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Preclinical report

Synthesis and cytotoxic activity of thiazolyl indolequinones

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A number of thiazolyl indolequinones have been prepared and evaluated for their antitumor properties. The compounds were synthesized from the appropriate indole, building up the thiazole ring using the Hantzsch reaction. Cytotoxic activity was determined in the human breast cancer SKBr3 cell line. Selected compounds were also studied in human lung carcinoma A549 and PV9 cell lines. In addition, some compounds were evaluated for their possible bioreductive action by determining their cytotoxicity towards V79 Chinese hamster lung fibroblasts in air and under anerobic (hypoxic) conditions. [© **1999 Lippincott Williams & Wilkins.**]

Key words: Bioreductive, cytotoxicity, indole, quinone, thiazole.

Introduction

The thiazole ring is present in a number of important biologically active compounds, ranging from vitamin B_1 (thiamine) to the epothilones, one of the most studied series of natural products in recent years.[1] The epothilones possess marked anticancer activity as do other thiazole containing compounds: the DNA-cleaving ability of bleomycin is well known[2] and dolastatin-10 is a powerful antineoplastic agent.[3] In connection with our own interest in biologically active thiazoles[4,5] and in indolequinones with anticancer properties,[6,7] we recently completed a synthesis of the naturally occurring quinone BE10988 **1**,[8] a compound which contains both thiazole and indolequinone moieties. This natural product, isolated from culture broths by Japanese workers,[9–11] was reported to have anticancer properties and to act as an inhibitor of topoisomerase (Topo) II, and although we were unable to confirm the reported potent Topo II inhibiting activity of BE10988 and related quinones,[12] some compounds did show sufficient cytotoxicity over and above the analogous compounds lacking the thiazole ring to merit further investigation. Thus a series of simple indolyl thiazoles, the 2-(indol-3-yl)thiazoles **2** and the 4-(indol-3-yl)- and 2-(indol-2-yl)-isomers **3** and **4**, was prepared and evaluated for their cytotoxic activity in a human breast tumor cell line.[13] Again, the compounds did not show any activity as inhibitors of Topo II, although they did exhibit modest cytotoxicity, with the 3-substituted series **2** being the most potent. We now report the completion of this study with the synthesis and detailed evaluation of the indolequinones **5–7**, corresponding to the simple thiazolyl indoles **2–4**.

Materials and methods

Chemistry

'Light petroleum' refers to the fraction boiling between 40 and 60°C, and ether refers to diethyl ether; solvents were dried using standard methods. Analytical thin-layer chromatography was carried out using aluminum backed plates coated with Merck Kieselgel 60 GF254. Plates were visualized under UV light (at 254 and/or 360 nm) or by staining with Ehrlich's reagent or phosphomolybdic acid reagent, followed by heating. Flash chromatography was

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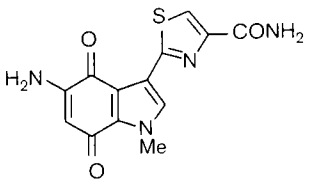

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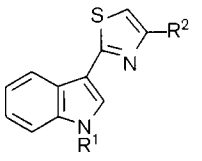
BE10988



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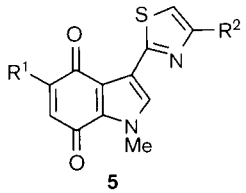

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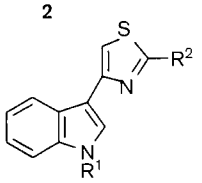

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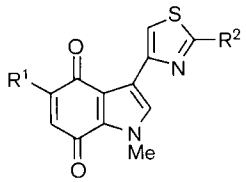

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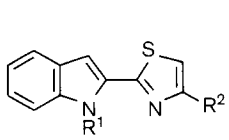

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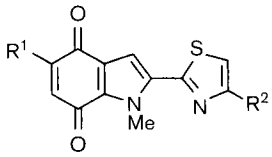

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carried out using Merck Kieselgel 60 H silica or Matrex silica 60; samples were applied pre-adsorbed on silica or as a saturated solution in an appropriate solvent. In most cases, gradient elution with an increasing proportion of the more polar solvent was used.

IR spectra were recorded in the range 4000–600 cm^{-1} using a Nicolet FT-205 spectrometer, with internal calibration. Spectra were recorded as solutions in chloroform, as KBr disks, or as Nujol mulls. ^{1}H- and ^{13}C-NMR spectra were recorded using a Bruker AC-250 instrument. High- and low-resolution mass spectra were recorded on a Kratos MS80 instrument or on a VG Analytical ZAB-E instrument (EPSRC Mass Spectrometry Service, Swansea). Compounds characterized by high-resolution mass spectrometry were chromatographically homogeneous.

Compounds **6a**, **6b**, **8** and **9** were prepared as previously described.[8,12]

2-(3-Indolyl)thiazoles

Ethyl 2-(5-(aziridin-1-yl)-1-methyl-4, 7-dioxoindol-3-yl)thiazole-4-carboxylate ***5a***. Ethyl 2-(5-methoxy-1-methyl-4, 7-dioxoindol-3-yl) thiazole-4-carboxylate **8**[8] (15.2 mg, 0.044 mmol), aziridine (0.5 ml) [**CAUTION**] and methanol (3 ml) were stirred at room temperature for 16 h. The reaction mixture was evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclusion chromatography (Sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (14.3 mg, 95%) as a red amorphous solid, m.p. 203–206°C (found: M^+, 357.0783; $C_{17}H_{15}N_3O_4S$ requires M, 357.0800); ν_{max} ($CHCl_3$)/ cm^{-1} 1711, 1664, 1632, 1592, 1400, 1215 and 755; δ_H (250 MHz; $CDCl_3$) 1.35 (3H, t, *J* 7.2 Hz, OCH_2Me), 2.21 (4H, s, $N(CH_2)_2$), 3.95 (3H, s, NMe), 4.34 (2H, q, *J* 7.2 Hz, OCH_2Me), 5.82 (1H, s, H6), 7.75 (1H, s) and 8.09 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.4, 28.0, 37.0, 61.4, 106.8, 117.9, 120.1, 127.6, 130.3, 131.2, 146.7, 157.7, 160.0, 161.6, 178.7 and 179.1; *m/z* 357 (M^+, 55%), 284 (25), 274 (30), 217 (20), 155 (15), 113 (20), 84 (30), 57 (25), 51 (60) and 42 (100).

2-(5-(Aziridin-1-yl)-1-methyl-4, 7-dioxoindol-3-yl)-4-methylthiazole ***5b***. 2-(5-Methoxy-1-methyl-4, 7-dioxoindol-2-yl)-4-methylthiazole **9**[12] (10.2 mg, 0.034 mmol), aziridine (0.5 ml) [**CAUTION**] and methanol (3 ml) were stirred at room temperature for 16 h. The reaction mixture was evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclusion chromatography (sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (9.3 mg, 92%) as a red amorphous solid (found: M^+, 299.0727; $C_{15}H_{13}N_3O_2S$ requires M, 299.0745); δ_H (250 MHz; $CDCl_3$) 2.25 (4H, s, $N(CH_2)_2$), 2.47 (3H, s, Me), 4.01 (3H, s, NMe), 5.88 (1H, s, H6), 6.93 (1H, s) and 7.62 (1H, s); δ_C (100 MHz; $CDCl_3$) 17.1, 27.8, 36.7, 114.5, 116.7, 119.0, 119.9, 130.0, 130.2, 152.5, 157.9, 158.6, 178.8 and 176.9; *m/z* 299 (M^+, 100%), 288 (30), 272 (25), 259 (10), 244 (10), 230 (10), 215 (10), 203 (10), 174 (10), 161 (5), 91 (20) and 42 (30).

