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Synthesis and *In Vitro* Cytotoxicity of 3- or 4-Dialkylaminomethyl-1-azaanthraquinones

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Six 3-dialkylaminomethyl-1-azaanthraquinones and five 4-dialkylaminomethyl-1-azaanthraquinones were synthesized and evaluated *in vitro* cytotoxicity against four human cancer cell lines. The compounds retained much of their cytotoxic activity against the multi-drug-resistant cell line (KB-V-1) as shown by resistance index.

Key words : Antitumor agents, Azaanthraquinone, Cytotoxic activity, Doxorubicin, Mitoxantrone

INTRODUCTION

The anthracycline antibiotics are commonly used intercalating agents in the treatment of cancer (Wakelin and Waring, 1990). Doxorubicin and Daunorubicin are the best known members of the anthracyclines. Doxorubicin (Fig. 1.) is particularly efficacious against solid tumors. However its cardiotoxicity limits the clinical usefulness (Priebe, 1995; Surato *et al.*, 1990). Synthetic analogues, mitoxantrone and ametantrone (Fig. 1.), resulted from efforts to produce anthracycline analogues that lack cardiac toxicity. Although mitoxantrone is endowed with an improved tolerability profile compared with doxorubicin and other anthracylines, this drug is not devoid of significant toxic side effects, especially those associated with myelosuppression (Gandolfi *et al.*, 1995). A number of studies have identified anthracyclines as intercalating agents, all indications being that their biological activity are attributable to their DNA-binding properties. DNA intercalation and interference with the DNA-topoisomerase II activity resulting in protein-associated DNA strand breaks have been proposed as critical events that lead to anthracycline-induced cell death (Wakelin and Waring, 1990). The search for new analogues having better therapeutic efficacy without undesirable side effects of the anthracycline analogues is of extreme interest and numerous analogues have been reported (Priebe, 1995).

We recently reported synthesis and *in vitro* evaluation of various 1-azaanthraquinones (Fig. 1.) (Lee *et al.*, 1996; Lee *et al.*, 1997; Lee *et al.*, 1998) and continued our effort to search for better antitumor intercalating agents that can overcome the shortcomings of the anthracycline analogues. In the present study, we report synthesis and *in vitro* evaluation of 3- or 4-dialkylaminomethyl-1-azaanthraquinones to probe the effect of the incorporation of tertiary amine into the side chain of the azaanthraquinones on their cytotoxic activity. The position of substituent of the target compounds was chosen based on the structure of mitoxantrone and doxorubicin. Considering the factor that may be needed to potentiate the antitumor activity of the intercalating agents, we designed the target compounds bearing H-bonding functional group. The tertiary amine substituents would increase the residence time of the azaanthraquinones within DNA through additional hydrogen bonding with sugar-phosphate backbone of DNA.

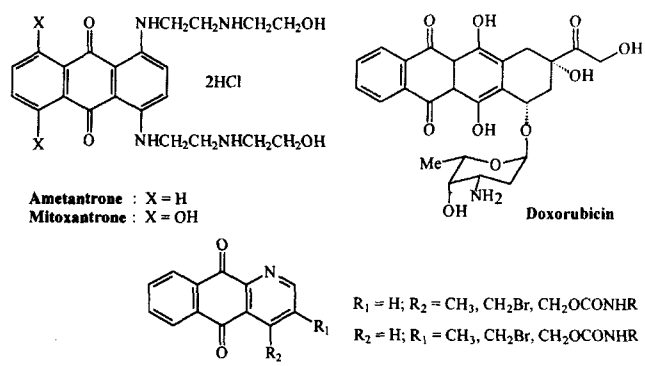

Fig. 1.

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MATERIALS AND METHODS

Melting points were recorded on a Electrothermal IA9100 digital melting point apparatus and are uncorrected. IR spectra were determined with a Jasco FT/IR-300E spectrophotometer and reported in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker DPS300 NMR spectrometer using TMS as an internal standard and chemical shifts are reported as ppm units. Thin-layer chromatography was performed on E. Merck silica gel GF-254 precoated plates and the identification was done with UV light and colorization with spray of concentrated sulfuric acid followed by heating. Column chromatography was carried out on silica gel 60 (230~400 mesh ASTM). Commercially available regents and solvents were used without additional purification unless otherwise stated. RPMI1640 media was obtained form Gibco BRL. Dimethyl sulfoxide (DMSO) and other chemicals were purchased from Sigma.

