromophenyl)amino-2-methyl-4,7-dioxobenzothiazole (3n)

Dark brown powder. mp: 233.5°C. IR (KBr): 3250 (NH),

3060 (w, aromatic ring), 2360, 1690 (s, C=O), 1600-1480 cm^{-1} (benzene ring). ^{1}H NMR (DMSO.d_6):δ 9.4 (s, 1H, NH), 7.4-7.5 (m, 4H, benzene), 6.2 (s, 1H, H6), 2.8 (s, 3H, -CH$_3$). MS (m/z): 348 (M$^+$-1), 269 (M$^+$-80, -Br), 241 (C$_{13}$H$_7$NO$_2$S), 126, 77 (C$_6$H$_5$$^+$). Anal. Calcd for C$_{14}H_9BrN_2O_2$S (349.20): C, 48.15; H, 2.60; N, 8.02; S, 9.18. Found: C, 55.88; H, 1.70; N, 6.76; S, 13.69.

5-(3,4-Dimethylphenyl)amino-2-methyl-4,7-dioxobenzo-thiazole (3o)

Black powder. mp: 202.4°C. IR (KBr): 3260 (NH), 3050 (w, aromatic ring), 2356, 1694 (s, C=O), 1590-1470 cm^{-1} (benzene ring). ^{1}H NMR (DMSO.d_6):δ 9.3 (s, 1H, NH), 7.1-7.2 (m, 3H, benzene), 5.8 (s, 1H, H6), 2.8 (s, 3H, -CH$_3$), 2.2-2.3 (m, 6H, di-CH$_3$). MS (m/z): 298 (M$^+$), 283 (M$^+$-15, -CH$_3$), 269 (M$^+$-29, -C$_2$H$_5$), 241 (C$_{13}$H$_7$NO$_2$S), 126, 77 (C$_6$H$_5$$^+$). Anal. Calcd for C$_{16}H_{14}N_2O_2$S (298.36): C, 64.41; H, 4.73; N, 9.39; S, 10.75. Found: C, 56.64; H, 2.52; N, 7.5; S, 12.98.

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 compound (3a-3o) 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% and filtrated. 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

Modulation of 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 1h at 4°C, and the cytosolic super-

natants 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-dichlorophenol-indophenol), cytosolic fractions for NQO1 sources (10 μL, 0.0013 mg/mL) and 0.1mM 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 4,7-dioxobenzothiazoles 3j-3o (Table I) is shown in Scheme 1. 5-Methoxy-2-methyl-4,7-dioxobenzothiazole (4) was prepared according to the previously reported method (Ryu et al, 2000). The 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 3j-3o were newly synthesized from the compound 4. The 5-arylamino-2-methyl-4,7-dioxobenzothiazoles 3j-3o were formed by regioselective nucleophilic substitution of the compound 4 with the appropriate arylamines. The nucleophilic displacement of the 6-methoxy group with the amines produced the 4,7-dioxobenzothiazoles 3j-3o. Most of these substitutions were produced as expected, and had overall high yields of 80-95%.

Cytotoxic potential against human cancer cells

The in vitro cytotoxic potential of compounds 3a-3o against human cancer cells was determined by the sulfo-rhodamine 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, SK-MEL-2, XF498 and HCT-15. The IC$_{50}$ values of 3a-3o were compared with those of cisplatin and quinoid antibiotic streptonigrin as a reference agent. As indicated in Table I, the 4,7-dioxobenzothiazoles 3a-3o showed generally potent cytotoxic activities against all cancer cell lines tested. Indeed, activities of the compounds 3a, 3b, 3f, 3g, 3h, 3i, 3n and 3o were superior with the range of IC$_{50}$ values of 0.16-0.42 μg/mL, or comparable to those of cisplatin against all cell lines. These results were much consistent with the previous reports tested with other cancer cell lines (Ryu et al., 1999). Unexpected, substitutions of Br, Cl, OCF$_3$, n-C$_6$H$_{13}$ in R$_2$ position of the 4,7-dioxobenzothiazoles 3

were decreased the cytotoxic activities.

