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Synthesis and cytotoxic activity of indolyl thiazoles

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A number of indolyl thiazoles 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 measured in the human breast cancer cell line SKBr3 using the MTT assay.

Key words: Cytotoxicity, indole, thiazole.

Introduction

Compounds containing the thiazole ring system, some of which (e.g. vitamin B_1—thiamine) occur quite widely in nature, possess a wide range of biological properties. For example, the DNA-cleaving ability of bleomycin is well known[1] and other thiazoles, both natural and unnatural, also exhibit marked anticancer activity.[2–4] In connection with our interest in indole derivatives as potential anticancer agents,[5,6] we were intrigued to see that the thiazolyl indolequinone BE 10988 **1**, isolated from culture broths by Japanese workers,[7,8] was reported to have anticancer properties and to act as an inhibitor of topoisomerase II.[7,8] We recently completed a synthesis of the natural product **1**,[9–11] together with some related indolequinones,[12] and although we were unable to confirm the reported potent topoisomerase II inhibitory activity of BE 10988 **1**, some of the indolyl thiazoles did show sufficient cytotoxicity to merit further investigation.

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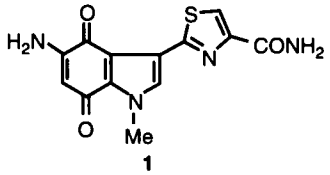
This fact, coupled with the knowledge that certain benzothiazoles exhibit potent activity against human breast tumor cell lines,[13–15] prompted a wider investigation of indolyl thiazoles as potential anticancer agents. Therefore a series of simple 3-thiazolyl indoles **2** and **3**, together with some 2-thiazolyl derivatives **4**, was prepared and evaluated for their cytotoxic activity in a human breast tumor cell line, and the results are described herein.



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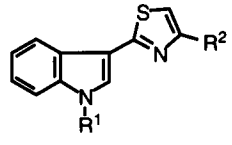

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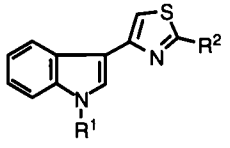

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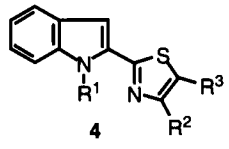

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Materials and methods

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 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 or as Nujol mulls. ^1H 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, UK). Compounds characterized by high resolution mass spectrometry were chromatographically homogeneous.

3-(2-Thiazolyl)indole (**2a**)

A solution of methyl iodide (3.37 g, 23.7 mmol) in ether (10 ml) was added over a 5 min period to magnesium turnings (420 mg, 17.5 mmol) and a few crystals of iodine, under a nitrogen atmosphere. The mixture was heated under reflux for 1 h, then the solvent was removed by increasing the nitrogen flow and benzene (20 ml) added. A solution of indole (1.74 g, 14.8 mmol) in benzene (8 ml) was added and the solution stirred for 10 min. 2-Bromothiazole (1.23 g 7.5 mmol) was added and the mixture heated under reflux, under a nitrogen atmosphere, for 65 h. Ethyl acetate (60 ml) was added and the mixture washed with ammonium chloride (2 × 20 ml), brine (2 × 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum: acetone elution) gave (i) the *title compound* (**2a**) (896 mg, 30%) as a pale brown solid, m.p. 134–138°C; (lit.,[16] 134–137°C); (found: M^+, 200.0489. $C_{11}H_8N_2S$ requires M, 200.0482); ν_{max} (Nujol)/cm^{-1} 1456, 1377 and 1243; δ_H (250 MHz; $CDCl_3$) 7.30 (3H, m), 7.44 (1H, m), 7.83 (1H, m), 7.88 (1H, m), 8.24 (1H, m) and 8.57 (1H, broad, NH); δ_C (62.9 MHz; $CDCl_3$) 111.69, 112.57, 116.01, 118.52, 120.43, 121.39, 123.12, 124.65, 136.44, 142.37 and 164.43; *m/z* 200 (M^+, 100%), 142 (30), 115 (15), 100 (10) and 58 (30), and (ii) 1-acetyl-3-(2-thiazolyl)indole (**2b**) (270 mg, 10%) as colorless crystalline solid, m.p. 117.5°C; (lit.,[16] 116–118°C); (found: M^+, 242.0511. $C_{13}H_{10}N_2OS$ requires M, 242.0513); ν_{max} (Nujol)/cm^{-1} 1712, 1450, 1376 and 1248; δ_H (250 MHz; $CDCl_3$) 2.73 (3H, s, $COCH_3$), 7.16 (1H, s), 7.42 (2H, m), 7.92 (1H, m), 8.06 (1H, m), 8.23 (1H, s) and 8.53 (1H, m); δ_C (62.9 MHz; $CDCl_3$) 24.09, 116.81, 116.97, 117.81, 120.69, 124.38, 124.62, 126.16, 127.24, 136.65, 143.04 and 169.02; one quaternary C unobserved; *m/z* 242 (M^+, 30%), 200 (100), 142 (20), 115 (10), 58 (30) and 43 (10).

1-Methyl-3-(2-thiazolyl)indole (**2c**)

3-(2-Thiazolyl)indole (**2a**) (100 mg, 0.49 mmol) was added to a stirred solution of potassium hydroxide (111 mg, 1.98 mmol) and DMSO (1 ml). The solution was stirred at room temperature for 45 min then methyl iodide (141 mg, 1.0 mmol) was added and the mixture stirred for a further 45 min. Water (5 ml) was added and the mixture extracted with ethyl acetate (3 × 10 ml). The organic extracts were combined, washed with water (3 × 10 ml), brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Recrystallization (hexane) gave the *title compound* (**2c**) (82 mg, 78%) as a brown solid, m.p. 69–70°C; (found: M^+, 214.0575. $C_{12}H_{10}N_2S$ requires M, 214.0564); ν_{max} (Nujol)/cm^{-1} 1552, 1468 and 1376; δ_H (250 MHz; $CDCl_3$) 3.68 (3H, s, NCH_3), 7.15 (1H, s), 7.28 (3H, m), 7.65 (1H, s), 7.77 (1H, m) and 8.23 (1H, s); δ_C (62.9 MHz; $CDCl_3$) 33.04, 109.75, 110.37, 115.43, 120.67, 121.10, 122.66, 125.31, 128.96, 137.42, 142.43 and 163.82; *m/z* 214 (M^+, 100%), 156 (40), 107 (10) and 58 (10).

