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Research Article

Synthesis and Evaluation of Anti-Depressant like Activity of Some Novel Thieno 1, 2, 3 – triazine 4 – ones

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ABSTRACT

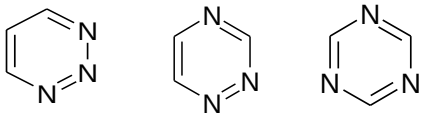
Five derivatives of Thieno 1, 2, 3 triazine 4 – ones were synthesized by a well known Gewald reaction followed by Diazotization. The chemical structures of the compounds were proved by Physical and Spectral data. Compounds were screened for its antidepressant activity by using Tail Suspension Test (TST), Reserpine Induced Hypothermia and Forced Swim Test (FST). In Tail suspension test **JSV 2a** (3- furfuryl–5,6–dihydrocyclohexathieno [2,3-d][1,2,3] triazine-4(3H)-one) and **JSV 2b** (3-cyclohexyl-5,6-dihydrocyclohexathieno [2,3-d][1,2,3] triazine -4(3H)-one) showed significant activity ($p<0.001$) by decreasing immobility time of mice at higher dose levels (50mg/kg). At lower dose levels (25 mg/kg) only **JSV 2a** had decreased the immobility time and shown significant activity ($p<0.001$), while **JSV 2c** (3- furfuryl-5,6-dihydrocyclopentathieno [2,3-d][1,2,3] triazine -4(3H)-one), **JSV 2d** (3-cyclohexayl-5,6-dihydrocyclopentathieno [2,3-d][1,2,3] triazine -4(3H)-one) and **JSV 2e** (3-cyclohexayl-5,6-dimethylthieno [2,3-d][1,2,3] triazine -4(3H)-one) have not shown any activity at both dose levels. **JSV 2b** at 25mg/kg did not show any activity. Based on the results of TST out of five only two compounds were used for other two models as they have shown significant results. In Reserpine induced hypothermia test in Rats **JSV 2a** and **JSV 2b** at 50mg/kg showed significant activity ($p<0.001$). At lower dose levels (25 mg/kg) only **JSV 2a** has shown significant activity ($p<0.001$). **JSV 2b** at 25mg/kg did not show any activity. In Forced Swim Test **JSV 2a** and **JSV 2b** at 50 mg/kg have shown significant activity ($p<0.001$). **JSV 2a** at 25 mg/kg has shown significant activity ($p<0.001$) whereas; **JSV 2b** did not show any activity at 25 mg/kg. These results indicate that compounds possessed Anti-depressant activity. Anti-depressant activity of both the compounds at both the dose levels (25mg/kg and 50mg/kg) was comparable to standard drug Imipramine (20mg/kg).

Keywords: Anti-Depressant Activity; Thieno 1, 2, 3 Triazine – 4 – ones; Forced swim test; Reserpine Induced Hypothermia; Tail Suspension test.

1. INTRODUCTION

Depression is a chronic, recurring and potentially life-threatening illness that affect up to 20% of the population across the globe. This disease is one of the top ten causes of morbidity and mortality worldwide and represents a high cost to countries economy. (Posser et al., 2009)

Triazines are a 6 membered ring containing 3 nitrogen atoms. Theoretically three triazines are possible.



1,2,3-triazine 1,2,4-triazine 1,3,5-triazine

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1, 3, 5-triazines are amongst the oldest known organic molecules. Originally they were called as symmetric triazines. Cyanuric acid, melamine, ammeline, acetoguanide, acetoguanamine are some of the important compounds under this class.1, 2, 4-triazines are well known compounds and a large number of 1, 2, 4-triazines from natural and synthetic sources show biological activity and have been used for various purposes. (Benzoguanamine et. al., 1953)

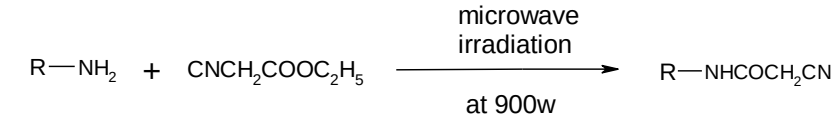
Of the three possible triazine system, the 1, 2, 3-triazines are by far the least studied class. In addition of the name 1, 2, 3-triazine, V-triazine or beta triazine can also be found in older literature. 1, 2, 3- triazines a novel class of heterocycles and only few papers dealing with thieno 1, 2, 3-triazines have been published and the number of known compounds of this type seems to be limited. (Benzoguanamine et. al., 1963)

Evidence suggests that triazine derivatives possess broad spectrum of biological activities such as Analgesic, Anti-inflammatory, antiviral and anti-tumour activity.

