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Hybrid pharmacophore design and synthesis of isatin–benzothiazole analogs for their anti-breast cancer activity

V. Raja Solomon [a,b], Changkun Hu [a], Hoyun Lee [a,b,*]

[a] Tumour Biology Group, Northeastern Ontario Regional Cancer Program at the Sudbury Regional Hospital, 41 Ramsey Lake Road, Sudbury, Ontario, Canada P3E 5J1
[b] Department of Biology, Laurentian University, Sudbury, Ontario, Canada P3E 2C6

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ABSTRACT

A hybrid pharmacophore approach was used to design and synthesize isatin–benzothiazole analogs to examine their anti-breast cancer activity. The cytotoxicity of these compounds were determined using three different human breast tumor cell lines, MDA-MB231, MDA-MB468, MCF7, and two non-cancer breast epithelial cell lines, 184B5 and MCF10A. Although all compounds examined were quite effective on all the cancer cell lines examined, the compounds 4-bromo-1-diethylaminomethyl-1*H*-indole-2,3-dione (**2l**) and 4-chloro-1-dimethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (**5e**) emerged as the most active compounds of this series. Importantly, the cytotoxic effect of **2l** was 10–15-fold higher on cancer than non-cancer cells, suggesting that this compound can be very effective for the control of breast cancer with low side effects. Since **2l** showed effective cytotoxicity on MCF7 cells and arrested the cells at G2/M at a similar concentration, these two phenomena may be closely correlated. We conclude that the isatin-linked benzothiazole analog can serve as a prototype molecule for further development of a new class of anti-breast cancer agents.

1. Introduction

The relative mortality rate caused by cancer is still very high in the developed countries, as it accounts for more than 20% of all deaths. Breast cancer is one of the most commonly diagnosed cancers and causes the second leading deaths in women.[1] Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited mainly due to undesirable side effects and a limited choice of available anticancer drugs. This clearly underscores the need of developing novel chemotherapeutic agents for more effective cancer treatments.[2] Recently, we have demonstrated that 10 μM chloroquine (CQ) significantly increases cancer cell-killing effects when used in combination with radiation or Akt inhibitors.[3,4] Importantly, the CQ-mediated enhancement of cell-killing by Akt inhibitors is cancer-specific.[4] We also synthesized several CQ-analogs and examined their cytotoxic effects on MDA-MB 468 and MCF-7 breast cancer cell lines, and found that some of these compounds are potentially more effective than CQ.[5] The present work is an extension of our ongoing efforts toward developing new, effective anticancer agents by a hybrid pharmacophore approach.

Isatin (1*H*-Indole-2,3-dione) is one of the most promising new class of heterocyclic molecules having many interesting activity profiles and well-tolerated in human subjects.[6,7] The 2-oxoindoles derivatives of SU-5416 (semaxanib, Fig. 1) and SU-11248 (Sunitinib, Fig. 1) were reported having tyrosine kinase inhibitory and antiangiogenic properties.[8] Besides, the structurally relevant SU9516 (Fig. 1) was reported a potential inhibitor of cyclin-dependent kinases (CDKs) that can induce apoptosis in colon carcinoma cells.[9] In addition, CDK inhibitory properties in isatin derived phenylhydrazones have also been reported (Fig. 1, **I**).[11] Based on this information, Abadi et al. synthesized several 2-indolone imino derivatives (Fig. 1, **II**) to examine their antitumor and antiangiogenic properties.[10] Vine et al. also synthesized several substituted isatin analogs (Fig. 1, **III**), and screened against a panel of five human cancer cell lines.[7] These authors concluded that substituted isatins could be effective anticancer drugs.

Numerous papers have shown that the benzothiazole nucleus possesses a potent anticancer activity against human cancer (C.H., unpublished data).[11–17] Importantly, we found that certain compounds containing the benzothiazole nucleus killed cells in a tumor-specific manner (C.H., unpublished data). Bradshaw et al. also showed that the Phortress benzothiazole prodrug (Fig. 2) had effective anticancer activity against xenografts in two different rodent models.[18]

Based on these prior observations, we postulated that a compound containing both isatin and benzothiazole pharmacophores could be very effective for anticancer activity. The synthesis of isatin–benzothiazole conjugates could be possible by a pharmacophore hybrid approach. Hybridization of two different bioactive molecules with complementary pharmacophoric functions or with different

* Corresponding author. Tel.: +1 705 522 6237x2703; fax: +1 705 523 7326.
E-mail address: hlee@hrsrh.on.ca (H. Lee).

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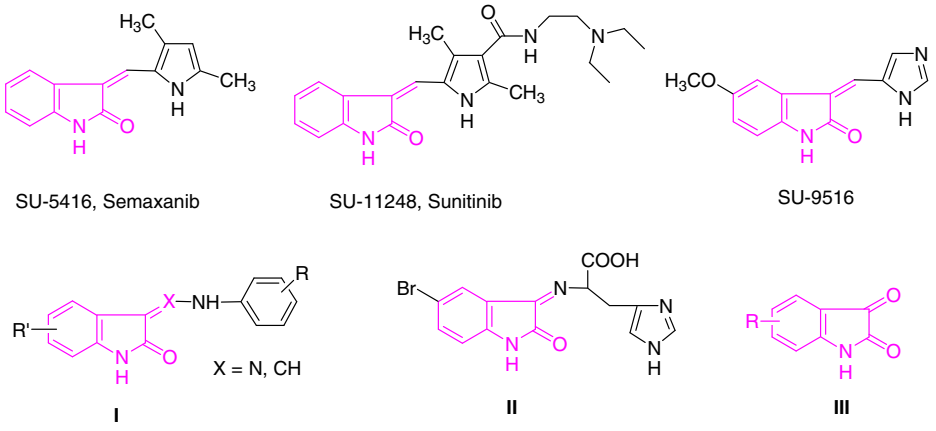


Figure 1. Isatin analogs with anticancer activity.

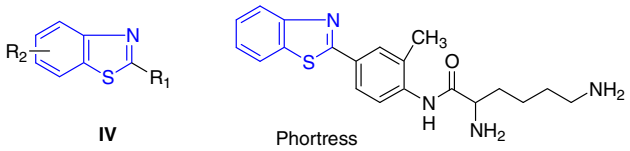


Figure 2. Benzothiazole analogs with anticancer activity.

mechanisms of action often showed synergistic effects.[19–23] Therefore, we synthesized hybrid compounds by linking the main structural unit of the isatin ring system with the benzothiazole ring system by a Schiff base reaction (Fig. 3, **5a–o**), and then examined their cytotoxic effects on three human breast tumor and two matching non-cancer cell lines.

