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

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A number of analogues of the naturally occurring thiazolylindolequinone 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 IIα 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 thiazolylindolequinones 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 $IC_{50}(air)/IC_{50}(N_2)$ substantially greater than unity would suggest that $O_2$-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 $O_2$-free radicals is not an important mechanism for the toxicity of these compounds in air.

In a related study,[14] when $R^1 = OCH_3$ and $R^2 = H$, ratios of aerobic:hypoxic toxicity in V79 cells were approximately 1.0 and absolute values of $IC_{50}$ were about 200 μM. 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 IIα gene.[15] Values of $IC_{50}$ 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 thiazolylindolequinones with compound **2** being least toxic. However, there are no obvious structural correlates with the values of $IC_{50}$ 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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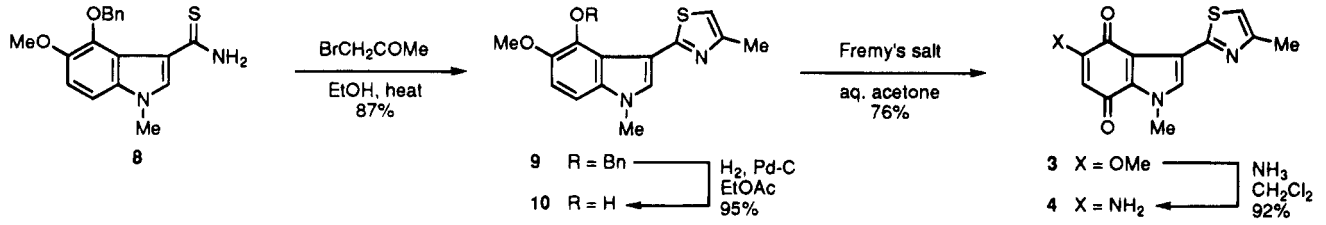
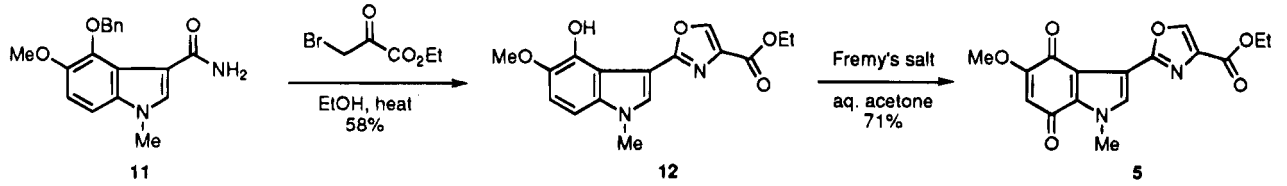
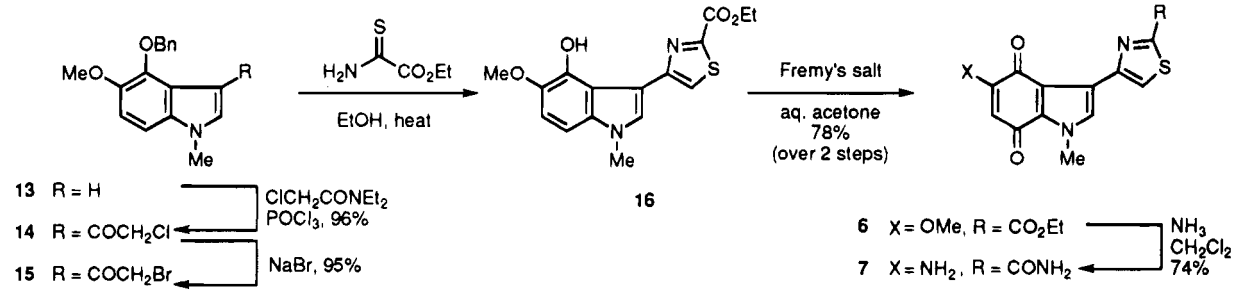
**Scheme 1.** Synthesis of the Thiazolylindolequinones **3** and **4**




