



# PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 4 | Issue 6 | November-December 2013 | Pages 1152-1161

Original Research Article

## SYNTHESIS AND EVALUATION OF NEW 2-CHLORO-N-[(Z)-(2-OXO-5 SULFAMOYL - INDOLIN-3-YLIDENE) AMINO] ACETAMIDE DERIVATIVES FOR THEIR ANTIMICROBIAL AND ANTI- INFLAMMATORY ACTIVITY

SRIKANTH LINGALA\*, RAGHUNANDAN NERELLA, KIRAN POUDALA

Department of Pharmaceutical Chemistry, Balaji Institute of Pharmaceutical Sciences, Warangal.

**Author for Correspondence:** srikanth802@gmail.com

Received: 13-08-2013

Revised: 04-09-2013

Accepted: 15-09-2013

Available online: 01-11-2013

### ABSTRACT

In view of various biological activities and enormous importance of indoles, isatins and their derivatives, it was our interest to synthesize and characterize some new 5 - Sulfamoyl Isatin derivatives and evaluate them for antimicrobial and anti-inflammatory activity. An appropriate quantity of isatin hydrazone was heated under reflux with chloroacetyl chloride to give 2-Chloro-N-[(Z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene) amino]acetamide which was then heated under reflux with various secondary amines to give 2-chloro-n-[(z)-(2-oxo-5 sulfamoyl -indolin-3-ylidene) amino] acetamide derivatives. The intermediates and final compounds were purified and their chemical structures have been confirmed by IR, <sup>1</sup>H NMR, and Mass spectral data. All the synthesized compounds were screened for antibacterial activity against *B. subtilis*, *B.cereus*, *S. epidermidis*, *S. typhi*, *P. aeruginosa* and *K. pneumoniae*, antifungal activity against *A.flavus*, *F.oxysporium* and *P. notatum* and anti-inflammatory activity using carrageenan induced rat paw edema model. Most of the compounds tested have shown promising activities when compared with the standard drugs.

**Key words:** Isatins, Antimicrobial activity, Anti inflammatory activity, Amikacin, Amphoterecin.

## INTRODUCTION

Isatins are an important group of heterocyclic compounds which are biologically active and of significant importance in medicinal chemistry. A variety of biological activities are associated with isatin including CNS activities as potentiation of pentobarbitone induced necrosis, analgesic, anticonvulsant, antidepressant, anti-inflammatory, antimicrobial and effects on the central nervous system<sup>1,2</sup>. Isatins are capable of crossing the blood-brain-barrier<sup>3,4</sup>. Isatin, a heterocyclic compound was identified in animals as a major component of the endogenous monoamine oxidase inhibitor<sup>5</sup>. Isatin (1H-indole-2,3-dione) is a synthetically versatile substrate, where it can be used for the synthesis of a large variety of heterocyclic compounds, such as indoles and quinolines, and as a raw material for drug synthesis. Isatin has also been found in mammalian tissues, and its function as a modulator of biochemical processes has been the subject of several discussions<sup>6</sup>. A survey of literature reveals the advances in the use of isatin for organic synthesis during the last twenty-five years, as well as enormous importance of its biological and pharmacological properties. In view of these valid observations in our present study, we reported the synthesis of new 5 - Sulfamoyl Isatin derivatives and the synthesized compounds were screened for their antibacterial, antifungal and anti inflammatory activity.

## MATERIALS AND METHODS

The chemicals and solvents used for the experimental work were commercially procured from E. Merck, India, S.D. Fine Chem, India and Qualigens, India. Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chem, India. Melting points were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. The proton magnetic resonance spectra (<sup>1</sup>H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/CDCl<sub>3</sub> using TMS as internal standard. Chemical shifts (δ) are expressed in ppm.

The infrared spectra of compounds were recorded in KBr on a FTIR 8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer.

Mass spectra were recorded on LC-MS/MS (API-4000 TM), Applied BioSystems, MDS SCIEX (Canada).

