Indolequinone Antitumor Agents: Correlation between Quinone Structure, Rate of Metabolism by Recombinant Human NAD(P)H:Quinone Oxidoreductase, and in Vitro Cytotoxicity[1]

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A series of indolequinones bearing various functional groups has been synthesized, and the effects of substituents on the metabolism of the quinones by recombinant human NAD(P)H: quinone oxidoreductase (NQO1) were studied. Thus 5-methoxyindolequinones were prepared by the Nenitzescu reaction, followed by functional group interconversions. The methoxy group was subsequently displaced by amine nucleophiles to give a series of amine-substituted quinones. Metabolism of the quinones by NQO1 revealed that, in general, compounds with electron-withdrawing groups at the indole 3-position were among the best substrates, whereas those with amine groups at the 5-position were poor substrates. Compounds with a leaving group at the 3-indolyl methyl position generally inactivated the enzyme. The toxicity toward non-small-cell lung cancer cells with either high NQO1 activity (H460) or no detectable activity (H596) was also studied in representative quinones. Compounds which were good substrates for NQO1 showed the highest selectivity between the two cell lines.

Introduction

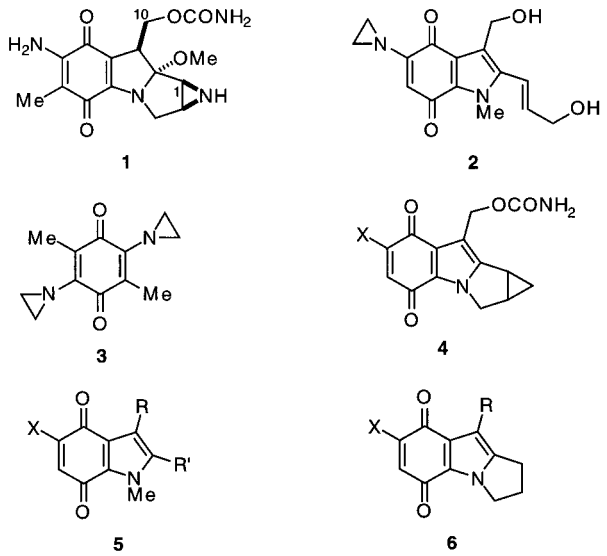
NAD(P)H:quinone oxidoreductase (NQO1; EC 1.6.99.2), also known as DT-diaphorase, is an obligate 2-electron reductase that is characterized by its ability to use either NADH or NADPH as cofactor.[2,3] The enzyme, a flavoprotein, exists as a homodimer with 1 mol of FAD/mol of NQO1,[4] and the human enzyme has been cloned and sequenced.[5] The crystal structure of the rat liver enzyme, which shows about 85% homology with human NQO1, has been determined to 2.1-Å resolution.[6]

NQO1 catalyzes the 2-electron reduction of quinones and can protect cells against the toxic effects of quinones. However NQO1 is also involved in the reductive activation of anticancer agents such as mitomycin C (MMC) (**1**) (Chart 1) which operate by the so-called bioreductive mechanism,[7−9] and although other enzymes have also been implicated in the reductive bioactivation process,[9] NQO1 has generated considerable interest because of its elevated levels in many tumors and tumor cell lines.[8,10]

It has historically been problematic to identify those compounds which are the most efficient substrates for NQO1. One approach has been to attempt to correlate reduction potentials with rates of reduction. A relationship between 1-electron reduction potential and rates of reduction by cytochrome P-450 reductase[11,12] and cytochrome b_5 reductase[12] has been reported. For the 1-electron reductase, xanthine oxidase, Clarke et al. determined that there was a good correlation between 1-electron reduction potentials and rates of reduction of nitroimidazoles.[13,14] However, when a similar approach was attempted for NQO1, no correlation was found between reduction potential and rates of reduction by NQO1 for a series of naphthoquinones[15] or a series of aziridinylbenzoquinones.[16] In the naphthoquinone study,[15] rates of reduction by NQO1 also did not correlate with octanol−water partition coefficients which further limits the ability to predict substrate efficiency for NQO1.

The role of NQO1 in the bioactivation of MMC (**1**) is somewhat controversial, although recently a clear cor-

Chart 1



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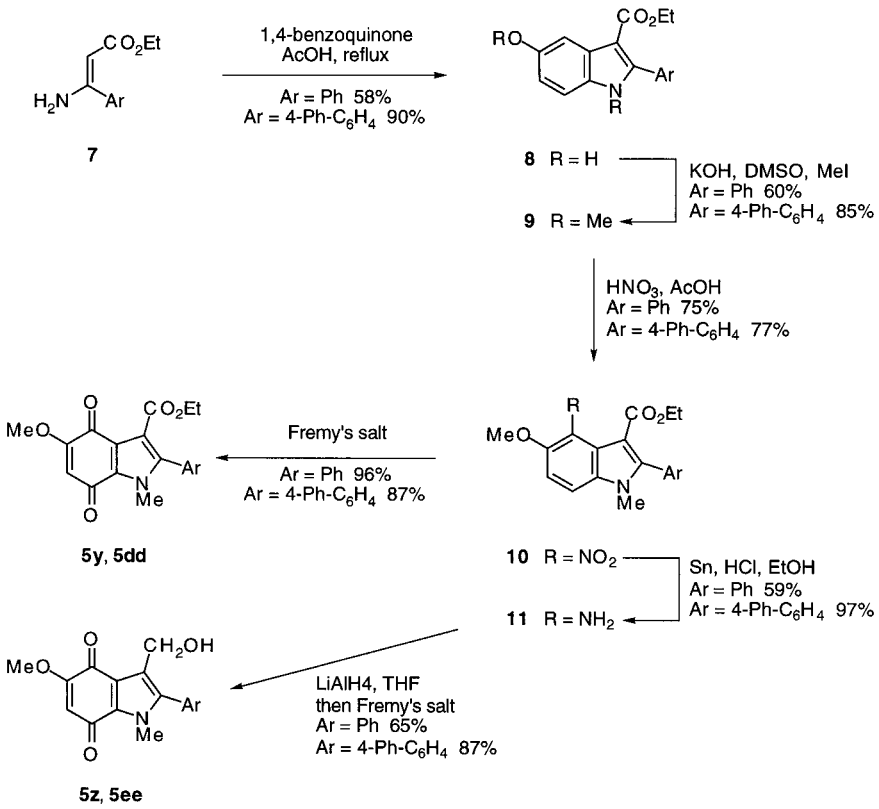
Scheme 1[a]



[a] For compounds **7**−**11**: **a**, Ar = Ph; **b**, Ar = 4-Ph-C_6H_4.

relation between NQO1 activity and MMC sensitivity in human lung and breast cancer cell lines has been demonstrated.[17] On the other hand, the importance of NQO1 in the bioactivation of other cytotoxic indolequinones such as EO9 (**2**),[18−20] aziridinylbenzoquinones such as MeDZQ (**3**),[16,17] and the novel cyclopropamitosenes **4**[21] has been established. However there has been no systematic attempt to correlate quinone structure with rate of metabolism by NQO1 and toxicity toward human tumor cell lines, and therefore we report in detail the results of such a study in a series of indolequinones **5** and mitosenes **6**.[1]

Results and Discussion

Chemistry. The synthesis of the 1,2-dimethylindolequinones **5a**−**5w** and the mitosenes **6** has already been developed in our laboratory, and the preparation of compounds **5a**−**5c**, **5e**, **5v**, **5w**, **6a**, **6b**, **6d**, **6e**, and **6h**−**6j** has been described previously.[22,23] Several new compounds were included in the present study with the aim of investigating the substituent effects at the 2-, 3-, and 5-positions. Hence a range of compounds containing a greater variety of amine substituents at C-5 was prepared, and the C-3 position was unsubstituted or substituted with methyl, hydroxymethyl, or (carbamoyloxy)methyl groups. Compounds containing electron-withdrawing groups (CHO or CO_2Et) were also included in the study. Finally on the basis that streptonigrin, an excellent substrate for NQO1,[17] contains a bulky aromatic substituent at C-2 of the quinolinequinone, we prepared both 2-phenylindolequinones and 2-(4-biphenylyl)indolequinones for direct comparison with their 2-methyl counterparts.

Of the new compounds, the indolequinone ester **5d** was obtained from commercially available ethyl 5-hydroxy-2-methylindole-3-carboxylate.[24] Reduction of ester **5d** also provided an alternative route to the 3-(hydroxymethyl)indolequinone **5e**, and reduction under more forcing conditions gave the 3-methyl derivative **5f**. A range of indolequinones containing amino substituents was prepared by reaction of the corresponding methoxy quinone with the appropriate amine, a substitution reaction widely used in the mitomycin series.[25,26] Thus indolequinones **5g**, **5h**, and **5j**−**5s** were obtained from the 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**5e**) by reaction with the amine in DMF. The quinone **5i** was prepared similarly from **5d**. The acetoxymethyl quinone **5t** was prepared by acetylation of the hydroxymethyl compound **5e**,[24] and the (4-nitrophenoxy)methyl derivative **5u** was obtained by carrying out a Mitsunobu reaction on the hydroxymethyl compound **5e** with 4-nitrophenol. Compound **5x** was prepared by oxidation of methyl 5,7-dimethoxy-1,3-dimethylindole-2-carboxylate[27] with ceric ammonium nitrate.

The 3-(hydroxymethyl)indolequinones **5y**, **5z**, **5dd**, and **5ee** containing an aromatic group at C-2 were prepared as shown in Scheme 1. Thus Nenitzescu reaction of the 3-amino-3-arylpropenoate **7** with 1,4-benzoquinone gave the 5-hydroxyindole **8**.[28] Methylation of the hydroxy group and the indole nitrogen was followed by nitration to give the 4-nitroindole **10**, which was readily reduced to the corresponding amino indole **11**. The 4-aminoindole **11** was either directly oxidized with Fremy's salt (potassium nitrosodisulfonate) to give the indolequinones **5y** and **5dd** or reduced with lithium

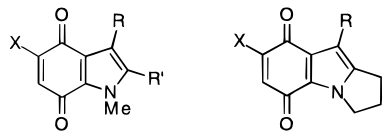
aluminum hydride and then oxidized with Fremy's salt to give the 3-(hydroxymethyl)indolequinones **5z** and **5ee**. The 2-phenylindolequinones **5aa**−**5cc** with amine substituents at C-5 were prepared from the 5-methoxy compound **5z** by reaction with the corresponding amine. In the series of pyrrolo[1,2-*a*]indolequinones **6**, the new amine derivative **6c** was prepared from **6a**, **6f** and **6g** from **6e**, and **6k**−**6m** from **6h**. Compounds **5e**, **5m**, **5n**, **5z**, and **5cc** have also been studied independently by another research group with a view to establishing their activity under hypoxic conditions.[29,30]

Electrochemical studies were performed on a number of the indolequinones using DMF as solvent and tetra-*n*-butylammonium tetrafluoroborate as the supporting electrolyte. E_{redox} values, determined from the (at least) quasi-reversible voltammograms recorded for the 1-electron reduction of the indolequinones, show little variation over the range of potential sweep rates used. Thus, the E_{redox} values given in Table 1 are averages of the values determined from voltammograms recorded at potential sweep rates of 25, 50, 100, 200, 300, 400, and 500 mV s^{-1}. The E_{redox} values, tabulated with reference to ferrocene (Fc), are shown in Table 1 and show substituent effects in line with our previous observations.[23]

Biology. Metabolism of the indolequinones **5** (Table 2) and mitosenes **6** (Table 3) by recombinant human NQO1 was studied using an HPLC system designed to quantify both NADH oxidation and quinone reduction. NADH oxidation is irreversible in this assay and is therefore used for comparison of reduction velocities. An NQO1 inactivation assay was used to determine enzyme activity remaining. The indolequinones were incubated in the presence of NQO1 and NADH, and residual enzyme activity was determined spectrophotometrically. In the 1,2-dimethylindolequinone series **5a**−**5w**, measurable NADH oxidation was observed for compounds **5a**−**5s** with the exception of the 5-pyrrolidinyl compound **5p**. Compounds **5b** and **5m**, which have an aziridinyl substituent at the 5-position, were better substrates for human NQO1 than the corresponding compounds **5a** and **5e**, which have a methoxy group at the 5-position. Compound **5n**, the methylaziridinyl analogue of **5m**, was reduced at a slightly slower rate than the aziridine **5m**, though it was still a better substrate than the methoxy compound **5e**. Compounds containing a basic nitrogen substituent at C-5 were poor substrates for human NQO1, and one, the pyrrolidinyl derivative **5p**, was not metabolized at all. Reduction rates for those compounds with hydroxymethyl substituents at the 3-position, for example, **5e** and **5m**, were somewhat lower than those for the corresponding unsubstituted derivatives, **5a** and **5b**.

