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Original Research Article

Synthesis and *in vitro* evaluation of novel isatin-incorporated thiadiazole hybrids as potential anti-breast cancer agents

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Abstract

Purpose: *To synthesis and characterize some novel isatin-incorporated thiadiazoles and screen them for anti-breast cancer activity in human breast adenocarcinoma cells (MCF-7).*
Method: *A series of isatin incorporated Schiff bases of thiadiazoles (3a-3l) was synthesized by reaction of substituted thiadiazoles (1a-1d) with isatin (2a) and N-alkyl substituted isatin (2b-2c) and characterized by elemental analysis, IR, 1H NMR, 13C NMR and LCMS. The newly synthesized compounds were screened for their in-vitro cytotoxicity against MCF-7 cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric and Sulforhodamine B (SRB) methods.*
Results: *Compounds 3a, 3c, 3d, 3g and 3j showed anticancer activity in both MTT and SRB assay. Compound 3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)-1-ethylindolin-2-one (3g) showed most potent cytotoxic activity against MCF-7 cell lines.*
Conclusion: *The novel isatin incorporated thiadiazoles synthesized and characterized in this study possess anti-cancer activities in human breast adenocarcinoma cells (MCF-7). This can possibly lead to emergence of new anti-breast cancer agents.*

Keywords: *Thiadiazoles, Isatin, In-vitro cytotoxicity, Human breast adenocarcinoma cells (MCF-7), SRB assay*

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INTRODUCTION

After cardio-vascular diseases, cancer is the second major cause of mortality in the world and accounting for 8.2 million deaths in 2012. Furthermore, breast cancer is a most common form of invasive cancer in women throughout the world and remains the most frequent cause of malignancy associated deaths among women. The effective treatment of this disease is restricted due to the development of breast cancer resistance against chemotherapy and endocrine therapy [1-3]. At the same time, isatin derivatives have also been reported as cytotoxic agents [4,5]. Some of the isatin incorporated thiazolines and benzthiazoles have shown significant anti breast cancer activity [6,7]. Therefore, there is essential to search and develop novel and potent chemotherapeutic agents that could overcome this health problem.

Thiadiazole, a promising heterocyclic template that has versatile pharmacological activity constitutes major class of agents for development of novel drugs. Numerous studies demonstrated that thiadiazole scaffold shows anticancer activity against various tumor cell lines [8-10]. Various reports suggested that various substituted thiadiazole derivatives exhibited significant anticancer activity against MCF-7 cell lines [11-13].

In this work, the objective was to incorporate isatins to thiadiazole moiety by using pharmacophore hybridization approach as it was expected that these two active anticancer pharmacophores may have synergistic effect.

EXPERIMENTAL

Materials

Human breast adenocarcinoma cells (MCF-7) were purchased from NCCS Pune (India). MTT reagent, 10 % foetal bovine serum (FBS), Dulbacco's minimum essential medium (DMEM) media and sulphorhodamine solution were purchased from Sigma Aldrich (USA). Antimycotic solution and TPVG solution were obtained from Himedia. Tissue culture flasks, 96 well microculture plates obtained from Tarson and Nunc (USA). All analytical grade reagents and solvents were purchased from Merck Chemical Co (Germany) and dried in a desiccator as appropriate.

Synthetic procedures

Synthesis of 2-amino-5-aryl-1,3,4-thiadiazoles (1a-1d)

The thiosemicarbazide (0.1 mol) was added in aryl carboxylic acid (0.1 mol) in an equipped assembly with a stirring bar. The reaction mixture was poured slowly over 0.5 h to polyphosphoric acid (10 times the weight of carboxylic acid) at 80-90 °C with stirring. The reaction mixture was stirred at 80-90 °C for 2-4 h and then cooled to room temperature; further water/ice was added. Finally, the mixture was made alkaline by NH_3 (0.88 g/ml), filtered and dried. The desired product was eluted from EtOH and purified by using column chromatography [14] (Figure 1).

