Anti-depressant like activity of N-n-butyl-3-methoxyquinoxaline-2-carboxamide (60) a 5-HT3 receptor antagonist

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The compound 60 (at 0.5, 1 and 2 mg/kg, ip) with optimum log P and pA2 value, was subjected to forced swim test (FST) and tail suspension test (TST). The compound 60 significantly reduced the duration of immobility in mice without affecting the base line locomotion in actophotometer. Moreover, 60 (2 mg/kg, ip), potentiated the 5-hydroxytryptophan (5-HTP)-induced head twitch responses in mice and at 1 and 2 mg/kg, ip antagonized the reserpine-induced hypothermia (RIH) in rats. In interaction studies with various standard drugs/ligands using FST, 60 (1 and 2 mg/kg, ip) potentiated the anti-depressant effect fluoxetine (5 mg/kg, ip) and reversed the depressant effect of parthenolide (1 mg/kg, ip) by reducing the duration of immobility. Furthermore, 60 (1 and 2 mg/kg, ip) potentiated the effect of bupropion (10 mg/kg, ip) in TST. The behavioural anomalies of the olfactory bulbectomised (OBX) rats were augmented by chronic 60 (1 and 2 mg/kg) treatment as observed from the modified open field test (parameters: ambulation, rearing, fecal pellet). The results suggest that compound 60 exhibited anti-depressant like effect in rodent models of depression.

Keywords: 5-HT3 receptor antagonists, Head twitches, Olfactory bulbectomy, Reserpine induced hypothermia, Tail suspension test

The serotonin, 5-hydroxytryptamine (5-HT) neuro-effector system constituted by its 14 (sub classes 5-HT1-7) receptors and their downstream targets has been the most important area for many recent drug screening strategies targeting various neuro-pathological conditions, especially depression.

In serotonin receptor subtypes 5-HT3 receptors are members of the Cys-loop superfamily of ligand-gated ion channels that includes nicotinic acetylcholine (nACh)-, γ-aminobutyric acid (GABA)A- and glycine receptors and a Zn2+-activated cation channel2,3.

The profound existence of 5-HT3 receptors in the chemoreceptor triggering zone (CTZ) has qualified them as major site for antinauseants/antiemetics4,5. Selective 5-HT3 receptor antagonists, for example, ondansetron, tropisetron and granisetron and dolasetron are now well accepted as drugs of choice in managing cancer chemotherapy-induced and postoperative nausea and vomiting. The promising outcomes from preliminary behavioural tests on 5-HT3 receptor antagonists, their excellent safety data and the complementary effectual regional distribution of 5-HT3 receptors in the CNS have urged further inquiries to establish their potential usage in a range of CNS disorders6,7.

First confirmation of a 5-HT3 receptor expression in the rat brain came from binding studies using the selective 5-HT3 receptor antagonist [3H]GR656308. Studies on humans using selective 5-HT3 receptor ligands revealed heterogeneous distribution throughout the brain within the brainstem e.g. nucleus tractus solitarius, area postrema and spinal trigeminal nucleus9,10 as well as the forebrain, e.g. hippocampus, amygdala, nucleus accumbens, putamen, caudate11,12. The research so far suggests: (i) the localization of 5-HT3 receptors in limbic regions of the rodent and human brain; (ii) the anti-depressant-like effects of some of the 5-HT3 receptor antagonists in rodent behavioural tests; (iii) the 5-HT3 receptor antagonistic property of anti-depressants; and (iv) the genetic association of the 5-HT3 receptor with bipolar disorder.

The involvement of 5-HT3 receptors in depression is complemented by studies of 5-HT3 knockout mice which revealed that 5-HT3 regulates depression- and anxiety-related behaviours12. It is reasonable to conclude that 5-HT3 receptors are involved in the modulation of depression-like behaviour and that pharmacotherapy targeting 5-HT3 receptors could be an alternative option for the treatment of depression and anxiety.

The proposed hypothetical mechanism for anti-depressant effect of 60 is postsynaptic 5-HT3 receptor

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antagonism in serotonergic neurons and facilitation of serotonin binding to other postsynaptic receptors such as 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C}, thereby aiding in serotonergic transmission\textsuperscript{13}.

Previous findings reveal the importance of 5-HT\textsubscript{3} antagonists in the treatment of depression and complex disorders such as fibromyalgia and bulimia showed improvement of the comorbid depression\textsuperscript{14,15}.

