Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 1623–1628
Bioorganic & Medicinal Chemistry

Synthesis and cytotoxicity evaluation of 6,11-dihydro-pyridazo- and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-diones

Hyun-Jung Lee,[a] Jin Sung Kim,[a] Se-Young Park,[a] Myung-Eun Suh,[a,*] Hwa Jung Kim,[a] Eun-Kyung Seo[a] and Chong-Ock Lee[b]

[a]*Division of Medicinal Chemistry, College of Pharmacy, Ewha Woman's University, Seoul 120-750, South Korea*
[b]*Pharmaceutical Screening Division, Korea Research Institute of Chemical Technology, TaeJon 305-606, South Korea*

Received 7 November 2003; revised 20 January 2004; accepted 20 January 2004

Abstract—The 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione derivatives were synthesized from 6,7-dichloro-5,8-phthalazinedione and 6,7-dichloro-5,8-quinolinedione, respectively, producing a series of new anticancer drugs. The cytotoxic activities of the prepared compounds were evaluated by a SRB (Sulforhodamine B) assay against the following tumor cell lines: A459 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF498 (human CNS), and HCT 15 (human colon). Almost all the derivatives of the 6,11-dihydro-pyridazo[2[,3-*b*]phenazine-6,11-dione and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione, tetracyclic heteroquinone analogues with four or three nitrogen atoms, exhibited excellent cytotoxicity on almost all the human tumor cell lines tested. Specifically, 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione (**4a**) exhibited potent activity against all the tumor cell lines, and in particular, its cytotoxic effect against HCT 15 ($ED_{50} = 0.004$ μg/mL) was 25 times greater than that of doxorubicin ($ED_{50} = 0.093$ μg/mL).

1. Introduction

Heterocyclic quinones containing nitrogen atoms are known to possess excellent antitumor[1,2] and other biological activities.[3–5] In addition, it has been proposed that the cytotoxic mechanism of action is that they act as a topoisomerase inhibitor via a DNA-intercalation.[6–8]

According to Moore and Pindure's thesis,[9,10] in order for a compound to be a DNA-intercalator, the structure should have a planar tetaracyclic ring and *p*-conjugated ketone groups containing a nitrogen atom, which enables hydrogen bonding with the DNA. Moreover, Johnson et al. reported that the presence and position of a nitrogen atom are important for determining the redox potential of the quinone moiety and have the ability to effect DNA strand cleavage, and the introduction of a second nitrogen atom increases both.[11]

In a previous paper,[12] 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione derivatives were synthesized and some exhibited excellent cytotoxicity against the human CNS tumor cell lines. As a continuous effort to develop novel anticancer agents, based on nitrogen-containing heterocyclic quinones, 6,7-dichloro-5,8-phthalazinedione (**1**), which have a second nitrogen atom in the 6,7-dichloro-5,8-quinolinedione (**5**), could be an useful intermediate. Based on these considerations, a series of 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione (**4a–g**), tetracyclic heteroquinone analogues with four nitrogen atoms, and a series of 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione (**9a–f**),[13] starting from 6,7-dichloro-5,8-quinolinedione (**5**), were newly designed and synthesized. The prepared compounds were evaluated for their cytotoxic activity using a SRB (Sulforhodamine B) assay[14,15] against the following cancer cell lines: A459 (human non-small cell lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF498 (human CNS), and HCT 15 (human colon). Their activities were compared with other clinically available anticancer agents, such as, doxorubicin.

2. Results and discussion

2.1. Synthetic chemistry

6,7-Dichloro-5,8-phthalazinedione (**1**), which was prepared according to the literature,[16] was reacted with several aryl amines, to give the 6-arylamino-7-chloro-

Keywords: 6,11-Dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione; 6,11-Dihydro-pyrido[2,3-*b*]phenazine-6,11-diones; Cytotoxicity.
* Corresponding author. Tel.: +82-2327-73040; fax: +82-232-773051; e-mail: suh@ewha.ac.kr

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.01.029

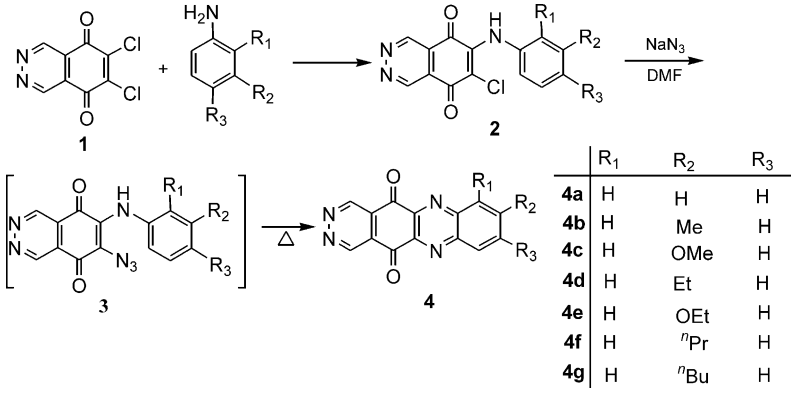
5,8-phthalazinediones (**2a**–**g**). In analogy, 6,7-dichloro-5,8-quinolinedione (**5**), prepared according to the literature,[17] was reacted with several arylamines, to give the 6-arylamino-7-chloro-5,8-quinolinediones (**7a**–**f**). 6,7-Dichloro-5,8-quinolinedione (**5**) has two asymmetric chlorine atoms at the C6 and C7 positions. When compound 5 reacts with arylamines, it is thought that the C6 and/or C7 position of the compound can be substituted. With the addition of $CeCl_3.7H_2O$, the electron density of C-6 can be reduced, so that preferential nucleophilic substitution takes place at the C-6 position due to chelate-complex forming of the Ce (III) with the oxygen atom at the C-8 position (6, Scheme 2). The synthesized 6-arylamino-7-chloro-derivatives (**2a**–**g** and **7a**–**f**) were reacted with sodium azide in DMF at 90–95 °C to give 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione derivatives (**4a**–**g**) and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione derivatives (**9a**–**f**), respectively. The overall synthetic method is outlined in Schemes 1 and 2.

