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SYNTHESIS AND CYTOTOXIC ACTIVITIES OF 6-CHLORO-7-ARYLAMINO-5,8-ISOQUINOLINEDIONES

Chung-Kyu Ryu[a*], In-Kyung Lee[a], Sung-Hee Jung[a] and Chong-Ock Lee[b]

[a] *College of Pharmacy, Ewha Womans University, Seodaemunku, Seoul 120-750, Korea and*

[b] *Korea Research Institute of Chemical Technology, Yusongku, Taejon 305-343, Korea*

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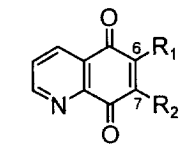
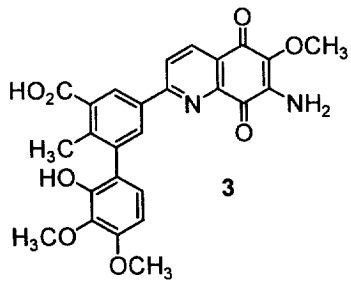
Abstract: 6-Chloro-7-arylamino-5,8-isoquinolinediones were newly synthesized and evaluated for *in vitro* cytotoxic activities against five human solid tumor cell lines. Among them, **5b, 5c** and **5d** exhibited potent activities against the cell lines HCT-15 and SK-MEL-2.

6,7-Disubstituted-5,8-quinolinediones **1** were frequently studied because of their wide spectrum of biological activities such as antitumor, antifungal and antimalarial agents.[1] 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.[2] The moiety **2** cleaves PM2 phage circular DNA of tumor cells.[3] Many structural variants of **2** showed that the bioreductive 5,8-quinolinedione ring seems to be required for antitumor activity.[3] Substituents such as halogen and amino groups of the synthetic quinone derivatives increase their cytotoxicities.[2,4,5]



1 : R_1, R_2 = X, NH-R_3, OH, OCH_3, NH_2,..
2 : R_1 = H, R_2 = NH_2



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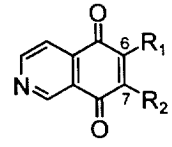

4 : R_1= Cl, R_2 = NH-R_3

It was interesting to synthesize 6,7-disubstituted-5,8-isoquinolinediones **4**, which may be a bioisostere of **1**, and to compare their cytotoxicities with those of the quinolinediones **1**. Studies on the cytotoxic activity of heterocyclic quinones containing nitrogen atom showed that the position of nitrogen are important for the

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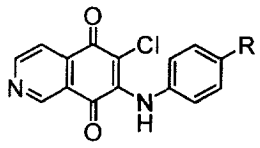
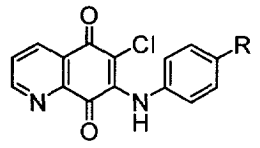
cytotoxicity.[6,7] The 5,8-isoquinolinedione moieties are more active to cleave the DNA than the corresponding 5,8-quinolinediones.[7] The presence of substituents such as chlorine and substituted amino groups of quinones improves their cytotoxicity.[2,5] Therefore, we synthesized newly 6-chloro-7-arylamino-5,8-isoquinolinediones **5a-5l** to evaluate their cytotoxic activity.



5a-5l : R = H, OH, Cl,...

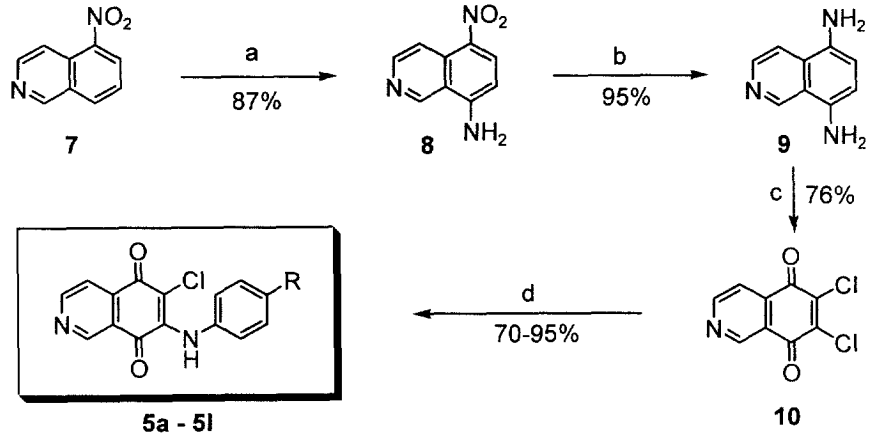


6a-6l : R = H, OH, Cl,...

