Serotonin or 5-hydroxytryptamine (5-HT) is a key neurotransmitter that modulates many neuronal functions and has been linked with pathophysiology of many disease conditions. Serotonin may possibly be imperative in psychological/psychiatric illnesses, such as depression, anxiety, schizophrenia, eating disorders, obsessive-compulsive disorder, panic disorders and migraines\(^1\)-\(^4\). The 5-HT\(_{1A}\) and 5-HT\(_{1B}\) receptors...
were the first serotonergic receptors targeted to treat anxiety and depression due to their pre-and/or postsynaptic localization\(^7\). However, the attention has been shifted towards the recognition of 5-HT\(_3\) role in psychological illness such as sleep, pain and migraine, as well as in the pathophysiology of many psychiatric disorders including depression and anxiety\(^7\). The 5-HT\(_3\) receptors are the only ionotropic or ligand-gated ion channel of the 5-HT receptor family that alters synaptic neurotransmission. The 5-HT\(_3\) receptors are found in median raphae, hypothalamus, hippocampus and amygdala, which have neural correlates of depression and anxiety\(^8\). Activation of 5-HT\(_3\) receptor in the brain leads to the release of monoamines like dopamine and serotonin. Since 5-HT\(_3\) receptor antagonists delivered central effects equivalent to those of antipsychotics and anxiolytics, in the early nineties schizophrenia and anxiety were considered as potential indications\(^8\). Redrobe and Bourin\(^9\) have demonstrated that 5-HT\(_3\) receptors play a partial role in the effectiveness of anti-depressants during the forced swim test (FST). The deletion of the 5-HT\(_3\) receptor gene exhibited anxiolytic behaviour in mice\(^10\).

Utilizing the three-component pharmacophore model\(^11\) for the 5-HT\(_3\) receptor antagonists as a guide, N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) was designed and synthesized. Our previous study showed the preliminary effect of novel 5-HT\(_3\) molecule in behavioural model\(^11\), the focus was to evaluate the new series of the 5-HT\(_3\) receptor antagonists for their antidepressant/anxiolytic activity at a minimal dose. In our previous studies, various 3-substituted quinoxalin-2-carboxamides (consisting of Mannich base as a linking unit for piperazine moiety and quinoxaline nucleus) were assessed as 5-HT\(_3\) receptor antagonists; however, none of the compounds showed encouraging preliminary effect in depression and anxiety\(^11\). Anticipating a dual use in managing both depression and anxiety, in this study the potential effect of QCF-21, a novel 5-HT\(_3\) receptor antagonist was investigated in animal models of depression and anxiety.

**Material & Methods**

Male Swiss albino mice (20-25 g) and Wistar rats (180-200 g) were purchased from Hisar Agricultural University (Hisar, Haryana, India) and kept under standard lighting (lights on: 0700-1900 h), temperature (23°C ± 2°C) and room humidity (60 ± 10%) conditions. The rodents were housed in standard polycarbonate cages and provided with free access to food (standard pellet chow, Amrit pellets, Bengaluru) and filtered water. The animals were used only once for each experiment and were acclimated to the experimental room for 1 h before the initiation of experiment. Each experimental group had 6-8 animals. The study protocol was approved by the Institutional Animal Ethics Committee of Birla Institute of Technology and Science, Pilani, India (IAEC/RES/14/04). Acute studies for depression and anxiety were performed in Swiss Albino mice, and chronic antidepressant study was done in male Wistar rats.

**Drugs & chemicals:** Escitalopram (ESC) and bupropion (BPN) hydrochloride (HCl) were obtained from Glenmark Pharmaceuticals, Mumbai, and Ranbaxy Research Laboratories, Gurgaon, India, as a gift sample. Diazepam (DZM) was procured from Ranbaxy, Gurgaon, India. Pargyline and 5-HTP were purchased from Sigma Chemicals, USA. Reserpine was purchased from Sisco Research Laboratories, Mumbai, India. Ketamine and xylazine were purchased from Neon laboratory and Indian Immunological, India, respectively. All drugs were freshly prepared in distilled water and administered perorally (p.o.) and intraperitoneally (i.p.). Dose-response studies were performed by using the mouse locomotor activity test, FST and tail suspension test (TST) to determine the QCF-21 doses that significantly exhibited antidepressant-like activity without affecting the baseline locomotion. QCF-21 (0.25-2 mg/kg) was administered to mice 30 min before starting the locomotor activity test, FST, TST, light and dark test, elevated plus maze (EPM) test and hole board test. In acute and chronic models, low and high doses, respectively, were selected for screening QCF-21. ESC (10 mg/kg) and BPN (20 mg/kg) were used as reference standard in the depression study, whereas DZM was used in anxiety studies. The dose of reference drugs was selected from the pilot studies in the laboratory.

