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# Ligand-Based Design, Synthesis, and Pharmacological Evaluation of 3-Methoxyquinoxalin-2-carboxamides as Structurally Novel Serotonin Type-3 Receptor Antagonists

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Employing a ligand-based approach, 3-methoxyquinoxalin-2-carboxamides were designed as serotonin type-3 (5-$HT_3$) receptor antagonists and synthesized from the starting material *o*-phenylenediamine in a sequence of reactions. The structures of the synthesized compounds were confirmed by spectral data. These carboxamides were investigated for their 5-$HT_3$ receptor antagonisms in longitudinal muscle myenteric plexus preparations from guinea-pig ileum against a standard 5-$HT_3$ agonist, 2-methy-5-HT, and their antagonism activities are expressed as p$A_2$ values. Compounds **6a** (p$A_2$: 7.2), **6e** (p$A_2$: 7.0), **6f** (p$A_2$: 7.5), **6g** (p$A_2$: 7.5), **6n** (p$A_2$: 7.0), and **6o** (p$A_2$: 7.2) exhibited antagonism greater than that of the standard 5-$HT_3$ antagonist, ondansetron (p$A_2$: 6.9).

**Keywords:** 3-Methoxyquinoxalin-2-carboxamides / 5-$HT_3$ receptor antagonists / Ligand-based drug design / Quinoxalines / Serotonin

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## Introduction

Serotonin is a major neurotransmitter identified first from serum and chemically known as 5-hydroxytryptamine (5-HT). More than 90% of the body serotonin is located in the peripheral system such as enterochromaffin cells, platelets, cardiovascular system, and kidney, and to some extent is also present in the central nervous system [1, 2]. It is implicated in various physiological and patho-physiological conditions, by acting through its receptor subtypes (5-$HT_{1-7}$) [3]. Among these receptor subtypes, 5-$HT_3$ is unique, a ligand gated ion channel receptor [4], whereas other receptor subtypes belong to the superfamily of G-protein coupled receptors (GPCR). Antagonists of this 5-$HT_3$ receptor displayed anti-emetic action in cancer chemo-/radiotherapy induced nausea and vomiting. Nausea and vomiting are the most common adverse effects of the existing chemotherapeutic agents and these side-effects occur in a substantial number of patients who are undergoing surgery or radiation therapy [5–8]. The clinically existing 5-$HT_3$ receptor antagonists substantially increase the patient compliance for anti-cancer treatment [7] by decreasing their side-effects (nausea and vomiting), but unfortunately these antagonists are ineffective in 10–30% of patients [9, 10]. Further they possess chiral center(s), which increases the synthetic cost of these drugs [11]. This discussion clearly stresses the requirements of new 5-$HT_3$ receptor antagonists. Based on the Hibert et al. [12] pharmacophore model (Fig. 1), several new chemical entities have been reported so far as 5-$HT_3$ receptor antagonists [13–16].

In our previous studies [13, 16] various 3-substituted quinoxalin-2-carboxamides (consisting of Mannich base as linking unit for piperazine moiety and quinoxaline nucleus, Fig. 2) were assessed as 5-$HT_3$ receptor antagonists; unfortunately none of the compounds exhibited antagonism greater than or equal to ondansetron, a standard 5-$HT_3$ receptor antagonist. Recently, we have demonstrated Mannich base free quinoxalin-2-carboxamides as 5-$HT_3$ receptor antagonists, where piperazines are directly connected with quinoxaline via amide carbonyl group, which yielded several potent antagonists [17, 18]. So in the current study to discover new 5-$HT_3$ receptor antagonists without chiral center, current attempts were focused on developing 3-methoxyquinoxlin-2-carboxamides

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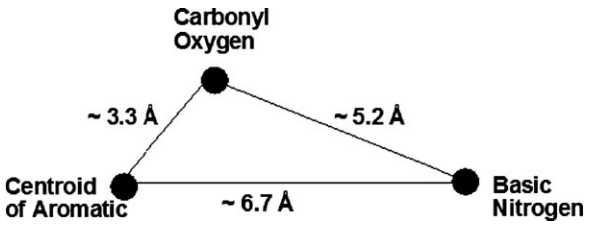
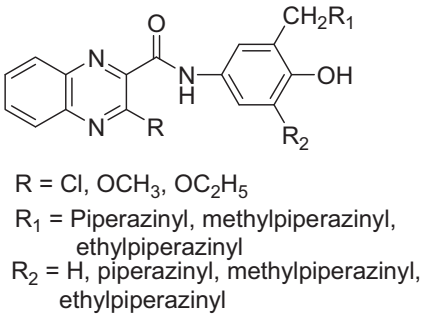



**Figure 1.** Pharmacophore model of 5-$HT_3$ receptor antagonists.




R = Cl, $OCH_3$, $OC_2H_5$
$R_1$ = Piperazinyl, methylpiperazinyl, ethylpiperazinyl
$R_2$ = H, piperazinyl, methylpiperazinyl, ethylpiperazinyl

**Figure 2.** Basic structure of Mannich base compounds.

devoid of Mannich base, as potential 5-$HT_3$ receptor antagonists.

## Results and discussion

### Drug design

The 3-methoxyquinoxalin-2-carboxamides were designed based on the Hibert et al. [12] pharmacophoric model. The minimum energy conformation (three least energy conformations for each compound) of the designed molecules were generated by ACDLABS-10.0/3D Viewer (CHARMM parameterization) and the pharmacophoric distances were measured from centroid of quinoxaline ring to oxygen of the carbonyl group, carbonyl oxygen to basic nitrogen atom ($N^4$ of piperazines, heterocyclic nitrogen, and nitrogen of tertiary amines), and centroid of quinoxaline residue to basic nitrogen. The distances between the pharmacophoric elements of the designed compounds complied with the above-mentioned pharmacophoric model, except compounds tryptamine and *N*,*N*-diethyl-*p*-phenylenediamine-based carboxamide, which showed slight variation in the distances between centroid of quinoxaline to basic nitrogen. Results are summarized in Table 1 and represented in Fig. 3. In order to attain the better pharmacokinetic (absorption, distribution, metabolism, and excretion) profile, during the design of the new compounds Lipinski's rules were considered, i.e., hydrogen bond donor atoms not more than 5, hydrogen bond acceptor atoms not more than 10, log *P*-value not higher than 5 and molecular weight not higher than 500 [19].

