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Research Article

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Synthesis and antitumor evaluation *in vitro* of 5-bromo-N-phenyl substituted isatin derivatives

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ABSTRACT

Our previous research have identified a series of N-phenyl substituted isatin derivatives as antitumor agents in vitro. 5-Bromo isatin derivatives have been found to increase their cytotoxic activity and selectivity. A series of 5-bromo-N-phenyl isatin analogues were designed and synthesized about 40-80% overall yields. All of synthesized compounds have not been reported before. Their structures were characterized by ^{1}H and ^{13}C NMR. The cytotoxic activities of all the synthesized derivatives were evaluated against two human tumor cell lines HepG2 and HT-29. Results showed that all bromo-substituted compounds exhibited potential anti-tumor activities against HepG2 and HT-29.

Keywords: Isatin, Cytotoxic activity, MTT, Structure-activity relationship

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INTRODUCTION

In recent years, a large number of isatins have marked anticancer effects toward various types of cancer cell lines *in vitro* [1-3]. Some of them had been successfully developed to treatment human cancers diseases in some therapeutic areas. Sunitinib (marketed as Sutent by Pfizer), a 5-fluoro-3-substituted isatin derivative, was approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST) on 2006[4].

Isatin is an indole derivative widely present endogenously in both human and other mammalian tissues and fluids [5]. It was reported that 6-bromo isatin exhibited anticancer activity against a human lymphoma cell line. And the 4-bromo isatin derivatives possess a wide range of pharmacological activities and biological activities [6-7], including anticancer activity [8]. However, the synthesis and antitumor of 5-bromo 1, 3-disubstituted derivatives have not been reported yet.
Here, we would like to report the synthesis and antitumor activity of a series of 5-bromo 1, 3-disubstituted isatin derivatives against two human cancer cell lines, human colon cancer HT-29 and human liver cancer HepG2.

EXPERIMENTAL SECTION

2.1 General Methods.
All reagents and solvents were purchased from commercially suppliers and used without further purification. ^{1}H NMR spectra were recorded with a Bruker AM-400 NMR spectrometer with $CDCl_3$-d_6 or DMSO-d_6 as the solvent. ^{13}C NMR spectra were recorded at 100 MHz. All chemical shifts (δ_H and δ_C) were reported in parts per million (ppm)

and the coupling constants were measured in hertz (Hz). Thin layer chromatography was performed using silica gel 60 F_{254} plates (Merck) with observation under UV when necessary. Chromatography was performed on 230-400 mesh silica gel.

