Synthesis and *In Vitro* Evaluation of Some Isatin-Thiazolidinone Hybrid Analogues as Anti-Proliferative Agents

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Abstract: A range of isatin-thiazolidinone hybrid analogues were synthesized and their cytotoxicity was evaluated against several cancer cell lines *in vitro*. The acute toxicity studies in mice models revealed that these analogues possess low systemic toxicity and are safe up to 1600mg/Kg. Among the compounds synthesized, 5-(2-nitrobenzylidene)-2-(isatin-3-azino)-thiazolidin-4-one (CI) has been shown to be the most active, highly promising compound which induced S phase arrest in cell cycle in a time dependent manner. Our initial analysis indicate that incorporation of electron withdrawing group at ortho position of the ring favors over the meta and para positions for eliciting its cytostatic effect. Overall, the *in vitro* biological evaluation suggests that the growth inhibitory effect of CI is promising and can be studied further.

Keywords: Isatin, thiazolidinone, cancer, cytotoxicity, cell cycle arrest.

1. INTRODUCTION

Surgical removal of cancerous tissue followed by radiotherapy and chemotherapy is the standard front line treatment modality of cancer diseases. However, this approach is always not feasible depending on severity of the disease and, in such conditions, the patients mainly rely on long term chemotherapy to maintain the quality of life. Though chemotherapy with conventional anti-tumor drugs offers promising control over tumor associated problems, by inhibiting its progression and metastasis, the drug itself, at most of the times, renders undesirable systemic toxicity. These undesirable effects are usually because of the indiscriminate cytotoxic effect of these drugs to normal cells. Drug resistance is another well known hurdle in chemotherapy. Use of combination chemotherapy, which is the administration of several drugs with different and complementary mechanism of action, is regarded as an effective approach to evade drug resistance. However administration of multiple cytotoxic drugs at a time brings in more serious deleterious systemic effects. Therefore design and synthesis of new anti-cancer agents, with low systemic toxicity and ability to evade different drug resistance mechanisms, is highly desirable and demanding in the field of medicinal chemistry.

Phosphorylation of serine / threonine / tyrosine residues is identified to be one of the main post translational modifications employed by cells to fine tone their metabolic and regulatory pathways [1,2]. Abnormal phosphorylation may sometimes become a cause of initiation or progression of different diseases like cancer, Alzheimer's and atherosclerosis [3,4]. Excessive activity of cyclin-dependent kinases or aberrant functions of other kinases has been observed to be one of the responsible mechanisms underlying pathological hyperproliferation [5]. Consequently, inhibition of these kinases is considered to be one of the therapeutic options for combating cancer [6,7]. The success of "bcr-abl" kinase inhibitor, imatinib, in chronic myeloid leukemia (CML) therapy has already proven the importance of kinase inhibitors in cancer chemotherapy.

1H-indole-2,3-diones (Isatins) and its derivatives have shown a variety of biological effects including antibacterial, antifungal and antiviral activities [8]. In addition, antiproliferative and proapoptotic actions of isatin have been reported in different cancer cell lines at concentrations lower than 10^{-6}mol/L [9]. These previous studies have shown good correlation between the anti-proliferative effects of synthetic oxindoles with their inhibitory effects on ATP binding pockets associated with biomolecules. To be more specific, some of the derivatives were earlier reported to target protein kinases associated with growth factor receptors [10-12] to elicit the anti-proliferative effects. C_3-substituted synthetic oxindoles are a major research focus area and, so far, they were developed as specific inhibitors of protein tyrosine kinases (PTKs) associated epidermal growth factor, platelet derived growth factor, vascular endothelial growth factor and insulin like growth factor receptors by different research groups [13]. The synthetic feasibility of isatin has led to an extensive use of this compound in organic synthesis to conjugate isatin with a variety of other potent molecules to improve the efficacy and versatility of action.

Strikingly, a large number of literatures indicate presence of thiazole moiety in the structure of several naturally occurring molecules with important antibiotic, immunosuppressive and antitumor activities [14]. Studies have shown that thiazolidinone moiety has potential to inhibit tyrosine kinases [15] and its synthetic analog 5-benzylidene-2-

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1573-4064/10 $55.00+.00

phenylimnio-1,3-thiazolidin-4-one has been shown to induce apoptosis in cancer cells sparing normal ones [16]. More interestingly, thiazolidinone analogs are not p-glycoprotein substrates and thus may act as promising molecules in treating p-glycoprotein over-expressing refractory cancers.

Cytotoxic π electron delocalized lipophilic cations (DLCs) possess marked and selective anti-tumor activity. This effect of DLCs is because of their selective accumulation in negatively charged mitochondria in carcinoma cells due to electrochemical proton gradient [17-23]. Structural requirements of 4-oxothiazolidine and double conjugate system has been reported to be essential for the π electron delocalization activity [24,25]. Altogether, considering the tumor therapeutic potential of oxindole and thiazole groups, in the present study, a hybrid molecule has been synthesized, combining these two central molecules and maintaining the unique double conjugate structure. This was followed by synthesis of a range of hybrid analogues and their pharmacological evaluation was done for their cytotoxic and cytostatic activities.

