Synthesis, Electrochemistry, and Bioactivity of the Cyanobacterial Calothrixins and Related Quinones

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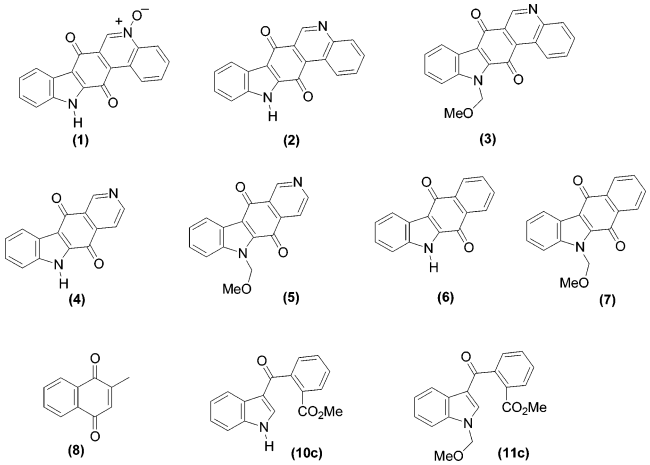
The calothrixins are quinone-based natural products isolated from *Calothrix* cyanobacteria which show potent antiproliferative properties against several cancer cell lines. Preliminary mechanistic studies suggest that the biological mode of action of the calothrixins may be linked to their ability to undergo redox cycling. In this study we compare the bioactivities of the calothrixins with those of structurally related quinones in order to identify the structural features in the calothrixins essential for biological activity. In particular, the reduction potentials of the calothrixins and some related quinones were measured electrochemically. Our studies indicate that while there is no direct correlation between the reduction potentials and biological activities of the studied compounds, in all cases quinones with EC_{50} values <1.6 μM undergo reduction to their respective semiquinones readily, with their $E_{1/2}$ values being more positive than −0.5 V versus the standard hydrogen electrode (SHE).

Introduction

The quinones are one of the most important classes of antitumor agents.[1,2] Their biological modes of action differ according to their particular structure, and they can act as DNA intercalators, bioreductive alkylators of biomolecules, and/or generators of reactive oxygen species through redox cycling.[3−5] Biological redox activity is recognized as playing a key role in a number of processes, including the triggering of cellular events that can be exploited for therapeutic uses.[6] For a number of quinone systems, a relationship between biological activity and redox potential has been suggested,[7] as, for example, between the redox potentials of azaanthraquinones and their inhibitory effects on Epstein−Barr virus early antigen activation,[8] or the cytotoxicity of naturally occurring quinones against human breast adenocarcinoma.[9] Other examples include the higher cytotoxic potency of mitomycin A versus mitomycin C, which has been tentatively correlated with the large difference in the quinone redox potentials of the two drugs.[10]

In 1999, a new class of quinones known as calothrixins A (**1**) and B (**2**) was isolated from the cyanobacteria *Calothrix*.[11] Calothrixin A (**1**) and B (**2**) both inhibited the in vitro growth of the chloroquine-resistant strain of the human parasite, *Plasmodium falciparum* in a dose-dependent manner.[11] They were also found to kill the human HeLa cancer cell line, with IC_{50} values at nanomolar levels.[11] Interestingly, subsequent investigations have shown that calothrixin A is redox-active and induces the intracellular formation of reactive oxygen species.[12] The present study was designed to investigate structure−activity relationships of the calothrixins and closely related quinones. In view of the apparent redox-active propensities of calothrixin A, we were interested in assessing whether there was a correlation between the redox potentials and the biological activities. To assess the contribution of the overall structure of the calothrixins, the reduction potential and biological activities of a number of structural analogues were also compared. This is the first reported structure−activity study of calothrixins and their analogues. In addition, to our knowledge, this paper also reports the first electrochemical study of the calothrixins **1**−**3** as well as that of their tetracyclic analogues, the ellipticine quinones **4** and **5**.



Results and Discussion

Chemistry. For structure−activity studies, calothrixins A (**1**) and B (**2**) and related derivative **3**, as well as structurally related analogues **4**−**8**, were investigated.

