Synthesis and Evaluation of 3-Aryloxymethyl-1,2-dimethylindole-4,7-diones as Mechanism-Based Inhibitors of NAD(P)H:Quinone Oxidoreductase 1 (NQO1) Activity

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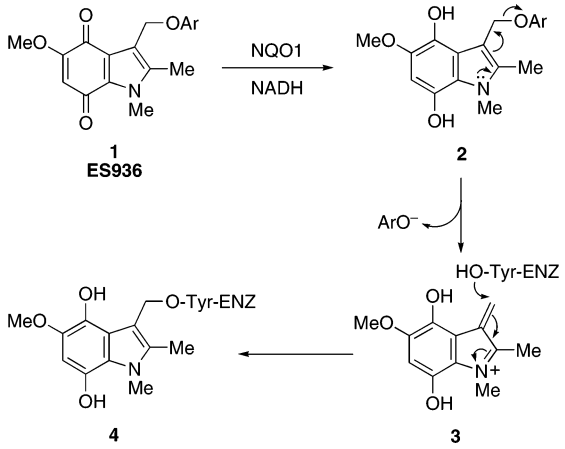
NAD(P)H:quinone oxidoreductase 1 is a proposed target in pancreatic cancer. We describe the synthesis of a series of indolequinones, based on the 5- and 6-methoxy-1,2-dimethylindole-4,7-dione chromophores with a range of phenolic leaving groups at the (indol-3-yl)methyl position. The ability of these indolequinones to function as mechanism-based inhibitors of purified recombinant human NQO1 was evaluated, as was their ability to inhibit both NQO1 and cell growth in human pancreatic MIA PaCa-2 tumor cells. The inhibition of rhNQO1 was related to the pK_a of the leaving group: compounds with poorer phenolic leaving groups were poor inhibitors whereas those with more acidic leaving groups were more efficient inhibitors. These inhibition data also correlated with the inhibition NQO1 in MIA PaCa-2 cells. However, the data demonstrate that NQO1 inhibition does not correlate with growth inhibitory activity, at least in the MIA PaCa-2 cell line, suggesting that targets in addition to NQO1 need to be considered to explain the potent growth inhibitory activity of this series of indolequinones in human pancreatic cancer cells.

Introduction

The enzyme NQO1 (EC 1.6.99.2), also known as DT-diaphorase, is an obligate two-electron reductase characterized by its ability to use either NADH or NADPH as cofactor. NQO1 catalyzes the two-electron reduction of quinones and, hence, can protect cells against the toxic effects of quinones.[1−5] However, NQO1 is also involved in the reductive activation of anticancer agents, such as mitomycin C and other cytotoxic quinones that operate by the so-called bioreductive mechanism,[4,6−8] and continues to generate interest because of its elevated levels in many tumors and tumor cell lines.[3] During our long-standing interest in NQO1, we have undertaken a detailed study on the substrate specificity of the enzyme and the correlation of quinone structure with rate of metabolism by the enzyme and toxicity toward human tumor cell lines.[9−13]

As part of our studies on quinone substrates for NQO1, we have recently developed a highly specific, potent, mechanism-based (suicide substrate) inhibitor 5-methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione **1**, a compound also known as **ES936**, and have fully characterized its interaction with the enzyme using biochemistry, mass spectrometry, and crystallography.[14] In cellular studies using human breast and colon cancer cell lines, it inhibits the enzyme at low nanomolar concentrations.[15] We rationalize the action of **1** by the mechanism shown in Scheme 1, whereby two-electron reduction of the quinone by NQO1 gives the corresponding hydroquinone **2**. In this compound, the indole nitrogen lone pair is no longer conjugated with the quinone carbonyl group and, hence, "normal" indole reactivity takes over, resulting in the elimination of the aryloxy group, in the case of **1** 4-nitrophenoxide, from the 3-indolylmethyl position to generate a highly electrophilic iminium ion **3**. Because this occurs in the enzyme active site, the generation of **3** leads to irreversible alkylation of the enzyme,

Scheme 1. Proposed Mechanism-Based Inhibition of NQO1 by the 3-(4-Nitrophenoxy)methylindolequinone **1**[a]



[a] Ar = 4-nitrophenyl.

shown by our mass spectrometric studies to be at Tyr-127 or Tyr-129[11] and, hence, its inhibition.

It was of considerable interest when it was recently reported that inhibition of NQO1 in pancreatic tumor cells using dicumarol resulted in inhibition of cell growth of the malignant in vitro phenotype of cells.[16] Although it only accounts for less than 10% of all cancers, pancreatic cancer has one of the worst prognoses. It is rapidly fatal; data from Cancer Research UK show that one-year survival rates are less than 15% (five-year survival, ca. 2%). Surgery and radiation are the common treatments because, to date, chemotherapy has made little or no impact. It was proposed that the effect of dicumarol on pancreatic tumor cell growth was mediated by inhibition of NQO1, causing a rise in superoxide levels.[16] We have recently shown that NQO1 can directly scavenge superoxide, and in cells containing high levels of the enzyme (i.e., most solid tumors) scavenging by NQO1 competes with superoxide dismutase (SOD).[17] These data provide support for the hypothesis that inhibition of NQO1 could lead to increased levels of superoxide and, given the low levels of SOD in pancreatic cancer cell lines,

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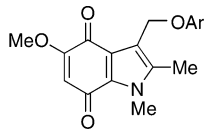
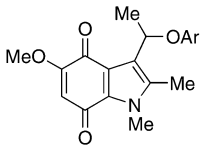
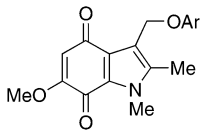


1 Ar = 4-NO_2-C_6H_4 **(ES936)**
5 Ar = C_6H_5
6 Ar = 3-NO_2-C_6H_4
7 Ar = 2-NO_2-C_6H_4
8 Ar = 2,4-$(NO_2)_2$-C_6H_3
9 Ar = 2-F-4-NO_2-C_6H_3
10 Ar = 2,4,6-F_3-C_6H_2
11 Ar = 2-pyridyl
12 Ar = 3-pyridyl
13 Ar = 4-pyridyl



14 Ar = 4-NO_2-C_6H_4



15 Ar = 4-NO_2-C_6H_4
16 Ar = C_6H_5
17 Ar = 4-NH_2-C_6H_4
18 Ar = 3-NO_2-C_6H_4
19 Ar = 2-NO_2-C_6H_4
20 Ar = 2,4-$(NO_2)_2$-C_6H_3
21 Ar = 2-F-4-NO_2-C_6H_3
22 Ar = 4-CN-C_6H_4
23 Ar = 4-CF_3-C_6H_4
24 Ar = 4-F-C_6H_4
25 Ar = 2,4-F_2-C_6H_3
26 Ar = 2,4,6-F_3-C_6H_2
27 Ar = 2-pyridyl
28 Ar = 3-pyridyl
29 Ar = 4-pyridyl

Figure 1. Structures of NQO1 inhibitors.

renders the contribution of NQO1, the levels of which are known to be elevated, potentially extremely important in regulating superoxide in these cells. Although the interaction of dicumarol with NQO1 has been characterized crystallographically,[18] the major problem with this inhibitor, however, is its lack of selectivity, because it is known to inhibit many other enzymes.[6] The indolequinone **1**, on the other hand, is a mechanism-based inhibitor of NQO1, and we have confirmed that, like dicumarol, it is a potent inhibitor of human pancreatic cancer cell growth in vitro. The compound is also effective in vivo; MIA PaCa-2 xenografts in mice grow significantly slower after treatment with **1**.[19] However, in the case of **1**, our studies showed that the cytotoxicity was independent of superoxide generation, and therefore raises the question of whether NQO1 inhibition is the only mechanism operating in pancreatic cancer cells.

To gain additional insights, we initiated a much wider study of quinone based inhibitors of NQO1, and we now describe the synthesis of a number of new analogs of **1**, their biological evaluation as mechanism-based inhibitors of NQO1 and their toxicity toward pancreatic tumor cell lines. Some preliminary data on six of these quinones have been included in our previous publications.[11,19,20]

