Arch Pharm Res Vol 23, No 1, 42-45, 2000

Cytotoxic Activities of 6-Arylamino-7-halo-5,8-quinolinediones against Human Tumor Cell Lines

Chung-Kyu Ryu[a*], Hye-Young Kang[a], Yu-Jin Yi[a] and Chong-Ock Lee[b]

[a] *College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea and*
[b] *Korea Research Institute of Chemical Technology, Yusongku, Taejon 305-343, Korea*

(Received September 24, 1999)

6-Arylamino-7-halo-5,8-quinolinediones (**4a-4k, 5a-5b**) were tested for *in vitro* cytotoxicity against human solid tumor cell lines such as A 549 (non-small cell lung), SK-OV-3 (ovarian), SK-MEL-2 (melanoma), HCT-15 (colon) and XF 498 (CNS) by SRB assay. The arylamino-7-chloro-5,8-quinolinediones **4** were also evaluated for cyclin-dependent kinase (CDK2 and CDK4) inhibitory effect. Among them, the 5,8-quinolinediones **4a** and **5a** with 7-(4-fluorophenyl)amino group were found to be potent cytotoxic against HCT 15, SKOV-3 and XF 498, and the compounds **4f** and **4i** showed inhibitory activities for the CDK4.

Key words: 6-Arylamino-7-halo-5,8-quinolinediones, Cytotoxicity, Human tumor cell lines, CDK4

INTRODUCTION

5,8-Quinolinediones 1 were frequently studied because of a variety of biological activities such as antitumor (Boger *et al.*, 1987; Rao *et al.*, 1991; Ryu *et al.*, 1999a; Yasuda *et al.*, 1987), antifungal (Ryu *et al.*, 1994; Jeschke *et al*, 1993), antimalarial agents and nitric oxide synthase inhibitor (Ryu *et al.*, 1999a). The 7-amino-5,8-quinolinedione moiety 2 of streptonigrin (**3**), streptonigrone and lavendamycin has been proposed to be important in determining their antitumor activity (Boger *et al.*, 1987). The moiety 2, common to a number of quinone antibiotics, cleaves PM2 phage circular DNA of tumor cells (Lown *et al.*, 1976; Shaikh *et al.*, 1986). Many structural variants of 2 showed that the bioreductive 5,8-quinolinedione ring seems to be required for antitumor activity. The presence of substituents such as halogen and substituted amino groups of quinones improves their antitumor activity (Rao *et al.*, 1991 and 1996; Ryu *et al.*, 1999a). Thus, synthesized 6-arylamino-7-halo-5,8-quinolinediones (**4a-4k, 5a-5b**) might be expected to have antitumor activity (Fig. 1 and Table I).

There have been a few reports (Ryu *et al.*, 1994; Jeschke *et al.*, 1993) on the antifungal activities of 6-arylamino-7-halo-5,8-quinolinediones (**4, 5**). However, their cytotoxicities of 6-arylamino-7-halo-5,8-quinolinedione derivatives (**4a-4k, 5a-5b**) on human tumor cell lines were not reported. Therefore, we determined the cytotoxicities of the 5,8-quinolinediones (**4a-4k, 5a-5b**) against human tumor cell lines by sulforhodamine B (SRB) assay (Skehan *et al.*, 1990).

In addition, we also evaluated for cyclin-dependent kinases (CDKs) inhibitory activities of the quinones **4a-4k**. CDKs play essential roles in cell cycle regulation (Collins

