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6-Arylamino-7-chloro-quinazoline-5,8-diones as novel cytotoxic and DNA topoisomerase inhibitory agents

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Abstract—A series of 6-arylamino-7-chloro-quinazoline-5,8-diones were prepared and evaluated for their in vitro cytotoxicity in cultured human cancer cell lines A549 (lung cancer), Col2 (colon cancer), and SNU-638 (stomach cancer). The preliminary structure–activity relationship has been described for providing further development of potent antitumor agents. To further investigate the cytotoxic mechanism, the effects of test compounds on DNA topoisomerase I and II activities have been assessed.

In our previous studies we reported that heterocyclic quinones including naphthoquinones, quinolinequinones, and quinoxalinequinones showed cytotoxic activities against human cancer cells.[1–4] Structure–activity studies from these compounds indicated that the number and position of nitrogen (N) atoms substituted in the heterocyclic ring were considerably important factors to affect the cytotoxic potential. Generally, the increasing numbers of substituent nitrogen atoms in the ring enhanced the cytotoxic activities. Based on the information, we further extended to design and synthesize quinazolinequinone derivatives for developing novel antitumor agents. Especially, we designed the synthesis of 6-arylamino-7-chloro-quinazoline-5,8-dione analogs, starting from quinazolinequinone with several arylamines. In this study we evaluated the cytotoxic potential of synthesized 6-arylamino-7-chloro-quinazoline-5,8-dione analogs in cultured human solid tumor cell lines including human lung (A549), colon (Col2), and stomach (SNU-638) cancer cells. Further, the cytotoxic mechanism shown by the quinone derivatives has been extensively studied, and one possible mechanism of action has been suggested as a DNA topoisomerase inhibitor via DNA-intercalation, or reducing quinone moiety by oxidoreductase.[5–10] Since a variety of antitumor agents currently used in chemotherapy or evaluated in clinical trials are known to inhibit DNA topoisomerase I (topo I) or II (topo II) and also in our previously synthesized compounds 6,11-dihydro-pyrido- and 6,11-dihydro-benzo[2,3-*b*] phenazine-6,11-dione derivatives were considered to be a new class of cytotoxic DNA intercalators and topo I and II inhibitors we applied to evaluate the newly synthesized compounds against topoisomerase I and II activities.[11]

Indeed, DNA topoisomerases (topo) are considered to have important roles in replication, recombination, transcription, chromosome condensation, and the maintenance of genome stability by catalyzing the passage of individual DNA strands (topo I) or double helices (topo II) through one another. In accordance, topoisomerase activities are activated in cancer cell growths, and thus are good targets for antineoplastic drugs.[12,13] The antitumor drugs camptothecin, doxorubicin, and etoposide are representative topo I or topo II inhibitors. Therefore, in this study, to investigate one possible mechanism of action of the cytotoxic activity of 6-arylamino-7-chloro-quinazoline-5,8-dione analogs, we evaluated their ability to inhibit topo I or topo II activities with DNA relaxation and a DNA decatenation assay, respectively. We report here that 6-arylamino-7-chloro-quinazoline-5,8-dione analogs show potential cytotoxicity against cancer cell lines with topo I or II inhibitory activity.

Keywords: 6-Arylamino-7-chloro-quinazoline-5,8-diones; Cytotoxicity; Topoisomerase inhibitor.
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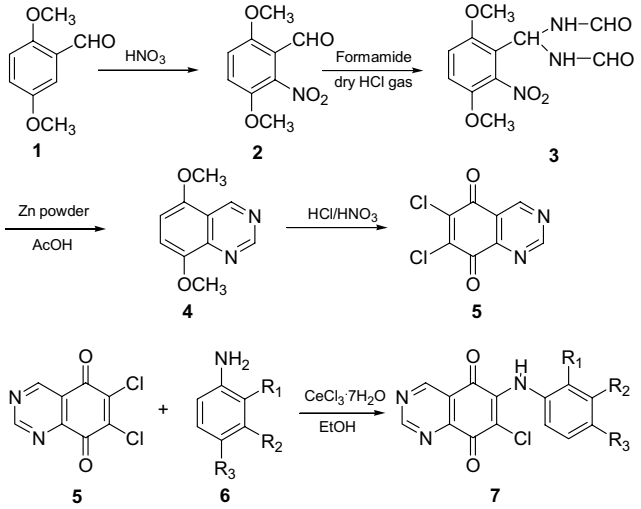
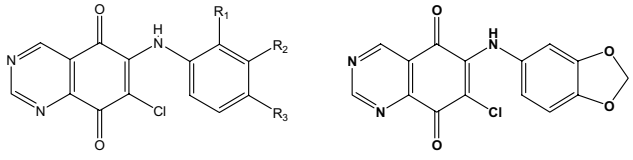


Scheme 1. Synthetic approach employed for the preparation of 6-arylamino-7-chloro-quinazoline-5,8-diones described in Tables 1 and 2.