Methyl 1-methylindole-3-carboxylate

Dry DMF (23 ml) was added to potassium hydride (1.17 g, 29.1 mmol) under a nitrogen atmosphere at 0°C. A solution of indole-3-carboxylic acid (0.94 g, 5.8 mmol) in DMF (13 ml) was added dropwise and the solution stirred at 0°C for 5 min, then at room temperature for 45 min. The solution was cooled to 0°C and methyl iodide (4.15 g, 29.2 mmol) added, the mixture was warmed to room temperature and stirred for a further 45 min. A saturated solution of ammonium chloride (50 ml) was added and the mixture extracted with ether (3 × 50 ml). The extracts were combined, washed with water (2 × 50 ml), brine (50 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* (1.09 g, 95%) as an off-white solid, m.p. 85–87°C; (found: C, 69.82; H, 5.80; N, 7.38. $C_{11}H_{11}NO_2$ requires C, 69.83; H, 5.86; N, 7.40%); (found: M^+, 189.0800. $C_{11}H_{11}NO_2$ requires M, 189.07897; ν_{max}

(Nujol)/cm^{-1} 1687, 1554 and 1232; δ_H (250 MHz; $CDCl_3$) 3.83 (3H, s, NCH_3), 3.91 (3H, s, OCH_3), 7.33 (3H, m), 7.78 (1H, s) and 8.15 (1H, m); δ_C (62.9 MHz; $CDCl_3$) 33.51, 50.90, 107.12, 109.77, 121.58, 122.73, 127.13, 135.16, 136.91 and 166.78; one quaternary C unobserved; *m/z* 189 (M^+, 70%) and 158 (100).

1-Methylindole-3-carboxamide (5)

Trimethyl aluminum (2 M solution in toluene, 7.95 ml, 15.9 mmol) was added dropwise to a mixture of ammonium chloride (850 mg, 15.9 mmol) and benzene (15.9 ml) at 5°C under a nitrogen atmosphere. The mixture was warmed to room temperature and stirred for 1 h. A solution of methyl 1-methylindole-3-carboxylate (1.00 g, 5.3 mmol) in benzene (50 ml) was added and the solution stirred at 60°C for 12 h. Hydrochloric acid (2 M, 30 ml) was added and the solution extracted with ethyl acetate (3 × 50 ml). The extracts were combined, washed with brine (50 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* (**5**) (680 mg, 74%) as a pale yellow solid, m.p. 178–179°C; (found: M^+, 174.0794. $C_{10}H_{10}N_2O$ requires M, 174.0793); ν_{max} (Nujol)/cm^{-1} 1604, 1465 and 1377; δ_H (250 MHz; $CDCl_3$) 3.83 (3H, s, NCH_3), 5.77 (2H, broad, NH_2), 7.36 (3H, m), 7.7 (1H, s) and 7.96 (1H, m); δ_C (62.9 MHz; $CDCl_3$) 33.12, 109.56, 109.66, 121.1, 121.24, 122.22, 126.47, 132.56, 136.97 and 167.15; *m/z* 174 (M^+, 80%), 158 (100), 130 (10) and 77 (10).

1-Methylindole-3-thiocarboxamide (6)

A solution of 1-methylindole-3-carboxamide (**5**) (120 mg, 0.68 mmol), Lawesson's reagent (163 mg, 0.4 mmol) and benzene (10 ml) was heated under reflux for 1 h. The crude reaction mixture was evaporated under reduced pressure and flash column chromatography (light petroleum:ether elution) gave the *title compound* (**6**) (120 mg, 60%) as a yellow solid, m.p. 125–126°C; (found: M^+, 190.2651. $C_{10}H_{10}N_2S$ requires M, 190.2646); ν_{max} (Nujol)/cm^{-1} 3496, 2968 and 1596; δ_H (250 MHz; $CDCl_3$) 3.85 (3H, s, NCH_3), 7.36 (5H, m), 7.86 (1H, m) and 8.07 (1H, s); δ_C (62.9 MHz; $CDCl_3$) 33.45, 110.58, 116.08, 119.89, 122.49, 122.88, 123.75, 137.14, 137.69 and 194.88; *m/z* 190 (M^+, 60%), 156 (100).

3-(4-Methyl-2-thiazolyl)-1-methylindole (2d)

A mixture of 1-methylindole-3-thiocarboxamide (**6**) (115 mg, 0.61 mmol), chloroacetone (113 mg, 1.2 mmol) and ethanol (28 ml) was heated under reflux for 3 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum:ethyl acetate elution) gave the *title compound* (**2d**) 60 mg, 43%) as a pale yellow solid, m.p. 74–76°C; (found: M^+, 228.0718. $C_{13}H_{12}N_2S$ requires M, 228.0721); δ_H (250 MHz; $CDCl_3$) 2.52 (3H, s, CH_3), 3.34 (3H, s, NCH_3), 6.76 (1H, s), 7.35 (3H, m), 7.56 (1H, s) and 8.19 (1H, m); δ_C (62.9 MHz; $CDCl_3$) 15.01, 33.69, 106.14, 109.69, 110.66, 119.55, 122.38, 123.41, 124.68, 133.79, 137.67, 146.94 and 163.68; *m/z* 228 (M^+, 100%), 156 (90), 114 (20) and 72 (30).

Ethyl 2-(1-methylindol-3-yl)thiazole-4-carboxylate (2e)

A mixture of 1-methylindole-3-thiocarboxamide (**6**) (100 mg, 0.53 mmol), ethyl bromopyruvate (155 mg, 0.79 mmol) and ethanol (25 ml) was heated under reflux for 0.5 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Recrystallization (methanol/water) gave the *title compound* (**2e**) (100 mg, 66%) as pale brown crystals, m.p. 117–118°C; (found: C, 63.07; H, 4.73; N, 9.51. $C_{15}H_{14}N_2OS$ requires C, 62.94; H, 4.90; N, 8.79%); (found: M^+, 286.0785. $C_{15}H_{14}N_2O_2S$ requires M, 286.0776); ν_{max} (Nujol)/cm^{-1} 3050, 2930 and 1730; δ_H (250 MHz; $CDCl_3$) 1.44 (3H, t, J 7.2 Hz, OCH_2CH_3), 3.85 (3H, s, NCH_3), 4.43 (2H, q, J 7.12 Hz, OCH_2CH_3), 7.34 (3H, m), 7.90 (1H, s), 8.05 (1H, s) and 8.19 (1H, m); δ_C (62.9 MHz; $CDCl_3$) 14.32, 23.18, 61.26, 109.88, 110.06, 120.390, 121.35, 122.75, 124.06, 125.03, 129.96, 137.19, 146.52, 162.32 and 163.52; *m/z* 286 (M^+, 100%), 214 (40), 174 (10), 77 (50), 158 (30) and 120 (12).

