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Antidepressant-like activity of 2-(4-phenylpiperazin-1-yl)-1, 8-naphthyridine-3-carboxylic acid (7a), a 5-HT$_3$ receptor antagonist in behaviour based rodent models: Evidence for the involvement of serotonergic system

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ABSTRACT

The present study was designed to investigate the putative antidepressant-like activity of **7a,** a 5-HT$_3$ receptor antagonist, (although indirect evidence of 5-HT$_3$ antagonism) with an optimal log P (3.35) and pA$_2$ value (7.6) greater than ondansetron (pA$_2$ — 6.6) using behavioural tests battery of depression. Acute treatment of 7a (0.5-2 mg/kg, i.p.) in mice produced antidepressant-like effects in forced swim test (FST) and tail suspension test (TST) without affecting the baseline locomotion in actophotometer test in mice. Moreover, the combination of a sub-effective dose of 7a (0.25 mg/kg, i.p.) and fluoxetine (5 mg/kg, i.p.) produced an anti-immobility effect in mouse FST. Pre-treatment of mice with p-chlorophenylalanine methyl ester (PCPA; 100 mg/kg, i.p., an inhibitor of serotonin (5-HT) synthesis, for 4 consecutive days) and 1-(m-Chlorophenyl)-biguanide (mCPBG, 10 mg/kg, i.p., a 5-HT$_3$ receptor agonist) prevented the anti-immobility effects of 7a (2 mg/kg, i.p.) in the mouse FST. In addition, 7a (0.5–2 mg/kg, i.p.) treatment also potentiated the 5-hydroxytryptophan (5-HTP) and pargyline induced head twitch response in mice. Furthermore, sub-chronic treatment (14 days) with 7a (0.5–2 mg/kg, i.p.) and paroxetine (10 mg/kg, i.p.) significantly attenuated the behavioural anomalies induced by bilateral olfactory bulbectomy in rats in a modified open field paradigm. These results suggest that the antidepressant-like action of 7a may be mediated by an interaction with the serotonergic system and this molecule should be further investigated as an alternative therapeutic approach for the treatment of depression.

1. Introduction

Depression is a severe psychiatric disorder with lifetime prevalence as high as 20% (Manji et al., 2001). According to World Health Organization by the year 2020, it will be the second largest global burden of disease, illustrating the severity and impact of the disorder (Manji et al., 2001; Nestler et al., 2002). Several promising attempts at explaining the neurobiology of depression (Davidson et al., 2002; Nestler et al., 2002) have focused on the serotonergic neurotransmitter system (Deakin, 1991) as a principal target for several antidepressant agents (Adell et al., 2005).

Serotonin type-3 (5-HT$_3$) receptor antagonists are currently used in the management of nausea and vomiting associated with cancer chemotherapy (Mahesh et al., 2004, 2005). Interestingly, in the last decade, these molecules have been extensively evaluated for their neuro-psychopharmacological potential in various pre-clinical and few clinical studies (Wolf, 2000; Israili, 2001). The electrophysiologically characterized 5-HT$_3$ receptors (Peters et al., 1992) are found in median raphe, hypothalamus, hippocampus and amygdala (Kilpatrick et al., 1987; Waeber et al., 1988; Laporte et al., 1992; Kidd et al., 1993). Several pre-clinical (behavioural, neurochemical and genetic) studies have provided evidence linking 5-HT$_3$ receptors and depression (Ramamoorthy et al., 2008; Rajkumar and Radhakrishnan, 2010). It has been well documented that 5-HT$_3$ receptor antagonists reverse escape deficits in rat learned helplessness test, a sensitive antidepressant screening method (Martin et al., 1992).

