

Synthesis and Biological Activity of Thiazolyindolequinones, Analogues of the Natural Product BE 10988

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A number of analogues of the naturally occurring thiazolyindolequinone BE 10988, a reported potent inhibitor of topoisomerase II, have been prepared and evaluated. The compounds were synthesized from 4-(benzyloxy)-5-methoxy-1-methylindole by appropriate substitution at the indole 3-position followed by standard thiazole ring-forming reactions. The toxicity of these potentially bioreductively activated indolequinones was measured in Chinese hamster V79 cells under aerobic and hypoxic conditions. In addition, toxicity was measured in a human breast cancer cell line that shows amplification of the topo II α gene and hypersensitivity to known topo II inhibitors such as mAMSA and mitoxantrone. Using a DNA decatenation assay, a comparison was also made of the inhibitory effects of BE 10988 and mitoxantrone on topo II activity.

Introduction

Topoisomerases, DNA-modifying enzymes, are becoming increasingly important as biological targets for potential anticancer agents,¹⁻³ and in the search for new compounds of this type, natural products have produced interesting leads.^{4,5} One example of this is the compound designated BE 10988, recently isolated from culture broths by Japanese workers.^{6,7} Although no detailed biological evaluation was described, BE 10988 was reported to be a potent inhibitor of topoisomerase II. The structure of BE 10988 (**1**) contains a novel thiazole-substituted indolequinone, and in connection with our interest in indolequinones as potential bioreductive anticancer agents,^{8,9} we recently completed a synthesis of the natural product **1**.¹⁰⁻¹² We now report the synthesis and preliminary biological evaluation of other thiazolyl (and oxazolyl) indolequinones, (**2-7**), analogues of BE 10988, together with our attempts to clarify the mechanism of action of BE 10988 itself.

Results and Discussion

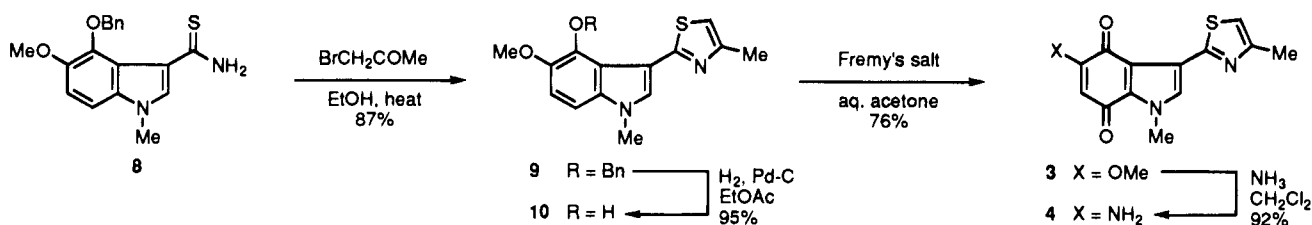
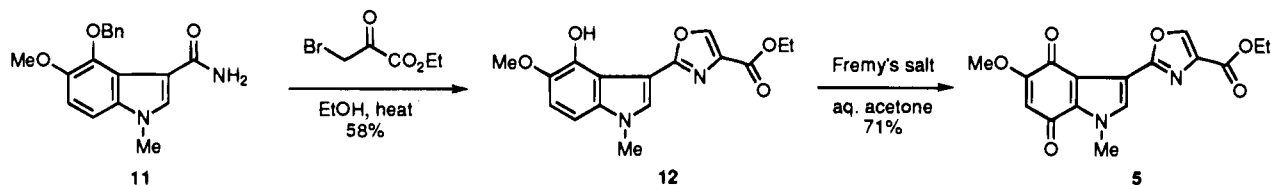
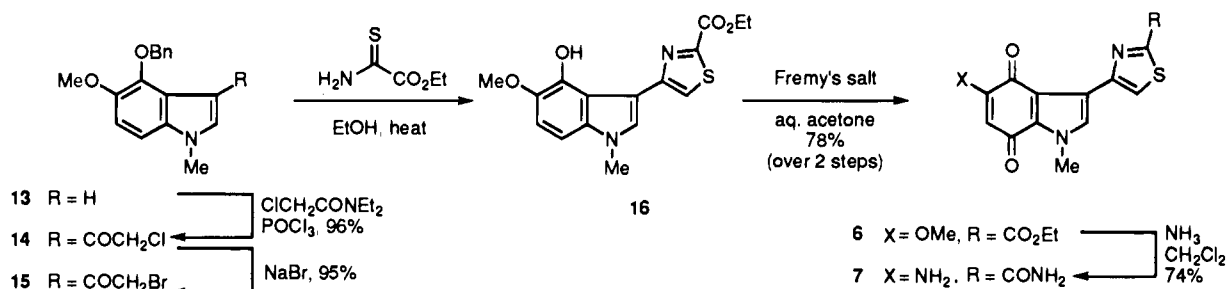
Chemistry. The work was designed to examine the structure-activity relationships within a small series of thiazolyindolequinones and to establish whether these indolequinones possessed any bioreductive activity in addition to their (supposed) activity as inhibitors of topoisomerase II. From the chemical point of view, the synthesis of the new indolequinones **3-7** rested largely on methods previously developed for the synthesis of the natural product BE 10988 (**1**) itself and is summarized in Schemes 1-3; this chemistry is discussed in detail elsewhere.¹¹

