Cross-Linking and Sequence-Specific Alkylation of DNA by Aziridinylquinones. 3. Effects of Alkyl Substituents

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The cytotoxicities and DNA cross-linking abilities of several alkyl-substituted diaziridinylquinones have been investigated. The cytotoxicities were determined in DT-diaphorase-rich (H460 and HT29) and -deficient (H596 and BE) cell lines. It was shown that the cytotoxicities in these cell lines correlated with the relative rates of reduction by the purified human enzyme and with the cross-linking efficiencies. The rates of reduction by DT-diaphorase were more dependent on the structures of the compounds than the reduction potentials, as determined by cyclic voltammetry. A computer model was also used to explain high efficiency of cross-linking and the GNC sequence selectivity of the reduced methyl-substituted diaziridinylquinones.

Introduction

Several aziridinylquinones have undergone clinical trials as potential antitumor drugs.[1−3] These types of compounds can be activated toward alkylation as a result of bioreduction by the one-electron reducing enzymes (e.g. NADPH:cytochrome P450 reductase, cytochrome b_5 reductase, xanthine oxidase) or by the two-electron reducing enzyme (NAD(P)H:oxidoreductase (DT-diaphorase, E.C. 1.6.99.2)).[4−6]

Our previous studies on diaziridinylquinones concentrated on determining the structural requirements for the compounds to be substrates for DT-diaphorase (DTD) and the mechanisms whereby they alkylate DNA.[7−9] During the course of these studies, MeDZQ (2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone (**1**)) was identified as being an excellent substrate for DTD and a good correlation was obtained for the toxicity of MeDZQ and the DTD levels in several non-small-cell lung cancer (NSCLC) and breast cell lines.[10] MeDZQ also inhibits the growth of DTD-rich human cell animal xenografts (unpublished results). As a consequence of these and other studies, an analogue of MeDZQ is currently being considered for phase I/II clinical trials by the NCI and the CRC.

The previous works showed that diaziridinylbenzoquinones with a hydrogen in the 6-position cross-link DNA at TGC sequences after reduction.[9,11,12] This is essentially because the hydrogen is small enough to be accommodated between the stacked bases of DNA. In contrast, MeDZQ cross-links the bases in DNA at 5′-GNC-3′ sequences after reduction.[13] However, the interactions responsible for the selectivity of reduced MeDZQ have not, as yet, been studied.

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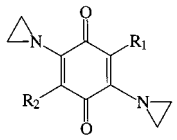
Table 1. Quinones Used in This Study and Reduction Potentials



compd	R_1	R_2	$E_2(Q/Q^{2-})$ (mV)[a]
1	CH_3	CH_3	−1330
2	CH_3	C_2H_5	−1355
3	CH_3	n-C_3H_7	−1344
4	CH_3	i-C_3H_7	−1396
5	C_2H_5	C_2H_5	−1350
6	n-C_3H_7	n-C_3H_7	−1407
7	i-C_3H_7	i-C_3H_7	−1396
8	H	H	−1065

[a] Values ± 30 mV.

The aim of the present work was to study the biological and physical properties of a series of alkyl-substituted MeDZQ analogues in order to establish the structural requirements which are important for determining the cytotoxic properties of MeDZQ.

Results

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

In Vitro Cytotoxicities. The cytotoxicities for the quinones **1**−**9** are shown in Table 2.

Cyclic Voltammetry. The two-electron reduction potentials, as measured by cyclic voltammetry, are included in Table 1. The trends in reduction potentials are as would be expected from the Hammett functions of the substituted groups.[14] Thus, as the σ_p values for the alkyl groups are relatively similar, the reduction potentials are also very similar. This is in contrast to the more positive σ_p value for a hydrogen which means that the reduction potentials for DZQ are more positive (DZQ is more readily reduced).

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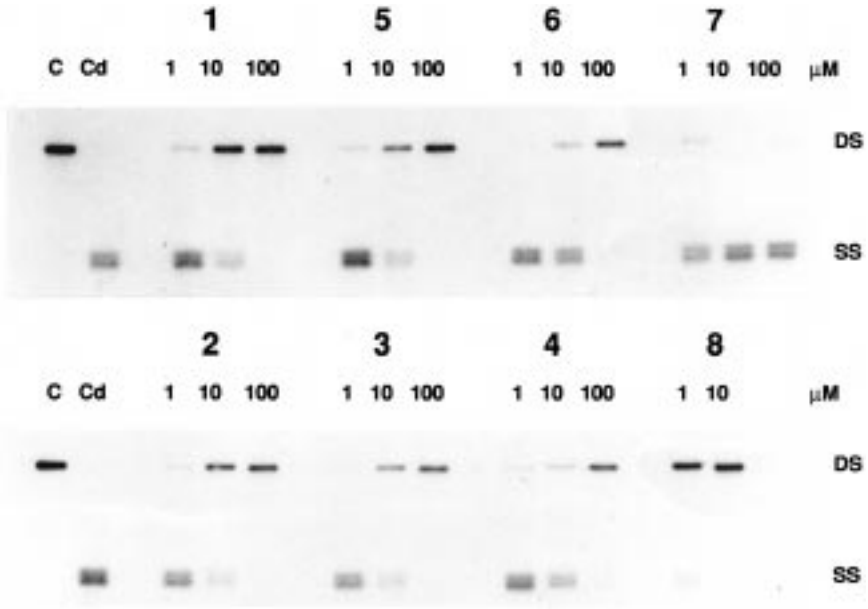

Figure 1. DNA interstrand cross-linking of plasmid DNA by the reduced quinones determined by the agarose gel assay. Compounds were reacted at the concentration shown over 2 h at 37 °C, pH 4, following reduction with dithiothreitol. C and Cd are nondenatured and denatured controls, respectively, DS is double-stranded DNA, and SS is single-stranded DNA.

