Controlling the rates of reductively-activated elimination from the (indol-3-yl)methyl position of indolequinones

Steven A. Everett,*[a] Matthew A. Naylor,[a] Paola Barraja,[b] Elizabeth Swann,[b] Kantilal B. Patel,[a] Michael R. L. Stratford,[a] Anna R. Hudnott,[b] Borivoj Vojnovic,[a] Rosalind J. Locke,[a] Peter Wardman[a] and Christopher J. Moody[b]

[a] *Gray Laboratory Cancer Research Trust, Mount Vernon Hospital, PO Box 100, Northwood, Middlesex, UK HA6 2JR*
[b] *School of Chemistry, University of Exeter, Stocker Road, Exeter, UK EX4 4QD*

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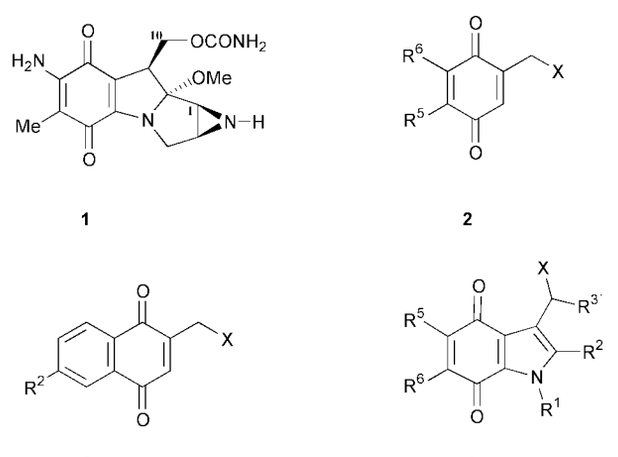
A series of substituted 3-(4-nitrophenyloxy)methylindole-4,7-diones (Q) were synthesised. The effects of substitution patterns on the indole core on rates of elimination of 4-nitrophenol as a model for drug release following fragmentation of a phenolic ether linker were studied. After reduction to either the radical anion ($Q^{\bullet -}$) or hydroquinone (QH_2) elimination of 4-nitrophenol occurred from the (indol-3-yl)methyl position. The half-lives of $Q^{\bullet -}$ radicals at $[O_2] \approx 5$ μmol dm^{-3}, typical of tumour hypoxia, were $t_{\frac{1}{2}} \approx 0.3$–1.8 ms, the higher values associated with higher reduction potentials. Half-lives for the autoxidation of the QH_2 were markedly longer at the same oxygen concentration ($t_{\frac{1}{2}} \approx 8$–102 min) and longer still in the presence of 4 μmol dm^{-3} superoxide dismutase ($t_{\frac{1}{2}} \approx 8$–19 h). Although the indolequinones were able to eliminate 4-nitrophenol with high efficiency only $Q^{\bullet -}$ radicals of the 3-carbinyl substituted derivatives did so with sufficiently short half-lives ($t_{\frac{1}{2}} \approx 41$–2 ms) to compete with electron transfer to oxygen and therefore have the potential to target the leaving group to hypoxic tissue. The hydroquinones are not sufficiently oxygen sensitive to prevent the elimination of 4-nitrophenol ($t_{\frac{1}{2}} \approx 1.5$–3.5 s) even at oxygen concentrations expected in normal tissue. By incorporating electron rich substituents at the indolyl carbinyl position it is possible to control the rate of reductive fragmentation. This may prove an important factor in the design of an indolequinone-based bioreductive drug delivery system.

Introduction

We have recently reported on the potential of indolequinones to act as a reductively-activated drug delivery system.[1–3] This class of compound was shown to be able to efficiently eliminate a variety of leaving groups from the (indol-3-yl)methyl position upon reduction.[2] Subsequent studies on the oxygen sensitive reduction chemistry of this class of compound revealed that the rate of reductive fragmentation is probably an important determinant of prodrug selectivity for hypoxic tissues.[4]

Indolequinones have been considered of particular importance in this field, because of the potential of indol-3-ylcarbinyl substituents in such compounds to undergo an elimination process upon reductive activation. This elimination chemistry, through the intervention of 'normal' indole reactivity, is suppressed in the quinone through delocalization into the quinonoid vinylogous amide system. The archetypal indolequinone-based bioreductive drug is mitomycin C (**1**, Fig. 1), which has been widely studied in this context, eliminating a carbamate group from the equivalent position.[5–7] Such processes have been demonstrated previously with simpler benzoquinones[8] and naphthoquinones[9] bearing halide leaving groups (*e.g.* **2** and **3**, Fig. 1), but not with biologically useful leaving groups. As depicted in Scheme 1 there are two possible reductive pathways for fragmentation involving either one-electron reduction to the intermediate semiquinone radical ($Q^{\bullet -}$) catalysed in biological systems by for example, NADPH: cytochrome c (P450) reductase[10] and/or two-electron reduction to the hydroquinone (QH_2). The latter occurs biologically following reduction by DT-diaphorase (NQO1),[11–16] where QH_2 is formed directly *via* hydride transfer, by-passing $Q^{\bullet -}$ radical formation.[17] From a chemical kinetic point of view the selectivity of indolequinones for hypoxic tissue will require establishing



1 **2** **3** **4**

Fig. 1 Structures of bioreductive quinones: compound **1** = mitomycin C; **2** = 2-CH_2X-1,4-benzoquinone (X = leaving group); **3** = 2-CH_2X-1,4-napthoquinone (X = leaving group); **4** = substituted indolequinone.

a balance between the one-electron reduction potential (which governs the rate of $Q^{\bullet -}$ radical scavenging by oxygen) and the rate of reductive elimination from the (indol-3-yl)methyl position. If the reactivity of the $Q^{\bullet -}$ radical with oxygen is much faster than the rate of reductive elimination then the half-life of the $Q^{\bullet -}$ radical may be too short to allow efficient release of a leaving group even under severe hypoxia. Conversely, should the rate of reductive elimination be much greater than the rate of $Q^{\bullet -}$ radical reactivity with oxygen then the release of the leaving group may also occur in normoxic tissue. Both scenarios would limit the hypoxia-selectivity of these indolequinones which would rely entirely on two-electron reducing

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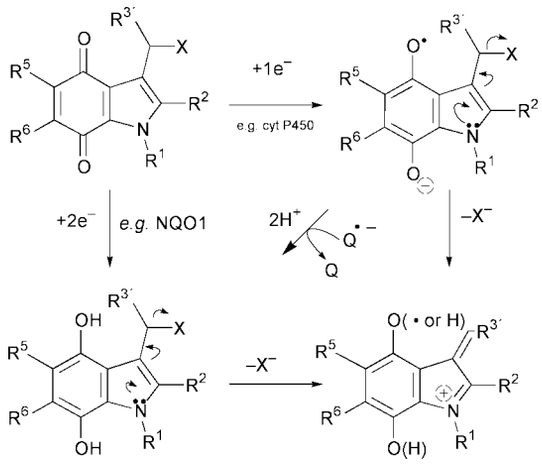


Scheme 1 One- and two-electron reduction pathways leading to the elimination of a leaving group (X) from 3-carbinyl-substituted indolequinones.

enzymes such as NQO1 to promote reductive elimination directly from the hydroquinone.[4,18]

A series of indolequinones (**4**, Fig. 1) with varying alkyl and aryl substituents were prepared. The derivatives studied included those substituted on the exocyclic (indol-3-yl)carbinyl group and bearing as a leaving group (X), the chromophoric 4-nitrophenol moiety (a model for drugs eliminated through a phenolic ether linkage). The indolequinones were reduced in a controlled and quantifiable manner using radiolytically-produced reducing radicals. The rates of reductive elimination of the model leaving group from both $Q^{\bullet -}$ radical and QH_2 were determined by pulse radiolysis. These rates were then compared with the corresponding reactivities of the $Q^{\bullet -}$ radical and QH_2 with oxygen with a view to controlling drug delivery over the range of oxygen concentrations present in hypoxic tumours.

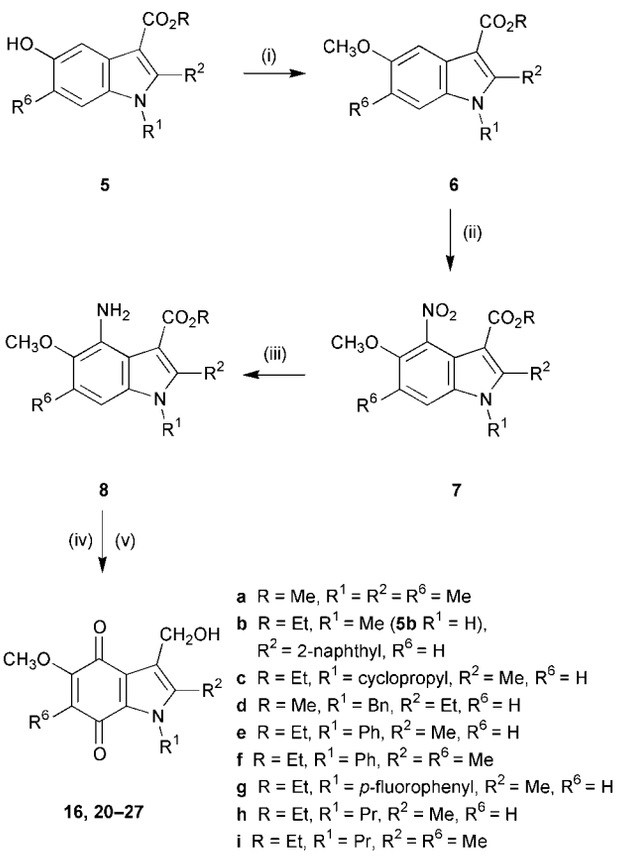
Results

Synthesis

A number of indolequinones had been prepared previously. New derivatives were prepared from 5-hydroxyindole-3-carboxylates **5** obtained from the Nenitzescu reaction of 1,4-benzoquinones with aminoalkenoates. Subsequent methylation to 5-methoxyindole-3-carboxylates **6** and nitration gave 4-nitroindoles **7** in good yield, after chromatographic separation of the minor 6-nitro isomer in some cases. Reduction of the nitro group to the amine **8** was followed by further reduction of the ester, and oxidation to the indolequinones using Fremy's salt (Scheme 2) except for **9** where the sequence of the ultimate step was reversed. The starting materials for the indolequinones **28**, **30** and **31** were the 3-acylindoles **10** prepared *via* their *N*-bromomagnesyl (Grignard) indole derivatives. Treatment with the appropriate acyl chloride then furnished the required 3-acylindoles **10**, which were then converted into the indolequinones **28**, **30** and **31** by way of the nitro compounds **11** and acylquinones **12** (Scheme 3) using established methods.[2,4] In general, the 3-(hydroxymethyl)indolequinones **13**–**31** (Table 1) were converted into the corresponding 3-(4-nitrophenoxy) derivatives **32**–**50** by coupling to 4-nitrophenol under standard conditions (Scheme 4).

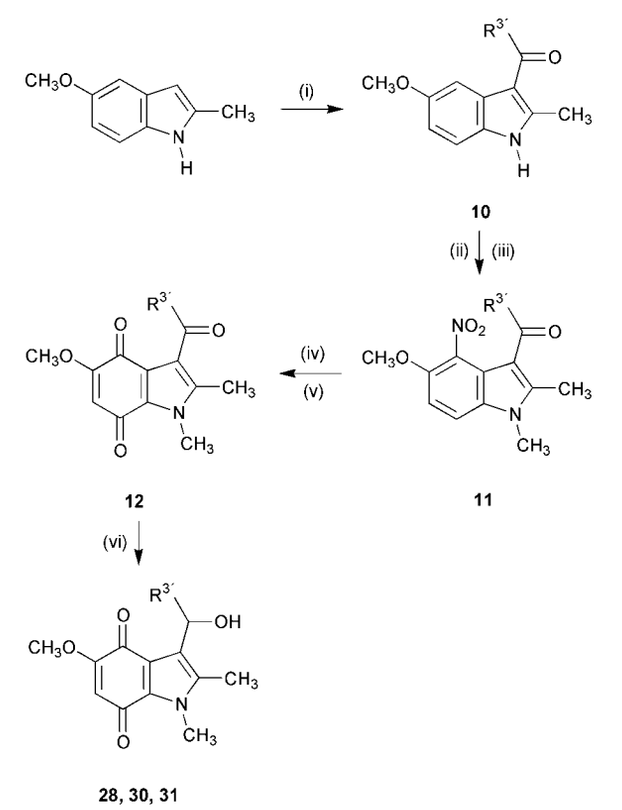
Chemical kinetics

Table 2 contains the one-electron reduction potentials and rate constants for the reaction of semiquinone radicals with oxygen, and rates of hydroquinone autoxidation for the corresponding alcohols **13**–**31**. Table 3 contains the rate constants for the reductive elimination of 4-nitrophenol plus the leaving group



a R = Me, $R^1 = R^2 = R^6$ = Me
b R = Et, R^1 = Me (**5b** R^1 = H), R^2 = 2-naphthyl, R^6 = H
c R = Et, R^1 = cyclopropyl, R^2 = Me, R^6 = H
d R = Me, R^1 = Bn, R^2 = Et, R^6 = H
e R = Et, R^1 = Ph, R^2 = Me, R^6 = H
f R = Et, R^1 = Ph, $R^2 = R^6$ = Me
g R = Et, R^1 = *p*-fluorophenyl, R^2 = Me, R^6 = H
h R = Et, R^1 = Pr, R^2 = Me, R^6 = H
i R = Et, R^1 = Pr, $R^2 = R^6$ = Me

Scheme 2 *Reagents and conditions*: (i) KOH, MeI, DMSO; (ii) HNO_3, AcOH; (iii) Sn, HCl; (iv) $LiAlH_4$, THF; (v) Fremy's salt, Me_2CO, NaH_2PO_4 buffer.



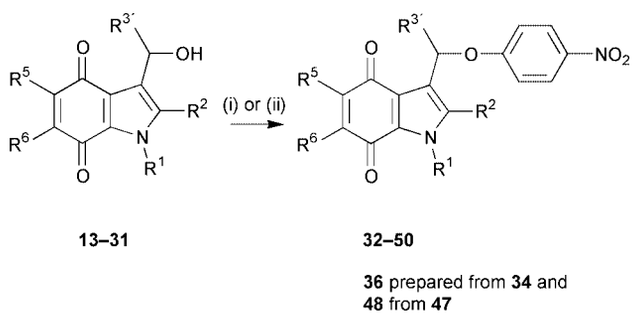
Scheme 3 *Reagents and conditions*: (i) EtMgBr, Et_2O then $R^{3'}COCl$; (ii) NaH, MeI, THF; (iii) HNO_3, AcOH; (iv) Sn, HCl; (v) Fremy's salt; (vi) $NaBH_4$ then air.

efficiencies of the 3-carbinyl substituted indolequinones under study **32**–**50**. The indolequinone alcohols **13**–**31** exhibit much poorer leaving group ability [leaving group (H_2O) pK_a = 15.7]

Table 1 3-Hydroxymethylindole-4,7-diones **13**–**31** and their corresponding 3-(4-nitrophenoxy)alkyl indolequinones **32**–**50**

CH_2OH indole	R^1	R^2	$R^{3'}$	R^5	R^6	CH_2OAr indole
13[a]	Me	H	H	MeO	H	**32**
14[b]	-$(CH_2)_3$-	—	H	MeO	H	**33**
15[b]	Me	Me	H	MeO	H	**34**
16	Me	Me	H	MeO	Me	**35**
17	Me	Me	H	Morpholino	H	**36**
18	Me	Ph	H	MeO	H	**37**
19	Me	4-Ph-C_6H_4	H	MeO	H	**38**
20	Me	2-Naphthyl	H	MeO	H	**39**
21	*c*-Pr	Me	H	MeO	H	**40**
22	CH_2Ph	Et	H	MeO	H	**41**
23	Ph	Me	H	MeO	H	**42**
24	Ph	Me	H	MeO	Me	**43**
25	4-F-C_6H_4	Me	H	MeO	H	**44**
26	*n*-Pr	Me	H	MeO	H	**45**
27	*n*-Pr	Me	H	MeO	Me	**46**
28	Me	Me	Me	MeO	H	**47**
29	Me	Me	Me	4-Methylpiperazin-1-yl	H	**48**
30	Me	Me	Ph	MeO	H	**49**
31	Me	Me	2-Thienyl	MeO	H	**50**

[a] Ref. 2. [b] Ref. 13.



Scheme 4 *Reagents and conditions*: (i) $SOCl_2$–CH_2Cl_2 then 4-NO_2-C_6H_4-OH–NaH–DMF; (ii) 4-NO_2-C_6H_4-OH–Ph_3P–DEAD–THF.

than their corresponding 4-nitrophenol conjugates [leaving group (4-nitrophenol) $pK_a = 7.8 \pm 0.1$] but their redox properties do not differ appreciably. The oxygen sensitivity of the semiquinone radical and hydroquinone autoxidation of **13**–**31** will parallel that of their 4-nitrophenoxy derivatives **32**–**50**, but measurements could be made without interference from the reductive elimination of the leaving group.

Rates of reductive elimination of 4-nitrophenol from substituted indolequinones. Elimination of 4-nitrophenol could feasibly occur either from the one-electron reduction of the parent indolequinones to the semiquinone radical ($Q^{\bullet -}$) or two-electron reduction to the hydroquinone (QH_2) as depicted in Scheme 1. Indolequinones were rapidly reduced by the propan-2-ol $(CH_3)_2C^{\bullet}OH$ radical ($k_1 \approx 10^9$ dm^3 mol^{-1} s^{-1}) to generate $Q^{\bullet -}$ radicals *via* reaction (1).

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

Fig. 2 shows the absorption spectra obtained by pulse radiolysis of an N_2O-saturated propan-2-ol–water mixture (50%, v/v) containing 40 μmol dm^{-3} **34**, in potassium phosphate buffer (4 mmol dm^{-3}) at pH 7.4. The initial spectrum observed ~200 μs after the electron pulse is characteristic of the $Q^{\bullet -}$ radical generated in reaction (1) and was virtually identical to that obtained on reduction of the alcohol **15**. Similar spectra were previously observed on the reduction of (5-methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl derivatives.[2] The rate constant for the reduction of the alcohol **15** by the $(CH_3)_2C^{\bullet}OH$ radical was determined to be $k_1 = (4.1 \pm 0.1) \times 10^9$ dm^3 mol^{-1} s^{-1}, over one order of magnitude faster than the reduction of 4-nitrophenol $k = (1.5 \pm 0.1) \times 10^8$ dm^3 mol^{-1} s^{-1} to the nitro anion radical

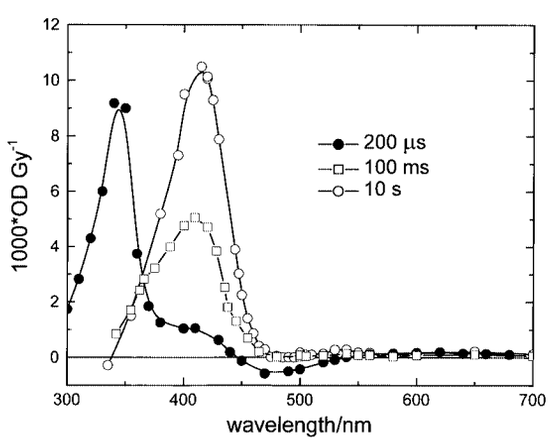


Fig. 2 Absorption spectra obtained on the reduction of **34** (40 μmol dm^{-3}) by the $(CH_3)_2C^{\bullet}OH$ radical at pH 9.3: (–●–) 200 μs, (–□–) 100 ms and (–○–) 10 s after the pulse. All absorbances were normalized to a dose of 16 Gy corresponding to $[(CH_3)_2C^{\bullet}OH] \approx 11$ μmol dm^{-3} in a 2 cm path length optical cell.

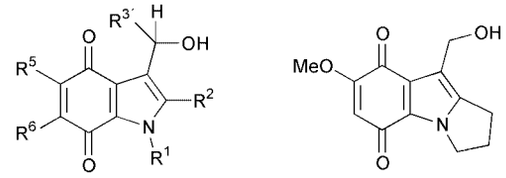
($RNO_2^{\bullet -}$), indicating that the reduction of **34** generates predominantly (~96%) $Q^{\bullet -}$ radical. The rate constant for the reduction of 4-nitrophenol by $(CH_3)_2C^{\bullet}OH$ was determined from the first-order build-up of the $RNO_2^{\bullet -}$ anion radical at 300 nm at [4-nitrophenol] ≈ 25–200 μmol dm^{-3} in propan-2-ol–water (50%, v/v) at pH 7.4 and was slower than that previously determined in propanol–water (95/5%, v/v) by conductivity detection.[19] From the pH dependence of the absorption at 345 nm, a pK_a ($QH^{\bullet}/Q^{\bullet -}$) = 5.2 ± 0.1 for **15** was obtained indicating that the semiquinone radical is deprotonated at physiological pH, which is expected to be the case for all 4-nitrophenol conjugates **32**–**50**.

