Indolequinone antitumour agents: correlation between quinone structure and rate of metabolism by recombinant human NAD(P)H:quinone oxidoreductase†

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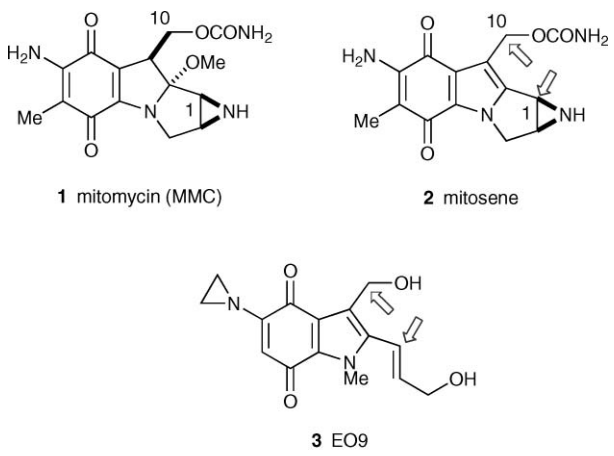
A series of indolequinones bearing a range of substituents at the (indol-2-yl)methyl position has been synthesized. The ability of these indolequinones to act as substrates for recombinant human NAD(P)H:quinone oxidoreductase (NQO1), a two-electron reductase upregulated in tumour cells, was determined, along with their toxicity to an isogenic tumour cell line pair that is differentiated as either NQO1-expressing cells (BE-NQ) or NQO1-null cells (BE-WT). Overall, the 2-substituted indolequinones were relatively poor substrates for NQO1. Hydroxymethyl groups at C-2 led to higher rates of reduction, a finding that was observed previously with 3-hydroxymethylated indolequinones. Predictably, the best substrate had an electron-withdrawing ester group at the indole-2-position. The indolequinones were generally non-toxic to both cell lines with the exception of those quinones that had methylaziridine groups at the indole-5-position. These compounds could form DNA cross-links when activated by reduction and were up to 3-fold more toxic to the BE-NQ cells than the BE-WT cells.

Introduction

The term bioreductive drug describes a range of anticancer agents that are inactive in their own right, but upon metabolic reduction are transformed into a cytotoxic species. The drugs act as substrates for one or more of the reductases that are present in most cells, and could be effective against tumours in which the activating reductase enzymes are upregulated. Bioreductive drugs based on *N*-oxides and on nitroarenes are known, but it is the quinones that form the best studied group of compounds. Quinones are readily reduced *in vitro* and *in vivo*, and the selective activation of agents such as mitomycin C **1** (MMC), a clinically used antitumour antibiotic and the archetypical quinone bioreductive alkylating agent, has been widely studied.[2–5] Upon enzymatic reduction the quinone forms a semiquinone or hydroquinone that can subsequently form a reactive electrophilic intermediate by elimination of an appropriate leaving group. In the case of MMC **1**, the loss of methanol occurs first to form, after reoxidation, the indolequinone (mitosene) derivative **2**, which upon further reductive activation can generate electrophilic centres at C-1 and C-10, the indole-2-carbinyl and 3-carbinyl positions as outlined in Scheme 1. This sequential generation of electrophilic centres can hence lead to cross-linking of DNA. Likewise, the indolequinone diol EO9 **3** is also a potential bi-functional alkylating agent after loss of water from both the indole 3-carbinyl and vinylogous indole 2-carbinyl positions (Scheme 1).[6,7] The compound has recently re-entered phase I clinical trials.[8]



Scheme 1

As a result of their structural relationship to MMC, the indolequinones have been widely studied, particularly because of the ability of 3-indolyl carbinyl substituents X in such compounds to undergo an elimination process upon either one- or two-electron reductive-activation. The resulting iminium species is then a potential electrophile capable of DNA-alkylation or other cellular-damaging events (Scheme 2).[6,7,9–12] However, bioreductive

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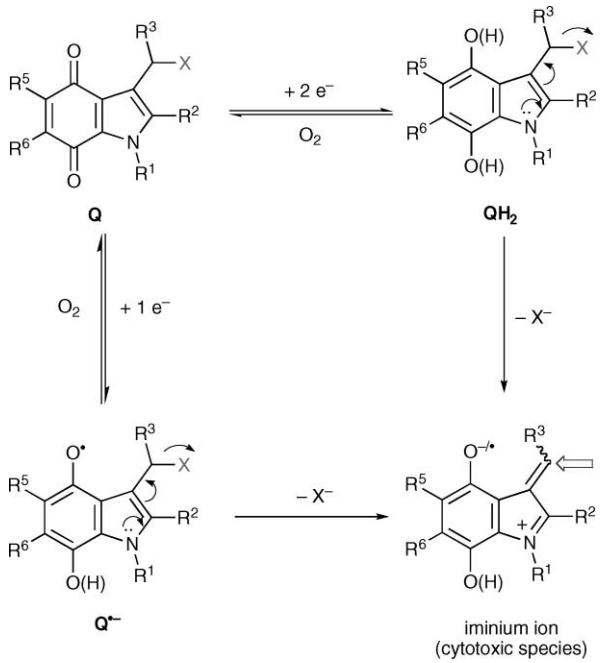
† Part 3. For part 2, see reference 1. Electronic supplementary information (ESI) available: experimental details and characterization data for synthetic intermediates. See DOI: 10.1039/b703370b

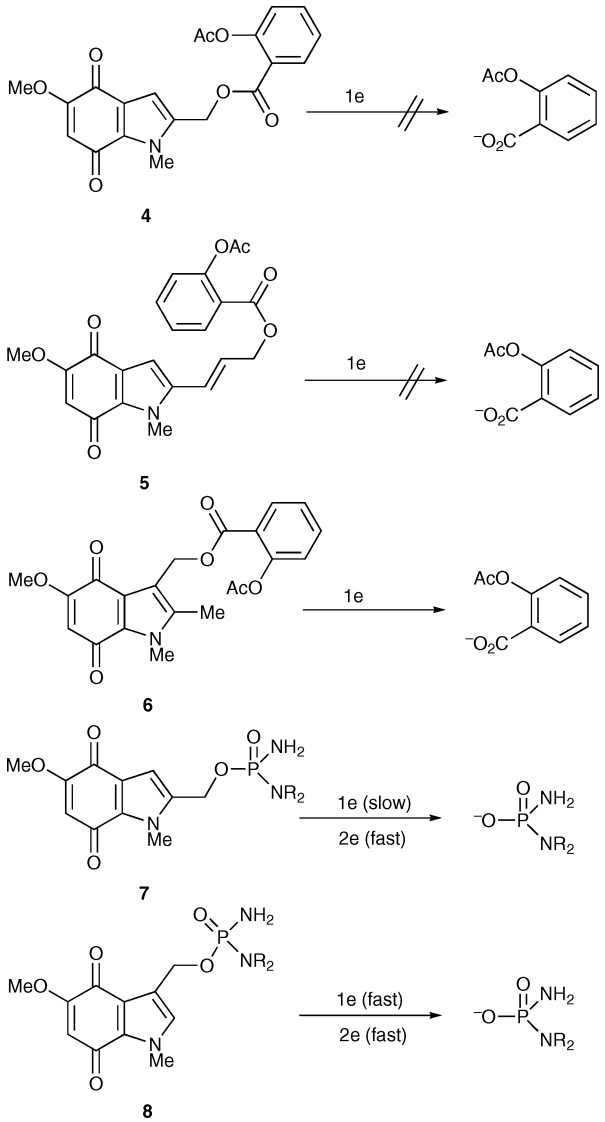


Scheme 2

drugs such as indolequinones are also of interest since they could act as reductively activated drug delivery vehicles by releasing a variety of leaving groups X in a reductive environment.[13] Thus they may have secondary biological effects due to the eliminated molecule XH, in addition to the cytotoxic iminium derivative formed on reduction and elimination (Scheme 2).[14–17]

As outlined in Scheme 2, indolequinones **Q** are capable of efficiently eliminating a range of leaving groups X from the (indolyl-3-yl)methyl position following two-electron reduction to the hydroquinone (**QH_2**) or one-electron reduction to the semiquinone radical (**$Q^{\bullet-}$**).[15] Such one- or two-electron reductions would be catalyzed in biological systems by, for example, NADPH:cytochrome *c* (P450) reductase[11] or NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) respectively.[1,18–21]

To date the focus has been the fragmentation of groups from the (indol-3-yl)methyl position of indolequinones (Scheme 2), notwithstanding the fact that in both MMC **1** and EO9 **3** elimination from (indol-2-yl)methyl position also occurs. The role of 2-indolyl substituents in other indolequinones is less clear, since relatively few studies have been reported thus far. For example, no elimination of the carboxylate leaving group – 2-acetoxybenzoate (aspirin) – occurred upon (one-electron) radiolytic reduction of the 2-substituted indolequinone **4** or the corresponding 'vinylogous' derivative **5**. On the other hand, the 3-substituted analogue **6** underwent efficient fragmentation upon reduction (Scheme 3).[22] Similar results were observed with indolequinone phosphoramidate prodrugs: upon one-electron reduction, elimination from the 2-substituted compound **7** was slow in comparison to the 3-substituted analogue **8** (Scheme 3). In contrast, the phosphoramidate was rapidly released from both the 2- and 3-substituted indolequinones **7** and **8** upon two-electron reduction suggesting that potential drug delivery mechanisms from the indolequinone 2-position proceed better from the hydroquinone QH_2.[23,24] In order to investigate this suggestion more fully, we have prepared a series



Scheme 3

of indolequinones bearing a range of substituents at the 2-position and investigated their metabolism by the two-electron reductase NQO1.