Biology. The activity of indolequinones **1-7** was initially evaluated in V79 cells under aerobic or hypoxic conditions, and results are given in Table 1. Ratios of IC₅₀(air)/IC₅₀(N₂) substantially greater than unity would suggest that O₂-dependent bioreductive processes are operational for the action of these compounds.¹³ Clearly, for none of the compounds in this series is this apparent. Further, the similarity in toxicity under aerobic and hypoxic conditions also suggests that production of O₂-free radicals is not an important mechanism for the toxicity of these compounds in air.

In a related study,¹⁴ when R¹ = OCH₃ and R² = H, ratios of aerobic:hypoxic toxicity in V79 cells were approximately 1.0 and absolute values of IC₅₀ were about 200 μ M. Only compound **2** in the present series shows such low toxicity. The remainder shows up to a 50-fold higher level of toxicity, e.g., compounds **1** and **7**, which suggests that these compounds may have other properties that contribute to their toxicity. This additional property could be inhibition of topo II activity, as was originally suggested might be the action of BE 10988 (compound **1**).^{6,7} To evaluate this, the compounds were tested for activity against a human breast cancer cell line (SKBr3) which over expresses the topo II α gene.¹⁵ Values of IC₅₀ for each compound are given in Table 1, and comparison is made with the toxicity of the known topo II inhibitor mitoxantrone. There is a 30-fold range in activity of the thiazolyindolequinones with compound **2** being least toxic. However, there are no obvious structural correlates with the values of IC₅₀ in this cell line. All the compounds are substantially less effective than mitoxantrone. In view of this and the claim^{6,7} that BE 10988, compound **1**, shows topo II inhibitory activity, preliminary experiments have been carried out to compare the action of **1**, **3**, **5**, and **6** with mitoxantrone in their ability to effect topo II-mediated

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Scheme 1. Synthesis of the Thiazolyndolequinones **3** and **4****Scheme 2.** Synthesis of the Oxazolyndolequinone **5****Scheme 3.** Synthesis of the Thiazolyndolequinones **6** and **7**

decatenation of kDNA. Results for **1** and mitoxantrone are shown in Figure 1. The maximum concentration of **1** tested is 150 μM , and this concentration has no effect on the decatenating activity of topo II. Similar concentrations of **3**, **5**, and **6** also showed no activity. In contrast, 10 μM mitoxantrone completely inhibits the action of topo II.

Further experiments have been carried out to elucidate the possible mechanism of action of compound **1**. Chinese hamster ovary (CHO) cell lines ADR-1 and ADR-4, that have been characterized as hypersensitive to topo II inhibitors,^{16,17} have been exposed to compound **1** and their sensitivity compared to wild type CHO-K1 cells. Values of IC₅₀ derived after a 3 h exposure in air are 5.9, 14.6, and 8.7 μM for CHO-K1, ADR-1, and ADR-4 cells, respectively. This contrasts to the findings with mitoxantrone and other topo II inhibitors^{16,17} that show the ADR-1 and ADR-4 cells to be substantially more sensitive than the CHO-K1 cells. Taken together with the results of the decatenation assay, it raises the possibility that the original contention^{6,7} that compound **1** (BE 10988) operates via a topo II-mediated mechanism may not be wholly correct.