Compounds were prepared as following sequential solution phase synthetic pathways (Scheme 1). Briefly, 2,5-dimethoxybenzaldehyde **1** was reacted with concd HNO_3 to afford 2,5-dimethoxy-6-nitrobenzaldehyde **2**. Subsequently, *N*,*N*-diformamidoacetal form **3** of **2** was produced by passing dry HCl gas at 80 °C in the presence of formamide. After standard aqueous workup, Zn powder was added to **3** in ice/acetic acid, and the reaction was continued for overnight. The reaction mixtures were filtered over 50% NaOH solution, extracted with ether and washed with water and dehydrated with $MgSO_4$, and then evaporated to give **4**. 6,7-Dichloro-quinazoline-5,8-dione **5** was produced by adding concd HNO_3 to **4** in the presence of concd HCl according to known method[14] with minor modification. Consequently, 6-arylamino-7-chloro-quinazoline-5,8-diones **7** were prepared by reacting with **5** and several arylamines **6** in the presence of a catalyst $CeCl_3{\cdot}7H_2O$ in ethanol.

Primarily, the cytotoxic activity of 6-arylamino-7-chloro-quinazoline-5,8-dione derivatives was evaluated in cultured three human cancer cell lines including lung (A549), colon (Col2), and stomach (SNU-638) carcinoma cells using sulforhodamine B (SRB) protein dye staining method, as described previously.[15] As shown in Table 1, the cytotoxicity was dependent on the position of substitution in arylamino groups. Generally, the cytotoxic effects of all test compounds were potent, judged by IC_{50} value of less than 10 μM in active criteria, and also stomach cancer cells were more susceptible compared to lung and colon cancer cells with two to five times. The substitution of R_1 or R_3 position still maintains the cytotoxic activity, but that of R_2 position with bromide or methyl group such as **7d**,**f**, and **7h** decreased the activity, indicating that the introduction of bulkiness at R_2 position might be inverse effect to the cytotoxic activity. Especially, compounds **7a**–**c**,**g**, and **7j** showed the potential cytotoxic activity in the range of IC_{50} with 0.6–1.2 μM against colon and stomach cancer cells. In another variation, methylenedioxy-arylamino analog **7o** enhanced the cytotoxic activity with more than twofold

Table 1. Effects of 6-arylamino-7-chloro-quinazoline-5,8-dione derivatives on cytotoxicity in human cancer cells[a]



7a-7n **7o**

Compounds	Structure			Cytotoxicity[a] (IC_{50}, μM)[b]		
	R_1	R_2	R_3	A549	Col2	SNU-638
7a	H	H	H	3.54	1.19	0.77
7b	H	H	C_2H_5	3.44	0.70	0.64
7c	OCH_3	H	OCH_3	2.02	1.07	0.61
7d	H	Br	H	3.68	5.07	1.18
7e	C_2H_5	H	H	4.65	4.37	0.70
7f	H	CH_3	H	5.17	5.67	0.97
7g	H	H	CH_3	5.81	0.87	0.73
7h	H	CH_3	CH_3	4.49	3.73	0.99
7i	Br	H	CH_3	4.07	3.04	0.89
7j	OC_2H_5	H	H	3.28	0.73	0.69
7k	Br	H	H	4.47	3.40	0.99
7l	H	Cl	H	4.22	3.55	0.81
7m	CH_3	H	H	3.60	3.77	0.90
7n	H	H	Br	4.31	3.02	0.69
7o				1.67	0.88	0.55
Ellipticine				1.22	1.22	2.00
Doxorubicin				0.30	0.52	0.69

[a] Cytotoxicity was measured as described previously.[15]

[b] The IC_{50} values were determined from triplicate tests.

against lung cancer cells. These active compounds were comparable in cytotoxic potential with the positive controls ellipticine and doxorubicin in the IC_{50} range of 0.3–2.0 μM.

Further, to investigate whether 6-arylamino-7-chloro-quinazoline-5,8-diones-mediated cytotoxicity is related to topoisomerase activity, the catalytic activity of topo I and II was measured using relaxation and decatenation assay, respectively, as described previously.[16] As illustrated in Figure 1, supercoiled pHOT1 DNA (lane 1) was relaxed in the presence of topo I enzyme (lane 2). The inhibitory effects of test compounds at the initial test concentration of 300 μM were depicted in Figure 1 and Table 2.

