



Synthesis and Characterization of Spiro Compounds Containing Phenothiazine Moiety and their Anticancer Potential Towards Breast Cancer Cell Lines

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ABSTRACT

The 1,3-dipolar cycloaddition reaction is a three-component reaction used for the synthesis of structurally complex and biologically active spiro-heterocycles. In the present work a two steps synthesis of the target compounds (7-22) are presented. 2-acetyl phenothiazine was converted into a chalcone 1 which was treated with various azomethine ylides to yield the compounds 7-22. The diversity in the azomethine ylides was made by using a variety of diones 2a-2d and the different aminoacids 3-6. The anti-cancer activity of the compounds 7-22 was evaluated by testing against breast cancer MCF-7 cell lines by MTT assay. Compounds 7, 12, 16 and 20 exhibited potent anticancer activity against MCF-7 breast cancer cell lines with an IC₅₀ values of 83.08, 84.68, 95.68 and 114.23 µg/mL respectively and non toxic towards normal fibroblast L929 cells. In the Cell cycle analysis of compound 7 arrests the cell cycle in MCF-7 cells.

Keywords: Spiro compounds, Breast cancer, MCF-7, Cell cytometry.

INTRODUCTION

Heterocyclic compounds with nitrogen atom are fascinating synthetic targets because of their wide uses the medicinal chemistry area and so attempts are being made continuously to prepare them.¹ Among the various methods available to prepare them, the strategy involving cycloaddition of dipolarophiles with ylidic species is considered as an attractive one.^{2,3} Interests are shown for the preparation of pyrrolidine compounds since they exhibited antidiabetic activity, antiviral activity, antibacterial activity

and anticancer activity, in addition to the glucosidase inhibitory activity⁴. Heterocycles with phenothiazine moiety are ubiquitous and their derivatives are very often found as bioactive compounds and also behave as a vasodilator⁵. The oxindole frames possessing spiro centres are considered to be privileged scaffolds and often seems to present in plants as alkaloids and also acts as potent inhibitors of p53-MDM2 interaction^{6,7}. Therefore, synthesising compounds possessing spiro-oxindole and acetyl phenothiazine rings through a convenient strategy is a great challenge.



In the present work we aimed at identifying potential anticancer agents, and efforts were made in search of novel potent molecules using dipolar cycloaddition strategy. In the present work a series of spirooxindole/pyrrolidine scaffolds were synthesized in a fully controlled regio- and stereo-selective cycloaddition fashion⁸⁻¹¹. Condensation of isatin derivatives, acenaphthoquinone and ninhydrins with L-proline, sarcosine, phenyl alanine and phenyl glycine to generate a series of azomethine ylides which subsequently reacted with phenothiazine chalcones afforded the target spiro-compounds in a single-pot process in good yields and are also structurally complex, biologically relevant spiro-heterocycles¹²⁻¹⁵.

MATERIALS AND METHODS

Chemicals were obtained from Merck & Co. and SD fine chemicals (India). Open capillary tubes were used to determine the melting points and is therefore uncorrected. TLC plates coated with 2mm thickness silica gel G were used to run TLC with ethyl acetate: *n*-hexane (7:3) solvent system in order to check the purity of the compounds. Iodine vapours were used to detect the compounds on the TLC plates. FTIR spectrophotometer (Jasco 4100 model) was used to record IR spectra. PMR data was obtained using a (400MHz FTNMR)- Bruker model DRX instrument and elemental analysis by a Perkin-Elmer 240 C instrument.

General method of preparation for the compounds 7-22

About 0.01 M of the dione (**2a-2d**) like acenaphthoquinone **2a**, isatine **2b**, bromo isatine **2c** or ninhydrine **2d** and 0.01M amino acid 3-6 like sarcosine **3**, proline **4**, phenyl alanine **5** or phenyl glycine **6** were taken in a reaction flask and dissolved in 100 mL of methanol. 0.012M of Chalcone **1** in methanol was added followed by the addition of 50 mL of DMF (N-N Di methyl formamide) and refluxed for 72 hours. The yielded compounds **7-22** were purified with column chromatography. The column was packed with silica gel and a mixture of chloroform and methanol in different proportions in the order of increasing polarity was used as eluting solvent.

Compound 7

Yield: 86%, light Yellow in colour, m.p. 260°C, Molecular formulae C₃₅H₂₆N₂O₂S, Mol. Wt.: 538.66, m/z: 539.17 [M+H]⁺. Elemental analysis:

Found: C, 78.64; H, 4.89; N, 5.28; Calc: C, 78.04; H, 4.87; N, 5.20; IR (KBr, cm⁻¹) ν_{\max} : 3243, 3076, 2964, 2872, 1723, 1679, 1617, 1587, 1569, 1485, 1473, 1438, 1387, 1328, 1291, 1207, 1134, 1112, 1091, 843, 724, 614. ¹H-NMR (400MHz FTNMR; TMS) δ : 4.39 (1H, d, J = 7.6 Hz, H-3), 4.56 (1H, m, H-4), 3.55, 3.65 (2H, dd, J = 7.6 Hz, H-5), 2.01 (3H, s, N-CH₃), 9.10(1H, br.s., NH), 6.28 (1H, s, H-1'), 6.31 (2H, d, H-6', H-8'), 6.53 (1H, d, J = 7.56, H-4'), 6.70 (2H, m, H-5', H-7'), 7.36 (1H, d, J = 7.6Hz, H-3'), 6.77 (1H, m, H-4''), 6.96 (2H, m, H-2'', H-6''), 7.60 (2H, m, H-3'', H-5''), 7.70–7.91 (6H, Ar-H of acenaphthoquinone) ¹³C-NMR (125MHz FTNMR; TMS) δ : 34.6 (N-CH₃), 76.5 (C-2), 62.42 (C-3), 43.93 (C-4), 60.31 (C-5), 111.6 (C-1'), 114.4 (C-12'), 144.9 (C-10'), 120.5 (C-6'), 120.9 (C-8'), 122.0 (C-3'), 122.01 (C-4), 131.0 (C-5'), 135.0 (C-4'), 135.5 (C-2'), 140.9 (C-9'), 141.1 (C-11') 128.37 (C-2'', C-6''), 129.5 (C-3'', C-5''), 142.0 (C-1''), 126.0 (C-4''), 122.9, 124.9, 125.1, 128.0, 128.4, 129.9, 132.19, 134.1, 135.9, 146.0, (C-acenaphthoquinone), 192.7(C=O) and 196.3 (C=O).

