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ORIGINAL ARTICLE

New spiroindolinones bearing 5-chlorobenzothiazole moiety

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Abstract

In this study, 5-chloro-3*H*-spiro-[1,3-benzothiazole-2,3′-indole]-2′(1′*H*)-one derivatives **3a**–**l** were synthesized by the reaction of 1*H*-indole-2,3-diones **1a**–**l** with 2-amino-4-chlorothiophenol **2** in ethanol. **3a**–**l** were tested for their abilities to inhibit lipid peroxidation (LP), scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, and to reduce Fe^{3+} to Fe^{2+}. Most of the tested compounds exhibited potent scavenging activities against ABTS$^{\bullet+}$ radical, reducing powers and strong inhibitory capacity on LP. **3 a**, **3 d**, **3e, 3h, 3j** and **3 k** chosen as prototypes were evaluated in the National Cancer Institute's *in vitro* primary anticancer assay. The greatest growth inhibitions were observed against a non-small cell lung cancer cell line HOP-92 for R_1-fluoro substituted **3 d** and a renal cancer cell line RXF-393 for R-chloro substituted **3 e** in the primary screen.

Keywords

Anticancer activity, antioxidant activity, benzothiazole, spiroindolinone, synthesis

History

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Introduction

Free radicals are constantly formed as a result of normal organ functions or excessive oxidative stress[1]. High levels of free radicals can damage cells, and may play a role in heart disease, cancer and other diseases[2–4]. Antioxidant agents may be promising alternative in the treatment of diseases involving free radicals and oxidative damage. There have been several reports on the antioxidant and cytotoxic activity of several indole derivatives. Melatonin (**I**) has gained significantly as a highly effective molecule in defense against oxidative damage and carcinogenesis. Indole-3-propionic acid, a deamination product of tryptophan, with a structure similar to that of melatonin, is present in biological fluids and is an effective free radical scavenger[5,6]. Some indoline-2-one, indoline-2-thione, indole-3-carboxamide and indolilbenzimidazole derivatives showed very good *in vitro* free-radical scavenging properties as shown by determination of their capacity to scavenge superoxide anion formation[7–10]. Depending on the analyses of structure–activity relationships and electrochemical studies, the indol ring is the reactive center dealing with oxidants due to its high resonance stability and very low activation energy barrier toward free radical reactions[4].

Phytoalexins are secondary metabolites produced by plants as a response to pathogen attack. Several indole phytoalexins isolated from the plant family cruciferae, brassinin (**II**), spirobrassinin (**III**), 1-methoxyspirobrassinin (**IV**), 1-methoxyspirobrassinol (**V**), 1-methoxyspirobrassinol methyl ether (**VI**) and camalexin (**VII**) have been found to possess significant antiproliferative activity against cancer cells. **II** and **III** also inhibit the growth of human cancer cells[11–14]. SU9516 (**VIII**) inhibits selectively cyclin-dependent kinase activity, decreases ligand-dependent and -independent cell cycle progression and increases apoptosis[15]. Semaxanib (SU5416) (**IX**) has been developed as a potent inhibitor of receptor tyrosine kinase for vascular endothelial growth factor receptor[16]. Sunitinib malate (SU11248) (**X**), the first oral drug acting as a multitarget tyrosine kinases inhibitor, has been approved for the treatment of gastrointestinal stromal tumors, renal cell carcinoma and several authors recall how the combination of SU11248 represent a promising novel treatment strategy against tumors (Figure 1)[17].

Benzothiazole derivatives are highly selective and potent antitumor agents[18–22]. Analysis of their structure–activity relationships shows that most of these compounds possess a second aromatic ring linked directly to the 2-position of benzothiazole ring or through an amine, an amide or an urea moiety. The presence of hydrophobic moieties in molecule is essential for cytotoxic activity of benzothiazole derivatives against cancer cell lines. The presence of electron withdrawing groups both in the benzothiazole nucleus and on the other aromatic ring improves the *in vitro* activity and metabolic stability of the compounds[23,24]. Chloro substituted benzothiazoles were found to have promising sensitivity to cancer cell lines[25,26]. Benzothiazole derivatives have also industrial applications as antioxidants[27–29].

Promoted by the earlier observations, we synthesized spiroindolinones by incorporating the 5-chlorobenzothiazole moiety. All compounds were tested *in vitro* for their abilities to inhibit lipid peroxidation (LP), induced by Fe^{3+}/ascorbate system, scavenge DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, and reducing power. The cytotoxic effects of the selected compounds were evaluated *in vitro* against the full panel of 60 human tumor cell lines derived from nine neoplastic diseases. The structure–activity relationships of the derivatives were also investigated.

Experimental protocols

Chemistry

2-Amino-4-chlorothiophenol, ascorbic acid, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$) diammonium

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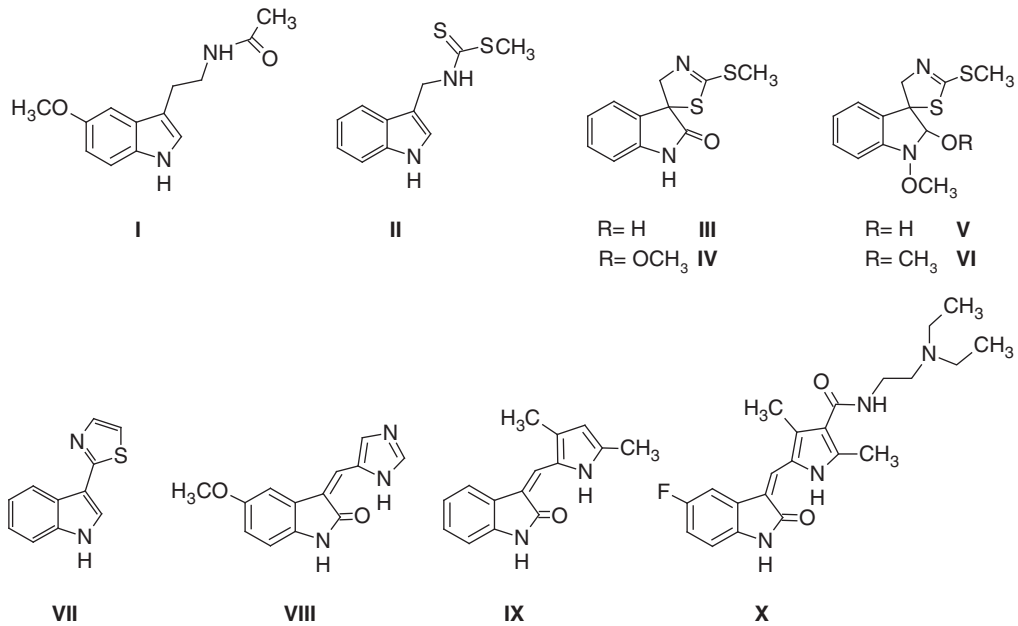


Figure 1. Structures of melatonin (**I**), brassinin (**II**), spirobrassinin (**III**), 1-methoxyspirobrassinin (**IV**), 1-methoxyspirobrassinol (**V**), 1-methoxyspirobrassinol methyl ether (**VI**), camalexin (**VII**), SU9516 (**VIII**), SU5416 (**IX**) and SU11248 (**X**).

salt, 5-bromoisatin, butylated hydroxytoluene (BHT), 5-chloroisatin, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), dimethylformamide (DMF), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 5-methylisatin, 5-nitroisatin, soybean lecithin (L-α-phosphatidylcholine Type IV-S), α-tocopherol and 5-(trifluoromethoxy)isatin were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO) were purchased from BDH Chemicals Ltd. (Poole, England). Ferric chloride, potassium ferricyanide, thiobarbituric acid (TBA) and trichloroacetic acid (TCA) were purchased from Merck (Darmstadt, Germany). All other chemicals used were of analytical grade. Melting points were estimated with a Buchi 540 melting point apparatus in open capillaries and were uncorrected. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 elemental analyzer. IR spectra were recorded on KBr discs, using a Perkin-Elmer Model 1600 FT-IR spectrometer. ^{1}H-NMR, ^{13}C-NMR (APT), HSQC-2D and HMBC-2D spectra were obtained on VarianUNITY INOVA 500 spectrophotometers using DMSO-d_6. Mass spectra were determined on Finnigan TM LCQ TM and AGILENT 1100 MSD instruments.

*General procedure for the synthesis of 1-methyl-1H-indole-2,3-diones (**1h–l**)*

A suspension of 5-substituted 1*H*-indole-2,3-diones **1 b**–**g** (5 mmol) and NaH (50% dispersion in mineral oil) (0.2 g) in anhydrous DMF (5 ml) was stirred for 30 min. at room temperature. After addition of iodomethane (15 mmol), the mixture was refluxed for 4 h. The product was poured onto ice and water, then was filtered.

*General procedure for the synthesis of 3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-ones (**3 a–l**)*

To a solution of 1*H*-indole-2,3-diones **1 a**–**l** (3.5 mmol) in ethanol (15 ml) was added 2-amino-4-chlorothiophenol **2** (3.5 mmol). The mixture was refluxed on a water bath for 3–6 h. The product formed after cooling was filtered and recrystallized from ethanol.

*5-Chloro-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3 a**)*

Yield 86%; m.p. 241 °C; IR (KBr) cm^{-1}: υ 3281, 3149 (NH), 1728 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.50 (1H, d, $J = 1.96$ Hz, benzothia. C_4–H); 6.62 (1H, dd, $J = 8.30$, 1.95 Hz, benzothia. C_6–H); 6.84 (1H, d, $J = 7.81$ Hz, ind. C_7–H); 7.03 (1H, d, $J = 8.30$ Hz, benzothia. C_7–H); 7.05 (1H, t, $J = 7.81$ Hz, ind. C_5–H); 7.29 (1H, ddd (td), $J = 7.81$, 7.32, 1.47 Hz, ind. C_6–H); 7.55 (1H, d, $J = 2.92$ Hz, ind. C_4–H); 7.56 (1H, s, benzothia. NH); 10.39 (1H, s, ind. NH). HSQC-2D (DMSO-d_6, 125 MHz) δ (ppm): 75.64 (spiro C); 108.40 (benzothia. C_4); 110.88 (ind. C_7); 118.52 (benzothia. C_6); 122.70 (benzothia. C_7); 123.28 (ind. C_5); 123.99 (ind. C_{3a}); 126.38 (ind. C_4); 129.86 (benzothia. C_{7a}); 130.92 (benzothia. C_5); 131.43 (ind. C_6); 142.03 (ind. C_{7a}); 149.43 (benzothia. C_{3a}); 176.56 (ind. C=O). LCMS-ESI (+) *m/z* (%): 289, 291 (MH^+; 35, 9.2); 287, 289 (100, 35); 261, 263 (25, 11). Analyses (%) Cald for $C_{14}H_9ClN_2OS$ (288.75): C, 58.23; H, 3.14; N, 9.70. Found: C, 58.06; H, 3.14; N, 9.52.

