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Potential antidepressants: Pharmacology of 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile in rodent behavioural models

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Serotonin type 3 (5-HT_3) antagonists, which find an unflinching place in the management of nausea and emesis are presently screened for their neuro-pharmacological potential in various animal models. In the present study, 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-2) with an optimal log P and pA_2 value comparable to that of ondansetron was screened in rodent models of depression. The acute and chronic (14 days) treatment of the synthetic compound exhibited antidepressant-like effects at the lower dose levels in mice forced swim test (FST). A typical and similar dose-immobility profile was observed in both mice FST and tail suspension test (TST). Interaction studies in FST revealed the reversal of mCPP induced immobility, attenuation of antidepressant effects of fluoxetine and desipramine. Chronic NA-2 treatment restored the behavioural deficits in olfactory bulbectomized (OBX) rats as indicated by reduction in hyperactivity in novel open field test. This preliminary study points to a serotonergic mechanism behind the antidepressant-like effects of NA-2 and invigorates further investigation of analogous compounds in various other models of depression.

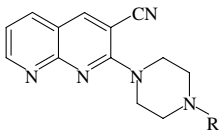
1. Introduction

Serotonin type-3 receptor antagonists (5-HT_3 RAs) are currently promising in the management of nausea and vomiting associated with cancer chemotherapy (Jantunen et al. 1997; Mahesh et al. 2005). Interestingly, in the last decade, these molecules have been extensively screened for their neuro-psychopharmacological potentials in various pre-clinical and few clinical studies (Israili 2001; Wolf 2000). The involvement of 5-HT_3 receptors in the neural pathways of depression has prompted research, considering these receptors as possible antidepressant drug targets (Costall and Naylor 2004). However, the complete neurobiological aspects behind depression are still anticipated and inconsistent results have been obtained with 5-HT_3 RAs (Greenshaw 1993). These receptors seem to play a partial yet notable role in depression (Bhatnagar et al. 2004; Redrobe and Bourin 1997). Depression affects 15% of the population worldwide (Hirschfeld et al. 1997) and is associated with both cancer and cancer chemotherapy (Pirl 2004; Massie 2004). Nevertheless, no special treatment modes have been devised or adopted for the management of cancer related depression, incidence of which is three times more than the depressive disorder itself (Fisch 2004).

Supported by the three component pharmacophore model (Hibert et al. 1990) of 5-HT_3 RAs, several series of compounds have been designed and screened for 5-HT_3 antagonism. Taking into account the cost involved in synthesis of 5-HT_3 RAs (compounds with pharmacophore design suggested by Hibert et al.) with chiral centers heteroaryl piperazines were designed and synthesized. Microwave assisted synthesis of 2-(4-substitued piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles was carried out giving a series of 12 compounds which were characterized by spectral (IR, ^{1}H NMR and mass) and elemental analysis. The compounds were tested for their 5-HT_3 antagonistic property in isolated guinea pig ileum and the pA_2 values were determined against 2-methyl-5 hydroxytryptamine (Mahesh et al. 2004). Anticipating a dual use in managing both vomiting and depression associated with cancer, we investigated the antidepressant potential of 5-HT_3 antagonists.

Forced swim test (Porsolt et al. 1977; Bourin et al. 2002) and tail suspension test (Steru et al. 1985; Thierry et al. 1986) are the most extensively used mice behavioural models for antidepressant screening. More than 25 years of immense research has strengthened the validities of these models, positioning it vitally among the various other models in whole animal antidepressant test battery (Bourin et al. 2001, 2005). Bilateral olfactory bulb ablation in rats induces various behavioural deficits as a result of secondary structural and functional neural alterations (Song and Leonard 2005; Cairncross et al. 1979) and antidepressants are proved to restore the normal behaviour (Van Reizen and Leonard 1990). The hyperactivity exhibited by olfactory bulbectomised rats in a novel open field arena is reversed by repeated administration of conventional antidepressants. The olfactory bulbectomy (OBX) model has also revealed the antidepressant-like effects of agents affecting the 5-HT receptor subtypes (Kelly et al. 1997).

Table 1: Data showing the pA2 and log P values of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile series of compounds (NA series)



Compd.	R	pA_2	log P
NA-1	$-H$	6.5	1.39
NA-2*	$-CH_3$	7.4	1.84
NA-3	$-C_2H_5$	7.1	2.27
NA-4	$-CH_2-CH=CH_2$	8.2	1.68
NA-5	$-C_6H_5$	5.7	2.35
NA-6	$-CH_2-C_6H_5$	5.1	2.38
NA-7	o-$OCH_3-C_6H_4$	5.4	2.44
NA-8	m-$OCH_3-C_6H_4$	6.0	2.46
NA-9	p-$OCH_3-C_6H_4$	4.9	2.53
NA-10	p-$NO_2-C_6H_4$	3.8	1.53
NA-11	p-$Cl-C_6H_4$	<3.0	2.97
NA-12	2-pyridyl	3.9	1.91
Ondansetron		6.9	2.58

* 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile, screened for antidepressant potential

In the present study, 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-2) which exhibited an optimal log P and pA_2 value comparable to ondansetron (Mahesh et al. 2004) was selected for preliminary antidepressant screening in the standard rodent models of depression viz. forced swim and tail suspension test in mice and open field behaviour of olfactory bulbectomised (OBX) rats. Interaction studies with fluoxetine (FLX), desipramine (DMI) and meta-chlorophenyl piperazine (mCPP) were carried out in mice FST.