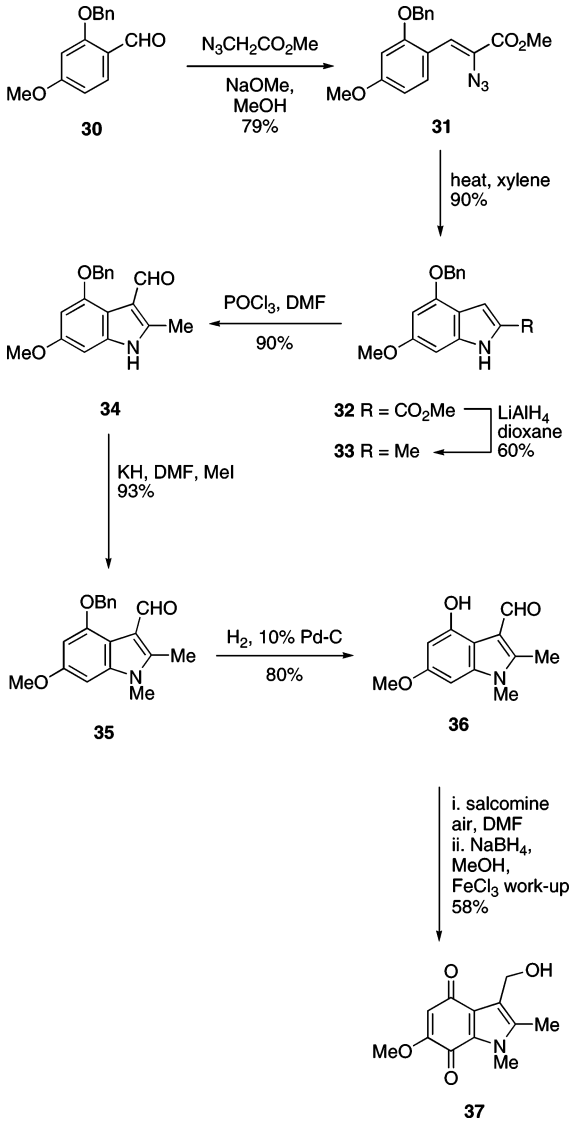
Results and Discussion

Chemistry. The structures of the new NQO1 inhibitors are shown in Figure 1. 5-Methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione **1** was prepared as previously described,[9] and nine further analogs **5**−**13** were synthesized, in which the 4-nitrophenoxy leaving group (4-nitrophenol pK_a = 7.2) was replaced by a range of other phenolic leaving groups. These were chosen to explore both the effect of leaving group ability, as evidenced by the pK_a of the corresponding phenol, and the steric effects of the 3-aryloxymethyl group. We were also conscious of the need to identify possible alternative electron-withdrawing groups on the phenolic moiety, because nitro (NO_2) groups are not without problems in potential drug molecules due to their metabolism. Hence, the choice of 2,4,6-trifluorophenoxy group was made because 2,4,6-trifluorophenol has a similar pK_a to 4-nitrophenol (7.5 and 7.2, respectively). Of these nine compounds, the phenoxy-,[21] the 2,4-dinitrophenoxy-,[22] and the 2-fluoro-4-nitrophenoxy-compounds,[22] **5**, **8**, and **9**, had been prepared previously. In general, two methods were used to prepare the new 3-aryloxylmethylindolequinones, both starting from the known 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione.[23] First, the primary alcohol was treated with thionyl chloride, and the resulting chloride reacted with a phenol in the presence of a base, usually potassium carbonate. The 3-aryloxylmethylindolequinones **6**, **7**, and **10** were prepared in this manner. Alternatively, the 3-hydroxymethyl compound was reacted with the phenol under the conditions of the Mitsunobu reaction in the presence of a triarylphosphine and a dialkyl azodicarboxylate. The indolequinones **11**−**13** were thus obtained: in the case of 2-hydroxypyridine, both *N*- and *O*-linked products were formed. The last analog in this series of compounds was the 3-[1-(4-nitrophenoxy)ethyl]indolequinone **14**;[24] in principle, the additional methyl group should stabilize the intermediate iminium ion and, thereby, affect rates of fragmentation of the hydroquinone **2** (Scheme 1).

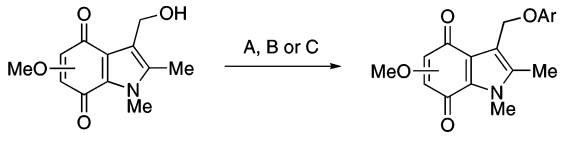
We have also investigated an isomeric series of NQO1 inhibitors based on the 6-methoxyindole-4,7-quinone ring system. Although this may appear a trivial change, the switch of the electron-releasing methoxy group from the 5- to the 6-position has an effect on the electronic properties of the quinone ring system and, hence, on the reductive elimination of phenoxide as required for NQO1 inhibition (cf. Scheme 1). Hence, it was by no means obvious that such analogs would be potent inhibitors because the position of the methoxy group affects the electrophilicity of the alkylating species formed upon loss of the leaving group—the 6-methoxy group, but not the 5-methoxy, can donate electron density toward the key exocyclic carbon. The synthesis of the 6-methoxyindolequinone series is summarized in Scheme 2. Thus, 2-benzyloxy-4-methoxybenzaldehyde **30**[25] was condensed with methyl azidoacetate, and the resulting azidocinnamate **31** was cyclized to the indole **32** in a Hemetsberger indole synthesis.[26−28] Treatment of the ester **32** with lithium aluminum hydride under forcing conditions resulted in reduction to the 2-methylindole **33** in satisfactory yield. Formylation under Vilsmeier−Haack conditions gave the indole-3-carbaldehyde **34** in excellent yield, and this was followed by *N*-methylation to give the indole **35**. Thereafter, the indole-3-carboxaldehyde was progressed to the corresponding 3-hydroxymethylindolequinone **37**, as outlined in Scheme 2.

The attachment of phenolic leaving groups to the 3-hydroxymethyl-6-methoxyindolequinone **37** was for the most part accomplished by conversion to the 3-chloromethylindole, followed by reaction with the corresponding phenol in the presence of base, as described above for the isomeric 5-methoxy series. The indolequinones **15**, **16**, **18**, **19**, **21**−**26**, and **28** were all prepared in this manner. The 2,4-dinitrophenoxy compound **20** was prepared by reaction of the hydroxymethyl compound **37**

Scheme 2. Preparation of 3-Hydroxymethyl-6-methoxy-1,2-dimethylindole-4,7-dione



Scheme 3. Preparation of 3-Aryloxymethylindolequinones from Corresponding 5- or 6-Methoxy-3-hydroxymethyl Derivatives



Method A: (i) $SOCl_2$, CH_2Cl_2; (ii) ArOH, K_2CO_3, acetone
Method B: ArOH, Ph_3P, $RO_2CN{=}NCO_2R$, THF
Method C (for Ar = 2,4-$(NO_2)_2$-C_6H_3): 2,4-$(NO_2)_2$-C_6H_3F, TBAF, THF

with 2,4-dinitrofluorobenzene, and the 2- and 4-pyridyl derivatives **27** and **29** were obtained using the Mitsunobu reaction (Scheme 3). The 3-(4-aminophenoxy)methyl compound **17** was prepared from 4-azidophenol[29] by coupling to the hydroxymethyl compound **37** followed by reduction of the azide group.

Biology. All 26 indolequinones **1** and **5**−**29** were assayed for their ability to inhibit the quinone reductase NQO1 using a series of assays employing both purified recombinant human NQO1 and NQO1-rich cells. Initially, experiments were carried out to determine whether members of this series of indolequinones were mechanism-based inhibitors of NQO1 (Table 1, Figure 2). For these experiments, indolequinones were incubated with rhNQO1[a] in the absence and presence of NADH. At the concentrations of indolequinones used in this assay, only minor inhibition of NQO1 activity was observed in the absence of NADH, indicating that inhibition of NQO1 by these indolequinones occurred following catalytic turnover of the indolequinone by NQO1. Representative examples of NADH-dependent inhibition of NQO1 by indolequinones are shown in Figure 2.

The dependence on NADH for inhibition was taken as preliminary evidence for mechanism-based inactivation of NQO1,[14] and compounds showing such properties were studied further. An important term used to describe the efficiency of inactivation for mechanism-based enzyme inhibitors is the partition ratio. The partition ratio is defined as the number of catalytic cycles required to inhibit one molecule of enzyme. In these experiments, partition ratios were determined by incubating defined molar ratios of indolequinone and rhNQO1 in the presence of NADH and measuring the resultant NQO1 activity. Partition ratios for the inhibition of NQO1 by this series of indolequinones are shown in Table 1. Many of the indolequinones in this study had partition ratios near 1, indicating very efficient inactivation of NQO1, and 13 out of 26 indolequinones in this series had partition ratios less than 5. The 2,4-dinitrophenoxy derivatives **8** and **20** displayed poor solubility characteristics and precipitated out of solution, so partition ratios could not be determined.

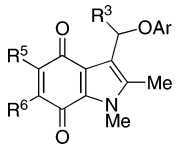
The ability of these indolequinones to inhibit NQO1 in cells was examined using the human pancreatic cancer cell line MIA PaCa-2 (NQO1 activity; 1000 nmol 2,6-dichlorophenol-indophenol (DCPIP)/min/mg). For these studies, MIA PaCa-2 cells were treated with indolequinones (1−10 000 nM) for 1 h, the cells were then washed free of drug and NQO1 activity was measured in cell sonicates. Results from these experiments clearly demonstrated that these indolequinones could enter cells and inactivate NQO1 (Figure 3 and Table 1). For most indolequinones in this study greater than 95% of NQO1 activity could be inhibited at indolequinone concentrations between 10 and 100 nM (Table 1). However, indolequinones with large partition ratio such as **23** or **24** required much higher concentrations to achieve 95% inhibition of NQO1 (Figure 3, Table 1).

Finally, the ability of these indolequinones to induce cytotoxicity was measured in MIA PaCa-2 cells using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) growth inhibition assay (Table 1). Because previous studies with mechanism-based inhibitors **1**,**15**, **23**, and **29** have demonstrated near complete inhibition of NQO1 catalytic activity after 4 h, this time point, as well as a longer time point of 72 h, were selected for growth inhibition studies in the MIA PaCa-2 cells. The IC_{50} values are reported for this extended series of compounds for both 4 and 72 h treatments with the appropriate indolequinone (Table 1). The growth inhibitory potency of these compounds after 4 h of incubation indicates that compounds **1**, **15**, **17**, **6**, **18**, **7**, **19**, **21**, **22**, **23**, **25**, **10**, and **26** were effective inhibitors of the growth of MIA PaCa-2 cells (IC_{50}, 4 h, <650 nM), while compounds **5**, **16**, **8**, **20**, **9**, **24**, **11**, **27**, **12**, **28**, **13**, **29**, and **14** were relatively ineffective at inducing growth inhibition (IC_{50}, 4 h, >900 nM).

The phenol and pyridine-based leaving groups derived from each of the indolequinones tested were also examined for growth inhibitory potency. The compounds tested included phenol, 2-, 3-, and 4-nitrophenol, 2-fluoro-4-nitrophenol, 4-fluorophenol,

[a] Abbreviations: rhNQO1, recombinant human NAD(P)H:quinone oxidoreductase 1; DCPIP, 2,6-dichlorophenol-indophenol; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; TBAF, tetra-*n*-butylammonium fluoride.