Synthesis of Thieno 1, 2, 3 triazine 4 – ones

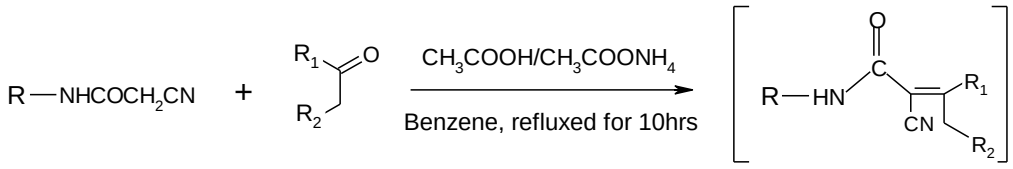
Scheme - I

Step-I



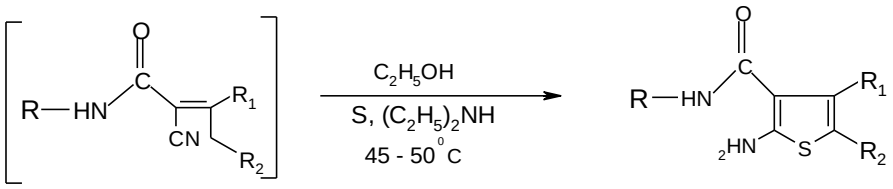
Substituted amines | ethyl cyanoacetate | substituted cyanoacetamides (JSV - A-C)

Step- II



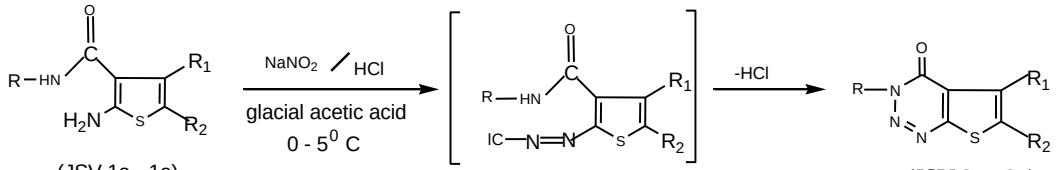
(JSV - A-C) | methylenic ketone | 2-cyano –2-(methylidene)- N-substituted carboxamides

Step-III



2–amino–3–N- substituted carboxamido – 4,5-disubstituted thiophenes (JSV 1a – 1e)

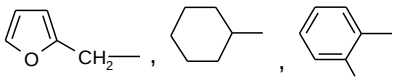
Scheme – II



(JSV 1a - 1e) | Via Diazonium Chloride intermediate | (JSV 2a - 2e)

Where:-

$R_1 = R_2 = -CH_3, -(CH_2)_3, -(CH_2)_4$.

R = 

Triazine derivatives are reported to have antidepressant like activity, when tested by conventional animal models of Depression. (Ferrand et al., 1987) According to studies conducted by Suzuki et al., Triazine derivatives have shown anti-depressant like activity in animal models of depression by decreasing immobility time in forced swim test. (Suzuki et al., 1982) Literature review on synthetic compounds screened for its CNS effects especially neuro-psychopharmacological aspects has clearly revealed a growing interest in this area.

The aim of the present study was to synthesise few derivatives of Thieno 1, 2, 3 triazine – 4 – ones and to evaluate its possible antidepressant effect, using various animal paradigms of depression.

