

Anti-depressant - Like Effect of Novel 5-HT₃ Receptor Antagonist, (4-benzylpiperazin-1-yl) (3-methoxyquinoxalin-2-yl)methanone (6g) in Acute and Chronic Animal Models of Depression

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ABSTRACT

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A novel 5-HT₃ receptor antagonist '6g' with a good log P and pA₂ value identified from a series of compounds synthesized in our laboratory was subjected to forced swim test (FST) (1 and 2 mg/kg, i.p) and tail suspension test (TST) (0.5–2 mg/kg, i.p.). Compound **6g** significantly reduced the duration of immobility in mice without affecting the base line locomotion. Moreover, **6g** (1 and 2 mg/kg, i.p.), potentiated the 5-hydroxytryptophan (5-HTP)-induced head twitch responses in mice and reversed the reserpine-induced hypothermia (RIH) in rats. In interaction studies of **6g** with various standard drugs/ligands using FST, **6g** (1 mg/kg, i.p.) potentiated the anti-depressant effect of venlafaxine, desipramine and fluoxetine. Moreover, **6g** (1 and 2 mg/kg, i.p.) influenced the effect of 8-OH DPAT and harmaline as well as reverse the effect of parthenolide by reducing the duration of immobility in FST. However, **6g** (1 and 2 mg/kg, i.p.) has no influence on mCPP induced increase in duration of immobility in FST. Furthermore, **6g** (1 mg/kg, i.p.) potentiated the effect of bupropion in TST. Chronic **6g** treatment attenuated the behavioral anomalies in olfactory bulbectomy (OBX) rats. In conclusion, these various findings reiterated the anti-depressant-like effects of **6g** in behavioral models of depression.

Keywords: Serotonin, quinoxaline, 5-HT₃ receptor antagonists, anti-depressant, forced swim test, tail suspension test.

INTRODUCTION

Depression is a serious disorder that, according to the World Health Organization (WHO), is one of the leading causes of disability, worldwide. It is one of the most prevalent and costly psychiatric disorder of the developed world with a lifetime prevalence of between 7.5 and 17%.^{1,2}

The untreated, chronic or recurrent depression may affect the normal physical, mental social life of the depressives and their family members and in worst cases depressives get the suicidal idea and behavior³ and hence, prompt diagnosis and treatment is important to treat this disorder. Several anti-depressant drugs are available to treat this disorder. Regardless of the chemical structures, most of the clinically existing anti-depressant drugs exert their action by elevating the level of monoamine(s) in synapse, either directly or indirectly.³ The older anti-depressant drugs such as monoamine oxidase inhibitors and tricyclic anti-depressants are unfortunately well known for their drug-food/-drug interaction and side-effects rather than their therapeutic efficacy, respectively.^{4,5} Introduction of 'low side-effect anti-depressants' such as SSRIs for the treatment of depression,

enhance the patient's compliance towards pharmacotherapy of depression.⁶ Moreover these molecules are less effective than the older drug molecules and take 4-6 weeks to produce therapeutic effects.⁷

Targeting 5-HT₃ receptor will be of a notable interest for the development of newer anti-depressants. The beneficial effects of, 5-HT₃ receptor antagonists (ondansetron, granisetron etc.) are well established for treatment of nausea and vomiting (with fewer or negligible side-effects profile) in several pre-clinical studies.^{8,9,10,11} The beneficial effects of 5-HT₃ receptor antagonists were demonstrated in depression, similarly other researchers have also proved the same^{12,13,14}. Serotonin, a neurotransmitter which has many important implications in the control of numerous behavioral and physiological processes both in peripheral and central nervous system, is known to modulate mood, emotion, sleep, and appetite.¹⁵ The 5-HT₃ receptors are members of the Cys-loop superfamily of ligand-gated ion channels.^{16,17} 5-HT₃ receptor activation either leads to fast excitatory responses or the modulation of neurotransmitter release depending on their neuronal localisation. The involvement of 5-HT₃ receptors in depression and anxiety is complemented by studies of 5-HT₃ knock out mice which revealed the regulation of 5-HT₃ (3A subtype) in depression- and anxiety-related behaviors¹⁸. It is reasonable to conclude that 5-HT₃ receptors are involved in the modulation of anxiety and depression-related behavior

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and pharmacotherapy targeting 5-HT₃ receptors could be an alternative option for the treatment of depression and anxiety disorders. Evidence for the relevance of 5-HT₃ 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.^{19,20}

Using the three-component pharmacophore model²¹ a series of 5-HT₃ receptor antagonists have been 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 ondansetron as a reference drug.^{22,23}

Animal models of depression have been utilized vigorously to screen the novel compounds which were originally designed as screening tests to assess the efficacy of anti-depressant drugs.²⁴ These tests neglect the aspect of face validity but have a strong predictive validity to aid in the identification of

efficient anti-depressant molecules.²⁵ Hence a battery of behavioral tests were adopted for the study which included acute models like Forced Swim Test (FST),²⁶ Tail Suspension Test (TST)^{27,28} mechanistic models like 5-hydroxytryptophan (5-HTP) induced head twitch response in mice and Reserpine Induced Hypothermia.¹⁰ Evaluation of chronic effect of the compound was studied on olfactory bulbectomised rats^{29,30} provide significant information on anti-depressant activity of **6g**, which was identified for this study based on pA₂ and log P values.

-In the present study, compound **6g** (4-benzylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)methanone which exhibited good log P and pA₂ value (pA₂-7.5) greater than the standard 5-HT₃ receptor antagonist, ondansetron (OND) (pA₂-6.9)²³ was selected for the preliminary anti-depressant screening in the standard rodent models of depression as mentioned above.

