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SYNTHESIS AND *IN VITRO* EVALUATION OF 3-SUBSTITUTED-1-AZAANTHRAQUINONES

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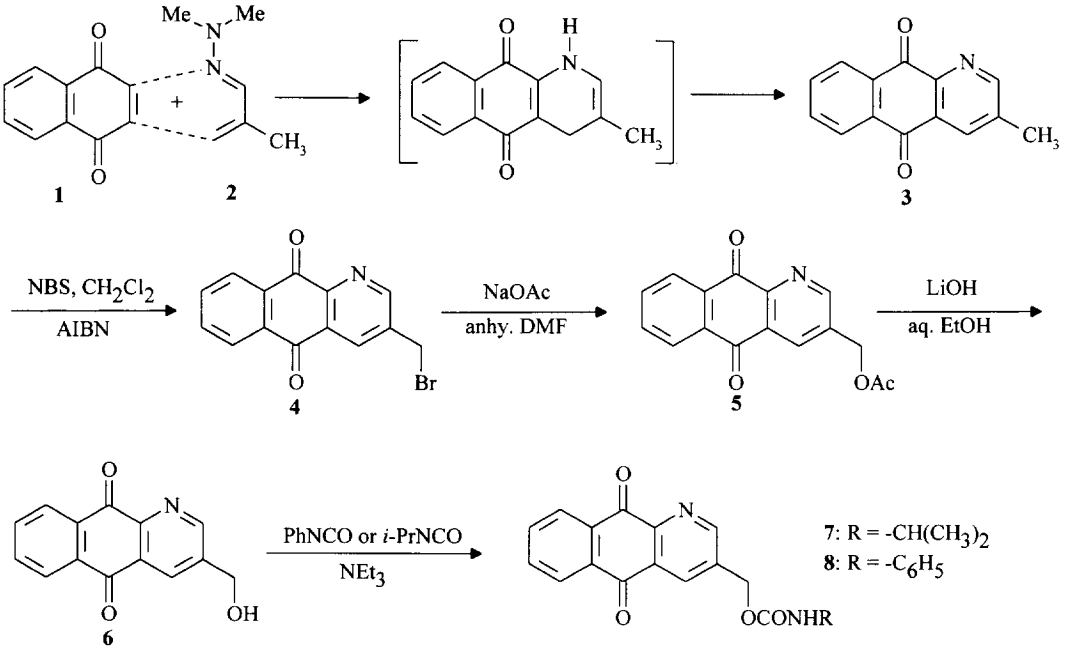
Abstract: In the course of developing novel antitumor agents, we synthesized 3-substituted-1-azaanthraquinones, incorporating the alkylating or latent alkylating substituents as potential antitumor agents. The most active comound **4** exhibited cytotoxic activity comparable to that of doxorubicin. The compounds **3-8** retained much of their activity against the doxorubicin-resistant cell line (MCF7/R).

The anthracycline antibiotics play an important role in the treatment of many human lymphomas, leukemia, and solid tumors[1,2]. Doxorubicin and daunorubicin are the best known members of the anthracycline antibiotics and are the most commonly used intercalating agents in the treatment of cancer[3]. Doxorubicin has a broad spectrum of antitumor activity, being particularly efficacious against solid tumors. However, the clinical usefulness of anthracyclines is limited mainly by two major problems that are cumulative cardiotoxicity, and the appearance of an acquired resistance[3,4]. The search for new analogues having better therapeutic efficacy without undesirable side effects is of extreme interest and numerous analogues have been synthesized[3].

In an effort to develop novel antitumor intercalating agents that can overcome the shortcomings of anthracyclines, 3-substituted-1-azaanthraquinones were designed, synthesized and evaluated for the cytotoxic activity. These compounds contained side chains that would play a role to increase a residence time of drug within DNA by forming covalent bond with DNA. Several attempts were made to incorporate an alkylating functionality into some intercalating chromophores[5-8]. These approaches have been based on the concept of targeting alkylating agents to DNA by attaching them to DNA-intercalating ligands as DNA-affinic carriers. We, herein, wish to report the synthesis and *in vitro* evaluation of 3-substituted-1-azaanthraquinones. The position of substituents at azaanthraquinone was chosen based on the examination of the structure of doxorubicin. 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 bio-reductive activation for the allyic acetate or carbamate to function as an electrophilic center.