Table 1. Inhibition of hNQO1 by Indolequinones **1** and **5**−**29** and Inhibition of Cell Growth in the MIA PaCa-2 Cell Line



No.	R^3	R^5	R^6	Ar	mechanism-based inhibition[a]	partition ratio[b]	>90% inhibition of NQO1 in MIA PaCa-2 cells[b] nM	IC_{50} MIA PaCa-2 4 h nM[c]	IC_{50} MIA PaCa-2 72 h nM[c]
1	H	OMe	H	4-NO_2-C_6H_4	yes	3.5	10−100	629 ± 17	508 ± 5
15	H	H	OMe	4-NO_2-C_6H_4	yes	3.7	10−100	638 ± 15	355 ± 3
5	H	OMe	H	C_6H_5	yes	4000	nd	1385 ± 24	962 ± 18
16	H	H	OMe	C_6H_5	yes	3800	nd	4563 ± 26	409 ± 30
17	H	H	OMe	4-NH_2-C_6H_4	no	nd	nd	504 ± 4	160 ± 4
6	H	OMe	H	3-NO_2-C_6H_4	yes	2.3	10−100	352 ± 16	258 ± 21
18	H	H	OMe	3-NO_2-C_6H_4	yes	3.5	10−100	351 ± 25	257 ± 22
7	H	OMe	H	2-NO_2-C_6H_4	yes	1.0	nd	345 ± 20	104 ± 18
19	H	H	OMe	2-NO_2-C_6H_4	yes	1.0	10−100	363 ± 9	178 ± 4
8	H	OMe	H	2,4-$(NO_2)_2$-C_6H_3	see text	nd	nd	NC	NC
20	H	H	OMe	2,4-$(NO_2)_2$-C_6H_3	see text	nd	nd	NC	NC
9[d]	H	OMe	H	2-F-4-NO_2-C_6H_3	yes	0.8	nd	4654 ± 48	3556 ± 31
21	H	H	OMe	2-F-4-NO_2-C_6H_3	yes	0.8	10−100	529 ± 5	417 ± 5
22	H	H	OMe	4-CN-C_6H_4	yes	37	nd	463 ± 6	192 ± 3
23	H	H	OMe	4-CF_3-C_6H_4	yes	652	5000−10 000	496 ± 3	311 ± 5
24	H	H	OMe	4-F-C_6H_4	yes	>100 000	5000−10 000	905 ± 25	493 ± 32
25	H	H	OMe	2,4-F_2-C_6H_4	yes	21.3	10−100	255 ± 5	75 ± 4
10	H	OMe	H	2,4,6-F_3-C_6H_4	yes	1.9	nd	427 ± 5	86 ± 3
26	H	H	OMe	2,4,6-F_3-C_6H_4	yes	1.7	10−100	452 ± 4	212 ± 3
11	H	OMe	H	2-pyridyl	yes	nd	<10	NC	NC
27	H	H	OMe	2-pyridyl	yes	1.1	10−100	9579 ± 48	2393 ± 28
12	H	OMe	H	3-pyridyl	yes	6.1	10−100	904 ± 26	562 ± 24
28	H	H	OMe	3-pyridyl	yes	nd	nd	2475 ± 40	1824 ± 19
13	H	OMe	H	4-pyridyl	yes	1.3	nd	2007 ± 16	2172 ± 25
29	H	H	OMe	4-pyridyl	yes	0.9	10−100	2560 ± 7	3271 ± 15
14	Me	OMe	H	4-NO_2-C_6H_4	yes	9.1	nd	1829 ± 14	1696 ± 23

[a] Classification of mechanism-based inhibition refers to dependence (or not) on NADH. [b] nd = not determined. [c] NC denotes no convergence. The IC_{50} value cannot be determined as profile is a straight line. [d] Compound **9** was found to be unstable in DMSO and was tested immediately for NQO1 mechansim-based inhibition.

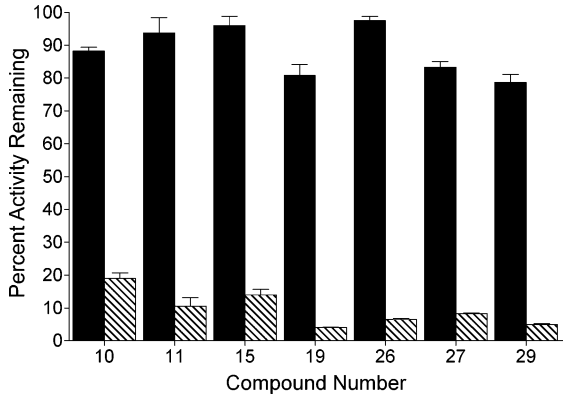


Figure 2. Mechanism based inhibition of NQO1 by representative indolequinones. NQO1 activity was assayed following the incubation of indolequinone (100 nM) with rhNQO1 (1 μg) in the absence (solid bars) and presence (striped bars) of 0.2 mM NADH. Incubations (0.5 mL) were performed for 5 min at 32 °C. Results are the mean ± standard deviation of three separate determinations.

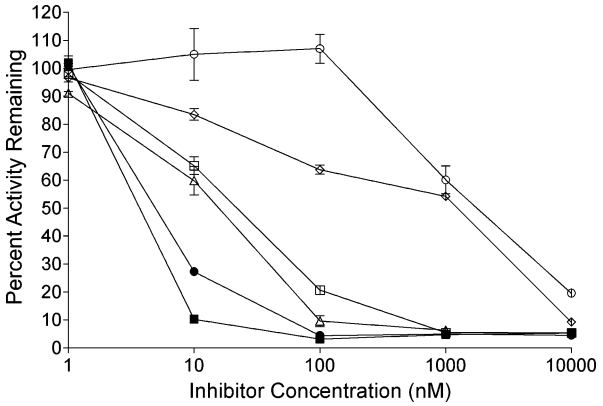


Figure 3. Inhibition of NQO1 activity in MIA PaCa-2 cells treated with indolequinones. NQO1 activity was measured in MIA PaCa-2 cells following treatment with indolequinones (1 nM−10 μM) for 1 h. Results are the mean ± standard deviation of three separate determinations. Open circles, **24**; open diamonds, **23**; open squares, **22**; open triangles, **12**; closed circles, **6**; closed squares, **19**.

2,4-difluorophenol, 2,4,6-trifluorophenol, 4-trifluoromethylphenol, 4-cyanophenol, 4-aminophenol, 2-, 3-, and 4-hydroxypyridine. All compounds were relatively nontoxic (data shown in Supporting Information). For example, all IC_{50} values obtained by MTT analysis were greater than 12 900 nM after 72 h of incubation of these compounds with MIA PaCa-2 cells. These data indicate that growth inhibitory properties of the indolequinones are unlikely to be related to the liberation of phenols or pyridines from the parent molecule.

Interestingly, the indolequinones **27**, **12**, **28**, **13**, **29**, and **14** were potent mechanism-based inhibitors of NQO1 activity, however displayed poor growth inhibitory profiles. This finding indicates dissociation of NQO1 inhibitory activity and the ability to induce growth inhibition in MIA PaCa-2 cells for these indolequinone compounds and implies a function via an NQO1-independent mechanism to inhibit cell proliferation.

In addition, compounds **5**, **16**, and **24**, which are very poor inhibitors of NQO1 as indicated by high partition ratios

demonstrated decreases in IC_{50} value after 72 h relative to 4 h of incubation with cells and the change with compound **4** was dramatic from 4563 nM to 409 nM. This observation also argues for an NQO1-independent mechanism of toxicity in MIA PaCa-2 cells.

Discussion. To make a more detailed comparison of the efficiency of NQO1 inactivation, the partition ratios of the indolequinones were examined. Within the series of analogs of **1** bearing a 5-methoxy group, there was a loose correlation of the partition ratio with the leaving group ability of the aryloxy group at the 3-indolylmethyl position, as evidenced by the pK_a of the corresponding phenol. Certainly, compound **9** with the most acidic phenol leaving group, 2-fluoro-4-nitrophenol (pK_a = 5.7), has the lowest partition ratio, while the higher are exhibited by the indolequinones **5** and **24**, which have the poorest leaving group (phenol pK_a = 9.9, 4-fluorophenol pK_a = 9.9). It is also noteworthy that a nitrophenolate leaving group is not essential because the indolequinone **10** bearing a 2,4,6-trifluorophenoxy group (2,4,6-trifluorophenol pK_a = 7.5) is a highly efficient inhibitor. Likewise, the compounds **12** and **13** with 3-hydroxypyridine and 4-hydroxypyridine (4-pyridone) leaving groups (3-hydroxypyridine pK_a = 8.51, 4-hydroxypyridine pK_a = 5.2) were also efficient inhibitors of NQO1. Finally, compound **14** bearing the additional methyl group at the 3-indolylmethyl position is a poorer inhibitor and less cytotoxic than its des-methyl analog **1**, notwithstanding the fact that the additional methyl group should stabilize the intermediate iminium ion and, thereby, affect rates of fragmentation of the hydroquinone **2** (Scheme 1).

Although it is not obvious that the isomeric series of NQO1 inhibitors based on the 6-methoxyindole-4,7-quinone ring system would be inhibitors of NQO1 due to the electronic effect of the switching of the methoxy group from the 5- to the 6-position, the indolequinones **15**, **16**, and **18**−**29** inactivated the enzyme. In fact, the 6-methoxy analog **15** of **1** is equipotent with the 5-methoxy isomer. Likewise, in the five pairs of compounds where the leaving groups are phenoxide (compounds **5** and **16**), 2-nitrophenoxide (**7** and **19**), 2-fluoro-4-nitrophenoxide (compounds **9** and **21**), 2,4,6-trifluorophenoxide (compound **10** and **26**), or the 4-pyridyloxy (compounds **13** and **29**), the 5- and 6-methoxy isomers exhibit very similar biological profiles as inhibitors of NQO1, as evidenced by their similar partition ratios. Therefore, surprisingly, the position of the electron releasing methoxy group appears to have little effect on the ability of the indolequinones to undergo reduction by NQO1, followed by elimination of the aryloxy group from the 3-indolylmethyl position with the ensuing inactivation of the enzyme.

