



Research Article

ISSN : 0975-7384  
CODEN(USA) : JCPRC5

## Design, synthesis and biological evaluation of 3-benzylidene 4-bromo isatin derivatives

Yao Zhou<sup>1,2</sup>, Biao Shi<sup>1,2</sup>, Kailin Han<sup>1,2</sup>, Qiannan Guo<sup>1,2</sup>, Yao Yang<sup>1,2</sup>, Binbin Song<sup>1,2</sup>,  
Yuou Teng<sup>1,2\*</sup> and Peng Yu<sup>1,2\*</sup>

<sup>1</sup>Key Laboratory of Industrial Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, P. R. China

<sup>2</sup>Tianjin Key Laboratory of Industry Microbiology, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, P. R. China

---

### ABSTRACT

4-Bromo substitution on isatin based molecules has been found to increase their cytotoxic activity and selectivity. So the studies on synthesis and pharmacological activities of 4-Bromo isatin and its derivatives have been attracted more and more attention. In this paper, we would like to report the design, synthesis and biological evaluation of a series of 3-benzylidene 4-Bromo isatin derivatives. Eight 3-benzylidene 4-bromo isatin derivatives were designed and synthesized in 4 steps in 44-75% overall yields. Six of the eight newly synthesized compounds have not been reported before. Their structures were characterized by <sup>1</sup>H-NMR. The anticancer activity of these new 4-Bromo isatin derivatives against two human tumor cell lines, K562 and HepG2, were evaluated by MTT assay in vitro. MTT results suggests that larger groups at the 3-position could increase the antitumor potency

**Keywords:** 4-Bromo isatin derivatives, Synthesis, Antitumor activity, MTT, K562

---

### INTRODUCTION

Isatin is an endogenous a natural product which was identified in many organisms and possesses variety of biological activities [1-2]. It was reported that 6-bromo isatin exhibited anticancer activity against a human lymphoma cell line. And the 4-Bromo isatin derivatives possess a wide range of pharmacological activities and biological activities [3-4], such as anticancer [5], antibacterial [6-7], anti-inhibitor, anticonvulsant, anti-fungal [8-9] and sedative-hypnotic [10-12], et al [13-16]. Recently Hoyun Lee's research group has studied a series of thiazolidinone's pharmacological effects and found out that bromo group in the 4th position of 1-diethylaminomethyl-1H-indole-2,3-dione leads to an increase in cytotoxic activity on MDA-MB231, MDA-MB468, and MCF7 cells and this compound's cytotoxic activity on cancer cells was 5.6-fold to 14.7-fold higher than non-cancer cells [17]. But the systematically study on 3-benzylidene-4-bromo indolin-2-one derivatives have not been reported yet.

Because the 4-Bromo isatin derivatives exhibited many pharmacological properties, therefore, it is very important to further study the origin of the side-effects and develop new generation of low toxic isatin derivatives to extend the application of this very potent natural product anticancer drug.

As part of our research program on the SAR study of isatin derivatives' anticancer property, herein we would like to report the synthesis and antitumor activity of a series of 3-benzylidene 4-Bromo isatin derivatives against two human cancer cell lines, including human leukemia K562, and human liver cancer HepG2.

