



## Research Article

### EFFICIENT SYNTHESIS AND CYTOTOXIC SCREENING OF 3,3 DISUBSTITUTED OXINDOLE DERIVATIVES

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#### ABSTRACT

3,3-disubstituted oxindole derivatives were synthesised by treating isatins with electron rich benzene derivatives at room temperature by using  $\text{BF}_3\text{O}(\text{Et})_2$  as catalyst which reduced the synthesis time. The compounds were evaluated for cytotoxic activity against human breast cancer cells (MCF7) and human ovarian carcinoma cells (SKVO3) by using MTT assay. Compounds **1** ( $7.2 \pm 0.22 \mu\text{M}$  and  $11.80.21 \pm \mu\text{M}$ ), **2** ( $7.10.24 \pm \mu\text{M}$  and  $9.8 \pm 0.27 \mu\text{M}$ ), exhibited relatively higher cytotoxic activity against both MCF7 and SKVO3 cell lines, respectively.

**Keywords:** Isatins, Methoxy Benzenes, Boron Trifluoride Diethyl Etherate, MCF7 and SKVO3

#### INTRODUCTION

Cancer is characterised by a change in controlled mechanisms that manage cell proliferation, differentiation and is continuing to be a major health problem in developing as well as undeveloped countries. Malignancy is caused by abnormalities in cells, which might be due to inherited genes or caused by exogenous agents including chemicals, radiation and some infectious agents <sup>1</sup>.

Organic compounds with an oxindole framework are represented in a large family of a pharmaceutically active compounds and bioactive natural products. Particularly, spirooxindole and 3, 3-disubstituted derivatives were present in a number of alkaloids which possess significant biological activities. Such as anticancer<sup>2</sup>, antidepressant<sup>3</sup>, anticonvulsant<sup>4</sup>, antifungal<sup>5</sup>, anti-HIV <sup>6</sup>, anti-inflammatory<sup>7</sup>. During past decades, researchers have embarked on the development of new oxindole based anticancer agents<sup>8-10</sup>. 5-fluoro-3-substituted-2-oxindole derivative compound SU11248 [Sutent] received FDA approval for the treatment of gastrointestinal stromal tumors and advanced renal cell carcinoma<sup>11</sup>. Due to the importance of oxindole motifs, several methods have been developed for the construction such structural motifs<sup>12-19</sup>. Despite of effectiveness, these methods involve use of expensive catalysts<sup>20-22</sup>, long reaction time<sup>16,17,19,23</sup>, tedious work up, vigorous reaction conditions<sup>15</sup> and poor yield<sup>19</sup>. Therefore there is need to develop efficient, convenient and practical protocol to synthesize oxindole scaffolds.

In the present investigation, we have developed a rapid and efficient method for synthesis of methoxy benzene substituted oxindoles using boron trifluoride diethyl etherate as catalyst. The newly synthesized 3,3-disubstituted oxindole are evaluated for their cytotoxic potentials against wild type human breast cancer cell line (MCF7) and human ovarian carcinoma cells (SKVO3).

#### MATERIALS AND METHODS

##### Chemistry

All the starting materials procured from Sigma Aldrich and used without further purification. Solvents were of analytical grade. All the reactions were carried out with the use of standard techniques and were monitored by analytical TLC performed on pre-coated silica.

##### General procedure for synthesis of 3,3-disubstituted oxindoles

To 1.0 equivalent of isatin 1.5 equivalent of tri and di methoxy benzene was added in DCM in a RBF, stirred it for 2min, 0.2ml of  $[\text{BF}_3\text{O}(\text{Et})_2]$  was added and stirred it for another 5-10 min (**Scheme 1**). Reaction was monitored by TLC, excess of reagent was quenched with solid sodium bicarbonate and directly loaded on to column. The structure of the compounds were confirmed by <sup>1</sup>H NMR, <sup>13</sup>CNMR, IR and Mass-spectroscopy. Elemental analysis: Calculated: C, 70.71; H, 5.34; N, 8.25, Found : C, 70.72; H, 5.31; N, 8.27

