Indian J Med Res 144, October 2016, pp 614-621
DOI: 10.4103/0971-5916.200893

Quick Response Code:

Antidepressant & anxiolytic activities of N-(pyridin-3-yl) quinoxalin-2-carboxamide: A novel serotonin type 3 receptor antagonist in behavioural animal models

Dilip Kumar Pandey[1], Thangraj Devadoss[2], Neha Modak[3] & Radhakrishnan Mahesh[3]

[1]Pharmacology Department, Novel Drug Discovery and Development (NDDD), Lupin Research Park, Pune, [2]Department of Pharmaceutical Chemistry, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, & [3]Department of Pharmacy, Birla Institute of Technology & Science, Pilani, India

Received February 25, 2014

***Background & objectives*: Alteration in the serotonin leads to the psychological illness, such as depression, anxiety, schizophrenia, eating disorders, obsessive-compulsive disorder, panic disorders and migraines. The objective of the current study was to investigate the antidepressant and anxiolytic activities of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21), a novel 5-HT$_3$ receptor antagonist in preclinical models of depression and anxiety.**

***Methods*: Antidepressant activity was evaluated in preliminary tests such as forced swim and tail suspension tests (FST & TST). Anti-anxiety effect of QCF-21 was investigated by employing elevated plus maze (EPM), light/dark and hole board tests. Olfactory bulbectomy (OBX) in rats was used as chronic model of depression. Mechanistic test of QCF-21 was evaluated by reserpine-induced hypothermia and 5-hydroxytryptophan (5-HTP)-induced head-twitch response.**

***Results*: The dose-response study revealed an initial antidepressant-like effect of QCF-21(0.25-1 mg/kg, i.p.) in the FST and TST and anxiolytic-like effect in EPM, light and dark and hole board tests. QCF-21 potentiated the 5-HTP-induced head-twitches response in mice and reversed reserpine-induced hypothermia in rats. QCF-21 significantly reversed the behavioural anomalies post-OBX in rats.**

***Interpretation & conclusions*: The present findings indicate the potential antidepressant-like and anxiolytic-like effects of QCF-21 at low doses in rodent behavioural models of depression and anxiety. Further studies need to be done to understand the underlying mechanism.**

Key words Anxiety - behavioural tests - depression - N-(pyridin-3-yl) quinoxalin-2-carboxamide - novel 5-HT3 antagonist - serotonin

Serotonin or 5-hydroxytryptamine (5-HT) is a key neurotransmitter that modulates many neuronal functions and has been linked with pathophysiology of many disease conditions. Serotonin may possibly be imperative in psychological/psychiatric illnesses, such as depression, anxiety, schizophrenia, eating disorders, obsessive-compulsive disorder, panic disorders and migraine[1-4]. The 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors

were the first serotonergic receptors targeted to treat anxiety and depression due to their pre-and/or post-synaptic localization[5,6]. However, the attention has been shifted towards the recognition of 5-HT_3 role in psychological illness such as sleep, pain and migraine, as well as in the pathophysiology of many psychiatric disorders including depression and anxiety[7-9]. The 5-HT_3 receptors are the only ionotropic or ligand-gated ion channel of the 5-HT receptor family that alters synaptic neurotransmission. The 5-HT_3 receptors are found in median raphae, hypothalamus, hippocampus and amygdala, which have neural correlates of depression and anxiety[8]. Activation of 5-HT_3 receptor in the brain leads to the release of monoamines like dopamine and serotonin. Since 5-HT_3 receptor antagonists delivered central effects equivalent to those of antipsychotics and anxiolytics, in the early nineties schizophrenia and anxiety were considered as potential indications[8]. Redrobe and Bourin[9] have demonstrated that 5-HT_3 receptors play a partial role in the effectiveness of anti-depressants during the forced swim test (FST). The deletion of the 5-HT_3 receptor gene exhibited anxiolytic behaviour in mice[10].