2.2 Synthesis route of isatin derivatives

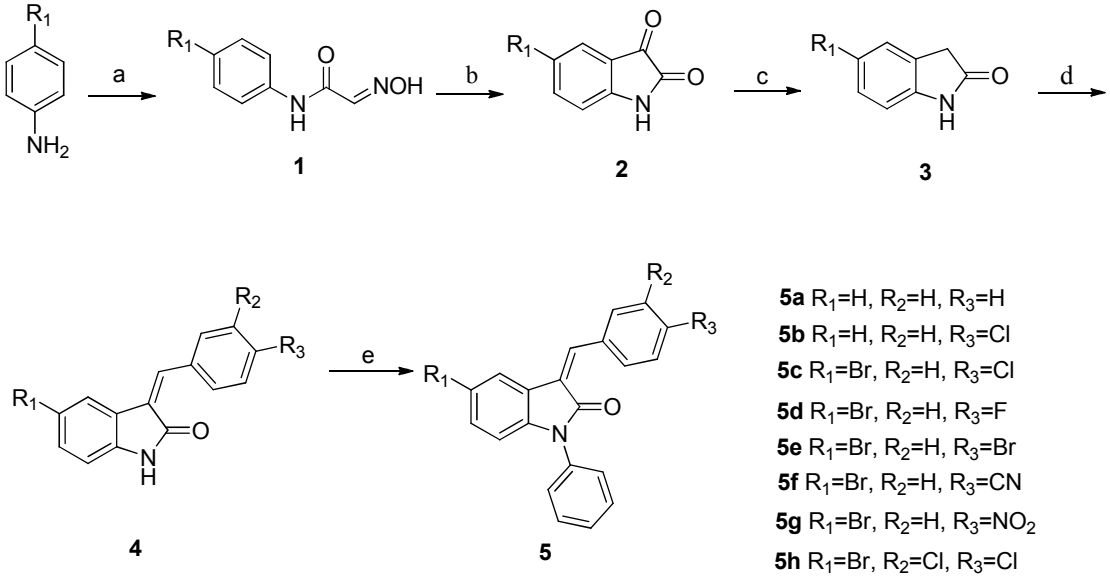


Fig 1: synthesis route of isatin derivatives

Reagents and conditions：(a)Na_2SO_4, $NH_2OH{\cdot}HCl$, $CCl_3CH(OH)_2$, 2 M HCl, H_2O, 90 ºC；(b) conc.H_2SO_4, 65 ºC；(c) Ethanol, $NH_2NH_2{\cdot}H_2O$, NaOH, 65 ºC, 82%；(d) benzaldehyde, Ethanol, Piperidine, 75 ºC; (e) DCM, $Cu(OAc)_2$, CH_3COOK, rt, 36-43%.

2.2.1 Synthesis of (Z)-3-benzylidene-1-phenylindolin-2-one (5a)

To a solution of isatin (5.00 g, 34.00 mmol) in ethanol was added 85% hydrazine hydrate (3.00g, 51.00 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was quenched with H_2O (100 mL). The solids were collected and filtrated to afford indolin-2-one as yellow solids (4.10 g, yield 91%).

To a solution of indolin-2-one (1.00 g, 7.50 mmol) in ethanol was added piperidine (0.30 mL) and benzaldehyde (0.96 g, 9.00 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-3-benzylideneindolin-2-one was purified by flash chromatography eluting with PE:EA (5:1). The product was a bright yellow solid (1.45 g, 87%).

To a solution of (Z)-3-benzylideneindolin-2-one (0.30 g, 1.36 mmol) in DCM was added copper acetate (0.49 g, 2.70 mmol), phenylboronic acid (0.32 g, 2.70 mmol) and triethylamine (0.70 mL, 4.10 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE: EA (5:1) as the eluent to afford the title compound **5a** (0.20 g, 50%).

^{1}H NMR (d_6-DMSO, 400 MHz): δ/ppm 6.80(d, *J*=8Hz, 1H), 7.17(t, *J* =7.6Hz, 1H), 7.45 (t, *J* =8Hz, 1H), 7.51(t, *J* =8.8Hz, 3H), 7.61(t, *J* =7.6Hz, 2H), 7.68(m, 5H), 8.71(s, 1H).

2.2.2 Synthesis of (Z)-3-(4-chlorobenzylidene)-1-phenylindolin-2-one (5b) [9]

To a solution of indolin-2-one (1.00 g, 7.50 mmol) in ethanol was added piperidine (0.30 mL) and 4-chlorobenzaldehyde (1.26 g, 9.00 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-3-(4-chlorobenzylidene) indolin-2-one was purified by flash chromatography eluting with PE:EA (3:1). The product was a bright yellow solid (1.45 g, 87%).

To a solution of (Z)-3-(4-chlorobenzylidene)indolin-2-one (0.30 g, 1.18 mmol) in DCM was added copper acetate (0.44 g, 2.40 mmol), phenylboronic acid (0.30 g, 2.40 mmol) and triethylamine (0.60 mL, 3.60 mmol) below 20℃.

After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-5:1) as the eluent to afford the title compound **5b** (0.20 g, 50%).

^{1}H NMR (d_6-DMSO,400 MHz): δ/ppm 6.80(d, *J*=8Hz,1H), 7.17(t, *J* =7.6Hz,1H), 7.45 (t, *J* =8Hz,1H), 7.51(t, *J* =8.8Hz,3H),7.61(t, *J* =7.6Hz,2H), 7.68(d, *J* =8Hz,2H), 8.05 (d, *J* =8Hz, 3H),8.71(s, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ/ppm 110.36, 116.41, 123.95, 127.38, 128.90, 129.42, 129.90, 130.17, 131.09, 132.70, 134.06, 134.24, 137.38, 146.48, 150.03, 160.10, 162.95.