## EXPERIMENTAL

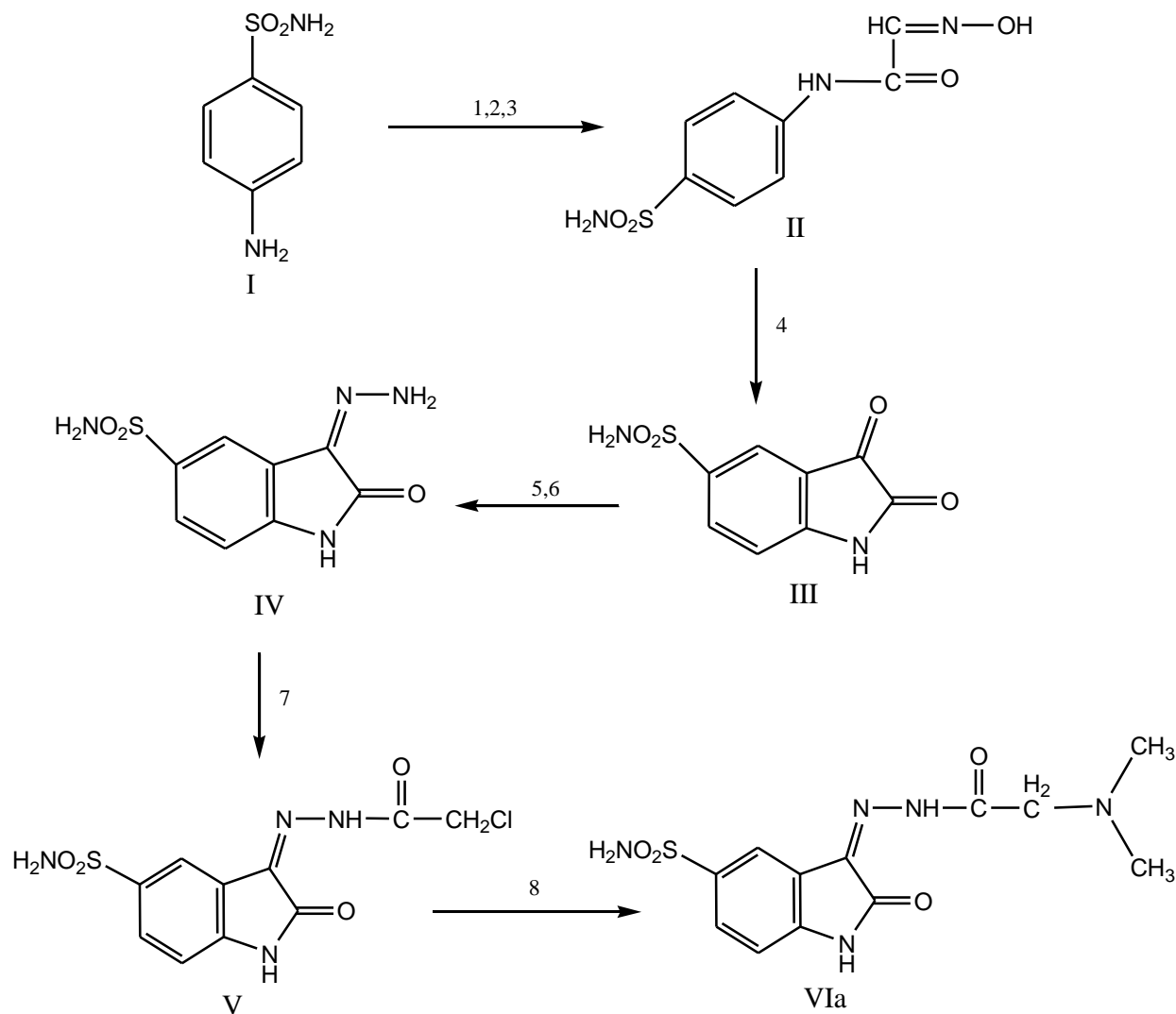
### Synthesis of 5- Sulphamoyl Isonitroso acetanilide (II):

**General procedure:** 5gm of Sulfanilamide was taken and added to 30 ml of Distilled water and 5ml of concentrated Hydrochloric acid (HCl), which gave a clear solution in the beaker. (solution I). In another beaker 9gms of Chloral hydrate and 30 ml of water was taken and this solution was made up to 150ml. (solution II). The solution I and II were mixed. To this mixed solution

anhydrous Sodium Sulphate ( $\text{Na}_2\text{SO}_4$ ) was added and the solution was stirred until precipitate was formed. 12gms of Hydroxylamine Hydrochloride was taken and added to 150ml of water (Solution III).

The above the solutions were mixed and the solution was heated on a water bath for 45 minutes and kept side for 12-24 hours<sup>7</sup> to give compound II. M.P : 170-173 °C, Yield : 75%.