### Synthesis of 3-methoxyquinoxalin-2-carboxamides

The synthetic protocol of 3-methoxyquinoxalin-2-carboxamides is depicted in Scheme 1. The penultimate intermediate, i.e., 3-chloroquinoxalin-2-carboxylic acid (**4**) was synthesized in multi-gram quantity from the starting material, *o*-phenylenediamine, as per literature method (with slight modification) [20]. The quinoxaline skeleton, ethyl 3-oxo-3,4-dihydroquinoxalin-2-carboxylate (**2**) was constructed from the starting material *o*-phenylenediamine by condensing with diethyl ketomalonate, which on chlorination with

**Table 1.** Distances between the pharmacophoric elements of **6a–o**[a)]

| Compound | Centroid of quinoxaline-carbonyl oxygen | Carbonyl oxygen-basic nitrogen | Centroid of quinoxaline-basic nitrogen |
|---|---|---|---|
| **6a** | 3.710 ± 0.003 | 4.954 ± 0.011 | 6.616 ± 0.019 |
| **6b** | 3.710 ± 0.004 | 4.940 ± 0.029 | 6.632 ± 0.016 |
| **6c** | 3.709 ± 0.006 | 4.953 ± 0.035 | 6.646 ± 0.033 |
| **6d** | 3.708 ± 0.002 | 4.970 ± 0.011 | 6.665 ± 0.026 |
| **6e** | 3.708 ± 0.002 | 4.958 ± 0.010 | 6.651 ± 0.023 |
| **6f** | 3.710 ± 0.001 | 4.959 ± 0.017 | 6.645 ± 0.017 |
| **6g** | 3.703 ± 0.007 | 4.922 ± 0.010 | 6.726 ± 0.031 |
| **6h** | 3.704 ± 0.004 | 4.950 ± 0.011 | 6.725 ± 0.062 |
| **6i** | 3.710 ± 0.005 | 4.961 ± 0.010 | 6.713 ± 0.037 |
| **6j** | 3.713 ± 0.001 | 4.969 ± 0.009 | 6.713 ± 0.045 |
| **6k** | 3.704 ± 0.010 | 5.130 ± 0.034 | 7.639 ± 0.003 |
| **6l** | 3.704 ± 0.007 | 5.088 ± 0.030 | 7.654 ± 0.027 |
| **6m** | 3.708 ± 0.001 | 6.496 ± 0.053 | 9.475 ± 0.025 |
| **6n** | 3.713 ± 0.003 | 6.222 ± 0.025 | 9.317 ± 0.025 |
| **6o** | 3.720 ± 0.006 | 5.027 ± 0.018 | 7.539 ± 0.019 |

[a)] Distances calculated for 3D optimized structures for at least three conformations of each compound, using CHARMM Parameterization (ACDLABS-10.0/3D Viewer) and the values are represented as mean ± SD in Å.

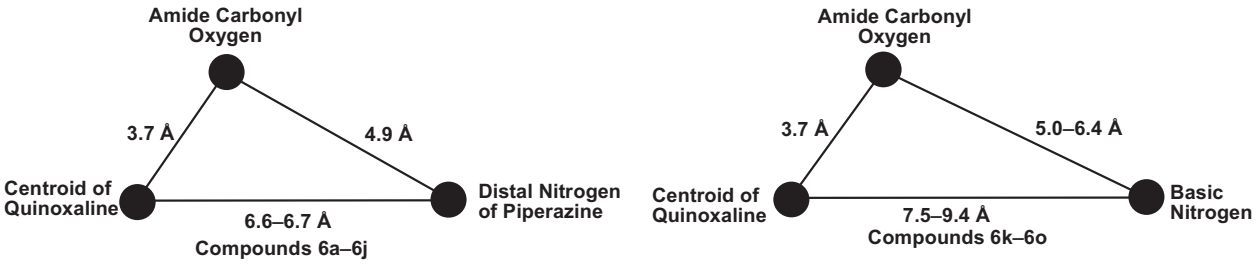
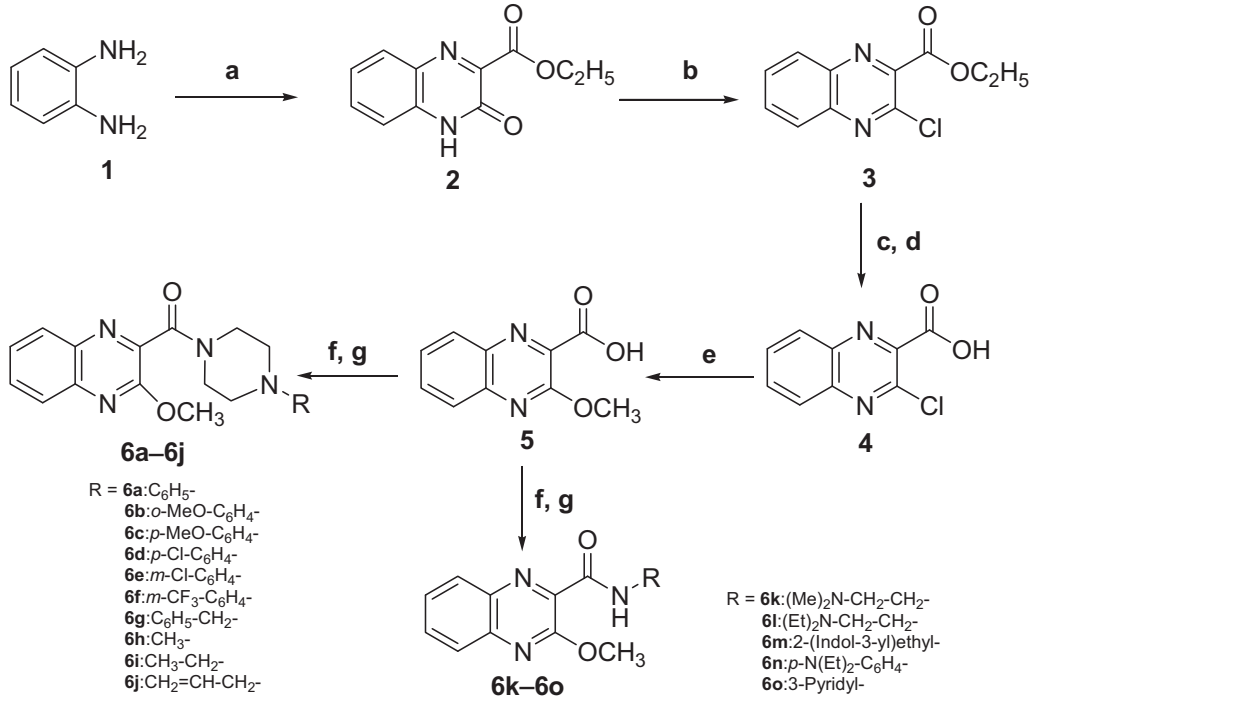