General procedure for dialkylamine substitution

This reaction was carried out by a modification of the reported procedure (Luzzio, M. J. *et al.* 1995). 3- or 4-Bromomethyl-1-azaanthracene-9,10-dione (0.17 mmol) was treated with corresponding dialkylamine (0.14 mmol) in DMF (10 mL) for 24 h. The solvent was removed *in vacuo* and the resulting residue was purified by flash column chromatography (5% methanol in dichloromethane).

3-(*N,N*-Dimethylamino)methyl-1-azaanthracene-9,10-dione (2a)

The product was obtained in 96% yield; mp 126°C; IR (KBr) 3500~3300 (br), 2939, 2856, 2760, 1685, 1665, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.63 (m, 6H), 2.49 (t, *J*=5 Hz, 4H), 3.73 (s, 2H), 7.85 (m, 2H), 8.32 (dd, *J*=6 Hz, *J*=3 Hz, 1H), 8.41 (m, 1H), 8.59 (d, *J*=2 Hz, 1H), 9.05 (d, *J*=2 Hz, 1H).

3-(*N,N*-Diethanolamino)methyl-1-azaanthracene-9,10-dione (2b)

The product was obtained in 96% yield; mp 137~138°C; IR (KBr) 3410, 2940, 2892, 2840, 1690, 1667, 1590, 1330, 1300 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.63 (m, 6H), 2.49 (t, *J*=5 Hz, 4H), 3.73 (s, 2H), 7.85 (m, 2H), 8.32 (dd, *J*=6 Hz, *J*=3 Hz, 1H), 8.41 (m, 1H), 8.59 (d, *J*=2 Hz, 1H), 9.05 (d, *J*=2 Hz, 1H).

3-Pyrrolidinomethyl-1-azaanthracene-9,10-dione (2c)

The product was obtained in 93% yield; mp 123°C; IR (KBr) 3500~3300 (br), 2960, 2920, 2790, 1685, 1660, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.85 (m, 4H), 2.60 (t, *J*=3 Hz, 6H), 3.87 (s, 2H), 7.85 (m, 2H), 8.33 (dd, *J*=6 Hz, *J*=3 Hz, 1H), 8.42 (t, *J*=4 Hz, 1H), 8.61 (d, *J*=2 Hz, 1H), 9.08 (d, *J*=2 Hz, 1H).

3-Piperidinomethyl-1-azaanthracene-9,10-dione (2d)

The product was obtained in 96% yield; mp 126°C; IR (KBr) 3500~3300 (br), 2939, 2856, 2760, 1685, 1665, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 1.63 (m, 6H), 2.49 (t, *J*=5 Hz, 4H), 3.73 (s, 2H), 7.85 (m, 2H), 8.32 (dd, *J*=6 Hz, *J*=3 Hz, 1H), 8.41 (m, 1H), 8.59 (d, *J*=2 Hz, 1H), 9.05 (d, *J*=2Hz, 1H).

3-Morpholinomethyl-1-azaanthracene-9,10-dione (2e)

The product was obtained in 93% yield; mp 147~148°C; IR (KBr) 3500~3300 (br), 2960, 2920, 2850, 2800, 1685, 1670, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 2.52 (t, *J*=5 Hz, 4H), 3.72 (s, 2H), 3.74 (t, *J*=4 Hz, 4H), 7.86 (t, *J*=5 Hz, 2H), 8.33 (dd, *J*=6 Hz, *J*=3 Hz, 1H), 8.42 (dd, *J*=6 Hz, *J*=3 Hz, 1H), 8.60 (d, *J*=2 Hz, 1H), 9.08 (d, *J*=2 Hz, 1H).

3-(1-Methylpiperazino)methyl-1-azaanthracene-9,10-dione (2f)

The product was obtained in 92% yield; mp 171~172°C; IR (KBr) 3500~3300 (br), 2810, 2800, 2770, 1685, 1668, 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 2.17 (s, 3H), 2.36 (m, 4H), 2.43 (m, 4H), 3.72 (s, 2H), 7.95 (t, *J*=5 Hz, 2H), 8.22 (m, 2H), 8.44 (d, *J*=2 Hz, 1H), 8.99 (d, *J*=2 Hz, 1H).

4-(*N,N*-Dimethyl)aminomethyl-1-azaanthracene-9,10-dione (4a)

The product was obtained in 88% yield; mp. 157~158°C; IR (KBr) 2939, 1685, 1660, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.22 (d, *J*=5 Hz, 1H), 8.42 (m, 1H), 8.29 (m, 1H), 8.02 (d, *J*=5 Hz, 1H), 7.88 (m, 2H), 4.99 (s, 2H), 3.49 (s, 6H).