1,2-Dimethylindolequinones **5t**−**5w** bearing a leaving group at the 3-indolyl methyl position were apparently not substrates for human NQO1, i.e., no NADH oxidation was observed. Inactivation studies showed that for compounds **5t**−**5v**, there was 0% enzyme activity remaining following incubation with NQO1 and NADH (Table 2). Inactivation was NADH-dependent suggesting that it is the reductively activated indolequinone which inactivates the enzyme, although no hydroquinones could be detected by HPLC due to their very low molar absorptivity and the rapid regeneration of the quinones by redox cycling. Blank experiments established that NADH alone did not reduce the quinones **5t**−**5v**. Because of the relative concentrations involved (more than a 1500-fold excess of quinone to NQO1 and more than a 6000-fold excess of NADH to NQO1), an efficient inactivator could be reduced by the enzyme and inactivate the enzyme completely and NADH oxidation would be undetectable. In other words, compounds **5t**−**5w** may actually be substrates for NQO1, but the analytical methods are not sensitive enough to detect the small amount of NADH oxidation required to facilitate inactivation of the enzyme by the quinones. Mitomycin C also has a (carbamoyloxy)methyl group at the 3-indolyl methyl position, and mitomycin C inactivation of rat NQO1 has been shown to be mechanism-based.[31]

Table 1. Electrochemical Reduction Potentials[a] (DMF) of Indolequinones **5** and Mitosenes **6** versus Ferrocene

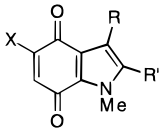


5 **6**

compd	R′	R	X	E_{redox} (V) vs Fc
5a	Me	H	OMe	−1.39[b]
5b	Me	H	aziridinyl	−1.39[b]
5c	Me	CHO	OMe	−1.19
5d	Me	CO_2Et	OMe	−1.32
5e	Me	CH_2OH	OMe	−1.28
5f	Me	Me	OMe	−1.46
5g	Me	CH_2OH	NHMe	−1.46
5h	Me	CH_2OH	NMe_2	−1.40
5i	Me	CO_2Et	$NHCH_2CH_2OH$	−1.47
5j	Me	CH_2OH	$NHCH_2CH_2OH$	−1.45
5k	Me	CH_2OH	$NHCH_2CH_2OMe$	−1.43
5l	Me	CH_2OH	cyclopropylamino	−1.43
5m	Me	CH_2OH	aziridinyl	−1.26
5n	Me	CH_2OH	methylaziridinyl	−1.27
5o	Me	CH_2OH	azetidinyl	−1.43
5p	Me	CH_2OH	pyrrolidinyl	−1.48
5r	Me	CH_2OH	morpholinyl	−1.31
5s	Me	CH_2OH	*N*-methylpiperazinyl	−1.34
5t	Me	CH_2OAc	OMe	−1.36
5v	Me	CH_2OCONH_2	OMe	−1.39[b]
5w	Me	CH_2OCONH_2	aziridinyl	−1.37[b]
5x	CO_2Me	Me	OMe	−1.29
5y	Ph	CO_2Et	OMe	−1.29
5z	Ph	CH_2OH	OMe	−1.23
5bb	Ph	CH_2OH	cyclopropylamino	−1.37
5cc	Ph	CH_2OH	methylaziridinyl	−1.22
5dd	4-Ph-C_6H_4	CO_2Et	OMe	−1.28
5ee	4-Ph-C_6H_4	CH_2OH	OMe	−1.22
6a		H	OMe	−1.39
6c		H	pyrrolidinyl	−1.61
6d		CHO	OMe	−1.19
6e		CH_2OH	OMe	−1.29
6f		CH_2OH	methylaziridinyl	−1.28
6g		CH_2OH	pyrrolidinyl	−1.50
6h		CH_2OCONH_2	OMe	−1.37
6i		CH_2OCONH_2	aziridinyl	−1.38[b]
6j		CH_2OCONH_2	methylaziridinyl	−1.39[b]
6k		CH_2OCONH_2	pyrrolidinyl	−1.59
6l		CH_2OCONH_2	piperidinyl	−1.46
6m		CH_2OCONH_2	cyclopropylamino	−1.53

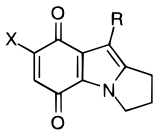
[a] E_{redox} (±0.005 V) values calculated as $(E_{pc} + E_{pa})/2$ are averages of the values determined from voltammograms recorded at potential sweep rates of 25, 50, 100, 200, 300, 400, and 500 mV s^{-1}; E_{pc}, cathodic peak potential; E_{pa}, anodic peak potential. [b] Reference 23.

Table 2. Metabolism of Indolequinones **5** by Recombinant Human NQO1



compd	R′	R	X	metabolism (μmol/min/mg) NADH oxidation	% enzyme activity remaining
5a	Me	H	OMe	5.31 ± 0.93	
5b	Me	H	aziridinyl	11.6 ± 0.5	94 ± 5
5c	Me	CHO	OMe	8.78 ± 1.91	49 ± 4
5d	Me	CO_2Et	OMe	14.3 ± 4.9	100
5e	Me	CH_2OH	OMe	1.25 ± 0.03	96 ± 4
5f	Me	Me	OMe	0.20 ± 0.03	100
5g	Me	CH_2OH	NHMe	0.49 ± 0.06	100
5h	Me	CH_2OH	NMe_2	0.46 ± 0.04	92 ± 4
5i	Me	CO_2Et	$NHCH_2CH_2OH$	0.32 ± 0.03	
5j	Me	CH_2OH	$NHCH_2CH_2OH$	0.22 ± 0.02	98 ± 2
5k	Me	CH_2OH	$NHCH_2CH_2OMe$	0.10 ± 0.01	100
5l	Me	CH_2OH	cyclopropylamino	0.23 ± 0.02	96 ± 4
5m	Me	CH_2OH	aziridinyl	3.35 ± 0.65	97 ± 4
5n	Me	CH_2OH	methylaziridinyl	2.01 ± 0.43	
5o	Me	CH_2OH	azetidinyl	0.22 ± 0.03	98 ± 2
5p	Me	CH_2OH	pyrrolidinyl	not detected	
5q	Me	CH_2OH	piperidinyl	0.22 ± 0.06	81 ± 2
5r	Me	CH_2OH	morpholinyl	0.69 ± 0.08	100
5s	Me	CH_2OH	*N*-methylpiperazinyl	0.45 ± 0.04	99 ± 2
5t	Me	CH_2OAc	OMe	not detected	0
5u	Me	$CH_2OC_6H_4NO_2$	OMe	not detected	0
5v	Me	CH_2OCONH_2	OMe	not detected	0
5w	Me	CH_2OCONH_2	aziridinyl	not detected	
5x	CO_2Me	Me	OMe	0.36 ± 0.04	100
5y	Ph	CO_2Et	OMe	23.4 ± 2.3	
5z	Ph	CH_2OH	OMe	11.0 ± 0.9	
5aa	Ph	CH_2OH	$NHCH_2CH_2OH$	0.075 ± 0.002	
5bb	Ph	CH_2OH	cyclopropylamino	0.09 ± 0.01	
5cc	Ph	CH_2OH	methylaziridinyl	0.74 ± 0.06	
5dd	4-Ph-C_6H_4	CO_2Et	OMe	0.17 ± 0.03	
5ee	4-Ph-C_6H_4	CH_2OH	OMe	5.40 ± 0.83	

Table 3. Metabolism of Mitosenes **6** by Recombinant Human NQO1



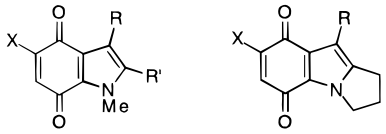
compd	R	X	metabolism (μmol/min/mg) NADH oxidation	% enzyme activity remaining
6a	H	OMe	5.62 ± 0.71	93 ± 2
6b	H	methylaziridinyl	2.37 ± 0.06	77 ± 5
6c	H	pyrrolidinyl	0.04 ± 0.01	84 ± 5
6d	CHO	OMe	8.77 ± 1.22	44 ± 5
6e	CH_2OH	OMe	4.80 ± 0.38	100
6f	CH_2OH	methylaziridinyl	2.22 ± 0.41	91 ± 2
6g	CH_2OH	pyrrolidinyl	0.06 ± 0.01	90 ± 5
6h	CH_2OCONH_2	OMe	not detected	0
6i	CH_2OCONH_2	aziridinyl	0.06 ± 0.02	0
6j	CH_2OCONH_2	methylaziridinyl	not detected	0
6k	CH_2OCONH_2	pyrrolidinyl	not detected	
6l	CH_2OCONH_2	piperidinyl	not detected	45 ± 5
6m	CH_2OCONH_2	cyclopropylamino	not detected	16 ± 4

However, compounds containing an electron-withdrawing group such as an ester at C-3 were excellent substrates; within the 5-methoxyindolequinones the rate of metabolism by the enzyme decreased as the 3-substituent was altered in the order: CO_2Et > CHO > H > CH_2OH > CH_3 > CH_2OCONH_2. Switching the electron-withdrawing ester group to the 2-position, as in indolequinone **5x**, resulted in poor metabolism by the enzyme.

As anticipated on the basis of the properties of streptonigrin, the 2-phenylindolequinones **5y** and **5z** were excellent substrates for NQO1, better than their corresponding 2-methyl derivatives **5d** and **5e**. As expected this was modified by the incorporation of amine substituents at C-5 as in compounds **5aa** and **5bb**. The methylaziridine derivative **5cc** was surprisingly a rather poor substrate. Incorporation of the larger biphenyl substituent at C-2 resulted in a compound (**5ee**) that was a better substrate for the enzyme than the corresponding 2-methyl compound **5e** but worse than the corresponding 2-phenyl derivative, though, surprisingly, the corresponding 3-ester **5dd** was a poor substrate.

