Design, Synthesis and Pharmacological Screening of Potential 5-HT_3 Receptor Antagonists for Co-morbid Disorders Like Depression and Anxiety

Synopsis

Submitted in partial fulfilment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

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BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI

2015

Synopsis:

Depression and anxiety are the most common psychiatric disorders affecting a large section of people in the world; particularly in the subcontinent and western countries. According to World Health Organization's (WHO) prediction, depression would be second leading cause of premature death or disability worldwide by the year 2020. Anxiety also represents one of the most common psychiatric disorders in the world, affecting 1/8 of the total population worldwide. Studies have also indicated that depression and anxiety are highly co-morbid conditions. Individuals suffering from both depression and anxiety disorders concurrently have generally shown greater levels of functional impairment, reduced quality of life, and poorer treatment outcomes as compared to individuals suffering from one disease. Despite the availability of a number of anti-depressant as well as anxiolytic agents, prevalence of these diseases still persists. This may be due to inadequate efficacy of currently available drugs with a number of side-effects and lack of understanding of pathophysiology of these diseases. This prompted the exploration for new anti-depressant and anxiolytic agents. Antagonists for the 5-HT_3 receptor, such as ondansetron, granisetron, tropisetron display antiemetic action in cancer chemo-/radiotherapy induced nausea, vomiting and are in clinical use. Interestingly, 5-HT_3 receptor antagonists have been comprehensively investigated for their neuro-psychopharmacological potential. Numerous preclinical studies have indicated that 5-HT_3 receptors are expressed in the areas of the brain implicated in depression and anxiety disorders. Moreover, several studies also have identified 5-HT_3 receptor antagonists as prospective anti-depressant and anxiolytic agents and endorsed the role of 5-HT_3 receptor in the neurobiology of depression and anxiety. Studies have provided evidence to support the hypothesis that depression and anxiety disorders may be associated with an alteration or dysfunction of the serotonergic system in the CNS. Thus, modulation of the serotonergic system through 5-HT_3 receptor may be implicated as a promising mechanism for the anti-depressant and anxiolytic action. Hence in the present study, attempts were focused on developing novel 5-HT_3 receptor antagonists which would be beneficial in treating co-morbid disorders like depression and anxiety.

New chemical entities (NCEs) were designed using ligand based approach based on two different pharmacophoric templates proposed for 5-HT_3 receptor antagonists. Series of piperazine analogs of 1,8 naphthyridine carboxylic acid (Series 1 NACA 1-15; figure 1) were designed based on the literature reported aryl piperazine template, where the pharmacophore consists of a hetero aromatic ring connected to piperazine via pseudoamidinic bond.

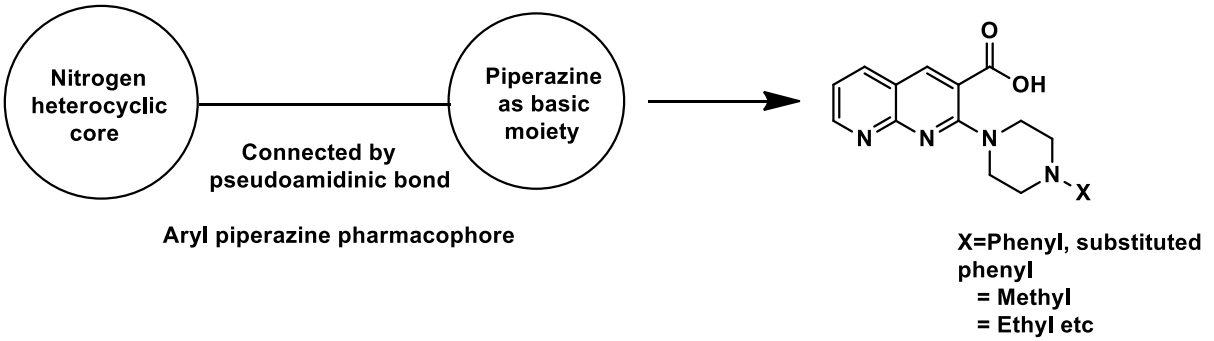


Figure 1: Compounds designed based on aryl piperazine pharmacophore for 5-HT_3 receptor antagonists

Literature survey revealed another pharmacophore (figure 2) for 5-HT_3 receptor antagonists which consists of an aromatic moiety, intervening hydrogen bond acceptor mostly a carbonyl group and basic nitrogen arranged at specific distances. The earlier studies have indicated that nitrogen containing fused six membered aromatic ring (aromatic moiety) may constitute a suitable template in the design of novel 5-HT_3 receptor antagonists. In recent years, several researchers have been engaged in the preparation and screening of compounds based on the above pharmacophoric pattern and 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.