2-(1-Methylindol-3-yl)thiazole-4-carboxamide (2f)

Ethyl 2-(1-methylindol-3-yl)thiazole-4-carboxylate (**2e**) (218 mg, 0.77 mmol), ammonium chloride (10.5 mg, 0.19 mmol) and ammonium hydroxide (15 ml, of a 0.88 solution) were heated at 100°C in a Young's tube for 48 h. The crude mixture was extracted with ethyl acetate (3 × 20 ml), the extracts were combined and washed with brine (20 ml),

dried ($MgSO_4$) and evaporated under reduced pressure. Column chromatography (ethyl acetate elution) gave the *title compound* (**2f**) (142 mg, 73%) as a pale brown solid, m.p. 184–185°C; (found: M^+, 257.0617. $C_{13}H_{11}N_3OS$ requires M, 257.0617); ν_{max} ($CHCl_3$)/cm^{-1} 3019, 1680, 1422, 1216 and 929; δ_H (250 MHz; CD_3OD) 3.62 (3H, s, NCH_3), 7.12 (3H, m), 7.54 (1H, s), 7.80 (1H, s) and 7.98 (1H, m); amide NH_2 unobserved; δ_C (62.9 MHz; CD_3OD) 32.65, 109.56, 109.67, 120.19, 120.83, 121.08, 122.66, 124.71, 129.58, 137.12, 148.52, 163.44 and 164.22; *m/z* 257 (M^+, 100%), 174 (10), 157 (60), 101 (20) and 44 (18).

3-Chloroacetylindole

Chloroacetyl chloride (11.29 g, 100 mmol) was added to a well stirred solution of pyridine (7.91 g, 100 mmol), indole (11.7 g, 100 mmol) and toluene (250 ml) over 1 h at 60°C. The mixture was stirred for 1 h, then water (300 ml) and methanol (50 ml) were added. The mixture was stirred at 60°C for 1 h, whereupon a brown solid separated. The brown solid was removed by filtration and recrystallization (ethanol) gave the *title compound* (13.92 g, 72% yield) as a pale brown solid, m.p. 230–232°C (lit.,[17] 230–232°C); ν_{max} (Nujol)/cm^{-1} 1645, 1459, 1436, 1377 and 751; δ_H (250 MHz; CD_3OD) 4.72 (2H, s, $COCH_2Cl$), 7.25 (2H, m), 7.49 (1H, m) and 8.24 (2H, m); δ_C (62.9 MHz; CD_3OD) 45.09 ($COCH_2Cl$), 111.83, 120.95, 120.97, 121.92, 122.94, 122.97, 133.35, 133.76 and 202.37; *m/z* 193 (M^+, 30%), 144 (100), 89 (20) and 148 (20).

3-Bromoacetylindole

3-Chloroacetylindole (1 g, 5.2 mmol), sodium bromide (5.35 g, 52 mmol) and acetone (30 ml) were heated reflux for 24 h. The reaction mixture was filtered through a glass sinter and the residue washed with acetone (100 ml); the filtrate was evaporated under reduced pressure and recrystallization (ethyl acetate) gave the *title compound* (780 mg, 62%) as a brown solid, m.p. 192–194°C (lit.,[18] 229°C); (found: M^+, 238.977. $C_{10}H_8{}^{80}BrNO$ requires M, 238.9779; found: M^+, 236.9787. $C_{10}H_8{}^{78}BrNO$ requires M, 236.9799); δ_H (250 MHz; $CDCl_3$:CD_3OD) 4.21 (2H, s, CH_2Br), 7.08 (2H, m), 7.30 (1H, m, Ar), 7.90 (1H, m) and 8.09 (1H, m); δ_C (62.9 MHz; $CDCl_3$:CD_3OD) 31.39, 104.32, 11.83, 114.62, 121.56, 122.47, 123.44, 125.01, 133.92 and 188.91; *m/z* 239 (M^+, 20%), 237 (10), 144 (100) and 89 (20).

3-(4-Thiazolyl)indole (**3a**)

A mixture of 3-bromoacetylindole (1070 mg, 4.5 mmol), thioformamide[19] (640 mg, 9 mmol) and ethanol (30 ml) was heated under reflux for 3 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Column chromatography (light petroleum:ether elution) gave the *title compound* (**3a**) (252 mg, 28%) as a pale yellow solid, m.p. 139–140°C; (found: M^+, 200.0372. $C_{11}H_8N_2S$ requires M, 200.0408); ν_{max} ($CHCl_3$)/cm^{-1} 3066, 1522, 1476, 1423, 1225 and 929; δ_H (250 MHz; $CDCl_3$) 7.24 (3H, m, Ar), 7.42 (1H, d, J 1.90 Hz), 7.80 (1H, d, J 2.7 Hz), 8.04 (1H, m), 8.45 (1H, s, NH) and 8.91 (1H, d, J 1.88 Hz); δ_C (62.9 MHz; $CDCl_3$) 109.66, 111.75, 111.80, 119.36, 120.18, 122.07, 124.23, 124.40, 137.29, 151.03 and 152.75; *m/z* 200 (M^+, 40%), 155 (30), 113 (30), 70 (25) and 51 (100).

1-Methyl-3-(4-thiazolyl)indole (**3b**)

3-(4-Thiazolyl)indole (**3a**) (50 mg, 0.25 mmol) was added to a stirred solution of potassium hydroxide (56 mg, 1 mmol) and DMSO (1 ml). The solution was stirred at room temperature for 45 min then methyl iodide (141 mg, 1.0 mmol) was added and the mixture stirred for a further 45 min. Water (5 ml) was added and the mixture extracted with ethyl acetate (3 × 10 ml). The extracts were combined, washed with water (3 × 10 ml), brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Column chromatography (ethyl acetate elution) gave the *title compound* (**3b**) (44 mg, 83%) as a brown solid, m.p. 54–55°C; (found: M^+, 214.0562. $C_{12}H_{10}N_2S$ requires M, 214.0564); ν_{max} ($CHCl_3$)/cm^{-1} 3020, 1522, 1421, 1228 and 929; δ_H (250 MHz; $CDCl_3$) 3.69 (3H, s, NCH_3), 7.19 (3H, m), 7.26 (1H, d, J 1.75 Hz), 7.55 (1H, s), 7.92 (1H, m) and 8.75 (1H, d, J 1.62 Hz); δ_C (62.9 MHz; $CDCl_3$) 32.86, 108.94, 109.66, 110.88, 120.07, 120.26, 122.07, 125.48, 128.53, 137.36, 151.65 and 152.03; *m/z* 214 (M^+, 40%), 186 (30), 155 (40), 113 (40) and 51 (100).