2. Investigations and results

The log P values of a series of 12 compounds are depicted in Table 1. NA-2 possessed log P and pA_2 value of 1.84 and 7.4 respectively.

2.1. Spontaneous locomotor activity

Acute and chronic treatment of NA-2 and paroxetine (PAR) did not influence the locomotion at tested dose levels. However, chronic NA-2 (1 and 10 μg/kg) treatment decreased the locomotor activity of mice compared to vehicle treatment (Fig. 1), however the results were statistically insignificant. Chronic mCPP (1 mg/kg) treatment decreased the locomotion significantly as compared to vehicle control.

2.2. Dose response study in FST and TST

Acute and chronic treatment with NA-2 showed significant ($p < 0.05$) anti-immobility effects in both the models of depression with peak activity at 0.01 μg/kg, but the effects were lesser compared to PAR (15 mg/kg). In the FST, NA-2 exhibited a decreased anti-immobility effect and decreased swimming episodes with an increase in dose. Chronic treatment with NA-2 slightly decreased the duration of immobility (Fig. 2A) and increased the swimming episodes (Fig. 2B) as compared to the acute treatment. NA-2 treatment exhibited a similar dose-immobility pattern in TST (Fig. 3).

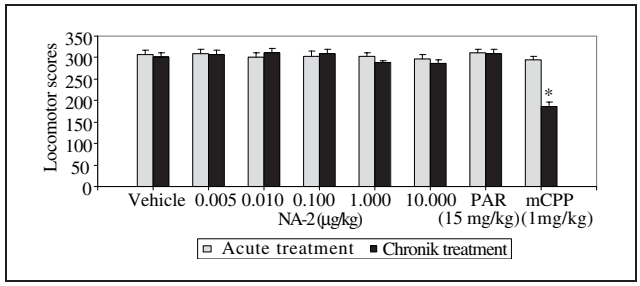


Fig. 1: Influence of NA-2, PAR or mCPP treatment on spontaneous locomotor activity. Results are expressed as mean Locomotor scores recorded in a period of 10 minutes. Error bars represent S.E.M. * $p < 0.05$ compared to corresponding vehicle treated group ($n = 6$)

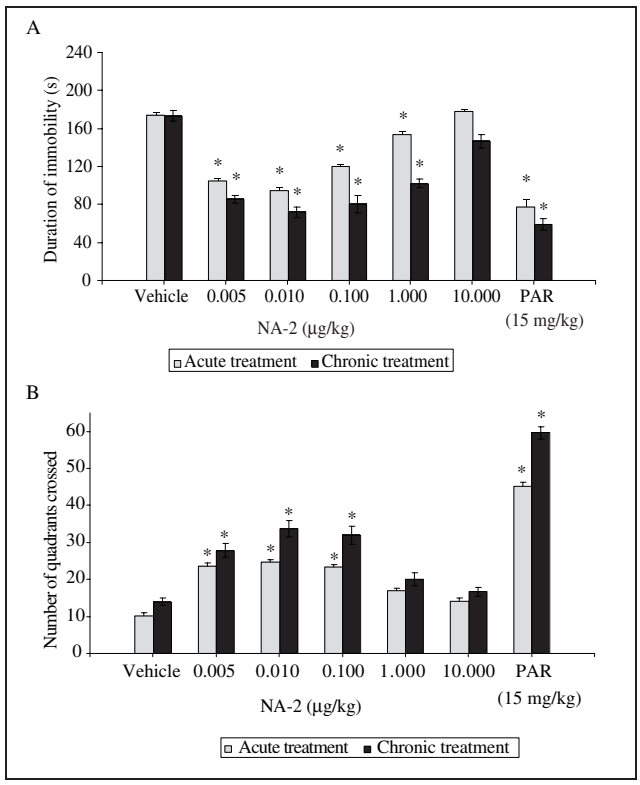


Fig. 2: A. Effects of acute and chronic treatment of NA-2 and PAR on duration of immobility in mice FST.Results are expressed as mean duration of immobility. Error bars represent S.E.M. * $p < 0.05$ compared to corresponding vehicle treated group ($n = 12$)
B. Effects of acute and chronic treatment of NA-2 and PAR on swimming behaviour in Mice FST.Results are expressed as mean number of quadrants crossed. Error bars represent S.E.M. * $p < 0.05$ compared to corresponding vehicle treated group ($n = 12$)