Experimental Section

Chemistry. For general experimental details, see ref 11. **2-[4-(Benzyloxy)-5-methoxy-1-methylindol-3-yl]-4-methylthiazole (9).** 4-(Benzyloxy)-5-methoxy-1-methylindole-3-thiocarboxamide (**8**) (0.76 g, 2.3 mmol) and bromoacetone (0.63 g, 4.6 mmol) were refluxed for 30 min in EtOH (50 mL). The crude mixture was concentrated and the residue purified by column chromatography (EtOAc–2-propanol elution) and recrystallized (dichloromethane/light petroleum) yielding the thiazole **9** (0.73 g, 87%) as a yellow crystalline solid: mp 93–95 °C; IR (CHCl₃) 2959, 2872, 1463 cm⁻¹; ¹H NMR (CDCl₃) δ 9.50 (1H, s), 7.33 (5H, m), 7.10 (2H, s), 6.63 (1H, s), 5.16 (2H, s, CH₂Ph), 3.95 (3H, s, OMe), 3.90 (3H, s, NMe), 2.68 (3H, s,

Me); ¹³C NMR (CDCl₃) δ 164.76, 147.83, 138.03 (CH), 136.46, 134.23, 128.73 (CH), 128.42 (CH), 128.32 (CH), 119.93, 112.68 (CH), 111.93 (CH), 106.46 (CH), 102.55, 76.30 (CH₂Ph), 57.30 (OMe), 34.29 (NMe), 13.98 (Me); HRMS found MH⁺ 365.1324, C₂₁H₂₀N₂O₂S requires M 365.1324.

2-(4-Hydroxy-5-methoxy-1-methylindol-3-yl)-4-methylthiazole (10). 2-[4-(Benzyloxy)-5-methoxy-1-methylindol-3-yl]-4-methylthiazole (**9**) (0.52 g, 1.43 mmol) was dissolved in dichloromethane (20 mL). A catalytic quantity of palladium/carbon 10% was added. The reaction mixture was shaken under a hydrogen pressure of 45 psi for 12 h. The catalyst was removed by filtering the crude mixture through Celite. The mixture was concentrated and the crude product purified by column chromatography (ether elution) to yield the phenol **10** (0.37 g, 95%) as a yellow crystalline solid: mp 151–152 °C; IR (CHCl₃) 3377, 3055, 2988, 1556 cm⁻¹; ¹H NMR (CDCl₃) δ 13.36 (1H, s, OH), 7.41 (1H, s, NCH=), 7.02 (1H, d, *J* = 8.7 Hz), 6.66 (1H, d, *J* = 8.7 Hz), 6.58 (1H, d, *J* = 1.0 Hz, SCH=), 3.94 (3H, s, OMe), 3.71 (3H, s, NMe), 2.42 (3H, d, *J* = 1.0 Hz); ¹³C NMR (CDCl₃) δ 164.91, 150.31, 141.91, 141.88, 135.09, 128.52 (CH), 116.87, 113.13 (CH), 111.03, 109.17 (CH), 98.99 (CH), 58.27 (OMe), 33.42 (NMe), 16.39 (CMe); HRMS found M⁺ 274.0776, C₁₄H₁₄N₂O₂S requires M 274.0776.

