2-Cyclopropylindoloquinones and Their Analogues as Bioreductively Activated Antitumor Agents: Structure−Activity *in Vitro* and Efficacy *in Vivo*

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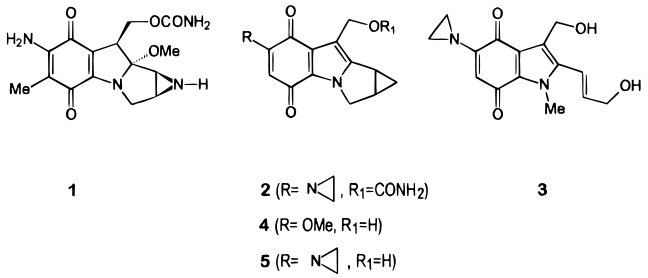
A series of 2-cycloalkyl- and 2-alkyl-3-(hydroxymethyl)-1-methylindoloquinones and corresponding carbamates have been synthesized and substituted in the 5-position with a variety of substituted and unsubstituted aziridines. Cytotoxicity against hypoxic cells *in vitro* was dependent upon the presence of a 5-aziridinyl or a substituted aziridinyl substituent for 3-hydroxymethyl analogues. The activity of 5-methoxy derivatives was dependent upon the presence of a 3-(carbamoyloxy)methyl substituent. Increasing the steric bulk at the 2-position reduced the compounds' effectiveness against hypoxic cells. A 2-cyclopropyl substituent was up to 2 orders of magnitude more effective than a 2-isopropyl substituent, suggesting possible radical ring-opening reactions contributing to toxicity. Nonfused 2-cyclopropylmitosenes were more effective than related fused cyclopropamitosenes reported previously. The reduction potentials of the quinone/semiquinone one-electron couples were in the range −286 to −380 mV. The semiquinone radicals reacted with oxygen with rate constants $2-8 \times 10^8$ dm^3 mol^{-1} s^{-1}. The involvement of the two-electron reduced hydroquinone in the mediation of cytotoxicity is implicated. The most effective compounds *in vitro* were the 2-cyclopropyl and 5-(2-methylaziridinyl) derivatives, and of these, 5-(aziridin-1-yl)-2-cyclopropyl-3-(hydroxymethyl)-1-methylindole-4,7-dione (**21**) and 3-(hydroxymethyl)-5-(2-methylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (**54**) were evaluated *in vivo*. Both compounds showed antitumor activity both as single agents and in combination with radiation, with some substantial improvements over EO9 (**3**) at maximum tolerated doses and as single agents against the RIF-1 tumor model and comparable efficacy in the KHT tumor model.

Introduction

Fused cyclopropamitosenes and closely related indoloquinones have recently been evaluated as novel bioreductive anticancer agents targeted toward both solid tumors with defined hypoxic fractions and tumor tissues that may be rich in the required activating enzymes.[1−3] The reduction of quinones bearing appropriate leaving groups to generate alkylating species, originally termed bioreductive alkylation,[4] is now well established, although the novel cyclopropamitosenes were originally designed as analogues of the prototype quinone bioreductive alkylating agent mitomycin *c* (MMC, **1**, Figure 1).[5] These compounds were expected to exhibit much reduced electophilicty at C-1 due to the inertness of the 1,2-cyclopropane compared to the aziridine in MMC. Certain indoloquinones (e.g. **2,** Figure 1) in this series were found to be highly potent cytotoxins compared with MMC and in some cases with substantially higher hypoxic cytotoxicty ratios (HCR).[1−3] The cyclopropamitosenes were found to be more rapidly reduced than MMC by DT-diaphorase (NAD(P)H: (quinone acceptor) oxidoreductase (EC 1.6.99.2)),[3] an important activator of mitosenes and an enzyme hyperexpressed in some tumors.[6−8] However, this could not explain fully the greater potency (aerobic and hypoxic)



1 **2** (R= N△, R_1=$CONH_2$) **3**

4 (R= OMe, R_1=H)

5 (R= N△, R_1=H)

Figure 1. Structures of known lead compounds.

of the compounds compared to the current lead clinical agent of this general type EO9 (**3,** Figure 1),[9] which is reduced 2 orders of magnitude more rapidly than **2** by DT-diaphorase.[3,10] The nature of the 5-substituent (in particular aziridines and substituted aziridines) and of the potential leaving group on the 3-methylene substituent was therefore identified as an important feature both in terms of oxic and hypoxic potency and ability to act as substrates for reductase enzymes. The reduction potential does not vary greatly among 5-aziridinyl and 5-methoxy derivatives.[2,11] However, the relative rates of reduction of this type of compound by one-electron reductases, which will be important under hypoxic conditions, is unknown. This may be related to the one-electron reduction potential of the quinone/semiquinone couple (E(Q/ Q$^{\bullet-}$)), but there are little published data on indoloquinones in aqueous solution. Such data would also be an indicator of the redox-cycling propensities of such compounds and thus be related to aerobic toxicity

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mediated through reactive oxygen species as well as a measure of ease of reduction by one-electron reducing enzymes such as NADPH:cytochrome P-450 reductase and xanthine oxidase.

Intramolecular ionic ring opening of the fused cyclopropane was considered unlikely,[3] but radical ring opening of the cyclopropane following one-electron reduction to give a reactive H atom abstractor has been suggested as a possible explanation for hypoxic potency, although there was no direct evidence for this mechanism.[3] We have therefore designed and synthesized the analogues of **2** in which the cyclopropane rings are not fused with the indoloquinone ring system and for which isopropyl analogues could be synthesized that are structurally very closely related but unable to undergo radical ring-opening reactions. These derivatives were also designed as closer analogues of EO9 (**3**), initially retaining a hydroxymethyl substituent at the 3-position together with an alkyl substituent at the 2-position, to give a series of compounds which have remained unevaluated to date. We have sought to obtain further structure−activity data for this series of bioreductively activated drugs as hypoxic cell cytotoxins with the aim of optimizing the hypoxic−cytotoxicity ratios and achieving activity *in vivo*, hitherto unseen with the fused cyclopropamitosenes such as **2**. The relationship of these biological effects to the redox properties of the drugs was also studied.

Synthetic Chemistry

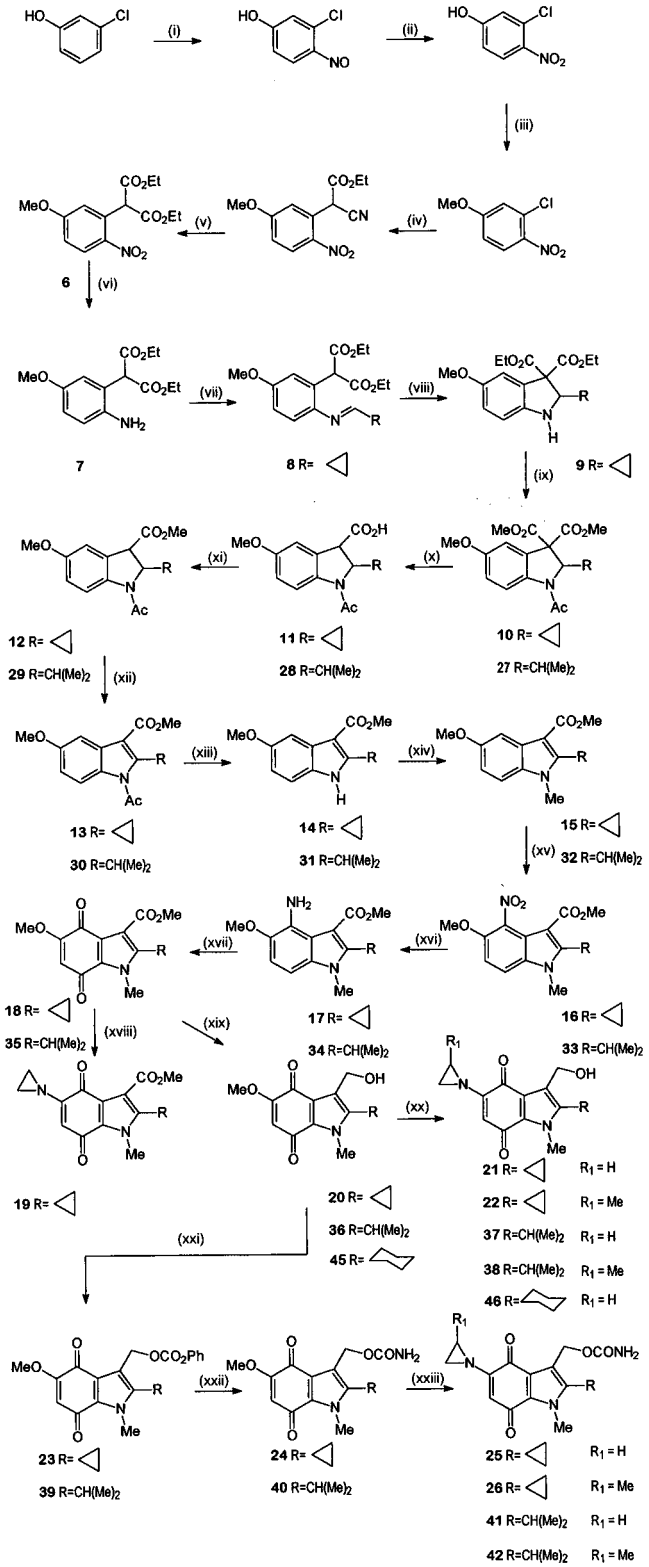
2-Alkyl- and 2-cycloalkyl-substituted indoles were synthesized in 14 steps from the common precursor 3-chlorophenol as shown in Scheme 1. The crucial step was the 1,5-electrocyclization of the imine (e.g. **8**) to the 2,3-dihydroindole derivatives, which was successfully carried out using isobutyraldehyde or cycloalkane carboxaldeydes, but not with acrolein in a proposed alternative route, using zinc acetate in methanol as has been employed in the synthesis of **3** *via* a 2-acrylate derivative.[9] Nitration at the desired 4-position could be achieved only subsequent to the *N*-methylation step in order to avoid increasing yields of the 6-nitro isomer. The subsequent six steps, including nitration, oxidations (DDQ and Fremy's salt), and reductions (Sn/HCl, $Na_2S_2O_4$, and DIBAL-H or $LiAlH_4$), left the 2-cyclopropyl moiety intact, and the desired indoloquinones were obtained. Substitution of the 5-methoxy substituent was successful in high-yielding reactions with aziridine and 2-methylaziridine.

Comparable 1,2-dimethyl analogues **52**−**56** were synthesized from commercially available 2-methyl-5-methoxyindole in seven steps (Scheme 2). Substitution of the 5-position with aziridine and 2-methylaziridine was again successful in high yield, as was the substitution with *cis*-2,3-dimethylaziridine. However, 2,2-dimethylaziridine reacted with difficulty, and the resulting 5-(2,2-dimethylaziridinyl) analogue was unstable in aqueous solution and on silica gel, ring opening *via* an S_N1 mechanism to give **57** (Scheme 2). The 2-unsubstituted analogues **62**−**66** were obtained in six steps from 5-methoxyindole-3-carboxaldehyde (Scheme 3).

Results and Discussion

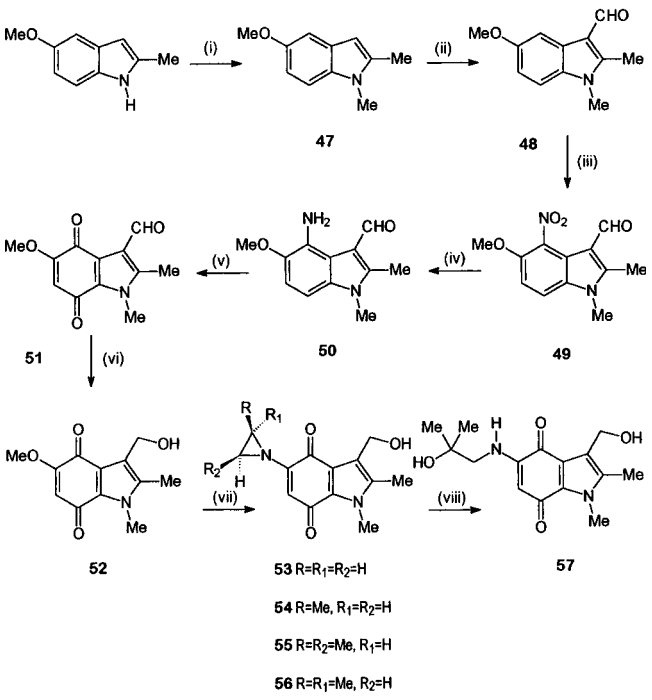
In previous studies the presence of a hydroxymethyl substituent at the 3-position of indoloquinone anticancer

Scheme 1. Synthesis of 2-Cycloalkyl- and 2-Alkylindoloquinones[a]



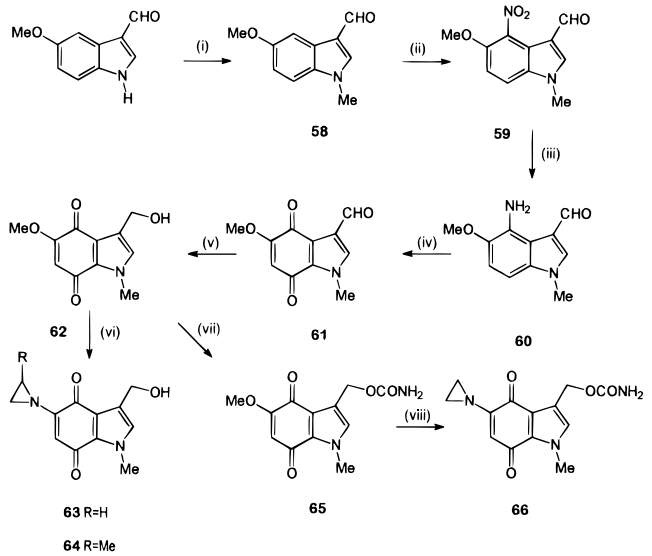
[a] Reagents: (i) $H_2SO_4/NaNO_2/H_2O/C_5H_5N$; (ii) $K_3Fe(CN)_6/KOH/H_2O$; (iii) NaH/THF/$(MeO)_2SO_2$; (iv) $NCCH_2CO_2Et$; (v) HCl/EtOH/H_2O; (vi) H_2/PtO_2/PhMe/EtOH; (vii) RCHO/MeOH; (viii) $Zn(OAc)_2$/MeOH; (ix) Ac_2O; (x) KOH/EtOH/H_2O/0 °C; (xi) $(MeO)_2SO_2$/DMF/K_2CO_3; (xii) DDQ/PhMe/reflux; (xiii) 4%KOH/MeOH; (xiv) NaH/DMF/MeI/60 °C; (xv) fuming HNO_3/AcOH/4 °C; (xvi) Sn/HCl/EtOH/H_2O; (xvii) Fremy's salt/$Me_2CO/NaH_2PO_4/Na_2HPO_4$/pH 6.0; (xviii) 1*H*-aziridine; (xix) $Na_2S_2O_4/H_2O$/EtOH/$CHCl_3$ then $LiAlH_4$/THF/30 °C (DIBAL-H/PhMe/−30 °C then 0 °C for **20**) then $FeCl_3$/HCl/H_2O/0 °C; (xx) R_1-$\overline{CHCH_2NH}$; (xxi) $PhCO_2Cl/C_5H_5N$/0 °C; (xxii) NH_3/CH_2Cl_2/−78 °C; (xxiii) R_1-$\overline{CHCH_2NH}$.

