



Antidepressant Potential of 5-HT₃ Receptor Antagonist, N-n-propyl-3-ethoxyquinoxaline-2-carboxamide (6n)

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ABSTRACT

The present study was designed to evaluate the antidepressant potential of 5-HT₃ receptor antagonist N-n-propyl-3-ethoxyquinoxaline-2-carboxamide (6n). The compound '6n' with optimum log P and pA₂ value identified from a series of compounds synthesized in our laboratory was subjected to forced Swim Test (FST) (1, 2, and 4 mg/kg, i.p.) and Tail Suspension Test (TST) (1, 2, and 4 mg/kg, i.p.). The compound '6n' significantly reduced the duration of immobility in mice without affecting the baseline locomotion. Moreover, '6n' (2 mg/kg, i.p.) potentiated the 5-hydroxytryptophan (5-HTP)-induced head twitch responses in mice and '6n' at tested dose (1 and 2 mg/kg, i.p.) reversed the reserpine-induced hypothermia in rats. In interaction studies of '6n' with various standard drugs/ligands using FST, '6n' (1 mg/kg, i.p.) potentiated the antidepressant effect of venlafaxine (4 and 8 mg/kg, i.p.) and fluoxetine (10 and 20 mg/kg, i.p.). Additionally, '6n' (1 and 2 mg/kg, i.p.) influenced the effect of harmaline (5 mg/kg, i.p.) as well as reversed the effect of parthenolide (1 mg/kg, i.p.) by reducing the duration of immobility in FST. Furthermore, '6n' (1 mg/kg, i.p.) potentiated the effect of bupropion (10 and 20 mg/kg, i.p.) in TST. Chronic '6n' (1 and 2 mg/kg, i.p.) treatment attenuated the behavioral abnormalities in olfactory bulbectomized rats. In conclusion, these various findings reiterated the antidepressant-like effects of '6n' in behavioral models of depression.

Key words: 5-HT₃ receptor antagonists, forced swim test, head twitch, reserpine, tail suspension test

INTRODUCTION

Depression is a chronic, recurring, and potentially life-threatening illness that affects up to 20% of the population across the globe.^[1-4] It is one of the top ten causes of morbidity and mortality worldwide, based on

a survey by the World Health Organization. Despite a steady increase in the number of antidepressants over the years, the prevalence of the disorder remains stable which may be due to unclear pathophysiology or the inconsistent efficacy of currently available antidepressants with undesirable side effects. However, there is a direct correlation between the catecholaminergic neuronal systems and depression. Serotonin is the major neurotransmitter involved in the depression. Till now, seven superfamilies of serotonin receptors are identified; in that, serotonin type 3 (5-HT₃) receptors are pentameric ligand-gated ion channels belonging to the superfamily of Cys-loop receptors. 5-HT₃ receptors are known to be expressed in the central nervous system in regions

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involved in the vomiting reflex, processing of pain, the reward system, cognition, depression and anxiety control. The abundance of 5-HT₃ receptors in the chemoreceptor triggering zone has qualified them as primary targets for anti-emetic agents. Selective 5-HT₃ receptor antagonists, such as; ondansetron (OND) and tropisetron are now recognized as drugs of choice in managing cancer chemotherapy-induced and postoperative nausea and vomiting.^[5,6] The motivating outcomes from preliminary behavioral tests on 5-HT₃ receptor antagonists, their good safety profile, and the complementary effectual regional distribution of 5-HT₃ receptors in the central nervous system have urged further research to establish their potential usage in a range of central nervous system disorders. Studies on human beings using selective 5-HT₃ receptor antagonists discovered heterogeneous distribution throughout the brain within the brainstem, e.g., nucleus tractus solitarius, area postrema, and spinal trigeminal nucleus as well as the forebrain, e.g., hippocampus, amygdala, nucleus accumbens, putamen, caudate.^[7,8]

The role of 5-HT₃ receptors in anxiety is confirmed by studies of 5-HT_{3A} knockout mice which revealed that 5-HT_{3A} receptor subtypes regulate depression- and anxiety-related behaviors.^[9] Evidence for the relevance of 5-HT₃ receptor antagonists in the treatment of depression stems from clinical trials in which patients suffering from complex disorders such as fibromyalgia and bulimia showed improvement of the comorbid depression.^[10,11]

According to proposed hypothesis by Rajkumar and Mahesh (2010), postsynaptic 5-HT₃ receptor antagonism in serotonergic neurons can facilitate specific binding of 5-HT to other postsynaptic receptors such as 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C}, thereby aiding in serotonergic transmission.^[12] Thus, the paucity of evidence on the direct influence of 5-HT₃ receptor antagonist treatment on rodent depression-like behavior triggered the present study with objectives of which are (i) to screen the molecule '6n', preclinically in various behavioral and mechanistic rodent models of depression (ii) to conduct drug interaction studies with conventional antidepressants/ligands in order to propose the plausible mechanism underlying the above effect.

