



TO SYNTHESIZE, CHARACTERIZATION AND PHARMACOLOGICAL EVALUATION OF NOVEL SUBSTITUTED ISATIN DERIVATIVES

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ABSTRACT:

Series of novel Schiff bases of Isatin the equimolar amines and 5-Dicrboxymethyl (R=COO₂Me) substituted isatins (1 mmol of each) were added to 96% w/w ethanol (20 mL) containing 8 drops of glacial acetic acid. The mixture was heated under reflux for 5 h and then cooled to room temperature. The resulting solid was collected by filtration, washed with cold ethanol and dried in open air. The derivatives thus prepared had sufficient analytical purity. anticonvulsant activity performed by method as Animals were weighed and numbered. Mice were divided into 7 groups of six animals each. Group 1 served as control which was treated with vehicle (2% v/v Tween 80), group 2 was treated with standard drug phenytoin (25 mg/kg, i.p.) and groups 3– 7 were treated with newly synthesized oxadiazole derivatives (25 mg/kg, i. p.). One hour after injection, the animals were subjected to electro shock through ear electrodes of 80 mA for 0.2 sec by electroconvulsimeter AND ANTI-inflammatory activity measured by Weigh the animals and number them. Mark the animals with picric acid for individual animal identification. Divide rats into 5 groups of 6 rats each. Note the initial paw volume of each rat by dipping just beyond tibio-tarsal junction by mercury displacement method. The pharmacological screening of the synthesized compounds showed anti convulsant activity ranging from 56.2 % to 76.3 % inhibition of epileptic seizures in mice, where as the standard drug Phenytoin showed 83.95 % inhibition of epileptic seizures in mice. The compound **iiih4** from each group was found to be nearly potent to Phenytoin which is used as standard drug. Anti-inflammatory activity ranging from 31.09 to 63.11 % inhibition of rat paw edema volume after 3 hours, whereas the standard drug Indomethacin showed 62.06 % inhibition of rat paw edema volume after 4 hours. The compound **iiih3** was found to be nearly more potent then indomethacin which is used as standard drug

Keywords: Isatin; Schiff bases; Anti convulsant activity; Anti-inflammatory activity; Isatin.

1. INTRODUCTION

Isatin (1H-indole-2,3-dione, Figure 1) was first obtained by Erdman and Laurent in 1841 as a product from the oxidation of indigo by nitric and chromic acids.

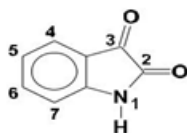


Figure 1: 1H-indole-2,3-dione

The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis. Three reviews have been published regarding the chemistry of this compound: the first by Sumpster, in 1954 [1], a second by Popp in 1975 [2], and the third on the utility of isatin as a precursor for the synthesis of other heterocyclic compounds [3].

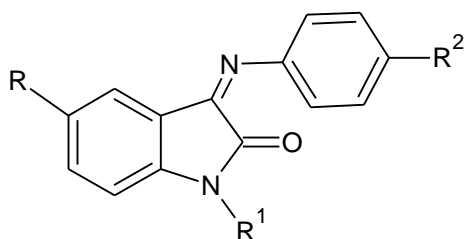
In nature, isatin is found in plants of the genus *Isatis* [4], in *Calanthe discolor* LINDL. [5] and in *Couroupita guianensis* Aubl. [6], and has also been found as a component of the secretion from the parotid gland of *Bufo* frogs [7], and in humans as it is a metabolic derivative of adrenaline [8-10]. Substituted isatins are also found in plants, for example the melosatin alkaloids (methoxy phenylpentyl isatins) obtained from the Caribbean tumorigenic plant *Melochia tomentosa* [11-13] as well as from fungi: 6-(3'-methylbuten-2'-yl)isatin was isolated from *Streptomyces albus* [14] and 5-(3'-methylbuten-2'-yl)isatin from *Chaetomium globosum* [15]