3-(2-Methyl-4-thiazolyl)indole (**3c**)

A solution of thioacetamide (1.15 g, 15.2 mmol) in ethanol (60 ml) was added dropwise to a stirred solution of 3-chloroacetylindole (1.5 g, 7.7 mmol) in ethanol (100 ml) at reflux. The solution was heated under reflux for 3 h. The reaction mixture was

cooled and sodium hydroxide (20%; 50 ml) added. The mixture was extracted with ethyl acetate (2 × 200 ml), washed with brine (50 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (light petroleum:ether elution) gave the *title compound* (**3c**) (650 mg, 43%) as a pale brown solid, m.p. 122–124°C (lit.,[20] 124°C); (found: M^+, 214.0564. $C_{12}H_{10}N_2S$ requires M, 214.0559); ν_{max} ($CHCl_3$)/cm^{-1} 3448, 1617, 1575, 1482 and 1360; δ_H (250 MHz; $CDCl_3$) 2.28 (3H, s, CH_3), 7.22 (3H, m), 7.38 (1H, m), 7.74 (1H, m), 7.99 (1H, m) and 8.45 (1H, broad, NH); δ_C (62.9 MHz; $CDCl_3$) 19.06, 109.57, 111.64, 112.22, 119.82, 120.44, 122.35, 123.96, 125.01, 136.58, 150.29 and 165.47; m/z 214 (M^+, 100%), 173 (70), 98 (50), 91 (40) and 84 (40).

3-(2-Methyl-4-thiazolyl)-1-methylindole (**3d**)

3-(2-Methyl-4-thiazolyl)indole (**3c**) (200 mg, 0.93 mmol) was added to a stirred solution of potassium hydroxide (209 mg, 3.73 mmol) and DMSO (2 ml). The resulting red solution was stirred at room temperature for 45 min, then methyl iodide (246 mg, 1.86 mmol) was added and the mixture stirred for a further 45 min. Water (5 ml) was added and the mixture extracted with ethyl acetate (3 × 10 ml). The extracts were combined, washed with water (3 × 10 ml), brine (10 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Recrystallization (hexane) gave the *title compound* (**3d**) (160 mg, 75%) as a yellow solid, m.p. 120–121°C; (found: C, 68.48; H, 5.24; N, 12.18. $C_{13}H_{12}N_2S$ requires C, 68.39; H, 5.3; N, 12.27%); (found: M^+, 228.0707. $C_{13}H_{12}N_2S$ requires M, 228.0721); δ_H (250 MHz; $CDCl_3$) 2.77 (3H, s, CH_3), 3.82 (3H, s, NCH_3), 7.19 (1H, s), 7.31 (3H, m), 7.96 (1H, s) and 8.0 (1H, m); m/z 228 (M^+, 100%), 187 (50), 172 (30), 98 (25) and 84 (25).

Ethyl 4-(indol-3-yl)thiazole-2-carboxylate (**3e**)

A mixture of 3-bromoacetylindole (500 mg, 2.1 mmol), ethyl thiooxamate (290 mg, 2.15 mmol) and acetonitrile (30 ml) was heated under reflux for 3 h, then more ethyl thiooxamate (60 mg, 0.45 mmol) was added and the mixture heated under reflux for a further 2 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Column chromatography (light petroleum:ether elution) gave the *title compound* (**3e**) (417 mg, 74%) as pale yellow solid, m.p. 170–171°C; (found: M^+, 272.0619. $C_{14}H_{12}N_2O_2S$ requires M, 272.0619); ν_{max} (Nujol)/cm^{-1} 3683, 3019, 1719, 1522, 1422, 1221 and 929; δ_H (250 MHz; $CDCl_3$) 1.44 (3H, t, J 7 Hz, OCH_2CH_3), 4.42 (2H, q, J 7 Hz, OCH_2CH_3), 7.23 (2H, m), 7.47 (1H, s), 7.64 (1H, s), 7.86 (1H, d, J 2.71 Hz), 8.09 (1H, m) and 8.98 (1H, s, NH); δ_C (62.9 MHz; $CDCl_3$) 16.65, 66.02, 115.23, 119.58, 123.12, 123.76, 125.31, 125.58, 127.21, 128.66, 128.71, 140.91, 160.09 and 164.82; m/z 272 (M^+, 90%), 198 (10), 172 (10) and 141 (100).

Ethyl 4-(1-methylindol-3-yl)thiazole-2-carboxylate (**3f**)

Dry DMF (1.5 ml) was added to potassium hydride (44 mg, 1.83 mmol), under a nitrogen atmosphere, at 0°C. A solution of ethyl 4-(indol-3-yl)thiazole-2-carboxylate (**3e**) (100 mg, 0.37 mmol) in DMF (2 ml) was added dropwise and the solution stirred at 0°C for 5 min, then at room temperature for 45 min. The solution was cooled to 0°C and methyl iodide (261 ml, 1.84 mmol) added, the mixture was warmed to room temperature and stirred for a further 45 min. A saturated solution of ammonium chloride (10 ml) was added and the mixture extracted with ether (3 × 20 ml); the extracts were combined, washed with water (2 × 20 ml), brine (20 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (ether elution) gave the *title compound* (**3f**) (95 mg, 90%) as an off-white solid, m.p. 131–132°C; (found: M^+, 286.0777. $C_{15}H_{14}N_2O_2S$ requires M, 286.0776); ν_{max} (Nujol)/cm^{-1} 1687, 1554 and 1232; δ_H (250 MHz; $CDCl_3$) 1.44 (3H, t, J 7 Hz, OCH_2CH_3), 4.01 (3H, s, NCH_3), 4.42 (2H, q, J 7 Hz, OCH_2CH_3), 7.25 (3H, m), 7.56 (1H, s), 7.73 (1H, s) and 7.98 (1H, m); δ_C (62.9 MHz; $CDCl_3$) 14.24, 32.87, 62.40, 109.73, 111.76, 115.42, 119.64, 120.44, 122.12, 124.99, 129.37, 137.30, 153.39, 157.08 and 160.21; m/z 286 (M^+, 100%), 172 (18), 154 (20) and 29 (20).

