

Synthesis of Novel Isatin-Thiazoline and Isatin-Benzimidazole Conjugates as Anti-Breast Cancer Agents

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A series of new isatin-thiazoline **3a-h** and isatin-benzimidazole **4a-h** derivatives were synthesized via condensation of isatin Mannich bases **2a-h** with either 2-aminothiazoline or 2-aminobenzimidazole. The structures of the newly synthesized compounds were characterized by spectral data. The anti-breast cancer activity of some of the synthesized compounds was assessed in the MCF-7 human breast cancer cell line. The results showed that compounds **4b**, **4d** and **4g** possess significant antiproliferative activity against MCF-7 cells.

Key words: Isatin, Benzimidazole, Thiazoline, Breast cancer, Anticancer activity

INTRODUCTION

Cancer is a major health concern worldwide. The relative mortality rate caused by cancer is very high in developing countries. Furthermore, breast carcinoma is the most common cancer in women worldwide and remains the most frequent cause of malignancy-associated deaths among women (Smith et al., 2009). Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited due to undesirable side effects as well as the increasing incidence of drug resistance to cancer chemotherapeutic agents, which is a serious medical problem (Rojo et al., 2008). Therefore, there is an urgent need to develop new classes of chemotherapeutic agents to treat cancer. Isatin (1H-indole-2,3-dione) is one of the most promising heterocyclic molecules that have interesting active profiles, and importantly, it is well tolerated by humans (Pandeya et al., 2005; Vine et al., 2009). Numerous publications have reported that the isatin scaffold shows anticancer activity against various human tumor cell lines (Bramson et al., 2001; Solomon et al., 2010). Significant interest in 2-oxoindole derivatives as kinase inhibitors came after the disclosure of the tyrosine kinase inhibitory properties and anti-

angiogenic properties of SU-5416 (Semaxanib) and SU-11248 (Sunitinib) (Matesic et al., 2008) (Fig. 1). The latter compound has received FDA approval for the treatment of gastrointestinal stromal tumors and advanced renal cell carcinoma (Motzer et al., 2006).

Besides the exploitation of new targets, there is another approach that combines two or more pharmacophores with anticancer activity, each with a different mode of action, into a single molecule (Matesic et al., 2008; Mayur et al., 2009). In our ongoing search for new pharmacophores as anti-breast cancer agents, benzimidazole-based compounds (I, II, Fig. 2) demonstrated high cytotoxic activity against hormone-dependent breast cancer cells (MCF-7) (Ramla et al., 2006). Benzimidazole derivatives selectively inhibit endothelial cell growth and suppress angiogenesis *in vitro* and *in vivo* (Hori et al., 2002). Furthermore, other authors have reported antiproliferative or anticancer potential of certain thiazoline derivatives (Rostom, 2006; Johnson