2. PHARMACOLOGICAL ACTIVITY OF ISATIN

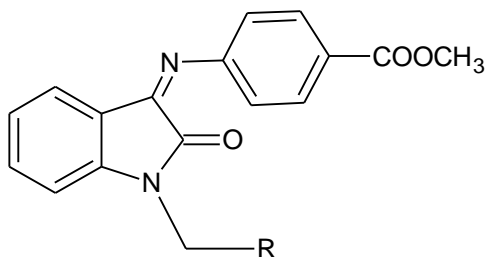
2.1 Anti-Convulsant: A large number of populations of different age groups and sex are affected by this disease. Mainly, two kinds of epilepsy have been identified, one with grand mal and the other with petit mal. Anticonvulsant drugs with MES (maximal electroshock) activity are generally useful in grand mal,

while ScMet (subcutaneous metrazole) antagonists are effective in petit mal. Many drugs have been marketed recently for the treatment of epilepsy. [16] These include miconazole, zonisamide, lamotrigine, felbamate and tiagabine. Recently, semicarbazones are emerging as novel anticonvulsant drugs. 4-Bromobenzaldehyde semicarbazone and 4-bromophenyl semicarbazones have shown promising activities [17-21]. The proposed pharmacophoric requirements in the semicarbazone molecules are:

- (i) aryl binding site with a hydrophobic group;
- (ii) hydrogen bonding domain exemplified by the presence of the -NHCO- grouping;
- (iii) two electron donor system;
- (iv) hydrophobic binding site whose size determines the type of activity.

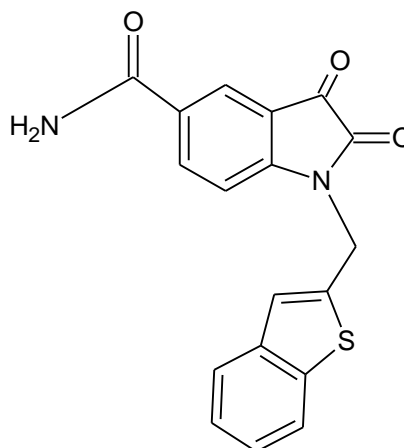


2.2 Anti-Microbial Activity: series of novel Schiff bases of Isatin (V) and (X) were synthesized by refluxing Isatin with p-amino ethyl benzoate (IV) and 4-(4'-amino phenyl)-6-substituted phenyl pyrimidine-2-thiol (IX). Mannich bases of ethyl-4-(2-oxindolin-3-ylidene amino) benzoate (VI) were synthesized by using various aromatic secondary amines synthesis of 1-(substituted amino methyl)-4-ethyl-4-(2-oxindolin-3-ylidene amino) benzoate.[22]

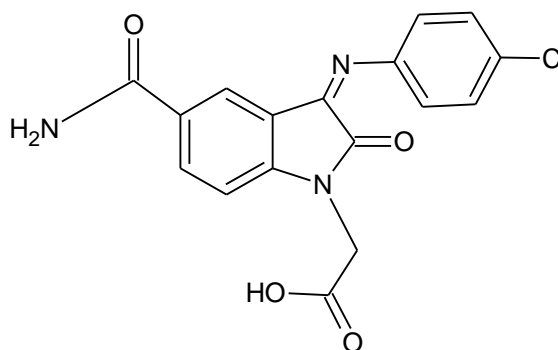


2.3 Protease Inhibitors: N-Substituted isatin derivatives were prepared from the reaction of isatin and various bromides via two steps. Bioactivity assay results (in vitro tests) demonstrated that some of these compounds are potent and selective inhibitors against SARS coronavirus 3Cl protease with IC₅₀ values ranging from 0.95 to 17.50 IM. Additionally, isatin 4o exhibited more potent inhibition for SARS coronavirus protease than for other

proteases including papain, chymotrypsin, and trypsin. [23]



2.3 Antioxidant and Cytotoxic Activities: new series of Schiff's bases of carbohydrazone coupled with isatins. Yields of all synthesized compounds were good. The synthesized derivatives were screened for their antioxidant and cytotoxic activities. N'1, N'3-bis [2-oxo-2,3-dihydro-1H-indoli-3-ylidene]isophthalic hydrazide derivatives were used for the evaluation of antioxidant activity. These 12 different novel derivatives were prepared by treating isophthalohydrazide with different isatin derivatives.[23]

