

## SYNTHESIS AND CHARACTERIZATION OF NOVEL THIAZOLO-ISOXAZOLE FUSED ISATIN AS ANALGESIC AND ANTI-INFLAMMATORY AGENT

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### ABSTRACT

A novel derivative of indole, containing the thiazole and isoxazole moieties, have been synthesized by isatin and evaluated for its possible analgesic, antipyretic and anti-inflammatory activities. The analgesic and anti-inflammatory activities were performed by tail flick and carrageenan induced oedema methods respectively. The compound showed significant analgesic and anti-inflammatory activity.

**Keywords:** Isatin, Analgesic, Anti-inflammatory

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## 1.0 INTRODUCTION

In spite of tremendous advance made in the modern medicine, there are still a large number of ailments for which suitable drugs are yet to be found. Today, there is an urgent need to develop safer drug for the treatment of pain, fever and inflammatory disorders.

Indole derivatives are an important class of organic heterocycles because of their potential activity as well as a part of several alkaloids. Indole derivatives are reported to be effective in CNS disorders such as convulsion<sup>1</sup> and depressions<sup>2</sup>. Indole and its analogs constitute the active class of the compounds possessing wide spectrum of biological activities<sup>3-4</sup>. Various indole derivatives exhibited antidepressive<sup>5</sup>, anti-inflammatory<sup>6</sup>, fungicidal<sup>7</sup>, bactericidal<sup>8</sup> and tuberculostatic activities<sup>9</sup>. Further azetidinones and thiazolidinones are well famed for their antimicrobial<sup>10-13</sup> activities. Schiff and Mannich bases of Isatin (Indole-2,3-dione) are reported to show antibacterial<sup>14</sup> and antifungal<sup>15</sup> activities. In addition, istains being synthetically versatile substrates are very useful for the synthesis of a large variety of heterocyclic compounds, as raw material for drug synthesis and can also function as suitable building blocks to synthesize some bioactive natural products. Hence a significant rising research interest in the design of oxindoles as drugs is currently observed

in the field of medicinal chemistry<sup>16</sup>. In the light of the above pharmacological activities, it was envisage to prepare biologically potent new isatin derivative carrying thiazole and isoxazole pharmacophores with the hope to possess better anti-inflammatory and analgesic activity. Isatin-3-p-chlorophenylimine (1) was synthesized from Isatin and the parachloroaniline which is then converted to its corresponding thiazolidine (2) on treatment with thioglycolic acid. Thiazolidine compound was then converted to corresponding arylidene on treatment with furfuraldehyde (3). Final isoxazole derivative was prepared by the third step arylidene compound and hydroxyl amine hydrochloride. Chromatographic analysis (TLC) of the compounds was used to check the completion of reaction. The structure of the final compound was stabilized on the basis of the IR and <sup>1</sup>H-NMR data. In IR spectra some significant stretching bands due NH between a range of 3378.67-3237.90cm<sup>-1</sup>, C=O~1711.51cm<sup>-1</sup>, C=N~1617.98cm<sup>-1</sup>, CH Ar~3064.33 cm<sup>-1</sup> & 1468.53 cm<sup>-1</sup>, C-N~1394.28 cm<sup>-1</sup> were found. In the <sup>1</sup>H-NMR spectra, the signal due to NH proton were observed at 8.3 ppm while the signals due to aromatic protons were observed at 7.6 – 6.4 ppm. All the synthesized compounds were screened for their analgesic and anti-

inflammatory activity using the nimesulide as reference standard.

## EXPERIMENTAL

All melting points were determined in open capillaries and are uncorrected. The progress of the reaction and the purity of compounds were checked by TLC on percolated silica gel plates using chloroform-ethyl acetate mixture (3:1). Physical characteristic data of the compounds are shown in table 1.

### Isatin-3-p-chlorophenylimine (1):

Equimolar mixture of Isatin and the p-chloro aniline was dissolved in warm ethanol and water (3:1) was refluxed on a steam bath for 6 hrs. After standing for 24hrs. at room temperature, the product were separated by filtration and re-crystallized from warm ethanol.

### p-chlorophenyl spiro [3H-indole-3, 2<sup>1</sup>-thiozolidine]-2-(1H), 4<sup>1</sup>-(5<sup>1</sup>H)-dione (2) :

In the prepared Isatin-3-p chlorophenylimine (1), equimolar solution of 1, 4-dioxan and thioglycolic acid was added and the mixture was refluxed for 10 hrs. Excess solvent was removed under pressure and liquefied residue was poured in ice-cold water. The solid

obtained was washed with sodium bicarbonate solution and re-crystallized from ethanol.