4-(*N,N*-Diethanol)aminomethyl-1-azaanthracene-9,10-dione (4b)

The product was obtained in 48% yield; mp. 110~111°C; IR (KBr) 3380, 2940, 1690, 1667, 1590, 1300 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.04 (d, *J*=5 Hz, 1H), 8.37 (m, 1H), 8.30 (m, 1H), 8.25 (m, 1H), 7.85 (m, 2H), 4.47 (s, 2H), 3.73 (m, 4H), 2.83 (m, 4H).

4-Piperidinomethyl-1-azaanthracene-9,10-dione (4c)

The product was obtained in 76% yield; mp. 127~128°C; IR (KBr) 2939, 1685, 1664, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.03 (d, *J*=5 Hz, 1H), 8.38 (m, 1H), 8.23 (m, 2H), 7.85 (m, 2H), 4.18 (s, 2H), 2.53 (m, 4H), 1.80~1.35 (m, 6H).

4-Morpholinomethyl-1-azaanthracene-9,10-dione (4d)

The product was obtained in 95% yield; mp. 177~

179°C; IR (KBr) 2960, 2920, 1685, 1665, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.01 (d, J=5 Hz, 1H), 8.34 (m, 1H), 8.21 (m, 2H), 7.83 (m, 2H), 4.21 (s, 2H), 3.81 (t, J=4 Hz, 4H), 2.59 (t, J=4 Hz, 4H).

4-(4-Methylpiperazino)methyl-1-azaanthracene-9,10-dione (4e)

The product was obtained in 73% yield; mp. 139~141°C; IR (KBr) 2810, 1685, 1665, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.30 (d, J=5 Hz, 1H), 8.36 (m, 1H), 8.24 (m, 1H), 8.16 (m, 1H), 7.84 (m, 2H), 4.26 (s, 2H), 2.70 (m, 8H), 2.42 (s, 3H).

Cell culture

Human breast cancer MCF7 and human liver cancer SNU-354 cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum and 2 mM L-glutamine. Human oral epidermoid cancer KB-3-1 cells and KB-V1 cells were grown in Dulbecco's modified Eagles medium (DMEM) containing 2 mM L-glutamine and 10% fetal calf serum. KB-V1 cells were maintained in continuous presence of 1 μM vinblastine. All cells were grown at 37°C in humidified atmosphere with 5% CO_2 and 95% air.

In vitro cytotoxicity

Cells in exponential growth were trypsinized, dispersed in single cell suspension and dispensed in 100 μl volume into 96 well plates. Cells (5×10^3 MCF7, 3×10^3 SNU 354, 2.5×10^3 KB-3-1, 5×10^3 KB-V1) were allowed to attach and grow overnight. One hundred microliters of medium containing test sample were added and further incubated for 48 h. Drugs were dissolved in small amounts of DMSO before dilution with medium (final concentration 0.1%). Cytotoxicity was measured by the SRB method (Skehan, 1990) and the IC_{50} value was calculated using Probits method. In brief, cells were fixed by gently layering 50 μl of cold 50% trichloroacetic acid on the top of the growth medium in each well and incubated at 4°C for 1 h and then washed five times with tap water. Plates were air-dried and stained with 0.4% (w/v) sulforhodamine B in 1% acetic acid for 15~30 min and rinsed four times with 1% acetic acid to remove unbound dye. Plates were airdried and bound dye was solubilized with 10 mM unbuffered Tris base (pH 10.5). Absorbasnce was read with microtiter plate reader at 570 nm. The IC_{50} value was the conncentration of drug that reduced absorbance to the 50% that of vehicle-treated controls.

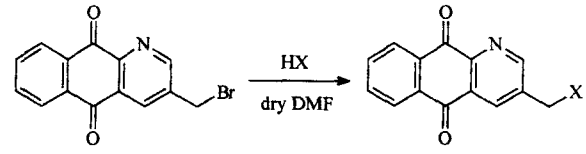
RESULTS AND DISCUSSION

The synthesis of **1** and **3** was accomplished as described in our previous reports (Lee *et al.*, 1996; Lee *et al.*, 1997; Lee *et al.*, 1998) using Diels-Alder reaction of 1,4-naphthaquinone with 1-dimethylamino-3-methyl-1-aza-1,3-butadiene and 1-dimethylamino-1-aza-1,3-pentadiene respectively followed by benzylic bromination. The compounds **1** and **3** were then converted to the taget compounds (**2a-f** and **4a-e**) bearing tertiary amines by displacement of the bromide (Luzzio, M. J. *et al.* 1995) (Table I and Table II).