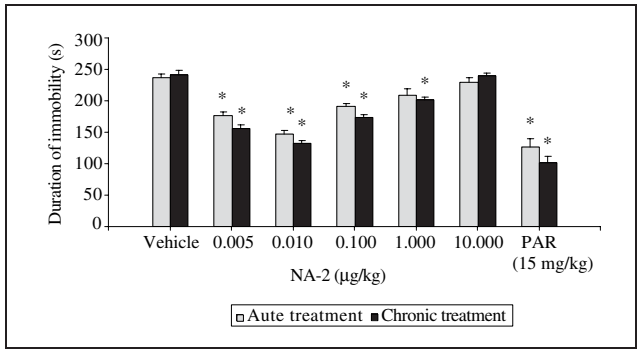


Fig. 3: Effects of acute and chronic treatment of NA-2 and PAR on duration of immobility in Mice TST. Results are expressed as mean duration of immobility. Error bars represent S.E.M. * $p < 0.05$. Compared to corresponding vehicle treated group ($n = 10$)

Table 2: Effect of NA-2 pre-treatment on mCPP, FLX and DMI modulated depressive states in mice FST

Group (n)	Treatment	Duration of Immobility (Sec)	Swimming Episodes (Number of quadrants crossed)
1 (12)	Vehicle (Distilled water)	168.17 ± 3.14	11.33 ± 1.03
2 (12)	NA-2 (0.01 μg/kg)	95.25 ± 3.40[a, c]	22.25 ± 1.62[a, c]
3 (12)	NA-2 (0.01 μg/kg for 14 days)	93.12 ± 4.15[a, c]	24.75 ± 1.75[a, c]
4 (12)	mCPP (1 mg/kg)	197.08 ± 3.09[a]	7.25 ± 0.59
5 (12)	FLX (10 mg/kg)	72.33 ± 1.82[a, b]	33.08 ± 1.54[a, b]
6 (12)	DMI (10 mg/kg)	101.17 ± 2.57[a]	20.33 ± 1.04[a]
7 (12)	NA-2 (0.01 μg/kg) + mCPP (1 mg/kg)	169.00 ± 4.13[b, c]	8.50 ± 0.82
8 (12)	NA-2 (0.01 μg/kg) + FLX (10 mg/kg)	118.75 ± 3.90[a, b, d]	22.75 ± 1.01[a, d]
9 (12)	NA-2 (0.01 μg/kg) + DMI (10 mg/kg)	123.67 ± 2.39[a, b, e]	16.17 ± 0.68[a, b]
10 (8)	NA-2 (0.01 μg/kg for 14 days) + mCPP (1 mg/kg)	165.25 ± 3.25[b, c]	12.50 ± 0.74[b, c]
11 (8)	NA-2 (0.01 μg/kg for 14 days) + FLX (10 mg/kg)	89.38 ± 5.46[a]	29.50 ± 3.35[a]
12 (8)	NA-2 (0.01 μg/kg for 14 days) + DMI (10 mg/kg)	135.88 ± 4.78[a, e]	17.13 ± 1.87

Values represent mean ± S.E.M. [a] $p < 0.05$ compared to vehicle group, [b] $p < 0.05$ compared to NA-2 treated group, [c] $p < 0.05$ compared to mCPP treated group. [d] $p < 0.05$ compared to FLX treated group. [e] $p < 0.05$ compared to DMI treated group

2.3. Interaction study

A single dose of NA-2 (0.01 μg/kg) showed a significant ($p < 0.05$) decrease in the duration of immobility and a significant ($p < 0.05$) increase in swimming episodes. The antidepressant-like effects of NA-2 (acute and chronic) were weaker than that of fluoxetine (FLX 10 mg/kg) and desipramine (DMI 10 mg/kg). Acute pretreatment of NA-2 significantly ($p < 0.05$) attenuated the antidepressant effects of FLX, whereas both acute and chronic pretreatment significantly ($p < 0.05$) reduced the antidepressant effect of DMI. The mCPP induced immobility was reversed by acute and chronic pretreatment with NA-2 (0.01 μg/kg). Results are depicted in Table 2.

2.4. Olfactory bulbectomy induced hyperactivity

The lower dose levels of NA-2 (0.01 and 0.1 μg/kg) were selected for screening in the olfactory bulbectomy model of depression in rats based on the data obtained from the FST, TST and pilot study. Chronic (14 days) treatment with NA-2 showed statistically significant ($p < 0.05$) reduction in ambulation (depicted as horizontal activity in Fig. 4), rearing (depicted as vertical activity in Fig. 5) and defecation (Fig. 6) in OBX rats compared to the vehicle treated group. NA-2 (0.1 μg/kg) showed antidepressant-like effects and PAR was the most effective among all treatments. NA-2 (0.1 μg/kg) also showed statistically significant increase in the time spent in grooming, scratching and licking (Fig. 7). None of the treatment showed any statistically significant change in the behaviour of sham operated animals.

