SYNTHESIS AND EVALUATION OF ACUTE TOXICITY STUDIES AND ANALGESIC CHARACTERS OF SOME NOVEL INDOLE DERIVATIVES

Nilanjan Pahari[1], Dibyajyoti Saha[2], Vibhor K. Jain[2]*, Bindu Jain[2], Debajyoti Mridha[3]

1. Calcutta Institute of Pharmaceutical Technology & AHS, Banitabla, Uluberia, W.B

2. School of pharmacy, Chauksey Engineering College, Bilaspur C.G.

3. Bharat Technology, Banitabla, Uluberia, W.B

Abstract:

The present study was carried out with an objective to synthesis the novel indole derivative from istatin and to evaluate its acute toxicity studies to estimate its safety dose level and evaluate its analgesic activity. The synthesis was carried out following the scheme and all the chemicals were purified according to established method prior to use. The synthesized compound was subjected to estimation of acute toxicity studies on albino rat (swiss model). Analgesic activity was evaluated by hot plate method. The results reveal that the synthesized novel indole derivative contains Indole, Thiazolidone and Isoxazole nucleus and by the probit scale analysis the LD50 of the Novel Indole derivative was found to be 1271 mg/Kg body weight. The results of analgesic activity reveals that the compound possesses significant analgesic activity when compared to standard.

Keywords: Novel indole derivative, acute toxicity, analgesic activity, istatin.

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 disorder etc.

Herbal drugs have played a vital role in curing so many ailments through out the history of medicines as well as the existence of mankind. But natural drug contain a mixture of components. In a number of natural drugs, the active principle is indole nucleus, as in case of reserpine, strychnine, ergotamine and several other alkaloids and is contributed towards the major biological activity of this natural drugs.2

Indole derivatives are an important class of organic heterocyclic because of there potential activity as well as a part of several alkaloid. Indole derivatives are reported to be effective in CNS disorders such as convulsion[3] and depressions[4], Para substituted any thiosemicarbazide derivatives[4] of isatins were reported to be non-toxic and pshychotropic. Arylpropanoloxyamines are known to possess a number of biological activities which induce local anasthetic[4], muscle relaxant and hypertensive[5], antibacterial[6], antifungal[7], antiviral[8], anti-HIV[9], antiprotozoal[10], antihelminthic[11], antineoplastic[12] and analgesic, antipyretic and anti-inflammatory.

Thiazole ring[14] bearing compounds show varied biological activities. There are a large number of synthetic compounds with thiazole nucleus having application as anticancer agent, analgesics, hypnotics, insecticides and pesticides. Thiazole ring bearing compounds shows antimicrobial activity. Some derivatives of thiazolidinones have displayed significant biological activity like antibacterial, antifungal, antihistaminic and anti-inflammatory[14].

Isoxazoles possess various biological and pharmacological activities such as being useful as CNS activity[14], antimicrobial, antiviral[15], anthelmintic[15], hypoglycemic[15], diabetic[15], neuroleptic[15], antitumour, antipyretic[15], anti-inflammatory[15,16], analgesic[15,16] and in Chemotherapy[17].

In view of various important pharmacological property of the above pharmacophores, it is speculated in our study to "synthesize and screen pharmacologically an Indole derivative named 3^1–(p-chlorophenyl) 6^1–

Furyl–cis–5[1]a, 6[1]–Dihydrospiro [3H–indole–3, 4[1]–thiazolo(5[1],1[1]–c) isoxazolo–2 (1H)-one]" to establish its various pharmacological actions in different animal models.

In last few decades various approaches were done for evaluation of biological activities of indole derivatives[18-24], thiazolidine derivatives, isoxazle derivatives[24], and istatin derivatives[19].

So, this work provides an empirical approach towards the synthesis of indole derivative and its oral acute toxicity studies were performed. The synthesized compound was subjected to spectral analysis to reveal its structure. The aim of acute toxicity studies is to establish a therapeutic index for drug.

Material and Methods:

The chemicals used in the present Project were of AR grade and LR grade purchased from sigma, Renchem, Rotex, Merck and Hi-media. Dehydrated ethanol used during the synthesis were distilled and purified following the standard protocol[55].

