NEUROPHARMACOLOGICAL PROFILE OF SOME NOVEL COUMARIN DERIVATIVES IN MICE

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Abstract: The discovery of new compounds which act on CNS processes will stimulate not only their clinical use but will also contribute useful information for the validation of animal models. This, in turn, will permit the investigation of new compounds and a better understanding of physio-pathological and neurochemical processes that are involved. The present study was aimed to evaluate the newly synthesized Benzopyran-2-one derivatives which includes 7-(5'-(p-chlorophenyl)-[1,3,4]thiadiazol-2'-yl-amino)-4-methyl benzopyran-2-one (comp- I), 4-methyl-7-(5’-p-nitrophenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-benzopyran-2-one (comp-II) and7-(5’-o-hydroxyphenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-4-methyl benzopyran-2-one possess (comp-III). For their neuropharmacological properties which includes analgesic activities spontaneous motor, anxiolytic, skeletal muscle relaxant and anti-convulsant activity.

Keywords: Neuropharmacology, Benzopyran-2-one derivatives, PTZ, GABA

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INTRODUCTION

Neurological disorders were first dated as far back as antiquity and thus have long troubled the society. Even today there are increased incidences of mental illness across the globe. World Health Organization data suggest that neurological and psychiatric disorders are an important and growing cause of morbidity. Unfortunately, the burden of these disorders in developing countries generally goes unknown. The causes for the disorders often remain unknown, modern life style, stress, environmental conditions and genetics play a role in determining the susceptibility of an individual to disease. Thus, there is a surge for research on the brain and the need to intensify our ability to provide better pharmacotherapy for known CNS disorders.

Path breaking research in psychopharmacology has flooded the market place with drugs for specification. For instance, benzodiazepines (diazepam, nitrazepam, lorazepam, etc.) are the most frequently prescribed synthetic drugs for variety of condition particularly anxiety, depression, epilepsy and insomnia amongst many patients. But these psychoneural drugs offer poor drug compliance due to their ineffectiveness and side effects and also these classes of drugs are also associated with some serious ill effects such as physical dependence and tolerance. Along with addiction liabilities, they adversely affect the respiratory, digestive and immune system of the body upon chronic use. In this context, a resurgence of interest of alternative medicine with significantly lesser side effects and higher efficacy would be desirable.

Neuropharmacology is the medical science field that examines the effects of pharmacological agents and is concerned with drug induced changes in the functioning of cells in the nervous system. Also, it is concerned with the study of the neurochemical interactions of neuropeptides, neurohormones, neuromodulators and enzymes, secondary messenger systems of the central nervous system, co-transporters, ion channels, receptor proteins and more. Ultimately we can say that neuropharmacology is nothing but, study of drug-induced changes in mood, thinking and behaviour. These drugs may originate from plants, minerals or synthetic derivatives. Changes in mood, thinking and behavior may be mediated by interaction of these drugs with particular target sites or receptors found in the nervous system.

Review of literature shows that natural plants containing coumarins as an active constituent such as *Leucas inflata*, *Torresencearensis*, *Eclipta alba*, *Pterodonpolygaliflorous* and *Hybanthusipecacuana* a group of Brazilian medicinal plants, *Careya arborea*, *Morus alba L* (mulberry) and prenyloxycoumarins from edible vegetables*Angelica gigas* and *Ficus platyphyl*[a] have been reported for CNS activities.

Chemically synthesized benzodiazepine derivatives from coumarins have been reported for their antianxiety activity. Some bi–heterocyclic coumarin derivatives are reported to have...
analgesic and anti-inflammatory activities\textsuperscript{11}. Thiosemicarbazido derivatives of coumarin for a potential anticonvulsant and analgesic activity have also been reported earlier\textsuperscript{12}. Some substituted coumarins are also reported for anti-convulsant activity\textsuperscript{13}.

Therefore, we can conclude that any plant extract or chemically synthesized derivatives containing coumarin may show neuropharmacological effects. Literature survey reveals no reports on newly synthesized benzopyran-2-one (coumarin) derivatives. Hence, the present study deals with the investigation of the neuropharmacological effects such as, analgesic effect, anxiolytic, spontaneous motor activity (SMA), skeletal muscle relaxant and anticonvulsant activities of some novel Benzopyran-2-one (coumarin) derivatives i.e., 7-(5'-(p-chlorophenyl)-[1,3,4]thiazol-2'-yl-amino)-4-methyl benzopyran-2-one (compound-I), 4-methyl-7-(5’-p-nitrophenyl)-[1,3,4]-thiadiazol-2'-yl-amino)-benzopyran-2-one (compound-II) 7-(5’-o-hydroxyphenyl)-[1,3,4]-thiadiazol-2'-yl-amino)-4-methyl benzopyran-2-one (compound-III) in mice.

**OBJECTIVES:**

a. To perform acute toxicity studies of the newly synthesized Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III.

b. To investigate the analgesic activity of Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III by Eddy’s hot plate method and Haffner’s tail clip method in mice.

c. To investigate the effect of Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III on spontaneous motor activity (SMA) by actophotometer test in mice.

d. To investigate the anxiolytic activity of Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III by evasion test in mice.

e. To investigate the skeletal muscle relaxant activity of Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III by rotarod test in mice.

f. To investigate the anti-convulsant effects of Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III by Pentylenetetrazole (PTZ) induced seizure model in mice.