Compound 8

Yield: 84%, light Yellow in colour, m.p. 292°C, Molecular formulae C₃₇H₂₈N₂O₂S, Mol. Wt.: 564.19, m/z: 564.66 [M+H]⁺; Elemental analysis: Found: C, 78.64; H, 4.89; N, 5.02; Calc: C, 78.70; H, 5.00; N, 4.96. IR (KBr, cm⁻¹) ν_{\max} : 3235, 3069, 2949, 2858, 1721, 1687, 1616, 1581, 1557, 1483, 1476, 1437, 1383, 1327, 1294, 1209, 1135, 1119, 1078, 839, 725, 615. ¹H-NMR (400MHz FTNMR; TMS) δ : 1.80 & 1.89 (2H each), 2.56 & 2.37 (each 1H), 3.65 & 3.98 (1H each), 4.79 (1H), 6.26-6.31 (3H), 6.53 (1H), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.70–7.91 (6H). ¹³C-NMR (125MHz FTNMR; TMS) δ : 72.53, 71.41, 62.59, 51.95, 29.19, 26.89, 47.39, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 122.9, 124.9, 125.1, 128.0, 128.4, 129.9, 132.19, 134.1, 135.9, 146.0, (C-acenaphthoquinone), 192.8 (C=O) and 196.6 (C=O)

Compound 9

Yield: 82%, light Yellow in colour, m.p. 292°C, Molecular formulae C₄₁H₃₀N₂O₂S, Mol. Wt.: 614.19, m/z: 615.20 [M+H]⁺; Elemental analysis: Found: C, 80.19; H, 4.94; N, 4.58; Calc: C, 80.10; H, 4.92; N, 4.56. IR (KBr, cm⁻¹) ν_{\max} : 3343, 3289, 3043, 2978, 2879, 1726, 1689, 1626, 1578, 1557, 1485, 1471, 1436, 1383, 1322, 1297, 1212, 1156, 1114, 1083

856, 743, 654. ¹H-NMR (400MHz FTNMR; TMS) δ: 2.70 and 2.91 (each for 1H), 3.766 (1H), 4.11 & 4.45 (1H each), 6.28-6.31 (3H), 6.53 (1H), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.70–7.97 (11H), 9.92(1H,N-H). ¹³C-NMR (125MHz FTNMR; TMS) δ: 34.6, 76.5, 62.42, 43.93, 60.31, 41.3 (C-6), 111.6 (C-1'), 114.4 (C-12'), 144.9 (C-10'), 120.5 (C-6'), 120.9 (C-8'), 122.0, 122.01, 131.0, 135.0, 135, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 122.9, 124.9, 125.1, 126.4 (2C), 128.0(2C), 128.4(2C), 129.9 (2C), 132.19, 132.21, 134.1, 135.9, 146.0, (C-acenaphthoquinone), 181.7(C=O) and 197.2 (C=O).

Compound 10

Yield: 92%, Yellow in colour, m.p. 292°C, Molecular formulae C₄₀H₂₈N₂O₂S, Mol. Wt.: 600.19, m/z: 601.20 [M+H]⁺. Elemental analysis: Found: C, 79.92; H, 4.77; N, 4.61; Calc: C, 79.97; H, 4.70; N, 4.66. IR (KBr, cm⁻¹) ν_{max}: 3262, 3089, 2987, 2891, 1718, 1689, 1614, 1588, 1567, 1484, 1472, 1431, 1382, 1324, 1295, 1201, 1133, 1115, 1099, 847, 726, 611. ¹H-NMR (400MHz FTNMR; TMS) δ: 3.76 (1H), 4.11 and 4.45 (1H each), 9.92(N-H), 6.28-6.31 (3H), 6.53 & 7.56 (1H each), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.70–7.97 (11H), ¹³C-NMR (125MHz FTNMR; TMS) δ: 34.6, 76.5, 62.42, 43.93, 60.31, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 122.9, 124.9, 125.1, 126.4, 128.0, 128.4, 129.9, 132.19, 132.21, 134.1, 135.9, 146.0, (C-acenaphthoquinone), 181.7(C=O) and 197.2 (C=O).

Compound 11

Yield: 72%, dark Yellow in colour, m.p. 286°C, Molecular formulae C₃₁H₂₅N₃O₂S, Mol. Wt.: 503.13, m/z: 504.14 [M+H]⁺. Elemental analysis: Found: C, 73.99; H, 5.03; N, 8.38; Calc: C, 73.93; H, 5.00; N, 8.34. IR (KBr, cm⁻¹) ν_{max}: 3256, 3048, 2972, 2864, 1717, 1679, 1637, 1597, 1558, 1484, 1472, 1437, 1383, 1327, 1279, 1201, 1126, 1106, 1076, 849, 732, 643. ¹H-NMR (400MHz FTNMR; TMS) δ: 2.01(3H), 3.55, 3.65 (1H each), 4.39 & 4.56 (1H each), 9.2(NH), 8.87(NH) 6.28 - 6.31 (3H), 6.53 (1H) 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.38-7.57 (4H), ¹³C-NMR (125MHz FTNMR; TMS) δ : 34.6, 76.5, 62.42, 43.93, 60.31, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 111.75, 114.8, 126.25, 127.94, 129.27, 154.1 (C-phenyl ring of isatin), 192.4(C=O) and 175.3 (C=O).

Compound 12

Yield: 87%, orange red in colour, m.p. 236°C, Molecular formulae C₃₃H₂₇N₃O₂S, Mol. Wt.: 529.18, m/z: 530.14 [M+H]⁺. Elemental analysis: Found: C, 74.88; H, 5.19; N, 7.94; Calc: C, 74.83; H, 5.14; N, 7.93. IR (KBr, cm⁻¹) ν_{max}: 3236, 3068, 2948, 2857, 1722, 1686, 1617, 1582, 1556, 1482, 1477, 1436, 1384, 1326, 1295, 1208, 1134, 1117, 1076, 837, 724, 614. ¹H-NMR (400MHz FTNMR; TMS) δ: 4.79 (1H, d, J = 7.6 Hz, H-3), 3.98 (1H, m, H-4), 3.95, 3.65 (1H, m, H-5), 2.56 & 2.37 (each 1H, m, H-8), 1.89 (2H, m, H-7) 1.80 (2H, m, H-6), 9.2(1H, br.s.,NH), 8.87(1H, br.s.,NH), 6.57 (1H, s, H-1'), 6.59 (2H, d, H-6', H-8'), 7.39 (1H, d, J = 7.56, H-4'), 7.32 (2H, m, H-5', H-7'), 6.85 (1H, d, J = 7.6Hz, H-3'), 6.85 (2H, m, H-4'', H-6''), 6.93 (1H, m, H-2'), 7.68 (2H, m, H-3'', H-5''), 7.86–7.91 (4H, Ar-H of Isatin). ¹³C-NMR (125MHz FTNMR; TMS) δ : 72.53 (C-2), 71.41 (C-3), 62.59 (C-4), 51.95 (C-5), 29.19 (C-6), 26.89 (C-7), 47.39 (C-8). 111.7 (C-1'), 114.5 (C-12'), 140.8 (C-10'), 121.4 (C-6'), 122.1 (C-8'), 123.9 (C-3'), 125.8 (C-4), 132.0 (C-5'), 135.0 (C-4'), 135.7 (C-2'), 140.9 (C-9'), 141.1 (C-11'), 128.44 (C-2'', C-6''), 129.83 (C-3'', C-5''), 146.82 (C-1''), 126.14 (C-4''), 111.75, 114.8, 126.25, 127.94, 129.27, 154.1 (C- phenyl ring of isatin), 192.4(C=O) and 174.3 (C=O).