#### SCHEME-1



1. Chloralhydrate
2. Hydroxylamine hydrochloride
3. Sodium sulphate
4. Conc. Sulphuric acid

5. Absolute Alcohol
6. Hydrazinehydrate
7. Chloroacetylchloride
8. Dimethylamine (Secondary amines)

### Synthesis of 5-Sulfamoyl-1H-Indole-2,3-dione (III):

**General procedure:** From the above crystals one gram was taken in dry beaker and 4ml of concentrated Sulphuric acid was added by stirring and the temperature was maintained at 60 °C. The beaker was kept aside for 12 hrs then this solution was poured into the crushed ice to give 5-Sulfamoyl-1H-Indole-2,3-dione as a coloured precipitate<sup>8</sup>. M.P : 198-201 °C, Yield: 70%.

### Synthesis of 5-Sulfamoyl isatin hydrazones (IV)

An appropriate quantity of isatin (III) (0.01 mol) was dissolved in alcohol (20ml) and hydrazinehydrate (0.01 mol) with shaking. The reaction mixture was stirred well, refluxed for 3 hours. The resultant crystalline solid was filtered, washed repeatedly with small portions of cold water and finally with a small portions of alcohol<sup>9</sup>. The product was dried and purified by recrystallization from chloroform. M.P : 198 °C, Yield: 68%. **IR (KBr)(cm<sup>-1</sup>):** 3250(N-H str.), 3155 (Ar-H str.), 1650-1540 (C=C & C=N str.), 1345, 1160 (SO<sub>2</sub>), 1240 (C-N). **<sup>1</sup>H NMR (DMSO-d<sub>6</sub>):**  $\delta$  7.2 (s, 2H, N-NH<sub>2</sub>), 2.3 (s, 2H, S-NH<sub>2</sub>), 10 (s, 1H, -NH), 7.8 – 8.2 (d, s, 3H, Ar-H). **EI-MS: m/z** = 240(M<sup>+</sup>).

### Synthesis of 2-Chloro-N-[(Z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]acetamide (V)

An appropriate quantity of isatin hydrazone (IV) (0.01mol) was heated under reflux with chloroacetyl chloride (0.01mol) in dry

acetone under anhydrous conditions using calcium chloride guard tube for 2 hrs. The product thus formed was filtered and washed with small portions of acetone to remove any unreacted chloroacetyl chloride<sup>10</sup>.

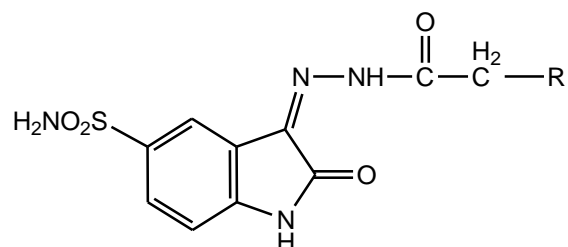
It was purified by recrystallization with ethanol. M.P; 178 °C Yield; 72 %. **IR (KBr)(cm<sup>-1</sup>):** 3235(N-H str.), 3145 (Ar-H str.), 1640-1550 (C=C & C=N str.), 1340, 1165 (SO<sub>2</sub>), 1260 (C-N), 745 (C-Cl). **<sup>1</sup>H NMR (DMSO-d<sub>6</sub>):**  $\delta$  7.4 (s, 1H, N-NH-C), 2.5 (s, 2H, S-NH<sub>2</sub>), 10.3 (s, 1H, -NH), 7.6 – 8.1 (d, s, 3H, Ar-H), 4.3(s, 2H, -CH<sub>2</sub>-). **EI-MS: m/z** = 316(M<sup>+</sup>).

### Synthesis of 2-(dimethylamino)-N-[(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]acetamide (VIa).

2-Chloro-n-[(z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]acetamide (V) (0.01mol) was heated under reflux with various secondary amines (dimethyl amine) (0.01mol) in dry acetone under anhydrous conditions using calcium chloride guard tube for 2 hrs. The product thus formed was filtered and washed with small portions of acetone to remove any unreacted secondary amines it was purified by recrystallization with ethanol<sup>11</sup>. The physical data of all the newly synthesized derivatives is given in Table 1. M.P; 212 – 214 °C Yield; 75 %.