**MATERIALS AND METHODS:**

**Animals:** Swiss albino mice weighing 20-25g were procured from the Venkateshwara enterprises Bengaluru, Karnataka and were used for the study. The animals were housed for one week to acclimatize to laboratory conditions before starting the experiment under standard 12-h light/dark cycle. They were given free access to water and standard feed, 12 h prior to an experiment; the mice were deprived of food but not water.
Chemicals and reagent:

Diazepam, Pentazocine, Pentylene tetrazole (PTZ), Sodium Carboxymethyl cellulose and Coumarin derivatives: 7-(5’-(p-chlorophenyl)-[1,3,4]thiadiazol-2’-yl-amino)-4-methyl benzopyran-2-one (comp-I), 4-methyl-7-(5’-p-nitrophenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-benzopyran-2-one (comp-II) and 7-(5’-o-hydroxyphenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-4-methyl benzopyran-2-one (comp-III).

Acute toxicity study:

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, revised from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Approval of the Institutional Animal Ethical Committee was obtained prior to the experimentation on animals. Acute toxicity studies were performed on female albino mice weighing between 20-25 gm. Mice were fasted overnight prior to the experimental up and down procedure.

PHARMACOLOGICAL SCREENING

I. Analgesic Activity:

1) Eddy’s Hot Plate method: 

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, of six each as follows:

Group-I: Control – 1 % Sodium CMC (5 ml/kg p.o.)

Group-II: Standard - Pentazocine (10 mg/kg body weight, i.p.)

Group-III: Compound-I (20 mg/kg body weight, p.o.)

Group-IV: Compound-II (20 mg/kg body weight, p.o.)

Group-V: Compound-III (20 mg/kg body weight, p.o.)

The test was carried out using Eddy’s hot plate apparatus, where the temperature was set at 55 ± 1°C. Mice of either sex weighing between 20-25 g which showing cut off time below 15 sec were selected for test and divided randomly into five groups. The mice were placed on hot plate and recorded the reaction time in second/s for licking of hind paw or jumping with cut off time of 15 seconds to avoid tissue injury. The reaction time following the administration of the
test derivatives, standard drug (pentazocine) and control saline vehicle were measured at 0, 30, 60, 90 and 120 minutes respectively.

2) Tail clip method:  

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, of six each as follows:

Group-I: Control – 1 % Sodium CMC (5 ml/kg p.o.)

Group-II: Standard - Pentazocine (10 mg/kg body weight, s.c.)

Group-III: Compound-I (20 mg/kg body weight, p.o.)

Group-IV: Compound-II (20 mg/kg body weight, p.o.)

Group-V: Compound-III (20 mg/kg body weight, p.o.)

All the mice were screened by applying a metal artery clip to the base of the tail with its jaws sheathed with rubber tubing. The pressure exerted by the clip was so adjusted that it was just sufficient to make all control mice respond. Those animals that did not show efforts to dislodge the clip within 15 sec were not used for the experiment. The mice showing positive response were divided into 5 groups of 6 each. The tail clip was applied 0, 30, 60, 90 and 120 min after oral administration of the Benzopyran-2-one derivatives i.e., comp-I, comp-II and comp-III at a dose of 20 mg/kg each or pentazocine (10 mg/kg). 1% Sodium CMC (5 ml/kg p.o.) was used as control. It was considered a positive analgesic response if there was no attempt to dislodge the clip within 15 sec in any of four consecutive trials.

II. Spontaneous Motor Activity (SMA):

Actophotometer test:  

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, of six each as follows:

Group-I: Control – 1 % Sodium CMC (5 ml/kg p.o.)

Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.)

Group-III: Compound-I (20 mg/kg body weight, p.o.)

Group-IV: Compound-II (20 mg/kg body weight, p.o.)

Group-V: Compound-III (20 mg/kg body weight, p.o.)
Spontaneous motor activity was evaluated using actophotometer. Mice were grouped of 6 each and treated with 1% sodium CMC (5 ml/kg p.o.) or the Benzopyran-2-one test drugs i.e. comp-I, comp-II and comp-III (20 mg/kg p.o.) or received diazepam as standard drug (1 mg/kg p.o.). Activity (number of counts) was recorded 30 min after treatment for 10 min. SMA measurements (number of counts) started 30 min after the administration of the test drug and the results were compared with those of control. The experiments were repeated at an interval of 30 min, for a total of 120 mins. (i.e. at 0, 30, 60, 90 and 120 mins). Results of the treated groups were compared with those of control group at each time interval.

III. Anxiolytic Effect:

Evasion test:76,77

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, of six each as follows:

Group-I: Control – 1% Sodium CMC (5 ml/kg p.o.)

Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.)

Group-III: Compound-I (20 mg/kg body weight, p.o.)

Group-IV: Compound-II (20 mg/kg body weight, p.o.)

Group-V: Compound-III (20 mg/kg body weight, p.o.)

