

## Research Article

# Synthesis, characterization and Evaluation for Anxiolytic activities of some Novel 4-Thiazolidinone Derivatives

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**Abstract:** The objective of the present work is to synthesis of N-[2-(4-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]-2-(naphthalene-2-yloxy) acetamide and evaluate for anxiolytic activity. Based on this a new series of compounds have been planned to synthesize by reacting  $\beta$ -naphthol, ethyl chloroacetate, hydrazinemonohydrate, ethyl alcohol and various aromatic aldehydes. The Anxiolytic activity of the test drugs were evaluated using the experimental Elevated Plus Maze (EPM) and Light-Dark Model (LDM) in mice. The result exhibit the anxiolytic activity among the three synthesized compounds at varying degree. Some compounds also exhibited skeletal muscle relaxant activity .

**Keywords:** 4-Thiazolidinone Derivatives, Anxiolytic activity, Elevated Plus Maze, Light Dark Model.

## INTRODUCTION

4-thiazolidinones are derivatives of thiazolidine with a carbonyl group at the 4 position. Several methods for syntheses are available. The synthesis of 2-imino-4-thiazolidinones-4-C has been reported by using thiourea and sodium salt of labeled monochloroacetic acid [1]. Another method of synthesis of 4-thiazolidinones is by use of thiocyanate, alkyl isothiocyanate with hydrazide/acetamide followed by the treatment with ethyl bromoacetate and sodium acetate[2].

The literature survey revealed that 4-thiazolidinone and their derivatives were possessed a wide range of pharmacological activities such as anti-inflammatory , analgesic, anticonvulsant, antimicrobial (antibacterial and antifungal), local and spinal anesthetics, CNS stimulants, hypnotics, anti HIV, hypoglycemic, anticancer, FSH receptor agonist and CFTR inhibitor etc[3-4].

The objective of the present work is to synthesis of N-[2-(4-substituted phenyl)-4-oxo-1,3-thiazolidine-3-yl]-2-(naphthalene-2-yloxy) acetamide and evaluate for anxiolytic activity. Based on this a new series of compounds have been planned to synthesize by reacting  $\beta$ -naphthol, ethyl chloroacetate, hydrazinemonohydrate, ethyl alcohol and various aromatic aldehydes.

## MATERIALS AND METHODS

The all chemicals used for the synthesis were of laboratory grade and analytical grade. The melting points of newly synthesized thiazolidinone compounds were determined by open capillary method. The IR

spectra of synthesized compounds were recorded by ABB Bomen FT-IR spectrometer MB 104 IR spectra recorder with KBr pellets. The <sup>1</sup>H-NMR spectra of synthesized compounds were recorded by BRUKER NMR spectrometer in DMSO. The Mass spectra of synthesized compounds were recorded by JEOL GCmate. The purification of newly synthesized compounds were done by TLC method. TLC plates are pre-coated silicagel (HF254-200 mesh) aluminium plate using ethyl acetate and n-hexane as an solvent system and spots were visualized under U.V chamber. The IR, <sup>1</sup>H-NMR and Mass spectra were assigned to elucidate the structure of synthesized compounds (V1-V3).

### Animals

Male mice weighing about 25-35 g were used for the study of anti-depressant activity.

### Steps involved in the Synthesis of Compounds

#### Step 1: Preparation of ethyl-2-naphthalene-6-yloxy acetate

2-naphthol (1.44 gm, 10 mmol), anhydrous potassium carbonate (1gm) and ethylchloroactate (1.67gm, 10mmol) in 50ml of anhydrous acetone were refluxed on oil bath for 6 hours. The reaction mixture was filtered and the excess solvent was removed by distillation under pressure.

