Cross-Linking and Sequence Specific Alkylation of DNA by Aziridinyl Quinones. 2. Structure Requirements for Sequence Selectivity

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The cytotoxicities and DNA sequence selectivity for guanine-N7 alkylation of 22 mono- and disubstituted 2,5-diaziridinyl-1,4-benzoquinones have been investigated. Several quinones produced patterns of alkylation following reduction with a selectivity for 5′-TGC-3′ sequences. This sequence selectivity appeared to be dependent only on the presence of a hydrogen in position-6 of the quinone. A computer model, based on published crystallographic data, was used to explain this selectivity. The sequence selective quinones were generally more cytotoxic than the quinones which reacted randomly.

Introduction

Several aziridinylbenzoquinones have undergone clinical trials as potential antitumour drugs.[1−3] These types of compounds can be activated toward alkylation as a result of bioreduction by one-electron or two-electron reducing enzymes.[4−6] In the case of diaziridinylbenzoquinones, these reactions result in the production of activated aziridine groups which cross-link DNA strands.[7−9]

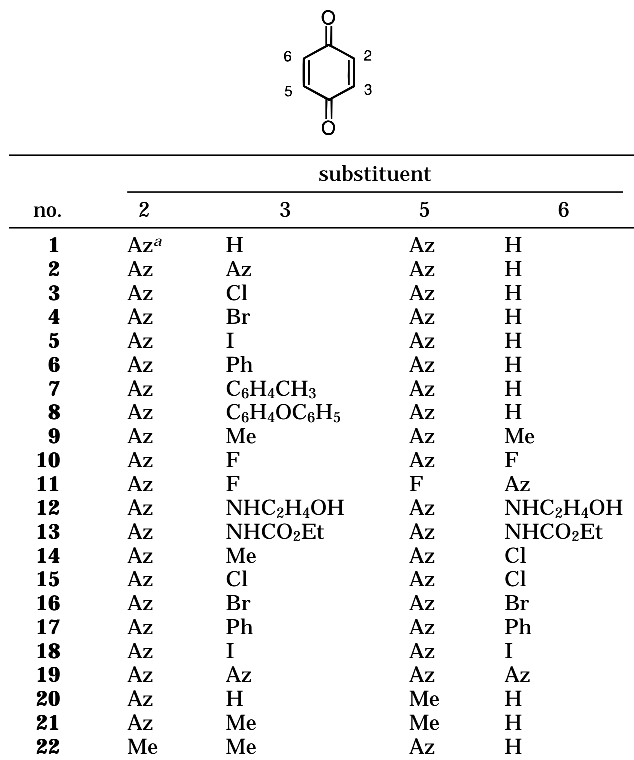
The recent attempts at developing new aziridinyl quinones have tended to focus on the ability of the quinone to act as substrates for the reducing enzymes.[5,9,10] However, our previous studies have indicated that the sequence specificity and structure of the resulting quinone−DNA cross-link may be equally important in determining the cytotoxicity.

It has previously been shown that the clinically relevant diaziridinyl quinones, AZQ (2,5-diaziridinyl-3,6-bis(ethoxycarbonyl)amino)-1,4-benzoquinone (**13**)) and BZQ (2,5-diaziridinyl-3,6-bis(bis(2-hydroxyethyl)-amino)-1,4-benzoquinone (**12**)), react with all guanine-N7 positions in DNA with a sequence selectivity similar to other chemotherapeutic alkylating agents such as the nitrogen mustards.[8,11] Nonreduced DZQ (2,5-diaziridinyl-1,4-benzoquinone (**1**)) showed similar alkylating selectivity whereas reduced DZQ selectively alkylated DNA at 5′-GC-3′ sequences and in particular at 5′-TGC-3′ sites.[8] Interstrand cross-linking occurred in this 5′-TGC sequence spanning two base pairs.[9] In contrast, MeDZQ (2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone (**9**)), which is an excellent substrate for the two-electron reducing enzyme, DT-diaphorase,[10] reacts at 5′-GNC-3′ sequences after reduction.[9]

We have recently shown that some reduced methylated monoaziridinyl benzoquinones can also cross-link DNA via the formation of a quinone methide, and in each of these compounds, the TGC sequence selectivity is necessary for the cross-linking.[12]

The results from these previous studies on sequence selectivity also suggested that a simple hydrogen in the 6-position on the benzoquinone is important in order to maintain the TGC sequence selectivity. The present work essentially confirms the necessity of the hydrogen and also shows that even when relatively bulky groups are in the 3-position, the sequence selectivity is maintained. We have also now investigated the relevance of the selectivity on the cytotoxicity of the quinones.