As with the 5-methoxy series of indolequinones, there also appears to be a relationship between the pK_a of the aryloxy leaving group at the 3-indolylmethyl position and the efficiency of enzyme inhibition, as measured by the partition ratio, in the 6-methoxyindolequinones. This is nicely illustrated by the series of fluorophenoxy compounds **24**−**26**, where the compounds become more potent inhibitors as the pK_a of the leaving group decreases as the degree of fluorine substitution increases (4-fluorophenol pK_a = 9.9; 2,4-difluorophenol pK_a = 8.7; 2,4,6-trifluorophenol pK_a = 7.5).

Next, the inhibition of NQO1 in cellular systems by representative indolequinones was examined to verify inhibition of NQO1, the proposed intracellular target of these compounds, and ensure that quinones were taken up into cells. Importantly, there was a general relationship between the partition ratio measured using purified NQO1 and the ability of the indolequinones to inhibit NQO1 in MIA PaCa-2 cells. Hence, it was shown that the indolequinones **1**, **6**, **15**, **21**, **25**, **26**, **27**, and **29** all caused >95% inhibition of enzyme activity in the human pancreatic MIA PaCa-2 solid tumor cell line after 1 h. The data for compounds **1**, **15**, **29**, **5**, **6**, **13**, and **29** were included in our previous paper,[20] and the data for the remaining compounds are shown in Figure 3.

In previous studies, we showed that **1** inhibited the growth of human pancreatic MIA PaCa-2 cancer cells, and therefore, it was of interest to evaluate this indolequinone series in the same cell line. Although greater than 95% inhibition of NQO1 occurs after a 1 h exposure to indolequinones, MIA PaCa-2 cells were treated with indolequinones for 4 h to maintain a prolonged period of NQO1 inhibition because, once these inhibitors are removed, NQO1 activity slowly returns due to the synthesis of new NQO1 protein. A longer exposure period (72 h) was also used for comparative purposes, and the IC_{50} values are reported for both 4 and 72 h treatments in Table 1. The 26 indolequinones tested exhibited IC_{50} values in the range of about 0.1−9.5 μM (100−9500 nM) with the 2- and 3-nitrophenyl (**6**, **7**, **18**, **19**), with the di- and tri-fluorophenyl (**10**, **25**, **26**) derivatives being among the most potent growth inhibitors, and the pyridyloxy derivatives (**13**, **27**−**29**) the least effective. Hence, it is clear that potent NQO1 inhibitors such as the pyridyloxy derivatives do not exhibit the highest levels of cytotoxicity. Conversely, relatively poor inhibitors such as the 4-aminophenoxy **17** and 4-trifluoromethylphenoxy **23** compounds demonstrated potent cell growth inhibition at both time periods. In addition, the relatively poor inhibitors, the phenoxy derivatives **5** and **16** and the 4-fluorophenoxy **24** compound, demonstrated potentiated cell growth inhibition with increasing drug exposure. These data on a large set of indolequinones reinforce our earlier observations[20] that NQO1 inhibition can be divorced from cell growth inhibition in the human pancreatic MIA PaCa-2 cell line at least.

In summary, we have examined a series of 26 indolequinones, most of them previously unreported, and evaluated their capacity to inhibit NQO1 in cell-free and cell-based systems, together with their ability to inhibit cell proliferation in the human pancreatic MIA PaCa-2 cancer cell line. We have characterized new mechanism-based inhibitors of NQO1 and shown that their partition ratios reflect their ability to inhibit NQO1 in tumor cells. Our data demonstrate that NQO1 inhibition does not correlate with growth inhibitory activity, at least in the MIA PaCa-2 cell line, suggesting that targets in addition to NQO1 need to be considered to explain the potent activity of this series of indolequinones in human pancreatic cancer cells.

Experimental Section

Chemistry. General Experimental Details. Commercially available reagents were used throughout without purification unless otherwise stated. Light petroleum refers to the fraction with a bp 40−60 °C and was distilled before use. Ether refers to diethyl ether. Reactions were routinely carried out under a nitrogen or argon atmosphere. Analytical thin layer chromatography was carried out on aluminum-backed plates coated with Merck Kieselgel 60 GF_{254} and visualized under UV light at 254 and/or 360 nm. Chromatography was carried out using Merck Kieselgel 60 H silica or Matrex silica 60. Fully characterized compounds were chromatographically homogeneous.

UV/vis spectra were recorded on a Phillips PU 8700 series spectrophotometer. Infrared spectra were recorded in the range 4000−600 cm^{-1} using Nicolet Magna FT-550, Perkin-Elmer 1600 series, or Avatar 320 FT-IR spectrometers. NMR spectra were carried out on Jeol EX270, Bruker AC300, AV400, and DRX500 instruments (operating at ^1H frequencies of 270, 300, 400, and 500 MHz, respectively; corresponding ^{13}C frequencies are 67.5, 75, 100,

and 125 MHz). Chemical shifts are quoted in ppm with TMS as internal standard. *J* values are recorded in Hz. In the ^{13}C spectra, signals corresponding to CH, CH_2, or CH_3 groups, as assigned from DEPT, are noted; all others are C. High and low-resolution mass spectra were recorded on Micromass GCT TDF, Bruker MicroTof, or VG Autospec spectrometers or at the EPSRC Mass Spectrometry Center (Swansea).

General Method A. Coupling of 3-(Hydroxymethyl)indolequinones with Substituted Phenols via the 3-Chloromethylindole. The following general procedure was used unless otherwise stated: To a stirred solution of the 5-methoxy or 6-methoxy 3-hydroxymethyl-1,2-dimethylindole-4,7-dione (0.1−0.2 mmol, 1 equiv) in dichloromethane (5 mL) at 0 °C was added thionyl chloride (50 equiv) dropwise. The reaction mixture was stirred at room temperature for 1 h. The solvent and excess thionyl chloride were removed in vacuo and the crude product was used directly in the next step without further purification. A mixture of the crude 3-chloromethylindolequinone, the phenol (3 equiv), and the potassium carbonate (3 equiv) was stirred in DMF (5 mL) for 16 h. The solvent was removed in vacuo and the crude product was dissolved in dichloromethane (50 mL), washed with NaOH (2 M; 20 mL), HCl (2 M; 20 mL), and water (20 mL), dried ($MgSO_4$), and filtered, and the filtrate was evaporated in vacuo. The crude product was purified by chromatography.

General Method B. Coupling of 3-(Hydroxymethyl)indolequinones with Substituted Phenols via the Mitsunobu Reaction. The following general procedure was used unless otherwise stated: the dialkyl azodicarboxylate (4 equiv) was added to a solution of the 5-methoxy or 6-methoxy 3-(hydroxymethyl)-1,2-dimethylindole-4,7-dione (100 mg, 0.4 mmol), triarylphosphine (3 equiv), and the phenol (3 equiv) in THF (15 mL). The solution was stirred for 1 h. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate and washed with sodium hydroxide (1 M), hydrochloric acid (1 M), and water, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography.

5-Methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione 1 (ES936). Prepared as previously described.[9]

5-Methoxy-1,2-dimethyl-3-(phenoxymethyl)indole-4,7-dione 5. Prepared as previously described.[21]

5-Methoxy-1,2-dimethyl-3-(3-nitrophenoxymethyl)indole-4,7-dione 6. Using method A, 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione[23] (30 mg, 0.13 mmol), thionyl chloride (0.75 mL, 10.3 mmol), 3-nitrophenol (53 mg, 0.38 mmol), and potassium carbonate (53 mg, 0.38 mmol) gave after chromatography (elution with 20% ethyl acetate/light petroleum) the title compound (33 mg, 44%) as an orange crystalline solid: mp 219−227 °C; ^1H NMR δ 7.82 (2 H, m, ArH), 7.43 (1 H, t, *J* 8.4, ArH), 7.29 (1 H, m, ArH), 5.64 (1 H, s, 6-H), 5.36 (2 H, s, CH_2), 3.91 (3 H, s, Me), 3.82 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.8 (C), 178.2 (C), 160.0 (C), 159.1 (C), 149.2 (C), 138.0 (C), 130.0 (CH), 129.0 (C), 121.6 (CH), 121.4 (C), 115.9 (overlapping CH and C), 109.9 (CH), 106.8 (CH), 61.1 (CH_2), 56.5 (Me), 32.4 (Me), 9.9 (Me); MS (ESI) 379 (M + Na^+, 100%), 218 (71); found, M + Na^+, 379.0902. $C_{18}H_{16}N_2O_6$ + Na requires 379.0906.

5-Methoxy-1,2-dimethyl-3-(2-nitrophenoxymethyl)indole-4,7-dione 7. Using method A, 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (30 mg, 0.13 mmol), thionyl chloride (1.2 mL, 16.5 mmol), 2-nitrophenol (124.3 mg, 0.89 mmol), and potassium carbonate (123.5 mg, 0.89 mmol) gave after chromatography (elution with ethyl acetate) the title compound (24.6 mg, 54%) as an orange crystalline solid: mp 204−205 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (1 H, dd, *J* 1.6, 8.0, ArH); 7.50 (1 H, dt, *J* 1.2, 8.4, ArH), 7.41 (1 H, dd, *J* 0.6, 8.4, ArH), 7.00 (1 H, dt, *J* 0.6, 8.0, ArH), 5.63 (1 H, s, 6-H), 5.50 (2 H, s, CH_2), 3.89 (3 H, s, Me), 3.82 (3 H, s, Me), 2.26 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.6 (C), 178.5 (C), 159.5 (C), 151.6 (C), 140.5 (C), 139.0 (C), 134.2 (CH), 128.7 (C), 125.4 (CH), 121.2 (C), 120.7 (CH), 116.4 (CH), 115.9 (C), 106.8 (CH), 62.1 (CH_2), 56.2 (Me), 32.4 (Me), 9.9 (Me); MS (ESI) 379 (M + Na^+, 100); found, M + Na^+, 379.0883. $C_{18}H_{16}N_2O_6$ + Na requires 379.0906.