MATERIALS AND METHODS

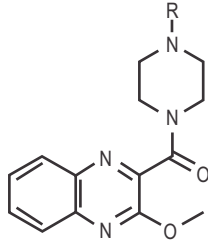
Animals

Albino mice (25±2 g), Wistar rats (250±20 g) and Dunkin Hartley guinea pigs (370±20 g) were obtained from Agricultural University, Hissar, Haryana, 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 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 (4-benzylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)methanone (**6g**)

The synthetic protocol of the target compound is illustrated in the (Fig 1). The title compound (**6g**) was synthesized from the starting material, *o*-phenylenediamine (**1**) in a sequence of reactions. Initial condensation with diethyl ketomalonate followed by chlorination using phosphorous oxychloride, furnished the chloro ester compound (**3**) which on saponification followed by nucleophilic displacement with sodium methoxide afforded the key intermediate, 3-methoxyquinoxalin-2-carboxylic acid in quantitative yield (**5**). The carboxylic acid group of the key intermediate (**5**) was coupled with benzylpiperazine in the presence of EDC·HCl and HOBT, to afford the desired product **6g** in good yield. The compound **6g** was converted into hydrochloride salt by using ethanolic hydrochloric acid³¹. The structure of the synthesized compound **6g** was confirmed by spectral data; ¹H NMR (CDCl₃, 400 MHz): δ 13.34 (s, 1H, salt proton), 7.87 (d, 1H,

Table 1: log P and pA₂ values of 3-methoxyquinoxalin-2-carboxamides

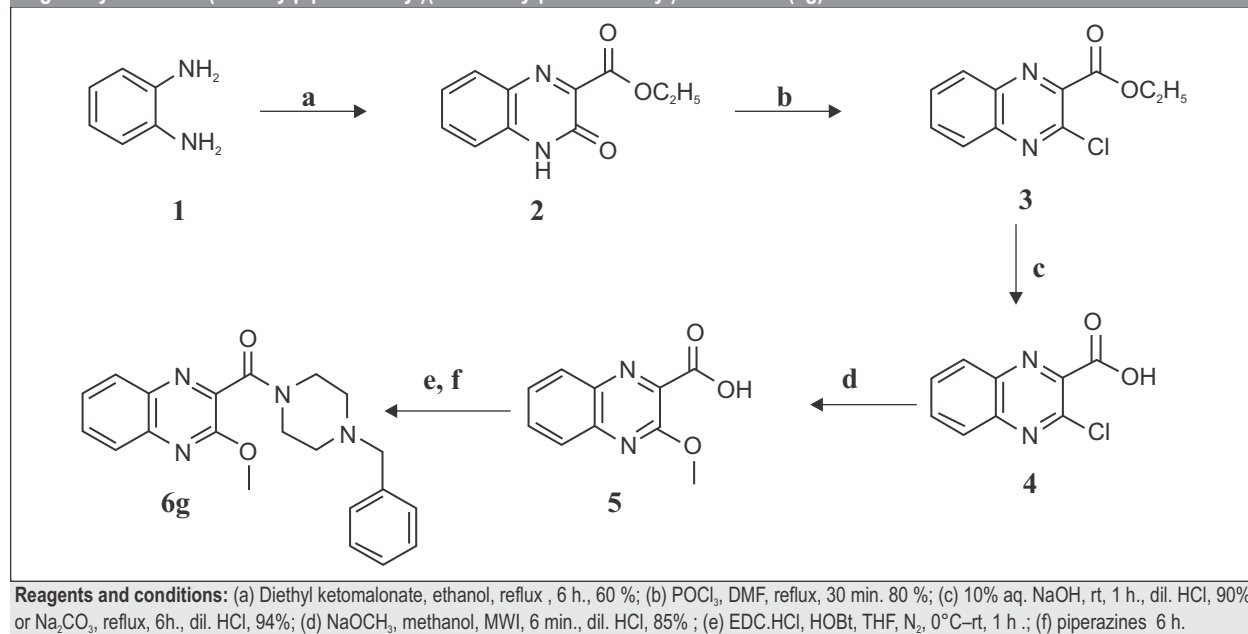


Compd	R	logP ^a	pA ₂ ^b
6a	C ₆ H ₅ -	3.43	7.2
6b	<i>o</i> -MeO-C ₆ H ₄ -	3.30	6.9
6c	<i>p</i> -MeO-C ₆ H ₄ -	3.30	5.3
6d	<i>p</i> -Cl-C ₆ H ₄ -	3.98	6.7
6e	<i>m</i> -Cl-C ₆ H ₄ -	3.98	7.0
6f	<i>m</i> -CF ₃ -C ₆ H ₄ -	4.35	7.5
6g	C ₆ H ₅ -CH ₂ -	3.08	7.5
6h	CH ₃ -	1.35	6.4
6i	CH ₃ -CH ₂ -	1.69	5.9
6j	CH ₂ =CH-CH ₂ -	2.04	4.1
6k	(Me) ₂ N-CH ₂ -CH ₂ -	1.36	6.1
6l	(Et) ₂ N-CH ₂ -CH ₂ -	2.04	6.4
6m	2-(Indol-3-yl)ethyl-	2.91	5.8
6n	<i>p</i> -N(Et) ₂ -C ₆ H ₄ -	3.98	7.0
6o	3-Pridyl-	1.69	7.2

^alog P values are calculated by using ChemBioDraw Ultra 11 (Cambridge Software)