Although less compounds were investigated, a similar pattern of quinone metabolism was observed in the mitosene series **6**. Thus measurable NADH oxidation was observed for methoxyquinones **6a** and **6e**, methylaziridines **6b** and **6f**, aziridine **6i**, and pyrrolidines **6c** and **6g**, although the latter three compounds were only slightly above the limits of detection. Interestingly in the mitosenes **6** the C-7 methoxy compounds were better substrates than the corresponding methylaziridines. Again the substituent at the indole 3-position (9-position in mitosene numbering) had an effect, with the unsub-

Table 4. Cytotoxicity of Representative Indolequinones **5** and Mitosenes **6** toward Non-Small-Cell Lung Cancer (NSCLC) Cell Lines[a]



5 **6**

compd	R′	R	X	cytotoxicity IC_{50} (μM) H460	cytotoxicity IC_{50} (μM) H596	selectivity ratio
5a	Me	H	OMe	>25	>25	1
5b	Me	H	aziridinyl	0.96 ± 0.24	>25	>26
5d	Me	CO_2Et	OMe	>25	>25	1
5e	Me	CH_2OH	OMe	>25	>25	1
5m	Me	CH_2OH	aziridinyl	0.018 ± 0.004	9.22 ± 1.51	510
5n	Me	CH_2OH	methylaziridinyl	0.11 ± 0.02	>25	>220
5o	Me	CH_2OH	azetidinyl	>25	>25	1
5u	Me	$CH_2OC_6H_4NO_2$	OMe	2.81 ± 0.52	2.00 ± 0.20	0.7
5v	Me	CH_2OCONH_2	OMe	7.45 ± 0.37	2.16 ± 0.47	0.3
5w	Me	CH_2OCONH_2	aziridinyl	0.15 ± 0.01	0.24 ± 0.03	1.6
5cc	Ph	CH_2OH	methylaziridinyl	8.0 ± 5.1	>25	>3
6e		CH_2OH	OMe	>25	>25	1
6f		CH_2OH	methylaziridinyl	0.093 ± 0.018	>25	>270
6h		CH_2OCONH_2	OMe	5.40 ± 0.58	6.48 ± 1.58	1.2
6i		CH_2OCONH_2	aziridinyl	0.36 ± 0.08	2.71 ± 0.37	7.5

[a] H460 has high NQO1 activity, and H596 has no measurable NQO1 activity.

stituted compounds **6a** and **6b** being more rapidly metabolized than their 9-hydroxymethyl counterparts **6e** and **6f**. The 9-[(carbamoyloxy)methyl]-substituted mitosenes **6h**−**6m** were either very poor substrates for NQO1 (**6i**) or not substrates for NQO1 (**6h**, **6j**−**6m**). As with the indolequinone series **5**, this was likely due to NADH-dependent inactivation of NQO1 by the reduced quinone. Enzyme activity remaining was 0% for compounds **6h**−**6j**, 45% for compound **6l**, and 16% for compound **6m**. The compound **6d** containing the electron-withdrawing formyl group was an excellent substrate, and as before, most of the compounds containing the (carbamoyloxy)methyl side chain were not substrates for human NQO1. Quinone reduction was undetectable for all of the compounds (with the exception of **6f** which was removed at a rate of 0.7 μmol/min/mg) suggesting that the reduced quinones (hydroquinones) reoxidize rapidly in air to regenerate the parent quinones.

It is clear that the substituent on the quinone ring (5-position in **5**, 7-position in **6**) has a marked effect on the rate of metabolism of the indolequinones by NQO1. In general those compounds bearing amine substituents (other than aziridines) at C-5(7) are not substrates for the enzyme, either as a result of steric effects (in the case of substituents such as pyrrolidine or piperidine) or because such substituents render the quinone more difficult to reduce by virtue of the donation of the nitrogen lone pair into the quinone. The fact that these substrates are more difficult to reduce is clearly evidenced by electrochemical experiments (Table 1). Conversely the indolequinones that are easiest to reduce electrochemically, i.e., those bearing an aziridine substituent, are among the best substrates for NQO1, with the methylaziridine and methoxy compounds being somewhat less efficient. The aziridine ring, because of its inability to donate electron density into the quinone which would involve an unfavorable flattening of the aziridine nitrogen, has a similar electronic effect to the methoxy group. However, although the ease of reduction is a key factor, just as with other quinones,[15,16] there is no *overall* correlation between reduction potential and rate of metabolism by NQO1.

The substituent at the indole 3-position (9-position in mitosene numbering in **6**) also has a considerable effect on metabolism by NQO1. The carbamates and other compounds bearing a leaving group at the 3-indolyl methyl position are generally much poorer substrates, although this is possibly due to inactivation of the enzyme by these compounds, since elimination of the leaving group would generate a reactive electrophile capable of alkylating the enzyme.[24,31] Compounds with an electron-withdrawing substituent at this position are the best substrates of those 1,2-dimethylindolequinones and mitosenes studied, although the 3(or 9)-unsubstituted compounds and the hydroxymethyl derivatives are also efficiently metabolized. The fact that the 2-arylindolequinones **5y**, **5z**, and **5ee** were all good substrates was anticipated (see above) and presumably reflects the fact that the enzyme can accommodate a large aromatic group in its active site; further studies to probe this aspect are underway.

However, being an efficient substrate for NQO1 does not necessarily mean that the quinone will be selectively cytotoxic to cells containing elevated NQO1 activity. Representative compounds were therefore tested in vitro against tumor cell lines. Cytotoxicity (cell survival) was measured using the MTT colorimetric assay. Two non-small-cell lung cancer (NSCLC) cell lines were used: H460 with high NQO1 activity (1360 nmol/min/mg) and H596 which has no measurable NQO1 activity due to a polymorphism in the NQO1 gene.[32] Activities of other relevant reductases have been measured and found to be similar as reported previously.[17] IC_{50} values (concentration at which cell survival equals 50% of control) are reported for the indolequinones **5** and the mitosenes **6** in Table 4. A compound with an IC_{50} value which is lower in the H460 cells than in the H596 cells suggests

a compound which is selectively toxic to the cell line with elevated NQO1 activity. The compounds with the greatest degree of selective toxicity were the 1,2-dimethylindolequinones **5b**, **5m**, and **5n**, each with greater than a 25-fold difference in IC_{50} values between the two cell lines, with **5m** being more than 500-fold selective. All three compounds were good substrates for human NQO1, and each has either an aziridinyl substituent or a methylaziridinyl substituent at the 5(7)-position. The only mitosenes which exhibited selective toxicity were **6f** and **6i**, with **6f** being greater than 250-fold selective for the H460 cell line. Both compounds have aziridinyl substituents.

Overall toxicity in non-small-cell lung cancer cell lines was greatest for those indolequinones that, in principle, can act as bifunctional alkylating agents, namely, the hydroxymethyl and (carbamoyloxy)methyl aziridines **5m**, **5n**, **5w**, **6f**, and **6i**. Compounds that are likely to function as monoalkylating agents were less cytotoxic, and **5a** and **5d**, which have no alkylating centers, were not toxic, although they were efficient redox-cycling compounds when activated by NQO1. In agreement with their ability to act as efficient substrates for NQO1, the aziridinyl quinones were usually the most selective agents in each series, exhibiting much greater toxicity toward the high-NQO1 H460 cells than to the NQO1-deficient H596 cells.

In conclusion in studying over 40 indolequinones, we have established the relationship between quinone structure, rate of metabolism by human NQO1, and cytotoxicity toward cells containing elevated levels of NQO1. Some of the compounds identified by this study may have potential uses as, for example, irreversible inactivators of NQO1 (e.g., **5t**−**5v**) or highly selective NQO1-directed antitumor agents (e.g., **5m**, **5n**, **6f**).

Experimental Section

Chemistry. Commercially available reagents were used throughout without further purification; solvents were dried by standard procedures. Analytical thin-layer chromatography was carried out using aluminum-backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualized under UV light (at 254 and/or 360 nm). Flash chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Pressure was applied at the column head with hand bellows. Samples were applied preadsorbed on silica. Fully characterized compounds were chromatographically homogeneous. ^1H and ^{13}C NMR spectra were recorded at 250, 300, or 400 MHz (^1H frequencies) on Bruker instruments. Full spectroscopic data are given for quinones; data for intermediates are provided in the Supporting Information.

General Method for Substitution Reactions at C-5 Position with Amines. The following general method was used unless otherwise stated: To a stirred solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**5e**) (0.080 g, 34.0 mmol) in DMF (5 mL) was added the amine (40 equiv), and the mixture was stirred for 48 h at room temperature. Water was added (30 mL), the crude product was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with water (12 × 30 mL). The organic layer was dried (Na_2SO_4). The crude material was purified by column chromatography (EtOAc/light petroleum, 1:1) to yield the product.

5-Methoxy-1,2-dimethylindole-4,7-dione, 5a. Prepared as previously described.[23]

5-(Aziridin-1-yl)-1,2-dimethylindole-4,7-dione, 5b. Prepared as previously described.[23]

3-Formyl-5-methoxy-1,2-dimethylindole-4,7-dione, 5c. Prepared as previously described.[23]

Ethyl 5-Methoxy-1,2-dimethyl-4,7-dioxoindole-3-carboxylate, 5d. Prepared as previously described.[24]

3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione, 5e. (a) Prepared as previously described.[22] (b) Alternative method: To a solution of ethyl 5-methoxy-1,2-dimethyl-4,7-dioxoindole-3-carboxylate (**5d**) (3.4 g, 12.3 mmol) in chloroform (370 mL) and EtOH (125 mL) was added a solution of sodium dithionite (25 g) in water (160 mL). The biphasic mixture was stirred vigorously overnight. The organic layer was separated, washed with brine, dried (Na_2SO_4), and concentrated to give ethyl 4,7-dihydroxy-5-methoxy-1,2-dimethylindolecarboxylate as a solid that was used directly.

To a stirred suspension of the hydroquinone in dichloromethane (500 mL) was added DIBAL (1 M solution in dichloromethane; 13.9 g, 97.9 mmol), keeping the temperature below −30 °C. The mixture was stirred at this temperature for a further 2 h. The reaction mixture was quenched by dropwise addition of an iron(III) chloride solution (1 M $FeCl_3$/0.1 M HCl, 125 mL) while keeping the temperature below −30 °C. The mixture was filtered through Celite and the residue washed with hot dichloromethane. The organic layer was separated, washed with saturated ammonium chloride, dried (Na_2SO_4), and concentrated. The crude product was recrystallized (dichloromethane/light petroleum) to yield the title compound as an orange/red crystalline solid (2.0 g, 71%): mp 200−202 °C (lit.[23] mp 199−200 °C); ^1H NMR ($CDCl_3$) δ 5.60 (1 H, s, 6-H), 4.59 (2 H, d, J = 6.7 Hz, CH_2OH), 3.87 (1 H, t, J = 6.7 Hz, OH), 3.85 (3 H, s, OMe), 3.80 (3 H, s, NMe), and 2.21 (3 H, s, Me).

5-Methoxy-1,2,3-trimethylindole-4,7-dione, **5f.** If the above reaction mixture in the reduction of **5d** was allowed to warm to 0 °C, the product was 5-methoxy-1,2,3-trimethylindole-4,7-dione, **5f**: mp 225−226 °C (dichloromethane/light petroleum); UV (MeOH) 473 (log ϵ 3.31), 361 (3.42), 286 (4.24), and 227 (4.15) nm; IR (KBr) 2981, 2944, 1664, 1654, 1636, and 1622 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.46 (1 H, s, 6-H), 3.75 (3 H, s, OMe), 3.70 (3 H, s, NMe), 2.14 (3 H, s, Me), and 2.06 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 178.7 (CO), 178.5 (CO), 160.0, 135.5, 128.4, 122.2, 118.9, 107.4 (CH), 56.6 (OMe), 32.6 (NMe), 10.3 (Me), and 9.2 (Me); HRMS found (M^+) 219.0895, $C_{12}H_{13}NO_3$ requires M 219.0895. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

3-(Hydroxymethyl)-5-(methylamino)-1,2-dimethylindole-4,7-dione, 5g. Purple crystalline solid in 93% yield: mp 235−237 °C; UV (MeOH) 524 (log ϵ 3.34), 316 (4.19), and 238 (4.27) nm; IR (CH_2Cl_2) 3474, 3270, 1663, 1597, and 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.85 (1 H, br s, NH), 5.13 (1 H, s, 6-H), 4.60 (2 H, d, J = 6.7 Hz, CH_2), 3.99 (1 H, t, J = 7.1 Hz, OH), 3.90 (3 H, s, NMe), 2.87 (3 H, d, J = 5.4 Hz, *Me*NH), and 2.20 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 179.7 (CO), 178.7 (CO), 149.1, 132.5, 131.9, 121.7, 120.0, 96.9 (CH), 56.1 (CH_2), 32.3 (Me), 29.4 (Me), and 9.4 (Me); HRMS found (M^+) 234.1003, $C_{12}H_{14}N_2O_4$ requires M 234.1004.