Scheme 2. Synthesis of 2-Methyl Analogues[a]



[a] Reagents: (i) NaH/DMF/MeI/60 °C; (ii) HCON(Me)Ph/$POCl_3$ then NaOAc/H_2O; (iii) fuming HNO_3/AcOH/4 °C; (iv) Sn/HCl/H_2O/EtOH; (v) Fremy's salt/Me_2CO/NaH_2PO_4/Na_2HPO_4/pH 6.0; (vi) $NaBH_4$/MeOH/Ar then air; (vii) R(R_1)CCH(R_2)NH; (viii) H_2O/45 °C.

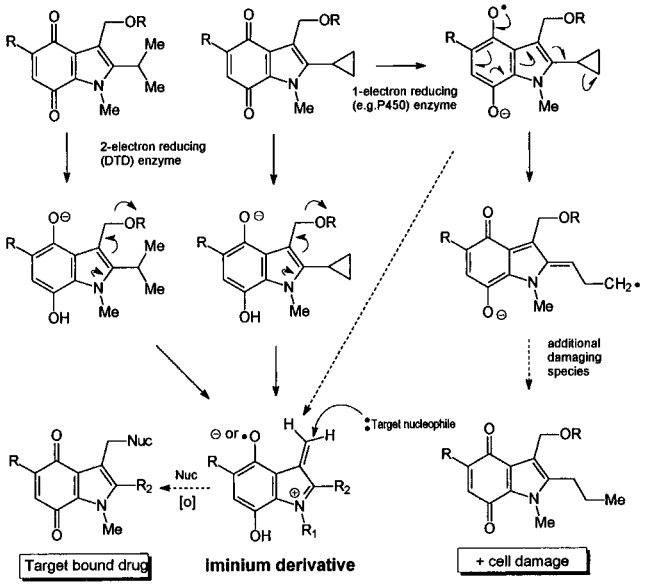
Scheme 3[a]



[a] Reagents: (i) NaH/DMF/MeI; (ii) concentrated HNO_3/AcOH/0 °C; (iii) Sn/HCl/H_2O/EtOH; (iv) Fremy's salt/Me_2CO/NaH_2PO_4/Na_2HPO_4/pH 6.0; (v) $NaBH_4$/MeOH/Ar; (vi) R-CHCH$_2$NH; (vii) $PhCO_2Cl/C_5H_5N$/0 °C; (viii) 1*H*-aziridine.

drugs has been thought to give inactive drugs, with the majority of compounds evaluated consequently possessing carbamate or acetate derived leaving groups.[12−14] The success of the mitosenediol EO9 (**3**) and earlier *in vivo* activity of related compounds, however,[7,9,14−16] suggests the importance of this class of compound, the structure of which has yet to be optimized. The *in vitro* biological data presented in this study (Table 1) add credence to this suggestion, indeed substitution with the carbamate moiety has in many cases actually reduced

Scheme 4. Potential Mechanisms of Toxicity for 2-Cyclopropyl- and 2-Alkyl-Substituted Indoloquinones

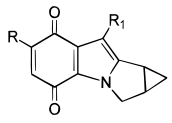


potency (see compounds **21** and **25**, **38** and **42**) while in other cases potency shows the expected increase (e.g. compounds **20** and **24**, **22** and **26**, **61** and **66**). Exceptional compounds are always 5-aziridinyl derivatives, suggesting that the dominance of aziridine-mediated toxicity may outweigh effects due to the nature of the potential leaving group at the 3-position in many cases. 5-Methoxy derivatives generally showed improved potency and HCR when carbamate replaced hydroxy on the 3-methylene substituent, indicating that the nature of this potential leaving group[1−3,12−14] can dominate the bioreductive properties of non-aziridinyl analogues. That the 3-hydroxymethyl substituent is required for hypoxia-selectivity is indicated when comparing the 5-aziridinyl-3-methyl derivative **44** (HCR = 1.77) with the corresponding 3-hydroxymethyl analogue **37** (HCR = 32.5).

There is a clear trend of increasing aerobic and hypoxic potency on reducing the steric bulk of substituent R_2 (see series of compounds **21**, **37**, **46**, and **53** for example). Thus, the least potent 5-aziridinyl analogue tested (both hypoxic and oxic) was the 2-cyclohexyl derivative **46** while the most potent compound tested (both hypoxic and oxic) was **66**, which has both aziridine and carbamate functionalities and no 2-alkyl substituent. The low HCR for this latter compound, however, demonstrates the clear need for these structure−activity studies to obtain both optimal potency and optimal hypoxia-selectivity.

Certain aziridinyl 2-cyclopropyl analogues do not fit into this pattern and also show much increased potency, particularly under hypoxic conditions, compared to corresponding isopropyl derivatives (e.g. compare **21** with **37** and **25** with **41**). Reactions of the semiquinone radical can dominate under severe hypoxia following one-electron reduction, and these data provide some indirect, though compelling, evidence for a contribution from semiquinone induced radical ring opening of the cyclopropane under such hypoxic conditions, possibly leading to further cytotoxic reactions. The full elucidation of the mechanism of such toxicity, hypothesized in Scheme 4, clearly requires further study.

Table 1. Structures of Compounds and *in Vitro* Biological Data Comparing Differential Aerobic/Hypoxic Cytotoxicities



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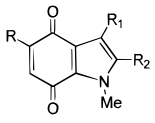


B

compd	type	R	R_1	R_2	C_{50} (air), μM	C_{50} (N_2), μM	HCR[a]
1	MMC				0.8[b]	0.4[b]	**2.0**[b]
2	A	Az[c]	CH_2OCONH_2		0.003[d]	0.003[d]	**1.0**[d]
3	EO9				0.19 ± 0.027	0.0038 ± 0.00057	**50.3**
4	A	MeO	CH_2OH		108.4 ± 5.9	60.6 ± 7.4	**1.8**
5	A	Az	CH_2OH		0.965 ± 0.11	0.073 ± 0.011	**13.2**
18	B	MeO	CO_2Me	c-Pr	103.5 ± 5.2	59.8 ± 11.4	**1.7**
20	B	MeO	CH_2OH	c-Pr	540 ± 52	820 ± 83	**0.65**
24	B	MeO	CH_2OCONH_2	c-Pr	29 ± 5.6	0.44 ± 0.023	**65.9**
19	B	Az	CO_2Me	c-Pr	1.72 ± 0.18	0.93 ± 0.067	**1.8**
21	B	Az	CH_2OH	c-Pr	0.603 ± 0.099	0.0058 ± 0.0013	**103.5**
25	B	Az	CH_2OCONH_2	c-Pr	3.33 ± 0.75	0.074 ± 0.015	**45.0**
22	B	2-Me-Az	CH_2OH	c-Pr	130 ± 9.8	5.6 ± 0.6	**23.4**
26	B	2-Me-Az	CH_2OCONH_2	c-Pr	12.5 ± 2.9	0.459 ± 0.096	**27.23**
35	B	MeO	CO_2Me	$CH(Me)_2$	150 ± 15	150 ± 15	**1.0**
36	B	MeO	CH_2OH	$CH(Me)_2$	800 ± 80	200 ± 20	**4.0**
37	B	Az	CH_2OH	$CH(Me)_2$	25.7 ± 3.0	0.79 ± 0.14	**32.5**
38	B	Az	CH_2OH	$CH(Me)_2$	127.8 ± 13.5	13.9 ± 1.5	**9.2**
40	B	MeO	CH_2OCONH_2	$CH(Me)_2$	20.9 ± 1.67	0.117 ± 0.018	**178.6**
41	B	Az	CH_2OCONH_2	$CH(Me)_2$	47.5 ± 14.6	5.46 ± 0.43	**8.7**
42	B	2-Me-Az	CH_2OCONH_2	$CH(Me)_2$	20.6 ± 2.5	0.87 ± 0.215	**23.7**
43	B	MeO	Me	$CH(Me)_2$	1.236 ± 0.189	0.657 ± 0.063	**1.88**
44	B	Az	Me	$CH(Me)_2$	0.333 ± 0.014	0.188 ± 0.011	**1.77**
45	B	MeO	CH_2OH	c-Hex	122.4 ± 17.5	20.5 ± 5.3	**6.0**
46	B	Az	CH_2OH	c-Hex	34.5 ± 6.0	0.93 ± 0.078	**37.0**
52	B	MeO	CH_2OH	Me	1077 ± 44	284.8 ± 37.6	**3.78**
53	B	Az	CH_2OH	Me	0.149 ± 0.011	0.0116 ± 0.0008	**12.8**
54	B	2-Me-Az	CH_2OH	Me	94 ± 7.6	0.5 ± 0.07	**188**
55	B	2,3-Me_2-Az	CH_2OH	Me	202 ± 11	14.2 ± 1.7	**14.2**
57	B	$Me_2C(OH)CH_2NH$-	CH_2OH	Me	1260 ± 126	500 ± 120	**2.5**
62	B	MeO	CH_2OH	H	220 ± 20	240 ± 23	**0.92**
63	B	Az	CH_2OH	H	0.153 ± 0.013	0.0086 ± 0.0009	**15.4**
64	B	2-Me-Az	CH_2OH	H	4.42 ± 1.17	0.0179 ± 0.02	**24.7**
65	B	MeO	CH_2OCONH_2	H	3.1 ± 0.24	0.037 ± 0.007	**83.8**
66	B	Az	CH_2OCONH_2	H	0.00019 ± 0.000032	0.00013 ± 0.000025	**1.46**

[a] HCR = hypoxic cytotoxicity ratio (C_{50} (air)/C_{50} (N_2)). [b] Reference 32. [c] Az = aziridin-1-yl. [d] Reference 2.

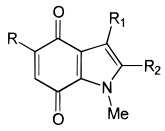
There is evidence in the present results for clear advantages in terms of both hypoxia-selectivity and hypoxic potency to compounds with a 2-cyclopropyl substituent rather than a 1,2-fused cyclopropane system (compare compounds **5** and **21**). This effect seems to be lessened when more potent leaving groups are present such as in carbamate **24**, which is comparable in its potencies to its fused analogue[2] (compare **2** with **25**).

An increase in HCR upon alkyl substitution of the aziridinyl moiety has been demonstrated in this study with some (compare **41** and **42**, **53** and **54**, **63** and **64**) but not all types of compound (compare **21** and **22**, **25** and **26**). Significantly, the compounds which do not fall into this pattern are again the 2-cyclopropane derivatives, where toxicity is not dominated by the reactivity of the aziridine moiety to the same degree as other 2-alkyl derivatives, possibly due to a contribution from the cyclopropane ring (e.g. Scheme 4). Generally, a single methyl substituent on the 5-aziridine ring is optimal. With multiple substitution, the potency and HCR is much reduced due to progressive deactivation of the aziridine counteracting increasing pK_a.

Redox chemical studies (Table 2) demonstrated that analogues containing an aziridine group exhibited a similar reactivity toward oxygen as EO9 (**3**)[17] (k_2 (see Experimental Redox Chemistry Section, eq 2) was generally found to be in the order of 2×10^8 M^{-1} s^{-1}). However EO7[9] (the 5-methoxy analogue of EO9 (**3**)) and other 5-methoxy derivatives selected from this study **20**, **24**, **40**, and **62** were less electron-affinic than corresponding 5-aziridinyl compounds and reacted typically 2−4 times faster with oxygen. Thus 5-aziridinyl semiquinone radicals will be longer-lived than 5-methoxy analogues in the presence of oxygen, which, in addition to reductive aziridine activation, may also contribute to their greater effectiveness compared to 5-methoxyindoloquinones following one-electron reduction. This will also be relevant following two-electron reduction since it will slow down the rate of reoxidation of the hydroquinone in sequential one-electron steps, and influence the lifetime of semiquinones formed through a comproportionation reaction between an indoloquinone and its hydroquinone.