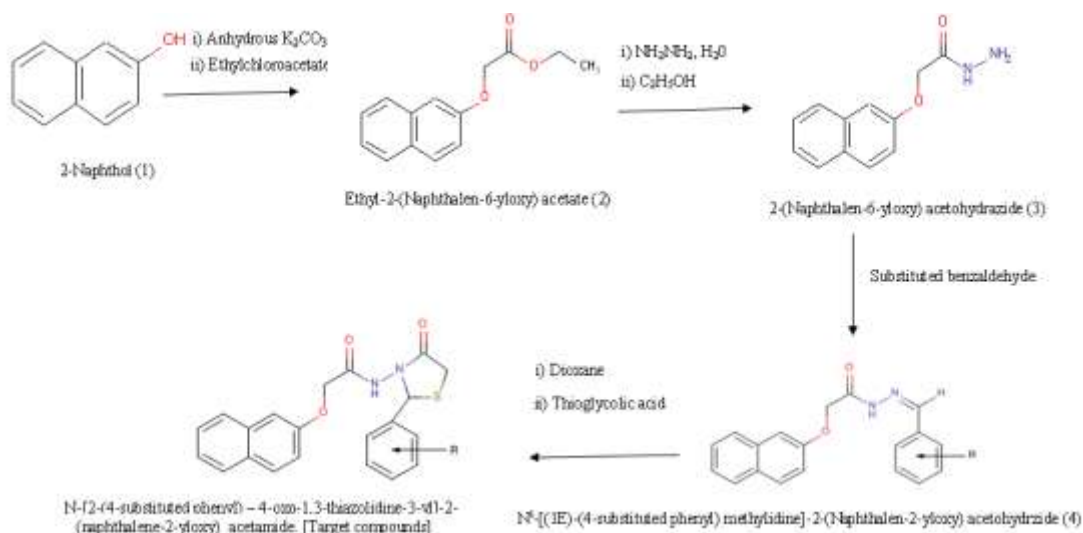
#### Step 2: Preparation of 2-(naphthalene-6-yloxy) acetohydrazide

The residue and 1gm hydrazine monohydrate (20 mmol) were dissolved in 50 ml of absolute ethanol and refluxed on a steam bath for 1 hour. The solute

must was filtered and dried and recrystallized from ethanol.

### Step 3: Preparation of substituted benzaldehyde derivatives

0.01mol of substituted benzaldehyde and 0.01mol of substance and 2-3 drops of glacial acetic acid and 20ml of ethanol were taken in round bottom flask and reflux for 6 hours on water bath. After cooling add ice cold water to the mixture to give solid white mass. Filtered and dried. Recrystallized from chloroform-methanol mixture.



## Pharmacological Evaluation

### Acute Oral Toxicity Study

In the present study acute oral toxicity of the synthesized compounds were performed by acute toxic class method 423 Guideline. In this method the toxicity of synthesized compounds were tested using a step wise procedure, each step using three mice of single sex (female). The mice were fasted prior to dosing (food but water should be withheld) for three to four hours. Following the period of fasting the animal should be weighed and synthesized compounds were administered initially at a dose of 2000mg/kg b.w. and 1% CMC (p. o.) and were observed for 14 days for acute toxicity.

### Evaluation of Anxiolytic Activity

The Anxiolytic activity of the test drugs were evaluated using the experimental Elevated Plus Maze (EPM) and Light-Dark Model (LDM) in mice.

### Elevated plus Maze Model [5]

All the rodents have aversion for height and open space, they prefer to hide in enclosed arm therefore, spend greater amount of time in enclosed arm. Anxiolytic effect statistically increase in open time or open entries. The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof. The drugs and vehicle were administered orally at their respective doses. After

### Step 4: General method of synthesis of thiazolidinone derivatives

A mixture of Schiff base (0.001mmol) and Thioglycolic acid (0.001mol) dissolved in 1,4-dioxane (20ml), anhydrous zinc chloride (0.5mg) was added and refluxed for 8 hours. The reaction was then cooled to 30°C and the result solid was washed with sodium bicarbonate solution. The compound recrystallized from absolute ethanol.

proper treatment with drugs each mouse was placed at the center of the maze with its head facing the open arm. During the 5 min experiment, the behavior of the mouse was recorded as the number of entries into the open or closed arms and time spent by the mouse in each of the arms. An arm entry was defined as the entry of all four paws into the arm.