There have been a few reports[8,9,10] on the cytotoxicities of some 5,8-isoquinolinedione compounds, which showed activities against Ehrlich carcinoma[8], murine leukemia L1210[8,9] or L5178Y[10]. However, their cytotoxicities against various human tumor cell lines were not evaluated. The *in vitro* cytotoxicities of the new 5,8-isoquinolinediones **5a-5l** against human tumor cell lines were determined and compared with those of the corresponding 6-chloro-7-arylamino-5,8-quinolinediones **6a-6l**.

Chemistry

A convenient method for the synthesis of 6-chloro-7-arylamino-5,8-isoquinolinediones **5a-5l** (**Table 1**) from commercially available 5-nitro-isoquinoline (**7**) is shown in **Scheme**.



Scheme a) $HONH_2$/EtOH/KOH, 5h, 60°C b) H_2/Pd-C/EtOH, RT, 30psi, 4h
c) $KClO_3$/HCl, 1h, 65°C d) Arylamine/EtOH, 2-5h, reflux

Table 1. Structures and *in vitro* cytotoxic activities

Compound	R	Cytotoxicity[a] IC_{50} (μg/mL)				
		A 549[b]	SK-OV-3	SK-MEL-2	XF 498	HCT-15
5a	H	1.28	0.86	0.52	0.96	0.10
5b	OH	0.36	0.30	0.06	0.32	0.05
5c	OCH_3	0.50	0.28	0.02	0.31	0.02
5d	OC_2H_5	0.42	0.34	0.04	0.27	0.03
5e	CH_3	1.22	1.54	0.17	1.06	0.11
5f	C_2H_5	0.62	0.34	0.07	0.33	0.05
5g	C_2H_5OH	0.61	0.63	0.08	0.06	0.18
5h	F	0.66	0.17	0.13	0.49	0.07
5i	Cl	1.27	1.86	1.22	1.54	0.31
5j	Br	1.10	0.97	0.66	1.28	0.18
5k	I	1.96	0.29	0.23	0.22	0.10
5l	CN	0.80	0.21	0.22	0.30	0.13
6a	H	2.30	1.34	1.42	1.33	0.74
6b	OH	1.56	1.27	0.47	0.97	0.86
6c	OCH_3	1.27	1.30	1.33	1.26	0.66
6d	OC_2H_5	0.96	0.33	0.34	0.55	0.32
6e	CH_3	1.14	1.27	0.42	1.26	0.33
6f	C_2H_5	1.35	1.35	1.97	0.66	1.39
6g	C_2H_5OH	1.11	0.42	0.36	0.52	0.48
6h	F	1.25	0.50	1.39	0.36	0.18
6i	Cl	5.03	1.33	1.30	3.31	0.34
6j	Br	4.28	0.92	1.26	2.83	0.34
6k	I	4.29	1.62	3.30	3.61	1.27
6l	CN	1.70	0.55	0.60	1.47	1.45
Cisplatin		1.80	2.07	1.38	2.74	2.90
Streptonigrin		0.33	0.28	0.02	0.31	0.02

a) Cytotoxicity screening: SRB assay according to the NCI protocols[18,19]

b) Human solid tumor cell lines: A 549 (non-small cell lung), SK-OV-3 (ovarian), SK-MEL-2 (melanoma), HCT-15 (colon) and XF 498 (CNS) from National Cancer Institute (NCI) in USA