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

Fig. 1: Synthesis of (4-benzylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)methanone (6g)



quinoxaline), 7.80 (d, 1H, quinoxaline), 7.67 (t, 1H, aromatic), 7.55 (q, 3H, aromatic), 7.39 (s, 3H, aromatic), 4.80 (s, 1H, aliphatic), 4.19 (s, 3H, OCH₃), 4.02 (s, 3H, aliphatic), 3.81 (d, 1H, aliphatic), 3.40 (t, 3H, aliphatic), 2.91 (s, 2H, aliphatic); IR (KBr): cm⁻¹ 2981, 2701, 2601, 2551, 1651, 1579, 1469, 1336, 1284, 1222, 1157, 952, 750; Mass spectra (ESI) of the compound (free base) exhibited the molecular ion peak at m/z 363 (M+1)⁺. The basic pharmacophore of 5-HT₃ receptor antagonists is shown in (Fig.2)

Drugs and chemicals

Fluoxetine (FLX), paroxetine (PAR), venlafaxine (VLA) and Bupropion (BUP) were obtained as gift samples from Cipla Pharmaceuticals & IPCA Laboratories Private Limited, India respectively. Escitalopram (ESC) & Ondansetron (OND) were obtained as generous gift samples from Glenmark Pharmaceuticals & Natco Pharmaceuticals, India. Desipramine (DMI) & pargyline were purchased from Sigma chemicals, USA. Harmane and 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH DPAT) were purchased from Tocris Bioscience, U.K. meta-Chlorophenylpiperazine

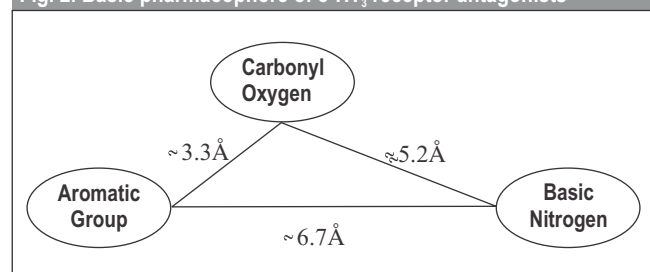
(mCPP) was purchased from Lancaster chemicals, USA. The drugs for anaesthesia namely, ketamine and xylazine were purchased from Reidel Neon Labs, Indian Immunologicals (Mumbai, India). The drugs were freshly prepared in distilled water and administered per oral (p.o.) or intra-peritoneally (i.p.) (as specified) in a constant volume of 10 ml/kg. For interaction studies, the anti-depressants/ligands and standards were administered i.p., 45 and 30 min, 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 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.^{32,33} In order to assess the 5-HT₃ receptor antagonistic activity, guinea pigs were sacrificed with ketamine (40 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). 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

Fig. 2. Basic pharmacophore of 5-HT₃ receptor antagonists



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_2 values, which were graphically determined. The pA_2 values of the test compound were compared with the standard antagonist, ondansetron.

Forced Swim Test (FST)

The FST was carried out as described elsewhere²⁶ 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 in this test, an immobile posture 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 min of the 6 min test. The mice were subjected to 15 minute training under similar conditions, 24 h before the test.

Tail Suspension Test (TST)

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.^{27,28} 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.³⁴ The animals were individually placed in a square arena (30 cm×30 cm) with walls painted black and are 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 trails.

5-Hydroxytryptophan (5-HTP) induced head twitch response

The method mentioned elsewhere³⁵ was adopted with slight modifications. The vehicle/drug treated mice were injected with pargyline (75 mg/kg i.p) 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). 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, FLX (10 and 20 mg/kg, i.p.), a serotonin re-uptake inhibitor, DMI (10 and 20 mg/kg, i.p.), a nor-epinephrine reuptake inhibitor, VLA (4 and 8 mg/kg, i.p.), a serotonin nor-epinephrine reuptake, harmaline (5mg/kg, i.p.), a monoamine oxidase inhibitor, 8-OH DPAT (1 mg/kg i.p.), 5-HT_{1A} and 5-HT₇ partial agonist, parthenolide (1 mg/kg i.p.), a serotonin release inhibitor, mCPP (2 mg/kg, i.p.), a 5-HT_{2A/2C} agonist, a tryptophan hydroxylase inhibitor. Interaction of bupropion (10 and 20 mg/kg, i.p), a nor-epinephrine and dopamine re-uptake inhibitor was carried out in TST since it is more sensitive than that of FST.^{36,37} All the standard drug doses were adopted from the previous work and standard responses recorded.^{36,37}

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, i.p.) was recorded at 30, 60, 90 and 120 min after the drug administration. The difference in the rectal temperature between the baseline and 60th min values were tabulated. On the day preceding of experimentation, the rectal temperature of the rats were assessed in a similar manner in order to habituate the animals to the experimental procedures.³⁸

Olfactory bulbectomy

Surgery

Bilateral olfactory bulbectomy (OBX) was carried out as described.^{29,30} In brief, the rat was anaesthetized 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. 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, 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 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 behavioral screening of OBX/sham rats were carried out based on the customized schedule previously reported.²⁹

Open field test (OFT) behavior

OBX and sham control rats were subjected to the OFT on 29th day after surgery. The open field exploration was conducted as described elsewhere²⁹ with substantial modifications. The apparatus consisted of a circular (90-cm diameter) arena with 75-cm high aluminium wated 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

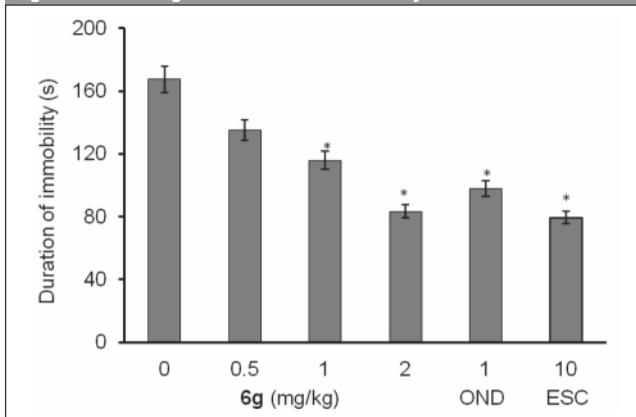
Data were expressed as the mean $\pm$ SEM. The single treatment studies were analyzed using a one-way analysis of variance followed by a *post-hoc* Dunnett test. The interaction studies were analyzed using a two-way analysis of variance followed by a *post-hoc* Tukey test. The level of statistical significance was fixed at $p < 0.05$.