Ethyl 2-(1-methyl-5-(2-methylaziridin-1-yl)-4, 7-dioxoindol-3-yl)thiazole-4-carboxylate ***5c***. Prepared in an analogous manner to **5a** from methylaziridine and **8**; a red amorphous solid, m.p. 189–190°C (found: M^+, 371.0938; $C_{18}H_{17}N_3O_4S$ requires M, 371.0956); ν_{max} ($CHCl_3$)/cm^{-1} 1731, 1659, 1632, 1590, 1396, 1210 and 753; δ_H (400 MHz; $CDCl_3$) 1.36 (3H, t, *J* 7.2 Hz, OCH_2Me), 1.39 (3H, d, *J* 6.8 Hz, CHMe), 2.07 (2H, m, $N(CH_2)$), 2.28 (1H, m, CH), 4.33 (3H, s, NMe), 4.39 (2H, q, *J* 7.2 Hz, OCH_2Me), 5.89 (1H, s, H6), 7.74 (1H, s) and 8.11 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.8, 28.3, 30.1, 37.2, 57.2, 61.8, 107.1, 117.2, 119.7, 120.4,

128.1, 130.3, 131.4, 146.9, 160.3, 161.9, 179.0 and 179.5; *m/z* 371 (M^+, 5%), 229 (15), 172 (50), 127 (60), 101 (65), 58 (100) and 44 (55).

2-(1-Methyl-5-(2-methylaziridin-1-yl)-4, 7-dioxoindol-3-yl)-4-methylthiazole ***5d***. Prepared in an analogous manner to **5b** from **9** and methylaziridine; a red amorphous solid, m.p. 181–184°C (found: M^+, 313.0885; $C_{16}H_{15}N_3O_2S$ requires M, 313.0902); ν_{max} ($CHCl_3$)/cm^{-1} 1661, 1634, 1589, 1500, 1397, 1216 and 753; δ_H (400 MHz; $CDCl_3$) 1.38 (3H, d, *J* 5.5 Hz, CHMe), 2.08 (2H, m, $N(CH_2)$), 2.23 (1H, m, CH), 2.33 (3H, s, Me), 3.96 (3H, s, NMe), 5.70 (1H, s, H6), 6.83 (1H, s) and 7.52 (1H, s); δ_C (100 MHz; $CDCl_3$) 16.0, 18.1, 35.1, 36.6, 37.1, 107.0, 114.9, 116.4, 119.2, 130.2, 130.6, 152.8, 158.2, 160.7, 179.1 and 179.3; *m/z* 313 (M^+, 25%), 267 (30), 217 (30), 155 (60), 144 (70), 51 (90) and 31 (100).

4-(3-Indolyl)thiazoles

4-(4-Benzyloxy-5-methoxy-1-methylindol-3-yl)-2-methylthiazole ***11***. To a stirring suspension of 4-benzyloxy-3-(α-chloroacetyl)-5-methoxy-1-methylindole **10**[12] (0.21 g, 0.62 mmol) and potassium hydrogen carbonate (0.5 g, 5.0 mmol) in ethanol (20 ml) was added thioacetamide (0.14 g, 1.8 mmol). After 15 min the mixture was cooled to 0°C and solution of trifluoroacetic anhydride (0.52 g, 2.5 mmol) and pyridine (0.15 g, 1.9 mmol) in ethanol (5 ml) was added dropwise. The mixture was allowed to warm up to room temperature. The volatile components were removed under reduced pressure and the residue was extracted with dichloromethane and washed with water. The organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography (ether elution) to yield the *thiazole* (0.2 g, 90%) as an off-white solid, m.p. 73–75°C, ν_{max} (KBr)/cm^{-1} 3011, 2950 and 1567; δ_H (250 MHz; $CDCl_3$) 2.70 (3H, s, CMe), 3.73 (3H, s, NMe), 3.91 (3H, s, OMe), 4.90 (2H, s, OCH_2Ph), 7.01 (2H, s), 7.32 (5H, m, Ph), 7.56 (1H, s) and 7.62 (1H, s); δ_C (62.9 MHz; $CDCl_3$) 19.1 (CMe), 33.0 (NMe), 58.1 (OMe), 76.4 (CH_2Ph), 105.2 (CH), 110.5, 111.3 (CH), 112.6 (CH), 127.8 (CH), 128.1 (CH), 128.9 (CH), 130.6 (CH), 134.9, 137.4, 141.8, 146.3, 149.2 and 164.1; *m/z* (EI) 365 (MH^+, 8%), 364 (M^+, 30), 272 (34), 273 (100), 218 (27) and 91 (61).

4-(4-Hydroxy-5-methoxy-1-methylindol-3-yl)-2-methylthiazole ***12***. 4-(4-Benzyloxy-5-methoxy-1-methylindol-3-yl)-2-methylthiazole **11** (0.2 g, 0.55 mmol) was dissolved in dichloromethane (15 ml). A catalytic quantity of palladium/carbon 10% was added. The reaction was stirred under a hydrogen atmosphere overnight. 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 *alcohol* (0.11 g, 76%) as an off-white solid, m.p. 121–122°C (found: M^+, 274.0776; $C_{14}H_{14}N_2O_2S$ requires M, 274.0793); ν_{max} (KBr)/cm^{-1} 3400, 3086 and 2910; δ_H (250 MHz; $CDCl_3$) 2.78 (3H, s, CMe), 3.73 (3H, s, NMe), 3.95 (3H, s, OMe), 6.67 (1H, d, *J* 8.7 Hz), 6.96 (1H, s, =CHS), 7.00 (1H, d, *J* 8.7 Hz), 7.27 (1H, s, NCH=) and 13.17 (1H, broad s, OH); δ_C (62.9 MHz; $CDCl_3$) 18.5 (CMe), 33.1 (NMe), 58.2 (OMe), 98.8 (CH), 106.1 (CH), 109.5, 112.6 (CH), 115.2, 125.8 (CH), 134.8, 141.1, 141.3, 149.4 and 165.6; *m/z* (EI) 275 (MH^+, 18%), 274 (M^+, 92), 260 (19), 259 (100), and 231 (31).

4-(5-Methoxy-1-methyl-4, 7-dioxoindol-3-yl)-2-methylthiazole ***13***. A solution of potassium nitrosodisulfonate (Fremy's salt) (0.34 g, 1.2 mmol) in water (12 ml) was added to a stirred solution of 4-(4-hydroxy-5-methoxy-1-methylindol-3-yl)-2-methylthiazole **12** (0.11 g, 0.4 mmol) in acetone (20 ml), buffered with sodium dihydrogen phosphate (0.17 M, 12 ml). After 2 h the mixture was concentrated and the resulting residue was extracted with dichloromethane and washed with water. The organic layer was dried ($MgSO_4$) and purified by column chromatography (ether elution) to give the *quinone* (0.092 g, 80%) as an orange crystalline solid, m.p. 216–218°C (found: C, 58.6; H, 4.0; N, 9.6; $C_{14}H_{12}N_2O_3S$ requires C, 58.3; H, 4.2; N, 9.7%) (found: M^+ 288.0568; $C_{14}H_{12}N_2O_3S$ requires M, 288.0585); ν_{max} (KBr)/cm^{-1} 3123, 2964, 2930, 1661, 1644, 1629 and 1599; λ_{max} (MeOH/nm 466 (4638), 288 (15634), 254 (16943) and 223 (28800); δ_H (400 MHz; DMSO) 2.71 (3H, s, CMe), 3.84 (3H, s, OMe), 3.99 (3H, s, NMe), 5.71 (1H, s), 7.53 (1H, s) and 8.58 (1H, s); δ_C (100.6 MHz; DMSO) 19.0 (CMe), 36.7 (NMe), 70.0 (OMe), 106.3 (CH), 117.0 (CH), 119.4, 120.2, 130.4 (CH), 146.6, 156.4, 160.6, 164.7, 176.8 (CO) and 178.9 (CO); *m/z* (EI) 289 (MH^+, 19%), 288 (M^+, 100), 273 (23), 259 (20), 69 (23) and 42 (46).