At pH 4.5 the semiquinone radical absorption of **34** recorded at 345 nm decayed *via* pure second order kinetics with a half-life which decreased with increasing radiation dose or initial concentration of reducing radicals ([$(CH_3)_2C^{\bullet}OH$] ≈ 3–30 μmol dm^{-3}), indicating that the semiquinone radical decays predominantly by a radical–radical reaction to generate the hydroquinone *via* reaction (2).

$$Q^{\bullet -} + Q^{\bullet -} + 2H^+ \rightleftharpoons QH_2 + Q \qquad (2)$$

The reciprocal of the first half-life of the semiquinone radical varied linearly with the initial radical concentration, and from the slope of the fitted straight line, the rate constant $2k_2$ =

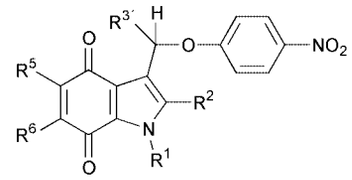
Table 2 One-electron reduction potentials, rate constants for the reaction of semiquinone radicals with oxygen and rates of hydroquinone autoxidation for selected indolequinones



Q	Type	R^1	R^6	R^5	R^2	$R^{3'}$	K	$E(Q/Q^{\bullet -})$/mV	$10^{-8}\ k_6(Q^{\bullet -} + O_2)$/dm^3 mol^{-1} s^{-1}	Apparent k_5/dm^3 mol^{-1} s^{-1} [f]	
										−SOD	+SOD[g]
13	A	Me	H	MeO	H	H	6.8 ± 0.4	−332 ± 9[a]	4.4 ± 0.1	~120–200	~4
14	B	—	—	—	—	—	137.6 ± 8.6	−329 ± 9[b]	2.1 ± 0.1	~170–280	~5
15	A	Me	H	MeO	Me	H	17.5 ± 1.4	−376 ± 9[d]	5.2 ± 0.1	~170–280	~5
16	A	Me	Me	MeO	Me	H	217 ± 9	−317 ± 8[a]	1.2 ± 1.2	~22–121	~30
17	A	Me	H	Morpholino	Me	H	36.5 ± 1.1	−365 ± 8[c]	5.1 ± 0.1	~65–238	~6
18	A	Me	H	MeO	Ph	H	8.0 ± 0.3	−315 ± 8[e]	1.2 ± 0.1	~40–100	~2
19	A	Me	H	MeO	4-Ph-C_6H_4	H	—	—	1.3 ± 0.2	~43–280[h]	—
20	A	Me	H	MeO	2-Naphthyl	H	—	—	1.5 ± 0.3	~70–320[h]	—
21	A	*c*-Pr	H	MeO	Me	H	4.4 ± 0.3	−336 ± 5[a]	1.3 ± 0.4	~21–253	~20
22	A	CH_2Ph	H	MeO	Et	H	6.6 ± 0.1	−326 ± 3[a]	2.2 ± 0.3	~42–262	~2
23	A	Ph	H	MeO	Me	H	4.4 ± 0.3	−336 ± 5[a]	1.4 ± 0.4	~16–318	~16
24	A	Ph	Me	MeO	Me	H	4.3 ± 0.3	−346 ± 5[a]	4.6 ± 0.5	~14–320	~16
25	A	*p*-F-C_6H_4	H	MeO	Me	H	12.0 ± 0.6	−310 ± 4[a]	1.8 ± 0.6	~20–116	~22
26	A	*n*-Pr	H	MeO	Me	H	6.4 ± 0.5	−318 ± 4[a]	2.1 ± 0.3	~45–220	~2
27	A	*n*-Pr	Me	MeO	Me	H	6.5 ± 0.5	−324 ± 4[a]	2.4 ± 0.3	~48–204	~4
28	A	Me	H	MeO	Me	Me	398.4 ± 24.8	−302 ± 9[b]	0.9 ± 0.1	~173–376	~2
29	A	Me	H	4-Methylpiperazin-1-yl	Me	Me	31.6 ± 2.1	−285 ± 5[a]	1.1 ± 0.1	~55–160	~15
30	A	Me	H	MeO	Me	Ph	32.1 ± 1.0	−290 ± 4[a]	0.9 ± 0.1	~147–143	~83
31	A	Me	H	MeO	Me	Thienyl	44.2 ± 1.4	−277 ± 4[a]	0.8 ± 0.1	~49–177	~60

[a] Redox potentials *vs.* $E(BV^{2+}/BV^{\bullet +}) = -374 \pm 3$ mV. [b] Potentials *vs.* $E(MV^{2+}/MV^{\bullet +}) = -448 \pm 3$ mV (corrected for 0.7 mol dm^{-3} propan-2-ol). [c] Potentials *vs.* $E(MV^{2+}/MV^{\bullet +}) = -450 \pm 7$ mV. [d] Ref. 2. [e] Ref. 4. [f] Where a range is given, lower and upper values refer to mixing air or O_2-saturated solutions respectively; single value: O_2-saturated solution used. [g] [SOD] ≈ 4 μmol dm^{-3}. [h] Concentration of propan-2-ol ~ 4.6 mol dm^{-3}.

Table 3 Rate constants for the reductive elimination of a leaving group (LG = 4-nitrophenol) from both semiquinone radical and hydroquinone plus relative leaving group ability of selected indolequinones at pH 7.4



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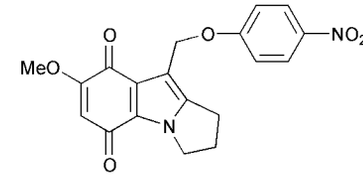

B

Q	Type	R^1	R^6	R^5	R^2	$R^{3'}$	$10^{-7}\ 2k_2(Q^{\bullet -} + Q^{\bullet -})$/ $dm^3\ mol^{-1}\ s^{-1}$	$k_3((Q - LG)^{\bullet -} \longrightarrow Q^{\bullet +} + LG)/s^{-1}$	$k_4(QH_2 \longrightarrow Q^+ + LG^-)/s^{-1}$	$G(-Q)^a$/ μmol J^{-1}	$G(LG)^a$/ μmol J^{-1}
32	A	Me	H	MeO	H	H	1.1 ± 0.1	27.3 ± 0.5	1.62 ± 0.05	1.6 ± 0.1	1.5 ± 0.1
33	B	—	—	—	—	—	1.2 ± 0.1	5.3 ± 0.5	0.31 ± 0.02	1.8 ± 0.1	1.6 ± 0.1
34	A	Me	H	MeO	Me	H	1.1 ± 0.1	25.2 ± 0.1	1.50 ± 0.03	2.3 ± 0.1	1.4 ± 0.1
35	A	Me	Me	MeO	Me	H	1.4 ± 0.1	23.1 ± 0.4	0.95 ± 0.1	2.2 ± 0.4	1.7 ± 0.2
36	A	Me	H	Morpholino	Me	H	3.1 ± 0.1	4.2 ± 0.5	0.18 ± 0.02	1.1 ± 0.1	0.8 ± 0.1
37	A	Me	H	MeO	Ph	H	1.7 ± 0.1	2.1 ± 0.1	0.23 ± 0.02	1.5 ± 0.1	1.2 ± 0.1
38	A	Me	H	MeO	4-Ph-C_6H_4	H	1.1 ± 0.1	1.9 ± 0.1	0.23 ± 0.05	1.6 ± 0.1	2.2 ± 0.1
39	A	Me	H	MeO	2-Naphthyl	H	0.7 ± 0.1	1.7 ± 0.1	0.35 ± 0.01	2.1 ± 0.4	1.9 ± 0.1
40	A	*c*-Pr	H	MeO	Me	H	1.2 ± 0.1	6.2 ± 0.1	0.61 ± 0.04	1.4 ± 0.1	1.2 ± 0.1
41	A	CH_2Ph	H	MeO	Et	H	0.8 ± 0.1	5.4 ± 0.1	0.33 ± 0.01	1.1 ± 0.1	1.0 ± 0.1
42	A	Ph	H	MeO	Me	H	1.4 ± 0.1	5.8 ± 0.2	0.22 ± 0.01	1.0 ± 0.1	0.8 ± 0.1
43	A	Ph	Me	MeO	Me	H	0.7 ± 0.1	6.3 ± 0.1	0.32 ± 0.02	1.1 ± 0.1	1.1 ± 0.2
44	A	4-F-C_6H_4	H	MeO	Me	H	0.9 ± 0.1	2.9 ± 0.1	0.26 ± 0.02	1.2 ± 0.3	0.7 ± 0.2
45	A	*n*-Pr	H	MeO	Me	H	1.0 ± 0.1	11.7 ± 0.1	0.61 ± 0.1	1.3 ± 0.3	1.2 ± 0.2
46	A	*n*-Pr	Me	MeO	Me	H	0.8 ± 0.1	13.1 ± 0.1	1.47 ± 0.1	1.4 ± 0.1	2.0 ± 0.2
47	A	Me	H	MeO	Me	Me	0.8 ± 0.1	354 ± 5	—	2.1 ± 0.1	1.4 ± 0.1
48	A	Me	H	4-Methylpiperazin-1-yl	Me	Me	1.2 ± 0.1	320 ± 5	—	1.4 ± 0.1	0.5 ± 0.1
49	A	Me	H	MeO	Me	Ph	1.1 ± 0.1	17.6 ± 0.5	0.2 ± 0.05	0.5 ± 0.01	0.8 ± 0.1
50	A	Me	H	MeO	Me	Thienyl	1.3 ± 0.1	126 ± 5	—	1.0 ± 0.1	0.9 ± 0.1

[a] Propan-2-ol–water (50%, v/v); $G((CH_3)_2C^{\bullet}OH = 0.67$ μmol J^{-1}; dose rate ≈ 6.0–6.5 Gy min^{-1}.

$(8.2 \pm 0.1) \times 10^7$ dm^3 mol^{-1} s^{-1} was obtained at pH 4.5. At pH 7.4 (where the $Q^{\bullet -}$ radical is fully deprotonated), the rate of second-order decay decreases by an order of magnitude to $2k_2 = (1.1 \pm 0.1) \times 10^7$ dm^3 mol^{-1} s^{-1} and the plot of the reciprocal of the first half-life of the semiquinone radical *versus* the initial radical concentration did not pass through the origin but gave an intercept indicative of a competing first-order process. The decay kinetics of the $Q^{\bullet -}$ radical were independent of the concentration of **34** ≈ 30–100 μmol dm^{-3} confirming a unimolecular first-order decay pathway which was ascribed to intramolecular fragmentation of the indolequinone conjugate and elimination of the leaving group (LG = 4-nitrophenol) as shown in reaction (3). This gave an estimate of $k_3 \approx 20$–50 s^{-1} which was more accurately determined from observing the release of the 4-nitrophenol chromophore.

$$(Q\text{–}LG)^{\bullet -} \longrightarrow Q^{\bullet +} + LG \qquad (3)$$

In propan-2-ol–water (50%, v/v) 4-nitrophenol has a $pK_a = 7.8 \pm 0.1$ and when deprotonated exhibits an absorption maximum at ~420 nm. At pH > 6 the decay of the $Q^{\bullet -}$ radical is associated with an increase in absorption in the 350–500 nm region ascribed to the reductive elimination of the 4-nitrophenoxide anion as shown in Fig. 2. At pH < 6, 4-nitrophenol does not absorb above 400 nm and the only absorption observed is that of the $Q^{\bullet -}$ radical. The reductive elimination of 4-nitrophenol from **34** is biphasic; the first phase complete by ~100 ms and a much slower second phase which is complete by 10 s after reduction to the $Q^{\bullet -}$ radical. The fact that the $Q^{\bullet -}$ radical of the alcohol **15** decayed by pure second-order kinetics over a broad pH range 3–9.5 (even at low $[Q^{\bullet -}] \approx 1.5$ μmol dm^{-3}) confirmed that the position of the equilibrium reaction (2) lay well to the side of the hydroquinone and that negligible quantities of the $Q^{\bullet -}$ radicals would be generated by the back reaction on the timescale of 4-nitrophenol release. At low initial radical concentrations (when $[Q^{\bullet -}] \approx 1.5$ μmol dm^{-3}), the first phase of 4-nitrophenol release could be fitted to first-order kinetics and gave an estimate for $k_3 \approx 40$ s^{-1} falling in the range of that determined from the decay of the $Q^{\bullet -}$ radical. Nevertheless, even under optimal experimental conditions approximately 10% of $Q^{\bullet -}$ radicals would be expected to decay *via* reaction (2) and as a consequence the rate constant $k_3 \approx 40$ s^{-1} would be an over-estimation of the actual value. The half-life for the disproportionation of $Q^{\bullet -}$ radicals *via* reaction (2) at pH 7.4 and 12 Gy ≈ $[Q^{\bullet -}] \approx 8$ μmol dm^{-3} is $t_{\frac{1}{2}} \approx (1.1 \times 10^7 \times 8 \times 10^{-6})^{-1} \approx 11$ ms which can compete with the elimination of 4-nitrophenol directly from the $Q^{\bullet -}$ radical $t_{\frac{1}{2}} \approx (0.7/40) \approx 15$ ms *via* reaction (3). This second slower phase of 4-nitrophenol release was ascribed to the reductive elimination of chromophore from the hydroquinone QH_2 in reaction (4).

$$(QH_2\text{–}LG) \longrightarrow QH_2^{+} + LG^{-} \qquad (4)$$

The slower elimination of 4-nitrophenol occurred on a timescale of seconds and the observed rate of 4-nitrophenol release was $k_4 \approx 0.3$ s^{-1}, significantly slower than from the $Q^{\bullet -}$ radical. Further evidence for this designation was obtained by comparing the effect of the initial radical concentrations on the radiation chemical yields of 4-nitrophenol at different pHs. Radiation chemical yields *G*(4-nitrophenol)/μmol J^{-1} were determined from absorbance measurements at 420 nm and some 10 s after the initial reduction of **34**, by which time semiquinone radicals had completely decayed and the reductive elimination of 4-nitrophenol was complete. The corresponding extinction coefficients for 4-nitrophenol in propan-2-ol–water (50%, v/v) at pH 9.3 and 7.4 were determined to be $\varepsilon_{420} = 8.72 \times 10^3$ and 3.54×10^3 dm^3 mol^{-1} cm^{-1} respectively. At pH 9.3, as the initial radical concentration decreased ($[(CH_3)_2C^{\bullet}OH] \approx 16$–$1.5$ μmol dm^{-3}) the radiation chemical yield of 4-nitrophenol increased (*G*(4-nitrophenol) ≈ 0.3–0.54 μmol J^{-1}). When $[(CH_3)_2C^{\bullet}OH] > 16$ μmol dm^{-3} the semiquinone radical decays predominantly *via* reaction (2) and *G*(4-nitrophenol) ≈ 0.3 μmol J^{-1} correlates well with the expected $G(QH_2) = 0.33$ μmol J^{-1} *i.e.* one-half of $G(Q^{\bullet -}) = 0.67$ μmol J^{-1}. At low $[(CH_3)_2C^{\bullet}OH] \approx 5$ μmol dm^{-3} the elimination of 4-nitrophenol directly from the $Q^{\bullet -}$ radical begins to compete with reaction (2) and as a consequence the radiation chemical yield of 4-nitrophenol increases to *G*(4-nitrophenol) ≈ 0.54 μmol J^{-1} and the contribution of the QH_2 to the overall yield of 4-nitrophenol decreases from 70 to 25%.

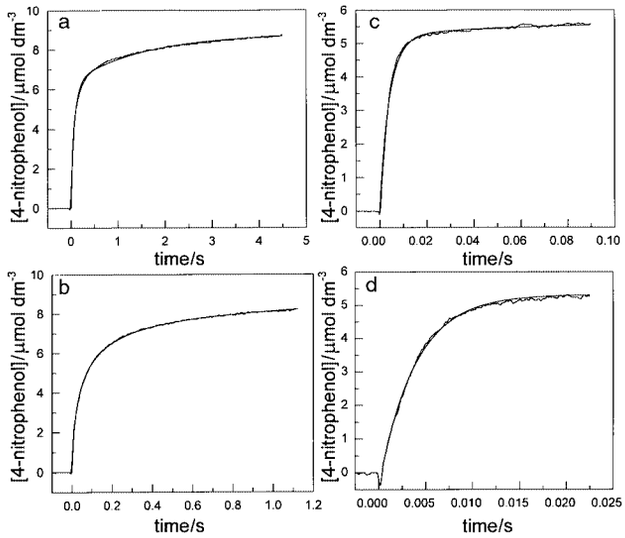

Fig. 3 Transient absorption traces recorded at 410 nm on the pulse radiolysis of either **35** or **48** (50 μmol dm^{-3}) in an N_2O-saturated propan-2-ol–water mixture (50%, v/v) at pH 9.3 and 7.4 respectively. Panels a and b show the biphasic reductive elimination of 4-nitrophenol on two different timescales when a dose per pulse of 15.5 Gy equivalent to $[(CH_3)_2C^{\bullet}OH] = 10.4$ μmol dm^{-3} was used. Panels c and d show the reductive elimination of 4-nitrophenol from the semiquinone radical of **48** on two different timescales when $[(CH_3)_2C^{\bullet}OH] = 5.4$ μmol dm^{-3}. Each of the kinetic traces is overlapped by a simulated trace which best fits the experimental data.

More accurate determinations of the rates of reductive elimination of 4-nitrophenol from both the $Q^{\bullet -}$ radical and QH_2 were made using a data fitting model in FACSIMILE. A model comprising reactions (2)–(4) was used to give 'best' fits to kinetic traces of 4-nitrophenol *versus* time traces obtained by pulse radiolysis. Fig. 3a and 3b show typical fits to kinetic data obtained 5 s and 1.1 s respectively, after the reduction of **35**. Rate constants for the reductive elimination of 4-nitrophenol from both the semiquinone radical and hydroquinone for all the 3-carbinyl substituted indolequinones **32**–**50** at pH 7.4 are displayed in Table 3. Rates of elimination from the $Q^{\bullet -}$ radical varied by over 2 orders of magnitude from $k_3 \approx 3$–354 s^{-1} and were always significantly faster than the corresponding rates of elimination from the hydroquinone. It was not possible to determine k_4 for the indolequinones **47**, **48** and **50** since even at high $[(CH_3)_2C^{\bullet}OH] \approx 30$ μmol dm^{-3} the release of 4-nitrophenol from the $Q^{\bullet -}$ radical could easily out-compete the formation of QH_2 by reaction (2). Fig. 3c and 3d show fits to kinetic data obtained from the reduction of **48** after 10 and 2.5 ms respectively. As expected the reduction of **48** to the $Q^{\bullet -}$ radical results in stoichiometric release of the leaving group *i.e.* $[Q^{\bullet -}] \approx$ [4-nitrophenol] = 5.4 μmol dm^{-3}.

Efficiencies of 4-nitrophenol release. The leaving group chemistry of the indolequinones was investigated by product analysis (HPLC) following γ-radiolysis of N_2O-saturated solutions containing quinones (100 μmol dm^{-3}) and propan-2-ol (8.3 mol dm^{-3}, 50%, v/v) at pH 7.4. The radiation chemical yield of the $(CH_3)_2C^{\bullet}OH$ radical in N_2O-saturated propan-2-ol–water mixtures was determined by ferricyanide reduction to be

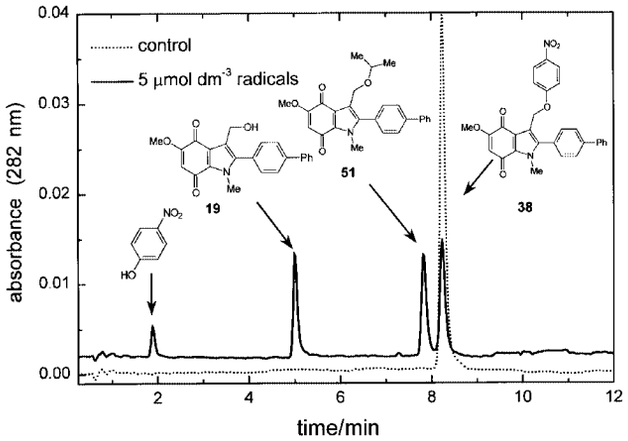

Fig. 4 HPLC chromatogram showing a typical product profile obtained by the γ-radiolysis (8 Gy) of **38** (40 μmol dm^{-3}) in an N_2O-saturated propan-2-ol–water mixture (50%, v/v) containing phosphate buffer (4 mmol dm^{-3}) at pH 7.4.