In the reaction of 6-arylamino-7-chloro-derivatives (**2a**–**g** and **7a**–**f**) with sodium azide, the reaction concentration was a very important point and if reacted in high concentration, the yield is much lower. Therefore, the reaction was conducted at a 0.02 molar concentration. It is assumed that the intramolecular and intermolecular reaction were entered into competition after the formation of 6-arylamino-7-azido-5,8-phthalazinedione (**3**) or 6-arylamino-7-azido-5,8-quinolinedione (**8**). The structure was identified by NMR and IR analysis and there was no doubt about the structure because X-ray crystallographic analysis of 6,11-dihydro-3-ethoxy-pyrido[2,3-*b*]phenazine-6,11-dione was carried out previously.[12]

2.2. In vitro antitumor activity evaluation by SRB assay

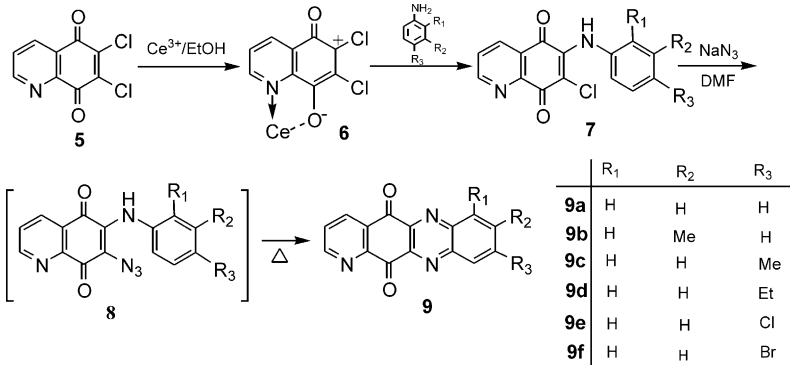
The prepared 6,7-dichloro-5,8-phthalazinedione (**1**), 6-arylamino-7-chloro-5,8-phthalazinedione (**2a**–**g**), 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione (**4a**–**g**), and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione (**9a**–**9f**) were evaluated for their cytotoxic activity against the cancer cell lines at the Korea Research Institute of Chemical Technology using a sulforhodamine B (SRB) assay.[14,15] This method was developed to measure the cellular culture protein concentration, and involved the tumor cell lines representing the five different cancer types, namely, A549 (human non-small cell lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), XF 498 (human CNS), and HCT 15 (human colon). The cells were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco). The cultures were passaged once or twice per week using trypsin-EDTA in order to detach the cells from their culture flasks.

The rapidly growing cells were harvested, counted, and incubated at the required concentration ($1–2\times10^4$ cells/well) in 96-well micro plates. After incubation for 24 h,



	R_1	R_2	R_3
4a	H	H	H
4b	H	Me	H
4c	H	OMe	H
4d	H	Et	H
4e	H	OEt	H
4f	H	^{n}Pr	H
4g	H	^{n}Bu	H

Scheme 1.



	R_1	R_2	R_3
9a	H	H	H
9b	H	Me	H
9c	H	H	Me
9d	H	H	Et
9e	H	H	Cl
9f	H	H	Br

Scheme 2.

the compounds, which were dissolved in the culture medium, were applied to the culture wells in triplicate and incubated for 48 h at 37 °C under a 5% CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After dissolving the bound stain with 100 μL of the unbuffered tris base solution (pH 10.5) using a gyratory shaker, the absorbance at 520 nm was measured using a microplate reader (Molecular Devices E-max, Sunnyvale, USA). The cytotoxic activity was evaluated by measuring the concentration needed to inhibit protein synthesis by 50% (i.e., ED_{50}). Each value shown in Table 1 represents the mean of triplicate experiments.

