Indolequinone Antitumor Agents: Correlation between Quinone Structure and Rate of Metabolism by Recombinant Human NAD(P)H:Quinone Oxidoreductase. Part 2[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. Indolequinones were selected for study on the basis of the X-ray crystal structure of the human enzyme, and were designed to probe the effect of substituents particularly at N-1. 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, and that groups larger than methyl at N-1 are clearly tolerated. Compounds with a leaving group at the 3-indolyl methyl position generally inactivated the enzyme. The toxicity toward human colon carcinoma cells with either no detectable activity (BE-WT) or high NQO1 activity (BE-NQ) was also studied in representative quinones. The most toxic compounds were those with a leaving group at the C-3 position; these compounds were 1.1– 5.3-fold more toxic to the BE-NQ than the BE-WT cells.

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] NQO1 catalyzes the 2-electron reduction of quinones and can protect cells against the toxic effects of quinones.[4] However, NQO1 is also involved in the reductive activation of anticancer agents such as mitomycin C (MMC) which operate by the so-called bioreductive mechanism,[5–7] and a clear correlation between NQO1 activity and MMC sensitivity in human lung and breast cancer cell lines has been demonstrated.[8] The importance of NQO1 in the bioactivation of other cytotoxic quinones such as EO9,[9–11] azidiridinylbenzoquinones such as MeDZQ,[8,12] and the novel cyclopropamitosenes[13] is now well established, and the subject has been reviewed recently.[14,15]

We recently reported the results of a study designed to correlate quinone structure with the rate of metabolism by NQO1 and toxicity toward human tumor cell lines,[1] and NQO1 continues to generate interest because of its elevated levels in many tumors and tumor cell lines.[6,16] Thus, other researchers have studied the metabolism of quinones by NQO1,[17–20] and the induction and inhibition of the enzyme.[21–23] Advances have also been made in the immunohistochemical detection of NQO1 in normal and tumor tissue,[24–26] in understanding NQO1 gene expression,[27–29] and the polymorphism, resulting from a C to T base change at position 609, that occurs.[30,31]

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Recently the 3-dimensional structure of human NQO1 has been solved by two research groups, and resolved to 2.3 Å[32] and 1.7 Å,[33] respectively. The structure of the complex of enzyme with duroquinone has also been solved,[33] and the studies highlight the structural changes that are necessary for control of access to the catalytic site that is required by the ping-pong mechanism in which NAD[P]H enters the catalytic site and reduces the flavin. NAD[P] leaves, thereby allowing the substrate to enter and be subsequently reduced by FADH. With the structure of the human enzyme available, molecular modeling studies will become important for the design of new quinone substrates; to date most studies have used a model of the human enzyme based on its ca. 85% homology to the previously determined structure of the rat enzyme.[17,32,34–36]

In continuation of our own interest in the metabolism of quinones by NQO1,[1,37] we now report the results of a further study on a range of indolequinones **2–35**.
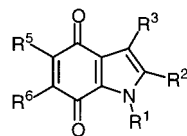
Results and Discussion

Most of the compounds in our previous study were based on the general *N*-methylindolequinone structure **1**.[1] The main conclusions from the work were that, in general, compounds with an electron-withdrawing substituent at C-3 were better substrates. Hence, the esters **1a** and **1e** were metabolized faster than the corresponding hydroxymethyl compounds **1b** and **1f**. A large substituent such as phenyl is preferred at C-2, the 2-phenyl quinones **1e** and **1f** being metabolized faster than the corresponding 2-methyl compounds **1a** and **1b**. Compounds with an amino substituent at C-5 were poor substrates (e.g., **1c**), even with the preferred phenyl substituent at C-2 (e.g., **1g**), with the exception of some aziridines which were generally better substrates (see below). Finally compounds containing a CH₂X substitu-

Table 1. Electrochemical Reduction Potentials^a (DMF) (versus Ferrocene) of Indolequinones and Their Metabolism by Recombinant Human NQO1

	compd	R^1	R^2	R^3	R^5	R^6	E_{redox} (V vs Fc)	metabolism (μmol min^{-1} mg^{-1}), NADH oxid	% enzyme activity remaining
1	**1a**	Me	Me	CO_2Et	OMe	H	1.32^b	14.3 ± 4.9^b	100^b
2	**1b**	Me	Me	CH_2OH	OMe	H	1.28^b	1.25 ± 0.03^b	96 ± 4^b
3	**1c**	Me	Me	CH_2OH	NMe_2	H	1.40^b	0.46 ± 0.04^b	92 ± 4^b
4	**1d**	Me	Me	CH_2OAc	OMe	H	1.36^b	$nd^{b,d}$	0^b
5	**1e**	Me	Ph	CO_2Et	OMe	H	1.29^b	23.4 ± 2.3^b	
6	**1f**	Me	Ph	CH_2OH	OMe	H	1.23^b	11.0 ± 0.9^b	
7	**1g**	Me	Ph	CH_2OH	NMepip^c	H		0.15 ± 0.06	
8	**2**	H	Me	COMe	OMe	H		29.8 ± 2.6	
9	**3**	H	Me	CHMeOH	OMe	H		27.7 ± 3.9	
10	**4**	n-Pr	Me	CO_2Et	OMe	H	1.28	77.6 ± 15.4	
11	**5**	n-Pr	Me	CH_2OH	OMe	H	1.24	1.3 ± 0.1	
12	**6**	c-Pr	Me	CO_2Et	OMe	H	1.23	36.6 ± 3.3	
13	**7**	c-Pr	Me	CH_2OH	OMe	H	1.30	14.0 ± 2.4	
14	**8**	Bn	Et	$CO2Me$	OMe	H	1.29	9.3 ± 0.9	
15	**9**	Bn	Et	CH_2OH	OMe	H	1.25	0.9 ± 0.2	
16	**10**	$CH_2CH_2R^e$	Me	CO_2Et	OMe	Me		39.4 ± 2.4	
17	**11**	$CH_2CH_2R^e$	Me	CH_2OH	OMe	Me		7.6 ± 1.3	
18	**12**	Ph	Me	CO_2Et	OMe	H	1.31	16.4 ± 2.2	
19	**13**	Ph	Me	CH_2OH	OMe	H	1.26	6.0 ± 0.8	
20	**14**	$4\text{-}FC_6H_4$	Me	CO_2Et	OMe	H	1.29	3.9 ± 1.0	
21	**15**	$4\text{-}FC_6H_4$	Me	CH_2OH	OMe	H	1.35	9.2 ± 0.6	
22	**16**	Me	H	CH_2OH	OMe	H		13.6 ± 3.5	96
23	**17**	Me	Ar^f	CO_2Et	OMe	H	1.28	0.23 ± 0.05	
24	**18**	Me	Ar^f	CH_2OH	OMe	H	1.22	4.0 ± 0.7	
25	**19**	Me	Me	CHMeOH	OMe	H	1.23	10.4 ± 2.4	
26	**20**	Me	Me	CHMeOH	R^e	H		0.27 ± 0.05	
27	**21**	Me	H	$CH_2O(4\text{-}NO_2C_6H_4)$	OMe	H		0.29 ± 0.01	52
28	**22**	Me	Me	CH_2OPh	OMe	H		0.28 ± 0.02	
29	**23**	Me	Me	$CH_2O(4\text{-}AcNHC_6H_4)$	OMe	H		0.38 ± 0.04	
30	**24**	Me	Me	$CH_2O(2\text{-}MeO\text{-}5\text{-}NO_2C_6H_3)$	OMe	H		0.05 ± 0.03	13
31	**25**	Me	Me	$CH_2O(2\text{-}MeO\text{-}4\text{-}NO_2C_6H_3)$	OMe	H		0.05 ± 0.01	
32	**26**	Me	Me	$CH_2O(2\text{-}F\text{-}4\text{-}NO_2C_6H_3)$	OMe	H		0.05 ± 0.02	0
33	**27**	Me	Me	$CH_2OCOCH_2(4\text{-}PhC_6H_4)$	OMe	H		0.09 ± 0.07	0
34	**28**	Me	Me	$CH_2OCONHCH_2CO_2Et$	OMe	H		0.11 ± 0.04	19
35	**29**	Me	Me	$CH_2SCSOEt$	OMe	H		1.1 ± 0.3	
36	**30**	Me	Me	CO_2Me	OMe	Me	1.27	13.7 ± 3.9	
37	**31**	Me	Me	CH_2OH	OMe	Me	1.25	3.8 ± 1.1	
38	**32**	n-Pr	Me	CO_2Et	OMe	Me	1.35	18.6 ± 4.8	
39	**33**	n-Pr	Me	CH_2OH	OMe	Me		1.7 ± 0.1	
40	**34**	Ph	Me	CO_2Et	OMe	Me	1.26	7.9 ± 2.6	
41	**35**	Ph	Me	CH_2OH	OMe	Me	1.22	3.0 ± 1.2	

