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Regioselective Substitution of 6,7-Dichloroquinoline-5,8-dione: Synthesis and X-ray Crystal Structure of 4a,10,11-Triazabenzo[3,2-*a*]fluorene-5,6-diones

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Abstract—6,7-Dichloroquinoline-5,8-dione (**1**) was reacted with a number of 2-aminopyridine derivatives. Of the several possible products of this reaction, 4a,10,11-triazabenzo[3,2-a]fluorene-5,6-dione (**6**), produced by condensation and rearrangement, was obtained as the major product, and its structure was subsequently unambigously determined by X-ray crystallographic study. *Ortho*-quinones were produced via nucleophilic substitution at position C7, which was unexpected, considering that *para*-quinones were produced via C6 substitution in the reaction between compound **1** and ethyl acetoacetate in our previous work. Such unexpected nucleophilic substitution at C7 provides an effective, yet simple route, to the preparation of biologically active *ortho*-quinone derivatives.

Introduction

In previous papers, we showed that 6,7-dichloroquinoline-5,8-dione (**1**) reacts at the C6 position with ethyl acetoacetate to give compound **2**, which in turn reacts with several amines to yield the *N*-substituted-pyridino[2,3-*f*]indole-4,9-dione derivatives **3** (Scheme 1).[1]

The compound **3** contains the 7-aminoquinoline quinone moiety (**5**), which has been proposed as an essential group in the antitumor agent, streptonigrine (**4**) (Fig. 1).[2,3] In human clinical trials, streptonigrine (**4**) proved active against malignant lymphomas, mycosis fungoids, and Hodgkin's disease.[4,5] Suh et al. reported that some of these *N*-substituted-pyridino[2,3-*f*]indole-4,9-dione derivatives do exhibit significant antitumor activity against various human cancer cell lines.[6] On the other hand, it is known that streptonigrine is one of the most potent inhibitors of avian myeloblastosis virus reverse transcriptase (AMV-RT).[7] Likewise, heterocyclic quinones consisting of *o*- and *p*-quinoline quinones have been reported to inhibit AMV-RT in retroviruses. It is also known that *o*-quinoline quinones are generally more potent inhibitors of AMV-RT than *p*-quinoline quinones.[7]

In this paper, we report upon an easy and efficient synthesis of *ortho*-quinoline quinone derivatives, which are useful potent antitumor agents.

Results and Discussion

Chemistry

Compound **1** has two asymmetric chlorine atoms in the C6 and C7 positions. When this compound reacts with 2-aminopyridine derivatives, two possible reaction pathways should be considered. Firstly, the two nitrogen atoms in the 2-aminopyridine may substitute the chlorine atoms of **1** to produce the *para*-quinones (**A**).[8] Secondly, the pyridyl nitrogen atom may substitute one chlorine atom of **1**, and allow the amine group of pyridine to react with the neighboring quinone oxygen

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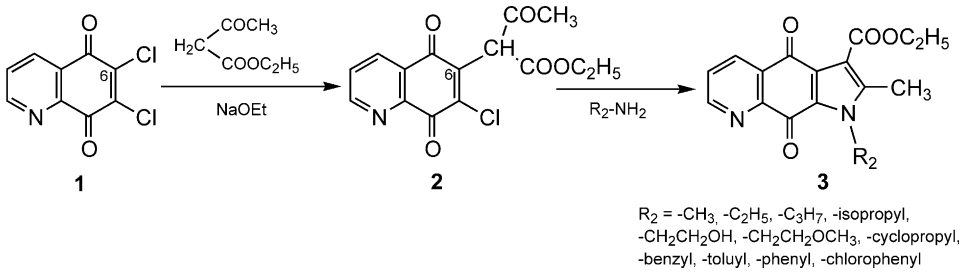


Scheme 1. Nucleophilic substitution at C 6 position of 6,7-dichloroquinoline-5,8-dione.

