European Journal of Pharmacology 735 (2014) 59–67

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural pharmacology

Antidepressant and anti-anxiety like effects of 4i (*N*-(3-chloro-2-methylphenyl) quinoxalin-2-carboxamide), a novel 5-HT_3 receptor antagonist in acute and chronic neurobehavioral rodent models

Deepali Gupta [a,*], Mahesh Radhakrishnan [a], Devadoss Thangaraj [b], Yeshwant Kurhe [a]

[a] Department of Pharmacy, Birla Institute of Technology and Science, Pilani, Rajasthan 333031, India
[b] KVSR Siddhartha College of Pharmaceutical Sciences, Vijaywada, Andhra Pradesh 520001, India

ARTICLE INFO

Article history:
Received 9 September 2013
Received in revised form
11 January 2014
Accepted 8 April 2014
Available online 18 April 2014

Keywords:
Depression
Anxiety
5-HT_3 receptor antagonist
Forced swim test
Hole board test
Olfactory bulbectomy

Chemical compounds studied in this article:
Fluoxetine (PubChem CID: 3386)
Serotonin (PubChem CID: 5202)

ABSTRACT

Depression and anxiety are the most debilitating mood disorders with poor therapeutic recovery rates. In the last decades, 5-HT_3 receptor antagonists have been identified as potential agents for mood disorders. The current investigation focuses on evaluating the, antidepressant and anti-anxiety like effects of a novel 5-HT_3 antagonist, 4i (*N*-(3-chloro-2-methylphenyl) quinoxalin-2-carboxamide). Preliminary, in vitro 5-HT_3 receptor binding affinity was performed in isolated longitudinal muscle-myenteric plexus from the guinea pig ileum. Consequently, neurobehavioral effects of 4i in acute and chronic rodent models were evaluated. In addition, involvement of serotonergic system in the postulated effects of the compound was analyzed by in vivo assay. in vitro, 4i demonstrated high 5-HT_3 receptor antagonistic activity (pA2, 7.6). in vivo acute study, 4i exhibited decreased duration of immobility in forced swim and tail suspension tests, and increased exploratory parameters as number and duration of nose-poking in hole board test and latency and time spent in aversive brightly illuminated light chamber in light–dark model. Moreover, in chronic model of depression, i.e., olfactory bulbectomy with behavioral deficits, 4i reversed depressive anhedonia in sucrose preference test and anxious hyperactive behavior in open field test in rats. Furthermore, synergistic effect of 4i with fluoxetine (a selective serotonin reuptake inhibitor) and inhibitory effect of 1-(m-chlorophenyl)-biguanide (a 5-HT_3 receptor agonist) revealed serotonergic modulation by 4i mediated 5-HT_3 receptor antagonism, which was further confirmed by potentiation of 5-hydroxytryptophan (a serotonin synthesis precursor) induced head twitch response. These findings suggest the potential antidepressant and anti-anxiety like effects of 4i, which may be related to the modulation of serotonergic system.

1. Introduction

Depression is a mood disorder that is pervasive and affects almost every part of the world. Globally, it ranked fourth among the leading causes of disability (Maes et al., 2009) and by 2030; it is expected to be the largest contributor to disease burden (Mathers et al., 2008). Depression is often associated with anxiety that share many overlapping symptoms including fatigue, impaired concentration, irritability, sleep disturbance, experiences of nervousness, worry and restlessness (Ressler and Nemeroff, 2000). Depression ranks among the top most co-existing disorders with anxiety and approximately 39% of the patients with mood disorder meet criteria for both generalized anxiety disorder and major depressive disorder (Abramowitz and Landy, 2013; Bromet et al., 2011; Bruce et al., 2001).

The co-existence of these mood disorders suggests that they may also share a common pathophysiology. According to the classic monoamine hypothesis, an imbalance in the levels of monoamines or more specifically serotonin (5-HT) deficiency in discrete areas of brain results in depression and anxiety (Barchas and Altemus, 1999; Castren, 2005; Duman et al., 2000; Ressler and Nemeroff, 2000). The effects of several antidepressants currently in the market have also been hypothesized to result from the correction of endogenous 5-HT inadequacy (Delgado, 2000). Adhering to the orthodox theory, in recent years, 5-HT_3 receptor has been identified as a potential target for these mood disorders.

5-HT_3 receptors are unique among the serotonergic receptor class and belong to the ligand-gated cation channel receptor superfamily (Rajkumar and Mahesh, 2010). Although, preliminary

* Corresponding author. Mobile: +91 9352011573; fax: +91 1596244183.
E-mail addresses: deepaligupta2010@gmail.com (D. Gupta), rmaheshbits@gmail.com (M. Radhakrishnan), tdevadoss@gmail.com (D. Thangaraj), yashkurhe@gmail.com (Y. Kurhe).

http://dx.doi.org/10.1016/j.ejphar.2014.04.008
0014-2999/

identified in the peripheral nervous system, 5-HT_3 receptors are widely distributed in the central nervous system. 5-HT_3 receptors are ubiquitously expressed in several brain stem nuclei and higher cortical areas such as the amygdala, hippocampus and cortex, which are involved in regulation of mood and emotional behavior (Thompson and Lummis, 2007). This certainly provides the possible role of 5-HT_3 receptors in the regulation of behavioral activity and in the pathophysiology of depression and anxiety like deficits.

Antagonism of 5-HT_3 receptors has shown promising effects in preclinical models in alleviating depression and anxiety behaviors. Ondansetron, a selective 5-HT_3 receptor antagonist has been reported to produce antidepressant like effects in animals. Previous studies demonstrate that ondansetron decreases duration of immobility in mouse forced swim test (FST) and tail suspension test (TST) (Ramamoorthy et al., 2008). Moreover, pretreatment with ondansetron potentiates the antidepressant effects of selective serotonin reuptake inhibitors (SSRIs) (Redrobe and Bourin, 1997). In addition, ondansetron has shown modified behavior in elevated plus maze, light–dark box and other anxiolytic testing paradigms, substantially demonstrating the anti-anxiety activity (Bourin and Hascoet, 2003; Rodgers et al., 1997; Roychoudhury and Kulkarni, 1997). Furthermore, MDL 72222 (bemesetron) and ICS 205930 (tropisetron), the potential 5-HT_3 receptor antagonists have shown to reduce the duration of immobility in FST and TST in mice and increased exploratory activity in a more aversive light compartment in light–dark box test, considerably indicating the antidepressant and anxiolytic like activity (Bilkei-Gorzo et al., 1998; Bill et al., 1992; Bourin and Hascoet, 2003; Bravo and Maswood, 2006; Kos et al., 2006). Other than 'Setron'-class of drugs, the novel 5-HT_3 receptor antagonists have also shown to exhibit antidepressant and anxiolytic like effects in preclinical settings (Devadoss et al., 2010; Gautam et al., 2013; Mahesh et al., 2013; Mork et al., 2012).

In addition to the fact that, several antidepressants currently in market exhibit 5-HT_3 antagonistic potential (in example; fluoxetine, imipramine, desipramine, reboxetine and mirtazapine) (Anttila and Leinonen, 2001; Eisensamer et al., 2003; Kent, 2000) and presence of electrophysiologically characterized 5-HT_3 receptors in neuronal areas (such as median raphe, amygdala, hippocampus and hypothalamus) concerned with regulation of mood and behavioral activity (Thompson and Lummis, 2007), the involvement of 5-HT_3 receptors in the pathophysiology of depression and anxiety seems to be significant. However, the exact correlation of 5-HT_3 receptors in mood disorders is uncertain and still remains an area of interest.

A series of carboxamide derivatives were synthesized as 5-HT_3 receptor antagonists using a ligand based approach employing a three-point pharmacophore model (consists of an aromatic residue, a linking carbonyl group and a basic nitrogen) (Mahesh et al., 2010). The target new chemical entities were preliminary screened for their antidepressant potential using FST. 4i, [*N*-(3-chloro-2 methylphenyl) quinoxalin-2-carboxamide, Fig. 1] was selected because of its strong antidepressant like effects in preliminary testing (Mahesh et al., 2010). In the present study, a detailed investigation of antidepressant and anti-anxiety like effects of 4i was performed by using acute and chronic neurobehavioral rodent models. In the experiment first, the 5-HT_3 receptor antagonistic potential was evaluated in longitudinal muscle myenteric plexus preparation from guinea pig ileum against 5-HT_3 agonist, 2-methyl-5-HT and was expressed as pA2 value (MacKay, 1978; Mahesh et al., 2004). In the second experiment, the effective doses were determined using dose response study. Consequently, the effects of 4i in validated neurobehavioral rodent models such as FST and TST (as acute preclinical models of depression), hole board test and light–dark box test (as acute rodent models of anxiety) and olfactory bulbectomy (OBX, as a chronic model with depression and anxiety like behavioral deficits) were assessed (third experiment). In the fourth experiment, the possible implication of serotonergic neurotransmission in the postulated effects of the compound was examined.