RESULTS

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

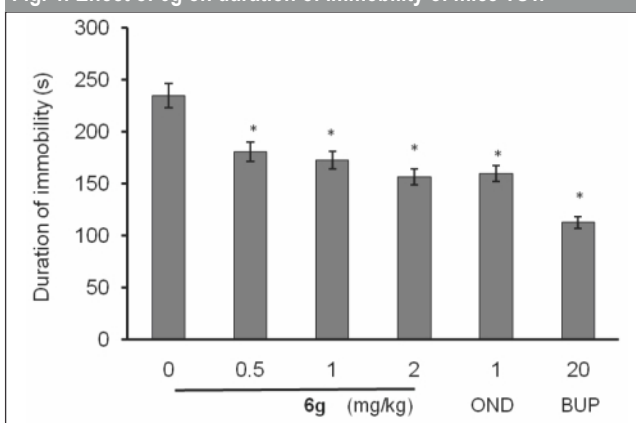
In FST, the acute treatment with **6g** (1 and 2 mg/kg, i.p.) significantly decreased the duration of immobility as compared to vehicle treatment (Fig. 3). The anti-depressant effect of **6g** was dose dependent, i.e. the lower dose (0.5 mg/kg, i.p.) failed to reach the level of significance. Likewise, a significant decrease in duration of immobility was evident at **6g** (0.5-2 mg/kg) in the TST (Fig.4). The standard anti-depressants, escitalopram (10 mg/kg) and bupropion (20 mg/kg) also significantly reduced the immobility duration in FST and TST respectively, as compared to the control group. Furthermore, ondansetron (1 mg/kg), significantly reduced the immobility duration in FST and TST as compared to the control group. None of the tested doses influenced the locomotion of mice in actophotometer. Depletion of brain serotonin induced by reserpine affects the central nervous system as demonstrated by reserpine induced hypothermia. Administration of reserpine (1 mg/kg i.p) elicited a

Fig. 3: Effect of **6g** on duration of immobility of mice FST.



The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 compared with vehicle treated group. OND = Ondansetron and ESC = Escitalopram

Fig. 4: Effect of **6g** on duration of immobility of mice TST.



The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 compared with vehicle treated group. OND = Ondansetron and BUP = Bupropion

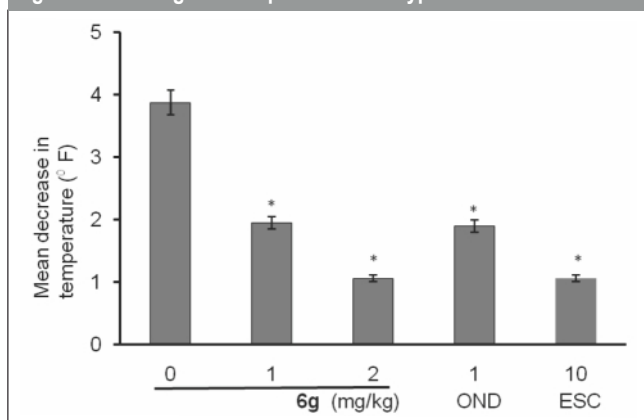
pronounced decrease in core body temperature of rats. This effect was significantly ($p < 0.05$) reversed by **6g**, ondansetron (1 mg/kg) and escitalopram (10 mg/kg) treatments (Fig. 5).

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. **6g** (1 and 2 mg/kg, i.p.), ondansetron (1 mg/kg, i.p.) and fluoxetine (20 mg/kg, i.p.) significantly potentiated the 5-HTP/pargyline induced head twitches, respectively (Fig. 6).

The peak dose of **6g** (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. **6g** (1 and 2 mg/kg, i.p.) significantly ($P < 0.05$) affect the duration of immobility throughout the interaction study as compared to control.

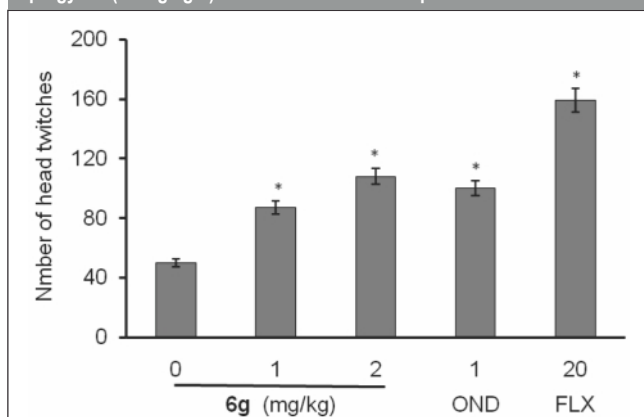
For a conclusive evaluation of anti-depressant potential of 5-HT₃ receptor antagonists, interaction studies with SSRIs were carried out. **6g** pre-treatment (1mg /kg, i.p.) was found to enhance anti-depressant-like effects of FLX (10 and 20 mg/kg, i.p.), VLA (4 & 8 mg/kg, i.p.) and DMI (10 and 20 mg/kg, i.p.) as shown in Fig. 7-9 respectively. Moreover, 8-OH-DPAT (1 mg/kg, i.p.), showed anti-depressant effects in the FST and **6g** (1 mg/kg, i.p.) potentiated the anti-depressant activity of 8-OH-DPAT as shown in (Fig. 10). Pre-treatment with **6g** (1 and 2 mg/kg, i.p.) enhanced the anti-depressant-like effects of harmaline (5 mg/kg, i.p.) (Fig. 11). Although **6g** (1 and 2 mg/kg, i.p.) failed to reverse the depression-like effects of mCPP (2 mg/kg, i.p.) as shown (Fig. 12). **6g** significantly reversed the depressant-like effect of parthenolide (1 mg/kg, i.p.) as shown (Fig. 13). Further **6g** (1 mg/kg) significantly enhanced the anti-depressant activity of BUP (10 and 20 mg/kg) in mice TST (Fig. 14).

