Design, Synthesis and Pharmacological Evaluation of Novel Serotoninergic 5-HT_3 Receptor Antagonists as Potential Agents for the Treatment of Cancer Chemotherapy-Induced Emesis

THESIS

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

by

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Under the Supervision of

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Dedicated

to my

Mother

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN) INDIA

CERTIFICATE

This is to certify that the thesis entitled "**Design, Synthesis and Pharmacological Evaluation of Novel Serotoninergic 5-HT_3 Receptor Antagonists as Potential Agents for the Treatment of Cancer Chemotherapy-Induced Emesis**" and submitted by **Venkatesha Perumal R., ID.No. 2001PHXF021**, for the award of Ph.D. degree of the Institute, embodies the original work done by him under my supervision.

Signature in full of the supervisor

Name in capital block letters: **Dr. R. MAHESH**

Date: 6/6/05

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R. Venu

Venkatesha Perumal R.

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List of Abbreviations / Symbols

^{1}H-NMR	Proton nuclear magnetic resonance
2-Me-5-HT	2-Methyl-5-hydroxytryptamine
5-CT	5-Carboxamidotryptamine
5-HIAA	5-Hydroxyindoleacetic acid
5-HT	5-Hydroxytryptamine
8-OH DPAT	8-Hydroxy-2-(dipropylamino)tetralin
Å	Angstrom (10^{-10} meter)
Ach	Acetyl choline
ANOVA	Analysis of variance
ANV	Anticipatory nausea and vomiting
AP	Area postrema
BJ	Benzold Jarisch
br s	Broad singlet
CINV	Chemotherapy-induced nausea and vomiting
CNS	Central nervous system
CoMFA	Comparative molecular field analysis
CSF	Cerebrospinal fluid
CTZ	Chemoreceptor trigger zone
d	Doublet
dd	Doublet of doublet
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulphoxide
DOI	1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane
EPM	Elevated plus-maze
fmol	Femtomole (10^{-15} M)
GABA	Gamma aminobutyric acid
GIT	Gastro intestinal tract
GPCR	G-protein coupled receptor

hr	Hour
i.c.v.	Intra cerebroventricular
i.p.	Intraperitoneal
i.v.	Intravenous
IBS	Irritable bowel syndrome
IR	Infra red
K_d	Equilibrium constant for dissociation
kg	Kilogram
K_i	Dissociation constant of an inhibitor
LGIC	Ligand gated ion channel
LMMP	Longitudinal muscle myenteric plexus
LSD	Lysergic acid diethylamide
m	Multiplet
M	Mole / molar
m.m.p.	Mixed melting point
m.p.	Melting point
m/z	Mass/charge
MAO	Mono amino oxidase
m-CPBG	1-(*m*-Chlorophenyl)-biguanide
mCPP	1-(3-Chlorophenyl)piperazine
mg	Milligram
min	Minute
MS	Mass spectra
MWI	Microwave irradiation
N&V	Nausea and vomiting
NK	Neurokinin
nM	Nanomole (10^{-9} M)
NMQ	*N*-Methylquipazine
NTS	Nucleus tractus solitarius

p.o.	Per oral
PONV	Post-operative nausea and vomiting
ppm	Parts per million
q	Quartet
RAs	Receptor antagonists
RMS	Root mean square
s	Singlet
s.c.	Subcutaneous
SAR	Structure Activity Relationship
SEM	Standard error of the mean
SI	Social interaction
SP	Substance P
t	Triplet
TEA	Triethylamine
TLC	Thin layer chromotography
TMS	Tetra methyl silane
VC	Vomiting centre
µM	Micromole (10^{-6} M)

Compound Index

AS 8112	(*R*)-5-Bromo-*N*-(1-ethyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxy-6-methylamino-3-pyridinecarboxamide
BRL 15572	3-[4-(4-Chlorophenyl)piperazine-1-yl]-1,1-diphenyl-2-propanol
BRL 24682	4-Amino-3-chloro-*N*-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)benzamide
BRL 24682	(2α,6β,9aα)-4-Amino-5-chloro-2-methoxy-*N*-tropanylbenzamide
BRL 25594	4-Amino-*N*-(8-benzyl-8-azabicyclo[3.2.1]oct-3-yl)-3-chlorobenzamide
BRL 43694	1-Methyl-*N*-(endo-9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide (Granisetron)
BRL 46470A	*endo*-*N*-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3,3-dimethylindole-1-carboxamide
BRL 54443	5-Hydroxy-3-(1-methylpiperidin-4-yl)-1*H*-indole
BW 723C86	α-Methyl-5-(2-thienylmethoxy)-1*H*-indole-3-ethanamine
CP 93129	1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-*b*]pyridine-5-one
CP 96345	(2*S*,3*S*)-*cis*-2-(Diphenylmethyl)-*N*-(2-methoxy-phenyl)methyl-1-azabicyclo[2.2.2]octan-3-amine
CP 99994	(+)-(2*S*,3*S*)-3-(2-Methoxybenzylamino)-2-phenyl-piperidine
CP 99994	(2*S*,3*S*)-*N*-(2-Methoxybenzyl)-2-phenylpiperidin-3-amine
DAIZAC	(*S*)-5-Chloro-3-iodo-2-methoxy-*N*-(1-azabicyclo-[2.2.2]oct-3-yl)benzamide
DAT 582	*N*-{(1*R*)-[3-(3-Methylbenzyl)-6-methylcycloheptyl]}-1*H*-indazole-3-carboxamide
DAU 6215	*N*-(8-Methyl-8-azabicyclo[3.2.1]oct-3-yl)-2-oxo-2,3-dihydro-1*H*-benzimidazole-1-carboxamide

FK 1052	8,9-Dihydro-10-methyl-7-[(5-methyl-1*H*-imidazol-4-yl)methyl]pyrido[1,2-a]indol-6(7*H*)-one
FK 888	*N*-2-[(4*R*)-4-Hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-*L*-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide
GR 113808	1-{[2-(Methylsulfonylamino)ethyl]-4-piperidinylmethyl}-1-methyl-1*H*-indole 3-carboxylate
GR 125743	*N*-[4-Methoxy-3-(4-methylpiperazin-1-yl)phenyl]-3-methyl-4-(4-pyridyl)benzamide
GR 127935	*N*-[4-Methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)1,1'-biphenyl-4-carboxamide
GR 38032F	1,2,3,9-Tetrahydro-9-methyl-3-[(2-methyl-1*H*-imidazo-1-yl)methyl]-4*H*-carbazol-4-one (Ondansetron)
GR 55562	3-[3-(Dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide
GR 65630	3-(5-Methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)propan-1-one
ICI 169369	2-(2-Dimethylaminoethylthio)-3-phenylquinoline
ICS 205930	8-Methyl-8-azabicyclo[3.2.1]oct-3-yl-1*H*-indole-3-carboxylate (Tropisetron)
LAS 30451	4-Amino-5-chloro-2-(cyclopropylmethoxy)-*N*-(quinuclidin-3-yl)benzamide (Pancopride)
LY 334370	5-(4-Flurobenzoyl)amino-3-(1-methylpiperidin-4-yl)-1*H*-indole
MCI 225	4-(2-Fluorophenyl)-6-methyl-2-(1-piperazinyl)-thieno[2,3-*d*]pyrimidine
mCPP	1-(3-Chlorophenyl)piperazine
MDL 100907	*R*(+)-α-(2,3-Dimethoxyphenyl)-1- [2-(4- fluoro-phenylethyl)]-4-piperidine-methanol
MDL 72222	Tropanyl-3,5-dichlorobenzoate (Bemesetron)

MK 212	6-Chloro-2-(1-piperazinyl)pyrazine
ML 10302	2-(1-Piperidinyl)ethyl-4-amino-5-chloro-2-methoxy-benzoate
NAN 190	1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)butyl]-piperazine
RG 12915	4-[*N*-(1-Azabicyclo[2.2.2.]octan-3-(*S*)-yl)]-2-chloro-*cis*-5a*S*,9a*S*-5a,6,7,8,9,9a-hexahydrobenzo-furancarboxamide
RG 12915	(5a*S*,9a*S*)-*N*-1-Azabicyclo[2.2.2]oct-3-yl-2-chloro-5a,6,7,8,9,9a-hexahydrodibenzo[*b*,*d*]furan-4-carboxamide
Ro 046790	4-Amino-*N*-[2,6-bis(methylaminopyrimidin-4-yl)]-benzene sulfonamide
Ro 630563	4-Amino-*N*-[2,6-bis(methylaminopyridin-4-yl)]-benzene sulfonamide
RP 67580	(3a*R*,7a*R*)-Octahydro-2-[1-imino-2-(2-methoxy-phenyl)ethyl]-7,7-diphenyl-4*H*-isoindole
RS 100235	1-(8-Amino-7-chloro-1,4-benzodioxan-5-yl)-3-{[(3,4-dimethoxyphenyl)prop-1-yl]piperidin-4-yl}propan-1-one
RS 102221	8-[5-(2,4-Dimethoxy-5-(4-trifluoromethylphenyl-sulphonamido)phenyl-5-oxopentyl]-1,3,8-triazaspiro[4.5]decane-2,4-dione
RS 67506	1-(4-Amino-5-chloro-2-methoxyphenyl)-3-[1-(2-methylsulphonylamino)ethyl-4-piperidinyl]-1-propanone
RU 24969	5-Methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole
SB 204070	1-Butyl-4-piperidinylmethyl-8-amino-7-chloro-1,4-benzoioxan-5-carboxylate
SB 207710	1-(-Butyl-4-piperidinylmethyl)-8-amino-7-iodo-1,4-benzodioxane-5-carboxylate
SB 216641	*N*-[3-(3-Dimethylamino)ethoxy-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1'-biphenyl]-4-carboxamide

SB 242084	6-Chloro-5-methyl-1-[6-(2-methylpiridin-3-yloxy)-pyridin-3-ylcarbamoyl]indoline
SB 258719	(*R*)-3-*N*-Dimethyl-*N*-[1-methyl-3-(4-methylpiperidin-1-yl)propyl]benzene sulfonamide
SC 53116	(1*S-cis*)-4-Amino-5-chloro-*N*-[(hexahydro-1*H*-pyrrolizin-1-yl)methyl]-2-methoxybenzamide
SKF 38393	(±)-1-Phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol
SKF 38393	1-Phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diol
UH 301	5-Fluro-8-hydroxy-2-(dipropylamino)tetralin
WAY 100289	*endo*-*N*-{[(8-Methyl-8-azabicyclo[3.2.1]octan-3-yl)-amino]carbonyl}-2-(cyclopropylmethoxy)-benzamide
WAY 100635	*N*-{2-[4-(2-Methoxyphenyl)-1-piperazinyl]}ethyl-*N*-(2-pyridinyl)cyclohexanecarboxamide
Y 25130	*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chloro-4-methyl-3-oxo-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (Azasetron)
YM 060	(*R*)-5-[(1-Methyl-3-indolyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole (Ramosetron)
YM 114	5-(2,3-Dihydro-1*H*-indol-1-ylcarbonyl)-4,5,6,7-tetrahydro-1*H*-benzimidazole

Abstract

The present work embodies the design and synthesis of three series of heterocyclic compounds, namely, 3-substituted-2-quinoxalinecarboxamides, 3-methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-ones, and 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles, which were designed according to the pharmacophoric requirements for serotoninergic 5-HT_3 receptor antagonists, using Tripos-Alchemy 2000 software. The molecules were synthesized by conventional heating and also by microwave assisted synthesis in liquid medium and in solid state. The structures of the synthesized compounds were confirmed by spectral and elemental analysis. All the compounds were evaluated for 5-HT_3 receptor antagonistic activities in isolated guinea-pig ileum, against 5-HT_3 agonist, 2-methyl-5-HT, as a standard reference. Most of the compounds showed varying degrees of 5-HT_3 receptor antagonistic activities. Among the compounds tested, three of the heteroaryl piperazines, 2-(4-methylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (**NA-2**), 2-(4-ethylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (**NA-3**) and 2-(4-allyl-piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (**NA-4**), exhibited more potent 5-HT_3 receptor antagonism than the standard 5-HT_3 receptor antagonist, ondansetron. The most potent molecule, 2-(4-allylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (**NA-4**) and ondansetron were studied for anxiolytic activity in mice using elevated plus maze, light/dark exploration test and hole board test. Both the test molecule and standard drug showed anxiolytic activity at higher dose level (10 mg/kg, *i.p.*), but failed to show anxiolytic activity at lower doses (0.1 and 1.0 mg/kg, *i.p.*). The compound **NA-4** showed equipotent anxiolytic activity when compared to ondansetron in all the three animal models of anxiety.

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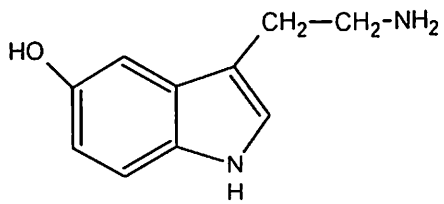
CHAPTER I

INTRODUCTION

Serotonin (5-Hydroxytryptamine, 5-HT)

Page and his colleagues isolated and chemically characterized a vasoconstrictor substance released from platelets in clotting blood (Rapport et al. 1948). The substance was called serotonin (**1.1**) (Page 1976). Subsequent studies revealed that this agent is present in central nervous systems (CNS) of a variety of animal species and is found in enterochromaffin cells in gastro-intestinal tract (GIT) and platelets of humans (Zifa and Fillion 1992). Serotonin acts as a neurotransmitter in the GIT as well in the CNS and also as a local hormone in the peripheral vascular system (Rang et al. 1999).



1.1

1.1. Distribution

5-HT occurs in the highest concentrations in three locations in the body.

In GIT. About 90% of the total amount in the body is present in the enterochromaffin cells, mainly in the stomach and the small intestine.

In Blood. 5-HT is present in high concentration in platelets that gets accumulated from the plasma by an active transport system, and is released when they aggregate at the sites of tissue damage.

In the CNS. 5-HT is a neurotransmitter in the CNS and is present in high concentrations in localized regions of the midbrain.

1.2. Biosynthesis

5-HT is an indole monamine and is synthesized from the essential amino acid tryptophan. Its synthesis, storage, release and inactivation are similar to the process occurring in tissues, which synthesize the other monamines, such as adrenaline, noradrenaline and dopamine (Mawe and Gershon 1993). The biosynthesis of 5-HT follows a pathway similar to that of noradrenaline except that the precursor amino acid is tryptophan instead of tyrosine (Fig. 1.2). 5-HT is present in the diet, but most of it is metabolised before entering the blood stream. Tryptophan is converted to 5-hydroxytryptamine (in chromaffin cells and neurons, but not in platelets) by the action of tryptophan hydroxylase (an enzyme confined to 5-HT producing cells). The 5-hydroxytryptophan is then decarboxylated to 5-HT, by the non-specific aromatic L-amino acid decarboxylase (Erspamer 1976), which also participates in the synthesis of catecholamine and histamine. Since the mechanism of synthesis, storage, release and reuptake of 5-HT are very similar to those of noradrenaline, many drugs affect both processes, indiscriminately.