4-(5-(Aziridin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)-2-methylthiazole ***6c***. 4-(5-Methoxy-1-methyl-4,7-dioxoindol-3-yl)-2-methylthiazole **13** (17.2 mg, 0.059 mmol), aziridine (0.5 ml) [**CAUTION**] and methanol (3 ml) were stirred at room temperature for 16 h. The reaction mixture was evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclu-

sion chromatography (sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (14 mg, 87%) as a red amorphous solid, m.p. 210–214°C; ν_{max} ($CHCl_3$)/cm^{-1} 1636, 1588, 1490, 1389, 1215 and 755; δ_H (250 MHz; $CDCl_3$) 2.24 (4H, s, $N(CH_2)_2$), 2.72 (3H, s, Me), 4.0 (3H, s, NMe), 5.85 (1H, s, H6), 7.52 (1H, s) and 8.61 (1H, s); δ_C (100 MHz; $CDCl_3$) 19.4, 28.2, 37.0, 106.8, 117.0, 117.4, 120.5, 130.8, 147.1, 158.6, 161.2, 165.1, 179.1 and 179.4.

4-(1-Methyl-5-(2-methylaziridin-1-yl)-4,7-dioxoindol-2-yl)-2-methylthiazole ***6d***. Prepared in an analogous manner to **6c** from **13** and methylaziridine; a red amorphous solid, m.p. 151–153°C (found: M^+, 313.0889; $C_{16}H_{15}N_3O_2S$ requires M, 313.0902); ν_{max} ($CHCl_3$)/cm^{-1} 1658, 1633, 1589, 1490, 1387, 1172 and 752; δ_H (400 MHz; $CDCl_3$) 1.38 (3H, d, *J* 5.7 Hz, CHMe), 2.05 (2H, m, $N(CH_2)$), 2.25 (1H, m, N(CH)), 2.60 (3H, s, Me), 3.93 (3H, s, NMe), 5.80 (1H, s, H6), 7.37 (1H, s) and 8.60 (1H, s); δ_C (100 MHz; $CDCl_3$) 18.1, 19.5, 34.5, 35.1, 36.7, 116.3, 117.4, 120.4, 120.5, 130.6, 131.0, 147.3, 158.7, 165.1, 179.2 and 179.5; *m/z* 313 (M^+, 5%), 249 (10), 125 (20), 98 (10), 84 (50), 70 (25), 56 (30) and 41 (100).

2-(2-Indolyl)thiazoles

4-Benzyloxy-5-methoxy-1-methylindole-2-carboxamide ***15***. Ammonium hydroxide (0.88; 15 ml) was added to methyl 4-benzyloxy-5-methoxy-1-methyl-2-carboxylate **14**[6] (410 mg, 1.32 mmol) and ammonium chloride (18 mg, 0.336 mmol), in a Young's pressure tube. The tube was sealed and the contents stirred at 100°C for 48 h. The crude reaction mixture was evaporated under reduced pressure to give a brown oil. Dichloromethane (100 ml) was added and the mixture was washed with brine (2 × 40 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* (264 mg, 68%) as a pale brown solid, m.p. 148–150°C (found: C, 69.3; H, 5.8; N, 8.9; $C_{18}H_{18}N_2O_3$ requires C, 69.65; H, 5.8; N, 9.0%); ν_{max} (Nujol)/cm^{-1} 1678, 1462, 1377 and 1229; δ_H (250 MHz; $CDCl_3$) 3.88 (3H, s, NMe), 4.01 (3H, s, OMe), 5.23 (2H, s, OCH_2Ar), 5.72 (2H, broad, NH_2), 6.83 (1H, s), 7.05 (1H, d, *J* 8.9 Hz, ArH), 7.09 (1H, d, *J* 8.9 Hz, ArH), 7.33 (4H, m, ArH) and 7.50 (1H, m, ArH); δ_C (62.5 MHz; $CDCl_3$) 31.7 (NMe), 58.1 (OMe), 75.1 (OCH_2Ar), 102.3, 105.4, 115.0, 121.4, 127.3, 127.8, 128.1, 128.3, 130.7, 136.5, 138.0, 145.1 and 164.2; *m/z* 310 (M^+, 70%), 267 (30), 148 (55), 91 (100) and 65 (30).

4-Benzyloxy-5-methoxy-1-methylindole-2-thiocarboxamide ***16***. A solution of 4-benzyloxy-5-methoxy-1-methylindole-3-carboxamide **15** (462 mg, 1.49 mmol), Lawesson's reagent (356 mg, 0.88 mmol) and toluene (25 were heated under reflux for 1 h. The crude reaction mixture was evaporated under reduced pressure and flash column chromatography (dichloromethane:diethyl ether elution) gave the title compound (318 mg, 65%) as a yellow solid, m.p. 176–177°C (found: C, 66.5; H, 5.4; N, 8.4; $C_{18}H_{18}N_2O_2S$ requires C, 66.2; H, 5.6; N, 8.6%); ν_{max} (Nujol)/cm^{-1} 1642, 1596, 1523 and 1463; δ_H (250 MHz; $CDCl_3$) 3.89 (3H, s, NMe), 4.03 (3H, s, OMe), 5.21 (2H, s, OCH_2Ar), 6.77 (1H, s), 7.03 (3H, m), 7.12 (3H, m) and 7.49 (3H, m); δ_C (62.5 MHz; $CDCl_3$) 32.3 (NMe), 58.2 (OMe), 75.2 (OCH_2Ar), 100.4, 105.6, 115.1, 122.3, 127.8, 128.1, 128.3, 128.7, 138.1, 138.1, 142.0, 146.0 and 192.0; *m/z* 326 (M^+, 40%), 235 (75), 201 (30), 148 (20), 91 (100) and 65 (30).

Ethyl 2-(4-benzyloxy-5 methoxy-1-methylindol-2-yl) thiazole-4-carboxylate ***17a***. 4-Benzyloxy-5-methoxy-1-methylindole-3-thiocarboxamide **16** (674 mg, 2.06 mmol), bromopyruvic acid (725 mg, 4.34 mmol) and ethanol (90 ml) were heated under reflux for 0.5 h. The crude reaction mixture was evaporated under reduced pressure, to give an oil, water (30 ml) was added and the mixture extracted with ethyl acetate (3 × 50 ml), washed with brine (30 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (dichloromethane: ethyl acetate elution) gave 2-(4-benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylic acid (509 mg, 62% yield) as a pale yellow solid, m.p. 143–145°C; δ_H (250 MHz; $CDCl_3$) 3.92 (3H, s, NMe), 4.11 (3H, s, OMe), 5.26 (2H, s, OCH_2Ar), 6.99 (1H, s), 7.07 (2H, m), 7.35 (3H, m), 7.50 (2H, m) and 8.24 (1H, s), carboxylic acid OH unobserved. The acid was used without further purification.

The above acid (471 mg, 1.19 mmol), ethanol (20 ml) and sulfuric acid (0.5 ml, of a concentrated solution) were heated under reflux for 14 h. Brine (20 ml) was added and the mixture extracted with dichloromethane (3 × 40 ml). The extracts were combined and washed with brine (30 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) gave the *title compound* (428 mg, 85%) as a pale yellow solid, m.p. 113–114°C (found: M^+, 422.1257; $C_{23}H_{22}N_2O_4S$ requires M, 422.1317); ν_{max} ($CHCl_3$)/cm^{-1} 1722, 1553, 1421, 1321, 1215 and 757; δ_H (250 MHz; $CDCl_3$) 1.43 (3H, t, *J* 7.2 Hz, $OCH_2\mathit{Me}$), 3.97 (3H, s, NMe), 4.15 (3H, s, OMe), 4.42 (2H, q, *J* 7.15, OCH_2Me), 5.25 (2H, s, OCH_2Ar), 6.96 (1H, s), 7.07

(2H, s), 7.35 (4H, m), 7.50 (1H, m) and 8.11 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 14.3 (OCH_2Me), 31.9 (NMe), 58.3 (OMe), 61.4 (OCH_2Me), 75.1 (OCH_2Ar), 102.9, 105.2, 114.0, 122.5, 126.6, 127.8, 128.1, 128.3, 128.7, 131.6, 136.5, 138.0, 145.3, 147.7, 161.1 and 161.2; *m/z* 422 (M^+, 5%), 332 (70), 317 (45), 111 (30), 97 (40), 83 (50), 69 (70) and 55 (100).

Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate ***18a***. Ethyl 2-(4-benzyloxy-5-methoxy-1-methylindol-2-yl)thiazole-4-carboxylate **17a** (0.6 g, 1.42 mmol), ethanol (10 ml) and palladium hydroxide (Pearlman's catalyst) (0.2 g, 0.14 mmol) were stirred under a hydrogen atmosphere at atmospheric pressure for 48 h. The reaction mixture was filtered, washed with dichloromethane (100 ml), and the filtrate and washings were combined and evaporated under reduced pressure. Acetone (20 ml) and sodium dihydrogen phosphate (0.17 M, 20 ml) were added and the mixture stirred at room temperature for 10 min. Fremy's salt (1.9 g, 2.5 mmol) and water (20 ml) were added and the mixture stirred at room temperature for 16 h. The mixture was extracted with dichloromethane (3 × 30 ml). The extracts were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) gave the *title compound* (0.317 g, 64%) as an orange solid (found: M^+, 346.0645; $C_{16}H_{14}N_2O_5S$ requires M, 346.0640); δ_H (250 MHz; $CDCl_3$) 1.42 (3H, t, *J* 7.2 Hz, OCH_2Me), 3.80 (3H, s, OMe), 4.39 (3H, 3H, s, NMe), 4.42 (2H, q, *J* 7.2 Hz, OCH_2Me), 7.78 (1H, s, H6), 7.96 (1H, s) and 8.2 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 14.2, 35.2, 56.6, 61.6, 108.1, 110.8, 116.1, 121.0, 124.3, 127.4, 127.9, 129.2, 148.2, 160.1, 178.9 and 179.6; *m/z* 346 (M^+, 10%), 97 (40), 81 (50), 69 (85), 55 (60) and 41 (100).

2-(4-Benzyloxy-5-methoxy-1-methylindol-2-yl)-4-methylthiazole ***17b***. 4-Benzyloxy-5-methoxy-1-methylindole-3-thiocarboxamide **16** (173 mg, 0.53 mmol), bromoacetone (145 mg, 1.06 mmol) and ethanol (20 ml) were heated under reflux for 1 h. The reaction mixture was evaporated under reduced pressure and flash column chromatography (dichloromethane : ethyl acetate elution) gave the *title compound* as a pale yellow solid (133 mg, 69%), m.p. 115–117°C (found: M^+, 364.1261; $C_{21}H_{20}N_2O_2S$ requires M, 364.1262); ν_{max} ($CHCl_3$)/cm^{-1} 1503, 1415, 1341, 1215, 1003 and 771; δ_H (250 MHz; $CDCl_3$) 2.32 (3H, s, Me), 3.92 (3H, s, NMe), 4.01 (3H, s, OMe), 5.25 (2H, s, OCH_2Ar), 6.85 (1H, s), 6.94 (1H, s), 7.04 (2H, s), 7.33 (3H, m) and 7.55 (2H, m); δ_C (62.5 MHz; $CDCl_3$) 17.3 (Me), 31.9 (NMe), 58.3 (OMe), 52.0, 75.0 (OCH_2Ar), 101.7, 102.2, 105.1, 113.1, 113.4, 113.7, 117.1, 122.5, 127.7, 128.1, 128.3, 132.8, 136.4, and 145.3; *m/z* 311 (40%), 364 (20), 273 (100), 230 (20) and 91 (40).

2-(5-Methoxy-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole ***18b***. 2-(4-Benzyloxy-5-methoxy-1-methylindol-2-yl)-4-methylthiazole **17b** (0.58 g, 1.60 mmol), ethanol (10 ml) and palladium hydroxide (Pearlman's catalyst) (0.223 g, 0.16 mmol) were stirred under a hydrogen atmosphere at atmospheric pressure for 48 h. The reaction mixture was filtered, washed with dichloromethane (100 ml), and the filtrate and washings were combined and evaporated under reduced pressure. Acetone (20 ml) and sodium dihydrogen phosphate (0.17 M, 20 ml) was added and the mixture stirred at room temperature for 10 min. Fremy's salt (2.14 g, 8.0 mmol) and water (20 ml) were added and the mixture stirred at room temperature for 16 h. The mixture was extracted with dichloromethane (3 × 30 ml). The extracts were combined, washed with brine (30 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) gave the *title compound* (0.272 g, 59%) as an orange solid, m.p. 210–213°C (found: M^+, 288.0573; $C_{14}H_{12}N_2O_3S$ requires M, 288.0585); ν_{max} ($CHCl_3$)/cm^{-1} 1741, 1692, 1569, 1481, 1208 and 741; δ_H (250 MHz; $CDCl_3$) 2.04 (3H, s, Me), 3.85 (3H, s, OMe), 4.36 (3H, NMe), 5.75 (1H, s, H6), 6.93 (1H, s) and 7.02 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 17.2, 35.0, 56.5, 107.9, 109.9, 113.4, 114.1, 125.0, 131.2, 154.1, 156.9, 160.0, 178.8 and 179.2.

Ethyl 2-(5-(aziridin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate ***7a***. Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate **18a** (25.6 mg, 0.074 mmol), aziridine (0.5 ml) [**CAUTION**] and methanol (3 ml) were stirred at room temperature for 16 h. The reaction mixture was evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclusion chromatography (Sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (11.2 mg, 42%) as a red amorphous solid (found: M^+, 357.0817; $C_{17}H_{15}N_3O_4S$ requires M, 357.0800); ν_{max} ($CHCl_3$)/cm^{-1} 1717, 1674, 1641, 1398, 1355, 1260 and 756; δ_H (250 MHz; $CDCl_3$) 1.42 (3H, t, *J* 7.2 Hz, OCH_2Me), 2.24 (4H, s, $N(CH_2)_2$), 4.4 (3H, s, NMe), 4.45 (2H, q, *J* 7.2 Hz, OCH_2Me), 5.9 (1H, s, H6), 7.06 (1H, s) and 8.17 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.3, 29.2, 35.2, 61.7, 110.8, 118.2, 125.3, 127.4, 128.4, 131.9, 141.8, 148.2, 157.4, 162.5, 179.0 and 179.7; *m/z* 357 (M^+, 10%), 267 (10), 217 (10), 148 (100), 77 (60) and 51 (40).

2-(5-(Aziridin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole **7b**. 2-(5-Methoxy-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole **18b** (25.6 mg, 0.074 mmol), aziridine (0.5 ml) [**CAUTION**] and methanol (3 ml) were stirred at room temperature for 16 h. The reaction mixture was evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclusion chromatography (Sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (11.2 mg, 42%) as a red amorphous solid (found: M^+, 299.0732; $C_{15}H_{13}N_3O_2S$ requires M, 299.0745); ν_{max} ($CHCl_3$)/cm^{-1} 1676, 1640, 1587, 1480, 1351, 1203 and 782; δ_H (250 MHz; $CDCl_3$) 2.24 (4H, s, $N(CH_2)_2$), 2.50 (3H, s, Me), 4.35 (3H, s, NMe), 5.88 (1H, s, H6), 6.97 (1H, s) and 6.99 (1H, S); δ_C (62.5 MHz; $CDCl_3$) 17.7, 28.1, 35.4, 110.3, 114.5, 118.6, 125.2, 131.5, 133.7, 154.5, 157.6, 157.9, 179.0 and 179.4; *m/z* 299 (M^+, 100%), 272 (20), 244 (10), 215 (10), 176 (10), 71 (20) and 45 (15).