Preclinical screening of new chemical entity (NCE) in depression have been utilized vigorously to evaluate the novel compounds\textsuperscript{16}. These tests neglect the aspect of face validity but have a strong predictive validity to aid in the identification of efficient anti-depressant molecules\textsuperscript{17}. Hence, a battery of behavioural tests were adopted for the study which included acute models like the forced swim test (FST)\textsuperscript{18}, tail suspension test (TST)\textsuperscript{19,20} mechanistic models like 5-hydroxytryptophan (5-HTP) induced head twitch response in mice and reserpine induced hypothermia in rats\textsuperscript{21}. Evaluation of chronic effect of the compound was done on olfactory bulbectomised rats\textsuperscript{22,23} provide significant information on anti-depressant activity of \textit{6o}, which was identified for this study based on \textit{pA}{\textsubscript{2}} and log P values.

In the present study, compound \textit{N}-n-butyl-3-methoxyquinoxaline-2-carboxamide (\textit{6o}) which exhibited an optimum log P (2.60) and \textit{pA}{\textsubscript{2}} value (7.7) greater than the standard 5-HT\textsubscript{3} receptor antagonist, ondansetron (OND) (\textit{pA}{\textsubscript{2}} -6.9)\textsuperscript{24,25} has been selected for the preliminary anti-depressant screening in the standard rodent models of depression as mentioned above.

Materials and Methods

\textit{Animals}—Albino mice (25±2 g), Wistar rats (250±20 g) and Dunkin Hartley guinea pigs (370±20 g) were obtained from Hissar Agricultural University, Hissar, India. All procedures were in adherence to Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/4/1, dated 13.08.08). The animals were kept for at least one week before the experiments, at optimum temperature (23 ± 2 °C) and humidity-controlled (50-60 %) animal rooms under a 12:12 h light/dark cycle (light on 06:00–18:00 hrs) with free access to food and water. Behavioural studies were carried out during the light phase (09.00 - 14.00 hrs). The animals were used only once for each experiment.

\textit{Chemistry of \textit{6o}}—The target compound \textit{6o} (\textit{N}-n-butyl-3-methoxyquinoxaline-2-carboxamide), was synthesized by coupling the 3-methoxyquinoxaline-2-carboxylic acid with n-butylamine in presence of coupling agent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) under nitrogen atmosphere. The key intermediate, 3-methoxyquinoxalin-2-carboxylic acid was prepared from the starting material \textit{o}-phenylenediamine in a sequence of reactions. The initial condensation with diethyl ketomalonate, followed by chlorination with phosphorous oxychloride then saponification and finally nucleophilic displacement with sodium methoxide afforded the 3-methoxyquinoxaline-2-carboxylic acid; the synthetic route of \textit{6o} is depicted in Fig.1. The basic pharmacophore of 5-HT\textsubscript{3} receptor antagonists is shown in Fig.2.

\textit{Drugs and chemicals}—Fluoxetine (FLX), paroxetine (PAR) and bupropion (BUP) were obtained as

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**Fig. 1**—Synthesis of \textit{6o} (\textit{N}-n-butyl-3-methoxyquinoxaline-2-carboxamide) (Reagents and conditions: (a) Diethyl ketomalonate, ethanol, reflux, 6 h, 60%; (b) POCl\textsubscript{3}, DMF, reflux, 30 min 80%; (c) Na\textsubscript{2}CO\textsubscript{3}, reflux, 6 h (d) dil. HCl, 94%; (e) NaOCH\textsubscript{3}, methanol, MW, 6 min, dil. HCl, 85%; (f) EDC·HCl, HOBt, THF, N\textsubscript{2}, 0°C–rt, 1h; (g) butylamine, rt, 6 h)

**Fig. 2**—Basic pharmacophore of 5-HT\textsubscript{3} receptor antagonists
gift samples from Cipla Pharmaceuticals and IPCA Laboratories Private Limited, India, respectively. Escitalopram (ESC) was obtained as generous gift samples from Glenmark Pharmaceuticals Ltd., India. Parthenolide was obtained from Tocris chemicals (UK). The drugs for anaesthesia namely, ketamine and xylazine were purchased from Reidel Neon Labs, Indian Immunologicals (Mumbai, India). The drugs were freshly prepared in distilled water with 10% PEG and administered per oral (po) or intra-peritoneally (ip) (as specified) in a constant volume of 10 mL/kg. For interaction studies, the anti-depressants/ligands and standards were administered ip, 45 and 30 min, respectively before testing in FST and TST as per the protocol adopted. The drugs were administered po once a day for 14 days in the chronic treatment schedule.