As already mentioned, NQO1 (EC 1.6.99.2) is an obligate 2-electron reductase that is characterized by its ability to use either NADH or NADPH as cofactor,[25,26] and is involved the reductive activation of anticancer agents such as mitomycin C (MMC).[19,27–29] Recently the 3-dimensional structure of human NQO1 has been solved by two research groups, and resolved to 2.3 Å[30] and 1.7 Å[31] respectively. The structures of the complex of enzyme with duroquinone[31] and potentially chemotherapeutic quinones[32] have also been solved. We have investigated the correlation between quinone structure and rate of metabolism by NQO1 and toxicity towards human tumour cell lines, and have studied both quinolinequinones,[33,34] and indolequinones bearing a range of substituents at the 3-position.[1,20] The present studies on indolequinones bearing a range of substituents at the 2-position extend and complement our earlier work.

Results and discussion

Synthetic chemistry

The key indolequinones for the present study are shown in Fig. 1. Indolequinone **9** was obtained from the known 4-benzyloxy-5-methoxy-1-methylindole-2-methanol[10] by hydrogenolysis of the benzyl protecting group followed by oxidation with potassium nitrosodisulfonate (Fremy's salt) (Scheme 4). Similarly indolequinone **10** was prepared from the known 4-benzyloxy-5-methoxy-1-methylindole-2-carboxaldehyde[35] by addition of methylmagnesium bromide, hydrogenolysis of the benzyl group and Fremy's salt oxidation (Scheme 4).

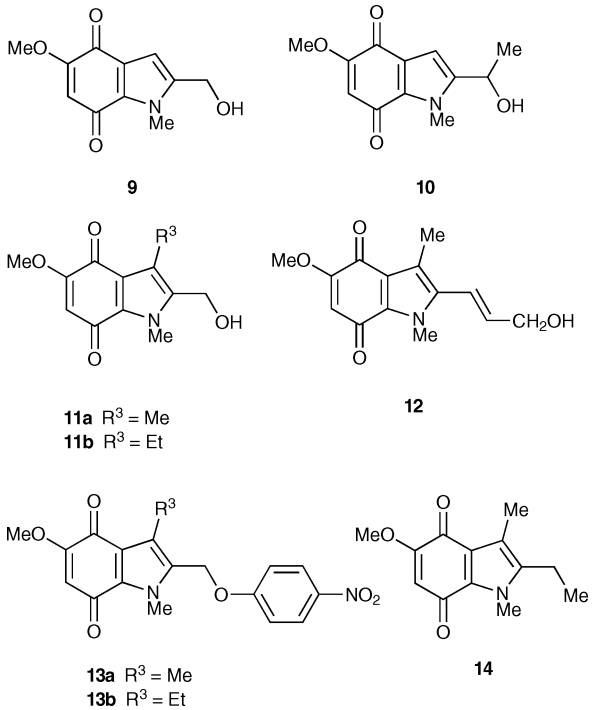
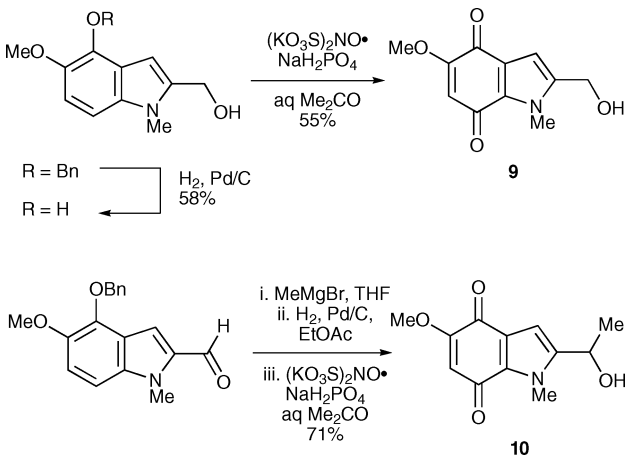
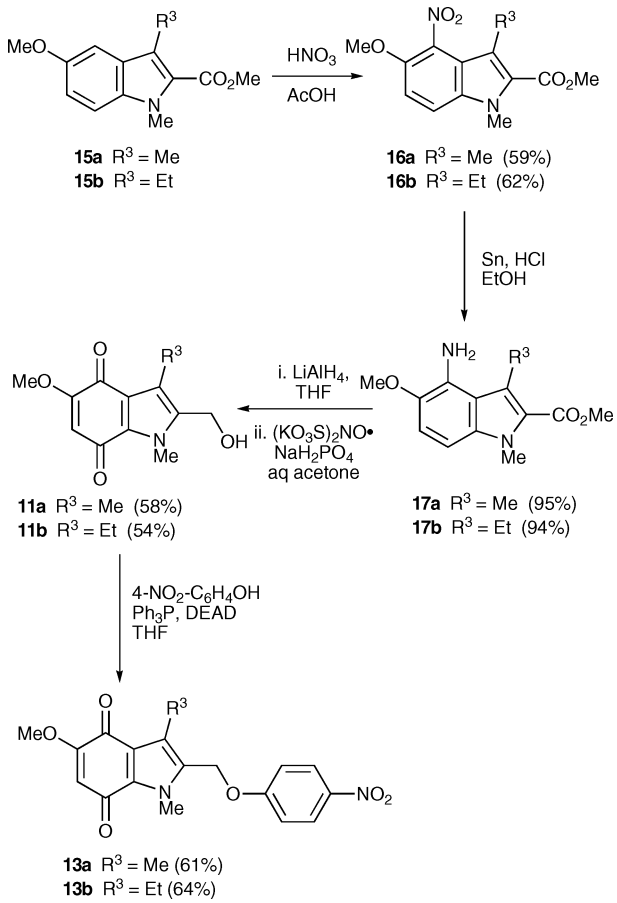


Fig. 1



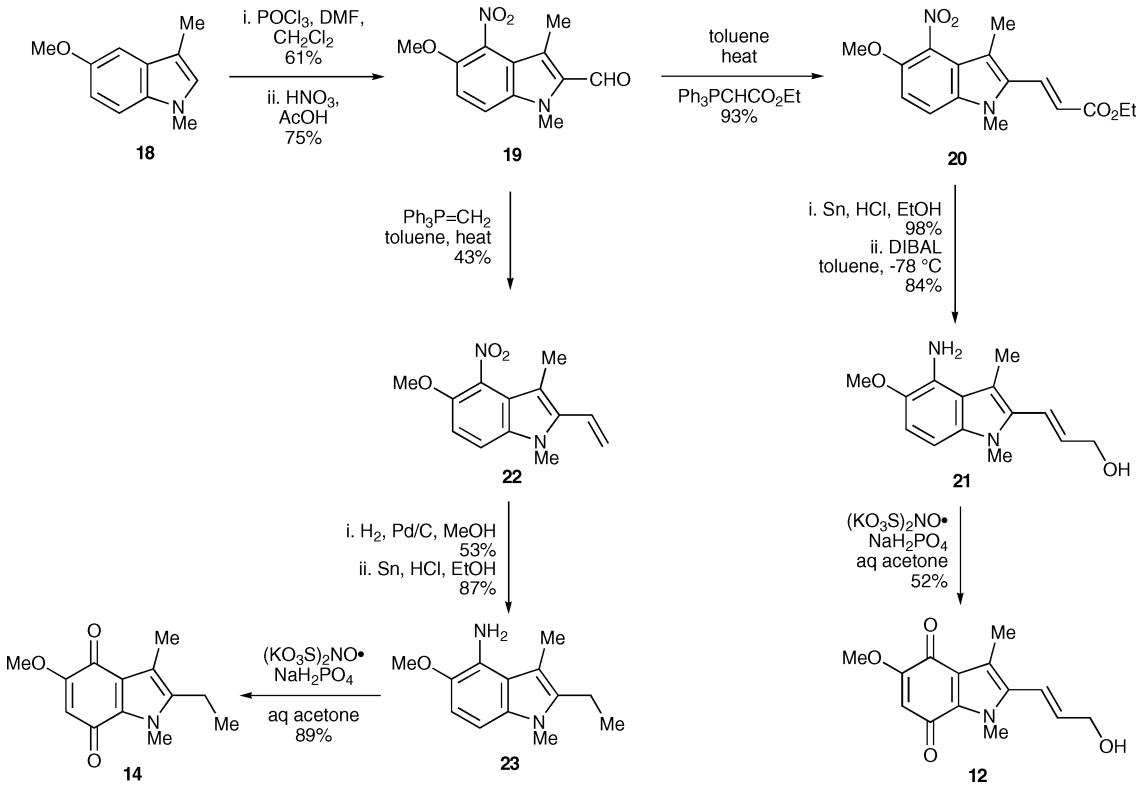
Scheme 4

The 3-alkyl indolequinones **11** were obtained from the corresponding 3-alkyl-5-methoxy-1-methylindole-2-esters **15**, prepared using our modified version of the Bischler indole synthesis.[36,37] Nitration occurred selectively at the indole-4-position to give the nitroindoles **16**, reduction of which gave the 4-aminoindoles **17**. Reduction of the ester group with lithium aluminium hydride gave the 4-amino-3-hydroxymethylindoles that, without purification, were oxidized to the quinones **11a** and **11b** in good yield (Scheme 5). The 2-aryloxymethyl derivatives were prepared in an analogous manner to their 3-substituted analogues. Thus Mitsunobu reaction of the alcohols with 4-nitrophenol gave the 2-(4-nitrophenoxymethyl) quinones **13a** and **13b**.



