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

THESIS

Submitted in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY
by

Arghya Kusum Dhar

Under the Supervision of

Prof. R. Mahesh

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE, PILANI
2015

CERTIFICATE

This is to certify that the thesis entitled **"Design, Synthesis and Pharmacological Screening of Potential 5-HT_3 Receptor Antagonists for Co-morbid Disorders Like Depression and Anxiety**" and submitted by **Arghya Kusum Dhar, ID No. 2009PHXF414P**, for the award of Ph.D. Degree of the Institute, embodies the original work done by him under my supervision.

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Date:

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Dedicated

To

My loving Family

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Acknowledgment

First and foremost, I wish to express my profound sense of admiration to my supervisor **Prof. Radhakrishnan Mahesh**, Professor Pharmacy Department & Dean, Faculty Affair Division, BITS, Pilani, for giving me the opportunity to join in his research group. I am privileged to have his constant guidance, encouragement and untiring help during the course of my PhD. since the day I began working in the lab, he has been very supportive in every aspect of the scientific process like forming hypotheses, designing experiments, analyzing results, writing and submitting papers for publications. His in-depth knowledge on a broad spectrum of different subjects has been extremely beneficial for me. He had given me more than enough freedom during my research work, which probably helped me to learn to think independently, to become more responsible and confident. He has always been nice to me. I will always remember his calm and relaxed personality and sympathetic nature. I consider myself lucky for having him as mentor.

I am truly grateful to **Prof. V.S. Rao**, Vice-Chancellor(acting), BITS, Pilani, **Prof. B.N. Jain**, ex-Vice-Chancellor, BITS, Pilani, **Prof. A.K. Sarkar**, Director, BITS Pilani, Pilani campus and **Prof. G. Raghurama**, ex-Director, BITS Pilani, Pilani campus for the excellent academic support and the research facilities provided to carry out the work at the Institute.

I express my sincere thanks to **Prof S.K. Verma**, Dean, ARD, BITS, Pilani, Pilani campus and **Prof. A.K. Das** (ex-Dean, ARD) for their motivating words throughout my period of stay in Pilani.

I express my gratitude to **Prof. R.N. Saha**, Director, BITS Pilani, Dubai Campus for his encouragement, constructive suggestions and untiring support throughout.

I am extremely thankful to **Dr. Hemant Jadhav,** Associate Dean, ARD and **Dr. Shrikant Charde** (Department of Pharmacy, Hyderabad campus), for their constructive comments and support during my work.

I amm thankful to my thesis Doctoral Advisory Committee members, and **Dr. S. Murugesan**, Head, Department of Pharmacy and **Dr. Hemant Jadhav** Associate Dean, ARD the valuable advice and intellectual guidance.

I owe my deepest gratitude to my seniors **Dr. Thangaraj Devadoss, Dr. Dilip Kumar Pandey** and my colleagues **Dr. Ankur jindal, Dr.Shvetank Bhatt, Mr. Baldev Gautam** and **Dr. Muthu V. Sudali, Mr. Sourabh, Mr. Yeshwant** and **Ms Deepali** for the enthusiasm and

support laid by them during all stages of my work and for their timely guidance, which boosted me to complete my work and thesis. **Dr. Ankur jindal, Dr. Devadoss, Dr.Shvetank, Mr. Baldev** and **Dr. Dilip Pandey** have all been incredibly patient and provided plenty of laughs along the way. I also thank to them for their advice and willingness to share their bright thoughts with me, which were very fruitful for shaping up my ideas and research. I feel very lucky to have been a part of this team and I hope to keep in touch with everyone.

I am grateful to **Mr. Aavtar Singh**, SAIF, Punjab University, Chandigargh, for the spectral analysis of my compounds.

I would also like to acknowledge **Dr. R P. Parekh**, **Dr. Rajeev Taliyan, Dr. Atish T. Paul** (DRC convener), **Dr. Anil Gaikwad, Dr. Anil Jindal, Dr. Deepak Chitkara, Dr. Anupama Mittal, Dr. M.M. Pandey**, **Dr. Jaipal A**, **Dr. Gautam, Dr. Sunil**, **Mr. Mahaveer, Ms. Priti** and **Ms. Archana** who made my stay in Pilani, a pleasant experience.

During my stay in BITS, Pilani weekends were usually the times I revived myself by intermingling with the friends around or playing cricket and badminton. These interactions aided in improving my debating and culinary skills. I would like to thank all my friends **Dr. Dipaloy Datta, Mr.Sourabh Sharma,** for the same. I express my sincere thanks to my Colleagues **Mr. Satish, Mr. Subhash, Kuldeep, Arpit, Dr. Kamesh, Dr.Manoj, Emil, Prashant, Senthil, Vadiraj**, **Garima, Anuradha, Shruti**, **Almesh, Pankaj, Shridhar, Santosh** and **Satish** for their companionship and much enriched help during all times whenever needed.

I express my thanks to **Dr. Sushil Yadav** (Veterinarian In-charge) and other BITS animal lab facility staffs **Mr. Vishal, Mr. Shyam, Mr. Mukesh** and **Mr. Shiv**, those also took care of the animals during the behaviour-based animal studies. I thank the non-teaching staff **Mr. Tarachand, Mr. Naveen, Mr. Puran, Mr. Hari, Mr. Pradeep, Mr. Sitaram, Mr. Ram Suthar, Mr. Laxman, Mr. Mahender,** and **Mr. Mahipal** and all the students of Pharmacy Department for the encouragement provided by them in each stage of the research work.

I am also thankful to **Indian council of medical research (ICMR),** New Delhi and **University grants commission (UGC)**, New Delhi, India, for giving me all the necessary funds to carry out the research and fellowship.

Acknowledgement

It would be injustice if I don't mention about those guinea pigs that, I had sacrificed for my work and those mice to which I had given pain during behavioral studies. I pay homage to those experimental animals that have sacrificed their lives in making my endeavour successful. May God grant them eternal peace.

I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

At last but not least, I am great thankful to God with whose mercy I reached through my endeavour so far.

Date: Arghya Kusum Dhar

List of Abbreviations/Symbols

^{1}H NMR	Proton nuclear magnetic resonance
2-Me-5-HT	2-Methyl-5-hydroxytryptamine
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
Å	Angstrom (10^{-10} meter)
ACTH	Adreno-corticotrophic hormone
ADR	Adverse drug reaction
ADs	Anti-depressants
ANOVA	Analysis of variance
ASHP	American society of health-system pharmacists
BAPTA	1,2-Bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid
BDI	Beck depression inventory
BDNF	Brain derived neurotrophic factor
B-J	Bezold-Jarisch
BZDs	Benzodiazepines
bs	Broad singlet
CINV	Chemotherapy-Induced Nausea and Vomiting
COMT	Catechol-*O*-methyltransferase
CBT	Cognitive behavioural therapy
CRF	Corticotrophin releasing factor
CNS	Central nervous system
pCREB	cAMP response element-binding protein

CSF	Cerebro spinal fluid
CTZ	Chemoreceptor trigger zone
δ	Delta
d	Doublet
dd	Doublet of doublet
DA	Dopamine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DSM-IV/V	Diagnostic and statistical manual of mental disorder-IV/V
EDC	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
ECT	Electroconvulsive treatment
EPM	Elevated plus-maze
EQZ	Ethoxyquinazoline.
eqv.	Equivalent
FT-IR	Fourier transform-Infra Red
GABA	Gamma amino butyric acid
GAD	Generalized anxiety disorder
GIT	Gastro-intestinal tract
GPCR	G-protein coupled receptor
h	Hour
HEK	Human embryonic kidney
HPA	Hypothalamic-pituitary-adrenal
HOBt	1-Hydroxybenzotriazole
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
ID_{50}	Median infective dose

i.p.	Intra-peritoneal
i.v.	Intravenous
IBS	IrriTable bowel syndrome
IR	Infra red
Ki	Dissociation constant of inhibitors
L/D	Light-dark
LGIC	Ligand-gated ion channel
LMMP	Longitudinal muscle myenteric plexus
MDD	Major depressive disorder
µM	Micromolar
MQZ	Methoxy quinazoline
M	Mole/molar
M_1	Muscarinic type-1
m	Multiplet
MASCC	Multinational association of supportive care in cancer
mCPP	*meta*-Chlorophenylpiperazine
mCPBG	m-Chlorophenylbiguanidine
mmol	Millimole
mL	Milliliter
m.p.	Melting point
m/z	Mass/charge
MAOIs	Monoamine oxidase inhibitors
mg	Milligram
min	Minute(s)
MS	Mass spectra
MW	Molecular weight

MWI	Microwave irradiation
NA	Nor-adrenaline
NBIC	N-benzyl indole carboxamide
NCCN	National comprehensive cancer network
NEIC	N-ethyl indole carboxamide
N&V	Nausea and vomiting
nm	Nanomolar
NMDA	*N*-methyl-D-aspartate
NMIC	N-methyl indole carboxamide
NK	Neurokinin
NPY	Neuropeptide Y
NTS	Nucleus tractus solitarious
OBX	Olfactory bulbectomized
OCD	Obsessive compulsive disorder
OFT	Open field test
pA_2	Negative logarithm of molar concentration of antagonist producing a 2-fold shift of the agonist concentration-activity curve
PD	Panic disorder
PND	Post natal depression
PONV	Post operative nausea and vomiting
PTSD	Post traumatic stress disorder
ppm	Parts per million
q	Quartet
RIMAs	Reversible inhibitors monamine oxidase A
rt	Room temperature
s	Singlet

SAD	Seasonal affective disorder
SD	Standard deviation
SEM	Standard error of mean
SERT	Serotonin transporter
SNARIs	Selective nor-adrenaline re-uptake inhibitors
SSRIs	Selective serotonin reuptake inhibitors
SNRIs	Serotonin nor-adrenaline reuptake inhibitors
SP	Social phobia
SSRE	Selective serotonin reuptake enhancer
SLA	Spontaneous locomotor activity
t	Triplet
THF	Tetrahydrofuran
TCAs	Tricyclic anti-depressants
TEA	Triethylamine
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TSH	Thyroid stimulating hormone
TST	Tail suspension test
WHO	World Health Organization

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Abstract:

Depression and anxiety are highly debilitating and commonly occuring psychiatric disorders. Depression affects 340 million people globally with lifetime prevalance of 21% and anxiety affects approximately 1/8 of the total population worldwide. World Health Organization has predicted that depression would be the second largest burdensome disorder of society by the year 2020 only behind ischemic heart disease. Studies have 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 only one disease. Despite the availability of a number of anti-depressant as well as anxiolytic agents, prevalence of these diseases still persists.Studies have indicated that depression and anxiety disorders might 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 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.

The pharmacophoric requirements for 5-HT_3 receptor antagonists derived from quipazine type aryl piperazines include a) a hetero aromatic ring and b) basic moiety in the form of piperazine. On the basis of the above proposed pharmacophore, a series of piperazine analogs of naphthyridine carboxylic acids were designed and sythesized. Three series of piperazine analogs of naphthyridine, indole and quinazoline carboxamides were designed and synthesized as per three point pharmacophoric requirements for 5-HT_3 antagonists that consist of hetero aromatic ring, hydrogen bond acceptor and a basic moiety arranged at particular distances.In-silico molecular properties of all the designed compounds were calculated using Qik–prop module of Schrodinger. The values were found to be within the acceptable range.

Initially 5-HT_3 receptor antagonism of all the synthesized compounds were evaluated using longitudinal muscle myenteric plexus (LMMP) of guinea pig ileum assay method as described in literature. Structure activity relationship with respect to 5-HT_3 receptor antagonism, antidepressant and anxiolytic activity was derived for each series of compounds.

SAR study demonstrated that in case of piperazine analogs of 1,8 naphthyridine carboxylic acids, compounds bearing unsubstituted phenyl (NACA 1), *p*-methoxyphenyl (NACA 9) and *m*-chlorophenyl (NACA 5) substituent at the N^4 piperazine exhibited better antagonistic activity as compared to the compounds bearing other substituents. In case of piperazine analogs of carboxamides, compounds bearing *m*-methoxyphenyl substituent (MN 8, EN 8 of naphthyridine carboxamide series; MQZ 6, EQZ 6 of quinazoline carboxamide series; and NMIC 6, NEIC 6, NBIC 6 of indole carboxamide series) at the N^4 piperazine were found to be most promising compounds among the compounds bearing other substituents. Whereas, in general, replacement of the phenyl ring and/or substituted phenyl ring at the N4 of piperazine with aliphatic alkyl group resulted in lesser antagonistic activity.

After evaluating their 5-HT_3 antagonistic activity, selected compounds were screened for their anti-depressant potentialusing various rodents' test battery like forced swim test (FST) and tail suspension test (TST) in mice model. Compounds NACA 1, NACA 2, NACA 6, NACA 8 and NACA 9 of piperazine analogs of 1,8 naphthyridine carboxylic acid series;compounds MN 7, MN 8, MN 9, EN 7, EN 8, EN 9 of piperazine analogs of 1,8 naphthyridine carboxamide series; compounds MQZ 4, MQZ 5, MQZ 6, EQZ 4, EQZ 5, EQZ 6 of piperazine analogs of quinazoline carboxamide series;compounds NMIC 5, NMIC 6, NEIC 4, NEIC 6, NEIC 7, NBIC 1 and NBIC 6 of piperazine analogs of Indole carboxamide series showed prominent anti-depressant like activity as indicated by considerable ($P < 0.05$) reduction in immobility time in comparison to the vehicle control group.

The compounds with promising 5-HT_3 antagonistic potential and significant anti-depressant activity were selected for anxiolytic screening using elevated plus-maze (EPM) test, light-dark(L/D) test and open field test (OFT).Compounds NACA 1, NACA 5, NACA 6, and NACA 8, of piperazine analogs of naphthyridine carboxylic acid series, compounds MN 7, MN 8, and EN 8, of 1,8 naphthyridine carboxamide series; compounds MQZ 4, and EQZ 4 of Quinazoline carboxamide series; and compounds NMIC 5, NBIC 1, and NBIC 6 of indole carboxamide series showed significant anxiolytic effect as compared to the vehicle treated group in all the three tests. Compounds NACA 1, NACA 6, NACA 8 of naphthyridine carboxylic acid series, compounds MN 7, MN 8, EN 8 of naphthyridine carboxamide series, compounds MQZ 4, EQZ 4 of quinazoline carboxamide series and compound NBIC 6 of indole carboxamide series with higher pA_2 values showed promising anti-depressant as well as anxiolytic activity. Hence, this study indicates the beneficial effects of these NCEs as putative 5-HT_3 receptor antagonists in co-morbid disorders like depression and anxiety.

Chapter 1: Introduction

1. INTRODUCTION

1.1. Serotonin

Erspamer and Vialli had explained 5-Hydroxytryptamine (5-HT) (**figure 1**) as an enteramine found in the gut (Erspamer and Vialli, 1937). Because of the vasoconstrictive nature of 5-Hydroxytryptamine the name serotonin was derived from Latin word *serum* and Greek word *tonic*, the term *serum* represents, it is isolated from serum and *tonic* refers to vasoconstrictor effect (Rapport et al.,1948).

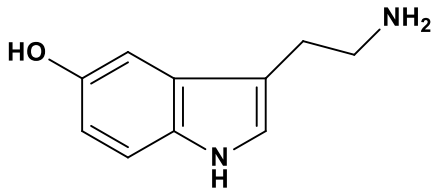


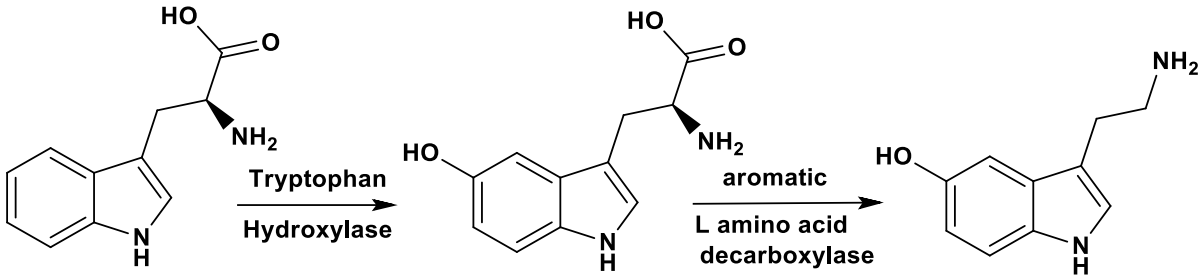
Figure 1: Structure of serotonin or 5-HT.

1.1.1. Biosynthesis, storage and distribution of serotonin

Serotonin is biosynthesized from the essential amino acid, L-tryptophan in 2 steps as represented schematically in **figure 2** (Fitzpatrick, 1999; Nichols and Nichols, 2008). It is largely synthesized in enterochromaffin cells followed by brain, heart and kidney and to some extent in the platelets (Tyce, 1990). The synthesized serotonin is stored in vesicles along with macromolecules known as serotonin binding proteins (Standford, 2001). In CNS the synthesized serotonin is accumulated in the pre-synaptic neurons (Mohamed-Zadeh, 2008).

The release of serotonin occurs through exocytosis and controlled by the auto and heteroreceptor of serotonin, 5-HT_{1A} and 5-$HT_{1B}/_{1D}$, respectively (Standford, 2001). Major amount of the serotonin (>90% of the body's serotonin) is found in the kidney, enterochromaffin cells of gastro-intestinal tract (GIT) (Tyce, 1990), cardiovascular system, platelets and all the regions of brain (Settembrini and Villar, 2004).

The 99% serotonin of body is found intracelluarly. The availability of serotonin in tissues is mainly concerned with synthesis and metabolic rate of serotonin (Tyce, 1990), where only 5% of tryptophan is converted into serotonin due to limited availability of tryptophan hydroxylase enzyme.



L-tyrptophan 5-hydroxytryptophan serotonin/5-HT

Figure 2: Biosynthesis of serotonin/5-HT.

1.1.2. Serotonin Transporter and Inactivation

Serotonin Transporter (SERT) located on the pre-synaptic membrane (Standford, 2001; Mohamed-Zadeh, 2008) and can be extensively found in the central nervous system (CNS), GIT, platelets, pulmonary and peripheral blood vessels (Ni and Watts, 2006). Serotonin re-uptake transporters are dependent on extracellular Na^+ and extracellular Cl^-.The serotonin transporter first binds with a Na^+ ion, followed by serotonin and then a Cl^- ion. Then the transporter protein spins inside the cell, and release serotonin. Subsequently, a K^+ ion binds, and the transporter protein flicks back out and readily receives another molecule of serotonin.SERT diminishes the function of serotonin at it's extracellular receptors by removing the extracellular serotonin by re-uptake process.The termination of pharmacological actions of serotonin by this process is known as inactivation (Standford, 2001).

1.1.3. Metabolism

Serotonin is metabolized by monoaminooxidase (MAO) enzyme to 5-hydroxy indole acetic acid (5-HIAA). 5-HIAA is the major metabolite of serotonin which is pharmacologically inert and is completely excreted in urine unchanged (Tyce, 1990; Standford, 2001; McIsaac and Page, 1959). Although major metabolic route arises from monoamine oxidase (MAO) activity and 5-hydroxy indole acetic acid is found as the major metabolite, it represents only 33 per cent in the rat, 20 per cent in human, and 1.5 per cent in the rabbit of the metaboilites of serotonin. Besides this pathway, glucuronidation, sulfation and *N*-methylation also occur as other metabolic pathways of serotonin (Tyce, 1990). Acetylation and conjugation are also important alternative metabolic pathways. Oxidation is also a possibility (McIsaac and Page, 1959). In the pineal gland, serotonin is metabolized into melatonin.

1.1.4. Serotonin Receptors

Mammals employ 5-HT as a neurotransmitter in CNS and peripheral nervous systems, and also as a local hormone in numerous other tissues, including GIT, the cardiovascular system and immune cells. This multiplicity of function implicates 5-HT in a vast array of physiological and pathological processes. Part of the ability of 5-HT to mediate a wide range of actions arises from the imposing number of 5- HT receptors (Barnes and Sharp, 1999). Based on the signal transduction and amino acid sequence, the serotonin receptors are classified into 7 major types (5-HT_{1-7}) (Nichols and Nichols, 2008). The serotonin type 3 (5-HT_3) receptor is a cation selective ligand-gated ion channel whereas all other serotonin receptors belong to G-protein coupled receptor (GPCR) class. (Hartig et al., 1992; Nicholas and Nicholas, 2008; Doly et al., 2008; Launay et al., 2002; Wong et al., 1995; Pan and Gallignan, 1999; Thomas, 2006; Rajkumar and Mahesh, 2008). Among serotonin receptors, 5-HT_3 receptor is unique, not only with respect to signal transduction and amino acid sequence but it is also distinct with respect to the involvement in various physiological and patho-physiological conditions. Since, antagonism of this ligand gated ion channel receptor in various pre-clinical studies expressed beneficial effects in depression, anxiety, schizophrenia, cognition, pain, etc (Machu, 2011; Walstab et al., 2010), these beneficial effects are evidence of the possible involvement of 5-HT_3 receptor in patho-etiology of aforementioned conditions.

5-HT_3 receptors are present exclusively on neurons of both central and peripheral nervous system and pre-synaptically as well as in post-synaptic regions. In the periphery, 5-HT_3 receptors were found on the autonomic pre and postganglionic neurons and on the sensory neurons and myenteric and submucosal plexus of enteric nervous system (Faerber et al., 2007). In CNS, high concentrations of 5-HT_3 receptors have been identified at several parts of brain, namely: amygdala, frontal cortex, hippocampus, entorhinal cortex, substantia nigra, cingulated cortex, ventral tegmental area and nucleus accumbens.

There are a range of selective agonists and antagonists for these receptors and the pharmacophore is resonably well understood. Studies have suggested many diverse potential disease targets that might be amenable to alleviation by 5-HT_3 receptor selective compounds but till date only two applications have been fully realised in the clinic, these are the treatment of emesis and irritable bowel syndrome. 5-HT_3 receptors are known to be expressed in the central nervous system in regions involved in the vomiting reflex, processing of pain, the reward system, cognition and anxiety control. In the periphery they are present on a variety of neurons and immune cells.

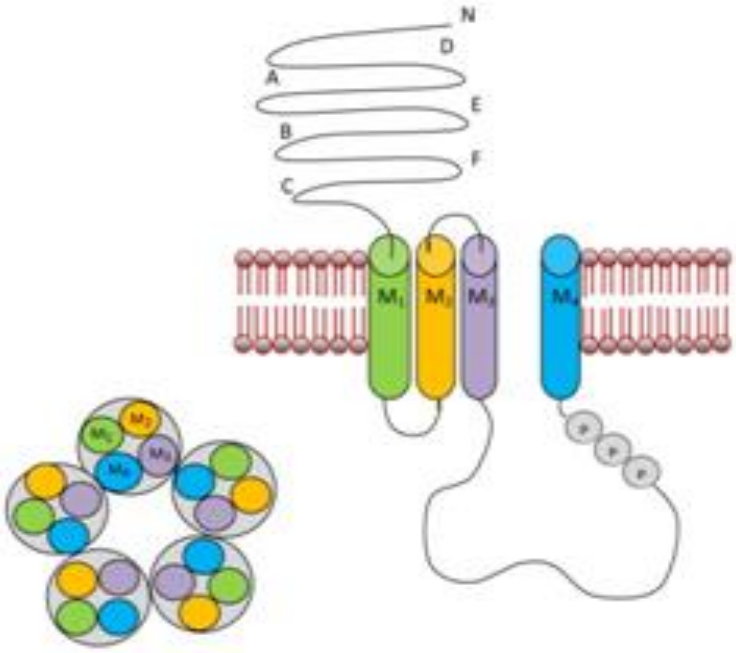
1.1.5. Types, location and functions of various serotonin receptors

Receptor Type	Locations	Functions
5-HT$_1$ **5-HT$_{1A}$**	CNS; widespread in brain (mainly hippocampus, cortex, raphe nuclei)	Neuronal inhibition, behavioral effects (sleep, depression, anxiety and thermoregulation)
5-HT$_{1B}$	CNS; Widespread in brain (mainly basal ganglia, cortex)	Behavioral effects, Vascular: pulmonary vasoconstriction
5-HT$_{1D}$	CNS; Basal ganglia, hippocampus, cortex	Behavioral effects, Vascular: cardiac function and movement
5-HT$_{1E}$	Location: CNS; Cortex, caudate putamen, claustrum	Cognition, memory process
5-HT$_{1F}$	CNS; Dorsal raphe nucleus, hippocampus, cortex, claustrum, caudate nucleus, brainstem	Regulates the cerebrovascular functions and dural inflammation
5-HT$_2$ **5-HT$_{2A}$**	CNS; Cortex, claustrum, hippocampus, hypothalamus and basal ganglia	Excitation of neuron, behavioral effects; contraction of Smooth muscle, vasoconstriction or vasodilatation, aggregation of platelets
5-HT$_{2B}$	CNS; Cerebellum, septum, hypothalamus and amygdala	Post-synaptic inhibition, behavioral effect Vascular: pulmonary vasoconstriction Heart: regulate the cardiac functions and structure
5-HT$_{2C}$	CNS; Choroid plexus, cortex, hippocampus, amygdala, striatum, substantia nigra;	Choroid plexus, CSF secretion, behavioral effects
5-HT$_3$	CNS; Widespread in brain (mainly area postrema, nucleus tractus solitarius, dorsal vagal complex, limbic structures)	Behavioral effects, emesis. Location: PNS: function: neuronal excitation, emesis, GIT motility
5-HT$_4$	CNS; Basal ganglia, cortex, septum, hippocampus	Behavioral effects, GIT motility. Location: Heart: regulate the ion of cardiac functions and structure
5-HT$_5$	CNS; Hippocampus, hypothalamus, olfactory bulb, cortex, thalamus, striatum, pons;	Behavioral effects; motor control, anxiety, depression, learning, memory consolidation, adaptive behavior
5-HT$_6$	CNS; Widespread in brain (mainly striatum, amygdala, hippocampus, cortex)	Behavioral effects; cognition, mood
5-HT$_7$	CNS; Thalamus, hippocampus, cortex, amygdala, suprachiasmatic nucleus	Behavioral effects; sleep, circadian rhythms, mood

1.1.6. Structure of the 5-HT_3 receptor

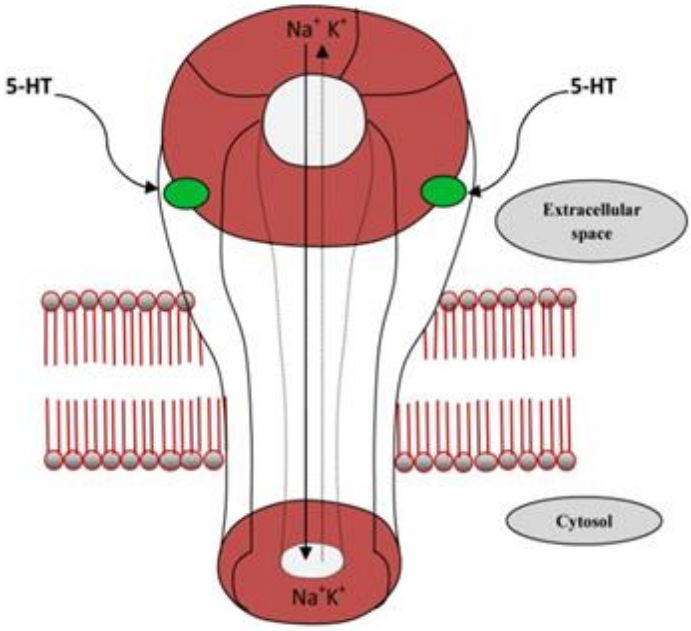
The 5-HT_3 is a ligand-gated ion channel (LGIC) which is selective to cations. Similar to nicotinic acetylcholine (*n*ACh), glycine and $GABA_A$ receptors, 5-HT_3 receptor belongs to LGICs of Cys-loop family. The 5-HT_3 receptor is a pentamer composed of five pseudo-symmetrically arranged subunits to form a cylindrical body structure which is permeable to small cations such as Na^+ and K^+ (**figure 3a**, **3b**). This pentamer may be formed by a combination of up to 5 different subunits, named 5-HT_{3A-E}, although at present only the 5-HT_{3A} and 5-HT_{3B} subunits have been studied in detail. Complete activation of the receptor takes place when three molecules of the agonist bind with homomeric 5-HT_{3A} receptors (Mott et al., 2001; Rayes et al., 2009). In case of heteromeric receptor 5-HT_{3AB}, activation takes place when it binds with its ligands stoichiometrically (Walstab et al., 2010).

These subunits can be homopentameric subunits made up of same 5-HT_{3A} receptors or heteropentameric subunits comprising of both 5-HT_{3A} and 5-HT_{3B} receptors. It has been found that functional homomeric 5-HT_3 receptors are made up of only 5-HT_{3A} subunit. It has also been found that essentially at least one 5-HT_{3A} subunit is present in the heteromeric receptors (Thompson and Lummis, 2007).



3a

Figure 3a: Structure of 5-HT_3 receptor (adopted from Thompson & Sarah Lummis 2007).



3b

Figure 3b: Structure of 5-HT_3 receptor (adopted from Thompson and Sarah Lummis 2007).

Binding sites for serotonin and antagonists at the 5-HT_3 receptor

Each subunit is composed of three parts namely, an extracellular, a transmembrane and an intracellular domain (**figure 3b**). Serotonin, competitive antagonists and agonists all bind to the same site which is located in the extracellular N-terminal domain. The 5-HT binding site within the 5-HT_3 receptor complex is constructed by two adjacent N-termini from neighboring subunits in the pentameric complex.There are reports suggesting the existence of the heteromeric 5-HT_{3AB} receptor (with a subunit composition BA-B-B-A) (Lochner and Lummis, 2010).

Structural analysis has indicated that the agonist and antagonist binding spot is formed at the edge of two adjoining subunits by the union of three peptide loops (three α-helix; designated A, B and C) from one principal subunit and three pepetide loops (three β-strands; designated D, E and F) from the neighbouring complementary subunit (**figure 4**) (Thompson et al., 2005; Reeves et al., 2003; Yan and white, 2005; Maksay, 2003). Homology modeling and docking of serotonin and several other 5-HT_3 receptor antagonists together with radioligand binding studies have revealed the importance of several amino acid residues in all the six loops and their involvement in ligand binding.

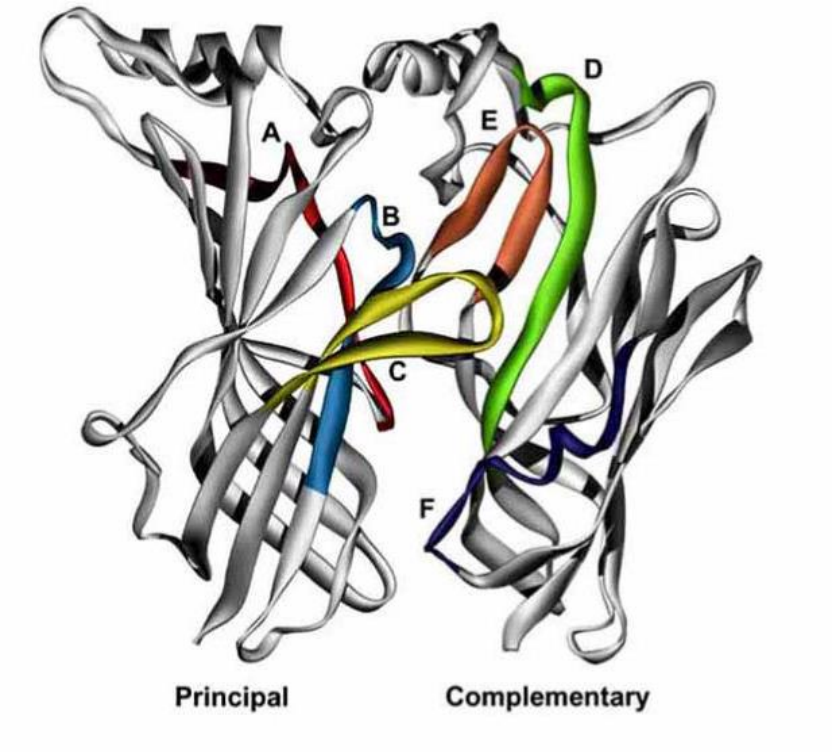


Figure 4: The extracellular domains of 5-HT_3 receptor composed of one principal subunit and one adjacent complementary subunit. This homology model was built on the basis of the crystal structure of AChBP (PDB ID; 1i9b) at 2.7 Å. This model shows 3 α-helix; A, B, C and 3 β-strands, D, E, and F, total six loops that unite to form the ligand binding site. Only two out of the five subunits are shown above. (Adopted from Reeves et al., 2003; Thompson and Lummis, 2006).

Glutamic acid-129 of loop A has been found to be involved in a hydrogen bonding interaction with the hydroxyl group of serotonin (Price et al., 2008). Tryptophan**-**183 of loop B has been found to be critical**,** which is involved in a pi-cation interaction with the 1^{o}amine group of serotonin (Spier and Lummis 2000; Beene et al., 2002). Tyrosine-234 of loop C is critical for both serotonin and binding of antagonists such as granisetron and tropisetron, which forms part of the aromatic box found in all Cys loop receptors (Lester et al., 2004).

Aromatic residue of Tryptophan-90 of Loop D was found to be involved in an interaction with the antagonist within the binding pocket. Studies have indicated that the aromatic rings of the competitive antagonists such as granisetron and tropisetron are positioned near to Tryptophan-90 and that the azabicyclic rings located near to Arginine-92 (Yan and White, 2005). Tyr-141, Tyr-143, Gly-148, Glu-149, Val-150, Gln-151, Asn-152, Tyr-153, and Lys-154 of Loop E may all be important for antagonists binding and perhaps function (Sullivan et al., 2006; Thompson et al., 2006; Thompson et al., 2006).

1.1.7. Location and function of 5-HT_3 receptor

5-HT_3 receptors are present exclusively on neurons of both central and peripheral nervous system and pre-synaptically as well as in post-synaptic regions. In CNS, a high concentration of 5-HT_3 receptors has been identified at several parts of brain, namely: amygdala, frontal cortex, hippocampus, entorhinal cortex, substantia nigra, cingulated cortex, ventral tegmental area, and nucleus accumbens. The highest amount of receptors was found in the brain stem, particularly in the area postrema and the nucleus tractus solitaries which are involved in the vomiting reflex (Hoyer et al., 1994; Tecott et al., 1993; Thompson and Lummis, 2007). In the periphery, 5-HT_3 receptors were found on the autonomic pre- and postganglionic neurons, sensory neurons, myenteric and submucosal plexus of enteric nervous system (Faerber et al., 2007).

Activation of the 5-HT_3 receptor modulates release of various neurotransmitters, including a facilitation of dopamine, GABA and 5-HT release, although the receptor is not thought to be expressed by 5-HT neurons (De Deurwaerdere et al 1998, Martin et al., 1992). Conversely, the 5-HT_3 receptor has an inhibitory effect on acetylcholine release in the cortex (Barnes et al., 1989b), this is likely to be mediated via GABAergic interneurons (Diez-Ariza et al., 2002; Morales and Bloom, 1997). Activation of the 5-HT_3 receptor leads to rapid depolarization of peripheral/central neuron which enhances the cytosolic concentration of Ca^{2+} ion by stimulating the Ca^{2+}influx as well as the release of intracellular Ca^{2+}stores.

This rapid neuronal depolarization also modulates the release pattern of neurotransmitters such as dopamine, GABA, nor-adrenaline and neuropeptide release such as glutamate, substance P, and cholecystokinenin (Greenshaw and Silverstone, 1997; Funahashi et al., 2004). The post-synaptic activation of 5-HT_3 receptors causes depolarization of Na^+ ion and K^+ ion influx (Ronde and Nichols, 1998).

5-HT_3 receptors are known to be involved in emesis, pain disorders, drug addiction, psychiatric and GI disorders. Studies have suggested many diverse potential disease targets that might be amenable to alleviation by 5-HT_3 receptor selective compounds, including addiction, pruritis and neurological phenomena such as anxiety, psychosis, nociception and cognitive function (Tell et al., 1984; Silverstone et al., 1992; Johnson et al., 2000; Jones et al., 1988; Grant, 1995). However, the main therapeutic targets are currently irritable bowel syndrome (IBS) and emesis resulting from cancer chemotherapy (Tyers and Freeman, 1992; Humphrey et al., 1999).

1.1.8. Depression

Major depression disorder (MDD) is a common incapacitating and life-threatening psychiatric disorder. Globailly it affects 340 million people that include all ages of people and gender, where females are more susceptible than males. (Poongothai et al., 2009). It is periodic and recurrent in nature, with partial improvement between episodes. Globally, it ranks fourth among the leading causes of disability (Nemeroff, 2007; Maes et al., 2009). The morbidity and mortality rates for MDD patients are high; two thirds of MDD patients contemplate suicide and 10-15% succeeds. World Health Organization has predicted that MDD will be the second largest contributor to global burden of the disease by the year 2020 (Aan het Rot et al., 2009), following ischemic heart disease. Therefore, depression represents a major medical and social problem, illustrating the severity and impact of this disorder on society.The untreated, chronic or recurrent depression may affect the normal physical, mental societal life of patients and their family members. So, early diagnosis and treatment is important to treat this disorder.

1.1.9. Epidemiology

Depression is the second highest cause of disability worldwide. Indians are one among most depressed population of the world. According to a study conducted by World Health Organization (WHO) around 9% of Indian population had an extended period of depression within their lifetime, whereas about 36% of Indian population had suffered from Major Depressive disorder (MDD). 31.9 years and 22.7 years are the average ages at which Indian population and US population respectively start experiencing depression. 6.7% of adults and 11.2% of 13-18 years olds in the US are reported to have one or the other form of depression. In general, women are affected twice as often than men.

1.1.10. Diagnostic criteria of depression

- Saddened mood – bad temper.
- Low self regard sensations of despair, insignificance and guiltiness.
- Diminished capability to concentrate and imagine.
- Declined or raised appetite.
- Weight loss or weight gain.
- Insomnia or hypersomnia short of energy, fatigue or increased agitation.
- Diminished interest in delightful activities such as sex, food, social interactions.
- Recurring suicidal thoughts and feelings of passing away.

Major depression is diagnosed when a certain number of the above symptoms persist for more than 2 weeks of time period, and when the symptoms interrupt usual social and occupational functioning (DSM IV, 1994).

1.2. Types of depression

Depression is characterized as per criteria given by Diagnostic & Statistical Manual of Mental Disorders-IV/V (DSM-IV/V). The DSM manuals are a series of official publications of the American Psychiatric Association (APA) that gives a standardized view on the treatment, diagnosis and evaluation of mental disorders. The DSM series started with DSM-I (1968) and the latest one is DSM-V (2013). DSM-IV series was structured into a five-part axial system. According to the diagnostic and statistical manual of mental disorder-IV (DSM-IV), depression is broadly classified into following categories.

1. Unipolar depression

Unipolar disorder is also known as major depressive disorder (MDD). Major depression disorder (MDD) is a common debilitating and severe psychiatric disorder with lifetime incidence approaching 21% and related to significant morbidity and mortality (McKenna et al., 2005; Schechter, 2005; Nemeroff, 2007; Maes et al., 2009). MDD is episodic and recurrent with atleast partial recovery between episodes (Ryan and Williamson, 1996). According to DSM-IV, a person is diagnosed to suffer from unipolar disorder when he or she had five or more of the above mentioned symptoms present during the same 2-week period and signify a change from earlier functioning, at least one of the symptoms is either (1) saddened mood or (2) loss of attention or enjoyment. Based on the severity and number of symptoms of depression, MDD is divided into four subcategories viz. psychotic, catatonic, melancholic and atypical (DSM-IV, 1994).

(a) Psychotic depression

This type of depression is combined with psychosis. The patient experiences either delusion which is a belief that depression is a punishment for past errors or hallucination i.e listening voices thatdepression is merit.

(b) Catatonic depression

It's a difficulty with psychomotor activity which means a person is having problem in controlling their voluntary movements who is suffering from catatonic depression.

(c) Melancholic depression

Melancholic depression is portrayed by terror, limited affect, typecast thought and similar changes in autonomic and neuroendocrine function.Typical symptoms of this type of depression include the failure to find enjoyment in positive activities, termed as anhedonia, or mood does not recover in reaction to positive incidents, termed as deficient mood reactivity.

(d) Atypical depression

This type of depression is combined with labile mood, hypersomnia, increased hunger, and increase in weight.

2. Bipolar depression

Persons suffering from this type of illness change back and forth between episodes of depression and episodes of mania (an extreme high). Bipolar disorder is characterized by chronic and cyclic episodes of elevated and depressed mood. It is a complex disorder (Frank et al described it as a disorderly disorder).

Bipolar disorder is now recognized as being heterogeneous such as bipolar disorder I and bipolar disorder II. In DSM-V bipolar disorder is a separate category. In order to increase the accuracy of diagnosis and for easy and early detection in clinical settings, in DSM-V, a criterion for manic and hypomanic episodes comprises an emphasis on alterations in activity and energy as well as mood has been incorporated.

In DSM-V a new specified criteria, “with mixed features,” has been incorporated that can be related to incidents of mania or hypomania when depressive characteristics are present.

Mania is described by following symptoms:

Fewer requirements of sleep, impudence, competitive thoughts, irresponsible behavior, raised energy, gradual occurrence of mood slump but this can also be sudden.

In DSM-IV bipolar disorder comes under mood disorder category. As per DSM-IV, diagnosis of bipolar disorder I, mixed episode, requires that the individual simultaneously meet full criteria for both mania and major depressive episode.

3. Seasonal effective disorder

This type of depression originates from seasonal changes. It is mostly initiated in the fall or winter, or when there is diminished sunlight. Symptoms of SAD include hypersomnia, increase in hunger with carbohydrate craving, increase in weight, diminished energy and exhaustion (APA, 2000).

4. Dysthymia

Persons suffering from this disease experience mild depression for few years. On a day to day basis they function quite well but their relationships go through trouble over time. Dysthymic disorder is a common class of mood disorder, according to the DSM-IV-TR; it is a low-grade and chronic depression which persists for about 2 years.

5. Post partum depression

Post partum depression (PPD) is a severe health concern across cultures. Nearly 10–15% of women are affected by this disease within a short time after the child birth, mostly within the first year. Women who suffer from this type of depression are more likely to have incidence of successive episodes of depression in their later part of life.

DSM-IV does not recognize postpartum depression as a separate diagnosis; rather, patients with a diagnosis of postpartum depression must meet the criteria for both major depressive episode and the criteria for the postpartum onset specifier.According to DSM-IV PPD exists as a part of the spectrum of major depression, coded with a modifier for postpartum onset, which must be within 4 weeks of delivery of a neonate.

Postpartum depression usually begins between two weeks to a month after delivery. Recent studies have shown that fifty percent of postpartum depressive episodes actually begin prior to delivery (Yonkers et al., 2001).Therefore, in the DSM-V postpartum depression now termed as peripartum depression. In DSM-V "peripartum onset" is defined as anytime either during pregnancy or within the four weeks following delivery.

1.2.1. Pathophysiology of depression

The etiology of the depression remains unclear, however several mechanisms have been proposed for the pathogenesis of depression, of which the important mechanisms are mentioned below.

1.2.1.2. Monoamine hypothesis

The earliest hypothesis of depression which was proposed near about 30 years ago was monoamine hypothesis. According to the monoamine hypothesis decrease in the monoaminergic neuro transmitters, such as, 5-HT, norepinephrine (NE) and/or dopamine (DA) in the brain cause main symptoms of depression (Schildkraut, 1965; Matussek, 1972, Coppen, 1967).

The monoaminergic system symbolizes one of the important targets in the pathophysiology and management of depression (Elhwuegi, 2004; Millan, 2004; Hamon and Blier, 2013). Over the last 50 years the chemical underpinnings of depression have been termed as the monoamine hypothesis. The monoamine theory of depression indicates that the etiology and pathogenesis of depression occurs from depletion in monoamine neurotransmitters such as serotonin (5-HT), noradrenaline, and/or dopamine in the CNS (Cardoso et al., 2009; Heninger et al., 1996; Schechter et al., 2005; Delgado and Moreno, 2000; Ardis et al., 2008).

This hypothesis originated from the studies performed in late 1950s which showed that effective anti-depressants such as monoamine oxidase inhibitors (MAOIs) and tricyclic anti-depressants (TCAs), raised the concentrations of monoamines by blocking their metabolism and preventing their reuptake respectively (Eriksson, 2000).The hypothesis is further approved by large amount of neurochemical investigations (Booij et al., 2003; Ruhé et al., 2007; O'Leary et al., 2007; Salomon 1993; Booij et al., 2005; Leyton et al.,2000) and also by the current pharmacotherapies e.g., SSRIs, SNRIs that boost the monoaminergic neurotransmission process (Nemeroff and Owens, 2002; Papakostas et al., 2007; Bymaster et al., 2003; Bond et al., 2008).

The monoamine hypothesis was also supported by some clinical studies and animal studies which showed that the concentrations of NE, 5-HT and DA at the presynaptic region were reduced by the antihypertensive drug reserpine and thereby depressions like effect was observed.

Iproniazid, an antituberculer drug, inhibits the metabolic enzyme MAO resulting in increased concentrations of NE and 5-HT in brain. In some patients, euphoria and hyperactive behavior were observed who were treated with Iproniazid.

Most current ADs act by increasing the amount of monoamines (5HT or NE released & retained in the synapse) or by restoring the normal functioning of monoamines. Regulations of serotonergic and nor-epinephrinergic circuits are known to indirectly modulate the dopaminergic system, which is implicated in anhedonia behavior (Dunlop and Nemeroff, 2007). Although, this hypothesis does not elucidate, what structures are affected in brain and how does deficiency in NTs affect the underlying function of these structures. Moreover, the synaptic monoamine levels are quickly augmented by ADs, the therapeutic effects require weeks to months to appear (Andrade and Rao, 2010).

1.2.1.2. HPA axis hyperactivity

The hypothalamic pituitary adrenal (HPA) axis is activated in response to a stress (Bao et al., 2008). There are many studies which indicate that major depression is characterized by hyperfunction of the hypothalamic–pituitary–adrenal (HPA) axis resulting in hypersecretion of corticotropin-releasing factor (CRF), inadequate glucocorticoid negative feedback function and increased level of cortisol (Twardowska and Rybakowski, 1996; Charlton and Ferrier, 1989; Owens and Nemeroff, 1993; Swaab et al., 2005; Kathol et al., 1989).

All these above mentioned abnormalities can be seen in patients suffering from one or recurrent major depressions (unipolar disorder), but these abnormalities may also be seen in the reduction phase of patients suffering from repeated incidents of both major depression and mania or hypomania (bipolar affective disorder) as well as in depressive phase and manic phase (Nemeroff, 1988; Schmider et al., 1995, Watson, et al., 2004). Psychotic major depression, a subtype of major depression has been found to be connected with high rates of HPA axis hyperactivity (Risch et al., 1992; Schatzberg et al., 2000; Arborelius et al., 1999; Schüle et al., 2009).Numerous studies have indicated an association between abnormalities in HPA axis responsiveness and depression state. (Carroll et al., 1976; Gold et al., 1986; Halbreich et al., 1985; Holsboer et al., 1984; Nemeroff et al., 1984; Holsboer, 1988). It was suggested that the alterations of the HPA axis can be reversed by successful anti-depressant treatment (Nikisch, 2009; Budziszewska, 2002; Barden, 2004).

Neuroendocrine abnormalities including a compressed diurnal cortisol rhythm (Young et al., 1993) increased serum and 24 h urinary cortisol levels (Kathol et al., 1989; Deuschle et al., 1997) and adrenal gland hyperplasia (Amsterdam et al., 1987) have been found in persons suffering from severe depression (Thomson and Craighead, 2008). Studies have indicated that impaired corticosteroid receptor signaling mechanism might be engaged in the pathophysiology of depression (Holsboer, 2000; Juruena et al., 2004; Pariante 1995, Pariante and Miller, 2001, Pariante, 2004, Boyle et al., 2005).

The precise pathophysiology of the HPA axis hyperactivity in major depression has not been traced out yet. However, two important mechanisms of HPA axis hyperactivity have been suggested, namely: the glucocorticoid receptor (GR) theory and the hyper-drive of corticotrophin releasing factor (CRF) (Thomson and Craighead, 2008a; Steckler et al., 1999).

1.2.1.3. Neurotropic factor hypothesis

Another hypothesis for the pathophysiology and treatment of depression indicates asignificant role of neurotrophic factors (Duman et al., 1997; Altar, 1999) and alteration of plasticity of neuronal systems. It is believed that Depression arises from an incapability to make proper adaptive reactions to stress or additional aversive stimuli. Anti-depressants possibly work by rectifying this dysfunction or by directly bringing to mind the appropriate adaptive responses (Malberg and Schechter, 2005; Duman et al., 1999). In brain, the brain-derived neurotrophic factor (BDNF) regulates growth and maintenance of neurons, and use-dependent plasticity systems such as long-term potentiation (LTP) and learning.

According to the neurotrophic factor hypothesis, a shortage of BDNF may add to hippocampal pathology in the advancement of depression, and anti-depressant treatments may improve the symptoms of depression by reversing this insufficiency. It has been found that severe stress causes reduction in BDNF expression which results in reduction in dendritic arborizations and several other changes in hippocampal pyramidal neuron and its innervation by glutamatergic, monoaminergic and other neurons. It has been found that excessive glucocorticoids hinder the normal CREB mediated transcriptional mechanisms that control the expression of BDNF resulting in reduction of BDNF level. Anti-depressants therapy increases expression of BDNF and arborizations of the dendrite of the neurons of hippocampus. This consequence is thought to be intervened by stimulation of CREB. Thus, anti-depressants actually quash and check the actions of stress on the hippocampus, and improve certain depressesive symptoms.

Chronic administration of BDNF has been found to exert beneficial results on serotonergic neuronal growth and rejuvenation, and additionally promote the development of the nerve endings (Altar, 1999). Similar to anti-depressant treatment, in animal experiments, BDNF administration was found to improve learned helplessness (Frank et al., 1997; Siuciak et al., 1997). Further studies have revealed that treatment with anti-depressants, specific 5-HT or NE uptake inhibitors and MAOIs, raises the levels of BDNF mRNA in the hippocampus region of rats possibly through 5-HT_{2A} and the β-adrenoceptor subtypes, and averts the stress-induced decline in BDNF mRNA (Vaidya et al., 1997; Duman et al., 1997). In a recent postmortem study it was found that patients who underwent anti-depressant treatment had increased BDNF expression in the brain (Chen et al., 1999).This further supported the results of these animal experiments and strengthened the BDNF hypothesis of depression.

1.2.2. Role of 5-HT_3 receptor antagonist in depression

The presumed mechanism of anti-depressant activity of 5-HT_3 receptor antagonists is based on the rodent behavioural and neuropharmacological research. According to the monoamine theory of depression, it is proposed that anti-depressant drug produces it's effect through enhancement in serotonergic neurotransmission (Rajkumar and Mahesh; 2008). Studies have indicated that, in addition to the atypical anti-depressants such as fluoxetine, reboxetine, tricyclic anti-depressants like imipramine, desipramine and doxepin block the agonist-stimulated currents via 5-HT_3 receptorsin a noncompetitive manner (Fan, 1994). Beside, atypical tetracyclic anti-depressants such as mianserin and mirtazapine were found to inhibit 5-HT_3 receptors in nanomolar concentration rangesin a competitive manner. (Montgomery, 2005; Eisensamer et al., 2003).This suggests that, 5-HT_3 receptor antagonists facilitate 5-HT neurotransmission and thus have a possible role in the management of depression. Fluoxetine has been found to act as a 5-HT_3 receptor antagonist and block inhibitory neurotransmissions, thereby increasing the excitatory synaptic potential. Thus, presynaptic 5-HT_3 receptors blockade of the inhibitory interneurons can produce anti-depressant effects (Fan, 1994; Rajkumar and Mahesh, 2008).

It is proposed 5-HT_3 receptor antagonists, at low concentrations, inhibit the 5-HT_3 receptors located at the postsynaptic region which arbitrates a fast excitatory potential in the limbic regions of the brain (Sugita et al., 1992). Although the fast transmission blockade which leads to the cascade of cellular events remains unclear, an overall anti-depressant-like behaviour is plausible. Blockade of postsynaptic 5-HT_3 receptor in serotonergic neurons promotes the specific binding of serotonin to other postsynaptic receptors such as 5-HT_{1B} (Bourin et al., 1998), 5-HT_{2A} and 5-HT_{2C} thereby improving serotonergic neuro transmission (**Fig. 5**). Novel anti-depressant, such as mirtazapine has been found to exert it's effect in similar fashion (Anttila and Leinonen, 2001; Berendsen and Broekkamp, 1997; Fawcett and Barkin, 1998; Sambunaris et al., 1997). It has been found that, at higher dose levels, the 5-HT_3 receptor antagonists block the pre-synaptic and somatodendritic 5-HT_3 receptor which inhibit the release of serotonin, eventually causing a decrease in the synaptic serotonin level resulting in depression-like effects (Ramamoorthy et al., 2008). Numerous preclinical animal studies as well as clinical studies with standard 5-HT_3 receptor antagonists also have supported the involvement of 5-HT_3 receptor in depression.

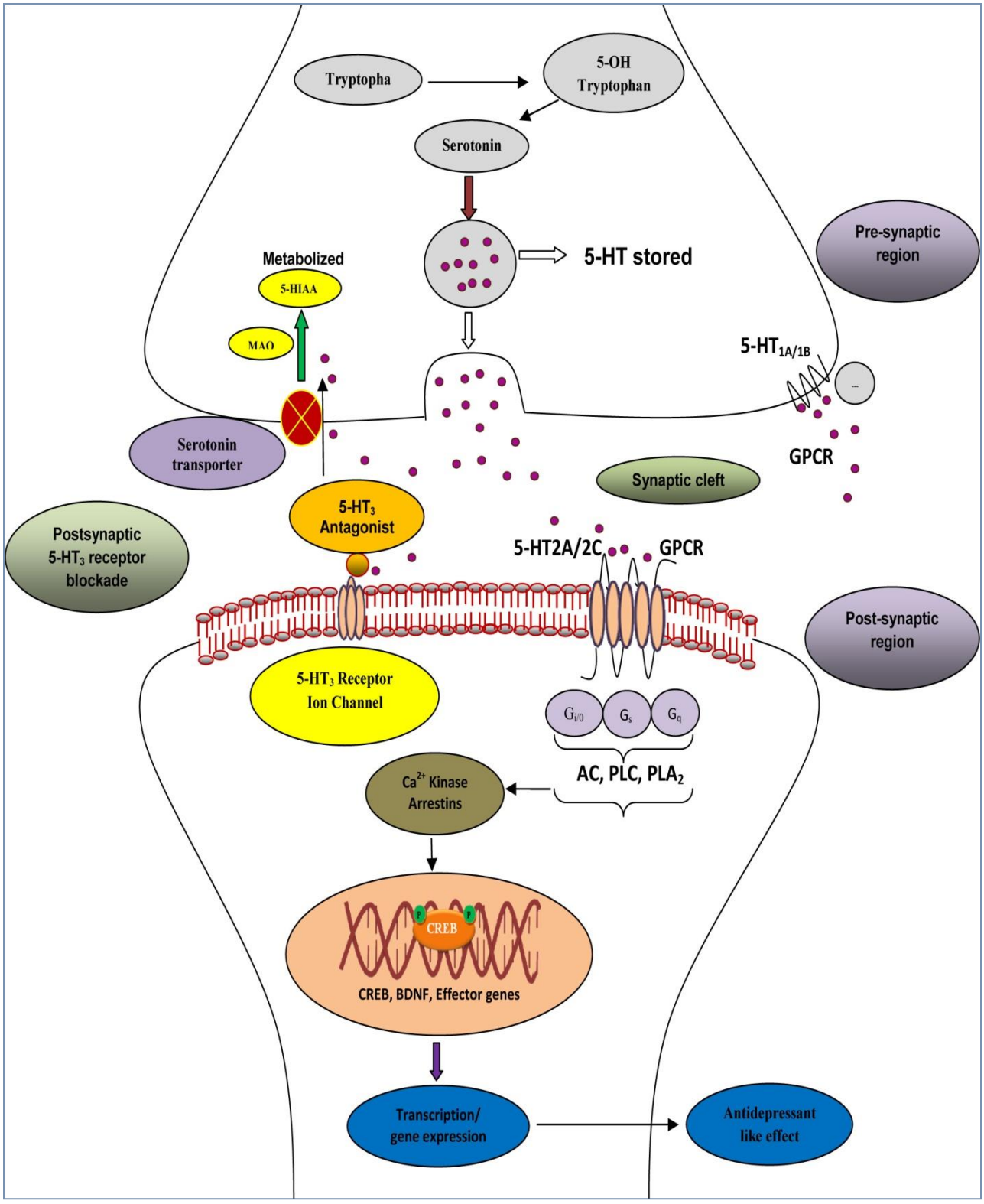


Figure 5: Probable mechanism of anti-depressant like activity with respect to serotonin type-3 ($5\text{-}HT_3$) receptor antagonism (Rajkumar and Mahesh; 2008).

1.2.3. Anxiety

Anxiety, a normal response to stress, is a cardinal symptom of many psychiatric illnesses closely allied with appropriate fear that in many cases can become an important pathological, disabling, and lasting disorder (Barkow et al., 2004; Airaksinen et al., 2005). Anxiety disorders are the most common psychiatric diseases in the US, with life span occurrence in around 30% of the population. Moreover, studies report that anxiety disorders associate with significant disability and contribute to constantly rising health load worldwide, affecting 1/8 thof the total population worldwide (Kessler et al., 2005). Anxiety is the most prevalent disorder which is co-morbid with depression and in line, both these conditions are also co-morbid with other complex diseases such aseating disorders, fibromyalgia, Parkinson's Disease and functional GI disorders such as irritable bowel syndrome (IBS) (Fairburn and Harrison, 2003; Cole et al., 2006; Nutt and Stein, 2006; Martinez- Martin and Damian, 2010). In spite of a stable enhancement in the number of people who received treatment for anxiety disorder, it's occurrence of the disease remains stable, which may be due to lack of neurobiological knowledge of patho-physiology or unpredicTable effectiveness of the currently available therapy. The anxiolytic effects of most of the currently available drugs are intervened via an activation of brain neurotransmitter GABA at the GABA A receptor complex (Fraser, 1998). GABA is an important neurotransmitter involved in the patho-physiology of anxiety disorders.

1.2.4. Classification and diagnosis of anxiety disorder

Pathological symptoms of anxiety are common, chronic, and disabling. Anxiety is a response to an unknown, internal, vague, or chronic threat. Anxiety disorders include generalized anxiety disorder (GAD), PTSD, panic disorder, social anxiety disorder, specific phobia and OCD. These disorders are characterized by shortness of breath, chest pain, motor tension, autonomic hyperactivity and increased vigilance. Diagnosis and classification of anxiety disorders are based on DSM-IV (1994) criteria. (1) According to the DSM-V (2013), social Phobia is now called as Social Anxiety Disorder DSM-V No longer includes OCD under Anxiety disorder.

Panic Disorder and Agoraphobia are unlinked in DSM-V, as many patients experience Agoraphobia without panic symptoms. Separation Anxiety Disorder and Selective Mutism now fall under the Anxiety Disorders chapter in DSM-V.

Based on the symptoms; anxiety is classified into following categories (**figure 6**).

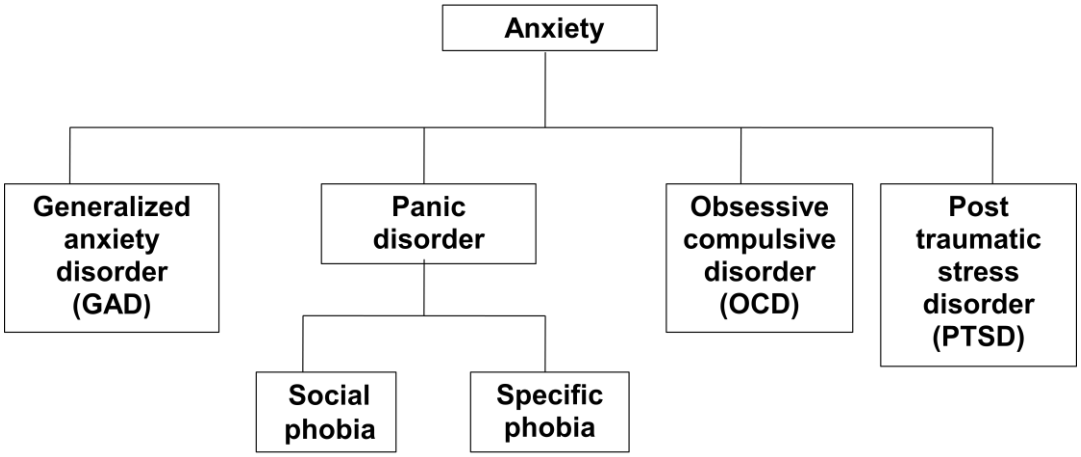


Figure 6: Classification of anxiety disorders.

1.2.4.1 Generalized anxiety disorder (GAD)

As per DSM-IV criteria, the Generalized anxiety disorder (GAD) is related to three or more of the following six symptoms with at least some symptoms present for the past 6 months and for children only one of the following symptom is required to be categorizedGAD (Andrews et al., 2010).(1) agitation or feeling tensed (2) fatigue feeling (3) trouble in making concentration or mind leaving empty (4) bad temper (5) muscle strain (6) problematic sleep i.e, difficulty in falling or staying asleep, or restless, inadequate sleep.

1.2.4.2 Panic Attack

The panic attack is characterized by a period of acute terror, with few bodily signs such as shortening of breathing, sticky sweat, jagged heartbeat, faintness, feelings of worthlessness and wanting to run away from the place, where the attack struck. During the attacks, many people experience anticipatory anxiety, which may terminate in agoraphobia. The occurrence of panic disorder is about 1 to 2%. Women are two times more likely to develop panic disorder than men. Panic attacks usually occur along with social phobia, GAD and major depressive disorder (MDD) as well.

Expected and unexpected are the new terms to differentiate the type of panic attack. Panic attacks play a prognostic role for harshness of diagnosis, course and co-morbidity across many anxiety and other disorders and thus, can be listed as a specifier that is valid for all DSM-V disorders.

1.2.4.3 Post-Traumatic Stress Disorder

PTSD is described by relentless escaping of stimuli combined with trauma and insensibility to current life events. Additional symptoms are self-destructive and reckless behavior, dissociative symptoms, somatic complaint, feelings of uselessness, disgrace, dejection, or hopelessness, loss of previously sustained beliefs, hostility and social withdrawal, along with a constant sense of being threatened and changes in personality characteristics (DSM-V, 2013). PTSD is diagnosed when exposure to perceived or actual threat of death or serious injury results in intense fear, helplessness, or horror for at least one month. Combat veterans, victims of natural disasters and victims of criminal violence are at risk for PTSD. Among these groups, lifetime prevalence rates are community and situational based.

1.2.5. Pathophysiology of anxiety:

Various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology and pathophysiology of anxiety disorders (Cates et al., 1996). These are said to be biopsychosocial factors such as heredity, Neurotransmitter imbalance, personality traits, low self-esteem, adverse Life experiences etc that contribute to anxiety disorders (Pies, 1994; White, 2005; Wong, 2006). The pathophysiology and precise mechanism of anxiety remains unclear. Nevertheless, it has been hypothesized that low levels of neurotransmitter GABA that lessens the central nervous system activity, contributes to anxiety. Numerous anxiolytics modulate the GABA receptor and thereby exert the effect (Lydiard, 2003; Nemeroff, 2003 a, b; Enna, 1984).

Brain imaging and functional studies have shown that several neurotransmitters are linked to the neurobiology of anxiety (Cates et al., 1996; Sandford et al., 2000; Millan, 2003; Augustin, 2005). It has been hypothesized that, monoaminergic system (serotonin (5-HT) and nor-epinephrine (NE)) in the brain, play an important part in the pathophysiology of anxiety disorders (Zhang et al. 2004). Few pre-clinical as well as clinical studies also provide evidence to hold up the hypothesis that anxiety may be associated with an alteration or dysfunction of the serotonergic system in the central nervous system (CNS) (Aghajanian et al., 1990; Revelli et al., 1998; Jindal et al., 2013).

1.2.6. Role of 5-HT_3 receptor antagonist in anxiety

Numerous animal experiments have led to the assumption that 5-HT_3 receptor antagonists block limbic hyperactivity response and thereby show anxiolytic like effects (Rajkumar and Mahesh, 2010; Millan, 2003; Griebel, 1995).

The association of 5-HT_3 receptor with anxiety is also supported by several studies with 5-HT_{3A} knock-out (KO) mice which revealedthat 5-HT_{3A} controls the behavior related to depression and anxiety (Kelley et al., 2003). Thus, it is rational to state that 5-HT_3 receptor is involved in the intonation of anxiety-related behavior.