Synthesis of 1-alkyl-3-(5-aryl-1,3,4-thiadiazol-2-ylimino)indolin-2-ones (3a-3l)

Equimolar quantities of an appropriate 2-amino-5-aryl-1,3,4-thiadiazoles (**1a-1d)** and isatins (**2a-2c)** (0.001 mol each) were dissolved in absolute ethanol (15 ml). Further, glacial acetic acid (0.2 ml) was added to the reaction mixture with continuous stirring. The reaction mixture was refluxed for 10-12 h with stirring, concentrated under reduced pressure to remove absolute ethanol and acetic acid and leave the mixture to cool at room temperature. The crude solid mixture was filtered and eluted from ethanol (70 %) to furnish **3a**-**3l** (Figure 1).

Characterization of synthesized compounds

All the reactions were followed by thin layer chromatography (TLC) analysis which performed on silica gel G for TLC (Merck) and visualized with iodine vapour. Melting points (ºC) were determined in open capillary tubes and were uncorrected. Infra-red absorption spectra were recorded on Jasco FT/IR-470 PLUS (Japan), KBr diffuse reflectance (υ_{max} in cm^{-1}). The ^{1}H and ^{13}C chemical shifts were recorded on the field strength 400 and 100 MHz specified by Bruker DPX-400 instrument, England and measured as parts per million (ppm) downfield from TMS (Me_4Si). The LCMS of the synthesized compounds were recorded on Shimadzu 8201PC spectrometer, Japan. IR, ^{1}H NMR, ^{13}C NMR and LCMS were consistent with the proposed structures. Elemental analysis was done on a CHN rapid analyzer and showed satisfactory results within ± 0.4 % of the theoretical values.

Determination of *in-vitro* cytotoxic activity

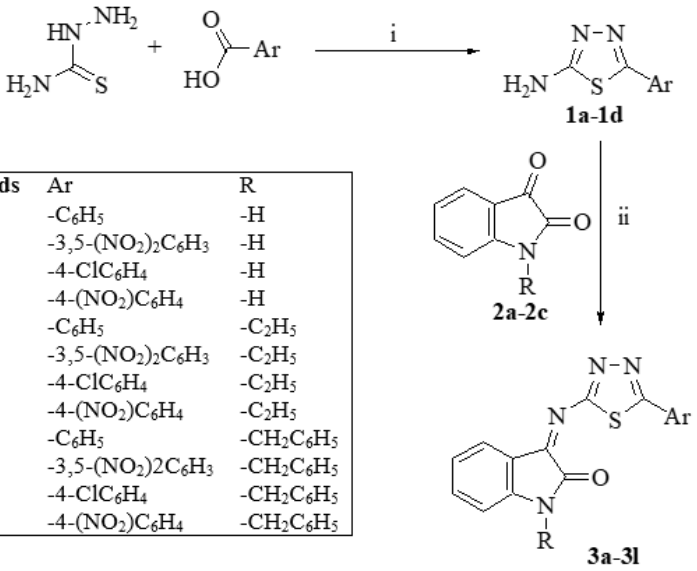
The isatin incorporated thiadiazoles (**3a-3l**) were screened for *in vitro* cytotoxicity against MCF-7 cell lines by MTT and SRB models.

Maintenance of cell lines

MCF-7 cells were grown in 75 cm^2 tissue culture flasks containing DMEM media supplemented with 10 % FBS, TPVG solution at 37 ºC in CO_2 incubator in an atmosphere of humidified 95 % air and 5 % CO_2. Further, they were maintained by sub culturing in 75 cm^2 tissue culture flasks.

MTT assay

The MTT was reduced to a colored product due to activity of NAD (P) H-dependent deshydrogenases enzyme. It indicates the level of energy metabolism in cells. The cells were seeded in 96-well microplates in DMEM medium supplemented (0.1 ml) with FBS (10 %). These cells were cultured routinely in a humidified incubator in 5 % CO_2 at 37 °C for 24 h. Test compounds were added in various concentrations (5-25 µg/ml) and further incubated for 24 h.