Ethyl 2-(1-methyl-5-(2-methylaziridin-1-yl)-4,7-dioxoindol-2-yl)thiazole-4-carboxylate **7c**. Prepared in an analogous manner to **7a** from methylaziridine and **18a**; a red amorphous solid, m.p. 196–199°C (found: M^+, 371.0905; $C_{18}H_{17}N_3O_4S$ requires M, 371.0956); ν_{max} ($CHCl_3$)/cm^{-1} 1715, 1668, 1620, 1591, 1397, 1242 and 747; δ_H (250 MHz; $CDCl_3$) 1.35 (3H, t, *J* 7.3 Hz, OCH_2Me), 1.53 (3H, d, *J* 6.7 Hz, CHMe), 3.12 (2H, m, NCH_2), 3.98 (1H, m, NCH), 4.35 (3H, s, NMe), 4.41 (2H, q, *J* 7.3 Hz, OCH_2Me), 6.01 (1H, s, H6), 6.97 (1H, s) and 8.09 (1H, s); *m/z* 371 (M^+, 5%), 317 (15), 267 (25), 217 (20), 155 (50) and 51 (100).

2-(1-Methyl-5-(2-methylaziridin-1-yl)-4,7-dioxoindol-2-yl)-4-methylthiazole **7d**. Prepared in an analogous manner to **7b** from **18b** and methylaziridine; a red amorphous solid, m.p. 190–193°C (found: M^+, 313.0891; $C_{16}H_{15}N_3O_2S$ requires M, 313.0902); ν_{max} ($CHCl_3$)/cm^{-1} 1669, 1594, 1552, 1503, 1393, 1258 and 753; δ_H (400 MHz; $CDCl_3$) 1.48 (3H, d, *J* 6.8 Hz, CHMe), 2.46 (3H, s, Me), 3.29 (2H, m, CH_2), 4.16 (1H, m, CH), 4.29 (3H, NMe), 5.81 (1H, s, H6), 6.82 (1H, s) and 6.89 (1H, s); δ_C (100 MHz; $CDCl_3$) 17.9, 23.7, 36.9, 47.3, 49.3, 110.2, 114.2, 129.2, 133.0, 133.5, 135.7, 147.1, 154.5, 158.1, 178.0 and 179.3; *m/z* 313 (M^+, 50%), 286 (100), 176 (15), 142 (15), 71 (70) and 45 (60).

2-(5-(2,3-cis-Dimethylaziridin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole **7e**. Prepared in an analogous manner to **7b** from **18b** and *cis*-2,3-dimethylaziridine; a red amorphous solid, m.p. 218–220°C (found: M^+, 327.1039; $C_{17}H_{17}N_3O_2S$ requires M, 327.1058); ν_{max} ($CHCl_3$)/cm^{-1} 1669, 1642, 1586, 1552, 1395 and 783; δ_H (400 MHz; $CDCl_3$) 1.32 (6H, d, *J* 6.6 Hz, $(Me)_2$), 2.15 (2H, m, $(CH)_2$), 3.89 (3H, s, Me), 4.37 (3H, s, NMe), 5.81 (1H, s, H6), 6.95 (1H, s) and 8.11 (1H, s); δ_C (100 MHz; $CDCl_3$) 13.1, 35.5, 40.6, 52.9, 111.1, 117.1, 125.1, 127.8, 132.2, 148.2, 158.6, 159.4, 161.8, 179.0 and 179.6; *m/z* 327 (M^+, 5%), 270 (10), 70 (20), 57 (15) and 41 (100).

2-(5-(Azetidin-1-yl)-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole **7f**. Prepared in an analogous manner to **7b** from **18b** and azetidine; a purple amorphous solid, m.p. 183–185°C (found: M^+, 313.0887; $C_{16}H_{15}N_3O_2S$ requires M, 313.0902); ν_{max} ($CHCl_3$)/cm^{-1} 1663, 1622, 1576, 1549, 1384, 1258 and 777; δ_H (400 MHz; $CDCl_3$) 2.11 (3H, s, Me), 2.86 (6H, m), 4.73 (3H, s, NMe), 5.72 (1H, s, H6), 7.24 (1H, s) and 7.63 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 17.3, 17.8, 29.7, 34.9, 109.4, 113.5, 122.7, 128.3, 131.9, 133.6, 148.8, 153.9, 157.9, 177.4 and 179.4; *m/z* 313 (M^+, 100%), 284 (30), 257 (20), 229 (20), 176 (20), 71 (45), 53 (20) and 45 (35).

Ethyl 2-(1-methyl-5-(pyrrolidin-1-yl)-4,7-dioxoindol-2-yl)thiazole-4-carboxylate **7g**. Ethyl 2-(5-methoxy-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate **18a** (15 mg, 0.043 mmol), pyrrolidine (0.5 ml) and methanol (3 ml) were stirred at room temperature for 16 h. Water (10 ml) was added and the mixture extracted with dichloromethane (3 × 10 ml). The extracts were combined, washed with hydrochloric acid (2 M, 3 × 10 ml), brine (10 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclusion chromatography (Sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (13 mg, 81%) as a purple amorphous solid, m.p. 175–178°C (found: M^+, 385.1103; $C_{19}H_{19}N_3O_4S$ requires M, 385.1113); ν_{max} ($CHCl_3$)/cm^{-1} 1725, 1667, 1613, 1545, 1385, 1272, 1205 and 752; δ_H (250 MHz; $CDCl_3$) 1.42 (3H, t, *J* 7.2 Hz, OCH_2Me), 1.65 (4H, m, $(CH_2)_2$), 3.45 (4H, m, $N(CH_2)_2$), 4.39 (3H, s, NMe), 4.47 (2H, q, *J* 7.2 Hz, OCH_2Me), 5.61 (1H, s, H6), 6.98 (1H, s) and 8.14 (1H, s); *m/z* 385 (M^+, 25%), 267 (10), 217 (10), 155 (25), 113 (20), 70 (20) and 51 (50).

2-(1-Methyl-5-(pyrrolidin-1-yl)-4,7-dioxoindol-2-yl)-4-methylthiazole **7h**. 2-(5-Methoxy-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole **18b** (10 mg, 0.052 mmol), pyrrolidine (0.5 ml) and methanol (3 ml) were stirred at room temperature for 16 h. Water (10 ml) was added and the mixture extracted

with dichloromethane (3 × 10 ml). The extracts were combined, washed with hydrochloric acid (2 M, 3 × 10 ml), brine (10 ml), dried (Na_2SO_4) and evaporated under reduced pressure. Flash column chromatography (dichloromethane:ethyl acetate elution) followed by size exclusion chromatography (Sephadex LH 20 gel filtration dichloromethane:methanol elution) gave the *title compound* (7.1 mg, 63%) as a purple amorphous solid, m.p. 224–226°C (found: M^+, 327.1048; $C_{17}H_{17}N_3O_2S$ requires M, 327.1058); ν_{max} ($CHCl_3$)/cm^{-1} 1663, 1606, 1541, 1383, 1274 and 773; δ_C (100 MHz; $CDCl_3$) 17.6, 35.5, 51.5, 53.8, 103.4, 110.2, 113.8, 123.8, 132.3, 133.2, 149.3, 154.2, 158.4, 178.2 and 180.1; *m/z* 327 (M^+, 100%), 284 (15), 229 (10), 155 (20), 113 (15), 70 (30) and 51 (40).

