Synthesis and evaluation of neuropharmacological profile of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones

Venkateshwar Rao Jupally[a], Venkateshwarlu Eggadi[b,c], Sharavana Bhava Bandaru Sheshagiri[b], Umasankar Kulandaivelu[b]

[a]Department of Pharmaceutical Chemistry, Talla Padmavathi College of Pharmacy, Urus, Warangal, [b]Neuropharmacology Divison, Vaagdevi College of Pharmacy, Warangal and [c]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India

Correspondence to Venkateshwar Rao J., M. Pharm., PhD, Talla Padmavathi College of Pharmacy, Urus, Warangal - 506 002, India
Tel: +91 984 883 5092; fax: +91 870 246 0108; e-mail: eggadivenkey@gmail.com

Received 22 July 2014
Accepted 17 August 2014

Egyptian Pharmaceutical Journal
2015, 14:130–138

Background and objective
Isatin and its derivatives are versatile lead molecule for potential bioactive agents and are reported to possess a wide spectrum of activities such as central nervous system (CNS), antibacterial, antifungal, anticonvulsant, anti-HIV, antidepressant, anti-inflammatory, etc. In this study, we evaluated neuropharmacological profile of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazone derivatives using well-defined preclinical models.

Materials and methods
The isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazone derivatives (**Va–Vj**) were synthesized and characterized using spectral data. Neuropharmacological profile of **Va–Vj** (10 and 100 mg/kg, orally) was investigated by gross behavioural profile, hole board, locomotor activity, hypnotic activity, forced swim test, tail suspension test and rota rod test in mice. Imipramine (10 mg/kg) and diazepam (2 mg/kg) were used as standard for antidepressant and sedative as well as a hypnotic drug, respectively.

Results and conclusion
Isatin derivatives showed dose-dependent neuropharmacological action on the CNS, such as anxiolytic, sedative, hypnotic and depressant action. Among all the derivatives, halogen-substituted compounds (**Vh** and **Vi**) at 100 mg/kg showed significant ($P < 0.001$) action when compared with the control group (0.1% sodium carboxy methylcellulose (CMC)), and other substituted isatin derivatives **Va, Vb, Vc, Vd, Ve, Vf, Vg** and **Vj** also proved to possess a significant ($P < 0.05$) action at 100 mg/kg compared with the control group. From these results, we concluded that **Va–Vj** compounds possessed a wide range of CNS activities.

Keywords:
anxiolytic activity, central nervous system activities, depressant activity, isatin, muscle-relaxant activity, sedative and hypnotic activity

Egypt Pharm J 14:130–138

1687-4315

Introduction

Neurophysiology is a branch of physiology that deals with the functions of the central nervous system (CNS), and neuropharmacology deals with the drug effects on the CNS. In the early 20th century, the explanation of mental illness changed from a disease of the 'mind' to a proper brain dysfunction that adversely affects the physiological functions and leads to depression, anxiety, psychosis, sleep and neurodegenerative disorders, which are mainly because of the imbalances of neurotransmitters such as 5-HT, dopamine, GABA, noradrenaline, etc [1,2]. Globally, 5–20% of children and adolescents suffer with anxiety disorder. A high rate of comorbid depression and anxiety ranging from 15.9 to 61.9% was also documented [3]. According to an analysis of sleep problems in African and Asian countries, an estimation of 150 million adults suffer with sleep-related problems and 16.6% of the population reported insomnia with other severe sleep disturbances [4]. Hence, treatment for CNS disorders and development of molecules for these disorders played a challenging role in the research field.

Isatin is a naturally occurring indole derivative (indole-2, 3-dione) that is found in the brain, body fluids and other tissues [5]. Isatin readily crosses the blood–brain barrier, suggesting its possible action on the CNS [6], and it possesses a wide range of activities such as selective inhibitor of monoamine oxidase-B [7], antipsychotic [8], anticonvulsant [9], anxiolytic [10], sedative–hypnotic [11], apoptosis [12], antimicrobial [13], anticancer and cytotoxic [14], analgesics and anti-inflammatory [15], antidiabetic [16] and diuretic activities [17].

The present investigation was to screen our isatin derivatives on their pharmacological profiles by using well-defined preclinical models.

Materials and methods

Chemicals

Imipramine hydrochloride was purchased from Torrent Pharmaceuticals Pvt Ltd (Ahmedabad, India); diazepam was obtained as a gift sample from Ranbaxy

Laboratories (New Delhi, India); and pentobarbital sodium was procured from Sigma-Aldrich (Bangalore, India). Isatin derivatives synthesis shown in Scheme 1 and physical data are given in Table 1.

Synthesis of isatin derivatives (Va–Vj).

General procedure for synthesis of isatin hydrazones [18].

General procedure for synthesis of isatin-3-[N^2-(chloroacetyl)] hydrazones [19].

General procedure for synthesis of isatin-3-[N^2-(2-aminothiazol-4-yl)] hydrazones [20].

Synthesis of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones [9,16,17].

Animals

Male Swiss albino mice (20–25 g) were selected for this study, procured from Mahaveer Enterprises (Hyderabad, India), and were acclimatized for 1 week under standard laboratory conditions (25 ± 2°C, relative humidity of 45–55% and 12 : 12 h light and dark cycle), fed with standard rodent pellet diet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC NO: 1047/Ac/07/CPCSEA). Experiments were carried out between 9:00 and 17:00 h.

