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Synthesis of DNA-Directed Pyrrolidinyl and Piperidinyl Confined Alkylating Chloroalkylaminoanthraquinones: Potential for Development of Tumor-Selective *N*-Oxides

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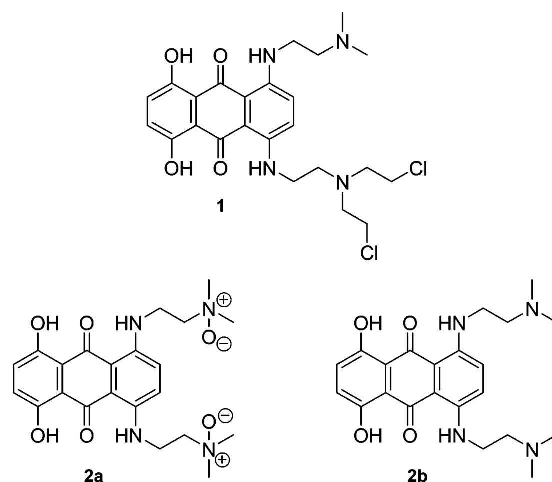
A novel series of 1,4-disubstituted chloroethylaminoanthraquinones, containing alkylating chloroethylamino functionalities as part of a rigid piperidinyl or pyrrolidinyl ring-system, have been prepared. The target compounds were prepared by *ipso*-displacement of halides of various anthraquinone chromophores by either hydroxylated or chlorinated piperidinyl- or pyrrolidinyl-alkylamino side chains. The chloroethylamino-anthraquinones were shown to alkylate guanine residues of linearized pBR322 (1−20 μM), and two symmetrically 1,4-disubstituted anthraquinones (compounds 14 and 15) were shown to interstrand cross-link DNA in the low nM range. Several 1,4-disubstituted chloroethylaminoanthraquinones were potently cytotoxic (IC_{50} values: $\leq$40 nM) in human ovarian cancer A2780 cells. Two agents (compounds 18 and 19) exhibited mean GI_{50} values of 96 nM and 182 nM, respectively, in the NCI human tumor cell line panel. Derivatization of the potent DNA cross-linking agent 15 to an *N*-oxide resulted in loss of the DNA unwinding, DNA interstrand cross-linking and cytotoxic activity of the parent molecule.

Introduction

DNA intercalating topoisomerase inhibitors are an important class of clinically useful antitumor agents. The anthraquinone pharmacophore is an essential structural feature in this class of therapeutics identified to date, notably in daunorubicin, doxorubicin, epirubicin, and mitoxantrone.[1] Substantial work has identified a well-defined relationship between the basic amino-alkylamino side chains and the configuration of functional groups attached to the 1,4-disubstituted pharmacophore of mitoxantrone.[2−7] The clinical success of the anthraquinone-based anticancer drugs is tempered by their failure in resistant tumors expressing the ABCB1 (MDR1) gene[8,9] or downregulation/mutation[10] or phosphorylation[11] of topoisomerase II (topo II). In an attempt to overcome these resistance mechanisms, we have reported on 1,4-disubstituted chloroethylaminoanthraquinones as a novel class of intercalators with alkylating functionalities,[12] of which one agent, alchemix (ZP281M), possesses substantial anticancer activity against doxorubicin (A2780AD) and cisplatin resistant (A2780/cp70) xenografted tumors in mice.[13] Alchemix (**1**) is a nonsymmetrical (mixed side chains) 1,4-disubstituted anthraquinone with a bisalkylating functionality confined to one sidearm.



We suggest that *N*-chloroethylamino- moieties tethered to anthraquinones irreversibly bind in a drug−DNA-topo II ternary complex thereby preventing topo II dissociation and cell efflux. Paradoxically, the high DNA affinity of the anthraquinone based intercalators, while essential to their efficacy, also promotes normal tissue sequestration limiting their intratumoral distribution.[14] Inevitably, slow release from normal tissue will have a major negative effect on an available drug reaching the tumor as well as promoting organ specific toxicity. Sequestration in tumor tissue close to the vasculature and its subsequent removal will limit the duration and extent of tumor exposure. This could substantially diminish the antitumor efficacy despite the known potency of both noncovalent and covalent DNA binding anthraquinones. In recognition of the improved pharmacology of anthraquinones that covalently complex DNA/topo II, but potential limitations of the nonspecific chemical reactivity of the *N*-chlorethylamino moieties, we are exploring more stable DNA-affinic alkylating agents.

In a novel series of compounds, we have prepared the *N*-chloroethylamino moiety with alkylating potential as part of a rigid piperidinyl or pyrrolidinyl ring-system. We wanted to explore if the ring-constraint would curtail the reactivity and lead to a more controlled and specific interaction with DNA. A further feature of these agents is their potential for *N*-oxide formation. We have pioneered the *N*-oxides of DNA binding agents as prodrugs that can be bio-activated in the hypoxic fraction of tumors to potent DNA intercalating topo II inhibitors. As a result of this, AQ4N (banoxantrone, 1,4-bis-[[2-(dimethyl-amino-*N*-oxide)ethyl]amino]5,8-dihydroxyanthracene-9,10-di-

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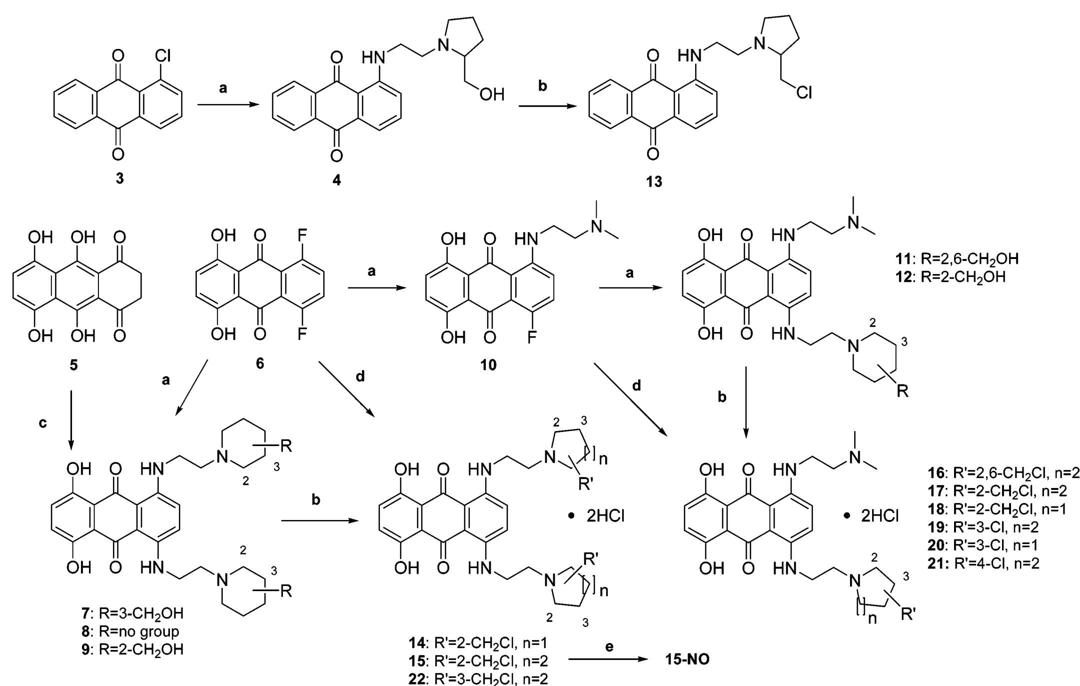
Scheme 1



Reactants and conditions: (a) aminoalkylamino side chain, pyridine, 90 °C, $^1/_2$−1 h; (b) $(Ph)_3P$, CCl_4, CH_2Cl_2, reflux, 3−10 h, ethereal HCl; (c) aminoalkylamino side chain, reflux, EtOH; (d) chlorinated aminoalkylamino side chain (see Scheme 2), pyridine, 30−60 °C, 2−5 h, ethereal HCl; (e) *m*-CPBA, −15 °C, 6 h (see Figure 5 for structure).

one, **2a**) is in advanced phase I trials against a range of solid tumors. No normal tissue toxicity has been demonstrated in the patients treated, but significantly, AQ4N has been shown to release the active agent AQ4 (1,4-bis-[[2-(dimethylamino)ethyl]-amino]5,8-dihydroxyanthracene-9,10-dione, **2b**) at cytotoxic concentrations within tumor tissue.[15]

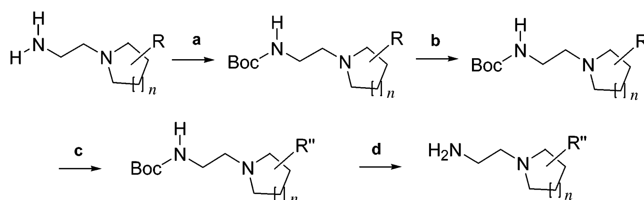
Here we report on the synthesis, DNA interstrand cross-linking, DNA sequence selectivity, and cytotoxicity of novel symmetrical and nonsymmetrical 1,4-disubstituted piperidinyl and pyrrolidinyl ring-constrained chloroalkylaminoanthraquinones. We describe also the influence of *N*-oxide formation on this biological activity.

Results and Discussion

Chemistry. The synthetic route to the target compounds involved preparation of the heterocyclic (piperidinyl or pyrrolidinyl) alkylamino side chains by alkylating the heterocyclic moiety with bromoacetonitrile, followed by reduction of the nitrile to the respective primary amine, as previously described.[16] The heterocyclic alkylamino side chains were then substituted onto the anthraquinone moiety by *ipso* displacement of the halide groups of the chromophores **3**, **6**, and **10** (Scheme 1). The exception to this was compound **7**, which was prepared by condensation of 5,8-dihydroxyleucoquinizarine[17] **5** with an excess amount of [1-(2-aminoethyl)-piperidin-2-yl]methanol.