2.2.3 Synthesis of (Z)-5-bromo-3-(4-chlorobenzylidene)-1-phenylindolin-2-one (5c)

To a solution of 5-bromoindoline-2,3-dione (5.00 g, 22.10 mmol) in ethanol was added 85% hydrazine hydrate (1.95 g, 33.20 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was quenched with H_2O (100 mL). The solids were collected and filtrated to afford 5-bromoindolin-2-one as yellow solids (4.20 g, yield 89.4%).

To a solution of 5-bromoindolin-2-one (1.00 g, 4.70 mmol) in ethanol was added piperidine (0.30 mL) and 4-chlorobenzaldehyde (0.79 g, 5.66 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-5-bromo-3-(4-chlorobenzylidene) indolin-2-one was purified by flash chromatography eluting with PE:EA (3:1). The product was a bright yellow solid (1.35 g, 86%).

To a solution of (Z)-5-bromo-3-(4-chlorobenzylidene)indolin-2-one (0.30 g, 0.90 mmol) in DCM was added copper acetate (0.33 g, 1.80 mmol), phenylboronic acid (0.22 g, 1.80 mmol) and triethylamine (0.50 mL, 3.60 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (5:1) as the eluent to afford the title compound **5c** (0.21 g, 56%).

^{1}H NMR ($CDCl_3$, 400 MHz)：δ/ppm 7.91 (s, 1H), 7.77 (d, *J* = 1.5 Hz, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.59-7.48 (m, 4H), 7.43 (t, *J* = 9.3 Hz, 3H), 7.32 (dd, *J* = 8.4, 1.7 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 1H).； ^{13}C NMR (d_6-DMSO, 100 MHz): 29.72, 111.10, 114.98, 122.72, 125.59, 126.38, 126.63, 128.34, 129.27, 129.79, 130.65, 132.51, 132.83, 134.12, 136.17, 137.95, 143.17,167.11.

2.2.4 Synthesis of (Z)-5-bromo-3-(4-fluorobenzylidene)-1-phenylindolin-2-one (5d)

To a solution of 5-bromoindolin-2-one (1.00 g, 4.70 mmol) in ethanol was added piperidine (0.30 mL) and 4-Fluorobenzaldehyde (0.70 g, 5.66 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-5-bromo-3-(4-fluorobenzylidene) indolin-2-one was purified by flash chromatography eluting with PE:EA(3:1). The product was a bright yellow solid (1.20 g, 80%).

To a solution of (Z)-5-bromo-3-(4-fluorobenzylidene)indolin-2-one (0.30 g, 0.94 mmol) in DCM was added copper acetate (0.34 g, 1.90 mmol), phenylboronic acid (0.23 g, 1.90 mmol) and triethylamine (0.50 mL, 3.80 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-4:1) as the eluent to afford the title compound **5d** (0.13 g, 35%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 7.91 (s, 1H), 7.75 (d, *J* = 20.7 Hz, 1H), 7.62 (d, *J* = 8.3 Hz, 2H), 7.59-7.47 (m, 4H), 7.43 (t, *J* = 9.3 Hz, 3H), 7.32 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.72 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ/ppm 111.10, 114.98, 122.72, 125.59, 126.20, 126.38, 126.63, 128.34, 129.27, 129.79, 130.65, 132.51, 132.83, 134.12, 136.17, 137.95, 143.17, 167.11.

2.2.5 Synthesis of (Z)-5-bromo-3-(4-bromobenzylidene)-1-phenylindolin-2-one (5e)

To a solution of 5-bromoindolin-2-one (1.00 g, 4.70 mmol) in ethanol was added piperidine (0.30 mL) and 4-Bromobenzaldehyde (1.00 g, 5.66 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-5-bromo-3-(4-bromobenzylidene) indolin-2-one was purified by flash chromatography eluting with PE:EA(5:1). The product was a red solid (1.50 g, 84%).

To a solution of (Z)-5-bromo-3-(4-bromobenzylidene)indolin-2-one (0.30 g, 0.80 mmol) in DCM was added copper acetate (0.28 g, 1.60 mmol), phenylboronic acid (0.19 g, 1.60 mmol) and triethylamine (0.45 mL, 3.20 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-5:1) as the eluent to afford the title compound **5e** (0.13 g, 35%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 7.88 (s, 1H), 7.77 (d, *J* = 1.7 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.60-7.50 (m, 4H), 7.48-7.37 (m, 3H), 7.32 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ/ppm 111.11, 114.99, 122.71, 124.50, 125.61, 126.41, 126.62, 128.35, 129.79, 130.82, 132.23, 132.55, 133.28, 134.11, 137.95, 143.18, 167.10.