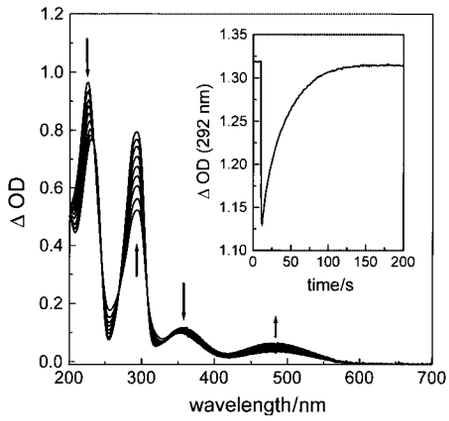

Fig. 5 Autoxidation of the hydroquinone of **15** in oxygen-saturated 0.1 mol dm^{-3} propan-2-ol and 200 μmol dm^{-3} phosphate buffer at pH 7.4. Spectrum measured at 10 s intervals. Insert: restoration of the ground-state absorption of **7** at 292 nm following the autoxidation of the hydroquinone in the presence of 650 μmol dm^{-3} oxygen.

$G((CH_3)_2C^{\bullet}OH) = 0.67 \pm 0.02$ μmol J^{-1} in propan-2-ol–water (50%, v/v) and 0.72 ± 0.01 μmol J^{-1} in 1 mol dm^{-3} propan-2-ol. Fig. 4 shows the product profile obtained on the reduction of **38** by the $(CH_3)_2C^{\bullet}OH$ radical and is characteristic of that obtained for all the 4-nitrophenol conjugates under study. Loss of the parent quinone **38** ($G(-Q) = 2.2 \pm 0.01$ μmol J^{-1}) paralleled the formation of the 4-nitrophenol leaving group (LG) with G(4-nitrophenol) $= 1.6 \pm 0.15$ μmol J^{-1} (~ 73% efficiency), both more than double the input of reducing (single-electron) equivalents. The two remaining major peaks in Fig. 4 were derived from the reaction of the resultant iminium derivative with water to generate the alcohol **19** and with the propan-2-ol to generate the isopropyl ether **51** (also synthesised independently). Both of these quinones are generated by autoxidation of their respective hydroquinones *via* reaction (5) following the unavoidable introduction of oxygen during HPLC sampling.

$$QH_2 + O_2 \longrightarrow Q + H_2O_2 \qquad (5)$$

As expected, the relative yields of **19** and isopropyl ether **51** were dependent on the alcohol concentration, with the alkylation product **51** virtually disappearing when radiolysis was performed in 1 mol dm^{-3} propan-2-ol. As shown in Table 3 all the indolequinones reductively eliminated 4-nitrophenol with high efficiency (typically >70%) although both $G(-Q)$/μmol J^{-1} and G(4-nitrophenol)/μmol J^{-1} were significantly greater ($G(-Q) = 1.1$–2.2 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 which occurred beyond the timescale of 4-nitrophenol release observed by pulse radiolysis. This chain reaction was previously observed for the reduction of (5-methoxy-1-methyl-4,7-dioxoindol-3-yl)methyl derivatives under similar experimental conditions.[2,4]

Semiquinone and hydroquinone reactivities with oxygen. The one-electron reduction potentials for selected indolequinone alcohols **13**–**31** are displayed in Table 2 and vary by ~100 mV, falling in the range $E(Q/Q^{\bullet-}) \approx -376$ to -277 mV. The corresponding rates of electron transfer from the $Q^{\bullet-}$ radical to oxygen in reaction (6) remain rather fast $k_6 \approx 1.3$–6.4×10^8 dm^3 mol^{-1} s^{-1}.

$$Q^{\bullet-} + O_2 \longrightarrow Q + O_2^{\bullet-} \qquad (6)$$

This is consistent with the fact that for quinones in general where reduction potentials are lower than -200 mV, manipulation of the redox potential will prove unsuccessful in modifying significantly the rate of reaction (6).[20]

Hydroxymethyl compounds **13**–**31** exhibit much poorer leaving group behavior than 4-nitrophenol and were therefore considered good candidates for the study of hydroquinone autoxidation without the added complication of reductive elimination of leaving groups. HPLC confirmed that under the conditions employed for radiolytic reduction (0.1 mol dm^{-3} propan-2-ol and 0.2 mmol dm^{-3} phosphate buffer) the hydroquinones derived from the isopropyl ethers or monophosphates (both indicative of H_2O lost) would make a negligible contribution to the observed rates of autoxidation. Fig. 5 shows the spectral changes which occur when **15** is reduced incrementally (5 Gy) by increasing doses of between 0–55 Gy or 0–40 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals. The insert in Fig. 5 shows a typical trace recorded at 292 nm showing the autoxidation of the hydroquinone and regeneration of the parent indolequinone **15** absorption in the presence of oxygen $[O_2] \approx 650$ μmol dm^{-3}. The autoxidation of the hydroquinone of **15** was not pure first-order in oxygen concentration, but in every case the half-life did decrease significantly with increasing oxygen concentration. Thus for the hydroquinone of **15**, first-order rate constants of $k_5 \approx 170$ and ≈ 280 s^{-1} were measured for $[O_2] = 110$ and 650 μmol dm^{-3} respectively. In marked contrast to the very rapid reactivity of the semiquinone radicals with oxygen in reaction (6), the autoxidation of the corresponding hydroquinones (QH_2) *via* reaction (5) occurs many orders of magnitude slower, with apparent values falling in the range $k_5 \approx 40$–300 dm^3 mol^{-1} s^{-1} (see Table 2). It is noted that rates of autoxidation for hydroquinones corresponding to **13**–**31** are all slower than that previously determined for both radiolytic[4] and enzymatic reduction[21] of the drug EO9 where $k_5 \approx 2.4 \times 10^3$ dm^3 mol^{-1} s^{-1}.

Reaction (5) represents the overall stoichiometry of QH_2 autoxidation but is an oversimplification of a more complex kinetic system involving the $O_2^{\bullet-}$ radical as an intermediate including reactions (2), (6)–(8).[22,23]

$$O_2^{\bullet-} + QH_2 \longrightarrow Q^{\bullet-} + H_2O_2 \qquad (7)$$

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad (8)$$

Our previous studies have shown that in the absence of oxygen the equilibrium reaction (2) favours the formation of the hydroquinone.[4] However, $Q^{\bullet-}$ exists in equilibrium with both QH_2 and Q and the rapid reactivity of $Q^{\bullet-}$ with oxygen produces the $O_2^{\bullet-}$ radical *via* reaction (6). In the absence of superoxide dismutase (SOD) which catalyses the disproportionation

of $O_2^{\bullet -}$ radicals in cells *via* reaction (8), reaction (7) may represent the rate determining step in QH_2 autoxidation when Q is reduced radiolytically. Under our experimental conditions <50% of Q is reduced to QH_2 prior to mixing with oxygen and this would be expected to influence the position of the equilibrium reaction (2) and therefore the measured rate of QH_2 autoxidation. The mixing of SOD (~4 μmol dm^{-3} after mixing, a biologically representative concentration) with QH_2 resulted in a significant reduction in the apparent rate constant of **15** QH_2 autoxidation, to $k_5 \approx 2$–83 $dm^3\ mol^{-1}\ s^{-1}$ (see Table 2). This is consistent with the involvement of $O_2^{\bullet -}$ radicals in the overall autoxidation.[24]

Discussion

In this work we have determined the rates of elimination and release of a model leaving group from indolequinone semiquinone and hydroquinone intermediates and also their corresponding rates of reaction with molecular oxygen. From a chemical kinetic point of view the selectivity of indolequinones for hypoxic environments will rely on establishing a balance between the one-electron reduction potential (which governs the rate of semiquinone radical reactivity with oxygen) and the rate of reductive elimination from these 3-carbinyl substituted derivatives. In most normal tissues, values of $pO_2 < 10$ mmHg or 1.3% O_2 are rarely observed, but such levels are common to many solid tumours.[25] If the reactivity of the semiquinone radical towards oxygen is faster than the rate of reductive elimination, the half-life of the semiquinone radical at tumour relevant oxygen tensions will be too short to allow efficient drug release even under severe hypoxia. It is immediately obvious that in hypoxic tumour cells where $[O_2] \approx 5$ μmol dm^{-3}, the semiquinone radical of **15** reacts with oxygen with a first-order rate constant of $k_6 = 5.2 \times 10^8 \times 5 \times 10^{-6} \approx 2.6 \times 10^3\ s^{-1}$ (half-life, $t_{\frac{1}{2}} = 0.7/k_6[O_2] \approx 270$ μs). Elimination of 4-nitrophenol from the semiquinone radical of **34** is therefore too slow (half-life, $t_{\frac{1}{2}} = 0.7/k_3 \approx 28$ ms) to compete effectively with reaction (6). As expected the reductive elimination of 4-nitrophenol from **34** was completely inhibited by 5 μmol dm^{-3} O_2 during pulse radiolysis and steady-state γ-radiolysis. Conversely, the rate of autoxidation of the hydroquinone of **15** at the same oxygen concentration $k_5 \approx 240$ (average) $\times 5 \times 10^{-6} \approx 1.2 \times 10^{-3}\ s^{-1}$ ($t_{\frac{1}{2}} \approx 580$ s), indicating that reaction of the hydroquinone derived from **34** with oxygen will be too slow to compete with reductive elimination of 4-nitrophenol ($t_{\frac{1}{2}} \approx 2$ s). Indeed, the rate of autoxidation could be significantly slower *in vivo* if SOD rapidly removes $O_2^{\bullet -}$ radicals *via* reaction (8), rate $k_5 \approx 5 \times 5 \times 10^{-6} \approx 2.5 \times 10^{-5}\ s^{-1}$ ($t_{\frac{1}{2}} \approx 8$ h). Thus, although the reduction of 3-carbinyl substituted indolequinones to their hydroquinones, which may be effected *in vivo via* two-electron reducing enzymes such as NQO1, results in efficient elimination of the substituent, this reaction is not inhibited by even normal tissue oxygen concentrations. Therefore based on kinetic arguments, such compounds would not be expected to be hypoxia-selective *per se*, and will only target such environments *in vivo* if the corresponding enzymes are actually up-regulated, which is the case for some solid tumours.[15] However, there is now compelling evidence that indolequinones substituted at the (indol-3-yl)methyl position are exceptionally poor substrates for NQO1, and in certain cases the resultant iminium derivative formed on reductive elimination from the hydroquinone actually inhibits the enzyme.[12,13] For example, the indolequinone alcohol **18** is efficiently metabolised by NQO1 while the corresponding 4-nitrophenoxy conjugate **37** is not.[13] The exploitation of the oxygen-sensitive reduction chemistry of these indolequinones to the semiquinone radical is therefore most likely to be the only way to attain hypoxia-selectivity in tumour cells.

The redox potentials of these compounds are not changed sufficiently by chemical modification to significantly lengthen the half-life of the semiquinone radicals under hypoxia. This would imply that the only strategy capable of controlling release over a range of oxygen tensions would require modifying the rate of release of the leaving group (4-nitrophenol in this study). Many of the substitutions at the 1- and 2-positions of the indolequinone 'core' reduced the rate of reductive elimination 10-fold (*e.g.* **37**–**39**). However, the 3-carbinyl substituted analogues **47**, **48** and **50** exhibited faster rates of 4-nitrophenol release than **34**. Parallels can be drawn with the reduction of nitrobenzyl halides where α-substitution with methyl increased the rate of fragmentation and release of halide ion by decreasing the bond dissociation energy of the linker through stabilization of the resultant nitrobenzyl radical.[26] Interestingly, although phenyl substitution in analogue **49** was expected to facilitate fragmentation by stabilizing the resultant radical cation, the rate of 4-nitrophenol release was slower than that of the unsubstituted indolequinone **34**. This may reflect distortion from planarity and impaired p-orbital overlap.

Conclusion

The semiquinone radical derived from the $R^{3'}$-methyl substituted analogue **47** exhibits the fastest rate of elimination of 4-nitrophenol ($t_{\frac{1}{2}} \approx 2$ ms) and is therefore capable of competing against electron transfer to oxygen ($t_{\frac{1}{2}} \approx 1.6$ ms) at $[O_2] \approx 5$ μmol dm^{-3} (a value typical of tumour hypoxia). However, 5 μmol dm^{-3} O_2 prevented elimination of 4-nitrophenol from **34**. Therefore 3-carbinyl substituted compounds with simple alkyl (*e.g.* methyl, **47**) or electron rich heterocyclic groups, (*e.g.* 2-thienyl, **50**) eliminate the model drug at a rate that can compete with oxygen at this concentration. It should be noted that 3-carbinyl substitution may not be the only structural feature which determines the rate of fragmentation on reduction. The use of 4-nitrophenol as a model leaving group in this study has facilitated structural optimisation of the indolequinone moiety but clearly the nature of the leaving group itself must also be considered in the design of novel drugs which are capable of selective fragmentation in hypoxic tissue.

Experimental

General procedures

NMR spectra: *J* values are given in Hz. UV–VIS spectra: ε values are given in $dm^3\ mol^{-1}\ cm^{-1}$. Elemental analyses were determined at the University of Exeter or by MEDAC Ltd. (Egham, Surrey, UK) and all compounds characterized by HRMS were chromatographically homogeneous. Solutions in organic solvents were dried by standard procedures, and dimethylformamide, toluene and tetrahydrofuran were anhydrous commercial grades. Silica gel for flash column chromatography was Merck Kieselgel 60 H grade (230–400 mesh) or Matrex silica 60.

Materials

4-Nitrophenol, 1,1′-dimethyl-4,4′-bipyridinium dichloride (methyl viologen, MV^{2+}), 1,1′-dibenzyl-4,4′-bipyridinium dichloride (benzyl viologen, BV^{2+}), propan-2-ol and superoxide dismutase (SOD) were obtained from Sigma-Aldrich Chemical Company Ltd (Gillingham, Dorset, UK). Nitrous oxide, oxygen and mixtures thereof were obtained from the British Oxygen Company (Gillingham, Kent, UK). The following compounds were obtained from Maybridge Chemical Company Ltd (Tintagel, Cornwall, UK): methyl 3-(methyl)-aminobut-2-enoate, ethyl 3-(phenyl)aminobut-2-enoate, ethyl 3-(4-fluorophenyl)aminobut-2-enoate, ethyl 1-cyclopropyl-5-hydroxy-2-methylindole-3-carboxylate **5c**, methyl 1-benzyl-2-ethyl-5-hydroxyindole-3-carboxylate **5d** and ethyl 5-hydroxy-2-methyl-1-phenylindole-3-carboxylate **5e**.

Pulse radiolysis

The redox properties of the indolequinones and the kinetic characteristics of their semiquinone radicals ($Q^{\bullet -}$) were investigated by pulse radiolysis. Semiquinone radicals were generated following reduction of the parent indolequinone by the propan-2-ol radical $(CH_3)_2C^{\bullet}OH$. Kinetic spectrophotometry with submicrosecond time resolution was used to monitor the reactions of $Q^{\bullet -}$ radical and the reductive elimination of 4-nitrophenol. Experiments were performed using a 6 MeV linear accelerator as described previously.[2] The absorbed radiation dose per electron pulse (typically 1–30 Gy) was determined by the thiocyanate dosimeter.[27]

The potentials were determined by establishing redox equilibria with a viologen (V^{2+}) of known reduction potential. Typically, solutions consisted of N_2O-saturated propan-2-ol (1–6.5 mol dm^{-3}) and phosphate buffer (NaH_2PO_4–Na_2HPO_4, 4 mmol dm^{-3}, pH 7.4–8.5) with Q (0–30 μmol dm^{-3}) and V^{2+} (0–5 mmol dm^{-3}). The alcohol converts the radiolytically-generated $^{\bullet}OH$ and $H^{\bullet}$ radicals in <2 μs to the propan-2-ol radical ($(CH_3)_2C^{\bullet}OH$) which rapidly reduces both the indolequinones and V^{2+} to the $Q^{\bullet -}$ radical or viologen radical-cation ($V^{\bullet +}$). Absorbances were measured at 600 nm at a dose per pulse of 3 Gy (or ~2 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals). Redox equilibration usually occurred within ~100 μs (during which time there was negligible decay of either the $Q^{\bullet -}$ radical or $V^{\bullet +}$ radical-cation *via* self-disproportionation reactions) and it was possible to determine the equilibrium constant K from the absorbance at equilibrium.[28] One-electron reduction potentials ($E(Q/Q^{\bullet -})$) for new indolequinones displayed in Table 2 are quoted relative to $E(BV^{2+}/BV^{\bullet +}) = -368 \pm 7$ mV in 1 mol dm^{-3} propan-2-ol, $E(BV^{2+}/BV^{\bullet +}) = -374 \pm 7$ mV in 0.2 mol dm^{-3} propan-2-ol, $E(MV^{2+}/MV^{\bullet +}) = -450 \pm 7$ mV in 0.2 mol dm^{-3} propan-2-ol and $E(MV^{2+}/MV^{\bullet +}) = -448 \pm 7$ mV in 0.7 mol dm^{-3} propan-2-ol.[29]

The reactivity of semiquinone radicals with oxygen were determined by gassing solutions with N_2O–O_2 mixtures (0.2–2.1% O_2, British Oxygen Company, UK). At pH > 7 the $Q^{\bullet -}$ radical absorption monitored at 345 nm exhibited negligible decay in the absence of oxygen up to 500 μs after an electron pulse of 1 Gy. In the presence of oxygen the $Q^{\bullet -}$ 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 and are displayed in Table 3. The latter were corrected for oxygen solubility in propan-2-ol (1–6.5 mol dm^{-3}) from literature data.[30]

For measurements of the reductive elimination of 4-nitrophenol from indolequinones over longer timescales up to 10 s, a solid-state light source was developed to minimise possible sample photobleaching. The source uses a number of narrow-band (15–30 nm) light-emitting diodes (LEDs) which cover the range ~430–900 nm. Thus 12 LEDs were positioned in front of an optical fibre and positioning was achieved using a rotating wheel servo system. The output end of the fibre was at the focus of an aspheric lens, producing a highly collimated beam to illuminate the sample cell. This novel system was utilised in combination with the traditional tungsten lamp and photodiode detector to determine the rates of 4-nitrophenol release displayed in Table 3. For indolequinones where the disproportionation of semiquinone radicals could compete with the release of 4-nitrophenol a simulated data fitting model (FACSIMILE)[31] provided estimates of rate constants from experimental data.

Steady-state γ-radiolysis

HPLC analysis was carried out using indolequinone solutions (50–100 μmol dm^{-3}) which were saturated with N_2O gas in gas-tight vials before irradiation in a ^{60}Co source. An absorbed dose of 1 Gy = 0.67 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals in N_2O-saturated propan-2-ol–water (50%, v/v) as determined by ferricyanide reduction. Dose rates of 5.9 to 6.5 Gy min^{-1} were used, as determined by Fricke dosimetry.[32] For studies on the autoxidation of the reduced forms of selected indolequinones, steady-state γ-radiolysis of N_2-saturated solutions of Q (50 μmol dm^{-3}) in 0.1 mol dm^{-3} propan-2-ol and phosphate buffer (NaH_2PO_4–Na_2HPO_4, 0.2 mmol dm^{-3}, pH 7.4) was performed in 20 cm^3 hypodermic syringes (Popper & Sons, USA). In this case, an absorbed dose of 1 Gy = 0.72 μmol dm^{-3} $(CH_3)_2C^{\bullet}OH$ radicals and a dose rate of 5.9 Gy min^{-1} was employed.

High-performance liquid chromatography

Product analysis following γ-radiolysis of indolequinone solutions was performed by gradient separation on a 100 mm × 3.2 mm base-deactivated reversed-phase column (Hichrom RPB, Hichrom, Reading, UK) at a flow rate of 1 cm^3 min^{-1}. The solvents and gradients used in the separation of products are displayed in Table 4. Detection was at 228 nm using a Waters 486 detector (Watford, UK) and concentrations were determined from peak areas using Waters Maxima Software.