General behavioural profiles

Behavioural effects of novel isatin derivatives were studied by following the method of Taber *et al.* [21]. The experimental animals were divided into different groups (n = 6). The test compounds were administered orally at a dose of 10 and 100 mg/kg body weight and the animals were observed after 30 min of administration up to 2 h for behavioural changes. The behavioural change of the vehicle administered group was also studied. The observed parameters were food intake, lacrimation, tremors, mortality, sedation, reaction to touch and pain.

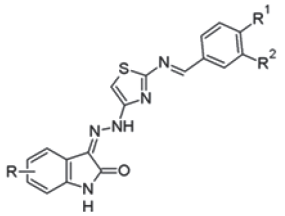
Anxiolytic activity

The anxiety level was evaluated in mice using hole board apparatus, which consists of wooden box (40 × 40 × 25 cm) with 16 holes (diameter, 3 cm) and elevated to a height of 25 cm, evenly distributed on the floor. The test was performed 60 min after administration of test substances (10 and 100 mg/kg orally), control (0.1% sodium CMC) and standard drug (diazepam 2 mg/kg, intraperitoneally). The number of head pokings during 5 min period was recorded [22]. For evaluation of anxiolytic activity, an increase in the hole poking response reveals a positive anxiolytic effect [23].

Locomotor activity

Locomotor activity was measured by digital actophotometer. Mice were divided into different groups (n = 6) and the test groups received the test substances at a dose of 10 and 100 mg/kg body weight orally The control and standard groups received 0.1% sodium CMC and diazepam (2 mg/kg intraperitoneally), respectively. Each mouse was placed individually in actophotometer for 10 min and basal reaction time was noted. Scoring was performed 60 min after administration of different doses of test substances and standard drug [24].

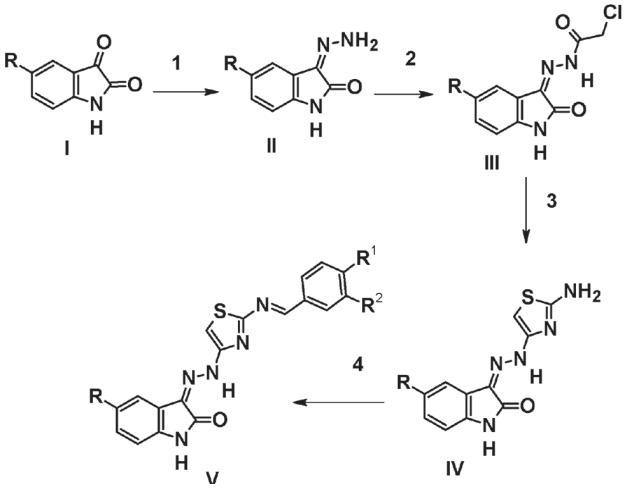
Table 1 Physical data of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones



Compound	R	R^1	R^2	MF	MW
Va	H	H	H	$C_{18}H_{13}N_5OS$	347
Vb	H	Cl	H	$C_{18}H_{12}ClN_5OS$	381
Vc	H	$N(CH_3)_2$	H	$C_{20}H_{18}N_6OS$	390
Vd	H	OH	OCH_3	$C_{19}H_{15}N_5O_3S$	393
Ve	5-CH_3	Cl	H	$C_{19}H_{14}ClN_5OS$	395
Vf	5-CH_3	OH	OCH_3	$C_{20}H_{17}N_5O_3S$	407
Vg	5-CH_3	H	H	$C_{19}H_{15}N_5OS$	361
Vh	5-Cl	OH	OCH_3	$C_{19}H_{14}ClN_5O_3S$	427
Vi	5-Cl	Cl	H	$C_{18}H_{11}Cl_2N_5OS$	416
Vj	5-NO_2	OH	OCH_3	$C_{19}H_{14}N_6O_5S$	438

MF, molecular formula; MW, molecular weight.

Scheme 1



(1) $NH_2NH_2.H_2O$ (80%), EtOH, Conc. H_2SO_4, reflux; (2) chloroacetyl chloride, EtOH, reflux; (3) thiourea, EtOH, reflux; (4) aryl aldehyde EtOH, Conc. H_2SO_4.

Hypnotic activity

The hypnotic activity was determined by pentobarbital-induced sleeping time. Mice were divided into different groups (n = 6). The test group received test substances at doses of 10 and 100 mg/kg body weight orally. The control and standard groups received 0.1% sodium CMC and diazepam (2 mg/kg intraperitoneally), respectively. After 60 min, all animals received 40 mg/kg pentobarbital (intraperitoneally). The time that elapsed between the loss and recovery of the righting reflex was recorded, both for control and for drug pretreated animals [25].

Antidepressant activity

The assessment of antidepressant activity was determined using two different models – that is, tail suspension test and forced swim test in mice.

Tail suspension test in mice

The tail suspension test (TST) is a behaviour despair model to screen antidepressant activity in rodents by decreasing the immobility measured by Steru *et al.* [26]. Animals were divided into different groups (n = 6), 60 min before testing and treated with different doses (10 and 100 mg/kg, orally) of test substances; the standard group received imipramine (10 mg/kg, intraperitoneally) and the control group received 0.1% sodium CMC. For this study, each mouse was individually suspended to the edge of a table, 50 cm above the floor, with the help of adhesive tape placed ~1 cm from the tip of the tail. The immobility was recorded for a period of 6 min. Animals were considered immobile when they hang passively and completely motionless for at least 1 min [27,28].