The one-electron reduction potentials at pH 8.5 for the aziridinylindoloquinones **53** (−294 mV) and **21** (−286 mV) were similar to that of EO9 (**3**), where E (Q/$Q^{\bullet-}$) = −265 mV (lit.[17] −253 mV). The redox potentials of 5-methoxyindoloquinones were generally 25−50 mV lower than the corresponding 5-aziridinyl analogues. This is consistent with recent half-wave potential reduction data on related indoloquinones[2] (e.g. compound **2** E_{redox} = −1.360 V and its 5-MeO analogue E_{redox} = −1.395 V). These differences in reduction potential were reflected in the corresponding rates of reaction of semiquinone radicals with oxygen (see Table 2). How-

Table 2. One-Electron Reduction Potentials at pH 8.5 (E $Q/Q^{\bullet-}$) for Representative Compounds and Rate Constants for Reaction of Semiquinone Radicals with Oxygen



compd	R	R_1	R_2	K_3[a]	E ($Q/Q^{\bullet-}$), mV	$10^8 k_2(Q^{\bullet-} + O_2)$, $dm^3\ mol^{-1}\ s^{-1}$
EO9(**3**)	Az[b]	CH_2OH	$CH{=}CHCH_2OH$	39.6 ± 5.7	-265 ± 5[c]	1.7 ± 0.1
21	Az	CH_2OH	c-Pr	41.2 ± 1.4	-286 ± 4[c]	2.3 ± 0.1
53	Az	CH_2OH	Me	30.1 ± 1.5	-294 ± 4[c]	2.8 ± 0.1
54	2-Me-Az	CH_2OH	Me	19.7 ± 1.7	-305 ± 4[c]	2.4 ± 0.1
EO7	MeO	CH_2OH	$CH{=}CHCH_2OH$	15.5 ± 1.5	-309 ± 5[c]	4.5 ± 0.1
62	MeO	CH_2OH	H	6.8 ± 0.4	-332 ± 4[d]	4.4 ± 0.1
20	MeO	CH_2OH	c-Pr	6.2 ± 0.2	-334 ± 4[c]	5.1 ± 0.2
40	MeO	CH_2OCONH_2	$CH(CH_3)_2$	20.3 ± 0.7	-380 ± 4[d]	6.3 ± 0.1
24	MeO	CH_2OCONH_2	c-Pr	19.8 ± 1.7	-377 ± 9[d]	8.2 ± 0.2

[a] Means of measurements using five different concentrations of indoloquinones. [b] Az = aziridin-1-yl. [c] Potentials *vs* $E(BV^{2+}/BV^{\bullet-}) = -374$ mV at pH 8.5. [d] Potentials *vs* $E(MV^{2+}/MV^{\bullet-}) = -450$ mV at pH 8.5 (will be the same at pH 7.4).

ever, 5-methoxy compounds bearing a good 3-methylene leaving group also have exquisite hypoxia-selectivity (e.g. **40** and **65**); therefore there appears to be little relationship between reduction potential and HCR as determined by the MTT assay in this study, particularly in view of the low reduction potentials determined for the hypoxia-selective carbamates **24** and **40** compared with EO9 (**3**). However, it should be noted that under conditions of greater oxygen concentration, reduction potential is likely to have a greater influence on reduced drug reactivity. The trends between k_2 and E ($Q/Q^{\bullet-}$) for the indoloquinones in this study are comparable with those published for quinones of similar redox potential.[18] Thus although the indoloquinones should be easily reduced by enzymes such as NADPH-cytochrome P-450 and DT-diaphorase, the high values measured for k_2 would indicate that even under modest tumor hypoxia (*ca.* 10 μmol dm^{-3} O_2) the half-life of the semiquinone radicals $\{\sim 0.7/(k_3[O_2]\}$ will be short (~0.4 ms).

Lead compounds **21** and **54** with HCR values in excess of 100 (2−3-fold higher than was obtained for the lead clinical candidate EO9 (**3**)) were evaluated *in vivo* in the RIF-1 and KHT rodent tumor models. Both compounds exhibited substantial cell killing both as single agents, with substantially greater effectiveness than EO9 (**3**) in both tumor models at drug MTDs (an MTD dose of **21** alone was as effective as a radiation dose of 15 Gy alone in the KHT tumor) and in particular after a single dose (up to 15 Gy) of radiation (up to 3.5 log cell kills against the RIF-1 tumor, Table 3) at drug doses well below MTDs (MTD **21** = 100 mg kg^{-1} and MTD **54** = 90 mg kg^{-1}). A lesser effect in the RIF-1 tumor with EO9 (**3**) could only be achieved at its MTD. These compounds are therefore now being evaluated against human tumor xenografts. A full study of the *in vivo* biological effects of these compounds will be reported elsewhere.

Since despite the oxygen reactivity of the semiquinone radicals the lead indoloquinone compounds **21** and **54** showed antitumor effects *in vivo* against hypoxic cell populations, and these effects compare favorably with EO9 (**3**) (Table 3), little relationship between bioreductive cytotoxicity and the oxygen reactivity of one-electron-reduced radicals is evident. This provides some evidence that the antitumor bioreductive effect is likely to be mediated principally through the hydroquinone,

Table 3. Comparison of Indoloquinones **21** and **54** with EO9 (**3**) When Used in Combination with X-rays (20 and 50 mg kg^{-1}) and Alone (MTD) in the RIF-1 and KHT Tumors

tumor	X-ray dose, Gy	drug	drug dose, mg kg^{-1}	MTD, mg kg^{-1}	mean relative surviving fraction
control					1.0
RIF-1	15				2.0×10^{-3}
RIF-1		**21**	100	100	5.0×10^{-3}
RIF-1	15	**21**	20	100	2.4×10^{-5}
RIF-1	15	**21**	50	100	$<10^{-5}$
RIF-1		**54**	90	90	2.0×10^{-2}
RIF-1	15	**54**	20	90	$<10^{-5}$
RIF-1	15	**54**	50	90	$<10^{-5}$
RIF-1		EO9 (**3**)	15	15	0.7
RIF-1	15	EO9 (**3**)	5	15	1.2×10^{-3}
RIF-1	15	EO9 (**3**)	10	15	1.6×10^{-3}
RIF-1	15	EO9 (**3**)	15	15	1.0×10^{-4}
KHT	10				9×10^{-3}
KHT		**21**	100	100	6.0×10^{-2}
KHT	10	**21**	20	100	8.0×10^{-3}
KHT	10	**21**	50	100	7.0×10^{-4}
KHT		**54**	90	90	1.5×10^{-1}
KHT	10	**54**	20	90	8.0×10^{-3}
KHT	10	**54**	50	90	5.0×10^{-3}
KHT		EO9 (**3**)	15	15	0.6[a]
KHT	10	EO9 (**3**)	5	15	1.0×10^{-2} [a]
KHT	10	EO9 (**3**)	10	15	3.0×10^{-3} [a]
KHT	10	EO9 (**3**)	15	15	1.0×10^{-4} [a]

[a] Data taken from ref 33.

generated by a two-electron reducing enzyme (such as DT-diaphorase) and/or by sequential one-electron reductions by other enzymes and/or disproportionation reactions. In this case reductive cytotoxicity will be less oxygen dependent since hydroquinones oxidize in air much less rapidly than do semiquinone radicals, and therefore hypoxia-selectivity is still evident against these experimental tumors even when the oxygen concentration is sufficiently high to render the lifetime of the semiquinone extremely short. The likely involvement of the hydroquinone species has also been suggested following the study of reduction of some mitosenes in aqueous and nonaqueous environments by electron spin resonance and cyclic voltammetry.[11] However, a recent study indicates that an *o*-aziridinyl group may actually be deactivated as an electrophilic center following reduction to a hydroquinone through a competing 1,5-sigmatropic rearrangement.[19] Further, other studies have previously indicated that hydroquinone-generating enzyme DT-diaphorase may protect cells

against damage by this type of agent under hypoxic conditions, and this has been demonstrated for EO9 (**3**),[20] again suggesting the probable importance of other, possibly radical-mediated mechanisms of cytotoxicity under such conditions.

Experimental Section

NMR spectra were obtained at 90 MHz with a JEOL FX90Q spectrometer using $SiMe_4$ as internal standard. Elemental analyses were determined by Butterworth Laboratories Ltd., Teddington, Middlesex, U.K. Solutions in organic solvents were dried by treatment with $MgSO_4$ or Na_2SO_4 and filtration. Dichloromethane (CH_2Cl_2) was dried over calcium chloride, and chloroform ($CHCl_3$) was passed through neutral alumina prior to use. Dimethylformamide (DMF), toluene, and tetrahydrofuran (THF) were anhydrous commercial grades. Silica gel for flash column chromatography was Merck grade (230−400 mesh). Melting points were determined on a Thomas-Hoover melting point apparatus and on a Thermogallen microscope and hot stage apparatus and are uncorrected. Stereochemically pure *cis*-2,3-dimethylaziridine and 2,2-dimethylaziridine were synthesized from the appropriately substituted 2-aminoethanols by *O*-sulfation and elimination with KOH.[21] Fused cyclopropamitosenes **4** and **5** were synthesized as described previously.[1,2,22] EO9 (**3**) and its 5-methoxy analogue EO7 were synthesized as described previously.[9] 5-Methoxy-2-methylindole, 5-methoxyindole-3-carboxaldehde, methyl viologen, and benzyl viologen were purchased from Sigma-Aldrich, U.K.

Diethyl (5-Methoxy-2-nitrophenyl)malonate (6). A solution of 17 g (0.064 mol) of ethyl (5-methoxy-2-nitrophenyl)-cyanoacetate, prepared in four steps from 3-chlorophenol as previously described,[9] in EtOH (100 mL), was saturated with HCl gas on cooling in an ice/salt bath. The solution was stirred for 2 days, ice/water (100 mL) added, and the solution stirred for a further 24 h at 4 °C before the crystalline solid formed was collected by filtration, dried, and recrystallized from EtOH to give 17 g (85%) of **6** as a white solid: mp 92−93 °C (lit.[9] mp 92−93 °C).

2-Cyclopropyl-3,3-bis(ethoxycarbonyl)-2,3-dihydro-5-methoxyindole (9). Compound **6** (5.0 g, 16.1 mmol) was dissolved in a mixture of toluene (62.5 mL) and EtOH (3.75 mL) and reduced with H_2 at atmospheric pressure over PtO_2 (75 mg) catalyst. After 7 h at room temperature the solution was filtered through Celite and evaporated to dryness below 30 °C, and the resulting pale green oil (**7**) dissolved immediately in MeOH (75 mL). To this methanolic solution was added cyclopropanecarboxaldehyde (1.3 g, 18.6 mmol) dissolved in MeOH (10 mL) and the solution stirred for 15 min. The resulting solution of imine **8** was treated *in situ* with $Zn(OAc)_2{\cdot}2H_2O$ (1.13 g, 5.15 mmol) and stirred for 18 h at room temperature. The solution was evaporated to dryness at 30 °C, HCl (2.0 M, 50 mL) was added, and the mixture was extracted with CH_2Cl_2 (2 × 150 mL), washed with saturated $NaHCO_3$ (aqueous, 150 mL) and saturated NaCl (aqueous, 100 mL), dried, and evaporated. The residue was purified on silica, eluting with hexane/EtOAc (1:1, R_f = 0.75) to give **9** (3.3 g, 68%) as a yellow oil: ^{1}H-NMR ($CDCl_3$) δ 0.48−0.52 (m, 4 H, 2 × cyclopropyl-C*H*$_2$), 1.03−1.08 (m, 1 H, cyclopropyl-*H*), 1.2 (t, 6 H, J = 7.2 Hz, CH_2CH_3), 1.3 (t, 6 H, J = 7.2 Hz, CH_2CH_3), 3.75 (s, 3 H, C*H*$_3$O-), 3.85 (d, 1 H, J = 2.7 Hz, 2-*H*), 4.1−4.35 (m, 4 H, C*H*$_2$CH$_3$), 6.63 (s, 1 H, Ar-4*H*), 6.71 (d, 1 H, J = 1.5 Hz, Ar-6*H*), and 7.03 (d, 1 H, J = 1.5 Hz, Ar-7*H*).

1-Acetyl-2-cyclopropyl-3,3-bis(ethoxycarbonyl)-2,3-dihydro-5-methoxyindole (10). Compound **9** (3.0 g, 10 mmol) was dissolved in Ac_2O (5 mL) and stirred for 3 h at room temperature. The anhydride was then evaporated *in vacuo* and the residue purified on silica, eluting with hexane/EtOAc (2:1, R_f = 0.4) to give a pale yellow oil which was redissolved in Et_2O and evaporated. Trituration of the resulting oil with Et_2O gave a white solid which was collected by filtration and washed with cold Et_2O to give **10** (3.0 g, 87%) as a white solid: mp 104−105 °C; ^{1}H-NMR ($CDCl_3$) δ 0.5−0.57 (m, 4 H, 2 × cyclopropyl-C*H*$_2$)), 1.0−1.15 (m, 1 H, cyclopropyl-*H*), 1.2 (t, 3 H, J = 7.2 Hz, CH_2CH_3), 1.3 (t, 3 H, J = 7.2 Hz, CH_2CH_3), 3.81 (s, 3 H, C*H*$_3$O), 4.05−4.3 (m, 4 H, C*H*$_2$CH$_3$), 4.5 (d, 1 H, J = 2.7 Hz, 2-*H*), and 6.9−7.2 (m, 3 H, Ar-4,6,7*H*).