Stopped-flow experiments

Effective rate constants for hydroquinone autoxidation were measured using a 1 cm flow cell (Optiglass Ltd, Essex, UK) and absorbance changes measured using a Hewlett Packard 8452A Diode Array Spectrophotometer. Irradiated samples and air or oxygen-saturated solutions were mixed just before the mixing cell *via* 20 cm^3 hypodermic syringes and a capillary t-junction, and passed through the flow cell to a stopping syringe in a conventional arrangement. The absorbance changes at specific wavelengths were monitored 0–500 s after mixing, with measurements every 0.5–2 s. The effect of SOD on rates of hydroquinone autoxidation was studied by mixing irradiated samples with SOD (~4 μmol dm^{-3} after mixing) in oxygen-saturated solutions containing 0.2 mmol dm^{-3} phosphate buffer at pH 7.4. All experiments were performed at ambient room temperature (25 ± 2 °C).

Chemical synthesis

Ethyl 3-amino-3-(2-naphthyl)propenoate. Ethyl (2-naphthoyl)-acetate (4.6 g, 0.019 mol), ammonium acetate (14.6 g, 0.19 mol), benzene (150 cm^3) and acetic acid (30 cm^3) were refluxed under Dean–Stark conditions for 24 h. The cooled reaction mixture was washed with sodium hydrogen carbonate and the benzene layer dried (Na_2SO_4) and concentrated. The crude material was purified by column chromatography (70% dichloromethane–30% light petroleum) to give the title compound (3.2 g, 70%) as a pale yellow oil; ν_{max} (film)/cm^{-1} 3483, 3442, 3334, 3058, 2981, 1680, 1655, 1619; δ_H (300 MHz; $CDCl_3$) 8.02 (1H, m), 7.87–7.83 (3H, m), 7.59 (1H, dd, *J* 8.6, *J* 1.8), 7.55–7.50 (2H, m), 5.12 (1H, s), 4.21 (2H, q, *J* 7.1), 1.33 (3H, t, *J* 7.1); NH_2 not observed; δ_C (100 MHz; $CDCl_3$) 170.4, 160.4, 134.9, 134.1, 133.0, 128.6, 128.5, 127.7, 127.1, 126.8, 125.8, 123.6, 85.1, 59.0, 14.6; *m/z* (EI, relative intensity) 241 (M^+, 40%), 196 (40), 169 (100); *m/z* (HRMS) 241.1088 ($C_{15}H_{15}NO_2$ requires *M* 241.1103).

General method for the preparation of the 5-hydroxyindole-3-carboxylates 5 by the Nenitzescu reaction

(a) 1,4-Benzoquinone (5.5 mmol) and the aminoalkenoate (4.6 mmol) were refluxed for 1 h in acetic acid (50 cm^3). The acetic acid was removed *in vacuo*. The crude product was purified by column chromatography (80% light petroleum–20% acetone) and recrystallised (acetone–light petroleum) to yield the product.

(b) Alternatively the reaction was carried out in nitromethane at room temperature as previously described.[33]

Table 4 Solvents used in HPLC analysis of indolequinones (Q)

Q	Aqueous buffer (mmol dm^{-3})	Organic phase	Gradient (% organic phase)
37	KH_2PO_4 (5), H_3PO_4 (5)	75%MeCN	50–95%, 6 min
32, **33**, **34**	KH_2PO_4 (5), H_3PO_4 (5)	75%MeCN	35–80%, 8 min
38, **45**	KH_2PO_4 (5) H_3PO_4 (5)	75%MeCN	50–95%, 6 min, hold 2.5 min
36, **47**	KH_2PO_4 (5), H_3PO_4 (5)	75%MeCN	45–95%, 6 min
40, **48**, **46**	KH_2PO_4 (5), H_3PO_4 (5)	75%MeCN	40–85%, 5 min
41, **49**	KH_2PO_4 (5), H_3PO_4 (5)	75%MeCN	50–95%, 8 min, hold 1 min
50	KH_2PO_4 (5), H_3PO_4 (5)	75%MeCN	45–90%, 5 min, hold 1 min
39, **42**, **43**, **44**	KH_2PO_4 (5), H_3PO_4 (5)	MeOH	50–95%, 8 min
35	KH_2PO_4 (5), H_3PO_4 (5)	MeOH	40–85%, 5 min, hold 1 min

Methyl 5-hydroxy-1,2,6-trimethylindole-3-carboxylate 5a. Prepared from 2-methyl-1,4-benzoquinone and methyl 3-(methyl)aminobut-2-enoate in nitromethane as an inseparable mixture which was used directly in the next step.

Ethyl 5-hydroxy-2-(2-naphthyl)indole-3-carboxylate 5b. Prepared from 1,4-benzoquinone and ethyl 3-amino-3-(2-naphthyl)propenoate in acetic acid in 78% yield; mp 197–199 °C (Found: C, 76.2; H, 5.2; N, 4.2. $C_{21}H_{17}NO_3$ requires C, 76.1; H, 5.2; N, 4.2%); ν_{max} (KBr disc)/cm^{-1} 3377, 3323, 3049, 2985, 1666, 1628; δ_H (400 MHz; $(CD_3)_2CO$) 10.89 (1H, br s), 8.23 (1H, d, *J* 1.0), 7.96–7.93 (4H, m), 7.86 (1H, dd, *J* 8.6, *J* 1.8), 7.72 (1H, d, *J* 2.4), 7.55 (2H, m), 7.35 (1H, dd, *J* 8.6, *J* 0.5), 6.85 (1H, dd, *J* 8.6, *J* 2.4), 4.23 (2H, q, *J* 7.1), 1.22 (3H, t, *J* 7.1); δ_C (100 MHz; $(CD_3)_2CO$) 164.8, 152.9, 144.5, 133.4, 132.9, 130.6, 130.3, 129.3, 128.8, 128.2, 127.9, 127.6, 127.0, 126.6, 126.3, 112.8, 112.0, 106.2, 103.7, 58.8, 13.8; *m*/*z* (EI, relative intensity) 331 (M^+, 95%), 286 (84), 259 (100); *m*/*z* (HRMS) 331.1208 ($C_{21}H_{17}NO_3$ requires *M* 331.1208).

Ethyl 5-hydroxy-2,6-dimethyl-1-phenylindole-3-carboxylate 5f. Prepared from 2-methyl-1,4-benzoquinone and ethyl 3-(phenyl)aminobut-2-enoate in nitromethane as an inseparable mixture which was used directly in the next step.

Ethyl 1-(4-fluorophenyl)-5-hydroxy-2-methylindole-3-carboxylate 5g. Prepared from 1,4-benzoquinone and ethyl 3-(*N*-4-fluorophenyl)aminobut-2-enoate in nitromethane in 22% yield; mp 220–222 °C (Found: C, 67.3; H, 5.1; N, 4.4. $C_{18}H_{16}FNO_3 \cdot 0.4\ H_2O$ requires C, 67.4; H, 5.3; N, 4.4%); ν_{max} (CH_2Cl_2)/cm^{-1} 3257, 1659; δ_H(300 MHz; $(CD_3)_2SO$) 7.54–7.42 (5H, m), 6.77 (1H, d, *J* 8.8), 6.64 (1H, dd, *J* 8.8, *J* 2.4), 4.34 (2H, d, *J* 7.0), 2.50 (3H, s), 1.37 (3H, t, *J* 7.0); OH not observed; δ_C (75 MHz; $(CD_3)_2SO$) 165.1, 163.3 (d, J_{CF} 246.2), 132.3 (d, J_{CF} 2.9), 131.6, 130.3 (d, J_{CF} 9.0), 127.1, 116.8 (d, J_{CF} 22.9), 112.1, 110.7, 105.5, 103.7, 59.0, 14.2, 12.8; *m*/*z* (EI, relative intensity) 313 (M^+, 43%), 268 (24), 241 (10), 83 (100), 58 (56); *m*/*z* (HRMS) 313.1116 ($C_{18}H_{16}FNO_3$ requires *M* 313.1114).

Ethyl 5-hydroxy-2-methyl-1-propylindole-3-carboxylate 5h. Prepared from 1,4-benzoquinone and ethyl 3-(propylamino)-but-2-enoate in nitromethane in 47% yield; mp 180–182 °C (lit.[34] 176–177 °C).

Ethyl 5-hydroxy-2,6-dimethyl-1-propylindole-3-carboxylate 5i. Prepared from 2-methyl-1,4-benzoquinone and ethyl 3-(propylamino)but-2-enoate in nitromethane in 52% yield; mp 195–198 °C (lit.[35] 193.5–195.0 °C)

General method for the synthesis of 5-methoxyindole-3-carboxylates 6

To a stirring solution of the 5-hydroxyindole **5** (2.3 mmol) in DMSO (15 cm^3) was added potassium hydroxide (0.52 g, 9.3 mmol). After 30 min, iodomethane (1.30 g, 9.2 mmol) was added drop-wise. The mixture was stirred at room temperature for 3 h. The crude mixture was diluted with ethyl acetate and washed thoroughly with 1 mol dm^{-3} hydrochloric acid. The organic layer was separated, dried ($MgSO_4$) and concentrated. The crude material was purified by column chromatography (70% light petroleum–30% ethyl acetate) and recrystallised (ethyl acetate–light petroleum) to yield the title compound as a colorless crystalline solid.

Methyl 5-methoxy-1,2,6-trimethylindole-3-carboxylate 6a. (80%), mp 162–164 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 1685; δ_H (300 MHz; $CDCl_3$) 7.55 (1H, s), 7.02 (1H, s), 3.99 (6H, s), 3.60 (3H, s), 2.70 (3H, s), 2.29 (3H, s); δ_C (75 MHz; $CDCl_3$) 166.7, 154.2, 144.0, 131.1, 125.2, 122.3, 110.6, 103.3, 101.7, 55.7, 50.2, 29.6, 17.1, 12.0; *m*/*z* (EI, relative intensity) 247 (M^+, 100%), 232 (64), 216 (34); *m*/*z* (HRMS) 247.1206 ($C_{14}H_{17}NO_3$ requires *M* 247.1208).

Ethyl 5-methoxy-1-methyl-2-(2-naphthyl)indole-3-carboxylate 6b. (90%), mp 130–131 °C (Found: C, 76.6; H, 5.8; N, 3.8. $C_{23}H_{21}NO_3$ requires C, 76.9; H, 5.9; N, 3.9%); ν_{max} (KBr disc)/cm^{-1} 3047, 3012, 2973, 2946, 2898, 2833, 1699, 1617, 1599; δ_H (300 MHz; $CDCl_3$) 8.00–7.88 (4H, m), 7.82 (1H, d, *J* 2.5), 7.59–7.50 (3H, m), 7.30 (1H, dd, *J* 8.8, *J* 0.3), 7.00 (1H, dd, *J* 8.8, *J* 2.5), 4.16 (2H, q, *J* 7.1), 3.94 (3H, s), 3.58 (3H, s), 1.07 (3H, t, *J* 7.1); δ_C (100 MHz; $CDCl_3$) 165.2, 156.0, 146.6, 133.3, 132.8, 132.1, 129.8, 129.2, 128.3, 128.0, 127.8, 127.6, 127.5, 126.8, 126.4, 113.2, 110.6, 105.2, 103.5, 59.2, 55.8, 31.1, 14.1; *m*/*z* (EI, relative intensity) 359 (M^+, 100%), 314 (40), 287 (38), 242 (21); *m*/*z* (HRMS) 359.1521 ($C_{23}H_{21}NO_3$ requires *M* 359.1521).

Ethyl 1-cyclopropyl-5-methoxy-2-methylindole-3-carboxylate 6c. (72%), mp 114–116 °C (Found: C, 70.1; H, 7.0; N, 4.9. $C_{16}H_{19}NO_3$ requires C, 70.3; H, 7.0; N, 5.1%); ν_{max} (CH_2Cl_2)/cm^{-1} 1679; δ_H (300 MHz; $CDCl_3$) 7.64 (1H, d, *J* 2.6) 7.43 (1H, d, *J* 8.8), 6.85 (1H, dd, *J* 8.8, *J* 2.6), 4.39 (2H, q, *J* 7.2), 3.88 (3H, s), 3.15–3.08 (1H, m), 2.81 (3H, s), 1.45 (3H, t, *J* 7.2), 1.25–1.19 (2H, m), 1.03–0.97 (2H, m); δ_C (75 MHz; $CDCl_3$) 166.0, 155.4, 147.4, 132.1, 127.2, 111.3, 111.2, 103.9, 103.4, 59.2, 55.6, 24.9, 14.5, 13.1, 7.5; *m*/*z* (EI, relative intensity) 273 (M^+, 100%), 244 (78), 228 (43), 200 (28); *m*/*z* (HRMS) 273.1370 ($C_{16}H_{19}NO_3$ requires *M* 273.1365).

Methyl 1-benzyl-2-ethyl-5-methoxyindole-3-carboxylate 6d. (90%), mp 114–116 °C (Found: C, 74.6; H, 6.7; N, 4.1. $C_{20}H_{21}NO_3$ requires C, 74.3; H, 6.55; N, 4.33%); ν_{max} (CH_2Cl_2)/cm^{-1} 1687; δ_H (300 MHz; $CDCl_3$) 7.53 (1H, d, *J* 2.6), 7.35 (1H, d, *J* 8.8), 7.30–7.21 (3H, m), 6.98 (2H, dd, *J* 7.6, *J* 1.09), 6.81 (1H, dd, *J* 8.8, *J* 2.6), 5.51 (2H, s), 3.84 (3H, s), 3.79 (3H, s), 3.11 (2H, q, *J* 7.3), 1.06 (3H, t, *J* 7.3); δ_C (75 MHz; $CDCl_3$) 165.2, 155.3, 150.9, 137.6, 131.1, 128.8, 127.4, 127.0, 126.0, 111.5, 111.5, 103.4, 102.3, 55.4, 55.6, 45.9, 18.8, 13.9; *m*/*z* (EI, relative intensity) 323 (M^+, 86%), 292 (22), 200 (33), 91 (100); *m*/*z* (HRMS) 323.1522 ($C_{20}H_{21}NO_3$ requires *M* 323.1521).

Ethyl 5-methoxy-2-methyl-1-phenylindole-3-carboxylate 6e. (82%), mp 87–88 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 1680.23; δ_H (400 MHz; $CDCl_3$) 7.71 (1H, d, *J* 2.5), 7.57–7.53 (3H, m), 7.30–7.33 (2H, m), 6.91 (1H, dd, *J* 8.8, *J* 0.5), 6.79 (1H, dd, *J* 8.8, *J* 2.5), 4.35

(2H, q, *J* 7.1), 3.90 (3H, s), 2.57 (3H, s), 1.47 (3H, t, *J* 7.1); δ_C (100 MHz; $CDCl_3$) 166.2, 155.9, 145.4, 136.7, 132.8, 129.7, 128.7, 128.2 (2 × CH), 127.4, 112.1, 111.1, 104.9, 104.4, 59.4, 55.8, 14.6, 13.1; *m*/*z* (EI, relative intensity) 309 (M^+, 28%), 152 (74), 84 (100), 55 (52); *m*/*z* (HRMS) 309.1370 ($C_{19}H_{19}NO_3$ requires *M* 309.1364).

Ethyl 5-methoxy-2,6-dimethyl-1-phenylindole-3-carboxylate 6f. (80%), mp 111–113 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 1680; δ_H (300 MHz; $CDCl_3$) 7.66 (1H, s), 7.60–7.48 (3H, m), 7.31 (2H, dd, *J* 7.5, *J* 2.2), 6.78 (1H, s), 4.44 (2H, t, *J* 7.0), 3.94 (3H, s), 2.56 (3H, s), 2.25 (3H, s), 1.48 (3H, t, *J* 7.0); δ_C (75 MHz; $CDCl_3$) 166.3, 154.5, 144.1, 136.8, 132.2, 129.7, 128.7, 128.2, 125.3, 122.8, 111.7, 104.8, 101.5, 59.4, 55.7, 16.94, 14.7, 13.1; *m*/*z* (EI, relative intensity) 323 (M^+, 100%), 308 (20), 278 (22); *m*/*z* (HRMS) 323.1529 ($C_{20}H_{21}NO_3$ requires *M* 323.1521).

Ethyl 1-(4-fluorophenyl)-5-methoxy-2-methylindole-3-carboxylate 6g. (70%), mp 113–115 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 1683; δ_H (300 MHz; $CDCl_3$) 7.70 (1H, d, *J* 2.4), 7.32–7.22 (4H, m), 6.87 (1H, d, *J* 9), 6.79 (1H, dd, *J* 9, *J* 2.4), 4.42 (2H, q, *J* 7.0), 3.90 (3H, s), 2.56 (3H, s), 1.47 (3H, t, *J* 7.0); δ_C (75 MHz; $CDCl_3$) 166.1, 162.4 (d, J_{CF} 249.2), 156.0, 145.4, 132.8, 132.6 (d, J_{CF} 3.3), 130.4, 130.0 (d, J_{CF} 8.8), 127.3, 116.8 (d, J_{CF} 22.8), 112.2, 110.9, 103.4, 59.5, 55.8, 14.6, 13.1; *m*/*z* (EI, relative intensity) 327 (M^+, 100%), 282 (33), 84 (38), 57 (38); *m*/*z* (HRMS) 327.1274 ($C_{19}H_{18}FNO_3$ requires *M* 327.1270).

Ethyl 5-methoxy-2-methyl-1-propylindole-3-carboxylate 6h. (68%), mp 88–89 °C (lit.[36] 87.5–88 °C).

Ethyl 5-methoxy-2,6-dimethyl-1-propylindole-3-carboxylate 6i. Ethyl 5-hydroxy-2,6-dimethyl-1-propylindole-3-carboxylate (2.0 g, 7.3 mmol) in DMF (20 cm^3) was added to a stirring suspension of sodium hydride (0.34 g, 14.2 mmol) in DMF (30 cm^3) at 0 °C. The mixture was stirred at room temperature for 45 min. Iodomethane (2.06 g, 14.5 mmol) was added drop-wise at 0 °C and the mixture allowed to warm to room temperature. After 2 h saturated ammonium chloride solution was added and the mixture extracted with ethyl acetate. The ethyl acetate layer was washed thoroughly with hydrochloric acid 1 mol dm^{-3}, dried ($MgSO_4$) and concentrated. The crude product was purified by column chromatography (10% ethyl acetate–hexane elution) to yield the title compound as colourless needles (1.69 g, 80%), mp 94–95 °C (from hexane) (Found: C, 70.7; H, 8.2; N, 4.8. $C_{17}H_{23}NO_3$ requires C, 70.5; H, 8.0; N, 4.8%); ν_{max} (KBr disc)/cm^{-1} 2971, 2935, 2894, 1685, 1572; δ_H (300 MHz; $CDCl_3$) 7.61 (1H, s), 7.04 (1H, s), 4.40 (2H, q, *J* 7.1), 4.01 (2H, t, *J* 7.4), 3.91 (3H, s), 2.73 (3H, s), 2.35 (3H, s), 1.78 (2H, sextet, *J* 7.4), 1.46 (3H, t, *J* 7.1), 0.96 (3H, t, *J* 7.4); δ_C (100 MHz; $CDCl_3$) 166.3, 154.1, 143.5, 130.5, 125.5, 122.2, 110.8, 103.5, 101.8, 59.2, 55.6, 44.8, 23.1, 17.1, 14.6, 12.0, 11.4; *m*/*z* (EI, relative intensity) 290 (MH^+, 100%).

General method for nitration

To a solution of indole **6** (2.06 mmol) in acetic acid (10 cm^3), cooled to −10 °C was added a mixture of nitric acid (0.14 cm^3) and acetic acid (0.54 cm^3). The mixture was stirred at room temperature for 2 h. A yellow suspension was obtained which was poured on to an ice–water mixture and the crystals obtained were filtered off and dried. The crude product was purified by column chromatography (50% dichloromethane–50% ethyl acetate) to yield the 4-nitro (major) and 6-nitro (minor) compounds. Spectroscopic data are given for the required 4-nitro isomer.

Methyl 5-methoxy-1,2,6-trimethyl-4-nitroindole-3-carboxylate 7a. (65%), mp 174–176 °C (Found: C, 57.1; H, 5.4; N, 9.6. $C_{14}H_{16}N_2O_5$ requires C, 57.5; H, 5.5; N, 9.6%); ν_{max} (CH_2Cl_2)/cm^{-1} 1695, 1536; δ_H (300 MHz; $CDCl_3$) 7.14 (1H, s), 3.84 (3H, s), 3.78 (3H, s), 3.62 (3H, s), 2.64 (3H, s), 2.40 (3H, s); δ_C (75 MHz; $CDCl_3$) 164.8, 146.7, 145.5, 138.2, 134.1, 126.6, 115.4, 112.9, 102.71, 62.7, 50.3, 29.9, 16.5, 11.9; *m*/*z* (EI, relative intensity) 292 (M^+, 43%), 215 (26), 69 (100); *m*/*z* (HRMS) 292.1061 ($C_{14}H_{16}N_2O_5$ requires *M* 292.1059).