Forced swim test in mice

Forced swim test (FST) study was carried out according to the method of Porsolt *et al.* [29]. Animals were divided into different groups (n = 6). The animals were individually forced to swim in glass cylinders (40 cm height and 18 cm in diameter) containing fresh water to a height of 15 cm and maintained at 25°C. After 15 min, mice were removed from the cylinder and allowed to dry in heated enclosure (32°C) at least 30 min before being returned to their cages. After 24 h, 1 h before the commencement of experiment, the test compounds were administered to the test group (dose 10 and 100 mg/kg, orally), the standard group (imipramine 10 mg/kg intraperitoneally) and the control group (0.1% sodium CMC), and the animals were placed in a cylinder and the immobility was recorded for a period of 6 min. A mouse was judged to be immobile when it floated in an upright position, making small movements to keep its head above water [30].

Motor coordination

The effect on motor coordination (H. L. Scientific, Ambala, Haryana, India) was assessed using rota rod apparatus. The grouped animals (n = 6) were trained to remain for 3 min on the rod rotating at a speed of 25 rpm, for a week. After training, the animals were treated with vehicle (0.1% sodium CMC), standard drug (diazepam 2 mg/kg, intraperitoneally) and test substances (10 and 100 mg/kg orally), and their ability to remain on the rotating rod was assessed before and 30 min after the administration. The time of fall off from the rod was observed and noted for each animal [31].

Statistical analysis

Results were expressed as mean ± SD; statistical significance was calculated by applying one-way analysis of variance. P value less than 0.05 was considered as significant (Newman–Keuls multiple comparison test).

Results

Synthesis of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones

The isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones have been synthesized by the following sequence of chemical reactions. The respective isatin was reacted with 99% hydrazine hydrate to form the isatin hydrazones. The isatin-3-[N^2-(chloroacetyl)] hydrazones were prepared by a reaction of respective isatin hydrazones with chloroacetyl chloride. The condensation of chloroacetyl derivatives of isatin hydrazones with thiourea in absolute ethanol gives isatin-3-[N^2-(2-aminothiazol-4-yl)] hydrazone. The intermediate isatin-3-[N^2-(2-aminothiazol-4-yl)] hydrazone was characterized by their spectral data. ^{1}H-NMR spectrum (DMSO-d_6, δ, ppm) of the compound exhibited characteristic absorption peaks at 13.48 (s, 1H, lactam), 11.31 (s, 1H, NHCO), 6.86–7.91 (m, 4H, Ar–H), 6.84 (s, 1H, thiazole-H) and 4.85 (s, 2H, –NH_2). IR spectrum (KBr, cm^{-1}) showed absorption frequencies at 3238 (NH_2), 3158 (NH), 1688 (C = O, lactam), 1591 (C = C, aromatic) and 1551 (C = N). Mass spectrum, m/z, were: 259 (72%), 245 (100%) and 160 (4%).

Finally, the title compounds prepared by respective isatin aminothiazolyl hydrazone were condensed with different aromatic aldehydes. All the final isatin derivatives were freely soluble in DMSO, methanol and ethanol. However, intermediates and final compounds have been further purified by recrystallization from appropriate solvents(s) and characterized by their physical and spectral data. The representative compound in the series **Va** [isatin-3-[N^2-(2-benzalaminothiazol-

4-yl)] hydrazones] was characterized by their spectral data. ^{1}H-NMR spectrum (DMSO-d_6, d, ppm) exhibited peaks at 10.94 (s, 1H, lactam), 8.67 (s, 1H, NNH), 7.91–8.07 (m, 9H, Ar–H), 7.47 (s, 1H, N = CH) and 6.97 (s, 1H, thiazole-H). Its IR spectrum (KBr, cm^{-1}) showed absorption frequencies at 3432 (NH), 3180 (NH), 1728 (C = O, lactam), 1618 (C = C, aromatic) and 1545 (C = N). Mass spectrum, m/z, were: 347.7 (7%), 272.7 (100%) and 244.7 (25%).

On the basis of the above spectral data, the **Va** has been characterized and confirmed as isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones.

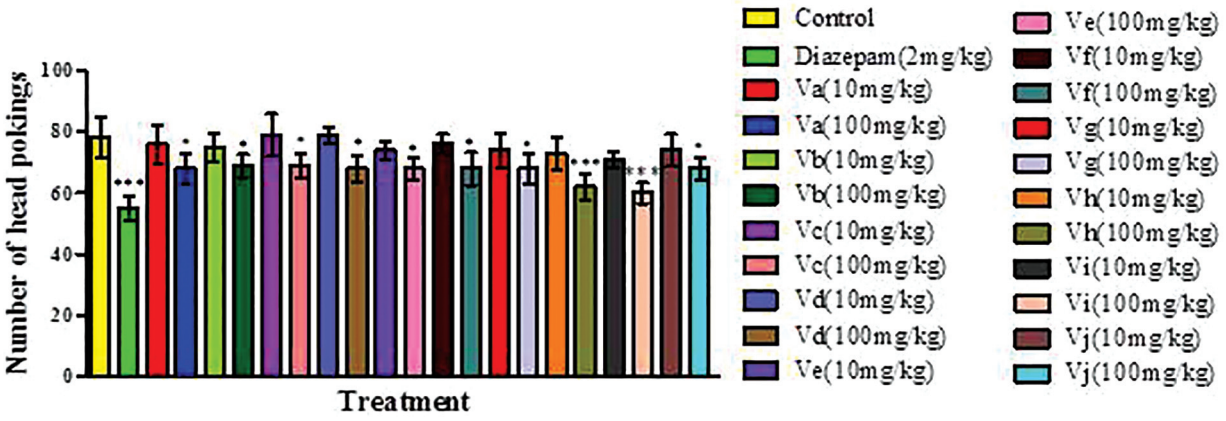
Effect on gross behavioural profile

The animals were observed for 2 h after oral administration of isatin derivatives (**Va–Vj** at a dose of 10 and 100 mg/kg), and the results obtained from the experiment are presented in Table 2. The animals treated with vehicle did not show any sedation, tremors, mortality and lacrimation of animals.