The target compound, QCF-21 was synthesized by coupling of 3-aminopyridine with quinoxaline-2-carboxylic acid in the presence of conventional coupling agents, \(1-(3\text{-dimethylaminopropyl})-3\text{-ethyl carbodiimide HCl}\) and 1-hydroxybenzotriazole under inert atmospheric nitrogen. The key intermediate, quinoxalin-2-carboxylic acid was synthesized in two steps as per the literature method\(^12\).

**Pharmacological studies**

**Locomotor activity:** Locomotor activity was evaluated using the actophotometer\(^13\), which contains a square
The procedure of Porsolt et al. was followed with certain modifications. In brief, the mice were forced to swim for 15 min on the pre-test day in glass cylinder. Post 24 h of training, each mouse (vehicle/drug treated) was placed into the water and forced to swim for six minutes after 30 min of i.p. treatment of QCF-21. The immobility time during the last four minutes was measured. The mouse was considered to be immobile when it stopped struggling and passively moved to remain floating and kept its head above water. After each trial water was changed, and temperature was maintained at 22°C ± 2°C.

Tail suspension test (TST): Behavioural despair was induced by a TST according to the procedure described by Steru et al. Mice were suspended individually from a horizontal bar 50 cm above the tabletop using an adhesive tape post 30 min of i.p. treatment of QCF-21. The point of attachment on the tail was 1 cm from the tip. The duration of immobility (seconds) during the six minutes observation period was recorded.

Reserpine-induced hypothermia (RIH): The procedure was adopted as mentioned by Devadoss et al. Male Wistar rats were treated with reserpine (1 mg/kg, i.p.) 30 min after oral administration of QCF-21. The effects of QCF-21 on reserpine-induced hypothermia (measured with digital thermometer) were recorded 30 min before administering reserpine and 30, 60, 90 and 120 min after administering reserpine. Hypothermia was measured by calculating the temperature difference between 120 and zero minute.

5-Hydroxy tryptophan induced head twitch response (5-HTP-HTR): The method has been described elsewhere. QCF-21 pretreated mice were treated (p.o.) with pargyline hydrochloride (75 mg/kg) and 5-HTP (5 mg/kg) 30 and 15 min before the observations began, respectively. The number of head twitches response was recorded post 15 min of 5-HTP administration.

Olfactory bullectomy (OBX) surgery: A bilateral OBX was performed in rats as described by Kelly et al. with substantial modifications. Briefly, the rats were anaesthetized with xylazine (5 mg/kg) and ketamine (75 mg/kg, i.p.). Burr holes (2 mm in diameter) were drilled 8 mm anterior to the bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the eye orbit. The olfactory bulbs were removed by suction, the holes were filled with haemostatic sponge to control excessive bleeding, and the scalp was sutured. Sham-operated rats went through the same procedure, including piercing of the dura mater, with their bulbs left intact.

Open field exploration: The OBX and sham rats were subjected to an open field test 29 days after the surgery and 15 days after starting the oral chronic drug/vehicle treatment. The open field exploration was conducted as described.

Anxiety tests

Elevated-plus maze (EPM): This test has been widely validated to measure anxiety in rodents. In brief, 30 min after the treatment, mice were placed for five minutes on an elevated-plus maze consisting of four arms (25 × 7 cm), two with high, black walls (15 cm high) and two without walls. The maze floor was constructed with plywood. Mice were placed in the intersection between the arms (7 × 7 cm), and the number of entries into, and the time spent in, the open arms were recorded. These two parameters were taken as measures of anxiety-related behaviour.

Light/dark box test: The light/dark test uses the rodent natural aversion to bright areas compared with darker ones. In a two-compartment box, rodents will prefer to remain in dark areas, whereas anxiolytics should increase the time spent in the lit arena. A 60 W bulb placed 25 cm above the light box provided the illumination. Mice were individually tested in five minutes sessions in this apparatus after 30 min of treatment.