2. Result and discussion

2.1. Chemistry

The compounds **2a–o** and **5a–o** described in this study were prepared as outlined in Scheme 1. The isatin Mannich base analogs 2a–**o** were prepared by condensing the active hydrogen atom of istain with formaldehyde and secondary amino function of amino component. A Mannich reaction can be performed via one pot multi-component protocol requiring isatin, an aldehyde component and an amine, or via a preformed iminium ion. Accordingly, molar equivalent amount of paraformaldehyde and an amino component

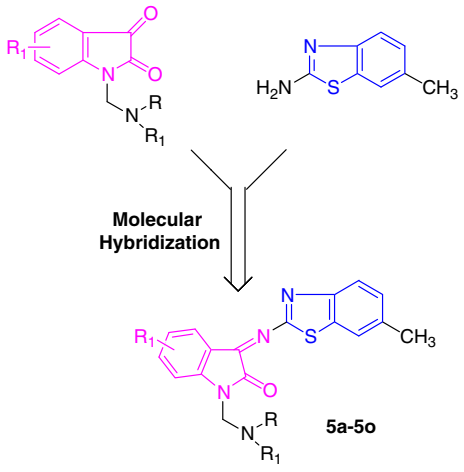


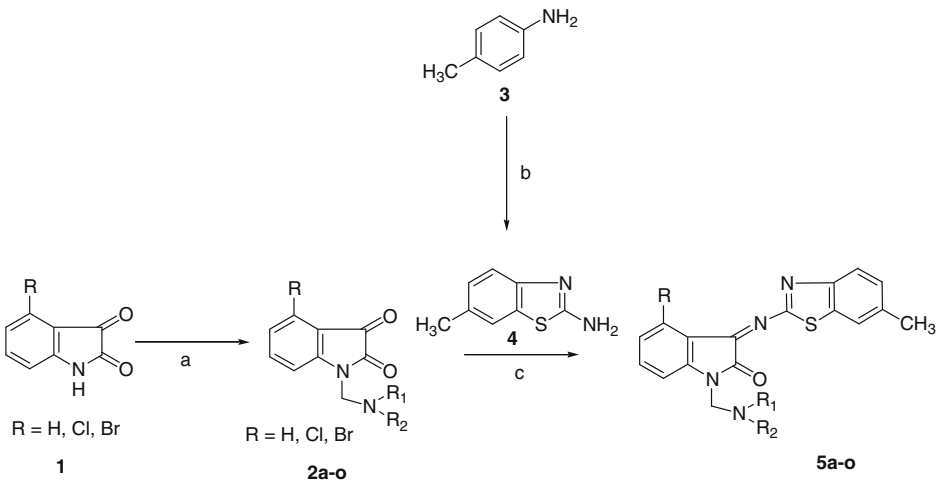
Figure 3. A design for synthesis of isatin–benzothiazole analogs (**5a–o**).

(such as dimethyl amine, diethyl amine, diphenyl amine, morpholine, and piperdine) were dissolved in ethanol and iminium ion formed in situ was then reacted with isatin and substituted isatin (Scheme 1) in ethanol reflux for 4 h to furnish the desired *N*-Mannich isatin derivatives (**2a–o**). The intermediate compound 6-methyl-benzothiazol-2-ylamine was synthesized from 4-methylaniline by a reported procedure, in which 4-methylaniline was treated with potassium thiocynate and bromine in glacial acetic acid.[24] The compounds **5a–o** were synthesized by a Schiff base reaction, in which an aromatic amine such as 6-methyl-benzothiazol-2-ylamine and a carbonyl compound (isatin Mannich base analog) undergoes nucleophilic addition, forming a hemiaminal. This reaction was followed by a dehydration to generate a stable imine. The compounds **5a–o** were synthesized by condensation of the Mannich base analogs **2a–o** with 6-methyl-benzothiazol-2-ylamine in ethanol reflux in the presence of glacial acetic acid. The final products reported in this study were thoroughly characterized by elemental analysis and spectral data.

2.2. In vitro cytotoxicity

Isatin Mannich bases were reported for a wide variety of biological activities such as antibacterial, antifungal, and anti-HIV activity. So far, there is no evidence in literature if isatin Mannich base analogs (**2a–o**) have anti-breast cancer activity. Therefore, we synthesized 15 isatin Mannich base analogs (**2a–o**) and 15 Schiff base compounds (**5a–o**), and then examined their potential as anticancer drugs.

All the compounds synthesized were evaluated for their cytotoxic effects on three breast cancer cell lines, MDA-MB468, MDA-MB231 and MCF7, and two non-cancer breast epithelial cell lines, 184B5 and MCF10A. Each compound stored at 20 mM was diluted from 200 μM to 0.0128 μM by fivefold serial dilutions. Cells were treated with each compound for 48 h, followed by measuring cell growth rates by SRB-based spectrophotometry as described previously.[4,25] The reading of SRB staining is known to accurately reflect the levels of total cellular macromolecules/cell growth/proliferation.[25] The GI_{50} concentration for each compound was calculated with reference to a control sample, which represents the concentration that results in a 50% decrease in cell growth after 48 h incubation in the presence of the drug. For each compound, 50% growth inhibition (GI_{50}) was calculated from Sigmoidal dose–response curves and presented in Table 1. The data for CQ and cisplatin were included as references. The resultant data showed that isatin Mannich base (**2a–o**) and Schiff base compounds (**5a–o**) had significant cytotoxic effects on the three breast cancer cell lines examined.



Scheme 1. Synthesis of isatin Mannich base and Schiff Base analogs (**2a–o** and **5a–o**). Reagents and conditions: (a) Mannich reaction (HCHO and secondary amine, ethanol reflux, 4–6 h (b) KSCN, Br_2/AcOH; (c) 1% acetic acid in ethanol reflux, 6–8 h.

Table 1
Cytotoxicity of novel Mannich isatin and Schiff base analogs (**2a–o** and **5a–o**) on human breast cancer and non-cancer cells[a]