^{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 50, 100, 200, 300, 400, and 500 mV s^{-1}. E_{pc} = cathodic peak potential. E_{pa} = anodic peak potential. ^{b} Reference 1. ^{c} NMepip = 4-methylpiperazin-l-yl. ^{d} nd = not detected. ^{e} R = 4-morpholinyl. ^{f} Ar = 2-naphthyl.

ent (X = leaving group) at C-3 (e.g., **1d**) were not metabolized by NQO1, and are possibly inhibitors of the enzyme. The inhibition of NQO1 by such indolequinones is the subject of a parallel study,[38] and the results will be reported separately. The metabolism data for compounds **1a**–**1g** is summarized in Table 1 (entries 1–7).
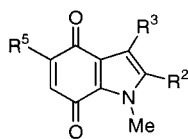

1a R^2 = Me, R^3 = CO_2Et, R^5 = OMe
1b R^2 = Me, R^3 = CH_2OH, R^5 = OMe
1c R^2 = Me, R^3 = CH_2OH, R^5 = NMe_2
1d R^2 = Me, R^3 = CH_2OAc, R^5 = OMe
1e R^2 = Ph, R^3 = CO_2Et, R^5 = OMe
1f R^2 = Ph, R^3 = CH_2OH, R^5 = OMe
1g R^2 = Ph, R^3 = CH_2OH, R^5 = NMe-piperazinyl
1h R^2 = Ph, R^3 = CH_2OH, R^5 = Me-aziridinyl

The present study builds on our previous results,[1] and on a consideration of the structure of the human enzyme.[33] This structure was not available at the time of our original study, although computer molecular modeling studies based mainly on the rat enzyme do rationalize some of the observed substituent effects on the rates of indolequinone metabolism by hNQO1. For example, one model suggests that both the N-1 and C-2 positions of indolequinones are oriented toward the active site entrance,[17] hence explaining the experimental observation that indolequinones with a large substituent such as a phenyl ring at C-2 are good substrates for the enzyme. The modeling also suggested that large substituents might also be accommodated at N-1, although there were no experimental data to support this. Our own work has significantly benefited from the recently determined crystal structure of the complexes of human NQO1 with 5-aziridinylindolequinones such as **1h** and EO9.[39] Although these two indolequinones

Scheme 1
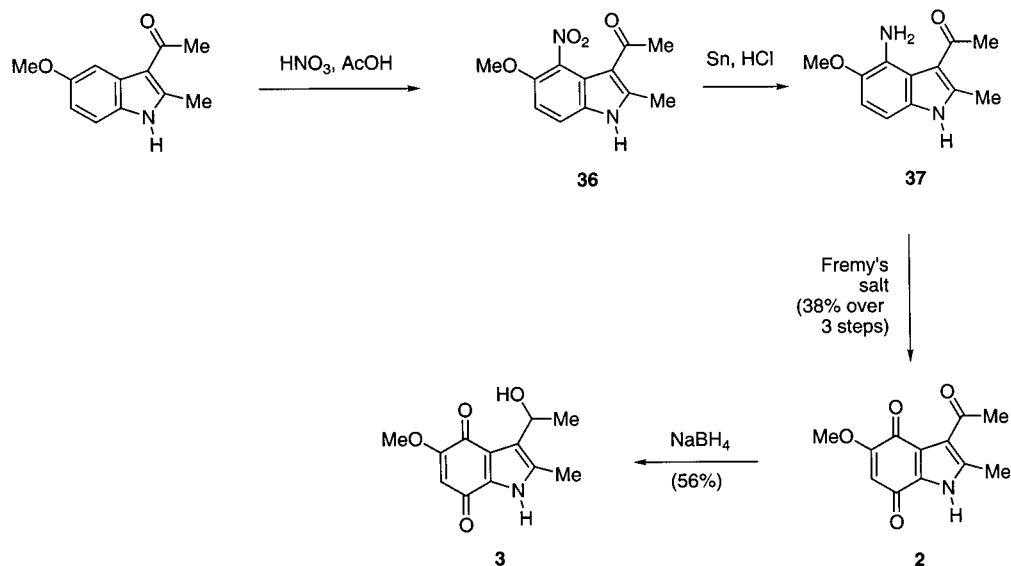
Scheme 2
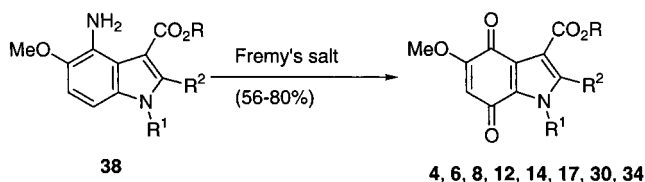
bind in different orientations, the accommodation of the large phenyl substituent at C-2 in **1h** is clearly seen. Additionally, the other orientation suggests that *N*-substituents larger than methyl should also be accommodated. Hence, we have studied the effect of a range of substituents at N-1, a greater range of groups at C-3, and the effect of a methyl group at C-6 in the indolequinones **2−35**.

Chemistry. The synthesis of indolequinones has been extensively investigated in our laboratory, and as a result, reliable methods are available.[1,40−45] Hence, the synthesis of indolequinones **5**, **7**, **9**, **13**, **15**, **16**, **18**, **19**, **21**, **22**, **24−27**, **29**, **31−33**, and **35** was achieved using previously published methods (see the Experimental Section). The *N*-unsubstituted indolequinones **2** and **3** were prepared from 3-acetyl-5-methoxy-1-methylindole as outlined in Scheme 1. The quinones **4**, **6**, **8**, **12**, **14**, **17**, **30,** and **34**, all of which bear an ester substituent at the 3-position, were synthesized by oxidation of the corresponding 4-aminoindole ester **38** (Scheme 2), readily obtained by the Nenitzescu reaction followed by functional group interconversions.[1,46] The Nenitzescu reaction was also the basis of the synthesis of the morpholinoethyl quinones **10** and **11**. Thus, methyl-1,4-benzoquinone and ethyl 3-[(2-morpholin-4-yl)ethyl]aminopropenoate (**39**) gave the 5-hydroxyindole **40**; methylation, nitration, reduction, and oxidation with Fremy's salt gave the quinone ester **10**, reduction of which gave the 3-hydroxymethyl compound **11** (Scheme 3). The quinones **1g** and **20** being amino substituents at C-5 were prepared from the corresponding 5-methoxy compounds by reaction with the corresponding amine. Finally the quinones **23** and **28** were prepared by functionalization of the 3-hydroxymethyl compound **1b** as shown in Scheme 4.

Electrochemical studies were performed on representative indolequinones in DMF as solvent with tetra-*n*-butylammonium tetrafluoroborate as supporting electrolyte as previously described.[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. Although electrochemical studies quickly identify compounds which are difficult to reduce, unfortunately, as discussed previously,[1] there is often little correlation between the reduction potential and rate of reduction by NQO1.

Biology. Metabolism of the indolequinones by recombinant human NQO1 was studied using an HPLC system designed to allow quantitation of both NADH oxidation and indolequinone removal as previously described.[1] NADH oxidation is used as the measure of indolequinone metabolism since 1 mol of NADH is oxidized for each mole of indolequinone that is reduced. Quinone reduction is reversible due to redox cycling of the hydroquinone, so results (Table 1) are reported as micromoles of NADH oxidized per minute per milligram of NQO1.