1.3. Degradation

The principal route of metabolism of 5-HT involves monoamine oxidase (MAO) forming an aldehyde, which is converted to 5-hydroxyindole acetic acid (5-HIAA) by an enzyme, aldehyde dehydrogenase. An alternative route, reduction of the acetaldehyde to an alcohol, 5-hydroxytryptophol, is normally insignificant (Udenfriend 1959). 5-HIAA is excreted in the urine, and serves as an indicator of 5-HT production in the body. The usual urinary excretion of 5-HIAA by a normal adult is 2 to 10 mg per day. In addition to the metabolism by MAO, 5-HT is also metabolised by an enzyme, 5-HT-N-acetylase, to N-acetyl-5-HT, which is converted to melatonin by an enzyme, hydroxyindole O-methyltransferase (Fig. 1.2) (Sanders-Bush and Mayer 1996).

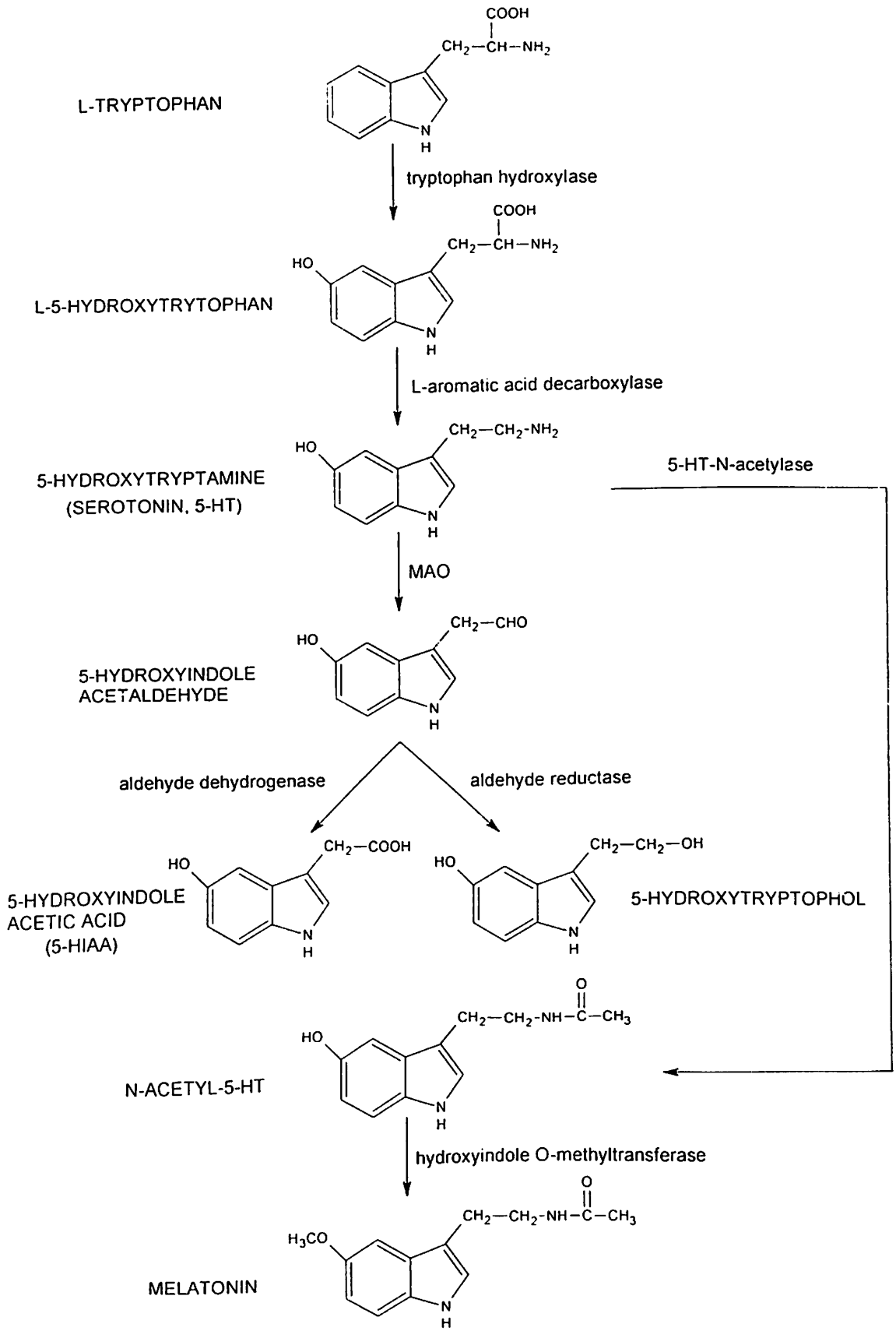


Fig. 1.2: Biosynthesis and degradation of 5-HT

1.4. Pharmacological Effects

The physiology and pharmacological actions of 5-HT are numerous and complex, and show considerable species variation. This complexity reflects a profusion of 5-HT receptor subtypes, which has been revealed in recent years. The main sites of action are as follows.

1.4.1. Gastrointestinal Tract

Enterochromaffin cells in the mucosa appear to be the location for the synthesis and most of the storage of 5-HT in the body and are the source of circulating 5-HT. 5-HT released from these cells enters the portal vein and is subsequently metabolised by MAO in the liver (Gillis 1985). 5-HT released by mechanical or vagal stimulation also acts locally to regulate gastrointestinal function. Motility of gastric and intestinal smooth muscle may either be enhanced or inhibited (Dhasmana et al. 1993) via the subtypes of 5-HT receptor. In esophagus, 5-HT acting at 5-HT_4 receptors causes either relaxation or contraction, depending on the species. Abundant 5-HT_3 receptors on vagal and other afferent neurons and on enterochromaffin cells play a pivotal role in emesis (Grunberg and Hesketh 1993). Serotoninergic terminals have been described in the myenteric plexus. Release of enteric 5-HT occurs in response to acetycholine, noradrenergic nerve stimulation, increase in intraluminal pressure, lowered pH (Gershon 1991) and by the triggering of peristaltic contraction.

1.4.2. Cardiovascular System

The classical response of blood vessels to 5-HT is contraction, particularly in the renal, pulmonary and cerebral vasculatures. This response also occurs in bronchial smooth muscles.

5-HT also induces a variety of responses in the heart as a result of the activation of 5-HT receptor subtypes, stimulation or inhibition of autonomic activity, dominance of reflex responses to 5-HT (Saxena and Villalon 1990). Thus, 5-HT has positive ionotropic and chronotropic actions on the heart that may by blunted by simultaneous stimulation of afferent nerves from baroreceptors and chemoreceptors. An effect on vagus nerve endings elicits the Benzold-Jarisch reflex (Ghuran and Camm 2001), causing extreme bradycardia and hypotension.

1.4.3. Platelets

5-HT is not synthesized in platelets but it is taken up from the circulation and stored in secretory granules by active transport similar to the uptake and storage of norepinephrine by sympathetic nerve terminals. At the sites of vascular injury, 5-HT is secreted by platelets that enhance platelet aggregation and also cause contraction of vascular muscle thereby aiding hemostasis (Gibbons and Dzau 1994). 5-HT causes platelet aggregation via 5-HT_{2A} receptors, and the platelets that collect in the blood vessels release more 5-HT. If the endothelium is intact, 5-HT released from adherent platelets causes vasodilatation, which helps to sustain blood flow; if it is damaged (e.g. by atherosclerosis), 5-HT causes constriction and impairs blood flow further. These effects of platelet derived 5-HT are thought to be important in vascular disease.

1.4.4. Central Nervous System

Tryptaminergic neurons are localized in the raphe region of pons and upper brain stem. The rostral nuclei appear to innervate forebrain regions, while the caudal raphe nuclei project into the brain stem and spinal cord. The median raphe nuclei contribute to tryptaminergic innervation of the limbic system. The dorsal raphe nuclei contribute towards the innervation of the cortical region (Palacious et al. 1990).

The multitude of sertotoninergic receptors can cause inhibitory or excitatory effects on tryptaminergic neurons. Many known centrally active drugs can affect tryptaminergic neuronal systems by influencing direct responses to 5-HT or its uptake, synthesis, storage, release or catabolism. Due to the complexity and wide distribution of 5-HT receptor subtypes in the CNS, a multitude of brain functions are influenced by 5-HT, including sleep, cognition, sensory perception, temperature regulation, nociception, appetite, sexual behaviour and hormone secretion (Barnes and Sharp, 1999). In addition, 5-HT has been implicated in etiology of numerous disease states including depression, anxiety, social phobia, schizophrenia, migraine, obsessive-compulsive and panic disorders (Jones and Blackburnz 2002). Different 5-HT receptor subtypes and different membrane mechanisms mediate these effects.

1.5. Classification of 5-HT Receptors

It has been suggested that 5-HT interacts with two different receptors in isolated tissues, namely smooth muscles and nervous tissue. Since dibenzyline selectively antagonized smooth muscle contraction, and morphine was selective for nervous tissue, these receptors were named as "D" and "M" receptors, respectively (Gaddum and Picarelli 1957). The radioligand-binding studies provided the first definitive evidence for two distinct recognition sites for 5-HT (5-HT_1 & 5-HT_2). 5-HT_1 receptors had a high affinity for [^{3}H]-5-HT, while 5-HT_2 receptors had a low affinity for [^{3}H]-5-HT and a high affinity for [^{3}H]-spiroperidol (Peroutka and Synder 1979). Initially, 5-HT receptor subtypes were characterized using pharmacological tools only. On the basis of the receptor binding profiles, four main subtypes of 5-HT receptors, viz., 5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4, were identified.

Recently, molecular biological techniques confirmed this classification, in which each subgroup has been found to have relatively dissimilar protein structures and led to the identification of novel 5-HT receptors types, 5-HT_{1-7}, of which types 1 and 2 are further subdivided into 3 or 4 sub-categories, denoted as A-D (Hoyer et al. 1994).

The current status for serotoninergic receptor classification has been established by the efforts of the serotoninergic nomenclature of the International Union of Pharmacology (IUPHAR) (Hoyer et al. 1994). IUPHAR now considers that it is essential to have "operational" (drug related characteristics), "transductional" (receptor-effector coupling events) and "structural" (gene and receptor structural sequences for nucleotide and amino acid components) characteristics as fingerprint criteria for receptor classification. None of these characteristics have precedence as has been described below. Fundamentally two receptor "superfamilies" can be distinguished: the ligand-gated ion channels (LGIC) as in the case of 5-HT_3 receptors and G-protein coupled receptors (GPCR), to which all the other 5-HT receptor subtypes belong. The 5-HT receptor subtypes within a class appear to display preferential coupling to the same type of effector.

The 5-HT_1 receptor family inhibits adenylate cyclase, while 5-HT_2 receptor subtypes favour the activation of phospholipase-C and intracellular calcium mobilization. The 5-HT_4 receptors stimulate adenylate cyclase and hence are not grouped with the 5-HT_1 class. It is also evident that receptors belonging to different classes can share the same transductional characteristics as in the case of 5-HT_4 as well as the 5-HT_6 and 5-HT_7. Hence, the transductional information, taken in isolation, is an insufficient basis for receptor classification. Though the ultimate proof for the existence of a receptor might be regarded as the identification of its gene, the amino acid sequence alone is insufficient to enable allocation to a particular neurotransmitter/hormone family. In the case of 5-HT_4, 5-HT_6 and 5-HT_7 receptors though they have the same transductional characteristics; they show very less homology (< 30%) with other 5-HT receptors and among themselves and hence are classified as different receptors.

In fact some 5-HT receptors are structurally more similar to histamine or dopamine receptors and hence structural characteristics cannot be solely taken into account for classification (Bard et al. 1993; Monsma et al. 1993). In a recent change of nomenclature of the 5-HT_{1B}, receptor prefixes for the species equivalent e.g. r5HT_{1B} for rodents and h5HT_{1B} for humans were introduced, as the pharmacology of 5-HT_{1B} receptor shows significant differences across species (Vanhoutte et al. 1996) even though they show a high degree of homology and have the same transductional characteristics, thereby adding a new dimension to the serotoninergic receptor nomenclature.

Demonstration of receptor mediated changes in some proximal biochemical events such as camp or phosphoinositol accumulation, especially when it is observed in transfected cells does not merit classification as such events may not have any physiological activity. It is at present recommended that a physiological role for a receptor can be accepted only when the activation in a native setting is shown to produce a robust and measurable effect, which is clearly interpretable as a physiological response, e.g. enzyme induction, modulation of ion conduction, smooth muscle contraction (Hoyer and Martin 1996). Polymorphism in population, as in the case of 5-HT_{2A} receptor in humans (Williams et al. 1997) also poses a challenge to receptor nomenclature. Recent developments in the completion of the human genome project in which serotonin genes are likely to comprise of 1-2% of 100,000 genes of the human genome, may lead to the re-classification of serotonin receptor sub-types (Harig et al. 1996; Hoyer and Martin 1997).

Cancer Chemotherapy-Induced Nausea and Vomiting.

Chemotherapy regimens for the treatment of cancer are unfortunately well known for their toxicity than for their efficacy. Although some of their toxic effects may be life threatening, patients are often most fearful of the nausea and vomiting (N&V) caused by cancer chemotherapy. N&V are distressing symptoms occurring due to motion sickness (disturbance in vestibular system), surgery, anaesthesia and a number of gastrointestinal disorders. Moreover, cancer chemotherapy, elicits an immediate emetic response. Usually, the emesis is severe during the first 24 hours [acute emesis] and often prolonged for several days after treatment with anticancer drugs or radiation [delayed emesis] (Wickham 1999; Coates et al. 1983).

1.6. General Risk Factors and Etiologies

All cancer patients receiving chemotherapy treatment may not experience nausea and/or vomiting. The most common causes are emetogenic chemotherapy drugs and radiation therapy to the gastrointestinal tract, liver or brain. Several patient characteristics have also been identified. These include: incidence and severity of N&V during the previous course of chemotherapy, history of chronic alcohol use, age, and gender. Patients with poor control of N&V during prior chemotherapy cycles are likely to experience N&V in subsequent cycles. N&V are more likely to occur in patients with a history of chronic, high alcohol intake (Sullivan et al. 1983) and in women (Tonato et al. 1991; Roila et al. 1987) and younger patients (less than 50 years) (Tonato et al. 1991). Other possible causes include fluid and electrolyte imbalances such as hypercalcemia, volume depletion or water intoxication; tumor invasion or growth in the gastrointestinal tract, liver, or CNS.

The psychological variables of anxious state, (level of anxiety during chemotherapy infusions), pretreatment expectations for nausea and vomiting have also been investigated as predictors of post-treatment nausea (Roscoe et al. 2000; Hickok et al. 2001).

At present, studies have found mixed results that vary due to different research methods. Better designed, more recent studies, have found anxiety state and patient expectations for nausea to be predictors of post-treatment nausea, even after controlling known physiological predictors (susceptibility to nausea during pregnancy and motion sickness) and emetogenic potential of the chemotherapy drugs (Roscoe et al. 2000). It is important to note that patient's fear and expectations about chemotherapy can vary and change over time (Passik et al. 2001). In a longitudinal study (Passik et al. 2001), patients' anticipatory fear for vomiting, decreased significantly from pretreatment, to a period 3 to 6 months later, particularly when their chemotherapy included anti-emetic medications.

The incidence and severity of emesis in persons receiving chemotherapy varies according to many factors, including the particular drug, dose and schedule of administration, route and individual patients.