Compound code	R	R_1R_2	GI_{50}[a,b] (μM)					Log *P*[c]
			MDA-MB231	MDA-MB468	MCF7	184B5	MCF10A	
2a	H	$(CH_3)_2$	65.63 ± 1.81	50.15 ± 1.75	40.48 ± 0.98	73.69 ± 1.56	150.43 ± 1.98	0.85
2b	H	$(CH_2CH_3)_2$	60.61 ± 1.53	35.92 ± 1.34	44.05 ± 1.12	41.69 ± 1.02	73.64 ± 1.36	1.53
2c	H	$(C_6H_5)_2$	24.82 ± 0.75	21.46 ± 0.44	23.73 ± 0.31	33.72 ± 0.84	73.01 ± 1.54	4.34
2d	H	Piperidinyl	38.03 ± 0.84	24.05 ± 0.53	41.25 ± 1.31	68.04 ± 1.24	41.75 ± 1.32	1.58
2e	H	Morpholinyl	57.64 ± 1.20	28.23 ± 0.38	52.30 ± 1.56	78.67 ± 1.34	72.08 ± 1.87	0.45
2f	Cl	$(CH_3)_2$	138.46 ± 2.02	107.12 ± 1.78	87.84 ± 1.89	130.41 ± 2.35	82.33 ± 1.98	1.41
2g	Cl	$(CH_2CH_3)_2$	44.48 ± 1.01	28.32 ± 0.32	34.05 ± 0.76	22.52 ± 0.19	34.99 ± 0.56	2.09
2h	Cl	$(C_6H_5)_2$	31.42 ± 0.92	14.35 ± 0.27	29.67 ± 0.52	96.81 ± 1.79	77.16 ± 1.42	4.90
2i	Cl	Piperidinyl	26.48 ± 0.65	82.84 ± 1.68	51.24 ± 1.44	93.72 ± 1.89	70.67 ± 1.24	2.14
2j	Cl	Morpholinyl	61.50 ± 1.76	61.50 ± 1.52	29.19 ± 0.16	79.82 ± 1.65	78.48 ± 1.56	1.01
2k	Br	$(CH_3)_2$	93.72 ± 1.59	49.02 ± 1.42	57.64 ± 0.98	77.92 ± 1.87	67.78 ± 1.01	1.68
2l	Br	$(CH_2CH_3)_2$	20.22 ± 0.52	11.68 ± 0.12	20.20 ± 0.15	171.85 ± 2.18	113.23 ± 1.98	2.36
2m	Br	$(C_6H_5)_2$	51.12 ± 1.24	63.25 ± 1.42	110.20 ± 2.04	98.45 ± 1.88	85.76 ± 1.34	5.17
2n	Br	Piperidinyl	30.15 ± 0.68	35.29 ± 0.84	31.45 ± 0.55	85.22 ± 1.55	66.90 ± 0.54	2.41
2o	Br	Morpholinyl	29.72 ± 0.72	54.03 ± 1.39	65.62 ± 0.89	79.51 ± 1.38	82.33 ± 1.08	1.28
5a	H	$(CH_3)_2$	19.15 ± 0.42	35.45 ± 0.75	29.18 ± 0.23	15.62 ± 0.21	13.41 ± 0.11	4.66
5b	H	$(CH_2CH_3)_2$	32.68 ± 0.93	63.53 ± 1.04	74.77 ± 0.85	27.54 ± 0.31	20.38 ± 0.23	5.33
5c	H	$(C_6H_5)_2$	34.09 ± 0.88	17.66 ± 0.21	39.15 ± 0.45	27.20 ± 0.35	34.04 ± 0.45	8.15
5d	H	Piperidinyl	34.49 ± 0.97	65.61 ± 1.55	37.83 ± 0.32	34.92 ± 0.36	38.92 ± 0.56	5.39
5e	H	Morpholinyl	19.76 ± 0.23	17.61 ± 0.19	14.56 ± 0.21	30.62 ± 0.29	38.49 ± 0.65	4.26
5f	Cl	$(CH_3)_2$	10.92 ± 0.11	28.09 ± 0.26	20.67 ± 0.34	5.77 ± 0.02	7.02 ± 0.01	5.21
5g	Cl	$(CH_2CH_3)_2$	20.43 ± 0.35	27.33 ± 0.34	19.17 ± 0.22	87.84 ± 1.87	43.89 ± 0.89	5.89
5h	Cl	$(C_6H_5)_2$	53.96 ± 1.05	85.04 ± 1.54	24.32 ± 0.39	67.78 ± 1.52	70.01 ± 1.02	8.70
5i	Cl	Piperidinyl	55.80 ± 1.23	19.78 ± 0.21	30.94 ± 0.40	82.33 ± 1.88	30.15 ± 0.44	5.95
5j	Cl	Morpholinyl	37.15 ± 0.69	24.03 ± 0.31	21.46 ± 0.15	63.52 ± 1.72	62.31 ± 0.89	4.81
5k	Br	$(CH_3)_2$	49.02 ± 1.22	27.35 ± 0.41	17.95 ± 0.29	74.77 ± 1.65	21.80 ± 0.12	5.48
5l	Br	$(CH_2CH_3)_2$	28.65 ± 0.82	43.06 ± 0.45	30.15 ± 0.56	34.32 ± 0.54	16.11 ± 0.21	6.16
5m	Br	$(C_6H_5)_2$	75.13 ± 1.89	82.33 ± 1.87	30.15 ± 0.59	70.25 ± 0.69	67.88 ± 0.89	8.98
5n	Br	Piperidinyl	38.25 ± 0.98	24.03 ± 0.24	39.07 ± .62	85.23 ± 1.25	18.37 ± 0.54	6.22
5o	Br	Morpholinyl	94.64 ± 1.85	24.82 ± 0.35	12.01 ± 0.11	29.93 ± 0.54	33.24 ± 0.23	5.08
Chloroquine			22.52 ± 1.44	28.58 ± 1.25	38.44 ± 1.20	76.13 ± 1.13	81.26 ± 1.45	–
Cisplatin			23.65 ± 0.23	31.02 ± 0.45	25.77 ± 0.38	25.54 ± 0.35	51.51 ± 0.65	–

GI_{50} was calculated from dose–response curves.
[a] Sigmoidal dose–response curves (variable slope) were generated using GraphPad Prism V. 4.02 (GraphPad Software Inc.).
[b] Values are the mean of triplicates of at least two independent experiments.
[c] Log *P* were calculated using ChemDraw Ultra V.8.0 (CambridgeSoft Corporation).

Among the thirty isatin–benzothiazole compounds examined, fourteen compounds showed GI_{50} in the range of 11.68–28.32 μM, nine compounds 35.29–61.50 μM, and remaining seven compounds showed above 63.25 μM on the MDA-MB468 breast cancer cells (Table 1). The GI_{50} of these 30 compounds on MDA-MB231 cells was as follows: 10 compounds showed between 10.92 and 30.15 μM, 15 compounds at 31.42–60.61 μM, the rest above 61.50 μM. As for MCF-7 cells, fifteen compounds showed GI_{50} in the range of 12.01–30.94 μM, 12 compounds at 31.45–57.64 μM, the remaining three above 65.62 μM. The differences in the GI_{50} values may be attributable to such factors as the nature of the N-substitution at the isatin ring system and the halogen substitution on the 4th position of isatin ring system, and the genetic and biochemical background of the cell lines.

The structure–activity relationship studies appeared to suggest that the introduction of a hydrophobic substituent of chloro (**2g**) and bromo (**2l**) group in the 4th position of 1-diethylaminomethyl-1*H*-indole-2,3-dione (**2b**) leads to an increase in cytotoxic activity on MDA-MB231, MDA-MB468, and MCF7 cells, in comparison to unsubstituted compound (**2b**). The most active compound among the isatin Mannich base series was **2l**, which exhibited GI_{50} values of 20.22, 11.68 and 20.2 μM on MDA-MB231, MDA-MB468, and MCF7 cells, respectively. This compound showed in vitro cytotoxicity similar to that of cisplatin. However, **2l** can be significantly more advantageous over cisplatin, since **2l** preferentially inhibited cancer cell growth. For example, GI_{50} values of **2l** for cancer cells were 20.22 (MDA-MB231), 11.68 (MDA-MB468, and 20.20 μM (MCF7), while its GI_{50} values for non-cancer cells were 171.85 (185B5) and 113.23 μM (MCF10A). Thus, the growth inhibition value of **2l** on cancer cells was 5.6-fold (i.e., 113.23 μM/20.22 μM) to 14.7-fold (i.e., 171.85 μM/20.20 μM) higher than non-cancer cells (Table 1). In contrast, the cytotoxic effects of cisplatin on cancer cells and non-cancer cells were similar (Table 1). This data certainly reveals the great potential of the compound **2l** as an effective anticancer agent, since it may have a similar antitumor activity with cisplatin but with much lower side effects. Considering the fact that undesirable side effect is often the rate limiting factor for chemotherapy, the compound **2l** shows great promise.

Comparing with the Mannich base compounds **2a–o**, the Schiff base compounds **5a–o** were generally more active. For example, the GI_{50} values of **2a** (Mannich base) and **5a** (Schiff base) were 65.63 versus 19.15 μM (MDA-MB231), 50.15 versus 35.45 μM (MDA-MB468), and 40.48 versus 29.18 μM (MCF7), respectively (Table 1). Likewise, the Schiff base compounds **5a**, **5e**, **5f**, **5g**, **5j** and **5k** were more active than isatin Mannich base compounds **2a**, **2e**, **2f**, **2g**, **2j** and **2k** on MDA-MB231, MDA-MB468, and MCF7 cells. This result suggests that hybridization of the isatin ring system with the benzothiazole ring system can lead to enhanced cytotoxic effects on human breast cancer cells. Interestingly, Schiff base compounds also showed stronger cytotoxic effects on non-cancer cells than Mannich base compounds.