2. MATERIALS AND METHODS

2.1. General

2.1.1. Chemistry

All solvents were of AR grade and compounds were purified by crystallization from absolute alcohol. Reactions were monitored using thin-layer chromatography (TLC) on aluminum backed precoated silica gel plates. In general isatins are highly colored and were visible on a TLC plate; colorless compounds were detected using UV light and/ or iodine vapor. All solvents ratio are quoted as vol/vol. Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infra red (IR) spectra were recorded using FT-IR spectrophotometer (6200 Jasco) in KBr. ^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Bruker (360 MHz) instrument. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard.

2.1.2. Cell Culture and Chemicals

Human cancer cell lines MDA MB 231, MDA MB 435, MCF 7, T47D and SiHa were purchased from ATCC, USA. NCI ADR Res cell line was obtained from National Cancer Institute (NCI, USA). The cells were maintained in DMEM (Sigma, USA) containing 10% heat inactivated FBS (GIBCO, USA) and 1% antibiotic-antimycotic cocktail (GIBCO, USA).

Propidium Iodide, RNAse, DMSO and MTT were purchased from Sigma, USA. All the compounds were dissolved in DMSO for the experiments. The final concentration of DMSO used for the cell culture experiment is 0.1%.

2.1.3. Animal Experiment

Acute toxicity studies were carried out in Swiss albino mice of both sexes weighing between 20-25g.

2.2. Chemistry

Synthesis of 5-arylidene-2-(isatin-3-azino)-thiazolidin-4-one derivatives were performed by the following steps.

2.2.1. Synthesis of Isatin-3-Thiosemicarbazone

Equimolar quantity of Isatin and Thiosemicarbazone was dissolved in warm ethanol. pH was adjusted to 4 - 5 with glacial acetic acid and heated under reflux for 40 minutes. The reaction mixture was allowed to stand for 24h at room temperature and the products were separated by filtration and recrystallized from 90% aqueous ethanol. Yield: 55%; mp: 198-200^{o}C; R_f [benzene: chloroform (5.5 : 4.5)] 0.81; UV λ_{max}: 220 nm in methanol; IR: (KBr νcm^{-1}) 3420cm^{-1}& 3162cm^{-1} NH str, 1700cm^{-1}CO str, 1131cm^{-1}CS str; ^{1}HNMR (DMSO) δ_{ppm} 6.4-6.1 (Ar-H, 9H); MS, *m/z*: 220(M^+).

2.2.2. Synthesis of 2-(Isatin-3-azino)-4-thiazolidinone

A mixture of Isatin-3-thiosemicarbazone (0.01 mol), monochloroacetic acid (0.01 mol) and anhydrous sodium acetate (0.02 mol) in ethanol (100 ml) was heated under reflux for 10 hrs with occasional shaking. The excess solvent was distilled off under vaccum and poured into crushed ice, filtered and recrystallized from 90% aqueous ethanol to give orange crystals. Yield: 60%; mp:210-212^{o}C; R_f [benzene: chloroform (5.5 : 4.5)] 0.85; UV λ_{max}: 220 nm in methanol; IR: (KBr νcm^{-1}) 3174cm^{-1} NH str, 1702cm^{-1} CO str; ^{1}HNMR (DMSO) δ_{ppm} 7.1-6.5 (Ar-H, 9H); MS, *m/z*: 260(M^+)

2.2.3. Synthesis of 5-Arylidene-2-(Isatin-3-azino)-thiazolidin-4-one Derivatives

2-(isatin-3-azino)-4-thiazolidinone (0.001 mol), appropriate aromatic aldehyde (0.3 gm) and anhydrous sodium acetate (0.006 mol) in glacial acetic acid (10 ml) were refluxed for 5h, cooled and poured into crushed ice and left overnight. The orange solid thus obtained was filtered, washed several times with cold distilled water and recrystallized from hot ethanol.

2.2.3.1. 5-(2-Nitrobenzylidene)-2-(Isatin-3-azino)-thiazolidin-4-one (CI)

The reaction of 2-(isatin-3-azino)-4-thiazolidinone with 2-nitrobenzaldehyde led to the target compound (CI) in 65% yield. R_f [benzene: methanol (9.4 : 0.6)] 0.71. Yield: 65%; mp: 345 - 347^{o}C; UV λ_{max}: 249 nm in methanol; IR: (KBr νcm^{-1}) 2918cm^{-1} C-H, Ar-H str, 1530cm^{-1}& 1350 cm^{-1} NO_2 str, 1709 cm^{-1} CO str, 1613 cm^{-1} CN str; ^{1}HNMR (DMSO) δ_{ppm} 8.5-7.2 (Ar-H, 9H); MS, *m/z*: 394(M^+).