Similarly, several pre-clinical studies have revealed the anxiolytic effect of 5-HT_3 receptor antagonist. Tropisetron proved good as an anxiolytic, ondansetron eliminated the emotion-potentiated startle response (Harmer et al., 2006). Ondansetron was found to potentiate the pentagastrin stimulated raised adreno-corticotrophic hormone (ACTH) levels and anxiety scores (McCann et al., 1997). Ondansetron was also found to decrease the scores of anxiety and depression in patients suffering from obsessive compulsive disorder (OCD) (Hewlett et al., 2003). The beneficial role of 5-HT_3 antagonists in the treatment of anxiety was demonstrated by several clinical studies: 5-HT_3 receptor antagonism by tropisetron has shown anxiolytic effects (Lecrubier et al., 1993). Several other pre-clinical as well as clinical studies with standard 5-HT_3 receptor antagonists also evidence the involvement of 5-HT_3 receptors in anxiety and that pharmacotherapy targeting 5-HT_3 receptors could be an alternative therapeutic approach for the treatment of anxiety disorders.

Co-morbidity in depression and anxiety

Depression and anxiety disorders are highly prevalent conditions that frequently co-occur. Individuals affected by both depression and anxiety disorders concurrently have generally shown greater levels of functional impairment, reduced quality of life and poorer treatment outcomes compared with individuals with only one disorder (Olfson et al.,1997; Brown et al., 1996; Kessler et al., 1998; Sherbourne et al., 1996). Major depressive disorder (MDD) is the most prevalent depression that is co-morbid with variety of anxiety disorders.High levels of co-morbidity were found among MDD and anxiety disorders (47.5% of those with MDD had co-morbid anxiety disorders; 26.1% of those with anxiety disorders had co-morbid MDD) (Aartjan et al., 2000).

Studies have shown that MDD is mostly co-morbid with panic disorders (50.0%) and OCD (44.4%) followed by GAD (30.3%) and social phobia (25.0%) (Aartjan et al., 2000; Douglas and Dolnak, 2006).

Consequences of co-morbidity:

Patients with co-morbid depression and anxiety have:

- Higher severity of illness (Aartjan et al., 2000).
- Slow recovery and greater chances of recurrence once the patient has recovered (Andreescu et al., 2007) due to higher chronicity.
- Increased risk of withdrawal from the treatment, and a decreased response to the treatment (Andreescu et al., 2007).
- Significantly greater impairment in work functioning, psycho-social functioning and quality of life as compared with patients not suffering from co-morbidity.
- Increased rate of psychiatric hospitalization.
- Increased rate of suicide attempts as compared to patients without co-morbidity.

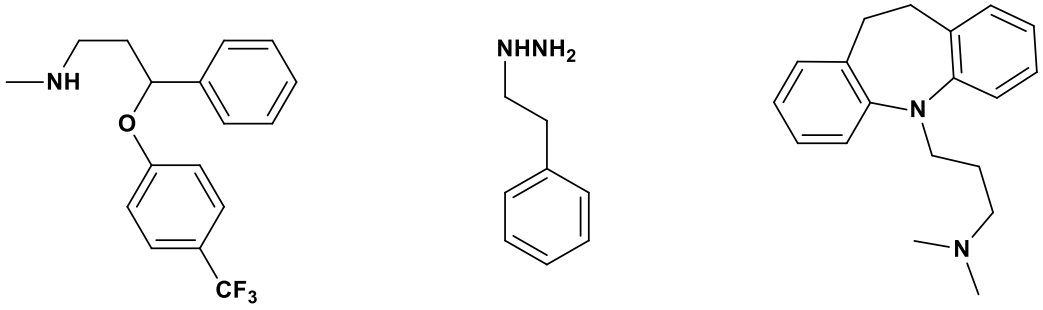
1.2.7. Current pharmacotherapy of depression and anxiety disorders

The most of the clinically existing anti-depressant and antianxiety drugs exert their therapeutic effect by elevating at least one of the endogenous monoamine such as serotonin (5-HT), nor-adrenaline (NA), nor-epinephrine (NE) or dopamine (DA). Irrespective of the chemical structures, target site and mechanism of action, all the clinically available drugs, elevate the levels of above mentioned monoamine(s) in synaptic cleft either directly or indirectly (Pinder and Wieringa, 1993). Early theories implicated a direct correlation between the monoamine neuronal systems and depression disorder, where, decreased monoamine availability as a biological substrate of depression (Schildkraut, 1965). Conventional ADs used for depression pharmacotherapy directly affect monoamine turnover in brain and engage in the restoring of normal function of monoamine associated signaling pathway (Schildkraut and Kety, 1967; Millan, 2006). Monoamines currently monopolize the treatment of depressive states in the sense that they are engaged by all currently available AD agents (Millan, 2004). Based on the mechanism of action and chemical structure, anti-depressant and anti-anxiety drugs are described below. The pharmacologic choices include anti-depressants, benzodiazepines, azapirones (non-benzodiazepine anxiolytics e.g. buspirone), or combined treatment.

1.2.7.1. Selective Serotonin Re-uptake Inhibitors (SSRIs)

These classes of drugs produce their therapeutic effect by selectively blocking the reuptake of serotonin over other monoamines by blocking SERT in the neuronal and platelet membrane. SSRIs such as fluoxetine (**figure 7a**), fluvoxamine, paroxetin, sertaline are chemically aryl or aryloxyalkylamine containing compounds (Blackburn and Wasley, 2007).

SSRIs are used as first line drugs for the management of various depression and anxiety disorders (Ballenger, 1999; Kasper and Resinger, 2001). These agents are useful in anxiety co-morbid with depression, which is a major advantage over benzodiazepines and azapirones (Kasper and Resinger, 2001; Standford, 2001). SSRIs are commonly used for the treatment of co-morbid OCD and depression (Kulhan et al., 2012; Douglas and Dolnak, 2006). SSRIs are the first-line treatment option for co-morbid panic disorder and MDD (Ballenger, 1986), social phobia and MDD (Van Ameringen et al., 1993) and also routinely being used to treat co-morbid GAD and MDD in combination with benzodiazepines (Greco, and Zajecka, 2000; Zajecka and Ross, 1995).



(a) Fluoxetine (b) Phenelzine (c) Imipramine

Figure 7: Structures of (a) SSRI, (b) MAOI and (c) TCA.

1.2.7.2. Monoamine oxidase inhibitors (MAOIs)

MAOIs produce their therapeutic effects by elevating the level of endogenous amines (serotonin, nor-adrenaline and dopamine) in the synaptic cleft by blocking the degradation process of monoamines by inhibiting the monoamine oxidase enzyme (Feighner, 1999), responsible for the normal metabolism of endogenous amines. All the MAO inhibitors such as tranylcypromine, phenelzine (**figure 7b**), isocarboxazide, meclobemide are used for the treatment of depression. Among the MAOs, phenelzine was found to be a promising

anxiolytic used in the treatment of panic disorder, SAD and second line drug for OCD and refractory OCD (Ballenger, 1999).

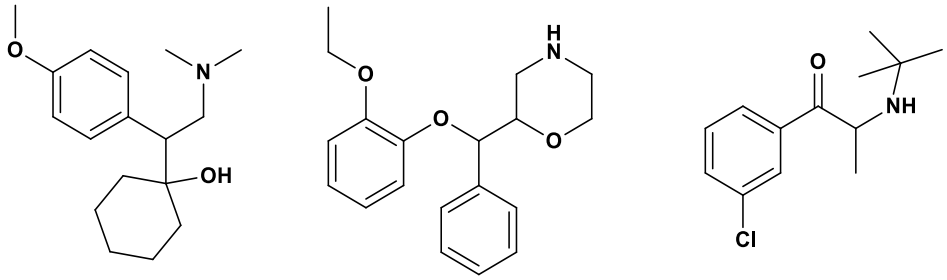
Iproniazid is another monoamine oxidase inhibitor which shows anxiolytic property (Standford, 2001). MAOs are also commonly used to treat co-morbid panic disorder and depression (Ballenger, 1986).

1.2.7.3. Tricyclic anti-depressants (TCAs)

Tricyclic anti-depressants such as Imipramine (**figure 7c**), clomipramine are considered to be gold standard for the treatment of depression. These are also very popularly being used to treat co-morbid panic disorder and depression (Ballenger, 1986). The therapeutic effects of these agents are due to the blockade of re-uptake of monoamines (NA and 5-HT) in neurons following the release of these neurotransmitters into synaptic cleft (Parrot et al., 2004; Blackburn and Wasley, 2007). Imipramine was the first tricyclic anti-depressant agent that showed prominent anxiolytic activity in panic disorder, effective in treating PTSD and GAD (Ballenger, 1999). Clomipramine was found to be efficient in the treatment of panic disorder with significant anxiolytic effects in OCD (Currie, 2003). Generally these agents are preferred when anxiety is co-morbid with depression.

1.2.7.4. Serotonin and Nor-adrenaline Reuptake Inhibitors (SNRIs)

This class of drugs such as venlafaxine (**figure 8a**), duloxetine, milnacipran, elicit their pharmacological response by elevating the level of both 5-HT and NA by blocking both the monoamine transporters. Because of this dual re-uptake inhibitor nature, SNRIs are equipotent with TCAs with devoid of several side-effects as compared to TCAs (Blackburn and Wasley, 2007; Sussman, 2003; Frazer, 1997).



(a) Venlafaxine (b) roboxetine (c) Bupropion

Figure 8: Structures of (a) SNRI (b) SNARI (c) NDRI.

1.2.7.5. Selective Nor-adrenaline Re-uptake Inhibitors (SNARIs)

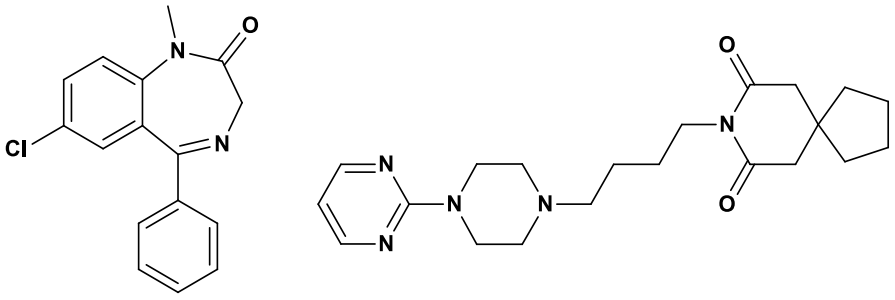
Roboxetine (**figure 8b**), viloxazine and atomoxetine belong to the class of SNARIs (Leonard, 1997; Fish et al., 2009). These drugs selectively enhance the nor-adrenergic function by specifically blocking the nor-adrenaline transporter (Brunello et al., 2002). These molecules do not affect the other transporters and receptors system; therefore these molecules are devoid of SSRIs and TCAs side-effects (Brunello et al., 2002).

1.2.7.6. Nor-adrenaline and Dopamine Reuptake Inhibitor (NDRI)

Bupropion **(figure 8c)**, enhances the nor-adrenergic and dopaminergic function by blocking the nor-adrenaline and dopamine transporter (Parrot et al., 2004). Bupropion is the only anti-depressant that does not have affinity towards serotonergic system. It is more effective intreating severely depressed patients (Pitts et al., 1983; Reimherr et al., 1998) and it does not precipitate sexual dysfunction.

1.2.7.7. Benzodiazepines (BZDs) and Azaspirones

Benzodiazepines such as diazepam, chlordiazepoxide, alprazolam, oxazepam are drugs of choice for the treatment of anxiety particularly GAD, facilitating GABA minergic transmission by acting on the $GABA_A$ complex (Gogas et al., 2007). Benzodiazepines are routinely being used to treat co-morbid GAD and MDD in combination with SSRIs (Greco and Zajecka, 2000; Zajecka and Ross, 1995). Buspirone and gepirone belong to the class of aspirones. Buspirone is a 5-HT_{1A} receptor partial agonist, widely used for the treatment of GAD (Ballenger, 1999). Chemical structures of diazepam and buspirone are shown in **figure 9a, 9b**.



(a) Diazepam (b) Buspirone

Figure 9: Structures of (a) Benzodiazepine and (b) Azaspirone.

1.2.7.8. Non-pharmacological treatments of depression and anxiety

1.2.7.8.1. Electroconvulsive treatment (ECT)

ECT is recognized to be one of the most efficient treatment options for patients suffering from severe depression. It is found to be mainly effective in patients suffering from refractory depression and melancholia (Broquet, 1999; McDonald et al., 2004; Sackeim et al., 2001; Sonawalla and Fava, 2001). Electroconvulsive treatment is rapid acting and most effective somatic treatment for depression (Salzman et al., 2002; Nemeroff, 2007; Machado-Vieira et al., 2010). Generally it is not regarded as first-line therapy for treating severe depression. However, it is a valuable alternative for patients with pharmacological treatment resistant depression and those who are unable to bear the pharmacotherapy (Nemeroff, 2007). It is also used in the treatment of co-morbid depression and anxiety.

1.2.7.8.2. Transcranial magnetic stimulation (TMS)

Repetitive TMS (rTMS) is a non-invasive technique which presumably exerts it's anti-depressant action utilizing single or rhythmic electrical current pulses and generating magnetic energy over particular areas in the brain (Nemeroff, 2007). TMS has been found to alter the blood flow in the cerebral region. The increase or decrease in cortical excitability associated with TMS depends on the stimulation frequency (Bohning et al., 2000; Catafau et al., 2001; George et al., 2000).There are reports from number of studies which have indicated that rTMS was preferred over ECT in the management of severely depressed treatment-resistant patients (Grunhaus et al., 2000; Pridmore, 2000).

1.2.7.8.3. Yoga and Meditation

Yoga and meditation are used as either adjuncts or alternative therapies for treating depression and anxiety disorders. Numerous studies have showed the beneficial outcome of yoga and meditation in depression (da Silva et al., 2009).These therapies significantly reduced the self-reported symptoms of depression and anxiety.

1.2.7.8.4. Phototherapy:

Phototherapy, also known as light therapy is an effective first line therapy for the treatment of SAD (Even et al., 2008; Bauer et al., 2002).This light therapy is effective in the morning time, further supplementary therapy may require for 5-10 minutes between 3 pm and 7 pm.

Recent study suggests that, phototherapy is also helpful for treating unipolar depression, bipolar depression, post partum depression, drug and electroconvulsive therapy resistant depression (Terman, 2007).

1.2.7.8.5. Psychotherapy

Psychotherapy is another important treatment methodfor depression and anxiety; Cognitive behavioral therapy (CBT) is the most often used treatment and well studied treatment for depression and anxiety (Quilty et al., 2008) as an adjunct in combination with anti-depressant drugs and anti-anxiety drugs (NHS, 2003; Hariri et al., 2000). CBT along with anti-depressants is also an effective means to treat co-morbid panic disorder and depression (Barlow, 1988).

1.2.8. Limitations of the existing pharmacotherapy of depression and anxiety

Despite of the fact that a number of anti-depressant and anxiolytic agents are available, these disorders are still prevalent. The reason may be due to insufficient efficacy of presently available drugs with side-effects and lack of in-depth knowledge of pathophysiology of depression and anxiety.

- All currently approved drugs for example, SSRIs have delayed onset of action, usually 3-4 weeks (Cryan et al., 2002). With an exception of ECT, all other non-pharmacological treatments also take longer times to show their effects such as psychotherapy, yoga and meditation.
- All the clinically useful agents produce withdrawal syndromes, except agomelatin (Montgomery et al., 2004).
- Classical drugs like MAO inhibitor and TCAs produce drug-drug/-food interactions, anti-cholinergic, and cardiovascular side effects (Pinder and Wieringa, 1993; Pinder, 1990).
- Benzodiazepine (BZDs) (diazepam and related drugs) class of drugs have a wide variety of side effects, such as sedation and addiction; impairment of cognitive function and psychomotor performance (Lader and Morton, 1991; Whiting, 2006).
- Change in body weight, insomnia, lethargy or sedation, headache, and sexual dysfunction (Dording et al., 2002; Priest et al., 1992) are reported side-effects associated with SSRIs and MAO inhibitors.
- High co-morbidity of depression with anxiety can lead to changes in diagnosis during the course of the illness.

The challenges of meeting all these facts demand a requirement for development of novel and effective anti-depressant and anti-anxiety agents with not many side-effects. Standard 5-HT_3 receptor antagonists such as ondansetron, granisetron, tropisetron, etc have been comprehensively investigated for their neuro-psychopharmacological potential (Wolf 2000, Israili 2001). Moreover, numerous pre-clinical studies (Bravo and Maswood, 2006; Martin et al., 1992; Costall and Naylor, 1992; Zhang et al., 2001; Kos et al., 2006) as well as earlier studies (Devadoss et al., 2010; Mahesh et al., 2007; Rajkumar et al., 2008; Mahesh et al., 2010; Mahesh et al., 2011) have recognized 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 (Rajkumar and Mahesh, 2010). Thus, in this study attempts were focused on developing novel 5-HT_3 receptor antagonists for co-morbid disorders like depression and anxiety.

Chapter 2: Literature Review

2. LITERATURE REVIEW

From the synthetic works involved related to the development of 5-HT_3 receptor ligands, the 5-HT_3 receptor antagonists were classified to the following chemical classes (Cappelli et al., 2002).

a. Arylpiperazine derivatives related to quipazine.

b. Tropane and quinuclidine derivatives related to the "Classical" 5-HT_3 receptor antagonists ('Setrons').

c. Mixed structures (Modica et al., 2010).

2.1 a) Arylpiperazine derivatives related to quipazine

The literature survey reveals quipazine, 2-(1-piperazinyl) quinoline (Figure-10), an arylpiperazine derivative, as a widely used pharmacological tool and, more importantly, an interesting lead compound which can be used to develop novel serotoninergic drugs. Quipazine was found to exhibit similar pharmacological profile to that of imipramine, a tricylic anti-depressant (Rodriguez and Pardo, 1971).Several authors have reported successful medicinal chemistry effortin which quipazine was used as a molecular template for the design of novel CNS agents (Hino et al., 1980).Quipazine was used as a molecular template to design and develop new anti-depressant agents (Alhaider et al., 1985).

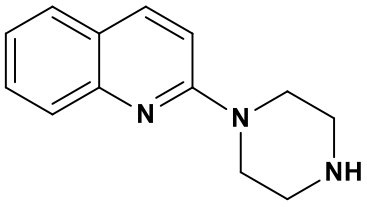
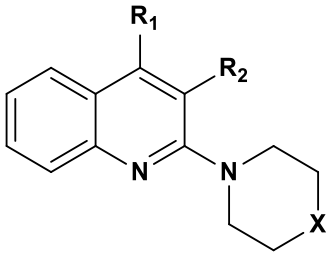


Figure 10: Structure of Quipazine.

Several studies with quipazine related compounds were done to understand the structure-activity relationships of these compounds with emphasis on the interaction with 5-HT_3 receptor.

During the last decade Cappelli and co-workers have worked extensively to develop high affinity ligands related to quipazine (general structure **2.2**) for 5-HT_3 receptor. The most significant structure activity relationship study and the interaction of these arylpiperazines with 5-HT_3 receptor were reported (Modica et al., 2010; Cappelli et al., 2002).



R1, R2= Ph, H, $COOC_2H_5$

X= N, O

2.2

Figure 11: Structure of arylpiperazine ligands related to Quipazine.

The following molecular modification strategies with quipazine derivatives were used, (a) the aromatic rings of quipazine derivatives were deleted and saturated, (b) aromatic or saturated rings of various sizes were incorporated, (c) various conformational constraints were incorporated, (d) substituents with various stereoelectronic properties such as side chains with different length and volume were introduced (e) the piperazinyl moiety of the quipazine derivatives were replaced with similar molecular fragments but with different stereo electronic properties, and finally (f) the quinoline nitrogen of the quipazine derivative was replaced by hydrogen (Cappelli et al., 2002a).

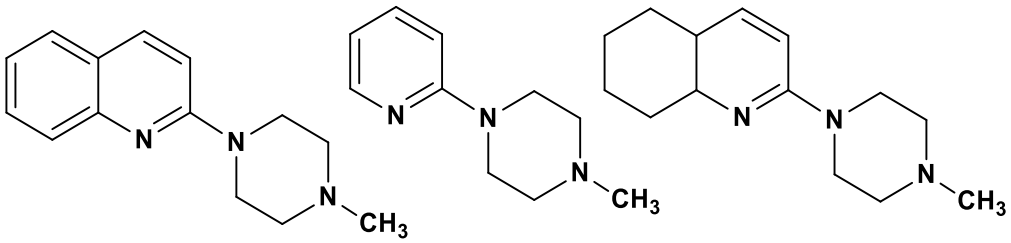
2.1.2 Structure activity relationship study and various molecular modifications of the class of quipazine derivatives

All these structural modifications on quipazine analogs were done by cappelli and group (Cappelli et al., 2002b).

(a) Modification of the Benzene Ring of the Quinoline Nucleus

The deletion of the fused benzene ring of N-methyl quipazine (NMQ) (**2.3**) to get **2.4** and its saturation to get compound **2.5.**This strategy had led to substantial lessening of the affinity towards the receptor indicating the importance of the fused benezene ring of this type of compounds in the interaction with the serorotonin type 3 receptor.Based on the above study, it was suggested that, the fused benzene skeleton of quinoline nucleus might have played dual role such as (1) π- π or π-charge interaction with specific amino acid residues located at the receptor and (2). The N atom of the quinoline ring was believed to be involved in hydrogen bond interaction with an amino acid of the receptor.

Similar activity was found in chloro and iodo derivatives of compound **2.4** with halogens at 2^{nd} position of the pyridine ring, since the halogen atoms on the pyridine ring increased both the ability to donate electron density through orbitals and the propensity of the pyridine nitrogen atom to behave as a hydrogen bonding acceptor.

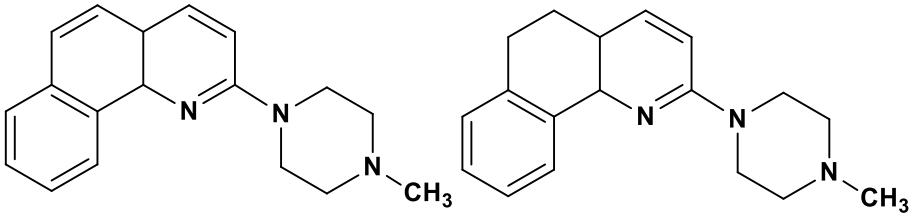


2.3 **2.4** **2.5**

Figure 12: Structure of N-methyl quipazine and it's modified derivatives.

(b) Incorporation of Additional Rings on the Quinoline Nucleus

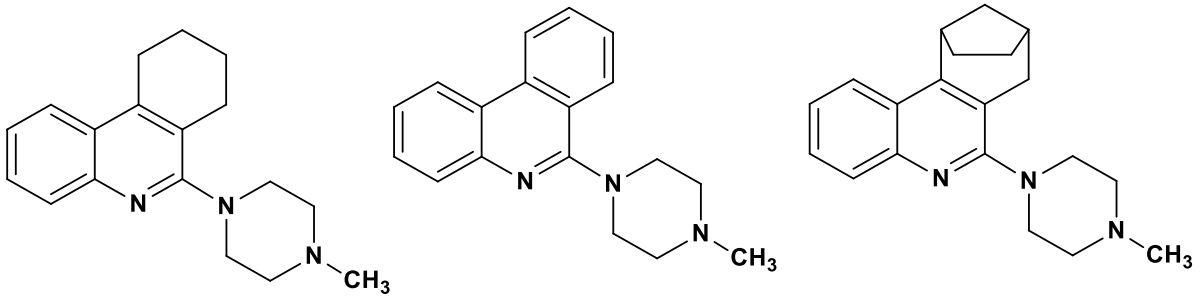
Benz-fusion on the quinoline nucleus of NMQ and **2.5** yielded compounds **2.6** and **2.7** respectively. Both compounds showed significantly decreased affinity for 5-HT_3 receptors.



2.6 **2.7**

Figure 13: Structure of NMQ and it's derivatives bearing additional rings.

The combination of a cyclohexane ring, a benzo ring, or a saturated bicyclic system on the other periphery of the quinoline skeleton of NMQ generatedcompounds **2.8**, **2.9** and **2.10** respectively.This approach had variably enhanced the compound's affinity towards the 5-HT_3 receptor.Benz-fused analog,compound **2.9** showed a 5-HT_3 receptor affinity slightly higher than that reported for NMQ, while **2.10** is found to be slightly more potent than **2.9**, and compound **2.8** showed about one time higher affinity than N-methyl quipazine(NMQ).



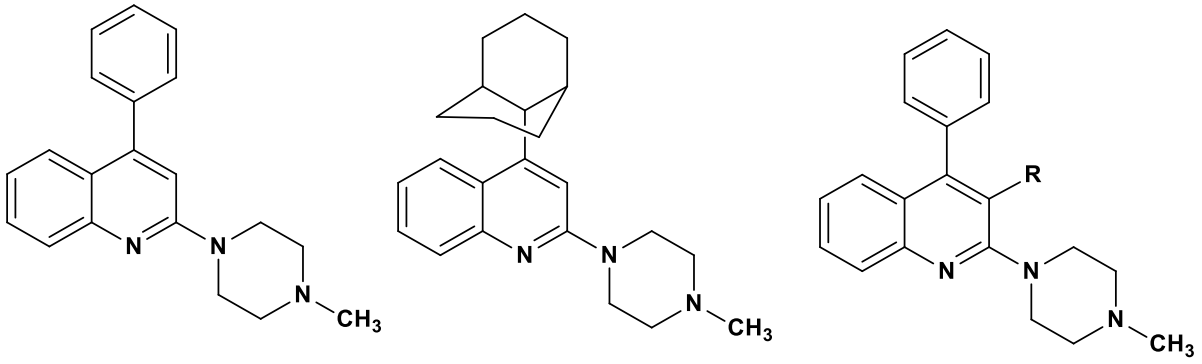
2.8 **2.9** **2.10**

Figure 14: Structure of NMQ analogs bearing additional rings on the other periphery of quinoline nucleus.

These results suggested that, larger substituents in the region of the receptor arround the edges of the quinoline ring of NMQ were not tolerated.

(d) Modification of the substituents located at the positions 3 and position 4 of the Quinoline skeleton

Compound **2.12** was generated when the phenyl group of the compound **2.11** was replacedwith 1-adamantyl group. This had led to a decrease in the affinity by 15 times. Furthermore, varieties of substituents were incorporated at position 3 of the quinoline ring of the compound **2.11** and these substituents were well tolerated and had fine tuned the affinity towards the 5-HT_3 receptor.Therefore, incorporation of a methyl group, hydroxymethyl group, or hydroxyethyl group at position 3 of the quinoline ring of the compound **2.11** hadincreased the affinity. Actually, the methyl analog ofcompound **2.13** showed a slightly higher affinity as compared to compound **2.11**, whereas the hydroxymethyl analog ofcompound **2.13** was more potent than **2.11** and hydroxyethyl derivative of **2.13** showed subnanomolar affinity and was about one time more potent than compound **2.11**.



2.11 **2.12** **2.13**

Figure 15: Structure of derivatives of NMQ bearing substituents at the position 3 and 4 of the quinoline nucleus.

(e) Modification of the Quinoline Nitrogen Atom

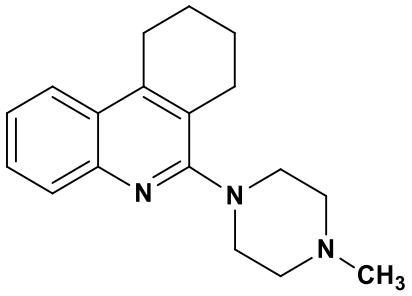
The work performed by Glennon and Colleagues on simple quipazine derivatives demonstrated that, the quinoline nitrogen of quipazine was involved in an important interaction with the 5-HT_3 receptor (Alhaider et al., 1987).

(f) Modifications of the piperazinyl Substructure

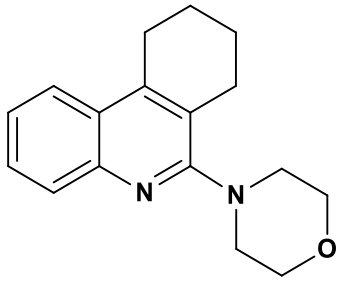
Glennon et al. studied the influence of Modifications of the piperazinyl substructure. The terminal (N4) nitrogen of piperazine of quipazine was replaced by a methylene group which resulted in drastic reduction in the affinity for the 5-HT_3 receptor, indicating that the terminal

(N4) nitrogen of piperazine of quipazine was involved in a key interaction with 5-HT_3 receptors (Dukat et al., 1996).

In order to understand whether the N atom of piperazine of compound **2.8** interacts with the 5-HT_3 receptor in it's protonated form, the terminal (N^4) nitrogen of piperazine of compound **2.8** was replaced by an isosteric oxygen atom since oxygen is not protonated at physiological pH to obtain compound **2.14**. Compound **2.14** was about five times less active than compound **2.8**.This effect showed the importance of the protonation of terminal (N4) nitrogen of piperazine in the interaction with the receptor.



a. 2.8



b. 2.14

Figure 16: a. Structure of derivatives of NMQ bearing additional cyclohexane ring; b.structure of the same derivative with modification at the piperazinyl substructure.

Quaternization in the form of N-methylation of the N^4 nitrogen of piperazine of compound **2.8** resulted in about 56 times reduction in the affinity towards 5-HT_3 receptor, which was in accordance with the previous experiments on other aryl piperazine derivatives (Dukat et al., 1996; Anzini et al., 1993; Rault et al., 1996).

Above mentioned studies on various quipazine derivatives generated a considerable amount of data on structure activity relationship showed the significance of the distal nitrogen of piperazine, the bicyclic aromatic ring, and the N atom of aromatic moiety that acts as a hydrogen bond acceptor for interaction and high-affinity binding with the 5-HT_3 receptor.

Based on the above mentioned studies, Cappelli and group derived a three-component model (**figure 17**) for the interaction of quipazine type aryl piperazine type of compounds with 5-HT_3 receptor (Cappelli et al., 2002).

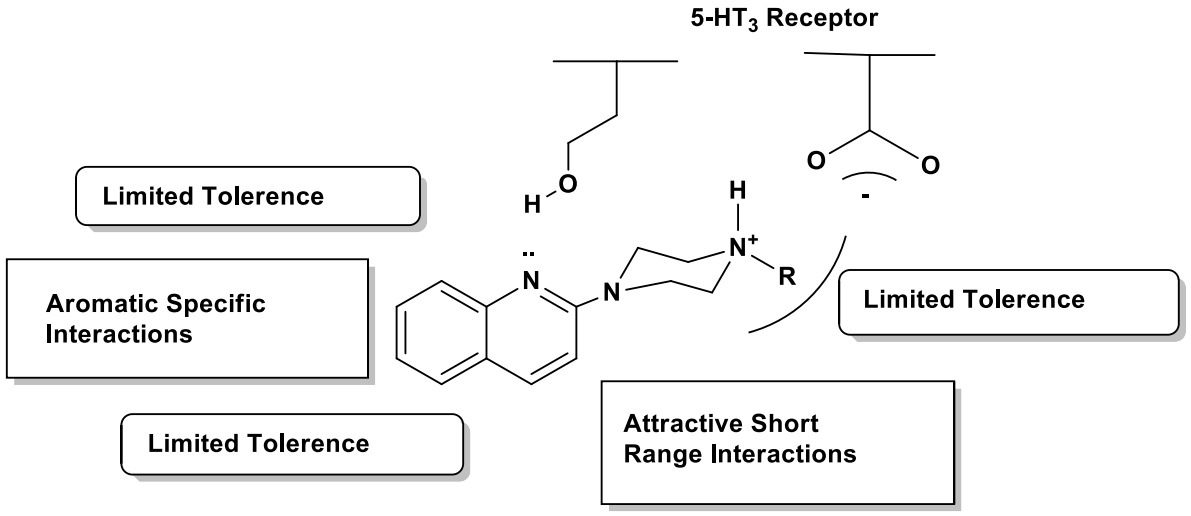


Figure 17: Interaction model of arylpiperazines with 5-HT_3 receptor proposed by Cappelli et al., 2002.

According to the above mentioned model, the arylpiperazine type of compounds interacted with the central 5-HT_3 receptor in the following manner:

(1) The terminal (N^4) nitrogen of piperazine in it's protonated form and negatively charged carboxylic group of an amino acid residue located at the receptor, were involved in a charge-assisted hydrogen bond interaction.

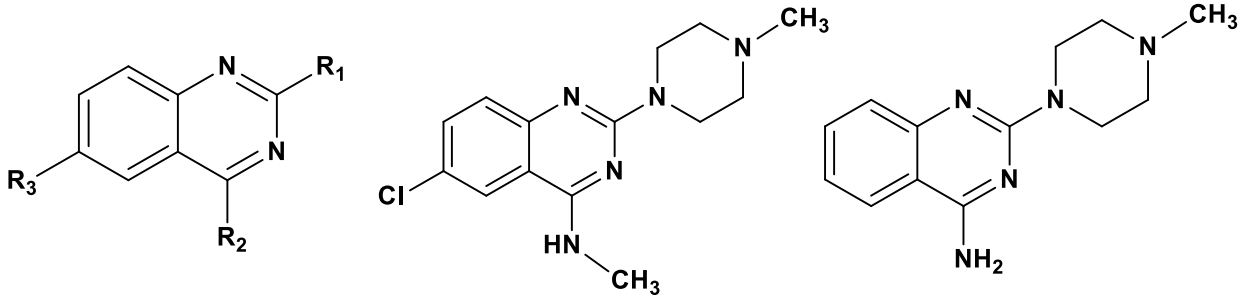
(2) The nitrogen atom of a heterocylic ring and an appropriate hydrogen bond donor located at the receptor took part in a hydrogen bond interaction.

(3) The aromatic moiety and a specific amino acid of the receptor were involved in an aromatic interaction (Cappelli et al., 2002).

In the completed pharmacophore model, around the fused benzene ring as well as the N^4 of piperazine of quipazine, restricted tolerance to bulky substituents were observed (Cappelli et al., 1998). Aryl piperazines which can be considered as quipazine analogs are discussed below. Quipazine analogues were classified as multicyclic aryl piperazine 5-HT_3 receptor ligands by Anzini and co-workers (Cappelli et al., 2002d).The structure of these arylpiperazine derivatives were divided into two categories namely: a) the N^4 of piperazine ring which was protonated at the physiological pH and b) the aromatic moiety, which was mostly a nitrogen containing heterocyclic ring connected to the piperazine ring through a "pseudoamidinic" bond. All these pharmacophoric requirements were fulfilled by the all these classes of ligands which are discussed below.

2.1.3. Quinazoline based aryl pipearzines

Recently, Verheij et al., (2012) have synthesized series of quinazoline based compounds (general structure **2.15**) and screened for 5-HT_3 receptor affinity using radioligand competition assay using [^{3}H] granisetron. The study reported quite a few high affinity ligands among which compound **2.17** displayed the maximum affinity (pK_i 10.29) followed by compound **2.16** (pK_i 8.95) for the 5-HT_3 receptor (Verheij et al., 2012).



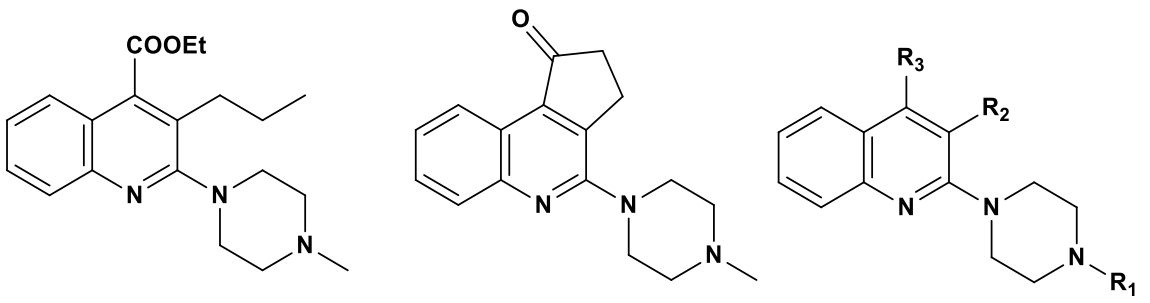
2.15 **2.16** **2.17**

Figure 18: Structure of quinazoline scaffold based aryl piperazines.

2.1.4. Quinoline based aryl pipearzines

Cappelli et.al,, (2005) had synthesized some structurally novel aryl-piperazine containing lipophilic moieties and determined their affinity for the 5-HT_3 receptor. In competitive radioligand binding experiment most of the synthesized compounds displayed sub-nanomolar binding affinity towards 5-HT_3 receptors. **2.18** was found to be the most active compound in picomolar concentration (K_i= 0.080 ± 0.02 nM) (Cappelli et al., 2005).

Most of the synthesized compounds (general structures **2.19** and **2.20)** showed sub-nanomolar 5-HT_3 receptor affinity. Compound **2.19** (**2.19a**, ,K_i = 2.1 nM) exhibited better results with respect to the affinity 5-HT_3 receptor with an increase of about one fold when compared with the compounds of general structure **2.20**. Compound **2.20a** having methyl substitution at N^4 of piperazine and carboxamide linkage at the 4th position of quinoline ring showed moderate affinity towards 5-HT_3 receptor. Among the **2.20** series compound **2.20c** having methyl substitution at N^4 of piperazine and ethyl ester group at 4rd position of quinoline ring (K_i = 0.080 nM) was found to be the most potent 5-HT_3 receptor ligand.



2.18

2.19

2.19a: X=N; R=CH_3

2.20

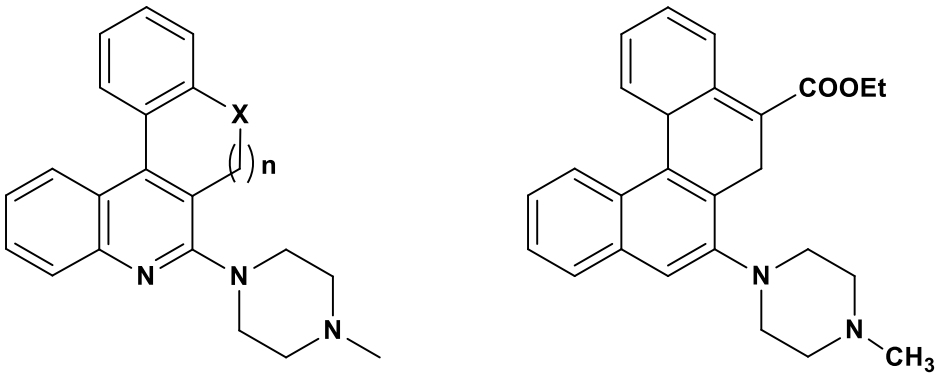
2.20a: R_1=R_2=CH_3
R_3=CON (n-C_3H_7)$_2$

2.20b: R_1=CH_3; R_2=C_2H_5

2.20c: R_1=CH_3; R_2=n-C_3H_7
R_3=$COOC_2H_5$

Figure 19: Structure of quinoline scaffold based aryl piperazines.

Cappelli et al., (2002) also had synthesized a series of compounds in which the phenyl ring at the position 4 of the quinoline ring of quipazine was bridged to the 3-position by means of different atomic groups spanning one to three atoms and tried to constrain the phenyl group in different orientations with respect to the quinoline moiety. The approach had yielded few potent and selective 5-HT_3 receptor antagonists **2.21a-c** and **2.22** shoarm very similar affinities with respect to that of quipazine and, with an improved selectivity (Cappelli et al., 2002c; Anzini et al., 1995).



2.21

2.22

2.21a: X = O, n = 1

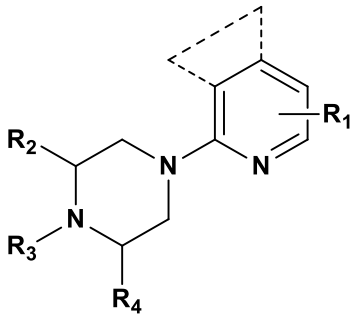
2.21b: X = O, n = 2

2.21c: X = CH_2, n = 2

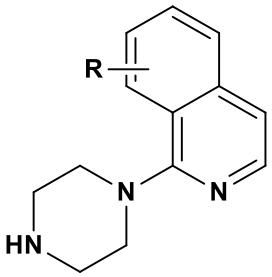
Figure 20: Structure of quipazine analogs bridged to the 3-position of quinoline ring.

2.1.5. Isoquinoline based aryl piperazines

Scientists at ASKA Pharmaceutical Co. had synthesized a new series of piperazinylpyridine analogs with common structure **2.23**. These compounds had close resemblance to quipazine, in which an isoquinoline scaffold was incorporated instead of quinoline ring.



2.23



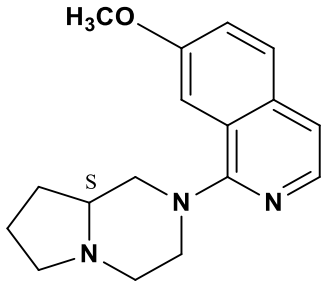
2.24

2.24a: R=H; **2.24b:** R=7-OCH_3

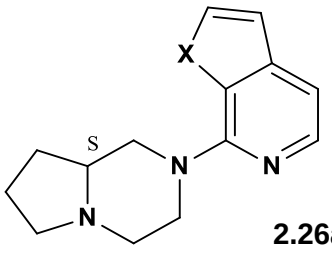
2.24c: R=7-Cl

Figure 21: Structure of quipazine analogs with isoquinoline ring.

These derivatives exhibited dual activity as 5-HT_{1A} receptor agonists and 5-HT_3 receptor antagonists and are indicated in the management of irriTable bowel syndrome (IBS). Compound **2.24a**, showed 74.6% inhibition for 5-HT_{1A} receptor and 95.6% inhibition for 5-HT_3 receptor at 10^{-7} M concentration,using [3H]-8-OH-DPAT and [3H]-BRL-43694 in radioligand experiments. Subsequently, this compound was selected as a lead for further optimization. Introduction of substituent at the position 7 of isoquinoline ring increased the binding to 5-HT_{1A} receptor as well as 5-HT_3 receptor.Methoxy substituent or halogen substituent were better as evidenced from compounds **2.24b** and compound **2.24c**. The diazabicyclo-type compound **2.25** showed promising affinity towards both 5-HT_{1A} as well as 5-HT_3 receptors (80.2% and 91.8% inhibition at 10-8 M for 5-HT_{1A} and 5-HT_3, respectively).



2.25



2.26

2.26a: X=O; TZB-20810

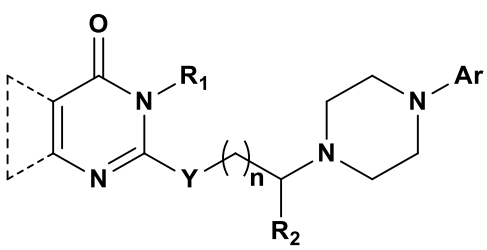
2.26b: X=S

Figure 22: Structure of diazabicyclo-type quipazine analogs with isoquinoline ring.

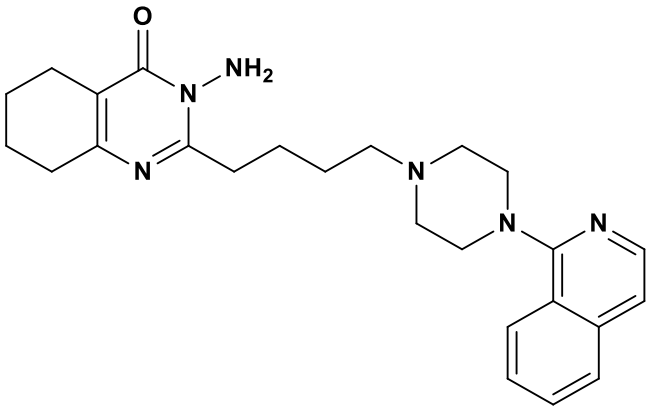
When isoquinoline moiety was replaced by different other aryl groups, compounds with lesser affinity were obtained. Whereas furano derivative compound **2.26a** or thieno [2,3-*c*]pyridine derivative compound **2.26b** were the exceptions (Sato et al., 2005; Asagarasu et al., 2009).

2.1.6. Pyrimidine based aryl piperazines

The same ASKA Pharmaceutical Co., research group also filed a patent which claimed a series of novel pyrimidines (general structure **2.27**) as potential 5-HT_3 receptor antagonists. They synthesized more than one hundred compounds and screened those compounds. Most of the compounds exhibited more than 90% 5-HT_3 receptor antagonism at 10^{-7} nM concentration.However, the most promising compounds was found to be **2.28** or TBZ-30878 (Sato et al., 2005a).



2.27

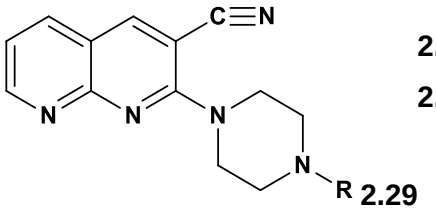


2.28; TZB 30878

Figure 23: Structure of Pyrimidine scaffold based aryl piperazines.

2.1.7. 1,8 naphthyridine based aryl piperazines

Mahesh and Perumal had synthesized a series of novel 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (general structure **2.29**) by microwave irradiation and conventional heating. The intermediate, 2-oxo-1,2-dihydro-1,8-naphthyridine-3-carbonitrile 3, was prepared from 2-aminonicotinaldehyde 1 and ethyl cyanoacetate 2 in the presence of piperidine under solvent free condition. The synthesized compounds were evaluated for 5-HT3 antagonisms in longitudinal muscle-myenteric plexus (LMMP) preparation from guinea pig ileum against 5-HT_3 agonist, 2-methyl-5-HT. The compounds **2.29a** and **2.29b** (pA_2 = 7.4 and 8.2, respectively) showed prominent antagonism when compared with ondansetron (pA_2 = 6.9) (Mahesh et al., 2004).



2.29

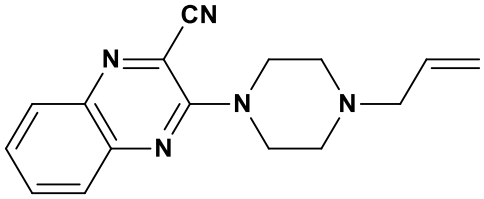
2.29a: R=CH_3

2.29b: R=$CH_2CH=CH_2$

Figure 24: Structure of 1,8 naphthyridine scaffold based aryl piperazines.

2.1.8. Quinoxaline based arylpiperazines

Monge and Colleagues (1993) had synthesized and evaluated the 5-HT_3 receptor antagonistic potential of piperazinyl quinoxalines where, cyano quinoxalines bearing alkyl substituent on the distal nitrogen of piperazine showed better antagonism. Compound **2.30** was found to be the most active agent among the synthesized compounds.Compound **2.30** showed greater antagonism than that of standard drugs tropisetron and ondansetron in *in-vitro* functional assays in guinea pig ileum (Monge et al., 1993).

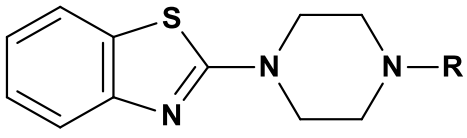


2.30

Figure 25: Structure of quinoxaline scaffold based aryl piperazines.

2.1.9. Benzothiazole based aryl piperazines

Monge et.al., (1994) had synthesized a series of 2-piperazinylbenzothiazole (general structure **2.31**) derivatives as 5-HT_3 antagonists.Compounds, **2.31a** and **2.31b** displayed greater antagonism than that of standard drug, ondansetron in functional assay in the LMMP preparation of the guinea-pig ileum. On the other hand, the binding affinity profile of these two compounds in radioligand binding assay and 5-HT_3 receptor antagonism in B-J reflex test were less potent than ondansetron (Monge et al., 1994).



2.31

2.31a: R=Benzyl

2.31b: R=piperonyl

Figure 26: Structure of benzothiazole scaffold based aryl piperazines.

2.2. Tropane and quinuclidine ligands related to the "classical" 5-HT_3 receptor antagonists

Compounds illustrated in this segment, consist of three general pharmacophoric features such as: a carbonyl group located between an aromatic/heteroaromatic skeleton and a basic nitrogen part. Hibert and co-workers had performed a conformational-activity relationship study for all the well known 5-HT_3 receptor antagonists like SDZ 206-792, BRL 43694, SDZ-206-830, ICS 205-930, GR 65630, GR 38032, BRL 24924, MDL 73147, metaclopramide, MDL 72222 and MDL 72422 (Hibert et al.,1990). Based on these studies, the generally recognised pharmacophore of 5-HT_3 receptor antagonists was defined by Hibert and group, which comprised of an aromatic group, an intervening carbonyl group and a basic part located at specific distances.The approximate distances between the pharmacophoric elements were identified; ~3.3 Å for centroid of aromatic to the oxygen of carbonyl group, ~5.2 Å for oxygen of carbonyl to basic centre and ~6.7 Å for centroid of aromatic to basic centre (nitrogen) (**figure 27**).The study also revealed that, the quaternarization of basic nitrogen centre results in improved 5-HT_3 receptor antagonism indicating that the 5-HT_3 receptor antagonists are more likely to bind with 5-HT_3 receptor in the protonated form. This model was behind the emergence of most of the well known 5-HT_3 receptor antagonists, named as "classical" or "setron" class of compounds (**figure 28**). These compounds were found to possess three common pharmacophoric features such as: a carbonyl group which was coplanar to the aromatic or heteroaromatic scaffold usually of 6- or 5-member nucleus size or a heterobicyclic ring and basic nitrogen of amine part.

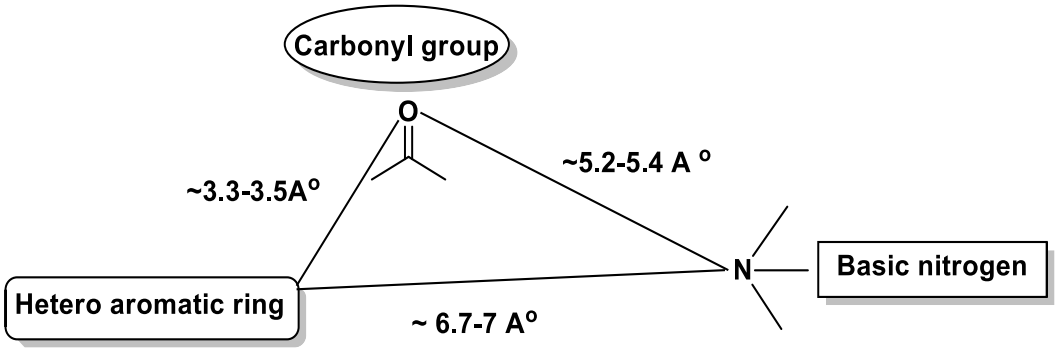


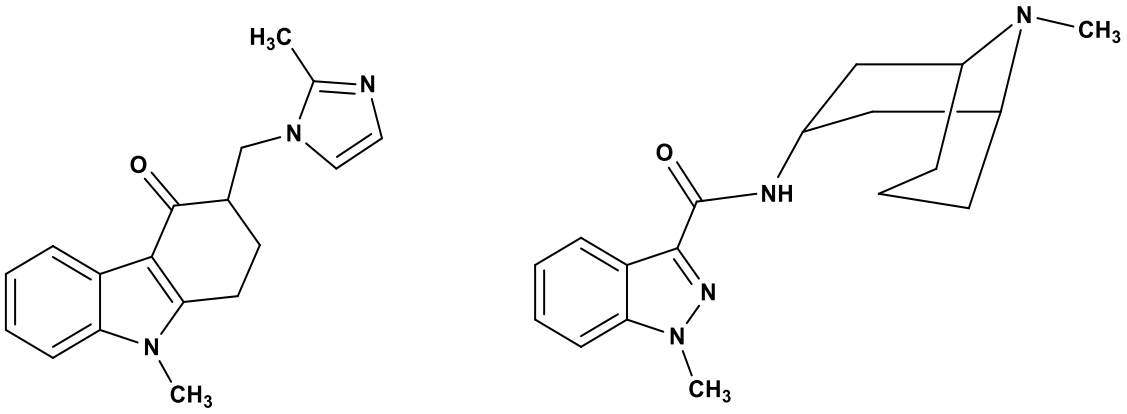
Figure 27: Hibert proposed pharmacophore model (Hibert et al.1990).

Over the years several computational studies mainly superimposition and docking studies and extensive structure-activity relationships (SARs) studies of 5-HT_3 receptor antagonists were carried out which had supported the above mentioned pharmacophore model for 5-HT_3

receptor antagonists (Gaster and King, 1997; Thompson and Lummis, 2006; Morreale et al., 2002; Reeves and Lummis, 2002). The interactions between these above mentioned pharamacophoric elements and specific amino acid residues of the 5-HT_3 receptor were reported (Maksay et al., 2003). Docking of 5-HT_3 receptor antagonists such as Granisetron, Tropisetron, Ondansetron, and Dolasetron into the homology modelled 5-HT_3 receptor were carried out by several researchers (Verheij et al., 2012; Lummis, 2012; Andrew et al., 2005; Andrew et al., 2006; Sullivan et al., 2006; Joshi et al., 2006).

5-HT_3 antagonists such as ondansetron, granisetron, and tropisetron (Figure 3) all were docked into the homology model of the 5-HT_3 receptor. All these molecules contain aromatic ring, carbonyl group, and basic nitrogen. The carbonyl groups of all these molecules were fixed in coplanar conformation with the aromatic rings in accordance with the energy minimizations and also with the proposed pharmacophore model. The lowest energy of docking of granisetron, ondansetron, tropisetron, and dolasetron showed that, the interactions of these compounds with 5-HT_3 receptor were analogous. The planer aromatic rings of these 5-HT_3 receptor antagonists form π–π interactions with tyrosine (Y) 143, tyrosine (Y) 153, tyrosine (Y) 234 of 5-HT_3 receptor and especially with Tryptophan (W) 183 (Morreale et al., 2002a; Spier and Lummis, 2000).

The carbonyl groups of the ‘setrons’ are involved in H-bonding interaction with serine (S) 227. The basic aza-bicyclic rings and/or imidazole ring (in case of ondansetron) were found to interact with glutamic acid (E) 236, glutamic acid (E) 129 or asparagine (N) 128, and/or tryptophan (W) 90.The docking conformations of the ’setrons were analogous to the hibert proposed pharmacophore model of 5-HT_3 receptor antagonists (Morreale et al., 2002b). In accordance with the above mentioned pharmacophore model, the distance between the hetero aromatic ring centre and the basic nitrogen atoms is about 7Å; between the oxygen of the carbonyl group and the basic nitrogen is about 5Å; whereas the carbonyl oxygen is within 3Å above the plane of the aromatic rings. These values were found to be valid for all favorable dockings in 5-HT_3 receptor model (Morreale et al., 2002c). Presently, these setrons/ classical 5-HT_3 receptor antagonists have attracted much attention in the treatment of chemotherapy-induced nausea and vomiting (CINV), and are the gold standards with few adverse side effects (Hesketh, 2008; Gan, 2007; Herrstedt and Dombernowsky, 2007). CINV has been classified into two main classes: acute phase which happens within the first 24 hours of chemotherapy and delayed phase which starts from 24 to 120 hours following the chemotherapy.



Ondansetron Granisetron

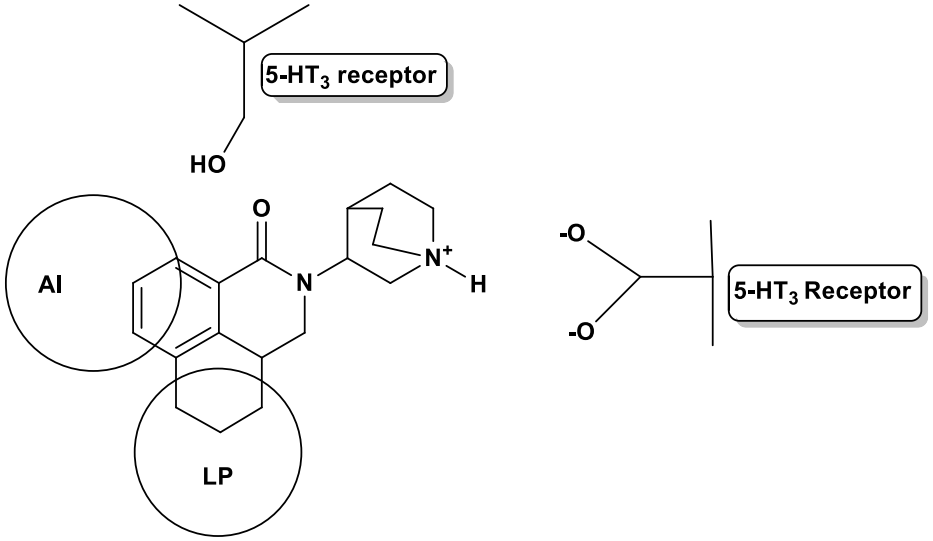
Figure 28: Structures of commercially available 'Classical' 5-HT_3 receptor antagonists- 'setrons'.

Ondansetron(Zofran®), tropisetron(Navoban®), granisetron(Kytril®), dolasetron(Anzemet®), and recently developed palonosetron(Aloxi®) (**figure 28**) marketed in India as well as USA and a numerous other countries, whereas ramosetron(Nozia®) and azasetron(Serotone®) marketed only in Japan are being used to treat Acute phase CINV (Modica et al., 2010).

Delayed phase nausea remains a highly under treated side effect of cancer chemotherapy. Till date, all the commercially available 5-HT_3 receptor antagonists, except palonosetron, are used to prevent only acute phase CINV. Palonosetron, the latest 5-HT_3 receptor antagonist which is approved to treat delayed phase CINV.Palonosetron is probably effective in treating delayed phase CINV because of it's long serum half-life (Eisenberg et al., 2003; Navari, 2003).

From a structural point of view the quinuclidine derivatives described by Clark can be considered as classical 5-HT_3 receptor antagonists since they contain the generally recognized pharmacophore, i. e. basic nitrogen, carbonyl group, and aromatic ring. Clark and co-workers had developed a pharmacophoric model for quinuclidine derivatives related to Palonosetron **2.32** (Clark et al., 1993). The structure of palonosetron and the proposed interactions with 5-HT_3 receptor are shown in **figure 29**. Several modifications and design strategies were used in Clark's model. In particular, the following molecular modification strategies were adopted (a) introduction of aromatic rings, (b) introduction of heteroaromatic nitrogen atom, which could behave as an additional hydrogen bonding acceptor, (c) introduction of substituents showing various stereoelectronic properties such as side chains showing different length.

(d) Replacement of the quinuclidine moiety with a similar molecular fragment showing different stereoelectronic properties (e. g. the tropane moiety).

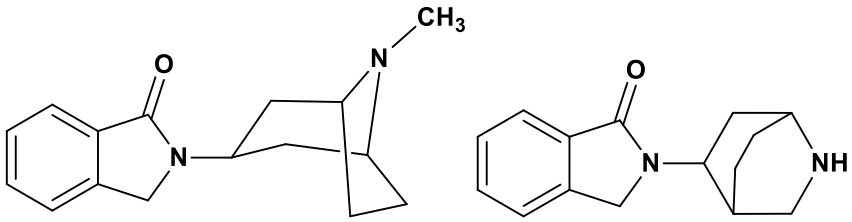


2.32 AI= Aromatic Interaction, LP=Lipophilic pocket

Figure 29: Interaction model for quiniclidine derivatives with 5-HT_3 receptor proposed by Clark et al., 1993.

2.2.1. Structure activity relationship study and various molecular modifications of the class of tropane and quinuclidine derivatives.

The simplest tropane derivative **2.33** and quinuclidine derivative **2.34** exhibited a relatively high 5-HT_3 receptor affinity.The structure of these compounds can be formally divided into two substructures: a) the heteroaryl moiety,which incorporated the carbonyl group (H-bond acceptor) and b) the azabicyclic moiety,which incorporates a nitrogen atom protonated at physiological pH.



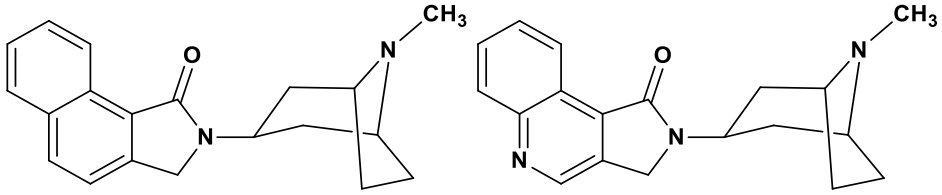
a. 2.33 **b. 2.34**

Figure 30: a. Structure of tropane derivative b. structure of quinuclidine derivative.

Following molecular modification, strategies were employed by Cappelli and group (Cappelli et al., 2002): (a) introduction of aromatic rings, (b) introduction of heteroaromatic nitrogen atom, which behaved as a supplementary hydrogen bonding acceptor, (c) introduction of substituents with different stereoelectronic properties such as side chains with different length and (d) change of the quinuclidine skeleton with the tropane moiety which had similar molecular segment but with different stereoelectronic properties.

(a) The Introduction of Aromatic Rings.

The incorporation of an extra benzene ring on the various edges of the homocyclic part of the bicyclic ring of tropane derivative **2.33** (benz-fusion), enhanced the 5-HT_3 receptor affinity. For example, compound **2.35** was found to be about 2 times more active than compound **2.33**.



2.35 **2.36**

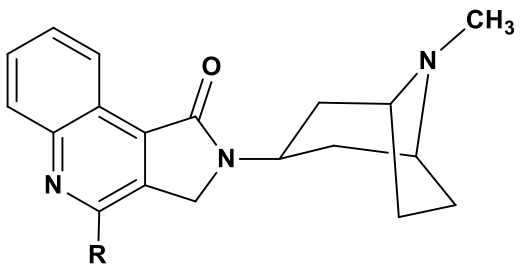
Figure 31: Structure of a.tropane derivative with additional aromatic ring b. structure of quinuclidine derivative with an additional aromatic ring.

(b) The Introduction of the Hetero aromatic Nitrogen Atom.

The incorporation of a heterocyclic nitrogen atom into the benz[e]isoindol-1-one nucleus of **2.35** to obtain the pyrrolo [3,4-c] quinolin-1-one heterocycle of **2.36** did not alter the 5-HT_3 receptor affinity significantly.

(c) The Introduction of substituents showing various stereoelectronic properties.

The incorporation of different substituents at the position 4 of the pyrrolo [3, 4 c] quinolin-1-one skeleton of compound **2.36** (compounds **2.37a-d**) produced very minor variations in the affinity for 5-HT_3 receptor. This suggested that lipophilic substituents were easily accommodated into the receptor area interacting with 4-position of the tricyclic system. This area could be identified either with the lipophilic pocket proposed by Clark (Clark et. al., 1993) or with the attractive short-range interaction zone proposed in the model for arylpiperazine ligands.



2.37

2.37a: R=Cl.

2.37b: R=OCH_3.

2.37c: R=OC_2H_5.

2.37d: R=OH.

Figure 32: Structure of a.tropane derivative with additional aromatic ring and nitrogen atom bearing various substituents.

(d) Modification of azabicyclic moiety.

Effects of the replacement of the quinuclidine azabicyclic system with the tropane moiety were found to be governed by the stereoisomery of the quinuclidine moiety attachment to the heteroaryl substructure. The tropane endo-stereochemistry was favorable for the interaction with the 5-HT_3 receptor, since the alteration of the endo-stereochemistry of **2.33** into the exo-stereo chemistry produced a 29 times reduction in affinity (Hayashi et al., 1993).

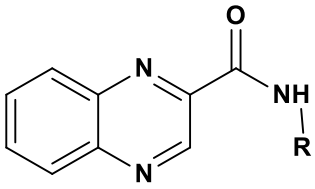
2.2. Arylcarboxamide derivatives as 5-HT_3 receptor antagonists

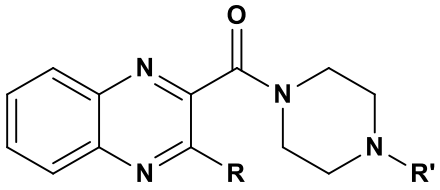
5-HT_3 receptor antagonists described below can be considered as tropane and quiniclidine derivatives related to classical 5-HT_3 receptor antagonists and these are aryl carboxamides bearing heterocyclic nucleus, among which benzimidazole, quinoline, indole and quinoxaline and benzoxazole are most common representatives.

2.2.2.1 Quinoxaline carboxamides

Mahesh et.al, (2010) had synthesized some quinoxaline carboxamides (general structures **2.38** and **2.39**). All these compounds have quinoxaline aromatic core, carbonyl group and basic moiety in the form of piperazine and aniline carboxamides as pharmacophoric elements. Four compounds (**2.38a**; pA_2 7.6, **2.38b:** pA_2 7.3; **2.39a:** pA_2 7.6, **2.39b:** pA_2 7.0) showed promising 5-HT_3 receptor antagonism in guinea-pig longitudinal muscle myenteric plexus preparation using 2-methyl 5-HT as agonist. All the compounds were evaluated for their anti-depressant potential in mouse model of forced swim test.