*5-Chloro-5′-methyl-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3 b**)*

Yield 72%; m.p. 242 °C; IR (KBr) cm^{-1}: υ 3288, 3161 (NH), 1722 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.26 (3H, s, CH_3); 6.50 (1H, d, $J = 1.95$ Hz, benzothia. C_4–H); 6.62 (1H, dd, $J = 8.29$, 1.95 Hz, benzothia. C_6–H); 6.73 (1H, d, $J = 8.30$ Hz, ind. C_7–H); 7.03 (1H, d, $J = 8.29$ Hz, benzothia. C_7–H); 7.09 (1H, dd, $J = 7.81$, 1.95 Hz, ind. C_6–H); 7.37 (1H, br. s, ind. C_4–H); 7.54 (1H, s, benzothia. NH); 10.29 (1H, s, ind. NH). HSQC-2D (DMSO-d_6, 125 MHz) δ (ppm): 21.18 (ind. 5-CH_3); 75.75 (spiro C); 108.30 (benzothia. C_4); 110.66 (ind. C_7); 118.46 (benzothia. C_6); 122.74 (ind. C_6); 123.98 (ind. C_{3a}); 126.82 (ind. C_4); 129.97 (ind. C_5); 130.87 (benzothia. C_{7a}); 131.67 (benzothia. C_7); 132.38 (benzothia. C_5); 139.44 (ind. C_{7a}); 149.41 (benzothia. C_{3a}); 176.56 (ind. C=O). LCMS-APCI (+) *m/z* (%): 303, 305 (MH^+; 35, 1.9); 302, 304 (17, 6); 301, 303 (100, 35). Analyses (%) Cald for $C_{15}H_{11}ClN_2OS$ (302.78): C, 59.50; H, 3.66; N, 9.25. Found: C, 59.35; H, 3.68; N, 9.07.

*5-Chloro-5′-trifluoromethoxy-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3c**)*

Yield 54%; m.p. 244 °C; IR (KBr) cm^{-1}: υ 3291, 3180 (NH), 1727 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.55 (1H, d, $J = 1.95$ Hz, benzothia. C_4–H); 6.65 (1H, dd, $J = 7.81$, 1.95 Hz, benzothia. C_6–H); 6.95 (1H, d, $J = 8.30$ Hz, ind. C_7–H); 7.06 (1H, d, $J = 8.30$ Hz, benzothia. C_7–H); 7.31 (1H, dd, $J = 8.30$, 1.95 Hz, ind. C_6–H); 7.53 (1H, d, $J = 1.95$ Hz, ind. C_4–H); 7.61 (1H, s, benzothia. NH); 10.60 (1H, s, ind. NH). LCMS-ESI (–) *m/z* (%): 371, 373 (MH^-; 100, 40). Analyses (%) Cald for $C_{15}H_8ClF_3N_2O_2S$ (372.75): C, 48.33; H, 2.16; N, 7.52. Found: C, 48.26; H, 2.56; N, 7.47.

*5-Chloro-5′-fluoro-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3d**)*

Yield 56%; m.p. 238 °C; IR (KBr) cm^{-1}: υ 3277, 3179, 3138 (NH), 1724 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.53 (1H, d, $J = 1.95$ Hz, benzothia. C_4–H); 6.64 (1H, dd, $J = 7.81$, 1.95 Hz, benzothia. C_6–H); 6.85 (1H, dd, $J = 8.79$, 4.14 Hz, ind. C_7–H); 7.05 (1H, d, $J = 7.81$ Hz, benzothia. C_7–H); 7.15 (1H, ddd (td), $J = 8.79$, 2.92 Hz, ind. C_6–H); 7.43 (1H, dd, $J = 7.81$, 2.44 Hz, ind. C_4–H); 7.60 (1H, s, benzothia. NH); 10.42 (1H, s, ind. NH). HMBC-2D (DMSO-d_6, 125 MHz) δ (ppm): 75.77 (spiro C, d, $J = 1.44$ Hz); 108.56 (benzothia. C_4); 111.98 (ind. C_7, d, $J = 7.67$ Hz); 113.79 (ind. C_4, d, $J = 24.93$ Hz); 117.96 (ind. C_6, d, $J = 23.49$ Hz); 118.70 (benzothia. C_6); 122.76 (benzothia. C_7); 123.75 (benzothia. C_{7a}); 131.18 (benzothia. C_5); 131.66 (ind. C_{3a}, d, $J = 7.67$ Hz); 138.13 (ind. C_{7a}, d, $J = 1.92$ Hz); 149.21 (benzothia. C_{3a}); 159.04 (ind. C_5, d, $J = 238.68$); 176.66 (ind. C=O). LCMS-ESI (+) *m/z* (%): 307, 309 (MH^+; 40, 9); 306, 308 (20, 7); 305, 307 (100, 40); 279, 281 (25, 13). Analyses (%) Cald for $C_{14}H_8ClFN_2OS$ (306.74): C, 54.82; H, 2.63; N, 9.13. Found: C, 54.52; H, 2.79; N, 9.11.

*5,5′-Dichloro-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3e**)*

Yield 63%, m.p. 238 °C, IR (KBr) cm^{-1}: υ 3276, 3168, 3133 (NH), 1725 (C=O). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.53 (1H, d, $J = 1.95$ Hz, benzothia. C_4–H); 6.65 (1H, dd, $J = 8.30$, 1.95 Hz, benzothia. C_6–H); 6.86 (1H, d, $J = 8.30$ Hz, ind. C_7–H); 7.06 (1H, d, $J = 7.80$ Hz, benzothia. C_7–H); 7.35 (1H, dd, $J = 8.29$, 2.44 Hz, ind. C_6–H); 7.55 (1H, d, $J = 2.44$ Hz, ind. C_4–H); 7.58 (1H, s, benzothia. NH); 10.54 (1H, s, ind. NH). HMBC-2D (DMSO-d_6, 125 MHz) δ (ppm): 75.54 (spiro C); 108.62 (benzothia. C_4); 112.51 (ind. C_7); 118.76 (benzothia. C_6); 122.78 (benzothia. C_7); 123.72 (benzothia. C_{7a}); 126.21 (ind. C_4); 127.16 (ind. C_5); 131.05 (ind. C_6); 131.29 (benzothia. C_5); 132.11 (ind. C_{3a}); 140.86 (ind. C_{7a}); 149.12 (benzothia. C_{3a}); 176.31 (ind. C=O). LCMS-ESI (–) *m/z* (%): 321, 323, 325 (MH^-; 100, 65, 13); 293, 295, 297 (11, 6.5, 1.2). Analyses (%) Cald for $C_{14}H_8Cl_2N_2OS$ (323.20): C, 52.03; H, 2.49; N, 8.67. Found: C, 51.97; H, 2.75; N, 8.63.

*5′-Bromo-5-chloro-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3f**)*

Yield 56%; m.p. 238 °C; IR (KBr) cm^{-1}: υ 3276, 3168, 3133 (NH), 1725 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.53 (1H, d, $J = 2.44$ Hz, benzothia. C_4–H); 6.65 (1H, dd, $J = 7.81$, 1.95 Hz, benzothia. C_6–H); 6.82 (1H, d, $J = 8.29$ Hz, ind. C_7–H); 7.06 (1H, d, $J = 8.29$ Hz, benzothia. C_7–H); 7.48 (1H, dd, $J = 8.30$, 1.95 Hz, ind. C_6–H); 7.58 (1H, s, benzothia. NH); 7.65 (1H, d, $J = 1.96$ Hz, ind. C_4–H); 10.55 (1H, s, ind. NH). LCMS-ESI (–) *m/z* (%): 365, 367, 369 (MH^-; 74, 100, 25). Analyses (%) Cald for $C_{14}H_8BrClN_2OS$ (367.65): C, 45.74; H, 2.19; N, 7.62. Found: C, 45.83; H, 2.60; N, 7.50.

*5-Chloro-5′-nitro-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3g**)*

Yield 58%; m.p. 227 °C; IR (KBr) cm^{-1}: υ 3312, 3149 (NH), 1730 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.60 (1H, d, $J = 2.13$ Hz, benzothia. C_4–H); 6.69 (1H, dd, $J = 8.23$, 2.13 Hz, benzothia. C_6–H); 7.06 (1H, d, $J = 8.85$ Hz, ind. C_7–H); 7.10 (1H, d, $J = 8.24$ Hz, benzothia. C_7–H); 7.60 (1H, s, benzothia. NH); 8.25 (1H, dd, $J = 8.24$, 2.44 Hz, ind. C_6–H); 8.28 (1H, d, $J = 2.44$ Hz, ind. C_4–H); 11.12 (1H, s, ind. NH). ^{13}C-NMR (APT, DMSO-d_6, 125 MHz) δ (ppm): 75.08 (spiro C); 108.90 (benzothia. C_4); 111.40 (ind. C_7); 119.06 (benzothia. C_6); 121.57 (ind. C_4); 122.95 (benzothia. C_7); 123.46 (benzothia. C_{7a}); 128.31 (ind. C_6); 131.21 (benzothia. C_5); 131.38 (ind. C_{3a}); 143.44 (ind. C_5); 148.33 (ind. C_{7a}); 148.82 (benzothia. C_{3a}); 176.88 (ind. C=O). Analyses (%) Cald for $C_{14}H_8ClN_3O_3S$ (333.75): C, 50.38; H, 2.42; N, 12.59. Found: C, 50.48; H, 2.27; N, 12.27.

*5-Chloro-1′,5′-dimethyl-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3h**)*

Yield 45%; m.p. 224-226 °C; IR (KBr) cm^{-1}: υ 3292 (NH), 1701 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 2.29 (3H, s, CH_3); 3.09 (3H, s, N-CH_3); 6.51 (1H, d, $J = 2.01$ Hz, benzothia. C_4–H); 6.64 (1H, dd, $J = 8.05$, 2.01 Hz, benzothia. C_6–H); 6.93 (1H, d, $J = 8.05$ Hz, ind. C_7–H); 7.04 (1H, d, $J = 8.05$ Hz, benzothia. C_7–H); 7.20 (1H, dd, $J = 8.05$, 1.68 Hz, ind. C_6–H); 7.42 (1H, $J = 1.68$ Hz, ind. C_4–H); 7.51 (1H, s, benzothia. NH). LCMS-ESI (-) *m/z* (%): 315, 317 (MH^-; 100, 35). Analyses (%) Cald for $C_{16}H_{13}ClN_2OS.1/2\ H_2O$ (325.82): C, 58.98; H, 4.33; N, 8.59. Found: C, 59.65; H, 4.08; N, 8.59.

