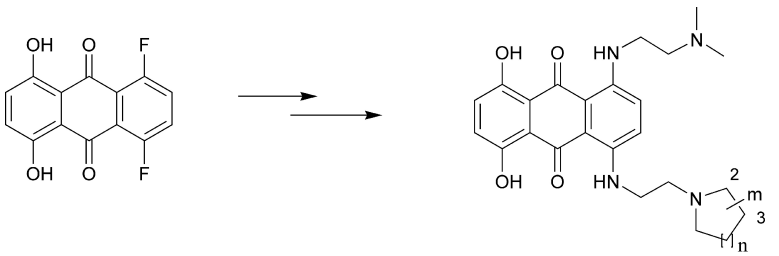
Journal of Medicinal Chemistry

Article

Development of Nonsymmetrical 1,4-Disubstituted Anthraquinones That Are Potently Active against Cisplatin-Resistant Ovarian Cancer Cells

Klaus Pors, Jane A. Plumb, Robert Brown, Paul Teesdale-Spittle, Mark Searcey, Paul J. Smith, and Laurence H. Patterson

J. Med. Chem., **2005**, 48 (21), 6690-6695• DOI: 10.1021/jm050438f • Publication Date (Web): 15 September 2005



n=1,2; m=OH, CH_2OH, CH_2Cl

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Development of Nonsymmetrical 1,4-Disubstituted Anthraquinones That Are Potently Active against Cisplatin-Resistant Ovarian Cancer Cells

Klaus Pors, Jane A. Plumb,† Robert Brown,† Paul Teesdale-Spittle,‡ Mark Searcey, Paul J. Smith,§ and Laurence H. Patterson*

Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29−39 Brunswick Square, London, WC1N 1AX, United Kingdom, CRUK Department of Medical Oncology, University of Glasgow, Glasgow, G61 1BD, United Kingdom, and Department of Pathology, School of Medicine, Cardiff University, Cardiff, CF14 4XN, United Kingdom

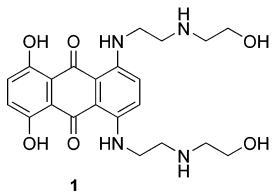
Received May 9, 2005

A novel series of 1,4-disubstituted aminoanthraquinones were prepared by ipso-displacement of 1,4-difluoro-5,8-dihydroxyanthraquinones by hydroxylated piperidinyl- or pyrrolidinylalkylamino side chains. One aminoanthraquinone (**13**) was further derivatized to a chloropropylamino analogue by treatment with triphenylphosphine−carbon tetrachloride. The compounds were evaluated in the A2780 ovarian cancer cell line and its cisplatin-resistant variants (A2780/cp70 and A2780/MCP1). The novel anthraquinones were shown to possess up to 5-fold increased potency against the cisplatin-resistant cells compared to the wild-type cells. Growth curve analysis of the hydroxyethylaminoanthraquinone **8** in the osteosarcoma cell line U-2 OS showed that the cell cycle is not frozen, rather there is a late cell cycle arrest consistent with the action of a DNA-damaging topoisomerase II inhibitor. Accumulative apoptotic events, using time lapse photography, indicate that **8** is capable of fully engaging cell cycle arrest pathways in G2 in the absence of early apoptotic commitment. **8** and its chloropropyl analogue **13** retained significant activity against human A2780/cp70 xenografted tumors in mice.

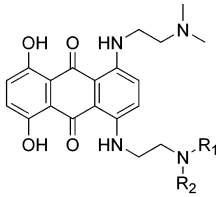
Introduction

Intrinsic or acquired resistance to chemotherapy is a major obstacle in the curative progress of patients. Patients are often highly responsive to initial treatment but relapse with tumors exhibiting a phenotype that is cross-resistant to not only the used chemotherapeutic agents but also to a wide variety of other anticancer drugs with different mechanisms of action.[1] Increasingly, there is a need for agents that can circumvent resistance arisen from exposure to first-line chemotherapeutic treatment.

Over the past 25 years, there have been extensive investigations on noncovalent DNA binding 1,4-disubstituted aminoanthraquinones.[2−7] Mostly, this has concerned symmetrically (identical side chains) substituted agents typified by mitoxantrone (**1**), a clinically utilized aminoanthraquinone that is a DNA intercalator and topo II inhibitor.[8] Compound **2** (AQ6) is the first nonsymmetrically 1,4-disubstituted aminoanthraquinone and was initially described by Patterson and coworkers.[9] **2** has been shown to possess enhanced cytotoxic potential under conditions of overexpression of topo II.[10] Krapcho et al. have shown **2** to be more cytotoxic than mitoxantrone in a colon carcinoma cell line (LoVo) and its doxorubicin-resistant variant (LoVo/Doxo).[7] The parent cell line has been shown to be deficient in MMR activity and also exhibits microsatelite instability (MSI).[11]



