Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1709–1714

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis and Cytotoxicity of 6,11-Dihydro-pyrido- and 6,11-Dihydro-benzo[2,3-*b*]phenazine-6,11-dione Derivatives

Young-Shin Kim,[a] Se-Young Park,[a] Hyun-Jung Lee,[a] Myung-Eun Suh,[a,*] Dieter Schollmeyer[b] and Chong-Ock Lee[c]

[a]*Division of Medicinal Chemistry, College of Pharmacy, Ewha Woman's University, Seoul 120-750, South Korea*
[b]*Department of Chemistry and Pharmacy, Institute of Organic Chemistry, University of Mainz, Duesbergweg 10-14, 55099 Mainz, Germany*
[c]*Pharmaceutical Screening Division, Korea Research Institute of Chemical Technology, TaeJon 305-606, South Korea*

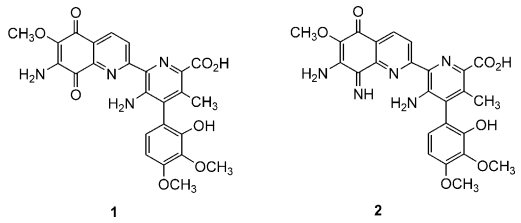
Received 5 November 2002; revised 27 December 2002; accepted 28 December 2002

Abstract—6,11-Dihydro-pyrido[2,3-*b*]phenazine-6,11-diones and 6,11-dihydro-benzo[2,3-*b*]phenazine-6,11-diones were synthesized from 6,7-dichloro-5,8-quinolinedione and 2,3-dichloro-1,4-naphthoquinone. The study on the cytotoxicity on these products revealed that the pyridophenazinediones, tetracyclic heteroquinone analogues with three nitrogen atoms exhibited a high cytotoxicity on several human tumor cell lines. Compound **9c** and **9e** showed in vitro antitumor activity comparable or superior to doxorubicin against the human ovarian tumor cells (SK-OV-3) and the human CNS cells (XF 498). The IC_{50} value for compound **9e** was 0.06 μM against the human CNS cells (XF 498), which was 2.6 times higher than that (0.16 μM) of doxorubicin. In addition, the X-ray crystallographic analysis of two phenazinedione derivatives (**9b**,**c**) showed clearly the exact position of the nucleophilic substitution of 6,7-dichloro-5,8-quinolinedione.

Introduction

Streptonigrin (**1**), isolated from *Streptomyces floccules*, is an alkaloid possessing extraordinary antitumor and antibiotic activities,[1] but its use has been limited because of its great toxicity.[2,3] However, interest in this compound is still increasing because of its ability to degrade DNA and that is an ability common to a number of quinone antibiotics like mitomycin C, actinomycin, rifamycin, etc.[4] Recently, Johnson reported that the 7-amino-6-methoxy-5,8-quinolinedione moiety is responsible for the antitumor activity of streptonigrin (**1**).[5] The methoxy group of quinone ring, the pyridyl group, and its phenyl ring were found not to be essential for the activity of murine tumors. The synthetic analogues without the 7-amino-quinoline moiety, as in azastreptonigrin (**2**), were inactive as antitumor agents (Fig. 1).

The cytotoxic mechanism exhibited by the quinone derivatives has been extensively studied, and the mechanism of action has been proposed that they act as a toposiomerase inhibitor via DNA-intercalation,[8–10] reducing quinone moiety by oxidoreductase.[11–13] The structure of phenazine-6,11-dione (**5a–e**, **9a–e**) has a planar tetracyclic ring and *p*-conjugated ketone groups containing a nitrogen atom which enables hydrogen bonding with DNA. Therefore, the present compounds seem to possess the appropriate properties for intercalation process, as suggested by Moor[14] and Pindur.[15] We have, therefore, concentrated on this essential aminoquinone moiety and have in recent years systematically studied the functionalization of this quinoline-5,8-dion (**6**)[6,7] with several arylamines to give 6-arylamino-7-chloro-quinoline-5,8-dione derivatives (**8a–e**). Also, the final compounds, 6,11-dihydro-pyrido[2,3-*b*]-phenazine-6,11-dione derivatives (**9a–e**) were synthesized



1 2

Figure 1. Streptonigrin (**1**) and azastreptonigrin (**2**).