Cytotoxicity studies were also performed on representative quinones, with cell survival being measured using the MTT colorimetric assay. In our previous work, we have used the nonsmall cell lung cancer (NSCLC) cell lines H460 (with high NQO1 activity) and H596 (with no measurable NQO1 activity). A better model has recently been developed by Winski et al. that utilizes the BE human colon carcinoma cell line stably transfected with human NQO1 cDNA.[27] Like the H596 cell line, the BE cells have no measurable NQO1 activity, whereas activity in the transfected cells was 555 ± 23 nmol min^{-1} mg^{-1} of total cell protein using dichlorophenolindophenol as the standard electron acceptor. For this report, we have compared quinone toxicity (Table 2) in the wild-type BE cells (BE-WT) and the NQO1-transfected BE cells (BE-NQ).

Although only two *N*-unsubstituted indolequinones were investigated, both are good substrates for hNQO1 (Table 1, entries 8 and 9), and where a direct comparison is possible, the *N*-unsubstituted hydroxyethyl compound **3** is a better substrate then the corresponding *N*-
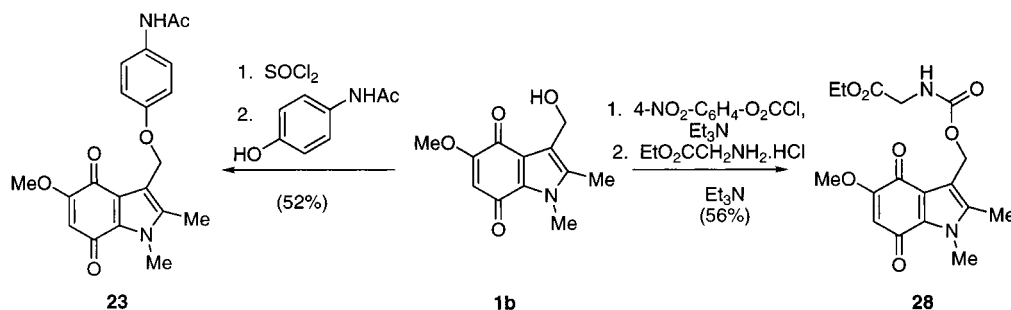
Scheme 3
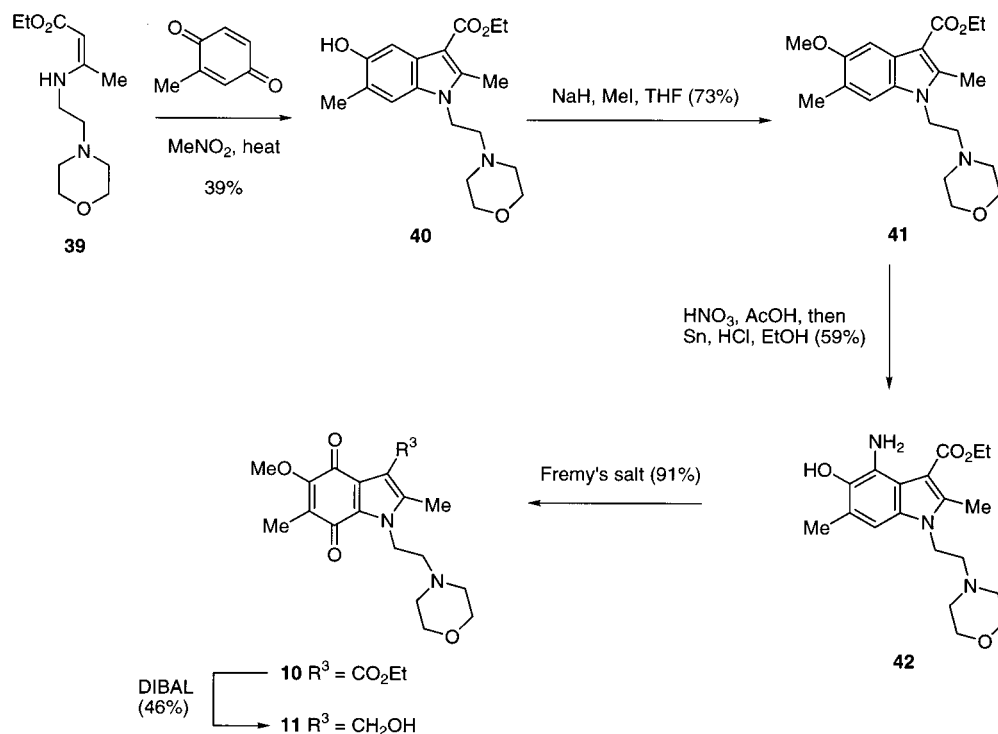
Scheme 4

Table 2. Cytotoxicity of Representative Indolequinones toward BE Human Colon Carcinoma Cell Lines BE-WT (No NQO1 Activity) and BE-NQ (High NQO1 Activity)

	compd	R^1	R^2	R^3	R^5	IC$_{50}$ (μM) (BE-WT)	IC$_{50}$ (μM) (BE-NQ)
1	**16**	Me	H	CH$_2$OH	OMe	>50	>50
2	**17**	Me	2-naphthyl	CO$_2$Et	OMe	49 ± 4	>50
3	**18**	Me	2-naphthyl	CH$_2$OH	OMe	>50	>50
4	**21**	Me	H	CH$_2$O(4-NO$_2$C$_6$H$_4$)	OMe	1.05 ± 0.05	0.44 ± 0.04
5	**23**	Me	Me	CH$_2$O(4-AcNHC$_6$H$_4$)	OMe	10.3 ± 2.2	6.6 ± 2.0
6	**24**	Me	Me	CH$_2$O(2-MeO-5-NO$_2$C$_6$H$_3$)	OMe	34.4 ± 4.4	6.5 ± 1.0
7	**25**	Me	Me	CH$_2$O(2-MeO-4-NO$_2$C$_6$H$_3$)	OMe	1.5 ± 0.2	0.51 ± 0.06
8	**26**	Me	Me	CH$_2$O(2-F-4-NO$_2$C$_6$H$_3$)	OMe	1.5 ± 0.2	1.2 ± 0.3
9	**27**	Me	Me	CH$_2$OCOCH$_2$(4-PhC$_6$H$_4$)	OMe	5.5 ± 0.4	5.1 ± 1.5
10	**28**	Me	Me	CH$_2$OCONHCH$_2$CO$_2$Et	OMe	3.8 ± 0.3	3.2 ± 0.2
11	**29**	Me	Me	CH$_2$SCSOEt	OMe	>50	>50

methylindolequinone **19** (entry 25). For the other *N*-substituted indolequinones **4**−**15**, there is clearly a dependence on the size of the *N*-substituent, and in all cases, with the exception of the *N*-4-fluorophenyl compounds **14** and **15**, the 3-esters are metabolized faster than the corresponding 3-hydroxymethyl compounds (entries 10−21). This effect of an electron-withdrawing substituent at C-3 has been noted previously; cf. entries 1, 2, 5, and 6.[1] *N*-Substituents larger than methyl are clearly tolerated by the enzyme, and the 3-esters bearing *n*-propyl, cyclopropyl, morpholinoethyl, or aryl groups are all excellent substrates. Clearly the indolequinones

bind in the active site in an orientation which allows space for a large substituent in the vicinity of the indole nitrogen, i.e., at N-1 (this study) or at C-2 as found in our previous study, and further evidenced by the results on the 2-(2-naphthyl) compound **18**. However, like the previously studied 2-(4-biphenylyl) compounds, and the *N*-(4-fluorophenyl) compounds referred to above, the naphthyl ester **17** surprisingly is a worse substrate than the corresponding hydroxymethyl compound **18**.