**Figure 3.** Distances between the pharmacophoric elements of **6a–o**.

phosphorous oxychloride afforded the ethyl 3-chloroquinoxalin-2-carboxylate (**3**) in quantitative yield. The obtained chloro ester compound (**3**) upon alkaline hydrolysis followed by acidification furnished the 3-chloroquinoxalin-2-carboxylic acid (**4**) in good yield. This penultimate intermediate's chlorine atom was replaced by methoxy group under microwave condition, which resulted in the formation of key intermediate (**5**). The target 3-methoxyquinoxalin-2-carboxamides were synthesized by coupling the key intermediate, 3-methoxyquinoxalin-2-carboxylic acid with appropriate amines in the presence of coupling agents EDC · HCl and HOBt under inert atmosphere, nitrogen gas. In large scale, synthesis of ethyl 3-oxo-3,4-dihydroquinoxalin-2-carboxylate (**2**) was carried out in the presence of citric acid to improve the yield [21].

Purity of the synthesized compounds was assessed by running TLC with two different solvent systems using three different detection (UV, $I_2$, and ninhydrin) methods. Homogeneity of one of the most active compounds (**6f**) was confirmed by elemental data. In IR spectra, the piperazine-based carboxamides showed strong absorption bands for carbonyl groups at $1640 \pm 10\ cm^{-1}$, whereas the other amine-based carboxamides showed an absorption band at $1680 \pm 15\ cm^{-1}$. Secondary carboxamides showed absorption




**Reagents and conditions:** (**a**) Diethyl ketomalonate, ethanol, reflux, 6 h, 60%; (**b**) $POCl_3$, DMF, reflux, 30 min 80%; (**c**) $Na_2CO_3$, reflux, 6 h; (**d**) dil. HCl, 94%; (**e**) $NaOCH_3$, methanol, MWI (420 Watt), 6 min, dil. HCl, 85%; (**f**) EDC·HCl, HOBt, THF, $N_2$, 0 °C–rt, 1 h; (**g**) amines, rt, 6 h.

**Scheme 1.** Synthetic route of 3-methoxyquinoxalin-2-carboxamides.

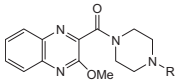
 

at 3260 ± 30 $cm^{-1}$ for N–H stretching and this absorption band was absent in tertiary carboxamides. Compound **6m** showed two N–H stretching absorption bands, viz. one for the amide N–H and the other for the indole N–H group. In proton NMR spectra, the chemical shift values of the amide N–H proton appeared between $\delta$ 10.03 and 8.05. The aromatic amine-based carboxamides, **6n** and **6o**, showed signals for the amide N–H proton at $\delta$ 10.03 and $\delta$ 9.86, respectively. The amides obtained from the aliphatic amines showed signal for amide proton in the range of $\delta$ 8.12–8.05, which (signal) was absent in piperazine-based carboxamides. The piperazine-based carboxamides displayed piperazine protons at ~$\delta$ 4.00, 3.46, 3.35, and 3.10 as triplet with two protons in each signal, when the distal nitrogen was attached to a phenyl residue. On the other hand, when the distal nitrogen was attached to aliphatic group containing compounds, the chemical shift value of piperazine protons was observed as a triplet in the region of ~$\delta$ 3.80, 3.25, 2.45, and 2.30 (two protons in each signal). The signals at ~$\delta$ 2.45 and 2.30 corresponded to piperazine protons vicinal to the distal nitrogen. The low chemical shift values of these protons were due to the positive inductive effect of the alkyl residue present on the distal nitrogen. The splitting patterns of the piperazine protons were greatly altered when the distal nitrogen was protonated, i.e., in hydrochloride salt form, e.g.: NMR spectra of compounds **6g** and **6i** as hydrochloride salts showed piperazine protons in the region between ~$\delta$ 4.80 and 2.00. The salt proton of the synthesized compounds showed a broad singlet signal at ~$\delta$ 13.00.