Table 1. Quinones Used in This Study



no.	substituent 2	3	5	6
1	Az[a]	H	Az	H
2	Az	Az	Az	H
3	Az	Cl	Az	H
4	Az	Br	Az	H
5	Az	I	Az	H
6	Az	Ph	Az	H
7	Az	$C_6H_4CH_3$	Az	H
8	Az	$C_6H_4OC_6H_5$	Az	H
9	Az	Me	Az	Me
10	Az	F	Az	F
11	Az	F	F	Az
12	Az	NHC_2H_4OH	Az	NHC_2H_4OH
13	Az	$NHCO_2Et$	Az	$NHCO_2Et$
14	Az	Me	Az	Cl
15	Az	Cl	Az	Cl
16	Az	Br	Az	Br
17	Az	Ph	Az	Ph
18	Az	I	Az	I
19	Az	Az	Az	Az
20	Az	H	Me	H
21	Az	Me	Me	H
22	Me	Me	Az	H

[a] Az, aziridinyl.

Results

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

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

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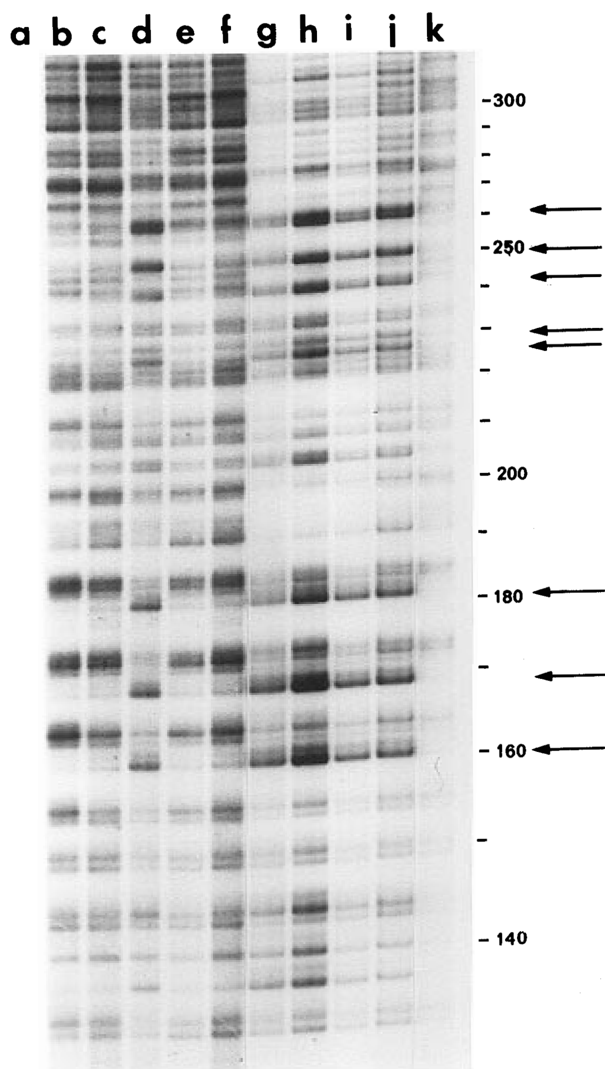

Figure 1. Sequence selectivity of guanine-N7 alkylation by several aziridinylbenzoquinones. Drug reactions were for 1 h at 20 °C at either pH 7 (lanes 2,3) or pH 4 (lanes 4−11): (a) control unalkylated DNA, (b) 100 μM melphalan, (c) 100 μM **1**, (d) 1 μM **1** + 2 mM ascorbic acid, (e) 100 μM **11** + 2 mM ascorbic acid, (f) 100 μM **10** + 2 mM ascorbic acid, (g) 50 μM **20** + 2 mM ascorbic acid, (h) 50 μM **22** + 2 mM ascorbic acid, (i) 50 μM **3** + 2 mM ascorbic acid, (j) 50 μM **2** + 2 mM ascorbic acid, and (k) 100 μM **19** + 2 mM ascorbic acid. The positions of the 5′-TGC sequences are indicated with arrows, and the sequence position in pBR322 is given.