*5-Chloro-1′-methyl-5′-trifluoromethoxy-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3i**)*

Yield 46%; m.p. 88-90 °C; IR (KBr) cm^{-1}: υ 3301 (NH), 1727 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.13 (3H, s, N-CH_3); 6.58 (1H, d, $J = 1.95$ Hz, benzothia. C_4–H); 6.67 (1H, dd, $J = 8.05$, 1.95 Hz, benzothia. C_6–H); 7.07 (1H, d, $J = 8.30$ Hz, ind. C_7–H); 7.16 (1H, d, $J = 8.79$ Hz, benzothia. C_7–H); 7.43 (1H, dd, $J = 8.30$, 1.95 Hz, ind. C_6–H); 7.54 (1H, s, benzothia. NH); 7.58 (1H, br. s, ind. C_4–H).^{13}C-NMR (APT, DMSO-d_6, 125 MHz) δ (ppm): 27.17 (N-CH_3); 75.12 (spiro C); 108.74 (benzothia. C_4); 111.10 (ind. C_7); 118.85 (benzothia. C_6); 119.54 (ind. C_4); 120.83 (q, $J = 255.78$ Hz, OCF_3); 122.82 (benzothia. C_7); 123.40 (benzothia. C_{7a}); 124.85 (ind. C_6); 131.11 (benzothia. C_5); 131.32 (ind. C_{3a}); 142.68 (ind. C_{7a}); 144.80 (q, $J = 1.92$ Hz, ind. C_5); 148.91 (benzothia. C_{3a}); 174.91 (ind. C=O). Analyses (%) Cald for $C_{16}H_{10}ClF_3N_2O_2S$ (386.78): C, 49.69; H, 2.61; N, 7.24. Found: C, 49.39; H, 2.95; N, 7.13.

*5-Chloro-5′-fluoro-1′-methyl-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3j**)*

Yield 77%; m.p. 173-176 °C; IR (KBr) cm^{-1}: υ 3307 (NH), 1701 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.11 (3H, s, N-CH_3); 6.55 (1H, d, $J = 2.01$ Hz, benzothia. C_4–H); 6.66 (1H, dd, $J = 8.06$, 2.02 Hz, benzothia. C_6–H); 7.06 (1H, dd, $J = 8.73$, 4.03 Hz, ind. C_7–H); 7.07 (1H, d, $J = 8.06$ Hz, benzothia. C_7–H); 7.26 (1H, ddd (td), $J = 8.73$, 2.68 Hz, ind. C_6–H); 7.48 (1H, dd, $J = 7.71$, 2.68 Hz, ind. C_4–H); 7.55 (1H, s, benzothia. NH). HSQC-2D (DMSO-d_6, 125 MHz) δ (ppm): 27.12 (N-CH_3); 75.34 (d, $J = 1.92$ Hz, spiro C); 108.66 (benzothia. C_4); 111.02 (d, $J = 8.15$ Hz, ind. C_7); 113.71 (d, $J = 24.92$ Hz, ind. C_4); 117.87 (d, $J = 23.48$ Hz, ind. C_6); 118.78 (benzothia. C_6); 122.79 (benzothia.

C_7); 123.73 (benzothia. C_{7a}); 131.06 (benzothia. C_5); 131.20 (d, $J = 8.14$ Hz, ind. C_{3a}); 139.70 (d, $J = 1.91$ Hz, ind. C_{7a}); 149.05 (benzothia. C_{3a}); 159.52 (d, $J = 239.16$ Hz, ind. C_5); 174.84 (d, $J = 1.44$ Hz, ind. C=O). Analyses (%) Cald for $C_{15}H_{10}ClFN_2OS$ (320.77): C, 56.17; H, 3.14; N, 8.73. Found: C, 55.82; H, 3.13; N, 8.55.

*5,5′-Dichloro-1′-methyl-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3k**)*

Yield 68%; m.p. 200-203 °C; IR (KBr) cm^{-1}: υ 3300 (NH), 1701 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.11 (3H, s, N-CH_3); 6.56 (1H, d, $J = 2.01$ Hz, benzothia. C_4–H); 6.67 (1H, dd, $J = 8.05$, 2.01 Hz, benzothia. C_6–H); 7.07 (1H, d, $J = 8.06$ Hz, benzothia. C_7–H); 7.09 (1H, d, $J = 8.39$ Hz, ind. C_7–H); 7.47 (1H, dd, $J = 8.39$, 2.35 Hz, ind. C_6–H); 7.53 (1H, s, benzothia. NH); 7.60 (1H, d, $J = 2.35$ Hz, ind. C_4–H). LCMS-ESI (−) *m/z* (%): 335, 337, 339 (MH^-; 100, 64, 12). Analyses (%) Cald for $C_{15}H_{10}Cl_2N_2OS.1/2\ H_2O$ (346.24): C, 52.03; H, 3.20; N, 8.08. Found: C, 52.58; H, 3.29; N, 8.05.

*5′-Bromo-5-chloro-1′-methyl-3H-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′H)-one (**3l**)*

Yield 45%; m.p. 228-233 °C; IR (KBr) cm^{-1}: υ 3300 (NH), 1701 (C=O); ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.11 (3H, s, N-CH_3); 6.56 (1H, d, $J = 2.44$ Hz, benzothia. C_4–H); 6.66 (1H, dd, $J = 8.29$, 1.95 Hz, benzothia. C_6–H); 7.03 (1H, d, $J = 8.29$ Hz, ind. C_7–H); 7.07 (1H, d, $J = 8.30$ Hz, benzothia. C_7–H); 7.52 (1H, s, benzothia. NH); 7.60 (1H, dd, $J = 8.30$, 1.95 Hz, ind. C_6–H); 7.70 (1H, d, $J = 2.44$ Hz, ind. C_4–H). ^{13}C-NMR (APT, DMSO-d_6, 125 MHz) δ (ppm): 27.12 (N-CH_3); 75.08 (spiro C); 108.73 (benzothia. C_4); 112.04 (ind. C_7); 115.40 (ind. C_5); 118.85 (benzothia. C_6); 122.82 (benzothia. C_7); 123.69 (benzothia. C_{7a}); 128.57 (ind. C_4); 131.08 (benzothia. C_5); 131.89 (ind. C_{3a}); 134.15 (ind. C_6); 142.79 (ind. C_{7a}); 148.91 (benzothia. C_{3a}); 174.47 (ind. C=O). Analyses (%) Cald for $C_{15}H_{10}BrClN_2OS$ (381.68): C, 47.20; H, 2.64; N, 7.34. Found: C, 46.98; H, 2.71; N, 7.42.

Biological assay

In vitro *evaluation of antioxidant activity*

Antioxidant activity on liposome peroxidation. Antioxidative effects of the compounds were measured using the inhibition of the Fe^{3+}/ascorbate induced LP in liposomes. LP has attracted much attention in relation to oxidative damage of biological membranes, due to the formation of lipid hydroperoxides which in the presence of cellular iron containing compounds, can break down to yield oxygen radicals. LP products were measured by quantification of TBA reactive substances (TBARS, including malondialdehyde), during incubation period, which would be produced by complete peroxidation of the oxidizable polyunsaturated fatty acids (PUFA) present in biological membranes using TBA method. Phosphatidylcholine (lecithin), a phospholipid believed to be present in high amounts in cell membranes, was used as liposome-like substrate.

The LP assay was based on the method described by Duh et al.[30]. Lecithin (300 mg) was suspended in 30 ml phosphate buffer (10 mmol/l, pH 7.4). This suspension was sonicated with a rod using an ultrasonic homogenizer (Bandelin, Berlin, Germany) at 30 s intervals for 10 min until an opalescent suspension was obtained.

The sonicated solution (10 mg/ml), $FeCl_3$, ascorbic acid and the indole derivatives (0.025–0.4 mM) or the reference antioxidant α-tocopherol (0.1–0.4 mM) were mixed to produce a final concentration of 3.08 mg liposome/ml, 123.2 µmol $FeCl_3$ and 123.2 µmol ascorbic acid. After 1 h incubation at 37 °C, the formation of LP products was assayed by the measurement of malondialdehyde (MDA) levels on the basis that MDA reacted with TBA at 532 nm according to Buege and Aust[31]. Briefly, 500 µl of this reaction mixture was mixed with 1000 µl TCA-TBA reagent (consisting of 15% w/v TCA and 0.375% TBA in 0.25 N HCl) and 14 µl BHT (2% in absolute ethanol). The mixture was vortexed and heated for 10 min in a boiling water bath. After cooling, an equal volume of *n*-butanol was added and the mixture was shaken vigorously. The *n*-butanol layer was separated by centrifugation at 3000 rpm for 5 min. The absorbance of the sample was measured at 532 nm against a blank, which contained all reagents except lecithin. The percentage inhibition of LP was calculated by comparing the results of the samples with those of controls non-treated with the extract using the following equation:

$$\text{Inhibition effect (\%)} = \left[1 - \left(\frac{\text{Absorbance of sample at 532 nm}}{\text{Absorbance of control at 532 nm}}\right)\right] \times 100$$

DPPH$^\bullet$ radical-scavenging activity. The DPPH radical scavenging capacities of the compounds were determined according to the method described by Brand-Williams et al.[32]. A purple-colored DPPH is a stable free radical and it is reduced to yellow colored diphenyl-picryl hydrazine by reacting with an antioxidant. A 0.1-ml aliquot of each indole derivative (0.2–6.25 mM) in DMSO, ascorbic acid (0.1–0.8 mM) or α-tocopherol (0.1–0.8 mM) in absolute ethanol was added to 3.9 ml of 6×10^{-5} M methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured spectrophotometrically at 517 nm against methanol. All measurements were made in triplicate and averaged. Two type of controls were used for this test, a negative control (containing all reagents except the test sample) and positive controls (using the reference antioxidants). The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \left(\frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}}\right)\right] \times 100$$

Total radical-antioxidant potential (TRAP). The total radical antioxidant potential of indole derivatives was measured using TEAC assay as described by Re et al.[33] with minor modifications.