Utilizing the three-component pharmacophore model[11] for the 5-HT_3 receptor antagonists as a guide, N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) was designed and synthesized. Our previous study showed the preliminary effect of novel 5-HT_3 molecule in behavioural model[11], the focus was to evaluate the new series of the 5-HT_3 receptor antagonists for their antidepressant/anxiolytic activity at a minimal dose. In our previous studies, various 3-substituted quinoxalin-2-carboxamides (consisting of Mannich base as a linking unit for piperazine moiety and quinoxaline nucleus) were assessed as 5-HT_3 receptor antagonists; however, none of the compounds showed encouraging preliminary effect in depression and anxiety[11]. Anticipating a dual use in managing both depression and anxiety, in this study the potential effect of QCF-21, a novel 5-HT_3 receptor antagonist was investigated in animal models of depression and anxiety.

Material & Methods

Male Swiss albino mice (20-25 g) and Wistar rats (180-200 g) were purchased from Hisar Agricultural University (Hisar, Haryana, India) and kept under standard lighting (lights on: 0700-1900 h), temperature (23°C ± 2°C) and room humidity (60 ± 10%) conditions. The rodents were housed in standard polycarbonate cages and provided with free access to food (standard pellet chow, Amrit pellets, Bengaluru) and filtered water. The animals were used only once for each experiment and were acclimated to the experimental room for 1 h before the initiation of experiment. Each experimental group had 6-8 animals. The study protocol was approved by the Institutional Animal Ethics Committee of Birla Institute of Technology and Science, Pilani, India (IAEC/RES/14/04). Acute studies for depression and anxiety were performed in Swiss Albino mice, and chronic antidepressant study was done in male Wistar rats.

Drugs & chemicals: Escitalopram (ESC) and bupropion (BPN) hydrochloride (HCl) were obtained from Glenmark Pharmaceuticals, Mumbai, and Ranbaxy Research Laboratories, Gurgaon, India, as a gift sample. Diazepam (DZM) was procured from Ranbaxy, Gurgaon, India. Pargyline and 5-HTP were purchased from Sigma Chemicals, USA. Reserpine was purchased from Sisco Research Laboratories, Mumbai, India. Ketamine and xylazine were purchased from Neon laboratory and Indian Immunological, India, respectively. All drugs were freshly prepared in distilled water and administered perorally (p.o.) and intraperitoneally (i.p.). Dose-response studies were performed by using the mouse locomotor activity test, FST and tail suspension test (TST) to determine the QCF-21 doses that significantly exhibited antidepressant-like activity without affecting the baseline locomotion. QCF-21 (0.25-2 mg/kg) was administered to mice 30 min before starting the locomotor activity test, FST, TST, light and dark test, elevated plus maze (EPM) test and hole board test. In acute and chronic models, low and high doses, respectively, were selected for screening QCF-21. ESC (10 mg/kg) and BPN (20 mg/kg) were used as reference standard in the depression study, whereas DZM was used in anxiety studies. The dose of reference drugs was selected from the pilot studies in the laboratory.

The target compound, QCF-21 was synthesized by coupling of 3-aminopyridine with quinoxalin-2-carboxylic acid in the presence of conventional coupling agents, 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide HCl and 1-hydroxybenzotriazole under inert atmospheric nitrogen. The key intermediate, quinoxalin-2-carboxylic acid was synthesized in two steps as per the literature method[12].

Pharmacological studies

Locomotor activity: Locomotor activity was evaluated using the actophotometer[13], which contains a square

arena (30 cm × 30 cm) with walls that are fitted with photocells just above the floor level. The locomotor activity was evaluated for 10 min post 30 min of i.p. drug treatment.

Forced swim test (FST): The procedure of Porsolt *et al*[14] was followed with certain modifications. In brief, the mice were forced to swim for 15 min on the pre-test day in glass cylinder. Post 24 h of training, each mouse (vehicle/drug treated) was placed into the water and forced to swim for six minutes after 30 min of i.p. treatment of QCF-21. The immobility time during the last four minutes was measured. The mouse was considered to be immobile when it stopped struggling and passively moved to remain floating and kept its head above water. After each trial water was changed, and temperature was maintained at 22°C ± 2°C.