2-(5-Methoxy-1-methyl-4,7-dioxindol-3-yl)-4-methylthiazole (3). A solution of potassium nitrosodisulfonate (0.99 g, 3.7 mmol) in water (15 mL) was added to a stirred solution of 2-(4-hydroxy-5-methoxy-1-methylindol-3-yl)-4-methylthiazole (**10**) (0.34 g, 1.2 mmol) in acetone (20 mL), buffered with sodium dihydrogen phosphate (0.17 M, 15 mL). The mixture was stirred overnight. The mixture was concentrated and the resulting oil extracted with dichloromethane. The organic layer was dried (MgSO₄) and purified by chromatography (ether elution) to give the quinone **3** (0.26 g, 76%) as an orange crystalline solid: mp 227–229 °C; IR (CHCl₃) 3634, 3020, 1711, 1674, 1644, 1605 cm⁻¹; UV (MeOH) λ 461 (ϵ 3692), 278 (22 966), 209 (14 068) nm; ¹H NMR (CDCl₃) δ 7.67 (1H, s, NCH=), 6.92 (1H, d, *J* = 1.0 Hz, SCH=), 5.75 (1H, s, CH=C(OMe)), 4.03 (3H, s, OMe), 3.86 (3H, s, NMe), 2.48 (3H, s, CMe); ¹³C NMR (CDCl₃) δ 178.99 (CO), 176.96 (CO), 160.51, 158.8, 152.29, 130.41 (CH), 129.76, 119.20, 119.20, 118.80, 115.04 (CH), 106.62 (CH), 56.80 (OMe), 36.94 (NMe), 16.82 (CMe);

Table 1

Compound	R ¹	R ²	V79 (μM)			SKBr3 (μM)
			IC ₅₀ (Air)	IC ₅₀ (N ₂)	Ratio	IC ₅₀
1 (BE 10988)	NH ₂		6.2	5.1	1.2	8.7
2	OCH ₃		423	345	1.2	27.8
3	OCH ₃		72.9	47.7	1.5	1.4
4	NH ₂		10.3	6.0	1.7	0.73
5	OCH ₃		51.7	46.0	1.1	4.8
6	OCH ₃		38.7	60.9	0.64	16.9
7	NH ₂		5.5	4.1	1.3	4.1
Mitoxantrone			-	-	-	0.016

HRMS found M⁺ 288.0569, C₁₄H₁₂N₂O₃S requires M⁺ 288.0568. Anal. (C₁₄H₁₂N₂O₃S) C, H, N.

2-(5-Amino-1-methyl-4,7-dioxindol-3-yl)-4-methylthiazole (4). A solution of 2-(5-methoxy-1-methyl-4,7-dioxindol-3-yl)-4-methylthiazole (3) (0.027 g, 0.094 mmol) in dichloromethane (5 mL) was placed in a Young's tube, and liquid ammonia was bubbled through the solution at -78 °C. When a final volume of approximately 30 mL was obtained, the tube was sealed and the mixture allowed to stir overnight. Prior to opening the tube, the mixture was cooled to -78 °C and then allowed to warm up slowly over a period of a few hours. The crude mixture was purified by column chromatography (EtOAc elution) yielding the desired aminoquinone **4** (0.023 g, 92%) as a dark red crystalline solid: mp 265–268 °C; IR (KBr) 3427, 3295, 3250, 1626, 1611, 1590 cm⁻¹; UV (MeOH) λ 510 (ε 2787), 401 (6370), 276 (21 146), 236 (15 982) nm; ¹H NMR (DMSO-*d*) δ 7.78 (1H, s, NCH=), 7.23 (1H, s, SCH=), 7.16 (2H, br s, NH₂), 5.37 (1H, s, CH=CNH₂), 3.94 (3H, s, NMe), 2.38 (3H, s, CMe); ¹³C NMR (DMSO-*d*) δ 178.40 (C=O), 177.80 (C=O), 158.30, 151.95, 151.14, 131.50, 129.20, 117.13, 116.86, 114.76, 98.29, 36.47 (NMe), 16.94 (Me); HRMS found MH⁺ 273.0572, C₁₃H₁₁N₃O₂S requires 273.0572.