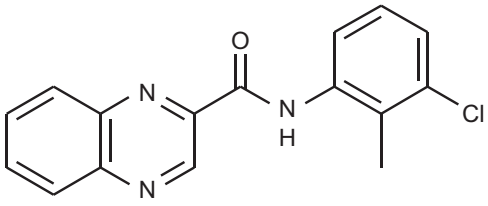

Fig. 1. The structure of 4i.

2. Materials and methods

2.1. Animals

Swiss Albino mice (22–25 g, of either sex), Wistar rats (200–250 g, of either sex) and male Dunkin Hartley guinea-pigs (350–400 g) were obtained from Hisar Agricultural University, Haryana, India. Animals were group housed in cages and were maintained in standard laboratory conditions with alternating light–dark cycle of 12 h each, temperature 23 ± 4 °C and humidity conditions $62 \pm 5\%$ relative humidity in the housing unit. Animals had free access to food (standard pellet chow feed) and filtered water ad libitum. Behavioral testing was done during the light cycle with a separate group of the animals being used for all behavioral assays. Animals were treated according to the guidelines of the *Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA, Registration number:* 417/01/*a*/*CPCSEA)* and all experiments were conducted in adherence to the approved protocol of the Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology & Science, Pilani, India (Protocol number: IAEC/RES/14/11, August-2011).

2.2. Drugs

4i, *N*-(3-chloro-2-methylphenyl) quinoxalin-2-carboxamide (Fig. 1) was synthesized and its structure was confirmed with Infrared (IR) spectroscopy, Mass spectrometry (MS) and Proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy by the Medicinal Chemistry Group, BITS-Pilani, India. The chemical synthesis scheme and spectral data are given as Supplementary material. Fluoxetine (FLX, a SSRI) and bupropion (BUP, norepinephrine and dopamine reuptake inhibitor) were obtained from Ranbaxy Research Laboratories, India. Diazepam (DIA) was purchased from Cipla Laboratories, Ltd. India. Ondansetron was procured from Indian Pharmaceutical Combine Association Labs, India. 1-(m-Chlorophenyl)-biguanide (mCPBG, a 5-HT_3 receptor agonist) and 2-methyl-5-HT (a 5-HT_3 receptor agonist) were purchased from Tocris Biosciences, UK. 5-Hydroxy-L-tryptophan (5-HTP, a 5-HT synthetic precursor) and pargyline (PAR, a monoamine oxidase inhibitor) were purchased from Sigma-Aldrich, Chemicals, USA.

2.3. Experiment 1: 5-HT_3 receptor antagonistic activity

For 5-HT_3 receptor antagonistic activity, guinea-pigs were sacrificed by mild ether anesthesia followed by cervical dislocation. The abdomen was cut open and a length of ileum was excised about 2 cm from the ileo-caecal junction. The longitudinal muscle-myenteric plexus (LMMP), 3–4 cm in length, was removed and mounted as per method described elsewhere (Paton and Aboo Zar, 1968). The tissue was equilibrated for 30 min under a resting

tension of 500 mg and constant aeration in a 40 mL organ bath containing Tyrode solution maintained at 37 °C. Non-cumulative concentrations (10^{-8}–10^{-4} M) 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. A fixed 2-methyl-5-HT concentration (10^{-5} M), approximately ED_{50} was used for antagonism studies. To study the antagonist effect of the test compound 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 quantitatively expressed in the form of pA2 values, defined as negative logarithm of molar concentration of antagonist producing a 2-fold shift of the agonist concentration–activity curve (MacKay, 1978; Mahesh et al., 2004).

2.4. Experiment 2: dose response study

The dose response profile of 4i was assessed in the mouse spontaneous locomotor activity (SLA) and further confirmed by FST. The SLA was assessed using the actophotometer (Boissier and Simon, 1965; Ramamoorthy et al., 2008). It consisted of a dark square chamber (30 cm × 30 cm) with inside walls painted black. Mice were individually placed in the chamber and after an initial 2 min familiarization period, the digital locomotor scores were recorded for the next 8 min period. The chamber was cleaned with dilute (70% v/v) alcohol and dried between trials. Two separate sets of experiments with 4i (0.25, 0.5, 1, 2, 4 and 8 mg/kg) were carried out to assess reproducibility of the results. In the single dose study, 30 min after 4i (0.25–8 mg/kg, i.p.) mice were subjected to locomotor activity.

2.5. Experiment 3: neurobehavioral assays

2.5.1. Acute studies

2.5.1.1. Antidepressant assay

2.5.1.1.1. Forced swim test. FST was carried out as described elsewhere with slight modifications (Porsolt et al., 1977). Mice were given 4i (0.5–1 mg/kg, i.p.) or FLX (10 mg/kg, i.p.) 30 min before the test and dropped individually into a plexiglass cylinder (height: 30 cm, diameter: 22.5 cm) filled with water to a depth of 15 cm and maintained at 23–25 °C. In this test, after an initial vigorous activity of 2 min, 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 (s), was recorded during the last 4 min of the 6 min test. The mice were subjected to 15 min training session under similar conditions, 24 h before the test.

2.5.1.1.2. Tail suspension test. After 30 min of 4i (0.5–1 mg/kg, i.p.) or FLX (10 mg/kg, i.p.) dosing, 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 escapes-oriented behavior interspersed with temporally increasing bouts of immobility (Steru et al., 1985) The duration of immobility (s) during the 6 min test session was recorded.

2.5.1.2. Anti-anxiety assay

2.5.1.2.1. Hole board test. The hole board model was first described by Takeda et al. (1998). It comprised of a square wooden box measuring 26 × 26 cm^2 with equally spaced 16 holes in the floor 2.2 cm in diameter. The experiment was carried out in a dimly lit, noise free room and exploratory parameters namely, number of nose-poking (head dipping through the holes) and duration of nose-poking (cumulative time the mouse spent with its head dip in the hole) were recorded for a 5 min period, after 30 min of 4i (0.5–1 mg/kg, i.p.) or DIA (2 mg/kg, i.p.) dosing. The apparatus was cleaned after every trial by dilute alcohol to avoid any residual effects.

2.5.1.2.2. Light–dark box test. The method of Crawley and Goodwin (1980) was adopted with slight modifications (Hascoet et al., 2001). In light–dark model, 30 min before the commencement of the experiment, mice were given 4i (0.5–1 mg/kg, i.p.) or DIA (2 mg/kg, i.p.) and individually kept in a polypropylene chamber (44 × 21 × 21 cm^3) in which 2/3rd of the light chamber was separated from 1/3rd dark chamber with 13 cm long block having 5 cm high openings. The light chamber was illuminated with a 60 W bulb. The animal was keenly observed for latency of the first entry into dark chamber and time spent in the light chamber over a time period of 5 min. The apparatus was cleaned as mentioned above.

2.5.2. Chronic studies

2.5.2.1. Olfactory bulbectomy. Bilateral ablation of the olfactory bulb was performed as described elsewhere (Kelly et al., 1997; Van Riezen and Leonard, 1990), with slight modifications. Rats were anesthetized with ketamine/xylazine (75/5 mg/kg, i.p.) and head was shaved. The rat was then fixed in a stereotaxic frame and the cranium was exposed by a midline sagittal incision. Two 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 olfactory bulbs were ablated by suction, avoiding damage to the frontal cortex, and the dead space was filled with hemostatic sponge to prevent excessive bleeding and the scalp was sutured. To prevent post surgical infection, the rats were given Sulprim injections (each milliliter containing sulfadiazine 200 mg and trimethoprim 40 mg), intramuscularly (0.2 ml/300 g) once a day for 4 days. The sham operation was performed in a similar manner but the bulbs were left intact. During the 14-day recovery period, the animals were handled regularly to avoid aggressive behavior (Leonard and Tuite, 1981), which might have developed otherwise. 15th day after the surgery, drug (4i at 0.5–1 mg/kg or FLX at 10 mg/kg, p.o.) or vehicle treatment was started and was continued once a day for 14 days. Behavioral assays were performed 24 h after the last dosing of the drug/vehicle. Only one behavioral test was performed on each day to avoid the residual effects.