Ethyl 5-methoxy-1-methyl-2-(2-naphthyl)-4-nitroindole-3-carboxylate 7b. (74%), mp 217–219 °C (Found: C, 67.8; H, 4.9; N, 6.7. $C_{23}H_{20}N_2O_5$ requires C, 68.3; H, 5.0; N, 6.9%); ν_{max} (KBr disc)/cm^{-1} 3012, 2985, 2931, 1691, 1621, 1596; δ_H (300 MHz; $CDCl_3$) 7.93 (4H, m), 7.57 (2H, m), 7.48 (2H, m), 7.11 (1H, d, *J* 9.0), 4.07 (2H, q, *J* 7.12), 3.98 (3H, s), 3.60 (3H, s), 0.98 (3H, t, *J* 7.2); δ_C (75 MHz; $CDCl_3$) 163.6, 148.6, 147.3, 133.4, 133.0, 132.6, 129.9, 128.3, 127.9, 127.9, 127.8, 127.4, 127.1, 126.7, 118.2, 112.6, 109.2, 104.7, 60.1, 57.8, 31.4, 13.7 (1 C not observed); *m*/*z* (EI, relative intensity) 404 (M^+, 10%), 149 (19), 105 (50), 44 (100); *m*/*z* (HRMS) 404.1372 ($C_{23}H_{20}N_2O_5$ requires *M* 404.1372).

Ethyl 1-cyclopropyl-5-methoxy-2-methyl-4-nitroindole-3-carboxylate 7c. (70%), mp 177–179 °C (Found: C, 58.9, H, 5.5, N, 9.1. $C_{16}H_{18}N_2O_5 \cdot 0.5\ H_2O$ requires C, 58.7; H, 5.8; N, 8.8%); ν_{max} (CH_2Cl_2)/cm^{-1} 1696, 1510; δ_H (300 MHz; $CDCl_3$) 7.60 (1H, d, *J* 9.0), 6.93 (1H, d, *J* 9.0), 4.27 (2H, q, *J* 7.2), 3.89 (3H, s), 3.18–3.11 (1H, m), 2.75 (3H, s), 1.34 (3H, t, *J* 7.2), 1.29–1.20 (2H, m), 0.93–0.90 (2H, m); δ_C (75 MHz; $CDCl_3$) 164.5, 149.5, 146.9, 133.4, 133.3, 117.8, 113.4, 108.0, 103.3, 60.1, 57.7, 25.2, 14.2, 13.2, 7.8; *m*/*z* (EI, relative intensity) 318 (M^+, 100%), 272 (19), 84 (26); *m*/*z* (HRMS) 318.1225 ($C_{16}H_{18}N_2O_5$ requires *M* 318.1216).

Methyl 1-benzyl-2-ethyl-5-methoxy-4-nitroindole-3-carboxylate 7d. (70%), mp 178–180 °C (Found: C, 65.2; H, 5.4; N, 7.4. $C_{20}H_{20}N_2O_5$ requires C, 65.2; H, 5.5; N, 7.6%); ν_{max} (CH_2Cl_2)/cm^{-1} 1701, 1507; δ_H (300 MHz; $CDCl_3$) 7.31–7.26 (3H, m), 7.21 (1H, d, *J* 9.0), 6.92 (2H, m), 6.88 (1H, d, *J* 9.0), 5.37 (2H, s), 3.87 (3H, s), 3.82 (3H, s), 3.11 (2H, q, *J* 7.2), 1.18 (3H, t, *J* 7.2); δ_C (75 MHz; $CDCl_3$) 164.5, 152.9, 147.1, 135.7, 133.8, 132.3, 129.1 (2 × CH), 128.0, 125.7, 118.1, 112.7, 108.5, 102.6, 57.7, 50.5, 46.8, 19.3, 13.8; *m*/*z* (EI, relative intensity) 368 (M^+, 10%), 319 (2), 201 (3); *m*/*z* (HRMS) 368.1373 ($C_{20}H_{20}N_2O_5$ requires *M* 368.1372).

Ethyl 5-methoxy-2-methyl-4-nitro-1-phenylindole-3-carboxylate 7e. (75%), mp 129–131 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 1696, 1516; δ_H (400 MHz; $CDCl_3$) 7.60–7.58 (3H, m), 7.30–7.04 (2H, m), 7.03 (1H, d, *J* 9.0), 6.89 (1H, d, *J* 9.0), 4.35 (2H, q, *J* 7.2), 3.92 (3H, s), 2.53 (3H, s), 1.39 (3H, t, *J* 7.2); δ_C (100 MHz; $CDCl_3$) 164.6, 147.6, 147.4, 135.7, 133.8, 131.3, 130.0, 129.5, 128.2, 118.0, 113.1, 108.6, 104.2, 60.3, 57.8, 14.2, 13.1; *m*/*z* (EI, relative intensity) 354 (M^+, 100%), 263 (40), 221 (35), 77 (57); *m*/*z* (HRMS) 354.1227 ($C_{19}H_{18}N_2O_5$ requires *M* 354.1216).

Ethyl 5-methoxy-2,6-dimethyl-4-nitro-1-phenylindole-3-carboxylate 7f. (61%), mp 122–124 °C (Found: C, 64.9; H, 5.4; N, 7.6. $C_{20}H_{20}N_2O_5$ requires C, 65.2; H, 5.5; N, 7.6%); ν_{max} (CH_2Cl_2)/cm^{-1} 1706, 1536; δ_H (300 MHz; $CDCl_3$) 7.64–7.55 (3H, m), 7.28 (2H, dd, *J* 7.0, *J* 2.2), 6.88 (1H, s), 4.34 (2H, q, *J* 7.0), 3.88 (3H, s), 2.51 (3H, s), 2.34 (3H, s), 1.39 (3H, t, *J* 7.0); δ_C (75 MHz; $CDCl_3$) 164.6, 146.9, 146.10, 138.2, 135.7, 135.3, 130.1, 129.5, 128.2, 127.3, 115.6, 114.2, 104.3, 62.7, 60.3, 16.5, 14.2, 13.1; *m*/*z* (EI, relative intensity) 368 (M^+, 1%), 295 (8), 149 (37), 84 (60), 69 (100); *m*/*z* (HRMS) 368.1379 ($C_{20}H_{20}N_2O_5$ requires *M* 368.1372).

Ethyl 1-(4-fluorophenyl)-5-methoxy-2-methyl-4-nitroindole-3-carboxylate 7g. (60%), mp 153–155 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 1700, 1512; δ_H (300 MHz; $CDCl_3$) 7.32–7.26 (4H, m), 7.0 (1H, d, *J* 9.0), 6.89 (1H, d, *J* 9.0), 4.32 (2H, q, *J* 7.0), 3.90 (3H, s),

2.51 (3H, s), 1.37 (3H, t, *J* 7.0); δ_C (75 MHz; $CDCl_3$) 162.8 (d, J_{CF} 249.6), 147.6, 147.4, 133.8, 131.6 (d, J_{CF} 3.2), 131.2, 130.0 (d, J_{CF} 8.9), 117.9, 117.2 (d, J_{CF}23.0), 113.0, 108.7, 104.6, 104.3, 60.3, 57.7, 14.2, 13.1.

Ethyl 5-methoxy-2-methyl-4-nitro-1-propylindole-3-carboxylate 7h. (72%), mp 162–164 °C (Found: C, 59.9; H, 6.3; N, 8.7. $C_{16}H_{20}N_2O_5$ requires C, 60.0; H, 6.3; N, 8.7%); ν_{max} (KBr disc)/cm^{-1} 3093, 2975, 2929, 2903, 1695; δ_H (300 MHz; $CDCl_3$) 7.34 (1H, d, *J* 9.0), 6.95 (1H, d, *J* 9.0), 4.30 (2H, q, *J* 7.1), 4.07 (2H, t, *J* 7.4), 3.92 (3H, s), 2.70 (3H, s), 1.76 (2H, sextet, *J* 7.4), 1.36 (3H, t, *J* 7.1), 0.96 (3H, t, *J* 7.4); δ_C (100 MHz; $CDCl_3$) 164.6, 146.93, 146.91, 133.7, 132.2, 118.0, 111.9, 108.2, 103.2, 60.1, 57.8, 45.1, 23.0, 14.1, 12.0, 11.3; *m*/*z* (EI, relative intensity) 343 (M + Na, 100%), 275 (26).

Ethyl 5-methoxy-2,6-dimethyl-4-nitro-1-propylindole-3-carboxylate 7i. (60%), mp 99–101 °C (Found: C, 60.7; H, 6.6; N, 8.6. $C_{17}H_{22}N_2O_5$ requires C, 61.0; H, 6.6; N, 8.4%); ν_{max} (KBr disc)/cm^{-1} 3416, 3324, 2980, 2924, 1695, 1659, 1602; δ_H (300 MHz; $CDCl_3$) 7.20 (1H, s), 4.30 (2H, *J* 7.1), 4.05 (2H, t, *J* 7.4), 3.88 (3H, s), 2.71 (3H, s), 2.45 (3H, s), 1.77 (2H, sextet, *J* 7.4), 1.37 (3H, t, *J* 7.1), 0.98 (3H, t, *J* 7.5); δ_C (100 MHz; $CDCl_3$) 164.6, 146.2, 145.6, 138.3, 133.6, 126.5, 115.7, 113.1, 103.3, 62.6, 60.0, 45.0, 22.9, 16.6, 14.2, 12.0, 11.3; *m*/*z* (EI, relative intensity) 335 (MH^+, 43%), 263 (40), 241 (100).

General method for reduction of the nitro group

To a suspension of the 4-nitroindole **7** (1.16 mmol) in ethanol (30 cm^3) were added tin powder (0.62 g, 5.21 mmol) and hydrochloric acid (3.0 mol dm^{-3}, 9 cm^3). The mixture was refluxed for 30 min. Upon cooling the solution was decanted from the excess tin and neutralized with saturated aqueous sodium hydrogen carbonate. The suspension obtained was added to an equal volume of water. The precipitate and aqueous layer were stirred overnight with dichloromethane, and filtered through Celite and the layers separated. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (80% light petroleum–20% ethyl acetate) and recrystallised (light petroleum–ethyl acetate).

Methyl 4-amino-5-methoxy-1,2,6-trimethylindole-3-carboxylate 8a. (80%), mp 169–171 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3473–3334, 1675; δ_H (300 MHz; $CDCl_3$) 6.38 (1H, s), 5.78 (2H, s), 3.88 (3H, s), 3.76 (3H, s), 3.54 (3H, s), 2.60 (3H, s), 2.38 (3H, s) δ_C (75 MHz; $CDCl_3$) 167.4, 143.1, 139.6, 134.8, 133.9, 126.5, 113.0, 104.3, 98.5, 59.1, 51.2, 29.9, 16.7, 12.9; *m*/*z* (EI, relative intensity) 262 (M^+, 41%), 247 (37), 215 (100); *m*/*z* (HRMS) 262.1323 ($C_{14}H_{18}N_2O_3$ requires *M* 262.1317).

Ethyl 4-amino-5-methoxy-1-methyl-2-(2-naphthyl)indole-3-carboxylate 8b. (64%), mp 165–167 °C (Found: C, 73.6; H, 5.9; N, 7.5. $C_{23}H_{22}N_2O_3$ requires C, 73.8; H, 5.9; N, 7.5%); ν_{max} (KBr disc)/cm^{-1} 3479, 3312, 2974, 2931, 2899, 2845, 1659, 1595; δ_H (300 MHz; $CDCl_3$) 7.94–7.86 (3H, m), 7.81 (1H, s), 7.56 (2H, m), 7.43 (1H, dd, *J* 8.4, *J* 1.6), 7.00 (1H, d, *J* 8.7), 6.63 (1H, d, *J* 8.7), 5.87 (2H, br s), 3.91 (5H, m), 3.44 (3H, s), 0.59 (3H, t, *J* 7.1); δ_C (75 MHz; $CDCl_3$) 166.6, 146.2, 141.1, 134.3, 133.0, 132.7, 131.7, 130.6, 129.4, 128.1, 127.9, 127.8, 127.4, 126.7, 126.5, 114.6, 110.7, 105.8, 96.8, 59.7, 57.7, 31.2, 13.4; *m*/*z* (EI, relative intensity) 374 (M^+, 74%), 314 (23), 313 (100); *m*/*z* (HRMS) 3374.1630 ($C_{23}H_{22}N_2O_3$ requires 374.1630).

Ethyl 4-amino-1-cyclopropyl-5-methoxy-2-methylindole-3-carboxylate 8c. (70%), mp 140–142 °C (Found: C, 66.2; H, 7.0; N, 9.6. $C_{16}H_{20}N_2O_3$ requires C, 66.6; H, 7.0; N, 9.7%); ν_{max} (CH_2Cl_2)/cm^{-1} 3334–3462, 1670; δ_H (300 MHz; $CDCl_3$) 6.86 (1H, d, *J* 8.6), 6.81 (1H, d, *J* 8.6), 5.73 (2H, br s), 4.36 (2H, q, *J* 7.2), 3.87 (3H, s), 3.08–3.02 (1H, m), 2.73 (3H, s), 1.41 (3H, t, *J* 7.2), 1.24–1.18 (2H, m), 1.0–0.95 (2H, m); δ_C (75 MHz; $CDCl_3$) 167.0, 146.1, 141.0, 134.5, 131.1, 114.3, 109.4, 104.8, 98.2, 60.1, 57.6, 25.1, 14.5, 14.3, 8.0; *m*/*z* (EI, relative intensity), 288 (M^+, 41%), 263 (100), 227 (52), 83 (96); *m*/*z* (HRMS) 288.1466 ($C_{16}H_{20}N_2O_3$ requires *M* 288.1474)

Methyl 4-amino-1-benzyl-2-ethyl-5-methoxyindole-3-carboxylate 8d. (75%), mp 150–152 °C (Found: C, 70.4; H, 6.6; N, 8.1. $C_{20}H_{22}N_2O_3$ requires C, 70.1; H, 6.6; N, 8.3%); ν_{max} (CH_2Cl_2)/cm^{-1} 3329–3432, 1675; δ_H (400 MHz; $CDCl_3$) 7.27–7.25 (3H, m), 6.98–6.95 (2H, m), 6.83 (1H, d, *J* 8.8), 6.45 (1H, d, *J* 8.8), 5.73 (2H, br s), 5.28 (2H, s), 3.91 (3H, s), 3.86 (3H, s), 3.02 (2H, q, *J* 7.2), 1.16 (3H, t, *J* 7.2); δ_C (100 MHz; $CDCl_3$) 167.3, 149.6, 141.4, 136.7, 133.7, 130.8, 128.9, 127.5, 125.8, 114.8, 109.9, 103.9, 97.5, 57.5, 51.3, 46.6, 20.1, 14.1; *m*/*z* (EI, relative intensity) 338 (M^+, 52%), 291 (27), 83 (100); *m*/*z* (HRMS) 338.1630 ($C_{20}H_{22}N_2O_3$ requires *M* 338.1630).

Ethyl 4-amino-5-methoxy-2-methyl-1-phenylindole-3-carboxylate 8e. (80%), mp 161–162 °C (Found: C, 68.4; H, 5.9; N, 8.3. $C_{19}H_{20}N_2O_3 \cdot 0.5\ H_2O$ requires C, 68.4; H, 6.4; N, 8.4%); ν_{max} (CH_2Cl_2)/cm^{-1} 3472–3329, 1700; δ_H (400 MHz; $CDCl_3$) 7.57–7.47 (2H, m), 7.30–7.26 (3H, m), 6.78 (1H, d, *J* 8.8), 6.19 (1H, d, *J* 8.8), 5.81 (2H, br s), 4.39 (2H, q, *J* 7.0), 3.86 (3H, s), 2.46 (3H, s), 1.42 (3H, t, *J* 7.0); δ_C (100 MHz; $CDCl_3$) 167.1, 144.1, 141.2, 137.0, 135.2, 131.0, 129.7, 128.7, 128.6, 114.4, 109.8, 105.6, 97.9, 60.2, 57.6, 14.5, 14.3; *m*/*z* (EI, relative intensity) 324 (M^+, 65%), 309 (28), 263 (100), 77 (14); *m*/*z* (HRMS) 324.1470 ($C_{19}H_{20}N_2O_3$ requires *M* 324.1474).

Ethyl 4-amino-5-methoxy-2,6-dimethyl-1-phenylindole-3-carboxylate 8f. (60%), mp 125–127 °C; ν_{max} (CH_2Cl_2)/cm^{-1} 3473–3340, 1675; δ_H (400 MHz; $CDCl_3$) 7.59–7.48 (3H, m), 7.29–7.26 (2H, m), 6.04 (1H, s), 5.85 (2H, br s), 4.38 (2H, q, *J* 7.2), 3.76 (3H, s), 2.43 (3H, s), 2.26 (3H, s), 1.41 (3H, t, *J* 7.2); δ_C (100 MHz; $CDCl_3$) 167.2, 143.3, 140.0, 137.0, 136.0, 133.3, 129.7, 128.8, 128.6, 126.9, 113.1, 105.8, 100.1, 60.3, 59.1, 16.5, 14.5, 14.1; *m*/*z* (EI, relative intensity) 338 (M^+, 25%), 277 (49), 83 (100); *m*/*z* (HRMS) 338.1635 ($C_{20}H_{22}N_2O_3$ requires *M* 338.1630).

Ethyl 4-amino-1-(4-fluorophenyl)-5-methoxy-2-methylindole-3-carboxylate 8g. (65%), mp 121–123 °C (Found: C, 66.5; H, 5.6; N, 7.8. $C_{19}H_{19}FN_2O_3$ requires C, 66.7; H, 5.6; N, 8.2%); ν_{max} (CH_2Cl_2)/cm^{-1} 3452–3324, 1672; δ_H (400 MHz; $CDCl_3$) 7.26–7.21 (4H, m), 6.78 (1H, d, *J* 8.6), 6.16 (1H, d, *J* 8.6), 5.78 (2H, br s), 4.39 (2H, q, *J* 7.2), 3.86 (3H, s), 2.44 (3H, s), 1.42 (3H, t, *J* 7.2); δ_C (100 MHz; $CDCl_3$) 167.0. 162.4 (d, J_{CF} 249.2), 144.0, 141.3, 135.3, 133.0, 131.0, 130.4 (d, J_{CF} 8.8), 116.7 (d, J_{CF} 22.6), 114.3, 109.8, 105.8, 97.6, 60.3, 57.5, 14.4, 14.2; *m*/*z* (EI, relative intensity) 342 (M^+, 40%), 327 (15), 281 (26) 83 (100); *m*/*z* (HRMS) 342.1382 ($C_{19}H_{19}FN_2O_3$ requires *M* 342.1380).

Ethyl 4-amino-5-methoxy-2-methyl-1-propylindole-3-carboxylate 8h. (88%), mp 105–107 °C (Found: C, 66.1; H, 7.7; N, 9.5. $C_{16}H_{22}N_2O_3$ requires C, 66.2; H, 7.6; N, 9.6%); ν_{max} (KBr disc)/cm^{-1} 3457, 3308, 2929, 2868, 2817, 1674, 1602; δ_H (400 MHz; $CDCl_3$) 6.86 (1H, d, *J* 8.7), 6.54 (1H, d, *J* 8.7), 5.77 (2H, br s), 4.37 (2H, q, *J* 7.1), 3.97 (2H, t, *J* 7.4), 3.87 (3H, s), 2.64 (3H, s), 1.76 (2H, sextet, *J* 7.4), 1.41 (3H, t, *J* 7.1), 0.95 (3H, t, *J* 7.4); δ_H (100 MHz; $CDCl_3$) 167.0, 143.4, 140.9, 133.4, 131.2, 114.7, 109.6, 104.4, 96.7, 60.0, 57.6, 44.9, 22.7, 14.5, 13.0, 11.4; *m*/*z* (CI, relative intensity) 291 (MH^+, 100%).

Ethyl 4-amino-5-methoxy-2,6-dimethyl-1-propylindole-3-carboxylate 8i. (82%), mp 86–88 °C (Found: C, 67.1; H, 8.1; N, 9.2. $C_{17}H_{24}N_2O_3$ requires C, 67.1; H, 8.0; N, 9.2%); ν_{max} (KBr disc)/cm^{-1} 3416, 3324, 2980, 2924, 1695, 1659, 1602; δ_H (400 MHz; $CDCl_3$) 6.39 (1H, s), 5.06 (2H, br s), 4.36 (2H, q, *J* 7.1), 3.95 (2H, t, *J* 7.5), 3.76 (3H, s), 2.63 (3H, s), 2.38 (3H, s), 1.76

(2H, sextet, *J* 7.5), 1.41 (3H, t, *J* 7.1), 0.97 (3H, t, *J* 7.5); δ_C (100 MHz; $CDCl_3$) 167.0, 142.5, 139.5, 134.2, 133.9, 126.3, 113.2, 104.7, 98.8, 60.0, 59.0, 44.8, 22.8, 16.7, 14.5, 12.9, 11.4; *m*/*z* (ES, relative intensity) 305 (MH^+, 26%), 219 (100).