*Corresponding author. Tel.: +82-2-3277-3040; fax: +82-2-3277-3051; e-mail: suh@ewha.ac.kr

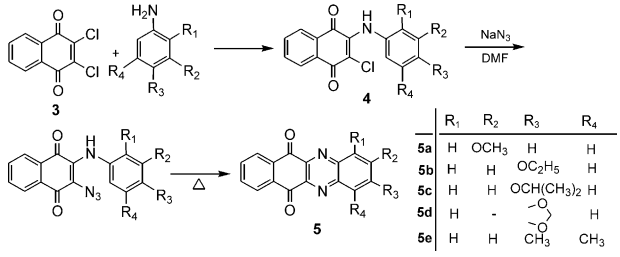
0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0968-0896(03)00028-2

via intramolecular cyclization with sodium azide. We also synthesized the analogue compounds, 6,11-dihydro-benzo[2,3-*b*]phenazine-6,11-dione derivatives (**5a–e**), starting from 1,4-naphtoquinone (**3**) with several arylamines. In addition, X-ray structure analyses of phenazinedione derivatives were carried out. This X-ray crystallographic study showed clearly the structure of 6-arylamino-7-chloro-quinoline-5,8-dione.

Results and Discussion

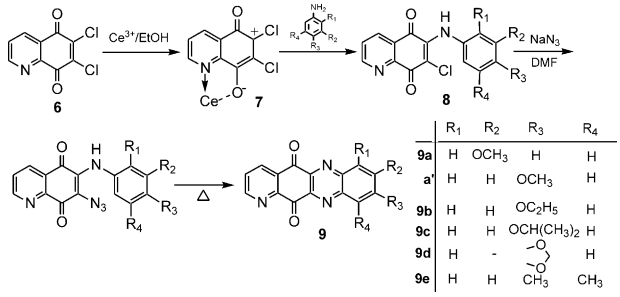
Synthetic chemistry

The 2,3-dichloro-1,4-naphthoquinone (**3**) was reacted with several arylamines in ethanol to give 2-arylamino-3-chloro-1,4-naphthoquinones (**4a–e**). In analogy, 6,7-dichloro-5,8-quinolinedione (**6**), prepared according to the literature,[16] was reacted with several aryl amines, to give 6-arylamino-7-chloro-5,8-quinolinediones (**8a–e**). 6,7-Dichloro-5,8-quinolinedione (**6**) has two asymmetric chlorine atoms at the C6 and C7 positions. When compound **6** reacts with arylamines, it is thought that the C6 and/or C7 position of the compound can be substituted.



	R_1	R_2	R_3	R_4
5a	H	OCH_3	H	H
5b	H	H	OC_2H_5	H
5c	H	H	$OCH(CH_3)_2$	H
5d	H	-	O⌒O	H
5e	H	H	CH_3	CH_3

Scheme 1.



	R_1	R_2	R_3	R_4
9a	H	OCH_3	H	H
a'	H	H	OCH_3	H
9b	H	H	OC_2H_5	H
9c	H	H	$OCH(CH_3)_2$	H
9d	H	-	O⌒O	H
9e	H	H	CH_3	CH_3

Scheme 2.

With the addition of $CeCl_3 \cdot 7H_2O$, 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 (**7**, Scheme 2). The synthesized aryl amino-derivatives (**4a–e** and **8a–e**) were reacted with sodium azide in DMF at 90–100 °C to give 6,11-dihydro-benzo[2,3-*b*]phenazine-6,11-dione derivatives (**5a–e**) and 6,11-dihydro-pyrido[2,3-*b*]phenazine-6,11-dione derivatives (**9a–e**), respectively. The overall reaction strategy is outlined in Schemes 1 and 2.

However, the exact position of substitution in products **8a–e** could not be identified by NMR analysis. This was only possible from an X-ray crystallographic analysis of the subsequence products, namely the phenazinedione derivatives. From the X-ray studies, the exact position of nucleophilic substitution at C6 was clearly identified. Suitable crystals for X-ray analysis of phenazinedione derivatives **9b** and **9c** were obtained through slow evaporation of dichloromethane/petrol ether solution of these compounds. In Table 1, the crystallographic data are summarized for compound **9b** and **9c**. Both structures were solved by direct methods using SIR 92[17] and refined against F^2 by using full matrix refinement (SHELXL 93).[18] Figures 2 and 3 show a view[19] of the molecules with the numbering system. As expected, these compounds were subsequence products of nucleophilic substitution of aryl amines at C6 position of compound **6**.