Compound 13

Yield: 79%, light Yellow in colour, m.p. decomposes at 296°C, Molecular formulae C₃₇H₂₉N₃O₂S, Mol. Wt.: 579.18, m/z: 580.14 [M+H]⁺. Elemental analysis: Found: C, 76.61; H, 5.07; N, 7.29; Calc: C, 76.66; H, 5.04; N, 7.25. IR (KBr, cm⁻¹) ν_{max}: 3349, 3276, 3041, 2992, 2876, 1725, 1693, 1634, 1576, 1553, 1486, 1470, 1434, 1386, 1329, 1296, 1218, 1159, 1123, 1084 859, 741, 658. ¹H-NMR (400MHz FTNMR; TMS) δ : 2.70 and 2.91 (each for 1H), 3.76 & 4.11 (1H each), 4.45 (1H), 9.92(N-H), 8.67(NH), 6.28-6.31 (3H), 6.53 (1H), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.10, 7.17, 7.26 and 7.68 (4H, Ar-H of Isatin ring). ¹³C-NMR (125MHz FTNMR; TMS) δ: 34.6, 76.5, 62.42, 43.93, 60.31, 41.3, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 111.75, 114.8, 126.25, 127.94, 129.27, 134.1, 122.9 (2C), 124.9, 126.4 (2C), 154.1, (C-phenyl ring of isatin), 186.4(C=O) and 173.7 (C=O).

Compound 14

Yield: 93%, light Yellow in colour, m.p. 291°C, Molecular formulae $C_{36}H_{27}N_3O_2S$, Mol. Wt.: 565.18, m/z: 566.14 $[M+H]^+$. Elemental analysis: Found: C, 76.47; H, 4.84; N, 7.48; Calc: C, 76.44; H, 4.81; N, 7.43. IR (KBr, cm^{-1}) ν_{max} : 3254, 3088, 2989, 2887, 1728, 1679, 1613, 1585, 1563, 1489, 1471, 1438, 1386, 1329, 1291, 1205, 1137, 1119, 1093, 842, 727, 618. 1H -NMR (400MHz FTNMR; TMS) δ : 3.766 (1H), 4.11 (1H), 4.45 (1H), 9.92(1H, N-H), 8.76(1H, NH), 6.28 (1H), 6.31 (2H), 6.53 (1H), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.10, -7.30 and 7.68 (9H). ^{13}C -NMR (125MHz FTNMR; TMS) δ : 34.6, 76.5, 62.42, 43.93, 60.31, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 111.75, 114.8, 126.25, 127.94, 129.27, 134.1, 122.9 (2C), 124.9, 126.4 (2C), 154.1, (C-phenyl ring of isatin), 186.4(C=O) and 176.2 (C=O).

Compound 15

Yield: 94%, light Yellow red in colour, m.p. 243°C, Molecular formulae $C_{31}H_{24}BrN_3O_2S$, Mol. Wt.: 607.08, m/z: 608.12 $[M+H]^+$. Elemental analysis: Found: C, 63.91; H, 4.19; N, 7.28; Calc: C, 63.93; H, 4.15; N, 7.21. IR (KBr, cm^{-1}) ν_{max} : 3234, 3072, 2949, 2857, 1723, 1683, 1634, 1579, 1559, 1483, 1469, 1429, 1378, 1333, 1271, 1213, 1129, 1101, 1067, 848, 754, 665. 1H -NMR (400MHz FTNMR; TMS) δ : 2.01(3H), 3.55, 3.65 (2H), 4.39 (1H), 4.56 (1H), 9.2(1H, NH), 8.56, 6.28(1H), 6.31 (2H), 6.53 (1H), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.32 7.41 and 7.57 (3H, Ar-H of bromo isatin ring). ^{13}C -NMR (125MHz FTNMR; TMS) δ : 34.6, 76.5, 62.42, 43.93, 60.31, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 121.75, 118.8, 126.25, 127.94, 129.27, 154.1 (C-phenyl ring of isatin), 192.4(C=O) and 169.9 (C=O).

Compound 16

Yield: 74%, dark Yellow in colour, m.p. 226°C, Molecular formulae $C_{33}H_{26}BrN_3O_2S$, Mol. Wt.: 581.08, m/z: 582.14 $[M+H]^+$. Elemental analysis: Found: C, 65.18; H, 4.39; N, 6.97; Calc: C, 65.13; H, 4.31; N, 6.90. IR (KBr, cm^{-1}) ν_{max} : 3234, 3066, 2946, 2855, 1727, 1687, 1615, 1583, 1557, 1483, 1478, 1437, 1385, 1327, 1294, 1207, 1133, 1119, 1077, 836, 723, 613. 1H -NMR (400MHz FTNMR; TMS) δ : 1.80 (2H), 1.89 (2H), 2.56 & 2.37 (each 1H), 3.65 (1H), 3.95, 3.98 (1H), 4.79 (1H), 8.87(1H, NH), 6.57

(1H), 6.59 (2H), 7.39 (1H), 7.32 (2H), 6.85 -6.93 (4H), 7.68 (2H), 7.32 7.41 and 7.57 (3H, Ar-H of bromo isatin ring). ^{13}C -NMR (125MHz FTNMR; TMS) δ : 72.53, 71.41, 62.59, 51.95, 29.19, 26.89, 47.39, 111.7, 114.5, 140.8, 121.4, 122.1, 123.9, 125.8, 132.0, 135.0, 135.7, 140.9, 141.1, 128.44, 129.83, 146.82, 126.14, 121.75, 118.8, 126.25, 127.94, 129.27, 154.1 (C-phenyl ring of isatin), 192.4(C=O) and 174.8 (C=O).