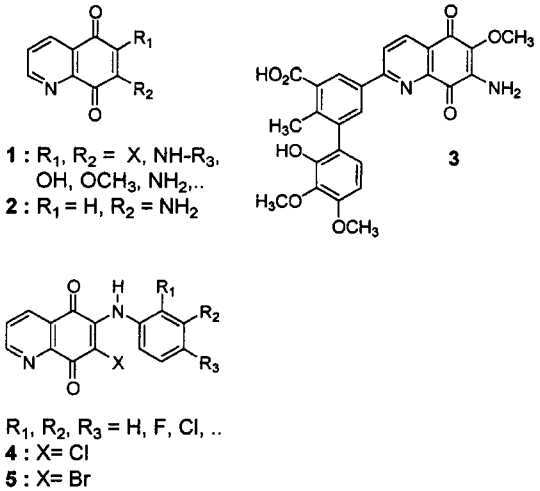
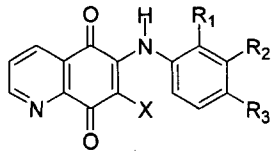


Fig. 1. Streptonigrin and 5,8-quinolinediones

Correspondence to: Chung-Kyu Ryu, College of Pharmacy, Ewha Womans University, Sodaemunku, Seoul 120-750, Korea
E-mail: ckryu@mm.ewha.ac.kr

Table I. 6-Arylamino-7-halo-5,8-quinolinediones



Compound	X	R_1	R_2	R_3
4a	Cl	H	H	F
4b	Cl	H	F	F
4c	Cl	H	H	Br
4d	Cl	H	H	I
4e	Cl	H	CN	H
4f	Cl	H	Cl	Cl
4g	Cl	Cl	H	Cl
4h	Cl	H	Cl	CH_3
4i	Cl	CF_3	H	Br
4j	Cl	H	H	Cl
4k	Cl	H	CH_3	Br
5a	Br	H	H	F
5b	Br	H	H	Cl

et al., 1997). Alternation and deregulation of CDKs activity are pathogenic hallmarks of neoplasia (Dunphy *et al.*, 1997). The CDK inhibitors have been shown for antitumor activities in animal models and would be of interest to explore as novel therapeutic agents in cancers and other hyperproliferative disorders (Webster *et al.*, 1998; Sausville *et al.*, 1999). Quinones were frequently studied as antitumor agents, but their inhibitory activities of CDKs have not been reported. Based on these considerations, 6-arylamino-7-chloro-5,8-quinolinedione derivatives **4** were evaluated for their inhibitory activities for CDKs (CDK2 and CDK4).

We report herein the cytotoxic potential of 6-arylamino-7-halo-5,8-quinolinediones (**4a-4k, 5a-5b**) as well as the CDK inhibitory activities of compounds **4**.

MATERIALS AND METHODS

All melting points were measured in open capillary tubes with Thomas Hoover Capillary Apparatus Model and were uncorrected. IR spectra were taken from Perkin Elmer 1420r IR spectrometer with KBr pellets. ^{1}H-NMR spectra were recorded on Brucker DPX 250 MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvent, and chemical shifts are given in d ppm with TMS as standard. Mass spectra were obtained on JMS AX 505 WA spectrometer (electronic impact at 70 eV). 8-Hydroxyquinoline, cerium(III) chloride heptahydrate ($CeCl_3 \cdot 7H_2O$), arylamines, dimethylsulfoxide (DMSO) and other reagents were obtained from Aldrich Chemical Co.

RPMI 1640 media was obtained from Gibco BRL and other chemicals were purchased from Sigma.

Preparation of the 6-arylamino-7-halo-5,8-quinolinediones (4a-4k and 5a-5b)

The 6-arylamino-7-chloro-5,8-quinolinediones **4a-4k** (Table I) were prepared according to the previously reported method (Ryu *et al.*, 1994). 6-Arylamino-7-bromo-5,8-quinolinediones **5a-5b** were synthesized by the known method (Schellhammer *et al.*, 1959).

General procedure for synthesis of 5a-5b

Briefly, 6,7-bromo-5,8-quinolinedione was prepared according to the known procedure (Schellhammer *et al.*, 1959). A solution of 6,7-bromo-5,8-quinolinedione (2.27 g, 0.01 mol) and $CeCl_3 \cdot 7H_2O$ (3.73 g, 0.01 mol) in 100 ml of 95% EtOH was added to a solution of the arylamine (0.011 mol) in 50 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 150 ml of ice water, the precipitate was collected by filtration. Crystallization from aq. EtOH afforded the 6-arylamino-7-bromo-5,8-quinolinediones (**5a-5b**).