2. MATERIALS AND METHODS

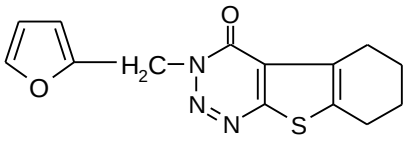
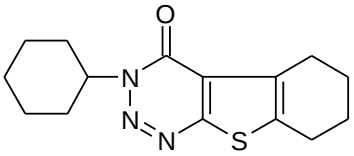
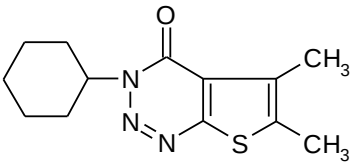
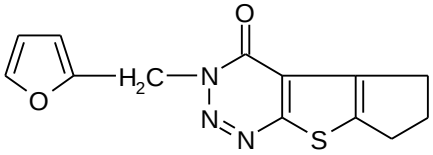
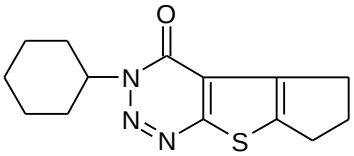
2.1 Drugs and Chemicals

All the chemicals and solvents obtained from local firms (India) and were of analytical grade.

1. Reserpine (Helix Lab Tech, Bangalore, India.)
2. Ethyl cyanoacetate (Sisco Research Laboratories Pvt. Ltd., India.)
3. Benzene (Sisco Research Laboratories Pvt. Ltd., India.)
4. Acetic acid (Sisco Research Laboratories Pvt. Ltd., India.)
5. Glacial acetic acid (Sisco Research Laboratories Pvt. Ltd., India.)
6. Sodium Nitrite (Helix Lab Tech, Bangalore, India.)
7. Sulphur (Helix Lab Tech, Bangalore, India.)

8. Imipramine (AntiDep (2mg/kg) Torrent Pharmaceuticals Ltd.)

Table 1: Physical Data

Sr. No.	Comp. No.	Structure	M.W. (g)	M. P. (^{O}C)	% Yield	TLC	
						Solvent System	Rf
1	JSV-2a		287	140	67.24	CCl_4: $CHCl_3$ (1: 9)	0.66
2	JSV-2b		289	123	62.55	CCl_4: $CHCl_3$ (1: 9))	0.72
4	JSV-2c		263	160	61.12	CCl_4:$CHCl_3$ (1: 9)	0.75
5	JSV-2d		273	178	69.54	CCl_4:$CHCl_3$ (1: 9)	0.65
6	JSV-2e		275	122	63.21	CCl_4:$CHCl_3$ (1: 9)	0.71

2.2 Animals

Swiss albino mice weighing between 18 – 25 g and Wistar Rats weighing 150 – 200 g were procured from In vivo Biosciences, Kachohalli, Bangalore for experimental purpose. Then all the animals were acclimatized at least under standard husbandry conditions, i.e.; room temperature of 24 ± 1° C; relative humidity 45 – 55% and a 12 : 12 h light/dark cycle. The animals had free access to standard rat pellet (Pranav Agro Industry, Bangalore), with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed anywhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any non- specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of P.E.S. College of Pharmacy Bangalore (Karnataka) was taken prior to the start of experiments. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines by committee for the purpose of control and Supervision of Experimental on Animals (CPCSEA).

2.3 Experimental Design

The required JSV 1a – 1e as starting materials were synthesized by adapting a well known Gewald reaction where, the appropriate amine was condensed with ethyl cyanoacetate followed by treatment with Sulphur.

Later, JSV 1a – 1e were subjected to Diazotization reaction to obtain the desired final compounds JSV 2a – 2e respectively in 60 – 70 %.

2.4 Grouping and drug treatment

Animals were divided into six groups, each consisting of 6 animals, as follows;

Group I: Control/Vehicle treated.

Group II: Standard drug (Imipramine 20mg/kg) i.p.

Group III: JSV 2a (25 mg/kg.) i.p.

Group IV: JSV 2a (50mg/kg) i.p.