The evaluations of the biological activity for the compounds were performed *in vitro* following the protocols developed by the National Cancer Institute (Skehan *et al.*, 1990). The *in vitro* activities against human cancer cell lines originated from liver (SNU-354), breast (MCF7), epidermoid carcinoma (KB-3-1) and a multi-drug resistant subline (KB-V-1) for the azaanthraquinone derivatives (**2a-f** and **4a-e**) along with comparative data for doxorubicin are listed in Table III.

All of the analogues were less cytotoxic than doxorubicin against the sensitive cell lines. Dialkylaminomethyl-1-azaanthraquinones were in general more potent than azaanthraquinones bearing carbamate (Lee *et al.*, 1997; Lee *et al.*, 1998). This suggests that the basic side chain of the intercalating chromophore plays an important role for the activity. The compounds **2e, 2f, 4d,** and

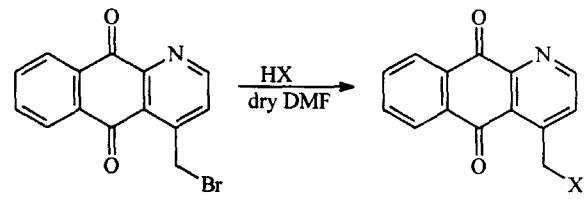
Table I. Synthesis of 3-dialkylaminomethyl-1-azaanthraquinones



1 2

	X	Yield (%)
2a	$-N(CH_3)_2$	96
2b	$-N(CH_2CH_2OH)_2$	96
2c	-Pyrrolidin	93
2d	-Piperidine	96
2e	-Morpholine	93
2f	(N-Methyl)piperazine	92

Table II. Synthesis of 4-dialylaminomethyl-1-azaanthraquinones



3 4

	X	Yield (%)
4a	$-N(CH_3)_2$	88
4b	$-N(CH_2CH_2OH)_2$	48
4c	-Piperidine	76
4d	-Morpholine	95
4e	-(N-Methyl)piperazine	73

Table III. *In vitro* cytotoxic activity of 3- or 4-dialkylaminomethyl-1-azaanthraquinones

	IC_{50}[a] (μM) of Cell Line[b]				
	SNU-354	MCF7	KB-3-1	KB-V-1	RI[c]
2a	18.9	11.7	3.7	6.7	1.8
2b	15.1	8.6	10.9	60.0	5.5
2c	5.5	4.1	3.64	5.1	1.4
2d	6.2	9.9	6.7	7.7	1.2
2e	21.3	20.2	27.8	35.3	1.3
2f	29.5	31.4	23.4	51.8	2.2
4a	11.68	1.26	4.47	6.33	1.4
4b	19.30	5.97	9.31	17.10	1.8
4c	16.52	1.20	3.15	6.81	2.2
4d	>100	1.24	37.25	>100	-
4e	36.09	13.2	20.8	39.15	1.9
DXR	1.10	0.80	0.37	19.2	52

IC_{50}[a]=concentration of compound (μM) required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by the SRB assay. Each experiment was run at least three times, and the results are presented as an average value. [b]Human cancer cell lines: SNU-354 (liver cancer cell), MCF7 (brest cancer cell), KB-3-1 (human epidermoid carcinoma cell), KB-V-1 (human epidermoid carcinoma multidrug-resistant cell) cResistance index: IC_{50} of resistance cell line/IC_{50} of sensitive cell line.

4e showed less potent cytotoxic activity than other amine analogues. These compounds possess additional hetero atom in the side chain. The incorporation of additional hetero atom into the heterocyclic ring system increases the hydrophilicity of the compounds. This suggests that DNA binding site of the compounds near the heterocyclic ring may be hydrophobic. The compounds **2e, 2f, 4d**, and **4e** exhibited 2 to 20-fold less cytotoxic activity compared with doxorubicin against sensitive tumor cell lines. However, the compounds were more potent than doxorubicin against doxorubicin-resistant cell line. The compounds retained much of their activity against the multi-drug-resistant cell line as shown by resistance index.

In summary, six 3-dialkylaminomethyl-1-azaanthraquinones and five 4-dialkylaminomethyl-1-azaanthraquinones were designed and synthesized as potential antitumor agents. The compounds **2a, c, d** and **4a, c** may need further in depth biological evaluation and may have potential for the treatment of resistant tumors. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

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