The 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-diones (**4a–g**) and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-diones (**9a–f**), tetracyclic heteroquinone analogues with four or three nitrogen atoms exhibited excellent cytotoxicity towards almost all the human tumor cell lines tested. In particular, the cytotoxicity of the 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-diones (**4a–g**) against human colon tumor cells (HCT 15) (ED_{50} = 0.004–0.014 μg/mL) was 9–25 times greater than that of doxorubicin (ED_{50} = 0.093 μg/mL). Also the ED_{50} values (0.007–0.061 μg/mL) of the 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-diones (**9a–f**) to human ovarian tumor cells (SK-OV-3) were approximately 3–22 lower than that of doxorubicin (ED_{50} = 0.160 μg/mL). In addition, the 6,11-dyhydro-pyridazo[2,3-*b*]phenazine-6,11-dione (**4a**) and 6,11-dyhydro-pyrido[2,3-*b*]phenazine-6,11-dione (**9a**), which have no substituent ($R_1 = R_2 = R_3 = H$, Schemes 1 and 2), exhibited a much greater cytotoxic activity upon the growth of all the human tumor cell lines tested in vitro than those of doxorubicin. Furthermore, the antitumor activities were considerably enhanced, as more heterocyclic rings were annulated to the heteroquinone ring. For example, the ring-closed compounds **4a–g**, which contains of four coplanar annulated heterocyclic rings, exhibited better antitumor activity than 6-arylamino-7-chloro-5,8-phthalazinediones (**2a–g**).

Table 1. In vitro anticancer activity against human lung tumor cell line (A549), human ovarian tumor cell line (SK-OV-3), human melanoma tumor cell line (SK-MEL-2), human CNS tumor cell line (XF 498), and human colon tumor cell line (HCT 15)

Compd	ED_{50} (μg/mL)				
	A549	SK-OV-3	SK-MEL-2	XF 498	HCT 15
Doxorubicin	0.095	0.16	0.079	0.34	0.093
1	0.98	1.69	0.22	2.71	0.34
2a	0.44	0.4	0.31	0.32	0.28
2b	5.61	2.18	2.06	3.96	3.86
2c	2.59	2.3	0.85	2.37	1.59
2d	7.38	2.44	1.52	4.08	2.36
2e	2.55	1.66	0.86	2.58	1.87
2f	3.47	1.62	2.43	7.26	4.65
4a	0.061	0.081	0.019	0.116	0.004
4b	0.156	0.097	0.037	0.106	0.005
4c	0.167	0.091	0.072	0.106	0.007
4d	0.095	0.102	0.031	0.106	0.006
4e	0.179	0.131	0.048	0.172	0.008
4f	0.128	0.204	0.031	0.172	0.007
4g	0.067	0.17	0.042	0.361	0.014
9a	0.028	0.007	0.026	0.041	0.027
9b	0.047	0.008	0.052	0.037	0.062
9c	0.028	0.007	0.039	0.03	0.031
9d	0.047	0.061	0.104	0.188	0.062
9e	0.033	0.007	0.041	0.071	0.021
9f	0.237	0.052	0.052	0.188	0.217

However, the concept that there is an increasing cytotoxic effect with more nitrogen atoms did not correspond to this study on the cytotoxicity. When compared the cytotoxicities between the 6,11-dyhydro-pyridazo[2,3-*b*]phenazine-6,11-diones (**4a–g**) and 6,11-dyhydro-pyrido[2,3-*b*]phenazine-6,11-diones (**9a–f**), it was hard to determine which one is more potent. In general, compounds **4a–g**, with four nitrogen atoms, showed especially potent cytotoxicities against SK-MEL-2 and HCT 15 and compounds **9a–f**, with three nitrogen atoms, exhibited potent activities against SK-OV-3 and XF 498.

3. Conclusion

6,7-Dichloro-5,8-phthalazinedione (**1**) and 6,7-dichloro-5,8-quinolinedione (**5**) were reacted with several aryl amines to give the 6-arylamino-7-chloro-5,8-phthalazinediones (**2a–g**) and 6-arylamino-7-chloro-5,8-quinolinedione (**7a–f**). The synthesized 6-arylamino-7-chloro-derivatieves (**2a–g** and **7a–f**) were reacted with sodium azide via an intramolecular cyclization, to yield the 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-diones (**4a–g**) and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-diones (**9a–f**), respectively.

The cytotoxicity of the prepared compounds was evaluated by a SRB assay versus doxorubicin. The 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-diones (**4a–g**) and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-diones (**9a–f**), tetracyclic heteroquinone analogues with four or three nitrogen atoms exhibited excellent cytotoxicity towards almost all the human tumor cell lines tested. The ring-closed compounds, 6,11-dihydro-pyridazo[2,3-*b*]phenazine-6,11-diones (**4a–g**) that consist of four coplanar annulated heterocyclic rings, showed a better antitumor activity than the 6-arylamino-7-chloro-5,8-phthalazinediones (**2a–g**).

4. Experimental

4.1. Materials and methods

All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Buechi) and were not corrected. The IR spectra were recorded on a FT-Infrared spectrometer (Bio-Rad. Co., USA) using KBr pellet. ^1H NMR spectra were recorded on a 400 MHz Varian FT-NMR spectrometer facility by using trimethylsilane as an internal standard. Samples were dissolved in acetone-d_6, DMSO-d_6 or $CDCl_3$. Elemental analyses were performed using Thermo Quest (CE Instruments) EA 1110. Most of the reagents were purchased from Aldrich Chemical Company and Merck Company.