2.2.6 Synthesis of (Z)-4-((5-bromo-2-oxo-1-phenylindolin-3-ylidene)methyl) benzonitrile (5f)

To a solution of 5-bromoindolin-2-one (1.00 g, 4.70 mmol) in ethanol was added piperidine (0.30 mL) and 4-cyanobenzaldehyde (0.74g, 5.66 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-4-((5-bromo-2-oxoindolin-3-ylidene) methyl) benzonitrile was purified by flash chromatography eluting with PE:EA(3:1). The product was a bright yellow solid (0.80 g, 53%).

To a solution of (Z)-4-((5-bromo-2-oxoindolin-3-ylidene)methyl)benzonitrile (0.30 g, 0.90 mmol) in DCM was added copper acetate (0.33g, 1.80 mmol), phenylboronic acid (0.22 g, 1.80 mmol) and triethylamine (0.50 mL, 3.60 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-1:1) as the eluent to afford the title compound **5f** (0.20 g, 54%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 8.34 (d, *J* = 8.3 Hz, 2H), 7.72 (dd, *J* = 14.2, 5.0 Hz, 3H), 7.59-7.50 (m, 3H), 7.47-7.33 (m, 4H), 6.72 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): 18.45, 58.49, 111.07, 113.60, 115.43, 118.55, 122.94, 125.37, 126.52, 127.74, 128.47, 129.78, 131.92, 132.23, 132.49, 133.87, 136.10, 137.32, 141.69, 164.72.

2.2.7 Synthesis of (Z)-5-bromo-3-(4-nitrobenzylidene)-1-phenylindolin-2-one (5g)

To a solution of 5-bromoindolin-2-one (1.00 g, 4.70 mmol) in ethanol was added piperidine (0.30 mL) and 4-Nitrobenzaldehyde (0.85 g, 5.66 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-5-bromo-3-(4-nitrobenzylidene)indolin-2-one was purified by flash chromatography eluting with PE:EA (3:1). The product was a red solid (1.00 g, 63%).

To a solution of (Z)-5-bromo-3-(4-nitrobenzylidene)indolin-2-one (0.30 g, 0.87 mmol) in DCM was added copper acetate (0.31 g, 1.74 mmol), phenylboronic acid (0.21 g, 1.74 mmol) and triethylamine (0.50 mL, 3.4 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE: EA (5:1) as the eluent to afford the title compound **5g** (0.15 g, 42%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 8.38 (d, *J* = 8.6 Hz, 2H), 7.93 (s, 1H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.60 (t, *J* = 4.1 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 2H), 7.49-7.38 (m, 3H), 7.35 (dd, *J* = 8.5, 1.6 Hz, 1H), 6.73 (dd, *J* = 8.2, 4.1 Hz, 1H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ/ppm 111.41, 115.16, 122.09, 124.24, 125.83, 126.55, 128.54, 128.63, 129.88, 129.98, 133.38, 133.85, 135.69, 141.04, 143.67, 148.29, 166.58.