2.2.3.2. 5-(4-Fluorobenzylidene)-2-(Isatin-3-azino)-thiazolidin-4-one (CII)

The reaction of 2-(isatin-3-azino)-4-thiazolidinone with 4-fluorobenzaldehyde led to the target compound (CII) in 67% yield. R_f [benzene: methanol (9.4 : 0.6)] 0.72. Yield: 67%; mp:348-50 ^{o}C; UV λ_{max}: 249 nm in methanol; IR: (KBr νcm^{-1}) 2918cm^{-1} C-H, Ar-H str, 1530cm^{-1}& 1350 cm^{-1} NO_2 str, 1709 cm^{-1} CO str, 1613 cm^{-1} CN str; ^{1}HNMR (DMSO) δppm 8.0-7.1 (Ar-H, 9H); MS, *m/z*: 366(M^+).

2.2.3.3. 5-(4-Hydroxybenzylidene)-2-(Isatin-3-azino)-thiazolidin-4 -one (CIII)

The reaction of 2-(isatin-3-azino)-4-thiazolidinone with 4-hydroxybenzaldehyde led to the target compound (CIII) in 65% yield. R_f [benzene: methanol (9.4 : 0.6)] 0.74. Yield: 65%; mp:294-95 ^{o}C;UV λ_{max}: 251 nm in methanol; IR: (KBr νcm^{-1}) 3182cm^{-1} OH str, 1711cm^{-1} CO str, 1614cm^{-1} CN str;

^{1}HNMR (DMSO) δ_{ppm} 8.5-7.6 (Ar-H, 9H); MS, *m/z*: 364(M^+).

2.2.3.4. 5-(2,4-Dihydroxybenzylidene)-2-(Isatin-3-azino)-thiazolidin-4-one (CIV)

The reaction of 2-(isatin-3-azino)-4-thiazolidinone with 2,4-dihydroxybenzaldehyde led to the target compound (CIV) in 65% yield. R_f [benzene: methanol (9.4 : 0.6)] 0.71. Yield: 68%; mp:298-300 ^oC;UV λ_{max}: 249 nm in methanol; IR: (KBr νcm^{-1}) 2917cm^{-1} C-H, Ar-H str, 1701cm^{-1} CO str, 1615cm^{-1} CN str, 1182cm^{-1} CS str; ^{1}HNMR (DMSO) δ_{ppm} 8.1-7.0 (Ar-H, 9H); MS, *m/z*: 380(M^+).

2.2.3.5. 5-(4-Dimethylaminobenzylidene)-2-(Isatin-3-azino)-thiazolidin-4-one (CV)

The reaction of 2-(isatin-3-azino)-4-thiazolidinone with 4-dimethylaminobenzaldehyde led to the target compound (CV) in 66% yield. R_f [benzene: methanol (9.4 : 0.6)] 0.77. Yield: 66%; mp:278-80^oC;UV λ_{max}: 249 nm in methanol; IR: (KBr νcm^{-1}) 2917cm^{-1} C-H, Ar-H str, 1719cm^{-1} CO str, 1166cm^{-1} CS str; ^{1}HNMR (DMSO) δ_{ppm} 8.2-7.4 (Ar-H, 9H); MS, *m/z*: 391(M^+).

2.3. Biological Assays

2.3.1. Acute Toxicity Assay

The new derivatives obtained from the reaction sequence were evaluated for acute toxicity studies [26-28] using stair-case method [29,30]. Albino mice of both sexes, weighing between 20 and 25g were starved overnight. They were divided randomly into 6 groups of two mice each. The first group served as the control and was given 0.1% CMC orally at a dose of 10ml/Kg body weight. The selected analogues at doses of 60mg/Kg, 120mg/Kg, 240mg/Kg, 480mg/Kg, 960mg/Kg and 1600mg/Kg body weight was given orally as a suspension in CMC (0.1%) to the remaining groups. All the animals were observed continuously for 2 hours, then intermittently for another 4 hours and also at the end of 24 hours. All the experiments were carried out according to the protocols approved by the Institutional Animal Ethical Committee.

2.3.2. Cell Viability Assay

Briefly, cells (5000 cells/well) were seeded into a 96 well plate and incubated for 24h. After 24 h, cells were replenished with fresh medium containing different concentration of compounds or 0.1% DMSO. After 48h of incubation, MTT (500µg/ml final concentration) was added to each well and incubated for 4h in order to allow the conversion of MTT to formazan crystals. Finally, the formazan crystals formed was dissolved in isopropyl alcohol and the OD measured at 570 nm using ELISA microplate reader (Bio Rad). The amount of formazan crystals formation is directly proportional to the viability of cells and the percentage growth inhibition by the compounds was calculated and tabulated.

2.3.3. Cell Cycle Analysis

Cell cycle status was identified by measuring the DNA content. Briefly, cells after treatment (CI; 100µM) were trypsinized, washed twice in PBS and fixed using 70% of ice cold ethanol for 30 minutes. Fixed cells were washed twice and treated with 200 µg/ml of RNase A at 37°C for 1h. Finally propidium iodide was used to stain the cells. Analysis was done in FACS Aria (BD, Mountain View, CA, USA) using BD FACS Diva software.