**5-chloro-3,3-bis(2,4,6-trimethoxyphenyl)indolin-2-one (1):** Light red solid; Mp 180-182°C; <sup>1</sup>H NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.54 (s, 3H), 3.68 (s, 3H), 6.72 (s, 3H), 3.80 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 5.14 (s, 1H), 6.09 (dd,  $J = 2.13$  Hz and 8.39 Hz, 1H), 6.20 (dd,  $J = 2.13$  Hz and 5.95 Hz, 2H), 6.22 (d,  $J = 2.28$  Hz, 1H), 6.74 (s, 1H), 7.00 (s, 1H), 8.68 (br s, 1H) ppm. <sup>13</sup>CNMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  842.5, 55.2, 55.3, 55.7, 55.9, 89.6, 89.8 90.6, 90.8, 91.6, 105.7, 108.7, 109.9, 113.2, 124.3, 127.1, 128.0, 131.0, 132.3, 139.8, 158.2, 158.3, 158.8, 159.3, 161.0, 180.1 ppm. IR (KBr):  $\nu = 809, 1116, 1153, 1225, 1458, 1609, 1707, 2936 \text{ cm}^{-1}$ . MS-ESI:  $m/z = 500 [\text{M}+\text{H}]^+$ , 522  $[\text{M}+\text{Na}]^+$ . Elemental analysis: Calculated: C, 62.46; H, 5.24; Cl, 7.09; O, 22.40, Found : C, 62.44; H, 5.23; Cl, 7.08; O, 22.40.

**5-bromo-3,3-bis(2,4,6-trimethoxyphenyl)indolin-2-one (2):**

Light red solid; Mp 233-235 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.56 (s, 3H), 3.69 (s, 3H), 3.73 (s, 3H), 3.81 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 5.14 (s, 1H), 6.08 (d,  $J = 6.02$  Hz, 1H), 6.19-6.23 (m, 3H), 6.73 (s, 1H), 7.18 (s, 1H), 8.01 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  42.3, 55.3, 55.7, 55.9, 90.6, 90.7, 91.6, 111.9, 112.9, 127.4, 131.3, 134.5, 140.4, 158.1, 159.3, 161.0, 179.4 ppm. IR (KBr):  $\nu = 782, 818, 1029, 1155, 1267, 1456, 1521, 1735, 3291$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 544$   $[\text{M}]^+$ . Elemental analysis: Calculated: C, 57.36; H, 4.81; Br, 14.68; N, 2.57; O, 20.57. Found: C, 57.37; H, 4.83; Br, 14.69; N, 2.56; O, 20.59.

**3,3-bis(2,4-dimethoxyphenyl)indolin-2-one (3):**

Light yellow solid; Mp 180-182 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.46 (s, 3H), 3.63 (s, 3H), 3.77 (s, 6H), 6.36 (d,  $J = 8.39$  Hz, 1H), 6.40-6.48 (m, 3H), 6.81 (t,  $J = 7.78$  Hz, 2H), 6.88 (td,  $J = 0.91$  Hz and 7.62 Hz, 1H), 7.11 (td,  $J = 1.06$  Hz and 7.62 Hz, 1H), 7.21 (d,  $J = 7.93$  Hz, 2H), 8.25 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.1, 55.2, 55.6, 59.2, 99.7, 100.1, 104.1, 104.2, 108.8, 120.2, 120.5, 121.6, 125.2, 127.1, 125.5, 127.1, 129.6, 130.8, 135.3, 140.6, 158.5, 158.9, 160.0, 180.9 ppm. IR (KBr):  $\nu = 760, 1035, 1134, 1210, 1310, 1468, 1609, 1709$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 406$   $[\text{M}+\text{H}]^+$ , 428  $[\text{M}+\text{Na}]^+$ . Elemental analysis: Calculated: C, 71.10; H, 5.72; N, 3.45; O, 19.73. Found: C, 71.12; H, 5.74; N, 3.44; O, 19.75.