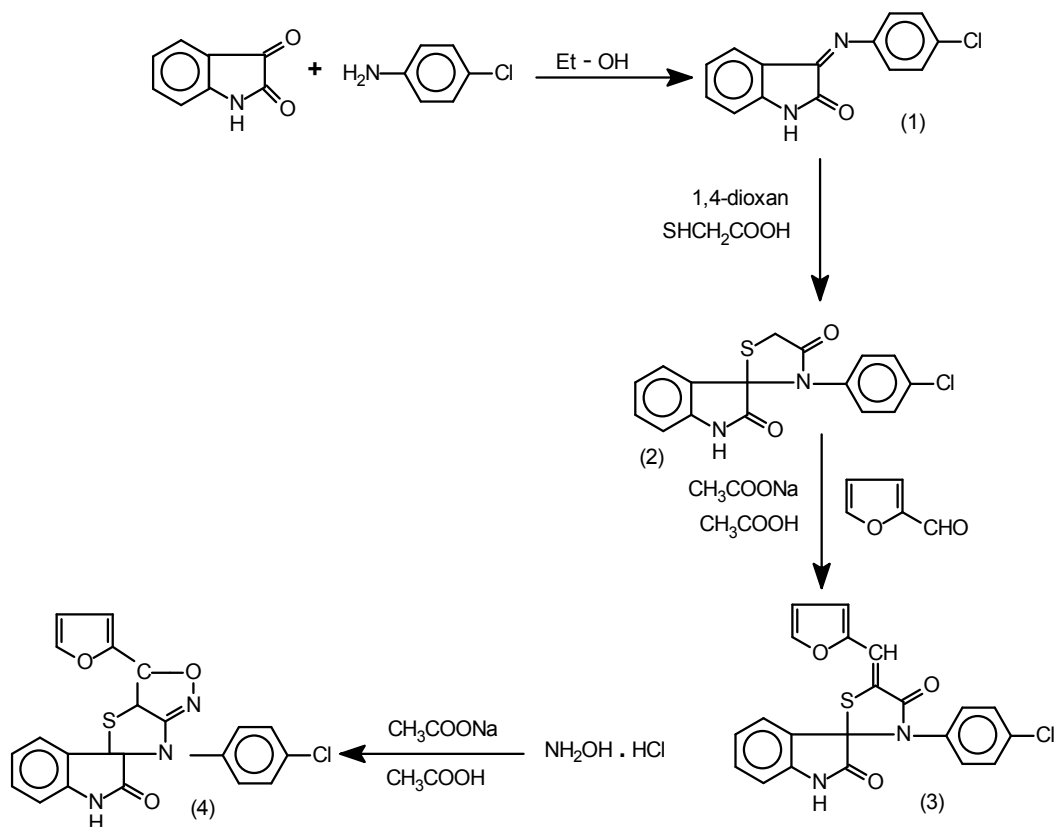
### 3<sup>1</sup>-p-chlorophenyl 5<sup>1</sup>-phenyl spiro [3H-indole 3, 2<sup>1</sup>-thiazolidine]-2- (1H), 4<sup>1</sup>-(5<sup>1</sup>H)-diones(3) :

Equimolar mixture of thiazolidine compound (2), furfuraldehyde and anhydrous sodium acetate in glacial acetic acid was refluxed on a heating mantle for 3hrs. The reaction mixture was concentrated, cooled and poured into ice-cold water. The solid thus separated, was filtered, washed with water and re-crystallized from glacial acetic acid.

### 3<sup>1</sup>-(p-chlorophenyl) 6<sup>1</sup>-Furyl-cis- 5<sup>1</sup>a, 6<sup>1</sup>-dihydro spiro [3H-indole 3, 4<sup>1</sup>-thiazolo(5<sup>1</sup>, 1<sup>1</sup>-c) isoxazolo-2(1H)-one](4) :

Equimolar mixture of 3<sup>1</sup>-p-chlorophenyl 5<sup>1</sup>-phenyl spiro [3H-indole 3, 2<sup>1</sup>-thiazolidine]-2-(1H), 4<sup>1</sup>-(5<sup>1</sup>H)-dione (3) in ethanol and anhydrous sodium acetate dissolved in minimum amount of glacial acetic acid was added to the solution of hydroxylamine hydrochloride. The reaction mixture was refluxed for 8 hrs, product was isolated and re-crystallized from warm ethanol.

## SCHEME



## PHARMACOLOGICAL ACTIVITY

### ANIMALS:

Healthy adult albino wistar rats weighing 180-200gm and healthy swiss albino mice weighing 25-30gm were taken for the study. The animal house was well ventilated  $12 \pm 1$  hour day and night schedule with average temperature  $25^\circ\text{C}$ . The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed hygienic food and water ad libitum.