Among the Schiff base analogs, the compound **5e** was the most effective as its GI_{50} values were 19.76, 17.61 and 14.56 μM on MDA-MB468, MDA-MB231 and MCF7 cells, respectively (Table 1). This data demonstrates that **5e** is more potent than cisplatin. Like other isatin–benzothiazole hybrid compounds, the growth inhibition by **5e** on non-cancer cells was also quite high (30.62 and 38.49 μM for 184B5 and MCF10A cell lines, respectively). Nevertheless, the cytotoxic effect of **5e** on cancer cells was approximately twofold greater than non-cancer cell lines (Table 1).

We did not find any significant correlation between GI_{50} values and log *P* values of the 30 compounds **2a–o** and **5a–o** (Table 1). Therefore, the difference in liphophilicity may not be a significant factor for the difference in cytotoxicity of the hybrid compounds examined in our work.

We carried out cell cycle analysis by flow cytometry of the two most promising compounds **2l** and **5e** to gain insight into their mechanism of action. MCF7 and MCF10A cells were treated with either the compound **2l** or **5e** at two different concentrations (20 or 40 μM) for 24 h. MCF7 cells treated with 20 μM of compound **2l** showed 38% and 8% in G2/M and S phase, respectively, while a non-treat control showed 12% and 17% in G2/M and S phase, respectively (Fig. 4, panels a and b). In contrast, the treatment of the non-cancer MCF10A with 20 μM of **2l** did not alter cell cycle distribution, compared to the non-treated control (f vs g in Fig. 4). Since **2l** could effectively arrest cell cycle and inhibit cancer cell growth at a similar concentration, our data suggests that the **2l**-mediated growth inhibition is directly relevant to its ability to arrest cell cycle progression. This data is also consistent with previous reports that isatin and its analogs induced G2/M arrest.[26–28]

Unlike **2l**, **5e** did not induce cell cycle arrest at 20 μM on either MCF7 or MCF10A, although it did result in accumulation of small number of MCF7 cells in G2/M at 40 μM (Fig. 4, panel e). Since GI_{50} values of the compound **5e** for cancer cells were observed at below 20 μM (Table 1), the cytotoxic effects by **5e** may not be caused by cell cycle perturbation.

3. Conclusion

Here, we describe synthesis and examination of a new series of isatin Mannich base and isatin-linked benzothiazole Schiff base analogs. Some of these derivatives exhibited promising anti-breast cancer activity. In particular, the compounds **2l** (4-bromo-1-diethylaminomethyl-1*H*-indole-2,3-dione) and **5e** (4-Chloro-1-dimethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydroindol-2-one) emerged as promising compounds. The compound **2l** is particularly promising, since it could kill cancer cells 10–15 times more effectively than non-cancer cells. This property of **2l** may enable us to effectively control tumors with low side effects. The differential killing of cancer and non-cancer cells by **2l** may be, at least in part, due to its differential effects on cell cycle progression of cancer and non-cancer cells. The compound **5e** also showed a promise as it requires lower concentration to achieve GI_{50} than **2l**, although its differential cytotoxic effects on cancer and non-cancer cells was not as large as **2l**. To further improve efficacy and specificity for cancer cell killing, additional structural modifications of isatin–benzothiazole compounds are in progress.

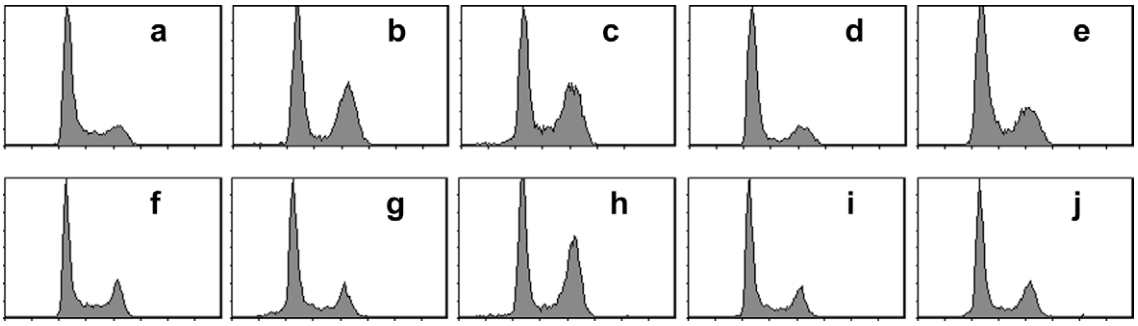


Figure 4. The compounds **2l** and **5e** arrest cell cycle progression at G2/M phase. Cells collected at 24 h post-compound treatment, fixed in 70% ethanol, stained with 100 μg/mL propidium iodide, and cell cycle progression was measured by cytometry (Beckmann Coulter Cytomics FC500). Panels a–e and f–j show data generated from MCF7 and MCF10A cells, respectively. Panels a and f are negative controls (DMSO treated). Samples were treated with **2l**: b (20 μM), c (40 μM), g (20 μM), and h (40 μM). The following samples were treated with **5e**: d (20 μM), 2 (40 μM), i (20 μM) and j (40 μM).

4. Experimental

Melting points (mp) were taken in open capillaries on the Complab melting point apparatus. Elemental analysis was performed on a Perkin–Elmer 2400 C, H, N analyzer and values were within the acceptable limit of the calculated values. The ^{1}H spectra were recorded on a DPX-200 MHz Bruker FT-NMR spectrometer using $CDCl_3$ and DMSO-d_6 as solvent. The chemical shifts were reported as parts per million (δ ppm) tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). The progress of the reaction was monitored on readymade silica-gel plates (Merck) using chloroform/methanol (9:1) as solvent. Iodine was used as a developing agent or by spraying with the Dragendorff's reagent. Chromatographic purification was performed over a silica gel (100–200 mesh). All chemicals and reagents obtained from Aldrich (USA) were used without further purification.

4.1. General synthetic procedure for the preparation of compounds 2a–o

To the solution of isatin or substituted isatin (0.9 g, 4.08 mmol) in 5 mL of absolute ethanol was added to a mixture of secondary amino compound (0.27 g, 1.35 mmol) and aqueous formaldehyde 37% (0.5 mL) also dissolved in 10 mL of absolute ethanol. The reaction mixture was stirred for 3 h at room temperature, and then refrigerated for 48 h to form crystals. The crystalline products were separated by filtration, washed with hexane, and vacuum dried. Recrystallization from ethanol rendered desired products in pure form.

4.1.1. 1-Dimethylaminomethyl-1*H*-indole-2,3-dione (2a)

This compound was obtained as gummy matter in 80% yield. Mp 108–110 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.31 (s, 6H, N(CH_3)$_2$), 4.36 (s, 2H, CH_2), 6.36–6.43 (m, 1H, Ar-*H*), 7.05–7.24 (m, 1H, Ar-*H*), 7.47–7.71 (m, 1H, Ar-*H*), 8.11 (s, 1H, Ar-*H*); FAB-MS *m/z* 205 [M+H]$^+$. Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.71; H, 5.95; N, 13.70.