1-Acetyl-3-carboxy-2-cyclopropyl-2,3-dihydro-5-methoxyindole (11). The acetylindole **10** (1.0 g, 2.9 mmol) was dissolved in EtOH (10 mL) and cooled to 0 °C in an ice/salt bath, and a cold (0 °C) solution of KOH (aqueous, 10%, 10 mL) was added. The solution was stirred at −5 °C for 4 h and then for 18 h at 4 °C. The solution was poured onto ice/water (25 mL) and washed with Et_2O. The aqueous layer was then acidified with 2.0 M HCl, extracted with $CHCl_3$ (6 × 50 mL), dried, and evaporated to give 0.68 g (97%) of **11** as a yellow oil (R_f = 0.45, Me_2CO), which was used in the next step without further purification: ^{1}H-NMR ($CDCl_3$) δ 0.5−0.7 (m, 4 H, 2 × cyclopropyl-C*H*$_2$), 1.25−1.4 (m, 1 H, cyclopropyl-*H*), 2.33 (s, 3 H, COC*H*$_3$), 3.78 (s, 3 H, C*H*$_3$O), 3.85 (br, 1 H, 3-*H*), 4.5 (br s, 1 H, 2-*H*), 6.9−7.0 (m, 2 H, Ar-4,6*H*), 8.05−8.15 (m, 1 H, Ar-7*H*), and 10.81 (s, 1 H, CO_2H).

Methyl 1-Acetyl-2-cyclopropyl-2,3-dihydro-5-methoxyindole-3-carboxylate (12). To a solution of **11** (0.68 g, 2.8 mmol) in DMF (10 mL) was added K_2CO_3 (0.83 g, 6 mmol) and $(MeO)_2SO_2$ (2 g, 15.8 mmol) and the solution stirred at room temperature for 4 h. The solution was then poured onto HCl (2.0 M, 20 mL), extracted with $CHCl_3$ (4 × 25 mL), washed with saturated NaCl, dried, and evaporated. The residue was purified on silica, eluting with EtOAC/hexane (1:2, R_f = 0.7 (EtOAc)) to give a pale yellow oil of **12** (0.7 g, 98%), which solidified on standing: ^{1}H-NMR ($CDCl_3$) δ 0.58−0.64 (m, 4 H, 2 × cyclopropyl-C*H*$_2$), 1.25−1.33 (m, 1 H, cyclopropyl-*H*), 2.33 (s, 3 H, COC*H*$_3$), 3.68 (s, 3 H, CO_2CH_3), 3.79 (s, 3 H, C*H*$_3$O), 3.85 (br, 1 H, 3-*H*), 4.54 (br, 1 H, 2-*H*), 6.78−6.95 (m, 2 H, Ar-4,6*H*), and 8.2 (br s, 1 H, Ar-7*H*).

Methyl 1-Acetyl-2-cyclopropyl-5-methoxyindole-3-carboxylate (13). A solution of **12** (1.0 g, 4 mmol) was stirred under reflux with DDQ (0.96 g, 4.2 mmol) in toluene (12.5 mL) for 7 h. The $DDQH_2$ was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified on silica, eluting with hexane/EtOAc (1:1, R_f = 0.7) to give **13** (0.88 g, 87%) as a pale yellow oil: ^{1}H-NMR ($CDCl_3$) δ 0.73−0.79 (m, 2 H, cyclopropyl-C*H*$_2$), 1.22−1.3 (m, 2 H, cyclopropyl-C*H*$_2$), 2.15−2.25 (m, 1 H, cyclopropyl-*H*), 2.83 (s, 3 H, COC*H*$_3$), 3.86 (s, 3 H, CO_2CH_3), 3.97 (s, 3 H, C*H*$_3$O), 6.99 (dd, 1 H, J = 2.7 and 9 Hz, Ar-6*H*), 7.5 (d, 1 H, J = 2.7 Hz, Ar-4*H*), and 7.96 (d, 1 H, J = 9 Hz, Ar-7*H*).

Methyl 2-Cyclopropyl-5-methoxyindole-3-carboxylate (14). A solution of **13** (0.88 g, 3.46 mmol) in KOH (4% in MeOH, 50 mL) was stirred at room temperature for 1.5 h, neutralized with 6.0 M HCl, and extracted with EtOAc (3 × 25 mL). The organic layer was washed with H_2O (50 mL), dried, and evaporated to give **14** (0.51 g, 70%) as a white solid: mp 128−131 °C; ^{1}H-NMR ($CDCl_3$) δ 0.82−0.92 (m, 2 H, cyclopropyl-C*H*$_2$), 1.05−1.2 (m, 2 H, cyclopropyl-C*H*$_2$), 2.2−3.0 (m, 1 H, cyclopropyl-*H*), 3.85 (s, 3 H, CO_2CH_3), 3.95 (s, 3 H, C*H*$_3$O), 6.79 (dd, 1 H, J = 2.7 and 9 Hz, Ar-6*H*), 7.22 (d, 1 H, J = 6.3 Hz, Ar-7*H*), 7.58 (d, 1H, J = 2.7 Hz, Ar-4*H*), and 8.2 (br s, 1 H, N*H*).

Methyl 2-Cyclopropyl-5-methoxy-1-methylindole-3-carboxylate (15). Compound **14** (9.0 g, 42.6 mmol) was added under argon to a stirred suspension of NaH (6.0 g, 0.13 mol) in DMF (150 mL). The solution was heated at 45 °C for 0.5 h and cooled to 0−10 °C and MeI (33 mL, 0.23 mol) added. The solution was then gradually heated to 60 °C, stirred at this temperature for 1 h, cooled, poured onto cold (0 °C) $NaHSO_4$ (aqueous, 10%, 500 mL), and extracted with EtOAc (5 × 75 mL). The organic extracts were washed with saturated NaCl (150 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:2, R_f = 0.45) to give **15** (8.3 g, 86%) as a pale yellow waxy solid: ^{1}H-NMR ($CDCl_3$) δ 0.77−0.84 (m, 2 H, cyclopropyl-C*H*$_2$), 1.21−1.29 (m, 2 H, cyclopropyl-C*H*$_2$), 1.85−2.05 (m, 1 H, cyclopropyl-*H*), 3.8 (s, 3 H, C*H*$_3$N), 3.86 (s, 3 H, CO_2CH_3), 3.9 (s, 3 H, C*H*$_3$O), 6.86 (dd, 1 H, J = 2.7 and 9 Hz, Ar-6*H*), 7.23 (d, 1 H, J = 9 Hz, Ar-7*H*), and 7.62 (d, 1 H, J = 2.7 Hz, Ar-4*H*).

Methyl 1-Methyl-2-cyclopropyl-5-methoxy-4-nitroindole-3-carboxylate (16). To a solution of **15** (8.0 g, 34.66 mmol) in AcOH (150 mL) cooled to 0 °C was added dropwise a cold (0 °C) mixture of fuming HNO_3 (27 mL) in AcOH (100

mL). The solution was stirred for 3 h while warming to room temperature, poured onto 300 g of crushed ice, and after 15 min the resulting yellow solid was collected by suction filtration. The dried residue was purified on silica, eluting with EtOAc/hexane (1:1, $R_f = 0.45$) to give 7.5 g (71%) of **16** as a yellow solid, recrystallized from EtOAc: mp 129−131 °C; ^{1}H-NMR ($CDCl_3$) δ 0.74−0.81 (m, 2 H, cyclopropyl-CH_2), 1.22−1.3 (m, 2 H, cyclopropyl-CH_2), 1.85−2.05 (m, 1 H, cyclopropyl-*H*), 3.8 (s, 3 H, CH_3N-), 3.81 (s, 3 H, CO_2CH_3), 3.89 (s, 3 H, CH_3O), 6.94 (d, 1 H, $J = 9$ Hz, Ar-7*H*), and 7.32 (d, 1 H, $J = 9$ Hz, Ar-6*H*).

Methyl 4-Amino-2-cyclopropyl-5-methoxy-1-methylindole-3-carboxylate (17). To a suspension of 2.5 g (9.25 mmol) of **16** in EtOH (180 mL) were added tin powder (5.25 g, 44.2 mmol) and HCl (3.0 M, 70 mL), and the solution was stirred at room temperature for 1 h. The solution was then decanted from the excess tin and neutralized with saturated $NaHCO_3$ (aqueous), the resulting red suspension was added to an equal volume of H_2O and extracted with $CHCl_3$ (5 × 50 mL) and then EtOAc (5 × 50 mL), and the combined extracts were evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:1, $R_f = 0.5$) to give **17** (2.2 g, 87%) as a white solid which was used immediately in the next step: mp 49−50 °C; ^{1}H-NMR ($CDCl_3$) δ 0.65−0.72 (m, 2 H, cyclopropyl-CH_2), 1.22−1.3 (m, 2 H, cyclopropyl-CH_2), 1.8−1.9 (m, 1 H, cyclopropyl-*H*), 3.73 (s, 3 H, CH_3N), 3.85 (s, 3 H, CO_2CH_3), 3.9 (s, 3 H, CH_3O-), 5.6 (br s, 1 H, NH_2), 6.5(d, 1 H, $J = 9$ Hz, Ar-7*H*), and 6.9 (d, 1 H, $J = 9$ Hz, Ar-6*H*).

2-Cyclopropyl-3-(methoxycarbonyl)-5-methoxy-1-methylindole-4,7-dione (18). To a solution of **17** (2.0 g, 7.2 mmol) in Me_2CO (250 mL) was added a solution of potassium nitrosodisulfonate ($(KSO_3)_2NO$, Fremy's salt, 9.7 g, 36.2 mmol)) in NaH_2PO_4/Na_2HPO_4 buffer (250 mL, 0.3 M, pH 6.0) and the solution stirred at room temperature for 1 h. The Me_2CO was removed *in vacuo* and the resulting orange precipitate collected by suction filtration, washed with H_2O, and dried in a vacuum oven at 45 °C to afford **18** as an orange solid which was recrystallized from EtOAc: mp 169−170 °C; ^{1}H-NMR ($CDCl_3$) δ 0.69−0.77 (m, 2 H, cyclopropyl-CH_2), 1.1−1.2 (m, 2 H, cyclopropyl-CH_2), 1.48−1.58 (m, 1 H, cyclopropyl-*H*), 3.80 (s, 3 H, CH_3N), 3.90 (s, 3 H, CO_2CH_3), 4.01 (s, 3 H, CH_3O), and 5.64 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{15}NO_5$) C, H, N.

5-(Aziridin-1-yl)-2-cyclopropyl-3-(methoxycarbonyl)-1-methy1indole-4,7-dione (19). Compound **18** (0.29 g, 1 mmol) was dissolved and stirred in freshly redistilled 1*H*-aziridine (3 mL, ca.70 mmol, **CAUTION!**) for 1 h and evaporated *in vacuo* and the residue redissolved in EtOAc. The solvent was then partially evaporated until a red precipitate appeared. The red solid was then collected by suction filtration and recrystallized from EtOAc to afford **19** (0.25 g, 83%) as a red solid: mp 138−140 °C; ^{1}H-NMR ($CDCl_3$) δ 0.65−0.72 (m, 2 H, cyclopropyl-CH_2), 1.2−1.35 (m, 2 H, cyclopropyl-CH_2), 1.7−1.8 (m, 1 H, cyclopropyl-*H*), 2.18 (s, 4 H, 2 × azir-CH_2), 3.92 (s, 3 H, CH_3N), 3.99 (s, 3 H, CO_2CH_3), and 5.77 (s, 1 H, 6-*H*). Anal. ($C_{16}H_{16}N_2O_4$) C, H, N.

2-Cyclopropyl-3-(hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione (20). To a solution of **18** (0.3 g, 1.03 mmol) in $CHCl_3$ (30 mL) and EtOH (11 mL) was added a solution of $Na_2S_2O_4$ (2.1 g, 12 mmol) in H_2O (13 mL). The solution was stirred at room temperature for 0.5 h and the organic layer separated, washed with saturated NaCl (50 mL), dried, and evaporated. The crude hydroquinone was then dissolved in anhydrous CH_2Cl_2 (30 mL) under argon and cooled to −30 °C, and DIBAL-H (5 mL of a 1.5 M solution in toluene) was added dropwise such that the solution temperature remained below −30 °C. The solution was then allowed to reach 0 °C and stirred for 2.5 h at this temperature, and a solution of $FeCl_3$ (9 mL, 1.0 M (0.1 M HCl)) was added. The solution was stirred for 10 min at 0 °C, and then $CHCl_3$ (150 mL) and H_2O (150 mL) were added. The aqueous layer was extracted with $CHCl_3$ (5 × 50 mL) and then EtOAc (5 × 50 mL), and the combined organic phases were washed with saturated NaCl (250 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc ($R_f = 0.5$) to give **20** as an orange solid after recrystallization from EtOAc (125 mg, 47%): mp 200−202 °C; ^{1}H-NMR ($CDCl_3$) δ 0.71−0.82 (m, 2 H, cyclopropyl-CH_2), 1.2−1.33 (m, 2 H, cyclopropyl-CH_2), 1.61−1.71 (m, 1 H, cyclopropyl-*H*), 3.81 (s, 3 H, CH_3N), 3.98 (s, 3 H, CH_3O), 4.0 (br s, 1 H, CH_2OH), 4.69 (br d, 2 H, CH_2OH), and 5.64 (s, 1 H, 6-*H*). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

5-(Aziridin-1-yl)-2-cyclopropyl-3-(hydroxymethyl)-1-methylindole-4,7-dione (21). Compound **20** (0.1 g, 0.38 mmol) was dissolved, stirred in freshly distilled 1*H*-aziridine (3 mL, ca.70 mmol, **CAUTION!**) for 0.75 h, and evaporated *in vacuo*, and the residue was redissolved in EtOAc and evaporated until a red precipitate appeared and the solid collected. The red solid was recrystallized from EtOAc to give **21** (80 mg, 77%): mp 177−179 °C; ^{1}H-NMR ($CDCl_3$) δ 0.7−0.8 (m, 2 H, cyclopropyl-CH_2), 1.22−1.3 (m, 2 H, cyclopropyl-CH_2), 1.6−1.7 (m, 1 H, cyclopropyl-*H*), 2.18 (s, 4 H, 2 × azir-CH_2), 3.97 (s, 3 H, CH_3N), 4.73 (s, 2 H, CH_2OH), and 5.77 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{16}N_2O_3$) C, H, N.