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Scheme 1

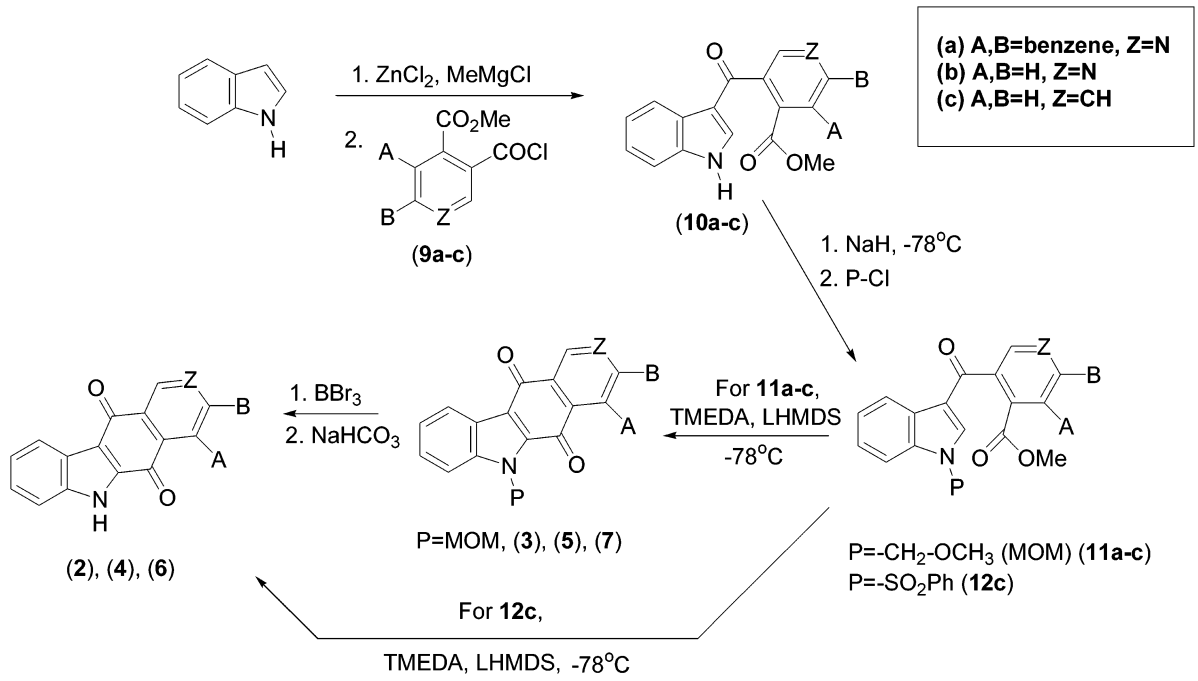

Table 1. Electrochemical Reduction Potentials and ln *K* Values of Quinones[a]

compound	$E_{1/2}$ (V) vs Fc^+/Fc wave I	wave II	$\Delta E_{1/2}$ (V)[b]	ln *K*[c]
calothrixin A (**1**)	−0.80	−1.46	0.66	26.1
calothrixin B (**2**)	−0.87	−1.54	0.67	26.5
N-MOM-calothrixin B (**3**)	−0.82	−1.54	0.72	28.5
ellipticine quinone (**4**)	−1.09	−1.67	0.58	23.0
N-MOM-ellipticine quinone (**5**)	−0.95	−1.65	0.70	27.7
benzocarbazoledione (**6**)	−1.17	−1.84	0.67	26.5
N-MOM-benzocarbazoledione (**7**)	−1.15	−1.86	0.71	28.1
menadione (**8**)	−1.09	−1.83	0.74	29.3

[a] Determined by ac and/or cyclic voltammetry at scan rates of 20 mV/s for ac and 50 mV/s for cyclic voltammetry experiments. All measurements were carried out at 20 °C in high grade DMSO, in the presence of tetrabutylammonium hexafluorophosphate (0.1 molar) as supporting electrolyte. Values vs the SHE may be estimated by addition of +0.77 to the Fc/Fc^+ values quoted. [b] $\Delta E_{1/2} = E_{1/2}$(wave I) − $E_{1/2}$ (wave II). [c] $\ln K = (F/RT)(\Delta E_{1/2})$.

Although calothrixin A and B can be sourced from cyanobacteria, access to sufficient quantities of the calothrixins **1** and **2** and derivative **3** was conveniently achieved through chemical synthesis using a route reported by us (Scheme 1).[13,14] The related indoloquinones, ellipticine quinone (**4**) and benzocarbazoledione (**6**) and their derivatives, were also conveniently synthesized following the route described in Scheme 1.

Electrochemical studies were performed on the quinones dissolved in anhydrous dimethyl sulfoxide as solvent, with tetra-*n*-butylammonium hexafluorophosphate as the supporting electrolyte and a platinum disk electrode as the working electrode. In these studies, both cyclic voltammetry (CV) and alternating current voltammetry (acV) were utilized. The $E_{1/2}$ values for the first reduction (wave I) and the second reduction (wave II) are tabulated with reference to ferrocene (Fc) in Table 1.

The electrochemical behavior of menadione (**8**) has been well studied in both organic and aqueous media.[15−17] In aprotic solvents menadione is observed to undergo reversible one-electron reduction to its semiquinone radical anion, followed by a separate one-electron reduction to its dianion. In DMF, the first reduction process has been observed to occur at a potential of −1.14 V relative to Fc^+/Fc, while in DMSO this reduction was seen to occur at −1.05 V. This behavior was observed as expected in the current work, and a reversible wave was evident in its CV trace at an $E_{1/2}$ of −1.09 V.

The calothrixins and synthetic tetracyclic analogues exhibited behavior similar to that observed for menadione. All of the quinones that were studied displayed electrochemically reversible one-electron reductions to their respective semiquinones, at potentials within 0.3 V of that of menadione. The observed CV of benzocarbazoledione (**6**) is consistent with that recently reported in the literature ($E_{1/2}$ = −1.18 V relative to ferrocene) using a glassy carbon working electrode.[18]

As a group, calothrixin A (**1**), calothrixin B (**2**), and *N*-MOM-calothrixin B (**3**) were found to have similar redox potentials, displaying relatively early reductions. Calothrixin A was the easiest to reduce, while calothrixin B was less facile by 70 mV. *N*-MOM-calothrixin B (**3**) was easier to reduce than calothrixin B (**2**) by approximately 50 mV. This may reflect the weakly electron-withdrawing nature of the indolic methoxymethyl substituent. Comparison of the first reduction potential of *N*-MOM ellipticine quinone (**5**) with that of ellipticine quinone (**4**), and also those of *N*-MOM-benzocarbazoledione (**7**) and benzocarbazoledione (**6**), showed similar trends in that the *N*-MOM derivative was easier to reduce than the corresponding *N*-H compound.

