Indolequinone Antitumor Agents: Reductive Activation and Elimination from (5-Methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl Derivatives and Hypoxia-Selective Cytotoxicity in Vitro

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A series of indolequinones bearing a variety of leaving groups at the (indol-3-yl)methyl position was synthesized by functionalization of the corresponding 3-(hydroxymethyl)indolequinone, and the resulting compounds were evaluated in vitro as bioreductively activated cytotoxins. The elimination of a range of functional groups—carboxylate, phenol, and thiol—was demonstrated upon reductive activation under both chemical and quantitative radiolytic conditions. Only those compounds which eliminated such groups under both sets of conditions exhibited significant hypoxia selectivity, with anoxic:oxic toxicity ratios in the range 10−200. With the exception of the 3-hydroxymethyl derivative, radiolytic generation of semiquinone radicals and HPLC analysis indicated that efficient elimination of the leaving group occurred following one-electron reduction of the parent compound. The active species in leaving group elimination was predominantly the hydroquinone rather than the semiquinone radical. The resulting iminium derivative acted as an alkylating agent and was efficiently trapped by added thiol following chemical reduction and by either water or 2-propanol following radiolytic reduction. A chain reaction in the radical-initiated reduction of these indolequinones (not seen in a simpler benzoquinone) in the presence of a hydrogen donor (2-propanol) was observed. Compounds that were unsubstituted at C-2 were found to be up to 300 times more potent as cytotoxins than their 2-alkyl-substituted analogues in V79-379A cells, but with lower hypoxic cytotoxicity ratios.

Introduction

There is currently much interest in the antitumor potential of indolequinone bioreductively activated cytotoxic drugs.[1−8] Compounds of note include the diol EO9 (**1**; Figure 1),[7,8] recently evaluated in clinical trials,[9] and related mitosenes[3,4,10,11] which show improved properties over the naturally occurring mitomycins such as the archetypal quinone bioreductive anticancer agent mitomycin C (**2**; Figure 1)[12−15] and the large number of analogues reported in the past 20 years.[16−20] The fact that these compounds require reductive activation to form electrophilic species toxic to cells is well-known, and the drugs act as substrates for one or more of the reductases present in most cells and can be targeted toward both solid tumors with defined hypoxic fractions and tumor tissues rich in the required activating enzymes. Thus, bioreductive drugs are assuming increasing importance as agents effective against these targets.[21−28]

The mechanism of action of mitomycin C and the mitosenes has been the subject of numerous studies,[29] and these have established the role of C-1 and C-10 in the alkylation and cross-linking of DNA after bioreductive activation. Our own studies have focused on the role of C-10 in the alkylation process by the design and synthesis of the cyclopropamitosenes such as **3** (Scheme 1) in which the electrophilicity at C-1 is reduced by the presence of a cyclopropane ring in place of the naturally occurring aziridine.[1,2,5,30] In particular we established that the carbamate group could be eliminated from C-10 of the cyclopropamitosenes upon chemical reduction

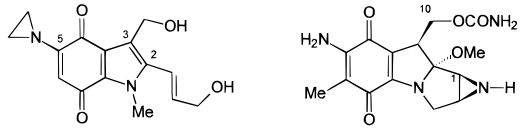

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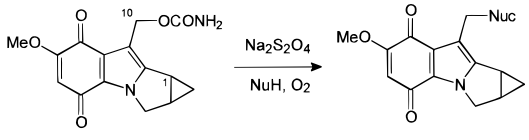
Figure 1. Structures of archetypal bioreductive quinones EO9 and mitomycin C.

Scheme 1. Reductive Elimination and Nucleophilic Trapping with Cyclopropamitosenes



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4a Nu = SCSOEt
4b Nu = NHC_6H_4-*p*-Me

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with sodium dithionite and the resulting intermediate trapped by added nucleophiles such as potassium ethyl xanthate or 4-toluidine (**4a,b**; Scheme 1).[5] No displacement of the carbamate by the nucleophile occurs in the absence of the reducing agent.

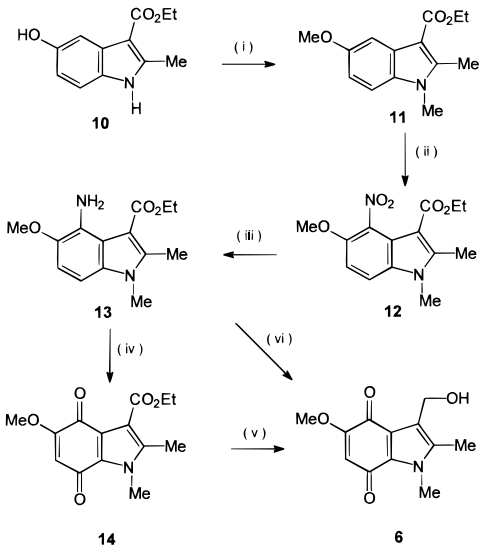
Our subsequent studies centered on further 2-alkyl derivatives more closely related to **1**, including the 2-cyclopropylindolequinones.[6] The most effective compounds, in terms of both hypoxic potency and hypoxia selectivity in vitro as established by anoxic:oxic cytotoxicity ratio (commonly referred to as hypoxic cytotoxicity ratio (HCR)) and in model tumor systems in vivo, were either 5-aziridinyl-3-hydroxymethyl derivatives or 5-methoxy-3-carbamoyloxy(methyl) derivatives.[6] Some of the hydroxymethyl derivatives were more effective than corresponding carbamates, which are normally the more potent species. The carbamate group is also present in naturally occurring mitomycins. Therefore, since variation in the 5-substituent of indolequinones and the equivalent positions of some mitosenes has been studied extensively[1,2,6,8,16,18] and only a limited variation in C-10 leaving group in mitosenes and EO9 (principally carbamates and acetoxy derivatives) has been examined,[1−4,6−8,10,11,16,17] there is an urgent need to clarify the role of both the 2- and 3-substituents in the indolequinones and their contribution toward the antitumor effects of these pharmacologically active classes of drugs. Of particular importance is the ability of 3-indolylcarbinyl substituents to undergo elimination, well-known in indole chemistry.[31] Such reactivity is only observed upon reductive activation of the indolequinone, through the participation of the 1-nitrogen lone pair electrons which are deactivated in the quinone parent prodrug where they are partially delocalized into the quinone carbonyl at C-4. The resulting iminium species is then a potential electrophilic DNA-alkylating or other cellular-damaging species.

It is not known whether water may be able to act as the leaving group in 3-hydroxymethyl analogues, although there is some evidence for the involvement of this substituent in cytotoxicity.[6] Compounds with more efficient leaving groups such as carbamate and acetoxy derivatives have generally shown greater potency and DNA cross-linking abilities,[3,4] but there have been no studies on the chemical and biological properties of compounds with more diverse leaving groups. Thus, the useful range of leaving groups is still unclear for mitosene analogues and completely unknown for 2-alkyl- and 2-cycloalkylindolequinones. To date the 2-substituent and (indol-3-yl)methyl substituent of the indolequinones have not yet been optimized for hypoxia-selective cytotoxicity.

We therefore report a study of the effect of (indol-3-yl)methyl leaving group variation upon hypoxia selectivity and potency of this series of indolequinones. The mechanism and scope of (indol-3-yl)methyl leaving group elimination has also been studied. The effect of the 2-substituent on leaving group-mediated activation has also been examined following preliminary evidence that this substituent has a pronounced effect on potencies of related compounds.[6]

The compounds evaluated were 5-methoxy-1,2-dialkylindolequinones and 2-unsubstituted analogues, substituted in the (indol-3-yl)methyl position with hydroxy, halo, acyloxy, arylalkyloxy, aroyloxy, aryloxy, arylthio, or aryl substituents.

Scheme 2. Synthetic Route to Critical Parent Compound and Precursor **6**[a]

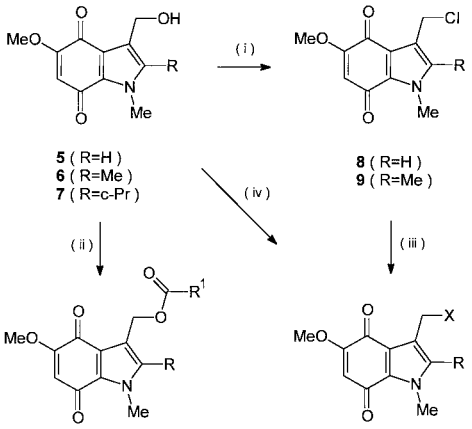


[a] Reagents: (i) KH/MeI/DMF (81%); (ii) HNO_3/AcOH (63% + 14% 6-nitro isomer); (iii) Sn/HCl (80%); (iv) $(KO_3S)_2NO$ (97%); (v) $Na_2S_2O_4$ then DIBAL-H then $FeCl_3$ (71%); (vi) $LiAlH_4$/THF (77%) then $(KO_3S)_2NO$ (75%).

Synthetic Chemistry

A more convenient route to our previously reported 3-(hydroxymethyl)indolequinone precursor compound **6**[1] has now been developed. Thus ethyl 5-hydroxy-2-methylindole-3-carboxylate, which is commercially available or readily obtained by the Nenitzescu reaction, was dimethylated to give **11**, nitration and reduction of which gave **13** (Scheme 2). Oxidation of the 4-aminoindole **13** with Fremy's salt gave the quinone **14**, which was reduced to the hydroquinone, and the ester group was immediately further reduced with DIBAL-H, to give, on oxidative workup, the desired 3-(hydroxymethyl)indolequinone **6**. In an alternative method, **13** was reduced with $LiAlH_4$ prior to oxidation with Fremy's salt to give **6**. The corresponding 2-desmethyl analogue **5** and the cyclopropane **7** were prepared according to our previously published method.[6] A range of indolequinones bearing different groups was prepared from the hydroxymethyl compounds **5**−**7**. The corresponding 3-chloromethyl derivatives **8** and **9** were obtained by treatment with $SOCl_2$ at room temperature. The carboxylic esters **15**−**23** and **25** were readily obtained from the appropriate (hydroxymethyl)indolequinone by direct coupling with either the corresponding acyl chloride or with the carboxylic acids using Mitsunobu or carbodiimide methodology. Aryl ethers **26**−**28** and **31** were obtained directly from the appropriate chloromethyl derivatives. Treatment of the (chloromethyl)indolequinone **9** with the less acidic 2- or 4-fluoro and unsubstituted phenols gave either C-alkylated products **29** and **30** or the required O-alkylated products **27** and **28** depending on the solvent used, with DMF favoring O-alkylation. The thioether **32** was obtained by DMF−acetal-mediated coupling to 2-mercaptopyrimidine. The chloromethyl compound **8** was treated directly with