6-(4'-Fluorophenyl)amino-7-bromo-5,8-quinolinedione (5a)

Yield: 72%; color: red purple powder; m.p. 201-204 °C; IR (KBr, cm^{-1}): 3560 (s, NH) 3200, 1660 (s, C=0), 1560, 1480; ^{1}H-NMR (DMSO-d_6): d ppm=7.1-7.2 (4H, m, benzene ring), 7.7-7.9 (4H, C_5H_3N), 9.3 (1H, s, NH).

6-(4'-Chlorophenyl)amino-7-bromo-5,8-quinolinedione (5b)

Yield: 79%; color: purple powder; m.p.223-225°C; IR (KBr, cm^{-1}): 3500 (s, NH), 3200, 1660 (s, C=0), 1550, 1470; ^{1}H-NMR (DMSO-d_6): d ppm=7.2-7.4 (4H, m, benzene ring), 8.4-8.9 (3H, m, C_5H_3N), 9.3 (1H, s, NH).

Cytotoxicity assay

Cytotoxicity potential was determined according to the NCI protocols (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 RPMI 1640 (Gibco BRL, USA) medium 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 (**4a-4k** and **5a-5b**) was dissolved in DMSO and diluted with the above medium at various concentrations

with the range of 0.01-30 mg/ml. The DMSO concentration was set to be below 0.5% and filtrated. After removing the well medium by aspiration, a portion 200 ml 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). These 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).

CDK2 and CDK4/cyclin D1 inhibition assay

CDK2/cyclin D1 inhibition assay was proceeded according to the standard method (Dunphy *et al.*, 1997). CDK4/cyclin D1 enzyme inhibition assay was proceeded to the following method. Briefly, CDK-4 and cyclin D1 gene were subcloned into pBacPaK8 vector (Clon Tech, USA) respectively. In addition, Glutathione-S-transferased gene was fused to N-terminal of CDK4 gene for affinity purification of CDK4/cyclin D1 complex. Active GST-CDK4/cyclin D1 enzyme was purified by using Glutathione Sepharose 4B beads (Pharmicia, Sweden) from sf21 insect cells cotransfected with 1 : 1 ratio of GST-CDK4 and cyclic D1 bacculoviral stocks. Enzyme assay was done in 20 μmL reaction mixture containing 100 ng of purified GST-CDK4/cyclin D1 enzyme, 4 μg of human RB c-terminal (amino acids 769-928) as substrate, 100 μM ATP, 1.2 μCi of P^{32}-ATP, 10 mM $MgCl_2$, 1 mM DTT in 50 mM Tris-HCl (pH 7.5) buffer. The reaction was proceeded for 10 min at 30°C in the presence of inhibitor **4** and stopped by adding 80 μl of 12% phosphoric acid. The stopped mixtures were transferred to 96-well PVDF filter (Millipore, USA) prewetted with 50% EtOH and drained with mild suction. Each well was washed five times with 10 mM Tris-HCl (pH 8.0) containing 0.1 M NaCl to remove free P^{32}-ATP. The filter was briefly dried and exposed to Phosphoimager (Molecular Dynamics, USA) to measure radioactivity.

RESULTS AND DISCUSSION

The potential of cytotoxicity assay against human cancer cells

The *in vitro* cytotoxic activities of compounds **4a-4k** and **5a-5b** were evaluated by SRB assay according to the NCI (National Cancer Institute, USA) protocol (Skehan *et al*, 1990). The IC_{50} values of **4a-4k** and **5a-5b** were compared with those of cisplatin as standard drug.