4.2. Synthesis

4.2.1. General procedure for the preparation of 6-Arylamino-7-chloro-5,8-phthalazinediones (2a–g). Arylamine (4.37 mmol) was added to a solution of 6,7-dichloro-5,8-phthalazinedione (500 mg, 2.19 mmol) in ethanol (20 mL) and stirred at the room temperature. The reaction mixture was cooled and then filtered. The filtered precipitate was crystallized from 95% ethanol.

4.2.2. 6-Phenylamino-7-chloro-5,8-phthalazinedione (2a). The general procedure was followed for 20 h with aniline (0.4 mL, 4.37 mmol) and the filtered precipitate was crystallized from ethanol to give 600 mg (96%) of purple powder. Mp: 245–246 °C; IR (KBr, cm^{-1}): 1553, 1684 (C=O), 3289 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.93 (s, 1H), 9.78 (s, 1H), 7.72 (br. s, 1H), 7.40 (t, $J=7.6$ Hz, 2H), 7.30 (t, $J=7.6$ Hz, 1H), 7.11 (d, $J=7.6$ Hz, 2H).

4.2.3. 6-(4-Methylphenylamino)-7-chloro-5,8-phthalazinedione (2b). The general procedure was followed for 18 h with *p*-toluidine (468 mg, 4.37 mmol) and the filtered precipitate was crystallized from ethanol to give 610 mg (93%) of purple powder. Mp: 218–219 °C; IR (KBr, cm^{-1}): 1552, 1686 (C=O), 3219 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.85 (d, $J=0.8$ Hz, 1H), 9.70 (d, $J=0.8$ Hz, 1H), 7.63 (br, s, 1H), 7.12 (d, $J=8.4$ Hz, 2H), 6.94 (d, $J=8.4$ Hz, 2H), 2.32 (s, 3H).

4.2.4. 6-(4-Methoxyphenylamino)-7-chloro-5,8-phthalazinedione (2c). The general procedure was followed for 22 h with *p*-anisidine (538 mg, 4.37 mmol), and the filtered precipitate was crystallized from ethanol to give 640 mg (93%) of red brown powder. Mp: 179–180 °C; IR (KBr, cm^{-1}): 1552, 1637, 1681 (C=O), 3289 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.85 (d $J=0.8$ Hz, 1H), 9.70 (d, $J=0.8$ Hz, 1H), 7.60 (br s, 1H), 7.00 (dd, $J=6.8$ and 2.4 Hz, 2H), 6.84 (dd, $J=6.8$ and 2.4 Hz, 2H), 3.78 (s, 3H).

4.2.5. 6-(4-Ethylphenylamino)-7-chloro-5,8-phthalazinedione (2d). The general procedure was followed for 19 h with 4-ethylanilne (529 mg, 4.37 mmol), and the filtered precipitate was crystallized from ethanol to give 650 mg (95%) of purple powder. Mp: 152–153 °C; IR (KBr, cm^{-1}): 1552, 1686 (C=O), 3213 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.90 (d, $J=1.2$ Hz, 1H), 9.74 (d, $J=1.2$ Hz, 1H), 7.66 (br s, 1H), 7.19 (d, $J=8.4$ Hz, 2H), 7.01 (d, $J=8.4$ Hz, 2H), 2.66 (q, $J=7.6$ Hz, 2H), 1.24 (t, $J=7.6$ Hz, 3H).

4.2.6. 6-(4-Ethoxyphenylamino)-7-chloro-5,8-phthalazinedione (2e). The procedure was followed for 18 h with *p*-phenetidine (599 mg, 4.37 mmol), and the filtered precipitate was crystallized from ethanol to give 690 mg (96%) of red brown powder. Mp: 191–192 °C; IR (KBr, cm^{-1}): 1520, 1553, 1639, 1695 (C=O), 3257 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.89 (d, $J=1.2$ Hz, 1H), 9.73 (d, $J=1.2$ Hz, 1H), 7.64 (br s, 1H), 7.04 (dd, $J=6.8$ and 2.0 Hz, 2H), 6.86 (dd, $J=6.8$ and 2.0 Hz, 2H), 4.04 (q, $J=6.8$ Hz, 2H), 1.42 (t, $J=6.8$ Hz, 3H).

4.2.7. 6-(4-*n*-Propylphenylamino)-7-chloro-5,8-phthalazinedione (2f). The procedure was followed for 18 h with 4-*n*-propylaniline (0.65 mL, 4.37 mmol), and the filtered precipitate was crystallized from ethanol to give 680 mg (95%) of red brown powder. Mp: 146–147 °C; IR (KBr, cm^{-1}): 1552, 1632, 1686 (C=O), 3207 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.89 (d, $J=1.2$ Hz, 1H), 9.74 (d, $J=1.2$ Hz, 1H), 7.67 (br s, 1H), 7.16 (d, $J=8.4$ Hz, 2H), 7.00 (d, $J=8.4$ Hz, 2H), 2.60 (t, $J=7.2$ Hz, 2H), 1.61–1.66 (m, 2H), 0.93 (t, $J=6.8$ Hz, 3H).