Scheme 3. General Synthetic Routes to (4,7-Dioxoindol-3-yl)methyl Carboxylate Esters and Aryl Ethers[a]



5 (R=H)
6 (R=Me)
7 (R=c-Pr)

8 (R=H)
9 (R=Me)

15 (R= Me, R^1=CH(NHCbz)$CHNMe_2$
16 (R=Me, R^1=$CH_2C_6H_4$-*p*-Ph, 87%)
17 (R=H, R^1=C_6H_4-*o*-F, 46%)
18 (R=Me, R^1=C_6H_4-*o*-F, 84%)
19 (R=H, R^1=C_6H_4-*p*-F, 46%)
20 (R=Me, R^1=C_6H_4-*p*-F, 63%)
21 (R=H, R^1=C_6H_5, 48%)
22 (R=Me, R^1=C_6H_5, 69%)
23 (R=c-Pr, R^1=C_6H_5, 68%)
25 (R=Me, R^1=CH_3, 69%)

24 (R=Me, X=$OCH_2C_6H_5$, 53%)
26 (R=H, X=OC_6H_2-2,4,6-Cl, 61%)
27 (R=Me, X=OC_6H_5, 65%)
28 (R=Me, X=OC_6H_4-*p*-F), 43%)
29 (R=Me, X=C_6H_3-2-OH-5-F, 50%)
30 (R=Me, X=C_6H_3-3-F-4-OH, 49%)
31 (R=Me, X=OC_6H_4-*m*-NHAc, 62%)
32 (R=Me, X=S-2-Pyrimidyl, 71%)
33 (R=Me, X=SCSOEt, 85%)

[a] Reagents: (i) $SOCl_2$; (ii) R^1COCl/pyridine/EtOAc or CH_2Cl_2, or R^1CO_2H/DEAD/Ph_3P/THF, or R^1CO_2H/1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide/4-(dimethylamino)pyridine; (iii) XH/EtOAc/K_2CO_3, or XH/NaH, or K_2CO_3/DMF; (iv) XH/DEAD/Ph_3P/THF, or XH/$(Me)_2NCH[OCH_2C(Me)_3]_2$/$CH_3CN$, or XH/MsCl/$CH_2Cl_2$/$Et_3N$.

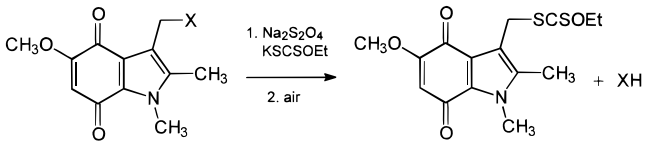
2,4,6-trichlorophenol to give **26**, and **9** was treated with benzyl alcohol to give **24** (Scheme 3).

Results and Discussion

A variety of chemical reductive conditions (catalytic hydrogenation, electrochemical, Cr(II), sodium dithionite) have been used to activate mitomycin C, and sodium dithionite had emerged as a convenient method.[32,33] Thus, the activation of the indolequinones was initially carried out using this reagent. However, radiolytic reduction methods have also been used so that the efficiency of leaving group loss could be quantified following reduction by radiolytically generated radicals and the kinetic properties of putative radical intermediates in reduction characterized. In particular, the reactivity toward oxygen of the semiquinone radicals, potentially formed under hypoxic conditions following reduction by one-electron reducing enzymes, and the effect of oxygen on leaving group release were studied.

Thus, to address the key question as to whether the diverse leaving groups X (Table 1) in some representative compounds of the series **15**−**32** are eliminated upon reductive activation, the activation of the indolequinones **6**, **15**, **16, 19, 20, 27, 28**, and **31**−**32** was first carried out using sodium dithionite as reducing agent. The reduction was carried out in the presence of a thiol to trap the electrophilic iminium intermediate; not only are thiols good nucleophiles, but the quenching of the reactive electrophile by a thiol would mimic the scavenging role of cellular thiols such as glutathione in biological systems. Initially *tert*-butylthiol was used as nucleophile, but the water-soluble potassium *O*-ethyl xanthate proved more satisfactory. The reduction of the

Table 1. Chemical Reductive Release from Indolequinones



33

indolequinone	X	yield (%) 33	yield (%) XH
6	OH	58	*a*
15	$OCOCH(NHCbz)CHMe_2$	72	67
16	$OCOCH_2C_6H_4$-*p*-Ph	78	52
19	$OCOC_6H_4$-*p*-F	73[c]	46
20	$OCOC_6H_4$-*p*-F	91	*b*
27	OC_6H_5	55	*b*
28	OC_6H_4-*p*-F	81	*b*
31	OC_6H_4-*m*-NHAc	66	33
32	S-2-pyrimidyl	58	*b*

[a] Product is water; not detected. [b] Product identified by TLC but not isolated. [c] Product is *O*-ethyl (5-methoxy-1-methyl-4,7-dioxo-indol-3-yl)methyl dithiocarbonate (**34**).

indolequinone was carried out using a large excess of sodium dithionite in the presence of the thiol (5 equiv) in degassed aqueous THF. In all cases, the elimination of X^- occurred and was observed by the appearance of XH in the TLC analysis of the reaction mixture. Concurrent with the elimination of X^-, the formation of the thiol-trapped product **33** (**34** in the case of the 2-desmethyl analogue) was also observed by TLC in all cases. Oxidative workup of the reaction mixture allowed the isolation of **33**, the identity of which was confirmed by independent synthesis, and, in most cases, the released compound, XH.

The results are summarized in Table 1 and show that a range of functional groups—carboxylic acids, phenols, and a thiol—can be eliminated from the (4,7-dioxoindol-3-yl)methyl derivatives upon reductive activation. The yields of thiol-trapped product **33** (55−91%, also synthesized independently) and the released XH are reasonably consistent (33−67%). Blank experiments established that the dithiocarbamate **33** was not formed in the absence of the reducing agent, i.e., no *direct* nucleophilic substitution occurs; the starting quinone was recovered (ca. 90%) on workup with exposure to air. The fact that water can function as a leaving group under these conditions (compound **6**) has implications for the cytotoxicity of indolequinones containing the 3-hydroxymethyl substituent, although interestingly this poorer leaving group was not efficiently eliminated under the more controlled radiolytic reducing conditions (Table 2). The HCR data (Table 3) also suggests that water may not be as effective a leaving group following cellular reduction, although compound **6** showed some hypoxia selectivity (HCR = 4) as do some other 3-(hydroxymethyl)indolequinones and certain mitosenes.[3,4,6]

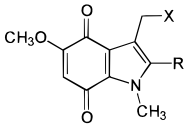
Representative compounds of the series were evaluated for hypoxia-selective cytotoxicity, and the 3-[(aroyloxy)methyl]-2-methylindole-4,7-diones **18**, **20**, and **22** exhibited hypoxic cytotoxicity ratios (HCR) of ∼80−90. The HCR was generally in the range ∼10−90 when the leaving group was a carboxylic acid and also in the case of aryl ethers with the best compound (**31**) having a HCR of 93. Structurally similar analogues with poorer leaving groups showed lower or no hypoxia selectivity (compounds **5**, **6**, **7, 24**, **29**, and **30**). This indicates that

Table 2. Rate Constants, One-Electron Reduction Potentials, Semiquinone Radical pK_a's, and Relative Leaving Group Ability of Representative Indolequinones at pH 7.4

Q	K	E(Q/Q$^{\bullet-}$) (mV)[a]	$10^{-8}k_3$ (Q$^{\bullet-}$ + O_2) (M^{-1} s^{-1})	$10^{-7}2k_2$ (Q$^{\bullet-}$ + Q$^{\bullet-}$) (M^{-1} s^{-1})	QH$^{\bullet}$ pK_a	G(−Q) (μmol J^{-1})[c]	G(LG) (μmol J^{-1})[c]
6	17.5 ± 1.4	−376 ± 9	5.2 ± 0.1	0.3 ± 0.1	4.51 ± 0.07	0.25 ± 0.01[d]	
19	4.8 ± 1.6	−397 ± 25	6.6 ± 0.1	0.8 ± 0.1	5.09 ± 0.07	0.85 ± 0.10	0.65 ± 0.02
20	6.0 ± 1.2	−387 ± 12	6.8 ± 0.1	1.7 ± 0.1	5.32 ± 0.08	1.65 ± 0.01	1.71 ± 0.15
21	48.5 ± 3.9	−350 ± 9	6.5 ± 0.1	0.6 ± 0.1	5.03 ± 0.05	0.90 ± 0.12	0.73 ± 0.04
22	17.3 ± 5.4	−376 ± 15	6.7 ± 0.1	1.6 ± 0.1	5.09 ± 0.10	1.47 ± 0.07	1.56 ± 0.08
26	25.8 ± 6.6	−447 ± 14[b]	8.2 ± 0.1	0.6 ± 0.1	5.07 ± 0.02	3.05 ± 0.10	2.64 ± 0.10
27	5.9 ± 0.4	−403 ± 9	5.3 ± 0.1	1.1 ± 0.1	4.97 ± 0.07	1.34 ± 0.10	1.32 ± 0.10
28	3.1 ± 0.2	−420 ± 9	4.4 ± 0.1	1.3 ± 0.1	5.13 ± 0.03	1.59 ± 0.10	1.54 ± 0.10
29	4.6 ± 0.1	−410 ± 8	7.4 ± 0.1	0.9 ± 0.1	5.93 ± 0.03	0.00	0.00

[a] Redox potentials vs E(MV^{2+}/$MV^{\bullet+}$) = −442 ± 7 mV (corrected for 2 M 2-propanol). [b] Potential vs E(TQ^{2+}/$TQ^{\bullet+}$) = −523 ± 7 mV. [c] 2-Propanol/water (50%, v/v); G($(CH_3)_2C^{\bullet}OH$) = 0.67 μmol J^{-1}; dose rate ~ 6.0−6.5 Gy min^{-1}. [d] Loss of quinone did not generate a significant quantity of the isopropyl ether **35**, indicative of the elimination of water.