3. Discussion

Alkyl substitutions at the 4[th] position of the piperazine, increased the log P value whereas allyl substitution reduced it. Increase in hydrophobicity was most prominent with aromatic substitutions. A log P around 2 is considered optimum for a centrally (blood brain barrier permeable) act-

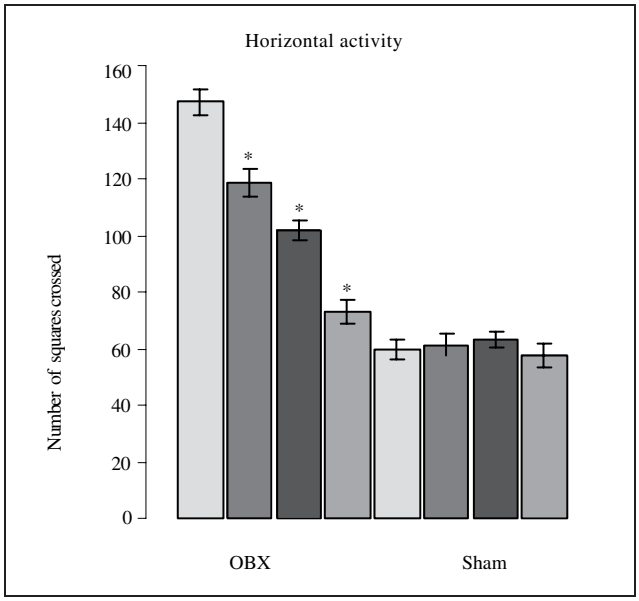


Fig. 4: Effect of NA-2 and PAR on the horizontal activity of OBX and sham rats in the open field test. All drug/vehicle administrations were done once a day for 14 days. Results are expressed as mean number of squares crossed. Error bars represent S.E.M; * $p < 0.05$ compared to vehicle treated OBX group (n = 6)
OBX + vehicle ■ OBX + NA − 2 (0.01 μg/kg)
■ OBX + NA − 2 (0.1 μg/kg) ■ OBX + PAR (10 mg/kg)
Sham + vehicle ■ Sham + NA − 2 (0.01 μg/kg)
■ Sham + NA − 2 (0.1 μg/kg) ■ Sham + PAR (10 mg/kg)

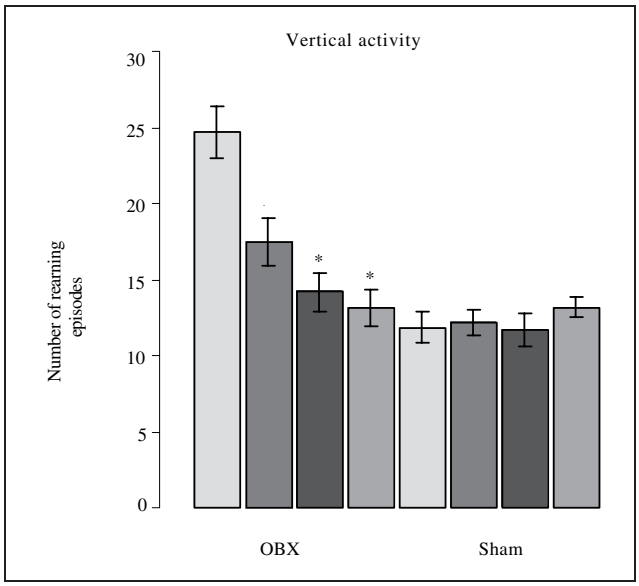


Fig. 5: Effect of NA-2 and PAR on the vertical activity of OBX and sham rats in the open field test. All drug/vehicle administrations were done once a day for 14 days. Results are expressed as mean number of rearing episodes. Error bars represent S.E.M. * $p < 0.05$ compared to vehicle treated OBX group (n = 6)
OBX + vehicle ■ OBX + NA − 2 (0.01 μg/kg)
■ OBX + NA − 2 (0.1 μg/kg) ■ OBX + PAR (10 mg/kg)
Sham + vehicle ■ Sham + NA − 2 (0.01 μg/kg)
■ Sham + NA − 2 (0.1 μg/kg) ■ Sham + PAR (10 mg/kg)

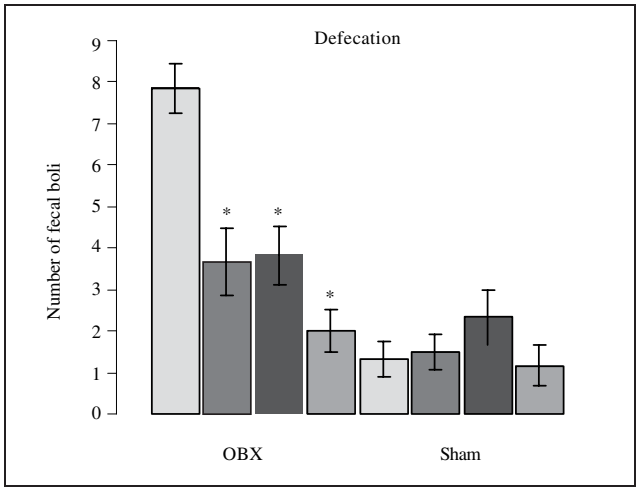


Fig. 6: Effect of NA-2 and PAR on defecation of OBX and Sham rats in the open field test. All drug/vehicle administrations were done once a day for 14 days. Results are expressed as mean number of fecal pellets. Error bars represent S.E.M. * $p < 0.05$ compared to vehicle treated OBX group (n = 6)
OBX + vehicle ■ OBX + NA − 2 (0.01 μg/kg)
■ OBX + NA − 2 (0.1 μg/kg) ■ OBX + PAR (10 mg/kg)
Sham + vehicle ■ Sham + NA − 2 (0.01 μg/kg)
■ Sham + NA − 2 (0.1 μg/kg) ■ Sham + PAR (10 mg/kg)