Modulation of NQO1 activity

The 4,7-dioxobenzothiazoles **3a-3o** were evaluated for their modulation of 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 tested 4,7-dioxobenzothia-zoles **3a-3o** were in the range of approximately 20~50 % of control of NQO1 activity, and thus comparable with streptonigrin as shown in the Table I. This suggests that the synthesized 5-arylamino-2-methyl-4,7-dioxonenzothia-zoles **3** will be good substrates for NQO1, and thus possibly be activated for their cytotoxic potential. Further, the results might encourage the synthesis of new 5,6-di-substituted-4,7-dioxobenzothiazoles analogs for improving NQO1 modulators and potent cytotoxic agents.

ACKNOWLEDGEMENTS

REFERENCES

Beall, H. D., Liu, Y., Siegel, D., Bolton, E. M., Gilson, N. W. and Ross, D., Role of NAD(P)H: quinone oxido-reductase (DT-diaphorase) in cytotoxicity and induction of DNA damage by streptonigrin. *Biochem. Pharmacol.*, 51, 645-652 (1996).

Beall, H. D., Murphy, A. M., Siegel, D., Hargreaves, R. H. J., Butler, J. and Ross, D., Nicotinamide adenine dinucleo-tide (Phosphate): Quinone oxidoreductase (DT-diaphorase) as a target for bioreductive antitumor quinones: qui-none cytotoxicity and selectivity in human lung and breast cancer cell lines. *Mol. Pharmacol.*, 48, 499-504

(1995).

Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein-dye binding. *Anal. Biochem.*, 72, 248-254 (1976).

Ernster, L., DT-diaphorase. *Methods Enzymol.*, 10, 309-317 (1967).

Fryatt, T., Goroski, D. T., Nilson, Z. D., Moody, C. J. and Beall, H. D., Novel quinolinequinone antitumor agents: structure-metabolism studies with NAD(P)H: quinone oxidoreductase (NQO1). *Bioorg. Med. Chem. Lett.*, 9 (15), 2195-2198 (1999).

Lee, S. K., Cui, B., Metha, R. R., Kinghorn, A. D. and Pezzuto, J. M., Cytostatic mechanism and antitumor potential of novel 1H-cyclopenta[b]benzofuran lignans isolated from *Aglaia elliptica*. *Chem.-Biol. Interact.*, 115, 215-228 (1998).

Phillips, R. M., Inhibition of DT-diaphorase [NAD(P)H: quinone oxidoreductase, EC 1.6.99.2] by 5,6-dimethyl-xanthenone-4-acetic acid (DMXAA) and flavone-8-acetic acid (FAA): implications for bioreductive drug develop-ment. *Biochem. Pharmacol.*, 58, 303-310 (1999).

Rao, K. V. and Beach, J. W., Streptonigrin and related compounds 5. Synthesis and evaluation of some iso-quinolin analogues. *J. Med. Chem.*, 34, 1871-1879 (1991).

Rao, K. V. and Rock, C. P., Streptonigrin and related com-pounds. 6. Synthesis and activity of some quinoxaline analogues. *J. Heterocycl. Chem.*, 33, 447-458 (1996).

Ryu, C. K., Lee, I. K., Jung, S. H. and Lee, C. O., Synthesis and cytotoxic activities of 6-chloro-7-arylamino-5,8-isoquinolinediones. *Bioorg. Med. Chem. Lett.*, 9(8), 1075-1080 (1999).

Ryu, C. K., Lee, Kang, H. Y., Lee, S. K., Nam, K. A., Hong, C. Y., Ko, W. G. and Lee, B. H., 5-Arylamino-2-methyl-4,7-dioxobenzothiazoles as inhibitors of cyclin-dependent kinase 4 and cyctotoxic agents. *Bioorg. Med. Chem. Lett.*, 10(5), 461-464 (2000)

Shaikh, I. A., Johnson, F. and Grollman, A. P., Structure-activity relationship among simple bicyclic analogues. Rate dependent of DNA degradation on quinone reduc-tion potential. *J. Med. Chem.*, 29, 1329-1340 (1986).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, T. W., Bokesch, H., Kenney, S. and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*; 82, 1107-1112 (1990).