Selective 5-HT$_3$ receptor antagonist, ondansetron (OND) has been reported to produce antidepressant-like effect in rodents (Ramamoorthy et al., 2008) and potentiated the anti-immobility effects of selective serotonin reuptake inhibitors (SSRIs) (Redrobe and Bourin, 1997) indicating the role played by 5-HT$_3$ receptors in depression. Furthermore, ICS 205930 (5-HT$_3$ receptor antagonist) has demonstrated antidepressant - like activity in forced swim test in mice (Nakagawa et al., 1998).

Furthermore, MDL72222 (bemesetron) and tropisetron, selective 5-HT$_3$ receptor antagonists, have been shown to reduce the duration of immobility in the mouse tail suspension test (Kos et al., 2006) and forced swim test respectively (Bravo and Maswood, 2006). Moreover, several commercially available antidepressants like fluoxetine, imipramine, phenelzine and iproniazid have been reported to produce antidepressant-like activity by blocking 5-HT$_3$ receptor (Fan, 1994).

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Recently, QCF-3, a novel 5-HT_3 receptor antagonist has demonstrated antidepressant-like activity in FST and TST (Devadoss et al., 2010).

A series of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids were designed as 5-HT_3 receptor antagonists using ligand-based approach and these molecules were synthesized from the staring material, nicotinamide, in a sequence of reactions as depicted in scheme 1 (supplementary data). 5-HT_3 receptor antagonism was determined in the form of pA_2 value against agonist 2-methyl 5-HT in longitudinal muscle myenteric plexus preparation from guinea pig ileum as per method described previously (Mahesh et al., 2004, 2005).

Animal models of depression have been utilized vigorously to screen novel compounds (Bourin, 1990) and were originally designed as screening tests to assess the efficacy of various antidepressants. Hence, we utilized a battery of behavioural tests viz. forced swim test (Porsolt et al., 1977), tail suspension test (Steru et al., 1985), 5-hydroxytryptophan (5-HTP) induced head twitch response in mice (Martin et al., 1989) and olfactory bulbectomy (Song and Leonard, 2005) in rats to investigate antidepressant-like activity of 7a.

In the present study, compound 7a was selected from a series of compounds based on the optimal log p (3.35) and pA_2 value (7.6) greater than 5-HT_3 receptor antagonist, ondansetron (pA_2-6.6). Hence, the present investigation was designed to (i) assess the antidepressant prospect of 7a (a 5-HT_3 receptor antagonist), in a battery of *in-vivo* behavioural tests of depression (ii) clarify the possible involvement of putative 5-HT_3 receptors in rodent depressive states and (iii) to elucidate the possible implication of serotonergic neurotransmitter system in mediating the antidepressant-like activity of 7a.

2. Materials and methods

2.1. Experimental animals

Swiss Albino mice (25–30 g; either sex) and male Wistar rats (225–300 g) were procured from Lala Lajpat Rai University of Veterinary and Animal Science, Hisar, Haryana, India. All procedures were in adherence to Institutional Animal Ethics Committee of Birla Institute of Technology & Science (BITS), Pilani, India (Protocol No. — IAEC/RES/14/4). The animals were housed in laboratory cages and maintained under standard light/dark cycle (light on 6:00–18:00 h), temperature (23 ± 2 °C) and humidity (50–60%) conditions in the housing unit for at least one week before the commencement of experiments. The animal was given free access to food (standard food pellets) and filtered water. All the behavioural studies were carried out during the light phase (9.00 h–14.00 h).

2.2. Chemistry of 2-(4-phenylpiperazin-1-yl)-1, 8-naphthyridine-3-carboxylic acid (7a)

The target molecule 2-(4-phenylpiperazin-1-yl)-1,8-naphthyridine-3-carboxylic acid (7a) was synthesized via sequence of reactions as shown in scheme 1 (supplementary data). The structure of the synthesized compound was confirmed by the spectral data; ^{1}H NMR, DMSO-d_6, δ (ppm): 8.90–8.94 (dd, 1H, J = 4.8, 2 Hz, naphthyridine), 8.40(s, 1H, naphthyridine), 8.10–8.12 (dd, 1H, J = 8, 2Hz, naphthyridine), 7.31–7.27 (m, 3H, phenyl), 6.97–6.95 (m, 2H, phenyl), 6.93–6.89 (dd, 1H, J = 10, 7.2 Hz, naphthyridine), 3.88–3.86 (m, 4H, piperazine), 3.38–3.35 (m, 4H, piperazine). FT-IR (KBr, cm^{-1}): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1350, 1266. The structure of 7a is shown in Fig. 1.