5-Methoxy-1,2-dimethyl-3-(2,4-dinitrophenoxymethyl)indole-4,7-dione 8. Prepared as previously described.[22]

3-(2-Fluoro-4-nitrophenoxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione 9. Prepared as previously described.[22]

5-Methoxy-1,2-dimethyl-3-(2,4,6-trifluorophenoxymethyl)indole-4,7-dione 10. Using method A, 3-(hydroxymethyl)-5-methoxy-1,2-dimethylindole-4,7-dione (30 mg, 0.13 mmol), thionyl chloride (0.85 mL, 11.7 mmol), 2,4,6-trifluorophenol (56.7 mg, 0.38 mmol), and potassium carbonate (52.9 mg, 0.38 mmol) gave after chromatography (elution with ethyl acetate) the title compound (28.9 mg, 62%) as an orange-red crystalline solid: mp 201−202 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 6.67 (2 H, t, *J* 8.0, ArH), 5.62 (1 H, s, 6-H), 5.33 (2 H, s, CH_2), 3.93 (3 H, s, Me), 3.81 (3 H, s, Me), 2.35 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8 (C), 176.7 (C), 156.3 (dt, J_{CF} 245, 14, CF), 155.5 (ddd, J_{CF} 250, 14, 8, CF), 155.0 (C), 137.9 (C), 130.8 (td, J_{CF} 14, 6, C), 127.7 (C), 120.5 (C), 115.2 (C), 105.6 (C), 99.5 (ddd, J_{CF} 27, 27, 8, CH), 64.9 (CH_2), 55.4 (Me), 31.4 (Me), 8.5 (Me); MS (ESI) 388 (M + Na^+, 100%), 363 (4); found, M + Na^+, 388.0773. $C_{18}H_{14}F_3NO_4$ + Na requires 388.0773; Anal. ($C_{18}H_{14}F_3NO_4$) C, H, N.

5-Methoxy-1,2-dimethyl-3-(pyridin-2-yloxymethyl)indole-4,7-dione 11. Using method B, 2-hydroxypyridine (25 mg, 0.26 mmol), triphenylphosphine (101 mg, 0.39 mmol), diethyl azodicarboxylate (0.047 mL, 0.30 mmol), and 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (30 mg, 0.13 mmol) gave after chromatography (ethyl acetate elution) the title compound (20 mg, 50%) as an orange solid, mp 138−140 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.18 (1 H, ddd, *J* 0.8, 2.0, 5.2, PyrH), 7.54 (1 H, ddd, *J* 2.0, 7.2, 8.4, PyrH), 6.86 (1 H, ddd, *J* 0.8, 5.2, 6.8, PyrH), 6.72 (1 H, dt, *J* 0.8, 8.4, PyrH), 5.61 (1 H, s, 6-H), 5.51 (2 H, s, CH_2), 3.90 (3 H, s, Me), 3.81 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.9 (C), 177.7 (C), 163.7 (C), 159.8 (C), 146.8 (C), 138.5 (CH), 138.0 (CH), 121.9 (C), 117.2 (2 C), 116.8 (CH), 111.2 (CH), 106.6 (CH), 58.0 (CH_2), 56.4 (Me), 32.4 (Me), 9.7 (Me); MS (ESI) 335 (M + Na^+, 100%), 218 (82); found, M + Na^+, 335.1002. $C_{17}H_{16}N_2O_4$ + Na requires 335.1008.

Also formed was **5-methoxy-1,2-dimethyl-3-(2-pyridon-1-ylmethyl)indole-4,7-dione** (8.4 mg, 21% yield) as an orange solid: mp 226−232 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (1 H, dd, *J* 2.0, 6.8, PyrH), 7.26 (1 H, ddd, *J* 2.0, 6.4, 8.8, PyrH), 6.50 (1 H, d, *J* 8.8, PyrH), 6.10 (1 H, td, *J* 1.2, 6.8, PyrH), 5.62 (1 H, s, 6-H), 5.22 (2 H, s, CH_2), 3.88 (3 H, s, Me), 3.82 (3 H, s, Me), 2.47 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.5 (2 C), 158.5 (C), 138.6 (CH), 138.4 (C), 138.3 (CH), 131.2 (C), 128.1 (C), 120.4 (CH), 119.5 (C), 115.2 (C), 105.8 (CH), 104.9 (CH), 55.5 (Me), 41.4 (CH_2), 31.6 (Me), 9.1 (Me); MS (ESI) 335 (M + Na^+, 100%), 218 (50); found, M + Na^+, 335.1006. $C_{17}H_{16}N_2O_4$ + Na requires 335.1008.

5-Methoxy-1,2-dimethyl-3-(3-pyridyloxymethyl)indole-4,7-dione 12. Using method B, 3-hydroxypyridine (40.8 mg, 0.43 mmol), triphenylphosphine (169 mg, 0.64 mmol), diethyl azodicarboxylate (0.079 mL, 0.50 mmol), and 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (50 mg, 0.21 mmol) gave after chromatography (ethyl acetate elution) the title compound (34 mg, 51%) as an orange solid: mp 158−162 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 8.38 (1 H, bs, PyrH), 8.25 (1 H, d, *J* 0.8, PyrH), 7.45 (1 H, dd, *J* 2.0, 8.4, PyrH), 7.30 (1 H, dd, *J* 4.0, 8.4, PyrH), 5.66 (1 H, s, 6-H), 5.39 (2 H, s, CH_2), 3.93 (3 H, s, Me), 3.84 (3 H, s, Me), 2.34 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7 (C), 178.2 (C), 159.6 (C), 154.8 (C), 141.9 (CH), 138.4 (CH), 138.1 (C), 128.9 (C), 124.1 (CH), 121.8 (CH), 121.3 (C), 116.3 (C), 106.8 (CH), 60.8 (CH_2), 56.5 (Me), 32.4 (Me), 9.9 (Me); MS (ESI) 313 (MH^+, 100%), 218 (76); found, MH^+, 313.1174. $C_{17}H_{16}N_2O_4$ + H requires 313.1188.

5-Methoxy-1,2-dimethyl-3-(pyridin-4-yloxymethyl)indole-4,7-dione 13. Using method B, 4-hydroxypyridine (40.8 mg, 0.43 mmol), triphenylphosphine (169 mg, 0.64 mmol), diethyl azodicarboxylate (0.079 mL, 0.50 mmol), and 3-hydroxymethyl-5-methoxy-1,2-dimethylindole-4,7-dione (50 mg, 0.21 mmol) gave after chromatography (ethyl acetate elution) the title compound (25.5 mg, 38%) as an orange solid: mp 195−201 °C; ^1H NMR

(400 MHz, $CDCl_3$) δ 8.40 (2 H, br s, PyrH), 6.89 (2 H, d, *J* 4.8, PyrH), 5.62 (1 H, s, 6-H), 5.34 (2 H, s, CH_2), 3.89 (3 H, s, Me), 3.80 (3 H, s, Me), 2.29 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.7 (C), 178.3 (C), 164.5 (C), 159.6 (C), 151.1 (CH), 138.1 (C), 128.9 (C), 121.2 (C), 115.9 (C), 110.6 (CH), 106.8 (CH), 60.1 (CH_2), 56.5 (Me), 32.4 (Me), 9.9 (Me); MS (ESI) 218 (MH^+, 100%), 313 (9); found, MH^+, 313.1190. $C_{17}H_{16}N_2O_4$ + H requires 313.1188.

5-Methoxy-1,2-dimethyl-3-[1-(4-nitrophenoxy)ethyl]indole-4,7-dione 14. Prepared as previously described.[24]

6-Methoxy-1,2-dimethyl-3-(4-nitrophenoxymethyl)indole-4,7-dione 15. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (0.045 g, 0.19 mmol), thionyl chloride (0.70 mL, 9.57 mmol), 4-nitrophenol (0.080 g, 0.57 mmol), and potassium carbonate (0.079 g, 0.57 mmol) gave after chromatography (gradient elution with 30−60% ethyl acetate/light petroleum) the title compound (0.057 g, 85%) as a yellow/orange crystalline solid: mp 213−215 °C (decomp, from methanol); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 (2 H, d, *J* 9.3, ArH), 7.06 (2 H, d, *J* 9.3, ArH), 5.68 (1 H, s, 5-H), 5.43 (2 H, s, CH_2), 3.92 (3 H, s, Me), 3.83 (3 H, s, Me), 2.34 (3 H, s, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 184.9 (C), 171.8 (C), 163.9 (C), 160.4 (C), 142.0 (C), 140.8 (C), 127.5 (C), 126.3 (CH), 124.3 (C), 116.4 (C), 115.3 (CH), 107.1 (CH), 61.3 (CH_2), 57.1 (Me), 33.1 (Me), 10.6 (Me); MS (FI) 356 (M^+, 10%), 219 (28), 139 (100); found, M^+, 356.1006. $C_{18}H_{16}N_2O_6$ requires 356.1008; Anal. ($C_{18}H_{16}N_2O_6$) H, N, C: calcd, 60.67; found, 60.11.