3-(Hydroxymethyl)-5-(dimethylamino)-1,2-dimethylindole-4,7-dione, 5h. Purple solid in 59% yield: mp 138−139 °C (EtOAc/light petroleum); UV (MeOH) 534 (log ϵ 3.41), 324 (4.04), and 246 (4.18) nm; IR (CH_2Cl_2) 3440, 3062, 1654, 1620, 1560, and 1276 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.16 (1 H, s, 6-H), 4.51 (3 H, m, CH_2OH), 3.81 (3 H, s, NMe), 3.08 (6 H, s, 2 × NMe), and 2.19 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 181.3 (CO), 177.3 (CO), 152.8, 135.6, 129.1, 121.6, 121.1, 107.1 (CH), 53.9 (CH_2), 42.9 (NMe), 32.0 (NMe), and 9.6 (Me); HRMS found (M^+) 248.1154, $C_{13}H_{16}N_2O_3$ requires M 248.1161. Anal. ($C_{13}H_{16}N_2O_3 \cdot 0.5H_2O$) C, H, N.

Ethyl 5-[(2-Hydroxyethyl)amino]-1,2-dimethyl-4,7-dioxoindole-3-carboxylate, 5i. Purple crystalline solid in 61% yield: mp 161−162 °C (EtOAc/light petroleum); UV (MeOH) 506 (log ϵ 3.21), and 312 (4.02) nm; IR (CH_2Cl_2) 3396, 3338, 2981, 1731, 1665, 1598, 1578, 1506, 1440, 1245, and 1214 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.22 (1 H, br s, NH), 5.21 (1 H, s, 6-H), 4.37 (2 H, q, J = 7.1 Hz, CH_2), 3.93 (3 H, s, NMe), 3.88 (2 H, q, J = 5.3 Hz, CH_2), 3.31 (2 H, q, J = 5.5 Hz, CH_2), 2.43 (3 H, s, Me), 1.94 (1 H, t, J = 5.4 Hz, OH), and 1.39 (3 H, t, J = 7.1 Hz, Me); ^{13}C NMR ($CDCl_3$) δ 181.2 (CO), 179.0 (CO), 157.2, 138.8, 130.6 (2 × CH), 130.2, 129.3 (CH), 128.8 (2 × CH), 128.6,

123.9, 122.8, 116.8 (CH), 56.2 (CH_2OH), 36.3 (CH), 34.7 (CH_2), 33.9 (NMe), and 17.7 (Me); HRMS found (M^+) 306.1216, $C_{15}H_{18}N_2O_5$ requires M 306.1217. Anal. ($C_{15}H_{18}N_2O_5 \cdot 0.4H_2O$) C, H, N.

3-(Hydroxymethyl)-5-[(2-hydroxyethyl)amino]-1,2-dimethylindole-4,7-dione, 5j. Purple solid in 54% yield: mp 211−213 °C (EtOAc/light petroleum); UV (MeOH) 524 (log ϵ 3.26), 314 (4.13), and 244 (4.30) nm; IR (CH_2Cl_2) 3472, 3359, 1654, 1612, and 1274 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.13 (1 H, br s, NH), 5.17 (1 H, s, 6-H), 4.60 (2 H, d, J = 6.8 Hz, CH_2), 4.03 (1 H, t, J = 6.8 Hz, OH), 3.89 (5 H, br s, NMe, CH_2), 3.31 (2 H, q, J = 5.5 Hz, CH_2), 2.20 (3 H, s, Me), and 1.92 (1 H, br s, OH); ^{13}C NMR ($CDCl_3$) δ 179.4 (CO), 178.6 (CO), 149.3, 135.2, 130.7, 120.4, 119.6, 95.2 (CH), 58.8 (CH_2), 54.0 (CH_2), 44.5 (CH_2), 31.2 (NMe), and 7.8 (Me); HRMS found (M^+) 264.1116, $C_{13}H_{16}N_2O_4$ requires M 264.1110. Anal. ($C_{13}H_{16}N_2O_4 \cdot 0.5H_2O$) C, H, N.

3-(Hydroxymethyl)-5-[(2-methoxyethyl)amino]-1,2-dimethylindole-4,7-dione, 5k. Purple crystalline solid in 93% yield: mp 141−142 °C (EtOAc/light petroleum); UV (MeOH) 526 (log ϵ 3.46), 314 (4.20), and 244 (4.36) nm; IR (CH_2Cl_2) 3690, 3380, 2996, 1661, 1627, 1607, 1506, and 1276 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.07 (1 H, br s, NH), 5.12 (1 H, s, 6-H), 4.58 (2 H, d, J = 3.7 Hz, C*H*$_2$OH), 3.99 (1 H, s, OH), 3.87 (3 H, s, OMe), 3.60 (2 H, t, J = 5.3 Hz, MeOC*H*$_2$), 3.38 (3 H, s, NMe), 3.27 (2 H, q, J = 5.4 Hz, NHC*H*$_2$), and 2.18 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 179.6 (CO), 178.8 (CO), 148.0, 132.6, 131.6, 121.8, 120.1, 97.2 (CH), 69.4 (CH_2), 58.9 (OMe), 56.0 (CH_2), 42.4 (CH_2), 32.2 (NMe), and 9.34 (Me); HRMS found (M^+) 278.1265, $C_{14}H_{18}N_2O_4$ requires M 278.1267. Anal. ($C_{14}H_{18}N_2O_4 \cdot 0.2H_2O$) C, H, N.

5-(Cyclopropylamino)-3-(hydroxymethyl)-1,2-dimethylindole-4,7-dione, 5l. Purple crystalline solid in 51% yield: mp 192−193 °C (EtOAc/light petroleum); UV (MeOH) 528 (log ϵ 3.39), 316 (4.22), and 242 (4.38) nm; IR (CH_2Cl_2) 3697, 3393, 2989, 1627, 1593, 1506, and 1283 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.89 (1 H, br s, NH), 5.50 (1 H, s, 6-H), 4.58 (2 H, d, J = 6.7 Hz, C*H*$_2$OH), 3.98 (1 H, t, J = 6.7 Hz, OH), 3.88 (3 H, s NMe), 2.44 (1 H, m, NHC*H*), 2.18 (3 H, s, Me), 0.85 (2 H, m, CH_2), and 0.65 (2 H, m, CH_2); ^{13}C NMR ($CDCl_3$) δ 180.0 (CO), 178.9 (CO), 148.9, 132.5, 131.6, 121.7, 120.2, 99.1 (CH), 56.0 (CH_2), 32.2 (Me), 24.2 (CH), 9.4 (Me), and 7.1 (2 × CH_2); HRMS found (M^+) 260.1166, $C_{14}H_{16}N_2O_3$ requires M 260.1161. Anal. ($C_{14}H_{16}N_2O_3 \cdot 0.2H_2O$) C, H, N.

5-(Aziridin-1-yl)-3-(hydroxymethyl)-1,2-dimethylindole-4,7-dione, 5m. To a stirred solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**5e**) (0.037 g, 0.15 mmol) in DMF (2 mL) was added aziridine (**CAUTION**) (0.13 g, 3.1 mmol), and the mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane (20 mL) and washed with water (5 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the crude material was purified by column chromatography (EtOAc) to yield the title compound (0.029 g, 77%) as a red solid: mp 164−166 °C (lit.[28] mp 173−174 °C); UV (MeOH) 483 (log ϵ 3.26), 305 (4.15), and 231 (4.23) nm; IR (KBr) 3422, 1636, 1588, and 1501 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.7 (1 H, s, 6-H), 4.62 (2 H, d, J = 7.1 Hz, C*H*$_2$OH), 4.12 (1 H, t, J = 7.1 Hz, OH), 3.87 (3 H, s, NMe), 2.22 (3 H, s, 2-Me), and 2.20 (4 H, s, azir-CH_2); ^{13}C NMR ($CDCl_3$) δ 181.4 (CO), 179.0 (CO), 157.2, 134.7, 130.0, 123.0, 122.9, 117.8 (CH), 56.3 (CH_2), 32.6 (CH_2), 28.0, and 9.9.

3-(Hydroxymethyl)-1,2-dimethyl-5-(2-methylaziridin-1-yl)indole-4,7-dione, 5n. To a stirred solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**5e**) (0.04 g, 0.17 mmol) in DMF (2 mL) was added 2-methylaziridine (0.19 g, 3.4 mmol), and the mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane (20 mL) and washed with water (5 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the crude material was purified by column chromatography (EtOAc) to yield the title compound (0.024 g, 56%) as a red solid: mp 124−125 °C (lit.[28] mp 120−122 °C); UV (MeOH) 484 (log ϵ 3.26), 305 (4.15), and 232 (4.24) nm; IR (KBr) 3422, 2980, 2732, 1660, 1629, and 1582 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.72 (1 H, s, 6-H), 4.61 (2 H, d, J = 6.5 Hz, C*H*$_2$OH), 4.16 (1 H, t, J = 6.5 Hz, OH), 3.86 (3 H, s, NMe), 2.21 (4 H, m, azir-CH, 2-Me), 2.10 (2 H, m, azir-CH_2), and 1.42 (3 H, d, J = 5.4 Hz, NCH*Me*); ^{13}C NMR ($CDCl_3$) δ 181.6 (CO), 179.1 (CO), 157.2, 134.4, 130.1, 123.0, 122.9, 117.0 (CH), 56.3 (CH_2), 36.5, 34.9 (CH_2), 32.6, 30.0, 18.0, and 9.8.

5-(Azetidin-1-yl)-3-(hydroxymethyl)-1,2-dimethylindole-4,7-dione, 5o. Purple crystalline solid in 88% yield: mp 190−191 °C (EtOAc/light petroleum); UV (MeOH) 548 (log ϵ 3.64), 328 (4.25), and 244 (4.36) nm; IR (CH_2Cl_2) 3474, 2980, 1670, 1604, 1578, and 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.87 (1 H, s, 6-H), 4.58 (4 H, m, CH_2), 4.40 (1 H, t J = 7.0 Hz, OH), 4.04 (2 H, t, J = 7.4 Hz, CH_2), 3.89 (3 H, s, NMe), 2.48 (2 H, m, CH_2), and 2.17 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 181.5 (CO), 177.4 (CO), 148.6, 132.0, 131.9, 121.4, 120.6, 97.7 (CH), 56.1 (CH_2), 55.8 (br CH_2), 52.0 (br CH_2), 32.1 (NMe), 17.7 (CH_2), and 9.4 (Me); MS *m*/*z* (EI) 260 (M^+, 75%), 215 (81), 199 (28), and 159 (20); HRMS found (M^+) 260.1166, $C_{14}H_{16}N_2O_3$ requires M 260.1161. Anal. ($C_{14}H_{16}N_2O_3 \cdot 0.5H_2O$) C, H, N.

3-(Hydroxymethyl)-1,2-dimethyl-5-(pyrrolidin-1-yl)indole-4,7-dione, 5p. To a stirred solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**5e**) (0.038 g, 0.16 mmol) in DMF (2 mL) was added pyrrrolidine (0.23 g, 3.24 mmol), and the mixture was stirred at room temperature overnight. The mixture was diluted with dichloromethane (20 mL) and washed with water (5 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the crude material was purified by column chromatography (EtOAc) to yield the title compound (0.038 g, 84%) as a purple solid: mp 196−198 °C; UV (MeOH) 547 (log ϵ 3.45), 328 (4.02), 250 (4.23), and 224 (4.18) nm; IR (KBr) 3422, 2980, 2732, 1639, 1612, and 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.11 (1 H, s, 6-H), 4.51 (2 H, s, C*H*$_2$-OH), 3.82 (3 H, s, NMe) 3.55 (4 H, br s, NCH_2), 2.12 (3 H, s, 2-Me), and 1.89 (4 H, m, CH_2); ^{13}C NMR ($CDCl_3$) δ 184.5 (CO), 180.5 (CO), 151.2, 134.7, 133.5, 124.0, 123.7, 104.2 (CH), 53.6 (CH_2), 50.3 (CH_2), 34.4 (NMe), and 11.8 (Me); HRMS found (M^+) 274.1317, $C_{15}H_{18}N_2O_3$ requires M 274.1317.