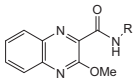
## Pharmacology and preliminary SAR of 3-methoxyquinoxalin-2-carboxamides

All the synthesized molecules showed 5-$HT_3$ receptor antagonistic activity; the pharmacological data are presented in Table 2. *N*-Phenylpiperazine was coupled with 3-methoxyquinoxalin-2-carboxylic acid to give product **6a** which displayed stronger antagonism with a p$A_2$ value of 7.2, greater than the standard drug, ondansetron (p$A_2$: 6.9). In order to get more potent compounds several modifications were carried out; initially a methoxy substituent was introduced on the 3-position of the phenyl ring of piperazine; the resultant compound exhibited antagonism equal to ondansetron, but lesser than **6a** (p$A_2$: 6.9). In contrast, the corresponding regioisomer of **6b**, i.e., amide **6c** bearing methoxy substituent at 4-position of phenyl ring of piperazine, markedly lost its antagonistic potential (p$A_2$: 5.3). Next, methoxy group was replaced with an electron withdrawing substituent, a chlorine atom, which lead to the formation of potent carboxamides **6d**

**Table 2.** Physical constants and pharmacological data of 3-methoxyquinoxalin-2-carboxamides




**6a–6j**




**6k–6o**

| Compound | R | % Yield[a)] | mp (°C) | log *P*-value[b)] | Antagonism to 2-Me-5-HT (p$A_2$)[c)] |
|---|---|---|---|---|---|
| **6a** | $C_6H_5$– | 80 | 160–162 | 3.43 | 7.2 |
| **6b** | *o*-MeO–$C_6H_4$– | 82 | 136–140 | 3.30 | 6.9 |
| **6c** | *p*-MeO–$C_6H_4$– | 84 | 130–132 | 3.30 | 5.3 |
| **6d** | *p*-Cl–$C_6H_4$– | 82 | 186–188 | 3.98 | 6.7 |
| **6e** | *m*-Cl–$C_6H_4$– | 86 | 96–98 | 3.98 | 7.0 |
| **6f** | *m*-$CF_3$–$C_6H_4$– | 76 | 96–100 | 4.35 | 7.5 |
| **6g** | $C_6H_5$–$CH_2$– | 84 | 198–200[d)] | 3.08 | 7.5 |
| **6h** | $CH_3$– | 78 | 84–86 | 1.35 | 6.4 |
| **6i** | $CH_3$–$CH_2$– | 78 | 208–210[d)] | 1.69 | 5.9 |
| **6j** | $CH_2$=CH–$CH_2$– | 74 | semisolid | 2.04 | 4.1 |
| **6k** | $(Me)_2N$–$CH_2$–$CH_2$– | 69 | semisolid | 1.36 | 6.1 |
| **6l** | $(Et)_2N$–$CH_2$–$CH_2$– | 72 | semisolid | 2.04 | 6.4 |
| **6m** | 2-(Indol-3-yl)ethyl– | 92 | 208–210 | 2.91 | 5.8 |
| **6n** | *p*-$N(Et)_2$–$C_6H_4$– | 85 | 108–110 | 3.98 | 7.0 |
| **6o** | 3-Pyridyl– | 89 | 176–178 | 2.40 | 7.2 |
| Ondansetron | – | – | – | – | 6.9 |

[a)] Yields refers to isolated pure product.
[b)] Log *P*-values were calculated using ChemBioDraw Ultra 11 (Cambridge Software).
[c)] p$A_2$ values are the means of two separate experiments. SE was less than 10% of the mean.
[d)] Melting points were recorded as hydrochloride salt and are uncorrected.

($pA_2$: 6.7). Changing the position of the chlorine atom from 4-position to 3-position was well tolerated and the resulting compound **6e** displayed good antagonism ($pA_2$: 7.0). Changing chlorine atom with trifluromethyl group, another electron withdrawing substituent, increased the hydrophobicity and potency of the resultant compound **6f** ($pA_2$ value of 7.5). Incorporation of a methylene group between the distal nitrogen and the phenyl group of piperazine furnished another potent compound **6g** ($pA_2$: 7.5).

Next, modification was accomplished by replacing aryl residue (phenyl) with aliphatic moiety (methyl) on the distal nitrogen of piperazine, which resulted in amide **6h** with moderate antagonism ($pA_2$: 6.4). Higher homologation, i.e., methylpiperazine into ethylpiperazine, afforded a mild antagonist **6i** with $pA_2$ value of 5.9; further increment in hydrophobicity by increasing the chain length led to the formation of the least active compound **6j** ($pA_2$: 4.1). These results indicated that the presence of a phenyl group is necessary to display better 5-$HT_3$ receptor antagonism than the alkyl group alone on the piperazine moiety, with an exception of compound **6c**.

Finally, various primary amines, viz. *N*,*N*-dimethylethylenediamine, *N*,*N*-diethylethylenediamine, tryptamine, *N*,*N*-diethyl-*p*-phenylenediamine, and 3-aminopyridine, were coupled with 3-methoxyquinoxalin-2-carboxylic acid to structurally mimic the role of piperazine. The *N*,*N*-dimethylethylenediamine-based carboxamide **6k** showed moderate antagonism ($pA_2$: 6.1) and slight improvement in the antagonistic profile was observed for its corresponding higher homologue (**6l**, $pA_2$: 6.4). The tryptamine and *p*-phenylenediamine-based carboxamides, **6m** and **6n**, showed moderate ($pA_2$: 5.8) and strong antagonism ($pA_2$: 7.0), respectively. The carboxamide **6o** formed as a result of coupling of 3-aminopyridine with 3-methoxyquinoxalin-2-carboxylic acid showed antagonism ($pA_2$: 7.2) greater than the reference standard drug. The compounds (**6k–o**) with slight deviation in the distances between carbonyl oxygen to basic nitrogen/aromatic to basic nitrogen pharmacophore element showed moderate to strong antagonism. These results indicated that these compounds (**6k–o**) may interact with the receptor in an allosteric site or the interacting sites of the receptor may have the favorable flexibility (conformational changes) toward carbonyl oxygen/basic nitrogen atom, when these molecules approach the 5-$HT_3$ receptors.

## Conclusion

The results of the current study indicated that the chiral center is not necessary for a compound to show strong 5-$HT_3$ receptor antagonism, since several synthesized compounds, **6a** ($pA_2$: 7.2), **6e** ($pA_2$: 7.0), **6f** ($pA_2$: 7.5), **6g** ($pA_2$: 7.5), **6n** $pA_2$: 7.0), and **6o** ($pA_2$: 7.2) which are devoid of a chiral center, showed antagonism greater than the standard 5-$HT_3$ antagonist, ondansetron ($pA_2$: 6.9). So, extensive studies are planned to be carried out further on these molecules to furnish clinically useful 5-$HT_3$ receptor antagonists, which is the future extension of the present work.