In terms of substituents at the indole 3-position, there are too few examples of compounds containing the 1-hydroxyethyl group to draw any firm conclusions, although it would appear that such compounds are better substrates for the enzyme than the corresponding hydroxymethyl derivatives. On the other hand, quinones **21**−**29**, all of which contain a 3-CH₂X (X = potential leaving group) substituent, are all apparently uniformly poor substrates for hNQO1; i.e., little or no NADH oxidation was observed. Inactivation studies on representative examples showed that there was little or no enzyme activity remaining following incubation with NQO1 and NADH. Hence, such quinones appear to inactivate the enzyme; similar results were found in our previous study for quinones containing acetate, 4-nitrophenolate, or carbamate leaving groups,[1] and the exact nature of this apparently mechanism-based inactivation has been studied in detail in the case of the 4-nitrophenoxy derivative.[38] Finally, the effect of a methyl group at C-6 was investigated on the basis that many natural or semisynthetic mitomycin/mitosene derivatives contain such a substituent, whereas many synthetic compounds do not. For compounds containing an ester group at C-3, those with the 6-methyl substituent are metabolized slower than the corresponding 6-unsubstituted quinones: **30** vs **1a**, **32** vs **4**, and **34** vs **12**. For the 3-hydroxymethyl derivatives the effect of the 6-methyl substituent is less clear. Overall, the 6-methyl substituent has a negative effect on metabolism rates, especially for the better substrates. This is possibly due to steric hindrance between the quinone and the active site of the enzyme.

Cytotoxicity data were obtained for selected indolequinones including compounds **21** and **23**−**29**, which have potential leaving groups at C-3. These compounds inactivate NQO1 to varying degrees and are toxic to both the BE-WT and BE-NQ cell lines (Table 2). All of the compounds with measurable IC₅₀ values (<50 μM) were more toxic to the NQO1-expressing BE-NQ cells than the NQO1-deficient BE-WT cells. Indolequinone **24** was the most selective compound; it was more than 5 times more toxic to the BE-NQ cells than the BE-WT cells. In contrast, the 3-hydroxymethyl compound **16**, which does not inactivate NQO1, is nontoxic to both cell lines. It is a much better substrate for NQO1 than the others, but it lacks a good leaving group at C-3. Consistent with this finding, the 2-naphthylindolequinones **17** and **18** were also not toxic to either cell line.

In conclusion, these findings further advance our understanding of the relationship between indolequinone structure and metabolism by NQO1. Our results are generally consistent with the recently solved structure of hNQO1 and previously published modeling studies. Expanded use of this technology should facilitate the design of new NQO1-directed antitumor agents.

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 GF₂₅₄. 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 ¹³C NMR spectra were recorded at 300 or 400 MHz (¹H frequencies) on Bruker instruments.

The following compounds were prepared as previously described: **5**,[46] **7**,[46] **9**,[46] **13**,[46] **15**,[46] **16**,[47,48] **18** and **19**,[46] **21**,[46] **22**,[45] **24**−**26**,[49] **27**,[45] **29**,[45] **31**−**33**,[46] and **35**.[46]

3-Acetyl-5-methoxy-1-methylindole-4,7-dione (2). To a solution of 3-acetyl-2-methyl-5-methoxyindole[46] (0.218 g, 1.1 mmol) in acetic acid (3 mL) cooled to 0 °C was added a mixture of concentrated nitric acid (0.27 mL) and acetic acid (4.3 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was poured into an ice/water mixture and the resulting precipitate filtered off and dried to give 3-acetyl-5-methoxy-1-methyl-4-nitroindole (**36**). The nitro compound was used directly in the next step without purification. To a suspension of the nitro compound in ethanol (53 mL) were added tin powder (1.28 g, 11.0 mmol) and hydrochloric acid (3 M; 23 mL). The mixture was stirred at room temperature for 1 h. 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 mixture was extracted with ethyl acetate. The organic layer was dried (MgSO₄) and concentrated to give 3-acetyl-4-amino-5-methoxy-1-methylindole (**37**). The amino compound was used directly in the next step without purification. To a solution of the amino compound in acetone (85 mL) was added a solution of potassium nitrosodisulfonate (0.86 g, 0.003 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 85 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 (Na₂SO₄) and concentrated. The crude product was purified by column chromatography (50% ethyl acetate/50% dichloromethane elution) and recrystallized (ethyl acetate/dichloromethane) to yield the title compound as an orange/red solid (0.095 g, 38%): mp 280 °C dec; UV (DMF) 440 (log ε 3.16), 328 (3.61), 284 (4.15) nm; IR (KBr) 3211, 3088, 1680, 1660, 1634, 1598 cm⁻¹; ¹H NMR (DMSO-*d*) δ 12.88 (1H, br s, NH), 5.87 (1H, s, 6-H), 3.80 (3H, s, OMe), 2.53 (3H, s, Me), 2.33 (3H, s, Me); ¹³C NMR (DMSO-*d*) δ 196.7 (CO), 178.4 (CO), 176.9 (CO), 161.3, 140.7, 130.9, 120.7, 120.3, 105.2 (CH), 57.2 (OMe), 31.6 (Me), 13.1 (Me); MS *m/z* (EI) 234 (MH⁺, 14), 233 (M⁺, 85), 218 (85), 176 (45), 43 (100). Anal. (C₁₂H₁₁NO₄) C, H, N.

3-(1-Hydroxyethyl)-5-methoxy-1-methylindole-4,7-dione (3). To a degassed solution of 3-acetyl-5-methoxy-1-methylindole-4,7-dione (0.08 g, 0.3 mmol) in methanol (50 mL) was added sodium borohydride (0.061 g, 1.6 mmol). The solution was stirred for 1 h. The reaction was quenched with FeCl₃ (1 M solution in 0.1 M HCl; 5 mL) and extracted with dichloromethane. The organic layer was dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography (20% ethyl acetate/80% dichloromethane elution) and recrystallized (hexane/ethyl acetate) to yield the title compound (0.045 g, 56%) as a red crystalline solid: mp 197−199 °C; UV (DMF) 468 (log ε 3.28), 348 (3.42), 284 (4.23) nm; IR (KBr) 3437, 3191, 3103, 2945, 1672, 1635, 1595 cm⁻¹; ¹H NMR (CDCl₃) δ 9.88 (1H, br s, NH), 5.67 (1H, s, 6-H), 4.80 (1H, m, CH(Me)OH), 4.58 (1H, m, OH), 3.86 (3H, s, OMe), 2.30 (3H, s, Me), 1.46 (3H, d, *J* = 6.6 Hz, CH(Me)OH); ¹³C NMR (DMSO-*d*) δ 179.1, 178.0, 161.0, 131.2, 130.7, 128.5, 120.9, 105.6 (CH),

63.3 (CH), 56.8 (OMe), 24.6 (NMe), 11.2 (Me); MS m/z (CI) 235 (M$^+$, 55), 220 (100). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

General Method for Oxidation of 4-Aminoindole 3-Esters. To a solution of the 4-aminoindole ester **38**[1,46] (0.22 mmol) in acetone (10 mL) was added a solution of potassium nitrosodisulfonate (0.66 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 10 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, and the residue was purified by column chromatography (9:1 dichloromethane/ethyl acetate) and recrystallized from ethanol (unless otherwise stated).

Ethyl 5-Methoxy-2-methyl-4,7-dioxo-1-propylindole-3-carboxylate (4). The crude product was purified by column chromatography (1:1 hexane/ethyl acetate elution) and recrystallized (hexane/ethyl acetate) to yield the title compound as an orange crystalline solid (0.056 g, 56%): mp 122−123 °C; UV (MeOH) 436 (log ϵ 3.12), 332 (2.52), 280 (4.00) nm; IR (KBr) 3058, 2971, 2930, 2873, 1685, 1646, 1604 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.63 (1H, s, 6-H), 4.36 (2H, q, J = 7.1 Hz, CH$_2$Me), 4.30 (2H, m, NCH$_2$CH$_2$Me), 3.81 (3H, s, OMe), 2.45 (3H, s, Me), 1.72 (2H, sextet, J = 7.4 Hz, NCH$_2$CH$_2$Me), 1.40 (3H, t, J = 7.1 Hz, CH$_2$Me), 0.97 (3H, t, J = 7.4 Hz, NCH$_2$CH$_2$Me); ^{13}C NMR (CDCl$_3$) δ 178.7, 175.5, 164.5, 159.9, 140.7, 129.0, 121.8, 113.2, 106.1 (CH), 60.9 (CH$_2$), 56.5 (OMe), 46.8 (CH$_2$), 23.6 (CH$_2$), 14.1 (Me), 11.0 (Me), 10.5 (Me); MS m/z (CI) 306 (MH$^+$, 100). Anal. ($C_{16}H_{19}NO_5$) C, H, N.