**5-chloro-3,3-bis(2,4-dimethoxyphenyl)indolin-2-one (4):**

Light yellow solid; Mp 152-154 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.43 (s, 3H), 3.67 (s, 3H), 3.77 (s, 6H), 6.36-6.51 (m, 4H), 6.72 (d,  $J = 8.24$  Hz, 1H), 6.81 (d,  $J = 8.54$  Hz, 1H), 7.01 (dd,  $J = 2.13$  and 8.24 Hz, 1H), 7.23 (d,  $J = 8.54$  Hz, 1H), 7.27 (d,  $J = 1.98$  Hz, 1H), 8.61 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.0, 55.2, 55.5, 59.6, 99.8, 104.2, 104.4, 109.7, 118.9, 119.7, 125.8, 126.8, 129.6, 130.9, 137.2, 139.3, 158.4, 158.6, 160.3, 180.9 ppm. IR (KBr):  $\nu = 819, 1030, 1208, 1306, 1465, 1504, 1610, 1713, 2934$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 440$   $[\text{M}+\text{H}]^+$ , 462  $[\text{M}+\text{Na}]^+$ . Elemental analysis: Calculated: C, 65.53; H, 5.04; Cl, 8.06; N, 3.18; O, 18.19. Found: C, 65.53; H, 5.06; Cl, 8.08; N, 3.17; O, 18.17.

**5-bromo-3,3-bis(2,4-dimethoxyphenyl)indolin-2-one (5):**

White solid; Mp 202-204 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.43 (s, 3H), 3.68 (s, 3H), 3.77 (s, 6H), 6.38 (d,  $J = 8.69$  Hz, 1H), 6.44 (d,  $J = 9.15$  Hz, 2H), 6.48 (s, 1H), 6.81 (d,  $J = 8.69$  Hz, 1H), 7.21 (dd,  $J = 1.98$  Hz,  $J = 8.24$  Hz, 2H), 7.24 (d,  $J = 8.54$  Hz, 1H), 7.41 (d,  $J = 1.98$  Hz, 1H), 8.62 (s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  54.9, 55.3, 55.5, 59.6, 99.8, 104.2, 110.3, 114.3, 128.6, 129.7, 131.0, 137.6, 139.8, 158.4, 158.6, 160.3, 180.7 ppm. IR (KBr):  $\nu = 768, 812, 1021, 1144, 1263, 1451, 1515, 1724, 3288$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 484$   $[\text{M}+\text{H}]^+$ , 506  $[\text{M}+\text{Na}]^+$ . Elemental analysis: Calculated: C, 59.52; H, 4.58; Br, 16.50; N, 2.89; O, 16.52. Found: C, 59.54; H, 4.59; Br, 16.50; N, 2.87; O, 16.54.

**3, 3-bis(2,4-dimethoxyphenyl)-5-methoxyindolin-2-one (6):**

White solid; Mp 110-112 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.46 (s, 3H), 3.66 (s, 3H), 3.68 (s, 3H), 3.77 (s, 6H), 6.36 (d,  $J = 8.69$  Hz, 1H), 6.43 (d,  $J = 8.54$  Hz, 2H), 6.46 (s, 1H), 6.65 (dd,  $J = 2.59$  Hz,  $J = 8.39$  Hz, 1H), 6.72 (d,  $J = 8.39$  Hz, 1H), 6.82 (d,  $J = 8.54$  Hz, 1H), 6.90 (d,  $J = 2.44$  Hz, 1H), 7.21 (d,  $J = 8.69$  Hz, 1H), 8.16 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.1, 55.2, 55.6, 59.8, 99.7, 100.0, 104.1, 104.3, 108.8, 111.5, 112.8, 120.1, 120.5, 129.6, 130.8, 134.3, 136.9, 155.1, 158.5, 158.8, 160.0, 160.1, 180.7 ppm. IR (KBr):  $\nu = 731, 823, 1040, 1215, 1519, 1721, 3192$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 436$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 68.95; H, 5.79; N, 3.22; O, 22.04. Found: C, 68.97; H, 5.77; N, 3.23; O, 22.06.

**3,3-bis(2,4-dimethoxyphenyl)-5-methylindolin-2-one (7):**

Light yellow solid; Mp 177-179 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):