2-Cyclopropyl-3-(hydroxymethyl)-5-(2-methylaziridin-1-yl)-1-methylindole-4,7-dione (22). Compound **20** (0.1 g, 0.38 mmol) was dissolved and stirred in freshly distilled 2-methylaziridine (3 mL, ca.50 mmol) for 2.5 h. The solution was evaporated *in vacuo* and the residue redissolved in EtOAc, evaporated until a red precipitate appeared and the solid collected. The red solid was recrystallized from EtOAc to give **22** (85 mg, 78%): mp 130−131 °C; ^{1}H-NMR ($CDCl_3$) δ 0.68−0.74 (m, 2 H, cyclopropyl-CH_2), 1.08−1.2 (m, 2 H, cyclopropyl-CH_2), 1.42 (d, 3 H, $J = 4.5$ Hz, azir-CH_3), 1.5−1.6 (m, 1 H, cyclopropyl-*H*), 2.01−2.15 (m, 3 H, azir-C*H*CH_2), 3.97 (s, 3 H, CH_3N), 4.73 (s, 2 H, CH_2OH), and 5.74 (s, 1 H, 6-*H*). Anal. ($C_{16}H_{18}N_2O_3$) C, H, N.

2-Cyclopropyl-5-methoxy-1-methyl-3-[[(phenoxycarbonyl)oxy]methyl]indole-4,7-dione (23). To a solution of **20** (0.1 g, 0.38 mmol) in anhydrous pyridine (6 mL) at 0 °C was added dropwise phenyl chloroformate (0.1 g, 0.64 mmol) and the solution then allowed to reach room temperature and stirred for 2 h. The solution was then extracted with CH_2Cl_2 (25 mL), washed with H_2O (25 mL) and saturated NaCl (25 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc ($R_f = 0.75$) to give **23** as an orange solid which was used without further purification: mp 136−139 °C; ^{1}H-NMR ($CDCl_3$) δ 1.02−1.18 (m, 2 H, cyclopropyl-CH_2), 1.21−1.28 (m, 2 H, cyclopropyl-CH_2), 1.78−1.88 (m, 1 H, cyclopropyl-*H*), 3.79 (s, 3 H, CH_3N), 4.01 (s, 3 H, CH_3O), 5.27 (s, 2 H, CH_2OCOPh), 5.51 (s, 1 H, 6-*H*), and 7.15−7.3 (m, 5 H, Ar).

3-[(Carbamoyloxy)methyl]-2-cyclopropyl-5-methoxy-1-methylindole-4,7-dione (24). The phenyl carbonate **23** (0.3 g, 0.78 mmol) was dissolved in anhydrous CH_2Cl_2 (38 mL) and the solution cooled to −78 °C. The solution was then saturated with NH_3 and stirred at −78 °C until reaction was complete (ca. 2 h). The solution was then allowed to reach room temperature and evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2 (100 mL), washed with H_2O (2 × 100 mL) and saturated NaCl (50 mL), dried, and evaporated, and the residue was recrystallized from EtOAc to afford 220 mg (92%) of **24** as an orange solid: mp 240−242 °C dec; ^{1}H-NMR ($CDCl_3$) δ 1.02−1.13 (m, 2 H, cyclopropyl-CH_2), 1.22−1.26 (m, 2 H, cyclopropyl-CH_2), 1.51−1.55 (m, 1 H, cyclopropyl-*H*), 3.78 (s, 3 H, CH_3N), 3.99 (s, 3 H, CH_3O), 4.79 (br s, 2 H, NH_2), 5.3 (s, 2 H, CH_2OCONH$_2$), and 5.62 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{16}N_2O_5$) C, H, N.

5-(Aziridin-1-yl)-3-[(carbamoyloxy)methyl]-2-cyclopropyl-1-methylindole-4,7-dione (25). The carbamate **24** (0.3 g, 1.0 mmol) was stirred at room temperature in 1*H*-aziridine (2 mL, **CAUTION!**) for 15 min, evaporated, and redissolved in EtOAc (5 mL). The solution was then evaporated to 50% volume and the resulting red precipitate filtered and washed well with cold EtOAc to afford **25** (210 mg, 63%) as a red solid: mp 235−238 °C dec; ^{1}H-NMR ($(CD_3)_2SO$) δ 0.65−0.73 (m, 2 H, cyclopropyl-CH_2), 1.05−1.15 (m, 2 H, cyclopropyl-CH_2), 1.73−1.85 (m, 1 H, cyclopropyl-*H*), 2.18 (s, 4 H, 2 × azir-CH_2), 3.93 (s, 3 H, CH_3N), 5.06 (s, 2 H, CH_2OCONH$_2$), 5.78 (s, 1 H, 6-*H*), and 6.42 (br s, 2 H, NH_2). Anal. ($C_{16}H_{17}N_3O_4$) C, H, N.

3-[(Carbamoyloxy)methyl]-2-cyclopropyl-1-methyl-5-(2-methylaziridin-1-yl)indole-4,7-dione (26). The carbamate **24** (0.05 g, 0.164 mmol) was stirred at room temperature in 2-methylaziridine (1.5 mL) for 4 h, evaporated, and redissolved in EtOAc (5 mL). The solution was then evaporated to

50% volume and the resulting red precipitate filtered, washed well with cold EtOAc, and then recrystallized from EtOAc to afford **26** (30 mg, 56%) as a red solid: mp 209−211 °C dec; ^{1}H-NMR ($(CD_3)_2SO$) δ 0.63−0.72 (m, 2 H, cyclopropyl-CH_2), 1.01−1.09 (m, 2 H, cyclopropyl-CH_2), 1.29 (d, 3 H, J = 5.4 Hz, azir-CH_3), 1.75−1.85 (m, 1 H, cyclopropyl-*H*), 1.98−2.05 (m, 3 H, azir-C*H*CH_2), 3.93 (s, 3 H, CH_3N), 5.06 (s, 2 H, CH_2-OCONH$_2$), 5.76 (s, 1 H, 6-*H*), and 6.41 (br s, 2 H, NH_2). Anal. ($C_{17}H_{19}N_3O_4$) C, H; N: calcd,12.76; found, 12.24.

1-Acetyl-3,3-bis(ethoxycarbonyl)-2,3-dihydro-2-isopropyl-5-methoxyindole (27). This compound was prepared (56%) by the method described for compound **9** but using isobutyraldehyde in place of cyclopropanecarboxaldehyde. The residue after workup was dissolved in Ac_2O (5 mL) and stirred for 3 h at room temperature. The anhydride was then evaporated *in vacuo* and the residue purified on silica, eluting with hexane/EtOAc (1:1, R_f = 0.5) to give **27** (87%) as a white solid: mp 77.5−78.5 °C; ^{1}H-NMR ($CDCl_3$) δ 0.6 (d, 3 H, J = 7.2 Hz, CHCH_3), 0.9 (d, 3 H, J = 7.2 Hz, CHCH_3), 1.2 (t, 6 H, J = 7.2 Hz, CH_2CH_3), 1.3 (t, 6 H, J = 7.2 Hz, CH_2CH_3), 2.12−2.28 (m, 1 H, C*H*$(CH_3)_2$), 2.36 (s, 3 H, COCH_3), 3.81 (s, 3 H, CH_3O), 4.1−4.33 (m, 4 H, 2 × CH_2CH_3), 4.8 (br, 1 H, 2-*H*), and 6.9−7.8 (m, 3 H, Ar-4,6,7*H*).

1-Acetyl-3-carboxy-2,3-dihydro-2-isopropyl-5-methoxyindole (28). The acetylindole **27** was hydrolyzed as described for the preparation of **11** to give **28** (90%) as an off-white foam (R_f = 0.45, Me_2CO), which was used in the next step without further purification: ^{1}H-NMR ($CDCl_3$) δ 0.59 (d, 3 H, J = 7.2 Hz, CHCH_3), 0.9 (d, 3 H, J = 7.2 Hz, CHCH_3), 2.15−2.22 (m, 1 H, C*H*$(CH_3)_2$), 2.2 (s, 3 H, COCH_3), 3.72 (s, 3 H, CH_3O), 3.98 (br, 1 H, 3-*H*), 4.62 (br, 1 H, 2-*H*), 6.8−6.98 (m, 2 H, Ar-4,7*H*), and 7.78−7.85 (m, 1 H, Ar-6*H*).

Methyl 1-Acetyl-2,3-dihydro-2-isopropyl-5-methoxyindole-3-carboxylate (29). In a procedure identical to that carried out on **11**, compound **29** was prepared as a pale brown oil (93%): ^{1}H-NMR ($CDCl_3$) δ 0.71 (d, 3 H, J = 7.2 Hz, CHCH_3), 1.0 (d, 3 H, J = 7.2 Hz, CHCH_3), 2.1−2.2 (m, 1 H, C*H*$(CH_3)_2$), 2.32 (s, 3 H, COCH_3), 3.69 (s, 3 H, CO_2CH_3), 3.79 (s, 3 H, CH_3O), 3.8 (br, 1 H, 3-*H*), 4.6 (br, 1 H, 2-*H*), 6.8−6.98 (m, 2 H, Ar-4,7*H*), and 7.78−7.85 (m, 1 H, Ar-6*H*).

Methyl 1-Acetyl-2-isopropyl-5-methoxyindole-3-carboxylate (30). A solution of **29** (1.0 g, 4.0 mmol) was stirred under reflux with DDQ (0.96 g, 4.2 mmol) in toluene (12.5 mL) for 2 days. The $DDQH_2$ was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified on silica, eluting with hexane/Me_2CO (3:1, R_f = 0.65) to give **30** (0.44 g, 44%) as a pale red oil: ^{1}H-NMR ($CDCl_3$) δ 1.45 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 2.78 (s, 3 H, COCH_3), 3.87 (s, 3 H, CO_2CH_3), 3.96 (s, 3 H, CH_3O), 3.88−3.98 (m, 1 H, C*H*$(CH_3)_2$), 6.89 (dd, 1 H, J = 2.7 and 9 Hz, Ar-6*H*), 7.44 (d, 1 H, J = 9 Hz, Ar-7*H*), and 7.55 (d, 1 H, J = 2.7 Hz, Ar-4*H*).

Methyl 2-Isopropyl-5-methoxyindole-3-carboxylate (31). This compound was prepared from **30** as described for **14** (70%) as a pale red oil: ^{1}H-NMR ($CDCl_3$) δ 1.43 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 3.87 (s, 3 H, CO_2CH_3), 3.92 (s, 3 H, CH_3O), 4.08−4.25 (m, 1 H, C*H*$(CH_3)_2$), 6.9 (dd, 2 H, J = 2.7 and 9 Hz, Ar-6*H*), 7.1−7.6 (m, 2 H, Ar-4,7*H*), and 8.2 (br s, 1 H, N*H*).

Methyl 2-Isopropyl-5-methoxy-1-methylindole-3-carboxylate (32). In a procedure identical to that carried out on **14**, compound **32** was prepared (80%) and purified on silica, eluting with EtOAc/hexane (1:2, R_f = 0.74) as a pale yellow solid: mp 105−106 °C; ^{1}H-NMR ($CDCl_3$) δ 1.45 (d, 6H, J = 7.2 Hz, CH(CH_3)$_2$), 3.79 (s, 3 H, CH_3N), 3.88 (s, 3 H, CO_2CH_3), 3.92 (s, 3 H, CH_3O), 4.22−4.45 (m, 1 H, C*H*$(CH_3)_2$), 6.9 (dd, 2 H, J = 2.7 and 9 Hz, Ar-6*H*), and 7.1−7.64 (m, 1 H, Ar-4,7*H*).

Methyl 2-Isopropyl-5-methoxy-1-methyl-4-nitroindole-3-carboxylate (33). To a solution of **32** (8.0 g, 34.66 mmol) in AcOH (150 mL) cooled to 0 °C was added dropwise a cold (0 °C) mixture of fuming HNO_3 (27 mL) in AcOH (100 mL). The solution was stirred for 3 h while warming to room temperature and then poured onto 300 g of crushed ice, and the resulting yellow solid was collected by suction filtration. The dried residue was purified on silica, eluting with EtOAc/hexane (1:2, R_f = 0.26) to give 7.5 g (71%) of **33** as a yellow solid, recrystallized from EtOAc: mp 149−150.5 °C; ^{1}H-NMR ($CDCl_3$) δ 1.44 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 3.78 (s, 3 H, CH_3N), 3.82 (s, 3 H, CO_2CH_3), 3.91 (s, 3 H, CH_3O), 3.95−4.15 (m, 1 H, C*H*$(CH_3)_2$), 6.97 (d, 1 H, J = 9 Hz, Ar-6*H*), and 7.35 (d, 1 H, J = 9 Hz, Ar-7*H*).