2.2.8 Synthesis of (Z)-5-bromo-3-(3, 4-dichlorobenzylidene)-1-phenylindolin -2-one (5h)

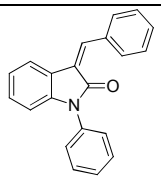
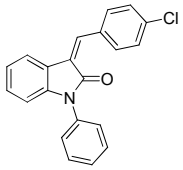
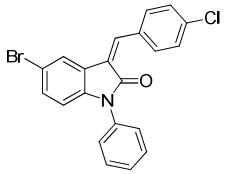
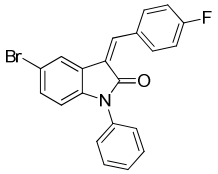
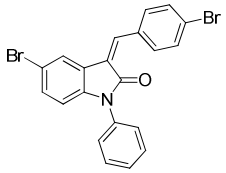
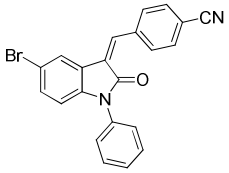
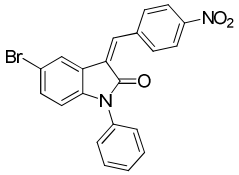
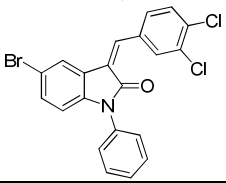
To a solution of 5-bromoindolin-2-one (1.00 g, 4.70 mmol) in ethanol was added piperidine (0.30 mL) and 3,4-Dichlorobenzaldehyde (0.99 g, 5.66 mmol) below 20℃. After complete addition, the reaction was stirred under reflux for 6 h. The reaction mixture was poured into 50 mL of ice water and the precipitate collected and washed with water. The crude (Z)-5-bromo-3-(3, 4-dichlorobenzylidene) indolin-2-one was purified by flash chromatography eluting with PE:EA (3:1). The product was a bright yellow solid (1.40 g, 80%).

To a solution of (Z)-5-bromo-3-(3,4-dichlorobenzylidene)indolin-2-one (0.30 g, 0.80 mmol) in DCM was added copper acetate (0.28 g, 1.60 mmol), phenylboronic acid (0.19g, 1.60 mmol) and triethylamine (0.45 mL, 3.20 mmol)

below 20℃. After complete addition, the reaction was stirred under reflux for 36 h. The reaction mixture was poured into 50 mL of ice water and extracted the mixture with dichloromethane three times. The combined organic layers were dried over $MgSO_4$, filtered and the ether removed. Further purification was performed with silica gel chromatography using PE:EA (10:1-5:1) as the eluent to afford the title compound **5h** (0.14 g, 39%).

^{1}H NMR ($CDCl_3$, 400 MHz): δ/ppm 8.50 (d, *J* = 2.0 Hz, 1H), 8.12 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.86-7.78 (m, 2H), 7.74 (d, *J* = 1.8 Hz, 1H), 7.60 (t, *J* = 9.2 Hz, 2H), 7.56-7.45 (m, 2H), 7.39 (dd, *J* = 8.4, 1.9 Hz, 1H), 6.76 (d, *J* = 8.4 Hz, 1H).

Table 1 Inhibition activity of isatin derivatives

Compd.	Structures	IC_{50} (μM)	
		HT-29	HepG2
5a		37.92	>100
5b		82.80	5.54
5c		16.10	63.29
5d		17.87	19.00
5e		16.31	>100
5f		21.52	33.53
5g		25.07	19.03
5h		30.21	33.40

2.3 Biological assay.

The cell lines HepG2 and HT-29 were plated in 96-well plates at a density of 5 × 10^3 cells per well and cultured at 37℃ in 5% CO_2 for 24 h. Cells were treated with different concentrations of compounds and incubated at 37 °C for

an additional 48 h. MTT assay was performed using Thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The IC_{50} values were obtained by nonlinear regression using GraphPad Prism 4.0. IC_{50} measurements for each compound were done three times.

RESULTS AND DISCUSSION

All the above compounds were tested for their cytotoxic activity against HepG2 and HT-29 by MTT assay. The results were presented in Table 1. Results showed that all bromo-substituted compounds exhibited potential anti-tumor activities against HepG2 and HT-29.

As shown in the Table 1, substitution of bromo group at C-5 position on isatin in analogues **5c**-**5h** increase in activity compared with the mother structure analogue **5a** and **5b**. Compounds **5c**, **5d** and **5e**, possessing halogen at *para*-position of 3-phenly side chain, exhibited better anticancer activity, with the IC_{50} values of 16.10, 17.87 and 16.13 μM (HT-29). This finding has prompted us to further investigate anticancer activity of 5-bromo derivatives.

CONCLUSION

A series of 5-bromo 1, 3-disubstituted isatin derivatives were synthesized and tested for their *in vitro* antitumor activity against two strains of cancer cell lines HepG2 and HT-29. The SAR study of these compounds led to the identification of a serious of new isatin analogues. 5-bromo substituted isatins exhibited potential anti-tumor activities against HT-29. Further chemo-biological studies of these compounds are ongoing in this laboratory.

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