Ethyl 5-methoxy-2,6-dimethyl-1-propyl-4,7-dioxoindole-3-carboxylate 9

To a solution of the ethyl 4-amino-5-methoxy-2,6-dimethyl-1-propylindole-3-carboxylate (0.03 g, 0.098 mmol) in acetone (10 cm^3) was added a solution of potassium nitrosodisulfonate (0.079 g, 0.29 mmol) in sodium dihydrogen phosphate buffer (0.3 mol dm^{-3}, 10 cm^3). The mixture was stirred at room temperature for 1 h. The excess acetone was removed *in vacuo*. The resulting residue was extracted with dichloromethane and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (column 50% ethyl acetate–50% hexane elution) to yield the title compound as an orange solid (0.03 g, 96%), mp 75–76 °C (from hexane) (Found: C, 63.7; H, 6.6; N, 4.2. $C_{17}H_{21}NO_5$ requires C, 63.9; H, 6.6; N, 4.4%); λ_{max} (MeOH)/nm 440 (log ε 2.97), 332 (3.61), 288 (4.13); ν_{max} (KBr disc)/cm^{-1} 2986, 2950, 1732, 1671, 1640, 1609; δ_H (300 MHz; $CDCl_3$) 4.37 (2H, q, *J* 7.1), 4.26 (2H, m), 4.01 (3H, s), 2.43 (3H, s), 1.94 (3H, s), 1.71 (2H, sextet, *J* 7.4), 1.39 (3H, t, *J* 7.1), 0.97 (3H, t, *J* 7.4); δ_C (100 MHz; $CDCl_3$) 179.3, 177.2, 164.4, 16.6, 140.4, 128.9, 127.2, 122.5, 113.0, 61.0, 60.8, 46.9, 23.6, 14.1, 11.0, 10.6, 8.5; *m*/*z* (CI, relative intensity) 320 (MH^+, 100%).

General method for the preparation of 3-hydroxymethylindole-4,7-diones

To a suspension of lithium aluminum hydride (0.079 g, 2.08 mmol) in tetrahydrofuran (30 cm^3) at 0 °C was added a solution of 4-aminoindole-3-ester **8** (0.52 mmol) in tetrahydrofuran (15 cm^3). 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 cm^3), sodium hydroxide (1.0 mol dm^{-3}, 0.5 cm^3) 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 indole in acetone (20 cm^3) was added a solution of potassium nitrosodisulfonate (0.70 g, 2.6 mmol) in sodium dihydrogen phosphate buffer (0.3 mol dm^{-3}, 20 cm^3). The mixture was stirred at room temperature for 1 h. The excess acetone was removed *in vacuo*. The resulting residue was extracted with dichloromethane and washed with water. The organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by column chromatography (5% ethyl acetate–95% dichloromethane) and recrystallised (ethyl acetate–light petroleum).

3-Hydroxymethyl-5-methoxy-1-methylindole-4,7-dione 13. Prepared as previously described.[37]

7-Methoxy-9-hydroxymethyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]-indole-5,8-dione 14. Prepared as previously described.[13]

3-Hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione 15. Prepared as previously described.[13,38]

3-Hydroxymethyl-5-methoxy-1,2,6-trimethylindole-4,7-dione 16. (68%), mp 163–165 °C; λ_{max} (MeOH)/nm 465 (log ε 2.37), 350 (2.79), 288 (3.41); ν_{max} (CH_2Cl_2)/cm^{-1} 2948, 1637, 1638; δ_H (400 MHz; $CDCl_3$) 4.60 (2H, d, *J* 7.0), 4.19 (1H, t, *J* 7.0), 3.98 (3H, s), 3.87 (3H, s), 2.22 (3H, s), 1.97 (3H, s); δ_C (100 MHz; $CDCl_3$) 181.0, 178.9, 156.0, 134.7, 129.8, 122.6, 122.4, 114.0, 61.1, 56.0, 32.4, 9.6, 8.90; *m*/*z* (EI, relative intensity) 249 (M^+, 100%), 234 (62), 56 (56); *m*/*z* (HRMS) 249.1001 ($C_{13}H_{15}NO_4$ requires *M* 249.1001).

3-Hydroxymethyl-1,2-dimethyl-5-(morpholin-1-yl)indole-4,7-dione 17. Prepared as previously described.[13]

3-Hydroxymethyl-5-methoxy-1-methyl-2-phenylindole-4,7-dione 18. Prepared as previously described.[13]

2-(Biphenyl-4-yl)-3-hydroxymethyl-5-methoxy-1-methyl-indole-4,7-dione 19. Prepared as previously described.[13]

3-Hydroxymethyl-5-methoxy-1-methyl-2-(2-naphthyl)indole-4,7-dione 20. (73%), mp 223–225 °C (Found: C, 72.4; H, 4.8; N, 4.1. $C_{21}H_{17}NO_4$ requires C, 72.6; H, 4.9; N, 4.0%); λ_{max} (MeOH)/nm 454 (log ε 3.39), 350 (3.62), 274 (4.45); ν_{max} (KBr disc)/cm^{-1} 3404, 3060, 2953, 2840, 1675, 1636, 1597; δ_H (400 MHz; $CDCl_3$) 7.93 (3H, m), 7.81 (1H, m), 7.61–7.55 (2H, m), 7.39 (1H, dd, *J* 8.4, *J* 1.7), 5.75 (1H, s), 4.56 (2H, d, *J* 7.2), 4.08 (1H, t, *J* 7.2), 3.87 (3H, s), 3.83 (3H, s); δ_C (100 MHz; $CDCl_3$) 179.3, 178.9, 156.0, 139.2, 133.3, 133.0, 130.5, 130.0, 128.7, 128.2, 127.9, 127.3, 127.2, 127.0, 125.6, 124.3, 122.2, 107.4, 56.7, 56.2, 34.2; *m*/*z* (EI, relative intensity) 347 (M^+, 100%), 346 (67), 331 (28), 286 (26), 189 (29), 165 (32); *m*/*z* (HRMS) 347.1156 ($C_{21}H_{17}NO_4$ requires 347.1157).

1-Cyclopropyl-3-hydroxymethyl-5-methoxy-2-methylindole-4,7-dione 21. (60%), mp 217–219 °C (Found: C, 64.1; H, 5.8; N, 5.0. $C_{14}H_{15}NO_4$ requires C, 64.4; H, 5.8; N, 5.4%); λ_{max} (MeOH)/nm 454 (log ε 3.34), 346 (3.57), 286 (4.35); ν_{max} (CH_2Cl_2)/cm^{-1} 3429, 1638, 1601; δ_H (400 MHz; $CDCl_3$) 5.61 (1H, s), 4.56 (2H, d, *J* 7.0), 3.99 (1H, t, *J* 7.0), 3.79 (3H, s), 3.17–3.09 (1H, m), 2.32 (3H, s), 1.28–1.21 (2H, m), 0.86–0.80 (2H, m); δ_C (100 MHz; $CDCl_3$) 179.5, 177.0, 159.0, 136.8, 130.9, 122.5, 122.3, 107.5, 56.5, 55.7, 27.9, 11.2, 10.0; *m*/*z* (EI, relative intensity) 261 (M^+, 100%), 246 (47), 220 (10); *m*/*z* (HRMS) 261.0993 ($C_{14}H_{15}NO_4$ requires *M* 261.1001).

1-Benzyl-2-ethyl-3-hydroxymethyl-5-methoxyindole-4,7-dione 22. (65%), mp 117–119 °C (Found: C, 69.9; H, 5.9; N, 4.1. $C_{19}H_{19}NO_4$ requires C, 70.1; H, 5.9; N, 4.3%); λ_{max} (MeOH)/nm 462 (log ε 3.30), 344 (3.48), 286 (4.31); ν_{max} (CH_2Cl_2)/cm^{-1} 3454, 1639, 1602; δ_H (400 MHz; $CDCl_3$) 7.30–7.20 (3H, m), 7.00–6.98 (2H, m), 5.63 (2H, s), 5.61 (1H, s), 4.62 (2H, d, *J* 7.0), 4.07 (1H, t, *J* 7.0), 3.80 (3H, s), 2.56 (2H, q, *J* 7.0), 0.98 (3H, t, *J* 7.0); δ_C (100 MHz; $CDCl_3$) 179.5, 178.3, 159.5, 146.6, 136.3, 129.0, 128.8, 127.7, 126.1, 122.9, 122.6, 107.4, 56.6, 55.7, 48.3, 17.1.14.6; *m*/*z* (EI, relative intensity) 325 (M^+, 38%), 234 (66), 91 (100); *m*/*z* (HRMS) 325.1312 ($C_{19}H_{19}NO_2$ requires *M* 325.1314).

3-Hydroxymethyl-5-methoxy-2-methyl-1-phenylindole-4,7-dione 23. (68%), mp 270–272 °C (Found: C, 67.0; H, 4.9; N, 4.4. $C_{17}H_{15}NO_4 \cdot 0.4\ H_2O$ requires C, 67.0; H, 5.2; N, 4.6%); λ_{max} (MeOH)/nm 450 (log ε 3.28), 344 (3.48), 286 (4.31); ν_{max} (CH_2Cl_2)/cm^{-1} 3462, 1653, 1646; δ_H (400 MHz; $CDCl_3$) 7.53–7.50 (3H, m), 7.24–7.19 (2H, m), 5.56 (1H, s), 4.68 (2H, d, *J* 7.0), 4.06 (1H, t, *J* 7.0), 3.81 (3H, s), 2.01 (3H, s); δ_C (100 MHz; $CDCl_3$) 179.8, 176.9, 159.5, 136.9, 135.3, 130.4, 129.4, 129.3, 127.1, 122.6, 122.4, 107.1, 56.6, 56.0, 10.1; *m*/*z* (EI, relative intensity) 297 (M^+, 38%), 282 (18), 84 (100), 77 (21); *m*/*z* (HRMS) 297.0998 ($C_{17}H_{15}NO_4$ requires *M* 297.1001).

3-Hydroxymethyl-5-methoxy-2,6-dimethyl-1-phenylindole-4,7-dione 24. (65%), mp 135–137 °C; λ_{max} (MeOH)/nm 460 (log ε 3.04), 342 (3.64), 286 (4.23); ν_{max} (CH_2Cl_2)/cm^{-1} 3417, 1637, 1612; δ_H (300 MHz; $CDCl_3$) 7.53–7.50 (3H, m), 7.21–7.18 (2H, m), 4.67 (2H, d, *J* 7.0), 4.23 (1H, t, *J* 7.0), 3.99 (3H, s), 2.07 (3H, s), 1.77 (3H, s); δ_C (100 MHz; $CDCl_3$) 181.4, 177.4, 156.0, 137.1, 135.4, 130.1, 130.0, 129.4, 129.3, 127.1, 123.0, 122.4, 61.1, 56.0, 10.1, 8.8; *m*/*z* (EI, relative intensity) 311 (M^+, 100%), 296 (69), 222 (15), 118 (22), 77 (71); *m*/*z* (HRMS) 311.1156 ($C_{18}H_{17}NO_4$ requires *M* 311.1157).

1-(4-Fluorophenyl)-3-hydroxymethyl-5-methoxy-2-methylindole-4,7-dione 25. (66%), mp 265–267 °C; λ_{max} (MeOH)/nm 454 (log ε 3.20), 344 (3.40), 286 (4.22); ν_{max} (CH_2Cl_2)/cm^{-1} 3436, 1647, 1607; δ_H (400 MHz; $CDCl_3$) 7.20–7.18 (4H, m), 5.55 (1H, s), 4.66 (2H, d, *J* 7.0), 4.03 (1H, t, *J* 7.0), 3.80 (3H, s), 2.00 (3H, s); δ_C (100 MHz; $CDCl_3$) 179.7, 176.9, 162.7 (d, J_{CF} 249.7), 159.5, 135.3, 132.8 (d, J_{CF} 3.4), 130.4, 128.9 (d, J_{CF} 8.9), 122.6, 122.5, 116.5 (d, J_{CF} 23.1), 107.1, 56.6, 55.9, 10.1; *m*/*z* (EI, relative intensity) 315 (M^+, 34%), 300 (18), 277 (51), 84 (100); *m*/*z* (HRMS) 315.0909 ($C_{17}H_{14}FNO_4$ requires *M* 315.0907).

3-Hydroxymethyl-5-methoxy-2-methyl-1-propylindole-4,7-dione 26. (39%), mp 156–158 °C (Found: C, 62.3; H, 6.3; N, 4.9. $C_{14}H_{17}NO_4 \cdot 0.4\ H_2O$ requires C, 62.1; H, 6.6; N, 5.2%); λ_{max} (MeOH)/nm 460 (log ε 3.18), 352 (3.39), 280 (4.00); ν_{max} (KBr disc)/cm^{-1} 3400, 3053, 2960, 2919, 1676, 1650, 1592; δ_H (300 MHz; $CDCl_3$) 5.60 (1H, s), 4.60 (2H, d, *J* 6.5), 4.23 (2H, t, *J* 7.4), 4.08 (1H, br t), 3.81 (3H, s), 2.23 (3H, s), 1.70 (2H, sextet, *J* 7.4), 0.95 (3H, t, *J* 7.4); δ_C (100 MHz; $CDCl_3$) 179.3, 178.2, 159.5, 134.0, 129.0, 122.9, 122.2, 107.2, 56.5, 55.9, 46.8, 23.6, 11.0, 9.4; *m*/*z* (EI, relative intensity) 263 (M^+, 65%), 248 (50), 221 (91), 206 (100).

3-(Hydroxymethyl)-5-methoxy-2,6-dimethyl-1-propylindole-4,7-dione 27. To a solution of ethyl 5-methoxy-2,6-dimethyl-1-propyl-4,7-dioxoindole-3-carboxylate **9** (0.31 g, 0.98 mmol) in water–dichloromethane–ethanol (20 cm^3) was added sodium dithionite (1.7 g, 9.7 mmol) and the mixture stirred overnight. The organic layer was separated, washed with saturated ammonium chloride, dried (Na_2SO_4) and concentrated, and used directly in the next step. To a stirred suspension of the crude dihydroxyindole in dichloromethane (30 cm^3) was added DIBAL (1 mol dm^{-3} solution in hexane, 1.38 g, 9.7 mmol) at −10 °C and the mixture was allowed to warm to 0 °C for 2 h. The reaction was quenched by drop-wise addition of iron(III) chloride (1 mol dm^{-3} in 0.1 mol dm^{-3} hydrochloric acid, 30 cm^3). The crude mixture was purified by column chromatography (1 : 1, hexane–ethyl acetate) to yield the title compound (0.103 g, 38%) as a red crystalline material, mp 81–83 °C (from hexane) (Found: C, 64.8; H, 6.9; N, 4.8. $C_{15}H_{19}NO_4$ requires C, 65.0; H, 6.9; N, 5.0%); λ_{max} (MeOH)/nm 468 (log ε 3.08), 352 (3.52), 288 (4.14); ν_{max} (KBr disc)/cm^{-1} 3457, 2969, 2942, 1633, 1606, 1503; δ_H (300 MHz; $CDCl_3$) 4.60 (2H, d, *J* 7.0), 4.23 (3H, m), 3.98 (3H, s), 2.23 (3H, s), 1.97 (3H, s), 1.71 (2H, sextet, *J* 7.4), 0.97 (3H, t, *J* 7.4); δ_C (100 MHz; $CDCl_3$) 181.1, 178.4, 156.0, 134.2, 129.8, 128.8, 122.8, 122.7, 61.0, 56.0, 46.9, 23.6, 11.1, 9.5, 8.9; *m*/*z* (CI, relative intensity) 278 (MH^+, 89), 277 (M^+, 47), 262 (100), 260 (39); *m*/*z* (HRMS) 277.1313 ($C_{15}H_{19}NO_4$ requires 277.1314).

General method for the preparation of 3-acylindoles 10

5-Methoxy-2-methylindole (3.2 g, 20 mmol) was dissolved in diethyl ether (anhydrous, 15 cm^3) and added drop-wise, with vigorous stirring, to a solution of ethylmagnesium bromide (3.0 mol dm^{-3}, 6.8 cm^3, 20 mmol) in diethyl ether (anhydrous, 10 cm^3) under a dry nitrogen atmosphere. The solution was heated under reflux for 0.5 h, cooled to 0 °C and the appropriate acyl chloride (20 mmol in ether (10 cm^3)) added with vigorous stirring. The solution was heated under reflux for a further 1 h, cooled and saturated ammonium chloride (100 cm^3) added. The solution was extracted with ethyl acetate (500 cm^3) and washed with saturated sodium bicarbonate solution (150 cm^3) and brine (150 cm^3) then evaporated by 50% and the resulting precipitate collected and washed with ether to give a white solid which was used without further purification.

3-Acetyl-2-methyl-5-methoxyindole 10a. (62%), mp 219–224 °C; δ_H (60 MHz; $(CD_3)_2SO$–$CDCl_3$) 7.57 (3H, m), 3.82 (3H, s), 2.72 (3H, s), 2.54 (3H, s).

3-Benzoyl-5-methoxy-2-methylindole 10b. (19%), mp 185–186 °C; δ_H (60 MHz; $CDCl_3$) 6.65–7.57 (8H, m), 3.63 (3H, s), 2.42 (3H, s).

5-Methoxy-2-methyl-3-(2-thienylcarbonyl)indole 10c. (46%), mp 198–200 °C; δ_H (60 MHz; $CDCl_3$) 6.63–7.88 (6H, m), 3.69 (3H, s), 2.5 (3H, s).

General method for the preparation of 3-acyl-5-nitroindoles 11

The crude 3-acylindole (12.3 mmol) was added to a stirred suspension of sodium hydride (60% dispersion in oil, 26 mmol) in tetrahydrofuran (anhydrous, 50 cm^3). The solution was stirred for 0.5 h at 50 °C, cooled and iodomethane (15 cm^3, 105 mmol) added. The solution was heated under reflux for 1 h, cooled and added to a cold solution of sodium bisulfate (10%, 50 cm^3). The solution was extracted with ethyl acetate (150 cm^3), washed with saturated sodium bicarbonate solution (100 cm^3) and evaporated. The residue was purified by column chromatography (ethyl acetate–hexane, 1 : 1) to yield the *N*-methyl derivative. This material (2.3 mmol) was then dissolved in glacial acetic acid (7.5 cm^3) and cooled to 0–4 °C. Fuming nitric acid (1.5 cm^3) in acetic acid (4.5 cm^3) was then added slowly with stirring at 0–4 °C. After 1 h crushed ice (50 g) was added and the solution stirred for 0.5 h. The resulting pale yellow solid was filtered, washed with water and dried in a vacuum oven at 45 °C over potassium hydroxide pellets for 12 h to give the *title compound.*

3-Acetyl-1,2-dimethyl-5-methoxy-4-nitroindole 11a. 3-Acetyl-1,2-dimethyl-5-methoxyindole (80%), mp 123–124 °C; δ_H (60 MHz; $(CD_3)_2SO$–$CDCl_3$) 7.49 (3H, m), 3.85 (3H, s), 3.58 (3H, s), 2.72 (3H, s), 2.54 (3H, s) and the *title compound* (83%) sufficiently pure for use in the next step, mp 183–186 °C (dec.); δ_H (60 MHz; $(CD_3)_2SO$–$CDCl_3$) 7.15 (1H, d, *J* 9.0), 7.69 (1H, d, *J* 9.0), 3.87 (3H, s), 3.79 (3H, s), 2.67 (3H, s), 2.28 (3H, s).

3-Benzoyl-5-methoxy-1,2-dimethyl-4-nitroindole 11b. *N*-Methyl derivative (89%) as an off-white solid after column chromatography (dichloromethane); δ_H (60 MHz; $CDCl_3$) 6.84–7.70 (8H, m), 3.66 (6H, s), 2.47 (3H, s); which was used in the next step without further purification, and the *title compound* (75%), mp 180–184 °C; δ_H (60 MHz; $(CD_3)_2SO$–$CDCl_3$) 7.72 (1H, d, *J* 9.0), 7.51 (5H, m), 7.14 (1H, d, *J* 9.0), 3.88 (3H, s), 3.76 (3H, s), 2.21 (3H, s).