Experimental details for this procedure are given in the **References and Notes.**[11-14] 5-Nitro-8-amino-isoquinoline (**8**) was synthesized by the amination of the isoquinoline **7** with $HONH_2$ and KOH in EtOH in 87 % yield. The compound **8** was reduced to 5,8-diaminoisoquinoline (**9**) by catalytic hydrogenation. The 6,7-dichloro-5,8-isoquinolinedione (**10**) was synthesized by oxidizing **9** with the $NaClO_3$/HCl variation in 76% yield. The key intermediate **10** was prepared in three steps with an overall yield of 63% from **7**. Also, the compound **10** could be prepared, according to another known procedure[7] from 5-hydroxyisoquinoline in 11% yield. The 5,8-isoquinolinediones **5a-5l** were synthesized by nucleophilic substitution of the dione **10** with appropriate arylamines. In the substitution reaction, a single compound[15] was contained, to which we ascribe structure **5a-5l**. This regioselectivity was based mainly on the speculation[16] that the C-5 carbonyl group, which is para to the nitrogen, is more electron deficient than the C-8 carbonyl group as depicted in the resonance structure **10a**. Thus, the electron deficiency led to substitute at the C-7 position. Most of these nucleophilic substitutions went as expected and had overall high yields of 70-95%.

The 5,8-quinolinedione derivatives **6a-6l** for comparison with the cytotoxicities of the 5,8-isoquinolinediones **5a-5l** were prepared according to the reported method.[17]

Cytotoxicities

The *in vitro* cytotoxic activities of **5a-5l** and **6a-6l** were evaluated by SRB (sulforhodamine B) assay according to the NCI protocols.[18,19] 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 IC_{50} values of **5a-5l** and **6a-6l** were compared with those of **3** and cisplatin.

As indicated in **Table 1**, the 5,8-isoquinolinediones **5a-5l** showed generally potent cytotoxic activities against all tested tumor cell lines, and especially potent activity against HCT-15 with the IC_{50} values of 0.02-0.18μg/mL. Also, the **5a-5l** showed mostly potent cytotoxicities against SK-MEL-2. The compounds **5b, 5c** and **5d**, which contain 7-(4-hydroxyphenyl)- or 7-(4-alkoxyphenyl)amino groups, exhibited the remarkable cytotoxicities against HCT-15 and SK-MEL-2. The activities of these compounds are superior or comparable to those of **3** and approximately 50-150 times more potent than cisplatin. Actually, activities of the quinoinediones **6a-6l** were superior or comparable to those of cisplatin against many cell lines.

The isoquinolinedione skeletons **5a-5l** had, in general, more potent activities than quinoinedione skeletons **6a-6l**. However, no structure-activity relationship would exist between properties of substituent (R) of 7-arylamino groups in the diones **5a-5l** and **6a-6l**.

In conclusion, the results of this study suggest that 6-chloro-7-arylamino-5,8-isoquinolinediones are potent cytotoxic agents against HCT-15 and SK-MEL-2. Moreover, the results should encourage the synthesis of new 5,8-isoquinolinedione derivatives for improving cytotoxic properties.

Acknowledgement.