3. RESULTS AND DISCUSSION

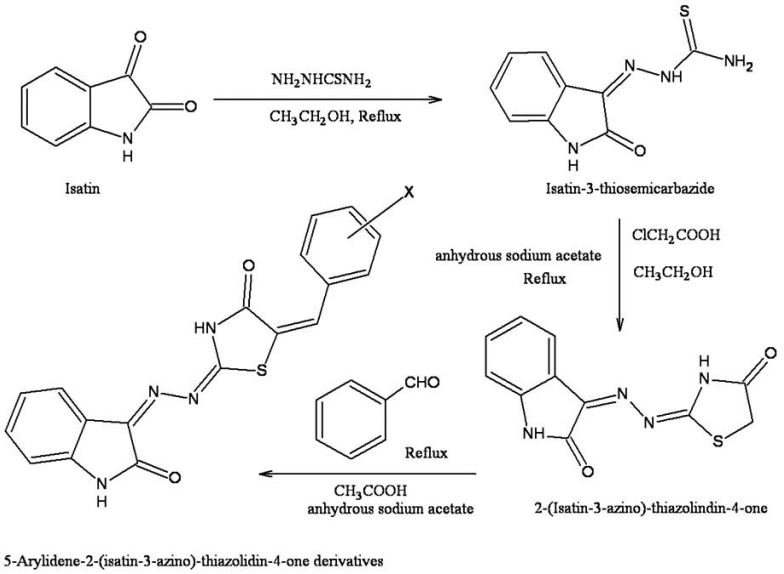
3.1. Chemistry

The schematic representation of the synthesis of compounds is shown in Scheme **1**. Isatin-3-thiosemicarbazide was synthesized by condensation reaction between isatin and thiosemicarbazide leading to hydrazone formation. pH was adjusted ranging between 4-5 with glacial acetic acid to get 2, 3-dihydro-2-oxo-3-substituted indole. Further, S-alkylation followed by cyclocondensation of this product with monochloroacetic acid yielded 2-(Isatin-3-azino)-thiazolidin-4-one. Different aromatic aldehydes were reacted with 2-(Isatin-3-azino)-thiazolidin-4-one to attain the desired 5-arylidene-2-(Isatin-3-azino)-thiazolidin-4-one derivatives (CI-CV). Thin layer chromatography (TLC) was run throughout the reaction for checking the purity and completion. Melting point, R_f value, UV, IR, ^{1}H NMR and mass spectral analysis were done to characterize the compounds. Physical data of these synthesized hybrid compounds are given in Table **1**.

3.2. Biological activity and SAR

Acute toxicity studies were performed in mice for all the analogues and all of them were found to be safe up to the dose of 1600 mg/Kg.

Cytotoxic effects of all the thiazolidinone derivatives synthesized (CI-CV) was assessed in five different cell lines using MTT assay (Table **2** & Fig. (**1**)). Among the derivatives, 5-(2-nitrobenzylidene)-2-(isatin-3-azino)-thiazolidin-4-one (CI) induced potent dose dependent and reproducible cytotoxicity in all the cell lines tested compared to others. CI was more effective in breast cancer cell lines (MDA MB 231 and MDA MB 435) with IC_{50} values ranging from 51µM to 63µM whereas in T47D it was observed to be slightly higher than 100µM. Similarly, the cytotoxic potential was observed to be lower in drug resistant NCI ADR Res as well as SiHa cells. However, the cytotoxic effect was dose dependant in these cell lines as well. Introduction of electron withdrawing nitro group at ortho position of benzylidene ring in CI might have resulted in greater dose dependent response due to the selective accumulation of DLCs in negatively charged mitochondria in carcinoma cells because of the electrochemical proton gradient. Further, introduction of electron withdrawing substituent in CI established a highly stable conjugated system and subsequently the inhibitory activity. CIV showed good cytotoxic effect in MDA MB 231 cells but failed to elicit a cytotoxic response in other cell lines. However, a moderate and dose dependant cytotoxic effect was observed with CIV in MDA MB 435 and T47D cells. Presence of OH radical in ortho and para position of benzylidene ring in CIV appeared to confer greater potency than CIV with OH at para position alone. Interestingly, potent growth stimulatory effect was observed with the compounds CII, CIII and CV in SiHa cells. The growth stimulatory effect of CII and CV was evident in MDA MB 435 cells as well. Electron rich halogen



Scheme 1. Scheme for synthesis of 5-Arylidene-2-(isatin-3-azino)-thiazolidin-4-one derivatives (CI - CV).