The synthesis of 1,4-disubstituted chloroalkylaminoanthraquinones from **6** was carried out by two different routes. The first route involved treatment of the previously[16] synthesized anthraquinone alcohols **9**, **11**, and **12** with triphenylphosphine−carbon tetrachloride complex (PPh_3−CCl_4) to obtain **15** (symmetrical congener), **16**, and **17** (nonsymmetrical congeners) in an overall yield of 20−30% (from **6**). In an attempt to improve the overall yield, a second route was devised that involved preparation of chlorinated side chains in a 4-step procedure (Scheme 2). This was comprised of (i) Boc-protection of the hydroxylated heterocyclic alkylamino side chains, (ii) mesylation

Scheme 2



Reactants and conditions: aminoalkylamino side chain, R = OH, *n* = 1, 2, synthesized as previously described;[16] (a) Boc_2O, Et_3N, 45 °C, 16−20 h, R = OH, *n* = 1, 2; (b) MsCl, Et_3N, dry CH_2Cl_2, 0 °C, 1 h, R′ = OMs, *n* = 1, 2; (c) tetra-*n*-butylammonium chloride, dry DMF, 90 °C, 30 min, R″ = Cl, *n* = 1, 2; (d) 4 M HCl in EtOAc, 1 h, R″ = Cl, *n* = 1, 2.

of the hydroxyl group, (iii) conversion to the chloride with tetra-*n*-butylammonium chloride, and (iv) deprotection of the Boc group without required purification of any of the intermediates. The deprotected side chain was then reacted with either **6** or **10** (Scheme 1) to obtain the symmetrical compound **14** (yield 39%, 1 step) and the nonsymmetrical compounds **18**−**21** (yield 6−28%, 2 steps). By employing route 2, the overall yield from **6** (apart from **20**) was increased by approximately 10%.

The di-*N*-oxide derivative **15-NO** was synthesized from **15** using *m*-CPBA (yield 69%).

DNA Sequence Selectivity. The design and synthesis of the novel chloroalkylaminoanthraquinones was based on using pyrrolidinyl and piperidinyl building blocks racemic in nature. However, due to the commercial availability of the (*S*)-(+)-2-pyrrolidinylmethanol moiety, we also decided to include the enantiomeric compounds **13**, **14**, and **18** in our structure−activity relationship studies.

The chloroalkylaminoanthraquinones were investigated for their ability to alkylate double-stranded DNA, using the *Taq* polymerase stop assay.[18] A synthetic 20-base oligonucleotide primer (SRM) that binds to the sequence 3303−3284 of pBR322 was used to analyze alkylation sites on the complementary strand, following linear amplification. To select the lowest

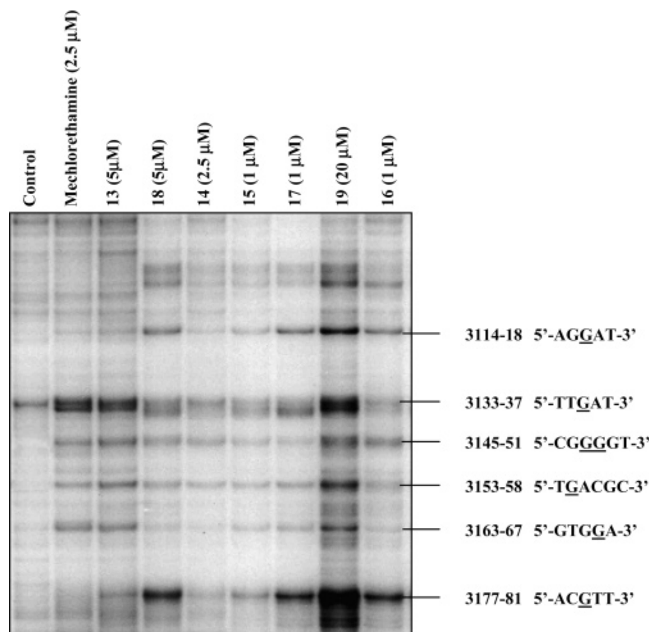


Figure 1. DNA strand alkylation pattern of chloroethylanthraquinones. Alkylation is denoted by an underscore. See Experimental Section for experimental details.

concentration that afforded alkylation, each *N*-chloroethyl and *N*-chloropropyl compound was first examined in a wide concentration range (1 nM−10 μM). Subsequently, all chloroethylaminoanthraquinones were compared on the same gel alongside unmodified DNA and mechlorethamine. The unmodified DNA (pBR322, not treated with agent) showed elongation with only a few background sites of early termination (Figure 1).

It was evident that the *N*-chloropropyl analogues **21** and **22** did not alkylate DNA after a 24 h incubation at 10 μM (data not shown), consistent with their inability to form an azirdinium intermediate.[19] In contrast, the *N*-chloroethyl analogues were shown to be equipotent with mechlorethamine at alkylating guanine, although some difference in sequence selectivity was observed. There were no marked differences in selectivity of DNA alkylation in the series of chloroethylaminoanthraquinones examined, despite the positional variation in their reactive moieties. The molecules showed a marked preference for 5′-A*G*GAT-3′ and AC*G*TT-3′ compared with mechlorethamine. This suggests that the planar aromatic anthraquinone chromophore, common to all the compounds, is directing the site of alkylation. Agents with a primary chloride leaving group (**13**−**18**) were shown to be significantly more potent alkylating agents than secondary chlorides (**19** and **20**).

DNA Interstrand Cross-Linking. Three chloroethylaminoanthraquinones (**14**−**16**) and one chloropropyl analogue (**22**) were investigated for their ability to interstrand cross-link DNA at a range of concentrations (0.01−10 μM) using an agarose gel based assay.[20] The chloropropyl analogue **22** did not interstrand cross-link DNA either in concentration- (up to 100 μM) or in time-dependent (up to 24 h) experiments (results not shown). The nonsymmetrically disubstituted compound **16**, with a bis-*N*-chloroethyl functionality confined to one sidearm, alkylated but did not interstrand cross-link DNA, indicating that this was the only compound of the series where the intramolecular distance between the alkylating functionalities required to cross-link adjacent strands was not met. This phenomenon was previously shown for Alchemix, an alkylating agent also with a bis-*N*-chloroethylamino functionality confined to one sidearm.[12]

A concentration- (Figures 2 and 3) and time-dependent (Figure 4) increase in formation of DNA interstrand cross-links was observed for both **14** and **15**, although the latter was a more potent cross-linker. Compound **15** contains a racemic piperidinyl *N*-chloroethyl moiety, while **14** is specifically the *S*-pyrrolidinyl enantiomer. Whether the ring size or the spatial conformation of the ring-constrained alkylating group is contributing to this difference in potency is not clear, but modeling suggests that the *R*-configuration may have an important role to play in DNA cross-linking, as described further below. Previously, individual enatiomers of racemic bispiperidine alkylating agents[21] with no intrinsic DNA affinity did not reveal any difference in reactivity in regards to DNA interstrand cross-linking. Hence, it would appear that the planar aromatic chromophore influences the orientation toward and, hence, the reactivity with DNA of these conformationally restricted alkylating moieties.

Significantly, we have shown that the alkylation capability can be totally inhibited by oxidation of the piperidinyl nitrogen, as in **15-NO** (Figure 5A). Furthermore, using an agarose gel based supercoiled plasmid pBR322 DNA mobility assay, it was also shown that **15-NO** did not induce DNA unwinding (cf. **15**, Figure 5B), consistent with the inability of *N*-oxides of DNA intercalators to bind to DNA.[22]

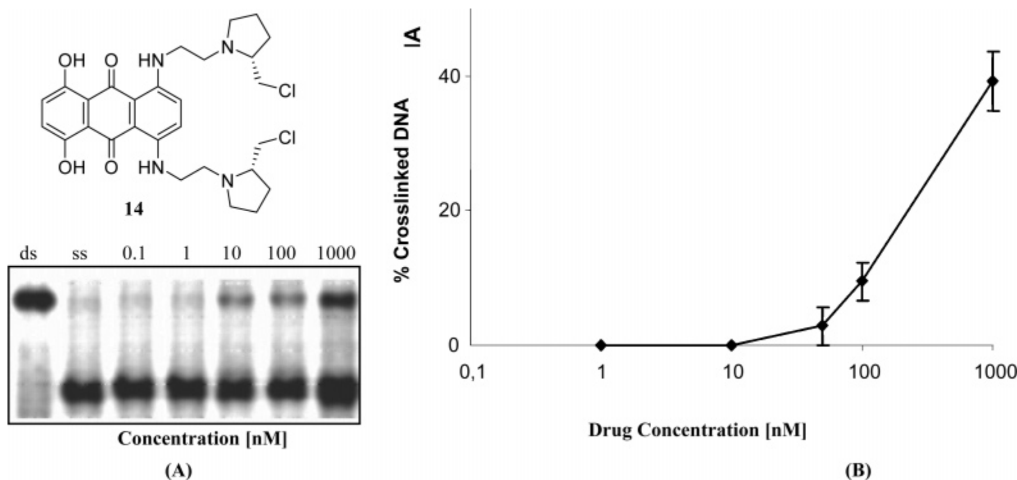


Figure 2. DNA cross-linking by **14**. (A) Autoradiograph of an agarose gel showing a concentration-dependent cross-link formation for **14** (1 h drug treatment). (B) Concentration−response curve: the cross-linked DNA was determined by densitometry and is expressed as the mean of three replicates (±s.d.). See Experimental Section for experimental detail.