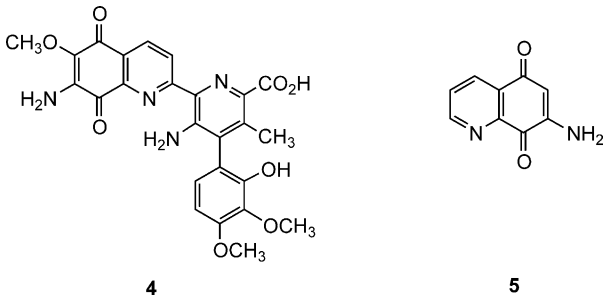
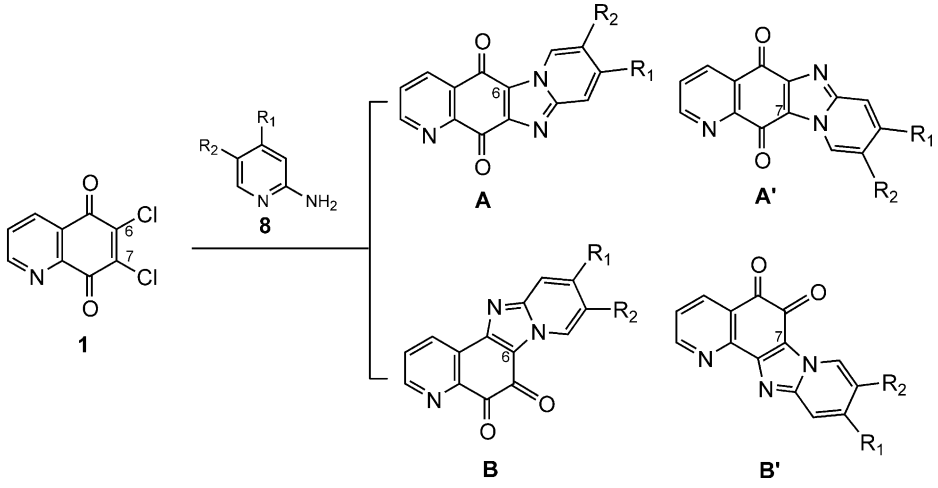


Figure 1. Streptonigrin (**4**) and 7-aminoquinoline quinone (**5**).

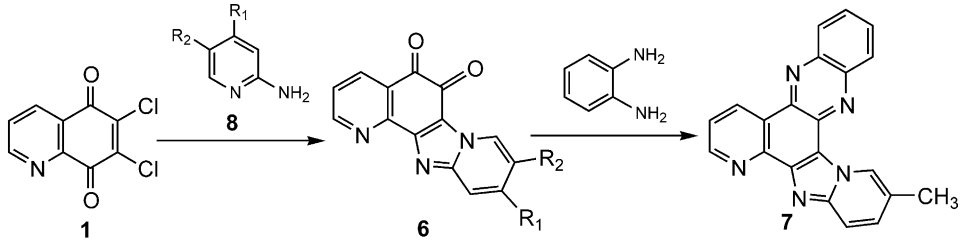
forming a Schiff's base, which is followed by *ortho*-quinone formation (**B**).[9,10] In both pathways, four possible structures can be produced (Scheme 2).

From the four possible structures mentioned, the linear heterocyclic structures **A** and **A′** could be disregarded due to the formation of **7**, caused by the subsequence reaction of the first product (**6b**) with *o*-phenylendiamine (Scheme 3).

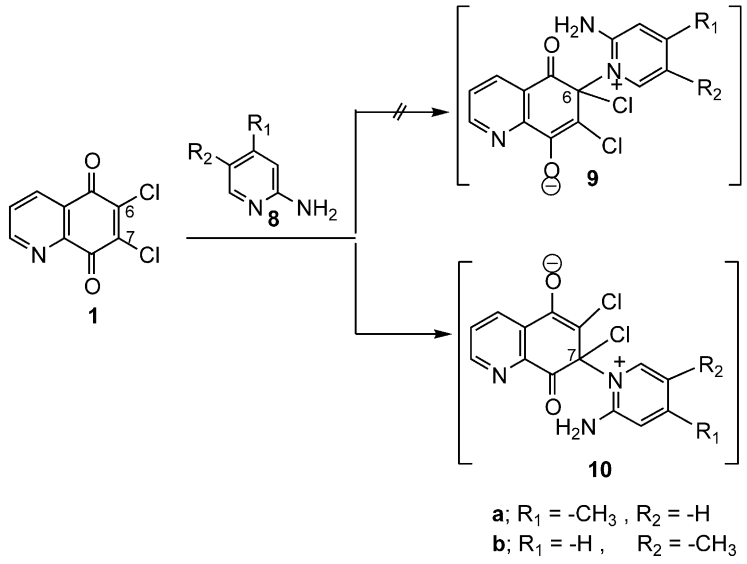
This leaves the *ortho*-quinone systems **B** and **B′**. It has previously been reported that strong nucleophiles substitute both chlorine atoms, while weak nucleophiles substitute only one chorine atom at C6 under mild conditions.[11] Our previous study of reactions between compound **1** and ethyl acetoacetate also showed that nucleophilic substitution takes place at C6 position to afford *para*-quinone derivatives.[1] However, from comparisons of the values of the heats of formation of the products of the C6- and C7-substitution reactions (**9** and **10**, respectively, Scheme 4), we expected to observe a preferential attack of nucleophiles at the C7 position (Table 1).[12] To calculate the heats of formation, the lowest geometry optimized energy conformers of these compounds were taken, and the heats were determined