### ANALGESIC ACTIVITY

Albino rats (180-200gms) were selected, weighed and divided into four groups of six animals each.

Control group received 0.5% sodium CMC (1mg/kg) orally whereas the standard reference group received Nimesulide (50mg/kg) orally. The rest two groups were treated as test groups and received indole derivative in dose level of 100mg/kg and 150mg/kg respectively. The animals which showed reaction time of 2-3s, were selected for the experiment and analgesic activity of the compound was studied by tail flick

method<sup>17</sup>. After the administration of the solvent Nimesulide and synthetic drug in different dose level was over, the basal reaction time was noted at 0, 1, 2, 3 and 4 hours by immersing the tail tips of the rats (last 1-2cm) in hot water heated at  $55^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ . The actual flick response of rats i.e. time taken (in sec) to withdraw tail from hot water source was calculated and compare with control group. The particulars are presented in Table 2.

#### **ANTI PYRETIC ACTIVITY**

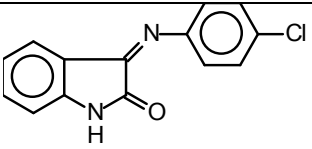
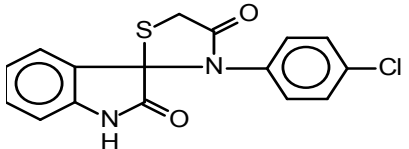
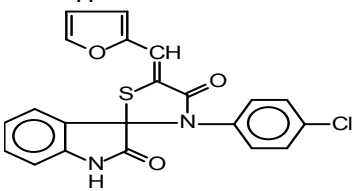
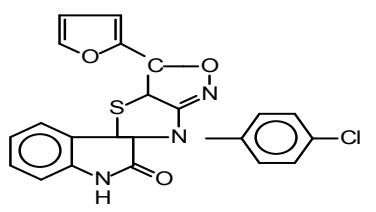
The male healthy albino rats (180-200gm) were randomly distributed in control and test groups of six animals each. The animals were kept 12/12hr dark-light cycle. One hour after starvation, the rectal temperature was recorded and animals having temperature between  $37.5^{\circ}\text{C}$  and  $38.5^{\circ}\text{C}$  were selected. Then they were injected intramuscularly with 20% suspension of Brewer's yeast with 0.9% NaCl. The animals developed  $0.5^{\circ}\text{C}$  or more rise in rectal temperature 18hrs after injection of Brewer's yeast in 0.9% NaCl suspension, were divided into four groups of six animals each similarly as for analgesic activity. The rectal temperature was recorded at 0, 1/2 1, 2, 3 and 4 hours after administration of solvent, Nimesulide and the novel indole derivative in different dose level. The reduction in temperature at the end of every record was calculated and showed in Table 3.

#### **ANTI-INFLAMMATORY ACTIVITY**

The anti-inflammatory activity was studied by Carrageenan induced paw oedema<sup>18</sup> method. The male healthy albino rats (180-200gm) were selected, weighed and divided into four groups into six animals each. All the groups were treated similarly as for analgesic activity. All these animals were fasted for 18 hrs. before the beginning of the experiment and water was given ad libitum. In animals of all the groups, acute inflammation was produced by subplantar injection of  $20\mu\text{l}$  of freshly prepared 1% suspension of carrageenan in normal saline in right hind paw of rat. The paw thickness was measured using a slide caliper before and after 3 hours carrageenan challenge in each group.

In the above model the degree of oedema formation was assayed as increase in paw thickness. The increase in paw thickness was obtained by subtracting the initial thickness from thickness at time 't'. Percentage of inhibition were calculated by the formula percent inhibition =  $(P_C - P_T) / P_C \times 100$ , where  $P_C$  is the increase in paw thickness in control and  $P_T$  is the increase in paw thickness in test. The increase in paw thickness were measured, inhibition were calculated by comparing with control group. The results were observed and are recorded in table 4.

**Table No. 1: Physical data of compounds**

S. No.	Structure	Mol. Formula	Mol. Wt.	Yield (%)	M. P. (°C)
1.		$C_{14}H_9N_2OCl$	256.5	78	236
2.		$C_{14}H_9N_2OCl$	330.5	60	162
3.		$C_{21}H_{13}N_2O_2ClS$	392.5	70	121
4.		$C_{21}H_{14}N_2O_3ClS$	409.5	60	147

**Table No. 2: Results of Analgesic Activity**

S.No.	Treatment	Tail flick response (sec)				
		0 hr	1 hr	2hr	3 hr	4 hr
1.	Control (0.5% Sodium CMC)	0.483±0.028	0.908±0.058	1.116±0.059	0.808±0.141	0.866±0.036
2.	Test- 1 (100 mg/Kg)	0.615*±0.063	0.833±0.071	1.216±0.234	1.733±0.437	2.041*±0.148
3.	Test- 2 (150 mg/Kg)	0.518±0.008	1.358**±0.040	1.556**±0.017	1.961±0.016	1.308±0.169
4.	Standard Nimusulide (50 mg/Kg)	0.568±0.014	0.846±0.014	1.566**±0.065	0.75±0.075	1.786 *±0.034

Results are expressed as mean ± SEM from five observations.