6-Methoxy-1,2-dimethyl-3-(phenoxymethyl)indole-4,7-dione 16. To a solution of 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (0.04 g, 0.17 mmol) in dichloromethane (5 mL) at 0 °C was added thionyl chloride (0.62 mL, 8.51 mmol) dropwise, and the reaction mixture was stirred at room temperature for 1 h. The solvent was removed in vacuo, and the crude product was used directly in the next step without further purification.

A solution of the crude chloride in DMF (10 mL) was added dropwise to a stirring suspension of phenol (0.032 g, 0.34 mmol) and sodium hydride (60% in oil; 0.014 g, 0.34 mmol) in DMF (10 mL) at 0 °C. After the addition, the mixture was allowed to stir at room temperature for 5 h. The reaction mixture was quenched with a saturated solution of ammonium chloride (10 mL), washed with NaOH (2 M, 10 mL), HCl (2 M, 10 mL), water (2 × 20 mL), and dried ($MgSO_4$). The solvent was removed in vacuo, and the crude product was purified by chromatography (30% ethyl acetate/light petroleum) to give the title compound (23 mg, 47%) as a yellow crystalline solid: mp 173−175 °C (decomp, from ethyl acetate/light petroleum); ^{1}H NMR (500 MHz, DMSO-*d*) δ 7.29 (2 H, m, ArH), 6.99 (2 H, m, ArH), 6.94 (1 H, m, ArH), 5.77 (1 H, s, 5-H), 5.20 (2 H, s, CH_2) 3.87 (3 H, s, Me), 3.78 (3 H, s, Me), 2.29 (3 H, s, Me); ^{13}C NMR (125 MHz, DMSO-*d*) δ 184.4 (C), 171.0 (C), 160.3 (C), 158.9 (C), 141.5 (C), 129.9 (CH), 126.9 (C), 123.7 (C), 121.0 (CH), 116.7 (C), 115.0 (CH), 106.9 (CH), 59.9 (CH_2), 57.1 (Me), 32.9 (Me), 10.0 (Me); MS (ES) 334 (M + Na^+, 100%), 218 (11); found, M + Na^+, 334.1056. $C_{18}H_{17}NO_4$ + Na requires 334.1055.

3-(4-Aminophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione 17. (a) To a solution of DMSO (10 mL) were added 4-iodophenol (1 g, 4.55 mmol), sodium azide (355 mg, 5.46 mmol), copper(I) iodide (87 mg, 0.46 mmol), L-proline (105 mg, 0.91 mmol), and sodium hydroxide (36.4 mg, 0.91 mmol). The reaction mixture was degassed and then stirred under an argon atmosphere at 60 °C for 20 h. On cooling, the reaction mixture was diluted with water (10 mL) and extracted into ethyl acetate (2 × 25 mL). The solvent was removed in vacuo to give 4-azidophenol as a brown/red oil (300 mg, 49%; lit.,[29] pale red oil) that was taken through to the next step without further purification.

(b) Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (60 mg, 0.26 mmol), thionyl chloride (0.93 mL, 12.77 mmol), 4-azidophenol (103 mg, 0.77 mmol), and potassium carbonate (106 mg, 0.77 mmol) gave after column chromatography (gradient elution with 20−30% ethyl acetate/light petroleum) 3-(4-azidophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione (50 mg, 59%) as an impure yellow crystalline solid (ca. 5% impurity): mp 182−184 °C (not recrystallized); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99−6.97 (2 H, m, ArH), 6.94−6.92 (2 H, m, ArH), 5.65 (1 H, s, 5-H), 5.35 (2 H, s, CH_2), 3.90 (3 H, s, Me), 3.81 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.5 (C), 171.4 (C), 160.0 (C), 155.9 (C), 140.4 (C), 138.3 (C), 132.6 (C), 127.1 (C), 124.0 (C), 120.0 (CH), 116.3 (CH), 106.8 (CH), 60.7 (CH_2), 56.6 (Me), 32.7 (Me), 10.2 (Me); MS (ES) 375 (M + Na^+, 100%), 218 (65); found, M + Na^+, 375.1060. $C_{18}H_{16}N_4O_4$ + Na requires 375.1069.

(c) To a solution of 3-(4-azidophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione (25 mg, 0.07 mmol) in THF/water (4 mL, 9:1) was added polymer supported triphenylphosphine (0.047 g, 0.14 mmol). The reaction mixture was stirred at room temperature for 48 h and then diluted with dichloromethane (25 mL), washed with water (10 mL), dried ($MgSO_4$), and purified by column chromatography (gradient elution with 30−60% ethyl acetate/light petroleum) to give a solid. Further purification by preparative reverse phase HPLC (250 × 21.2 mm Polaris C18 column; gradient elution 5−95% MeCN/water) gave the title compound (6 mg, 26%) as a red crystalline solid: mp 211−213 °C (from MeCN/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86−6.82 (2 H, m, ArH), 6.65−6.62 (2 H, m, ArH), 5.64 (1 H, s, 5-H), 5.23 (2 H, s, CH_2), 3.90 (3 H, s, Me), 3.81 (3 H, s, Me), 3.42 (2 H, bs, NH_2), 2.31 (3 H, s, Me); ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.4 (C), 171.3 (C), 159.8 (C), 151.6 (C), 140.4 (C), 140.2 (C), 126.9 (C), 124.0 (C), 118.1 (C), 116.3 (CH), 116.2 (CH), 106.7 (CH), 61.0 (CH_2), 56.5 (Me), 32.6 (Me), 10.2 (Me); MS (ES) 349 (M + Na^+, 100%), 327 (45); found, M + Na^+, 349.1156. $C_{18}H_{18}N_2O_4$ + Na requires 349.1164.

6-Methoxy-1,2-dimethyl-3-(3-nitrophenoxymethyl)indole-4,7-dione 18. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (40 mg, 0.17 mmol), thionyl chloride (0.62 mL, 8.51 mmol), 3-nitrophenol (71 mg, 0.51 mmol), and potassium carbonate (71 mg, 0.51 mmol) gave after chromatography (elution with 30% ethyl acetate/light petroleum) the title compound (0.047 g, 78%) as a yellow crystalline solid: mp 203−205 °C (from ethyl acetate/light petroleum); ^{1}H NMR (500 MHz, DMSO-*d*) δ 7.82−7.79 (2 H, m, ArH), 7.58 (1 H, t, *J* 8.0, ArH), 7.44 (1 H, ddd, *J* 8.0, 2.5, 1.5, ArH), 5.76 (1 H, s, 5-H), 5.32 (2 H, s, CH_2) 3.86 (3 H, s, Me), 3.77 (3 H, s, Me), 2.30 (3 H, s, Me); ^{13}C NMR (125 MHz, DMSO-*d*) δ 183.8 (C), 170.6 (C), 159.8 (C), 159.0 (C), 148.8 (C), 141.1 (C), 130.7 (C), 126.6 (C), 123.3 (C), 122.2 (CH), 115.6 (CH), 115.2 (C), 108.8 (CH), 106.4 (CH), 60.5 (CH_2), 56.7 (Me), 32.5 (Me), 9.5 (CH_3); MS (ES) 379 (M + Na^+, 100%), 218 (16); found, M + Na^+, 379.0906. $C_{18}H_{16}N_2O_6$ + Na requires 379.0901; Anal. ($C_{18}H_{16}N_2O_6$) H, N, C: calcd, 60.67; found, 59.99.

6-Methoxy-1,2-dimethyl-3-(2-nitrophenoxymethyl)indole-4,7-dione 19. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (20 mg, 0.21 mmol), thionyl chloride (0.78 mL, 10.64 mmol), 2-nitrophenol (89 mg, 0.64 mmol), and potassium carbonate (88 mg, 0.64 mmol) gave after column chromatography (elution with 30% ethyl acetate/light petroleum) the title compound (33 mg, 44%) as an orange crystalline solid: mp 222−224 °C (from ethyl acetate); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (1 H, dd, *J* 8.1, 1.7, ArH), 7.49 (1 H, ddd, *J* 8.5, 7.4, 1.7, ArH), 7.39 (1 H, dd, *J* 8.5, 0.9, ArH), 7.00, (1 H, m, ArH), 5.66 (1 H, s, 5-H), 5.53 (2 H, s, CH_2), 3.91 (3 H, s, Me), 3.82 (3 H, s, Me), 2.38 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 185.1 (C), 171.6 (C), 160.4 (C), 151.9 (C), 141.6 (C), 140.7 (C), 134.5 (CH), 127.3 (C), 125.7 (CH), 124.1 (C), 120.9 (CH), 116.6 (C), 116.3 (CH), 106.9 (CH), 62.1 (CH_2), 57.0 (Me), 33.0 (Me), 10.6 (Me); MS (ES) 379 (M + Na^+, 100%); found, M + Na^+, 379.0901. $C_{18}H_{16}N_2O_6$ + Na requires 379.0906; Anal. ($C_{18}H_{16}N_2O_6$) H, N, C: calcd, 60.67; found, 60.07.