Melting point of the synthesized compounds were determined using melting point apparatus(SISCO, India) and were found uncorrected.

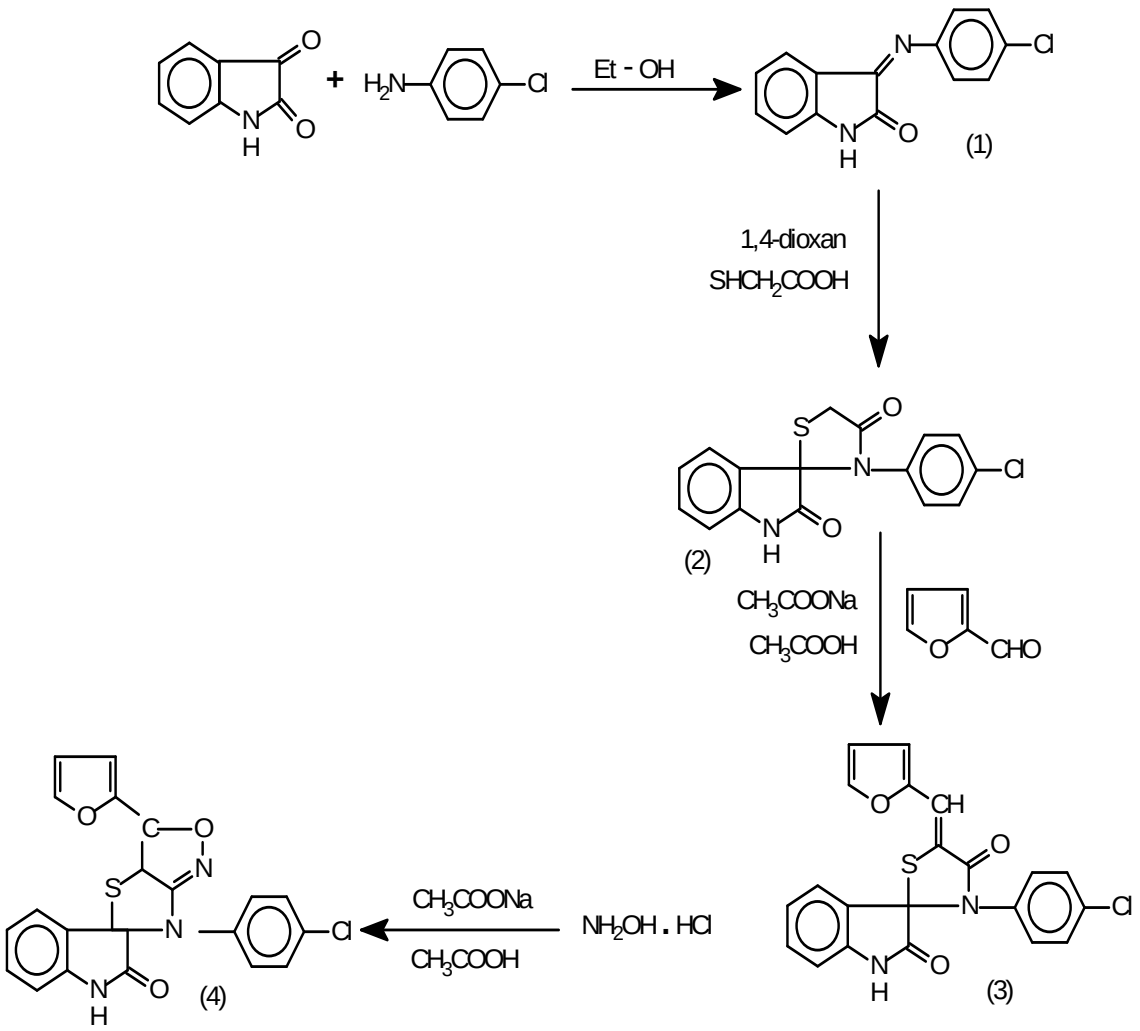
Purity of the compounds was checked by the Thin layer chromatography using silica gel G as stationary phase and various combination of Benzene : Ethyl acetate as mobile phase. The spots resolved were visualized as brown colored spots by using Iodine chamber.

The IR spectra of synthesized compounds were recorded using KBr pellets in range of 400-500cm^{-1} on a Fourier Transform IR spectrometer(Ft-IR 410, Jasco, IICB, Cal) the frequencies are recorded in wave numbers.