DNA for many chemotherapeutic agents. The DNA sequence selectivity for guanine-N7 alkylation by the aziridinylbenzoquinones was examined as previously described.[8,12] A portion of a typical gel for several quinones is shown in Figure 1. The patterns of alkylation produced were compared at doses that gave at most **1** alkylation per DNA molecule.

For quinone **1**, the pattern of guanine-N7 alkylation without reduction (Figure 1, lane c) was similar to melphalan (lane b) with alkylation at all guanine sites, with a general preference for runs of guanines. Following reduction (lane d), **1** produced an altered pattern with the reactions occurring almost exclusively at 5′-G̲C sites with a strong preference for 5′-TG̲C sequences (indicated by the arrows in Figure 1). All of the quinones produced an increase in DNA alkylation after reduction. For some quinones, the pattern of alkylation was unchanged following reduction, e.g. **10** (lane f), **11** (lane e), and **19** (lane k). For other quinones, the alkylation pattern on reduction was altered to produce the 5′-TG̲C selectivity, e.g. **20** (lane g), **22** (lane h), **3** (lane i), and **2** (lane j). All of the quinones that produce this 5′-TG̲C selectivity are indicated in Table 2. It is clear that the requirement for this selectivity is a hydrogen in position 6 of the quinone and that this selectivity is maintained even with relatively bulky groups in position 3.

Table 2. *In Vitro* Cytotoxicity against Human Chronic Leukemia K562 Cells and Sequence Selectivity of Compound for 5′-TG̲C Sites following Reduction

quinone	IC_{50}/nM[a]	5′-TGC selectivity
1	8.3	+
2	2.9	+
3	5.3	+
4	7.3	+
5	12.6	+
6	3.1	+
7	4.8	+
8	170	+
9	1.8	
10	10	
11	39	
12	158	
13	63	
14	76	
15	46	
16	42	
17	67	
18	50	
19	5.8	
20	216	+
21	476	+
22	239	+

[a] Errors within ±15%.

***In Vitro* Cytotoxicities.** The cytotoxicities for the quinones **1**−**22** after a continuous challenge are shown in Table 2. The K562 cell line was chosen as it has been shown to be sensitive to the redox reactions of diaziridinyl quinones, and the levels of the main reducing enzymes have been determined.[7,13]

Discussion

The results, summarized in Table 2, clearly show that all of the hydroquinones with a hydrogen in position 6 are capable of reacting selectively at 5′-TG̲C sequences. Essentially this is because only a hydrogen is small enough to fit in between the bases. A simulation showing the association between the GC bases is shown in Figure 2. It should be noted that this diagram is different than that reported previously[8] as it is now based on a true crystal structure of an oligonucleotide[17] as opposed to a computer-generated model. This structure now clearly shows that the hydroquinones can fit into the major groove. The associations involving hydrogen bonds between the OH groups of the hydroquinones and the O-2 and C4-NH_2 of the cytosine are still maintained as in the previously derived structure.[8]

The 5′-GC as opposed to 5′-CG sequence selectivity of these compounds can now be explained using the results of Dickerson and colleagues[18,19] who have studied the local geometry of several B-DNA oligonucleotides. Essentially, it has been shown that the GC step in oligonucleotides has a "high twist profile". This means that the sequence has a high twist, a low rise, a positive cup, and a negative roll. The positive cup ensures that there is more room in the center space

