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SYNTHESIS AND *IN VITRO* CYTOTOXICITY OF 3-SUBSTITUTED-1,8-DIAZAANTHRAQUINONES PRODUCED BY LEWIS-ACID CATALYZED HETERO DIELS-ALDER REACTION

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Abstract : A hetero Diels-Alder reaction of quinoline-5,8-dione with 1-(*N,N*-dimethylamino)-3-methyl-1-aza-1,3-butadiene proceeded to give 3-methyl-1,8-diazaanthraquinone (100% regioselectivity) in the presence of Lewis-acid catalyst ($ZnCl_2$ or $ZnBr_2$). Subsequent functionalizations of the benzylic methyl group resulted in the 1,8-diazaanthraquinone analogues as potential antitumor agents. The most active compound, **8**, exhibited *in vitro* cytotoxic activity comparable to that of doxorubicin.

The azaanthraquinones are a new class of antitumor agents that exhibit promising *in vitro* and *in vivo* activity against a wide spectrum of tumor cell lines[1-3]. These are the chromophore-modified analogues of mitoxantrone. In an effort to develop novel antitumor intercalating agents that could overcome the shortcomings of anthracyclines, we recently reported the synthesis and biological evaluation of 3- or 4-substituted-1-azaanthraquinones[4-6] and continued our efforts to design the related compounds. Herein, we wish to report the regioselective synthesis and biological evaluation of 3-substituted-1,8-diazaanthraquinones. These analogues were designed to explore the effect of incorporation of an additional nitrogen atom into the mono-azaanthraquinone chromophore.

It was envisioned that the target compounds could be obtained by a hetero Diels-Alder reaction of quinoline-5,8-dione (**1**) with 1-dimethylamino-3-methyl-1-aza-1,3-butadiene (**2**) and subsequent manipulations of the benzylic methyl group. However, the cycloaddition of dienophile **1** with diene **2** in dichloromethane afforded a mixture of the both regioisomers (3:2 determined by the integration of ^{1}H-NMR). For the synthesis of diazaanthraquinone derivatives, an efficient hetero Diels-Alder reaction methodology was required because of the formation of the undesired regioisomer as described above and the difficulty to separate the mixture. There were two conflicting reports on the same reaction. One reported the formation of a mixture of the both regioisomers (the minor isomer in 11 %)[7], and the other concerned with the formation of the major isomer as

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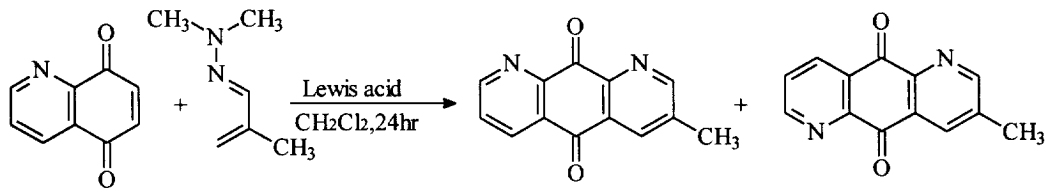
the sole adduct[8]. Repetitive trials of the same reaction condition failed to give satisfactory results, and a variation of the reaction temperature from benzene reflux to 25°C had little effect on the yield and regioselectivity of the hetero Diels-Alder reaction. However, addition of Lewis acid catalysts had dramatic effects. The effects of various Lewis acids on the Diels-Alder reaction of quinoline-5,8-dione (**1**) with 1-dimethylamino-3-methyl-1-aza-1,3-butadiene (**2**) are outlined in **Table 1**. The bidentate Lewis acids ($ZnBr_2$ and $ZnCl_2$) produced the desired regioisomer **3** in high yields as the sole product, but with the monodentate Lewis acid ($AlCl_3$), no cycloaddition product was obtained. This suggests that the reaction may proceed *via* the tight complex (**Table 2**) between the bidentate Lewis acid and **1** to give a single regioisomer. The complex between the monodentate Lewis acid and **1** might exchange **1** with the 1-azadiene containing an electron donating dimethylamino substituent, thereby diminishing the electron donating ability of the amino substituent.

Table 1. Effects of Lewis acids on Diels-Alder Reaction of Quinoline-5,8-dione (**1**)



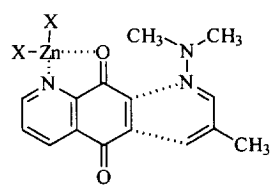
1 **2** **3** **4**

				Yield	
Entry	**2**	Lewis acid	**1**	**4**	**3**
1	2.0 eq	No	1.0 eq	27%	50%
2	1.2 eq	$ZnBr_2$ (1.2 eq)	1.0 eq		73%
3	1.2 eq	$ZnCl_2$ (1.2 eq)	1.0 eq		74%
4	1.2 eq	$AlCl_3$ (1.2 eq)	1.0 eq		no reaction

Effects of the bidentate Lewis acid ($ZnBr_2$) on a hetero Diels-Alder reaction were also examined by varying its molar equivalence (**Table 2**). The best regioselectivity and yield were obtained with 1.2 eq of $ZnBr_2$. With 0.5 eq of $ZnBr_2$, a trace amount of the minor regioisomer was also obtained. With 2.0 eq of $ZnBr_2$, the regioselectivity of the major isomer remained high, but the yield was poor. This supports the above explanation that the formation of a complex between a Lewis acid and **2** prevents the cycloaddition.