The animals were introduced into a rectangular box with an inclined plane by which the mice can escape from the box and the mice that escaped within 5 min from the rectangular box were selected for this test. 30, 60, 90 and 120 minutes after administration of normal saline or diazepam (standard drug) or Benzopyran-2-one derivatives, the animals (n=6) were placed in the box and the number of mice remaining in the box after 5 min of test duration in each group was noted.

IV. Skeletal Muscle Relaxant Activity:

Rotarod test:54,78

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, of six each as follows:

Group-I: Control – 1% sodium CMC (5 ml/kg p.o.)

Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.)
Group-III: Compound-I (20 mg/kg body weight, p.o.)

Group-IV: Compound-II (20 mg/kg body weight, p.o.)

Group-V: Compound-III (20 mg/kg body weight, p.o.)

Mice were placed on a horizontal wooden rod (32 mm diameter) rotating at a speed of 16 rpm. The animals remaining on the rod for 3 min or more in two successive trials were selected for the test and were divided into 5 groups of 6 animals each. The animals were treated with 1% sodium CMC (5 ml/kg p.o.) or standard drug diazepam (1 mg/kg p.o.) or Benzopyran-2-one derivatives (20 mg/kg p.o.) and after 30 min of treatment animals were placed on the rod to note the time taken for the mice to fall from the rotating rod.

V. Anti-convulsant activity:

Pentylenetetrazole (PTZ) induced seizures:

Wistar albino mice of either sex weighing between 20-25 g were divided into five groups, of six each as follows:

Group-I: Control – 1 % sodium CMC + PTZ (5 ml/kg p.o. + 90 mg/kg body weight i.p.)

Group-II: Standard - Diazepam (1 mg/ kg body weight, p.o.)

Group-III: Compound-I (20 mg/kg body weight, p.o.)

Group-IV: Compound-II (20 mg/kg body weight, p.o.)

Group-V: Compound-III (20 mg/kg body weight, p.o.)

Mice were treated with either Benzopyran-2-one derivatives i.e. comp-I, comp-II and comp-III (20 mg/kg p.o.) or normal (1% sodium CMC, 5 ml/kg p.o.) or standard drug Diazepam (1mg/kg p.o.). They were all given PTZ (90 mg/kg i.p.) 60 min later and observed for the seizures. The time taken for the onset of seizures and the duration of seizures was noted. The percentage of animals protected (not showing convulsion) within 60 min after PTZ administration was recorded.

Statistical Analysis:

The results are expressed as the mean ± S.E.M. The results obtained from the present study were analyzed by using one-way Analysis of Variance (ANOVA) followed by Dunnett’s multiple comparison tests. Data was computed for statistical analysis by using Graph Pad Prism 5.0.
software and compared with the vehicle control group. P values less than 0.05 were considered statistically significant

RESULTS

Acute Toxicity Study:

Following up and down method it has been found that all three Benzopyran-2-one (coumarin) derivatives i.e. comp-I, comp-II and comp-III were toxic above a dose of 200 mg/kg. Hence, 20 mg/kg dose was selected for all the neuropharmacological activities.

I. Analgesic Activity:

1. Eddy’s Hot Plate Method:

The reaction time of the mice is shown in Table No 1. at different time interval. In hot plate method pentazocine was used as standard drug which showed significant increase in reaction time at 60 min (4.80±0.52 sec), 90 min (6.17±0.56 sec) and 120 min (5.24±0.50 sec) after treatment, when compared with control (1.90±0.15, 1.87±0.12, 2.38±0.27, 2.25±0.25 and 2.16±0.23) sec after 30, 60, 90 and 120 min respectively).

Table 1: Effect of Benzopyran-2-one derivatives in mice by Eddy’s hot plate test

<table>
<thead>
<tr>
<th>Sr.No</th>
<th>Treatment</th>
<th>Dose (p.o.)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (1% sodium CMC)</td>
<td>5 ml/kg</td>
<td>1.90±0.1</td>
<td>1.87±0.12</td>
<td>2.38±0.27</td>
<td>2.25±0.25</td>
<td>2.16±0.23</td>
</tr>
<tr>
<td>2</td>
<td>Compound -I</td>
<td>20</td>
<td>1.53±0.1</td>
<td>2.15±0.35</td>
<td>1.75±0.17</td>
<td>3.58±0.18*</td>
<td>2.19±0.15</td>
</tr>
<tr>
<td>3</td>
<td>Compound -II</td>
<td>20</td>
<td>1.52±0.1</td>
<td>1.56±0.15</td>
<td>1.91±0.14</td>
<td>2.04±0.16</td>
<td>1.83±0.20</td>
</tr>
<tr>
<td>4</td>
<td>Compound -III</td>
<td>20</td>
<td>1.44±0.1</td>
<td>1.5±0.11</td>
<td>1.81±0.12</td>
<td>1.93±0.15</td>
<td>1.92±0.12</td>
</tr>
<tr>
<td>5</td>
<td>Pentazocine</td>
<td>10</td>
<td>1.53±0.1</td>
<td>3.13±0.44 *</td>
<td>4.80±0.52**</td>
<td>6.17±0.56**</td>
<td>5.24±0.50**</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± S.E.M. (n=6). ***p< 0.001 as compared to control (ANOVA followed by Dunnett’s test)
Effect of Benzopyran-2-one derivatives in mice by Eddy’s hot plate test

From the results it was found that comp-I increased the reaction time at 90 min (p<0.05) but the effect was not significant as compared to control.