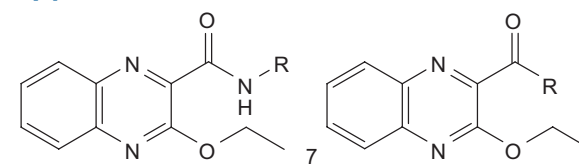
Using the three-component pharmacophore mode,^[13] a series of 5-HT₃ receptor antagonists were designed, synthesized, and screened for their 5-HT₃ antagonist potential [Table 1]. The compounds were tested for their ability to inhibit the 5-HT₃ receptor in isolated guinea pig ileum, and the pA₂ values were determined against

2-methyl- 5-hydroxytryptamine with OND as the reference drug.^[6,14]

Preclinical screening of new chemical entity (NCE) in depression have been utilized vigorously to evaluate the novel compounds.^[15] These tests neglect the aspect of face validity but have a strong predictive validity to aid in the identification of efficient antidepressant molecules.^[16] Hence, a battery of behavioral tests were adopted for the study which included acute models like Forced Swim Test (FST),^[17] Tail Suspension Test (TST),^[18,19] mechanistic models like 5-hydroxytryptophan (5-HTP)-induced head twitch response in mice, and reserpine-induced hypothermia (RIH).^[20] Evaluation of chronic effect of the compound was studied on olfactory bulbectomized (OBX) rats^[21,22] to provide significant information on antidepressant activity of 6n, which was identified for this study based on pA₂ and log P values.

In the present study, compound *N*-*n*-propyl-3-ethoxyquinoxaline-2-carboxamide (6n) which exhibited an optimum log P and pA₂ values (pA₂-7.6) greater than the standard 5-HT₃ receptor antagonist, OND (pA₂-6.9),^[23,24] was selected for the preliminary antidepressant screening in the standard rodent models of depression as mentioned above.

Table 1: Log P and pA₂ values of series of 3-ethoxyquinoxali-2-carboxamides



Compound	R	Log P ^a	pA ₂ ^b
6a	C ₆ H ₅ -	3.36	7.8
6b	4-Me-C ₆ H ₄ -	3.85	5.7
6c	4-MeO-C ₆ H ₄ -	3.23	6.1
6d	C ₆ H ₅ -CH ₂ -	3.43	6.2
6e	C ₆ H ₅ -NH-	2.88	5.8
6f	3-Ac-C ₆ H ₄ -	2.67	6.1
6n	3-Cl-C ₆ H ₄ -	3.92	5.4
6h	4-NO ₂ -C ₆ H ₄ -	3.36	5.5
6i	2-pyridinyl-	2.74	6.2
6j	3-Cl-2-CH ₃ -C ₆ H ₃ -	4.41	7.4
6k	Benzothiazol-2-yl-	4.56	6.8
6l	4-Benzamido-phenyl-	4.17	5.3
6m	2-Benzamido-phenyl-	4.17	6.2
6n	CH ₃ CH ₂ CH ₂ -	2.52	7.6
6o	CH ₃ CH ₂ CH ₂ CH ₂ -	2.94	7.6
7	Pyrrolidinyl	2.25	4.9
6p	Cyclopentyl-	2.83	4.8
6q	Cyclohexyl-	3.25	7.8
-	Ondansetron	1.7	6.9

^alogP values were calculated using ChemBioDraw Ultra 11 (Cambridge Software)

^bpA₂ values are the means of two separate experiments. SE was less than 10% of the mean

MATERIALS AND METHODS

Animals

Albino mice (25 ± 2 g), Wistar rats (250 ± 20 g), and Dunkin Hartley guinea pigs (370 ± 20 g) were obtained from Hissar Agriculture University, Haryana, India. All procedures were in adherence to protocol approved by Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology and Science, Pilani, India (Protocol No. IAEC/RES/14/04, dated 07.09.11). The animals were kept for at least one week before the experiments, at optimum temperature ($23 \pm 2^\circ\text{C}$) and humidity-controlled (50-60%) animal rooms under a 12:12-h light/dark cycle (light on 6:00–18:00 h) with free access to food and water *ad libitum*. Behavioral studies were carried out during the light phase (9.00 a.m. - 2.00 p.m.). The animals were used only once for each experiment.

Chemistry of 6n

The target compound 6n (*N*-*n*-propyl-3-ethoxyquinoxaline-2-carboxamide) was synthesized by coupling the 3-ethoxyquinoxalin-2-carboxylic acid with *n*-propylamine in the presence of coupling agents 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC•HCl) and 1-hydroxybenzotriazole (HOBT) under nitrogen atmosphere. The key intermediate, 3-ethoxyquinoxalin-2-carboxylic acid, was synthesized from the starting material *o*-phenylenediamine in a sequence of reactions, involving cyclic condensation with diethyl ketomalonate, chlorination with phosphorous oxychloride followed by saponification then nucleophilic displacement with sodium ethoxide, the synthetic route of 6n is depicted in Figure 1.

The basic pharmacophore of 5-HT₃ receptor antagonists is shown in Figure 2.