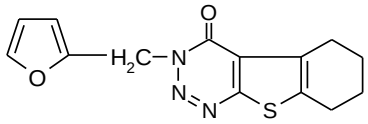
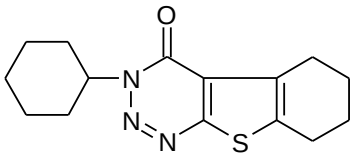
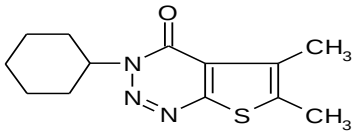
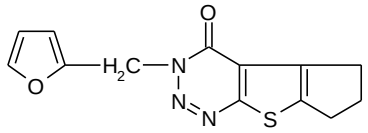
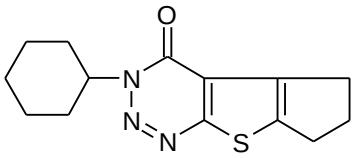
Group V : JSV 2b (25 mg/kg) i.p.

Group VI: JSV 2b (50 mg/kg.) i.p.

Group VII: JSV 2c (25mg/kg) i.p.

Group VIII: JSV 2c (50 mg/kg) i.p.

Table 2: Spectral Data

Comp. No.	Structure	λ_{max} (nm)	IR (KBr) cm^{-1}	^1H NMR
JSV-2a		322	2977 (Ali-CH); 3099 (Ar-CH); 1694 (C=O); 1459 (C=C); 739 (C-S); 837 (C-N).	-------
JSV-2b		325	2931 (Ali-CH); 1688 (C=O); 1477 (C=C); 771 (C-S); 822.6 (C-N).	δ (ppm) = 3.1 (t, 4H, CH_2, cyclohexane, at 5, 8); 1.24-2 (m, 10H, ali-CH_2,at 13 to 17); 1.61 (s, 4H, CH_2, cyclohexane at 6, 7); 3.54 (s, 1H, CH, at 12).
JSV-2c		317	2914.1 (Ali-CH); 1697.2 (C=O); 1496 (C=C); 742 (C-S).	--------
JSV-2d		334	2947 (Ali-CH); 3071(Ar-CH); 1701 (C=O); 1477 (C=C); 721 (C-S); 1079 (C-O)	δ (ppm) = 3.2 (s, 2H, CH_2, cyclopentane, at b); 2.62 (t, 2H, CH_2 cyclopentane, at a); 3.88 (t, 2H, CH_2, cyclopentane, at c); 6.52 (d, 1H, Ar- CH, at e); 6.39 (m, 1H, Ar-CH, at f); 7.47 (d, 1H, Ar-CH, at g).
JSV-2e		332	2922 (Ali-CH); 1687.17 (C=O); 1523 (C=C); 750 (C-S); 837.44 (C-N).	----------

Group IX: JSV 2d (25 mg/kg) i.p.

Group X : JSV 2d (50 mg/kg) i.p.

Group XI: JSV 2e (25 mg/kg) i.p.

Group XII: JSV 2e (50 mg/kg) i.p.

Vehicle used was normal Saline (0.1ml/100g). Test compounds were dissolved in 0.1% DMSO and Imipramine (reference standard drug) was suspended in distilled water for administration. Both the test compounds and Standard were injected intra peritoneally.

2.5 Acute Toxicity Test

Acute toxicity of the preparation was determined using female albino mice. The animals were fasted for 3 h prior to the experiment according to the recommended procedure (OECD guideline no. 425).[6] As per the guidelines, the animals were observed for 48 h for any mortality following oral administration of the different doses of preparation.