4.2.8. 6-(4-*n*-Butylphenylamino)-7-chloro-5,8-phthalazinedione (2g). The procedure was followed for 21 h with 4-*n*-butylaniline (0.96 mL, 4.37 mmol), and the filtered precipitate was crystallized from ethanol to give 700 mg (94%) of purple powder. Mp: 153–154 °C; IR (KBr, cm^{-1}): 1552, 1687 (C=O), 3213 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.89 (d, $J=1.2$ Hz, 1H), 9.74 (d, $J=1.2$ Hz, 1H), 7.67 (br s, 1H), 7.16 (d, $J=8.4$ Hz, 2H), 7.00 (d, $J=8.4$ Hz, 2H), 2.60 (t, $J=7.2$ Hz, 2H), 1.61–1.66 (m, 2H), 0.93 (t, $J=6.8$ Hz, 3H).

4.2.9. General procedure for the preparation of 6,11-Dihydro-pyridazo[2,3-*b*]phenazine-6,11-diones (4a–g). To the solution of **2a–g** (2.0 mmol) in DMF (100 mL), sodium azide (200 mg, 3.08 mmol) which was suspended in little amount of distilled water, was added. The mixture was heated at 90–95 °C on a steam bath overnight. The reaction mixture was cooled, the filtered precipitate was extracted with ethylacetate. The organic layer was washed with water, dried with anhydrous $MgSO_4$, concentrated, and then the residue was purified by recrystallization or column chromatography.

4.2.10. 6,11-Dihydro-pyridazo[2,3-*b*]phenazine-6,11-dione (4a). The general procedure was followed for 26 h with 6-phenylamino-7-chloro-5,8-phthalazionedione **2a** (570 mg, 2.0 mmol), and the concentrated residue was purified by recrystallization with methanol to give 150 mg (23%) of dark brown powder. Mp: >300 °C; ^1H NMR (DMSO-d_6): δ 9.36 (s, 2H), 7.71 (m, 2H), 7.31 (m, 2H); IR (CH_2Cl_2) 1697 cm^{-1}. Anal. calcd for $C_{14}H_6N_4O_2$: C, 64.12; H, 2.31; N, 21.37. Found: C, 63.62; H, 2.50; N, 20.71.

4.2.11. 6,11-Dihydro-2-methyl-pyridazo[2,3-*b*]phenazine-6,11-dione (4b). The general procedure was followed for 25 h with 6-(4-methylphenylamino)-7-chloro-5,8-phthalazinedione **2b** (600 mg, 2.0 mmol), and the concentrated residue was purified by recrystallization with ethanol to give 93 mg (16.3%) of dark brown powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1703 (C=O); ^1H NMR (DMSO-d_6, δ): 9.99 (s, 2H), 8.32 (d, $J=8.4$ Hz, 1H), 8.22 (br s, 1H), 7.99 (dd, $J=8.4$ and 1.6 Hz, 1H), 2.65 (s, 3H). Anal. calcd ($C_{15}H_8N_4O_2$): C, 65.22; H, 2.92; N, 20.28. Found: C, 64.92; H, 2.95; N, 19.84.

4.2.12. 6,11-Dihydro-2-methoxy-pyridazo[2,3-*b*]phenazine-6,11-dione (4c). The general procedure was followed for 26 h with 6-(4-methoxyphenylamino)-7-chloro-5,8-phthalazinedione **2c** (630 mg, 2.0 mmol), and the concentrated residue was purified by recrystallization with ethanol to give 110 mg (19%) of brown powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1692 (C=O); ^1H NMR (DMSO-d_6, δ): 9.97 (s, 2H), 8.31 (d, $J=9.2$ Hz,

1H), 7.75–.82 (m, 2H), 4.04 (s, 3H). Anal. calcd ($C_{15}H_8N_4O_3$): C, 61.65; H, 2.76; N, 19.17. Found: C, 61.31; H, 2.78; N, 18.81.

4.2.13. 6,11-Dihydro-2-ethyl-pyridazo[2,3-*b*]phenazine-6,11-dione (4d). The general procedure was followed for 30 h with 6-(4-ethylphenylamino)-7-chloro-5,8-phthalazinedione **2d** (630 mg, 2.0 mmol), and the concentrated residue was purified by column chromatography (hexane/ethylacetate/methanol, 10:10:1) to give 80 mg (14%) of yellow powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1697 (C=O); ^1H NMR (DMSO-d_6, δ): 10.03 (s, 2H), 8.38 (d, $J=8.8$ Hz, 1H), 8.26 (br s, 1H), 8.08 (br d, $J=8.8$ Hz, 1H), 2.99 (q, $J=7.6$ Hz, 2H), 1.37 (t, $J=7.6$ Hz, 3H). Anal. calcd ($C_{16}H_{10}N_4O_2$): C, 66.20; H, 3.47; N, 19.30. Found: C, 65.87; H, 3.36; N, 18.98.