Table 2. In Vitro Cytotoxicity Values (nM) for the Different Cell Lines and Cytotoxic Differentials[a]

compd	H460	H596	HT29	BE
1	0.77	48.8 (63.4)	3.4	25.9 (7.6)
2	1.6	98.5 (61.6)	3.9	84 (21.5)
3	3.3	228 (69.1)	5.5	67 (12.2)
4	3.6	1230 (341.7)	33	1300 (39.4)
5	4.1	1160 (282.9)	21.8	290 (13.3)
6	20.1	1290 (64.2)	58.2	590 (10.1)
7	170	2620 (15.4)	2740	3130 (1.1)
8	4.6	12.9 (2.8)	5.1	10.2 (2)

[a] Errors within ±15%. The cytotoxicity differentials (H596/H460 and HT29/BE) are in parentheses.

Table 3. Rates of Reduction by Purified DTD and BE Cell Extracts

compd	DTD[a]	BE extracts[b]
1	21.0	9.2
2	12.5	4.0
3	7.9	3.4
4	2.7	1.1
5	3.0	2.3
6	1.0	1.7
7	0.22	ND[c]
8	35.6	25.3

[a] μM/min/mg of protein. [b] nM/min/mg of protein. [c] ND, not detected.

Reduction Rates by DTD. The rates of reduction of the compounds by human recombinant DTD and in the BE cell extracts are shown in Table 3.

DNA Interstrand Cross-Linking. The abilities of the reduced forms of the quinones to produce interstrand cross-links in the plasmid DNA are shown in Figure 1, and the relative cross-link efficiencies are given in Table 4.

Discussion

The cytotoxicity values for MeDZQ (**1**) and DZQ (**8**), given in Table 2, are of similar magnitude to those reported previously.[8,11] Interestingly, although DZQ is the best substrate for DTD (Table 3) and is the best cross-linking agent (Table 4), it shows the poorest differential between the DTD-rich and -deficient cell lines. This is probably because DZQ has a more positive one-electron reduction potential and thus can be more readily reduced by other enzymes.[15,16] Furthermore, as DZQ has labile hydrogens, it can be detoxified by undergoing Michael additions with other cell components.[17] We have observed similar trends with other diaziridinylquinones.[10]

Table 4. Relative DNA Interstrand Cross-Linking Efficiencies of the Reduced Quinones

	% double-stranded (cross-linked) DNA	
compd	10 μM	100 μM
1	79.8	100
2	69.0	100
3	58.4	100
4	28.1	87.8
5	60.9	100
6	16.4	95.6
7	0	0
8	100	100

The cytotoxicity data shows that there is a trend such that as the size of the alkyl group is increased, the toxicities in all of the cell lines generally decrease (compare **1**, **5**, **6**, and **7**). A similar trend is found for the DNA cross-linking efficiencies (Table 4). However, the cross-linking data was obtained after chemical reduction by dithiothreitol and hence is not influenced by the relative rates of reduction by DTD. Indeed, the conditions of the reduction were chosen such that all of the quinones were reduced to the same extent. Hence, the implication is that it is the size or shape of the hydroquinones which determines the cross-linking efficiencies.

The results in Table 4 show that the cross-linking efficiencies decrease as the size of the side chain increases (compare **8, 1, 5, 6**, and **7**). It is also apparent that if a compound contains a methyl group, it can cross-link DNA more efficiently than the disubstituted analogue (compare **2** and **5**, **3** and **6**, **4** and **7**). Quinone **7**,

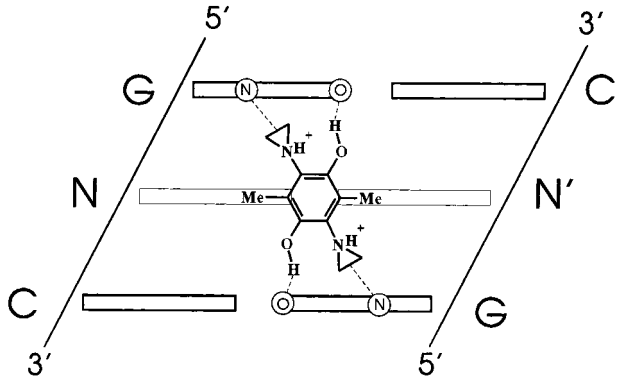

Figure 2. Initial interactions between MeDZQ hydroquinone and DNA at a 5′-GNC-3′ sequence. The dotted lines indicate the hydrogen bonds between the hydroquinone OH's and the guanine O-6's and the interactions between the protonated aziridines and the guanine N-7's.