Ethyl 2-(1-methyl-5-(piperidin-1-yl)-4,7-dioxoindol-2-yl)thiazole-4-carboxylate **7i**. Prepared in an analogous manner to **7g** from **18a** and piperidine; a purple amorphous solid, m.p. 153–155°C (found: M^+, 399.1262; $C_{20}H_{21}N_3O_4S$ requires M, 399.1269); ν_{max} ($CHCl_3$)/cm^{-1} 1724, 1668, 1628, 1548, 1391, 1244 and 757; δ_H (250 MHz; $CDCl_3$) 1.41 (3H, t, *J* 7.2 Hz, OCH_2Me), 1.61 (2H, m, CH_2), 1.65 (4H, m, $(CH_2)_2$), 2.95 (4H, m, $N(CH_2)_2$), 4.39 (3H, s, NMe), 4.44 (2H, q, *J* 7.2, OCH_2Me), 5.61 (1H, s, H6), 6.99 (1H, s) and 8.1 (1H, s); δ_C (100 MHz; $CDCl_3$) 14.7, 24.6, 29.9, 35.3, 51.3, 61.7, 109.9, 111.4, 125.2, 127.3, 128.4, 131.5, 148.5, 154.3, 159.6, 161.4, 178.8 and 179.2; *m/z* 399 (M^+, 100%), 176 (10), 155 (15), 103 (20), 84 (40), 51 (35) and 41 (40).

2-(1-Methyl-5-(piperidin-1-yl)-4,7-dioxoindol-2-yl)-4-methylthiazole **7j**. Prepared in an analogous manner to **7h** from **18b** and piperidine; a purple amorphous solid, m.p. 166–168°C (found: M^+, 341.1204; $C_{18}H_{19}N_3O_2S$ requires M, 341.1215); ν_{max} ($CHCl_3$)/cm^{-1} 1663, 1632, 1547, 1385, 1244 and 782; δ_H (250 MHz; $CDCl_3$) 1.69 (6H, m), 2.49 (3H, s, Me), 3.44 (4H, m, $N(CH_2)_2$), 4.35 (3H, s, NMe), 5.59 (1H, s, H6), 6.88 (1H, s) and 6.92 (1H, s); δ_C (100 MHz; $CDCl_3$) 17.6, 24.7, 26.2, 35.2, 51.3, 110.0, 110.4, 114.0, 125.3, 132.2, 132.8, 154.3, 154.3, 158.2, 179.0 and 180.3; *m/z* 341 (M^+, 100%), 284 (10), 257 (10), 229 (10), 205 (10), 84 (30), 55 (10) and 41 (25).

2-(1-Methyl-5-(morpholin-1-yl)-4,7-dioxoindol-2-yl)-4-methylthiazole **7k**. Prepared in an analogous manner to **7h** from **18b** and morpholine; a purple amorphous solid; ν_{max} ($CHCl_3$)/cm^{-1} 1664, 1629, 1547, 1386 and 778; δ_H (250 MHz; $CDCl_3$) 2.51 (3H, s, Me), 3.46 (4H, m, $N(CH_2)_2$), 3.85 (4H, m, $O(CH_2)_2$), 4.35 (3H, s, NMe), 5.59 (1H, s, H6), 6.91 (1H, s) and 6.94 (1H, s).

Ethyl 2-(5-cyclopropylamino-1-methyl-4,7-dioxoindol-2-yl)thiazole-4-carboxylate **7l**. Prepared in an analogous manner to **7g** from **18a** and cyclopropylamine; a purple amorphous solid, m.p. 210–214°C (found: M^+, 371.0938; $C_{18}H_{17}N_3O_4S$ requires M, 371.0956); ν_{max} ($CHCl_3$)/cm^{-1} 1725, 1674, 1620, 1593, 1504, 1398, 1257 and 780; δ_H (400 MHz; $CDCl_3$) 0.81 (4H, m, $(CH_2)_2$), 1.36 (3H, t, *J* 7.2 Hz, OCH_2Me), 2.39 (1H, m, CH), 4.36 (3H, s, NMe), 4.39 (2H, q, *J* 7.2 Hz, OCH_2Me), 5.60 (1H, s, H6), 5.87 (1H, br, NH), 6.94 (1H, s) and 8.09 (1H, s); δ_C (100 MHz; $CDCl_3$) 7.4, 14.7, 24.6, 35.6, 62.0, 100.9, 110.9, 122.7, 127.3, 127.6, 131.4, 148.5, 149.6, 159.4, 161.4, 178.3 and 179.4; *m/z* 371 (M^+, 70%), 217 (20), 186 (20), 155 (30), 113 (25), 80 (40) and 51 (100).

2-(5-Cyclopropylamino-1-methyl-4,7-dioxoindol-2-yl)-4-methylthiazole **7m**. Prepared in an analogous manner to **7h** from **18b** and cyclopropylamine; a purple amorphous solid, m.p. 248–251°C (found: M^+, 313.0892; $C_{16}H_{15}N_3O_2S$ requires M, 313.0902); ν_{max} ($CHCl_3$)/cm^{-1} 1664, 1585, 1551, 1496, 1394, 1215 and 753; δ_H (400 MHz; $CDCl_3$) 0.54 (2H, m), 0.77 (2H, m), 2.37 (1H, m, CH), 2.38 (3H, s, Me), 4.30 (3H, s, NMe), 5.56 (1H, s, H6), 5.85 (1H, br, NH), 6.81 (1H, s) and 6.86 (1H, s); δ_C (100 MHz; $CDCl_3$) 6.5, 16.7, 23.7, 34.5, 99.8, 109.1, 113.1, 121.8, 131.8, 132.8, 148.6, 153.5, 157.2, 177.6 and 180.3; *m/z* 313 (M^+, 100%), 296 (30), 284 (35), 71 (25) and 51 (50).

Biology

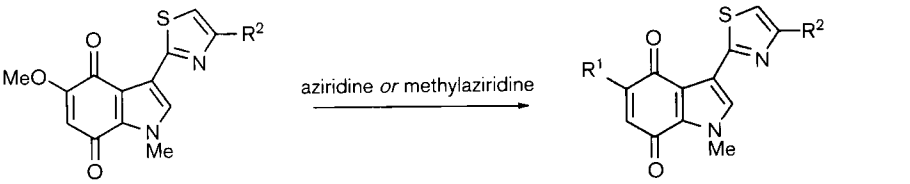
The thiazolyl indolequinones were tested against the human breast cancer cell line SKBr3, in order to aid comparison with the previously prepared compounds which were also tested against this cell line.[12,13] Selected compounds were also studied in human lung carcinoma A549 and PV9 cell lines. Aliquots of cells were placed into 24-well microtiter tissue culture plates at a seeding density of 10^4 cells/well (SKBr3) and 3×10^3 cells/well (A549 and PV9). These cell numbers allow exponential growth for the subsequent 4 days. Two hours after the initial plating, varying concentrations of each compound were added to the cells (four wells per concentration). Cells were then incubated continuously for 4 days prior to determination of cell growth by the MTT assay.[14–16] Each experiment was repeated at least twice and values of IC_{50}, the concentration of compound required to reduce optical density (i.e. cell growth) by 50% compared to untreated controls, were derived from the cumulative data.

Selective toxicity towards hypoxic V79 cells was determined also using the MTT assay as described elsewhere.[15] 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) versus IC_{50} (N_2) hypoxic cytotoxicity ratio (HCR) enables quantitative comparison to be made of the O_2-dependent bioreductive activities of these compounds.

Results

The 3-(2-thiazolyl)indolequinones **5a–5d** were readily obtained by displacement of the methoxy group in the previously prepared quinones **8**[8] and **9**[12] by aziridine and methylaziridine (Scheme 1).