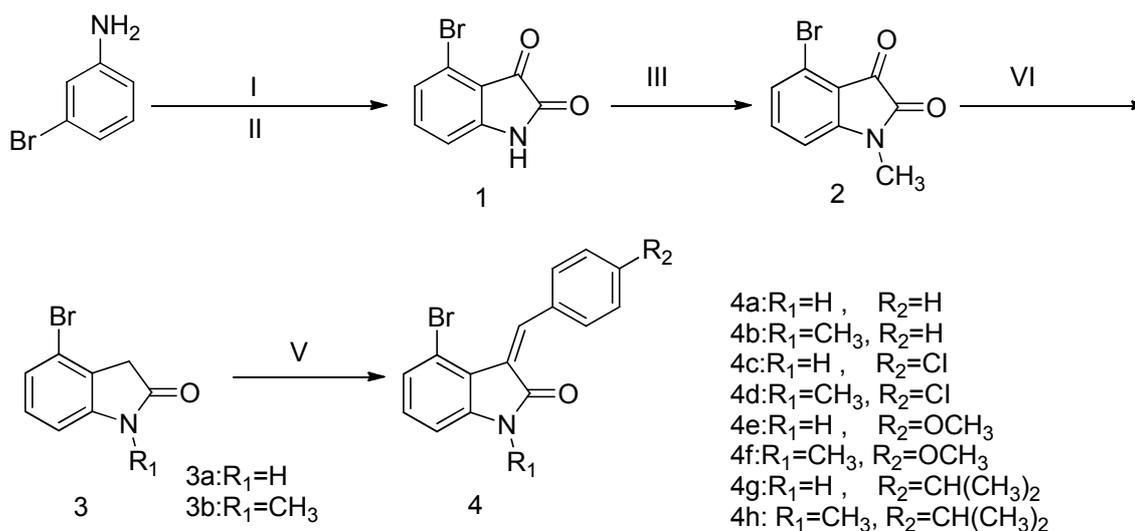
## EXPERIMENTAL SECTION

**2.1 Materials and measurements**

Used in this article, all reagents and solvents were of analytical grade. The reaction temperature control uses the oil bath temperature modulator. Thin layer chromatography (TLC) with silica gel 60 GF254 E.Merck precoated plates (0.25 mm) was visualized using UV. 0.1 for flash chromatography on silica gel (particle size 100-200 mesh). <sup>1</sup>H-NMR spectra were recorded on Bruker AM-400 NMR spectrometers in deuterated chloroform and deuterated DMSO. The chemical shifts are reported in  $\delta$  (ppm) relative to tetramethylsilane as internal standard.

**2.2 Chemistry**

Fig. 1 showed synthesis route of the target compounds. Compound **2** is 3-bromoaniline synthesized from compound **1** by the methylation reaction of iodide, hydrazine to give compound **3**, compound through aldol condensation of compound **4**:



Reagents and conditions: (i) Na<sub>2</sub>SO<sub>4</sub>, NH<sub>2</sub>OH; CCl<sub>3</sub>CH(OH)<sub>2</sub>; (ii) conc. H<sub>2</sub>SO<sub>4</sub>; (iii) CH<sub>3</sub>I, DMF;

(vi) NH<sub>2</sub>NH<sub>2</sub> H<sub>2</sub>O Ethanol; (v) benzaldehyde Ethanol

Fig 1: synthesis route of 3-substituted 4-Bromo isatin derivatives

**2.2.1 Synthesis of 4-bromoindoline-2, 3-dione (1)**

i: Fetch 3-bromoaniline 5.00 g (0.029 mol) into 500 mL round bottom flask with 250 mL water. Dried over anhydrous sodium sulfate 31.78 g (0.22 mol) and hydroxylamine hydrochloride 6.62 g (0.096 mol) were added under stirring, then add 2 mol / L hydrochloric acid solution 5 mL, stir at room temperature for 5 min, finally add chloral hydrate 5.30 g (0.032 mol). The reaction mixture was stirred at room temperature for 15 min, and then reacted at 90 °C for 2 h. The reaction was monitored by TLC after 2 h to confirm whether the starting material was disappeared, then cooled to room temperature, filtered, dried in vacuum to give a yellow solid 6.80 g.

ii: Fetch 20 mL concentrated sulfuric acid into 100 mL round bottom flask. The yellow solid 6.80 g was slowly added to concentrated sulfuric acid at 50 °C, and then reacted for 30 min at 65 °C. The reaction was cooled to room temperature then poured into ice-water mixture, stirred for 30 min. Suction filtration as a red solid was dried under vacuum oven to give 4-bromoindolinone-2, 3-dione (**1**) (6.19 g, 91.1% two steps).