1



2 R_1 = H, R_2 = CH_2CH_2OH
3 R_1 = CH_3, CH_2CH_2OH
4 R_1 = R_2 = CH_2CH_2OH

In a study on novel aminoanthraquinones, we observed that two nonsymmetrical aminoanthraquinones, **3** and **4**, had considerable cytotoxic activity in a panel of ovarian cancer cell lines.[12,13] They were shown to be weakly cross-resistant with doxorubicin in 2780AD, a P-glycoprotein overexpressing ovarian cancer cell line.[12] Interestingly, **3** and **4** retained cytotoxic potency in the cisplatin-resistant ovarian adenocarcinoma A2780/cp70 subline, which has been characterized with elevated levels of glutathione,[14] alterations in drug uptake/efflux[15] and DNA repair mechanisms, and notably MMR deficiency.[16−18] MMR proteins are involved in recognition of DNA adducts induced by a variety of chemotherapeutic agents. Deficiency in either MHL1 or MSH2 has frequently been associated with resistance to a wide variety of conventional cytotoxic agents with different mechanism of action, including monofunctional alkylating agents, thiopurines and cisplatin.[19]

The combined data of **2**−**4** prompted us to develop a novel series of 1,4-disubstituted aminoanthraquinones and explore if other nonsymmetrically configured aminoanthraquinones would possess potent activity against cisplatin-resistant ovarian cancer cells. Here we disclose

* Corresponding author. Phone: +44 (0)1274 233226 (p.a.). Fax: +44 (0)1274 233234. E-mail: l.h.patterson@bradford.ac.uk.
† University of Glasgow.
‡ Present address: School of Biological Sciences, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand.
§ Cardiff University.

10.1021/jm050438f CCC: $30.25
Published on Web 09/15/2005

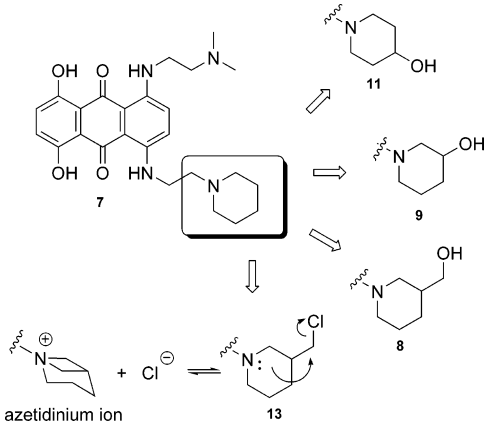
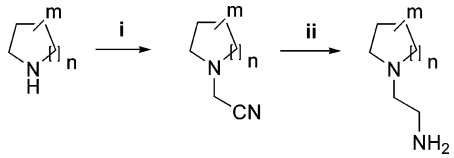


Figure 1. Attachment of a functional group on the piperidine.

Scheme 1[a]



[a] (i) $BrCH_2CN$, Et_3N, dry THF, 45−50 °C, (ii) $LiAlH_4$, dry THF, reflux; m = OH, CH_2OH, n = 1, 2.

the first report of novel agents that are significantly more toxic to cisplatin-resistant cells in culture and also maintain potent activity in human xenografted tumors in mice.

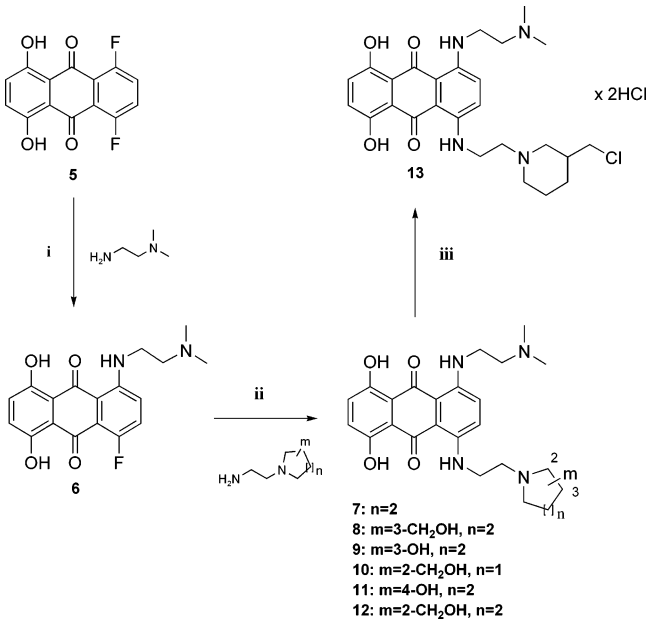
Results

Design of Nonsymmetrical 1,4-Disubstituted Aminoanthraquinones. 2−4 are compounds in which one of the two hydroxyethylaminoethyl side chains of mitoxantrone is replaced by a *N*,*N*-dimethylaminoethyl functionality. In the present study, we report on a number of agents that have retained the structural features important to the cytotoxicity of **2−4**. In addition, we decided to incorporate the hydroxyl group as part of a more rigid pyrollidinyl- or piperidinyl-containing sidearm in an attempt to obtain a more specific interaction with DNA and/or DNA-processing enzymes. In an attempt to explore the influence of the substituents on cellular uptake and drug accumulation, we decided to derivatize **8** to the chloropropyl analogue **13**, which has the potential of forming the stable quaternary bicyclic azetidinium ion (see Figure 1). The intramolecular transformation from ω-haloalkylamine to the corresponding quaternary ammonium compound has previously been investigated as a way to improve drug concentration at receptor sites.[20] Our synthesis of the compounds of interest was accomplished in two stages.