Compound 17

Yield: 79%, dark Yellow in colour, m.p. 278°C, Molecular formulae $C_{37}H_{28}BrN_3O_2S$, Mol. Wt.: 657.11, m/z: 658.14 $[M+H]^+$. Elemental analysis: Found: C, 67.42; H, 4.31; N, 6.31; Calc: C, 67.48; H, 4.29; N, 6.38. IR (KBr, cm^{-1}) ν_{max} : 3326, 3256, 3029, 2991, 2877, 1729, 1689, 1634, 1575, 1552, 1487, 1473, 1437, 1385, 1324, 1286, 1232, 1156, 1125, 1074 834, 751, 648. 1H -NMR (400MHz FTNMR; TMS) δ : 4.45 (1H, d, J = 10.4 Hz, H-3), 4.11 (1H, m, H-4), 3.766 (1H, d, J = 7.6 Hz, H-5), 2.70 and 2.91 (each for 1H, J = 7.4 Hz) 9.92(1H, br.s, N-H), 8.87(1H, br.s.,NH), 6.28 (1H, s, H-1'), 6.31 (2H, d, H-6', H-8'), 6.53 (1H, d, J = 7.56, H-4'), 6.70 (2H, m, H-5', H-7'), 7.36 (1H, d, J = 7.6Hz, H-3'), 6.77 (1H, m, H-4''), 6.96 (2H, m, H-2'', H-6''), 7.60 (2H, m, H-3'', H-5''), 7.32 7.41 and 7.57 (3H, Ar-H of bromo isatin ring). ^{13}C -NMR (125MHz FTNMR; TMS) δ : 34.6 (N-CH₃), 76.5 (C-2), 62.42 (C-3), 43.93 (C-4), 60.31 (C-5), 41.3 (C-6), 111.6 (C-1'), 114.4 (C-12'), 144.9 (C-10'), 120.5 (C-6'), 120.9 (C-8'), 122.0 (C-3'), 122.01 (C-4), 131.0 (C-5'), 135.0 (C-4'), 135.5 (C-2'), 140.9 (C-9'), 141.1 (C-11') 128.37 (C-2'', C-6''), 129.5 (C-3'', C-5''), 142.0 (C-1''), 126.0 (C-4''), 121.75, 118.8, 126.25, 127.94, 129.27, 154.1 (C-phenyl ring of isatin), 192.4(C=O) and 172.3 (C=O).

Compound 18

Yield: 87%, Yellow red in colour, m.p. 243°C, Molecular formulae $C_{36}H_{26}BrN_3O_2S$, Mol. Wt.: 643.09, m/z: 644.16 $[M+H]^+$. Elemental analysis: Found: C, 67.03; H, 4.12; N, 6.57; Calc: C, 67.08; H, 4.07; N, 6.52. IR (KBr, cm^{-1}) ν_{max} : 3255, 2999, 2817, 1734, 1692, 1624, 1591, 1573, 1484, 1476, 1431, 1389, 1324, 1293, 1209, 1135, 1117, 1091, 618. 1H -NMR (400MHz FTNMR; TMS) δ : 3.76 (1H), 4.11, 4.45 (1H each), 9.92(N-H), 8.87(N-H), 6.28-6.31 (3H), 6.53-6.70 (3H), 7.36, 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.32 7.41 and 7.57 (3H, Ar-H of bromo isatin ring). ^{13}C -NMR (125MHz FTNMR; TMS) δ : 34.6, 76.5, 62.42, 43.93, 60.31, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5,

140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 121.75, 118.8, 126.25, 127.94, 129.27, 154.1 (C-phenyl ring of isatin), 192.4(C=O) and 176.3 (C=O).

Compound 19

Yield: 88 %, Yellow in colour, m.p. decomposes at 265°C, Molecular formulae $C_{32}H_{26}N_2O_3S$, Mol. Wt.: 518.17, m/z: 519.16 [M+H]⁺. Elemental analysis: Found: C, 74.15; H, 5.09; N, 5.47; Calc: C, 74.11; H, 5.05; N, 5.40. IR (KBr, cm^{-1}) ν_{max} : 3304, 3056, 2929, 2874, 1731, 1694, 1687, 1642, 1576, 1552, 1489, 1461, 1422, 1374, 1337, 1278, 1232, 1132, 1117, 1059, 847, 753, 646. ¹H-NMR (400MHz FTNMR; TMS) δ : 2.01(3H), 3.55, 3.65 (2H), 4.39 & 4.56 (1H each), 6.28-6.31 (3H), 6.53-6.70 (3H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.57-8.01 (4H, Ar-H of ninhydrin ring), 9.2 (1H, NH). ¹³C-NMR (125MHz FTNMR; TMS) δ : 34.6, 76.5, 62.42, 43.93, 60.31, 111.1, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 129.27, 133.7 and 144.1 (C-ninhydrin system), 192.4, 195.3 & 197.3 (3 C=O).

Compound 20

Yield: 94 %, light Yellow in colour, m.p. decomposes at 263°C, Molecular formulae $C_{34}H_{28}N_2O_3S$, Mol. Wt.: 544.17, m/z: 545.16 [M+H]⁺. Elemental analysis: Found: C, 74.89; H, 5.11; N, 5.19; Calc: C, 74.98; H, 5.18; N, 5.14. IR (KBr, cm^{-1}) ν_{max} : 3314, 3045, 2936, 2885, 1724, 1683, 1678, 1654, 1584, 1561, 1478, 1457, 1434, 1375, 1341, 1276, 1239, 1125, 1126, 1063, 846, 748, 623. ¹H-NMR (400MHz FTNMR; TMS) δ : 1.80 (2H), 1.89 (2H), 2.56 & 2.37 (each 1H), 3.95, 3.65 (1H), 3.98 (1H), 4.79 & 6.57 (1H each), 6.59 (2H), 6.85 (1H), 6.85 (2H), 6.93 (1H), 7.39 (1H), 7.32 (2H), 7.68 (2H), 7.57-8.01 (4H, Ar-H of ninhydrin ring). ¹³C-NMR (125MHz FTNMR; TMS) δ : 72.53, 71.41, 62.59, 51.95, 29.19, 26.89, 47.39, 111.7, 114.5, 140.8, 121.4, 122.1, 123.9, 125.8, 132.0, 135.0, 135.7, 140.9, 141.1, 128.44, 129.83, 146.82, 126.14, 129.27, 133.7 and 144.1 (C-ninhydrin system), 192.4(C=O), 195.3 (C=O), 197.3 (C=O).

Compound 21

Yield: 81 %, light Yellow in colour, m.p. decomposes at 248°C, Molecular formulae $C_{38}H_{28}N_2O_3S$, Mol. Wt.: 592.18, m/z: 593.12 [M+H]⁺. Elemental analysis: Found: C, 77.07; H, 4.78; N, 4.71; Calc: C, 77.00; H, 4.76; N, 4.73. IR (KBr, cm^{-1}) ν_{max} : 3323, 3033, 2924, 2876, 1731, 1686, 1679, 1653, 1583, 1559, 1476, 1456, 1431, 1372, 1347, 1275, 1238, 1123, 1123, 1065, 845, 741, 629. ¹H-NMR (400MHz FTNMR; TMS) δ : 2.70 and 2.91 (each

for 1H), 3.76 (1H), 4.11 (1H), 4.45 (1H), 9.92(1H, N-H), 6.28 (1H), 6.31 (2H), 6.53 (1H), 6.70 (2H), 7.36 (1H), 6.77 (1H), 6.96 (2H), 7.60 (2H), 7.57-8.01 (4H, Ar-H of ninhydrin ring). ¹³C-NMR (125MHz FTNMR; TMS) δ : 34.6 (N-CH₃), 76.5 (C-2), 62.42, 43.93, 60.31, 41.3, 111.6, 114.4, 144.9, 120.5, 120.9, 122.0, 122.01, 131.0, 135.0, 135.5, 140.9, 141.1, 128.37, 129.5, 142.0, 126.0, 121.75, 118.8, 126.25, 144.1 (C-phenyl ring), 129.27, 133.7 and 144.1 (C-ninhydrin system), 192.4, 195.3 & 197.3 (3 C=O).