Table 2. Effects of $ZnBr_2$ on Diels-Alder Reaction of 3-Methyl-1-azadiene (**2**)

		Lewis acid		Yield	
Entry	Diene	($ZnBr_2$)	Dienophile	**4**	**3**
1	2.0 eq	No	1.0 eq	27%	50%
2	1.2 eq	0.5 eq	1.0 eq	Trace	65%
3	1.2 eq	1.2 eq	1.0 eq		73%
4	1.2 eq	2.0 eq	1.0 eq		36%
5	2.0 eq	1.2 eq	1.0 eq		69%

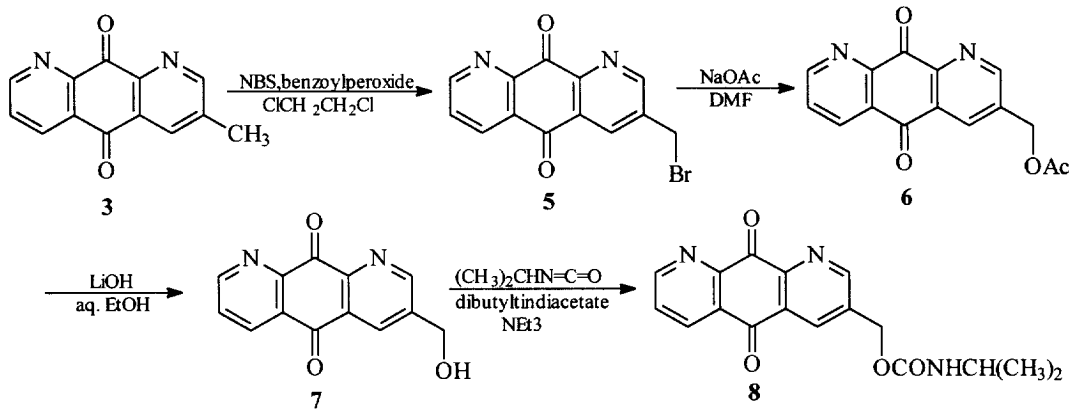


X = Cl or Br

Proposed Transition State

Having obtained the required 3-methyl-1,8-diazaanthraquinone (**3**), it was treated with *N*-bromosuccinimide (NBS) and a catalytic amount of benzoyl peroxide in anhydrous 1,2-dichloroethane at reflux for 48 h with irradiation of a tungsten lamp to give the monobromo product **5** in 30% yield. Treatment of **5** with anhydrous sodium acetate in anhydrous DMF provided the acetate **6** in 96 % yield. Hydrolysis of **6** (purified with FC) with LiOH in aqueous ethanol afforded 3-hydroxymethyl-1,8-diazaanthraquinone (**7**) (79 %). The hydroxymethyl product **7** was also obtained without isolation of the intermediate **6** (73 % in two steps). Treatment of **7** with isopropylisocyanate, triethylamine and a catalytic amount of dibutyltin diacetate (2 drops) in anhydrous dichloromethane afforded the *N-i*-propylcarbamoyloxymethyl analogue **8** in 76% yield (**Scheme 1**).

Scheme 1. Synthesis of 3-Substituted-1,8-diazaanthraquinones



Evaluation of *in vitro* cytotoxicity was performed following the protocols developed by the National Cancer Institute[9]. *In vitro* cytotoxic activity of the 1,8-diazaanthraquinone derivatives (**3**, **5**, **7**, and **8**) against human cancer cell lines, which originated from the lung (HOP62), ovary (SK-OV-3), colon (HCT-15) and CNS (SF295) is listed in **Table 3** along with that for doxorubicin.

Table 3. *In Vitro* Cytotoxic Activity of the 3-Substituted-1,8-diazaanthraquinones.

	IC_{50} (μM)[a] of cell lines[b]			
Compound	HOP62	SK-OV-3	HCT15	SF295
3	0.015	0.04	0.35	0.25
5	0.055	0.3	0.4	0.5
7	0.1	0.2	0.4	0.3
8	0.006	0.02	0.02	0.1
Doxorubicin	0.004	0.02	0.1	0.035

[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: HOP62 (lung cancer), SK-OV-3 (ovarian carcinoma), HCT-15 (colon cancer), SF295 (CNS carcinoma).

The novel 1,8-diazaanthraquinones, with the exception of compound **8**, were generally 2 to 20 folds less potent than doxorubicin in the four tumor cells. The most active compound, **8**, exhibited cytotoxic activity comparable to that of doxorubicin in HOP62, SK-OV-3 and SF295 cells. The 1,8-diazaanthraquinone chromophore was designed to make a 1-azaanthraquinone system more π-deficient. This would bring about a favorable π-π interaction with the base-pairs of electron-rich DNA. The incorporation of an additional nitrogen atom into the monoazaanthraquinone ring system would increase the residence time of the compounds within the DNA and/or affect the interaction with topoisomerase II. In our opinion, this effect of the 1,8-diazaanthraquinone analogues improved their cytotoxic activity when compared to those of the monoazaanthraquinones[4]. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

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References and Notes

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