Synthesis of the Aminoalkylamino Side Chains. Synthesis of the 1-(2-aminoethyl)pyrrolidine and piperidine side chains was a two-step procedure. The cyclic secondary amine was alkylated by bromoacetonitrile (Et_3N, THF, 45−50 °C, 30 min, 63−92%) and the nitrile was converted to a primary amine by reduction with $LiAlH_4$ (THF, reflux, 5 h, 27−76%) (Scheme 1).

Synthesis of Aminoanthraquinones 7−13. Treatment of 1,4-difluoro-5,8-dihydroxyanthraquinone **5** with *N*,*N*-dimethylethylenediamine led to a mixture of the di- and monosubstituted anthraquinones from which the intermediate **6** was isolated by flash chromatography

Scheme 2[a]



[a] (i) Pyridine, rt, 24 h; (ii) pyridine, 90 °C, 0.5−1 h; (iii) Ph_3P, CCl_4, dry CH_2Cl_2, reflux, 5 H, ethereal HCl.

Table 1. Inhibition of Cell Growth by Pyrollidinyl- and Piperidinyl-Substituted Aminoanthraquinones

compound	A2780[b]: IC_{50}[a] (nM)	A2780/cp70[b] IC_{50}[a] (nM)	A2780/cp70[b] RF[c]	MCP1[b] IC_{50} (nM)	MCP1[b] RF
4	3.05 ± 0.56	1.53 ± 0.19	0.5	1.34 ± 0.04	0.4
7	102.0 ± 13.3	32.63 ± 5.64	0.3	21.01 ± 8.55	0.2
8	8.38 ± 1.14	1.59 ± 0.52	0.2	1.92 ± 0.18	0.2
9	10.8 ± 1.5	5.01 ± 0.82	0.5	4.97 ± 0.77	0.5
10	3.54 ± 0.6	1.16 ± 0.25	0.3	1.05 ± 0.20	0.3
11	6.08 ± 0.25	2.77 ± 0.51	0.5	4.24 ± 0.42	0.7
12	1.89 ± 0.6	0.51 ± 0.23	0.3	nd[d]	nd
13	101 ± 12	35.4 ± 2.1	0.4	36.8 ± 3.1	0.4
cisplatin	253 ± 26	1440 ± 111	5.7	348 ± 16	1.4
doxorubicin	4.98 ± 1.07	13.3 ± 0.9	2.6	nd	nd

[a] IC_{50} is the concentration of drug (nM) required to inhibit cell growth by 50% and is recorded as the mean ± the standard error (n = 3). [b] A2780 is the wild-type ovarian cell line; A2780/cp70 and MCP1 are cisplatin-resistant variants. [c] RF = resistance factor (IC_{50} in resistant cell line/IC_{50} in parent cell line). [d] nd = not determined.

(*N*,*N*-dimethylethylenediamine, C_5H_5N, 22 °C, 24 h, 34%) (Scheme 2). Pure **6** was then treated with the piperidine or pyrrolidine amine side chains to afford the deshydroxylated analogue **7** and the target compounds **8−12** and in good yield after purification (amine, C_5H_5N, 90 °C, 0.5−1 h, 51−65%). Preparation of the chloropropylaminoanthraquinone **13** was carried out by treating the precursor alcohol **8** with triphenylphosphine−carbon tetrachloride, a commonly employed complex reagent for conversion of alcohols to corresponding halides[21,22] (Ph_3P, CCl_4, CH_2Cl_2, reflux, N_2, 5 h, 68−81%). It should be noted that all compounds, with exception of **10** (*S*-enantiomer), are racemic mixtures.

Biological Evaluation. Table 1 compares the cytotoxicity of a series of nonsymmetrical 1,4-disubstituted anthraquinones in the A2780 ovarian cancer cell line and its independent cisplatin-resistant variants (A2780/cp70 and A2780/MCP1), which have demonstrated several resistance mechanisms, including deficiency in the MMR protein hMLH1.[18] Compound **4** was included since this was the first agent previously to suggest that

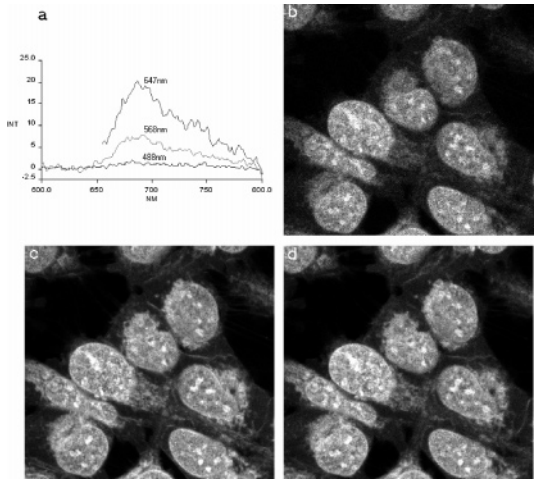


Figure 2. Fluorescence analysis of **8** in live cells to identify nuclear localization. Panel a shows the emission spectra for different excitation wavelengths showing poor excitation efficiency at blue versus red wavelengths. The montage of confocal images (far red emission) of live cells exposed to 10 μM **8** (1 h) and then excited at different wavelengths: 488 nm (b), 568 nm (c), and 647 nm (d). The confocal (BioRad 1024MP system) images were derived using 40× oil objective and 1.5× zoom. Each image has been normalized for peak pixel intensity to provide comparisons of drug distributions analyzed at suboptimal (488 nm) and optimal (647 nm) excitation wavelengths.

the nonsymmetrical 1,4-disubstitution pattern of cytotoxic anthraquinones has an interesting antitumor profile.[12,13] Compound **4** has been shown to possess high DNA-affinity, as determined by DNA thermal denaturation,[13] and is also a potent inhibitor of topo IIα.[12] The archetypical topo II inhibitor, doxorubicin, was >2.5-fold less active in the A2780/CP70 cell line.