3-(Hydroxymethyl)-1,2-dimethyl-5-(piperidin-1-yl)indole-4,7-dione, 5q. Purple glassy solid in 44% yield: UV (MeOH) 534 (log ϵ 3.30), 324 (3.96), and 240 (4.20) nm; IR (CH_2Cl_2) 3455, 2945, 1624, 1556, 1503, and 1268 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.48 (1 H, s, 6-H), 4.57 (2 H, s, CH_2), 4.10 (1 H, m, OH), 3.86 (3 H, s, NMe), 3.39 (4 H, m, 2 × NCH_2), 2.20 (3 H, s, Me), and 1.68 (6 H, m, 3 × CH_2); ^{13}C NMR ($CDCl_3$) δ 182.3 (CO), 178.4 (CO), 154.1, 133.3, 129.9, 122.8, 122.1, 109.5 (CH), 55.9 (CH_2), 51.1 (2 × CH_2), 32.0 (Me), 25.7 (2 × CH_2), 24.2 (CH_2), and 9.4 (Me); HRMS found (MH^+) 289.1552, $C_{16}H_{20}N_2O_3$ + H requires M 289.1552.

3-(Hydroxymethyl)-1,2-dimethyl-5-(morpholin-1-yl)indole-4,7-dione, 5r. Purple crystalline solid in 65% yield: mp 199−200 °C (EtOAc/light petroleum); UV (MeOH) 522 (log ϵ 3.40), 326 (4.05), and 240 (4.28) nm; IR (CH_2Cl_2) 3427, 1639, 1577, 1515, 1267, and 1221 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.50 (1 H, s, 6-H), 4.58 (2 H, d, J = 7.0 Hz, CH_2), 3.97 (1 H, t, J = 7.0 Hz, OH), 3.87 (3 H, s, NMe), 3.84 (4 H, t, J = 4.8 Hz, OCH_2), 3.40 (4 H, t, J = 4.8 Hz, NCH_2), and 2.22 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 182.0 (CO), 178.3 (CO), 153.3, 133.9, 129.6, 123.0, 122.4, 111.0 (CH), 66.5 (2 × CH_2), 55.9 (CH_2), 49.9 (2 × CH_2), 32.1 (NMe), and 9.4 (Me); HRMS found (M^+) 290.1262, $C_{15}H_{18}N_2O_4$ requires M 190.1267. Anal. ($C_{15}H_{18}N_2O_4 \cdot 0.3H_2O$) C, H, N.

3-(Hydroxymethyl)-1,2-dimethyl-5-(4-methylpiperazin-1-yl)indole-4,7-dione, 5s. Purple crystalline solid in 75% yield: mp 166−167 °C (EtOAc/light petroleum); UV (MeOH) 522 (log ϵ 3.41), 326 (4.07), and 240 (4.28) nm; IR (CH_2Cl_2) 3460, 2993, 1664, 1637, 1571, 1512, and 1269 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.49 (1 H, s, 6-H), 4.57 (2 H, s, CH_2), 3.86 (3 H, s, NMe), 3.42 (4 H, m, 2 × CH_2), 2.54 (4 H, m, 2 × CH_2), 2.33 (3 H, s, NMe), and 2.20 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 182.1 (CO), 178.3 (CO), 153.5, 133.7, 129.7, 122.9, 122.2, 110.8 (CH), 55.9 (CH_2), 54.6 (2 × CH_2), 49.6 (2 × CH_2), 46.0 (NMe), 32.1 (NMe), and 9.4 (Me); HRMS found (M^+) 303.1587, $C_{16}H_{21}N_3O_3$ requires M 303.1583. Anal. ($C_{16}H_{21}N_3O_3$ $0.1H_2O$) C, H, N.

3-(Acetoxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione, 5t. To a stirred solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (0.084 g, 34.0 mmol) in pyridine (24 mL) was added acetic anhydride (3.5 mL), and the mixture was stirred for 24 h at room temperature. Water was added (30 mL), the crude product was washed with 1 M HCl and extracted with dichloromethane (2 × 40 mL), and the combined extracts were washed with water (2 × 20 mL). The organic layer was dried (Na_2SO_4). The crude material was purified by column chromatography (EtOAc/light petroleum, 1:1) to yield the title compound (0.040 mg, 40%) as an orange crystalline solid: mp 177−178 °C (EtOAc/light petroleum) (lit.[24] mp 185−186 °C); UV (MeOH) 450 (log ϵ 3.32), 340 (3.60), and 286 (4.37) nm; IR (CH_2Cl_2) 1736, 1657, 1637, 1564, 1269, and 1229 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.62 (1 H, s, 6-H), 5.25 (2 H, s, CH_2), 3.90 (3 H, s, OMe), 3.80 (3 H, s, NMe), 2.28 (3 H, s, Me), and 2.04 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 178.9 (CO), 177.6 (CO), 171.0, 159.7, 137.8, 129.2, 121.8, 155.8, 56.6 (CH_2), 56.4 (OMe), 32.4 (NMe), 22.7 (Me), and 14.1 (Me).

5-Methoxy-1,2-dimethyl-3-[(4-nitrophenoxy)methyl]indole-4,7-dione, 5u. 3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (0.46 g, 1.96 mmol), triphenylphosphine (1.0 g, 3.8 mmol), diethyl azodicarboxylate (0.68 g, 3.9 mmol), and 4-nitrophenol (0.46 g, 3.3 mmol) were stirred overnight in THF at 50 °C. Excess solvent was removed and the majority of the product triturated out with ether and filtered off. The residue was dissolved in dichloromethane, washed with water, dried (Na_2SO_4), and concentrated. The crude residue was purified by column chromatography (light petroleum/EtOAc, 1:1) to yield the title compound as an orange crystalline solid (0.4 g, 57%): mp 227−288 °C; UV (MeOH) 454 (ϵ log 2.82), 282 (3.91), and 227 (3.82) nm; IR (KBr) 3242, 3043, 2991, 1753, 1695, 1662, 1637, 1594, and 1482 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.18, 7.05 (4 H, AA′XX′, ArH), 5.64 (1 H, s, 6-H), 5.40 (2 H, s, CH_2OAr), 3.91 (3 H, s, OMe), 3.82 (3 H, s, NMe), and 2.31 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 178.7 (CO), 178.28 (CO), 163.6, 159.6, 141.7, 138.1, 129.0, 125.9 (CH), 121.3, 115.8, 115.0 (CH), 106.8 (CH), 61.1 (CH_2OAr), 56.5 (OMe), 32.4 (NMe), and 9.9 (Me); HRMS found (M^+) 356.1013, $C_{18}H_{16}N_2O_6$ requires M 356.1008.

3-[(Carbamoyloxy)methyl]-5-methoxy-1,2-dimethylindole-4,7-dione, 5v. Prepared as previously described.[23]

5-(Aziridin-1-yl)-3-[(carbamoyloxy)methyl]-1,2-dimethylindole-4,7-dione, 5w. Prepared as previously described.[23]

Methyl 5-Methoxy-1,3-dimethyl-4,7-dioxoindole-2-carboxylate, 5x. To a stirring solution of methyl 5,7-dimethoxy-1,3-dimethylindole-2-carboxylate[27] (0.56 g, 0.21 mmol) in acetonitrile (20 mL) at 0 °C was added a solution of cerium(IV) ammonium nitrate (1.2 g, 2.1 mmol) in water (8 mL) dropwise. After the addition, the mixture was allowed to stir at room temperature for 1 h. Saturated ammonium chloride was added and the mixture extracted with dichloromethane. The organic layer was separated, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (dichloromethane/EtOAc, 19:1) and recrystallized (dichloromethane/light petroleum) to yield the title compound (0.012 g, 21%) as a yellow crystalline solid: mp 218−220 °C; UV (MeOH) 418 (ϵ log 3.09), 348 (3.24), and 266 (4.32) nm; IR (KBr) 3046, 2967, 1723, 1683, 1650, 1604, and 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.75 (1 H, s, 6-H), 4.26 (3 H, s, NMe), 3.92 (3 H, s, OMe), 3.83 (3 H, s, OMe), and 2.58 (3 H, s, Me); ^{13}C NMR ($CDCl_3$) δ 179.4, 177.5, 161.8, 160.7, 131.7, 130.2, 126.8, 121.1, 107.8 (CH), 56.6 (OMe), 51.7 (OMe), 35.1 (NMe), and 11.7 (Me); HRMS found (M^+) 263.0794, $C_{13}H_{13}NO_5$ requires M 263.0793. Anal. ($C_{13}H_{13}NO_5 \cdot 0.3H_2O$) C, H, N.

Ethyl 5-Hydroxy-2-phenylindole-3-carboxylate, 8a. Ethyl 3-amino-3-phenylpropenoate (**7a**) (1.03 g, 5 mmol) in acetic acid was added to a solution of benzoquinone (0.54 g, 5 mmol) in acetic acid (3 mL) and heated under reflux for 2.5 h. The excess acetic acid was removed in vacuo, the residue was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with water (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried (Na_2SO_4) and evaporated. The product was purified by flash column chromatography (EtOAc/light petroleum, 4:6) to give the title compound **8a** (0.82 g, 58%) as a colorless crystalline solid: mp 179−181 °C (lit.[28] mp 179 °C).

Ethyl 5-Methoxy-1-methyl-2-phenylindole-3-carboxylate, 9a. Potassium hydroxide (0.18 g, 3.2 mmol) was added to a stirring solution of ethyl 5-hydroxy-2-phenylindole-3-carboxylate (**8a**) (0.30 g, 1.1 mmol) in DMSO (10 mL); the reaction was stirred for 0.5 h. Methyl iodide (0.68 g, 4.8 mmol) was added, and the reaction was stirred for 2 h. Water was added (20 mL), and the mixture was extracted with EtOAc (3 × 15 mL), washed with 1 M hydrochloric acid (4 × 15 mL) and water (2 × 15 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by flash column chromatography (light petroleum/EtOAc, 4:1) to give the title compound **9a** (0.20 g, 60%) as a colorless crystalline solid: mp 107−108 °C. Anal. ($C_{19}H_{19}NO_3$) C, H, N.

Ethyl 5-Methoxy-1-methyl-6-nitro-2-phenylindole-3-carboxylate, 10a. Ethyl 5-methoxy-1-methyl-2-phenylindole-3-carboxylate (**9a**) (0.56 g, 1.7 mmol), stirred in glacial acetic acid (7 mL), was cooled to −10 °C. A mixture of glacial acetic acid (4 mL) and nitric acid (1.5 mL) was added at −10 °C, and the reaction was stirred at room temperature for 2 h. The yellow suspension was poured onto an ice/water mixture. The reaction mixture was stirred in dichloromethane (30 mL) for 1 h. The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 30 mL); the combined extracts were washed with saturated sodium bicarbonate (3 × 40 mL) and water (2 × 40 mL), dried (Na_2SO_4), and evaporated under reduced pressure. Flash column chromatography (light petroleum/EtOAc, 7:3) gave the 6-nitro and 4-nitro compounds in the ratio 1:7. Ethyl 5-methoxy-1-methyl-6-nitro-2-phenylindole-3-carboxylate (66 mg, 11%) was a yellow crystalline solid: mp 167−168 °C. Anal. ($C_{19}H_{18}N_2O_5 \cdot 0.2H_2O$) C, H, N. Ethyl 5-methoxy-1-methyl-4-nitro-2-phenylindole-3-carboxylate (**10a**) (0.45 g, 75%) was a yellow crystalline solid: mp 154−155 °C. Anal. ($C_{19}H_{18}N_2O_5 \cdot 0.2H_2O$) C, H, N.