$\delta$  2.21 (s, 3H), 3.48 (s, 3H), 3.63 (s, 3H), 3.77 (s, 6H), 6.36 (d,  $J = 8.39$  Hz, 1H), 6.43 (d,  $J = 8.54$  Hz, 1H), 6.45 (d,  $J = 2.44$  Hz, 2H), 6.70 (d,  $J = 7.78$  Hz, 1H), 6.79 (d,  $J = 8.69$  Hz, 1H), 6.92 (d,  $J = 8.69$  Hz, 1H), 6.98 (s, 1H), 7.17 (d,  $J = 8.54$  Hz, 1H), 8.16 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  21.2, 55.1, 55.2, 55.7, 59.2, 99.7, 100.2, 104.1, 104.3, 108.5, 120.5, 120.7, 126.3, 127.4, 129.6, 130.7, 130.8, 135.1, 138.2, 158.4, 159.1, 159.8, 160.1, 180.8 ppm. IR (KBr):  $\nu = 727, 801, 1027, 1206, 1501, 1703, 3178$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 420$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 71.58; H, 6.01; N, 3.34; O, 19.07. Found: C, 71.59; H, 6.03; N, 3.36; O, 19.08.

**3,3-bis(3,4-dimethoxyphenyl)indolin-2-one (8):**

White solid; Mp 178-180 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.75 (s, 6H), 3.84 (s, 6H), 6.77 (d,  $J = 7.17$  Hz, 4H), 6.85 (s, 2H), 6.96 (br d,  $J = 7.78$  Hz, 1H), 7.06 (td,  $J = 0.96$  Hz,  $J = 7.62$  Hz, 1H), 7.20 (br d,  $J = 7.17$  Hz, 1H), 7.24 (td,  $J = 1.22$  Hz,  $J = 7.78$  Hz, 1H), 8.38 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.8, 55.9, 62.0, 110.1, 110.6, 111.9, 120.6, 122.7, 126.1, 128.1, 133.8, 139.8, 148.3, 148.7, 180.0 ppm. IR (KBr):  $\nu = 760, 1018, 1153, 1258, 1514, 1614, 1716, 3294$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 406$   $[\text{M}+\text{H}]^+$ , 428  $[\text{M}+\text{Na}]^+$ . Elemental analysis: Calculated: C, 71.10; H, 5.72; N, 3.45; O, 19.73. Found: C, 71.10; H, 5.74; N, 3.44; O, 19.75.

**5-chloro-3,3-bis(3,4-dimethoxyphenyl)indolin-2-one (9):**

Light yellow solid; Mp 185-187 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.76 (s, 6H), 3.85 (s, 6H), 6.73 (dd,  $J = 2.13$  and  $J = 8.39$  Hz, 2H), 6.78 (d,  $J = 8.39$  Hz, 2H), 6.83 (d,  $J = 1.98$  Hz, 2H), 6.89 (d,  $J = 8.24$  Hz, 1H), 7.15 (d,  $J = 1.98$  Hz, 1H), 7.21 (dd,  $J = 1.98$  Hz,  $J = 8.24$  Hz, 1H), 8.98 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.8, 55.9, 62.3, 110.7, 111.3, 111.8, 120.4, 126.3, 128.0, 128.2, 133.0, 135.6, 138.5, 148.6, 148.9, 180.0 ppm. IR (KBr):  $\nu = 821, 1027, 1221, 1315, 1470, 1521, 1623, 1715, 2951$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 440$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 65.53; H, 5.04; Cl, 8.06; N, 3.18; O, 18.19. Found: C, 65.55; H, 5.07; Cl, 8.08; N, 3.19; O, 18.17.

**5-bromo-3,3-bis(3,4-dimethoxyphenyl)indolin-2-one (10):**

White solid; Mp 181-183 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.76 (s, 6H), 3.85 (s, 6H), 6.73 (dd,  $J = 2.13$  Hz,  $J = 8.39$  Hz, 2H), 6.79 (d,  $J = 8.39$  Hz, 2H), 6.83 (d,  $J = 2.13$  Hz, 2H), 6.85 (s, 1H), 7.28 (d,  $J = 1.98$  Hz, 1H), 7.36 (dd,  $J = 1.98$  Hz,  $J = 8.24$  Hz, 1H), 8.91 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.8, 55.9, 62.2, 110.7, 111.8, 115.3, 120.4, 129.1, 131.1, 133.0, 135.9, 139.0, 148.6, 148.9, 179.9 ppm. IR (KBr):  $\nu = 765, 810, 1022, 1140, 1262, 1465, 1513, 1721, 3280$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 483$   $[\text{M}]^+$ , 485  $[\text{M}+2\text{H}]^+$ . Elemental analysis: Calculated: C, 59.52; H, 4.58; Br, 16.50; N, 2.89; O, 16.52. Found: C, 59.54; H, 4.59; Br, 16.51; N, 2.87; O, 16.54.

