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Synthesis and cytotoxicity of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives

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Abstract—A series of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives **8** was synthesized from 6,7-dichlorophthalazine-5,8-dione **5** and evaluated for in vitro cytotoxicity against several human tumor cell lines. Most of the tested compounds showed potential cytotoxic activity considerably higher than that of the reference compounds, ellipticine and doxorubicin.

1. Introduction

DNA intercalating agents bearing heterocyclic quinones are well known as antiproliferative and anticancer agents, including streptonigrin.[1–3] The antitumor activity of the quinone moiety has been thoroughly studied and it is known that they act as topoisomerase inhibitors via the DNA intercalation and the reduction of the quinone moiety by quinone oxidoreductase. According to Moore and Pindure,[4,5] a DNA-intercalating molecule must have three to four coplanar rings with a length of 3–4 Å and a width of 6–8 Å. The molecule must also have a *para*-conjugated quinone ring containing nitrogen atoms, as this enables hydrogen bonding with DNA.

Studies on the structure–activity relationship of heterocyclic quinones containing nitrogen showed that the number and position of nitrogen are very important for the cytotoxicity. When Johnson and co-workers examined the antitumor activity of 2,3-dichloro-1,4-naphthoquinone **1**, 6,7-dichloroquinoline-5,8-dione **2**, 6,7-dichloroisoquinoline-5,8-dione **3**, 6,7-dichloroquinazoline-5,8-dione **4** and 6,7-dichlorophthalazine-5,8-dione **5**, compound **5** exhibited the highest activity[6] (Fig. 1).

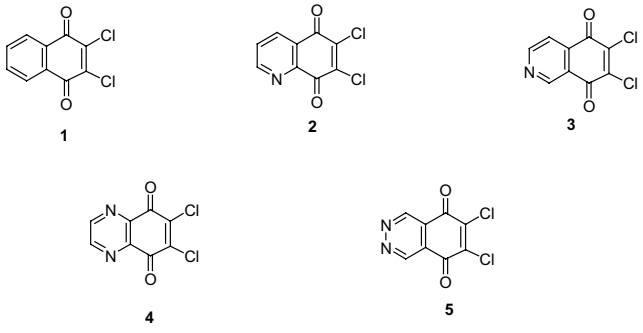

Figure 1.

In the previous papers,[7,8] we reported the antitumor activity of nitrogen-containing heterocyclic quinones; 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]quinoxaline-4,9-dione derivatives **6** and 3-substituted 2-methyl-3*H*-imidazo[4,5-*g*]quinoline-4,9-dione derivatives **7** (Fig. 2).

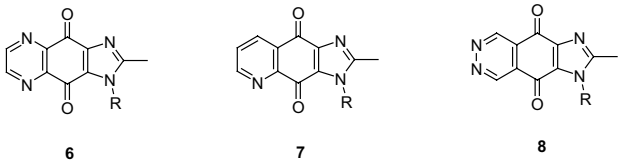

Figure 2.

Keywords: Imidazophthalazine; Cytotoxicity.
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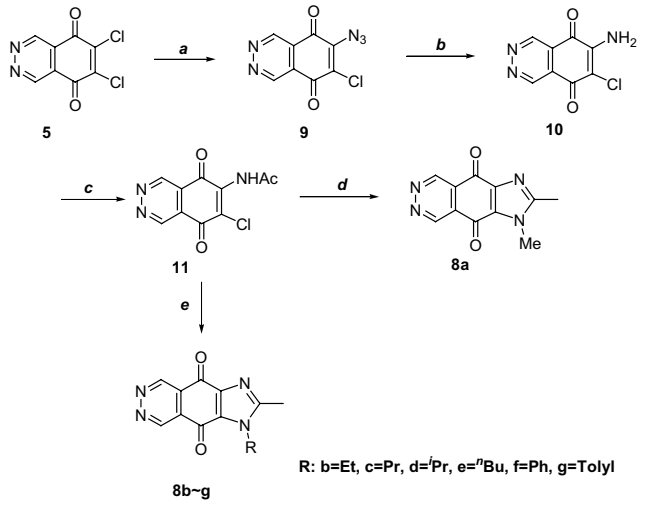
In our continuing development of novel DNA intercalators based on nitrogen-containing heterocyclic quinones, we envisioned that 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives **8** (Fig. 2) would be a good DNA intercalator. Now, we wish to disclose the synthesis and antitumor activities of imidazophthalazinedione derivatives.