Tail suspension test (TST): Behavioural despair was induced by a TST according to the procedure described by Steru *et al*[15]. Mice were suspended individually from a horizontal bar 50 cm above the tabletop using an adhesive tape post 30 min of i.p. treatment of QCF-21. The point of attachment on the tail was 1 cm from the tip. The duration of immobility (seconds) during the six minutes observation period was recorded.

Reserpine-induced hypothermia (RIH): The procedure was adopted as mentioned by Devadoss *et al*[16]. Male Wistar rats were treated with reserpine (1 mg/kg, i.p.) 30 min after oral administration of QCF-21. The effects of QCF-21 on reserpine-induced hypothermia (measured with digital thermometer) were recorded 30 min before administering reserpine and 30, 60, 90 and 120 min after administering reserpine. Hypothermia was measured by calculating the temperature difference between 120 and zero minute.

5-Hydroxy tryptophan induced head twitch response (5-HTP-HTR): The method has been described elsewhere[17]. QCF-21 pretreated mice were treated (p.o.) with pargyline hydrochloride (75 mg/kg) and 5-HTP (5 mg/kg) 30 and 15 min before the observations began, respectively. The number of head twitches response was recorded post 15 min of 5-HTP administration.

Olfactory bulbectomy (OBX) surgery: A bilateral OBX was performed in rats as described by Kelly *et al*[18] with substantial modifications. Briefly, the rats were anaesthetized with xylazine (5 mg/kg) and ketamine (75 mg/kg, i.p.). Burr holes (2 mm in diameter) were drilled 8 mm anterior to the bregma and 2 mm on either side of the midline at a point corresponding to the posterior margin of the eye orbit. The olfactory bulbs were removed by suction, the holes were filled with haemostatic sponge to control excessive bleeding, and the scalp was sutured. Sham-operated rats went through the same procedure, including piercing of the dura mater, with their bulbs left intact.

Open field exploration: The OBX and sham rats were subjected to an open field test 29 days after the surgery and 15 days after starting the oral chronic drug/vehicle treatment. The open field exploration was conducted as described[18,19].

Anxiety tests

Elevated-plus maze (EPM): This test has been widely validated to measure anxiety in rodents. In brief, 30 min after the treatment, mice were placed for five minutes on an elevated-plus maze consisting of four arms (25 × 7 cm), two with high, black walls (15 cm high) and two without walls. The maze floor was constructed with plywood. Mice were placed in the intersection between the arms (7 × 7 cm), and the number of entries into, and the time spent in, the open arms were recorded. These two parameters were taken as measures of anxiety-related behaviour[20].

Light/dark box test: The light/dark test uses the rodent natural aversion to bright areas compared with darker ones[21]. In a two-compartment box, rodents will prefer to remain in dark areas, whereas anxiolytics should increase the time spent in the lit arena. A 60 W bulb placed 25 cm above the light box provided the illumination. Mice were individually tested in five minutes sessions in this apparatus after 30 min of treatment.

Hole-board test: The exploratory activity of the QCF-21 in mice following administration was determined using the hole-board test[22]. The apparatus used consisted of a white wooden board (60 × 30 cm) with 16 evenly spaced holes (1 cm diameter x 2 cm depth). Each mouse was placed singly at one corner of the board. Dipping of the head into a hole is a typical behaviour of the mouse indicating a certain degree of curiosity. The number of dips in five minutes was recorded. The test was carried out 30 min after i.p. treatment of QCF-21.

Statistical analysis: The single treatment data were analyzed using one-way analysis of variance followed by Dunnett's *post hoc* test.