**Scheme 2.** Synthesis of the Oxazolylindolequinone **5**




**Scheme 3.** Synthesis of the Thiazolylindolequinones **6** and **7**




decatenation of kDNA. Results for **1** and mitoxantrone are shown in Figure 1. The maximum concentration of **1** tested is 150 $\mu$M, 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 $\mu$M 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 $IC_{50}$ derived after a 3 h exposure in air are 5.9, 14.6, and 8.7 $\mu$M 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

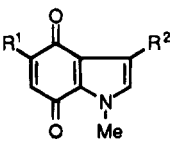
**Chemistry.** For general experimental details, see ref 11.

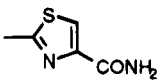
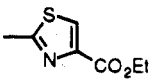
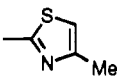
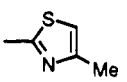
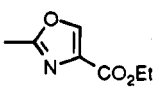
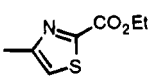
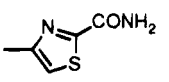
**2-[4-(Benzyloxy)-5-methoxy-1-methylindol-3-yl]-4-methylthiazole (9).** 4-(Benzyloxy)-5-methoxy-1-methylindole-3-thiocarboxamide (**8**) (0.76 g, 2.3 mmol) and bromoacetone (0.63 g, 4.6 mmol) were refluxed for 30 min in EtOH (50 mL). The crude mixture was concentrated and the residue purified by column 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 ($CHCl_3$) 2959, 2872, 1463 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 9.50 (1H, s), 7.33 (5H, m), 7.10 (2H, s), 6.63 (1H, s), 5.16 (2H, s, $CH_2Ph$), 3.95 (3H, s, OMe), 3.90 (3H, s, NMe), 2.68 (3H, s, Me); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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 ($CH_2Ph$), 57.30 (OMe), 34.29 (NMe), 13.98 (Me); HRMS found $MH^+$ 365.1324, $C_{21}H_{20}N_2O_2S$ 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 ($CHCl_3$) 3377, 3055, 2988, 1556 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 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); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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, $C_{14}H_{14}N_2O_2S$ requires M 274.0776.

**2-(5-Methoxy-1-methyl-4,7-dioxoindol-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 dihyrogen 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 ($MgSO_4$) 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 ($CHCl_3$) 3634, 3020, 1711, 1674, 1644, 1605 $cm^{-1}$; UV (MeOH) $\lambda$ 461 ($\epsilon$ 3692), 278 (22 966), 209 (14 068) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 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); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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) $IC_{50}$(Air) | V79 (μM) $IC_{50}(N_2)$ | V79 (μM) Ratio | SKBr3 (μM) $IC_{50}$ |
|---|---|---|---|---|---|---|
| **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, $C_{14}H_{12}N_2O_3S$ requires $M^+$ 288.0568. Anal. ($C_{14}H_{12}N_2O_3S$) C, H, N.

**2-(5-Amino-1-methyl-4,7-dioxoindol-3-yl)-4-methylthiazole (4).** A solution of 2-(5-methoxy-1-methyl-4,7-dioxoindol-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) $\lambda$ 510 ($\epsilon$ 2787), 401 (6370), 276 (21 146), 236 (15 982) nm; $^1H$ NMR (DMSO-*d*) $\delta$ 7.78 (1H, s, NCH=), 7.23 (1H, s, SCH=), 7.16 (2H, br s, $NH_2$), 5.37 (1H, s, CH=$CNH_2$), 3.94 (3H, s, NMe), 2.38 (3H, s, CMe); $^{13}C$ NMR (DMSO-*d*) $\delta$ 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, $C_{13}H_{11}N_3O_2S$ 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 ($CHCl_3$) 3403, 3020, 1719 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 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, $CH_2CH_3$), 3.96 (3H, s, OMe), 3.76 (3H, s, NMe), 1.39 (3H, t, $J$ = 7.1 Hz, $CH_2CH_3$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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 ($CH_2CH_3$), 58.27 (OMe), 33.66 (NMe), 14.35 ($CH_2CH_3$); HRMS found $M^+$ 316.1059, $C_{16}H_{16}N_2O_5$ requires M 316.1059.

