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Regio- and Stereoselective Synthesis of a New Series of Spirooxindole Pyrrolidine Grafted Thiochromene Scaffolds as Potential Anticancer Agents

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Abstract: A series of new spiro-heterocycles engrafted spirooxindole/pyrrolidine/thiochromene scaffolds was synthesized by the three-component 1,3-dipolar cycloaddition reactions in a fully controlled regio- and stereo-selective fashion. Condensation of several substituted isatin derivatives with L-proline generated the azomethine ylides which subsequently reacted with chalcones based thiochromene scaffold, and finally afforded the target spiro-compounds. This simple protocol furnished a structurally complex, biologically relevant spiro-heterocycles in good yields through a one-pot process. All synthesized chalcone-based thiochromene, along with the spirooxindole/pyrrolidine/thiochromene scaffolds, were tested for their anticancer activity against four cancer cell lines (PC3, HeLa, MCF-7, and MDA-MB231). Toxicity of these compounds was also evaluated against human fibroblast BJ cell line, and they appeared to be not cytotoxic. For the prostate cancer (PC3) cell line, the most active hybrid, among synthesized series, was compound (**7f**, IC₅₀ = 8.7 ± 0.7 μM). The most potent spirooxindole/pyrrolidine/thiochromene hybrid against cervical (HeLa) cancer cells was compound (**7k**, IC₅₀ = 8.4 ± 0.5 μM) having chlorine and *p*-trifluoromethyl substituents attached to phenyl rings. Finally, against the MCF-7 and MDA-MB231 breast cancer cell lines, compound (**7d**) was the most active member of this series (IC₅₀ = 7.36 ± 0.37, and 9.44 ± 0.32 μM, respectively).

Keywords: spirooxindole; pyrrolidine; thiochromene; 1,3-dipolar cycloaddition reaction; anticancer activity

1. Introduction

The three-component 1,3-dipolar cycloaddition reaction is a powerful methodology for robust synthesis of structurally complex and biologically active spiro-heterocycles [1]. This method has been widely used for the synthesis of many compounds, engrafted spiro-centers, with diverse biological activities, and suitability to the integral structure rigidity. Spirooxindole/pyrrolidine heterocycles are ubiquitous in many natural products, such as tryprostatins A and B [2,3], formosanine [4], coerulecine [5], elacomine [6], horsfiline [7], pteropodine, isopteropodine [8], alstonisine [9], rychno-phyilline [10], strychnofoline [11], and other many alkaloids (Figure 1). These spiro-heterocycles with oxindole and pyrrolidine scaffolds possesses wide spectrum pharmacological activities, such as

antimycobacterial [12,13], antimicrobial [14], local anesthetic [15], and anticancer [16–23] agents, and in acetylcholinesterase inhibition [24,25].

Figure 1. Bioactive compounds incorporating spirooxindole, and thiochromane skeletons.

Barakat et al. reported the synthesis and biological activities of several spirooxindoles they exhibited interesting pharmacological activities as MDM2–p53 protein–protein interaction inhibitors, phosphodiesterase 1, and as selective cyclooxygenase COX-1 with TNF- α and IL-6 inhibitors. These spirooxindoles were reported to have potential hypoglycemic activity with inhibitory activities against α -amylase and α -glucosidase, and other pharmacological targets [26–34].

Another interesting motif is thiochromane and its analogues. Thiochromanone, thiochromane, and thiochromene derivatives are known to exhibit anticancer [35], antiviral [36], non-steroidal estrogen downregulation [36], human steroid sulfatase inhibition [37–40], α -adrenergic antagonism [41], and has antiparasitic [42], and antifungal [43] properties.

There are many reports on different approaches in the literature on the construction of spirooxindole systems [44,45]. For example, Mannich reactions and related transformations were successfully employed in the construction of naturally occurring spirooxindole alkaloid, ($\pm$)-elacomine [46]. An oxidative rearrangement approach of tetrahydro- β -carboline and related core structures [47], an intra-molecular Heck reaction and similar transformations approach were reported by L. Overman and M. Rosen for the total synthesis

of spiro-trypro-statins B [48]. Transition metal-catalyzed synthesis of the spirooxindole scaffold [49] and 1,3-dipolar cycloaddition reactions are regarded as useful approaches for the construction of the spirooxindole scaffold [50]. However, successful approaches for the synthesis of thio-chromenyl/spirooxindole systems are still limited, although replacement of the oxygen atom by sulfur could be a rational strategy for improving pharmacological activity in drug discovery [51–53]. The design and synthesis of new compounds as potent and safe anti-cancer agents with low side effects is still a challenge.

Based on literature reports which highlight the biological importance of thiochromene sub-structure and spirooxindole/pyrrolidine scaffolds, our aim was to synthesize a new hybrids comprising three pharmacophores, spirooxindole/pyrrolidine/thiochromene employing three components 1,3-dipolar cycloaddition reaction. This protocol is based on multiple bond formations [54] and hence were able to generate compounds with structural complexity for possible use in agrochemical, drug discovery, and pharmaceutical industries.

2. Materials and Methods

2.1. General

“Thiochroman-3-one, acetophenone derivatives, substituted isatins and L-proline were purchased from Aldrich or TCI, and used as received. All solvents were used as received when experiments were conducted in air. Flash chromatography was performed on 100–200 mesh silica gel. The ^1H - and ^{13}C -NMR spectra of the synthesized compound were recorded on a JEOL 400-MHz spectrometer (JEOL, Ltd., Tokyo, Japan) at ambient temperature. The solvents used were $\text{DMSO}-d_6$, and CDCl_3 ; the chemical shifts (δ) were given in ppm. Single-crystal X-ray data of compound **7m** were collected on a Rigaku Oxford Diffraction Supernova diffractometer at 120 K. Melting points were determined using Mel-Temp apparatus and are uncorrected. Thin Layer Chromatography (TLC) was conducted on silica gel (Kiesel gel G, E.Merck) and spots were detected under UV light at 254 nm. FT-IR spectra were measured on a Perkin Elmer, Spectrum 100 FT-IR spectrometer (FT-IR, Perkin Elmer, USA). Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV”.

2.2. Synthesis of 4-Chloro-2H-thiochromene-3-carbaldehyde (2)

Phosphorus oxychloride (32 mL, 350 mmol) was added dropwise at a steady rate to a cold solution (0–5 °C) of *N,N*-dimethylformamide (11.5 mL, 150 mmol). After 30 min, thiochroman-3-one (8.2 g, 50 mmol) was added. The resulting mixture was allowed to heat to 100 °C for 1.5 h. The reaction mixture was poured slowly into a cold water (300 mL), and the precipitated product was filtered and dried in vacuum at 40 °C for 24 h to obtain a light yellow solid compound 4-chloro-2H-thiochromene-3-carbaldehyde (2) [49].

Yield (8.5 g, yield: 81%); m.p. 139–141 °C; ^1H -NMR (400 MHz, $\text{DMSO}-d_6$): δ (ppm) = 10.22 (d, J = 2.2 Hz, 1H, CHO), 7.96–7.88 (m, 1H, Ar-H), 7.51–7.40 (m, 2H, Ar-H), 7.37 (dd, J = 8.5, 6.3 Hz, 1H, Ar-H), 3.72 (d, J = 2.3 Hz, 2H, SCH_2); ^{13}C -NMR (101 MHz $\text{DMSO}-d_6$): δ (ppm) = 189.0- (CHO), 145.0, 137.0-, 132.0-, 131.0-, 128.6-, 127.7-, 127.0-, 126.5-, 22.8- (SCH_2); [Anal. Calcd. for $\text{C}_{10}\text{H}_7\text{ClOS}$: C, 57.01; H, 3.35; Found: C, 57.13; H, 3.24]; LC/MS (ESI, m/z): found 211.22 [$\text{M} + \text{H}^+$]. All the analytical data were in agreement with the reported literature [37].

2.3. Synthesis of Thiochromene Chalcones (4a-e)

General procedure (GP1): 4-Chloro-2H-thiochromene-3-carbaldehyde (2) (1.05 g, 5.0 mmol) and acetophenone derivatives (3a-e) (5.0 mmol) were dissolved fully (by heating if necessary) in 30 mL ethanol and then NaOH solution in water (30 mL, 20 mmol) was added. The reaction mixture was left for stirring overnight. Light yellow solid precipitates emerged which were filtered and washed with water and ethanol to afford pure chalcone (4a-e).

(E)-3-(4-Chloro-2H-thiochromen-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (4a)

Following GP1, 4-chloro-2*H*-thiochromene-3-carbaldehyde (**2**) (1.05 g, 5.0 mmol) reacted with 4-chloroacetophenone (**3b**) (0.77 g, 5.0 mmol) to produce the chalcone (**4b**) (yield 1.51 g, 87%); m.p. 118–119; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.24 (d, *J* = 15.4 Hz, 1H, CH = CH), 7.96–7.89 (m, 2H, Ar-H), 7.86–7.81 (m, 1H, Ar-H), 7.47 (d, *J* = 8.7 Hz, 2H, Ar-H), 7.36–7.31 (m, 1H, Ar-H), 7.25–7.19 (m, 2H, Ar-H), 7.10 (d, *J* = 15.4 Hz, 1H, CH = CH), 3.72 (s, 2H, SCH₂); ¹³C{¹H}-NMR (126 MHz, CDCl₃): δ (ppm) = 189.2 (CO), 141.0-, 139.5-, 138.0-, 136.4-, 135.1-, 132.7, 130.0-, 130.0-, 129.1-, 128.9-, 127.4, 126.3, 126.2-, 124.1, 26.9 (SCH₂); IR (KBr, cm^{−1}) ν_{max} = 3087, 2363, 2340, 1654, 1592, 1577, 1568, 1540, 1488, 1456, 1431, 1412, 1401, 1325, 1308, 1294, 1261, 1245, 1212, 1180, 1090, 1029, 1014, 964, 911, 845, 822, 759, 752, 720, 649, 608; [Anal. Calcd. for C₁₈H₁₂Cl₂OS: C, 62.26; H, 3.48; Found: C, 62.33; H, 3.39]; LC/MS (ESI, *m/z*): found 347.21 [*M* + H⁺].