Anxiolytic activity

Anxiolytic activity of isatin derivatives was shown in Fig. 1. In the present study, isatin derivatives (**Va–Vj**) at a dose of 10 mg/kg, significantly (P < 0.001) increased

Figure 1



Number of hole poking by mice. *P < 0.05, ***P < 0.001. All data were compared with the control using one-way ANOVA followed by Newman–Keuls multiple comparison test. ANOVA, analysis of variance.

Table 2 Behavioural assessment of isatin-3-[*N*^{2}-(2-benzalaminothiazol-4-yl)] hydrazones in mice

Treatment	Dose (mg/kg)	Gross behavioural signs					
		Food intake	Lacrimation	Reactivity to touch and stimuli	Tremors	Mortality	Alertness
Control	0.1% sodium CMC	√	–	√	–	–	–
Va	10	√	–	√	–	–	–
	100	↓	–	↓	–	–	+
Vb	10	√	–	√	–	–	–
	100	↓	–	↓	–	–	+
Vc	10	√	–	√	–	–	–
	100	↓	–	↓	–	–	+
Vd	10	√	–	√	–	–	–
	100	↓	–	↓	–	–	+
Ve	10	√	–	√	–	–	–
	100	↓	–	√	–	–	+
Vf	10	√	–	√	–	–	–
	100	↓	–	√	–	–	+
Vg	10	√	–	√	–	–	–
	100	↓	–	√	–	–	+
Vh	10	√	–	√	–	–	–
	100	↓	–	↓	–	–	+
Vi	10	√	–	√	–	–	–
	100	↓	–	↓	–	–	+
Vj	10	√	–	√	–	–	–
	100	↓	–	√	–	–	+

–, none; √, normal; ↓, decreased; **+**, respond mice were pretreated orally with isatin derivatives and behavioural profile was observed for 2 h.

the number of head poking in mice and at 100 mg/kg significantly ($P < 0.001$) decreased the number of head poking, when compared with normal control. The standard drug diazepam significantly ($P < 0.001$) decreases the number of head pokings. From the above results, it was concluded that the isatin derivatives at low doses anxiogenic and at high doses showing anxiolytic property.

Locomotor activity

Isatin derivatives at 100 mg/kg dose and standard diazepam (2 mg/kg) significantly ($P < 0.05$ and $P < 0.001$, respectively) reduced the locomotor activity when compared with the control group (Table 3). At 10 mg/kg dose, isatin derivative does not show any reduction in locomotor activity.

Hypnotic activity

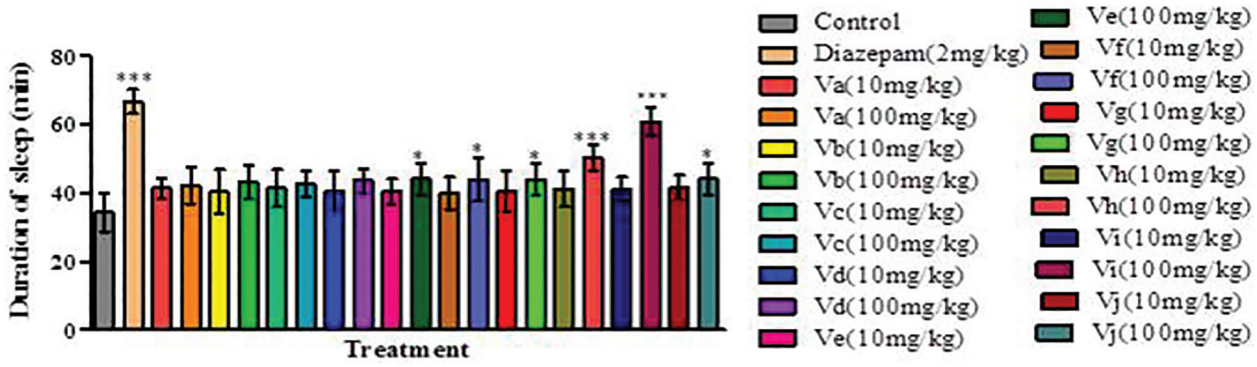
The pentobarbital-induced sleeping time of isatin derivatives (10 and 100 mg/kg) doses is given in Fig. 2. The isatin derivatives (100 mg/kg) and standard diazepam (2 mg/kg) dose significantly ($P < 0.05$ and $P < 0.001$, respectively) increased the duration of pentobarbital-induced sleeping time

Table 3 Effect of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones and diazepam on muscle-relaxant activity and locomotor activity