Compounds with higher pA_2 values were subjected to anxiolytic screening in mouse model of elevated plus maze and open field test.Compounds **2.38b** and **2.39b** exhibited prominent anxiolytic activity in both model (Mahesh et al., 2010).





2.38

2.38a: R=3-Cl-2-CH_3-C_6H_3

2.38b: R= $CH_3CH_2CH_2CH_2$

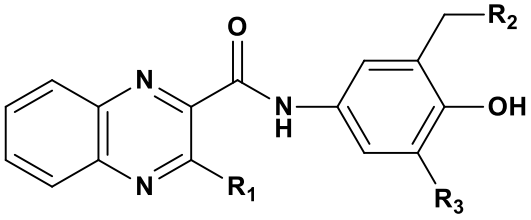
2.39

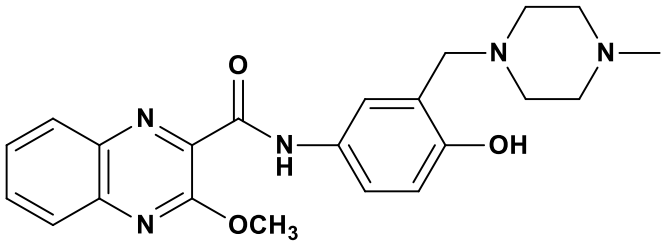
2.39a: R=OC_2H_5**;** R'=2-OCH_3-C_6H_4

2.39b: R=OC_2H_5; R'=CH_3

Figure 33: Structure of quinoxaline carboxamides as 5-HT_3 receptor antagonists.

Mahesh and perumal had synthesized 3-substituted quinoxaline-2-carboxamides derivatives General structure **2.40**. The carboxamides were formed using quinoxaline carboxylic acids and appropriate anilines and mannich bases of the anilines. The synthesized molecules were designed based on the three point pharmacophoric requirements of 5-HT_3 receptor antagonists. In the compounds synthesized, aromatic and basic nitrogen pharmacophore are linked via Mannich base (paminophenol derivatives). In the guinea pig LMMP functional assay, all the screened compounds displayed moderate to weak antagonist activity.Compound **2.41** was found to be the most active compound among the synthesized carboxamides, with a pA_2 value of 5.7 (Perumal and Mahesh, 2006).





2.40

2.41

R_1= OCH_3, OC_2H_5

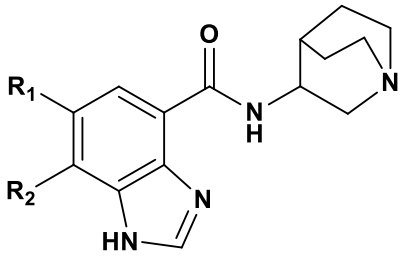
R_2= Piperazine, substituted piperazines

R_3= H, CH_3, OCH_3

Figure 34: Structure of quinoxaline carboxamides as 5-HT_3 receptor antagonists.

2.2.2.2. Benzimidazole carboxamides

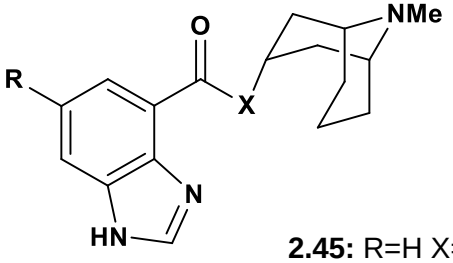
Lopez-Rodriguez and his colleagues had synthesized some benzimidazole-4-carboxylic derivatives, amides and esters as 5-HT_3 receptor antagonists. In structure-activity relationship study, compounds with no substitution or halogen substituent at position 6 and NO_2 substitution at position 7 on the benzimidazole nucleus exhibited better 5-HT_3 receptor affinity and selectivity profiles. Among all the synthesized compounds, compounds **2.42**, **2.43** and **2.44** showed better affinity (K_i= 2.6, 0.13, and 1.7 nM, respectively) and antagonistic profile (pA_2 = 9.6, 9.9, and 9.1, respectively) in the guinea-pig ileum preparation (Lopez-Rodriguez et al., 1999).



2.42: R_1= R_2 = H

Figure 35: Structure of benzimidazole4carboxamides as 5-HT_3 receptor antagonists.

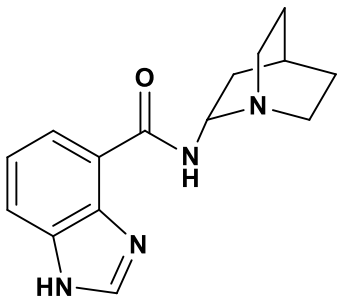
Same group had also reported the synthesis and 5-HT_3 and 5-HT_4 receptor affinities of some benzimidazole-4-carboxylic acids, esters and amides. All the compounds showed moderate to high affinity towards 5-HT_3 receptors with not much affinity for 5-HT_4 receptors. Compounds, **2.45, 2.46** and **2.47** exhibited greater affinity towards 5-HT_3 receptors (K_i= 6.1, 3.7 and 4.9 nM, respectively). These compounds did not exhibit significant affinity towards other subtypes of 5-HT receptor, 5-HT_4 and 5-HT_{1A} (K_i> 1000 nm and K_i > 10,000 nM, respectively) in radioligand binding studies. The compound **2.47** displayed 5-HT_3 receptor antagonism in the light-dark test (Lopez-Rodriguez et al., 1996).



2.45; 2.46

2.45: R=H X=NH

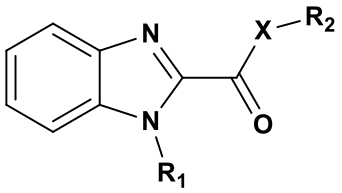
2.46: R=Cl, X=O



2.47

Figure 36: Structure of benzimidazole 4- carboxamides and ester analogs as 5-HT_3 receptor antagonists.

Researchers at Faes Farma pharmaceutical company synthesized a new class of benzimidazole-2-carboxamide and ester derivatives with the general structure **2.48**, selective towards 5-HT_3 receptor over 5-HT_4 receptor and D_2 receptor (Orjales et al., 1999). Particularly, compounds **2.49** (*K*i = 12.7 nM), **2.50**(*K*i =18.4, nM)**, 2.51**(*K*i =20.2 nM) showed good affinity for 5-HT_3 receptor.While considering the substituent effect on benzimidazole ring, the isopropyl substituent **2.49** was found to be better than the benzyl substituent **2.50** and, with respect to the carbonyl linker, the carboxamide **2.49** was found to be better than the ester counterpart **2.51**. In Bezold Jarisch reflex (BZR) assay these compounds showed moderate 5-HT_3 receptor antagonism.

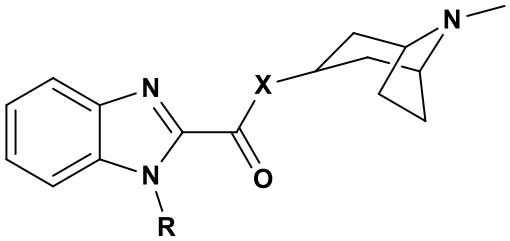


2.48

R_1= Isopropyl, benzyl

X=NH, O

R_2= Tropane



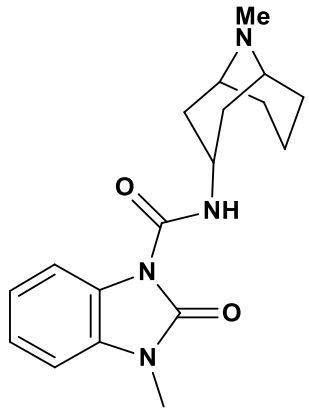
2.49: X=NH, R=isopropyl

2.50: X=NH, R=benzyl

2.51: X=O, R=isopropyl

Figure 37: Structure of benzimidazole 2-carboxamides and ester analogs as 5-HT_3 receptor antagonists.

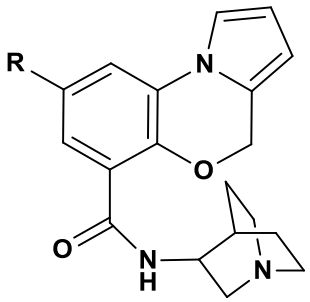
Turconi et al., (1990) had synthesized some 2, 3-dihydro-2-oxo-1Hbenzimidazole-1-carboxamides and esters with basic azacyclo or azabicycloalkyl moieties as 5-HT_3 antagonists.The carboxamides were found to be more active as compared to the corresponding ester analogs. In competitive radioligand binding study as well as in 5-HT-induced B-J reflex test, compound **2.52** (K_i= 0.89 ± 0.42 nM, ED_{50} = 1.5 nM/kg, i.v.) was found to be potent as compared to that of standard antagonists, MDL-72222 (K_i= 24.4 ± 5.8 nM, ED_{50}= 195 nM/kg, i.v.) and ICS 205930 (K_i= 1.71 ± 0.29 nM, ED50 = 2.1 nM/kg, i.v.) (Turconi et al., 1990).



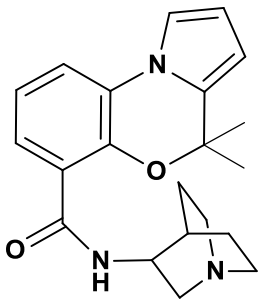
2.52

Figure 38: Structure of 2, 3-dihydro-2-oxo-1H benzimidazole- 1-carboxamide as 5-HT_3 receptor antagonists.

Kato et al., (1995) had synthesized novel heterocyclic carboxamides, general structure **2.53** and evaluated the 5-HT_3 receptor antagonistic potential.Compounds with small and lipophilic substituents (chloro, compound **2.54**/methyl, compound **2.55**) on the 8th position of aromatic ring maintained high potency. However, compounds with bulky substituent did not show activity. A gemdimethyl group at the 4th position of aromatic ring, compound **2.56** slightly decreased the antagonistic potency. Compounds derived from the 1 azabicyclo[2.2.2] octan-3-amine showed most potent activity compared to other amine derivatives. Compound **2.53** was found to be the most potent antagonist (ED_{50}:0.4 µg/kg i.v.) and 40 times more potent than ondansetron (ED_{50} = 17.5 µg/kg i.v.) in B-J reflex test (Kato et al., 1995b).



2.53



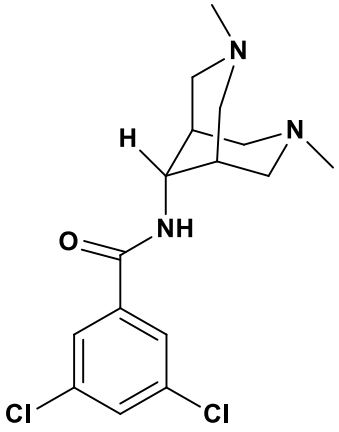
2.56

2.54: R=Cl

2.55: R=CH_3

Figure 39: Structure of benzoxazine-8-carboxamide as 5-HT_3 receptor antagonists.

Fernandez et al., (1995) synthesized a series of amide based compounds as 5-HT_3 receptor antagonists which were developed from 3, 7-dimethyl-3, 7-bicyclo [3.3.1] nonan-9-amines. Among the synthesized compounds, **2.57**was found to be the most potent compound, which was equipotent to MDL-72222, in radioligand binding assay.This test compound also showed 5-HT_3 receptor antagonism in B-J reflex test at a dose of 25mg/kg. X-ray diffraction study revealed that, the compound existed in chair conformation and N-methyl groups lie in equatorial position (Fernandez et al., 1995).



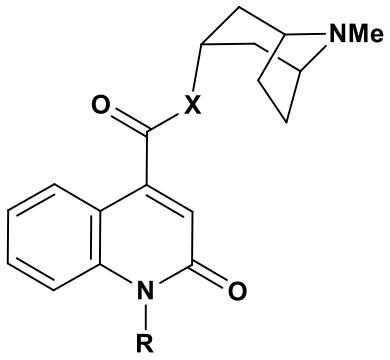
2.57

Figure 40: Structure of amide based compound as 5-HT_3 receptor antagonist which was derived from 3, 7-dimethyl-3,7-bicyclo [3.3.1]nonan-9-amines.

2.2.2.3. Quinoline carboxamides

Hayashi et al., (1992) prepared a number of esters and amides of 1-alkyl-2-oxo-1,2 dihydroquinoline-4-carboxylic acid or 2-alkoxyquinoline-4-carboxylic acid having a basic azabicycloalkyl residue. They evaluated their affinities for the [3H] quipazine-labeled 5-HT_3 receptors. 5-HT_3 receptor antagonism was evaluated using serotonin-induced B-J reflex test. Most of the esters showed ten fold more affinity as compared to the ondansetron (K_i= 7.6 nM). An increase in the affinity towards the receptor was observed when hydrophobic substituents were at position 1st or 2nd of the quinoline nucleus. Compounds **2.58** *(*K_i= 0.32 nM) and **2.59** *(*K_i=0.31 nM) exhibited the best affinity among all other compounds. Molecular modelling studies revealed that, the carbonyl group inester **2.58** or amide **2.61** was not coplanar (over 20° deviation was observed) to the plane of the aromatic moiety.

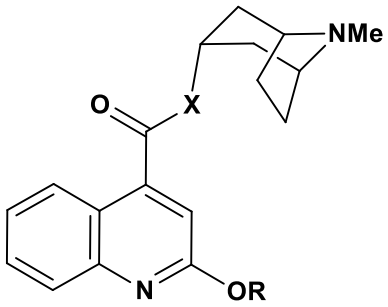
Although, a number of compounds showed potent activities in the B-J reflex experiment, proper relationship between the affinity for the 5-HT_3 receptors and the *in-vivo* activity in the B-J reflex experiment was not observed (Hayashi et al., 1992).



2.58-2.60

2.58: R = i-Pro, X = O

2.59: R = Me, X = **2.60:** R = Me, X = NH

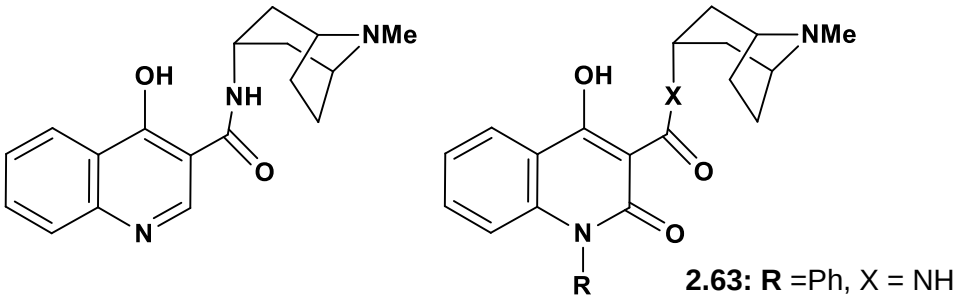


2.61

2.61: R = i-Pro, X = O

Figure 41: Structure of 1-alkyl-2-oxo-1,2 dihydroquinoline-4-carboxamide and 2-alkoxyquinoline-4-carboxamide containing a basic azabicycloalkyl residue.

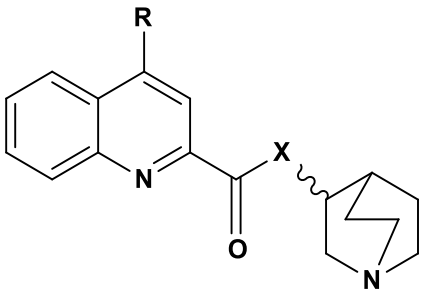
The same group also had synthesized a series of 4-hydroxy-3-quinolinecarboxamide, compound **2.62** and 4-hydroxy-2-oxo-1,2-dihydro-3-quinolinecarboxamides, compounds **2.63**, **2.64** as potential 5-HT_3 receptor antagonists.The molecular modelling studies showed that, the carbonyl group present in the 4-hydroxy-3-quinolinecarboxylic acids were coplanar with aromatic residue, whereas in the case of 2-oxo-quinoline derivatives, 30° deviation was observed. Compound **2.62** without a 2nd carbonyl group in the quinoline ring and without any substitution at the quinoline nitrogen was found to be the most active compound in B-J reflex test (ED50 = 0.1 µg/kg, i.v.). Compounds **2.63** and **2.64** having an extra carbonyl group at 2nd position of quinoline nucleous and phenyl and butyl substitutions respectively at the quinoline nitrogen turned out to be less active as compared to compound **2.62**. Although compounds such as **2.63** and **2.64** (K_i= 0.48 nM) exhibited greater affinities for the 5-HT_3 receptor in binding assay as compared to that of ondansetron with K_i=7.6 nM or granisetron with *Ki* = 2.1 nM. The later compounds displayed lesser activity in the B-J reflex experiment as compared to the reference compound (Hayashi et al., 1993).



2.62 **2.63; 2.64**

Figure 42: Structures of 4-hydroxy-3-quinolinecarboxamide and 4-hydroxy-2-oxo-1,2-dihydro-3-quinolinecarboxamides as potential 5-HT_3 receptor antagonists.

A novel class of quinoline carboxamides (general structure **2.65**) was discovered by Orjales et al., (2000). Derivatives **2.65a** and **2.65b** showed good affinity for 5-HT_3 receptor (K_i = 12.3 and 9.9 nM, respectively), and selectivity over 5-HT_4 receptor and D_2 receptor and a reasonable 5-HT_3 receptor antagonism in BJR assay (Orjales et al., 2000).



2.65a: X=NH, R=OCH3.*=R/S

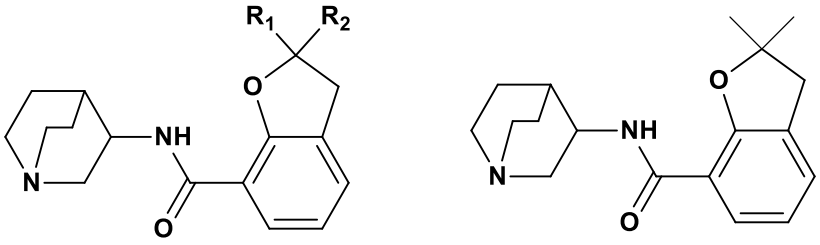
2.65b: X=NH, R=OCH3.*=S

2.65

Figure 43: Structures of 4-methoxy-2-quinolinecarboxamide as potential 5-HT_3 receptor antagonists.

2.2.2.4. Benzofuran carboxamides

Kuroita et al., (1994) had synthesized a series of *N*-(azabicyclo-3-yl)-2,3-dihydrobenzofuran-7-carboxamides (general structure **2.66**) as 5-HT_3 receptor antagonists.The 5-HT_3 receptor affinity was evaluated by radioligand binding assay and 5-HT_3 receptor antagonism in B-J reflex test. Incorporation of methyl substitution (compound **2.69**, compound **2.68**) as well as dimethyl substitution (compound **2.70**) at 2nd position of the dihydrobenzofuran ring of compound **2.67** resulted in increased 5-HT_3 antagonistic activity and the order of antagonistic activity was dimethyl compound **2.70**> (2S)-methyl compound **2.69**> (2R)-methyl, compound 2.68 > dihydro, compound 2.67. Compound **2.70** showed highest affinity for the 5-HT_3 receptors (K_i= 0.055 nM) in B-J reflex test (Kuroita et al., 1994).



2.66 **2.70**

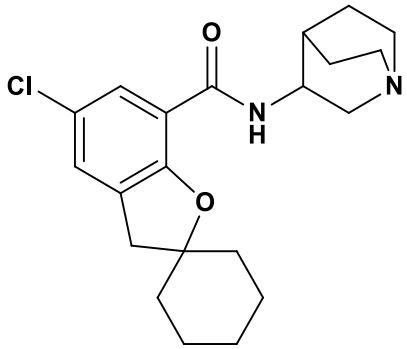
2.67: R_1=H, R_2=H

2.68: R_1=H, R_2=CH_3

2.69: R_1=CH_3, R_2=H

Figure 44: Structure of benzofuran carboxamide as potential 5-HT_3 receptor antagonists.

Youssefyeh et al., (1992) had synthesized a series of novel benzamides as 5-HT_3 receptor antagonists. S-isomer of compound **2.71** was identified as a more selective antagonist, compared to that of standard antagonists such as GR 38032F, BRL 43694, and metaclopramide. With respect to the inhibition of the binding to the binding sites of the 5-HT_3 receptor present in rat entorhinal cortex, the S-isomer of compound **2.71** (*Ki:* 0.19 nM) was the most active one. It also blocked the cisplatin-induced emesis in the ferret (ED_{50}:9 mg/kg p.o.) (Youssefyeh et al., 1992).

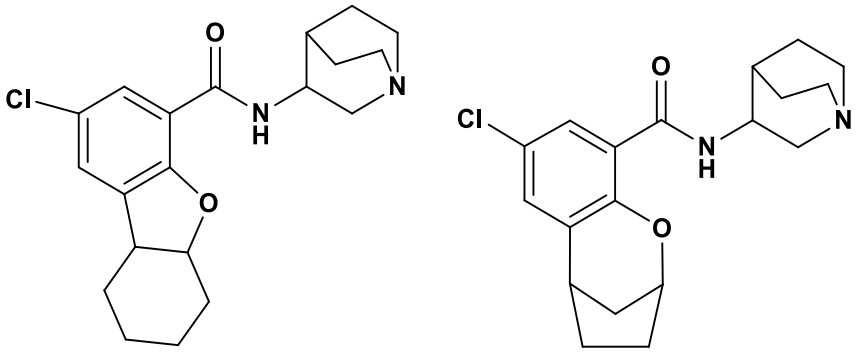


2.71

Figure 45: Structure of benzamide as potential 5-HT_3 receptor antagonists.

They also reported two series of tricyclic carboxamides as 5-HT_3 receptor antagonists. Compounds **2.72** and **2.73** showed most promising antagonistic effects in both *in-vitro* and *in-vivo* studies and these molecules also prevented the chemotherapeutic agent-induced emesis.

Compound **2.72** showed better affinity (*Ki* = 0.17 nM) in radioligand binding, antagonism (1 kg/kg i.v. in B-J reflex test, 10-9 M in 5-HT-provoked guinea-pig ileum contraction assay) as compared to standard 5-HT_3 receptor antagonists, GR 38032F, zacopride, BRL 43694, and ICS 205-930 in and *in-vitro/in-vivo* functional assays, respectively.

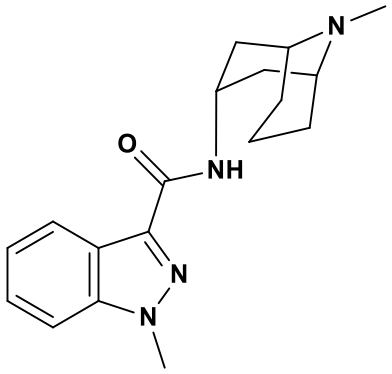


2.72 **2.73**

Figure 46: Structure of tricyclic carboxamides as potential 5-HT_3 receptor antagonists.

2.2.2.5. Indazole carboxamides

Bermudez et al., (1990) reported the synthesis of indazole carboxamides as 5-HT_3 receptor antagonists. The molecules were designed based on the pharmacophoric requirements of metaclopramide with some modification such as conformational restriction on the side chain by replacing (diethylamino) ethyl group by azabicyclic tropane and replacement of phenyl group with aforementioned heterocyclic nucleus. The obtained compounds exhibited potent 5-HT_3 receptor antagonistic activity with no dopaminergic antagonism or stimulation of gastric motility. Replacement of tropane nucleus with other azabicyclic moiety such as quinoclidine and isoquinoclinide retained the 5-HT_3 receptor antagonism in resultant compounds. Compound **2.74** was found to be the most potent and selective agent among the synthesized compounds which also showed promising anti-emetic effect in both ferret and man (Bermudez et al., 1990).

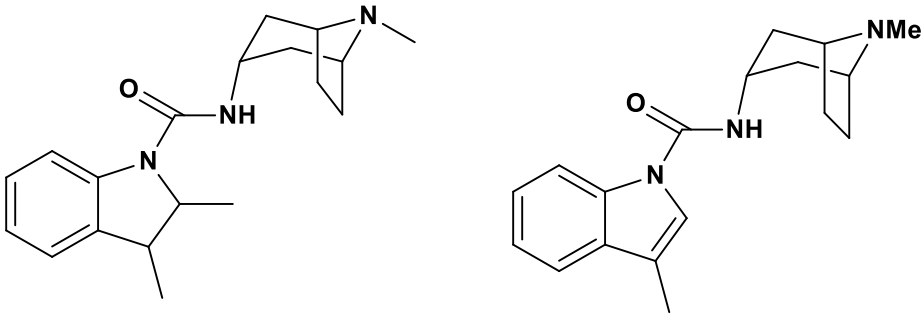


2.74

Figure 47: Structure of indazole carboxamides as potential 5-HT_3 receptor antagonists.

2.2.2.6. Indole carboxamides

Bermudez et al., (1990) had synthesized two series of Indoline carboxamides and indole carboxamides as potential 5-HT_3 receptor antagonists. The antagonistic potential of the synthesized compounds were assessed by examining their capability to inhibit the serotonin-induced B-J reflex test in rats. Indoline carboxamide **2.75** (ID_{50} = 0.5 µg/kg) and indolecarboxamide **2.76** (ID_{50} = 1.6 µg/kg) were the most active compounds. These results suggested that, the 5-membered ring's aromaticity is not required for potency given that carbonyl group is in a favouarble "in plane" orientation (Bermudez et al., 1990).

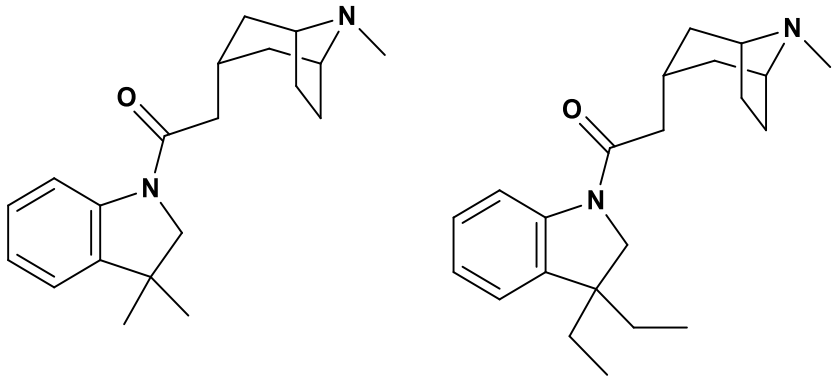


2.75 **2.76**

Figure 48: Structure of indole carboxamides as potential 5-HT_3 receptor antagonists.

Kato et al., (1995) had synthesized and explored the SAR of a series of azabicycloalkaneacetic acid derivatives as 5-HT_3 receptor antagonists. Compounds with 2,3-dihydroindole aromatic nucleus bearing gem-dimethyl or gem-diethyl groups on the 3rd position displayed potent 5-HT_3 receptor antagonism in B-J reflex test. The compounds **2.77** and **2.78** exhibited strong 5-HT_3 receptor antagonism among the synthesized compounds.

Compounds **2.77** (ED_{50} = 2.3 µg/kg i.v.) and **2.78** (ED_{50} = 1.7 µg/kg i.v.) displayed 10 fold morepotent antagonism than the standard drug, ondansetron (ED50 = 17.5 µg/kg i.v.). This study indicates that the azabicycloalkane acetyl group is a new bioisosterefor basic nitrogen region and a linking carbonyl group (Kato et al., 1995).



2.77 **2.78**

Figure 49: Structures of dihydro indole carboxamides as potential 5-HT_3 receptor antagonists.

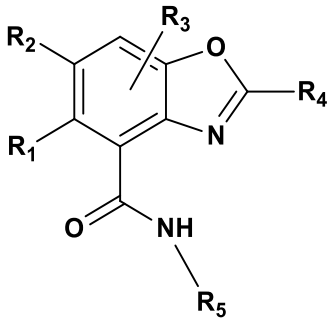
2.2.2.7. Benzoxazole carboxamides

Researchers of Albany Molecular Res. Inc. (AMRI) disclosed a patent, claiming a series of novel 2-arylbenzoxazole-4 or 7-carboxamide derivatives (general structure **2.79**) as 5-HT_3 receptor antagonists for the treatment of CINV and IBS-D. (Fairfax and Yang, 2006). The binding affinity K_i of a compound is the concentration of the compound (antagonist which has to compete competitively with radio tagged agonist for the receptor) K_i values of all the synthesized compounds were calculated using equation; $K_i=IC_{50}/(1+ ([L]/K_d))$, where IC_{50} is the concentration of compound which displace 50% of the specific binding of the radioligand [L] is the concentration of the radioligand used and K_d is the dissociation constant of the radioligand.More than 150 compounds were reported to exhibit K_i value less than 300 nM when screened in competitive radioligand binding study using mouse and human 5-HT_3 receptor.

Furthermore, the functional 5-HT_3 receptor antagonism of some selected compounds was explored using the Bezold-Jarish reflex (BJR) assay (Fairfax and Yang, 2006a; Beer et al., 2007). Later on, the same research group had also reported a new derivative of benzoxazole carboxamides (common structure **2.79**) in which the aryl substituent at 2nd position was exchanged with an alkyl residue.

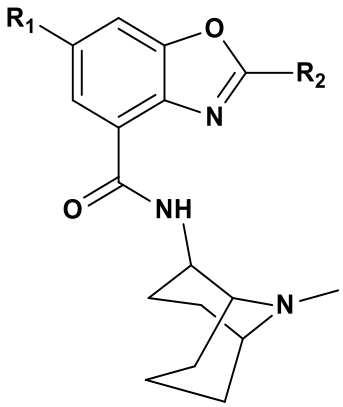
Quinuclidine and granatane in the axial or endo configuration were selected as basic components. In general granatane derivatives (general structure **2.80**) produced better affinities.

SAR study of the synthesized compounds indicated that modifications on the alkyl substituent at the position 2 of the benzoxazole scaffold significantly influenced the K_i values. Bulky substituents were not well tolerated and better results were shown by smaller alkyl rings, for example cyclopentyl (**2.80a**) and cyclopropyl (**2.80b**, Ki = 4.1 nM).



2.79

R_1=R_2=R_3=H



2.80

2.80a: R_1=H, R_2=cyclopentyl

R_4=Cyclopentyl, cyclopropyl

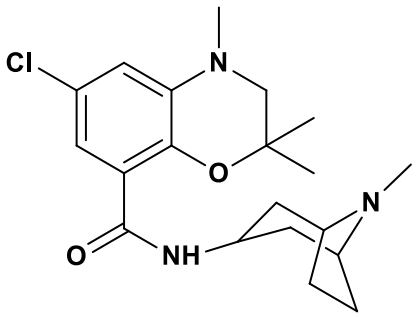
2.80b:R_1=H, R_2=cyclopropyl

R_5=Tropane

Figure 50: Structures of benzoxazole carboxamides as potential 5-HT_3 receptor antagonists.

2.2.2.8. Benzoxazine carboxamides

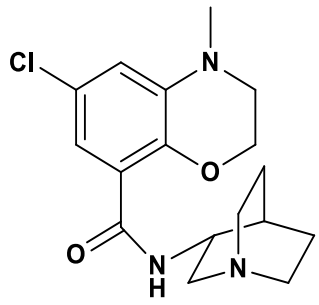
Kuroita et al., (1995) had synthesized a series of 3,4-dihydro-2-H-1,4-benzoxazine-8 carboxamides as 5-HT_3 receptor antagonists. In this series, replacement of the 1,4-benzoxazine ring with a 1,4-benzothiazine or seven-membered ring resulted in reduced affinity for 5-HT_3 receptors. Introduction of substituent on 2nd position of 1,4-benzoxazine enhanced the antagonistic activity.The compounds bearing 9-methyl-9-azabicyclo [3.3.1] non-3-yl moiety as the basic part were equipotent to those bearing 1-azabicyclo [2.2.2] oct-3-yl moiety. Among the synthesized compounds, **2.81** showed highest affinity (K_i= 0.019 nM) for 5-HT_3 receptors and long lasting (3h) 5-HT_3 receptor antagonism in B-J reflex test (Kuroita et al., 1996).



2.81

Figure 51: Structures of benzoxazine carboxamides as potential 5-HT_3 receptor antagonists.

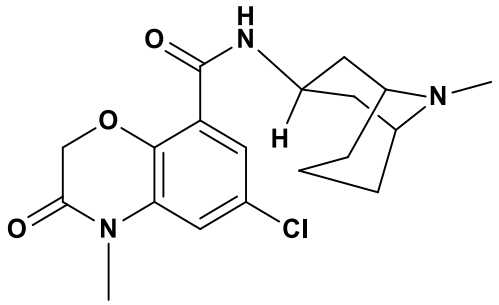
Later on, the same group had reported the synthesis and pharmacological evaluation of 6-chloro-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxamides as 5-HT_3 receptor antagonists. Among the synthesized compounds, compound **2.82** was more potent with a 5-HT_3 receptor binding affinity (K_i= 0.051nM) higher than the corresponding R-isomer (K_i= 0.54 nM), whereas granisetron, zacopride and azasetron displayed K_i= 0.41, 0.18 and 0.54 nM, respectively, in radioligand binding assays.The S-isomer exhibited potent antagonism (ED_{50} = 0.089 µg/kg, i.v.) in B-J reflex test, whereas the respective R-isomer showed an ED_{50}value of 0.73 µg/kg, i.v. The reference compounds, granisetron, zacopride and azasetron showed ED_{50} values of 0.74, 0.5 and 1.3 µg/kg, i.v., respectively (Kuroita et al., 1996).



2.82

Figure 52: Structures of benzoxazine-8-carboxamides as potential 5-HT_3 receptor antagonists.

A series of novel substituted benzoic acid tropan-3-yl amides having 5-HT_3 receptor antagonistic activity were reported by Zhang and coworkers In particular, compound **2.83** a benzooxazine carboxamide with a chloro at position 6 and a methyl group at 4th position of the benzooxazine ring exhibited a pA_2 of 6.2. The basic part of the pharmacophore was served by the azabicyclononane moiety (Zhang et al., 2008).



2.83

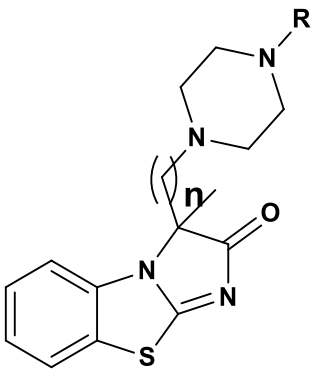
Figure 53: Structures of benzoic acid tropan-3-yl amide as potential 5-HT_3 receptor antagonists.

2.3. Mixed structures as 5-HT_3 receptor antagonists

Compounds described in this section neither belong to the category of neither arylpiperazine derivatives nor tropane and quinuclidine derivatives. These structures are quite different from the structures described above and can be considered as mixed structures (Modica et al., 2010).

Mahesh and venkatesh Perumal had synthesized and screened a series of 3[(4-substituted-piperazin-1-yl)alkyl] imidazo[2,1-*b*][1,3] benzothiazol-2(3*H*)ones (general structure **2.84**) for their 5-HT_3 receptor antagonistic potential.

Compound **2.84a** (pA_2 = 6.7) was found to the most promising compound among all the compounds when tested in 2-Me-5-HT induced contraction in guinea pig LMMP (Mahesh et al., 2005).

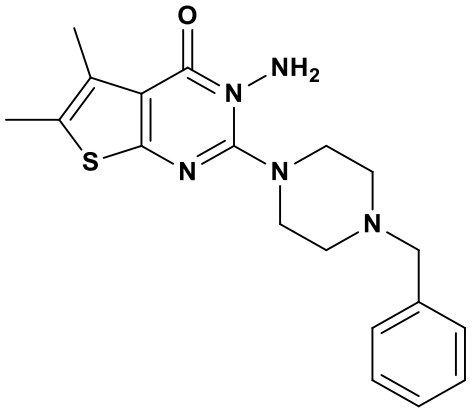


2.84

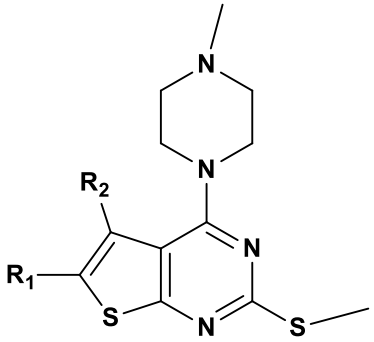
2.84a: R=CH_3**;** n=2

Figure 54: Structures of 3[(4-substituted-piperazin-1-yl) alkyl] imidazo[2,1-b][1, 3] benzothiazol-2(3H)ones as potential 5-HT_3 receptor antagonist.

Modica et al., (2001, 2004, 2008) had synthesized a series of novel thieno [2,3-*d*] pyrimidine nucleus based 5-HT_3 receptor ligands. Three compounds (**2.85**, *K*i = 3.92; **2.86a**, *K*i = 33 and **2.86b**, *K*i = 67 nM) showed good binding affinity for 5-HT_3 receptor. Moreover, compound **2.86a** was found to act as a non-competitive antagonist, since in *in vitro* 2-Me-5-HT-induced contraction in guinea pig colon functional assay it did not cause any alteration in the dose response curve. However compound **2.86a** brought a reduction in maximum effect of 2-Me-5-HT. Compound **2.86b**, when screend using the similar functional assay, exerted reasonable competitive antagonistic like acitivity (Modica et al., 2001, 2004, 2008).



2.85



2.86

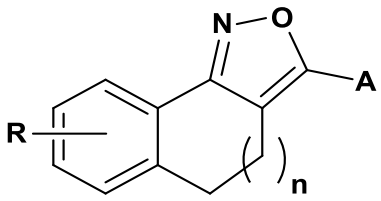
2.86a:$R_1=R_2=(CH_2)_3$

2.86b: $R_1=C_2H_5$; $R_2=H$

Figure 55: Structures of novel thieno [2, 3-d] pyrimidine nucleus based 5-HT_3 receptor ligands.

Reseacher at Hoechst Marion Roussel, Inc. synthesized and patented a novel class of 4,5-dihydronaphth[1,2-*c*] isoxazole derivatives (common structure **2.87**) as 5-HT_3 receptor antagonists with CNS activity. Promising activity was obtained from compounds with an unsubstituted 1st ring of 4,5-dihydronaphthalene nucleous,2nd ring of 4,5-dihydronaphthalene nucleous being six memebered (n=1) and having either a piperazine or homopiperazine ring attached to the isoxazole nucleous.

Three compounds **2.87a** *K*i = 868; **2.87b** *K*i =83; **2.87c***K*i = 56 nM were found to possess promising binding affinity in radio ligand binding assay using [3H]-GR65630 in rat entorhinal cortex membranes.Compounds **2.87b** and **2.87c**, also found to possess functional antagonism at 5-HT_3 receptor when tested in BJR assay (Hrib, 1997,1999).



2.87

2.87a: A=4-(2-hydroxyethyl)-1-piperazinyl, R=H, n=1

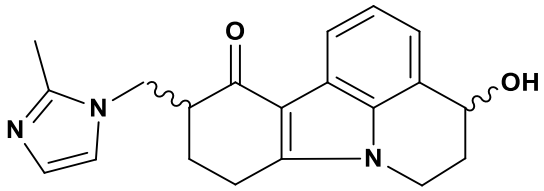
2.87b: A=1-piperazinyl, R=H, n=1

2.87c: A=3-(1-homopiperazinyl), R=H, n=1

Figure 56: Structures of novel 4,5-dihydronaphth[1,2-c] isoxazole derivatives as 5-HT_3 antagonists.

Researchers of Solvay Pharmaceuticals GmbH synthesized and patented 5,6,9,10-tetrahydro-4-hydroxy-10-[(2-methyl-1*H*-imidazol-1-yl)methyl]-4*H*pyrido[3,2,1*jk*]carbazol-11(8*H*)-one and its diastereoisomers (compounds **2.88a-2.88d**) (Brueckner et al., 2004) related to cilansetron (Haeck et al., 1989). Synthesized compounds (**2.88a** *K*i = 3.0; **2.88b** *K*i =10nM, **2.88c** *K*i =8.8 nM and **2.88d** *K*i =38 nM) exhibited nanomolar range affinities towards the 5-HT_3 receptor.

Compounds **2.88a** (pA_2 = 6.73) and **2.88b** (pA_2 = 6.45) showed 5-HT_3 receptor antagonistsic activity when screened on 2-Me-5-HT-induced contractions in guinea pigs ileal preparations.



2.88

2.88a: 4*S*, 10*R*;

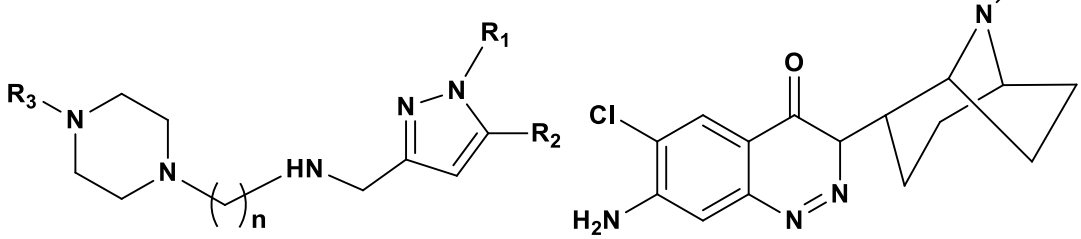
2.88b:4*R***2.88c**: 4*S*, 10*S*;

2.88d: 4*R*, 10S

Figure 57: Structures of 5, 6, 9, 10-tetrahydro-4-hydroxy-10-[(2-methyl-1H-imidazol-1-yl)methyl]-4H pyrido[3,2,1jk]carbazol-11(8H)-one as 5-HT_3 antagonists.

Nam et al., (2008) had synthesized novel derivatives of pyrazole (common structure **2.89**). All the screened compounds displayed 5-HT_3 receptor antagonism. In radio ligand binding assay, IC_{50} values of these compounds were found to be in the micromolar range (Nam et al., 2008).

Another series of benzodiazinones were synthesized by King et al., as 5-HT_3 receptor antagonists. Compound **2.90** (ID_{50} 0.17 ± 0.08) was found to be the most active in B-J reflex test, among the synthesized compounds (King et al., 1990).



2.89 **2.90**

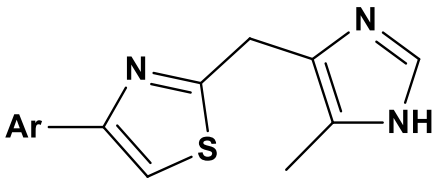
2.89a: n=0, 1, 2; R_1=R_2=R_3=H

2.89b: n=0, 1, 2; R1=R_2=R_3=CH_3

2.89b: n=0, 1, 2; R_1=R_2=R_3=etc

Figure 58: Structures of novel pyrazole derivatives as 5-HT_3 antagonists.

Synthesis of a series of thiazole based compounds (general structure **2.91**) as 5-HT_3 receptor antagonists were reported by Rosen et al., (1990). Thiazole ring was inserted between the aromatic part and the basic nitrogen as a linker.Thiazole ring mimicked the role of carbonyl group for the interaction with 5-HT_3 receptors.Compounds **2.91a** and **2.91b** showed promising binding affinities for 5-HT_3 receptors in radioligand binding assay as well as good 5-HT_3 antagonism in B-J reflex test (Rosen et al.,1990).



2.91

2.91a: Ar=indol-3-yl;

2.91b: Ar=2-methoxyphenyl

Figure 59: novel thiazole based mixed structures as 5-HT_3 antagonists.

2.4. 5-HT_3 receptor antagonists and anti-depressant like activity

Preclinical studies with 5-HT_3 receptor antagonists

Recently, a number of novel 3-ethoxyquinoxalin-2-carboxamides and quinoxalin-2-carboxamides were synthesized as 5-HT_3 receptor antagonists and evaluated for their anti - depressant potential in mice model of forced swim test (Mahesh et al., 2010; 2011). Further two compounds from the above mentioned series n-Butylquinoxalin-2-carboxamide (**4n**), and (4-phenylpiperazin-1-yl) (quinoxalin-2-yl) methanone (**4a**) were studied in 5-hydroxytryptophan (5-HTP) mediated head twitch response model.

These compounds were also tested in reserpine induced hypothermia (RIH) and olfactory bulbectomy models in male wistar rats. Both **4a** and **4n** antagonized RIH in rats and also potentiated the 5-HTP induced head twitches response in mice.

In modified open field experiment, compounds **4a** and **4n** considerably attenuated the behavioural irregularities of olfactory bulbectomized rats after sub-chronic (14 days) treatment (Kumar et al., 2012, Mahesh et al., 2012).

In house synthesized compound, (4-benzylpiperazin-1-yl) (quinoxalin-2-yl) methanone (QCF-3) (Mahesh et al., 2010) as 5-HT_3 receptor antagonist, displayed anti-depressant like activity both alone and in association with fluoxetine (Devadoss et al., 2010).

The anti-depressant effect of standard 5-HT_3 receptor antagonist, ondansetron was also evaluated by Mahesh and group (Ramamoorthy et al., 2008). Acute and chronic treatment of ondansetron in mice, showed anti-depressant-like effects in FST and TST, with no affect on the baseline locomotion. In interaction studies, ondansetron pre-treatment potentiated the anti-depressant effects of fluoxetine and venlafaxine in FST and TST, whereas, it failed to potentiate the anti-depressant effects of desipramine, thus indicating the involvement of serotonergic system in anti-depressant like effect of ondansetron (Ramamoorthy et al., 2008).Chronic ondansetron treatment quashed the hyperactivity of the OBX rats in a bi-phasic manner in the open field test. In elevated plus maze test, ondansetron treated OBX rats increased the percentage of open/total arm entry ratio and percentage of time spent in open arm as compared to that of sham group (Ramamoorthy et al., 2008).

In house synthesized, novel 5-HT_3 receptor antagonist 2-(4-methylpiperazin-1-yl)-1,8-napthyridine-3-carbonitrile (NA-2) (Mahesh et al., 2004) demonstrated anti-depressant-like effect in animal models of depression. In acute and chronic treatment, NA-2 displayed anti-depressant-like effect in FST at lower dose levels.

It also reduced the duration of immobility in TST. In interaction studies, NA-2 reversed the mCPP-induced immobility, lessening of anti-depressant effects of fluoxetine and desipramine.In chronic treatment, it also reduced the hyperactivity of olfactory bulbectomized (OBX) rats in novel open field test (Mahesh et al., 2007).

Following studies by other researchers also supported the anti-depressant like activity of 5-HT_3 receptor antagonists.

Studies were carried out to find out the role of 5-HT_3 receptors in depression using 5-HT_3 knockout mice especially in relation to its role in sex differences in behaviour.

Three behavioural tests namely open field exploration, FST and defensive withdrawal tests were conducted on male and female mice lacking the 5-HT_3 receptor (knock-out) and their wild-type litter-mates. In FST, sex differences were abolished by removal of the 5-HT_3 receptor while deletion had no effect in the habituation of locomotor activity to repeated exposure to an open field. In the defensive withdrawal experiment, removal of the 5-HT_3 receptor had more complicated effects although these effects were in the opposite direction in males and females. The above results suggested that the 5-HT_3 receptor was involved in differential regulation of behaviour-related to depression and anxiety in males and females (Bhatnagar et al., 2004).

(Srivastava, 1998) demonstrated the anti-depressant effect of ondansetron in mice forced swim test in comparison with normal saline, as a control. Ondansetron was administered to mice through intra-peritoneal route at the doses of 0.25, 0.1, 1.0 and 2.0 mg/kg. In all the tested doses, ondansetron significantly reduced the immobility of mice in forced swim test as compared to that of control group. Ondansetron reduced the immobility of mice in a dose-dependent manner. Based on these results, it was suggested that the anti-depressant effect of ondansetron may be due to direct blockade of 5-HT_3 receptor or increased release of nor-epinephrine as the result of 5-HT_3 receptor blockade.

Kos et al., (2006) evaluated the 5-HT_3 receptor antagonist, MDL 72222 on behavior induced by ketamine in rodents. In TST, ketamine and MDL 72222 (50-66 and 3 mg/kg, respectively) reduced the duration of immobility in mice when given alone or in combination (12.5-25 and 1mg/kg, respectively). This study indicated that the 5-HT_3 receptor antagonism did not reverse the ketamine behavior deficit, but potentiated some of its effect such as anti-depressant-like effects.

(Martin and Puech, 1992) showed anti-depressant properties of some standard 5-HT_3 antagonists, ondansetron, zacopride and tropisetron ISO 205-930 in rodent model of depression. In learned helplessness test, low to moderate daily doses of antagonists reduced the number of escape failure, which was absent in higher doses of 5-HT_3 receptor antagonists. These results indicated that the anti-depressant effect of these 5-HT_3 antagonists may affect like those of standard anti-depressant agents.

Nakagawa et al., (1998) evidenced the involvement of 5-HT_3 receptor antagonism in anti-depressant property of standard mood elevators, imipramine, desipramine and mianserin. They adopted forced swim test in rats to evaluate the anti-depressant potential. The standard anti-depressants were administered through intra-peritoneal route on the first and second day, 15 minutes after and 5 minutes before the experiment, respectively. These drugs reduced the duration of immobility in dose-dependent manner. The co-administration of *m*-chlorophenylbiguanidine (mCPBG) reduced the anti-depressant effects (attenuates the reduction of immobility) of standard mood elevators. However, mCPBG did not show reduction in immobility, when given alone. The 5-HT_3 receptor antagonist, tropisetron, dose-dependently reduced the immobility in FST on the second day. The anti-depressant effect of tropisetron was weakened when co-administerd with mCPBG, which indicated the involvement of 5-HT_3 receptor antagonism in beneficial effects of depression (Nakagawa et al., 1998).

Bravo and Maswood evaluated the anti-depressant potential of standard 5-HT_3 receptor antagonist tropisetron using rat forced swim test. On the test day, 30 minutes prior to the start of FST experiment, saline (1.0 or 2.0 mg/kg i.p) and tropisetron (1.0 or 2.0 mg/kg i.p) were administered to rats. In FST, the parameters observed were duration of immobility, swimming episodes and struggling behaviour for 5 minutes. Tropisetron (2.0 mg/kg i.p) considerably reduced the immobility duration and no significant struggling behavior was observed. These result suggested that the beneficial effect of 5-HT_3 receptor antagonist in depression (Bravo and Maswood, 2006).

2.5 Clinical studies with 5-HT_3 receptor antagonists

Haus et.al, had studied the effect of tropisetron in fibromalgia patients. In a randomized, double-blind study, tropisetron was administered to 418 fibromalgia patients for the period of 10 days and 28 days and functional symptoms such as sleep disturbances and dizziness were compared with the results from 10^{th} day. Sleep disturbances and dizziness were found to be significantly improved in both 10 days and 28 days studies.

In 28 days study, better improvement in the above symptoms was observed as compared to that of 10 days study. Psychometric test exhibited considerable developments in depression and anxiety condition scores. These results indicated that the chronic treatment of tropisetron was well tolerated, improved the clinical beneficial and it also evidenced the anti-depressant effects of 5-HT_3 receptor antagonist (Haus et al., 2000).

A randomized, placebo controlled, double-blind trial was conducted by Piche et.al., to study the effect of ondansetron (administered orally) on chronic hepatitis patients, associated with fatigue.Using a validated self report feedback form fatigue and depression parameters such as fatigue impact scale and beck depression inventory, were determined on 0^{th}, 15^{th}, 30^{th}, and 60^{th} day in 36 patients.Ondansetron treatment caused considerable reduction in the fatigue score on the day 15, 30 and 60, whereas the placebo failed to show beneficial effect. Ondansetron treatment also resulted in a notable reduction in the depression scores as compared to control placebo.This report indicated the anti-depressant effect of ondansetron (Piche et al., 2005).

The effect of ondansetron was studied on human subjects, to explore the involvement of 5-HT_3 receptors in emotional processing.5-HT_3 receptor antagonist, ondansetron (12 mg, oral) or placebo was given in a randomized, double-blind manner to healthy volunteers. The emotional processing were evaluated using three tasks viz. affective modulation of the startle reflex, emotional categorization, memory and facial recognition. In healthy volunteers the well being, anxiety and mood behaviors were not affected with ondansetron treatment. However, ondansetron abolished the emotion potentiated effects in healthy volunteers. These results suggested that the $5HT_3$ receptors could be involved in certain phases of fear processing in humans (Harmer et al., 2006).

2.6. 5-HT_3 receptor antagonists and anxiolytic activity

Preclinical studies with 5-HT_3 receptor antagonists

The anxiolytic effects of several 5-HT_3 antagonists were evaluated in several experimental paradigms of anxiety. The tests which have convincingly demonstrated the anxiolytic activity of 5-HT_3 receptor antagonists are the light-dark test (Crawley, 1981), the social interaction test (File and Hyde 1978) and the elevated plus maze test (Montgomery, 1958).

The parameters measured were (1) reduction of aversion to bright light in mice using two compartments in light-dark test (2) disinhibiting suppressed behaviour measured in social

interaction test. (3) Decrease in fear-induced inhibition of open alley exploration in elevated plus maze test.

5-HT_3 receptor antagonists such as Granisetron, ondansetron, MDL 72222, tropisetron, zacopride and WAY 100289 were studied in these behavioural tests.

Both the standard anxiolytic Diazepam and 5-HT_3 receptor antagonists enhanced the exploratory behaviour and social interaction in all the three paradigms in rodents.

The 5-HT_3 receptor antagonists such as ondansetron, tropisetron, bemesetron, itasetron, zacopride, (DAU 6215), RS-42385-197, WAY 100289 and WAY SEC-579 were examined in L/D test and found to produce effects similar to the benzodiazepines (Costall et al., 1987, 1993; Kilfoil et al., 1989; Onavi and Martin, 1989; Bill et al., 1992; Borsini et al., 1993; Middlefell et al., 1996).

The anxiolytic activity of the 5-HT_3 receptor antagonists such as ondansetron, zacopride, tropisetron, bemesetron, granisetron and RS 42358-197 (Costall et al., 1993) were also explored by Costall et al., (Costall et al., 1989a, 1989b) using EPM test. All these compounds showed an increase in time spent in the open arms of an elevated plus-maze apparatus. The same group had alsoinvestigated the anxiolytic effect of 5-HT_3 receptor antagonist, zacopride in a three different rodent models of anxiety such as light-dark test, social interaction test. In all those tests zacopride was found to be 100 times more potent than of standard anxiolytic agent, diazepam (Costall et al., 1998).

Zhang and coworker had reported anxiolytic activity of a high-affinity and selective 5-HT_3 receptor antagonist, desamino-3-iodozacopride (DAIZAC). The compound was examined in the mouse EPM test using diazepam, as a positive control (Zhang et al., 2001).The 5-HT_3 receptor antagonist, ondansetron increased social interaction (Jones et al., 1988; Piper et al., 1988; Dunn et al., 1991). Bhatt et al., (2010) reported anti anxiety activity of novel 5-HT_3 receptor antagonists, quinoxaline carboxamides **6g** and **6o** in a behavioral test battery of anxiety. EPM, L&D test, HB and OFT were conducted using diazepam as positive control.

During early nineties, several patents were also filed for possible anxiolytic activity of 'setron' class of 5-HT_3 receptor antagonists by several pharmaceutical companies. Alosetron and GR-67330 was patented by Glaxo Smithkline (EP-306323 1989; US4859662 1989). Zatosetron was patented by Eli Lilly Co. (EP-307172 1989). RS-56532 and RS-66331 were patented by Syntex & Co. Novel 5-HT_3 antagonists YMll4 KAE-393 and YM 060 were patented by Yarnanouch & Co. (EP-381422 1990).

2.7. Clinical studies with 5-HT_3 receptor antagonists

Testing in humans provides the final evidence for putative anxiolytic effects of a novel drug. Several clinical studies were conducted to assess the usefullness of 5-HT_3 receptor antagonists in the treatment of anxiety. Most of the clinical studies in human volunteers were done on ondansetron.

In clinical studies, ondansetron abolished emotion-potentiated startle response (Harmer et al., 2006) and it was reported to potentiate penta gastrin induced elevated adreno corticotrophic hormone (ACTH) levels and anxiety scores (McCann et al., 1997).

In some cases, treatment with ondansetron reduced depression as well as anxiety scores in patients suffering from obsessive compulsive disorder (OCD) (Hewlett et al., 2003).

5-HT_3 receptor antagonist, tropisetron, was found to show anxiolytic effects (Lecrubier et al., 1993). Tropisetron was given for three weeks at three dose levels (0.5, 5 and 25 mg daily) in a placebo-controlled double-blind study to assess the effect of tropisetron in the treatment of patients with generalized anxiety disorders. At day twenty-one, tropisetron showed significant effects on all anxiety-related outcome measures (Lecrljbier et al., 1993).

A multicentred double-blind placebo-controlled phase II/III study with ondansetron (1 mg to 4 mg t.i.d. for four weeks) in patients with generalized anxiety disorders was conducted by Lader (Lader, 1991). After four weeks, treatment had significantly lowered anxiety scores than patients in the placebo group.

Another multicentre, double-blind, placebo-controlled study conducted by Metz and group showed that ondansetron, 1 to 2 mg b.i.d. for ten weeks, significantly reduced total panic attack frequency and improved anticipatory anxiety and functional impairment (Metz et al., 1994). A multicentric double blind study was conducted by Bell and De Veaugh-Gees in which ondansetron (0.25 mg b.i.d. for ten weeks) treatment improved symptoms of social phobia to a greater extent as compared with placebo treated group (Bell and De Veaugh-Gees, 1994).Thus, there are some preclinical as well clinical evidences for anti-depressant and anxiolytic activity of 5-HT_3 receptor antagonists available in literature. These pilot studies investigating 5-HT_3 antagonists in the treatment of depression and anxiety were promising, particularly; the data on chronic study appear promising. This strongly suggests the design, synthesis and pharmacological evaluation of new chemical entities as 5-HT_3 receptor antagonists for co-morbid disorders like depression and anxiety.

Chapter 3: Objectives and Plan of Work

3. OBJECTIVES and PLAN of WORK

3.1. Objectives

Depression and anxiety are serious mental health issues that affect the life of most people at any age point during their lifetime. It has been found that depression and anxiety disorders commonly occur together in patients. High co-morbidity of depression with anxiety can lead to changes in diagnosis during the course of the illness. Co-morbid depression and anxiety has been found to be more resistant to pharmacological treatment and patients with these coexisting disorders have a poorer medical prognosis as compared with patients with either disease alone.

Significant research attempts have been made in the understanding of co-morbid depression and anxiety disorders. Despite a constant increase in the advancement in research, incidence of these diseases still persist which may be due to lack of clear understanding of the pathophysiology/or the inconsistent efficacy of current pharmacotherapy. Current treatment recommendations for co-morbid depression and anxiety are same as with the treatment of depression and anxiety disorders when they occur independently. A common problem associated with the current anti-depressant and anxiolytic therapies are several side effects. Conventional drugs used for depression and anxiety disorders pharmacotherapy act directly affecting monoamine (5-HT, NE and DA) turnover in brain and engage in restoring of normal function of monoamine associated signaling pathways (Schildkraut and Kety, 1967; Millan, 2006). However, no single agent has come out as a gold standard or a first-line treatment in clinical studies and their ability to improve daily performance and productivity in patients with both depressed and anxiety states is questionable.Thus, the search for an agent for the treatment of co-morbid disorders like depression and anxiety with lesser side effects has become a need of the hour.

During last four decades several researchers have reported a large number of new chemical entities (NCEs) as potential 5-HT_3 receptor antagonists. They also have identified two basic pharmacophores for 5-HT_3 receptor antagonists. However, the neuropharmacological potential in terms of anti-depressant and anxiolytic activity of NCEs as 5-HT_3 receptor antagonists are still not well explored. Previous investigations (Devadoss and pandey, 2010, Rajkumar et al., 2008, Mahesh et al., 2007) have identified 5-HT_3 receptor antagonist as potential neuropharmacological agent and acknowledged the role of 5-HT_3 receptor in the pathophysiological processes of depression and anxiety (Rajkumar and Mahesh, 2010).

Thus, it seemed appropriate to design and develop novel 5-HT_3 receptor antagonist as prospective agent, which could be effective in both depression and anxiety states.

Keeping all these aspects in consideration, following prime objectives are set for the present study.

- To design NCEs as 5-HT_3 receptor antagonists by ligand-based approach based on different pharmacophoric templates.
- To synthesize the designed NCEs using appropriate organic synthetic chemistry approach and spectral characterization of all the synthesized compounds.
- To examine the 5-HT_3 receptor antagonistic potential of all the synthesized NCEs using isolated LMMP of guinea pig.
- To evaluate anti-depressant and anxiolytic potential of the synthesized NCEs in various validated animal models of depression and anxiety.

3.2 PLAN OF WORK:

3.2.1 Design and synthesis of new chemical entities

Based on the earlier discussed literature review, two pharmacophore models for 5-HT_3 receptor antagonists were identified namely, pharmacophore model for aryl piperazines related to quipazine and three point pharmacophore model for tropane and quiniclidine derivatives related to classical 5-HT_3 receptor antagonists. New chemical entities were designed based on both the above mentioned pharmacophores.

In order to attain better pharmacokinetic profile, molecules were designed according to the 'Lipinski's rule of five'. Lipophilicity is an essential criterion to be regarded when designing compound to manifest drug-like behaviour, particularly CNS drugs. Hence, logP values of all the compounds were calculated using JME molecular editor (courtesy of Peter Ertl, Novartis).

The basic structures of the proposed new chemical entities are depicted below. The proposed new chemical entities were synthesized via appropriate synthetic routes.

Piperazine analogs of 1,8 naphthyridine 3-carboxylic acids were designed Based on pharmacophore model for aryl piperazine type 5-HT_3 receptor antagonists (Figure 60) proposed by cappelli and coworkers (cappelli et al., 2002), which composed of a hetero aromatic mostly nitrogen containing core which is connected with a basic moiety mostly a piperazine ring through a pseudoamidinic bond.

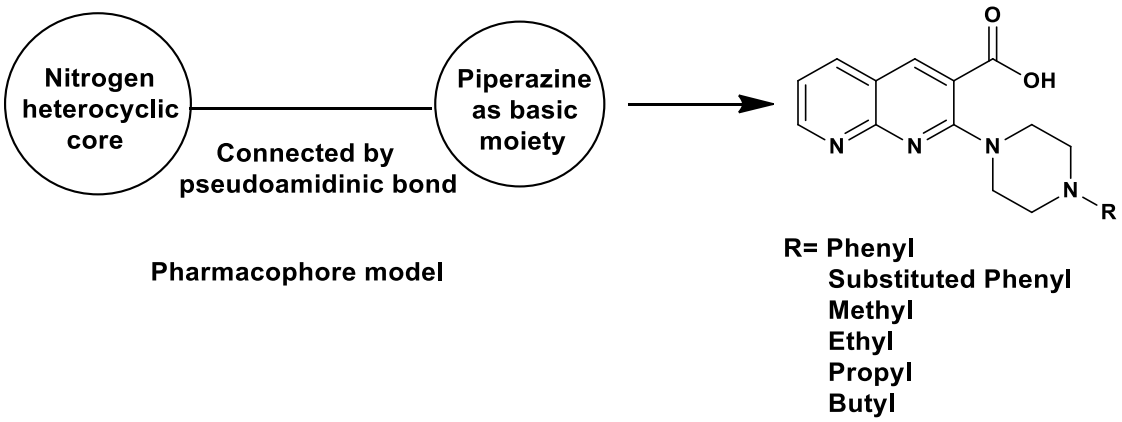


Figure 60: Compounds designed based on aryl piperazine pharmacophore model.

In the previous study (Mahesh and perumal, 2004), nitrile derivatives of 1,8 naphthyridines were synthesized based on the above pharmacophoric model and assessed their 5-HT_3 receptor antagonistic potential. The encouraging 5-HT_3 receptor antagonistic activity of 1,8 naphthyridine derivatives prompted us to further synthesize few more analogous compounds with a similar approach.

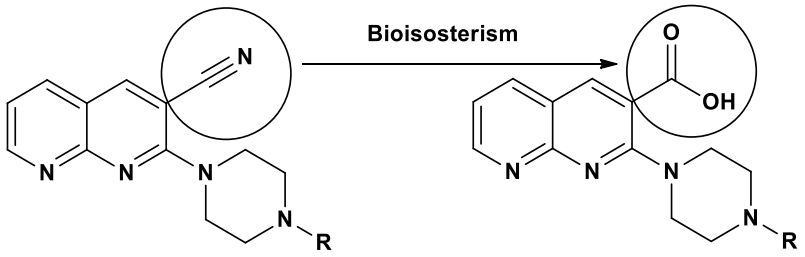


Figure61: Nitrile based and Carboxylic acid based 1,8 naphthyridines.

Thus, in the present study, 1,8-naphthyridine-3-carboxylic acids as a novel congener series of compounds were proposed as bioisosteric analogues, by substituting the nitrile group of the previously synthesized compound with it's non classical bioisoster carboxylic acid group (Lima and Barreiro, 2005).

Various piperazine analogues of carobxamides with three different heteroaromatic scaffolds were designed.