2.5.2.2. Open field test. OBX and sham control rats were subjected to open field test (OFT) on the 15th day of chronic drug/vehicle administration. The test was conducted as described by Kelly et al. (1997) with slight modifications. The apparatus consisted of a circular (90-cm diameter) arena with 75-cm high aluminum walls and floor equally divided into 10 cm squares (Gray and Lalljee, 1974). 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 rat was individually placed in the center of the open field apparatus and the following parameters were observed for 5 min. Ambulation scores (number of squares crossed) and number of rearing episodes were noted as horizontal and vertical activity, respectively. Crossing of a square was scored only when the hind limbs of the animal moved to the next square. The number of fecal pellets was counted at the end of each 5-min trial. The apparatus was cleaned with 70% ethyl alcohol and dried between trials.

2.5.2.3. Sucrose preference test. Sucrose preference test was carried out as described previously (Willner et al., 1987). It is conducted in three phases as follows: phase 1 habituation, phase 2 sucrose

preference baseline, phase 3 sucrose preference testing. In phase 1, tap water in the home cage was replaced with 1% w/v sucrose in tap water for 24 h to habituate rats to the novel solution. In phase 2, each rat was transferred to single cage and was exposed to both tap water and sucrose solution consequently for 3 days to attain the sucrose preference baseline. Sucrose preference was then determined by a two-bottle choice test using standard bottles, one filled with tap water and one with 1% sucrose solution, supplied to rats for 24 h (phase 3). The locations of water and sucrose (left/right) were counterbalanced across the study. The tap water and sucrose solution intake was quantified by subtracting the final weight of bottles after 24 h exposure period from their initial weight and averaged for 2 days. The preference was then calculated as % preference = [(sucrose intake/total intake) × 100].

2.6. Experiment 4

2.6.1. Interaction studies

The interaction studies were carried out in mouse FST. To assess the possible involvement of the 5-HT system in the postulated effect of 4i, the combination of sub-effective dose of 4i (0.25 mg/kg, i.p.) and sub-effective dose of FLX, a SSRI (5 mg/kg, i.p.) was tested. FLX and 4i were given 60 min and 30 min before being tested in FST, respectively. To determine the possible contribution of the 5-HT_3 receptor in mediating the effect of 4i, the animals were pretreated with mCPBG (10 mg/kg, i.p.) or vehicle and after 30 min they received 4i (1 mg/kg, i.p.). The duration of immobility was recorded after 30 min and 60 min from the 4i and mCPBG dosing respectively. The administration schedule and the doses of the drugs used were chosen on the basis of experiments previously conducted in our laboratory and literature data confirming the efficacy of the above mentioned protocols (Bourin et al., 2002; Gautam et al., 2013; Machado et al., 2009; Mahesh et al., 2011; Ramamoorthy et al., 2008).

2.6.2. 5-HTP induced head twitch response

The study was carried out as mentioned elsewhere (Martin et al., 1989) with minor modifications (Devadoss et al., 2010). Briefly, mice were treated with PAR (75 mg/kg, i.p) 30 min before 5-HTP (5 mg/kg, i.p.) treatment. 4i (0.5–1 mg/kg, i.p.) or FLX (10 mg/kg, i.p.) were injected 15 min prior 5-HTP administration. After 15 min of 5-HTP administration, mice were placed individually in clear glass chambers and the number of head twitches exhibited by each mouse (vehicle or drug treated) during the next 20 min was recorded as head twitch score. The head twitch response was characterized by abrupt lateral movements, which may or may not be accompanied by body twitches and hind limb retraction.

2.6.3. Statistical analysis

Data of the present study were analyzed by GraphPad Prism software (version 3.0), USA. All values were expressed as mean ± standard error of mean (S.E.M.). The data of single drug treatment were analyzed by Student's *t*-test. The results obtained from behavioral models of depression were statistically analyzed by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's Test. The data obtained from behavioral models of anxiety were statistically analyzed using Kruskal–Wallis Test followed by post hoc Dunn's Multiple Comparison test. Analysis of the results obtained from OBX study was performed using one-way ANOVA followed by Tukey's Multiple Comparison Test. The results of interaction studies were subjected to two-way ANOVA followed by post hoc Bonferroni Test.

3. Results

3.1. 5-HT_3 receptor antagonistic activity of 4i

The ligand, 4i was synthesized as 5-HT_3 receptor antagonist; therefore an examination of the antagonist affinities of 4i at 5-HT_3 receptors in guinea pig ileum was carried out. The study confirmed the 5-HT_3 antagonistic activity of the compound as indicated by the pA2 value. The data indicate that 4i affinity for guinea-pig 5-HT_3 receptors was more than that observed for ondansetron (Table 1).

3.2. Dose response curve and dose selection

The spontaneous locomotor activity was assessed to determine the effect of 4i on generalized increase in locomotor scores. While assessing the behavioral activity on the basis of duration of immobility or the exploratory behavior of rodents, the effect of a general increase in the locomotor activity may possibly give false positive results. Therefore, the doses of 4i were selected on the basis of insignificant effect of 4i on the SLA. The results showed

Table 1
5-HT_3 receptor antagonistic activity (as pA2 value) of 4i.

Compound	*n*	Guinea pig ileum (pA2)
4i	3	7.6
Ondansetron	3	6.9

n = number of subjects used per group.

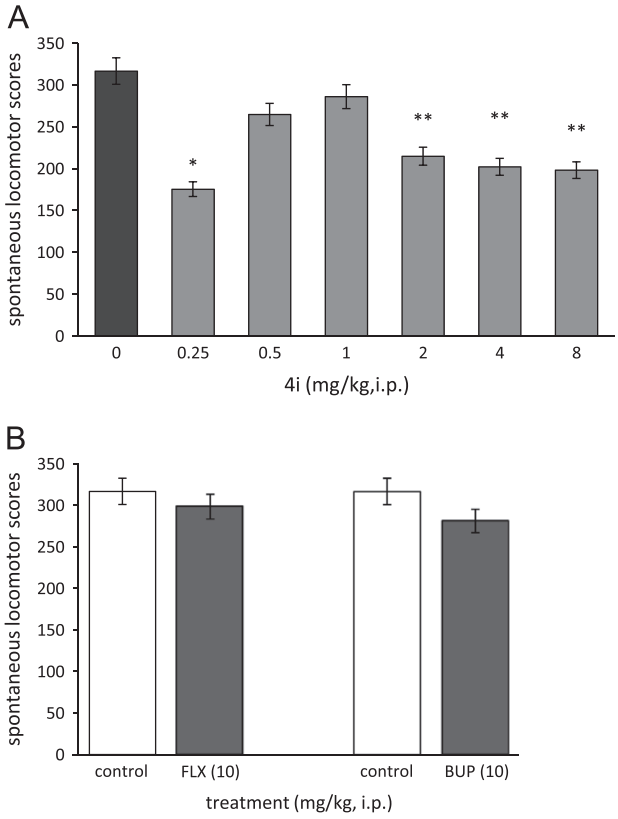

Fig. 2. The effects of different doses of 4i and active doses of FLX (fluoxetine) and BUP (bupropion) on spontaneous locomotor activity. The columns represent mean values of spontaneous locomotor scores, while error bars show S.E.M. The results from post hoc Dunnett's tests are indicated in the figure; *$P < 0.01$, **$P < 0.05$ as compared to the respective control groups, $n = 7$ mice/group.

that, i.p. administration of 4i at 0.5 and 1 mg/kg had no effect on the SLA [one-way ANOVA, F (8, 54)=3.16, $P>0.05$]. Whereas, 4i (0.25, 2, 4, 8 mg/kg) produced a reduction in the SLA [one-way ANOVA, F (8, 54)=3.16, P=0.0058] that differed significantly from vehicle values. In mice, neither FLX (10 mg/kg) nor BUP (10 mg/kg) used in the study, induced modulatory effect on the SLA following i.p. administration [one-way ANOVA, F (8, 54)=3.16, $P>0.05$]. Therefore, the doses, 0.5 and 1 mg/kg, i.p. of 4i were selected for behavioral assays (Fig. 2).