Results

Locomotor scores: Lower doses of QCF-21 (0.25-1 mg/kg, i.p.) had no influence on baseline locomotion when

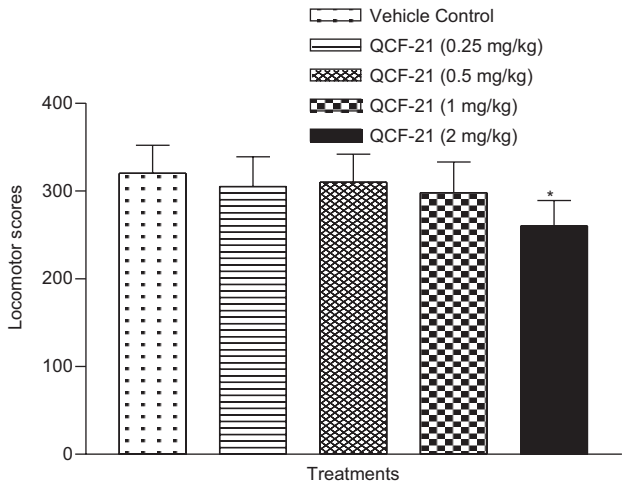

Figure. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) on locomotor activity in mice. The columns represent the mean locomotor scores recorded in a 10 min observation period. Values are given as mean ± SEM, $^{*}P<0.05$ compared to vehicle-treated group, n=8 mice/group.

compared to the control group, whereas a higher QCF-21 (2 mg/kg) showed significant ($P<0.05$) decrease in locomotor activity compared to the control group (Figure).

FST and TSTs: QCF-21 (0.5-1 mg/kg) induced a significant ($P<0.05$) reduction in immobility time in mice during the FST as compared to the control group (Table I). The positive control, ESC (10 mg/kg), also induced a significant ($P<0.01$) change in immobility as compared to the control group. In the TST, QCF-21 (0.5-1 mg/kg) treatment significantly ($P<0.05$) decreased the duration of immobility as compared to the vehicle control group, and BPN was used as a standard in TST (Table I).

Reserpine-induced hypothermia: Administration of reserpine (1 m/kg, i.p.) to rats exhibits a pronounced decrease ($P<0.05$) in body temperature. Oral administration of QCF-21 (1 mg/kg) significantly ($P<0.05$) reversed the reserpine-induced hypothermia in rats as compared to vehicle-treated group. Similarly, ESC (10 mg/kg) reversed the hypothermic effect of reserpine (Table II).

5-HTP-induced head-twitch response: The combined administration of pargyline and 5-HTP (75 + 5 mg/kg) induced the characteristic head-twitch response. Pre-treatment with QCF-21 (1 mg/kg, $P<0.05$) and ESC significantly ($P<0.01$) potentiated the head-twitch response as compared to a combination of pargyline and 5-HTP alone (Table II).

Open field test post OBX: The effect of different treatments on the behavioural anomalies of the OBX/sham rats was investigated in open field test (Table III). OBX rats exhibited characteristic hyperactivity behaviour during the open field test as compared to sham rats. Chronic (14 days) administration of QCF-21 (1 mg/kg) significantly ($P<0.05$) reduced the ambulation and rearing behaviour in the OBX rats as compared to the vehicle-treated OBX rats. QCF-21 exhibited antidepressant-like effects, while ESC (10 mg/kg) was the most effective antidepressant among all treatments. QCF-21 had no effect in the sham rats, but ESC had a moderate effect in the behaviour of sham rats.

EPM test: Table IV displays the behavioural effect in EPM test. In EPM test, untreated mice preferred to be in the closed arm. The QCF-21 (1 mg/kg) significantly ($P<0.05$) increased the per cent time spent and entry in open arm as compared to vehicle-treated group. DZM (2 mg/kg)-treated animals showed more number of entries and time spent in open arm when compared to vehicle-treated mice group.

