Cross-Linking and Sequence Specific Alkylation of DNA by Aziridinylquinones. 1. Quinone Methides

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The cytotoxicities and DNA cross-linking abilities of 16 1,4-benzoquinones have been investigated. All of the alkylmonoaziridinyl-1,4-benzoquinones were able to interstrand cross-link DNA after reduction and were cytotoxic *in vitro*. Compounds lacking an aziridine group were unable to cross-link DNA and were less cytotoxic. The methyl analogues were shown to preferentially react at TGC sequences. From comparing the structural requirements for cross-linking and the cytotoxicities, a mechanism has been proposed wherein some hydroquinones can associate and react at TGC sequences in DNA. These hydroquinones can subsequently autoxidize to form a reactive quinone methide which reacts at the opposite strand to form a cross-link.

Introduction

Several aziridinylbenzoquinones have undergone clinical trials as potential antitumor drugs.[1−3] These types of compounds can be activated toward alkylation as a result of bioreduction which is mainly brought about by NADPH:cytochrome P-450 reductase (E.C. 1.6.2.3), a one-electron-reducing enzyme which produces a semiquinone radical, or via NAD(P)H:oxidoreductase (DT-diaphorase, E.C. 1.6.99.2), which is a two-electron-reducing enzyme, and results in the formation of an activated hydroquinone.[4−6] The semiquinones and the hydroquinones can be in equilibrium.[7] In the case of aziridinylbenzoquinones, these reactions result in a change of electron distribution in going from a nonaromatic quinone to an aromatic hydroquinone or semiquinone. This effectively changes the p*K* of the aziridine rings such that they can protonate and become activated toward nucleophilic attack. The diaziridinylbenzoquinones can undergo two such activation processes and cross-link DNA strands.[6,8,9]

In a previous series of studies we have been able to show that whereas nonreduced DZQ (2,5-diaziridinyl-1,4-benzoquinone) reacted with all guanine N7-positions in DNA with a sequence selectivity similar to other chemotherapeutic alkylating agents such as the nitrogen mustards, reduced DZQ was found to react exclusively at 5′-GC-3′ sequences and in particular at 5′-TGC-3′ sites.[8] Further, independent studies using synthetic DNAs showed that DZQ forms covalent cross-links at dG-to-dG.[10] Reduced DZQ selectively cross-links DNA at TGC sequences with the cross-link spanning two base pairs, while reduced MeDZQ (2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone) preferably cross-links across three base pairs in the sequence 5′-GNC-3′.[11]

While attempting to assess the structural requirements necessary to maintain sequence selectivity in a variety of different novel aziridinylquinones, we have observed that some methylated monoaziridinylbenzoquinones can also cross-link DNA. The mechanisms of the cross-linking reactions have been investigated together with the structural requirements.

Results

Chemistry. The quinones used in this study are shown in Table 1.

DNA Interstrand Cross-Linking. DNA interstrand cross-linking was measured using a sensitive agarose gel-based assay.[9,11] A typical cross-link gel is shown in Figure 1 for **7** and **16**. The 2,5-diaziridinyl-1,4-benzoquinone (**16**) is a highly efficient cross-linking agent. Cross-linking is more efficient at lower pH and is enhanced markedly following reduction with ascorbic acid. Previous studies[9] have demonstrated that under the conditions used, ascorbic acid does not significantly alter the pH of the reaction mixture. The 2-aziridinyl-5-methyl-1,4-benzoquinone (**7**) can also cross-link DNA but is at least 10-fold less efficient at cross-linking than **16** under identical conditions. Again, cross-linking by **7** is more efficient at lower pH, and significant levels of cross-linking are only observed following reduction. The relative DNA interstrand cross-linking yields for **1**−**16**, as measured by microdensitometry, are shown in Table 2. Only **16** and the alkyl-containing monoaziridinylquinones **7**−**11** could cross-link DNA to any extent. The 5-methyl-containing compound **7** was more efficient at cross-linking than the 5,6- or 3,5-methyl compounds **8** and **9**. Cross-linking also decreased with the increase in length of the methylene chain at the 5-position in compounds **7**, **10**, and **11**.

The time courses for the cross-linking of 2-aziridinyl-5-methylbenzoquinone, **7**, and **16** were examined. Consistent with the previous results,[9,11] DZQ (**16**) achieved maximum cross-linking by 2 h under the conditions employed. In contrast, cross-links by **7** form slowly and are continuing to increase at 12 h (data not shown). At longer time points, accurate measurement of the percent cross-linking is not possible due to the production of single-strand breaks in the DNA which interfere with the assay.