Scheme 2. Possible reaction pathways of 6,7-dichloroquinoline-5,8-dione (**1**) with 2-aminopyridine derivatives.



Scheme 3. Nucleophilic substitution at C7 position of 6,7-dichloroquinoline-5,8-dione.[11]



Scheme 4. Possible formation of the imaginary initial substitution products.

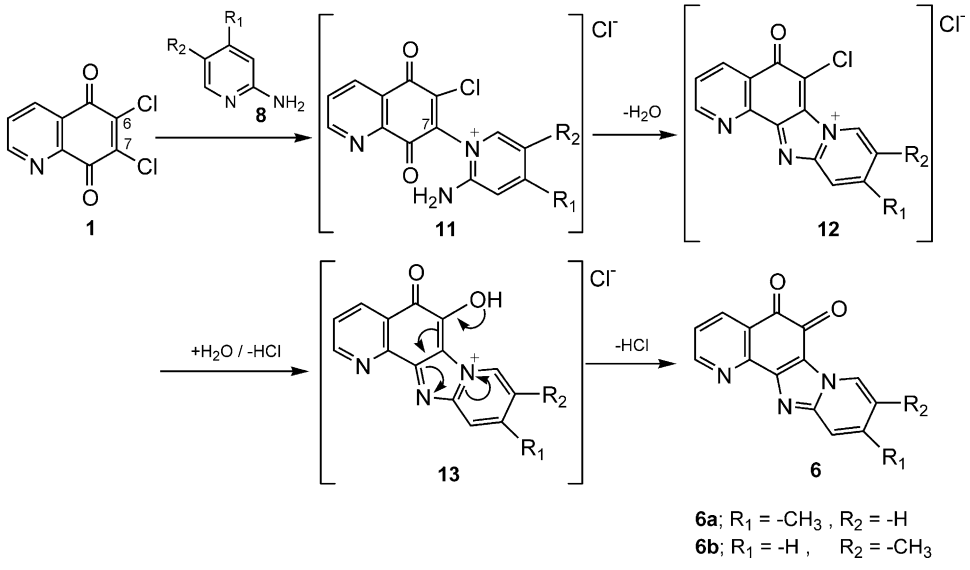
Table 1. Heats of formation of the imaginary transition states (AM1)

Transition state	Heat of formation (kcal/mol)
9a	44.99
9b	63.95
10a	43.29
10b	44.10

using the Sybyl 6.8 version energy minimizer, MAXIMIN2 and a Random Search program. In addition, of the two nucleophilic centers of the 2-aminopyridine derivatives (**8**), the considerable greater dipole-moment (1.5–1.7 Debye on the basis of AM1 charge calculations) at N1 should give rise to a preferential nucleophilic attack at C7 position of **1** (Scheme 4, Table 1).

Thus, according to the AM1 calculations the structure type **B′** should be favored. Structures **6** are not discriminated by customary analytical methods, and X-ray crystallographic structure analysis was found necessary to identify these species. X-ray crystallographic study[13] showed that the nucleophilic substitution actually took place at C7, and that this resulted in structure type **B′** and not **B** (Scheme 2), according to AM1 calculations (Table 1).

In appears that the mechanism, is as follows: the chlorine in the initial cyclization products (**12**), which were subsequently obtained from the initial substitution products (**10**, Scheme 4), was replaced by a hydroxyl group, with the loss of hydrogen chloride, giving structure **13**, the electronic configuration of which favors the formation of the final product **6**, and thus **B′** formation (Scheme 5).