After addition of 990 µl of $ABTS^{\bullet+}$ solution to 10 µl of indole derivatives (0.2–6.25 mM), ascorbic acid (0.2–3.1 mM), α-tocopherol (0.2–3.1 mM) or Trolox standards (final concentration 0–20 µM in ethanol), the decrease in absorbance at 734 nm was monitored exactly 6 min after the initial mixing. Appropriate ethanol blanks were run in each assay. All determinations were carried out in triplicate. The ability to scavenge $ABTS^{\bullet+}$ radical was calculated by the following equation:

$$\text{ABTS radical scavenging activity (\%)} = \left[1 - \left(\frac{\text{Absorbance of sample at 734 nm}}{\text{Absorbance of control at 734 nm}}\right)\right] \times 100$$

Reducing power. The reducing powers of the indole derivatives, ascorbic acid and α-tocopherol were determined according to the method described by Chung et al.[34]. A 0.1-ml aliquot of each indole derivative (1.6–6.25 mM), ascorbic acid (0.4–3.1 mM) or

α-tocopherol (0.4–6.25 mM) were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50 °C for 20 min. About 0.25 ml of 1% TCA was added to the mixture to stop the reaction, and then the mixture was centrifuged at 2790 *g* for 10 min. The supernatant (0.25 ml) was mixed with 0.25 ml of distilled water and 0.1% of $FeCl_3$ (0.5 ml) and then the absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the absorbance values.

Statistical analysis

Results were expressed as the mean ± SD of triplicate analysis. Statistical comparisons were performed using the Student's *t*-test. Differences were considered significant at $p < 0.05$. The correlation coefficient (r^2) between the parameters tested was established by regression analysis.

In vitro *evaluation of cancer activity.* The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 µl at plating densities ranging from 5000 to 40 000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed *in situ* with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (time zero, Tz). Experimental drugs were solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final test concentration with complete medium containing 50 µg/ml gentamicin. Aliquots of 100 µl of these drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentration (10^{-5} M). Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air-dried. Sulphorhodamine B solution (100 µl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air-dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was measured on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80% TCA (final concentration, 16% TCA). Using the seven absorbance measurements [Tz, control growth (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. The percentage growth inhibition (GI) was calculated as:

$$[(\mathrm{Ti} - \mathrm{Tz})/(\mathrm{C} - \mathrm{Tz})] \times 100 \text{ for concentrations for which } \mathrm{Ti} \geq \mathrm{Tz}$$

$$[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 \text{ for concentrations for which } \mathrm{Ti} < \mathrm{Tz}.$$

Results and discussion

Chemistry

The reaction of 1*H*-indole-2,3-diones with 2-aminothiophenol have been investigated in dry xylene in the presence of anhydrous zinc chloride under reflux and some interesting results have been obtained. The products formed were an indolobenzothiazide (**XI**) (15%), a benzothiazinone (**XII**) (20%) and a spirobenzothiazoline (**XIII**) (40%). In the case of 1-methyl-1*H*-indole-2,3-dione, only **XIII** was obtained under the same conditions (Figure 2)[35–37]. The reaction of 1*H*-indole-2,3-dione with 2-aminothiophenol was reported to result in the formation of **XIII** in ethanol[38–40].

In the present article, 1-methyl-1*H*-indole-2,3-diones **1 h–l** were prepared by reacting 1*H*-indole-2,3-diones **1 b–g** with sodium hydride in DMF followed by treatment with iodomethane. 5-Chloro-3*H*-spiro[1,3-benzothiazole-2,3′-indol]-2′(1′*H*)-ones **3 a–l** were synthesized by the reaction of 2-amino-4-chlorothiophenol **2** with 1*H*-indole-2,3-diones **1 a–l** in ethanol (Figure 3)[39,40]. The structures of spiroindolinones were confirmed by analytical and spectral (IR, ^{1}H-NMR, ^{13}C-NMR (APT), HSQC-2D, HMBC-2D, ESMS and LCMS-APCI) data.

Analysis data of all synthesized compounds were in full agreement with the suggested molecular structures. The IR spectra of **3 a–l** showed bands resulting from the NH stretchings of the indole and benzothiazoline rings in the 3312–3133 cm^{-1} regions. Amide and ketone C=O bands of 1*H*-indole-2,3-diones **1 a–l** show two strong absorptions at 1755–1765 and 1734–1744 cm^{-1} regions. Observation of only the lactam C=O band (1701–1730 cm^{-1}) assigned to the 2-indolinone moiety in the IR spectra of **3 a–l** and the absence of the SH (δ 3.40 ppm) and NH_2 (δ 6.70 ppm) resonances of 4-chloro-2-aminothiophenol **2** in the ^{1}H-NMR **3 a–l** proved the aimed cyclization[37,41,42]. ^{1}H-NMR spectra of R_2-nonsubstituted derivatives **3 a-g** displayed the NH protons of the indole (δ 10.29–11.12 ppm) and benzothiazoline (δ 7.55–7.61 ppm) rings as two separate singlets[43]. The NH resonances (δ 7.51–7.55 ppm) indicative of the benzothiazoline structure appeared, the indole NH resonances disappeared and R_2-methyl resonances (δ 3.09–3.20 ppm) provided evidence for the methylation in the spectra of R_2-methylated derivatives **3 h–l**. Benzothiazoline C_4–H, C_6–H and C_7–H being shielded due to the electron donating effect of benzothiazoline NH function, appeared at δ 6.50–6.60, 6.63–6.69 and 7.03–7.16 ppm as a doublet, a double doublet and a doublet, respectively. Indole C_7–H showed as a doublet due to the mesomeric effect of the NH-C=O function at δ 6.73–7.07 ppm. Indole C_6–H and C_4–H signals being shielded or deshielded due to the substituent at 5-position of indole ring were shifted to upfield when compared to that of the other aromatic protons. Indole C_6–H displayed as a triple doublet or a double doublet at 7.09–8.25 ppm, while indole C_4–H signals were observed as a broad singlet or a doublet at δ 7.37–8.28 ppm. The indole protons and carbons which showed the ^{1}H-^{19}F and ^{13}C-^{19}F couplings in the spectra of 5-fluorosubstituted **3 d** and **3j** resonated as separate doublets. The ^{13}C-NMR (APT) spectra of

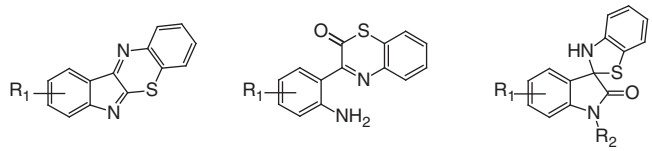


Figure 2. Structures of indolobenzothiazide (**XI**), benzothiazinone (**XII**) and spirobenzothiazole (**XIII**).

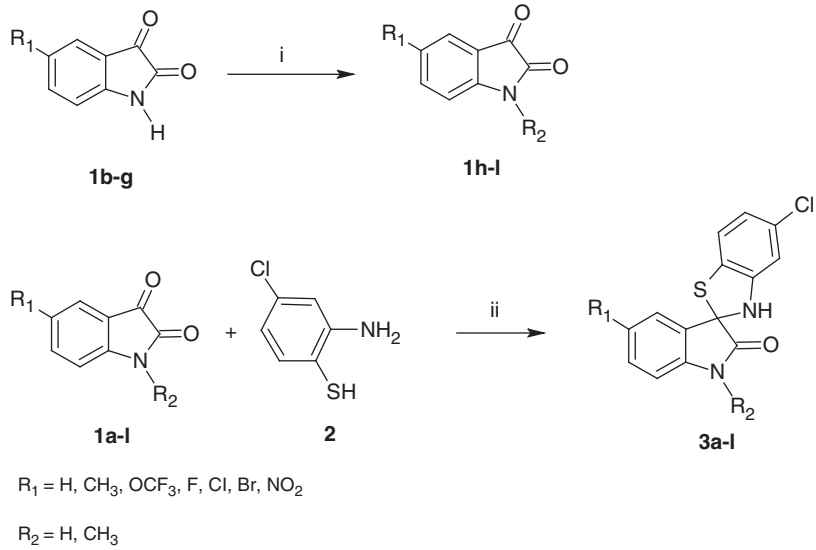


Figure 3. Synthesis of **3a–l**. Reagent and condition: (i) NaH, anhyd. DMF, stirred, 0.5 h, CH_3I, reflux, 4 h and (ii) EtOH, reflux, 3–6 h.

3 g, **3i** and **3 l**, the HSQC spectra of **3 a**, **3 b** and **3f**, and the HMBC spectra of **3 d** and **3 e** displayed the spiro C (δ 75.08–75.77 ppm) and the indolinone C=O (δ 174.47–176.88 ppm) peaks which verified the spiroindolinone structure[44]. Upfield shifts observed in the ^{13}C-NMR resonances assigned to the indole C_{7a} (δ 138.13–148.33 ppm) and the benzothiazoline C_{3a} (δ 148.82–149.13 ppm) and cross peaks observed in the HMBC spectrum of **3 d** and **3 e** further supported the structure of **3 a–l**. The OCF_3 (δ 120.83 ppm) and the indole C_5 (δ 144.80 ppm) signals which showed the ^{13}C-^{19}F coupling in the APT spectra of **3i** displayed as quartets[45,46]. In the mass spectra of **3 a–f**, **3 h** and **3 k,** MH^+ or MH^- peaks were observed which confirmed their molecular weights. The X-ray data of **3 a** were also determined in order to confirm the assigned spiro structure[40].

The antioxidant activity

The antioxidant activities of spiroindolinones were determined by measuring their ability of inhibiting LP induced by Fe^{3+}-ascorbate, their reducing power, hydrogen-donor and radical scavenging activities. In the assessment of antioxidant activity, both synthetic and biologically relevant free radicals were used. The synthetic nitrogen-centered DPPH$^\bullet$ and ABTS$^{\bullet+}$ radicals are not biologically relevant, but are often used as indicator compounds in testing hydrogen transfer capacity that is related to antioxidant activity. In order to quantify the antioxidant activity, the EC_{50} values were further calculated and summarized in Table 1. The high antioxidant potential is characterized by low EC_{50} value and high reducing power.