^{1}H NMR(300Mhz) spectra were recorded in chloroform-d in BRUKER DPX-300(IICB, Cal)NMR Spectrometer. Chemical shift(□)are reported in parts per million downfield from internal reference Tetramethyl Silane (TMS)

Synthesis was carried out according to the scheme given as follows:

4. SCHEME


Et - OH
(1)
1,4-dioxan
SHCH2COOH
(2)
CH3COONa
CH3COOH
CHO
(3)
NH2OH . HCl
CH3COONa
CH3COOH
(4)

1. Preparation of Isatin-3-p-chlorophenylimine(1) :

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

Percentage Yield = 78

Melting point ^{0}C= 236

2. Preparation of p-chlorophenyl spiro [3H-indole-3, 2^1-thiozolidine]-2-(1H), 4^1-(5^1H)-dione(2) :

Equimolar to a solution of Isatin-3-p chlorophenylimine is 1, 4-dioxan and thioglycolic acid was added and the mixture was refluxed for 10hrs. Excess solvent was removed under pressure and liquefied residue was poured in ice-cold water. The solid was obtained washed with sodium bicarbonate solution and re-crystallized from ethanol.

Percentage Yield = 60

Melting point ^{0}C= 162

3. Preparation of 3^1-p-chlorophenyl 5^1-phenyl spiro [3H-indole 3, 2^1-thiazolidine]-2- (1H), 4^1-(5^1H)-diones(3) :

Equimolar mixture of thiazolidine compound, 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 crystallized from glacial acetic acid.

Percentage Yield = 70

Melting point ^{0}C= 121

4. Preparation of 3^1-(p-chlorophenyl) 6^1-Furyl-cis- 5^1a, 6^1- dihydro spiro [3H-indole 3, 4^1-thiazolo(5^1, 1^1-c) isoxazolo-2(1H)-one](4) :

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

Percentage Yield = 60

Melting point ^{0}C= 147

SPECTRAL STUDIES :