Scheme 1.

The synthesis of the target compounds utilized hetero Diels-Alder reaction of 1-dimethylamino-3-methyl-1-aza-1,3-butadiene with 1,4-naphthoquinone as a key step and outlined in **scheme 1**. Sercks-Poncin's cycloaddition procedure[9] of 1,4-naphthoquinone with diene **2** was modified to avoid the use of excess naphthoquinone. The cycloaddition of dienophile **1** and diene **2** followed by stirring in the presence of silica gel afforded aromatized 3-methyl-1-azanthraquinone **3** (51 %) with the simultaneous elimination of dimethylamine from initial 1:1 addition adduct. The use of MnO_2 as oxidizing agent in this reaction did not improve the yield. The functionalization of allylic methyl of **3** to introduce the potential electrophile was achived by allylic bromination with NBS in the presence of catalytic amount of azobisisobutyronitrile (AIBN). The reaction afforded 3-bromomethyl-1-azanthraquinone **4** (36 %) along with the starting material **3** (54 %). The recovered starting material was reused. Treatment of **4** with anhydrous sodium acetate in anhydrous DMF provided **5** in 87 % yield. Hydrolysis of acetate of **5** with LiOH in aqueous ethanol afforded 3-hydroxymethyl-1-azaanthraquinone **6** (85 %). Treatment of **6** with corresponding isocyanates and triethylamine in anhydrous dichloromethane afforded *N-i*-propyl- and *N*-phenylcarbamoyloxymethyl derivatives **7** (55 %) and **8** (60 %) respectively.

Table 1. *In Vitro* Cytotoxic Activity of 3-Substituted-1-azaanthraquinones against Human cancer cell lines.

	IC_{50} (μg/ml)[a] of cell lines[b]	
compound	SNU-1	SNU-354
3	13.9	>20.0
4	0.56	0.89
5	8.4	11.8
6	8.1	4.5
7	>20.0	>20.0
8	14.9	15.2
doxorubicin	0.27	0.54

[a] IC_{50} = concentration of compound (μg/ml) 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).

The evaluations of the biological activity for the compounds[10] were performed *in vitro* following the protocols developed by the National Cancer Institute[11]. The *in vitro* activities against human cancer cell lines originated from stomach (SNU-1) and liver (SNU-354) for the azaanthraquinone derivatives (**3**, **4**, **5**, **6**, **7**, and **8**) along with comparative data for doxorubicin are listed in **Table 1**. Except for the compound **4**, the novel azaanthraquinones are in general 40-100 fold less cytotoxic than doxorubicin. The most active compound **4** containing bromomethyl substituent at 3-position exhibited cytotoxic activity comparable to that of doxorubicin.

Table 2. *In Vitro* Cytotoxic Activity of 3-substituted-1-azaanthraquinones against Human Breast Cancer Cells (MCF7) and a Subline Resistant to Doxorubicin (MCF7/R).

compound	IC_{50} (μg/ml)[a]		RI[b]
	MCF7	MCF7/R	
3	18.33	69.08	3.8
4	2.49	3.08	1.2
5	6.70	49.89	7.4
6	6.66	17.73	2.7
7	>100.0	>100.0	
8	4.58	57.29	12.5
doxorubicin	0.15	37.57	250

[a] IC_{50} = concentration of compound (μg/ml) 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] Resistance index: IC_{50} of resistant cell line/IC_{50} of sensitive cell line.

The analogues were also screened *in vitro* against a human breast cancer cell line (MCF7) and a subline (MCF7/R) with 250-fold resistant to doxorubicin (**Table 2**). All of the analogues were less cytotoxic than doxorubicin against the sensitive cell line with the bromomethyl compound, **4**, again being the most potent. It is noteworthy that the compounds retained much of their activity against the doxorubicin-resistant cell line. The compound, **4**, possesed the most favorable resistance index and was the most cytotoxic.

Poor cytotoxic activity of the compound, **7**, may be ascribed to the poor aqeous solubility. Alkylating 3-substituted-1-azaanthraquinones may have potential for the treatment of tumors resistant to the doxorubicin. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems

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