**IR (KBr)(cm<sup>-1</sup>):** 3230 (N-H str.), 3140 (Ar-H str.), 1645-1553 (C=C & C=N str.), 1342, 1168 (SO<sub>2</sub>), 1255 (C-N). **<sup>1</sup>H NMR (DMSO-d<sub>6</sub>):**  $\delta$  7.3 (s, 1H, N-NH-C), 2.2 (s, 2H, S-NH<sub>2</sub>), 10.1 (s, 1H, -NH), 7.6 – 8.3 (d, s, 3H, Ar-H), 3.6 (s, 6H, N-(CH<sub>3</sub>)<sub>2</sub>), 4.5 (s,

2H, -CH<sub>2</sub>-). **EI-MS:**  $m/z$  = 325(M<sup>+</sup>),  
326(M+1).



**Table.1.Physical data of synthesized compounds (VIa-VIe)**

Comp.no.	R	Molecular formula	Mol. Wt.	% yield	Melting Point (°C)	R <sub>f</sub> Value
<b>VIa</b>		C <sub>12</sub> H <sub>15</sub> N <sub>5</sub> O <sub>4</sub> S	325.34	75.00%	212-214	0.51
<b>VIb</b>		C <sub>14</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub> S	353.39	71.00%	219-221	0.49
<b>VIc</b>		C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O <sub>4</sub> S	367.09	70.00%	200-202	0.63
<b>VIId</b>		C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub> S	365.40	68.00%	180-185	0.66
<b>VIe</b>		C <sub>14</sub> H <sub>18</sub> N <sub>6</sub> O <sub>4</sub> S	366.39	65.00%	188-189	0.70

**Solvent system used for TLC :** Pet.ether : Ethylacetate (3:2)

## BIOLOGICAL EVALUATION

### ANTIBACTERIAL ACTIVITY

Antibacterial activity of the synthesized compounds was determined, using a slightly modified cup plate method<sup>12</sup>. Muller Hinton agar was used for the growth of bacterial strains (*B.subtilis* (MTCC 121), *B.cereus* (ATCC 14579), *S.epidermidis* (ATCC 25923), *S.typhi* (MTCC 733), *P.aeruginosa* (MTCC 741) and *K.pneumoniae* (ATCC 29212). Each organism was suspended in normal saline solution and transmittance (T) of 75 to 77% at 530 nm was made, which is equal to  $10^6$

CFU/ml. All the test compounds were dissolved in DMSO at a concentration of 2 mg/ml. Each plate was inoculated with 20  $\mu$ l of microbial suspension. 100  $\mu$ l of the test compounds was added to each cup. The plates containing bacteria were incubated at 37° C for 24 hrs, the positive antimicrobial activity were read based on the growth inhibition zone and compared with the solvent as a negative control and Amikacin as comparative drug, and the results are presented in the Table 2. All the synthesized compounds have shown antibacterial activity.

**Table.2.Antibacterial activity of synthesized derivatives. (VIa - VIe)**

Comp.	Zone of inhibition (mm)					
	<i>B.subtilis</i>	<i>B.cereus</i>	<i>S.epidermidis</i>	<i>S.typhi</i>	<i>P.aeruginosa</i>	<i>K.pneumoniae</i>
<b>VIa</b>	6	8	12	8	11	7.8
<b>VIb</b>	9.2	7	13	10	10	8.8
<b>VIc</b>	12	10.2	12	11	10.1	9.6
<b>VIId</b>	14	15	18	17.4	18.2	12
<b>VIe</b>	10	13	11	13.8	14	11
Negative Ctrl.	--	--	--	--	--	--
Standard (Amikacin)	22	19	20	22	20	18

**-- No activity, Negative Control – DMSO**

### ANTIFUNGAL ACTIVITY

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121°C /15lbs for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160°C for 1 ½

hours. 30 ml of sterile SDA was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (40°C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 0.1 ml of

the solution of synthesized compounds at 2mg/ml concentration in DMF were added to respective bores and 0.1ml of the standard Amphotericin B at a concentration of 200 µg/0.1ml was used as standard as shown in Table 3. The Petri plates seeded with fungal organisms, containing solution of synthesized compounds and the

standard drug were kept in a refrigerator at 4 °C for 1 hour to facilitate the diffusion of the compounds and the standard in to the media. After diffusion the Petri plates were incubated at 28 °C for one week and later the zone of inhibition was observed and measured using a scale<sup>13</sup> and the results are presented in the Table 3.