Compound 22

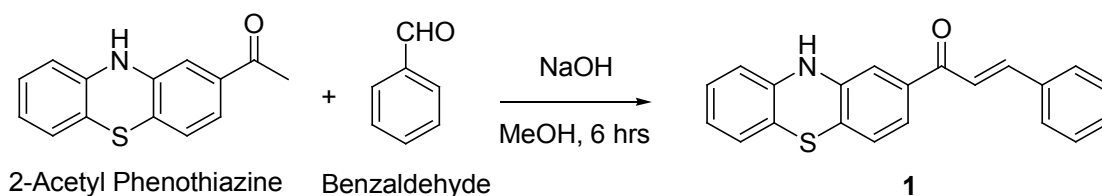
Yield: 83%, light Yellow in colour, m.p. decomposes at 223°C, Molecular formulae $C_{37}H_{26}N_2O_3S$, Mol. Wt.: 578.18, m/z: 579.19 [M+H]⁺. Elemental analysis: Found: C, 76.85; H, 4.57; N, 4.78; Calc: C, 76.79; H, 4.53; N, 4.84. IR (KBr, cm^{-1}) ν_{max} : 3343, 3098, 2997, 2887, 1732, 1689, 1671, 1649, 1578, 1546, 1477, 1455, 1436, 1379, 1341, 1276, 1233, 1128, 1128, 1064, 844, 743, 621. ¹H-NMR (400MHz FTNMR; TMS) δ : 4.45 (1H, d, J = 10.4 Hz, H-3), 4.11 (1H, m, H-4), 3.76 (1H, d, J = 7.6 Hz, H-5), 9.92 (1H, br.s, N-H), 6.28 (1H, s, H-1'), 6.31 (2H, d, H-6', H-8'), 6.53 (1H, d, J = 7.56, H-4'), 6.70 (2H, m, H-5', H-7'), 7.36 (1H, d, J = 7.6 Hz, H-3'), 6.77 (1H, m, H-4''), 6.96 (2H, m, H-2'', H-6''), 7.60 (2H, m, H-3'', H-5''), 7.57-8.01 (4H, Ar-H of ninhydrin ring). ¹³C-NMR (125MHz FTNMR; TMS) δ : 34.6 (N-CH₃), 76.5 (C-2), 62.42 (C-3), 43.93 (C-4), 60.31 (C-5), 111.6 (C-1'), 114.4 (C-12'), 144.9 (C-10'), 120.5 (C-6'), 120.9 (C-8'), 122.0 (C-3'), 122.01 (C-4), 131.0 (C-5'), 135.0 (C-4'), 135.5 (C-2'), 140.9 (C-9'), 141.1 (C-11') 128.37 (C-2'', C-6''), 129.5 (C-3'', C-5''), 142.0 (C-1''), 126.0 (C-4''), 121.75 (2C), 118.8 (2C), 126.25, 144.1 (C-phenyl ring), 129.27 (2C), 133.7 (2C) and 144.1(2C) (C-ninhydrin system), 192.4 (C=O), 195.3 (C=O), 197.3 (C=O).

In-vitro anticancer effect: and 1 mg of each of the compounds **7-22** were dissolved in DMEM (1 mL) medium to prepare solution of different concentration with 6.25 μ g, 12.5 μ g, 25 μ g, 50 μ g, and 100 μ g each in 500 μ L medium. MTT assay was carried out and IC₅₀ value (μ g/ML) was calculated by the procedure we have reported¹⁶ to determine the *In-vitro* cytotoxic effect of the compounds **7-22** against MCF-7 Human Breast cancer cells. The cells which are not treated served as a control and doxorubicin acted as a positive control. The fluorescence activated cell sorting (FACS) Analysis of compound **7** on MCF-7 cells was carried out as reported by us earlier¹⁷. The percentage of calculated cells in the G2/M, S and G0/G1 phases were analysed.

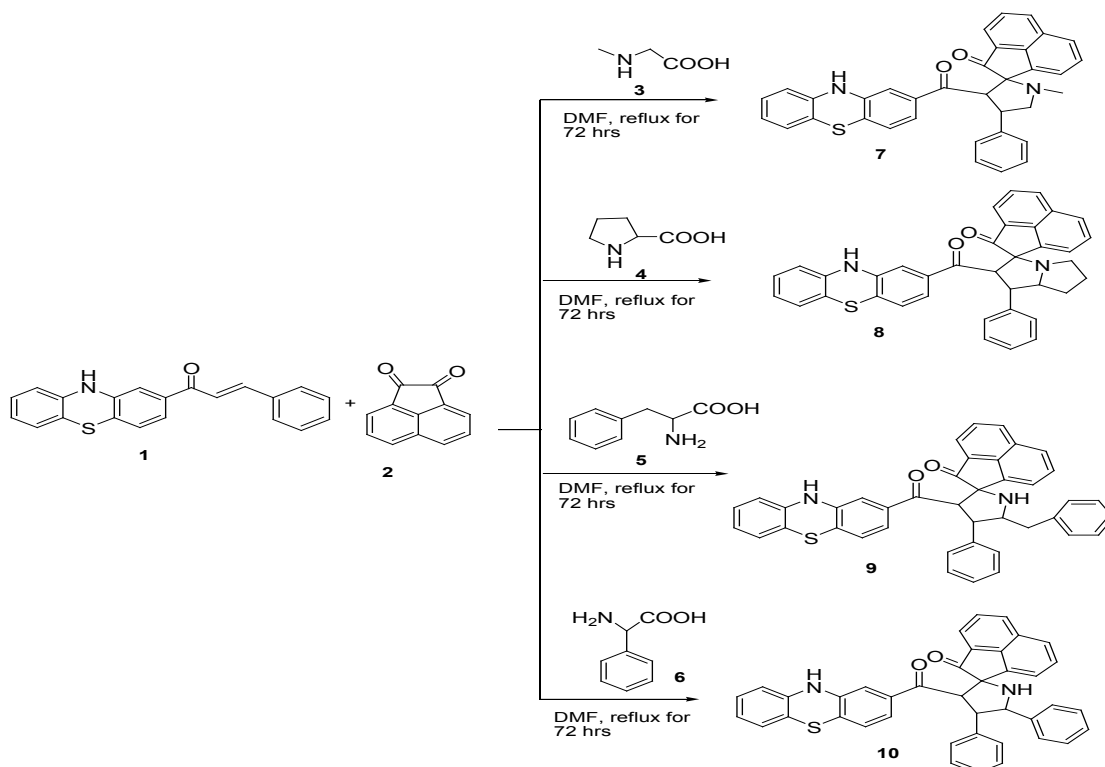
RESULT AND DISCUSSION

The 1,3-dipolar cycloaddition reaction is a three-component reaction and used to spiro-heterocycles¹²⁻¹⁵. In the present work a two steps synthesis of the target compounds (**7-22**) are presented in Schemes 1-5. The first step was to synthesize the chalcone **1** which is required to prepare the target compounds (Scheme 1). It was prepared and characterised by spectral data as reported¹⁶. The second step is a reaction between the chalcone and the azomethine ylides (Scheme 2-5). The azomethine ylides were generated from the acenaphthoquinone **2a** and amino acids like sarcosine **3**, proline **4**, phenyl alanine **5** and phenyl glycine **6** (Scheme 2), isatin **2b** and amino acids like sarcosine, proline, phenyl alanine and phenyl