**3,3-bis(3,4-dimethoxyphenyl)-5-methoxyindolin-2-one (11):**

Yellow solid; Mp 180-182 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.74 (s, 3H), 3.76 (s, 6H), 3.85 (s, 6H), 6.77 (s, 6H), 6.85 (s, 3H), 8.19 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.7, 55.8, 55.9, 62.4, 110.4, 110.7, 112.0, 112.6, 113.3, 120.6, 113.2, 113.8, 135.3, 148.4, 148.8, 155.8, 179.8 ppm. IR (KBr):  $\nu = 721, 810, 1032, 1212, 1502, 1713, 3181$   $\text{cm}^{-1}$ . MS-ESI:  $m/z = 436$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 68.95; H, 5.79; N, 3.22; O, 22.04. Found: C, 68.97; H, 5.77; N, 3.23; O, 22.06.

**3,3-bis(3,4-dimethoxyphenyl)-5-methylindolin-2-one (12):**

Light yellow solid; Mp 170-172 °C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.30 (s, 3H), 3.76 (s, 6H), 3.85 (s, 6H), 6.74-6.79 (m, 4H), 6.83-6.87 (m, 3H), 6.98 (s, 1H), 7.03 (d,  $J = 7.78$  Hz, 1H), 8.50 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  21.2, 55.7, 55.9, 62.1, 109.9, 110.6, 112.0, 120.6, 126.6, 128.5, 132.1, 133.9, 137.4, 148.3, 148.7, 180.2 ppm. IR (KBr):  $\nu = 729, 804, 1031, 1201,$

1505, 1716, 3181  $\text{cm}^{-1}$ . MS-ESI:  $m/z = 420$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 71.58; H, 6.01; N, 3.34; O, 19.07, Found: C, 71.59; H, 6.03; N, 3.36; O, 19.09.

**3,3-bis(2,5-dimethoxyphenyl)indolin-2-one (13):** Light yellow solid; Mp 238-240  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.45 (s, 3H), 3.58 (s, 3H), 3.64 (s, 3H), 3.69 (s, 3H), 6.51 (d,  $J = 2.74$  Hz, 1H), 6.75-6.79 (m, 2H), 6.80-6.86 (m, 3H), 6.87 (d,  $J = 2.74$  Hz, 1H), 6.90 (t,  $J = 7.62$  Hz, 1H), 7.13 (t,  $J = 7.62$  Hz, 1H), 7.23 (d,  $J = 7.47$  Hz, 1H), 7.99 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.5, 55.7, 56.0, 56.4, 60.1, 108.9, 112.5, 112.6, 113.3, 114.0, 115.6, 116.9, 121.9, 125.8, 127.5, 129.2, 129.4, 134.2, 140.7, 151.7, 152.5, 153.3, 153.6, 179.6 ppm. IR (KBr):  $\nu = 772$ , 1030, 1157, 1256, 1522, 1631, 1719, 3284  $\text{cm}^{-1}$ . MS-ESI:  $m/z = 406$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 74.02; H, 5.95; N, 3.60; O, 16.43, Found: C, 74.04; H, 5.97; N, 3.62; O, 16.45.

**3,3-bis(2,5-dimethoxyphenyl)-5-methoxyindolin-2-one (14):** Brown solid; Mp 135-137  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  3.44 (s, 3H), 3.60 (s, 3H), 3.64 (s, 3H), 3.68 (s, 6H), 6.54 (d,  $J = 2.74$  Hz, 1H), 6.66 (dd,  $J = 2.59$  and 8.54 Hz, 1H), 6.73 (d,  $J = 8.39$  Hz, 1H), 6.77 (d,  $J = 8.85$  Hz, 2H), 6.82 (d,  $J = 8.85$  Hz, 2H), 6.88 (d,  $J = 2.59$  Hz, 1H), 6.92 (d,  $J = 2.44$  Hz, 1H), 8.64 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  55.5, 55.9, 56.4, 60.3, 60.8, 109.2, 111.9, 112.5, 113.0, 113.2, 113.8, 114.5, 11.5, 116.9, 129.0, 129.4, 134.6, 135.7, 151.8, 152.4, 153.2, 153.6, 155.2, 180.0 ppm. IR (KBr):  $\nu = 728$ , 801, 1027, 1231, 1495, 1704, 3171  $\text{cm}^{-1}$ . MS-ESI:  $m/z = 436$   $[\text{M}+\text{H}]^+$ , 458  $[\text{M}+\text{Na}]^+$ . Elemental analysis: Calculated: C, 71.58; H, 6.01; N, 3.34; O, 19.07, Found: C, 71.59; H, 6.03; N, 3.36; O, 19.09.