Table 3. In Vitro Cytotoxicity of Indolequinones



compd	R	X	IC_{50}(air) (μM)[a]	IC_{50}(N_2) (μM)[a]	HCR
5	H	OH	220	240	1
6	Me	OH	1077	285	4
7	c-Pr	OH	540[c]	820[c]	0.7[c]
8	H	Cl	304	33	9
16	Me	$OCOCH_2C_6H_4$-*p*-Ph	14.1	0.66	21
17	H	$OCOC_6H_4$-*o*-F	0.5	0.04	13
18	Me	$OCOC_6H_4$-*o*-F	164	2	82
19	H	$OCOC_6H_4$-*p*-F	0.7	0.05	14
20	Me	$OCOC_6H_4$-*p*-F	27	0.3	90
21	H	$OCOC_6H_5$	1.4	0.04	35
22	Me	$OCOC_6H_5$	16	0.2	80
23	c-Pr	$OCOC_6H_5$	198	1	198
24	Me	$OCH_2C_6H_5$	225	410	0.6
25	Me	OAc	27	2.5	11
26	H	OC_6H_3−2,4,6-Cl	0.7	0.08	9
27	Me	OC_6H_5	12	0.7	17
28	Me	OC_6H_4-*p*-F	31	0.85	36
29	Me	C_6H_3−2-OH-5-F	200	110	2
30	Me	C_6H_3−3-F-4-OH	470	280	2
31	Me	OC_6H_4-*m*-NHAc	3.7	0.04	93
33	Me	SCSOEt	67	3	22
36	H	$OCONH_2$	3[c]	0.04[c]	75[c]
37	Me	$OCONH_2$	25[b]	0.3[b]	83[b]

[a] Mean of three experiments. Typical errors ±10%. [b] Reference 1. [c] Reference 6.

bioreductively induced elimination of the leaving group at C-3 is likely to be central to the cytotoxic action of the drugs and that in the compounds evaluated to date, biologically useful leaving groups are carboxylic acids and phenols. Thiols remain unevaluated in vitro but would be expected to be effective based on the data obtained thus far.

Elimination of the leaving group acid or phenol could feasibly occur either from the one-electron-reduced semiquinone radical (Q$^{\bullet-}$; Scheme 4) produced by single-electron reduction or via the hydroquinone (QH_2; Scheme 4) produced directly by two-electron reduction or the disproportionation of Q$^{\bullet-}$ radicals. Radiolytic reducing conditions using a one-electron reductant were appropriate for probing this mechanism. The indolequinones were rapidly reduced by the $(CH_3)_2C^{\bullet}OH$ radical ($k_1 \sim 10^9$ M^{-1} s^{-1}) to generate the semiquinone radicals via reaction 1.

$$Q + (CH_3)_2C^{\bullet}OH \rightarrow Q^{\bullet-} + (CH_3)_2CO + H^+ \quad (1)$$

Scheme 4. Reductive Activation Pathways Leading to Elimination of Leaving Group Acid and Alkylation by the Resulting Iminium Derivative

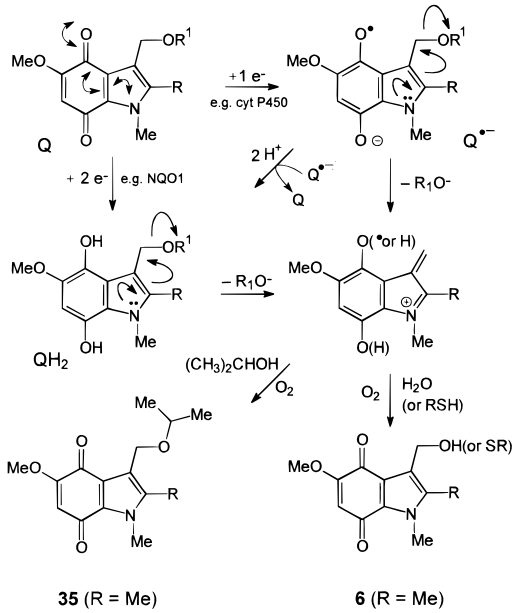


Figure 2 shows the absorption spectra of the semiquinone radical generated by the reduction of **20** by $(CH_3)_2C^{\bullet}OH$ in the pH range of 5−11. These spectra are typical for the indolequinones in this study, all of which exhibited differential absorption maxima in the 340−400 nm region. The radical spectra were similar at pH 7.4 and 10 suggesting that the semiquinone radical is deprotonated at pH 7.4 and above (typical semiquinones have pK_a ~4−6).[34] The insert in Figure 2 displays the variation with pH of the semiquinone radical absorption at 360 nm, which corresponds to pK_a = 5.32 ± 0.08 for the semiquinone radical of **22**. Semiquinone radical pK_a's for the range of indolequinones under study are displayed in Table 2 and show little variation, falling in a narrow range of pK_a = 5−6.

At pH 3.9 the semiquinone radical absorption (345 nm) decayed via second-order kinetics (Figure 3) with a half-life which decreased with increasing radiation dose (i.e., with initial concentration of radicals, [$(CH_3)_2C^{\bullet}$OH] ~ 3−18 μM) indicating that the semiquinone radical decays predominantly by a radical−radical reaction to generate the hydroquinone via reaction 2.

$$Q^{\bullet-} + Q^{\bullet-} + 2H^+ \rightarrow QH_2 + Q \quad (2)$$

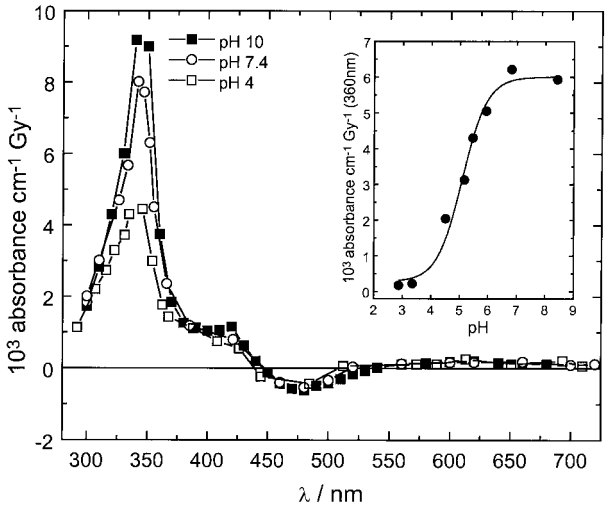


Figure 2. Absorption spectra of the semiquinone radicals of **20** obtained by pulse radiolysis of an N_2O-saturated 2-propanol/water mixture (50%, v/v) containing phosphate buffer (4 mM, pH 10, 7.4, and 4). Insert: pK_a curve showing the effect of pH on the absorption of the semiquinone radical of **22** (40 μM **22** in 2 M 2-propanol after a dose of 3 Gy).

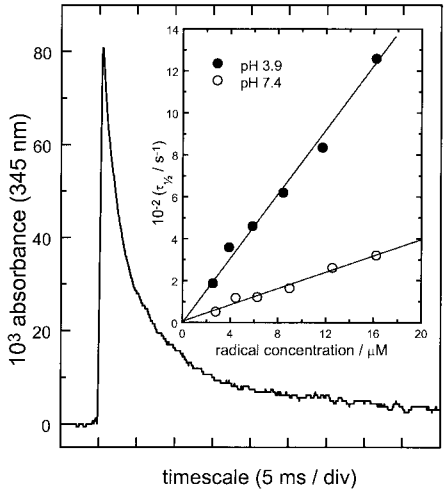


Figure 3. Transient absorption at 345 nm observed on the reaction of $(CH_3)_2C^{\bullet}OH$ with **20** (50 μM) in an N_2O-saturated 2-propanol/water mixture (50%, v/v) containing phosphate buffer (4 mM, pH 7.4) initiated by a pulse of 18.7 Gy, corresponding to $[(CH_3)_2C^{\bullet}OH] \sim 12.5$ μM. Inset: dependence of the reciprocal of the first half-life of the semiquinone radical on initial radical concentration at pH 3.9 and 7.4.

The reciprocal of the first-half-life of the semiquinone radical of **20** varied linearly with the initial radical concentration, and from the slope of the fitted straight line, the rate constant $2k_2 = 7.4 \pm 0.1 \times 10^7$ M^{-1} s^{-1} was obtained at pH 3.9 (see insert Figure 3). At pH 7.4, where the semiquinone radical is almost fully deprotonated, the rate of decay decreases to $2k_2 = 1.7 \pm 0.1 \times 10^7$ M^{-1} s^{-1}. In the case of the drug EO9 (**1**), reaction 2 is a reversible equilibrium;[35] however, the semiquinone radicals of all the indolequinones in this study decay completely (even at pH > 9) suggesting that any possible equilibrium lies well to the side of hydroquinone formation. Rate constants for the bimolecular decay of semiquinone radicals ($2k_2$) at pH 7.4 are displayed in Table 2. Some evidence was obtained for a first-order component attributable to unimolecular elimination of a leaving group via intramolecular electron transfer in the semiquinone species. However, for all of the indolequinones studied, direct elimination of the leaving group from the $Q^{\bullet-}$ radical must be slow ($k \ll 100$ s^{-1}) based on the small intercepts obtained from plots exemplified by Figure 3. Such processes are unknown in other classes of quinones, such as 1,4-naphthoquinones,[36] but have been reported in the dehalogenation of nitroaromatic anion radicals.[37] The one-electron reduction potentials ($E(Q/Q^{\bullet-})$) at pH 7.4 for the indolequinones are also displayed in Table 2. The nature of the (indol-3-yl)methyl substituent had little effect on the electron affinity of benzoate esters, which exhibited redox potentials similar to that of **6,** whereas the phenols were markedly less electron affinic. The one-electron reduction potentials fell within a good range (−350 to −447 mV) for reduction by enzymes such as NADPH cytochrome *c* (P450) reductase.

In the presence of oxygen the semiquinone radicals decayed faster with increasing oxygen concentrations (ca. 2−20 μM O_2), presumably via electron-transfer according to reaction 3.

$$Q^{\bullet-} + O_2 \rightarrow Q + O_2^{\bullet-} \qquad (3)$$

Absolute rate constants for k_3 were determined from the slope of the linear plots of the observed first-order rate constants for radical decay versus oxygen concentration and are shown in Table 2. Values for k_3 were found to be in the order of 10^8 M^{-1} s^{-1}, and hence the new compounds exhibit similar reactivity toward oxygen as related indolequinones determined previously.[6]

The marked pH dependence of the radical stability in anoxia is important in the possible competition between semiquinone disproportionation (reaction 2) and oxygen inhibition (reaction 3) in reductive activation. It may thus be possible to modify p$K_a(QH^{\bullet})$ to alter the sensitivity to oxygen, of toxicity through one-electron reduction. Further work is needed to ascertain whether p$K_a(QH^{\bullet})$ and $2k_2$ are linked to the variation in toxicity with oxygen tension.