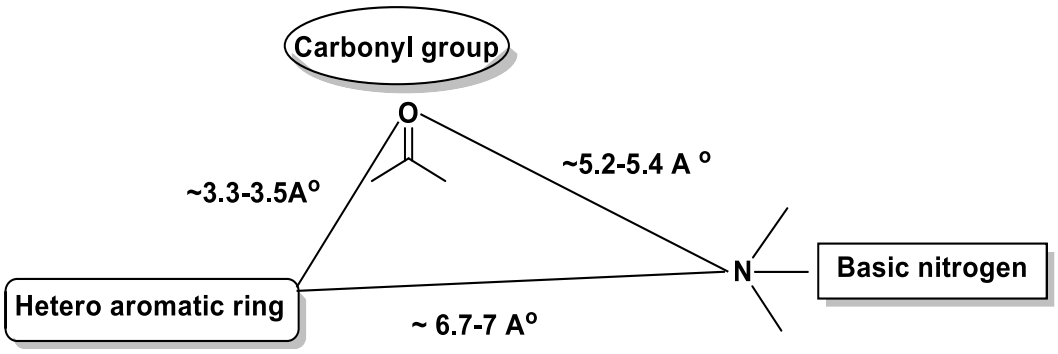


Figure 2: The three point pharmacophore of 5-HT_3 receptor antagonists

Thus, the attention turned toward the nitrogen containing three different heteroaromatic rings such as naphthyridine, quinazoline and indole as aromatic part of the pharmacophore. Hence, in this study it was proposed to attach the 1,8 naphthyridine ring, quinazoline ring and indole rings to piperazines (basic moiety of the pharmacophore) through the carbonyl group of carboxamide linkage (hydrogen bond acceptor moiety) to generate series of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides, piperazine analogs of 2-alkoxy 1,3 quinazoline carboxamides and piperazine analogs of 1 alkyl/benzyl indole carboxamides respectively (figure 3).

Till date, the role of aromatic part and basic moiety within the pharmacophore of the 5-HT_3 receptor antagonists is not completely explored. So, identifying the role of hetero aromatic moiety and basic moiety within the pharmacophore of the 5-HT_3 receptor antagonists, may give an idea for developing a new pharmacophore model for 5-HT_3 receptor antagonists, which may direct the identification of potent 5-HT_3 receptor antagonists that would be beneficial in treating co-morbid disorders like depression and anxiety.

Thus, the objective was to investigate the influence of the aromatic part and basic moiety on activity. Keeping similar hydrogen bond acceptor moiety, variations were made to the heteroaromatic core and distal nitrogen (N^4) of the piperazine moiety with the intention of exploring the structure activity relationship (SAR) associated with such changes. Therefore, three different nitrogen containing fused heterocycle rings were alternately attached to various N^4 substituted piperazines through a common carbonyl group of carboxamide linkage, resulting in the construction of these carboxamides (figure 3).

Average Distances between the aromatic core to carbonyl group, carbonyl group to the piperazine N^4 and aromatic core to piperazine N^4 of 3D optimized structures (at least for each three conformations) of each designed compounds were calculated using CHARMM Parametrization (ACDLABS-10.0/3D Viewer). All the distances were found to be in close proximity of the proposed pharmacophoric distances. Three point pharmacophore was generated with reference to standard 5-HT_3 receptor antagonists, ‘setrons’ such as granisetron, ondansetron etc (ten ‘setrons’) using Discovery Studio (Accelrys) version 3.5. The Pharmacophore from each series of designed carboxamides were superimposed over the pharmacophore of setons, in order to find the best fit among compounds. All the designed compounds were found to have good fit values. In silico molecular properties of each compound were calculated to determine their ‘drug-likeness’. All the values were found to be within the acceptable range.