which has two isopropyl side chains, did not produce any measurable cross-links, even at the highest dose used in the assay. Our previous studies have shown that MeDZQ cross-links DNA at GNC sequences after reduction.[13] Computer models were therefore generated in order to study the initial interaction between the hydroquinones of the alkyl-substituted diaziridinylbenzoquinones and DNA. A simplified diagram for the hydroquinone of MeDZQ is shown in Figure 2. In this model, the two hydroquinone OH groups hydrogen bond to the two O-6's of the guanine residues. In this position, the two protonated aziridines can interact with the two N-7's of the same guanines (the positions where the GNC alkylations will occur). The overall interactions occur in the major groove of DNA. However, the model also predicts that due to the helical structure of the DNA, the hydroquinone has to be twisted in the major groove. This twisting forces one of the methyl groups to point slightly into the DNA and the other group points slightly out. It is this slight twisting which can account for the increased cross-linking efficiencies of the methyl-substituted analogues. The models predict that the hydroquinones with a methyl group can fit closer to the DNA and have less steric hindrance with the N bases. If this side chain is increased, the GNC cross-linking abilities of these compounds will decrease. The models also predict that as the second side chain can point away from the DNA, all of the methyl-substituted quinones can be accommodated at this alkylation site. Hence the analogues **2**−**4** are relatively efficient cross-linkers.

The rates of reduction of the different quinones by the human recombinant DTD (Table 3) show that DZQ is the best substrate for the enzyme and there is a decrease in the rates as the alkyl groups are extended. DZQ has the most positive two-electron reduction potential (Table 1) and thermodynamically should be the most readily reduced. The two-electron potentials of the alkyl-substituted quinones are very similar. Hence, although there is an indication that rates of reduction by DTD follow a similar trend as the two-electron reduction potentials, it is probable that the dramatic changes in the rates of reduction are due to the changes in structure of the quinones. A recent study has shown that indolequinones behave similarly.[18]

Interestingly, the rates of reduction by DTD follow a similar trend as the cross-linking efficiencies (Table 4). Unfortunately, the crystal structure of the human DTD enzyme is not available, but the rat enzyme with bound duroquinone has been published and is believed to be similar to that of the human.[19] The rat enzyme structure shows that a methyl group from duroquinone can be accommodated close to the Y126, Y128, and F178 amino acids, with the −OH group of the hydroquinone being hydrogen bonded to the −OH groups of Y126 and Y128. It would appear that the methyl group in the para position to this methyl is free to rotate around the C−C bond. It can be speculated from these positions that groups larger than a simple hydrogen or methyl group will have difficulty in locating the space available around the Y126, Y128, and F178 amino acids. If this is true, it would explain why the ethyl and propyl groups of compounds **5** and **6** are much poorer substrates than **1**. Similarly, the methyl groups of compounds **2**−**4** can be accommodated as with duroquinone with the more bulkier alkyl groups at the para positions having a much less effect on steric hindrance. This general concept wherein only one side of the quinone has to interact with the electron-transfer sites of DTD could explain why other apparently more bulkier quinones (e.g. certain indolequinones such as EO9 analogues and streptonigrin[11,18,20]) can also be good substrates for the enzyme and also would explain why adriamycin and other anthraquinones are poor substrates for DTD.[21]

Although **1** is the best substrate for DTD, the best DNA cross-linker, and the most cytotoxic of the alkyl-substituted quinones, it does not produce the highest cytotoxic differential (Table 2). It is generally believed that these differentials are due to the differences in activation of the quinones by DTD in the DTD-rich cell lines (H460 and HT29) compared to the amount of activation by the one-electron reducing enzymes in the DTD-deficient cell lines (H596 and BE).

The relative rates of reduction by DTD and the reduction in the BE extracts generally follow similar trends (Table 3). However, it can be seen from Table 2 that **4** and **5** are inordinately less toxic in the BE and H596 cell lines and hence produce relatively high differentials. It would appear that these quinones or their semiquinones are more readily detoxified in these DTD-deficient cell lines. At the present time, we cannot explain these findings. However, the possible mechanisms of detoxification involving glutathione addition, phenol UDP glucuronosyltransferases, sulfotransferases, and MRP1 are currently being investigated for all of these quinones.

Experimental Section

Chemicals. The quinones used in the study are shown in Table 1. 1,4-Diacetyl-2,5-dimethoxybenzene was purchased from Apin Chemicals Ltd., Abingdon, Oxon, U.K.

Aziridine (ethylenimine) was prepared according to the flash distillation method of Reeves et al.[22] Initial stock solutions for the cross-linking assays 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 pBR322 plasmid DNA was obtained from Northumbria Biologicals. All other reagents were of the highest purity commercially available.

2,5-Diaziridinyl-3,6-dimethyl-1,4-benzoquinone, MeDZQ (**1**),[23] and 2,5-diaziridinyl-1,4-benzoquinone (**8**)[24] were prepared according to previous methods.

