INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

A FACILE AND AN EFFICIENT SYNTHESIS OF 3, 3-DISUBSTITUTED OXINDOLE SCAFFOLDS AND THEIR CYTOTOXIC PROPERTIES

Sasikala Maadwar [1,2]*, Rajitha Galla [1], Santhosh K. G [1,2]
[1]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam, Tirupati, India
[2]Medicinal Chemistry and Pharmacology Division, Indian Institute of Chemical Technology, Hyderabad, India
*Corresponding Author Email: maadwarsasikala@gmail.com

Article Received on: 13/02/19 Approved for publication: 02/04/19

DOI: 10.7897/2230-8407.1004148

ABSTRACT

3,3-disubstituted oxindole derivatives were synthesised by treating isatins with electron rich benzene derivatives at room temperature by using $BF_3O(Et)_2$ 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±0.22µM and 11.80.21±µM), **2**(7.10.24±µM and 9.8±0.27µM), 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 [1].

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[2], antidepressant[3], anticonvulsant[4], antifungal[5], anti-HIV [6], anti-inflammatory[7]. During past decades, researchers have embarked on the development of new oxindole based anticancer agents[8-10]. 5-fluoro-3-substituted-2-oxoindole derivative compound SU11248 [Sutent] received FDA approval for the treatment of gastrointestinal stromal tumors and advanced renal cell carcinoma[11]. Due to the importance of oxindole motifs, several methods have been developed for the construction such structural motifs[12-19]. Despite of effectiveness, these methods involve use of expensive catalysts[20-22], long reaction time[16,17,19,23], tedious work up, vigorous reaction conditions[15] and poor yield[19]. 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-disubstited 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 $[BF_3O(Et)_2]$ 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 ^{1}H NMR, ^{13}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^{o}C;^{1}H NMR (300 MHz, $CDCl_3$): δ 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. ^{13}CNMR (75 MHz, $CDCl_3$): δ42.5, 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): ν = 809, 1116, 1153, 1225, 1458, 1609, 1707, 2936 cm^{-1}. MS-ESI: m/z = 500 $[M+H]^+$, 522 $[M+Na]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}C NMR (75 MHz, $CDCl_3$): δ42.3,55.3, 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): ν = 782, 818, 1029, 1155, 1267, 1456, 1521, 1735, 3291 cm^{-1}.MS-ESI: m/z = 544 $[M]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 760, 1035, 1134, 1210, 1310, 1468, 1609, 1709 cm^{-1}. MS-ESI: m/z = 406 $[M+H]^+$, 428 $[M+Na]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 819, 1030, 1208, 1306, 1465, 1504, 1610, 1713, 2934 cm^{-1}. MS-ESI: m/z = 440 $[M+H]^+$, 462 $[M+Na]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 768, 812, 1021, 1144, 1263, 1451, 1515, 1724, 3288 cm^{-1}. MS-ESI: m/z = 484 $[M+H]^+$, 506 $[M+Na]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 731, 823, 1040, 1215, 1519, 1721, 3192 cm^{-1}. MS-ESI: m/z = 436 $[M+H]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 727, 801, 1027, 1206, 1501, 1703, 3178 cm^{-1}. MS-ESI: m/z = 420 $[M+H]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 760, 1018, 1153, 1258, 1514, 1614, 1716, 3294 cm^{-1}. MS-ESI: m/z = 406 $[M+H]^+$, 428 $[M+Na]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 821, 1027, 1221, 1315, 1470, 1521, 1623, 1715, 2951 cm^{-1}. MS-ESI: m/z = 440 $[M+H]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ3.76 (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}C NMR (75 MHz, $CDCl_3$): δ55.8, 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): ν = 765, 810, 1022, 1140, 1262, 1465, 1513, 1721, 3280 cm^{-1}. MS-ESI: m/z = 483 $[M]^+$, 485 $[M+2H]^+$. Elemental analysis: Calculated: C, 59.52; H,4.58; Br,16.50; N,2.89; O16.52, Found: C,59.54; H,4.59; Br,16.51; N,2.87; O16.54.