Comparisons of the $E_{1/2}$ values of the first wave potentials of *N*-MOM-calothrixin B (**3**) and *N*-MOM-ellipticine quinone (**5**) showed that the former was easier to reduce by 130 mV. The relative ease of reduction of *N*-MOM-calothrixin B, compared with *N*-MOM-ellipticine quinone, is interesting and can be taken to reflect measurable lowering of the redox-active

LUMO. The extended conjugated π-system of *N*-MOM-calothrixin B may provide for greater delocalization of the unpaired electron of its semiquinone radical anion and consequently have a stabilizing effect compared to that of *N*-MOM-ellipticine quinone.

N-MOM-ellipticine quinone (**5**) was found to be significantly easier to reduce than its benzo analogue, *N*-MOM-benzocarbazoledione (**7**), by 200 mV. The putative semiquinone radical anion of *N*-MOM-ellipticine quinone can be better stabilized through delocalization on to the nitrogen atom of the pyridine ring system than can the radical anion of *N*-MOM-benzocarbazoledione. Thus the influence of introducing the pyrido-nitrogen atom into the benzo ring system of benzocarbazoledione is to dramatically facilitate reduction. Similar trends in the ease of reduction are also observed in the parent quinones, calothrixin B (**2**), ellipticine quinone (**4**) and benzocarbazoledione (**6**).

The first one-electron reductions of benzocarbazolediones **6** and **7** were both seen to be more negative (i.e. harder to reduce) than for menadione. All of the other quinones studied, however, were significantly easier to reduce than menadione (**8**), whose clinically relevant anticancer properties are believed to be dependent to a certain extent on its redox potential.

The reduction of the semiquinones to their respective dianions occurs at much more negative potentials (ranging from -1.46 to -1.86 V). The effect of *N*-MOM substitution on the second reduction of the quinones was much less pronounced. In general, the reduction of the semiquinone to the dianion occurred more readily for the calothrixin semiquinone than for the semiquinone of ellipticine quinone, which in turn was more readily reduced than the benzocarbazoledione semiquinone. The stabilities of the semiquinone radical anions $Q^{-\bullet}$ toward disproportionation to Q^0 and Q^{2-} can be readily assessed from the ln K values,[18] directly related to the gap in the electrode potentials so long as the reductions are free of complication.[19] From Table 1, we see that the three *N*-MOM derivatives, the *N*-MOM-calothrixin B (**3**), *N*-MOM-ellipticine quinone (**5**), and *N*-MOM-benzocarbazoledione (**7**), are essentially indistinguishable in this respect with $\Delta E \sim 0.7$ V.

Biology. In this study, the MTT colorimetric assay[20] was used to measure cell viability of HeLa cells. The antiproliferative assays performed in this work involved a three-day period of incubation of the cells with the test compound before determination of the proportion of metabolically active cells in each sample.

The EC_{50} value of each compound was determined from a plot of the log of concentration against absorbance at 590 nm (A_{590}), in which nonlinear regression analysis was used to fit a sigmoidal concentration−response curve through the data points. The GraphPad Prism 2.0 software package was used to fit the curves from quadruplicate sets of data and to calculate the corresponding EC_{50} values.

The value obtained for calothrixin B (**2**) ($EC_{50} = 0.24$ μM; Table 2) was in agreement with that from earlier work, whereas the value obtained for calothrixin A (**1**) differed from the earlier report.[11] In our hands, calothrixin A was approximately twice as active ($EC_{50} = 0.12$ μM) as calothrixin B, whereas the earlier report found an almost 10-fold difference in activity. The presence of the methoxymethyl (MOM) substituent on the indolic nitrogen of *N*-MOM-calothrixin B (**3**) ($EC_{50} = 0.42$ μM) had the effect of decreasing the potency of the calothrixin B derivative 2-fold.

Table 2. Cytotoxicity of Compounds toward HeLa Cell Lines

compound	EC_{50} (μM)
calothrixin A (**1**)	0.12 ± 0.01
calothrixin B (**2**)	0.24 ± 0.04
N-MOM-calothrixin B (**3**)	0.42 ± 0.02
ellipticine quinone (**4**)	0.15 ± 0.09
N-MOM-ellipticine quinone (**5**)	0.37 ± 0.08
benzocarbazoledione (**6**)	0.43 ± 0.1
N-MOM-benzocarbazoledione (**7**)	1.6 ± 1.0
menadione (**8**)	3.7 ± 0.3
uncyclized precursors to benzocarbazoledione and *N*-MOM benzocarbazoledione (**10c**) and (**11c**)	>50

Interestingly, the biological activity of the tetracyclic quinone, ellipticine quinone (**4**), was comparable to that of calothrixin B (**2**). These two compounds differ structurally only in the presence of the fifth fused benzene ring in the calothrixin molecule. The equipotency of the pentacyclic calothrixin B and the tetracyclic ellipticine quinone suggests that the E-ring in the calothrixins is not crucial for activity per se, although it might of course have subtle effects on its activity. Comparison of ellipticine quinone (**4**) ($EC_{50} = 0.15$ μM) and benzocarbazoledione (**6**) ($EC_{50} = 0.43$ μM) revealed a small decrease in potency upon replacement of the heteroaromatic D-ring of ellipticine quinone with its carbocyclic aromatic analogue. The presence of the MOM substituent on the indolic nitrogen atom of *N*-MOM-benzocarbazoledione was observed to cause a decrease in the activity, compared with the unsubstituted benzocarbazoledione, by a factor of 4. A similar trend is observed with calothrixin B, ellipticine quinone, and their *N*-MOM derivatives.