**3,3-bis(2,5-dimethoxyphenyl)-5-methylindolin-2-one (15):** Brown solid; Mp 230-232  $^{\circ}\text{C}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  2.21 (s, 3H), 3.47 (s, 3H), 3.58 (s, 3H), 3.64 (s, 3H), 3.69 (s, 3H), 6.49 (d,  $J = 2.74$  Hz, 1H), 6.71 (d,  $J = 7.78$  Hz, 1H), 6.74-6.79 (m, 2H), 6.80-6.86 (m, 3H), 6.94 (br d,  $J = 7.47$  Hz, 1H), 7.00 (br s, 1H), 7.91 (br s, 1H) ppm.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  21.2, 55.5, 55.7, 56.1, 56.5, 60.1, 108.6, 112.4, 113.4, 114.1, 115.5, 117.0, 126.6, 128.0, 129.6, 131.2, 134.0, 138.2, 151.6, 152.7, 153.2, 153.6, 179.6 ppm. IR (KBr):  $\nu = 725$ , 797, 1035, 1222, 1507, 1701, 3172  $\text{cm}^{-1}$ . MS-ESI:  $m/z = 420$   $[\text{M}+\text{H}]^+$ . Elemental analysis: Calculated: C, 74.42; H, 6.25; N, 3.47; O, 15.86, Found: C, 74.44; H, 6.26; N, 3.49; O, 15.88.

#### In vitro Cytotoxic Activity of Novel 3,3-disubstituted oxindoles

Cell viability of the test compounds were determined on the basis of measurement of in vitro growth inhibition of cell lines by cell mediated reduction of tetrazolium salt to form water insoluble formazan crystals. The compounds were evaluated for cytotoxic activity against various cancer cell lines such as human breast cancer cells (MCF7) and human ovarian carcinoma cells (SKVO3) by using MTT assay <sup>24</sup>. Briefly, the exponential growing cells were harvested and plated ( $1 \times 10^4$ ) in 96-well microtiter plates and grown for a period of 24 h. The cells were treated with different concentrations of test compounds and incubated for 48 h. Later, the cells were incubated again for 2 h with 250  $\mu\text{g mL}^{-1}$  of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After incubation, the medium was replaced with 100  $\mu\text{L}$  of DMSO and the absorbance was measured at 570 nm. The  $\text{IC}_{50}$  values of the compounds were calculated from the dose-response curves. Doxorubicin was used as a positive control for both cancer and normal cell lines. Each experiment was performed in triplicates and the  $\text{IC}_{50}$  values were expressed in mean  $\pm$  SD.

## RESULTS AND DISCUSSION

Compounds were synthesised by reacting different substituted isatins with 1,3,5-trimethoxy benzenes, 1,3-dimethoxy benzenes, 1,2-dimethoxy benzenes and 1,4-dimethoxy benzenes using boron trifluoride diethyl etherate as catalyst. The completion of reaction was identified by TLC and all the compounds were purified by column chromatography and the synthesised compounds were confirmed by  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, IR and Mass spectroscopy. In the  $^1\text{H}$  NMR spectra of newly synthesised compounds, the secondary amine peak was found at the range of 7.91-8.98 ppm. All the methoxy protons were found at the range of 3.26-3.85 ppm as a characteristic singlet signal. All the remaining aromatic and aliphatic proton peaks were observed at their expected regions.  $^{13}\text{C}$  NMR of all the compounds showed appropriate signals. Synthesised compounds were analysed by mass spectra under ESI, molecular ions were analysed in the form of  $\text{M}+1$ . The data indicates that as such there is no difference in the fragmentation pattern among the derivatives.