Scheme 5

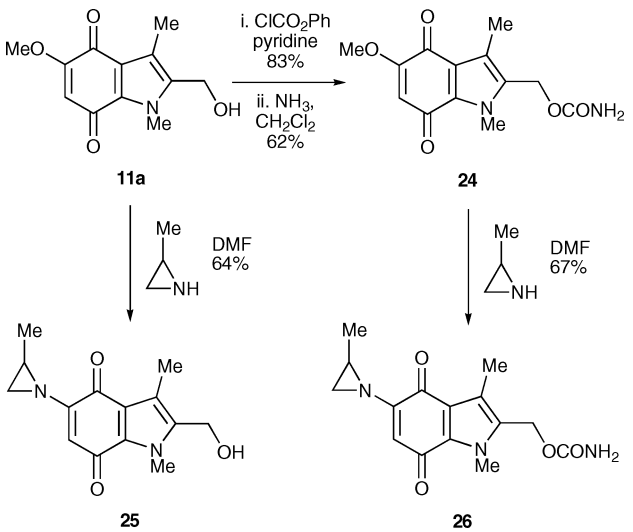
The synthesis of quinones **12** and **14** started with the known 5-methoxy-1,3-dimethylindole **18**, readily available from a Bischler indole synthesis.[38] Vilsmeier formylation at the 2-position was followed by nitration under controlled conditions to prevent over reaction to give the 4-nitroindole **19** (Scheme 6). Wittig reaction of **19** gave the *trans*-alkene **20** in excellent yield. Reduction of the nitro and ester groups with tin and hydrochloric acid and di-isobutylaluminium hydride (DIBAL) respectively gave the 4-aminoindole **21** which was oxidized to the indolequinone **12** using Fremy's salt (Scheme 6), although some over-oxidation (21%) of the allylic alcohol to the corresponding aldehyde also occurred. The allylic alcohol **12** was selected for study because of its resemblance to EO9 **3**. Indolequinone **14** was also prepared from the 4-nitroindole-2-carboxaldehyde **19** as shown in Scheme 6. Wittig reaction to give the vinyl derivative **22** was followed by a



Scheme 6

2-step reduction to give the 4-aminoindole **23**, and oxidation to the quinone **14** with Fremy's salt.

Compounds **24–26** were prepared from the quinone **11a** by standard transformations as outlined in Scheme 7. The quinone **29** bearing an extended linker that can eliminate $^{-}OC_6H_4CH_2OCONH_2$ (that can further fragment to give a *p*-quinonemethide, CO_2 and ammonia)[39] was also prepared from quinone **11a** as shown in Scheme 8. Thus Mitsunobu reaction with 4-(*tert*-butyldimethylsiloxymethyl)phenol[40] to give **27**, was followed by removal of the silicon protecting group and conversion of the alcohol **28** into the carbamate **29**. As a comparison, the corresponding 3-substituted derivative **33** was also prepared from the known indolequinone **30**[20] by way of the Mitsunobu product **31** and the alcohol **32** as shown in Scheme 8. Finally three further indolequinones were prepared starting from ethyl 5-methoxy-1-methylindole-2-carboxylate **34**. Vilsmeier formylation to give the 3-carboxaldehyde **35** was followed by nitration at C-4, both steps proceeding in high yield (Scheme 9). Reduction of the nitro compound **36** gave the corresponding amine **37** the structure of which was confirmed by X-ray crystallography (Fig. 2).[41] Reduction of both aldehyde and ester groups using lithium aluminium hydride was followed by oxidation to the 2,3-bis(hydroxymethyl)indolequinone **38**, a compound that has been prepared previously by a different route.[42] Displacement of the



Scheme 7

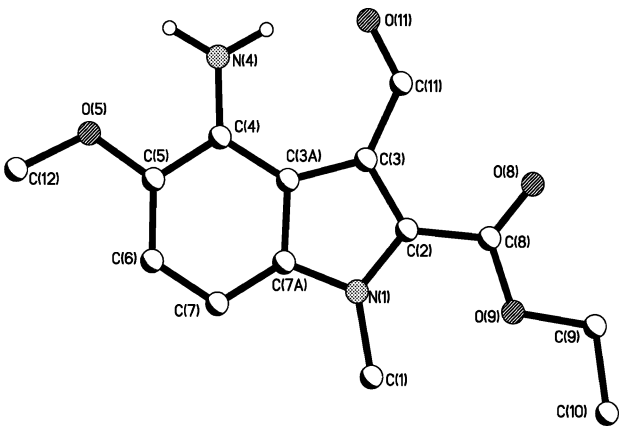
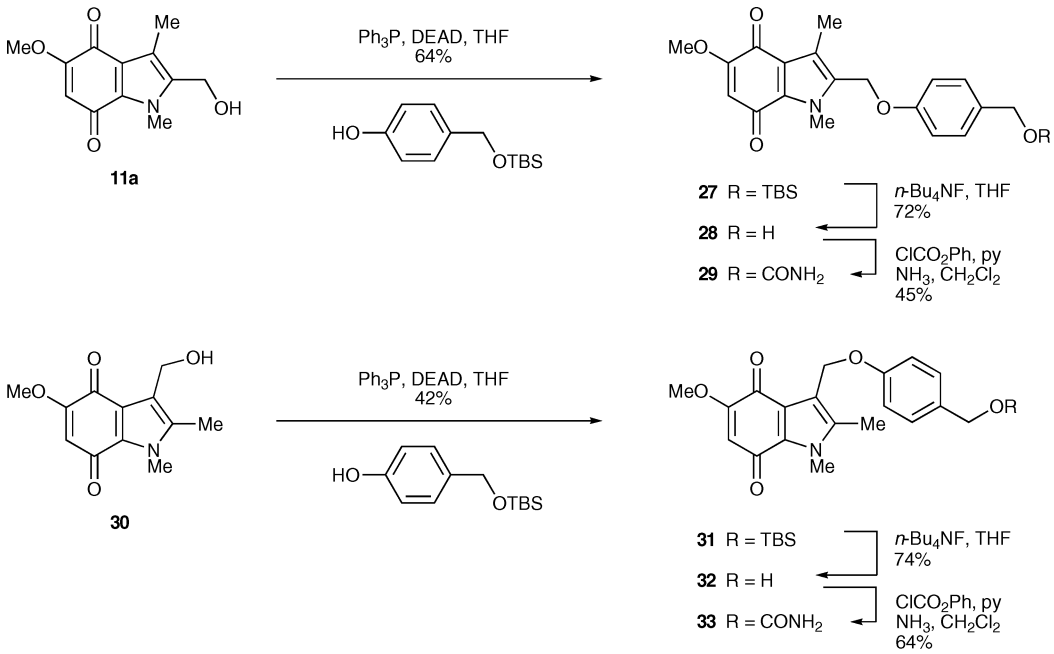
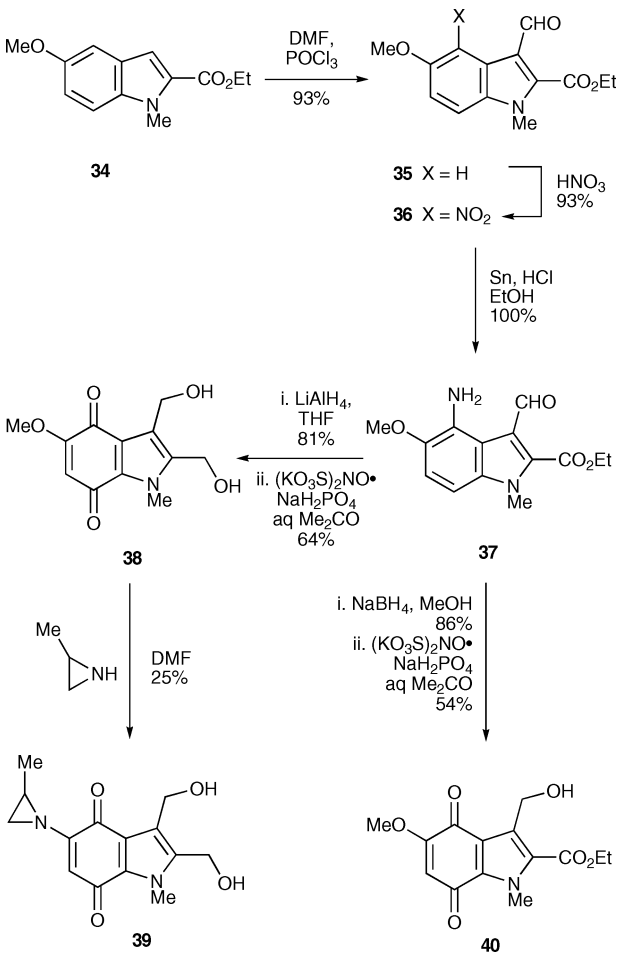


Fig. 2 X-ray crystal structure of ethyl 4-amino-3-formyl-5-methoxy-1-methylindole-2-carboxylate **37**.



Scheme 8



Scheme 9

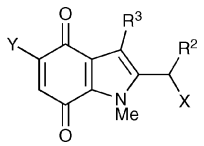
5-methoxy group with 2-methylaziridine gave the corresponding aziridinylindolequinone **39**. Amine **37** was also converted into the indolequinone **40** by reduction of the aldehyde and oxidation to the quinone (Scheme 9).

