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Full Paper

# Piperazine Analogs of Naphthyridine-3-carboxamides and Indole-2-carboxamides: Novel 5-$HT_3$ Receptor Antagonists with Antidepressant-Like Activity

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Series of piperazine analogs of naphthyridine-3-carboxamides and indole-2-carboxamides were designed using a ligand-based approach with consideration of the pharmacophoric requirements for 5-$HT_3$ receptor antagonists. The title carboxamides were synthesized using appropriate synthetic routes. Initially, the 5-$HT_3$ receptor antagonistic activity of all the compounds was determined on isolated guinea pig ileum tissue against the 5-$HT_3$ agonist, 2-methyl-5-hydroxytryptamine, which was denoted in the form of p$A_2$ values. The structure–activity relationship regarding the influence of the aromatic part and basic moiety as features in the 5-$HT_3$ pharmacophore was derived. Among all the compounds screened, the piperazine derivatives of indole-2-carboxamide **13i** and naphthyridine-3-carboxamide **8h** exhibited prominent 5-$HT_3$ receptor antagonism with p$A_2$ values of 7.5 and 7.3, respectively. Subsequent investigation of the antidepressant activities of selected compounds in the mouse forced swim test (FST) led to the identification of the piperazine analogs of indole-2-carboxamide **13i** and naphthyridine-3-carboxamide **8h** as the most promising compounds. Both **13i** and **8h** demonstrated significant reduction in the duration of immobility as compared to the control. Importantly, none of the tested compounds affected the baseline locomotion of mice at the tested dose levels.

**Keywords:** Antidepressant / Forced swim test / 5-$HT_3$ receptor antagonist / Indole-2-carboxamide / Naphthyridine-3-carboxamide

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## Introduction

Depression is a psychiatric disorder associated with considerable impairment in a person's quality of life and lack of efficiency, and triggers a major public and economic health problem affecting a large section of people, particularly in the western world [1–3]. Typical symptoms of depression are anhedonia or the loss of interest or pleasure in normal daily activities and feelings of sadness [4]. World Health Organization (WHO) has predicted that depression will become the second leading cause of premature death or disability worldwide by the year 2020 [5]. Although a number of antidepressant agents are available, prevalence of the disease still persists. In addition, a significant proportion of depressed patients do not respond to the current treatment, or show only partial response. A common problem associated with the current antidepressant therapies is the several side effects, e.g., clinically useful antidepressant agents produce withdrawal syndromes, except agomelatine [6]. Classical antidepressants like monoamine oxidase (MAO) inhibitors and tricyclic antidepressants produce drug-drug/-food interactions, anticholinergic, and cardiovascular side effects [7, 8]. Besides all currently approved antidepressant drugs for example, selective serotonin reuptake inhibitors (SSRIs) have slow onset of action, since there is a delay of about 4–6 weeks to alleviate the symptoms [9]. Thus, the search for a novel and effective antidepressant agent with less side effects has become a need. The 5-$HT_3$ receptor, which is a pentameric ligand-gated ion channel (pLGIC), mediates neurotransmission and controls the release of monoamine neurotransmitters in the central nervous system (CNS) [10, 11] and is considered to be an important therapeutic target. The 5-$HT_3$ receptor antagonist

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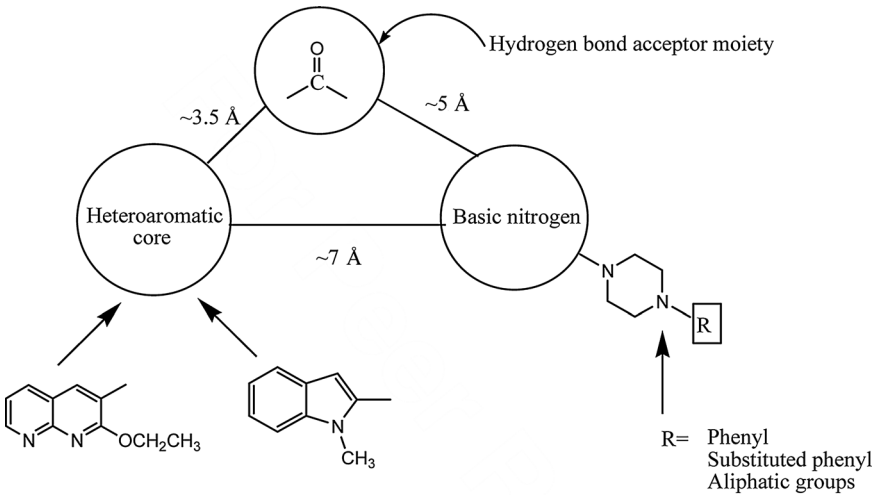


**Figure 1.** Proposed pharmacophore of 5-HT$_3$ receptor antagonists; naphthyridine and indole piperazine carboxamides designed on the basis of the pharmacophore.

ondansetron is in clinical use as antiemetic for the treatment of cancer chemo-/radiotherapy induced nausea, vomiting [12]. Interestingly, 5-HT$_3$ receptor antagonists have been extensively evaluated for their neuro-psychopharmacological potential [13, 14]. Moreover, numerous pre-clinical studies [15–18] as well as previous studies by our group [19–22] have identified 5-HT$_3$ receptor antagonists as potential antidepressant agents and recognized the role of 5-HT$_3$ receptor in the pathophysiological processes of depression [23]. Thus, it seemed appropriate to design and develop novel 5-HT$_3$ receptor antagonists as antidepressants. The key elements of the three-component pharmacophoric model (Fig. 1) proposed for the interaction of 5-HT$_3$ receptor antagonists with the 5-HT$_3$ receptor-binding site: consist of a heteroaromatic core, a hydrogen-bond acceptor moiety, and a basic nitrogen located at a specific distance [24–26]. In recent years, we have been engaged in the preparation and screening of compounds based on the above pharmacophoric pattern and our earlier studies have indicated that nitrogen-containing fused aromatic rings (heteroaromatic core) may serve as a suitable starting point for the design of novel 5-HT$_3$ receptor antagonists [27–30]. In addition, compounds based on the indolyl aromatic core were reported as being potential 5-HT$_3$ receptor antagonists [31–33]. Thus, our attention turned toward the naphthyridine and indole core as aromatic part and substituent effects on the basic moiety. In this work, the objective was to study the influence of the aromatic part and basic moiety on 5-HT$_3$ receptor antagonistic and antidepressant activity. Keeping similar hydrogen-bond acceptor moiety, variations were made to the heteroaromatic core and distal nitrogen (N4) of the basic moiety of the pharmacophore (Fig. 1) with the intention of exploring the structure–activity relationship (SAR) associated with such changes. Therefore, two different nitrogen-containing fused heterocyclic rings (aromatic part) were alternately attached to various N4-substituted piperazines (basic moiety) through a carbonyl group of carboxamide linkage (hydrogen-bond acceptor), resulting in the construction of two new series of piperazine analogs of naphthyridine-3-carboxamides and of indole-2-carboxamides. The minimum energy conformation (three least energy conformations for each compound) of each designed molecule was generated by ACDLABS-10.0/3D Viewer (CHARMM parameterization). The pharmacophoric distances were measured from the centroid of the heteroaromatic ring to the oxygen of the carbonyl group (naphthyridine carboxamides ~3.71 Å, indole carboxamides ~3.41 Å), the carbonyl oxygen to N4 of piperazines (basic nitrogen) (naphthyridine carboxamides ~4.90 Å, indole carboxamides ~4.75 Å) and the centroid of the heteroaromatic ring to the basic nitrogen (naphthyridine carboxamides ~6.50 Å, indole carboxamides ~6.28 Å). The calculated distances between the pharmacophoric elements of the designed compounds comply with the proposed pharmacophore model (Fig. 1) for 5-HT$_3$ receptor antagonists. Lipophilicity is an important criterion to be considered when designing compound to manifest drug-like behavior, particularly CNS drugs. Hence, log p values (Tables 1 and 2) of all the compounds were calculated using JME molecular editor (courtesy of Peter Ertl, Novartis). Synthesis of various N4-substituted piperazine analogs of naphthyridine-3-carboxamides and indole-2-carboxamides (Tables 1 and 2) was accomplished using the procedure outlined in Schemes 1 and 2, respectively.