Treatment	Dose (mg/kg)	Muscle-relaxant activity		Locomotor activity	
		Number of falls in 3 min			
		Basal reading	After treatment	Before treatment[a]	After 60 min treatment[a]
Control	0.1% sodium CMC	99.20 ± 4.52	98.12 ± 3.62	180.32 ± 8.25	178.32 ± 7.25
Diazepam	2	101.56 ± 6.34	65.84 ± 5.20	178.35 ± 7.63	95.56 ± 5.63***
Va	10	94.46 ± 4.26	90.89 ± 5.82	176.28 ± 5.38	150.28 ± 5.88
	100	95.12 ± 6.57	86.10 ± 3.89*	170.67 ± 9.23	137.67 ± 8.27*
Vb	10	98.95 ± 4.16	90.43 ± 5.86	175.83 ± 10.33	145.66 ± 7.39
	100	96.62 ± 3.42	85.56 ± 6.43*	169.28 ± 7.56	133.58 ± 6.56*
Vc	10	97.64 ± 2.65	89.43 ± 4.97	173.38 ± 6.37	146.38 ± 7.39
	100	94.83 ± 6.21	86.00 ± 5.42*	168.67 ± 8.23	136.64 ± 7.25*
Vd	10	98.23 ± 4.25	93.45 ± 6.94	179.83 ± 10.33	144.54 ± 9.56
	100	96.24 ± 5.32	85.87 ± 6.12*	165.28 ± 7.56	134.45 ± 6.89*
Ve	10	97.32 ± 6.64	92.65 ± 4.64	173.65 ± 8.38	146.65 ± 10.38
	100	93.53 ± 5.63	86.38 ± 7.64*	166.68 ± 6.78	138.56 ± 9.78*
Vf	10	100.23 ± 3.41	94.34 ± 5.71	176.67 ± 8.23	145.50 ± 9.35
	100	99.43 ± 5.31	86.98 ± 7.56*	169.83 ± 9.33	138.66 ± 8.78*
Vg	10	96.54 ± 4.31	90.54 ± 4.26	175.28 ± 7.56	144.45 ± 8.35
	100	97.24 ± 8.64	87.56 ± 7.82*	166.28 ± 5.48	138.85 ± 8.75*
Vh	10	99.82 ± 7.63	91.62 ± 5.96	174.67 ± 7.23	149.76 ± 5.38
	100	97.56 ± 6.53	70.72 ± 5.15***	162.83 ± 11.33	125.53 ± 8.72**
Vi	10	98.56 ± 5.43	90.34 ± 5.62	169.28 ± 6.56	141.68 ± 10.88
	100	99.92 ± 4.82	67.34 ± 4.76***	160.28 ± 5.38	122.48 ± 9.58**
Vj	10	102.65 ± 7.82	93.56 ± 6.31	173.67 ± 7.23	156.28 ± 5.68
	100	99.98 ± 7.42	86.16 ± 7.46*	168.83 ± 9.56	138.97 ± 8.67*

[a]Movements/min; values are mean ± SD, $n = 6$ in each group. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$ when compared with the control group (Newman–Keuls multiple comparison test).

Figure 2



Duration of sleeping time using pentobarbital. *$P < 0.05$, ***$P < 0.001$. All data were compared with the control using one-way ANOVA followed by Newman–Keuls multiple comparison test. ANOVA, analysis of variance.

when compared with control. However, 10 mg/kg dose did not alter the sleeping time in mice.

Tail suspension test

The depressant activity of isatin derivatives was also studied by TST. Table 4 shows depressant percentage variation of isatin derivatives. All the derivatives **Va**, **Vb**, **Vc**, **Vd**, **Ve**, **Vf**, **Vg**, **Vh**, **Vi** and **Vj** at 100 mg/kg showed significantly increased duration of immobility time in comparison with the vehicle-treated group with variation 15.89, 16.33, 15.73, 16.26, 15.29, 14.78, 13.87, 33.20, 39.57 and 18.92%, respectively. All the isatin derivatives at 10 mg/kg dose do not alter the immobility time. It is indicated in all our isatin derivatives **Vh** and **Vi** showing more prodepressant activity. Imipramine significantly decreased the duration of immobility and exhibited antidepressant activity (Fig. 3).

Forced swim test

The depressant activity of isatin derivatives was studied by the FST. Table 4 shows depressant percentage variation of isatin derivatives. All the derivatives **Va**, **Vb**, **Vc**, **Vd**, **Ve**, **Vf**, **Vg**, **Vh**, **Vi** and **Vj** at 100 mg/kg showed significantly increased duration of immobility time in comparison with the vehicle-treated group with variation 17.79, 19.04, 17.69, 20.08, 17.58, 17.69, 17.77, 38.39, 43.94 and 19.16%, respectively. All the isatin derivatives at 10 mg/kg dose do not alter the immobility time. It is indicated in all our isatin derivatives **Vh** and **Vi** showing more prodepressant activity. Imipramine significantly decreased the duration of immobility and exhibited antidepressant activity (Fig. 4).

Motor coordination

The mice treated with isatin derivatives showed a significant loss of motor coordination at 100 mg/kg tested. The results obtained from the rota rod test showed that the isatin derivatives (100 mg/kg) and diazepam (2 mg/kg) significantly ($P < 0.05$ and $P < 0.001$, respectively) reduced the motor coordination of the test animals (Table 3). However, 10 mg/kg did not show muscle-relaxant activity in tested animals.

Discussion

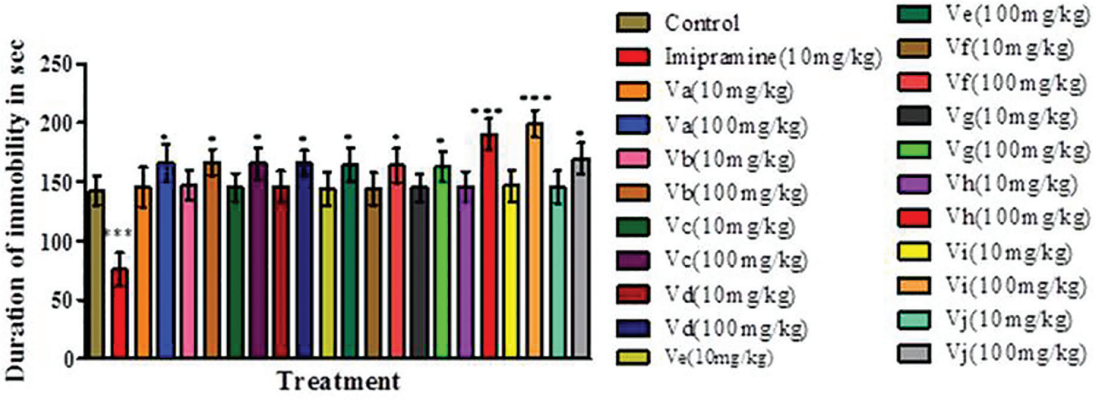
The isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones (**Va–Vj**) have been synthesized as per our previously published papers [9,16,17]. Anxiety may be