Antioxidant activity on liposome peroxidation

Measurement of LP using phospholipid model system was the first line of tests to establish the potential antioxidant action of compounds under study. **3 a–l** were capable of inhibiting the formation of TBA reactive substances (TBARS) in a concentration dependent manner, therefore were capable of inhibiting degradation of phospholipids. α-Tocopherol was used as reference antioxidant. α-Tocopherol has been defined as a radical-chain breaker, which delays LP by reacting with chain-propagating peroxyl radicals faster than these radicals can react with fatty acid side-chains. As reported in Table 1, the whole of the tested R_1-nonsubstituted **3 a** (0.15 ± 0.004 mM), R_1-methyl substituted **3 b** (0.12 ± 0.007 mM), R_1-trifluoromethoxy substituted **3 c** (0.16 ± 0.005 mM), R_1-fluoro substituted **3 d** (0.11 ± 0.006 mM), R_1-chloro substituted **3 e** (0.13 ± 0.004 mM), R_1-bromo substituted **3f** (0.12 ± 0.006 mM) and R_1-nitro substituted **3 g** (0.18 ± 0.006 mM) showed similar degrees of efficacy, which were comparable to the EC_{50} value obtained for α-tocopherol (0.15 ± 0.04 mM). Introduction of a methyl group at R_2 did not induce an increase in activity. R_1-methyl substituted **3 h** (0.12 ± 0.005 mM), R_1-fluoro substituted **3j** (0.12 ± 0.01 mM), R_1-chloro substituted **3 k** (0.12 ± 0.005 mM) and R_1-bromo substituted **3 l** (0.12 ± 0.005 mM) exhibited similar activity in comparison with the corresponding R_2-nonsubstituted compounds, **3 b**, **3 d**, **3 e** and **3f**. In R_1-trifluoromethoxy substituted **3i** (0.22 ± 0.015 mM), and R_1-nitro substituted **3 m** (0.30 ± 0.01 mM), the activity decreased somewhat when compared with the corresponding **3 c** and **3 g**.

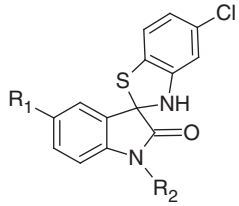
DPPH radical scavenging activity

DPPH radical scavenging activities of all the compounds were significantly lower than those of both ascorbic acid (0.36 ± 0.04 mM) and α-tocopherol (0.35 ± 0.007 mM). R_2-methylation increased slightly the DPPH$^\bullet$ scavenging activity of **3 h–m** in comparison with R_2-nonsubstituted **3 a–g**. From the EC_{50} values, it was seen that **3 h** and **3j–l** showed the highest DPPH radical scavenging activity. As shown in Table 1, the EC_{50} values of these compounds were 5.31 ± 0.6, 5.89 ± 0.18, 4.19 ± 0.05 and 5.63 ± 0.7 mM, respectively.

ABTS radical scavenging activity

ABTS radical cation scavenging activities of the compounds were measured also as the Trolox Equivalent Antioxidant Capacity (TEAC) (The concentration of Trolox solution having an antioxidant activity equivalent to 1 mM concentration of the tested compound). The TEAC involves the generation of the long-lived specific radical cation chromophore (ABTS) by controlled chemical oxidation and reflects the ability of hydrogen or electron-donating antioxidants to scavenge the ABTS$^{\bullet+}$ compared with that of Trolox[32]. As can be seen in Table 1, R_1-methyl substituted **3 b** (1.16 ± 0.03 mM) and R_1-flouro substituted **3 d** (0.99 ± 0.01 mM) showed similar ($p > 0.05$) degrees of efficacy in their scavenging activities against ABTS$^{\bullet+}$, which were the highest among the tested compounds. The EC_{50} values of these compounds were lower than the values observed for ascorbic acid (1.20 ± 0.03 mM) and α-tocopherol (1.20 ± 0.01 mM). R_1-trifluoromethoxy substituted **3 c** (1.21 ± 0.08 mM) and

Table 1. Antioxidant properties of **3 a–l**.



EC_{50} (mM)						
Compound	R_1	R_2	Anti-LP	DPPH	ABTS	Reducing power
3 a	H	H	0.15 ± 0.004^{a}	12.1 ± 1.23^{a}	1.35 ± 0.05^{a}	1.15 ± 0.01^{a}
3 b	CH_3	H	$0.12 \pm 0.007^{b,d}$	8.33 ± 0.85^{b}	1.16 ± 0.03^{b}	2.44 ± 0.18^{b}
3 c	OCF_3	H	0.16 ± 0.005^{a}	12.1 ± 1.17^{c}	1.21 ± 0.08^{a}	$1.37 \pm 0.10^{a,c}$
3 d	F	H	$0.11 \pm 0.006^{b,d}$	13.0 ± 0.73^{c}	0.99 ± 0.01^{c}	0.99 ± 0.12^{a}
3 e	Cl	H	0.13 ± 0.004^{b}	$6.96 \pm 0.35^{b,d}$	1.28 ± 0.03^{a}	1.00 ± 0.10^{a}
3 f	Br	H	$0.12 \pm 0.006^{b,d}$	7.19 ± 0.62^{b}	1.30 ± 0.02^{a}	1.26 ± 0.07^{a}
3 g	NO_2	H	0.18 ± 0.006^{c}	$8.05 \pm 0.13^{b,d}$	1.42 ± 0.08^{a}	1.13 ± 0.10^{a}
3 h	CH_3	CH_3	$0.12 \pm 0.005^{b,d}$	$5.31 \pm 0.6^{f,g}$	$1.44 \pm 0.09^{a,h}$	N.d.
3 i	OCF_3	CH_3	0.22 ± 0.015^{e}	$6.60 \pm 0.3^{g,h}$	2.74 ± 0.026^{d}	N.d.
3 j	F	CH_3	$0.12 \pm 0.01^{b,d}$	$5.89 \pm 0.18^{f,h}$	1.65 ± 0.07^{e}	3.30 ± 0.17^{f}
3 k	Cl	CH_3	$0.12 \pm 0.005^{b,d}$	4.19 ± 0.05^{f}	$1.55 \pm 0.18^{a,e,h}$	N.d.
3 l	Br	CH_3	$0.12 \pm 0.005^{b,d}$	$5.63 \pm 0.7^{f,g,h}$	1.40 ± 0.045^{h}	N.d.
Ascorbic acid	–	–	–	0.36 ± 0.04^{e}	1.20 ± 0.03^{b}	0.65 ± 0.10^{d}
α-Tocopherol	–	–	0.15 ± 0.04^{a}	0.35 ± 0.007^{e}	1.20 ± 0.01^{b}	1.95 ± 0.04^{c}

EC_{50} value: the effective concentration at which the antioxidant activity was 50%; $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals were scavenged by 50% and the absorbance was 0.5 for reducing power. EC_{50} value was obtained by interpolation from linear regression analysis. Values were the means of three replicates ± SD. Values with different letters in the same row were significantly different ($p < 0.05$).
N.d.: not determined.

R_1-chloro substituted **3 e** (1.28 ± 0.03 mM) were slightly less active than ascorbic acid and α-tocopherol. Nevertheless, R_2-nonsubstituted **3 a–g** were slightly more active than R_2-methyl substituted **3 h–m**. R_2-methylation especially decreased the activities of R_1-trifluoromethoxy substituted **3i** and R_1-nitro substituted **3 m**.

Reducing power

The electron donation capacities (reflecting the electron transfer ability) of **3 a–m** were determined according to the ferric to ferrous reduction assay[34]. The EC_{50} values (the effective concentration at which the absorbance was 0.5) of **3 a** (1.15 ± 0.01 mM), **3 c** (1.37 ± 0.10 mM), **3 d** (0.99 ± 0.12 mM), **3 e** (1.00 ± 0.10 mM), **3f** (1.26 ± 0.07 mM) and **3 g** (1.13 ± 0.10 mM) were found to be significantly lower than the values observed for α-tocopherol (1.95 ± 0.04 mM) but higher than that of the ascorbic acid (0.65 ± 0.10 mM). As shown in Table 1, in R_1-fluoro substituted **3 d** and R_1-chloro substituted **3 e**, the activity was two times higher than α-tocopherol, whereas the substitution of the methyl groups at R_1 or/and R_2 caused significantly decrease in reducing power. This modification led to complete loss of the reducing powers of the R_2-methyl substituted **3 h**, **3i**, **3 k**, **3 l** and **3 m**.

Anticancer activity

3 a, **3 d**, **3 e**, **3 h**, **3j** and **3 k** chosen as prototypes were evaluated in the National Cancer Institute (NCI). A primary anticancer assay was performed according to the protocol of the Drug Evaluation Branch of the NCI, Bethesda[47–50]. The cytotoxic and/or growth inhibitory effects of the compounds were tested *in vitro* at a single dose (10^{-5} M) against the full panel of 60 human tumor cell lines derived from nine neoplastic diseases. The growth percentage was evaluated spectrophotometrically and compared to controls not treated with test agents. A 48-h continuous drug exposure protocol was followed and a sulphorhodamine B protein assay was used to estimate cell viability or growth. For each compound, the GI was obtained for all the cell lines. The cell lines used in the NCI screen were leukemia, non-small cell lung cancer (NSCL), colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer cell lines. Mean Graphs were constructed for each effect, with bars depicting the deviation of individual tumor cell lines from the overall mean value for all the cells tested. In the Mean Graph the center point is the mean of all GI percentages over all cell lines. Bars that point to the right are cell lines where the inhibition is greater than the average, while bars that point to the left are cell lines where the inhibition is less than the average.