As indicated in Table II, the 5,8-quinolinediones **4a-4k** and **5a-5b** showed generally cytotoxic activities against all tested tumor cell lines, and especially potent activity against XF 498 with the IC_{50} values of 0.14-1.73 μg/ml. Also, the compounds **4a-4k** and **5a-5b** showed mostly potent cytotoxicities against SK-MEL-2. The compounds **4a** and **5a**, which contain 7-(4-fluorophenyl) amino groups, exhibited the remarkable cytotoxicities against HCT 15 and XF 498. The activities of the compounds **4a** and **5a** are superior to those of cisplatin with 2-5 times more potency. Actually, activities of the quinoinediones **4b** and **4i** were superior or comparable to those of cisplatin against various cell lines. Structure-activity relationship (SAR) may not exist between properties of substituents (R) of 7-(substituted-phenyl)amino moiety of **4a-4l** and **5a-5b**. The SAR certainly needs to be studied further.

CDKs inhibitory activities

The 6-arylamino-7-chloro-5,8-quinolinediones **4** were tested for their CDKs/cyclins (CDK2 and CDK4) inhibitory activities according to the procedure described in the reference (Dunphy *et al.*, 1997). The IC_{50} values were determined by comparison with those of olomoucine as positive control. As indicated in the Table II, the tested 5,8-quinolinediones **4e**, **4f** and **4i** showed generally moderate inhibitory activities for CDK4/cyclin

Table II. Cytotoxic and CDKs inhibitory activities of 6-aryl-amino-7-halo-5,8-quinolinediones

Compounds	Cytotoxicity[a] IC_{50} (mg/ml)					IC_{50} (mM/mM)[b]	
	A549[c]	SKOV-3	SKMEL-2	HCT 15	XF 498	CDK2	CDK4
4a	0.85	0.50	1.39	0.36	0.18	50>	45
4b	1.74	0.49	1.25	1.60	0.21	NT[d]	NT
4c	4.28	0.92	1.26	2.84	0.34	50>	50
4d	4.29	1.62	3.29	3.61	1.27	NT	NT
4e	1.39	0.38	0.28	1.66	0.33	50>	14
4f	3.28	1.36	2.80	4.14	0.61	50>	5
4g	7.53	3.67	4.40	4.94	1.73	50>	50>
4h	5.23	4.15	4.43	7.90	1.28	50>	50>
4i	1.99	0.57	0.88	1.84	0.55	50>	9
4j	2.10	1.33	1.30	3.31	0.34	NT	NT
4k	1.83	1.77	1.73	3.18	0.59	50>	45
5a	2.92	0.44	1.27	0.32	0.14	NT	NT
5b	3.45	1.29	1.98	0.53	0.76	NT	NT
cisplatin	0.78	0.46	0.46	0.64	1.10	NT	NT
Olomoucine	57.00	>100	87.00	50.00	57.00	7	>50

[a] Cytotoxicity evaluation: SRB assay according to the NCI protocols (Skehan *et al.*, 1990)
[b] CDK4/cyclin D1 and CDK2/cyclin A inhibition assay according to the reference (Dunphy *et al.*, 1997)
[c] Human solid tumor cell lines: A 549 (non-small cell lung), SKOV-3 (human ovarian), SK-MEL-2 (melanoma), HCT-15 (colon) and XF498 (CNS) from National Cancer Institute (NCI) in USA
[d] NT: not tested

D1 in concentration range of 5-14 μM, but no inhibitory activities for CDK2 up to 50 μM. In contrast, the olomoucine in the concentration of 7.0 μM showed 50% inhibitory activities for CDK2, but at the concentration of 100 mM exhibited no 50% inhibitory activities for CDK4. The result indicates that the 5,8-quinolinediones **4f** and **4i** could be the selective CDK4 inhibitors. Many compounds such as olomoucine, roscovitine, flavopiridol and staurosporine are potent and/or relatively selective inhibitor of CDK1 and CDK2 but none are selective inhibitor of CDK4 (Webster *et al*, 1998).