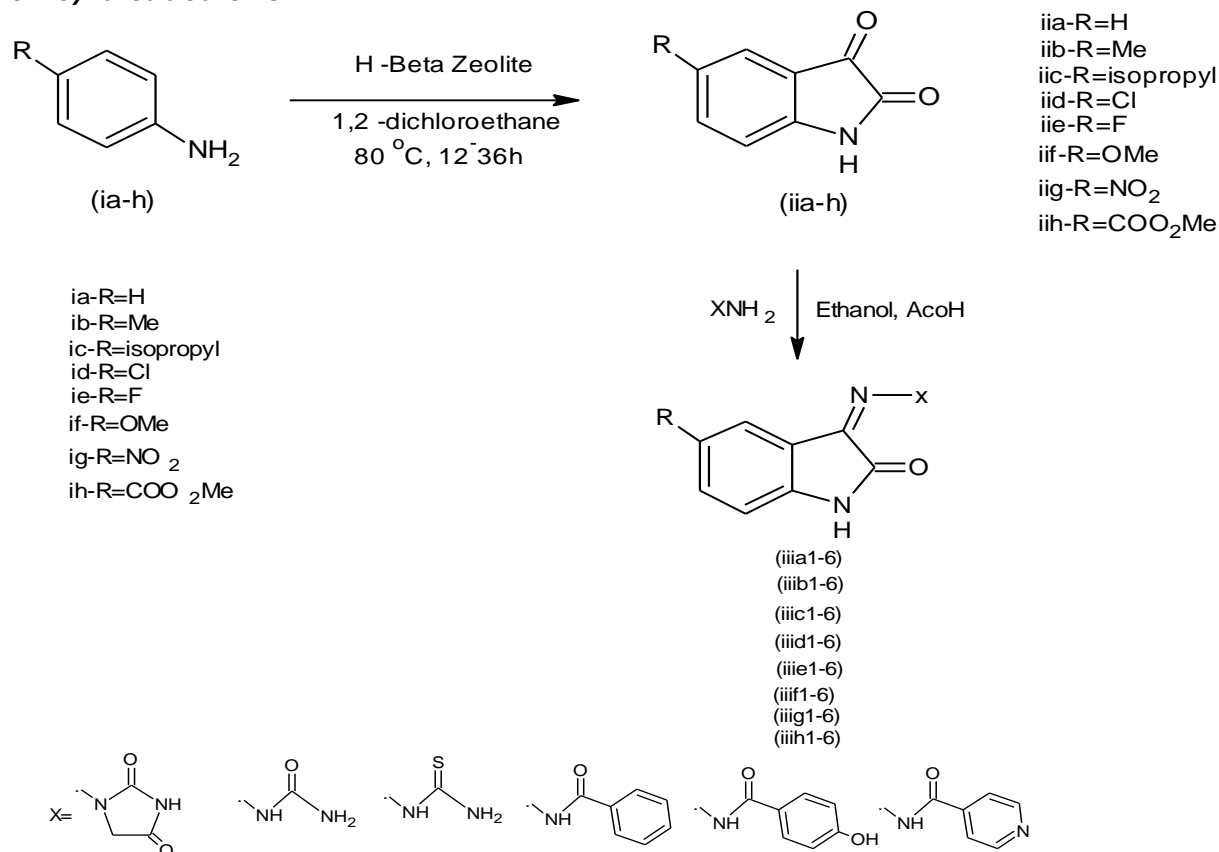


3. MATERIAL AND METHODS:

3.1 Chemical and reagents:

All chemicals were provided from our college. All solvents were redistilled before use. Reactions were routinely monitored by thin layer chromatography and spots were visualized by exposure to iodine vapour or UV light. All the synthesized compounds were purified by recrystallization. Melting points were determined by using open capillary method.

3.2 synthetic Scheme:

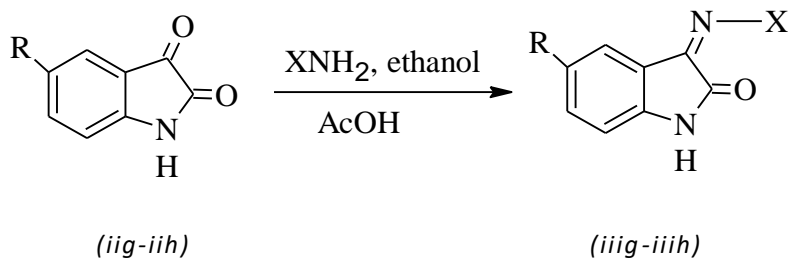


3.3 Instrumentation:

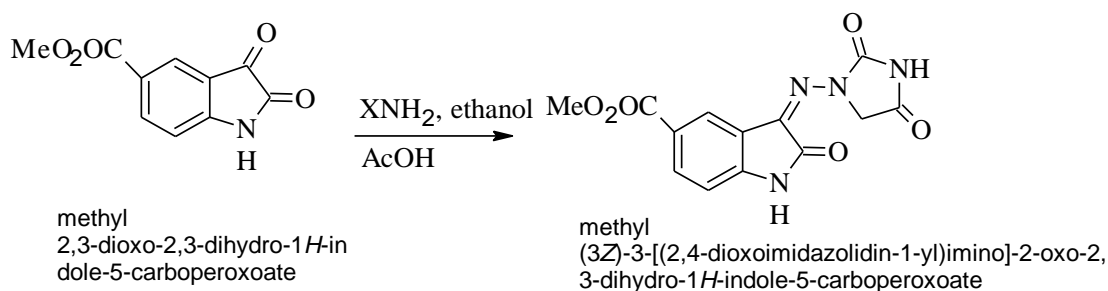
Spectroscopic data were recorded with following instrument; Fourier Transform Infra-Red spectra (FTIR) were recorded on Shimadzu FTIR-8400S spectrophotometer using potassium bromide pellets and sodium chloride cell. Nuclear Magnetic Resonance spectra (¹H-NMR) were recorded on JEOL-300 MHz spectrophotometer in CDCl₃ using TMS as an internal standard. Chemical shifts (δ) are expressed in parts per million (ppm). Mass spectra were recorded on HEWLETT 180017, PACKARD GCD System mass spectrophotometer using electron ionization detector. Anticonvulsant activity checked by Electroconvulsometer (LABTECH).

3.4 General procedure for the synthesis of Schiff bases:

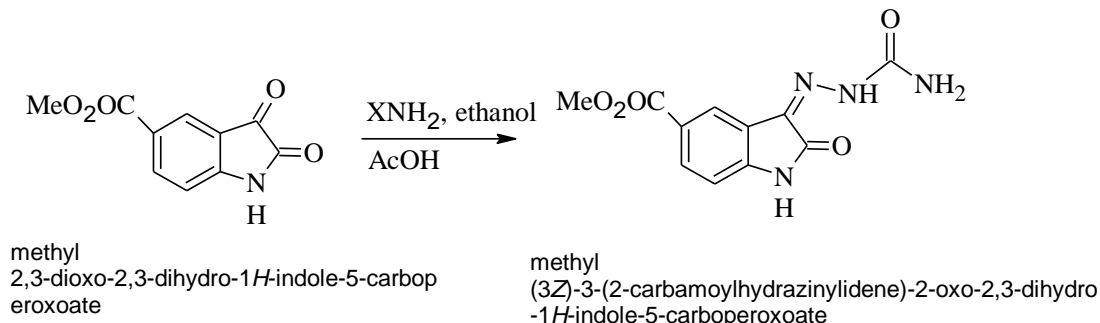
Equimolar amines and 5-Dicrboxymethyl (R=COO₂Me) substituted isatins (1 mmol of each) were added to 96% w/w ethanol (20 mL) containing 8 drops of glacial acetic acid. The mixture was heated under reflux for 5 h and then cooled to room temperature. The resulting solid was collected by filtration, washed with cold ethanol and dried in open air. The derivatives thus prepared had sufficient analytical purity.[24-29]