4.1.2. 1-Diethylaminomethyl-1*H*-indole-2,3-dione (2b)

It was obtained as a reddish orange solid in 82% yield. Mp 176–178 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.05–1.12 (t, J = 7.0 Hz, 6H, N(CH_2CH_3)$_2$), 2.62–2.73 (q, 4H, N(CH_2CH_3)$_2$), 4.51 (s, 2H, CH_2), 7.09–7.16 (m, 2H, Ar-*H*), 7.55–7.63 (m, 2H, Ar-*H*); FAB-MS *m/z* 233 [M+H]$^+$. Anal. Calcd for $C_{13}H_{16}N_2O_2$: C, 67.22; H, 6.94; N, 12.06. Found: C, 67.22; H, 6.95; N, 12.07.

4.1.3. 1-[(Diphenylamino)-methyl]-1*H*-indole-2,3-dione (2c)

It was was obtained as a pale orange solid in 72% yield. Mp 138–140 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.16 (s, 2H, CH_2), 6.39–6.93 (m, 2H, Ar-*H*), 7.05–7.24 (m, 5H, Ar-*H*), 7.48–7.69 (m, 6H, Ar-*H*), 8.05 (s, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 63.40, 112.19, 112.67 (2C), 117.76 (2C), 118.27 (2C), 123.22 (2C), 123.99 (2C), 124.99 (2C), 125.15 (2C), 150.73, 151.18, 158.09, 183.99 (2C); FAB-MS *m/z* 329 [M+H]$^+$. Anal. Calcd for $C_{21}H_{16}N_2O_2$: C, 76.81; H, 4.91; N, 8.53. Found: C, 76.80; H, 4.95; N, 8.55.

4.1.4. 1-Piperidin-1-ylmethyl-1*H*-indole-2,3-dione (2d)

This compound was obtained as a orange red solid in 76% yield. Mp 126–128 °C; IR (KBr, cm^{-1}): 1745, 1481, 1357, 970, 820, 767; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.39–1.52 (m, 6H, CH_2 piperidinyl), 2.52–2.57 (m, 4H, CH_2 piperidinyl), 4.39 (s, 2H, CH_2), 7.09–7.24 (m, 1H, Ar-*H*), 7.53–7.68 (m, 2H, Ar-*H*), 8.14 (s, 1H, Ar-*H*); FAB-MS *m/z* 245 [M+H]$^+$. Anal. Calcd for $C_{14}H_{16}N_2O_2$: C, 68.83; H, 6.60; N, 11.47. Found: C, 68.80; H, 6.58; N, 11.45.

4.1.5. 1-Morpholin-4-ylmethyl-1*H*-indole-2,3-dione (2e)

This compound was obtained as a yellowish orange solid in 72% yield. Mp 194–196 °C; IR (KBr, cm^{-1}): 1744, 1344, 1440, 1029, 997, 771, 731, 689; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.55–2.62 (m, 4H, CH_2 morpholinyl), 3.60–3.64 (m, 4H, CH_2 morpholinyl), 4.43 (s, 2H, CH_2), 7.11–7.25 (m, 1H, Ar-*H*), 7.54–7.67 (m, 2H, Ar-*H*), 8.09 (s, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 51.00 (2C), 62.58(2C), 66.65, 111.73, 117.57, 123.97, 125.30, 138.40, 151.43, 158.84, 183.08; FAB-MS *m/z* 247 [M+H]$^+$. Anal. Calcd for $C_{13}H_{14}N_2O_3$: C, 63.40; H, 5.73; N, 11.38. Found: C, 63.42; H, 5.75; N, 11.42.

4.1.6. 4-Chloro-1-dimethylaminomethyl-1*H*-indole-2,3-dione (2f)

This compound was obtained as gummy matter in 63% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.36 (s, 6H, N(CH_3)$_2$), 4.40 (s, 2H, CH_2), 7.02–7.22 (m, 2H, Ar-*H*), 7.54–7.58 (m, 1H, Ar-*H*); FAB-MS *m/z* 240 [M+H]$^+$. Anal. Calcd for $C_{11}H_{11}ClN_2O_2$: C, 55.36; H, 4.65; N, 11.74. Found: C, 55.39; H, 4.68; N, 11.78.

4.1.7. 4-Chloro-1-diethylaminomethyl-1*H*-indole-2,3-dione (2g)

This compound was obtained as a dark brownish red solid in 79% yield. Mp 98–100 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.04–1.11 (t, J = 7.0 Hz, 6H, N(CH_2CH_3)$_2$), 2.61–2.72 (q, 4H, N(CH_2CH_3)$_2$), 4.51 (s, 2H, CH_2), 7.03–7.09 (m, 2H, Ar-*H*), 7.45–7.46 (m, 1H, Ar-*H*); FAB-MS *m/z* 268 [M+H]$^+$. Anal. Calcd for $C_{13}H_{15}ClN_2O_2$: C, 58.54; H, 5.67; N, 10.50. Found: C, 58.52; H, 5.65; N, 13.27.

4.1.8. 4-Chloro-1-[(diphenylamino)-methyl]-1*H*-indole-2,3-dione (2h)

This compound was obtained as a yellowish orange solid in 72% yield. Mp 210–212 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.18 (s, 2H, CH_2), 6.83–6.87 (m, 2H, Ar-*H*), 6.97–7.01 (m, 2H, Ar-*H*), 7.08–7.12 (m, 2H, Ar-*H*), 7.17–7.22 (m, 2H, Ar-*H*), 7.44–7.48 (m, 2H, Ar-*H*), 7.52–7.63 (m, 2H, Ar-*H*), 7.90–7.92 (m, 1H, Ar-*H*); FAB-MS *m/z* 364 [M+H]$^+$. Anal. Calcd for $C_{21}H_{15}ClN_2O_2$: C, 69.52; H, 4.17; N, 7.72. Found: C, 69.57; H, 4.15; N, 7.77.

4.1.9. 4-Chloro-1-piperidin-1-ylmethyl-1*H*-indole-2,3-dione (2i)

This compound was obtained as a dark brownish red solid in 75% yield. Mp 154–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.35–1.39 (m, 2H, CH_2 piperidinyl), 1.46–1.49 (m, 4H, CH_2 piperidinyl), 2.43–2.54 (m, 4H, CH_2 piperidinyl), 4.31 (s, 2H, CH_2), 7.01–7.08 (m, 2H, Ar-*H*), 7.48–7.51 (m, 1H, Ar-*H*); FAB-MS *m/z* 280 [M+H]$^+$. Anal. Calcd for $C_{14}H_{15}ClN_2O_2$: C, 60.33; H, 5.42; N, 10.05. Found: C, 60.29; H,12.70; N, 10.03.

4.1.10. 4-Chloro-1-morpholin-4-ylmethyl-1*H*-indole-2,3-dione (2j)

This compound was obtained as a yellowish orange solid in 69% yield. Mp 184–186 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.51–2.55 (m, 4H, CH_2 morpholinyl), 3.56–3.61 (m, 4H, CH_2 morpholinyl), 4.34 (s, 2H, CH_2), 6.99–7.12 (m, 2H, Ar-*H*), 7.48–7.51 (m, 1H, Ar-*H*); FAB-MS *m/z* 282 [M+H]$^+$. Anal. Calcd for $C_{13}H_{13}ClN_2O_3$: C, 55.62; H, 4.67; N, 9.98. Found: C, 55.65; H, 4.69; N, 10.00.