## Experimental

### Chemistry

The minimum energy conformations of the designed molecules were generated by ACDLABS-10.0/3D Viewer (CHARMM parameterization). All the chemicals and reagents were obtained from Spectrochem Pvt. Ltd. (India), S. D. Fine Chem Limited (India), and Aldrich (USA). Microwave irradiations were carried out in a CATA-R microwave synthesizer. Reactions were monitored by TLC, which was performed with 0.2 mm Merck pre-coated silica gel 60 $F_{254}$ aluminum sheets. Compounds were detected by UV, iodine chamber, and by dipping the TLC plates in ethanolic solution of ninhydrin and heating. Melting points (uncorrected) were determined on a Buchi 530 melting point apparatus. IR spectra ($\nu_{max}$ in $cm^{-1}$) were recorded on a Jasco IR Report-100 infrared spectrophotometer or a Shimadzu IR Prestige-21 FT-IR spectrophotometer, $^1H$ NMR spectra were recorded at 400 MHz on a Bruker Avance-II, FT NMR spectrometer using TMS as internal standard (chemical shifts in $\delta$, ppm), elemental analysis (C, H, N) on a CHNS-O analyzer (Flash EA 1112).

#### *Synthesis of ethyl 3-oxo-3,4-dihydroquinoxalin-2-carboxylate (**2**)*

*o*-Phenylenediamine (20 g, 0.184 mol) was placed in a 1 L three-neck round bottom flask equipped with a mechanical stirrer and a thermocouple. Ethanol (200 mL) was added to the reactor, and the mixture was stirred for 30 min. To the above reaction mass, diethyl ketomalonate (33.90 g, 0.194 mol) was added in portions over 30 min and the mixture was refluxed for 6 h. Solvent was removed under vacuum; the obtained residue was recrystallized from ethanol to afford the desired compound **2** in 60% yield. mp: 164–167°C; IR (KBr, $cm^{-1}$): 3440 (O–H lactim OH), 3040 (aromatic C–H str.), 1750 (ester C=O str.), 1660 (lactam C=O str.), 1570, 1490 (C=C, C=N ring str.), 1465 ($CH_2$ bend), 1375 ($CH_3$ bend), 1300, 1100 (C–O str.); $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ 12.95 (s, 1H, *NH*), 7.93 (m, 1H, quinoxaline), 7.61 (m, 1H, quinoxaline), 7.47 (m, 1H, quinoxaline), 7.39 (m, 1H, quinoxaline), 4.54 (q, 2H, $OCH_2CH_3$), 1.46 (t, 3H, $OCH_2CH_3$).

#### *Synthesis of ethyl 3-chloro-3,4-dihydroquinoxalin-2-carboxylate (**3**)*

Ethyl 3-oxo-3,4-dihydroquinoxalin-2-carboxylate (10 g, 0.045 mol) was stirred with phosphorous oxychloride (32.20 g, 1.072 mol) in a 1 L three-necked flask equipped with a mechanical stirrer, reflux condenser, and a thermocouple, at ice bath temperature; 10 drops of dimethylformamide were added and the reaction mass was refluxed for 30 min. Resultant mixture was diluted with ethyl acetate and washed with aqueous sodium hydroxide (2 × 100 mL), saturated sodium chloride solution (2 × 75 mL), and dried over anhydrous sodium sulfate. The obtained product was then evaporated under reduced pressure to afford the desired compound **3** in 70–80% yield. mp: 34–36°C; IR (KBr, $cm^{-1}$): 3040 (aromatic C–H str.), 2950, 2890 (aliphatic C–H str.)

1740 (ester C=O str.) 1570, 1490 (C=C, C=N ring str.), 1465 ($CH_2$ bending), 1375 ($CH_3$ bending) 1300, 1100 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.09–8.07 (m, 1H, quinoxaline), 7.97–7.94 (m, 1H, quinoxaline), 7.81–7.72 (m, 2H, quinoxaline), 4.49 (q, 2H, $OCH_2CH_3$), 1.40 (t, 3H, $OCH_2CH_3$).

### *Synthesis of 3-chloroquinoxalin-2-carboxylic acid (**4**)*

Ethyl 3-chloro-3,4-dihydroquinoxalin-2-carboxylate (10 g, 0.042 mol) was dissolved in aqueous methanol (80%, 285 mL). To this solution, sodium carbonate (4.4 g, 0.042 mol) was added and the mixture was refluxed for 6 h. Ethanol was removed under vacuum. The resultant solution was acidified with 2 N HCl and the obtained solids were filtered, washed with water, and dried in an oven overnight at 45°C. mp: 146–150°C; $^1$H NMR ($CDCl_3$) $\delta$: 8.21 (dd, 1H, quinoxaline), 8.15 (dd, 1H, quinoxaline), 8.02–7.98 (m, 1H, quinoxaline), 7.95–7.91 (m, 1H, quinoxaline); IR (KBr, cm$^{-1}$): 3460 (broad O–H str.COOH), 1745 (C=O str.), 1590, 1490 (C=C, C=N ring str.), 1300, 1100 (C–O str.). Mass spectra of the compound showed molecular ion peak at *m*/*z* 207 $(M-1)^+$, 208 $(M)^+$, 209 $(M+1)^+$.

### *Synthesis of 3-methoxyquinoxalin-2-carboxylic acid (**5**)*

To a suspension of 3-chloroquinoxalin-2-carboxylic acid (5 g, 0.023 mol) in methanol, excess of sodium methoxide (2.8 g, 0.121 mol of sodium metal in methanol) was added and refluxed for 6 min under microwave conditions (420 Watt). Methanol was removed under vacuum; the resultant residue was diluted with water and then acidified with 2 N HCl. The obtained solids were filtered, washed, and dried to afford the product **5** in quantitative yield. mp: 115–119°C; FT-IR (KBr, cm$^{-1}$): 3485 (broad O–H str. COOH), 3143 (aromatic C–H str.), 2967, 2856 (aliphatic C–H str.), 1730 (C=O str.), 1570, 1450 (C=C, C=N ring str.), 1225, 1093 (C–O str.); $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.69 (s, 1H, *COOH*), 8.05 (dd, 1H, quinoxaline), 7.91 (dd, 1H, quinoxaline), 7.86–7.82 (m, 1H, quinoxaline), 7.73–7.69 (m, 1H, quinoxaline), 4.08 (s, 3H, $OCH_3$). Mass spectra of the compound showed molecular ion peak at *m*/*z* 203 $(M-1)^+$, 204 $(M)^+$, 205 $(M+1)^+$.