Cytotoxicity measurement by SRB assay

The IC_{50} values listed in Table 2 support the SAR suggested by Johnson[5] and give further validity to our strategy. Johnson has reported that the number and positions of nitrogen atoms are important for cytotoxicity. Likewise, in our study pyridophenazinediones (**9a–e**) possesing three nitrogen atoms show much better cytotoxicity than benzophenazinediones (**5a–e**) possesing two nitrogen atoms. According to the results, it seems that the activity was dependent on the number of the nitrogen atoms. And the antitumor activities were considerably enhanced, as more heterocyclic rings were annulated to the heteroquinone ring. For example, the ring-closed compounds, phenazine-6,11-dione derivatives (**9a′**, **9b**) that consist of four coplanar annulated heterocyclic rings, showed higher antitumor activity

Table 1. Crystallographic data for compound **9b** and **9c**

Identification code	**9b**	**9c**
Empirical formula	$C_{17}H_{11}N_3O_3$	$C_{18}H_{13}N_3O_3$
Formula weight	305.29	319.32
Crystal system,	$P2_1/c$ (monoclinic)	$P2_1/c$ (monoclinic)
Unit cell dimensions (Å)	a = 3.9362(16)	a = 8.988(2)
	b = 14.9462(8)	b = 15.2084(5)
	c = 23.838(8)	c = 23.835(6)
	β = 93.377(18)	β = 111.874(12)
Packing: V (Å^3)	1400.0(7)	3024(1)
d_{calc} (g cm^{-3})	1.448	1.403
Reflections meas.	2821	6793
Final R indices [I > 2σ (I)]	R1 = 0.0507, wR2 = 0.1250	R1 = 0.0524, wR2 = 0.1426
R indices (all data)	R1 = 0.0837, wR2 = 0.1420	R1 = 0.0867, wR2 = 0.1651

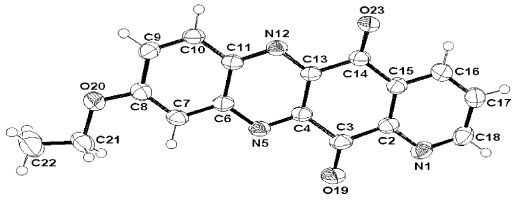


Figure 2. ORTEP drawing of **9b** with an atomic labeling (the numbering scheme shown does not correspond to that of the IUPAC nomenclature).

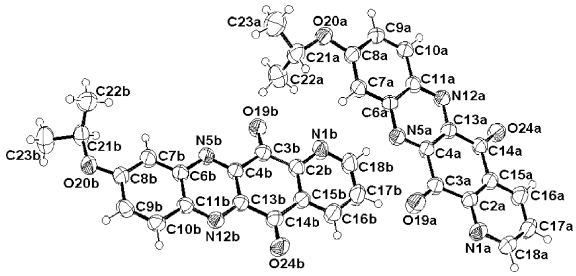


Figure 3. ORTEP drawing of **9c** with an atomic labeling (the numbering scheme shown does not correspond to that of the IUPAC nomenclature).

Table 2. In vitro anticancer activity against human lung tumor cell lines (A 549), human ovarian tumor cell lines (SK-OV-3), human melanoma tumor cell lines (SK-MEL-2), human brain tumor cell lines (XF 498), and human colon tumor cell lines (HCT 15)

Compounds	IC_{50} (μmol)				
	A 549	SK-OV-3	SK-MEL-2	XF 498	HCT 15
Doxorubicin	0.04	0.32	0.05	0.16	0.05
5a	60.11	>100	>100	81.71	>100
5b	3.28	50.73	47.94	19.38	42.39
5c	66.78	>100	>100	52.22	>100
5d	1.64	16.59	16.40	2.26	14.59
5e	10.41	49.67	77.37	6.45	60.67
8a′	2.75	2.37	2.20	2.37	2.23
8b	2.49	2.17	2.15	2.20	1.68
9a	0.24	1.37	0.68	0.30	0.75
9a′	0.24	0.89	0.61	0.51	0.20
9b	0.42	2.09	0.65	0.85	1.01
9c	0.12	0.16	0.21	0.16	0.12
9d	0.19	0.29	0.26	0.09	0.13
9e	0.13	0.20	0.20	0.06	0.03

than 6-arylamino-7-chloro-quinoline-5,8-diones (**8a′**, **8b**). In particular, compound **9c** and **9e** showed in vitro antitumor activity comparable or superior to doxorubicin against the human ovarian tumor cells (SK-OV-3) and the human CNS cells (XF 498). The IC_{50} value for compound **9e** was 0.06 μM against the human CNS cells (XF 498), which was 2.6 times higher than that (0.16 μM) of doxorubicin.