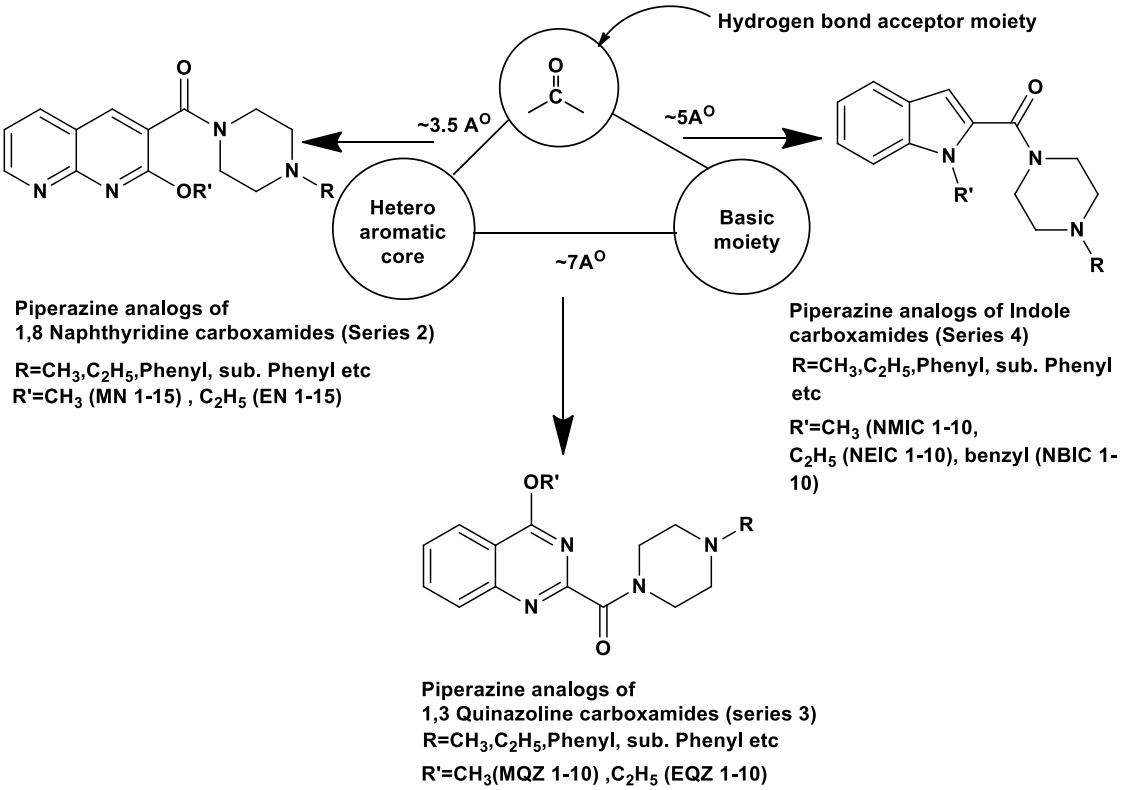


Figure 3: Compounds designed based on three point pharmacophore model for 5-HT_3 receptor antagonists.

All the designed compounds were synthesized via appropriate synthetic route. In the synthetic scheme leading to desired piperazine analogs of 1,8 naphthyridine carboxylic acids (Scheme I; NACA 1-15; series1) and 1,8 naphthyridine-3-carboxamides (Scheme I; MN1-15, EN1-15; Series 2) nicotinamide and ammonium sulphamate were heated under solvent free condition to generate pyrido-pyrimidine derivative (2). The pyirido-pyrimidine derivative was reacted with dilute hydrochloric acid to obtain 2-aminonicotinaldehyde (3). A mixture of 2-aminonicotinaldehyde, diethylmalonate, and drops of piperidine in alcohol was refluxed to obtain dihydronaphthyridinone ester derivative (4). The dihydronaphthyridinone ester derivative was further refluxed with phosphorous oxychloride in presence of catalytic amount of DMF to generate the common intermediate, 1,8 naphthyridine 2-chloro derivative (5). The chloro derivative was subjected to nucleophilic substitution reaction with various substituted piperazines to obtain (6 a-o) followed by hydrolysis to obtain the title piperazine analogs of 1,8 naphthyridine carboxylic acids (NACA1-15; series 1; figure 1). Whereas, the 1,8 naphthyridine 2-chloro derivative was further reacted with sodium alkoxide in alcohol (sodium ethoxide/ethanol for ethoxy derivative, EN1-15; sodium methoxide/methanol for methoxy derivative, MN 1-15 series 2) to obtain the corresponding 2-alkoxy1,8 naphthyridine 3-ethyl ester (6).

SCHEME-I: Synthetic routes of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids (NACA 1-15; Series 1) and (2-alkoxy-1, 8-naphthyridin-3-yl) (4-substituted piperazin-1-yl) methanones (MN1-15, EN 1-15; Series 2)