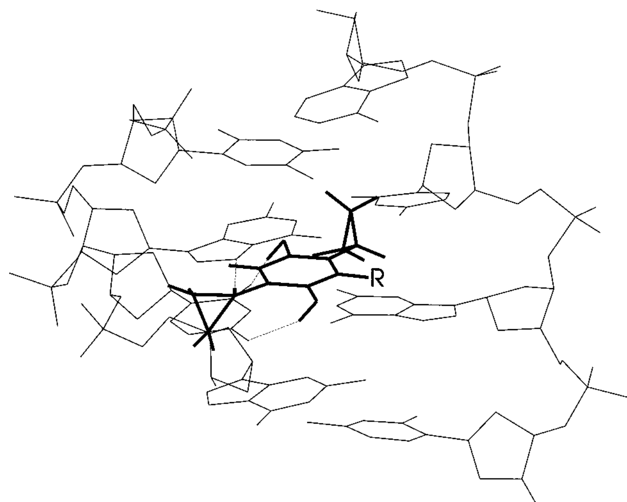

Figure 2. Computer simulation for the cross-linking of reduced diaziridinylhydroquinones in the GC step in TGCC sequences. The OH groups of the hydroquinone are associated at the O2 and C4-NH_2 groups of cytosine and the protonated aziridine associates with the N7 of guanine on the same strand.

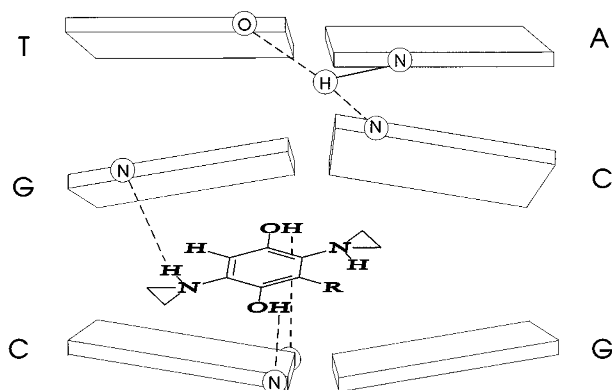

Figure 3. Schematic representation of the TGC sequence and its interactions with a reduced diaziridinylquinone showing the cup and roll of the GC step. The three-center hydrogen bond between the N6-H of the adenine and the O4 of the thymine and N4 of the cytosine is indicated by the dashed line. The hydrogen bonding of the hydroquinone is as in Figure 2.

between the base pairs and the negative roll means that these bases are tilted open toward the major groove. Thus the GC step is a more open site of attack. The CG step on the other hand has a low twist profile with a negative cup and a positive roll and thus is more compact. The extension of the sequence to 5′-TGC, and thus 5′-GCA on the complementary strand, can be explained by the occurrence of three center hydrogen bonds between adjacent base pairs in the major groove. These were found to frequently occur at CA steps.[18] In our sequences, these hydrogen bonds could cause the middle cytosine to be pulled further up, causing the space between the GC step to be even larger. A schematic diagram showing consequences of the high twist profile and a three centre hydrogen bond involving the T, A, and C bases in the TGC sequence is shown in Figure 3.

Quinones can be cytotoxic due to a variety of reasons which include redox cycling and the production of superoxide and other reactive oxygen radicals, reactions with thiols and amines, drug-uptake, and DNA alkylations of the type described herein.[14,15] It is therefore difficult to isolate any one factor that will determine the cytotoxicities within a series of different quinones.

However, apart from a few exceptions, the results in Table 2 show that the quinones with a hydrogen in position 6 are generally more cytotoxic than the corresponding di-substituted quinones (compare the monohalides **3**−**5** with the dihalides **15**, **16**, and **18**, the monomethyl monochloro quinone **14** with **3**, and the monophenyl **6** with the diphenyl **17**). This can be explained by the ability of the hydroquinones of the monosubstituted compounds to alkylate at TGC sequences. If the reduced quinones can locate specifically between G and C bases within DNA (Figure 2), then the aziridines are in a favorable position to form a cross-link. The hydroquinones which cannot associate in these positions are expected to react randomly at guanines and form less cytotoxic monalkylations. Similarly, consistent with our previous results,[12] although we have shown that the methylmonoaziridine compounds, **20**, **21**, and **22** can alkylate at TGC sequences, they are significantly less toxic than the diaziridinyl quinones which also react at this sequence.