ing, orally bioactive compound (Ter Laak et al. 1994). NA-2, which possessed log P and pA_2 values of 1.84 and 7.4 respectively, was hence found suitable for antidepressant assays.

Acute and chronic treatment of NA-2 showed antidepressant-like effect with similar dose-immobility profiles in both models of depression. Assessment of anti-depressant potential is interfered by possible hyper-locomotive property of a test substance leading to false positive results (Porsolt et al. 1978). The antidepressant-like effects of NA-2 in the FST and TST are not due to hyper-locomotive

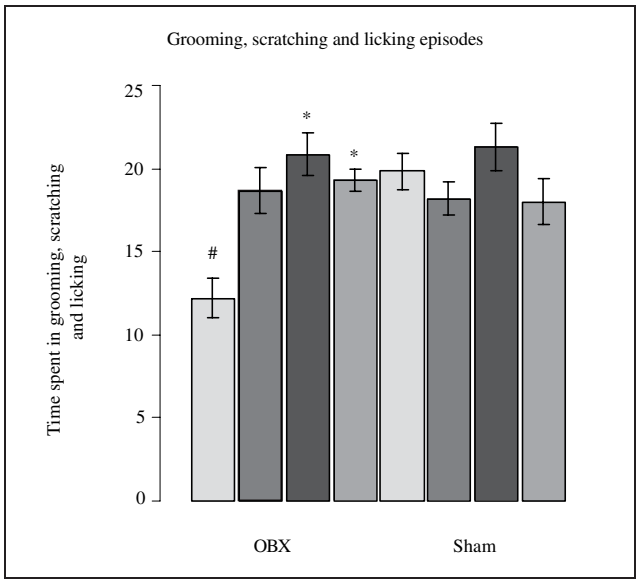


Fig. 7: Effect of NA-2 and PAR on the time spent by OBX and sham rats in grooming, scratching and licking in the open field test. All drug/vehicle administrations were done once a day for 14 days. Results are expressed as mean of time spent in Grooming, scratching and licking. Error bars represent S.E.M. * $p < 0.05$ compared to vehicle treated OBX group. #$p < 0.05$ compared to vehicle treated sham group (n = 6)
OBX + vehicle ■ OBX + NA − 2 (0.01 μg/kg)
■ OBX + NA − 2 (0.1 μg/kg) ■ OBX + PAR (10 mg/kg)
Sham + vehicle ■ Sham + NA − 2 (0.01 μg/kg)
■ Sham + NA − 2 (0.1 μg/kg) ■ Sham + PAR (10 mg/kg)

effects as indicated by the spontaneous locomotor activity test. The characteristic dose-immobility profile and partial effects (at lower doses) on swimming behaviour needs focus. The 5-HT_3 receptor is claimed as one of the substrates for behavioural effects of antidepressants and for definitive evaluation of antidepressant potential of any 5-HT_3 RAs, interaction study with selective serotonin reuptake inhibitors (SSRIs), is essential (Cryan et al. 2005). Ondansetron pretreatment (single dose) was found to enhance antidepressant effects of FLX and it has been demonstrated in mice FST that antagonism of 5-HT_3 receptors may have a partial role in anti-immobility effect of SSRIs and antagonism of 5-$HT_{2A/C}$ is vital for antidepressant effects of tricyclic antidepressants (Redrobe and Bourin 1997).

In the current study, acute pre-treatment of NA-2 significantly attenuated the anti-depressant effects of FLX and DMI. mCPP, a non selective 5-HT_2 agonist induces immobility in rat FST possibly mediated by 5-HT_{2C} or 5-HT_{1B} receptors (Cryan and Lucki 2000). Acute mCPP (1 mg/kg) treatment increased the duration of immobility and decreased the swimming behaviour but did not affect spontaneous locomotor activity and hence was used in the interaction study. However, chronic treatment showed significant decrease in locomotor scores. Pretreatment (chronic slightly effective than acute) with NA-2 (0.01 μg/kg) reversed mCPP(acute) induced immobility indicating the probable serotonergic mechanism involving 5-HT_2 and 5-HT_{1B} receptors, behind the antidepressant-like effect of NA-2.