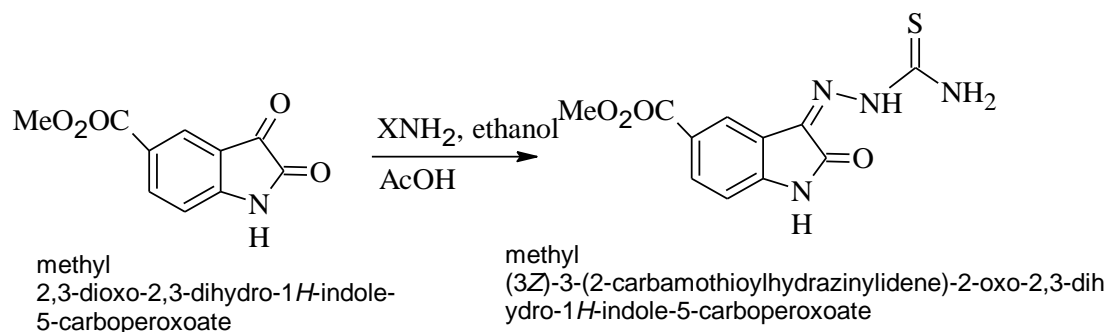
A. methyl (3Z)-3-[(2,4-dioximidazolidin-1-yl)imino]-2-oxo-2,3-dihydro-1H-indole-5-carboxperoxoate



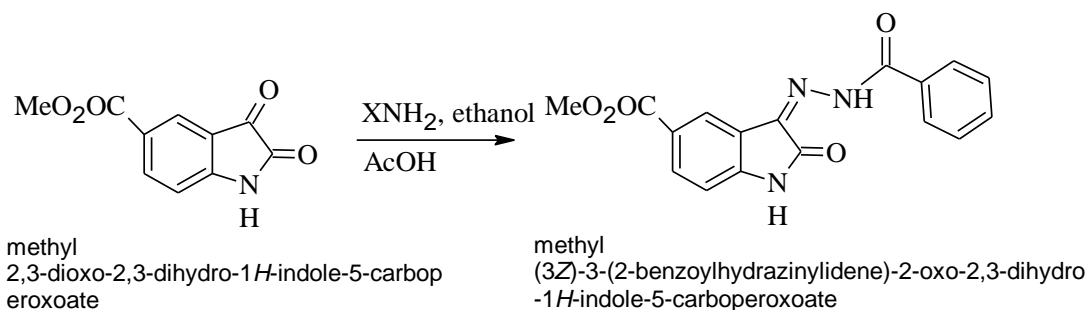
B. methyl (3*Z*)-3-(2-carbamoylhydrazinylidene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylate



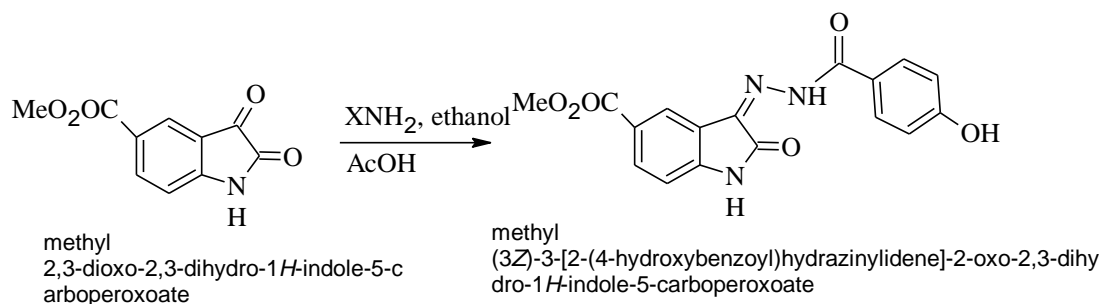
C. methyl (3*Z*)-3-(2-carbamothioylhydrazinylidene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylate



D. methyl (3*Z*)-3-(2-benzoylhydrazinylidene)-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylate



E. methyl (3*Z*)-3-[2-(4-hydroxybenzoyl)hydrazinylidene]-2-oxo-2,3-dihydro-1*H*-indole-5-carboxylate



F. methyl (3Z)-2-oxo-3-[2-(pyridin-4-ylcarbonyl)hydrazinylidene]-2,3-dihydro-1H-indole-5-carboxylate

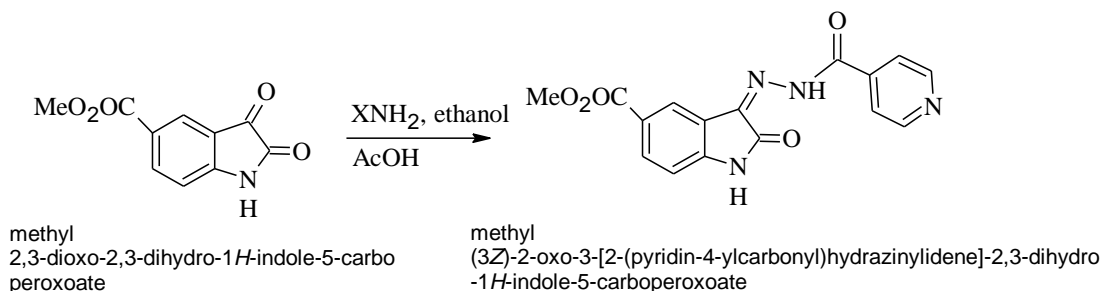


Table 1: physicochemical data of synthesized compound (iiih1-iiih6)

Compound	% yield	R _f value	Mol. formula	Mol. Mass
iiih1	58.12	0.63	C ₁₃ H ₁₀ N ₄ O ₆	318.24
iiih2	69.89	0.72	C ₁₁ H ₁₀ N ₄ O ₅	278.22
iiih3	57.66	0.84	C ₁₁ H ₁₀ N ₄ O ₄ S	294.28
iiih4	86.30	0.51	C ₁₇ H ₁₃ N ₃ O ₅	339.30
iiih5	78.63	0.90	C ₁₇ H ₁₃ N ₃ O ₆	355.30
iiih6	63.51	0.79	C ₁₆ H ₁₂ N ₄ O ₅	340.29

4. ANTI-CONVULSANT ACTIVITY:

All antiepileptic drugs (AEDs) are rigorously study in animals, particularly rodents, before they are given to patients. Understanding how drugs are screened in animals is useful to the clinician, since the screening process is valuable in predicting the type of seizure in which the drug would be efficacious, as well as determining the mechanism of the drug's anti-seizure action. Indeed, the dramatic discovery of the anti-seizure properties of phenytoin was identified by Merritt

and Putnam in 1938 using the electroshock-induced seizure model.[30-31]

4.2 Animals:

Animals Albino mice, weighing 18-30 g, were used for experiments. The animals were kept in colony cages (6 mice each), maintained on a standard pellet diet with water, and left for 2 days for acclimatization before the experimental session. The food was withdrawn on the day before the experiment, but free access to water was

allowed. All experiments were carried out according to the suggested ethical guidelines for the care of laboratory animals.[32]

4.3 Selection of experimental animals:

Healthy Albino wistar male rats weighing between 18-30 g were used for the evaluation of anti-convulsant activity.

4.4 Laboratory conditions:

The rats were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top. Environmental room should be 22°C (± 3°C) relative humidity was kept at least 30 % and preferably not exceed 70 % other than during room cleaning the aim was maintained between 50-60%. Lighting was artificial, the sequence being 12 hours light and 12 hours.

4.5 Food and water: All animals were allowed free access to water and standard palletized laboratory animal diet.

4.6 Bedding: In the present study animals were provided with clean paddy husk bedding. Bedding was changed every alternate day to maintain proper hygienic conditions.

The anticonvulsant activity was carried out by maximal electrical shock induced convulsion method in albino mice.

Animals to be use: - Albino mice

No. of animals used per group:-6 mice

Dose of test compound:-0.5ml/100g

Dose of standard drug:-0.5ml/100 (Phenytoin)

Route of administration:-Intra peritoneal (suspended in 1% tween-80 solution)

4.7 Requirements:-

Instruments: - Electroconvulsimeter

Chemicals:-Tween-80

Standard drug: - Phenytoin (25 mg/kg) aqueous suspension was prepared using solution of tween-80 as a suspending agent.

4.8 Test compounds:-suspension of compounds were prepared and administered intra peritoneal similar to that of standard drug. Apparatus: -Syringes (1 ml, 2 ml), sample tubes.

4.9 Experimental design and procedure:

Animals were weighed and numbered. Mice were divided into 7 groups of six animals each. Group 1 served as control which was treated with vehicle (2% v/v Tween 80), group 2 was treated with standard drug

phenytoin (25 mg/kg, i.p.) and groups 3– 7 were treated with newly synthesized oxadiazole derivatives (25 mg/kg, i. p.). One hour after injection, the animals were subjected to electro shock through ear electrodes of 80 mA for 0.2 sec by electroconvulsimeter and the duration of time for extensor response was noted and the activity was expressed in terms of % protection.[33]

All the results are expressed as mean ± SEM. The % inhibition of epileptic seizures was calculated by using the formula,

$$\text{Percent (\%)} \text{ protection} = \frac{VC - VT}{VC} \times 100,$$

Where, VT- Mean time in test group, VC-Mean time in control group.

