Anxiolytic-like effect of N-n-butyl-3-methoxyquinoxaline-2-carboxamide (6o) in experimental mouse models of anxiety

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The present research was designed to explore the anxiolytic-like activity of a novel 5-HT3 receptor antagonist (6o) in experimental mouse models of anxiety. The anxiolytic activity of '6o' at (1 and 2 mg/kg, ip) was evaluated in mice by using a battery of behavioural tests of anxiety such as elevated plus maze (EPM), light/dark aversion test, hole board (HB) and open field test (OFT) with diazepam (2 mg/kg, ip) as a standard anxiolytic. None of the tested doses of '6o' affected the base line locomotion. Compound '6o' (2 mg/kg, ip) and diazepam (2mg/kg, ip) significantly increased the percentage of both time spent and open arm entries in the EPM test. Compound '6o' in (1 mg/kg, ip) dose was only able to affect the percentage time spent in open arm significantly in the EPM test. In the light and dark test, compound '6o' (2 mg/kg, ip) and diazepam (2mg/kg, ip) significantly increased the total time spent in light compartment as well as number of transitions from one compartment to other and number of square crossed. Compound '6o' (1 and 2 mg/kg, ip) and diazepam (2mg/kg, ip) also significantly increased number of head dips and number of squares crossed, whereas significantly decreased the head dipping latency in HB test as compared to vehicle control group. In addition, '6o' in both the doses and diazepam (2mg/kg, ip) significantly increased the ambulation scores (squares crossed) in OFT however, there was no significant effect of '6o' (1 and 2 mg/kg, ip) and diazepam (2 mg/kg, ip) on rearing scores. To conclude compound '6o' exhibited an anxiolytic-like effect in animal models of anxiety.

Keywords: Anxiolytic, Elevated plus maze, 5-HT3 receptor antagonists, Light and dark, Open field test

Anxiety is a physiological state characterized by somatic, emotional, cognitive and behavioral components1. Anxiety is considered to be a normal reaction to a stressor. It may help an individual to deal with a demanding situation by prompting them to cope with it. However, when anxiety becomes overwhelming, it may fall under the classification of an anxiety disorder. Moreover, anxiety disorders associate with significant disability which has a negative impact on the quality of life with an incidence of 18.1% and prevalence of 28.8%2.

Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety, despite these associated with a large number of unwanted side effects such as sedation, muscle relaxation, ataxia, amnesia, ethanol and barbiturate potentiation and tolerance3.

5-HT3 is the only type of serotonergic receptor that belongs to superfamily of ligand gated ion channel. Many 5-HT3 receptor antagonists have been developed and widely used in the clinic. The main therapeutic use of 5-HT3 receptor antagonists is for chemotherapy-induced emesis. The 5-HT3 receptors are well renowned to be expressed in the brain regions involved in the vomiting reflex, perception of pain, the reward system, cognition, depression and anxiety control. In the periphery they are present on a variety of neurons and immune cells4,5.

The involvement of 5-HT3 receptors in depression and anxiety is complemented by studies of 5-HT3 knockout mice which revealed the regulation of 5-HT3A (3A subtype) in anxiety-related behaviours6,7. Serotonergic transmission in prefrontal cortex plays a key role in regulating emotion and cognition under normal and pathological conditions. Previous studies have reported that increased availability of serotonin on 5-HT2 and 5-HT3 receptors increase anxiety, while 5-HT3 receptor blockade has anxiolytic effect8,9.

Despite increased interest among the clinical neurological studies, information regarding the anxiolytic activity of 5-HT3 receptor antagonists is still insufficient. Thus, selecting the test sensitive to anxiolytic drugs, the present study has been designed...
to investigate the anxiolytic potential of ’6o’ in rodent models of anxiety.

In the present study, compound ’6o’ (Fig. 1) which exhibited good log P (2.60) and pA2 value (7.7) greater than the standard 5-HT1 receptor antagonist, ondansetron (OND) (pA2-6.9) has been selected for the preliminary anxiolytic screening in the standard rodent models of anxiety as mentioned above (unpublished data).