Data for Ethyl 1-Cyclopropyl-5-methoxy-2-methyl-4,7-dioxoindole-3-carboxylate (6): obtained in 73% yield; mp 184−186 °C; UV (MeOH) 424 (log ϵ 3.17), 326 (3.53), 286 (4.26) nm; IR (CH$_2$Cl$_2$) 1697, 1683, 1646 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.62 (1H, s, H-6), 4.33 (2H, q, J = 7.2 Hz, CH$_2$), 3.79 (3H, s, CH$_3$), 3.18−3.14 (1H, m, CH), 2.50 (3H, s, CH$_3$), 1.36 (3H, t, J = 7.2 Hz, CH$_3$), 1.31−1.21 (2H, m, CH$_2$), 0.87−0.82 (2H, m, CH$_2$); ^{13}C NMR (CDCl$_3$) δ 177.5, 175.6, 164.4, 159.5, 143.4, 131.0, 121.9, 112.9, 106.4 (CH), 61.0 (CH$_2$), 56.5 (CH$_3$), 28.2 (CH), 14.1 (CH$_3$), 12.5 (CH$_3$), 10.2 (2 × CH$_2$); MS m/z (EI) 304 (MH$^+$, 19), 303 (M$^+$, 100), 257 (77), 242 (47), 230 (50), 69 (57); HRMS found (M$^+$) 303.1109, $C_{16}H_{17}NO_5$ requires M 303.1107. Anal. ($C_{16}H_{17}NO_5$) C, H, N.

Data for Methyl 1-Benzyl-2-ethyl-5-methoxy-4,7-dioxoindole-3-carboxylate (8): obtained in 72% yield; mp 169−171 °C; UV (MeOH) 430 (log ϵ 3.07), 328 (3.48), 286 (4.22) nm; IR (CH$_2$Cl$_2$) 1708, 1679, 1634 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.33−7.22 (3H, m, ArH), 6.99−6.97 (2H, m, ArH), 5.69 (2H, s, H-6), 5.27 (1H, s, CH$_2$), 3.90 (3H, s, CH$_3$), 3.80 (3H, s, CH$_3$), 2.80 (2H, q, J = 7.2 Hz, CH$_2$), 1.09 (3H, t, J = 7.2 Hz, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 178.8, 175.7, 165.7, 164.6, 160.0, 147.2, 136.0, 129.1, 128.9 (2 × CH), 127.8 (CH), 126.0 (2 × CH), 122.2, 106.2 (CH), 56.6 (CH$_3$), 51.9 (CH$_3$), 48.3 (CH$_2$), 18.1 (CH$_2$), 13.9 (CH$_3$); MS m/z (EI) 354 (MH$^+$, 5), 353 (M$^+$, 23), 321 (27), 230 (36), 91 (100); HRMS found (M$^+$) 353.1264, $C_{20}H_{19}NO_5$ requires M 353.1263. Anal. ($C_{20}H_{19}NO_5$) C, H, N.

Data for Ethyl 5-Methoxy-2-methyl-4,7-dioxo-1-phenylindole-3-carboxylate (12): obtained in 70% yield; mp 206−208 °C; UV (MeOH) 424 (log ϵ 3.10) 326 (3.50) 284 (4.19) nm; IR (CH$_2$Cl$_2$) 1701, 1680, 1650 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.53−7.51 (3H, m, ArH), 7.21−7.18 (2H, m, ArH), 5.55 (1H, s, H-6), 4.39 (2H, q, J = 7.2 Hz, CH$_2$), 3.79 (3H, s, CH$_3$), 2.19 (3H, s, CH$_3$), 1.42 (3H, t, J = 7.2 Hz, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 177.3, 175.7, 164.4, 160.0, 142.2, 136.6, 130.5, 129.5 (3 × CH), 127.1 (2 × CH), 121.7, 113.1, 105.9 (CH), 61.1 (CH$_2$), 56.6 (CH$_3$), 14.1 (CH$_3$), 11.4 (CH$_3$); MS m/z (EI) 340 (MH$^+$, 9), 339 (M$^+$, 9), 293 (100), 118 (42), 77 (53); HRMS found (M$^+$) 339.1106, $C_{19}H_{17}NO_5$ requires M 339.1107.

Data for Ethyl 1-(4-Fluorophenyl)-5-methoxy-2-methyl-4,7-dioxoindole-3-carboxylate (14): obtained in 73% yield; mp 219−221 °C; UV (MeOH) 416 (log ϵ 3.16), 330 (3.58), 284 (4.29) nm; IR (CH$_2$Cl$_2$) 1694, 1650, 1609 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.26−7.06 (4H, m, ArH), 5.60 (1H, s, H-6), 4.38 (2H, q, J = 7.0 Hz, CH$_2$), 3.80 (3H, s, CH$_3$), 2.19 (3H, s, CH$_3$), 1.41 (3H, t, J = 7.0 Hz, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 177.3, 175.6, 164.2, 162.8 (d, J_{CF} = 250 Hz), 160.0, 142.2, 132.4, 130.5, 129.0

(d, J_{CF} = 9 Hz), 116.7 (d, J_{CF} = 23 Hz), 113.2, 105.9 (CH), 61.1 (CH$_2$), 56.7 (CH$_3$), 14.1 (CH$_3$),11.4 (CH$_3$); MS m/z (EI) 358 (MH$^+$, 3), 357 (M$^+$, 33), 311 (78), 136 (32), 91 (100); HRMS found (M$^+$) 357.1006, $C_{19}H_{16}FNO_5$ requires M 357.1012. Anal. ($C_{19}H_{16}FNO_5$·0.8H$_2$O) C, H, N.

Ethyl 5-Methoxy-1-methyl-2-(2-naphthyl)-4,7-dioxo-indole-3-carboxylate (17). The crude product was purified by column chromatography and recrystallized (ethyl acetate/light petroleum) to yield the title compound as an orange solid (70%): mp 174−177 °C; UV (MeOH) 432 (log ϵ 3.21), 332 (3.62) nm; IR (KBr) 3054, 2980, 2942, 1729, 1681, 1636, 1597 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.96−7.88 (4H, m, ArH), 7.61−7.54 (2H, m, ArH), 7.44 (1H, dd, J = 8.4, 1.7 Hz, ArH), 5.76 (1H, s, 6-H), 4.16 (2H, q, J = 7.1 Hz, CH$_2$CH$_3$), 3.87 (3H, s, OMe), 3.84 (3H, s, NMe), 1.22 (3H, t, J = 7.1 Hz, CH$_2$CH$_3$); ^{13}C NMR (CDCl$_3$) δ 179.2, 175.6, 163.8, 160.3, 142.5, 133.5, 132.8, 130.3 (CH), 129.6, 128.3 (CH), 127.8 (CH), 127.3 (CH), 127.0 (CH), 126.8 (CH), 125.9, 121.6, 115.1, 106.5 (CH), 61.1 (CH$_2$), 56.6 (OMe), 34.3 (NMe), 13.8 (Me); MS m/z (EI) 389 (M$^+$, 14), 344 (11), 189 (42), 168 (64), 69 (100); HRMS found (M$^+$) 389.1263, $C_{23}H_{19}NO_5$ requires M 389.1263. Anal. ($C_{23}H_{19}NO_5$) C, H, N.