Table 1. Physical Data of 5-Arylidene-2-(isatin-3-azin)-thiazolidin-4-one Derivatives (CI - CV)

Code No	X	Yield (%)	MP(°C)	Mol. Formula	Mol. weight
CI	o-NO_2	65	345	$C_{18}H_{11}N_5O_4S$	394.384
CII	p-F	67	348	$C_{18}H_{11}FN_4O_2S$	366.377
CIII	p-OH	65	294	$C_{18}H_{12}N_4O_3S$	364.386
CIV	o,p-OH	68	300	$C_{18}H_{12}N_4O_4S$	380.385
CV	p-$N(CH_3)_2$	66	278	$C_{20}H_{17}N_5O_2S$	391.456

Table 2. Percentage Growth Inhibition Induced by the Derivatives in Different Cell Lines Based on MTT Assay

Cell lines	Conc. in µM	CI	CII	CIII	CIV	CV
MDA MB 231	25	26.1	-3.96	10.02	-3.13	7.6
	50	49.24	2.59	8.41	-0.28	14.75
	100	70.36	-0.73	12.65	54.36	17.97
MDA MB 435	25	2.63	-30.31	-0.91	-12.1	-28.27
	50	19.89	-1.1	-2.45	7.4	-5.99
	100	74.69	8.69	-6.69	20.17	7.51
NCI ADR Res	25	4.34	-0.98	-0.07	-18.04	-12.62
	50	10.93	3.15	2.15	-6.5	-5.68
	100	29.55	8.98	3.01	-2.79	1.12
T47D	25	19.56	-1.34	-9.8	-6.85	-11.63
	50	33.04	3.55	-8.81	5.91	-7.09
	100	46.11	5.41	4.3	18.42	1.53
SiHa	25	-3.24	-16.02	-18.89	-12.65	-20.35
	50	3.91	-57.29	-27.78	-13.78	-26.3
	100	28.27	-63.3	-46.55	-31.24	-55.72

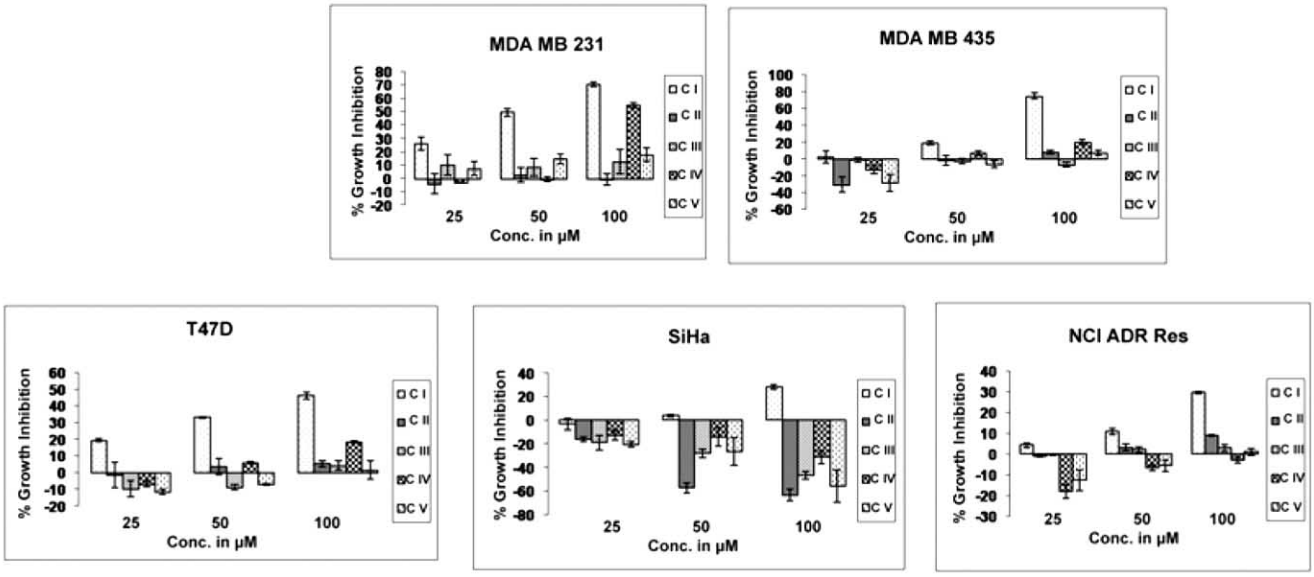


Fig. (1). Graphical representation of MTT assay results**.** Cells were treated with different concentration of compounds or 0.1% DMSO for 48h and the percentage growth inhibition was calculated and the corresponding graph was plotted using %growth inhibition *vs* concentration.

substitution at para position of benzylidene ring in CII and introduction of electron rich (N $(CH_3)_2$) at the para position of benzylidene ring in CV might have resulted in compounds with potent growth stimulatory effects.