Ethyl 4-Amino-5-methoxy-1-methyl-2-phenylindole-3-carboxylate, 11a. To a stirred solution of ethyl 5-methoxy-1-methyl-4-nitro-2-phenylindole-3-carboxylate (**10a**) (1.47 g, 4 mmol) in EtOH (100 mL) was added tin powder (2.94 g, 24 mmol) in hydrochloric acid (3 M; 35 mL), and the mixture was heated under reflux for 2 h. After cooling, the solution was decanted and neutralized with saturated sodium bicarbonate (500 mL), water (100 mL) and dichloromethane (300 mL) were added, and the mixture was stirred vigorously overnight. The aqueous layer was removed and the organic layer washed with water (2 × 200 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash column chromatography (light petroleum/EtOAc, 7:3) to give the title compound **11a** (0.79 g, 59%) as a colorless crystalline solid: mp 125−126 °C. Anal. ($C_{19}H_{20}N_2O_3 \cdot 0.4H_2O$) C, H, N.

Ethyl 5-Methoxy-1-methyl-4,7-dioxo-2-phenylindole-3-carboxylate, 5y. Ethyl 4-amino-5-methoxy-1-methyl-2-phenylindole-3-carboxylate (**11a**) (0.21 g, 0.64 mmol) was stirred in acetone (30 mL). Potasium nitrosodisulfonate (0.36 g, 0.14 mmol) in dihydrogen phosphate buffer (0.3 M; 30 mL) was added, and reaction mixture was stirred at room temperature for 3 h. The excess acetone was removed in vacuo, water was added (40 mL), and the mixture was extracted with dichloromethane (2 × 20 mL). The combined extracts were washed with water (2 × 20 mL) and brine (2 × 20 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was purified by flash column chromatography (light petroleum/EtOAc, 1:1) to give the title compound (0.21 g, 96%) as an orange crystalline solid: mp 138−140 °C (EtOAc/light petroleum); UV (MeOH) 430 (log ϵ 3.12) and 326 (3.53) nm; IR (CH_2Cl_2) 2930, 1726, 1629, 1603, 1450, 1260, 1194, and 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.47 (3 H, m, ArH), 7.36 (2 H, m, ArH), 5.73 (1 H, s, 6-H), 4.19 (2 H, q, J = 7.1 Hz, CH_2), 3.85 (3 H, s, OMe), 3.78 (3 H, s, NMe), 1.16 (3 H, t, J = 7.1 Hz, Me); ^{13}C NMR ($CDCl_3$) δ 179.2, 175.5, 163.7, 160.3, 142.5, 130.3 (2 × CH), 129.6, 129.6 (CH), 128.6, 128.5 (2 × CH), 121.6, 114.8, 106.5, (CH), 61.0 (CH_2), 56.6 (OMe), 34.1 (Me), and 13.8

(Me); HRMS found (M^+) 339.1111, $C_{19}H_{17}NO_5$ requires M 339.1107. Anal. ($C_{19}H_{17}NO_5 \cdot 0.9H_2O$) C, H, N.

3-(Hydroxymethyl)-5-methoxy-1-methyl-2-phenylindole-4,7-dione, 5z. THF (5 mL) was added to $LiAlH_4$ (0.11 g, 3 mmol) under a nitrogen atmosphere. A solution of ethyl 4-amino-5-methoxy-1-methyl-2-phenylindole-3-carboxylate (**11a**) (0.20 g, 0.06 mmol) in THF (10 mL) was added to the stirring suspension. The reaction was stirred at room temperature for 2 h. The reaction was cooled to 0 °C; water (0.5 mL) followed by 15 mol % NaOH (1 mL) were added dropwise. Silica and EtOAc (40 mL) were then added, the reaction mixture was filtered through Celite, and the aqueous layer was removed. The organic layer was washed with water (2 × 15 mL) and brine (2 × 15 mL), dried ($MgSO_4$), and evaporated under reduced pressure, giving 4-amino-3-(hydroxymethyl)-5-methoxy-1-methyl-2-phenylindole (0.13 g, 76%) as a brown solid: mp 135−136 °C (EtOAc/light petroleum); IR (CH_2Cl_2) 3391, 3191, 2991, 1506, 1491, 1440, and 1276 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (3 H, m, ArH), 7.36 (2 H, m, ArH), 6.98 (1 H, d, J = 8.7 Hz, ArH), 6.70 (1 H, d, J = 8.7 Hz, ArH) 4.78 (2 H, s, CH_2), 3.91 (3 H, s, OMe), and 3.51 (3 H, s, NMe); ^{13}C NMR ($CDCl_3$) δ 140.6, 138.7, 134.7, 131.1, 130.8 (2 × CH), 128.4 (2 × CH), 128.3 (CH), 117.4, 111.9, 110.9 (CH), 98.6 (CH), 58.2 (OMe), 57.5 (CH_2), and 30.9 (Me); HRMS found (M^+) 282.1373, $C_{17}H_{18}N_2O_2$ requires M 282.1368. Anal. ($C_{17}H_{18}N_2O_2 \cdot 0.2H_2O$) C, H, N.

Potassium nitrosodisulfonate (1.95 g, 7 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 40 mL) was added to a stirring solution of the above compound (0.79 g, 2.8 mmol) in acetone (40 mL). The reaction mixture was stirred at room temperature for 1 h. The excess acetone was removed in vacuo, water (30 mL) was added, and the mixture was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with water (2 × 20 mL) and brine (2 × 20 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The crude product was recrystallized (EtOAc/light petroleum) giving the title compound **5z** (0.35 g, 65% yield over two steps) as a red crystalline solid: mp 206−208 °C (lit.[29] mp 150−152 °C); UV (MeOH) 450 (log ϵ 3.25) and 344 (3.48) nm; IR (CH_2Cl_2) 3519, 3421, 3053, 2986, 1639, 1593, 1491, 1265, and 1209 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (3 H, m, ArH), 7.30 (2 H, m, ArH), 5.71 (1 H, s, 6-H), 4.50 (2 H, s, CH_2) 3.98 (1 H, br s, OH), 3.85 (3 H, s, OMe), and 3.78 (3 H, s, NMe); ^{13}C NMR ($CDCl_3$) δ 179.2 (CO), 178.9 (CO), 160.0, 139.2, 130.6 (2 × CH), 129.9, 129.4 (CH), 128.8 (2 × CH), 128.3, 124.1, 122.2, 107.4 (CH), 56.6 (OMe), 56.1 (CH_2), and 34.0 (Me).

3-(Hydroxymethyl)-5-[(2-hydroxyethyl)amino]-1-methyl-2-phenylindole-4,7-dione, 5aa. Purple crystalline solid in 58% yield: mp 192−194 °C (dichloromethane); UV (MeOH) 524 (log ϵ 3.40) and 314 (4.21) nm; IR (CH_2Cl_2) 3365, 3309, 2934, 1669, 1583, 1506, 1435, 1214, and 1163 cm^{-1}; ^1H NMR (DMSO-*d*) δ 7.50 (5 H, m, ArH), 7.02 (1 H, t, J = 5.8 Hz, NH), 5.21 (1 H, s, 6-H), 4.86 (1 H, t, J = 5.7 Hz, OH, D_2O exch), 4.55 (1 H, t, J = 5.2 Hz, OH, D_2O exch), 4.37 (2 H, d, J = 5.2 Hz, CH_2OH), 3.75 (3 H, s, NMe), 3.58 (2 H, m, CH_2), and 3.19 (2 H, m, CH_2); ^{13}C NMR (DMSO-*d*) δ 178.9 (CO), 178.3 (CO), 149.4, 138.8, 131.4, 131.0 (2 × CH), 129.3 (CH), 129.0 (2 × CH), 122.5, 119.7, 111.6, 96.5 (CH), 58.8 (CH_2), 53.9 (CH_2), 45.3 (CH_2), and 34.2 (NMe); HRMS found (M^+) 326.1272, $C_{18}H_{18}N_2O_4$ requires M 326.1267. Anal. ($C_{18}H_{18}N_2O_4 \cdot 2.4H_2O$) C, H, N.

5-(Cyclopropylamino)-3-(hydroxymethyl)-1-methyl-2-phenylindole-4,7-dione, 5bb. Purple crystalline solid in 86% yield: mp 188−189 °C (EtOAc/light petroleum); UV (MeOH) 520 (log ϵ 3.30) and 318 (4.07) nm; IR (CH_2Cl_2) 3503, 3360, 2950, 1655, 1614, 1588, 1491, and 1178 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (3 H, m, ArH), 7.29 (2 H, m, ArH), 5.94 (1 H, br s, NH), 5.62 (1 H, s, 6-H), 4.50 (2 H, d, J = 7.1, CH_2OH), 4.00 (1 H, t, J = 7.1 Hz, OH), 3.81 (3 H, s, NMe), 2.49 (1 H, m, C*H*NH), 0.88 (2 H, m, CH_2), and 0.65 (2 H, m, CH_2); ^{13}C NMR ($CDCl_3$) δ 180.1 (CO), 179.0 (CO), 149.2, 137.4, 132.1, 130.7 (2 × CH), 129.2 (CH), 128.8 (2 × CH), 123.3, 120.4, 99.6 (CH), 56.3 (CH_2OH), 34.0 (NMe), 24.3 (CH), and 7.1 (2 × CH); HRMS found (M^+) 322.1322, $C_{19}H_{18}N_2O_3$ requires M 322.1317. Anal. ($C_{19}H_{18}N_2O_3$) C, H, N.

3-(Hydroxymethyl)-5-(2-methylaziridin-1-yl)-1-methyl-2-phenylindole-4,7-dione, 5cc. Red crystalline solid in 79% yield: mp 163−164 °C (EtOAc/light petroleum) (lit.[29] mp 147−149 °C); UV (MeOH) 478 (log ϵ 3.32) and 312 (4.14) nm; IR (CH_2Cl_2) 3370, 3047, 2976, 1619, 1578, 1486, 1450, and 1260 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (3 H, m, ArH), 7.29 (2 H, m, ArH), 5.82 (1 H, s, 6-H), 4.52 (2 H, d, J = 7.3 Hz, CH_2OH), 4.19 (1 H, t, J = 7.2 Hz, OH), 3.78 (3 H, s, NMe), 2.30 (1 H, m, CH), 2.09 (2 H, m, CH_2), and 1.44 (3 H, d, J = 5.5 Hz, Me); ^{13}C NMR ($CDCl_3$) δ 181.2 (CO), 179.0 (CO), 157.2, 138.8, 130.6 (2 × CH), 130.2, 129.3 (CH), 128.8 (2 × CH), 128.6, 123.9, 122.8, 116.8 (CH), 56.2 (CH_2OH), 36.3 (CH), 34.7 (CH_2), 33.9 (NMe), and 17.7 (Me).

Ethyl 3-Amino-3-(4-biphenylyl)propenoate, 7b. Prepared using a modified procedure for enamine formation from 1,3-dicarbonyl compounds.[33] Ethyl (4-phenylbenzoyl)acetate (3.59 g, 13.4 mmol), ammonium acetate (10.75 g, 0.138 mol), benzene (110 mL), and acetic acid (22 mL) were heated under reflux using Dean−Stark conditions for 24 h. The cooled reaction mixture was washed with saturated sodium hydrogen carbonate (3 × 75 mL); the benzene layer was dried (Na_2SO_4) and concentrated to give the title compound (3.48 g, 97%) as an off-white crystalline solid: mp 90−92 °C (EtOAc/hexane). Anal. ($C_{17}H_{17}NO_2$) C, H, N.