All the agents possessed significantly increased potency against the cisplatin resistant cells compared to parent cells with the primary alcohol derivatives of piperidinyl or pyrollidinyl compounds **8**, **10**, and **12** demonstrating the most increase in activity. Compounds **7** and **13** respectively demonstrate that removal of the hydroxyl group from or substitution of hydroxymethyl for chloromethyl in the piperidinyl ring diminished cytotoxic activity some 10−25-fold, although the increased sensitivity to resistant cells was still maintained.

We decided to further investigate **8** because it exhibited the highest increase in potency against the resistant cells compared with the parent cells. We sought to establish the biological characteristics of **8** in tumor cells with functional capacity to express cellular stress responses. The characteristics studied were nuclear targeting and cytotoxicity (apoptosis induction). We further studied the cytostatic (cell cycle arrest) properties, as cisplatin-treated cells deficient in MMR have been reported to show reduced G2 arrest. The human osteosarcoma cell line U-2 OS was used because it is known to be functional for DNA-damage-induced transcriptional activation of p53. The tracking of nuclear targeting in live cells was achieved by exploiting the natural fluorescence signature of **8** (Figure 2) and the spatial resolution of confocal laser scanning microscopy. The emission spectra indicate optimal excitation at 647 nm and far-red emission. Figure 2 demonstrates clear nuclear localization of **8**, consistent with the properties

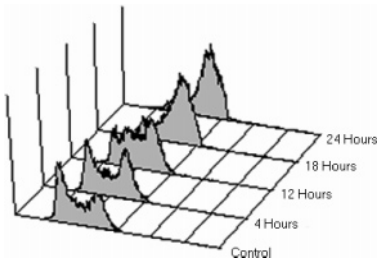


Figure 3. Frequency versus DNA content histogram demonstrating the effect of 100 nM **8** on the cell cycle of U-2 OS cells. Cells were treated for 4, 12, 18, and 24 h with the drug, stained with the DNA dye DRAQ5, and analyzed by flow cytometry (10 000 events were collected). The drug dose was selected from growth curve analysis to yield maximal loss of mitotic potential

Table 2. Tumor Doubling Times (days)[a]

tumor	control	**8**[b]	**13**
A2780	2.94 ± 0.34	5.23 ± 0.39 177%[c]	5.83 ± 0.40 198%
A2780/cp70	2.52 ± 0.17	4.33 ± 0.16 172%	4.18 ± 0.27 166%

[a] Calculated as time taken for tumor to reach twice the initial volume. [b] Percent increase in median life span (calculated as time taken for tumor to reach twice the initial volume). [c] **8** (20 mg/kg) and **13** (16 mg/kg) were both administered as a single-dose ip.

of a DNA-binding agent. Figure 3 shows the frequency versus DNA content histograms for cells treated with **8** for up to 24 h. The drug dose used was selected from growth curve analysis to yield maximal cytostatic action (i.e. loss of mitotic potential). Here we show that the cell cycle is not frozen, rather there is a late cell cycle arrest consistent with the action of a DNA-damaging topo II inhibitor. To track any coexpression of cell death events, we have used visual observation of time-lapse microscopy images to count the number of cells undergoing apoptosis to provide cumulative values for apoptotic events over a 24-h drug exposure period. Apoptotic events for **8** expressed as mean percent (±SD) of total cells counted were 1.5 ± 0.5 (control), 10.4 ± 3.7 (0.1 nM), 7.5 ± 2.8 (1.0 nM), 1.2 ± 2.1 (10 nM), and 3.0 ± 1.2 (100 nM). The cumulative apoptotic events indicate that **8** acts as a cell cycle arresting agent in G2 with low levels of apoptosis. The significantly ($p < 0.05$) lower levels cell death for high (10−100 nM) compared with low (0.1−1 nM) doses may reflect nonarrested cells undergoing mitotic catastrophe.

8 and its chloropropyl congener **13** were investigated in mice bearing human xenografted tumors (A2780 and A2780/cp70). Table 2 shows that both agents produced a significant antitumor effect, albeit no enhanced cell-killing effect was observed against the cisplatin-resistant xenografted tumor.

Discussion

Despite a high initial response rate (up to 80%) to chemotherapeutic treatment of ovarian cancer, the majority of the patients relapse eventually with drug-resistant disease.[23] In vitro, resistance to cisplatin-treated ovarian cancer cells have been characterized with elevated levels of glutathione[14] and alterations in drug uptake/efflux[15] and DNA repair mechanisms including MMR deficiency.[16−18]

An attractive approach to overcoming resistance caused by first-line chemotherapeutic agents is the

development of agents that exploit acquired resistant mechanisms. Currently, second-line therapy against ovarian carcinomas includes epirubicin, etoposide, and gemcitabine, which at best produces a palliative effect for the patients.[23] We are raising the question whether it is possible to develop second-line agents that not only circumvent the resistance mechanisms acquired through treatment with first-line chemotherapeutic agents but also can reveal processes that increase the potency of selected agents.