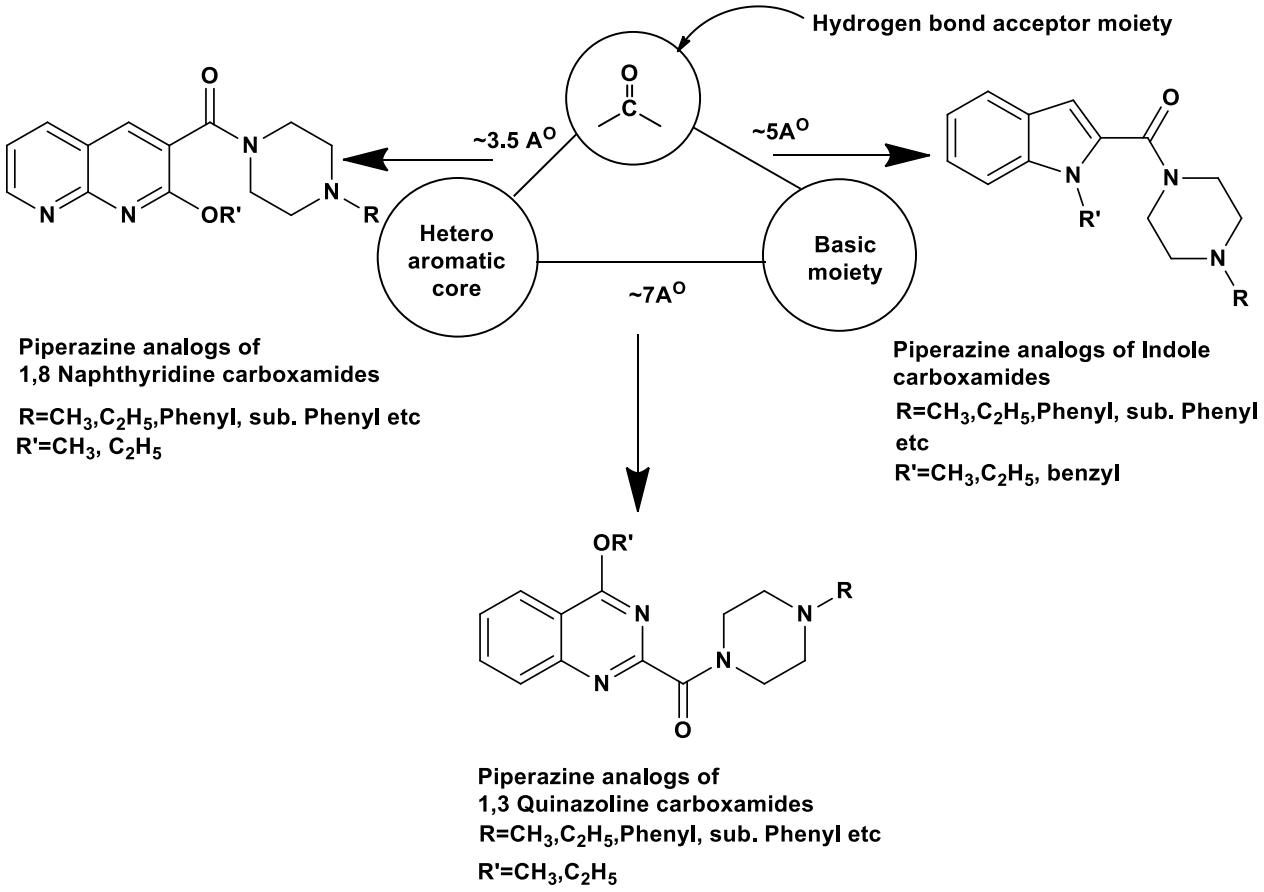


Figure 62: Compounds designed based on three point pharmacophore model for 5-HT_3 receptor antagonists.

The key elements of the three-component pharmacophoric model (**figure 62**) proposed for the interaction of 5-HT_3 receptor antagonists with the 5-HT_3 receptor-binding site: consist of an aromatic moiety, a hydrogen bond acceptor moiety, mostly a caebonyl group and a basic moiety located at a specific distance. In recent years, researchers have been engaged in the preparation and screening of compounds on the basis of the above pharmacophoric model and the previous studies have also indicated that fused aromatic rings containing nitrogen (heteroaromatic core) may serve as a suitable starting point for the design of novel 5-HT_3 receptor antagonists (Mahesh et al., 2010; 2011a,b; 2012). Thus, the attention turned towards, the naphthyridine, quinazoline and indole core as aromatic part and substituent effects on the piperazine as a basic moiety.

In this work, the plan was to study 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 of the pharmacophore with the intention of exploring the structure activity relationship (SAR) associated with such changes.Therefore, three different nitrogen containing fused heterocycle rings; naphthyridine, quinazoline, and indole (aromatic part) were alternately attached to various N^4 substituted piperazines (basic moiety) through a carboxamide linkage of a carbonyl group (hydrogen bond acceptor), resulting in the construction of three new series of piperazine analogues of naphthyridine-3-carboxamides, quinazoline-2-carboxamides and of indole-2-carboxamides. The three least energy conformations of each designed molecule were created using ACDLABS-10.0/3D Viewer (CHARMM parameterization). The average pharmacophoric distances were calculated from centroid of heteroaromatic ring to O of the carbonyl group, carbonyl O to basic N (N^4 of piperazines) and centroid of heteroaromatic ring to basic nitrogen.The calculated distances between the pharmacophoric components of the designed compounds were found to conform to the suggested pharmacophore model for 5-HT_3 receptor antagonists.

3.2.2. Determination of 5-HT_3 receptor antagonism

The NCEs were screened for their 5-HT_3 receptor antagonistic potential in longitudinal muscle myenteric plexus preparation from guinea pig ileum against 5-HT_3 agonist, 2-methyl 5-HT. 5-HT_3 receptor antagonism of all the compounds were denoted in the form of pA_2 values.pA_2 value is the negative logarithm of molar concentration of the antagonist which produces a 2-fold change in the agonist concentration-activity curve, which was calculated using graphical method as described in the literature.

3.2.3. Behavioural Assay

3.2.3.1. Spontaneous Locomotor Activity (SLA)

The SLA test was used for the selection of appropriate doses of the test drugs to avoid the false positive and false negative AD effect. The SLA of mice was assessed using the actophotometer (Boissier & Simon, 1965).

3.2.3.2. Anti depressant Screening

3.2.3.2.1. Forced swim test (FST)

Compounds were screened in mice model forced swim test (FST) to assess the anti depressant potential. The fundamental basis of this experiment is on the assumption that stress is also a causative factor of depression. The immobile condition has been termed as behavioral despair on the assumption that the animal has given up the expectation of 'escaping from the situation' a condition which reflects the clinical symptoms of depressive disorders.

The procedure reported elsewhere (Porsolt et al., 1977) was adopted with small variations in glass cylinder dimension, number of quadrants and water temperature (Pandey et al., 2008).The vehicle/drug treated mice were positioned in the water and compelled to swim for 6 min. The immobility period which mirrors the condition of depression was traced during the last 4 min of the 6 min experiment. A mouse was deemed to be immobile, when it stopped struggling & inertly moved to stay floated & kept its head above the level of water.

3.2.3.2.2. Tail Suspension test (TST)

TST is another most widely used behavior despair model of depression. Behavioral despair was induced by TST protocol of Steru et al. (1985). In brief, the mice were suspended on the edge of a shelf 50 cm above a Table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility during the 6 min observation period was tracked and recorded using stop watch. The state of immobility has been named 'behavioral despair' on the statement that rodent has given up hope of 'escaping' a symptom, that reflects clinical feature of depressive disorder. Mice were considered immobile only when they were totally motionless. The parameter recorded was duration of immobility.

3.2.4.1 Anxiolytic Screening

The compounds were also tested for their anxiolytic-like potential in investigational models of anxiety such as

3.2.4.1.1 Ligh-Dark aversion Test (L/D Test):

Latency time to depart from the light chamber, time spent in light chamber and number of crossings between the light and dark chambers were measured. Increase in all the above parameters is indicative of anxiolytic activity.

3.2.4.1.2. Elevated Plus Maze Test (EPM Test):

Percentage of both open arm entry (OAE) and total time spent in open arm (TSOA). Increase in open arm entry and time spent in open arm is an indicator of anxiolytic activity.

3.2.4.1.3. Open Field Test (OFT):

The number of square traversed and rearing were recorded for a period of 5 minutes. Movement away from the (walls) periphery towards centre, were considered to be anxiolytic activity.

Chapter 4: Experimental Work

4. EXPERIMENTAL WORK

4.1. Materials and Methods

4.1.1 Chemistry

All the chemicals and reagents were procured from S. D. Fine Chem Limited (India), Spectrochem Pvt. Ltd. (India), and Aldrich (USA). Reactions were monitored usingTLCwith 0.2 mm Merck pre-coated silica gel 60 F254 aluminum sheets. Compounds were visualized using UV chamber, exposing to iodine vapour and by using ethanolic solution of ninhydrin followed by heating. All melting points were found using Buchi 530 melting point apparatusand were uncorrected. Schimadzu IR Prestige-21 FT-IR spectrophotometer was used to obtain the IR spectra (nmax in cm-1). Bruker Avance-II, 400 MHz NMR spectrometer was used to obtain the ^{1}H NMR spectra. TMS was used as internal standard, and chemical shifts were in, ppm scale. Mass spectra were obtained on a 'Hewlett-Packard' HP GS/MS 5890/5972 electron spray ionization (ESI) technique (positive and negative mode). Silica gels of 60-120/100-200 mesh size as an adsorbent and ethyl acetate/hexane or dichloromethane/ethanol as eluent were used for purification of compounds using column chromatography.

4.1.1.1 Synthetic schemes

Series of synthetic scheme designed, reaction conditions are described below. Synthetic routes for title carboxamides are as follows

SCHEME-I: Synthesis of 2-[4-(substituted piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 1-15).

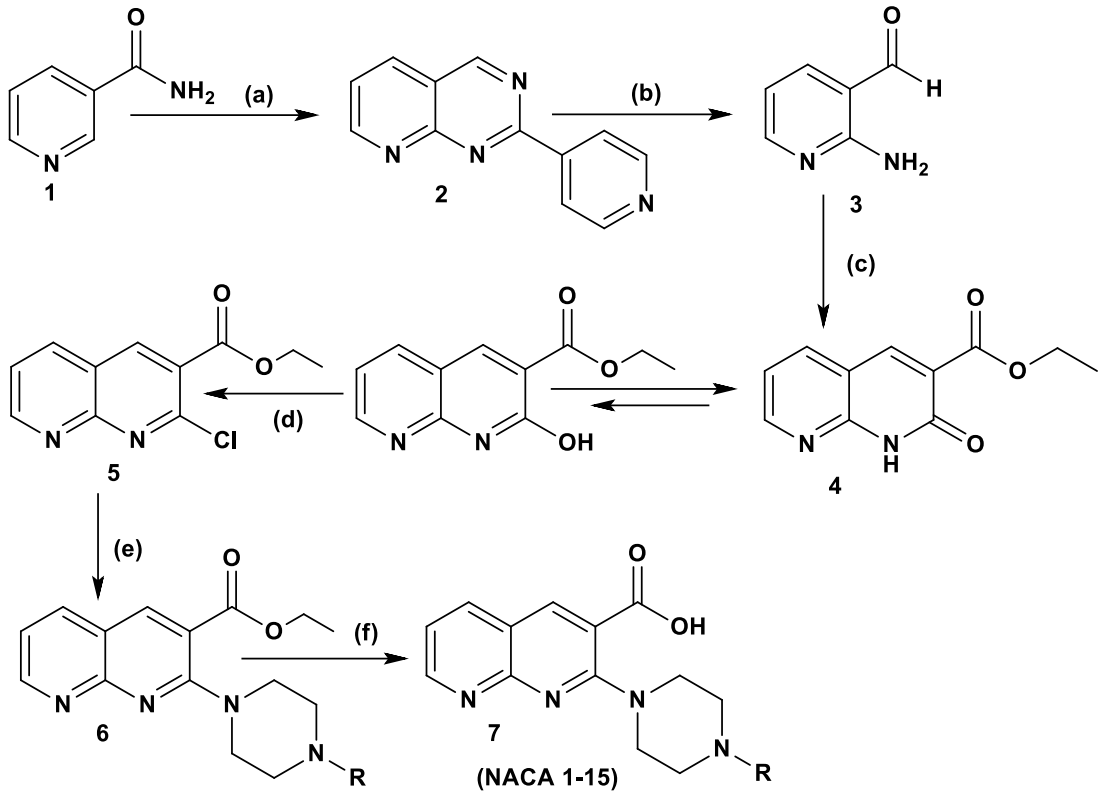
SCHEME-II: Synthesis of 2-alkoxy 1, 8 naphthyridine 3-carboxamides (MN 1-15 and EN 1-15).

SCHEME-III: Synthesis of (4-alkoxyquinazolin-2-yl)(4-substitutedpiperazin-1-yl) methanone (MQZ 1-10 and EQZ 1-10).

SCHEME-IV: Synthesis of (1-substituted-1*H*-indol-2-yl) (4-substituted piperazin-1-yl) methanone (NMIC1-10, NEIC1-10 and NBIC 1-10).

Synthetic routes of compounds are as follows:

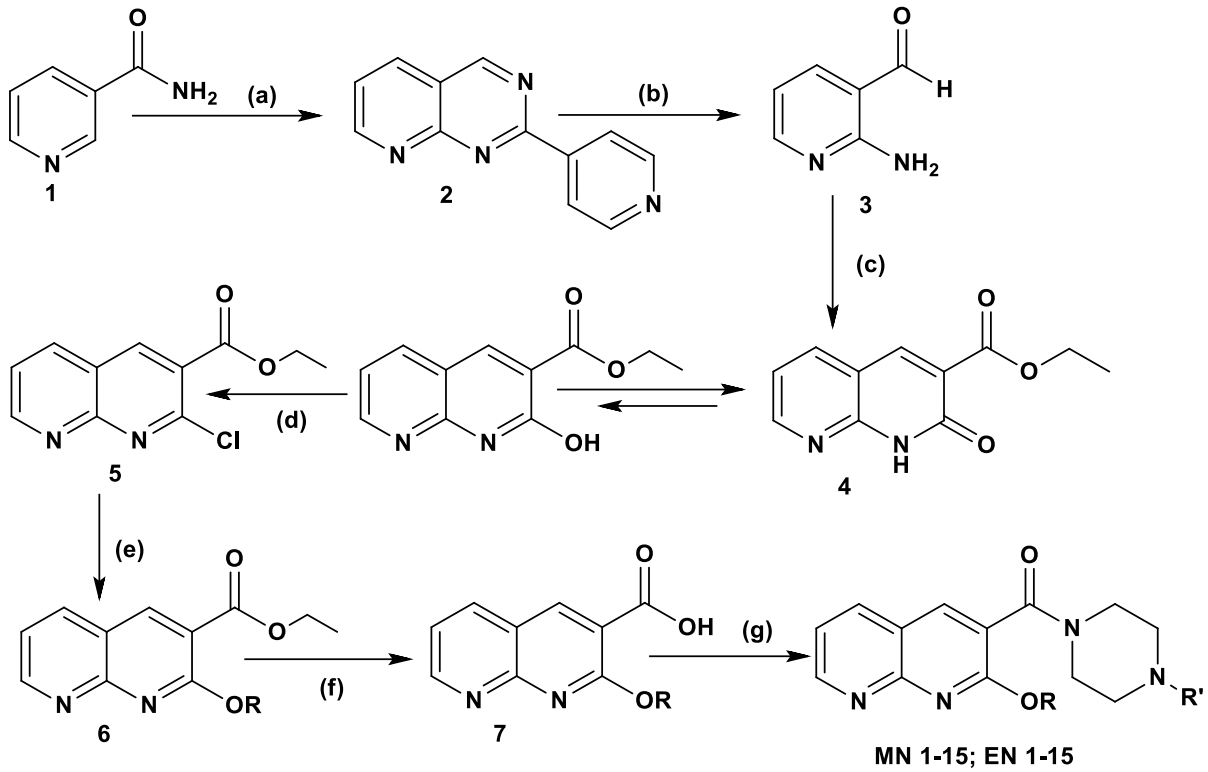
SCHEME-I: Synthetic route of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids (NACA 1-15; Series 1)



R'=Ph, sub. Phenyl, benzyl, methyl, ethyl, propyl, etc.

Figure 63: 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, r.t, 1h, aq. citric acid.

Scheme II: Synthetic route to (2-alkoxy-1, 8-naphthyridin-3-yl) (4-substituted piperazin-1-yl) methanone derivatives (MN1-15; EN 1-15; Series 2):

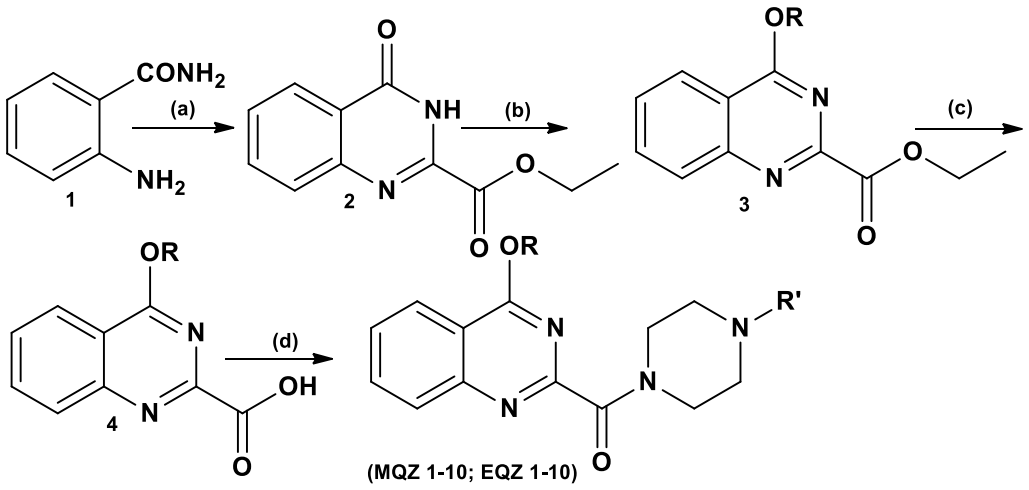


R= CH_3 (MN1-15), C_2H_5 (EN1-15)

R'=Ph, sub. Phenyl, benzyl, methyl, ethyl, propyl, etc.

Figure 64: Scheme II: Reagents & Conditions: (a) ammonium sulphamate, neat, 200^{o}C, 28h, 64% (b) 4N HCl, 1h, 38% (c) Diethyl malonate, EtOH, reflux, 6h, 60% (d) $POCl_3$, DMF, 1h, 67% (e) NaOR, EtOH, rt, 1h, 68% (f) 10% aq.NaOH, r.t, 1h, dil.HCl, 70% (g) EDC.HCl, HOBt, 0^{o}C-r.t, 1h, piperazines, 4h.

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

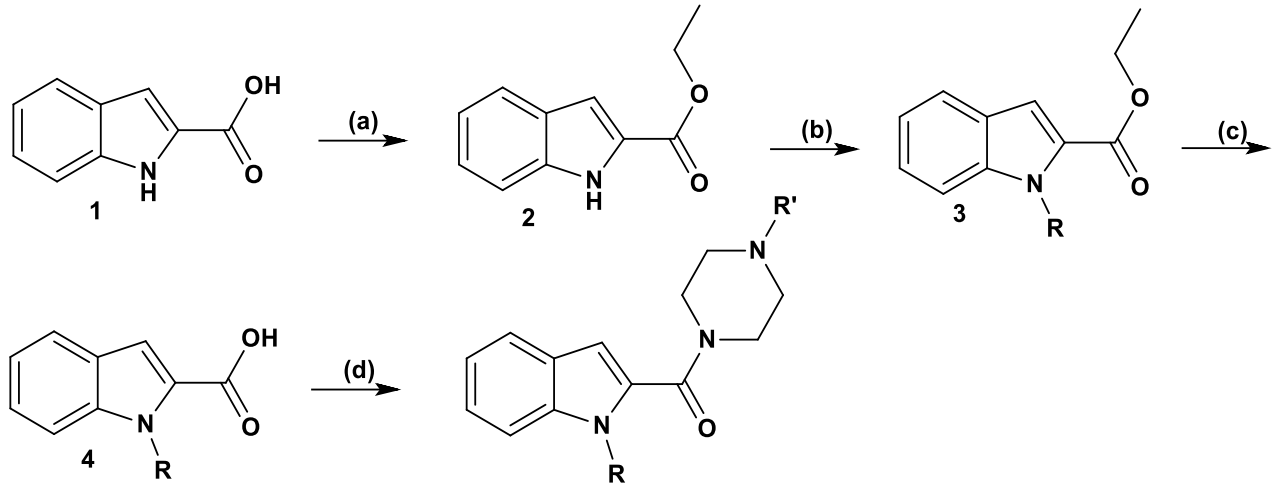


R= CH_3 (MQZ1-10), C_2H_5 (EQZ1-10)

R'=Ph, sub. Phenyl, methyl, ethyl, propyl, etc.

Figure 65: Scheme III:Reagents & conditions: (a) diethyl oxalate, sodium ethoxide, ethanol, reflux, 3h, 84% (b) RI, K_2CO_3, DMF,1.5h, 0^oC 68% (c) LiOH, THF: water (3:1), 30-45 min, 60%. (d) Oxalyl chloride, DCM, 0^oC-rt, Et_3N, piperazines, r.t, 1h.

Scheme IV: 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=CH_3 (NMIC 1-10), C_2H_5 (NEIC 1-10), $CH_2C_6H_5$ (NBIC 1-10)

R'=Ph, sub. Phenyl, methyl, ethyl, propyl, etc.

Figure 66: Scheme IV: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^oC-r.t, 1h, piperazines, 1h.

Scheme I: Synthesis of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids (NACA 1-15) (Series 1)

Synthesis of 2-(pyridin-4-yl) pyrido [2,3-d]pyrimidine (2)

A mixture of nicotinamide **1** (1 mmol) and ammonium sulphamate (3.5 mmol) was heated at 200^{o}C, in solvent free condition, with stirring for 20-24h. As the reaction progressed, the mixture solidified.The reaction was monitored by TLC using EtOAc: n-Hexane (70%) as solvent system.On completion of reaction, water was added to the solidified mixture and crushed; light brown solid thus obtained was filtered. The solid was washed thoroughly with water, followed by diethyl ether. Yield: 64%; m.p.: 167-168 ^{o}C; FT-IR (KBr, cm^{-1}): 3059, 2962, 1602, 1591, 1579, 1546, 1527, 1462, 1361, 1336, 1190, 1097, 1080, and 1022.

Synthesis of 2-amino nicotinaldehyde (3) (Majewicz and Caluwe, 1974)

The pyridopyrimidine intermediate (2) was dissolved in 100 ml of 4N HCl, and the mixtureheated at 100^{o}C, for 1-2h. The progress of the reaction was checked using TLC using EtOAc: n-Hexane (7:3) mixture as solvent system. The reaction mixture was cooled after completion and basified using 10% aq. NaOH. The reaction mixture was extracted with dichloromethane; the organic portion was washed several times with water and evaporated in vacuo to obtain the 2-amino nicotinaldehyde as yellow solid. Yield: 38%, melting point 98^{o}C.FT-IR (KBr, cm^{-1}): 3414, 3290, 3172, 2831, 2750, 2644, 2357, 1680, 1625, 1587, 1462, 1350, 1296, 1271, 1195.^{1}HNMR, $CDCl_3$, δ (ppm): 9.86 (s, 1H, aldehyde), 8.27-8.25 (dd, 1H, *J*=4.8Hz, 2Hz, naphthyridine), 7.82-7.80 (m, 1H), 6.84-6.73 (dd, 1H, *J*=10Hz, 7.2Hz).

Synthesis of Ethyl 2-oxo-1, 2-dihydro-1, 8-naphthyridine-3-carboxylate (4) 2-aminonicotinaldehyde **3** (1 mmol) was dissolved in sufficient amount of ethanol. To this solution, piperidine in drops and diethyl malonate (1.5 mmol) were added. The reaction mixture was refluxed for about 5 hrs, following completion of the reaction (monitored by TLC using ethylacetate: hexane 1:1 by volume), ethanol was removed under reduced pressure to obtain a pale yellow solid. The crude compound was recrystalized from ethanol to obtain the pure compound. Yield: 60%, melting point 170^{o}C-172 ^{o}C.FT-IR (KBr, cm^{-1}): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1650, 1570, 1462, 1350, ^{1}HNMR, $CDCl_3$, δ (ppm):12.2 (s, 1H, enol OH); 8.81-8.79 (dd, 1H, *J*=4.8Hz, 2Hz, naphthyridine); 8.4(s, 1H, naphthyridine); 7.98-7.95 (m, 1H); 7.22-7.19 (dd, 1H, *J*=10Hz, 7.2Hz). **(**Xiao et al., 2010)

Synthesis of Ethyl 2-chloro-1, 8-naphthyridine-3-carboxylate (5)

A reaction mixture of compound **4** (0.3g, 1.57 mmol), few drops of DMF and 5mL $POCl_3$ was refluxed for 1-2h. Once the reaction was complete as indicated by TLC using ethylacetate / hexane (7:3), the reaction mixture was carefully poured onto crushed ice kept in a large vessel. To this mixture, saturated sodium bicarbonate solution was added very carefully till the solution became slightly basic to obtain a pale yellow solid. Precipitated compound was filtered and washed thoroughly with cold water.

Yield: 67%, melting point 170^o-172 C. FT-IR (KBr, cm^{-1}): 3471, 3034, 2922, 2358, 2241, 1969, 1907, 1625, 1510, 1462, 1350, 1296, 1250, and 1090. ^{1}HNMR, $CDCl_3$, δ (ppm):8.81-8.79 (dd, 1H, *J*=4.8Hz, 2Hz, naphthyridine); 8.4(s, 1H, naphthyridine); 7.98-7.95 (m, 1H); 7.22-7.19 (dd, 1H, *J*=10Hz, 7.2Hz).

General procedure for the synthesis of Ethyl 2-(4-substituted piperazin-1-yl)-1, 8-naphthyridine-3-carboxylate (6a-o)

Compound **5** (1 mmol) was dissolved in dry acetonitrile (5ml). To this K_2CO_3 (2 mmol), and substituted piperazine (eg. phenyl piperazine) (1 mmol) were added. The reaction mixture was stirred and simultaneously heated at 80 ^{o}C for 1-2h. Once the reaction was complete, crushed ice was added to the reaction mixture and stirred to result in a solid compound that precipitated out, which was filtered and washed with water. Alternately, for some compounds, solvent was removed in vacuo and the residue thus obtained was dissolved in ethyl acetate. Organic portion was washed twice with water and dried over Na_2SO_4 and concentrated in vacuo to obtain a pale yellow residue which was purified using column chromatography using silica (60-120) as stationary phase.

^{1}H NMR, spectra of one representative compound is as follows

Ethyl 2-(4-phenyl piperazin-1-yl)-1, 8-naphthyridine-3-carboxylate

DMSO-d_6, δ (ppm): 8.95-8.94 (dd, 1H, *J*=4.8, 2 Hz, naphthyridine), 8.41(s, 1H, naphthyridine), 8.14-8.11 (dd, 1H, *J*=8, 2Hz, naphthyridine), 7.31-7.27 (m, 3H, phenyl), 7.01-6.99 (m, 2H, phenyl), 6.93-6.89 (dd, 1H, *J*=10, 7.2 Hz, naphthyridine), 4.47-4.41 (q, 2H,-CH_2, ethyl ester), 3.88-3.86 (m, 4H, piperazine), 3.38-3.35 (m, 4H, piperazine), 1.46-1.42 (t, 3H, -CH_3-, ethyl ester).

General procedure for the synthesis of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids (NACA 1-15)

Ester compound 6 was dissolved in 4-5 ml ethanol. To this 10% aqueous NaOH (1-2ml) was added and stirred for 1h at room temperature. On completion of reaction alcohol was evaporated and the reaction mixture acidified very carefully at 0^oC with a weak acid, (citric acid) up to pH 4-5 to avoid protonation at the N^4 nitrogen of the piperazine ring, and this furnished the title compounds NACA 1-15. The solid mass that separated out was filtered and dried. The solid carboxylic acids thus obtained were further purified by column chromatography and (or) recrystalization.

Scheme II: Synthesis of (2-alkoxy-1, 8-naphthyridin-3-yl) (4-substituted piperazin-1-yl) methanone derivatives (MN1-15; EN1-15) (Series 2):

The intermediates 2-(pyridin-4-yl) pyrido [2,3-d] pyrimidine **(2)** to ethyl 2-chloro-1, 8-naphthyridine-3-carboxylate **(5)** were synthesized as mentioned above in scheme I (series 1)

Synthesis of Ethyl 2-alkoxy-1,8-naphthyridine-3-carboxylate (6)

Compound (**5**) 2 gm (8.45mmol) was taken in a round bottomed flask (100ml), to this 10 ml alcohol, sodium alkoxide (0.45g, 8.45 mmol) were added and the reaction mixture refluxed for 2h. The progress of the reaction was checked using TLC with EtOAc: n-Hexane (8:2) as solvent system. Once the reaction was completed the reaction mixture was evaporated in vacue, to obtain a dark brown thick liquid, to this liquidEtOAc (30ml) was added and washed with water, organic portion separated and evaporated to get the alkoxy derivative, which was used for next reaction without purification. Yield=70%.

Synthesis of 2-alkoxy-1, 8-naphthyridine-3-carboxylic acid (7)

The ester **(6)** (2 g, 8.45 mmol) was dissolved in 10 ml ethanol and to this equimolar aqueous NaOH was added. The reaction mixture was stirred at room temperature for 1h, during which time the progress of the reaction was checked using TLC with EtOAc: n-Hexane (1:1) as solvent system.

On completion of reaction, the mixture was evaporated on vacue to obtain a dark brown thick liquid. Crushed ice was added to the liquid and acidified with dilute HCl to pH 3.

Brown solid which precipitated out was filtered, washed with ice cold water to obtain pure acid. ^{1}HNMR, $DMSOd_6$, δ (ppm): 9.07-9.05 (m, 1H, naphthyridine), 8.89 (s, 1H, naphthyridine), 8.73-8.70 (m, 1H, naphthyridine), 7.70-7.67 (m, 1H, naphthyridine), 4.42 (s, 1H, $-OCH_3$).

General procedure for synthesis of (2-alkoxy-1, 8-naphthyridin-3-yl) (4-substituted piperazin-1-yl) methanone derivatives (MN1-15; EN1-15):

2-alkoxy 1, 8 naphthyridine 3-Carboxylic acid; compound **7** was dissolved in DCM, in a round bottomed flask, under nitrogen condition. To this 1.2 equivalent ofEDC.HCl, followed by 1.5 equivalent of HOBt were added and stirred at 0^oC, for 5-10 min. To this reaction mixture various commercially available substituted piperazines were added in equimolar quantities.The reaction mixture was further stirred for 1h at room temperature. On completion, the reaction mixture was evaporated and washed with water, and compounds purified by recrystalization and (or) column chromatography.

Scheme III: (4-substituted piperazine-1-yl) (4-alkylquinazoline-2-yl) methanone (MQZ1-10; EQZ 1-10) (Series 3)

Synthesis of Ethyl 3, 4-dihydro-4oxoquinazoline-2-carboxylate (2) (Nakanishi and Massett, 1980)

A mixture of anthranilamide **1** (1 mmol), sodium ethoxide and diethyl oxalate (3.5 mmol), in ethanol were heated at 100^oC, and stirred for 3h. As the reaction progressed, the mixture became semisolid. The reaction was monitored using TLC using EtOAc: n-Hexane (70%) as solvent system. Upon completion of the reaction, glacial acetic acid and water were added to the reaction mixture, the pale yellow solid thus obtained was filtered. The solid was washed thoroughly with water and recrystalized from methanol. Yield: 64%.

Synthesis of Ethyl 3, 4-dihydro-4-alkyl quinazoline-2-carboxylate (3) (Bogentoft et al., 1969)

To a stirred solution of quinazolinone **3** (1 mmol) in sufficient quantities of dimethyl formamide, anhydrous K_2CO_3 (3 mmol) and alkyl halide (methyl iodide for methoxy quinazoline, ethyl iodide for ethoxy quinazoline) (1.5 mmol) were added at 0^oC. The reaction mixture was refluxed for 4-5 h. completion of the reaction was monitored using TLC using ethyl acetate: hexane 1:1 by volume. Ethanol was removed in vacuo to obtain a pale yellow solid. The crude compound was recrystalized from ethanol to obtain the pure compound.

^{1}HNMR, $CDCl_3$, δ (ppm): 8.26–8.24 (m, 1H, quinazoline ring), 7.76–7.71 (m, 2H, quinazoline), 7.53–7.48 (m, 1H, quinazoline), 4.54–4.49 (q, 2H, -CH_2- of ester), 3.62 (s, 3H,-OCH_3), 1.48–1.44 (t, 3H, -CH_3- of ester).

Synthesis of Ethyl 3, 4-dihydro-4-alkylquinazoline-2-carboxylic acid (4)

To a stirred solution of ester **(3)** (1 mmol) in THF: water (3:1) was added lithium hydroxide (1.5 mmol). After striing for about 30 min at r.t, dil HCl was added dropwise to the reaction mixture to make it slightly acidic.The aqueous reaction mixture was then extracted with ethyl acetate in portions. The combined organic portions was dried over Na_2SO_4 and evaporated in vacuo to obtain the solid acid.

^{1}HNMR, DMSO-d_6, δ (ppm): 8.18–8.15 (m, 1H, quinazoline ring), 7.80–7.75 (m, 1H, quinazoline), 7.66–7.64 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 3.53 (s, 3H,-OCH_3).

Synthesis of (4-substituted piperazine-1-yl) (4-alkylquinazoline-2-yl) methanone (MQZ1-10; EQZ 1-10)

To a stirred solution of acid **(4)** (1 mmol) in DCM catalytic amount of DMF, followed by oxalyl chloride (3 mmol) were added at 0°C. After completion of the reaction in about 1h, the excess dichloromethane and oxalyl chloride was removed in vacuo to obtain the solid acid chloride. The acid chloride thus obtained was redissolved in DCM. To this, triethylamine (1 mmol) and various substituted piperazines (1 mmol) were added and the kept under continuous stirring at room temperature for about 1 h. Once the reaction was complete, the solvent was removed under vacuo to obtain a semi solid mass. The semisolid mass was dissolved in ethylacetate and washed several times with water and saturated sodium bicarbonate solution.The ethylacetate portion was dried over Na_2SO_4 and evaporated in vacuo to obtain the desired carboxamides.

Scheme-IV:(1-methyl-1H-indol-2-yl)(4-substitutedpiperazin-1-yl)methanone derivatives (NMIC, NEIC and NBIC series)

Synthesis of ethyl 1H-indole-2-carboxylate (2)

Indole-2-carboxylic acid was dissolved in sufficient amount of ethanol in a round bottomed flask. To this, 2 ml of conc. H_2SO_4 was added.

The reaction mixture was refluxed for 6 hrs. Upon completion of the reaction, the reaction mixture was poured onto crushed ice and neutralised using sodium bicarbonate.The solid thus obtained was filtered, washed with water and used for further reaction without purification.

Synthesis of ethyl 1-alkyl/benzyl-1H-indole-2-carboxylate (3) (Sechi et al., 2004)

The indole-2-carboxylic acid ethyl ester (**2**) was dissolved in anhydrous DMSO, to this KOH (3 eqv.) and alkyl halide or benzyl bromide (1.5 eqv.) were added and the reaction mixture stirred at room temperature for 1hr.The reaction was monitored using TLC. Upon completion of the reaction, the reaction mixture was poured on to crushed ice. The solid thus obtained was washed with water and filtered.

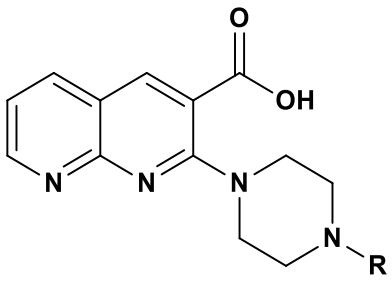
Synthesis of 1-methyl-1H-indole-2-carboxylic acid (4)

A reaction mixture of the ester (**3**) (2 g, 8.45 mmol), equimolar aq. NaOH and 10ml ethanol was stirred at room temperature for 1h.The progress of the reaction was monitored using TLC using EtOAc: n-Hexane (1:1) as solvent system.Once the reaction was completed, the reaction mixture was evaporated in vacuo.To the dark brown thickliquid obtained, crushed ice was added and acidified with dilute HCl upto pH 3. Brown solid thus precipitated out was filtered and washed with ice cold water to obtain the pure acid.IR (KBr)γcm^{-1}: 2987, 2946, 2821, 1878, 1740, 1494, 1430, 1370, 1271. ^{1}HNMR, DMSOd_6, δ (ppm): 9.07-9.05 (m, 1H, naphthyridine), 8.89 (s, 1H, naphthyridine), 8.73-8.70 (m, 1H, naphthyridine), 7.70-7.67 (m, 1H, naphthyridine), 4.42 (s, 1H, -OCH_3).

General procedure for thesynthesis of (1-methyl-1H-indol-2-yl)(4-substitutedpiperazin-1-yl)methanone derivatives (NMIC, NEIC and NBIC series):

N-substituted indole 2-Carboxylic acid **(4)**, 1 mmol was dissolved in DCM, in a round bottomed flask, under nitrogen condition. To this 1.2 equivalent EDC.HCl, followed by 1.5 equivalent HOBt were added and stirred at 0^{o}C, for 5-10 min. Finally, various commercially available substituted piperazines were added in equimolar quantities. The reaction mixture was further stirred for 1h at room temperature. On completion, the reaction mixture was evaporated and washed with water. The crude compounds were purified by recrystalization and (or) column chromatography.

Table 1: Physical constants of piperazine analogs of 1,8-naphthyridine-3-carboxylic acids (NACA 1-15;Series 1)

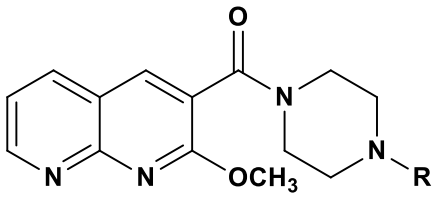


NACA 1-15

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
NACA 1	C_6H_5-	334	58	180-182	3.35
NACA 2	C_6H_5-CH_2-	348	56	160-162	2.89
NACA 3	$C_6H_5CH_2CH_2$-	362	71	98-100	3.05
NACA 4	*p*- NO_2-C_6H_4-	379	62	220-222	3.24
NACA 5	*o*-Cl-C_6H_4-	368	75	116-118	4.01
NACA 6	*m*-Cl-C_6H_4-	368	69	138-140	4.01
NACA 7	*p*-Cl-C_6H_4-	368	54	165-167	4.01
NACA 8	*o*-$OCH_3C_6H_4$-	364	76	200-202	3.26
NACA 9	*m*-OCH_3-C_6H_4-	364	58	120-122	3.26
NACA 10	*p*-OCH_3-C_6H_4-	364	67	158-160	3.26
NACA 11	*o*-CH_3-C_6H_4-	348	61	178-180	3.57
NACA 12	*p*-CH_3-C_6H_4-	348	81	118-120	3.57
NACA 13	CH_3-	272	65	88-90	1.18
NACA 14	CH_3CH_2-	286	62	120-122	1.60
NACA 15	$(CH_3)_2CH$-	300	66	135-137	2.06

[a] yields refer to isolated pure compounds; [b] log P values are calculated using JME molecular editor (courtesy of Peter Ertl, Novartis) *all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 2: Physical constants of synthesized piperazine analogs of 2-methoxy-1,8-naphthyridine carboxamides (MN1-MN15;Series 2)

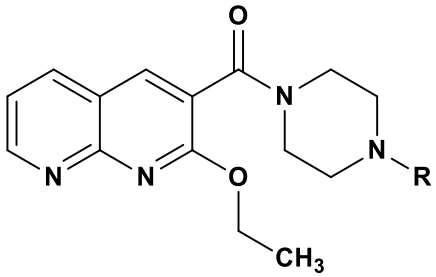


MN 1-15

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
MN 1	C_6H_5-	348	53	156-159	2.98
MN 2	C_6H_5-CH_2-	362	56	160-162	2.53
MN 3	*p*- NO_2-C_6H_4-	391	62	202-204	2.88
MN 4	*o*-Cl-C_6H_4-	382.5	67	148-150	3.16
MN 5	*m*-Cl-C_6H_4-	382.5	54	165-167	3.16
MN 6	*p*-Cl-C_6H_4-	382.5	80	196-198	3.16
MN 7	*o*-$OCH_3C_6H_4$-	378	76	160-162	2.90
MN 8	*m*$OCH_3C_6H_4$-	378	77	123-125	2.90
MN 9	*p*-$OCH_3C_6H_4$-	378	58	120-122	2.90
MN 10	*o*-CH_3-C_6H_4-	362	89	129-131	3.21
MN 11	*p*-CH_3-C_6H_4-	362	63	200-202	3.21
MN 12	CH_3-	286	65	114-116	0.82
MN 13	CH_3-CH_2-	300	62	120-122	1.24
MN 14	$CH_3CH_2CH_2$-	314	76	135-137	1.60
MN 15	$CH_3(CH_2)_3$-	328	64	143-145	1.96

[a] yields refer to isolated pure compounds; [b] log P values are calculated using JME molecular editor (courtesy of PeterErtl, Novartis) *all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 3: Physical constants of piperazine analogs of 2-ethoxy-1, 8-naphthyridin-3-carboxamides (EN 1-15; Series 2)

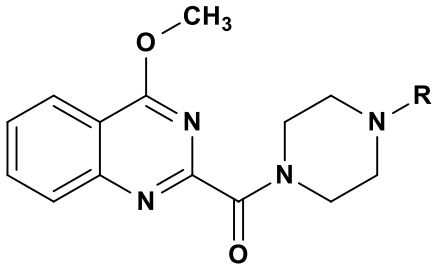


EN1-15

Compound	R	MW	% Yield[a]	m.p.*	Log P [b]
EN 1	C_6H_5-	362	84	126-128	3.41
EN 2	C_6H_5-CH_2-	376	65	118-120	2.95
EN 3	*p*- NO_2-C_6H_4-	405	67	196-198	3.30
EN 4	*o*-Cl-C_6H_4-	396.5	75	134-136	3.58
EN 5	*m*-Cl-C_6H_4-	396.5	83	138-140	3.58
EN 6	*p*-Cl-C_6H_4-	396.5	76	145-147	3.58
EN 7	*o*-OCH_3-C_6H_4-	392	71	130-132	3.32
EN 8	*m*-OCH_3-C_6H_4-	392	68	112-114	3.32
MN 9	*p*-$OCH_3C_6H_4$-	392	54	120-122	3.32
EN 10	*o*-CH_3-C_6H_4-	376	71	90-92	3.63
EN 11	*p*-CH_3-C_6H_4-	376	76	146-148	3.63
EN 12	CH_3-	300	67	114-116	1.24
EN 13	CH_3CH_2-	314	66	120-122	1.66
EN 14	$CH_3CH_2CH_2$	328	57	135-137	2.02
EN 15	$CH_3(CH_2)_3$-	342	52	131-133	1.66

[a] yields refer to isolated pure compounds; [b] log P values are calculated using JME molecular editor (courtesy of PeterErtl, Novartis) *all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 4: Physical constants of synthesized piperazine analogs of 4-methoxy-quinazoline carboxamide (MQZ1-10; Series 3)

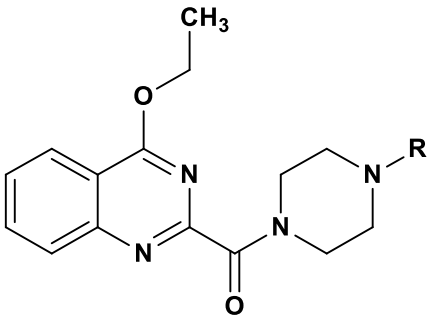


MQZ 1-10

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
MQZ 1	C_6H_5-	348	53	156-159	2.98
MQZ 2	*o*-Cl-C_6H_4-	382.5	67	148-150	3.16
MQZ 3	*m*-Cl-C_6H_4-	382.5	54	165-167	3.16
MQZ 4	*p*-Cl-C_6H_4-	382.5	80	196-198	3.16
MQZ 5	*o*-$OCH_3C_6H_4$-	378	76	160-162	2.90
MQZ 6	*m*$OCH_3C_6H_4$-	378	77	123-125	2.90
MQZ 7	*p*-$OCH_3C_6H_4$-	378	58	120-122	2.90
MQZ 8	CH_3-	286	65	114-116	0.82
MQZ 9	CH_3-CH_2-	300	62	120-122	1.24
MQZ10	$CH_3CH_2CH_2$-	314	76	135-137	1.60

[a] yields refer to isolated pure compounds; [b] log P values are calculated using JME molecular editor (courtesy o fPeter Ertl, Novartis) *all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 5: Physical constants of piperazine analogs of 4-ethoxy-1,3 quinazoline 2-carboxamides (EQZ1-10;Series 3)

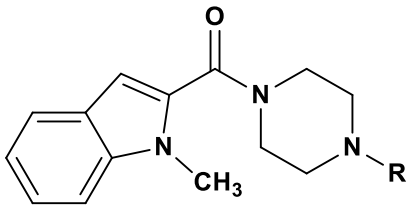


EQZ 1-10

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
EQZ 1	C_6H_5-	362	53	126-128	3.41
EQZ 2	*o*-Cl-C_6H_4-	396.5	67	134-136	3.58
EQZ 3	*m*-Cl-C_6H_4-	396.5	54	138-140	3.58
EQZ 4	*p*-Cl-C_6H_4-	396.5	80	145-147	3.58
EQZ 5	*o*-OCH_3-C_6H_4-	392	76	130-132	3.32
EQZ 6	*m*-OCH_3-C_6H_4-	392	77	112-114	3.32
EQZ 7	*p*-$OCH_3C_6H_4$-	392	58	120-122	2.90
EQZ 8	CH_3-	300	65	114-116	1.24
EQZ 9	CH_3CH_2-	314	62	120-122	1.66
EQZ 10	$CH_3CH_2CH_2$	328	76	135-137	2.02

[a] yields refer to isolated pure compounds; [b] log P values are calculated using JME molecular editor (courtesy of Peter Ertl, Novartis)*all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 6: Physical constants of piperazine analogs of 1-methyl-1H-indol-2-carboxamides (NMIC 1-10; Series 4)

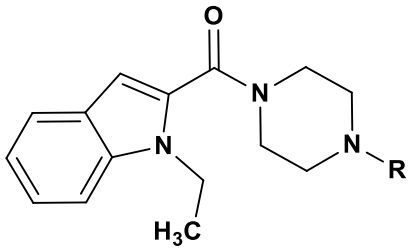


NMIC 1-10

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
NMIC 1	C_6H_5-	319	67	120-122	2.98
NMIC 2	*o*-Cl-C_6H_4-	353.5	63	148-150	3.16
NMIC 3	*m*-Cl-C_6H_4-	353.5	58	136-138	3.16
NMIC 4	*p*-Cl-C_6H_4-	353.5	69	112-114	3.16
NMIC 5	o-$OCH_3C_6H_4$-	349	68	160-162	2.90
NMIC 6	*m*$OCH_3C_6H_4$-	349	74	108-110	2.90
NMIC 7	*p*$OCH_3C_6H_4$-	349	65	122-124	2.90
NMIC 8	CH_3-	257	78	91-93	0.82
NMIC 9	CH_3CH_2-	271	61	100-102	1.24
NMIC 10	$CH_3CH_2CH_2$	285	71	125-127	1.60

[a] yields refer to isolated pure compounds; [b] Log P values of all the compounds were calculated by using JME molecular editor (courtesy of PeterErtl,Novartis) *all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 7: Physical constants of piperazine analogs of 1-ethyl-1H-indol-2-carboxamides (NEIC 1-10; Series 4)

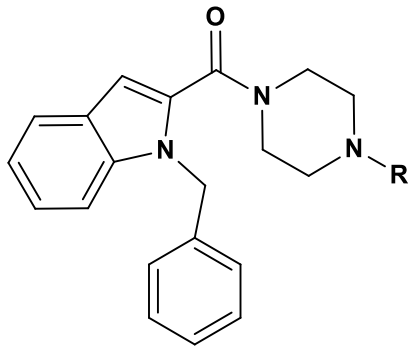


NEIC 1-10

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
NEIC 1	C_6H_5-	333	63	105-107	3.02
NEIC 2	*o*-Cl-C_6H_4-	367	60	138-140	3.21
NEIC 3	*m*-Cl-C_6H_4-	367	53	142-144	3.21
NEIC 4	*p*-Cl-C_6H_4-	367	69	132-134	3.21
NEIC 5	o-$OCH_3C_6H_4$-	363	68	150-152	2.98
NEIC 6	*m*$OCH_3C_6H_4$-	363	64	143-145	2.98
NEIC 7	*p*$OCH_3C_6H_4$-	363	54	140-142	2.98
NEIC 8	CH_3-	271	71	98-100	0.91
NEIC 9	CH_3CH_2-	285	60	110-112	1.28
NEIC10	$CH_3CH_2CH_2$	299	65	115-117	1.70

[a] yields refer to isolated pure compounds; [b]Log P values of were calculated using JME molecularedítor(courtesy of Pete rErtl, Novartis).*all melting points taken in duplicate are expressed in ^oC and are uncorrected.

Table 8: Physical constants of piperazine analogs of 1-benzyl-1H-indol-2-carboxamides NBIC 1-10; Series 4)



NBIC 1-10

Compound	R	MW	% Yield[a]	m.p. *	Log P [b]
NBIC 1	C_6H_5-	395	55	120-122	3.16
NBIC 2	*o*-Cl-C_6H_4-	430	75	148-150	3.33
NBIC 3	*m*-Cl-C_6H_4-	430	70	136-138	3.33
NBIC 4	*p*-Cl-C_6H_4-	430	61	112-114	3.33
NBIC 5	*o*-OCH_3-C_6H_4-	425	67	160-162	3.10
NBIC 6	*m*-OCH_3-C_6H_4-	425	58	108-110	3.10
NBIC 7	*p*-OCH_3-C_6H_4-	425	71	130-132	3.10
NBIC 8	CH_3-	333	54	91-93	1.21
NBIC 9	CH_3CH_2-	347	62	100-102	1.32
NBIC 10	$CH_3CH_2CH_2$-	361	60	125-127	1.78

[a] yields refer to isolated pure compounds; [b] log P values are calculated using JME molecular editor (courtesyofPeterErtl,Novartis)*all melting points taken in duplicate are expressed in ^oC and uncorrected.

Spectral Data

Individual spectral data of the synthesized compounds are described below.

Scheme I (Series 1)

2-(4-phenylpiperazin-1-yl)-1,8-naphthyridine-3-carboxylic acid (NACA 1)

FT-IR (KBr, cm-1): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1350, and 1266. ^{1}H NMR, (400 MHz, DMSO-d_6): δ (ppm): 8.94-8.90 (dd, , J=4.8, 2 Hz, 1H naphthyridine), 8.40 (s, 1H, naphthyridine), 8.10-8.12 (dd, 1H, J=8, 2Hz, naphthyridine), 7.31-7.27 (m, 3H, phenyl), 6.97-6.95 (m, 2H, phenyl), 6.93-6.89 (dd, 1H, J=10, 7.2 Hz, naphthyridine), 3.88-3.86 (m, 4H, piperazine), 3.38-3.35 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 333.1 (M-H$^+$, 100), 335.1 (M+H$^+$, 100).

2-(4-benzylpiperazin-1-yl)-1,8-naphthyridine-3-carboxylic acid (NACA 2)

FT-IR (KBr, cm-1): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1450, 1396, 1250, 1032.^{1}H NMR, (400 MHz, $CDCl_3$): δ (ppm): 8.92-8.90 (m, 1H, naphthyridine), 8.46 (s, 1H, naphthyridine), 8.08-8.06 (m, 1H, naphthyridine), 7.37-7.35 (m, 3H, naphthyridine), 7.29-7.26 (m, 3H, phenyl), 4.01-3.69 (m, 6H, piperazine), 3.13-3.08 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 347.3 (M-H$^+$, 100) 349.1 (M+H$^+$, 100).

2-[4-(2-phenylethyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 3)

FT-IR (KBr, cm-1): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1350, 1296, 1250, 1060. ^{1}H NMR, (400 MHz, $CDCl_3$): δ (ppm): 8.90-8.88 (m, 1H, naphthyridine), 8.30 (s, 1H, naphthyridine), 8.18-8.16 (m, 1H, naphthyridine), 7.37-7.35 (m, 3H, naphthyridine), 7.29-7.26(m, 3H, phenyl), 4.01-3.69 (m, 6H, piperazine), 3.60-3.48 (m, 2H, -CH_2-), 3.12-3.06 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 361.1 (M-H$^+$, 100); 363.1(M+H$^+$, 100).

2-[4-(4-nitrophenyl) piperazin-1-yl]-1, 8-naphthyridine-3-carboxylic acid (NACA 4)

FT-IR (KBr, cm-1): 3580, 3172, 2931, 2850, 2644, 2357, 1749, 1625, 1510, 1462, 1350, 1296, 1240. ^{1}H NMR, (400 MHz,DMSO-d_6): δ (ppm): 8.75-8.73 (dd, 1H, J=4.8, 2 Hz, naphthyridine), 8.12 (s, 1H, naphthyridine), 8.07-8.04 (m, 2H, naphthyridine, phenyl), 7.98-7.96 (m, 1H, naphthyridine), 7.18-7.15 (m, 1H, phenyl), 6.84-6.82 (m, 2H, phenyl), 4.0-3.95 (m, 4H, piperazine), 3.62-3.58 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 378.32 (M-H$^+$, 100) 380.12 (M+H$^+$, 100).

2-[4-(2-chlorophenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 5)

FT-IR (KBr, cm-1): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1350, 1296, 1250, and 1010. ^{1}H NMR, (400 MHz, $CDCl_3$): δ (ppm): 9.05-9.04 (m, 1H, naphthyridine), 8.88 (s, 1H, naphthyridine), 8.33-8.30 (m, 1H, naphthyridine), 7.49-7.45 (m, 1H, naphthyridine), 7.33-7.30 (m, 1H, phenyl), 7.20-7.15 (m, 1H, phenyl), 7.01-6.99 (m, 1H, phenyl), 6.99-6.93 (m, 1H, phenyl), 3.64-3.62 (m, 4H, piperazine), 3.24-3.22 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 367.0 ($M-H^+$, 100) 369.0 ($M+H^+$, 100).

2-[4-(3-chlorophenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 6)

FT-IR (KBr, cm-1): 3290, 3172, 2831, 2750, 2644, 2358, 2328, 1749, 1625, 1510, 1462, and 1350. ^{1}H NMR, (400 MHz, $CDCl_3$):δ (ppm): 8.92-8.91 (m, 1H, naphthyridine); 8.44 (s, 1H, naphthyridine), 8.01-7.99 (m, 1H, naphthyridine), 7.27-7.24 (m, 1H, naphthyridine), 7.19-7.10 (m, 1H, phenyl), 6.90-6.88 (m, 1H, phenyl), 6.83-6.73 (m, 1H, phenyl), 6.72-6.68 (m, 1H, phenyl), 3.50-3.45 (m, 4H, piperazine), 3.34-3.28 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 367.1 ($M-H^+$, 100); 369.0 ($M+H^+$, 100).

2-[4-(4-chlorophenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylicacid (NACA 7)

FT-IR (KBr, cm-1): 3290, 3172, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1288, 1222. ^{1}H NMR, (400 MHz, DMSO-d_6):δ (ppm): 8.86-8.84 (dd, 1H, J=4.8, 2 Hz, naphthyridine), 8.42 (s, 1H, naphthyridine), 8.14-8.12 (dd, 1H, J=8, 2Hz, naphthyridine), 7.28-7.25 (m, 1H, naphthyridine), 7.20-7.17 (m, 2H, phenyl), 6.90-6.88 (m, 2H, phenyl), 3.84-3.81 (m, 4H, piperazine), 3.31-3.29 (m, 4H, piperazine); MS (ESI-ve mode, ESI+ve mode): m/z (%) = 367.2 ($M-H^+$, 100) 369.2 ($M-H^+$, 100).

2-[4-(2-methoxyphenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 8)

FT-IR (KBr, cm-1): 3500, 2872, 2831, 2750, 2644, 2357, 1749, 1625, 1510, 1462, 1350, 1296, 1250. ^{1}HNMR, (400 MHz, DMSO-d_6): δ (ppm): 8.98-8.96 (m, 1H, naphthyridine), 8.21 (s, 1H, naphthyridine), 7.48-7.46 (m, 1H, naphthyridine), 7.19-7.14 (m, 1H, naphthyridine), 6.76-6.68 (m, 4H, phenyl), 3.68 (s, 3H, -OCH_3), 3.62-3.60 (m, 4H, piperazine), 3.19-3.17 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 363.1 ($M-H^+$, 100) 365.0 ($M+H^+$, 100).

2-[4-(3-methoxyphenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 9)

FT-IR (KBr, cm-1): 3460, 3172, 2831, 2750, 2644, 2357, 1749, 1635, 1540, 1462, 1350, 1296, 1250, 1180. ^{1}H NMR, (400 MHz, $CDCl_3$): δ (ppm): 8.81-8.83 (m, 1H, naphthyridine), 8.17 (s, 1H, naphthyridine), 7.84-7.82 (m, 1H, naphthyridine), 7.19-7.14 (m, 1H, naphthyridine), 6.76-6.68 (m, 4H, phenyl), 3.68 (s, 3H, -OCH_3), 3.66-3.63 (m, 4H, piperazine), 3.19-3.17 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 363.1 (M-H^+, 100) 365.1 (M+H^+, 100).

2-[4-(4-methoxyphenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 10)

FT-IR (KBr, cm-1): 3480, 3472, 2531, 2750, 2644, 2457, 1749, 1615, 1510, 1462, 1350, 1296, 1250, 1810. ^{1}H NMR, (400 MHz, $CDCl_3$): δ (ppm): 8.84-8.83 (m, 1H, naphthyridine), 8.35 (s, 1H, naphthyridine), 7.94-7.92 (m, 1H, naphthyridine), 7.19-7.14 (m, 1H, naphthyridine), 6.76-6.68 (m, 4H, phenyl), 3.74 (s, 3H, -OCH_3), 3.66-3.63 (m, 4H, piperazine), 3.09-3.07 (m, 4H, piperazine). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 363.1 (M-H^+, 100) 365.1 (M+H^+, 100).

2-[4-(4-methylphenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 11)

FT-IR (KBr, cm-1): 3490, 3272, 2531, 2750, 2544, 2357, 1749, 1645, 1510, 1462, 1350, 1296, 1250, 1080. ^{1}H NMR, (400 MHz, $CDCl_3$): δ (ppm): 8.98-8.96 (dd, 1H, J=4.8, 2 Hz, naphthyridine), 8.80 (s, 1H, naphthyridine), 8.12-8.10 (dd, 1H, J=8, 2Hz, naphthyridine), 7.35-7.32 (m, 1H, naphthyridine), 6.94-6.92 (m, 2H, phenyl), 6.73-6.71 (m, 2H, phenyl), 3.48-3.46 (m, 4H, piperazine), 3.24-3.22 (m, 4H, piperazine), 2.12 (s, 3H, -CH_3). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 347.3 (M-H^+, 100) 349.3 (M+H^+, 100).

2-[4-(2-methylphenyl) piperazin-1-yl]-1,8-naphthyridine-3-carboxylic acid (NACA 12)

FT-IR (KBr, cm-1): 3290, 2820, 2831, 2750, 2644, 2480, 1749, 1625, 1510, 1462, 1350, 1296. 1H NMR, (400 MHz DMSO-d_6); δ (ppm): 8.88-8.86 (dd, 1H, J=4.8, 2 Hz, naphthyridine), 8.51 (s, 1H, naphthyridine), 8.12-8.10 (dd, 1H, J=8, 2Hz, naphthyridine), 7.55-7.53 (m, 1H, naphthyridine), 6.90-6.92 (m, 2H, phenyl), 6.63-6.61 (m, 2H, phenyl), 3.48-3.46 (m, 4H, piperazine), 3.24-3.22 (m, 4H, piperazine), 2.18 (s, 3H, -CH_3). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 347.1 (M-H^+, 100) 349.1 (M+H^+, 100).

2-(4-methylpiperazin-1-yl)-1,8-naphthyridine-3-carboxylic acid (NACA 13)

FT-IR (KBr, cm-1): 3490, 3172, 2831, 2750, 2490, 2357, 1750, 1625, 1510, 1462, 1350, 1296, 1250, 1032.^{1}H NMR, (400 MHz DMSO-d_6) : δ (ppm): 8.78-8.76 (dd, 1H, J=4.8, 2 Hz, naphthyridine), 8.28 (s, 1H, naphthyridine), 8.02-7.99 (dd, 1H, J=8, 2Hz, naphthyridine), 7.20-7.17 (dd, 1H, J=10, 7.2 Hz, naphthyridine), 3.80-3.78 (m, 4H, piperazine), 2.86-2.84 (m, 4H, piperazine), 2.47 (s, 3H, -CH_3). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 271.1 (M-H^+, 100) 273.1 (M+H^+, 100).

2-(4-ethyl piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acid (NACA 14)

FT-IR (KBr, cm-1): 3590, 3172, 2831, 2750, 2644, 2357, 1749, 1655, 1510, 1462, 1450, 1396, 1250, 1010. ^{1}H NMR, (400 MHz DMSO-d_6): δ (ppm): 8.98-8.96 (m, 1H, naphthyridine), 8.48 (s, 1H, naphthyridine), 7.90-7.88 (m, 1H, naphthyridine), 7.18-7.16 (m, 1H, naphthyridine), 3.18-3.16 (m, 4H, piperazine), 2.46-2.44 (m, 4H, piperazine), 2.10-2.08 (q, 2H, -CH_2-), 1.15-1.13 (t, 3H,-CH_3-). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 285.0 (M-H^+, 100) 287.0 (M+H^+, 100).

2-(4-isopropylpiperazin-1-yl)-1, 8-naphthyridine-3-carboxylic acid (NACA 15)

FT-IR (KBr, cm-1): 3500, 2998, 2831, 2750, 2644, 1749, 1625, 1510, 1462, 1350, 1296, 1250, 1010. ^{1}H NMR, (400 MHz, DMSO-d_6): δ (ppm): 8.72-8.71 (m, 1H, naphthyridine), 8.06 (s, 1H, naphthyridine), 7.99-7.97 (m, 1H, naphthyridine), 7.17-7.14 (m, 1H, naphthyridine), 3.80-3.75 (m, 4H, piperazine), 2.75-2.74 (m, 1H, isopropyl), 2.66-2.56 (m, 4H, piperazine), 1.07-1.05 (d, J=8 Hz, 6H, isopropyl). MS (ESI-ve mode, ESI+ve mode): m/z (%) = 299.2 (M-H^+, 100) 301.1 (M+H^+, 100).

Spectral data of series 2

2-methoxy-1,8-naphthyridin-3-yl(4-phenylpiperazine-1-yl)methanone (MN 1)

IR (KBr) ⁄cm^{-1}: 3125, 2954, 1633, 1500, 1485, 1232, 1157. ^{1}HNMR, (400 MHz DMSOd_6): δ (ppm): 9.04–8.45 (m, 1H, naphthyridine), 8.41–8.32 (m, 2H, naphthyridine), 7.54–7.52 (m, 1H, naphthyridine), 7.51–7.22 (m, 2H, phenyl),7.21–7.01(m, 2H, phenyl), 6.96–6.79(m, 1H, phenyl), 4.09(s, 3H,-OCH_3), 3.88–3.82(m, 2H, piperazine), 3.38–3.34 (m, 2H, piperazine), 3.33–3.24 (m, 2H, piperazine), 3.10–3.09 (m, 2H, piperazine). ESI-MS: m/z 349 (M + 1) 100%.

2-methoxy-1,8-naphthyridin-3-yl (4-benzylpiperazine-1-yl) methanone (MN 2)

IR (KBr) ⁄cm^{-1}: 3120, 3050, 1610, 1554, 1483, 1274. 1HNMR, (400 MHz DMSOd_6): δ (ppm): 9.04–8.45(m, 1H, naphthyridine), 8.41–8.32 (m, 2H, naphthyridine), 7.54–7.52 (m, 1H, naphthyridine), 7.51–7.22 (m, 2H, phenyl), 7.21–7.01(m, 2H, phenyl), 6.96–6.79(m, 1H, phenyl),

4.09(s, 3H,-OCH_3), 3.38–3.34 (m, 2H, piperazine), 3.33–3.24 (m, 2H, piperazine), 3.60–3.48 (m, 2H, -CH_2-), 3.12–3.06 (m, 4H, piperazine). ESI-MS: m/z 363 (M + 1) 100%.