## Results and discussion

### 5-HT$_3$ receptor antagonistic activity

The 5-HT3 receptor antagonistic activity data of all the compounds are represented in Table 3.

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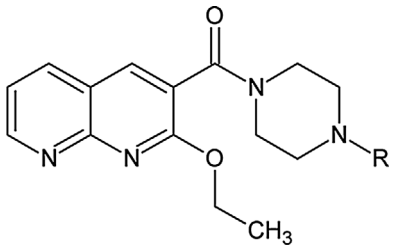
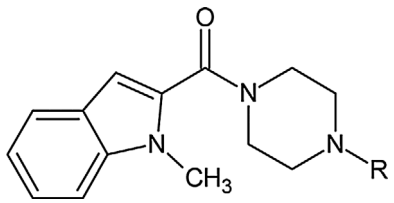
**Table 1.** **Physical constants of (2-ethoxy-1,8-naphthyridin-3-yl)(4-substituted piperazin-1-yl)methanone derivatives.**



| Compound | R | MW | % Yield[a] | m.p. in °C | Log *P*[b] |
|---|---|---|---|---|---|
| **8a** | $C_6H_5$- | 362 | 53 | 126–128 | 3.41 |
| **8b** | $C_6H_5$-$CH_2$- | 376 | 56 | 118–120 | 2.95 |
| **8c** | *p*- $NO_2$-$C_6H_4$- | 405 | 62 | 196–198 | 3.30 |
| **8d** | *o*-Cl-$C_6H_4$- | 396.5 | 67 | 134–136 | 3.58 |
| **8e** | *m*-Cl-$C_6H_4$- | 396.5 | 54 | 138–140 | 3.58 |
| **8f** | *p*-Cl-$C_6H_4$- | 396.5 | 80 | 145–147 | 3.58 |
| **8g** | *o*-$OCH_3$-$C_6H_4$- | 392 | 76 | 130–132 | 3.32 |
| **8h** | *m*-$OCH_3$-$C_6H_4$- | 392 | 77 | 112–114 | 3.32 |
| **8i** | *o*-$CH_3$-$C_6H_4$- | 376 | 89 | 90–92 | 3.63 |
| **8j** | *p*-$CH_3$-$C_6H_4$- | 376 | 63 | 146–148 | 3.63 |
| **8k** | $CH_3$- | 300 | 65 | 114–116 | 1.24 |
| **8l** | $CH_3CH_2$- | 314 | 62 | 120–122 | 1.66 |
| **8m** | $CH_3CH_2CH_2$ | 328 | 76 | 135–137 | 2.02 |

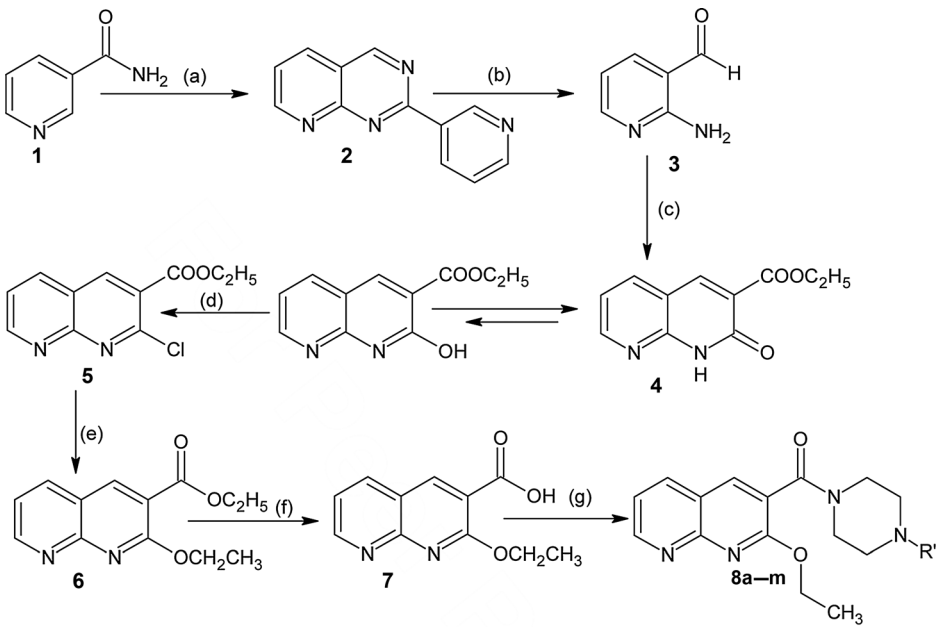
[a]Yields refer to isolated pure compounds.
[b]Log *p* values are calculated by using JME molecular editor (courtesy of Peter Ertl, Novartis).

**Table 2.** **Physical constants of (1-methyl-1*H*-indol-2-yl)(4-substituted piperazin-1-yl)methanone derivatives.**

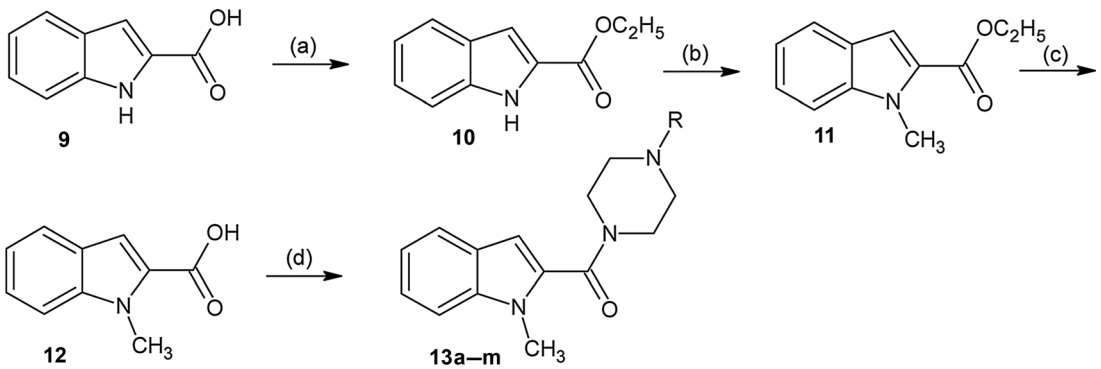


| Compound | R | MW | % Yield[a] | m.p. in °C | Log *P*[b] |
|---|---|---|---|---|---|
| **13a** | $C_6H_5$- | 319 | 67 | 120–122 | 2.98 |
| **13b** | $C_6H_5$-$CH_2$- | 333 | 50 | 118–120 | 2.53 |
| **13c** | *p*- $NO_2$-$C_6H_4$- | 364 | 58 | 176–178 | 2.88 |
| **13d** | *o*-Cl-$C_6H_4$- | 353.5 | 63 | 148–150 | 3.16 |
| **13e** | *m*-Cl-$C_6H_4$- | 353.5 | 58 | 136–138 | 3.16 |
| **13f** | *p*-Cl-$C_6H_4$- | 353.5 | 69 | 112–114 | 3.16 |
| **13g** | *o*-$OCH_3$-$C_6H_4$- | 349 | 68 | 160–162 | 2.90 |
| **13h** | *m*-$OCH_3$-$C_6H_4$- | 349 | 74 | 108–110 | 2.90 |
| **13i** | *o*-$CH_3$-$C_6H_4$- | 333 | 58 | 130–132 | 3.21 |
| **13j** | *p*-$CH_3$-$C_6H_4$- | 333 | 69 | 98–100 | 3.21 |
| **13k** | $CH_3$- | 257 | 78 | 91–93 | 0.82 |
| **13l** | $CH_3CH_2$- | 271 | 61 | 100–102 | 1.24 |
| **13m** | $CH_3CH_2CH_2$ | 285 | 71 | 125–127 | 1.60 |

[a]Yields refer to isolated pure compounds.
[b]Log *P* values of all the synthesized indole derivatives were calculated by using JME molecular editor (courtesy of Peter Ertl, Novartis).