1,4-Diethyl-2,5-dimethoxybenzene. A stirred solution of 1,4-diacetyl-2,5-dimethoxybenzene (2.5 g, 11.3 mmol), hydrazine (80% solution in water, 9.4 mL, 0.23 mol) and potassium hydroxide (4.9 g, 87.5 mmol) in ethylene glycol (18 mL) was refluxed for 13 h. The solution was then distilled until the reaction temperature reached 195 °C. The distillate was then extracted with ether. The ether extracts were dried (Na_2SO_4), and the solvent was removed in vacuo to yield a colorless liquid which solidified to colorless crystals (0.9 g, 41%). A further crop was obtained by extracting the reaction mixture in the same way (0.8 g, 36%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.67 (s, 2H, Ar−H), 3.75 (s, 6H, OCH_3), 2.60 (q, J = 7 Hz, 4H, CH_2), 1.19 (t, J = 7 Hz, 6H, CH_3); MS EI m/z 194 (M^+), 193, 179, 165, 135.

(2,5-Dimethoxy-4-methylphenyl)ethene. To a stirred suspension of methyltriphenylphosphonium bromide (6.54 g, 18.3 mmol) in dry THF (120 mL) was added butyllithium (11.5 mL, 1.6 M solution in hexanes, 18.4 mmol). After 20 min, a solution of 2,5-dimethoxy-4-methylbenzaldehyde[25] (3 g, 16.7 mmol) in dry THF (20 mL) was added and the stirring continued. After a further 30 min, the reaction was quenched with water (150 mL) and extracted three times with diethyl ether. The ether extract was washed twice with water and once with brine and dried (Na_2SO_4) and the solvent removed in vacuo. The product was then passed down a short silica column (eluent, 9:1 petroleum ether 40/60:EtOAc) to remove triphenylphosphonium oxide, and the solvent was again removed in vacuo (2.49 g, 84%): MS EI m/z 178 (M^+), 163, 135.

1-(2,5-Dimethoxy-4-methylphenyl)prop-1-ene. To a stirred suspension of ethyltriphenylphosphonium bromide (6.8 g, 18.3 mmol) in dry THF (120 mL) was added butyllithium (11.5 mL, 1.6 M solution in hexanes, 18.4 mmol). After 20 min, a solution of 2,5-dimethoxy-4-methylbenzaldehyde (3 g, 16.7 mmol) in dry THF (20 mL) was added and the stirring continued. After a further 30 min, the reaction was quenched with water (150 mL) and extracted three times with diethyl ether. The ether extract was washed twice with water and once with brine and dried (Na_2SO_4) and the solvent removed in vacuo. The product was then passed down a short silica column (eluent, 9:1 petroleum ether 40/60:EtOAc) to remove any triphenylphosphonium oxide, and the solvent was again removed in vacuo (2.53 g, 79%): MS EI m/z 192 (M^+), 177, 149, 115, 91.

2-Ethyl-5-methyl-1,4-dimethoxybenzene. To a stirred solution of (2,5-dimethoxy-4-methylphenyl)ethene (1 g, 5.6 mmol) and 1,4-cyclohexadiene (10.61 mL, 8.98 g, 112 mmol) in ethanol (30 mL) under N_2 was added 10% palladium on charcoal (1 g, 1 wt equiv). After 1 h, the resulting mixture was filtered through Celite and the solvent removed in vacuo. The compound was not purified further (837 mg, 83%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.61 (s, 2H, Ar−H), 3.73 (s, 6H, OCH_3), 2.60 (q, J = 7.5 Hz, 2H, CH_2), 2.16 (s, 3H, $ArCH_3$), 1.17 (t, J = 7.5 Hz, 3H, CH_2CH_3); MS EI m/z 180 (M^+), 179, 165, 151, 135.

2-Methyl-5-propyl-1,4-dimethoxybenzene. To a stirred solution of 1-(2,5-dimethoxy-4-methylphenyl)prop-1-ene (1 g, 5.2 mmol) and 1,4-cyclohexadiene (9.8 mL, 8.3 g, 104 mmol) in ethanol (30 mL) under N_2 was added 10% palladium on charcoal (1 g, 1 wt equiv). After 1 h, the resulting mixture was filtered through Celite and the solvent removed in vacuo. The compound was not purified further (914 mg, 91%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.62 (s, 2H, ArH), 3.74 (s, 6H, OCH_3), 2.56 (t, J = 8 Hz, 2H, $ArCH_2$), 2.18 (s, 3H, $ArCH_3$), 1.61 (m, 2H, $CH_2CH_2CH_3$), 0.93 (t, J = 7 Hz, 3H, CH_2CH_3); MS EI m/z 194 (M^+), 179, 165, 151, 135.

2,5-Diethyl-1,4-benzoquinone. To a stirred solution of 1,4-diethyl-2,5-dimethoxybenzene (0.8 g, 4.12 mmol) in acetonitrile (15 mL) was added a solution of ceric ammonium nitrate (6.8 g, 12.36 mmol) in water (10 mL) over a period of 5 min. After 30 min, water (100 mL) was added to the reaction mixture and the resulting precipitate was filtered and dried to give a yellow solid (593 mg, 88%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.48 (s, 2H, C−H), 2.43 (q, J = 7 Hz, 4H, CH_2), 1.12 (t, J = 7 Hz, 6H, CH_3); MS EI m/z 164 (M^+), 149, 120, 91, 73.