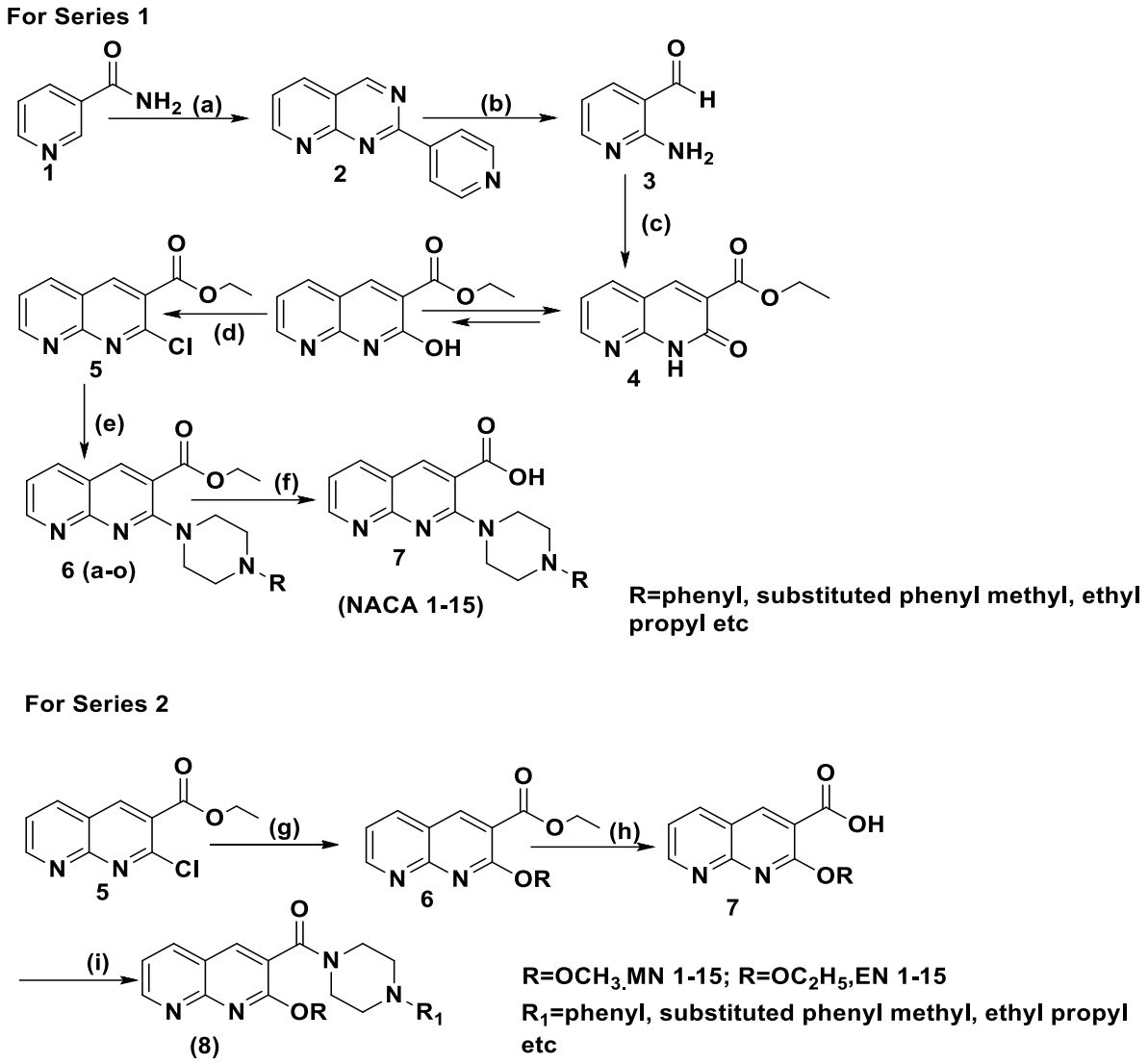
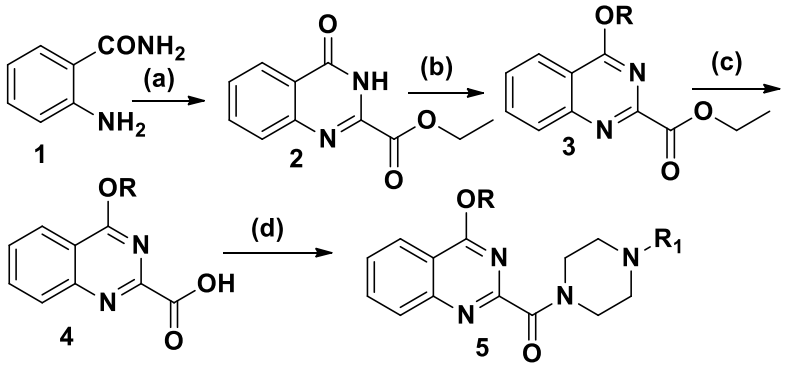


Figure 4: Scheme I: Reagents and conditions: (a) ammonium sulphamate, neat, 200^{o}C, 20-24h, 64%; (b) 4N HCl, 100˚C, 1-2h, 38%; (c) Diethyl malonate, EtOH, reflux, 4-5 h, 60%; (d) $POCl_3$, cat. DMF, 1-2 h, 67%; (e) K_2CO_3, acetonitrile, Piperazines, 80^{o}C, 1-2 h (f) 10% aq. NaOH, rt, 1h, aq. citric acid. (g) $NaOCH_3$ (for methoxy naphthyridine analogs) $NaOCH_2CH_3$ (for ethoxy naphthyridine analogs), EtOH, rt, 1h, 68% (h) 10% aq.NaOH, r.t, 1h, dil.HCl, 70% (i) EDC.HCl, HOBt, 0^{o}C-rt, 1h, piperazines,4h.

Scheme II: Synthetic route to (4-substituted piperazine-1-yl) (4-alkylquinazoline-2-yl) methanones (MQZ 1-10; EQZ1-10; Series 3):

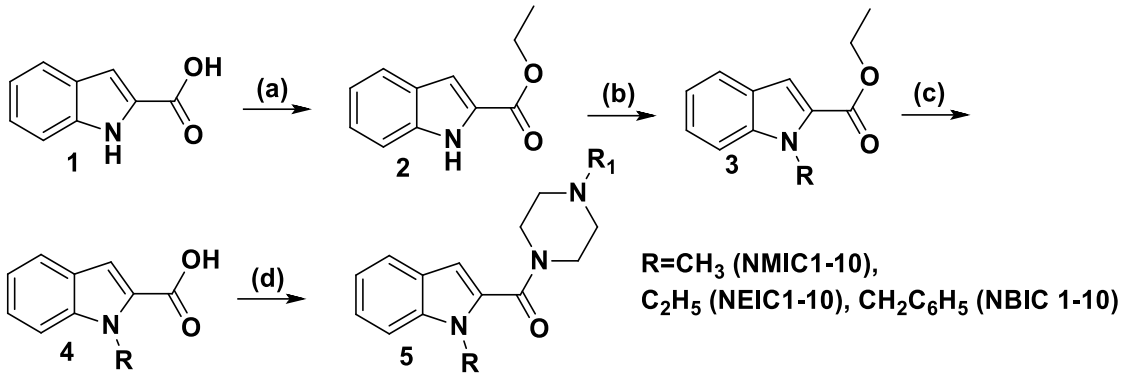


(MQZ 1-10; EQZ 1-10)

R= CH_3 (MQZ1-10), C_2H_5 (EQZ1-10) **R_1**=Ph, sub. Phenyl, methyl, ethyl, propyl, etc.

