Design, synthesis, and QSAR study of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-phenylacetamide derivatives as cytotoxic agents

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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and QSAR study of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-phenylacetamide derivatives as cytotoxic agents

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Abstract This study deals with the synthesis of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-phenylacetamide derivatives (**6a–j**) from isatin (**3**) and 5,7-dibromoisatin (**4**). All newly synthesized compounds were characterized using IR, ^{1}H NMR, MS, and elemental analysis followed by evaluation of their cytotoxic activity by XTT assay on breast cancer cell line MCF-7 and non-cancer African green monkey cell line VERO. Correlation study for QSAR and in vitro assay was performed. The outcomes indicated that electron withdrawing substitutions at para position of phenyl ring and 5, 7 positions of isatin ring and increasing lipophilicity of the compound increased the cytotoxic activity. The 2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-(4-nitrophenyl)acetamide (**6b**) was found to be the most active compound in the series and demonstrated higher selectivity toward MCF-7 cell line. The IC_{50} values were 1.96 and 1.90 μM for test compound (**6b**) and vinblastin (reference drug), respectively. This indicates compound (**6b**) may possess equipotent cytotoxic activity to vinblastine. The compound (**6b**) is particularly promising, since it could kill cancer cells 19–20 times more effectively than the non-cancer cells. This property of (**6b**) may enable us to effectively control tumors with low side effects. Hence, we propose that 2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-(4-nitrophenyl)acetamide may be used as lead for further development.

Keywords Cytotoxic assay · Isatin · QSAR · XTT assay

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Introduction

The isatin molecule (1*H*-indole-2,3-dione) is a versatile moiety that displays diverse biological activities, including anticancer activity (Pandeya *et al.*, 1999; Cane *et al.*, 2000; Torres *et al.*, 2004; Pandeya *et al.*, 2005; Abadi *et al.*, 2006; Vine *et al.*, 2007a, b; Uddin *et al.*, 2007; Hung *et al.*, 2008; González *et al.*, 2009; Sirisoma *et al.*, 2009). The synthetic flexibility of isatin led to synthesis of a variety of substituted derivatives; however, the susceptibility of isatin to attack by nucleophiles at C3 resulted in the generation of a large number of 3-substituted isatins in particular (Bramson *et al.*, 2001; Andreani *et al.*, 2005; Chen *et al.*, 2005). Despite the significant amount of anticancer research being devoted to 3-substituted indolin-2-ones, it was not proven that 3-substituted isatins are better in bioactivity than other 1*H*-indole-2,3-dione derivatives. *N*-alkylated indoles have also been reported to exhibit anticancer activity. For example, the indolyl amide D-24851 has been found to block cell cycle progression in a variety of malignant prostate, brain, breast, pancreas, and colon cell lines (Bacher *et al.*, 2001). Structurally related other compounds have also been reported to activate caspases in a cytochrome *c*-dependent manner and, therefore, induce apoptosis in cancer cell lines but not in normal cells

(Nguyen and Wells, 2003). Furthermore, Liu *et al.* (2003) identified a class of isatin *O*-acyl oximes that selectively inhibited neuronal ubiquitin *C*-terminal hydrolase (UCH-L1) in a H1299 lung cancer cell line, although very few studies have described structural activity relationship (SAR) attributable to modifications of benzene ring of isatin. Moreover, Vine *et al.* (2007a, b) showed that the introduction of electron-withdrawing groups to the benzene ring of *N*-alkyl-substituted isatins is associated with increased biological activity. Based on this background, the current synthetic scheme was designed, synthesized, and screened for their cytotoxic activity.

QSAR analysis

In order to identify the effect of substitutions on cytotoxic activity, QSAR study using a reported data set of 32 compounds of *N*-substituted isatin derivatives (Vine *et al.*, 2007a, b; Matesic *et al.*, 2008; Solomon *et al.*, 2009) was performed, and outcomes are presented in Tables 1 and 2. The molar concentration was converted to logarithmic values for undertaking the QSAR study. The structure of all the compounds were built on workpspace of Chem 3D version 8.0.3 (Cambridge Softcorporation, http://www.cambridgesoft.com/software/ChemOffice/), and energy minimization of the molecules was done using MM2 force field followed by semi empirical PM3 method available in MOPAC module until the RMS gradient value becomes smaller than 0.01 kcal/mol. The most stable structure for each compound was generated and used for calculating various physio-chemical descriptors like thermodynamic, steric, and electronic values of descriptors. The 21 descriptors were calculated by Chem3D version 8.0.3 considered as independent variable and biological activity as dependent variable. The total data set was divided into training (Table 1) and test (Table 2) sets. The quality of the model was assessed by cross-validated q^2 in the training set, and external validation was performed by calculating predictive r^2 (Pred_r^2) from the test-set compounds. Medicinal Chemistry Regression Machine, Biosoft (http://www.biosoft.com/w/downloads.html) (Sharma *et al.*, 2008) software was used to generate QSAR model. Multiple linear regression analysis yields two statistically significant QSAR models:

Model 1

$$\mathrm{BA} = [2.07992(\pm 0.45753)] + \mathrm{PC}\ [-0.13681(\pm 0.07642)] + \mathrm{BE}[-0.03512(\pm 0.02604)]$$

$n = 22$, $r = 0.81122$, $r^2 = 0.65807$, variance $= 0.03176$, std $= 0.17821$, $F = 18.28420$, FIT $= 140.64800$, $q^2 = 0.54294$, Spress $= 0.20605$, SDEP $= 0.19149$

Contribution of parameter PC to model is 49.34760%

Contribution of parameter BE to model is 50.65240%

Model 2

$$\mathrm{BA} = [1.24081(\pm 0.42753)] + \mathrm{Ed}[0.03967(\pm 0.04083)] + \mathrm{VDW}\ [-0.06858(\pm 0.03194)]$$

$n = 22$, $r = 0.80813$, $r^2 = 0.64580$, variance $= 0.02919$, std $= 0.17084$, $F = 20.73510$, FIT $= 159.5000$, $q^2 = 0.51191$, Spress $= 0.20293$, SDEP $= 0.19088$

Contribution of parameter Ed to model is 25.7219%

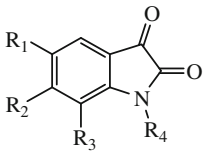
Contribution of parameter VDW to model is 74.2781%

where BA $=$ LogIC_{50}, is the molar logarithmic concentration of the drug leading to 50% cell death, BE $=$ bending energy, PC $=$ partition coefficient (Hansch and Leo, 1995), Ed $=$ dipole–dipole energy, and VDW $=$ van der waals force. Both models are statistically significant. There is only a slight difference in regression coefficient. Model 1 was selected as the best model because, on the basis of the partition coefficient, the drug–receptor interaction and drug kinetics can be better explained. Ed and VDW both show electronic property, which is why they should not be used in one model. The calculated and predicted (LOO) activities of the compounds by the above model are shown in Table 1, and the variables used in the selected model have no mutual correlation.

The most effective model has approximately equal contribution of PC and BE i.e., 49.35 and 50.65%, respectively. Therefore, it was inferred that electronic (BE) and lipophilic parameter (PC) have important roles in the activity of the compounds of series. The above model is validated by predicting the biological activities of the test molecules, as indicated in Table 2. The plot of observed versus predicted activities for the test compounds is represented in Fig. 1. From Table 2, it is evident that the predicted activities of all the compounds in the test set are in good agreement with their corresponding experimental activities and optimal fit is obtained. The external predictability of the above QSAR model using the test set was determined by Pred_r^2, which is 0.56688. Therefore, the above results indicate that QSAR model for cytotoxic activity generates 54.29 and 56.68% internal and external model prediction, respectively.

The term BE represents the energy associated with deforming bond angles from their optimal values. The term PC represents the lipophilicity. Its negative value in the model indicates that cytotoxic activity increases as bending energy and lipopholicity of the compound increase. Thus, we conclude that, when substituents that bring about these changes in the molecule are introduced, the biological activity will increase. On the basis of QSAR study, the proposed molecules were designed (**6a–j**) and synthesized.

Table 1 Structure of *N*-substituted isatin derivatives (**1–22**) with observed and predicted (LOO) activities of the training-set compounds using model 1 and physicochemical properties



(1-22)

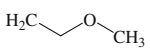
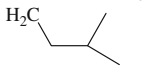
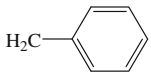
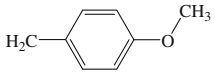
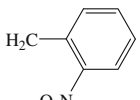
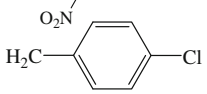
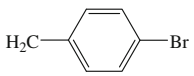
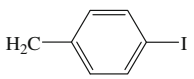
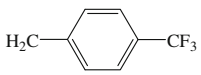
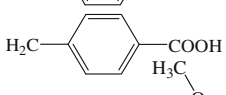
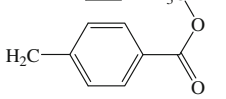
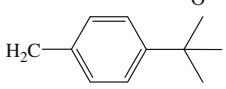
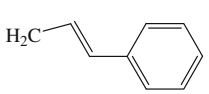
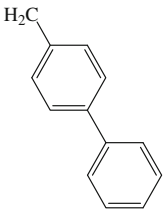
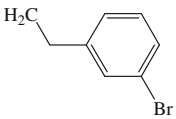
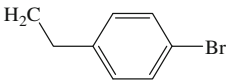
Comp. no.	R_1	R_2	R_3	R_4	Observed activity ($LogIC_{50}$)	PC	BE	Predicted activity(LOO) ($LogIC_{50}$)
1	H	Br	Br	H	1.313	2.554	11.897	1.257
2	Br	H	Br	H	1.327	2.354	12.270	1.334
3	I	H	I	H	1.242	3.074	11.874	1.237
4	Br	Br	Br	H	1.221	3.017	12.674	1.242
5	Br	H	Br		1.058	2.475	19.450	1.139
6	Br	H	Br		0.873	3.981	18.862	0.872
7	Br	H	Br		0.840	4.292	18.581	0.839
8	Br	H	Br		0.767	4.791	18.726	0.752
9	Br	H	Br		0.816	4.011	20.362	0.827
10	Br	Br	H		0.758	4.211	21.239	0.808
11	Br	H	Br		0.731	5.005	18.904	0.740
12	Br	H	Br		0.705	5.155	19.067	0.704
13	Br	H	Br		0.669	5.415	19.061	0.673
14	Br	H	Br		0.688	5.175	19.476	0.676
15	Br	H	Br		0.835	4.035	19.717	0.808
16	Br	H	Br		0.709	4.261	22.421	0.661
17	Br	H	Br		0.529	6.118	20.317	0.548
18	Br	H	Br		0.935	4.917	13.442	0.943
19	Br	H	Br		0.770	6.321	12.670	0.831