Drugs and chemicals

Fluoxetine (FLX), paroxetine (PAR), venlafaxine (VLA), and bupropion (BUP) were obtained as gift samples from Cipla Pharmaceuticals and IPCA Laboratories Private Limited, India, respectively. Escitalopram (ESC) was obtained as generous gift samples from Glenmark Pharmaceuticals. Harmane was purchased from Tocris Bioscience, U.K. The drugs for anesthesia, namely ketamine and xylazine, were purchased from Reidel Neon Labs, Indian Immunologicals (Mumbai, India). The drugs were freshly prepared in distilled water and administered per os (p.o.) or intra-peritoneally (i.p.) (as specified) in a constant volume of 10 ml/kg. For interaction studies, the antidepressants/ligands and standards were administered i.p., 45 and 30 minutes, respectively, before testing in FST and TST as per the protocol adopted, earlier in our laboratory. The drugs were administered p.o. once a day for 14 days in the chronic treatment schedule.

5-HT₃ receptor antagonistic activity

The compounds '6n' was tested for its ability to inhibit the 5-HT₃ receptor in isolated guinea-pig ileum, and the pA₂ value was determined against 2-methyl-5-hydroxytryptamine.^[23,24] In order to assess the 5-HT₃ 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, 3-4 cm in length, was prepared and mounted as cited in the literature. The tissue was equilibrated for 30 minutes under

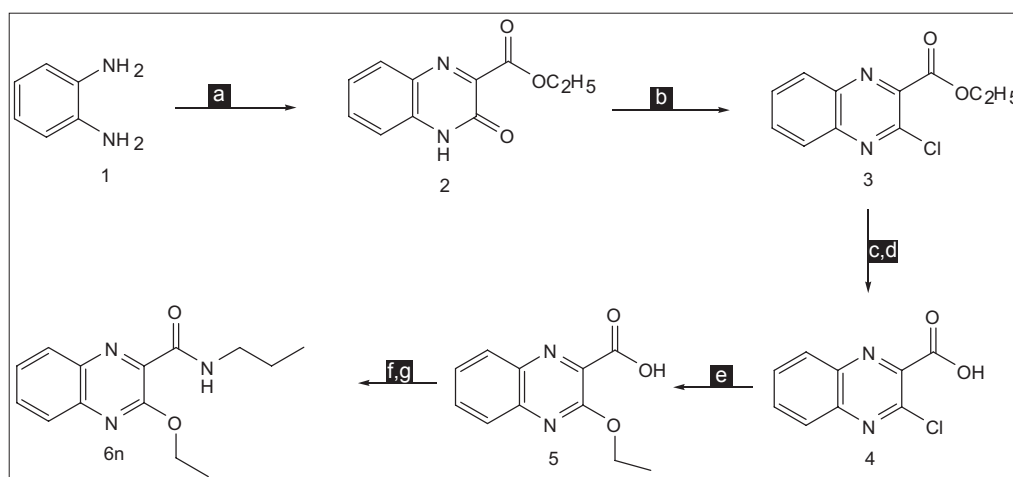


Figure 1: Synthesis of 6n (*N*-*n*-propyl-3-ethoxyquinoxaline-2-carboxamide), Reagents and conditions: (a) Diethyl ketomalonate, ethanol, reflux, 6 h., 60%; (b) POCl₃, DMF, reflux, 30 min. 80%; (c) Na₂CO₃, reflux, 6 h. (d) dil. HCl, 94%; (e) NaOCH₂CH₃, ethanol, MW, 6 min., dil. HCl, 85%; (f) EDC•HCl, HOBT, THF, N₂, 0°C–rt, 1h.; (g) propylamine, rt, 6h

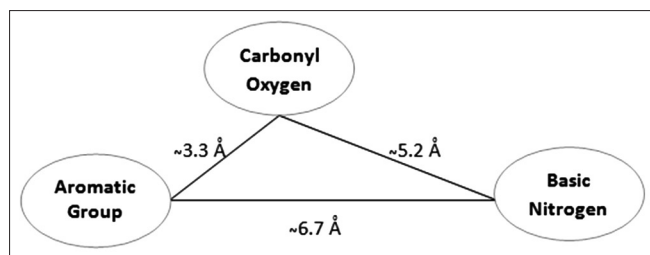


Figure 2: Basic pharmacophore of 5-HT₃ receptor antagonists

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-minute 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 minutes 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_2 values, which were graphically determined. The pA_2 values of the test compound were compared with the standard antagonist, OND.

Forced swim test

The FST was carried out as described elsewhere,^[17] 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 minutes), the mice acquired an immobile posture in this test, which was characterized by motionless floating in the water, 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 minutes of the 6-minute test. The mice were subjected to 15-minute training under similar conditions, 24 hours before the test.

Tail suspension test

Mice were individually suspended by the tail to a horizontal bar (distance from floor was 50 cm) using scotch tape (distance from tip of tail was approximately 1 cm). Typically, mice exhibited several escape-oriented behavior interspersed with temporally increasing bouts of

immobility.^[18,19] The duration of immobility (in seconds) during the 6-minute test session was recorded.

Spontaneous locomotor activity

The spontaneous locomotor activity was assessed using an actophotometer.^[25] The animals were individually placed in a square arena (30 cm × 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 two minute familiarization period, the digital locomotor scores were recorded for the next 10 minutes in a dimly lit room. The arena was cleaned with dilute alcohol and dried between trails.