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-fluorophenyl)prop-2-en-1-one (**4b**)

Following GP1, 4-chloro-2*H*-thiochromene-3-carbaldehyde (**2**) (1.05 g, 5.0 mmol) reacted with 4-fluoroacetophenone (**3c**) (0.69 g, 5.0 mmol) to produce the chalcone (**4c**) (yield 1.47 g, 89%); m.p. 104–105; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.24 (d, *J* = 15.8 Hz, 1H, CH = CH), 8.06–7.99 (m, 2H, Ar-H), 7.86–7.81 (m, 1H, Ar-H), 7.36–7.31 (m, 1H, Ar-H), 7.25–7.21 (m, 2H, Ar-H), 7.18 (t, *J* = 8.6 Hz, 2H, Ar-H), 7.13 (d, *J* = 15.8 Hz, 1H, CH = CH), 3.73 (s, 2H, SCH₂); ¹³C-NMR (126 MHz, CDCl₃): δ (ppm) = 188.9 (CO), 166.9 & 164.0 (d, *J*_{C1-F} = 238.96 Hz), 140.7, 137.7, 135.1, 134.5, 132.7, 131.3 & 131.2 (d, *J*_{C3-F} = 9.32 Hz), 123.0, 129.0, 127.4, 126.3, 124.2, 116.0 & 116.0 (d, *J*_{C2-F} = 21.67 Hz), 26.9 (SCH₂); IR (KBr, cm^{−1}) ν_{max} = 3059, 2360, 2336, 1652, 1596, 1572, 1564, 1537, 1502, 1432, 1415, 1404, 1223, 1312, 1296, 1262, 1223, 1213, 1152, 1029, 1009, 969, 947, 909, 861, 852, 827, 805, 762, 603; [Anal. Calcd. for C₁₈H₁₂ClFOS: C, 65.35; H, 3.66; Found: C, 65.47; H, 3.59]; LC/MS (ESI, *m/z*): found 331.14 [*M* + H⁺].

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**4c**)

Following GP1, 4-chloro-2*H*-thiochromene-3-carbaldehyde (**2**) (1.05 g, 5.0 mmol) reacted with 4-nitroacetophenone (**3f**) (0.83 g, 5.0 mmol) to produce the chalcone (**4f**) (yield 1.48 g, 83%); m.p. 180–181; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.35 (d, *J* = 8.7 Hz, 2H, Ar-H), 8.28 (d, *J* = 16.1 Hz, 1H, CH = CH), 8.15–8.08 (m, 2H, Ar-H), 7.88–7.81 (m, 1H, Ar-H), 7.37–7.33 (m, 1H, Ar-H), 7.28–7.24 (m, 3H, Ar-H), 7.10 (d, *J* = 15.5 Hz, 1H, CH = CH), 3.74 (s, 2H, SCH₂); ¹³C-NMR (101 MHz, CDCl₃): δ (ppm) = 189.1 (CO), 150.3, 143.0, 142.2, 139.0, 135.3, 132.6, 130.2, 129.6, 129.1, 127.4, 126.4, 126.0, 124.0, 123.8, 26.8 (SCH₂); IR (KBr, cm^{−1}) ν_{max} = 3107, 2360, 1664, 1602, 1563, 1539, 1519, 1433, 1407, 1344, 1323, 1308, 1264, 1243, 1209, 1032, 1011, 965, 912, 899, 835, 759, 704; [Anal. Calcd. for C₁₈H₁₂ClNO₃S: C, 60.42; H, 3.38; N, 3.91; Found: C, 60.31; H, 3.27; N, 3.84]; LC/MS (ESI, *m/z*): found 358.17 [*M* + H⁺].

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-bromophenyl)prop-2-en-1-one (**4d**)

Following GP1, 4-chloro-2*H*-thiochromene-3-carbaldehyde (**2**) (1.05 g, 5.0 mmol) reacted with 4-bromoacetophenone (**3d**) (1.0 g, 5.0 mmol) to produce the chalcone (**4d**) (yield 1.66 g, 85%); m.p. 141–142; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.24 (d, *J* = 15.5 Hz, 1H, CH = CH), 7.88–7.80 (m, 3H, Ar-H), 7.64 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.33 (d, *J* = 4.4 Hz, 1H, Ar-H), 7.24 (dd, *J* = 8.8, 4.3 Hz, 2H, Ar-H), 7.09 (d, *J* = 16.0 Hz, 1H, CH = CH), 3.72 (s, 2H, SCH₂); ¹³C-NMR (101 MHz, CDCl₃): δ (ppm) = 189.4 (CO), 141.0, 138.0, 136.9, 135.2, 132.7, 132.1, 130.2, 130.0, 129.0, 128.2, 127.4, 126.3, 126.2, 124.0, 26.9 (SCH₂); IR (KBr, cm^{−1}) ν_{max} = 3083, 2372, 1653, 1584, 1576, 1539, 1484, 1430, 1324, 1308, 1294, 1244, 1212, 1181, 1068, 1028, 1008, 964, 945, 911, 844, 819, 758, 752, 718, 608; [Anal. Calcd. for C₁₈H₁₂BrClOS: C, 55.19; H, 3.09; Found: C, 54.97; H, 3.02]; LC/MS (ESI, *m/z*): found 391.19 [*M* + H⁺].

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4e**)

Following GP1, 4-chloro-2*H*-thiochromene-3-carbaldehyde (**2**) (1.05 g, 5.0 mmol) reacted with 4-(trifluoromethyl)acetophenone (**3e**) (0.94 g, 5.0 mmol) to produce the chalcone (**4e**) (yield 1.52 g, 80%); m.p. 149–150; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.27 (d, *J* = 16.0 Hz, 1H, CH = CH), 8.07 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.86–7.81 (m, 1H, Ar-H), 7.77 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.37–7.32 (m, 1H, Ar-H), 7.28–7.21 (m, 2H, Ar-H), 7.11 (d, *J* = 16.0 Hz,

1H, CH = CH), 3.74 (s, 2H, SCH₂); ¹³C-NMR (101 MHz, CDCl₃): δ (ppm) = 189.6 (CO), 141.6, 141.0, 138.4, 135.2, 132.6, 130.1, 129.0, 129.0, 127.4, 126.3, 126.1, 125.9, 125.9, 124.0, 26.8 (SCH₂); IR (KBr, cm⁻¹) ν_{max} = 3055, 1656, 1590, 1579, 1569, 1543, 1433, 1409, 1319, 1306, 1266, 1247, 1210, 1188, 1175, 1123, 1111, 1065, 965, 943, 912, 829, 757, 718, 681; [Anal. Calcd. for C₁₉H₁₂ClF₃OS: C, 59.93; H, 3.18; Found: C, 60.14; H, 3.13]; LC/MS (ESI, *m/z*): found 381.13 [M + H⁺].

2.4. Synthesis of Spirooxindole/Pyrrolidine/Thiochromene (7a-m)

Substituted (*E*)-3-(4-chloro-2*H*-thiochromen-3-yl)-1-phenylprop-2-en-1-one (**4a-e**) (0.5 mmol), isatin derivatives (**6a-e**) (0.5 mmol), and L-proline **5** (57.5 mg, 0.5 mmol) were dissolved in methanol (20 mL) and the reaction mixture was refluxed for 3–6 h. Finally, the products were isolated by flash column chromatography using 100–200 mesh silica gel and MeOH/CH₂Cl₂ (2:98) as an eluent to afford pure 4-chloro-2*H*-thiochromen spirooxindoles (**7a-m**).