Scheme 5. The predicted reaction mechanism for the formation of 4a,10,11-triazabenzo[3,2-*a*]fluorene-5,6-dione.

The products 4a,10,11-triazabenzo[3,2-*a*]fluorene-5,6-dione derivatives (**6**) were obtained as major products. The crystal of compound **6b** (Fig. 2) for X-ray crystallographic study,[13] was obtained by slowly evaporating dichloromethane/petrol ether solution. The molecule is completely flat, and showed a typical C=O bond distance [1.217(2) Å and 1.228(2) Å for C(7A)–O(18A) and C(8A)–O(19A), respectively] and a long C(7A)–C(8A) bond length [1.546(3) Å]. In the packed molecular structure, π–π* interactions were observed, which might be important in the interaction between these moieties and DNA during the intercalation process.

Crystal structure analysis

Crystal data for **6b**. $C_{15}H_9N_3O_2$, monoclinic, space group, $P2_1/n$, $a = 13.0706(5)$, $b = 16.6820(5)$, $c = 13.9852(5)$ Å, $\beta = 90.109(1)°$, $V = 2318.2(3)$ Å^3, $Z = 8$, $D_{calcd} = 1.509\ gcm^{-3}$, $\mu = 0.104\ \mu^{-1}$, $2\theta_{max} = 57°$; of 17 782 observed reflections, 5731 were unique [$I > 2\sigma(I)$] and were used in the final refinement, which converged to $R1 = 0.0434$, $wR2 = 0.1153$. Reflection data were collected on a Enraf-Nonius, Turbo CAD4 diffractometer. SIR 92 and SHELXL 93 were used for the stucture solution and for the full-matrix least squares refinedment of F^2.

Cytotoxicity by SBR assay

The antitumor activity of these compounds was assayed in vitro against various human tumor cell lines, and the results obtained are listed in Table 2. The IC_{50} values of compound **6a** for SK-OV-3 and HCT-15 were 0.08 and 0.09 μg/mL, respectively, which are superior or comparable to that of doxorubicine (0.13 and 0.07 μg/mL under the same conditions, respectively). The activity of compound **6a** was higher than **6b** by a factor of about 10. Such a difference in cytotoxicity seems to be due to the steric or electronic effect resulting from the altered methyl position.

Table 2. Cytotoxicity data on various human tumor cell lines

Compd	IC_{50} (μg/mL)				
	A549	SK-OV-3	SK-mel-2	XF-498	HCT-15
Doxorubicine	0.02	0.13	0.03	0.06	0.06
6a	0.12	0.08	0.09	0.11	0.07
6b	0.97	0.72	0.89	1.03	0.86
7	0.78	0.91	0.85	0.72	0.91

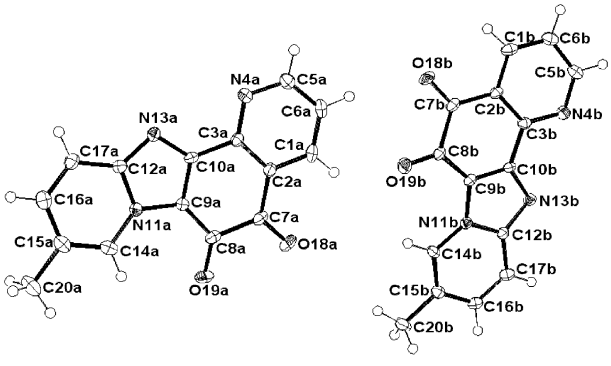


Figure 2. ORTEP drawing of **6b** with atomic labeling scheme. The labeling does not correspond to IUPAC nomenclature.

Conclusion

6,7-Dichloroquinoline-5,8-dione (**1**) was reacted with 2-aminopyridine derivatives. Because of the similarities of the four possible structures, which involved *ortho*- and *para*-quienone systems and their regioisomers, the actual structure of the products obatined could only unambigously be identified by X-ray crystallography. Accordingly, the products were found to have the 4a,10,11-triazabenzo[3,2-*a*]fluorene-5,6-dione structure, an *ortho*-quinone system (**B′**), which results from nucleophilic substitution at the C7-position, and the subsequently rearangement of the initial product. A reaction mechanism is proposed for the formation of these products. In addition, the compound **6a** was found to exhibit antitumor activity superior or comparable to doxorubicine.