Prevention and control of N&V are paramount in the treatment of cancer patients. N&V can result in serious metabolic derangements, nutritional depletion and anorexia, esophageal tear, deterioration of patients' physical and mental status, which prompts the patient to discontinue the potentially useful and curative antineoplastic treatment (Craig et al. 1987; Passik et al. 2001).

1.7. Physiology of Emesis

Nausea and vomiting (N&V) are natural protective reflexes designed to eliminate toxins from the gastrointestinal tract (GIT) and to prevent further ingestion of the same substance. The process of vomiting is coordinated by vomiting centre (VC) located in the medulla oblongata. Chemoreceptor trigger zone (CTZ), located in the area postrema (AP) of the fourth ventricle and nucleus tractus solitarius (NTS) of the vagus nerve, are the most important relay centres for afferent impulses arising in the periphery (pharynx and GIT). CTZ can also detect circulating toxins directly from the blood and cerebrospinal fluid (CSF), as it is unprotected by the blood brain barrier. Vagal afferents from the liver may also play a role in the relay of information to the CTZ.

Two additional areas, which send impulses to the VC, are the cerebral cortex (particularly in anticipatory N&V) and vestibular labyrinthine system (in motion sickness). In response to afferent impulses, VC sends efferent impulses to nuclei responsible for respiratory, salivary and vasomotor activity besides the striated and smooth muscles, involved in the process of vomiting (Brunton 1996; Karim et al. 1996). In contrast to vomiting, the mechanism of nausea is not clearly defined and it has been believed that nausea may be due to low level activation of the vomiting pathway. With respect to chemotherapy and radiation therapy, the CTZ, the GIT (particularly enterochromaffin cells of the small intestinal mucosa are likely to be involved) and the cerebral cortex have been identified as sources of afferent input to the VC (Gregory and Ettinger 1998) where the GIT is connected to the VC, via the CTZ and NTS. Cerebral cortex stimulates the VC, in anticipatory N&V (Craig et al. 1987). The CTZ has high concentrations of serotonin (5-HT_3), dopamine (D_2) and opioid receptors, while the NTS which is rich in enkephalin, histamine and cholinergic receptors, also contains 5-HT_3 receptors (Brunton 1996). The neuronal pathways involved in the N&V are represented in Fig.1.3.

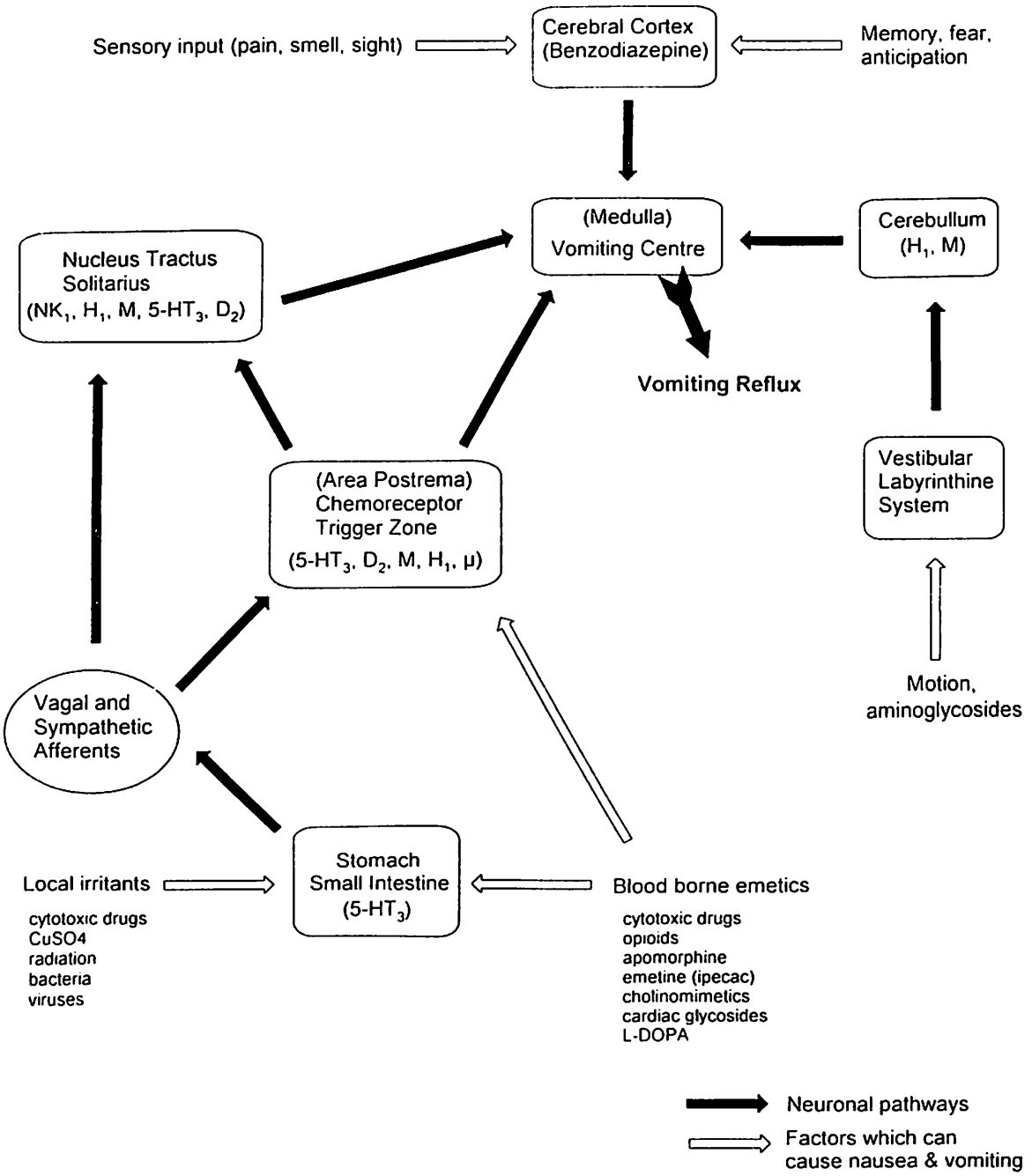


Fig.1.3. Neuronal pathways involved in nausea and vomiting (Brunton 1996)

1.8. Classification of Chemotherapy-Induced Nausea and Vomiting

Anticipatory nausea and vomiting (ANV): ANV is nausea and/or vomiting which occur prior to the beginning of new cycle of chemotherapy, in response to conditioned stimuli such as the smell, sight and sound of the treatment room. ANV is a classically conditioned response that typically occurs after 3 - 4 prior chemotherapy treatments, following which the person experiences acute or delayed N&V.

Acute nausea and vomiting: N&V experienced during the first 24-hour period, following chemotherapy administration.

Delayed (or late) nausea and vomiting: N&V that occurs beyond 24 hours, following chemotherapy administration. This is considered as delayed or late N&V. Delayed N&V is associated with drugs used in the treatment of cancer such as cisplatin, Cyclophosphamide, doxorubicin and ifosfamide, given at high doses or on 2 or more consecutive days.

Chronic nausea and vomiting: N&V that occur in advanced cancer patients, which is associated with a variety of potential etiologies. A definite understanding of the cause is not well known, nor well researched, but potential causal factors include gastrointestinal, cranial, metabolic, drug-induced (e.g. morphine), cytotoxic chemotherapy and radiation-induced mechanisms.

Breakthrough Nausea and vomiting: N&V that occur despite patients being treated with preventive therapy.

Refractory nausea and vomiting: N&V that occur during subsequent cycles of chemotherapy when anti-emetic prophylaxis or rescue therapy has failed in earlier cycles.

1.9. Serotonin 5-HT_3 Receptors

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in various pharmacological effects in several peripheral and central nervous system (CNS) (Boess and Martin 1994). Fifteen 5-HT receptor subtypes belonging to 7 major classes (5-HT_1-5-HT_7) have been identified so far (Hoger and Martin 1997). The majority of 5-HT receptors belong to the *G protein-coupled* receptor (GPCR) family. The 5-HT_3 receptors on the other hand, act through pentameric cation channel that are present within the peripheral and CNS (Derkach et al. 1989). Since the last decade, 5-HT_3 receptors have gained much attention because of the clinical use of 5-HT_3 receptor antagonists (RAs) in the treatment of chemotherapy-induced nausea and vomiting (CINV) (Karim et al. 1996; Watters et al. 2001) and also in post-operative nausea and vomiting (PONV) (Jones and Blackburn 2002). Besides, a number of preclinical studies have suggested that 5-HT_3 RAs can be used in the treatment of various CNS disorders such as anxiety and cognitive dysfunctions (Jones and Blackburn 2002).

1.9.1. Involvement of 5-HT_3 Receptors in CINV

In early 1980s, the best available treatment for CINV was high dose metoclopramide (Gralla et al. 1981; Homesley et al. 1982), combined with a number of other drugs, such as lorazepam and dexamethasone. However, this treatment regimen was effective only in less than 60% patients and these drugs produce extra pyramidal side effects, which added to the patient's problem. The dose of metoclopramide was very much in excess of those required for gut motility effects, and other prokinetic agents failed to control CINV. Since dopamine antagonists are known to be useful anti-emetics for a number of conditions, it was assumed that metoclopramide was effective by blocking the dopamine receptor activity. However, other dopamine receptor antagonists did not appear to be as effective as metoclopramide against CINV.

From these observations it can be tentatively concluded that the anti-emetic action of metoclopramide against CINV, is not due to gut motility stimulation of dopamine antagonism (Andrews and Bhandari 1993). Metoclopramide was known to have other pharmacological properties including interactions with muscarinic receptors, α-adrenoreceptors and 'M' receptors (5-HT_3 receptors). Serotonin had not previously been implicated as a part of the vomiting reflex pathway, but the discovery of ondansetron, a selective 5-HT_3 RA without affinity for dopamine receptors, proved the involvement of 5-HT_3 receptors in the CINV (Tyers et al. 1993).

1.9.2. Mechanism of the Anti-Emetic Action of 5-HT_3 Receptor Antagonists

Serotonin is found in high concentrations within the enterochromaffin cells in the gut (Gregory and Ettinger 1998; Rapepart and Sanger 1988), and is also located in the CNS and platelets. In the CNS, highest densities of 5-HT_3 receptors are present in the NTS, AP where the CTZ is located and where vagal afferents enter the brain and in dorsovagal nucleus (Kilpatrick et al. 1987).

1.9.2.1. Peripheral site of action

It has been revealed that cisplatin caused the release of serotonin from enterochromaffin cells in the GIT (Guinning et al. 1987; Cubeddu et al. 1990), possibly as a result of free radical generation (Matsuki et al. 1993). The released serotonin, then activates the 5-HT_3 receptors on vagal afferent fibres, which stimulates the CNS that mediate emetic response. 5-HT_3 RAs, by blocking the afferent vagal pathways would prevent vomiting (Andrews et al. 1990).

It has been shown that urinary excretion and plasma concentrations of the major metabolite of serotonin, 5-hydroxyindole acetic acid (5-HIAA), is increased after the administration of highly emetogenic chemotherapy (Alfieri and Cebeddu 1995) and is not affected by the administration of 5-HT_3 RAs. Thus, peripherally, 5-HT_3 RAs do not prevent the release of serotonin, but bind to the 5-HT_3 receptors and prevent CINV by preventing agonism of the 5-HT_3 receptors by serotonin.

1.9.2.2. Central site of action

Autoradiographic studies have revealed that the 5-HT_3 receptor ligands bind with much higher density to the dorsal vagal complex (AP, NTS, dorsal motor nucleus of the vagus nerve) than to other brain regions, in ferrets (Higgins et al. 1989; Fozard 1987). These studies suggested that central 5-HT_3 receptors are also involved in CINV.

When 5-HT_3 receptor agonist, 2-methyl-5-HT was injected by intracerebroventricular (*i.c.v.*) route, it has been observed that it induced emesis in ferrets (Miller and Nonaka 1990). Cisplatin-induced emesis is fully prevented by surgical ablation of CTZ (Bhandari et al. 1989). Administration of ondansetron or other 5-HT_3 RAs into the area postrema or the fourth cerebral ventricle has been reported to inhibit the emesis induced by peripherally or centrally administered cisplatin (Higgins et al. 1989; Smith et al. 1988; Kamato et al. 1993). These findings supported that the 5-HT_3 RAs act at CTZ in the AP of the fourth ventricle.

1.9.3. Developments of 5-HT_3 Receptor Antagonists

In 1957, a neuronally mediated, 'M" receptor response to serotonin in the guinea-pig isolated ileum preparation was reported (Gaddum and Picarelli 1957). After this, many research groups worked on this receptor to explore its physiological functions.

It has been reported that the blockade of serotonin 'M' receptor was facilitated by metoclopramide (Fozard and Mobarek Ali 1978). The pharmacology of MDL-72222 (Fozard 1984), which was a structural hybrid of two compounds, cocaine and metoclopramide, which were known to have 'M' receptor-blocking properties. It has also been published that some tropane substituted indoles showed potent and selective 'M' receptor antagonist activities (Richardson et al. 1985). In 1986, the 'M' receptor was renamed as the 5-HT_3 receptor (Bradley et al. 1986).

For many years, metoclopramide, a benzamide derivative has been widely used to treat the emesis induced by anticancer therapy, besides being used as a prokinetic agent (Pinder et al. 1976; Harrington et al. 1983). Pharmacologically, metoclopramide effects are believed to be due to a combination of relatively weak serotonin 5-HT_3 and dopamine D_2 receptors antagonism and serotonin 5-HT_4 receptor agonism. The weak affinity and lack of selectivity of metoclopramide for these receptors may be due to large number of conformations arising from the flexibility of 2-(diethylamino)ethyl moiety. To develop potent 5-HT_3 receptor antagonists and/or 5-HT_4 receptor agonists that are devoid of significant D_2 receptor antagonistic activity, several research groups have modified the 2-(diethylamino)ethyl moiety of metoclopramide. Accordingly, benzamide with conformationally rigid amines such as piperidine, quinuclidine and quinolizidine have been reported (Kato et al. 1999). The structures of these benzamide derivatives are shown in Fig.1.4. Thus, cisapride, mosapride, zacopride, renzapride, compound **1.4.1**, BRL 24682, SC 53116 and compound **1.4.2**, were found to exhibit good affinity for 5-HT_3 and/or 5-HT_4 receptors, whereas clebopride, BRL 25594 and compound **1.4.3**, having a benzyl group on the nitrogen atom in the amine moiety have shown high affinity for the D_2 receptors (Harada et al. 1995; Hirokawa et al. 2003; Hirokawa et al. 1998a; Hirokawa et al. 1998b). The structures of these compounds are given in Fig.1.4.