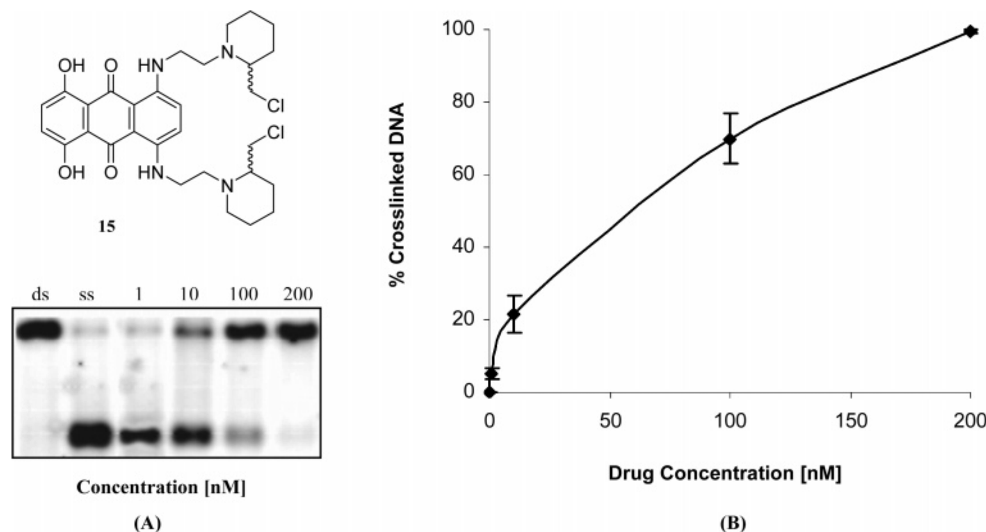


Figure 3. DNA cross-linking by **15**. (A) Autoradiograph of an agarose gel showing a concentration-dependent cross-link formation for **15** (1 h drug treatment). (B) Concentration−response curve: the cross-linked DNA was determined by densitometry and is expressed as the mean of three replicates (±s.d.). See Experimental Section for experimental detail.

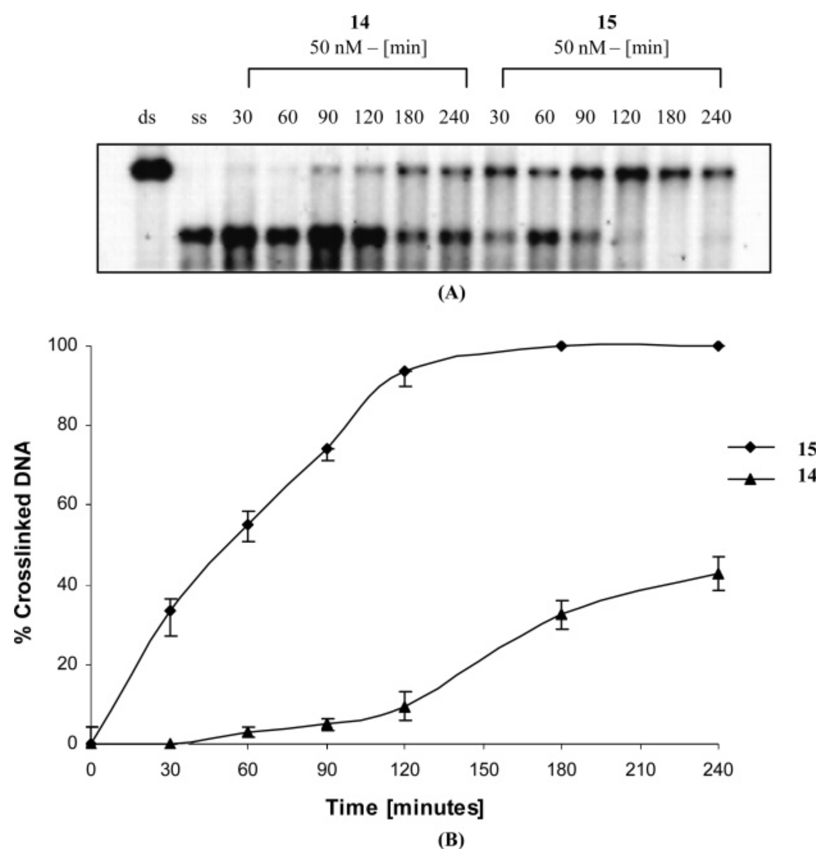


Figure 4. Time course of **14** and **15** cross-linking of pBR322 plasmid DNA. (A) Autoradiograph of an agarose gel showing time course of DNA cross-link formation following treatment with **14** or **15**, both at 50 nM. (B) Time course of DNA cross-linking was quantitated by densitometry and is expressed as the mean of three replicates (±s.d.). See Experimental Sectionfor experimental detail.

DNA Modeling Studies. The studies on the DNA sequence specificity of the chloroethylaminoanthraquinones did not reveal significant differences in terms of the potency and the pattern of alkylation. However, the DNA interstrand cross-linking studies demonstrated a considerable difference in reactivity between the racemate **15** and the *S*-enantiomer **14**. Molecular modeling of the *R* and *S* enantiomers of the cross-linking compounds **14** and **15** and the monoalkylating compounds **17**−**19** was carried out to investigate whether their relative spatial geometries were predictive of DNA reactivity.

A set of five lowest-energy conformations of **14**, **15**, and **17**−**19** were chosen to examine their preference for N^7-guanine alkylation, as it has been demonstrated that the principal sites of attack by mechloroethamine on DNA are N^7-guanine and N^3-adenine in a ratio of about 86:16.[23] Docking was performed using short sequences containing guanine as a part of the intercalation site shown to be alkylated, as shown in Figure 7. In every simulation, the ligand was shown to dock into the intercalation site and with energies of interaction that differed according to the base sequence investigated. Modeling also

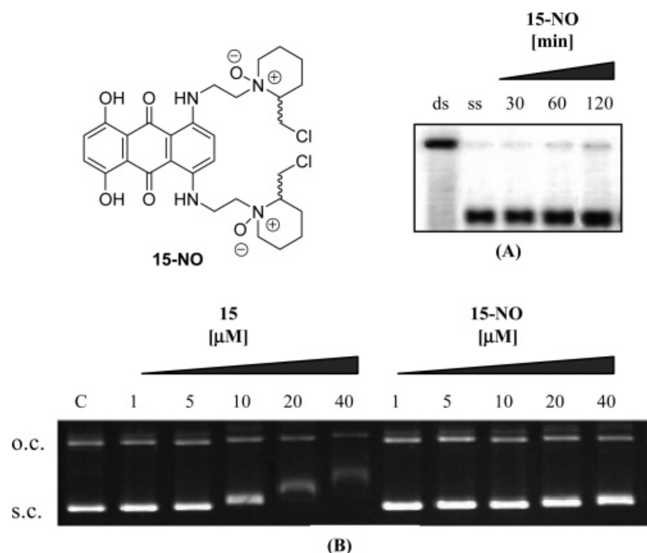


Figure 5. DNA binding by **15-NO**. (A) Autoradiograph of time-dependent DNA interstrand cross-linking experiment by **15-NO** (100 nM). (B) Concentration-dependent effect of **15** and **15-NO** on supercoiled plasmid pBR322 DNA. Results are expressed as the mean of three replicates (±s.d.).

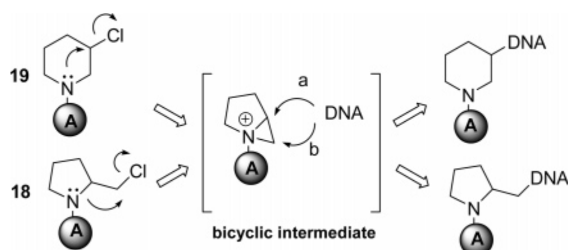


Figure 6. Compounds **18** and **19** will form the same bicyclic aziridinium ion intermediate prior to DNA alkylation (*A* = anthraquinone moiety). Due to the less sterically hindered site (b), the favored alkylation product is assumed to be the DNA−pyrrolidine adduct.

revealed that each ligand had a range of potential orientations with respect to the intercalation site. The criteria used to determine the probability of alkylation were based on the proximity of respective pyrrolidinyl or piperidinyl β-carbons from the nitrogen to N^7-guanine The data in Table 1 show that the energies of interaction with DNA are very similar for all three sets of enantiomers, although there appears to be slight differences in the probability of alkylation. A possible explanation for the similarity in DNA-binding, irrespective of the nature of the piperidinyl or pyrollidinyl side arm, may be due to the free rotation of the tertiary nitrogen of these 5- and 6-membered ring structures. The energy-minimized structures of the enantiomeric pairs of **18** and **19**, capable of forming the same bicyclic aziridinium intermediate (1-azoniabicyclo[2.1.0]hexane, Figure 6), are shown covalently adducted to N^7-guanine in Figure 7.

In contrast, the enantiomeric configuration of the interstrand DNA cross-linking agents **14** and **15** appeared to play a more important role on their interaction with DNA, presumably because two alkylation events are necessary for the interstrand DNA cross-linking observed in Figures 2−4. The modeling showed that the *R*-enantiomer is favored, in particular at 5′-CTG_ACG sequences, which perhaps explains why **15**, composed of 50% *R*-enantiomer, is shown to be more reactive than the pure *S*,*S*-enantiomer **14** (Table 2).

Cytotoxicity. Several agents (**16**−**19**) were shown to be potent cytotoxins ($IC_{50} \leq 40$ nM) in wild-type A2780 cells. However, the majority of compounds were significantly less-active, and some (**4**, **8**, **13**, and **22**) had IC_{50} values greater than 1 μM. Compound **13** shows that conjugation of a monosubstituted alkylating functionality to an anthraquinone chromophore is not sufficient to create a potent cytotoxin, despite its DNA binding and alkylating properties. However, the addition of a *N*,*N*′-dimethylethylenediamine side arm to produce the bis-substituted compound **19** results in a significant increase in cytotoxicity, This confirms the importance of two basic amino-alkylamino side chains, irrespective of alkylating potential, in determining the potency of this class of agent.

The effect of these agents on doxorubicin- (A2780AD) and cisplatin-resistant (A2780/cp70) cell lines was also explored. The A2780AD cell line is shown to have elevated P-gp[24] and a diminution of topo IIα,[25] while A2780/cp70 cells exhibit elevated levels of glutathione,[26] alterations in drug uptake/efflux,[27] and diminished DNA repair mechanisms, including mismatch repair (hMLH1) deficiency.[28−30] Generally, these anthraquinones were less-active in the resistant cell line variants, although **17** and **19** were equipotent with doxorubicin in the A2780AD cells. The compounds with alkylating potential (**16**−**21**) were also more affected by the resistance mechanism present in the A2780/cp70 cell line, but notably were still shown to be at least 100-fold more cytotoxic than cisplatin.

Compound **18**, which potentially can form the bicyclic aziridinium ion intermediate common to **19** (Figure 6), was less cytotoxic in these ovarian cancer cell lines (Table 3). However, when screened against the NCI-60 human tumor cell line panel (data not shown), **18** (mean $GI_{50} = 96$ nM) was shown to be approximately twice as cytotoxic as **19** (mean $GI_{50} = 182$ nM). It is an interesting observation that **18**, possessing a primary chloride in the alkylating subunit, exhibits no significant advantage over **19** in DNA reactivity and cytotoxic activity, in spite of the latter only comprising a secondary chloride as a leaving group in the alkylating subunit. This may be important in the future design of alkylating chloroethylaminoanthraquinones.