Guanine N7-Alkylation by Aziridinylquinones. The guanine N7-position is the most reactive site on

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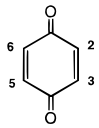
Table 1. Quinones Used in This Study and the *in Vitro* Cytotoxicity against Human Chronic Leukemia K562 Cells



drug	2	3	5	6	IC_{50} (μM)[a]
1	Me	H	H	H	2.75
2	Me	Me	H	H	1.4
3	Me	H	Me	H	0.75
4	Me	H	H	Me	1.25
5	Me	Me	Me	H	3.6
6	Me	Me	Me	Me	>50
7	Az[b]	H	Me	H	0.1
8	Az	H	Me	Me	1.3
9	Az	Me	Me	H	1.2
10	Az	H	Et	H	0.6
11	Az	H	Pr	H	0.6
12	Azet	H	Azet	H	>40
13	Azet	H	Me	H	>25
14	NH_2	H	Me	H	>15
15	NH_2	H	NH_2	H	>25
16	Az	H	Az	H	0.009

[a] Drug treatment was for 2 h at 37 °C. Errors are within ±15%. [b] Az = aziridinyl. Azet = azetidinyl.

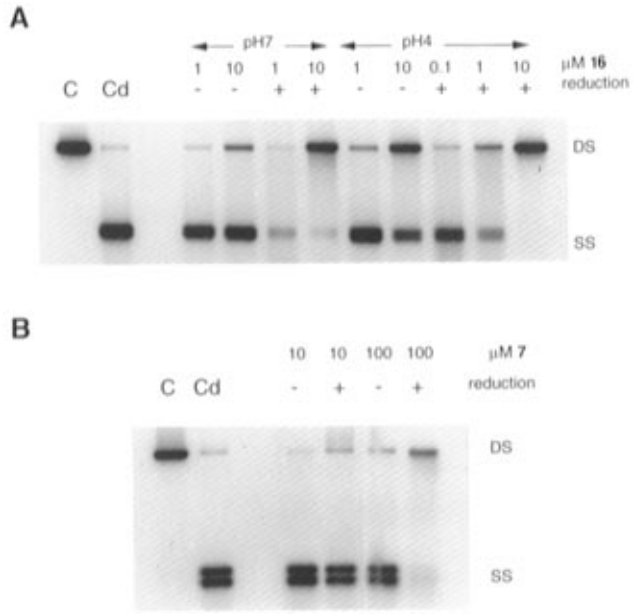


Figure 1. DNA interstrand cross-linking by **16** (A) and **7** (B) determined by the agarose gel assay. The compounds were reacted over a 2 h period at the pH indicated for **16** and at pH 4 for **7**. The reductions were carried out using 2 mM ascorbic acid. C and Cd are nondenatured and denatured controls, repectively. DS is double stranded, and SS is single stranded.

DNA for many chemotherapeutic agents. The DNA sequence selectivity for guanine N7-alkylation by the aziridinylbenzoquinones was examined as previously described.[9] A portion of a typical gel is shown in Figure 2. The patterns of alkylation produced were compared qualitatively under conditions of comparable alkylation and at doses that gave at most 1 alkylation/DNA molecule. Consistent with the previous results, DZQ (**16**) was able to weakly alkylate the guanines within the DNA sequence under nonreducing conditions. However, under these conditions alkylation was observed at all guanine bases with little sequence selectivity and was similar to that of melphalan. Upon reduction, the extent of overall alkylation was increased and the pattern was markedly altered. Both DZQ (**16**) and **7** following reduction were found to alkylate at the same subset of guanines within the sequence, consistent with alkylation primarily at 5′-TGC-3′ sequences. No alkylation of guanine N7 could be detected for compounds lacking an aziridinyl group (data not shown).

Table 2. DNA Interstrand Cross-Linking by **1**−**16** in Plasmid DNA

	cross-linking (%)[a]	
compd	10 μM	100 μM
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	12.2	81.0
8	1.2	4.7
9	3.0	9.0
10	6.9	57.1
11	2.2	13.2
12	0	0
13	0	0
14	0	0
15	0	0
16	100	100

[a] Drug treatment was for 2 h, pH 4, with reduction at the doses shown. Results are from a single representative experiment.

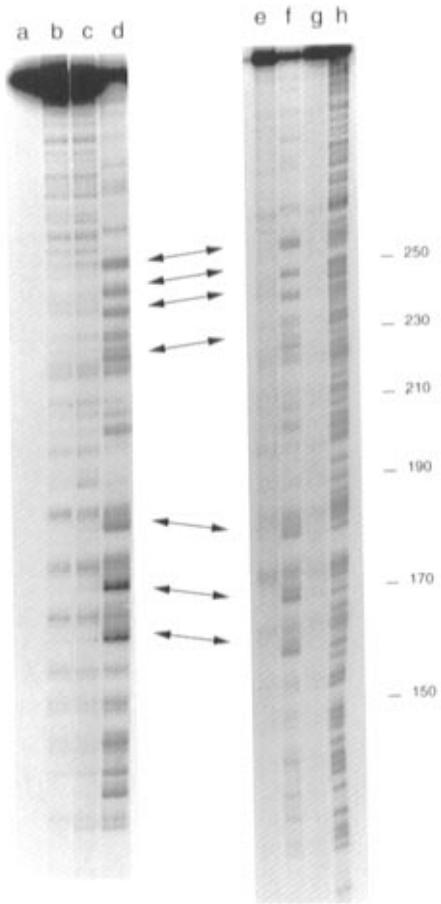


Figure 2. Sequence selectivity of guanine N7-alkylation by **16** and **7**. Reactions were performed at pH 4: (a) control, (b) 100 μM melphalan, (c) 100 μM **16**, (d) 1 μM **16** + 2 mM ascorbic acid, (e) 100 μM **7**, (f) 100 μM **7** + 2 mM ascorbic acid, (g) control, and (h) formic acid purine marker lane. The positions of the 5′-TGC sequences are indicated with arrows, and the sequence position in pBR322 is given.