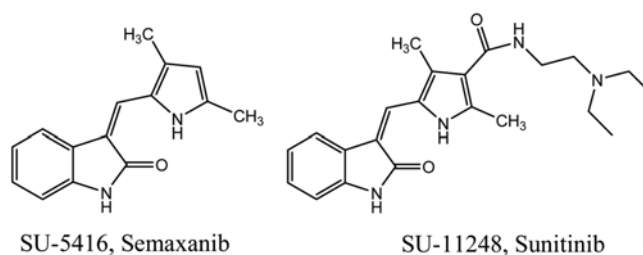


Fig. 1. Chemical structures of isatin analogues reported for anticancer activity

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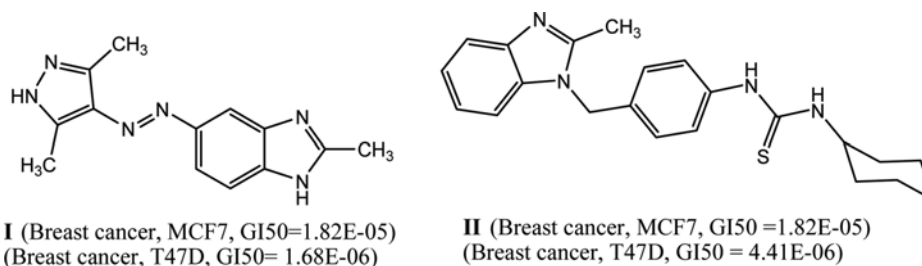
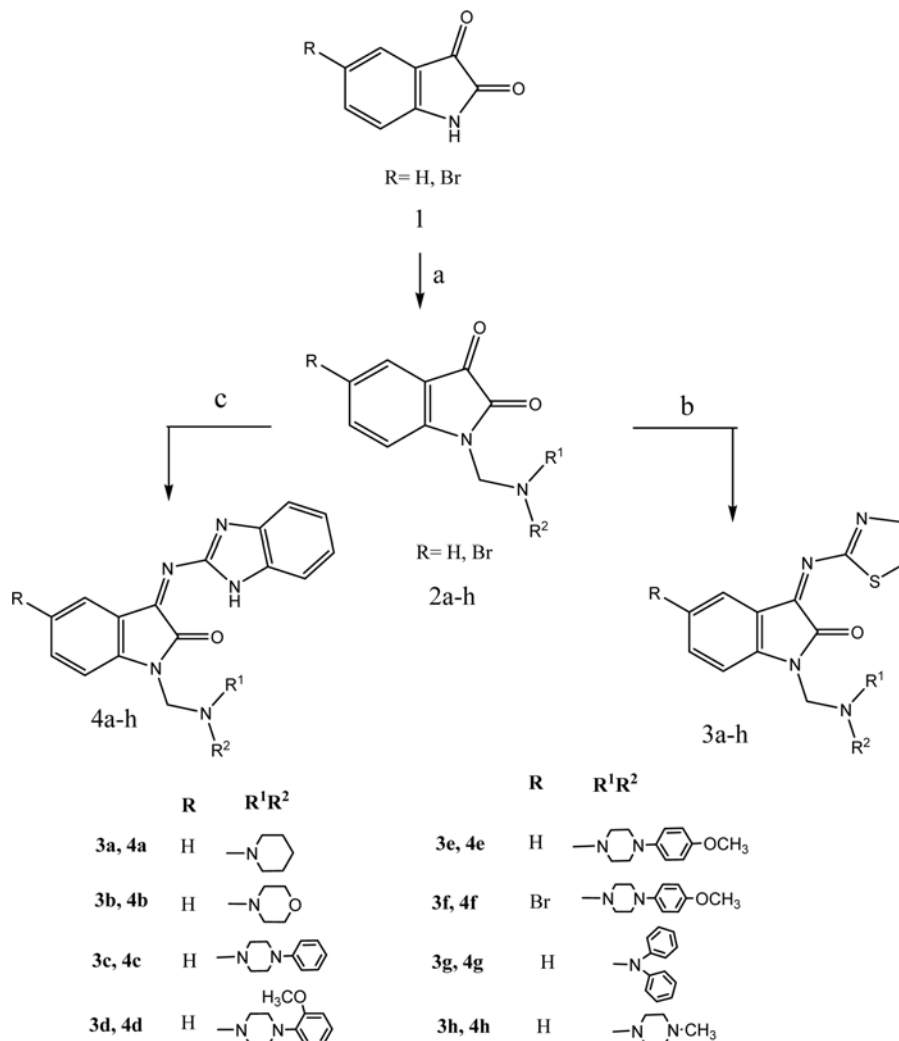


Fig. 2. Structures of previously synthesized benzimidazole analogues with anti-breast cancer activity

et al., 2007).

Due to the great potential of both moieties, and encouraged by these previous reports, we postulated that a compound containing both isatin and benzimidazole or thiazoline pharmacophores could have synergistic effects as an anticancer agent.

Herein we report the synthesis of isatin conjugates with either benzimidazole scaffold or thiazoline moiety by a Schiff base reaction **3a-h** and **4a-h** (Scheme 1), and the *in vitro* cytotoxic effects of some of the synthesized compounds on a human breast carcinoma cell line (MCF-7).



Scheme 1. Synthesis of isatin Mannich bases and Schiff base analogues. Reagents and conditions: (a) Mannich reaction (HCHO and secondary amine, ethanol); (b) 2-Aminothiazoline, ethanol, AcOH, 13-15 h; (c) 2-Aminobenzimidazole, ethanol, AcOH, 13-15 h

MATERIALS AND METHODS

Chemistry

All melting points were determined on a Stuart apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrophotometer using potassium bromide discs. ^1H -NMR spectra were recorded using a Varian Gemini 200 MHz Spectrophotometer and Varian Mercury-300 (300 MHz) Spectrophotometer using TMS as an internal standard. Chemical shift values were recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt. Mass spectra were recorded on a GCMP-QP1000EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were performed on a Perkin-Elmer 2400 C, H, N analyzer at the Microanalytical Center, Cairo University, Egypt, and values were within ± 0.4 of theoretical percentages. Progress of the reactions was monitored by TLC. TLC sheets were precoated with UV fluorescent silica gel Merck 60 F 254; CHCl_3 - CH_3OH (9.5:0.5) was used as a solvent and products were visualized using UV lamp.

General procedure for the preparation of 1-(substitutedmethyl)-1H-indole-2,3-diones (2a-h)

To a solution of isatin or 5-bromoisatin (4.08 mmol) in absolute ethanol (10 mL) was added a mixture of secondary amino compound (1.35 mmol) and formaline 37% (7 mmol) in absolute ethanol (15 mL). The reaction mixture was stirred for 6 h at 50°C and then refrigerated for 48 h. The crystalline products were separated by filtration, washed with cold methanol, dried and recrystallized from ethanol. Compounds **2a**, **b**, **c**, **g**, and **h** were prepared as reported (Solomon et al., 2009).