Cisplatin is one of the most commonly employed anticancer drugs in the clinic; thus, we have initially concentrated on screening novel agents against cisplatin-resistant cells. In this study, we have identified agents that are significantly more active in vitro against ovarian cancer cells resistant to cisplatin (A2780/CP70 and A2780/MCP1) than against the parent nonresistant cells (A2780). The equivalent activity of **8** against A2780 and A2780/CP70 ovarian cancer xenografts after a single dose is encouraging and suggests that more selective agents can be identified. Our results suggest that the presence of cisplatin-resistance mechanisms in the ovarian cancer cells actually sensitizes them to treatment with the novel nonsymmetrically substituted anthraquinones described in this paper. It has been shown previously that loss of either hMSH2 or hMLH1 in human colorectal cancer cell lines is associated with resistance to the intercalating topo II inhibitors doxorubicin, epirubicin, and mitoxantrone.[24] On this basis, it is intriguing that, for example, **4**,[12] which we have shown to inhibit topo IIα, is more cytotoxic in hMLH1-deficient ovarian cancer cells. We believe that the nonsymmetrical configuration on which these anthraquinone analogues are based is vital for this unique activity and are continuing investigations to understand their mechanism of action.

Experimental Section

Proton and carbon NMR were analyzed on a Bruker AM 250 MHz nuclear magnetic resonance spectrometer. Chemical shifts are reported in ppm downfield from internal TMS. Fast atom bombardment (FAB+) mass spectra sample identification using 20 kV Cs^+ ion bombardment was accomplished on a ZAB SE instrument. Microelemental analysis was performed using a Carlo-Erba EA 1108 instrument with a PC-based data system, Eager 200 for Windows, and Sartorious ultramicro balance 4504 MP8. IR spectra were recorded on a Nicolet 205 FTIR spectrophotometer.

3-Hydroxypiperidin-1-ylacetonitrile. $BrCH_2CN$ (3.19 g, 25.82 mmol) was added dropwise to a solution of 3-hydroxypiperidine (6.53 g, 64.55 mmol) in dry THF (25 mL) under N_2, while the temperature was maintained between 45 and 50 °C. Following addition of $BrCH_2CN$, the solution was refluxed for 30 min, before allowing the solution to cool to room temperature. The solvent was removed in vacuo and the residual oil was purified by flash chromatography using CH_2Cl_2:CH_3OH (9:1) as eluent. The title compound was obtained as a straw-colored oil (3.04 g, 83%).

3-Hydroxymethylpiperidin-1-ylacetonitrile. The method follows that of 3-hydroxypiperidinylacetonitrile using 3-piperidinemethanol (4.79 g, 41.59 mmol), $BrCH_2CN$ (1.99 g, 16.64 mmol), and dry THF (25 mL). The title compound was yielded as a straw-colored oil (2.35 g, 92%) after flash chromatography using CH_2Cl_2/CH_3OH (9:1) as eluent.

4-Hydroxypiperidin-1-ylacetonitrile. $BrCH_2CN$ (28.55 g, 0.238 mmol) was added dropwise to a solution of 4-hydroxypiperidine (24.98 g, 0.216 mol) and Et_3N (33.18 mL, 0.238 mmol) in dry THF (100 mL) under N_2, while the temperature was maintained between 45 and 50 °C. After addition of $BrCH_2CN$, the solution was refluxed for 30 min, before allowing the solution to cool to room temperature. The title compound was afforded as a straw-colored oil (18.97 g, 63%) after purification by flash chromatography using ether/CH_3OH (19:1) as eluent.

2-Hydroxymethylpiperidin-1-ylacetonitrile. The method follows that of 4-hydroxypiperidinylacetonitrile using 2-piperidinemethanol (26.50 g, 0.230 mol), $BrCH_2CN$ (30.35 g, 0.253 mol), Et_3N (35.27 mL, 0.253 mol), and dry THF (150 mL). The crude product was purified by flash chromatography using ether/CH_3OH (19:1) as eluent. The title compound was crystallized from ether, yielding cream-colored crystals (31.46 g, 89%).

2-Hydroxymethylpyrrolidin-1-ylacetonitrile. The method follows that of 4-hydroxypiperidinylacetonitrile using (*S*)-(+)-2-pyrrolidinemethanol (24.27 g, 0.241 mol), $BrCH_2CN$ (31.81 g, 0.265 mol), Et_3N (37 mL, 0.265 mol), and dry THF (150 mL). The product was obtained as a straw-colored oil (21.60 g, 64%) after purification by flash chromatography using ether/CH_3OH (19:1).

1-(2-Aminoethyl)piperidin-3-ol. $LiAlH_4$ (2.44 g, 64.2 mmol) was added to dry THF (20 mL) at 0 °C in a three-neck round-bottom flask under N_2. The solution was stirred for 15 min before the 3-hydroxypiperidin-1-ylacetonitrile (3 g, 21.4 mmol), diluted in dry THF (5 mL), was added slowly via syringe. The reaction mixture was then refluxed for 5 h before allowing the solution to cool to room temperature. Excess $LiAlH_4$ was destroyed by dropwise addition of 2.4 mL of H_2O and 2.4 mL of NaOH (15%), and finally EtOAc was added dropwise until no effervesence was observed. The formed granular precipitate (lithium hydroxide and aluminum hydroxide) was filtered off and washed several times with CH_2Cl_2 and EtOAc. The organic layer was dried ($MgSO_4$) and the solvent was removed in vacuo to yield a thick yellowish oil. The title-compound was purified by Kugelrohr distillation (172 °C, 0.05 mbar) and obtained as a straw colored oil (1.95 g, 63%).