Elimination following Radiolytic Reduction to the Semiquinone Radical. The leaving group chemistry of the indolequinones was investigated by product analysis (HPLC) following γ-radiolysis of N_2O-saturated solutions containing quinones (100 μM) and 2-propanol (8.3 M, 50%, v/v) at pH 7.4. The radiation chemical yield (G) of the $(CH_3)_2C^{\bullet}OH$ radical in N_2O-saturated 2-propanol/water mixtures was determined by ferricyanide reduction[38] to be $G((CH_3)_2C^{\bullet}OH) = 0.67 \pm 0.02$ μmol J^{-1} in 2-propanol/water (50%, v/v) and 0.72 ± 0.03 μmol J^{-1} in 1 M 2-propanol, respectively. Figure 4 shows the product profile obtained on the reduction of **20** by the $(CH_3)_2C^{\bullet}OH$ radical. Loss of the parent quinone **20** ($G(-Q) = 1.65 \pm 0.01$ μmol J^{-1}) paralleled the formation of the fluorobenzoate leaving group (LG) with $G(LG) = 1.71 \pm 0.15$ μmol J^{-1}, both more than double the input of reducing (single-electron) equivalents. The $Q^{\bullet-}$ radicals decay rapidly via reaction 2; therefore, since the first-order elimination route is slow, the fluorobenzoic acid is predominantly derived from the hydroquinone (QH_2; Scheme 4) and not via electron transfer within the $Q^{\bullet-}$ radicals.

The two remaining major peaks in Figure 4 were derived from the reaction of the resultant iminium

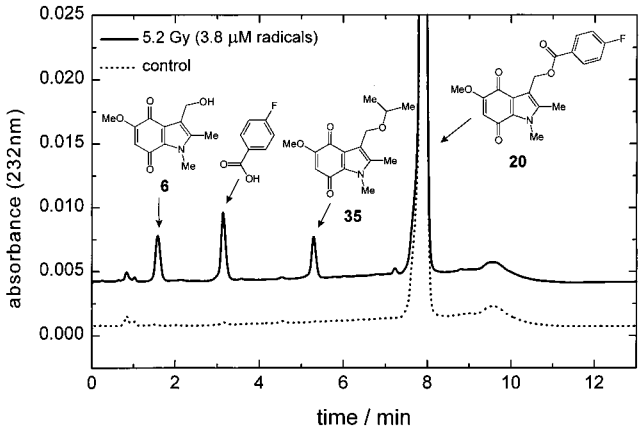


Figure 4. HPLC chromatogram showing a typical product profile obtained by the γ-radiolysis (5.2 Gy) of 100 μM **20**, in an N_2O-saturated 2-propanol/water mixture (50%, v/v) containing phosphate buffer (4 mM, pH 7.4).

derivative (Scheme 4) with water to generate **6** and with the 2-propanol to generate the isopropyl ether **35** (also synthesized independently). Both of these quinones are generated by autoxidation of their respective hydroquinones via reaction 4 following the unavoidable introduction of oxygen during HPLC sampling.

$$QH_2 + O_2 \rightarrow Q + H_2O_2 \qquad (4)$$

As expected, the relative yields of **6** and **35** were dependent on the alcohol concentration, with the alkylation product virtually disappearing when radiolysis was performed in 1 M 2-propanol. Other indolequinone benzoate esters including **19, 21,** and **22** also exhibited equally efficient leaving group ability. The aryl ethers **26**−**28** and **31** which contained the phenoxy moieties also eliminated from their hydroquinones. The generation of the isopropyl ether **35** following the radiolytic reduction of **6** was indicative of the formation of the iminium derivative following the elimination of water. As expected, the loss of **6** ($G(-Q) = 0.25 \pm 0.01$ μmol J^{-1}) was lower than for other indolequinones exhibiting leaving group chemistry since the iminium derivative would also trap water to regenerate **6**. Nevertheless, the relatively low yield of **35** reflected an inefficient elimination of water from **6** on reduction. Under the more forcing chemical reducing conditions of excess sodium dithionite, water was effectively eliminated from **6** and the iminium derivative trapped with xanthate to give **33**. This poorer leaving group behavior is reflected in the poorer hypoxia selectivity of the hydroxymethyl compounds (HCR ranging from 1 to 4, Table 3). From Table 2, for the indolequinones which exhibited leaving group chemistry, both $G(-Q)$ and $G(LG)$ were significantly greater ($G(-Q) = 0.9-3.05$ μmol J^{-1}) than expected from the bimolecular decay of $Q^{\bullet-}$ radicals via reaction 2 where the expected $G(-Q) = 0.33$ μmol J^{-1} (i.e., one-half of $G((CH_3)_2C^{\bullet}OH) = 0.67$ μmol J^{-1} determined by ferricyanide reduction). Reduction of 2,6-dimethylbenzoquinone to its hydroquinone under the same experimental conditions gave the expected $G(-Q) = 0.37 \pm 0.01$ μmol J^{-1} and $G(QH_2) = 0.32 \pm 0.03$ μmol J^{-1} and confirmed the presence of a chain reaction in the reduction of the indolequinones. A pH-dependent chain reaction has also been reported in the reduction of mitomycin C by radiolytically produced formate radicals.[39]

A comparison of the leaving group abilities of representative indolequinones in Table 2 with HCR in Table 3 shows that compounds which eliminated leaving groups upon reduction also exhibited the greatest HCRs, in the range 10−100. Structurally similar analogues with poorer leaving groups such as **5**−**7** and **24** showed little or no hypoxia selectivity and no leaving group chemistry (under radiolysis conditions) together with substantially lower potency. Similarly, the compounds in which the fluorophenyl moiety was linked through the aromatic ring rather than through the phenolic oxygen (**29** and **30**) exhibited no hypoxia selectivity and no leaving group chemistry. Although the aerobic cytotoxicity of these compounds that do not possess leaving groups can be explained by redox cycling (reaction), their residual hypoxic cytotoxicity (100−300 μM) must be attributed to other unknown mechanisms.

The aerobic and anoxic cytotoxic potencies (Table 3) of this series of indolequinones were markedly influenced by 2-substitution, with 2-unsubstituted compounds being up to 300 times more cytotoxic against V79-379A cells in some cases (Table 3, cf. **17** and **18, 19** and **20**). The data for the benzoate esters **21**−**23** (Table 3) show that there is an order of magnitude decrease in aerobic cytotoxicity upon progressive substitution at the 2-position (2-unsubstituted to 2-methyl and again to 2-cyclopropyl substitution). With the exception of the known carbamates **36** and **37**, which show the same trend, the differences under hypoxia are slightly less dramatic, and therefore HCR also increases across the series. Similar trends in related indolequinones have been observed previously, as has the exceptional hypoxic potency of some 2-cyclopropanes also seen for compound **23** (HCR = 198).[6] An increase in C-2 sidechain length is known to reduce the potencies of compounds closely related to those in this study,[6] although the differences between methyl and unsubstituted analogues were not as great as has been seen in the present study. The reported increased rate of reduction by NAD(P)H:quinone oxidoreductase (DT-diaphorase, E.C. 1.6.99.2, NQO1) of 2-unsubstituted compounds compared with 2-substituted analogues may be a significant factor leading to the observed trends in cytotoxicity for such compounds.[40] For example, if R is a substituent that results in slower enzymatic reduction, then low oxygen tension would be required for hydroquinone buildup. This might explain increasing HCR with certain C-2 substituent (R) groups and clearly merits further study.

The present study has established that a range of groups can be eliminated from the (indol-3-yl)methyl position upon reductive activation of the quinone moiety. The reduction-initiated release of a range of compounds from the indolequinone parallels the work of Denny and co-workers who have used the reduction of aromatic nitro groups to initiate the release of cytotoxic leaving groups.[41−43] However unlike the work of Denny and co-workers in which the releasing moiety is unreactive and only the released fragment in cytotoxic, the present work results in the formation of a highly reactive electrophilic species from the releasing moiety that is cytotoxic, together with a leaving group that can be

varied at will either to be cytotoxic in its own right or to exhibit other biological properties. This concept of bioreductive release from indolequinones is being actively investigated, and further results will be reported in due course.

Experimental Section

NMR spectra were obtained at 60 MHz with a JEOL MY60 spectrometer, at 90 MHz with a JEOL FX90Q spectrometer, and at 250, 300, or 400 MHz using Bruker instruments and $SiMe_4$ as internal standard. Elemental analyses were determined at the University of Exeter or by Butterworth Laboratories Ltd., Teddington, Middlesex, U.K., and all compounds characterized by HRMS were chromatographically homogeneous. Solutions in organic solvents were dried by standard procedures, and dimethylformamide (DMF), toluene, and tetrahydrofuran (THF) were anhydrous commercial grades. Silica gel for flash column chromatography was Merck Kieselgel 60 H grade (230−400 mesh) or Matrex silica 60. Melting points were determined on a Thomas-Hoover melting point apparatus and on a Thermogallen microscope and hot stage apparatus and are uncorrected. The 3-(hydroxymethyl)-indolequinones **5** and **7** required as precursors were synthesized as described previously.[6] 1,1′-Dimethyl-4,4′-bipyridinum dichloride (methyl viologen, MV^{2+}) and 2,6-dimethylbenzoquinone were obtained from Sigma-Aldrich, and 1,1′-propylene-2,2′-bipyridinum dibromide (Triquat, TQ^{2+}) was a gift from Dr. Peter O'Neill (MRC Radiation and Genome Stability Unit, Chilton, U.K.).

3-(Chloromethyl)-5-methoxy-1-methylindole-4,7-dione (8) and 3-(Chloromethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (9). The appropriate alcohol (**5** or **6**; 2 mmol) was stirred at room temperature with $SOCl_2$ (5 mL) for 0.5 h. The solution was then evaporated in vacuo, redissolved in EtOAc (25 mL), and evaporated to dryness. This procedure was repeated twice to coevaporate $SOCl_2$ residues, and the crude orange solid (75−85% yield) of the corresponding 3-(chloromethyl)-5-methoxyindole-4,7-dione (**8** or **9**) was used in the next step without further treatment. A small sample of compounds **8** and **9** was recrystallized from EtOAc for NMR and CHN analysis. **8**: mp 202−204 °C dec; ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, s, 2-*H*), 5.68 (1H, s, 6-*H*), 4.80 (2H, s, CH_2Cl), 3.94 (3H, s, OCH_3), 3.80 (3H, s, NCH_3). Anal. ($C_{11}H_{10}NO_3Cl$) C, H, N. **9**: mp 204−205 °C dec; ^{1}H NMR ($CDCl_3$) δ 5.62 (1H, s, 6-*H*), 4.86 (2H, s, CH_2Cl), 3.89 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.28 (3H, s, 2-CH_3). Anal. ($C_{12}H_{12}NO_3Cl\cdot0.3H_2O$) C, H, N.