The rat olfactory bulbectomy model of chronic depression (with adequate face and predictive validity) is known to espial novel agents that are unmarked by more conventional models of depression (Bourin et al. 2001). The OBX rats exhibited a specific abnormal behavioural pattern in the brightly lit, circular, open field arena (Song and Leonard 2005; Kelly and Leonard 1994). The various behavioural changes included increased horizontal and vertical activity, increased defecation and decreased grooming compared to sham controls (Slotkin et al. 1999). Such changes are reversed by antidepressants of many pharmacological classes (Van Riezen and Leonard 1990), and in the present study PAR (10 mg/kg) significantly ($p < 0.05$) reversed the behavioural changes. Olfactory bulbectomy was proposed as a model for agitated hyposerotonergic depression since the antidepressant-responsive changes in locomotor activity in bulbectomised rats are associated with reduction in serotonin neurotransmission (Lumia et al. 1992). In addition, 5-HT_2 receptor density is increased in OBX rats, which are normalized by chronic antidepressant treatment (Earley et al. 1994). NA-2 at both doses (0.1 μg/kg being more effective) reduced the hyperactivity in bulbectomised rats but the restoration of behavioural deficits was weaker than that of PAR.

Based on this behavioural study, though viewed as a 5-HT_3 RA, it is speculative to attribute 5-HT_2 receptor involvement in the antidepressant-like effect of NA-2 indicated by the reversal of both mCPP induced immobility in mice FST and behavioural anomalies in OBX rats. However, studies probing the affinity of NA-2 on 5-HT_{1B}, 5-HT_2, 5-HT_3 receptor subtypes and serotonin transporters are required to confirm its precise mechanism of action.

To conclude, the results from three reliable antidepressant behavioural models indicate that 2-(4-methylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile, exhibits antidepressant-like effects at the dose levels mentioned. The study also encourages analogues molecules for antidepressant screening. Though no abnormal behaviour was observed

at the tested dose levels, assessment of the safety profile of the molecule is essential.

4. Experimental

4.1. Animals

Experiments on animals were approved by the Institutional Animal Ethics Committee of Birla Institute of Technology and Science, Pilani, India (Protocol No.IAEC/RES/4/1, dated 22. 09. 04; IAEC/RES/7/1, dated 24. 04. 06). Albino mice of either sex (18–25 g) and male Wistar rats (250–300 g) were obtained from Hissar Agricultural University, Hissar, Haryana, India and maintained in standard laboratory conditions with food (standard pellet chow feed) and filtered water *ad libitum*. The animals were used only once for each experiment.

4.2. Compound administration

Fluoxetine (FLX) and paroxetine (PAR) were obtained from Sun Pharmaceuticals, and Ipca Laboratories, India respectively. Desipramine (DMI) was obtained from Sigma Chemicals and meta-chlorophenyl piperazine (mCPP) was obtained from Lancaster chemicals, USA. Chloral hydrate was obtained from Reidel (India) Chemicals Pvt. Ltd. The hydrochloride salt of NA-2 (Mahesh et al. 2004) or drugs were solublised in sterile distilled water and were freshly prepared for use.

In the acute study, groups were treated individually with a single dose of NA-2 (0.005, 0.01, 0.1, 1, 10 μg/kg). All administrations were made intraperitoneally. Thirty minutes after treatment, the mice were subjected to locomotor or antidepressant screening. In the chronic study, NA-2 (0.005, 0.01, 0.1, 1, 10 μg/kg) was administered orally, once a day for 14 days. On the 14th day, 1 h after the last dose the animals were subjected to locomotor or antidepressant assays. For interaction studies NA-2 and antidepressants/mCPP were administered intraperitoneally, 45 and 30 min respectively before testing as per the procedure mentioned elsewhere (Redrobe and Bourin 1997; Bourin et al. 2002). The acute and chronic dose response profiles of NA-2 were studied in both FST and TST. Interaction studies of NA-2 with conventional antidepressants/mCPP were carried out in the FST.

In OBX rat model of depression, NA-2 (0.01 and 0.1 μg/kg), vehicle or PAR (10 mg/kg) was administered once a day for 14 days to OBX rats, after a post-surgical rehabilitation period of 21 days. The OBX rats were then subjected to open field test, 20 h after the last dose to avoid the possible acute effects of the drug treatment. The doses of standard antidepressants were selected from the pilot studies conducted in our laboratory. All administrations and testing were done between 10–14 h. To avoid bias, all behavioural observations were carried out by trained experimenters, who had no information on the treatment.

4.3. Chemistry

Na-2 was synthesized according to Mahesh et al. (2004). Briefly, a mixture of 2 aminonicotinaldehyde (Majewicz and Caluwe 1974), ethyl cyanoacetate and piperidine was triturated at room temperature for about 10 min. The completion of reaction was confirmed by TLC. The solid thus obtained was treated with water, filtered, and recrystallised from dimethyl formamide (DMF)-water mixture to give 2-oxo-1,2-dihydro-1,8 naphthyridine-3-carbonitrile. This was refluxed with phosphorus oxychloride for 1 h in the presence of DMF, cooled to room temperature and treated with ice water. The resulting solution was slowly basified with aqueous sodium hydroxide (40%) with cooling. The separated product was washed with water and recrystallised to give 2-chloro-1,2-dihydro-1,8 naphthyridine-3-carbonitrile. Microwave irradiation of this intermediate with 1-methyl piperazine in the presence of potassium carbonate in DMF for 5 min gave 2-(4-methyl piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-2). The compound (m.r. 152–154 °C) was characterized by Spectral (IR, NMR, and MS) and elemental analysis. This free base was dissolved in chloroform and hydrochloride gas was purged to obtain the hydrochloride salt. The freely water soluble compound was stored in a cool dry place until use.