3,3-bis(3,4-dimethoxyphenyl)-5-methoxyindolin-2-one (11): Yellow solid;Mp 180-182 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 721, 810, 1032, 1212, 1502, 1713, 3181 cm^{-1}.MS-ESI: m/z = 436 $[M+H]^+$. 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 ºC;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 729, 804, 1031, 1201,

1505, 1716, 3181 cm^{-1}. MS-ESI: m/z = 420 $[M+H]^+$. Elemental analysis: Calculated: C, 71.58; H, 6.01; N,3.34; O,1907, 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 °C;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 772, 1030, 1157, 1256, 1522, 1631, 1719, 3284 cm^{-1}. MS-ESI: m/z = 406 $[M+H]^+$. 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 °C;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 728, 801, 1027 1231, 1495, 1704, 3171 cm^{-1}. MS-ESI: m/z = 436 $[M+H]^+$, 458 $[M+Na]^+$. 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 °C;^{1}H NMR (300 MHz, $CDCl_3$): δ 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}CNMR (75 MHz, $CDCl_3$): δ 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): ν = 725, 797, 1035, 1222, 1507, 1701, 3172 cm^{-1}. MS-ESI: m/z = 420 $[M+H]^+$. 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 [24]. Briefly, the exponential growing cells were harvested and plated (1×10^4) 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 µg $mL^{-1}$of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). After incubation, the medium was replaced with 100 µL of DMSO and the absorbance was measured at 570 nm. The 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 IC_{50} values were expressed in mean ± 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}H NMR, ^{13}C NMR, IR and Mass spectroscopy. In the ^{1}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}C NMR of the all the compounds showed appropriate signals. Synthesised compounds were analysed by mass spectra under ESI, molecular ions were analysed in the form of M+1. The data indicates that as such there is no difference in the fragmentation pattern among the derivatives.

Formation of 3,3-disubstited oxindole motif is a Lewis acid catalysed nucleophilic addition elimination type of reaction (Figure 1). In this reaction, carbonyl oxygen present on 3rd carbon of isatin molecule, donates an electron pair to Boron trifluoridediethyletherate 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 (3rd position) which makes it possible for one more methoxy benzene to attack resulting in the formation of 3,3 di-substituted oxindoles.

In vitro Cytotoxic Activity

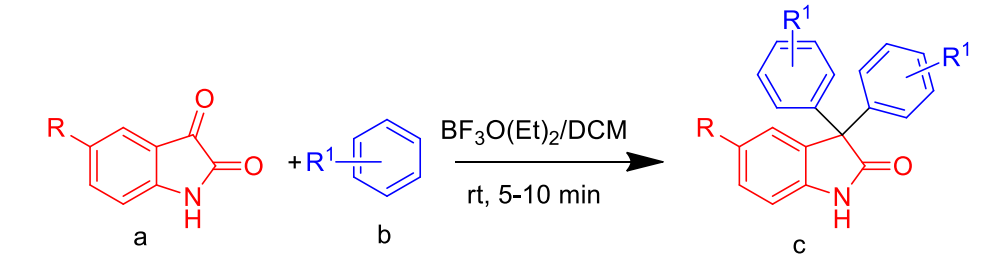
Novel 3,3-disubstituted oxindoles were evaluated for cytotoxicity against human breast cancer cells (MCF7) and human ovarian carcinoma cell lines (SKVO3) using MTT assay, with doxorubicin as standard. Results (Table 1) revealed that both MCF7 and SKVO3 cell lines were susceptible to the evaluated compounds.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

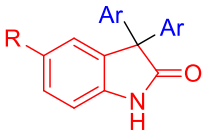
1,3,5 trimethoxybenzene substituted isatins have showed good activity compared to dimethoxy benzene compounds which indicates the significance of additional methoxy group on benzene. Compounds **1** and **2** having chloro and bromo substitution respectively at 5th position showed good activity which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

CONCLUSION

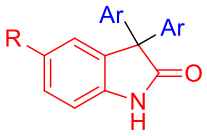
In the current investigation, a efficient method was developed for synthesis of a series of novel 3,3-disubstituted oxindoles using Boron trifluoride diethyl etherate as catalyst. This process is simple to operate, less time consuming with no work up and reaction conditions are mild. The newly developed 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

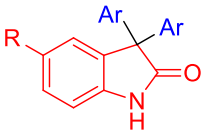




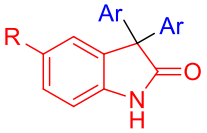
Ar = 1,3,5-trimethoxy benzene
1: R = Cl, 93%
2: R = Br, 87%



Ar = 1,3-dimethoxy benzene
3: R = H, 82%
4: R = Cl, 78%
5: R = Br, 80%
6: R = OMe, 88%
7: R = Me, 87%



Ar = 1,2-dimethoxy benzene
8: R = H, 77%
9: R = Cl, 79%
10: R = Br, 72%
11: R = OMe, 76%
12: R = Me, 83%