Conclusion

We synthesized a series of phenazinediones (**5a–e**, **9a–e**) which were given by interacting 2-arylamino-3-chloro-naphtho-1,4-diones (**4a–e**) and 6-arylamino-7-chloro-quinoline-5,8-diones (**8a–e**), respectively, with sodium azide. The cytotoxicity of these compounds were tested, in vitro, and were compared with that of doxorubicin. Compound **9c** and **9e** showed cytotoxic activity comparable or superior to doxorubicin against all human tumor cell lines tested. The IC_{50} values of compound **9e** was 0.06 μM against the human CNS cells (XF 498), whereas that of doxorubicin was 0.16 μM. The X-ray crystallographic analysis of phenazinedione derivatives showed clearly the exact position of the nucleophilic substitution of 6,7-dichloro-5,8-quinolinedione by several arylamines.

Experimental

Materials and methods

All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Buechi). 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.

Synthesis

General procedure for the preparation of 2-arylamino-3-chloro-1,4-naphthoquinones (4a–e). Arylamine (0.05 mmol) was added to a solution of 2,3-dichloro-1,4-naphthoquinone (1.135 g, 0.5 mmol) in ethanol (100 mL) and heated under reflux. The reaction mixture was cooled and then filtered. The filtered precipitate was crystallized from 95% ethanol.

2-(3-Methoxyphenylamino)-3-chloro-1,4-naphthoquinone (4a). The general procedure was followed for 3 h using 0.56 mL (0.5 mmol) of 3-methoxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.29 g (82%) of purple powder. Mp: over 144 °C; IR (KBr, cm^{-1}): 1727 (C=O), 3331 (s, NH); ^1H NMR (acetone-d_6, δ): 9.2 (br. s, 1H, −NH), 8.0 (m, 2H, C5 and C8), 7.8 (m, 2H, C6 and C7), 7.2 (t, 1H, phenyl), 6.6 (m, 3H, phenyl), 3.7 (s, 3H, $-OCH_3$).

2-(4-Ethoxyphenylamino)-3-chloro-1,4-naphthoquinone (4b). The general procedure was followed for 3 h using 0.65 mL (0.5 mmol) of 4-ethoxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.47 g (90%) of red-brown powder. Mp: 172 °C; IR (KBr, cm^{-1}): 1716 (C=O), 3297 (s, NH); ^1H NMR ($CDCl_3$, δ): 8.1 (m, 2H, C5 and C8), 7.7 (m, 2H, C6 and C7), 7.6 (br, s, 1H, −NH), 7.0 (m, 2H, phenyl, 6.8 (m, 2H, phenyl), 4.0 (q, 2H, $-O\underline{CH_2}CH_3$), 1.4 (t, 3H, $-OCH_2\underline{CH_3}$).

2-(4-Isopropoxyphenylamino)-3-chloro-1,4-naphthoquinone (4c). The general procedure was followed for 2.5 h using 0.73 mL (0.5 mmol) of 4-isopropoxyaniline, and the filtered precipitate was crystallized from ethanol to give

1.35 g (79%) of red-brown powder. Mp: 168–171 °C; IR (KBr, cm^{-1}): 1702 (C=O), 3297 (s, NH); ^1H NMR ($CDCl_3$, δ): 8.1 (m, 2H, C5 and C8), 7.7 (m, 2H, C6 and C7), 7.6 (br, s, 1H, −NH), 7.0 (m, 2H, phenyl), 6.8 (m, 2H, phenyl), 4.6 (m, 1H, −CH(CH_3)$_2$), 1.4 (s, 6H, −OCH(CH_3)$_2$).

2-(3,4-Methylenedioxyphenylamino)-3-chloro-1,4-naphthoquinone (4d). The general procedure was followed for 6 h using 0.69 g (0.5 mmol) of 3,4-methylenedioxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.25 g (76%) of purple powder. Mp: 238–240 °C; IR (KBr, cm^{-1}): 1715 (C=O), 3304 (s, NH); ^1H NMR ($CDCl_3$, δ): 8.1 (m, 2H, C5 and C8), 7.7 (m, 2H, C6 and C7), 7.5 (br, s, 1H, −NH), 6.8 (d, 1H, phenyl), 6.6 (m, 2H, phenyl), 6.0 (s, 2H, −CH_2).