6-Methoxy-1,2-dimethyl-3-(2,4-dinitrophenoxymethyl)indole-4,7-dione 20. To a solution of 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (40 mg, 0.17 mmol) and 2,4-dinitrofluorobenzene (128 μL, 1.02 mmol) in THF (5 mL) at 0 °C was added a solution of TBAF (1 M in THF, 1.02 mmol). The reaction mixture was stirred for 18 h at room temperature. The

solvent was removed in vacuo, and the crude product was purified by chromatography (elution with dichloromethane then 50% dichloromethane/ethyl acetate) to give the title compound (54 mg, 79%) as a yellow crystalline solid: mp 184−186 °C (from ethyl acetate/light petroleum); ^{1}H NMR (400 MHz, DMSO-*d*) δ 8.68 (1 H, d, *J* 2.8, ArH), 8.37 (1 H, dd, *J* 2.8, 9.2, ArH), 7.60 (1 H, d, *J* 9.2, ArH), 5.69 (1 H, s, 5-H), 5.67 (2 H, s, CH_2) 3.91 (3 H, s, Me), 3.84 (3 H, s, Me), 2.38 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.9 (C), 171.3 (C), 160.1 (C), 156.0 (C), 141.4 (C), 140.1 (C), 139.3 (C), 129.0 (CH), 127.0 (C), 123.6 (C), 121.7 (CH), 115.9 (CH), 114.7 (C), 106.6 (CH), 62.5 (CH_2), 56.8 (Me), 32.8 (Me), 10.3 (Me); MS (ES) 424 (M + Na^+, 100%); found, M + Na^+, 424.0735. $C_{18}H_{15}N_3O_8$ + Na requires 424.0756; Anal. ($C_{18}H_{15}N_3O_8$) H, C: calcd, 53.87; found, 53.43; N: calcd, 10.47; found, 9.75.

3-(2-Fluoro-4-nitrophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione 21. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (0.040 g, 0.17 mmol), thionyl chloride (0.62 mL, 8.51 mmol), 2-fluoro-4-nitrophenol (0.080 g, 0.51 mmol), and potassium carbonate (0.071 g, 0.51 mmol) gave after chromatography (elution with 20% ethyl acetate/light petroleum) the title compound (0.040 g, 63%) as a yellow crystalline solid: mp 229−231 °C (from chloroform/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (1 H, ddd, *J* 8.8, 2.8, 1.6, ArH), 7.96 (1 H, dd, *J* 10.8, 2.8, ArH), 7.34 (1 H, dd, *J* 8.8, 8.4, ArH), 5.68 (1 H, s, 5-H), 5.53 (2 H, s, CH_2) 3.92 (3 H, s, Me), 3.83 (3 H, s, Me), 2.36 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.6 (C), 171.4 (C), 160.0 (C), 151.5 (d, J_{CF} 249, CF), 152.2 (d, J_{CF} 11.3, C), 140.9 (C), 140.7 (C), 127.2 (C), 123.9 (C), 121.0 (d, J_{CF} 3.8, CH), 115.6 (C), 114.1 (CH), 112.3 (d, J_{CF} 23.8, CH), 106.7 (CH), 61.8 (CH_2), 56.7 (Me), 32.7 (Me), 10.2 (Me); MS (ES) 397 (M + Na^+, 79%), 218 (100); found, M + Na^+, 397.0788. $C_{18}H_{15}FN_2O_6$ + Na requires 397.0812; Anal. ($C_{18}H_{15}FN_2O_6$) C, H, N: calcd, 7.44; found, 6.92.

3-(4-Cyanophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione 22. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (60 mg, 0.26 mmol), thionyl chloride (0.93 mL, 12.77 mmol), 4-hydroxybenzonitrile (91.1 mg, 0.77 mmol), and potassium carbonate (106 mg, 0.77 mmol) gave after column chromatography (gradient elution with 20−30% ethyl acetate/light petroleum) the title compound (53 mg, 62%) as a yellow crystalline solid: mp 184 °C (from ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (2 H, dd, *J* 7.0, 1.8, ArH), 7.05 (2 H, dd, *J* 7.0, 1.8, ArH), 5.67 (1 H, s, 5-H), 5.38 (2 H, s, CH_2), 3.92 (3 H, s, Me), 3.83 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4 (C), 171.4 (C), 161.7 (C), 159.9 (C), 140.3 (C), 134.0 (CH), 127.1 (C), 123.9 (C), 119.2 (C), 116.2 (C), 115.6 (CH), 106.7 (CH), 104.1 (C), 60.5 (CH_2), 56.6 (Me), 32.7 (Me), 10.2 (Me); MS (ES) 359 (M + Na^+, 80%), 218 (100); found, M + Na^+, 359.1017. $C_{19}H_{16}N_2O_4$ + Na requires 359.1007; Anal. ($C_{19}H_{16}N_2O_4$) H, N, C: calcd, 67.85; found, 66.67.

6-Methoxy-1,2-dimethyl-3-[4-(trifluoromethyl)phenoxymethyl]-indole-4,7-dione 23. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (60 mg, 0.26 mmol), thionyl chloride (0.93 mL, 12.77 mmol), 4-trifluoromethylphenol (124 mg, 0.77 mmol), and potassium carbonate (106 mg, 0.77 mmol) gave after column chromatography (gradient elution with 20−40% ethyl acetate/hexane) the title compound (75 mg, 78%) as an orange crystalline solid: mp 150 °C (from ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (2 H, d, *J* 8.8, ArH), 7.05 (2 H, d, *J* 8.8, ArH), 5.66 (1 H, s, 5-H), 5.37 (2 H, s, CH_2), 3.91 (3 H, s, Me), 3.82 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4 (C), 171.4 (C), 160.9 (C), 159.9 (C), 140.3 (C), 127.1 (C), 126.8 (q, J_{CF} 3.7, CH), 124.4 (q, J_{CF} 271, CF_3), 123.0 (q, J_{CF} 32.7, C), 123.9 (C), 116.7 (C), 114.8 (CH), 106.7 (CH), 60.4 (CH_2), 56.6 (Me), 32.6 (Me), 10.2 (Me); MS (ES) 402 (M + Na^+, 49%), 218 (100); found, M + Na^+, 402.0930. $C_{19}H_{16}F_3NO_4$ + Na requires 402.0929; Anal. ($C_{19}H_{16}F_3NO_4$) C, H, N.

3-(4-Fluorophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione 24. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (60 mg, 0.21 mmol), thionyl chloride (0.78 mL, 10.6 mmol), 4-fluorophenol (71.6 mg, 0.64 mmol), and potassium carbonate (88 mg, 0.64 mmol) gave after column chromatography (gradient elution with 20−40% ethyl acetate/hexane) the title compound (30 mg, 43%) as a yellow crystalline solid: mp 171 °C (from ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96−6.93 (4 H, m, ArH), 5.65 (1 H, s, 5-H), 5.28 (2 H, s, CH_2), 3.91 (3 H, s, Me), 3.82 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4 (C), 171.4 (C), 159.9 (C), 157.4 (d, J_{CF} 238, CF), 154.6 (C), 140.3 (C), 127.0 (C), 124.0 (C), 117.4 (C), 116.0 (d, J_{CF} 8.1, CH), 115.8 (d, J_{CF} 23.0, CH), 106.7 (CH), 60.9 (CH_2), 56.6 (Me), 32.6 (Me), 10.2 (Me); MS (ES) 352 (M + Na^+, 100%), 218 (56); found, M + Na^+, 352.0959. $C_{18}H_{16}FNO_4$ + Na requires 352.0961.

3-(2,4-Difluorophenoxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione 25. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (60 mg, 0.26 mmol), thionyl chloride (0.93 mL, 12.77 mmol), 2,4-difluorophenol (99.5 mg, 0.77 mmol), and potassium carbonate (106 mg, 0.77 mmol) gave after column chromatography (gradient elution with 20−40% ethyl acetate/hexane) the title compound (42 mg, 47%) as a yellow crystalline solid: mp 173 °C (from ethyl acetate); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (1 H, td, *J* 9.2, 5.4, ArH), 6.82 (1 H, ddd, *J* 11.2, 8.3, 2.8, ArH), 6.79−6.73 (1 H, m, ArH), 5.65 (1 H, s, 5-H), 5.34 (2 H, s, CH_2), 3.91 (3 H, s, Me), 3.81 (3 H, s, Me), 2.35 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4 (C), 171.4 (C), 159.9 (C), 156.7 (dd, J_{CF} 242, 10.3, CF), 152.9 (dd, J_{CF} 249, 12.1, CF), 142.8 (dd, J_{CF} 10.8, 3.3, C), 140.7 (C), 127.0 (C), 124.0 (C), 117.0 (dd, J_{CF} 9.5, 2.9, CH), 116.9 (C), 110.4 (dd, J_{CF} 22.4, 3.8, CH), 106.6 (CH), 104.7 (dd, ^2J 26.7, ^2J 22.3, CH), 62.3 (CH_2), 56.6 (Me), 32.6 (Me), 10.1 (Me); MS (ES) 370 (M + Na^+, 80%), 218 (100); found, M + Na^+, 370.0863. $C_{18}H_{15}F_2NO_4$ + Na requires 370.0867; Anal. ($C_{18}H_{15}F_2NO_4$) C, H, N.

6-Methoxy-1,2-dimethyl-3-(2,4,6-trifluorophenoxymethyl)indole-4,7-dione 26. Using method A, 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (60 mg, 0.26 mmol), thionyl chloride (0.93 mL, 12.77 mmol), 2,4,6-trifluorophenol (113 mg, 0.77 mmol), and potassium carbonate (106 mg, 0.77 mmol) gave after column chromatography (gradient elution with 20−40% ethyl acetate/hexane) the title compound (70 mg, 75%) as a yellow crystalline solid: mp 221 °C (from ethyl acetate/hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.67 (2 H, t, *J* 8.2, ArH), 5.60 (1 H, s, 5-H), 5.32 (2 H, s, CH_2), 3.92 (3 H, s, Me), 3.79 (3 H, s, Me), 2.35 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.9 (C), 171.5 (C), 159.7 (C), 157.3 (dt, J_{CF} 246, 14.3, CF), 156.5 (ddd, J_{CF} 250, 14.8, 7.7, CF), 141.0 (C), 131.9 (dd, J_{CF} 15.3, 5.0, C), 127.0 (C), 124.2 (C), 116.5, (C), 106.6 (CH), 100.6 (t, J_{CF} 26.7, CH), 65.9 (CH_2), 56.5 (Me), 32.6 (Me), 9.7 (Me); MS (ES) 388 (M + Na^+, 57%), 218 (100); found, M + Na^+, 388.0766. $C_{18}H_{14}F_3NO_4$ + Na requires 388.0773; Anal. ($C_{18}H_{14}F_3NO_4$) C, H, N.