### *General procedure for the synthesis of 3-methoxyquinoxalin-2-carboxamides (**6a–o**)*

To 0.5 g (0.0024 mol) of 3-methoxyquinoxalin-2-carboxylic acid taken in a 100 mL round bottom flask, 2 equivalents (0.92 g, 0.0047 mol) of EDC · HCl was added and stirred with 5 mL of dry THF in an inert atmosphere (nitrogen) at 0°C for 15 min. To the above reaction mixture, 1-hydroxybenzotriazole (0.74 g, 0.0047 mol) was added and stirred for another 45 min. To the above mixture, 1 equivalent of appropriate amines was added and stirring continued for 6 h. The reaction mixture was concentrated under reduced pressure, the resultant mass was diluted with dichloromethane or ethyl acetate, washed with aqueous sodium bicarbonate (2 × 50 mL), saturated sodium chloride solution (2 × 50 mL), and dried over anhydrous sodium sulfate, which were then evaporated under reduced pressure to afford the desired compounds.

### *(3-Methoxyquinoxalin-2-yl)(4-phenylpiperazin-1-yl)-methanone (**6a**)*

Yield: 80%; mp: 160–162°C; FT-IR (KBr, cm$^{-1}$): 3052, 3003, 2991, 2945, 2856 (C–H str.), 1643 (C=O str.), 1598, 1485 (C=C, C=N ring str.), 1149, 1053 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.03 (dd, 1H, quinoxaline), 7.88 (dd, 1H, quinoxaline), 7.74–7.70 (m, 1H, quinoxaline), 7.62–7.58 (m, 1H, quinoxaline), 7.30–7.26 (m, 2H, phenyl), 6.92 (q, 3H, phenyl), 4.13 (s, 3H, $OCH_3$), 4.03 (t, 2H, piperazine), 3.46 (t, 2H, piperazine), 3.33 (t, 2H, piperazine), 3.17 (t, 2H, piperazine).

### *(4-(2-Methoxyphenyl)piperazin-1-yl)-(3-methoxyquinoxalin-2-yl)methanone (**6b**)*

Yield: 82%; mp: 136–140°C; FT-IR (KBr, cm$^{-1}$): 3061, 2922, 2852, 2833 (C–H str.), 1643 (C=O str.), 1151, 1091 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.04 (d, 1H, quinoxaline), 7.88 (d, 1H, quinoxaline), 7.72 (t, 1H, quinoxaline), 7.60 (t, 1H, quinoxaline), 7.06 (quin, 1H, phenyl), 6.94 (d, 2H, phenyl), 6.89 (d, 1H, phenyl), 4.15 (s, 3H, $OCH_3$), 4.07 (s, 2H, piperazine), 3.87 (s, 3H, $OCH_3$), 3.49 (s, 2H, piperazine), 3.21 (s, 2H, piperazine), 3.06 (s, 2H, piperazine).

### *(4-(4-Methoxyphenyl)piperazin-1-yl)-(3-methoxyquinoxalin-2-yl)methanone (**6c**)*

Yield: 84%; mp: 130–132°C; FT-IR (KBr, cm$^{-1}$): 3043, 3004, 2956, 2906, 2833 (C–H str.), 1642 (C=O str.), 1594, 1473 (C=C, C=N ring str.), 1372 ($CH_2$ bend), 1249, 1018 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.96 (d, 1H, quinoxaline), 7.81 (d, 1H, quinoxaline), 7.65 (quin, 1H, quinoxaline), 7.53 (quin, 1H, quinoxaline), 6.85 (d, 2H, phenyl), 6.78 (d, 2H, phenyl), 4.07 (s, 3H, $OCH_3$), 3.98 (t, 2H, piperazine), 3.70 (s, 3H, $OCH_3$), 3.39 (t, 2H, piperazine), 3.14 (t, 2H, piperazine), 2.98 (t, 2H, piperazine).

### *(4-(4-Chlorophenyl)piperazin-1-yl)-(3-methoxyquinoxalin-2-yl)methanone (**6d**)*

Yield: 82%; mp: 186–188°C; FT-IR (KBr, cm$^{-1}$): 3046, 3000, 2956, 2835 (C–H str.), 1639 (C=O str.), 1573, 1496 (C=C, C=N ring str.), 1477, 1388 ($CH_3$, $CH_2$ bend), 1020 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.03 (dd, 1H, quinoxaline), 7.88 (dd, 1H, quinoxaline), 7.73 (td, 1H, quinoxaline), 7.61 (quin, 1H, quinoxaline), 7.23 (d, 2H, phenyl), 6.85 (d, 2H, phenyl), 4.14 (s, 3H, $OCH_3$), 4.03 (t, 2H, piperazine), 3.46 (t, 2H, piperazine), 3.30 (t, 2H, piperazine), 3.14 (t, 2H, piperazine).

### *4-(3-Chlorophenyl)piperazin-1-yl)-(3-methoxyquinoxalin-2-yl)methanone (**6e**)*

Yield: 86%; mp: 96–98°C; FT-IR (KBr, cm$^{-1}$): 3061, 2999, 2960, 2912, 2827 (C–H str.), 1643 (C=O str.), 1595 (C=C, C=N ring str.), 1435, 1390 ($CH_3$, $CH_2$ bend); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.95 (dd, 1H, quinoxaline), 7.81 (dd, 1H, quinoxaline), 7.65 (sep, 1H, quinoxaline), 7.53 (sep, 1H, quinoxaline), 7.11 (t, 1H, phenyl), 6.81–6.78 (m, 2H, phenyl), 6.73–6.71 (m, 1H, phenyl), 4.06 (s, 3H, $OCH_3$), 3.94 (t, 2H, piperazine), 3.38 (t, 2H, piperazine), 3.26 (t, 2H, piperazine), 3.10 (t, 2H, piperazine).