\*  $\longrightarrow$  P<0.05

\*\*  $\longrightarrow$  P<0.001

**Table No. 3: Results of Antipyretic effect on Yeast induced Hyperpyrexia**

S. No.	Treatment	Initial temp in °c	Temp. After 18hr of yeast administration in °c	Temperature after treatment in °c				
				30 min	1 hr	2 hr	3 hr	4 hr
1.	Control (0.5% Sodium CMC)	38.216± 0.047	38.566 ±0.042	38.675 ± 0.103	38.3 ±0.127	38.116 ±0.217	38.583 ±0.228	38.016 ±0.217
2.	Test-1 (100 mg/Kg)	38.066 ± 0.162	38.35 ±0.076	37.665 ± 0.248 0.685a	37.233 ±0.293 1.117a	36.951 ±0.217 1.399a	37.245 ±0.258 1.05a	37.308 ±0.147 1.042a
3.	Test 2 (150 mg/Kg)	37.866 ±0.114	38.625 ± 0.044	37.275 ±0.0389 1.35a	36.766 ±0.208 1.859a	36.805 ±0.201 1.82a	37.208 ±0.198 1.417a	38.05 ±0.185 0.575a
4.	Standard Nimusulide (50 mg/Kg)	38.316 ±0.027	38.8±0.036	37.95 ±0.158 0.85a	37.483 ±0.313 1.317a	37.616 ±0.429 1.184a	37.626 ±0.358 1.174a	37.748 ±0.053 1.052a

Each value is mean ± SEM of 5 observation  
 'a' represent mean reduction in temperature

**Table No.4 Results of Anti-Inflammatory activity**

S.No.	Treatment	Initial Paw Thickness in mm	Mean Paw Thickness		% Inhibition at the end of hr	
			1.5 Hr	3 Hr	1.5Hr	3 Hr
1.	Control (0.5% Sodium CMC)	0.518± 0.018	0.791± 0.031	0.815±0.035	-	-
2.	Test -1 (100 mg/Kg)	0.555± 0.012	0.663 *± 0.018	0.568 **± 0.022	60.439	91.582
3.	Test-2 (150 mg/Kg)	0.588± 0.022	0.676 *± 0.014	0.646 **± 0.026	67.765	80.471
4.	Standard Nimusulide (50 mg/Kg)	0.57± 0.02	0.588 *± 0.024	0.6 **± 0.023	93.406	89.898

Results are expressed as mean + SEM from four observations.

\* → P<0.05

\*\* → P<0.001

## RESULTS AND DISCUSSION

All the physical characteristics of the final compound as well as the intermediates are shown in table. The purified products were screened for their analgesic, antipyretic and anti-inflammatory activities. The final compound 3<sup>1</sup>-(p-chlorophenyl) 6<sup>1</sup>-Furyl-cis-5<sup>1</sup>a, 6<sup>1</sup>- dihydro spiro [3H-indole 3, 4<sup>1</sup>-thiazolo(5<sup>1</sup>, 1<sup>1</sup>-c) isoxazolo-2(1H)-one](4) was obtained in good yield and has the melting point of 147°C. In IR spectra some significant stretching bands due to N-H between a range of 3378.67-3237.90 cm<sup>-1</sup>, C=O~1711.51, C=N~1617.98 cm<sup>-1</sup>, C-HAr~3064.33cm<sup>-1</sup>, C-N~1394.28 cm<sup>-1</sup>, C-O-N ~1215.90cm<sup>-1</sup> were observed. In the <sup>1</sup>H-NMR spectra, the signal due to NH proton were observed at 8.3 ppm, while the signals due to aromatic protons were observed at 7.6–6.4 ppm. The signal due to isoxazole and 2-furan were observed at 3.3–3.0 and 8.0–7.7 ppm respectively.

The new indole derivative compound under test shows a remarkable analgesic, antipyretic and anti-inflammatory activity. The antipyretic effect of compound is probably due to reduction in Bradikinin synthesis and other peptide synthesis. Further studies are necessary to established and confirm the above mechanisms by following the in vivo and in vitro methods.

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