Ethyl 5-Methoxy-1,2-dimethylindole-3-carboxylate (11). Ethyl 5-hydroxy-2-methylindole-3-carboxylate (**10**) (6.04 g, 27.5 mmol) in DMF (50 mL) was added to a stirring suspension of KH (3.3 g, 82.5 mmol) in DMF (200 mL) at 0 °C. The mixture was stirred at room temperature for 45 min. Iodomethane (11.7 g, 82.4 mmol) was added dropwise at 0 °C and the mixture allowed to warm to room temperature. The reaction was monitored by TLC and was completed within 2 h. Saturated ammonium chloride solution was added and the mixture extracted with EtOAc. The EtOAc layer was washed twice with water, dried ($MgSO_4$), and concentrated. The crude product was purified by column chromatography (eluting with EtOAc) to give the title compound as a colorless solid (5.5 g, 81%): mp 119−121 °C; IR (KBr) 2979, 2947, 1680, 1621, 1584 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.66 (1H, d, J = 2.5 Hz, 4-*H*), 7.17 (1H, d, J = 8.8 Hz, 7-*H*), 6.87 (1H, dd, J = 8.8 Hz, J = 2.5 Hz, 6-*H*), 4.39 (2H, q, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.88 (3H, s, OCH_3), 3.66 (3H, s, NCH_3), 2.74 (3H, s, CH_3), 1.45 (3H, t, J = 7.1 Hz, CO_2CH_2 CH_3); ^{13}C NMR ($CDCl_3$) δ 166.0 (CO), 155.5, 145.1, 131.5, 127.3, 111.4 (CH), 109.6 (CH), 103.6 (CH), 59.1 (OCH_2), 55.6 (OCH_3), 29.4 (NCH_3), 14.5 (CH_3), 11.8 (CH_3); HRMS found M^+ 247.1208, $C_{14}H_{17}NO_3$ requires M 247.1208. Anal. ($C_{14}H_{17}NO_3$) C, H, N.

Ethyl 5-Methoxy-1,2-dimethyl-4-nitroindole-3-carboxylate (12). To a solution of ethyl 5-methoxy-1,2-dimethylindole-3-carboxylate (**11**) (5.1 g, 20.6 mmol) in AcOH (80 mL), cooled to −10 °C, was added a mixture of concentrated HNO_3 (11 mL) and AcOH (41 mL). The mixture was stirred at room temperature for 2 h. A yellow suspension was obtained which was poured onto an ice/water mixture, and the crystals obtained were filtered off and dried. The crude product was purified by column chromatography (EtOAc/light petroleum) to yield (i) the title compound (3.8 g, 63%): mp 189−192 °C; IR (KBr) 2942, 1696, 1625, 1573, 1540 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.30 (1H, d, J = 9.1 Hz, 6/7-*H*), 6.93 (1H, d, J = 9.1 Hz, 7/6-*H*), 4.29 (2H, q, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.91 (3H, s, OCH_3), 3.66 (3H, s, NCH_3), 2.67 (3H, s, CH_3), 1.36 (3H, t, J = 7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR (DMSO-d_6) δ 163.2 (CO), 146.1, 145.6, 132.1, 131.4, 116.5, 110.6 (CH), 106.7 (CH), 101.5, 58.7 (OCH_2), 56.5 (OCH_3), 28.7 (NCH_3), 13.0 (CH_3), 10.7 (CH_3); HRMS found M^+ 292.1059, $C_{14}H_{16}N_2O_5$ requires M 292.1059. Also obtained was (ii) ethyl 5-methoxy-1,2-dimethyl-6-nitroindole-3-carboxylate (0.8 g, 14%): mp 139−141 °C; IR (KBr) 2981, 2944, 1689, 1626, 1583, 1558 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.96 (1H, s, 4/7-*H*), 7.79 (1H, s, 7/4-*H*), 4.40 (2H, q, J = 7.0 Hz, $CO_2CH_2CH_3$), 4.02 (3H, s, OCH_3), 3.73 (3H, s, NCH_3), 2.79 (3H, s, CH_3), 1.46 (3H, t, J = 7.0 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 167.0 (CO), 152.1, 151.5, 137.3, 133.3, 131.4, 109.5 (CH), 106.5 (CH), 106.3, 61.7 (OCH_2), 58.7 (OCH_3), 32.0 (NCH_3), 16.4 (CH_3), 14.3 (CH_3); HRMS found M^+ 292.1059, $C_{14}H_{16}N_2O_5$ requires M 292.1059.

Ethyl 4-Amino-5-methoxy-1,2-dimethylindole-3-carboxylate (13). To a suspension of ethyl 5-methoxy-1,2-methyl-4-nitroindole-3-carboxylate (**12**) (2.18 g, 7.5 mmol) in EtOH (190 mL) were added tin powder (4.0 g, 33.6 mmol) and HCl (3 M, 54 mL). The mixture was heated under reflux for 30 min. Upon cooling the solution was decanted from the excess tin and neutralized with $NaHCO_3$ (aqueous). The suspension obtained was added to an equal volume of water. The precipitate and aqueous layer were stirred overnight with CH_2Cl_2 and filtered through Celite to separate the layers. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (EtOAc) to yield the title compound as an off-white crystalline solid (1.56 g, 80%): mp 96−98 °C; IR (KBr) 3459, 3336, 3289, 2928, 1663, 1598, 1561 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.88 (1H, d, J = 8.7 Hz, 6/7-*H*), 6.52 (1H, d, J = 8.7 Hz, 7/6-*H*), 5.68 (2H, br s, NH_2), 4.36 (2H, q, J = 7.0 Hz, $CO_2CH_2CH_3$), 3.87 (3H, s, OCH_3), 3.58 (3H, s, NCH_3), 2.64 (3H, s, CH_3), 1.41 (3H, t, J = 7.0 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 167.3 (CO), 144.3, 141.4, 134.4, 131.6, 114.8, 110.0 (CH), 104.6, 96.8 (CH), 59.5 (OCH_2), 58.0 (OCH_3), 30.2 (NCH_3), 14.9 (CH_3), 13.3 (CH_3); HRMS found M^+ 262.1317, $C_{14}H_{18}N_2O_3$ requires M 262.1317. Anal. ($C_{14}H_{18}N_2O_3$) C, H, N.

Ethyl 5-Methoxy-1,2-dimethyl-4,7-dioxoindole-3-carboxylate (14). To a solution of ethyl 4-amino-5-methoxy-1,2-dimethylindole-3-carboxylate (**13**) (3.2 g, 12.2 mmol) in Me_2CO (400 mL) was added a solution of potassium nitrosodisulfonate (5 g, excess) in sodium dihydrogen phosphate buffer (0.3 M, 400 mL). The mixture was stirred at room temperature for 1 h. The excess Me_2CO was removed in vacuo. The resulting residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was recrystallized (CH_2Cl_2/light petroleum) to give an orange/yellow crystalline solid (3.3 g, 97%); mp 202−204 °C; UV (MeOH) 429 (ϵ 1004), 328 (2614), 288 (12 673), 217 nm (12 984); IR (KBr) 2980, 2942, 1686, 1639, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.64 (1H, s, 6-*H*), 4.36 (2H, q, J = 7.1 Hz, $CO_2CH_2CH_3$), 3.91 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 2.44 (3H, s, CH_3), 1.40 (3H, t, J = 7.1 Hz, $CO_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$) δ 179.5 (CO), 175.7 (CO), 164.7 (CO), 141.8, 129.8, 121.9, 113.4 106.3 (CH), 61.3 (OCH_2), 56.9 (OCH_3), 32.8 (NCH_3), 14.4 (CH_3), 10.9 (CH_3); HRMS found M^+ 227.0950, $C_{14}H_{15}NO_5$ requires M 277.0950.

3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (6). To a solution of ethyl 5-methoxy-1,2-dimethyl-4,7-dioxoindole-3-carboxylate (**14**) (3.4 g, 12.3 mmol) in $CHCl_3$ (370 mL) and EtOH (125 mL) was added a solution of sodium dithionite (25 g) in water (160 mL). The biphasic mixture was stirred vigorously overnight. The organic layer was separated,

washed with brine, dried (Na_2SO_4), and concentrated to give ethyl 4,7-dihydroxy-5-methoxy-1,2-dimethylindolecarboxylate as a solid that was used directly.

To a stirred suspension of the hydroquinone in CH_2Cl_2 (500 mL) was added DIBAL-H (1 M solution in CH_2Cl_2; 13.9 g, 97.9 mmol), keeping the temperature below −30 °C. The mixture was stirred at this temperature for a further 2 h. The reaction was quenched by dropwise addition of an iron(III) chloride solution (1 M $FeCl_3$/0.1 M HCl, 125 mL) while keeping the temperature below −30 °C. The mixture was filtered through Celite and the residue washed with hot CH_2Cl_2. The organic layer was separated, washed with saturated ammonium chloride, dried (Na_2SO_4), and concentrated. The crude product was recrystallized (CH_2Cl_2/light petroleum) to yield the title compound as an orange/red crystalline solid (2.0 g, 71%): mp 200−202 °C (lit.[1] mp 199−200 °C); ^{1}H NMR ($CDCl_3$) δ 5.60 (1H, s, 6-*H*), 4.59 (2H, d, J = 6.7 Hz, CH_2OH), 3.87 (1H, t, J = 6.7 Hz, O*H*), 3.85 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.21 (3H, s, CH_3).

3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (6) (Alternative Method). To a suspension of $LiAlH_4$ (1.28 g, 33.7 mmol) in THF (50 mL) at 0 °C was added a solution of ethyl 4-amino-5-methoxy-1,2-dimethylindole-3-carboxylate (**13**) (2.21 g, 8.4 mmol) in THF (25 mL). The mixture was allowed to warm to room temperature and stirred for 0.5 h. The mixture was cooled to 0 °C and the reaction quenched by the addition of water (1 mL), 1 M sodium hydroxide (1 mL), and silica gel (10 g). The granular precipitate was filtered off through a pad of Celite. The filtrate was dried ($MgSO_4$) and concentrated in vacuo to yield 4-amino-3-(hydroxymethyl)-5-methoxy-1,2-methylindole (1.42 g, 77%) as a dark-brown solid, which was used directly in the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 6.86 (1H, d, J = 8.7 Hz, Ar*H*), 6.59 (1H, d, J = 8.7 Hz, Ar*H*), 4.78 (2H, s, 3-CH_2), 3.88 (3H, s, OCH_3), 3.50 (3H, s, NCH_3), 2.25 (3H, s, CH_3).

To a solution of 4-amino-3-(hydroxymethyl)-5-methoxy-1,2-methylindole (1.42 g, 5.4 mmol) in Me_2CO (400 mL) was added a solution of potassium nitrosodisulfonate (5.8 g, 21.6 mmol) in sodium dihydrogen phosphate buffer (0.3 M, 400 mL). The mixture was stirred at room temperature for 1 h. The excess Me_2CO was removed in vacuo. The resulting residue was extracted with CH_2Cl_2 and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (EtOAc/CH_2Cl_2, 4:1) and recrystallized (CH_2Cl_2/light petroleum) to yield the title compound as an orange/red solid (0.96 g, 75%); data given above.