Table 1 continued

Comp. no.	R_1	R_2	R_3	R_4	Observed activity ($LogIC_{50}$)	PC	BE	Predicted activity(LOO) ($LogIC_{50}$)
20	Br	H	Br		0.627	5.484	20.007	0.629
21	Br	H	Br		0.625	5.484	20.063	0.610
22	Br	H	Br		0.660	5.466	19.141	0.670

PC partition coefficient and *BE* bending energy

Chemistry

This synthetic strategy began with the synthesis of isatin (**3**) (Scheme 1) by the reaction of dry isonitrosoacetanilide (**2**) with concentrated sulfuric acid (Marvel and Hiers, 1941). In the IR spectrum of isatin (**3**), the carbonyl stretching frequency was observed at 1718 cm^{-1}, whereas the amide carbonyl stretching appeared at 1660 cm^{-1}. In the ^1H NMR spectrum of compound (**3**), a singlet was observed at δ 8.00 ppm due to N–H proton. The aromatic protons resonated in the range of δ 7.52–7.83 ppm. The synthesis of 5,7dibromoisatin (**4**) (Scheme 1) was carried out using a reported method of Lindwall (Lindwall *et al.*, 1931). In this reaction, isatin (**3**) reacts with bromine in methanol and yields 5,7dibromoisatin (**4**). The IR spectrum of 5,7dibromoisatin (**4**) showed stretching frequencies for carbonyl, amide carbonyl, and –C–Br groups at 1718, 1665, and 575 cm^{-1}, respectively.The ^1H NMR spectrum of (**4**) exhibited a singlet which was observed at δ 8.00 ppm due to N–H proton. The aromatic protons resonated in the range of δ 7.86–7.95 ppm.

Final products (**6a–j**) were synthesized (Torres *et al.*, 2004) by reactions of isatin (**3**) and 5,7dibromoisatin (**4**) with appropriate 2-chloro-N-phenylacetamide derivatives (**5a–e**) (Farag *et al.*, 2008) in the presence of K_2CO_3, KI, and DMF as a solvent at temperature of 80°C (Scheme 2). The yields of synthesized products are presented in Table 3. We hereby report the synthesis of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-phenylacetamide derivatives and their cytotoxic potentials.

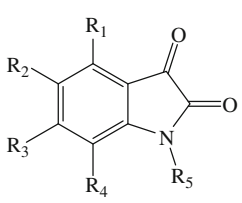
Pharmacology

All the synthesized compounds were screened for their cytotoxic activity by XTT assay (cell proliferation kit; Roche Molecular Biochemicals, Mannheim, Germany) (Skehan *et al.*, 1990) on breast cancer cell line MCF-7, and non-cancer African green monkey cell line VERO (Marcsek *et al.*, 2007). MCF-7 and VERO cell cultures used in the experiments were derived from National Centre for Cell Science, Pune, India. Stock cells of these cell lines were cultured in DMEM, supplemented with fetal calf serum (FCS; 5%), gentamycin sulfate (0.004%), glucose (0.57%), and $NaHCO_3$ (0.12%), in a humidified atmosphere with 5% CO_2 at 37°C until confluence reached. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock cultures were grown initially in 25 cm^2 tissue culture flasks, then in 75 cm^2, and finally in 150 cm^2 tissue culture flask, and all the cytotoxicity experiments were carried out in 96-well microtiter plates. Vinblastine was used as a reference standard for cytotoxic activity. The test compounds were prepared with different concentrations using DMSO, and IC_{50} valueswere determined. IC_{50} is a drug concentration causing a 50% inhibition of cell proliferation.

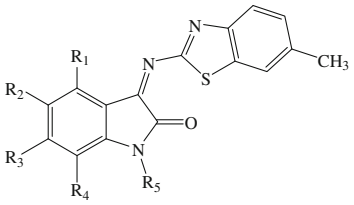
Results and discussion

The best model 1 predicts that increasing the PC and BE would increase the biological activity of the compound.