2. Tail Clip Method:

The reaction time of the mice at different time interval is shown in Table No 2. In tail clip method pentazocine was used as standard drug which showed significant increase in reaction time to the pain stimulus(p<0.001). The values were found to be significant at 60 min (6.34±0.19 sec), 90 min (7.04±0.13 sec) and 120min (7.39±0.16 sec) when compared with control (2.32±0.24, 2.36±0.31, 2.39±0.16, 1.97±0.21, 2.13±0.24)

Table 2: Effect of Benzopyran-2-one derivatives in mice by Haffner’s tail clip test

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (1 % sodium CMC)</td>
<td>5 ml/kg (p.o.)</td>
<td>2.13±0.2</td>
<td>1.97±0.21</td>
<td>2.39±0.16</td>
<td>2.36±0.31</td>
<td>2.32±0.24</td>
</tr>
<tr>
<td>2</td>
<td>Comp-I</td>
<td>20</td>
<td>2.66±0.3</td>
<td>3.05±0.38</td>
<td>3.48±0.29</td>
<td>4.04±0.50*</td>
<td>3.80±0.44*</td>
</tr>
<tr>
<td>3</td>
<td>Comp-II</td>
<td>20</td>
<td>2.45±0.1</td>
<td>2.73±0.22</td>
<td>3.13±0.39</td>
<td>3.60±0.45</td>
<td>3.46±0.65</td>
</tr>
<tr>
<td>4</td>
<td>Comp-III</td>
<td>20</td>
<td>1.60±0.1</td>
<td>3.20±0.56</td>
<td>3.23±0.60</td>
<td>3.67±0.45</td>
<td>3.24±0.25</td>
</tr>
<tr>
<td>5</td>
<td>Pentazocine</td>
<td>10</td>
<td>2.36±0.2</td>
<td>5.25±0.19*</td>
<td>6.34±0.19*</td>
<td>7.04±0.13*</td>
<td>7.39±0.16*</td>
</tr>
</tbody>
</table>
All values are expressed as mean ± S.E.M. (n=6). **p < 0.01, ***p < 0.001 as compared to control (ANOVA followed by Dunnett’s test)

Effect of Benzopyran-2-one derivatives in mice by Haffner’s tail clip test

![Haffner's tail clip test graph]

However, not all three compounds showed a significant analgesic activity in tail clip method. Comp-I showed increase in the reaction time to the pain stimulus as compared to control at 90 min (4.04±0.50 p<0.05)

II. Spontaneous Motor Activity (SMA): Actophotometer Test

Results of SMA are shown in Table No 3. For SMA Diazepam (1 mg/kg, p.o) was used as a standard drug. Diazepam showed a significant decrease in locomotor activity after 30 min (37.17±2.6 counts) and continued its effect at 60 min (13.34±2.67 counts), 90 min (11.83±3.62 counts) and 120 min (9.167±2.151 counts) when compared with control at 30 min (270.0±4.0 counts), 60 min (254.7±6.42 counts), 90 min (250.8±4.23 counts) and 120 min (246.3±3.75 counts).

- Comp-I showed a significant decrease in the locomotor activity in which the SMA (no of counts) were found to be 146.7±2.48, 123.7±1.80, 99.0±2.40 and 68.34±2.72 at 30, 60, 90 and 120 min respectively after drug administration when compared with control at 30 min (270.0±4.0 counts), 60 min (254.7±6.42 counts), 90 min (250.8±4.23 counts) and 120 min (246.3±3.75 counts).

- Comp-II showed a significant decrease in the locomotor activity in which the SMA (no of counts) were found to be 158.0±2.79, 145.8±3.35, 124.7±3.09 and 129.0±2.74 at 30, 60, 90 and 120 min respectively after drug administration when compared with control at 30 min...
(270.0± 4.0 counts), 60 min (254.7±6.42 counts), 90 min (250.8±4.23 counts) and 120 min (246.3±3.75 counts).

- Comp-III also showed a significant decrease in the locomotor activity in which the no of counts were found to be 192.3±4.17, 168.7±3.30, 143.2±4.0 and 126.2±4.86 at 30, 60, 90 and 120 min respectively after drug administration when compared with control at 30 min (270.0± 4.0 counts), 60 min (254.7±6.42 counts), 90 min (250.8±4.23 counts) and 120 min (246.3±3.75 counts).