3.3. Effect of 4i in acute studies

3.3.1. Antidepressant like effect of 4i

A pronounced effect in duration of immobility in mouse FST was observed in 4i treatment group as compared to vehicle treated group [F (3, 28)=36.09, $P<0.0001$]. Further, post-hoc Dunnett's test revealed that 4i at the doses of 0.5 or 1 mg/kg significantly decreased the duration of immobility ($P<0.01$ vs. vehicle for the dose of 0.5 and 1 mg/kg). Similarly, FLX (10 mg/kg, i.p.), the positive control used in this study, significantly reduced the duration of immobility ($P<0.01$) (Fig. 3A) in mice during FST.

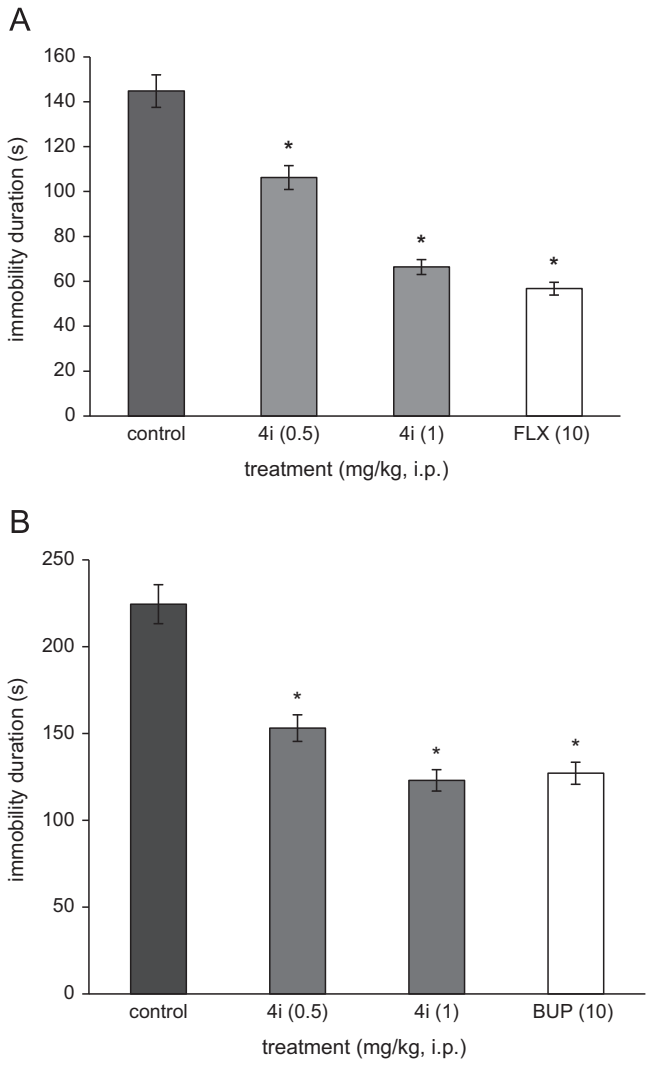

Fig. 3. The effects of 4i and FLX (fluoxetine) on behavioral despair effects in FST (A) and TST (B). The columns represent mean values of duration of immobility (s), while error bars show S.E.M. The Results from post hoc Dunnett's test are indicated in the figure; *$P<0.01$ as compared to the respective control groups, n=8 mice/group.

Similarly in TST, the duration of immobility was decreased significantly in 4i (0.5 or 1 mg/kg) treated group as compared to vehicle group [F (3, 28)=16.25, $P<0.0001$]. Further, post hoc analysis revealed a dose dependent decrease in duration of immobility after 4i treatment (0.5–1 mg/kg) (Dunnett's test, $P \ll 0.01$). In TST, BUP also reduced the duration of immobility significantly ($P<0.01$ vs. vehicle group at 10 mg/kg) (Fig. 3B).

3.3.2. Anti-anxiety like effect of 4i

The anti-anxiety effect of 4i was evaluated by hole board and light–dark box test. Compared with vehicle control, DIA (2 mg/kg) significantly increased the number and duration of nose-poking in mice during hole board test, indicating the validation of the model. Acute treatment with 4i similarly produced significant increase in number of nose-poking and duration of nose-poking in mice [Kruskal–Wallis test, Kruskal–Wallis statistic=13.9, P=0.003 and Kruskal–Wallis statistic=12.1, P=0.0071, respectively], (Table 2).

During, light–dark box test, 4i demonstrated significant increase in latency to enter first time into dark chamber from light chamber [Kruskal–Wallis test, Kruskal–Wallis statistic=11.38, n=7, P=0.0098]. Moreover, the time spent by mice in light chamber was measured. Compared with control group, 4i treatment significantly increased the cumulative time spent in light chamber [Kruskal–Wallis test, Kruskal–Wallis statistic=14.31, n=7, P=0.0025] in mice. Similarly, DIA as positive control, elicited pronounced increase in latency and time spent in light chamber in mice ($P<0.01$) when subjected to the test (Table 2).

3.4. Effect of 4i in chronic studies

The OBX rats were evaluated for neurobehavioral derangements in OFT and sucrose preference test as the indices of anxiety and depressive behavior in rodents respectively, after 24 h of last dosing. In OFT, compared with sham rats, control OBX rats manifested a significantly increased number of crossings (as a measure of horizontal activity) and rearings (as a measure of vertical activity) after 4-weeks of ablation of olfactory bulb, indicating that the model was successfully developed. Further, as positive control, chronic FLX treatment (10 mg/kg, p.o., for 14 days) reversed increased the number of crossings and rearings, demonstrating the high predictive validity of the model. Daily oral administration of 4i (0.5–1 mg/kg) showed evident effect on the open filed behavior in OBX rats, as compared with OBX control rats [one-way ANOVA, F (7, 48)=6.00, $P<0.0001$]. Further, post hoc analysis revealed that 4i significantly reduced number of crossings and rearings as compared to OBX control group (Tukey's Multiple Comparison Test, $P<0.5$ vs. OBX control rats for 0.5 mg/kg and $P<0.01$ vs. OBX control rats for 1 mg/kg). However, in sham rats neither FLX nor 4i exhibited significant

Table 2
Effects of 4i and DIA (diazepam) in hole board and light–dark box test models for anxiety.

Treatment (dose, mg/kg)	Hole board test		Light–dark test	
	Number of nose-poking	Duration of nose-poking	Latency (s)	Time spent in light chamber (s)
Control	15.43 ± 1.94	22.60 ± 1.72	22.96 ± 3.33	64.62 ± 5.02
4i (0.5)	31.14 ± 2.38[b]	39.47 ± 3.87[b]	48.17 ± 6.48[b]	121.13 ± 11.49[b]
4i (1)	35.14 ± 3.71[a]	43.3 ± 6.13[b]	48.21 ± 4.12[b]	119.98 ± 3.04[b]
DIA (2)	32.29 ± 4.63[b]	40.31 ± 4.48[b]	49.25 ± 6.45[b]	133.60 ± 11.21[a]

The columns represent mean values ± S.E.M. The results from post hoc Kruskal–Wallis test are indicated as (a) $P<0.01$ and (b) $P<0.05$ as compared to control group, n=7 mice/group.