Enzyme studies

In related work, using physical chemical kinetic studies, we have demonstrated that reductive elimination from the (indol-2-yl)methyl position occurs most readily *via* the hydroquinone,[43] which in biological systems is most readily accessed by two-electron reductive activation by the enzyme NQO1. Therefore we have examined the ability of a number of our novel 2-substituted indolequinones to act as substrates for NQO1. Previous studies have demonstrated that indolequinones bearing good leaving groups at the (indol-3-yl)methyl position are poor substrates for the two-electron reducing enzyme NQO1.[20,21] In fact the indolequinone analogous to compound **13a** with the 4-nitrophenoxy group at the (indol-3-yl)methyl position, *i.e.* 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxy)methylindole-4,7-dione, a compound known as ES936, is a potent mechanism-based inhibitor of the enzyme.[44–46]

In our earlier studies on indolequinone metabolism by recombinant human NQO1, we used an HPLC system that is capable of quantifying both NADH oxidation and quinone reduction.[1,20] To simplify comparisons with these earlier compounds, we have also used this method in the present study. Quinone reduction is reversible due to redox cycling of the hydroquinone, so results (Table 1) are reported as μmol NADH oxidized min^{-1} mg^{-1} NQO1. This HPLC method gives average rates of reduction over a 30–40 minute period. An alternative spectrophotometric method for determining quinone metabolism uses cytochrome *c* as the terminal electron acceptor and gives initial rates of reduction.[34] This assay generally gives higher reduction rates than the HPLC

Table 1 Metabolism of indolequinones by recombinant human NQO1 and cytotoxicity of representative indolequinones to BE-NQ (NQO1-rich) and BE-WT (NQO1-deficient) human colon carcinoma cell lines



Cpd	X	Y	R^2	R^3	Metabolism (μmol min^{-1} mg^{-1})	BE-NQ IC_{50} (μM)	BE-WT IC_{50} (μM)
9	OH	OMe	H	H	20.4 ± 2.7		
10	OH	OMe	Me	H	0.44 ± 0.08		
11a	OH	OMe	H	Me	2.49 ± 1.27	>50	>50
11b	OH	OMe	H	Et	0.19 ± 0.02		
12	[a]	OMe	[a]	Me	0.35 ± 0.06		
13a	OAr[b]	OMe	H	Me	0.23 ± 0.04		
13b	OAr[b]	OMe	H	Et	0.09 ± 0.02		
14	H	OMe	Me	Me	0.41 ± 0.08		
24	$OCONH_2$	OMe	H	Me	0.30 ± 0.02	>50	>50
25	OH	MeAz[c]	H	Me	0.27 ± 0.03	29.8 ± 8.5	>50
26	$OCONH_2$	MeAz[c]	H	Me	0.70 ± 0.09	17.6 ± 2.1	>50
29	H	OMe	H	CH_2OAr[d]	0.49 ± 0.08		
33	OAr[d]	OMe	H	Me	0.53 ± 0.08		
3b8	OH	OMe	H	CH_2OH	12.0 ± 2.0	>50	>50
39	OH	MeAz[c]	H	CH_2OH	0.61 ± 0.14	4.93 ± 1.34	15.4 ± 3.8
40	[e]	OMe	[e]	CH_2OH	65.8 ± 5.0		

[a] Indole 2-substituent is $CH{=}CHCH_2OH$. [b] Ar = 4-nitrophenyl. [c] MeAz = 2-methylaziridinyl. [d] Ar = 4-carbamoylmethylphenyl. [e] Indole 2-substituent is COOEt.

method, but the relative order of metabolism is essentially the same with two methods (data not shown). Cytotoxicity studies were also performed on representative quinones with cell survival being measured using the MTT colorimetric assay. As in our previous work,[1] we have used the BE human colon carcinoma cell line stably transfected with human NQO1 cDNA.[47] The wild type BE cells have no measurable NQO1 activity whereas activity in the transfected cells was 664 ± 49 nmol min^{-1} mg^{-1} total cell protein using dichlorophenolindophenol as the standard electron acceptor. Hence, we have compared quinone toxicity (Table 1) in the wild-type BE cells (BE-WT) and the NQO1-transfected BE cells (BE-NQ).

The enzyme data show that the new indolequinones are generally quite poor substrates for NQO1, although compounds with leaving groups are substrates as opposed to inhibitors. Compounds **13a** and **24** with leaving groups at the (indol-2-yl)methyl position had no inhibitory effect on NQO1 (data not shown) whereas analogous 3-substituted indolequinones (including the aforementioned ES936) completely inactivated the enzyme.[20] Still, these two compounds, **13a** and **24**, were relatively poor substrates for NQO1. The NQO1 active site can accommodate a range of substituents at the indole-2-position since these substituents are normally oriented towards the entrance to the active site.[21,32] Other factors appear to be more important than steric bulk at C-2 to the metabolism of these compounds. For example, most of the better substrates have hydroxymethyl groups at C-2, a finding that was also observed with the C-3 substituted indolequinones[20] suggesting the possibility that hydrogen bonding with active site residues may be important. The best substrate, **40**, has an electron-withdrawing ester group at C-2, demonstrating the importance of electronic effects on quinone reduction by NQO1. With regard to the indole-3-position, steric effects do appear to be more important here since ethyl substituents lead to much lower rates of reduction than methyl substituents.

A selection of indolequinones was chosen for cytotoxicity evaluation in the isogenic cell lines BE-NQ and BE-WT. As expected, only indolequinones **25**, **26** and **39** showed detectable toxicity to either cell line, since they contain methylaziridine alkylating groups at C-5. With potential leaving groups at C-2 and/or C-3, they also have the ability to form DNA cross-links, a more toxic lesion than simple alkylation. All three methylaziridinyl indolequinones were selectively toxic to the NQO1-transfected BE-NQ cells. Indolequinones **26** and **39** were >3 times more toxic to the BE-NQ cells than the BE-WT cells whereas **25** was *ca.* 1.6 times more toxic. Selectivity was related to substrate efficiency as **26** and **39** were also better substrates for NQO1 than **25**.

The results presented here complement our previous work on bioreductive activation of indolequinone antitumour agents by NQO1.[1,20] Novel indolequinones have been synthesized, characterized and studied biologically as substrates for recombinant human NQO1 and for their toxicity to NQO1-rich tumour cells. These data will be valuable for the design and development of NQO1-directed antitumour agents that are either intended to act directly as cytotoxic agents or to release a secondary cytotoxic metabolite by reductive fragmentation.

Experimental

Chemistry

General procedures. Commercially available reagents were used throughout without purification unless otherwise stated. Light petroleum refers to the fraction with bp 40–60 °C and was distilled before use. Ether refers to diethyl ether. Reactions were

routinely carried out under a nitrogen or argon atmosphere. Analytical thin layer chromatography was carried out on aluminium-backed plates coated with Merck Kieselgel 60 GF_{254}, and 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. Fully characterized compounds were chromatographically homogeneous. Infrared spectra were recorded in the range 4000–600 cm^{-1} using Nicolet Magna FT-550 or Perkin Elmer FT-1600 spectrometers. NMR spectra were carried out on Bruker 300 and 400 MHz instruments (^1H frequencies, corresponding ^{13}C frequencies are 75 and 100 MHz). Chemical shifts are quoted in ppm with TMS as internal standard. *J* values are recorded in Hz. In the ^{13}C spectra, signals corresponding to CH, CH_2 or CH_3 groups, as assigned from DEPT, are noted; all others are C. High and low resolution mass spectra were recorded on a Micromass GCT TDF High Resolution mass spectrometer, or at the EPSRC Mass Spectrometry Service (Swansea).

The synthesis of the 16 compounds described in Table 1 is included below. The experimental details and characterization data for all other compounds are in the Electronic Supplementary Information†.

2-Hydroxymethyl-5-methoxy-1-methylindole-4,7-dione 9. (a) A catalytic quantity of palladium/carbon (10%; *ca.* 25 mg) was added to a solution of 4-benzyloxy-5-methoxy-1-methylindole-2-methanol[10] (0.235 g, 0.79 mmol) in methanol (30 ml). The reaction mixture was stirred under an atmosphere of hydrogen for 12 h. The catalyst was removed by filtering the crude mixture through a pad of Celite. The filtrate was evaporated *in vacuo* and the residue purified by chromatography (9/1 dichloromethane/ethyl acetate) to yield *4-hydroxy-5-methoxy-1-methylindole-2-methanol* as an off white crystalline solid, (0.095 g, 58%), mp 168–171 °C; (Found: M^+, 207.0898. $C_{11}H_{13}NO_3$ requires 207.0895); ν_{max} (KBr)/cm^{-1} 3447, 2947, 1514; δ_H (300 MHz; d_6-acetone) 7.60 (1 H, s, ArO*H*), 6.94 (1 H, d, *J* 8.7, ArH), 6.80 (1 H, dd, *J* 8.7, 0.8, ArH), 6.42 (1 H, d, *J* 0.6, 3-H), 4.74 (2 H, d, *J* 5.5, CH_2OH), 4.14 (1 H, t, *J* 5.5, CH_2O*H*), 3.82 (3 H, s, OMe), 3.75 (3 H, s, NMe); δ_C (100 MHz; d_6-acetone) 139.5, 139.4, 139.2, 135.7, 117.8, 110.6 (CH), 99.6 (CH), 97.3 (CH), 58.0 (Me), 56.4 (CH_2), 29.2 (Me); *m*/*z* (EI) 207 (M^+, 67%), 192 (98), 164 (34).