2-Isopropyl-3-(methoxycarbonyl)-5-methoxy-1-methylindole-4,7-dione (35). Compound **33** was reduced as described for **17** to give **34** (85%) and the crude material oxidized with Fremy's salt as described for **18.** After workup the resulting orange precipitate was collected by suction filtration, washed with H_2O, and dried in a vacuum oven at 45 °C to afford **35** as an orange solid (75%), recrystallized from EtOAc: mp 192−194 °C; ^{1}H-NMR ($CDCl_3$) δ 1.34 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 3.12−3.28 (m, 1 H, C*H*$(CH_3)_2$), 3.81 (s, 3 H, CH_3N), 3.90 (s, 3 H, CO_2CH_3), 3.97 (s, 3 H, CH_3O), and 5.64 (s, 1 H, 6-H). Anal. ($C_{15}H_{17}NO_5$) H, N, C; calcd., 61.86; found, 62.31.

3-(Hydroxymethyl)-2-isopropyl-5-methoxy-1-methylindole-4,7-dione (36). To a solution of **35** (0.5 g, 1.7 mmol) in $CHCl_3$ (50 mL) and EtOH (18 mL) was added a solution of $Na_2S_2O_4$ (3.5 g, 20 mmol) in H_2O (22 mL). The solution was stirred at room temperature for 1 h, and the organic layer was separated, washed with saturated NaCl (50 mL), dried, and evaporated. The crude hydroquinone was dried over 18 h *in vacuo* and then dissolved in anhydrous THF (5 mL) under argon and added to a solution of $LiAlH_4$ (12 mL of a 1.0 M solution in THF) dropwise at room temperature and under argon. The solution was then stirred for 1 h at 30 °C and cooled to 0 °C, and H_2O (15 mL) was added dropwise, followed by a solution of $FeCl_3$ (12 mL, 1.0 M (0.1 M HCl)) added at 0 °C. The solution was stirred for 10 min at 0 °C, and then EtOAc (150 mL) and H_2O (150 mL) were added. The aqueous layer was extracted with EtOAc (5 × 50 mL) and the organic phase washed with saturated NaCl (250 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:1, R_f = 0.22) to give, after recrystallization from EtOAc, **36** as an orange solid (140 mg, 31%): mp 160−161 °C; ^{1}H-NMR ($CDCl_3$) δ 1.37 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 3.1−3.28 (m, 1 H, C*H*$(CH_3)_2$), 3.82 (s, 3 H, CH_3N), 3.97 (s, 3 H, CH_3O), 4.71 (br, 2 H, CH_2OH), and 5.64 (s, 1 H, 6-*H*). Anal. ($C_{14}H_{17}NO_4$) C, H, N.

5-(Aziridin-1-yl)-3-(hydroxymethyl)-2-isopropyl-1-methylindole-4,7-dione (37). A solution of **36** (50 mg, 0.19 mmol) in 1*H*-aziridine (0.5 mL, ca.11.7 mmol, **CAUTION!**) was stirred for 0.5 h at room temperature and then evaporated to dryness, and the residue was purified on silica, eluting with EtOAc (R_f = 0.55) to a give, after recrystallization from EtAOc, **37** (42 mg, 81%) as a red solid: mp 144−145 °C; ^{1}H-NMR ($CDCl_3$) δ 1.37 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 2.18 (s, 4 H, 2 × azir-CH_2), 3.1−3.28 (m, 1 H, C*H*$(CH_3)_2$), 3.95 (s, 3 H, CH_3N), 4.68 (br s, 1 H, CH_2OH), 4.76 (br, 2 H, CH_2OH), and 5.76 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

3-(Hydroxymethyl)-2-isopropyl-5-(2-methylaziridin-1-yl)-1-methylindole-4,7-dione (38). Compound **36** (0.1 g, 0.38 mmol) was dissolved and stirred in freshly distilled 2-methylaziridine (3 mL, ca.50 mmol) for 2.5 h. The solution was evaporated *in vacuo* and the residue redissolved in EtOAc, evaporated, and purified on silica (eluting with EtOAc) to afford a red glass (**38**, 85 mg, 78%): ^{1}H-NMR ($CDCl_3$) δ 1.37 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 1.42 (d, 3 H, J = 4.5 Hz, azir-CH_3), 2.01−2.15 (m, 3 H, azir-C*H*CH_2), 3.1−3.28 (m, 1 H, C*H*$(CH_3)_2$), 3.95 (s, 3 H, CH_3N), 4.68 (br s, 1 H, CH_2OH), 4.76 (br, 2 H, CH_2OH), and 5.76 (s, 1 H, 6-*H*). Anal. ($C_{16}H_{20}N_2O_3$·0.5H_2O) C, H, N.

2-Isopropyl-5-methoxy-1-methyl-3-[[(phenoxycarbonyl)oxy]methyl]indole-4,7-dione (39). To a solution of **36** (0.55 g, 2.1 mmol) in anhydrous pyridine (25 mL) at 0 °C was added dropwise phenyl chloroformate (0.5 g, 3.2 mmol), the solution was then allowed to reach room temperature and stirred for 1 h, and then a further 0.25 g (1.6 mmoL) of phenyl chloroformate was added. After a further 1 h, H_2O (200 mL) was added, and the solution was extracted with EtOAc (4 × 50 mL), washed with H_2O (100 mL) and saturated NaCl (100 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc (R_f = 0.8) to give **39** (0.6 g, 75%) as an orange solid: mp 126−127 °C; ^{1}H-NMR ($CDCl_3$) δ 1.37 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 3.1−3.28 (m, 1 H, C*H*$(CH_3)_2$), 3.79

(s, 3 H, CH_3N), 4.01 (s, 3 H, CH_3O), 5.27 (s, 2 H, CH_2OCOPh), 5.51 (s, 1 H, 6-*H*), and 7.15−7.3 (m, 5 H, Ar).

3-[(Carbamoyloxy)methyl]-2-isopropyl-5-methoxy-1-methylindole-4,7-dione (40). The phenyl carbonate **39** (0.1 g, 0.26 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and the solution cooled to −78 °C. The solution was then saturated with NH_3 and stirred at −78 °C until reaction was complete (ca. 2.5 h). The solution was then allowed to reach room temperature and evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2 (50 mL), washed with H_2O (2 × 50 mL) and saturated NaCl (25 mL), dried, and evaporated, and the residue was recrystallized from EtOAc to afford 55 mg (69%) of **40** as an orange solid: mp 244−246 °C dec; ^{1}H-NMR ($CDCl_3$) δ 1.37 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 3.1−3.28 (m, 1 H, CH(CH$_3$)$_2$), 3.78 (s, 3 H, CH_3N), 3.99 (s, 3 H, CH_3O), 4.79 (br s, 2 H, NH_2), 5.3 (s, 2 H, CH_2OCONH$_2$), and 5.62 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{18}N_2O_5$) C, H, N.

5-(Aziridin-1-yl)-3-[(carbamoyloxy)methyl]-2-isopropyl-1-methylindole-4,7-dione (41). The carbamate **40** (0.1 g, 0.33 mmol) was stirred at room temperature in 1*H*-aziridine (2 mL, **CAUTION!)** for 25 min, evaporated, and redissolved in EtOAc (5 mL). The solution was then evaporated to 50% volume, and the resulting red precipitate was filtered and washed well with cold EtOAc to afford **41** (55 mg, 53%) as a red solid: mp 230−233 °C dec; ^{1}H-NMR ((CD$_3$)$_2$SO) δ 1.37 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 2.18 (s, 4 H, 2 × azir-CH_2), 3.1−3.28 (m, 1 H, CH(CH$_3$)$_2$), 3.93 (s, 3 H, CH_3N), 5.06 (s, 2 H, CH_2OCONH$_2$), 5.78 (s, 1 H, 6-*H*), and 6.42 (br s, 2 H, NH_2). Anal. ($C_{16}H_{19}N_3O_4$) C, H, N.

3-[(Carbamoyloxy)methyl]-2-isopropyl-1-methyl-5-(2-methylaziridin-1-yl)indole-4,7-dione (42). The carbamate **40** (0.1 g, 0.32 mmol) was stirred at room temperature in 2-methylaziridine (1.5 mL) for 2.5 h, evaporated, and redissolved in EtOAc (5 mL). The solution was then evaporated to 50% volume, and the resulting red precipitate was filtered, washed well with cold EtOAc, and then recrystallized from EtOAc to afford **42** (55 mg, 52%) as a red solid: mp 204−205 °C dec; ^{1}H-NMR ((CD$_3$)$_2$SO) δ 1.37 (d, 6 H, J = 7.2 Hz, CH-(CH_3)$_2$), 1.42 (d, 3 H, J = 5.4 Hz, azir-CH_3), 1.98−2.05 (m, 3 H, azir-CHCH_2), 3.1−3.28 (m, 1 H, CH(CH$_3$)$_2$), 3.93 (s, 3 H, CH_3N), 5.06 (s, 2 H, CH_2OCONH$_2$), 5.76 (s, 1 H, 6-*H*), and 6.41 (br s, 2 H, NH_2). Anal. ($C_{17}H_{21}N_3O_4$) C, H, N.

1,3-Dimethyl-2-isopropyl-5-methoxyindole-4,7-dione (43). To a solution of **35** (0.5 g,1.7 mmol) in $CHCl_3$ (50 mL) and EtOH (18 mL) was added a solution of $Na_2S_2O_4$ (3.5 g, 20 mmol) in H_2O (22 mL). The solution was stirred at room temperature for 1 h, and the organic layer was separated, washed with saturated NaCl (50 mL), dried, and evaporated. The crude hydroquinone was dried over 18 h *in vacuo*, dissolved in anhydrous THF (5 mL) under argon, and added to a solution of DIBAL-H (10 mL of a 1.5 M solution in toluene) dropwise at −30 °C and under argon. The solution was then stirred for 18 h at 4 °C and cooled to 0 °C, and H_2O (15 mL) was added dropwise, followed by a solution of $FeCl_3$ (12 mL, 1.0 M (0.1 M HCl)) added at 0 °C. The solution was stirred for 10 min at 0 °C, and then EtOAc (150 mL) and H_2O (150 mL) were added. The aqueous layer was extracted with EtOAc (5 × 50 mL), and the organic phase was washed with saturated NaCl (250 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1: 2, R_f = 0.44) to give, after recrystallization from EtOAc, **43** as an orange solid (48 mg, 11%): mp 168−169 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 2.38 (s, 3 H, 3-CH_3), 3.1−3.28 (m, 1 H, CH(CH$_3$)$_2$), 3.78 (s, 3 H, CH_3N), 3.95 (s, 3 H, CH_3O), and 5.57 (s, 1 H, 6-*H*). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

5-(Aziridin-1-yl)-1,3-dimethyl-2-isopropylindole-4,7-dione (44). A solution of **43** (30 mg, 0.12 mmol) in 1*H*-aziridine (0.5 mL, ca.11.7 mmol, **CAUTION!**) was stirred for 0.5 h at room temperature and then evaporated to dryness and the residue purified on silica, eluting with EtOAc/hexane (1:2, R_f = 0.32) to a give, after recrystallization from EtAOc, **44** (5.5 mg, 18%) as a red solid: mp 110−112 °C; ^{1}H-NMR ($CDCl_3$) δ 1.35 (d, 6 H, J = 7.2 Hz, CH(CH_3)$_2$), 2.15 (s, 4 H, 2 × azir-CH_2), 2.38 (s, 3 H, 3-CH_3), 3.1−3.28 (m, 1 H, CH(CH$_3$)$_2$), 3.94 (s, 3 H, CH_3N), and 5.72 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{18}N_2O_2$) H, N; C: calcd, 65.22; found, 65.74.

2-Cyclohexyl-3-(hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione (45). The corresponding 3-methoxycarbonyl precursor compound was prepared as described for **18** and **35** but using cyclohexanecarboxaldehyde in the imine cyclization step, and the subsequent oxidation step with DDQ required a much prolonged reaction time of 12 h. The crude 4-amino compound was again oxidized with Fremy's salt as described for **18**. After workup the resulting orange precipitate was collected by suction filtration, washed with H_2O, and dried in a vacuum oven at 45 °C to give 2-cyclohexyl-3-(methoxycarbonyl)-5-methoxy-1-methylindole-4,7-dione as an orange solid (75%) recrystallized from EtOAc: mp 190−192 °C; ^{1}H-NMR ($CDCl_3$) δ 1.27 (m, 6 H, 6 × cyclohexyl-*H*), 1.83 (m, 5 H, 5 × cyclohexyl-*H*), 3.79 (s, 3 H, CH_3N), 3.91 (s, 3 H, CO$_2$CH_3), 3.96 (s, 3 H, CH_3O), and 5.63 (s, 1 H, 6-H). This compound was then reduced with $LiAlH_4$ as described for **36** to give **45** as an orange solid (59%): mp 214−215 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (m, 6 H, 6 × cyclohexyl-*H*), 1.78 (m, 5 H, 5 × cyclohexyl-*H*), 3.81 (s, 3 H, CH_3N), 3.97 (s, 3 H, CH_3O), 4.76 (s, 2 H, CH_2-OH), and 5.63 (s, 1 H, 6-*H*). Anal. ($C_{17}H_{21}NO_4$) C, H, N.

5-(Aziridin-1-yl)-2-cyclohexyl-3-(hydroxymethyl)-1-methylindole-4,7-dione (46). A solution of **45** (30 mg,0.1 mmol) in freshly distilled 1*H*-aziridine (0.5 mL, ca.11.7 mmol, **CAUTION!**) was stirred for 0.5 h at room temperature and then evaporated to dryness and the residue purified on silica, eluting with EtOAc/hexane (1:1, R_f = 0.3) to give, after recrystallization from EtOAc, **46** (22 mg, 70%) as a red solid: mp 128−130 °C; ^{1}H-NMR ($CDCl_3$) δ 1.26 (m, 6 H, 6 × cyclohexyl-*H*), 1.78 (m, 5 H, 5 × cyclohexyl-*H*), 2.18 (s, 4 H, 2 × aziridine-CH_2), 3.95 (s, 3 H, CH_3N), 4.76 (s, 2 H, CH_2OH), and 5.77 (s, 1 H, 6-*H*). Anal. ($C_{18}H_{22}N_2O_3$·1.5H_2O) C, H, N.