**2.2.2 Synthesis of 4-bromo-1-methylindoline-2, 3-dione (2)**

Fetch 4-bromoindolinone-2, 3-dione (**1**) 2.00 g (0.0088 mol) into 50 mL round bottom flask with 6 mL N, N-dimethylformamide. Until dissolved, sodium hydride 0.63 g (0.026 mol) and 1.88 g (0.013 mol) iodomethane were slowly added into the ice bath then reacted for 4 h at room temperature. When the reaction completed, the reaction mixture was added 15 mL water, collected by filtration, to give 4-bromo-1-methylindoline-2, 3-dione (**2**) (1.96 g, 92.0%).

**2.2.3 Synthesis of 4-bromoindolin-2-one (3a)**

Fetch 4-bromoindolin-2-one (1) 2.00 g (0.0088 mol) into 50 mL round bottom flask with 6 mL N,N-dimethylformamide. Until dissolved, sodium hydride 0.63 g (0.026 mol) and 1.88 g (0.013 mol) iodomethane were slowly added into the ice bath then reacted at room temperature for 4 h. When the reaction was completed, the reaction mixture was added 15 mL water. Precipitate was collected by filtration to give 4-bromo-indole-dione 2.00 g (0.0083 mol). It was put into 50 mL round bottom flask with 15 mL anhydrous ethanol and 0.62 g (0.010 mol) 85% hydrazine hydrate to reflux for 3 h. The reaction was cooled to room temperature and added 0.30 g sodium hydroxide to reflux for another 3 h., When the reaction was complete, the reaction mixture was poured into ice-water mixture, then the precipitate was collected by filtration and dried under vacuum to give 4-bromoindolin-2-one (3a) (1.60 g, 91.3%).

**2.2.4 Synthesis of 4-bromo-1-methylindolin-2-one (3b)**

Fetch 4-bromo-1-methylindoline-2,3-dione (2) 2.00 g (0.0088 mol) into 50 mL round bottom flask with 6 mL N,N-dimethylformamide. Until dissolved, sodium hydride 0.63 g (0.026 mol) and 1.88 g (0.013 mol) iodomethane were slowly added into the ice bath then reacted at room temperature for 4 h. The reaction mixture was added 15 mL water then precipitate was collected by filtration to give 1 - methyl - 4 - bromo-indole-dione 2.00 g (0.0083 mol). It was put into 50 mL round bottom flask with 15 mL anhydrous ethanol and 0.62 g (0.010 mol) 85% hydrazine hydrate, to reflux for 3 h. The reaction was cooled to room temperature then added 0.30 g sodium hydroxide to reflux for another 3 h. The reaction mixture was poured into ice-water mixture, then the precipitate was collected by filtration and dried under vacuum to give 4-bromo-1-methylindolin-2-one (3b) (1.75 g, 93.1%).

**2.2.5 Synthesis of (Z)-4-bromo-3-benzylidene-1,3-dihydro-indol-2-one (4a)**

Fetch 4-bromoindolin-2-one (3a) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine, and 0.56 g (0.0053 mol) benzaldehyde. The reaction mixture was refluxed for 6 h. The reaction was cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent. Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-benzylidene-1,3-dihydro-indol-2-one (4a) (0.80 g, 60.7%).

**2.2.6 Synthesis of (Z)-4-bromo-3-benzylidene-1-methyl-1,3-dihydro-indol-2-one (4b)**

Fetch 4-bromo-1-methylindolin-2-one (3b) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine and 0.56 g (0.0053 mol) benzaldehyde. The reaction mixture was refluxed for 6 h then cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent. Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-benzylidene-1-methyl-1,3-dihydro-indol-2-one (4b) (0.84 g, 61.3%).

**2.2.5 Synthesis of (Z)-4-bromo-3-(4-chlorobenzylidene) indolin-2-one (4c)**

Fetch 4-bromoindolin-2-one (3a) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine, and 0.56 g (0.0053 mol) chlorobenzaldehyde. The reaction mixture was refluxed for 6 h. The reaction was cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent. Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-(4-chlorobenzylidene) indolin-2-one (4c) (1.55 g, 97.8%).