4.4. Behavioural screening

4.4.1. Spontaneous locomotor activity

The spontaneous locomotor activity was assessed using an actophotometer (Boisser and Simon 1965). The photocells of the actophotometer were checked before use. The animals were individually placed in a square arena (30 cm × 30 cm, with walls painted black) and after an initial 2 min familiarization period, the digital locomotor scores were recorded for the next 10 min in a dimly lit room. The arena was cleaned with ethyl alcohol and dried between trails.

4.4.2. Forced swim test

The forced swim test (FST) was carried out according to Porsolt et al. (1977). Mice were dropped individually into a glass cylinder (height: 30 cm, diameter: 22.5 cm) containing a depth of 15 cm of water maintained at 23–25 °C and were left in the water for 6 min. A mouse was judged immobile if it floated in the water in an upright position and exhibited only small movements to keep its head above the water or made other passive movements. The duration of immobility was recorded during the last 4 min of the 6 min test. The swimming episodes were recorded as the number of quadrants (demarcated at the base of the cylinder) crossed.

4.4.3. Tail suspension test

The method mentioned elsewhere (Steru et al. 1985, Rodrigues et al. 2002) with slight modifications was adopted. To mention in brief, 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 demonstrated several escape-oriented behaviors interspersed with temporally increasing bouts of immobility. The 6-min test session was manually observed. The parameter recorded was the number of seconds spent immobile.

4.4.4. Olfactory bulbectomy

4.4.4.1. Surgery

Bilateral olfactory bulb ablation was performed in rats anesthetized with chloral hydrate (400 mg/kg) as described elsewhere (Kelly et al. 1997; Van Riezen and Leonard 1990), with slight modifications. The skull was exposed by a midline incision and 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 removed by suction, the holes were then filled with haemostatic sponge in order to control excessive bleeding and the scalp was sutured. To prevent infection, the animals were given Sulprim injection (each ml containing 200 and 40 mg of sulphadiazine and trimethoprim respectively), intramuscularly (0.2 ml/300 g) once a day for 3 days, post-surgery. Sham-operated animals received the same surgical treatment, but the bulbs were left intact. The animals were given 21 days recovery period following the surgery prior to antidepressant screening. All the animals were handled daily by the experimentor throughout the recovery period to reduce aggressive behavior (Leonard and Tuite 1981). Animals which exhibited any other abnormal behaviour were excluded from the study. The drug treatment was started on the 21st day after surgery.

4.4.4.2. Openfield behaviers

Olfactory bulbectomized (OBX) and sham control rats were subjected to an open field test on the 14th day of chronic drug/vehicle administration. The open field exploration 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. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. Each animal was individually placed in the center of the open field apparatus and the following parameters were noted for 5 min by 2 trained observers. Ambulation scores (number of squares crossed) and number of rearing episodes were noted as horizontal and vertical activity, respectively. The time spent in grooming/scratching/licking and number of fecal pellets were also measured. The apparatus was cleaned with ethyl alcohol and dried between trials.

4.5. Statistical analysis

The locomotor scores, duration of immobility, swimming episodes and the behavioural scores in the open field test were expressed as mean ± SEM. The data was subjected to one-way ANOVA followed by post hoc dunnett's 'T3' test and the level of statistical significance was fixed at $p < 0.05$.

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References

Bhatnagar S, Nowak N, Babich L, Bok L (2004) Deletion of the 5-HT$_3$ receptor differentially affects behavior of males and females in the porsolt forced swim and defensive withdrawal tests. Behav Brain Res 153: 527–535.

Boissier JR, Simon P (1965) Action of caffeine on the spontaneous motility of the mouse. Arch Int Pharmacodyn Ther 158: 212–221.

Bourin M, Chenu F, Ripoll N, David DJP (2005) A proposal of decision tree to screen putative antidepressants using forced swim and tail suspension tests. Behav Brain Res 164: 266–269.

Bourin M, Fiocco AJ, Clenet F (2001) How valuable are animal models in defining antidepressant activity? Hum Psychopharmacol Clin Exp 16: 9–21.

Bourin M, Hascoët M, Colombel MC, Coutts RT, Baker GB (2002) Clonidine potentiates the effects of tranylcypromine, phenelzine and two analogues in the forced swimming test in mice. J Psychiatry Neurosci 27: 178–185.

Cairncross KD, Cox B, Forster C, Wren AF (1979) Olfactory projection systems, drugs and behaviour: A review. Psychoneuroendocrinol 4: 253–272.