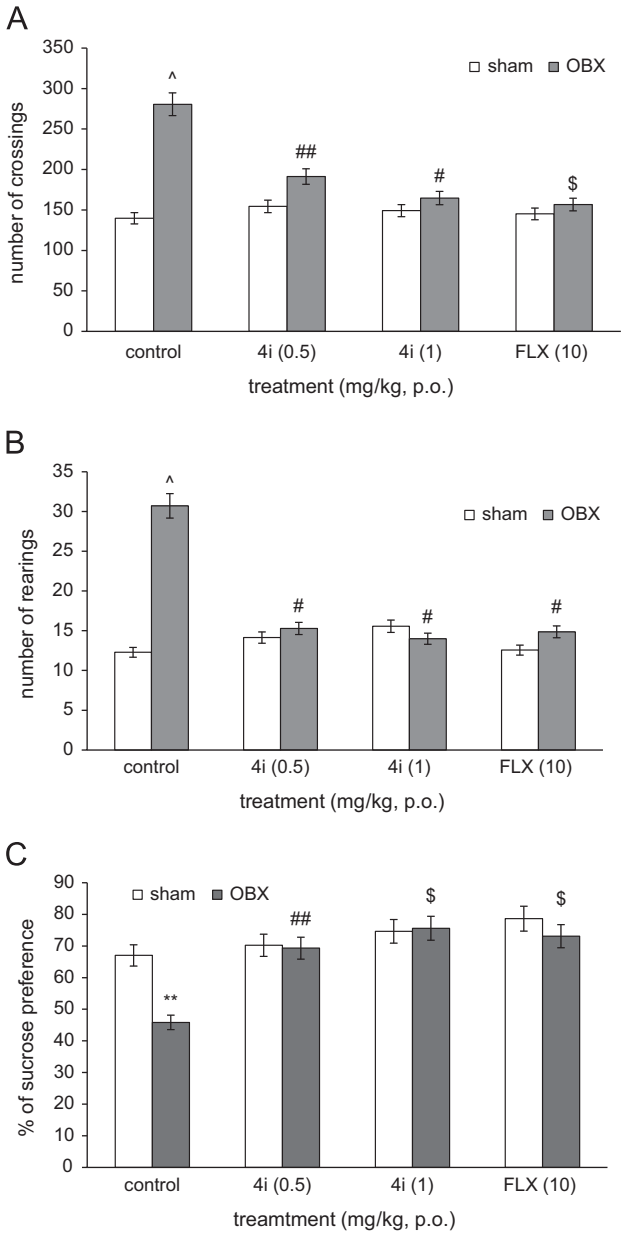

Fig. 4. The effects of 4i and FLX (fluoxetine) on OBX induced behavioral deficits, evaluated as number of crossings (A) and number of rearings (B) in OFT and % of sucrose preference/intake in sucrose preference test (C). The columns represent mean values, while error bars show S.E.M. The results from post hoc Tukey's tests are indicated in the figure; $^{\wedge}P<0.001$, $^{**}P<0.05$ as compared to sham control rats and $^{\#}P<0.01$ $^{\#\#}P<0.05$, $^{\$}P<0.001$ as compared to OBX control rats, $n=7$ rats/group.

alteration of the behavioral activity [one-way ANOVA, F (7, 48)= 6.00, $P>0.05$], Fig. 4A and B.

In sucrose preference test, after four weeks of ablation of olfactory bulb, OBX rats exhibited decreased % of sucrose preference as compared to sham rats, demonstrating the successful development of OBX model in rats. As positive control, FLX increased % of sucrose preference in OBX rats, which is thought to be an indication of predictive validity of OBX model. One-way ANOVA revealed the significant effect of 4i in % of sucrose preference in OBX rats [F (7, 48)=4.571, $P<0.001$]. Further, post hoc Tukey's Multiple Comparison Test showed that, chronic 4i treatment (0.5–1 mg/kg) elicited a pronounced increase in the % of sucrose preference in OBX rats ($P<0.5$ vs. OBX control rats for 0.5 mg/kg and $P<0.01$ vs. OBX control rats for 1 mg/kg). Importantly, in sham rats neither FLX nor 4i produced evident changes in % of sucrose preference over water intake [one-way ANOVA, F (7, 48)=4.571, $P>0.05$] Fig. 4C.

3.5. Effect of 4i in interaction studies

To assess the possible involvement of 5-HT system, interaction study of 4i with FLX (at sub-therapeutic doses), a SSRI was carried out in mouse FST. 4i (0.25 mg/kg) elicited a significant decrease in duration of immobility in mice pretreated with FLX (5 mg/kg) as compared to vehicle control [two-way ANOVA, F (7, 24)=4.395, $P<0.05$]. While, neither FLX (5 mg/kg) nor 4i (0.25 mg/kg) produced significant effect on duration of immobility when compared to control mice. Moreover, to evaluate the involvement of 5-HT_3 receptors in the effect of 4i, interaction study with mCPBG, a 5-HT_3 receptor agonist was conducted. Pretreatment with mCPBG (10 mg/kg), effectively reduced the effect of 4i (1 mg/kg) [two-way ANOVA, F (7, 24)=17.651, $P<0.001$] as indicated by insignificant change in duration of immobility in mCPBG plus 4i treated mice ($P>0.05$ vs. control group for 4i, 0.25 mg/kg and mCPBG, 10 mg/kg treated group). While, 4i alone (1 mg/kg) produced significant decrease in duration of immobility in mice compared with control [F (7, 24)=17.651, $P<0.01$]. Also, mCPBG (10 mg/kg) alone did not affect the duration of immobility in mice during FST ($P>0.05$ vs. control mice), Table 3.

3.6. Effect of 4i in 5-HTP induced head twitch response

The confirmatory study for the involvement of 5-HT in the postulated effect of 4i was depicted using 5-HTP induced head

Table 3
Effects of 4i in interaction studies with fluoxetine (FLX, a SSRI) and mCPBG (1-(m-chlorophenyl)-biguanide, a 5-HT_3 receptor agonist) in mouse FST.

Treatment (dose, mg/kg)	Duration of immobility (s)
Control	144.75 ± 8.54
4i (0.25)	148.63 ± 10.80
4i (1)	66.38 ± 4.52[ac]
FLX (5)	130.50 ± 12.47
4i (0.25)+FLX (5)	94.88 ± 9.40[b]
mCPBG (10)	157.13 ± 8.70
mCPBG (10)+4i (1)	124.00 ± 11.48[d]

The columns represent mean values ± S.E.M. of duration of immobility (s). The results from two-way ANOVA post hoc Bonferroni Test are indicated as (a) $P<0.001$, (b) $P<0.05$ as compared to control group, (c) $P<0.001$as compared to mCPBG treated group and (d) $P<0.001$ as compared to 4i (1 mg/kg) treated group, $n=8$ mice/group.

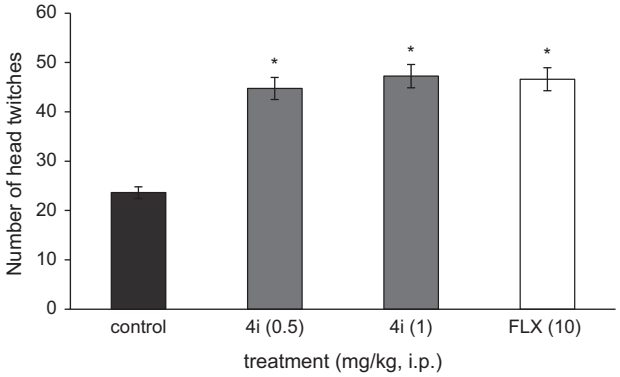

Fig. 5. The effects of 4i and FLX (fluoxetine) on head twitch response produced by 5-HTP (5-hydroxy-L-tryptophan) and PAR (pargyline). The columns represent mean values of number head twitches, while error bars show S.E.M. The Results from post hoc Dunnett's test are indicated in the figure; $^{*}P<0.01$ compared with control mice received only 5-HTP plus PAR, $n=8$ mice/group.

twitch response potentiation [$F(3, 48) = 10.39$, $P < 0.0001$]. In this study, FLX (10 mg/kg) significantly increased the 5-HTP induced head twitch scores in mice ($P < 0.01$ vs. control group), indicating the predictive validity of the model. Further, post hoc analysis revealed that, 4i (0.5 and 1 mg/kg) significantly elevated the head twitch response induced by 5-HTP as shown by markedly increase in head twitch scores in 4i treated group in mice ($P < 0.01$ vs. control group for 0.5–1 mg/kg), Fig. 5.

4. Discussion

The complex pathophysiology of mood disorders (depression and anxiety) and inconsistent therapeutic efficacy of present antidepressants necessitate the research of new targets and novel compounds with better effectiveness and promising treatment. In the present study, 4i, a novel ligand with potential 5-HT_3 receptor antagonistic activity, demonstrated evident antidepressant and anti-anxiety like effects in a series of acute and chronic rodent behavioral assays.

In the preliminary study, the 5-HT_3 receptor antagonistic activity of 4i (in the form of pA2) was evaluated using guinea pig-ileum and was found to be higher than the standard drug, ondansetron, indicating potential 5-HT_3 receptor affinity and antagonistic action of 4i (MacKay, 1978; Mahesh et al., 2004).