Data for Methyl 5-Methoxy-1,2,6-trimethyl-4,7-dioxoindole-3-carboxylate (30): obtained in 80% yield; mp 164−166 °C; UV (MeOH) 441 (log ϵ 2.95) 327 (3.63), 285 (4.12) nm; IR (CH$_2$Cl$_2$) 1701, 1670, 1639 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.0 (3H, s, CH$_3$), 3.88 (6H, s, 2 × CH$_3$), 2.41 (3H, s, CH$_3$), 1.91 (3H, s, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 179.7, 177 1, 164.7, 156.7, 141.5, 129.4, 127.1, 122.1, 112.2, 61.1 (CH$_3$), 51.8 (CH$_3$), 32.5 (CH$_3$), 10.8 (CH$_3$), 8.5 (CH$_3$); MS m/z (EI) 278 (MH$^+$, 9), 277 (M$^+$, 57), 245 (100), 217 (45), 56 (53); HRMS found (M$^+$) 277.0951, $C_{14}H_{15}NO_5$ requires M 277.0950. Anal. ($C_{14}H_{15}NO_5$) C, H, N.

Data for Ethyl 5-Methoxy-2,6-dimethyl-4,7-dioxo-1-phenylindole-3-carboxylate (34): obtained in 80% yield; mp 165−166 °C; UV (MeOH) 430 (log ϵ 3.14), 336 (3.74), 286 (4.30) nm; IR (CH$_2$Cl$_2$) 1712, 1672, 1650 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.54−7.52 (3H, m, ArH), 7.21−7.18 (2H, m, ArH), 4.40 (2H, q, J = 7.0 Hz, CH$_2$), 4.03 (3H, s, CH$_3$), 2.19 (3H, s, CH$_3$), 1.84 (3H, s, CH$_3$), 1.41 (3H, t, J = 7.0 Hz, CH$_3$) ^{13}C NMR (CDCl$_3$) δ 178.1, 177.3, 164.2, 156.8, 141.9, 136.8, 130.4, 129.6 (2 × CH), 129.5 (CH), 129.5 (CH), 127.3, 127.1 (2 × CH), 122.4, 113.0, 61.1 (CH$_3$), 61.0 (CH$_2$), 14.2 (CH$_3$), 11.6 (CH$_3$), 8.5 (CH$_3$); MS m/z (EI) 354 (MH$^+$, 4), 353 (M$^+$, 17), 307 (35), 118 (28), 77 (100); HRMS found (M$^+$) 353.1262, $C_{20}H_{19}NO_5$ requires M 353.1263. Anal. ($C_{20}H_{19}NO_5$) C, H, N.

Functionalization of the 3-Hydroxymethyl Group. 3-[(4-Acetamidophenoxy)methyl]-5-methoxy-1,2-dimethylindole-4,7-dione (23). To a stirred solution of 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (**1b**)[1] (0.122 g, 0.5 mmol) in dichloromethane (10 mL) was added thionyl chloride (3.1 g, 25 mmol) dropwise. The solution was stirred at room temperature for 1 h. The solvent was removed and the crude material used directly in the next step. The crude chloride, 4-acetamidophenol (0.23 g, 1.5 mmol), and potassium carbonate (0.21 g, 1.5 mmol) were stirred in DMF (10 mL) overnight. The DMF was evaporated in vacuo, and the residue was dissolved in dichloromethane. The dichloromethane was washed with sodium hydroxide (1 M), hydrochloric acid (1 M), and water, dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography (10% dichloromethane/90% ethyl acetate) to give the title compound as a yellow/orange crystalline solid (0.1 g, 52%): mp 229−231 °C; UV (MeOH) 459 (log ϵ 3.28), 348 (3.53), 272 (4.16), 240 (4.20) nm; IR (KBr) 3449, 3324, 1665, 1629, 1598, 1546, 1509 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.42 (1H, br s, NH), 7.35 (2H, d, J = 7.2 Hz, ArH), 6.92 (2H, d, J = 7.2 Hz, ArH), 5.60 (1H, s, 6-H), 5.25 (2H, s, IndOCH$_2$), 3.87 (3H, s, OMe), 3.79 (3H, s, NMe), 2.28 (3H, s, Me), 2.12 (3H, s, NCOCH$_3$); ^{13}C NMR (CDCl$_3$) δ 179.1 (CO), 178.5 (CO), 168.6, 160.0, 155.7, 138.4, 129.2, 122.3 (CH), 121.7, 117.6, 115.8 (CH), 107.1 (CH), 61.2 (CH$_2$), 56.8 (OMe), 32.7 (NMe), 24.6 (NCOCH$_3$), 10.2 (Me); MS m/z (EI) 368 (M$^+$, 5), 267 (7), 218 (100); HRMS found (M$^+$) 368.1368, $C_{20}H_{20}N_2O_5$ requires M 368.1372. Anal. ($C_{20}H_{20}N_2O_5$·0.5H$_2$O) C, H, N.

(1,2-Dimethyl-5-methoxy-4,7-dioxoindol-3-yl)methyl (Ethoxycarbonylmethyl)carbamate (28). 3-Hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (**1b**)[1] (0.06 g, 0.25 mmol), 4-nitrophenyl chloroformate (0.103 g, 0.51 mmol), and triethylamine (4 mL) in dichloromethane (20 mL) were stirred for 2 h at room temperature. The crude mixture was washed with brine. The organic layer was dried (Na_2SO_4) and concentrated. The crude mixture was purified by column chromatography (ethyl acetate elution) to yield the carbonate as an orange solid (0.079 g, 77%), used directly without characterization. The carbonate (0.04 g, 0.1 mmol), glycine ethyl ester hydrochloride (0.042 g, 0.3 mmol), and triethylamine (4 mL) in dichloromethane (30 mL) were stirred at a room temperature overnight. The solvent was removed under reduced pressure and the crude product purified by column chromatography (ethyl acetate elution) to yield the title compound as an orange crystalline solid (0.02 g, 56%): mp 123−124 °C; UV (MeOH) 450 (log ϵ 2.67), 336 (3.00), 295 (2.63) nm; IR (KBr) 3333, 2978, 2944, 1734, 1705, 1677, 1640, 1599 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.61 (1H, s, 6-H), 5.28 (2H, s, CH$_2$O), 5.20 (1H, br m), 4.19 (2H, q, $J = 7.1$ Hz, CO$_2$C*H*$_2$CH$_3$), 3.94 (2H, br d, $J = 5.3$ Hz, HNC*H*$_2$), 3.89 (3H, s, OMe), 3.80 (3H, s, NMe), 2.30 (3H, s, Me), 1.26 (3H, t, $J = 7.1$ Hz, CO$_2$CH$_2$C*H*$_3$); ^{13}C NMR (CDCl$_3$) δ 178.9 (CO), 177.7 (CO), 170.0 (CO), 159.7 (CO), 156.4, 138.1, 129.1, 121.8, 116.2, 106.6 (CH), 61.4 (CH$_2$), 57.3 (CH$_2$), 56.4 (OMe), 42.8 (CH$_2$), 32.4 (NMe), 14.1 (Me), 9.6 (Me); MS *m/z* (EI) 364 (M$^+$, 2), 320 (32), 233 (38), 218 (94), 131 (11); HRMS found (M$^+$) 364.1315, C$_{17}$H$_{20}$N$_2$O$_7$ requires M 364.1270.