### *(3-Methoxyquinoxalin-2-yl)(4-(3-(trifluoromethyl)-phenyl)piperazin-1-yl)methanone (**6f**)*

Yield: 76%; mp: 96–100°C; FT-IR (KBr, cm$^{-1}$): 3062, 3035, 2958, 2912, 2833 (C–H str.), 1643 (C=O str.), 1610, 1477 (C=C, C=N ring str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.03 (dd, 1H, quinoxaline), 7.90 (dd, 1H, quinoxaline), 7.74 (td, 1H, quinoxaline), 7.61 (sep, 1H, quinoxaline), 7.38 (t, 1H, phenyl), 7.13 (t, 2H, phenyl), 7.09 (t, 1H, phenyl), 4.14 (s, 3H, $OCH_3$), 4.05 (t, 2H, piperazine), 3.49 (t, 2H, piperazine), 3.39 (t, 2H, piperazine), 3.23 (t, 2H, piperazine). Anal. Calcd. for $C_{21}H_{19}F_3N_4O_2$: C, 60.57; H, 4.60; N, 13.46; O, 7.68. Found: C, 60.59; H, 4.59; N, 13.49.

### *(4-Benzylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)-methanone hydrochloride (**6g**)*

Yield: 84%; mp: 198–200°C; FT-IR (KBr, cm$^{-1}$): 2981, 2701, 2601, 2551 (C–H str.), 1651 (C=O str.), 1579, 1469 (C=C, C=N ring str.), 1336, 1284, 1222, 1157 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 13.34 (s, 1H, salt proton), 7.87 (d, 1H, quinoxaline), 7.80 (d, 1H, quinoxaline), 7.67 (t, 1H, aromatic), 7.55 (q, 3H, aromatic), 7.39 (s, 3H, aromatic), 4.80 (s, 1H, aliphatic), 4.19 (s, 3H, $OCH_3$), 4.02 (s, 3H, aliphatic), 3.81 (d, 1H, aliphatic), 3.40 (t, 3H, aliphatic), 2.91 (s, 2H, aliphatic).

### *(4-Methylpiperazin-1-yl)(3-methoxyquinoxalin2-yl)-methanone (**6h**)*

Yield: 78%; mp: 84–86°C; IR ($CCl_4$, cm$^{-1}$): 3000, 2950, 2840 (C–H str.), 1640 (C=O str.), 1560 (C=C, C=N ring str.), 1450, 1360 ($CH_3$, $CH_2$ bend), 1320, 1250, 1020 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.94 (dd, 1H, quinoxaline), 7.79 (dd, 1H, quinoxaline), 7.63 (sep, 1H, quinoxaline), 7.51 (sep, 1H, quinoxaline), 4.05 (s, 3H, O$CH_3$), 3.81 (t, 2H, piperazine), 3.24 (t, 2H, piperazine), 2.47 (t, 2H, piperazine), 2.31 (t, 2H, piperazine).

### *(4-Ethylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)-methanone hydrochloride (**6i**)*

Yield: 78%; mp: 208–210°C; FT-IR (KBr, cm$^{-1}$): 3020, 3000, 2956, 2922, 2661, 2503, 2348 (C–H str.), 1651 (C=O str.), 1573, 1479 (C–C, C=N ring str.), 1390 ($CH_2$ bend), 1215, 1128, 1091 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 12.95 (s, 1H, salt proton), 7.89 (dd, 1H, quinoxaline), 7.82 (dd, 1H, quinoxaline), 7.68 (sep, 1H, quinoxaline), 7.54 (sep, 1H, quinoxaline), 4.84 (s, 1H, piperazine), 4.06 (s, 3H, O$CH_3$, 1H, piperazine), 3.77–3.27 (m, 4H, piperazine), 3.11 (q, 2H, $CH_2CH_3$), 2.92 (s, 2H, piperazine), 1.44 (t, 3H, $CH_2CH_3$).

### *(4-Allylpiperazin-1-yl)(3-methoxyquinoxalin-2-yl)-methanone (**6j**)*

Semisolid, hygroscopic; yield: 74%; IR ($CCl_4$, cm$^{-1}$): 3050, 3000, 2950, 2925, 2860 (C–H str.), 1650 (C=O str.), 1580, 1470 (C=C, C=N ring str.), 1360 ($CH_2$ bend), 1220 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.94 (dd, 1H, quinoxaline), 7.79 (dd, 1H, quinoxaline), 7.64 (sep, 1H, quinoxaline), 7.52 (sep, 1H, quinoxaline), 5.80–5.72 (m, 1H, olefinic), 5.12 (sep, 2H, olefinic), 4.06 (s, 3H, O$CH_3$), 3.81 (t, 2H, piperazine), 3.24 (t, 2H, piperazine), 2.96 (d, 2H, methylene), 2.52 (t, 2H, piperazine), 2.36 (t, 2H, piperazine).

### *N-(2-(Dimethylamino)ethyl)-3-methoxyquinoxalin-2-carboxamide (**6k**)*

Semisolid, hygroscopic; yield: 69%; IR ($CCl_4$, cm$^{-1}$): 3241 (sharp N–H str.), 2925, 2850 (C–H str.), 1640 (C=O str.), 1460, 1320 ($CH_3$, $CH_2$ bend), 1320, 1060 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.12 (s, 1H, amide), 8.08 (dd, 1H, quinoxaline), 7.87 (dd, 1H, quinoxaline), 7.76–7.72 (m, 1H, quinoxaline), 7.63–7.59 (m, 1H, quinoxaline), 4.20 (s, 3H, O$CH_3$), 3.67 (q, 2H, NH$CH_2$CH$_2$NMe$_2$), 2.72 (t, 2H, NHCH$_2$$CH_2$NMe$_2$), 2.41 (s, 6H, NHCH$_2$CH$_2$N$Me_2$).