(b) To a solution of the above phenol (0.078 g, 0.37 mmol) in acetone (10 ml) was added a solution of potassium nitrosodisulfonate (0.31 g, 1.1 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 10 ml). The mixture was stirred at room temperature for 1 h, extracted with ethyl acetate, the organic layer washed with water, dried ($MgSO_4$) and concentrated. The crude product was purified by chromatography (9/1 dichloromethane/ethyl acetate) to yield the *title compound* as an orange crystalline solid (0.46 g, 55%), mp 208–210 °C (from dichloromethane/ether); (Found: C, 59.6; H, 4.9; N, 6.2. $C_{11}H_{11}NO_4$ requires C, 59.7; H, 5.0; N, 6.3%); λ_{max} (MeOH) 440 (log ε 2.98), 336 (3.32), 280 (3.87) nm; ν_{max} (KBr)/cm^{-1} 3380, 2930, 1679, 1632, 1587; δ_H (300 MHz; $CDCl_3$) 6.53 (1 H, s, 3-H), 5.64 (1 H, s, 6-H), 4.66 (2 H, d, *J* 4.2, CH_2OH), 4.00 (3 H, s, OMe), 3.82 (3 H, s, NMe), 2.01 (1 H, br t, CH_2O*H*); δ_C (100 MHz; $CDCl_3$) 179.2, 177.1, 160.1, 139.6, 130.8, 123.5, 107.6 (CH), 107.2 (CH), 56.5 (Me), 56.3 (CH_2), 32.8 (Me); *m*/*z* (EI) 221 (M^+, 8%), 150 (16), 122 (24), 69 (100).

2-(1-Hydroxyethyl)-5-methoxy-1-methylindole-4,7-dione 10. To a solution of 4-benzyloxy-5-methoxy-1-methylindole-2-carboxaldehyde[35] (0.135 g, 0.46 mmol) in THF (20 ml) at −10 °C was added methylmagnesium bromide (3 M; 0.4 ml, 1.2 mmol). The mixture was stirred for 1 h, quenched with saturated ammonium chloride solution and extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and concentrated. The crude product was used directly in the next step without purification. To a solution of the benzyl ether in ethyl acetate was added a catalytic quantity of palladium on carbon (5%; 15 mg) and stirred overnight under an atmosphere of hydrogen. The crude mixture was filtered through a pad of Celite. The solvent was removed *in vacuo* and used directly in the next step without purification. To a solution of the phenol in acetone (20 ml) was added a solution of potassium nitrosodisulfonate (0.35 g, 1.3 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 20 ml). The mixture was stirred at room temperature overnight. The acetone was removed *in vacuo*, and the residue was extracted with ethyl acetate and washed with saturated ammonium chloride solution. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by chromatography (ethyl acetate) and recrystallized (ethyl acetate/hexane) to yield the *title compound* (0.76 g, 71%), as an orange solid, mp 166–168 °C; (Found: C, 60.3; H, 5.5; N, 5.6. $C_{12}H_{13}NO_4.0.2H_2O$ requires C, 60.3; H, 5.6; N, 5.9%); (Found: MH^+, 236.0924. $C_{12}H_{13}NO_4$ + H requires 236.0923); λ_{max} (DMSO)/nm 444 (log ε 3.18), 336 (3.52), 280 (4.04); ν_{max} (KBr)/cm^{-1} 3483, 2976, 2930, 1671, 1634, 1588; δ_H (300 MHz; $CDCl_3$) 6.48 (1 H, s, 3-H), 5.55 (1 H, s, 6-H), 4.85 (1 H, q, *J* 6.5, C*H*Me), 3.97 (3 H, s, OMe), 3.78 (3 H, s, NMe), 1.60 (3 H, d, *J* 6.5, CH*Me*); OH not observed; δ_C (100 MHz; $CDCl_3$) 179.2, 177.1, 160.0, 143.5, 130.5, 123.5, 107.2 (CH), 105.0 (CH), 61.9 (CH), 56.4 (Me), 32.9 (Me), 22.1 (Me); *m*/*z* (EI) 235 (M+, 33%), 220 (40), 192 (17).

2-Hydroxymethyl-5-methoxy-1,3-dimethylindole-4,7-dione 11a. To a suspension of lithium aluminium hydride (0.174 g, 4.58 mmol) in THF (30 ml) at 0 °C was added a solution of methyl 4-amino-5-methoxy-1,3-dimethylindole-2-carboxylate **17a** (0.379 g, 1.52 mmol) in THF (15 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 4-aminoindole in acetone (100 ml) was added a solution of potassium nitrosodisulfonate (1.23 g, 4.59 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 100 ml). The mixture was stirred at room temperature for 1 h. The acetone was removed *in vacuo*, the 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 chromatography, eluting with dichloromethane/ethyl acetate (3:1), to give the *title compound* as an orange solid (0.210 g, 58%), mp 223–224 °C (from ethyl acetate/hexane); (Found: C, 59.9; H, 5.7; N, 5.8. $C_{12}H_{13}NO_4.0.3H_2O$ requires C, 59.9; H, 5.7; N, 5.8%); (Found: M^+, 235.0845. $C_{12}H_{13}NO_4$ requires 235.0844); λ_{max} (MeOH)/nm 448 (log ε 3.26), 356 (3.43), 280 (4.05); ν_{max} (KBr)/cm^{-1} 3452, 3053, 2935, 1665, 1642, 1606, 1503; δ_H (300 MHz; $CDCl_3$) 5.63 (1 H, s, 6-H), 4.65 (2 H, d, *J* 5.4, CH_2OH), 4.02 (3 H, s, OMe), 3.81 (3 H, s, NMe), 2.33 (3 H, s, Me), 1.67

(1 H, t, *J* 5.4, CH_2OH); δ_C (75 MHz; $CDCl_3$) 179.1, 178.0, 160.1, 136.2, 129.6, 121.4, 120.7, 107.0 (CH), 56.5 (Me), 53.1 (CH_2), 32.8 (Me), 9.9 (Me); *m/z* (CI) 236 (MH^+, 96%), 220 (22), 206 (40), 200 (100).

3-Ethyl 2-hydroxymethyl-5-methoxy-1-methylindole-4,7-dione 11b. To a suspension of lithium aluminium hydride (0.056 g, 1.5 mmol) in THF (20 ml) at 0 °C was added a solution of methyl 4-amino-3-ethyl-5-methoxy-1-methylindole-2-carboxylate **17b** (0.15 g, 0.59 mmol) in THF (15 ml). The mixture 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. To a solution of the amino indole in acetone (40 ml) was added a solution of potassium nitrosodisulfonate (0.47 g, 1.7 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 40 ml). The mixture was stirred at room temperature for 1 h. The acetone was removed *in vacuo*, the 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 chromatography, eluting with ethyl acetate/dichloromethane (1 : 4), to yield the *title compound* as an orange solid (0.081 g, 54%), mp 192–194 °C (from ethyl acetate/hexane); (Found: C, 62.2; H, 6.0, N, 5.4. $C_{13}H_{15}NO_4 \cdot 0.1H_2O$ requires C, 62.2; H, 6.1, N, 5.6%); (Found: M^+, 249.1002. $C_{13}H_{15}NO_4$.requires 249.1001); λ_{max} (MeOH)/nm 448 (log ε 3.21), 360 (3.38), 280 (4.07); ν_{max} (KBr)/cm^{-1} 3406, 2966, 2925, 1667, 1644, 1601; δ_H (300 MHz; $CDCl_3$) 5.60 (1 H, s, 6-H), 4.63 (2 H, d, *J* 4.4, CH_2OH), 4.00 (3 H, s, OMe), 3.79 (3 H, s, NMe), 2.75 (2 H, q, *J* 7.5, CH_2Me), 1.99 (1 H, br t, CH_2OH), 1.12 (3 H, t, *J* 7.5, CH_2Me); δ_C (75 MHz; $CDCl_3$) 179.2, 177.6, 160.1, 135.8, 129.8, 127.7, 120.7, 106.9 (CH), 56.5 (Me), 53.0 (CH_2), 32.8 (Me), 17.9 (CH_2), 15.7 (Me); *m/z* (EI) 250 (MH^+, 100%), 236 (27), 234 (48), 220 (47), 206 (52).

(*E*)-2-(3-Hydroxypropenyl)-5-methoxy-1,3-dimethylindole-4,7-dione 12. To a solution of (*E*)-3-(4-amino-5-methoxy-1,3-dimethylindol-2-yl)prop-2-en-1-ol **21** (416 mg, 1.69 mmol) in acetone (95 ml) was added a solution of potassium nitrosodisulfonate (1.86 g, 6.77 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 76 ml). The mixture was stirred at room temperature for 1 h. The acetone was removed *in vacuo*, and the residue was stirred at room temperature in a 1 : 1 mixture of hydrochloric acid (2 M) and acetone (200 ml) for 1 h. The acetone was removed *in vacuo* and the residue was extracted with dichloromethane. The organic layer was washed with water, dried ($MgSO_4$) filtered and evaporated under reduced pressure. The crude material was purified by chromatography eluting with ethyl acetate/light petroleum (1 : 1) to yield the *title compound* (262 mg, 52%) as an orange crystalline solid, mp 206–207 °C; (Found: M^+, 261.0991. $C_{14}H_{15}NO_4$ requires 261.1001); λ_{max} (acetonitrile)/nm 472 (log ε 3.37), 351 (3.42), 285 (4.04), 258 (4.09); ν_{max} (KBr)/cm^{-1} 3423, 2926, 2849, 1671, 1639, 1595, 1491, 1451, 1338, 1226, 1170, 1150, 1098, 1030; δ_H (300 MHz; $CDCl_3$) 6.50 (1 H, dt, *J* 12.1, 1.3, =CH), 6.21 (1 H, dt, *J* 12.1, 3.7, =CH), 5.63 (1 H, s, 6-H), 4.41 (2 H, dd, *J* 3.7, 1.3, CH_2OH), 3.95 (3 H, s, NMe), 3.80 (3 H, s, OMe), 2.41 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 179.8, 178.2, 159.9, 135.44 (CH), 135.40, 132.6, 122.1, 120.8, 117.0 (CH), 107.2 (CH), 63.4 (CH_2), 56.4 (Me), 33.0 (Me), 11.3 (Me); *m/z* (EI) 261 (M^+, 100%), 244 (31), 230 (53), 218 (80), 187 (29), 176 (32), 158 (34).

