

Screening of the Anticonvulsant Activity of Some Isatin Derivatives in Experimental Seizure Models and Its Effect on Brain GABA Levels in Mice

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Abstract The modern therapeutic approaches of antiepileptic agents against epileptic patients are showing many side effects, dose related effects and chronic toxicity. The aim of our present work is to evaluate anticonvulsant activity of isatin derivatives (**Ia-Ij**) to reduce the side-effects and increase the percentage protection from various stages of convulsions. No animals showed toxic effects even up to 2000mg/kg. It is evident that **Ib, Ie, Ih, Ii** and **Ij** showed anticonvulsant effect at the dose levels of 10 and 100mg/kg in MES test and except **Ij** all other derivatives exhibited antiepileptic effect at 10 and 100mg/kg in PTZ induced convulsions test. The isatin derivatives (**Ib, Ie, Ih** and **Ii**) which proved antiepileptic effect in both MES and PTZ induced convulsion models are selected and GABA levels in brain were estimated. They showed significant increase of GABA levels in brain.

Keywords: *isatin, anticonvulsant activity, pentylene tetrazole, maximum electric shock, GABA*

1. Introduction

Epilepsy is the most common neurological disorder and affects around 50 million people worldwide [1] and it is characterized by recurrent unprovoked seizures. Seizures are induced mainly due to imbalance between inhibitory and excitatory neurotransmitters [2]. They are characterized by uncontrolled involuntary shaking which may be partial seizures involving only one part of the body or generalized seizures involving entire body and may accompanied by loss of consciousness. There are twice as many people living with epilepsy in low and lower-middle income countries than higher income nations and more than 60 per cent of those affected in these regions are not accessed with any appropriate treatment [3]. The incidence rate of epilepsy in the developed and developing countries approximately ranges from 25-50 and 30-115 per one lakh people per year respectively [4]. The prevalence of epilepsy in the developed countries ranges from 4-10 and 14-57 per thousand people in developing countries per year respectively [5]. Antiepileptic drugs are effective in controlling seizures in about 70-80% of patients, but their use is often limited by side effects. In addition, these are used to treat or prevent convulsions induced by other brain diseases like trauma, brain infections, brain tumours and cerebral infarction [2]. The treatment of seizures is always a challenge for clinical practitioners and researchers to avoid the therapeutic failure in 20-25% of patients. This stimulated an intensive research to develop novel antiepileptic drugs.

Isatin has an indole moiety which exhibits variety of biological activities like anticonvulsant [6], anxiolytic [7], anticancer [8], anti-inflammatory, analgesic, antipyretic [9], antifungal [10], antiviral [11], anti-angiogenic [12] and anti-parkinsonian effects [13] etc.

From the above observations, the present work was carried out to synthesize some new isatin-3-[N²-(2-benzalaminothiazol-4-yl)] hydrazones and these are screened for anticonvulsant activity.

2. Materials and Methods

Chemicals

Diazepam (Ranbaxy Laboratories, India), Phenytoin (Sun Pharmaceuticals India. Ltd, India). Pentylene tetrazole, (PTZ) (Sigma-Aldrich, India). Isatin derivatives (the structure and physical properties of **Ia-Ij** are addressed in Table 1).

Animals

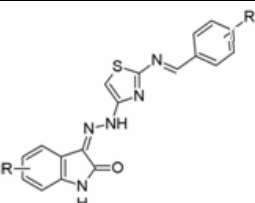
Swiss albino mice (20-25gm) were selected and procured from Mahaveer enterprises, Hyderabad, India. They were acclimatized for one week under standard laboratory conditions (25±2 °C, relative humidity of 45 to 55% and 12:12 hr light and dark cycle) and fed with standard rodent pellet diet and water *ad libitum*. Each group contains six animals and are used for the mentioned research activities. The study protocol was approved by Institutional Animal Ethics Committee (IAEC NO: 1047/ac/07/CPCSEA).

2.1. Acute Toxicity Studies

Healthy adult female Swiss albino mice were fasted overnight and test compounds in 0.1% sodium carboxy methyl cellulose (CMC) were administered *i.p.*, in doses up to 2000mg/kg. The control group received only vehicle

(0.1% CMC). The animals were observed for one month from the time of administration of test compounds and mortality was recorded [14].