**2.2.6 Synthesis of (Z)-4-bromo-3-(4-chlorobenzylidene)-1-methylindolin-2-one (4d)**

Fetch 4-bromo-1-methylindolin-2-one (3b) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine and 0.56 g (0.0053 mol) chlorobenzaldehyde. The reaction mixture was refluxed for 6 h then cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent. Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-(4-chlorobenzylidene)-1-methylindolin-2-one (4d) (1.48 g, 96.3%).

**2.2.7 Synthesis of (Z)-4-bromo-3-(4-methoxybenzylidene) indolin-2-one (4e)**

Fetch 4-bromoindolin-2-one (3a) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine and 0.56 g (0.0053 mol) p-methoxybenzaldehyde. The reaction mixture was refluxed for 6 h then cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-(4-methoxybenzylidene)indolin-2-one (4e) (1.42 g, 98.2%).

**2.2.8 Synthesis of (Z)-4-bromo-3-(4-methoxybenzylidene)-1-methylindolin-2-one (4f)**

Fetch 4-bromo-1-methylindolin-2-one (3b) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine and 0.56 g (0.0053 mol) Methoxybenzaldehyde. The reaction mixture was refluxed for 6 h then cooled to room temperature, added 20mL anhydrous ethanol under reduced pressure to remove the solvent. Using

petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-(4-methoxybenzylidene)-1-methylindolin-2-one (**4f**) (1.46 g, 96.9%).

### 2.2.9 Synthesis of (Z)-4-bromo-3-(4-isopropylbenzylidene) indolin-2-one (**4g**)

Fetch 4-bromoindolin-2-one (**3a**) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine and 0.56 g (0.0053 mol) 4-isopropylbenzaldehyde. The reaction mixture was refluxed for 6 h then cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent. Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-(4-isopropylbenzylidene) indolin-2-one (**4g**) (0.94 g, 57.6%).

### 2.2.10 Synthesis of (Z)-4-bromo-3-(4-isopropylbenzylidene)-1-methylindolin-2-one (**4h**)

Fetch 4-bromo-1-methylindolin-2-one (**3b**) 1.00 g (0.0044 mol) into 50 mL round bottom flask with 10 mL ethanol, 0.5 mL dry pyridine and 0.56 g (0.0053 mol) 4-isopropylbenzaldehyde. The reaction mixture was refluxed for 6 h then cooled to room temperature, added 20 mL anhydrous ethanol under reduced pressure to remove the solvent. Using petroleum ether: ethyl acetate = 15:1, 200-300 mesh silica gel column to give (Z)-4-bromo-3-(4-isopropylbenzylidene)-1-methylindolin-2-one (**4h**) (1.21 g, 71.7%).

### 2.3 Biological assay.

Cells (100  $\mu$ L) were cultured in 96-well plates at a density of  $5 \times 10^4$  cells/mL for 2 hours (K562) or overnight (HepG2). Compounds (DMSO solution of 0.5  $\mu$ L) were added to each well to culture for another 48 hours. MTT assay was performed using Thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The IC<sub>50</sub> values were obtained by nonlinear regression using GraphPad Prism 4.0. IC<sub>50</sub> measurements for each compound were done three times.

## RESULTS AND DISCUSSION

### 3.1 Characterize isatin derivatives by <sup>1</sup>H-NMR

#### 3.1.1 (Z)-4-bromo-3-(4-chlorobenzylidene) indolin-2-one (**4c**) by <sup>1</sup>H-NMR

<sup>1</sup>H-NMR(DMSO, 400MHz).  $\delta$ /ppm 10.87(s, 1H), 8.54(d, 1H), 8.10-8.12(d, 2H), 7.49-7.51 (d, 2H), 7.13-7.19(t, 1H), 7.21(s, 1H), 6.85-6.87(d, 1H).