In summary the quinones showed micromolar EC_{50} values against HeLa cells. The most active of these quinones were the calothrixins and the ellipticine quinone, followed by the benzocarbazolediones. In contrast, menadione (**8**) also showed micromolar EC_{50} values against HeLa cells ($EC_{50} = 3.7$ μM) compared to the value of 0.43 μM for benzocarbazoledione **6**.

The uncyclized precursors, **10c** and **11c**, lack the connectivity of the central quinone ring system. Their lack of activity, even at 10 times the EC_{50} values of their cyclized products, suggests that the tetracyclic core structure is an important aspect of bioactivity. This perhaps suggests that the rigid geometry of the tetracyclic compounds is a defining factor; for example, their planar geometry may confer an ability to intercalate into DNA or to bind with high affinity to a particular cleft in a protein or a DNA−enzyme complex. Alternatively, it might simply reflect the loss of the quinone functionality.

Cytotoxicity and Reduction Potentials. A comparison of the $E_{1/2}$ values with the EC_{50} of the quinones (**1**−**8**) reveals no direct correlation. It is, however, clear that all these quinones (especially **1**−**3**) are distinctively easily reduced to the semiquinones, with $E_{1/2}$ values more positive than -0.5 V versus the standard hydrogen electrode (SHE). It has previously been documented that this threshold is significant for physiologically active substances to permit electron acceptance from biological

donors.[7] The ease of reduction and the pattern for bioactivity of these quinones are consistent with preliminary mechanistic studies on calothrixins which suggest that the production of reactive oxygen species maybe an important factor in the manifestation of their biological activities.[12] Further studies are in progress.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were acquired on a Varian Gemini 2 spectrometer operating at 300 MHz and 75 MHz, respectively. The spectra were obtained in deuterated solvents with chemical shifts (δ), reported in parts per million, referenced to the respective trace solvent peak.

Electron impact mass spectra (EIMS) and high-resolution mass spectra (HRMS) were obtained by the Research School of Chemistry Mass Spectrometry Unit, ANU, using a VG Micromass 7070F double focusing mass spectrometer and positive ion impact techniques.

Infrared (IR) spectra were measured on a Perkin-Elmer Spectrum One Fourier transform infrared spectrometer, with samples analyzed as KBr disks.

Melting points were measured using a Reichert hot-stage microscope and are reported uncalibrated. Flash column chromatography was performed using Merck silica gel (Kieselgel 60, 0.040−0.063 mm), with positive pressure applied by nitrogen flow.

Menadione (2-methyl-1,4-naphthoquinone, **8**) and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), used in the bioassays, were purchased from Sigma. Natural calothrixin A (**1**) was kindly donated by Professor Rod Rickards. Calothrixin A (**1**), calothrixin B (**2**), and *N*-MOM calothrixin B (**3**) were synthesized as described previously and are identical to the authentic product (for the former) and reported data (for the latter).

The syntheses of quinones **4**−**7** via Scheme 1 have not been reported previously, and experimental procedures and data are presented below.

3-(1-Methoxycarbonylbenzene-2-yl)carbonylindole (10c). To a stirred mixture of indole (0.86 g, 7.3 mmol) and anhydrous zinc chloride (2.0 g, 14.6 mmol, 2 equiv) in dry CH_2Cl_2 (40 mL) at room temperature under nitrogen was added dropwise MeMgCl (2.6 mL of 3.0 M solution, 1 equiv). After stirring for 1 h, the reaction mixture was added in one portion to a solution of acid chloride **9c** (1.21 g, 6 mmol, 0.82 equiv) in dry CH_2Cl_2 (20 mL). The reaction mixture was stirred overnight at room temperature under nitrogen, before being quenched by the addition of saturated aqueous NH_4Cl (20 mL). The aqueous phase was extracted with $CHCl_3$ (3 × 20 mL), and the organic layers were combined and washed with saturated aqueous $NaHCO_3$ (25 mL) and saturated brine (25 mL). The organic layer was then dried over $MgSO_4$ and filtered and the solvent removed to give an orange solid. The crude product was triturated with hot ether to yield **10c** as a fine white powder (1.30 g, 4.65 mmol, 76% yield).