4.1.11. 4-Bromo-1-dimethylaminomethyl-1*H*-indole-2,3-dione (2k)

This compound was obtained as a reddish orange solid in 76% yield. Mp 152–154 °C; IR (KBr, cm^{-1}): 1735, 1445, 1331, 1015, 859, 782, 292; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 6H, N(CH_3)$_2$), 5.19–5.23 (m, 2H, CH_2), 7.21–7.30 (m, 2H, Ar-*H*), 7.31–7.49 (m, 1H, Ar-*H*); FAB-MS *m/z* 284 [M+H]$^+$. Anal. Calcd for $C_{11}H_{11}BrN_2O_2$: C, 46.66; H, 3.92; N, 9.89. Found: C, 46.68; H, 3.96; N, 9.91.

4.1.12. 4-Bromo-1-diethylaminomethyl-1*H*-indole-2,3-dione (2l)

This compound was obtained as a yellowish orange solid in 77% yield. Mp 128–130 °C; IR (KBr, cm^{-1}): 1741, 1446, 1326, 1160, 1442, 906, 870, 770, 693; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.04–1.11 (t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$), 2.61–2.71 (q, 4H, $N(CH_2CH_3)_2$), 4.51 (s, 2H, CH_2), 7.19–7.31 (m, 2H, Ar-*H*), 7.36–7.40 (m, 1H, Ar-*H*); FAB-MS *m/z* 312 $[M+H]^+$. Anal. Calcd for $C_{13}H_{15}BrN_2O_2$: C, 50.18; H, 4.86; N, 9.00. Found: C, 50.16; H, 4.88; N, 8.98.

4.1.13. 4-Bromo-1-[(diphenylamino)-methyl]-1*H*-indole-2,3-dione (2m)

This compound was obtained as a yellowish orange solid in 71% yield. Mp 230–232 °C; IR (KBr, cm^{-1}): 1721, 1439, 1388, 970, 793, 676; ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.20–5.24 (m, 2H, CH_2), 6.89–6.91 (m, 2H, Ar-*H*), 7.20–7.31 (m, 2H, Ar-*H*), 7.33–7.37 (m, 2H, Ar-*H*), 7.41–7.48 (m, 2H, Ar-*H*), 7.44–7.48 (m, 2H, Ar-*H*), 7.52–7.63 (m, 2H, Ar-*H*), 7.64–7.66 (m, 1H, Ar-*H*); FAB-MS *m/z* 408 $[M+H]^+$. Anal. Calcd for $C_{21}H_{15}BrN_2O_2$: C, 61.93; H, 3.71; N, 6.88. Found: C, 61.91; H, 3.69; N, 6.86.

4.1.14. 4-Bromo-1-piperidin-1-ylmethyl-1*H*-indole-2,3-dione (2n)

This compound was obtained as a reddish orange solid in 68% yield. Mp 136–138 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.42–1.45 (m, 2H, CH_2 piperidinyl), 1.49–1.57 (m, 4H, CH_2 piperidinyl), 2.43–2.54 (m, 4H, CH_2 piperidinyl), 5.19–5.22 (s, 2H, CH_2), 7.13–7.31 (m, 2H, Ar-*H*), 7.44–7.51 (m, 1H, Ar-*H*); FAB-MS *m/z* 324 $[M+H]^+$. Anal. Calcd for $C_{14}H_{15}BrN_2O_2$: C, 52.03; H, 4.68;; N, 8.67. Found: C, 52.05; H,4.64; N, 8.70.

4.1.15. 4-Bromo-1-morpholin-4-ylmethyl-1*H*-indole-2,3-dione (2o)

This compound was obtained as a orange red solid in 65% yield. Mp 184–186 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.59–2.64 (m, 4H, CH_2 morpholinyl), 3.62–3.67 (m, 4H, CH_2 morpholinyl), 4.35 (s, 2H, CH_2), 7.15–7.31 (m, 2H, Ar-*H*), 7.45–7.53 (m, 1H, Ar-*H*); FAB-MS *m/z* 326 $[M+H]^+$. Anal. Calcd for $C_{13}H_{13}BrN_2O_3$: C, 48.02; H, 4.03; N, 8.62. Found: C, 48.07; H, 4.01; N, 8.59.

4.2. General synthetic procedure for the preparation of compounds 5a–o

Equimolar quantities of isatin derivatives **2a–o** (3.65 mmol) and 6-methyl-benzothiazol-2-ylamine (3.65 mmol) were dissolved in 50 mL of absolute ethanol containing 0.5 mL of glacial acetic acid. The reaction mixture was refluxed for 12–14 h, and was cooled to room temperature, and then concentrated to dryness under reduced pressure. The residue was taken up in dichloromethane and washed with 5% aqueous sodium hydrogen carbonate (**2x**) and then with brine solution. The organic phase was then dried over sodium sulfate, the filtrate was concentrated to dryness under reduced pressure and the crude product was purified by column chromatography on silica gel using dichloromethane–methanol.

4.2.1. 1-Dimethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5a)

This compound was obtained as a orange solid in 65% yield. Mp 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 6H, $N(CH_3)_2$), 2.39 (s, 3H, CH_3), 5.38–5.45 (m, 2H, CH_2), 7.04–7.12 (m, 2H, Ar-*H*), 7.36–7.40 (m, 2H, Ar-*H*), 7.52–7.62 (m, 2H, Ar-*H*), 8.65 (s, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 21.24, 48.93 (2C), 66.75, 112.67, 120.93, 123.09, 123.83, 124.80, 125.00, 126.92, 131.03, 131.36, 138.52, 138.55, 149.96, 150.43, 151.17, 164.89; FAB-MS *m/z* 351 $[M+H]^+$. Anal. Calcd for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N, 15.99. Found: C, 65.15; H, 5.16; N, 15.96.

4.2.2. 1-Diethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5b)

This compound was obtained as a dark yellowish orange solid in 60% yield. Mp 202–204 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.19–1.25 (t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$), 2.40 (s, 3H, CH_3), 2.49–2.53 (q, 4H, $N(CH_2CH_3)_2$), 5.30–5.32 (m, 2H, CH_2), 6.91–6.94 (m, 2H, Ar-*H*), 6.96–7.40 (m, 2H, Ar-*H*), 7.51–7.62 (m, 2H, Ar-*H*), 8.65 (s, 1H, Ar-*H*); FAB-MS *m/z* 379 $[M+H]^+$. Anal. Calcd for $C_{21}H_{22}N_4OS$: C, 66.64; H, 5.86; N, 14.80. Found: C, 66.62; H, 5.88; N, 14.81.

4.2.3. 1-[(Diphenylamino)-methyl]-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5c)

This compound was obtained as a reddish orange solid in 58% yield. Mp 158–160 °C; IR (KBr, cm^{-1}): 1731, 1573, 1460, 1344, 920,910, 790, 760; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 5.39–5.43 (s, 2H, CH_2), 6.88–6.93 (m, 2H, Ar-*H*), 7.04–7.15 (m, 6H, Ar-*H*), 7.37–7.63 (m, 6H, Ar-*H*), 7.93–7.97 (m, 2H, Ar-*H*), 8.65 (s, 1H, Ar-*H*); FAB-MS *m/z* 475 $[M+H]^+$. Anal. Calcd for $C_{29}H_{22}N_4OS$: C, 73.39; H, 4.67; N, 11.81. Found: C, 73.41; H, 4.71; N, 11.80.