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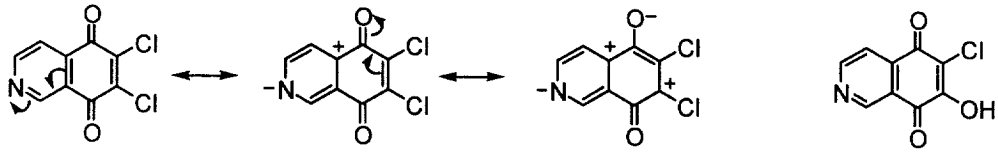
11. 5-Nitro-8-amino-isoquinoline (**8**) – the isoquinoline **7** (2g, 11mmol) and $HONH_2$·HCl (5g, 70mmol) were dissolved in 120mL of 95% EtOH which was heated at 50-60°C. A solution of 10g NaOH in 65mL MeOH was added gradually to the mixture with stirring over a period of 90min, and the reaction solution was poured into 700mL ice water. The precipitate was filtered and recrystallized from 95% EtOH: yellow crystals **8** (1.88g, 87%): mp 332-333°C ; ^{1}H-NMR ($CDCl_3$) 9.47 (s, 1 H, H1), 8.75 (d, J = 5.2Hz, 1 H, H3), 8.20 (d, J=5.4Hz, 1 H, H7), 8.16 (d, J=5.2Hz, 1 H, H4), 6.87(d, J=5.4Hz, 1 H, H6), 4.1(s, 2 H, NH_2); MS, m/z 189 (M^+), 173, 143.
12. 5,8-Diaminoisoquinoline (**9**) - A suspension of **8** (2g, 10mmol), 10% Pd on carbon (0.5g), 300ml EtOH was shaken under 30psi H_2 for 2h. The product was filtered through Celite and recrystallized from EtOH.: yellow crystalline **9** (1.8g, 95%): mp 139-140°C (Lit.[8], 138-140°C).
13. 6,7-Dichloro-5,8-isoquinolinedione (**10**) - $KClO_3$ (5.50g) was added over 30min to a mixture of the compound **9** (6,36g, 40mol) in 69mL C-HCl at 60°C and was heated at 50-60°C for 30min. The mixture was poured into 500mL ice water. The precipitate was filtered and recrystallized from n-BuOH.: pale-green crystals **10** (1.0g, 76%): mp 179-181°C (Lit.[7], 180-181°C) ; ^{1}H-NMR ($CDCl_3$) 9.45 (s,1 H, H1), 9.13 (d, J = 5.0 Hz, 1 H, H3), 7.98 (d, J=5.0Hz, 1 H, H4); MS, m/z 231(M^+), 229, 227, 201,199, 166.
14. *General procedure for synthesis of 6-chloro-7-arylamino-5,8-isoquinolinediones* **5a-5l**: A mixture of **10** (2.27g, 10mmol) and appropriate arylamine (11mmol) in 95% EtOH (100mL) was refluxed for 4-10h. After

the mixture was kept overnight in the refrigerator or was poured into 150mL ice water, the precipitate was collected by filtration. The precipitate was filtered and recrystallized from 95% EtOH or MeOH. And the recrystallized **5a-5l** were filtered, washed with cold EtOH and dried (**Scheme, Table 1**).

15. Purity of reaction products **5a-5l** was determined both by to TLC and GC, and the results showed that a single compound was contained. TLC was performed on precoated silica gel (60G 254, Merck) using $CHCl_3$ for solvent. The compounds were detected under UV light (254nm) or by heating at 110°C after spraying 30% H_2SO_4 vanillin solution. The purity of compounds **5a-5l** was also verified by GC (Hewlett Packard 5890A, HP-5 capillary column at 260 °C, N_2, 17mL/mim as carrier gas, FID).
16. Shaikh *et al*[7] reported that treatment of the compound **10** with aqueous NaOH gave exclusively 6-chloro-7-hydroxy-5,8-isoquinolinedione (**11**) and suggested a mechanism based on a resonance structure **10a** in a positive charge was place at C-7 position. The **10a** is the most stable among possible resonance structures, due to the lowest energy of its dipole moment.



10 **10a** **11**

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19. Cytotoxicity screening according to the NCI protocols[18]: The cells were grown at 37°C in RPMI 1640 medium supplemented with 10% FBS and separated using PBS containing 0.25% trypsin and 3mM EDTA. 5×10^3 - 2×10^4 cells were added to each well of 96 well plate and incubated at 37°C for 24h. Each compound (**5a-5l** and **6a-6l**) was dissolved in DMSO and diluted with the above medium at different concentrations with the range of 0.01-30μg/mL. The DMSO concentration was set to be below 0.5% and filtrated. After removing the well medium by aspiration, a portion 200mL of the solution was added to above well plates, which were placed in 5% CO_2 incubator for 48 hrs. The protein stain assay was performed according to SRB assay method.[18]