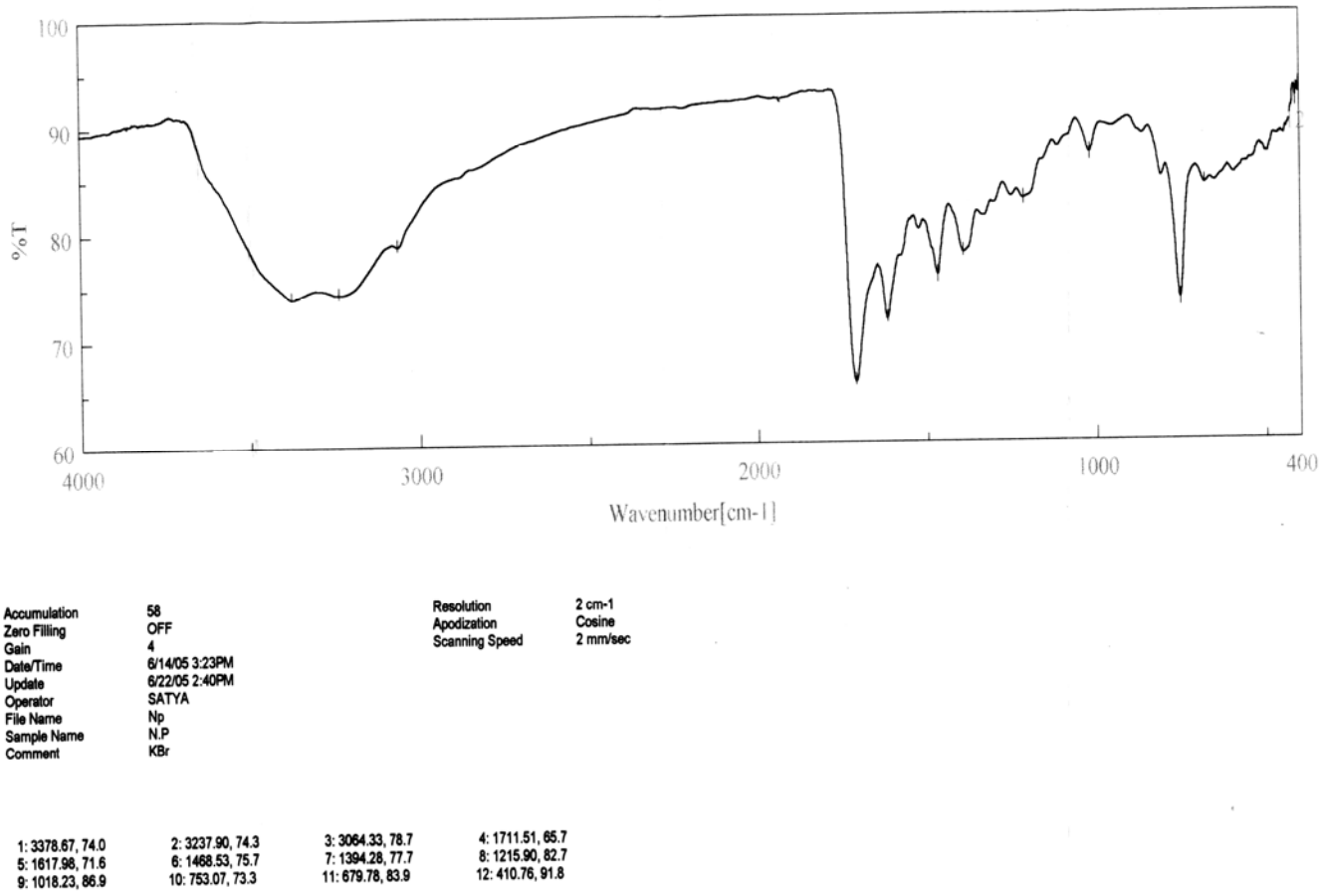


FIG. I- IR SPECTRA

Compound Name – 3^1-(p-chlorophenyl) 6^1-Furyl-cis- 5^1a, 6^1- Dihydro spiro [3H-indole 3, 4^1-thiazolo(5^1, 1^1-c) isoxazolo-2(1H)-one]
3378.67, 3237.90(NH Str)
1711.51(C=O Str)
1617.98 (C=N)
1394.28((C–N)
1215.90(C–O–N)
3064.33, 1468.53(Ar–CH–Str)
1018.23(C–O–C)
753.07(C–Cl–Str)

^{1}H NMR SPECTRA :