**Scheme 1.** Synthetic route of (2-ethoxy-1,8-naphthyridin-3-yl)(4-substituted piperazin-1-yl)methanone derivatives. Reagents and conditions: (a) Ammonium sulfamate, neat, 200°C, 14 h, 64%; (b) 4 N HCl, 1 h, 38%; (c) diethyl malonate, EtOH, reflux, 6 h, 60%; (d) $POCl_3$, DMF, reflux, 1 h, 67%; (e) $NaOCH_2CH_3$, EtOH, rt, 30 min, 68%; (f) 10% aq. NaOH, rt, 1 h, dil. HCl, 70%; (g) EDC·HCl, HOBt, 0°C to rt, piperazines, 1 h.



**Scheme 2.** Synthetic route of (1-methyl-1*H*-indol-2-yl)(4-substituted piperazin-1-yl)methanone derivatives. Reagents and conditions: (a) Ethanol, cat $H_2SO_4$ reflux, 4–6 h, 80%; (b) $CH_3I$, KOH pellets, DMSO, rt, 1 h, 88%; (c) 10% aq. KOH, reflux, 1 h, dil. HCl, 73%; (d) EDC·HCl, HOBt, 0°C to rt, 1 h, piperazines, 1 h.

Naphthyridine-3-carboxamide (compound **8a**, p$A_2$: 5.6) and indole-2-carboxamide (compound **13a**, p$A_2$: 6.25) with phenyl ring at the distal (N4) nitrogen of piperazine displayed moderate antagonistic activity.

Increasing the spacer length between the distal nitrogen of piperazine and the phenyl from 0 to 1 methylene (naphthyridine-3-carboxamide, compound **8b** p$A_2$: 5.0 and indole-2-carboxamide, compound **13b** p$A_2$: 5.8) led to decrease in 5-HT$_3$ receptor antagonism.

We also surveyed a variety of electron withdrawing as well as electron donating substituents appended at different positions on the N4 phenyl ring of both naphthyridine and indole (piperazino) carboxamides. However, the groups (e.g., *o*-methoxyphenyl, *o*-chlorophenyl, *m*-chlorophenyl, methyl, ethyl, etc.) on the basic moiety (N4) of the piperazine ring were kept similar in case of both the carboxamide series.

The presence of electron withdrawing *p*-$NO_2$ group in the N4 phenyl ring of both naphthyridine-3-carboxamide and

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**Table 3. 5-$HT_3$ receptor antagonistic activity of (2-methoxy-1,8-naphthyridin-3-yl)(4-substituted piperazin-1-yl)methanones and (1-methyl-1*H*-indol-2-yl)(4-substituted piperazin-1-yl)methanones.**

| Compound | Antagonism to 2-Me-5-HT ($pA_2$)[a] | Compound | Antagonism to 2-Me-5-HT ($pA_2$)[a] |
|---|---|---|---|
| **8a** | 5.60 | **13a** | 6.25 |
| **8b** | 5.00 | **13b** | 5.80 |
| **8c** | 5.00 | **13c** | 5.20 |
| **8d** | 5.90 | **13d** | 5.60 |
| **8e** | 6.20 | **13e** | 6.60 |
| **8f** | 5.30 | **13f** | 5.40 |
| **8g** | 6.60 | **13g** | 6.60 |
| **8h** | 7.30 | **13h** | 6.80 |
| **8i** | 6.25 | **13i** | 7.50 |
| **8j** | 5.20 | **13j** | 6.40 |
| **8k** | 3.36 | **13k** | 4.50 |
| **8l** | 3.50 | **13l** | 4.80 |
| **8m** | 3.67 | **13m** | 4.20 |
| | | Ondansetron | 6.60 |

[a]$pA_2$ values are the means of two separate experiments. SE was less than 10% of the mean.

indole-2-carboxamide decreased the antagonistic activity as evidenced by compound **8c** ($pA_2$: 5.0) and compound **13c** ($pA_2$: 5.2).

Naphthyridine-3-carboxamide, compound **8d** ($pA_2$: 5.9) with electron withdrawing *o*-Cl group showed better 5-$HT_3$ antagonistic activity than compound **8f** ($pA_2$: 5.3) with -Cl at *p*-position. However, *m*-Cl substitution in the N4 phenyl ring appeared to be more favorable with compound **8e** ($pA_2$: 6.2) showing higher antagonistic activity than both compounds **8d** and **8f**.

Similar types of results were found in piperazine analogs of indole-2-carboxamides with respect to chloro substitution on the N4 phenyl ring wherein *m*-Cl derivative **13e** ($pA_2$: 6.6) showed better 5-$HT_3$ receptor antagonistic activity compared to the *o*-Cl derivative **13d** ($pA_2$: 5.6) and *p*-Cl derivative **13f** ($pA_2$: 5.4).

Introduction of *o*-$OCH_3$ group in the N4 phenyl ring resulted in moderate antagonistic activity as evident by naphthyridine-3-carboxamide **8g** ($pA_2$: 6.0) and indole-2-carboxamide **13g** ($pA_2$: 6.0). However, naphthyridine-3-carboxamide compound **8h** ($pA_2$: 7.5) and indole-2-carboxamide **13h** ($pA_2$: 6.8) with *m*-$OCH_3$ group in the phenyl ring showed appreciable 5-HT3 receptor antagonism.

Naphthyridine-3-carboxamide, compound **8i** ($pA_2$: 6.25) bearing *o*-$CH_3$ group in the N4 phenyl ring showed moderate activity and the indole counterpart **13i** ($pA_2$: 7.3) displayed substantial antagonistic activity. While, naphthyridine-3-carboxamide, compound **8j** ($pA_2$: 5.2) with *p*-$CH_3$ group in the phenyl ring showed less 5-HT3 receptor antagonistic activity and the corresponding indole analog **13j** ($pA_2$: 6.20) showed moderate activity.

Incorporation of aliphatic methyl group at the N4 piperazine resulted in the lessening of 5-$HT_3$ receptor antagonism, as seen with naphthyridine-3-carboxamide **8k** ($pA_2$: 3.36), indole-2-carboxamide **13k** ($pA_2$: 4.50).

Further incorporation of ethyl as well as propyl substituent at the N4 of piperazine also was not tolerated, as illustrated by the reduced antagonistic activities of naphthyridine-3-carboxamides compound **8l** ($pA_2$: 3.50), compound **8m** ($pA_2$: 3.67) and indole-2-carboxamides compound **13l** ($pA_2$: 4.80), compound **13m** ($pA_2$: 4.20).

However, indole-2-carboxamides were found to be comparatively more tolerant of aliphatic substitutions at the N4 nitrogen than those of their naphthyridine counterparts.

It was observed that within both naphthyridine-3-carboxamide and indole-2-carboxamide series the 5-$HT_3$ receptor antagonistic activity was influenced by the nature of the N4 substituent of the piperazine. With respect to structure–activity relationship, the results demonstrated that compounds bearing methoxyphenyl group at the N4 piperazine showed better activity than compounds with nitrophenyl group and chlorophenyl group. Whereas, replacement of phenyl ring and/or substituted phenyl ring with aliphatic group was detrimental to the activity. It seemed, therefore, the role of N4 substituent in these compounds for the interaction with 5-$HT_3$ receptor might be due to aromatic interaction.

It was also observed that compounds **13a**, **13e**, **13i**, and **13j** with indole core displayed relatively better activity than compounds **8a**, **8e**, **8i**, and **8j** with naphthyridine core bearing similar substituents in the N4 phenyl ring. However, naphthyridine carboxamide compound **8h** showed better activity in comparison to its indole counterpart **13h**.