#### 3.1.2 (Z)-4-bromo-3-(4-chlorobenzylidene)-1-methylindolin-2-one (**4d**) by <sup>1</sup>H-NMR

<sup>1</sup>H-NMR(DMSO, 400MHz).  $\delta$ /ppm 8.60(d, 1H), 8.10-8.12(d, 2H), 7.49-7.52(d, 2H), 7.25-7.28 (d, 1H), 7.22(s, 1H), 7.05-7.07 (t, 1H), 3.32(s, 3H).

#### 3.1.3 (Z)-4-bromo-3-(4-methoxybenzylidene)indolin-2-one (**4e**) by <sup>1</sup>H-NMR

<sup>1</sup>H-NMR(DMSO, 400MHz).  $\delta$ /ppm 10.82(s, 1H), 8.60(s, 1H), 8.28-8.30(d, 2H), 7.17-7.20 (d, 1H), 7.09-7.13(t, 1H), 7.02-7.04(d, 2H), 6.84-6.86(d, 1H), 3.85(s, 3H).

#### 3.1.4 (Z)-4-bromo-3-(4-methoxybenzylidene)-1-methylindolin-2-one (**4f**) by <sup>1</sup>H-NMR

<sup>1</sup>H-NMR (DMSO, 400MHz).  $\delta$ /ppm 8.64(s, 1H), 8.29-8.31(d, 2H), 7.18-7.26(m, 2H), 7.02-7.05 (d, 3H), 3.85(s, 3H), 3.32(s, 3H).

#### 3.1.5 (Z)-4-bromo-3-(4-isopropylbenzylidene) indolin-2-one (**4g**) by <sup>1</sup>H-NMR

<sup>1</sup>H-NMR(DMSO, 400MHz).  $\delta$ /ppm 10.83(s, 1H), 8.59(s, 1H), 8.08-8.10(d, 2H), 7.32-7.34(d, 2H), 7.18-7.21(d, 1H), 7.11-7.15(t, 1H), 6.85-6.87(d, 1H), 2.93-2.96(m, 1H), 1.22-1.24(d, 6H).

#### 3.1.6 (Z)-4-bromo-3-(4-isopropylbenzylidene)-1-methylindolin-2-one (**4h**) by <sup>1</sup>H-NMR

<sup>1</sup>H-NMR(DMSO, 400MHz).  $\delta$ /ppm 9.96(s, 1H), 8.63(s, 1H), 8.09-8.11(d, 2H), 7.82-7.84 (d, 1H), 7.46-7.48(d, 1H), 7.32-7.34 (d, 2H), 7.21-7.26(m, 2H), 7.03-7.04(d, 2H), 2.93-2.96(m, 1H), 1.22-1.24(d, 6H).

### 3.2 Anticancer activity assay.

All the above compounds were tested for their in vitro anticancer activity against K562 and HepG2 cells by MTT based assay. The results were presented in Table 1. MTT results suggests that larger groups at the 3-position could increase the antitumor potency.

**Table 1** Inhibition Activity of isatin derivatives

Tested cells	Compounds (IC <sub>50</sub> , μM)									
	1	2	4a	4b	4c	4d	4e	4f	4g	4h
HepG2	>10	>10	>10	>10	>10	5.47	>10	>10	4.39	>10
K562	>10	>10	>10	>10	8.69	>10	>10	>10	6.18	>10

### CONCLUSION

Here we reported design, synthesis and biological evaluation of a series of 3-benzylidene 4-Bromo isatin derivatives. When we substituted the 3-position of the 4-Bromo isatin, we found that different group has a great influence on the result. After we have done the parallel experiment using chlorobenzaldehyde, p-methoxybenzaldehyde, and 4-isopropylbenzaldehyde we found the different substituent did not influence reaction yield.

According to the results that shown in the table 1, we can concluded that the compounds of 4-Bromo isatin derivatives have substituted on 3-position, such as compound **4g** has better antitumor activity against K562 and HepG2 cells. So we deduce that the group was substituted by big space branch and 1-position has no group substituted will have better antitumor activity against K562 and HepG2 cells.