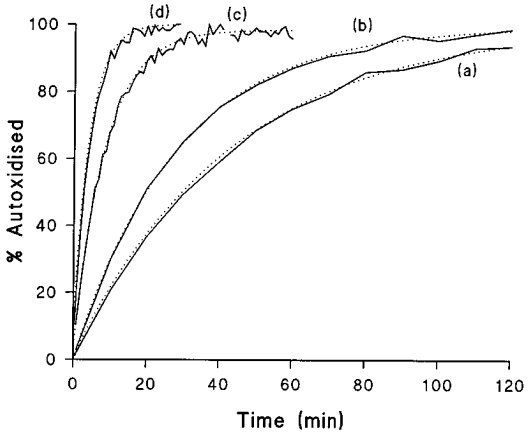


Figure 3. Rates of autoxidation of hydroquinones: (a) DZQ, **16**, (b) 2-aziridinyl-5-methyl-1,4-benzoquinone, **7**, (c) 2-[(2-chloroethyl)amino]-5-methyl-1,4-benzoquinone (ring-opened **7**), and (d) 2,5-bis[(2-chloroethyl)amino]-1,4-benzoquinone (aziridine ring-opened **16**). The dotted lines are the least-squares fits for the the calculated rates.

Autoxidation of Hydroquinones. The percent autoxidation rates for four hydroquinones are shown in Figure 3. From a least-squares analysis of these plots, the rates were calculated to be 0.022, 0.035, 0.12, and 0.23 min^{-1} for **16**, **7**, 2,5-bis[(2-chloroethyl)amino]-1,4-benzoquinone (ring-opened **7**), and 2-[(2-chloroethyl)-amino]-5-methyl-1,4-benzoquinone (ring-opened **16**), respectively.

***In Vitro* Cytotoxicities.** The cytotoxicities for the quinones **1**−**16** are included in Table 1. The K562 cell line was chosen as it has been shown to be sensitive to the redox reactions of diaziridinylquinones, and the levels of the main reducing enzymes have been determined.[8,12] DZQ (**16**) was highly cytotoxic with IC_{50} < 0.01 μM in the cell line. Compounds lacking an aziridine group were generally less toxic, particularly the azetidinyl- and amino-containing compounds **12**−**15.** The alkyl-containing monoaziridinyl quinones **7**−**11** were cytotoxic. In particular **7**, the most efficient cross-linker, had an an IC_{50} of 0.1 μM. The methyl-containing analogues **1**−**5** showed activities similar to **8**−**11** despite their inability to produce interstrand cross-links.

Discussion

The ability of aziridines to alkylate DNA has been exploited in a variety of antitumor drugs including AZQ, BZQ, mitomycin C, and triethylenemelamine. However, aziridines are inherently very reactive and prone to react with other cellular components. In an earlier study using ^{3}H-labeled BZQ, it was observed that only 5−10% of the label was found on the DNA of K562 cells while the remaining 90−95% reacted with protein (Hoey and Butler, unpublished data). It is our intention to exploit the sequence selectivity of the aziridinylbenzoquinones and subsequent larger DNA specific analogues (essentially DZQ analogues with large sequence selective side groups on position 3 of the quinone) to increase the DNA targeting of aziridinylquinones.

We initially synthesized the methylmonoaziridinylbenzoquinones **7** in order to study the structural requirements for bioreductive TGC sequence selectivity. It was assumed that these compounds would only monoalkylate. However, these studies clearly demonstrate (Table 2) that the methyl compounds are capable

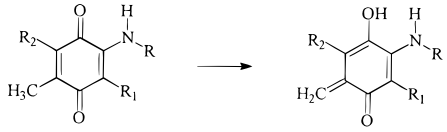


Figure 4. Methyl monoamine 1,4-benzoquinones and quinone methide formation.