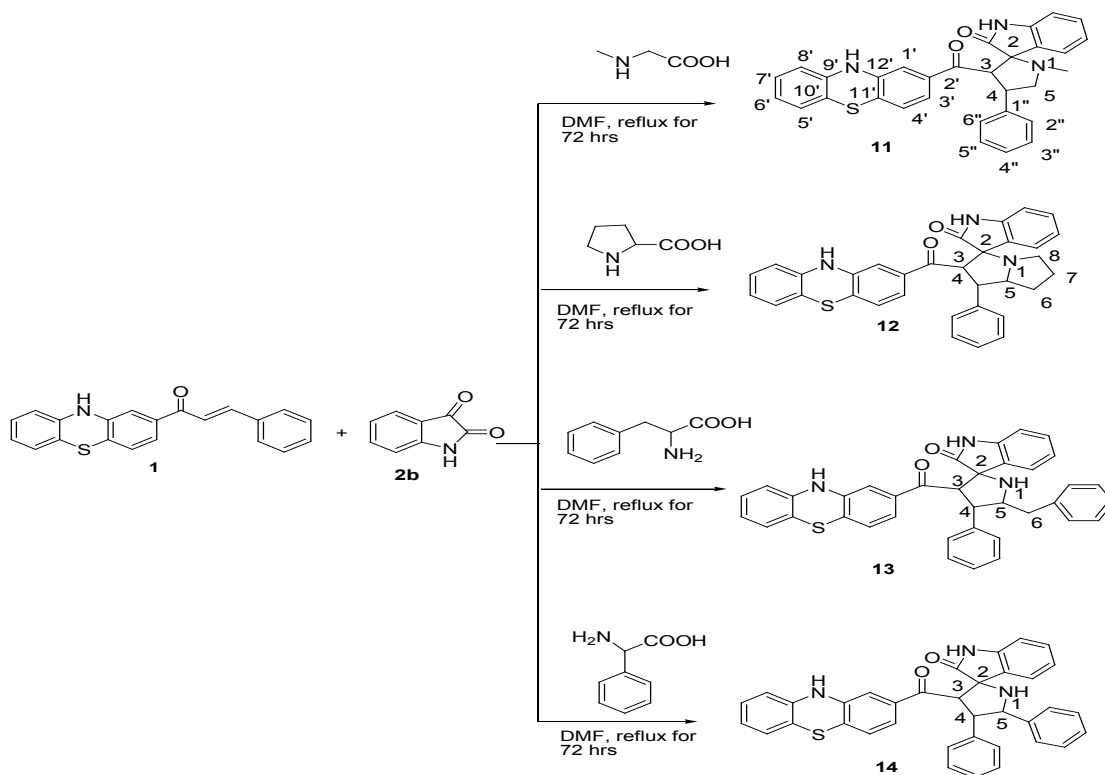
glycine (Scheme 3), bromoisatin **2c** and amino acids like sarcosine, proline, phenyl alanine and phenyl glycine (Scheme 4) and by the ninhydrine **2d** and amino acids like sarcosine, proline, phenyl alanine and phenyl glycine (Scheme 5). The diversity exists among the compounds **7-22** due to the diones like acenaphthoquinone **2a**, isatin **2b**, bromo isatin **2c** and ninhydrine **2d** and the different aminoacids **3-6** together which forms the azomethine ylides. All the three component reactions were done by refluxing an equimolar mixture of the chalcone **1**, dione **2a-2d** and aminoacids **3-6** in DMF for 72 hours. After checking the reaction completion by TLC and subsequent evaporation of the solvent resulted **7-22**. Column chromatography was used to purify the resulted cycloadducts in good yield and were characterised by spectral and elemental analysis.