Hole-board test: The exploratory activity of the QCF-21 in mice following administration was determined using the hole-board test. The apparatus used consisted of a white wooden board (60 × 30 cm) with 16 evenly spaced holes (1 cm diameter x 2 cm depth). Each mouse was placed singly at one corner of the board. Dipping of the head into a hole is a typical behaviour of the mouse indicating a certain degree of curiosity. The number of dips in five minutes was recorded. The test was carried out 30 min after i.p. treatment of QCF-21.

Statistical analysis: The single treatment data were analyzed using one-way analysis of variance followed by Dunnett’s post hoc test.

Results

Locomotor scores: Lower doses of QCF-21 (0.25-1 mg/kg, i.p.) had no influence on baseline locomotion when
ESC (10 mg/kg) reversed the hypothermic effect of reserpine (Table II).

5-HTP-induced head-twitch response: The combined administration of pargyline and 5-HTP (75 + 5 mg/kg) induced the characteristic head-twitch response. Pre-treatment with QCF-21 (1 mg/kg, \( P<0.05 \)) and ESC significantly (\( P<0.01 \)) potentiated the head-twitch response as compared to a combination of pargyline and 5-HTP alone (Table II).

Open field test post OBX: The effect of different treatments on the behavioural anomalies of the OBX/sham rats was investigated in open field test (Table III). OBX rats exhibited characteristic hyperactivity behaviour during the open field test as compared to sham rats. Chronic (14 days) administration of QCF-21 (1 mg/kg) significantly (\( P<0.05 \)) reduced the ambulation and rearing behaviour in the OBX rats as compared to the vehicle-treated OBX rats. QCF-21 exhibited antidepressant-like effects, while ESC (10 mg/kg) was the most effective antidepressant among all treatments. QCF-21 had no effect in the sham rats, but ESC had a moderate effect in the behaviour of sham rats.

EPM test: Table IV displays the behavioural effect in EPM test. In EPM test, untreated mice preferred to be in the closed arm. The QCF-21 (1 mg/kg) significantly (\( P<0.05 \)) increased the per cent time spent and entry in open arm as compared to vehicle-treated group. DZM (2 mg/kg)-treated animals showed more number of entries and time spent in open arm when compared to vehicle-treated mice group.

Light and dark test: In light and dark test, animals treated with three doses of QCF-21 (0.25, 0.5 and 1 mg/kg) and DZM showed increase in the time that mice spent in the light area and increased number of crossing (Table IV). DZM (2 mg/kg) significantly (\( P<0.01 \)) increased the time spent in light arena of light and dark boxes. Animals treated with high dose

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**Table I.** Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on forced swim and tail suspension tests

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg, i.p.)</th>
<th>FST Duration (sec)</th>
<th>TST Duration (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>160.8±7.5</td>
<td>233.6±8.3</td>
</tr>
<tr>
<td>QCF-21</td>
<td>0.25</td>
<td>137.6±3.7</td>
<td>200.3±12.0</td>
</tr>
<tr>
<td>QCF-21</td>
<td>0.5</td>
<td>128.6±4.3*</td>
<td>187.4±6.5*</td>
</tr>
<tr>
<td>QCF-21</td>
<td>1</td>
<td>117.6±13.3*</td>
<td>164.3±8.3*</td>
</tr>
<tr>
<td>ESC (FST)/BPN (TST)</td>
<td>10/20</td>
<td>78.8±5.9*</td>
<td>143.8±10.5*</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SEM, \( P<0.05 \), \( *<0.01 \) compared to vehicle control; \( n=8 \) mice/group. SEM, standard error of mean; ESC, escitalopram; BPN, bupropion; FST, forced swim test; TST, tail suspension test.
Table II. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on temperature and head twitch response evaluation

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg, i.p.)</th>
<th>Mean decrease in temp. (°F)</th>
<th>Number of head twitches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>2.9±0.2</td>
<td>22.9±1.8</td>
</tr>
<tr>
<td>QCF-21 0.25</td>
<td>2.3±0.3</td>
<td>26.4±2.7</td>
<td></td>
</tr>
<tr>
<td>QCF-21 0.5</td>
<td>1.9±0.2</td>
<td>35.6±5.3</td>
<td></td>
</tr>
<tr>
<td>QCF-21 1</td>
<td>1.3±0.1*</td>
<td>53.4±5.5∑</td>
<td></td>
</tr>
<tr>
<td>ESC 10</td>
<td>1.1±0.2**</td>
<td>91.9±6.2*</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SEM, *P<0.05, **<0.01 when compared to the vehicle control; n=8 mice/group. SEM, standard error of mean; ESC, escitalopram.

