Synthesis and *In vitro* Evaluation of 4-Substituted-1-azaanthraquinones

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Doxorubicin and daunomycin are the best known members of the anthracycline antibiotics and the most commonly used intercalating agents in the treatment of cancer (Wakelin and Waring, 1990). Doxorubicin (Fig. 1.) has a broad spectrum of activity, being particularly efficacious against solid tumors. However its clinical usefulness is limited by cardiotoxicity that develops after extended therapy, and the appearance of an acquired resistance (Priebe, 1995; Surato *et al.*, 1990). Synthetic analogues, mitoxantrone and ametantrone (Fig. 1), resulted from efforts to produce anthracycline analogues that lack cardiac toxicity. Mitoxantrone in particular currently plays an important role in the clinical management of hematological malignancies such as chronic myelogenous leukemia, acute nonlymphoblastic leukemia, and non-Hodgkin's lymphoma, as well as in combination therapy of refractory ovarian and breast cancers (Wakelin and Waring, 1990). 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 mitoxantrone as an intercalating agent, all indications being that its biological activity is attributable to its 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 mitoxantrone-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).

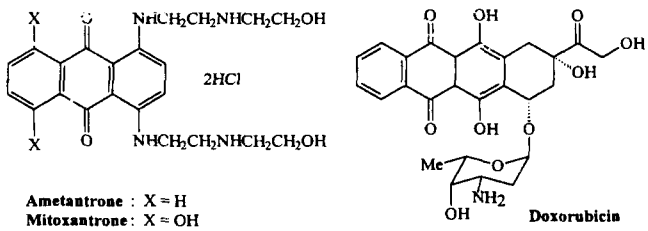

Fig. 1.

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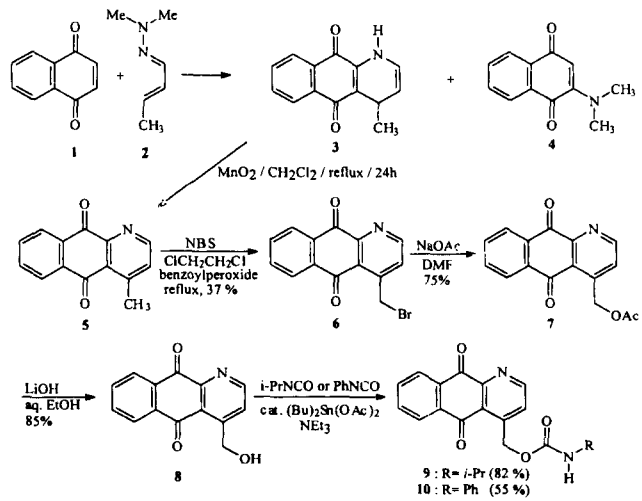
We recently reported synthesis and *in vitro* evaluation of 3-substituted-1-azaanthraquinones (Lee *et al.*, 1996) and continued our efforts to search for better antitumor intercalating agents that can overcome the shortcomings of the anthracycline analogues. In the present study, we report synthesis and *in vitro* evaluation of 4-substituted-1-azaanthraquinones to examine the effect of the position of the side chains on the cytotoxic activity. The position of substituent of the target compounds was chosen based on the structure of mitoxantrone. Considering the factor that may be needed to potentiate the antitumor activity of the intercalating agents, we designed the target compounds bearing alkylating or latent alkylating functionality. The azaanthraquinones containing carbamoyloxymethyl substituents were designed based on the possible metabolic activation process. In the hypoxic core of solid tumors, the quinone compound could undergo bioreductive activation for the allylic acetate or carbamate to function as an electrophilic center.



Sheme 1. Synthesis of 4-Substituted-1-azaanthraquinones.

Table I. *In Vitro* Cytotoxic Activity of 4-Substituted-1-azaanthraquinones

	IC_{50} (μM)[a] of cell lines[b]				
	SNU-1	SNU-354	MCF7	MCF7/R	RI[c]
5	45.3	>50	27.7	54.5	2.0
6	14.9	5.38	5.8	14.1	2.4
7	49.8	>50	10.9	59.0	5.4
8	45.7	>50	3.8	27.3	7.2
9	26.1	3.91	10.5	33.6	3.2
10	>50	>50	1.09	41.7	3.8
Doxorubicin	0.5	1.0	0.28	69.1	247

[a]IC_{50}=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-1 (stomach cancer cell), SNU-354 (liver cancer cell), MCF7 (Human Breast Cancer Cell) and MCF7/R (Subline Resistant to Doxorubicin). [c]Resistance index: IC_{50} of resistant cell line/IC_{50} of sensitive cell line.