There are a few exceptions to the correlation between sequence selectivity and cytotoxicity. MeDZQ (**9**) and the tetraaziridinyl quinone **19** are very cytotoxic but do not have a hydrogen in position-6. However, MeDZQ is an excellent substrate for DT-diaphorase,[10] and it has been previously shown that it efficiently cross-links at GNC positions in DNA after reduction.[9] MeDZQ may therefore be abnormally cytotoxic due to the efficient bioreduction in the K562 cell line.[13] Our previous studies on quinone methides[12] and the similar reductive capability of methyl and aziridinylquinones[14] would suggest that **19** could react in a similar manner as MeDZQ.

The ether-substituted quinone, **8**, has a hydrogen in position 6 but is not very cytotoxic whereas the phenyl (**6**) and tolylquinones (**7**) are more than 30 times more toxic. We have previously shown[10] that **6** is an excellent substrate for DT-diaphorase and the one-electron reducing enzymes. However, **8** is also an excellent substrate for the enzyme (data not shown), and hence the reduced cytotoxicity of this quinone cannot be explained simply by the bioreductive step. Our computer modeling of the hydroquinone of **8** into the TGC sequences of DNA shows that whereas the hydroquinone can be initially inserted between the G and C bases as in Figure 2, the rotation of the phenyl group around the ether oxygen is severely restricted by the phosphate backbone of the DNA, particularly between the GC bases. This hindered rotation dramatically increases the energy minimisation. Hence, it would appear that this compound should not readily react at TGC sequences. Analysis of the pattern of alkylation by **8** following reduction revealed that although alkylation at TGC was observed, the overall extent of alkylation was much less than that of **6** or **7** at equimolar doses (data not shown).

Nonetheless, further computer modeling of the quinones with a hydrogen in position 6 have shown that if long-chain aliphatic groups are placed at position 3, the sequence selectivity can still be maintained. Indeed, if other sequence selective groups are incorporated at the end of these aliphatic groups, then extended sequence selectivity could be formed. Studies of this type will be the subject of future work.

Experimental Section

Chemicals. The quinones used in the study are shown in Table 1. Phenyl-1,4-benzoquinone was obtained from Aldrich Chemical Co. (4-Methylphenyl)-1,4-benzoquinone and 2,6-dibromo-1,4-benzoquinone were made from the oxidation of (4-methylphenyl)hydroquinone (TCI) and 2,6-dibromohydroquinone (Apin) using silver(I) oxide (Aldrich).

Aziridine (ethylenimine) was prepared according to the flash distillation method of Reeves et al.[20]

Initial quinone stock solutions for 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 purchased from BRL, and piperidine and ascorbic acid were purchased from BDH. [γ-^{32}P]ATP (5000 Ci/mmol) was from Amersham and pBR322 plasmid DNA from Northumbria Biologicals.

The following quinones were prepared according to previous methods: 2,5-Diaziridinyl-1,4-benzoquinone (**1**), 2,5-diaziridinyl-3-chloro-1,4-benzoquinone (**3**), 2,5-diaziridinyl-3,6-dichloro-1,4-benzoquinone (**15**), 2,5-diaziridinyl-3,6-dibromo-1,4-benzoquinone (**16**), 2-aziridinyl-5-methyl-1,4-benzoquinone (**20**), and 5-aziridinyl-2,3-dimethyl-1,4-benzoquinone (**22**);[21] 2,3,5-triaziridinyl-1,4-benzoquinone (**2**);[22] 2,5-diaziridinyl-3-phenyl-1,4-benzoquinone (**6**);[10] 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone (**9**);[23] 2,5-diaziridinyl-3,6-bis[bis(2-hydroxyethyl)-amino]-1,4-benzoquinone (BZQ, **12**) and 2,5-diaziridinyl-3,6-difluoro-1,4-benzoquinone (**10**);[24] 2,6-Diaziridinyl-3,5-diflouro-1,4-benzoquinone (**11**);[32] 2,5-diaziridinyl-3,6-bis[(ethoxycarbonyl)-amino]-1,4-benzoquinone (AZQ, **13**);[25] 2,5-diaziridinyl-3-chloro-6-methyl-1,4-benzoquinone (**14**), and 2,5-diaziridinyl-3,6-diphenyl-1,4-benzoquinone (**17**);[26] 2,3,5,6-tetraaziridinyl-1,4-benzoquinone (**19**);[27] and 2-aziridinyl-3,5-dimethyl-1,4-benzoquinone (**21**).[12] HREIMS were measured on a VG-ZAB-E instrument at the EPSRC National Mass Spectrometry Service Centre, Swansea, U.K.