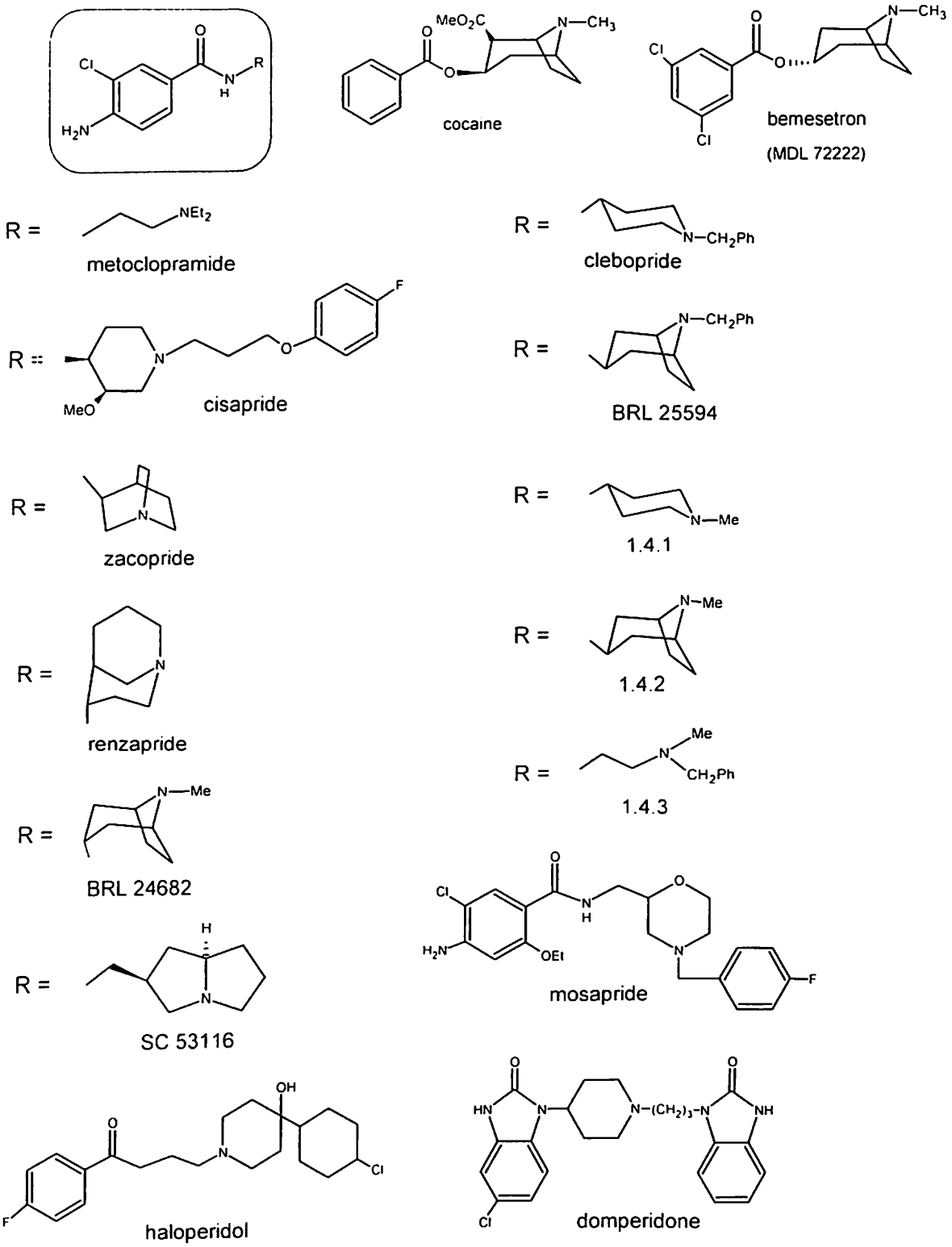


Fig. 1.4. Serotonin 5-HT$_3$, 5-HT$_4$ and/or dopamine D$_2$ receptor antagonists

In due course, the most familiar and clinically used 5-HT_3 RA, ondansetron, was introduced in 1990 by Glaxo, followed by granisetron in 1991 by Smithkline Beecham, tropisetron in 1992 by Sandoz and dolasetron by Marion Merrell Dow. All these drugs are currently available in many countries, whereas dolasetron is available in the UK and is now approved in the USA. Other 5-HT_3 RAs, alosetron, azasetron, BRL-46470A, DAT-582, DAU-5215, pancopride, RG-12915, WAY-100289, YM-060, zatosetron are yet to be commercialized. Structures of these compounds are given in Fig. 1.5.

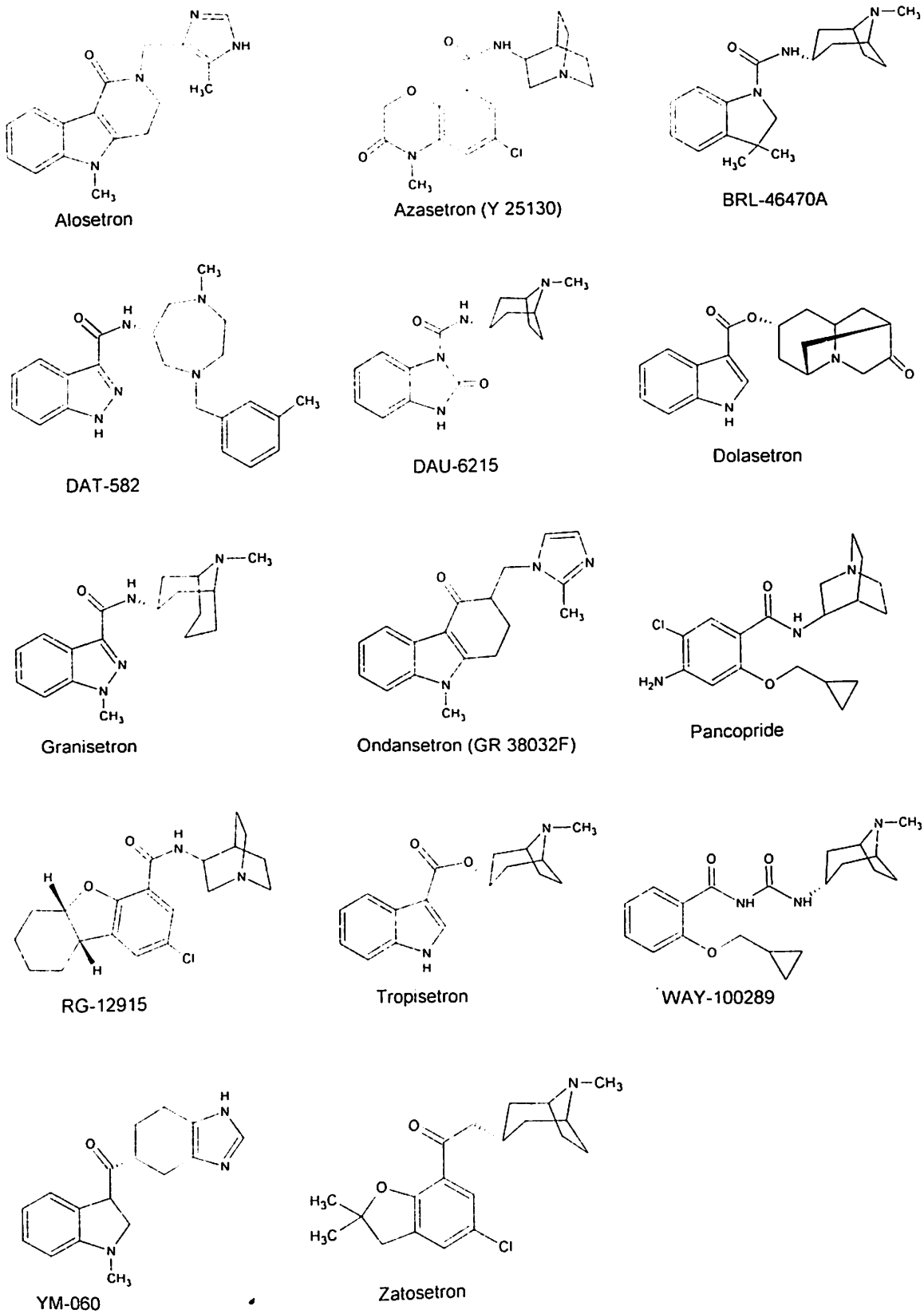
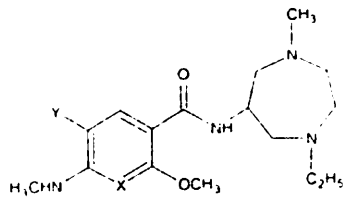


Fig. 1.5. 5-HT$_3$ receptor antagonists

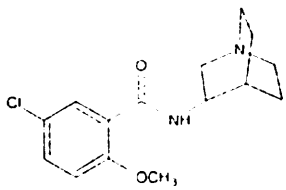
1.9.4. Pharmacophore of 5-HT_3 Receptor Antagonists

Computer-based three-dimensional steric molecular models of the 5-HT_3 receptor pharmacophore have been developed on the basis of radio-ligand binding data using potent agents like ondansetron and granisetron. Perhaps the most well known and generally accepted 5-HT_3 pharmacophore, consists of three components: an aromatic ring, a carbonyl group containing linking moiety and an out of plane basic centre preferably nitrogen in a specific spatial arrangement (Hibert et al. 1990).

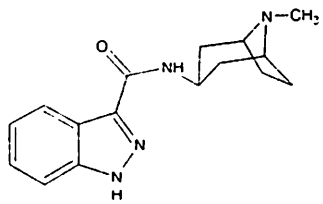
Since Hibert published his model in 1990, a number of molecules have been reported based on this model and also some refinements and extensions to this model have been proposed. Many research groups have developed molecules based on this model. Beginning with the aromatic component, various allowable modifications have been achieved; namely: substitution with phenyl (**1.6.1**) (Hirokawa et al. 1998a), (**1.6.3**) (Youssefyeh et al. 1992), pyridyl (**1.6.2**) (Hirokawa et al. 2003), indazoles (**1.6.4**) (Robertson et al. 1990), indole (**1.6.5**) (Rosen et al. 1990), indolizine (**1.6.6**) (Bermudez et al. 1990b), indoline (**1.6.7**) (Bermudez et al. 1990a), quinoline (**1.6.8**) (Hayashi et al. 1992), (**1.6.9**) (Hayashi et al. 1993), dihydrobenzofuran (**1.6.10**) (Kuroita et al. 1994), benzoxazine (**1.6.11**) (Kato et al. 1995a) and benzimidazole (**1.6.12**) (Lopez-Rodriguez et al. 1996). Concerning the basic amine component, a number of sterically hindered and conformationally rigid aliphatic azabicyclics like tropane (**1.6.4**) (Robertson et al. 1990), (**1.6.6a**) (Bermudez et al. 1990b), (**1.6.7**) (Bermudez et al. 1990a), (**1.6.8**) (Hayashi et al. 1992), (**1.6.9**) (Hayashi et al. 1993), granatane (**1.6.6b**) (Bermudez et al. 1990b), (**1.6.7b**) (Bermudez et al. 1990a), quinuclidine (**1.6.3**) (Youssefyeh et al. 1992), (**1.6.10**) (Kuroita et al. 1994), (**1.6.11**) (Kato et al. 1995) and monocyclics like 1,4-diazepine (**1.6.1**) (Hirokawa et al. 1998a; Hirokawa et al. 1998b), (**1.6.2**) (Hirokawa et al. 2003) have been studied. Amides (**1.6.8a**) (Hayashi et al. 1992), (**1.6.9a**) (Hayashi et al. 1993) and esters (**1.6.8b**) (Hayashi et al. 1992) (**1.6.9b**) (Hayashi et al. 1993) have been studied for carbonyl linking moiety. Structures of these compounds are given in Fig. 1.6.



1.6.1: X = CH, Y = Cl; 1.6.2: X = N, Y = Br



1.6.3



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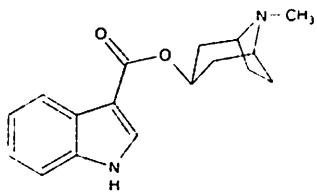

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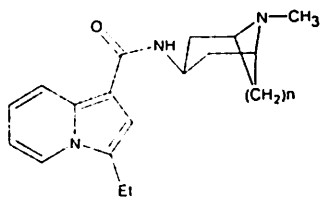
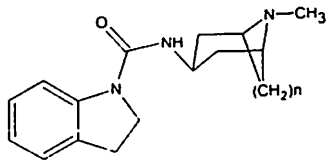


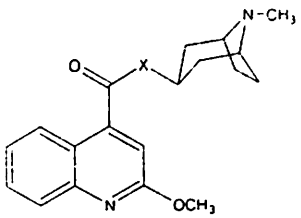
1.7.6

1.6.6a: n = 2, 1.6.6b: n = 3



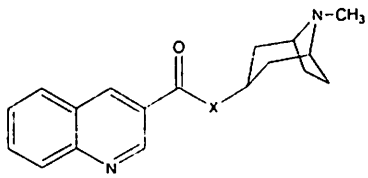
1.7.7

1.6.7a: n = 2, 1.6.7b: n = 3



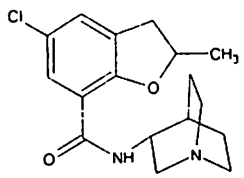
1.7.8

1.6.8a: X = NH, 1.6.8b: X = O

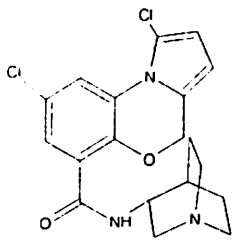


1.7.9

1.6.9a: X = NH, 1.6.9b: X = O



1.6.10



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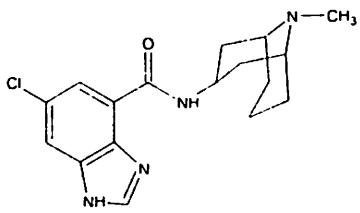


1.6.12

Fig. 1.6. Structures of 5-HT_3 receptor antagonists based on the pharmacophore (Hibert et al. 1990)

1.9.5. Receptor Binding and Affinity

Granisetron and dolasetron have been identified as selective 5-HT_3 RAs with extremely low or no affinity towards other receptors. Granisetron, has 4000 to 40000 times higher binding affinity for the 5-HT_3 receptors than any other receptor type, which have been studied. The major metabolite of dolasetron (hydrodolasetron), has an affinity for the 5-HT_3 receptor that is 23 to 64 times greater than that of the parent compound (Gregory and Ettinger 1998). In addition to 5-HT_3 receptor binding, ondansetron binds at 5-HT_{1B}, 5-HT_{1C}, α_1-adrenergic and opioid μ receptors (Wijngaarden et al. 1990). The affinity of ondansetron for the 5-HT_3 receptor is 250 to 500 times than that of other receptors. Despite the differences in chemical structures, receptor binding and affinity, these properties do not appear to have an effect on the therapeutic activity or adverse effect profiles of the 5-HT_3 RAs. In addition, several prospective, randomized comparison trials have shown bio-equivalence between ondansetron and granisetron (Navari et al. 1995) as well as between ondansetron and dolasetron (Hesketh et al. 1996) and granisetron and dolasetron (Audhuy et al. 1996). The same level of therapeutic efficacy has been observed among these agents. The specific binding of [^{3}H]-GR 65630 at homogenates of discrete areas of ferret brain is given in Table 1.1 and the effect of a number of drugs on specific [^{3}H]-BRL 43694 binding to rat cortical membranes are given in Table 1.2.