5-Methoxy-1,2-dimethyl-4-nitro-3-(2-thienylcarbonyl)indole 11c. *N*-Methyl derivative (76%) as an off-white solid after column chromatography (ethyl acetate–hexane, 1 : 1), mp 106–107 °C; δ_H (60 MHz; $CDCl_3$) 6.74–7.64 (6H, m), 3.73 (3H, s), 3.66 (3H, s), 2.53 (3H, s); which was used in the next step without further purification, and the *title compound* (43%), purified by column chromatography (ethyl acetate–hexane, 1 : 1), mp 183–184 °C; δ_H (60 MHz; $(CD_3)_2SO$–$CDCl_3$) 7.81 (1H, d, *J* 9.0), 7.1–7.91 (3H, m), 7.07 (1H, d, *J* 9.0), 3.88 (3H, s), 3.75 (3H, s), 2.39 (3H, s).

General method for the preparation of 3-acylindolequinones 12

The nitro compound **11** (1.91 mmol) was dissolved in ethanol (75 cm^3) and tin (powder, 2.2 g, 18.5 mmol) added, followed by hydrochloric acid (3.0 mol dm^{-3}, 30 cm^3). The solution was stirred for 0.5 h at 20 °C, decanted and ethyl acetate (250 cm^3) added. The organic solution was then washed with saturated sodium bicarbonate solution (2 × 100 cm^3) and brine (sat., 50 cm^3), dried and evaporated. The residue was dissolved in acetone (60 cm^3) and Fremy's salt (0.25 g in 60 cm^3 Na_2HPO_4–NaH_2PO_4 buffer (0.3 mol dm^{-3}, pH 6.0)) added. The solution was stirred at 20 °C for 0.3 h and the acetone removed *in vacuo*. The resulting precipitate was collected by suction filtration,

washed with water and dried *in vacuo* for 12 h to give the *title compound* as an orange solid.

3-Acetyl-5-methoxy-1,2-dimethylindole-4,7-dione 12a. (64%), mp 227–228 °C; δ_H (60 MHz; $CDCl_3$) 5.64 (1H, s), 3.89 (3H, s), 3.84 (3H, s), 2.62 (3H, s), 2.36 (3H, s).

3-Benzoyl-5-methoxy-1,2-dimethylindole-4,7-dione 12b. (71%), mp 178–180 °C; δ_H (60 MHz; $CDCl_3$) 7.23–7.88 (5H, m), 5.62 (1H, s), 3.94 (3H, s), 3.72 (3H, s), 2.29 (3H, s).

5-Methoxy-1,2-dimethyl-3-(2-thienylcarbonyl)indole-4,7-dione 12c. (78%), mp 194–196 °C; δ_H (60 MHz; $CDCl_3$) 6.94–7.66 (3H, m), 5.63 (1H, s), 3.94 (3H, s), 3.75 (3H, s), 2.29 (3H, s).

General method for the preparation of hydroxymethyl derivatives 28, 30, 31

Compound **12** (1 mmol) was dissolved in methanol (anhydrous, N_2-degassed, 100 cm^3) and sodium borohydride (0.5 g, 26.5 mmol) added with stirring. After 10 min water (50 cm^3) was added followed by ethyl acetate (50 cm^3) and the solution shaken vigorously. The solution was then extracted with ethyl acetate (4 × 100 cm^3) and washed with saturated sodium bicarbonate solution (100 cm^3) and brine (100 cm^3), dried and evaporated. The residue was purified by column chromatography (ethyl acetate–hexane, 2 : 1) to give the *title compound* as an orange solid, recrystallised from ethyl acetate–hexane.

3-(1-Hydroxyethyl)-5-methoxy-1,2-dimethylindole-4,7-dione 28. (56%), mp 148–150 °C (Found: C, 62.8; H, 6.0; N, 5.6. $C_{13}H_{15}NO_4$ requires C, 62.6; H, 6.1; N, 5.6%); δ_H (60 MHz; $CDCl_3$) 5.58 (1H, s), 4.80 (1H, m), 3.86 (3H, s), 3.79 (3H, s), 2.17 (3H, s), 1.45 (3H, d, *J* 4.8) (OH not observed); *m*/*z* (EI, relative intensity) 248.9 (M^+, 22%), 234.0 (100), 215.9 (8), 205.8 (22).

3-(1-Hydroxy-1-phenylmethyl)-5-methoxy-1,2-dimethylindole-4,7-dione 30. (38%), mp 138–140 °C (Found: C, 67.3; H, 5.8; N, 4.0. $C_{18}H_{17}NO_4 \cdot 0.5\ H_2O$ requires C, 67.5; H, 5.7; N, 4.4%); δ_H (60 MHz; $CDCl_3$) 7.28 (5H, m), 5.78 (1H, br), 5.58 (1H, s), 3.89 (3H, s), 3.77 (3H, s), 2.28 (3H, s); *m*/*z* (EI, relative intensity) 310.8 (M^+, 100%), 294.7 (54), 217.8 (39), 205.8 (67).

3-[1-Hydroxy-1-(2-thienyl)methyl]-5-methoxy-1,2-dimethylindole-4,7-dione 31. (46%), mp 175–176 °C (Found: C, 58.8; H, 4.8; N, 4.1. $C_{16}H_{15}NO_4S \cdot 0.5\ H_2O$ requires C, 58.8; H, 4.9; N, 4.3%); δ_H (60 MHz; $CDCl_3$) 7.17 (1H, m), 6.84 (2H, m), 5.93 (1H, br), 5.88 (1H, d, *J* 4.8), 5.59 (1H, s), 3.90 (3H, s), 3.78 (3H, s), 2.27 (3H, s); *m*/*z* (EI, relative intensity) 317 (M^+, 100%), 301 (76), 286 (25), 233 (58), 218 (30), 206 (39).

Preparation of 3-(4-nitrophenoxy)alkyl indolequinones: 7-methoxy-9-[(4-nitrophenoxy)methyl]-2,3-dihydro-1*H*-pyrrolo-[1,2-*a*]indole-5,8-dione 33

To a stirred solution of the hydroxymethylindolequinone **14** (0.046 g, 0.18 mmol) in dichloromethane (10 cm^3) was added thionyl chloride (1.6 g, 13.4 mmol). The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*. The chloride was used directly in the next step without purification. 4-Nitrophenol (0.054 g, 0.39 mmol) in dimethylformamide (5 cm^3) was added to a stirring suspension of sodium hydride (0.009 g, 0.37 mmol) in dimethylformamide (10 cm^3) at 0 °C. The mixture was stirred at room temperature for 45 min. A solution of the chloride in dimethylformamide (5 cm^3) was added dropwise at 0 °C and the mixture stirred at room temperature for 2 h. Saturated ammonium chloride solution was added and the mixture extracted with ethyl acetate. The ethyl acetate layer was washed twice with water, dried ($MgSO_4$) and concentrated. The crude material was purified by column chromatography (90% dichloromethane–10% ethyl acetate) to yield the *title compound* (0.017 g, 25%) as a orange solid, mp 237–239 °C (from dichloromethane–light petroleum) (Found: C, 60.9; H, 4.0; N, 7.5. $C_{19}H_{16}N_2O_6 \cdot 0.3H_2O$ requires C, 61.0; H, 4.5; N, 7.5%); λ_{max} (MeOH)/nm 450 (log ε 2.74), 290 (3.85), 226 (3.83); ν_{max} (KBr disc)/cm^{-1} 3119, 2970, 2919, 1670, 1644, 1593; δ_H (300 MHz; $CDCl_3$) 8.18, 7.02 (4H, m), 5.64 (1H, s), 5.39 (2H, s), 4.23 (2H, m), 3.83 (3H, s), 2.87 (2H, m), 2.57 (2H, m); δ_C (100 MHz; $CDCl_3$) 178.0, 177.8, 163.5, 160.5, 143.6, 141.7, 126.6, 125.9, 124.0, 114.7, 111.6, 105.8, 62.8, 56.6, 47.0, 27.3, 23.5; *m*/*z* (EI, relative intensity) 368 (M^+, 1%), 231 (9), 230 (67), 44 (100).

General method for the Mitsunobu reaction

The 3-hydroxymethylindolequinone (1.96 mmol), triphenylphosphine (3.8 mmol), diethyl azodicarboxylate (3.9 mmol) and 4-nitrophenol (3.3 mmol) were stirred overnight in tetrahydrofuran at 50 °C. Excess solvent was removed and the majority of the product triturated out with ether, and filtered off. The residue was dissolved in dichloromethane washed with water, dried (Na_2SO_4) and concentrated. The crude residue was purified by column chromatography (light petroleum–ethyl acetate 1 : 1) to yield the title compound as an orange crystalline solid. The following compounds were thus prepared.

5-Methoxy-1-methyl-3-(4-nitrophenoxy)methylindole-4,7-dione 32. (67%) as an orange crystalline solid; mp 207–209 °C; λ_{max} (MeOH)/nm 284 (log ε 1.02), 224 (0.99); ν_{max} (Nujol)/cm^{-1} 3058, 1675, 1644, 1588, 1516, 1342, 1184; δ_H (300 MHz; $CDCl_3$) 8.21 (2H, d, *J* 9.3, ArH), 7.04 (2H, d, *J* 9.3), 6.88 (1H, s), 5.71 (1H, s), 5.38 (2H, s), 3.97 (3H, s), 3.84 (3H, s); δ_C (75 MHz; $CDCl_3$) 178.9, 177.7, 163.4, 160.3, 141.8, 130.0, 128.5, 125.9, 121.0, 119.9, 114.8, 107.0, 63.1, 56.6, 36.4; *m*/*z* (EI, relative intensity) 342 (M^+, 3%), 205 (38), 204 (100), 174 (8), 161 (14), 139 (24); *m*/*z* (HRMS) 342.0853 ($C_{17}H_{14}N_2O_6$ requires *M* 342.0851).

5-Methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione 34. Prepared as previously described [39] in 57% yield.

5-Methoxy-1,2,6-trimethyl-3-(4-nitrophenoxymethylindole-4,7-dione 35. (72%), mp 193–195 °C (Found: C, 60.6; H, 4.8; N, 7.2. $C_{19}H_{18}N_2O_6 \cdot 0.3\ H_2O$ requires C, 60.7; H, 5.0; N, 7.4%); λ_{max} (MeOH)/nm 458 (log ε 2.21), 318 (3.24), 290 (3.43); ν_{max} (CH_2Cl_2)/cm^{-1} 1665, 1642, 1592; δ_H (300 MHz; $CDCl_3$) 8.18 (2H, d, *J* 9.2), 7.05 (2H, d, *J* 9.2), 5.37 (2H, s) 3.98 (3H, s), 3.89 (3H, s), 2.30 (3H, s), 1.96 (3H, s); δ_C (100 MHz; $CDCl_3$) 180.0, 179.1, 163.6, 156.1, 141.6, 138.2, 129.2, 128.8, 125.9, 121.6, 115.4, 114.9, 61.2, 61.1, 32.5, 9.9, 8.8; *m*/*z* (EI, relative intensity) 232 (100%), 127 (40), 57 (77); *m*/*z* (HRMS) 370.1162 ($C_{19}H_{18}N_2O_6$ requires *M* 370.1165).

5-Methoxy-1-methyl-3-[(4-nitrophenoxy)methyl]-2-phenylindole-4,7-dione 37. (84%) as an orange–yellow solid, mp 229–230 °C (Found: C, 65.1; H, 4.2; N, 6.5. $C_{23}H_{18}N_2O_6 \cdot 0.3\ H_2O$ requires C, 65.2; H, 4.4; N, 6.6%); λ_{max} (MeOH)/nm 444 (log ε 3.10), 284 (4.06); ν_{max} (KBr disc)/cm^{-1} 3114, 3068, 3017, 2965, 2934, 1665, 1639, 1598; δ_H (300 MHz; $CDCl_3$) 8.14 (2H, d, *J* 9.2), 7.49 (3H, m), 7.35 (2H, m), 6.92 (2H, d, *J* 9.2), 5.74 (1H, s), 5.16 (2H, s), 3.85 (3H, s), 3.83 (3H, s); δ_C ($CDCl_3$) 179.1, 177.8, 163.7, 160.0, 142.1, 141.6, 130.4, 129.8, 129.7, 128.9, 128.2, 125.8, 121.6, 116.6, 114.9, 107.0, 61.4, 56.6, 34.1; *m*/*z* (EI, relative intensity) 418 (M^+, 6%), 388 (13), 311 (89), 296 (100); *m*/*z* (HRMS) 419.1243 ($C_{23}H_{19}N_2O_6$ requires *M*H 419.1243).

2-(Biphenyl-4-yl)-5-methoxy-3-[(4-nitrophenoxy)methyl]-1-methylindole-4,7-dione 38. (89%) as an orange–red solid, mp 128–129 °C (Found: C, 68.8; H, 4.5; N, 5.4. $C_{29}H_{22}N_2O_6 \cdot 0.6\ H_2O$ requires C, 68.9; H, 4.6; N, 5.5%); λ_{max} (MeOH)/nm 496

(log ε 3.19) and 280 (4.56); ν_{max} (KBr disc)/cm^{-1} 3083, 3027, 2925, 1742, 1680, 1644, 1598; δ_H (300 MHz; $CDCl_3$) 8.16 (2H, d, *J* 9.2), 7.71 (2H, m), 7.62 (2H, m), 7.51–7.40 (5H, m), 6.96 (2H, d, *J* 9.2), 5.75 (1H, s), 5.21 (2H, s), 3.90 (3H, s), 3.85 (3H, s); δ_C (100 MHz; $CDCl_3$) 179.0, 177.8, 163.7, 160.0, 142.5, 141.8, 141.6, 139.8, 130.8, 129.9, 129.0, 128.0, 127.5, 127.1, 127.0, 125.8, 121.7, 116.8, 114.9, 107.0, 61.4, 56.6, 34.2; *m/z* (CI, relative intensity) 495 (MH^+, 2%), 373 ($M - NO_2C_6H_4OH$, 100), 357 (25), 298 (10), 138 (90).

5-Methoxy-1-methyl-2-(2-naphthyl)-3-[(4-nitrophenoxy)-methyl]indole-4,7-dione 39. (77%) as an orange solid, mp 165–167 °C (Found: C, 66.9; H, 4.2; N, 6.0. $C_{27}H_{20}N_2O_6 \cdot 0.9\ H_2O$ requires C, 66.9; H, 4.5; N, 5.8%); λ_{max} (MeOH)/nm 460 (log ε 3.31), 286 (4.58), 266 (4.59); ν_{max} (KBr disc)/cm^{-1} 2952, 1677, 1642, 1591; δ_H (300 MHz, $CDCl_3$) 8.14 (2H, d, *J* 9.2), 7.89 (2H, m), 7.83 (2H, m), 7.59 (2H, m), 7.42 (1H, dd, *J* 8.4, *J* 1.7), 6.91 (2H, d, *J* 9.2), 5.76 (1H, s), 5.21 (2H, s), 3.88 (3H, s), 3.86 (3H, s); δ_C (75 MHz; $CDCl_3$) 179.1, 177.9, 163.7, 160.0, 142.2, 141.5, 133.4, 132.9, 130.5, 129.8, 128.7, 128.2, 127.9, 127.5, 127.1, 126.9, 125.8, 125.4, 121.7, 116.9, 114.9, 107.1, 61.4, 56.6, 34.3, 10.7; *m/z* (EI, relative intensity) 331 ($MH^{2+} - HOC_6H_4NO_2$, 29%), 330 ($MH^+ - HOC_6H_4NO_2$, 100), 139 ($HOC_6H_4NO_2$, 52), 109 (63), 65 (72).

1-Cyclopropyl-5-methoxy-2-methyl-3-[(4-nitrophenoxy)-methyl]indole-4,7-dione 40. (80%), mp 217–219 °C (Found: C, 61.1; H, 4.5; N, 6.9. $C_{20}H_{18}N_2O_6 \cdot 0.6\ H_2O$ requires C, 61.1; H, 4.9; N, 7.1%); λ_{max} (MeOH)/nm 450 (log ε 3.04) 318 (3.90), 294 (4.21); ν_{max} (CH_2Cl_2)/cm^{-1} 1674, 1641, 1592; δ_H (400 MHz; $CDCl_3$) 8.17 (2H, d, *J* 9.2), 7.03 (2H, d, *J* 9.2), 5.64 (1H, s), 5.36 (2H, s), 3.78 (3H, s), 3.23–3.15 (1H, m), 2.42 (3H, s), 1.33–1.21 (2H, m), 0.97–0.86 (2H, m); δ_C (100 MHz; $CDCl_3$) 178.5, 177.1, 163.7, 159.0, 141.6, 140.3, 130.5, 125.9, 121.7, 115.3, 114.9, 107.2, 61.0, 56.4, 28.1, 11.6, 10.0 (2 × CH_2); *m/z* (EI, relative intensity) 244 (100%), 139 (15), 65 (26); *m/z* (HRMS) 382.1163 ($C_{20}H_{18}N_2O_6$ requires *M* 382.1165).

1-Benzyl-2-ethyl-5-methoxy-3-[(4-nitrophenoxy)methyl]-indole-4,7-dione 41. (76%), mp 177–179 °C; λ_{max} (MeOH)/nm 454 (log ε 2.08), 316 (2.96), 290 (3.22); ν_{max} (CH_2Cl_2)/cm^{-1} 1665, 1638, 1597; δ_H (300 MHz; $CDCl_3$) 8.19 (2H, d, *J* 9.2), 7.33–7.26 (3H, m), 7.06 (2H, d, *J* 9.2), 7.08–6.99 (2H, m), 5.67 (2H, s), 5.65 (1H, s), 5.41 (2H, s), 3.81 (3H, s), 2.67 (2H, q, *J* 7.5), 1.07 (3H, t, *J* 7.5); δ_C (100 MHz; $CDCl_3$) 178.4, 178.0, 163.6, 159.5, 144.0, 141.6, 136.3, 128.9, 128.7, 127.7, 126.0 (2 × CH), 126.0 (2 × CH), 121.8, 115.8, 114.9, 107.1, 61.0, 56.6, 48.4, 17.5, 14.2; *m/z* (EI, relative intensity) 308 ($M^+ - HOC_6H_4NO_2$, 26%), 91 (100).

5-Methoxy-2-methyl-3-[(4-nitrophenoxy)methyl]-1-phenyl-indole-4,7-dione 42. (80%), mp 249–251 °C; λ_{max} (MeOH)/nm 444 (log ε 2.87), 288 (4.06); ν_{max} (CH_2Cl_2)/cm^{-1} 1676, 1643, 1590; δ_H (400 MHz; $CDCl_3$) 8.21 (2H, d, *J* 8.8) 7.54–7.52 (3H, m), 7.26–7.24 (2H, m), 7.10 (2H, d, *J* 8.8), 5.57 (1H, s), 5.44 (2H, s), 3.81 (3H, s), 2.10 (3H, s); δ_C (100 MHz; $CDCl_3$) 178.6, 177.0, 163.7, 159.5, 141.6, 138.8, 136.7, 130.0, 129.5, 127.1, 126.0, 121.7, 115.6, 114.9, 106.8, 61.2, 56.6, 10.5 (one C not observed); *m/z* (EI, relative intensity) 418 (M^+, 0.2%), 280 (58), 81 (85), 55 (100); *m/z* (HRMS) 418.1160 ($C_{23}H_{18}N_2O_6$ requires *M* 418.1165).

5-Methoxy-2,6-dimethyl-3-[(4-nitrophenoxy)methyl]-1-phenylindole-4,7-dione 43. (70%), mp 134–136 °C; λ_{max} (MeOH)/nm 452 (log ε 2.40), 322 (3.51), 294 (3.63); ν_{max} (CH_2Cl_2)/cm^{-1} 1662, 1649, 1593; δ_H (400 MHz; $CDCl_3$) 8.22 (2H, d, *J* 9.2), 7.54–7.52 (3H, m), 7.26–7.23 (2H, m), 7.09 (2H, d, *J* 9.2), 5.42 (2H, s), 3.99 (3H, s), 2.09 (3H, s), 1.88 (3H, s); δ_C (100 MHz; $CDCl_3$) 180.3, 177.6, 163.7, 156.1, 141.7, 139.0, 136.9, 129.8, 128.5, 129.4, 129.0, 127.1, 126.0, 122.0, 115.4, 114.9, 61.3, 61.1, 10.4, 8.7; *m/z* (EI, relative intensity) 432 (M^+, 4%), 295 (100), 251 (33), 118 (30), 77 (36); *m/z* (HRMS) 432.1327 ($C_{24}H_{20}N_2O_6$ requires *M* 432.1321).