1'-(4-Chloro-2*H*-thiochromen-3-yl)-2'-(4-chlorobenzoyl)-1',2',5',6',7',7*a*'-hexahydro-spiro[indoline-3,3'-pyrrolizin]-2-one (**7a**)

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (**4a**) (174 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and isatin (**6a**) (74 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7a**) (149 mg, 86%); m.p. 75–76 °C; ¹H-NMR (500 MHz, CDCl₃): δ (ppm) = 8.22 (s, 1H, NH), 7.73 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.38 (dd, *J* = 8.6, 1.8 Hz, 2H, Ar-H), 7.26–7.23 (m, 2H, Ar-H), 7.19–7.14 (m, 3H, Ar-H), 7.12 (t, *J* = 7.4 Hz, 2H, Ar-H), 6.99 (t, *J* = 7.4 Hz, 1H, Ar-H), 6.62 (d, *J* = 8.0 Hz, 1H, Ar-H), 4.72 (d, *J* = 11.1 Hz, 1H, COCH), 4.64 (t, *J* = 10.0 Hz, 1H, NCHCH), 4.15 (td, *J* = 9.0, 8.1, 3.4 Hz, 1H, NCH), 3.61 (d, *J* = 14.7 Hz, 1H, SCH_{2(a)}), 3.53 (d, *J* = 14.7 Hz, 1H, SCH_{2(b)}), 2.81–2.72 (m, 1H, NCH_{2(a)}), 2.67–2.60 (m, 1H, NCH_{2(a)}), 2.14–2.08 (m, 1H, CH₂), 2.01–1.92 (m, 2H, CH₂), 1.92–1.85 (m, 1H, CH₂); ¹³C NMR (126 MHz, CDCl₃): δ (ppm) = 195.6 (CO), 180.6 (CO), 140.7, 139.7, 135.3, 133.7, 133.0, 130.2, 130.0, 129.4, 128.7, 128.7, 128.4, 127.8, 127.8, 127.1, 125.9, 124.7, 122.8, 110.4, 68.3, 59.6, 53.6, 50.7, 48.6, 30.2, 26.9, 26.7 (CH₂); IR (KBr, cm⁻¹) ν_{max} = 3201, 3060, 2964, 2873, 1721, 1681, 1618, 1588, 1569, 1486, 1471, 1435, 1400, 1387, 1328, 1291, 1254, 1232, 1207, 1178, 1134, 1112, 1091, 1012, 942, 901, 843, 794, 755, 724, 614, 532; [Anal. Calcd. for C₃₀H₂₄Cl₂N₂O₂S: C, 65.81; H, 4.42; N, 5.12; Found: C, 65.93; H, 4.52; N, 5.01]; LC/MS (ESI, *m/z*): found 547.16 [M + H⁺].

6-Chloro-1'-(4-chloro-2*H*-thiochromen-3-yl)-2'-(4-chlorobenzoyl)-1',2',5',6',7',7*a*'-hexahydro-spiro[indoline-3,3'-pyrrolizin]-2-one (**7b**)

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (**4a**) (174 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 6-chloroisatin (**6b**) (91 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7b**) (253 mg, 87%); m.p. 110–111 °C; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 9.53 (s, 1H, NH), 7.96 (d, *J* = 5.9 Hz, 1H, Ar-H), 7.69 (d, *J* = 7.3 Hz, 1H, Ar-H), 7.43 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.19 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.13 (t, *J* = 7.0 Hz, 3H, Ar-H), 6.92 (d, *J* = 8.1 Hz, 1H, Ar-H), 6.69 (s, 1H, Ar-H), 4.70 (d, *J* = 11.0 Hz, 1H, COCH), 4.61 (t, *J* = 10.6 Hz, 1H, NCHCH), 4.18–4.12 (m, 1H, NCH), 3.60 (d, *J* = 15.4 Hz, 1H, SCH_{2(a)}), 3.52 (d, *J* = 14.7 Hz, 1H, SCH_{2(b)}), 2.76–2.76–2.69 (m, 1H, NCH_{2(a)}), 2.66–2.57 (m, 1H, NCH_{2(b)}), 2.15–2.01 (m, 1H, CH₂), 1.95–1.84 (m, 3H, CH₂); ¹³C-NMR (101 MHz, CDCl₃): δ (ppm) = 195.4 (CO), 180.2 (CO), 174.3, 162.8, 142.7, 139.8, 135.6, 135.0, 133.6, 132.7, 130.2, 129.3, 128.8, 128.3, 127.6, 126.9, 125.8, 122.8, 122.3, 111.2, 72.9, 67.9, 58.9, 50.7, 48.5, 31.5, 29.7, 26.4 (SCH₂); IR (KBr, cm⁻¹) ν_{max} = 3064, 2972, 1730, 1614, 1588, 1486, 1458, 1437, 1401, 1234, 1091, 1074, 1012, 943, 845, 757, 724, 668, 608, 530; [Anal. Calcd. for C₃₀H₂₃Cl₃N₂O₂S: C, 61.92; H, 3.98; N, 4.81; Found: C, 61.84; H, 4.05; N, 4.93]; LC/MS (ESI, *m/z*): found 581.23 [M + H⁺].

1'-(4-Chloro-2*H*-thiochromen-3-yl)-2'-(4-chlorobenzoyl)-5-fluoro-1',2',5',6',7',7*a*'-hexahydro-spiro[indoline-3,3'-pyrrolizin]-2-one (**7c**)

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-chlorophenyl)prop-2-en-1-one (**4a**) (174 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 5-fluoroisatin (**6c**) (83 mg, 0.5 mmol) were

reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7c**) (229 mg, 81%); m.p. 150–1151 °C; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ (ppm) = 9.35 (s, 1H), 7.69 (d, J = 7.5 Hz, 1H), 7.44 (d, J = 8.6 Hz, 2H), 7.22 (d, J = 7.3 Hz, 1H), 7.17 (d, J = 8.3 Hz, 2H), 7.14–7.06 (m, 2H), 6.98 (dd, J = 8.0, 2.7 Hz, 1H), 6.81 (td, J = 8.6, 2.7 Hz, 1H), 6.59 (dd, J = 8.7, 4.3 Hz, 1H), 4.72 (d, J = 11.0 Hz, 1H, COCH), 4.60 (t, J = 10.3 Hz, 1H, NCHCH), 4.19–7.12 (m, 1H, NCH), 3.60 (d, J = 14.6 Hz, 1H, $\text{SCH}_{2(a)}$), 3.52 (d, J = 14.8 Hz, 1H, $\text{SCH}_{2(a)}$), 2.79–2.73 (m, 1H, $\text{SCH}_{2(a)}$), 2.67–2.60 (m, 1H, $\text{SCH}_{2(b)}$), 2.13–2.08 (m, 1H, CH_2), 1.99–1.83 (m, 3H, CH_2); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ (ppm) = 195.3 (CO), 159.8 (CO), 157.9, 140.0, 136.6, 135.2, 133.7, 133.0, 130.4, 129.5, 130.0, 128.5, 128.3, 127.9, 127.1, 126.0, 116.7, 116.5, 115.8, 115.6, 111.0, 110.9, 68.3, 53.6, 50.8, 48.5, 29.8, 27.0, 26.7 (SCH_2); IR (KBr, cm^{-1}) ν_{max} = 3213, 3061, 2962, 2860, 1724, 1707, 1683, 1652, 1588, 1569, 1486, 1461, 1436, 1400, 1303, 1283, 1249, 1189, 1113, 994, 900, 852, 820, 763, 752, 721, 687, 660, 628, 593, 535; [Anal. Calcd. for $\text{C}_{30}\text{H}_{23}\text{Cl}_2\text{FN}_2\text{O}_2\text{S}$: C, 63.72; H, 4.10; N, 4.95; Found: C, 63.57; H, 4.21; N, 4.84]; LC/MS (ESI, m/z): found 565.21 [$\text{M} + \text{H}^+$].

1'-(4-Chloro-2H-thiochromen-3-yl)-5-fluoro-2'-(4-fluorobenzoyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**7d**)

(*E*)-3-(4-Chloro-2H-thiochromen-3-yl)-1-(4-fluorophenyl)prop-2-en-1-one (**4b**) (164 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 5-fluoroisatin (**6c**) (83 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7d**) (209 mg, 76%); m.p. 200–201 °C; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) = 8.45 (s, 1H, NH), 7.73 (dd, J = 7.9, 1.5 Hz, 1H, Ar-H), 7.54 (dd, J = 8.9, 5.3 Hz, 2H, Ar-H), 7.27–7.23 (m, 1H, Ar-H), 7.19–7.15 (m, 1H, Ar-H), 7.13 (dd, J = 7.5, 1.6 Hz, 1H, Ar-H), 7.03 (dd, J = 8.1, 2.5 Hz, 1H, Ar-H), 6.92–6.87 (m, 2H, Ar-H), 6.87–6.83 (m, 1H, Ar-H), 6.59 (dd, J = 8.5, 4.2 Hz, 1H, Ar-H), 4.74 (d, J = 10.8 Hz, 1H, COCH), 4.60 (dd, J = 11.2, 9.7 Hz, 1H, NCHCH), 4.15–4.11 (m, 1H, NCH), 3.58 (d, J = 14.5 Hz, 1H, $\text{SCH}_{2(a)}$), 3.52 (d, J = 14.5 Hz, 1H, $\text{SCH}_{2(b)}$), 2.77–2.71 (m, 1H, $\text{NCH}_{2(a)}$), 2.68–2.61 (m, 1H, $\text{NCH}_{2(b)}$), 2.09–2.15 (m, 1H, CH_2), 2.03–1.85 (m, 3H, CH_2); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ (ppm) = 194.8 (CO), 181.00 (CO), 166.9 & 164.8 (d, $J_{\text{C1-F}}$ = 256.54 Hz, Ph ring), 159.9 & 157.9 (d, $J_{\text{C1-F}}$ = 242.8 Hz, indole ring), 136.6, 133.6, 133.3 & 133.3 (d, $J_{\text{C4-F}}$ = 3.96 Hz, Ph ring), 132.9, 130.8 & 130.7 (d, $J_{\text{C3-F}}$ = 9.45 Hz, Ph ring), 130.4, 128.4, 127.8, 127.1, 126.7 & 126.6 (d, $J_{\text{C6-F}}$ = 7.43 Hz, indole ring), 126.0, 116.6 & 116.4 (d, $J_{\text{C2-F}}$ = 23.56 Hz, indole ring), 115.8 & 115.6 (d, $J_{\text{C2-F}}$ = 21.92 Hz, Ph ring), 115.6, 111 & 110.8 (d, $J_{\text{C5-F}}$ = 7.81 Hz, indole ring), 68.3, 59.5, 50.8, 48.4, 30.2, 27.1, 26.7 (SCH_2); IR (KBr, cm^{-1}) ν_{max} = 3179, 3074, 2968, 2877, 2862, 1718, 1688, 1634, 1596, 1505, 1487, 1472, 1459, 1298, 1260, 1230, 1207, 1189, 1156, 1137, 1106, 1078, 952, 857, 822, 813, 759, 722, 686; [Anal. Calcd. for $\text{C}_{30}\text{H}_{23}\text{ClF}_2\text{N}_2\text{O}_2\text{S}$: C, 65.63; H, 4.22; N, 5.10; Found: C, 65.72; H, 4.37; N, 4.97]; LC/MS (ESI, m/z): found 549.17 [$\text{M} + \text{H}^+$].