R_f (1:1EtOAc/light petroleum): 0.48. Mp: 197−198 °C. ^{1}H NMR (*d*-DMSO): δ 3.59 (s, 3H), 7.24 (m, 2H), 7.49−7.73 (m, 5H), 7.90 (d, J = 8 Hz, 1H), 8.13 (d, J = 8 Hz, 1H), 12.01 (s, 1H). ^{13}C NMR (*d*-DMSO): 52.1, 112.3, 116.4, 121.2, 121.9, 123.1, 125.6, 128.0, 129.43, 129.46, 129.52, 132.0, 135.4, 136.8, 142.6, 166.8, 190.1. IR (KBr disk) cm^{-1}: 3214, 1721, 1604. EIMS *m*/*z* (relative abundance): 279 ($M^{+\bullet}$, 65%), 248 (M − OCH_3, 22), 220 (M − CO_2CH_3, 13), 163 (13), 144 (100), 116 (29), 89 (24). HRMS: calcd 279.0895 for $C_{17}H_{13}NO_3$, found 279.0896. Anal. ($C_{17}H_{13}NO_3$) C, H, N.

1-Methoxymethyl-3-(1-methoxycarbonylbenzene-2-yl)-carbonylindole (11c). To a stirred solution of **10c** (350 mg, 1.25 mmol) in dry THF (35 mL) at 0 °C under nitrogen was added NaH (60 mg of a 60% dispersion in mineral oil, 1.5 mmol, 1.2 equiv). The reaction mixture was stirred for 20 min at 0 °C, upon which MOMCl (0.14 mL, 1.8 mmol, 1.5 equiv) was added and the color dissipated. After the mixture was allowed to warm to room temperature, the reaction was monitored by TLC (1:1 EtOAc/light petroleum) for the disappearance of starting material (R_f = 0.48 in this eluent). The reaction mixture was stirred at room temperature for 16 h, and the THF was removed in vacuo before the addition of saturated aqueous $NaHCO_3$ (25 mL), followed by the addition of CH_2Cl_2 (25 mL). The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated to give a pale yellow solid (420 mg). Trituration with ether and *n*-hexane, followed by recrystallization from hot acetone and *n*-hexane, gave **11c** as a fine white solid (380 mg, 1.2 mmol, 93% yield) in excellent purity. The product was thoroughly dried in vacuo before further use.

R_f (1:1 EtOAc/light petroleum): 0.52. Mp: 128 °C. ^{1}H NMR ($CDCl_3$): δ 3.23 (s, 3H), 3.62 (s, 3H), 5.41 (s, 2H), 7.31 (m, 3H), 7.50−7.65 (m, 4H), 8.01 (d, J = 6 Hz, 1H), 8.34 (t, J = 4 Hz, 1H). ^{13}C NMR ($CDCl_3$): 52.2, 56.2, 78.1, 110.4, 118.0, 122.6, 123.2, 124.1, 126.8, 127.8, 129.3, 129.4, 130.1, 132.0, 136.3, 137.0, 142.8, 167.1, 191.3. IR (KBr disk) cm^{-1}: 1712, 1640. EIMS *m*/*z* (relative abundance): 323 ($M^{+\bullet}$, 100%), 292 (M − CH_3O, 55), 247 (15), 188 (79), 163 (44), 158 (23), 77 (15). HRMS: calcd 323.1158 for $C_{19}H_{17}NO_4$, found 323.1153. Anal. ($C_{19}H_{17}NO_4$) C, H, N.

1-Phenylsulfonyl-3-(1-methoxycarbonylbenzene-2-yl)-carbonylindole (12c). To a stirred solution of **10c** (260 mg, 0.94 mmol) in dry THF (40 mL) at −78 °C under nitrogen was added NaH (0.055 g of a 60% dispersion in mineral oil, 1.4 mmol, 1.5 equiv). The reaction mixture was stirred for 30 min at −78 °C before being warmed to room temperature, upon which the previously clear, colorless solution became translucent orange in color. The reaction mixture was stirred for a further 20 min at room temperature before being cooled to −78 °C, upon which $PhSO_2Cl$ (0.24 mL, 1.87 mmol, 2 equiv) was added and the color dissipated. The reaction mixture was warmed to room temperature and stirred for 16 h before removal of the solvent in vacuo. To the residue was added saturated aqueous $NaHCO_3$ (25 mL), followed by CH_2Cl_2 (40 mL). The organic layer was separated, dried over Na_2SO_4, and filtered, and the filtrate was concentrated in vacuo to leave a yellow oil. The residue was then subjected to flash column chromatography with 2:3 EtOAc/light petroleum to give **12c** in 73% yield, as a fine white solid (268 mg, 0.69 mmol). The product was thoroughly dried in vacuo before further use.

R_f (2:3 EtOAc/light petroleum): 0.52. Mp: 106−108 °C. ^{1}H NMR ($CDCl_3$): δ 3.51 (s, 3H), 7.40−8.39 (m, 14H). ^{13}C NMR ($CDCl_3$): δ 52.2, 113.1, 122.2, 123.0, 125.0, 126.0, 127.1, 127.2, 127.61, 127.64, 129.2, 129.6, 129.6, 130.0, 130.3, 132.4, 133.3, 134.5, 135.0, 137.3, 141.6, 166.6, 191.6. IR (KBr disk) cm^{-1}: 1720, 1664. EIMS *m*/*z* (relative abundance): 419 ($M^{+\bullet}$, 100%), 388 (4), 283 (22), 250 (13), 235 (25), 234 (18), 219 (24), 190 (12), 163 (16), 144 (22), 115 (13), 77 (57). HRMS: calcd 419.0830 for $C_{23}H_{17}NO_5S$, found 419.0827.