The lowest growth percentages of R_1 and R_2-nonsubstituted **3 a** were 39.68% and 41.96% for leukemia cell lines K-562 and RPMI-8226, 44.51% for a prostate cancer cell line PC-3, and 42.36% for a breast cancer cell line T-47D (Figure 4). Introduction of a fluoro or a chloro group at the C-5 position of the indole ring resulted in compounds (**3 d** and **3 e**) with different anticancer activities. In R_1-fluoro substituted **3 d**, the greatest GI showed against a NSCL cancer cell line HOP-92. The growth percentage of **3 d** was −5.66% for a NSCL cancer cell line HOP-92. The substitution of the chlorine group at R_1 caused a significant decrease in the growth inhibitory effect against a NSCL cancer cell line HOP-92. Whereas R_1-chloro substituted **3 e** was highly selective in growth inhibiting against a renal cancer cell line RXF 393. For **3 e**, a growth percentage of −8.23% was obtained for the renal cancer cell line. For R_1-flouro substituted **3 d**, the other growth percentages were 48.60%, 32.94% and 39.95% for leukemia cell lines K-562, RPMI-8226 and a breast cancer cell line T-47D, respectively. The other growth percentages of R_1-chloro substituted **3 e** were 42.61%, 39.16%, 38.25% and 44.27% for leukemia cell lines HL-60(TB), K-562, MOLT-4 and RPMI-8226, 47.07% for a NSCL cancer cell line NCI-H522, 43.42% and 33.80% for breast cancer cell lines MCF7 and

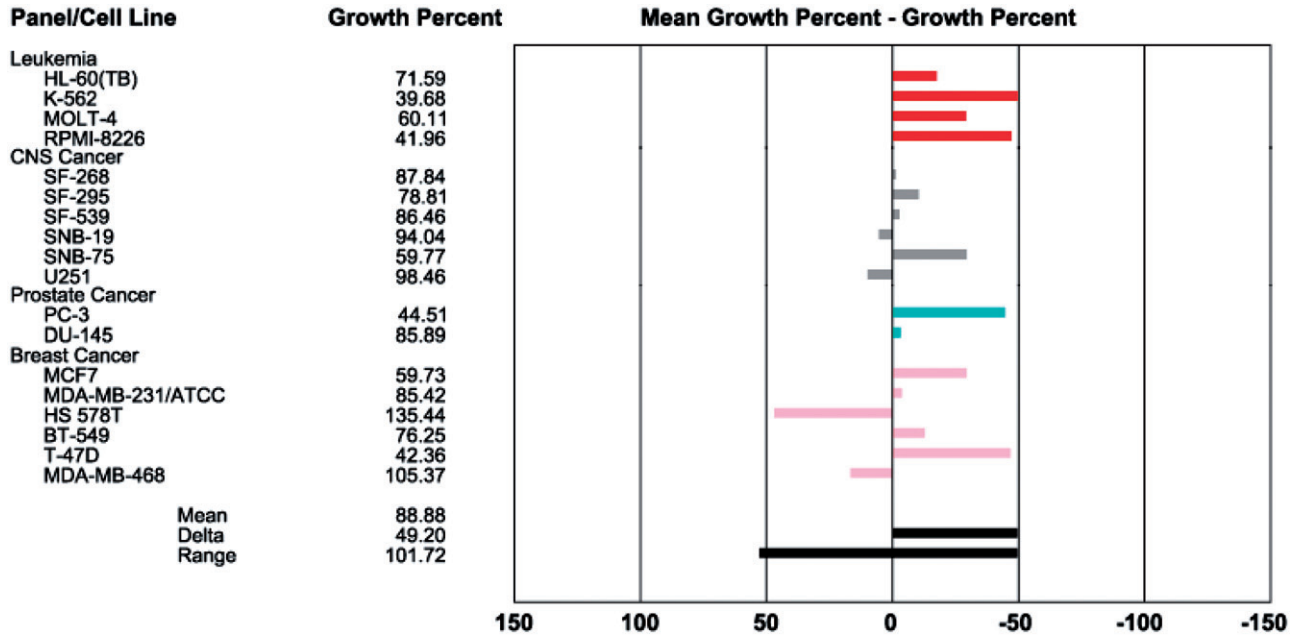


Figure 4. One dose mean graph of **3 a**.

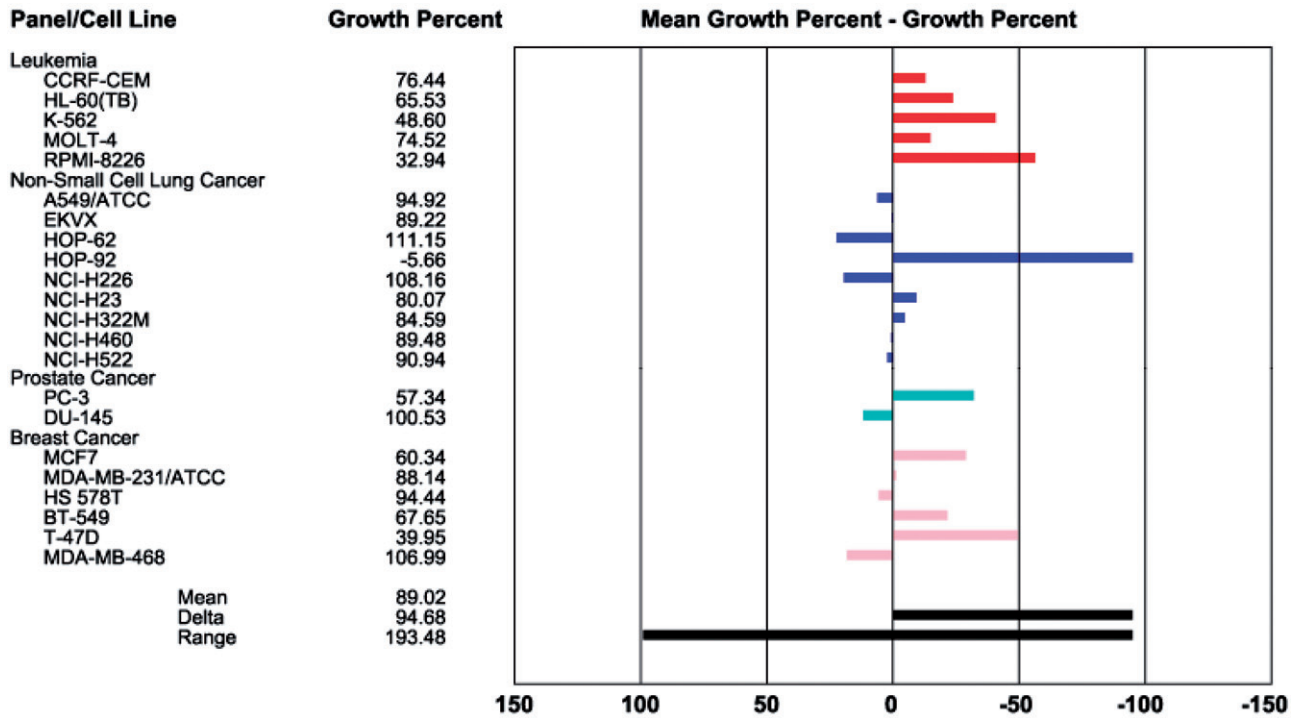


Figure 5. One dose mean graph of **3 d**.

T-47D, respectively. As shown, **3 d** and **3 e** had similar degrees of growth inhibitory effects against leukemia and breast cancer cell lines (Figures 5 and 6). When the results of R_2-methyl substituted **3 h**, **3j** and **3 k** were compared with R_2-nonsubstituted compounds **3 a**, **3 d** and **3 e**, it was observed that the growth inhibitory effects of the compounds significantly decreased by R_2-methylation (Figures 7–9).

Conclusion

New spiroindolinones by incorporating the 5-chlorobenzothiazoline moiety were synthesized, their structures were confirmed by analytical and spectral data. The new derivatives **3 b**–**m**, along with previously reported **3 a**, were evaluated for antioxidant activity. The results evidenced that most of the tested compounds had a high degree of potency in inhibiting LP. The compounds demonstrated strong scavenging activity against the $ABTS^{\bullet+}$ radical and reducing power, indicating possible value as antioxidants in medicine and industry. In the anti-LP assay, there were only minor variations in activity of R_2-nonsubstituted **3 a**–**g** and R_2-methyl substituted **3 h**–**m**, whereas R_2-methylation increased slightly the $DPPH^{\bullet}$ scavenging activity of **3 h**–**m** in comparison with **3 a**–**g**. **3 a**–**m** were found to be more effective in scavenging $ABTS^{\bullet+}$ radical cation when compared to the $DPPH^{\bullet}$ radical, since EC_{50} values were found to be lower in $ABTS^{\bullet+}$ scavenging method. In R-fluoro substituted **3 d** and R-chloro substituted **3 e**,

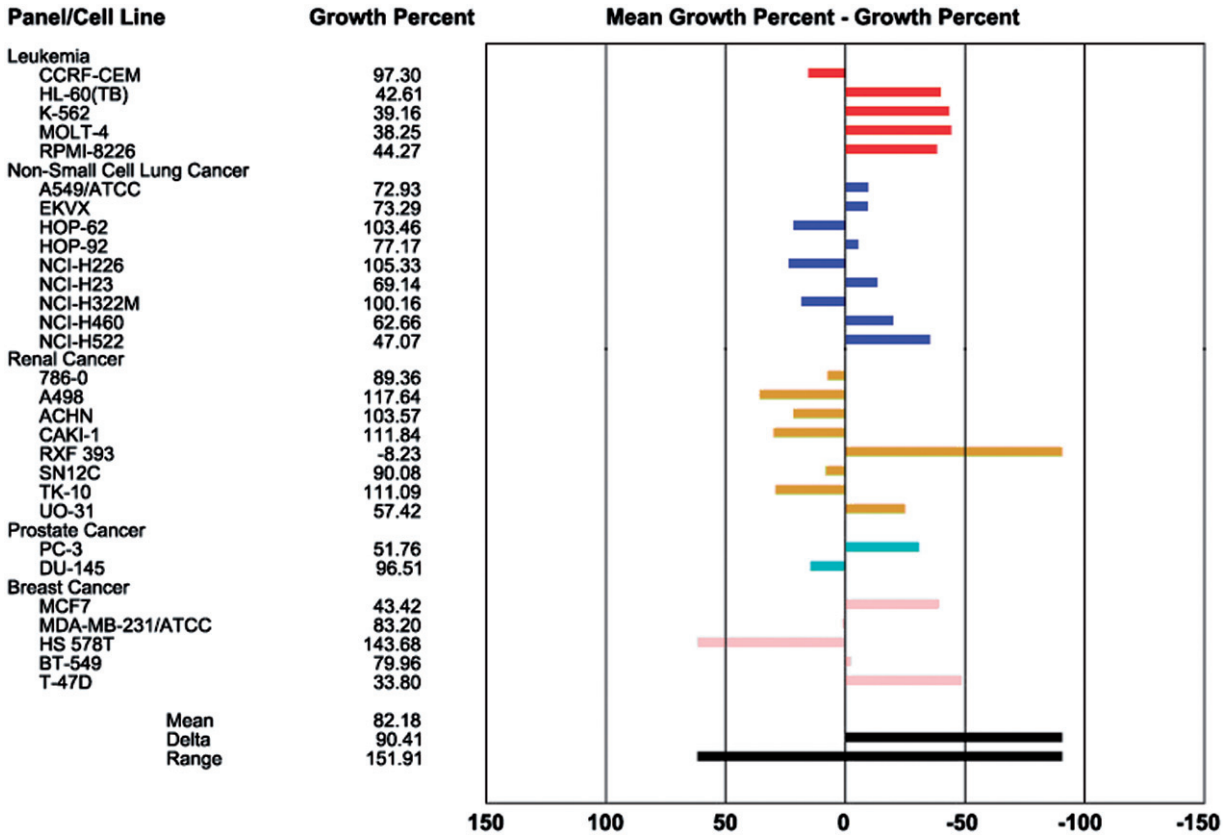


Figure 6. One dose mean graph of **3e**.