Especially, compounds **7b**,**f**,**h**,**i**, and **7k** potently inhibited DNA relaxation induced by topo I with more than 80% compared to control DNA. The inhibitory activity of compounds **7h** and **7i** was much more potent than that of positive control camptothecin at 100 μM. In terms of structure–activity relationships, no considerable functional groups were taken to exhibit topo I-mediated inhibitory activity, but electron-donating groups at R_2 (**7f** and **7h**) and R_3 (**7b**,**h**, and **7i**) seem to potentiate the activity. In addition, for topo II activity, the topo II-mediated KDNA decatenation activity was evaluated. When tested at a concentration of 150 μM, most of the test compounds showed weak inhibitory activity, but compounds **7e**,**i**,**k**, and **7n** were found to potentially inhibit the catenated KDNA to the decatenated form by topo II-mediated catalytic activity (Fig. 2 and Table 2).

Dose-dependent inhibitory activity by **7i** (IC_{50} = 40.5 μM), **7k** (IC_{50} = 17.3 μM) and **7n** (IC_{50} = 15.1 μM) was shown in topo II-mediated decatenation of DNA (Fig. 3), and also compound **7i** and **7k** seem to be active against both topo I and topo II. The substitution at R_1 (**7e**,**i**, and **7k**) or R_3 (**7i** and **7n**) position enhanced the activity. No obvious correlation was observed between the cytotoxicity of individual compound and the inhibitory activity of topoisomerase inhibitory activity. However, the inhibitory activity against topo I or II by active compounds might be one possible mechanism for

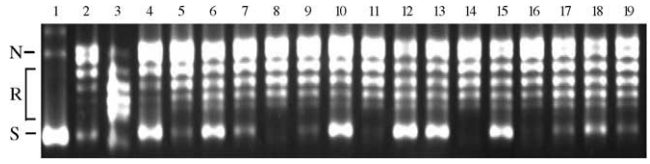


Figure 1. Effects of 6-arylamino-7-chloro-quinazoline-5,8-dione analogs on the relaxation of supercoiled DNA by topo I. Plasmid DNA (pHOT1, 0.25 μg) was incubated with topo I (1 unit) in the presence of test compounds and then depicted on an agarose gel. Lane 1: supercoiled DNA without enzyme; Lane 2: supercoiled DNA with topo I (relaxed form); Lane 3: Relaxed DNA marker; Lane 4: Supercoiled DNA with topo I in the presence of 100 μM camptothecin; Lane 5–19 in the presence of compounds **7a**–**o** at a concentration of 300 μM. N: nicked, R: relaxed, S: supercoiled form.

Table 2. Effects of 6-arylamino-7-chloro-quinazoline-5,8-dione derivatives on topo I and II activities

Compounds	Topo I[a] (% inhibition)	Topo II[b] (% inhibition)
7a	27	41
7b	88	4
7c	35	20
7d	9	2
7e	20	82
7f	96	2
7g	12	1
7h	100	20
7i	100	100
7j	9	1
7k	89	99
7l	12	2
7m	29	6
7n	58	94
7o	27	2
Camptothecin	50	
Etoposide		48

[a] Relaxation assay for topo I catalytic activity (at 300 μM excepting 100 μM for comptothecin).

[b] Decatenation assay for topo II catalytic activity (at 150 μM excepting 100 μM for etoposide) was determined as described previously.[15]

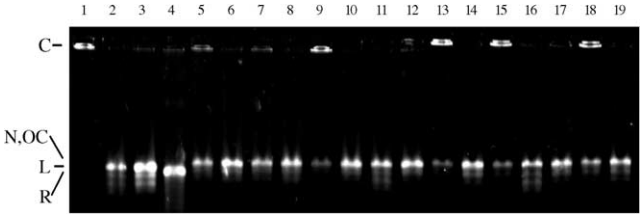


Figure 2. Effects of 6-arylamino-7-chloro-quinazoline-5,8-quinone analogs on the decatenation of KDNA by topo II. Plasmid DNA (KDNA, 0.2 μg) was incubated with topoisomerase II (1 unit) in the presence of test agents and then seen on an agarose gel. Lane 1: KDNA without enzyme (catenated form); Lane 2: KDNA with 1 unit of topoisomerase II (decatenated form); Lane 3: Decatenated KDNA marker; Lane 4: Linear KDNA marker; Lane 5–19; KDNA with 1 unit of topoisomerase II in the presence of compound **7a**–**o** at a concentration of 150 μM, respectively. C: catenated, N: nicked, OC: open circular, L: linear, R: relaxed form.