Compounds	Ar	R
3a	$-C_6H_5$	-H
3b	$-3,5-(NO_2)_2C_6H_3$	-H
3c	$-4-ClC_6H_4$	-H
3d	$-4-(NO_2)C_6H_4$	-H
3e	$-C_6H_5$	$-C_2H_5$
3f	$-3,5-(NO_2)_2C_6H_3$	$-C_2H_5$
3g	$-4-ClC_6H_4$	$-C_2H_5$
3h	$-4-(NO_2)C_6H_4$	$-C_2H_5$
3i	$-C_6H_5$	$-CH_2C_6H_5$
3j	$-3,5-(NO_2)2C_6H_3$	$-CH_2C_6H_5$
3k	$-4-ClC_6H_4$	$-CH_2C_6H_5$
3l	$-4-(NO_2)C_6H_4$	$-CH_2C_6H_5$

Figure 1: Reaction scheme for the synthesis of **3a-3l**: (*i*) Polyphosphoric acid**;** (*ii*) EtOH, CH_3COOH

The medium was discarded, tetrazolium dye (MTT) solution (0.1 ml, 1 mg/ml in PBS) was added to every well and re-incubated for 4 h. The formazan crystals formed was dissolved by adding of 0.1 ml DMSO. The plate was then read on a microplate reader at 540 nm [15,16].

SRB assay

Cell suspension (0.1 ml) of optimum density was added into each well of 96-well plates. Various concentrations (5-25 µg/ml) of test compounds were made in the culture medium. Each concentration of test compounds (0.1 ml) was added to the wells containing the cells and 0.1 ml of medium only to the control wells. Then these cells were incubated with the test compounds for 48 h and fixed with ice-cold trichloroacetic acid (TCA) at 4 °C for 1 h. Then these plates were washed 5 times in water and dried in the air. Further SRB solution (0.05 ml) was added to each well of the dried 96-well plates and kept for staining at room temperature for half an hour. Then SRB solution was removed by washing the plates with acetic acid (1 % v/v) 5 times to remove dye. Bound SRB was solubilized using unbuffered Tris Base (0.1 ml, 10 mM, pH 10.5) to each well and shaked for 5 min. Plates were read using a 96-well plate reader at the working wavelength 492 nm [17].

RESULTS

Synthesized compounds

The following compounds were successfully synthesized:

*5-phenyl-1,3,4-thiadiazol-2-amine (**1a**).* Yield, 65 %; mp 226-228°C. IR u_{max}/cm^{-1} 3488 (NH), 3074 (Aromatic C–H), 1596 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 7.26–7.59 (m, 5H, Ar-H), 5.13 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 172.1 (C_2), 167.9 (C_5), 133.7 (C_1-Ar), 131.7 (C_4-Ar), 129.4 ($C_{3,5}$-Ar), 126.7 ($C_{2,6}$-Ar). LCMS *m/z*; 177.2, $[M+1]^+$. Anal. Calcd for $C_8H_7N_3S$ (%): C, 54.22; H, 3.98; N, 23.71. Found: C, 54.34; H, 4.02; N, 23.54.

*5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-amine (**1b**).* Yield, 62 %; mp 242-246°C. IR u_{max}/cm^{-1} 3488 (NH), 3058 (Aromatic C–H), 1607 (C=N), 1469 (C-NO_2). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 7.69-7.86 (m, 3H, Ar-H), 6.13 (s, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 171.6 (C_2), 163.2 (C_5), 136.4 ($C_{3,5}$-Ar), 135.7 (C_1-Ar), 129.5 ($C_{2,6}$-Ar), 119.7 (C_4-Ar). LCMS *m/z*; 267.1, $[M+1]^+$. Anal. Calcd for $C_8H_5N_5O_4S$ (%): C, 35.96; H, 1.89; N, 26.21. Found: C, 35.75; H, 1.96; N, 26.33.