2-methoxy-1,8-naphthyridin-3-yl)[4-(4-nitrophenyl)piperazine-1-yl]methanone (MN 3)

IR (KBr)/cm^{-1}: 3025, 2854, 1625, 1597, 1336, 1244. 1HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.96–8.94 (m, 1H, naphthyridine), 8.10–8.05 (m, 4H, phenyl), 7.37–7.34 (m, 1H, naphthyridine), 6.80–6.74 (m, 2H, naphthyridine), 4.13 (s, 3H,-OCH_3), 3.98–3.90 (m, 2H, piperazine), 3.60–3.50 (m, 2H, piperazine), 3.43–3.25 (m, 4H, piperazine).ESI-MS: m/z 392 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(3-chlorophenyl)piperazine-1-yl]methanone (MN 4)

IR (KBr)/cm^{-1}: 3100, 2997, 2858, 1627, 1527, 1483, 1398, 1238. 1HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.01–9.00 (m, 1H, naphthyridine), 8.15–8.13 (m,1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 7.94–7.40 (m, 1H, naphthyridine), 7.21–7.17(m, 1H, phenyl), 6.89–6.86 (m, 2H, phenyl), 6.81–6.78 (m, 1H, phenyl), 4.20 (s, 3H,-OCH_3), 4.02–3.95 (m, 2H, piperazine), 3.46–3.40 (m, 2H, piperazine), 3.31–3.30 (m, 2H, piperazine), 3.14–3.12 (m, 2H, piperazine). ESI-MS: m/z 383 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(4-chlorophenyl) piperazine-1-yl] methanone (MN 5)

IR (KBr)/cm^{-1}: 3105, 3060, 1627, 1526, 1481,1238.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.01–9.00 (m, 1H, naphthyridine), 8.15–8.13 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 7.94–7.40 (m, 1H, naphthyridine), 7.21–7.17(m, 1H, phenyl), 6.89–6.86 (m, 2H, phenyl), 6.81–6.78 (m,1H, phenyl), 4.20 (s, 3H,-OCH_3), 4.02–3.95 (m, 2H, piperazine), 3.46–3.40 (m, 2H, piperazine), 3.31–3.30 (m, 2H, piperazine), 3.14–3.12 (m, 2H, piperazine). ESI-MS: m/z 383.18 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(2-chlorophenyl) piperazine-1-yl]methanone (MN 6)

IR (KBr)/cm^{-1}: 3100, 2990, 1633, 1556, 1479, 1396. ^{1}HNMR, (400 MHz, $CDCl_3$): δ (ppm): 9.01–9.00 (m, 1H, naphthyridine), 8.15–8.13 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 7.94–7.40 (m, 1H, naphthyridine),7.21–7.17(m, 1H, phenyl), 6.89–6.86 (m, 2H, phenyl), 6.81–6.78 (m, 1H, phenyl), 4.20 (s, 3H,-OCH_3 of quinazoline), 4.02–3.95 (m, 2H, piperazine), 3.46–3.40 (m, 2H, piperazine), 3.31–3.30 (m, 2H, piperazine), 3.14–3.12 (m, 2H, piperazine). ESI-MS: m/z 383 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(2-methoxyphenyl)piperazine-1-yl]methanone (MN 7)

IR (KBr)ν/cm^{-1}: 3150, 3050, 1643, 1558, 1473, 1242, 1018; ^{1}HNMR (400 MHz,$CDCl_3$):δ (ppm): 9.01–8.92 (m, 1H, naphthyridine), 8.15–8.08 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.35–7.30 (m, 1H, naphthyridine), 6.99–6.80 (m, 1H, phenyl), 6.88–6.87 (m, 2H, phenyl), 6.84–6.79 (m, 1H, phenyl), 4.12 (s, 3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.81 (s, 3H, -OCH_3 of phenyl ring), 3.42–3.33 (m, 2H, piperazine), 3.10–2.99 (m, 2H, piperazine) 2.93–2.86 (m, 2H, piperazine). ESI-MS: m/z 379.1 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(3-methoxyphenyl)piperazine-1-yl]methanone (MN 8)

IR (KBr)ν/cm^{-1}: 3050, 2949, 1676, 1500, 1481, 1398, 1250. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.98–8.90 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.30 (m, 1H, naphthyridine), 6.89–6.80 (m, 1H, phenyl), 6.78–6.77 (m, 2H, phenyl), 6.81–6.79 (m, 1H, phenyl), 4.26 (s, 3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.96 (s, 3H, -OCH_3), 3.42–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine) 2.83–2.80 (m, 2H, piperazine). ESI-MS: m/z 379 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(4-methoxyphenyl) piperazine-1-yl]methanone (MN 9)

IR (KBr)ν/cm^{-1}: 3100, 3050, 2949, 1626, 1530, 1475, 1358, 1232. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 9.01–8.97 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.30 (m, 1H, naphthyridine), 6.99–6.80 (m, 1H, phenyl), 6.88–6.87 (m, 2H, phenyl), 6.84–6.79 (m, 1H, phenyl), 4.2 (s,3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.91 (s, 3H, -OCH_3), 3.42–3.33 (m, 2H, piperazine), 3.10–2.99 (m, 2H, piperazine) 2.93–2.86 (m, 2H, piperazine). ESI-MS: m/z 379 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(4-methyl phenyl) piperazine-1-yl] methanone (MN 10)

IR (KBr)ν/cm^{-1}: 3100, 2956, 1620, 1517, 1483, 1398, 1280. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.98–8.90 (m, 1H, naphthyridine), 8.25–8.18 (m,1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.34 (m, 1H, naphthyridine), 6.89–6.80 (m, 1H, phenyl), 6.78–6.77 (m, 2H, phenyl), 6.81–6.79 (m, 1H, phenyl), 4.23 (s, 3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.42–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine), 2.96 (s, 3H, -CH_3), 2.83–2.80 (m, 2H, piperazine). ESI-MS: m/z 363 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)[4-(2-methyl phenyl) piperazine-1-yl] methanone (MN 11)

IR (KBr)ν/cm^{-1}: 3100, 3050, 1633, 1500, 1469, 1224. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.58–8.56 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine),

7.36–7.34 (m, 1H, naphthyridine), 7.10–6.95 (m, 2H, phenyl), 6.93–6.88 (m, 2H, phenyl), 4.16 (s, 3H,-OCH_3), 4.08–3.97 (m, 2H, piperazine), 3.42–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine), 2.25 (s, 3H, -CH_3), 2.88–2.76 (m, 2H, piperazine). ESI-MS: m/z 363 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)(4-methylpiperazine-1-yl) methanone (MN 12)

IR (KBr)/cm^{-1}: 3130, 3080, 1627, 1560, 1435, 1344. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.98–8.90 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.34 (m, 1H, naphthyridine), 4.26 (s, 3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.42–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine), 2.96 (s, 3H, -CH_3), 2.83–2.80 (m, 2H, piperazine). ESI-MS: m/z 287 (M+1)100%.

(2-methoxy-1,8-naphthyridin-3-yl) (4-ethyl piperazine-1-yl) methanone (MN 13)

IR (KBr)/cm^{-1}: 3000, 2980, 1620, 1520, 1429, 1294. ^{1}HNMR, (400 MHz, $CDCl_3$): δ (ppm): 8.98–8.90 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.34 (m, 1H, naphthyridine), 4.21 (s, 3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.42–3.38 (m, 2H, piperazine), 3.12–3.10 (m, 2H, piperazine), 2.93-2.90 (q, 2H, -CH_2), 2.86 (t, J=2.8 Hz, 3H, -CH_3), 2.83–2.80 (m, 2H, piperazine).ESI-MS: m/z 301 (M + 1) 100%.

(2-methoxy-1,8-naphthyridin-3-yl)(4-propylpiperazine-1-yl) methanone (MN 14)

IR (KBr)/cm^{-1}: 3100, 3050, 1625, 1580, 1449, 1244. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.98–8.90 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.34 (m, 1H, naphthyridine), 4.23 (s, 3H,-OCH3), 3.98–3.97 (m, 2H, piperazine), 3.42–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine), 2.90 (t, 2H, -CH_2), 2.86–2.83 (m, 2H, -CH_2), 2.80–2.77 (t, 3H, -CH_3), 2.73–2.70 (m, 2H, piperazine).ESI-MS: m/z 315 (M + 1) 100%.

(2-methoxy-1, 8-naphthyridin-3-yl) (4-butyl piperazine-1-yl) methanone (MN 15)

IR (KBr)/cm^{-1}: 3120, 3060, 1630, 1500, 1469, 1224. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.98–8.90 (m, 1H, naphthyridine), 8.25–8.18 (m, 1H, naphthyridine), 8.0 (s, 1H, naphthyridine), 7.36–7.34 (m, 1H, naphthyridine), 4.20 (s, 3H,-OCH_3), 3.98–3.97 (m, 2H, piperazine), 3.42–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine), 2.95 (t, 2H, -CH_2), 2.90–2.88 (m, 2H, -CH_2), 2.86–2.85 (m, 2H, -CH_2), 2.83–2.80 (m, 2H, piperazine), 2.79 (t, J=2.4 Hz, 3H, -CH_3). ESI-MS: m/z 329 (M + 1) 100%.

2-ethoxy-1, 8-naphthyridin-3-yl (4-phenylpiperazin-1-yl) methanone (EN 1)

IR (KBr)ν/cm^{-1}: 3130, 3080, 1648, 1560,1470, 1232, 1040.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.06-9.04 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 8.16-8.0 (m, 1H, naphthyridine), 7.45-7.42 (m, 1H, naphthyridine), 7.31-7.26 (m, 2H, phenyl), 7.0-6.89 (m, 3H, phenyl), 4.13-3.90 (m, 2H, piperazine), 3.58-3.40 (m, 2H,-piperazine), 3.34-3.32 (m, 2H, piperazine), 3.28-3.24 (q, 2H,-OCH_2), 3.12-3.05 (m, 2H, piperazine), 1.48 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 363.1 (M + 1) 100%.

2-ethoxy-1, 8-naphthyridin-3-yl (4-benzyl piperazin-1-yl) methanone (EN 2)

IR (KBr)ν/cm^{-1}: 3120, 3050, 1650, 1554, 1470, 1035. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.03-9.01 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 8.17-8.15 (m, 1H, naphthyridine), 7.47-7.45 (m, 2H, naphthyridine, phenyl), 7.31-7.19 (m, 4H, phenyl), 3.96-3.90 (m, 2H, piperazine), 3.60-3.58 (m, 2H, benzylic CH_2), 3.43-3.37 (m, 2H, piperazine), 3.28-3.24 (q, 2H,-OCH_2), 2.69-2.48 (m, 2H, piperazine), 2.42-2.30 (m, 2H, piperazine), 1.50 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 377.26 (M + 1) 100%.

2-ethoxy-1,8naphthyridin-3-yl)[4-(4-nitrophenyl)piperazin1yl]methanone (EN 3)

IR (KBr)ν/cm^{-1}: 3025, 2854, 1650, 1597, 1336, 1244. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.07-8.98 (m, 1H, naphthyridine), 8.21-8.18 (m, 3H, naphthyridine, phenyl), 8.12 (s, 1H, naphthyridine), 7.49-7.40 (m, 1H, naphthyridine), 6.90-6.78 (m, 2H, phenyl), 4.17-3.84 (m, 2H, piperazine), 3.65-3.60 (m, 2H,-piperazine), 3.49-3.44 (m, 4H, piperazine), 3.28-3.24 (q, 2H,-OCH_2), 1.48 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 406 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-(2-chlorophenyl) piperazin-1yl] methanone (EN 4)

IR (KBr)ν/cm^{-1}: 3100, 3080, 1648, 1556, 1479, 1037.^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 9.01-8.98 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 8.17-7.93 (m, 1H, naphthyridine), 7.38-7.26 (m, 2H, naphthyridine, phenyl), 7.20-7.14 (m, 1H, phenyl), 7.05-6.86 (m, 2H, phenyl), 4.09-3.83 (m, 2H, piperazine), 3.49-3.46 (m, 2H,-piperazine), 3.28-3.24 (q, 2H,-OCH_2), 3.15-3.07 (m, 2H, piperazine), 3.04-2.84 (m, 2H, piperazine), 1.50 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 397 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-(3-chlorophenyl) piperazin-1yl] methanone (EN 5)

IR (KBr)ν/cm^{-1}: 3100, 3050, 1648, 1546, 1473, 1142, 1037. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.04-8.95 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 8.18-8.08 (m, 1H, 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), 4.09 -3.87 (m, 2H, piperazine), 3.57-3.40 (m, 2H,-piperazine), 3.26-3.24 (q, 2H,-OCH_2), 3.29-3.19 (m, 2H, piperazine), 3.12-3.03 (m, 2H, piperazine), 1.50 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 397.1 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-(4-chlorophenyl) piperazin1yl] methanone (EN 6)

IR (KBr)ν/cm^{-1}: 3150, 3050, 1648, 1566, 1470, 1234, 1040. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 9.03-9.0 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 8.16-8.12 (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), 4.10-3.90 (m, 2H, piperazine), 3.52-3.40 (m, 2H,-piperazine), 3.32-3.30 (m, 2H, piperazine), 3.28-3.24 (q, 2H,-OCH_2), 3.15-3.05 (m, 2H, piperazine), 1.50 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 397.1 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3yl)[4-(2-methoxyphenyl) piperazin1yl] methanone (EN 7)

IR (KBr)ν/cm^{-1}: 3130, 3050, 1650, 1558, 1473, 1242, 1037. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.04-9.0 (m, 1H, naphthyridine), 8.19-8.06 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.45-7.35 (m,1H, naphthyridine), 7.15-7.12 (m,1H, phenyl), 6.90-6.85 (m, 3H, phenyl), 4.18-4.16 (m, 2H, piperazine), 3.80 (s, 3H, OCH_3), 3.38-3.36 (m, 2H, piperazine), 3.26-3.24 (q, 2H,-OCH_2), 3.21-3.13 (m,2H, piperazine), 3.11-2.93 (m, 2H, piperazine), 1.48 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 393.1 (M +1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-(3-methoxyphenyl)piperazin-1yl]methanone (EN 8)

IR (KBr)ν/cm^{-1}: 3150, 3050, 1650, 1560, 1470, 1142, 1035. ^{1}HNMR (400 MHz, $CDCl_3$): δ ppm: 9.05-9.0 (m, 1H, naphthyridine), 8.18-8.14 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.45-7.40 (m, 1H, naphthyridine), 7.26-7.22 (m,2H, phenyl), 6.90-6.85 (m, 2H, phenyl), 4.83-4.63 (m, 2H, piperazine), 4.12-3.93 (m, 2H, piperazine), 3.80 (s, 3H, -OCH_3), 3.53-3.43 (m, 2H, piperazine), 3.28-3.24 (q, 2H, -OCH_2 of ethoxy), 3.18-3.03 (m, 2H, piperazine), 1.49 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). MS (ESI): m/z 393 (M+H) + 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-methoxyphenyl) piperazin1yl] methanone (EN 9)

IR (KBr)ν/cm^{-1}: 3100, 3050, 2949, 1650, 1560, 1470, 1458, 1035. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.06-9.04 (m, 1H, naphthyridine), 8.17-8.15 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.42-7.40 (m, 1H, naphthyridine), 6.92-6.85 (m, 2H, phenyl), 6.82-6.71 (m, 2H, phenyl), 3.21-3.18 (q, 2H,-OCH_2),4.06-3.84 (m, 2H, piperazine),3.80 (s, 3H, OCH_3), 3.51-3.31 (m, 2H, piperazine), 3.19-3.10 (m, 2H, piperazine), 3.01-2.85 (m, 2H, piperazine), 1.48 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 393.1 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-(2-methylphenyl) piperazin-1-yl]methanone (EN 10)

IR (KBr)ν/cm^{-1}: 3120, 3050, 1646, 1500, 1460, 1350, 1037. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.99-8.92 (m, 1H, naphthyridine), 8.19-8.16 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.41-7.36 (m, 1H, naphthyridine), 7.21-7.14 (m, 2H, phenyl), 7.04-6.96 (m, 2H, phenyl), 3.26-3.24 (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 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 377.1 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)[4-(4-methylphenyl) piperazin-1yl] methanone (EN 11)

IR (KBr)ν/cm^{-1}: 3120, 3050, 1646, 1550, 1430, 1340, 1037. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.09-8.93 (m, 1H, naphthyridine), 8.23-8.18 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.50-7.48 (m, 1H, naphthyridine), 7.21-7.02 (m, 2H, phenyl), 6.94-6.77 (m, 2H, phenyl), 4.13-3.92 (m, 2H, piperazine), 3.56-3.37 (m, 2H, piperazine), 3.30-3.28 (m, 2H,-piperazine), 3.26-3.24 (q, 2H,-OCH_2), 3.17-2.98 (m, 2H, piperazine), 2.29 (s, 3H, CH_3), 1.50 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 377.1 (M + 1) 100%.

(2-ethoxy-1, 8-naphthyridin-3-yl) (4-methyl piperazin-1-yl) methanone (EN 12)

IR (KBr)ν/cm^{-1}: 3120, 3070, 1648, 1550, 1435, 1340, 1037. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.06-8.98 (m, 1H, naphthyridine), 8.25-8.18 (m, 1H, naphthyridine), 8.10 (s, 1H, naphthyridine), 7.41-7.38 (m, 1H, naphthyridine), 4.10-3.95 (m, 2H, piperazine), 3.58-3.34 (m, 2H, piperazine), 3.36-3.32 (m, 2H,-piperazine), 3.28-3.26 (q, 2H,-OCH_2), 3.10-2.97 (m, 2H, piperazine), 2.96 (s,3H,CH_3), 1.50 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 301.1 (M + 1) 100%.

(2-ethoxy-1, 8-naphthyridin-3-yl) (4-ethyl piperazin-1-yl) methanone (EN 13)

IR (KBr)ν/cm^{-1}: 3010, 3070, 1648, 1520, 1430, 1290, 1037. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.03-8.96 (m, 1H, naphthyridine), 8.15-8.03 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.35-7.30 (m, 1H, naphthyridine), 3.98-3.93 (m, 2H, piperazine), 3.70-3.58 (m, 2H, piperazine), 3.42-3.40 (m, 2H,-piperazine), 3.28-3.26 (q, 2H,-OCH_2), 3.12-3.10 (m, 2H, piperazine), 2.97-2.80 (m, 2H,CH_2) 2.70-2.50 (m, 3H, CH_3), 1.52 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 315.1 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl)(4-propylpiperazin-1-yl) methanone (EN 14)

IR (KBr)ν/cm^{-1}: 3130, 3030, 1648, 1560, 1470, 1038. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 9.03-8.93 (m, 1H, naphthyridine), 8.30-8.20 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.40-7.35 (m, 1H, naphthyridine), 3.98-3.94 (m, 2H, piperazine), 3.70-3.58 (m, 2H, piperazine), 3.42-

3.38 (m, 2H,-piperazine), 3.28-3.26 (q, 2H,-OCH_2), 3.18-3.16 (m, 2H, piperazine), 2.88-2.70 (m, 2H,-CH_2), 2.68-2.64 (m, 2H, -CH_2), 2.60-2.52 (m, 3H, -CH_3), 1.52 (t, J=2.8 Hz,3H, -CH_3 of ethoxy). ESI-MS: m/z 329.1 (M + 1) 100%.

(2-ethoxy-1,8-naphthyridin-3-yl) (4-butyl piperazin-1-yl) methanone (EN 15)

IR (KBr)νcm^{-1}: 3130, 3038, 1648, 1530, 1470, 1037. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm):9.03-8.93 (m, 1H, naphthyridine), 8.30-8.20 (m, 1H, naphthyridine), 8.11 (s, 1H, naphthyridine), 7.42-7.38 (m, 1H, naphthyridine), 3.98-3.90 (m, 2H, piperazine), 3.70-3.62 (m, 2H, piperazine), 3.42-3.33 (m, 2H,-piperazine), 3.28-3.26 (q, 2H,-OCH_2), 3.12-3.10 (m, 2H, piperazine), 2.93 (t, J=2.6 Hz, 2H, -CH_2), 2.88–2.86 (m, 2H, -CH_2), 2.80–2.78 (m, 2H, -CH_2), 2.83–2.80 (m, 2H, piperazine), 2.78–2.76 (t, J=2.4 Hz, 3H, -CH_3), 1.52 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 343.1 (M + 1) 100%.

Spectral data of Series 3

(4-phenyl piperazine-1-yl) (4-methoxy quinazoline-2-yl) methanone (MQZ 1)

IR (KBr)νcm^{-1}: 3125, 3050, 1633, 1500, 1485, 1252, 1157. ^{1}HNMR, (400 MHz DMSO-*d6*): δ (ppm): 8.24-8.18 (m, 1H, quinazoline), 7.74-7.70 (m, 1H, quinazoline), 7.66–7.60 (m, 1H, quinazoline), 7.54–7.50 (m, 1H, quinazoline), 7.46–7.28 (m, 2H, phenyl), 7.20–7.0 (m, 2H, phenyl), 6.90–6.80 (m, 1H, phenyl), 3.78–3.72 (m, 2H, piperazine), 3.98 (s, 3H,-OCH_3), 3.45–3.40 (m, 2H, piperazine), 3.30–3.28 (m, 2H, piperazine), 3.16–3.10 (m, 2H, piperazine). ESI-MS: m/z 349 (M + 1) 100%.

{4-(2-chloro phenyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 2)

IR (KBr)νcm^{-1}: 3100, 2990, 1633, 1556, 1479, 1396. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.34–8.28 (m, 1H, quinazoline), 7.75–7.68 (m, 1H, quinazoline), 7.60-7.51 (m, 1H, quinazoline), 7.52–7.38 (m, 1H, quinazoline), 7.20–7.17(m, 1H, phenyl), 6.80–6.76 (m, 2H, phenyl), 6.76–6.70 (m, 1H, phenyl), 4.08–3.95 (m, 2H, piperazine), 3.98 (s, 3H,-OCH_3), 3.48–3.38 (m, 2H, piperazine), 3.30–3.26 (m, 2H, piperazine), 3.16–3.11 (m, 2H, piperazine). ESI-MS: m/z 383 (M + 1) 100%.

{4-(3-chloro phenyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 3)

IR (KBr)νcm^{-1}: 3100, 3050, 2858, 1627, 1527, 1483, 1398, 1238. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.34–8.28 (m, 1H, quinazoline), 7.75–7.68 (m, 1H, quinazoline), 7.60-7.51 (m, 1H, quinazoline), 7.52–7.38 (m, 1H, quinazoline), 7.18–7.10 (m, 1H, phenyl), 6.80–6.76 (m, 2H, phenyl), 6.81–6.78 (m, 1H, phenyl), 3.96 (s, 3H,-OCH_3), 3.90–3.80 (m, 2H, piperazine), 3.42–

3.38 (m, 2H, piperazine), 3.31–3.26 (m, 2H, piperazine), 3.18–3.12 (m, 2H, piperazine). ESI-MS: m/z 383 (M + 1) 100%.

{4-(4-chloro phenyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 4)

IR (KBr)ν/cm^{-1}: 3105, 3060, 1620, 1566, 1481, 1234, 1040. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.34–8.28 (m, 1H, quinazoline), 7.75–7.68 (m, 1H, quinazoline), 7.60-7.51 (m, 1H, quinazoline), 7.52–7.38 (m, 1H, quinazoline), 7.21–7.18 (m,1H, phenyl), 6.83–6.76 (m, 2H, phenyl), 6.71–6.68 (m,1H, phenyl), 3.96 (s, 3H,-OCH_3), 3.88–3.85 (m, 2H, piperazine), 3.58–3.50 (m, 2H, piperazine), 3.31–3.25 (m, 2H, piperazine), 3.18–3.14 (m, 2H, piperazine). ESI-MS: m/z 383.18(M + 1) 100%.

{4-(2-methoxy phenyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 5)

IR (KBr)ν/cm^{-1}: 3150, 3050, 1636, 1558, 1473, 1242, 1018; ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32–8.28 (m, 1H, quinazoline), 7.77–7.68 (m, 1H, quinazoline), 7.60-7.50 (m, 1H, quinazoline), 7.41–7.38 (m, 1H, quinazoline), 6.90–6.84 (m, 1H, phenyl), 6.76–6.67 (m, 2H, phenyl), 6.61–6.59 (m, 1H, phenyl), 3.98 (s, 3H, -OCH_3 of quinazoline ring), 3.90–3.87 (m, 2H, piperazine), 3.80 (s, 3H,-OCH_3 of phenyl ring), 3.40–3.33 (m, 2H, piperazine), 3.11–2.95 (m, 2H, piperazine), 2.88–2.84 (m, 2H, piperazine). ESI-MS: m/z 379.1 (M + 1) 100%.

{4-(3-methoxy phenyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 6)

IR (KBr)ν/cm^{-1}: 3050, 2949, 1636, 1500, 1481, 1398, 1250. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32–8.26 (m, 1H, quinazoline), 7.77–7.68 (m, 1H, quinazoline), 7.60-7.52 (m, 1H, quinazoline), 7.43–7.38 (m, 1H, quinazoline), 6.90–6.83 (m, 1H, phenyl), 6.70–6.65 (m, 2H, phenyl), 6.60–6.55 (m, 1H, phenyl), 3.98 (s, 3H, -OCH_3 of quinazoline ring), 3.96–3.93 (m, 2H, piperazine), 3.80 (s, 3H,-OCH_3), 3.39–3.33 (m, 2H, piperazine), 3.10–2.93 (m, 2H, piperazine), 2.89–2.80 (m, 2H, piperazine). ESI-MS: m/z 379 (M + 1) 100%.

{4-(4-methoxy phenyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 7)

IR (KBr)ν/cm^{-1}: 3100, 3050, 2949, 1626, 1530, 1475, 1358, 1232. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32–8.26 (m, 1H, quinazoline), 7.77–7.68 (m, 1H, quinazoline), 7.60-7.52 (m, 1H, quinazoline), 7.43–7.38 (m, 1H, quinazoline), 6.95–6.85 (m, 1H, phenyl), 6.80–6.76 (m, 2H, phenyl), 6.68–6.60 (m, 1H, phenyl), 3.98 (s, 3H, -OCH_3 of quinazoline ring),3.93–3.90 (m, 2H, piperazine), 3.50 (s, 3H,-OCH_3 of phenyl ring), 3.40–3.33 (m, 2H, piperazine), 3.10–2.95 (m, 2H, piperazine), 2.90–2.84 (m, 2H, piperazine). ESI-MS: m/z 379 (M + 1) 100%.

{4-(4-methyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 8)

IR (KBr)ν/cm^{-1}: 3130, 3080, 1627, 1560, 1435, 1344. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.32–8.26 (m, 1H, quinazoline), 7.74–7.68 (m, 1H, quinazoline), 7.56-7.52 (m, 1H, quinazoline), 7.43–7.36 (m, 1H, quinazoline), 4.21–4.05 (m, 2H, piperazine), 3.96 (s, 3H,-OCH_3 of quinazoline ring), 3.42–3.36 (m, 2H, piperazine), 3.0–2.95 (m, 2H, piperazine), 2.86 (s, 3H, -CH_3 of N-methyl group), 2.83–2.78 (m, 2H, piperazine). ESI-MS: m/z 287 (M+1)100%.

{4-(4-ethyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 9)

IR (KBr)ν/cm^{-1}: 3130, 2980, 1620, 1520, 1429, 1294. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32–8.26 (m, 1H, quinazoline), 7.76–7.68 (m, 1H, quinazoline), 7.56-7.52 (m, 1H, quinazoline), 7.43–7.36 (m, 1H, quinazoline), 3.98 (s, 3H,-OCH_3), 3.90–3.86 (m, 2H, piperazine), 3.40–3.33 (m, 2H, piperazine), 3.10–2.97 (m, 2H, piperazine), 2.90-2.88 (q, 2H, -CH_2), 2.84 (t, J=2.4 Hz, 3H, CH_3), 2.78–2.76 (m, 2H, piperazine). ESI-MS: m/z 301 (M + 1) 100%.

{4-(4-propyl piperazine-1-yl)} (4-methoxy quinazoline-2-yl) methanone (MQZ 10)

IR (KBr)ν/cm^{-1}: 3100, 3050, 1620, 1520, 1449, 1244. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32–8.28 (m, 1H, quinazoline), 7.77–7.68 (m, 1H, quinazoline), 7.56-7.52 (m, 1H, quinazoline), 7.43–7.36 (m, 1H, quinazoline) 3.98 (s, 3H,-OCH_3), 3.90–3.86 (m, 2H, piperazine), 3.42–3.33 (m, 2H, piperazine), 3.10–2.95 (m, 2H, piperazine), 2.90 (t, J=2.8 Hz, 2H, -CH_2), 2.85–2.83 (m, 2H, -CH_2), 2.80 (t J=2.4 Hz, 3H, -CH_3) 2.71–2.63 (m, 2H, piperazine). ESI-MS: m/z 315 (M + 1) 100%.

(4-phenyl piperazine-1-yl) (4-ethoxy quinazoline-2-yl) methanone (EQZ 1)

IR (KBr)ν/cm^{-1}: 3010, 2940, 1628, 1530,1445, 1343, 1257. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.36-8.28 (m, 1H, quinazoline), 7.74-7.70 (m, 1H, quinazoline), 7.66–7.60 (m, 1H, quinazoline), 7.54–7.50 (m, 1H, quinazoline), 7.35-7.28 (m, 2H, phenyl), 7.09-6.83 (m, 3H, phenyl), 3.86-3.81 (m, 2H, piperazine), 3.78-3.76 (q, 2H,-OCH_2), 3.48-3.40 (m, 2H,-piperazine), 3.30-3.24 (m, 2H, piperazine), 3.18-3.05 (m, 2H, piperazine), 1.53 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 363 (M + 1) 100%.

{4-(2-chloro phenyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 2)

IR (KBr)ν/cm^{-1}: 3020, 2940, 1620, 1536, 1455, 1375, 1240.^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.34-8.28 (m, 1H, quinazoline), 7.74-7.70 (m, 1H, quinazoline), 7.66–7.62 (m, 1H, quinazoline), 7.53–7.48 (m, 1H, quinazoline), 7.38-7.26 (m, 2H, phenyl), 6.98-6.86 (m, 2H, phenyl), 3.78-3.76 (q, 2H,-OCH_2), 3.67-3.60 (m, 2H, piperazine), 3.53-3.45 (m, 2H,-piperazine), 3.25-3.21 (m, 2H, piperazine), 3.15-3.05 (m, 2H, piperazine), 1.50 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 397 (M + 1) 100%.

{4-(3-chloro phenyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 3)

IR (KBr)ν/cm^{-1}: 3020, 2856, 1625, 1536,1455, 1375, 1248. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.34-8.28 (m, 1H, quinazoline), 7.73-7.70 (m, 1H, quinazoline), 7.66–7.60 (m, 1H, quinazoline), 7.53–7.48 (m, 1H, quinazoline), 7.30-7.26 (m, 1H, phenyl), 7.17-7.08 (m, 2H, phenyl), 6.72-6.64 (m, 1H, phenyl), 3.78-3.76 (q, 2H,-OCH_2), 3.63-3.58 (m, 2H, piperazine), 3.50-3.43 (m, 2H,-piperazine), 3.26-3.21 (m, 2H, piperazine), 3.11-3.03 (m, 2H, piperazine), 1.50 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 397.1 (M + 1) 100%.

{4-(4-chloro phenyl piperazine-1-yl)}(4-ethoxy quinazoline-2-yl) methanone (EQZ 4)

IR (KBr)ν/cm^{-1}: 3020, 2850, 1625, 1532,1455, 1371, 1248. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.34-8.28 (m, 1H, quinazoline), 7.74-7.70 (m, 1H, quinazoline), 7.66–7.62 (m, 1H, quinazoline), 7.53–7.48 (m, 1H, quinazoline), 7.34-7.26 (m, 2H, phenyl), 6.98-6.83 (m, 2H, phenyl), 3.78-3.74 (q, 2H,-OCH_2), 3.67-3.58 (m, 2H, piperazine), 3.53-3.43 (m, 2H,-piperazine), 3.26-3.21 (m, 2H, piperazine), 3.15-3.03 (m, 2H, piperazine), 1.50 (t, J=2.8 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 397.1 (M + 1) 100%.

{4-(2-methoxy phenyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 5)

IR (KBr)ν/cm^{-1}: 3167, 3050, 1640, 1558, 1471, 1242, 1018; ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32-8.28 (m, 1H, quinazoline), 7.72-7.68 (m, 1H, quinazoline), 7.62–7.58 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 7.35-7.26 (m, 1H, phenyl), 6.90-6.85 (m, 3H, phenyl), 3.76-3.74 (q, 2H,-CH_2 of ethoxy), 3.60-3.52 (m, 2H, piperazine), 3.43 (s, 3H, OCH_3 of phenyl), 3.36-3.28 (m, 2H,-piperazine), 3.21-3.18 (m, 2H, piperazine), 3.11-3.05 (m, 2H, piperazine), 1.52 (t, 3 J=2.4 Hz, H, -CH_3 of ethoxy). ESI-MS: m/z 393.1 (M + 1) 100%.

{4-(3-methoxy phenyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 6)

IR (KBr)/cm^{-1}: 3167, 3052, 1640, 1550, 1471, 1231, 1018; ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32-8.26 (m, 1H, quinazoline), 7.72-7.66 (m, 1H, quinazoline), 7.62–7.60 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 7.35-6.21 (m, 2H, phenyl), 6.92-6.81 (m, 2H, phenyl), 3.76-3.74 (q, 2H,-OCH_2), 3.60-3.54 (m, 2H, piperazine), 3.43 (s, 3H, OCH_3 of phenyl ring), 3.31-3.28 (m, 2H,-piperazine), 3.20-3.18 (m, 2H, piperazine), 3.12-3.05 (m, 2H, piperazine), 1.52 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 393.1 (M + 1) 100%.

{4-(4-methoxy phenyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 7)

IR (KBr)/cm^{-1}: 3160, 3052, 1638, 1550, 1471, 1227, 1021. (400 MHz, $CDCl_3$):δ (ppm): 8.32-8.28 (m, 1H, quinazoline), 7.72-7.68 (m, 1H, quinazoline), 7.62–7.60 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 7.35-6.21 (m, 2H, phenyl), 6.92-6.88 (m, 2H, phenyl), 3.76-3.74 (q, 2H,-OCH_2), 3.60-3.54 (m, 2H, piperazine), 3.40 (s, 3H, OCH_3 of phenyl ring), 3.31-3.28 (m, 2H,-piperazine), 3.20-3.18 (m, 2H, piperazine), 3.10-3.05 (m, 2H, piperazine), 1.52 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 393.1 (M + 1) 100%.

{4-(4-methyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 8)

IR (KBr)/cm^{-1}: 3135, 3070, 1621, 1560, 1438, 1344. (400 MHz, $CDCl_3$):δ (ppm): 8.32-8.28 (m, 1H, quinazoline), 7.72-7.68 (m, 1H, quinazoline), 7.62–7.58 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 3.78-3.76 (q, 2H,-CH_2 of ethoxy), 3.60-3.55 (m, 2H, piperazine), 3.38-3.34 (m, 2H, piperazine), 3.24-3.14 (m, 2H,-piperazine), 3.10-3.05 (m, 2H, piperazine), 2.90 (s, 3H, CH_3), 1.50 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 301 (M + 1) 100%.

{4-(4-ethyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 9)

IR (KBr)/cm^{-1}: 3120, 2980, 1620, 1560, 1438, 1314.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.32-8.28 (m, 1H, quinazoline), 7.72-7.68 (m, 1H, quinazoline), 7.62–7.58 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 3.78-3.76 (q, 2H,-CH_2 of ethoxy), 3.60-3.53 (m, 2H, piperazine), 3.38-3.34 (m, 2H, piperazine), 3.24-3.16 (m, 2H,-piperazine), 3.10-3.06 (m, 2H, piperazine), 2.90-2.83 (q, 2H,CH_2), 2.72-2.65 (m, 3H, CH_3),1.50 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 315.1 (M + 1) 100%.

{4-(4-propyl piperazine-1-yl)} (4-ethoxy quinazoline-2-yl) methanone (EQZ 10)

IR (KBr)νcm^{-1}: 3100, 2980, 1620, 1560, 1440, 1344.^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 8.32-8.28 (m, 1H, quinazoline), 7.72-7.68 (m, 1H, quinazoline), 7.62–7.58 (m, 1H, quinazoline), 7.52–7.48 (m, 1H, quinazoline), 3.78-3.76 (q, 2H,-CH_2 of ethoxy), 3.58-3.50 (m, 2H, piperazine), 3.38-3.34 (m, 2H, piperazine), 3.24-3.16 (m, 2H,-piperazine), 3.15-3.05 (m, 2H, piperazine), 2.88-2.78 (m, 2H,-CH_2), 2.70 (t, J=2.8 Hz, 2H,-CH_2), 2.57-2.50 (m, 2H, -CH_2), 1.50 (t, J=2.4 Hz, 3H, -CH_3 of ethoxy). ESI-MS: m/z 329.1 (M + 1) 100%.

Spectral data of Series 4

(1-methyl-1*H*-indol-2-yl) (4-phenylpiperazin-1-yl) methanone (NMIC 1)

IR (KBr) /cm: 3100, 3080, 1628, 1540, 1230, 1135. 1HNMR (400 MHz, $CDCl_3$): 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-methyl-1*H*-indol-2-yl) (2-chloro phenylpiperazin-1-yl) methanone (NMIC 2)

IR (KBr)/cm: 3120, 3050, 1625, 1530, 1240, 1125. 1HNMR (400 MHz, $CDCl_3$): 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, CH3) 3.14-2.84 (m, 4H, piperazine). ESI-MS: m/z 354.1 (M + 1) 100%.

(1-methyl-1*H*-indol-2-yl) (3-chloro phenylpiperazin-1-yl) methanone (NMIC 3)

IR (KBr/cm): 3120, 3080, 1625, 1550, 1240, 1035. 1HNMR (400 MHz, $CDCl_3$): 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, CH3) 3.35-3.19 (m, 4H, piperazine). ESI-MS: m/z 354 (M + 1) 100%.

(1-methyl-1*H*-indol-2-yl) (4-chlorophenylpiperazin-1-yl) methanone (NMIC 4)

IR (KBr/cm): 3100, 3080, 1625, 1550, 1240, 1035. 1HNMR (400 MHz, $CDCl_3$): 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, CH3) 3.34-3.08 (m, 4H, piperazine). ESI-MS: m/z 354 (M + 1) 100%.

(1-methyl-1*H*-indol-2-yl) (2-methoxy phenylpiperazin-1-yl) methanone (NMIC 5)

IR (KBr/cm): 3130, 3050, 1626, 1550, 1240, 1035. 1HNMR (400 MHz, $CDCl_3$): 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, -CH3 of N-CH3), 3.79 (s, 3H, OCH3 of phenyl), 3.19-2.87 (m, 4H, piperazine). ESI-MS: m/z 350 (M + 1) 100%

(1-methyl-1*H*-indol-2-yl) (3-methoxy phenylpiperazin-1-yl) methanone (NMIC 6)

IR (KBr/cm): 3130, 3050, 1628, 1550, 1230, 1035. 1HNMR (400 MHz, $CDCl_3$): 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, CH3 of N-CH3), 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-1*H*-indol-2-yl) (4-methoxy phenylpiperazin-1-yl) methanone (NMIC 7)

IR (KBr)/cm^{-1}: 3150, 3090, 2853, 1725, 1626, 1526, 1462, 1431, 1350.^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 7.65-7.60 (m, 1H, indole ring), 7.46-7.36 (m, 1H, indole, phenyl), 7.34-7.30 (m, 1H, indole), 7.12-7.08 (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-1*H*-indol-2-yl) (4-methyl piperazin-1-yl) methanone (NMIC 8)

IR (KBr)/cm: 3130, 3010, 1620, 1580, 1244, 1150. 1HNMR (400 MHz, $CDCl_3$) 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, CH3 of N-CH3), 3.68-3.52 (m, 4H, piperazine). 2.60 (s, 3H, -CH3). ESI-MS: m/z 258.1 (M + 1) 100%.

(1-methyl-1*H*-indol-2-yl) (4-ethyl piperazin-1-yl) methanone (NMIC 9)

IR (KBr)/cm: 3120, 3030, 1628, 1580, 1244, 1150. 1HNMR (400 MHz, $CDCl_3$): 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), 2.90-83 (q, 2H, -CH_2), 2.80 (t, J=2.4 Hz, 3H, -CH3). ESI-MS: m/z 272 (M + 1) 100%.

(1-methyl-1*H*-indol-2-yl) (4-propyl piperazin-1-yl) methanone (NMIC 10)

IR (KBr)/cm: 3130, 3050, 1625, 1580, 1244, 1149. 1HNMR (400 MHz, $CDCl_3$): 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 (t, J=2.4 Hz, 2H, -CH_2), 2.86–2.83 (m, 2H, -CH_2), 2.70 (t, J=2.4 Hz, 3H, -CH_3). ESI-MS: m/z 286.1 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (4-phenylpiperazin-1-yl) methanone (NEIC 1)

IR (KBr)/cm^{-1}: 3109, 2930, 2660, 2652, 2500, 2475, 1630, 1523, 1450, 1432. ^{1}HNMR (400 MHz, $CDCl_3$):δ (ppm): 7.65-7.60 (m, 1H, indole), 7.56-7.43 (m, 1H, indole), 7.38-7.33 (m, 1H, indole), 7.31-7.25 (m, 1H, indole), 7.20-7.12 (m, 2H, phenyl) , 7.05-6.98 (m, 3H, phenyl), 6.64 (s, 1H, indole), 4.68-4.52 (q, 2H,-CH_2 of ethyl group), 4.13-43.99 (m, 4H, piperazine), 3.40-3.21 (m, 4H, piperazine), 1.46 (t, J=2.8 Hz,3H, -CH_3 of ethyl group). ESI-MS: m/z 334 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (2-chlorophenyl piperazin-1-yl) methanone (NEIC 2)

IR (KBr)/cm^{-1}: 3170, 2868, 2762, 1766, 1714, 1627, 1531, 1442, 1313. ^{1}HNMR (400 MHz, $CDCl_3$) δ (ppm): 7.66-7.59 (m, 1H, indole ring), 7.56-7.43 (m, 1H, indole), 7.45-7.38 (m, 2H, indole, phenyl), 7.33-7.23 (m, 1H indole), 7.19-7.12 (m, 1H phenyl), 7.08-6.97 (m, 2H, phenyl), 6.61 (s,1H, indole), 4.39-4.28 (q, 2H,-CH_2 of ethyl group), 4.04-3.90 (m, 4H, piperazine), 3.19-3.01 (m, 4H, piperazine), 1.43 (t, J=2.4 Hz, 3H, -CH_3 of ethyl). ESI-MS: m/z 368 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (3-chlorophenyl piperazin-1-yl) methanone (NEIC 3)

IR (KBr)/cm^{-1}: 3170, 3050, 2762, 1766, 1714, 1627, 1531, 1442, 1313. ^{1}HNMR (400 MHz, $CDCl_3$):, δ (ppm): 7.66-7.59 (m, 1H, indole ring), 7.56-7.43 (m, 1H, indole), 7.45-7.38 (m, 2H, indole, phenyl), 7.33-7.23 (m, 1H indole), 7.19-7.12 (m, 1H phenyl), 7.08-6.97 (m, 2H, phenyl), 6.61 (s,1H, indole), 4.39-4.28 (q, 2H,-CH_2 of ethyl group), 4.04-3.90 (m, 4H, piperazine), 3.19-3.01 (m, 4H, piperazine), 1.43 (t, J=2.4 Hz, 3H, -CH_3 of ethyl). ESI-MS: m/z 368 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (4-chlorophenyl piperazin-1-yl) methanone (NEIC 4)

IR (KBr)/cm^{-1}: 3170, 3050, 2760, 1766, 1730, 1627, 1531, 1442, 1313. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 8.71-8.54 (m, 1H, indole ring), 7.50-7.36 (m, 1H, indole), 7.29-7.26 (m, 2H indole), 7.19-7.09 (m, 2H phenyl), 6.94-6.80 (m, 2H, phenyl), 6.64 (s,1H, indole), 4.44-4.36 (q, 2H,-CH_2 of ethyl group), 4.09-3.81 (m, 4H, piperazine), 3.47-3.30 (m, 4H, piperazine), 1.43 (t, J=2.4 Hz, 3H, -CH_3 of ethyl group). ESI-MS: m/z 368 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (2-methoxy phenylpiperazin-1-yl) methanone (NEIC 5)

IR (KBr)ν/cm^{-1}: 3108, 2836, 1726, 1630, 1520, 1462, 1435, 1350.^{1}HNMR (400 MHz, $CDCl_3$): 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), 6.65 (s, 1H, indole), 4.08-3.83 (m, 4H, piperazine), 4.40-4.28 (q, 2H,-CH_2 of ethyl group), 3.79 (s, 3H, OCH_3 of phenyl), 3.19-2.87 (m, 4H, piperazine). 1.46 (t, J=2.8 Hz, 3H, -CH_3 of ethyl group). ESI-MS: m/z 364.1 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (3-methoxy phenylpiperazin-1-yl) methanone (NEIC 6)

IR (KBr)ν/cm^{-1}: 3108, 2853, 1720, 1628, 1530, 1462, 1431, 1350.^{1}HNMR (400 MHz, $CDCl_3$): 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), 4.40-4.28 (q, 2H,-CH_2 of ethyl group), 4.02-3.93 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3 of phenyl), 3.33-3.12 (m, 4H, piperazine).1.46 (t, J=2.8 Hz, 3H, -CH_3 of ethyl group). ESI-MS: m/z 364 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (4-methoxy phenylpiperazin-1-yl) methanone (NEIC 7)

IR (KBr)ν/cm^{-1}: 3105, 3050, 1720, 1628, 1528, 1462, 1431, 1350.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 7.67-7.60 (m, 1H, indole ring), 7.46-7.38 (m, 1H, indole, phenyl), 7.34-7.29 (m, 1H, indole), 7.14-7.11 (m, 2H phenyl), 6.64 (s, 1H, indole), 6.61-6.58 (m, 1H phenyl), 6.49-6.45 (m, 2H phenyl), 4.42-4.30 (q, 2H,-CH_2 of ethyl group), 4.02-3.93 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3 of phenyl), 3.33-3.12 (m, 4H, piperazine), 1.46 (t, J=2.8 Hz, 3H, -CH_3 of ethyl group). ESI-MS: m/z 364.1 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (4-methyl piperazin-1-yl) methanone (NEIC 8)

IR (KBr)ν/cm^{-1}: 3125, 2980, 1621, 1560, 1438, 1344.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 7.65-7.60 (m, 1H, indole), 7.56-7.43 (m, 1H, indole), 7.38-7.33 (m, 1H, indole), 7.31-7.25 (m, 1H, indole), 6.64 (s, 1H, indole), 4.40-4.30 (q, 2H,-CH_2 of ethyl group), 4.05-3.88 (m, 4H, piperazine), 3.11-2.9 (m, 4H, piperazine), 2.86 (s, 3H, CH_3). 1.46 (t, J=2.8 Hz, 3H, -CH_3 of ethyl group).ESI-MS: m/z 272 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (4-ethyl piperazin-1-yl) methanone (NEIC 9)

IR (KBr)ν cm^{-1}: 3120, 3050, 1620, 1560, 1440, 1314.^{1}HNMR $CDCl_3$, δ (ppm): 7.65-7.60 (m, 1H, indole), 7.56-7.43 (m, 1H, indole), 7.38-7.33 (m, 1H, indole), 7.31-7.25 (m, 1H, indole), 6.64 (s, 1H, indole), 4.40-4.32 (q, 2H,-CH_2 of ethyl group), 4.05-3.88 (m, 4H,-piperazine), 3.11-2.9 (m, 4H, piperazine), 2.90 (t, J=2.4 Hz, 2H, CH_2), 2.70-2.65 (m, 3H, CH_3). 1.46 (t, J=2.8 Hz, 3H, -CH_3 of ethyl group). ESI-MS: m/z 286 (M + 1) 100%.

(1-ethyl-1*H*-indol-2-yl) (4-propyl piperazin-1-yl) methanone (NEIC 10)

IR (KBr)ν cm^{-1}: 3120, 3050, 1620, 1560, 1440, 1314.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm):7.67-7.60 (m, 1H, indole), 7.56-7.43 (m, 1H, indole), 7.40-7.33 (m, 1H, indole), 7.31-7.25 (m, 1H, indole), 6.64 (s, 1H, indole), 4.40-4.28 (q, 2H,-CH_2 of ethyl group), 4.05-3.88 (m, 4H,-piperazine), 3.11-2.9 (m, 4H, piperazine), 2.88 (t, J=2.6 Hz, 2H, CH_2), 2.80-2.78 (m, 2H, CH_2), 2.70-2.65 (m, 3H, CH_3). 1.46 (t, J=2.8 Hz, 3H, -CH_3 of ethyl group). ESI-MS: m/z 300.1 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (4-phenyl piperazin-1-yl) methanone (NBIC 1)

IR (KBr)ν cm^{-1}: 2930, 2660, 2652, 2500, 2475, 1639, 1523, 1450, 1432. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.44-7.42 (m, 1H, indole), 7.33-7.31 (m, 4H indole, phenyl), 7.30-7.22 (m, 4H indole, phenyl), 7.20-7.08 (m, 2H phenyl), 7.06-6.92 (m, 1H phenyl), 6.90-6.89 (m, 1H phenyl), 6.64 (s, 1H, indole), 5.53 (s, 2H, benzyl), 3.84-3.75 (m, 4H, piperazine), 2.87-2.82 (m, 4H, piperazine). ESI-MS: m/z 396 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (2-chlorophenyl piperazin-1-yl) methanone (NBIC 2)

IR (KBr)ν cm^{-1}: 2916, 2829, 1732, 1628, 1506, 1456, 1435, 1369. ^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.45-7.43 (m, 1H, indole), 7.34-7.31 (m, 1H indole, phenyl), 7.29-7.23 (m, 6H indole, phenyl), 7.20-7.18 (m, 2H phenyl), 6.98-6.96 (m, 1H phenyl), 6.86-6.84 (m, 1H phenyl), 6.64 (s, 1H, indole), 5.54 (s, 2H, benzylic -CH_2), 3.87-3.86 (m, 4H, piperazine), 3.70-3.65 (m, 4H, piperazine). ESI-MS: m/z 431 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (3-chlorophenyl piperazin-1-yl) methanone (NBIC 3)

IR (KBr)ν cm^{-1}: 3150, 2829, 1732, 1626, 1506, 1456, 1420, 1369. ^{1}HNMR, (400 MHz, $CDCl_3$):δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.45-7.43 (m, 1H, indole), 7.34-7.31 (m, 1H indole, phenyl), 7.29-7.23 (m, 6H indole, phenyl), 7.20-7.18 (m, 2H phenyl), 6.98-6.96 (m, 1H phenyl), 6.86-6.84 (m, 1H phenyl), 6.64 (s, 1H, indole), 5.54 (s, 2H, benzylic CH_2), 3.87-3.86 (m, 4H, piperazine), 3.70-3.65 (m, 4H, piperazine). ESI-MS: m/z 431.1 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (4-chlorophenyl piperazin-1-yl) methanone (NBIC 4)

IR (KBr)ν cm^{-1}: 2985, 2910, 2825, 1886, 1628, 1494, 1433, 1371, 1273.^{1}HNMR, (400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.45-7.43 (m, 1H, indole), 7.34-7.31 (m, 1H indole, phenyl), 7.29-7.23 (m, 6H indole, phenyl), 7.20-7.18 (m, 2H phenyl), 6.76-6.73 (m, 2H phenyl), 6.64 (s, 1H, indole), 5.52 (s, 2H, benzylic CH_2), 3.87-3.86 (m, 4H, piperazine), 3.73-3.71 (m, 4H, piperazine). ESI-MS: m/z 431 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (2-methoxyphenyl piperazin-1-yl) methanone (NBIC 5)

IR (KBr)ν cm^{-1}: 3126, 2829, 1732, 1624, 1512, 1450, 1427, 1369.^{1}HNMR (400 MHz, $CDCl_3$): δ (ppm): 7.73-7.71 (m, 1H, indole ring), 7.44-7.41 (m, 1H, indole, phenyl), 7.29-7.27 (m, 5H indole, phenyl), 7.27-7.25 (m, 2H phenyl), 7.23-7.20 (m, 1H phenyl), 7.19-7.17 (m, 1H phenyl), 7.16-7.01 (m, 1H phenyl), 6.93-6.84 (m, 1H phenyl), 6.63 (s, 1H, indole), 5.51 (s, 2H, benzyl), 3.87-3.86 (m, 4H, piperazine), 3.76 (s, 3H, OCH_3 of phenyl), 3.73-3.71 (m, 2H, piperazine), 2.68-2.59 (m, 2H, piperazine). ESI-MS: m/z 426 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (3-methoxyphenyl piperazin-1-yl) methanone (NBIC 6)

IR (KBr)ν cm^{-1}: 3126, 2829, 1732, 1625, 1494, 1456, 1435, 1369. ^{1}HNMR (400 MHz, $CDCl_3$):, δ (ppm): 7.65-7.63 (m, 1H, indole ring), 7.44-7.41 (m, 1H, indole, phenyl), 7.29-7.27 (m, 5H indole, phenyl), 7.27-7.25 (m, 2H phenyl), 7.23-7.20 (m, 1H phenyl), 7.19-7.17 (m, 1H phenyl), 7.16-7.01 (m, 1H phenyl), 6.93-6.84 (m, 1H phenyl), 6.64 (s, 1H, indole), 5.54 (s, 2H, benzylic CH_2), 3.87-3.86 (m, 4H, piperazine), 3.80 (s, 3H, OCH_3 of phenyl), , 3.73-3.71 (m, 2H, piperazine), 2.68-2.59 (m, 2H, piperazine). ESI-MS: m/z 426 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (4-methoxyphenyl piperazin-1-yl) methanone (NBIC 7)

IR (KBr)ν/cm^{-1}: 3126, 2829, 1732, 1624, 1512, 1450, 1427, 1369.^{1}HNMR,(400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.44-7.42 (m, 1H, indole, phenyl), 7.30-7.29 (m, 1H, indole), 7.28-7.26 (m, 4H, phenyl), 7.22-7.20 (m, 2H, phenyl), 7.02-6.89 (m, 4H phenyl), 6.63 (s, 1H, indole), 5.55 (s, 2H, benzyl), 3.76 (s, 3H, OCH_3 of phenyl), 3.72-3.70 (m, 4H, piperazine), 3.69-3.58 (m, 2H, piperazine), 2.93-2.80 (m, 2H, piperazine). ESI-MS: m/z 426 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (4-methyl piperazin-1-yl) methanone (NBIC 8)

IR (KBr)ν/cm^{-1}: 2966, 2897, 2804, 1625, 1529, 1492, 1465, 1435, 1313. ^{1}HNMR, (400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.44-7.42 (m, 1H, indole), 7.30-7.29 (m, 1H indole), 7.28-7.26 (m, 4H indole, phenyl), 7.22-7.20 (m, 2H phenyl), 6.62 (s,1H, indole), 5.54 (s, 2H, benzyl), 3.82-3.79 (m, 4H, piperazine), 3.09-2.89 (m, 4H, piperazine), 2.65 (s, 3H,-CH_3). ESI-MS: m/z 334.1 (M + 1) 100%.

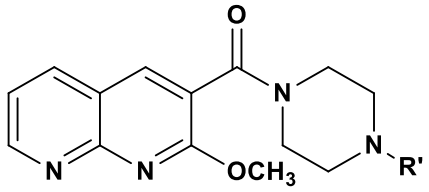
(1-benzyl-1*H*-indol-2-yl) (4-ethyl piperazin-1-yl) methanone (NBIC 9)

IR (KBr)ν/cm^{-1}: 3120, 2897, 2804, 1625, 1529, 1492, 1465, 1435, 1313. ^{1}HNMR, (400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.44-7.42 (m, 1H, indole), 7.30-7.29 (m, 1H indole), 7.28-7.26 (m, 4H indole, phenyl), 7.22-7.20 (m, 2H phenyl), 6.62 (s,1H, indole), 5.54 (s, 2H, benzyl), 4.38-3.82-3.79 (m, 4H, piperazine), 3.09-2.80 (m, 4H, piperazine), 2.88-2.86 (m, 2H, CH_2), 2.68-2.65 (t, J=2.8 Hz, 3H, CH_3 of ethyl). ESI-MS: m/z 348.1 (M + 1) 100%.

(1-benzyl-1*H*-indol-2-yl) (4-propyl piperazin-1-yl) methanone (NBIC 10)

IR (KBr)ν/cm^{-1}: 3120, 2897, 2804, 1625, 1529, 1492, 1465, 1435, 1313. ^{1}HNMR, (400 MHz, $CDCl_3$): δ (ppm): 7.66-7.64 (m, 1H, indole ring), 7.44-7.42 (m, 1H, indole), 7.30-7.29 (m, 1H indole), 7.28-7.26 (m, 4H indole, phenyl), 7.22-7.20 (m, 2H phenyl), 6.62 (s,1H, indole), 5.54 (s, 2H, benzyl), 4.38-3.82-3.79 (m, 4H, piperazine), 3.09-2.80 (m, 4H, piperazine), 2.88-2.86 (t, J=2.6 Hz, 2H,-CH_2), 2.80-2.76 (m, 2H, -CH_2). 2.70-2.60 (t, J=2.4 Hz, 3H, CH_3). ESI-MS: m/z 362.1 (M + 1) 100%.

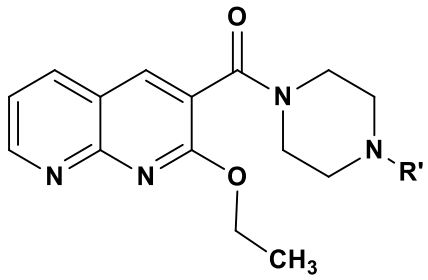
Table 9: Distances between the pharmacophoric elements of compounds MN 1– MN15 [(a)]



compd	R'	Centroid of Naphthyridine to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Naphthyridine ring to basic nitrogen
MN1	C_6H_5	3.71-3.72	4.76-4.761	6.44-6.441
MN2	C_6H_5-CH_2	3.70-3.71	4.16-4.20	5.94-5.96
MN3	*p*-$NO_2C_6H_4$	3.70-3.701	4.95-4.96	6.49-6.59
MN4	*o*-Cl-C_6H_4	3.71-3.73	4.73-4.74	6.43-6.431
MN 5	*m*-Cl-C_6H_4	3.71-3.714	4.81-4.82	6.48-6.481
MN 6	*p*-Cl-C_6H_4	3.72-3.726	4.79-4.80	6.45-6.480
MN 7	*o*$OCH_3C_6H_4$	3.71-3.72	4.38-4.40	6.23-6.231
MN 8	*m*$OCH_3C_6H_4$	3.70-3.702	4.76-4.79	6.36-6.361
MN 9	*pO*$CH_3C_6H_4$	3.71-3.712	4.82-4.83	6.39-6.391
MN10	*o*-CH_3-C_6H_4-	3.71-3.72	5.04-5.06	6.73-6.740
MN11	*p*-CH_3-C_6H_4-	3.71-3.72	5.02-5.04	6.70-6.710
MN12	CH_3-	3.70-3.701	4.97-4.99	6.70-6.720
MN13	CH_3-CH_2	3.70-3.701	4.66-4.69	6.46-6.470
MN14	$CH_3CH_2CH_2$	3.70-3.702	4.78-4.79	6.46-6.470
MN15	$CH_3(CH_2)_3$-	3.71-3.712	4.59-4.62	6.19-6.210

[(a)] Average 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.

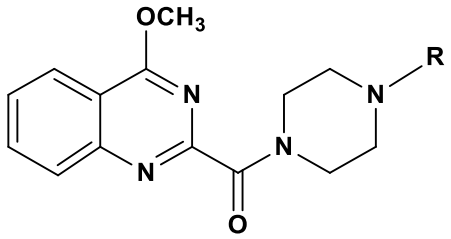
Table 10: Distances between the pharmacophoric elements of compounds EN1–EN15 [(a)]



compd	R'	Centroid of Naphthyridine to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Naphthyridine ring to basic nitrogen
EN 1	C_6H_5	3.73-3.77	**4.88-4.890**	**6.52-6.560**
EN 2	C_6H_5-CH_2	3.71-3.711	4.78-4.840	6.45-6.550
EN 3	*p*$NO_2C_6H_4$	3.71-3.710	4.88-4.890	6.48-6.490
EN 4	*o*-ClC_6H_4	3.70-3.701	5.00-5.010	6.77-6.780
EN 5	*m*-ClC_6H_4	3.71-3.710	4.89-4.960	6.57-6.710
EN 6	*p*-ClC_6H_4	3.70-3.701	4.89-4.960	6.51-6.520
EN 7	*o*$OCH_3C_6H_4$	3.71-3.710	5.03-5.050	6.51-6.520
EN 8	*m*$OCH_3C_6H_4$	3.72-3.730	5.03-5.050	6.78-6.820
EN 9	*p*$OCH_3C_6H_4$	3.71-3.710	5.03-5.05	6.70-6.710
EN 10	*o*-$CH_3C_6H_4$	3.71-3.711	4.99-5.03	6.82-6.850
EN 11	*p*-$CH_3C_6H_4$	3.70-3.71	5.01-5.03	6.79-6.810
EN 12	CH_3	3.71-3.711	4.91-4.920	6.70-6.730
EN 13	CH_3CH_2	3.71-3.711	4.97-4.980	6.68-6.690
EN 14	$CH_3CH_2CH_2$	3.72-3.721	4.98-4.990	6.65-6.651
EN 15	$(CH_3)_3CH_2$	3.71-3.712	4.98-4.981	6.66 -6.670

[(a)] Average 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.

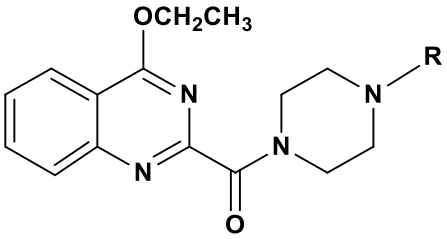
Table 11: Distances between the pharmacophoric elements of compounds MQZ1–MQZ10 [(a)]



Compd.	R	Centroid of Naphthyridine to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Naphthyridine ring to basic nitrogen
MQZ 1	C_6H_5	3.71-3.711	4.76-4.770	6.44-6.441
MQZ 2	*o*-Cl-C_6H_4	3.71-3.710	4.73-4.740	6.43-6.440
MQZ 3	*m*-Cl-C_6H_4	3.71-3.711	4.81-4.820	6.48-6.490
MQZ 4	*p*-Cl-C_6H_4	3.72 -3.721	4.79 -4.800	6.45-6.480
MQZ 5	*o*$OCH_3C_6H_4$	3.71-3.711	4.38-4.390	6.23-6.231
MQZ 6	*m*$OCH_3C_6H_4$	3.70-3.701	4.76-4.790	6.36-6.361
MQZ 7	*pO*$CH_3C_6H_4$	3.71-3.712	4.82-4.830	6.39-6.391
MQZ 8	CH_3-	3.70-3.701	4.97-4.990	6.70-6.720
MQZ 9	CH_3-CH_2	3.70-3.701	4.66-4.690	6.46-6.470
MQZ 10	$CH_3CH_2CH_2$	3.70-3.701	4.78 -4.790	6.46-6.471

[(a)] Average 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.

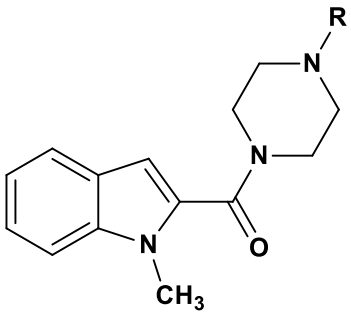
Table 12: Distances between the pharmacophoric elements of compounds EQZ 1- EQZ 15[(a)]



Compd.	R	Centroid of Naphthyridine to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Naphthyridine ring to basic nitrogen
EQZ 1	C_6H_5	3.71-3.711	4.76-4.770	6.44-6.441
EQZ 2	*o*-Cl-C_6H_4	3.71-3.710	4.73-4.740	6.43-6.431
EQZ 3	*m*-Cl-C_6H_4	3.71-3.711	4.81-4.820	6.48-6.490
EQZ 4	*p*-Cl-C_6H_4	3.72-3.721	4.79-4.801	6.45-6.480
EQZ 5	*o*$OCH_3C_6H_4$	3.71-3.711	4.38-4.401	6.23-6.231
EQZ 6	*m*$OCH_3C_6H_4$	3.70-3.710	4.76-4.790	6.36-6.361
EQZ 7	*pO*$CH_3C_6H_4$	3.71-3.720	4.82-4.830	6.39-6.391
EQZ 8	CH_3-	3.70-3.710	4.97-4.990	6.70-6.720
EQZ 9	CH_3-CH_2	3.70-3.701	4.66-4.690	6.46-6.471
EQZ 10	$CH_3CH_2CH_2$	3.70-3.701	4.78-4.790	6.46-6.471

[(a)] Average 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.