2-(3,4-Dimethylphenylamino)-3-chloro-1,4-naphthoquinone (4e). The general procedure was followed for 5 h using 0.61 g (0.5 mmol) of 3,4-dimethylaniline, and the filtered precipitate was crystallized from ethanol to give 1.33 g (85%) of red-brown powder. Mp: 177–180 °C; IR (KBr, cm^{-1}): 1724 (C=O), 3335 (s, NH); ^1H NMR ($CDCl_3$, δ): 8.1 (m, 2H, C5 and C8), 7.7 (m, 2H, C6 and C7), 7.6 (br, s, 1H, −NH), 7.1 (d, 1H, −CH, C5), 6.9 (s, 1H, phenyl), 6.8 (d, 1H, phenyl), 2.3 (s, 6H, 2×CH_3).

General procedure for the preparation of 6,11-dihydrobenzo[2,3-*b*]phenazine-6,11-diones (5a–e). A mixture of 0.5 mmol of **4a**–**e**, 50 mL of DMF and 0.65 g (0.01 mol) of sodium azide, 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.

6,11-Dihydro-2-methoxy-benzo[2,3-*b*]phenazine-6,11-dione (5a). The general procedure was followed for 20 h using 1.57 g (0.5 mmol) of **4a**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:4) to give 0.59 g (41%) of yellow-brown powder. Mp: 330–331 °C; IR (KBr, cm^{-1}): 1705 (C=O); ^1H NMR ($CDCl_3$, δ): 8.5 (m, 2H, C7 and C10), 8.3 (d, 1H, −CH, C4), 7.9 (m, 2H, C8 and C9), 7.7 (s, 1H, −CH, C1), 7.6 (d, 1H, −CH, C3), 4.0 (s, 3H, −OCH_3). Anal. calcd for $C_{17}H_{10}N_2O_3$: C, 70.34; H, 3.47; N, 9.65. Found: C, 70.30; H, 3.64; N, 9.63.

6,11-Dihydro-3-ethoxy-benzo[2,3-*b*]phenazine-6,11-dione (5b). The general procedure was followed for 20 h using 1.64 g (0.5 mmol) of **4b**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:3) to give 0.57 g (40%) of yellow-brown powder. Mp: 254–255 °C; IR (KBr, cm^{-1}): 1701 (C=O); ^1H NMR ($CDCl_3$, δ): 8.5 (m, 2H, C7 and C10), 8.3 (d, 1H, −CH, C1), 7.9 (m, 2H, C8 and C9), 7.7 (s, 1H, −CH, C4), 7.6 (d, 1H, −CH, C2), 4.3 (q, 2H, −O$CH_2$$CH_3$), 1.6 (t, 3H, −$OCH_2$$CH_3$). Anal. calcd for $C_{18}H_{12}N_2O_3$: C, 71.05; H, 3.97; N, 9.21. Found: C, 70.65; H, 4.05; N, 9.22.

6,11-Dihydro-3-isopropoxy-benzo[2,3-*b*]phenazine-6,11-dione (5c). The general procedure was followed for 22 h using 1.71 g (0.5 mmol) of **4c**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:5) to give 0.34 g (22%) of yellow-brown powder. Mp: 242–244 °C; IR (KBr, cm^{-1}): 1670 (C=O); ^1H NMR ($CDCl_3$, δ): 8.5 (m, 2H, C7 and C10), 8.3 (d, 1H, −CH, C1), 7.9 (m, 2H, C8 and C9), 7.7 (s, 1H, −CH, C4), 7.6 (d, 1H, −CH, C2), 4.8 (m, 1H, −OCH(CH_3)$_2$), 1.5 (d, 6H, −OCH(CH_3)$_2$). Anal. calcd for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.19; H, 4.69; N, 8.97.

6,11-Dihydro-2,3-methylenedioxy-benzo[2,3-*b*]phenazine-6,11-dione (5d). The general procedure was followed for 20 h using 1.64 g (0.5 mmol) of **4d**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:4) to give 0.28 g (19%) of yellow-brown powder. Mp: 306–309 °C; IR (KBr, cm^{-1}): 1657 (C=O); ^1H NMR (acetone-d_6, δ): 8.4 (m, 2H, C8 and C11), 8.0 (m, 2H, C9 and C10), 7.6 (s, 2H, C1 and C5), 6.5 (s, 2H, −CH_2, C3). Anal. calcd for $C_{17}H_8N_2O_4$: C, 67.11; H, 2.65; N, 9.21. Found: C, 66.77; H, 2.77; N, 9.29.