Fig. 5: Effect of **6g** on reserpine induced hypothermia in rats.



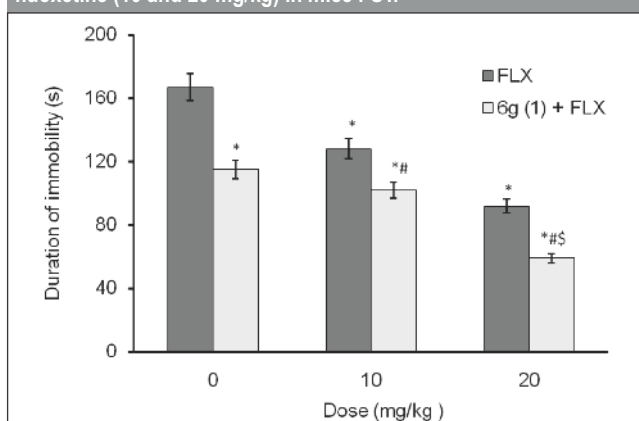
The columns represent mean decrease in temperature (°C) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 compared with vehicle treated group. OND = Ondansetron and ESC = Escitalopram

Fig. 6: Effect of **6g** and fluoxetine (FLX) on 5-hydroxytryptophan (5 mg kg⁻¹) + pargyline (75 mg kg⁻¹)-induced head twitch response in mice.



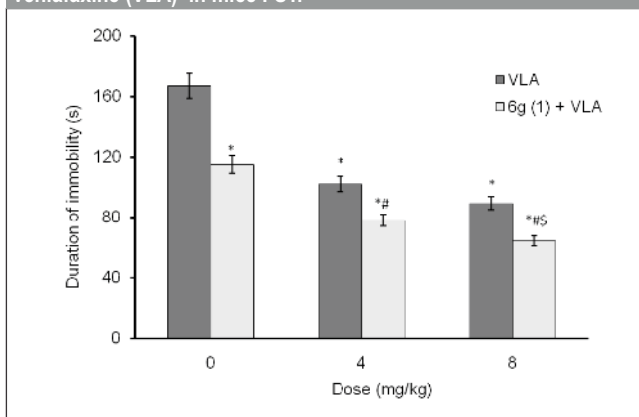
The columns represent mean number of head twitches and error bars indicate S.E.M. n = 6 per group. *P < 0.05 compared with vehicle-treated group. OND = Ondansetron and FLX = Fluoxetine

Fig. 7: Effect of **6g** (1 mg/kg) on anti-depressant activity of fluoxetine (10 and 20 mg/kg) in mice FST.



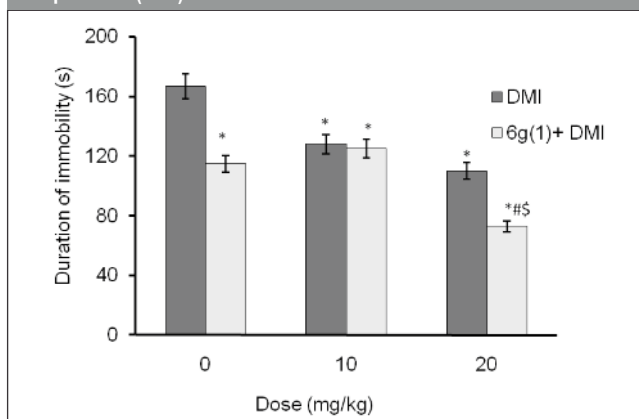
The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 6 per group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with fluoxetine (FLX) treated group and S P < 0.05 compared with alone **6g** treated group.

Fig. 8: Effect of **6g** (1 mg/kg) on anti-depressant activity of venlafaxine (VLA) in mice FST.



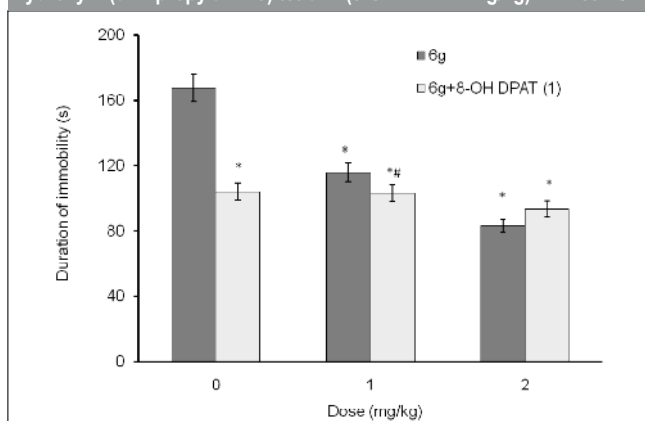
The columns represent mean duration of immobility (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with venlafaxine (4 and 8 mg/kg) treated group and S P < 0.05 compared with alone **6g** treated group.