Table 1.1. Specific binding of [^{3}H]-GR 65630 at homogenates of discrete areas of ferret brain

Brain Region	Bound (fmol/mg protein)	Brain Region	Bound (fmol/mg protein)
Entorhinal cortex	0.4 ± 0.1	Hippocampus	0.5 ± 0.2
Retrosplinic cortex	0.1 ± 0.04	Nucleus accumbens/ olfactory tubercle	2.8 ± 0.7
Frontal cortex	0.4 ± 0.1	Septum	1.4 ± 0.3
Cingulate cortex	0.3 ± 0.1	Thalamus	0.4 ± 0.02
Temporal cortex	0.3 ± 0.1	Hypothalamus	0.8 ± 0.2
Occipital cortex	0.2 ± 0.05	Striatum	2.9 ± 0.11
Parietal cortex	0.3 ± 0.1	Cerebellum	0.1 ± 0.03
Amygdala	1.0 ± 0.4	Area postrema	37.1 ± 12.3

Results are the means ± standard error of mean (SEM) of three separate experiments.

Table 1.2. Effect of a number of drugs on specific [^{3}H]-BRL 43694 binding to rat cortical membranes

Drug	Mean K_i (nM)
Zacopride	0.10 ± 0.01
BRL 43694	0.26 ± 0.04
BRL 24682	0.33 ± 0.07
ICS 205-930	0.40 ± 0.06
GR 38032F	0.87 ± 0.12
BRL 47204	1.07 ± 0.13
Quipazine	1.23 ± 0.15
MDL 72222	5.3 ± 0.4
Mianserin	5.8 ± 0.9
Cisapride	134 ± 13
5-HT	160 ± 75
Metoclopramide	160 ± 35
2-Methyl-5-HT	620 ± 73
ICI 169,369	990 ± 170
Methiothepine	3000 ± 810
Ketanserin	5300 ± 1100
Metergoline	7400 ± 1700

Drug	Mean K_i (nM)
7-HO-DPAT	>10,000
Buspirone	>10,000
Methylsergide	>10,000
Spiperone	>10,000
Mepyramine	2400 ± 190
Domperidone	3900 ± 1700
Noradrenaline	>10,000
Prazosin	>10,000
Yohimbine	>10,000
Idazoxan	>10,000
Alprenolol	>10,000
Cyanopindolol	>10,000
Dopamine	>10,000
Sulpiride	>10,000
GABA	>10,000
Diazepam	>10,000

Results are the mean ± SEM of three separate experiments except for those Ki values > 10,000 nM which is the mean of two separate experiments.

1.9.6. Pharmacokinetic Differences

Granisetron and tropisetron have long elimination half-life as compared to that of ondansetron. Dolasetron, as the parent compound, has a short elimination half-life (0.13 to 0.24 hrs), but it is rapidly metabolished to its active metabolite, which has an elimination half-life similar to that of granisetron and tropisetron. Some of the pharmacokinetic parameters of the 5-HT_3 RAs are summarized in Table 1.7. Early clinical studies took these differences in half-life into consideration for the dosage regimens. Thus ondansetron was initially administered two times daily compared to once daily for the other 5-HT_3 RAs. It has now been demonstrated that ondansetron, as well as the other 5-HT_3 RAs, can be effectively administered once daily and that their anti-emetic efficacy persists long after their plasma concentrations are undetectable. This indicates that interactions at the receptor site and not plasma pharmacokinetics are important criteria for defining efficacy (Gregory and Ettinger, 1998). Other than difference in half-life, other pharmacokinetic parameters of the 5-HT_3 RAs are very similar. Thus, pharmacokinetic differences among these drugs are unlikely to contribute significantly to clinical differences in activity. Pharmacokinetics data of some 5-HT_3 RAs are given in Table 1.3

Table 1.3. Comparative pharmacokinetics of 5-HT_3 RAs in adults with cancer

	Ondansetron	Granisetron	Tropisetron	Dolasetron[a]
Dose	0.15 mg/kg, *i.v.*	40 µg/kg, *i.v.*	10 mg *i.v.*	0.6-3.0 mg/kg, *i.v*
$t_{1/2}$ (h)	3.9	9.0-11.6	7.3	7.9
CL (L/min)	0.398	0.24-0.43	0.96	0.42
Vd (L)	160	154-228	554	109

[a] *Pharmacokinetics values reported for the active metabolite of dolasetron*

Abbreviations: CL = clearance; i.v. = intravenous; $t_{1/2}$ = half life; Vd = apparent volume of distribution.

1.10. Dopamine Receptors

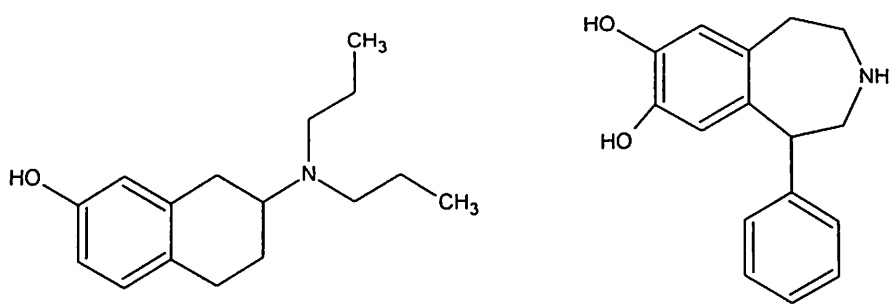
Over the last few decades, substantial evidence have been obtained indicating that central dopaminergic systems play an important role in the regulation of emesis in humans, ferrets, and dogs. The availability of selective agonists towards dopamine receptor subtypes may prove useful in understanding the role of individual dopamine receptor subtypes in emesis. It is well known that, dopamine agonist, apomorphine causes emesis in ferrets, dogs and humans (King 1990). Experiments with dogs have revealed that apomorphine-induced emesis may be mediated by dopamine D_2 receptors in the CTZ, located in the AP (King 1990). However, some studies have also reported that apomorphine has equal affinity for dopamine D_2, D_3 and D_4 receptor sites (Seeman and Van Tol 1994). Thus, it is not clear whether that apomorphine-induced emesis is mediated by dopamine D_2 receptors and/or dopamine D_3 and D_4 receptors.

Administration of *R*(+) enantiomer of 7-hydroxy-*N,N*-di-n-propyl-2-aminotetralin (7-OH-DPAT), caused emesis in ferrets, whereas, *S*(-) enantiomer of 7-OH-DPAT, even at higher doses, failed to induce emesis in ferrets (Yoshikawa et al. 1996). It has been reported that *R*(+)-7-OH-DPAT had about 40-70 fold more affinity for dopamine D_3 receptors than *S*(-)-7-OH-DPAT. Moreover, the affinity of *R*(+)-7-OH-DPAT for the dopamine D_3 receptor was approximately 60-200 fold higher than that for the D_2 receptor (Damsma et al. 1993; Rivert et al. 1994). In addition, the emesis induced by *R*(+)-7-OH-DPAT was found to be abolished by *S*(-)-eticlopride, a dopamine D_2 and D_3 receptor antagonist. Thus, these findings suggested that D_2 receptors may be involved in *R*(+)-7-OH-DPAT induced emesis, in ferrets.

There are no reports as to whether dopamine D_1 receptor agonists can induce emesis in experimental animals. In an isolated single study (Yoshikawa et al. 1996), the selective dopamine D_1 receptor agonist, SKF 38393, did not cause emesis even at higher doses, in ferrets.

It has been reported that the affinity of *R*(+)-7-OH-DPAT for the dopamine D_1 receptor was approximately 10000 fold lower than that for the D_3 receptor (Levesque et al. 1992). These results clearly indicate that the dopamine D_1 receptors are probably not involved in *R*(+)-7-OH-DPAT induced emesis, in ferrets.

Pretreatment with dopamine D_2 and D_3 receptor antagonists, *S*(-)-eticlopride and domperidone, inhibited the retching and emetic episodes induced by *R*(+)-7-OH-DPAT in ferrets. However, pretreatment with clozapine, a dopamine D_4 receptor antagonist, did not inhibit the emesis induced by *R*(+)-7-OH-DPAT in ferrets and *R*(+)-7-OH-DPAT exhibited 1000 fold lower affinity for dopamine D_4 receptor than for dopamine D_3 receptor (Levesque et al. 1992). Hence, these results indicated that the *R*(+)-7-OH-DPAT induced emesis is not mediated via the dopamine D_4 receptor subtype. The structure of the dopamine receptor ligands are given in Fig.1.7.



7-OH-DPAT SKF 38393

Fig. 1.7. Dopamine receptor ligands

1.11. Substance P (SP) Receptor Antagonists

The principal members of the mammalian peptide tachykinins are Substance P (SP), Neurokinin A and Neurokinin B, which bind selectively to the three corresponding neurokinin receptors, NK_1, NK_2 and NK_3. Among these tachykinins, SP has gained much attention due to its agonistic action on NK_1 receptor, which is believed to be involved in the emetic pathway.

SP is found in neurons, particularly in vagal afferent fibers innervating the brainstem NTS (which send impulses to VC) and the AP, when exogenous SP applied to cells in the NTS induces emesis (Gardner et al. 1996). SP acts via the G-Protein coupled NK_1 receptors to generate an inositol phosphate second messenger and thereby exert its biological effects (Otsuka and Yoshioka 1993).

Although early antagonists based on modifications of SP itself did provide peptide reagents useful in the laboratory, a real breakthrough in this field came with the discovery of nonpeptide antagonists. The first of these resulted from SAR studies around a lead structure discovered by empirical file screening with the optimization of this template resulting in the potent and NK_1 -selective compound CP 96,345 (IC_{50} = 0.77 nM in human IM-9 cells). The disclosure of a structurally related piperidine with increased potency at the NK_1 receptor CP 99,994 indicated an additional optimization in this series of antagonists. Subsequently, several structurally unrelated nonpeptide SP antagonists have also been reported including RP-67,580 (IC_{50} = 4.16 nM for NK_1 binding in rat brain) and CGP 47,899 (IC_{50} = 10 nM). Fermentation screening has also yielded structurally novel NK_1 antagonists with cyclic peptide FK 224 showing a mixed spectrum of NK_1 (IC_{50} = 37 nM) and NK_2 (IC_{50} = 72 nM) antagonism. Modification of FK 224 resulted in the compound FK-888 with enhanced potency and selectivity at the NK_1 receptor (Snider and Lowe 1997). Structures of NK_1 RAs are given in Fig. 1.8.

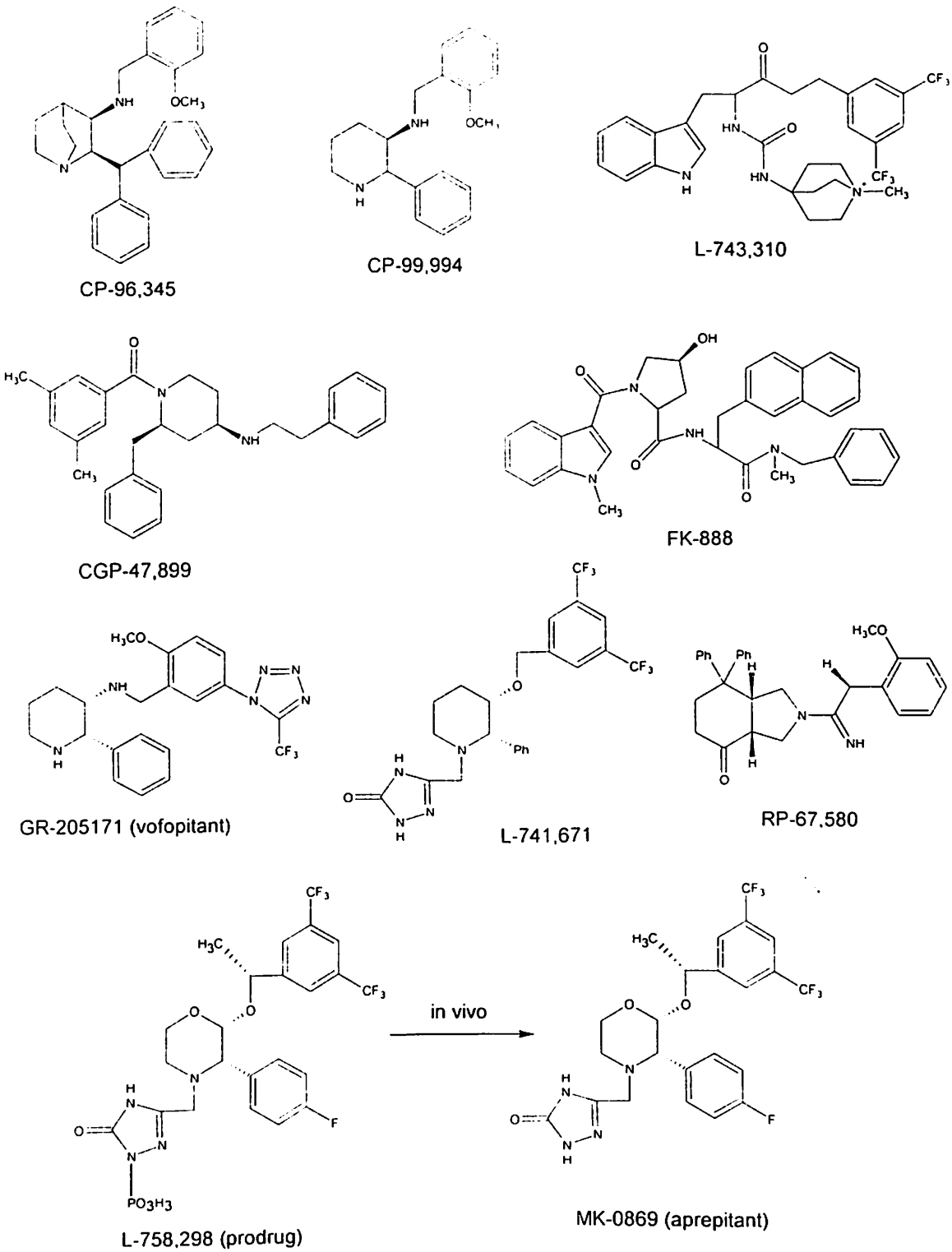


Fig. 1.8. NK_1 receptor antagonists

In a clinical trial (Hesketh et al. 2003), it has been observed that patients who received 5-HT_3 RAs had better protection of N&V that occurred within 12 hrs, whereas patients who received NK_1 RAs (aprepitant) had better protection of N&V that occurred after 12 hrs. Patients who received both drugs had superior control of symptoms compared with patients who received one or the other. This study provides substantial evidence for the involvement of separate pathophysiological mechanisms in CINV. Serotonin mediates the early vomiting processes that occur within 12 hrs following cisplatin-based chemotherapy, after which time substance P acting at NK_1 receptors becomes the dominant mediator for vomiting.

These results suggest that serotonin may heavily influence the 'early' acute emesis while Substance P is a primary mediator of 'later' acute and delayed emesis. Currently, the combination of a 5-HT_3 receptor antagonist, NK_1 receptor antagonist and dexamethasone was shown to provide better control of acute and delayed emesis among the existing therapy options.

Although the efficacy of aprepitant (a NK_1 receptor antagonist) has been proven, there is a need for more study on the effectiveness in subsequent cycles of chemotherapy. As the treatment guidelines often include metoclopramide for the prevention of delayed emesis, aprepitant should be compared with such regimens. At present aprepitant can only be used with highly emetogenic chemotherapy, including high-dose cisplatin.