Mitsunobu reactions: general method. Diethyl azodicarboxylate (64 μl, 0.41 mmol) was added to a stirred solution of the 2-hydroxyalkylindolequinone (0.12 mmol), 4-nitrophenol (44 mg, 0.32 mmol) and triphenylphosphine (84 mg, 0.32 mmol) in dry THF (10 ml) at 0 °C. The reaction mixture was stirred at room temperature overnight. The mixture was evaporated and the residue was dissolved in ethyl acetate and washed with hydrochloric acid (1 M; 10 ml) and sodium hydroxide (1 M; 10 ml), dried ($MgSO_4$) and concentrated. The crude material was purified by chromatography.

5-Methoxy-1,3-dimethyl-2-(4-nitrophenoxy)methylindole-4,7-dione 13a. Purified by chromatography eluting with hexane/ethyl acetate (1:1) to give the *title compound* (61%) as a yellow solid, mp 230–232 °C (from ethyl acetate/hexane); (Found: C, 60.3; H, 4.3; N, 7.5. $C_{18}H_{16}N_2O_6$ requires C, 60.6; H, 4.5; N, 7.9%); λ_{max} (MeOH) 436 (log ε 3.39), 284 (4.28) nm; ν_{max} (KBr)/cm^{-1} 3114, 3068, 290, 2909, 2838, 1676, 1646, 1596; δ_H (300 MHz; $CDCl_3$) 8.25 (2 H, d, *J* 9.1, ArH), 7.06 (2 H, d, *J* 9.1, ArH), 5.70 (1 H, s, 6-H), 5.08 (2 H, s, CH_2), 4.00 (3 H, s, OMe), 3.83 (3 H, s, NMe), 2.40 (3 H, s, Me); δ_C (75 MHz; $CDCl_3$) 179.2, 177.8, 162.8, 160.3, 142.2, 130.9, 130.3, 126.1 (CH), 122.5, 121.4, 114.7 (CH), 107.1 (CH), 59.1 (CH_2), 56.6 (Me), 32.9 (Me), 10.2 (Me); *m/z* (CI) 357 (MH^+, 95), 298 (22).

3-Ethyl-5-methoxy-1-methyl-2-(4-nitrophenyl)oxymethyl)indole-4,7-dione 13b. Purified by chromatography eluting with hexane/ethyl acetate (5:1) to yield the *title compound* (64%) as a yellow solid, mp 219–221 °C (from ethyl acetate/hexane); (Found: C, 61.4; H, 4.8, N, 7.4. $C_{19}H_{18}N_2O_6$ requires C, 61.6; H, 4.9, N, 7.6%; λ_{max} (MeOH)/nm 440 (log ε 3.26), 288 (4.45); ν_{max} (KBr)/cm^{-1} 3114, 3073, 2960, 2934, 1677, 1642, 1592; δ_H (300 MHz; $CDCl_3$) 8.26 (2 H, d, *J* 9.1, ArH), 7.07 (2 H, d, *J* 9.1, ArH), 5.70 (1 H, s, 6-H), 5.06 (2 H, s, CH2), 4.00 (3 H, s, OMe), 3.84 (3 H, s, NMe), 2.82 (2 H, q, *J* 7.5, CH_2Me), 1.16 (3 H, t, *J* 7.5, CH_2Me); δ_C (75 MHz; acetone-d) 179.7, 177.5, 164.4, 161.3, 142.8, 132.3, 131.0, 129.2, 126.6 (CH), 121.4, 116.1 (CH), 107.7 (CH), 60.0 (CH_2), 56.9 (Me), 33.0 (Me), 18.3 (CH_2), 15.7 (Me); *m/z* (CI) 371 (MH^+, 8%), 234 (14), 206 (14), 127 (28), 110 (100).

2-Ethyl-5-methoxy-1,3-dimethylindole-4,7-dione 14. To a solution of 4-amino-2-ethyl-5-methoxy-1,3-dimethylindole **23** (32 mg, 0.15 mmol) in acetone (9 ml) was added a solution of potassium nitrosodisulfonate (159 mg, 0.58 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 7.3 ml). The mixture was stirred at room temperature for 1 h. The acetone was removed *in vacuo*, and the residue was stirred at room temperature in a 1:1 mixture of hydrochloric acid (3 M) and acetone for 1 h. The acetone was removed *in vacuo*, and the resulting residue was extracted with dichloromethane. The organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated under reduced pressure. The crude material was purified by chromatography eluting with ethyl acetate/light petroleum (1 : 1) to yield the *title compound* (30 mg, 89%) as a red crystalline solid, mp 200–201 °C; (Found: M^+, 233.1050. $C_{13}H_{15}NO_3$ requires 233.1052); λ_{max} (acetonitrile)/nm 461 (log ε 3.35), 356.5 (3.44), 273 (3.99); ν_{max} (KBr)/cm^{-1} 3434, 2967, 2922, 2851, 1666, 1636, 1596, 1556, 1506, 1456, 1338, 1221, 1156; δ_H (300 MHz; $CDCl_3$) 5.55 (1 H, s, 6-H), 3.87 (3 H, s, OMe),

3.77 (3 H, s, NMe), 2.58 (2 H, q, *J* 7.6, C*H*$_2$Me), 2.24 (3 H, s, Me), 1.11 (3 H, t, *J* 7.6, CH$_2$*Me*); δ_C (75 MHz; $CDCl_3$) 178.5, 178.3, 159.6, 140.7, 128.1, 122.0, 118.3, 106.8 (CH), 56.3 (Me), 32.2 (Me), 16.7 (Me), 13.4 (CH_2), 9.8 (Me); *m*/*z* (EI) 233 (M^+, 80%), 218 (100), 203 (16), 190 (34).

2-Carbamoyloxymethyl-5-methoxy-1,3-dimethylindole-4,7-dione 24. (a) To a solution of 2-hydroxymethyl-5-methoxy-1,3-dimethylindole-4,7-dione **11a** (81 mg, 0.37 mmol) in dry pyridine (6.1 ml) at 0 °C was added phenyl chloroformate (184 µl, 1.48 mmol). The reaction mixture was stirred at 0 °C for 2 h and at room temperature overnight. The reaction mixture was evaporated under reduced pressure and the residue was purified by chromatography eluting with ethyl acetate/light petroleum (1 : 3) to yield the *phenyl carbonate* (103 mg, 83%) as a yellow crystalline solid, mp 124–126 °C, used without further purification; ν_{max} (KBr)/cm^{-1} 3438, 2926, 1757, 1685, 1641, 1597, 1505, 1269, 1225, 1161; δ_H (300 MHz; $CDCl_3$) 7.38 (2 H, dd, *J* 8.0, 7.5, ArH), 7.24 (1 H, t, *J* 8.0, ArH), 7.16 (2 H, d, *J* 7.5, ArH), 5.67 (1 H, s, 6-H), 5.27 (2 H, s, CH_2), 4.03 (3 H, s, NMe), 3.81 (3 H, s, OMe), 2.40 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 179.2, 177.7, 160.4, 153.4, 150.9, 130.8, 130.2, 129.6 (CH), 126.3 (CH), 123.6, 121.4, 120.9 (CH), 107.1 (CH), 58.2 (CH_2), 56.5 (Me), 32.8 (Me), 10.0 (Me).

(b) A solution of the above phenyl carbonate (84 mg, 0.28 mmol) in dry dichloromethane (14 ml) was cooled to −78 °C. Ammonia gas was bubbled into the solution for 20 min. The mixture was stirred for 6 h whilst allowing it to warm up to room temperature The reaction mixture was evaporated under reduced pressure and the residue was purified by chromatography eluting with ethyl acetate to yield the *title compound* (47 mg, 62%) as an orange crystalline solid, mp 246–247 °C; (Found: MH^+, 279.0981. $C_{13}H_{14}N_2O_5$ + H requires 279.0981); λ_{max} (acetonitrile)/nm 428 (log ε 3.15), 344 (3.34), 288 (3.96); ν_{max} (KBr)/cm^{-1} 3436, 3300, 2916, 1734, 1634, 1596, 1500, 1392, 1325, 1254, 1225, 1158, 1037; δ_H (300 MHz; $CDCl_3$) 6.63 (2 H, bs, NH_2), 5.79 (1 H, s, 6-H), 5.00 (2 H, s, CH_2), 3.89 (3 H, s, NMe), 3.74 (3 H, s, OMe), 2.23 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 179.0, 177.7, 160.2, 156.6, 134.1, 129.3, 121.1, 121.0, 107.5 (CH), 57.0 (Me), 53.8 (CH_2), 32.8 (Me), 10.1 (Me); *m*/*z* (CI) 279 (MH^+, 25%), 192 (55), 112 (65), 98 (100), 84 (60).