**Ethyl 2-(5-Methoxy-1-methyl-4,7-dioxoindol-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 ($MgSO_4$) 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 ($CHCl_3$) 3020, 1733, 1685, 1646, 1606 $cm^{-1}$; UV (MeOH) $\lambda$ 348 ($\epsilon$ 3529), 278 (11 233), 231 (13 591) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 8.29 (1H, s), 7.56 (1H, s), 5,74 (1H, s), 4.40 (2H, q, $J$ = 7.12 Hz, $CH_2CH_3$), 4.02 (3H, s, OMe), 3.84 (3H, s, NMe), 1.38 (3H, t, $J$ = 7.12 Hz, $CH_2CH_3$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 178.84 (CO),

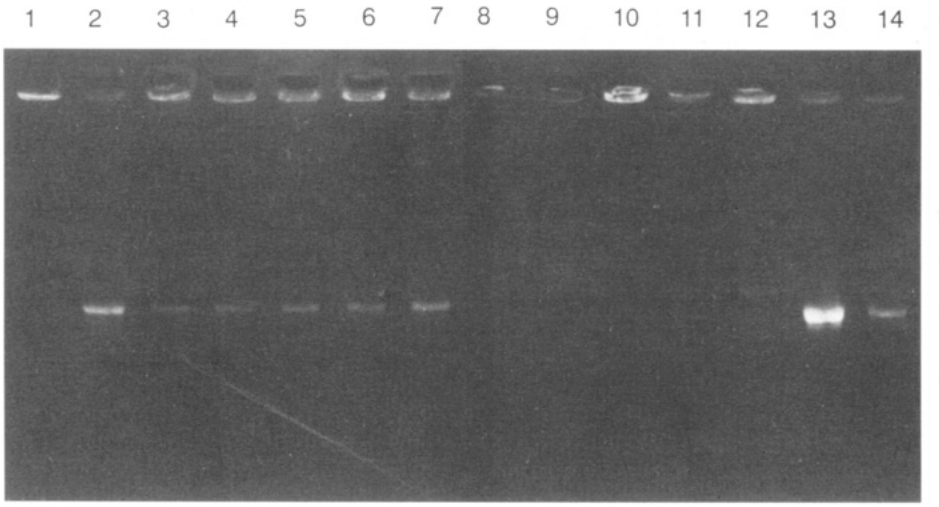



**Figure 1.** Decatenation of kDNA: effects of varying concentrations of compound **1** and mitoxantrone. Lane 1: kDNA only. Lane 2: kDNA plus nuclear extract (topo II). Lane 3: same as lane 2 plus 150 $\mu$M compound **1**. Lane 4: same as lane 2 plus 75 $\mu$M compound **1**. Lane 5: same as lane 2 plus 30 $\mu$M compound **1**. Lane 6: same as lane 2 plus 3 $\mu$M compound **1**. Lane 7: same as lane 2 plus 0.3 $\mu$M compound **1**. Lane 8: same as lane 2 plus 100 $\mu$M mitoxantrone. Lane 9: same as lane 2 plus 50 $\mu$M mitoxantrone. Lane 10: same as lane 2 plus 20 $\mu$M mitoxantrone. Lane 11: same as lane 2 plus 10 $\mu$M mitoxantrone. Lane 12: same as lane 2 plus 5 $\mu$M mitoxantrone. Lane 13: same as lane 2 plus 1 $\mu$M mitoxantrone. Lane 14: same as lane 2 plus 0.1 $\mu$M 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 ($CH_2$-$CH_3$), 56.62 (OMe), 36.92 (NMe), 14.22 ($CH_2CH_3$); HRMS found $M^+$ 330.0852, $C_{16}H_{14}N_2O_6$ 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 102−103 °C; IR ($CHCl_3$) 3023, 3013, 2940, 1656 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.75 (1H, s), 7.49 (2H, m), 7.36 (3H, m), 7.07 (2H), 5.29 (2H, s, $CH_2$Ph), 4.71 (2H, s, $CH_2$Cl), 3.93 (3H, s, OMe), 3.78 (3H, s, NMe); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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 ($CH_2$Ph), 57.72 (OMe), 48.61 ($CH_2$-Cl), 33.68 (NMe). Anal. ($C_{19}H_{18}NO_3Cl$) 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 in acetone (5 mL) for 2 days. The acetone was removed 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 105−106 °C; IR ($CHCl_3$) 3023, 3013, 2940, 1656 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 7.75 (1H, s), 7.48 (2H, m), 7.35 (3H, m), 7.14 (2H), 5.14 (2H, s, $CH_2$Ph), 4.52 (2H, s, $CH_2$Br), 3.94 (3H, s, OMe), 3.79 (3H, s, NMe); $^{13}C$ NMR ($CDCl_3$) $\delta$ 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 ($CH_2$Ph), 57.77 (OMe), 35.01 ($CH_2$Br), 33.63 (NMe). Anal. ($C_{19}H_{18}NO_3Br$) 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 ($CDCl_3$) $\delta$ 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, $C_{16}H_{16}N_2O_4S$ requires M 332.0830.

**Ethyl 4-(5-Methoxy-1-methyl-4,7-dioxoindol-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 225−227 °C; IR (KBr) 2919, 2850, 1709, 1670, 1660, 1636, 1604 $cm^{-1}$; UV (MeOH) $\lambda$ 455 ($\epsilon$ 671), 379 (631), 326 (625), 287 (2920), 223 (4798) nm; $^1H$ NMR ($CDCl_3$) $\delta$ 9.02 (1H, s), 7.72 (1H, s), 5.75 (1H, s), 4.49 (2H, q, $J$ = 7.2 Hz, $CH_2$-$CH_3$), 4.03 (3H, s, OMe), 3.87 (3H, s, NMe), 1.45 (3H, t, $J$ = 7.2 Hz, $CH_2CH_3$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 178.75 (CO), 176.98 (CO), 160.50 ($CO_2$Et), 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, $C_{16}H_{14}$-$N_2O_5S$ requires M 346.0623.

**Ethyl 4-(5-Amino-1-methyl-4,7-dioxoindol-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 285−287 °C; IR (KBr) 3445, 3310, 3119, 2924, 1693, 1673, 1651, 1625, 1615 $cm^{-1}$; UV (MeOH) $\lambda$ 511 ($\epsilon$ 1418), 398 (3466), 304 (10 307), 234 (14 424) nm; $^1H$ NMR (DMSO-$d$) $\delta$ 8.74 (1H, s, SCH=C), 8.15, 7.93 (2H, 2 br s, $CONH_2$), 7.73 (1H, s, NCH=C), 7.12 (2H, br s, $NH_2$), 5.38 (1H, s, $(H_2N)C$=CH), 3.95 (3H, s, NMe); $^{13}C$ NMR (DMSO-$d$) $\delta$ 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, $C_{13}H_{10}N_4O_3S$ 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 ($N_2$) 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 $IC_{50}$ 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 $N_2$). The ratio of $IC_{50}$(air) vs $IC_{50}(N_2)$ enables quantitative comparison to be made of the $O_2$-dependent bioreductive activities of these compounds.

The human breast cancer cell lines SKBr3 overexpresses topo IIα[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 $10^4$ 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 $IC_{50}$ 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 μL of nuclear extract and kDNA (4 μL, 0.1 μg/μL, from *Crithidia fasciculata*; Topogen Inc., Columbus, OH) made up to a final volume of 50 μL. 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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