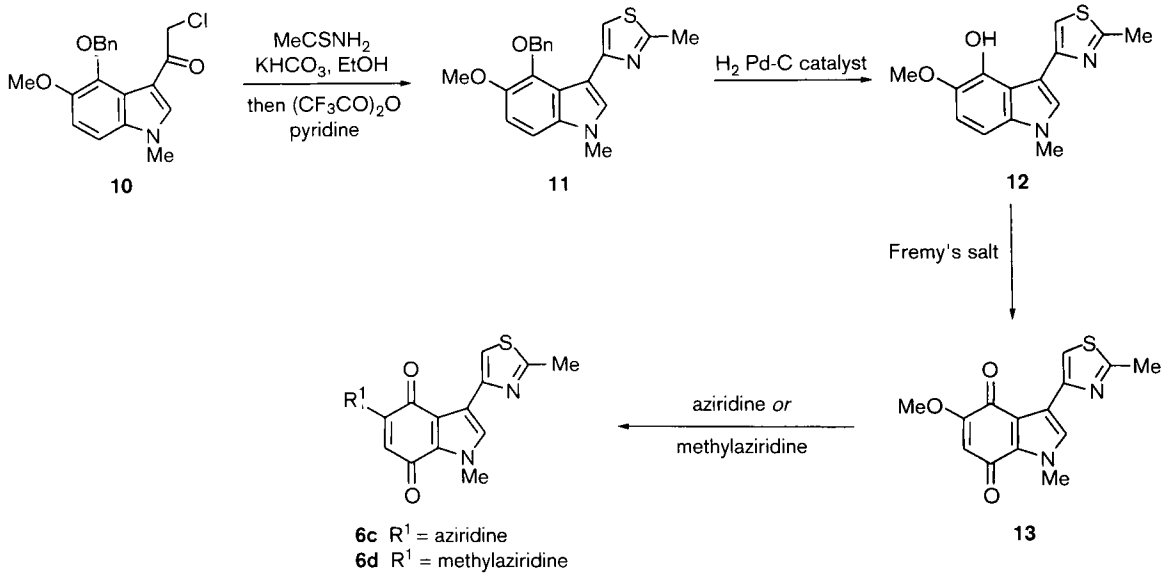
The 4-thiazolyl isomers **6a** and **6b** were obtained as previously described,[12] whilst the analogs **6c** and **6d** were prepared from the corresponding 5-methoxyindolequinone **13** (Scheme 2). Thus the chloroacetylindole **10**[12] was reacted with thioacetamide in a modified Hantzsch reaction to give the thiazole **11** in excellent yield (90%). Hydrogenolysis of the benzyl group (76%) was followed by oxidation of the phenol **12** with Fremy's salt to give the required quinone **13**



8 $R^2 = CO_2Et$
9 $R^2 = Me$

5a R^1 = aziridinyl, $R^2 = CO_2Et$
5b R^1 = aziridinyl, R^2 = Me
5c R^1 = methylaziridinyl, $R^2 = CO_2Et$
5d R^1 = methylaziridinyl, R^2 = Me

Scheme 1



10 **11** **12** **13**

6c R^1 = aziridine
6d R^1 = methylaziridine

Scheme 2

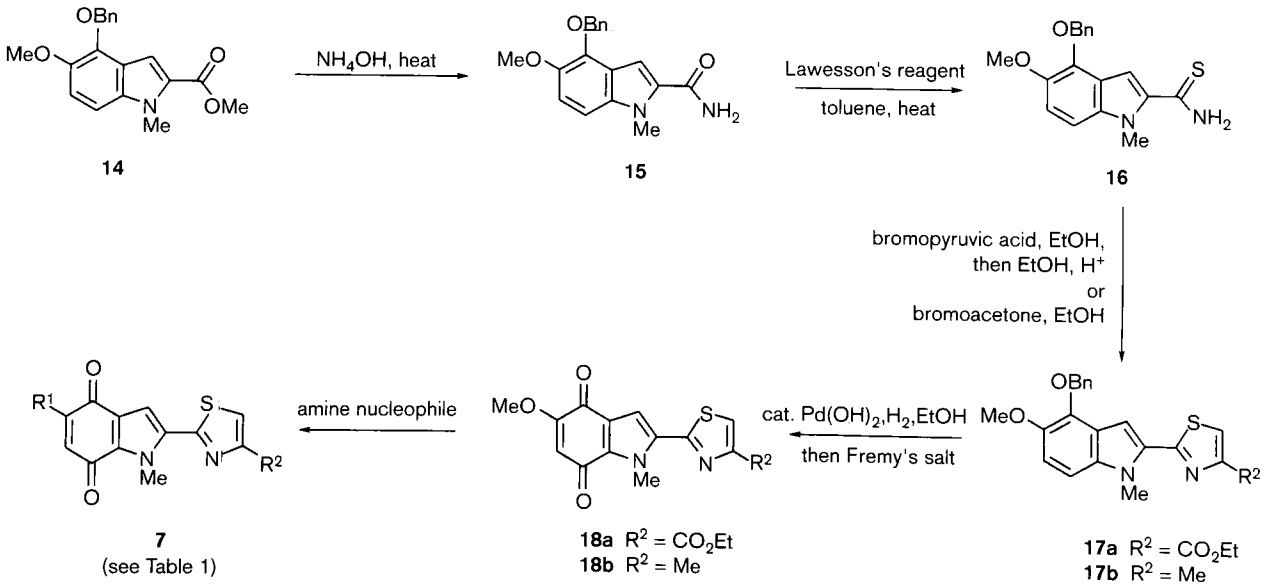
(80%) (Scheme 2). Finally displacement of the methoxy group with aziridine or methylaziridine gave the substituted indolequinones **6c** and **6d**.

The 2-(2-thiazolyl)indolequinones **7** were prepared using the Hantzsch reaction. The indole ester **14**[6] was converted into the thioamide **16** (68%) via the corresponding amide **15** (Scheme 3). Hantzsch reaction of the thioamide **16** with either bromopyruvic acid, followed by esterification, or with bromoacetone gave the 2-(2-thiazolyl)indoles **17a** and **17b**. Hydrogenolysis of the benzyl group followed by oxidation with Fremy's salt gave the 5-methoxyindolequinones **18** (Scheme 3). Displacement of the methoxy group with a range of nitrogen nucleophiles gave the series of indolequinones **7**.

The cytotoxic activity of the thiazolyl indolequinones was determined in the human breast cancer SKBr3 cell line, in order to aid comparison with the previously prepared compounds which were also tested against this cell line.[12,13] This line, which over-expresses Topo IIα,[17] is well characterized with respect to estrogen and EGF receptors.[18] Selected compounds were also studied in human lung carcinoma A549 and PV9 cell lines. A549 cells also show high expression of both the Topo IIα and IIβ genes and also show sensitivity to conventional Topo II inhibitors such as etoposide, mitoxantrone and amsacrine. Houlbrook *et al.* generated a variant of A549 by continuous culture of the cells in etoposide.[19] A549 cells were first exposed to 58 nM etoposide, allowed to grow to confluence and then re-seeded in twice the concentration of drug. This procedure was followed for nine passages so that the final population of cells (PV9) were able to grow in 15 mM etoposide. The PV9 cells show a 15-fold resistance to etoposide compared to A549 wild-type cells and a 5-fold increase in resistance to adriamycin and amsacrine. The resistance of the PV9 cells to the Topo II inhibitors was thought to be due to a 5.8- and 3.5-fold down-regulation of the Topo IIα and Topo IIβ gene expression, respectively. Sensitivity of the SKBr3, A549 and PV9 cells to the thiazolyl indolequinones was assessed by the MTT assay. In addition, some compounds were evaluated for their possible bioreductive action by determining their cytotoxicity towards V79 Chinese hamster lung fibroblasts in air and under anerobic (hypoxic) conditions. The biological results are presented in Table 1.