**Table.3.Antifungal activity of synthesized derivatives. (VIa - VIe)**

Comp.	Zone of inhibition in(mm) (200µg/100µl)		
	<i>A.flavus</i>	<i>F.oxysporium</i>	<i>P.notatum</i>
<b>VIa</b>	--	--	--
<b>VIb</b>	4	6	6
<b>VIc</b>	7	5.8	3.2
<b>VId</b>	12	14	10
<b>VIe</b>	--	11	11
Negative Control(DMF)	--	--	--
Standard (Amphotericin B)	19	16	19

-- No activity; the antifungal activity was assayed by cup plate method; 100 µl/cup (disc diameter is 6mm) Amphotericin B (100µl/cup) was used as +ve ctrl.

#### ANTI-INFLAMMATORY ACTIVITY

Wistar strain albino rats weighing between 180-250 gm fasted 24 hrs before the test, were divided into four groups of six animals each. The volume of the right hind paw was measured using a plethysmometer. This constituted the initial reading compounds were tested in the dose of 50 mg/kg body weight. Diclofenac 20 mg/kg was used as standard.

The compounds were administered as suspensions in sodium CMC (0.1% w/v) intraperitoneally 30 min before the injection of carrageenan. Control group of animals received a suspension of sodium CMC only 0.1ml of 1.0% w/v carrageenan suspension in normal saline was injected into the plantar region of the right hind paw. The swelling produced after injection of the phlogistic agent was measured<sup>14</sup> at hourly intervals for 6 hrs.

Percentage inhibition of edema was calculated using the formula given below and the results are presented in the Table 4.

$$\% \text{ inhibition of edema} = \frac{\text{Mean edema of control group} - \text{Mean edema of treated group}}{\text{Mean edema of control group}} \times 100$$

**Table.4.Anti-inflammatory activity of synthesized derivatives. (VIa - VIe)**

Compound 50mg/kg	Dose (mg/kg)	%Inhibition
<b>VIa</b>	<b>50</b>	23.89 ± 0.24
<b>VIb</b>	<b>50</b>	21.24 ± 0.28
<b>VIc</b>	<b>50</b>	56.13 ± 0.37
<b>VIId</b>	<b>50</b>	53.97 ± 0.39
<b>VIe</b>	<b>50</b>	29.40 ± 0.31
<b>Diclofenac sodium</b>	<b>20</b>	84.29 ± 0.43

**Values (edema inhibition) represent mean values ± SD, n = 6.**

## RESULTS AND DISCUSSION

From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed as indicated in Table 2. The compound **VIId** has shown good activity against both the Gram-positive and Gram-negative bacteria. The compound showed maximum zone of inhibition (18.2 mm) against *P.aeruginosa* and zone of inhibition (18 mm) against *S.epidermidis*. Compounds **VIc** and **VIe** have shown good antibacterial activity, compound **VIe** has shown zone of inhibition (14 mm) against *P.aeruginosa* and zone of inhibition (13.8 mm) against *S. typhi*. The compound **VIb** has shown moderate activity against all the organisms. Compound **VIa**

has shown less activity among all the synthesized compounds. But all the derivatives have shown less antibacterial activity when compared to the standard drug Amikacin. The antifungal activity of the compounds studied against *A.flavus*, *F.oxysporium* and *P.notatum* is shown in Table 3. Amphotericin B was used as reference for inhibitory activity against fungi. It was observed that the compound **VIId** showed maximum zone of inhibition (14mm) against *F.oxysporium* and zone of inhibition of 12 mm and 10 mm against *A.flavus* and *P.notatum*. Compound **VIe** has shown good activity against *F.oxysporium* and *P.notatum* with zone of inhibition (11mm and 11mm) but the compound **VIe**



has not shown any activity against *A.flavus*. The compounds **Vlb** and **Vlc** have shown moderate activity against all the three strains employed for the study. The compound **Vla** has not shown any activity on any of the test organism employed. The preliminary studies on anti-inflammatory activity of the new title compounds ie., 2-Chloro-N-[(Z)-(2-oxo-5-sulfamoyl-indolin-3-ylidene)amino]acetamide derivatives (Vla - Vle) generated some interesting data. The test compounds were evaluated for anti-inflammatory activity and data are presented in Table 4 using diclofenac sodium (20 mg/kg) as the standard. The close observation of anti-inflammatory activity of all the test compounds shows that all the test compounds showed mild to moderate anti-inflammatory activity. Compound **Vlc** exhibited maximum activity with percentage inhibition of  $56.13 \pm 0.37$  which was followed by compound **Vld** with percentage inhibition of  $53.97 \pm 0.39$ . The compounds **Vle** and **Vla** were found to be next in the order with percentage inhibition of  $29.40 \pm 0.31$  and  $23.89 \pm 0.24$ . The compound **Vlb** has shown least activity with percentage inhibition of  $21.24 \pm 0.28$ .