1.1. Results and discussion

1.1.1. Chemistry. The synthetic route for the 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-diones **8** illustrated in Scheme 1 was a slight modification of the previously reported method.[7] The 6,7-dichlorophthalazine-5,8-dione **5**, a starting compound in the synthesis of **8**, was obtained from phthalazine.[9] Treatment of 6,7-dichlorophthalazine-5,8-dione **5** with sodium azide in AcOH at room temperature yielded the 6-azido-7-chlorophthalazine-5,8-dione **9**, which was converted into 6-amino derivative **10** by reducing of the azide group with sodium borohydride in EtOH. The amino group of 6-amino-7-chlorophthalazine-5,8-dione **10** was acetylated with acetic anhydride in the presence of H_2SO_4 to give 6-acetamido-7-chlorophthalazine-5,8-dione **11**. The required 1,2-dimethyl-1*H*-imidazo[4,5-*g*]-phthalazine-4,9-dione **8a** was prepared directly by the reaction of **11** with methylamine in ethanol. However, the preparation of 1-ethyl-2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione **8b** under this condition was not successful. When **11** was treated with ethylamine in ethanol, TLC indicated the presence of several inseparable compounds. Thus, another condition was tried; **11** was first treated with ethylamine in THF to give 6-acetamido-7-ethylaminophthalazine-5,8-dione, which was then cyclized with 2 N NaOH in ethanol, giving **8b**. Compounds **8c**–**g** were also prepared using the same reaction conditions with different amines. These alkyl and aryl groups were introduced to mimic streptonigrin.



Scheme 1. Reagents: (a) NaN_3/AcOH (100%); (b) $NaBH_4$/EtOH (45%); (c) concd H_2SO_4/Ac_2O (89%); (d) methyl amine/EtOH (30%); (e) 1. Corresponding amines/THF, 2. 2N NaOH/EtOH (5–30%).

1.1.2. Cytotoxicity by SRB assay. The in vitro cytotoxic activities were evaluated by SRB assay method. The following human tumor cell lines were used; A549 (lung cancer), Col2 (colon carcinoma), SNU-638 (stomach carcinoma), HT1080 (fibrosarcoma), HL-60 (myeloid leukemia).

The cell growth inhibitory potential was determined as described by other researchers.[10] Briefly, cells (5×10^4 cells/mL) were treated with various concentrations of compound for 3 days. After treatment, the cells were fixed with trichloroacetic acid and viability was determined with a SRB (sulforhodamine B) protein staining method. The results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using nonlinear regression analysis (percent survival concentration). The IC_{50} values evaluated were compared with those of ellipticine (Table 1).

All the synthesized compounds showed more potent cytotoxicity than ellipticine, clinically used agent for the treatment of solid tumor. Especially compound **8e** showed significantly increased activity in all cell lines tested. The IC_{50} value of **8e** was 0.001 μM against the human stomach cancer cell line (SNU 638), which was 2200 times more potent than that (2.20 μM) of ellipticine. The cytotoxicity of **8e** against other cell lines was also much higher than that of ellipticine. Apparently, the introduction of aryl groups at the N-1 position (*R*) seemed to reduce the cytotoxicity compared to alkyl groups. For example, the compound having phenyl (**8f**) was 30–100 times less active than that of **8e**.

To compare the activity of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-diones **8** and 3-substituted 2-methyl-3*H*-imidazo[4,5-*g*]quinoline-4,9-diones **7**, three compounds with methyl (**8a**), *i*-propyl (**8d**) and phenyl (**8f**) substitution were evaluated for cytotoxic activity using SK-OV-3 (ovarian cancer), SK-MEL-2 (melanoma cancer), XF 498 (CNS), and HCT 15 (colon cancer).

Table 1. Cytotoxicity (IC_{50}) of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives

Compound	IC_{50} (μM)				
	A549	Col2	SNU-638	HT1080	HL-60
Ellipticine	1.88	2.30	2.20	5.80	4.30
8a	0.07	1.03	0.07	0.07	0.23
8b	0.07	0.11	0.07	0.07	0.10
8c	0.09	0.11	0.04	0.04	0.03
8d	0.02	0.09	0.08	0.09	0.08
8e	0.01	0.06	0.001	0.01	0.05
8f	1.07	6.27	0.11	0.39	1.67
8g	0.11	2.76	0.77	0.13	0.80

A549: Human lung cancer cell line.
Col2: Human colon cancer cell line.
SNU-638: Human stomach cancer cell line.
HT1080: Human fibrosarcoma cancer cell line.
HL-60: Human myeloid leukemic cell line.

Table 2. Cytotoxicity (IC_{50}) of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives

Compound	*R*	IC_{50} (μM)			
		SK-OV-3	SK-MEL-2	XF 498	HCT 15
Doxorubicin		0.39	0.20	0.34	0.35
8a	Me	0.04	0.04	0.01	0.34
8d	^{i}Pr	0.03	0.02	0.003	0.03
8g	Ph	0.19	0.02	0.01	0.06

SK-OV-3: Human ovarian cancer cell line.
SK-MEL-2: Human melanoma cancer cell line.
XF 498: Human CNS cancer cell line.
HCT 15: Human colon cancer cell line.