Table III. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on the behaviour of olfactory bulbectomy rats in the modified open field test

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>Ambulation</th>
<th>Rearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>87.30±10.09</td>
<td>8.32±0.86</td>
<td></td>
</tr>
<tr>
<td>Sham + QCF-21 0.5</td>
<td>91.8±7.21</td>
<td>9.23±0.96</td>
<td></td>
</tr>
<tr>
<td>Sham + QCF-21 1</td>
<td>95.89±6.19</td>
<td>8.45±0.85</td>
<td></td>
</tr>
<tr>
<td>Sham + ESC 10</td>
<td>100.23±6.67</td>
<td>7.45±0.67</td>
<td></td>
</tr>
<tr>
<td>OBX control</td>
<td>155.21±23.45</td>
<td>24.23±2.85</td>
<td></td>
</tr>
<tr>
<td>OBX + QCF-21 0.5</td>
<td>132.75±8.84</td>
<td>20.21±1.04</td>
<td></td>
</tr>
<tr>
<td>OBX + QCF-21 1</td>
<td>112.56±8.9</td>
<td>15.41±1.53</td>
<td></td>
</tr>
<tr>
<td>OBX + ESC 10</td>
<td>98.40±6.45</td>
<td>13.69±0.81</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SEM. The drug(vehicle)/treatments were administered once a day for 14 days. *P<0.05 compared to sham control; †P<0.05 compared to vehicle-treated OBX group. n=6 rats/group. SEM, standard error of mean; OBX, olfactory bulbectomy; ESC, escitalopram.

Table IV. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on Elevated plus maze, Light and dark, and Hole board Tests

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg, i.p.)</th>
<th>EPM test</th>
<th>Light and dark test</th>
<th>Hole board test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Open arm entry</td>
<td>% Time spent in open arm</td>
<td>Time spent in lit area (sec)</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>-</td>
<td>13.9±1.1</td>
<td>32.1±2.8</td>
<td>23.9±2.3</td>
</tr>
<tr>
<td>QCF-21 0.25</td>
<td>12.0±0.9</td>
<td>34.5±3.7</td>
<td>34.3±3.0</td>
<td>43.4±5.6</td>
</tr>
<tr>
<td>QCF-21 0.5</td>
<td>42.9±3.3*</td>
<td>60.7±5.1*</td>
<td>51.0±4.7*</td>
<td>26.5±1.6*</td>
</tr>
<tr>
<td>QCF-21 1</td>
<td>60.3±4.4**</td>
<td>70.6±4.4**</td>
<td>76.1±11.1*</td>
<td>39.8±3.5**</td>
</tr>
<tr>
<td>DZM 2</td>
<td>13.9±1.1</td>
<td>32.1±2.8</td>
<td>23.9±2.3</td>
<td>12.5±2.4</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±SEM, *P<0.05, **<0.01 compared to vehicle control; n=8 mice/group. SEM, standard error of mean; ESC, escitalopram; DZM, diazepam.

(1 mg/kg) showed more significant results when compared with low dose.

Hole board test: The hole board test is a measure of exploratory behaviour in animals. The QCF-21 dose dependently increased the number and duration of head dipping in the hole board experiment (Table IV).

The increase was significantly (P<0.05) different from vehicle control and DZM which were used as controls.

Discussion

The present results revealed that the novel 5-HT₁₃ antagonist, QCF-21 possessed antidepressant-like

and anxiolytic-like activities. The assessment of the probable antidepressant activity of QCF-21 was evaluated in the preliminary depression models such as FST and TST. The primary assessment in the tests is the duration of immobility. QCF-21 exhibited antidepressant-like effects in the FST and TST without having any role in the baseline locomotion. QCF-21 significantly reduced the immobility time in behavioural paradigm of FST and TST in mice.