4-(Indol-3-yl)thiazole-2-carboxamide (**3g**)

A mixture of ethyl 4-(indol-3-yl)thiazole-2-carboxylate (**3e**) (100 mg, 0.4 mmol), ammonium chloride (5 mg, 0.1 mmol) and ammonium hydroxide (5 ml, of a 0.88 solution) was heated at 100°C in a Young's tube for 48 h. The crude mixture was extracted with ethyl acetate (3 × 10 ml), the extracts were com-

bined and washed with brine (15 ml), dried ($MgSO_4$), and evaporated under reduced pressure. Column chromatography (ethyl acetate elution) gave the *title compound* (**3g**) (43 mg, 47%) as a pale brown solid, m.p. 215–216°C; ν_{max} (Nujol)/cm^{-1} 3683, 3019, 1719, 1522, 1422, 1221 and 929; δ_H (250 MHz; CD_3OD:$CDCl_3$) 7.13 (2H, m), 7.33 (1H, m), 7.36 (1H, s), 7.64 (1H, s) and 7.90 (1H, m); δ_C (62.9 MHz; CD_3OD:$CDCl_3$) 114.81, 115.34, 119.02, 123.35, 123.79, 125.64, 125.81, 128.40, 128.72, 140.92, 157.30 and 166.12; *m/z* 243 (M^+, 100%), 225 (30), 173 (50), 141 (40) and 44 (30).

4-(1-Methylindol-3-yl)thiazole-2-carboxamide (**3h**)

A mixture of ethyl 4-(1-methylindol-3-yl)thiazole-2-carboxylate (**3f**) (233 mg, 0.82 mmol), ammonium chloride (20 mg, 0.37 mmol) and ammonium hydroxide (10 ml, of a 0.88 solution) was heated at 100°C in a Young's tube for 48 h. The crude mixture with ethyl acetate (3 × 10 ml), the extracts were combined and washed with brine (15 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Column chromatography (ethyl acetate elution) gave the *title compound* (**3h**) (120 mg, 58%) as a pale brown solid, m.p. 186–187°C; (found: M^+, 257.0617. $C_{13}H_{11}N_3OS$ requires M, 257.0623); ν_{max} (Nujol)/cm^{-1} 1687, 1554 and 1232; δ_H (250 MHz; $CDCl_3$) 1.44 (3H, t, J 7 Hz, OCH_2CH_3), 4.01 (3H, s, NCH_3), 4.42 (2H, q, J 7 Hz, OCh_2CH_3), 7.25 (3H, m), 7.56 (1H, s), 7.73 (1H, s) and 7.98 (1H, m); δ_C (62.9 MHz, $CDCl_3$) 14.24, 32.87, 62.40, 109.73, 111.76, 115.42, 119.64, 120.44, 122.12, 124.99, 129.37, 137.30, 153.39, 157.08 and 160.21; *m/z* 257 (M^+, 100%), 187 (25), 172 (20), 155 (15) and 69 (20).

Methyl-1-methylindole-2-carboxylate (**7**)

Dry DMF (70 ml) was added to potassium hydride (5.542 g, 138 mmol) under a nitrogen atmosphere, at 0°C. A solution of indole-2-carboxylic acid (4.44 g, 27.5 mmol) in DMF (70 ml) was added dropwise. The solution was stirred at 0°C for 5 min and then stirred at room temperature for 45 min. The solution was cooled to 0°C and methyl iodide (19.45 g, 138 mmol) added. The mixture was warmed to room temperature and stirred for a further 45 min. A saturated solution of ammonium chloride (150 ml) was added and the mixture extracted with diethyl ether (3 × 150 ml), the extracts were combined washed with water (2 × 150 ml), brine (150 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Recrystallization (hexane) gave the *title compound* (**7**) (4.64 g, 90%) as a colorless solid, m.p. 95–97°C; (found: C, 70.3; H, 6.06; N, 7.46. $C_{11}H_{11}NO_2$ requires C, 69.84; H, 5.82; N, 7.41%); (found: M^+, 189.0785. $C_{11}H_{11}NO_2$ requires M, 189.0789); ν_{max} (Nujol)/cm^{-1} 1704, 1516, 1467, 1253 and 752; δ_H (250 MHz; $CDCl_3$) 3.90 (3H, s, OMe), 4.07 (3H, s, NMe), 7.12 (1H, m), 7.31 (1H, s), 7.34 (2H, m) and 7.66 (1H, dt, J 7.9, 1.0 Hz); δ_C (62.5 MHz; $CDCl_3$) 31.51, 51.45, 110.16, 110.17, 120.5, 122.55, 124.95, 125.84, 127.61, 139.92 and 163.32; *m/z* 189 (M^+, 100%), 158 (40), 89 (50), 57 (48) and 43 (42).

1-Methylindole-2-carboxamide (**8**)

Trimethyl aluminum (2 M solution in toluene, 19.87 ml, 39.75 mmol) was added dropwise to a solution of ammonium chloride (2.125 g, 39.75 mmol) and benzene (39.75 ml) at 5°C, under a nitrogen atmosphere. The mixture was warmed to room temperature and stirred for 1 h. A solution of methyl-1-methylindole-2-carboxylate (2.5 g, 13.25 mmol) in benzene (125 ml) was added and the solution stirred at 60°C for 12 h. Hydrochloric acid (2 M, 200 ml) was added and the solution extracted with ethyl acetate (3 × 150 ml). The extracts were combined, washed with brine (200 ml), dried ($MgSO_4$) and evaporated under reduced pressure. Flash column chromatography (ethyl acetate elution) gave the *title compound* (**8**) (1.32 g, 58% yield) as a colorless solid, m.p. 167–169°C; (found: C, 68.88; H, 5.71; N, 16.13. $C_{10}H_{10}N_2O$ requires C, 68.97; H, 5.75; N, 16.09%); (found: M^+, 174.0776. $C_{10}H_{10}N_2O$ requires M, 174.0793); ν_{max} (Nugol)/cm^{-1} 1652, 1616, 1464 and 1332; δ_H (250 MHz; $CDCl_3$) 4.07 (3H, s, NMe), 5.91 (2H, broad, NH_2), 6.93 (1H, s), 7.16 (1H, m), 7.34 (2H, m) and 7.66 (1H, m); *m/z* 174 (M^+, 100%), 158 (40), 130 (40) and 89 (50).

1-Methylindole-2-thiocarboxamide (**9**)

A solution of 1-methylindole-2-carboxamide (**8**) (300 mg, 1.74 mmol) and Lawesson's reagent (351 mg, 0.87 mmol) in benzene (30 ml) were heated under reflux for 1 h. The crude reaction mixture was evaporated under reduced pressure and flash column chromatography (light petroleum: diethyl ether elution) gave the *title compound* (**9**) (40) (252 mg, 77% yield) as a yellow solid, m.p.