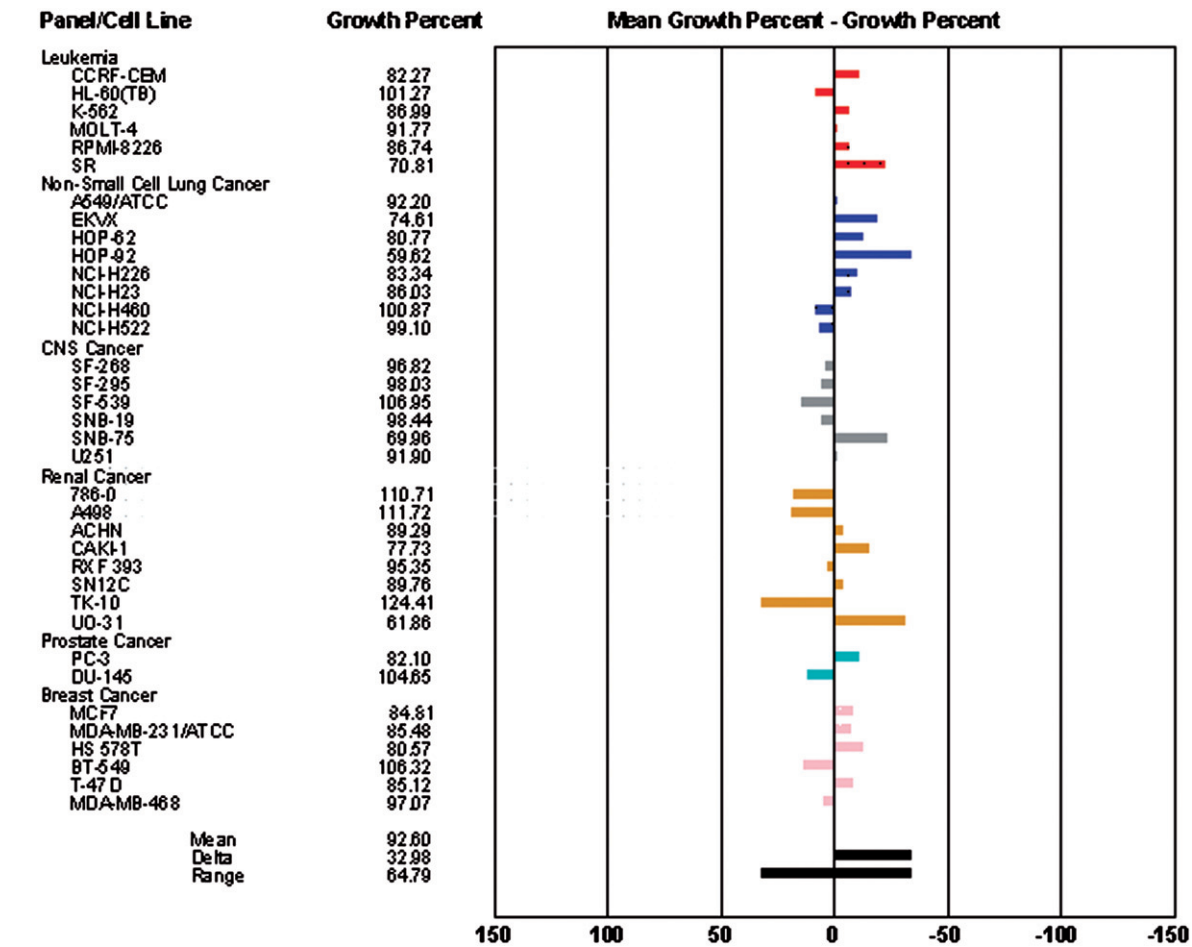


Figure 7. One dose mean graph of **3h**.

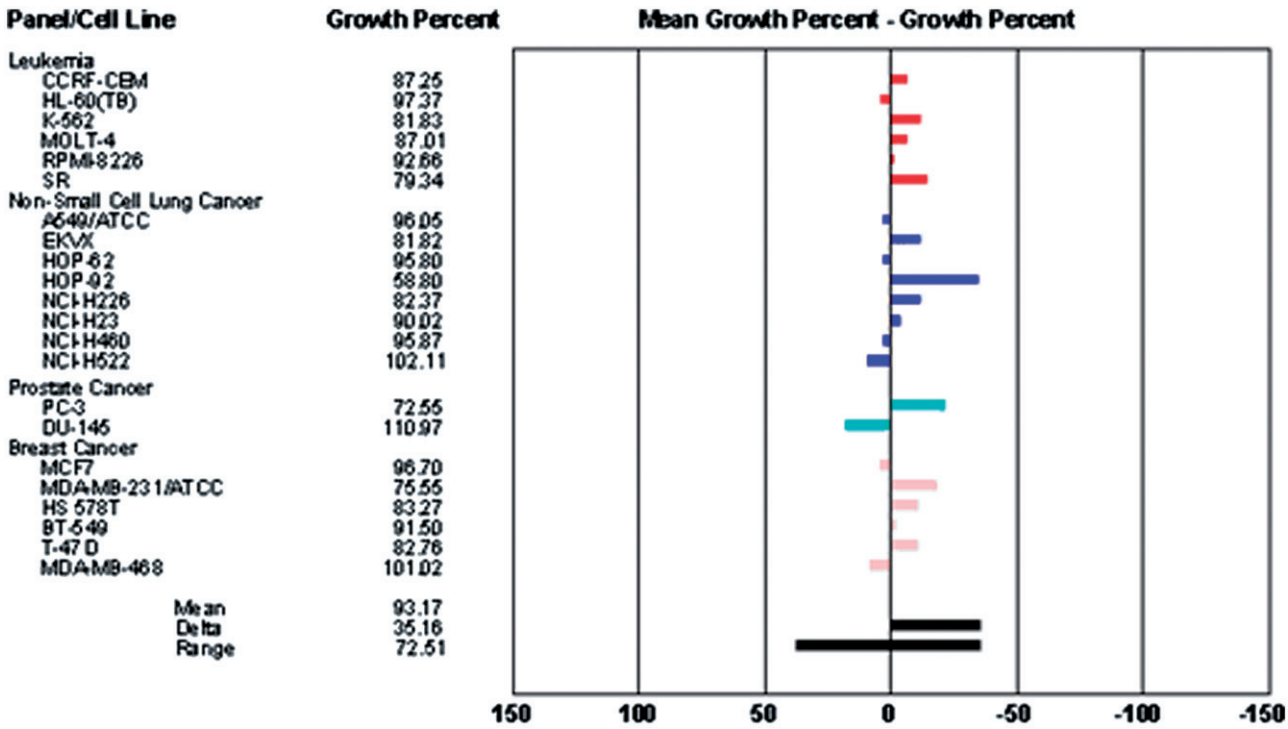


Figure 8. One dose mean graph of **3j**.

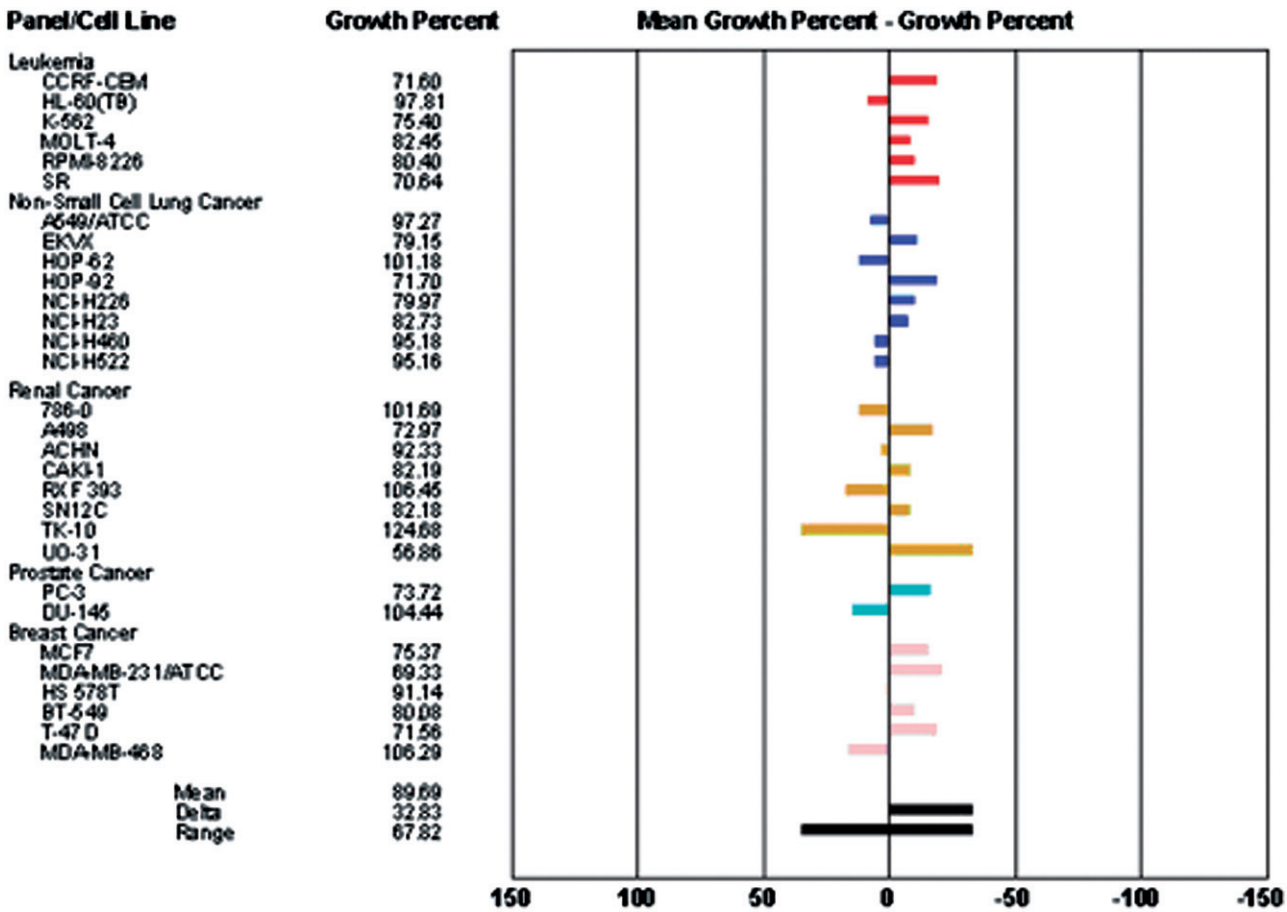


Figure 9. One dose mean graph of **3k**.

the reducing powers were two times higher than α-tocopherol, whereas the substitution of the methyl groups at R_1 or/and R_2 caused significant decrease in reducing power. **3a**, **3d**, **3e**, **3h**, **3j** and **3k** chosen as prototypes were evaluated in the National Cancer Institute *in vitro* primary anticancer assay. The anticancer activity increased by substitution of a fluoro or a chloro group at R_1. Moreover, the substitution of the methyl group at R_2 caused significant decrease in GI. The greatest GIs showed against a NSCL cell line HOP-92 for R_1-fluoro substituted **3d** and a renal cancer cell line RXF-393 for R-chloro substituted **3e** in the primary screen. As shown, there is a correlation between the growth inhibitory effects and the antioxidant activities of spiroindolinones by incorporating the 5-chlorobenzothiazoline moiety. The presence of a halogen group at C_5 of the indole ring and the absence of the methylation at N_1 of the indole ring play a very important role in both the antioxidant and anticancer activities of the compounds. On the other hand, the introduction of a chlorine group at C_5 of the benzothiazoline ring significantly increased the growth inhibitory effect of **3e**, whereas this modification decreased especially the inhibition of LP and the $DPPH^{\bullet}$ scavenging activity compared to other entries without the substituent which had been previously reported[34]. These preliminary results show that the spiroindolinones by incorporating the benzothiazoline moiety are promising and deserve a more detailed investigation.