[1-(2-Aminoethyl)-3-piperidin-2-yl]methanol. The method follows that of 1-(2-aminoethyl)piperidin-3-ol using 3-(hydroxymethyl)piperidinylacetonitrile (2.95 g, 19.1 mmol), $LiAlH_4$ (2.18 g, 57.3 mmol), and dry THF (15 mL). The title compound (2.30 g, 76%) was afforded as a colorless oil by Kugelrohr distillation at (164 °C, 0.01 mbar).

1-(2-Aminoethyl)piperidin-4-ol. The method follows that of 1-(2-aminoethyl)piperidin-3-ol using 4-hydroxypiperidinylacetonitrile (18.97 g, 0.136 mol), $LiAlH_4$ (15.48 g, 0.408 mol), and dry THF (150 mL). The title compound (8.56 g, 44%) was afforded as a straw-colored oil after Kugelrohr distillation (178 °C, 0.05 mbar).

[1-(2-Aminoethyl)piperidin-2-yl]methanol. The method follows that of 1-(2-aminoethyl)piperidin-3-ol using 2-(hydroxymethyl)piperidin-1-ylacetonitrile (31.96 g, 0.208 mol), $LiAlH_4$ (23.68 g, 0.624 mol), and dry THF (200 mL). The title compound (8.74 g, 27%) was afforded as a straw-colored oil by Kugelrohr distillation (225 °C, 0.13 mbar).

[1-(2-Aminoethyl)pyrrolidin-2-yl]methanol. The method follows that of 1-(2-aminoethyl)piperidin-3-ol using 2-(hydroxymethyl)pyrrolidin-1-ylacetonitrile (19.5 g, 0.139 mol), $LiAlH_4$ (15.84 g, 0.417 mol), and dry THF (150 mL). The title compound (12.5 g, 63%) was afforded as a straw-colored oil by Kugelrohr distillation (142 °C, 0.3 mbar).

1-[2-(Dimethylamino)ethylamino]-4-fluoro-5,8-dihydroxyanthracene-9,10-dione (6). 1,4-Difluoro-5,8-dihydroxyanthraquinone (0.50 g, 1.812 mmol), *N*,*N*-dimethylethylenediamine (0.16 g, 1.812 mmol), and pyridine (3 mL) were stirred for 24 h at room temperature. The mixture was quenched in cold brine (50 mL) and left for 3 h before the crude product was isolated by filtration. The crude product was chromatographed using a gradient elution from 1 to 5% CH_3OH in CH_2Cl_2. The product **8** was afforded as a purple powder (0.24 g, 38%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(piperidin-1-yl)-ethylamino]-5,8-dihydroxyanthracene-9,10-dione (7). 1,4-Difluoro-5,8-hydroxyanthraquinone (62 mg, 0.18 mmol) and 1-(2-aminoethyl)piperidine (250 mg, 1.953 mmol) were stirred

in pyridine (2 mL) at 90 °C for 1 h. The reaction mixture was added to ice-cold brine and set aside at 4 °C overnight. The precipitated solid was isolated by filtration and lyophilized. The desired product was purified by flash chromatography, initially eluting with CH_2Cl_2/CH_3OH (95:5) to remove nonpolar impurities, followed by a gradual increase of CH_3OH to $CH_2Cl_2/CH_3OH:NH_3$ (93.5:6:0.5). The product was afforded as a dark blue powder (42.9 mg, 65%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(3-hydroxymethylpiperidin-1-yl)ethylamino]-5,8-dihydroxyanthracene-9,10-dione (8). The method follows that of **7** using **6** (200 mg, 0.581 mmol), [1-(2-aminoethyl)piperidin-3-yl]-methanol (350 mg, 2.215 mmol), and pyridine (2 mL), at 90 °C, for 30 min. The product was afforded as a dark blue powder (190 mg, 68%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(3-hydroxypiperidin-1-yl)ethylamino]-5,8-dihydroxyanthracene-9,10-dione (9). The method follows that of **7** using **6** (120 mg, 0.349 mmol), 1-(2-aminoethyl)piperidin-3-ol (150 mg, 1.047 mmol), and pyridine (1 mL), for 30 min, at 90 °C. The product was afforded as a dark blue powder (95 mg, 58%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(2-hydroxyethylpyrrolidin-1-yl)ethylamino]-5,8-dihydroxyanthracene-9,10-dione (10) The method follows that of **7** using **6** (75 mg, 0.218 mmol), [1-(2-aminoethyl)-pyrrolidin-2-yl-]methanol (650 mg, 4.114 mmol), and pyridine (2 mL), for 1 h, at 90 °C. The product was afforded as a dark blue powder (52 mg, 51%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(4-hydroxypiperidin-1-yl)ethylamino]-5,8-dihydroxyanthracene-9,10-dione (11) The method follows that of **7** using **6** (18 mg, 0.0523 mmol), *N*-(2-aminoethyl)piperidin-4-ol (140 mg, 0.97 mmol), and pyridine (1 mL), for 1 h, at 90 °C. The product was afforded as a dark blue powder (16.1 mg, 65%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(2-hydroxyethylpiperidin-1-yl)ethylamino]-5,8-dihydroxyanthracene-9,10-dione (12). The method follows that of **7** using **6** (30 mg, 0.0872 mmol), [1-(2-aminoethyl)-piperidin-2-yl-]methanol (700 mg, 4.43 mmol), and pyridine (1 mL), for 30 min, at 100 °C. The product was afforded as a dark blue powder (21.3 mg, 51%).