Consequently, the current study utilized standard rodent behavioral models to assess the effects of 4i, in vivo. With high predictive validity, behavioral despair tests such as FST and TST are the most widely used models across the laboratories to evaluate the antidepressant activity of numerous compounds (Cryan and Slattery, 2007; Millan et al., 2001; Petit-Demouliere et al., 2005). The increased duration of immobility reflects a state of hopelessness, which simulates the depressive symptoms seen in humans (Castagne et al., 2011). 4i significantly reduced the duration of immobility in mice after acute treatment in FST and TST, a behavioral profile shown by several antidepressants in clinical use (Millan et al., 2001) and several 5-HT_3 receptor antagonists (Bravo and Maswood, 2006; Devadoss et al., 2010; Kos et al., 2006; Ramamoorthy et al., 2008). Since drugs with central nervous system stimulant effects can also reduce the duration of immobility in FST and TST, and may possibly give false positive results (Boissier and Simon, 1965; Petit-Demouliere et al., 2005), the effect of 4i on the SLA in mice was determined and it was found that the antidepressant like effect of 4i (0.5 and 1 mg/kg) in these behavioral despair tests were independent of a generalized increase in motor activity.

Anxiety is one of the most common mood disorders associated with depression (Ressler and Nemeroff, 2000). Several antidepressants such as fluoxetine and escitalopram, with serotonergic modulatory activity have shown to be effective in both depression and associated anxiety disorders (David et al., 2009; Davidson et al., 2004; Dulawa et al., 2004; Pedersen, 2005). Therefore, the anti-anxiety like effect of 4i in anxiety testing paradigms was estimated.

Hole board and light–dark box test are exploratory behavior based, extensively used models for estimation of anxiety/anti-anxiety effects (Bourin and Hascoet, 2003; Crawley, 1985; Takeda et al., 1998; Van Gaalen and Steckler, 2000). In hole board test, the number and duration of nose-poking (head dipping) are putative indices of anxiety like behavior, which are shown to be increased by various anti-anxiety agents (Takeda et al., 1998). In the present study, similar to the positive control, 4i significantly increased number of nose-poking as well as cumulative time spent in nose-poking (duration of nose-poking) in mice. This is in agreement with the previous findings that 5-HT_3 receptor antagonists have anti-anxiety like effects in rodents (Costall and Naylor, 1992).

Mice subjected to light–dark box avoid the aversive light illumination and prefer the dark chamber, which reflect the anxious response of the animal, while increased exploration in light chamber and latency to enter dark chamber are associated with anti-anxiety effects (Hascoet et al., 2001). Acute treatment with 4i increased latency to first passage in dark chamber and time spent in light chamber, which corroborates the previous reports, that 5-HT_3 receptor antagonists increase animal exploration in light chamber when exposed to light–dark box, evidently demonstrating the anti-anxiety like effect of 4i (Costall et al., 1989). Although acute behavioral studies substantially demonstrate the pharmacological activity of the compounds, the tested doses do not correspond to the clinical time course of their action. Therefore, in the present study the effects of 4i in chronic behavioral model namely, OBX was evaluated. Bilateral ablation of olfactory bulb results in several neurobehavioral changes that correspond to both depression and anxiety like symptoms (Glinka et al., 2012; Kelly et al., 1997). In example, hyperactivity is one of the putative indices of agitation like behavior in anxious patients whereas, decreased reward related behavior reflects anhedonia or inability to experience pleasure, which is one of the cardinal signs of depression in humans (Kelly et al., 1997; Song and Leonard, 2005; Wang et al., 2007). In the current study, after 4-weeks of bulbectomy, OBX rats exhibited abnormal behavioral pattern in sucrose preference test and OFT used as the testing paradigms of depression and anxiety respectively (Kelly et al., 1997; Wang et al., 2007). Furthermore, FLX as a reference drug reversed the OBX induced behavioral defects demonstrating high predictive validity of the model (Zueger et al., 2005). In the sucrose preference test, OBX rats showed pronounced anhedonic behavior (as indicated by increased preference for sucrose consumption over water), which was curtailed by chronic 4i treatment, the effect similar to that shown by several antidepressants (Song and Leonard, 2005). It demonstrated the potential influence of 4i in reward related activity, which is specific to the diseased condition (since 4i had no influence on hedonic behavior of sham rats) and revealed antidepressant like behavior following chronic treatment of 4i, in agreement with its acute action (Romeas et al., 2009). In OFT, OBX rats elicited the increased number of ambulation and rearing (as a measure of horizontal and vertical locomotor activity, respectively) which showed hyperactive behavior following bulbectomy and revealed anxiety condition in association with depression like behavior in rats (Song and Leonard, 2005; Wang et al., 2007). Chronic 4i treatment on the other hand, reversed the hyperlocomotor performance in OBX rats without any influence on the motor behavior of sham rats, which revealed specificity of 4i treatment in diseased condition. This is in agreement with the activity of several antidepressants, including reference drug used in the present study and several 5-HT_3 antagonists (Devadoss et al., 2010; Gautam et al., 2013; Song and Leonard, 2005; Ramamoorthy et al., 2008), indicating the anti-anxiety like effect of 4i on chronic treatment. Taking all the behavioral results into account, 4i, when administered either acutely or chronically, displayed robust antidepressant and anti-anxiety like effects in multiple animal models in mice and rats.

While considering the behavioral effects in rodents as well in humans, several antidepressants act by enhancing synaptic concentration of monoamines particularly serotonin. Therefore an estimate of serotonergic modulatory activity of 4i was evaluated through interaction studies with an antidepressant (FLX, a SSRI) in clinical use. Combination of sub-active doses of 4i (0.25 mg/kg) and FLX (5 mg/kg) significantly reduced the duration of immobility in FST, which showed a synergistic action of 4i with FLX and possibly demonstrated that, similar to FLX, 4i increased serotonergic neurotransmission in mice. The results are in line with the previous report that 5-HT_3 receptor antagonists potentiate the

effects of SSRIs (Ramamoorthy et al., 2008; Redrobe and Bourin, 1997). In addition, pretreatment with mCPBG a 5-HT_3 receptor agonist, abolished the response of 4i at the active dose (1 mg/kg), although mCPBG alone was ineffective in FST. Similarly, in the previous study it was shown that mCPBG attenuates the anti-immobility effect of ICS 205930 (a 5-HT_3 antagonist) (Nakagawa et al., 1998). This suggested that the 5-HT_3 receptor antagonistic activity of 4i is actively involved in the postulated effect of the compound. Therefore, a confirmatory assay for 4i to mediate serotonin neurotransmission was performed by an in vivo study in mice.

Head twitch is a characteristic response produced due to enhanced synaptic serotonin neurotransmission in the brain. It has been reported that drugs with facilitatory effects on serotonergic system potentiate head twitch response produced by 5-HTP (a serotonin synthesis precursor) (Ramamoorthy et al., 2008; Rajkumar and Mahesh, 2010). In the present study, 5-HTP and PAR (a monoamine oxidase inhibitor) produced head twitches in mice, which were potentiated by 4i as well as FLX. This suggested that the test compound 4i, similar to fluoxetine, facilitates serotonergic neurotransmission. The results are in accordance with the previous findings that 5-HT_3 receptor antagonists increase head twitch behavior in rodents (Devadoss et al., 2010; Gautam et al., 2013). This study is further supported by the clinical view that 5-HT_3 receptor antagonists produce serotonin syndrome (a side-effect of drugs that enhance serotonin contents in the brain) in patients on antidepressant treatment (Turkel et al., 2001).

At this juncture it is worth noting that 4i has antidepressant and anti-anxiety like effects. Although the exact mechanism of action involved in the postulated effect of the compound is yet to be known, in vivo behavioral assays revealed that 4i may act by enhancing the synaptic serotonergic neurotransmission as indicated by enhanced FLX (a SSRI) action and potentiated 5-HTP induced head twitch response, which may be mediated by antagonism of 5-HT_3 receptors (as mCPBG, a 5-HT_3 receptor agonist blocked the action of 4i). This is in agreement with the previous reports that 5-HT_3 receptor antagonists may have facilitatory action at serotonergic system (Ramamoorthy et al., 2008). Moreover, currently existing drugs with antidepressant and anxiolytic profile support the proposed mechanism of action of the compound, that enhanced serotonergic activity ameliorates the depression and anxiety like behavior both in rodents and humans (David et al., 2009; Davidson et al., 2004; Dulawa et al., 2004; Pedersen, 2005).