2-Ethyl-5-methyl-1,4-benzoquinone. To a stirred solution of 1,4-dimethoxy-2-ethyl-5-methylbenzene (0.75 g, 4.17 mmol) in acetonitrile (15 mL) was added a solution of ceric ammonium nitrate (6.88 g, 12.5 mmol) in water (15 mL) over a period of 5 min. After a further 30 min, the solution was extracted with diethyl ether and dried (Na_2SO_4) and the solvent removed in vacuo, yielding a yellow solid. This solid was then dissolved in dichloromethane and passed down a small silica column; the solvent was again removed in vacuo to yield yellow crystals (379 mg, 61%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.53 (m, 2H, CH), 2.45 (q, J = 7 Hz, 2H, CH_2CH_3), 2.01 (d, J = 1.5 Hz, 3H, CH_3), 1.11 (t, J = 7 Hz, 3H, CH_2CH_3); MS EI m/z 150 (M^+), 122, 107, 79.

2-Methyl-5-propyl-1,4-benzoquinone. To a stirred solution of 1,4-dimethoxy-2-propyl-5-methylbenzene (0.8 g, 4.12 mmol) in acetonitrile (15 mL) was added a solution of ceric ammonium nitrate (6.8 g, 12.36 mmol) in water (15 mL) over a period of 5 min. After a further 30 min the solution was extracted with diethyl ether and dried (Na_2SO_4) and the solvent removed in vacuo to yield a brown oil. This oil was then dissolved in dichloromethane and passed down a small silica column; the solvent was again removed in vacuo to yield a yellow oil (601 mg, 89%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.53 (m, 2H, CH), 2.39 (t, J = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 2.00 (d, J = 1.5 Hz, 3H, CH_3), 1.46 (m, 2H, $CH_2CH_2CH_3$), 0.96 (t, J = 7 Hz, 3H, $CH_2CH_2CH_3$); MS EI m/z 164 (M^+), 149, 137, 121, 91.

2,5-Dipropyl-1,4-benzoquinone. To a stirred solution of 2,5-diallylhydroquinone (1 g, 5.3 mmol) and 1,4-cyclohexadiene (10.0 mL, 8.47 g, 106 mmol) in ethanol (40 mL) under N_2 was added 10% palladium on charcoal (2 g, 2 wt equiv). After 13 h, the resulting mixture was filtered through Celite and the solvent removed in vacuo to yield 2,5-dipropylhydroquinone as a white solid (1.01 g, 99%). This solid was redissolved in diethyl ether (50 mL), and silver(I) oxide (2.39 g, 10.3 mmol) was added. This was stirred as a slurry for 12 h after which time the silver residue was filtered off and the solvent removed in vacuo to yield a yellow oil (0.92 g, 93%): MS EI m/z 192 (M^+), 177, 164, 163, 149, 135, 121.

2,5-Diaziridinyl-3,6-diethyl-1,4-benzoquinone (5). To a solution of 2,5-diethyl-1,4-benzoquinone (420 mg, 2.56 mmol) in ethanol (15 mL) under N_2 was added aziridine (0.40 mL, 0.33 g, 7.68 mmol). This was then placed in a refrigerator for 72 h. The resulting red precipitate was then filtered and recrystallized from THF to yield dark-red needles (203 mg, 32%): mp 183−184 °C (lit. mp 179 °C[26]); ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.55 (q, J = 7 Hz, 4H, CH_2CH_3), 2.27 (s, 8H, Az), 1.09 (t, J = 7 Hz, 6H, CH_2CH_3); MS EI m/z 246 (M^+), 231, 203, 189, 175, 94, 80.

2,5-Diaziridinyl-3,6-dipropyl-1,4-benzoquinone (6). To a solution of 2,5-dipropyl-1,4-benzoquinone (0.9 g, 4.7 mmol) in dry ethanol (30 mL) under N_2 was added aziridine (0.73 mL, 0.61 g, 14.1 mmol). This was placed in a refrigerator for 24 h. The resulting orange precipitate was filtered, washed with cold ethanol, and dried (324 mg, 25%): mp 121−122 °C (lit. mp 119 °C[26]); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.50 (t, J = 8 Hz, 4H, $CH_2CH_2CH_3$), 2.39 (s, 8H, Az), 1.51 (m, 4H, $CH_2CH_2CH_3$), 0.99 (t, J = 7.5 Hz, 6H, $CH_2CH_2CH_3$); MS EI m/z 274 (M^+), 259, 245, 231, 217, 194, 189, 175, 165, 108, 91, 80.