Ethyl 2-(4-Hydroxy-5-methoxy-1-methylindol-3-yl)oxazole-4-carboxylate (12). 4-(Benzyloxy)-5-methoxy-1-methylindole-3-carboxamide (11) (0.057 g, 0.18 mmol) and ethyl bromopyruvate (0.072 g, 0.37 mmol) were refluxed for 30 min in EtOH (10 mL). The crude mixture was concentrated and

the residue purified by column chromatography (ether elution) yielding the oxazole **12** (0.034 g, 58%) as a colorless crystalline solid: mp 149–150 °C; IR (CHCl₃) 3403, 3020, 1719 cm⁻¹; ¹H NMR (CDCl₃) δ 11.99 (1H, s, OH), 8.11 (1H, s, OCH=), 7.68 (1H, s, NCH=), 7.04 (1H, d, *J* = 8.8 Hz), 6.72 (1H, d, *J* = 8.8 Hz), 4.38 (2H, q, *J* = 7.1 Hz, CH₂CH₃), 3.96 (3H, s, OMe), 3.76 (3H, s, NMe), 1.39 (3H, t, *J* = 7.1 Hz, CH₂CH₃); ¹³C NMR (CDCl₃) δ 161.31 (C=O), 160.76 (C=N), 142.02 (CH), 141.87, 141.17, 134.62, 132.89, 129.74 (CH), 114.98, 113.38 (CH), 101.58, 99.70 (CH), 61.26 (CH₂CH₃), 58.27 (OMe), 33.66 (NMe), 14.35 (CH₂CH₃); HRMS found M⁺ 316.1059, C₁₆H₁₆N₂O₅ requires M 316.1059.

Ethyl 2-(5-Methoxy-1-methyl-4,7-dioxindol-3-yl)oxazole-4-carboxylate (5). A solution of potassium nitrosodisulfonate (0.086 g, 0.32 mmol) in water (2 mL) was added to a stirred solution of the 4-hydroxyindole **12** (0.033 g, 0.10 mmol) in acetone (5 mL), buffered with sodium dihydrogen phosphate (0.17 M, 2 mL), and stirred overnight. The mixture was concentrated and the resulting residual oil extracted with dichloromethane. The organic layer was dried (MgSO₄) and purified by column chromatography (ether) to give the quinone **5** (0.025 g, 71%) as a yellow/orange solid: mp 186–188 °C; IR (CHCl₃) 3020, 1733, 1685, 1646, 1606 cm⁻¹; UV (MeOH) λ 348 (ε 3529), 278 (11 233), 231 (13 591) nm; ¹H NMR (CDCl₃) δ 8.29 (1H, s), 7.56 (1H, s), 5.74 (1H, s), 4.40 (2H, q, *J* = 7.12 Hz, CH₂CH₃), 4.02 (3H, s, OMe), 3.84 (3H, s, NMe), 1.38 (3H, t, *J* = 7.12 Hz, CH₂CH₃); ¹³C NMR (CDCl₃) δ 178.84 (CO),