1.12. Other Receptors / Neurotransmitters Involved in CINV

1.12.1. Corticosteroids

The anti-emetic action of glucocorticoids to reduce chemotherapy-induced emesis has been hypothesized to involve partly a reduction of prostaglandin synthesis via the inhibition of phospholipase A_2 and by preventing cyclo-oxygenase-2-expression (Sam et al. 2001). This hypothesis is supported by studies in piglets showing the anti-emetic activity of cyclo-oxygenase inhibitors to reduce cisplatin-induced emesis (Girod et al. 2002); in addition to reports that depict a reduction of radiation-induced emesis in dogs (Carpenter et al. 1986).

Steroids are sometimes used as a single agent against mild to moderate emetogenic chemotherapy, but are more often used in anti-emetic drug combinations (Chiara et al. 1987). Dexamethasone is the drug of choice in N&V in patients receiving radiation to the brain as it reduces cerebral edema. Anti-emetic efficacy in patients receiving cisplatin rises from 40-50% to 80% when dexamethasone is co-administered with a 5-HT_3 RA or metoclopramide. With moderately emetogenic agents such as cyclophosphamide, the rate of complete control rises to over 90% when a 5-HT_3 RA is co-administered with dexamethasone (Carpenter et al. 1986).

1.12.2. Benzodiazepines

Benzodiazepines such as lorazepam and alprazolam, by themselves are not very effective anti-emetics, but their sedative, amnesic and anti-anxiety effects are helpful in reducing the anticipatory component of N&V (Greenberg et al. 1987). In addition, they markedly decrease the severity of extra pyramidal side effects, associated with dopaminergic receptor antagonists.

1.12.3. Cannabinoids

Cannabinoids presumably target higher CNS centres to prevent mild to moderate N&V. Dronabinol (δ-9-tetrahydro cannabinol) may be used as a prophylactic agent in patients receiving chemotherapy when other anti-emetic medications are not effective. Generally cannabinoids are not useful as first line anti-emetics because of their side effects: dysphoria, hallucination, sedation, etc. It has been proposed that synthetic cannabinoids may activate μ-opiate receptor in a so-called 'anti-emetic' centre, which is supposed to keep the VC in an inhibited state (Vincent et al. 1983; Costello and Borison 1977).

1.13. Alternative Methods for the Management of CINV

1.13.1. Natural Remedies for CINV

Powdered rhizome of ginger (*Zingiber officinale* Roscoe, Zingiberaceae) has long been used in traditional medicine for alleviating the symptoms of gastrointestinal disorders. It has been reported that ginger was used in the treatment of excessive and uncontrolled vomiting that occur in the first trimester of pregnancy (Fisher-Rasmussen et al. 1990), experimentally-induced motion sickness (Stewart et al. 1990) and Cisplatin, cyclophosphamide-induced emesis in *Suncus murinus* (Yamahara et al. 1989). It has been published that the acetone extract of ginger contains a diterpenoid, galanolactone (Fig. 1.9), which possesses 5-HT_3 receptor antagonist activity (Huang et al. 1991). However, it is not known whether the antagonism of galanolactone is selective or non-selective for the receptor. The free radical scavenging activity of gingerol (one of the main constituent of ginger) has also been reported (Krishnakantha and Lokesh 1993). Free radicals play an important role in chemotherapy-induced emesis; antioxidants, e.g., glutathione, tiopronin, vitamin C and vitamin E, prevented cisplatin-induced emesis in dogs (Gupta and Sharma 1996).

Hence, the anti-emetic action of ginger against cisplatin-induced emesis is probably due to 5-HT_3 receptor blocking action and/or free radical scavenging activity. As a well-accepted traditional medicine, ginger is worth considering for further clinical evaluation as an anti-emetic against CINV.

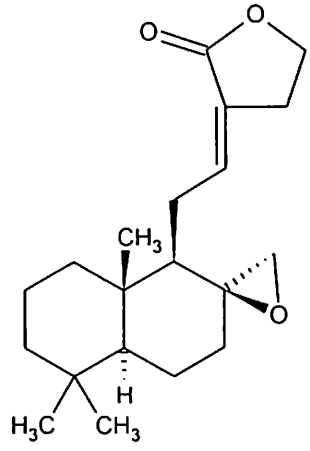


Fig. 1.9. Galanolactone

1.13.2. Adjunct Therapy for CINV

Evidences support that the stimulation of acupuncture points, particularly the *Neiguan* (P6) acupuncture point (located on the inside of the wrist) is helpful in controlling N&V (Mayer 2000; Roscoe and Matterson 2002), while no theory that is generally accepted by the scientific community adequately explains as how the stimulation of P6 acupuncture point reduces N&V. However, studies have shown that the efficacy of needling (acupuncture), mild electrical stimulation (acustimulation) or constant pressure (acupressure) to the P6 acupuncture point reduces N&V associated with motion sickness (Hu et al. 1995; Bertolucci and Didario 1995), anesthesia (Fan et al. 1997; Harmon et al. 1999), pregnancy (Dealoysio and Penacchioni 1992; Slotnick 2001) and chemotherapy (Dundee et al. 1991; Roscoe et al. 2002).

The efficacy of acupressure and acustimulation wristbands for the relief of CINV was studied by the Behavioral Medicine Unit, University of Rochester Cancer Centre, New York, USA. In this study (Roscoe et al. 2003), ninety participants were randomly assigned to receive standard treatment (control group), standard treatment with acupressure band or standard treatment with acustimulation band. Patients, who received acustimulation showed better control of N&V, compared to control group. Patients, who received acupressure band showed better tolerance to nausea on the day of treatment, compared to control group. However, these bands didn't show significant effect in delayed N&V. It cannot be ascertained from this study as why these bands were helpful on the day of treatment but not on the following days. It may be related to the fact that the acute and delayed N&V have different etiologies, as evidenced by the fact that the 5-HT_3 class of anti-emetics is more effective than the old line anti-emetics for CINV but are less effective than the older drugs for the control of delayed N&V.

5-HT_3 Receptors and Anxiety

Traditionally, pharmacological research in the area of anxiety and stress treatment is very much influenced by the availability of anxiolytic drugs. Throughout recorded history ethanol was the standard drug for the (self) treatment of feelings of discomfort, tension, anxiety and stress. Mankind has sought purposefully for substances that modify these feelings and in the last century compounds such as bromide salts, paraldehyde and chloral hydrate were used in medical practice for such symptoms.

In the early 1900's, the barbiturates were introduced, which were by far the dominant anxiolytic agents throughout the first half of the twentieth century. But considerable concern arose about their safety, leading to the search for better alternatives. This search resulted in the development of compounds like meprobamate, but these compounds, which were still considered 'risky', were rapidly abandoned when the benzodiazepines were discovered and introduced to the market in the late fifties and early sixties. The benzodiazepines (BDZ) have been, and still are, the drugs of choice for the treatment of anxiety and stress over the last three decades because of their effectiveness and relative safety. As with most other drugs, continued use revealed side effects, the most serious of them being tolerance and both psychological and physical dependence.

Once again this has led to the search for new and better anxiolytics without the side effects of the BDZ. Serotoninergic agents seem to be promising in this regard. The accidental finding of buspirone, a 5-HT_{1A} receptor agonist having anxiolytic effects (Goldberg and Finnerty 1979), focused on the role of serotonin in anxiety processes, leading to the development of various 5-HT_{1A}-anxiolytics, like ipsapirone, gepirone, flesinoxan, etc. (Yocca 1990; Glennon et al. 1991).

It has been classically accepted that the 5-HT neurons promote anxiety in humans as well as in animal models and hence it has been hypothesized that drugs that reduce 5-HT function will be effective anxiolytic agents (Traber and Glaser 1987; Kennet 1991). Yet results indicate that the selective serotonin reuptake inhibitors (SSRIs), which primarily increase synaptic 5-HT levels are effective in anxiety disorders, with a broader spectrum of action than the benzodiazepines (Handley 1995). The 5-HT neurons originate from the raphe nuclei that occupy the midline of the midbrain and medulla, the proposed origins of the behavioural inhibition system (functions to suppress behaviour that is under the threat of contingent punishment or of non-reward) (Lovick et al. 1993). An important element controlling 5-HT release throughout the brain is the serotoninergic auto receptors. The 5-HT_{1A} auto receptor is present on the soma and dendrites of 5-HT neurons and exerts a negative feedback influence on their firing activity (Dourish et al. 1986). The other post-synaptic serotoninergic receptors that have been investigated for their role in effective disorders include 5-HT_{1A}, 5-HT_{2C}, and 5-HT_3 receptors (Chopin and Briley 1987; Handley 1991).

Halfway through the eighties, 5-HT_3 receptors were identified and this was soon followed by the synthesis of 5-HT_3 RAs. In 1987, the first reports apperared describing the anxiolytic effects of the 5-HT_3 RA, ondansetron, in different animal species and models (Costall et al. 1987), followed by numerous investigations on this and other 5-HT_3 RAs, like tropisetron (ICS 205930), bemesetron (MDL 72222), granisetron (BRL 43694) and others (Kilpatrick et al. 1990).

At least nine murine studies using the elevated plus-maze model (Andrews and File 1993b; Artaiz et al. 1995; Blackburn et al. 1993; Costall and Naylor 1991; Costall et al. 1989, 1993; Dunn et al. 1991; Filip et al. 1992; Piper et al. 1988) have demonstrated anxiolytic-like actions of 5-HT_3 RAs. Consistent with these findings, one study using this model, found anxiogenic-like activity in rats treated with the 5-HT_3 receptor agonist, *m*-CPBG (Andrews and File 1993a).

There have been also conflicting findings. At least four elevated plus-maze studies have found no anxiolytic effects (File and Johnson 1989; Griebel et al. 1997; Piper et al., 1988; Wright et al. 1992) and three studies found anxiolytic effects for some 5-HT_3 antagonists, but not for others (Artaiz et al. 1995; Filip et al. 1992; Pollack and Gould 1996). Numerous studies using murine light-dark box (Artaiz et al. 1995; Barnes et al. 1990, Bill et al. 1992; Costall et al. 1988a, 1993; Eglen et al. 1994; Gao and Cutler 1992, Jones et al. 1988; Kilfoil et al. 1989; Young and Johnson 1991a,b) and social-interaction (Costall et al. 1988, 1993; Gao and Cutler 1992) models of anxiety have found anxiolytic-like activity for 5-HT_3 antagonists; however, negative findings have also been reported in these models as well (Andrew and File 1993a; Barnes et al. 1990; File and Johnson 1989; Morinan 1989; Mos et al. 1989). Dosing may have been a factor in some of the negative findings. Additionally, many methodological factors are known to affect outcomes in these models. Such factors include age, species and strain differences, circadian factors and the nature of the behavioral measurements recorded (Griebel et al. 2000; Rodgers et al. 1992). Physical and procedural variables such as manipulation of the animals prior to testing, prior exposure to novel environments and presence of the experimenter during testing have been reported to alter or even abolish the anxiolytic-like effects of active compounds, including 5-HT_3 RAs (Andrew and File 1993a; Rodgers and Shepherd 1993).

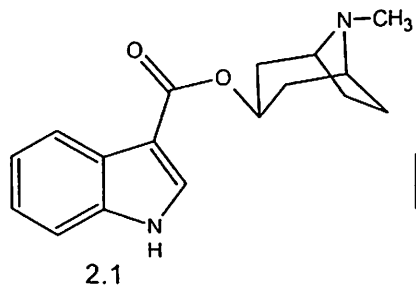
Chapter II
Literature Review

CHAPTER II

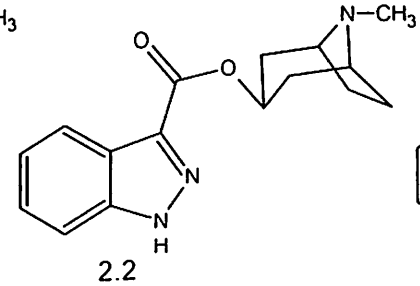
LITERATURE REVIEW

A large number of 5-HT_3 RAs have been prepared with the aim to develop possible anti-emetic drugs for the treatment of cancer chemotherapy-induced nausea and vomiting. Cocaine and metoclopramide were the first compounds discovered to exhibit the 5-HT_3 receptor antagonistic activities. Metoclopramide is also the dopamine D_2 antagonist and 5-HT_4 agonist. Since metoclopramide has the affinity towards different receptors, it produces various side-effects, in particular, extra pyramidal side-effects. Since then, many aromatic acid esters and amides having bridged bicyclic amine structures have been synthesized and tested for 5-HT_3 receptor antagonistic activities.

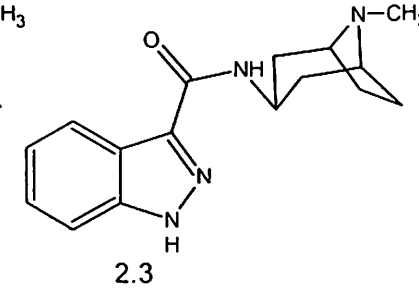
2.1. Fludzinski et al. (1987) synthesized Indazole and Indole derivatives for 5-HT_3 antagonistic activities. In this series, the compounds **2.1** and **2.2** showed ED_{50} of 0.004 & 0.003 mg/kg, *i.v.*, respectively in the inhibition of the Benzold-Jarisch (BJ) reflex in the rats (a method to evaluate 5-HT_3 RAs) whereas the corresponding amide derivative, compound **2.3**, showed ED_{50} 0.002 mg/kg, *i.v.* In another study (Robertson et al. 1990), compound **2.3** showed binding affinity K_i of 4.26 nM, whereas ondansetron showed K_i of 2.46 nM in the rat cortical membranes.



2.1



2.2



2.3

2.2. Smith et al. (1988) studied the emetic activity of centrally administered cisplatin in cats and its antagonism by zacopride. Cisplatin administered by either the *i.v.* or *i.c.v.* route produced emesis in cats. The average time of onset of emesis was decreased significantly (4.0 min Vs 100.6 min) when cisplatin was administered *i.c.v.* Zacopride administered either *i.c.v.* (0.02 mg) or *i.v.* (0.1 mg/kg) completely blocked the emesis due to cisplatin given by either route. These results showed that cisplatin possessed a central emetic component and that was blocked by zacopride.

2.3. Butler et al. (1988) reported the pharmacological properties of GR 38032F, a novel antagonist on 5-HT_3 receptors. On the isolated vagus nerve and superior cervical ganglion of the rat, (*R*)(*S*)-GR 38032F behaved as a reversible competitive antagonist of 5-HT induced depolarization with pK_B values of 8.61 and 8.13, respectively. The resolved *R*- and *S*-isomers showed pK_B values of 8.95 and 8.63, respectively. On the longitudinal muscle of the guinea-pig ileum, (*R*)(*S*)-GR 38032F showed potent 5-HT_3 antagonistic activity followed by *R*- and *S*-isomers (pK_B 7.31, 7.20 and 6.30, respectively) against 2-methyl-5-HT. On the isolated heart of rabbit, low concentrations of (*R*)(*S*)-GR 38032F ($3 \times 10^{-11} - 1 \times 10^{-9}$ M) antagonized the positive chronotropic effect of 5-HT and 2-Me-5-HT. In the BJ reflex in rats (measuring the ability to antagonize the bradycardic response to 2-Me-5-HT, 100 µg/kg, *i.v.*) (*R*)(*S*)-GR 38032F showed ED_{50} value of 0.4 µg/kg, *i.v.* and 7.0 µg/kg, *p.o.*

2.4. Jones et al. (1988) reported the potential anxiolytic activity of GR 38032F, a 5-HT_3 receptor antagonist. In the social interaction test in rats and in a light/dark exploration test in mice, GR 38032F dose-dependently released the suppressed behavior, without modifying locomotor activity. In the cynomolgus monkey and the marmoset, GR 38032F reduced anxiety-related symptoms without causing sedation. In the marmoset, the effects were clearly dose-related. In the water-lick conflict test in rats, GR 38032F did not show any detectable activity. This study revealed that GR 38032F was a potential anxiolytic agent without sedative, anticonvulsant or hypnotic activity.