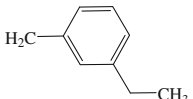
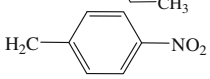
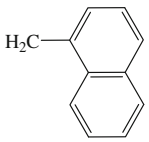
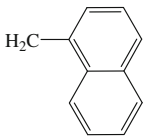
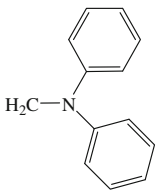
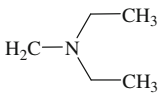
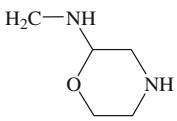
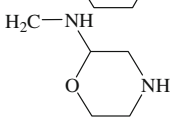
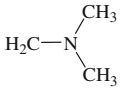
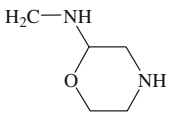
Table 2 Structure of *N*-substituted isatin derivatives (**23–32**) with observed and QSAR derived predicted activities of the test-set compounds using model 1 and physicochemical properties



(23-28)



(29-32)

Comp. no.	R_1	R_2	R_3	R_4	R_5	Observed activity ($LogIC_{50}$)	PC	BE	Predicted activity ($LogIC_{50}$)
23	H	Br	H	Br		0.365	5.466	22.345	0.547
24	H	Br	H	Br		0.635	4.211	20.925	0.769
25	H	Br	H	Br		0.887	4.182	18.906	0.844
26	H	Br	H	Br		0.544	5.466	21.489	0.577
27	H	H	H	H		1.375	3.670	16.864	0.985
28	Br	H	H	H		1.305	3.035	18.854	1.002
29	H	H	H	H		1.163	3.632	18.888	0.920
30	Cl	H	H	H		1.332	2.766	18.095	1.065
31	Br	H	H	H		1.254	3.777	17.113	0.962
32	Br	H	H	H		1.080	3.371	17.530	1.003

PC partition coefficient and *BE* bending energy

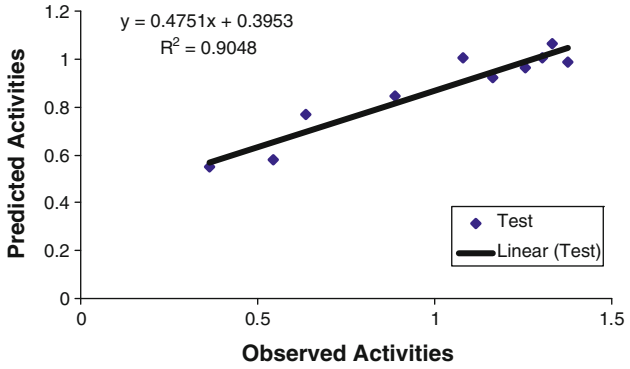


Fig. 1 Observed versus predicted activities according to model 1

Thus, we conclude that, when substituents that bring about these changes in the molecule are introduced, the biological activity will increase. On the basis of model 1, a series of ten compounds (**6a–j**) were designed with the objective of finding higher potent molecules than the existing N-substituted isatin derivatives (Tables 1 and 2). The independent variables were calculated and put in model 1 to obtain predicted biological activities of newly synthesized compounds (**6a–j**). The predicted activities of the newly designed series of compounds are shown in Table 4. From Tables 1, 2, and 4, it is observed that the introduction of amide functional group at *N*-1 position into exiting *N*-substituted isatin derivatives increases the PC and BE. The predicted activities of the newly designed series of compounds (**6a–j**) showed that they have predicted activities of IC_{50} = 1.59–3.65 μM whereas, the reported series of N-substituted isatin derivatives have the highest IC_{50} value of 23.71 μM.

Based on in vitro cytotoxic assay, graphs of Log conc. (μM) vs. % inhibition of different compounds (**6a–e**) and vinblastine were plotted (Fig. 2). IC_{50} values on MCF-7 and VERO cell lines for the in vitro cytotoxic activity of the compounds (**6a–e**) and the standard are presented in Table 4. Active compounds (**6f–j**) (with greater than 50% survival) after an exposure time of 72 h were not further evaluated for finding IC_{50} values. Based on in vitro results, electron withdrawing substitutions at para position of phenyl ring and 5,7 positions of isatin ring and increasing lipophilicity of the compound increased the activity as predicted by QSAR study. Graphs of predicted versus observed activities of the synthesized compounds (**6a–e**) were plotted (Fig. 3). The most active compound in the synthetic series was compound 2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-(4-nitrophenyl)acetamide (**6b**). The IC_{50} values were 1.96 and 1.90 μM for test compound (**6b**) and vinblastine, respectively. Hence, the compound (**6b**) may be considered almost equipotent to that of vinblastine.