Table 1. Effect of isatin-3-[N2-(2-benzalaminothiazol-4-yl)] hydrazones on PTZ induced convulsions



Treatment	R	R ¹	Dose (mg/kg)	Latency (sec/0.5hr)	Onset of tonic convulsion (sec/ 0.5hr)	Status of animal after 0.5hr		Status of animal after 24 hrs	
						No of alive animals	% protection	No of alive animals	% protection
Control	---	---	----	48.26 ± 5.62	412.32 ± 4.84	0/6	0.00	0/6	0.00
Diazepam	---	---	5	No clonus	No tonic	6/6	100.00	6/6	100.00
Ia	H	H	10	56.80 ± 2.36*	418.44 ± 2.75*	1/6	16.67	1/6	16.67
			100	175.68 ± 3.28***	789.66 ± 1.46***	2/6	33.33	2/6	33.33
Ib	H	4-Cl	10	56.04 ± 4.65*	418.52 ± 2.65*	2/6	33.33	2/6	33.33
			100	200.56 ± 5.42***	915.43 ± 3.52***	4/6	66.66	4/6	66.66
Ic	H	4-N(CH ₃) ₂	10	58.14 ± 1.98*	417.23 ± 3.65*	1/6	16.67	1/6	16.67
			100	175.54 ± 2.68***	800.45 ± 2.98***	3/6	50.00	3/6	50.00
Id	H	4-OH, 3-OCH ₃	10	56.90 ± 6.34*	418.84 ± 2.79*	1/6	16.67	1/6	16.67
			100	175.84 ± 5.83***	810.66 ± 1.86***	2/6	33.33	2/6	33.33
Ie	5-CH ₃	4-Cl	10	54.34 ± 4.56*	418.74 ± 1.94*	2/6	33.33	2/6	33.33
			100	210.34 ± 1.98***	923.48 ± 2.93***	3/6	50.00	3/6	50.00
If	5-CH ₃	4-OH, 3-OCH ₃	10	56.79 ± 6.14*	417.62 ± 3.56*	1/6	16.67	1/6	16.67
			100	185.84 ± 5.33***	795.34 ± 2.57***	3/6	50.00	3/6	50.00
Ig	5-CH ₃	H	10	56.39 ± 6.84*	418.02 ± 2.86*	1/6	16.67	1/6	16.67
			100	193.84 ± 5.63***	850.43 ± 1.99***	3/6	50.00	3/6	50.00
Ih	5-Cl	4-OH, 3-OCH ₃	10	55.34 ± 2.64*	418.35 ± 3.68*	3/6	50.00	3/6	50.00
			100	230.28 ± 3.85***	930.45 ± 2.56***	5/6	83.33	5/6	83.33
Ii	5-Cl	4-Cl	10	54.02 ± 2.46*	417.77 ± 5.84*	03/6	50.00	03/6	50.00
			100	286.12 ± 4.28***	945.65 ± 3.62***	05/6	83.33	05/6	83.33
Ij	5-NO ₂	4-OH, 3-OCH ₃	10	49.23 ± 2.56	414.34 ± 2.45	0/6	0.00	0/6	0.00
			100	100.34 ± 2.63	518.56 ± 2.26	0/6	0.00	0/6	0.00

% protection= no of animals alive/no of animals used x 100; Values are Mean ± SD, n=6 in each group. *P<0.05, **P<0.01, ***P<0.001 when compared with control group (Newman-Keuls multiple comparison test)

2.2. Anticonvulsant Activity Against PTZ Induced Seizures

Animals were assigned into three groups viz., control, standard and test groups. The control group received 0.1% CMC *i.p.*, standard group animals were given diazepam 5mg/kg *i.p.* and test groups were injected with isatin derivatives (Ia-Ij) at dose levels of 10 and 100mg/kg *i.p.* One hour after the administration of these substances to the respective groups, all the animals of assigned groups were administered with PTZ (80mg/kg; *s.c.*) [15]. Each

animal was observed up to 0.5 hr for onset of clonus, tonic convulsions and status of animals were also recorded. After 24hrs the percentage protection of animals was calculated [16].

2.3. Anticonvulsant Activity Against MES Induced Seizures

Animals were checked for sensitivity to electric shock 24hr prior to administration of test compounds and those animals which fail to show hind limb tonic extension were rejected. Animals were assigned into three groups viz.,

control, standard and test groups. The control group received 0.1% CMC *i.p.*, standard group animals were given phenytoin 25mg/kg *i.p.* [17] and test groups were injected with isatin derivatives (**Ia-Ij**) at dose levels of 10 and 100mg/kg *i.p.* A 60Hz alternating current of 150mA intensity for 0.2 sec using corneal electrodes elicited maximal seizures. A drop of lignocaine was applied to the corneal electrodes which ensure better contact to the animal [18,19]. This current intensity elicited complete tonic extension of the hind limbs in control group. A clear rectangular polypropylene cage with an open top which permits full view of the animal to observe various phases of convulsions like tonic, flexion, extension, stupor and mortality due to convulsions was used.