4.2.14. 6,11-Dihydro-2-ethoxy-pyridazo[2,3-*b*]phenazine-6,11-dione (4e). The general procedure was followed for 20 h with 6-(4-ethoxyphenylamino)-7-chloro-5,8-phthalazinedione **2e** (660 mg, 2.0 mmol), and the concentrated residue was purified by recrystallization with hexane and ethylacetate to give 210 mg (34%) of yellow powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1703 (C=O); ^1H NMR (DMSO-d_6, δ): 10.01 (s, 2H), 8.33 (d, $J=9.2$ Hz, 1H), 7.71–7.95 (m, 2H), 4.37 (q, $J=6.9$ Hz, 2H), 1.47 (t, $J=6.9$ Hz, 3H). Anal. calcd ($C_{16}H_{10}N_4O_3$): C, 62.74; H, 3.29; N, 18.29 Found: C, 63.19; H, 3.26; N, 18.06.

4.2.15. 6,11-Dihydro-*n*-propyl-pyridazo[2,3-*b*]phenazine-6,11-dione (4f). The general procedure was followed for 22 h with 6-(4-*n*-propylphenylamino)-7-chloro-5,8-phthalazinedione **2f** (660 mg, 2.0 mmol), and the concentrated residue was purified by column chromatography (hexane/ethylacetate/methanol, 5:5:1) to give 120 mg (20%) of dark yellow powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1703 (C=O); ^1H NMR (DMSO-d_6, δ): 9.99 (s, 2H), 8.33 (d, $J=8.8$ Hz, 1H), 8.21 (br s, 1H), 8.03 (br d, $J=8.8$ Hz, 1H), 2.90 (t, $J=7.6$ Hz. 2H), 1.75 (m, 2H), 0.94 (t, $J=7.2$ Hz, 3H). Anal. calcd ($C_{17}H_{12}N_4O_2$): C, 67.10; H, 3.97; N, 18.41. Found: C, 67.09; H, 4.06; N, 18.57.

4.2.16. 6,11-Dihydro-2-*n*-butyl-pyridazo[2,3-*b*]phenazine-6,11-dione (4g). The general procedure was followed for 28 h with 6-(4-*n*-butylphenylamino)-7-chloro-5,8-phthalazinedione **2g** (680 mg, 2.0 mmol), and the concentrated residue was purified by column chromatography (hexane/ethylacetate, 1:9) to give 140 mg (22%) of yellow brown powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1699 (C=O); ^1H NMR (DMSO-d_6, δ): 9.99 (s, 2H), 8.33 (d, $J=8.8$ Hz, 1H), 8.21 (br s, 1H), 8.03 (br d, $J=8.8$ Hz, 1H), 2.93 (t, $J=7.6$ Hz, 2H), 1.71 (m, 2H), 1.34 (m, 2H), 0.91 (t, $J=7.2$ Hz, 3H). Anal. calcd ($C_{18}H_{14}N_4O_2$): C, 67.91; H, 4.43; N, 17.60. Found: C, 68.01; H, 4.42; N, 17.61.

4.2.17. General procedure for the preparation of 6-Arylamino-7-chloro-5,8-quinolinediones (7a–f). Arylamine (5.0 mmol) was added to a mixture of 6,7-dichloro-5,8-naphthoquinone (1.14g, 5.0 mmol) and $CeCl_3.7H_2O$ (cerium chloride hepatahydrate) (0.5 g) in ethanol (100 mL) and heated under reflux. The reaction mixture was cooled and then filtered. The filtered precipitation was crystallized from 95% ethanol.

4.2.18. 6-Phenylamino-7-chloro-5,8-quinolinedione (7a). This compound was synthesized as described in the literature.[13] Mp: 204 °C; IR (KBr, cm^{-1}): 1694 (C=O), 3233 (s, NH); ^1H NMR (Acetone-d_6, δ): 9.0 (d, 1H, –CH, C2), 8.5 (br. s, 1H, –NH), 8.4 (d, 1H, –CH, C4), 7.8 (dd, 1H, –CH, C3), 7.4 (t, 2H, phenyl), 7.1 (m, 3H, phenyl).

4.2.19. 6-(3-Methylphenylamino)-7-chloro-5,8-quinolinedione (7b). The general procedure was followed for 5.5 h using 3-methylaniline (0.54 mL, 5.0 mmol), and the filtered precipitate was crystallized from ethanol to give 1.18 g (79%) of purple powder. Mp: 188–189 °C; IR (KBr, cm^{-1}): 1678 (C=O), 3175 (s, NH); ^1H NMR (Acetone-d_6, δ): 9.0 (d, 1H, –CH, C2), 8.5 (br, s, 1H, –NH), 8.4 (d, 1H, –CH, C4), 7.8 (dd, 1H, –CH, C3), 7.2 (m, 1H, phenyl), 7.0 (m, 3H, phenyl), 2.5 (s, 3H, $–CH_3$).