Light and dark test: In light and dark test, animals treated with three doses of QCF-21 (0.25, 0.5 and 1 mg/kg) and DZM showed increase in the time that mice spent in the light area and increased number of crossing (Table IV). DZM (2 mg/kg) significantly ($P<0.01$) increased the time spent in light arena of light and dark boxes. Animals treated with high dose

Table I. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on forced swim and tail suspension tests

Treatments	Dose (mg/kg, i.p.)	Duration of immobility (sec)	
		FST	TST
Vehicle control	-	160.8±7.5	233.6±8.3
QCF-21	0.25	137.6±3.7	200.3±12.0
QCF-21	0.5	128.6±4.3*	187.4±6.5*
QCF-21	1	117.6±13.3*	164.3±8.3*
ESC (FST)/BPN (TST)	10/20	78.8±5.9**	143.8±10.5**

Values are expressed as the mean±SEM, $P^{*}<0.05$, $^{**}< 0.01$ compared to vehicle control; n=8 mice/group. SEM, standard error of mean; ESC, escitalopram; BPN, bupropion; FST, forced swim test; TST, tail suspension test

Table II. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on temperature and head twitch response evaluation

Treatments	Dose (mg/kg, i.p.)	Mean decrease in temp. (°F)	Number of head twitches
Vehicle control	-	2.9±0.2	22.9±1.8
QCF-21	0.25	2.3±0.3	26.4±2.7
QCF-21	0.5	1.9±0.2	35.6±5.3
QCF-21	1	1.3±0.1*	53.4±5.5*
ESC	10	1.1±0.2**	91.9±6.2**

Values are expressed as the mean±SEM, *P<0.05, **<0.01 when compared to the vehicle control; n=8 mice/group. SEM, standard error of mean; ESC, escitalopram

Table III. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on the behaviour of olfactory bulbectomy rats in the modified open field test

Treatments	Dose (mg/kg)	Ambulation	Rearing
Sham control		87.30±10.09	8.32±0.86
Sham + QCF-21	0.5	91.8±7.21	9.23±0.96
Sham + QCF-21	1	95.89±6.19	8.45±0.85
Sham + ESC	10	100.23±6.67	7.45±0.67
OBX control		155.21±23.45*	24.23±2.85*
OBX + QCF-21	0.5	132.75±8.84	20.21±1.04
OBX + QCF-21	1	112.56±8.9#	15.41±1.53#
OBX + ESC	10	98.40±6.45#	13.69±0.81#

Values are expressed as the mean±SEM. The drug/vehicle treatments were administered once a day for 14 days. *P<0.05 compared to sham control; #P<0.05 compared to vehicle-treated OBX group. n=6 rats/group. SEM, standard error of mean; OBX, olfactory bulbectomy; ESC, escitalopram

Table IV. Effect of N-(pyridin-3-yl) quinoxalin-2-carboxamide (QCF-21) and escitalopram on Elevated plus maze, Light and dark, and Hole board Tests

Treatments	Dose (mg/kg, i.p.)	EPM test		Light and dark test		Hole board test	
		% Open arm entry	% Time spent in open arm	Time spent in lit area (sec)	No. of crossing	No. of nose poking	Duration of nose poking (sec)
Vehicle control	-	13.9±1.1	32.1±2.8	23.9±2.3	12.5±2.4	20.8±2.4	19.8±2.2
QCF-21	0.25	12.0±0.9	34.5±3.7	27.7±4.6	10.3±0.8	26.9±3.0	21.3±1.8
QCF-21	0.5	34.3±3.0	43.4±5.6	39.0±4.7	19.4±1.3	29.4±3.7	25.3±2.8
QCF-21	1	42.9±3.3*	60.7±5.1*	51.0±4.3*	26.5±1.6*	34.8±3.2*	30.6±2.68
DZM	2	60.3±4.4**	70.6±4.4**	76.1±11.1**	39.8±3.5**	50.4±3.2**	39.4±4.0**

Values are expressed as the mean±SEM, *P<0.05, **<0.01 compared to vehicle control; n=8 mice/group. SEM, standard error of mean; ESC, escitalopram; DZM, diazepam

(1 mg/kg) showed more significant results when compared with low dose.