Figure 5: Scheme III:Reagents & conditions: (a) diethyl oxalate, sodium ethoxide, ethanol, reflux, 3h, 84% (b) RI, K_2CO_3, DMF,1.5h, 0°C 68% (c) LiOH, THF: water (3:1), 30-45 min, 60%. (d) Oxalyl chloride, DCM, 0°C-rt, Et_3N, piperazines, rt, 1h.

Scheme III: Synthetic route to (1-alkyl/benzyl-1H-indol-2-yl)(4-substituted piperazin-1-yl) methanone derivatives (NMIC 1-10; NEIC 1-10; NEIC 1-10; Series 4):



R_1=Ph, sub. Phenyl, methyl, ethyl, propyl, etc.

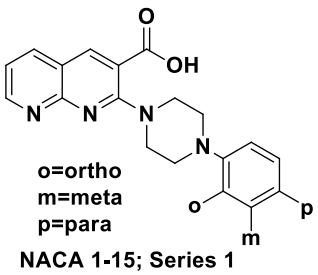
Figure 6: Scheme III: Reagents and conditions: (a) ethanol, cat H_2SO_4 reflux, 4-6h, 80% (b) RI, KOH pellets, DMSO, rt, 1h, 88% (c) 10% aq.KOH, reflux, 1h, dil.HCl,73% (d) EDC.HCl, HOBt, 0°C-rt, 1h, piperazines, 1h.

The 2-alkoxy 1,8 naphthyridine 3-ethyl ester derivative was hydrolyzed to get the corresponding acid derivative (7). The acid was subjected to EDC.HCl/HOBt coupling reaction with various piperazines to obtain the title 2-alkoxy 1,8 naphthyridine carboxamides (8) (MN 1-15, EN 1-15; series 2).

The starting material of the synthetic route to piperazine analogs of quinzoline carboxamides (Scheme II, MQZ 1-10, EQZ 1-10; series 3) was anthranilamide (1) which was reacted with sodium ethoxide in ethanol to obtain the quinazolinone ester derivative (2). The quinazolinone ester derivative was reacted with methyl iodide in DMF and ethyl iodide in DMF to obtain the methoxy quinazoline and ethoxy quinazoline derivative (3) respectively. The alkoxy quinazoline ester derivative was hydrolyzed to obtain the acid (4). The acid was subjected to EDC.HCl/HOBt coupling with various substituted piperazines to obtain the title quinazoline carboxamides (5).

In the synthetic scheme of piperazine analogs of indole-2-carboxamide (Scheme III, NMIC 1-10, NEIC 1-10, NBIC 1-10, series 4) the starting material indole-2-carboxylic acid was esterified using conc. Sulphuric acid and ethanol. The ester derivative (2) was N-alkylated/benzylated using alkyl iodide/benzyl bromide in NaOH/DMF. The N-alkylated/benzylated derivative (3) was hydrolyzed to obtain the corresponding acid derivative (4). The acid was subjected to EDC.HCl/HOBt coupling with various substituted piperazines to obtain the title indole carboxamides (5).

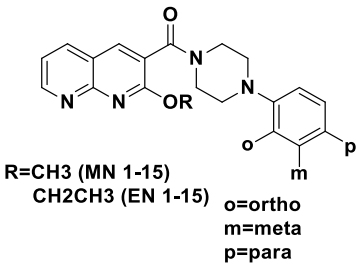
The compounds were purified using column chromatography and (or) recrystalization and characterized using spectral analysis (IR, NMR, MS). 5-HT_3 receptor antagonistic potential of all the compounds were determined in longitudinal muscle myenteric plexus preparation from guinea pig ileum against standard 5-HT_3 agonist, 2-methyl 5-HT. 5-HT_3 receptor antagonism was expressed in the form of pA_2 values, which was determined according to literature reported methods.



NACA 1-15; Series 1

In case of piperazine analogs of 1,8 naphthyridine carboxylic acids (NACA 1-15 series 1), compounds NACA 1(pA_2 7.6), NACA 4 (pA_2 7.2), NACA 6 (pA_2 7.3) and NACA 10 (pA_2 7.0) disclosed better 5-HT_3 receptor antagonistic activity than ondansetron (pA_2 6.6) and compounds NACA 2 (pA_2 6.7), NACA 3 (pA_2 6.5), NACA 7 (pA_2 6.0), and NACA 8 (pA_2 6.6) showed moderate antagonism as compared to ondansetron (pA_2 6.6).

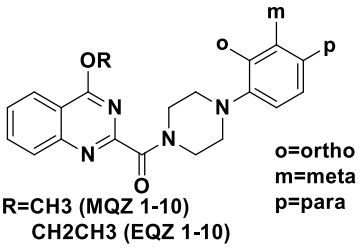
With respect to SAR, the results demonstrated that compounds bearing unsubstituted phenyl ring, OCH_3 group o and *p* position and Cl group at *m* position of the phenyl ring of N^4 piperazine showed better activity than compounds with all other substituents at the N^4.