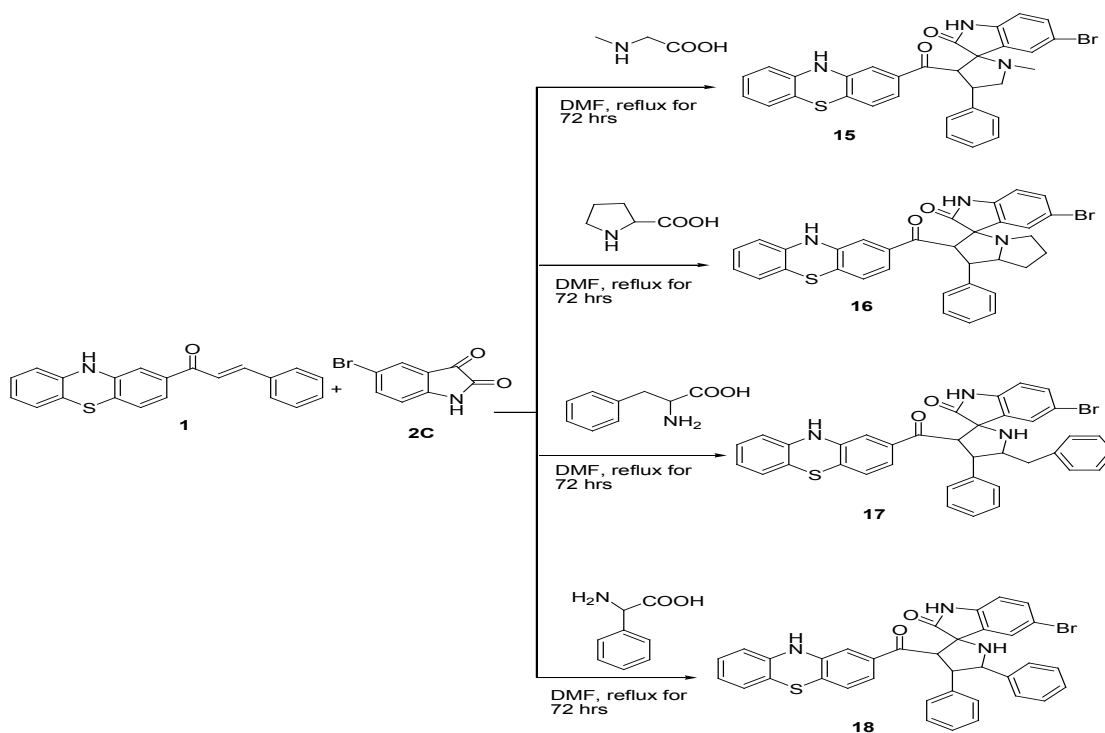
Scheme 1. Synthesis of chalcone **1**



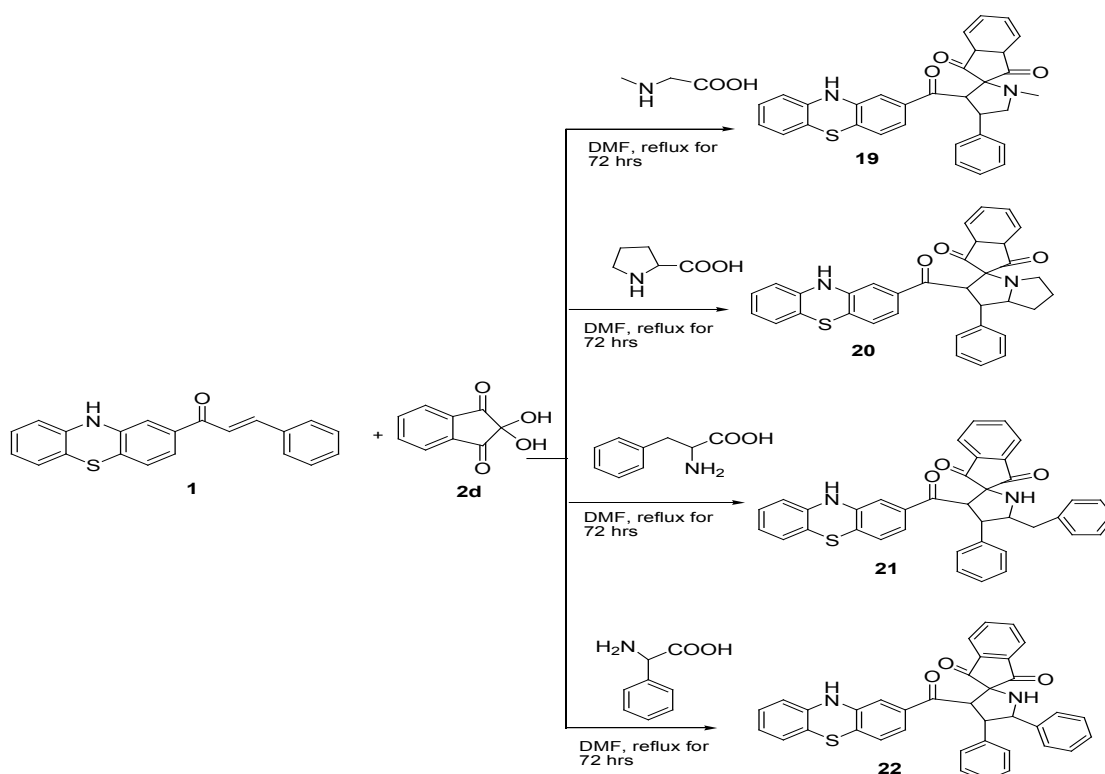
Scheme 2. Synthesis of Spiro compounds using acenaphthoquinone and the chalcone **1**



Scheme 3. Synthesis of Spiro compounds using isatin and the chalcone 1



Scheme 4. Synthesis of Spiro compounds using bromo isatin and the chalcone 1



Scheme 5. Synthesis of Spiro compounds using ninhydrin and the chalcone 1

The compound **7**, showed characteristic absorption bands in its IR spectrum at 1716 cm^{-1} and at 1618 cm^{-1} due to the carbonyl group in phenothiazine ring and oxindole ring respectively.

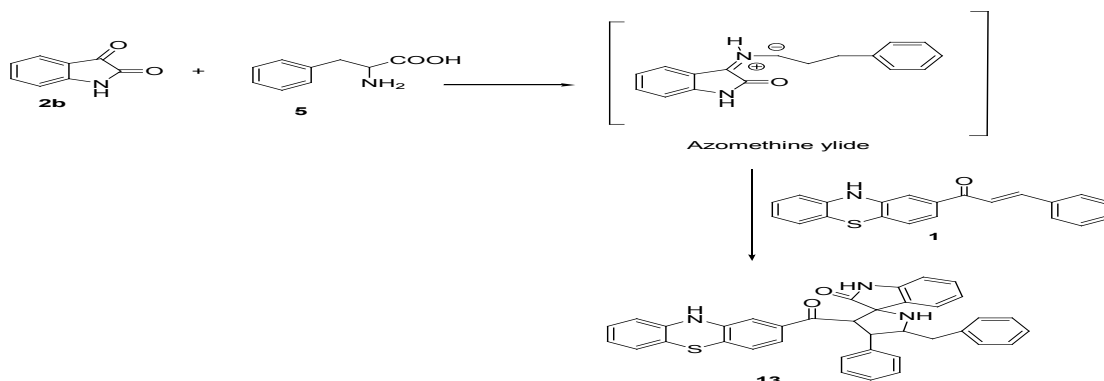
The benzylic proton (H-4) of **7** appeared as a multiplet signal at δ 4.56 in its PMR data. The proton at C-3 present in the pyrrolidine ring exhibited as a doublet at δ 4.39 ($J = 10.8\text{ Hz}$). The H-5 proton of pyrrolidine moiety resonated at 3.55 and 3.65 (2H, dd, $J = 7.6\text{ Hz}$), as doublet of doublets due to the diastereostopic impact at C-5. The bunch of multiplets between δ 6.28 and 7.91 were attributed to aromatic protons. The $-\text{NH}$ proton present in the phenothiazine moiety and the $\text{N}-\text{CH}_3$ group of pyrrolidine ring resonated as singlets at δ 9.10 and δ 2.01 respectively. These data were supported by ^{13}C -NMR spectra of **7**. The spiro carbon resonated at δ 76.5. The carbonyl functions in acenaphthoquinone and phenothiazine rings resonated at δ 192.7 and 196.3 respectively. These data confirmed the proposed structures.