Data in Table 2 showed that compounds **8** are highly cytotoxic on all the tested tumor cell lines. Especially these coplanar tricyclic quinones with four nitrogen atoms exhibited potent activities against A549 and XF 498. All three compounds (**8a**, **8d**, **8f**) were more active than doxorubicin clinically used for the treatment of solid tumor. These compounds showed 2–400 times higher activity than doxorubicin in the all tested cancer cell lines. In contrast, the previous results performed on compounds in **7** series showed 3–30 times lower activity than doxolubicin.[8] Combined results suggest compounds **8** showed superior activity.

1.2. Conclusion

A series of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione derivatives **8** were synthesized from 6,7-dichlorophthalazine-5,8-dione **5**. The cytotoxicity of these compounds was tested in vitro against various human tumor cell lines. All synthesized compounds showed superior cytotoxic effect in comparison with ellipticine and doxorubicin.

2. Experimental

2.1. Materials and methods

All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Buchi). The IR spectra were recorded on a FT-Infrared spectrometer (Bio-Rad. Co., USA) using KBr pellet. ^{1}H NMR spectra were recorded on a Bruker Avance 300 and a 400 MHz Varian FT-NMR spectrometer using trimethylsilane as an internal standard. Samples were dissolved in $CDCl_3$, CD_3OD or DMSO-d_6. Mass spectra were obtained on a Tandem Mass spectrometer JMS-HX110/110A (Jeol). Elemental analyses were performed using Thermo Quest (CE Instruments) EA 1110.

2.1.1. 6-Azido-7-chlorophthalazine-5,8-dione (9). Sodium azide (7.11 g, 109 mmol) was added to a solution of **5** (8.35 g, 36.5 mmol) in AcOH (200 mL) at rt, and then the reaction mixture was stirred for 1.5 h. Water was poured to the reaction mixture and the precipitate was collected by filtration. The filtrate was washed again with water, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The residue was combined with above obtained precipitate to give **9** (8.58 g, 100%) as a purple solid: mp 135–136 °C (decomposed); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.93 (d, $J = 1.0$ Hz, 1H), 9.86 (d, $J = 1.0$ Hz, 1H); IR (CH_2Cl_2) 2105, 1659, 1547 cm^{-1}; HR-FABMS Calcd for $C_8H_3O_2N_5Cl$ (M^++H): 235.9975. Found: 235.9974.

2.1.2. 6-Amino-7-chlorophthalazine-5,8-dione (10). To a solution of 6-azido-7-chlorophthalazine-5,8-dione **9** (6.73 g, 28.6 mmol) in EtOH (150 mL) was added $NaBH_4$ (1.19 g, 31.4 mmol) at 0 °C. The reaction mixture was stirred for 2 h at room temperature, quenched by addition of saturated aq NH_4Cl. The solvent was evaporated under reduced pressure, and the residue was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was washed with Et_2O and cold EtOH to give **10** (2.69 g, 45%) as a purple solid: mp > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (d, $J = 1.2$ Hz, 1H), 9.63 (d, $J = 1.2$ Hz, 1H), 8.11 (br s, 1H), 7.94 (br s, 1H); IR (CH_2Cl_2) 3426, 3278, 1611 cm^{-1}; HR-FABMS Calcd for $C_8H_5O_2N_3Cl$ (M^++H): 210.0070. Found: 210.0068.

2.1.3. 6-Acetamido-7-chlorophthalazine-5,8-dione (11). The solution of **10** (2.50 g, 11.9 mmol) in Ac_2O (150 mL) was stirred for 2 h at 0 °C in the presence of concd H_2SO_4 (0.50 mL). The reaction mixture was quenched by addition of 25% aq NaOAc, and extracted with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized with MeOH to give **11** (2.67 g, 89%) as a purple solid: mp 200–201 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 9.79 (s, 1H), 9.78 (s, 1H), 2.25 (s, 3H); IR (CH_2Cl_2) 3391, 1703, 1677, 1525 cm^{-1}. HR-FABMS Calcd for $C_{10}H_7O_3N_3Cl$ (M^++H): 252.0176. Found: 252.0175.

2.1.4. 1,2-Dimethyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8a). Methylamine (2.0 M solution in THF; 1.30 mL, 2.62 mmol) was added to a solution of **11** (300 mg, 1.19 mmol) in EtOH (4.00 mL) at 70 °C. The reaction mixture was heated to refluxed for 30 min, and cooled. The solvent was evaporated off, and obtained residue was purified by column chromatography (EtOAc:MeOH = 10:1) gave **8a** (81.0 mg, 30%) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.93 (d, $J = 1.0$ Hz, 1H), 9.87 (d, $J = 1.0$ Hz, 1H), 4.07 (s, 3H), 2.63 (s, 3H); IR (CH_2Cl_2) 1662, 1519 cm^{-1}; Anal. Calcd for $C_{11}H_8N_4O_2$: C, 57.89; H, 3.53; N, 24.55. Found: C, 58.08; H, 3.47; N, 24.27.