### *N-(2-(Diethylamino)ethyl)-3-methoxyquinoxalin-2-carboxamide (**6l**)*

Semisolid, hygroscopic; yield: 72%; IR ($CCl_4$, cm$^{-1}$): 3243 (sharp N–H str.), 3050, 2950, 2925, 2850, 2800 (C–H str.), 1680 (C=O str.), 1565, 1500 (C=C, C=N ring str.), 1460, 1380 ($CH_3$, $CH_2$ bend) 1320, 1060 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.05 (s, 1H, amide), 8.00 (dd, 1H, quinoxaline), 7.80 (dd, 1H, quinoxaline), 7.67 (sep, 1H, quinoxaline), 7.54 (sep,1H, quinoxaline), 4.13 (s, 3H, O$CH_3$), 3.51 (q, NH$CH_2$CH$_2$N(CH$_2$CH$_3$)$_2$), 2.64 (t, 2H, NHCH$_2$$CH_2$N(CH$_2$CH$_3$)$_2$), 2.54 (q, 4H, NHCH$_2$CH$_2$N($CH_2$CH$_3$)$_2$), 1.00 (t, 6H, NHCH$_2$CH$_2$N(CH$_2$$CH_3$)$_2$).

### *N-(2-(1H-Indol-3-yl)ethyl)-3-methoxyquinoxalin-2-carboxamide (**6m**)*

Yield: 92%; mp: 208–210°C; FT-IR (KBr, cm$^{-1}$): 3390 (sharp, N–H str.), 3273 (sharp N–H str.), 3053, 2943, 2922 (C–H str.), 1666 (C=O str.), 1571 (C=C, C=N ring str.), 1440, 1390 ($CH_3$, $CH_2$ bend), 1330, 1226 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.11 (dd, 1H, amide), 7.90 (dd, 1H, quinoxaline), 7.86 (dd, 1H, quinoxaline), 7.80 (s, 1H, *NH*), 7.75–7.70 (m, 2H, aromatic), 7.61–7.57 (m, 2H, aromatic), 7.40 (d, 1H, aromatic), 7.24–7.20 (m, 1H, aromatic), 7.15–7.11 (m, 2H, aromatic), 4.12 (s, 3H, O$CH_3$), 3.88 (q, 2H, NH$CH_2$CH$_2$), 3.16 (t, 2H, NHCH$_2$$CH_2$).

### *N-(4-(Diethylamino)phenyl)-3-methoxyquinoxalin-2-carboxamide hydrochloride (**6n**)*

Yield: 85%; mp: 108–110°C; FT-IR (KBr, cm$^{-1}$) (free base): 3251 (sharp N–H str.), 3062, 3000, 2924, 2854 (C–H str.), 1681 (C=O str.), 1542 (N–H bend), 1471, 1361 ($CH_3$, $CH_2$ bend), 1320, 1220 (C–O str.); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 13.74 (s, 1H, salt proton), 10.03 (s, 1H, amide), 8.11 (d, 1H, aromatic), 8.04 (d, 2H, aromatic), 7.91 (d, 1H, aromatic), 7.80 (q, 4H, quinoxaline), 7.67 (t, 1H, aromatic), 4.25 (s, 3H, O$CH_3$), 3.72 (t, 2H, N$CH_2$CH$_3$), 3.36 (d, 2H, N$CH_2$CH$_3$), 1.26 (t, 6H, N(CH$_2$$CH_3$)$_2$).

### *3-Methoxy-N-(pyridin-3-yl)quinoxalin-2-carboxamide (**6o**)*

Yield: 89%; mp: 176–178°C; FT-IR (KBr, cm$^{-1}$): 3236 (sharp N–H str.), 3097, 3057, 2951 (C–H str.), 1693 (C=O str.), 1587, 1485, (C=C, C=N ring str.); $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 9.86 (s, 1H, amide), 8.83 (d, 1H, pyridine), 8.47 (dd, 1H, pyridine), 8.45 (dd, 1H, pyridine), 8.08 (dd, 1H, quinoxaline), 7.90 (dd, 1H, quinoxaline), 7.79 (sep, 1H, quinoxaline), 7.65 (sep, 1H, quinoxaline), 7.35 (q, 1H, pyridine).

## Pharmacology

All the animals were obtained from Hissar Agricultural University, Hissar, Haryana, India, and were maintained in colony cages at 23 ± 2°C, relative humidity of 45–55% under a 12-h light/dark cycle, fed with standard animal feed, and water ad libitum. The Institutional Animal Ethics Committee of the Birla Institute of Technology & Science, Pilani, India, approved the experimentation on animals (Protocol No. IAEC/RES/04/01/Rev 01, dated 13.08.08). Compounds were assessed for their serotonin type-3 receptor antagonism in male Dunkin Hartley guinea-pigs (350–400 g).

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

For serotonin type-3 receptor antagonistic activity, guinea-pigs were sacrificed by mild ether anesthesia followed by cervical dislocation. The abdomen was cut open and a length of ileum was excised about 2 cm from the ileo-caecal junction. The longitudinal muscle myenteric plexus (LMMP), 3–4 cm in length, was removed and mounted as per the method described elsewhere [22]. The tissue was equilibrated for 30 min under a resting

tension of 500 mg and constant aeration in a 40 mL organ bath containing Tyrode solution maintained at 37°C.

Non-cumulative concentrations ($10^{-8}$–$10^{-4}$ M) of 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. A fixed 2-methyl-5-HT concentration ($10^{-5}$ M), approximately $ED_{80}$ was used for antagonism studies. To study the antagonist effect of the test compounds on the response evoked by 2-methyl-5-HT, 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). Antagonism was expressed in the form of $pA_2$ values (negative logarithm of antagonist concentration producing a twofold shift of the agonist concentration–activity curve), which were graphically determined [13, 16, 22, 23]. The $pA_2$ values of the test compounds were compared with that of the standard antagonist ondansetron (Natco Pharma, Hyderabad, India).

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