2-Hydroxymethyl-1,3-dimethyl-5-(2-methylaziridinyl)indole-4,7-dione 25. To a solution of 2-hydroxymethyl-5-methoxy-1,3-dimethylindole-4,7-dione **11a** (100 mg, 0.46 mmol) in DMF (16 ml) was added 2-methylaziridine (1.61 ml, 22.8 mmol). The reaction mixture was stirred at room temperature for 4 days. The reaction mixture was diluted with dichloromethane, washed with brine (3 × 150 ml) and deionized water (2 × 150 ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield the *title compound* (140 mg, 64%) as a red crystalline solid, mp 148–150 °C; (Found: M^+, 260.1160. $C_{14}H_{16}N_2O_3$ requires 260.1161); λ_{max} (acetonitrile)/nm 468 (log ε 3.30), 356 (3.46), 308 (3.99); ν_{max} (KBr)/cm^{-1} 3360, 2921, 1660, 1624, 1580, 1492, 1463, 1272, 1151, 1007; δ_H (300 MHz; $CDCl_3$) 5.68 (1 H, s, 6-H), 4.59 (2 H, s, CH_2), 3.96 (3 H, s, NMe), 2.30 (3 H, s, Me), 2.23 (1 H, m, CH), 2.08 (1 H, d, *J* 3.7, CH), 2.04 (1 H, d, *J* 5.9, CH), 1.42 (3 H, d, *J* 5.5, CH*Me*); OH not observed; δ_C (100 MHz; $CDCl_3$) 179.7, 178.9, 157.2, 136.1, 129.7, 121.9, 120.5, 116.8 (CH), 53.0 (CH_2), 36.2 (CH), 34.5 (CH_2), 32.7 (Me), 17.7 (Me), 9.9 (Me); *m*/*z* (EI) 260 (M^+, 61%), 243 (35), 196 (32), 149 (100), 91 (46), 71 (48), 57 (68).

2-Carbamoyloxymethyl-5-(2-methylaziridinyl)-1,3-dimethylindole-4,7-dione 26. To a solution of 2-carbamoyloxymethyl-5-methoxy-1,3-dimethylindole-4,7-dione **24** (41 mg, 0.15 mmol) in DMF (5 ml) was added 2-methylaziridine (517 µl, 7.3 mmol). The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was diluted with dichloromethane, washed with brine (2 × 150 ml) and deionized water (2 × 150 ml), dried ($MgSO_4$), filtered and evaporated under reduced pressure to yield the *title compound* (30 mg, 67%) as a red crystalline solid, mp 160–162 °C; (Found: M^+, 303.1214. $C_{15}H_{17}N_3O_4$ requires 303.1219); λ_{max} (acetonitrile)/nm 464 (log ε 3.37), 344 (3.55), 312 (4.06); ν_{max} (KBr)/cm^{-1} 3436, 3288, 2924, 1729, 1682, 1639, 1586, 1498, 1405, 1321, 1167; δ_H (300 MHz; $CDCl_3$) 5.75 (1 H, s, 6-H), 5.09 (2 H, s, CH_2), 4.71 (2 H, bs, NH_2), 3.96 (3 H, s, NMe), 2.37 (3 H, s, Me), 2.25 (1 H, m, CH), 2.10 (1 H, d, *J* 3.7, CH), 2.06 (1 H, d, *J* 5.8, CH), 1.43 (3 H, d, *J* 5.5, CH*Me*); δ_C (100 MHz; $CDCl_3$) 179.8, 179.2, 157.6, 156.0, 131.7, 130.2, 122.6, 121.9, 116.6 (CH), 54.9 (CH_2), 36.1 (CH), 34.5 (CH_2), 32.6 (Me), 17.7 (Me), 9.9 (Me); *m*/*z* (EI) 303 (M^+, 8%), 279 (16), 196 (28), 167 (30), 149 (100), 104 (16), 91 (26).

(5-Methoxy-1,3-dimethyl-4,7-dioxoindol-2-yl)methyloxyphenyl-4-methylcarbamate 29. (a) To a stirred solution of 2-[(4-hydroxymethyl)phenoxymethyl]-5-methoxy-1,3-dimethyl-indole-4,7-dione 28 (33 mg, 0.097 mmol) in pyridine (5 ml) was added an excess of phenyl chloroformate (500 µl). The reaction mixture was stirred at room temperature overnight and quenched by addition of water. The reaction mixture was extracted with dichloromethane, washed twice with aqueous copper sulfate (10%), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue obtained was purified by chromatography, eluting with ethyl acetate–light petroleum (1 : 3) to yield the *carbonate intermediate*, 2-(4-phenylcarbonatemethyl-phenoxymethyl)-5-methoxy-1,3-dimethyl-indole-4,7-dione, as an orange semi solid which was used in the next step with no further purification.

(b) The *carbonate intermediate* was dissolved in dry dichloromethane (7 ml) and the solution was cooled down to −78 °C. Ammonia was bubbled into the solution until the flask is filled up with liquid ammonia (*ca.* 25 ml). The reaction mixture was left to warm up to room temperature until all the ammonia has disappeared. The reaction mixture was evaporated off under reduced pressure and purified by chromatography, eluting with ethyl acetate/dichloromethane (1 : 2) to yield the *title compound* (15 mg, 45%) as an orange solid; mp 212–213 °C; λ_{max} (acetonitrile)/nm 280 (log ε 4.11), 348 (3.37), 432 (3.17); ν_{max} (KBr)/cm^{-1} 3413, 3258, 3207, 2915, 1666, 1692, 1637, 1592, 1493, 1333, 1222, 1175, 1160; δ_H (300 MHz; $CDCl_3$) 7.34 (2 H, d, *J* 8.6, ArH), 6.96 (2 H, d, *J* 8.6, ArH), 5.67 (1 H, s, 6-H), 5.05 (2 H, s, OCH_2), 4.98 (2 H, s, OCH_2), 4.68 (2 H, brs, NH_2), 3.99 (3 H, s, NMe), 3.82 (3 H, s, OMe), 2.37 (3 H, s, Me); δ_C (100 Hz; $CDCl_3$) 179.5, 178.2, 160.5, 158.3, 156.9, 132.5, 130.5 (CH), 129.9, 129.2, 122.4, 121.7, 115.1 (CH), 107.3 (CH), 66.9 (CH_2), 58.8 (CH_2), 56.8 (Me), 33.2 (Me), 10.4 (Me).

(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyloxyphenyl-4-methylcarbamate 33. (a) To a stirred solution of 5-methoxy-3-(4-hydroxymethylphenoxy)methyl-1,2-dimethylindole-4,7-dione

32 (74 mg, 0.22 mmol) in dry pyridine (10 ml) was added an excess of phenyl chloroformate (1 ml). The reaction mixture was stirred at room temperature overnight and quenched by addition of water. The reaction mixture was extracted with dichloromethane, washed twice with aqueous copper sulfate (10%), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by chromatography, eluting with ethyl acetate–light petroleum (1 : 3) to yield (5-methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyloxyphenyl-4-methylphenyl carbonate (66 mg, 67%) as an orange solid; mp 41–43 °C; (Found: M + NH_4^+, 479.1818. $C_{26}H_{23}NO_7$ + NH_4 requires 479.1818); λ_{max} (acetonitrile)/nm 280 (log ε 4.21), 336 (3.55), 452 (3.30); ν_{max} (KBr)/cm^{-1} 3426, 2922, 2853, 1764, 1675, 1637, 1598, 1510, 1460, 1229, 1179, 1156; δ_H (400 MHz; $CDCl_3$) 7.38 (4 H, m, ArH), 7.24 (1 H, m, ArH), 7.15 (2 H, d, *J* 8.0, ArH), 7.00 (2 H, d, *J* 8.0, ArH), 5.60 (1 H, s, 6-H), 5.30 (2 H, s, OCH_2), 5.18 (2 H, s, OCH_2), 3.86 (3 H, s, NMe), 3.79 (3 H, s, OMe), 2.28 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 179.1, 178.6, 160.0, 159.4, 154.1, 151.5, 138.5, 131.0 (CH), 130.0 (CH), 129.1, 127.5, 126.7, 126.4, 121.7, 121.5 (CH), 117.3, 115.5 (CH), 107.1 (CH), 70.7 (CH_2), 60.8 (CH_2), 56.9 (Me), 32.7 (Me), 10.3 (Me); *m*/*z* (ES) 479 (M + NH_4^+, 100), 232 (20), 213 (20).

(b) The above carbonate (65 mg, 0.14 mmol) was dissolved in dry dichloromethane (10 ml) and the solution was cooled down to −78 °C. Ammonia was bubbled into the solution until the flask was filled up with liquid ammonia (*ca.* 40 ml). The reaction mixture was left to warm up to room temperature until all the ammonia had disappeared. The reaction mixture was evaporated under reduced pressure and the residue purified by chromatography, eluting with ethyl acetate/dichloromethane (1 : 2) to yield the *title compound* (51 mg, 95%) as a red solid; mp 222–224 °C; (Found: M + NH_4^+, 402.1661. $C_{20}H_{20}N_2O_6$ + NH_4 requires 402.1665); λ_{max} (acetonitrile)/nm 280 (log ε 4.11), 340 (3.41), 452 (3.17); ν_{max} (KBr)/cm^{-1} 3433; 2921, 1732, 1682, 1632, 1598, 1509, 1455, 1328, 1224, 1159; δ_H (300 MHz; $CDCl_3$) 7.29 (2 H, d, *J* 6.6, ArH), 6.98 (2 H, d, *J* 6.6, ArH), 5.61 (1 H, s, 6-H), 5.30 (2 H, s, OCH_2), 5.02 (2 H, s, OCH_2), 4.62 (2 H, bs, NH_2), 3.88 (3 H, s, NMe), 3.81 (3 H, s, OMe), 2.30 (3 H, s, Me); δ_C (100 MHz; $CDCl_3$) 178.8, 178.2, 159.6, 158.6, 156.7, 138.1, 130.0 (CH), 128.7, 128.5, 121.3, 117.0, 115.0 (CH), 106.7 (CH), 66.8 (CH_2), 60.4 (CH_2), 56.5 (Me), 32.4 (Me), 9.9 (Me); *m*/*z* (ES) 402 (M + NH_4^+, 100), 276 (15).