Piperazine analogs of 1,8 naphthyridine carboxamides (Series 2)

In case of piperazine analogs of 1,8 naphthyridine carboxamides (MN 1-15; EN 1-15 series 2), compounds MN 5 (pA_2: 7.33), MN 7 (pA_2: 7.67), MN 8 (pA_2: 7.33) and EN 8 (pA_2: 7.30) showed better 5-HT_3 receptor antagonistic activity and compounds MN 1 (pA_2: 6.67), MN 4 (pA_2: 6.11), MN 6 (pA_2: 6.30), EN 5 (pA_2: 6.20), EN 7 (pA_2: 6.60) and EN 10 (pA_2: 6.60) exhibited moderate antagonistic activity as compared to ondansetron (pA_2: 6.25).

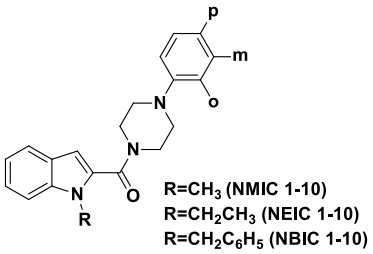
With respect to SAR, the results demonstrated that compounds bearing OCH_3 group (particularly at *o* and *m* position) and Cl at *m* position of the phenyl ring of N^4 piperazine showed good activity.



Piperazine analogs of 1,3 quinazoline carboxamides (Series 3)

Among the piperazine analogs of 1,3 quinazoline carboxamides (MQZ 1-10, EQZ 1-10; Series 3) compounds MQZ 6 (pA_2: 6.70), EQZ 6 (pA_2: 6.75) showed better antagonistic activity and compounds MQZ 4 (pA_2: 6.10), MQZ 5 (pA_2: 6.30), EQZ 4 (pA_2: 6.30) and EQZ 5 (pA_2: 6.00) showed moderate activity as compared to ondansetron (pA_2: 6.60).

With respect to SAR, the results demonstrated that compounds bearing OCH_3 group at *m* position) and Cl group at *p* position at the phenyl ring of N^4 piperazine showed better activity than compounds with all other substituents at the N^4.



Piperazine analogs of indole carboxamides (Series 4)

In case of piperazine analogs of 1-alkyl/benzyl indole carboxamides (NMIC, NEIC, NBIC; Series 4), compounds NMIC 6 (pA_2: 6.80), NEIC 6 (pA_2: 7.10) and NBIC 6 (pA_2: 7.50) showed prominent antagonistic activity whereas compounds NMIC 1 (pA_2: 6.25), NMIC 3 (pA_2: 6.60), NMIC 5 (pA_2: 6.60), NEIC 4 (pA_2: 6.50), NEIC 5 (pA_2: 5.50), NBIC 4 (pA_2: 6.30) and NBIC 5 (pA_2: 6.00) showed moderate 5-HT_3 antagonistic activity.

The SAR study indicated that compounds bearing *o*-OCH_3 phenyl group and *m*-OCH_3 phenyl group at the N^4 piperazine showed promising 5-HT_3 receptor antagonistic activity.

In general, analogs that contain alkyl substituents (methyl, ethyl, propyl) at the N^4 position of piperazine (compounds NACA-11 to 15, MN 12-15, EN 12-15, NMIC 8,10, NEIC 8-10, NBIC 8, 9, MQZ 8, 9 and EQZ 8-10), showed lesser 5-HT_3 receptor antagonism.

To ascertain the possible occurrence of drug induced changes (stimulation/suppression) in the locomotor activity of mice, which may contribute to their behavior in forced swim test (FST) and Tail suspension Test (TST) all the compounds were subjected to spontaneous locomotor activity study. The spontaneous locomotor activity in mice was evaluated using the actophotometer.

The compounds which have shown higher pA_2 values were screened for their anti-depressant potential using standard mice model of depression, *i.e.* the FST and the TST. The parameter measured was duration of immobility in seconds for duration of 4 minutes and 6 minutes respectively. Decrease in duration of immobility was considered as anti-depressant like effect.

Compounds NACA 1, NACA 3, NACA 4, NACA 6, NACA 8, NACA 9 and NACA 10 of piperazine analogs of 1,8 naphthyridine carboxylic acids (Series 1) significantly ($P < 0.05$) reduced the duration of immobility in mice as compared to control in FST and TST.

It was found that, analogs with unsubstituted phenyl, *m*-Cl phenyl, *o*-OCH_3 phenyl and *p*- OCH_3 phenyl substituents at the N^4 of piperazine showed promising anti-depressant like activity in FST and TST.

In case of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides (series 2), treatment with compounds MN 1, MN 4 to MN 9 and EN 4 to EN 10 significantly ($P < 0.05$) decreased the duration of immobility in mice as compared to control in FST and TST.