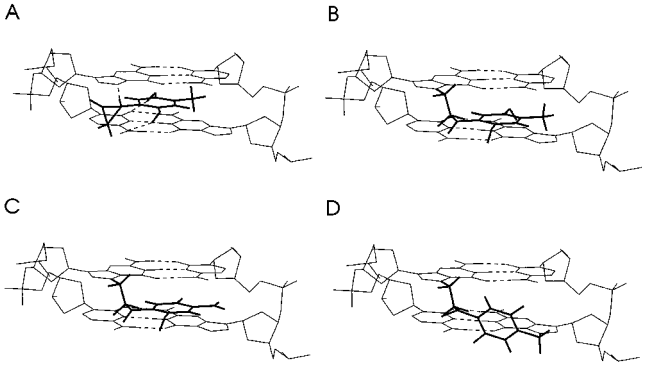


Figure 5. Computer simulations for the cross-linking of 2-aziridinyl-5-methyl-1,4-benzoquinone, **7**, between DNA strands. (A) Hydroquinone associates at the O2 and C4-NH_2 groups of cytosine and the N7 of guanine on the same strand. (B) The aziridine ring opens and reacts at the N7 of guanine. (C) The hydroquinone autoxidizes, the associations at O2 and C4-NH_2 of cytosine are lost, and a quinone methide can be formed near the guanine N7 on the opposite strand. (D) The methide reacts at the N7 and completes the cross-link.

of forming cross-links in DNA. It is proposed that this reaction can occur as a consequence of the formation of a quinone methide intermediate (Figure 4). Compounds of this type have been widely implicated in the activation of several anticancer drugs including mitomycin C, daunorubicin, and aclacinomycin and are essential intermediates in melanization and insect cuticle sclerotization.[13] As the methylaziridinylbenzoquinones react preferentially at TGC sequences and require the quinone to be initially reduced to a hydroquinone, the initial association of the hydroquinone into the DNA must be similar to that previously proposed for DZQ.[9] Computer simulations showed that the two hydroxyl groups of the hydroquinone can hydrogen bond to the O2 and C4-NH_2 groups of cytosine while the protonated aziridine associates with the N7 of the guanine on the same strand. A similar configuration can be assumed for the hydroquinones of the methylaziridinylbenzoquinones. Figure 5A shows a simulation for the hydroquinone of 2-aziridinyl-5-methyl-1,4-benzoquinone (**7**) associating in the same position. It can be seen from Figure 5B that when the protonated aziridine ring opens and reacts with the N7-position of one of the guanines, the methyl group moves closer toward the N7-position of the guanine on the opposite strand. However, at this stage, the methyl group cannot react with the guanine as the compound is still in the form of a hydroquinone, and a reactive quinone methide cannot readily be formed.

Figure 3 shows that aziridine ring-opened analogues are more susceptible to autoxidation reactions than the parent hydroquinones. This is consistent with the increase in electron donation from the nitrogen into the aromatic hydroquinone after the strained aziridine ring is opened. The increase in electron donation would

essentially lower the reduction potential of the hydroquinones and hence increase the rates of oxidation by oxygen.[14]

We therefore propose that the DNA-bound hydroquinones of the methyl aziridinyl analogues can autoxidize to reform quinones. Once the quinones are produced, they can rearrange to give reactive quinone methides (Figures 4 and 5C). The relatively negative reduction potential of the quinone would also favor methide formation.[15] The methide can then react with the guanine at the N7-position and complete the cross-link. (Figure 5D). The dimethylmonoaziridinylbenzoquinones **8** and **9** can form similar configurations; although as **9** has the methyl group in the same position as the hydrogens in **7** and **8**, it will be sterically constrained from entering at the same configuration. Simulations suggest that **9** may have to initially alkylate the guanines on the opposite strand in order to form a cross-link.

The above mechanism relies on the fact that the hydroquinones of the methyl aziridines have to autoxidize in order to form a cross-link. This is also consistent with the different time courses for the formation of cross-links by DZQ and 2-aziridinyl-5-methyl-1,4-benzoquinone, **7**. As the DNA-bound hydroquinone of DZQ still has a very reactive aziridine, it does not have to autoxidize in order to react with the other guanine and complete the cross-link. Indeed, if the singly bound DZQ hydroquinone were to oxidize back to a quinone, then the remaining aziridine would be less reactive toward nucleophilic attack.[8,9] Our failure at attempting to isolate the "half-ring-opened" analogue of DZQ (see experimental) illustrates the reactivity of the remaining aziridine. We propose that the cross-linking by the methylmonoaziridinylquinones is much slower than that of DZQ because they require autoxidation and the formation of a quinone methide. The alkylation reactions of the quinone methide are also expected to be slower than that of an aziridine.

The ethyl and propyl analogues **10** and **11** alkylate DNA and also cross-link but at lower rates than the methyl analogues. Computer simulations suggest that although the ethyl and propyl groups can associate between the bases in a similar manner as methyl, they are initially more sterically hindered. Furthermore, the resulting quinone methides would be less reactive than those of the methylquinones as it had been demonstrated that methylene-substituted quinone methides generally have reduced reactivities with nucleophiles.[13]

The simple mono-, di-, tri-, and tetramethyl quinones **1**−**6** can readily form quinone methides. Indeed, it well known that these compounds react via quinone methides with amines and CH-acidic compounds to form adducts.[16,17] However, the present study shows that they do not alkylate or cross-link DNA. It is therefore apparent that the association of the protonated aziridine group with the N7-position of the guanine (Figure 5A) is necessary in order to hold the hydroquinones in the correct position for alkylation.