Material and Methods

Animals—Behaviour based experiments were carried out using male Swiss albino mice (20–25 g), procured from Hisssar Agricultural University, Hisssar, India. Animals were kept in polypropylene boxes under standard laboratory conditions (23 ± 2 °C and room humidity 60 ± 10% RH), maintained on 12:12 h light/dark cycle. Standard diet and filtered water were given ad libitum. All the experiments were carried out between 09.00-14.00 hrs in accordance with the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/14/04).

Drugs and treatments—Diazepam was purchased from Cipla Ltd. India. Compound ’6o’ and diazepam were prepared freshly before use in distilled water. ’6o’ (1 and 2 mg/kg, ip) and diazepam (2 mg/kg, ip) were administered intraperitoneally (ip) to respective groups, 30 min prior to testing in each test.

Elevated plus maze (EPM)—The EPM test was first evaluated for rats10 and later adapted for mice11. In brief, the apparatus consisted of a wooden maze with two enclosed arm (30 × 5 ×15 cm3) and two open arms (30 × 5 × 0.25 cm3) that extended from a central platform (5 × 5 cm3) to form a plus sign. The plus-maze apparatus was elevated to a height of 45 cm and placed inside a sound-attenuated room. The trial was started by placing a mouse on the central platform of the maze facing its head towards an open arm. The behavioural performances recorded during a 5 min test were: percentage open arm entries, percentage time spent in open arm and total entries12. Entry into an arm was considered valid only when all four paws of the mouse were inside that arm13. The animal activities were tracked and recorded via an overhead video camera linked to a monitor with computer software smart version 2.5 (Panlab co., USA). The apparatus was thoroughly cleaned with 70% ethanol after each trial.

Light/dark aversion test—The L and D apparatus comprised of a box divided into two separate compartments, occupying two-thirds and one-third of the total size, respectively. The larger compartment (light compartment) was illuminated by a 60 Watt bulb, while the smaller (dark compartment) was entirely black and enclosed under a dark cover. The light and dark compartments were separated by a partition with a tunnel to allow passage from one compartment to the other14. At the beginning of the test, the mouse was placed individually at the center of the light compartment facing towards the tunnel and was allowed to explore the entire apparatus for 5 min. The behavioural parameters such as latency time for the first crossing to the light compartment, total time spent in the light compartment and number of transitions between the light and dark compartments were tracked and recorded using computer software smart version 2.5 (Panlab co., USA). A compartment entry was considered valid when the animal’s all four paws were inside that chamber. The apparatus was thoroughly cleaned with 70% ethanol after each trial.

Hole board (HB) test—The HB apparatus consisted of a grey Plexiglas platform (40 × 40 cm2) raised to a height of 15 cm from the floor of a gray wooden box (40 × 40 × 40 cm3). The grey Plexiglas platform consisted of 16 equivalent square compartments (12 peripheral and 4 central), each featuring a central circular hole (3 cm diam.). Test session was started by placing each animal in the center of the HB and allowed to freely explore on the apparatus for 5 min. The behavioural performances such as number of head dipping, total time spent in head dipping and latency to the first head dipping10 were tracked and recorded using computer software smart version 2.5 (Panlab co., USA).

Open field test—The apparatus consisted of a wooden box (60 × 60 × 30 cm3) with the floor divided into 16 squares (15 × 15 cm2) squares by black parallel and intersecting lines. The apparatus was illuminated with 60 Watt bulb.

Fig.1—Structure of compound ’6o’ (N-n-butyl-3-methoxyquinoxaline-2-carboxamide)
suspended 100 cm above. At the beginning of the test, the mouse was placed individually at the center of the square arena. The ambulation scores (number of square crossed) and rearing number (standing upright on the hind legs) were recorded using computer software smart version 2.5 (Panlab co., USA) for 5 min. After each individual test session the floor was thoroughly cleaned with 70% ethano11.