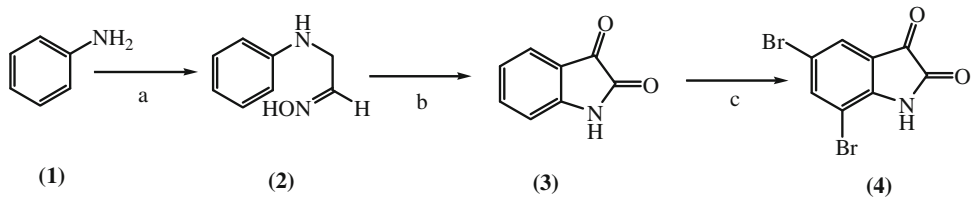
Conclusion

In conclusion, a series of 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-phenylacetamide (**6a–j**) was synthesized and screened for their in vitro cytotoxic activity. Result of bioassay indicated that the compound (**6b**) possesses equivalent cytotoxic activity to standard drug and the remaining compounds showed good-to-moderate activity. The compound (**6b**) is particularly promising, since it could kill cancer cells 19–20 times more effectively than the non-cancer cells. This property of (**6b**) may enable us to effectively control tumors with low side effects. Furthermore, on the basis of quantitative structure–activity relationship study, it can be concluded that the presence of electron withdrawing group substitutions at *para* position of phenyl ring and 5,7 positions of isatin ring and increasing lipophilicity of the compound increase the cytotoxic activity. Therefore, 2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-(4-nitrophenyl)acetamide may be further evaluated in other cell lines and in vivo animal models in the line of further development, and can serve as a prototype molecule of new class of anti-breast cancer agents.

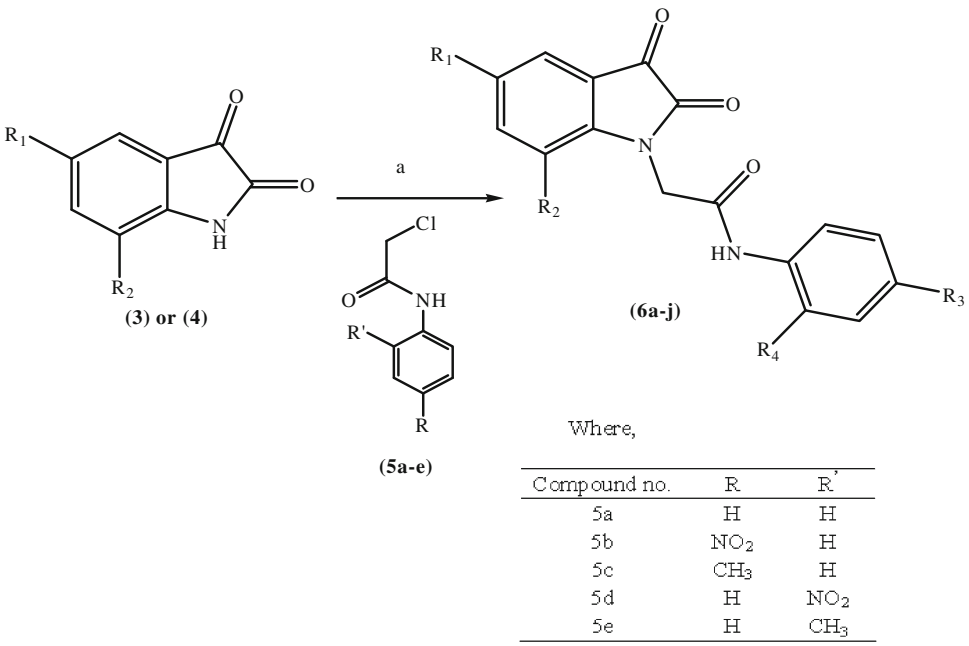
Experimental protocol

Chemistry

Melting points of the synthesized compounds were determined in open capillaries using Veego Melting Point



Scheme 1 Synthesis of isatin (**3**) and 5,7dibromoisatin (**4**). Reagents and condition: **a** chloral hydrate, sodium sulfate, concentrated hydrochloric acid, and hydroxylamine hydrochloride 40–45 min **b** concentrated sulfuric acid, 50–70°C and **c** ethanol, bromine, 70–75°C



Compound no.	R	R'
5a	H	H
5b	NO_2	H
5c	CH_3	H
5d	H	NO_2
5e	H	CH_3

Scheme 2 Schematic representation for the synthesis of novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*phenylacetamide derivatives (**6a–j**). Reagents and condition: **a** K_2CO_3, KI, anhydrous DMF, stirred at 80°C for 5–24 h