Similarly for the spiropyrrolizidine oxindoles **12** and **16**, acenaphthylene spiro pyrrolidinones **9** and **10**, spiro[acenaphthylene-1,2'-pyrrolizidin]-2-

one derivative **8**, spiropyrrolidine oxindoles **11**, **13**-**15**, **17** and **18** spiroindan pyrrolidines (**19**, **21**, **22**) and spiroindan-pyrrolizidine (**20**), ^1H -NMR spectrum showed the assigned protons and ^{13}C -NMR spectrum showed the characteristic carbon signals matched with the proposed structures. As a representative compound **12** for spiropyrrolizidines the broad singlets at δ 9.21 and 8.68 were assigned to the NH proton of phenothiazine and oxindole nucleus. The signals occurred at δ 6.57 and 7.91 were assigned for the aromatic protons while the protons of the fused pyrrolidine ring were assigned between δ 1.80 and 4.79. The multiplet at δ 4.56 due to benzylic proton (H-4) of pyrrolizidine ring. The pyrrolizidine ring proton of C-3 was obtained at δ 4.79 ($J = 10.8\text{ Hz}$) as a doublet. The H-5 proton of pyrrolizidine moiety resonated as a multiplet at δ 3.65. The multiplets at 2.56 and 2.37 (each 1H, m, H-8) are due to NCH_2 protons due the diastereostopic impact at C-8. The protons in the methylene groups H-7 and H-6 appeared at δ 1.89 (2H, m, H-7) and 1.80 (2H, m, H-6) respectively. In the ^{13}C -NMR spectrum the signal at δ 72.5 was assigned to spiro carbon. The two carbonyl groups were showed at δ 174.3 and 192.4. All other ascertained chemical shift values confirm the expected structures.

The mechanism for the formation of a spirooxindole-pyrrolidine derivative **13** was discussed in scheme 6. Two π -electrons of the dipolarophile and four electrons of the dipole participate in a concerted, pericyclic shift. The addition is a [2S+4S] cycloaddition and stereo conservative (suprafacial)

where the reaction occurs through the interaction between HOMO of azomethine ylide and LUMO of the chalcone, followed by a cyclisation reaction to yield a regioselective product **13**. The same mechanism is followed for formation of all other compounds **7-22**.



Mechanism of the reaction between the phenothiazine chalcone 1, isatin 2b and phenylalanine 5 to form the compound 13

Antiproliferative activity by MTT Assay

The anti-proliferative activity of **7-22** was tested (Table 1) against cell line MCF-7 by means of MTT assay. Five doses of 100 μ L each of 100, 50, 25, 12.5 and 6.25 μ g in 500 μ L of DMEM were used for the assay. The IC_{50} values of **7-22** were

summarized in Table 1 and the result showed that compounds **7**, **12**, **16** and **20** exhibited good activity with an IC_{50} values of 83.08, 84.68, 95.68 and 114.23 μ g/mL respectively. The IC_{50} value against MCF-7 cells showed a quantity dependent inhibition in cell proliferation at low concentration.

Table 1: *In-vitro* cytotoxic effect of 7-22 using L929 (Fibroblast) cells by MTT assay

Compound	IC_{50} value (μ g/ml)	Compound	IC_{50} value (μ g/mL)
7	270.17	15	180.23
8	196.34	16	214.09
9	160.45	17	176.54
10	164.78	18	156.40
11	181.23	19	178.65
12	239.56	20	190.03
13	172.94	21	179.12
14	181.21	22	185.26
		Doxorubicin	285.30

The cytotoxicity of **7-22** is also determined (Table 2) using normal fibroblast L929 cells and the results are shown in Table 2. Strikingly the IC_{50} value for the compounds **7**, **12**, **16** and **20** were 270.17, 239.56, 214.09 and 190.13 μ g/

mL respectively. The lower cytotoxicity against normal fibroblast L929 cells indicates a selective cytotoxic activity of compounds **7**, **12**, **16** and **20** for MCF-7 cell lines. Doxorubicin was used as the positive control.

Table 2: *In-vitro* anticancer effect of the compounds 7-22

Compound	IC_{50} value (μ g/mL)	Compound	IC_{50} value (μ g/mL)
7	83.08	15	129.34
8	132.94	16	95.68
9	130.21	17	134.65
10	123.87	18	138.38
11	128.95	19	132.78
12	84.68	20	114.23
13	131.23	21	141.24
14	142.04	22	121.59
		Doxorubicin	64.21

Cell cycle analysis by flow cytometry

After treatment of cells for 48 h with compound **7**, the compound was analysed to determine the effect on cell cycle phase using flow cytometry assay. The results showed that treated cells showed arrest at G0/G1 when compared with the control group. The percentage of untreated cells in the G2/M, S and phases are 45.00 %, 5.30% and 29.8% respectively

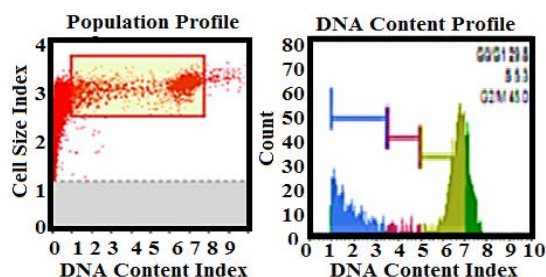


Fig. 1a. The Cell cycle analysis by flow cytometry with the untreated cells

whereas cells treated with compound **7** are 4.2%, 8.1% and 86.30% respectively (Fig. 1a & 1b). Hence compound **7** exhibited cell cycle arrest in MCF-7 cells. The subG1 step was not noticeable. Further after 48 h treatment to compound **7** it showed that the population in G0/G1 phase has increased. There was also a slight increase in the proportion of S however the G2/M phase percentage significantly decreases.

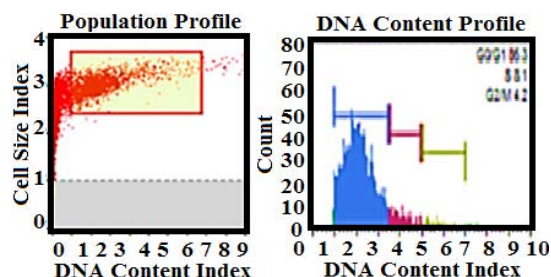


Fig. 1b. The Cell cycle analysis by flow cytometry with the treated cells.

CONCLUSION

The 1,3-dipolar cycloaddition reaction between the chalcone **1** and the azomethine ylides resulted in compounds **7-22**. The structure of the resulted compounds were elucidated by spectral and elemental analysis. The anti-proliferative activity of synthesized compounds **7-22** was evaluated against MCF-7 cell lines via MTT assay. Compounds **7**, **12**, **16** and **20** exhibited effective inhibitory activity against MCF-7 cell lines with an IC_{50} values of 83.08, 84.68, 95.68 and 114.23 $\mu\text{g}/\text{mL}$ respectively. The cytotoxicity of **7-22** was tested against normal fibroblast L929 cells and the IC_{50} value for the compounds **7**, **12**, **16** and **20**

were 270.17, 239.56, 214.09 and 190.13 $\mu\text{g}/\text{mL}$ respectively. In the Cell cycle analysis of compound **7** by flow cytometry it showed arrest cell cycle in MCF-7 cells.

ACKNOWLEDGMENT

It is our pleasure to thank the Central Instrumentation Facility in Karpagam Academy of Higher Education for recording the data required to characterise the synthesised compounds.

Conflict of Interest

There is no conflict of interest with respect to the present work.

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