*5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (**1c**).* Yield, 72 %; mp 230-234°C. IR u_{max}/cm^{-1} 3471 (NH), 3064 (Aromatic C–H), 1611 (C=N), 821 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.99-7.38 (m, 4H, ArH), 3.64 (s, 2H, NH2). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 174.2 (C_2), 163.7 (C_5), 134.3 (C_1-Ar), 132.8 (C_4-Ar), 128.3 ($C_{3,5}$-Ar), 125.7 ($C_{2,6}$-Ar). LCMS *m/z*; 211.1, $[M+1]^+$. Anal. Calcd for $C_8H_6ClN_3S$ (%): C, 45.39; H, 2.86; N, 19.85. Found: C, 45.53; H, 2.79; N, 19.97.

*5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (**1d**).* Yield, 69 %; mp 228-232°C. IR u_{max}/cm^{-1} 3463 (NH), 3053 (Aromatic C–H), 1619 (C=N), 1452 (C-NO_2). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 7.53-

7.69 (m, 3H, Ar-H), 5.92 (s, 2H, NH_2).^{13}C NMR (100 MHz, DMSO-d_6): δ: 173.8 (C_2), 164.3 (C_5), 143.3 (C_4-Ar), 137.8 (C_1-Ar), 126.6 ($C_{2,6}$-Ar), 125.2 ($C_{3,5}$-Ar).LCMS *m/z*;222.0, $[M+1]^+$. Anal. Calcd for $C_8H_6N_4O_2S$ (%): C, 43.24; H, 2.72; N, 25.21. Found: C, 43.43; H, 2.59; N, 25.28.

*3-(5-phenyl-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3a**).* Yield, 79 %; mp 232-236 °C. IR υ_{max}/cm^{-1} 3476 (NH), 3069 (Aromatic C–H), 1711 (C=O), 1608, 1586 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 8.32 (s, 1H, NH), 6.83-7.58 (m, 9H, Ar). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 174.4 (C_5-thia), 169.4 (C_2), 167.5 (C_2-thia), 149.4 (C_3), 145.9 (C_8), 135.7 (C_1-Ar), 134.7 (C_5), 132.9 (C_4-Ar), 129.0 ($C_{3,5}$-Ar), 126.8 (C_4), 125.6 ($C_{2,6}$-Ar), 124.1(C_6), 119.7 (C_9), 116.2 (C_7). LCMS *m/z*; 306.1, $[M+1]^+$. Anal. Calcd for $C_{16}H_{10}N_4OS$ (%): C, 62.73; H, 3.29; N, 18.29. Found: C, 62.56; H, 3.42; N, 18.42.

*3-(5-(3,5-dinitrophenyl)-1,3,4thiadiazol-2-ylimino)indolin-2-one (**3b**).* Yield, 71 %; mp 282-284 °C. IR υ_{max}/cm^{-1} 3462 (NH), 3077 (Aromatic C–H), 1694 (C=O), 1619, 1594 (C=N), 1482 (C-NO_2). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 8.43 (s, 1H, NH), 6.96-7.71 (m, 7H, Ar). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 172.6 (C_5-thia), 168.2 (C_2), 166.3 (C_2-thia), 149.5 (C_3), 146.6 ($C_{3,5}$-Ar), 144.5 (C_8), 135.6 (C_1-Ar), 132.7 (C_6), 126.2 ($C_{2,6}$-Ar), 125.1 (C_4), 123.3 (C_5), 117.8 (C_9), 117.6 (C_4-Ar), 115.8 (C_7). LCMS *m/z*; 396.0, $[M+1]^+$. Anal. Calcd for $C_{16}H_8N_6O_5S$ (%): C, 48.49; H, 2.03; N, 21.20. Found: C, 48.33; H, 2.09; N, 21.32.