Several N-alkylindole derivatives have been reported to trigger apoptosis [31] through induction of G2/M arrest [32-34]. Since CI possess potent cytotoxic activity compared to other derivatives, we profiled its effect during cell division cycle to investigate whether the cytotoxic effect precedes growth arrest at any particular phase of cell cycle. A time dependant FACS analysis was performed in all the five cell lines treatment with CI (Fig. (**2**)). Our results showed a time dependant accumulation of cells at S-phase, at the onset of 12hr post treatment, in all the tested cell lines. This selective and time dependent cell cycle arrest might be attributed to inhibition of cyclin dependent kinases by CI and supported by the previous literatures suggesting the ability of isatins and thiazolidinones to target different kinases. Interestingly, a reversal in the S phase arrest was quite evident in SiHa cells at 36h. The cells were observed to be relieved from S phase arrest and were shown gradually entering into M phase. This reversibility might be due to the predominant cytostatic, not cytotoxic, effect of the treatment in SiHa cells at early time intervals and attributed to the low toxicity observed in SiHa and NCI ADR Res cells in MTT assay. Therefore, it can be speculated that CI acts as more of cytostatic than cytotoxic agent at low doses or at highly resistant conditions. The predominant cytostatic property of CI at a single dose may benefit to reduce systemic toxicity under *in vivo* conditions as evidenced from the acute toxicity results. The cytostatic effect of CI on resistant cells is promising and can be utilized in combination therapy to circumvent advanced/ metastatic cancers.

C_3 substituted isatins have already been shown to selectively target and inhibit PTKs. SAR studies on previously synthesized different compounds have shown that the oxindole core occupies a site where the adenine of ATP binds, while substituent at C_3 of various isatins contact residues in the hinge region of growth factor receptors [35]. The *in vitro* growth inhibitory activity of CI and its ability to arrest cells at S phase indicate that CI may have an affinity to ATP binding pockets associated PTKs and may probably be the mode of action by which it elicits its biological effect.

4. CONCLUSIONS

A range of isatin derivatives have been synthesized and were characterized by their MS, ^{1}H NMR, UV and IR data. Cytotoxicity screening in different cell lines revealed CI to be most active among the different compounds synthesized. Cell cycle analysis revealed induction of S phase arrest in CI treated cells. CI acts as more of a cytostatic than cytotoxic agent, especially at low doses and resistant conditions. However, the structural similarity it shares with other isatins that competitively bind at ATP catalytic sites suggests that the molecular mode of action may be via inhibition of protein kinases associated with S phase transition. CI can be subjected to more detail pharmacological screening, under specific conditions, for elucidation of its specific target. Also structural modifications can be attempted in other analogues to improve their activity in order to convert them to prospective drug candidates.

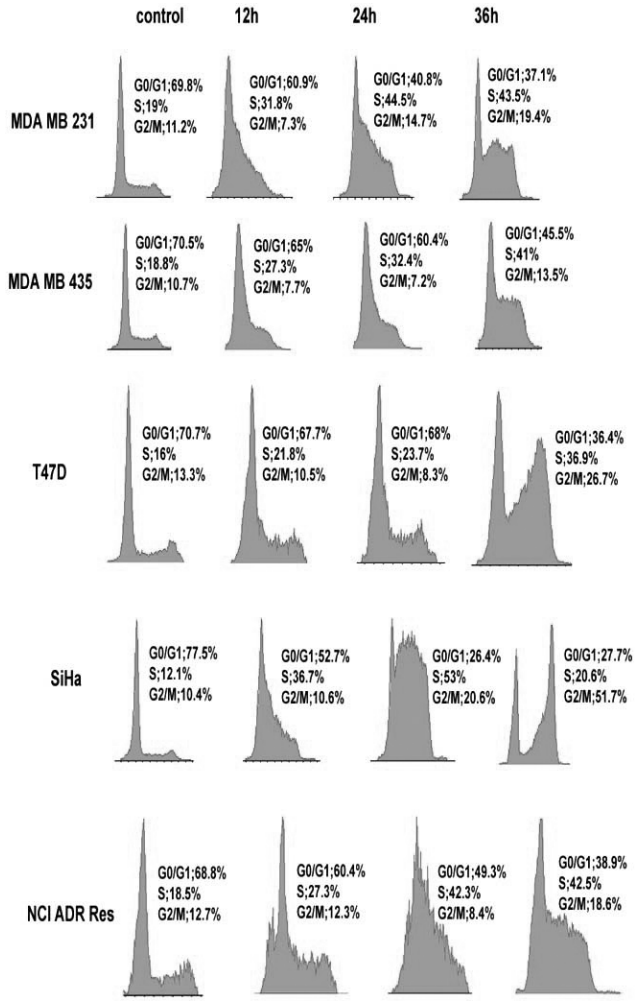


Fig. (2). Cell cycle analysis in CI treated cells**.** Cells were treated with 100μM CI for different time intervals and cell cycle status was assessed by measuring DNA content. Histogram was plotted using cell count vs PI-A (DNA content) and the %population at different phases was analyzed using BD FACS Diva software.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of Mr. Bineesh, National Institute of Interdisciplinary Science and Technology, Trivandrum, India for spectral analysis.