5-hydroxytryptophan-induced head twitch response

The method mentioned elsewhere^[26] was adopted with substantial modifications. The vehicle/drug treated mice were injected with pargyline (75 mg/kg i.p) and 5-HTP (5 mg/kg), 30 and 15 minutes prior to drug administration, respectively, and gently placed in separate, clear plexi-glass cages (12 × 12 × 10 cm). The total number of head twitches (characterized by abrupt lateral movements) episodes was recorded for the next 15 minutes.

Interaction studies in forced swim test and tail suspension test

The animals were treated either with vehicle or with one of the following test compounds namely, FLX (10 and 20 mg/kg, i.p.), a serotonin re-uptake inhibitor, VLA (4 and 8 mg/kg, i.p.), a serotonin norepinephrine (NE) reuptake, inhibitor harmine (5 mg/kg, i.p.), a monoamine oxidase inhibitor, parthenolide (1 mg/kg i.p.), a serotonin release inhibitor, Interaction of BUP (10 and 20 mg/kg, i.p), a NE and dopamine re-uptake inhibitor was carried out in TST since it is more sensitive than that of FST.^[27,28] All the standard drug doses were adopted from the previous work and standard responses recorded.^[27,28]

Reserpine-induced hypothermia 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, i.p.) was recorded at 30, 60, 90, and 120 minutes after the drug administration. The difference in the rectal temperature between the baseline and 60th minute values were recorded. On the day preceding to experimentation, the rectal temperature of the rats were assessed in a

similar manner in order to habituate the animals to the experimental procedures.^[29]

Olfactory bulbectomy

Bilateral olfactory bulbectomy (OBX) was carried out as described earlier.^[21,22] In brief, the rat was anesthetized with a combination of ketamine (75 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). 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. Hemostatic 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 sulfadiazine and trimethoprim, respectively), was administered (0.2 ml/300 g, i.m.) 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 OBX 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 behavioral screening of OBX/sham rats were carried out based on the customized schedule previously reported.^[21]

Open field test behavior

OBX and sham control rats were subjected to the Open field test (OFT) on the 29th day after surgery. The open field exploration was conducted as described elsewhere,^[21] with substantial modifications. The apparatus consisted of a circular (90 cm diameter) arena with 75 cm high aluminium-walled 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 minutes by a trained observer, unaware of the specific treatments (observer blind). After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly.^[22]

Statistical analysis

Data are expressed as the mean \pm SD. The single treatment studies were analyzed using a one-way analysis of variance followed by a *post-hoc* Dunnett's test and Tukey's test (FST and TST). The interaction studies were analyzed using a two-way analysis of variance followed by a *post-hoc* Bonferroni test. The level of statistical significance was fixed at $P < 0.05$.

RESULTS

Based on the pA_2 and log P values, compound 6n was taken for extensive behavioral pharmacological testing from amongst the series of compounds.

In FST, the acute treatment with 6n (1, 2, and 4 mg/kg, i.p.) significantly decreased the duration of immobility as compared with vehicle treatment [Figure 3]. Likewise, a significant decrease in duration of immobility was evident at 6n (1, 2, and 4 mg/kg) in the TST [Figure 4]. The positive control, ESC (10 mg/kg) and BUP (20 mg/kg), also significantly reduced the duration of immobility in FST and TST, respectively, as compared with the control group. None of the tested doses influenced the locomotion of mice in actophotometer [Figure 5]. Depletion of brain serotonin induced by reserpine affects the central nervous system as demonstrated by RIH. Administration of reserpine (1 mg/kg i.p) elicited a pronounced decrease in core body temperature of rats. This effect was significantly ($P < 0.05$) reversed by 6n, ESC (10 mg/kg) treatments [Figure 6].

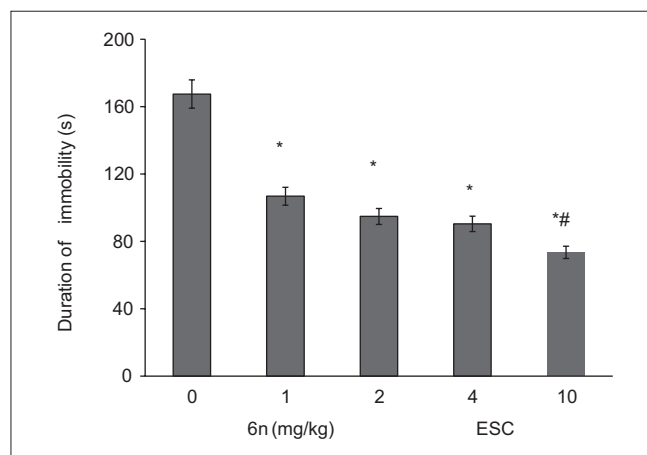


Figure 3: Effect of 6n on duration of immobility of mice FST. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ compared with vehicle treated group. # $P < 0.05$, ESC compared with 1 mg/kg treatment group. ESC = Escitalopram

5-HTP-induced head twitches were performed to confirm the involvement of serotonergic pathway. The combination of 5-HTP (5 mg/kg, i.p.) and pargyline (75 mg/kg, i.p.) induced the characteristic head twitch response. 6n (2 mg/kg, i.p.) and FLX (20 mg/kg, i.p.) significantly potentiated the 5-HTP/pargyline-induced head twitches, respectively [Figure 7].