6-Chloro-1'-(4-chloro-2H-thiochromen-3-yl)-2'-(4-fluorobenzoyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**7e**)

(*E*)-3-(4-Chloro-2H-thiochromen-3-yl)-1-(4-fluorophenyl)prop-2-en-1-one (**4b**) (164 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 6-chloroisatin (**6b**) (91 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7e**) (232 mg, 82%); m.p. 114–115 °C; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) = 8.35 (s, 1H, NH), 7.72 (dd, J = 7.9, 1.6 Hz, 1H, Ar-H), 7.53 (ddd, J = 8.9, 5.3, 1.4 Hz, 2H, Ar-H), 7.25 (q, J = 1.3 Hz, 1H, Ar-H), 7.20–7.11 (m, 3H, Ar-H), 7.00–6.97 (m, 1H, Ar-H), 6.92 (td, J = 8.6, 1.4 Hz, 2H, Ar-H), 6.67 (d, J = 1.8 Hz, 1H, Ar-H), 4.73 (d, J = 11.0 Hz, 1H, COCH), 4.62 (t, J = 10.5 Hz, 1H, NCHCH), 4.22–4.10 (m, 1H, NCH), 3.60 (d, J = 16.0 Hz, 1H, $\text{SCH}_{2(a)}$), 3.53 (d, J = 14.0 Hz, 1H, $\text{SCH}_{2(b)}$), 2.81–2.72 (m, 1H, $\text{NCH}_{2(a)}$), 2.68–2.60 (m, 1H, $\text{NCH}_{2(b)}$), 2.14–2.10 (m, 1H, CH_2), 2.00–1.85 (m, 3H, CH_2); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ (ppm) = 194.9 (CO), 180.4 (CO), 167.0 & 165.0 (d, $J_{\text{C1-F}}$ = 256.54 Hz), 141.9, 135.8, 133.7, 133.2, 132.9, 130.8 & 130.7 (d, $J_{\text{C3-F}}$ = 9.45 Hz), 130.4, 128.9, 128.5, 128.3, 127.8, 127.1, 125.9, 122.8, 115.9 & 115.8 (d, $J_{\text{C2-F}}$ = 21.80 Hz), 111.0, 73.0, 68.2, 59.1, 50.9, 48.6, 30.0, 26.8, 26.6 (SCH_2); IR (KBr, cm^{-1}) ν_{max} = 3231, 3066, 2964, 2868, 1727, 1681, 1613, 1597, 1506, 1485, 1459, 1411, 1324, 1235, 1207, 1157, 1133, 1074, 944, 921, 848, 813, 757, 625, 600; [Anal. Calcd. for $\text{C}_{30}\text{H}_{23}\text{Cl}_2\text{FN}_2\text{O}_2\text{S}$: C,

63.72; H, 4.10; N, 4.95; Found: C, 63.64; H, 4.28; N, 5.09]; LC/MS (ESI, m/z): found 565.19 $[M + H^+]$.

1'-(4-Chloro-2*H*-thiochromen-3-yl)-5-fluoro-2'-(4-nitrobenzoyl)-1',2',5',6',7',7*a*'-hexahydro-spiro[indoline-3,3'-pyrrolizin]-2-one (**7f**)

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**4c**) (179 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 5-fluoroisatin (**6c**) (83 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded yellow colored solid thio-chromen spirooxindole (**7f**) (213 mg, 74%); m.p. 165–166 °C; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) = 8.34 (s, 1H, NH), 8.15–8.04 (m, 2H, Ar-H), 7.74 (dd, J = 8.2, 1.9 Hz, 1H, Ar-H), 7.64 (d, J = 8.5 Hz, 2H, Ar-H), 7.30–7.26 (m, 1H, Ar-H), 7.22–7.10 (m, 2H, Ar-H), 6.99 (dd, J = 8.0, 2.7 Hz, 1H, Ar-H), 6.88 (td, J = 8.8, 2.3 Hz, 1H, Ar-H), 6.61 (dd, J = 8.6, 4.1 Hz, 1H, Ar-H), 4.79 (d, J = 11.0 Hz, 1H, COCH), 4.62 (t, J = 10.3 Hz, 1H, NCHCH), 4.30–4.16 (s, 1H, NCH), 3.64 (d, J = 14.7 Hz, 1H, $\text{SCH}_{2(a)}$), 3.59 (d, J = 14.6 Hz, 1H, $\text{SCH}_{2(b)}$), 2.93–2.78 (m, 1H, $\text{NCH}_{2(a)}$), 2.77–2.63 (m, 1H, $\text{NCH}_{2(b)}$), 2.18–2.14 (m, 1H, CH_2), 2.04–1.93 (m, 3H, CH_2); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ (ppm) = 195.6 (CO), 180.6 (CO), 159.9 & 157.9 (d, $J_{\text{C1-F}}$ = 243.56 Hz), 150.3, 141.4, 136.6, 133.6, 132.9, 130.6, 129.0, 128.6, 128.0, 127.9, 127.1, 126.3 & 126.3 (d, $J_{\text{C3-F}}$ = 6.93 Hz), 126.0, 123.7, 117.0 & 116.7 (d, $J_{\text{C2-F}}$ = 25.07 Hz), 115.7 & 115.5 (d, $J_{\text{C6-F}}$ = 23.56 Hz), 111.1 & 111.1 (d, $J_{\text{C5-F}}$ = 8.06 Hz), 68.2, 60.6, 50.7, 48.3, 30.2, 27.1, 26.7 (SCH_2); IR (KBr, cm^{-1}) ν_{max} = 3392, 3086, 2970, 2893, 2868, 2845, 1740, 1713, 1690, 1326, 1603, 1521, 1485, 1460, 1435, 1347, 1320, 1258, 1180, 1116, 1024, 1011, 988, 945, 900, 868, 856, 760, 720, 709, 683, 630, 594, 557; [Anal. Calcd. for $\text{C}_{30}\text{H}_{23}\text{ClFN}_3\text{O}_4\text{S}$: C, 62.55; H, 4.02; N, 7.29; Found: C, 62.75; H, 4.17; N, 7.13]; LC/MS (ESI, m/z): found 576.13 $[M + H^+]$.

6-Chloro-1'-(4-chloro-2*H*-thiochromen-3-yl)-2'-(4-nitrobenzoyl)-1',2',5',6',7',7*a*'-hexahydro-spiro[indoline-3,3'-pyrrolizin]-2-one (**7g**)

(*E*)-3-(4-Chloro-2*H*-thiochromen-3-yl)-1-(4-nitrophenyl)prop-2-en-1-one (**4c**) (179 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 6-chloroisatin (**6d**) (91 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded yellow colored solid thio-chromen spirooxindole (**7g**) (234 mg, 79%); m.p. 143–144 °C; $^1\text{H-NMR}$ (500 MHz, CDCl_3): δ (ppm) = 8.34 (s, 1H, NH), 8.08 (d, J = 8.8 Hz, 2H, Ar-H), 7.72 (d, J = 7.9 Hz, 1H, Ar-H), 7.60 (d, J = 7.6 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.19–7.10 (m, 3H, Ar-H), 7.01 (dd, J = 8.1, 1.9 Hz, 1H, Ar-H), 6.66 (d, J = 1.8 Hz, 1H, Ar-H), 4.77 (d, J = 11.1 Hz, 1H, COCH), 4.58 (t, J = 10.5 Hz, 1H, NCHCH), 4.15–4.07 (m, 1H, NCH), 3.59 (d, J = 14.9 Hz, 1H, $\text{SCH}_{2(a)}$), 3.53 (d, J = 14.7 Hz, 1H, $\text{SCH}_{2(b)}$), 2.73–2.65 (m, 1H, $\text{NCH}_{2(a)}$), 2.65–2.57 (m, 1H, $\text{NCH}_{2(b)}$), 2.14–2.07 (m, 1H, CH_2), 1.98–1.85 (m, 3H, CH_2); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ (ppm) = 195.7 (CO), 180.4 (CO), 150.4, 141.9, 141.4, 136.1, 133.7, 132.8, 130.6, 129.0, 128.8, 128.6, 127.9, 127.1, 126.0, 123.8, 123.0, 111.2, 72.9, 68.1, 60.4, 50.9, 48.5, 30.2, 27.0, 26.7 (SCH_2); IR (KBr, cm^{-1}) ν_{max} = 3112, 3083, 2967, 2902, 2865, 1745, 1718, 1695, 1612, 1568, 1518, 1484, 1459, 1405, 1343, 1319, 1277, 1244, 1204, 1132, 1077, 1040, 864, 816, 765, 706, 626, 599; [Anal. Calcd. for $\text{C}_{30}\text{H}_{23}\text{Cl}_2\text{N}_3\text{O}_4\text{S}$: C, 60.81; H, 3.91; N, 7.09; Found: C, 61.01; H, 4.12; N, 7.19]; LC/MS (ESI, m/z): found 592.15 $[M + H^+]$.