Fig. 9: Effect of **6g** (1 mg/kg) on anti-depressant activity of desipramine (DMI) in mice FST



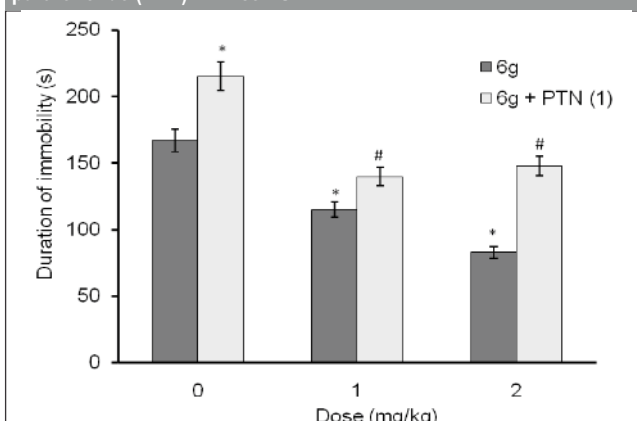
The columns represent mean duration of immobility (s) and error bars indicate S.E.M., n = 6 per group. *P < 0.05 compared with vehicle-treated group, # P < 0.05 compared with desipramine (10 and 20 mg/kg) treated group alone and S P < 0.05 compared with alone **6g** treated group

Fig. 10: Effect of **6g** (1 and 2 mg/kg) on anti-depressant activity of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH DPAT- 1mg/kg) in mice FST.



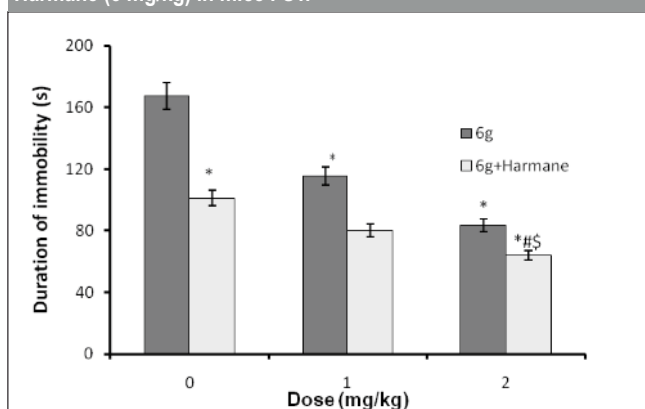
The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 vs. vehicle treated Group and #P < 0.05 compared with **6g** treated group alone.

Fig. 13: Effect of **6g** (1 and 2 mg/kg) on depressant activity of parthenolide (PTN) in mice FST



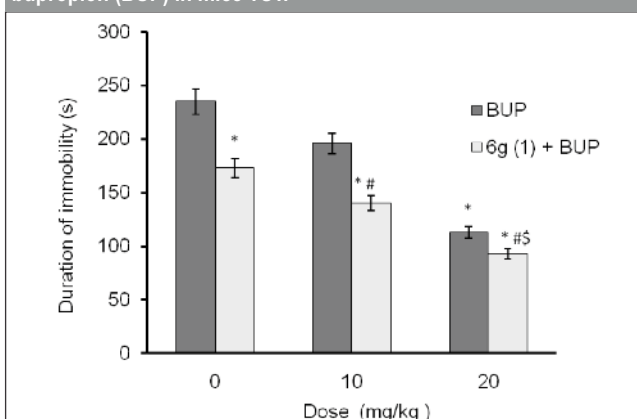
The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 vs. vehicle treated group and #P < 0.05 compared with PTN (1 mg/kg) treated group.

Fig. 11: Effect of **6g** (1 and 2 mg/kg) on anti-depressant activity of Harmaline (5 mg/kg) in mice FST.



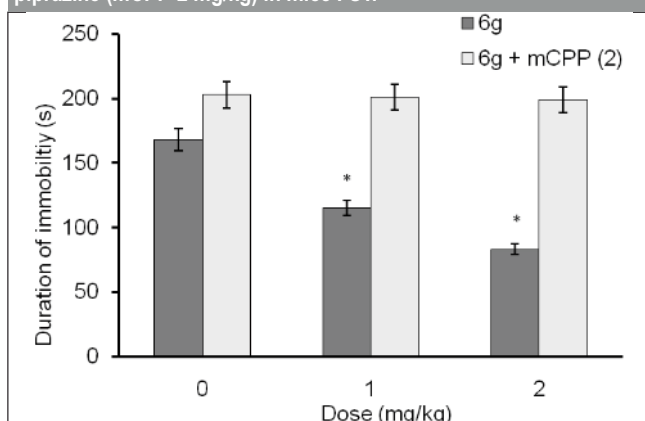
The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 vs. vehicle treated group, #P < 0.05 compared with harmaline (5 mg/kg) treated group and \$P < 0.05 compared with alone **6g** treated group.

Fig. 14: Effect of **6g** (1 mg/kg) on anti-depressant activity of bupropion (BUP) in mice TST.



The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 vs. vehicle treated group, #P < 0.05 compared with bupropion (10 and 20 mg/kg) treated group and \$P < 0.05 compared with alone **6g** treated group.

Fig. 12: Effect of **6g** on depressant activity of meta-chloro phenyl piperazine (mCPP-2 mg/kg) in mice FST.