Statistical analysis—All values are expressed as mean ± SE. The data obtained from various groups were statistically analyzed using one way analysis of variance (ANOVA) followed by the post-hoc Dunnett’s test in Graph pad prism 3. The P value <0.05 was considered to be statistically significant.

Results

Elevated plus maze—Acute treatment with ‘60’ (2 mg/kg, ip) and diazepam (2 mg/kg, ip) significantly increased the percentage of both open arm entries and time spent in open arm as compared to vehicle control group (Table 1). Compound ‘60’ (at 1 mg/kg, ip dose) also increased the percentage time spent in open arm significantly compared to vehicle control group.

Light/dark test—Compound ‘60’ (2 mg/kg, ip) and diazepam (2 mg/kg, ip) treatment significantly increased the number of transitions from one compartment to other, number of square crossed, as well as increase the total time spent in lit area (Table 2). However, lower dose of ‘60’ (1 mg/kg, ip) did not produce significant change in any of the parameters.

Hole board test—Both the doses of compound ‘60’ (1 and 2 mg/kg, ip) and diazepam (2 mg/kg, ip) treatment significantly increased the number of head dips and square crossed while decreased the head dipping latency compared to vehicle control group (Table 3).

Open field test—Compound ‘60’ in both the doses (1 and 2 mg/kg, ip) and diazepam (2 mg/kg, ip) significantly increased the number of square crossed as compared to vehicle treatment group (Table 4). However none of the tested dose of ‘60’ and diazepam significantly affect the rearing scores in OFT (Table 4).

Discussion

In present study, the anxiolytic effects of ‘60’ was examined in mouse models of anxiety such as the elevated plus maze, light and dark, hole board and open field tests15. The results of the present study verified the designed hypothesis that 5-HT3 receptor antagonist plays an important role in pathogenesis of

### Table 1—Effect of ‘60’ on behaviour of mice in elevated plus maze test

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>open arm</th>
<th>closed arm</th>
<th>OA+CA</th>
<th>OAE (%)</th>
<th>TSOA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of entries</td>
<td>Time spent (sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>1.50±0.18</td>
<td>13.67±1.33</td>
<td>15.17±1.53</td>
<td>8.00±0.75</td>
<td>292.00±15.50</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2</td>
<td>2.33±0.25*</td>
<td>3.67±0.80*</td>
<td>6.00±0.56*</td>
<td>31.75±4.23*</td>
<td>268.25±20.63*</td>
</tr>
<tr>
<td>‘60’</td>
<td>1</td>
<td>0.83±0.11</td>
<td>3.00±0.16*</td>
<td>3.83±0.27*</td>
<td>15.50±1.21*</td>
<td>284.50±23.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.50±0.14*</td>
<td>3.75±0.19*</td>
<td>5.25±0.41*</td>
<td>32.00±3.46*</td>
<td>268.00±21.3*</td>
</tr>
</tbody>
</table>

*P<0.05 when compared with vehicle-treated group (one way ANOVA followed by Dunnett’s test)

OAE= Open arm entries  TSOA= Time spent in open arm

### Table 2—Effect of ‘60’ on behavior of mice in Light/Dark test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time spent in lit area (sec)</th>
<th>No. of transitions</th>
<th>No of squares crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>44.83 ± 4.14</td>
<td>8.00 ± 0.62</td>
<td>19.41 ± 1.25</td>
</tr>
<tr>
<td>Diazepam (2mg/kg, ip)</td>
<td>101.83 ± 5.08*</td>
<td>15.73 ±1.33*</td>
<td>31.33 ± 1.91*</td>
</tr>
<tr>
<td>‘60’ (1 mg/kg, ip)</td>
<td>56.33 ± 5.17</td>
<td>13.00 ± 0.97</td>
<td>21.17 ± 2.47</td>
</tr>
<tr>
<td>(2 mg/kg, ip)</td>
<td>68.17 ± 3.95*</td>
<td>14.83 ± 1.35*</td>
<td>32.83 ± 2.63*</td>
</tr>
</tbody>
</table>