Hole board test: The hole board test is a measure of exploratory behaviour in animals. The QCF-21 dose dependently increased the number and duration of head dipping in the hole board experiment (Table IV). The increase was significantly (P<0.05) different from vehicle control and DZM which were used as controls.

Discussion

The present results revealed that the novel 5-HT_3 antagonist, QCF-21 possessed antidepressant-like

and anxiolytic-like activities. The assessment of the probable antidepressant activity of QCF-21 was evaluated in the preliminary depression models such as FST and TST. The primary assessment in the tests is the duration of immobility. QCF-21 exhibited antidepressant-like effects in the FST and TST without having any role in the baseline locomotion. QCF-21 significantly reduced the immobility time in behavioural paradigm of FST and TST in mice.

To elucidate the role in serotonergic modulation, QCF-21 was evaluated in the 5-HTP-induced head-twitch response and reserpine-induced hypothermia. The decrease in body temperature induced by reserpine was proved to be antagonized by antidepressants[23]. QCF-21 and ESC significantly reversed the hypothermic effect of reserpine pertaining to the antidepressant-like effect of QCF-21 in this model. Synaptic enhancement of monoamines, particularly serotonin, is one of the pharmacological mechanisms of antidepressants. 5-HTP, the immediate serotonin precursor, leads to an increase in serotonergic transmission causing characteristic head-twitch response in mice[19,23]. Pretreatment with ESC and QCF-21 significantly potentiated pargyline and 5-HTP induced head-twitch responses in mice indicating that the antidepressant-like effect of QCF-21 was modulated by serotonin concentrations at the synapse[20].

OBX has been reported as one of the chronic model of depression with adequate face and predictive validity and is used to investigate the antidepressant potential of novel agents[24]. Bilateral OBX results in changes in behaviour in the endocrine, immune and neurotransmitter systems that simulate many of the anomalies seen in patients with major depression. OBX rats displayed specific behavioural anomalies in the open field test[18], as evident by significant increase in the number of ambulation, rearing and faecal pellets in open field test[25], and this abnormal behaviour was reversed by antidepressants[26]. In the current study, QCF-21 chronic treatment significantly reversed the increased ambulation in bulbectomized rats, but the restoration of behavioural deficits was weaker than that of ESC. In sham group, QCF-21 had no effect, but ESC treatment slightly modulated the behaviour of rats in open field test although not significant.

Along with antidepressant evaluation, QCF-21 was investigated in various traditional anxiety tests (elevated plus-maze, light and dark, and hole board tests). Serotonin has long been viewed as a neurotransmitter involved in regulating emotional states[27]. Location of 5-HT_3 binding sites throughout cortical and limbic brain regions suggested the clinical application of their antagonists to treat anxiety. Blockade of central 5-HT_3 receptors was also discovered to be bound to an anxiolytic action[28]. The light/dark box is also a preferred model for evaluating anxiolytic or anxiogenic drugs, based on the innate aversion of rodents to brightly illuminated areas[29]. The assessment of the study showed that the treatment with QCF-21 significantly increased the time spent in the light area, suggesting the anxiolytic effect of QCF-21.

The hole-board test provides a simple method for evaluating the reaction of an animal to an unfamiliar environment and is widely used to assess emotionality and anxiety responses to stress in animals[30]. In the present study, QCF-21 increased counts and duration of head dip, indicating a significant anxiolytic effect of QCF-21.

In the treatment of anxiety disorders, benzodiazepines are now slowly replaced by antidepressants, which are efficacious not only in depression but also in the acute and long-term treatment of major anxiety disorders[31]. The current, neurobehavioural study showed antidepressant- and anxiolytic-like effects of QCF-21, a 5-HT_3 antagonist, in animal models of depression and anxiety, although the precise mechanism is not clear. A potentiation of the head-twitch response and reversal of reserpine-induced hypothermia suggested that QCF-21 produced an antidepressant-like effect by increasing the concentration of a neurotransmitter.