2'-(4-Bromobenzoyl)-1'-(4-chloro-2*H*-thiochromen-3-yl)-5-fluoro-1',2',5',6',7',7*a*'-hexahydro-spiro[indoline-3,3'-pyrrolizin]-2-one (**7h**)

(*E*)-1-(4-Bromophenyl)-3-(4-chloro-2*H*-thiochromen-3-yl)prop-2-en-1-one (**4d**) (196 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 5-fluoroisatin (**6c**) (83 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7h**) (241 mg, 79%); m.p. 132–133 °C; $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ (ppm) = 8.45 (s, 1H, NH), 7.72 (dd, J = 7.3, 2.0 Hz, 1H, Ar-H), 7.39 (s, 4H, Ar-H), 7.29–7.25 (m, 1H, Ar-H), 7.15 (dtd, J = 16.5, 7.5, 1.5 Hz, 2H, Ar-H), 6.98 (dd, J = 8.1, 2.7 Hz, 1H, Ar-H), 6.86 (td, J = 8.7, 2.7 Hz, 1H, Ar-H), 6.67 (dd, J = 8.8, 4.4 Hz, 1H, Ar-H), 4.72 (d, J = 10.8 Hz, 1H, COCH), 4.69 (t, J = 11.2 Hz, 1H, NCHCH), 4.43–4.22 (m, 1H, NCH), 3.64 (d, J = 14.8 Hz, 1H, $\text{SCH}_{2(a)}$), 3.58 (d, J = 14.8 Hz, 1H, $\text{SCH}_{2(b)}$), 3.02–2.88 (m, 1H, $\text{NCH}_{2(a)}$), 2.88–2.73 (m, 1H, $\text{NCH}_{2(b)}$), 2.24–2.16 (m, 1H, CH_2), 2.17–1.95 (m, 3H, CH_2); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3): δ (ppm) = 195.5 (CO), 180.8 (CO), 159.8 & 157.9 (d, $J_{\text{C1-F}}$ = 242.8 Hz), 136.7, 135.6, 133.6, 132.9, 131.9, 130.4, 129.5,

128.7, 128.4, 128.3, 127.8, 127.1, 126.5 & 126.5 (d, $J_{C3-F} = 7.31$ Hz), 126.0, 116.6 & 116.4 (d, $J_{C2-F} = 23.56$ Hz), 115.8 & 115.6 (d, $J_{C6-F} = 24.95$ Hz), 111.0 & 110.89 (d, $J_{C5-F} = 7.68$ Hz), 73.7, 68.3, 59.6, 50.8, 48.4, 30.2, 27.1, 26.6 (SCH₂); IR (KBr, cm⁻¹) $\nu_{max} = 3355, 3075, 2973, 2899, 2871, 2842, 1711, 1683, 1629, 1584, 1568, 1486, 1460, 1398, 1318, 1277, 1259, 1182, 1143, 1114, 1070, 1028, 1007, 988, 948, 899, 852, 814, 760, 721, 685, 631, 594$; [Anal. Calcd. for C₃₀H₂₃BrClFN₂O₂S: C, 59.08; H, 3.80; N, 4.59; Found: C, 58.97; H, 3.92; N, 4.43]; LC/MS (ESI, m/z): found 609.13 [M + H⁺].

2'-(4-Bromobenzoyl)-6-chloro-1'-(4-chloro-2H-thiochromen-3-yl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**7i**)

(E)-1-(4-Bromophenyl)-3-(4-chloro-2H-thiochromen-3-yl)prop-2-en-1-one (**4d**) (196 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 6-chloroisatin (**6b**) (91 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded white solid thio-chromen spirooxindole (**7i**) (266 mg, 85%); m.p. 105–106 °C; ¹H-NMR (500 MHz, CDCl₃): δ (ppm) = 8.36 (s, 1H, NH), 7.73 (dd, $J = 7.9, 1.6$ Hz, 1H, Ar-H), 7.43–7.34 (m, 4H, Ar-H), 7.27–7.25 (m, 1H, Ar-H), 7.19 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.17 (dd, $J = 7.7, 1.6$ Hz, 1H, Ar-H), 7.13 (td, $J = 7.4, 1.6$ Hz, 1H, Ar-H), 7.00 (dd, $J = 8.1, 1.9$ Hz, 1H, Ar-H), 6.69 (d, $J = 1.9$ Hz, 1H, Ar-H), 4.73 (d, $J = 11.1$ Hz, 1H, COCH), 4.61 (t, $J = 9.5$ Hz, 1H, NCHCH), 4.17–4.09 (m, 1H, NCH), 3.59 (d, $J = 14.7$ Hz, 1H, SCH_{2(a)}), 3.52 (d, $J = 14.6$ Hz, 1H, SCH_{2(b)}), 2.77–2.69 (m, 1H, NCH_{2(a)}), 2.65–2.60 (m, 1H, NCH_{2(b)}), 2.16–2.08 (m, 1H, CH₂), 1.98–1.86 (m, 3H, CH₂); ¹³C-NMR (126 MHz, CDCl₃): δ (ppm) = 195.5 (CO), 180.6 (CO), 141.8, 135.7, 135.5, 133.6, 132.9, 131.9, 130.4, 129.5, 128.8, 128.4, 128.3, 127.8, 127.0, 126.0, 123.1, 122.8, 111.0, 73.0, 68.2, 59.3, 50.9, 48.5, 30.1, 27.0, 26.6 (SCH₂); IR (KBr, cm⁻¹) $\nu_{max} = 3225, 2958, 2869, 1726, 1680, 1613, 1584, 1484, 1459, 1436, 1398, 1323, 1233, 1206, 1133, 1072, 1008, 944, 842, 809, 756, 726, 625, 599$; [Anal. Calcd. for C₃₀H₂₃BrCl₂N₂O₂S: C, 57.52; H, 3.70; N, 4.47; Found: C, 57.63; H, 3.62; N, 4.39]; LC/MS (ESI, m/z): found 625 [M(³⁵Cl/⁷⁹Br) + H]⁺; 627.22 [M + H⁺].

2'-(4-Bromobenzoyl)-1'-(4-chloro-2H-thiochromen-3-yl)-5-nitro-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**7j**)

(E)-1-(4-Bromophenyl)-3-(4-chloro-2H-thiochromen-3-yl)prop-2-en-1-one (**4d**) (196 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 5-nitroisatin (**6e**) (96 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded light yellow solid thio-chromen spirooxindole (**7j**) (255 mg, 80%); m.p. 120–121 °C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ (ppm) = 11.10 (s, 1H, NH), 8.11 (dd, $J = 8.7, 2.3$ Hz, 1H, Ar-H), 7.90 (d, $J = 2.4$ Hz, 1H, Ar-H), 7.68–7.64 (m, 1H, Ar-H), 7.53 (d, $J = 8.6$ Hz, 2H, Ar-H), 7.38 (d, $J = 8.6$ Hz, 2H, Ar-H), 7.33 (dd, $J = 7.1, 2.0$ Hz, 1H, Ar-H), 7.27–7.19 (m, 2H, Ar-H), 6.79 (d, $J = 8.7$ Hz, 1H, Ar-H), 4.83 (d, $J = 10.8$ Hz, 1H, COCH), 4.49 (t, $J = 10.3$ Hz, 1H, NCHCH), 4.05–4.00 (m, 1H, NCH), 3.78 (d, $J = 15.1$ Hz, 1H, SCH_{2(a)}), 3.71 (d, $J = 15.1$ Hz, 1H, SCH_{2(b)}), 2.68–2.63 (m, 1H, NCH_{2(a)}), 2.48–2.43 (m, 1H, NCH_{2(b)}), 2.06–1.98 (m, 1H, CH₂), 1.94–1.89 (m, 1H, CH₂), 1.84–1.74 (m, 2H, CH₂); ¹³C-NMR (126 MHz, DMSO-*d*₆): δ = 196.2 (CO), 179.1 (CO), 148.5, 141.7, 135.4, 133.6, 132.2, 131.6, 130.0, 129.6, 128.6, 128.3, 127.73, 127.1, 127.0, 126.9, 126.0, 125.3, 122.3, 110.1, 71.8, 67.2, 59.3, 50.3, 47.7, 29.1, 26.3 (SCH₂), 25.8; IR (KBr, cm⁻¹) $\nu_{max} = 3075, 2978, 2878, 2837, 1718, 1678, 1626, 1596, 1584, 1525, 1480, 1461, 1398, 1338, 1311, 1242, 1127, 1100, 1071, 997, 742, 856, 833, 755, 558$; [Anal. Calcd. for C₃₀H₂₃BrClN₃O₄S: C, 56.57; H, 3.64; N, 6.60; Found: C, 56.71; H, 3.55; N, 6.52]; LC/MS (ESI, m/z): found 636.10 [M + H⁺].