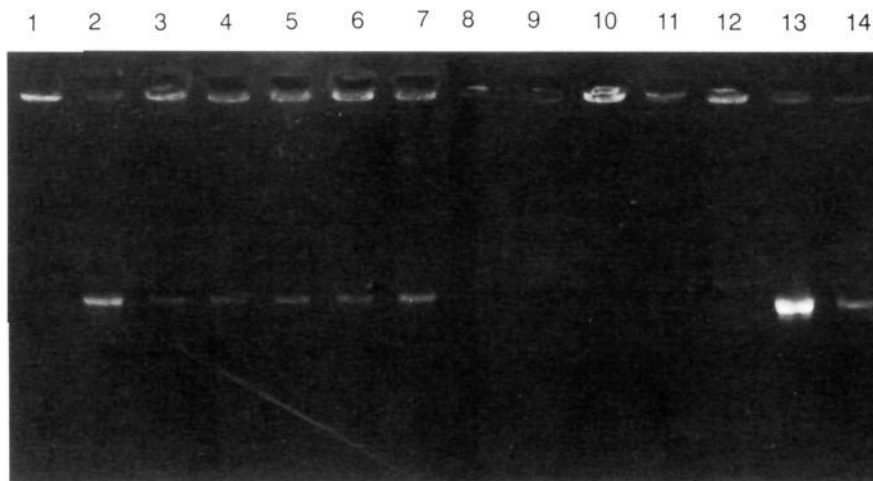


Figure 1. Decatenation of kDNA: effects of varying concentrations of compound **1** and mitoxantrone. Lane 1: kDNA only. Lane 2: kDNA plus nuclear extract (topo II). Lane 3: same as lane 2 plus 150 μM compound **1**. Lane 4: same as lane 2 plus 75 μM compound **1**. Lane 5: same as lane 2 plus 30 μM compound **1**. Lane 6: same as lane 2 plus 3 μM compound **1**. Lane 7: same as lane 2 plus 0.3 μM compound **1**. Lane 8: same as lane 2 plus 100 μM mitoxantrone. Lane 9: same as lane 2 plus 50 μM mitoxantrone. Lane 10: same as lane 2 plus 20 μM mitoxantrone. Lane 11: same as lane 2 plus 10 μM mitoxantrone. Lane 12: same as lane 2 plus 5 μM mitoxantrone. Lane 13: same as lane 2 plus 1 μM mitoxantrone. Lane 14: same as lane 2 plus 0.1 μM mitoxantrone.

175.32 (CO), 161.18, 160.60, 157.37, 143.60 (CH), 134.03, 132.06 (CH), 109.90, 106.73, 106.41, 106.15 (CH), 61.16 ($\text{CH}_2\text{-CH}_3$), 56.62 (OMe), 36.92 (NMe), 14.22 (CH_2CH_3); HRMS found M^+ 330.0852, $\text{C}_{16}\text{H}_{14}\text{N}_2\text{O}_6$ requires M 330.0852.

4-(Benzyloxy)-3-(chloroacetyl)-5-methoxy-1-methylindole (14). *N,N*-Diethylchloroacetamide (1.44 g, 9.6 mmol) and phosphorus oxychloride (1.48 g, 9.6 mmol) were stirred at -5°C for 30 min. A solution of the indole **13** (0.52 g, 1.9 mmol) in 1,2-dichloroethane (5 mL) was added slowly dropwise maintaining the temperature below 10°C . After addition was completed, the mixture was refluxed for 2 h. Additions of ice water (7 mL), sodium hydroxide solution (40%, 7 mL), and water (7 mL) were made, and the mixture was extracted with ether. The ether layer was dried (MgSO_4) and concentrated. The residue was purified by column chromatography (ether elution) yielding the (chloroacetyl)indole **14** (0.64 g, 96%) as a colorless crystalline solid: mp $102\text{--}103^\circ\text{C}$; IR (CHCl_3) 3023, 3013, 2940, 1656 cm^{-1} ; ^1H NMR (CDCl_3) δ 7.75 (1H, s), 7.49 (2H, m), 7.36 (3H, m), 7.07 (2H), 5.29 (2H, s, CH_2Ph), 4.71 (2H, s, CH_2Cl), 3.93 (3H, s, OMe), 3.78 (3H, s, NMe); ^{13}C NMR (CDCl_3) δ 187.92 (C=O), 148.18, 141.40, 137.67 (CH), 134.59, 128.78 (CH), 128.39 (CH), 128.08 (CH), 120.06, 114.06, 112.03 (CH), 105.66 (CH), 75.84 (CH_2Ph), 57.72 (OMe), 48.61 ($\text{CH}_2\text{-Cl}$), 33.68 (NMe). Anal. ($\text{C}_{19}\text{H}_{18}\text{NO}_3\text{Cl}$) C, H, N.

4-(Benzyloxy)-3-(bromoacetyl)-5-methoxy-1-methylindole (15). The 3-(chloroacetyl)indole **14** (0.18 g, 0.52 mmol) and sodium bromide (0.95 g, 9.2 mmol) were refluxed under reduced pressure. The residue was extracted with dichloromethane and washed with water. The organic layer was dried (MgSO_4) and concentrated. The crude product was purified by column chromatography (ether elution) yielding the (bromoacetyl)indole **15** (0.17 g, 95%) as a colorless crystalline solid: mp $105\text{--}106^\circ\text{C}$; IR (CHCl_3) 3023, 3013, 2940, 1656 cm^{-1} ; ^1H NMR (CDCl_3) δ 7.75 (1H, s), 7.48 (2H, m), 7.35 (3H, m), 7.14 (2H), 5.14 (2H, s, CH_2Ph), 4.52 (2H, s, CH_2Br), 3.94 (3H, s, OMe), 3.79 (3H, s, NMe); ^{13}C NMR (CDCl_3) δ 187.22 (C=O), 161.20, 148.20, 141.76, 137.47 (CH), 134.80, 128.73 (CH), 128.31 (CH), 127.98 (CH), 118.86, 114.06, 112.19 (CH), 105.62 (CH), 75.88 (CH_2Ph), 57.77 (OMe), 35.01 (CH_2Br), 33.63 (NMe). Anal. ($\text{C}_{19}\text{H}_{18}\text{NO}_3\text{Br}$) C, H, N.