5. ANTI-INFLAMMATORY ACTIVITY

5.1 Animals

For the biological evaluation Albino wistar rats, (200-300 g), were used. The animals were kept in colony cages (6 rats each), maintained on a standard pellet diet with water, and left for 2 days for acclimatization before the experimental session. They kept on fast for 16 hour before the experiment, but free access to water. Experiments were carried out according to the ethical guidelines for the care of laboratory animals.

5.2 Selection of experimental animals:

Healthy Albino wistar male rats weighing between 200-300 g. were used for the evaluation of anti-inflammatory activity. The animals were obtained from Zydu research centre, Ahmedabad.

5.3 Laboratory conditions:

The rats were housed comfortably in a group of six in a single clean plastic cage with a metal frame lid on its top. Environmental room should be 22°C (± 3°C) relative humidity was at least 30 % and preferably not exceed 70 % other than during room cleaning the aim was to maintain between 50-60%. Lighting was to be artificial, the sequence being 12 hours light and 12 hours.

5.4 Anti-inflammatory activity:

The anti-inflammatory activity of synthesized indole derivatives were carried out using carrageenan induced rat hind paw edema method.

Method: - carrageenan induced paw edema.

Animals used:-Albino wistar rats

No. of animals used per group:-6 rats

Dose of test compound:-3 mg/kg

Dose of standard drug:-3 mg/kg (Indomethacin)

Route of administration:-Intra peritoneal (suspended in 1% tween-80 solution)

5.5 Requirements:

Instruments:-Mercury displacement plethysmometer.

Inflammation inducing agent:-carrageenan solution (1%w/v) in saline solution was prepared and injected (0.1ml) in sub planter region to induce paw edema.

Chemicals:-Tween-80

Standard drug:-Indomethacin (3 mg/kg) aqueous suspension was prepared using solution of tween-80 as a suspending agent.

Test compounds:-suspension of compounds were prepared and administered intra peritoneal similar to that of standard drug. [34-36]

Apparatus: -Syringes (1 ml, 2 ml), sample tubes (to prepare suspension of test compounds).

5.6 Experimental design and procedure:

Weigh the animals and number them. Mark the animals with picric acid for individual animal identification. Divide rats into 5 groups of 6 rats each. Note the initial paw volume of each rat by dipping just beyond tibio-tarsal junction by mercury displacement method, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. The animals were deprived of food overnight (allowed free access to water) and synthetic compounds were administered once before 30 minutes the injection of carrageenan. Dose volume not exceeding 0.5ml/100gm intra peritoneal was administered.

Group I:-The solvent control received normal saline.

Group II:-Positive control received Indomethacin (3 mg/kg).

Group III:-Received indole derivative-4.7 at a dose of 3 mg/kg suspended in 1%w/v tween-80

Group IV:-Received indole derivative-4.8 at a dose of 3 mg/kg suspended in 1%w/v. tween-80

Group V:-Received indole derivative-4.9 at a dose of 3 mg/kg suspended in 1%w/v tween-80.

Group VI:-Received indole derivative-4.10 at a dose of 3 mg/kg suspended in 1%w/v tween-80.

After 30 minutes of test compound administration, 0.1ml of 1%w/v of carrageenan in normal saline was injected in to the sub planter region of the left hind paw of rat. Immediately after the carrageenan injection, the volume of its displacement was measured using plethysmometer.[37-39]. The reading was recorded at 0, ½, 1, 2, 3 hrs.

The % inhibition of edema was calculated at the end of 3 hrs by using the formula.^[30]

Percent (%) inhibition = $1 - V_t/V_c \times 100$,

Where V_t : - edema volume in test group,

V_c : -edema volume in control group

Results were expressed as mean \pm standard deviation.