5-Chloro-1'-(4-chloro-2H-thiochromen-3-yl)-2'-(4-(trifluoromethyl)benzoyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (**7k**)

(E)-3-(4-Chloro-2H-thiochromen-3-yl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (**4e**) (191 mg, 0.5 mmol), L-proline (**5**) (57.5 mg, 0.5 mmol) and 5-chloroisatin (**6d**) (91 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded light yellow solid thiochromen spirooxindole (**7k**) (237 mg, 77%); m.p. 141–142 °C; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.24 (s, 1H, NH), 7.74 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.61 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.53 (d, $J = 8.2$ Hz, 2H, Ar-H), 7.28 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.20–7.08 (m, 4H, Ar-H), 6.63 (d, $J = 8.5$ Hz, 1H, Ar-H), 4.77 (d, $J = 10.8$ Hz, 1H, COCH), 4.68 (t, $J = 10.4$ Hz, 1H, NCHCH), 4.44–4.22 (m, 1H,

NCH), 3.66 (d, $J = 14.8$ Hz, 1H, SCH_{2(a)}), 3.61 (d, $J = 14.8$ Hz, 1H, SCH_{2(b)}), 3.06–2.88 (m, 1H, NCH_{2(a)}), 2.88–2.72 (m, 1H, NCH_{2(b)}), 2.25–2.16 (m, 1H, CH₂), 2.12–1.95 (m, 3H, CH₂); ¹³C-NMR (126 MHz, CDCl₃): δ (ppm) = 196.0 (CO), 180.4 (CO), 139.7, 139.2, 133.6, 132.9, 130.5, 130.0, 128.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.1, 126.6, 126.0, 125.6, 125.6, 124.6, 122.4, 111.3, 73.2, 68.2, 60.1, 50.9, 48.5, 30.0, 27.0, 26.7 (SCH₂); IR (KBr, cm^{−1}) $\nu_{\max}$ = 3078, 2973, 2877, 2833, 1740, 1685, 1617, 1585, 1510, 1474, 1235, 1172, 1132, 1066, 1014, 1003, 950, 891, 863, 819, 761, 682, 626, 553; [Anal. Calcd. for C₃₁H₂₃Cl₂F₃N₂O₂S: C, 60.49; H, 3.77; N, 4.55; Found: C, 60.67; H, 3.85; N, 4.66]; LC/MS (ESI, m/z): found 615.18 [M + H⁺].

1'-(4-Chloro-2H-thiochromen-3-yl)-5-nitro-2'-(4-(trifluoromethyl)benzoyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (7l)

(E)-3-(4-Chloro-2H-thiochromen-3-yl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (4e) (191 mg, 0.5 mmol), L-proline (5) (57.5 mg, 0.5 mmol) and 5-nitroisatin (6e) (96 mg, 0.5 mmol) were reacted according to GP2 for 3–6 h and yielded light yellow solid thiochromen spirooxindole (7l) (235 mg, 75%); m.p. 139–140 °C; ¹H-NMR (500 MHz, DMSO-*d*₆): δ (ppm) = 11.06 (s, 1H, NH), 8.10 (dd, $J = 8.7, 2.3$ Hz, 1H, Ar-H), 7.91 (d, $J = 2.3$ Hz, 1H, Ar-H), 7.71–7.65 (m, 3H, Ar-H), 7.61 (d, $J = 8.3$ Hz, 2H, Ar-H), 7.34 (dd, $J = 7.1, 2.0$ Hz, 1H, Ar-H), 7.24 (ddd, $J = 9.0, 7.3, 1.7$ Hz, 2H, Ar-H), 6.74 (d, $J = 8.7$ Hz, 1H, Ar-H), 4.91 (d, $J = 10.8$ Hz, 1H, COCH), 4.54–4.45 (m, 1H, NCHCH), 4.08–4.01 (m, 1H, NCH), 3.82 (d, $J = 15.1$ Hz, 1H, SCH_{2(a)}), 3.71 (d, $J = 15.1$ Hz, 1H, SCH_{2(b)}), 2.67–2.61 (m, 1H, NCH_{2(a)}), 2.49–2.44 (m, 1H, NCH_{2(b)}), 2.08–1.99 (m, 1H, CH₂), 1.95–1.88 (m, 1H, CH₂), 1.86–1.74 (m, 2H, CH₂); ¹³C-NMR (126 MHz, DMSO-*D*₆) δ 196.6 (CO), 179.0 (CO), 148.5, 141.8, 139.6, 133.6, 132.2, 130.0, 128.6, 128.5, 128.4, 127.1, 127.0, 126.0, 125.4, 125.2, 124.6, 122.4, 122.2, 110.2, 71.7, 67.3, 59.8, 50.2, 47.6, 29.2, 26.4 (SCH₂), 25.8; IR (KBr, cm^{−1}) $\nu_{\max}$ = 3075, 2972, 2875, 2837, 1737, 1719, 1688, 1625, 1598, 1526, 1480, 1461, 1409, 1339, 1325, 1171, 1130, 1102, 1066, 1014, 943, 903, 861, 835, 755, 721, 628, 556; [Anal. Calcd. for C₃₁H₂₃ClF₃N₃O₄S: C, 59.47; H, 3.70; N, 6.71; Found: C, 59.38; H, 3.82; N, 8.63]; LC/MS (ESI, m/z): found 626.12 [M + H⁺].

1'-(4-Chloro-2H-thiochromen-3-yl)-2'-(4-fluorobenzoyl)-1',2',5',6',7',7a'-hexahydrospiro[indoline-3,3'-pyrrolizin]-2-one (7m)

(E)-3-(4-Chloro-2H-thiochromen-3-yl)-1-(4-fluorophenyl)prop-2-en-1-one (4b) (164 mg, 0.5 mmol), L-proline (5) (86 mg, 0.75 mmol) and isatin (6a) (74 mg, 0.5 mmol) were reacted according to GP2 for 2 h and yielded white solid thiochromen spirooxindole (7m) (226 mg, 85%); m.p.: 110–111 °C; ¹H-NMR (400 MHz, CDCl₃): δ (ppm) = 8.32 (s, 1H, NH), 7.74 (dd, $J = 7.8, 1.7$ Hz, 1H, Ar-H), 7.50 (dd, $J = 8.7, 5.3$ Hz, 2H, Ar-H), 7.30–7.21 (m, 2H, Ar-H), 7.15 (ddd, $J = 19.9, 9.2, 7.3$ Hz, 3H, Ar-H), 6.99 (t, $J = 7.7$ Hz, 1H, Ar-H), 6.89 (t, $J = 8.7$ Hz, 2H, Ar-H), 6.66 (d, $J = 7.9$ Hz, 1H, Ar-H), 4.73 (d, $J = 10.8$ Hz, 1H, COCH), 4.69 (t, $J = 10.8$ Hz, 1H, NCHCH), 4.30–4.20 (m, 1H, NCH), 3.64 (d, $J = 14.8$ Hz, 1H, SCH_{2(a)}), 3.56 (d, $J = 14.8$ Hz, 1H, SCH_{2(b)}), 2.93–2.80 (m, 1H, NCH_{2(a)}), 2.77–2.69 (m, 1H, NCH_{2(b)}), 2.17–2.12 (m, 1H, CH₂), 2.07–1.91 (m, 3H, CH₂); ¹³C-NMR (101 MHz, CDCl₃): δ (ppm) = 195.11 (CO), 181.00 (CO), 166.73 & 164.70 (d, $J_{C1-F} = 255.78$ Hz), 140.65, 133.62, 133.44, 133.04, 130.69 & 130.62 (d, $J_{C3-F} = 9.20$ Hz), 130.17, 129.80, 128.83, 128.34, 127.84, 127.78, 127.05, 125.91, 124.86, 122.78, 115.65 & 115.48 (d, $J_{C2-F} = 21.80$ Hz), 110.30, 68.42, 59.61, 50.74, 48.52, 30.29, 27.02, 26.68 (SCH₂); IR (KBr, cm^{−1}) $\nu_{\max}$ = 3187, 3145, 3074, 2963, 2872, 1718, 1689, 1620, 1596, 1504, 1471, 1461, 1435, 1408, 1388, 1322, 1297, 1263, 1234, 1207, 1184, 1155, 1105, 951, 853, 797, 761, 751, 722, 604; [Anal. Calcd. for C₃₀H₂₄ClFN₂O₂S: C, 67.85; H, 4.56; N, 5.28; Found: C, 67.73; H, 4.45; N, 5.16]; LC/MS (ESI, m/z): found 531.24 [M + H]⁺.

2.5. Biological Activity Assays Protocols

Cytotoxicity against BJ human fibroblast normal cell line against PC3, HeLa, MCF-7 and MDA-MB231 cancer cell lines were evaluated by following the procedure as described in the literature [55–59] (Supplementary Materials).