Ethyl 2-(4-Biphenylyl)-5-hydroxyindole-3-carboxylate, 8b. 1,4-Benzoquinone (1.71 g, 15.8 mmol) and ethyl 3-amino-3-(4-biphenylyl)propenoate (**7b**) (3.5 g, 9.8 mmol) were heated under reflux for 1 h in acetic acid (50 mL). After cooling, the crude product was precipitated out with light petroleum and filtered off. The crude product was purified by column chromatography (EtOAc) and recrystallized (EtOAc) to yield a colorless solid (4.21 g, 90%): mp 260−262 °C. Anal. ($C_{23}H_{19}NO_3 \cdot 0.1H_2O$) C, H, N.

Ethyl 2-(4-Biphenylyl)-5-methoxy-1-methylindole-3-carboxylate, 9b. To a stirring solution of indole **8b** (2.61 g, 7.3 mmol) in DMSO (20 mL) was added potassium hydroxide (1.64 g, 29.3 mmol). After 30 min, iodomethane (4.15 g, 29.2 mmol) was added dropwise. The mixture was stirred at room temperature for 3 h. The crude mixture was diluted with EtOAc and washed thoroughly with 1 M hydrochloric acid. The organic layer was separated, dried ($MgSO_4$), and concentrated. The crude material was purified by column chromatography (petroleum ether/EtOAc, 4:1) and recrystallized (EtOAc/hexane) to yield the title compound (2.39 g, 85%) as a colorless crystalline solid: mp 171−173 °C. Anal. ($C_{25}H_{23}NO_3$) C, H, N.

Ethyl 2-(4-Biphenylyl)-5-methoxy-1-methyl-4-nitroindole-3-carboxylate, 10b. To a solution of indole **9b** (1.41 g, 3.7 mmol) in acetic acid (15 mL), cooled to −10 °C, was added a mixture of concentrated nitric acid (2 mL) and acetic acid (7.5 mL). The mixture was stirred at room temperature for 2 h. A yellow suspension was obtained which was poured onto an ice/water mixture; the crystals obtained were filtered off and dried. The crude product was purified by column chromatography (light petroleum/EtOAc, 1:1) and recrystallized (light petroleum/EtOAc) to yield the title compound (1.22 g, 77%) as a pale yellow crystalline solid: mp 277−279 °C.

Ethyl 4-Amino-2-(4-biphenylyl)-5-methoxy-1-methylindole-3-carboxylate, 11b. To a suspension of indole **10b** (0.76 g, 1.76 mmol) in EtOH (44 mL) were added tin powder (0.94 g, 7.90 mmol) and hydrochloric acid (3 M; 13 mL). The mixture was heated under reflux for 30 min. Upon cooling the solution was decanted from the excess tin and neutralized with saturated aqueous sodium hydrogen carbonate. The suspension obtained was added to an equal volume of water. The precipitate and aqueous layer were stirred overnight with dichloromethane and filtered through Celite and the layers separated. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (dichloromethane/EtOAc, 7:3) and recrystallized (light petroleum/EtOAc) to yield the title compound as a pale

yellow crystalline solid (0.71 g, 97%): mp 204−205 °C. Anal. ($C_{25}H_{24}N_2O_3$) C, H, N.

Ethyl 2-(4-Biphenylyl)-5-methoxy-1-methyl-4,7-dioxoindole-3-carboxylate, 5dd. To a solution of indole **11b** (0.161 g, 0.4 mmol) in acetone (15 mL) was added a solution of potassium nitrosodisulfonate (0.54 g, 2.0 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 15 mL). The mixture was stirred at room temperature for 1 h. The excess acetone was removed in vacuo. The resulting residue was extracted with dichloromethane and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (EtOAc/dichloromethane, 1:1) and recrystallized (dichloromethane/light petroleum) to yield the title compound as an orange solid (0.14 g, 87%): mp 204−205 °C; UV (MeOH) 434 (log ϵ 3.19) and 278 (4.68) nm; IR (KBr) 3063, 2971, 2940, 1731, 1685, 1639, and 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.70 (2 H, m, ArH), 7.65 (2 H, m, ArH), 7.45 (4 H, m, ArH), 7.38 (1 H, m, ArH), 5.73 (1 H, s, 6-H), 4.22 (2 H, q, J = 7.1 Hz, CH_2Me), 3.84 (3 H, s, OMe), and 1.20 (3 H, t, J = 7.1 Hz, CH_2*Me*); ^{13}C NMR ($CDCl_3$) δ 179.2, 175.5, 163.8, 160.3, 142.4, 142.2, 140.1, 130.7 (CH), 129.6, 128.9 (CH), 127.9 (CH), 127.3, 127.2 (CH), 127.1 (CH), 121.6, 114.9, 106.5 (CH), 61.1 (CH_2), 56.6 (OMe), 43.2 (NMe), and 13.8 (Me); HRMS found (M^+) 415.1420, $C_{25}H_{21}NO_5$ requires M 415.1419. Anal. ($C_{25}H_{21}NO_5 \cdot 0.1H_2O$) C, H, N.

2-(4-Biphenylyl)-3-(hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione, 5ee. To a suspension of lithium aluminum hydride (0.41 g, 10.8 mmol) in THF (50 mL) at 0 °C was added a solution of aminoindole **11b** (1.12 g, 2.7 mmol) in THF (25 mL). The reaction was allowed to warm to room temperature and stirred for 30 min. The mixture was cooled to 0 °C and quenched by the addition of water (0.5 mL), sodium hydroxide (1 M; 0.5 mL), and silica gel (5 g). The granular precipitate was filtered off through a pad of Celite. The filtrate was dried ($MgSO_4$) and concentrated in vacuo to give the alcohol which was used directly in the next step without purification or characterization.

To a solution of the above compound in acetone (120 mL) was added a solution of potassium nitrosodisulfonate (4.2 g, 15.7 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 120 mL). The mixture was stirred at room temperature for 1 h. The excess acetone was removed in vacuo. The resulting residue was extracted with dichloromethane and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (EtOAc/dichloromethane, 3:7) and recrystallized (dichloromethane/light petroleum) to yield the title compound as an orange/red solid (0.87 g, 87%): mp 200−201 °C; UV (MeOH) 446 (log ϵ 3.35), 350 (3.56), and 280 (4.69) nm; IR (KBr) 3416, 3073, 2950, 1644, and 1598 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72 (2 H, m, ArH), 7.64 (2 H, m, ArH), 7.44 (5 H, m, ArH), 5.74 (1 H, s, 6-H), 4.57 (2 H, d, J = 7.3 Hz, CH_2OH), 4.05 (1 H, t, J = 7.3 Hz, OH), 3.87 (3 H, s, OMe), and 3.85 (3 H, s, NMe); ^{13}C NMR ($CDCl_3$) δ 179.2 (CO), 178.9 (CO), 160.0, 142.3, 140.0, 139.0, 131.1, 131.0 (CH), 130.0, 128.9 (CH), 127.9 (CH), 127.3 (CH), 127.1 (CH), 124.2, 122.2, 107.4 (CH), 56.6 (OMe), 56.1 (CH_2), and 34.1 (Me); HRMS found (M^+) 373.1314, $C_{23}H_{19}NO_4$ requires M 373.1314. Anal. ($C_{23}H_{19}NO_4 \cdot 0.4H_2O$) C, H, N.

9-(Hydroxymethyl)-7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6a. Prepared as previously described.[22]

7-(2-Methylaziridin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6b. Prepared as previously described.[23]

7-(Pyrrolidin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6c. To a stirred solution of 7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione (**6a**) (0.044 g, 0.20 mmol) in DMF (4 mL) was added pyrrolidine (0.170 g, 24 mmol), and the mixture was stirred at room temperature overnight. Water was added and the mixture extracted with dichloromethane. The dichloromethane extracts were washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and the residue purified by column chromatography (EtOAc/light petroleum, 7:3, to EtOAc) and recrystallized (dichloromethane/ether) to give the title compound as a purple solid (0.036 g, 74%): mp 260−262 °C; UV (MeOH) 546 (log ϵ 3.50), 330 (4.12), 248 (4.36), and 224 (4.28) nm; IR (KBr) 2973, 2875, 1677, 1624, and 1558 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.17 (1 H, s, 9-H), 5.16 (1 H, s, 6-H), 4.22 (2 H, t, J = 7.2 Hz, 3-CH_2), 3.61 (4 H, br s, NCH_2), 2.80 (2 H, t, J = 7.4 Hz, 1-CH_2), 2.54 (2 H, quintet, J = 7.4 Hz, 2-CH_2), and 1.94 (4 H, m, CH_2); ^{13}C NMR ($CDCl_3$) δ 180.2 (CO), 177.1 (CO), 149.9, 142.5, 128.4, 126.9, 100.5, 98.9, 51.2 (CH_2), 46.5 (CH_2), 27.8 (CH_2), 25.3 (CH_2), and 13.4; HRMS found (M^+) 256.1212, $C_{15}H_{16}N_2O_2$ requires M 256.1212. Anal. ($C_{15}H_{16}N_2O_2 \cdot 0.2H_2O$) C, H, N.

9-Formyl-7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6d. Prepared as previously described.[22]

9-(Hydroxymethyl)-7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6e. Prepared as previously described.[22]

9-(Hydroxymethyl)-7-(2-methylaziridin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6f. To a stirred solution of 9-(hydroxymethyl)-7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione (**6e**) (0.108 g, 0.44 mmol) in DMF (5 mL) was added 2-methylaziridine (0.81 g, 14.0 mmol), and the mixture was stirred at room temperature overnight. The reaction was shown to have gone to completion by TLC. The excess aziridine was blown off by bubbling nitrogen directly into the mixture. Water was added and the mixture extracted with dichloromethane. The dichloromethane extracts were washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and the residue purified by column chromatography (EtOAc/dichloromethane, 1:1) and recrystallized (dichloromethane/light petroleum) to give the title compound as a red solid (0.081 g, 68%): mp 133−135 °C; UV (MeOH) 486 (log ϵ 3.28), 362 (3.56), 312 (4.19), and 232 (4.32) nm; IR (KBr) 3572, 3375, 3276, 2980, 2927, 1624, and 1591 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.72 (1 H, s, 6-H), 4.60 (2 H, d, J = 6.9 Hz, 10-CH_2), 4.20 (2 H, t, J = 7.2 Hz, 3-CH_2), 4.03 (1 H, t, J = 6.9 Hz, OH), 2.85 (2 H, t, J = 7.2 Hz, 1-CH_2), 2.55 (2 H, m, 2-CH_2), 2.27 (1 H, m, azir-CH), 2.10 (2 H, m, azir-CH_2), and 1.43 (3 H, d, J = 5.5 Hz, NCH*Me*); ^{13}C NMR ($CDCl_3$) δ 181.0 (CO), 177.9 (CO), 157.8, 141.3, 127.4, 125.8, 117.8, 115.5 (CH), 56.6 (CH_2), 46.9 (CH_2), 36.3 (CH), 34.6 (CH_2), 27.4 (CH_2), 22.5 (CH_2), and 17.6 (Me); HRMS found (M^+) 272.1161, $C_{15}H_{16}N_2O_3$ requires M 272.1161. Anal. ($C_{15}H_{16}N_2O_3 \cdot H_2O$) C, H, N.