2.6 Tail suspension test

This method is based on the observation that a mouse suspended by the tail shows alternating agitation and immobility; the immobility is an indicative of a state of a depression. The tail suspension test was performed according to Steru et al. as a method of evaluating potential antidepressants with slight modification to suit our laboratory condition. Group of 6 animals were treated with test compounds JSV 2a to JSV 2e (25mg/kg and 50mg/kg) and with the Standard (Imipramine 20mg/kg) by the intraperitoneal injection 30 minutes prior to testing. Mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for the period of 6 minutes. Mice were considered immobile when they hang passively and completely motionless for at least 1 minute. (Steru et al., 1985)

2.7 Reserpine induced Hypothermia

Reserpine, an Anti-Hypertensive drug depletes neuronal storage granules of nor-epinephrine, serotonin and dopamine, causes clinically significant depression in human. In animals administration of Reserpine causes symptoms like catalepsy, ptosis and Hypothermia. Wistar rats (150-200 g) were used. Rats were injected with Reserpine (5mg/kg), after measuring their basic rectal temperature. The rectal temperature was determined by inserting an electronic thermometer to a constant depth of 3 cm. After 18 hours of the administration of Reserpine, once again rectal temperature

was measured. Test compounds JSV 2a, JSV 2b and Standard Imipramine were injected intra-peritoneally and rectal temperature was measured at 30, 60, 120 and 180 minutes. (Almeida et al., 2008)

2.8 Forced Swim test

In FST rats were forced to swim in a restricted space from which they cannot escape and which induces a characteristic behaviour of immobility. This behaviour reflects a state which is identical as a depression in human. FST was performed according to Porsolt et al. as a model to test for antidepressant activity with modification to suit our laboratory condition. Rats were individually placed in plastic cylinder (40 x 12 cm) containing of column of 25 cm water at 25 ± 1º C. The rats learned in a pre-test of 15 minutes that they could not escape from the cylinder. 24 hours later in the test period time the total immobility time of the rats was assessed in last 5 minutes of 6 minutes test swimming session.(Porsolt et al., 1979)

2.9 Statistical Analysis:

The values were expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one-way ANOVA followed by Tukey-Kramer test to calculate the significance difference if any among the groups. P<0.05 was considered as significant.

3. RESULTS

3.3 Effect of Thieno 1, 2, 3 triazine – 4 – ones on acute toxicity in mice

JSV 2a to **JSV 2e** administered at a dose of 550 mg/kg did not showed any death in any animal, indicating that these compounds were safe till 550 mg/kg. Whereas for **JSV 2a** to **JSV 2e** at 2000 mg/kg all animals died within 3 days after the administration. Based on these results the LD_{50} was computed and it was found to be 1098 mg/kg for all these compounds.

3.4 Tail Suspension Test

Administration of vehicle (Saline 0.1ml/100 g) treated mice showed mean immobility time of 189 seconds in a 6 minutes observation period. Imipramine (20 mg/kg) was used as reference standard showed significant antidepressant activity.

Test compounds **JSV 2a** and **JSV 2b** showed significant activity (p<0.001) by decreasing immobility time of mice at higher dose levels (50mg/kg). At lower dose levels (25 mg/kg) only **JSV 2a** had decreased the immobility time and shown significant activity (p<0.001), while **JSV 2c**, **JSV 2d** and **JSV 2e** have not shown any activity at both dose levels. **JSV 2b** at 25mg/kg did not show any activity (Table 3).

Table 3: Effect of Thieno 1, 2, 3 triazine -4-ones series of compounds on Tail suspension method

Sl.no	Groups	Dose (mg/kg)	Immobility time (in mins)
1	VEHICLE	0.1 ml/100 g	189.3±3.853
2	IMIPRAMINE	20	105.2±2.372***
3	JSV 2a	25	148.3±4.787***
4	JSV 2a	50	126.7±3.211***
5	JSV 2b	25	178.2±4.593ns
6	JSV 2b	50	143.5±2.693***
7	JSV 2c	25	181.5±3.112ns
8	JSV 2c	50	177.7±4.221ns
9	JSV 2d	25	180.4±2.354ns
10	JSV 2d	50	175±2.133ns
11	JSV 2e	25	177.2±3.623ns
12	JSV 2e	50	173.5±3.453ns

Values are expressed as mean ± SEM; n=6

****P*<0.001 compared with vehicle treated group using one way ANOVA followed by Tukey- Kramer test.

Table 3: Effect of 1,2,3 triazines-4-ones series of compounds in Reserpine induced hypothermia