3. Results and Discussion

3.1. Synthesis of (4a-e) and (7a-m)

Three steps synthesis of the target compounds are presented in Schemes 1 and 2. The first step was to synthesize 4-chloro-2H-thiochromene-3-carbaldehyde (**2**) which is required to prepare chalcone based thiochromene scaffolds (**4a-e**) (Scheme 1). The second step is a three-component reaction in which the 1,3-dipolar cycloaddition key-reaction between thiochromene based-chalcone and the azomethine ylides, generated the substituted isatins (**6a-e**) (Isatin (**6a**), 5-chloroisatin (**6b**), 6-chloroisatin (**6c**), 5-fluoroisatin (**6d**), and 5-nitroisatin (**6e**) and amino acid L-proline (**5**) (Scheme 2). Thus, the diversity points in our library are the substituents at the isatin ring, and the aromatic substituents in the thiochromene based-chalcone. All three component reactions were carried out by heating an equimolar mixture of the thiochromene based-chalcone, isatin derivative, and L-proline in MeOH under reflux conditions for 3–6 h. After completion of the reaction as checked by TLC, the solvent was evaporated and the cyclized spiro-compounds were purified by column chromatography to afford pure cycloadducts in a very good yield. The following aromatic substituents in the thiochromene based-chalcone in the *para*-position (Cl, F, NO₂, Br, and CF₃) were employed to explore the substrate scope, and to establish generality of this approach. The structures of the spirooxindoles (**7a-m**) were assigned based on spectrophotometric tools including ¹H- & ¹³C-NMR, MS and IR spectral analyses which have been found with a high constituency with the proposed chemical structures. Additionally, compound (**7m**) was obtained in crystalline form suited for single crystal X-ray diffraction analysis. The final cycloadducts were obtained in excellent regio-selectivity and diastereo-selectivity. The reaction mechanism assumed to proceed via two steps shown in Scheme 3. The first step is to generate the azomethine yields by the reaction of isatins with L-proline to afford the cyclic lactone, followed by decarboxylation. The cycloaddition step-2 occurs in a such way as to make the two carbonyls of the oxindole ring and the chalcone moiety trans to each other to minimize the steric repulsion in the final product. Besides, the stereochemistry of the stereo-genic centers is unambiguously confirmed by single crystal X-ray analysis for compound (**7m**) as a representative example. ¹H-NMR spectrum showed the assigned protons and matched with the proposed structure. A singlet at δ 8.32 was assigned to the NH proton. The signals occurred at δ 7.74–6.66 were assigned for the aromatic protons while the protons of the fused pyrrolidine ring were assigned at δ 4.73–1.91. The two protons of the methine group adjacent to the sulfur atom of the thiochromene ring were observed at δ 3.64 and 3.56. ¹³C-NMR spectrum showed the characteristic carbon signals of the proposed compound. IR spectrum showed the desired final compound's functionalities and the functional groups disappeared as active carbon in isatin derivatives, as well as the olefin functional group in the chalcones.

Scheme 1. Synthesis of chalcone grafted 2H-thiochromenes (**4a-e**).

Scheme 2. Synthesis of spirooxindoles (**7a-m**) based on the thiochromene scaffold.

Proposed approach of 1,3-dipole to dipolarophile, explaining the regio- and diastereoselective Synthesis.

Scheme 3. Proposed mechanism for the regio- and diastereo-selective synthesis of the spirooxindole (**7a-m**) based thiochromene scaffold.

3.2. X-ray Structure Description

The X-ray structure of (**7m**), crystallized in the monoclinic crystal system and of centrosymmetric $C2/c$ space group, with eight molecular units per unit cell was elucidated. The unit cell parameters are $a = 36.6524(7)$ Å, $b = 8.27830(10)$ Å, $c = 17.1963(3)$ Å, $102.216(2)^\circ$ and $V = 5099.55(15)$ Å³. The X-ray structure was in agreement with the spectral characterization and the formation of the spiro system of the compound (Figure 2). The two spiro-moieties (oxindole and pyrrolizine) are linked together via the C15 asymmetric center. The thiochromene moiety was also found to be connected to the pyrrolizine via the C10 chiral carbon. The molecular units of this compound form a dimer via noncovalent interactions (Figure 3). In this figure two molecules of (**7m**) are connected via N-H...O hydrogen bonding interactions. Crystal data and structure refinement for compound (**7m**) are listed in Table 1. Selected bond lengths [Å] and angles [°] for (**7m**) are summarized in Table 2.

Figure 2. Thermal ellipsoids are showing atom numbering for the X-ray structure of compound (**7m**).

Figure 3. Hydrogen bonding interactions in compound (**7m**).

Table 1. Crystal data and structure refinement for compound (7m). Hydrogen bond details: N(2)-H(2): 0.80(2) Å; H(2)...O(1)^{#1}: 2.08(2) Å; N(2)...O(1)^{#1}: 2.877(1) Å and N(2)-H(2)...O(1)^{#1}: 174(2)° and the symmetry code is ^{#1} −x + 1, −y + 1, −z + 1.

Empirical Formula	C ₃₀ H ₂₄ ClFN ₂ O ₂ S	
Formula weight	531.02	
Temperature	120(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	C2/c	
Unit cell dimensions	$a = 36.6524(7)$ Å $b = 8.27830(10)$ Å $c = 17.1963(3)$ Å	$\alpha = 90^\circ$ $\beta = 102.216(2)^\circ$ $\gamma = 90^\circ$
Volume	5099.55(15) Å ³	
Z	8	
Density (calculated)	1.383 Mg/m ³	
Absorption coefficient	0.271 mm ^{−1}	
F(000)	2208	
Crystal size	0.228 × 0.166 × 0.139 mm ³	
Theta range for data collection	2.274 to 32.514°	
Index ranges	$-54 \leq h \leq 44$, $-12 \leq k \leq 12$, $-24 \leq l \leq 25$	
Reflections collected	29,780	
Independent reflections	8550 [R(int) = 0.0261]	
Completeness to theta = 25.242°	100.00%	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.96869	
Refinement method	Full-matrix least-squares on F ²	
Data/restraints/parameters	8550/0/338	
Goodness-of-fit on F2	1.017	
Final R indices [I > 2sigma(I)]	R1 = 0.0445, wR2 = 0.1170	
R indices (all data)	R1 = 0.0543, wR2 = 0.1251	
Extinction coefficient	n/a	
Largest diff. peak and hole	1.063 and −0.491 e.Å ^{−3}	
CCDC	2092002	

Table 2. Selected bond lengths [Å] and angles [°] for compound (7m).

Atoms	Distance	Atoms	Distance
Cl(1)-C(7)	1.7480(13)	N(1)-C(15)	1.4555(17)
S(1)-C(1)	1.7554(14)	N(1)-C(14)	1.4765(17)
S(1)-C(9)	1.8027(14)	N(1)-C(11)	1.4919(16)
O(1)-C(22)	1.2272(16)	N(2)-C(22)	1.3534(17)
O(2)-C(24)	1.2169(16)	N(2)-C(21)	1.4052(17)
F(1)-C(28)	1.3556(18)		
Atoms	Angle	Atoms	Angle
C(1)-S(1)-C(9)	96.20(6)	C(5)-C(6)-C(1)	117.89(12)
C(15)-N(1)-C(14)	119.17(11)	C(5)-C(6)-C(7)	122.18(12)
C(15)-N(1)-C(11)	110.27(10)	C(1)-C(6)-C(7)	119.72(11)
C(14)-N(1)-C(11)	109.32(10)	C(8)-C(7)-C(6)	124.07(12)

3.3. Biological Activity

All chalcones-based-thiochromenes (4a-e), and the spirooxindole/pyrrolidine/thiochromenes (7a-m) were initially examined for their toxicity against the human fibroblast BJ normal cell line. The results depicted in Table 1 indicated that all the synthesized compounds were non-toxic, except compounds (7d) and (7k) which appeared to be slightly toxic at 30 µM concentration. The toxicities of these two compounds may be due to the

presence of F, CF₃ and Cl substituents at the phenyl ring-based chalcone, and the chlorine atom attached to C-5 incorporated isatin moiety.

The antiproliferative activity against four human cancer lines, including prostate PC-3, cervical HeLa, and breast (MCF-7 and MDA-MB231) were evaluated by MTT assay [55–59] and the results were compared with the standard anti-cancer drug doxorubicin as a reference.

MCF-7 is a human breast cancer cell line with glucocorticoid, progesterone and estrogen receptors it is widely used worldwide for in vitro anti-cancer assay. The cell line is known to retain mammary epithelial characteristics, particularly estrogen (the first hormone to respond to breast cancer) processing via estrogen receptors [60,61]. In contrast the MDA-MB-231 cell line is particularly used to model late stage cancer, and is considered to be a good triple negative model because of the lack of growth factor receptor HER2 and absence of ER, PR, and E-cadherin [62].

All chalcones-based thiochromenes (**4a–e**) were found to be inactive against PC-3, HeLa, MCF-7, and MDA-MB231 cell lines, except 4-fluoro and 4-nitro substituted phenyl moiety containing compounds (**4b**) (IC₅₀ = 27.7 ± 0.9 µM) and (**4c**) (IC₅₀ = 27.7 ± 0.9 µM), which appeared to be weakly active against PC-3 cervical cancer cell line.

The results of antiproliferative activity against the prostate cancer PC3 cell line by the spiro-oxindole/pyrrolidine/thiochromene series (**7a–m**) showed that the most active hybrid in these series was compound (**7f**) (IC₅₀ = 8.7 ± 0.7 µM), having *para*-NO₂ aromatic substituent, and the fluorine at C-5 of the isatin ring, whereas complete loss of activity was observed for compound (**7g**) having a chlorine atom at C-5 of the isatin ring. The replacement of *para*-NO₂ with *para*-bromo at the aromatic ring also contributed towards a decrease in activity, as observed for compounds (**7h**) (IC₅₀ = 16 ± 0.7 µM). Further decrease in activity was observed for compounds with 4-chloro (**7i**, IC₅₀ = 27.7 ± 0.9 µM) and 5-nitro (**7j**, IC₅₀ = 22.5 ± 0.4 µM) groups, attached to the isatin ring. Present of a chlorine atom at C-6 of the isatin ring, in combination with the *para*-fluoro-substituted benzene ring, decreased the activity of compounds (**7e**, IC₅₀ = 27.5 ± 0.5 µM), and (**7i**, IC₅₀ = 27.7 ± 0.9 µM) against PC-3 cells. On the other hand, compound (**7k**) with a chlorine atom at C-5 at the isatin ring and *p*-trifluoromethyl benzene appeared to be the second most active member of the series (IC₅₀ = 15.6 ± 0.3 µM), whereas replacement of the C-5 chlorine isatin ring with C-5 nitro isatin moiety resulted in a complete loss of activity, as observed in compound **7l**. Compounds (**7a–d**, **7g**, **7l**, and **7m**) were not active.