5-HT\textsubscript{3} receptor antagonistic activity—The compounds were tested for their ability to inhibit the 5-HT\textsubscript{3} receptor in isolated guinea pig ileum and the pA\textsubscript{2} values were determined against 2-methyl-5-hydroxytryptamine. In order to assess the 5-HT\textsubscript{3} receptor antagonistic activity, guinea pigs were sacrificed under mild anesthesia. The abdomen was cut open and a length of ileum excised about 2 cm from the ileo-caecal junction, the longitudinal muscle-myenteric plexus (LMMP), 3-4 cm in length, was prepared and mounted as cited in the literature. The tissue was equilibrated for 30 min under a resting tension of 500 mg with constant aeration in a 40 mL organ bath containing Tyrode solution, maintained at 37 °C.

Non-cumulative concentrations of 2-methyl-5-HT were added with a 15 min dosing cycle (to prevent desensitization) and left in contact with the tissue until the maximal contraction had developed. To study the antagonist effect of the test compounds on the response evoked by 2-methyl-5-HT, the compounds were added to the organ bath and left in contact with the tissue for at least 10 min prior to the addition of 2-methyl-5-HT. The contractions were recorded using a T-305 Force transducer coupled to a Student’s physiograph (Bio Devices, Ambala, India). Antagonism was expressed in the form of pA\textsubscript{2} values, which were graphically determined. The pA\textsubscript{2} values of the test compound were compared with the standard antagonist, ondansetron (Table 1).

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Log P\textsuperscript{a}</th>
<th>pA\textsubscript{2}\textsuperscript{b}</th>
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<tr>
<td>6a</td>
<td>C\textsubscript{6}H\textsubscript{5}</td>
<td>3.02</td>
<td>7.2</td>
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<tr>
<td>6b</td>
<td>4-Me-C\textsubscript{6}H\textsubscript{4}</td>
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<td>7.3</td>
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<tr>
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<td>3.58</td>
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<td>3.8</td>
</tr>
<tr>
<td>6o</td>
<td>CH\textsubscript{3}CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}</td>
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<td>7.7</td>
</tr>
<tr>
<td>7</td>
<td>Pyrrolidinyl-</td>
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<td>Cyclopentyl-</td>
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<td>Cyclohexyl-</td>
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</tr>
<tr>
<td>-</td>
<td>Ondansetron</td>
<td>-</td>
<td>6.9</td>
</tr>
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\textsuperscript{a}Log P values were calculated using ChemBioDraw Ultra 11 (Cambridge Software).
\textsuperscript{b}pA\textsubscript{2} values are the means of two separate experiments. SE was less than 10% of the mean.
Forced swim test (FST)—The FST was carried out as described elsewhere\textsuperscript{18} with slight modifications. Mice were dropped individually into a plexi-glass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water to a depth of 15 cm and maintained at 23–25 °C. After an initial vigorous activity (2 min), the mice acquired an immobile posture which was characterized by motionless floating in the water and making only those movements necessary to keep the head above the water. The duration of immobility which reflects the state of depression was recorded during the last 4 min of the 6 min test. The mice were subjected to 15 min training under similar conditions, 24 h before the test.

Tail suspension test (TST)—Mice were individually suspended by the tail to a horizontal bar (50 cm above the floor) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice exhibited several escape-oriented behaviour interspersed with temporally increasing bouts of immobility\textsuperscript{19,20}. The duration of immobility (in seconds) during the 6 min test session was recorded.

Spontaneous locomotor activity (SLA)—The SLA was assessed using an actophotometer\textsuperscript{26}. The animals were individually placed in a square arena (30×30 cm) with walls painted black and fitted with photocells just above the floor level. The photocells were checked before the beginning of the experiment. After an initial 2 min familiarization period, the digital locomotor scores were recorded for the next 10 min in a dimly lit room. The arena was cleaned with dilute alcohol and dried between trials.

5-Hydroxytryptophan (5-HTP) induced head twitch response—The method mentioned elsewhere\textsuperscript{21} was adopted with slight modifications. The vehicle/drug treated mice were injected with pargyline (75 mg/kg, ip) and 5-HTP (5 mg/kg). 30 and 15 min prior to drug administration respectively and gently placed in separate, clear plexi-glass cages (12×12×10 cm\textsuperscript{3}). The total number of head twitches (characterized by abrupt lateral movements) episodes were recorded for the next 15 min.

