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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

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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

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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.

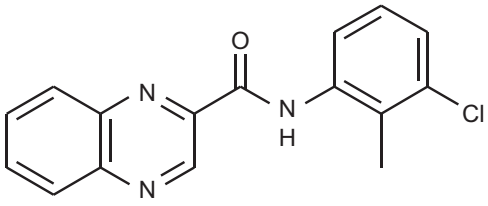

Fig. 1. The structure of 4i.

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