1-[4-(2-Methoxyphenyl)piperazin-1-yl]methyl-1H-indole-2,3-dione (2d)

Yield 76%; m.p. $144\text{--}145^\circ\text{C}$; IR (KBr, cm^{-1}): 3062, 3050 (C-H aromatic), 2943, 2831 (C-H aliphatic), 1750, 1735 (C=O); ^1H -NMR 300 MHz (DMSO- d_6): 2.71-2.74 (m, 4H, 2CH_2 piperazinyl), 2.92-2.94 (m, 4H, 2CH_2 piperazinyl), 3.77 (s, 3H, OCH_3), 4.48 (s, 2H, CH_2), 6.85-6.92 (m, 4H, Ar-H), 7.15-7.17 (m, 1H, Ar-H), 7.30-7.33 (m, 1H, Ar-H), 7.55-7.58 (m, 1H, Ar-H), 7.67-7.68 (m, 1H, Ar-H). Anal. calcd for $\text{C}_{20}\text{H}_{21}\text{N}_3\text{O}_3$ (351.40): C, 68.36; H, 6.02; N, 11.96. Found: C, 68.15; H, 5.92; N, 11.75.

1-[4-(4-Methoxyphenyl)piperazin-1-yl]methyl-1H-indole-2,3-dione (2e)

Yield 82%; m.p. $170\text{--}171^\circ\text{C}$; IR (KBr, cm^{-1}): 3086, 3035 (C-H aromatic), 2947, 2823 (C-H aliphatic), 1745, 1728 (C=O); ^1H -NMR 300 MHz (CDCl_3): 2.80-2.85 (m, 4H,

2CH_2 piperazinyl), 3.05-3.11 (m, 4H, 2CH_2 piperazinyl), 3.77 (s, 3H, OCH_3), 4.55 (s, 2H, CH_2), 6.82-6.94 (m, 4H, Ar-H), 7.15 (d, 2H, Ar-H), 7.62 (d, 2H, Ar-H). Anal. calcd for $\text{C}_{20}\text{H}_{21}\text{N}_3\text{O}_3$ (351.40): C, 68.36; H, 6.02; N, 11.96. Found: C, 68.55; H, 6.18; N, 11.70.

5-Bromo-1-[4-(4-methoxyphenyl)piperazin-1-yl]methyl-1H-indole-2,3-dione (2f)

Yield 68%; m.p. $130\text{--}131^\circ\text{C}$; IR (KBr, cm^{-1}): 3062, 3050 (C-H aromatic), 2947, 2831 (C-H aliphatic), 1750, 1732 (C=O); ^1H -NMR 300 MHz (CDCl_3): 2.78-2.83 (m, 4H, 2CH_2 piperazinyl), 3.05-3.12 (m, 4H, 2CH_2 piperazinyl), 3.77 (s, 3H, OCH_3), 4.53 (s, 2H, CH_2), 6.83-7.06 (m, 5H, Ar-H), 7.71-7.74 (m, 2H, Ar-H). Anal. calcd for $\text{C}_{20}\text{H}_{20}\text{BrN}_3\text{O}_3$ (430.30): C, 55.83; H, 4.68; N, 9.77. Found: C, 55.60; H, 4.55; N, 9.96.

General synthetic procedure for the synthesis of 3-(4,5-dihydro[1,3]thiazol-2-ylimino)-1-(substituted-1-ylmethyl)indol-2(3H)-ones (3a-h) and 3-(1H-benzimidazol-2-ylimino)-1-(substituted-1-ylmethyl)indol-2(3H)-ones (4a-h)

Equimolar quantities of an appropriate Mannich base **2a-h** and 2-amino-4,5-dihydrothiazole or 2-amino-1H-benzo[d]imidazole (3.65 mmol each) were dissolved in absolute ethanol (50 mL) containing glacial acetic acid (0.5 mL). The reaction mixture was heated under reflux for 13-15 h, concentrated under reduced pressure and left to cool. The precipitated solid was filtered and crystallized from ethanol (70%) to give **3a-h** or **4a-h**.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-(piperidin-1-ylmethyl) indol-2(3H)-one (3a)

Yield 70%; m.p. $160\text{--}161^\circ\text{C}$; IR (KBr, cm^{-1}): 3062, 3050 (C-H aromatic), 2932, 2855 (C-H aliphatic), 1728 (C=O), 1616 (C=N); ^1H -NMR 300 MHz (DMSO- d_6): 1.05-1.46 (m, 6H, 3CH_2 piperidinyl), 2.76-2.84 (m, 4H, 2CH_2 piperidinyl), 3.55 (t, 2H, CH_2 thiazoline), 4.36 (t, 2H, CH_2 thiazoline), 4.69 (s, 2H, CH_2), 6.59-7.58 (m, 4H, Ar-H). Anal. calcd for $\text{C}_{17}\text{H}_{20}\text{N}_4\text{OS}$ (328.43): C, 62.17; H, 6.14; N, 17.06. Found: C, 62.38; H, 6.18; N, 17.45.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-(morpholin-1-ylmethyl)indol-2(3H)-one (3b)

Yield 65%; m.p. $172\text{--}173^\circ\text{C}$; IR (KBr, cm^{-1}): 3101, 3050 (C-H aromatic), 2955, 2897 (C-H aliphatic), 1732 (C=O), 1612 (C=N); ^1H -NMR 200 MHz (DMSO- d_6): 2.41-2.49 (m, 4H, 2CH_2 morpholinyl), 2.56 (t, 2H, CH_2 thiazoline), 3.35-3.45 (m, 4H, 2CH_2 morpholinyl), 3.53 (t, 2H, CH_2 thiazoline), 4.39 (s, 2H, CH_2), 6.90-7.71 (m, 4H, Ar-H); MS (EI) m/z (% rel. Int.): 330 (45.97, M^+), 331 (16.93, $\text{M}+\text{H}$). Anal. calcd for $\text{C}_{16}\text{H}_{18}\text{N}_4\text{O}_2\text{S}$ (330.40): C, 58.16;