Ethyl 4-(4-Hydroxy-5-methoxy-1-methylindol-3-yl)thiazole-2-carboxylate (16). 4-(Benzyloxy)-3-(bromoacetyl)-5-methoxy-1-methylindole (**15**) (0.19 g, 0.5 mmol), ethyl thiooxamate (0.13 g, 1.0 mmol), and EtOH (10 mL) were refluxed for 2 h. The crude mixture was concentrated and the residue column chromatographed (ether elution), yielding the thiazole

16 (approximately 70% pure by NMR) which was carried directly to the next step: ^1H NMR (CDCl_3) δ 11.89 (1H, s, OH), 7.43 (1H, s), 7.02 (1H, d, $J = 8.7$ Hz), 6.69 (1H, d, $J = 8.7$ Hz), 4.50 (2H, q, $J = 7.2$ Hz, CH_2CH_3), 3.95 (3H, s, OMe), 3.37 (3H, s, NMe), 1.46 (3H, t, $J = 7.2$ Hz, CH_2CH_3); HRMS found M^+ 332.0831, $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_4\text{S}$ requires M 332.0830.

Ethyl 4-(5-Methoxy-1-methyl-4,7-dioxindol-3-yl)thiazole-2-carboxylate (6). A solution of potassium nitrosodisulfonate (0.44 g, 1.64 mmol) in water (7 mL) was added to a stirred solution of crude ethyl 4-(4-hydroxy-5-methoxy-1-methylindol-3-yl)thiazole-2-carboxylate (**16**) (0.18 g, 0.54 mmol) in acetone (20 mL), buffered with sodium dihydrogen phosphate (0.17 M, 7 mL), and stirred overnight. The mixture was concentrated and the resulting residual oil extracted with dichloromethane. The organic layer was dried (MgSO_4) and purified by column chromatography (ether elution) to yield the quinone **6** (0.13 g, 78%) from the (bromoacetyl)indole as an orange solid: mp $225\text{--}227^\circ\text{C}$; IR (KBr) 2919, 2850, 1709, 1670, 1660, 1636, 1604 cm^{-1} ; UV (MeOH) λ 455 (ϵ 671), 379 (631), 326 (625), 287 (2920), 223 (4798) nm; ^1H NMR (CDCl_3) δ 9.02 (1H, s), 7.72 (1H, s), 5.75 (1H, s), 4.49 (2H, q, $J = 7.2$ Hz, $\text{CH}_2\text{-CH}_3$), 4.03 (3H, s, OMe), 3.87 (3H, s, NMe), 1.45 (3H, t, $J = 7.2$ Hz, CH_2CH_3); ^{13}C NMR (CDCl_3) δ 178.75 (CO), 176.98 (CO), 160.50 (CO_2Et), 159.76, 156.72, 149.42, 131.11, 130.21, 122.90, 119.43, 118.95, 106.32, 62.42 (CH_2CH_3), 56.56 (OMe), 36.68 (NMe), 14.10 (CH_2CH_3); HRMS found M^+ 346.0623, $\text{C}_{16}\text{H}_{14}\text{-N}_2\text{O}_5\text{S}$ requires M 346.0623.