Diazetidine benzoquinone **12** does not alkylate DNA. This shows the greater stability of an azetidine ring system compared to that of the aziridine.[15] Nonetheless, it might have been expected that the methyl azetidine analogue **13** could have alkylated DNA via quinone methide formation as the azetidine in the hydroquinone would be protonated and associate at the N7-position in a similar manner as the aziridines (Figure 5A). The methyl azetidine analogues did not, however, produce any measurable alkylations. One plausible reason for the lack of reactivity could be due to the relative position of the resulting quinone methide. Figure 5C suggests that when the aziridine ring opens and reacts with the N7-position of guanine, the methide group moves closer to the guanine on the opposite strand. It is possible that although the azetidine group can mimic the aziridine by associating between the bases, as the azetidine ring does not similarly open, the methide is too far away to react with the opposite guanine. Alternatively, the hydrogen bonding from the protonated azetidine may be so weak that after autoxidation the quinone merely leaves the DNA before the methide can react with the guanine. A similar situation could occur for the amine analogue **14**.

The cytotoxicities in Table 1 vary between 0.009 and $>50\ \mu$M, with DZQ being the most toxic. Quinones can be cytotoxic due to a variety of reasons which include redox cycling, the production of superoxide and other reactive oxygen radicals, reactions with thiols and amines, and DNA alkylations of the type described herein.[7,19] Quinones undergo redox cycling after reduction to semiquinones by the one-electron-reducing enzymes. It has recently been shown that the rates of reduction by cytochrome P-450 reductase and xanthine oxidase are dependent on the ease of reduction of the quinone.[20,21] Hence compounds which have low one-electron reduction potentials are reduced by the enzymes at relatively low rates and are poor at redox cycling. The one-electron reduction potentials may also affect the reactions with thiols.[22] The azetidine and amino analogues are expected to have much lower reduction potentials than the other quinones as the amine nitrogens will donate electron density into the quinone ring. Hence, they are expected to be less toxic, and this is shown in Table 1.

The nitrogens on the aziridines only weakly donate electrons into the ring and the aziridine analogues have similar reduction potentials as the simple methylbenzoquinones.[23] It appears from the results in Table 1 that the dimethylmonoaziridinylquinones **8** and **9** and the ethyl and propyl analogues **10** and **11** have similar toxicity values as the simple methylated quinones **1**−**6**. Compounds **8**−**11** can at least monoalkylate DNA and redox cycle, but the cross-linking reactions are slow. The implications from the cytotoxicity data are that the main contribution toward the toxicity is from the redox cycling or thiol/amine adduct formation and the alkylation reactions are of minor importance.

The methylmonoaziridinylbenzoquinone **7** is the most efficient cross-linker and the most toxic but still more than 10 times less toxic than DZQ, **16**. In principle, the cross-linking efficiencies of the methylaziridinylbenzoquinones should be similar to that of DZQ as once the aziridine ring opens and reacts with one guanine, the quinone methide reaction at the other guanine will eventually follow. One plausible explanation for the lower toxicity could be in the relatively slow rate of forming the second alkylation to complete the cross-link. It was suggested above that the monoalkylating reactions of the other monoaziridinylquinones **8**−**11** do not significantly contribute toward the cytotoxicities al-

though all of these compounds are capable of alkylating DNA. The implication is that these types of adducts are readily repaired. As the cross-linking step is so slow and requires the initial alkylation step to fix the methide in the correct position, it is possible that repair processes can occur before the cross-link can be formed.

The present compounds therefore have limited use as potential antitumor agents. However, we are currently pursuing the quinone methide mechanism as it may be possible to exploit this reaction and avoid the use of at least one reactive aziridine in the design of effective bioreductive cross-linking agents. It is possible to increase the rate of autoxidation of a hydroquinone simply by lowering the reduction potentials of the quinone (see Figure 3). Hence, for example, if an amine is linked at position 3 of the quinone ring (e.g., 2-aziridinyl-3-(alkylamino)-5-methyl-1,4-benzoquinone), the amine group would lower the one-electron potential of the quinone such that one-electron reduction and the subsequent redox cycling would not readily occur. The reactions with DT-diaphorase do not appear to be dependent on the reduction potentials.[24] Hence, if compounds of this type could be reduced by this enzyme, the resulting hydroquinone would have the correct structure to associate into TGC sequences and readily autoxidize. The lower potential would also increase the reactivity of the resulting quinone methide.[13] We are currently synthesizing a series of compounds based on these ideas.

Experimental Section

Chemicals. The quinones used in the study are shown in Table 1. Quinones **1**−**4** and **6** and azetidine were obtained from Aldrich Chemical Co. Compounds **2** and **5** were made from the oxidation of 2,3-dimethylhydroquinone and trimethylhydroquinone (Aldrich) by silver oxide. Ethyl- and propyl-1,4-benzoquinone were prepared according to previous methods.[25]

Aziridine (ethyleneimine) was prepared according to the flash distillation method of Reeves et al.[26] Initial quinone stock solutions for cross-linking and sequence selectivity experiments were made up in DMSO at 10 mM. Electrophoresis-grade acrylamide and bis(acrylamide) were purchased from Sigma; ultrapure urea and agarose were from BRL and piperidine and ascorbic acid from BDH. [γ-^{32}P]ATP (5000 Ci/mmol) was from Amersham and pBR322 plasmid DNA from Northumbria Biologicals.