Interaction studies in FST and TST—The animals were treated either with vehicle or with one of the following test compounds namely, fluoxetine (5 mg/kg, ip), a selective serotonin re-uptake inhibitor, parthenolide (1 mg/kg, ip), a serotonin release inhibitor, Interaction of bupropion (10 mg/kg, ip), a nor-ephinephrine and dopamine re-uptake inhibitor was carried out in TST since it is more sensitive than that of FST\textsuperscript{27}.

Reserpine induced hypothermia (RIH) in rats—Rats were gently hand-restrained and the glycerol lubricated digital thermometer probe was inserted into the rectum. The rectal temperature of the rats treated with reserpine (1 mg/kg, ip) was recorded at 30, 60, 90 and 120 min after the drug administration. The difference in the rectal temperature between the baseline and 60\textsuperscript{th} min values were tabulated. On the day preceding of experimentation, the rectal temperature of the rats was assessed in a similar manner in order to habituate the animals to the experimental procedures\textsuperscript{28}.

Olfactory bulbectomy

Surgery—Bilateral olfactory bulbectomy was carried out as described earlier\textsuperscript{22,23}. In brief, the rat was anaesthetized with a combination of ketamine (75 mg/kg, ip) and xylazine (5 mg/kg, ip). The head was fixed to a stereotaxic frame (Inco, India) and the skull exposed by a midline incision. Burr holes (2 mm in diameter) were drilled 8 mm anterior to bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the orbit of the eye. The dura was punctured and the olfactory bulbs were removed by suction. Haemostatic sponge was used to prevent excessive bleeding and to fill the dead space. The scalp was then sutured and the wound was dabbed with antiseptic solution. Sham surgery was carried out in the same way (including the piercing of the dura matter) except that olfactory bulbs were left intact. Sulprim (each mL containing 200 and 40 mg of sulphadiazine and trimethoprim respectively), was administered (0.2 mL/300 g, im) once a day for the first 3 days for both OBX and sham operated rats. The animals were individually housed for the first 3 days following surgery and thereafter in groups of two (one sham and one OBX rat) until the end of the study. The olfactory bulbectomised and sham rats were allowed 14 day rehabilitation period during which they were handled by the same experimenter to prevent aggression which would have developed, otherwise. The surgery, rehabilitation, treatment and behavioural screening of OBX/sham rats were carried out based on the customized schedule\textsuperscript{22}.

Open field test (OFT) behaviour—OBX and sham control rats were subjected to OFT on 29\textsuperscript{th} day after surgery. The open field exploration was conducted as described elsewhere\textsuperscript{22} with subantial modifications. The apparatus consisted of a circular (90 cm dia.) arena with 75 cm high aluminium walls and floor equally divided into 10 cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which
was the only source of illumination in the testing room. Each animal was individually placed in the center of the OFT apparatus and the following parameters such as ambulation scores (number of squares crossed), number of rearing episodes (when the rat stands upright on its hind paws) and number of fecal pellets were noted for 5 min by a trained observer, unaware of the specific treatments. After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly.

Statistical analysis—Data were expressed as the mean ± SE. The single treatment studies were analyzed using a one-way analysis of variance followed by a post-hoc Dunnett test. The interaction studies were analyzed using a two-way analysis of variance followed by a post-hoc Tukey test. The level of statistical significance was fixed at $P < 0.05$.

Results

Based on the $pA_2$ and log P values, compound 6o was chosen from the series of compounds synthesized in our laboratory for extensive behavioural pharmacological studies.

In FST, the acute treatment with 6o (1 and 2 mg/kg, ip) significantly decreased the duration of immobility as compared to vehicle treatment (Fig. 3). Similarly, a significant decrease in duration of immobility was evident at 6o (1 and 2 mg/kg, ip) in the TST (Fig. 4). The standard drugs, escitalopram (10 mg/kg, ip) and bupropion (20 mg/kg, ip) also significantly reduced the immobility duration in FST and TST respectively, as compared to the control group. Locomotor activity of mice was not affected at three dose levels (0.5, 1.2 mg/kg, ip) except 4 and 8 mg/kg, ip as indicated by actophotometer study (Fig. 5). Depletion of brain neurotransmitters induced by reserpine affects the central nervous system as demonstrated by reserpine induced hypothermia. Administration of reserpine (1 mg/kg, ip) elicited a pronounced decrease in core body temperature of rats. This effect was significantly ($P < 0.05$) reversed by 6o (1 and 2 mg/kg, ip) and escitalopram (10 mg/kg) treatments (Fig. 6).