2,5-Diaziridinyl-3-bromo-1,4-benzoquinone (4). To a stirred solution of 2,6-dibromo-1,4-benzoquinone (0.5 g, 1.8 mmol) at 0 °C under N_2 in the dry methanol (30 mL) was added a solution of aziridine (0.6 mL, 11.5 mmol) in dry methanol (1 mL). After 1 h the resulting precipitate was filtered, washed with cold dry methanol, and then crystallized from ethanol to yield orange needles (160 mg, 32%): mp 181 °C dec; TLC R_f (petroleum ether (40:60)/EtOAc, 1:1) 0.33; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (1H, s, CH), 2.58 (4H, s, Az), 2.30 (4H, s, Az); MS EI m/z 268, 270 (M$^+$), 68, 67; λ_{max} (EtOH) 214, 336, 466 nm; ν_{max} (film) 1662, 1564, 1380, 1257, 1222, 1159 cm^{-1}; HREIMS found 267.9847, $C_{10}H_9N_2O_2Br$ requires 267.9847.

2,5-Diaziridinyl-3-iodo-1,4-benzoquinone (5). To a stirred solution of 2,6-diiodo-1,4-benzoquinone (0.25 g, 0.69 mmol) in dry methanol (25 mL) at 0 °C under N_2 was added aziridine (0.23 mL, 4.4 mmol). After 2 h the solvent was removed *in vacuo*, the resulting solid was chromatographed on silica (ethyl acetate/petroleum ether 40/60 (1:1)), and the second band was collected and yielded a dark red solid (15 mg, 4.9%): mp 139–40 °C; TLC R_f (petroleum ether (40:60)/EtOAc, 1:1) 0.3; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.22 (1H, s, CH), 2.53 (4H, s, Az), 2.22 (4H, s, Az); MS EI m/z 316 (M$^+$), 133, 105, 67; λ_{max} (EtOH) 222, 342, 486 nm; ν_{max} (film) 1675, 1621, 1552, 1342, 1253 cm^{-1}; HREIMS found 315.9717, $C_{10}H_9N_2O_2I$ requires 315.9711.

2,5-Diaziridinyl-3-(4-methylphenyl)-1,4-benzoquinone (7). To a stirred solution of (4′-methylphenyl)-1,4-benzoquinone (0.5 g, 2.2 mmol) at 0 °C under N_2 in dry ethanol (30 mL) was added a solution of aziridine (0.4 mL, 7.7 mmol) in dry ethanol (1 mL). After 2 h the resulting precipitate was filtered, washed with cold dry ethanol, and crystallized from methanol to yield purple crystals (51 mg, 7.2%): mp 185–6 °C; TLC R_f (petroleum ether (40:60)/EtOAc, 1:1) 0.35; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (4H, m, Ph), 6.02 (1H, s, H), 2.44 (3H, s, CH_3), 2.27 (4H, s, Az), 2.03 (4H, s, Az); MS EI m/z 280(M$^+$), 265, 251, 224, 129; λ_{max} (EtOH) 218, 328, 466 nm; ν_{max} (film) 1645, 1562, 1376, 1283, 1147, 1122 cm^{-1}. Anal. Found: C, 72.86; H, 5.66; N, 9.83. $C_{17}H_{16}N_2O_2$ requires: C, 72.84; H, 5.75; N, 9.99%.

2-(4′-Phenoxyphenyl))-1,4-benzoquinone. 2-(4′-Phenoxyphenyl)-1,4-benzoquinone was prepared as an intermediate according to the method of Kvalnes from 4-phenoxyaniline:[28] TLC R_f (petroleum ether (40:60)/EtOAc, 1:1) 0.63; ^{1}H NMR (300 MHZ, acetone-d_6) δ 7.74–7.14 (9H, m, PhOPh), 6.97–7.02 (3H, m, C=CH); MS EI m/z 276 (M$^+$), 194, 183, 165, 77.