It was found that, compounds with unsubstituted phenyl, *o*-Cl phenyl, *m*-Cl phenyl, *p*-Cl phenyl, *o*-OCH_3 phenyl, *m*-OCH_3 phenyl and *p*-OCH_3 phenyl substituents at the N^4 of piperazine showed promising anti-depressant like activity in FST and TST.

Compounds MQZ 1, MQZ 4, MQZ 5, MQZ 6, EQZ 1, EQZ 4, EQZ 5 and EQZ 6 of piperazine analogs of quinazoline carboxamides (series 3) showed prominent anti-depressant like activity as indicated by significantly ($p < 0.05$) decrease in immobility time compared to the control in FST and TST.

In case of piperazine analogs of indole carboxamides (series 4) treatment with compounds NMIC 5 and NMIC 6, NEIC 4, NEIC 6, NEIC 7, NBIC 1 and NBIC 6 expressed prominent anti-

depressant like activity as indicated by considerable ($p < 0.05$) reduction in immobility time in comparison to the control.

It was observed that, compounds having unsubstituted phenyl, *o*-OCH_3 phenyl, *m*-OCH_3 phenyl, *p*-OCH_3 phenyl and *p*-Cl phenyl substituents at the N^4 of piperazine showed distinct 5-HT_3 receptor antagonism and promising anti-depressant like activity in FST and TST.

The compounds which exhibited higher pA_2 values and significant anti-depressant activity were selected for the anxiolytic study using L/D test, EPM test and OFT. In L/D Test Parameters measured were latency to enter into the light chamber, No of transitions, Time spent in light chamber. In EPM Test parameters measured were no of open arm entries, time spent in open arm. In OFT Test parameters measured ambulation scores and rearing number. Increases in all these parameters indicate anxiolytic activity.

In case of piperazine analogs of naphthyridine carboxylic acids (series 1), it was observed that, analog that contain unsubstituted N^4 phenyl ring (compound NACA 1) and analogs that contain electron withdrawing substituents, such as *p*-Cl (compound NACA 6) and electron releasing substituent such as *o*-OCH_3 (compound NACA 8) groups at the N^4 phenyl ring, induced anxiolytic like activity in the L/D test, EPM test and OFT.

Piperazine analogs of 1,8 naphthyridine carboxamides (series 2), it was observed that, analogs that contain unsubstituted N^4 phenyl ring (compound MN 1) and analogs that contain electron releasing substituents at the N^4 phenyl ring, such as o-OCH_3 (compound MN 7) and m-OCH_3 (compound MN 8 and EN 8) induced anxiolytic activity in the L/D test, EPM test and OFT.

In case of piperazine analogs of quinazoline carboxamides (series 3), it was observed that, analogs that contain electron withdrawing *p*-Cl substituent (compounds MQZ 4 and EQZ 4) and electron releasing *m*-OCH_3 substituent (compounds MQZ 6, EQZ 6) at the N^4 phenyl ring induced anxiolytic activity in the L/D test, EPM test and OFT.

In case of piperazine analogs of indole carboxamides (series 4), it was found that analogs that contain electron releasing substituent at the N^4 phenyl ring, such as *o*-OCH_3 (compound NMIC 5), *m*-OCH_3 (NMIC 6, NEIC 6 and NBIC 6) compound and *p*-OCH_3 (compound NEIC 7) induced anxiolytic activity in the L/D test, EPM test and OFT It was observed that irrespective of naphthyridine, quinazoline and indole carboxamides, compounds with *m*-OCH_3 phenyl substituent at the N^4 piperazine displayed significant anxiolytic activity in all the three tests.