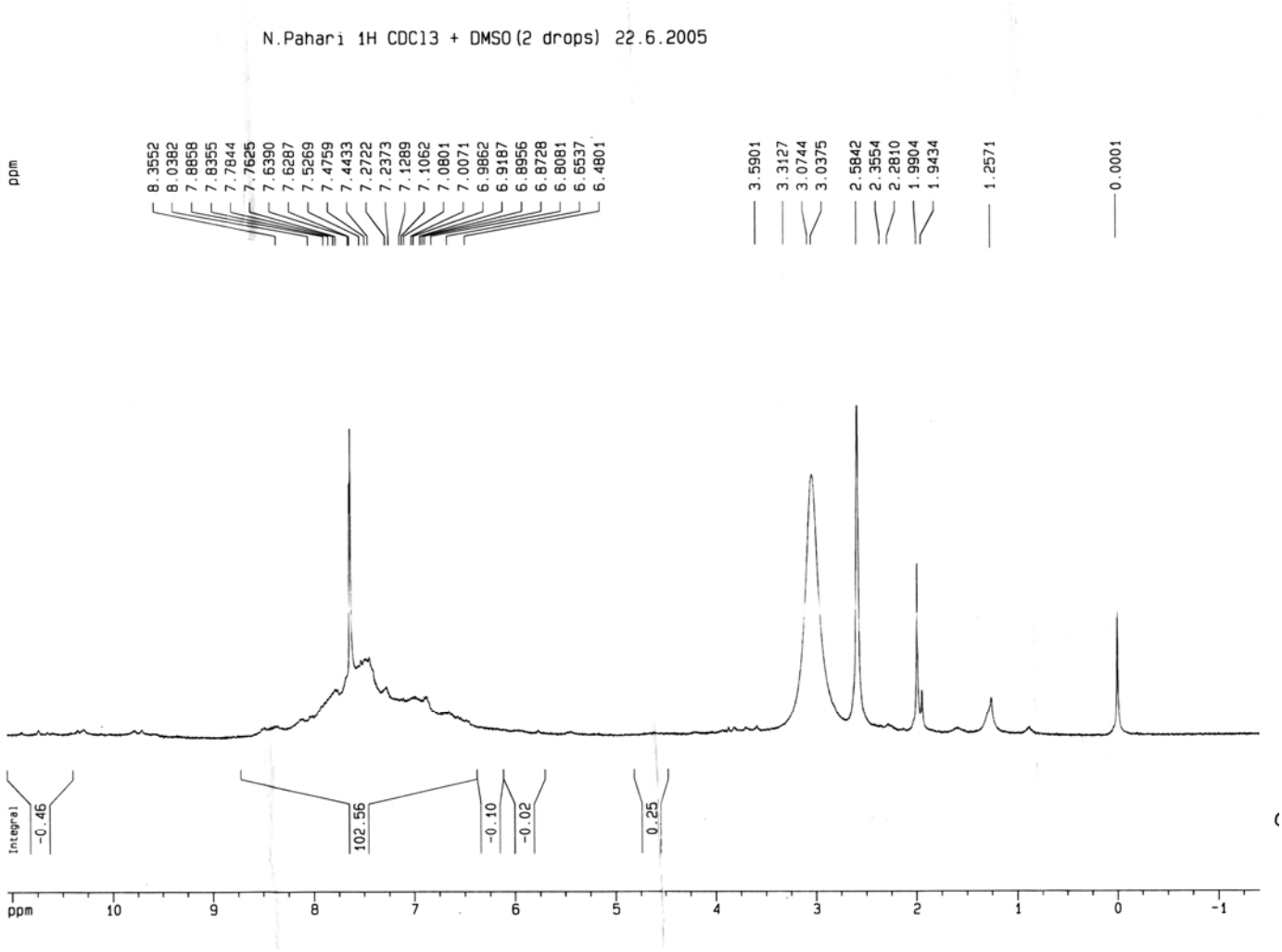


FIG. II- NMR SPECTRA

NH – 8.352 (Secondary Amine)

CH – 8.03 – 7.78 (2-Furan)

CH – 7.6390 – 6.4801 (Aromatic)

CH – 3.3172 – 3.0375 (Isoxazole)

Biological evaluation :

Acute toxicity studies[25]:

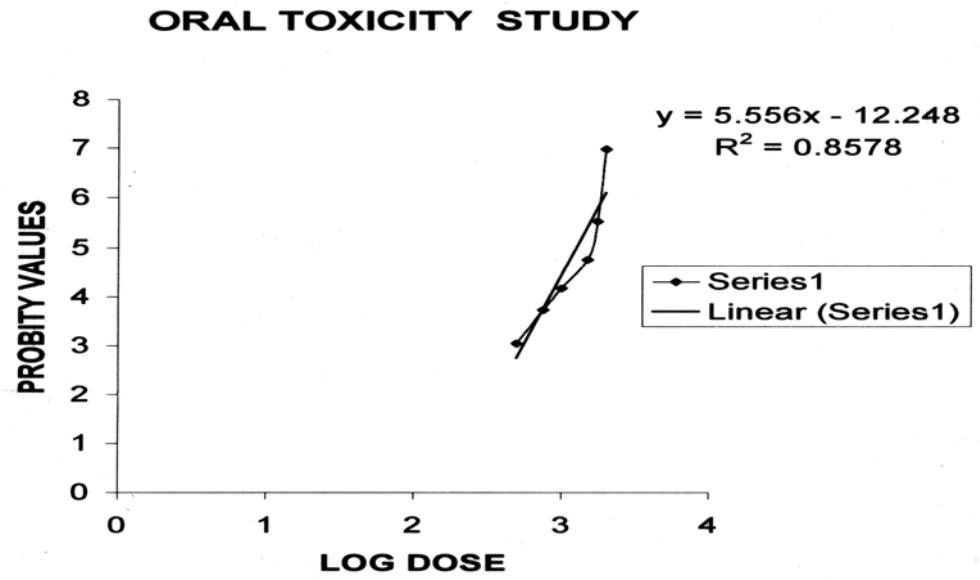
The adult albino mice (Swiss strain) of either sex weighing between 25-30gm were selected for studies before actual LD50 determination, a primary study was made on a small group of mice. The acute oral toxicity studies are carried out to determine the safety dose of the Novel Indole derivative. Mainly to select dose range for the subsequent studies.