2.5. Peroutka et al. (1988) evaluated the [^{3}H]-quipazine labeled 5-HT$_3$ recognition sites in rat cortical membranes. When increasing concentrations of [^{3}H]-quipazine (0.1 – 15 nM) were incubated with rat cortical membranes, the radioligand displayed high affinity (K_d = 1.3 ± 0.1 nM; n = 6) for a single population of binding sites. The density of sites in rat cortex was 2.9 ± 0.5 pmol/gm of wet weight tissues. Drug competition studies demonstrated that ICS 205-930 displayed high affinity for [^{3}H]-quipazine labeled sites (K_i value 0.55 ± 0.04 nM; n = 3). Quipazine was slightly potent and its apparent K_i value (1.0 ± 0.2 nM) was consistent with its K_d value obtained from saturation studies. MDL 72222 and mCPP displayed affinities of approximately 10 nM for [^{3}H]-quipazine labeled binding studies. 5-HT and metoclopramide were equipotent at these membrane recognition sites, with K_i values equal to 190 nM. By contrast, 8-OH-DPAT, *d*-LSD, mesulergine and ketanserin were significantly less potent at [^{3}H]-quipazine labeled sites in rat cortex.

2.6. Glennon et al. (1989) investigated the binding affinities of a series of arylpiperazine derivatives at [^{3}H]-quipazine-labeled central 5-HT$_3$ sites. Features determined to be important for binding of quipazine include the N^4 piperazine nitrogen atom (but not the N^1 piperazine nitrogen) and a quinolinyl group. The quinoline nitrogen atom of quipazine also contributes to the affinity and its replacement by carbon reduces the affinity by 20 fold. The entire quinoline nucleus is not necessary for binding; certain monocyclics with a chloro group *meta* to the piperazine ring (e.g. mCPP, MK-212) also bind at 5-HT$_3$ sites with one order of magnitude less than that of quipazine. Tertiary amine of quipazine i.e. *N*-methylquipazine (NMQ), binds very similar to that quipazine, however, unlike quipazine, NMQ showed very little affinity (IC_{50} >10,000 nM) for central 5-HT$_{1B}$ sites.

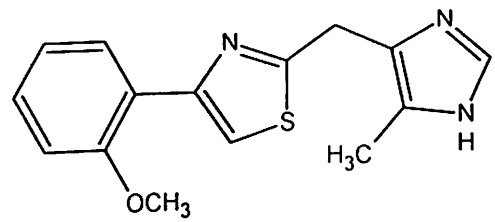
2.7. Nelson et al. (1989) studied the [^{3}H]-BRL 43694 (granisetron), a specific ligand for 5-HT_3 binding sites in rat brain cortical membranes. This study demonstrated that [^{3}H]-BRL 43694 labeled potently (K_d = 0.30 nM) 5-HT_3 recognition sites in rat cortical membranes where the number of binding sites was quite low (B_{max} = 1.29 fmol/mg, tissue) as compared with the central 5-HT_2 binding sites (B_{max} = 30.9 fmol/mg tissue). Drug competition studies showed that known 5-HT_3 receptor antagonists, GR 38032F, ICS 205-930, zacopride, quipazine, MDL 72222, BRL 43694 and BRL 24682 (K_i = 0.87 ± 0.12, 0.40 ± 0.06, 0.10 ± 0.01, 1.23 ± 0.15, 5.3 ± 0.4, 0.26 ± 0.04, 0.33 ± 0.07 nM, respectively), displaced potently specific [^{3}H]-BRL 43694 binding. Metoclopramide (K_i = 160 nM) was a moderate inhibitor of [^{3}H]-BRL 43694 binding. The agonists, 5-HT (K_i = 160 nM) and 2-Me-5-HT (K_i = 620 nM) also inhibited specific binding. Drugs known to interact with other 5-HT receptor subtypes, e.g. ritanserin (K_i = 5300 nM), ICI 169,369 (K_i = 990 nM), spiperone (K_i >10,000 nM), metergoline (K_i = 7400 nM), methylsergide (K_i > 10,000 nM), methiothepin (K_i = 3000 nM) and 8-OH-DPAT (K_i > 10,000 nM) displaced only weakly.

Indirect evidence suggest that [^{3}H]-BRL 4694 binding sites in rat cortex could represent functional receptors is provided by the good correlation (r = 0.98) between the inhibition of [^{3}H]-BRL 43694 binding to central tissue and the inhibition of the 5-HT-evoked BJ reflex in the rat (a functional assessment of peripheral 5-HT_3 receptor antagonism) for compounds tested in both systems.

2.8. Higgins et al. (1989) reported that the 5-HT_3 RAs injected into the area postrema inhibited the cisplatin-induced emesis in the ferrets. This study investigated the role of 5-HT_3 receptors in the area postrema in the control of cisplatin-induced emesis in the ferret. Homogenate binding and autoradiography using the high affinity 5-HT_3 receptor ligand, [^{3}H]-GR 65630, identified the presence of high concentration of 5-HT_3 receptors in the area postrema of the ferret.

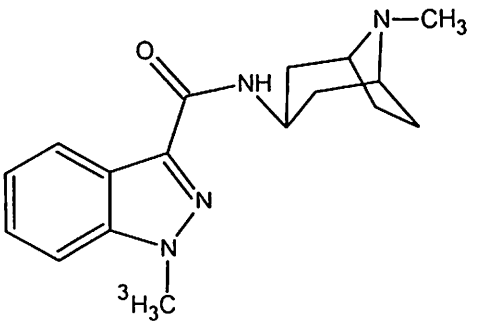
Intraperitoneal injection of 5-HT_3 RAs, GR 38032F (1 mg/kg), GR 65630A (0.1 mg/kg) and MDL 72222 (1 mg/kg), inhibited the cisplatin (9 mg/kg, *i.p.*) induced emesis. When 5-HT_3 RAs, GR 38032F (0.01 – 1 µg), GR 65630A (0.001 – 0.1 µg) and MDL 72222 (0.1 – 10 µg), were injected directly into the area postrema region, they were found to inhibit cisplatin-induced (9 mg/kg; *i.p.*) emesis. These results confirmed the role of 5-HT_3 receptors, in the control of cisplatin-induced emesis, and showed that at least one functional site for these receptors in modulating the emetic response in the area postrema, the locus of the CTZ.

2.9. Rosen et al. (1990) reported a novel class of highly potent and selective 5-HT_3 RAs. The compounds in this series contain a thiazole moiety linking an aromatic group and a nitrogen-containing basic region; where the thiazole group appeared to be acting as a carbonyl bioisoster in this system. An optimized member of this series, compound **2.4** (100 µg/kg, *i.v.*), inhibited the BJ reflex completely (100%) in rats induced by 5-HT (100 µg/kg, *i.v.*). At the same dose level, ondansetron and ICS 205-930 inhibited only 89.7% and 77.5%, respectively. Compound **2.4**, showed more potent binding affinity (K_i = 0.42 nM) to the 5-HT_3 receptors than ondansetron and ICS 205-930 (K_i = 16.2 and 2.7 nM, respectively) in the displacement of [^{3}H]-ICS 205-930 binding to neuroblastoma-Glioma NG-108-15 cells.



2.4

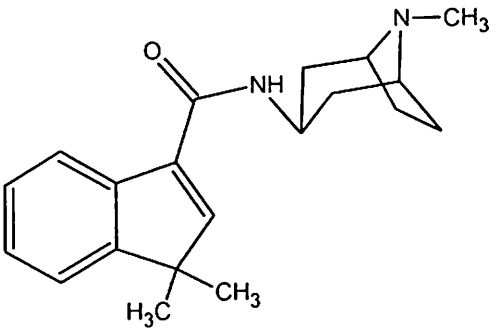
2.10. Robertson et al. (1990) synthesized tritium labeled indazole-3-carboxamide derivatives and evaluated for their binding affinity to 5-HT_3 receptors. Alkylation of *N*(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide with sodium hydride and tritium-labeled iodomethane resulted in **[^{3}H]-2.5**. This radioligand bound with higher affinity to a single class of saturable recognition sites in membranes, isolated from cerebral cortex of rat brain. The K_d was 0.69 nM and the B_{max} was 16.9 fmol/mg of protein. The specific binding was excellent, and accounted for 83-93% of total binding at concentrations of 2 nM or less. The potencies of known 5-HT_3 RAs as inhibitions of **[^{3}H]-2.5** binding, correlated well with their pharmacological receptor affinities as antagonists of 5-HT-induced decrease in heart rate and contraction of guinea-pig ileum. This study suggested that the central recognition site for this radioligand might be extremely similar to or identical with peripheral 5-HT_3 receptors.



[^{3}H]-2.5

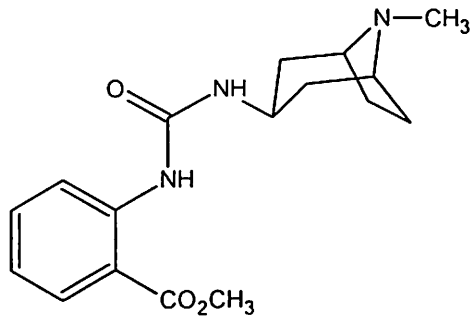
2.11. Bermudez et al. (1990a) synthesized Indazole and Indolizine-3-carboxylic acid derivatives for 5-HT_3 RAs. In this series, BRL 43694 (granisetron) antagonized the 5-HT evoked tachycardia on isolated heart of rabbit (pA_2 = 10.6). In addition, it showed a high affinity for central 5-HT_3 sites (K_i = 0.59 nM for displacement of [^{3}H]-GR 65630) but very little affinity for other receptor sites (5-HT_1; K_i >10,000 nM, 5-HT_2; K_i >1000 nM). In ferrets, BRL 43694 (2 x 0.5 mg/kg, *i.v.*) totally inhibited the emesis evoked either by cisplatin (10 mg/kg, *i.v.*) or by the combination of doxorubicin (6mg/kg, *i.v.*) and cyclophosphamide (8 mg/kg, *i.v.*).

2.12. Bermudez et al. (1990b) reported a novel series of potent 5-HT_3 RAs, 1-indolinecarboxamides. The activity of the indoline suggested that the aromaticity of the 5-membered ring was not an essential requirement for the potency whereas the "in plane" orientation of the carbonyl group favoured 5-HT_3 antagonistic activity. Based on this hypothesis, indene, compound **2.6**, was prepared in which the "in plane" orientation of the carbonyl group was maintained by conjugation with the aromatic ring through the sp^2 hybridized carbon. It was also found to be a potent 5-HT_3 receptor antagonist. In the inhibition of BJ reflex in rats, the compound **2.6** showed ID_{50} value of 1.0 µg/kg, *i.v.*, whereas BRL 43694 showed 0.7 µg/kg, *i.v.*



2.6

2.13. Bermudez et al. (1990c) reported a novel series of *ortho* substituted phenylureas as potent 5-HT_3 RAs. Five membered ring of indazoles (e.g. BRL 43694) and indolines have been replaced by an intramolecular hydrogen bond. In this series, different *ortho* substituted phenylureas and carbamates were prepared. The compound **2.7** exhibited potent 5-HT_3 receptor antagonist by inhibiting BJ reflex (ID_{50} 2.1 µg/kg, *i.v.*) evoked by 5-HT in rats, whereas ICS 205930 showed an ID_{50} value of 1.4 µg/kg, *i.v.*



2.7

2.14. Andrews et al. (1990) reviewed the response of various animals to different emetic stimuli. From the study conducted on different animals for various emetic stimuli, it was concluded that the ferrets were sensitive to a wide range of emetic stimuli including intragastric irritants, opiates, dopamine receptor agonists, cytotoxic drugs and radiation. It was proposed that (in the ferret) 5-HT_3 RAs have their main anti-emetic effect by acting on vagal afferent terminals in the walls of the upper gut with additional minor sites either in the NTS or presynaptically on the vagal afferent terminals in the medulla, where binding sites for 5-HT_3 receptor ligands have been demonstrated in this species.

2.15. Marty et al. (1990) studied the comparison of the 5-HT_3 RA, ondansetron (GR 32032F) with high dose metoclopramide, in the control of cisplatin-induced emesis. In a double-blind cross over study in 97 patients, cisplatin was administered (80 to 100 mg per square meter of body-surface area) for the treatment of cancer. None of the patients received chemotherapy before this trial. Ondansetron (8 mg, *i.v.*) was given as a loading dose before cisplatin administration and then as a continuous infusion (1 mg/hr) for 24 hours. Metoclopramide (3 mg/kg, *i.v.*) was given as a loading dose and infused (0.5 mg/kg/hr) for 8 hr; placebo was infused for the next 16 hours.

Among the 76 patients who satisfactorily completed both parts of the study (ondansetron & metoclopramide), ondansetron showed better control of emesis than metoclopramide (75% Vs 42%). There was significant preference among patients for ondansetron (55% Vs 26%). Dystonic reactions were observed during treatment with metoclopramide. From this study it was concluded that ondansetron was more effective than metoclopramide in the control of cisplatin-induced N&V, which also suggested that serotonin is an important mediator of these side-effects.

2.16. Yakel et al. (1990) studied the selectivity of the channel coupled to the 5-HT_3 receptor. The 5-HT_3 receptor was unusual among receptors of biogenic amines in that it was directly coupled to an ion channel that was highly permeable to Na^+, K^+. The authors have studied the permeation properties of this channel in order to achieve a more detailed understanding of its physiological function and to extend the comparison with other ligand gated ion channels. The 5-HT_3 receptor channel was found to be significantly permeable to the organic cations tris, choline, and *N*-methyl-glutamine, with permeability decreasing with size. The permeability ratios for tris and choline were similar to the nAch; the permeability ratio for tris was similar to the non-NMDA excitatory amino acid receptor. This suggested that the diameters at the narrowest parts of these three channels were similar. For 5-HT_3 receptor channels, the Ca^{2+} permeability and single channel conductance (determined by noise analysis) were lower than that of the nAch receptor. In these respects, the 5-HT_3 receptors were closer to non-NMDA excitatory amino acid receptors. The results were interpreted in terms of a model of the 5-HT_3 receptor channel in which the interior had a lower polarizability and possibly a greater length, in comparison with the nAch receptor channel.

2.17. Jones et al. (1990) extensively reviewed the 5-HT_3 RAs in anxiety and schizophrenia. The BDZ, used to treat anxiety are becoming notorious for their dependence liability and even the latest of the neuroleptics used to treat schizophrenia are bedeviled by side-effects. In autoradiography studies of the human brain, it was found that the highest concentrations of 5-HT_3 receptor were in cortical and limbic structures, where the underlying disturbances in anxiety and schizophrenia might be located. The area postrema of the hindbrain had a very high density of binding sites. This could be the possible site for activating 'vomiting centre' after chemotherapy.