H, 5.49; N, 16.96. Found: C, 58.25; H, 5.32; N, 16.68.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-[(4-phenylpiperazin-1-yl)methyl]indol-2(3H)-one (3c)

Yield 60%; m.p. 142–143°C; IR (KBr, cm^{-1}): 3086, 3059 (C-H aromatic), 2924, 2855 (C-H aliphatic), 1728 (C=O), 1616 (C=N); $^1\text{H-NMR}$ 300 MHz (DMSO- d_6): 2.73 (t, 2H, CH_2 thiazoline), 2.82–2.88 (m, 4H, 2 CH_2 piperazinyl), 3.00–3.10 (m, 4H, 2 CH_2 piperazinyl), 3.55 (t, 2H, CH_2 thiazoline), 4.75 (s, 2H, CH_2), 6.73–7.71 (m, 9H aromatic). Anal. calcd for $\text{C}_{22}\text{H}_{23}\text{N}_5\text{OS}$ (405.52): C, 65.16; H, 5.72; N, 17.27. Found: C, 64.95; H, 5.55; N, 17.05.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-[[2-(methoxyphenyl)piperazin-1-yl]methyl]indol-2(3H)-one (3d)

Yield 60%; m.p. 100–101°C; IR (KBr, cm^{-1}): 3080, 3059 (C-H aromatic), 2935, 2831 (C-H aliphatic), 1728 (C=O), 1616 (C=N); $^1\text{H-NMR}$ 300 MHz (CDCl_3): 2.77–2.81 (m, 4H, 2 CH_2 piperazinyl), 2.89 (t, 2H, CH_2 thiazoline), 2.95–3.04 (m, 4H, 2 CH_2 piperazinyl), 3.52 (t, 2H, CH_2 thiazoline), 3.76 (s, 3H, OCH_3), 4.53 (s, 2H, CH_2), 6.84–7.27 (m, 8H, Ar-H). Anal. calcd for $\text{C}_{23}\text{H}_{25}\text{N}_5\text{O}_2\text{S}$ (435.54): C, 63.43; H, 5.79; N, 16.08. Found: C, 63.15; H, 5.88; N, 15.90.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-[[4-(methoxyphenyl)piperazin-1-yl]methyl]indol-2(3H)-one (3e)

Yield 68%; m.p. 150–151°C; IR (KBr, cm^{-1}): 3086, 3059 (C-H aromatic), 2935, 2832 (C-H aliphatic), 1728 (C=O), 1616 (C=N); $^1\text{H-NMR}$ 300 MHz (DMSO- d_6): 2.60–2.68 (m, 4H, 2 CH_2 piperazinyl), 2.72 (t, 2H, CH_2 thiazoline), 2.85–2.98 (m, 4H, 2 CH_2 piperazinyl), 3.43 (t, 2H, CH_2 thiazoline), 3.66 (s, 3H, OCH_3), 4.45 (s, 2H, CH_2), 6.58–7.45 (m, 8H, Ar-H). Anal. calcd for $\text{C}_{23}\text{H}_{25}\text{N}_5\text{O}_2\text{S}$ (435.54): C, 63.43; H, 5.79; N, 16.08. Found: C, 63.12; H, 5.88; N, 16.35.

5-Bromo-3-(4,5-dihydro[1,3]thiazol-2-ylimino)-1-[[4-(4-methoxyphenyl)piperazin-1-yl]methyl]indol-2(3H)-one (3f)

Yield 73%; m.p. 120–121°C; IR (KBr, cm^{-1}): 3097, 3066 (C-H aromatic), 2931, 2831 (C-H aliphatic), 1724 (C=O), 1612 (C=N); $^1\text{H-NMR}$ 300 MHz (DMSO- d_6): 2.62 (t, 2H, CH_2 thiazoline), 2.68–2.71 (m, 4H, 2 CH_2 piperazinyl), 2.90–2.95 (m, 4H, 2 CH_2 piperazinyl), 3.43 (t, 2H, CH_2 thiazoline), 3.67 (s, 3H, OCH_3), 4.44 (s, 2H, CH_2), 6.58–7.45 (m, 7H, Ar-H); MS (EI) m/z (% rel. Int.): 515 (1.71, $\text{M}+2$). Anal. calcd for $\text{C}_{23}\text{H}_{24}\text{BrN}_5\text{O}_2\text{S}$ (514.44): C, 53.70; H, 4.65; N, 13.61. Found: C, 53.55; H, 4.70; N, 13.88.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-(N,N-diphenylamino-1-ylmethyl)indol-2(3H)-one (3g)

Yield 73%; m.p. 152–153°C; IR (KBr, cm^{-1}): 3100, 3062 (C-H aromatic), 2927, 2858 (C-H aliphatic), 1732 (C=O), 1616 (C=N); $^1\text{H-NMR}$ 300 MHz (DMSO- d_6): 2.88 (t, 2H, CH_2 thiazoline), 3.47 (t, 2H, CH_2 thiazoline), 4.66 (s, 2H, CH_2), 6.89–7.61 (m, 14H, Ar-H). Anal. calcd for $\text{C}_{24}\text{H}_{20}\text{N}_4\text{OS}$ (412.51): C, 69.88; H, 4.89; N, 13.58. Found: C, 69.68; H, 4.62; N, 13.72.