Experimental

Matrials and methods

All melting points were taken in Pyrex capillaries using electrothermal digital melting point apparatus (Buechi). 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 400 MHz Varian FT-NMR spectrometer facility using trimethylsilane as an internal standard. Samples were dissolved in CF_3COOD and $CDCl_3$. Elemental analyses were performed using Thermo Quest (CE Instruments) EA 1110. Most reagents were purchased from Aldrich Chemical Company and Merck Company. 6,7-Dichloroquinoline-5,8-dione (**1**) was prepared accoring to the literature.[14]

2-Methyl-4a,10,11-triazabenzo[3,2-*a*]fluorene-5,6-dione (6a). To a mixture of 2.28 g (0.01 mol) of 6,7-dichloroquinoline-5,8-dione (**1**) and 1.363 g of potassium carbonate anhydrous in 20 mL of ethanol, 2.30 g (0.02 mol) of 2-amino-4-methyl-pyridine was added. The reaction mixture was refluxed for 15 h. It was cooled and filtered under reduced pressure. The filtered precipitation was crystallized from *o*-dichlorobenzene to give 1.87 g (71%) of red precipitation. Mp: over 250 °C; IR (KBr, cm^{-1}): 1701 (–C=O); ^{1}H NMR (CF_3COOD, δ): 11.3 (d, 1H), 11.0 (m, 2H), 10.1 (m, 1H), 9.9 (s, 1H, C-1), 9.6 (d, 1H), 3.8 (s, 3H, $-CH_3$). Anal. calcd for $C_{15}H_9N_3O_2$: C, 68.44; H, 3.45; N, 15.96. Found: C, 67.99; H, 3.52; N, 15.85.

3-Methyl-4a,10,11-triazabenzo[3,2-*a*]fluorene-5,6-dione (6b). To a mixture of 2.28 g (0.01 mol) of 6,7-dichloroquinoline-5,8-dione (**1**) and 1.363 g of potassium carbonate anhydrous in 20 mL of ethanol, 2.30 g (0.02 mol) of 2-amino-5-methyl-pyridine was added.

The reaction mixture was refluxed for 15 h. It was cooled and filtered under reduced pressure. The filtered precipitation was crystallized from *o*-dichlorobenzene to give 2.05 g (78%) of orange precipitation. Mp: over 250 °C; IR (KBr, cm^{-1}): 1650 (–C=O); ^1H NMR (CF_3COOD, δ): 10.5 (d, 1H, C-9), 10.2 (m, 2H, C-7, C-8), 9.3 (m, 3H), 3.8 (s, 3H, -CH_3). Anal. calcd for $C_{15}H_9N_3O_2$: C, 68.44; H, 3.45; N, 15.96. Found: C, 68.22; H, 3.33; N, 15.75.

3 - Methyl - 4a,5,10,14,15 - pentazabenz[3,2 - *a*]indeno[3,2-c]anthracene (7). A suspension of 0.5 g (1.9 mmol) of compound **6b** and 10 mL of acetic acid as acid catalyst in 30 mL of ethanol were stirred for 30 min. To the mixture, 0.205 g (1.9 mmol) of 1,2-phenylendiamine was dropped and heated under reflux for 10 min. It was cooled and filtered under reduced pressure. The filtered precipitation was crystallized from *o*-dichlorobenzene to produce 0.228 g (36%) of golden yellow precipitation. Mp: over 250 °C; ^1H NMR ($CDCl_3$, δ): 9.9 (d, 1H), 9.5 (d, 1H), 9.2 (d, 1H), 8.3 (d, 2H), 7.9 (t, 1H), 7.8 (m, 2H), 7.7 (s, 1H), 7.4 (d, 1H), 2.6 (s, 3H, –CH_3). Anal. calcd for $C_{21}H_{13}N_5$: C, 75.21; H, 3.91; N, 20.88. Found: C, 74.76; H, 3.76; N, 20.39.

Supporting Information

Crystallographic date (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publications nos. CCDC.190701. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB21EZ, UK (fax: +44-1223-336-033; e-mail; deposit@ccdc.cam.ac.uk).

Acknowledgements

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