Few piperazine analogs of both naphthyridine and indole carboxamides (compound **8a**, compound **13a** containing N4 phenyl substituent and compound **8c** and **13c** containing N4 benzyl substituent) were found to have better 5-$HT_3$ antagonism compared to our earlier synthesized quinoxaline carboxamide counterparts [30]. In case of compounds containing *m*-chlorophenyl and *o*-methoxyphenyl substituents

at N4 piperazine, almost similar antagonistic activity was observed among naphthyridine, indole, and quinoxaline carboxamide derivatives. However, naphthyridine as well as indole carboxamides with aliphatic substituents at N4 piperazine were found to have less 5-HT$_3$ antagonistic activity as compared to the corresponding quinoxaline carboxamides [30].

## Antidepressant activity

On the basis of 5-HT3 receptor antagonistic activity shown by all the compounds, four naphthyridine-3-carboxamides (**8e–8i**) and six indole-2-carboxamides (**13a**, **13e**, **13g–13j**) were selected for antidepressant screening.

Initially to rule out the possible existence of any false positive and (or) false negative result in the forced swim test (FST) due to drug-induced stimulation/suppression in the locomotor activity of mice, the compounds were subjected to spontaneous locomotor activity study using actophotometer [34]. Neither ondansetron, nor test compounds were found to cause any significant changes in the locomotor activity of mice when compared to the control as shown in Table 4.

Subsequently, compounds were subjected to the mouse FST [35, 36] in order to evaluate the antidepressant potential. Based on the actophotometer study, compounds were examined at dose of 1 mg/kg (i.p.) (lower dose level) and 2 mg/kg (i.p) (higher dose level) in the mouse FST. Antidepressant activity was determined as mean immobility time in seconds. The antidepressant activity data from the FST is shown as mean ± S.E.M. in Table 5.

**Table 4. Spontaneous locomotor scores of (2-ethoxy-1,8-naphthyridin-3-yl)(4-substituted piperazin-1-yl)methanones and (1-methyl-1*H*-indol-2-yl)(4-substituted piperazin-1-yl)methanones.**

| Compound | Locomotor scores[a] (10 min) | |
|---|---|---|
| | Dose, mg/kg (i.p.) | |
| | 2 | 1 |
| **8e** | 535.33 ± 14.63 | 507.00 ± 18.91 |
| **8g** | 447.00 ± 08.33 | 435.50 ± 17.41 |
| **8h** | 487.00 ± 08.33 | 445.50 ± 7.86 |
| **8i** | 447.00 ± 24.60 | 362.67 ± 10.63 |
| **13a** | 541.67 ± 11.10 | 435.33 ± 15.76 |
| **13e** | 457.00 ± 07.34 | 438.00 ± 15.62 |
| **13g** | 467.00 ± 18.03 | 425.50 ± 10.41 |
| **13h** | 471.00 ± 10.33 | 464.00 ± 25.53 |
| **13i** | 571.00 ± 11.33 | 384.00 ± 25.53 |
| **13j** | 400.67 ± 48.90 | 300.00 ± 41.90 |
| Ondansetron | 447.00 ± 08.33 | 395.50 ± 1.41 |
| control | 489.00 ± 12.10 | |

[a]Water was used as a vehicle, the values are expressed as mean, $n = 8$ per group. Data were analyzed by Graph Pad Prism 3 software through one-way ANOVA followed by post hoc Dunnett's test.

**Table 5. Antidepressant activity of (2-ethoxy-1,8-naphthyridin-3-yl)(4-substituted piperazin-1-yl)methanones and (1-methyl-1*H*-indol-2-yl)(4-phenylpiperazin-1-yl)methanones in the forced swim test mice model.**

| Compound | Duration of immobility in seconds (FST)[a]; dose, mg/kg (i.p.) | |
|---|---|---|
| | 1 | 2 |
| **8e** | 128.33 ± 12.80 | 109.00 ± 05.18* |
| **8g** | 98.33 ± 07.12* | 121.33 ± 09.98 |
| **8h** | 90.67 ± 10.57* | 84.33 ± 08.51* |
| **8i** | 120.33 ± 11.45 | 64.33 ± 16.36* |
| **13a** | 145.33 ± 04.83 | 124.00 ± 14.40 |
| **13e** | 166.33 ± 07.56 | 117.00 ± 06.66* |
| **13g** | 100.43 ± 03.91* | 88.33 ± 06.53* |
| **13h** | 113.33 ± 13.86 | 88.67 ± 06.34* |
| **13i** | 105.00 ± 04.41* | 85.67 ± 05.06* |
| **13j** | 80.00 ± 07.76* | 79.00 ± 10.69* |
| Ondansetron | 105.10 ± 8.90* | 98.50 ± 05.50* |
| Control | 174.67 ± 5.21 | |

[a]Water was used as a vehicle, the values are expressed as mean, $n = 8$ per group.
Data were analyzed by Graph Pad Prism (3) software through one-way ANOVA followed by post hoc Dunnett's test.
*$p < 0.05$ as compared to vehicle treated group (control).

Among the compounds screened, three compounds (**8h, 13i,** and **13j**) showed prominent antidepressant-like activity as indicated by significant ($p < 0.05$) reduction in immobility time compared to the control, while two compounds (**8g** and **13g**) showed moderate antidepressant-like activity. Three compounds (**8h, 13i,** and **13j**) demonstrated a statistically significant reduction in immobility time compared to control in a dose-dependent manner. Compounds **8h**, **13i,** and **13j** emerged as most potential derivatives as they significantly ($p < 0.05$) reduced the immobility time compared to the control at both the tested dose levels (1 and 2 mg/kg). Compounds **8e**, **8i, 13e,** and **13h** showed significant antidepressant-like activity as compared to vehicle control only at higher (2 mg/kg) dose level.

## Conclusion

To summarize, the present study enabled us to extend the structure–activity relationship studies of the carboxamides with two different heteroaromatic cores in the proposed pharmacophore. In the indole series, promising compounds were obtained from methyl phenyl and methoxy phenyl substitution at the N4 of piperazine. Similar results were obtained with naphthyridine analogs bearing similar substituents. Supposedly, alteration in the heteroaromatic core

did not elicit substantial changes in the activity of the compounds. Nevertheless, few analogs from the indole series were found to be comparatively more active than their naphthyridine counterpart. Analysis of SAR study led to the assumption that within both naphthyridine and indole carboxamide series the activity is more sensitive to changes in the N4-substituent of piperazine. Thus, it is likely that an appropriate combination of heteroaromatic core and basic centre (substitution at N4 nitrogen of piperazine) of the pharmacophore produces potential 5-$HT_3$ receptor antagonistic activity. Studies in our laboratory are currently underway to further understand the role of aromatic core and basic moiety as 5-$HT_3$ receptor pharmacophoric criteria within a molecule in order to identify novel compounds with potential as antidepressant agents.

## Experimental

### Chemistry

Melting points (m.p.) were determined in open capillary tubes on a Buechi 530 melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was performed to monitor progress of the reaction and assess purity of the compounds; spots were detected by their absorption under UV light. IR spectra were recorded with IR prestige-21 (FT-IR, Shimadzu) and mass spectra were recorded using 'Hewlett-Packard' HP GS/MS 5890/5972. $^1$H NMR spectra were recorded with Bruker DPX operating at 400 MHz in $CDCl_3$ or DMSO-$d_6$ solution, with tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown as $\delta$ values (ppm), the *J* values are expressed in Hertz (Hz). Signals are represented as s (singlet), d (doublet), t (triplet), q (quintet), or m (multiplet). The substituted phenyl piperazine derivatives, and other chemicals were commercially available (Aldrich or Fluka). The synthetic route to the target carboxamides are illustrated in the scheme.