6,11-Dihydro-2,3-dimethyl-benzo[2,3-*b*]phenazine-6,11-dione (5e). The general procedure was followed for 18 h using 1.44 g (0.5 mmol) of **4e**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:5) to give 0.56 g (39%) of yellow-brown powder. Mp: 298–299 °C; IR (KBr, cm^{-1}): 1670 (C=O); ^1H NMR ($CDCl_3$, δ): 8.5 (m, 2H, C7 and C10), 8.2 (d, 1H, −CH, C4), 7.9 (m, 2H, C8 and C9), 7.8 (d, 1H, −CH, C1), 3.0 (s, 3H, −CH_3, C3), 2.6 (s, 3H, −CH_3, C4). Anal. calcd for $C_{18}H_{12}N_2O_2$: C, 74.99; H, 4.20; N, 9.72. Found: C, 74.62; H, 4.11; N, 9.62.

General procedure for the preparation of 6-arylamino-7-chloro-5,8-quinolinediones (8a–e). Arylamine (0.05 mmol) was added to a mixture of 6,7-dichloro-5,8-naphthoquinone (1.14 g, 0.5 mmol) and $CeCl_3 \cdot 7H_2O$ (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.

6-(3-Methoxyphenylamino)-7-chloro-5,8-quinolinedione (8a). The general procedure was followed for 8 h using 0.56 mL (0.5 mmol) of 3-methoxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.29 g (82%) of dark purple powder. Mp: 152–154 °C; IR (KBr, cm^{-1}): 1679 (C=O), 3284 (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 (t, 1H, phenyl), 6.7 (m, 3H, phenyl), 3.8 (s, 3H, −OCH_3).

6-(4-Ethoxyphenylamino)-7-chloro-5,8-quinolinedione (8b). The general procedure was followed for 4 h using 0.65 mL (0.5 mmol) of 4-ethoxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.50 g (91%) of dark brown powder. Mp: 197–199 °C; IR (KBr, cm^{-1}): 1713 (C=O), 3370 (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, 2H, phenyl), 6.9 (m, 2H, phenyl), 4.1 (q, 2H, −O$CH_2$$CH_3$), 1.4 (t, 3H, −$OCH_2$$CH_3$).

6-(4-Isopropoxyphenylamino)-7-chloro-5,8-quinolinedione (8c). The general procedure was followed for 6 h using 0.73 mL (0.5 mmol) of 4-isopropoxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.27g (74%) of dark brown powder. Mp: 182 °C; IR (KBr, cm^{-1}): 1693 (C=O), 3360 (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, 2H, phenyl), 6.9 (m, 2H, phenyl), 4.6 (m, 1H, $-O\underline{CH}(CH_3)_2$), 1.3 (s, 6H, $-OCH(\underline{CH_3})_2$).

6-(3,4-Methylenedioxyphenylamino)-7-chloro-5,8-quinolinedione (8d). The general procedure was followed for 8 h using 0.69 g (0.5 mmol) of 3,4-methylenedioxyaniline, and the filtered precipitate was crystallized from ethanol to give 1.02 g (62%) of dark brown powder. Mp: 190 °C; IR (KBr, cm^{-1}): 1625 (C=O), 3132 (s, NH); ^1H NMR (acetone-d_6, δ): 9,2 (br.s, 1H, −NH), 9.0 (d, 1H, −CH, C2), 8.4 (d, 1H, −CH, C4), 7.8 (dd, 1H, −CH, C3), 6.6 (m, 3H, phenyl), 6.0 (s, 2H, $-CH_2$).

6-(3,4-Dimethylphenylamino-7-chloro-5,8-quinolinedione (8e). The general procedure was followed for 8 h using 0.61 g (0.5 mmol) of 3,4-dimethylaniline, and the filtered precipitate was crystallized from ethanol to give 1.33 g (85%) of orange powder. Mp: 186–187 °C; IR (KBr, cm^{-1}): 1694 (C=O), 3182 (s, NH); ^1H NMR ($CDCl_3$, δ): 9.0 (d, 1H, −CH, C2), 8.4 (d, 1H, −CH, C4), 7.6 (dd, 1H, −CH, C3), 7.6 (br. s, 1H, −NH), 7.1 (d, 1H, phenyl), 6.9 (s, 1H, phenyl), 6.8 (d, 1H, phenyl), 2.3 (d, 6H, $2\times CH_3$).

General procedure for the preparation of 6,11-dihydropyrido[2,3-*b*]phenazine-6,11-diones (9a–e). A mixture of 0.5 mmol of **8a**–**e**, 50 mL of DMF and 0.65 g (0.01 mol) of sodium azide, 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.