The peak dose of 6n (1 and 2 mg/kg, i.p.) that induced a significant ($P < 0.05$) decrease in the duration of immobility was selected for the interaction studies. The reference compounds were tested individually to determine their effects in FST and TST. 6n (1 and 2 mg/kg, i.p.) significantly ($P < 0.05$) affected the duration of immobility throughout the interaction study as compared with control.

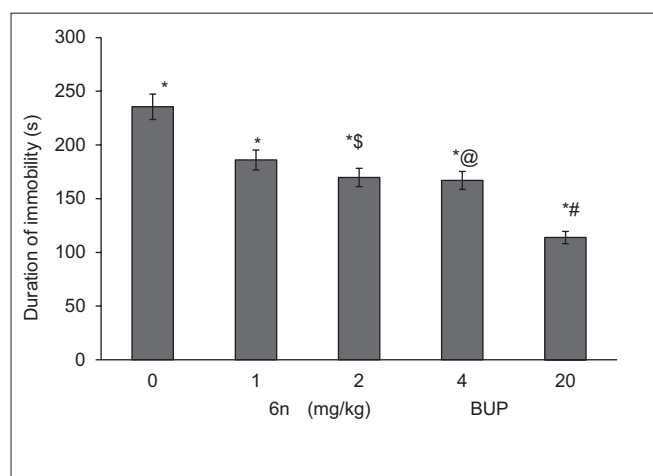


Figure 4: Effect of 6n on duration of immobility of mice TST. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ compared with vehicle treated group. # $P < 0.05$, BUP compared with all treatment group. § $P < 0.05$, 2 mg/kg compared with 1 mg/kg treated group. @ $P < 0.05$, 4 mg/kg compared with 1 mg/kg treated group. BUP = Bupropion

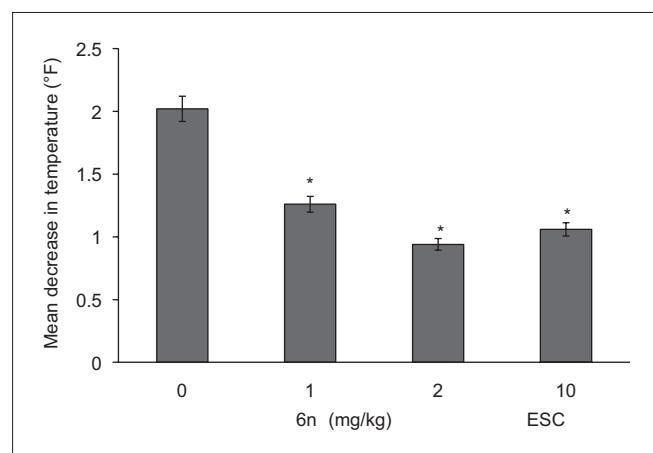


Figure 6: Effect of 6n on reserpine-induced hypothermia in rats. The columns represent mean decrease in temperature (°C) and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ compared with vehicle-treated group. ESC = Escitalopram

For a conclusive evaluation of antidepressant potential of 5-HT₃ receptor antagonists, interaction studies with SSRIs were carried out. Animals pretreated with 6n (1 mg/kg, i.p.) was found to enhance antidepressant-like effects of FLX (10 and 20 mg/kg, i.p.) and VLA (4 and 8 mg/kg, i.p.) as shown in Figures 8 and 9, respectively. Pre-treatment with 6n (1 and 2 mg/kg, i.p.) enhanced the antidepressant-like effects of harmaline (5 mg/kg, i.p.) [Figure 10] indicating the facilitatory effects of 6n on the serotonergic neurotransmission. 6n significantly reversed the depressant-like effect of parthenolide (1 mg/kg, i.p.) as shown in Figure 11. Further, 6n (1 mg/kg) significantly enhanced the antidepressant activity of BUP (10 and 20 mg/kg) in mice TST [Figure 12].

The effects of 6n on the behavior of OBX/sham rats

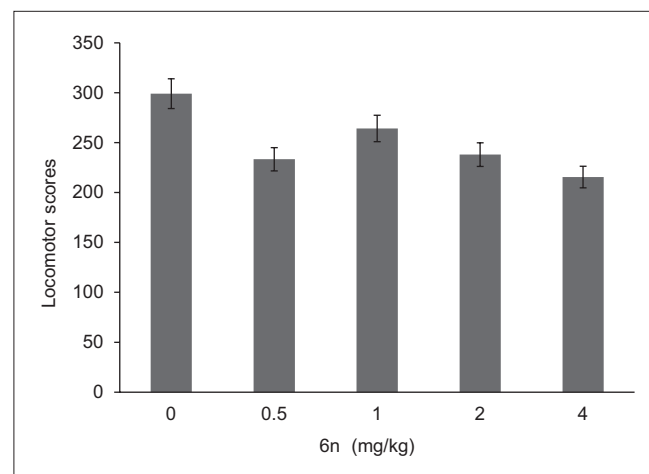


Figure 5: Effect of 6n on spontaneous locomotor activity of mice. The columns represent mean locomotor scores recorded in a 10-minute observation period. The error bars indicate S.D., $n = 8$ per group