2,5-Diaziridinyl-3-(4′-phenoxyphenyl)-1,4-benzoquinone (8). To a stirred solution of 2-(4′-phenoxyphenyl)-1,4-benzoquinone (310 mg, 1.12 mmol) in dry methanol (15 mL) at 0 °C under N_2 was added aziridine (0.12 mL, 2.32 mmol). After 1 h the solution was the concentrated *in vacuo* to ~5 mL and cooled on ice, and the resulting precipitate was collected and chromatographed on silica (petroleum ether (40:60)/EtOAc, 1:1) to yield a purple solid (41 mg, 10.2%): mp 168–9 °C; TLC R_f (petroleum ether (40:60)/EtOAc, 1:1) 0.43; ^{1}H NMR (300 MHz, acetone-d_6) δ 7.56–7.13 (9H, m, PhOPh), 6.07 (1H, s, C=CH), 2.32 (4H, s, Az), 2.07 (4H, s, Az); MS EI m/z 359 (M$^+$), 329, 302, 265, 114, 77; λ_{max} (EtOH) 214, 327, 477 nm; ν_{max}(film) 1649, 1566, 1489, 1371, 1286, 1236 cm^{-1}; HREIMS found 358.1319, $C_{11}H_{11}N_2O_2Cl$ requires 358.1317.

2,5-Diaziridinyl-3,6-diiodo-1,4-benzoquinone (18). To a solution of iodanil (0.5 g, 0.82 mmol) at 0 °C under N_2 in dry ethanol (15 mL) was added aziridine (0.27 mL, 4.92 mmol). The resulting solution was stirred for 2 h, and the brown precipitate was collected and crystallized from THF as purple needles (115 mg, 32%): mp 163 °C dec; TLC R_f ($CHCl_3$/MeOH, 9:1) 0.71; ^{1}H NMR (60 MHz,$CDCl_3$) δ 2.58 (s); MS EI m/z 442 (M$^+$), 274, 231, 193, 179, 165, 127, 94; λ_{max} (MeCN) 198, 220, 242, 364 nm; ν_{max} (film) 1653, 1552, 1363, 1227, 1165, 734 cm^{-1}. Anal. ($C_{10}H_8N_2O_2I_2$) C, H, N.

2,5-Diaziridinyl-3-fluoro-1,4-benzoquinone could not be synthesized. This was because, despite several attempts, the synthesis of the precursor, 2,6-difluoro-1,4-benzoquinone,[29] could not be repeated with any significant yield.

Enzymes. Restriction enzymes *Hin*dIII and *Bam*H1, 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. Alkylation stop solution is 0.6 M sodium acetate, 20 mM EDTA, 100 mg/mL tRNA. BAP buffer is 10 mM Tris-HCl and 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.[30] The DNA was labeled at the 5′ ends with T4 PNK as described by Maxam and Gilbert.[31] For measurement of guanine N7-alkylation the DNA was further cut with *Bam*H1 (2 units/mg, 37 °C, 1 h), and the 375 base fragment was purified from an agarose gel by electroelution.

Determination of Sites of Guanine-N7 Alkylation. Singly labeled and end-labeled DNA (~50 000 cpm/sample) was 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 three volumes of 95% ethanol. The DNA was resuspended in 0.3 M sodium acetate and 1 mM EDTA, ethanol was precipitated again, and the pellet was 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.[33] 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 approximately 3 h at 3000V, 55 °C. Gels were dried and autoradiographed and relative band intensities determined by microdensitometry as described above.

Toxicity Testing. Human chronic myeloid leukaemic 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. Continuous challenge cytotoxicity studies were carried out on these cells (600 per

well) in 96-well plates using the MTT method.[34] After 5 days, the absorbances were read on a multiscan plate reader at 540 and 640nm. Plots were then drawn for inhibition of cell growth as a function concentration. Each compound was tested in triplicate at each concentration. This method differs from that used in our previous study which involved 2-h challenges.[12] The continuous challenge method was used here to reduce variations in cytotoxicity due to differences in drug uptake.

Molecular Modeling. Molecular modeling was carried out on a Silicon Graphics Iris 4D/310GTX work station using QUANTA 4.0 software (including CHARMm 22.2) working under IRIS 4.0.5. The 5′-TGCG double strand structure was taken from the work of Edwards et al.[17] using the coordinates deposited at the Protein Data Bank, Upton, NY.

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