3-(4,5-Dihydro[1,3]thiazol-2-ylimino)-1-[(4-methylpiperazin-1-yl)methyl]indol-2(3H)-one (3h)

Yield 65%; m.p. 138–139°C; IR (KBr, cm^{-1}): 3120, 3050 (C-H aromatic), 2947, 2877 (C-H aliphatic), 1728 (C=O), 1604 (C=N); $^1\text{H-NMR}$ 300 MHz (CDCl_3): 2.65–2.83 (m, 4H, 2 CH_2 piperazinyl), 2.90–3.15 (m, 4H, 2 CH_2 piperazinyl), 3.36 (t, 2H, CH_2 thiazoline), 3.76 (s, 3H, CH_3), 3.84 (t, 2H, CH_2 thiazoline), 4.54 (s, 2H, CH_2), 6.84–7.61 (m, 4H, Ar-H). Anal. calcd for $\text{C}_{17}\text{H}_{21}\text{N}_5\text{OS}$ (343.45): C, 59.45; H, 6.16; N, 20.39. Found: C, 59.35; H, 6.44; N, 20.15.

3-(1H-Benzimidazol-2-ylimino)-1-(piperidin-1-ylmethyl)indol-2(3H)-one (4a)

Yield 74%; m.p. 220–221°C; IR (KBr, cm^{-1}): 3174, 3059 (C-H aromatic), 2931, 2858 (C-H aliphatic), 1735 (C=O), 1616 (C=N); $^1\text{H-NMR}$ 200 MHz (DMSO- d_6): 1.24–1.65 (m, 6H, 3 CH_2 piperidinyl), 2.95–3.05 (m, 4H, 2 CH_2 piperidinyl), 4.60 (s, 2H, CH_2), 6.98–7.89 (m, 8H aromatic), 11.06 (br s, 1H, NH, D_2O exchangeable); MS (EI) m/z (% rel. Int.): 359 (33, M^+). Anal. calcd for $\text{C}_{21}\text{H}_{21}\text{N}_5\text{O}$ (359.42): C, 70.17; H, 5.89; N, 19.48. Found: C, 70.25; H, 5.99; N, 19.30.

3-(1H-Benzimidazol-2-ylimino)-1-(morpholin-1-ylmethyl)indol-2(3H)-one (4b)

Yield 67%; m.p. 168–169°C; IR (KBr, cm^{-1}): 3109, 3050 (C-H aromatic), 2951, 2854 (C-H aliphatic), 1732 (C=O), 1612 (C=N); $^1\text{H-NMR}$ 200 MHz (DMSO- d_6): 2.40–2.56 (m, 4H, 2 CH_2 morpholinyl), 3.50–3.65 (m, 4H, 2 CH_2 morpholinyl), 4.39 (s, 2H, CH_2), 6.93–7.70 (m, 8H, Ar-H), 11.02 (br s, 1H, NH, D_2O exchangeable). Anal. calcd for $\text{C}_{20}\text{H}_{19}\text{N}_5\text{O}_2$ (361.40): C, 66.47; H, 5.30; N, 19.38. Found: C, 66.28; H, 5.45; N, 19.55.

3-(1H-Benzimidazol-2-ylimino)-1-[(4-phenylpiperazin-1-yl)methyl]indol-2(3H)-one (4c)

Yield 65%; m.p. 244–245°C; IR (KBr, cm^{-1}): 3059 (C-H aromatic), 2927, 2881 (CH aliphatic), 1735 (C=O), 1620 (C=N); $^1\text{H-NMR}$ 200 MHz (DMSO- d_6): 2.65–2.80 (m, 4H, 2 CH_2 piperazinyl), 3.05–3.18 (m, 4H, 2 CH_2 piperazinyl), 4.47 (s, 2H, CH_2), 6.60–7.60 (m, 13H, Ar-H), 11.05 (br s, 1H, NH, D_2O exchangeable); MS (EI) m/z (%)

rel. Int.): 434 (54.03, M-2). Anal. calcd for $C_{26}H_{24}N_6O$ (436.51): C, 71.54; H, 5.54; N, 19.25. Found: C, 71.25; H, 5.36; N, 19.15.