*P<0.05 when compared with vehicle-treated group (one way ANOVA followed by Dunnett’s test)

### Table 3—Effect of ‘60’ on behavior of mice in hole board test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of head dips</th>
<th>No. of squares crossed</th>
<th>Latency time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>7.25 ± 1.06</td>
<td>2.63 ± 0.87</td>
<td>9.47 ± 0.67</td>
</tr>
<tr>
<td>Diazepam (2 mg/kg, ip)</td>
<td>25.38 ±2.43*</td>
<td>19.50 ± 2.14 *</td>
<td>2.33 ± 0.42*</td>
</tr>
<tr>
<td>‘60’ (1 mg/kg, ip)</td>
<td>19.83 ±3.93*</td>
<td>9.00 ± 1.71*</td>
<td>3.67* ± 0.80*</td>
</tr>
<tr>
<td>(2 mg/kg, ip)</td>
<td>21.83 ±5.06*</td>
<td>9.17 ± 2.52*</td>
<td>3.80 ± 0.75*</td>
</tr>
</tbody>
</table>

*P<0.05 when compared with vehicle-treated group (one way ANOVA followed by Dunnett’s test)
anxiety disorder via increasing the availability of serotonin at postsynaptic receptors. Although, it is uncertain that any single animal model captures all of the components of the complex expression of anxiety, a battery of tests have been used to evaluate the potential anxiolytic effect of '60' in the present study.

The Light/dark test is another widely used animal model for screening of anti-anxiety compounds. The EPM test used to evaluate the psychomotor performance and emotional aspects of rodents. EPM is considered as a model for unconditioned anxiety to detect anxiolytic/anxiogenic-like activity by investigating aspects of physiological and pharmacological behaviour. In the EPM test, increase number of entries and time spent into the open arms is the most reliable indicators of decreased anxiety or indicating the anxiolytic-like activity of a compound, while anxiogenic substances have the opposite effect. In the present study treatment with '60' produced anxiolytic-like effects in the EPM test, as evidenced by increased percentages of both open arm entries and time spent in open arm. In addition, diazepam used as reference anxiolytic also showed the potential anxiolytic effects in EPM.

The Open field test is also widely used for the screening of anxiolytic/anxiogenic drugs. Normal aversion of a rodent to the brightly lit area produces the anxiety and fear, which is characterized by alteration in the behavioural parameters of animal in open field. Previous reports suggested that anxiolytic compound have a tendency to reduce the fearful behaviours of rodents in open field. '60' treatment increased the ambulation scores in open field test indicating the anxiolytic effect of '60'.

The hole board test has been recognized as a popular model for measuring the behavioural response of rodents to an unfamiliar environment. The head dipping behaviour of a rodent in hole board is sensitive to change in emotional state of the animal. The present results revealed that '60' treatment significantly increased the number of head dipping and latency of head dipping reflecting the anxiolytic activity of compound. This effect is in accordance with previous studies which suggest that increase in the head dipping number and decrease latency of head dipping reflected the anxiolytic like activity of a compound.

In summary, the results of present study suggest the anxiolytic activity of '60' in animal models of anxiety. However, further studies are required in order to better evaluate the possible mechanisms, underlying the anxiolytic-like effects of '60'.

Acknowledgement
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References

### Table 4—Effect of '60' on behavior of mice in open field test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambulation scores</th>
<th>Rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>145.67±4.08</td>
<td>4.40±0.64</td>
</tr>
<tr>
<td>Diazepam (2 mg/kg, ip)</td>
<td>267.23±5.09*</td>
<td>4.20±0.45</td>
</tr>
<tr>
<td>'60' (1 mg/kg, ip)</td>
<td>245.5±3.54*</td>
<td>3.76±0.56</td>
</tr>
<tr>
<td>'60' (2 mg/kg, ip)</td>
<td>292.5±3.25*</td>
<td>3.92±0.62</td>
</tr>
</tbody>
</table>

*P < 0.05 when compared with vehicle-treated group (one way ANOVA followed by Dunnett’s test)