The Novel Indole derivative as various dose level like (500,750, 1000, 1500, 1750 & 2000) mg/Kg body weight suspended in 0.5% sodium CMC were administered as a single dose to a pair of mice as per dose level. The morality was observed at 1750 and 2000 mg/Kg body weight. So for the actual LD 50 determination the dose range was narrowed and various dose level chosen are 500, 750, 1000, 1500, 1750 and 2000 mg/Kg body weight which are orally administered to a group of 10 mice of both sexes about equal in number which have the treated mice were observed continuously for 2 hours and then occasionally for 4 hours and finally overnight mortality was recorded. The behavior of the animal and any other toxic symptoms were also observed for 72 hours and then animal were kept on observation up to 14 days.

The percentage of mortality is different dose level of synthetic compound is cited in Table 1.

TABLE–1. ORAL TOXICITY STUDY BY PROBIT METHOD OF THE NOVEL INDOLE DERIVATIVE

Group	Dose (mg/Kg)	Log Dose	Dead Total	Dead %	Corrected %	Probit
1	500	2.698	0/10	0	2.5	3.035
2	750	2.875	1/10	10	10	3.72
3	1000	3	2/10	20	20	4.16
4	1500	3.176	4/10	40	40	4.75
5	1750	3.243	7/10	70	70	5.52
6	2000	3.301	10/10	100	97.5	6.965



At the dose level of 500mg/Kg body weight no mortality was observed, but when the dose increased, the mortality was increased. At higher dose of 1500 to 2000 mg/Kg body weight toxic symptoms like loss of muscle tone, loss of traction, mild drowsiness were observed, on repeated administration in higher dose level, mortality were observed. By the probit scale analysis the LD50 of the Novel Indole derivative was found to be 1271 mg/Kg body weight.

ANALGESIC ACTIVITY:

HOT PLATE METHOD :

METHOD[57] :

Glassman's method was used. Albino rats of either sex(180-200gm) were selected weighted and divided into four groups of six animals each. All the animals were fasted for 18hrs. before the beginning of the experiment and water was given adlibitum. The animals were placed in cages with grating to avoid coprophogy. The animals were treated as follows :

Group 1 : Control group received 0.5% sodium CMC(1mg/kg) orally.

Group 2 : Nimesulide 50mg/kg were administered orally.

Group 3 : The Novel Indole derivative in dose level of 100mg/kg was administered orally.

Group 4 : The Novel Indole derivative in dose level of 150mg/kg was administered orally.

Here, Group 1 is the control, group 2 is active control/standard and group 3 and group 4 is test.

After administration was over the time of reactions of pain stimulus of the rat placed on the plate heated at $55^0C \pm 0.5^0C$ was recorded at 0 hr., 1 hr., 2 hrs., 3 hrs. and 4 hrs., after the administration of solvent, Nimesulide and different dose level of synthetic drug. The increase in reaction time against solvent control group was calculated. The particulars are presented in Table -2.

TABLE–2. ANALGESIC ACTIVITY OF THE NOVEL INDOLE DERIVATIVE (HOT PLATE METHOD)