In our previous studies[12,13,17], 5-HT_3 receptor antagonist showed the antidepressant activity at higher dose without the anxiolytic effect. The possible anxiolytic and antidepressant activities of QCF-21 may result from its interaction with diversely localized 5-HT_3 receptors and/or from the indirect influence on neurotransmitter systems which are thought to contribute to the modulation of emotional states. Increased serotonergic neurotransmission through post-synaptic 5-HT_3 receptor antagonism leads to allosteric modulation of serotonergic system on other serotonin receptor[9] which could be an added mechanism of anxiolytic property of novel 5-HT_3 receptor antagonist as confirmed in the 5-HTP-induced head twitches and reserpine-induced hypothermia.

In conclusion, QCF-21, a novel 5-HT_3 antagonist, exhibited the antidepressant-like and anxiolytic-like activities in acute and chronic models of depression

and anxiety at a lower dose than the doses tested in earlier studies. One of the major shortcomings of all the marketed antidepressants, regardless of their mechanism of action, is a slower onset (2-4 wk) of therapeutic efficacy. Co-administration of 5-HT_3 antagonist could potentially accelerate the onset as well as the therapeutic potential of the antidepressant and anxiolytic agents. Future studies are needed to clarify the receptor systems responsible for the anxiolytic and antidepressant effects of QCF-21 in animal models.

Acknowledgment

The authors thank Glenmark Research Centre, Mumbai, and Ranbaxy Laboratory, for providing drug sample. Authors acknowledge the Council of Scientific and Industrial Research, New Delhi, India, for partly funding this work.