Ethyl 4-(5-Amino-1-methyl-4,7-dioxindol-3-yl)thiazole-2-carboxamide (7). A solution of the thiazole-2-carboxylate **6** (0.02 g, 0.057 mmol) in dichloromethane (5 mL) was placed in a Young's tube, and ammonia was bubbled through the solution at -78°C . When a final volume of approximately 30 mL was obtained, the tube was sealed and the mixture allowed to stir at room temperature for 3 days. Prior to opening, the tube was cooled to -78°C and then allowed to warm up slowly over a period of a few hours. Purification by column chromatography (EtOAc elution) yielded the desired aminoquinone **7** (0.013 g, 74%) as a dark red solid: mp $285\text{--}287^\circ\text{C}$; IR (KBr) 3445, 3310, 3119, 2924, 1693, 1673, 1651, 1625, 1615 cm^{-1} ; UV (MeOH) λ 511 (ϵ 1418), 398 (3466), 304 (10 307), 234 (14 424) nm; ^1H NMR ($\text{DMSO-}d_6$) δ 8.74 (1H, s, $\text{SCH}=\text{C}$), 8.15, 7.93 (2H, 2 br s, CONH_2), 7.73 (1H, s, $\text{NCH}=\text{C}$), 7.12 (2H, br s, NH_2), 5.38 (1H, s, $(\text{H}_2\text{N})\text{C}=\text{CH}$), 3.95 (3H, s, NMe); ^{13}C NMR ($\text{DMSO-}d_6$) δ 178.45 (CO), 177.94 (CO), 163.05 (CONH_2), 160.97, 151.44, 148.63, 132.01, 129.96, 121.12, 117.84, 117.28, 98.22, 36.50 (NMe); HRMS found M^+ 302.0474, $\text{C}_{13}\text{H}_{10}\text{N}_4\text{O}_3\text{S}$ requires M 302.0473.

Biology. Selective toxicity toward hypoxic V79 cells was determined for all compounds using the MTT assay as described elsewhere.¹⁸ Cells derived from an exponentially growing culture were treated with varying concentrations of drugs for 3 h at 37 °C under hypoxic (N₂) or aerobic conditions. Then, following the removal of drug, the cells were allowed to proliferate for 3 days prior to MTT assay. Data are expressed as values of IC₅₀ which are the concentrations required to kill 50% of the cells under the conditions of the initial treatment (i.e., exposure to drug in air or N₂). The ratio of IC₅₀(air) vs IC₅₀(N₂) enables quantitative comparison to be made of the O₂-dependent bioreductive activities of these compounds.

The human breast cancer cell lines SKBr3 overexpresses topo II α ¹⁵ and is extremely sensitive to known topo II inhibitors such as mAMSA and mitoxantrone. Since the indoloquinones described in this work have been synthesized as potential topo II inhibitors, all compounds have been assessed for their toxicity in SKBr3 cells. Aliquots of cells were placed into 24-well microliter tissue culture plates at a seeding density of 10⁴ cells/well, which will allow exponential growth for the subsequent 4 days. Two hours after the initial plating, varying concentrations of each compound were added to the cells (4 wells/concentration). Cells were then incubated continuously for 4 days prior to determination of cell growth by the MTT assay.¹⁸⁻²⁰ Each experiment was repeated at least twice, and values of IC₅₀ were derived from the cumulative data.

As a source of topo II, nuclear extracts of human HeLa cells were prepared as described previously.^{21,22} Protein concentration in the extracts was determined by the method of Bradford.²³ Extracts were stored at -20 °C and used within 1 week. Inhibition of topo II activity was determined by a modification to the decatenation assay described by Glisson.²² Various concentrations of each compound in reaction buffer were incubated with 5 μ L of nuclear extract and kDNA (4 μ L, 0.1 μ g/ μ L, from *Crithidia fasciculata*; Topogen Inc., Columbus, OH) made up to a final volume of 50 μ L. Incubation was carried out for 0.5 h at 30 °C and the reaction stopped by addition of 25% ficol/5% SDS/0.05% bromophenol blue. Samples were then electrophoresed through 1% agarose in 90 mM Tris borate/2 mM EDTA buffer at pH 8.0. After staining with ethidium bromide, gels were photographed under UV illumination. kDNA consists of a series of interlinked rings of DNA. During incubation with nuclear extracts that contain topo II, those links became separated. Electrophoresis of this decatenated DNA showed that it runs approximately one-half the distance of the dye front. An effective inhibitor of topo II activity will prevent the decatenation from occurring, and the electrophoretic pattern will appear the same as for kDNA alone.

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