Discussion

This work was designed to examine the structure-activity relationships within three series of thiazole-containing indolequinones, thereby extending our previous preliminary work.[12] The compounds chosen for study incorporated structural elements (e.g. aziridine ring) known to increase cytotoxicity in indolequinones and substituents on the thiazole rings (e.g. methyl and ester rather than carboxamide) found to be optimum in simple indolyl thiazole derivatives.[13]

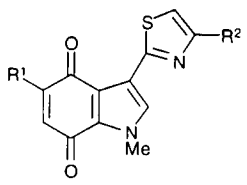


Scheme 3

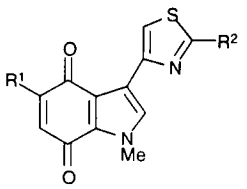
The compounds were prepared in satisfactory yield using the methods described above.

In the first instance the activity of compounds was evaluated against the human breast cancer cell line SKBr3 in order to aid comparison with previous compounds which were tested in the same cell line.[12,13] Values of IC_{50} for each compound are given in Table 1. This cell line, which over-expresses Topo IIα,[17] is sensitive to agents such as mitoxantrone, etoposide and amsacrine which are known to act via inhibition of Topo;[18] for comparison the IC_{50} values of the known Topo inhibitors are shown in Table 2. It is clear from the data that the majority of the thiazolyl indolequinones are at least 100 times less potent than

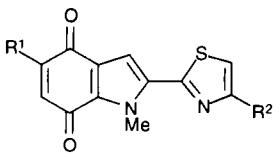
Table 1. Cytotoxicity of thiazolyl indolequinones



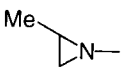
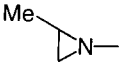
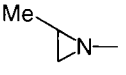
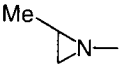
1, 5, 8, 9



6

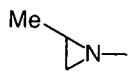
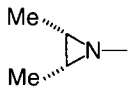
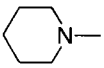
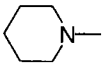
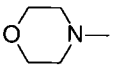


7, 18

Compound	R^1	R^2	IC_{50} (μM) against indicated cell line					
			SKBr3	A549	PV9	V79 (air)	V79 (N_2)	HCR[a]
1 (BR10988)	H_2N	$CONH_2$	8.7[b]	–	–	6.2[b]	5.1[b]	1.2[b]
8	MeO	CO_2Et	27.8[b]	–	–	420[b]	340[b]	1.2[b]
9	MeO	Me	1.4[b]	–	–	74[b]	48[b]	1.5[b]
5a		CO_2Et	0.10	0.41	1.3	1.0	1.0	1.0
5b		Me	0.12	0.31	0.76	1.5	0.75	2.0
5c		CO_2Et	0.33	1.80	2.0	2.0	2.0	1.0
5d		Me	0.66	1.1	2.3	3.0	3.0	1.0
6a	MeO	CO_2Et	16.9[b]	–	–	38.7[b]	60.9[b]	0.6[b]
6b	H_2N	$CONH_2$	4.1[b]	–	–	5.5[b]	4.1[b]	1.3[b]
6c		Me	0.12	0.18	0.52	3.0	1.5	2.0
6d		Me	4.4	2.4	3.2	20	5	4
18a	MeO	CO_2Et	3.9	–	–	200	20	10
18b	MeO	Me	3.2	–	–	54	76	0.7
7a		CO_2Et	0.42	–	–	10.8	2.2	5
7b		Me	0.2	0.7	0.5	17.4	3.4	5
7c		CO_2Et	4.2	–	–	112	7.9	14

Continued

Table 1. *Continued*

Compound	R^1	R^2	IC_{50} (μM) against indicated cell line					
			SKBr3	A549	PV9	V79 (air)	V79 (N_2)	HCR[a]
7d		Me	7.0	25.7	27.1	30	30	1
7e		Me	8.9	19.0	19.7			
7f		Me	30.1	26.1	36.3	100	25	4
7g		CO_2Et	11.3	13.9	24.3			
7h		Me	8.5	14.6	7.5			
7i		CO_2Et	21.5	20.1	31.4			
7j		Me	12.4	23.2	21.3			
7k		Me	–	–	–	300	200	1.5
7l	*c*-C_3H_5NH	CO_2Et	10.1	22.6	38.0			
7m	*c*-C_3H_5NH	Me	22.3	38.1	37.5			

[a]IC_{50} (air)/IC_{50} (nitrogen).
[b]see Moody *et al.*[12]

mitoxantrone against the SKBr3 cell line and therefore, in common with the previous compounds which were studied in detail,[12] we assume that most of the present compounds do not act by inhibition of Topo II. However, some of the aziridinyl compounds, e.g. **5b**, **6c** and **7b**, do exhibit significant cytotoxicity in the SKBr3 cells, comparable to or greater than both etoposide and amsacrine. Therefore additional tests were performed against the human lung carcinoma cell lines A549 and PV9. The A549 cell line also shows high expression of both the Topo IIα and IIβ genes, and has been shown to be hypersensitive to recognized Topo II inhibitors such as mitoxantrone.[19] The PV9 cells were selected as a stably resistant population of A549 cells following long-term etoposide exposure. The PV9 have decreased expression of Topo II, and showed resistance to mitoxantrone and all other agents thought to act through inhibition of Topo. Therefore Topo inhibitors are expected to show good activity towards both SKBr3 and A549 cell lines, but poor activity towards PV9. It is evident from the data in Table 1 that the compounds that possess good activity towards SKBr3 and A549 were similarly active in the PV9 cell line. Therefore it seems extremely unlikely that any of the thiazolyl indolequinones tested act via inhibition of Topo.

Table 2. Cytotoxicity of known Topo II inhibitors and other anticancer agents against the SKBr3 cell line

Compound	IC_{50} (μM)[a]
Mitoxantrone	0.016
Etoposide	0.60
Amsacrine	0.16
Cisplatin	7.4
Melphalan	13.0

[a]See Houlbrook *et al.*[20]

Nevertheless despite their apparent lack of Topo inhibitory activity, several of the quinones do possess good activity in the SKBr3 cell line, and exhibit comparable or greater potency than both cisplatin and melphalan in the same cell line (Table 2).[20] Many of the quinones are also significantly more potent than BE10988 **1**. The most striking observation is the high

potency of the aziridinyl indolequinones which were active in all the SKBr3, A549 and PV9 cell lines; other amine substitution at the indole 5-position resulted in much less potent compounds. Aziridine rings, which are potential alkylating centers, are known to increase the cytotoxicity of indolequinones in general.[6] Within the aziridinyl compounds, unsubstituted aziridines were more potent than the methyl derivatives with the one example of a dimethyl aziridine being least potent. A similar effect of methyl substitution on the cytotoxicity of aziridines has been noted previously in other compounds.[21] Other trends which emerge from the data are: the higher cytotoxicity of the indolequinones over the simple indolyl thiazoles previously studied,[13] the higher potency of the 3-indolylthiazoles over the derivatives and the increased activity of methyl substituted thiazoles over their ester counterparts. These last two trends were also observed in the simpler derivatives.[13]

Finally, since indolequinones have the potential to act as bioreductively activated anticancer drugs, we investigated the cytotoxicity of representative examples in V79 cells under aerobic or hypoxic conditions. The results are shown in Table 1, together with the HCR, the ratio of the IC_{50} (air)/IC_{50} (nitrogen). Values of HCR substantially greater than unity would suggest that O_2-dependent bioreductive processes are operational for the action of the compounds.[22] The results show that, perhaps not surprisingly, the aziridine derivatives are the most cytotoxic. However, an interesting difference between the series of indolylthiazoles emerged. Whereas in general the 3-substituted indolequinones showed little if any HCR, in agreement with previous preliminary results,[12] the 2-substituted analogs showed modest values for HCR suggesting that one-electron reductive activation might be responsible, at least in part, for their cytotoxicity.

Acknowledgments

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