### Table 3: Spontaneous Motor Activity (SMA): Actophotometer Test

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Treatment</th>
<th>Dose (mg/kg p.o)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5 ml/kg</td>
<td>324.7 ± 6.48</td>
<td>270.0 ± 4.0</td>
<td>254.7 ± 6.42</td>
<td>250.8 ± 4.23</td>
<td>246.3 ± 3.75</td>
</tr>
<tr>
<td>2</td>
<td>Compoun d-I</td>
<td>20</td>
<td>316.0 ± 3.17</td>
<td>146.7 ± 2.48 ***</td>
<td>123.7 ± 1.80 *</td>
<td>99.0 ± 2.40 **</td>
<td>68.34 ± 2.72 **</td>
</tr>
<tr>
<td>3</td>
<td>Compoun d-II</td>
<td>20</td>
<td>320.8 ± 3.34</td>
<td>158.0 ± 2.79 *</td>
<td>145.8 ± 3.35 **</td>
<td>124.7 ± 3.09 *</td>
<td>129.0 ± 2.74 **</td>
</tr>
<tr>
<td>4</td>
<td>Compoun d-III</td>
<td>20</td>
<td>319.3 ± 3.60</td>
<td>192.3 ± 4.17 *</td>
<td>168.7 ± 3.30 **</td>
<td>143.2 ± 4.0 **</td>
<td>126.2 ± 4.86 **</td>
</tr>
<tr>
<td>5</td>
<td>Diazepam</td>
<td>1</td>
<td>316.5 ± 4.21</td>
<td>37.17 ± 2.6 **</td>
<td>13.34 ± 2.67 **</td>
<td>11.83 ± 3.62 **</td>
<td>9.167 ± 2.151 **</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± S.E.M. (n=6). **p < 0.01, ***p < 0.001 as compared to control (ANOVA followed by Dunnett’s test)

**Effect of Benzopyran-2-one derivatives on spontaneous motor activity in mice by Actophotometer test**
All three Benzopyran-2-one derivatives i.e., comp-I, comp-II and comp-III showed significant decrease in spontaneous motor activity in mice (p<0.001). Where comp-I was found to be better than comp-II and comp-II better than comp-III. comp-III was found to be good.

III. Anxiolytic Activity: Evasion test

Observation: Number of animals (mice, n=6) remaining in the box during 5 min of test duration at various time interval after drug administration. Here mean of two values were taken.

Results of evasion test to test the exploratory behaviour is shown in Table No 4. For Evasion test diazepam (1 mg/kg, p.o.) was used as a standard drug, which showed a significant anxiolytic effect after 30 min (no of mice remaining in box – 5.5 i.e mean of two values 6 and 5), 60 min (5.5 mice remaining in box), 90 min (06 mice remaining in box) of drug administration when compared with control (0 mice remaining in box) throughout the test period.

- Comp-I showed a significant anxiolytic effect after 30 min (2.5 mice remaining in box) 60 min (2.5 mice remaining in box), 90 min (4.) and at 120 min (4 mice remaining in box) of treatment when compared with control (0 mice remaining in box throughout the test period).

- Comp-II showed a significant anxiolytic effect after 60 min (02 mice remaining in box) 90 min (4.0) and at 120 min (3.0 mice remaining in box) of treatment when compared with control (0 mice remaining in box).
• Comp-III also showed a significant anxiolytic effect after in which the number of animals remaining in box was found to be at 2.5 and 2.5 at 90 and 120 min respectively after treatment when compared with control (0 mice remaining in box).

**Table 4: Effect of Benzopyran-2-one derivatives on exploratory behavior in mice by Evasion test**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose (mg/kg p.o)</th>
<th>Number of animals (n=6) remaining in the box after 5 min (Mean of 2 values)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>1</td>
<td>Control (1% sodium CMC)</td>
<td>5 ml/kg</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Compound-I</td>
<td>20</td>
<td>2.5±0.5**</td>
</tr>
<tr>
<td>3</td>
<td>Compound-II</td>
<td>20</td>
<td>2.5±0.5**</td>
</tr>
<tr>
<td>4</td>
<td>Compound-III</td>
<td>20</td>
<td>2.0±0.0*</td>
</tr>
<tr>
<td>5</td>
<td>Diazepam</td>
<td>1</td>
<td>5.0±0.5***</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± S.E.M. (n=6). **p< 0.01, ***p< 0.001 as compared to control (ANOVA followed by Dunnett’s test)

**Effect of Benzopyran-2-one derivatives on exploratory behaviour in mice by Evasion test**

![Evasion test graph](image-link)
All three derivatives i.e., comp-I, comp-II and comp-III were found to possess the anti-anxiety activity in mice. All the test compounds were found to be statistically significant $p<0.001$ for comp-I at 90 and 120 min, for comp-II $p<0.001$ at 60 and 90 mins and $p<0.01$ for comp-III. Comp-I was found to be better and comp-II and comp-III were found to be good.

IV. Skeletal Muscle Relaxant Activity: Rotarod test

Observation: Time taken by the mice in sec to fall from the rod in 180 sec (3 min) of trial period after 60 min of drug administration.

Results of rotarod test to test the skeletal muscle relaxation is shown in Table No 5. For skeletal muscle relaxation diazepam (1 mg/kg, p.o) was used as a standard drug, which showed a significant relaxation of skeletal muscles after 30 min (11.17±1.07 sec required for mice to fall from rod) of drug administration when compared with the control (177.8±2.80 sec required for mice to fall from rod).