5-HTP-induced head twitches were performed to confirm the involvement of serotonergic pathway. The combination of 5-HTP (5 mg/kg, ip) and pargyline (75 mg/kg, ip) induced the characteristic head twitch response. 6o (2 mg/kg, ip) and fluoxetine (20 mg/kg, ip) significantly potentiated the 5-HTP/pargyline induced head twitches, respectively (Fig. 7).

The reference compounds were tested individually to determine their effects in FST and TST. Compound 6o (1 and 2 mg/kg, ip) significantly ($P < 0.05$) affected the duration of immobility throughout the interaction study as compared to control.

For a conclusive evaluation of anti-depressant potential of 5-HT$_3$ receptor antagonists, interaction studies with SSRIs were carried out. 6o pre-treatment (1 and 2 mg/kg, ip) was found to enhance anti-depressant-like effects of FLX (5 mg/kg, ip) (Fig. 8) and also significantly reversed the depressant-like effect of parthenolide (1 mg/kg, ip) as described.

![Fig. 3—Effect of 6o on duration of immobility of mice FST.](image)

![Fig. 4—Effect of 6o on duration of immobility of mice TST.](image)

![Fig. 5—Effect of 6o on spontaneous locomotor activity of mice.](image)
shown (Fig. 9). Further, 6o (1 and 2 mg/kg, ip) significantly enhanced the anti-depressant activity of BUP (10 mg/kg, ip) in mice TST (Fig. 10).

The effects of 6o on the behaviour of OBX/sham rats were analyzed in different circumstances as shown in Table 2. Chronic (14 days, po) treatment with 6o (1 and 2 mg/kg, ip) significantly ($P < 0.05$) reduced the number of ambulation, rearing and fecal pellets in OBX rats as compared to the vehicle treated OBX rats.

**Discussion**

Various preclinical studies have indicated that 5-HT$_3$ receptor antagonists exert anti-depressant effect by blocking limbic hyperactivity response$^{29}$. The results of this behavioural investigation divulge the antidepressant-like effects of 6o. Since 5-HT$_3$ receptors are expressed in nucleus tractus solitarius, area postrema and spinal trigeminal nucleus as well as the forebrain, e.g. hippocampus, amygdala, nucleus accumbens, putamen, caudate indicate their role in depression and anxiety$^{30,31}$. The forced swimming test (FST) takes advantage of the observation that rodents, following initial escape oriented movements in an inescapable situation (in a cylinder filled with water), rapidly adopt a characteristic immobile posture (indicative of despair). It is also a useful tool in the better understanding of the role of specific monoamines and receptor subtypes implicated in depressive states. The tail suspension test (TST) is a similar procedure in which anti-depressant like activity of a compound is determined by a decrease in the duration of immobility. Both of these models of depression are widely used to screen NCE for their anti-depressant potential$^{18,19}$. These tests are quite sensitive and relatively specific to all classes of anti-depressants. In the present study, 6o significantly
depressant activity on treated group. 

Table 2— Effects of paroxetine (10 mg/kg, po) and 6o (1 and 2 mg/kg, po) open field behaviour (ambulation/ Rearing/Fecal pellet) in OBX/sham rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ambulation score</th>
<th>Rearing score</th>
<th>Fecal Pellet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Control</td>
<td>91.17±6.88</td>
<td>10±1.24</td>
<td>2.17±0.47</td>
</tr>
<tr>
<td>Sham +6o (1)</td>
<td>103.00±8.87</td>
<td>9.3±1.05</td>
<td>2.33±0.71</td>
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<tr>
<td>Sham +6o (2)</td>
<td>102.17±7.34</td>
<td>8.3±1.15</td>
<td>2.00±0.86</td>
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<tr>
<td>Sham +Par (10)</td>
<td>99.67±9.37</td>
<td>8.67±7.1</td>
<td>2.00±5.8</td>
</tr>
<tr>
<td>OBX Control</td>
<td>206±7.48*</td>
<td>27.33±4.58*</td>
<td>7.00±0.76*</td>
</tr>
<tr>
<td>OBX +6o (1)</td>
<td>133.00±8.82#</td>
<td>13.50±1.01#</td>
<td>6.00±0.62#</td>
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<tr>
<td>OBX +6o (2)</td>
<td>113.5±8.95#</td>
<td>22.83±1.19#</td>
<td>5.00±0.65#</td>
</tr>
<tr>
<td>OBX +Par (10)</td>
<td>113±7.34#</td>
<td>11.83±5.9#</td>
<td>2.33±0.47#</td>
</tr>
</tbody>
</table>