The preparation of stable *N*-oxides of *N*,*N*′-bischloroethylaminoanthraquinones, including **1**, was not possible (Patterson and Pors, unpublished observations), most likely due to their potential to rearrange to a cyclic hydroxylamine, as has been observed with chlorambucil *N*-oxide.[31] However, these sterically constrained tertiary nitrogen-containing ring systems were rationalized to form stable *N*-oxides, and the successful synthesis and characterization of **15-NO** confirms this. Table 3 shows that **15-NO** was inactive ($IC_{50} > 1$ μM) against the ovarian cancer cell lines, which was consistent with its inability to intercalate and alkylate DNA (Figure 5).

Conclusion

This series of ring-constrained chloroalkylaminoanthraquinones were prepared in part to explore whether such agents would retain potent cytotoxicity, as observed previously with intrinsically less-stable *N*,*N*′-bis-chloroethylaminoanthraquinones, and also to explore their properties as *N*-oxides. The constraint on the alkylating subunits by the pyrrolidine and piperidine ring systems led to less DNA reactive and cytotoxic agents. However, several agents remained potently cytotoxic in the doxorubicin- and cisplatin-resistant ovarian cancer cell lines. In addition, we successfully derivatized the DNA interstrand cross-linking agent **15** to the corresponding di-*N*-oxide **15-NO**. The lack of reactivity of the latter suggests that such agents might have potential as molecular delivery devices, transporting otherwise reactive alkylating agents to tumors

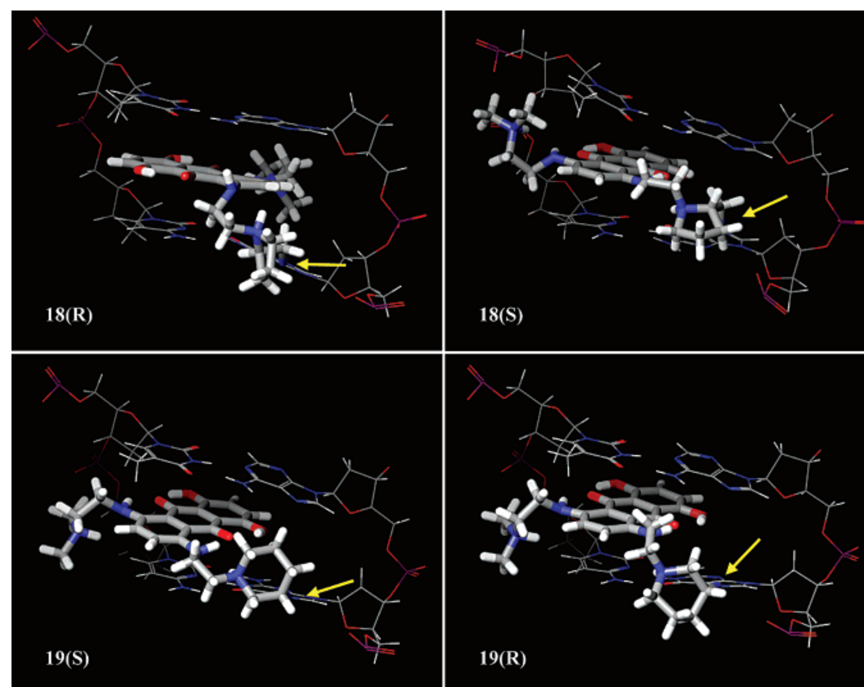


Figure 7. DNA modeling studies of the enantiomeric pairs of **18** and **19**. The images show the most favorable positions of the respective enantiomers docked into the intercalation sites TTG_ATC with the ligands covalently bound to N^7-guanine (indicated by yellow arrow).

Table 1. Ligand Interaction Energies (kcal/mol) and Alkylation Probabilities of Three Monoalkylating Chloroethylaminoanthraquinones

DNA	**17(*R*)**	P[a]	**17(*S*)**	P[a]	**18(*R*)**	P[a]	**18(*S*)**	P[a]	**19(*R*)**	P[a]	**19(*S*)**	P[a]
ACG_GGG	−14.926	m	−12.264	l	−14.241	m	−16.555	m	−15.17	l	−13.546	m
ACG_TTA	−11.131	l	−10.01	l	−10.830	l	−10.666	l	−11.384	l	−8.524	m
AGG_ATC	−13.422	l	−14.517	l	−15.308	m	−14.748	m	−16.569	l	−15.305	l
CTG_ACG	−24.948	m	−22.984	m	−21.332	h	−25.776	l	−26.107	l	−26.293	l
GGG_TCT	−7.3	l	−8.19	l	−8.268	l	−9.23	m	−9.888	l	−8.692	l
TGG_AAC	−5.695	l	−6.909	l	−5.682	m	−5.9	l	−6.111	l	−6.833	l
TTG_ATC	−22.118	l	−24.542	h	−23.501	h	−25.12	m	−24.601	l	−25.999	l

[a] P = probability; l = low (if the orientation of the ring is not in correct orientation to alkylate N^7-G), m = medium (the ring is in suitable orientation to interact with N^7-G, but the Cl atom was more than 3 Å away from N^7-G, h = high (the ring is in the correct orientation in respect to the N^7-G and distances between Cl and N^7-G were smaller than 3 Å.

Table 2. Ligand Interaction Energies (kcal/mol) and Interstrand DNA Cross-Linking Probabilities of Two Bisalkylating Chloroethylaminoanthraquinones

DNA	**14(*R*,*R*)**	P[a]	**14(*S*,*S*)**	P[a]	**15(*R*,*R*)**	P[a]	**15(*S*,*S*)**	P[a]
ACG_GGG	−13.117	l	−11.999	l	−12.013	l	−16.621	l
ACG_TTA	−6.216	l	−10.433	l	−8.845	l	−7.868	l
AGG_ATC	−10.467	l	−15.38	l	−13.822	l	−12.131	l
CTG_ACG	−17.794	m	−24.41	l	−24.064	m	−25.582	l
GGG_TCT	−6.116	l	−8.332	l	−8.492	l	−6.327	l
TGG_AAC	−5.244	l	−5.649	l	nd[b]	nd[b]	−6.741	l
TTG_ATC	−18.212	l	−19.749	l	−24.455	l	−19.579	l

[a] P = probability; l = low (if the orientation of the ring is not in correct orientation to alkylate N^7-G), m = medium (the ring is in suitable orientation to interact with N^7-G, but the Cl atom was more than 3 Å away from N^7-G, h = high (the ring is in the correct orientation in respect to the N^7-G and distances between Cl and N^7-G were smaller than 3 Å. [b] nd = interaction energy was not detected.

Table 3. Growth Inhibition (IC_{50}[a]) of Aminoanthraquinones and Chloroalkylaminoanthraquinones against Ovarian Cancer Cell Lines

	A2780[b]	2780AD		A2780/cp70	
compd	nM	nM	RF[c]	nM	RF[c]
4	>1000	>1000		>1000	
7	70	>1000		70	1
8	>1000	>1000		>1000	
9	740	>1000		>590	0.9
13	>1000	>1000		>1000	
14	428	782	1.8	442	1
15	178	>1000		>1000	
15-NO	>1000	>1000		>1000	
16	24	106	4.4	357	14.9
17	40	91	2.3	69	1.7
18	29	292	10.1	79	2.7
19	9	75	8.3	63	7
20	68	342	5.0	96	1.4
21	576	426	0.7	535	0.9
22	>1000	>1000		>1000	
cisplatin	900	2500	2.8	6600	2.6
doxorubicin	7.5	61.0	8.1	3.0	0.4

[a] IC_{50} is the concentration of drug (nM) required to inhibit cell growth by 50% ± standard error of the mean (n = 3). [b] A2780 is the wild type ovarian cell line; 2780AD and A2780/cp70 are doxorubicin and cisplatin resistant variants. [c] RF = resistance factor (IC_{50} in resistant cell line/IC_{50} in parent cell line).

without the associated systemic toxicity common to all conventional alkylating agents. Previously, we have identified *N*-oxides of DNA intercalator-based topo II inhibitors as bioreductively activated agents.[32] A lead compound, AQ4N, is currently in clinical trial as a tumor hypoxia-activated cytotoxin. AQ4N shows no systemic toxicity, and tumor tissue shows a significantly higher burden of reduction product AQ4 than normal tissue (for reviews, see refs 15 and 33). By analogy, *N*-oxides such as **15-NO** may remain inactive until reduced in the hypoxic regions of tumors to generate activated DNA alkylating agents. We are currently exploring this potential.

Experimental Section

The UV/vis absorbance of the compounds investigated biologically was recorded on a Beckman DU70 UV/vis spectrophotometer fitted with deuterium and tungsten lamps. The infrared absorbance was recorded on a Nicolet 205 FT-IR spectrometer. ^{1}H NMR (250 MHz) and ^{13}C NMR (69 MHz) spectra were obtained on a Bruker AC-250 spectrometer. Fast atom bombardment (FAB+) mass spectra sample identification was obtained on a V.G 70 SEQ mass spectrometer. Microelemental analysis was obtained using a Carlo-Erba EA 1108 instrument with a PC-based data system, Eager 200 for Windows, and Sartorious ultra microbalance 4504 MP8. Thin-layer chromatography was carried out on aluminum-backed silica plates (Merck, 60 F_{254}), and column chromatography was carried out on silica gel (particle size 35−70 μm and 20−35 μm).