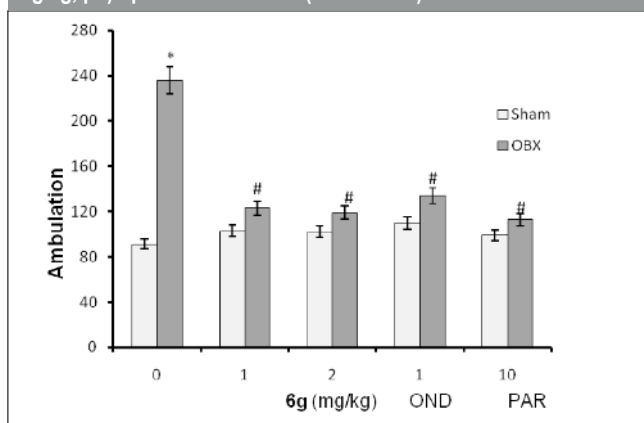
The columns represent mean duration of immobility in seconds (s) and error bars indicate S.E.M. n = 6 per group. *P < 0.05 vs. vehicle treated Group.

The effects of **6g** on the behavior of OBX/sham rats were analyzed in different circumstances as shown in (Fig. 15A, 15 B, 15 C). Chronic (14 days, p.o) treatment with **6g** (1 and 2 mg/kg) significantly ($p < 0.05$) reduced the number of ambulation, rearing and fecal pellets in OBX rats as compared to 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 to control group.

DISCUSSION

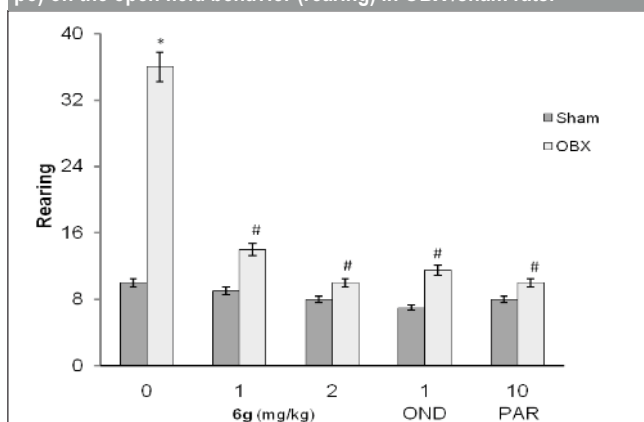
Animal studies led to the consensus that 5-HT₃ antagonists have anti-depressant effects by blocking limbic hyperactivity response.³⁹ Since 5-HT₃ receptors are expressed in brain areas implicated in anxiety and mood more over 5-HT₃ antagonists

Fig. 15 A: Effects of paroxetine (10 mg/kg, po) and 6g (1 and 2 mg/kg, po) open field behavior (ambulation) in OBX /sham rats.



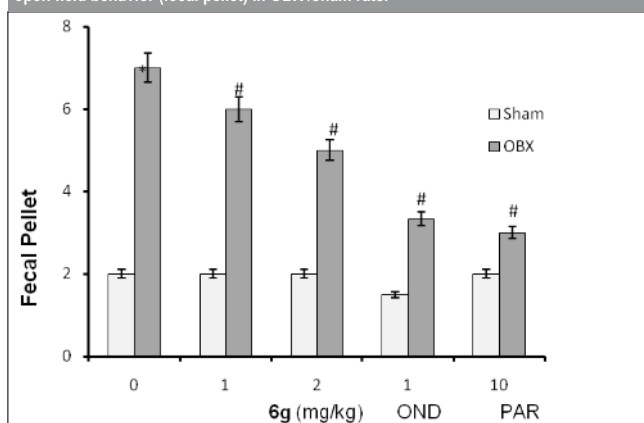
The columns represent ambulation score and error bars indicate S.E.M., * $p < 0.05$ when compared to the sham operated rats, # $p < 0.05$ when compared to the vehicle-treated OBX rats ($n = 6$ per group). OND = Ondansetron, PAR=Paroxetine

Fig. 15 B: Effects of paroxetine (10 mg/kg, po) and 6g (1 and 2 mg/kg, po) on the open field behavior (rearing) in OBX /sham rats.



The columns represent rearing score and error bars indicate S.E.M., * $p < 0.05$ when compared to the sham operated rats, # $p < 0.05$ when compared to the vehicle-treated OBX rats ($n = 6$ per group). OND = Ondansetron, PAR=Paroxetine

Figure 15 C. Effects of paroxetine (10 mg/kg, po) and 6g (1 and 2 mg/kg, po) on the open field behavior (fecal pellet) in OBX /sham rats.



The columns represent fecal pellet and error bars indicate S.E.M., * $p < 0.05$ when compared to the sham operated rats, # $p < 0.05$ when compared to the vehicle-treated OBX rats ($n = 6$ per group). OND = Ondansetron, PAR=Paroxetine

are able to pass the blood-brain barrier^{40,41} they represent excellent therapeutic candidates. In the present study, **6g**, a selective 5HT₃ receptor antagonist revealed the anti-depressant like effect. The psychomotor stimulation/sedation may increase/decrease the locomotor status (global motor activity) of mice in behavioral assays (FST and TST), when interpreting the depressant or anti-depressant-like effect of a new chemical entity (NCE). To rule out non-specific motor effects of **6g** in the present study that could influence the activity in the FST and TST, the locomotor activity of **6g** in mice was evaluated. None of the tested doses of **6g** influenced the locomotion of mice in actophotometer, which suggesting that the anti-depressant-like activity of **6g** was not associated with any global motor impairment.