Treatment	Basal temp	Basal temp after 18 hrs of Reserpine treatment	Temperature after treatment			
			30 min	60 min	120 min	180 min
Imipramine (20 mg/kg)	37.1± 0.08	35.15±0.08	35.35± 0.099	35.83± 0.11	36.6± 0.10	37.20± 0.090***
JSV 2a (25mg/kg)	37.18± 0.094	35.10±0.14	35.25± 0.084	35.75± 0.067	36.2± 0.051	37.06± 0.063***
JSV 2a (50mg/kg)	37.13± 0.12	35.12±0.11	35.20± 0.10	35.79± 0.05	36.4± 0.10	37.11± 0.09***
JSV 2b (25mg/kg)	37.1± 0.13	35.09±0.14	35.03± 0.08	35.02± 0.07	35.3± 0.08	35.88± 0.04ns
JSV 2b (50mg/kg)	37.17± 0.07	35.10±0.11	35.23± 0.11	35.53± 0.09	35.98± 0.05	36.47± 0.07**

Values are expressed as mean ± SEM; n=6

****P*<0.001 compared with vehicle treated group using one way ANOVA followed by Tukey- Kramer test

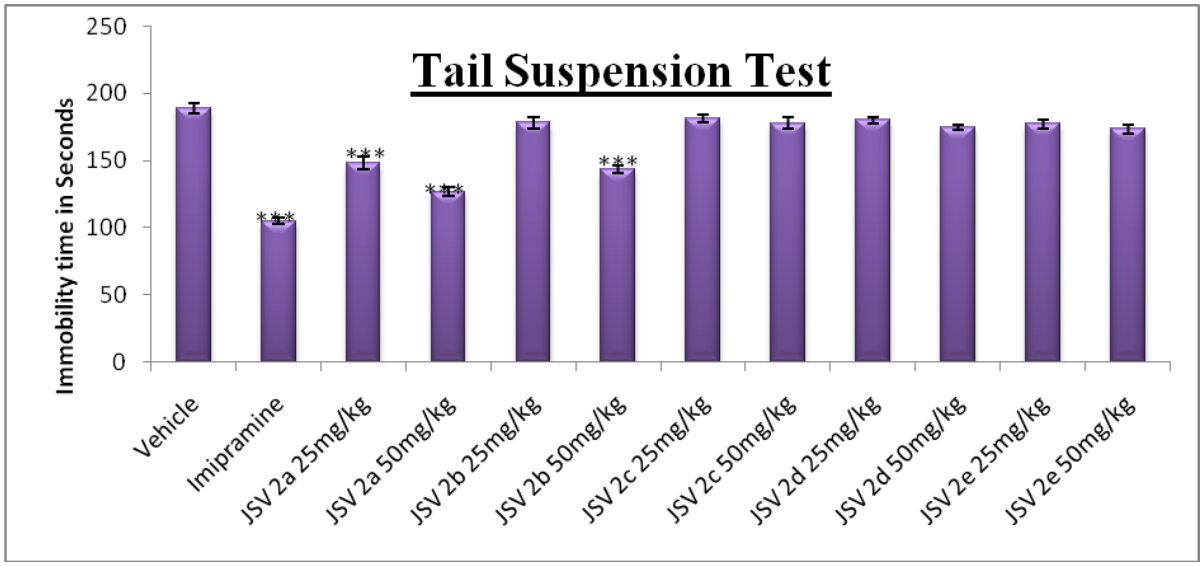


Figure 1: Effect of JSV 2a to JSV 2e on immobility time in Tail suspension method