- Comp-I showed a significant relaxation of skeletal muscles after 30 min (105.8±3.84 sec required for mice to fall from rod) of treatment when compared with the control (177.8±2.80 sec required for mice to fall from rod).

- Comp-II showed a significant relaxation of skeletal muscles after 30 min (128.3±7.2 sec required for mice to fall from rod) of treatment when compared with the control (177.8±2.80 sec required for mice to fall from rod).

- Comp-III showed a significant relaxation of skeletal muscles after 30 min (168.8±6.37 sec required for mice to fall from rod) of treatment when compared with the control (177.8±2.80 sec required for mice to fall from rod).

Table 5: Effect of Benzopyran-2-one derivatives on skeletal muscle relaxation in mice (Rotarod test)

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Treatment</th>
<th>Dose (mg/kg p.o.)</th>
<th>Time in seconds ( To fall from rotarod )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (1 % sodium CMC)</td>
<td>5 ml/kg</td>
<td>177.8±2.80</td>
</tr>
<tr>
<td>2</td>
<td>Compound-I</td>
<td>20</td>
<td>105.8±3.84***</td>
</tr>
<tr>
<td>3</td>
<td>Compound-II</td>
<td>20</td>
<td>128.3±7.2***</td>
</tr>
<tr>
<td>4</td>
<td>Compound-III</td>
<td>20</td>
<td>168.8±6.37</td>
</tr>
<tr>
<td>5</td>
<td>Diazepam</td>
<td>1</td>
<td>11.17±1.07***</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± S.E.M. (n=6). **$p< 0.01$, ***$p< 0.001$ as compared to control (ANOVA followed by Dunnett’s test)
Effect of Benzopyran-2-one derivatives on skeletal muscle relaxation in mice by Rotarod test

All three derivatives i.e., comp-I, comp-II and comp-III were found to possesses the skeletal muscle relaxant activity in mice, where comp-I and comp-II were found to be better (p<0.001) where as comp-III (p<0.01) was found to be good.

V. Anti-convulsant Activity: PTZ induced seizure model

Results of anticonvulsant activity in PTZ induced seizure model is shown in Table No 6. For anticonvulsant activity diazepam (1mg/kg, p.o) was used as a standard drug which showed a significant prolongation of onset of seizures to 554.5±18.96 sec and reduced the duration of seizures to 2.32±0.54 sec. Also, it showed a 100% protection from mortality when compared with the control which showed onset of seizure at 16.70±1.40 sec and duration of seizures for 18.82±0.83 sec with 0% mortality.

- Comp-I was found effective after 60 min of administration. It prolonged the time of onset of seizures to 59.84±1.79 sec and reduced the duration of seizure to 6.45±0.57 sec including 66.66 % of mortality protection when compared with the control which showed onset of seizure at 16.70±1.40 sec and duration of seizures for 18.82±0.83 sec with 0% mortality protection.

- Comp-II was found effective after 60 min of drug administration. Comp-II prolonged the onset of seizure to 53.11±2.94 sec and reduced the duration of seizure to 8.76±0.74 sec when compared with the control which showed onset of seizure at 16.70±1.40 sec and duration of seizures for 18.82±0.83 sec with 0% mortality protection. It only prolonged the
onset of seizures and decreased the duration of seizures but, it was not found to be effective in case of mortality protection(0 %).

- Comp-III was found effective after 60 min of drug administration. Comp-III prolonged the onset of seizure to 49.77±1.38 sec and reduced the duration of seizure to 9.53±1.82 sec when compared with the control which showed onset of seizure at 16.70±1.40 sec and duration of seizures for 18.82±0.83 sec with 0% mortality protection. It only prolonged the onset of seizures and decreased the duration of seizures but, it was not found to be effective in case of mortality protection(0 %).

Table 6: Effect of Benzopyran-2-one derivatives on pentylenetetrazole (PTZ) - induced seizure in mice

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Dose(mg/kg p.o.)</th>
<th>Onset of Seizures (sec)</th>
<th>Duration of seizure(sec)</th>
<th>Percentage of mortality protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (1 % sodium CMC) + PTZ</td>
<td>5 ml/kg+ 90 mg/kg</td>
<td>16.70±1.40</td>
<td>18.82±0.83</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Compound-I</td>
<td>20</td>
<td>59.84±1.79**</td>
<td>6.45±0.57***</td>
<td>66.66±0.0</td>
</tr>
<tr>
<td>3</td>
<td>Compound-II</td>
<td>20</td>
<td>53.11±2.94*</td>
<td>8.76±0.74***</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>4</td>
<td>Compound-III</td>
<td>20</td>
<td>49.77±1.38*</td>
<td>9.53±1.82***</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>5</td>
<td>Diazepam</td>
<td>1</td>
<td>554.5±18.96***</td>
<td>2.32±0.54***</td>
<td>100.00±0.0***</td>
</tr>
</tbody>
</table>

All value are expressed as mean ± S.E.M. (n=6). **p < 0.01, ***p < 0.001 as compared to control (ANOVA followed by Dunnett’s test)
All the test compounds were found to be statistically significant at $p<0.001$, $p<0.01$ and $p<0.05$. But anti-convulsant activity of comp-I was found to be superior to the other two derivatives i.e., comp-II and comp-III with regard to prolongation of onset of seizures, reduction of duration of seizures and % of mortality protection.