2,5-Diaziridinyl-3-ethyl-6-methyl-1,4-benzoquinone (2). To a solution of 2-ethyl-5-methyl-1,4-benzoquinone (370 mg, 2.47 mmol) in ethanol (15 mL) under N_2 was added aziridine (0.39 mL, 0.32 g, 7.4 mmol). This was then placed in a refrigerator for 72 h. The resulting orange precipitate was then filtered and passed down a silica column. Removal of the solvent in vacuo yielded red crystals (233 mg, 41%): mp 157−158 °C (lit. mp 156 °C[26]); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.53 (q, J = 7.5 Hz, 2H, CH_2CH_3), 2.27 (s, 4H, Az), 2.26 (s, 4H, Az), 1.95 (s, 3H, CH_3), 1.09 (t, J = 7.5 Hz, 3H, CH_2CH_3); MS EI m/z 232 (M^+), 217, 203, 189, 161, 94, 80.

2,5-Diaziridinyl-3-methyl-6-propyl-1,4-benzoquinone (3). To a solution of 2-methyl-5-propyl-1,4-benzoquinone (580 mg, 3.53 mmol) in ethanol (15 mL) under N_2 was added aziridine (0.55 mL, 0.45 g, 10.61 mmol). This was then placed in a refrigerator for 72 h. The resulting orange precipitate was

then filtered and dried (270 mg, 31%): mp 107−108 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.48 (t, J = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 2.28 (s, 4H, Az), 2.26 (s, 4H, Az), 2.00 (s, 3H, CH_3), 1.49 (m, 2H, $CH_2CH_2CH_3$), 0.98 (t, J = 7 Hz, 3H, $CH_2CH_2CH_3$); MS EI m/z 246 (M^+), 231, 217, 203, 189, 137, 80. Anal. ($C_{14}H_{18}N_2O_3$) C, N; H: calcd, 7.37; found, 8.09. HREIMS: found, 246.1368; $C_{14}H_{18}N_2O_3$ requires 246.1368.

2,5-Diaziridinyl-3-isopropyl-6-methyl-1,4-benzoquinone (2,5-Diaziridinylthymoquinone) (4). To a solution of thymoquinone (1 g, 6.09 mmol) in ethanol (30 mL) under N_2 was added aziridine (0.94 mL, 0.78 g, 18.27 mmol). This was then placed in a refrigerator for 72 h. The resulting red precipitate was then filtered and recrystallized from methanol to yield purple needles (317 mg, 21%): mp 119−120 °C (lit. mp 118 °C[26]); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.22 (sept, J = 6.5 Hz, 1H, $CH(CH_3)_2$), 2.27 (s, 8H, Az), 1.97 (s, 3H, CH_3), 1.39 (d, J = 6.5 Hz, 6H, $CH(CH_3)_2$); MS EI m/z 246 (M^+), 231, 203, 189, 175, 108, 94, 81.

2,5-Diaziridinyl-3,6-diisopropyl-1,4-benzoquinone (7). To a solution of 2,5-diisopropyl-1,4-benzoquinone (350 mg, 1.82 mmol) in ethanol (10 mL) was added aziridine (0.28 mL, 0.23 g, 5.34 mmol). This was placed in a refrigerator for 14 days. The resulting red/orange precipitate was filtered, washed with cold ethanol and dried (176 mg, 35%): mp 168−169 °C (lit. mp 170 °C[26]); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.23 (sept, J = 7 Hz, 2H, $CH(CH_3)_2$), 2.29 (s, 8H, Az), 1.32 (d, J = 7 Hz, 6H, $CH(CH_3)_2$); MS EI m/z 274 (M^+), 259, 245, 231, 216, 203, 201, 189, 175, 160.

Toxicity Testing. The cell lines used in this work were H596, H460, HT29 and BE. These were obtained from the American Tissue Type Culture Collection (ATTCC) or from the European Collection for Animal Cell Cultures (ECACC). HT29 is a human colon cancer cell line, and H460 is a human large-cell lung carcinoma line. These two cell lines were used as they express relatively high levels of DTD. The corresponding matched cell lines, H596 (human lung carcinoma) and BE (human colon carcinoma), were chosen because the H596 and BE lines do not have detectable DTD activity.[8,27] Continuous challenge cytotoxicity studies were carried out on these cells (600/well) in 96-well plates using the MTT method.[28] After 5 days, 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 on at least two occasions.

Cyclic Voltammetry. The voltametric studies were carried out using an EG and G K0264 microcell; current was applied by a PAR 362 scanning potentiostat operated by Condecon 310 software. All quinones were tested at 100 μM in dimethylformamide. Tetra-*n*-butylammonium perchlorate was used as the supporting electrolyte. Oxygen was removed from the system by purging with nitrogen. The cyclic voltammograms were recorded using potential sweep rates ranging from 10 to 500 mVs^{-1}, but it was routinely set at 100 mVs^{-1}.

Rates of Reduction of the Quinones by DTD. The reduction of the quinones by human DTD was followed by the corresponding reduction of cytochrome *c*.[29] The quinones (50 μM) were put into a 1-mL quartz cuvette containing 50 mM tris-HCl buffer (pH 7.4), 70 μM cytochrome *c*, and 10 μL of DTD (specific activity 653 μmol/min/mg of protein). The reaction was initiated by the addition of NADH (250 μM). The reduction of the cytochrome *c* was followed at 550 nm ($\Delta\alpha_{550nm}$ = 21 × 10^3 M^{-1} cm^{-1}). Each quoted value is the average of three determinations (typical errors ± 12%).