*3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3c**).* Yield, 74 %; mp 258-262°C. IR υ_{max}/cm^{-1} 3484 (NH), 3082 (Aromatic C–H), 1723 (C=O), 1611, 1590 (C=N), 819 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6); 8.38 (s, 1H, NH), 7.03-7.52 (m, 8H, Ar). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 171.4 (C_5-thia), 167.5 (C_2), 166.1 (C_2-thia), 148.8 (C_3), 144.6 (C_8), 136.4 (C_4-Ar), 133.8 (C_1-Ar), 132.9 (C_6), 129.1 ($C_{3,5}$-Ar), 127.3 ($C_{2,6}$-Ar), 125.8 (C_4), 123.3 (C_5), 117.9 (C_9), 113.2 (C_7). LCMS *m/z*; 341.1, $[M+1]^+$. Anal. Calcd for $C_{16}H_9ClN_4OS$ (%): C, 56.39; H, 2.66; N, 16.44.Found: C, 56.56; H, 2.72; N, 16.48.

*3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3d**).* Yield, 69 %; mp 268-272°C. IR υ_{max}/cm^{-1} 3475 (NH), 3063 (Aromatic C–H), 1698 (C=O), 1616, 1599 (C=N), 1466 (C-NO_2). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 8.41 (s, 1H, NH), 6.89-7.62 (m, 8H, Ar). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 172.1 (C_5-thia), 168.2 (C_2), 166.5 (C_2-thia), 148.5 (C_3), 147.4(C_4-Ar) , 143.9 (C_8), 139.7 (C_1-Ar), 132.9 (C_6), 126.8 ($C_{2,6}$-Ar), 125.4 (C_4), 124.3 ($C_{3,5}$-Ar), 123.3 (C_5), 117.8 (C_9), 113.7 (C_7). LCMS *m/z*; 352.1, $[M+1]^+$. Anal. Calcd for $C_{16}H_9N_5O_3S$ (%): C, 54.70; H, 2.58; N, 19.93. Found: C, 54.82; H, 2.73; N, 19.71.

*1-ethyl-3-(5-phenyl-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3e**).* Yield, 72 %; mp 224-226°C. IR υ_{max}/cm^{-1} 3081 (Aromatic C–H), 1718 (C=O), 1612, 1586 (C=N), 1349 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.97-7.52 (m, 9H, Ar), 3.58-3.62 (q, 2H, CH_2), 1.29-1.31 (t, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 170.7 (C_5-thia), 167.4 (C_2), 165.8 (C_2-thia), 153.3 (C_3), 146.6 (C_8), 133.7 (C_1-Ar), 133.6 (C_6), 132.0 (C_4-Ar), 129.2 ($C_{3,5}$-Ar), 128.4 (C_4), 126.8 ($C_{2,6}$-Ar), 123.4 (C_5), 117.3 (C_9), 113.3 (C_7), 39.3 (-CH_2-), 18.5 (-CH_3). LCMS *m/z*; 335.1, $[M+1]^+$. Anal. Calcd for $C_{18}H_{14}N_4OS$ (%): C, 64.65; H, 4.22; N, 16.75. Found: C, 64.83; H, 4.27; N, 16.62.

*1-ethyl-3-(5-(3,5-dinitrophenyl)-1,3,4thiadiazol-2-ylimino)indolin-2-one (**3f**).* Yield, 78 %; mp 208-212°C. IR υ_{max}/cm^{-1} 3076 (Aromatic C–H), 1705 (C=O), 1608, 1592 (C=N), 1474 (C-NO_2), 1352 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.73-7.77 (m, 7H, Ar), 3.63-3.66 (q, 2H, CH_2), 1.32-1.35 (t, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 172.4 (C_5-thia), 167.7 (C_2), 166.7 (C_2-thia), 152.7 (C_3), 147.5 (C_8), 146.7 ($C_{3,5}$-Ar), 136.2 (C_1-Ar), 134.3 (C_6), 125.5 (C_4), 124.9 ($C_{2,6}$-Ar), 124.6 (C_5), 119.2 (C_4-Ar), 118.6 (C_9), 114.3 (C_7), 40.6 (-CH_2-), 19.4 (-CH_3). LCMS *m/z*; 425.1, $[M+1]^+$. Anal. Calcd for $C_{18}H_{12}N_6O_5S$ (%): C, 50.94; H, 2.85; N, 19.80. Found: C, 50.81; H, 2.72; N, 19.67.