Table 4 Antidepressant activity of isatin-3-[N^2-(2-benzalaminothiazol-4-yl)] hydrazones by forced swim test and tail suspension test in mice

Treatment	Dose (mg/kg)	Variation (%)	
		Forced swim test	Tail suspension test
Control	0.1% Na CMC	–	–
Imipramine	10	−48.28	−47.11
Va	10	3.22	1.75
	100	17.79	15.89
Vb	10	5.72	3.03
	100	19.04	16.33
Vc	10	4.47	1.82
	100	17.69	15.73
Vd	10	4.99	2.49
	100	20.08	16.26
Ve	10	1.56	0.57
	100	17.58	15.29
Vf	10	1.35	0.92
	100	17.69	14.78
Vg	10	0.73	1.70
	100	17.77	13.87
Vh	10	6.67	1.98
	100	38.39	33.20
Vi	10	9.67	2.61
	100	43.94	39.57
Vj	10	2.11	2.03
	100	19.16	18.92

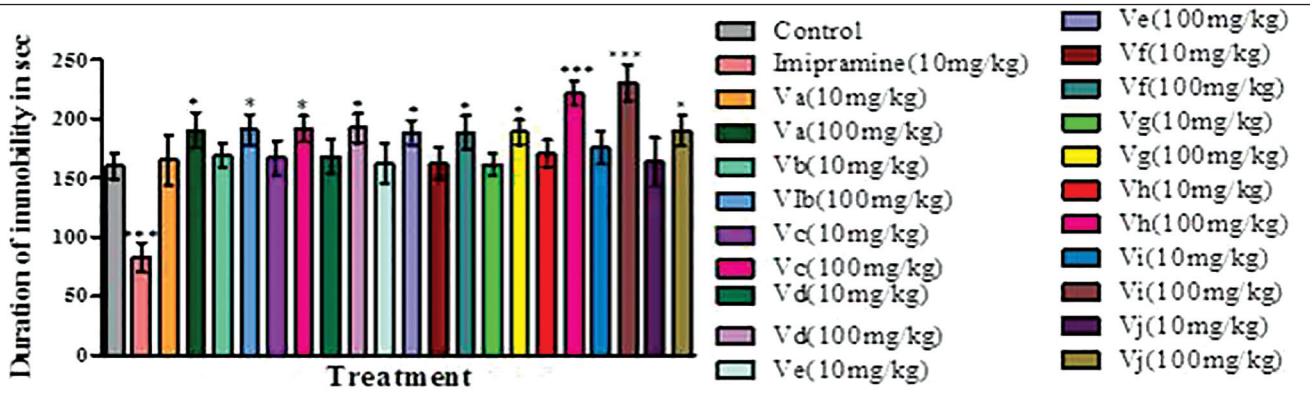
% Variation in immobility ($n = 6$) compared with control.

Figure 3



Duration of immobility using tail suspension. $^*P < 0.05$, $^{***}P < 0.001$. All data were compared with the control using one-way ANOVA followed by Newman–Keuls multiple comparison test. ANOVA, analysis of variance.

Figure 4



Duration of immobility using forced swim test. *$P < 0.05$, ***$P < 0.001$. All data were compared with the control using one-way ANOVA followed by Newman–Keuls multiple comparison test. ANOVA, analysis of variance.

regarded as a particular form of behavioural inhibition that occurs in response to environmental events. First, any abuse can be a factor in the development of anxiety disorders. Second, a chaotic home life (parent's divorce, substance abuse, neglect, etc.) can also be an influence. Benzodiazepines (BZDs), barbiturates and tricyclic antidepressants have been used since a long time to treat anxiety disorders. The serious side effects associated with these drugs, which have limited use in patient [32].

The hole board model indicates that head dipping behaviour is sensitive to changes in the emotional state of the animal and suggests that the expression of anxiolytic state in an animal may be reflected by an increase in head dipping behaviour [33]. The effect of most anxiolytic agents is to enhance the response to GABA, by facilitating the opening of the GABA-activated chloride channel. $GABA_A$ receptors were involved in anxiety and their direct activation would have an anxiolytic effect [34].

In the present study, both diazepam and isatin derivatives showed the sedative action of the highest dose, but anxiolytic activity was not witnessed, whereas the lowest dose (**Va–Vj**) showed better anxiolytic activity. These derivatives (**Va–Vj**) showed a pharmacological biphasic profile similar to that of diazepam, which in small doses causes anxiogenic effects, whereas sedative effects are observed at increasing doses. In this regard, it is well known that BZDs such as diazepam produces anxiolytic effect in humans and anxiolytic-like responses in most animal models through their interaction with the GABAergic system. Locomotor activity is an index of alertness and a decrease may lead to calming and sedation as a result of reduced excitability of the brain [35]. In the present study, isatin-treated mice showed decreased locomotor activity indicating mild CNS depressant activity.