REFERENCES

[1] Polychronopoulos, P.; Magiatis, P.; Skaltsounis, A.; Myrianthopoulos, V.; Mikros, E.; Tarricone, A.; Musacchio, A.; Roe, M.; Pearl, L.; Leost, M.; Greengard, P.; Meijer, L. Structural basis for the synthesis of indirubins as potent and selective inhibitors of glycogen synthase kinase -3 and cyclin-dependent kinases. *J. Med. Chem.*, **2004**, *47*, 935 -946.

[2] Cohen, P. The role of protein phosphorylation in human health and disease. The Sir Hans Krebs Medal Lecture. *Eur. J. Biochem.*, **2001**, *268*, 5001 -5010.

[3] Cohen, P. Protein kinases — the major drug targets of the twenty-first century? *Nat. Rev. Drug Discov.*, **2002**, *1*, 309-315.

[4] Sridhar, R.; Hanson-Painton, O.; Cooper, D.R. Protein kinases as therapeutic targets. *Pharm. Res.*, **2000**, *17*, 1345-1353.

[5] Malumbres, M.; Barbacid, M. To cycle or not to cycle: a critical decision in cancer. *Nat. Rev. Cancer*, **2001**, *1*, 222-231.

[6] Roublevskaia, I. N.; Polevoda, B.V.; Ludlow, J.W.; Haake, A.R. Induced G2/M arrest and apoptosis in human epidermoid carcinoma cell lines by semisynthetic drug Ukrain. *Anticancer Res.*, **2000**, *20*, 3163 - 3167.

[7] Knockaert, M.; Greengard, P.; Meijer, L. Pharmacological inhibitors of cyclin-dependent kinases. *Trends Pharmacol. Sci.*, **2002**, *23*, 417 - 425.

[8] Cerchiaro, G.; da Costa Ferreira, A. M. Oxindoles and copper complexes with oxindole-derivatives as potential pharmacological agents. *J. Braz. Chem. Soc.*, **2006**, *17*, 1473 - 1488.

[9] Cane, A.; Tournaire, M. C.; Barritault, D.; Crumeyrolle-Arias, M. The endogenous oxindoles 5-hydroxyoxindole and isatin are anti-proliferative and proapoptotic. *Biochem. Biophys. Res. Commun.*, **2000**, *276*, 379 - 384.

[10] Connell, R.D. The 2-oxindole chemotype and patent activity inspired by the SU5416 franchise. *Expert Opin. Ther. Patents*, **2003**, *13*, 737 - 749.

[11] Jianguo, M.A.; Shaolan, L.I.; Reed, K.; Guo, P.; Gallo, J. M. Pharmacodynamic-mediated effects of the angiogenesis inhibitor SU5416 on the tumor disposition of temozolomide in subcutaneous and intracerebral glioma xenograft models. *J. Pharmcol. Exp. Ther*., **2003**, *305*, 833 - 839.

[12] Marzola, P.; Degrassi, A.; Calderan, L.; Farace, P.; Crescimanno, C.; Nicolato, E.; Giusti, A.; Pesenti, E.; Terron, A.; Sbarbati, A.; Abrams, T.; Murray, L.; Osculati, F. *In vivo* assessment of antiangiogenic activity of SU6668 in an experimental colon carcinoma model. *Clin. Cancer Res.*, **2004**, *15*, 739 - 750.

[13] Sun, L; Tran, N.; Tang, F.; App, H.; Hirth, P.; Mc Mahon, G.; Tang, C. Synthesis and biological evaluations of 3-substituted indolin-2-ones: A novel class of tyrosine kinase inhibitors that exhibit selectivity toward particular receptor tyrosine kinases. *J. Med. Chem.*, **1998**, *41*, 2588 - 2603.

[14] Badorc, A.; Bordes, M. F.; de Cointet, P.; Savi, P.; Bernat, A.; Lale, A.; Petitou, M.; Maffrand, J.P.; Herbert, J. M. New orally active non-peptide fibrinogen receptor (GpIIb-IIIa) antagonists: Identification of ethyl 3-[N-[4-[4- [amino [(ethoxycarbonyl) imino]methyl]phenyl]-1,3-thiazol-2-yl]-N-[1-[(ethoxycarbonyl) methyl]piperid-4-yl]amino]propionate (SR 121787) as a potent and long-acting antithrombotic agent *J. Med. Chem.*, **1997**, *40*, 3393 - 3401.

[15] Lv, P.C.; Zhou, C. F.; Chen, J.; Liu, P. G.; Wang, K. R.; Mao, W. J.; Li, H.Q.; Yang, Y.; Xiong, J.; Zhu, H. L. Design, synthesis and biological evaluation of thiazolidinone derivatives as potential EGFR and HER-2 kinase inhibitors. *Bioorg. Med. Chem.*, **2010**, *18*, 314 - 319.

[16] Wu, S.; Guo, W.; Teraishi, F.; Pang, J.; Kaluarachchi, K.; Zhang, L.; Davis, J.; Dong, F.; Yan, B.; Fang, B. Anticancer Activity of 5-Benzylidene-2-Phenylimino-1, 3-Thiazolidin-4-one (BPT) Analogs. *Med. Chem.*, **2006**, *2*, 597 - 605.