4.2.4. 3-(6-Methyl-benzothiazol-2-ylimino)-1-piperidin-1-ylmethyl-1,3-dihydro-indol-2-one (5d)

This compound was obtained as viscous gummy matter in 59% yield; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.52–1.71 (m, 6H, CH_2 piperidinyl), 2.40 (s, 3H, CH_3), 2.52–2.57 (m, 4H, CH_2 piperidinyl), 5.53–5.55 (m, 2H, CH_2), 6.61–6.95 (m, 2H, Ar-*H*), 7.10–7.17 (m, 2H, Ar-*H*), 7.30–7.76 (m, 2H, Ar-*H*), 8.29 (s, 1H, Ar-*H*); FAB-MS *m/z* 391 $[M+H]^+$. Anal. Calcd for $C_{22}H_{22}N_4OS$: C, 67.67; H, 5.68; N, 14.35. Found: C, 67.65; H, 5.65; N, 14.34.

4.2.5. 3-(6-Methyl-benzothiazol-2-ylimino)-1-morpholin-4-ylmethyl-1,3-dihydro-indol-2-one (5e)

This compound was obtained as gummy matter in 45% yield. Mp 124–126 °C; ^{1}H NMR (200 MHz, $DMSOd_6$ + $CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.51–2.67 (m, 4H, CH_2 morpholinyl), 3.67–3.78 (m, 4H, CH_2 morpholinyl), 5.30–5.33 (m, 2H, CH_2), 6.63–6.77 (m, 2H, Ar-*H*), 7.15–7.22 (m, 2H, Ar-*H*), 7.37–7.48 (m, 2H, Ar-*H*), 8.65 (s, 1H, Ar-*H*); ^{13}C NMR ($DMSOd_6$ + $CDCl_3$): δ 21.62, 22.99, 23.76, 28.93, 30.38, 68.16, 112.58, 119.01, 121.98, 123.72, 125.78, 128.16, 130.88, 132.47, 135.05, 137.08, 149.31, 150.47, 167.76 (2C), 170.55; FAB-MS *m/z* 393 $[M+H]^+$. Anal. Calcd for $C_{21}H_{20}N_4O_2S$: C, 64.27; H, 5.14; N, 14.28. Found: C, 64.25; H, 5.16; N, 14.32.

4.2.6. 4-Chloro-1-dimethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5f)

This compound was obtained as a reddish orange solid in 57% yield. Mp 134–136 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.35 (s, 6H, $N(CH_3)_2$, 2.44 (s, 3H, CH_3), 5.48 (s, 2H, CH_2), 6.97–7.13 (m, 2H, Ar-*H*), 7.23–7.45 (m, 3H, Ar-*H*), 7.95 (s, 1H, Ar-*H*); FAB-MS *m/z* 386 $[M+H]^+$. Anal. Calcd for $C_{19}H_{17}ClN_4OS$: C, 59.29; H, 4.45; N, 14.56. Found: C, 59.27; H, 4.43; N, 14.54.

4.2.7. 4-Chloro-1-diethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5g)

This compound was obtained as a orange red solid in 53% yield. Mp 200–202 °C; IR (KBr, cm^{-1}): 1740, 1586, 1439, 1390, 921, 910, 877, 662; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.01–1.06 (t, J = 7.0 Hz, 6H, $N(CH_2CH_3)_2$), 2.35 (s, 3H, CH_3), 2.47–2.51 (q, 4H, $N(CH_2CH_3)_2$), 5.36 (s, 2H, CH_2), 6.80–6.96 (m, 2H, Ar-*H*), 7.33–7.57 (m, 3H, Ar-*H*), 7.98 (s, 1H, Ar-*H*); ^{13}C NMR ($CDCl_3$): δ 10.8 (2C), 21.25, 49.12 (2C), 66.51, 120.96, 123.77, 124.23, 124.76, 125.02, 131.05, 131.39, 132.42, 138.84, 139.03, 149.90, 151.56, 164.85 (2C), 181.30; FAB-MS *m/z* 414 $[M+H]^+$. Anal. Calcd for $C_{21}H_{21}ClN_4OS$: C, 61.08; H, 5.13; N, 13.57. Found: C, 61.06; H, 5.15; N, 13.55.

4.2.8. 4-Chloro-1-[(diphenylamino)-methyl]-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5h)

This compound was obtained as a yellowish orange solid in 61% yield. Mp 190–192 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.51 (s, 3H, CH_3), 5.38 (s, 2H, CH_2), 6.77–6.93 (m, 5H, Ar-*H*), 7.01–7.04 (m, 5H, Ar-*H*), 7.30–7.35 (m, 5H, Ar-*H*), 7.98 (s, 1H, Ar-*H*); FAB-MS *m/z* 510 $[M+H]^+$. Anal. Calcd for $C_{29}H_{21}ClN_4OS$: C, 68.43; H, 4.16; N, 11.01. Found: C, 68.45; H, 4.15; N, 11.04.

4.2.9. 4-Chloro-3-(6-methyl-benzothiazol-2-ylimino)-1-piperidin-1-ylmethyl-1,3-dihydro-indol-2-one (5i)

This compound was obtained as gummy matter in 50% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.55–1.60 (m, 2H, CH_2 piperidinyl), 1.66–1.69 (m, 4H, CH_2 piperidinyl), 2.35 (s, 3H, CH_3), 2.38–2.42 (m, 4H, CH_2 piperidinyl), 5.36 (s, 2H, CH_2), 7.15–7.46 (m, 2H, Ar-*H*), 7.62–7.99 (m, 3H, Ar-*H*), 8.35 (s, 1H, Ar-*H*); FAB-MS *m/z* 426 $[M+H]^+$. Anal. Calcd for $C_{22}H_{21}ClN_4OS$: C, 62.18; H, 4.98; N, 13.18. Found: C, 62.20; H, 5.01; N, 13.20.

4.2.10. 4-Chloro-3-(6-methyl-benzothiazol-2-ylimino)-1-morpholin-4-ylmethyl-1,3-dihydro-indol-2-one (5j)

This compound was obtained as a yellowish orange solid in 49% yield. Mp 218–220 °C; IR (KBr, cm^{-1}): 1741, 1590, 1439, 914, 793, 676; ^{1}H NMR (200 MHz, DMSOd_6): δ 2.18–2.27 (m, 4H, CH_2 morpholinyl), 2.35 (s, 3H, CH_3), 3.38–3.44 (m, 4H, CH_2 morpholinyl), 4.02–4.06 (m, 2H, CH_2), 7.15–7.34 (m, 2H, Ar-*H*), 7.47–7.51 (m, 3H, Ar-*H*), 8.15 (s, 1H, Ar-*H*); FAB-MS *m/z* 428 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}ClN4O_2S$: C, 59.08; H, 4.49; N, 13.12. Found: C, 58.06; H, 4.51; N, 13.10.

4.2.11. 4-Bromo-1-dimethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5k)

This compound was obtained as a yellowish orange solid in 56% yield. Mp 202–204 °C; IR (KBr, cm^{-1}): 1741, 1580, 1450, 1325, 970, 920, 787; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.27 (s, 6H, N(CH_3)$_2$, 2.51 (s, 3H, CH_3), 5.36–5.38 (m, 2H, CH_2), 7.00–7.20 (m, 2H, Ar-*H*), 7.32–7.40 (m, 3H, Ar-*H*), 8.57 (s, 1H, Ar-*H*); FAB-MS *m/z* 430 $[M+H]^+$. Anal. Calcd for $C_{19}H_{17}BrN_4OS$: C, 53.15; H, 3.99; N, 13.05. Found: C, 53.11; H, 3.96; N, 13.01.