1-[(2-Dimethylamino)ethylamino]-4-[2-(2-chloromethylpiperidin-1-yl)ethylamino]-5,8-dihydroxyanthracene-9,10-dione (13). Ph_3P (180 mg, 0.686 mmol) and CCl_4 (400 μL, 4.145 mmol) were stirred for 15 min before the mixture was added dropwise to a stirred solution of **8** (110 mg, 0.228 mmol) in dry CH_2Cl_2 (10 mL) under N_2 at reflux temperature. The reaction mixture was kept at reflux temperature for 5 h before it was cooled to room temperature. Ethereal HCl was added to the solution, and after 1 h of stirring, the precipitated solid was filtered off. To remove excess Ph_3P and Ph_3PO, the precipitated solid was dissolved in warm CH_3OH (10 mL). While the dark blue solution stirred at reflux, a mixture of EtOAc and EtOH (1:1) was added until precipitation of solid was observed. The solution was set aside for 1 h before the precipitated product was isolated by filtration; the excess Ph_3P and Ph_3PO remained in the EtOAc/EtOH solution. The title compound was afforded as a dark blue solid (78 mg, 60%).

Biology. In Vitro Studies. Cytotoxicity (IC_{50}) was investigated with ovarian carcinoma cell lines A2780, 2780/cp70 (also known as A2780CisR or CP70), and MCP1. Drug sensitivity was determined using a 96-well plate based MTT assay with a 24-h drug exposure period and a 3 day growth period. The human osteosarcoma cell line U-2 OS (ATCC HTB-96) was grown as a monolayer and inoculated into 6-well plates for time-lapse imaging or confocal imaging and cell cycle analysis.[25] All cells were maintained in RPMI-1640 medium containing glutamine (2 mM) and fetal calf serum (10%). The cisplatin-resistant sublines were obtained as previously described.[18]

In Vivo Studies. Monolayer cultures were harvested with trypsin/EDTA (0.25%/1 mM in PBS) and resuspended in PBS. Approximately 10^7 cells were injected subcutaneously into the right flank of athymic female nude mice (MF1 nu/nu mice from Harlan Olac). After 10−15 days, when the mean tumor diameter was at least 0.5 cm, animals were randomized into groups of six for experiments, and cytotoxic drugs were administered intraperitoneally. **8** and **13** were dissolved in DMSO and then diluted with sterile water to give a final concentration of 10%. Mice were weighed daily and tumor volumes were estimated by calliper measurements assuming spherical geometry (volume = $d^3\pi/6$).

Supporting Information Available: Spectroscopic data of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Baird, R. D.; Kaye, S. B. Drug Resistance Reversal—Are We Getting Closer? *Eur. J. Cancer* **2003**, *39*, 2450−2461.

(2) Stefanska, B.; Dzieduszycka, M.; Martelli, S.; Borowski, E. Synthesis of Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)-amino]anthracene-9,10-diones as Potential Antileukemic Agents. *J. Med. Chem.* **1989**, *32*, 1724−1728.

(3) Krapcho, A. P.; Landi, J. J.; Shaw, K. J.; Phinney, D. G.; Hacker, M. P.; McCormack, J. J. Synthesis and Antitumor Activities of Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones and Related Systems. *J. Med. Chem.* **1986**, *29*, 1370−1373.

(4) Johnson, R. K.; Zee-Cheng, R. K.; Lee, W. W.; Acton, E. M.; Henry, D. W.; Cheng, C. C. Experimental Antitumor Activity of Aminoanthraquinones. *Cancer Treat. Rep.* **1979**, *63*, 425−439.

(5) Zee-Cheng R. K. Y.; Mathew A. E.; Xu P.; Northcutt R. V.; Cheng C. C. Structural Modification Study of Mitoxantrone (DHAQ). Chloro-Substituted Mono- and Bis[(aminoalkyl)amino]anthraquinones. *J. Med. Chem.* **1987**, *30*, 1682−1686.

(6) Murdock, K. C.; Child, R. G.; Fabio, P. F.; Angier, R. B.; Wallace, R. E.; Durr, F. E.; Citarella, R. V. Antitumor Agents. 1,4-Bis-[(aminoalkyl)amino]-9,10-anthracenediones. *J. Med. Chem.* **1979**, *22*, 1024−1030.

(7) Krapcho, A. P.; Zelleka, G.; Avery, K. L.; Vargas, K. J.; Hacker, M. P. Synthesis and Antitumor Evaluations of Symmetrical and Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]-5,8-dihydroxyanthracene-9,10-diones. *J. Med. Chem.* **1991**, *34*, 2373−2380.