2.4. Estimation of Brain GABA Content

Following the methods of Lowe *et al.*, the GABA contents in the brains were estimated. Animals were scarified by decapitation, the brains were exposed and separated the forebrain region. It was blotted, weighed and placed in a beaker containing 5ml of ice cold TCA (10%w/v) and homogenised. The homogenated brain was centrifuged at 10,000rpm for 10 min at 0 °C. Then 0.1ml of supernatant was dissolved in 0.2ml of ninhydrin solution (0.15M) in a 0.5M of carbonate-bicarbonate buffer (0.5, pH 9.95). This mixture is kept in a water bath for 30min maintaining 60 °C and then cooled. The cooled mixture is treated with 5ml of copper tartarate reagent and kept aside for 10min. Fluorescence was observed at 377/455 nm by using spectrofluorimeter [20].

Statistical analysis

Results were expressed as Mean±SD. Statistical significance was calculated by applying one way ANOVA. P < 0.05 was considered as significant (Newman-Keuls multiple comparison test).

3. Results

3.1. Acute Toxicity Studies

The compounds used in our work were found to be safe and no toxic effects were seen even at higher doses of 2000mg/kg. Some behavioural changes were recorded but no toxic symptoms.

3.2. Anticonvulsant Activity

All the isatin derivatives (**Ia-Ij**) were evaluated for their anticonvulsant effect by performing MES and PTZ activities are most widely accepted models and the compounds are screened at two dose levels 10 and 100mg/kg. The output was given in Table 1 and Table 2. In MES model, the compounds **Ib**, **Ie**, **Ih**, **Ii** and **Ij** showed significant reduction in various phases of epileptic seizures when compared with control. In PTZ test, the compounds **Ia**, **Ib**, **Ic**, **Id**, **Ie**, **If**, **Ig**, **Ih** and **Ii** exhibited a significant anticonvulsant activity by increasing both latency time (onset of clonus) and onset of tonic convulsions. All the compounds exhibited anticonvulsant activity in dose dependent manner.

Table 2. Effect of isatin-3-[N2-(2-benzalaminthiazol-4-yl)] hydrazones on MES induced convulsions

Treatment	Dose (mg/kg)	Various phases of convulsions (time in sec)				% Protection
		Flexion	Extensor	Colonus	Stuper	
Control	0.1%Na CMC	8.30±0.35	15.24±0.40	15.32±0.32	9.25±0.30	NP
Phenytoin	25	3.25±0.15***	00±00***	8.32±0.34***	3.56±0.15***	100
Ia	10	NA	NA	NA	NA	NP
	100	NA	NA	NA	NA	NP
Ib	10	7.87±0.45*	14.33±0.54*	14.75±0.22*	8.38±0.42*	33.33
	100	6.22±0.41***	10.44±0.32***	9.95±0.43***	6.71±0.47***	50.00
Ic	10	NA	NA	NA	NA	NP
	100	NA	NA	NA	NA	NP
Id	10	NA	NA	NA	NA	NP
	100	NA	NA	NA	NA	NP
Ie	10	7.67±0.35*	14.47±0.56*	14.82±0.32*	8.49±0.52*	33.33
	100	5.62±0.21***	6.62±0.36***	9.73±0.33***	6.64±0.77***	50.00
If	10	NA	NA	NA	NA	NP
	100	NA	NA	NA	NA	NP
Ig	10	NA	NA	NA	NA	NP
	100	NA	NA	NA	NA	NP
Ih	10	7.69±0.38*	14.36±0.52*	14.73±0.12*	8.43±0.62*	50.00
	100	5.22±0.25***	6.12±0.32***	9.46±0.23***	6.26±0.67***	83.33
Ii	10	7.73±0.16*	14.34±0.93*	14.67±0.21*	8.48±0.12*	50.00
	100	4.32±0.24***	5.12±0.26***	9.12±0.56***	5.65±0.31***	83.33
Ij	10	7.68±0.43*	14.72±0.33*	14.91±0.25*	8.79 ±0.22*	33.33
	100	7.59±0.22*	14.63±0.32*	14.86±0.23*	8.63±0.41*	50.00

NA= No activity; NP= No protection at 10,100 mg/kg dose; Values are Mean±SD, n=6 in each group. *P<0.05, **P<0.01, ***P<0.001 when compared with control group (Newman-Keuls multiple comparison test)

3.3. Estimation of Brain GABA Content

For estimation of GABA levels in brain of screened animals, test compounds **Ib**, **Ie**, **Ih** and **Ii** were selected

which showed significant anticonvulsant activity in both models. In MES and PTZ models, GABA levels were significantly ($p < 0.001$) decreased in forebrain of epileptic control animals. The compounds **Ib**, **Ie**, **Ih** and **Ii** at the doses of 10 and 100mg/kg, standard drugs

Phenytoin and diazepam treated animals showed a significant ($p < 0.05$ & $p < 0.001$) increase in GABA levels in forebrain of animals. The results of estimation of GABA content in brain are tabulated in Table 3.