Chemistry. The preparation of the chromophores, 5,8-dihydroxy-leucoquinizarin (leuco-1,4,5,8,-tetrahydroxyanthraquinone **5**,[34] 1,4-difluoro-5,8-dihydroxyanthraquinone **6**,[35] and 1-[[2-(di-methyl-amino)ethyl]amino]-4-fluoro-5,8-dihydroxyanthracene-9,10-dione (**10**),[16] was carried out as previously described. The aminoalkyl-amino side chains and intermediate hydroxylaminoanthraquinones **9**, **11**, and **12** were prepared as described previously.[16]

1-{[2-(2-Hydroxyethylpyrrolidine)ethyl]amino}-anthracene-9,10-dione (4). [1-(2-Aminoethyl)-pyrrolidin-2-yl-]methanol (1.63 g, 11.30 mmol) in pyridine (10 mL) was added to 1-chloro-anthraquinone (1.37 g, 5.65 mmol), and the mixture was stirred at 65 °C for 24 h. Pyridine was removed in vacuo, and the resulting mixture of oil and solid was dissolved in CH_2Cl_2 and washed with H_2O (3 × 50 mL) to remove any unreacted amine. The separated organic layer was removed in vacuo, and the crude product was chromatographed using CH_2Cl_2/CH_3OH (97:3). The desired product was yielded as a red powder (0.21 g, 11%). Mp 113−115 °C; ^{1}H NMR ($CDCl_3$) δ 10.05 (s, 1H, C(1)N*H*), 8.20 (2 × d, H), 8.45 (2 × d, H), 7.65−7.81 (m, 2H), 7.45−7.62 (m, 2H), 7.01 (2 × d, 1H), 3.75−3.85 (2 × d, 1H), 3.35−3.55 (m, 4H), 3.15−3.30 (m, 2H), 2.60−2.83 (m, 2H), 2.25−2.35 (m, 1H), 1.75−2.02 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 184.89, 183.77, 151.49, 135.32, 133.81, 132.86, 126.57, 115.60, 113.15, 65.26, 62.61, 53.82, 52.99, 41.64, 27.15, 24.11; FAB MS *m/z* $(M + H)^+$ 351.

1,4-Bis-{[2-(3-piperidinemethanol)ethyl]amino}-5,8-dihydroxy-anthracene-9,10-dione (7). 1-(2-Aminoethyl)-3-piperidin-3-ol (1.0 g, 6.33 mmol) in EtOH (5 mL) was added to a suspension of tetrahydroxyleucoquinizarin (0.14 g, 0.53 mmol) in EtOH (25 mL) under N_2. After 8 h of stirring at reflux temperature, the reaction mixture was stirred at rt another 14 h. The EtOH was removed in vacuo, and the remaining residue was added to ice-cold brine. The precipitated solid was isolated by filtration and lyophilised. The dark blue solid was flash chromatographed using CH_2Cl_2 to remove nonpolar side-products, then by increasing the polarity gradually to CH_2Cl_2/CH_3OH (4:1) to obtain the desired product as a dark-blue solid (64 mg, 22%). Mp 180−183 °C; ^{1}H NMR ($CDCl_3$) δ 13.64 (s, 2H, C(5)O*H* and C(8)O*H*), 10.54 (t, 2H, C(1)N*H* and C(4)N*H*), 7.10 (s, 2H, C(2)*H* and C(3)*H*), 7.23 (s, 2H, C(6)*H* and C(7)*H*), 3.63−3.66 (m, 4H, 2 × CHC*H*$_2$OH), 3.45−3.58 (m, 4H, 2 × HNC*H*$_2$CH$_2$), 2.71 (t, 6H, 2 × HNCH$_2$C*H*$_2$N and 2 × ring-*H*), 2.95 (2 × d, 2H, 2 × ring-*H*), 2.15−2.35 (m, 4H, 4 × ring-*H*), 1.10−1.95 (m, 12H, 2 × O*H* and 10 × ring-*H*); ^{13}C NMR ($CDCl_3$) δ 182.36, 156.36, 146.60, 134.46, 132.09, 126.13, 123.87, 109.89, 65.70, 57.75, 56.95, 54.43, 40.52, 37.90, 26.92, 24.08; FAB MS, *m/z* $(M + H)^+$ 553; IR υ_{max}/cm^{-1}; 3400 (OH), 3225 (NH), 3100 (Ar−CH), 2960−2800 (CH_2, CH_3), 1650, 1625, 1575, 1480, 1370, 1225.

1,4-Bis-{[2-(piperidine)ethyl]amino}-5,8-dihydroxy-anthracene-9,10-dione (8). 1,4-Difluoro-5,8-hydroxyanthraquinone (50 mg, 0.18 mmol) and 1-(2-aminoethyl)-piperidine (232 mg, 1.81 mmol) were stirred in pyridine (1 mL) at 90 °C for 30 min. The reaction mixture was added to ice-cold brine and set aside at 4 °C overnight. The precipitated solid was isolated by filtration and lyophilised. 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 (85:15). The chromatographed solid was then crystallized from $CHCl_3$, affording the title compound as a dark blue powder (32 mg, 35%). Mp 219−221 °C; ^{1}H NMR ($CDCl_3$) δ 13.65 (s, 2H, C(5)O*H*, C(8)O*H*), 10.51 (t, 2H, C(1)N*H* and C(4)N*H*), 7.15 (s, 2H, C(6)*H* and C(7)*H*), 7.05 (s, 2H, C(2)*H* and C(3)*H*), 3.55 (q, 4H, 2 × HNC*H*$_2$CH$_2$N), 2.65 (t, 4H, 2 × HNCH$_2$C*H*$_2$N), 2.41−2.68 (m, 8H, 2 × NC*H*$_2$H$_2$), 1.55−1.72 (m, 8H, 8 × ring-*H*), 1.45−1.55 (m, 4H, 4 × ring-*H*); ^{13}C NMR ($CDCl_3$) δ 185.35, 155.46, 146.31, 124.63, 123.63, 115.48, 109.21, 57.63, 54.64, 40.72, 26.09, 24.37; FAB MS *m/z* $(M + H)^+$ 493.

1-{[2-(2-Chloroethylpyrrolidine)ethyl]amino}-anthracene-9,10-dione Hydrochloride (13). Ph_3P (260 mg, 0.99 mmol) and CCl_4 (100 μL, 9.86 mmol) were stirred for 15 min before the mixture was added dropwise to a stirred solution of **4** (115 mg, 0.329 mmol) in dry CH_2Cl_2 (5 mL) under N_2 at reflux temperature. The reaction mixture was kept at reflux temperature for 3 h before it was cooled down to rt. Ethereal HCl was added to the solution, and after 1 h of stirring, the precipitated solid was filtered off. To remove excess of Ph_3P and Ph_3PO, the precipitated solid was dissolved in warm CH_3OH (10 mL). While stirring the dark blue solution 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 product was afforded as an orange powder (92 mg, 69%). Mp 250−253 °C; ^{1}H NMR ($CDCl_3$) δ 9.95 (s, 1H, C(1)N*H*), 8.14−8.32 (m, 2H), 7.75−7.95 (m, 2H), 7.45−7.65 (m, 2H), 7.22 (2 × d, 1H), 3.8 (2 × d, 1H), 3.42−3.61 (m, 4H), 3.05−3.15 (m, 2H), 2.65−2.75 (m, 2H), 2.15−2.35 (m, 1H), 1.61−2.13 (m, 4H), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3$) δ 184.38, 183.65, 151.29, 135.26, 133.91, 132.81, 126.47, 115.51, 113.35, 65.26, 62.61, 55.82, 50.99, 41.74, 27.45, 24.31; FAB MS *m/z* $(M + H)^+$ 351. Anal. ($C_{21}H_{21}N_2O_2Cl\cdot HCl$) C, H, N.

1,4-Bis-{[2-(2-chloroethylpyrrolidine)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (14). Preparation of **14** involved 5 steps, where none of the intermediate products (i−iv) were isolated and purified: (i) Boc protection of monohydroxylated diamine side chain, (ii) mesylation of Boc-protected monohydroxylated diamine side chain, (iii) chlorination of monomesylated diamine side chain, (iv) deprotection of the Boc group of chlorinated diamine side chain, and (v) *ipso* substitution of the fluoride of 1-[[2-(dimethylamino)ethyl]amino]-4-fluoro-5,8-dihydroxyanthraquinone by chlorinated diamine.

Preparation of Side Chain: *tert*-Butyl 2-(2-(Hydroxymethyl)-pyrollidin-1-yl)ethylcarbamate (i). [1-(2-Aminoethyl)-pyrrolidin-2-yl-]methanol (7 g, 48.61 mmol) and Et_3N (8.12 mL, 58.33 mmol) were stirred together with CH_3OH (50 mL) for 5 min before Boc_2O (12.73 g, 58.33 mmol) in dissolved in CH_3OH (15 mL) was added dropwise over 15−20 min. The reaction mixture was then stirred 20 h at 45 °C before being concentrated in vacuo. The oil was diluted in EtOAc (40 mL) and washed with 2 × H_2O (20 mL) and brine (20 mL). The organic phase was dried with magnesium sulfate ($MgSO_4$) and, after filtration, was concentrated in vacuo, yielding a straw-colored oil that needed no further purification (9.36 g, 79%). FAB MS *m/z* $(M + H)^+$ 245. **(1-(2-(*tert*-Butoxycarbonylamino)-ethyl)pyrrolidin-2-yl)methyl Methanesulfonate (ii).** MsCl (3.81 mL, 49.20 mmol) was added dropwise to an ice-cold solution of the Boc-protected amine (8 g, 32.8 mmol) and Et_3N (6.85 mL, 49.2 mmol) in dry CH_2Cl_2 (50 mL) under N_2. After the reaction mixture was stirred for 1 h at 0 °C, the solution was diluted with cold CH_2Cl_2 and washed with ice-cold $NaHCO_3$ and ice-cold brine. The organic phase was dried with $MgSO_4$, filtered, and concentrated in vacuo at room temperature. The mesylated product was afforded as a crude straw-colored oil (9.06 g, 86%). FAB MS *m/z* $(M + H)^+$ 323. ***tert*-Butyl 2-(2-(Chloromethyl)pyrrolidin-1-yl)ethylcarbamate (iii).** Tetra-*n*-butylammonium chloride (2.0 g, 7.20 mmol) was added to a stirred solution of the crude mesylate (9 g, 28.01 mmol) in dry DMF (50 mL). The reaction mixture was heated at 90 °C for 30 min before DMF was removed in vacuo. The residual oil was taken up in CH_2Cl_2 and washed with ice-cold $NaHCO_3$ and ice-cold brine. The organic phase was dried ($MgSO_4$) and filtered, and the solvent

was concentrated in vacuo at room temperature. The crude product was yielded as a straw-/yellowish-colored oil (6.78 g, 61%). FAB MS *m/z* (M + H)$^+$ 263. **2-(2-(Chloromethyl)pyrrolidin-1-yl)-ethanamine (iv).** The crude chloride (6.78 g, 25.70 mmol) was stirred in 4 M HCl in EtOAc for an hour to remove the Boc group. To the acidic EtOAc solution, cooled in an ice-bath, was slowly added a solution of brine and NH_3 (pH = 12) until the aqueous phase was pH ~11. The chlorinated diamine was then extracted into the organic phase, which was dried with $MgSO_4$. The solvent was removed in vacuo, and the crude product was yielded as a brownish oil (1.98 g, 47%) that was used directly in the next step. FAB MS *m/z* (M + H)$^+$ 163.