139–140°C; (found: M^+, 190.0545. $C_{10}H_{10}N_2S$ requires M, 190.0565); ν_{max} (Nujol)/cm^{-1} 1615, 1464, 1377 and 1351; δ_H (250 MHz; $CDCl_3$) 4.09 (3H, s, NMe), 6.71 (1H, s), 7.21 (3H, m), 7.34 (2H, s) and 7.64 (1H, m); δ_C (62.5 MHz; $CDCl_3$) 32.23, 103.49, 110.44, 120.97, 122.22, 124.76, 125.57, 138.03, 140.52 and 193.24; *m/z* 190 (M^+, 90%), 157 (100) and 130 (50).

2-(4-Methyl-2-thiazolyl)-1-methylindole (**4a**)

1-Methylindole-2-thiocarboxamide (**9**) (79 mg, 0.4 mmol), chloroacetone (75 mg, 0.81 mmol), sodium bromide (414 mg, 4.03 mmol) and acetonitrile (5 ml) were heated under reflux for 2 h. The reaction mixture was cooled, filtered and washed with dichloromethane (20 ml). The filtrate was washed with brine (2 × 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum:-diethyl ether elution) gave the *title compound* (**4a**) (68 mg, 71% yield) as pale yellow solid, m.p. 83–84°C; (found: M^+, 228.0723. $C_{13}H_{12}N_2S$ requires M, 228.0721); ν_{max} ($CHCl_3$)/cm^{-1} 2957, 1522, 1224 and 884; δ_H (250 MHz; $CDCl_3$) 2.56 (3H, s, Me), 4.19 (3H, s, NMe), 6.87 (1H, s), 7.14 (1H, s), 7.20 (1H, m), 7.35 (2H, m) and 7.65 (1H, dd, J 7.8, 1.4 Hz); δ_C (62.5 MHz; $CDCl_3$) 17.32 (Me), 31.75 (NMe), 104.54, 109.85, 113.04, 120.22, 121.07, 123.16, 127.27, 132.76, 139.81, 153.58 and 159.96; *m/z* 228 (M^+, 100%), 213 (30), 130 (25), 98 (40) and 44 (15).

Ethyl 2-(1-methylindol-2-yl)thiazole-4-carboxylate (**4b**)

1-Methylindole-2-thiocarboxamide (**9**) (100 mg, 0.53 mmol), ethyl bromopyruvate (155 mg, 0.79 mmol) and ethanol (25 ml) were heated under reflux for 0.5 h. The reaction mixture was evaporated under reduced pressure to give a yellow solid. Flash column chromatography (light petroleum:ethyl acetate elution) gave the *title compound* (**4b**) (74 mg, 48% yield) as pale yellow solid, m.p. 122–123°C; (found: M^+, 286.0771. $C_{15}H_{14}N_2O_2S$ requires M, 286.0776); ν_{max} ($CHCL_3$)/cm^{-1} 1733, 1420, 1098 and 668; δ_H (250 MHz; $CDCl_3$) 1.47 (3H, t, J 7.15 Hz, CH_2Me), 4.44 (3H, s, NMe), 4.44 (2H, q, J 7.2 Hz, OCH_2Me), 7.24 (2H, m), 7.37 (2H, m), 7.61 (1H, m) and 8.29 (1H, s); δ_C (62.5 MHz; $CDCl_3$) 14.31 (OCH_2Me), 32.86 (NMe), 61.45 (OCH_2Me), 110.17, 120.19, 121.07, 124.97, 126.74, 127.42, 127.53, 138. 72, 143.21, 157.98, 161.67 and 187.07; *m/z* 286 (M^+, 100%), 214 (50), 1 57 (30) and 77 (15).

2-(1-Methylindol-2-yl)thiazole-4-carboxylate (**4c**)

Ethyl 2-(1-methylindol-2-yl)thiazole-4-carboxylate (**4b**) (15 mg, 0.057 mmol) was dissolved in dichloromethane (1 ml) and placed in a Young's tube. The mixture was cooled to −78°C and ammonia gas was bubbled into the mixture until the volume reached approximately 30 ml. The Young's tube was sealed and allowed to warm to room temperature. The mixture was stirred at room temperature for 4 days. The reaction mixture was cooled to −78°C and the Young's tube opened (**caution**!). The mixture was left to warm to room temperature over a 4 h period. Ethyl acetate (20 ml) was added and the mixture was washed with brine (2 × 20 ml), dried ($MgSO_4$) and evaporated under reduced pressure to give a pale brown solid. Flash column chromatography (ethyl acetate elution) gave the *title compound* (**4c**) (11 mg, 80%) as a pale brown solid, m.p. 190°C; (found: M^+, 257.0617. $C_{13}H_{11}N_3OS$ requires M, 257.0617); ν_{max} ($CHCl_3$)/cm^{-1} 1686, 1420, 1216 and 853; δ_H (250 MHz; CD_3OD) 4.04 (3H, s, NMe), 7.10 (3H, m), 7.21 (1H, m), 7.43 (1H, m) and 8.03 (1H, m); amide NH_2 unobserved; *m/z* 257 (M^+, 100%), 213 (40), 120 (20) and 77 (10).

2-(2-Benzothiazolyl)-1-methylindole (**4d**)

Trimethyl aluminum (2 M solution in toluene, 6.76 ml, 13.53 mmol) was added dropwise to toluene (25 ml) at 0°C, under a nitrogen atmosphere. 2-Aminothiophenol (504 mg, 4.02 mmol) was added dropwise maintaining the temperature at 0°C. The mixture was stirred at 0°C for 0.5 h and then warmed to room temperature. A solution of methyl-1-methylindole-2-carboxylate (**7**) (500 mg, 2.67 mmol) in toluene (10 ml) was added and the solution stirred at 60°C for 14 h. The mixture was cooled to 0°C and water (5 ml) followed by methanol (50 ml) were added. The mixture was filtered and washed with methanol (100 ml). Then the filtrate was evaporated under reduced pressure to give a brown solid. Recrystallization (diethyl ether) gave the *title compound* (**4d**) (401 mg, 57%) as a pale brown solid, m.p. 135–136°C; (found: M^+, 264.0721. $C_{16}H_{12}N_2S$ requires M, 264.0721); ν_{max}

($CHCl_3$)/cm^{-1} 1549, 1424, 1345, 1312, 975 and 929; δ_H (250 MHz; $CDCl_3$) 4.3 (3H, s, NMe), 7.19 (2H, m), 7.4 (3H, m), 7.54 (1H, m), 7.67 (1H, dd, J 7.9, 1.0 Hz), 7.9 (1H, dd, J 7.8, 1.3 Hz) and 8.07 (1H, dd, J 8.7, 1.4 Hz); δ_C (100 MHz; $CDCl_3$) 32.65, 107.96, 110.51, 120.97, 121.68, 121.96, 123.60, 124.51, 125.66, 126.65, 127.69, 132.62, 134.90, 140.22, 154.69 and 161.00; *m/z* 264 (M^+, 90%), 263 (100), 155 (20), 51 (20) and 28 (35).