2,3-Bis(hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione 38. (a) To a suspension of lithium aluminium hydride (200 mg, 5.22 mmol) in THF (7.7 ml) at 0 °C was added a solution of ethyl 4-amino-3-formyl-5-methoxy-1-methylindole-2-carboxylate **37** (360 mg, 1.31 mmol) in THF (3.5 ml). The mixture was allowed to warm up to room temperature, stirred for 30 min, and cooled to 0 °C before being quenched by the addition of water (1 ml), NaOH (1 M; 1 ml) and silica gel. The granular precipitate was filtered off through a pad of Celite. The filtrate was dried over $MgSO_4$, and concentrated *in vacuo* to yield 4-amino-5-methoxy-1-methylindole-2,3-dimethanol (249 mg, 81%) as a brown solid, which was used in the next step without any further purification.

(b) To a solution of 4-amino-5-methoxy-1-methylindole-2,3-dimethanol (242 mg, 1.03 mmol) in acetone (62 ml) was added a solution of potassium nitrosodisulfonate (1.10 g, 3.42 mmol) in sodium dihydrogen phosphate buffer (0.3 M; 49 ml). The reaction was stirred at room temperature for 1 h. The excess acetone was removed *in vacuo*. The residue was extracted with dichloromethane and concentrated. The residue was stirred at room temperature in a 1 : 1 mixture of hydrochloric acid (2 M) and acetone (120 ml) for 1 h. The acetone was removed *in vacuo*. The resulting residue was extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, filtered and evaporated under reduced pressure to yield the *title compound* (165 mg, 64%) as an orange crystalline solid, recrystallized from dichloromethane–pentane; mp 200–202 °C (lit.,[42] mp 191–193 °C); (Found: C, 57.2; H, 5.1; N, 6.0. $C_{12}H_{13}NO_5$ requires C, 57.4; H, 5.2; N, 5.6%); λ_{max} (acetonitrile)/nm 280 (log ε 3.97), 344 (3.38), 440 (3.11); ν_{max} (KBr)/cm^{-1} 3318, 2927, 1672, 1634, 1596, 1507, 1473, 1335, 1224, 1143, 1070; δ_H (400 MHz; d_6-DMSO) 5.81 (1 H, s, 6-H), 5.20 (1 H, t, *J* 5.4, OH), 4.71 (1 H, t, *J* 5.4, OH), 4.62 (2 H, d, *J* 5.4, OC*H*$_2$), 4.57 (2 H, d, *J* 5.4, OC*H*$_2$), 3.94 (3 H, s, NMe), 3.79 (3 H, s, OMe); δ_C (100 MHz; d_6-DMSO) 179.1, 177.8, 160.1, 139.7, 128.9, 123.4, 120.5, 107.3 (CH), 57.0 (Me), 53.5 (CH_2), 51.9 (CH_2), 32.9 (Me); *m*/*z* (EI) 251 (M^+, 10), 233 (65), 218 (20), 204 (10), 190 (25), 176 (25), 162 (20), 120 (25), 69 (100).

2,3-Bis(hydroxymethyl)-1-methyl-5-(2-methylaziridinyl)indole-4,7-dione 39. To a solution of 2,3-bis(hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione **38** (50 mg, 0.20 mmol) in DMF (7.4 ml) was added 2-methylaziridine (702 µl, 9.96 mmol). The reaction mixture was stirred at room temperature for 3 days. The reaction mixture was diluted with ethyl acetate and washed with brine (150 ml). The aqueous layer was re-extracted with ethyl acetate and was quenched with hydrochloric acid (2 M). The combined organic layer was dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude material was purified by chromatography on florisil, eluting with dichloromethane, then dichloromethane-ethyl acetate (1 : 1) to yield the *title compound* (14 mg, 25%) as a red crystalline solid; mp 77–79 °C; (Found: MH^+, 277.1185. $C_{14}H_{16}N_2O_4$ + H requires 277.1188); λ_{max} (acetonitrile)/nm 308 (log ε 4.04), 352 (3.58), 476 (3.18); ν_{max} (KBr)/cm^{-1} 3418, 2918, 1636, 1582, 1505, 1455, 1267, 1232, 1136; δ_H (300 MHz; $CDCl_3$) 5.72 (1 H, s, 6-H), 4.65 (2 H, s, OCH_2), 4.58 (2 H, s, OCH_2), 3.95 (3 H, s, NMe), 2.27 (1 H, m, CH), 2.10 (1 H, d, *J* 6.3, CH), 2.06 (1 H, d, *J* 11.1, CH), 1.41 (3 H, d, *J* 5.4, Me); OH not observed; δ_C (100 MHz; $CDCl_3$) 181.0, 179.2, 157.3, 136.5, 130.8, 123.7, 122.1, 117.1 (CH), 55.6 (CH_2), 53.0 (CH_2), 36.6 (CH), 34.9 (CH_2), 32.9 (Me), 17.9 (Me); *m*/*z* (ES) 299 (MNa^+, 10%), 277 (M^+, 40), 259 (10), 219 (10), 187 (10), 155 (20), 123 (40), 91 (100).

Ethyl 3-hydroxymethyl-5-methoxy-1-methyl-4,7-dioxoindole-2-carboxylate 40. (a) Sodium borohydride (18 mg, 0.45 mmol) was added portionwise to a solution of ethyl 4-amino-3-formyl-5-methoxy-1-methylindole-2-carboxylate **37** (50 mg, 0.18 mmol) in dry methanol (2 ml) at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 1 h. The mixture was cooled to 0 °C, quenched by the addition of water (1 ml), extracted with ether, dried over $MgSO_4$, filtrated and concentrated *in vacuo* to yield ethyl 4-amino-3-hydroxymethyl-5-methoxy-1-methylindole-2-carboxylate (44 mg, 86%) as a light orange solid; mp 136–139 °C; ν_{max} (KBr)/cm^{-1} 3359, 3124, 2980, 2875, 1705, 1521, 1403, 1255, 1224, 1128, 1001; δ_H (400 MHz; $CDCl_3$) 7.04 (1 H, d, *J* 6.6, ArH), 6.64 (1 H, d, *J* 6.6, ArH), 5.19 (2 H, s, C*H*$_2$OH), 4.39 (2 H, q, *J* 5.4, OC*H*$_2$Me), 3.86 (3 H, s, NMe), 3.84 (3 H, s, OMe), 1.42 (3 H, t, *J* 5.4, OCH$_2$*Me*); δ_C (100 MHz; $CDCl_3$) 162.4,

140.1, 136.2, 131.2, 124.9, 121.7, 117.0, 114.1 (CH), 98.8 (CH), 60.9 (CH_2), 58.0 (Me), 56.6 (CH_2), 32.1 (Me), 14.3 (Me); *m/z* (EI) 278 (M^+, 40%), 260 (30), 247 (25), 232 (50), 217 (100), 189 (15), 145 (25).

(b) To a solution of ethyl 4-amino-3-hydroxymethyl-5-methoxy-1-methylindole-2-carboxylate (321 mg, 1.16 mmol) in acetone (70 ml) was added a solution of potassium nitrosodisulfonate (1.24 g, 4.62 mmol) in sodium dihydrogen phosphate buffer (0.3 M, 56 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 evaporated. The residue was stirred at room temperature in a 1 : 1 mixture of hydrochloric acid (2 M) and acetone (140 ml) for 1 h. The acetone was removed *in vacuo.* The resulting residue was extracted with dichloromethane. The organic layer was washed with water, dried over $MgSO_4$, filtered and evaporated under reduced pressure. The residue was purified by chromatography, eluting with ethyl acetate/dichloromethane (1 : 20) to yield the *title compound* (181 mg, 54%) as a light orange crystalline solid, recrystallized from dichloromethane–pentane; mp 149–151 °C; (Found: C, 57.1; H, 5.0; N, 4.6. $C_{14}H_{15}NO_6$ requires C, 57.3; H, 5.2; N, 4.8%); (Found: M + NH_4^+, 311.1246. $C_{14}H_{15}NO_6$ + NH_4 requires 311.1243); λ_{max} (acetonitrile)/nm 250 (log ε 4.10), 336 (3.37), 408 (3.15); ν_{max} (KBr)/cm^{-1} 3433, 2949, 1708, 1673, 1646, 1604, 1493, 1435, 1374, 1266, 1178, 1047, 1016; δ_H (400 MHz; $CDCl_3$) 5.80 (1 H, s, 6-H), 5.00 (2 H, s, CH_2O), 4.40 (2 H, q, *J* 7.2, OCH_2Me), 4.26 (3 H, s, NMe), 3.86 (3 H, s, OMe), 1.41 (3 H, t, *J* 7.2, OCH_2Me); OH not observed; δ_C (100 MHz; $CDCl_3$) 179.1, 178.5, 160.7, 160.5, 132.8, 132.2, 126.3, 121.3, 108.2 (CH), 61.8 (CH_2), 56.8 (Me), 56.0 (CH_2), 35.2 (Me), 14.2 (Me); *m/z* (CI) 294 (MH^+, 100%), 278 (95), 265 (45), 250 (50), 236 (25).

Biology

Cell culture. BE-WT and BE-NQ cells were a gift from David Ross (University of Colorado Health Sciences Centre, Denver, CO). Cell culture components were obtained from Invitrogen (Grand Island, NY) unless otherwise noted. Cells were grown in minimum essential medium (MEM) with Earle's salts, non-essential amino acids, L-glutamine, penicillin/streptomycin and supplemented with 10% fetal bovine serum (HyClone Logan, UT). 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% to 80% B over 10 min, 80% B for 5 min, 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 Centre, Denver, CO). NADH oxidation was quantified at 340 nm following 30 min incubations at 22 °C.

Cytotoxicity assay. Cytotoxicity was determined using the MTT colorimetric assay.[48] Cells were plated in plates of 96 wells at a density of 1–2 × 10^4 cells ml^{-1} 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 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 plots of percent of control *vs.* concentration.

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