Isatin is anxiogenic at low doses (15–20 mg/kg) and sedative at higher doses (50 mg/kg or above). Similar to high doses of isatin (80 mg/kg) [36], these new derivatives produced sedation, which was observed by a decreased spontaneous locomotor activity of mice. The MAO inhibition cannot explain the behavioural effects of isatin because these effects were observed at doses that do not inhibit MAO-A and because MAO-B inhibitors are neither anxiogenic nor sedative [37]. The muscle-relaxant property was evaluated to strengthen the CNS depressant action in muscle relaxant evaluation; decrease in fall off time was due to the loss of muscle grip implying skeletal muscle relaxation [38].

Barbiturates are used as sedatives, inducing sleep in human beings and animals by depressing the CNS [39]. Isatin pretreatment significantly prolonged the duration of sleep in pentobarbital-induced mice in a dose-dependent manner.

Potentiation of pentobarbital-induced sleeping time by isatin indicated the anxiolytic or sedative property, thereby confirming its CNS depressant role in mice. Prolongation of pentobarbital-induced sleeping time might be due to tranquilizing action as well as CNS depressant action [40]. In our investigation, it is witnessed that low dose of isatin showed anxiogenic activity and high doses showed anxiolytic activity with less sedative and depression than diazepam. In the present study, the synthesized compounds are better anixiolytic, mild sedative and show depressive activity than standard diazepam.

The depressant properties of isatin derivatives were evaluated by TST and FST. A 100 mg/kg dose of **Vh** and **Vi** increased the immobility time when compared with the control group. This is not surprising, as C5 substitution has been associated with increased biological activity for a range of indole-based compounds. Previous

studies have showed that strong electronegative atom substitution such as chloro/bromo at the C5 position of the aromatic ring increased the liphophilicity of molecules and is responsible for CNS activity of isatin derivatives [41,42]. Motor coordination was also evaluated for neuromuscular blockade because most of the centrally acting skeletal muscle relaxants show sedative and anxiolytic activity; along with this result from the rota rod experiment and motor impairment assessment, a marked muscle-relaxant property was observed with higher doses of diazepam.

Conclusion

From the results, it can be concluded that isatin derivatives have CNS actions. These derivatives at higher doses have sedative/hypnotic, anxiolytic, depressant and muscle-relaxant properties. Among all the synthesized compounds, 5-halosubstituted compounds (**Vh** and **Vi**) were found to be most potent than other molecule to treat CNS disorders. Although the previous studies evidence the mechanism of action of isatin, the exact mechanism has to be elucidated, and MAO-B inhibitory actions also suggest that these derivatives may also have anti-Parkinsonian effect.

Acknowledgements

The authors are grateful to the Secretary, Vishwambhara Educational Society, Warangal for providing the necessary facilities to carry out the study.

Conflicts of interest

There are no conflicts of interest.

References

1 Kenemans JL, Kähkönen S. How human electrophysiology informs psychopharmacology: from bottom-up driven processing to top-down control. Neuropsypharmacology 2011; 36:26–51.

2 Rogers RD. The roles of dopamine and serotonin in decision making: evidence from pharmacological experiments in humans. Neuropsychopharmacology 2011; 36:114–132.

3 Su LK, Wang F, Fan Y, Su X, Gao X. Reliability and validity of the screen for child anxiety related emotional disorders (SCARED) in Chinese children. J Anxiety Disord 2008; 22:612–621.

4 Wood STS, Gómez-Olivé FX, Thorogood M, Kandala NB. Sleep problems: an emerging global epidemic? Findings from the INDEPTH WHO-SAGE study among more than 40,000 older adults from 8 countries across Africa and Asia Sleep 2012; 35:1173–1181.

5 Medvedev A, Buneeva O, Glover V. Biological targets for isatin and its analog implicationsfor therapy. Biologics 2007; 1:151–162.

6 Bhattacharya SK, Clow A, Przyborowska A, Halket J, Glover V, Sandler M. Effect of aromatic amino acids, pentylenetetrazole and yohimbine on isatin and tribulin activity in rat brain. Neurosci Lett 1991; 132:44–46.

7 Manley-King CI, Bergh JJ, Petzer JP. Inhibition of monoamine oxidase by selected C5- and C6-substituted isatin analogues. Bioorg Med Chem 2011; 19:261–274.

8 Kaur H, Kumar S, Kumar A. Synthesis, antipsychotic and anticonvulsant activity of some new pyrazolinyl/isoxazolinylindol-2-ones. Int J Chem Tech Res 2010; 2:1010–1019.

9 Venkateshwarlu E, Umasankar K, Sharavana Bhava BS, Venkateshwar Rao J. Screening of the anticonvulsant activity of some isatin derivatives in experimental seizure models and its effect on brain GABA levels in mice. Am J Pharmacol Sci 2013; 1:42–46.

10 Geronikaki A, Babaev E, Dearden J, Dehaen W, Filimonov D, Galaeva I, *et al.* Design, synthesis, computational and biological evaluation of new anxiolytics. Bioorg Med Chem 2004; 12:6559–6568.

11 Smitha S, Pandeya SN, Stables JP, Ganapathy S. Anticonvulsant and sedative-hypnotic activities of N-acetyl/methyl isatin derivatives. Sci Pharm 2008; 76:621–636.

12 Glaser M, Goggi J, Smith G, Morrison M, Luthra SK, Robins E, Aboagye EO. Improved radiosynthesis of the apoptosis marker ^{18}F-ICMT11 including biological evaluation. Bioorg Med Chem Lett 2011; 21:6945–6949.

13 Praveen C, Ayyanar A, Perumal PT. Practical synthesis, anticonvulsant, and antimicrobial activity of N-allyl and N-propargyl di(indolyl)indolin-2-ones. Bioorg Med Chem Lett 2011; 21:4072–4077.