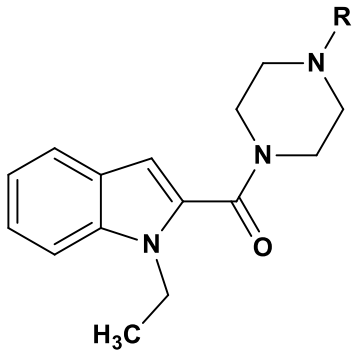
Table 13: Distances between the pharmacophoric elements of compounds NMIC1–NMIC10 [(a)]



Compd.	R	Centroid of Indole ring to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Indole ring to basic nitrogen
NMIC 1	C_6H_5	3.41-3.410	4.76 -4.830	6.26 -6.330
NMIC 2	*o*-Cl-C_6H_4	3.42-3.421	4.75-4.760	6.29 - 6.312
NMIC 3	*m*-Cl-C_6H_4	3.41-3.411	4.74 -4.750	6.28 - 6.310
NMIC 4	*p*-Cl-C_6H_4	3.40-3.401	4.78 - 4.850	6.27 - 6.330
NMIC 5	*o*$OCH_3C_6H_4$	3.41-3.420	4.73-4.750	6.26 -6.300
NMIC 6	*m*$OCH_3C_6H_4$	3.41-3.411	4.73 -4.810	6.27- 6.330
NMIC 7	*p*$OCH_3C_6H_4$	3.41-3.420	4.73-4.750	6.26-6.300
NMIC 8	CH_3-	3.41-3.411	4.81- 4.850	6.16-6.190
NMIC 9	CH_3-CH_2	3.40-3.410	4.73- 4.750	6.23- 6.250
NMIC 10	$CH_3CH_2CH_2$	3.41-3.420	4.77- 4.780	6.20- 6.210

[(a)] Average 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.

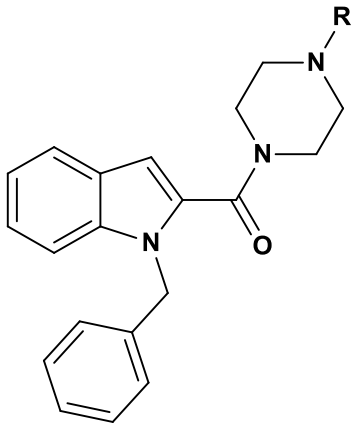
Table 14: Distances between the pharmacophoric elements of compounds NEIC1–NEIC 10[(a)]



Compd.	R	Centroid of Indole ring to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Indole ring to basic nitrogen
NEIC 1	C_6H_5	3.53-3.540	4.98- 4.990	6.44-6.441
NEIC 2	*o*-Cl-C_6H_4	3.71-3.711	4.73-4.740	6.43-6.431
NEIC 3	*m*-Cl-C_6H_4	3.71 -3.711	4.81-4.820	6.48-6.481
NEIC 4	*p*-Cl-C_6H_4	3.72 -3.721	4.79-4.810	6.45-6.480
NEIC 5	*o*$OCH_3C_6H_4$	3.71-3.711	4.38-4.40	6.23-6.231
NEIC 6	*m*$OCH_3C_6H_4$	3.70 - 3.701	4.76-4.790	6.36-6.361
NEIC 7	*pO*$CH_3C_6H_4$	3.71- 3.720	4.82-4.830	6.39- 6.40
NEIC 8	CH_3-	3.70 -3.710	4.97-4.990	6.70-6.720
NEIC 9	CH_3-CH_2	3.70 -3.710	4.66-4.690	6.46-6.470
NEIC 10	$CH_3CH_2CH_2$	3.70-3.710	4.78- 4.790	6.46-6.470

[(a)] Average 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.

Table 15: Distances between the pharmacophoric elements of compounds NBIC1–NBIC10 [(a)]



Compd.	R	Centroid of Naphthyridine to carbonyl oxygen	Carbonyl Oxygen to basic nitrogen	Centroid of Naphthyridine ring basic nitrogen
NBIC 1	C_6H_5	3.71-3.720	4.76-4.770	6.44-6.441
NBIC 2	*o*-Cl-C_6H_4	3.71-3.711	4.73-4.740	6.43-6.431
NBIC 3	*m*-Cl-C_6H_4	3.71-3.711	4.81-4.820	6.48-6.481
NBIC 4	*p*-Cl-C_6H_4	3.72-3.721	4.79-4.810	6.45-6.480
NBIC 5	*o*$OCH_3C_6H_4$	3.71-3.712	4.38-4.400	6.23-6.231
NBIC 6	*m*$OCH_3C_6H_4$	3.70-3.701	4.76-4.790	6.36-6.361
NBIC 7	*pO*$CH_3C_6H_4$	3.71-3.720	4.82-4.830	6.39-6.391
NBIC 8	CH_3-	3.70-3.710	4.97-4.990	6.70-6.720
NBIC 9	CH_3-CH_2	3.70-3.701	4.66-4.690	6.46-6.470
NBIC 10	$CH_3CH_2CH_2$	3.70-3.701	4.78-4.790	6.46-6.470

[(a)] Average 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.

4.1.2 Pharmacology:

4.1.2.1 Animals:

Behavioral screenings were conducted using Swiss Albino mice (22–30 g) of both sex and Guinea pig for 5-HT_3 antagonism assay procured from Hissar Agricultural University, Haryana, India. Animals were accommodated under standard laboratory circumstances (temperature 23 ± 2 °C & room humidity 60 ± 10%), maintained on 12:12 h light/dark cycle with free right to use of standard diet & filtered water. The experimental procedures on animals were in agreement with the Institutional Animal Ethics Committee of Birla Institute of Technology & Science, Pilani, India (ProtocolNo.IAEC/RES/14/04;IAEC/RES/Rev-1/14/04;IAEC/RES/18/19-1;IAEC/RES/Rev-1/19/33). All behavioural experimentations were carried out amid 09.00 a.m. and 03.00 p.m. Following a quarantine period of two weeks the animals were randomly assigned to different experimental groups.

4.1.2.2. Drugs:

Ondansetron was obtained from Natco Pharmaceuticals, Hyderabad, India. Diazepam was procured from Lancaster chemicals (UK). All of the other chemicals used for synthesis were of analytical grade. Diazepam and ondansetron were used as reference standards. All of the NCEs, diazepam, and ondansetron were freshly prepared before use by dissolving in distilled water. Diazepam (2 mg/kg body mass), ondansetron (2 mg/kg), and NCEs (1 mg/kg) were administered intraperitoneally (i.p.) to mice 30 min before starting the behavioural observation in each test.

4.1.2.3. Determination of 5-HT_3 receptor antagonism:

The NCEs were screened for their 5-HT_3 receptor antagonisms in longitudinal muscle myenteric plexus preparation from guinea pig ileum against standard 5-HT_3 agonist, 2-methyl 5-HT. Dunkin Hartley guinea pigs (250–300g) were sacrificed by cervical dislocation. The abdomen was cut open and a piece of ileum was removed about 2 cm from the ileo-caecal intersection.

The longitudinal muscle-myenteric plexus (LMMP), 3–4 cm in length, was isolated and mounted as described in the literature method (Paton and Zar, 1968).The tissue was kept for equilibration for 30 min. beneath a resting tension of 500 mg and with steady airing in a 40 ml organ bath holding Tyrode solution (composition for 1 lit NaCl 8g, KCl 0.2g, $CaCl_2$ 0.2 g, $MgCl_2$ 0.1g, NaH_2PO_4 0.05g, $NaHCO_3$ 1.00g, Glucose 1.0g, pH 6.7), maintained at 25 °C**.**

Non-cumulative concentrations (10^{-8}-10^{-4} M) of the agonist 2-methyl-5-HT (10^{-5} M), (Tocris, UK) were added with a 15 min dosing cycle (to prevent desensitization) and left in contact with the tissue until the maximum contraction had developed. To evaluate 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 no less than 10 min earlier than the addition of 2-methyl-5-HT.The contractions were documented using a T-305 Force transducer paired to a Student's physiograph procured from Bio Devices, Ambala, India. 5-HT_3 receptor antagonism was stated in the form of pA_2 values (negative logarithm of molar concentration of antagonist producing a 2-fold shift of the agonist concentration-activity curve), which were graphically determinedas cited in literature (Paton and Zar, 1968; Perumal and Mahesh, 2006; Mahesh et al., 2004; Mac Kay, 1978). The pA_2 values of the synthesized compounds were evaluated in comparison with the standard antagonist, ondansetron.

4.1.2.4. In-Vivo activity:

Spontaneous locomotor activity:

To ascertain the possible occurrence of drug induced changes (stimulation/suppression) in the locomotor activity of mice, which may contribute to their behavior in FST, all the compounds were subjected to spontaneous locomotor activity (Boissier and Simon, 1965) study at 1 mg/kg, i.p. dose level. The spontaneous locomotor activity in mice was evaluated using the actophotometer (Boissier and Simon, 1965a), which composed of a square area (30 × 30 cm) with walls that are fitted with photocells just over the floor level. The photocells were examined prior to commencement of the experiment. The drug/vehicle treated mice were then individually put in the arena. After a two minute acclimatization phase, the digital locomotor scores were recorded for the subsequent 8 min in a dimly lit room (Mahesh et al., 2007). All new chemical entities and ondansetron (1 mg/kg, i.p.) were given 30 min. earlier than testing.

4.1.2.5. Anti-depressant screening:

Forced swim test (FST):

Behavioral despair or forced swim test (FST) is recommended asa model to evaluate anti-depressant activity (Porsolt et al., 1977a). It wassuggested that mice when compelled to swim within a limited spacefrom where they cannot flee and hence, are developed with a distinctivebehavior of idleness.

This characteristic behavior demonstrates a condition of hopelessnesswhich can be diminished by numerous compounds which are therapeuticallyefficient in human depression. This behavioral despair test was employed to assess the anti-depressant activity of the NCEs.

The method described by Porsolt and coworkers (Porsolt et al., 1977) was adopted (Devadoss et al., 2010; Mahesh et al., 2011) and each mouse was positioned individually in a cylinder made up of glass (diameter: 22.5 cm, height: 30 cm) loaded with water up to 15 cm of height at 23±1°C. The mice were compelled to swim in water for 15 min on the pre-experiment day. Mice were then permitted to go back to their home cage. After 24 h from the pre-experiment day, each mouse (vehicle/drug treated) was positioned in water and compelled to swim for 6 min. The duration ofimmobilities through the final 4 min was calculated. The mouse was deemed to be motionless when it discontinued struggling and submissively moved to stay afloate and kept its head just above water. Water was replaced amid experiments and the temperature was kept at 23±1°C. Ondansetron (1 mg/kg, *i.p.*) and new chemical entities (1 mg/kg, *i.p.*) were given 30 min. earlier to the experiment.

Tail suspension test (TST):

The TST is a 'behavioral despair' test, in which the duration of immobility is measured. Behavioral despair was induced according to the procedure of Steru et al. (Steru et al., 1985). Mice were suspended individually from a horizontal bar 50 cm above the Tabletop using adhesive tape. The point of attachment on the tail was 1 cm from the tip. The duration of immobility during the 6-min observation period was recorded. Mice were considered immobile only when they were completely motionless. The parameter recorded was the number of seconds spent immobile. Compounds which exhibited higher antagonism were subjected to TST to further ascertain the anti-depressant activity. In the TST, ondansetron (1 mg/kg, *i.p.*) and new chemical entities (1 mg/kg, *i.p.*) were administered 30 min. prior to experiment.

4.1.2.6. Anxiolytic screening:

In the present study, anxiolytic effect of standard diazepam and synthesized compounds were examined in a behavioral test battery of anxiety, including

- The light-dark (L/D) test,
- Elevated plus maze (EPM) test, and
- The open field exploratory tests (OFT).

All of these models of anxiety are reasonably responsive and specific to anxiolytics, and have been widely used to screen NCEs for their anxiolytic potential (Borsini et al., 1989; Costall et al., 1989, 1993; Kilfoil et al., 1989; Barnes et al., 1992; Hogg, 1996).

For anxiolytic screening, the compounds from each series were selected based on their 5-HT_3 antagonistic activity, pA_2 value (particularly if it was superior to the standard 5-HT_3 receptor antagonist, ondansetron) and their anti-depressant activity in FST and TST.

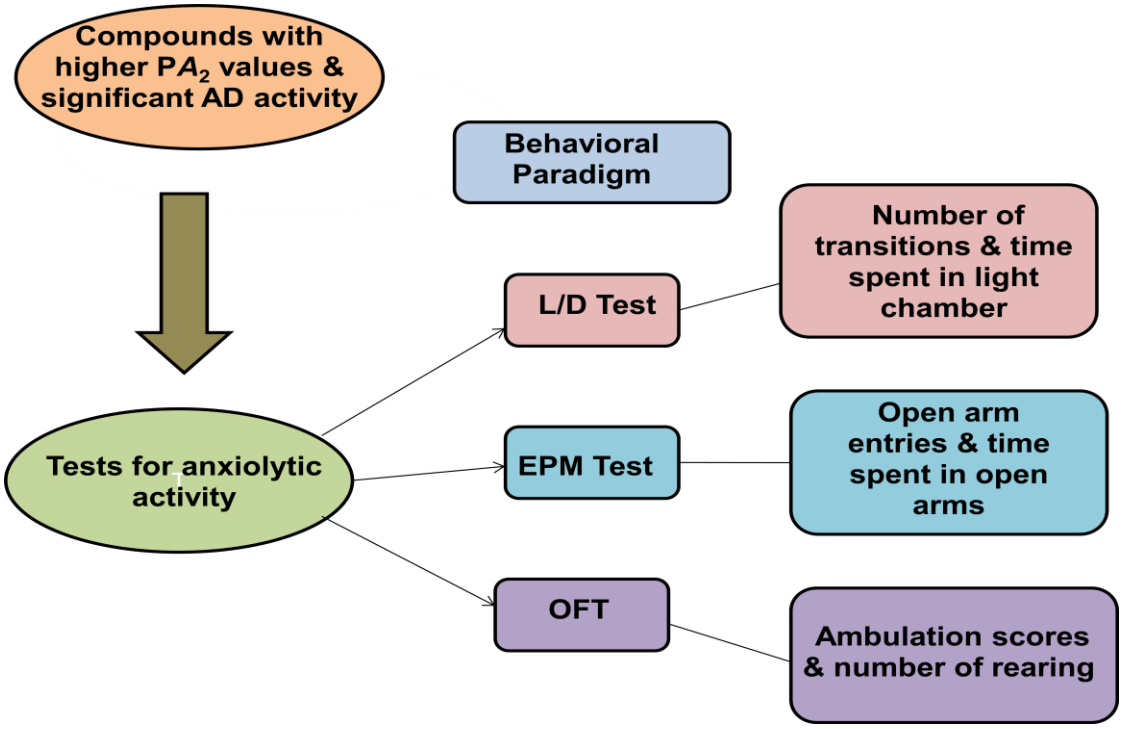


Figure 67: Selection criteria for anxiolytic screening.

Light–dark aversion test (L/D Test):

The L/D apparatus comprise of a box divided into 2 separate compartments, occupying 2/3 and 1/3 of the total size, respectively.The larger compartment (light compartment) was illuminated by a 60 W bulb, while the smaller (dark compartment) is entirely black and enclosed under a dark cover. The L/D compartments are separated by a partition with a tunnel to allow the mouse passage from one compartment to the other (Mi et al., 2005). At the beginning of the test, the mouse was put individually at the middle of the light compartment facing towards the tunnel and permitted to walk around the entire equipment for 5 min.

The behavioural parameters such as latency time to leave the light chamber, sum of the time spent in the light box, and amount of transitions between the light and dark compartments were observed and recorded. A compartment entry is considered valid when all 4 of the animal's paws are inside that chamber. The equipment was meticulously cleaned with 70% ethanol following every experiment.

Elevated plus maze test (EPM Test):

The EPM test was first evaluated for rats (Pellow et al., 1985) and later adapted for mice (Lister, 1987). In brief, the equipment consisted of a timber maze with 2 enclosed arms (30 cm × 5 cm ×15 cm) and 2 open arms (30 cm × 5 cm × 0.25 cm) that extended from a middle raised area (5 cm × 5 cm) to give a appearance of a “plus sign”.The plus-maze apparatus was raised to a stature of 25 cm and placed inside a sound-attenuated room. The experiment begun with placing of a mouse on the middle stage of the maze facing it’s head towards an open arm. The behavioral aspects recorded through a 5 min experiment phase were as follows: percentage open arm entries, percentage time spent in open arm, and total entries (Klodzinska et al., 2004). Entry into an arm was considered valid only when all 4 paws of the mouse were inside that arm (Biala and Kruk, 2008).The animal's activities were observed and readings recorded. The equipment was meticulously cleaned with 70% ethanol after every test.

Open field test (OFT):

The equipment is made up of a timber box (60 cm × 60 cm × 30 cm) with the floor segmentedinto 16 squares (15 cm×15 cm) by black parallel and intersecting lines. The equipment was lit up with 60 W bulb dangled 100 cm above it. At the beginning of the test, the mouse was positioned separately at the middle of the square arena.

The number of squares crossed (ambulation counts) and frequency of rearing (standing upright on the hind legs) were recorded for a 5 min period. After each individual test session the floor was thoroughly cleaned with 70% ethanol.

Statistical analysis:

Statistical analyses were performedusing Graph pad prism (version 3) software and Microsoft excel (MS Office 2007). The results of anti-depressant,locomotor and anxiolytic activity are expressed as the mean ± SEM. Experimental data were analyzed through one way ANOVA followed by post hoc Dunnett’s test. Statistical significance was set at $p < 0.05$.

Chapter 5: Results and Discussions

5. Results and discussion:

5.1. Pharmacophore and chemistry:

5.1.1. Pharmacophore design:

Piperazine analogs of naphthyridine, quinazolne and indole carboxamides were designed based on the pharmacophore related to classical 5-HT_3 receptor antagonists ('setrons') (figure 2).

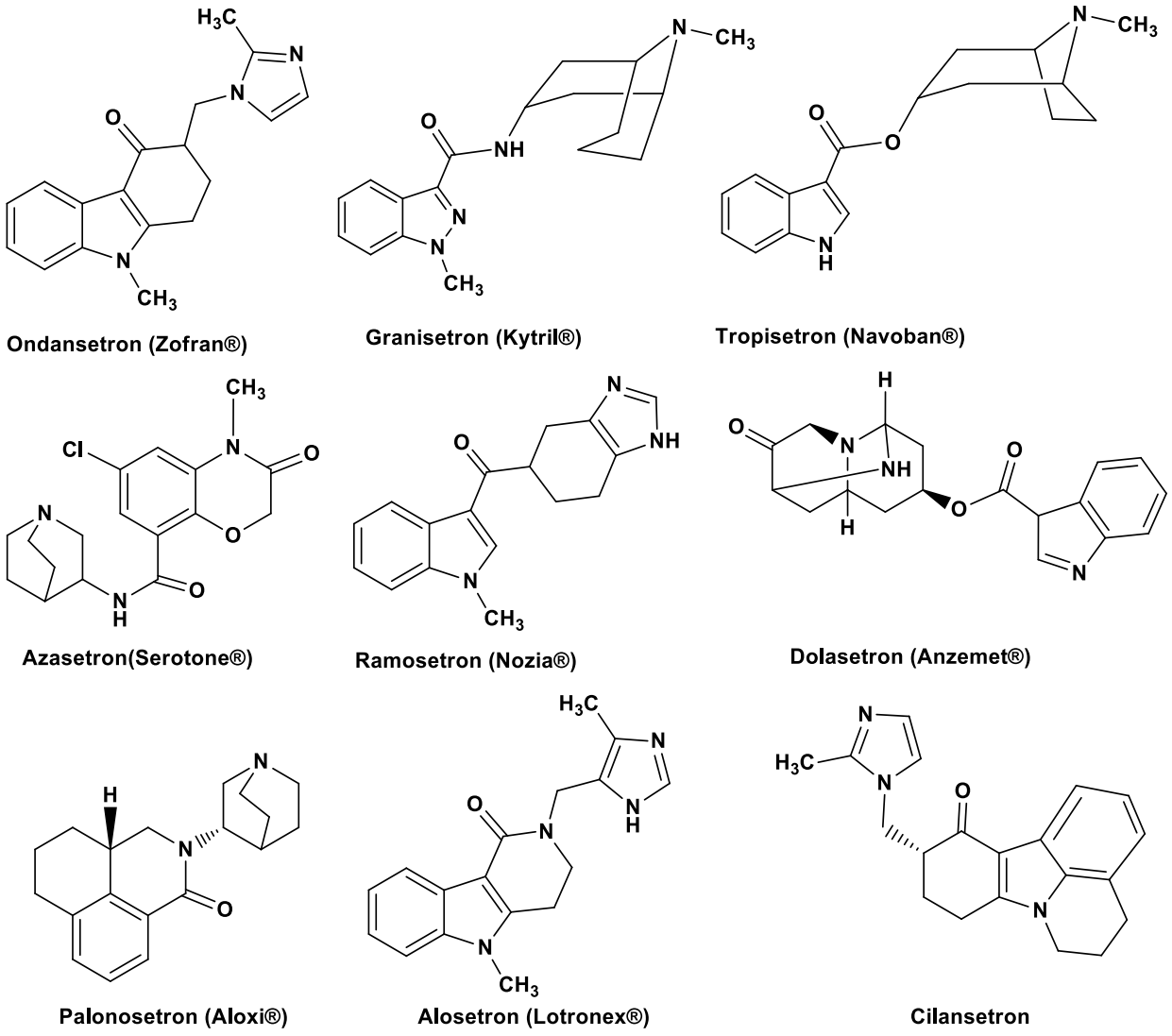


Figure 68: Structures of classical 5-HT_3 receptor antagonists, 'setrons.'

The common pharmacophoric features of classical 5-HT_3 receptor antagonists (Setrons), heteroaromatic ring, carbonyl group, and basic nitrogen were identified.The pharmacophoric distances were measured and considered for designing novel compounds. Calculations were performed using CHARMm force field in D.S ver 3.5 or ACDLABS-12.0/3D (**table 16**).

In this study, keeping similar hydrogen bond acceptor moiety (carbonyl group), variations were made to the heteroaromatic core and distal nitrogen (N^4) of the basic moiety of the pharmacophore (**figure 1**) with the intention of exploring the structure activity relationship (SAR) associated with such changes and explore the role of aromatic part and basic moiety within the pharmacophoric criteria of 5-HT_3 receptor antagonists. Therefore, three different nitrogen containing fused heterocycle rings (aromatic part) were alternately attached to various N^4 substituted piperazines (basic moiety) through a carbonyl group of carboxamide linkage (hydrogen bond acceptor), resulting in the construction of three new series of piperazine analogues of naphthyridine-3-carboxamides (Scheme II), quinazoline-2-carboxamides (Scheme III) and of indole-2-carboxamides (Scheme IV). However, the groups (e.g., *o*-methoxyphenyl, *o*-chlorophenyl, *m*-chlorophenyl, methyl, ethyl etc) on the basic moiety (N^4 of the piperazine ring) were kept similar, in case of all the carboxamides.

5.1.2. 3D Pharmacophore model

The aforementioned compounds were used as template for making a pharmacophore model for 5-HT_3 antagonists. The least energy conformation for each compound was generated and the pharmacophoric distances were measured by ACDLABS-12.0/3D Viewer (CHARMM Parameterizations). The important structural components that were included in the three-point pharmacophore model were the heteroaromatic group(s) [B]; planar scaffold with a hydrogen bond acceptor (HBA) atom [A] and a basic nitrogen [C].

The distances between the centroid of the heteroaromatic ring [B] and HBA atom which is a carbonyl group [A], carbonyl group [A] to basic nitrogen [C] and centroid of the heteroaromatic ring [B] to basic nitrogen [C] were measured, the obtained average distances between the pharmacophoric elements (**table 16**) were considered for building this model.

Table 16: Average Distances between the pharmacophoric units [BN], [CO], [CA]* of some standard 5-HT_3 antagonists

Compound	[CA]-[CO]	[CO]-[BN]	[CA]-[BN]
Ondansetron	3.424-3.521	5.762-5.821	7.136-7.187
Granisetron	3.396-3.401	6.373-6.420	7.108-7.134
Tropisetron	3.433-3.434	5.243-5.256	6.932-6.962
Dolasetron	3.441-3.462	5.962-6.101	6.106-6.622
Zatosetron	3.691-3.692	5.131-5.197	7.968-7.971
Ramosetron	3.696-3.703	5.516-5.537	6.787-6.817
Alosetron	3.705-3.707	5.514-5.517	6.789-6.801
Cilansetron	3.707-3.711	5.863-5.920	6.632-6.661
Palonosetron	3.520-3.541	5.321-5.342	6.612-6.624
Average Distances	3.463-3.598	5.792-6.365	6.971-7.644

*Average Distances calculated for 3D optimized structures at least for each three conformations using CHARMM Parametrization (ACDLABS-10.0/3D Viewer) and represented BN= Basic nitrogen; CO=Carbonyl oxygen;CA=Aromatic centre.

The pharmacophore model includes the interfeature distances between a) carbonyl group(CO) as hydrogen bond acceptor and the centroid of heteroaromatic ring system (~3.463 Å to ~3.598 Å), b) carbonyl group and basic nitrogen (~5.792 to ~6.365 Å) and c) the centroid of aromatic ring and basic nitrogen (CA-BN) (~6.971 to ~7.644 Å), respectively.

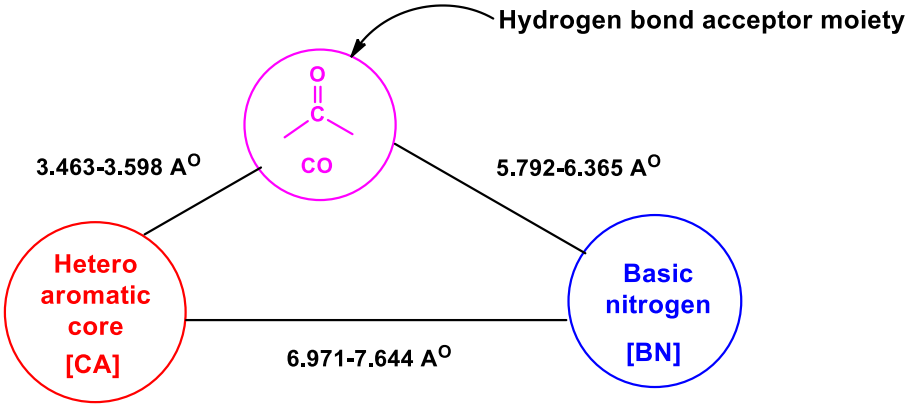


Figure 69: Pharmacophore model for designing novel 5-HT_3 anatgonists.

5.1.3. Design of compounds: Piperazine analogues of naphthyridine-3-carboxamides, quinazoline-2-carboxamides and indole-2-carboxamides (Series 2, 3, 4):

Once the pharmacophoric model was defined, several condensed heterocyclic ring systems such as naphthyridine, quinazoline and indole carboxamides were selected consisting of a heteroaromatic ring, a carobonyl group as hydrogen bond acceptors (HBA) and piperazine as basic moiety. The pharmacophoric distances were measured (**tables 9-15**). Using the tropane and quiniclidine derivatives related to classical 5-HT_3 receptor antagonists derived pharmacophore model, the proposed compounds were mapped, in order to find the best fit among compounds. The pharmacophoric superimposition was done using Discovery Studio (Accelrys) version 3.5. Three point pharmacophore was generated with reference to standard 5-HT_3 receptor antagonists, 'setrons' (ten 'setrons'; **table 16**). Three common structural features i.e., aromatic ring, hydrogen bond acceptor, and basic moiety were considered while generating the pharmacophore. Pharmacophore from each series of synthesized carboxamides were also generated and superimposed over the pharmacophore of 'setrons'. On comparison between 'setrons' and the designed series Methoxynaphthyridine carboxamides (MN) (RMSD~2.22); ethoxynaphthyridine carboxamides (EN) (RMSD~2.12); methoxy quinazoline carboxamides (MQZ) (RMSD~2.24) ethoxyquinazoline carboxamides (EQZ) (RMSD~1.99); Indole carboxamides; NMIC (RMSD~2.12); NEIC (RMSD~0.42); NBIC (RMSD~0.32), it was found that designed compounds showed high fit value (Figure 70-72).

Table 17: Average Distances between the pharmacophoric units [BN], [CO], [CA]* of designed series of compounds

Compd. code/series	**[CA]-[CO]**	**[CO]-[BN]**	**[CA]-[BN]**
MN Series 2	3.700-3.714	4.830-4.870	6.560-6.580
EN Series 2	3.710-3.713	4.950-4.970	6.630-6.670
MQZ Series 3	3.700-3.707	4.740-4.760	6.440-6.449
EQZ Series 3	3.710-3.713	4.740-4.760	6.440-6.448
NMIC Series 4	3.400-3.410	4.750-4.780	6.240-6.280
NEIC Series 4	3.680-3.690	4.760-4.780	6.440-6.448
NBIC Series 4	3.700-3.710	4.740-4.760	6.440-6.447
Average Distances	3.657-3.662	4.780-4.810	6.450-6.474

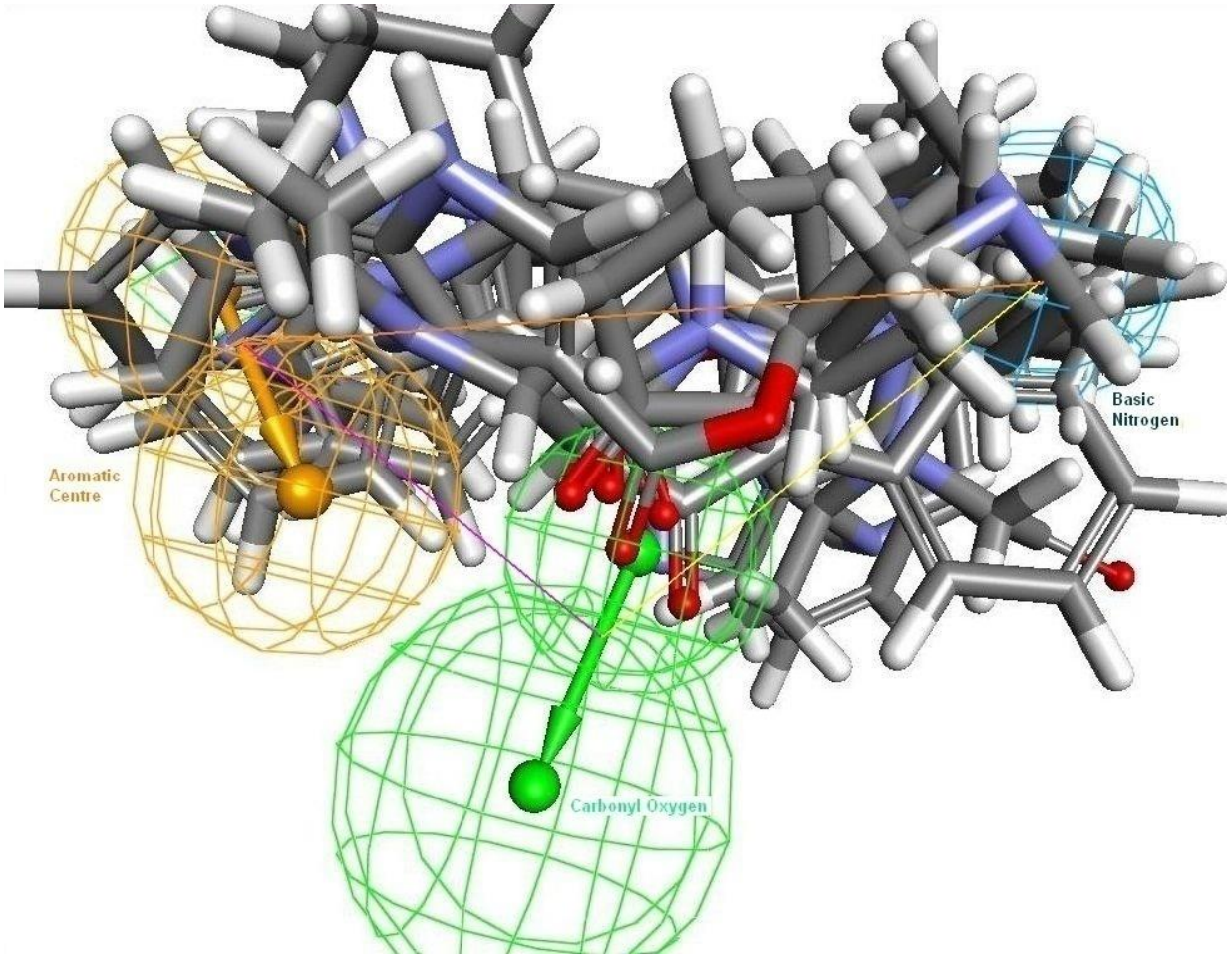
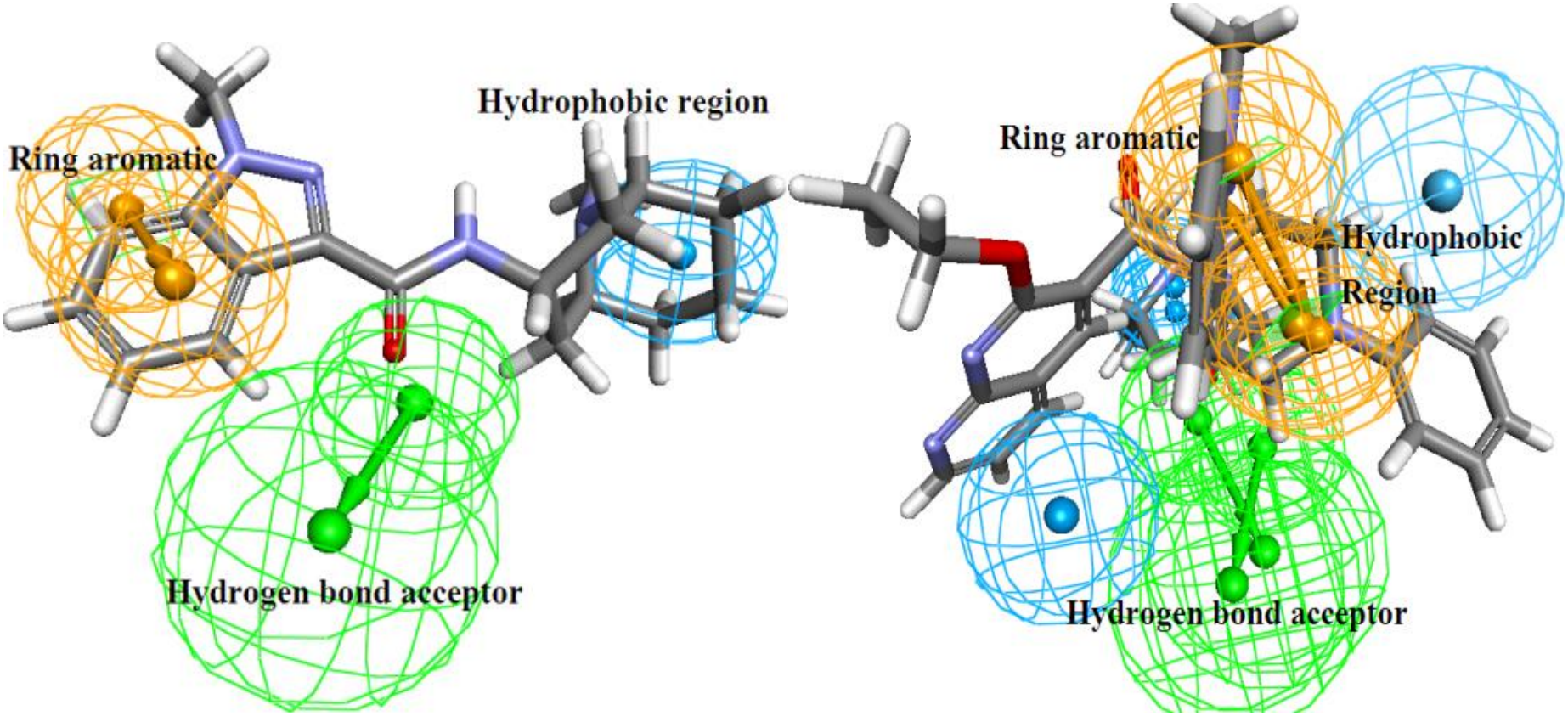
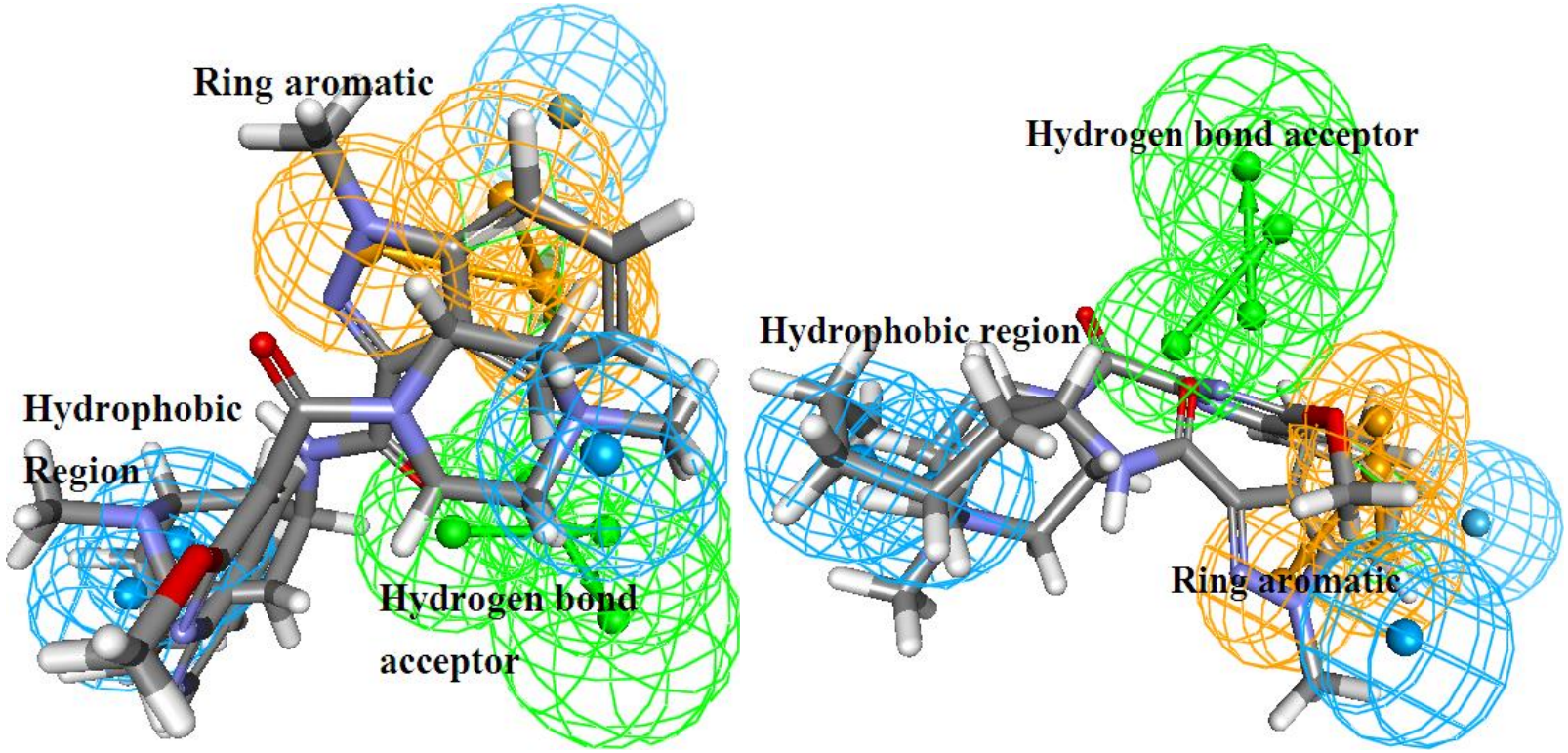


Figure 70: Pharmacophoric overlay of 'setrons' (figure 68; table 16),



A **B**

Figure 71a: A: pharmacophore of setrons (Table 1), only Granisetron shown here for easy visualisation; B: Pharmacophoric overlap of setrons and methoxynaphthyridine carboxamides.



C **D**

Figure 71b: C: Pharmacophoric overlap of setrons and ethoxynaphthyridine carboxamides; D: Pharmacophoric overlap of setrons and methoxyquinazoline carboxamides.

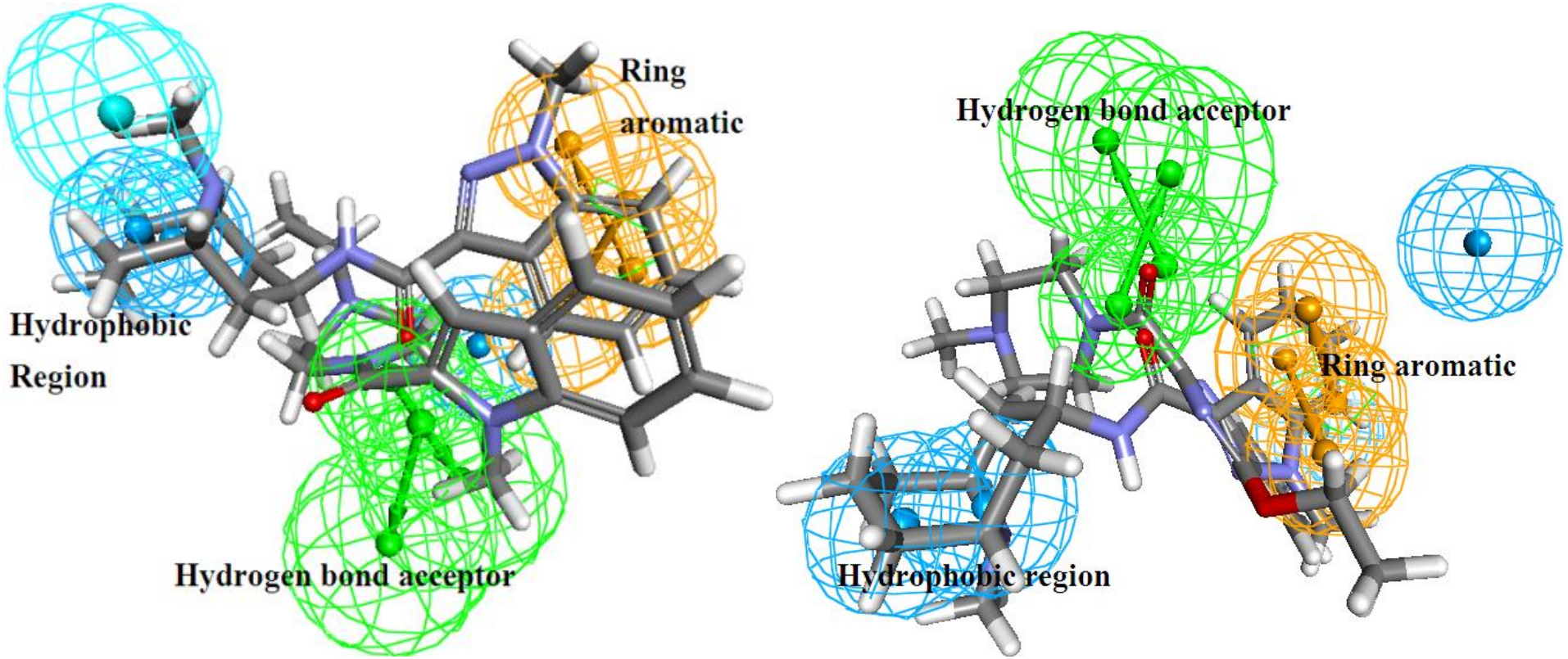


Figure 72a: E: Pharmacophoric overlap of setrons and ethoxyquinazoline carboxamides; F: Pharmacophoric overlap of setrons and N-methylindole carboxamides.

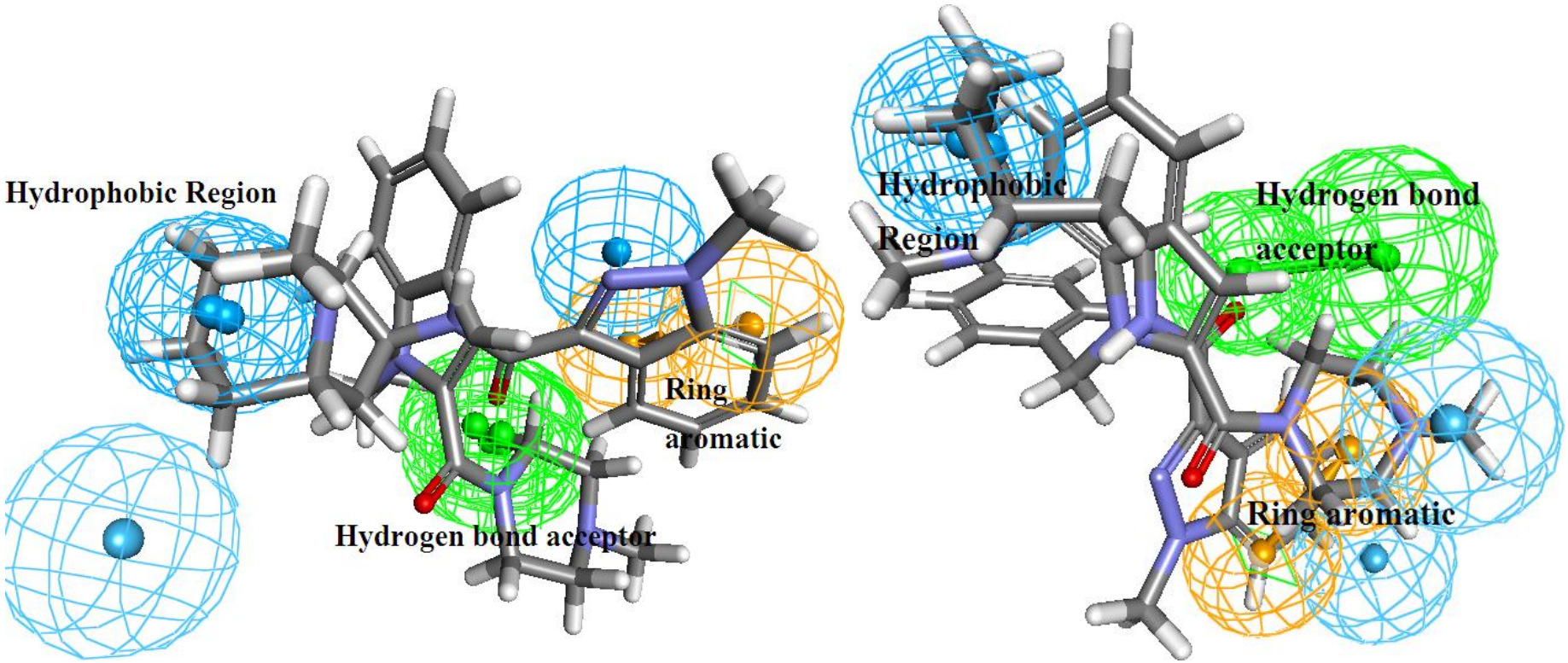


Figure 72b: G: Pharmacophoric overlap of setrons and N-ethylindole carboxamides; H: Pharmacophoric overlap of setrons and N-benzylindole carboxamide.

5.2. Calculation of Molecular properties:

The molecular properties which influence absorption, distribution, metabolism and excretion (ADME) are crucial for drug design. Following oral administration, a drug must pass through intestinal cell membranes via passive diffusion, carrier-mediated uptake or active transport processes before reaching the systemic circulation. The development of many potential drugs has been discontinued because of their poor pharmacokinetic properties, mainly absorption. Molecular properties, such as ClogP (≤5), Molecular weight (≤500), number of hydrogen bond acceptors (≤5) and number of hydrogen bond donors (≤10), and aqueous solubility have much impact on pharmacokinetic properties of a compound. Good pharmacokinetic properties are essential for any NCE to become a successful drug candidate. Thus, in-silico prediction of molecular properties of NCEs is gaining more importance in medicinal chemistry. 'Drug-likeness' is a qualitative concept used in drug design for how 'drug-like' a substance is with respect to factors like bioavailability. Overall, molecular properties determine the drug-likeness of a compound. Hence, In-silico molecular properties of all the designed compounds was calculated using Qik–prop module of Schrodinger.

All the designed compounds were predicted to have good aqueous solubility and human oral absorption. Properties based on ADMET analysis assessed for their chemical properties of these ligands with their molecular weights are < 500 Daltons with < 5 hydrogen bond donors, < 10 hydrogen bond acceptors and QPlogPo/w < 5; these properties are well within the acceptable range of the Lipinski rule for drug-like molecules. All the compounds were predicted to have blood/brain partition coefficient (-3.0 to +1.2) and CNS activity (-2 to +2) within the acceptable range. Partition coefficient (QPlogPo/w) ranged from - 2.0 to 6.5. Water solubility (QPlogS) (-6.5 good to 0.5 poor), critical for estimation of absorption and distribution of drugs within the body, ranged between -6.49 and -1.13. Cell permeability (QPPCaco) (<0.25 poor; >0.5 good), a key factor governing drug metabolism and its access to biological membranes ranged from 1.1 to 0.57. Overall, the percentage human oral absorption for the compounds tested ranged from 73% to 100%. All these pharmacokinetic parameters were found to be within the acceptable range. Compounds with aliphatic substitutions at N^4 of piperazine were found to possess lesser blood/brain partition coefficient (-3.0 to +1.2) and CNS activity (-3 to +2) as compared to other compounds. Compounds with aliphatic substitution at N^4 were also predicted to have lower aqueous solubility and percentage of human oral absorption than all other compounds.

Table 18: Calculation of molecular properties of 1,8 naphthyridine carboxylic acid derivative (Series 1)

Compd code NACA	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-4.14	-0.32	2.24	3	95.48	0	1	6	0.93	0.56	2.69
2	-2.68	-0.06	1.57	3	80.77	1	1	6	0.71	0.56	2.66
3	-3.08	-0.15	1.97	3	83.10	1	1	6	0.71	0.56	2.66
4	-4.31	-1.36	1.53	3	74.00	-2	1	7	0.57	0.05	2.47
5	-4.67	-0.17	2.61	3	73.03	0	1	6	0.87	0.35	2.66
6	-4.67	-0.17	2.61	3	74.51	0	1	6	0.87	0.35	2.66
7	-4.42	-0.22	2.46	3	57.12	0	1	6	0.85	0.35	2.66
8	-5.34	0.07	3.93	3	91.50	-1	1	6	1.05	0.67	2.39
9	-4.32	0.07	-4.32	3	95.00	0	1	6	1.17	0.63	2.36
10	-4.28	0.08	-4.28	3	95.00	0	1	6	1.17	0.63	2.36
11	-4.59	-0.34	2.48	3	96.00	0	1	6	0.87	0.78	2.73
12	-4.34	-0.32	2.38	3	96.26	0	1	6	0.96	0.82	2.68
13	-1.47	0.14	0.25	3	73.00	1	1	6	1.10	0.78	2.65
14	-1.64	0.06	0.50	3	74.50	1	1	6	0.71	0.45	2.58
15	-3.32	-0.30	-0.04	2	57.12	0	1	6	0.96	0.41	2.64

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated apparent human epithelial colorectal adenocarcinoma (Caco-2) cell permeability (nm/sec). [d] Calculated apparent Madin-Derby Canine Kidney Cells (MDCK cell) permeability (nm/sec), %HOA=human oral absorption, %HOA=percent human absorption via oral route,CNS= predicted CNS activity,HBD=hydrogen bond doner, HBA=hydrogen bond acceptor.

Table 19: Calculation of molecular properties of piperazine analogs 1, 8-naphthyridin-3-carboxamides (Series 2)

Compd code MN	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-4.56	-0.20	3.61	3	100	0	0	6	0.67	0.88	2.21
2	-2.68	0.05	2.85	3	92.33	1	0	7	0.73	0.75	2.34
3	-4.81	-1.31	2.89	3	100	-2	0	7	0.12	0.11	1.43
4	-5.31	-0.08	4.08	3	100	0	0	6	0.51	0.43	2.36
5	-5.21	-0.03	4.09	3	100	0	0	6	0.51	0.43	2.38
6	-5.33	0.04	4.12	3	100	0	0	6	0.51	0.43	2.38
7	-4.23	-0.24	3.59	3	100	0	0	6.75	0.71	0.62	2.35
8	-4.69	-0.28	3.67	3	100	0	0	6.75	0.71	0.62	2.51
9	-4.23	-0.31	3.77	3	100	0	0	6.75	0.71	0.66	2.54
10	-4.38	-0.17	3.74	3	100	0	0	6	0.76	0.73	2.42
11	-5.1	-0.22	3.94	3	100	0	0	6	0.76	0.73	2.42
12	-1.13	0.13	1.12	3	80.69	1	0	7	0.38	0.41	1.54
13	-1.83	0.11	1.67	3	85.49	1	0	7	0.41	0.48	1.76
14	-2.07	0.01	1.97	3	86.69	1	0	7	0.55	0.52	1.88
15	-2.53	-0.06	2.37	2	89.00	1	0	7	0.40	0.44	1.23

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated noticeable human epithelial colorectal adenocarcinoma(Caco-2) cell permeability (nm/sec). [d] Calculated apparent Madin-Derby Canine Kidney Cell (MDCK cell) permeability (nm/sec), CNS=predicted CNS activity, HOA=human oral absorption, %HOA=percent human oral absorption,HBD=hydrogen bond doner, HBA=hydrogen bond acceptor.

Table 20: Calculation of molecular properties of piperazine analogs of 1, 8-naphthyridin-3-carboxamides (Series 2)

Compd code EN	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-4.38	-0.21	2.80	3	100	0	1	7	0.67	0.80	2.25
2	-3.50	-0.009	2.37	3	88.67	1	1	7	0.54	0.71	2.30
3	-4.50	-1.26	2.07	3	100	-2	1	8	0.21	0.17	1.41
4	-4.65	-0.11	3.02	3	100	0	1	7	0.53	0.46	2.32
5	-4.93	-0.06	3.18	3	100	0	1	7	0.54	0.46	2.32
6	-4.93	-0.06	3.18	3	100	0	1	7	0.53	0.46	2.32
7	-4.42	-0.32	3.85	3	100	0	1	7.75	0.53	0.58	2.36
8	-4.50	-0.28	2.86	3	100	-1	1	7.75	0.72	0.58	2.36
9	-4.54	-0.29	2.85	3	100	-1	1	7.75	0.72	0.70	2.35
10	-4.56	-0.21	2.94	3	100	0	1	7	0.72	0.70	2.42
11	-4.84	-0.23	3.05	3	100	0	1	7	0.68	0.68	2.40
12	-1.59	0.23	0.77	3	79.34	1	1	8	0.68	0.68	1.45
13	-1.72	0.16	1.01	3	80.72	1	1	8	0.44	0.41	1.32
14	2.20	0.06	1.37	3	82.84	1	1	8	0.38	0.50	1.23
15	-2.51	-0.01	1.69	3	84.70	1	1	8	0.34	0.34	1.20

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated evident human epithelial colorectal adenocarcinoma (Caco-2) cell permeability (nm/sec). [d] Calculated apparent Madin-Derby Canine Kidney Cell (MDCK cell)permeability (nm/sec),%HOA=human absorption via oral route, %HOA=percent human absorption via oral route,CNS=predicted CNS activity, HBD=hydrogen bond doner, HBA=hydrogen bond acceptor.

Table 21: Calculation of molecular properties of piperazine analogs of 4-methoxy-quinazoline carboxamides (Series 3)

Compd code MQZ	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-4.684	-0.117	3.741	3	100	0	0	6	0.61	0.81	2.17
2	-5.213	0.017	4.169	3	100	1	0	6	0.43	0.34	2.32
3	-5.445	0.045	4.244	3	100	1	0	6	0.43	0.33	2.31
4	-5.445	0.046	4.248	3	100	1	0	6	0.43	0.34	2.41
5	-4.866	-0.193	3.869	3	100	0	0	6.75	0.65	0.60	2.32
6	-4.859	-0.206	3.81	3	100	0	0	6.75	0.65	0.60	2.43
7	-4.833	-0.193	3.802	3	100	0	0	6.75	0.65	0.61	2.45
8	-1.22	0.27	1.323	3	84.47	1	0	7	0.33	0.34	1.48
9	-1.638	0.224	1.721	3	87.41	1	0	7	0.40	0.42	1.52
10	-2.104	0.144	2.117	3	89.75	1	0	7	0.48	0.46	1.67

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated evident human epithelial colorectal adenocarcinoma Caco-2 cell permeability (nm/sec). [d] Calculated apparent Madin-Derby Canine Kidney Cell (MDCK cell)permeability (nm/sec),% HOA=human oral absorption, %HOA=percent human oral absorption,CNS=predicted CNS activity, HBD=hydrogen bond doner, HBA=hydrogen bond acceptor.

Table 22: Calculation of molecular properties of piperazine analogs of 4-ethoxy-quinazoline carboxamides (Series 3)

Compd code EQZ	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-5.27	-0.20	4.18	3	100	0	0	6	0.61	0.81	2.28
2	-5.77	-0.06	4.51	3	100	0	0	6	0.57	0.48	2.32
3	-6.06	-0.04	4.65	3	100	0	0	6	0.56	0.48	2.32
4	-6.07	-0.04	4.65	3	100	0	0	6	0.56	0.48	2.32
5	-4.99	-0.24	4.18	3	100	0	0	6.75	0.63	0.51	2.36
6	-5.44	-0.28	4.25	3	100	0	0	6.75	0.75	0.51	2.36
7	-5.44	-0.28	4.25	3	100	0	0	6.75	0.75	0.76	2.35
8	-5.55	-0.19	4.41	3	100	0	0	6	0.75	0.76	2.42
9	-2.239	0.13	2.17	3	90.04	1	0	7	0.41	0.35	1.32
10	-2.705	0.05	2.56	3	92.39	1	0	7	0.35	0.41	1.23

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated evident human epithelial colorectal adenocarcinoma Caco-2 cell permeability (nm/sec). [d] Calculated apparentMadin-Derby Canine Kidney Cell (MDCK cell)permeability (nm/sec),%HOA=human oral absorption, CNS=predicted CNS activity, %HOA=percent human absorption via oral route ,HBD=hydrogen bond doner, HBA=hydrogen bond acceptor.

Table 23: Calculation of molecular properties of piperazine analogs of 1-methyl-1H-indol-2-carboxamides (Series 4)

Compd code NMIC	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-4.69	0.14	3.90	3	100	1	0	6	0.55	0.18	2.22
2	-3.04	0.42	3.17	3	100	1	0	7	0.57	0.73	2.08
3	-4.74	-0.88	3.13	3	93.65	-2	0	7	0.57	0.73	2.08
4	-4.98	0.24	4.13	3	100	1	0	6	0.55	0.73	2.16
5	-5.28	0.30	4.29	3	100	1	0	6	0.53	0.46	2.64
6	-5.28	0.30	4.29	3	100	1	0	6	0.53	0.46	2.64
7	-4.84	0.07	3.96	3	100	1	0	6	0.53	0.46	2.64
8	-4.76	0.08	3.93	3	100	1	0	6	0.34	0.83	2.88
9	-4.91	0.14	4.05	3	87.10	1	0	6	0.33	0.83	2.73
10	-5.22	0.14	4.17	3	90.04	0	0	6	0.34	0.83	2.78

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated evident human epithelial colorectal adenocarcinoma(Caco-2) cell permeability (nm/sec). [d] Calculated apparentMadin-Derby Canine Kidney Cell (MDCK cell) permeability (nm/sec), CNS=predicted CNS activity, % HOA=human oral absorption, %HOA=percent human oral absorption,HBD=hydrogen bonddoner,HBA=hydrogenbondacceptor.

Table 24: Calculation of molecular properties of piperazine analogs of 1-ethyl 1-H indol-2-carboxamides (Series 4)

Compd code NEIC	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-4.86	0.11	4.15	3	100	1	0	6	0.45	0.24	2.11
2	-3.25	0.39	3.43	3	100	1	0	7	0.51	0.66	1.98
3	-4.91	-0.92	3.38	3	95.65	-1	0	7	0.51	0.66	1.98
4	-5.14	0.21	4.38	3	100	1	0	6	0.53	0.66	1.98
5	-6.22	0.30	5.30	3	100	1	0	6	0.58	0.41	2.43
6	-5.44	0.26	4.54	3	100	1	0	6	0.58	0.41	2.44
7	-5.00	0.04	4.21	3	100	1	0	6	0.58	0.41	2.43
8	-4.93	0.05	4.17	3	100	1	0	6	0.29	0.81	2.76
9	-5.07	0.11	4.30	3	100	1	0	6	0.23	0.76	2.71
10	-5.38	0.10	4.42	3	100	1	0	6	0.20	0.78	2.70

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated evident human epithelial colorectal adenocarcinoma(Caco-2) cell permeability (nm/sec). [d] Calculated apparent Madin-Derby Canine Kidney Cell (MDCK cell) permeability (nm/sec), CNS=predicted CNS activity, % HOA=human oral absorption, %HOA=percent human oral absorption,HBD=hydrogen bonddoner,HBA=hydrogenbondacceptor.

Table 25: Calculation of molecular properties of piperazine analogs of 1-benzyl-1H-indol-2-carboxamides (Series 4)

Compd code NBIC	LogS[a]	LogBB[b]	LogPo/w	HOA	% HOA	CNS	HBD	HBA	QPPCaco[c]	PMDCK[d]	Acute toxicity (LD50, mol/kg)
1	-6.44	0.11	6.03	1	100	1	0	6	0.68	0.21	2.77
2	-3.12	0.31	3.40	1	100	1	0	7	0.76	0.83	2.82
3	-6.49	-0.90	5.28	1	95.25	-1	0	7	0.76	0.83	2.82
4	-7.71	0.27	6.68	1	100	1	0	6	0.76	0.83	2.80
5	-7.91	0.33	6.74	1	100	1	0	6	0.65	0.67	2.54
6	-7.91	0.33	6.74	1	100	1	0	6	0.65	0.67	2.54
7	-7.47	0.09	6.39	1	100	1	0	6	0.65	0.67	2.54
8	-7.32	0.08	6.30	1	78	1	0	6	0.43	0.95	2.33
9	-7.30	0.09	6.30	1	83	1	0	6	0.48	0.91	2.31
10	-2.87	0.45	3.56	3	87	2	0	6	0.37	0.88	2.29

[a] Predicted aqueous solubility (moles/liter), [b] Predicted brain/blood partition coefficient, [c]Calculated evident human epithelial colorectal adenocarcinoma(Caco-2) cell permeability (nm/sec). [d] Calculated apparentMadin-Derby Canine Kidney Cell (MDCK cell) permeability (nm/sec), CNS=predicted CNS activity, % HOA=human oral absorption, %HOA=percent human oral absorption,HBD=hydrogen bonddoner,HBA=hydrogenbondacceptor.

5.3. **Chemistry of Piperazine analogs of 1, 8 naphthyridine carboxylic acids and 1,8 naphthyridine carboxamides (Series 1** and **Series 2):**

Piperazine analogs of 1,8 naphthyridine carboxylic acids were synthesized via Scheme I. Starting materials nicotinamide **(1)** and ammonium sulphamate **(a)** were heated at 200 °C in neat, solvent free condition to obtain pyrido-pyrimidine **(2)** intermediate, which was hydrolyzed using dil HCl with heating to furnish 2-aminonicotinaldehyde **(3) figure 73**, the possible mechanism of formation of 2-amino nicotinaldehyde is described below.