4.2.20. 6-(4-methylphenylamino)-7-chloro-5,8-quinolinedione (7c). This compound was synthesized as described in the lit.[13] Mp: 194–196 °C; IR (KBr, cm^{-1}): 1691 (C=O), 3254 (s, NH); ^1H NMR(Acetone-d_6, δ): 9.0 (d, 1H, –CH, C2), 8.5 (br, s, 1H, –NH), 8.4 (d, 1H, –CH, C4), 7.8 (dd, 1H, –CH, C3), 7.1 (m, 4H, phenyl), 2.4 (s, 3H, $–CH_3$).

4.2.21. 6-(4-Ethylphenylamino)-7-chloro-5,8-quinolinedione (7d). The general procedure was followed for 3 h using *p*-phenetidine (0.62 mL, 5.0 mmol), and the filtered precipitate was crystallized from ethanol to give 1.27g (81%) of orange-colored powder. Mp: 176–178 °C; IR (KBr, cm^{-1}): 1678 (C=O), 3275 (s, NH); ^1H NMR (Acetone-d_6, δ): 9.0 (d, 1H, –CH, C2), 8.5 (br. s, 1H, –NH), 8.4 (d, 1H, –CH, C4), 7.8 (dd, 1H, –CH, C3), 7.2 (m, 4H, phenyl), 2.6 (q, 2H, $–\underline{CH_2}CH_3$), 1.2 (t, 3H, $–CH_2\underline{CH_3}$),

4.2.22. 6-(4-Chlorophenylamino-7-chloro-5,8-quinolinedione (7e). This compound was synthesized as described in the lit.[13] Mp: 223 °C; IR (KBr, cm^{-1}): 1717 (C=O), 3336 (s, NH); ^1H NMR (Acetone-d_6, δ): 9.0 (d, 1H, –CH, C2), 8.6 (br. s, 1H, –NH), 8.4 (d, 1H, –CH, C4), 7.8 (dd, 1H, –CH, C3), 7.4 (m, 2H, phenyl), 7.2 (d, 2H, phenyl).

4.2.23. 6-(4-Bromophenylamino)-7-chloro-5,8-quinolinedione (7f). The general procedure was followed for 5 h using 4-bromoaniline (0.86 g, 5.0 mmol), and the filtered precipitate was crystallized from ethanol to give 1.64 g (90%) of red powder. Mp: 252 °C; IR (KBr, cm^{-1}): 1694 (C=O), 3331 (s, NH); ^1H NMR (Acetone-d_6, δ): 9.0 (d, 1H, –CH, C2), 8.4 (d, 1H, –CH, C4), 7.7 (dd, 1H, –CH, C3), 7.6 (br. s, 1H, –NH), 7.5 (m, 2H, phenyl), 7.0 (d, 2H, phenyl).

4.2.24. General procedure for the preparation of 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-diones (9a–e). A mixture of **7a**–**7f** (5.0 mmol), DMF (150 mL) and sodium azide (0.65 g, 0.01 mol), suspended in a little amount of water, was heated on the steam bath overnight. The

reaction mixture was chilled, the filtered precipitate was extracted with methylene chloride and concentrated, and then the residue was purified by column chromatography.

4.2.25. 6,11-Dihydro-pyrido[2,3-*b*]phenazine-6,11-dione (9a). This compound was synthesized as described in the lit.[13] Mp: >300 °C; IR (KBr, cm^{-1}): 1667 (C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, –CH, C8), 8.8 (d, 1H, –CH, C10), 8.5 (m, 2H, C1, C4), 8.0 (m, 2H, C2, C3) 7.8 (s, 1H, –CH, C9). Anal. calcd for $C_{15}H_7N_3O_2$: C, 68.97; H, 2.70; N, 16.09. Found: C, 68.63; H, 2.60; N, 16.03.

4.2.26. 6,11-Dihydro-2-methyl-pyrido[2,3-*b*]phenazine-6,11-dione (9b). The general procedure was followed for 20 h using **7b** (1.45 g, 5.0 mmol), and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:4) to give 0.29 g (21%) of yellow brown powder. Mp: 267 °C; IR (KBr, cm^{-1}): 1722 (C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, –CH, C8), 8.8 (d, 1H, –CH, C10), 8.3 (d, 1H, –CH, C1), 8.0 (t, 1H, –CH, C2), 7.9 (d, 1H, –CH, C3), 7.8 (dd, 1H, –CH, C9), 3.0 (s, 3H, $–CH_3$). Anal. calcd for $C_{16}H_9N_3O_2$: C, 69.81; H, 3.30; N, 15.27. Found: C, 69.31; H, 3.23; N, 14.95.

4.2.27. 6,11-Dihydro-3-methyl-pyrido[2,3-*b*]phenazine-6,11-dione (9c). This compound was synthesized as described in the literature.[13] Mp: >300 °C; IR (KBr, cm^{-1}): 1696 (–C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, –CH, C8), 8.8 (d, 1H, –CH, C10), 8.4 (d, 1H, –CH, C1), 8.3 (d, 1H, –CH, C4), 7.9 (d, 1H, –CH, C2), 7.8 (dd, 1H, –CH, C9), 2.7 (s, 3H, $–CH_3$). Anal. calcd for $C_{16}H_9N_3O_2$: C, 69.81; H, 3.30; N, 15.27. Found: C, 69.59; H, 3.20; N, 15.02.