14 Havrylyuk D, Kovach N, Zimenkovsky B, Vasylenko O, Lesyk R. Synthesis and anticancer activity of isatin-based pyrazolines and thiazolidines conjugates. Arch Pharm (Weinheim) 2011; 344:514–522.

15 Venkateshwarlu E, Venkateshwar Rao J, Umasankar K, Dheeraj G. Study of anti-inflammatory, analgesic and antipyretic activity of novel isatin derivatives. Asian J Pharm Clin Res 2012; 5:187–190.

16 Venkateshwarlu E, Venkateshwar Rao J. Evaluation of anti-diabetic and hypolipidemic activity of isatin derivatives in streptozotocin-nicotinamide induced type II diabetic rats. Adv Biol Res 2013; 7:288–295.

17 Venkateshwarlu E, Sharavana Bhava BS, Umasankar K, Venkateshwar Rao J. Evaluation of diuretic activity of isatin derivatives in wistar rats. J Pharm Nutr Sci 2013; 3:141–148.

18 Raghunandan N, Devi MA, Sriram D Sarangapani M. Synthesis and pharmacological screening of some new derivatives of (Z)-[2-(2-substituted benzalamino-4-yl)hydrazono]-indolin-2-ones. Der Pharma Chemica 2010; 96:106.

19 Sarangapani M, Reddy VM. Synthesis and screening of isatin-3-N^2-(alkoxyethyloxy) acetyl hydrazones. Indian drugs 1999; 36:357–362.

20 John J, Bobade AS, Khadse BG. Synthesis of some new triazole ring systems from 2, 4-disubstitued thiazole. Indian J Heterocycl Chem 2001; 10:295–298.

21 Taber RI, Irwin S, Fox JA, Roth FE. Comparison of perphenazine and fluphenazine enanthates in rats. Psychopharmacologia 1968; 12:441–447.

22 Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerulea*. Pharmacol Biochem Behav 1994; 47:1–4.

23 File SE, Pellow S. The effects of triazolobenzodiazepines in two animal tests of anxiety and in the holeboard. Br J Pharmacol 1985; 86:729–735.

24 Adnaik R, Pai P, Sapakal V, Naikwade N, Magdum C. Anxiolytic activity of *Vitex negundo* Linn. in experimental models of anxiety in mice. Int J Green Pharm 2009; 3:243–247.

25 Danjuma N, Abdu-Aguye I, Anuka J, Hussaini I, Zezi A. Evaluation of anticonvulsant activity of the hydroalcoholic stem bark extractof *Randia nilotica* Stapf in mice and chicks. Nig J Pharm Sci 2009; 8:36–45.

26 Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 1985; 85:367–370.

27 Pawar VS, Hugar S, Gawade B, Patil R. Evaluation of antidepressant like activity of *Frigonella foenum* graecum linn seeds in mice. Pharmacology Online 2008; 1:455–465.

28 Peng WH, Lo KL, Lee YH, Hung TH, Lin YC. Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice. Life Sci 2007; 81:933–938.

29 Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur J Pharmacol 1979; 57:201–210.

30 Zomkowski AD, Santos AR, Rodrigues AL. Evidence for the involvement of the opioid system in the agmatine antidepressant-like effect in the forced swimming test. Neurosci Lett 2005; 381:279–283.

31 Estrada-Reyes R, López-Rubalcava C, Rocha L, Heinze G, González Esquinca AR, Martínez-Vázquez M. Anxiolytic-like and sedative actions of *Rollinia mucosa*: possible involvement of the GABA/benzodiazepine receptor complex. Pharm Biol 2010; 48:70–75.

32 Kulkarni SK, Singh K, Bishnoi M. Comparative behavioural profile of newer antianxiety drugs on different mazes. Indian J Exp Biol 2008; 46:633–638.

33 Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behaviour in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 1998; 350:21–29.

34 Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. *Pharmacology*. New York: Elsevier, Churchill Livingstone; 2012. 531–532.

35 Singh N, Kaur S, Bedi PM, Kaur D. Anxiolytic effects of *Equisetum arvense* Linn extracts in mice. Indian J Exp Biol 2011; 49:352–356.

36 Abel EL. Behavioral effects of isatin on open field activity and immobility in the forced swim test in rats. Physiol Behav 1995; 57:611–613.

37 Medvedev AE, Clow A, Sandler M, Glover V. Isatin: a link between natriuretic peptides and monoamines?. Biochem Pharmacol 1996; 52:385–391.

38 Vogel GH. *Drug discovery and evaluation: pharmacological assays*. Germany: Springer Verlag; 2002. 396.

39 Tripathi KD. *Essentials of medical pharmacology*. New Delhi, India: Jaypee Brothers Medical Publishers Ltd; 2013. 398–399.

40 Mandal SC, Dhara AK, Maiti BC. Studies on psychopharmacological activity of *Andrographis paniculata* extract. Phytother Res 2001; 15: 253–256.

41 Rajyalakshmi G, Ramnarsimha Reddy A, Sarangapani M. Synthesis, characterization and anticancer activity of certain 3-{4-(5-mercapto-1,3,4-oxadiazole-2-yl)phenylimino}indolin-2-one derivatives. Saudi Pharma J 2011; 19:153–158.

42 Zapata-Sudo G, Pontes LB, Gabriel D, Mendes TC, Ribeiro NM, Pinto AC, *et al*. Sedative-hypnotic profile of novel isatin ketals. Pharmacol Biochem Behav 2007; 86:678–685.