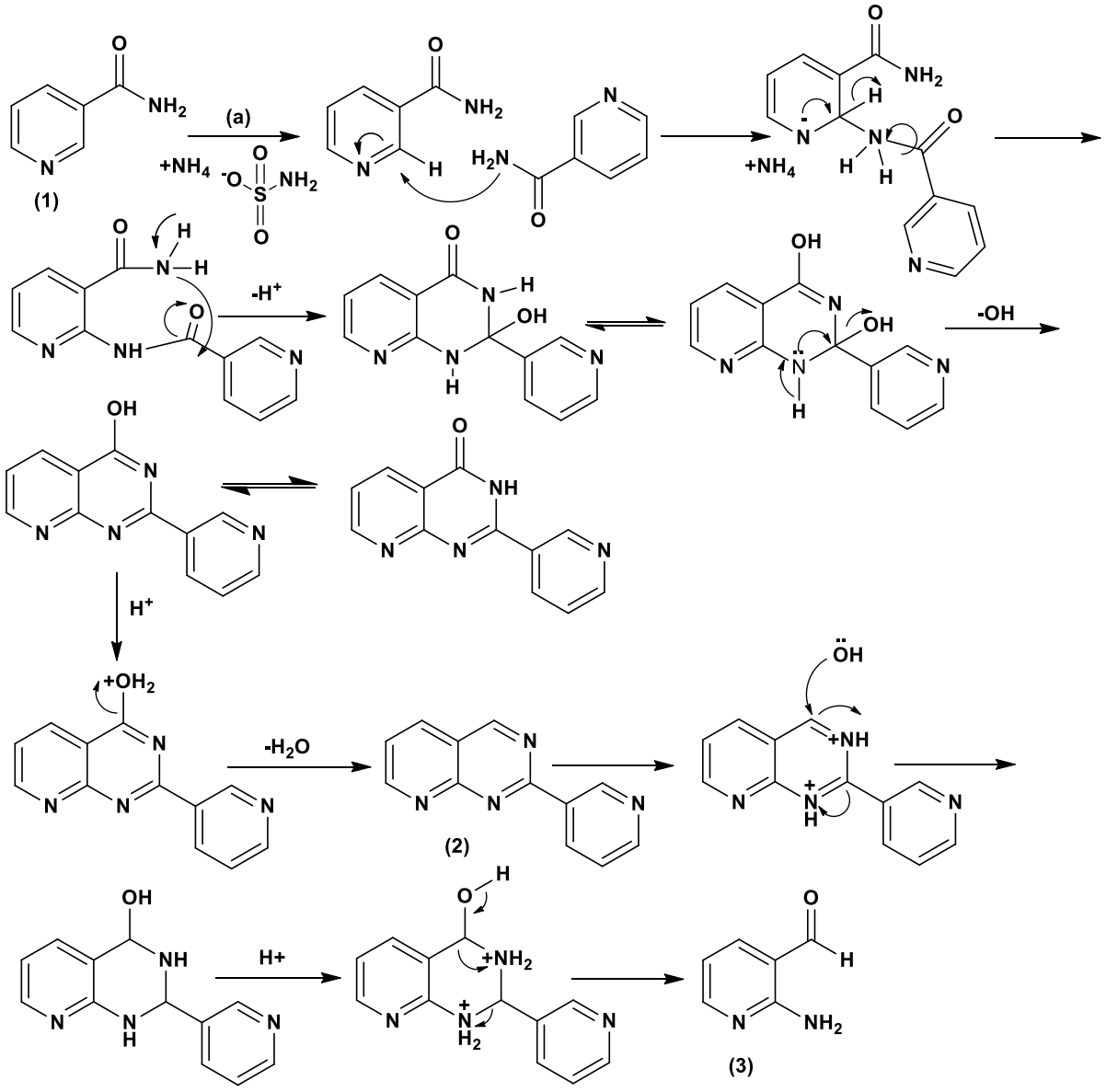


Figure 73: The possible mechanism for the formation of 2-amino nicotinaldehyde

Refluxing 2-aminonicotinaldehyde with diethyl malonate in ethanol and few drops of piperidine as catalyst, afforded the intermediate 1,2-dihydro-1,8-naphthyridinone-3-carboxylic acid ethyl ester (4). (Xiao et al., 2010). FT-IR spectra showed 1650 cm^{-1} (C=O of cyclic amide str) 1570 cm^{-1} (sec. cyclic amide –NH str) while NMR spectra showed 12.2 δ (s, 1H, cyclic amide NH); NMR signals for 1,8 naphthyridine moiety C_7-H: 8.81-8.79 δ (dd, 1H, *J*=4.8Hz, 2.0 Hz); C_4-H: 8.4 δ (s, 1H); C_6-H: 7.98-7.95 δ (m, 1H); C_5-H: 7.22-7.19 δ (dd, 1H, *J*=10.0 Hz, 7.2Hz). Compound **(4) figure 74**, was refluxed in $POCl_3$ with drops of DMF to furnish the chloro derivative of 1,8-naphthyridine-3-carboxylic acid ethyl ester **(5)**. The mechanism involved is described below.

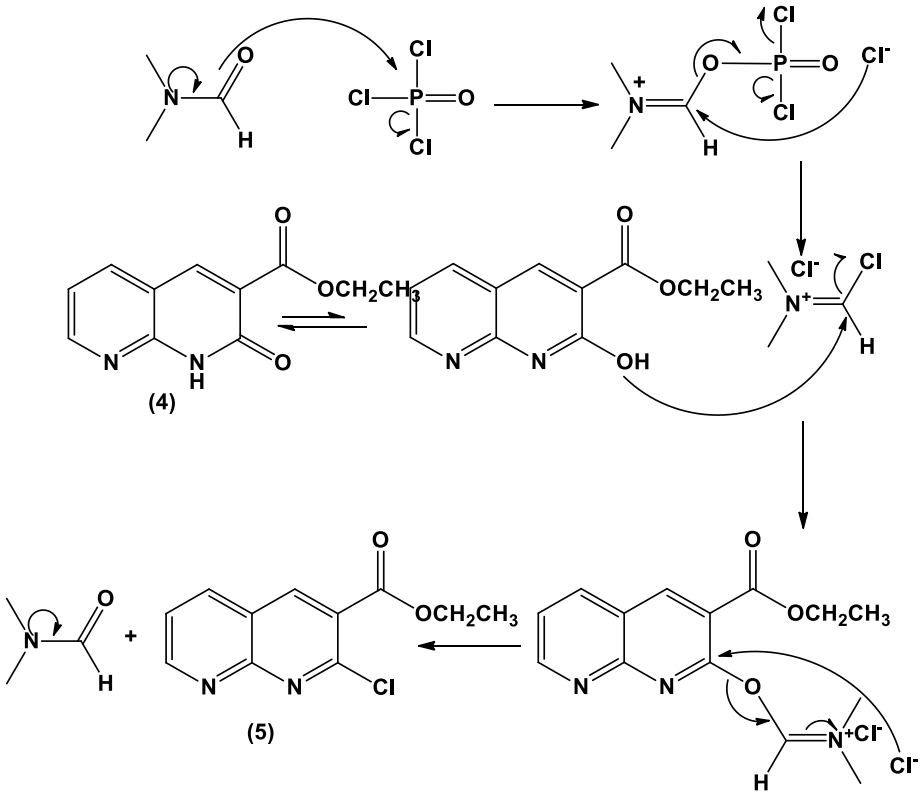


Figure 74: Mechanism involved in the synthesis of chloro derivative of 1,8-naphthyridine-3-carboxylic acid ethyl ester **(5).**

Nucleophilic displacement reaction of chlorine atom in Compound **(5)**, **figure 74**, was carried out with various substituted piperazines in refluxing acetonitrile with K_2CO_3 to obtain various piperazine analogs of 1,8-naphthyridine-3-carboxylic acid ethyl ester.

NMR spectra revealed signals at 4.47-4.41 δ (q, 2H,-CH_2, ethyl ester), 3.88-3.86 δ (m, 4H, methylene protons closest to N^1 of piperazine ring), 3.38-3.35 δ (m, 4H, methylene protons nearby N4 of piperazine ring), 1.46-1.42 δ (t, 3H, -CH_3-, ethyl ester). Finally the ester derivative (6a-o) was stirred with aq. NaOH in ethanol, upon completion of reaction, alcohol was removed in vacuo and a weak acid, like citric acid was used to acidify the reaction mixture up to pH 4-5 so as to avoid the protonation at N^4 nitrogen of piperazine ring and prevent the acid going into the solution. Finally, acidification furnished the title 1,8 naphthyridine carboxylic acids NACA 1-NACA 15. IR spectra of the title compounds showed C=O stretching vibrations of tertiary carboxamides at 1580±20 cm^{-1}.

5.4. Chemistry of 4-alkoxy 1,3 quinazoline carboxamides (Series 3):

4-alkoxy 1,3 quinazoline carboxamides described in Scheme III were designed as regioisomeric analogues of earlier described 2-alkoxy 1,8 naphthyridine carboxamides (**scheme III**). 1,3 quinazoline ring serves as a regioisomeric heteroaromatic core of earlier described 1,8 naphthyridine ring.

Variations were made to the heteroaromatic core (1,3 quinazoline in place of 1,8 naphthyridine). However, the hydrogen bond acceptor moiety (carbonyl group of carboxamide linkage), and the substituents at the N^4 of piperazine (the basic moiety of the pharmacophore) (**figure 1**) were kept similar to that of 1,8 naphthyridine carboxamides.

In **scheme III** 1,3 Quinazoline 4-one ring **(2)** was construted from anthranilamide (1) and diethyl oxalate in presence of sodium ethoxide under refluxing condition (Nakanishi and Massett 1980). The mechanism of quinazoline ring formation is explained in **figure 75**. The 4^{th} position keto group was O-alkylated using methyl iodide (to obtain methoxy derivatives), and ethyl iodide (to obtain ethoxy derivatives), in presence of canhydrous K_2CO_3, in DMF at 0^oC-5^oC. (Bogentoft et al., 1969). The meachanism behind the O alkylation is shown in **figure 76**.

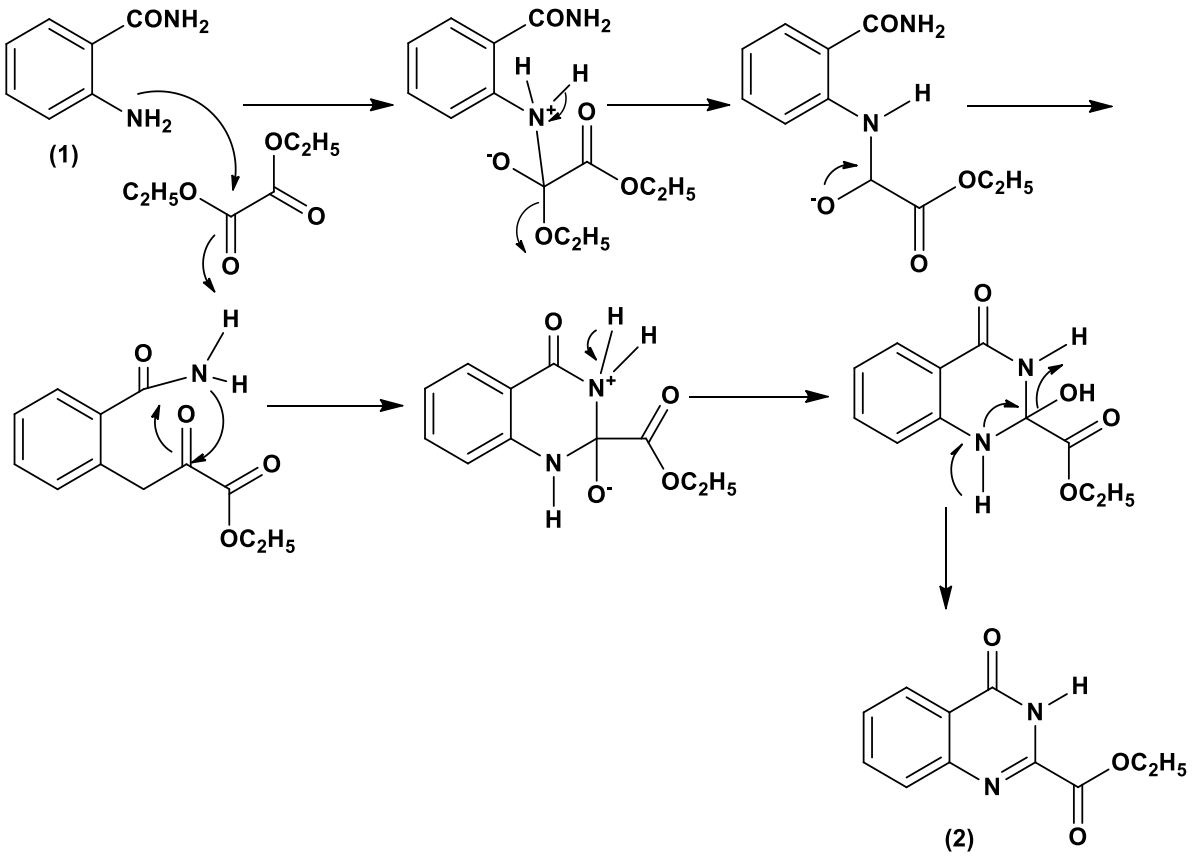


Figure 75: Mechanism of formation of quinazoline ring from anthranilamide

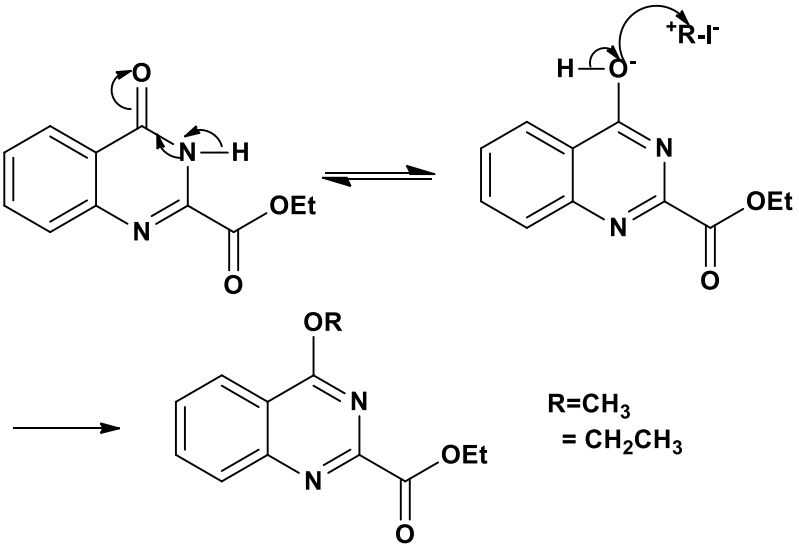


Figure 76: mechanism of alkylation

5.5. Chemistry of (1-alkyl/benzyl-1H-indol-2-yl) (4-substituted piperazin-1-yl) methanones (Series 4):

Another series of analogous indole-2-carboxamides were synthesized with indole as a heteroaromatic core and similar substitution pattern at the N^4 piperazine as described in the earlier two series (1,8 napthyridine carobxamides and 1,3 quinazoline carboxamides).

In this scheme indole carboxylic acid was converted to indole carboxylic acid ethyl ester. In order to modulate the overall lipophilicity of the carboxamides, the NH of the indole ring of the indole carboxylic acid ethyl ester was N-alkylated (with methyl iodide to obtain N-methyl analogues and ethyl iodide to obtain N-ethyl analogues) as well as benzylated (with benzyl bromide to obtain N-benzylated analogues) using KOH in DMSO.

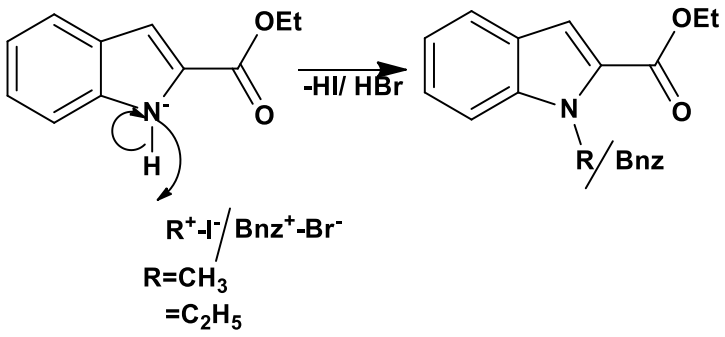
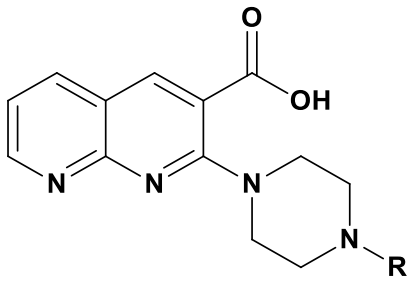


Figure 77: N alkylation and benzylation of indole

5.6. Evaluation of 5-HT_3 receptor antagonistic activity of piperazine analogues of 1,8 naphthyridine carboxylic acids (Series 1):



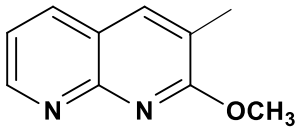
(NACA 1-15)

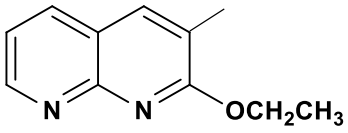
Table 26: 5-HT_3 receptor antagonism of piperazine analogues of 1,8 naphthyridine carboxylic acids/2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids

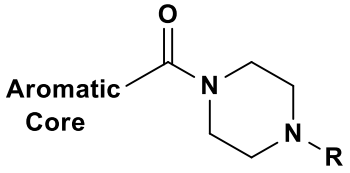
Compound	R	PA_2 Value	Compound	R	PA_2 Value
NACA 1	**C_6H_5-**	**7.6**	NACA 9	*m*-$OCH_3C_6H_4$	5.0
NACA 2	**C_6H_5-CH_2-**	**6.7**	**NACA 10**	***p*-$OCH_3C_6H_4$**	**7.0**
NACA 3	C_6H_5-CH_2-CH_2	6.5	NACA 11	*o*-$CH_3C_6H_4$	4.4
NACA 4	***p*- NO_2-C_6H_4-**	**7.2**	NACA 12	*p*-$CH_3C_6H_4$	5.9
NACA 5	*o*-Cl-C_6H_4-	4.6	NACA 13	CH_3-	6.3
NACA 6	***m*-Cl-C_6H_4-**	**7.3**	NACA 14	CH_3 CH_2-	5.4
NACA 7	*p*-Cl-C_6H_4-	6.0	NACA 15	$(CH_3)_2CH$-	5.2
NACA 8	***o*-$OCH_3C_6H_4$**	**6.6**	Ondansetron		6.6

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

5.7. Evaluation of 5-HT_3 receptor antagonistic activity of piperazine analogues of 1,8 naphthyridine carboxamides (Series 2):







Aromatic Core: **A** **B**

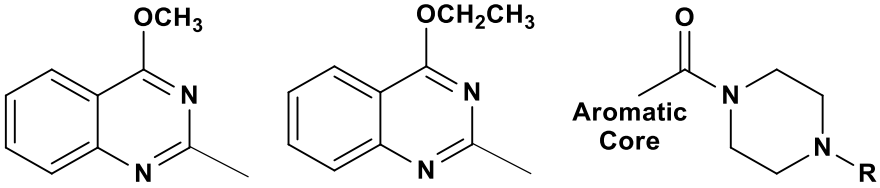
Series Code: **MN** **EN**

Table 27: 5-HT_3 receptor antagonism of piperazine analogues of 1,8 naphthyridine carboxamides/ (2-alkoxy-1, 8-naphthyridin-3-yl)(4-substituted piperazin-1-yl) methanones.

Compound Core A	**R**	**PA_2 Value**	**Compound Core B**	**R**	**PA_2 Value**
MN1	**C_6H_5-**	**6.67**	EN1	C_6H_5-	5.60
MN2	C_6H_5-CH_2-	4.67	EN2	C_6H_5-CH_2-	5.00
MN3	*p*- NO_2-C_6H_4-	4.53	EN3	*p*-NO_2-C_6H_4-	5.00
MN4	***o*-Cl-C_6H_4-**	**6.11**	EN4	*o*-Cl-C_6H_4-	5.90
MN5	***m*-Cl-C_6H_4-**	**7.33**	**EN5**	***m*-Cl-C_6H_4-**	**6.20**
MN6	***p*-Cl-C_6H_4-**	**6.30**	EN6	*p*-Cl-C_6H_4-	5.30
MN7	***o*-$OCH_3C_6H_4$**	**7.67**	**EN7**	***o*-$OCH_3C_6H_4$**	**6.60**
MN8	***m*-$OCH_3C_6H_4$**	**7.33**	**EN8**	***m*$OCH_3C_6H_4$**	**7.30**
MN9	*p*-$OCH_3C_6H_4$	5.00	EN9	*p*-$OCH_3C_6H_4$	5.50
MN10	*o*-CH_3-C_6H_4-	4.10	**EN10**	***o*-CH_3-C_6H_4-**	**6.25**
MN11	*p*-CH_3-C_6H_4-	5.56	EN11	*p*-CH_3-C_6H_4-	5.20
MN12	CH_3-	3.78	EN12	CH_3-	3.36
MN13	CH_3 CH_2-	4.40	EN13	CH_3 CH_2-	3.50
MN14	$CH_3CH_2CH_2$-	4.20	EN14	$CH_3CH_2CH_2$-	3.67
MN15	$CH_3(CH_2)_3$-	3.67	MN15	$CH_3(CH_2)_3$-	3.21
			Ondansetron		6.60

[a] pA_2 values are the means of two individual experiments. Mean± SE <10% of the mean.

5.8. Evaluation of 5-HT_3 receptor antagonism of piperazine analogues of 4-alkoxy-1,3 quinazolin-carboxamides (Series 3):



Aromatic Core: **A** **B**

Series Code: **MQZ** **EQZ**

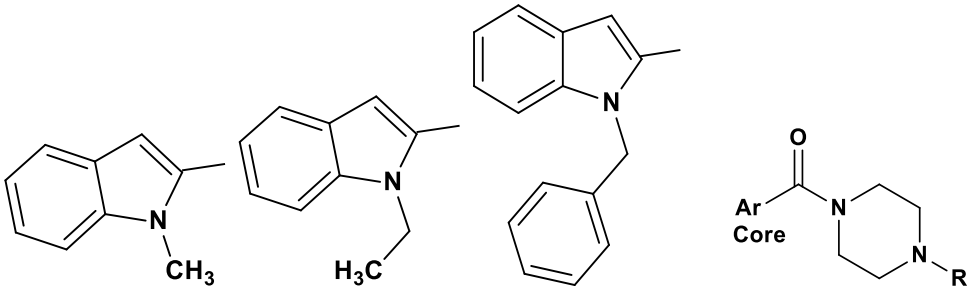
MQZ: Piperazine analogs of methoxy quinazoline carboxamides; EQZ: Piperazine analogs of ethoxy quinazoline carboxamides

Table 28: 5-HT_3 receptor antagonism of piperazine analogues of 4-alkoxy-1,3 quinazolin-carboxamides/{4-substituted piperazine-1-yl)} (4-alkoxy quinazoline-2-yl) methanones

Compound Core A	**R**	**P*A*$_2$ Value**	**Compound Core B**	**R**	**P*A*$_2$ Value**
MQZ1	C_6H_5-	5.68	C_6H_5-	EQZ1	6.00
MQZ2	*o*-Cl-C_6H_4-	5.00	*o*-Cl-C_6H_4-	EQZ2	5.43
MQZ3	*m*-Cl-C_6H_4-	5.80	*m*-Cl-C_6H_4-	EQZ3	6.10
MQZ4	***p*-Cl-C_6H_4-**	**6.10**	***p*-Cl-C_6H_4-**	**EQZ4**	**6.30**
MQZ5	***o*-$OCH_3C_6H_4$**	**6.30**	***o*-$OCH_3C_6H_4$**	**EQZ5**	**6.00**
MQZ6	***m*$OCH_3C_6H_4$**	**6.70**	***m*$OCH_3C_6H_4$**	**EQZ6**	**6.75**
MQZ7	*p*-$OCH_3C_6H_4$	5.57	*p*-$OCH_3C_6H_4$	EQZ7	5.38
MQZ8	CH_3-	3.50	CH_3-	EQZ8	3.80
MQZ9	CH_3-CH_2-	3.80	CH_3-CH_2-	EQZ9	3.56
MQZ10	$CH_3CH_2CH_2$-	3.76	$CH_3CH_2CH_2$-	EQZ10	3.78
	Ondansetron	6.60			

[a]pA_2 values are the means of two individual experiments. Mean±SE<10% of the mean.

5.9. Evaluation of 5-HT_3 receptor antagonistic activity of piperzine analogues of indole-2-carboxamides (Series 4):



Aromatic Core	**A**	**B**	**C**
Series Code	**NMIC**	**NEIC**	**NBIC**

NMIC: Piperazine analogs N-methyl indole carboxamides; **NEIC:** Piperazine analogs of N-ethyl indole carboxamides; **NBIC**: Piperazine analogs of N-benzyl indole carboxamides

Table 29: 5-HT_3 receptor antagonism of piperazine analogs of 1-alkyl/ benzyl-1H-indol-2-carboxamides/(1-alkyl/benzyl-1H-indol-2-yl) (4-substituted piperazin-1-yl) methanones

R	Compound Core A	PA_2	Compound Core B	PA_2	Compound Core C	PA_2
C_6H_5	**NMIC 1**	**6.25**	NEIC 1	6.00	**NBIC 1**	**6.80**
o-ClC_6H_4	NMIC 2	5.60	NEIC 2	5.50	NBIC 2	5.40
***m*-ClC_6H_4**	**NMIC 3**	**6.60**	NEIC 3	5.00	NBIC 3	5.00
p-ClC_6H_4-	NMIC 4	5.40	**NEIC 4**	**6.50**	**NBIC 4**	**6.30**
***o*-$OCH_3C_6H_4$**	**NMIC 5**	**6.60**	NEIC 5	5.50	**NBIC 5**	**6.00**
***m*$OCH_3C_6H_4$**	**NMIC 6**	**6.80**	**NEIC 6**	**7.10**	**NBIC 6**	**7.50**
p-$OCH_3C_6H_4$	NMIC 7	5.20	**NEIC 7**	**6.86**	NBIC 7	5.50
CH_3-	NMIC 8	4.00	NEIC 8	4.36	NBIC 8	5.00
CH_3-CH_2-	NMIC 9	4.80	NEIC 9	4.61	NBIC 9	4.70
$CH_3CH_2CH_2$	NMIC 10	4.50	NEIC 10	4.66	NBIC 10	4.40
	Ondansetron	6.60				

[a] pA_2 values are the means of two individual experiments. Mean ±SE <10% of the mean.

5.10. Evaluation of spontaneous locomotor activity and anti-depressant potential:

Table 30: Locomotor scores and anti-depressant activity of piperazine analogs of 1,8-naphthyridine 3-carboxylic acids (Series 1)

Compound	Locomotor Scores (10 min) Dose 1mg/kg (*i.p*)	Immobility period in second (FST)[a] Dose, 1 mg/kg (*i.p.)*	Immobility period in second (TST)[a] Dose, 1 mg/kg (*i.p.)*
NACA 1	347.00 ± 08.33	**65.25 ± 07.86***	**143.00 ± 11.39***
NACA 2	341.00 ± 17.54	96.00 ± 10.50*	200.00 ± 11.00
NACA 3	343.70 ± 02.91	**119.75 ± 11.33***	**149.00 ± 11.00***
NACA 4	346.70 ± 19.68	**109.50 ± 05.01***	**128.60 ± 07.00***
NACA 5	351.40 ± 12.29	101.00 ± 01.87*	204.00 ± 04.00
NACA 6	354.50 ± 12.89	**97.00 ± 12.40***	**169.00 ± 14.00***
NACA 7	356.50 ± 15.57	134.75 ±12.20	168.00 ± 13.00
NACA 8	347.66 ± 16.04	**62.25 ± 08.54***	**166.00 ± 18.00***
NACA 9	344.50 ±05.13	150.50 ± 10.30	118.00 ± 11.00*
NACA 10	338.56 ±13.85	**78.00 ± 03.66***	**160.00 ± 11.00***
NACA 11	328.34 ± 13.31	**110.50 ±06.23***	**166.00 ± 06.00***
NACA 12	366.70 ± 12.84	152.00 ± 12.42	144.00 ± 04.00*
NACA 13	329.70 ± 11.17	**100.50 ± 04.82***	**192.00 ± 13.00**
NACA 14	351.50 ± 14.38	153.50±11.83	225.00 ± 19.00
NACA 15	372.70 ± 17.55	133.00 ± 13.19	172.00 ± 16.00
Ondansetron	365.22 ± 08.42	105.10 ± 08.90*	155.00 ± 05.60*
Control	350.50 ± 08.19	165.23 ± 10.91	226.00 ± 08.14

*p< 0.05 as compared to vehicle treated group (control)[a] water was used as a vehicle, the values are stated as mean±SEM, n=6 per group. Data were evaluated using graph pad prism (3) software through one way ANOVA followed by post hoc Dunnett's test.

Table 31: Locomotor scores and anti-depressan activity of piperazine analogs of 2-methoxy 1,8 naphthyridine carboxamides (Series 2)

Compound	Locomotor scores[a] (10 min) Dose of 1 mg/kg (i.p.)	Immobility period in second (FST)[a] Dose, 1 mg/kg (i.p.)	Immobility period in second (TST)[a] Dose, 1 mg/kg (i.p.)
MN 1	436.25 ± 03.39	**108.33 ± 0.98***	**152.00 ± 09.39***
MN 2	456.75 ± 18.72	159.00 ± 03.53	198.49 ± 15.39
MN 3	460.00 ± 16.15	151.05 ± 13.5	146.32 ± 04.87*
MN 4	448.00 ± 19.50	**96.50 ± 09.54***	**132.55 ± 03.80***
MN 5	416.50 ± 13.50	**120.00 ± 01.87***	**165.80 ± 10.53***
MN 6	478.75 ± 10.68	**119.00 ± 02.40***	**161.00 ± 11.29***
MN 7	473.50 ± 04.91	**101.00±09.89***	**138.00 ± 11.76***
MN 8	457.75 ± 02.29	**104.00 ± 02.12***	**196.00 ± 16.70**
MN 9	466.00 ±12.76	**98.00 ± 20.88***	**150.00 ± 10.43***
MN 10	471.25 ±12.11	157.50 ± 01.30	158.00 ± 09.00*
MN 11	432.00 ± 12.37	152.00 ± 02.20	184.00 ± 14.00
MN 12	417.00 ± 14.93	144.00 ± 21.21	201.00 ± 16.00
MN 13	438.75 ± 17.42	152.50 ± 04.82	192.00 ± 16.32
MN 14	486.50 ± 07.41	153.50 ± 11.83	205.10 ± 09.65
MN 15	470.25 ± 6.19	143.00 ± 13.19	189.00 ± 13.89
Ondansetron	461.22 ± 10.42	105.10 ± 08.90*	155.00 ± 05.60*
Control	442.00 ± 15.50	158.33 ± 04.99	226.00 ± 08.14

*p< 0.05 as compared to vehicle treated group (control) [a] water used as a vehicle, the values are stated as mean, n=6 per group. Data were evaluated using graph pad prism (3) software through one way ANOVA followed by post hoc Dunnett's test.

Table 32: Locomotor scores and anti-depressant activity of piperazine analogs of 2-ethoxy 1,8 naphthyridine carboxamides (Series 2)

Compound	Locomotor scores[a] (10 min) Dose of 1 mg/kg (i.p.)	Immobility period in second (FST)[a] Dose, 1 mg/kg (i.p.)	Immobility period in second (TST)[a] Dose,1 mg/kg (i.p.)
EN 1	442.67± 07.65	150.67±13.01	192.83 ±11.00
EN 2	432.42± 05.32	152.83± 7.08	197.17±08.59
EN 3	467.59± 12.43	159.83±6.28	193.33±16.32
EN 4	482.00± 09.47	**130.00±9.59 ***	**112.50±13.93***
EN 5	507.00± 18.91	**136.50±9.25***	**114.00±09.56***
EN 6	458.45± 11.34	**140.50±7.45***	**140.33±14.45***
EN 7	435.50 ± 17.41	**98.33± 07.12***	**113.50±12.06***
EN 8	445.50 ± 07.86	**90.67 ±10.57***	**120.83±08.93 ***
EN 9	362.67 ± 10.63	**120.33 ±11.45***	**145.17±09.06***
EN 10	476.59± 03.87	**92.30 ±03.24***	**117.17±13.16***
EN 11	498.12± 07.75	150.50±08.37	193.83±12.92
EN 12	486.45± 10.42	154.17±08.28	200.17±09.20
EN 13	421.76± 15.34	150.00±06.01	194.33±06.12
EN 14	444.89± 13.65	153.33±02.78	200.17±05.80
EN 15	471.54± 07.53	155.33±06.46	196.83±08.11
Ondansetron	395.50 ± 1.41	105.10± 08.90*	148.50±06.78*
Control	489.00 ± 12.10	174.67 ± 05.21	236.33± 6.90

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean, n=6 per group. Data were evaluated using graph pad prism (3) software through one way ANOVA followed by post hoc Dunnett's test.

Table 33: Spontaneous locomotor activity and anti-depressant activity of piperazine analogue of 1,3 quinazoline 2-carboxamides (Series 3)

Compound	Locomotor Scores (10 min) Dose: 1 mg/kg (i.p.)	Duration of immobility in second (FST)[a] Dose: 1 mg/kg (i.p.)	Duration of immobility in second (TST)[a] Dose: 1 mg/kg (i.p.)
MQZ 1	371.00 ± 08.33	151.75 ±03.27	198.83±10.93
MQZ 3	387.23 ± 12.72	135.00 ± 12.50*	203.17±05.38
MQZ 4	333.70 ± 09.56	**98.75 ± 05.62***	**145.17±11.12***
MQZ 5	383.24 ± 12.55	**103.11 ± 0.65***	**150.50±10.34***
MQZ 6	378.87 ± 10.77	**87.81 ± 12.31***	**148.83±09.73***
MQZ 7	367.50 ± 01.89	143.12 ± 15.42	202.33±10.02
EQZ 1	388.12 ± 09.55	**97.25 ± 07.86***	**153.33±10.36***
EQZ 3	313.66 ± 09.45	132.10 ± 13.85	201.00± 09.71
EQZ 4	348.53± 10.31	**76.00 ± 02.76***	**163.50± 07.38***
EQZ 5	361.32 ± 05.66	**90.50 ± 09.72***	**170.17±06.07***
EQZ 6	378.29± 09.12	**100.07 ± 08.48***	**158.17± 09.47***
EQZ 7	384.19 ± 13.76	140.50 ±16.23	202.67± 08.44
Ondansetron	365.22 ± 08.42	105.10 ± 08.90*	148.50 ± 06.78*
Control	350.50 ± 08.19	165.23 ± 10.91	236.33 ± 6.90

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean, n=6 per group. Data were evaluated using graph pad prism (3) software through one way ANOVA followed by post hoc Dunnett's test.

Table 34: Spontaneous locomotor activity and anti-depressant activity of piperazine analogue of indole 2-carboxamides (Series 4)

Compound	Locomotor scores[a] (10 min) Dose 1 mg/kg (i.p.)	Duration of immobility in sec (FST)[a] Dose, 1 mg/kg (i.p.)	Duration of immobility in sec (TST)[a] Dose, 1 mg/kg (i.p.)
NMIC 1	435.33± 15.76	145.33 ± 04.83	198.67±13.94
NMIC 3	438.00± 15.62	166.33 ± 07.56	199.50±10.42
NMIC 5	425.50 ± 10.41	**100.43±03.91***	**149.33±08.60***
NMIC 6	464.00 ± 25.53	**113.33±13.86***	**119.67±05.83***
NEIC 1	401.40 ± 10.23	137.00 ± 06.78	155.67±07.94*
NEIC 4	454.14 ± 09.87	**90.50 ± 10.41***	**136.00±08.48***
NEIC 6	444.31 ± 11.56	**81.21 ±09.82***	**122.50±08.29***
NEIC 7	398.56 ± 05.47	**100.31 ± 08.54***	**122.33±12.44***
NBIC 1	387.56 ±11.45	**101.24 ± 10.53***	**140.50±07.45***
NBIC 4	462.50 ±15.13	151.50 ± 13.30	218.00±12.35
NBIC 5	436.70 ± 06.33	157.00 ± 12.42	203.33±13.70
NBIC 6	451.28 ± 09.44	**90.12 ±03.25***	**147.83±10.55***
Ondansetron	395.50 ± 1.41	105.10 ± 08.90*	148.50±06.78*
Control	489.00 ± 12.10	174.67 ± 05.21	236.33±06.90

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean± SEM, n=6 per group. Data were evaluated using graph pad prism (3) software through one way anova followed by post hoc dunnett's test.

Table 35: Effect of piperazine analogs of 1,8-naphthyridine 3-carboxylic acids, ondansetron, and diazepam on the behavior of mice in L/D test

Compound	Latency (s) Dose 1 mg/kg	Time spent in light chamber (s) Dose 1 mg/kg	Number of transitions Dose 1 mg/kg
NACA 1	40.5± 13.84	**116.83± 07.5***	**12.67±0.88***
NACA 2	27.17± 09.78	**131.75±22.15***	**8.83 ± 02.42***
NACA 3	7.17± 02.84	**107.17±26.33***	**15.6 ± 02.22***
NACA 4	26.17± 07.80	**124.33±10.79***	**13.83±01.12***
NACA 5	35.20±10.40	**129.33±10.44***	**15.33±01.37***
NACA 6	10.00 ± 01.83	**90.67 ±15.03***	**13.17±0.83***
NACA 8	19.76 ± 06.84	**87.76±16.04***	**11.00±02.19***
NACA 10	27.17 ±09.34	**96.33 ±20.09***	**10.00±2.29***
NACA 11	12.50 ± 04.05	71.33± 14.61	13.40±2.12*
NACA 13	09.83 ± 01.91	83.50 ± 10.21*	14.60±3.19*
Ondansetron	18.00± 05.21	123± 5.50*	11.23±04.00*
Diazepam	73.83 ± 08.90*	164.83± 5.50*	17.16±09.00*
Control	24.17 ± 03.06	63.40 ± 5.29	04.67 ± 0.87

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean± SEM, n=6 per group. Data were evaluated using graph pad prism (3) software through one way anova followed by post hoc dunnett's test.

Table 36: Effect of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides, ondansetron, and diazepam on the behavior of mice in L/D test

Compound	Latency (s) Dose 1 mg/kg	Time spent in light chamber (s) Dose 1 mg/kg	Number of transitions Dose 1 mg/kg
MN 1	27.33 ± 13.25	**91.00 ± 07.5***	**10.76±01.21***
MN 5	38 ± 03.88	**105.81±01.92***	07.00± 04.52
MN 6	15.56 ± 4.21	68.67±11.89	09.00± 05.86
MN 7	**36.20±03.42***	**118.33±15.43***	**14.53±03.77***
MN 8	**30.00±01.53***	**109.67±11.54***	**13.00±03.43***
EN 5	15.56 ± 04.21	80.70 ± 17.14	11.76± 08.61
EN 7	27.69 ± 09.70	98.72 ± 10.42*	09.00 ± 06.76
EN 8	**19.87±01.76***	**116.12±0.68***	**13.45±03.21***
EN 10	20.21 ± 08.96	71.61± 14.56	08.00 ± 04.87
Ondansetron	18.00± 05.21	123± 05.50*	11.23±04.00*
Diazepam	73.83 ± 08.90*	164.83±05.50*	17.16±09.00*
Control	24.17 ± 03.06	63.40 ±0.29	04.67 ± 0.87

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean± SEM, n=6 per group. Data were evaluated using graph pad prism (3) software through one way anova followed by post hoc dunnett's test.

Table 37: Effect of piperazine analogue of 4-alkoxy 1,3 quinazoline 2-carboxamides, ondansetron, and diazepam on the behavior of mice in L/D test

Compound	Latency (s) Dose 1 mg/kg	Time spent in light chamber (s) Dose 1 mg/kg	Number of transitions Dose 1 mg/kg
EQZ 1	13.00 ±08.38	62.00± 06.30	05.57±4.76
MQZ 4	**32.33±01.09***	**102.00± 11.50***	**13.37±0.94***
MQZ 5	18.00 ±01.88	87.81±16.87	07 ± 04.52
MQZ 6	18.16 ± 05.30	**118.67±07.89***	**15.00 ±01.25***
EQZ 4	**32.20±01.42***	**121.33±05.41***	**17.21±04.27***
EQZ 5	20.00±08.13	65.67±09.62	**13.00±03.43***
EQZ 6	24.00±05.49	**101.21±06.50***	**18.53±04.41***
Ondansetron	18.00± 05.21	123± 05.50*	11.23±04.00*
Diazepam	73.83 ± 08.90*	164.83± 05.50*	17.16±09.00*
Control	24.17 ± 03.06	63.40 ± 05.29	4.67 ± 00.87

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean± SEM, n=6 per group. Data were evaluated using graph pad prism (3) software through one way anova followed by post hoc dunnett's test.

Table 38: Effect of piperazine analogs of indole 2-carboxamides, ondansetron, and diazepamon the behavior of mice in L/D test

Compound	Latency (s) Dose 1 mg/kg	Time spent in light chamber (s) Dose 1 mg/kg	Number of transitions Dose 1 mg/kg
NMIC 5	**37.33±02.56***	**84.00± 03.50***	**13.16±05.28***
NMIC 6	**38.34±05.53***	**101.67±09.54***	**11.00±04.41***
NEIC 4	25.56 ±08.50	71.67±12.40	11.56± 09.86
NEIC 6	**46.20±06.42***	**105.33±08.43***	**17.21±03.32***
NEIC 7	28.00 ±13.88	98.81±07.42*	10.00± 08.52
NBIC 1	27.18±06.33	77.87±10.65	10.00±01.61*
NBIC 6	**36.34±05.17***	**103.67±07.39***	**12.50±02.53***
Ondansetron	18.00± 05.21	123.00 ± 05.50*	11.23±04.00*
Diazepam	73.83 ± 08.90*	164.83± 05.50*	17.16±09.00*
Control	24.17 ± 03.06	63.40 ± 05.29	04.67± 0.87

*p< 0.05 as compared to vehicle treated group (control) [a]water was used as a vehicle, the values are stated as mean± SEM, n=6 per group. Data were evaluated using graph pad prism (3) software through one way anova followed by post hoc dunnett's test.

Table 39: Effect of piperazine analogs of 1,8-naphthyridine-3-carboxylic acids, ondansetron and diazepam on the performance of mice in EPM test

Compounds	Dose (mg/kg)	No. of entries		% OAE	Time spent (sec)		% TSOA
		open arm	close arm		open arm	close arm	
NACA 1	1	**2.83± 00.51**	5.50±0 0.96	**33.97±02.56***	**105.17±06.90***	194.83±04.90	**35.05±03.64***
NACA 2	1	2.29±01.08	6.86±01.03	25.02±03.53	32.14±02.22	267.86±03.95	10.71±00.98*
NACA 3	1	1.21±0.67	5.50±01.01	18.03±07.51	32.13±10.14	267.87±10.11	10.71±04.04
NACA 4	1	2.00±0.57	3.33±00.56	30.73±07.64	26.33±07.80	273.67±10.11	08.78±02.60
NACA 5	1	2.83±02.67	7.10±01.51	28.49±07.51	44.83±02.04*	255.17±01.11	14.94±04.04
NACA 6	1	**2.33±0.67**	5.50±02.01	**29.75±07.51**	**37.83±00.04***	262.17±02.11	**12.61±02.04**
NACA 8	1	**3.11±0.67**	6.50±01.01	**32.36±07.51**	**54.83±01.14***	245.17±02.51	**18.27±04.04**
NACA 10	1	3.0±0.50	5.93±01.01	33.59 ±4.11	28.00±13.93	272.00±15.11	09.33±01.04
NACA 12	1	2.43±01.67	5.10±01.21	32.27±07.51	34.83±02.14	265.17±01.11	11.61±02.34
NACA 13	1	2.13±0.67	7.10±01.51	23.07±30.51	30.83±12.14	269.17±12.11	10.27±02.04
Ondansetron	1	4.83±0.37*	6.67±01.22	39.84±03.51	41.50±04.35*	258.50±02.32	13.83±01.44*
Diazepam	2	6.5±01.23*	4.33±0.71	60.01±05.53*	124.5±11.8*	175.5±08.15	41.50±04.38*
Control		2.29±0.68	4.4±0.73	34.23±02.53	26.30± 9.94	281.70±09.94	08.76±02.16

All values represent mean ± SEM. #p < 0.05 when compared with vehicle-treated group; n = 6/group; %OAE = percentage of open arm entries; % TSOA = percentage of time spent in open arm in seconds.

Table 40: Effect of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides, ondansetron and diazepam on the performence of mice in EPM test

Groups	Dose (mg/kg)	No. of entries		% OAE	Time spent (sec)		% TSOA
		open arm	close arm		open arm	close arm	
MN 1	**1**	**3.83± 1.87**	6.45± 1.23	**37.25±01.22***	**100.17±4.32***	199.83±3.56	**33.39±4.16***
MN 5	1	2.00±3.58	6.61±0.86	23.22±03.33	23.14±5.46	276.86±5.53	7.71±4.75
MN 6	1	1.21±0.67	4.37±1.68	21.68±01.45	28.67±9.57	271.33±11.21	9.55±5.51
MN 7	1	2.00±0.57	4.52±2.37	30.67±4.23	76.45±4.21*	223.55±6.73	25.48±6.64*
MN 8	**1**	**4.83±0.45***	5.75±2.58	**45.65±3.44***	**54.00±5.43***	246.00±3.45	**18.00±1.04***
EN 5	**1**	**3.69±2.11**	4.25±2.62	**46.47±3.77***	**27.33±5.04**	272.67±3.11	**9.11±2.46**
EN 7	1	2.56±2.44	6.43±1.53	28.47±3.32	34.83±6.89	265.17±3.51	11.61±2.24
EN 8	**1**	**5.0±1.23***	6.17±0.63	**44.76 ±1.11***	**68.00±3.12***	232.00±5.46	**22.66±3.76***
EN 10	1	3.00±2.17	7.11±0.76	29.67±6.10	24.83±0.55	275.17±2.31	8.27±5.22
Ondansetron	1	4.83±0.37*	6.67±1.22	39.84±3.51	41.50±4.35*	258.50±2.32	13.83±1.44*
Diazepam	2	6.5±1.23*	4.33±0.71	60.01±5.53*	124.5±11.8*	175.5±8.15	41.50±4.38*
Control		2.29±0.68	4.4±0.73	34.23±2.53	26.30± 9.94	281.70±9.94	8.76±2.16

All values represent mean ± SEM. #p < 0.05 when compared with vehicle-treated group; n = 6/group; %OAE = percentage of open arm entries; % TSOA = percentage of time spent in open arm in seconds.

Table 41: Effect of piperazine analogs of 4-alkoxy 1,3 quinazoline 2-carboxamides, ondansetron and diazepam on the performance of mice in EPM test

Groups	Dose (mg/kg)	No. of entries		% OAE	Time spent (sec)		% TSOA
		open arm	closed arm		open arm	closed arm	
EQZ 1	1	2.43±3.21	10.00±07.43	24.30±4.67	21.52±5.46	278.48±09.75	09.55±05.51
MQZ 4	1	3.76±2.14	7.20±00.75	21.68±01.45	**91.00±4.21***	209.00±6.32	**30.33±02.24***
MQZ 5	1	3.21±1.69	7.53±03.61	23.22±03.33	30.21±7.85	269.79±3.43	7.71±4.75
MQZ 6	1	4.31± 0.98	8.91± 02.65	37.25±01.22	**100.17±4.32***	199.83±3.56	**33.39±4.16***
EQZ 4	1	**5.00±0.57***	4.52±02.37	**52.52±4.23***	**83.47±6.72***	216.53±03.46	**25.48±6.64***
EQZ 5	1	**4.83±0.45***	5.75±02.58	**45.65±3.44***	**67.92±2.85***	232.08±01.32	**18.00±1.04***
EQZ 6	1	**9.83±3.15***	5.75±02.58	**45.65±3.44***	**78.32±4.21***	221.68±04.21	**26.10±4.14***
Ondansetron	1	4.83±0.37*	6.67±01.22	39.84±3.51	41.50±4.35*	258.50±2.32	13.83±1.44*
Diazepam	2	6.5±1.23*	4.33±0.71	60.01±5.53*	124.5±11.8*	175.5±8.15	41.50±4.38*
Control		2.29±0.68	4.4±0.73	34.23±2.53	26.30± 9.94	281.70±9.94	8.76±2.16

All values represent mean ± SEM. #p < 0.05 when compared with vehicle-treated group; n = 6/group; %OAE = percentage of open arm entries; % TSOA = percentage of time spent in open arm in seconds.

Table 42: Effect of piperazine analogs of indole 2-carboxamides, ondansetron and diazepam on the performance of mice in EPM test

Groups	Dose (mg/kg)	No. of entries		% OAE	Time spent (sec)		% TSOA
		open arm	closed arm		open arm	Closed arm	
NMIC 5	1	**5.83±1.07***	8.45± 0.98	**40.82±03.54***	**117.17±8.53***	182.83±06.41	**39.05±03.54***
NMIC 6	1	**7.18±0.81***	11.37±5.31	**38.70±0.56***	**96.45±07.48***	203.55±03.42	**32.15±0.52***
NEIC 4	1	3.00±4.58	7.61±3.86	28.27±03.73	34.04±06.87	265.96±10.32	11.34±09.45
NEIC 6	1	**8.33±3.14***	13.00±3.83	**39.05±1.41***	**87.47±08.17***	212.53±06.16	**29.15±04.51***
NEIC 7	1	4.86±0.81	15.80± 7.37	23.52±0.56	43.67±14.53	256.33±09.67	10.55±02.33
NBIC 1	1	4.20±3.57	18.52±0.37	18.42±2.77	46.45±13.51	253.55±10.64	15.48±09.71
NBIC 6	**1**	**8.20±1.20***	13.52±6.37	**37.75±4.23***	**102.15±02.55***	197.85±04.31	**34.05±01.55***
Ondansetron	1	4.83±0.37*	6.67±1.22	39.84±3.51	41.50±4.35*	258.50±2.32	13.83±01.44*
Diazepam	2	6.5±1.23*	4.33±0.71	60.01±5.53*	124.5±11.8*	175.5±8.15	41.50±04.38*
Control		2.29±0.68	4.4±0.73	34.23±2.53	26.30± 9.94	281.70±9.94	8.76±02.16

All values represent mean ± SEM. #p < 0.05 in comparison to the vehicle-treated group; n = 6/group; %OAE = percentage of open arm entries; % TSOA = percentage of time spent in open arm in seconds.

Table 43: Effect of piperazine analogs of 1,8-naphthyridine-3-carboxylic acids, ondansetron and diazepam on the behavior of mice in OFT

Compound	Ambulation score Dose 1 mg/kg	Rearing number Dose 1 mg/kg
NACA1	**319.00± 8.30***	14.83± 6.00
NACA 2	236.43± 3.30	08.26 ± 5.53
NACA 3	302.08± 8.39	15.17 ± 7.51
NACA 4	285.00 ± 7.36	18.17 ± 5.51
NACA 5	253.66 ± 2.35	11.17 ± 3.51
NACA 6	**280.16 ± 6.45**	**15.17± 3.11***
NACA 8	**313.58 ± 5.99***	**21.17± 7.51***
NACA 10	291.17 ± 7.80	11.17 ±7.51
NACA11	277.60 ± 5.89	05.17± 2.51
NACA13	279.30 ± 6.39	12.17 ± 7.91
Ondansetron	333.17± 25. 32	20.5± 3.31*
Diazepam	467.50 ± 15.19*	40.50 ± 1.02*
Control	276.50 ± 29.23	09.83 ± 2.18

All values are stated as mean± SEM.*p< 0.05 as compare to vehicle treated group (control); n=6/group.

Table 44: Effect of (2-alkoxy-1, 8-naphthyridin-3-yl) (4-substituted piperazin-1-yl) methanones, ondansetron and diazepam on the behavior of mice in OFT

Compound	Ambulation score (Dose 1 mg/kg)	Rearing number (Dose 1 mg/kg)
MN 1	**325.00± 4.10***	15.13±05.74
MN 5	217.00± 3.40	10.26±05.13
MN 6	277.08± 4.19	16.17±08.11
MN 7	**343.22 ± 5.36***	**28.17±03.51***
MN 8	**363.28 ± 5.34***	**30.00±02.21***
EN 5	233.16 ± 4.45	11.17±07.78
EN 7	253.26 ± 2.71	13.17±08.73
EN 8	**363.08 ± 1.69***	**31.17±0.51***
EN 10	231.66 ± 3.80	13.17 ±8.73
Ondansetron	333.17± 25. 32	20.5± 3.31*
Diazepam	467.50 ± 15.19*	40.50 ± 1.02*
Control	276.50 ± 29.23	09.83 ± 2.18

All values are expressed as mean± SEM.*p< 0.05 in comparison to vehicle treated group (control); n=6/ group.

Table 45: Effect of piperazine analogue of quinazoline 2-carboxamides, ondansetron and diazepam on the behavior of mice in OFT

Compound	Ambulation score Dose 1 mg/kg	Rearing number Dose 1 mg/kg
MQZ 4	**309.00± 03.29***	**12.35±09.47**
MQZ 5	201.41± 11.54	11.26±09.11
MQZ 6	**347.08± 03.42***	**26.17±01.11***
EQZ 4	**351.32 ± 08.31***	**32.51±07.32***
EQZ 5	263.28 ± 05.34	18.00±03.84*
EQZ 6	**303.28 ± 11.21***	**20.00±09.21***
Ondansetron	333.17± 25. 32	20.5± 03.31*
Diazepam	467.50 ± 15.19*	40.50 ± 01.02*
Control	276.50 ± 29.23	09.83 ± 02.18

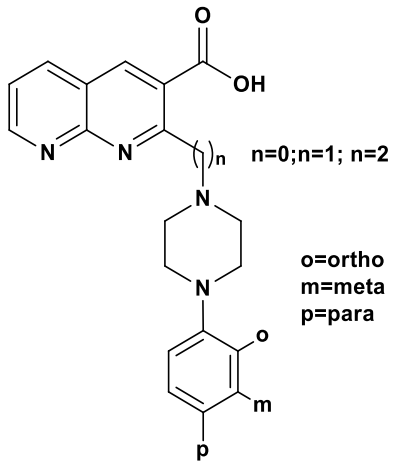
All values are expressed as mean ± SEM. *p< 0.05 in comparison to vehicle treated group (control), n=6/group.

Table 46: Effect of piperazine analogue of indole 2-carboxamides, ondansetron and diazepam on the behavior of mice in OFT

Compound	Ambulation score Dose 1 mg/kg	Rearing number Dose 1 mg/kg
NMIC 5	**318.00± 02.10***	**13.13±06.87**
NMIC 6	**342.00± 08.40***	**21.43±3.51***
NEIC 4	265.08± 08.20	11.17±07.35
NEIC 6	**350.51 ± 03.36***	**17.43±0.51***
NEIC 7	287.10± 11.58	14.32± 05.52
NBIC 1	227.10± 05.38	09.26±0.76
NBIC 6	**348.74 ± 09.34***	**20.60±02.65***
Ondansetron	333.17± 25.32	20.50± 03.31*
Diazepam	467.50 ± 15.19*	40.50 ± 01.02*
Control	276.50 ± 29.23	09.83 ± 02.18

All values are expressed as mean ± SEM. *p< 0.05 in comparison to vehicle treated group (control), n=6/group.

5.11. 5-HT$_3$ receptor anatgonism of piperazine analogs of 1,8 naphthyridine -3-carboxylic acids (Series 1):



The 5-HT$_3$ receptor antagonisms of compounds of this series are shown in **table 26**. Compound NACA 1 with a phenyl ring attached to the distal (N^4) nitrogen of piperazine (pA_2: 7.6) displayed prominent 5-HT$_3$ receptor antagonism. Incorporation of a methylene group between the N^4 of piperazine and the phenyl ring resulted in compound NACA 2 (pA_2:6.7), which showed lesser 5-HT$_3$ receptor antagonistic activity as compared to compound NACA 1 (pA_2:7.6).

In addition, the linker span between the N^4 of piperazine and the phenyl ring was increased by an additional methylene group to obtain compound NACA 3 (pA_2:6.5), the compound exhibited lesser antagonistic activity than both compound NACA 1 (pA_2:7.6) and compound NACA 2 (pA_2:6.7) as well as ondansetron.

With a view to explore the effects of different substituents on the N^4 phenyl ring and generate a SAR, a couple of analogs with electron withdrawing as well as electron donating groups attached at diverse positions in the phenyl ring were synthesized and their 5-HT$_3$ antagonistic potential examined. Incorporation of an electron withdrawing nitro group at para position of the phenyl ring, compound NACA 4 **(**pA_2: 7.2) resulted in a decrease in the antagonistic activity as compared to compound NACA 1.

Incorporation of the same electron withdrawing chloro group at dissimilar positions in the phenyl ring gave compound NACA 6 with chloro at meta position and compound NACA 7 with chloro at para position, but compound NACA 6 (pA_2: 7.3) and NACA 7 (pA_2: 6.0) exhibited diminished 5-HT$_3$ antagonistic activity than compound NACA 1 (pA_2: 7.6). When the point of attachment of chloro group was changed to ortho position to get compound NACA 5 (pA_2: 4.6), the compound showed lesser activity than it's position isomers NACA 6 and NACA 7.

Introduction of a methoxy group at para position of the phenyl ring, resulted in compound NACA 8 (pA_2: 6.6) which showed antagonistic activity comparable to ondansetron (pA_2: 6.6). When methoxy group was attached at para position of the phenyl ring to get compound NACA 10 (pA_2: 7.0), the compound showed better 5-HT_3 receptor antagonism than ondansetron (pA_2: 6.6). Though compounds NACA 8 and NACA 10 exhibited less antagonistic activity than compound NACA 1, yet these compounds retained promising 5-HT_3 receptor antagonistic activity. However, introduction of methoxy group at meta position of the phenyl ring (compound NACA 9, pA_2: 5.0) reduced the antagonistic activity.

Incorporation of weaker electron releasing methyl group instead of methoxy group in the phenyl ring resulted in a decline in antagonistic activity. The incorporation of methyl group at ortho position of the phenyl ring (compound NACA 11, pA_2: 4.4) and para position (compound NACA 12, pA_2: 5.9) caused a reduction in the 5-HT_3 receptor antagonistic activity.

When the phenyl ring at N^4 of piperazine was exchanged with an aliphatic methyl group to obtain compound NACA 13 (pA_2: 6.3), the compound exhibited lesser 5-HT_3 receptor antagonism than compound NACA 1. In addition, when higher homologation was accomplished with ethyl substituent to obtain compound NACA 14 (pA_2: 5.4) and branched homologation with isopropyl substituent to obtain compound NACA 15 (pA_2:5.2), both of these compounds displayed lesser antagonism than compound NACA 1 as well as ondansetron (pA_2: 6.6).

Among the compounds evaluated, compounds NACA 1, NACA 4, NACA 6 and NACA 10 disclosed better 5-HT_3 receptor antagonistic activity than ondansetron (pA_2 6.6), and compounds NACA 2, NACA 3, NACA 7, and NACA 8 showed moderate antagonism as compared to ondansetron.

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

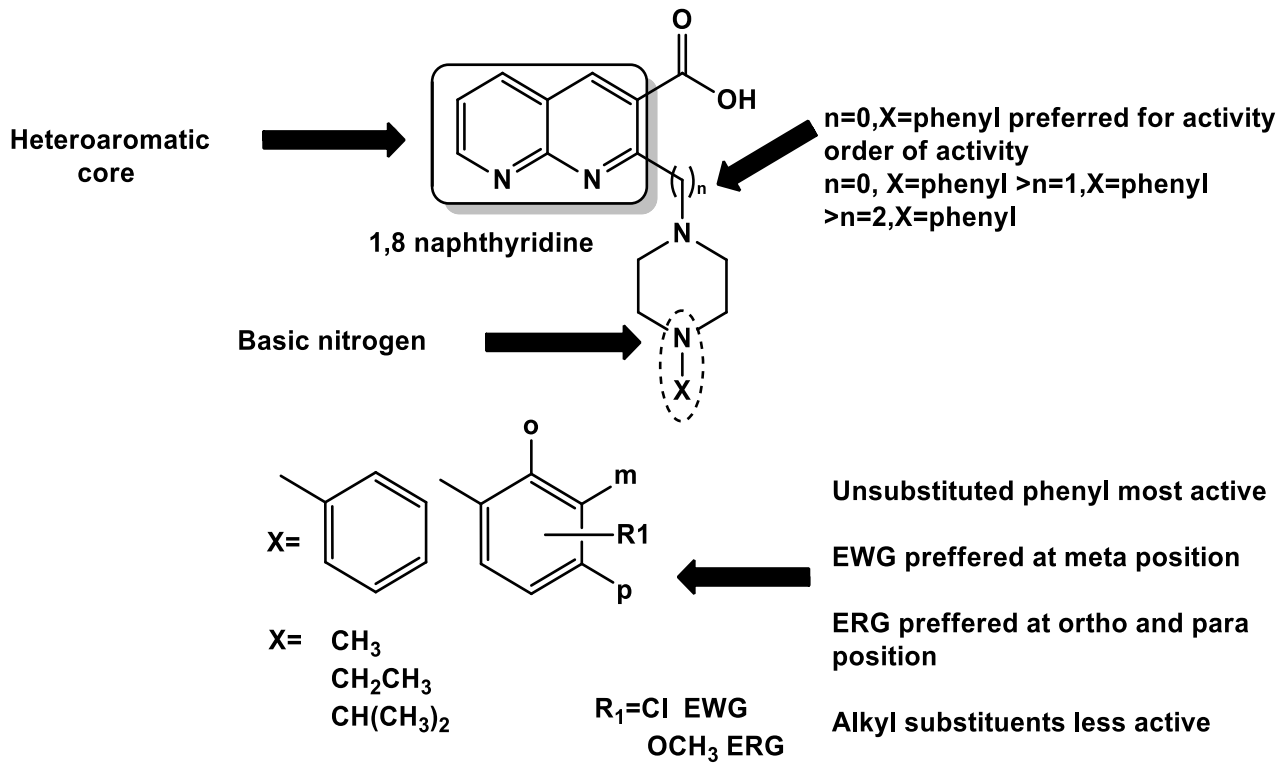
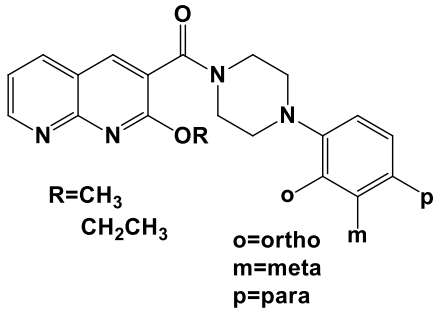


Figure 78: SAR of 5-HT_3 receptor antagonism of piperazine analogs of 1,8 naphthyridine-3-carboxylic acids (NACA 1-15; **Series 1**).

5.12. 5-HT_3 receptor anatgonism of Piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides (Series 2):



The 5-HT_3 receptor antagonistic activities of this series of compounds are shown in **table 27**. Methoxy naphthyridine compound MN 1 with a phenyl ring at the N^4 of piperazine (pA_2: 6.67) showed promising 5-HT_3 receptor antagonistic activity. Whereas ethoxy naphthyridine compound EN1, (pA_2: 5.6) with phenyl ring at the N^4 of piperazine displayed moderate antagonistic activity.

Increasing the spacer length between the distal nitrogen of piperazine and the phenyl from 0 to 1 methylene (methoxy naphthyridine compound MN 2 (pA_2: 4.67), ethoxy naphthyridine, compound EN 2 (pA_2: 5.0) led to decrease in 5-HT_3 receptor antagonism.

With a view to observe the consequence of attachment of different substituents at the phenyl ring at the N^4 of piperazineand create a structure activity relationship, a series of compounds with electron withdrawing as well as electron donating groups attached at different positions in the phenyl ring were synthesized and screened.

The presence of electron withdrawing *p*-NO_2 group in the N^4 phenyl ring of naphthyridin-3-carboxamides decreased the antagonistic activity as evidenced by methoxy analogue compound MN 3 (pA_2: 4.53) and ethoxy analogue compound EN 3 (pA_2: 5.0).

Methoxy naphthyridines compound MN 4 with chloro group at ortho position**,** and compound MN 5 with chloro at para position had the same electron withdrawing chloro group at different positions in the phenyl ring, but compound MN 4 (pA_2: 6.11) and MN 5 (pA_2: 6.30) exhibited less 5-HT_3 antagonism as compared to compound MN 1 (pA_2: 6.67). However, both compounds MN 4 and MN 5 retained promising activity.

Whereas, ethoxy naphthyridine compound EN 4 (pA_2: 5.9) with electron withdrawing *o*-Cl group showed moderate activity and compound EN 6 (pA_2: 5.3) with -Cl at *p*-position showed lesser antagonism. Both these compounds were less active than corresponding methoxy naphthyridine analogues.

However, *m*-Cl substitution in the N^4 phenyl ring appeared to be more favorable in case of both methoxy naphthyridine and ethoxy naphthyridine analogues.

When the point of attachment of chloro group was changed (chloro at *m*-position) to obtain methoxy naphthyridine; compound MN 5 (pA_2: 7.33), and ethoxy naphthyridine; compound EN 5 (pA_2: 6.2) were found to be more active than their respective position isomers MN 4, MN 6 and EN 4, EN 6.

In case of 2-methoxy naphthyridine, incorporation of a methoxy group at position 2 and position 3 of the phenyl ring resulted in compound MN 7 (pA_2: 7.67) and compound MN 8 (pA_2: 7.33), respectively. Both compounds exhibited better 5**-**HT_3 receptor antagonistic activity as compared to compound MN 1. However, introduction of methoxy group at position 4 of the phenyl ring (methoxy naphthyridine, compound MN 9 (pA_2: 5.0) decreased the 5-HT_3 antagonistic activity.