2.1.5. General procedure for the preparation of 1-substituted 2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8b–g). Corresponding amine (2.62 mmol) was added to a solution of **11** (300 mg, 1.19 mmol) in THF (0.3 M)

at 70 °C. The reaction mixture was refluxed for 30 min, and cooled. The solvent was evaporated off. 2 N NaOH was added to a suspension of the residue in EtOH at 70 °C, and the reaction mixture was refluxed for 30 min. The reaction mixture was cooled, and 2 N HCl added at 0 °C. The precipitate was collected by filtration, and purified by column chromatography (EtOAc:MeOH = 10:1) to give **8b–g**.

2.1.6. 1-Ethyl-2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8b). Compound **8b** (27.0 mg, 9%) was obtained from **11** (300 mg, 1.19 mmol) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.24 (d, $J = 0.8$ Hz, 1H), 9.92 (d, $J = 0.8$ Hz, 1H), 4.47 (q, $J = 7.2$ Hz, 2H), 2.64 (s, 3H), 1.49 (t, $J = 7.2$ Hz, 3H); IR (CH_2Cl_2) 1669, 1521 cm^{-1}; HR-FABMS Calcd for $C_{12}H_{11}O_2N_4$ (M^++H): 243.0882. Found: 248.0883.

2.1.7. 2-Methyl-1-*n*-propyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8c). Compound **8c** (61 mg, 20%) was obtained from **11** (300 mg, 1.19 mmol) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (d, $J = 1.2$ Hz, 1H), 9.82 (d, $J = 1.2$ Hz, 1H), 4.33 (t, $J = 7.6$ Hz, 2H), 2.59 (s, 3H), 1.77–1.85 (m, 2H), 0.99 (t, $J = 7.2$ Hz, 3H); IR (CH_2Cl_2) 1670, 1522 cm^{-1}; HR-FABMS Calcd for $C_{13}H_{13}O_2N_4$ (M^++H): 257.1039. Found: 257.1043.

2.1.8. 2-Methyl-1-isopropyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8d). Compound **8d** (81.0 mg, 27%) was obtained from **11** (300 mg, 1.19 mmol) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.90 (s, 1H), 9.89 (s, 1H), 5.12 (br s, 1H), 2.70 (s, 3H), 1.67(d, $J = 7.0$ Hz, 6H); IR (CH_2Cl_2) 1665, 1511 cm^{-1}; HR-FABMS Calcd for $C_{13}H_{13}O_2N_4$ (M^++H): 257.1039. Found: 257.1035.

2.1.9. 1-*n*-Butyl-2-methyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8e). Compound **8e** (30.0 mg, 9.3%) was obtained from **11** (300 mg, 1.19 mmol) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.86 (d, $J = 1.2$ Hz, 1H), 9.81 (d, $J = 1.2$ Hz, 1H), 4.36 (t, $J = 7.6$ Hz, 2H), 2.59 (s, 3H) 1.74–1.79 (m, 2H), 1.37–1.44 (m, 2H), 0.97 (t, $J = 7.2$ Hz, 3H); IR (CH_2Cl_2) 1668, 1515 cm^{-1}; HR-FABMS Calcd for $C_{14}H_{15}O_2N_4$ (M^++H): 271.1195. Found: 271.1198.

2.1.10. 2-Methyl-1-phenyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8f). Compound **8f** (85.0 mg, 24%) was obtained from **11** (300 mg, 1.19 mmol) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.97 (d, $J = 1.1$ Hz, 1H), 9.76 (d, $J = 1.1$ Hz, 1H), 7.63–7.67 (m, 2H), 7.28–7.38 (m, 3H), 2.47 (s, 3H); IR (CH_2Cl_2) 1671 cm^{-1}; Anal. Calcd for $C_{16}H_{10}N_4O_2$: C, 66.20; H, 3.47; N, 19.30. Found: C, 66.13; H, 3.45; N, 19.19.

2.1.11. 2-Methyl-1-tolyl-1*H*-imidazo[4,5-*g*]phthalazine-4,9-dione (8g). Compound **8g** (47.0 mg, 13%) was obtained from **11** (300 mg, 1.19 mmol) as a pale yellow solid: mp > 300 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.87 (s, 1H), 9.67 (s, 1H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.15 (d, $J = 8.0$ Hz, 2H), 2.44 (s, 3H), 2.38 (s, 3H); IR (CH_2Cl_2) 1669 cm^{-1}; HR-FABMS Calcd for $C_{17}H_{13}O_2N_4$ (M^++H): 305.1039. Found: 305.1038.

Acknowledgements

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