4.2.28. 6,11-Dihydro-3-ethyl-pyrido[2,3-*b*]phenazine-6,11-dione (9d). The general procedure was followed for 20 h using **7d** (1.56 g, 5.0 mmol), and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate/MeOH, 10:15:1) to give 0.41 g (28%) of yellow brown powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1701 (C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, –CH, C8), 8.8 (d, 1H, –CH, C10), 8.4 (d, 1H, –CH, C1), 8.3 (s, 1H, –CH, C4), 7.9 (d, 1H, –CH, C2), 7.8 (dd, 1H, –CH, C9), 3.0 (q, 2H, $–\underline{CH_2}CH_3$), 1.4 (t, 2H, $–CH_2\underline{CH_3}$). Anal. calcd for $C_{17}H_{11}N_3O_2$: C, 70.58; H, 3.83; N, 14.53. Found: C, 70.28; H, 3.74; N, 14.56.

4.2.29. 6,11-Dihydro-3-chloro-pyrido[2,3-*b*]phenazine-6,11-dione (9e). This compound was synthesized as described in the lit.[13]

4.2.30. 6,11-Dihydro-3-bromo-pyrido[2,3-*b*]phenazine-6,11-dione (9f). The general procedure was followed for 22 h using of **7f** (1.82 g, 5.0 mmol), and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:5) to give 0.62 g (36%) of yellow brown powder. Mp: >300 °C; IR (KBr, cm^{-1}): 1697 (C=O); ^1H NMR ($CDCl_3$, δ): 9.3 (d, 1H, –CH, C8), 8.9 (d, 1H, –CH, C10), 8.7 (d, 1H, –CH, C4), 8.4 (d, 1H, –CH, C1), 8.1 (d, 1H, –CH, C2), 7.9 (dd, 1H, –CH, C9). Anal. calcd for $C_{15}H_6N_3O_2Br$: C, 52.97; H, 1.78; N, 12.35. Found: C, 52.71; H, 1.53; N, 12.30.

Acknowledgements

The authors wish to thank the Korea Science and Engineering Foundation for the financial support (R04-2000-000-00055-0) and the Korea Research Foundation Grant (KRF-2001-005-F00023).

References and notes

1. Renault, S. G.; Renault, J.; Baron, M.; Servolles, P.; Paoletti, C.; Cros, S. *Eur. J . Med. Chem-Chim. Ther.* **1985**, *20*, 144.
2. Hayashi, T.; Smith, F. T.; Lee, K. H. *J . Med. Chem.* **1987**, *26*, 2005.
3. Pratt, Y. T.; Drake, N. L. *J. Am. Chem. Soc.* **1960**, *82*, 1155.
4. Crosby, A. H.; Lutz, R. E. *J. Am. Chem. Soc.* **1956**, *78*, 1233.
5. Katoh, A.; Ueda, S.; Ohkanda, J.; Hirota, M.; Komine, H.; Mitsuhashi, K. *Heterocycles* **1992**, *34*, 1965.
6. Denny, W. A.; Bagulet, B. C. In *Molecular Aspects of Anticancer Drug-DNA Interactions*, 2nd ed.; Waring, M. J.; Neidle. S., (Eds.), Macmillan: London, 1994; p 270.
7. Pommier, Y.; Capranico, G.; Orr, A.; Kohn, K. W. *Nucleic Acids Rep.* **1991**, *19*, 5973.
8. Fosse, P.; Rene, B.; Le Bret, M. C.; Paoletti, C.; Saucier, J. M. *Nucleic Acids Rep.* **1991**, *19*, 2861.
9. Moore, M. H.; Hunter, W. H.; Kennard, O. J. *J. Mol. Biol.* **1987**, *206*, 693.
10. Pindur, U.; Haber, M.; Sattler, K. *J. Chem. Education* **1993**, *70*, 263.
11. Shaikh, I. A.; Johnson, F.; Grollmann, A. P. *J. Med. Chem.* **1986**, *29*, 1329.
12. Kim, Y. S.; Park, S. Y.; Lee, H. J.; Suh, M. E.; Schollmeyer, D.; Lee, C. O. *Bio. Med. Chem.* **2003**, *11*, 1709.
13. Yanni, A. S. *Indian J. Chem.* **1991**, *30*, 808.
14. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J. B.; Vistica, D. T.; Warren, J. T.; Bokesc, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
15. Rubinstein, L. V.; Shoemaker, R. H.; Paull, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D.; Monks, A.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1113.
16. Kim, J. S.; Shin, K. J.; Kim, D. C.; Kang, Y. K.; Kim, D. J.; Yoo, K. H.; Park, S. W. *Bull. Korean Chem. Soc.* **2002**, *23*, 1425.
17. Suh, M. E.; Shin, S. H. *Yakhak Hoeji* **1997**, *41*, 575.