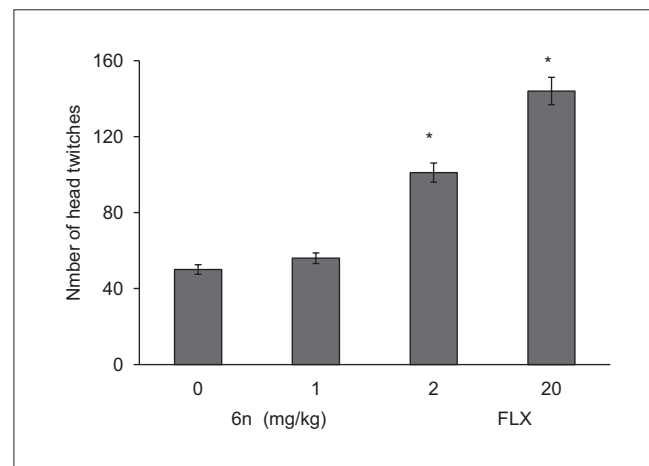


Figure 7: Effect of 6n and fluoxetine (FLX) on 5-hydroxytryptophan (5 mg/kg) + pargyline (75 mg/kg)-induced head twitch response in mice. The columns represent mean number of head twitches and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ compared with vehicle-treated group. FLX = Fluoxetine

were analyzed in different circumstances as shown in Table 2. Chronic (14 days, p.o) treatment with 6n (1 and 2 mg/kg) significantly ($P < 0.05$) reduced the number of ambulation, rearing, and fecal pellets in OBX rats as compared with the vehicle-treated OBX rats. Moreover, OND (1 mg/kg, i.p.) and PAR (10 mg/kg) also significantly reduced the number of ambulation, rearing, and fecal pellets as compared with control group.

DISCUSSION

Preclinical studies led to the consensus that 5-HT₃ receptor antagonists have antidepressant effects by blocking limbic hyperactivity response.^[12] The results of this behavioral

investigation divulge the antidepressant-like effects of 6n. Since 5-HT₃ 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] as the areas mentioned affected in these disorders. The 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 TST is similar and the antidepressant-like activity of a compound is determined by

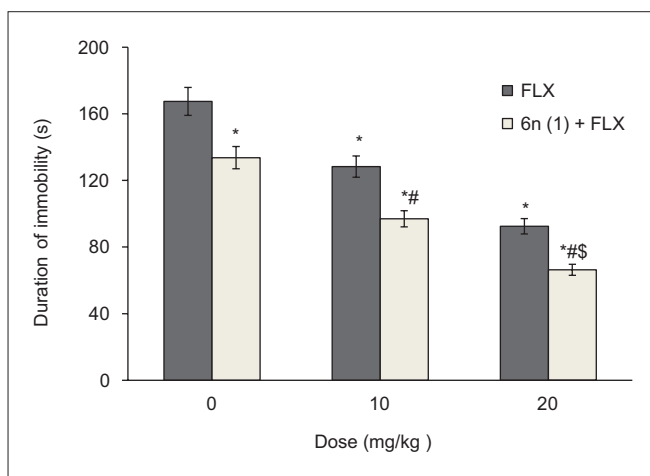


Figure 8: Effect of 6n (1 mg/kg) on antidepressant activity of fluoxetine (10 and 20 mg/kg) in mice FST. The columns represent mean duration of immobility (s) and error bars indicate S.D., $n = 8$ per group. * $P < 0.05$ compared with vehicle-treated group. # $P < 0.05$ compared with fluoxetine (FLX) treated group. \$ $P < 0.05$ compared with 6n treated group

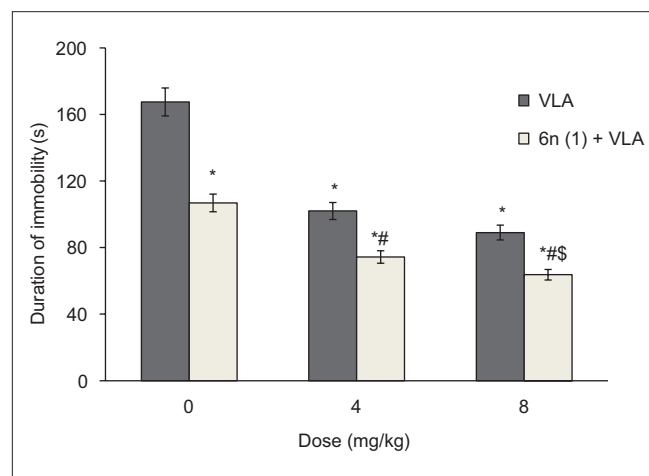


Figure 9: Effect of 6n (1 mg/kg) on antidepressant activity of venlafaxine (VLA) in mice FST. The columns represent mean duration of immobility (s) and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ compared with vehicle-treated group. # $P < 0.05$ compared with venlafaxine (4 and 8 mg/kg) treated group. \$ $P < 0.05$ compared with 6n-treated group