The piperazine analogs of naphthyridine-3-carboxamides were prepared as depicted in Scheme 1. 2-Aminonicotinaldehyde was synthesized as reported in the literature via sequence of reactions (Scheme 1) from the starting material nicotinamide (1), which upon solvent free heating with ammonium sulfamate followed by hydrolysis, gave 2-aminonicotinaldehyde **3** [37]. Condensation of 2-aminonicotinaldehyde with diethylmalonate in alcohol provided ethyl 2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxylate **4** [38].

#### Synthesis of ethyl 2-chloro-1,8-naphthyridine-3-carboxylate 5

To compound **4** (1 mmol), few drops of DMF and excess $POCl_3$ were added. The reaction mixture was refluxed for 1–2 h. After completion of the reaction (monitored by TLC using ethyl acetate/hexane 7:3 by volume), the reaction mixture was poured onto crushed ice kept in a large vessel. To this, saturated $NaHCO_3$ solution was added very carefully until slightly basic pH was attained. The pale yellow solid compound thus precipitated out was filtered and washed thoroughly with cold water.

Yield: 67%, melting point 170°–172°C. FT-IR (KBr, $cm^{-1}$): 3471, 3034, 2922, 2358, 2241, 1969, 1907, 1625, 1510, 1462, 1350, 1296, 1250, and 1090. $^1$H NMR, $CDCl_3$, $\delta$ (ppm): 8.78–8.76 (dd, 1H, $J = 4.8$ Hz, 2 Hz, naphthyridine); 8.5 (s, 1H, naphthyridine); 7.95–7.97 (m, 1H, naphthyridine); 7.20–7.17 (dd, 1H, $J = 10$ Hz, 7.2 Hz, naphthyridine); 4.38–4.35 (q, 2H, ester); 1.35–1.33 (t, 3H, ester).

#### Synthesis of ethyl 2-ethoxy-1,8-naphthyridine-3-carboxylate 6

To a solution of chloro derivative (**5**) (1 mmol) in a sufficient amount of alcohol, sodium ethoxide (2 mmol) was added; the reaction mixture was stirred at room temperature for 2 h. The reaction was monitored by TLC. On completion of the reaction the mixture was evaporated *in vacuo*, and the dark brown thick liquid so obtained was dissolved in EtOAc (30 mL), washed with saturated $NaHCO_3$ solution, dil. HCl solution, and brine. Organic portion was separated and evaporated to get the ethoxy derivative, which was used further without purification. Yield = 70%.

#### Synthesis of 2-ethoxy-1,8-naphthyridine-3-carboxylic acid 7

The ester **6** (1 mmol) was dissolved in sufficient amount of ethanol, to this 10% aqueous NaOH (3 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h, during which the reaction was monitored by TLC using EtOAc/*n*-hexane (1:1) as solvent system. On completion of reaction, the mixture was evaporated *in vacuo* to obtain a dark brown thick liquid, to this crushed ice was added and acidified with dilute HCl up to pH 3. Brown solid precipitated out which was filtered and washed with ice cold water to obtain pure acid. $^1$H NMR, DMSO-$d_6$, $\delta$ (ppm): 9.07–9.05 (m, 1H, naphthyridine), 8.90 (s, 1H, naphthyridine), 8.78–8.74 (m, 1H, naphthyridine), 7.70–7.67 (m, 1H, naphthyridine), 4.80–4.60 (q, 2H, -$OCH_2$), 1.48–1.34 (t, 3H, -$CH_3$ of ethoxy).

#### General procedure for the synthesis of compounds 8a–o

2-Ethoxy-1,8-naphthyridine-3-carboxylic acid (**7**) (1 mmol) was dissolved in THF. To this EDC·HCl (1.2 mmol) and HOBt (1.5 mmol) were added under nitrogen condition and stirred at 0°C, for 5–10 min, followed by various substituted piperazines were added in equimolar quantity. The reaction mixture was further stirred for 1 h at room temperature. On completion, the reaction mixture was evaporated and washed with water; and compounds were purified by recrystallization and (or) column chromatography. Physical constants of the title compounds are represented in the Table 1. Spectral data of the synthesized compounds are as follow:

*2-Ethoxy-1,8-naphthyridin-3-yl(4-phenylpiperazin-1-yl)methanone **8a**:* IR (KBr)/cm: 3125, 2954, 1620, 1500, 1485, 1232, 1157. $^1$H NMR, $CDCl_3$, $\delta$ (ppm): 9.06–8.96 (m, 1H, naphthyridine),

8.16–8.0 (m, 2H, naphthyridine), 7.45–7.35 (m, 1H, naphthyridine), 7.31–7.26 (m, 2H, phenyl), 7.0–6.89 (m, 3H, phenyl), 4.82–4.57 (q, 2H, -$OCH_2$), 4.13–3.90 (m, 2H, piperazine), 3.58–3.40 (m, 2H, -piperazine), 3.34–3.29 (m, 2H, piperazine), 3.22–3.05 (m, 2H, piperazine), 1.51–1.38 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 363 [M+H] 100%.