5-Methoxy-1,2-dimethylindole (47). 5-Methoxy-2-methylindole (10 g, 0.062 mol) was added gradually and under dry argon to a stirred suspension of NaH (2.73 g of a 60% dispersion, 0.068 mol) in DMF (150 mL). The suspension was heated at 45 °C for 10 min and cooled to room temperature, and MeI (33 mL, 0.23 mol) was added over 5 min. The solution was then heated at 60 °C for 1 h, cooled, poured onto cold (0 °C) $NaHSO_4$ (aqueous, 10%, 150 mL), extracted with EtOAc (3 × 100 mL), dried, and evaporated. The residue was purified on silica, eluting with 3%EtOAc/hexane (R_f = 0.5) to give 4.5 g (41%) of **47** as a pale brown solid: mp 73−74 °C; ^{1}H-NMR ($CDCl_3$) δ 2.37 (s, 3 H, 2-CH_3), 3.59 (s, 3 H, CH_3N), 3.82 (s, 3 H, CH_3O), 6.16 (s, 1 H, 3-*H*), and 6.9−7.28 (m, 3 H, Ar-4,6,7*H*).

5-Methoxy-1,2-dimethylindole-3-carboxaldehyde (48). *N*-Methylformanilide (0.95 g, 7.04 mmol) and $POCl_3$ (1.08 g, 7.05 mmol) were stirred at room temperature until the yellow solid chloroimmonium Vilsmeier compound formed. This yellow solid was then added to a solution of **47** (0.7 g, 4 mmol) in 1,2-dichloroethane (15 mL), the solution was heated under reflux for 1.5 h and cooled, NaOAc (1.0 M, 50 mL) was added, and the solution was stirred for 2.5 h. The solution was extracted with EtOAc (4 × 100mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1: 1, R_f = 0.5(EtOAc)) to give 0.35 g (43%) of **48** as an off-white solid: mp 108−110 °C; ^{1}H-NMR ($CDCl_3$) δ 2.62 (s, 3 H, 2-CH_3), 3.63 (s, 3 H, CH_3N), 3.89 (s, 3 H, CH_3O), 6.94−7.2 (m, 2 H, Ar-6,7*H*), 7.8 (d, 1 H, J = 2 Hz, Ar-4*H*), and 10.1 (s, 1 H, C*H*O).

5-Methoxy-1,2-dimethyl-4-nitroindole-3-carboxaldehyde (49). Compound **48** (6.0 g, 0.03 mol) was dissolved in AcOH (480 mL) and cooled to 5 °C and fuming HNO_3 (18 mL) in AcOH (72 mL) added dropwise with stirring over 5 min. The solution temperature was allowed to rise to 20 °C over 3 h, the mixture was poured onto crushed ice (500 g), and the yellow precipitate was collected by suction filtration and dried *in vacuo* at 50 °C. The yellow solid was purified on silica, eluting with EtOAc/hexane (2:1, R_f = 0.42 (EtOAc)) to give 4.37 g (59%) of **49** as a pale yellow solid: mp 236−238 °C dec; ^{1}H-NMR ((CD$_3$)$_2$SO) δ 2.71 (s, 3 H, 2-CH_3), 3.76 (s, 3 H, CH_3N), 3.89 (s, 3 H, CH_3O), 7.25 (d, 1 H, J = 9 Hz, Ar-7*H*), 7.75 (d, 1 H, J = 9 Hz, Ar-6*H*), and 9.9 (s, 1 H, C*H*O).

4-Amino-5-methoxy-1,2-dimethylindole-3-carboxaldehyde (50). Nitro compound **49** (0.18 g, 0.74 mmol) was dissolved in EtOH (15 mL), powdered tin (0.45 g, 3.8 mmol) was added, followed by HCl (3.0 M, 5.6 mL), and the solution was heated under gentle reflux for 1 h. Water (50 mL) was

added and the solution neutralized with $NaHCO_3$(aq), extracted with $CHCl_3$ (3 × 100 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc/hexane (1:1, R_f = 0.57 (EtOAc)) to give 0.1 g (62%) of **50** as a pale yellow solid: mp 152−153 °C dec; ^{1}H-NMR ($(CD_3)_2SO$) δ 2.61 (s, 3 H, 2-C*H*$_3$), 3.59 (s, 3 H, C*H*$_3$N), 3.75 (s, 3 H, C*H*$_3$O), 6.0 (br s, 2 H, N*H*$_2$), 6.56 (d, 1 H, J = 9 Hz, Ar-7*H*), 6.85 (d, 1 H, J = 9 Hz, Ar-6*H*), and 9.71 (s, 1 H, C*H*O).

3-Formyl-5-methoxy-1,2-dimethylindole-4,7-dione (51). To a solution of **50** (0.074 g, 0.34 mmol) in Me_2CO (14 mL) was added a solution of Fremy's salt (0.45 g, 1.68 mmol) in NaH_2PO_4/Na_2HPO_4 buffer (14 mL, 0.3 M, pH 6.0) and the solution stirred at room temperature for 1 h. The solution was evaporated at 30 °C to remove most of the Me_2CO and the resulting orange precipitate collected by suction filtration and washed well with H_2O and cold MeOH to give **51** (60 mg, 75%): mp 239−242 °C; ^{1}H-NMR ($(CD_3)_2SO$) δ 2.5 (s, 3 H, 2-C*H*$_3$), 3.82 (s, 3 H, C*H*$_3$N), 3.88 (s, 3 H, C*H*$_3$O), 5.89 (s, 1 H, 6-*H*), and 10.37 (s, 1 H, C*H*O).

5-Methoxy-3-(hydroxymethyl)-1,2-dimethylindole-4,7-dione (52). To a suspension of **51** (0.25 g, 0.94 mmol) in MeOH (100 mL, degassed by boiling under argon *in vacuo*) was added $NaBH_4$ (0.36 g, 9.7 mmol) while a dry argon atmosphere was maintained. The solution was stirred at room temperature for 1 h, aerated prior to the addition of H_2O (40 mL), extracted with CH_2Cl_2 (3 × 100 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc (R_f = 0.4) to afford **52** (0.1 g, 33%) as an orange solid, recrystallized from EtOAc: mp 215−216 °C (lit.[2] mp 199−200 °C); ^{1}H-NMR ($CDCl_3$) δ 2.21 (s, 3 H, 2-C*H*$_3$), 3.81 (s, 3 H, C*H*$_3$N), 3.86 (s, 3 H, C*H*$_3$O), 4.65 (br d, 2 H, J = 7.2 Hz, C*H*$_2$-OH), and 5.61 (s, 1 H, 6-*H*). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

5-(Aziridin-1-yl)-3-(hydroxymethyl)-1,2-dimethylindole-4,7-dione (53). A solution of **52** (235 mg, 1.0 mmol) in freshly redistilled 1*H*-aziridine (1.5 mL, ca. 35 mmol, **CAUTION!**) was stirred for 0.5 h at room temperature and evaporated *in vacuo*. The residue was redissolved in EtOAc and condensed by 75%, and the precipitate was collected and washed with cold EtOAc to give 225 mg (91%) of **53** as a dark red solid: mp 173−174 °C dec; ^{1}H-NMR ($CDCl_3$) δ 2.19 (s, 4 H, 2 × azir-C*H*$_2$), 2.22 (s, 3 H, 2-C*H*$_3$), 3.86 (s, 3 H, C*H*$_3$N), 3.95 (br, 1 H, CH$_2$O*H*), 4.65 (br d, 2 H, C*H*$_2$OH), and 5.76 (s, 1 H, 6-*H*). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

3-(Hydroxymethyl)-5-(2-methylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (54). A solution of **52** (235 mg, 1.0 mmol) in 2-methylaziridine (2 mL) was stirred at room temperature for 4 h and worked up as described for **53** to give **54** (195 mg, 75%) as a dark red solid: mp 120−122 °C; ^{1}H-NMR ($CDCl_3$) δ 1.42 (d, 3 H, J = 5.4 Hz, azir-C*H*$_3$), 2.08−2.21 (m, 3 H, azir-C*H*C*H*$_2$), 2.22 (s, 3 H, 2-C*H*$_3$), 3.89 (s, 3 H, C*H*$_3$N), 4.61 (br, 1 H, CH$_2$O*H*), 4.63 (br, 2H, C*H*$_2$OH), and 5.75 (s, 1 H, 6-*H*). Anal. ($C_{14}H_{16}N_2O_3$) H, N; C: calcd, 64.62; found, 65.09.

3-(Hydroxymethyl)-5-(*cis*-2,3-dimethylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (55). A solution of **52** (100 mg, 0.43 mmol) in *cis*-2,3-dimethylaziridine (2 mL) was stirred for 1 h at room temperature and then evaporated *in vacuo* at 30 °C. The residue was purified on silica, eluting with EtOAc (R_f = 0.6) to give **55** (65 mg, 55%) after recrystallization from EtOAc: mp 132−135 °C; ^{1}H-NMR ($CDCl_3$) δ 1.38 (d, 6 H, J = 5.4 Hz, 2 × azir-C*H*$_3$), 2.15−2.21 (m, 2 H, 2 × azir-C*H*), 2.22 (s, 3 H, 2-C*H*$_3$), 3.85 (s, 3 H, C*H*$_3$N), 4.24 (br, 1 H, CH$_2$O*H*), 4.63 (br, 2 H, C*H*$_2$OH), and 5.73 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{18}N_2O_3$) C, H, N.

3-(Hydroxymethyl)-5-(2,2-dimethylaziridin-1-yl)-1,2-dimethylindole-4,7-dione (56). A solution of **52** (100 mg, 0.43 mmol) in 2,2-dimethylaziridine (2 mL) was stirred at 95 °C for 5 h. After this time (reaction does not go to completion) the solution was cooled and evaporated *in vacuo*, and the residue redissolved in EtOAc and evaporated to precipitate **56** (25 mg, 21%) as a dark red solid, which was unstable on silica and in solution: mp 138−141 °C; ^{1}H-NMR ($CDCl_3$) δ 1.32 (s, 6 H, 2 × azir-C*H*$_3$), 2.07 (s, 2 H, azir-C*H*$_2$), 2.22 (s, 3 H, 2-C*H*$_3$), 3.87 (s, 3 H, C*H*$_3$N), 4.25 (br, 1 H, CH$_2$O*H*), 4.86 (br, 2 H, C*H*$_2$OH), and 5.68 (s, 1 H, 6-*H*). Anal. ($C_{15}H_{18}N_2O_3 \cdot {}^2/_3H_2O$) C, H, N.

3-(Hydroxymethyl)-5-(2,2-dimethyl-2-hydroxyethyl)-amino)-1,2-dimethylindole-4,7-dione (57). Compound **56** (25 mg, 0.09 mmol) was dissolved in 5 mL of H_2O with warming to 45 °C and then evaporated *in vacuo* at 50 °C. The residue was purified on silica, eluting with EtOAc (R_f = 0.35) to give **57** (25 mg, 95%) after recrystallization from EtOAc: mp 188−190 °C; ^{1}H-NMR ($(CD_3)_2SO$) δ 1.14 (s, 6 H, 2 × C*H*$_3$), 2.21 (s, 3 H, 2-C*H*$_3$), 3.02 (d, 2 H, J = 7.2 Hz, NHC*H*$_2$C(CH$_3$)$_2$), 3.84 (s, 3H, C*H*$_3$N), 4.58 (br, 2 H, C*H*$_2$OH), 4.65 (br, 1 H, CH$_2$O*H*), 5.14 (s, 1 H, 6-*H*), and 6.63 (br t, 1 H, N*H*). Anal. ($C_{15}H_{20}N_2O_4 \cdot {}^1/_3H_2O$) C, H, N.

5-Methoxy-1-methylindole-3-carboxaldehyde (58). 5-Methoxyindole-3-carboxaldehyde (2.0 g, 11.4 mmol) was added gradually to a suspension of NaH (0.55 g, 13.7 mmol) in DMF (50 mL) with stirring. The suspension was stirred for 0.5 h, MeI (1.94 g, 13.7 mmol) was added, and the mixture was stirred for 1 h at room temperature. The reaction mixture was then poured onto $NaHCO_3$ (10%, 300 mL), extracted with EtOAc (4 × 75 mL), washed with $NaHCO_3$ (10%, 3 × 50 mL) and saturated NaCl (3 × 100 mL), dried, and evaporated *in vacuo* to give **58** (1.70 g, 79%) as a white solid: mp 132−133 °C; ^{1}H-NMR ($CDCl_3$) δ 3.81 (s, 3 H, C*H*$_3$N), 3.89 (s, 3 H, C*H*$_3$O), 7.08 (dd, 1 H, J = 2 and 9 Hz, Ar-6H), 7.31 (d, 1 H, J = 9 Hz, Ar-7H), 7.59 (s, 1 H, 2-H), 7.8 (d, 1 H, J = 2Hz, Ar-4*H*), and 9.93 (s, 1H, C*H*O).

5-Methoxy-1-methyl-4-nitroindole-3-carboxaldehyde (59). To a solution of **58** (1.50 g 7.94 mmol) dissolved in AcOH (150 mL) was added a mixture of concentrated HNO_3 (4.5 mL) in AcOH (25 mL) dropwise at 0 °C over 3 h. After addition, the mixture was stirred at room temperature for 16 h, added to crushed ice (75 g), filtered, washed with H_2O (5 × 100mL), and dried to give **59** (1.56 g, 84%) as a pale yellow solid: mp 197−198 °C; ^{1}H-NMR ($(CD_3)_2SO$) δ 3.94 (s, 6 H, C*H*$_3$O and C*H*$_3$N), 7.32 (d, 1 H, J = 9 Hz, Ar-7*H*), 7.72 (d, 1 H, J = 9 Hz, Ar-6*H*), 8.27 (s, 1 H, 2-*H*), and 9.75 (s, 1 H, C*H*O).