Substitutions at C-5. 3-Hydroxymethyl-1-methyl-5-(4-methylpiperazin-1-yl)-2-phenylindole-4,7-dione (1g). To a stirred solution of 3-hydroxymethyl-5-methoxy-1-methyl-2-phenylindole-4,7-dione (**1f**)[1] (1 equiv) in acetonitrile (5 mL) was added 4-methylpiperazine (40 equiv), and the mixture was stirred at room temperature for 24 h. Water (30 mL) was added, the crude product was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with water (2 × 30 mL) and brine (2 × 30 mL), dried (Na_2SO_4), and evaporated under reduced pressure. Column chromatography, eluting with ethyl acetate/light petroleum (1:1) gave the title compound as a purple crystalline solid (62 mg, 62%): mp 184−186 °C (from ethyl acetate/light petroleum); UV (MeOH) 514 (log ϵ 3.44), 326 (4.04) nm; IR (CH$_2$Cl$_2$) 3457 (OH), 3053, 2945, 1650 (CO), 1618, 1568, 1501, 1250 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.47 (3H, m, ArH), 7.31 (2H, m, ArH), 5.60 (1H, s, 6-H), 4.48 (2H, s, OCH$_2$), 3.78 (3H, s, NMe), 3.48 (4H, m, 2·CH$_2$), 2.57 (4H, m, 2·CH$_2$), and 2.34 (3H, s, NMe); ^{13}C NMR (CDCl$_3$) δ 182.1 (CO), 178.5 (CO), 153.7, 138.4, 130.6 (2·ArCH), 130.2, 129.2 (ArCH), 128.8 (2·ArCH), 128.7, 123.6, 123.1, 110.8 (6-CH), 56.2 (CH$_2$OH), 54.2 (CH$_2$O), 49.6 (CH$_2$N), 46.0 (NMe), and 33.7 (Me); MS *m/z* (EI) 365 (M$^+$, 30), 347 (100), 331 (27), 304 (10), and 265 (8); HRMS found (M$^+$) 365.1740, C$_{21}$H$_{23}$N$_3$O$_3$ requires M 365.1739. Anal. (C$_{21}$H$_{23}$N$_3$O$_3$) C, H, N.

3-(1-Hydroxyethyl)-5-(morpholin-4-yl)-1,2-dimethylindole-4,7-dione (20). To a stirring solution of 3-(1-hydroxyethyl)-5-methoxy-1,2-dimethylindole-4,7-dione[46] (0.021 g, 0.08 mmol) in DMF (1 mL) was added morpholine (1 mL), and the mixture was allowed to stir overnight. The solvent was removed in vacuo and purified by column chromatography (80% light petroleum/20% acetone elution) and recrystallized (ethanol/light petroleum) to yield the title compound (0.023 g, 90%) as a purple solid: mp 159−161 °C; UV (DMF) 524 (log ϵ 3.31), 384 (3.57), 332 (4.00), 268 (3.62) nm; IR (KBr) 3425, 2926, 2781, 2706, 1632 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 5.50 (1H, s, 6-H), 4.98 (1H, d, $J = 11.4$ Hz, OH), 4.81 (1H, dq, $J = 11.5$, 6.6 Hz, C*H*MeOH), 3.9 (3H, s, NMe), 3.87−3.81 (4H, m, 2CH$_2$), 3.47−3.29 (4H, m, 2CH$_2$), 2.19 (3H, s, Me), 1.43 (3H, d, $J = 6.6$ Hz, CH*Me*OH); ^{13}C NMR (CDCl$_3$) δ 181.8, 178.2, 153.5, 132.4, 130.0, 127.8, 121.5, 111.0 (CH), 66.4 (CH$_2$), 63.5 (CH), 50.1 (CH$_2$), 32.1 (NMe), 24.6 (Me), 9.5 (Me); MS *m/z* (FAB) 327 ([M+Na]$^+$, 7), 305 (MH$^+$, 42), 287(47). Anal. (C$_{16}$H$_{20}$N$_2$O$_4$·0.2H$_2$O) C, H, N.

Ethyl 5-Methoxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethyl-4,7-dioxoindole-3-carboxylate (10). (a) Methyl-1,4-benzoquinone (1.0 g, 8.2 mmol) was dissolved in nitromethane

(15 mL), and ethyl 3-[(2-morpholin-4-yl)ethyl]aminopropenoate (**39**) (2.2 g, 9.1 mmol) was added. The mixture was allowed to stand at room temperature for 2 days. The resulting product was filtered off, washed thoroughly with hexane, recrystallized (ethanol/hexane) to yield ethyl 5-hydroxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethylindole-3-carboxylate (**40**) as an off-white crystalline solid (1.1 g, 39%): mp 206−207 °C; IR (KBr) 3286, 2948, 2914, 2851, 2811, 1644, 1526 cm^{-1}; ^{1}H NMR (DMSO-*d*) δ 8.93 (1H, s, OH), 7.40 (1H, s, ArH), 7.17 (1H, s, ArH), 4.23 (4H, m, CH$_2$, OC*H*$_2$Me), 3.55 (4H, m, 2CH$_2$), 2.68 (3H, s, Me), 2.53 (2H, m, CH$_2$), 2.42 (4H, m, 2CH$_2$), 2.23 (3H, s, Me), 1.35 (3H, t, $J = 7.1$ Hz, CH$_2$*Me*); ^{13}C NMR (DMSO-*d*) δ 165.6 (CH), 151.6, 144.4, 130.3, 125.6, 120.3, 111.4 (CH), 105.6 (CH), 102.5, 66.6 (CH$_2$), 59.0 (CH$_2$), 57.6 (CH$_2$), 54.0 (CH$_2$), 40.9 (CH$_2$), 17.3 (Me), 15.0 (Me), 12.1 (Me); MS *m/z* (ES) 347 (MH$^+$, 54), 301 (33), 114 (100). Anal. (C$_{19}$H$_{26}$N$_2$O$_4$) C, H, N.

(b) Ethyl 5-hydroxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethylindole-3-carboxylate (**40**) (1.94 g, 5.6 mmol) in THF (20 mL) was added to a stirring suspension of sodium hydride (0.27 g, 11.2 mmol) in THF (30 mL) at 0 °C. The mixture was stirred at room temperature for 45 min. Iodomethane (1.19 g, 8.4 mmol) was added dropwise at 0 °C and the mixture allowed to warm to room temperature. After 2 h the excess sodium hydride was quenched with water (3 mL), and the crude mixture was concentrated. The crude product was purified by column chromatography (10% ethyl acetate/hexane elution) and recrystallized (ethyl acetate/hexane) to yield ethyl 5-methoxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethylindole-3-carboxylate (**41**) as a colorless solid (1.47 g, 73%): mp 101−102 °C; IR (KBr) 2980, 2955, 2919, 2847, 1680 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.59 (1H, s, ArH), 7.05 (1H, s, ArH), 4.38 (2H, q, $J = 7.1$ Hz, C*H*$_2$Me), 4.18 (2H, m, CH$_2$), 3.90 (3H, s, OMe), 3.71 (4H, m, 2CH$_2$), 2.75 (3H, s, Me), 2.64 (2H, t, $J = 7.1$ Hz, CH$_2$), 2.51 (4H, m, 2CH$_2$), 2.34 (3H, s, Me), 1.45 (3H, t, $J = 7.1$ Hz, CH$_2$*Me*); ^{13}C NMR (CDCl$_3$) δ 166.2, 154.2, 143.6, 130.3, 125.6, 122.4, 110.5 (CH), 103.9, 101.9 (CH), 66.8 (CH$_2$), 59.2 (CH$_2$), 57.5 (CH$_2$), 55.6 (OMe), 54.0 (CH$_2$), 41.1 (CH$_2$), 17.1 (Me), 14.6 (Me), 12.0 (Me); MS *m/z* (FAB) 383 ([M + Na]$^+$, 10), 361 (M$^+$, 100). Anal. (C$_{20}$H$_{28}$N$_2$O$_4$) C, H, N.