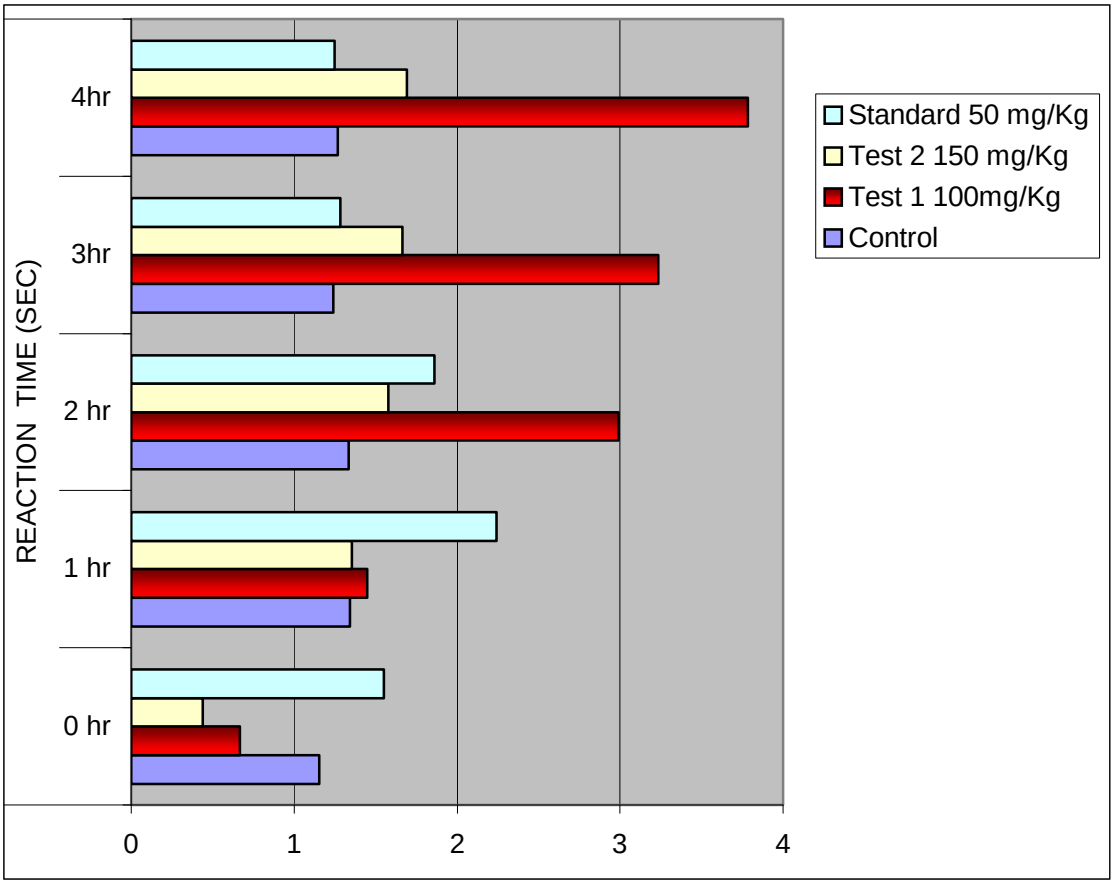
S. No.	TREATMENT	REACTION TIME (SEC)				
		0 hr	1 hr	2 hr	3hr	4hr
1	**Control (0.5% Sodium CMC)**	1.155 ± 0.018	1.341 ± 0.20	1.333 ± 0.140	1.241 ± 0.108	1.266 ± 0.091
2	**Test 1 The Novel Indole Derivative (100 mg/Kg)**	0.666 ** ± 0.042	1.45 ** ± 0.022	2.991** ± 0.220	3.233** ± 0.338	3.783** ± 0.370
3	**Test 2 The Novel Indole Derivative (150 mg/Kg**	0.441 ± 0.032	1.356 ± 0.112	1.578** ± 0.016	1.666 * ± 0.062	1.691 * ± 0.058
4	**Standard Nimusulide (50 mg/Kg)**	1.55 ± 0.191	2.241** ± 0.168	1.86 ** ± 0140	1.283 ± 0.094	1.25 ± 0.076

Results are expressed as mean ± SEM from five observations.

* ⟶ P<0.05

** ⟶ P<0.001

FIGURE III. ANALGESIC ACTIVITY OF THE NOVEL INDOLE DERIVATIVE (HOT PLATE METHOD)



Results (Table – 2) shows that there is a significant peripheral analgesic activity at a dose level of 100 to 150 mg/Kg body weight of Test1 & Test 2, when compared to the solvent control (05% Sodium CMC, 1 ml /Kg body weight) and reference standard drug Nimesulide (50 mg/Kg) body weight from 0 hour to 4 hours.

Result and discussion:

Following the scheme, novel indol derivative was synthesized which is ascertained by melting point determination and spectral analysis. This compound possesses an Indole, Thiazolidone and Isoxazole nucleus. Compound was than subjected to acute toxicity studies to determine the safety dose level, the probit scale analysis the LD50 of the Novel Indole derivative was found to be 1271 mg/Kg body weight. Compound is then subjected for evaluation of analgesic activity by tail flick method and results shows that compound synthesized posses significant analgesic activity when compared with nimuslide as standard.