### Light-Dark Model [6-8]

The light-dark model works on the principal that the light/bright environment works as source of anxiety and Anxiolytic effect statistically significant increase in light (movement) time or number of transition. The mice's light-dark box (40cm x 20cm x 20cm) consists of two parts, the light-compartment and the dark compartment. The box consists of a hole (5cm x 5cm) in the bottom of the clapboard between the two compartments. The mice were treated with drugs and vehicles as respective groups and after one hour of treatment each mice during the test the mice were put into the center of the light compartment with their back to dark compartment and then transition behavior over 5 min was observed. Number of crossings between the light and dark area and total time spent in the illuminated part of the box were calculated. Every time before placing each animal, the maze was cleaned with 5% alcohol to eliminate the possible bias due the odor left by the previous animal.

**Evaluation of skeletal muscle relaxant activity****Rota Rod test [9]**

The rota rod test is used to evaluate the activity of drugs interfering with motor coordination. The rotarod test was used to determine the effect of drugs on motor coordination. The instrument (a horizontal rotation device, Rota Rod, Edison) was set at a rate of 25 rpm. Each rat was placed on the rod and those animals that remained on the rod for 3 mins were selected for the study. The animals were then evaluated for motor coordination basal reading (the time each animal falls off from the rod/ time spent by animal in rotarod. After 60 min of administration of the tested drugs the each animal was kept on rotarod and the time each animal falls off from the rod each animal were recorded.

**Grip strength test [10]**

In grip strength test, the mice was allowed to hold with the forepaws a steel wire (2mm in diameter and 35 cm in length), placed at a height of 50 cm over a cushion support. The length of time the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength. After 60 min of administration of the tested drugs the each animal was kept on wire and the time each animal falls off from the wire of each animal were recorded.

**Statistical Analysis**

The data were expressed as mean  $\pm$  standard error mean (SEM). The data were analyzed by using Graph pad software version5 by one way analysis of variance (ANOVA). The test was followed by Dennett's  $t^2$ -test,  $p$  values less than 0.05 were considered as significance.

**RESULT AND DISCUSSION****Characterization of the synthesized Compounds**

**Compound N1:** N-[2-(4-hydroxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene-2-yloxy)acetamide, Molecular formula:  $C_{21}H_{18}N_2O_4S$ , Melting point: 180<sup>0</sup> C,  $R_f$  value: 0.55 (Ethyl acetate: n- hexane: 2:3); Freely soluble in DMF, DMSO, Yield: 62%, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 1624.11 $cm^{-1}$ (Ar-C=C), 3177.12 $cm^{-1}$ (aliph-N-

H), 1026.57 $cm^{-1}$ (N-N), 747.42 $cm^{-1}$ (C-S), 3610.57 $cm^{-1}$ (O-H-phe), 1689.24 $cm^{-1}$ (C=O), 1269.54 $cm^{-1}$ (C-N), 1728.62 $cm^{-1}$ (C=O-thiazolidine);  $^1H$ -NMR  $\delta$  (ppm): 8.0(1H, -NH-), 6.8-7.9(11H, Ar-H), 5.92(1H, -N-CH-S-), 5.21(1H, Ar-OH), 5.0(2H, -O-CH<sub>2</sub>-CO-), 3.8(2H, -S-CH<sub>2</sub>); Mass (m/e value): 394.5(30%) ( $M^+$ ).

**Compound N2:** N-[2(4-chlorophenyl-4-oxo-1,3-thiazolidin-3-yl)-2-(naphthalene-2-yloxy)acetamide; Molecular formula:  $C_{21}H_{17}ClN_2O_3S$ ; Melting point: 172<sup>0</sup>C,  $R_f$  value 0.46, Freely soluble in DMF, DMSO, Yield: 65.2%, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 1611.20 $cm^{-1}$ (Ar-C=C), 3186.99 $cm^{-1}$ (Aliph-N-H), 1086.99 $cm^{-1}$ (N-N), 695.56 $cm^{-1}$ (C-S), 1668.87 $cm^{-1}$ (C=O), 1267.68 $cm^{-1}$ (C-N), 750.35 $cm^{-1}$ (Ar-C-Cl), 1716.32 $cm^{-1}$ (C=O-thiazolidine);  $^1H$ -NMR  $\delta$  (ppm): 8.3 (1H, -NH-), 6.8-7.9 (11H, Ar-H), 5.80 (1H, -N-CH-S-), 5.0 (2H, -O-CH<sub>2</sub>-CO-), 3.3(2H, -S-CH<sub>2</sub>); Mass (m/e value): 412.9 (24%) ( $M^+$ ).