9-(Hydroxymethyl)-7-(pyrrolidin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6g. To a stirred solution of 9-(hydroxymethyl)-7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione (**6e**) (0.07 g, 0.28 mmol) in DMF (4 mL) was added pyrrolidine (0.17 g, 24 mmol), and the mixture was stirred at room temperature overnight. Water was added and the mixture extracted with dichloromethane. The dichloromethane extracts were washed with water and dried (Na_2SO_4). The solvent was removed in vacuo and the residue purified by column chromatography (EtOAc) and recrystallized (dichloromethane/ether) to give the title compound as a purple solid (0.07 g, 86%): mp 228−230 °C; UV (MeOH) 546 (log ϵ 3.56), 330 (4.16), 248 (4.38), and 224 (4.29) nm; IR (KBr) 3289, 2966, 2861, 1657, 1618, and 1558 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.17 (1 H, s, 6-H), 4.56 (2 H, d, J = 6.8 Hz, 10-CH_2), 4.20 (2 H, t, J = 7.2 Hz, 3-CH_2), 4.08 (1 H, t, J = 6.8 Hz, OH), 3.68 (4 H, br s, NCH_2), 2.79 (2 H, t, J = 7.2 Hz, 1-CH_2), 2.52 (2 H, quintet, J = 7.2 Hz, 2-CH_2), and 1.95 (4 H, m, CH_2); ^{13}C NMR ($CDCl_3$) δ 181.9 (CO), 176.8 (CO), 149.7, 139.5, 129.0, 124.4, 116.7, 100.7 (CH), 56.7 (CH_2), 51.4 (CH_2), 46.6 (CH_2), 27.5 (CH_2), 25.2 (CH_2), and 22.3 (CH_2); HRMS found (MH^+) 287.1396, $C_{13}H_{14}N_2O_3$ + H requires M 287.1395. Anal. ($C_{16}H_{18}N_2O_3 \cdot 0.1H_2O$) C, H, N.

9-[(Carbamoyloxy)methyl]-7-methoxy-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6h. Prepared as previously described.[22]

7-(Aziridin-1-yl)-9-[(carbamoyloxy)methyl]-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6i. Prepared as previously described.[23]

9-[(Carbamoyloxy)methyl]-7-(2-methylaziridin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6j. Prepared as previously described.[23]

9-[(Carbamoyloxy)methyl]-7-(pyrrolidin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6k. A solution of the carbamate **6h** (0.061 g, 0.21 mmol), pyrrolidine (3.4 g, 47.9 mmol), and DMF (5 mL) was stirred at room temperature for 16 h. The mixture was evaporated and the residue recrystallized (MeOH/light petroleum) to give the title compound (0.057 g, 82%) as a purple solid: mp >300 °C; UV (MeOH) 538 (log ϵ 3.52), 326 (4.09), 248 (4.32), and 222 (4.23) nm; IR (KBr) 3440, 3355, 3210, 2934, 2855, 1690, 1664, 1618, and 1552 cm^{-1}; ^1H NMR (DMSO-*d*) δ 6.44 (2 H, br s, NH_2), 5.04 (1 H, s, 6-H), 5.01 (2 H, s, 10-CH_2), 4.09 (2 H, m, 3-CH_2), 3.51 (4 H, br s, NCH_2), 2.78 (2 H, m, 1-CH_2), 2.44 (2 H, m, 2-CH_2), and 1.86 (4 H, m, CH_2); ^{13}C NMR (DMSO-*d*) δ 180.5, 175.9, 157.2, 149.7, 142.3, 127.9, 123.1, 111.6, 100.0 (CH), 57.8 (CH_2), 51.4 (CH_2), 46.9 (CH_2), 27.4 (CH_2), and 23.0 (CH_2); HRMS found (MH^+) 330.1454, $C_{17}H_{19}N_3O_4$ + H requires M 330.1454. Anal. ($C_{17}H_{19}N_3O_4 \cdot 0.4H_2O$) C, H, N.

9-[(Carbamoyloxy)methyl]-7-(piperidin-1-yl)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6l. A solution of the carbamate **6h** (0.061 g, 0.21 mmol), piperidine (3.4 g, 40.5 mmol), and DMF (5 mL) was stirred at room temperature for 16 h. The mixture was evaporated and the residue chromatographed (EtOAc) and recrystallized (dichloromethane/light petroleum) to give the title compound (0.049 g, 73%) as a dark purple solid: mp 231−232 °C; UV (MeOH) 534 (log ϵ 3.52), 328 (4.11), and 238 (4.35) nm; IR (KBr) 3421, 3309, 3210, 2940, 2855, 1749, 1710, 1670, 1618, and 1552 cm^{-1}; ^1H NMR (DMSO-*d*) δ 5.47 (1 H, s, 6-H), 5.21 (2 H, s, 10-CH_2), 4.69 (2 H, br s, NH_2), 4.20 (2 H, m, 3-CH_2), 3.40 (4 H, m, NCH_2), 2.89 (2 H, m, 1-CH_2), 2.53 (2 H, m, 2-CH_2), and 1.68 (6 H, m, CH_2); ^{13}C NMR (DMSO-*d*) δ 180.7, 177.8, 156.8, 155.2, 143.3, 127.3, 125.2, 111.3, 107.8 (CH), 58.5 (CH_2), 51.1 (CH_2), 46.8 (CH_2), 27.3 (CH_2), 25.8 (CH_2), 24.3 (CH_2), and 23.0 (CH_2); HRMS found (MH^+) 344.1610, $C_{18}H_{21}N_3O_4$ + H requires M 344.1610. Anal. ($C_{18}H_{21}N_3O_4 \cdot 0.5H_2O$) C, H, N.

9-[(Carbamoyloxy)methyl]-7-(*N*-cyclopropylamino)-1,2-dihydro-3*H*-pyrrolo[1,2-*a*]indole-5,8-dione, 6m. A solution of the carbamate **6h** (0.057 g, 0.19 mmol), cyclopropylamine (3.3 g, 57.8 mmol), and DMF (5 mL) was stirred at room temperature for 16 h. The mixture was evaporated and the residue chromatographed (EtOAc/2-propanol, 9:1) and recrystallized (MeOH/light petroleum) to give the title compound (0.050 g, 81%) as a purple solid: mp 257−258 °C; UV (MeOH) 524 (log ϵ 3.29), 316 (3.87), and 242 (4.32) nm; IR (KBr) 3427, 3348, 3309, 3204, 2980, 1703, 1664, 1611, and 1591 cm^{-1}; ^1H NMR (DMSO-*d*) δ 7.30 (1 H, d, J = 2.9 Hz, NH), 6.44 (2 H, br s, NH_2), 5.30 (1 H, s, 6-H), 5.00 (2 H, s, 10-CH_2), 4.10 (2 H, m, 3-CH_2), 2.78 (2 H, m, 1-CH_2), 2.42 (3 H, m, 2-CH_2, cycloprop-CH), 0.74 (2 H, m, cycloprop-CH_2), and 0.59 (2 H, m, cycloprop-CH_2); ^{13}C NMR (DMSO-*d*) δ 178.6, 177.1, 157.1, 151.1, 142.6, 128.4, 122.5, 111.6, 96.8 (CH), 57.5 (CH_2), 47.1 (CH_2), 27.4 (CH_2), 24.9 (CH), 22.9 (CH_2), and 6.8 (CH_2); HRMS found (M^+) 315.1219, $C_{16}H_{17}N_3O_4$ requires M 315.1219. Anal. ($C_{16}H_{17}N_3O_4 \cdot 0.4H_2O$) C, H, N.

Electrochemical Measurements. Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (Aldrich, 99%) was dried under vacuum at 70 °C. Ferrocene (Aldrich, 97%) was resublimed. Dimethylformamide (DMF) (Aldrich, 99.8%) was dried over 4-Å molecular sieves.

For each of the indolequinones studied, cyclic voltammograms were recorded over a range of potential sweep rates from 25 to 500 mV s^{-1}, using an E.G. & G. Princeton Applied Research model 273 potentiostat, controlled by an IBM-compatible PC using E.G. & G. Princeton Applied Research model 270/250 research electrochemistry software. A three-electrode cell was employed, with a silver quasi-reference electrode and a platinum disk working electrode. The working electrode was pretreated by polishing with 3-μm diamond paste, followed by anodization and then cathodization for 2−3 min each in 0.1 M H_2SO_4, before rinsing with deionized water and drying. Solutions for voltammetry were all 1 mM in the indolequinone, approximately 1 mM in ferrocene (added as an internal reference), and 0.1 M in Bu_4NBF_4 supporting electrolyte in DMF. Oxygen was removed from the solutions by purging with nitrogen. All measurements were performed at 21 ± 2 °C.

Biology. NADH and MTT were obtained from Sigma Chemical Co., St. Louis, MO. All other reagents were at least of analytical grade.

1. Cell Culture. H460 and H596 cells were grown in minimum essential medium (MEM) with Earle's salts, nonessential amino acids, L-glutamine, and penicillin/streptomycin and supplemented with 10% fetal bovine serum. Cell culture medium and supplements were obtained from Life Technologies, Inc., Grand Island, NY. The cells were incubated at 37 °C under a humidified atmosphere containing 5% CO_2.

2. Human Recombinant NQO1. Recombinant human NQO1 from *Escherichia coli* was expressed, purified, and characterized as previously described.[34]

3. HPLC Analysis. Reduction of the indolequinones was followed by HPLC using an Alltech C18 (5 μm, 250 mm × 4.6 mm) column with a Shimadzu HPLC system (SCL-6A controller, SPD-6AV UV−vis detector, two LC-6A pumps, and a C-R3A integrator). The solvent program used a linear gradient of 5−80% B over 10 min, 80% B for 5 min, then 80−5% B over 5 min (solution A, 10 mM potassium phosphate buffer, pH 6.0; solution B, methanol). Reactions were run in 25 mM Tris-HCl (pH 7.4) containing 200 μM NADH, 50 μM indolequinone, and recombinant human NQO1. NADH oxidation and quinone removal were quantified at 340 nm following 30-min incubations at 22 °C.

4. Cytotoxicity Assay. Cytotoxicity was determined using the MTT colorimetric assay.[35] Cells were plated in 96-well plates at a density of (1−2) × 10^4 cells/mL and allowed to attach overnight (16 h). Indolequinone solutions were applied in serumless media for 2 h. Indolequinone solutions were removed and replaced with complete media (with serum), and the 96-well plates were incubated for 5−7 days. MTT (50 μg) was added, and the cells were incubated for another 4 h. Media/MTT solutions were removed carefully by aspiration, the MTT formazan crystals were dissolved in 100 μL of DMSO, and absorbance was determined on a plate reader at 550 nm. IC_{50} values (concentration at which cell survival equals 50% of control) were determined from semilog plots of percent of control versus concentration.

5. Inactivation of NQO1 by Indolequinones. The ability of the indolequinones to inactivate NQO1 was determined using a previously reported procedure[31] with some modifications. Inactivation was determined spectrophotometrically following 30-min incubations at 22 °C in the presence (+) or absence (−) of NADH. Incubations (0.5 mL) contained (±) 100 μM NADH, 10 μM indolequinone, and 1 μg of recombinant hNQO1 in 25 mM Tris-HCl (pH 7.4) + 2 mg/mL Tween-20. After 30 min, reactions were stopped with 4 volumes (2 mL) of cold 25 mM Tris-HCl (pH 7.4) + 250 mM sucrose. Enzyme activity remaining was determined using a standard NQO1 activity assay.[2] The NQO1 assay (1 mL) contained a 50-μL sample from the incubation reaction, 40 μM 2,6-dichlorophenol-indophenol (DCPIP), and 200 μM NADH in 25 mM Tris-HCl (pH 7.4) + 0.7 mg/mL bovine serum albumin (BSA). DCPIP reduction was followed for 30 s at 600 nm. NQO1 activity from (+) NADH incubations was compared to (−) NADH controls for calculations of enzyme activity remaining (%).

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Supporting Information Available: Spectroscopic data for intermediates (4 pages). Ordering information is given on any current masthead page.

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