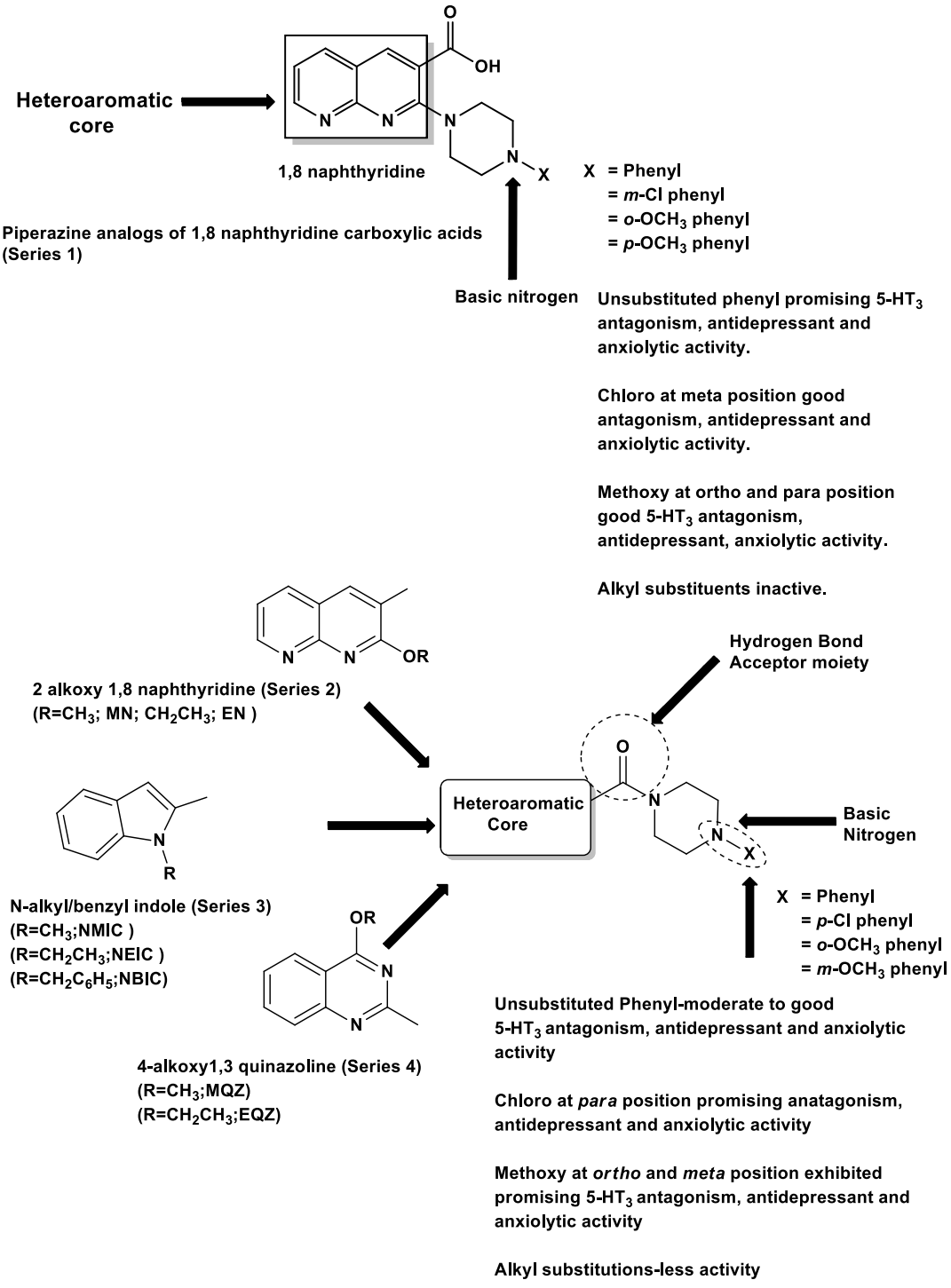


Figure 7: Combined SAR of 5-HT$_3$ anatgonism, antidepressant and anxiolytic activity of piperazine analogs of 1,8 naphthyridine carboxylic acids (Series 1) and piperazine analogs of naphthyridine, quinazoline and indole carboxamides (Series 2, 3 and 4).

In case of all the carboxamides, N^1 of piperazine is within a carboxamide linkage (tertiary amide) and the N^4 of the piperazine is the basic part of the proposed pharmacophore. Basicity of the compound is associated with the basicity of N^4 nitrogen of piperazine ring. The substituents at the N^4 determine the basicity of the N^4 nitrogen. In case of all the synthesized compounds, basicity of the N^4 of piperazine changes with the substituent attached to it. The N^4 nitrogen of the piperazine becomes comparatively more basic when the N^4 substituent is alkyl. The basicity of the N^4 increases because of the positive inductive effect of alkyl substituents. Whereas, basicity of the N^4 nitrogen of the piperazine is comparatively less when it has phenyl and or substituted phenyl substituent. The results indicated that increasing the basicity on the N^4 nitrogen of the piperazine (with alkyl substituents) reduced the of 5-HT_3 receptor antagonism. Hence, it is hypothesized that, extent of basicity of the N^4 of piperazine moiety of the pharmacophore could be an important factor contributing towards the 5-HT_3 receptor antagonistic activity.

The present study enabled us to explore the SAR of the carboxamides with three different hetero aromatic cores in the proposed three point pharmacophore of the 5-HT_3 receptor antagonists. Analysis of SAR indicated that across all the three heteroaromatic carboxamide series, the 5-HT_3 receptor antagonistic activity is more sensitive to changes in the N^4-substituent of piperazine than change in aromatic core. However, few naphthyridine-3-carboxamides showed better activity than the corresponding quinazoline carboxamides as well as indole carboxamides. Whereas, few indole analogs exhibited better activity than both their naphthyridine as well as quinazoline counterparts. Thus, it is likely that an appropriate combination of heteroaromatic core and basic centre (substitution at N^4 nitrogen of piperazine) of the pharmacophore produces potential activity.

With few exceptions, in general, compounds with higher pA_2 values exhibited significant anti-depressant-like effects as compared to the vehicle treated group. Compounds NACA 1, NACA 6, NACA 8 of piperazine analogs of naphthyridine carboxylic acid series, compounds MN 7, MN 8, EN 8 of 1,8 naphthyridine carboxamide series, compounds MQZ 6, and EQZ 6 of quinazoline carboxamide series and compound NMIC 6, NEIC 6 and NBIC 6 of indole carboxamide series displayed higher pA_2 values, significant anti-depressant activity in FST, TST and also showed significant anxiolytic activity in L/D test, EPM test and OFT. Hence, this study indicates the possible beneficial effects of these compounds as putative 5-HT_3 receptor antagonists in co-morbid disorders like depression and anxiety.