2.3. Drugs and chemicals

Fluoxetine and paroxetine were obtained from Sun Pharmaceuticals and Ipca Laboratories, India, respectively as generous gift samples. Bupropion was obtained from Ranbaxy Research Laboratories, India.

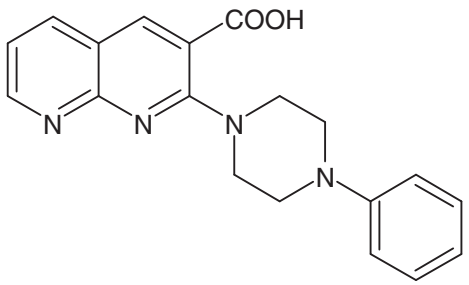

Fig. 1. Structure of 7a.

Pargyline, 5-Hydroxtryptophan (5-HTP) and para-chlorophenylalanine (PCPA) were procured from Sigma Chemicals, USA. 1-(m-Chlorophenyl)-biguanide (mCPBG) was purchased from Tocris Bioscience, UK. **7a** and standard antidepressants were dissolved immediately before use in distilled water. Ketamine and xylazine were purchased from Neon Laboratories Ltd. and Indian Immunologicals, India, respectively. Haemostatic sponge was purchased from Sri Gopal Krishan Lab Pvt. Ltd, India.

2.4. Dose selection

Based on the preliminary studies using FST in mice, the active doses of the 7a were found to be ranging from 0.5 to 8 mg/kg, i.p. Doses less than 0.5 mg/kg, i.p. didn't show any activity, whereas at dose above 2 mg/kg, saturation effect was observed. That's why dose range 0.5–2 mg kg/i.p was selected and tested in various animal models.

2.5. Behavioural tests

2.5.1. Spontaneous locomotor activity

The spontaneous locomotor activity of mice was assessed using actophotometer (Boissier and Simon, 1965). In brief, the mice were individually placed in the centre of the square arena (30 cm × 30 cm) of actophotometer. After an initial familiarization period (2 min), the digital locomotor scores were recorded for the next 8 min in a dimly lit room. The arena was cleaned with dilute alcohol and dried between trails. 7a (0.25–2 mg/kg, i.p.) was administered 30 min prior to testing.

2.5.2. Forced swim test (FST)

The FST was performed with minor modifications (Mahesh et al., 2011) from the originally described method (Porsolt et al., 1977). In brief, each mouse was placed individually in a glass cylinder (diameter: 22.5 cm, height: 30 cm) containing 15 cm of water at 23 ± 1 °C. The floor of the cylinder was demarcated into four equal quadrants. The mice (vehicle/drug treated) were placed in the water and forced to swim for 6 min. The duration of immobility, which reflects the state of depression was recorded during the last 4 min of the 6 min test. The swimming episodes were recorded as number of quadrants (demarcated at the base of the cylinder) crossed. A mouse was considered to be immobile when it stopped struggling and passively moved to remain floating and kept its head above water. Water was changed between trials and temperature was maintained at 23 ± 1 °C. 7a (0.25–2 mg/kg, i.p.) and fluoxetine (10 mg/kg, i.p.) were administered 30 min prior to testing.

2.5.3. Tail suspension test (TST)

The method reported by Steru et al. (1985) was followed with slight modifications (Mahesh et al., 2011). In brief, the mice were suspended on the edge of a shelf 50 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for 6 min observation period. Mice were