Ar = 1,4-dimethoxy benzene
13: R = H, 89%
14: R = OMe, 90%
15: R = Me, 82%

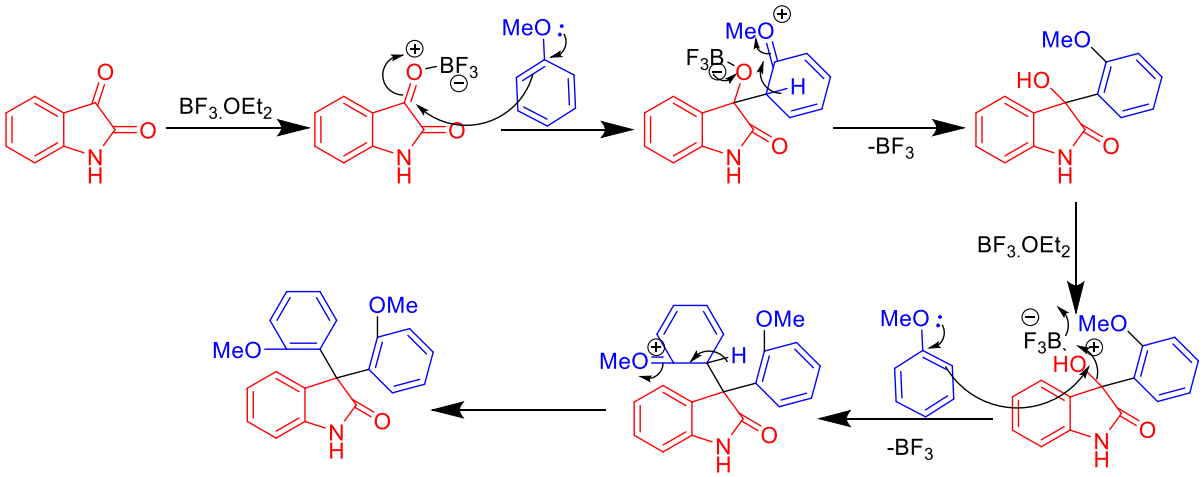


Figure 1: Probable Reaction Mechanism for the Formation of 3,3-Disubstituted Oxindole Scaffold

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

Compound No.	R	IC_{50} values in (μM)[a]	
		MCF7[b]	SKVO3[c]
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

[a] 50% cytotoxic concentration or compound concentration required to reduce viability of MCF7 or SKVO3 cell lines by 50%, using the MTT methodology, [b] Breast cancer cell lines,
[c] Ovarian cancer cell lines, MCF7 breast cancer (ATCC® HTB22™), SKOV3 ovarian cancer (ATCC® HTB 77™)

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Sunil Misra (Senior Scientist, Biology Division, CSIR-IICT, and Hyderabad) for providing biological laboratory facilities to perform biological evaluation. We also thank Mrs. Swetha and Mr. Devender, (Discovery Labs, CSIR-IICT, Hyderabad), for providing facilities to perform chemical reactions.