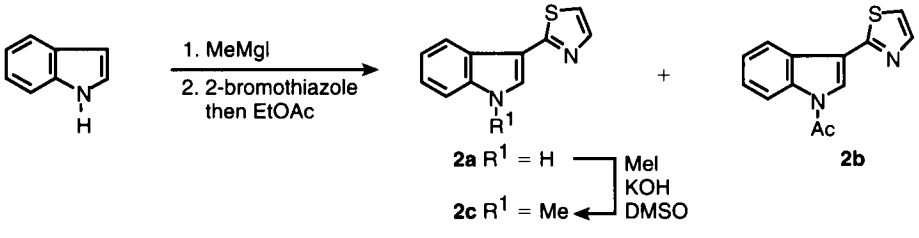
Biology

The indolyl thiazoles were tested against the human breast cancer cell line SKBr3, in order to aid comparison with the previously prepared compounds.[12] This line, which over-expresses topoisomerase IIα,[21] is well characterized with respect to estrogen and epidermal growth factor receptors and sensitivity to chemotherapeutic drugs.[22] Aliquots of cells were placed into 24-well microtiter 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 (four wells/concentration). Cells were then incubated continuously for 4 days prior to determination of cell growth by the MTT assay.[23–25] 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, was derived from the cumulative data.

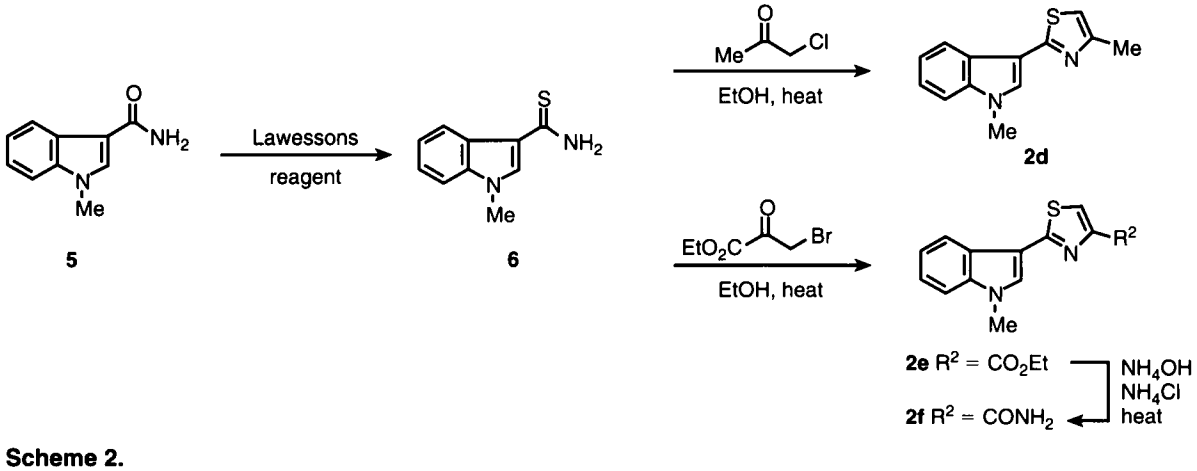
Results

The simplest compound 3-(2-thiazolyl) indole **2a** was prepared from 2-bromothiazole and indolylmagnesium bromide using the literature procedure.[16] Thiazolylindole **2a** (30%) was accompanied by the 1-acetyl derivative **2b** (10%), presumably formed by quenching of the reaction mixture with ethyl acetate as described in the original paper. 3-(2-Thiazolyl)indole **2a** is a natural occurring phytoalexin known as camalexin. Methylation of **2a** using the method of Heaney and Ley (MeI/KOH/DMSO)[26] gave the *N*-methyl derivative **2c** (78%). See Scheme 1.

The remaining 3-(2-thiazolyl)indoles were prepared from 1-methylindole-3-thiocarboxamide **6** using the Hantzsch reaction. Thus reaction of **6**, prepared from the amide **5** as shown in Scheme 2, with chloroacetone or ethyl bromopyruvate gave the indolyl thiazoles **2d** and **2e** in 43 and 66% yield,



Scheme 1.

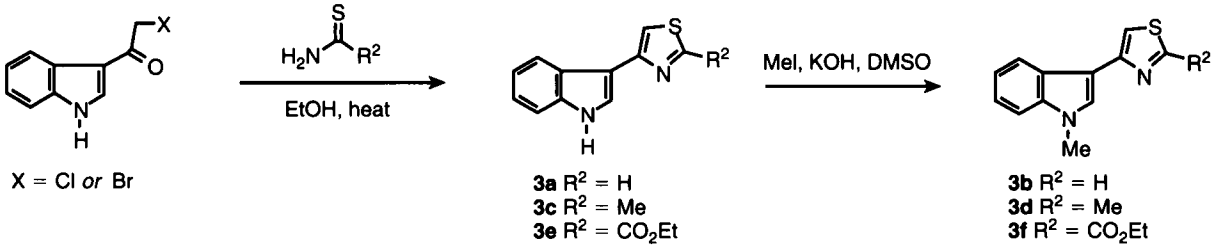


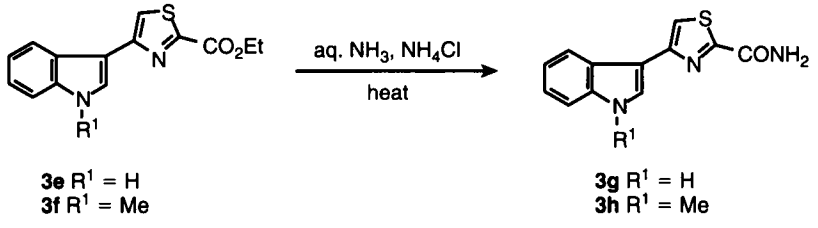
Scheme 2.

respectively. Treatment of ester **2e** with aqueous ammonia in a sealed tube gave the corresponding amine **2f** (73%).