2-Aziridinyl-5-methyl-1,4-benzoquinone (7) and 2-Aziridinyl-5,6-dimethyl-1,4-benzoquinone (8). These quinones were prepared according to previous methods.[27]

2-Aziridinyl-3,5-dimethyl-1,4-benzoquinone (9). To a stirred solution of 2,6-dimethyl-1,4-benzoquinone (0.5 g, 3.7 mmol) in dry methanol (40 mL) under nitrogen at 0 °C was added aziridine (0.25 mL, 4.7 mmol). The solution was then stirred for 16 h. The methanol was then removed *in vacuo*; the resulting solid was dissolved in dichloromethane (30 mL) and filtered. This was then chromatographed on silica gel using 3:1 petroleum ether (40−60)/ethyl acetate as the eluent. Evaporation of the solvent *in vacuo* afforded **9** as red crystals: yield 241 mg (37%); mp 105−106 °C; TLC R_f 0.45 (EtOAc/Pet (40−60), 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.45 (d, 1H, J = 1.5 Hz, H), 2.3 (s, 4H, Az), 2.09 (s, 3H, CH_3), 2.06 (d, 3H, J = 1.5 Hz, CH_3); MS (EI) m/z 177 (M^+), 176, 148, 134, 120, 107, 81, 58; UV (EtOH) λ_{max} 216, 274, 426 (no base shift) nm; IR (film) ν_{max} 1691, 1648, 1592, 1357 cm^{-1}. Anal. Found: C, 67.71; H, 6.17; N, 8.35. $C_{10}H_{11}NO_2$ requires C, 67.8; H, 6.21; N, 7.91.

2-Aziridinyl-5-ethyl-1,4-benzoquinone (10). To a stirred, ice-cooled solution of ethyl-1,4-benzoquinone (445 mg, 3.27 mmol) in THF (2.5 mL) at 0 °C under nitrogen was added aziridine (85 μL, 1.64 mmol) dropwise. The solution turned dark immediately and after 50 min was filtered through a pad of silica gel and eluted with ethyl acetate/petroleum ether (40−60) (1:3). The filtrate was evaporated *in vacuo*, and the residue was chromatographed on silica gel using ethyl acetate/petroleum ether (40−60) (1:3) as eluent. Two isomeric products were collected, **10** and 2-ethyl-6-aziridinyl-1,4-benzoquinone, with a combined yield of 46%. 5-Ethyl-2-aziridinyl-1,4-benzoquinone appeared as orange crystals: mp 66−68 °C; TLC R_f 0.35 (EtOAc/Pet (40−60), 1:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (t, 1H, J = 1.5 Hz, H-2), 6.07 (s, 1H, H-1), 2.49 (qd, 2H, J = 7, 1.5 Hz, CH_2), 2.24 (s, 4H, Az), 1.17 (t, 3H, J = 7 Hz, CH_3); MS (EI) m/z 177 (M^+), 162, 150, 135, 120, 107, 67, 53; UV (EtOH) λ_{max} 235, 275, 403 nm (no base shift); IR (film) ν_{max} 2981, 2910, 1670, 1645, 1602 cm^{-1}. Anal. Found: C, 67.0; H, 6.65; N, 7.40. $C_{10}H_{11}NO_2$·0.1EtOAc requires C, 67.17; H, 6.35; N, 7.53.

2-Aziridinyl-5-propyl-1,4-benzoquinone (11). To a solution of propyl-1,4-benzoquinone (669 mg, 4.46 mmol) in THF (5 mL) at 0 °C under N_2 was added aziridine (116 mL, 96 mg, 2.23 mmol). After 4 h, the mixture was filtered through a pad of silica gel and eluted with petroleum ether (40−60)/ethyl acetate (3:1). After the solvent was removed *in vacuo*, the crude mixture was chromatographed on silica gel using petroleum ether (40−60)/ethyl acetate (3:1) as eluent. Two isomeric products were collected, **11** (27 mg, 6% yield) as orange/brown crystals and 2-aziridinyl-6-propyl-1,4-benzoquinone (7 mg, 2% yield) as a brown solid: TLC R_f 0.26 (Pet (40−60)/EtOAc, 3:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51−6.49 (m, 1H, H-3), 6.06 (s, 1H, H-6), 2.43 (td, 2H, J = 7, 1 Hz, H-7), 2.24 (s, 4H, aziridine-H), 1.57 (sext, 2H, J = 7 Hz, H-8), 1.01 (t, 3H, J = 7 Hz, H-9); MS (EI) m/z 191 (M^+), 176, 149, 107; UV (EtOH) λ_{max} 212, 278, 405 (no base shift) nm; IR (film) ν_{max} 2356, 1681, 1644, 1592 cm^{-1}.