***(S)*-(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl 2-[(Benzyloxycarbonyl)amino]-3-methylbutanoate (15).** 3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (0.1 g, 0.42 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (0.098 g, 0.51 mmol), *N*-Cbz-L-valine (0.128 g, 0.51 mmol), and a catalytic quantity of 4-(dimethylamino)pyridine were stirred overnight in CH_2Cl_2 (10 mL) at room temperature. The crude mixture was washed with water, dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (EtOAc) to yield the title compound (0.14 g, 70%); mp 102−104 °C; UV (MeOH) 452 (ϵ 1430), 347 nm (2955); IR (KBr) 3347, 2964, 1724, 1673, 1642, 1601 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.32 (5H, m, Ar*H*), 5.62 (1H, s, 6-*H*), 5.29 (3H, m, N*H*, OCH_2Ph), 5.09 (2H, s, IndCH_2O), 4.27 (1H, dd, J = 9.1 Hz, J = 4.5 Hz, OCOC*H*), 3.89 (3H, s, OCH_3), 3.79 (3H, s, NCH_3), 2.26 (3H, s, CH_3), 2.14 (1H, m, C*H*(CH_3)$_2$), 0.92 and 0.85 (6H, 2 × d, J = 6.9 Hz, CH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$) δ 179.2 (CO), 177.9 (CO), 172.3 (CO), 160.1 (CO), 156.6, 138.5, 136.8, 129.5, 128.9 (CH), 128.5 (CH), 128.4 (CH), 122.1, 115.5, 107.1 (CH), 67.3 (CH_2), 59.5 (CH), 57.7 (CH_2), 56.8 (OCH_3), 32.8 (NCH_3), 31.7 (CH), 19.4 (CH_3), 17.7 (CH_3), 9.9 (CH_3); HRMS found M^+ 468.1881, $C_{25}H_{28}N_2O_7$ requires M 468.1896.

(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl (4-Phenyl)phenylacetate (16). 3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (0.085 g, 0.36 mmol), triphenylphosphine (0.19 g, 0.72 mmol), diethyl azodicarboxylate (0.125 g, 0.718 mmol), and 4-biphenylacetic acid (0.1 g, 0.47 mmol) were stirred overnight in THF (15 mL) at 50 °C. Excess solvent was removed and the majority of the product triturated out with ether and filtered off. The residue was dissolved in CH_2Cl_2, washed with water, dried (Na_2SO_4), and concentrated. The crude residue was purified by column chromatography (1:1 light petroleum/EtOAc) to give an orange crystalline solid (0.1 g, 87%): mp 163−165 °C; UV (MeOH) 454 (ϵ 846), 348 (1683), 243 nm (6876); IR (KBr) 3244, 3042, 2990, 1750, 1721, 1699, 1673, 1633, 1598 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.56 (5H, m, Ar*H*), 7.46 (2H, m, Ar*H*), 7.33 (2H, m, Ar*H*), 5.61 (1H, s, 6-*H*), 5.30 (2H, s, IndCH_2O-), 3.88 (3H, s, OCH_3), 3.97 (3H, s, NCH_3), 3.65 (2H, s, OCOCH_2), 2.22 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 179.2 (CO), 177.9 (CO), 171.9 (CO), 160.1, 141.2, 140.3, 138.3, 133.5, 130.1 (CH), 129.5, 129.1 (CH), 127.6 (CH), 127.4 (CH), 122.1, 116.1, 107.0 (CH), 57.5 (CH_2), 56.8 (OCH_3), 41.2 (CH_2), 32.7 (NCH_3), 9.9 (CH_3); HRMS found M^+ 429.1574, $C_{26}H_{23}NO_5$ requires M 429.1576.

(5-Methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl 2-Fluorobenzoate (17). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**5**) (0.47 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and 2-fluorobenzoyl chloride (0.87 g, 5 mmol) added. The solution was then stirred at room temperature for 2 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (1:1 hexane/EtOAc) to give an orange solid (328 mg, 46%), recrystallized from EtOAc: mp 175−176 °C; ^{1}H NMR ($CDCl_3$) δ 7.95 (2H, m, Ar*H*), 7.23 (2H, m, Ar*H*), 6.96 (1H, s, 2-*H*), 5.69 (1H, s, 6-*H*), 5.56 (2H, s, CH_2OAr), 3.96 (3H, s, OCH_3), 3.83 (3H, s, NCH_3). Anal. ($C_{18}H_{14}FNO_5$) C, H, N.

(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl 2-Fluorobenzoate (18). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**6**) (0.47 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and 2-fluorobenzoyl chloride (0.87 g, 5 mmol) added. The solution was then stirred at room temperature for 1.5 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (EtOAc) to give an orange solid (0.6 g, 84%), recrystallized from EtOAc: mp 166−168 °C; ^{1}H NMR ($CDCl_3$) δ 7.92 (2H, m, Ar*H*), 7.25 (2H, m, Ar*H*), 5.63 (1H, s, 6-*H*), 5.51 (2H, s, CH_2OAr), 3.90 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 2.35 (3H, s, CH_3). Anal. ($C_{19}H_{16}FNO_5 \cdot 0.3H_2O$) C, H, N.

(5-Methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl 4-Fluorobenzoate (19). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**5**) (0.44 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and 4-fluorobenzoyl chloride (0.87 g, 5 mmol) added. The solution was then stirred at room temperature for 2 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give an orange solid (316 mg, 46%), recrystallized from EtOAc: mp 210−212 °C; ^{1}H NMR ($CDCl_3$) δ 8.0 (2H, m, Ar*H*), 7.19 (2H, m, Ar*H*), 6.91 (1H, s, 2-*H*), 5.70 (1H, s, 6-*H*), 5.52 (2H, s, CH_2OAr), 3.96 (3H, s, OCH_3), 3.83 (3H, s, NCH_3). Anal. ($C_{18}H_{14}FNO_5$) C, H, N.

(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl 4-Fluorobenzoate (20). 3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (0.051 g, 0.22 mmol), triphenylphosphine (0.113 g, 0.43 mmol), diethyl azodicarboxylate (0.075 g, 0.43 mmol), and 4-fluorobenzoic acid (0.039 g, 0.27 mmol) were stirred overnight in THF (10 mL) at 50 °C. The solvent was evaporated and residue dissolved in CH_2Cl_2 and washed with 1 M HCl (20 mL) and NaOH (aqueous, 1 M, 20 mL). The CH_2Cl_2 layer was dried (Na_2SO_4) and concentrated

and the residue purified by column chromatography (EtOAc) and recrystallized (EtOAc/light petroleum) to yield the title compound as an orange crystalline solid (0.048 g, 63%): mp 212−214 °C; ^{1}H NMR ($CDCl_3$) δ 8.00 (2H, m, Ar*H*), 7.04 (2H, m, Ar*H*), 5.62 (1H, s, 6-*H*), 5.47 (2H, s, CH_2OAr), 3.91 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.34 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.8, 177.5, 167.4, 165.6, 164.0, 159.7, 138.0, 132.3 (CH), 132.2 (CH), 129.2, 126.6, 126.5, 121.9, 115.8, 115.5 (CH), 115.2 (CH), 106.7 (CH), 57.1 (OCH_2), 56.4 (OCH_3), 32.4 (NCH_3), 9.6 (CH_3); HRMS found M^+ 357.1013, $C_{19}H_{16}FNO_5$ requires M 357.1012.

(5-Methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl Benzoate (21). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**5**) (0.44 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and benzoyl chloride (0.78 g, 5 mmol) added. The solution was then stirred at room temperature for 16 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give an orange solid (312 mg, 48%), recrystallized from EtOAc: mp 134−135 °C; ^{1}H NMR ($CDCl_3$) δ 7.57 (5H, m, Ar*H*), 6.90 (1H, s, 2-H), 5.69 (1H, s, 6-*H*), 5.53 (2H, s, CH_2OAr), 3.93 (3H, s, OCH_3), 3.83 (3H, s, NCH_3). Anal. ($C_{18}H_{15}NO_5$·0.5H_2O) C, H, N.

(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl Benzoate (22). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**6**) (0.47 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and benzoyl chloride (0.78 g, 5 mmol) added. The solution was then stirred at 40 °C for 1.5 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (EtOAc) to give an orange solid (468 mg, 69%), recrystallized from EtOAc/Me_2CO: mp 168−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (2H, m, Ar*H*), 7.38 (3H, m, Ar*H*), 5.58 (1H, s, 6-*H*), 5.46 (2H, s, CH_2OAr), 3.86 (3H, s, OCH_3), 3.76 (3H, s, NCH_3), 2.31 (3H, s, CH_3). Anal. ($C_{19}H_{17}NO_5$) C, H, N.

(2-Cyclopropyl-5-methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl Benzoate (23). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**7**) (0.55 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and benzoyl chloride (5 mmol) added. The solution was then stirred at room temperature for 1.5 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (hexane/EtOAc, 1:1) to give an orange solid (496 mg, 68%), recrystallized from EtOAc/Me_2-CO: mp 221−223 °C; ^{1}H NMR ($CDCl_3$) δ 8.0 (2H, m, Ar*H*), 7.43 (3H, m, Ar*H*), 5.64 (1H, s, 6-*H*), 5.56 (2H, s, CH_2OAr), 4.03 (3H, s, OCH_3), 3.79 (3H, s, NCH_3), 1.66 (1H, m, c-Pr), 1.17 (2H, m, c-Pr). Anal. ($C_{21}H_{19}NO_5$) C, H, N.

3-(Benzyloxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (24). The 3-(chloromethyl)-5-methoxyindole-4,7-dione (**9**) (100 mg, 0.4 mmol) and K_2CO_3 (140 mg, 1 mmol) were dissolved in anhydrous EtOAc (20 mL), and benzyl alcohol (0.78 g, 5 mmol) was added with stirring. Stirring was continued at room temperature for 12 h and then water (20 mL) added. The organic layer was separated, washed with saturated $NaHCO_3$ (aqueous, 20 mL) and brine (aqueous, 20 mL), dried, and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc, 1:1) and recrystallized from EtOAc to give an orange solid (70 mg, 53%): mp 132−133 °C; ^{1}H NMR ($CDCl_3$) δ 7.32 (5H, m, Ar*H*), 5.60 (1H, s, 6-*H*), 4.74 (2H, s, IndCH_2O), 4.59 (2H, s, OCH_2-Ar), 3.86 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.25 (3H, s, CH_3). Anal. ($C_{19}H_{19}NO_4$·0.5H_2O) C, H, N.