*3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)-1-ethylindolin-2-one (**3g**).* Yield, 70 %; mp 242-246°C. IR υ_{max}/cm^{-1} 3058 (Aromatic C–H), 1687 (C=O), 1623, 1597 (C=N), 1343 (C-N), 824 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 7.06-7.65 (m, 8H, Ar), 3.57-3.60 (q, 2H, CH_2), 1.30-1.33 (t, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 170.8 (C_5-thia), 166.7 (C_2), 162.2 (C_2-thia), 155.3 (C_3), 146.7 (C_8), 136.5 (C_4-Ar), 132.2 (C_1-Ar), 131.6 (C_6), 129.1 ($C_{3,5}$-Ar), 127.2 ($C_{2,6}$-Ar), 124.4 (C_4), 121.6 (C_5), 116.8 (C_9), 113.5 (C_7), 41.3 (-CH_2-), 17.5 (-CH_3). LCMS *m/z*; 368.2, $[M+1]^+$. Anal. Calcd for $C_{18}H_{13}ClN_4OS$ (%): C, 58.61; H, 3.55; N, 15.19. Found: C, 58.68; H, 3.63; N, 15.31.

*1-ethyl-3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3h**).* Yield, 75 %; mp 272-274°C. IR υ_{max}/cm^{-1} 3065 (Aromatic C–H), 1723 (C=O), 1611, 1596 (C=N), 1478 (C-NO_2), 1338 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.83-7.68 (m, 8H, Ar), 3.61-3.65 (q, 2H, CH_2), 1.27-1.30 (t, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 172.4 (C_5-thia), 167.2 (C_2), 163.8 (C_2-thia), 153.5 (C_3), 147.4 (C_4-Ar), 144.3 (C_8), 137.5 (C_1-Ar), 130.6 (C_6), 129.3 (C_4), 126.3 ($C_{2,6}$-Ar), 124.7

($C_{3,5}$-Ar), 123.4 (C_5), 120.3 (C_9), 113.5(C_7), 42.3 (-CH_2-), 18.5 (-CH_3). LCMS *m/z*; 380.2, $[M+1]^+$. Anal. Calcd for $C_{18}H_{13}N5O_3S$ (%): C, 56.98; H, 3.45; N, 18.46. Found: C, 56.87; H, 3.53; N, 18.33.

*1-benzyl-3-(5-phenyl-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3i**).* Yield, 73 %; mp 288-292°C. IR υ_{max}/cm^{-1} 3087 (Aromatic C–H), 1709 (C=O), 1619, 1588 (C=N), 1346 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.67-7.72 (m, 14H, Ar), 4.29-4.33 (d, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 171.1 (C_5-thia), 167.4 (C_2), 164.2 (C_2-thia), 152.3 (C_3), 146.6 (C_8), 136.0 (C_1-Ar_2), 132.6 (C_1-Ar_1), 133.1 (C_6), 131.7 (C_4-Ar_1), 128.0 ($C_{3,5}$-Ar_1), 125.6 ($C_{3,5}$-Ar_2), 123.3 ($C_{2,6}$-Ar_2), 121.1 (C_4-Ar_2), 118.1 (C_4), 118.8 ($C_{2,6}$-Ar_1), 116.3 (C_5), 115.9 (C_9), 113.0 (C_7), 50.13 (-CH_2-). LCMS *m/z*; 397.1, $[M+1]^+$. Anal. Calcd for $C_{23}H_{16}N_4OS$ (%): C, 69.68; H, 4.07; N, 14.13. Found: C, 69.82; H, 4.15; N, 14.23.