Rates of Reduction of the Quinones by BE Cell Extracts. The extract used in these experiments was made from a suspension of 2 × 10^7 BE cells in 2 mL of phosphate-buffered saline. This was sonicated and then centrifuged to remove the cell debris. The quinones (100 μM) were placed in a 1-mL cuvette containing phosphate-buffered saline, acetylated cytochrome *c* (100 μM), and NADPH (200 μM). The reaction was initiated by the addition of 50 μL of cell extract. The reduction of acetylated cytochrome *c* was followed at 550 nm. Each quoted value is the average of three determinations (typical errors ± 15%). The concentration of protein was determined using the Biorad assay.

Determination of DNA Interstrand Cross-Linking. The method has been previously described in detail.[30] Briefly, 5′-end-labeled DNA (∼5000 cpm/sample) was incubated with drug in 25 mM triethanolamine, 1 mM EDTA (pH 4) with 2 mM dithiothreitol at 37 °C for 2 h. Reactions were terminated by the addition of an equal volume of 0.6 M sodium acetate, 20 mM EDTA, 100 μM/mL tRNA, and the DNA was immediately precipitated by the addition of 3 vol of 95% ethanol. Following centrifugation and removal of the supernatant, the DNA pellet was dried by lyophilization. Samples were dissolved in 10 mL of 30% dimethyl sulfoxide, 1 mM EDTA, 0.04% bromophenol blue, 0.04% xylene cylanol, heated at 90 °C for 2 min, and chilled immediately in an ice−water bath prior to gel 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 90 °C onto filter paper; an 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 was calculated.

Molecular Modeling. The computer 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.

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Supporting Information Available: Stereoview of the initial interactions of the protonated hydroquinone of MeDZQ (**1**) with DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Schold, S. C.; Friedman, H. S.; Bjornsson, T. D.; Falleta, J. M. Treatment of patients with recurrent primary brain tumours with AZQ. *Neurology* **1984**, *34*, 615−619.
(2) Bender, J. F.; Grillo-Lopez, A.; Posada, J. G. Diaziquone. *Invest. New Drugs* **1983**, *1*, 71−84.
(3) Obe, G.; Beek, B. Trenimon: Biochemical, physiological and genetic effects on cells and organisms. *Mutat. Res.* **1979**, *65*, 21−70.
(4) Szmigiero, L.; Kohn, K. W. Mechanisms of DNA strand breakage and interstrand cross-linking by diaziridinylbenzoquinone (Diaziquone) in isolated nuclei from human cells. *Cancer Res.* **1984**, *44*, 4453−4457.
(5) Siegel, D.; Gibson, N. W.; Preusch, P. C.; Ross, D. Metabolism of diaziquone by NAD(P)H:(quinone acceptor) oxireductase (DT-diaphorase); role in diaziquone-induced DNA damage and cytotoxicity in human colon carcinoma cells. *Cancer Res.* **1990**, *50*, 7293−7300.
(6) Butler, J.; Dzielendziak, A.; Lea, J. S.; Ward, T. H.; Hoey, B. M. Contrasting cytotoxic mechanisms of similar antitumour diaziridinybenzoquinones. *Free Radical Res. Commun.* **1990**, *8*, 231−239.
(7) Dzielendziak, A.; Butler, J.; Hoey, B. M.; Lea, J. S.; Ward, T. H. A comparison of the structural and cytotoxic activity of novel AZQ analogues. *Cancer Res.* **1990,** *5*, 2003−2007.
(8) Mayalarp, S. P.; Hargreaves, R. H. J.; Butler, J.; O'Hare, C. C.; Hartley, J. A. Cross-linking and sequence specific alkylation of DNA by diaziridinylquinones. 1. Quinone Methides. *J. Med. Chem.* **1996**, *39*, 531−537.
(9) Hargreaves, R. H. J.; Mayalarp, S. P.; Butler, J.; McAdam, S. R.; O'Hare, C. C.; Hartley, J. A. Cross-linking and sequence specific alkylation of DNA by aziridinylquinones. 2. Structure requirements for sequence selectivity. *J. Med. Chem.* **1997**, *40*, 357−362.
(10) Gibson, N. W.; Hartley, J. A.; Butler, J.; Seigel, D.; Ross, D. The relationship between DT-diaphorase mediated metabolism of a series of diaziridinylbenzoquinones and DNA damage and cytotoxicity. *Mol. Pharmacol.* **1992**, *42*, 531−536.

(11) Beall, H. D.; Murphy, A. M.; Siegel, D.; Hargreaves, R. H. J.; Butler, J.; Ross, D. Nicotinamide adenine dinucleotide (phosphate): Quinone oxireductase (DT-diaphorase) as a target for bioreductive antitumor quinones: Quinone cytotoxicity and selectivity in human lung and breast cancer cell lines. *Mol. Pharmacol*. **1995**, *48*, 499−504.

(12) Hartley, J. A.; Berardini, M.; Ponti, M.; Gibson, N. W.; Thompson, A. S.; Thurston, D. E.; Hoey, B. M.; Butler, J. DNA cross-linking and sequence selectivity of aziridinylbenzoquinones: A unique reaction at 5′-GC-3′ sequences with 2,5-diaziridinyl-1,4-benzoquinone upon reduction. *Biochemistry* **1991**, *30*, 11719−11724.