4.2.12. 4-Bromo-1-diethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5l)

This compound was obtained as a yellowish orange solid in 54% yield. Mp 235–237 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.23–1.26 (t, J = 7.0 Hz, 6H, N(CH_2CH_3)$_2$), 2.36 (s, 3H, CH_3), 2.41–2.47 (q, 4H, N(CH_2CH_3)$_2$), 5.40–5.43 (m, 2H, CH_2), 7.15–7.35 (m, 2H, Ar-*H*), 7.36–7.51 (m, 3H, Ar-*H*), 8.63 (s, 1H, Ar-*H*); FAB-MS *m/z* 458 $[M+H]^+$. Anal. Calcd for $C_{21}H_{21}BrN_4OS$: C, 55.14; H, 4.63; N, 12.25. Found: C, 55.17; H, 4.68; N, 12.29.

4.2.13. 4-Bromo-1-[(diphenylamino)-methyl]-3-(6-methyl-benzothiazol-2-ylimino)-1,3-dihydro-indol-2-one (5m)

This compound was obtained as a yellowish orange solid in 50% yield. Mp 228–230 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3), 5.34–5.38 (m, 2H, CH_2), 6.59–7.01 (m, 5H, Ar-*H*), 7.15–7.35 (m, 5H, Ar-*H*), 7.38–7.41 (m, 3H, Ar-*H*), 7.57–7.65 (m, 2H, Ar-*H*), 8.61 (s, 1H, Ar-*H*); FAB-MS *m/z* 554 $[M+H]^+$. Anal. Calcd for $C_{29}H_{21}BrN_4OS$: C, 62.93; H, 3.82; N, 10.12. Found: C, 62.90; H, 3.85; N, 10.09.

4.2.14. 4-Bromo-3-(6-methyl-benzothiazol-2-ylimino)-1-piperidin-1-ylmethyl-1,3-dihydro-indol-2-one (5n)

This compound was obtained as a yellowish orange solid in 52% yield. Mp 194–196 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.21–1.30 (m, 2H, CH_2 piperidinyl), 1.45–1.63 (m, 4H, CH_2 piperidinyl), 2.29–2.45 (m, 4H, CH_2 piperidinyl), 2.51 (s, 3H, CH_3), 5.51–5.54 (m, 2H, CH_2), 7.01–7.19 (m, 2H, Ar-*H*), 7.31–7.38 (m, 3H, Ar-*H*), 8.33 (s, 1H, Ar-*H*); FAB-MS *m/z* 470 $[M+H]^+$. Anal. Calcd for $C_{22}H_{21}BrN_4OS$: C, 56.29; H, 4.51; N, 11.94. Found: C, 56.31; H, 4.49; N, 11.92.

4.2.15. 4-Bromo-3-(6-methyl-benzothiazol-2-ylimino)-1-morpholin-4-ylmethyl-1,3-dihydro-indol-2-one (5o)

This compound was obtained as a yellowish orange solid in 44% yield. Mp 118–120 °C; IR (KBr, cm^{-1}): 1748, 1607, 1570, 1442, 937, 814, 780, 660; ^{1}H NMR (200 MHz, DMSOd_6): δ 2.29–2.39 (m, 4H, CH_2 morpholinyl), 2.40 (s, 3H, CH_3), 3.35–3.53 (m, 4H, CH_2 morpholinyl), 5.34–5.35 (m, 2H, CH_2), 7.14–7.28 (m, 2H, Ar-*H*), 7.34–7.50 (m, 3H, Ar-*H*), 8.41 (s, 1H, Ar-*H*); FAB-MS *m/z* 472 $[M+H]^+$. Anal. Calcd for $C_{21}H_{19}BrN_4O_2S$: C, 53.51; H, 4.06; N, 11.89. Found: C,53.55; H, 4.10; N, 11.92.

5. Materials and methods

5.1. Cell lines

The human MDA-MB468, MDA-MB231 and MCF-7 breast cancer cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Hyclone, Logan UT) and 2 mM L-glutamine. 184B5 and MCF10A immortalized breast cells were maintained in mammary epithelial basal medium supplemented with an MEGM mammary epithelial singlequot kit (Cambrex). Cells were grown at 37 °C with 5% CO_2, 95% air under the humidified conditions.

5.2. Reagents

Chloroquine diphosphate and cisplatin were purchased from Sigma–Aldrich Canada Ltd (Oakaville, ON, Canada). All the compounds were dissolved in 10–20 mM dimethyl sulfoxide (DMSO) and stored at −20 °C until use. The stock solution was diluted in culture medium (0.1–100 μM) immediately before use. The final concentration of DMSO in the SRB-based cytotoxicity assays did not exceed 0.1%. To rule out that the DMSO concentration used may affect cell cytotoxicity, culture medium containing equivalent concentration of DMSO was used as a negative control in all experiments. In all studies, the concentration of DMSO used did not notably show any cytotoxicity.

5.3. SRB assay

Cytotoxic effects were determined by a Sulphorhodamine B (SRB)-based protocol.[4,25] For a typical screening experiment, 5000–10,000 cells were inoculated into100 μL medium per well of a 96-well microtiter plate as described previously.[25,29] Briefly, after the inoculation, the microtiter plate was incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h, prior to addition of experimental drugs. Some of the sample wells were fixed with 25 μL of 50% trichloroacetic acid (TCA) as a control of the cell population for each cell line at the time of drug addition (*Tz*). An aliquot of the frozen stock was thawed and diluted to the desired final maximum test-concentration with complete medium. Two- to 10-fold serial dilutions were made to provide a total of seven drug concentrations (and a control [*C*]). Following the addition of drugs, the culture plate was incubated for additional 48 h. Cells were fixed in situ by slowly adding 25 μL of cold 50% (w/v) TCA (final concentration, 10% TCA), and were then incubated for 60 min at 4 °C. The supernatant was discarded, and the plate was washed five times with tap water, followed by air-dry. Fifty microliters of SRB solution at 0.4% (w/v) in 1% acetic acid was added to each well, and the plate was incubated for >30 min at room temperature. Unbound SRB was removed by five washes with tap water, followed

by air-drying. The cells 'stained' with SRB were solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515–564 nm. The relative growth rate (%) was calculated for each of the compound concentrations according to the following formula:

$$(Ti - Tz)/(C - Tz) \times 100$$

In the formula, time zero (Tz), control growth (C), and OD for different concentration of tested compounds (Ti). The GI_{50} for each compound was obtained from a non-linear Sigmoidal dose–response (variable slope) curve which is fitted by GraphPad Prism v.4.03 software. Values were calculated for each of these parameters if the level of activity was reached. However, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested.[25,29]

5.4. Flow cytometry

Cells (2.0×10^6) were harvested by centrifugation at 1000 rpm on a bench-top centrifuge for 5 min, followed by fixation with ice-cold ethanol (70%) for 30 min to overnight at −20 °C.[3,30] The ethanol was then removed by centrifugation, and cells were resuspended in 1× PBS solution, followed by centrifuge. The cell pellet was than stained with PI master mix (100 μg/mL RNase A, 100 μg/mL PI, 0.3% Nonidet P-40 and 0.1% sodium citrate in distilled water) for 30 min at 37 °C. DNA content was measured using a Beckmann Coulter Cytomics FC500 (Beckman Coulter, Fullerton, CA), and the proportion of cells in G0/G1, S, and G2/M phases of cell cycle was calculated on the basis of DNA distribution histograms using CXP software.

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