4-Amino-5-methoxy-1-methylindole-3-carboxaldehyde (60). To a suspension of **59** (1.0 g, 4.27 mmol) in EtOH (150 mL) was added tin (4.43 g, 37 mmol) followed by HCl (3.0 M, 60 mL). The mixture was stirred at room temperature for 2 h and decanted. The solution was added gradually to saturated $NaHCO_3$ (aqueous, 300 mL) and extracted with EtOAc (3 × 100 mL). The organic layer was separated, washed with saturated $NaHCO_3$ (aqueous, 2 × 175 mL) and saturated NaCl (3 × 75 mL), dried, and evaporated *in vacuo* to give **60** (0.79 g, 91%) as a dark yellow solid which was used in the next step without further purification: R_f = 0.64 (EtOAc); ^{1}H-NMR ($CDCl_3$) δ 3.75 (s, 3 H, C*H*$_3$N), 3.88 (s, 3 H, C*H*$_3$O), 5.79 (br s, 2 H, N*H*$_2$), 6.56 (d, 1 H, J = 9 Hz, Ar-7*H*), 7.53 (d, 1 H, J = 9 Hz, Ar-6*H*), 7.64 (s, 1 H, 2-*H*), and 9.60 (s, 1 H, C*H*O).

3-Formyl-5-methoxy-1-methylindole-4,7-dione (61). To **60** (0.75 g, 3.68mmol) dissolved in Me_2CO (75 mL) was added Fremy's salt (4.0 g, 14.9 mmol) in H_2O (20 mL) followed by a solution of Na_2HPO_4/NaH_2PO_4 buffer (0.3 M, pH 6, 20 mL). The mixture was stirred for 0.75 h, excess Me_2CO was removed, and the product was filtered, washed with H_2O (50 mL), dried, and recrystallized from EtOAc to give **61** (0.61 g, 76%) as a yellow solid: mp 188−190 °C; ^{1}H-NMR ($CDCl_3$) δ 3.87 (s, 3 H, C*H*$_3$N), 4.02 (s, 3 H, C*H*$_3$O), 5.78 (s, 1 H, 6-*H*), 7.44 (s, 1 H, 2-*H*), and 10.54 (s, 1 H, C*H*O).

3-(Hydroxymethyl)-5-methoxy-1-methylindole-4,7-dione (62). To a solution of **61** (0.5 g, 2.28 mmol) in anhydrous argon degassed MeOH (300 mL) was added $NaBH_4$ (0.65 g, 17 mmol). The solution was degassed with argon, stirred for 2 h under argon, and then evaporated *in vacuo* to give a solid which was diluted with CH_2Cl_2 (300 mL), washed with H_2O (2 × 100 mL) and saturated NaCl (100 mL), and condensed to give a **62** as an orange solid (0.2 g, 40%) after recrystallization from EtOAc: mp 185−186°C; ^{1}H-NMR ($CDCl_3$) δ 3.85 (s, 3 H, C*H*$_3$N), 3.94 (s, 3 H, C*H*$_3$O), 4.25−4.29 (m, 2 H, C*H*$_2$OH), 5.73 (s, 1 H, 6-*H*), and 6.88 (s, 1 H, 2-*H*). Anal. ($C_{11}H_{11}NO_4$) C, H, N.

5-(Aziridin-1-yl)-3-(hydroxymethyl)-1-methylindole-4,7-dione (63). A solution of **62** (200 mg, 0.9 mmol) in 1*H*-aziridine (1.5 mL, ca. 35 mmol, **CAUTION!**) was stirred at room temperature for 1.5 h. Excess aziridine was removed *in vacuo*, and the product was recrystallized from EtOAc to give

63 (130 mg, 62%) as an orange solid: mp 169−171 °C; ^{1}H-NMR ($CDCl_3$) δ 2.22 (s, 4 H, 2 × aziridine-CH_2), 3.91 (s, 3 H, CH_3N), 4.64 (s, 2 H, CH_2OH), 5.81 (s, 1 H, 6-*H*), and 6.69 (s, 1 H, 2-*H*). Anal. ($C_{12}H_{12}N_2O_3$) C, H, N.

3-(Hydroxymethyl)-5-(2-methylaziridin-1-yl)-1-methylindole-4,7-dione (64). A solution of **62** (200 mg, 0.9 mmol) in 2-methylaziridine (1 mL) was stirred at room temperature for 2.5 h. Excess 2-methylaziridine was removed *in vacuo*, and the product was purified on silica, eluting with EtOAc (R_f = 0.6) to give **64** (120 mg, 54%) as a red solid recrystallized from EtOAc: mp 89−90 °C; ^{1}H-NMR ($CDCl_3$) δ 1.47 (d, 3 H, J = 4.5 Hz, azir-CH_3), 2.04−2.2 (m, 3 H, azir-CHCH_2), 3.91 (s, 3 H, N-CH_3), 4.67 (m, 2 H, CH_2OH), 5.79 (s, 1 H, 6-*H*), and 6.70 (s, 1 H, 2-*H*). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

3-[(Carbamoyloxy)methyl]-5-methoxy-1-methylindole-4,7-dione (65). To a solution of **62** (0.1 g, 0.45 mmol) in anhydrous pyridine (5 mL) at 0 °C was added dropwise phenyl chloroformate (0.1 g, 0.64 mmol) and the solution then allowed to reach room temperature and stirred for 2 h. The solution was then extracted with CH_2Cl_2 (30 mL), washed with H_2O (35 mL) and saturated NaCl (35 mL), dried, and evaporated. The residue was purified on silica, eluting with EtOAc (R_f = 0.8) to give an orange solid of 5-methoxy-1-methyl-3-[[(phenoxycarbonyl)oxy]methyl]indole: mp 85−86 °C; ^{1}H-NMR ($(CD_3)_2SO/CDCl_3$) δ 3.79 (s, 3 H, CH_3N), 3.91 (s, 3 H, CH_3O), 5.43 (s, 2 H, CH_2OCOPh), 5.67 (s, 1 H, 6-*H*), 6.89 (s, 1 H, 2-*H*), and 7.15−7.3 (m, 5 H, Ar). This material (0.1 g, 0.3 mmol) was dissolved in anhydrous CH_2Cl_2 (18 mL) and the solution cooled to −78 °C. The solution was then saturated with NH_3 and stirred at −78 °C until the reaction was complete (0.75 h). The solution was then allowed to reach room temperature and evaporated *in vacuo*. The residue was redissolved in CH_2Cl_2 (100 mL), washed with H_2O (2 × 100 mL) and saturated NaCl (50 mL), dried and evaporated, and the residue recrystallized from EtOAc to afford 75 mg (98%) of **65** as an orange solid: mp 231−234 °C (dec.); ^{1}H-NMR ($(CD_3)_2SO/CDCl_3$) δ 3.78 (s, 3 H, CH_3N), 3.88 (s, 3 H, CH_3O), 5.04 (s, 2 H, CH_2OCONH$_2$), 5.77 (s, 1 H, 6-*H*), 6.42 (br s, 2 H, NH_2), and 7.09 (s, 1 H, 2-*H*). Anal. ($C_{12}H_{12}N_2O_5$) H, N; C: calcd, 54.55; found, 54.98.

3-[(Carbamoyloxy)methyl]-5-(aziridin-1-yl)-1-methylindole-4,7-dione (66). Compound **65** (50 mg, 0.2 mmol) was stirred for 0.5 h in 1*H*-aziridine (ca. 35 mmol, **CAUTION!**), evaporated *in vacuo*, and redissolved in EtOAc. The solution was evaporated again and the residue recrystallized from EtOAc to afford 35 mg (70%) of **66** as a red solid: mp 195−198 °C dec; ^{1}H-NMR ($(CD_3)_2SO$) δ 2.18 (s, 4 H, 2 × azir-CH_2), 3.90 (s, 3 H, CH_3N), 5.23 (s, 2 H, CH_2OCONH$_2$), 5.49 (br s, 2 H, NH_2) 5.76 (s, 1 H, 6-*H*), and 6.86 (s, 1 H, 2-*H*). Anal. ($C_{13}H_{13}N_3O_4$) C, H, N.

Redox Chemistry. The properties of representative semiquinone radicals were studied following reduction of the quinones by the $(CH_3)_2C^{\bullet}OH$ radical generated by pulse radiolysis.[23] Optical detection was used to determine the one-electron reduction potentials (E ($Q/Q^{\bullet-}$)) of the indoloquinones and the reactivities of the semiquinone radicals with oxygen. Experiments were performed with a 6 MeV linear accelerator as described previously.[24] Solutions at 23 °C, pH 8.5, contained 0.2 M 2-propanol and 10 mM phosphate buffer (NaH_2PO_4/Na_2HPO_4) with Q (0 or 60 μM) and either benzyl viologen, BV^{2+} (0−4 mM), or methyl viologen, MV^{2+} (0−4 mM). Absorbances at 600 nm were measured ~20 μs after a dose of ~2 Gy. Solutions were dearated by saturation with N_2O. The alcohol converts the radiolytically produced $^{\bullet}OH$ and $H^{\bullet}$ radicals in <0.2 μs to the reductant $(CH_3)_2C^{\bullet}OH$ which reduces the indoloquinones or viologens (V^{2+}) to the corresponding semiquinone $Q^{\bullet-}$ radicals or viologen radical cations $V^{\bullet+}$. All experiments were performed at pH 8.5 because the aziridinyl indoloquinones, including EO9 (**3**),[25] slowly hydrolyze at physiological pH. The absorption spectra of the radicals exhibited absorption maxima in the 340−400 nm region, similar to that of EO9 (**3**).[17] The radical spectra did not change between pH 7 and pH 10, and it was concluded that at pH 7.4 and above the semiquinone is deprotonated. Due to the instability of the aziridine at low pH it was not possible to determine the pK of the semiquinone radical. Previous studies indicate that most semiquinone radicals have a pK of around 4−6.[26]

At pH 8.5 the semiquinone radical absorption (720 nm) decayed with second-order kinetics and a half-life which decreased with increasing radiation dose (i.e. with initial concentration of radicals), indicating that the semiquinone radical decays predominantly by a radical−radical reaction to generate the hydroquinone as in reaction 1.

$$2Q^{\bullet-} + 2H^+ \rightarrow Q + QH_2 \quad (1)$$

The reciprocal of the first half-life of the semiquinone radical of **54** varied linearly with the initial radical concentration, and from the slope of the fitted straight line the rate constant $2k_2$ = (2.3 ± 0.1) × 10^7 M^{-1} s^{-1} was obtained, and this is comparable with that recorded for EO9 (**3**).[17]

The reactivities of semiquinone radicals with oxygen were determined by saturating solutions with N_2O/O_2 gas mixtures (British Oxygen Company, U.K.) which contained 0.2−2.1% O_2. In the presence of oxygen the semiquinone radicals decayed (eq 2) faster with increasing oxygen concentrations. Absolute rate constants k_2 determined from the slope of the linear plots of the first-order rate constants versus oxygen concentration are shown in Table 2.

$$Q^{\bullet-} + O_2 \rightarrow Q + O_2^{\bullet-} \quad (2)$$

The redox potentials of the one-electron couple E ($Q/Q^{\bullet-}$) were determined by establishing redox equilibria with viologens of known redox potential in reaction 3 according to methods descibed previously.[27]

$$Q^{\bullet-} + V^{2+} \rightleftharpoons Q + V^{\bullet+} \quad (3)$$

For V^{2+}/indoloquinone systems, redox equilibration usually occurred within ~20 μs (during which there was negligible decay of semiquinone radicals via reaction 1), and it was therefore possible to calculate the equilibrium constant K_3 from absorbances at equilibrium.

Estimates of K_3 and E ($Q/Q^{\bullet-}$) for the range of indoloquinones studied are shown in Table 2. All E ($Q/Q^{\bullet-}$) values quoted have been corrected for the effect of ionic strength (I = 0.004 = ca. 7 mV), utilizing the Debye−Hückel expression.[24]

Biological Evaluation *in Vitro*. Selective toxicity to hypoxic V79−379A cells was determined for all compounds using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay as has been described previously.[28−30] These results are presented in Table 1, where C_{50} (air) values, the concentration required to kill 50% of the aerobic cells under the conditions of the assay, are divided by C_{50} (N_2) values, concentrations required to kill hypoxic cells, to give hypoxic cytotoxicity ratios (HCR), which enable quantitative comparisons of bioreductive activities (hypoxic cytotoxicities) of drugs.

Biological Evaluation *in Vivo*. The RIF-1 and KHT murine sarcoma tumor models were used for the initial *in vivo* tumor toxicity evaluations of lead compounds. The maximum tolerated doses and tumor response experiments were carried out essentially as described previously.[30] Tumor-bearing mice were given doses of irradiation that were chosen to kill most of the oxic cells in the tumors. Thus, the measured response would reflect the survival of residual hypoxic clonogenic cells.[31] Indoloquinones were given immediately after X-rays such that any therapeutic response greater than that achieved by radiation alone is a reflection of residual hypoxic cell killing. These conditions were achieved by giving 10Gy to KHT tumors and 25Gy to RIF-1 tumors. Each experiment also included mice exposed to radiation without drug and drug alone. Results obtained with the selected lead compounds are displayed in Table 3.

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