## CONCLUSION

The proposed isatin derivatives were synthesized successfully. All the compounds were evaluated for antibacterial, antifungal and anti-inflammatory activity. All the synthesized compounds were found to have good activity, among all the active compounds of isatin derivatives, compound **Vld** has shown good activity against both the Gram-

positive and Gram-negative bacteria. The compound **Vld** has also shown significant antifungal activity with maximum zone of inhibition (14mm) against *F.oxysporium* and zone of inhibition of 12 mm and 10 mm against *A.flavus* and *P.notatum* and compound **Vlc** showed high antiinflammatory activity than all the other synthesized compounds with percentage inhibition of  $56.13 \pm 0.37$ .

## ACKNOWLEDGEMENTS

The authors are thankful to Dr. A. Rajendra Prasad Reddy, Chairman of Balaji Institute of Pharmaceutical Sciences, Warangal, for providing laboratory facilities and financial support.

## REFERENCES

1. Foye, Williams O. Principles of Medicinal chemistry. Fourth edition. 1995. 553.
2. Harish Rajak,, Ravichandran Veerasamy, Arun Kumar Gupta, Murli Dhar Kharya and Pradeep Mishra. Synthesis and Pharmacological Evaluation of New Indolyl-oxadiazoles as Anticonvulsant Agents. International Journal of Pharmaceutical Sciences and Nanotechnology. 2009; 2(3): 661-666.
3. J. Durell and w. Pollin. A Trial on Chronic Schizophrenic Patients of Oxypertine, a Psychotropic Drug with an Indole Ring. The British Journal of Psychiatry. 1963; 109: 687-691.
4. Medvedev AE, Ivanov AS, Veselovsky AV, Skvortsov VS, Archakov AI. QSAR analysis of indole analogues as monoamine oxidase inhibitors. J Chem Inf Comput Sci. 1996; 36(4): 664-71.
5. Bhanupriya Bhargu, Devender Pathak, Nadeem Siddiqui, ShamsheerAlam M,Waquar Ahsan. Search for Biological Active

- Isatins. A Short Review. International Journal of Pharmaceutical Sciences and Drug Research. 2010; 2(4): 229-235.
6. <http://preprint.chemweb.com/orgchem/0010004>
  7. Ankur Patel, Sanjay Bari, Gokul Talele, Jitendra Patel and Manda Sarangapani. Synthesis and Antimicrobial Activity of Some New Isatin Derivatives. Iranian Journal of Pharmaceutical Research. 2006; 4: 249-254.
  8. Marvel CS and Heirs GS. Organic Synthesis Collective. Volume -1. 2nd Ed. John Wiley & Sons. New York: 1941. 423.
  9. L.Srikanth, Usha Naik, Ramesh Jadhav, N.Raghunandan and J.Venkateshwar Rao. Synthesis and Evaluation of New Phenylaminothiadiazolo-Oxadiazolo-1,3 Benzoxazoles For their Antibacterial Activity. International Journal of Pharma and Biosciences. 2010; 1(4): 260-271.
  10. Vivek Daniel, Kratika Daniel, Swapnil Goyal, Mahendra S.Parihar, Mangal Singh. Synthesis and In-vitro Anthelmintic Activity of Some 4-aminophenol Derivatives. International Journal of Pharmaceutical Research. 2010; 2 (2): 21-24.
  11. Srikanth Lingala, Raghunandan Nerella, K.R.S.Sambasiva Rao. Synthesis, Antimicrobial and Anthelmintic Activity of some Novel Benzimidazole Derivatives. International Journal of Pharmaceutical Sciences Review and Research. 2011; 10(2): 100-105.
  12. K.F. Ansari, C. Lal. Synthesis, physicochemical properties and antimicrobial activity of some new benzimidazole derivatives. European Journal of Medicinal Chemistry. 2009; 44: 4028-4033.
  13. Ayhan-Kilcigil, G, Altanlar N. Synthesis and antimicrobial activities of some new benzimidazole derivatives. Farmaco. 2003; 58: 1345-1350.
  14. Bahaa G. Mohamed, Abdel-Alim M. Abdel-Alim, Mostafa A. Hussein. Synthesis of 1-acyl-2-alkylthio-1,2,4 triazolobenzimidazoles with antifungal, anti-inflammatory and analgesic effects. Acta Pharm. 2006; 56: 31-48.