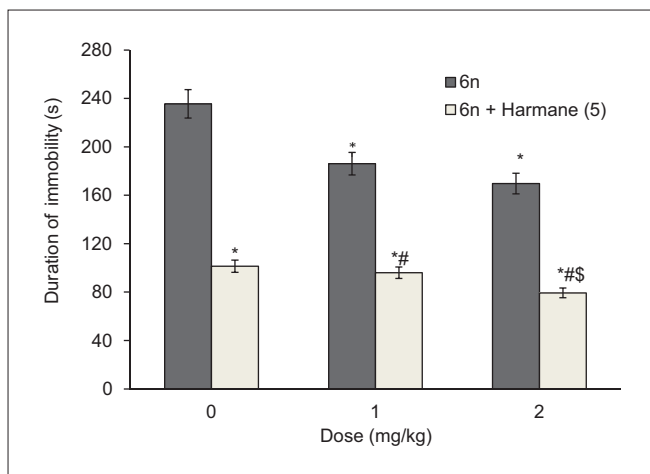


Figure 10: Effect of 6n (1 and 2 mg/kg) on antidepressant activity of Harmane (5 mg/kg) in mice FST. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ vs. vehicle treated Group. # $P < 0.05$ compared with 6n treated group. \$ $P < 0.05$ compared vs harmane (5 mg/kg)-treated group

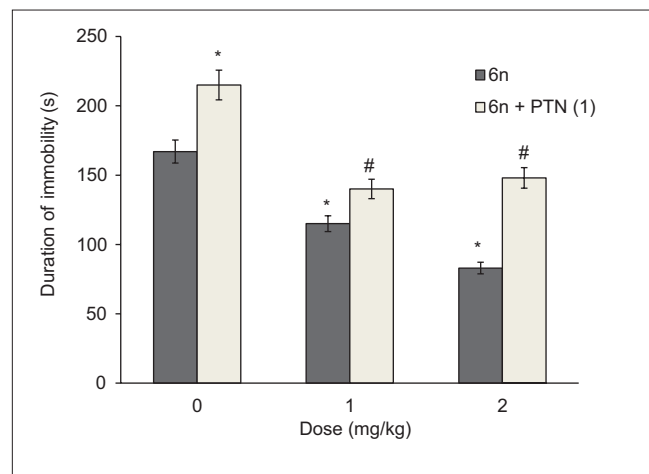


Figure 11: Effect of 6n (1 and 2 mg/kg) on depressant activity of parthenolide (PTN) in mice FST. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.D. $n = 8$ per group. * $P < 0.05$ vs vehicle-treated group. # $P < 0.05$ compared with PTN (1 mg/kg)-treated group

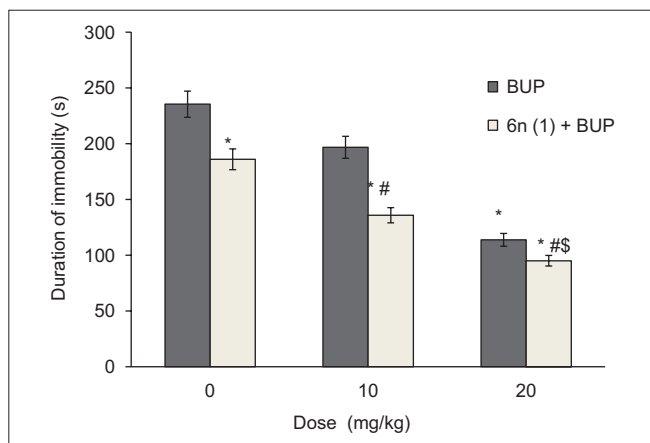


Figure 12: Effect of 6n (1 mg/kg) on antidepressant activity of bupropion (BUP) in mice TST. The columns represent mean duration of immobility in seconds (s) and error bars indicate S.D., *n* = 8 per group. **P* < 0.05 vs vehicle-treated group. #*P* < 0.05 compared with bupropion (10 and 20 mg/kg) treated group. \$*P* < 0.05 compared with 6n-treated group

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

Treatment	Ambulation score	Rearing score	Fecal pellet
Sham Control	91.17 ± 10.88	10 ± 2.24	2.17 ± .23
Sham+6n (1)	103.00 ± 12.72	9.33 ± 2.05	2.33 ± .87
Sham+6n (2)	102.17 ± 12.43	8.33 ± 2.18	2.00 ± 0.89
Sham+Par (10)	99.67 ± 12.94	8.67 ± 1.74	2.00 ± .81
OBX Control	225 ± 13.48*	30.67 ± 3.58*	4.33 ± 0.92*
OBX+6n (1)	140.00 ± 10.82 [#]	15.00 ± 2.01 [#]	3.80 ± 0.92 [#]
OBX+6n (2)	138 ± 12.95 [#]	11.4 ± 2.18 [#]	3.67 ± 0.97 [#]
OBX+Par (10)	113 ± 12.34 [#]	11.83 ± 1.43 [#]	2.33 ± .99 [#]

The value represent ambulation score, rearing score, and number of fecal pellet and error bars indicate S.D., **P* < 0.05 when compared with the sham-operated rats, [#]*P* < 0.05 when compared with the vehicle-treated OBX rats (*n* = 8 per group)

a decrease in the duration of immobility during the FST and TST. Both of these models of depression are widely used to screen NCEs for their antidepressant potential.^[17,18] These tests are quite sensitive and relatively specific to all classes of antidepressants. In the present study, 6n significantly decreased the duration of immobility in mice FST and TST. The decreased duration of immobility in mice FST and TST reflects the antidepressant-like activity of a compound.^[17,18]