P < 0.05 when compared with sham operated rats, #vehicle-treated OBX rats (n = 8 per group). Par-Paroxetine

decreased the duration of immobility in mice during both FST and TST. The decreased duration of immobility in mice FST and TST reflects the anti-depressant-like activity of a compound.18,19

In mechanistic models, reserpine is a non specific monoamine depleting agent, which acts by blocking the monoamine transport in to synaptic vesicle. The depletion of brain biogenic amines affect the central nervous system characterized by hypothermia.28 The decrease in body temperature induced by reserpine was reported to be antagonized by anti-depressants.28 6o and escitalopram significantly prevented the hypothermic effect of reserpine exhibiting anti-depressant effects in this sensitive model.

5-HTP being the immediate precursor of 5-HT, significantly increases the serotonergic transmission inducing a characteristic head twitch response in mice. 6o increased the head twitches in presence of pargyline, a MAO inhibitor. In this regard, the anti-depressant like effect of 6o appears to be modulated by an increase in monoamines; particularly 5-HT concentrations in the synapse.21 On the basis of results obtained in various mechanistic studies like potentiation of head twitch responses and reversal of reserpine induced hypothermia suggest that, compound 6o exhibited anti-depressant-like effect by increasing the concentration of neurotransmitter in the synapse. Increase in serotonin level through blockade of 5-HT3 receptor could possibly explain the overall anti-depressant-like effect of 6o. As these mechanistic studies hint that compound 6o possibly act through the modulation of serotonin in synaptic cleft, further interaction studies were performed.

Interaction studies with established anti-depressants in FST and TST were carried out not only to predict the probable mechanism (possible receptor targets) of anti-depressant like effects of 6o, but also to pharmacologically validate the 6o induced anti-depressant like behaviour in the above mentioned predictive tests. For a conclusive evaluation of anti-depressant potential of compound 6o interaction studies with fluoxetine, parthenolide and bupropion were carried out.

Compound 6o (1 and 2 mg/kg, ip), significantly enhanced the anti-depressant-like action of fluoxetine (5 mg/kg, ip) in FST and bupropion (10 mg/kg, ip) in TST. Though the exact mechanism behind anti-depressant-like effect of compound 6o is not clearly understood, nevertheless it could be due to the modulation of monoamnergic system. Further to explore the serotonergic influence of compound 6o, interaction study with parthenolide in FST was carried out. Parthenolide is a serotonin release inhibitor that produces depressant-like effect. A depressant-like effect induced by parthenolide was considered as a model to identify anti-depressants acting through serotonergic mechanisms.32 Reversal of parthenolide (1 mg/kg, ip) induced depressogenic effect by Compound 6o (1 and 2 mg/kg, ip) indicates that it possibly acting through increase in serotonin level.

Olfactory bulbectomy has been proposed to be an agitated hypo-serotonergic model of depression and is used to explore the anti-depressant potential of novel agents.32 OBX rats exhibited a specific, abnormal behavioural pattern in the open field test characterized by increased ambulation, rearing and fecal pellets, and this abnormal behaviour is reversed by anti-depressants.32 The Increased locomotor/exploratory behaviour of OBX rat in open field test may be due to
exposure to a novel environment. Moreover, other possible reason for hyperactivity of OBX rats in OFT may be due to decrease in activity in competing behaviour or delay/failure to habitate to novel environment. Compound 6o (1 and 2 mg/kg, ip) significantly ($P < 0.05$) reduced the number of ambulation, rearing and fecal pellets in OBX rats as compared to the vehicle treated OBX rats which may be due increase in serotonin level.

**Conclusion**

The present neuro-behavioural investigation showed anti-depressant-like effects of 6o, a novel 5-HT$_3$ antagonist in animal models of depression. Compound 6o, showed anti-depressant like effects in rodent models of depression, without affecting the locomotion of rodents. The present study indicate that compound 6o possibly acting by enhancing the level of monoamine in synapse through 5-HT$_3$ receptor antagonism. These results correlated the beneficial effects of 5-HT$_3$ receptor antagonist in depression. Compound 6o potentiates the effect of established anti-depressants, which indicate the combination of standard anti-depressants with 6o can potentially accelerate the onset of action. Further studies with compound 6o are required to furnish a clinically useful anti-depressant agent.

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**References**