Formation of 3,3-disubstituted oxindole motif is a Lewis acid catalysed nucleophilic addition elimination type of reaction (Figure 1). In this reaction, carbonyl oxygen present on 3<sup>rd</sup> carbon of isatin molecule, donates an electron pair to Boron trifluoride diethyl etherate which generates an electron deficiency on third carbon atom of isatin. Due to this the electron rich tri or dimethoxy benzenes as a nucleophile attacks at that position of isatin, followed by regeneration and reattack of Lewis acid at hydroxyl oxygen which again forms an electropositive centre on that carbon (3<sup>rd</sup> position) which makes it possible for one more methoxy benzene to attack resulting in the formation of 3,3-disubstituted oxindoles.

#### In vitro Cytotoxic Activity

Novel 3,3-disubstituted oxindoles were evaluated for cytotoxicity against human breast cancer cells (MCF7) and human ovarian carcinoma cell lines (SKVO3) using MTT assay, with doxorubicin as standard. Results (Table 1) revealed that both MCF7 and SKVO3 cell lines were susceptible to the evaluated compounds. Compounds **1** and **2** showed good activity with  $\text{IC}_{50}$  values  $7.2 \pm 0.22$ ,  $7.1 \pm 0.24$   $\mu\text{M}$  respectively against MCF7 and  $11.8 \pm 0.21$ ,  $9.8 \pm 0.27$   $\mu\text{M}$  respectively against SKVO3 cell lines, whereas, remaining all other compounds showed moderate activity against both cell lines.

1,3,5-trimethoxybenzene substituted isatins have showed good activity compared to dimethoxy benzene compounds which indicates the significance of additional methoxy group on benzene. Compounds **1** and **2** having chloro and bromo substitution respectively at 5<sup>th</sup> position showed good activity which may be due to negative inductive effect.

## CONCLUSION

In the current investigation, a efficient method was developed for synthesis of a series of novel 3,3-disubstituted oxindoles using Boron trifluoride diethyl etherate as catalyst. This process is simple to operate, less time consuming with no work up and reaction conditions are mild. The newly developed di and tri substituted oxindole resulted in considerable cytotoxic activity against both human breast cancer cell lines (MCF7) and human ovarian carcinoma cell lines (SKVO3). Of the developed set of molecules, compound **1** and **2** exhibited relatively higher cytotoxic activity against both MCF7 and SKVO3 cell lines.