Further, to investigate the plausible mechanism of 6n RIH, and 5-HTP induced head twitch was done. Reserpine being a non-specific monoamine depleting agent, acts by blocking the monoamine transport into synaptic vesicle. The depletion of brain biogenic amines affect the central nervous system characterized by hypothermia.^[29] The decrease in body temperature induced by reserpine was reported to be antagonized by antidepressants.^[29] 6n and ESC significantly prevented the hypothermic effect of reserpine-exhibiting antidepressant-like 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. 6n increased the head twitches in presence of pargyline, a MAO inhibitor. In this regard, the antidepressant-like effect of 6n appears to be modulated by an increase in monoamines, particularly 5-HT concentrations in the synapse.^[26]

Effect of 6n on aforementioned models gives us certain clue regarding the probable mechanism of 6n which could be mainly through modulation of serotonin in synaptic cleft; further interaction studies were performed to strengthen the mechanistic hypothesis of the molecule.

Interaction studies with ligands/conventional antidepressants in FST and TST were carried out not only to predict the probable mechanism (possible receptor targets) of antidepressant-like effects of 6n, but also to pharmacologically validate the 6n-induced antidepressant-like behavior in the above mentioned predictive tests. For a conclusive evaluation of antidepressant potential of 5-HT₃ receptor antagonists, interaction studies with standard ligands/conventional antidepressants were carried out.^[20] 6n (1 mg/kg, i.p.) significantly enhanced the antidepressant-like action of FLX (10 and 20 mg/kg, i.p.). VLA, an SNRI in a lower dose range (4–8 mg/kg), mainly influences the serotonergic system, whereas at higher doses the involvement of the NE system predominate.^[20,22] 6n (1 mg/kg, i.p.) significantly enhanced the antidepressant-like action of VLA (4 and 8 mg/kg, i.p.) as compared with alone treatment group.

Harmane, a β-carboline alkaloid, increases the monoamine levels by inhibiting the enzyme monoamine oxidase-A and B.^[32] 6n (1 and 2 mg/kg, i.p.) decreased the duration of immobility and influenced the effect of harmane (5 mg/kg, i.p.) in FST. Parthenolide, a serotonin release inhibitor^[33] that produces depressant-like effects,^[33] was tested with 6n to explore the serotonergic influence of 6n. A depressant-like effect induced by parthenolide was considered as a model to identify antidepressants acting through serotonergic mechanisms.^[33] 6n (1 and 2 mg/kg, i.p.) reversed the depressogenic effect of parthenolide (1 mg/kg, i.p.), possibly through enhancing serotonin release. 6n (1 mg/kg, i.p.) potentiated the effect of BUP (10 and 20 mg/kg, i.p.), possibly by interacting with the dopaminergic system, following the dopamine hypothesis of depression.^[20,22] Though the exact mechanism is not clear, it could be due to the release of dopamine through inhibition of 5-HT₃ receptors.^[20]

Olfactory bulbectomy has been proposed to be an agitated hyposerotonergic model of depression^[22] and used to explore the antidepressant potential of novel

agents.^[21] OBX rats exhibited a specific, abnormal behavioral pattern in the open field test characterized by increased ambulation, rearing, and fecal pellets. This abnormal behavior is reversed by antidepressants.^[21] The increased locomotor/ exploratory behavior of OBX rat in open field test may be due to exposure to a novel environment.^[34] Moreover, other possible reason for hyperactivity of OBX rats in OFT may be due to decrease in activity in competing behavior or delay/failure to habituate to novel environment.^[34]

The present neurobehavioral investigation showed antidepressant-like effects of 6n, a novel 5-HT₃ antagonist, in animal models of depression. The precise mechanism by which 6n produced antidepressant-like effect is not clear. However, on the basis of results obtained in various mechanistic and interaction studies like potentiation of head twitch responses and reversal of RIH, it is suggested that 6n exhibited antidepressant-like effect by increasing the concentration of monoamine neurotransmitter in the synapse. Increase in serotonin level through blockade of 5-HT₃ receptor could possibly explain the overall antidepressant-like effect of 6n. Reversal of parthenolide-induced depression-like behavior indicated the antidepressant-like action of 6n via serotonergic modulation.

CONCLUSION

Compound 6n showed antidepressant-like effects in rodents' models of depression, without affecting the locomotion of rodents *at tested* doses. The interaction studies with several standard ligands/antidepressants indicated that compound 6n expressed the antidepressant-like effect by enhancing the level of monoamine in synapse, probably through 5-HT₃ receptor antagonism. These results correlated the beneficial effects of 5-HT₃ receptor antagonist in depression, particularly comorbid disorders. 6n potentiate the effects of antidepressants, which indicated that the combination of antidepressant with 5-HT₃ receptor antagonist can potentially accelerate the onset of antidepressant action. Further studies on 6n are warranted to investigate the molecular effect of 6n and safety of the molecule.

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