***N*-Methoxymethyl-5*H*-benzo[*b*]carbazole-6,11-dione (7).** To a stirred solution of **11c** (110 mg, 0.34 mmol) and TMEDA (52 μL, 0.34 mmol, 1.1 equiv) in dry THF (25 mL) at −78 °C under nitrogen was added dropwise a solution of LHMDS (0.68 mmol, 2.2 equiv) in dry THF (2 mL) under N_2 at −78 °C. The reaction mixture was stirred for 2 h at −78 °C before being allowed to warm to room temperature and stirred overnight. The reaction mixture was concentrated under reduced pressure, following which saturated aqueous $NaHCO_3$ (10 mL) was added. The product was extracted with CH_2Cl_2 (3 × 25 mL). The organic layers were combined, washed with saturated brine, dried over $MgSO_4$, and filtered, and the solvent was evaporated to leave an orange solid. Column chromatography in 1:2 EtOAc/light petroleum, followed by recrystallization from ether/*n*-hexane, gave the desired compound as a yellow solid (87 mg, 0.30 mmol, 88% purified yield).

R_f (1:2 EtOAc/light petroleum): 0.58. Mp: 143−145 °C. ^{1}H NMR ($CDCl_3$): δ 3.39 (s, 3H), 6.18 (s, 2H), 7.43 (t, J = 7 Hz, 1H), 7.51 (t, J = 7 Hz, 1H), 7.65 (d, J = 8 Hz, 1H), 7.73 (m, 2H), 8.20 (m, 2H), 8.49 (d, J = 8 Hz, 1H). ^{13}C NMR ($CDCl_3$): 56.5, 75.3, 111.9, 120.2, 123.9, 124.0, 124.9, 126.3, 126.6, 127.9, 133.0, 133.5, 133.7, 133.9, 135.1, 139.8, 179.0, 181.6. IR (KBr disk) cm^{-1}: 1658. EIMS *m*/*z* (relative abundance): 291 ($M^{+\bullet}$, 100%), 276 (M − CH_3, 88), 260 (M − OCH_3, 47), 248 (M −

CH_2OCH_3, 94), 190 (22), 102 (28). HRMS: calcd 291.0895 for $C_{18}H_{13}NO_3$, found 291.0897. Anal. ($C_{18}H_{13}NO_3$) C, H, N.

5*H*-Benzo[*b*]carbazole-6,11-dione (6). Method 1: Cyclization of 12c.[21] To a stirred solution of **12c** (100 mg, 0.26 mmol) and TMEDA (44 μL, 0.29 mmol, 1.1 equiv) in dry THF (20 mL) at −78 °C under nitrogen was added dropwise a solution of LHMDS (0.57 mmol, 2.2 equiv). The reaction mixture was stirred for 2 h at −78 °C under nitrogen, before being allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, 5 mL of saturated aqueous $NaHCO_3$ was added to quench the reaction, and the product was extracted with CH_2Cl_2 (4 × 30 mL). The organic layers were combined, washed with saturated brine, dried over Na_2SO_4, and filtered, and the solvent was evaporated to leave a dark orange solid (100 mg). Trituration with cold $CHCl_3$ gave 4 mg (0.02 mmol, 6%) of fine orange crystals. The filtrate was subjected to flash column chromatography in 100% CH_2Cl_2, which afforded a further 8 mg of fine orange crystals. The combined yield of desired product was 18% (12 mg, 0.06 mmol).

Method 2: Deprotection of 7. To a stirred solution of **7** (42 mg, 0.14 mmol) in CH_2Cl_2 (10 mL) at −78 °C under nitrogen was added BBr_3 via a disposable syringe (0.28 mL of 1.0 M solution in hexanes, 0.28 mmol, 1 equiv). The reaction mixture was stirred at room temperature for 1 h. An equal volume of saturated aqueous NH_4Cl (10 mL) was added to the reaction mixture and stirred vigorously for 1 h at 60 °C. The aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic layers were dried over Na_2SO_4 and filtered, and the solvent was removed in vacuo to give an orange solid (30 mg). Purification by column chromatography (1:2 EtOAc/light petroleum), followed by recrystallization from ether/*n*-hexane, afforded the desired compound as a fine orange solid (20 mg, 0.08 mmol, 57%).

R_f (1:2 EtOAc/light petroleum): 0.34. Mp (lit.[22] 307−310 °C): 300−303 °C. ^{1}H NMR (*d*-DMSO): δ 7.36 (t, J = 7 Hz, 1H), 7.48 (t, J = 7 Hz, 1H), 7.58 (d, J = 8 Hz, 1H), 7.84 (m, 2H), 8.10 (t, J = 7 Hz, 2H), 8.21 (d, J = 8 Hz, 1H), 13.09 (s, 1H). ^{13}C NMR (*d*-DMSO): 113.9, 117.4, 122.4, 123.9, 124.0, 126.0, 126.1, 127.0, 132.6, 133.2, 134.1, 134.3, 137.2, 138.2, 177.6, 180.4. EIMS *m*/*z* (relative abundance): 247 ($M^{+\bullet}$, 64%), 228 (19), 219 (M − CO, 19), 201 (23), 199 (22), 190 (15), 91 (100). HRMS: calcd 247.0633 for $C_{16}H_9NO_2$, found 247.0637. Anal. ($C_{16}H_9NO_2$) C, H, N.