Apparatus (Janki impex private limited, Ahmedabad, India) and are uncorrected. Infrared spectra were recorded using KBr pellets on SHIMADZU-FT-IR 8400S instrument. Mass spectra were recorded on Perkin–Elmer LC–MS PE Sciex API/65 Spectrophotometer (Perkin–Elmer (India) Pvt. Ltd., Mumbai, India). The ^{1}H NMR spectra was recorded on Brucker Avance-300 (300 MHz) model in $CDCl_3$ and DMSO as solvent and TMSi as internal standard with 1H resonant frequency of 300 MHz. The chemical shifts were measured in δ ppm downfield from internal standard TMSi at $\delta = 0$. Purity of the synthesized compounds was checked by thin layer chromatography (TLC). The TLC was performed on alumina silica gel 60 F_{254} (Merck). The mobile phase was ethyl acetate and n-hexane (1:1), and detection was made using UV light and iodine spotting. The resulting compounds were purified by column chromatography. For column chromatography, Merck silica gel (0.040–0.063 mm) was used. The elemental analyses were done on Elementar Vario EL III Carlo Erba 1108 and were in well accordance with the structures assigned to the compounds. All the compounds gave C, H, and N analyses within ±0.5% of the theoretical values. Synthetic grade chemicals procured from SD fine Chemicals, Baroda, India, were used for the synthesis of the target compounds, only after purification. All the starting materials were prepared according to the established procedures with some minor modifications. General synthetic procedures used for the preparation of the target compounds (**6a–j**) are as follows:

General procedure for the preparation of compounds

Preparation of isatin (3) and 5,7dibromo isatin (4) It was done according to the already reported procedure, mentioned in Vine *et al.* 2007a, b.

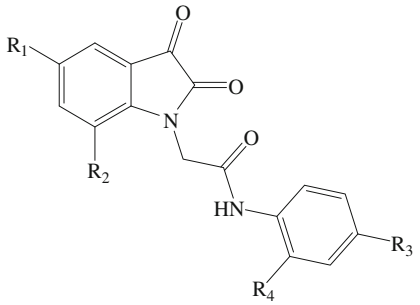
*General procedure for preparation of 2-chloro-N-phenylacetamide derivatives (**5a–e**)*

Aromatic amines (like aniline, *p*-nitroaniline, *p*-toluedine, *o*-nitroaniline, and *o*-toluedine (1 g)) were dissolved in a mixture of 25 ml of glacial acetic acid and 25 ml of saturated solution of sodium acetate. To this chloroacety chloride (1 ml, 8.8 mmol) was added dropwise to avoid the vigorous reaction. After half an hour, product (**5a–e**) was filtered. The product was washed with 50% acetic acid and finally with water. The product was crystallized from methanol.

*General procedure for preparation of final product (**6a–j**)*

The isatin (**3**) or 5,7-dibromoisatin isatin (**4**) (1 g) (47) was taken up in anhydrous DMF and ice cooled with stirring. Solid K_2CO_3 (1 g, 7.2 mmol) was added in one portion, and the dark colored suspension was raised to room temperature and stirred for a further 1 h. The appropriate 2-chloro-*N*-phenylacetamide derivatives (**5a–e**) (1 g) and KI (0.5 g, 6 mmol) were added, and the reaction mixture was stirred at 80°C for 5–24 h, until the reaction was over which was confirmed using TLC. The reaction mixture was

Table 3 Physical and analytical data of the novel 2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-*N*-phenylacetamide derivatives



Entry	Product[a]	R_1	R_2	R_3	R_4	Yield[b] (%)	m.p.[c] (°C)	Mol. formula/mol. wt.	Elem. analysis (cal./found)		
									C	H	N
1	**6a**	Br	Br	H	H	67.85	230–234	$C_{16}H_{10}Br_2N_2O_3$	43.87	2.30	6.39
								438	43.92	2.25	6.43
2	**6b**	Br	Br	NO_2	H	61.05	212–218	$C_{16}H_9Br_2N_3O_5$	39.78	1.88	8.70
								483	39.75	1.83	8.74
3	**6c**	Br	Br	CH_3	H	59.00	225–228	$C_{17}H_{12}Br_2N_2O_3$	45.16	2.68	6.20
								452	45.20	2.73	6.18
4	**6d**	Br	Br	H	NO_2	54.00	234–238	$C_{16}H_9Br_2N_3O_5$	39.78	1.88	8.70
								483	39.74	1.92	8.73
5	**6e**	Br	Br	H	CH_3	59.87	214–218	$C_{17}H_{12}Br_2N_2O_3$	45.16	2.68	6.20
								452	45.18	2.72	6.18
6	**6f**	H	H	H	H	62.35	215–219	$C_{16}H_{12}N_2O_3$	68.56	4.32	9.99
								280	68.60	4.28	9.95
7	**6g**	H	H	NO_2	H	56.87	222–225	$C_{16}H_{11}N_3O_5$	59.08	3.41	12.92
								325	59.12	3.45	12.98
8	**6h**	H	H	CH_3	H	57.60	225–228	$C_{17}H_{14}N_2O_3$	69.38	4.79	9.52
								294	69.35	4.83	9.55
9	**6i**	H	H	H	NO_2	51.64	215–221	$C_{16}H_{11}N_3O_5$	59.08	3.41	12.92
								325	59.06	3.38	12.88
10	**6j**	H	H	H	CH_3	47.90	222–226	$C_{17}H_{14}N_2O_3$	69.38	4.79	9.52
								294	69.33	4.83	9.55

[a] Products were characterized by IR, ^{1}H NMR, MS, and elemental analyses, [b] isolated yields, [c] melting points are uncorrected

poured into HCl (50 ml) and extracted with ethyl acetate (50 ml). The ethyl acetate layer was washed with brine and dried over $MgSO_4$. The solvent was removed under vacuum to yield products (**6a–j**).