**Compound N3:** N-[2-(4-fluorophenyl)-4-oxo-1,3-thiazolidin-3-yl]-2-(naphthalene) acetamide., Molecular formula:  $C_{21}H_{17}FN_2O_3S$ , Melting point: 175<sup>0</sup> C,  $R_f$  value: 0.48 (Ethyl acetate: n- hexane: 2:3); Freely soluble in DMF, DMSO, Yield: 55.7%, IR (KBr)  $\nu$  ( $cm^{-1}$ ): 1609.09 $cm^{-1}$ (Ar-C=C), 3194.42 $cm^{-1}$ (Aliph-N-H), 1026.76 $cm^{-1}$ (N-N), 1256.34 $cm^{-1}$ (C-N), 705.10 $cm^{-1}$ (C-S), 1662.09 $cm^{-1}$ (C=O), 1000.62 $cm^{-1}$ (Ar-C-F), 1721.94 $cm^{-1}$ (C=O-thiazolidine);  $^1H$ -NMR  $\delta$  (ppm): 8.20(1H, -NH-), 6.8-7.9(11H, Ar-H), 6.0(1H, -N-CH-S-), 4.90(2H, -O-CH<sub>2</sub>-CO-), 3.5(2H, -S-CH<sub>2</sub>-); Mass (m/e value): 396.5(13%) ( $M^+$ ).

**Acute oral toxicity studies**

No sign of toxicity observed at 2000 mg/kg b.w. in the experimental animals, the LD<sub>50</sub> value of the title compounds (V1-V3) expected to exceed 2000 mg/kg b.w. and represented as class 5 (2000 mg/kg < LD<sub>50</sub> < 2500 mg/kg). Thus, 100 mg/k.g. b.w. was considered as the dose for the further studies.

**Result of Anxiolytic activity**

The result of anxiolytic activity are given in Table and Figure respectable.

**Table-1: Effect of Test compounds for anxiolytic activity on EPM in mice**

Group	Treatment	No. of entries / 5min		Time spent (Sec)/5min	
		Open arm	Close arm	Open arm	Close arm
Group I	Control (Vehicle, 1% Tween 80; 10ml/kg, p.o)	4.50 $\pm$ 0.56	9.40 $\pm$ 1.34	26.17 $\pm$ 2.62	210.34 $\pm$ 10.11
Group II	Test1 (N1) (100mg/kg in 1% Tween 80, p.o)	7.333 $\pm$ 1.24*	8.45 $\pm$ 0.78	71.83 $\pm$ 6.23**	166.76 $\pm$ 9.27
Group III	Test2 (N2) (100mg/kg in 1% Tween 80, p.o)	5.50 $\pm$ 1.06 <sup>ns</sup>	9.87 $\pm$ 0.67	35.17 $\pm$ 4.97 <sup>ns</sup>	193.67 $\pm$ 9.76
Group IV	Test3 (N3) (100mg/kg in 1% Tween 80, p.o)	6.50 $\pm$ 0.76 <sup>ns</sup>	9.80 $\pm$ 0.59	55.17 $\pm$ 4.97**	174.67 $\pm$ 8.45
Group V	Standard (Diazepam; 10mg/kg, in 1% Tween 80, p.o)	7.66 $\pm$ 1.52**	8.45 $\pm$ 1.39	129.7 $\pm$ 6.66***	124.78 $\pm$ 4.14

(Values are in Mean  $\pm$  S.E.M (n=6); <sup>ns</sup> -Non Significant, \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001 when compared with Control using One way ANOVA followed by Dunnett's "t" test.).