Table 3. Effect of isatin derivatives on GABA levels in rat brain after MES and PTZ induced convulsion

Treatment	Dose (mg/kg body wt)	GABA Levels	
		MES	PTZ
Control	---	278.34 ± 1.34	288.32 ± 1.81
Disease control	---	230.12 ± 2.12***	200.14 ± 1.26***
Phenytoin	25	273.32 ± 1.15***	---
Diazepam	5	---	285.22 ± 1.54***
Ib	10	232.50 ± 2.23*	202.23 ± 1.92*
	100	266.43 ± 1.62***	258.21 ± 1.32***
Ie	10	233.00 ± 2.53*	202.53 ± 1.24*
	100	269.41 ± 1.92***	265.11 ± 1.13***
Ih	10	234.20 ± 2.13**	203.12 ± 1.51**
	100	272.63 ± 1.32***	275.18 ± 1.72***
Ii	10	234.50 ± 2.33**	203.53 ± 1.45**
	100	272.42 ± 1.12***	279.28 ± 1.42***

Values are Mean ± SD, n=6 in each group. *P<0.05, **P<0.01, ***P<0.001 when compared with disease control group (Newman-Keuls multiple comparison test). Units = pg/mg of wet tissue

4. Discussion

In the present investigation, it is evident that isatin derivatives were able to reduce the epileptic seizures induced by MES and PTZ in the laboratory animals. From the literature, GABAergic transmission is closely associated with the induction of epilepsy in animals [21]. In central nervous system, GABA is the major inhibitory neurotransmitter (NT) and slight deficiency in this transmitter may lead to hyper excitability and pathological neuronal discharge leading to epilepsy. In most of the antiepileptic drugs an enhancement of GABAergic inhibitory transmission is responsible which directly bind, activate GABA_A receptors or influence GABA release, transport and metabolize. GABA mediated chlorine channel opening is closely associated with induction and onset of seizures [22]. PTZ is non-competitive antagonist which blocks GABA mediated Cl influx and leads to enhanced release of excitatory neurons to release NTs such as α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and N-methyl-D-aspartic acid (NMDA). This results in neuronal excitotoxicity leading to seizures and convulsions characterized by clonic and tonic phases. The substitutions of different substituent's (H, CH₃, NO₂ and Cl) at 5th position in the isatin ring significantly shows the anticonvulsant activity [23]. Verma *et al.*, identified 5-Br isatin as anticonvulsant agent, here we replaced Br with Cl because of its bioisosterism [24]. In our investigation, the compounds having mild deactivator like chloride at the 5th position of isatin ring (Ih and Ii) shows a good anticonvulsant activity against

MES and PTZ test without any toxicity. Substitution of strong deactivator like nitro group at 5th position of isatin ring, showed blockade of PTZ induced convulsions [25] but in the present investigation the nitro derivative (Ij) exhibited less potency due to other peripheral part of molecule. In this series, the isatin ring substituted with electron donating and mild deactivating substituents shown potency but the strong deactivating group exhibited less potency.

The 5-methyl substituent's with the least negative inductive effect on the isatin ring was found to yield more active compounds [23], Ie, If and Ig also exhibited anticonvulsant activity due to the introduction of hydrophobic group i.e., increased lipophilic nature. Among all the compounds, Ie showed anticonvulsant activity in MES model because of the presence of methyl group on isatin and chlorine substitution in the phenyl ring at 4th position where as the unsubstituted (Ig) and substituted with strong electron donating group like OH and OCH₃ on phenyl ring (If) showed against to PTZ model. Unsubstituted isatin derivatives (Ia-Ic) showed anticonvulsant activity but the phenyl ring of side chain has strong electron donating group (N(CH₃)₂, OH) is less potent when compared with phenyl ring substituted with weak electron donating group (Cl; Ib) at 4th position against both models but remaining three compounds were active against only PTZ model and Pandeya *et al* also observed same kind of effect [25].

In present study, the established antiepileptic drugs such as phenytoin and diazepam restored the GABA levels in brain. Similarly in our investigation isatin derivatives (Ib, Ie, Ih and Ii) significantly ($p < 0.05$, $p < 0.01$ and $p < 0.001$) increased GABA levels in forebrain of animals.

5. Conclusion

In our investigation GABA is involved in both MES and PTZ induced convulsion models. Our findings support the hypothesis that decrease in GABA levels of mice brain after induction of convulsions. The animals treated with isatin derivatives (Ib, Ie, Ih and Ii) significantly restore the GABA levels in brain. We conclude that our isatin derivatives possess antiepileptic activity by restoring the GABA levels in mice brain.

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