To elucidate the role in serotonergic modulation, QCF-21 was evaluated in the 5-HTP-induced head-twitch response and reserpine-induced hypothermia. The decrease in body temperature induced by reserpine was proved to be antagonized by antidepressants. QCF-21 and ESC significantly reversed the hypothermic effect of reserpine pertaining to the antidepressant-like effect of QCF-21 in this model. Synaptic enhancement of monoamines, particularly serotonin, is one of the pharmacological mechanisms of antidepressants. 5-HTP, the immediate serotonin precursor, leads to an increase in serotonergic transmission causing characteristic head-twitch response in mice. Pretreatment with ESC and QCF-21 significantly potentiated pargyline and 5-HTP induced head-twitch responses in mice indicating that the antidepressant-like effect of QCF-21 was modulated by serotonin concentrations at the synapse.

OBX has been reported as one of the chronic model of depression with adequate face and predictive validity and is used to investigate the antidepressant potential of novel agents. Bilateral OBX results in changes in behaviour in the endocrine, immune and neurotransmitter systems that simulate many of the anomalies seen in patients with major depression. OBX rats displayed specific behavioural anomalies in the open field test, as evident by significant increase in the number of ambulation, rearing and faecal pellets in open field test, and this abnormal behaviour was reversed by antidepressants. In the current study, QCF-21 chronic treatment significantly reversed the increased ambulation in bulbectomized rats, but the restoration of behavioural deficits was weaker than that of ESC. In sham group, QCF-21 had no effect, but ESC treatment slightly modulated the behaviour of rats in open field test although not significant.

Along with antidepressant evaluation, QCF-21 was investigated in various traditional anxiety tests (elevated plus-maze, light and dark, and hole board tests). Serotonin has long been viewed as a neurotransmitter involved in regulating emotional states. Location of 5-HT<sub>3</sub> binding sites throughout cortical and limbic brain regions suggested the clinical application of their antagonists to treat anxiety. Blockade of central 5-HT<sub>1</sub> receptors was also discovered to be bound to an anxiolytic action. The light/dark box is also a preferred model for evaluating anxiolytic or anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas. The assessment of the study showed that the treatment with QCF-21 significantly increased the time spent in the light area, suggesting the anxiolytic effect of QCF-21.

The hole-board test provides a simple method for evaluating the reaction of an animal to an unfamiliar environment and is widely used to assess emotionality and anxiety responses to stress in animals. In the present study, QCF-21 increased counts and duration of head dip, indicating a significant anxiolytic effect of QCF-21.

In the treatment of anxiety disorders, benzodiazepines are now slowly replaced by antidepressants, which are efficacious not only in depression but also in the acute and long-term treatment of major anxiety disorders. The current, neurobehavioural study showed antidepressant- and anxiolytic-like effects of QCF-21, a 5-HT<sub>3</sub> antagonist, in animal models of depression and anxiety, although the precise mechanism is not clear. A potentiation of the head-twitch response and reversal of reserpine-induced hypothermia suggested that QCF-21 produced an antidepressant-like effect by increasing the concentration of a neurotransmitter.

In our previous studies, 5-HT<sub>3</sub> receptor antagonist showed the antidepressant activity at higher dose without the anxiolytic effect. The possible anxiolytic and antidepressant activities of QCF-21 may result from its interaction with diversely localized 5-HT<sub>3</sub> receptors and/or from the indirect influence on neurotransmitter systems which are thought to contribute to the modulation of emotional states. Increased serotonergic neurotransmission through postsynaptic 5-HT<sub>3</sub>, receptor antagonism leads to allosteric modulation of serotonergic system on other serotonin receptor which could be an added mechanism of anxiolytic property of novel 5-HT<sub>3</sub> receptor antagonist as confirmed in the 5-HTP-induced head twitches and reserpine-induced hypothermia.

In conclusion, QCF-21, a novel 5-HT<sub>3</sub> antagonist, exhibited the antidepressant-like and anxiolytic-like activities in acute and chronic models of depression.
and anxiety at a lower dose than the doses tested in earlier studies. One of the major shortcomings of all the marketed antidepressants, regardless of their mechanism of action, is a slower onset (2-4 wk) of therapeutic efficacy. Co-administration of 5-HT antagonist could potentially accelerate the onset as well as the therapeutic potential of the antidepressant and anxiolytic agents. Future studies are needed to clarify the receptor systems responsible for the anxiolytic and antidepressant effects of QCF-21 in animal models.

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Conflicts of Interest: None.

References


*Reprint requests*: Dr Dilip Kumar Pandey, Pharmacology Department, Novel Drug, Discovery and Development, Lupin Research Park, Pune 412 115, Maharashtra, India
e-mail: pandeysdl1408@gmail.com