It is interesting to note that, 5-HT_3 receptors are present both pre- as well as post-synaptically (Rajkumar and Mahesh, 2010; Thompson and Lummis, 2007) and hence, 4i may have affinity at both receptor levels. It is evident that antagonism of pre-synaptic 5-HT_3 receptors results in inhibition of 5-HT_3 receptor mediated excitation of inhibitory neurons. Previously, this hypothesis has been proposed for the pre-synaptic 5-HT_3 receptor mediated antidepressant-like effect of fluoxetine (Fan, 1994), which could possibly be defined as a mode of action of 4i. Alternatively, antagonism of post-synaptic 5-HT_3 receptors may provide a more specific 5-HT mediated action of 4i, which might be responsible for its postulated effects (Ramamoorthy et al., 2008). In addition, being hetero-receptor (regulating the synthesis and/or release of mediators other than its own ligand) (Thompson and Lummis, 2007), 5-HT_3 antagonists may have other possible pathways to regulate behavioral activities. A wide literature survey has shown that, besides 5-HT, 5-HT_3 receptors are located on nerve terminals and alter the release of other monoamines (like nor epinephrine and dopamine) and gamma aminobutyric acid (GABA). The accumulated evidence suggests that inhibition of 5-HT_3 receptors has a variable impact on synaptic levels of these neurotransmitters, consequently affecting the behavior (Rajkumar and Mahesh, 2010). Therefore, heterogeneity as well as existence at pre- and post-synaptic levels of 5-HT_3 receptors should be considered while explaining the overall mechanism of action of 4i.

Although, 5-HT_3 receptor antagonists have been proposed to have antidepressant and anti-anxiety like effects predominantly by facilitating serotonergic activity (similar to the SSRIs), the previous reports have shown that improvement in the behavioral symptoms occurs within 2–3 weeks of the treatment (unlike SSRIs) (Ramamoorthy et al., 2008; Rajkumar and Mahesh, 2010). Furthermore, the activity of 5-HT_3 receptor antagonists (as in the present study) was found at much lower dose ranges (Bill et al., 1992; Kos et al., 2006; Ramamoorthy et al., 2008). This offers the advantages over the currently existing clinical drugs. However, further studies are required to be done to get the potential therapeutic efficacy of 5-HT_3 receptor antagonists like 4i in clinical settings and if proved right it may provide the 4i as a potential agent for the therapy of depression and anxiety like mood disorders, which may overcome the drawbacks of existing pharmacotherapy.

5. Conclusion

Overall, the present neurobehavioral investigation demonstrates that, 4i a novel compound with 5-HT_3 receptor antagonistic action, exerts potential antidepressant and anti-anxiety like effects in multiple acute and chronic animal models. Based on the results of behavioral analysis performed, the modulation of serotonergic system is thought to be involved in the postulated effects of the investigated compound.

Acknowledgment

The authors are thankful to BITS-Pilani and University Grants Commission, India for providing support and research facilities to pursue this work.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ejphar.2014.04.008.

References

Abramowitz, J.S., Landy, L., 2013. Treatment of Comorbid Depression. In: Storch, E. A., McKay, D. (Eds.), Handbook of Treating Variants and Complications in Anxiety Disorders. Springer, New York, pp. 243–254.

Anttila, S.A., Leinonen, E.V., 2001. A review of the pharmacological and clinical profile of mirtazapine. CNS Drug Rev. 7, 249–264.

Barchas, J.D., Altemus, M., 1999. Monoamine Hypothesis of Mood Disorders. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Lippincott-Raven, Philadelphia, Available from: ⟨http://www.ncbi.nlm.nih.gov/books/NBK28257/⟩.

Bilkei-Gorzo, A., Gyertyan, I., Levay, G., 1998. mCPP-induced anxiety in the light–dark box in rats–a new method for screening anxiolytic activity. Psychopharmacology 136, 291–298.

Bill, D.J., Fletcher, A., Glenn, B.D., Knight, M., 1992. Behavioral studies on WAY100289, a novel 5-HT_3 receptor antagonist, in two animal models of anxiety. Eur. J. Pharmacol. 218, 327–334.

Boissier, J.R., Simon, P., 1965. Action of caffeine on the spontaneous motility of the mouse. Arch. Int. Pharmacodyn. Ther. 158, 212–221.

Bourin, M., Hascoet, M., 2003. The mouse light/dark box test. Eur. J. Pharmacol. 463, 55–65.

Bourin, M., Hascoet, M., Colombel, M.C., Coutts, R.T., Baker, G.B., 2002. Clonidine potentiates the effects of tranylcypromine, phenelzine and two analogues in the forced swimming test in mice. J. Psychiatry Neurosci. 27, 178–185.

Bravo, G., Maswood, S., 2006. Acute treatment with 5-HT_3 receptor antagonist, tropisetron, reduces immobility in intact female rats exposed to the forced swim test. Pharmacol. Biochem. Behav. 85, 362–368.

Bromet, E., Andrade, L.H., Hwang, I., Sampson, N.A., Alonso, J., de Girolamo, G., et al., 2011. Cross-national epidemiology of DSM-IV major depressive episode. BMC Med. 9, 90–105.