6,11-Dihydro-2-methoxy-pyrido[2,3-*b*]phenazine-6,11-dione (9a). The general procedure was followed for 20 h using 1.57 g (0.5 mmol) of **8a**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:5) to give 0.35 g (24%) of yellow-brown powder. Mp: 337 °C; IR (KBr, cm^{-1}): 1710 (C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, −CH, C8), 8.8 (d, 1H, −CH, C10), 8.4 (d, 1H, −CH, C4), 7.9 (dd, 1H, −CH, C9), 7.8 (s, 1H, −CH, C1), 7.7 (d, 1H, −CH, C3), 4.1 (s, 3H, $-OCH_3$). Anal. calcd for $C_{16}H_9N_3O_3$: C, 65.98; H, 3.11; N, 14.43. Found: C, 65.51; H, 2.74; N, 14.34.

6,11-Dihydro-3-methoxy-pyrido[2,3-*b*]phenazine-6,11-dione (9a′). This compound was synthesized as described in the literature.[20]

6,11-Dihydro-3-ethoxy-pyrido[2,3-*b*]phenazine-6,11-dione (9b). The general procedure was followed for 18 h using 1.64 g (0.5 mmol) of **8b**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:5) to give 0.64 g (42%) of yellow-brown powder. Mp: 333 °C; IR (KBr, cm^{-1}): 1682 (C=O); ^1H NMR ($CDCl_3$, δ): 9.1 (d, 1H, –CH, C8), 8.8 (d, 1H, −CH, C10), 8.3 (d, 1H, −CH, C1), 7.8 (dd, 1H, −CH, C9), 7.7 (s, 1H, −CH, C4), 7.6 (d, 1H, −CH, C2), 4.2 (q, 2H, $-O\underline{CH_2}CH_3$), 1.5 (t, 3H, $-OCH_2\underline{CH_3}$). Anal. calcd for $C_{17}H_{11}N_3O_3$: C, 66.88; H, 3.63; N, 13.76. Found: C, 67.04; H, 3.63; N, 13.81.

6,11-Dihydro-3-isopropoxy-pyrido[2,3-*b*]phenazine-6,11-dione (9c). The general procedure was followed for 18 h using 1.71 g (0.5 mmol) of **8c**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:5) to give 0.77 g (48%) of yellow-brown powder. Mp: 316–318 °C; IR (KBr, cm^{-1}): 1674 (−C=O); ^1H NMR (DMSO-d_6, δ): 9.1 (d, 1H, −CH, C8), 8.7 (d, 1H, −CH, C10), 8.3 (d, 1H, −CH, C1), 7.9 (dd, 1H, −CH, C9), 7.8 (s, 1H, −CH, C4), 7.7 (d, 1H, −CH, C2), 5.1 (m, 1H, $-O\underline{CH}(CH_3)_2$), 1.4 (s, 6H, $-OCH(\underline{CH_3})_2$). Anal. calcd for $C_{18}H_{13}N_3O_3$: C, 67.71; H, 4.10; N, 13.16. Found: C, 67.55; H, 4.33; N, 13.26.

6,11-Dihydro-2,3-methylenedioxy-pyrido[2,3-*b*]phenazine-6,11-dione (9d). The general procedure was followed for 20 h using 1.64 g (0.5 mmol) of **8d**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate/MeOH, 10:10:1) to give 0.28 g (18%) of yellow-brown powder. Mp: 280–281 °C; IR (KBr, cm^{-1}): 1670 (C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, −CH, C9), 8.8 (d, 1H, −CH, C11), 7.8 (dd, 1H, −CH, C10), 7.7 (s, 2H, C1 and C5), 6.4 (s, 2H, $-CH_2$, C3).

6,11-Dihydro-3,4-dimethyl-pyrido[2,3-*b*]phenazine-6,11-dione (9e). The general procedure was followed for 20 h using 1.56 g (0.5 mmol) of **8e**, and the concentrated residue was purified by column chromatography (*n*-hexane/ethyl acetate, 1:3) to give 0.39 g (27%) of yellow-brown powder. Mp: 282 °C; IR (KBr, cm^{-1}): 1710 (C=O); ^1H NMR ($CDCl_3$, δ): 9.2 (d, 1H, −CH, C8), 8.8 (d, 1H, −CH, C10), 8.2 (d, 1H, −CH, C1), 7.9 (d, 1H, −CH, C2), 7.8 (dd, 1H, −CH, C9), 3.0 (s, 3H, $-CH_3$), 2.6 (s, 3H, $-CH_3$). Anal. calcd for $C_{17}H_{11}N_3O_2$: C, 70.58; H, 3.83; N, 14.53. Found: C, 70.19; H, 3.82; N, 14.51.