3-(1H-Benzimidazol-2-ylimino)-1-[[4-(2-methoxyphenyl)-piperazin-1-yl]methyl]indol-2(3H)-one (4d)

Yield 58%; m.p. 160-161°C; IR (KBr, cm^{-1}): 3066 (C-H aromatic), 2935, 2827 (C-H aliphatic), 1743 (C=O), 1608 (C=N); 1H -NMR 300 MHz (DMSO- d_6): 2.64-2.73 (m, 4H, 2CH₂ piperazinyl), 2.96-3.04 (m, 4H, 2CH₂ piperazinyl), 3.79 (s, 3H, OCH₃), 3.84 (s, 2H, CH₂), 6.85-7.51 (m, 12H, Ar-H), 11.01 (br s, 1H, NH, D₂O exchangeable). Anal. calcd for $C_{27}H_{26}N_6O_2$ (466.53): C, 69.51; H, 5.62; N, 18.01. Found: C, 69.32; H, 5.44; N, 18.28.

3-(1H-Benzimidazol-2-ylimino)-1-[[4-(4-methoxyphenyl)-piperazin-1-yl]methyl]indol-2(3H)-one (4e)

Yield 72%; m.p. 185-186°C; IR (KBr, cm^{-1}): 3059 (C-H aromatic), 2931, 2850 (C-H aliphatic), 1732 (C=O), 1616 (C=N); 1H -NMR 200 MHz (DMSO- d_6): 2.68-2.81 (m, 4H, 2CH₂ piperazinyl), 2.90-3.15 (m, 4H, 2CH₂ piperazinyl), 3.67 (s, 3H, OCH₃), 4.47 (s, 2H, CH₂), 6.79-7.59 (m, 12H, Ar-H), 11.05 (br s, 1H, NH, D₂O exchangeable). Anal. calcd for $C_{27}H_{26}N_6O_2$ (466.53): C, 69.51; H, 5.62; N, 18.01. Found: C, 69.64; H, 5.35; N, 18.15.

3-(1H-Benzimidazol-2-ylimino)-5-bromo-1-[[4-(4-methoxyphenyl)piperazin-1-yl]methyl]indol-2(3H)-one (4f)

Yield 72%; m.p. 134-135°C; IR (KBr, cm^{-1}): 3066 (C-H aromatic), 2935, 2831 (C-H aliphatic), 1743 (C=O), 1612 (C=N); 1H -NMR 300 MHz (DMSO- d_6): 2.67-2.71 (m, 4H, 2CH₂ piperazinyl), 2.95-3.17 (m, 4H, 2CH₂ piperazinyl), 3.69 (s, 3H, OCH₃), 4.45 (s, 2H, CH₂), 6.71-7.72 (m, 11H, Ar-H), 11.15 (br s, 1H, NH, D₂O exchangeable). Anal. calcd for $C_{27}H_{25}BrN_6O_2$ (545.43): C, 59.46; H, 4.62; N, 15.41. Found: C, 59.65; H, 4.45; N, 15.65.

3-(1H-Benzimidazol-2-ylimino)-1-[N,N-diphenyl-amino-1-yl]-methyl]indol-2(3H)-one (4g)

Yield 75%; m.p. 174-175°C; IR (KBr, cm^{-1}): 3062 (C-H aromatic), 2943, 2827 (C-H aliphatic), 1743 (C=O), 1608 (C=N); 1H -NMR 300 MHz (DMSO- d_6): 4.38 (s, 2H, CH₂), 6.77-8.10 (m, 18H, Ar-H), 11.18 (br s, 1H, NH, D₂O exchangeable). Anal. calcd for $C_{28}H_{21}N_5O$ (443.50): C, 75.83; H, 4.77; N, 15.79. Found: C, 75.68; H, 4.52; N, 16.05.

3-(1H-Benzimidazol-2-ylimino)-1-[4-(4-methylpiperazin-1-ylmethyl]indol-2(3H)-one (4h)

Yield 62%; m.p. 166-167°C; IR (KBr, cm^{-1}): 3059 (C-H aromatic), 2924, 2854 (C-H aliphatic), 1732 (C=O),

1616 (C=N); 1H -NMR 300 MHz (DMSO- d_6): 2.80-2.92 (m, 4H, 2CH₂ piperazinyl), 3.15-3.22 (m, 4H, 2CH₂ piperazinyl), 3.77 (s, 3H, CH₃), 4.56 (s, 2H, CH₂), 6.83-7.62 (m, 8H, Ar-H), 11.10 (br s, 1H, NH, D₂O exchangeable). Anal. calcd for $C_{21}H_{22}N_6O$ (374.44): C, 67.36; H, 5.92; N, 22.44. Found: C, 67.15; H, 5.85; N, 22.65.

Cytotoxic activity studies

Anticancer activity studies were carried out at Cairo University, National Cancer Institute, Cancer Biology Department, Pharmacology Unit. All chemicals and solvents were purchased from Sigma and Aldrich. Eleven of the synthesized compounds **3a**, **3b**, **3c**, **3d**, **3g**, **4a**, **4b**, **4c**, **4d**, **4e** and **4g** were tested at concentrations between 1 and 10 mg/mL using the Sulfo-Rhodamine-B (SRB) assay for cytotoxic activity against a human breast adenocarcinoma cell line (MCF7).