2,5-Diazetidinyl-1,4-benzoquinone (12). This compound was prepared according to previous methods.[28,29]

2-Azetidinyl-5-methyl-1,4-benzoquinone (13). Methyl-1,4-benzoquinone (1.34 g, 10.95 mmol) dissolved in THF, under nitrogen, was cooled on an ice bath. To this was added azetidine (0.37 mL, 5.47 mmol), and the solution was stirred for 3 h. The resulting mixture was filtered through a pad of silica gel and eluted with ethyl acetate/petroleum ether (40−60) (1:1). The solvent was removed *in vacuo*, and the residue was chromatographed on silica gel using ethyl acetate/petroleum ether (40−60) (1:1) as eluent to yield **13** as purple microcrystals: yield 158 mg (16%); mp 115−116 °C; TLC R_f 0.33 (EtOAc/Pet (40−60), 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.33 (q, 1H, J = 1.5 Hz, H-1), 5.26 (s, 1H, H-2), 4.67−4.38 (bm, 2H, H-3a), 4.20−3.93 (bm, 2H, H-3b), 2.50 (quintet, 2H, J = 7.5 Hz, H-4), 2.08 (d, 3H, J = 1.5 Hz, CH_3); MS (EI) m/z 177 (M^+), 160, 148, 136, 122, 108, 53; UV (EtOH) λ_{max} 224, 275, 507 nm (no base shift); IR (film) ν_{max} 3052, 3018, 2939, 2871, 1662, 1641, 1594 cm^{-1}. Anal. Found: C, 67.50; H, 6.50; N, 8.09. $C_{10}H_{11}NO_2$ requires C, 67.80; H, 6.21; N, 7.91.

2-Amino-5-methyl-1,4-benzoquinone (14) and 2,5-Diamino-1,4-benzoquinone (15). These compounds were prepared according to the methods of Moore et al.[30,31]

2,5-Diaziridinyl-1,4-benzoquinone (DZQ) (16). This compound was prepared according to the previous method.[32]

2,5-Bis[(2-chloroethyl)amino]-1,4-benzoquinone. This compound was prepared according to the previous method.[33]

The "half-ring-opened" analogue, 2-aziridinyl-5-[(2-chloroethyl)amino]-1,4-benzoquinone, could be detected during the reaction using TLC and HPLC. However, it was unstable and could not be isolated.

2-[(2-Chloroethyl)amino]-5-methyl-1,4-benzoquinone. 2-Aziridinyl-5-methyl-1,4-benzoquinone (10 mg, 0.061 mmol) was dissolved in methanol (10 mL). To this was slowly added 1.0 mL of concentrated hydrochloric acid. The solvent was removed *in vacuo*, and the resulting solid was chromatographed on silica gel using petroleum ether (40−60)/ethyl acetate (3:1) as eluent. The first band was collected and on removal of the solvent *in vacuo* afforded a red solid: yield 3 mg (24%); mp 98−100 °C; TLC R_f 0.33 (EtOAc/Pet (40−60), 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (d, 1H, J = 1.5 Hz, H-6), 5.81 (bm, 1H, N-H), 5.47 (s, 1H, H-3), 3.67 (t, 2H, J = 6 Hz, H-8), 3.46 (q, 2H, J = 6 Hz, H-7), 2.05 (d, 3H, J = 1.5 Hz,

CH_3); MS (EI) *m/z* 201, 199 (M^+), 164, 150, 68; UV (EtOH) λ_{max} 216, 274, 470 nm (no base shift); IR (film) ν_{max} 3274, 1673, 1641, 1587, 1502 cm^{-1}. Anal. Found: C, 54.00; H, 5.25; N, 6.86. $C_9H_{10}NO_2Cl$ requires C, 54.13; H, 5.01; N, 7.02.

An attempt was made to synthesize 2-aziridinyl-5-(trifluoromethyl)-1,4-benzoquinone. (Trifluoromethyl)-1,4-benzoquinone was prepared according to the methods of Whalley[34] and Littell et al.[35] Upon addition of aziridine in THF, an orange compound was formed. However, it was too unstable to isolate. Similarly, despite several attempts, trimethylbenzoquinone did not form a stable compound when reacted with aziridine.

Enzymes. Restriction enzymes *Hin*dIII and *Bam*HI, T4 polynucleotide kinase (PNK), and bacterial alkaline phosphatase (BAP) were obtained from BRL.

Buffers. TEA is 25 mM triethanolamine, 1 mM EDTA, pH 7.2. TBE electrophoresis buffer is 90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.3. Tris-acetate electrophoresis buffer is 40 mM Tris, 20 mM acetic acid, 2 mM EDTA, pH 8.1. Alkylation stop solution is 0.6 M sodium acetate, 20 mM, EDTA, 100 mg/mL tRNA. Strand separation buffer is 30% dimethyl sulfoxide, 1 mM EDTA, 0.04% bromophenol blue, 0.04% xylene cylanol. BAP buffer is 10 mM Tris-HCl, 120 mM NaCl, pH 8. PNK buffer is 60 mM Tris-HCl, 15 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 35 mM ATP, pH 7.8.