(c) To a solution of ethyl 5-methoxy-1-[(2-morpholin-4-yl)-ethyl]-2,6-dimethylindole-3-carboxylate (**41**) (0.73 g, 2.0 mmol) in acetic acid (10 mL) cooled to −10 °C was added a mixture of concentrated nitric acid (0.13 mL) and acetic acid (5 mL). The mixture was stirred at room temperature for 1 h. The reaction mixture was poured into an ice/water mixture and the resulting precipitate filtered off and dried. The nitro compound was used directly in the next step without purification. To a suspension of the nitro compound in ethanol (120 mL) were added tin powder (2.4 g, 20.2 mmol) and hydrochloric acid (3 M; 48 mL). The mixture was stirred at room temperature for 1 h. 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 mixture was extracted with ethyl acetate. The organic layer was dried (MgSO$_4$) and concentrated. The crude product was purified by column chromatography (ethyl acetate elution) and recrystallized (ethyl acetate/hexane) to yield ethyl 4-amino-5-methoxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethylindole-3-carboxylate (**42**) as an off-white crystalline solid (0.45 g, 59% over two steps): mp 120−122 °C; IR (KBr) 3467, 3324, 2965, 2853, 2812, 1655, 1568 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 6.38 (1H, s, ArH), 5.78 (2H, br s, NH$_2$), 4.36 (2H, q, $J = 7.1$ Hz, C*H*$_2$Me), 4.14−4.09 (2H, m, CH$_2$), 3.75 (3H, s, OMe), 3.73−3.70 (4H, m, 2CH$_2$), 2.66 (3H, s, Me), 2.64−2.60 (2H, m, CH$_2$), 2.52−2.49 (4H, m, 2CH$_2$), 2.37 (3H, s, Me), 1.41 (3H, t, $J = 7.1$ Hz, CH$_2$*Me*); ^{13}C NMR (CDCl$_3$) δ 167.0, 142.5, 139.6, 134.1, 134.0, 126.5, 113.2, 105.0, 98.3 (CH), 66.9 (CH$_2$), 60.1 (CH$_2$), 59.0 (OMe), 57.2 (CH$_2$), 54.1 (CH$_2$), 41.1 (CH$_2$), 16.7 (Me), 14.5 (Me), 12.9 (Me); MS *m/z* (FAB) 375 (M$^+$, 100). Anal. (C$_{20}$H$_{29}$N$_3$O$_4$) C, H, N.

(d) To a solution of ethyl 4-amino-5-methoxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethylindole-3-carboxylate (**42**) (0.76. g, 0.002 mmol) in acetone (150 mL) was added a solution of potassium nitrosodisulfonate (1.63 g, 0.006 mmol) in sodium

dihydrogen phosphate buffer (0.3 M; 150 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 (5% ethyl acetate/95% dichloromethane elution) and recrystallized (hexane/ethyl acetate) to yield the title compound as an orange solid (0.72 g, 91%): mp 84–86 °C; UV (DMF) 444 (log ϵ 2.96), 336 (3.57), 288 (4.06), 256 (3.85) nm; IR (KBr) 2981, 2950, 2925, 2832, 2807, 1697 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.45–4.33 (4H, m, 2CH$_2$), 4.01 (3H, s, OMe), 3.69–3.66 (4H, m, 2CH$_2$), 2.63–2.59 (2H, m, CH$_2$), 2.54–2.51 (4H, m, 2CH$_2$), 2.47 (3H, s, Me), 1.94 (3H, s, Me), 1.39 (3H, t, $J = 7.1$ Hz, CH$_2$Me); ^{13}C NMR (CDCl$_3$) δ 179.3, 177.1, 164.3, 156.8, 140.9, 129.0, 127.1, 122.6, 113.1, 66.9 (CH$_2$), 61.0 (OMe), 60.9 (CH$_2$), 58.2 (CH$_2$), 54.0 (CH$_2$), 43.0 (CH$_2$), 14.1 (Me), 10.7 (Me), 8.6 (Me); MS m/z (FAB) 391 (MH$^+$, 45), 107 (100). Anal. (C$_{20}$H$_{26}$N$_2$O$_6$) C, H, N.

3-Hydroxymethyl-5-methoxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethylindole-4,7-dione (11). To a solution of ethyl 5-methoxy-1-[(2-morpholin-4-yl)ethyl]-2,6-dimethyl-4,7-dioxoindole-3-carboxylate (**10**) (0.228 g, 0.58 mmol) in water/dichloromethane/ethanol (50 mL) was added sodium dithionite (1.0 g, 5.75 mmol), and the mixture was stirred overnight. The organic layer was separated, washed with saturated ammonium chloride, dried (Na$_2$SO$_4$), concentrated, and used directly in the next step. To a stirred suspension of the crude dihydroxyindole in dichloromethane (50 mL) was added DIBAL (1 M solution in hexane; 0.83 g, 5.84 mmol) at −50 °C, and the mixture was stirred at this temperature for 2 h. The reaction was quenched by dropwise addition of FeCl$_3$ (1 M in 0.1 M HCl; 1 mL). The crude mixture was purified by column chromatography (2% ethyl acetate/98% dichloromethane) and recrystallized (ethyl acetate/hexane) to yield the title compound (0.093 g, 46%) as red crystals: mp 109–111 °C; UV (DMF) 468 (log ϵ 2.93), 356 (3.31), 288 (3.94) nm; IR (KBr) 3339, 2970, 2939, 2817, 1663, 1638, 1608 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 4.60 (2H, s, CH$_2$OH), 4.38 (2H, t, $J = 7.0$ Hz, CH$_2$), 3.98 (3H, s, OMe), 3.71–3.68 (4H, m, 2 × CH$_2$), 2.64–2.53 (6H, m, 3 × CH$_2$), 2.26 (3H, s, Me), 1.97 (3H, s, Me); ^{13}C NMR (CDCl$_3$) δ 181.1, 178.5, 156.1, 134.6, 129.7, 128.8, 123.8, 66.9, 61.1 (OMe), 58.3 (CH$_2$), 56.0 (CH$_2$), 53.9 (CH$_2$), 42.9 (CH$_2$), 9.5 (Me), 8.9 (Me); MS m/z (ES) 372([MH + Na]$^+$, 15), 349 (MH$^+$, 12), 342 (23), 241 (100). Anal. (C$_{18}$H$_{24}$N$_2$O$_5$·0.3H$_2$O) C, H, N.

Biology. **Cell Culture**. BE-WT and BE-NQ cells were a gift from David Ross (University of Colorado Health Sciences Center, Denver, CO). Cell culture components were obtained from Life Technologies, Inc. unless otherwise noted. 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 (HyClone Laboratories, Inc.). The cells were incubated at 37 °C under a humidified atmosphere containing 5% CO$_2$.

HPLC Analysis. Reduction of the indolequinones was followed by HPLC using an Alltech C18 (5 μm, 250 mm × 4.6 mm) column with a Waters HPLC system (2487 dual λ absorbance detector, two 515 HPLC pumps, 717plus autosampler, Millennium32 chromatography manager). The solvent program used a linear gradient of 5% B to 80% B over 10 min, 80% B for 5 min, and then 80% B to 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 (Sigma), 50 μM indolequinone, and recombinant human NQO1 (gift from David Ross, University of Colorado Health Sciences Center, Denver, CO). NADH oxidation was used to measure indolequinone metabolism since 1 mol of NADH is oxidized for every mole of indolequinone that is reduced. NADH oxidation was quantified at 340 nm following 30 min reactions at 22 °C. The indolequinone peaks were also monitored, but changes were minimal due to reoxidation of the indolehydroquinone (redox cycling).

Cytotoxicity Assay. Cytotoxicity was determined using the MTT colorimetric assay.[50] Cells were plated in 96-well plates at a density of $(1–2) \times 10^4$ cells/mL and allowed to attach overnight (16 h). Indolequinone solutions were applied in medium for 2 h. Indolequinone solutions were removed and replaced with medium alone, and the 96-well plates were incubated for 5–7 days. MTT (Sigma) was added to each well (50 μg), and the cells were incubated for another 4 h. Medium/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 the control) were determined from plots of the percent of the control vs concentration.

Inactivation of NQO1 by Indolequinones. The ability of selected indolequinones to inactivate NQO1 was determined using a previously reported procedure[51] 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 1–2 μg of recombinant hNQO1 in 25 mM Tris–HCl (pH 7.4) + 2 mg/mL Tween-20. After 30 min, the reactions were stopped with 4 volumes (2 mL) of cold 25 mM Tris–HCl/250 mM sucrose (pH 7.4). The 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-dichloroindophenol (DPIP; Sigma), and 200 μM NADH in 25 mM Tris–HCl (pH 7.4) + 0.7 mg/mL bovine serum albumin (BSA). DPIP reduction was followed for 30 s at 600 nm. NQO1 activity from (+) NADH incubations was compared to that of (−) NADH controls for calculations of the enzyme activity remaining (%).

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