SCHEME

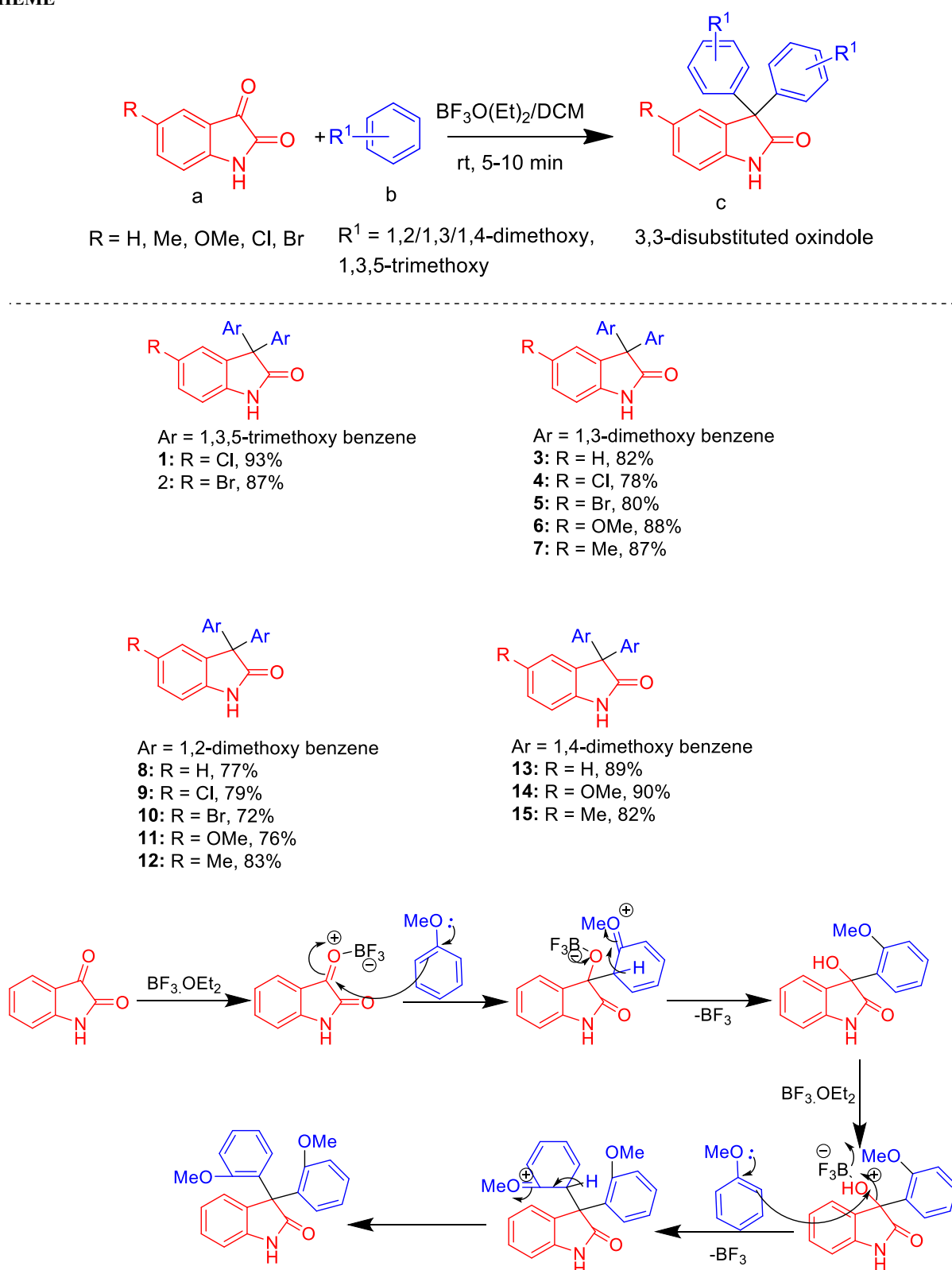


Figure 1: Probable Reaction Mechanism for the Formation of 3,3-Disubstituted Oxindole Scaffold

Table 1: *In Vitro* Cytotoxic Activity Of 3,3 disubstituted oxindoles By MTT Assay

| Compound No. | R   | IC <sub>50</sub> values in (μM) <sup>a</sup> |                    |
|--------------|-----|----------------------------------------------|--------------------|
|              |     | MCF7 <sup>b</sup>                            | SKVO3 <sup>c</sup> |
| 1            | Cl  | 7.2±0.22                                     | 11.8±0.21          |
| 2            | Br  | 7.1±0.24                                     | 9.8±0.27           |
| 3            | H   | 18.6±0.34                                    | 16.8±0.32          |
| 4            | Cl  | 29.5±0.29                                    | 11.0±0.29          |
| 5            | Br  | 21.4±0.31                                    | 21.7±0.33          |
| 6            | OMe | 34.8±0.68                                    | 15.1±0.26          |
| 7            | Me  | 40.8±0.57                                    | 35.2±0.41          |
| 8            | H   | 15.6±0.42                                    | 17.8±0.12          |
| 9            | Cl  | 11.1±0.44                                    | 12.4±0.42          |
| 10           | Br  | 11.5±0.22                                    | 14.5±0.27          |
| 11           | OMe | 17.1±0.26                                    | 15.9±0.37          |
| 12           | Me  | 18.1±0.23                                    | 11.8±0.61          |
| 13           | H   | 14.5±0.57                                    | 16.6±0.21          |
| 14           | OMe | 15.1±0.67                                    | 18.2±0.49          |
| 15           | Me  | 15.3±0.9                                     | 14.3±0.33          |
| Doxorubicin  |     | 1.6±0.28                                     | 1.8±0.47           |

<sup>a</sup> 50% cytotoxic concentration or compound concentration required to reduce viability of MCF7 or SKVO3 cell lines by 50%, using the MTT methodology, <sup>b</sup> Breast cancer cell lines,

<sup>c</sup> Ovarian cancer cell lines, MCF7 breast cancer (ATCC® HTB22™), SKOV3 ovarian cancer (ATCC® HTB 77™)

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