[17] Chen, L. B. Mitochondrial membrane potential in living cells. *Annu. Rev. Cell Biol.*, **1988**, *4*, 155 -181.

[18] Lampids, T. J.; Bernal, S. D.; Summerhayes, I. C.; Chen, L. B. Selective toxicity of rhodamine-123 in carcinoma cells *in vitro*. *Cancer Res.*, **1983**, *43*, 716 -720.

[19] Nadakavukaren, K. K.; Nadakavukaren, J. J.; Chen, L. B. Increased rhodamine 123 uptake by carcinoma cells. *Cancer Res.*, **1985**, *45*, 6093 -6099.

[20] Anderson, W. M.; Delinck, D. L.; Benniner, L.; Wood, J. M.; Smiley, S. T.; Chen, L. B. Cytotoxic effect of thiacarbocyanine dyes on human colon carcinoma cells and inhibition of bovine heart mitochondrial NADH-ubiquinone reductase activity via a rotenone-type mechanism by two of the dyes. *Biochem. Pharmacol.*, **1993**, *45*, 691 - 696.

[21] Sun, X.; Wong, J. R.; Song, K.; Hu, J.; Garlid, K. D.; Chen, L. B. AA1, a newly synthesized monovalent lipophilic cation, expresses potent *in vivo* antitumor activity. *Cancer Res.*, **1994**, *54*, 1465 - 1471.

[22] Modica-Napolitano, J. S.; Aprille, J. R. Basis for the selective cytotoxicity of rhodamine 123. *Cancer Res*., **1987**, *47*, 4361 - 4365.

[23] Dairkee, S. H.; Deng, G.; Stampfer, M. R.; Waldman, F. M.; Smith, H. S. Selective culture of primary breast carcinoma. *Cancer Res*., **1995**, *55*, 2516 - 2519.

[24] Kawakami, M.; Keizokoya, Ukai, T.; Tatsutu, N.; Kegawa, A. I.; Chen, L. B. Synthesis and evaluation of novel rhodacyanine dyes that exhibit antitumor activity. *J. Med. Chem.*, **1997**, *40*, 3151 - 3160.

[25] Kawakami, M.; Keizokoya, Ukai, T.; Tatsutu, N.; Kegawa, A. I.; Chen, L. B. . Structure−activity of novel rhodacyanine dyes as antitumor agents. *J. Med. Chem.*, **1998**, *41*, 130 - 142.

[26] OECD. Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24. **2000**

[27] Goyal, R.K. In: *Practicals in Pharmacology*, 2nd ed.; B S Shah Prakashan : Ahmedabad, **1999**.

[28] Kulkarni, S.K. In: *Hand Book of Experimental Pharmacology*, 3rd ed.; Vallabh Prakashan : New Delhi, **2005**.

[29] Ghosh, M. N. In: *Fundamentals of Experimental Pharmacology*, 3rd ed.; Hilton & Company : Kolkata, **2005**.

[30] Turner, R.A.; Hebborn, P. In: *Screening Methods in Pharmacology;* Academic Press: New York, **1971**, *Vol. 2.*

[31] Nguyen, J.T.; Wells, J.A. Direct activation of the apoptosis machinery as a mechanism to target cancer cells. *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 7533-7538.

[32] Bacher, G.; Nickel, B.; Emig, P.; Vanhoefer, U.; Seeber, S.; Shandra, D.; Klenner, T.; Beckers, T. D-24851, a novel synthetic microtubule inhibitor, exerts curative antitumoral activity *in vivo*, shows efficacy toward multidrug-resistant tumor cells, and lacks neurotoxicity. *Cancer Res.*, **2001**, *61*, 392 -399.

[33] Bramson, H. N.; Corona, J.; Davis, S.T.; Dickerson, S.H.; Edelstein, M. J. Oxindole-based inhibitors of cyclin-dependent kinase 2 (CDK2): design, synthesis, enzymatic activities, and X-ray crystallographic analysis. *Med. Chem.*, **2001**, *44*, 4339 - 4358.

[34] Andreani, A.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Garaliene, V.; Welsh, W.; Arora, S.; Farruggia, G.; Masotti, L. Antitumor activity of new substituted 3-(5-Imidazo[2,1-*b*]thiazolylmethylene)-2-indolinones and study of their effect on the cell cycle. *J. Med. Chem.*, **2005**, *48*, 5604 - 5607.

[35] Kara, L. V.; Julie, M. L.; Marie, R.; Stephen, G. P.; Benkendorff, K.; John, B. B. An investigation into the cytotoxicity and mode of action of some novel *N*-alkyl-substituted isatins. *Bioorg. Med. Chem.*, **2007**, *15*, 931 -938.

Received: June 20, 2010 **Revised: August 25, 2010** **Accepted: August 28, 2010**