In case of 2-ethoxy naphthyridine, introduction of *o*-OCH_3 group in the N^4 phenyl ring resulted in moderate antagonistic activity as evident by compound EN 7 (pA_2: 6.6) However, ethoxy naphthyridine compound EN 8 (pA_2: 7.3) with *m*-OCH_3 group in the phenyl ring showed appreciable 5**-**HT_3 receptor antagonism. Methoxy group at para position of the phenyl ring; compound EN 9 (pA_2: 5.5) decreased the 5-HT_3 antagonistic activity.

Substitution of methoxy group with another weaker electron releasing methyl group in the phenyl ring brought a reduction in the antagonistic activity. Incorporation of methyl group at position 2, methoxy naphthyridine compound MN 10 (pA_2: 4.10) and position 4 of the phenyl ring methoxy naphthyridine compound MN 11 (pA_2: 5.56) caused a reduction in the 5-HT_3 receptor antagonistic activity.

However, ethoxy naphthyridine compound EN 10 (pA_2: 6.25) bearing *o*-CH_3 group in the N4 phenyl ring showed moderate activity. While, compound EN 11 (pA_2: 5.2) with *p*-CH_3 group in the phenyl ring showed less 5-HT_3 receptor antagonistic activity as that of methoxy analogues.

Replacement of the phenyl ring at the N^4 nitrogen of piperazine with an aliphatic methyl group in methoxy naphthyridine; compound MN 12**;** pA_2: 3.78, ethoxy naphthyridine; compound EN 12; pA_2: 3.36 resulted in the reduction of 5-HT_3 receptor antagonism as compared to the corresponding phenyl analogues.

Further incorporation of ethyl as well as propyl substituent at the N^4 of piperazine were not tolerated, as illustrated by the reduced antagonistic activities of methoxy naphthyridine compound MN 13 (pA_2: 4.40), and compound MN 14 (pA_2:4.20) respectively.

Similarly, corresponding ethoxy analogues compound EN 13 (pA_2: 3.50)**,** compound EN 14 (pA_2: 3.67) exhibited much lesser 5-HT_3 receptor antagonism as compared to compound EN 1 as well as ondansetron (pA_2: 6.60).

Methoxy naphthyridine MN 15 (pA_2: 3.67) and ethoxy naphthyridine EN 15 (pA_2: 3.21) with butyl substituent also showed less antagonistic activity.

Among the compounds screened, compounds MN 5, MN 7, MN 8 and EN 8 showed better 5-HT_3 receptor antagonistic activity and compounds MN 1, MN 4, MN 6, EN 5, EN 7 and EN 10 exhibited moderate antagonistic activity as compared to ondansetron (pA_2: 6.60).

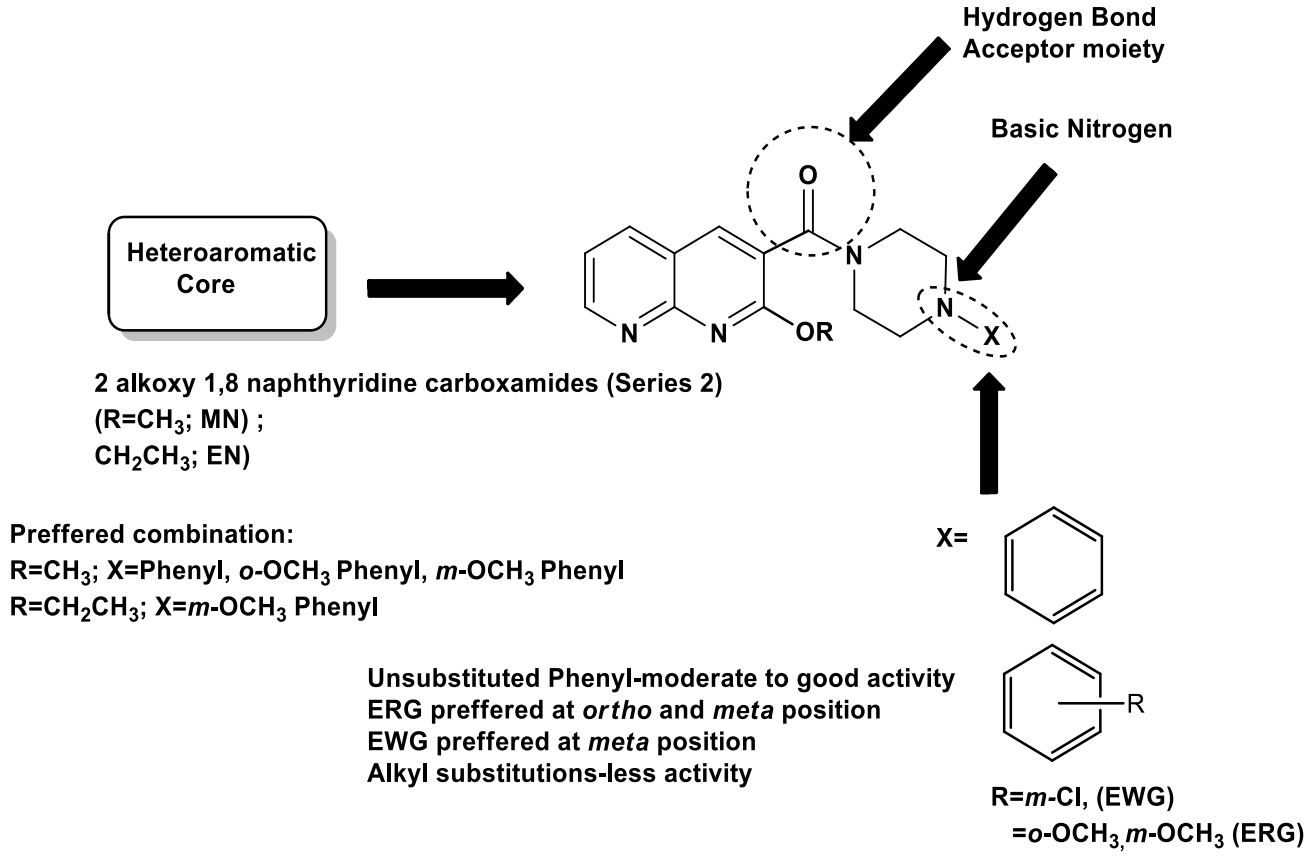
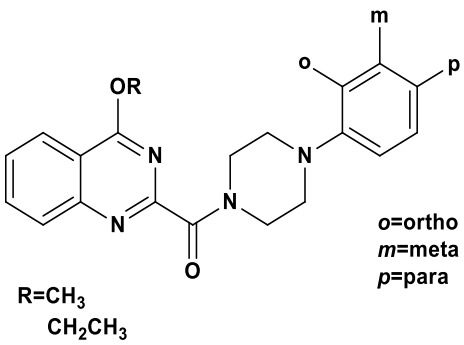


Figure 79: SAR of 5-HT_3 receptor antagonism of piperazine analogs of 2-alkoxy 1, 8 naphthyridine carboxamides (MN, EN; **Series 2**).

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

5.13. 5-HT_3 receptor anatgonism of Piperazine analogs of 4-alkoxy 1,3 quinazoline carboxamides (Series 3):



The 5-HT_3 receptor antagonism data of all the synthesized compounds are represented in **table 28**. Compounds with phenyl ring at the distal (N^4) nitrogen of piperazine, Methoxy quinazoline compound MQZ 1 (pA_2: 5.68) and ethoxy quinazoline compound EQZ 1 (pA_2: 6.00) displayed moderate 5-HT_3 receptor antagonism.

substituents on the distal nitrogen of piperazineand generate a structure activity relationship, a series of compounds were synthesized. Few Substituent patterns on the N^4 piperazine of these quinazoline carboxamides were chosen similar to that of earlier synthesized 1.8 naphthyridine carboxamides.

Methoxy quinazoline compound MQZ 2 (**-**Cl at *o*-position) (pA_2: 5.0), and compound MQZ 3 (-Cl at *m*-position) (pA_2: 5.8) showed moderate whereas MQZ 4 (-Cl at *p*-position) (pA_2: 6.1) showed better 5-HT_3 antagonistic potency than it's positional isomers.

Ethoxy quinazoline, compound EQZ 2 (pA_2: 5.43) with electron withdrawing *o*-Cl group showed moderate antagonism. Compound MQZ 3 (-Cl at *m*-position) (pA_2: 6.1) compound EQZ 4 (pA_2: 6.3) with -Cl at *p*-position showed better antagonism than it's position isomer as well as corresponding methoxy analogues.

In case of methoxy quinazoline, incorporation of methoxy group at ortho position of the phenyl ring and meta position of the phenyl ring, resulted in compound MQZ 5 (pA_2: 6.30) and compound MQZ 6 (pA_2: 6.70) respectively. Both these compounds showed better 5-HT_3 receptor antagonism than compound MQZ 1. However, introduction of methoxy group at para position of the phenyl ring (compound MQZ 7, pA_2: 5.57) decreased the 5-HT_3 antagonistic activity.

Similar results were obtained in case of ethoxy quinazoline derivatives where compound with o-methoxy phenyl substitution EQZ 5 (pA_2: 6.00), and compound with *m*-methoxy phenyl substitution EQZ 6 (pA_2: 6.75), exhibited better antagonism than the *p*-methoxyphenyl counterpart EQZ 7 (pA_2: 5.38).

Substitution of the phenyl ring at the N^4 nitrogen of piperazine with an aliphatic methyl group (methoxy quinazoline; compound MQZ 8; (pA_2: 3.5) and ethoxy quinazoline; compound EQZ 8; pA_2: 3.80), also resulted in lesser 5-HT_3 receptor antagonism as compared to the corresponding phenyl analogues.

Further incorporation of ethyl as well as propyl substituent at the N^4 of piperazine also was not tolerated, as illustrated by the reduced antagonistic activities of methoxy quinazoline compound MQZ 9 (pA_2: 3.8), compound MQZ10 (pA_2: 3.76) respectively. Similarly, corresponding ethoxy analogues compound EQZ 9 (pA_2: 3.56), compound EQZ 10 (pA_2: 3.78) exhibited much lesser 5-HT_3 receptor antagonism as compared to phenyl analogues as well as ondansetron (pA_2: 6.60).

Compounds MQZ 6, EQZ 6 showed better antagonistic activity and compounds MQZ 4, MQZ 5, EQZ 4 and EQZ 5 showed moderate activity as compared to ondansetron (pA_2: 6.60). With respect to SAR, the results demonstrated that compounds bearing methoxy group (particularly at *m* position) and chloro group (particularly at *p* position) at the phenyl ring of N^4 piperazine showed better activity than compounds with all other substituents at the N^4.

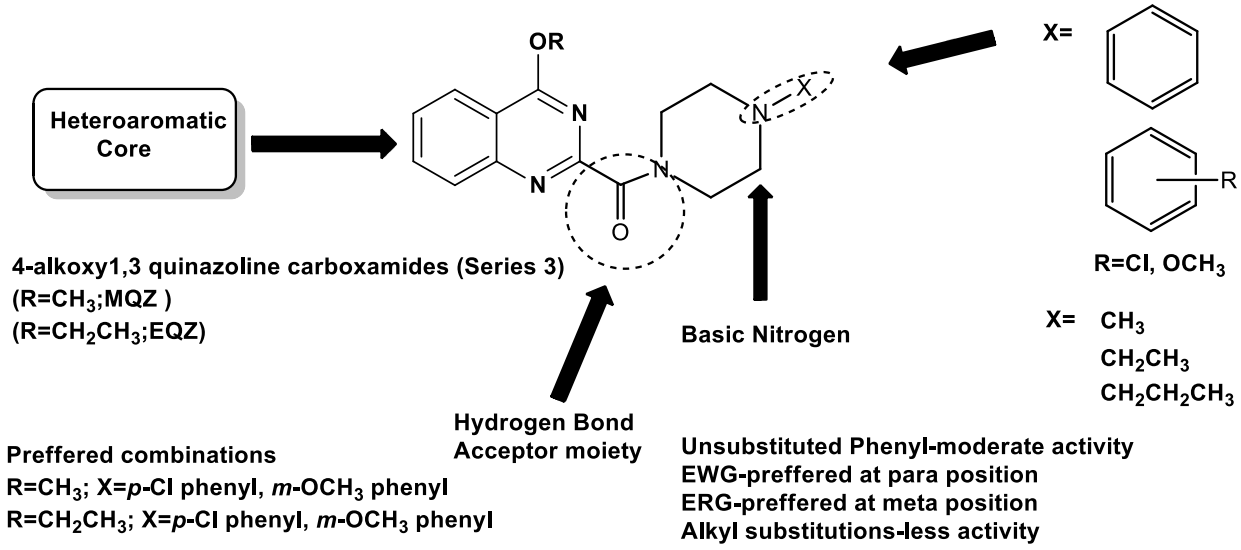
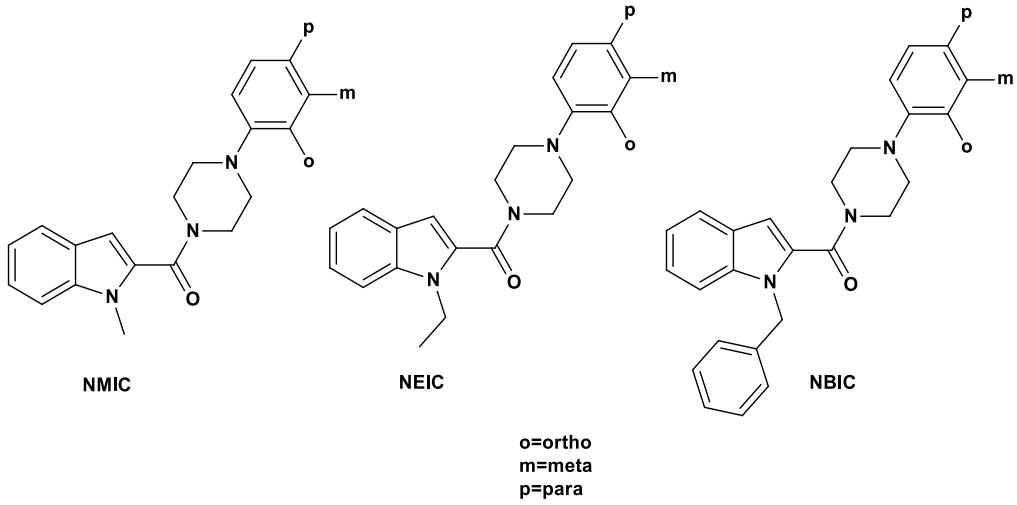


Figure 80: SAR of 5-HT_3 receptor antagonism of piperazine analogs of 4-alkoxy 1,3 quinazoline carboxamides (MQZ, EQZ; **Series 3**).

5.14. 5-HT_3 receptor anatgonism of piperazine analogs of 1-alkyl/benzyl indole carboxamides (Series 4):



The 5-HT_3 receptor antagonistic activity data of all the compounds of this series are represented in **table 29**. 1-methyl indole-2-carboxamide (compound NMIC 1, pA_2: 6.25) and N-ethyl indole-2-carboxamide compound NEIC 1, (pA_2: 6.00) with a phenyl ring at N^4 of piperazine displayed moderate antagonistic activity. Whereas, corresponding N-benzylated indole-2-carboxamide compound NBIC 1, (pA_2: 6.80) showed better antagonism than the N-methyl and N-ethyl counterparts.

Electron withdrawing as well as electron donating substituents appended at different positions on the piperazine N^4 phenyl rings of indole carboxamides was also surveyed. Few substituents (e.g., *o*-methoxyphenyl, *o*-chlorophenyl, *m*-chlorophenyl, methyl, ethyl etc) on the N^4 of the piperazine ring were kept similar to that of earlier synthesized carboxamides (1,8 naphthyridine & quinazoline) series.

In case of 1- methyl indole-2-carboxamides with respect to chloro substitution on the N^4 phenyl ring, *m*-Cl derivative NMIC 3 (pA_2: 6.6) showed better 5-HT_3 receptor antagonistic activity compared to the *o*-Cl derivative NMIC 2 (pA_2: 5.6) and *p*-Cl derivative NMIC 4 (pA_2: 5.4).

1-ethyl indole-2-carboxamides, compound NEIC 2 (pA_2: 5.5) with electron withdrawing *o*-Cl group on the N^4 phenyl ring showed lesser 5-HT_3 antagonistic activity than compound with-Cl at *p*-position NEIC 4 (pA_2: 6.5).

Similar results were observed in piperazine analogs of 1-benzyl indole-2-carboxamides (NBIC series) with respect to chloro substitution on the N^4 phenyl ring.

Wherein *o*-Cl analogue NBIC 2 (pA_2: 5.4) showed lesser antagonism than *p*-Cl analogue NBIC 4 (pA_2: 6.3) *m*-Cl substitution on the N^4 phenyl ring appeared to yield 1-ethyl indole-2-carboxamides compound NEIC 3 (pA_2: 5.0) and N-benzyl indole-2-carboxamides compound NBIC 3 (pA_2: 5.0) with lesser antagonistic activity than both their *o*-Cl and *p*-Cl counterparts NEIC 2, NEIC 4 and NBIC 2, NBIC 4 respectively.

Introduction of *o*-OCH_3 group in the N^4 phenyl ring resulted in prominent antagonistic activity as evident by 1-methyl indole-2-carboxamide NMIC 5 (pA_2: 6.6) and 1-benzyl indole-2-carboxamide 13g (pA_2: 6.0) however, the corresponding 1-ethyl analogue NEIC 5 (pA_2: 6.0) showed lesser activity.

Compounds, 1-methyl indole-2-carboxamide 13h (pA_2: 6.8), 1-ethyl indole-2-carboxamide 8g (pA_2: 7.10) and 1-benzyl indole-2-carboxamide 8h (pA_2: 7.5) with *m*-OCH_3 group on the N^4 phenyl ring showed appreciable 5-HT_3 receptor antagonism.

1-methyl indole-2-carboxamide, compound NMIC 7 (pA_2: 5.2), 1-benzyl analogue NBIC 7 (pA_2: 5.50) with *p*-OCH_3 group on the N^4 phenyl ring showed moderate antagonistic activity and the corresponding 1-ethyl analogue NEIC 7 (pA_2: 6.86) showed good antagonism.

Incorporation of aliphatic methyl group at the N^4 piperazine resulted in decreasing of 5-HT_3 receptor antagonism, as seen with 1-methyl indole-2-carboxamide NMIC 8 (pA_2: 4.0), 1-ethyl indole-2-carboxamide NEIC 8 (pA_2: 4.36) and 1-benzyl-indole-2-carboxamide NBIC 8 (pA_2: 5.0). Further incorporation of ethyl substituent at the N^4 of piperazine also was not tolerated, as illustrated by reduced antagonistic activities of 1-methyl indole-2-carboxamide compound NMIC 9 (pA_2: 4.80), 1-ethyl indole-2-carboxamide compound NEIC 9 (pA_2: 4.61) and 1-benzyl indole-2-carboxamide compound NBIC 9 (pA_2: 4.70). N^4 propyl analogues, compound NMIC 10 (pA_2: 4.50), compound NEIC 10 (pA_2: 4.67) and compound NBIC 10 (pA_2: 4.80) also turned out to be less active.

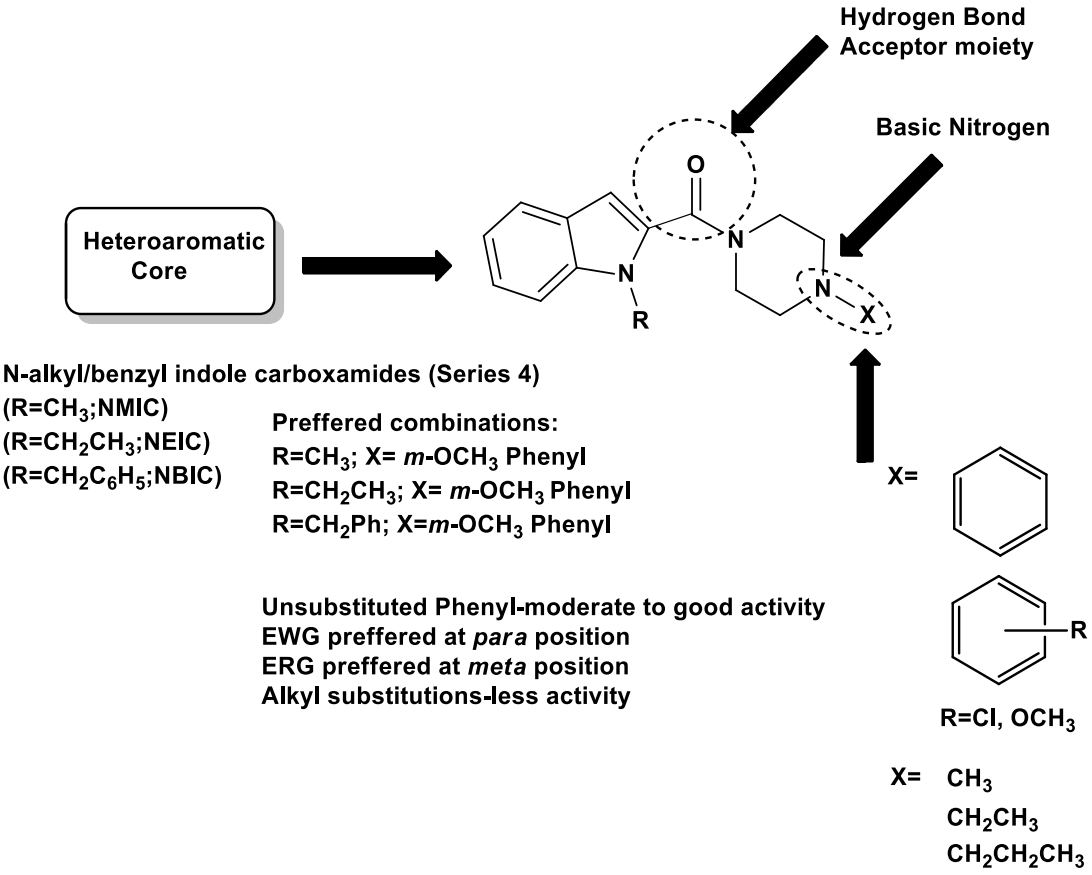


Figure 81: SAR of 5-HT_3 receptor antagonism of piperazine analogs of 1-alkyl/benzyl indole carboxamides (NMIC, NEIC, NBIC; **Series 4**).

Compounds NMIC 6, NEIC 6 and NBIC 6 shwed better antagonistic activity as compared to ondansetron, whereas compounds NMIC 1, NMIC 3, NMIC 5, NEIC 4, NEIC 5, NBIC 1, NBIC 4 and NBIC 5 showed moderate 5-HT_3 antagonistic activity. The SAR study demonstrated that regardless of the indole core, compounds bearing *o*-methoxyphenyl group and *m*-methoxy phenyl group at the N^4 piperazine showed promising 5-HT_3 receptor antagonistic activity.

5.15. Evaluation of spontaneous locomotor activity and anti-depressant potential:

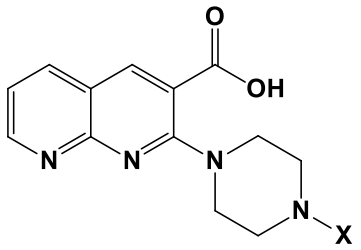
To detect the possible incidence of any drug induced stimulation/suppression in the locomotor activity of mice, which may add to their behavior in FST and TST, all the compounds were subjected to spontaneous locomotor activity study using actophotometer. Importantly, treatment with ondansetron as well as test compounds did not cause any significant change in the locomotor activity of mice as compare to the control group as depicted in Tables 30-34. Hence, the actophotometer study appear to have denied the possible occurrence of any false positive and (or) false negative result in FST and TST. Compounds which have shown higher 5-HT_3 receptor antagonism were subjected to FST and TST in mice to evaluate their anti-depressant potential. Anti-depressant activity is expressed as mean immobility time in seconds (**tables 30-34**).

5.15.1. Anti-depressant activity of piperazine analogs of 1,8 naphthyridine-3-carboxylic acid (NACA 1-15; Series 1):

In the anti-depressant screening by FST and TST, all the compounds and ondansetron were administered to mice via intra-peritoneal (*i.p.*) route at a dose of 1 mg/kg body weight of mice. The results of FST and TST are shown in **table 30**.

Treatment with ondansetron, compounds NACA 1, NACA 3, NACA 4, NACA 6, NACA 8, NACA 10 and NACA 11 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.



Compounds showed promising antidepressant activity in FST and TST

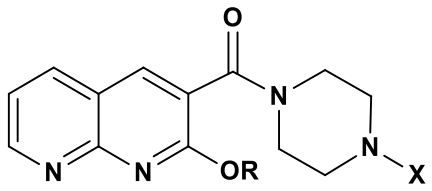
X = Phenyl
= *m*-Cl phenyl
= *o*-OCH_3 phenyl
= *p*-OCH_3 phenyl

Figure 82: SAR of anti-depressant activity of piperazine analogs of 1,8 naphthyridine-3-carboxylic acid (NACA 1-15; **Series 1**).

5.15.2. Anti-depressant activity of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides (MN1-15; EN1-15; Series 2):

Test compounds and ondansetron were administered to mice and examined in FST and TST as described earlier. The results are displayed in **table 31** and **table 32**. Treatment with ondansetron significantly decreased the duration of immobility in FST and TST. In case of 2-methoxy 1,8 naphthyridine carboxamides, treatment with compounds MN 1 and MN 4 to MN 9 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 showed promising antidepressant activity in FST and TST

R=CH_3 (MN); X= o-Cl, m-Cl and p-Cl phenyl = o-OCH_3, m-OCH_3 and *p*-OCH_3 phenyl

R=CH_2CH_3 (EN); X= o-Cl, m-Cl and p-Cl phenyl = o-OCH_3, m-OCH_3 and *p*-OCH_3 phenyl

Figure 83: SAR of anti-depressant activity of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides (MN1-15; EN1-15; **Series 2**).

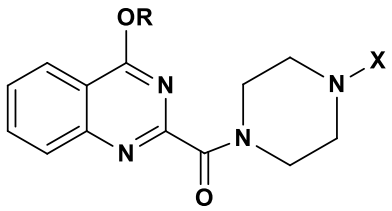
In case of 2-ethoxy 1,8 naphthyridine carboxamides, treatment with compounds EN 4 to EN 10 appeared as potential derivatives since these compounds showed significant ($p< 0.05$) reduction in the duration of immobility time as compared to control in FST and TST.

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

5.15.3. Anti-depressant activity of piperazine analogs of 4-alkoxy 1,3 quinazoline carboxamides (MQZ, EQZ; Series 3):

Test compounds and ondansetorn were administered to mice at a dose of 1 mg/kg (*i.p.*) body weight of mice and subjected to FST and TST. The results of FST and TST are shown in **table 33**. Treatment with ondansetron significantly decreased the duration of immobility in mice in FST and TST. Treatment with methoxy quinazoline carboxamides, compounds MQZ 1, MQZ 4, MQZ 5 and MQZ 6 and ethoxy quinazoline carboxamides; compounds EQZ 1, EQZ 4, EQZ 5 and EQZ 6 series showed prominent anti-depressant like activity as indicated by noteworthy ($p < 0.05$) decrease in immobility time compared to the control in FST and TST.

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



Compounds showed most promising antidepressant activity in FST and TST

$R=CH_3$ (MQZ); **X = Phenyl**
= *p*-Cl phenyl
= *o*-OCH_3, and *m*-OCH_3 phenyl

$R=CH_2CH_3$ (EQZ); **X = Phenyl**
= *p*-Cl phenyl
= *o*-OCH_3, and *m*-OCH_3 phenyl

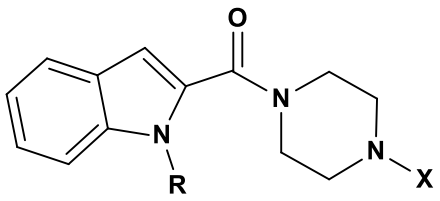
Figure 84: SAR of anti-depressant activity of piperazine analogs of 4-alkoxy 1,3 quinazoline carboxamides (MQZ, EQZ; **Series 3**).

5.15.4. Anti-depressant activity of piperazine analogs of 1-alkyl/benzyl indole carboxamides (NMIC, NEIC, NBIC; Series 4):

In case of piperazine analogs of indole carboxamides (Series 4; NMIC, NEIC, NBIC) mice were administered with synthesized compounds and subjected to FST and TST as described earlier. The results are shown in **table 34**.

Compounds NMIC 5 and NMIC 6 of N-methyl indole carboxamide series, compounds NEIC 4, NEIC 6 and NEIC 7 of N-ethyl indole carboxamide series, and compounds NBIC 1 and NBIC 6 of N-benzyl indole carboxamide series expressed prominent anti-depressant like activity as indicated by considerable ($p < 0.05$) reduction in immobility time in comparison to the control in FST and TST.

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.



N-alkyl/benzyl indole carboxamides (Series 4)

Compound showed most promising antidepressant activity in FST and TST

R=CH_3 (NMIC); X = *o*-OCH_3 phenyl
= *m*-OCH_3 phenyl

R=CH_2CH_3 (NEIC); X = *p*-Cl phenyl
= *m*-OCH_3 phenyl
= *p*-OCH_3 pheny

R=$CH_2C_6H_5$ (NBIC) X = Phenyl
= *m*-OCH_3 phenyl

Figure 85: SAR of anti-depressant activity of piperazine analogs of 1-alkyl/benzyl indole carboxamides (NMIC, NEIC, NBIC; **Series 4**).

5.16. Evaluation of anxiolytic potential:

Compounds which exhibited higher 5-HT_3 antagonistic activity and promising anti-depressant activity were selected for their anxiolytic activity screening in mouse using validated models such as L/D test, EPM and OFT.

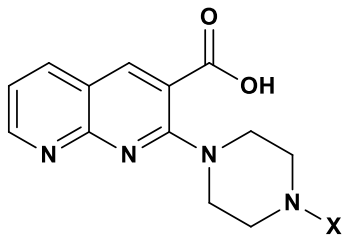
5.16.1. Anxiolytic activity of piperazine analogs of 1,8 naphthyridine-3-carboxylic acids (Series 1):

In the L/D test, compounds, ondansetron and diazepam were administered to mice intra-peritoneally (*i.p*) at a dose of 1 mg/kg, 1 mg/kg, and 2 mg/kg body weigth of mice respectively. The results of L/D test are shown in **table 35**. Diazepam and ondansetron, treatment significantly ($P < 0.05$) raised the time spent in the lit area and number of transitions as compared to vehicle control. In addition, diazepam treatment considerably ($P < 0.05$) increased the latency to enter into the dark chamber. Treatment with compounds NACA 1-NACA 6, NACA 8, NACA 9 and NACA 13 significantly ($P < 0.05$) increased the time spent in the lit area and number of transitions as compared to vehicle control.

In the EPM test, test compounds, ondansetron and diazepam were administered to mice via *i.p* route at a dose of 1 mg/kg, 1 mg/kg and 2 mg/kg body weight of mice respectively. Results of EPM test are summarized in **table 39**. Diazepam and ondansetron treatment appreciably ($P < 0.05$) raised the open arm entries, time spent in the open arm and percentage time spent in the open arm as compared to the vehicle control. Treatment with compounds NACA 1, NACA 5, NACA 6 and NACA 8 considerably ($P < 0.05$) enhanced the time spent in the open arm as compared to the control. Compound NACA 1 treatment significantly ($P < 0.05$) increased the percentage time spent in the open arm in comparison to vehicle.

In OFT, mice were administered with test compounds, ondansetron and diazepam via intra-peritoneal route at a dose of 1 mg/kg, 1 mg/kg and 2 mg/kg respectively. The results of OFT are shown in **table 43**. Treatment with diazepam considerably ($P < 0.05$) increased the ambulation scores and rearing number as compared to the vehicle control. Treatment with ondansetron, compound NACA 6 and NACA 8 appreciably ($P < 0.05$) increased the rearing number as compared to the vehicle control. Treatment with compound NACA 1 and NACA 8 significantly ($P < 0.05$) increased the ambulation scores as compared to the vehicle control.

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*-chloro (compound NACA 6) and electron releasing substituent such as ortho methoxy (compound NACA 8) groups at the N^4 phenyl ring, induced anxiolytic like activity in the L/D test, EPM test and OFT.



Compounds exhibited promising anxiolytic activity in L/D Test, EPM Test and OFT

X= Phenyl
= *m*-Cl phenyl
= *o*-OCH_3 phenyl

Figure 86: SAR of anxiolytic activity of piperazine analogs of 1,8 naphthyridine-3- carboxylic acids (**Series 1**).

5.16.2. Anxiolytic activity of piperazine analogs of 2-alkoxy 1,8 naphthyridine carboxamides (Series 2):

In the L/D test, mice were admisnistered with test compounds, ondansetron and diazepam as described earlier. The results of L/D experiment are shown in **table 36**. Diazepam and ondansetron treatment significantly ($P < 0.05$) increased the time spent in the lit area and number of transitions as compared to vehicle control. In addition, diazepam treatment considerably ($P < 0.05$) increased the latency to enter into the dark chamber. Treatment with Methoxy naphthyridines; compounds MN 7, MN 8 and ethoxy naphthyridine; EN 8 significantly ($P < 0.05$) increased the latency time to enter inside the dark chamber, time spent in the lit chamber and number of transitions between the lit and dark chambers as compared to vehicle control. Treatment with compound MN 1 significantly ($P < 0.05$) increased the time spent in the lit chamber and number of transitions between the lit and dark chambers as compared to vehicle control.

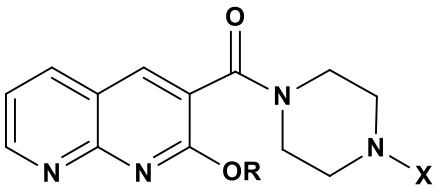
Treatment with compounds MN 5 and EN 7 significantly (P <0.05) increased the time spent in the lit chamber as compared to vehicle control.

In the EPM test, test compounds, ondansetron and diazepam were administered to mice as described earlier. Results of EPM test are shown in **table 40**. Diazepam and ondansetron treatment significantly (P <0.05) increased the open arm entries and time spent in the open arm in comparison to the vehicle control group. Treatment with compounds MN 8 and EN 8 significantly (P <0.05) increased the number of open arm entries, the percentage open arm entries, time spent in the open arm and percentage time spent in the open arm as compared to the vehicle. Treatment with compounds MN 1 and MN 7 significantly (P <0.05) increased the time spent in the open arm and percentage time spent in the open arm as compared to vehicle.

In the OFT, compounds, ondnasetron and diazepam were administered to mice as described earlier. Results of OFT are shown in **table 44**. Treatment with diazepam and ondansetron considerably (P <0.05) enhanced the ambulation scores, rearing number and rearing number respectively as compared to vehicle control.

Treatment with compounds MN 7, MN 8 and EN 8 significantly (P <0.05) raised the ambulation scores and rearing numbers as compared to vehicle control. Treatment with compound MN 1 significantly (P <0.05) increased the ambulation score as compared to the vehicle.

It was observed that, analogs that contain unsubstituted N^4 phenyl ring (compound MN 1) and analogs that contain electron releasing substituents at the N4 phenyl ring, such as *o*-methoxy (compound MN 7) and *m*-methoxy (compound MN 8 and EN 8) induced anxiolytic activity in the L/D test, EPM test and OFT.



Compounds exhibited most promising anxiolytic activity in L/D Test, EPM Test and OFT

R=CH_3 (MN); **X = Phenyl**
= *m*-OCH_3 phenyl

R=CH_2CH_3 (EN); **X = *m*-OCH_3 phenyl**

Figure 87: SAR of anxiolytic activity of piperazine analogs of 2-alkoxy1,8 naphthyridine carboxamides (**Series 2**).

5.16.3. Anxiolytic activity of piperazine analogs of 4-alkoxy1,3 quinazoline carboxamides (Series 3):

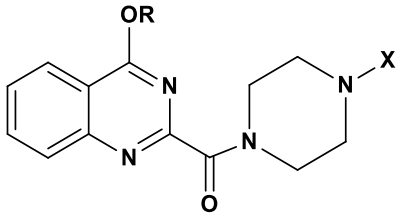
In the L/D experiment, test compounds, ondansetron and diazepam were administered to mice as stated earlier. The results of L/D test are depicted in **table 37**. Treatment with diazepam and ondansetron significantly (P <0.05) increased the time spent in the lit area and number of transitions as compared to vehicle control. In addition, diazepam treatment significantly (P <0.05) increased the latency to enter into the dark chamber. Treatment with compounds MQZ 4 and EQZ 4 significantly (P <0.05) increased the latency time to entar the dark chamber, time spent in the lit area and number of transitions as compared to vehicle control. Treatment with compounds MQZ 6 and EQZ 6 significantly (P <0.05) enhanced the time spent in the lit chamber and number of transitions as compared to vehicle control. Treatment with compounds EQZ 5 significantly (P <0.05) enhanced the number of transitions as compared to vehicle control.

In the EPM test, compounds, ondansetron and diazepam were administered to mice as described earlier. Results of EPM test are shown in **table 41**. Diazepam and ondansetron treatment significantly (P <0.05) increased the open arm entries and time spent in the open arm in comparison to the vehicle control group. Treatment with compound EQZ 4, EQZ 5 EQZ 6 significantly (P <0.05) enhanced the open arms entries and percentage open arm entries as well as time spent in the open arms and percentage time spent in the open arms. Treatment with compounds MQZ 4 and MQZ 6 significantly (P <0.05) increased the time spent in the open arms and percentage time spent in the open arm as compared to the vehicle.

In the OFT, compounds, ondnasetron and diazepam were administered to mice as described earlier. Results of OFT are shown in **table 45**. Treatment with diazepam, significantly (P <0.05) enhanced the ambulation scores and rearing numbers as compared to vehicle control.

Treatment with compounds MQZ 6, EQZ 6 and EQZ 4 significantly (P <0.05) increased the ambulation score and number of rearings as compared to vehicle control. Treatment with compound MQZ 4 increased the ambulation score as compared to vehicle control. Treatment with compounds EQZ 5 increased the rearing number as compared to vehicle control.

It was observed that, analogs that contain electron withdrawing *p*-chloro substituent (compounds MQZ 4 and EQZ 4) and electron releasing *m*-methoxy substituent (compounds MQZ 6, EQZ 6) at the N^4 phenyl ring induced anxiolytic activity in the L/D test, EPM test and OFT.



Compounds exhibited promising anxiolytic activity in L/D Test, EPM Test and OFT

R=CH_3 (MQZ); X= *p*-Cl phenyl
= *m*-OCH_3 phenyl

R=CH_2CH_3 (EQZ); X= *p*-Cl phenyl
= *m*-OCH_3phenyl

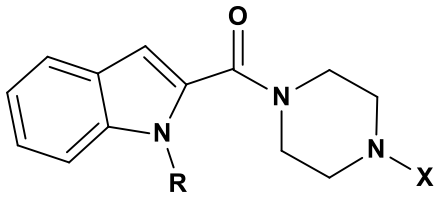
Figure 88: SAR of anxiolytic activity of piperazine analogs of 4-alkoxy1, 3 quinazoline carboxamides (**Series 3**).

5.16.4. Anxiolytic activity of piperazine analogs of 1-alkyl/benzyl indole carboxamides (Series 4):

In the L/D experiment, test compounds, ondansetron and diazepam were administered to mice as stated earlier. The results are shown in **table 38**. Treatment with diazepam and ondansetron significantly ($P < 0.05$) increased the time spent in the lit area and number of transitions as compared to vehicle control. In addition, diazepam treatment considerably ($P < 0.05$) enhanced the latency to enter into the dark chamber. Treatment with compounds NMIC 5, NEIC 6, NEIC 7 and NBIC 6 significantly ($P < 0.05$) increased the latency to enter into the dark chamber, increased the time spent in the lit area and number of transitions as compared to vehicle control. Treatment with compound NMIC 6 significantly ($P < 0.05$) increased the time spent in the lit area as compared to vehicle control.

In the EPM test, compounds, ondansetron and diazepam were administered to mice as described earlier. Results of EPM test are summarized in **table 42**. Treatment with diazepam and ondansetron significantly ($P < 0.05$) enhanced the open arm entries and time spent in the open arms as compared to the vehicle control. Treatment with compounds NMIC 5, NMIC 6,NEIC 6 and NBIC 6 significantly ($P < 0.05$) increased the number of open arm entries, percentage open arm entries, time spent in the open arm and percentage time spent in open arms as compared to the vehicle.

In the OFT, test compounds, ondnasetron and diazepam were administered to mice as described earlier. Results of OFT are shown in **table 46**. Treatment with diazepam considerably ($P < 0.05$) increased the ambulation scores and rearing number as compared to the vehicle control. Treatment with compounds NMIC 6, NEIC 6 and NBIC 6 significantly ($P < 0.05$) enhanced the ambulation scores and number of rearing as compared to the vehicle control. Treatment with compound NMIC 5 significantly increased the ambulation score as compared to the control.



Compounds exhibited promising anxiolytic activity in L/D Test, EPM Test and OFT

R=CH_3 (NMIC); **X= *o*-OCH_3 phenyl**
***m*-OCH_3 phenyl**

R=CH_2CH_3 (NEIC); **X= *m*-OCH_3 phenyl**

R=$CH_2C_6H_5$ (NBIC); **X= *m*-OCH_3 phenyl**

Figure 89: SAR of anxiolytic activity of piperazine analogs of 1-alkyl/benzyl indole carboxamides (**Series 4**).

It was found that analogs that contain electron releasing substituent at the N^4 phenyl ring, such as *o*-methoxy (compound NMIC 5), *m*-methoxy (NMIC 6, NEIC 6 and NBIC 6) compound and *p*-methoxy (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* methoxy phenyl substituent at the N^4 piperazine displayed significant anxiolytic activity in all the three tests.

6. Discussion:

In the present study, NCEs were designed based on two pharmacophoric templates for 5-HT_3 receptor antagonists. Initially 5-HT_3 receptor antagonism of all the synthesized NCEs were evaluated using Guinea pig ileum LMPP assay, subsequently compounds which exhibited significant pA_2 values were assessed for anti-depressant as well as anxiolytic potential.

The results of the present study indicate the AD-like and anxiolytic activity of the synthesized NCEs as 5-HT_3 receptor antagonists in the acute rodent models that predicts the efficacy of AD drugs and anxiolytic drugs. Acute treatment with the NCEs exhibited AD-like effects in the FST, TST and anxiolytic-like effect in EPM test, L/D test and OFT at selected doses.

6.1 Anti-depressant Potential of NCEs as 5-HT_3 receptor antagonists:

The psychomotor stimulation or sedation characteristic of a test drug imitates AD/depressant-like behavioral consequence of rodents in FST and TST (Porsolt et al., 1977; Steru et al., 1985). Hence, the affect of test compounds on the locomotor activity of animal is a leading apprehension.

In order to ascertain that non-specific locomotor effects donot impact the activity of test compounds in FST and TST, locomotor activities of all the NCEs and ondansetron were evaluated in mice. Interestingly, all the NCEs as well as ondansetron did not affect the basal locomotor scores as compared to normal control (mice) in SLA test at selected dose. All the NCEs were evaluated for their AD and anxiolytic-like potential following dose selection, as per findings from SLA test.

The preliminary AD-like effects of test drugs were evaluated in the FST, a behavioral despair test. Since 1977, when the original report by Porsolt and Colleagues was published, the FST has been widely used for assessing the effectiveness of candidate ADs, as well as to investigate the underlying mechanisms of action of ADs (Porsolt et al., 1977). Porsolt and Colleagues (1977) have been shown that decrease in the duration of immobility in FST, reflects the AD-like potential of molecules.

Effects of NCEs on depression-like behavior were also investigated using TST, another behavioral despair model of depression. TST is a well established screening paradigm for AD/depressant-like effect of molecules.

The test involves placing an animal in inescapable situation where initial attempts to escape are followed by prolonged duration of immobility. Decrease in the duration of immobility in TST is an indicative of AD-like effect (Steru et al., 1985; Lucki, 1997). The results of FST and TST strongly support the anti-depressant potential of NCEs as 5-HT_3 receptor antagonists. Moreover, the AD-like effects of NCEs in the FST and TST were not due to hyper-locomotive effects as indicated by the SLA test.

6.2. Anxiolytic Potential of NCEs as 5-HT_3 receptor antagonists:

The present behavioral investigation with various animal models of anxiety revealed the anxiolytic like effect of NCEs as 5-HT_3 receptor antagonists. The putative anxiolytic activities of the synthesized compounds were determined by L/D test, EPM test and OFT. The time spent in the lit chamber and in the open arm was measured during the L/D test and EPM test respectively, whereas the ambulation score and rearing frequency were recorded in the OFT. All these models of anxiety are quite sensitive and relatively specific to anxiolytics and have been widely used to screen NCEs for their anxiolytic potential (Borsini et al., 1989; Costall et al., 1989, 1993; Kilfoil et al., 1989; Barnes et al., 1992; Hogg, 1996).

The L/D experiment is based on the aversive property of light, which is a tendancy of rodent to avoid brightly lit area (Angelis and Furlan, 2000; Belzuing et al., 1987). The L/D experiment utilizes the time spent in the lit chamber as its indicator of anxiety as opposed to the time spent in the dark chamber. The data from this study is consistent with previous studies (Young and Johnson, 1991) showing that the best method to determine the consequence of treatment of an anxiolytic agent in mice is the time spent in the lit area, while reduction in time spent in the lit chamber with decrease in the number of moves between light and dark area are features of anxiogenic reaction (Lal and Emmett-Oglesby, 1983; De Angelis, 1992).

EPM is considered as a well established model of unconditioned anxiety for detecting anxiolytic/anxiogenic-like activity. In the test, increase in number of entries and time spent in the open arms of the maze are the most reliable indicators of decrease in anxiety or anxiolytic-like activity of a compound, while anxiogenic substances have the opposite effect (Fernandes and File, 1996; Dubinsky et al., 2002; Sienkiewicz-Jarosz et al., 2003).

In this study, treatment with few of the NCEs produced anxiolytic-like effects in the EPM test, as evidenced by a significant increase in both open-arm entries and time spent in open arms, as compared with the vehicle control. These results are in agreement with the earlier studies that suggest 5-HT_3 receptor antagonists enhanced the number of entries and time spent in the open arms, and exhibited anxiolytic activity in the EPM test (Blackburn et al., 1993; Artaiz et al., 1995; Hewlett et al., 2001; Zhang et al., 2001). In addition, diazepam was used as a standard reference, and it also showed an anxiolytic effect in the EPM test.

The anxiolytic-like effect of the synthesized NCEs was further confirmed using OFT. The OFT is widely used for the screening of anxiolytic/anxiogenic drugs. Normal aversion of a rodent to brightly lit open area produces anxiety and fear, which is characterized by alteration in the behavioral parameters of the animal in the open field. Previous reports have suggested that anxiolytic compounds have a tendency to reduce the fearful behaviors of rodents in the OFT (Mechan et al., 2002).

In conclusion, the results from this study demonstrate anti-depressant and anxiolytic activity of some NCEs as 5-HT_3 receptor antagonists in validated animal models of depression and anxiety. The precise mechanism behind the anti-depressant and anxiolytic activity of these NCEs remains to be fully explored. However, an increase in the level of serotonin through blockade of the 5-HT_3 receptor (Schafer, 1999) could possibly contribute to the anti-depressant as well as anxiolytic effect of these compounds (Greenshaw, 1992). The 5-HT_3 receptor located within a neuro-anatomical region might be indirectly regulating the 5-HT transmission (Kilpatrick et al., 1989). The blockade of 5-HT_3 receptors facilitates 5 HT neurotransmission (Rajkumar and Mahesh, 2010). 5-HT_3 receptor antagonists block the postsynaptic 5-HT_3 receptors that generate a fast excitatory potential in the limbic regions of brain (Sugita et al., 1992). Although the flow of cellular events that occur after this fast transmission blockade is still unclear, on the whole, anti-depressant as well as anti-anxiety-like behaviour is plausible (Rajkumar and Mahesh, 2010). Therefore, the current study reiterates the development of 5-HT_3 receptor antagonists as a possible alternative for the treatment of depression and anxiety disorders. Hopefully, future studies with molecular techniques will help in better understanding of the possible mechanism underlying the anti-depressant and anxiolytic like effect of these NCEs at the cellular level, which may lead to agents that are useful for controlling co-morbid disorders like depression and anxiety.

Chapter 6: Summary and Conclusions

7. Summary and conclusions:

In the present study, NCEs were designed as 5-HT_3 receptor antagonists based on two pharmacophoric templates. 5-HT_3 receptor antagonism of all the synthesized compounds were evaluated using Guinea pig ileum LMMP assay and those which exhibited significant pA_2 values were subsequently assessed for anti-depressant as well as anxiolytic potential.

Piperazine analogs of naphthyridine carboxylic acids (series 1) were designed based on the aryl piperazine pharmacophore, synthesized and screened. SAR study showed that compounds bearing unsubstituted phenyl (NACA 1), *p*-methoxy phenyl (NACA 10) and *m*-chlorophenyl (NACA 6) substituent at the N^4 piperazine were found to be most promising compounds. In general, replacement of the phenyl ring and/or substituted phenyl ring with aliphatic alkyl group resulted in lesser antagonistic activity.

Piperazine analogs of naphthyridine (series 2), quinazoline (series 3) and indole (series 4) carboxamides with three different hetero aromatic cores were designed based on the three point pharmacophore for 5-HT_3 anatgonists, synthesized and screened.

SAR study indicated that in case of 1,8 naphthyridine carboxamides compounds bearing *o* and *m*-methoxyphenyl (MN 7, EN 7, MN 8, EN 8) substituents at the N^4 piperazine were found to exhibit promising activity. Similar results were obtained with indole (NMIC 5, NMIC 6, NEIC 6, NBIC 6) and 1, 3 quinazoline counterparts (MQZ 5, MQZ 6, EQZ 5, EQZ 6). Compounds bearing m-chlorophenyl substituents (1,8 naphthyridine carboxamides; MN 5 and EN 5) and p-chlorophenyl substituent (quinazoline carboxamides ;MQZ 4, EQZ 4 and indole carboxamides; NEIC 4 and NBIC 4) also showed promising acivity.

In general, replacement of the phenyl ring and/or substituted phenyl ring with aliphatic alkyl group resulted in lesser antagonistic activity. However, indole-2-carboxamides were found to be comparatively more tolerant of aliphatic substitutions at the N^4 nitrogen than those of their 1, 8 naphthyridine and 1, 3 quinazoline counterparts.

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.

Basic moiety of the pharmacophore for all the synthesized compounds is associated with the of N^4 nitrogen of piperazine ring. The substituents at N^4 are responsible for changes in the basicity of the N^4 nitrogen. In case of all the synthesized compounds, basicity of the nitrogen changes with substitutions attached to the N^4 of the piperazine. 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 substituent. Whereas, basicity of the N^4 nitrogen of the piperazine is comparatively less when it has phenyl and or substituted phenyl substituent attached to it. The results indicated that increasing the basicity on the N^4 nitrogen of the piperazine (with alkyl substituents, such as methyl, ethyl propy, butyl) reduced the of 5-HT_3 receptor antagonism.

Hence, it is hypothesized that, 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. In general, compounds bearing *o* and *m*-methoxyphenyl and *m* and *p*-chlorophenyl substituents at the N^4 piperazine showed promising anti-depressant and anxiolytic activity. The combined SAR of all four series of compounds with respect to 5-HT_3 receptor antagonism as well as antidepressant and anxiolytic activity was derived which is shown below (**figure 90**).The results of the present study indicate the AD-like and anxiolytic activity of the synthesized NCEs as 5-HT_3 receptor antagonists in the acute rodent models. Acute treatment with the NCEs exhibited AD-like effects in the FST and TST and anxiolytic-like effect in EPM, L/D and OFT at selected dose.

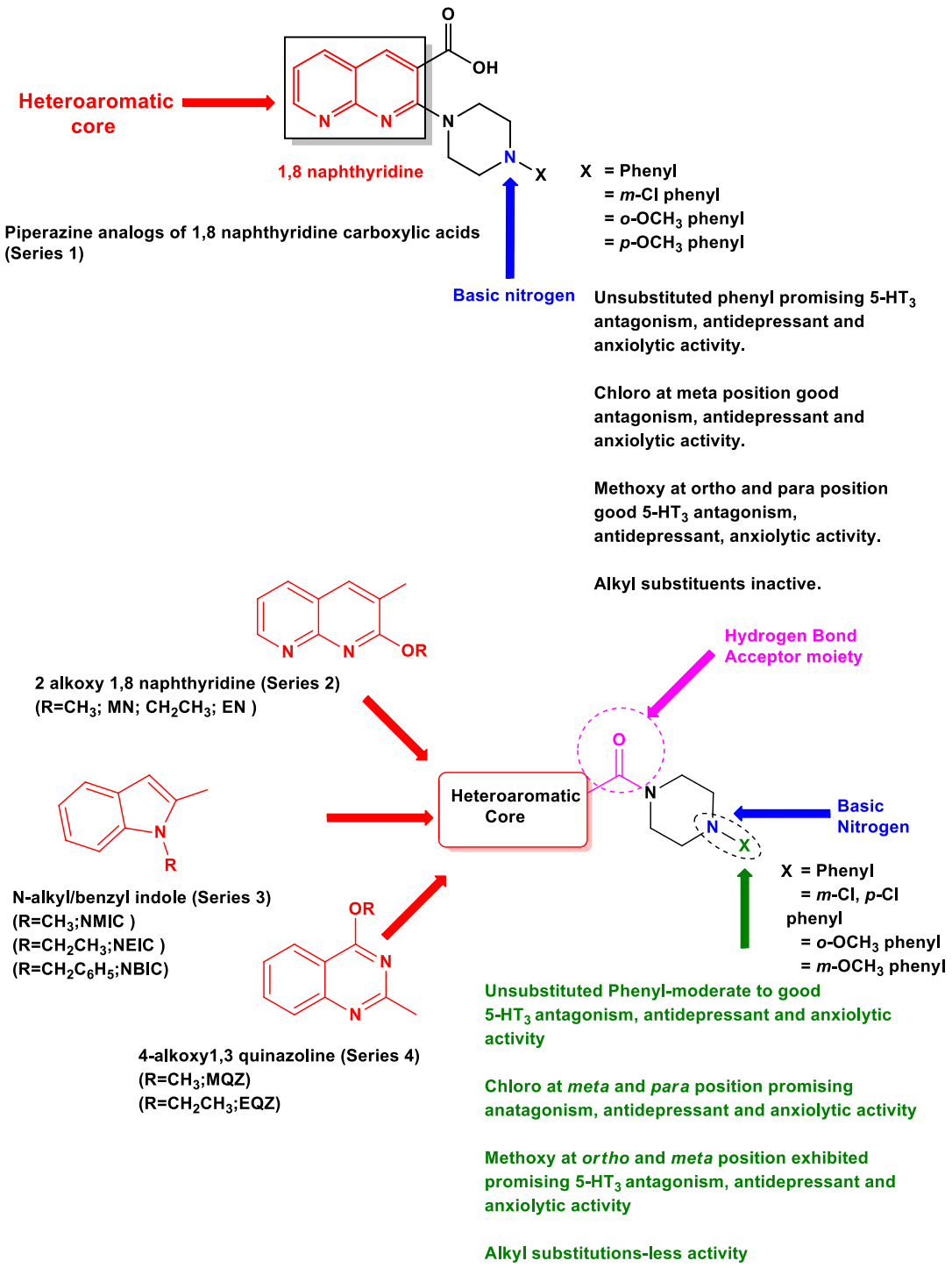


Figure 90: Combined SAR of 5-HT_3 anatgonism, antidepressant and anxiolytic activity of piperazine analogs of 1,8 naphthyridine carboxylic acids (**Series 1**) and piperazine analogs of naphthyridine, quinazoline and indole carboxamides (**Series 2, 3** and **4**).

Chapter 7: Salient Findings and Future Scopes

8. Salient Findings form the study and future scopes:

- It was observed that within three series of carboxamides, the 5-HT_3 receptor antagonisms of most of the compounds were more influenced by the nature of the N^4 substituent of the piperazine than change in the hetero aromatic core. However, few naphthyridine 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 a hydrogen bond acceptor moiety, heteroaromatic core and basic centre (substitution at the N^4 nitrogen of piperazine) of the pharmacophore, produces promising antagonistic activity.

- Investigations of anti-depressant and anxiolytic potential of these NCEs as 5-HT_3 receptor antagonists were performed. With few exceptions in general, compounds with higher pA_2 values exhibited significant anti-depressant and anxiolytic effects as compared to the vehicle treated group.

- Compounds NACA 1, NACA 3, NACA 4, NACA 6, NACA 8, NACA 9 and NACA 12 of piperazine analogs of 1,8 naphthyridine carboxylic acids (series 1) showed significant ($p< 0.05$) anti-depressant activity and compounds NACA 1, NACA 5, NACA 6, NACA 8 of the same series also showed significant ($p< 0.05$) anxiolytic activity as compared to the vehicle control.

- Compounds MN1, MN4, MN7, MN 8, MN 9 of methoxy naphthyridine carboxamide series and EN 7, EN 8, EN 9, EN 10 of ethoxy naphthyridine carboxamide series, compounds MQZ 4 MQZ 5, MQZ 6 and EQZ 4, EQZ 5, EQZ 6 of quinazoline carboxamide series and compounds NMIC 5, NMIC 6, NEIC 4, NEIC 6, NEIC 7, NBIC 1, and NBIC 6 from indole carboxamide series exhibited significant ($P < 0.05$) anti-depressant activity as compared to the vehicle control. Compounds MN 7, MN 8, EN 8 of naphthyridine carboxamide series, compounds MQZ 4, MQZ 6, EQZ 4, EQZ 5, of quinazoline carboxamide series, and compounds NMIC 5, NMIC 6, NEIC 6, NEIC 7 and NBIC 6 of indole carboxamide series exhibited significant ($P < 0.05$) anxiolytic activity as compared to the vehicle treated group.

- Compounds NACA 1, NACA 6, NACA 8 of naphthyridine carboxylic acid series, compounds MN 7, MN 8, EN 8 of naphthyridine carboxamide series, compounds MQZ 4, EQZ 4 of quinazoline carboxamide series and compounds NMIC 6, NEIC 6 and NBIC 6 of indole carboxamide series exhibited higher pA_2 values and significant anti-depressant as well as anxiolytic activity. Among all series of compounds (95) synthesized, the following were found to be most promising which could be used for further investigations.

Sl No	**Code/Series**	**5-HT$_3$ receptor antagonism p$A_2 \geq 6.6$**	**Significant antidepressant activity (p<0.05) in**		**Significant anxiolytic activity (p<0.05) in**		
			FST	**TST**	**L/D**	**EPM**	**OFT**
1	NACA 1 (Series 1)	√	√	√	√	√	√
2	NACA 6 (Series 1)	√	√	√	√	√	√
3	NACA 8 (Series 1)	√	√	√	√	√	√
4	MN 1 (Series 2)	√	√	√	√	√	√
5	MN 5 (Series 2)	√	√	√	√	--	--
6	MN 1 (Series 2)	√	√	√	√	√	√
7	MN 7 (Series 2)	√	√	√	√	--	√
8	MN 8 (Series 2)	√	√	√	√	√	√
9	EN 7 (Series 2)	√	√	√	√	--	--
10	EN 8 (Series 2)	√	√	√	√	√	√

11	MQZ 6 (Series 3)	√	√	√	√	--	√
12	EQZ 6 (Series 3)	√	√	√	√	--	--
13	NMIC 5 (Series 4)	√	√	√	√	√	√
14	NMIC 6 (Series 4)	√	√	√	√	√	√
15	NEIC6 (Series 4)	√	√	√	√	√	√
16	NEIC7 (Series 4)	√	√	√	√	--	--
17	NBIC1 (Series 4)	√	√	√	√	--	--
18	NBIC6 (Series 4)	√	√	√	√	√	√

Among 95 compounds synthesized compounds NACA 1, NACA 6, NACA 8 from series 1, compounds MN 8, EN 8 from series 2, MQZ 6 and EQZ 6 from series 3 and compound NBIC 6 from series 4 were found to be most promising compounds.

8.1. Future scope of the study:

- Competitive binding assay and cloned receptor study of the synthesized compounds, which would provide considerable amount of information regarding the selectivity towards 5-HT_3 receptor over other serotonergic receptors.
- Acute and chronic toxicity studies can be conducted to observe the safety profile, as an extension of the work on most promising compounds.
- Pharmacokinetic study of the compounds having good safety margin can be done.
- Further, molecular biology investigations are required to strengthen the proposed hypothesis, such as role of HPA axis, neurotrophic factor, monoamine system, oxidant/anti-oxidant system and their integration with each other in the development of co-morbid depression and anxiety.
- Further study may be undertaken to assess the effectiveness of some of the most promising compounds of the present study in CORT, TBI, OBX and CUMS models.

Chapter 8: References

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Appendix

Publications from thesis

Dhar A.K, Mahesh R, Jindal A, Devadoss T, Bhatt S. Design, synthesis and pharmacological evaluation of novel 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids as 5-HT(3) receptor antagonists for the management of depression. ***Chemical Biology & Drug Design,*** 2014,84(6):721-31.

Mahesh R, **Dhar A.K**, Jindal A, Bhatt S. Design, synthesis and evaluation of anti depressant activity of novel 2-methoxy 1, 8 naphthyridine 3-carboxamides as 5-HT(3) receptor antagonists. ***Chemical Biology & Drug Design***, 2014; 83, 583-591.

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Gautam BK, Jindal A, **Dhar A.K**, Mahesh R. Anti-depressant - Like Activity of 2-(4-phenylpiperazin-1-yl)-1, 8-naphthyridine-3-carboxylic acid (7a), a 5-HT(3) Receptor Antagonist in Behaviour Based Rodent Models: Evidence for the Involvement of Serotonergic System. ***Pharmacology Biochemistry Behaviour***, 2013; 109, 91–97.

Dhar A.K, Mahesh R, Jindal A, Bhatt S. piperazine analogues of naphthyridine-3-carboxamides and indole-2-carboxamides: Novel 5-HT_3 receptor antagonists with anti depressant like activity. ***Archiv der pharmazie.*** (Accepted, In press).

Dhar A.K, Mahesh R, Jindal A, Bhatt S. Evaluation of anti-depressant and anxiolytic activity of some Piperazine analogues of indole-2-carboxamides as Novel 5-HT3 receptor antagonists ***Journal of enzyme inhibition and medicinal chemistry***. (Under review)

Other Publications

Mahesh R., **Dhar A.K**, Sasank T.v.n.v., T., Thirunavukkarasu S., Devadoss T. Citric acid: An efficient and green catalyst for rapid one pot synthesis of quinoxaline derivatives at room temperature.Chinese Chemical Letters. 2011, 22(4): 389–392.

Mahesh R, Devadoss T, **Dhar A.K**, Venkatesh SM, Mundra S, Pandey DK, Bhatt S, Jindal AK. Ligand-Based Design, Synthesis, and Pharmacological Evaluation of 3-Methoxyquinoxalin-2-carboxamides as Structurally Novel Serotonin Type-3 Receptor Antagonists. ***Arch Pharm***, 2012; 345(9), 687-694.

Bhatt S, Mahesh R, Jindal A, Devadoss T, **Dhar A.K.** Neuropharmacological evaluation of a novel 5-HT3 receptor antagonist (6g) on chronic unpredicTable mild stress-induced changes in behavioural and brain oxidative stress parameters in mice. ***Indian Journal of Pharmacology***, 2014; 46, 191-196.

Mahesh R, Bhatt S, Devadoss T, Jindal A, Gautam B.K , **Dhar A.K**, Pandey D.K, Anti-depressant - Like Effect of Novel 5-HT Receptor Antagonist, (4-benzylpiperazin-1-yl) (3-methoxyquinoxalin-2-yl)methanone (6g) in Acute and Chronic Animal Models of Depression. ***Indian Journal of Pharmaceutical Education and Research***. 2013, 47(1): 71-81.

CONFERENCE PRESENTATION

Mahesh R, **Dhar A.K**, **Jindal A**, Bhatt S. 2-(4-substitutedpiperazin-1-yl)-1,8-naphthyridine-3-carboxylic acids: novel 5-HT3 receptor antagonists as potential anxiolytics. (**International Conference on Recent Advances in Molecular Mechanisms of Neurological Disorders, 21-23rd February 2013, AIIMS New Delhi).**

Mahesh R, **Dhar A.K**, Jindal A, Muthu VS, Mundra S. Design, synthesis and pharmacological evaluation of Novel 2-(4-substituted piperazine 1-yl)-1,8-naphthyridine 3-carboxylic acids as 5-HT3 receptor antagonists for the management of depression. **(Association of Pharmaceutical Teachers in India Conference, 17-18th March 2012, Manipal)**

Mahesh R, **Dhar A.K,** Singh S, Jindal A, Sharma A, Bhatt S. Evaluation of antianxiety like activity of 2-(-4-phenylpiperazine-1-yl) 1.8-Naphthridine-3-carboxylic acid(7a) A serotonin type-3(5-HT3) receptor antagonist: An investigation using behavioural test battery of anxiety. (**Indian Pharmacological Society Conference 19-21th December 2011, Manipal).**

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