*2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-phenylacetamide (**6a**)* Colorless crystals from DMF: Yield 67.85%; m.p. 230–234°C; IR (KBr, υ cm^{-1}): 1726 (>C=O of isatin), 1650 (–NH–CO), 552 (–C–Br); ^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.85–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 7.00–7.90 (m, 7H, Ar–H) ppm; and MS: m/z 439 (M + 1), 440 (M + 2), 442 (M + 4).

*2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(4-nitrophenyl)acetamide (**6b**)* Light red crystals from DMF: Yield 61.05%; m.p. 212–218°C; IR (KBr, υ cm^{-1}): 1730 (>C=O of isatin), 1655 (–NH–CO), 558 (–C–Br), 1355 (NO_2 of phenyl); ^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.90–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 7.20–7.85 (m, 6H, Ar–H) ppm; and MS: m/z 484 (M + 1), 485 (M + 2), 487 (M + 4).

*2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(4-methylphenyl)acetamide (**6c**)* Light brown crystals from DMF: Yield 59.00%; m.p. 225–228°C; IR (KBr, υ cm^{-1}): 1734 (>C=O of isatin), 1640 (–NH–CO), 550 (–C–Br);

Table 4 Predicted and observed activities of the synthesized compounds (**6a–j**) with physicochemical properties

Comp. no.	PC	BE	Predicted activity Log IC_{50}	Predicted activity IC_{50} (μM)	Observed activity MCF-7 IC_{50} (μM)[a]	Observed activity VERO IC_{50} (μM)
6a	6.128	20.417	0.538	3.45	3.91	28.06
6b	8.130	22.437	0.202	1.59	1.96	38.09
6c	7.258	21.318	0.356	2.27	2.43	24.98
6d	7.568	21.215	0.319	2.08	2.04	25.99
6e	6.538	20.851	0.468	2.94	3.85	29.87
6f	6.115	20.217	0.547	3.52	–	–
6g	6.121	20.316	0.542	3.48	–	–
6h	5.998	20.225	0.562	3.65	–	–
6i	6.105	19.998	0.556	3.60	–	–
6j	6.155	20.338	0.537	3.44	–	–
Vinblastine					1.90	27.06

MCF-7: human mammary gland adenocarcinoma (non-metastatic) cell line, VERO: African green monkey cell line (non-cancer cells)

"–": compounds (with greater than 50% survival) after an exposure time of 72 h were not further evaluated for finding IC_{50} values

PC partition coefficient and *BE* bending energy

[a] Values are the mean of triplicates of at least two independent experiments

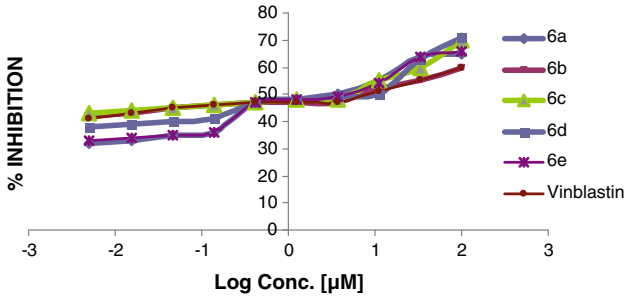


Fig. 2 Graph of Log conc. (μM) versus % inhibition of different compounds (**6a–e**) and vinblastine

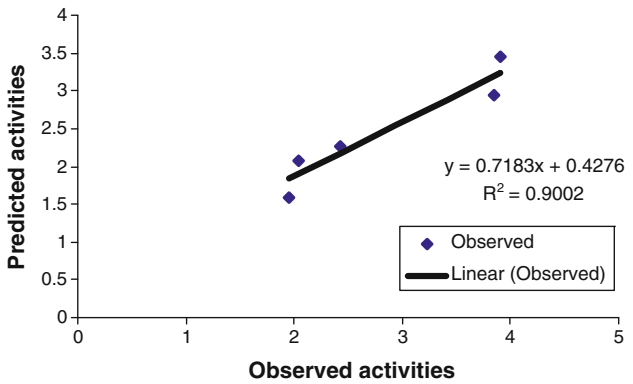


Fig. 3 Graph of observed versus predicted activities of synthesized compounds (**6a–e**)

^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.90–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 2.35–2.40 (m. 3H, Ar–CH_3), 7.00–7.90 (m, 6H, Ar–H) ppm; MS: m/z 453 (M + 1), 454 (M + 2), 456 (M + 4).