3-(4-Methoxycarbonylpyridine-3-yl)carbonylindole (10b). To a stirred mixture of indole (0.39 g, 3.33 mmol) and anhydrous zinc chloride (0.91 g, 6.66 mmol, 2 equiv) in dry CH_2Cl_2 (50 mL) at 0 °C was added dropwise MeMgCl (1.11 mL of 3.0 M solution, 3.33 mmol, 1 equiv). The ice bath was removed and the reaction mixture allowed to warm to room temperature. After being stirred for 1 h, the reaction mixture, which was opaque pink in color, was transferred in one portion to the orange solution of the acid chloride **9b** (0.55 g, 2.76 mmol, 0.83 equiv) in dry CH_2Cl_2 (50 mL). The reaction mixture was stirred overnight at room temperature under nitrogen, before being quenched by addition of saturated aqueous NH_4Cl (50 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL), and the organic layers were combined and washed with saturated aqueous $NaHCO_3$ and saturated brine (50 mL). The organic layer was then dried over $MgSO_4$ and filtered, and the solvent was removed to leave a solid residue. Trituration with ether gave the desired product as a white solid (0.28 g, 2.0 mmol, 72% yield).

Mp: 173−175 °C. ^{1}H NMR ($CDCl_3$): δ 3.44 (s, 3H), 7.2−7.3 (m, 2H), 7.41 (m, 1H), 7.56 (d, J = 4 Hz, 1H), 7.85 (d, J = 9 Hz, 1H), 8.79 (s, 1H), 9.16 (s, 1H). ^{13}C NMR ($CDCl_3$): 53.0, 111.7, 118.1, 122.3, 123.2, 124.4, 125.4, 134.2, 136.5, 137.9, 148.3, 150.5, 165.7, 188.0. EIMS *m*/*z* (relative abundance): 281 ($M^{+\bullet}$, 27), 280 (71), 144 (100), 116 (32), 89 (27). HRMS: calcd 280.0848 for $C_{16}H_{12}N_2O_3$, found 280.0854. Anal. ($C_{16}H_{12}N_2O_3$) C, H, N.

1-Methoxymethyl-3-(4-methoxycarbonylpyridine-3-yl-)carbonylindole (11b). To a stirred solution of **10b** (0.63 g, 2.2 mmol) in dry THF (100 mL) at 0 °C under nitrogen was added NaH (0.16 g of a 60% dispersion in mineral oil, 2.2 mmol). The reaction mixture was stirred for 30 min at 0 °C, after which MOMCl (0.34 mL, 4.4 mmol, 2 equiv) was added and the reaction mixture became orange in color. The reaction mixture was warmed to room temperature and stirred overnight. The THF was removed in vacuo before addition of saturated aqueous $NaHCO_3$ (20 mL). The product was extracted into CH_2Cl_2 (3 × 30 mL), dried over Na_2SO_4, and filtered. Concentration of the filtrate gave a pale brown solid, which upon flash chromatography with EtOAc gave the N-protected indole **11b** as a fine white solid (0.66 g, 2 mmol, 90% purified). The product was thoroughly dried in vacuo before further use.

R_f (1:1 EtOAc/light petroleum): 0.24. R_f (EtOAc): 0.40. Mp: 126 °C. ^{1}H NMR ($CDCl_3$): δ 3.26 (s, 3H), 3.71 (s, 3H); 5.41 (s, 2H), 7.38 (m, 3H), 7.51 (m, 1H), 7.80 (d, J = 5 Hz, 1H), 8.37 (t, J = 4 Hz, 1H), 8.85 (br s, 2H). ^{13}C NMR ($CDCl_3$): δ 52.9, 56.3, 78.2, 110.5, 117.7, 122.6, 123.1, 123.5, 124.5, 126.6, 136.1, 136.7, 137.0, 137.3, 148.9, 151.2, 165.7, 188.2. EIMS *m*/*z* (relative abundance): 324 ($M^{+\bullet}$, 100), 293 (48), 188 (72), 164 (26). HRMS: calcd 324.1110 for $C_{18}H_{16}N_2O_4$, found 324.1112. Anal. ($C_{18}H_{16}N_2O_4$) C, H, N.

***N*-Methoxymethyl-6*H*-pyrido[4,3-*b*]carbazole-5,11-dione (5).** To a stirred solution of **11b** (0.35 g, 1.1 mmol) and TMEDA (0.16 mL, 1.1 mmol) in dry THF (50 mL) at −78 °C under nitrogen was added dropwise a solution of LHMDS (2.2 mmol) at −78 °C. The reaction mixture was stirred for 2 h at −78 °C, before being allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure, following which saturated aqueous $NaHCO_3$ (50 mL) was added. The product was extracted with EtOAc (4 × 25 mL), and the combined organic layers were washed with saturated brine (50 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to leave a yellow solid (0.46 g). Column chromatography (1:1 EtOAc/light petroleum followed by pure EtOAc) afforded pure *N*-MOM-ellipticine quinone **5** (0.11 g, 35% purified yield).