Preparation of End-Labeled DNA. pBR322 DNA was linearized by reaction with *Hin*dIII (2 units/mg, 37 °C, 1 h), dephosphorylated with BAP (3 units/mg, 65 °C, 1 h), and purified by standard phenol/chloroform extraction and ethanol precipitation reactions.[36] The DNA was labeled at the 5′-ends with T4 PNK as described by Maxam and Gilbert.[37] For measurement of guanine N7-alkylation, the DNA was further cut with *Bam*HI (2 units/mg, 37 °C, 1 h).

Determination of DNA Interstrand Cross-Linking. The method has been described in detail.[38] Briefly, labeled DNA (~5000 cpm/sample) was incubated with drug in TEA buffer at 37 °C. Reactions were terminated by the addition of an equal volume of alkylation stop solution, and the DNA was immediately precipitated by the addition of 3 vol of 95% ethanol. Following centrifugation and removal of supernatant, the DNA pellet was dried by lyophilization.

Samples were dissolved in 10 mL of strand separation buffer, heated at 90 °C for 2 min, and chilled immediately in an ice−water bath prior to loading. Control undenatured samples were dissolved in 10 mL of 6% sucrose, 0.04% bromophenol blue and loaded directly. Electrophoresis was performed on 20 cm 0.8% submerged horizontal agarose gels at 40 V for 16 h with Tris-acetate running buffer.

Gels were dried at 80 °C onto filter paper, and autoradiography was performed at −70 °C. Quantitation was achieved by microdensitometry of the autoradiograph using a LKB Ultrascan-XL laser densitometer. For each lane the amount of single- and double-stranded DNA was determined and the percent cross-linked (double-stranded) DNA calculated.

Determination of Sites of Guanine N7-Alkylation. Singly and end-labeled DNA (~50 000 cpm/sample) were incubated with drug in TEA buffer in a total volume of 50 μL for 60 min at 20 °C. The reaction was terminated by the addition of 50 μL of cold alkylation stop solution and DNA recovered by precipitation with 3 vol of 95% ethanol. The DNA was resuspended in 0.3 M sodium acetate, 1 mM EDTA and ethanol precipitated again and the pellet washed with cold ethanol prior to vacuum drying.

The salt-free DNA pellet was resuspended in freshly diluted 1 M piperidine and incubated at 90 °C for 15 min to convert quantitatively sites of guanine N7-alkylation into strand breaks.[39] Samples were lyophilized, resuspended in formamide loading buffer, heated at 90 °C for 1 min, and chilled in an ice bath prior to loading onto the gel. Electrophoresis was achieved in 0.4 mm × 80 cm × 20 cm 6% polyacrylamide gels containing 8 M urea. Running time was ca. 3 h at 3000 V, 55 °C. Gels were dried and autoradiographed and relative band intensities determined by microdensitometry as described above.

Toxicity Testing. Human chronic myeloid leukemic K562 cells were maintained in log phase growth in suspension in complete RPMI 1640 medium supplemented with 10% horse serum (GIBCO BRL) at 37 °C, 5% CO_2; 2 h cytotoxicity studies were carried out on these cells (600/well) in 96-well plates using the MTT method.[40] The absorbances were read on a multiscan plate reader at 540 and 640 nm. Plots were then drawn for inhibition of cell growth as a function concentration. Each compound was tested in triplicate at each concentration.

Molecular Modeling. Molecular modeling was carried out on a Silicon Graphics Iris 4D/310GTX workstation using QUANTA 4.0 software (including CHARMm 22.2) working under IRIS 4.0.5.

Autoxidation of Hydroquinones. The rates of reaction for some of the hydroquinones with oxygen were determined by initially reducing argon-saturated solutions of the quinones (50 μM) in 25 mM Tris-HCl, pH 7.4, with sodium borohydride. After completion of the reaction, the solutions were rapidly shaken in air, and then the absorbance changes were measured as a function of time in a 3 mL stirred cuvette using a Hewlett Packard 8452A spectrophotometer. The percent autoxidation was calculated from the increase of the quinone absorptions at their λ_{max}. These were 324 nm for **16**, 344 nm for 2,5-bis[(2-chloroethyl)amino]-1,4-benzoquinone (aziridine ring-opened **16**), 412 nm for **7**, and 492 nm for 2-[(2-chloroethyl)amino]-5-methyl-1,4-benzoquinone (ring-opened **7**). As the absorbance changes for the latter two quinones were relatively low, percent autoxidations were calculated from the difference of absorbance at 412 or 492 nm and that at 600 nm. Neither the quinones nor the hydroquinones absorb at 600 nm, and hence this procedure compensates for the background noise levels of the absorbances.

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