Preparation of Title Compound 14 (v): A mixture of **10** (125 mg, 0.363 mmol) and the crude 2-(2-(chloromethyl)pyrrolidin-1-yl)ethanamine (198 mg, 12.1 mmol) was reacted in pyridine (5 mL) at 30 °C for 4 h. The reaction mixture was concentrated in vacuo, and the crude product was purified by initially eluting with CH_2Cl_2 to remove nonpolar impurities, followed by a gradual increase of CH_3OH to CH_2Cl_2/CH_3OH (97:3). The product was dissolved in CH_2Cl_2 (2 mL), and ethereal HCl was added to generate the hydrochloride salt **19**, which was isolated by filtration and dried under vacuum to afford the title compound as a dark blue solid (89 mg, 39%). Mp > 300 °C dec; ^{1}H NMR (DMSO) δ 13.45 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.15 (s, 2H, C(6)*H* and C(7)*H*), 7.05 (s, 2H, C(2)*H* and C(3)*H*), 4.10−4.19 (m, 2H, 2 × NCHC*H*$_2$Cl), 3.45−3.55 (q, 4H, 2 × HNC*H*$_2$-CH$_2$N), 3.15 (d, 2H, 2 × NCHC*H*$_2$Cl), 2.65−2.85 (m, 6H, 2 × HNCH$_2$C*H*$_2$N and 2 × ring-*H*), 2.15−2.35 (m, 6H, 6 × ring-*H*), 1.45−1.85 (m, 6H, 6 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR (DMSO) δ 183.28, 156.69, 144.33, 127.56, 124.33, 114.93, 107.91, 65.26, 60.52, 55.43, 50.83, 41.78, 35.05, 28.11; FAB MS *m/z* (M + H)$^+$ 561. Anal. ($C_{28}H_{34}N_4O_4Cl_2$·2HCl) C, H, N.

1,4-Bis-{[2-(2-chloroethylpiperidine)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (15). The method follows that of **13**, using **9** (60 mg, 0.11 mmol), Ph_3P (172 mg, 0.65 mmol), CCl_4 (190 μL, 1.96 mmol), and dry CH_2Cl_2 (5 mL). The reaction was stopped after 5 h of reflux. The product was afforded as a dark blue powder (50 mg, 78%). Mp > 300 °C dec; ^{1}H NMR (DMSO) δ 13.45 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.65 (s, 2H, C(6)*H* and C(7)*H*), 7.24 (s, 2H, C(2)*H* and C(3)*H*), 4.15 (2 × d, 4H, 2 × NCHC*H*$_2$Cl), 4.03−4.12 (q, 4H, 2 × HNC*H*$_2$CH$_2$N), 3.44−3.83 (m, 8H, 2 × HNCH$_2$C*H*$_2$N and 4 × ring-*H*), 1.75−2.15 (m, 10H, 10 × ring-*H*), 1.51−1.65 (m, 4H, 4 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR (DMSO) δ 184.28, 154.69, 145.93, 126.56, 125.00, 114.33, 108.41, 62.26, 60.32, 51.83, 50.78, 42.78, 37.05, 26.11; FAB MS *m/z* (M + H)$^+$ 589. Anal. ($C_{30}H_{38}N_4O_4Cl_2$·2HCl) C, H, N.

1,4-Bis-{[2-(2-chloroethylpiperidine-*N*-oxide)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione (15-NO). *m*-CPBA (25 mg, 0.15 mmol) dissolved in dry DCM (1 mL) was added dropwise to a stirred solution of **15** (33 mg, 0.06 mmol) in dry CH_2Cl_2 (5 mL) under N_2. After 15 min of stirring at −10 °C (acetone ice bath), the reaction was stirred 3 h at 4 °C. The crude product was chromatographed by initially eluting with CH_2Cl_2/CH_3OH (1:10), then followed by a gradual increase of polarity to $CH_2Cl_2/CH_3OH/NH_3$ (29:70:1). The desired product was afforded as a crude dark blue solid (24 mg, 69%). ^{1}H NMR (DMSO) δ 13.52 (s, 2H, C(5)O*H*, C(8)O*H*), 10.54 (t, 2H, C(1)N*H* and C(4)N*H*), 7.71 (s, 2H, C(6)*H* and C(7)*H*), 7.32 (s, 2H, C(2)*H* and C(3)*H*), 4.55 (2 × d, 4H, 2 × NCHC*H*$_2$Cl), 3.95−4.44 (m, 12H, 2 × HNCH$_2$C*H*$_2$N, 2 × HNC*H*$_2$CH$_2$N, and 4 × ring-*H*), 2.95−3.23 (m, 6H, 6 × ring-*H*), 1.82−2.02 (m, 6H, 6 × ring-*H*), 1.64−1.71 (m, 4H, 4 × ring-*H*), HCl salt was not observable in the spectrum; FAB MS *m/z* (M + H)$^+$ 623. Anal. ($C_{30}H_{38}N_4O_6Cl_2$) C, H, N.

1-{[(2-Dimethylamino)ethyl]amino}-4-{[2-(2,6-dichloroethylpiperidine)ethyl]-amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (16). The method follows that of **13**, using **11** (14 mg, 0.03 mmol), Ph_3P (43 mg, 0.164 mmol), CCl_4 (79 μL, 0.82 mmol), and dry CH_2Cl_2 (5 mL). The reaction was stopped after 5 h of reflux. The product was afforded as a dark blue powder (12 mg, 81%). ^{1}H NMR ($CDCl_3$) δ 13.65 (s, 2H, C(5)O*H*, C(8)O*H*), 10.55 (t, 2H, C(1)N*H* and C(4)N*H*), 7.12−7.18 (m, 2H, C(6)*H* and C(7)*H*), 7.10 (s, 2H, C(2)*H* and C(3)*H*), 3.81−3.94 (d, 4H, 2 × CHC*H*$_2$Cl), 3.35−3.45 (q, 4H, 2 × HNC*H*$_2$CH$_2$N), 3.02 (t, 2H, 1 × HNCH$_2$C*H*$_2$N), 2.81−2.93 (m, 2H, 2 × NC*H*CH$_2$), 2.72 (t, 2H, 1 × HNCH$_2$C*H*$_2$N), 2.35 (s, 6H, 2 × NC*H*$_3$), 1.30−1.65 (m, 6H, 6 × ring-*H*), HCl salt was not observable in the spectrum; FAB MS *m/z* (M + H)$^+$ 549. Anal. ($C_{28}H_{34}N_4O_4Cl_2$·2HCl) C, H, N.

1-{[(2-Dimethylamino)ethyl]amino}-4-{[2-(2-chloroethylpiperidine)ethyl]-amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (17). The method follows that of **13**, using **12** (105 mg, 0.21 mmol), Ph_3P (165 mg, 0.63 mmol), CCl_4 (500 μL, 5.25 mmol), and dry CH_2Cl_2 (10 mL). The reaction was stopped after 3 h of reflux. The product was afforded as a dark blue powder (89 mg, 73%). Mp 233−235 °C; ^{1}H NMR ($CDCl_3$) δ 13.35 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.64 (s, 2H, C(6)*H* and C(7)*H*), 7.15 (s, 2H, C(2)*H* and C(3)*H*), 4.20−4.24 (m, 1H), 3.92−4.15 (m, 4H), 3.61−3.75 (m, 2H), 3.24−3.35 (m, 4H), 2.81 (s, 6H, 2 × NC*H*$_3$), 1.55−2.10 (m, 8H, 8 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3$) δ 184.20, 154.65, 145.90, 145.83, 124.93, 124.81, 114.23, 108.34, 62.25, 54.85, 51.73, 42.21, 36.70, 26.03, 21.60; FAB MS *m/z* (M + H)$^+$ 501. Anal. ($C_{26}H_{33}N_4O_4Cl$·2HCl·3H_2O) C, H, N.

1-{[(2-Dimethylamino)ethyl]amino}-4-{[2-(2-chloroethylpyrrolidine)ethyl]-amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (18). The method follows that of **14**.

Preparation of Side Chain: *tert*-Butyl 2-(2-(Hydroxymethyl)-pyrollidin-1-yl)ethylcarbamate (i). [1-(2-Aminoethyl)-pyrrolidin-2-yl-]methanol (5 g, 34.72 mmol), Et_3N (5.8 mL, 41.67 mmol), CH_3OH (40 mL), and Boc_2O (9.10 g, 41.67 mmol) were dissolved in CH_3OH (10 mL). The reaction mixture was stirred 18 h. The product was afforded as a straw-colored oil that needed no further purification (6.9 g, 82%). **(1-(2-(*tert*-Butoxycarbonylamino)ethyl)-pyrrolidin-2-yl)methyl Methanesulfonate (ii).** Boc-protected amine (5.1 g, 20.90 mmol), MsCl (2.43 mL, 31.35 mmol), Et_3N (4.32 mL, 31.35 mmol), and dry CH_2Cl_2 (50 mL) were used. The mesylated product was afforded as a crude straw-colored oil (5.63 g, 84%). FAB MS *m/z* (M + H)$^+$ 245. ***tert*-Butyl 2-(2-(Chloromethyl)pyrrolidin-1-yl)ethylcarbamate (iii).** Crude mesylate (5.63 g, 17.48 mmol), tetra-*n*-butylammonium chloride (9.72 g, 11.26 mmol), and dry DMF (30 mL) at 90 °C for 30 min were the reaction conditions. The crude chloride was afforded as a straw/yellowish-colored oil (2.2 g, 48%). FAB MS *m/z* (M + H)$^+$ 323. **2-(2-(Chloromethyl)pyrrolidin-1-yl)ethanamine (iv).** Crude chloride (2.0 g, 7.58 mmol), 4 M HCl, and EtOAc for 1 h were the reaction conditions. The crude Boc-deprotected amine was afforded as a brownish oil (675 mg, 55%). FAB MS *m/z* (M + H)$^+$ 263.