(5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl Acetate (25). The 3-(hydroxymethyl)-5-methoxyindole-4,7-dione (**6**) (0.47 g, 2 mmol) was dissolved in CH_2Cl_2 (50 mL) together with pyridine (5 mL) and acetyl chloride (0.39 g, 5 mmol) added. The solution was then stirred at 40 °C for 0.5 h and EtOAc (150 mL) added followed by HCl (aqueous, 0.1 M, 150 mL). The organic layer was separated, washed again with HCl (aqueous, 0.1 M, 100 mL), $NaHCO_3$ (aqueous, 5%, 100 mL), and brine (aqueous, 100 mL), dried, and evaporated in vacuo. The residue was purified by column chromatography (EtOAc) to give an orange solid (0.38 g, 69%), recrystallized from EtOAc: mp 185−186 °C; ^{1}H NMR ($CDCl_3$) δ 5.62 (1H, s, 6-*H*), 5.24 (2H, s, CH_2OAc), 3.90 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 2.28 (3H, s, CH_3), 2.04 (s, 3H, COCH_3). Anal. ($C_{14}H_{15}NO_5$) C, H, N.

3-[(2,4,6-Trichlorophenyl)oxymethyl]-5-methoxy-1-methylindole-4,7-dione (26). The 3-(chloromethyl)-5-methoxyindole-4,7-dione (**8**) (90 mg, 0.4 mmol) and K_2CO_3 (140 mg, 1 mmol) were dissolved in anhydrous EtOAc (20 mL), and 2,4,6-trichlorophenol (0.9 g, 5 mmol) was added with stirring. Stirring was continued at room temperature for 15 h and then water (20 mL) added. The organic layer was separated, washed with saturated $NaHCO_3$ (aqueous, 20 mL) and brine (aqueous, 20 mL), dried, and evaporated to dryness. The residue was purified by column chromatography (EtOAc) and recrystallized from EtOAc to give an orange solid (104 mg, 61%): mp 222−223 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (2H, s, Ar*H*), 7.06 (s, 1H, 2-*H*), 5.67 (1H, s, 6-*H*), 5.26 (2H, s, IndCH_2O), 3.98 (3H, s, OCH_3), 3.82 (3H, s, NCH_3). Anal. ($C_{18}H_{12}Cl_3NO_5$) C, H, N.

5-Methoxy-3-(phenyloxymethyl)-1,2-dimethylindole-4,7-dione (27). To a stirring solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (78 mg, 0.33 mmol) in CH_2Cl_2 (30 mL) was added $SOCl_2$ (3.3 g, 27 mmol) dropwise. The solution was stirred at room temperature overnight. The solvent was removed and the crude material used directly in the next step. A solution of the crude chloride in DMF (20 mL) was added to a stirring suspension of phenol (62 mg, 0.66 mmol) and NaH (16 mg, 0.66 mmol) in DMF (25 mL) at 0 °C. After the addition the mixture was allowed to stir at room temperature for 3 h. The reaction was quenched by the addition of NH_4Cl (aqueous, saturated, 20 mL). The mixture was extracted with CH_2Cl_2, washed with NaOH (aqueous, 1 M), 1 M HCl, and water, dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography (EtOAc/petroleum ether, 1:1) and recrystallized (CH_2Cl_2/Et_2O) to give the title compound as an orange crystalline solid (67 mg, 65%): mp 183−185 °C; UV (MeOH) 456 (ϵ 1696), 342 (3110), 288 (18 363), 222 nm (25 148); IR (KBr) 3420, 2967, 2933, 1683, 1637, 1611, 1499 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.30 (2H, m, Ar*H*), 6.97 (3H, m, Ar*H*), 5.62 (1H, s, 6-*H*), 5.30 (2H, s, IndCH_2O), 3.89 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 2.31 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.7 (CO), 178.1 (CO), 159.6, 158.5, 138.0, 129.4 (CH), 128.7, 121.3, 120.9 (CH), 117.3, 115.0 (CH), 106.7 (CH), 60.4 (CH_2), 56.4 (OCH_3), 32.3 (NCH_3), 9.9 (CH_3); HRMS found M^+ 312.1236. $C_{18}H_{17}NO_4$ requires M 312.1236, Anal. ($C_{18}H_{17}NO_4$) C, H, N.

3-[(4-Fluorophenyl)oxymethyl]-5-methoxy-1,2-dimethylindole-4,7-dione (28). To a stirring solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (0.109 g, 0.46 mmol) in CH_2Cl_2 (30 mL) was added dropwise $SOCl_2$ (3.3 g, 27 mmol). The solution was stirred at room temperature overnight. The solvent was removed and the crude material used directly in the next step. A solution of the crude chloride in DMF (20 mL) was added to a stirring suspension of 4-fluorophenol (0.104 g, 0.92 mmol) and NaH (0.22 g, 0.92 mmol) in DMF (25 mL) at 0 °C. After the addition the mixture was stirred at room temperature for 3 h. The reaction was quenched by the addition of NH_4Cl (aqueous, saturated, 20 mL). The mixture was extracted with CH_2Cl_2, washed with NaOH (aqueous, 1 M), 1 M HCl, and water, dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography (EtOAc/petroleum ether, 1:1) and recrystallized (CH_2Cl_2/Et_2O) to give the title compound as an orange crystalline solid (59 mg, 43%): mp 191−192 °C; UV (MeOH) 458 (ϵ 1661), 342 (3043), 284 (19 691), 222 nm (22 191); IR

(KBr) 3401, 2934, 2848, 1677, 1637, 1604, 1505 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.94, 6.93 (4H, 2s, Ar*H*), 5.61 (1H, s, 6-*H*), 5.24 (2H, s, IndCH_2O), 3.88 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.29 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.7 (CO), 178.1 (CO), 159.6, 158.6, 156.2, 154.6, 138.0, 128.8, 121.3, 117.1, 116.3 (CH), 116.2 (CH), 115.8 (CH), 115.6 (CH), 106.7, 61.2 (CH_2), 56.4 (OCH_3), 32.3 (CH_3), 9.8 (CH_3); HRMS found M^+ 329.1063, $C_{18}H_{16}FNO_4$ requires M 329.1063. Anal. ($C_{18}H_{16}FNO_4$) C, H, N.

3-(5-Fluoro-2-hydroxybenzyl)-5-methoxy-1,2-dimethylindole-4,7-dione (29). The 3-(chloromethyl)-5-methoxyindole-4,7-dione (**6**) (94 mg, 0.4 mmol) and K_2CO_3 (140 mg, 1 mmol) were dissolved in anhydrous EtOAc (20 mL), and 4-fluorophenol (0.56 g, 5 mmol) was added with stirring. Stirring was continued at room temperature for 12 h and then water (20 mL) added. The organic layer was separated, washed with saturated $NaHCO_3$ (aqueous, 20 mL) and brine (aqueous, 20 mL), dried, and evaporated to dryness. The residue was purified by column chromatography (EtOAc) and recrystallized from EtOAc to give an orange solid (66 mg, 50%): mp 228−230 °C; ^1H NMR ($CDCl_3$) δ 6.86 (3H, m, Ar*H*), 5.62 (1H, s, 6-*H*), 4.99 (1H, br, ArO*H*), 4.01 (2H, s, IndCH_2-Ar), 3.89 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 2.33 (3H, s, CH_3). Anal. ($C_{18}H_{16}FNO_4$) C, H, N.

3-(3-Fluoro-4-hydroxybenzyl)-5-methoxy-1,2-dimethylindole-4,7-dione (30). The 3-(chloromethyl)-5-methoxyindole-4,7-dione (**6**) (94 mg, 0.4 mmol) and K_2CO_3 (140 mg, 1 mmol) were dissolved in anhydrous EtOAc (20 mL), and 2-fluorophenol (0.56 g, 5 mmol) was added with stirring. Stirring was continued at room temperature for 12 h and then water (20 mL) added. The organic layer was separated, washed with saturated $NaHCO_3$ (aqueous, 20 mL) and brine (aqueous, 20 mL), dried, and evaporated to dryness. The residue was purified by column chromatography (EtOAc) and recrystallized from EtOAc to give an orange solid (65 mg, 49%): mp 212−213 °C; ^1H NMR ($CDCl_3$) δ 6.89 (3H, m, Ar*H*), 5.59 (1H, s, 6-*H*), 4.92 (1H, br, ArO*H*), 4.02 (2H, s, IndCH_2-Ar), 3.89 (3H, s, OCH_3), 3.79 (3H, s, NCH_3), 2.21 (3H, s, CH_3). Anal. ($C_{18}H_{16}FNO_4$) C, H, N.

3-[(3-Acetamidophenyl)oxymethyl]-5-methoxy-1,2-dimethylindole-4,7-dione (31). To a stirring solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (0.307 g, 1.3 mmol) in CH_2Cl_2 (20 mL) was added dropwise $SOCl_2$ (7.77 g, 65 mmol). 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, 3-acetamidophenol (0.59 g, 3.9 mmol), and K_2CO_3 (0.54 g, 3.9 mmol) were stirred in DMF (15 mL) overnight. The DMF was evaporated in vacuo, and the residue was dissolved in CH_2Cl_2. The CH_2Cl_2 was washed with NaOH (aqueous, 1 M), 1 M HCl, and water, dried (Na_2SO_4), and concentrated. The crude material was purified by column chromatography (EtOAc/CH_2Cl_2, 9:1) to give the title compound as a yellow/orange crystalline solid (0.29 g, 62%): mp 264−266 °C; UV (MeOH) 444 (ϵ 1161), 433 (2102), 288 (14 597), 216 nm (29 064); IR (KBr) 3302, 3256, 3138, 2954, 1683, 1637, 1611, 1518 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.84 (1H, s, N*H*), 7.28 (1H, t, J = 2.0 Hz, Ar*H*), 7.16 (1H, t, J = 8.1 Hz, Ar*H*), 7.08 (1H, d, J = 8.8 Hz, Ar*H*), 6.65 (1H, dd, J = 8.1 Hz, J = 1.8 Hz, Ar*H*), 5.75 (1H, s, 6-*H*), 5.10 (2H, s, IndCH_2O), 3.85 (3H, s, OCH_3), 3.74 (3H, s, NCH_3), 2.24 (3H, s, CH_3), 2.01 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6) δ 178.7, 177.8, 168.7, 159.7, 159.2, 140.9, 139.0, 129.8 (CH), 128.5, 121.2, 116.4, 111.9 (CH), 109.4 (CH), 107.2 (CH), 106.2 (CH), 60.3 (CH_2), 57.0 (OCH_3), 32.6 (NCH_3), 24.5 ($NCOCH_3$), 9.7 (CH_3). Anal. ($C_{20}H_{20}N_2O_5$) C, H, N.