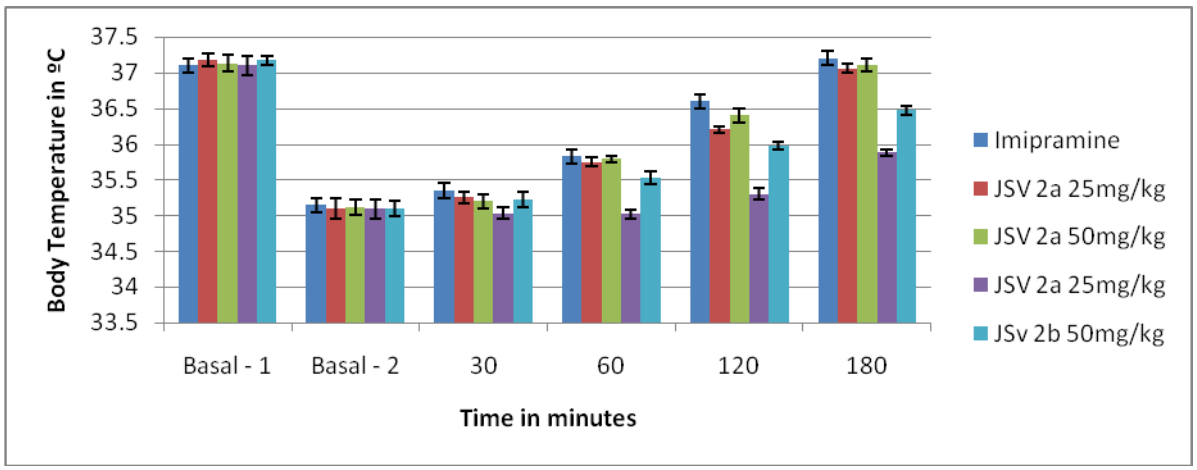


Figure 2: Effect of 1,2,3 triazines-4-ones series of compounds in Reserpine induced Hypothermia

Table 4: Effect of 1, 2, 3 triazines-4-ones series of compounds in Forced swim test

Sl.no	Groups	Dose (mg/kg)	Immobility time (in mins)
1	VEHICLE	0.1 ml/100 g	197±2.266
2	IMIPRAMINE	20	104.8±1.887***
3	JSV 2a	25	142.7±2.512***
4	JSV 2a	50	130.2±1.4***
5	JSV 2b	25	181.3±4.006ns
6	JSV 2b	50	141±2.38***

Values are expressed as mean ± SEM; n=6

****P*<0.001 compared with vehicle treated group using one way ANOVA followed by Tukey- Kramer test

3.5 Reserpine induced Hypothermia

In Reserpine induced Hypothermia test the Reserpine treated groups showed decrease in body temperature after 18 hours of administration. Imipramine (20mg/kg) used as standard drug significantly increase body temperature of Rats after 180 minutes the administration of Reserpine. Treatment of animals with **JSV 2a and JSV 2b** at (50 mg/kg) significantly increase ($p<0.001$) the body temperature of rats after 180 minutes of Reserpine administration. While at lower dose level **JSV 2a** (25mg/kg) significantly increase ($p<0.001$) the body temperature after 180 minutes of Reserpine administration but **JSV 2b** (25mg/kg) did not significantly increase the body temperature (Table 4).