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Declaration of interest

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References

1. Valko M, Rhodes CJ, Moncol J, et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem-Biol Interact 2006;160:1–40.
2. Dröge W. Free Radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95.
3. Ratnam DV, Ankola DD, Bhardwaj V, et al. Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. J Control Release 2006;113:189–207.
4. Suzen S. Antioxidant activities of synthetic indole derivatives and possible activity mechanisms. In: Khan MTH, ed. Topics in heterocyclic chemistry, bioactive heterocycles V. Berlin Heidelberg: Spinger-Verlag; 2007:145–78.
5. Peyrot F, Ducrocq C. Potential role of tryptophan derivatives in stress responses characterized by the generation of reactive oxygen and nitrogen species. J Pineal Res 2008;45:235–46.
6. Karbownik M, Reiter RJ, Garcia JJ, et al. Indole-3-propionic acid, a melatonin-related molecule, protects hepatic microsomal membranes from iron-induced oxidative damage: relevance to cancer reduction. J Cell Biochem 2001;81:507–13.
7. Aboul-Enein HY, Kladna A, Kruk I, et al. Scavenging of reactive oxygen species by novel indolin-2-one and indoline-2-thione derivatives. Biopolymers 2005;78:171–8.
8. Ölgen S, Kılıç Z, Ada AO, Çoban T. Synthesis and evaluation of novel N-H and N-substituted indole-2- and 3-carboxamide derivatives as antioxidants agents. J Enzym Inhib Med Chem 2007;22:457–62.
9. Ölgen S, Varol P, Çoban T, Nebioğlu D. Synthesis and evaluation of N-substituted indole-3-carboxamide derivatives as inhibitors of lipid peroxidation and superoxide anion formation. J Enzym Inhib Med 2008;23:334–40.
10. Ateş-Alagöz Z, Kuş C, Çoban T. Synthesis and antioxidant properties of novel benzimidazoles containing substituted indole or 1,1,4,4-tetramethyl-1,2,3,4-tetrahydro-naphthalene fragments. J Enzym Inhib Med 2005;20:325–31.
11. Jahangir M, Kim HK, Choi YH, Verpoorte R. Health-affecting compounds in Brassicacaae. Comp Rev Food Sci Food Safety 2009;8:31–43.
12. Mezencev R, Galizzi M, Kutschy P, Docampo R. Trypanosoma cruzi: antiproliferative effect of indole phytoalexins on intracellular amastigotes *in vitro*. Exp Parasitol 2009;122:66–9.
13. Mezencev R, Mojžiš J, Pilátová M, Kutschy P. Antiproliferative and cancer chemopreventive activity of phytoalexins: focus on indole phytoalexins from crucifers. Neoplasma 2003;50:239–45.
14. Pilátová M, Šarišský M, Kutschy P, et al. Cruciferous phytoalexins: antiproliferative effects in T-Jurkat leukemic cells. Leukemia Res 2005;29:415–21.
15. Lane ME, Yu B, Rice A, et al. A Novel cdk2-selective inhibitor, SU9516, induces apoptosis in colon carcinoma cells. Cancer Res 2001;61:6170–7.
16. O'Donnell A, Padhani A, Hayes C, et al. A Phase I study of the angiogenesis inhibitor SU5416 (semaxanib) in solid tumours, incorporating dynamic contrast MR pharmacodynamic end points. Br J Cancer 2005;93:876–83.
17. Chow LQM, Eckhardt SG. Sunitinib: from rational design to clinical efficacy. J Clin Oncol 2007;25:884–96.
18. Mortimer CG, Wells G, Crochard JP, et al. Antitumor benzothiazoles. 26.[1] 2-(3,4-Dimethoxyphenyl)-5-fluorobenzothiazole (GW 610, NSC 721648), a simple fluorinated 2-arylbenzothiazole, shows potent and selective inhibitory activity against lung, colon and breast cancer cell lines. J Med Chem 2006;49:179–85.
19. Kok SH, Chui HC, Lam WS, et al. Synthesis and structure evaluation of a novel cantharimide and its cytotoxicity on SK-Hep-1 hepatoma cells. Bioorg Med Chem Lett 2007;17:1155–9.
20. Ćaleta I, Kralj M, Marjanović M, et al. Novel cyano- and amidinobenzothiazole derivatives: synthesis, antitumor evaluation, and X-ray and quantitative structure – activity relationship (QSAR) analysis. J Med Chem 2009;52:1744–56.
21. Kamal A, Reddy KS, Khan MNA, et al. Synthesis DNA-binding ability and anticancer activity of benzothiazole/benzoxazole-pyrrolo[2,1-c][1,4]benzodiazepine conjugates. Bioorg Med Chem 2010;18:4747–61.
22. Caputo R, Calabro ML, Micale N, et al. Synthesis of benzothiazole derivatives and their biological evaluation as anticancer agents. Med Chem Res 2012;21:2644–51.
23. Das J, Moquin RV, Lin J, et al. Discovery of 2-amino-heteroarylbenzothiazole-6-anilides as Potent $p56^{lck}$ inhibitors. Bioorg Med Chem Lett 2003;13:2587–90.
24. Song EY, Kaur N, Park M, et al. Synthesis of amide and urea derivatives of benzothiazole as Raf-1 inhibitor. Eur J Med Chem 2008;43:1519–24.
25. Yadav PS, Devprakash, Senthilkumar GP. Benzothiazole: different methods of synthesis and diverse biological activities. Int J Pharm Sci Drug Res 2011;3:1–7.
26. Noolvi MN, Patel HM, Kaur M. Benzothiazoles: search for anticancer agents. Eur J Med Chem 2012;54:447–62.
27. De Brabander MJ, Lesage ASJ, Leysen JEMF. Use of fused benzothiazoles as neuroprotectants. From PCT Int Appl WO9625931 A2 19960829; 1996.
28. Singh J, Gurney ME, Burgin A, et al. Substituted benzoazole PDE4 inhibitors for treating pulmonary and cardiovascular disorders. US Patent Appl WO275164 A1 20090130076; 2009.
29. Davies PR, Herbert HF. Benzothiazoline antioxidants. United Kingdom Patent GB2150136; 1985.
30. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). LWT-Food Sci Technol 1999;32:269–77.
31. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302–10.
32. Brand-Williams W, Cuvelier ME. Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995;28:25–30.
33. Re R, Pellegrini N, Proteggente A, et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 1999;26:1231–7.
34. Chung YC, Chen SJ, Hsu CK, et al. Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther. Food Chem 2005;91:419–24.

35. Joshi KC, Dandia A, Khanna S. Studies in spiroheterocycles: Part XXIII-investigation on the reactions of indole-2,3-diones with 2-aminothiophenol and 2-aminophenol. Indian J Chem Soc 1990; 29B:824–9.
36. Dandia A, Khanna S, Joshi KC. Reactions of fluorinated isatin derivatives with 2-aminothiophenol. J Indian Chem Soc 1990;67: 824–6.
37. Silva JFM, Garden SJ, Pinto AC. The chemistry of isatins: a review from 1975 to 1999. J Braz Chem Soc 2001;12:273–324.
38. Allam YA, Nawwar GAM. Facile synthesis of 3-spiroindolines. Heteroatom Chem 2002;13:207–10.
39. Karalı N, Güzel Ö, Özsoy N, et al. Synthesis of new spiroindolinones incorporating a benzothiazole moiety as antioxidant agents. Eur J Med Chem 2010;45:1068–77.
40. Akkurt M, Karaca S, Ermut G, et al. 5-Chlorobenzothiazole-2-spiro-3′-indolin-2′-one. Acta Cryst 2010;E66:o399–400.
41. Naumov P, Anastasova F. Experimental and theoretical vibrational study of isatin, its 5-(NO_2, F, Cl, Br, I, CH_3) analogues and the isatinato anion. Spectrochim Acta Part A 2001;A57:469–81.
42. Silverstein RM, Bassler GC, Morrill TC. Spectrometric identification of organic. 4th ed. New York: John Wiley & Sons Inc; 1981:197–9.
43. Laatsch H, Thomson RH, Cox PJ. Spectroscopic properties of violacein and related compounds: crystal structure of tetramethylviolacein. J Chem Soc Perkin Trans II 1984;II:1331–9.
44. Jayashankaran J, Manian RDRS, Raghunathan R. A facile synthesis of novel dispiroheterocycles through solvent-free microwave-assisted [3+2] cycloaddition of azomethine ylides. Tetrahedron Lett 2004;45:7303–5.
45. SDBS. Available from: http://sdbs.riodb.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi SDBS No: 51367.
46. SDBS. Available from: http://sdbs.riodb.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi SDBS No: 51561.
47. Alley MC, Scudiero DA, Monks A, et al. Feasibility of drug screening with panels of human tumor cell lines using a microculture. Cancer Res 1988;48:589–601.
48. Grever MR, Schepartz SA, Chabner BA. The National Cancer Institute: Cancer Drug Discovery and Development Program. Sem Oncol 1992;19:622–38.
49. Boyd MR, Paull KD. Some practical considerations and applications of the national cancer institute in vitro anticancer drug discovery screen. Drug Dev Res 1995;34:91–109.
50. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. Nat Rev Cancer 2006;6:813–23.