5-Methoxy-1,2-dimethyl-3-[(pyrimidylthio)methyl]indole-4,7-dione (32). 3-(Hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (90 mg, 0.38 mmol), 2-mercaptopyrimidine (0.085 g, 0.75 mmol), and dimethylformamide dineopentyl acetal (0.32 g, 1.38 mmol) in CH_3CN (50 mL) were heated for 2 h at 80 °C. The solvent was removed under reduced pressure and the crude mixture purified on silica gel to give the desired product as an orange crystalline solid (90 mg, 71%): mp 238−239 °C; UV (MeOH) 464 (ϵ 2202), 348 nm (3353); IR (KBr) 3031, 1940, 1664, 1599, 1564, 1548 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.51 (2H, d, J = 5.0 Hz, NC*H*, pyrimidine), 6.95 (1H, t, J = 5.0 Hz, C*H*, pyrimidine), 5.60 (1H, s, 6-*H*), 4.59 (2H, s, CH_2S), 3.88 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.34 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 178.7 (CO), 177.7 (CO), 172.8, 159.7, 157.1 (CH), 136.6, 128.8, 121.3 (CH), 117.0, 116.3 (CH), 106.8 (CH), 56.4 (OCH_3), 32.4 (NCH_3), 25.2 (SCH_2), 9.8 (CH_3); HRMS found M^+ 329.0838, $C_{16}H_{15}N_3O_3S$ requires M 329.0834.

Typical Reduction Procedure. The indolequinone drug (20−30 mg) and potassium ethyl xanthate (5 equiv) in THF/H_2O (3:1) (20 mL) were degassed thoroughly by bubbling nitrogen directly into the stirring solution. Sodium dithionite ($Na_2S_2O_4$, sodium hydrosulfite; 10 equiv) was added in one portion with the nitrogen needle still in the stirring solution. After a few minutes the orange coloration was lost from the solution. The formation of released compound XH and the xanthate-captured product was followed by TLC, and the reaction was usually completed within 30 min. The solvent was removed and the residue dissolved in CH_2Cl_2 and washed with NH_4Cl (aqueous, saturated). The organic layer was dried (Na_2SO_4) and concentrated, and after analysis by TLC, the mixture was separated by column chromatography to give the trapped indolequinone **33** (or **34**) and the product of elimination.

***O*-Ethyl (5-methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)-methyl Dithiocarbonate (33):** data given below.

***O*-Ethyl (5-methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl dithiocarbonate (34):** mp 153−154 °C; UV (MeOH) 351 (ϵ 4000), 280 (33 500), 223 nm (28 750); IR (film) 3059, 2993, 1683, 1663, 1593, 1518 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.85 (1H, s, 2-*H*), 5.64 (1H, s, 6-*H*), 4.64 (2H, q, J = 7.1 Hz, CH_2CH$_3$), 4.51 (2H, s, IndCH_2), 3.91 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 1.40 (3H, t, J = 7.1 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 214.8 (CS), 178.8 (CO), 177.6 (CO), 160.3, 129.7, 129.4, 121.2, 120.3, 106.8 (CH), 70.0 (CH_2), 56.5 (OCH_3), 36.2 (NCH_3), 30.6 (CH_2), 13.8 (CH_3); HRMS found M^+ 325.0443, $C_{14}H_{15}NO_4S_2$ requires M 325.0442.

Independent Synthesis of *O*-Ethyl (5-Methoxy-1,2-dimethyl-4,7-dioxoindol-3-yl)methyl Dithiocarbonate (33). Methanesulfonyl chloride (0.033 g, 0.28 mmol) was added dropwise to a stirring solution of 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (**6**) (0.053 g, 0.22 mmol) in CH_2Cl_2 (10 mL) and Et_3N (0.029 g, 0.28 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min. The crude material was concentrated and the residue used directly in the next step without purification. To a stirring solution of the mesylate in CH_2Cl_2 (10 mL) was added potassium ethyl xanthate (0.11 g, 0.68 mmol). The mixture was stirred at room temperature for 3 h. The crude material was concentrated and the residue purified by column chromatography (EtOAc) to give the title compound as an orange crystalline solid (0.053 g, 85%): mp 205−207 °C; UV (MeOH) 459 (ϵ 2381), 348 (3907), 270 (17 187), 229 nm (13 662); IR (KBr) 2986, 1667, 1638, 1597, 1507 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.60 (1H, s, 6-*H*), 4.66 (2H, q, J = 7.7 Hz, OCH_2Me), 4.58 (2H, s, CH_2S), 3.88 (3H, s, OCH_3), 3.80 (3H, s, NCH_3), 2.29 (3H, s, CH_3), 1.43 (3H, t, J = 7.0, OCH$_2$CH_3); ^{13}C NMR ($CDCl_3$) δ 215.2 (CS), 178.9 (CO), 178.0 (CO), 160.0, 137.0, 129.3, 121.6, 116.3, 107.2 (CH), 70.3 (CH_2), 56.8 (OCH_3), 32.8 (NCH_3), 30.9 (CH_2), 14.2 (CH_3), 10.2 (CH_3); HRMS found M^+ 339.0606, $C_{15}H_{17}NO_4S_2$ requires M 339.0599. Anal. ($C_{15}H_{17}NO_4S_2$) C, H, N.

Independent Synthesis of 3-[(2-Methylethoxy)methyl]-5-methoxy-1,2-dimethylindole-4,7-dione (35). The 3-(chloromethyl)-5-methoxyindole-4,7-dione (**6**) (94 mg, 0.4 mmol) and K_2CO_3 (140 mg, 1 mmol) were dissolved in anhydrous EtOAc (20 mL), and 2-propanol (5 mL) was added with stirring. Stirring was continued at 90 °C for 1 h and then water (20 mL) added. The organic layer was separated, washed with saturated $NaHCO_3$ (aqueous, 20 mL) and brine (aqueous, 20 mL), dried, and evaporated to dryness. The residue was purified by column chromatography (hexane/EtOAc, 1:1) and recrystallized from EtOAc to give an orange solid (83 mg, 75%): mp 174−176 °C; ^1H NMR ($CDCl_3$) δ 5.54 (1H, s, 6-*H*),

4.57 (2H, s, IndCH_2O), 3.82 (3H, s, OCH_3), 3.73 (3H, s, NCH_3), 3.62 (1H, m, *i*-Pr), 2.24 (3H, s, CH_3), 1.11 (6H, d, J = 6 Hz, *i*-Pr). Anal. ($C_{15}H_{19}NO_4$) C, H, N.

Biological Evaluation in Vitro. Selective toxicity to hypoxic V79-379A cells was determined for all compounds using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay as has been described previously.[44−46] These results are presented in Table 3, where C_{50}(air), the concentration required to kill 50% of the aerobic cells under the conditions of the assay, is divided by $C_{50}(N_2)$, the corresponding concentration for hypoxic cell killing, to give hypoxic cytotoxicity ratios (HCR).

Pulse Radiolysis. The redox properties of the quinones and the kinetic characteristics of the associated semiquinone radicals ($Q^{\bullet-}$) were investigated by pulse radiolysis.[47] Semiquinone radicals were generated following reduction of the parent indolequinone by the 2-propanol radical ($(CH_3)_2C^{\bullet}OH$). Kinetic spectrophotometry with sub-microsecond resolution was used to monitor the reactions of quinone radicals. Experiments were performed using a 6-MeV linear accelerator as described previously.[48] The redox potentials of the one-electron couple $E(Q/Q^{\bullet-})$ were determined by establishing redox equilibria with a viologen (V^{2+}) of known redox potential according to methods described previously.[49] Solutions contained 2-propanol (1 M) and phosphate buffer (NaH_2PO_4/Na_2HPO_4, 4 mM, pH 7.4) with Q (0−60 μM) and MV^{2+} (0−4 mM). Absorbances were measured at 600 nm at a dose per pulse of 3 Gy. The alcohol converts the radiolytically produced $^{\bullet}OH$ and $H^{\bullet}$ radicals in <2 μs to the reductant $(CH_3)_2C^{\bullet}OH$ which reduces the indolequinones and the MV^{2+} to the corresponding semiquinone ($Q^{\bullet-}$) radicals or viologen radical-cation ($V^{\bullet+}$). Redox equilibration usually occurred within ~100 μs (during which time there was negligible decay of semiquinone radicals via bimolecular decay), and it was therefore possible to calculate the equilibrium constant K from absorbances at equilibrium. Values for $E(Q/Q^{\bullet-})$ are quoted relative to $E(MV^{2+}/MV^{\bullet+})$ = −442 ± 7 mV corrected for 1 M 2-propanol[50] and $E(TQ^{2+}/TQ^{\bullet+})$ = −523 ± 7 mV corrected for 2 M 2-propanol.

The reactivities of semiquinone radicals with oxygen were determined by gassing solutions with N_2O/O_2 mixtures (British Oxygen Co., U.K.) to give solutions which contained 0.1−0.5% O_2. In the presence of oxygen the semiquinone radicals decayed faster with increasing oxygen concentrations. Absolute rate constants were determined from the slopes of the linear plots of the observed first-order rate constants versus oxygen concentration. The latter was corrected for oxygen solubility in 2 M 2-propanol from published data.[51]

Steady-State γ-Radiolysis. Indolequinone solutions were saturated with N_2O gas in gastight vials before irradiation in a ^{60}Co source. An absorbed dose of 1 Gy = 0.67 μM $(CH_3)_2C^{\bullet}OH$ radicals in N_2O-saturated 2-propanol/water (50%, v/v). A dose rate of 6−6.5 Gy min^{-1} was used, as determined by Fricke dosimetry,[52] and radiation chemical yields were corrected for the absorbed dose in the various alcohol−water mixtures employed.

High-Performance Liquid Chromatography (HPLC). Product analysis following γ-radiolysis was performed by gradient HPLC separation on a 100 mm × 4.6 mm base-deactivated reverse-phase column (Hichrom RPB, Hichrom, Reading, U.K.). The eluents were (A) KH_2PO_4 (5 mM), H_3PO_4 (5 mM); (B) CH_3CN/H_2O (3:1, v/v), with a flow rate of 2 cm^3 min^{-1}. One of two linear gradients was used for each compound: (1) 35−80% B in 8 min or (2) 20−50% B in 5 min. Detection was at 232 nm using a Waters 486 detector (Watford, U.K.), and concentrations were determined from peak areas using Waters Maxima software.

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