**Table-2: Effect of drugs for Anxiolytic Activity on Dark-Light Model**

Groups	Treatment	Time spent in Light-chamber (Sec) Mean±SEM
Group I	Control (Vehicle, 1% Tween 80; 10ml/kg, p.o)	68.83 ± 4.62
Group II	Test1 (N1) (100mg/kg in 1% Tween 80, p.o)	89.33 ± 6.28*
Group III	Test2 (N2) ( 100mg/kg in 1% Tween 80, p.o)	78.83 ± 4.52ns
Group IV	Test3 (N3) (100mg/kg in 1% Tween 80, p.o)	109.0 ± 4.05***
Group V	Standard (Diazepam; 10mg/kg, in 1% Tween 80, p.o)	138.3 ± 5.35***

(Values are in Mean ± S.E.M (n=6); <sup>ns</sup> -Non Significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

**Table-3: Effect of drugs on skeletal muscle relaxant activity in Rota Rod Test**

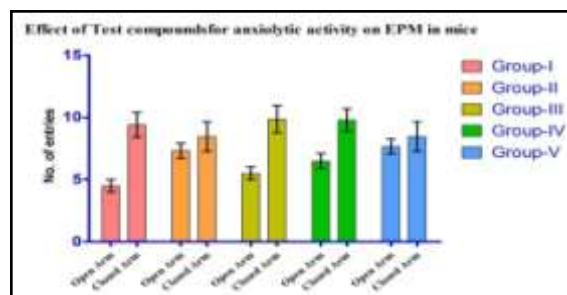
Groups	Treatment	Time spent in Rota Rod (Sec) Mean±SEM
Group I	Control (Vehicle, 1% Tween 80; 10ml/kg, p.o)	223.8± 5.48***
Group II	Test1 (N1) (100mg/kg in 1% Tween 80, p.o)	91.83± 6.04***
Group III	Test2 (N2) ( 100mg/kg in 1% Tween 80, p.o)	78.83± 4.52***
Group IV	Test3 (N3) (100mg/kg in 1% Tween 80, p.o)	183.2± 8.29***
Group V	Standard (Diazepam; 10mg/kg, in 1% Tween 80, p.o)	21.17± 3.55***

(Values are in Mean ± S.E.M (n=6); <sup>ns</sup> -Non Significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).

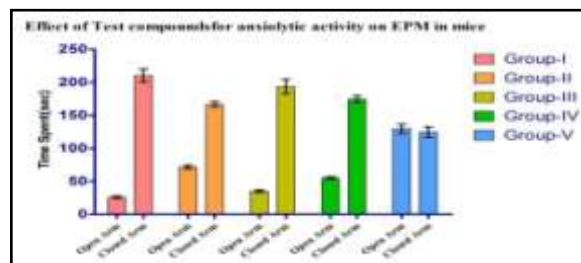
**Table-4: Effect of drugs on skeletal muscle relaxant activity on grip strengthTest**

Groups	Treatment	Time spent in thread (Sec) Mean±SEM
Group I	Control (Vehicle, 1% Tween 80; 10ml/kg, p.o)	73.17±5.51
Group II	Test1 (N1) (100mg/kg in 1% Tween 80, p.o)	46.17±4.31***
Group III	Test2 (N2) ( 100mg/kg in 1% Tween 80, p.o)	50.00±4.26*
Group IV	Test3 (N3) (100mg/kg in 1% Tween 80, p.o)	61.33±3.82 <sup>ns</sup>
Group V	Standard (Diazepam; 10mg/kg, in 1% Tween 80, p.o)	17.33±1.45***

(Values are in Mean ± S.E.M (n=6); <sup>ns</sup> -Non Significant, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test.).