Preparation of Title Compound 18: Compound **10** (95 mg, 0.28 mmol), crude 2-(2-(chloromethyl)pyrrolidin-1-yl)ethanamine (675 mg, 4.15 mmol), and pyridine (2 mL) for 2 h at 30 °C were the reaction conditions. The title compound was afforded as a dark blue solid (64 mg, 41%). FAB MS *m/z* (M + H)$^+$ 163. Mp 253−255 °C; ^{1}H NMR ($CDCl_3$) δ 13.55 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.12 (s, 2H, C(6)*H* and C(7)*H*), 7.05 (s, 2H, C(2)*H* and C(3)*H*), 3.38−4.47 (m, 5H, 2 × HNC*H*$_2$CH$_2$N and 1 × NCHC*H*$_2$Cl), 3.15 (d, 1H, 1 × NCHC*H*$_2$Cl), 2.62−2.83 (2 × t, 4H, 2 × HNCH$_2$C*H*$_2$N), 2.35 (s, 6H, 2 × NC*H*$_3$), 2−21.25 (m, 3H, 3 × ring-*H*), 1.51−1.85 (m, 4H, 4 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3$) δ 185.11, 155.32, 146.2, 146.01, 124.49, 123.69, 123.55, 115.40, 109.02, 61.43, 58.35, 56.27, 56.03, 55.78, 52.82, 45.59, 41.19, 40.33, 34.86, 24.93; FAB MS *m/z* (M + H)$^+$ 487. Anal. ($C_{25}H_{31}N_4O_4Cl$·2HCl·2H_2O) C, H, N.

1-{[(2-Dimethylamino)ethyl]amino}-4-{[2-(3-chloropiperidine)-ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (19). The method follows that of **14**.

Preparation of Side Chain: *tert*-Butyl 2-(2-(Hydroxymethyl)-piperidin-1-yl)ethylcarbamate (i). 1-(2-Aminoethyl)-piperidin-3-ol (1 g, 6.94 mmol), Et_3N (1.16 mL, 8.33 mmol), CH_3OH (10 mL), and Boc_2O (1.82 g, 8.33 mmol) dissolved in CH_3OH (5 mL) were

stirred 20 h at 45 °C. The product, a straw-colored oil, needed no further purification (1.35 g, 80%). FAB MS *m/z* (M + H)$^+$ 245. **(1-(2-(*tert*-Butoxycarbonylamino)ethyl)piperidin-2-yl)methyl Methanesulfonate (ii).** MsCl (420 μL, 5.41 mmol), Boc-protected amine (880 mg, 3.61 mmol), Et_3N (755 μL, 5.41 mmol), and dry CH_2Cl_2 (10 mL) were stirred for 1 h at 0 °C. The mesylated product was afforded as a crude straw-colored oil (965 mg, 83%). FAB MS *m/z* (M + H)$^+$ 323. ***tert*-Butyl 2-(2-(Chloromethyl)piperidin-1-yl)ethylcarbamate (iii).** Tetra-*n*-butylammonium chloride (2.0 g, 7.20 mmol), crude mesylate (965 mg, 3.01 mmol), and dry DMF (5 mL) were heated at 90 °C for 30 min. The crude product was yielded as a straw-/yellowish-colored oil (624 mg, 79%). FAB MS *m/z* (M + H)$^+$ 263. **2-(2-(Chloromethyl)piperidin-1-yl)ethanamine (iv).** The crude chloride (624 mg, 2.38 mmol) was stirred in 4 M HCl in EtOAc for an hour to remove the Boc group. The crude product was yielded as a brownish oil (175 mg, 45%) that was used directly in the next step. FAB MS *m/z* (M + H)$^+$ 163.

Preparation of Title Compound 19: A mixture of **10** (36 mg, 0.11 mmol) and the crude 2-(2-(chloromethyl)piperidin-1-yl)-ethanamine (175 mg, 1.08 mmol) was reacted in pyridine (2 mL) at rt for 2 h. The title compound was afforded as a dark blue solid (35 mg, 60%). Mp > 300 °C dec; ^{1}H NMR ($CDCl_3$) δ 13.51 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.15 (s, 2H, C(6)*H* and C(7)*H*), 7.05 (s, 2H, C(2)*H* and C(3)*H*), 4.10−4.14 (m, 1H, CH_2CHCl), 3.45 (t, 4H, 2 × $HNCH_2CH_2N$), 3.15−3.24 (2 × d, 1H, ring-*H*), 2.65−2.81 (2 × t, 4H, 2 × $HNCH_2CH_2N$), 2.35 (s, 6H, 2 × NCH_3), 2.15−2.25 (m, 3H, 3 × ring-*H*), 1.52−1.93 (m, 4H, 4 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3$) δ 185.35, 185.28, 155.41, 146.24, 146.05, 124.66, 123.78, 123.64, 115.39, 109.23, 61.43, 58.39, 56.28, 55.78, 52.85, 45.63, 41.26, 40.37, 34.88, 29.65, 24.95; FAB MS *m/z* (M + H)$^+$ 487. Anal. ($C_{25}H_{31}N_4O_4Cl \cdot 2HCl$) C, H, N.

1-{[(2-Dimethylamino)ethyl]amino}-4-{[2-(3-chloropyrrolidine)-ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (20). The method follows that of **14**.

Preparation of Side Chain: *tert*-Butyl 2-(3-Hydroxypyrrolidin-1-yl)ethylcarbamate (i). 1-(2-Aminoethyl)-pyrrolidin-3-ol (1 g, 7.94 mmol), Et_3N (1.32 mL, 9.52 mmol), CH_3OH (10 mL), and Boc_2O (2.08 g, 9.52 mmol) dissolved in CH_3OH (5 mL) were used. The reaction mixture was stirred 16 h. The product was afforded as a straw-colored oil that needed no further purification (1.54 g, 86%). FAB MS *m/z* (M + H)$^+$ 230. **1-(2-(*tert*-Butoxycarbonylamino)ethyl)pyrrolidin-3-yl Methanesulfonate (ii).** Boc-protected amine (960 mg, 4.16 mmol), MsCl (483 μL, 6.24 mmol), Et_3N (868 μL, 6.24 mmol), and dry CH_2Cl_2 (10 mL) were used. The crude product was afforded as a straw-colored oil (1.0 g, 78%). FAB MS *m/z* (M + H)$^+$ 308. ***tert*-Butyl 2-(3-Chloropyrrolidin-1-yl)ethylcarbamate (iii).** Crude mesylate (1 g, 3.24 mmol), tetra-*n*-butylammonium chloride (1.35 g, 4.86 mmol), and dry DMF (10 mL) at 100 °C for 30 min were the reaction conditions. The product was afforded as a straw-/yellowish-colored oil (705 mg, 81%). FAB MS *m/z* (M + H)$^+$ 248. **2-(3-Chloropyrrolidin-1-yl)ethanamine (iv).** Crude chloride (705 mg, 2.88 mmol), 4 M HCl, and EtOAc for 1 h were the reaction conditions. The crude Boc-deprotected amine was afforded as a brownish oil (124 mg, 30%). FAB MS *m/z* (M + H)$^+$ 148.

Preparation of Title Compound 20: Compound **10** (50 mg, 0.18 mmol), crude 2-(3-chloropyrrolidin-1-yl)ethanamine (124 mg, 0.86 mmol), and pyridine (2 mL) for 2 h at 60 °C were the reactions conditions. The product was afforded as a dark blue powder (15 mg, 15%). Mp > 300 °C dec; ^{1}H NMR (DMSO/$CDCl_3$ (1:1)) δ 13.55 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.21 (s, 2H, C(6)*H* and C(7)*H*), 7.11 (s, 2H, C(2)*H* and C(3)*H*), 3.81−4.12 (m, 5H), 2.85−3.05 (m, 7H), 2.35 (s, 6H, 2 × NCH_3), 2.31−2.35 (m, 1H, ring-*H*), 1.81−2.15 (m, 2H, 2 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3$) δ 184.44, 154.74, 146.09, 125.15, 124.91, 114.40, 107.65, 68.95, 62.05, 55.05, 52.01, 42.31, 37.19; FAB MS *m/z* (M + H)$^+$ 473. Anal. ($C_{24}H_{29}N_4O_4Cl \cdot 2HCl \cdot 4H_2O$) C, H, N.

1-{[(2-Dimethylamino)ethyl]amino}-4-{[2-(4-chloropiperidine)-ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (21). The method follows that of **14**.

Preparation of Side Chain: *tert*-Butyl 2-(4-Hydroxypiperidin-1-yl)ethylcarbamate (i). 1-(2-Aminoethyl)-piperidin-4-ol (2.0 g, 13.89 mmol), Et_3N (2.32 mL, 16.65 mmol), CH_3OH (20 mL), and Boc_2O (3.63 g, 16.65 mmol) were dissolved in CH_3OH (5 mL). The reaction mixture was stirred 18 h. The product was afforded as a straw-colored oil that needed no further purification (2.35 g, 69%). FAB MS *m/z* (M + H)$^+$ 245. **1-(2-(*tert*-Butoxycarbonylamino)ethyl)piperidin-4-yl Methanesulfonate (ii).** Boc-protected amine (1.70 g, 6.94 mmol), MsCl (810 μL, 10.41 mmol), Et_3N (1.45 mL, 10.41 mmol), and dry CH_2Cl_2 (20 mL) were the reactants. The mesylated product was afforded as a crude straw-colored oil (1.39 g, 62%). FAB MS *m/z* (M + H)$^+$ 323. ***tert*-Butyl 2-(4-chloropiperidin-1-yl)ethylcarbamate (iii).** Crude mesylate (1.39 g, 4.29 mmol), tetra-*n*-butylammonium chloride (2.38 g, 8.58 mmol), and dry DMF (10 mL) at 120 °C for 30 min were the reaction conditions. The crude chloride was afforded as a straw/yellowish-colored oil (0.73 g, 65%). FAB MS *m/z* (M + H)$^+$ 263. **2-(4-Chloropiperidin-1-yl)ethanamine (iv).** Crude chloride (0.73 g, 2.78 mmol), 4 M HCl, and EtOAc for 1 h were the reaction conditions. The crude Boc-deprotected amine was afforded as a brownish oil (160 mg, 35%). FAB MS *m/z* (M + H)$^+$ 163.