***Conflicts of Interest*:** None.

References

1. Murray CJ, Lopez AD. Global mortality, disability, and the contribution of risk factors: global burden of disease study. *Lancet* 1997; *349* : 1436-42.
2. Fava M, Rankin MA, Wright EC, Alpert JE, Nierenberg AA, Pava J, *et al*. Anxiety disorders in major depression. *Compr Psychiatry* 2000; *41* : 97-102.
3. Bakish D. The patient with comorbid depression and anxiety: the unmet need. *J Clin Psychiatry* 1999; *60* (Suppl 6): 20-4.
4. Merikangas K, Pine D. Genetic and other vulnerability factors for anxiety and stress disorders. In: Davis K, Charney D, Coyle J, Nemeroff C, editors. *Neuropsychopharmacology*. The Fifth Generation of Progress. Philadelphia: American College of Neuropsychopharmacology; 2002. p. 868-82.
5. Pauwels PJ, Van Gompel P, Leysen JE. Activity of serotonin (5-HT) receptor agonists, partial agonists and antagonists at cloned human 5-HT1A receptors that are negatively coupled to adenylate cyclase in permanently transfected HeLa cells. *Biochem Pharmacol* 1993; *45* : 375-83.
6. Lemonde S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, *et al*. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J Neurosci* 2003; *23* : 8788-99.
7. Darmani NA, Gerdes CF. Temporal differential adaptation of head-twitch and ear-scratch responses following administration of challenge doses of DOI. *Pharmacol Biochem Behav* 1995; *50* : 545-50.
8. Greenshaw AJ. Behavioural pharmacology of 5-HT3 receptor antagonists: A critical update on therapeutic potential. *Trends Pharmacol Sci* 1993; *14* : 265-70.
9. Redrobe JP, Bourin M. Partial role of 5-HT2 and 5-HT3 receptors in the activity of antidepressants in the mouse forced swimming test. *Eur J Pharmacol* 1997; *325* : 129-35.
10. Costall B, Naylor RJ. Astra award lecture. The psychopharmacology of 5-HT3 receptors. *Pharmacol Toxicol* 1992; *71* : 401-15.
11. Mahesh R, Devadoss T, Dhar AK, Venkatesh SM, Mundra S, Pandey DK, *et al*. Ligand-based design, synthesis, and pharmacological evaluation of 3-methoxyquinoxalin-2-carboxamides as structurally novel serotonin type-3 receptor antagonists. *Arch Pharm Chem Life Sci* 2012; *345* : 1-8.
12. Harms AE. An efficient synthesis of 2-quinoxalinecarboxylic acid. *Org Process Res Dev* 2004; *8* : 666-9.
13. Boissier JR, Simon P. Action of caffeine on the spontaneous motility of the mouse. *Arch Int Pharmacodyn Ther* 1965; *158* : 212-21.
14. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. *Arch Int Pharmacodyn Ther* 1977; *229* : 327-36.
15. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* (*Berl*) 1985; *85* : 367-70.
16. Devadoss T, Pandey DK, Mahesh R, Yadav SK. 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* 2010; *62* : 245-57.
17. Martin P, Massol J, Soubrie P, Puech AJ. Effects of triiodothyronine (T3) on the potentiation by antidepressants of L-5-hydroxytryptophan-induced head-twitches in mice. *Prog Neuropsychopharmacol Biol Psychiatry* 1989; *13* : 735-48.
18. Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* 1997; *74* : 299-316.
19. Pandey DK, Rajkumar R, Mahesh R, Radha R. Depressant-like effects of parthenolide in a rodent behavioural antidepressant test battery. *J Pharm Pharmacol* 2008; *60* : 1643-50.
20. Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 1985; *14* : 149-67.
21. Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav* 1980; *13* : 167-70.
22. Wolfman C, Viola H, Paladini A, Dajas F, Medina JH. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora caerulea*. *Pharmacol Biochem Behav* 1994; *47* : 1-4.
23. Ortmann R, Martin S, Radeke E, Delini-Stula A. Interaction of beta-adrenoceptor agonists with the serotonergic system in rat brain. A behavioral study using the L-5-HTP syndrome. *Naunyn Schmiedebergs Arch Pharmacol* 1981; *316* : 225-30.
24. Bourin M, Fiocco AJ, Clenet F. How valuable are animal models in defining antidepressant activity? *Hum Psychopharmacol* 2001; *16* : 9-21.

25. Slotkin TA, Miller DB, Fumagalli F, McCook EC, Zhang J, Bissette G, *et al*. Modeling geriatric depression in animals: biochemical and behavioral effects of olfactory bulbectomy in young versus aged rats. *J Pharmacol Exp Ther* 1999; *289* : 334-45.

26. van Riezen H, Leonard BE. Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacol Ther* 1990; *47* : 21-34.

27. Lucki I. Serotonin receptor specificity in anxiety disorders. *J Clin Psychiatry* 1996; *57* (Suppl 6) : 5-10.

28. Costall B, Naylor RJ. Anxiolytic effects of 5-HT3 antagonists in animals. In: Rodgers RJ, Cooper SJ, editors. *5-HT1A agonists, 5-HT3 antagonists and benzodiazepines, their comparative behavioural pharmacology*. Boca Raton: CRC; 1991. p. 133-57.

29. Imaizumi M, Suzuki T, Machida H, Onodera K. A fully automated apparatus for a light/dark test measuring anxiolytic or anxiogenic effects of drugs in mice. *Jpn J Psychopharmacol* 1994; *14* : 83-91.

30. Nolan NA, Parkes MW. The effects of benzodiazepines on the behaviour of mice on a hole-board. *Psychopharmacologia* 1973; *29* : 277-86.

31. Buller R, Legrand V. Novel treatments for anxiety and depression: hurdles in bringing them to the market. *Drug Discov Today* 2001; *6* : 1220-30.

Reprint requests: Dr Dilip Kumar Pandey, Pharmacology Department, Novel Drug, Discovery and Development, Lupin Research Park, Pune 412 115, Maharashtra, India
e-mail: pandeysdl1408@gmail.com