REFERENCES

1. Naresh Kumar R, Poornachandra Y, Nagender Y, Santhosh Kumar G, Krishna Swaroop D, Ganesh Kumar, et al. Synthesis of novelnicotino-Hydarzide and (1,3,4-oxadiazole-2-yl)-6-(Trifluro methyl)pyridine derivatives as potential Anticancer agents. Bioorg Med Chem Lett 2016; *26*: 4829-4831.
2. Ibrahim HS, Abou-seri SM, Tanc M, Elaasser MM, Abdel-Aziz HA, Supuran CT. Isatin-pyrazole benzenesulfonamide hybrids potently inhibit tumor-associated carbonic anhydrase isoforms IX and XII. Eur J Med Chem 2015; 103: 583-593.
3. Rohini R, Reddy PM, Shanker K, Kanthaiah K, Ravender V, Hu A. Synthesis of mono, bis-2-(2-arylideneaminophenyl) indole azomethines as potential antimicrobial agents. Arch Pharm Res 2011; 34: 1077-1084.
4. Verma M, Pandeya SN, Singh KN, Stables JP. Anticonvulsant activity of Schiff bases of isatin derivatives. Acta pharm 2004; 54: 49-56.
5. Pandeya SN, Yogeeshwari P, Sri Ram, Nath DG. Synthesis, antibacterial and antifungal activities of N-mannich bases of 3-[N2-pyrimethaminylimino] isatin. Indian J Pharm Sci 2002; 64: 209-212.
6. Selvam P, Murugesh N, Chandra mohan M, Debyser Z, Witvrouw M. Synthesis and antiHIV activity of Novel Isatin-Sulphonamides. Indian J Pharm Sci 2008; 70: 779-782.
7. Sridhar SK, Ramesh. Synthesis and pharmacological activities of hydrazones, Schiff and Mannich bases of isatin derivatives. Biol Pharm Bull 2001; 24: 1149-1152.
8. Eldehna WM, Altoukhy A, Maherous H, Abdel-Aziz HA. Design, synthesis and QSAR study of certain isatin-pyridine hybrids as potential anti-proliferative agents. Eur J Med Chem 2015; 90: 684-694.
9. Sharma P, Senwar KR, Jeengar MK, Reddy TS, Naidu VG, Kamal A, Shankaraiah N. H_2O-mediated isatin spiroepoxide ring opening with NaCN: Synthesis of novel 3-tetrazolylmethyl-3-hydroxy-oxindole hybrids and their anticancer evaluation. Eur J Med Chem 2015; 104: 11-24.
10. Pervez H, Ramzan M, Yaqub M, Khan KM. Cytotoxic and Phytotoxic Effects of Some New N4-Aryl Substituted Isatin-3-thiosemicarbazones. Med Chem 2012; 8: 505-514.
11. Hamdy AM, Al-Masoudi NA, Pannecouque C, Rahman Q, Villinger A, Langer P. Regioselective synthesis Suziki-Miyaura reaction of 4,7-dichloro-N-methylisatin. Synthesis, anti-HIV activity and modelling studies. Rsc Adv 2015; 5: 107360-107369.
12. Bandgar BP, Shaikh AK. Molecular iodine catalyzed efficient and highly rapid synthesis of bis(indolyl) methanes under mild conditions. Tetrahedron lett 2003; 44: 1959-1961.
13. Bandgar BP, Bettigeri SA, Joshi NS. Hexamethylenetetramine-Bromine Catalysed Rapid and Efficient synthesis of bis(indolyl) methanes. Monatshefte fur Chemie 2004; 135: 1265-1273.
14. Silveira CC, Mendes SR, Libero FM, Leonardao EJ, Perrin G. Glycerine and $CeCl_3$ $7H_2O$:A new and efficient recyclable medium for the synthesis of bis (indolyl) methanes. Tetrahedron lett 2009; 50: 6060-6063.
15. Xia M, Wang SB, Yuan WB. Lewis acid catalysed electrophillic substitution of indole with schiffs bases under microwave solvent free irradiation. Synth Commun. 2004; 34: 3175-3182.
16. Babu G, Sridhar N, Perumal PT. A convenient method of synthesis of bis(indolyl)methanes: Indium trichloride catalysed reaction of indoles with aldehydes and schiffs bases. Synth Commun 2000; 30: 1609-1614.
17. Bandgar BP, Shaikh KA. Organic reactions in aqueous media: In F3 catalysed synthesis of bis(indolyl)methanes in water under mild conditions. J Chem Res Synop 2004; 34: 72-74.
18. Reddy YT, Reddy PN, Kumar BS, Rajitha B. Efficient synthesis of Bis(indolyl)methanes catalysed by TiCl4. Indian J Chem 2005; 44B: 2393-2395.
19. Kamal A, Qureshi AA. Synthesis of some substitutrd Di Indolyl methanes in aqueous medium at room temperature. Tetrahedron lett 1963; 19: 513-520.
20. Chen D, Yu L, Wang PG. Lewis acid catalysed reactions in protic media, Lanthanides-catalyzed reaction of indoles with aldehydes or ketones. Tetrahedron lett 1996; 37: 4467-4470.

21. Ji SJ, Zhou MF, Gu DG, Wang SY, Loh TP. Effecient synthesis of Bis(indolyl)methanes catalysed by Lewis acids in ionic liquids. Syn lett 2003; 13: 2077-2079.
22. ME XL, Luo SZ, He JK, Cheng JP. Simple and efficient method for synthesis of Bis(indolyl)methanes with Cu(BF4).SiO2 Under mild conditions. Tetrahedron lett 2004; 45: 4567-4570.
23. Yadav JS, Reddy BVS, Sunitha S, Reddy KS. Novel use of selectfluor^{TM} for th synthesis of cis-fused pyrano- and furano-tetrahydro quinoline. Adv Synth Catal 2003; 345-349.
24. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65: 55-63.

Cite this article as:

Sasikala Maadwar *et al*. A facile and an efficient synthesis of 3, 3-disubstituted oxindole scaffolds and their cytotoxic properties. Int. Res. J. Pharm. 2019;10(4):190-195 http://dx.doi.org/10.7897/2230-8407.1004148

Source of support: Nil, Conflict of interest: None Declared