In conclusion, the results of this study suggest that 6-arylamino-7-halo-5,8-quinolinediones **4a** and **5a** should be potent cytotoxic agents against HCT-15 and SK-MEL-2, and the compounds **4f** and **4i** could be selective CDK4 inhibitors. Moreover, the results might encourage the synthesis of new 6,7-disubstituted-5,8-quinolinedione analogs for improving cytotoxic and CDK inhibitory potential.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the MOST through National R&D Program (97-N6-01-01-A-18) for Women's Universities. We thank Dr. Chang Yong Hong (Biotech Research Institute, LG Research Park, Taejon 305-380, Korea) for the testing of CDKs inhibitory activities.

REFERENCES

Boger, D. L., Yasuda, M., Mitscher, L. A., Drake, S. D., Kitos, P. A. and Thomson, S. C., Streptonigrin and lavandamycin partial structure. A probe for the minimum potent pharmacophore of streptonigrin, lavandamycin and synthetic quinoline-5,8-diones. *J. Med. Chem.*, 30, 1918-1928 (1987).

Collins, K., Jacks, T. and Pavletich, N. P., The cell cycle and cancer. *Proc. Natl. Acad. Sci. USA*, 94, 2776-2778 (1997).

Dunphy, W. G., *Methods in Enzymology*; Cell Cycle Control. Academic Press, San Diego, pp 1-205, 1997; Phelps, D. E. and Xiong, Y. Assay for activity of mammalian cyclin D-dependent kinases CDK4 and CDK6, 194-205; Meijer, L. and Kim, S-.H. Chemical inhibitors of cyclin-dependent kinases, 113-128.

Jeschke, P., Linder, W., Mueller, N., Harder, A. and Mencke, N., New 6(7)amino-substituted-5,8-quinolinediones to combat endoparasites. *Eur. Patent.*, Appl. EP 519, 290 (Cl. C07D215/38) (1992).

Lown, J. W. and Sim, S. K., Studies related to antitumor antibiotics. Synthesis of streptonigrin analogues and their single strand scission of DNA. *Can. J. Chem.*, 54, 2563-2572 (1976).

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

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

Ryu, C. K. and Kim, H. J., The synthesis of 6-(N-arylamino)-7-chloro-5,8-quinolinedione derivatives for evaluation of antifungal activities. *Arch. Pharm. Res.*, 17, 139-144 (1994).

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

Ryu, C. K., Jung, S. H., Lee, J. A., Kim, H. J., Lee, S. H. and Chung, J. H., 6-Arylamino-5,8-quinolinediones and 7-arylamino-5,8-isoquinolinediones as inhibitors of endothelium-dependent vasorelaxation. *Bioorg. Med. Chem. Lett.*, 9(17), 2466-2472 (1999b)

Sausville, E. A., Zahararevitz, D., Gussio, R., Meijer, L., Louarn-Leost, M., Kunick, C., Schultz, R., Lahusen, T., Headlee, D., Stinson, S., Arbuck, S. G. and Senderowicz, A., Cyclin-dependent kinases: Initial approaches to exploit a novel therapeutic target. *Pharmacol. Ther.*, 82, 285-292 (1999).

Schellhammer, C. W. and Petersen, S., Ueber Derivatives des 5,8-Chinolinchinone. *Ann. der Chem.*, 624, 108-119 (1959).

Shaikh, I. A., Johnson, F. and Grollman, A. P., Structure -activity relationship among simple bicyclic analogues. Rate dependent of DNA degradation on quinone reduction 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).

Webster, K. R., The therapeutic potential of targeting the cell cycle. *Exp. Opin. Invest. Drugs*, 7(6), 865-887 (1998).

Yasuda, M. and Boger, D. L., Streptonigrin and lavandamycin partial structure. A probe for the minimum potent pharmacophore of the natural occurring antitumor-anitibiotics. *J. Heterocycl. Chem.*, 24, 1253 -1259 (1987).