Next, the anticancer assay against cervical cancer HeLa cell line exhibited moderate to weak anticancer activity in comparison to the standard drug doxorubicin (IC₅₀ = 0.9 ± 0.14 µM). The most potent spirooxindole/pyrrolidine/thiochromene hybrid (**7k**) having C-5 chlorine incorporated isatin moiety with *p*-trifluoromethyl phenyl ring gave the best results with IC₅₀ = 8.4 ± 0.5 µM, followed by C-4 chlorine incorporated isatin and *p*-nitro phenyl moieties containing (**7g**, IC₅₀ = 10.4 ± 0.7 µM). The replacement of C-4 chlorine containing isatin moiety with C-5 fluoro isatin led to a decrease in anti-cancer potential of (**7f**, IC₅₀ = 22.6 ± 0.1 µM) against HeLa cell line, found to be further decreased in compound (**7h**, IC₅₀ = 25.3 ± 0.25 µM), containing *p*-bromo-phenyl ring instead of *p*-nitro phenyl moiety, whereas a sharp increase in activity was observed for adduct (**7j**, IC₅₀ = 12.0 ± 0.1 µM) having C-5 nitro isatin instead of C-5 fluoro isatin (**7h**, IC₅₀ = 25.3 ± 0.25 µM). Complete loss of activity was observed for compound (**7e**) having a *p*-fluorophenyl ring instead of *p*-nitrophenyl (**7g**, IC₅₀ = 10.4 ± 0.7 µM), (**7i**) having a C-5 chloro isatin ring instead of C-6 nitro isatin (**7j**, IC₅₀ = 12.0 ± 0.1 µM), and (**7l**) having a C-6 nitro isatin ring instead of C-6 chloro isatin moiety (**7k**, IC₅₀ = 8.4 ± 0.5 µM). Comparison of compound (**7a**) with (**7m**), which have IC₅₀ = 24.8 ± 0.3 µM and 18.5 ± 0.9 µM, respectively, indicated slight improvement in the reactivity due to the replacement of the chlorine atom with fluorine.

Many of the tested chalcones-based-thiochromenes (**4a–e**), and the spirooxindole/pyrrolidine/thiochromenes (**7a–m**) appeared to be inactive against MCF-7 and MDA-MB231 breast cancer cell lines, except (**7d–f**, **7h**, and **7k**). C-5 fluoro incorporated isatin moiety with *p*-fluoro phenyl ring containing (**7d**) appeared as the most potent against MCF-7 (IC₅₀ = 7.36 ± 0.37 µM) and MDA-MB231 (IC₅₀ = 9.44 ± 0.32 µM) breast cancer cell lines.

A slight decrease in activity against both cell lines was observed in compound (**7h**, MCF-7, $IC_{50} = 8.34 \pm 0.64 \mu M$, and MDA-MB231, $IC_{50} = 11.25 \pm 0.28 \mu M$) having a *p*-fluoro phenyl ring instead of a *p*-bromo phenyl ring. The observed anti-cancer potential in-case of compound (**7h**) was further decreased in compound (**7e**, MCF-7, $IC_{50} = 16.4 \pm 0.61 \mu M$ and MDA-MB231, $IC_{50} = 21.29 \pm 1.35 \mu M$) having C-4 chloro incorporated isatin moiety with *p*-fluoro phenyl ring and C-5 chloro isatin with *p*-trifluoromethyl phenyl moieties containing (**7k**, MCF-7, $IC_{50} = 26.04 \pm 1.07 \mu M$, and MDA-MB231, $IC_{50} = 25.28 \pm 0.77 \mu M$). On the other hand, substitution of *p*-nitro group on the phenyl ring together with C-5 fluoro incorporated isatin moiety contributed towards a drastic increase in anti-cancer potential against MDA-MB231 cell line ($IC_{50} = 9.29 \pm 0.34 \mu M$) and slight increase in anticancer activity against MCF-7 cell line ($IC_{50} = 23.27 \pm 0.80 \mu M$), as observed in (**7f**), whereas, in comparison to (**7f**), the replacement of C-5 fluoro incorporated isatin moiety with 4-chloro isatin ring contributed towards a considerable increase in activity of compound (**7g**) against MCF-7 ($IC_{50} = 14.3 \pm 0.16 \mu M$), and a complete loss of activity against MDA-MB231 breast cancer cell line. Compound (**7m**) of *p*-F-atom on the phenyl ring without any substitution on the isatin core structure showed a weak activity for both breast cancer cell lines (MCF-7; $IC_{50} = 24.16 \pm 0.25 \mu M$ and MDA-MB231; $IC_{50} = 21.09 \pm 0.1 \mu M$). All results are summarized in Table 3.

Table 3. Results of cytotoxicity assay against BJ (normal), and PC3, HeLa, MCF-7, and MDA-MB231 (cancer) cell lines for the synthesized chalcones (**4a–e**), and spirooxindoles (**7a–m**).

Compounds	Chemical Structure 4a–e/7a–m	Cancer Type/Cell Line (IC_{50} , μM)				
		Human Fibroblast BJ	Prostate PC3	Cervical HeLa	Breast MCF-7	Breast MDA-MB231
4a		NA	NA	NA	NA	NA
4b		NA	NA	19.3 ± 0.3	NA	NA
4c		NA	NA	19.7 ± 0.7	NA	NA
4d		NA	NA	NA	NA	NA
4e		NA	NA	NA	NA	NA

Table 3. Cont.

Compounds	Chemical Structure 4a-e/7a-m	Cancer Type/Cell Line (IC ₅₀ , μ M)				
		Human Fibroblast BJ	Prostate PC3	Cervical HeLa	Breast MCF-7	Breast MDA-MB231
7a		NA	NA	24.8 $\pm$ 0.3	NA	NA
7b		NA	NA	NA	NA	NA
7c		NA	NA	24.8 $\pm$ 0.2	NA	NA
7d		11.3 $\pm$ 0.17	NA	12.7 $\pm$ 0.2	7.36 $\pm$ 0.37	9.44 $\pm$ 0.32

Table 3. Cont.

Compounds	Chemical Structure 4a-e/7a-m	Cancer Type/Cell Line (IC ₅₀ , μ M)				
		Human Fibroblast BJ	Prostate PC3	Cervical HeLa	Breast MCF-7	Breast MDA-MB231
7e		NA	27.5 $\pm$ 0.5	NA	16.45 $\pm$ 0.61	21.29 $\pm$ 1.35
7f		NA	8.7 $\pm$ 0.7	22.6 $\pm$ 0.1	23.27 $\pm$ 0.80	9.29 $\pm$ 0.34
7g		NA	NA	10.4 $\pm$ 0.7	14.30 $\pm$ 0.16	NA
7h		NA	16.0 $\pm$ 0.7	25.3 $\pm$ 0.25	8.34 $\pm$ 0.64	11.25 $\pm$ 0.28

Table 3. Cont.

Compounds	Chemical Structure 4a-e/7a-m	Cancer Type/Cell Line (IC ₅₀ , μ M)				
		Human Fibroblast BJ	Prostate PC3	Cervical HeLa	Breast MCF-7	Breast MDA-MB231
7i		NA	27.7 $\pm$ 0.9	NA	NA	NA
7j		NA	22.5 $\pm$ 0.4	12.0 $\pm$ 0.1	NA	NA
7k		24.9 $\pm$ 0.4	15.6 $\pm$ 0.3	8.4 $\pm$ 0.5	26.04 $\pm$ 1.07	25.28 $\pm$ 0.77
7l		NA	NA	NA	NA	NA

Table 3. Cont.

Compounds	Chemical Structure 4a-e/7a-m	Cancer Type/Cell Line (IC ₅₀ , μ M)				
		Human Fibroblast BJ	Prostate PC3	Cervical HeLa	Breast MCF-7	Breast MDA-MB231
7m		NA	NA	18.5 $\pm$ 0.9	24.16 $\pm$ 0.25	21.09 $\pm$ 0.1
STD.	Doxorubicin	N/A	1.9 $\pm$ 0.4	0.9 $\pm$ 0.14	0.79 $\pm$ 0.05	0.32 $\pm$ 0.002

IC₅₀ (μ M) was evaluated using MTT assay and $\pm$ is the standard deviation from three independent experiments. NA: the tested compound did not show anticancer activity at 30 μ M.

4. Conclusions

During this study we have successfully achieved the synthesis of a new library of hybrid based spirooxindole/pyrrolidine/thiochromenes (**7a-m**) by three-component 1,3-dipolar cycloaddition reactions in complete regio- and stereo-selective fashion. The anti-cancer assay showed promising results as good candidates for further studies. Compounds (**7f**, IC₅₀ = 8.7 $\pm$ 0.7 μ M) exhibited more potent activity against PC3, whereas hybrid (**7k**) was most active against cervical cancer HeLa (IC₅₀ 8.4 $\pm$ 0.5 μ M) and for breast cancer MCF-7 cell lines (**7d**, IC₅₀ = 7.36 $\pm$ 0.37 μ M), whereas (**7d**, IC₅₀ = 9.44 $\pm$ 0.32 μ M) also appeared more active against MDA-MB231 breast cancer cell line. The mechanism of action and in vivo study will be considered in the near future to further validate results of in vitro assays.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/sym13081426/s1>, Figures S1–S18: ¹H-NMR and ¹³C-NMR for compounds **4a-e** and **7a-m** along with the X-ray structure determinations and biological activity assays protocols are provided in SI.

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