Measurement of cytotoxicity by SRB assay

The cytotoxic activity of the newly synthesized compounds was measured *in vitro* using the SRB assay according to the method of Skehan et al. (1990). Cells were plated in 96-multiwell microtiter plates (10⁴ cells/well) for 24 h before treatment with the compound(s) to allow attachment of cells to the walls of the plates. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 1, 2.5, 5, and 10 μ g/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h at 37°C in an atmosphere of 5% CO₂. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relationship between surviving fraction and drug concentration was plotted to get the survival curve. The concentration required for 50% inhibition of cell viability (IC₅₀) was calculated. The results are given in Table I and presented graphically in Table I. The survival curves for the most potent cytotoxic compounds, **4b** and **4g**, against MCF-7 cells are presented in Fig. 3.

RESULTS AND DISCUSSION

Compounds **2a-h**, **3a-h** and **4a-h** were prepared as outlined in Scheme 1. The isatin Mannich bases **2a-h** were prepared by condensing isatin with formaline and secondary amine of the amino compound. The 1H -NMR spectra of the new Mannich bases **2d**, **2e**, **2f**

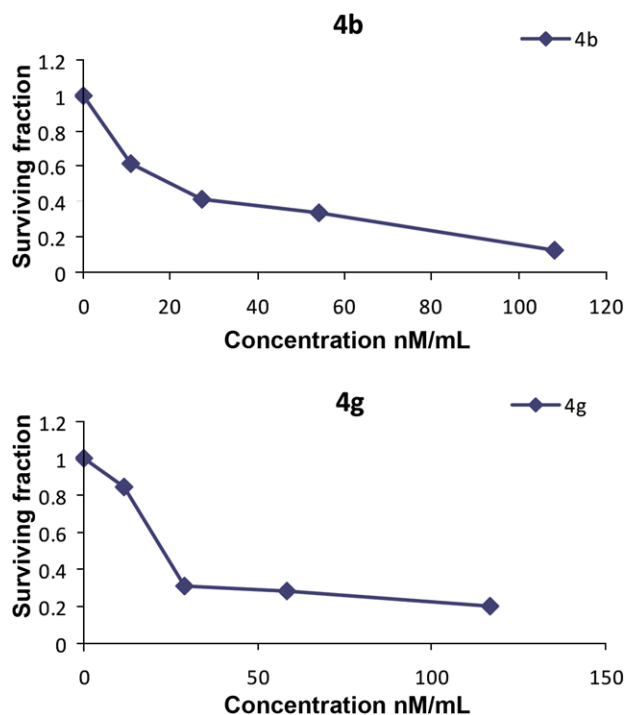


Fig. 3. Effect of different concentrations of compounds **4b** and **4g** on the viability of the MCF-7 cell line

demonstrated the characteristic singlet signals of CH_2 in the region of 4.48-4.55 ppm. Compounds **3a-h** and **4a-h** were synthesized by condensation of the Mannich base analogues **2a-h** with either 2-aminothiazoline or 2-aminobenzimidazole by refluxing in ethanol in the presence of glacial acetic acid. IR spectra in both derivatives **3a-h** and **4a-h** showed one strong band corresponding to carbonyl in the range of 1724-1743 cm^{-1} . 1H -NMR spectra of **3a-h** exhibited two triplets of two CH_2 protons of thiazoline in the range of 2.56-3.55 and 3.43-4.36 ppm. Compounds **4a-h** showed an exchangeable signal of NH proton around 11 ppm. All other aromatic and aliphatic protons were observed at the expected regions.

Cytotoxic activity

The cytotoxicity of compounds **3a**, **3b**, **3c**, **3d**, **3g**, **4a**, **4b**, **4c**, **4d**, **4e**, and **4g** was evaluated against a human breast adenocarcinoma cell line (MCF-7). For comparison purposes, the cytotoxicity of doxorubicin, a standard antitumor drug, was evaluated under the same conditions. The IC_{50} (the concentration required for 50% inhibition of cell viability) was calculated for each compound and the results are given in Table I.

Our results revealed that the title compounds showed inhibition of proliferation of MCF-7 human breast cancer cells with IC_{50} range from 22.59-64.14 nM (Table I). Generally, the isatin conjugates with benzimidazole

Table I. *In vitro* cytotoxic activity of some of the synthesized compounds against the human breast cancer cell line (MCF-7)

Compound No.	MCF7 (IC_{50}) ^{a,b} nM
3a	59.67
3b	47.81
3c	38.22
3d	50.97
3g	42.62
4a	34.50
4b	24.95
4c	64.14
4d	26.36
4e	42.86
4g	22.59
Dox.	5.46

^a IC_{50} : dose of the compound that inhibits tumor cell proliferation by 50%; ^bvalues are means of three experiments.

(compounds **4**) showed higher cytotoxic activity than isatin conjugates with thiazoline pharmacophore (compounds **3**). The best activity was obtained with compounds **4b**, **4d** and **4g**. On the other hand, from the isatin-linked thiazoline Schiff base series, compound **3c** displayed the highest anti-breast cancer activity with $IC_{50} = 38.22$ nM.