(8) Faulds, D.; Balfour, J. A.; Chrisp, P.; Langtry, H. D. Mitoxantrone, A Review of its Pharmacodynamic and Pharmacokinetic Properties, and Therapeutic Potential in the Chemotherapy of Cancer. *Drugs* **1991**, *41*, 400−449.

(9) Patterson, L. H. Anthraquinone Anti-Cancer Compounds with (Disubstituted-amino-*N*-oxide)alkylamino Substituent. UK Patent No. 2237283, 1991.

(10) Smith, P. J.; Blunt, N. J.; Desnoyers, R.; Giles, Y.; Patterson, LH. DNA Topoisomerase II-Dependent Cytotoxicity of Alkylaminoanthraquinones and their *N*-oxides. *Cancer Chemother. Pharmacol.* **1997**, *39*, 455−461.

(11) Watanabe, Y.; Haugen-Strano, A.; Umar, A.; Yamada, K.; Hemmi, H.; Kikuchi, Y.; Takano, S.; Shibata, Y.; Barrett, J. C.; Kunkel, T. A.; Koi, M. Complementation of an hMSH2 Defect in Human Colorectal Carcinoma Cells by Human Chromosome 2 Transfer. *Mol. Carcinog.* **2000**, *29*, 37−49.

(12) Pors, K.; Paniwnyk, Z.; Teesdale-Spittle, P. H.; Plumb, J. A.; Willmore, E.; Austin, C. A.; Patterson, L. H. Alchemix: A novel Alkylating Anthraquinone with Potent Activity against Anthracycline- and Cisplatin-Resistant Ovarian Cancer. *Mol. Cancer Ther.* **2003**, *2*, 607−610.

(13) Pors, K.; Paniwnyk, Z.; Ruparelia, K. C.; Teesdale-Spittle, P. H.; Hartley, J. A.; Kelland, L. R.; Patterson, L. H. Synthesis and Biological Evaluation of Novel Chloroethylaminoanthraquinones with Potent Cytotoxic Activity against Cisplatin Resistant Tumour Cells. *J. Med. Chem.* **2004**, *47*, 1856−9.

(14) Jansen B. A.; Brouwer J.; Reedijk J. Glutathione Induces Cellular Resistance against Cationic Dinuclear Platinum Anticancer Drugs. *J. Inorg. Biochem.* **2002**, *89*, 197−202.

(15) Parker R. J.; Eastman A.; Bostick-Bruton F.; Reed E. Acquired Cisplatin Resistance in Human Ovarian Cancer Cells Is Associated with Enhanced Repair of Cisplatin-DNA Lesions and Reduced Drug Accumulation. *J. Clin. Invest.* **1991**, *87*, 772−777.

(16) Kelland L. R.; Barnard C. F.; Mellish K. J.; Jones M.; Goddard P. M.; Valenti M.; Bryant A.; Murrer B. A.; Harrap K. R. A Novel Trans-Platinum Coordination Complex Possessing in Vitro and in Vivo Antitumour Activity. *Cancer Res.* **1994**, *54*, 5618−22.

(17) Strathdee, G.; MacKeen, M. J.; Illand, M.; Brown, R. A Role for Methylation of the hMLH1 Promoter in Loss of hMLH1 Expression and Drug Resistance in Ovarian Cancer. *Oncogene* **1999**, *18*, 2335−2341.

(18) Brown, R.; Hirst, G. L.; Gallagher, W. M.; McIlwrath, A. J.; Margison, G. P.; van der Zee, A. G.; Anthoney, D. A. hMLH1 Expression and Cellular Responses of Ovarian Tumour Cells to Treatment with Cytotoxic Anticancer Agents. *Oncogene* **1997**, *15*, 45−52.

(19) Stoijic, L.; Brun, R.; Jiricny J. Mismatch Repair and DNA Damage Signaling. *DNA Repair* **2004**, *3*, 1091−1101.
(20) Levine, R. R.; Weinstock, J.; Zirckle, C. S.; Mclean R. The Intestinal Absorption of Some *Omega*-Haloalkylamines and Their Quaternary Analogues. *J. Pharmacol. Exp. Ther*. **1961**, *131*, 334−340.
(21) Appel, R. *Angew. Chem. Int. Ed*. **1975**, *14*, 801−811.
(22) Jones, L. A.; Sumner, C. E.; Franzus, B.; Huang, T. T. S.; Snyder, E. I. The Intermediate from the Triphenylphosphine-Tetrachloromethane-Alcohol Reaction: Relative Rates of Intermediate Formation, Kinetics and Mechanism of Intermediate Decomposition. *J. Org. Chem*. **1978**, *43*, 2821−2826.
(23) Moss C.; Kaye S. B. Ovarian Cancer: Progress and Continuing Controversies in Management. *Eur. J. Cancer*. **2002**, *38*, 1701−1707.
(24) Fedier A.; Schwarz V. A.; Walt H.; Carpini R. D.; Haller U.; Fink D. Resistance to Topoisomerase Poisons Due to Loss of DNA Mismatch Repair. *Int. J. Cancer* **2001**, *93*, 571−576.
(25) Marquez, N.; Chappell, S. C.; Sansom, O. J.; Clarke, A. R.; Teesdale-Spittle, P, Errington, R. J.; Smith, P. J. Microtubule Stress Modifies Intra-Nuclear Location of Msh2 in Mouse Embryonic Fibroblasts. *Cell Cycle* **2004**, *3*, 662−71.

JM050438F