*2-Ethoxy-1,8-naphthyridin-3-yl(4-benzylpiperazin-1-yl)methanone* ***8b****:* IR (KBr)/cm: 3110, 3050, 1620, 1554, 1483, 1034. $^1$H NMR, $CDCl_3$, δ (ppm): 9.03–8.92 (m, 1H, naphthyridine), 8.17–7.99 (m, 2H, naphthyridine), 7.47–7.35 (m, 2H, naphthyridine, phenyl), 7.31–7.19 (m, 4H, phenyl), 4.81–4.54 (m, 2H, -$OCH_2$), 3.96–3.76 (m, 2H, piperazine), 3.64–3.48 (m, 2H, -piperazine), 3.43–3.17 (m, 2H, piperazine), 2.69–2.48 (m, 2H, piperazine), 2.42–2.30 (m, 2H, piperazine), 1.52–1.37 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 377.26 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(4-nitrophenyl)piperazin-1-yl]methanone* ***8c****:* IR (KBr/cm): 3100, 3060, 1628, 1570, 1460, 1142, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 9.07–8.98 (m, 1H, naphthyridine), 8.21–8.09 (m, 4H, naphthyridine, phenyl), 7.49–7.40 (m, 1H, naphthyridine), 6.90–6.78 (m, 2H, phenyl), 4.83–4.63 (q, 2H, -$OCH_2$), 4.17–3.84 (m, 2H, piperazine), 3.65–3.58 (m, 2H, -piperazine), 3.49–3.34 (m, 4H, piperazine), 1.49–1.42 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 406 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(2-chlorophenyl)piperazin-1-yl]methanone* ***8d****:* IR (KBr/cm): 3120, 3060, 1628, 1570, 1460, 1137, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 9.01–8.85 (m, 1H, naphthyridine), 8.17–7.93 (m, 2H, naphthyridine), 7.38–7.26 (m, 2H, naphthyridine, phenyl), 7.20–7.14 (m, 1H, phenyl), 7.05–6.86 (m, 2H, phenyl), 4.75–4.54 (q, 2H, -$OCH_2$), 4.09–3.83 (m, 2H, piperazine), 3.49–3.28 (m, 2H, -piperazine), 3.15–3.07 (m, 2H, piperazine), 3.04–2.84 (m, 2H, piperazine), 1.48–1.34 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 397 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(3-chlorophenyl)piperazin-1-yl]methanone* ***8e****:* IR (KBr/cm): 3100, 3060 (aromatic C–H str.), 1628 (C=O str.of amide), 1570, 1460 (C=C, C=N ring str.), 1137, 1035 (C–O–C str. of aryl alkyl ether). $^1$H NMR, $CDCl_3$, δ (ppm): 9.04–8.95 (m, 1H, naphthyridine), 8.18–8.08 (m, 2H, naphthyridine), 7.47–7.36 (m, 1H, naphthyridine), 7.25–7.13 (m, 1H, phenyl), 6.94–6.84 (m, 2H, phenyl), 6.82–6.76 (m, 1H, phenyl), 4.82–4.60 (q, 2H, -$OCH_2$), 4.09–3.87 (m, 2H, piperazine), 3.57–3.40 (m, 2H, -piperazine), 3.29–3.19 (m, 2H, piperazine), 3.12–3.03 (m, 2H, piperazine), 1.50–1.41 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 397.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(4-chlorophenyl)piperazin-1-yl]methanone* ***8f****:* IR (KBr/cm): 3100, 3060, 1628, 1570, 1460, 1142, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 9.03–8.96 (m, 1H, naphthyridine), 8.16–8.0 (m, 1H, naphthyridine), 7.45–7.38 (m, 1H, naphthyridine), 7.35–7.26 (m, 3H, naphthyridine, phenyl), 6.90–6.69 (m, 2H, phenyl), 4.80–4.60 (q, 2H, -$OCH_2$), 4.10–3.90 (m, 2H, piperazine), 3.52–3.40 (m, 2H, -piperazine), 3.30–3.20 (m, 2H, piperazine), 3.15–3.05 (m, 2H, piperazine), 1.50–1.37 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 397.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(2-methoxyphenyl)piperazin-1-yl]methanone* ***8g****:* IR (KBr/cm): 3150, 3050, 1630, 1560, 1478, 1135, 1038. $^1$H NMR, $CDCl_3$, δ (ppm): 9.04–8.92 (m, 1H, naphthyridine), 8.19–8.06 (m, 2H, naphthyridine), 7.45–7.33 (m, 1H, naphthyridine), 7.15–7.02 (m, 1H, phenyl), 6.99–6.85 (m, 3H, phenyl), 4.86–4.54 (q, 2H, -$OCH_2$), 4.18–3.96 (m, 2H, piperazine), 3.83 (s, 3H, -$OCH_3$), 3.61–3.36 (m, 2H, -piperazine), 3.31–3.13 (m, 2H, piperazine), 3.11–2.93 (m, 2H, piperazine), 1.54–1.40 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 393.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(3-methoxyphenyl)piperazin-1-yl]methanone* ***8h****:* IR (KBr/cm): 3150, 3050, 1630, 1560, 1470, 1142, 1038. $^1$H NMR, $CDCl_3$, δ (ppm): 8.98–8.92 (m, 1H, naphthyridine), 8.19–8.06 (m, 2H, naphthyridine), 7.45–7.30 (m, 1H, naphthyridine), 7.20–7.10 (m, 1H, phenyl), 6.59–6.45 (m, 1H, phenyl), 6.48–6.35 (m, 2H, phenyl), 4.66–4.50 (q, 2H, -$OCH_2$), 4.18–3.98 (m, 2H, piperazine), 3.73 (s, 3H, $OCH_3$), 3.48–3.36 (m, 2H, -piperazine), 3.31–3.20 (m, 2H, piperazine), 3.15–2.97 (m, 2H, piperazine), 1.48–1.40 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 393.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(2-methylphenyl)piperazin-1-yl]methanone* ***8i****:* IR (KBr)/cm: 3100, 3050, 1620, 1500, 1460, 1350. $^1$H NMR, $CDCl_3$, δ (ppm): 8.99–8.92 (m, 1H, naphthyridine), 8.19–8.06 (m, 2H, naphthyridine), 7.41–7.36 (m, 1H, naphthyridine), 7.21–7.14 (m, 2H, phenyl), 7.04–6.96 (m, 2H, phenyl), 4.78–4.62 (q, 2H, -$OCH_2$), 4.12–3.89 (m, 2H, piperazine), 3.76–3.65 (m, 2H, piperazine), 3.58–3.32 (m, 2H, -piperazine), 3.11–2.77 (m, 2H, piperazine), 2.33 (s, 3H, $CH_3$), 1.52–1.46 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 377.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)[4-(4-methylphenyl)piperazin-1-yl]methanone* ***8j****:* IR (KBr)/cm: 3100, 3060, 1620, 1550, 1430, 1340. $^1$H NMR, $CDCl_3$, δ (ppm): 9.09–8.93 (m, 1H, naphthyridine), 8.23–8.03 (m, 2H, naphthyridine), 7.50–7.38 (m, 1H, naphthyridine), 7.21–7.02 (m, 2H, phenyl), 6.94–6.77 (m, 2H, phenyl), 4.82–4.57 (q, 2H, -$OCH_2$), 4.13–3.92 (m, 2H, piperazine), 3.56–3.37 (m, 2H, piperazine), 3.30–3.24 (m, 2H, -piperazine), 3.17–2.98 (m, 2H, piperazine), 2.29 (s, 3H, $CH_3$), 1.50–1.39 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 377.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)(4-methyl)piperazin-1-yl)-methanone* ***8k****:* IR (KBr)/cm: 3100, 3070, 1620, 1550, 1435, 1340. $^1$H NMR, $CDCl_3$, δ (ppm): 9.06–8.98 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 7.98–7.86 (m, 1H, naphthyridine), 7.41–7.34 (m, 1H, naphthyridine), 4.75–4.57 (q, 2H, -$OCH_2$), 4.10–3.95 (m, 2H, piperazine), 3.58–3.34 (m, 2H, piperazine), 3.28–3.24 (m, 2H, -piperazine), 3.10–2.97 (m, 2H, piperazine), 2.96 (s, 3H, $CH_3$), 1.50–1.35 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 301 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)(4-ethylpiperazin-1-yl)methanone* ***8l****:* IR (KBr)/cm: 3010, 2960, 1620, 1520, 1430, 1290. $^1$H NMR, $CDCl_3$, δ (ppm): 9.03–8.96 (m, 1H, naphthyridine), 8.15–8.03 (m, 1H, naphthyridine), 7.98–7.86 (m, 1H, naphthyridine), 7.25–7.10 (m, 1H, naphthyridine), 4.53–4.25 (q, 2H, -$OCH_2$), 3.98–3.93 (m, 2H, piperazine), 3.70–3.58 (m, 2H, piperazine), 3.42–3.33 (m, 2H, -piperazine), 3.25–3.10 (m, 2H, piperazine), 2.97–2.80 (m, 2H,$CH_2$), 2.70–2.50 (m, 3H, $CH_3$), 1.52–1.38 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 315.1 [M+H] 100%.

*(2-Ethoxy-1,8-naphthyridin-3-yl)(4-propylpiperazin-1-yl)methanone* ***8m****:* IR (KBr)/cm: 3130, 3030, 1620, 1560, 1430, 1280. $^1$H NMR, $CDCl_3$, δ (ppm): 9.0–8.93 (m, 1H, naphthyridine), 8.30–8.20 (m, 1H, naphthyridine), 7.98–7.88 (m, 1H, naphthyridine), 7.30–7.20 (m, 1H, naphthyridine), 4.53–4.35 (q, 2H, -$OCH_2$), 3.98–3.80 (m, 2H, piperazine), 3.70–3.58 (m, 2H, piperazine), 3.42–3.33 (m, 2H, -piperazine), 3.25–3.10 (m, 2H, piperazine), 2.94 (t, 2H, -$CH_2$), 2.84–2.80 (m, 2H, -$CH_2$), 2.76–2.70 (t, 3H, -$CH_3$), 1.73–1.52 (t, 3H, -$CH_3$ of ethoxy). ESI-MS: *m/z* 329 [M+H] 100%.

The synthetic route to piperazine analogs of indole-2-carboxamides is illustrated in the Scheme 2. The compounds were synthesized in a sequence of reactions from the starting material indole-2-carboxylic acid **9**.

### Synthesis of ethyl 1*H*-indole-2-carboxylate 10

Indole-2-carboxylic acid (1 mmol) was dissolved in a sufficient amount of ethanol in a round-bottomed flask. To this 2–3 mL of conc. $H_2SO_4$ was added. The reaction mixture was refluxed for 6 h. Upon completion of the reaction the reaction mixture was poured onto crushed ice and neutralized using sodium bicarbonate. The solid thus obtained was filtered and washed with water and used for further reaction without purification.