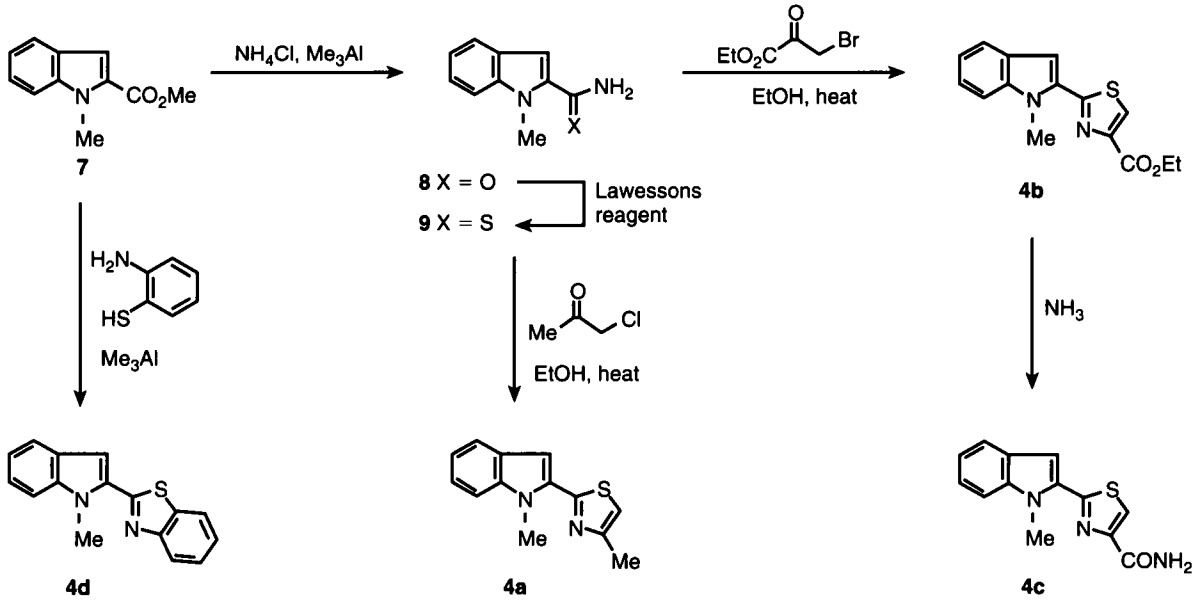
The 3-(4-thiazolyl)indoles **3** were prepared from 3-haloacetylindole by Hantzsch reaction with a thioamide (Scheme 3). Thus reaction of 3-bromoacetylindole with thioformamide[19] gave 3-(4-thiazolyl)indole **3a** itself, albeit in poor yield (28%). Similar reactions with thiacetamide (in this case 3-chloroacetylindole was used) and ethyl thiooxamate gave the thiazolylindoles **3c** (43%) and **3e** (74%). *N*-Methylation of indoles **3a**, **3c** and **3e** with MeI/KOH/DMSO gave the corresponding *N*-methyl derivatives **3b**, **3d** and **3f** in good yield. Finally treatment of the esters **3e** and **3f** with aqueous ammonia in a sealed tube gave the corresponding amides **3g** and **3h** (Scheme 3).

The 2-(2-thiazolyl)indoles **4a** and **4b** were prepared from 1-methylindole-2-thiocarboxamide **9**, obtained from the ester **7** by reaction with trimethylaluminum/ammonium chloride,[27] followed by treatment with Lawessons reagent, by Hantzsch reactions with chloroacetone and ethyl bromopyruvate, respectively (Scheme 4). The thiazole ester **4b** was converted into the amide **4c** by treatment with





Scheme 3.



Scheme 4.

ammonia. Finally, the benzothiazole **4d** was prepared by the reaction of the ester **7** with 2-aminothiophenol using the method recently described by Hudkins.[28]

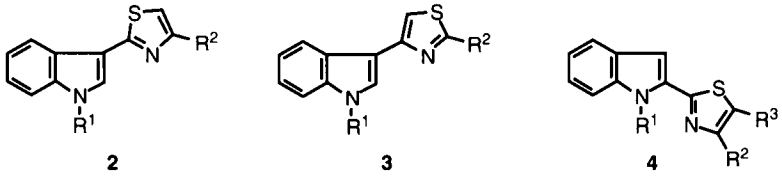
The cytotoxic activity of the indolyl thiazoles was determined in SKBr3 cells using the MTT assay. Values of IC_{50} for each compound are given in Table 1.

Discussion

The work was designed to examine the structure–activity relationships within a series of indolyl thiazoles and to establish whether they possessed any significant cytotoxicity in human breast tumor cell lines. In order to avoid any possible complication from bioreductive activity resulting from the indolequinone moiety,[12,29] a range of relatively simple indolyl thiazoles was chosen. The compounds were prepared in satisfactory yield using the methods described above.

The activity of the indolyl thiazoles **2a**–2f, **3a**–3h and **4a**–4d was evaluated 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] Values of IC_{50} for each compound are given in Table 1. This cell line is sensitive to agents such as mitoxantrone, etoposide and amsacrine which are known to act via inhibition of topoisomerase enzymes,[22] and as expected these three compounds exhibit high potency (0.016, 0.60 and 0.16 μM, respectively). The indolyl thiazoles are all at least 10 times less potent than the above topoisomerase II inhibitors, so presumably have little activity against this enzyme. However, they are reasonably cytotoxic, and exhibit comparable potency to both cisplatin and melphalan in the same cell line (Table 1).[30] There is a 12-fold range in activity of the indolyl thiazoles **2**, **3** and **4** with the simpler derivatives (R^2 = H or Me) being slightly more potent, and in general the 3-indolyl 2-substituted thiazoles **2** are more potent than the corresponding 4-substituted thiazoles **3**. The 2-indolyl thiazoles are less potent still with the exception of benzothiazole **4d**, which was the most cytotoxic of the novel compounds tested. Given the potent activity of other benzothiazoles,[13–15] this result suggests that indolyl benzothiazoles merit further attention as potential anticancer agents.

Table 1. Biological evaluation of indolyl thiazoles **2**–**4** in SKBr3 cells



2 **3** **4**

Compound	R^1	R^2	R^3	IC_{50} (μM)
2a	H	H	H	2.7
2b	Ac	H	H	3.6
2c	Me	H	H	3.8
3d	Me	Me	H	6.3
2e	Me	CO_2Et	H	19.6
2f	Me	$CONH_2$	H	32.0
3a	H	H	H	18.0
3b	Me	H	H	12.0
3c	H	Me	H	4.9
3d	Me	Me	H	9.5
3e	H	CO_2Et	H	35.5
3f	Me	CO_2Et	H	35.5
3g	H	$CONH_2$	H	32.3
3h	Me	$CONH_2$	H	30.0
4a	Me	Me	H	40.1
4b	Me	CO_2Et	H	20.0
4c	Me	$CONH_2$	H	30.0
4d	Me	—$(CH)_4$—		2.6
Mitoxantrone				0.016
Cisplatin				7.4
Melphalan				13.0

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