Preparation of Title Compound 21: Compound **10** (36 mg, 0.11 mmol), crude 2-(4-chloropiperidin-1-yl)ethanamine (160 mg, 0.98 mmol), and pyridine (2 mL) for 5 h at 45 °C were the reaction conditions. The title compound **21** was afforded as a dark blue solid (31 mg, 54%). Mp 200−202 °C; ^{1}H NMR ($CDCl_3$) δ 13.55 (s, 2H, C(5)O*H*, C(8)O*H*), 10.45 (t, 2H, C(1)N*H* and C(4)N*H*), 7.14 (s, 2H, C(6)*H* and C(7)*H*), 7.05 (s, 2H, C(2)*H* and C(3)*H*), 3.42−3.47 (m, 1H, CH_2CHCl), 3.31 (q, 4H, 2 × $HNCH_2CH_2N$), 2.62−2.84 (t, 4H, 2 × $HNCH_2CH_2N$), 2.35 (s, 6H, 2 × NCH_3), 1.95−2.12 (m, 2H, 2 × ring-*H*), 1.32−1.75 (m, 6H, 6 × ring-*H*), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3$) δ 185.29, 161.33, 155.34, 146.22, 146.12, 124.61, 123.95, 123.77, 115.31, 109.09, 58.96, 56.25, 50.79, 45.38, 34.69, 24.06; FAB MS *m/z* (M + H)$^+$ 487. Anal. ($C_{25}H_{31}N_4O_4Cl \cdot 2HCl \cdot 2H_2O$) C, H, N.

1,4-Bis-{[2-(3-chloroethylpiperidine)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione Hydrochloride (22). The method follows that of **13**, using Ph_3P (404 mg, 1.54 mmol), CCl_4 (447 μL, 4.63 mmol), and **7** (142 mg, 0.26 mmol) with stirring in dry $CHCl_3/CH_3CN$ (4:1; 5 mL) under N_2 at reflux temperature for 5 h. The title compound was afforded as a dark blue solid (117 mg, 68%). Mp > 300 °C dec; ^{1}H NMR ($CDCl_3/D_2O$ (10:1)) δ 7.01 (s, 2H, C(6)*H* and C(7)*H*), 6.96 (s, 2H, C(2)*H* and C(3)*H*), 3.43−3.83 (m, 16H, 2 × $HNCH_2CH_2N$, 2 × $HNCH_2CH_2$, and 8 × ring-*H*), 3.04 (m, 4H, ring-*H*), 1.85−2.31 (m, 8H, 8 × ring-*H*), 1.38−1.43 (m, 2H, 2 × CH_2CHCH_2OH), HCl salt was not observable in the spectrum; ^{13}C NMR ($CDCl_3/D_2O$(10:1)) δ 187.18, 156.32, 148.32, 127.37, 126.78, 117.38, 111.43, 58.40, 56.36, 48.97, 40.03, 38.45, 27.99; FAB MS *m/z* (M + H)$^+$ 589. Anal. ($C_{30}H_{38}N_4O_4Cl_2 \cdot 2HCl \cdot 2H_2O$) C, H, N.

DNA Modeling. Initial models of these selected chloroethylaminoanthraquinones were built using Maestro 6.05 graphic user interface (Schrodinger, Inc.). The conformational searches for all molecules were performed by Macromodel 8.5[36] using Monte Carlo simulation,[37] Amber force field,[38] and generalized Born/surface area (BD/SA) solvent representation.[39] Various models of duplex DNA with and without intercalation site were built using NAMOT software.[40]

The optimized structures of the selected chloroethylaminoanthraquinones were used as input files for GRID 22a software.[41] Whole molecules of target DNA and ligand were considered during calculations and all GRID parameters were kept at their default values. Docking of ligands into intercalation site of different DNA sequences were examined using the Glue method implemented in GRID software. All reported interaction energies were determined by GRID software.

Most favorable positions of ligands docked into the intercalation sites were used to model DNA−ligand conjugates, and in some

cases, the positions of ligands were adjusted manually to enable the generation of a bond between the alkylating moiety and N^7-guanine. The latter was assumed to be the preferred site of alkylation as the principal sites of attack by nitrogen mustard on DNA are N^7-guanine and N^3-adenine in a ratio of about 86:16.[42] Conjugates were minimized and subjected to short stochastic dynamics simulations of 10 ps at 300 K using Amber force field and GB/SA solvent representations, and final structures were minimized until convergence criteria was satisfied (0.5×10^{-2} kJ/mol/A). The hydrogen bonding between base pairs was preserved during simulations by using distance restraints between hydrogen acceptor and hydrogen donor of bases. Single ligand and DNA molecules were subjected to the same protocols.

Biology. Agarose Gel Cross-link Assay. The experiments followed the method of Hartley et al.[20] Briefly, linearized and 5′-end ^{32}P-radiolabelled plasmid pBR322 DNA (10 μL, ~100 ng), drug (25 μL to provide a range of final concentrations), and TEOA buffer (15 μL), to give a total final volume of 50 μL, were incubated at 37 °C for 1 h and subsequently terminated by the addition of DNA stop solution (50 μL) to each sample. Samples were precipitated, dried, and resuspended in strand separation buffer and heated for 2 min at 95 °C before being chilled in ice. The samples were electrophoresed in 0.8% agarose gel at 40 V overnight, and the resultant gel (covered in film wrap) was dried for 2 h at 80 °C onto one layer of Whatman 3MM paper and one layer of DE81 filter paper on a BIO-RAD 583 gel drier connected to a vacuum pump. Autoradiography was performed using Kodak Hyperfilm for 5 h at −70 °C using a cassette with an intensifying screen.

***Taq* Polymerase Stop Assay.** The following constituents were added to a PCR tube: 0.2% gelatine (5 μL), 25 mM $MgCl_2$ (10 μL), 10× buffer (10 μL), 2.5 mM dNTP mix (10 μL), drug-treated linearized DNA (50 μL), and dH_2O (8 μL). To the mixture (93 μL) was then added the labeled SRM primer (5 μL) and the *Taq* polymerase (2 μL, 5 U/μL), and the final mixture was vortexed. Each tube was covered with mineral oil, and the PCR was programmed to with the following steps: (1) 95 °C for 5 min, (2) 95 °C for 1 min, (3) 58 °C for 1 min, (4) 74 °C for 1 min and an additional 1 min per cycle. Steps 2−4 were repeated 29 times before denaturing at 94 °C for 5 min, followed by 10 min at 25 °C. The samples were then transferred into sterile eppendorfs, and the DNA was precipitated with three volumes of ethanol (95%, 300 μL) and NaOAc (3 M 2 μL), vortexed, and cooled in a dry ice bath for 10 min before being centrifuged for 10 min. The supernatant was removed, and the samples were washed with ethanol (70%, 150 μL), vortexed, and spun for 10 min, and the supernatant was removed. The wash was repeated before the samples were lyophilised. Each dried sample was resuspended in formamide dye (4 μL), heated to 95 °C for 3 min, and cooled in an ice-bath to denature the DNA. The samples were loaded into the wells of the gel, and electrophoresis was performed in TBE buffer at 1600−2000 V (approximately 2−3 h) at 55 °C using vertical glass electrophoresis plates. The resulting gel was then transferred onto one Whatman 3MM filter paper and one layer of DE81, covered in film wrap, and dried under pressure on a BIO-RAD 583 gel drier for approximately 2 h. Once dry, the gel was exposed to a Kodak Hyperfilm for 24 h before developing.

DNA Unwinding Assay. The 10× reaction buffer (includes 100 mM Tris-HCl, pH 7.9, 500 mM NaCl, 500 mM KCl, 50 mM $MgCl_2$, 1 mM EDTA, 150 μg/mL BSA, and 10 mM ATP; 2 μL), ddH_2O (15 μL), circular supercoiled pBR322 (250 ng, 1 μL), and **15** or **15-NO** (2 μL) were added to an eppendorf, mixed, and kept on ice. Each sample was carefully vortexed before incubation at 30 °C for 15 min. The reaction was stopped by addition of stop solution (2 μL). A sucrose loading buffer (2 μL) was added, and the mixture was vortexed and pulse spun. Electrophoresis was performed on 0.8% submerged horizontal agarose gels in 1 × TAE running buffer at 100 V for 3 h. Once the electrophoresis was completed, the agarose gel was submerged in dH_2O (enough to cover the gel) containing EtBr (30 μL) for 30 min. The EtBr containing water was discarded, and the gel was washed with water. The gel was observed under UV fluorescence, and the result was photographed.

Cytotoxicity Studies. The effect of the aminoanthraquinones on viability of human ovarian carcinoma cell lines was used as a measure of cytotoxicity. The parent cell line A2780 and the resistant variants A2780AD and A2780/cp70 were used to study the cytotoxic activity of the novel compounds. All cell lines were maintained as previously described.[43] The novel compounds were dissolved in DMSO and cell culture medium such that the final DMSO concentration was less than 0.1%. Aliquots of 20 μL were added to the cells to provide a range of concentrations from 0.1−1000 nM. The cell/drug combinations were incubated for 4 days at 37 °C. Cells were incubated with 0.5 mg ml^{-1} MTT solution for 4 h at 37 °C in a humidified 5% CO_2 atmosphere. The arrest of cell growth was determined as previously reported by Mosmann.[44]

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Supporting Information Available: Data of elemental analysis of aminoanthraquinones. This material is available free of charge via the Internet at http://pubs.acs.org

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