Costall B, Naylor NJ (2004) 5-HT_3 Receptors. Current Drug Targets – CNS & Neurological Disorders 3: 27–37.

Crayan JF, Lucki I (2000) Antidepressant-like behavioural effects of mediated by 5-hydroxytryptamine (2C) receptors. J Pharmacol Exp Ther 295: 1120–1126.

Cryan JF, Valentino RJ, Lucki I (2005) Assessing substrates underlying the behavioural effects of antidepressants using the modified rat forced swim test. Neurosci Biobehav Rev 29: 547–569.

Earley B, Glennon M, Lally M, Leonard BE, Junien JL (1994) Autoradiographic distribution of cholinergic Muscarinic receptors and serotonin (2) receptors in olfactory bulbectomised rats after chronic treatment with mianserin and desipramine. Hum Psychopharmacol 9: 397–407

Fisch M (2004) Treatment of depression in cancer. J Natl Cancer Inst Monographs 32: 105–111.

Greenshaw AJ (1993) Behavioural pharmacology of 5-HT_3 receptor antagonist: a critical update on therapeutic potential. TIPS 141: 265–270.

Hibert MF, Hoffmann R, Miller RC, Carr AA (1990) Conformation-activity relationship study of 5-HT_3 receptor antagonists and a definition of a model for this receptor site. J Med Chem 33: 1594–1600.

Hirschfeld RM, Keller MB, Panico S, Arons BS, Barlow D, Davidoff F (1997) The national depressive and manic-depressive association consensus statement on the under treatment of depression. JAMA 277: 333–340.

Israili ZH (2001) Clinical pharmacology of serotonin receptor type 3 (5-HT_3) Antagonists. Curr Med Chem – CNS Agents 1: 171–199.

Jantunen IT, Kataja VV, Muhonen TT (1997) An overview of randomised studies comparing 5-HT_3 receptor antagonists to conventional anti-emetics in the prophylaxis of acute chemotherapy-induced vomiting. 33: 66–74.

Kelly JP, Leonard BE (1994) The effect of tianeptine and sertraline in three animal models of depression. Neuropharmacol 33: 1011–1016.

Kelly JP, Wyrnn AS, Leonard BE (1997) Olfactory Bulbectomised rat as a model of depression: An update. Pharmac Ther 74: 299–316.

Leonard BE, Tuite M (1981) Anatomical, physiological and behavioural aspects of olfactory bulbectomy in the rat. Int Rev Neurobiol 22: 251–286

Lumia AR, Teicher MH, Salchli F, Ayers E, Possidente B (1992) Olfactory bulbectomy as a model for agitated hyposerotonergic depression. Brain Res 587: 181–185.

Mahesh R, Perumal RV, Pandi PV (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.

Mahesh R, Perumal RV, Pandi PV (2005) Cancer chemotherapy-induced nausea and vomiting: role of mediators, development of drugs and treatment methods. Pharmazie 60: 83–96.

Majewicz TG, Caluwe PJ (1974) A facile synthesis of 2-aminonicotinaldehyde. J Org Chem 39: 720–721.

Massie MJ (2004) Prevalence of depression in patients with cancer. J Natl Cancer Inst Monographs 32: 57–71.

Pirl WF (2004) Evidence report on the occurrence, assessment, and treatment of depression in cancer patients. J Natl Cancer Inst Monographs 32: 32–39.

Porsolt RD, Bertin A, Jalfre M (1978) "Behavioural despair" in rats and mice: strain differences and the effects of imipramine. Eur J Pharmacol 51: 291–294.

Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther 229: 327–336.

Redrobe JP, 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.

Rodrigues AL, da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, Calixto JB (2002) Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. Life Sci 70: 1347–1358.

Song C, Leonard BE (2005) The olfactory bulbectomised rat as a model of depression. Neurosci Biobehav Rev 29: 627–647.

Slotkin TA, Miller DB, Fumagali F, Mccook EC, Zhang J, Bissette G, Seidler FJ (1999) Modeling geriatric depression in animals: biochemical and behavioural effects of olfactory bulbectomy in young versus aged rats. J Pharmacol Exp Ther 289: 334–345.

Steru L, Chermat R, Thierry B and Simon P (1985) The tail suspension test: a new method for screening antidepressant drugs. Psychopharmacol 85: 367–370.

Ter Laak AM, Tsai RS, Donné-Op den Kelder GM, Carrupt PA, Testa B, Timmerman H (1994) Lipophilicity and hydrogen-bonding capacity of H_1-antihistaminic agents in relation to their central sedative side-effects.- Eur J Pharm Sci 2: 373–384.

Thierry B, Steru L, Simon P, Porsolt RD (1986) The tail suspension test: ethical considerations. Psychopharmacol 90: 284–285.

Van Reizen H, Leonard BE (1990) Effects of psychotropic drugs on the behaviour and neurochemistry of olfactory Bulbectomised rats. Pharmacol Ther 47: 21–34.

Wolf H (2000) Preclinical and clinical pharmacology of the 5-HT_3 receptor Antagonists. Scand J Rheumatol 29: 37–45.