1-(4-Fluorophenyl)-5-methoxy-2-methyl-3-[(4-nitrophenoxy)-methyl]indole-4,7-dione 44. (78%), mp 239–241 °C; λ_{max} (MeOH)/nm 446 (log ε 3.25), 288 (4.42); ν_{max} (CH_2Cl_2)/cm^{-1} 1675, 1634, 1592; δ_H (400 MHz; $CDCl_3$) 8.21 (2H, d, *J* 9.2), 7.26–7.17 (4H, m), 7.09 (2H, d, *J* 9.2), 5.57 (1H, s), 5.43 (2H, s), 3.81 (3H, s), 2.10 (3H, s); δ_C (100 MHz; $CDCl_3$) 178.5, 177.0, 163.6, 162.8 (d, J_{CF} 249.9), 159.5, 141.7, 138.8, 132.3, 129.0 (d, J_{CF} 9.0), 126.0, 121.7, 116.5, (d, J_{CF} 23.2), 115.7, 114.9, 106.8, 61.2, 56.6, 10.4; *m/z* (EI, relative intensity) 185 (8%), 155 (25), 127 (29), 69 (45), 57 (100).

5-Methoxy-2-methyl-3-(4-nitrophenoxymethyl)-1-propyl-indole-4,7-dione 45. (64%), mp > 250 °C (Found: C, 62.1; H, 5.2; N, 7.1. $C_{20}H_{20}N_2O_6$ requires C, 62.5; H, 5.2; N, 7.3%); λ_{max} (MeOH)/nm 456 (log ε 3.21), 316 (4.10), 292 (4.39); ν_{max} (KBr disc)/cm^{-1} 3063, 2965, 2940, 1676, 1637, 1592; δ_H (300 MHz; $CDCl_3$) 8.18 (2H, d, *J* 9.2), 7.05 (2H, d, *J* 9.2), 5.63 (1H, s), 5.39 (2H, s), 4.27 (2H, m), 3.81 (3H, s), 2.32 (3H, s), 1.73 (2H, sextet, *J* 7.5), 0.96 (3H, t, *J* 7.5); δ_C (100 MHz; $CDCl_3$) 178.34, 178.27, 163.6, 159.5, 141.6, 137.5, 128.6, 125.9, 121.4. 115.8, 114.9, 106.9, 61.2, 56.5, 46.9, 23.7, 11.0, 9.8; *m/z* (CI, relative intensity) 402 (M + NH_4^+, 10%), 385 (M^+, 5), 263 (55), 248 (100).

5-Methoxy-2,6-dimethyl-3-[(4-nitrophenoxy)methyl]-1-propylindole-4,7-dione 46. (64%) as an orange crystalline solid, mp 120–122 °C (Found: C, 62.9; H, 5.5; N, 6.8. $C_{21}H_{22}N_2O_6 \cdot 0.1\ H_2O$ requires C, 63.0; H, 5.6; N, 7.0%); λ_{max} (MeOH)/nm 452 (log ε 2.96), 288 (4.25); ν_{max} (KBr disc)/cm^{-1} 2966, 2930, 1657, 1637, 1606, 1591; δ_H (400 MHz; $CDCl_3$) 8.20 (2H, d, *J* 9.2), 7.07 (2H, d, *J* 9.2), 5.37 (2H, s), 4.25 (2H, m), 3.98 (3H, s), 2.31 (3H, s), 1.97 (3H, s), 1.74 (2H, sextet, *J* 7.5), 0.98 (3H, t, *J* 7.3); δ_C (100 MHz; $CDCl_3$) 180.1, 178.6, 163.7, 156.0, 141.6, 137.8, 129.2, 128.4, 125.9, 121.8, 115.5, 114.9, 61.2, 61.0, 47.0, 23.7, 11.1, 9.8, 8.8; *m/z* (EI, relative intensity) 398 (M^+, 3%), 260 ($M^+ - HOC_6H_4NO_2$, 100), 218 (37); *m/z* (HRMS) 398.1471 ($C_{21}H_{22}N_2O_6$ requires 398.1478).

5-Methoxy-1,2-dimethyl-3-[1-(4-nitrophenoxy)ethyl]indole-4,7-dione 47. (82%) as an orange–yellow solid, mp 178–179 °C (Found: C, 61.7; H, 5.0; N, 7.5. $C_{19}H_{18}N_2O_6$ requires C, 61.6; H, 4.9; N, 7.6%); λ_{max} (MeOH)/nm 458 (log ε 3.17), 290 (4.37), 236 (4.04); ν_{max} (KBr disc)/cm^{-1} 2976, 2925, 1665, 1634, 1593; δ_H (400 MHz, $CDCl_3$) 8.09 (2H, d, *J* 9.2), 6.91 (2H, d, *J* 9.2), 6.35 (1H, q, *J* 6.5), 5.64 (1H, s), 3.84 (3H, s), 3.81 (3H, s), 2.28 (3H, s), 1.66 (3H, d, *J* 6.5); δ_C (100 MHz; $CDCl_3$) 178.6, 178.4, 162.8, 159.5, 141.4, 135.2, 128.6, 125.8, 122.3, 120.0, 115.2, 106.9, 69.4, 56.5, 32.1, 22.0, 10.5; *m/z* (EI, relative intensity) 232 ($M^+ - NO_2C_6H_4OH$, 100%), 206 (10), 127 (8), 110 (48).

5-Methoxy-1,2-dimethyl-3-[1-(4-nitrophenoxy)-1-phenyl-methyl]indole-4,7-dione 49. (80%) as an orange solid, mp 208–209 °C (Found: C, 66.4; H, 4.5; N, 6.3. $C_{24}H_{20}N_2O_6$ requires C, 66.7; H, 4.7; N, 6.5%); λ_{max} (MeOH)/nm 456 (log ε 3.22), 290 (4.40); ν_{max} (KBr disc)/cm^{-1} 2926, 2851, 1666, 1658, 1631, 1599; δ_H (300 MHz; $CDCl_3$) 8.15 (2H, d, *J* 9.2), 7.49–7.44 (3H, m), 7.38–7.28 (3H, m), 7.29 (2H, d, *J* 9.2), 5.67 (1H, s), 3.86 (3H, s), 3.82 (3H, s), 2.20 (3H, s); δ_C (100 MHz; $CDCl_3$) 178.8, 178.4, 162.6, 159.6, 141.8, 139.5, 136.3, 128.7, 128.6, 127.9, 126.0, 125.9, 120.7, 120.5, 115.5, 107.0, 73.5, 56.6, 32.2, 10.7; *m/z* (EI, relative intensity) 295 ($MH^+ - HOC_6H_4NO_2$, 13%), 294 ($MH^+ - HOC_6H_4NO_2$, 40), 139 ($HOC_6H_4NO_2$, 20), 69 (100).

5-Methoxy-1,2-dimethyl-3-[1-(4-nitrophenoxy)-1-(2-thienyl)-methyl]-indole-4,7-dione 50. (49%) as an orange solid, mp 178–180 °C (from ethyl acetate) (Found: C, 60.3; H, 4.1; N, 6.4. $C_{22}H_{18}N_2O_6S$ requires C, 60.3; H, 4.1; N, 6.4%); δ_H (60 MHz;

$CDCl_3$) 8.12 (2H, m), 7.57 (1H, br d), 6.90–7.3 (4H, m), 6.96 (1H, s), 5.65 (1H, s), 3.84 (3H, s), 3.83 (3H, s), 2.3 (3H, s).

1,2-Dimethyl-5-(morpholin-1-yl)-3-(4-nitrophenoxy)methylindole-4,7-dione 36. Morpholine (1.00 g, 11 mmol) was added to a stirring solution of 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxy)methylindole-4,7-dione **34** (11 mg, 30 μmol) in acetonitrile (5 cm^3). The reaction mixture was stirred at room temperature for 5 days. Water (10 cm^3) was added, the mixture was extracted with dichloromethane (3 × 15 cm^3), washed with water (2 × 10 cm^3), brine (2 × 10 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure. The crude product was preabsorbed onto silica and purified by flash column chromotography, eluting with ethyl acetate–light petroleum (1 : 1) to give the *title compound* (9 mg, 73%) as a purple crystalline solid; mp 266–267 °C (Found: C, 60.8; H, 5.1; N, 10.1. $C_{21}H_{21}N_3O_6$·0.2 H_2O requires C, 60.8; H, 5.1; N, 10.1%); λ_{max} (MeOH)/nm 520 (log ε 4.03), 314 (3.97); ν_{max} (CH_2Cl_2)/cm^{-1} 3053, 2986, 1654, 1629, 1588, 1521, 1480, 1454, 1352, 1260, 1219; δ_H (200 MHz; $CDCl_3$) 8.20 (2H, d, *J* 9.3), 7.05 (2H, d, *J* 9.3), 5.50 (1H, s), 5.34 (2H, s), 3.91 (3H, s), 3.83 (4H, m), 3.40 (4H, m), 2.29 (3H, s), and 1.25 (2H, s); δ_C (100 MHz; $CDCl_3$) 180.9, 178.3, 163.8, 153.4, 141.6, 137.2, 129.3, 125.9 (2 × CH), 122.0, 115.3, 114.8, 110.4, 66.4, 61.3, 49.8, 32.2, 9.7; *m*/*z* (EI, relative intensity) 411 (M^+, 5%), 273 (100), 243 (24), 215 (18), 188 (18), 139 (30); *m*/*z* (HRMS) 411.1426 ($C_{21}H_{21}N_3O_6$ requires *M* 411.1430).

3-(1-Hydroxyethyl)-1,2-dimethyl-5-(4-methylpiperazin-1-yl)-indole-4,7-dione 29. Compound **28** (100 mg, 0.4 mmol) was dissolved in DMF (3 cm^3) and *N*-methylpiperazine (0.75 g, 7.5 mmol) added. The solution was stirred for 24 h and ethyl acetate (50 cm^3) added, followed by saturated $NaHCO_3$ (50 cm^3). The ethyl acetate layer was separated and the aqueous phase further extracted with ethyl acetate (50 cm^3) and the combined extracts washed with water (50 cm^3) and brine (50 cm^3), dried and evaporated. The residue was purified on silica, eluting with ethyl acetate–methanol (1 : 1) to give a red solid (50 mg, 39%); mp 147–149 °C (from ethyl acetate) (Found: C, 63.4; H, 7.3; N, 12.9. $C_{17}H_{23}N_3O_3$·0.3 H_2O requires C, 63.2; H, 7.3; N, 13.0%); δ_H (60 MHz; $CDCl_3$) 5.49 (1H, s), 4.85 (1H, q, *J* 6.5), 3.85 (3H, s), 3.47 (4H, m), 2.66 (4H, m), 2.32 (3H, s), 2.18 (3H, s), 1.53 (3H, d, *J* 6.5).

1,2-Dimethyl-5-(4-methylpiperazin-1-yl)-3-[1-(4-nitrophenoxy)-ethyl]indole-4,7-dione 48. The methoxyindolequinone **35** (250 mg, 0.68 mmol) was dissolved in dimethylformamide (10 cm^3) together with *N*-methylpiperazine (2.5 g, 25 mmol) and the solution stirred at 20 °C for 24 h. Ethyl acetate (100 cm^3) was added and the solution extracted with saturated sodium bicarbonate solution (2 × 50 cm^3), water (2 × 50 cm^3) and brine (2 × 50 cm^3), then dried and evaporated. The residue was purified on silica (ethyl acetate–methanol, 1 : 1) to give a purple glassy solid (130 mg, 44%) after evaporation, mp 142–144 °C (Found: C, 62.8; H, 6.0; N, 12.5. $C_{23}H_{26}N_4O_5$ requires C, 63.0; H, 6.0; N, 12.8%); δ_H (60 MHz; $CDCl_3$) 8.09 (2H, d, *J* 9.3), 6.95 (2H, d, *J* 9.3), 6.28 (q, *J* 6.5), 5.52 (1H, s), 3.80 (3H, s), 3.45 (4H, m), 2.53 (4H, m), 2.36 (3H, s), 2.26 (3H, s), 1.66 (3H, d, *J* 6.5); HPLC (97% pure); *m*/*z* (EI, relative intensity) 438 (M^+, 7%), 299 (100), 256 (42), 139 (79).

Independent synthesis of 3-isopropoxymethyl indolequinone: 2-(biphenyl-4-yl)-3-(isopropoxy)methyl-5-methoxy-1-methyl-indole-4,7-dione 51

To a stirring solution of the hydroxymethyl indolequinone **11** (0.020 g, 0.053 mmol) in dichloromethane (10 cm^3) was added thionyl chloride (1.6 g, 13.4 mmol). The reaction mixture was stirred at room temperature overnight. The solvent was removed *in vacuo*. The chloride was used directly in the next step without purification. To a solution of the chloride in tetrahydrofuran (15 cm^3) was added silver(I) oxide (0.075 g, 0.32 mmol) and propan-2-ol (0.5 cm^3, 6.5 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was filtered through a pad of Celite. The filtrate was concentrated and purified by column chromatography (95% dichloromethane–5% ethyl acetate) to yield the *title compound* as an orange crystalline solid (0.02 g, 91%), mp 158–159 °C (from ether–petroleum ether) (Found: C, 72.9; H, 5.7; N, 3.2. $C_{26}H_{25}NO_4$·0.8 H_2O requires C, 72.6; H, 6.2; N, 3.2%); λ_{max} (MeOH)/nm 450 (log ε 3.27), 346 (3.51), 278 (4.57); ν_{max} (KBr disc)/cm^{-1} 2971, 2930, 2848, 1670, 1644, 1598; δ_H (300 MHz; $CDCl_3$) 7.72 (2H, d, *J* 8.0), 7.65 (2H, d, *J* 7.1), 7.55–7.37 (5H, m), 5.70 (1H, s), 4.48 (2H, s), 3.87 (3H, s), 3.84 (3H, s), 3.75 (1H, septet, *J* 6.1), 1.20 (6H, d, *J* 6.1); δ_C (100 MHz; $CDCl_3$) 179.2, 177.7, 160.1, 141.9, 141.6, 140.2, 130.9, 129.9, 128.9, 127.8, 127.6, 127.2, 127.1, 120.3, 106.9, 71.5, 60.0, 56.5, 34.2, 22.2; *m*/*z* (EI, relative intensity) 415 (M^+, 14%), 373 (32), 372 (88), 356 (23), 91 (100); *m*/*z* (HRMS) 415.1784 ($C_{26}H_{25}NO_4$ requires *M* 415.1783).

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References

1 C. J. Moody and E. Swann, *Farmaco*, 1997, **52**, 271.
2 M. A. Naylor, E. Swann, S. A. Everett, M. Jaffar, J. Nolan, N. Robertson, S. D. Lockyer, K. B. Patel, M. F. Dennis, M. R. L. Stratford, P. Wardman, G. E. Adams, C. J. Moody and I. J. Stratford, *J. Med. Chem.*, 1998, **41**, 2720.
3 M. Jaffar, S. A. Everett, M. A. Naylor, S. G. Moore, S. Ulhaq, K. B. Patel, M. R. L. Stratford, J. Nolan, P. Wardman and I. J. Stratford, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 113.
4 S. A. Everett, M. A. Naylor, J. Nolan, K. B. Patel and P. Wardman, *Anti-Cancer Drug Des.*, 1998, **13**, 635.
5 B. M. Hoey, J. Butler and A. J. Swallow, *Biochemistry*, 1988, **27**, 2608.
6 L. D. Skarsgard, A. Vinczan, M. W. Skwarchuk and D. J. Chaplin, *Int. J. Radiat. Oncol. Biol. Phys.*, 1994, **29**, 363.
7 Y. Palcom, M. F. Belcourt, S. M. Musser, A. C. Sartorelli, S. Rockwell and M. Tomasz, *Chem. Res. Toxicol.*, 2000, **13**, 479.
8 H. W. Moore, *Science*, 1977, **197**, 527.
9 I. Wilson, P. Wardman, T.-S. Lin and A. C. Sartorelli, *J. Med. Chem.*, 1986, **29**, 1381.
10 M. P. Saunders, M. Jaffar, A. V. Patterson, J. Nolan, M. A. Naylor, R. M. Phillips, A. L. Harris and I. J. Stratford, *Biochem. Pharmacol.*, 2000, **59**, 993.
11 D. Ross, H. D. Beall, D. Siegel, R. D. Traver and D. L. Gustafson, *Br. J. Cancer*, 1996, **74**, S1.
12 R. M. Phillips, M. A. Naylor, M. Jaffar, S. W. Doughty, S. A. Everett, A. G. Breen, G. A. Choudry and I. J. Stratford, *J. Med. Chem.*, 1999, **42**, 4071.
13 H. D. Beall, S. Winski, E. Swann, A. R. Hudnott, A. S. Cotterill, N. O'Sullivan, S. J. Green, R. Bien, D. Siegel, D. Ross and C. J. Moody, *J. Med. Chem.*, 1998, **41**, 4755.
14 H. D. Beall, R. T. Mulcahy, D. Siegel, R. D. Traver, N. W. Gibson and D. Ross, *Cancer Res*, 1994, **54**, 3196.
15 P. Workman, *Oncol. Res.*, 1994, **6**, 461.
16 S. Bailey, A. Lewis, R. Knox, L. Patterson, G. Fisher and P. Workman, *Biochem. Pharmacol.*, 1998, **56**, 613.
17 L. Rongabo, M. A. Bianchet, P. Talalay and M. Amzel, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 8846.
18 P. Wardman, *Curr. Med. Chem.*, 2001, **8**, in the press.
19 V. Jagannadham and S. Steenken, *J. Phys. Chem.*, 1984, **106**, 6542.
20 P. Wardman, M. F. Dennis, S. A. Everett, K. B. Patel, M. R. L. Stratford and M. Tracy, in *Free Radicals and Oxidative Stress: Environment, Drugs and Food Additives (Biochemical Society*

Symposium No. 61), eds. C. Rice-Evans, B. Halliwell and G. G. Lunt, Portland Press, London, 1995, p. 171.
21 J. Butler, V. J. Spanswick and J. Cummings, *Free Radical Res.*, 1996, **25**, 141.
22 C. Giulivi and E. Cadenas, *Biochem. J.*, 1994, **301**, 21.
23 V. A. Roginsky, L. M. Pisarenko, W. Bors and C. Michel, *J. Chem. Soc., Perkin Trans. 2*, 1999, 871.
24 E. Cadenas, D. Mira, A. Brunmark, C. Lind, J. Segura-Augilar and L. Ernster, *Free Radical Biol. Med.*, 1998, **5**, 71.
25 P. Vaupel and M. Höckel, in *Blood Perfusion and Micro-Environment of Tumours*, eds. M. Molls and P. Vaupel, Springer, New York, 1998, p. 63.
26 J. P. Bays, S. T. Blumer, S. Baral-Tosh, D. Behar and P. Neta, *J. Am. Chem. Soc.*, 1983, **103**, 320.
27 B. H. J. Bielski, *Radiat. Phys. Chem.*, 1993, **41**, 527.
28 P. Wardman, *J. Phys. Chem. Ref. Data*, 1989, **18**, 1637.
29 P. Wardman and E. D. Clarke, *Br. J. Cancer*, 1987, **55 Supplement VIII**, 172.
30 J. Tokunaga, *J. Chem. Eng. Data.*, 1975, **20**, 41.
31 FACSIMILE for Windows version 3.0.30, 2000, AEA Technology, Winfrith, Dorset, UK.
32 K. Sehested, O. L. Rasmussen and H. Fricke, *J. Phys. Chem.*, 1968, **72**, 626.
33 J. B. Patrick and E. K. Saunders, *Tetrahedron Lett.*, 1979, 4009.
34 J. Wrotek, Z. Lukasiewicz and S. Sabiniewicz, *PL Pat.*, 54192 (*Chem. Abstr.*, 1968, **69**, 27245).
35 G. R. Allen Jr, C. Pidacks and M. J. Weiss, *J. Am. Chem. Soc.*, 1966, **88**, 2536.
36 J. Wrotek, S. Sabiniewicz and Z. Lukasiewicz, *PL Pat.*, 54806, 1968 (*Chem. Abstr.*, 1969, **70**, 68152).
37 M. Jaffar, M. A. Naylor, S. Lockyer, R. M. Phillips, S. A. Everett, G. E. Adams and I. J. Stratford, *Anti-Cancer Drug Des*, 1998, **13**, 105.
38 A. S. Cotterill, P. Hartopp, G. B. Jones, C. J. Moody, C. L. Norton, N. O'Sullivan and E. Swann, *Tetrahedron*, 1994, **50**, 7657.
39 C. J. Moody, N. O'Sullivan, I. J. Stratford, M. A. Stephens, P. Workman, S. M. Bailey and A. Lewis, *Anti-Cancer Drugs*, 1994, **5**, 367.