2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(2-nitrophenyl)acetamide (***6d***) Colorless crystals from DMF: Yield 54.00%; m.p. 234–238°C; IR (KBr, υ cm^{-1}): 1730 (>C=O of isatin), 1650 (–NH–CO), 555 (–C–Br), 1360 (NO_2 of phenyl); ^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.85–3.95 (m, 2H, H_2C–CO), 7.90 (s, H, NH), 7.00–7.90 (m, 6H, Ar–H) ppm; and MS: m/z 484 (M + 1), 485 (M + 2), 487 (M + 4).

2-(5,7-dibromo-2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(2-methylphenyl)acetamide (***6e***) Colorless crystals from DMF: Yield 59.87%; m.p. 214–218°C; IR (KBr, υ cm^{-1}): 1734 (>C=O of isatin), 1640 (–NH–CO), 550 (–C–Br); ^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.90–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 2.35–2.40 (m. 3H, Ar–CH_3), 7.00–7.90 (m, 6H, Ar–H) ppm; and MS: m/z 453 (M + 1), 454 (M + 2), 456 (M + 4).

2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-phenylacetamide (***6f***) Light red crystals from DMF: Yield 62.35%; m.p. 215–219°C; IR (KBr, υ cm^{-1}): 1726 (>C=O of isatin), 1650 (–NH–CO); ^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.85–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 7.00–7.70 (m, 9H, Ar–H) ppm; and MS: m/z 281 (M + 1).

2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(4-nitrophenyl) acetamide (***6g***) Colorless crystals from DMF: Yield 56.87%; m.p. 222–225 °C; IR (KBr, υ cm^{-1}): 1730 (>C=O of isatin), 1655 (–NH–CO), 1355 (NO_2 of phenyl); ^{1}H NMR (300 MHz, δ ppm, DMSO-d_6): 3.90–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 7.20–8.20 (m, 8H, Ar–H) ppm; and MS: m/z 326 (M + 1).

*2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(4-methylphenyl) acetamide (**6h**)* Colorless crystals from DMF: Yield 57.60%; m.p. 225–228°C; IR (KBr, υ cm^{-1}): 1734 (>C=O of isatin), 1640 (–NH–CO); ^1H NMR (300 MHz, δ ppm, DMSO-d_6): 3.90–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 2.35–2.40 (m. 3H, Ar–CH_3), 7.00–7.90 (m, 8H, Ar–H) ppm; and MS: m/z 295 (M + 1).

*2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(2-nitrophenyl) acetamide (**6i**)* Light brown crystals from DMF: Yield 51.61%; m.p. 215–221°C; IR (KBr, υ cm^{-1}): 1730 (>C=O of isatin), 1650 (–NH–CO), 1360 (NO_2 of phenyl); ^1H NMR (300 MHz, δ ppm, DMSO-d_6): 3.85–3.95 (m, 2H, H_2C–CO), 7.90 (s, H, NH), 7.00–7.90 (m, 8H, Ar–H) ppm; and MS: m/z 326 (M + 1).

*2-(2,3-dioxo-2,3-dihydro-1H-indol-1-yl)-N-(2-methylphenyl) acetamide (**6j**)* Light yellow crystals from DMF: Yield 47.90%; m.p. 222–226°C; IR (KBr, υ cm^{-1}): 1734 (>C=O of isatin), 1640 (–NH–CO); ^1H NMR (300 MHz, δ ppm, DMSO-d_6): 3.90–3.95 (m, 2H, H_2C–CO), 8.00 (s, H, NH), 2.35–2.40 (m. 3H, Ar–CH_3), 7.00–7.90 (m, 8H, Ar–H) ppm; and MS: m/z 295 (M + 1).

Cytotoxic assay

100 µl of exponentially growing MCF-7 (3×10^3 cells/well) and VERO (5×10^3 cells/well) cells were seeded in 96-well flat-bottomed microtiter plates, and incubated at 37°C in a humidified atmosphere of 5% CO_2/95% air for 24 h to allow the cells to attach to the plates. Then, cells were incubated with 25 µM concentration of the assayed compound at 37°C under the 5% CO_2/95% air atmosphere for 72 h. Cell proliferation was quantified using the XTT (30-[1-(phenylamino) carbonyl]-3,4-tetrazoliumbis (4-methoxy-6-nitro) benzene sulfonic acid sodium salt hydrate) cell proliferation kit according to the manufacturer's instructions. In brief, a freshly prepared mixture solution (50 µl) of XTT labeling reagent and PMS (*N*-methyldibenzopyrazine methyl sulfate) electron coupling reagent were added to each well. The resulting mixtures was further incubated for 4 h in a humidified atmosphere (37°C, 5% CO_2), and the absorbance of the formazan product generated was measured with ELISA reader at a test wavelength of 540 nm and a reference wavelength of 650 nm. Active samples (with less than 50% survival) after an exposure time of 72 h were serially diluted in different concentration ranges and tested adopting the similar procedure. The IC_{50} (50% inhibitory concentration) was then calculated as the drug concentration causing a 50% inhibition of cell proliferation. Data are shown as mean values of three independent experiments performed in triplicate. VERO cell line was used to find out cytotoxic effects of synthesized compound on non-cancer cells.

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