Bruce, S.E., Machan, J.T., Dyck, I., Keller, M.B., 2001. Infrequency of pure GAD: impact of psychiatric comorbidity on clinical course. Depress. Anxiety 14, 219–225.
Castagne, V., Moser, P., Roux, S., Porsolt, R.D., 2011. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Curr. Protoc. Neurosci. 55, 11–18.
Castren, E., 2005. Is mood chemistry. Nat. Rev. Neurosci. 6, 241–246.
Costall, B., Jones, B.J., Kelly, M.E., Naylor, R., Tomkins, D.M., 1989. Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol. Biochem. Behav. 32, 777–785.
Costall, B., Naylor, R.J., 1992. Anxiolytic potential of 5-HT_3 receptor antagonists. Pharmacol. Toxicol. 70, 157–162.
Crawley, J.N., 1985. Exploratory behavior models of anxiety in mice. Neurosci. Biobehav. Rev. 9, 37–44.
Crawley, J.N., Goodwin, F.K., 1980. Preliminary report of a simple animal behavior for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 13, 167–170.
Cryan, J.F., Slattery, D.A., 2007. Animal models of mood disorders: recent developments. Curr. Opin. Psychiatry 20, 1–7.
David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., et al., 2009. Neurogenesis-dependent and-independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479–493.
Davidson, J.R.T., Bose, A., Korotzer, A., Zheng, H., 2004. Escitalopram in the treatment of generalized anxiety disorder: double-blind, placebo controlled, flexible-dose study. Depress. Anxiety 19, 234–240.
Delgado, P.L., 2000. Depression: the case for a monoamine deficiency. J. Clin. Psychiatry 61 (Suppl. 6), S7–S11.
Devadoss, T., Pandey, D.K., Mahesh, R., Yadav, S.K., 2010. Effect of acute and chronic treatment with QCF-3 (4-benzylpiperazin-1-yl)(quinoxalin-2-yl) methanone, a novel 5-HT (3) receptor antagonist, in animal models of depression. Pharmacol. Rep. 62, 245–257.
Dulawa, S.C., Holick, K.A., Gundersen, B., Hen, R., 2004. Effects of chronic fluoxetine in animal models of anxiety and depression. Neuropsychopharmacology 29, 1321–1330.
Duman, R.S., Malberg, J., Nakagawa, S., D'Sa, C., 2000. Neuronal plasticity and survival in mood disorders. Biol. Psychiatry 48, 732–739.
Eisensamer, B., Rammes, G., Gimpl, G., Shapa, M., Ferrari, U., Hapfelmeier, G., et al., 2003. Antidepressants are functional antagonists at the serotonin type 3 (5-HT3) receptor. Mol. Psychiatry 8, 994–1007.
Fan, P., 1994. Effects of antidepressants on the inward current mediated by 5-HT3 receptors in rat nodose ganglion neurones. Br. J. Pharmacol. 112, 741–744.
Gautam, B.K., Jindal, A., Dhar, A.K., Mahesh, R., 2013. Antidepressant–like activity of 2-(4-phenylpiperazin-1-yl)-1, 8-naphthyridine-3-carboxylic acid (7a), a 5-HT_3 receptor antagonist in behavior based rodent models: evidence for the involvement of serotonergic system. Pharmacol. Biochem. Behav. 109, 91–97.
Glinka, M.E., Samuels, B.A., Diodato, A., Teillon, J., Mei, D.F., Shykind, B.M., et al., 2012. Olfactory deficits cause anxiety-like behaviors in mice. J. Neurosci. 32, 6718–6725.
Gray, J.A., Lalljee, B., 1974. Sex differences in emotional behavior in rat: correlation between open field defecation and active avoidance. Anim. Behav. 22, 856–861.
Hascoet, M., Bourin, M., Nic Dhonnchadha, B.A., 2001. The mouse ligth-dark paradigm: a review. Prog. Neuropsychopharmacol. Biol. Psychiatry 25, 141–166.
Kelly, J.P., Wyrnn, A.S., Leonard, B.E., 1997. Olfactory bulbectomized rat as a model of depression: an update. Pharmacol. Ther. 74, 299–316.
Kent, J.M., 2000. SNaRIs, NaSSAs, and NaRIs: new agents for the treatment of depression. Lancet 355, 911–918.
Kos, T., Popik, P., Pietraszek, M., Schafer, D., Danysz, W., Dravolina, O., et al., 2006. Effect of 5-HT_3 receptor antagonist MDL 72222 on behaviors induced by ketamine in rats and mice. Eur. Neuropsychopharmacol. 16, 297–310.
Leonard, B.E., Tuite, M., 1981. Anatomical, physiological and behavioral aspects of olfactory bulbectomy in the rat. Int. Rev. Neurobiol. 22, 251–286.
Machado, D.G., Bettio, L.E.B., Cunha, M.P., Capra, J.C., Dalmarco, J.B., Pizzolatti, M.G., et al., 2009. Antidepressant-like effect of the extract of *Rosmarinus officinalis* in mice: involvement of the monoaminergic system. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 642–650.
MacKay, D., 1978. How should values of pA2 and affinity constants for pharmacological competitive antagonists be estimated. J. Pharm. Pharmacol. 30, 312–313.
Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., et al., 2009. The inflammatory and neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab. Brain Dis. 24, 27–53.
Mahesh, R., Devadoss, T., Pandey, D.K., Bhatt, S., Yadav, S.K., 2010. Design, synthesis and structure–activity relationship of novel quinoxalin-2-carboxamides as 5-HT_3 receptor antagonists for the management of depression. Bioorg. Med. Chem. Lett. 20, 6773–6776.
Mahesh, R., Dhar, A.K., Jindal, A., Bhatt, S., 2013. 2-(4-substituted piperazin-1-yl)-1, 8-naphthyridine-3-carboxylic acids: Novel 5-HT3 receptor antagonists with anxiolytic-like activity in rodent behavioral models. Can. J. Physiol. Pharmacol. 91, 848–854.
Mahesh, R., Jindal, A., Gautam, B., Bhatt, S., Pandey, D., 2011. Evaluation of antidepressant-like activity of linezolid, an oxazolidinone class derivative — an investigation using behavioral tests battery of depression. Biochem. Biophys. Res. Commun. 409, 723–726.
Mahesh, R., Perumal, R.V., Pandi, P.V., 2004. Microwave assisted synthesis of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile as a new class of serotonin 5-HT_3 receptor antagonists. Bioorg. Med. Chem. Lett. 14, 5179–5181.
Martin, P., Massol, J., Soubrie, P., Puech, A.J., 1989. Effects of triiodothyronine (T3) on the potentiation by antidepressants of L-5-hydroxytryptophan-induced head-twitches in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 13, 749–764.
Mathers, C.D., Fat, D.M., Boerma, J.T., 2008. The Global Burden of Disease: 2004 Update. World Health Organization, Switzerland.
Millan, M.J., Dekeyne, A., Papp, M., La Rochelle, C.D., Mac Sweeny, C., Peglion, J.L., et al., 2001. S33005, a novel ligand at both serotonin and norepinephrine transporters: II. Behavioral profile in comparison with venlafaxine, reboxetine, citalopram and clomipramine. J. Pharmacol. Exp. Ther. 298, 581–591.
Mork, A., Pehrson, A., Brennum, L.T., Nielsen, S.M., Zhong, H., Lassen, A.B., et al., 2012. Pharmacological effects of Lu AA21004: a novel multimodal compound for the treatment of major depressive disorder. J. Pharmacol. Exp. Ther. 340, 666–675.
Nakagawa, Y., Ishima, T., Takashima, T., 1998. The 5-HT3 receptor agonist attenuates the action of antidepressants in the forced swim test in rats. Brain Res. 786, 189–193.
Paton, W.D.M., Aboo Zar, M., 1968. The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. J. Physiol. 194, 13–33.
Pedersen, A.G., 2005. Escitalopram and suicidality in adult depression and anxiety. Int. Clin. Psychopharmacol. 20, 139–143.
Petit-Demouliere, B., Chenu, F., Bourin, M., 2005. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology 177, 245–255.
Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.
Rajkumar, R., Mahesh, R., 2010. The auspicious role of the 5-HT3 receptor in depression: a probable neuronal target. J. Psychopharmacol. 24, 455–469.
Ramamoorthy, R., Radhakrishnan, M., Borah, M., 2008. Antidepressant-like effects of serotonin type-3 antagonist, ondansetron: an investigation in behaviour-based rodent models. Behav. Pharmacol. 19, 29–40.
Redrobe, J.P., Bourin, M., 1997. Partial role of 5-HT_2 and 5-HT_3 receptors in the activity of antidepressants in the mouse forced swimming test. Eur. J. Pharmacol. 325, 129–135.
Ressler, K.J., Nemeroff, C.B., 2000. Role of serotonergic and noradrenergic systems in the pathophysiology of depression and anxiety disorders. Depress. Anxiety 12 (S1), 2–19.
Rodgers, R.J., Perrault, G., Sanger, D.J., Griebel, G., 1997. Risk assessment Hewlettior: evaluation of utility in the study of 5-HT-related drugs in the rat elevated plus-maze test. Pharmacol. Biochem. Behav. 57, 817–827.
Romeas, T., Morissette, M.C., Mnie-Filali, O., Piñeyro, G., Boye, S.M., 2009. Simultaneous anhedonia and exaggerated locomotor activation in an animal model of depression. Psychopharmacology 205, 293–303.
Roychoudhury, M., Kulkarni, S.K., 1997. Anti-anxiety profile of ondansetron, a selective 5-HT_3 antagonist, in a novel animal model. Method Find. Exp. Clin. 19, 107–111.
Song, C., Leonard, B.E., 2005. The olfactory bulbectomized rat as a model of depression. Neurosci. Biobehav. Rev. 29, 627–647.
Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressant drugs. Psychopharmacology 85, 367–370.
Takeda, H., Tsuji, M., Matsumiya, T., 1998. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur. J. Pharmacol. 350, 21–29.
Thompson, A.J., Lummis, S.C., 2007. The 5-HT_3 receptor as a therapeutic target. Expert Opin. Ther. Targets 11, 527–540.
Turkel, S.B., Nadala, J.G., Wincor, M.Z., 2001. Possible serotonin syndrome in association with 5-HT_3 antagonist agents. Psychosomatics 42, 258–260.
Van Gaalen, M.M., Steckler, T., 2000. Behavioral analysis of four mouse strains in an anxiety test battery. Behav. Brain Res. 115, 95–106.
Van Riezen, H., Leonard, B.E., 1990. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. Pharmacol. Ther. 47, 21–34.
Wang, D., Noda, Y., Tsunekawa, H., Zhou, Y., Miyazaki, M., Senzaki, K., et al., 2007. Behavioral and neurochemical features of olfactory bulbectomized rats resembling depression with comorbid anxiety. Behav. Brain Res. 178, 262–273.
Willner, P., Towell, A., Sampson, D., Sophokleous, S., Muscat, R., 1987. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology 93, 358–364.
Zueger, M., Urani, A., Chourbaji, S., Zacher, C., Roche, M., Harkin, A., Gass, P., 2005. Olfactory bulbectomy in mice induces alterations in exploratory behavior. Neurosci. Lett. 374, 142–146.