### Synthesis of ethyl 1-methyl-1*H*-indole-2-carboxylate 11 [39]

The ethyl ester (1 mmol) was dissolved in anhydrous DMSO, to this KOH pellets (3 mmol) and methyl iodide (1.5 mmol) were added and the reaction mixture was stirred at room temperature for 1 h. During which the reaction was monitored using TLC using EtOAc/*n*-hexane (3:7). Upon completion of the reaction the reaction mixture was poured on crushed ice. The solid thus obtained was washed with water and filtered.

### Synthesis of 1-methyl-1*H*-indole-2-carboxylic acid 12

The ester (2 g, 8.45 mmol) was dissolved in 10 mL ethanol; to this aqueous NaOH, equimolar was added, and the reaction mixture was stirred at room temperature for 1 h, during which the reaction was monitored by TLC using EtOAc/*n*-hexane (1:1) as solvent system. On completion of reaction the mixture was evaporated *in vacuo* to obtain a dark brown thick liquid, to this crushed ice was added and acidified with dilute HCl up to pH 3. Brown solid precipitated out which was filtered and washed with ice cold water to obtain pure acid. $^1$H NMR, $CDCl_3$, δ (ppm): 7.77–7.60 (m, 1H, indole), 7.42–7.36 (s, 1H, indole), 7.30–7.26 (m, 1H, indole), 7.20–7.16 (m, 1H, indole), 6.6 (s, 1H, indole).

### General procedure for the synthesis of compounds 13a–m

*N*-substituted indole 2-carboxylic acid was dissolved in DCM, in a round-bottomed flask, under nitrogen condition. To this 1.2 equiv EDC·HCl, followed by 1.5 equiv HOBt were added and stirred at 0°C, for 5–10 min, followed by various commercially available substituted piperazines (for compounds **13a–m**) were added in equimolar quantity. The reaction mixture was further stirred for 1 h at room temperature. On completion, the reaction mixture was evaporated and washed with water; and compounds were purified by recrystalization and (or) column chromatography. Physical constants of the title compounds are represented in the Table 2. Spectral data of the synthesized compounds are as follow.

*(1-Methyl-1H-indol-2-yl)(4-phenylpiperazin-1-yl)methanone* ***13a****:* IR (KBr)/cm: 3100, 3080, 1628, 1540, 1230, 1135. $^1$H NMR, $CDCl_3$, δ (ppm): 7.72–7.59 (m, 1H, indole ring), 7.44–7.36 (m, 1H, indole, phenyl), 7.32–7.28 (m, 2H, indole), 7.23–7.14 (m, 1H, phenyl), 7.10–7.04 (m, 3H, indole, phenyl), 6.98–6.90 (m, 2H, phenyl), 6.64 (s, 1H, indole), 4.03–3.90 (m, 4H, piperazine), 3.86 (s, 3H, $CH_3$), 3.35–3.10 (m, 4H, piperazine). ESI-MS: *m/z* 320 [M+1] 100%.

*(1-Ethyl-1H-indol-2-yl)(4-benzylpiperazin-1-yl)methanone* ***13b****:* IR (KBr)/cm: 3000, 2063, 1620, 1523, 1240, 1155. $^1$H NMR, $CDCl_3$, δ (ppm): 7.70–7.58 (m, 2H, indole ring), 7.50–7.37 (m, 2H, indole), 7.45–7.36 (m, 3H, indole), 7.35–7.29 (m, 1H, phenyl), 7.23–7.09 (m, 1H, phenyl), 6.60 (s, 1H, indole), 4.28–4.26 (m, 2H), 4.22–3.96 (m, 4H, piperazine), 3.86 (s, 3H, $CH_3$), 3.42–3.38 (m, 4H, piperazine), ESI-MS: *m/z* 334.1 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(4-nitrophenylperazin-1-yl)methanone* ***13c****:* IR (KBr)/cm: 3010, 2050, 1620, 1525, 1240, 1155. $^1$H NMR, $CDCl_3$, δ (ppm): 8.14–8.07 (m, 2H, indole ring), 7.64–7.58 (m, 1H, indole, phenyl), 7.51–7.45 (m, 1H, indole), 7.36–7.24 (m, 1H, phenyl), 7.18–7.10 (m, 1H, phenyl), 6.97–6.95 (m, 2H, phenyl), 6.71 (s, 1H, indole), 3.96–3.85 (m, 4H, piperazine), 3.82 (s, 3H, $CH_3$ of N-$CH_3$), 3.68–3.52 (m, 4H, piperazine). ESI-MS: *m/z* 365 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(2-chlorophenylpiperazin-1-yl)methanone* ***13d****:* IR (KBr)/cm: 3120, 3050, 1625, 1530, 1240, 1125. $^1$H NMR, $CDCl_3$, δ (ppm): 7.54–7.52 (m, 1H, indole ring), 7.36–7.28 (m, 2H, indole, phenyl), 7.26–7.14 (m, 2H, indole), 7.13–7.04 (m, 1H, phenyl), 6.98–6.90 (m, 2H, phenyl), 6.60 (s, 1H, indole), 4.03–3.87 (m, 4H, piperazine), 3.76 (s, 3H, $CH_3$), 3.14–2.84 (m, 4H, piperazine). ESI-MS: *m/z* 354.1 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(3-chlorophenylpiperazin-1-yl)methanone* ***13e****:* IR (KBr/cm): 3120, 3080, 1625, 1550, 1240, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 7.72–7.56 (m, 1H, indole ring), 7.45–7.37 (m, 1H, indole, phenyl), 7.33–7.29 (m, 1H, indole), 7.26–7.22 (m, 3H, phenyl), 7.19–7.13 (m, 2H, phenyl), 6.64 (s, 1H, indole),