6-Methoxy-1,2-dimethyl-3-(pyridin-2-yloxymethyl)indole-4,7-dione 27. Using method B, diethyl azodicarboxylate (96 μL, 0.61 mmol), 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (40 mg, 0.17 mmol), tri(2-furyl)phosphine (122 mg, 0.51 mmol), and 2-hydroxypyridine (49.5 mg, 0.51 mmol) gave after column chromatography (gradient elution with 10−40% ethyl acetate/light petroleum) the title compound (22 mg, 41%) as an orange crystalline solid: mp 178−180 °C (from ethyl acetate/light petroleum); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (1 H, dd, *J* 5.0, 1.5, ArH), 7.57−7.54 (1 H, m, ArH), 6.88 (1 H, ddd, *J* 7.0, 5.0, 1.0, ArH), 6.74 (1 H, d, *J* 8.5, ArH), 5.66 (1 H, s, 5-H), 5.54 (2 H, s, CH_2), 3.92 (3 H, s, Me), 3.81 (3 H, s, Me), 2.35 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.9 (C), 171.5 (C), 163.6 (C), 159.7 (C), 146.8 (CH), 140.3 (C), 138.5 (CH), 127.3 (C), 124.5 (C), 117.5 (C), 116.8 (CH), 111.1 (CH), 106.8 (CH), 57.9 (CH_2), 56.5 (Me), 32.6 (Me), 10.0 (Me); MS (ES) 335 (M+Na^+, 72%), 218 (100%); found, M + Na^+, 335.1006. $C_{17}H_{16}N_2O_4$ + Na requires 335.1007; Anal. ($C_{17}H_{16}N_2O_4$) H, N; C: calcd, 65.38; found, 62.00.

3-Methoxy-1,2-dimethyl-3-(3-pyridyloxymethyl)indole-4,7-dione 28. To a solution of 3-hydroxypyridine (26.9 mg, 0.277 mmol) in DMF (3 mL) was added sodium hydride (60% dispersion in oil; 11 mg, 0.28 mmol) at 0 °C. This solution was stirred at room

temperature for 45 min. The crude chloride (0.213 mmol) (prepared as in method A) in DMF (4 mL) was added dropwise at 0 °C, and the reaction mixture was stirred at room temperature for an additional 3 h. The solvent was concentrated in vacuo, and the crude product was dissolved in dichloromethane (50 mL), washed with water (2 × 25 mL) and NaOH (25 mL), and dried ($MgSO_4$), and the solvent removed in vacuo. The crude product was purified by column chromatography (gradient elution with 30−80% ethyl acetate/light petroleum) to give the title compound (28 mg, 42%) as a yellow crystalline solid: mp 179−181 °C (from ethyl acetate/light petroleum); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (1 H, bs, ArH), 8.21 (1 H, apparent bd, ArH), 7.36 (1H, ddd, *J* 8.4, 1.6, 1.2, ArH), 7.22 (1 H, dd, *J* 8.4, 4.8, ArH), 5.67 (1 H, s, 5-H), 5.38 (2 H, s, CH_2), 3.92 (3 H, s, Me), 3.82 (3 H, s, Me), 2.33 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4 (C), 171.4 (C), 159.9 (C), 154.7 (C), 142.1 (CH), 140.4 (C), 138.6 (CH), 127.1 (C), 124.0 (C), 123.9 (CH), 121.4 (CH), 116.6 (C), 106.7 (CH), 60.5 (CH_2), 56.6 (Me), 32.6 (Me), 10.2 (Me); MS (EI) 313 (MH^+, 100%); found, MH^+, 313.1178. $C_{17}H_{16}N_2O_4$ + H requires 313.1188.

6-Methoxy-1,2-dimethyl-3-(pyridin-4-yloxymethyl)indole-4,7-dione 29. Using method B, diethyl azodicarboxylate (96 μL, 0.61 mmol), 3-(hydroxymethyl)-6-methoxy-1,2-dimethylindole-4,7-dione **37** (40 mg, 0.17 mmol), tri(2-furyl)phosphine (122 mg, 0.51 mmol), and 4-hydroxypyridine (49.5 mg, 0.51 mmol) gave after column chromatography (gradient elution with 10−40% ethyl acetate/light petroleum) the title compound (0.022 g, 41%) as an orange crystalline solid: mp 161−163 °C (from ethanol/light petroleum); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 (2 H, d, *J* 5.0, ArH), 6.92 (2 H, d, *J* 5.0, ArH), 5.67 (1 H, s, 5-H), 5.39 (2 H, s, CH_2), 3.92 (3 H, s, Me), 3.83 (3 H, s, Me), 2.32 (3 H, s, Me); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.5 (C), 171.4 (C), 164.7 (C), 160.0 (C), 150.8 (CH), 140.4 (C), 127.2 (C), 123.9 (C), 116.1 (C), 110.7 (CH), 106.7 (CH), 60.2 (CH_2), 56.6 (Me), 32.7 (Me), 10.2 (Me); MS (ES) 313 (MH^+, 17%), 218 (100); found, MH^+, 313.1177. $C_{17}H_{16}N_2O_4$ + H requires 313.1188.

Biology. Materials. NADH, FAD, DCPIP, MTT, and bovine serum albumin were obtained from Sigma Chemical (St. Louis MO). rhNQO1 was purified from *E. coli* using Cibacron blue affinity chromatography, as previously described,[30] and had a specific activity of 800 μmol DCPIP/min/mg protein. For all biological assays, stock concentrations of indolequinones (5 mM) were prepared in dimethyl sulfoxide and stored in the dark at 32 °C.

Cell Lines. MIA PaCa-2 human pancreatic carcinoma cells were obtained from ATCC (Manassas VA). MIA PaCa-2 cells were grown in Dulbecco's modified Eagle's medium adjusted to contain 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10% (v/v) fetal bovine serum, 2.5% (v/v) horse serum, 100 units/mL penicillin and 100 μg/mL streptomycin in a humidified incubator containing 5% carbon dioxide at 37 °C.

NQO1 Inhibition Studies. The mechanism-based inactivation of rhNQO1 by this indolequinone series was assayed using the following methods. In these reactions (0.5 mL final volume), rhNQO1 (2 μg/mL) was incubated with 0.1−5.0 μM of the indolequinone in the absence and presence of 0.2 mM NADH in 50 mM potassium phosphate buffer, pH 7.4, containing 125 mM NaCl and 1 mg/mL bovine serum albumin at 32 °C. After 5 min, a 50 μL aliquot was removed and diluted 100-fold in stop buffer (50 mM potassium phosphate buffer, pH 7.4, containing 250 mM sucrose, 5 μM FAD and 0.1% (v/v) Tween-20), and NQO1 activity was measured using the reduction of DCPIP. Briefly, 960 μL was transferred to a cuvette with 0.2 mM NADH. The reaction was started with the addition of 40 μM DCPIP (final volume 1 mL), and the linear decrease in absorbance was monitored at 600 nm for 1 min at 32 °C.

Partition ratios for the inactivation of rhNQO1 by the indolequinones, that demonstrated mechanism-based inhibition, were determined essentially as described above, except that the compounds and rhNQO1 were incubated in the presence of 0.2 mM NADH for 15 min, with defined molar ratios of indolequinone to rhNQO1 (range 0.2:1 to 1250:1).

Inactivation of NQO1 in MIA PaCa-2 cells in culture, by these indolequinones, was determined using the following procedure. MIA PaCa-2 cells (1×10^6) were plated in 60 mm plates with 5 mL of complete medium. The following day the growth medium was replaced with the indolequinone containing complete medium for 1 h, after which the medium was removed, the cells were washed with 10 mL of phosphate buffered saline, and the cells were then scraped into 0.5 mL of 50 mM potassium phosphate buffer, pH 7.4, containing 250 mM sucrose and 5 μM FAD and briefly sonicated on ice. NQO1 activity was determined in MIA PaCa-2 sonicates using the reduction of DCPIP. Briefly, sonicates (20 μL) were added to 50 mM potassium phosphate buffer, pH 7.4, containing 1 mg/mL bovine serum albumin and 0.2 mM NADH. The reaction was started with the addition of 40 μM DCPIP (final volume 1 mL), and the linear decrease in absorbance was monitored at 600 nm for 1 min at 32 °C.

Growth Inhibition Assays. Growth inhibition in the human pancreatic MIA PaCa-2 cancer cell line was measured using the MTT colorimetric assay.[20] In these studies, the MIA PaCa-2 cells were seeded at 2×10^3 cells/well in 96-well plates, in triplicate, for each indolequinone, and allowed to attach for 16 h. Medium was removed by aspiration, and the MIA PaCa-2 cells were treated with the appropriate indolequinone (6.25−3200 nM) in complete medium for 4 and 72 h time periods. The medium was removed, and MTT (50 μg) in medium (50 μL) was added to each well and incubated for an additional 4 h. Cell viability was determined by measuring the cellular reduction of MTT to the crystalline formazan product, dissolved by the addition of DMSO (100 μL). Optical density was determined at 550 nm using a Molecular Devices Thermomax microplate reader. The IC_{50} values were defined as the concentration of indolequinone that resulted in 50% reduction in cell number compared to the DMSO-treated control, determined from semilog plots of percentage of control versus indolequinone concentration.

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Supporting Information Available: General experimental details, experimental procedures for compounds **31**−**37**, elemental analyses, full characterization data for compounds **6**, **7**, **10**−**13**, and **15**−**29**, HPLC data, and cytotoxicity data on phenols and hydroxypyridines. This material is available free of charge via the Internet at http://pubs.acs.org.

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