**DISCUSSION**

The present study deals with the investigation of neuropharmacological profile of newly synthesized Benzopyran-2-one derivatives. The most frequent step in evaluating drug action on CNS is to observe the behavior of the test animals. The neuropharmacological profile Benzopyran-2-one derivatives were evaluated by performing the anti-convulsant activity by PTZ induced convulsion model, anxiolytic activity by evasion test, SMA by actophptometer test, motor coordination by rotarod test and analgesic activity by Eddy’ hot plate test and Haffner’s tail clip method.

Locomotor activity of the animals is one of the frequent steps to evaluate the effect of drugs on CNS. SMA was assessed by using Actophotometer test. This model has been used in laboratory animals to evaluate the gross behavioral effects of the drugs. Locomotor activity is considered as an index of alertness and a decrease in locomotion reveals sedative effect. The SMA is a measure of the levels of excitability of the CNS and decrease may be closely related to sedation resulting from depression of the CNS. The results obtained showed that the Benzopyran-2-one derivatives decreased the alertness and restlessness there were neither tremors, twitches, convulsions nor straub tail response. No effects were noticed on alarm reaction, body posture, limb position, gait, righting reflex, muscle tone and pinna and corneal reflexes. From such observations, it is possible to conclude that the depressant effect of the Benzopyran-2-one derivatives on locomotor activity was probably not due to a peripheral neuromuscular blockage. Diazepam, used as the positive control in this test, belongs to the benzodiazepine group of CNS depressant reduced spontaneous motor activity. Hence, we can say that all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) act like that of diazepam which is one of CNS depressant agent.

Evasion test was used to test the effect of Benzopyran-2-one derivatives on exploratory behavior. There was significant increase in the number of animals remaining in the box after treatment with Benzopyran-2-one derivatives. The results obtained from the evasion test of all three Benzopyran-2-one derivatives indicate that these derivatives have depressant action on the CNS. Diazepam was used as a standard reference drug for the evasion test. A significant reduction in exploratory behavior exemplified in the evasion test by all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) indicate a CNS depressant action and a strong indication of anxiolytic action as produced by diazepam. Anxiolytics are known to exert
pharmacological action by causing an increase in the GABA content in the cerebral hemisphere in mice.\textsuperscript{73} Hence, we can conclude that all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) used in present study act through BDZ-GABA receptors, i.e., test derivatives might involve an action on GABAergic transmission as like that of diazepam.

From the results by rotarod test, it is clear that all three Benzopyran-2-one derivatives (i.e., comp-I, comp-II and comp-III) used in present study showed a significant relaxation of skeletal muscles as like that of diazepam. Hence, we can conclude that all the Benzopyran-2-one derivatives exert their relaxant effect on skeletal muscle by acting through a centrally mediated GABA inhibition of polysynaptic reflexes to skeletal muscles\textsuperscript{84}.

PTZ induced seizure model was used for testing anti-convulsant effects of Benzopyran-2-one derivatives. Anti-convulsant drugs those are effective against the most common forms of epileptic seizures, namely partial and generalized tonic-clonic seizures, act either by;

- Reducing or limiting the sustained repetitive firing of neurons, an effect mediated by promoting or prolonging the inactivated state of voltage-activated Na\textsuperscript{+}-channels, thereby reducing the ability of neurons to fire at high frequencies.

- Enhancing and facilitating GABA-mediated synaptic transmission and inhibition, an effect mediated either by a pre- or post-synaptic action. In presence of GABA, the GABA\textsubscript{A} receptor is opened, thus allowing an influx of Cl\textsuperscript{-} ions, which in turn increases membrane polarization.

- Some anti-convulsant drugs also act by reducing the metabolism of GABA.

- Some act at GABA\textsubscript{A} receptors, enhancing Cl\textsuperscript{-} ion influx in response to GABA or by promoting GABA release.

- Anticonvulsant drugs that are effective against absence seizure, act by reducing or limiting the flow of Ca\textsuperscript{2+} through T-type of voltage-activated Ca\textsuperscript{2+} channels, thus reducing the pacemaker Ca\textsuperscript{2+} current\textsuperscript{85}.

Drugs that reduce PTZ induced seizures act by;

- Reducing T-type of Ca2+ currents.

- Enhancing GABA\textsubscript{A} receptor mediated inhibitory neurotransmission\textsuperscript{86}.

Results obtained from the PTZ induced seizure model clearly indicate significant prolongation in the onset of seizures and a significant reduction in the duration of seizures for all three Benzopyran-2-one derivatives used for present study, i.e., comp-I, comp-II and comp-III. Comp-I
showed a significant reduction in the mortality rate of mice, whereas, comp-II and comp-III did not show any protection of mortality in mice.