References :

[1] Sareen K., Kohli R. P., Amma M. K. P. and Gujral M. L., Indian J., Phys. Pharmacology, 6, 87, 1962.
[2] Shetty M. V., Parimoo P. and Chopra Y. M., European J. Med. Chem., Chim ther; 13, 581, 1978.
[3] Varma R. S. and Panday R. K., Indian J. Chem; 21B, 157, 1982.
[4] Ing H. R. and Ormerod W. E., J. Pharm. Pharmacol, 4, 21, 1952.
[5] Chizhivskaya II, Yakimovies B., Shadurski L. A. and Vestri K. S., Aead Navuk Belarus, USSR, Ser Fiz-Tekh naruk, 115, 1957.
[6] Sridhar S. K., Pandeya Surendra N., Bajpai S. K. and Manjula H., Indian Drugs ; 36, 412, 1999.
[7] Sarangapani M. Narayana Reddy A., Jayammay and Reddy V. M., Indian Drugs, 35, 336, 1998.
[8] Varma Rajendra S. and Lewis W. L., J. Med. Chem., 10, 972, 1967.
[9] Pandeya S. N., Yogeswari P., Sriram D., De Clereq E. and Witvouw M., Chemotherapy, 45, 192, 1999.
[10] Imam S. A. and Varma R. S., Experenzia, 31, 1287, 1975.
[11] Marie Elisabeth Sarciron, Patrick Audin, Isabelle Delebre, Clade Gabrion, Francoise Petavy and Joelle Paris, J. Pharm Sci., 82, 605, 1993.
[12] Popp Frank D., J. Med. Chem., 12, 182, 1968.
[13] Sacama M. A. and El-Lssa A. S., Indian J. of Chemistry, 40B, 678-681, 2001.
[14] Mandhare P. N, Rinthe S. S., Ratil L. R., Mane R. A., Ind. J. of Hetero. Chem., 12, 209-212, Jan-March 2003.
[15] Nagar D. N., Mehta T. and Shah V. H., Ind. J. of Hetero. Chem., 13, 173-174, Oct-Dec 2003.
[16] Eugster, Prog. Chem. Org. Nat. Prod., 27, 261, 1969.
[17] Reddy P. B., Reddy S. M., Rajanarendar E and Murthy A. K., Indian Phytopathology, 37, 370, 1984.
[18] Selvam P., Rajasekaran A., Murugesh N., Chandramohan M. and De Clerecq E., Indian Journal of Pharmaceutical Science, July-August-2004.
[19] Seshaiah K. S. and Ramesh A., Indian Drugs, 38(4), April-2001.
[20] Saundane A. R., K. Rudresh, N. D. Satyanarayanan and Hiremath S. P., Indian Journal of Pharmaceutical Sciences, Nov-Dec-1998.
[21] Sridhar S. K. and Ramesh A., Indian J. of Chem., 41B, 2002, pp.668-672.
[22] Baidya M., Kumar J., Srivastava A. K., Jyothi C., Gupta A. and Das A. K., Indian J. of Heterocyclic chem., vol.-14, July-Sept.-2004, pp.81-82.
[23] Pathak V. N., Gupta P., Garg M. and Kumar A., Indian J. of heterocyclic Chem., Vol.13, Oct-Dec-2003, pp.159-162.
[24] P. N. Mandhare, S. S, Rindhe, L. R. Patil and R. A. Mane., Indian J. of Heterocyclic Chem., Vol.12, Jan-Mar -2003.
[25] Ghosh M. N., Fundamental of Experimental Pharmacology, 2nd Edn., Scientific Book Agency, Calcutta, 153-158, 1984.
[26] Glassman J. M., Agent with analgesic activity and dependence liability, In : Twener R. A. and Hebborn P(eds)., Screening methods in Pharmacology, Academic press, New York and London, 11, 231-232, 1971.
[27] Kulkarni S. K., Hand Book of Experimental Pharmacology", Vallabh Prakashan, New Delhi, 49-50, 1997.