*1-benzyl-3-(5-(3,5-dinitrophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3j**).* Yield, 76 %; mp 320-324°C. IR υ_{max}/cm^{-1} 3081 (Aromatic C–H), 1693 (C=O), 1609, 1573 (C=N), 1471 (C-NO_2), 1359 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.83-7.89 (m, 12H, Ar), 4.29-4.33 (d, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 171.4 (C_5-thia), 168.6 (C_2), 163.2 (C_2-thia), 155.3 (C_3), 147.5 ($C_{3,5}$-Ar_1), 141.6 (C_8), 138.0 (C_1-Ar_2), 133.2 (C_1-Ar_1), 133.3 (C_6), 126.6 ($C_{3,5}$-Ar_2), 125.3 ($C_{2,6}$-Ar_2), 126.1 (C_4-Ar_2), 123.2 ($C_{2,6}$-Ar_1), 121.1 (C_4), 120.3 (C_5), 117.0 (C_4-Ar_1), 115.1 (C_9), 111.0 (C_7), 52.3 (-CH_2-). LCMS *m/z*; 486.4, $[M+1]^+$. Anal. Calcd for $C_{23}H_{14}N_6O_5S$ (%): C, 56.79; H, 2.90; N, 17.28. Found: C, 56.69; H, 2.98; N, 17.38.

*1-benzyl-3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3k**).* Yield, 73 %; mp 262-264°C. IR υ_{max}/cm^{-1} 3079 (Aromatic C–H), 1713 (C=O), 1614, 1574 (C=N), 1352 (C-N), 816 (C-Cl). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.92-7.72 (m, 13H, Ar), 4.29-4.33 (d, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 170.9 (C_5-thia), 167.4 (C_2), 164.2 (C_2-thia), 154.3 (C_3), 147.6 (C_8), 139.1 (C_1-Ar_2), 136.6 (C_4-Ar_1), 131.2 (C_1-Ar_1), 129.5 (C_6), 126.1 ($C_{3,5}$-Ar_1), 123.6 ($C_{3,5}$-Ar_2), 121.3 ($C_{2,6}$-Ar_2), 120.0 (C_4-Ar_2), 118.5 ($C_{2,6}$-Ar_1), 112.1 (C_4), 111.3 (C_5), 110.7 (C_9), 107.0 (C_7), 56.3 (-CH_2-). LCMS *m/z*; 431.1, $[M+1]^+$. Anal. Calcd for $C_{23}H_{15}ClN_4OS$ (%): C, 64.11; H, 3.51; N, 13.00. Found: C, 64.24; H, 3.62; N, 12.89.

*1-benzyl-3-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3l**).* Yield, 77 %; mp 294-296°C. IR υ_{max}/cm^{-1} 3068 (Aromatic C–H), 1722 (C=O), 1619, 1589 (C=N), 1484 (C-NO_2), 1361 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6); δ: 6.71-7.81 (m, 13H, Ar), 4.29-4.33 (d, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ: 171.0 (C_5-thia), 166.2 (C_2), 163.2 (C_2-thia), 157.3 (C_3), 148.1 (C_4-Ar_1), 142.6 (C_8), 139.5 (C_4-Ar_1), 137.0 (C_1-Ar_2), 133.1 (C_6), 128.7 ($C_{3,5}$-Ar_2), 126.3 ($C_{2,6}$-Ar_2), 122.1 (C_4-Ar_2), 120.7 ($C_{2,6}$-Ar_1), 118.4 (C_4), 115.7 ($C_{3,5}$-Ar_1), 111.3 (C_5), 106.9 (C_9), 102.0 (C_7), 53.13 (-CH_2-). LCMS *m/z*; 441.1, $[M+1]^+$. Anal. Calcd for $C_{23}H_{15}N_5O_3S$ (%): C, 62.43; H, 3.51; N, 15.94. Found: C, 69.59; H, 4.33.