4.02–3.88 (m, 4H, piperazine), 3.86 (s, 3H, $CH_3$), 3.35–3.19 (m, 4H, piperazine). ESI-MS: *m/z* 354 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(4-chlorophenylpiperazin-1-yl)methanone* ***13f****:* IR (KBr/cm): 3100, 3080, 1625, 1550, 1240, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 7.68–7.59 (m, 1H, indole ring), 7.44–7.29 (m, 3H, indole, phenyl), 7.26–7.22 (m, 2H, indole), 7.20–7.10 (m, 1H, phenyl), 6.95–6.81 (m, 1H, phenyl), 6.63 (s, 1H, indole), 4.01–3.89 (m, 4H, piperazine), 3.85 (s, 3H, $CH_3$), 3.34–3.08 (m, 4H, piperazine). ESI-MS: *m/z* 354 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(2-methoxyphenylpiperazin-1-yl)-methanone* ***13g****:* IR (KBr/cm): 3130, 3050, 1626, 1550, 1240, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 7.57–7.50 (m, 1H, indole ring), 7.36–7.21 (m, 2H, indole, phenyl), 7.21–7.18 (m, 1H, indole), 7.06–7.04 (m, 1H, phenyl), 6.95–6.91 (m, 3H, indole, phenyl), 6.55 (s, 1H, indole), 4.08–3.83 (m, 4H, piperazine), 3.88 (s, 3H, -$CH_3$ of N-$CH_3$), 3.79 (s, 3H, $OCH_3$ of phenyl), 3.19–2.87 (m, 4H, piperazine). ESI-MS: *m/z* 350 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(3-methoxyphenylpiperazin-1-yl)-methanone* ***13h****:* IR (KBr/cm): 3130, 3050, 1628, 1550, 1230, 1035. $^1$H NMR, $CDCl_3$, δ (ppm): 7.67–7.60 (m, 1H, indole ring), 7.46–7.36 (m, 1H, indole, phenyl), 7.34–7.29 (m, 1H, indole), 7.14–7.11 (m, 2H, phenyl), 6.64 (s, 1H, indole), 6.61–6.55 (m, 1H, phenyl), 6.49–6.45 (m, 2H, phenyl), 4.02–3.93 (m, 4H, piperazine), 3.86 (s, 3H, $CH_3$ of N-$CH_3$), 3.80 (s, 3H, $OCH_3$ of phenyl), 3.33–3.12 (m, 4H, piperazine). ESI-MS: *m/z* 350 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(2-methylphenylpiperazin-1-yl)methanone* ***13i****:* IR (KBr)/cm: 3150, 3050, 1628, 1530, 1230, 1135. $^1$H NMR, $CDCl_3$, δ (ppm): 7.69–7.55 (m, 1H, indole ring), 7.43–7.33 (m, 1H, indole, phenyl), 7.30–7.26 (m, 1H, indole), 7.25–7.13 (m, 3H, phenyl), 7.11–6.95 (m, 2H, phenyl), 6.64 (s, 1H, indole), 4.03–3.89 (m, 4H, piperazine), 3.87 (s, 3H, $CH_3$), 3.14–2.79 (m, 4H, piperazine), 2.35 (s, 3H, $CH_3$ of phenyl). ESI-MS: *m/z* 334 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(4-methylphenylpiperazine-1-yl)-methanone* ***13j****:* IR (KBr)/cm: 3150, 3050, 1625, 1530, 1230, 1135. $^1$H NMR, $CDCl_3$, δ (ppm): 8.14–8.07 (m, 2H, indole ring), 7.64–7.58 (m, 1H, indole), 7.51–7.45 (m, 1H, indole), 7.36–7.24 (m, 1H, phenyl), 7.18–7.10 (m, 1H, phenyl), 6.97–6.95 (m, 2H, phenyl), 6.71 (s, 1H, indole), 3.96–3.85 (m, 4H, piperazine), 3.82 (s, 3H, $CH_3$ of N-$CH_3$), 3.68–3.52 (m, 4H, piperazine). ESI-MS: *m/z* 334.1 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(4-methylpiperazine-1-yl)methanone* ***13k****:* IR (KBr)/cm: 3130, 3010, 1620, 1580, 1244, 1150. $^1$H NMR, $CDCl_3$, δ (ppm): 7.56–7.46 (m, 2H, indole ring), 7.38–7.34 (m, 1H, indole), 7.28–7.18 (m, 1H, indole), 6.73 (s, 1H, indole), 3.96–3.85 (m, 4H, piperazine), 3.80 (s, 3H, $CH_3$ of N-$CH_3$), 3.68–3.52 (m, 4H, piperazine), 2.60 (s, 3H, -$CH_3$). ESI-MS: *m/z* 258.1 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(4-ethylpiperazine-1-yl)methanone* ***13l****:* IR (KBr)/cm: 3120, 3030, 1628, 1580, 1244, 1150. $^1$H NMR, $CDCl_3$, δ (ppm): 7.50–7.46 (m, 2H, indole ring), 7.38–7.36 (m, 1H, indole), 7.28–7.18 (m, 1H, indole), 6.70 (s, 1H, indole), 3.96–3.85 (m, 4H, piperazine), 3.80 (s, 3H, -$CH_3$ of N-$CH_3$), 3.68–3.52 (m, 4H, piperazine), 2.90 (q, 2H, -$CH_2$), 2.80 (t, 3H, -$CH_3$). ESI-MS: *m/z* 272 [M+1] 100%.

*(1-Methyl-1H-indol-2-yl)(4-propylpiperazine-1-yl)methanone* ***13m****:* IR (KBr)/cm: 3130, 3050, 1625, 1580, 1244, 1149. $^1$H NMR, $CDCl_3$, δ (ppm): 7.50–7.46 (m, 2H, indole ring), 7.38–7.36 (m, 1H, indole), 7.28–7.18 (m, 1H, indole), 6.70 (s, 1H, indole), 3.96–3.85 (m, 4H, piperazine), 3.80 (s, 3H, $CH_3$ of N-$CH_3$), 3.68–3.52 (m, 4H, piperazine), 2.93–2.90 (t, 2H, -$CH_2$), 2.86–2.83 (m, 2H, -$CH_2$), 2.78–2.70 (t, 3H, -$CH_3$). ESI-MS: *m/z* 286.1 [M+1] 100%.

## Pharmacological evaluation

### Animals

All the animals were obtained from Hissar Agricultural University (Hissar, Haryana, India) and maintained under standard light/dark 12.00–12.00 h, temperature (23 ± 2°C), and room humidity (60 ± 10%) conditions. The rodents were housed in standard polycarbonate cages and fed with standard animal feed (standard pellet chow) and water *ad libitum*. The animals were used only once for each experiment and were acclimatized to the experimental room for 1 h before testing. Experiments on animals were approved by the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/14/04).

### Determination of 5-HT$_3$ receptor antagonism

The synthesized compounds were tested for 5-HT$_3$ receptor antagonistic activity in longitudinal muscle myenteric plexus preparation from guinea pig ileum against 5-HT$_3$ agonist, 2-methyl 5-HT. Dunkin Hartley guinea pigs (250–300 g) were sacrificed 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 prepared and mounted as described by the literature method [40]. The tissue was kept for equilibration for 30 min under a resting tension of 500 mg and with constant aeration in a 40 mL organ bath containing Tyrode solution (composition for 1 L NaCl 8 g, KCl 0.2 g, $CaCl_2$ 0.2 g, $MgCl_2$ 0.1 g, $NaH_2PO_4$ 0.05 g, $NaHCO_3$ 1.00 g, glucose 1.0 g, pH 6.7) maintained at 25°C. Non-cumulative concentrations of the agonist 2-methyl-5-HT (Tocris, UK) were added with a 15 min dosing cycle (to prevent desensitization) and left in contact with the tissue until the maximal contraction had developed. To assess the antagonistic effect of the synthesized compounds on the response incited by the agonist, 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). 5-HT$_3$ receptor antagonism was denoted in the form of p$A_2$ values, which was determined according to literature methods [40–43]. The p$A_2$ values of the

test compounds were compared with the standard antagonist ondansetron (Natco Pharma, Hyderabad, India). The results are represented in Table 5.

**Spontaneous locomotor activity (SLA)**

To ascertain the possible occurrence of drug-induced changes (stimulation/suppression) in the locomotor activity of mice, which may contribute to their behavior in the FST and TST, all the compounds were subjected to spontaneous locomotor activity [34] study using the actophotometer at different doses. The actophotometer [34] is composed of a square arena ($30 \times 30$ cm) with walls that are fitted with photocells just above the floor level. The photocells were checked before beginning of the experiment. The drug-/vehicle-treated mice were then individually placed in the square arena. After a 2 min adaptation period, the digital locomotor scores were recorded for the next 8 min in a dimly lit room. All new chemical entities and ondansetron (1 and 2 mg/kg, i.p.) were administered 30 min prior to the test.

**Antidepressant activity**

**Forced swim test:** Behavioral despair or forced swim test (FST) was proposed as a model to evaluate antidepressant-like activity by Porsolt et al. [35, 36]. It was suggested that mice when forced to swim within a restricted space from where they cannot escape and hence, are developed with a characteristic behavior of immobility. This characteristic behavior demonstrates a state of despair, which can be diminished by numerous agents that are therapeutically effective in human depression. This behavioral despair test was employed to assess the antidepressant activities of synthesized compounds. The FST described by Porsolt et al. [35, 36] was slightly modified [19, 44]: each mouse was placed individually in a glass cylinder (diameter: 22.5 cm, height: 30 cm) filled with water up to 15 cm of height at $23 \pm 1$°C. The mice were forced to swim in water for 15 min on the pre-experiment day. Mice were then allowed to return to their home cage. After 24 h from the pre-experiment day, each mouse (vehicle-/drug-treated) was placed in water and forced to swim for 6 min. The duration of immobility during the last 4 min was measured. The mouse was considered to be immobile when it stopped struggling and passively moved to remain floating and kept its head just above water. Water was replaced between trials and temperature was maintained at $23 \pm 1$°C. Ondansetron (1 and 2 mg/kg, i.p.) and new chemical entities (1 and 2 mg/kg, i.p.) were administered 30 min prior to experiment.

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