The anti-depressant-like activity of a compound is determined by a decrease in the duration of immobility during the FST and TST. Both of these models of depression are widely used to screen NCE is for their anti-depressant potential.^{26,27} These tests are quite sensitive and relatively specific to all classes of anti-depressants. In the present study, **6g** significantly decreased the duration of immobility in mice during the FST and TST. The decreased duration of immobility in mice FST and TST reflects the anti-depressant-like activity of a compound.^{26,27}

In mechanistic models, reserpine being a monoamine depleting agent, which acts by blocking the monoamine transport in synaptic vesicle. The depletion of brain biogenic amines affect the central nervous system characterized by hypothermia.³⁸ The decrease in body temperature induced by reserpine was reported to be antagonized by anti-depressants.³⁸ **6g** 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. **6g** increased the head twitches in presence of pargyline, a MAO inhibitor. In this regard, the anti-depressant-like effect of **6g** appears to be modulated by an increase in monoamines; particularly 5-HT concentrations in the synapse.³⁵

As these mechanistic models gives us certain clue regarding the mechanism of **6g** which could be mainly through modulation of serotonin in synaptic cleft, further interaction studies was performed.

Interaction studies with ligands/conventional 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 **6g**, but also to pharmacologically validate the **6g** induced anti-depressant

like behavior in the above mentioned predictive tests. For a conclusive evaluation of anti-depressant potential of 5-HT₃ receptor antagonists, interaction studies with standard ligands/conventional anti-depressants were carried out.⁴² **6g** (1 mg/kg, i.p.), significantly enhanced the anti-depressant-like action of FLX (10 and 20 mg/kg, i.p.). VLA, a SNRI in a lower dose range (2–8 mg/kg), mainly influences the serotonergic system, whereas at higher doses the involvement of the nor-epinephrine (NE) system predominate.^{43,44} **6g** (1 mg/kg, i.p.), significantly enhanced the anti-depressant-like action of VLA (4 and 8 mg/kg, i.p.). DMI a tricyclic anti-depressant inhibits the reuptake of nor-epinephrine and to a lesser extent that of serotonin.⁴⁵ **6g** (1 mg/kg, i.p.), potentiated the effect of desipramine (10 and 20 mg/kg, i.p.) by decreasing the duration of immobility, indicating the influence of nor-adrenergic system by **6g**.

Potentiation of anti-depressant effects of 8-OH-DPAT (1 mg/kg, i.p.), a 5-HT_{1A} and 5-HT₇ partial agonist¹⁰ in FST, points to tricyclic anti-depressant (TCA)-like activity of compound **6g** (1 mg/kg, i.p.).⁴⁶ Harmane, a β -carboline alkaloid, increases the monoamine levels by inhibiting the enzyme monoamine oxidase-A and B.⁴⁷ **6g** (1 and 2 mg/kg, i.p.) decreases the duration of immobility and influenced the effect of harmane (5 mg/kg, i.p.) in FST. Previous studies in our laboratory have shown that acute administration of mCPP (2 mg/kg, i.p.) induces a depressogenic-like effects in rodents, and this effect is primarily mediated by stimulation of 5-HT_{2A/2C} receptors.⁴⁸ **6g** (1 and 2 mg/kg, i.p.) incapable of reverse the depressogenic effect of mCPP (2 mg/kg, i.p.) which further indicates that **6g** has no effect on 5-HT_{2A/2C} receptors. Parthenolide, a serotonin release inhibitor⁴⁹ that produces depressant-like effects⁵⁰ was tested with **6g** to explore the serotonergic influence of **6g**. A depressant-like effect induced by parthenolide was considered as a model to identify anti-depressants acting through serotonergic mechanisms.⁴⁹ **6g** (1 and 2 mg/kg, i.p.) reverse the depressogenic effect of parthenolide (1 mg/kg, i.p.) possibly through serotonin release. **6g** (1 mg/kg, i.p.) potentiate the effect of BUP (10 and 20 mg/kg, i.p.) possibly by interacting with the dopaminergic system, following the dopamine hypothesis of depression.⁹ Though the exact mechanism is not clear, it could be due to the release of dopamine through inhibition of 5-HT₃ receptors.⁹

Olfactory bulbectomy has been proposed to be an agitated hypo-serotonergic model of depression⁹ and is used to explore the anti-depressant potential of novel agents.⁵¹ OBX rats exhibited a specific, abnormal behavioral pattern in the open field test characterized by increased ambulation, rearing and fecal pellets, and this abnormal behavior is reversed by anti-

depressants⁵¹. The Increased locomotor/exploratory behaviour of OBX rat in open field test may be due to exposure to a novel environment.⁵² 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 habituate to novel environment.⁵³

The present neuro-behavioral investigation showed anti-depressant-like effects of **6g**, a novel 5-HT₃ antagonist, in animal models of depression. Though the precise mechanism by which **6g** produced anti-depressant-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 reserpine induced hypothermia suggests that, **6g** 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 **6g**. Reversal of parthenolide induced depression-like behavior indicated the anti-depressant-like action of **6g** via serotonergic modulation.

CONCLUSION

In this study, we have designed and synthesized a series of structurally novel 3-methoxyquinoxalin-2-carboxamides as 5-HT₃ receptor antagonists using ligand-based approach from the starting material *o*-phenylenediamine. All the synthesized compounds displayed antagonism towards, 5-HT₃ receptor, compounds **6f** and **6g** were the most active compounds among the synthesized molecules. Compound **6g**, showed anti-depressant-like effects in rodents' models of depression, without affecting the locomotion of rodents. The interaction studies with several standard ligands/anti-depressants, indicates compound **6g** elicit the anti-depressant-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. **6g** potentiate the effects of anti-depressants, which indicate the combination of anti-depressant with 5-HT₃ receptor antagonist can potentially accelerate the onset of anti-depressant action. Further studies on **6g** are required to furnish a clinically useful anti-depressant agent, which would be futuristic extension of the present study.

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