6. RESULT AND DISCUSSION:

6.1 Screening of Anti-Convulsant Activity:

Table 2: Screening of Anti-convulsant activity in Albino Mice (By Maximal electro shock method)

Compound Code	Duration (Mean \pm SEM,Sec, sec)					% Protection
	Flexion	Extension	Clonus	Stupor	Recovery	
Control	12.75 \pm 0.3	15.85 \pm 0.23	27.50 \pm 0.19	96.0 \pm 0.09	Recovered	
Standard (Phenytoin)	9.80 \pm 0.08	11.83 \pm 0.19	3.07 \pm 0.05	1.91 \pm 0.03	Recovered	83.95 %
Comp-iiih1	9.5 \pm 0.12	11.5 \pm 0.13	5.5 \pm 0.06	20.4 \pm 0.10	Recovered	56.2%
Comp- iiih2	9.0 \pm 0.08	11.0 \pm 0.10	6.0 \pm 0.12	19.7 \pm 0.01	Recovered	69.0%
Comp- iiih3	10.2 \pm 0.05	14.4 \pm 0.01	15.0 \pm 0.05	21.5 \pm 0.11	Recovered	65.22%
Comp- iiih4	9.2\pm0.15	10.4\pm0.12	6.1\pm0.03	18.1\pm0.01	Recovered	73.3%
Comp- iiih5	10.0 \pm 0.09	12.0 \pm 0.15	5.0 \pm 0.14	26.7 \pm 0.01	Recovered	61.4%
Comp- iiih6	12.1 \pm 0.10	14.6 \pm 0.25	16.5 \pm 0.10	22.5 \pm 0.01	Recovered	63.66%

6.2 Screening of Anti-Inflammatory Activity:

Table 3: Screening of Anti-inflammatory activity in Albino wistar rat

Compound code	Inhibition of inflammation in cm					% inhibition			
	0 hr	1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Control	0.36±0.02	0.33±0.02	0.31±0.02	0.30±0.02	0.29±0.02				
Standard (Indome thacin)	0.31±0.02	0.27±0.02	0.17±0.02	0.14±0.09	0.11±0.009	18.18	16.12	53.33	62.06
Comp-iiih1	0.31±0.02	0.34±0.02	0.29±0.02	0.19±0.005	0.15±0.02	23.11	26.15	46.55	49.55
Comp- iiih2	0.31±0.02	0.34±0.02	0.25±0.01	0.16±0.004	0.15±0.02	18.20	20.14	38.16	46.55
Comp- iiih3	0.33±0.02	0.34±0.02	0.27±0.01	0.15±0.007	0.12±0.02	18.18	29.53	56.66	61.17
Comp- iiih4	0.31±0.02	0.32±0.02	0.25±0.01	0.15±0.002	0.14±0.02	15.06	19.21	29.60	42.82
Comp- iiih5	0.32±0.02	0.33±0.02	0.26±0.01	0.17±0.004	0.14±0.02	15.19	19.26	30.87	40.55
Comp- iiih6	0.33±0.02	0.33±0.02	0.28±0.01	0.18±0.003	0.14±0.02	19.38	26.45	53.92	52.16

No. of animals used in each Group (n) = 6, Values are expressed as Mean ± SEM Dose of test compound = 3 mg/kg, Dose of Indomethacin = 3 mg/kg

7. CONCLUSION:

The pharmacological screening of the synthesized compounds showed anti convulsant activity ranging from 56.2 % to 76.3 % inhibition of epileptic seizures in mice, where as the standard drug Phenytoin showed 83.95 % inhibition of epileptic seizures in mice. The compound **iiih4** from each group was found to be nearly potent to Phenytoin which is used as standard drug. Structure resembles in above compound have significant to standard drugs were structure having substitute isatin ring with **penta-cyclic ring with amine group** give better anti-convulsant activity.

The pharmacological screening of the synthesized compounds showed anti-inflammatory activity ranging from 31.09 to 63.11 % inhibition of rat paw edema volume after 3 hours, whereas the standard drug Indomethacin showed 62.06 % inhibition of rat paw edema volume after 4 hours. The compound **iiih3** was found to be nearly more potent then indomethacin which is used as standard drug. Except then above mention compound has shown less activity then indomethacin. **iiih3** compound was possess **5-methoxy (-OCH₃) with 4-hydroxy benzahydrazide** responsible for anti-inflammatory activity.

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