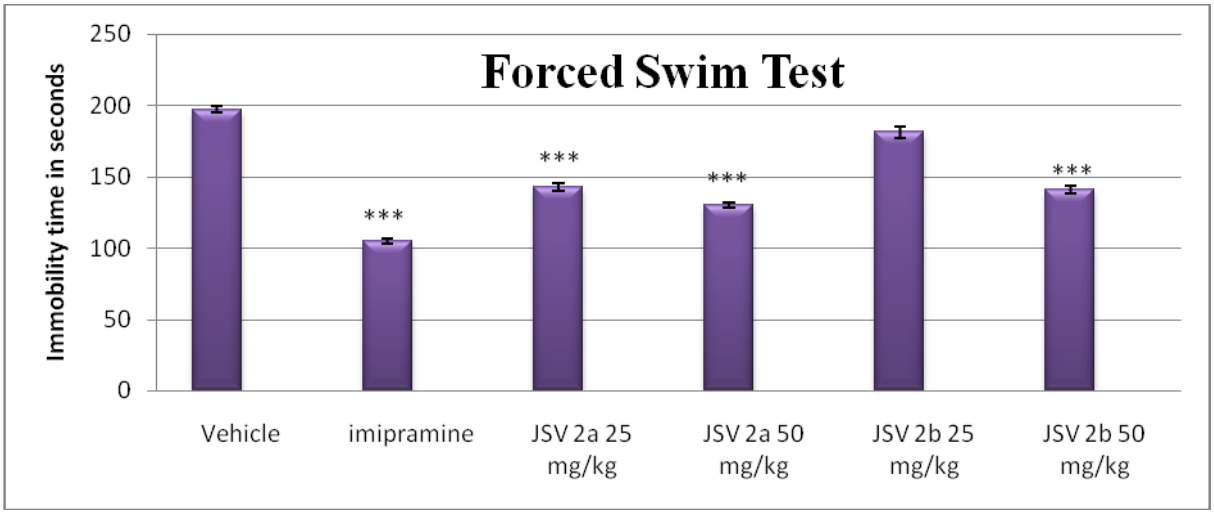


Figure 3: Effect of 1,2,3 triazines-4-ones series of compounds in Forced swim test

3.5 Forced swim test

Administration of low dose level **JSV 2a** (25mg/kg) significantly decreased ($p<0.001$) immobility time in last 5 min of 6 minutes observation period.**JSV 2b** (25mg/kg) did not significantly decrease immobility time in last 5 min of 6 minutes observation period. While **JSV 2a** and **JSV2b** at a dose of 50 mg/kg significantly decrease ($p<0.001$) the immobility time in last 5 min of 6 minutes observation period (Table 5).

DISCUSSION

In the present study five derivatives of Thieno 1, 2, 3 triazine –4 – ones were prepared by Gewald reaction followed by Diazotization reaction and screened for their anti-depressant activity. JSV 2a (25 & 50 mg/kg) and JSV 2b (50 mg/kg) produced significant anti-depressant like effect in all the three models of depression. JSV 2c, JSV 2d, JSV 2e at 25 and 50 mg/kg and JSV 2b at 25 mg/kg did not show any activity in TST. Based on the results of TST only JSV 2a and JSV 2b were used for other two models.

In FST rats are forced to swim in a restricted space from which they cannot escape, and are induced to a characteristic behaviour of Immobility. This behaviour reflects a state of despair that can be reduced by several agents, which are therapeutically effective in human depression. The TST also induces a state of immobility in animals like that in FST. This immobility, referred as behavioural despair in animals, which is claimed to reproduce a condition similar to human depression. (Steru et al., 1985; Willner et al., 1984) It has been argued that the TST is less stressful than FST and has greater Pharmacological Sensitivity. (Thierry et al., 1984)

Standard drug used in test was Imipramine. It is a tricyclic antidepressant and it is a monoamine uptake inhibitor. It inhibits the uptake of nor-adrenaline and/or 5-HT by monoaminergic nerve terminals, and thus it facilitates transmission. So, the possible mechanism of 1, 2, 3 triazine 4-ones to decrease the immobility time may be due to the inhibition of monoamine uptake. Reserpine causes depletion of biogenic amines in the brain and which not only induces catalepsy and ptosis but also induces hypothermia in rodents. Reserpine decreases the body temperature. Anti-depressant drugs like Imipramine increases the body temperature and have slow onset of action and action is long lasting. On the contrary drugs like amphetamine can also increase the body temperature in the model of Reserpine induced hypothermia but they differ from anti-depressants in a way that they have fast onset of action and shorter duration. (Vogel, H. G. 2002) Thieno 1, 2, 3 triazine 4-ones series of compounds have maintained the body temperature of rodents as compared to control animals.

CONCLUSION

Thieno 1,2,3 triazine -4 – ones series of compounds were synthesized by Gewald reaction followed by Diazotization reaction and their chemical structure was proved by Physical and Spectral analysis. These compounds than were screened for their anti-depressant activity and based on above observation it may be concluded that JSV 2a (25 & 50 mg/kg) and JSV 2b (50 mg/kg) may possess anti-depressant like activity.

However, Neurobiological basic research as well as clinical studies have revealed that the monoamines (5-HT, nor adrenaline and dopamine) (Ying et al., 2008) have a crucial role in the development of the depression syndrome. So, further studies are also required to confirm the influence of JSV 2a and JSV 2b on Monoamines level.

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