Of the 12 compounds synthesized, five compounds (**3a**, **3c**, **3d**, **3g** and **3j**) of the 1-alkyl-3-(5-aryl-1,3,4-thiadiazol-2-ylimino)indolin-2-ones (**3a-3l**) significantly decreased MCF-7 cell viability produced cytotoxic activity (IC_{50} for MCF-7 by MTT assay of 12,62-21.41µg/ml, Table 1).

Table 1: *In vitro* cytotoxic activity of isatin incorporated Schiff bases of thiadiazoles (**3a-3l**) against MCF-7

Compound	IC_{50} (µg/ml)	
	MTT assay	***SRB assay***
3a	13.72 ± 0.1058	16.41± 0.4179
3b	-	-
3c	12.62± 0.2586	15.14± 0.3925
3d	16.37± 0.3805	18.44± 0.5196
3e	-	-
3f	-	-
3g	10.46± 0.1637	13.04± 0.3435
3h	-	-
3i	-	-
3j	21.41± 0.4676	24.27± 0.2464
3k	-	-
3l	-	-

The results represent mean ± SEM of three independent experiments. IC_{50}values were obtained using a dose response curve by non-linear regression using a curve fitting program, Graph Pad Prism 5.0. IC_{50}> 25 µg/ml

DISCUSSION

In this work, 12 novel isatin incorporated thiadiazoles have successfully been synthesized and characterised. Five of the compounds demonstrated cytotoxic effects. In the synthetic procedure, thiosemicarbazide was reacted with substituted aromatic carboxylic acid in the presence of polyphosphoric acid to yield 2-amino-5-aryl-1,3,4-thiadiazoles (**1a-1d**). The proposed structures of these compounds were confirmed on the basis of spectroscopic data (IR and NMR) and elemental analysis. The IR spectra showed C=N absorption bands at 1619-1596 cm^{-1}.

In the last step, 2-amino-5-aryl-1,3,4-thiadiazoles (**1a-1d**) were reacted with isatin (**2a**) and N-alkyl substituted isatin (**2b-2c**) to formation of isatin incorporated Schiff bases of thiadiazoles (**3a-3l**). The proposed structures of (**3a-3l**) were confirmed by spectroscopic data (IR, ^{1}H and ^{13}C

NMR) and elemental analysis and data are shown in experimental protocol section. The structures of compounds **3a-3l** were confirmed by the disappearance of NH_2 peak in ^1H NMR spectra. The remaining protons appeared at the expected chemical shifts.

From the structure-activity relationship (SAR) study, it was found that the substitution at C-5 position of the 1,3,4-thiadiazole ring may affect the cytotoxic activity to the compounds. The compound **3c** and **3g,** possessing a 4-chlorophenyl ring at C-5 position, were found to be most active compounds. Replacement of 4-chloro with 4-nitro or 3,5-dinitro decreased cytotoxicity against MCF-7 cell lines. Compound 3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)-1-ethylindolin-2-one (**3g**) showed highest potency with IC_{50} **10.46** and **13.04** µg/ml in MTT and SRB assay, respectively, against MCF-7 cells.

CONCLUSION

A novel and simple method for the synthesis of isatin incorporated Schiff bases of thiadiazoles has been developed. Some of the synthesized compounds produced cytotoxic activity against MCF-7 cells; in particular, the compounds 3-(5-phenyl-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3a**), 3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)indolin-2-one (**3c**) and 3-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-ylimino)-1-ethylindolin-2-one (**3g**) were found as promising compounds and could serve as leads for further modification to develop clinically useful anticancer agents. These new findings might be useful for scientist in future research and development of isatin incorporated thiadiazoles nucleus as newer anti-breast cancer agents.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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