The analgesic activity of Benzopyran-2-one derivatives used for present study, i.e., (comp-I, comp-II and comp-III) was carried out using by Eddy’ hot plate test and Haffner’s tail clip method. The hot plate method was selected to investigate central analgesic activity because it had several advantages; particularly the sensitivity to strong anti-nociceptives and limited tissue damage. Pentazocine was used as a standard reference drug in hot plate method which acts through opioid receptors and inhibits the neurogenic pain releasing substance. The results of hot plate method showed that comp-II and comp-III did not produce analgesic effect where as comp-I did increase the reaction time to heat stimulus at time interval of 90 min but the analgesic effect was not significant as compared to control.

Haffner’s tail clip method is simple and has the advantage that the reflex mechanism on which it is based involves the higher centres. The animal has to identify exactly the place where the noxious stimulus is applied and it carries out coordinated movements to remove it. In Haffner’s tail clip method, animals significantly prolonged the latency to dislodge the clip from their tail in pentazocine group when compared with control group but the increase in latency to dislodge the clip by test compounds II and III was not statistically significant compared to control. Comp-I showed increase in the latency to dislodge the clip as compared to control however the effect was not significant. The results obtained from the analgesic tests for all three Benzopyran-2-one derivatives showed that Comp-I produces slight analgesia in both Eddy’s hot plate test and Haffner’s tail clip test but compounds II and III did not show significant analgesic effect.

**CONCLUSION**

The results obtained from the present study shows that the newly synthesized Benzopyran-2-one derivatives i.e., 7-(5’-(p-chlorophenyl)-[1,3,4]thiazol-2’-yl-amino)-4-methyl benzopyran-2-one (comp-I), 4-methyl-7-(5’-p-nitrophenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-benzopyran-2-one (comp-II) and 7-(5’-o-hydroxyphenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-4-methyl benzopyran-2-one possess (comp-III) neuropharmacological properties that include analgesic activity by hot plate and tailclip method. CNS depressant activity by SMA, anxiolytic activity, skeletal muscle relaxant activity and anticonvulsant activity. The overall effect of comp I was found to be better than that of comp II and comp III. Further evaluation of detail mechanism pathway involved in all the neuropharmacological profiles needs to be investigated.
SUMMARY

Newly synthesized Benzopyran-2-one (coumarin) derivatives i.e. 7-(5’-(p-chlorophenyl)-[1,3,4]thiadiazol-2’-yl-amino)-4-methyl benzopyran-2-one (comp-I), 4-methyl-7-(5’-p-nitrophenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-benzopyran-2-one (comp-II) and 7-(5’-o-hydroxyphenyl)-[1,3,4]-thiadiazol-2’-yl-amino)-4-methyl benzopyran-2-one possess (comp-III) were assessed for their effects on the central nervous system (CNS) using a series of established pharmacological tests including Eddy’s hot plate and Haffner’s tail clip method for analgesic activity, actophotometer test for spontaneous motor activity (SMA), evasion test for anxiolytic activity, rotarod test for skeletal muscle relaxation and pentylentetrazole (PTZ) induced convulsant model for anti-convulsant activity. Benzopyran-2-one derivatives were found to produce symptoms of CNS depression in conscious mice, viz. reduction in SMA and exploratory behaviour by evasion test and skeletal muscle relaxation. Benzopyran-2-one derivatives showed a significant prolongation of latency and reduced the duration of convulsions induced by PTZ. Further, only comp-I was found to be effective the other Benzopyran-2-one derivatives did not show a significant anti-nociceptive activity evaluated by centrally acting analgesic models i.e., Eddy’s hot plate and Haffner’s tail clip methods.

The results of the above neuropharmacological profiles suggest that all the three Benzopyran-2-one derivatives i.e., comp-I, comp-II and comp-III at 20 mg/kg possess CNS depressant and anticonvulsant activity. Comp-I was found to be more effective than comp-II whereas, comp-III was less effective as compared to comp-II.

CNS depressant actions of the test compounds i.e., reduction in SMA by actophotometer test, exploratory behaviour by evasion test may be due to the action of all the Benzopyran-2-one derivatives on the ion-channel of GABA receptors in the same manner as that of Diazepam i.e., test derivatives potentiate the action of GABA, which leads to opening of the channel pore and increase in the conduction of Cl⁻ ions through it leading further to inhibitory action by hyperpolarisation of the cell membrane. CNS depressant action was further supported by skeletal muscle relaxant activity by rotarod test, which indicates that muscle relaxation may be through a centrally mediated GABA inhibition of polysynaptic reflexes to skeletal muscles. Similarly, anti-convulsant activity of the test derivatives may be by enhancing the GABA receptor mediated inhibitory neurotransmission or by reducing T-type of Ca²⁺ currents or by inhibiting GABA transaminase enzyme.

REFERENCE

1. Disease control priorities related to mental, neurological, developmental and substance abuse disorders, Mental Health: Evidence and Research Department of Mental Health and Substance Abuse. World Health Organization: Geneva; 2006 p627.


65. Olsen RW. GABA. Neuropsychopharmacol 2002;5:159-68.
66. Olstad E. Glutamate and GABA; Major Players in Neuronal Metabolism. St. Olavs Hospital, Universitetssykehuset i Trondheim 2007;15-17.