Measurement of antitumor activity

Human cancer cell lines of the lung (A 549), the ovarian (SK-OV-3), the melanoma (SK-MEL-2), the brain (XF 498) and the colon (HCT 15) were used for cytotoxicity test in vitro using SRB (Sulforhodamine B) assay.[21,22] They were maintained as stocks in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco). Cell cultures were passaged once or twice weekly by using trypsin–EDTA to detach the cells from their culture flasks. The rapidely growing cells harvested, counted, and incubated at the appropriate concentration ($1-2\times10^4$ cells/well) in 96-well μ plates. After incubation for 24 h, the compounds dissolved in culture medium were applied to the culture wells in triplicate and incubated for 48 h at 37 °C under 5% CO_2 atmosphere. The cultures were fixed with cold TCA and stained with 0.4% SRB dissolved in 1% acetic acid. After solubilizing the bound stain with 100 μL of unbuffered tris base solution (pH 10.5) using gyratory shaker, the absorbance at 520 nm was measured with a

microplated reader. Cytotoxic activity was evaluated by measuring the concentration of a compound which was required to inhibit the protein synthesis by 50% (ED_{50}) as comparison with that of doxorubicine.

Supplementary material

Crystallographic date (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publications nos. CCDC 196625 (compound **9C**) and 1996626 (compound **9b**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

Acknowledgements

The authors wish to thank the Korea Science and Engineering Foundation for the financial support (R04-2000-000-00055-0).

References and Notes

1. Rao, K. V.; Cullen, W. P. *Antibiot. Ann.* **1960**, *1959–1960*, 950.
2. Ebert, P. S.; Chirigos, M. A.; Ellsworth, P. A. *Cancer Res.* **1968**, *28*, 363.
3. Kaung, D. T.; Whittington, R. M.; Spencer, H. H.; Patno, M. E. *Cancer* **1969**, *23*, 597.
4. Lown, J. W. In *The Molecular Mechanism of Antitumor Action of the Mitomycins in Mitomycin C, Current Status and New Developments*; Carter, S. T.; Crooke, S. T., Eds.; Academic: Orlando, FL, 1979; pp 5–26.
5. Shaikh, I. A.; Johnson, F.; Grollmann, A. P. *J. Med. Chem.* **1986**, *29*, 1329.
6. Suh, M. E.; Kang, M. J.; Yoo, H. W.; Park, S. Y.; Lee, C. O. *Bioorg. Med. Chem.* **2000**, *8*, 2079.
7. Suh, M. E.; Park, S. Y.; Lee, C. O. *Bioorg. Med. Chem.* **2001**, *9*, 2979.
8. 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; pp 270–311.
9. Pommier, Y.; Capranico, G.; Orr, A.; Kohn, K. W. *Nucleic Acids Rep.* **1991**, *19*, 5973.
10. Fosse, P.; Rene, B.; Le Bret, M. C.; Paoletti, C.; Saucier, J. M. *Nucleic Acids Rep.* **1991**, *19*, 2861.
11. Lin, A. J.; Lillis, B. J.; Sartorelli, A. C. *J. Med. Chem.* **1975**, *18*, 917.
12. Yamashita, Y.; Kawada, S.; Fujii, N.; Nakano, H. *Cancer Res.* **1990**, *50*, 5841.
13. Beall, H. D.; Siegel, D.; Liu, Y.; Bolton, E. M.; Gibson, N. W.; Ross, D. *Biochem. Pharmacol.* **1996**, *51*, 645.
14. Moore, M. H.; Hunter, W. H.; Kennard, O. J. *J. Mol. Biol.* **1987**, *206*, 693.
15. Pindur, U.; Haber, M.; Sattler, K. *J. Chem. Educ.* **1993**, *70*, 263.
16. Suh, M. E.; Shin, S. H. *Yakhak Hoeji* **1997**, *41* (5), 575.
17. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Cryst. Part 3* **1994**, *27*, 435.
18. Sheldrick, G. M. *Program for Crystal Structure Refinement*; University of Goettingen, Germany, 1993.
19. Keller, E. *A computer program for the graphic representation of molecular and crystallographic models*; University of Freiburg, Germany, 1992.
20. Yanni, A. S.; Indian, J. *Chem., Sec. B* **1991**, *30B* (8), 808.
21. 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.
22. 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.