In summary, the synthesis and characterization of two series of isatin conjugates, isatin-linked thiazoline Schiff base analogues **3a-h** and isatin-linked benzimidazole Schiff base analogues **4a-h**, has been described. All the tested compounds displayed antitumor activity against MCF-7. Some of these derivatives exhibited promising anti-breast cancer activity; in particular, the compounds 3-(1*H*-benzimidazol-2-ylimino)-1-(morpholin-1-ylmethyl)indol-2(3*H*)-one (**4b**), 3-(1*H*-benzimidazol-2-ylimino)-1-{[4-(2-methoxyphenyl)-piperazin-1-yl]methyl}indol-2(3*H*)-one (**4d**), and 3-(1*H*-benzimidazol-2-ylimino)-1-[*N,N*-diphenylamino-1-yl]-methyl}indol-2(3*H*)-one (**4g**) emerged as promising compounds and could therefore serve as lead compounds for further modification to render them clinically useful agents. To further improve the efficacy and specificity for cancer cell killing, additional structural modifications of isatin conjugates are in progress.

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REFERENCES

- Bramson, H. N., Corona, J., Davis, S. T., Dickerson, S. H., Edelstein, M., Frye, S. V., Gampe, R. T., Jr., Harris, P. A., Hassell, A., Holmes, W. D., Hunter, R. N., Lackey, K. E., Lovejoy, B., Luzzio, M. J., Montana, V., Rocque, W. J., Rusnak, D., Shewchuk, L., Veal, J. M., Walker, D. H., and Kuyper, L. F., Oxindole-based inhibitors of cyclin-dependent kinase 2 (CDK2): design, synthesis, enzymatic activities, and X-ray crystallographic analysis. *J. Med. Chem.*, 44, 4339-4358 (2001).
- Hori, A., Imaeda, Y., Kubo, K., and Kusaka, M., Novel benzimidazole derivatives selectively inhibit endothelial cell growth and suppress angiogenesis *in vitro* and *in vivo*. *Cancer Lett.*, 183, 53-60 (2002).
- Johnson, M., Younglove, B., Lee, L., LeBlanc, R., Holt, H., Jr., Hills, P., Mackay, H., Brown, T., Mooberry, S. L., and Lee, M., Design, synthesis, and biological testing of pyrazoline derivatives of combretastatin-A4. *Bioorg. Med. Chem. Lett.*, 17, 5897-5901 (2007).
- Matesic, L., Locke, J. M., Bremner, J. B., Pyne, S. G., Skropeta, D., Ranson, M., and Vine, K. L., N-phenethyl and N-naphthylmethyl isatins and analogues as *in vitro* cytotoxic agents. *Bioorg. Med. Chem.*, 16, 3118-3124 (2008).
- Mayur, Y. C., Peters, G. J., Prasad, V. V., Lemo, C., and Sathish, N. K., Design of new drug molecules to be used in reversing multidrug resistance in cancer cells. *Curr. Cancer Drug Targets*, 9, 298-306 (2009).
- Motzer, R. J., Michaelson, M. D., Redman, B. G., Hudes, G. R., Wilding, G., Figlin, R. A., Ginsberg, M. S., Kim, S. T., Baum, C. M., DePrimo, S. E., Li, J. Z., Bello, C. L., Theuer, C. P., George, D. J., and Rini, B. I., Activity of SU11248, a multi-targeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.*, 24, 16-24 (2006).
- Pandeya, S. N., Smitha, S., Jyoti, M., and Sridhar, S. K., Biological activities of isatin and its derivatives. *Acta Pharm.*, 55, 27-46 (2005).
- Ramla, M. M., Omar, M. A., El-Khamry, A. M., and El-Diwani, H. I., Synthesis and antitumor activity of 1-substituted-2-methyl-5-nitrobenzimidazoles. *Bioorg. Med. Chem.*, 14, 7324-7332 (2006).
- Rojo, F., Albanell, J., Rovira, A., Corominas, J. M., and Manzarbeitia, F., Targeted therapies in breast cancer. *Semin. Diagn. Pathol.*, 25, 245-261 (2008).
- Rostom, S. A., Synthesis and *in vitro* antitumor evaluation of some indeno[1,2-c]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems. *Bioorg. Med. Chem.*, 14, 6475-6485 (2006).
- Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, 82, 1107-1112 (1990).
- Smith, R. A., Cokkinides, V., and Brawley, O. W., Cancer screening in the United States, 2009: a review of current American Cancer Society guidelines and issues in cancer screening. *CA Cancer J. Clin.*, 59, 27-41 (2009).
- Solomon, V. R., Hu, C., and Lee, H., Hybrid pharmacophore design and synthesis of isatin-benzothiazole analogs for their anti-breast cancer activity. *Bioorg. Med. Chem.*, 17, 7585-7592 (2009).
- Solomon, V. R., Hu, C., and Lee, H., Design and synthesis of anti-breast cancer agents from 4-piperazinylquinoline: a hybrid pharmacophore approach. *Bioorg. Med. Chem.*, 18, 1563-1572 (2010).
- Vine, K. L., Matesic, L., Locke, J. M., Ranson, M., and Skropeta, D., Cytotoxic and anticancer activities of isatin and its derivatives: a comprehensive review from 2000-2008. *Anticancer Agents Med. Chem.*, 9, 397-414 (2009).