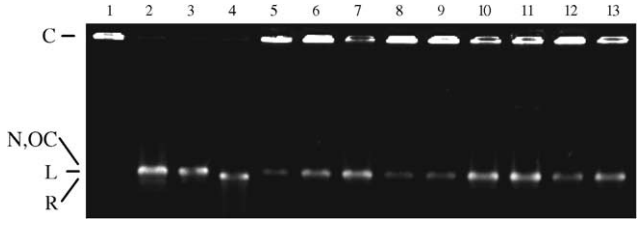


Figure 3. Concentration-dependent effects of compound **7i**,**k**, and **7n** on the decatenation of KDNA by topo II. Plasmid DNA (KDNA, 0.2 μg) was incubated with topo II (1 unit) in the presence of test agents and then seen on an agarose gel. Lane 1: KDNA without enzyme (catenated form); Lane 2: KDNA with 1 unit of topo II (decatenated form); Lane 3: Decatenated KDNA marker; Lane 4: Linear KDNA marker. Lane 5–7: KDNA with 1 unit of topoisomerase II in the presence of compound **7i**; Lane 8–10: Compound **7k**; Lane 11–13: Compound **7n** at a concentration of 30, 60, 120 μM, respectively. C: catenated, N: nicked, OC: open circular, L: linear, R: relaxed.

their cytotoxic effects, and also other mechanisms of action could not be excluded.

In conclusion, a series of 6-arylamino-7-chloro-quinazoline-5,8-dione derivatives has been prepared and evaluated for their cytotoxic activities with the goal of developing potent antitumor agents. We found that most of compounds synthesized in this study generally showed potent cytotoxic effects and also more susceptible to human stomach cancer cells compared to lung or colon cancer cells tested. Among them **7c** could serve as a good lead in these series of compounds. Further, based on the information of DNA intercalating potential of these series of compounds, the mechanism of action study was employed by topoisomerase activity. Several compounds exhibited the potential inhibitory activity against either topo I or topo II, suggesting that the inhibitory activity of topo I or II might be one possible mechanism for their cytotoxic activity against cancer cells. Further studies for more potent cytotoxic compounds, based on the above findings, are in progress in our laboratory.

Acknowledgements

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16. Park, H. J.; Lee, H.; Lee, E.; Hwang, H. J.; Shin, S.; Suh, M.; Kim, C.; Kim, H. J.; Seo, E.; Lee, S. K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1944. (a) *Measurement of topoisomerase I catalytic activity*: An assay was done using supercoiled pHOT1 DNA as a substrate according to the protocol provided by TopoGEN, Inc. (Columbus, USA). Supercoiled (form I) plasmid substrate DNA was used in a reaction volume of 20 μL containing the following: 10 mM Tris–HCl, pH 7.9, 1 mM ethylene diamine tetraacetic acid (EDTA), 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine, 5% glycerol, and 1 unit of purified human topo I (TopoGEN, Inc.). The appropriate inhibitor was added as indicated, and the reaction was started by the addition of the enzyme. After 10 min of incubation at 37 °C, the reaction was stopped by addition of 5 μL of stop buffer containing the loading dye (1% sarkosyl, 0.025% bromophenol blue, 5% glycerol), and then the reaction mixture was analyzed on a 1% agarose gel by running at 40 V for 3.5 h in TBE buffer (89 mM Tris, 89 mM borate, 2 mM Na-EDTA, pH 8.3). Gels were stained with SYBR Green I (Molecular Probes, Eugene, OR), and observed under UV illumination. For the quantitative determination of topo I activity, photographic negatives were densitometrically scanned using AlphaImager 2200 (AlphaEase version 5.5). The inhibition of topo I was calculated from the equation: %Inhibition = [Intensity of sample-treated DNA/Intensity of vehicle-treated control DNA]×100; (b) *Decatenation assay for topo II activity*: The total reaction volume of the topo II-mediated cleavage reaction was fixed at 20 μL. Briefly, assay buffer [50 mM Tris–HCl, pH 8, 120 mM KCl, 10 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM dithiothreitol, 30 μg/mL bovine serum albumin (BSA)] containing 200 ng of KDNA (TOPOgen), and a solution of the test drugs were added to 1 unit of the human recombinant topo II (the amount of enzyme, which resulted in the complete decatenation of 200 ng of KDNA). After 10 min of incubation at 37 °C, the reaction was stopped by addition of 5 μL of stop buffer containing the loading dye (1% sarkosyl, 0.025% bromophenol blue, 5% glycerol), and then the reaction mixture was analyzed on a 1% agarose gel by running at 40 V for 3.5 h in TBE buffer (89 mM Tris, 89 mM borate, 2 mM Na-EDTA, pH 8.3). Gels were made visible as described above for the relaxation assay. Gels were photographed, and remaining KDNA from photographic negatives was scanned and calculated the inhibition activity as mentioned above.