(13) Berardini, M. D.; Souhami, R. L.; Lee, C.-S.; Gibson, N. W.; Butler, J.; Hartley, J. A. Two structurally related diaziridinylbenzoquinones preferentially cross-link DNA at different sites upon reduction with DT-Diaphorase. *Biochemistry* **1993,** *32*, 3306−3312.

(14) Hansch, C.; Leo, A.; Taft, R. W. A survey of Hammett substituent constants and resonance and field parameters. *Chem. Rev.* **1991**, *91*, 165−195.

(15) Butler, J.; Hoey, B. M. The one electron reduction potential of several substrates can be related to their reduction rates by cytochrome P450 reductase. *Biochim. Biophys Acta* **1993**, *1161*, 73−77.

(16) Butler, J.; Redox Cycling antitumour drugs. In *DNA and Free Radicals*: *Techniques, Mechanisms and Applications*; Halliwell, B., Aruoma, O. I., Eds.; OICA International: London, 1998; pp 131−154.

(17) Butler, J.; Hoey, B. M. Reactions of glutathione and glutathione radicals with benzoquinones. *Free Radical Biol. Med*. **1992**, *12*, 337−345.

(18) Beall, D. H.; Winski, S.; Swann, E.; Hudnott, A. R.; Cottrill, A. S.; O'Sullivan, N.; Green, S. J.; Bien, R.; Siegel, D.; Ross, D.; Moody, C. J. Indoloquinone antitumour agenst: Correlation between quinone structure, rate of metabolism by rcombinant human NQO1 and in vitro cytoxicity. *J. Med. Chem*. **1998**, *41*, 4755−4766.

(19) Li, R.; Bianchet, M. A.; Talalay, P.; Amzel, L. M. The three-dimensional structure of NAD(P)H: quinone reductase, a flavoprotein involved in cancer chemoprotection and chemotherapy: mechanism of the two-electron reduction. *Proc. Natl. Acad. Sci. U.S.A*. **1995**, *92*, 8846−8850.

(20) Jaffar, M.; Naylor, M. A.; Robertson, N.; Lockyer, S. D.; Phillips, R. M.; Everett, S. A.; Adams, G. E.; Stratford, I. J. 5-Substituted analogues of 3-hydroxymethyl-5-aziridinyl-1-methyl-2-[1H-indole-4,7-dione]prop-2-en-1-ol (EO9, NSC 382459) and their regioisomers as hypoxia-selective agents: structure-cytotoxicity in vitro. *Anticancer Drug Des*. **1998**, *13*, 105−123.

(21) Wallin, R. Adriamycin and DT-diaphorase. *Cancer Lett*. **1986**, *30*, 97−101.

(22) Reeves, W. A.; Drake, G. L.; Hoffpauir, C. L. Ethylenimine by flash distillation. *J. Am. Chem. Soc*. **1951**, *73*, 3522.

(23) Cameron, D. W.; Giles, R. G. F. Photochemical formation of benzoxazoline derivatives from aminated quinones. *J. Chem. Soc. C* **1968**, 1461−1464.

(24) Petersen, S.; Gauss, W.; Urbschat, E. Synthesis of simple quinone derivatives with fungicidal, bacteriostatic and cytostatic properties. *Angew. Chem*. **1955**, *67*, 217−231.

(25) Standridge, R. T.; Howell, H. G.; Gylys, J. A.; Partyka, R. A.; Shulgin, A. T. Phenyalkylamines with potential psychotherapeutic utility. 1. 2-Amino-1-(2,5-dimethoxy-4-methylphenyl-butane. *J. Med. Chem*. **1976**, *19*, 1400−1404.

(26) Nakao, H.; Arakawa, M.; Nakamura, T.; Fukushima, M. Antileukemic agents. II. New 2,5-Bis(1-aziridinyl)-p-benzoquinone derivatives. *Chem. Pharm. Bull*. **1972**, *20*, 1968−1979.

(27) Traver, R. D.; Siegel, D.; Beall, H. D.; Phillips, R. M.; Gibson, N. W.; Franklin, W. A.; Ross, D. Characterization of a polymorphism in NAD(P)H: quinone oxidoreductase (DT-diaphorase) *Br. J. Cancer* **1997**, *75*, 69−75.

(28) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Michael, J. B. Evaluation of tetrazolium- based semiautomatic colorimetric assay; assessment of chemosensitivity testing. *Cancer Res*. **1987**, *47*, 936−942.

(29) Beall, H. D.; Mulcahy, R. T.; Siegel, D.; Traver, R. D.; Gibson, N. W.; Ross, D. Metabolism of bioreductive antitumor compounds by purified rat and human DT-diaphorase. *Cancer Res*. **1994**, *54*, 3196−3201.

(30) Hartley, J. A.; Berardini, M.; Souhami, R. L. An agarose gel method for the determination of DNA interstrand cross-linking applicable to the measurement of total and second-arm cross-link reactions. *Anal. Biochem*. **1991**, *193*, 131−134.

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