**Fig-1: Effect on no. of entries of Test compounds for anxiolytic activity on EPM in mice**



**Fig-2: Effect on time spent in arms of Test compounds for anxiolytic activity on EPM in mice**

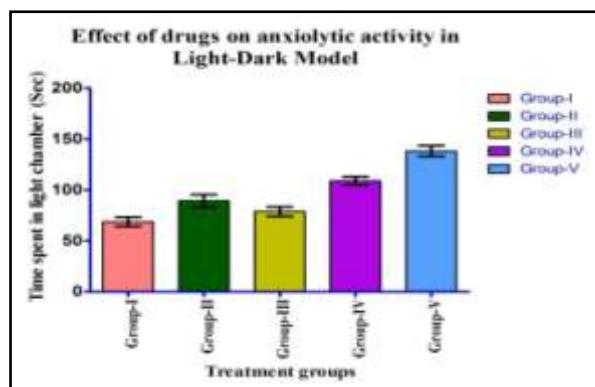


Fig-3: Effect of drugs for Anxiolytic Activity on Dark-Light Model

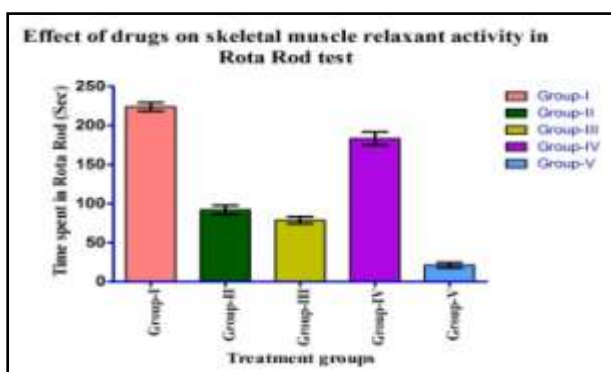


Fig-4: Effect of drugs on skeletal muscle relaxant activity in Rota Rod Test

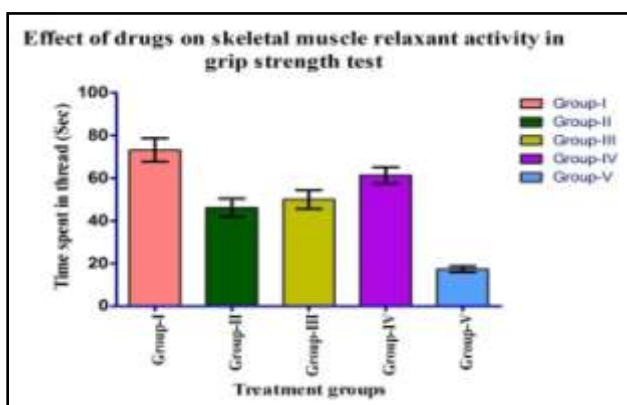


Fig-5: Effect of drugs on skeletal muscle relaxant activity on grip strength test

The statistical analysis of EPM model in mice have shown that the compound N1 exhibited significant result when compared with control. Where as the compound N2 & N3 has shown non significant result when compared with control, that is compound N1 possess the highest anxiolytic activity than the N2 & N3.

On the basis of statistical analysis on dark light model I have seen that that the N3 compound have shown the significant result when compared with standard. Where as N1 compound have shown significant result but less

than N2 it mean that N3 compound possessed highest anxiolytic activity, where as the N1 exhibited mild anxiolytic activity and the compound N2 shows non significant result.

The statistical analysis of rota rod test for skeletal muscle relaxant activity have shown that all compounds (N1,N2,N3) are exhibited mild, moderate to good skeletal muscle relaxant activity. Among these three test compounds (N1,N2,N3), N2 has shown the highest skeletal muscle relaxant activity when compared with control, N1 has shown mild to moderate whereas N3 has shown mild skeletal muscle relaxant activity.

Statistical analysis of GST has shown that the test compound N1 possessed highest skeletal muscle relaxant activity when compared with control. Where as N2 exhibited mild activity when compared with control. The compound N3 has shown non significant result.

## CONCLUSION

From the present study it can be concluded that- The test compounds N1 and N3 exhibit the highest anxiolytic activity among the three synthesized compounds. The test compound N2 exhibit the highest skeletal muscle relaxant activity among the three synthesized compound. The test compound N2 does not exhibit anxiolytic activity.

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