R_f (2:1 EtOAc/light petroleum): 0.47. Mp: (lit.[23] 196−197 °C): 195 °C. ^{1}H NMR ($CDCl_3$): δ 3.39 (s, 3H), 6.15 (s, 2H), 7.47 (t, J = 6 Hz, 1H), 7.56 (t, J = 6 Hz, 1H), 7.67 (d, J = 9 Hz, 1H), 7.99 (d, J = 9 Hz, 1H), 8.49 (d, J = 8 Hz, 1H), 9.06 (d, J = 5 Hz, 1H), 9.46 (s, 1H). ^{13}C NMR ($CDCl_3$): 56.6, 75.4, 112.0, 120.3, 123.7, 124.1, 125.4, 128.7, 134.4, 139.4, 140.2, 148.2, 154.9, 177.8, 180.9. IR (KBr disk) cm^{-1}: 1667, 1651. EIMS *m*/*z* (relative abundance): 292 ($M^{+\bullet}$, 100); 277 (60); 261 (25); 249 (60); 219 (10); 164 (13). HRMS: calcd 292.0842 for $C_{17}H_{12}N_2O_3$, found 292.0842. Anal. ($C_{17}H_{12}N_2O_3$) C, H, N.

6H-Pyrido[4,3-*b*]carbazole-5,11-dione[22] (4). The title compound was prepared from **5** by removal of the MOM group as described above and was purified by column chromatography (1:2 EtOAc/light petroleum), followed by recrystallization from diethyl ether and hexane to give the pure compound in 71% yield.

R_f (1:2 EtOAc/light petroleum): 0.29. Mp (lit.[22] 315−316): ≥300 °C. ^{1}H NMR (d_6-DMSO): δ 7.40 (t, J = 7 Hz, 1H), 7.49 (t, J = 7 Hz, 1H), 7.62 (d, J = 8 Hz, 1H), 7.95 (d, J = 5 Hz, 1H), 8.23 (d, J = 8 Hz, 1H), 9.08 (d, J = 5 Hz, 1H), 9.27 (s, 1H). Anal. ($C_{17}H_{12}N_2O_3$) H, N; C: calcd 72.58, found 71.95.

Voltammetric Experiments. Voltammetric experiments were performed using a three-electrode potentiostat, which consisted of a Princeton Applied Research electrochemistry system (PAR-170) or a PAR-273 system, both under computer control through a Maclab interface. A Tacussel platinum disk electrode was employed as the working electrode, together with a platinum wire quasi-reference electrode, a platinum wire as the auxiliary electrode, and tetra-*n*-butylammonium hexafluorophosphate (0.1 molar) as the supporting electrolyte. The ferrocene/ferrocenium couple was used as an internal standard. The dimethyl sulfoxide used as a solvent was of analytical grade and dried over 4 Å molecular sieves before use. The electrolyte solution was deoxygenated by vigorous purging with nitrogen for 30 min prior to the experiments, which were performed under nitrogen. All experiments were carried out at 20 °C. Typical scan rates of 20 mV/s were used for acV

experiments, and 50−1000 mV/s for CV experiments. The potentials quoted are the convergent results of 50 mV CV data and the acV data. The acV experiments were performed with the oscillating voltage modulation having an amplitude of 10 mV and frequency of 205 Hz.

Bioassays. Human cervical cancer cells (HeLa cell line) were used for all assays. The HeLa cells were cultured as monolayers in RPMI 1640 media with 10% fetal calf serum, at 37 °C with 5% CO_2. Progression of confluence was monitored by observation using an inverted light microscope. Cells were harvested according to the following protocol: the existing medium was decanted and the cells were washed twice with phosphate-buffered saline (PBS), before addition of sufficient Trypsin-EDTA solution to cover the cell monolayer and incubation for 5 min at 37 °C. The released cells were transferred to a centrifuge tube with 30 mL of fresh medium and spun at 800 rpm for 3 min. The media was decanted and the pellet resuspended in 5−10 mL of fresh media. The cell density of this suspension was determined by use of a haemocytometer, which had a volume of 10^{-4} mL and was transposed with a 5 × 5 grid. The number of cells present in the 25 squares of the grid were counted under a light microscope (Olympus, BHB, 20× magnification) and the concentration thereby determined.

Effects on cell proliferation and viability were measured using a colorimetric MTT assay. To wells 2−12 of a 96-well flat-bottomed microtiter plate was added 50 μL of fresh medium (RPMI 1640 with 10% FCS). To well 1 was added 100 μL of a solution of the sample compound (of known concentration in medium, made up from an initial stock solution in Hybrimax DMSO; Sigma), and this was serially diluted (1:1) along the plate, discarding after well 10. To wells 1−11 was added 50 μL of cell suspension, adjusted to achieve a final concentration of 10000 cells per well in 100 μL for a 3 day assay. The cells were incubated at 37 °C for 72 h, after which the solution was tipped from the plate and the cells were washed with PBS (2 × 100 μL). To each well were added 100 μL of fresh medium and 10 μL of MTT (as a solution in PBS, 5 mg/mL), followed by incubation at 37 °C for 5 h. After this time, 100 μL of solubilizing agent (10% sodium dodecyl sulfate in 0.01 M aqueous HCl) was added to each well before incubation overnight at 37 °C. The absorbance of each well of the plate was read at 590 nm by an ELISA automated spectrophotometric plate reader. Results were expressed as percentages of control (untreated) cells and were analyzed using GraphPad Prism 2.0 software. All determinations were performed in quadruplicate, and results were typical of multiple independent experiments.

Supporting Information Available: An appendix containing purity criteria data. This material is available free of charge via the Internet at http://pubs.acs.org.

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