In conclusion, a series of 3-benzylidene 4-bromo isatin derivatives were synthesized and tested for their *in vitro* antitumor activity against two strains of cancer cell lines K562, HepG2. The SAR study of these compounds led to the identification of a new isatin, **4g**, as higher potent anticancer compounds with IC<sub>50</sub>=4.39 μM, IC<sub>50</sub>= 6.18 μM, against human leukemia HepG2 and K562 cells, respectively. Further chemo-biological study of **4g** with regards to their antitumor pathway and *in vivo* investigation are ongoing in this laboratory.

### Acknowledgment

The authors sincerely thank the financial support from the National Natural Science Foundation of China (81241104, 31301142), the International Science & Technology Cooperation Program of China (2013DFA31160) and the Ministry of Education Changjiang Scholars and Innovative Research Team Development Plan (IRT1166).

### REFERENCES

- [1] R Sabet; M Mohammadpoura; A Sadeghi; A Fassihi, *European Journal of Medicinal Chemistry*, **2010**, 45(3), 1113-1118.
- [2] KL Han; Y Zhou; FX Liu; QN Guo; PF Wang; Y Yang; BB Song; W Liu; QW Yao; YO Teng; P Yu, *Bioorganic & Medicinal Chemistry Letters*, **2013**, (in press).
- [3] SN Pandeya; S Smitha; M Jyoti; SK Sridhar, *Acta Pharm.*, **2005**, 55(1), 27-46.
- [4] MJ Moon; SK Lee; JW Lee; WK Song; SW Kim; JI Kim; C Cho; SJ Choi; YC Kim, *Bioorg. Med. Chem.*, **2006**, 14(1), 237-246.
- [5] KL Vine; JM Locke; M Ranson; K Benkendorff; SG Pyne; JB Bremner, *Bioorg. Med. Chem.*, **2007**, 15(2), 931-938.
- [6] T Chandrasekhar; LV Kumar; AB Reddy, PJ Naik; GN Swamy, *J. Chem. Pharm. Res.*, **2012**, 4(5), 2795-2802.
- [7] BR Nathani; KS Pandya; MM Jeni; A Prasad, *J. Chem. Pharm. Res.*, **2011**, 3(6), 687-692.
- [8] ZH Chohan; H Pervez; A Rauf; KM Khan; CT Supuran, *J Enzym Inhib Med Chem.*, **2004**, 19(1), 417-423.
- [9] G Madhu; KN Jayaveera; LKR Nath; BS Kumar; PN Reddy, *J. Chem. Pharm. Res.*, **2012**, 4(6), 2928-2936.
- [10] KM Khan; M Khan; M Ali; M Taha; S Rasheed; S Perveen; MI Choudhary, *Bioorg Med Chem.*, **2009**, 17(22), 7795-7801.
- [11] M Verma; SN Pandeya; KN Singh; JP Stables, *Acta Pharm Turc.*, **2004**, 54(1), 49-56.
- [12] S Smitha; SN Pandeya; JP Stables; S Ganapathy, *Sci Pharm.*, **2008**, 76(4), 621-636.
- [13] T Tomasic; LP Masic. *Curr Med Chem.*, **2009**, 16(13), 1596-1629.
- [14] AP Savanur; SD Lamani; ST Nandibewoor; SA Chimatar, *J. Chem. Pharm. Res.*, **2011**, 3(6), 1061-1088.
- [15] P Singh; P Sharma; A Anand; PMS Bedi; T Kaur; AK Saxena; V Kumar, *European Journal of Medicinal Chemistry*, **2012**, 55(1), 455-461.
- [16] K Swathi; A Srinivas; M Sarangapani, *J. Chem. Pharm. Res.*, **2010**, 2(2), 220-225
- [17] VR Solomon; ChK Hu; H Lee, *Bioorganic & Medicinal Chemistry*, **2009**, 17(21), 7585-7592.