The synthesis of the target compounds utilized hetero Diels-Alder reaction of 1,4-naphthaquinone **1** with 1-dimethylamino-1-aza-1,3-pentadiene **2** as a key step and outlined in scheme 1. The hetero Diels-Alder reaction of 1-dimethylamino-1-aza-1,3-pentadiene **2** has been used in the construction of various heterocyclic systems (Chigr *et al.*, 1988; Lee and Anderson, 1990). The dienophile **1** (1 equiv.) was treated with diene **2** (2 equiv.) in dichloromethane for 12 h at room temperature to give the dihydropyridine **3** along with a minor amount of the side product **4**. While the formation of **3** is expected from this Diels-Alder reaction, the formation of the side product **4** can be accounted for by Michael addition of the liberated dimethylamine during the cycloaddition to the starting dienophile **1**. The formation of the side product was also reported by Chigr *et al.* Unlike hetero Diels-Alder reaction of 1,4-naphthaquinone with 1-dimethylamino-3-methyl-1-aza-1,3-butadiene (Lee *et al.*, 1996), the cycloaddition of naphthoquinone **1** with diene **2** required the purification of the dihydropyridine **3**. The subsequent oxidation of the intermediate **3** with manganese(IV)oxide (MnO_2) afforded 4-methyl-1-azaanthraquinone **5** (50% in two steps). Attempted one pot reaction using either MnO_2 or silica gel resulted in complex mixture by TLC.

After a great number of trial of the benzylic bromination of 4-methyl-1-azaanthraquinone **5**, the best result was obtained with 4.5 equiv. of *N*-bromosuccinimide (NBS) and 0.09 equiv. of benzoylperoxide in 1, 2-dichloroethane at reflux for 48 h with the irradiation of tungsten lamp. The reaction afforded the monobrominated **6** (37%) along with 3-dibromomethyl-1-azaanthraquinone (19%). The starting material was reused. A conventional benzylic bromination using NBS in carbon tetrachloride or benzene failed to give the desired product. The compound **5** was sparingly soluble in both benzene and CCl_4 so that required large amount of solvent. This might be the reason for the poor yield of the desired monobrominated **6** and the recovery of the starting material.

Having obtained the required bromomethyl product **6**, it was treated with anhydrous sodium acetate in anhydrous DMF at room temperature to give the acetate **7** in 75% yield. Hydrolysis of the acetate **7** (purified by flash chromatography) with LiOH in 80% aqueous ethanol afforded 4-hydroxymethyl-1-azaanthraquinone **8** (85%). Treatment of **8** with corresponding isocyanates, catalytic amount of dibutyltin diacetate and triethylamine in anhydrous dichloromethane afforded *N*-i-propyl analog **9** (82%) and 3-(*N*-phenyl) carbamoyloxymethyl-1-azaanthraquinone **10** (55%), respectively. The sturucture of the target compounds was determined by spectroscopic analysis.

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 stomach (SNU-1), liver (SNU-354), breast (MCF7) and a subline (MCF 7/R) with 247-fold resistant to doxorubicin for the azaanthraquinone derivatives (**5**, **6**, **7**, **8**, **9** and **10**) along with comparative data for doxorubicin are listed in Table 1. All of the analogues were less cytotoxic than doxorubicin against the sensitive cell lines with the bromomethyl compound **6** being the most potent as 3-substituted-1-anthraquinones. The most active compound **6** exhibited 5 to 10-fold less cytotoxic activity compared with doxorubicin against sensitive tumor cell lines. However, the compound was more potent than doxorubicin against doxorubicin-resistant cell line. The compounds retained much of their activity against the doxorubicin-resistant cell line as shown by resistance index.

In summary, six 4-subsituted-1-azaanthraquinones were designed and synthesized using hetero Diels-Alder reaction as a key step. Although a great number of reaction conditions for benzylic bromination were examined, this step need to be improved for the efficient synthesis of the related analogues. 4-Bromomethyl-1-azaanthraquinone **6** may have potential for the treatment of tumors resistant to the doxorubicin. The compounds **9** and **10** containing the latent alkylating functionality may need further in depth biological evaluation. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

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