Ondansetron, was very effective in a social interaction test (a model for studying anxiety/anxiolytic effect) in remarkably low doses. Unlike the BDZ, 5-HT_3 RAs were not active in conflict models of anxiety, where punishment is associated with reward. Furthermore, they did not have sedative, anticonvulsant or muscle-relaxant actions.

5-HT_3 RAs were inactive in the traditional test for antipsychotic activity. For example, they didn't reverse apomorphine- or amphetamine-induced stereotyped behavior and didn't induce catalepsy. However, when they were tested in sophisticated models, very exciting results were obtained. These models are simulations of the pathological disturbances that are believed to occur in schizophrenia, which is a selective overactivity of the mesolimbic dopaminegic system.

2.18. Butler et al. (1990) studied the pharmacological characterization of 5-HT_3 receptors in three isolated preparations derived from guinea-pig tissues. The tissues used were ileum (longitudinal muscle-myenteric plexus), colon and vagus nerve. In the guinea-pig isolated ileum, 5-HT ($1 \times 10^{-3} - 1 \times 10^{-5}$ M) and the selective 5-HT_3 agonist, 2-Me-5-HT ($3 \times 10^{-7} - 1 \times 10^{-4}$ M) caused concentration-related contractions. The EC_{50} for 2-Me-5-HT was 1.23×10^{-5} M.

Selective 5-HT_3 RAs caused rightward shifts of the 2-Me-5-HT dose-response curve. Neither ketanserin (1×10^{-6} M) nor methylsergide (1×10^{-5} M) antagonized the responses to 5-HT or 2-Me-5-HT.

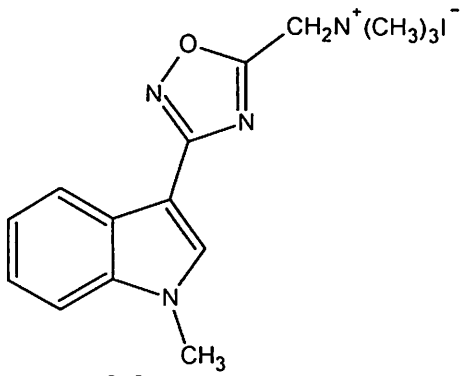
In the isolated guinea-pig colon, 5-HT ($3 \times 10^{-7} - 3 \times 10^{-5}$ M; EC_{50} 2.4×10^{-6} M) caused contractions, which were mimicked by 2-Me-5-HT ($1 \times 10^{-6} - 1 \times 10^{-4}$ M; EC_{50} 7.2×10^{-6} M). Selective 5-HT_3 receptor antagonists caused rightward displacements of the 5-HT dose-response curves. Neither ketanserin (1×10^{-6} M) nor methysergide (1×10^{-5} M) had any effect on responses to 5-HT or 2-Me-5-HT.

In the guinea-pig isolated vagus nerve, 5-HT ($1 \times 10^{-6} - 3 \times 10^{-4}$ M) and 2-Me-5-HT ($1 \times 10^{-5} - 1 \times 10^{-3}$ M; EC_{50} 7.6×10^{-5} M) caused depolarizations; at higher concentrations there were after-hyperpolarizations. The maximum response to 2-Me-5-HT was less than half as compared to that of 5-HT. Selective 5-HT_3 RAs caused rightward displacements of the 5-HT dose-response curves. Antagonists at other 5-HT receptors (ketanserin, 1×10^{-5} M and methysergide, 1×10^{-6} M) had no effect.

The estimated affinity values of 5-HT_3 RAs correlated well between the three models. Phenylbiguanide was inactive as an agonist or antagonist (upto 1×10^{-4} M) in each preparation.

2.19. Swain et al. (1991) reported the synthesis and biochemical evaluation of a series of indole oxadiazoles as 5-HT_3 RAs. The key pharmacophoric elements have been defined as basic nitrogen, a linking group capable of H-bonding interactions and an aromatic moiety. The steric limitations of the aromatic binding site have been determined by substitution about the indole ring. Variations of the heterocyclic linking group had shown that though two hydrogen-bonding interactions were possible, only one was essential for high affinity.

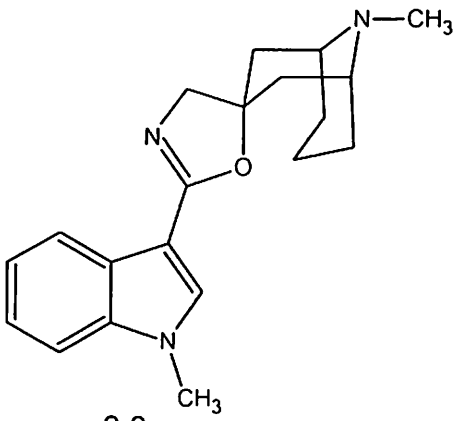
In this series, the compound **2.8** showed more potent activity (pIC_{50} 9.15) in the displacement of [^{3}H]-Q-ICS 205-930, binding to 5-HT_3 recognition sites in rat brain membranes.



2.8

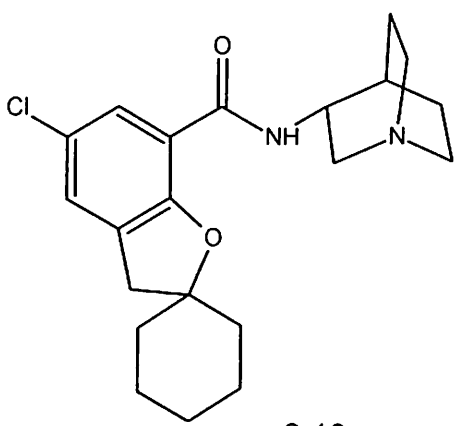
2.20. Swain et al. (1992) reported the synthesis and biochemical evaluation of a series of spirofused indole oxazoline 5-HT_3 RAs. In this series, oxazoline ring acted as a bioisosteric replacement for esters and amides. The effect of substitution about the indole ring has shown the steric limitations of the aromatic binding site. Incorporation of a variety of azabicyclic systems within the rigid spirofused framework allowed the definition of a binding model, which incorporated a number of known antagonists and agonists. In this model, steric constraints limited the substitution around the indole ring although there was some tolerance due to bulkiness of groups at the 1- and 2-postions. The importance of constraining the basic nitrogen within an azabicyclic system was underlined in comparison with monocyclic piperidine.

The highest affinity was observed for those compounds in which the basic nitrogen occupied a bridgehead position. The most potent analogue in this group being the azabicyclic [3.3.1] system, compound **2.9** (pIC_{50} = 8.95), suggested that the lipophilic interactions may play a role in increasing affinity.



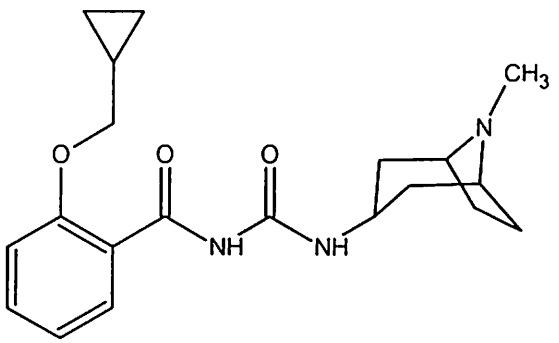
2.9

2.21. Youssefyeh et al. (1992) reported the development of novel benzamides, which were orally active, highly potent and specific antagonists of 5-HT_3 receptors. In radio-ligand binding assays, (*S*)-isomer of **2.10**, potently displaced [^{3}H]-GR 65630 binding with rat cortical tissues with a K_i value of 0.19 nM, while (*R*) isomer with 0.89 nM. BRL 43694, GR 38032F and ICS 205-930 showed K_i value of 1.72, 6.16 and 1.66 nM, respectively. (*S*)-**2.10** exhibited potent activity in blocking cisplatin-induced emesis in the ferrets with an ED_{50} value of 9 µg/kg, *p.o.*



2.10

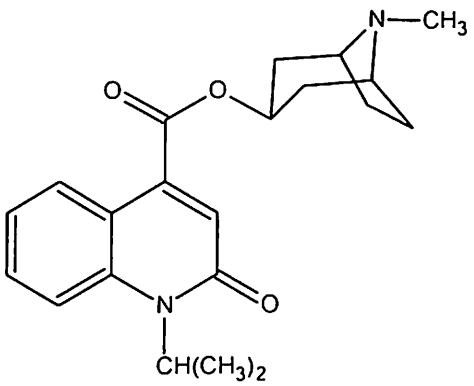
2.22. Bradley et al. (1992) synthesized a series of benzoylureas derived from bicyclic amines and evaluated for 5-HT_3 antagonistic activities on the rat isolated vagus nerve. In this series, those analogues, which were *ortho* substituted by an alkoxy group on the benzoyl function, were shown to be potent 5-HT_3 antagonists with similar or greater potency than the standard antagonist, ondansetron. NMR and X-ray crystallography studies showed that these O-alkoxy compounds existed as a planar, hydrogen-bonded and tricyclic ring system. In molecular modeling studies on *endo-N*-{[(8-methyl-8-azabicyclo[3,2,1]octan-3-yl)amino]-carbonyl}-2-(cyclopropylmethoxy)benzamide (**2.11**), the central hydrogen-bonded ring was able to mimic an aromatic ring proposed for 5-HT_3 RAs.



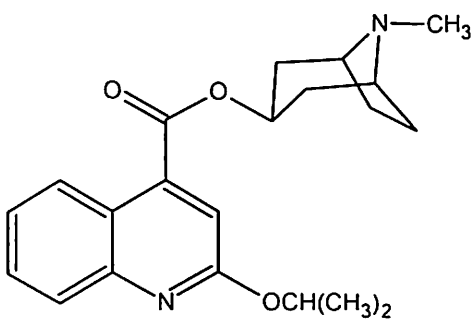
2.11

2.23. Hayashi et al. (1992) synthesized a series of esters and amides of substituted quinoline-4-carboxylic acid containing a basic azabicycloalkyl moiety and evaluated for their affinity towards [^{3}H]-quipazine-labeled 5-HT_3 receptors. In this series, most of the esters exhibited 10-fold more potent activity than that of ondansetron (K_i = 7.6 nM). Lipophilic substituents at the 1- or 2-position of the quinoline ring enhanced the affinity for the receptors. Compounds **2.12** and **2.13** showed the highest affinity (K_i = 0.32 and 0.31 nM, respectively). On the other hand, most of the amides showed 100 fold lower affinity than that of the esters. Although some of the selected compounds exhibited potent activity in the BJ reflex test, good correlation was not observed between the affinity for the 5-HT_3 receptors and the activity in the BJ reflex test (*in-vivo*).

From these data, it was suggested that quinoline derivatives in this series might interact with the 5-HT_3 receptors in a different way from that of the reported 5-HT_3 RAs presumably due to the presence of the heterogeneity of the 5-HT_3 receptors in the brain and the heart.



2.12



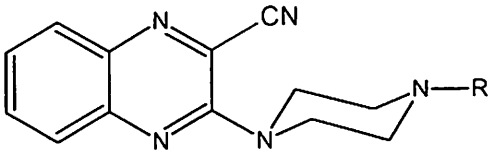
2.13

2.24. Martin et al. (1992) evaluated the opposing roles for 5-HT_{1B} and 5-HT_3 receptors in the control of 5-HT release, in rat hippocampus. Intracerebral microdialysis was performed to determine whether 5-HT release in the ventral hippocampus of rats anaesthetized with chloral hydrate was modulated by 5-HT_3 receptors. It was confirmed that RU 24969, a selective 5-HT_{1B} receptor agonist, decreased 5-HT release in a dose- and concentration-related manner when administered *i.p.* (1 & 5 mg/kg) in rats or via the dialysis probe (0.1 & 1 μM), respectively. When the selective 5-HT_3 agonist, 2-Me-5-HT (0.1 – 10 μM) was infused into the hippocampus for 15 min, the dialysate 5-HT levels increased in a concentration-related manner; this effect was abolished by concurrent infusion of MDL 72222 (1 μm), a selective 5-HT_3 antagonist. This study confirmed that 5-HT_3 and 5-HT_{1B} receptors have opposing roles in the control of 5-HT release in the hippocampus, with 5-HT_3 receptors facilitating and 5-HT_{1B} receptors inhibiting efflux, respectively.

2.25. Sato et al. (1992) evaluated the antagonistic activity of Y 25130 on 5-HT_3 receptors. This study described the 5-HT_3 antagonism of Y 25130 in the rat cerebral cortex, isolated rabbit heart and isolated guinea-pig ileum. In an *in vitro* binding assay, Y 25130 inhibited the specific binding of [^{3}H]-quipazine to 5-HT_3 receptors at the synaptic membranes of the rat cerebral cortex with a K_i value of 2.9 nM, the same as that of ondansetron. Y 25130 showed low affinity for histamine H_1 receptors (IC_{50} = 4.4 μM) but could not reveal any affinities for the other receptors (5-HT_{1A}, 5-HT_2, dopamine D_1, D_2, α_1-adrenoreceptor, α_2-adrenoreceptor, muscarine and benzodiazepine) even at 10 μM concentration. In the isolated rabbit heart, Y-25130 antagonized the indirect sympathomimetic responses to 5-HT (pA_2 = 10.06), which was more than that of metoclopramide. In the isolated longitudinal smooth muscle of the guinea-pig ileum, Y 25130 antagonized the effect of 5-HT (pA_2 = 7.04), which was more than that of metoclopramide. These results indicated that Y 25130 was a potent and selective 5-HT_3 receptor antagonist.

2.26. Monge et al. (1993) and Lasheras et al. (1996) synthesized a series of piperazinylquinoxalines and evaluated them for 5-HT_3 receptor antagonistic activities. Cyanoquinoxaline derivatives with an alkyl substituent on the piperazine moiety showed good antagonism to 5-HT_3 in the longitudinal muscle myenteric plexus of the guinea-pig ileum. The pA_2 values of some selected compounds against the 5-HT_3 agonist, 2-Me-5-HT in the guinea-pig ileum was in the range of tropisetron or ondansetron, and one of the compound **2.16**, was more potent (pA_2 = 10.2) than these reference compounds by approximately 2 or 3 orders of magnitude. However, these compounds were less potent (14c: K_i = 8 nM) than either tropisetron (K_i = 3.8 nM) or ondansetron (K_i = 8 nM) as displacers of [^{3}H]-BRL 43694 binding to rat cortical membranes or as antagonists of BJ reflex in rats. The compounds **2.14** and **2.15**, prevented retches and vomiting (72.6% inhibition) induced by 2-Me-5-HT and cisplatin in the ferrets.

From these data, piperazinlycyanoquinoxalines represented a new class of 5-HT_3 RAs with a selective effect on guinea-pig peripheral receptors.

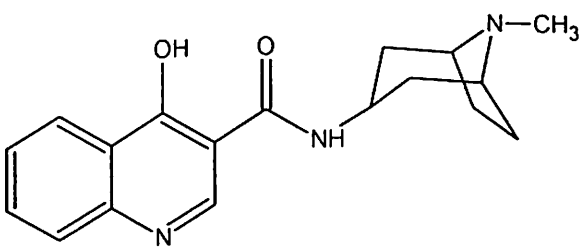


2.14: R = -H

2.15: R = $-C_2H_5$

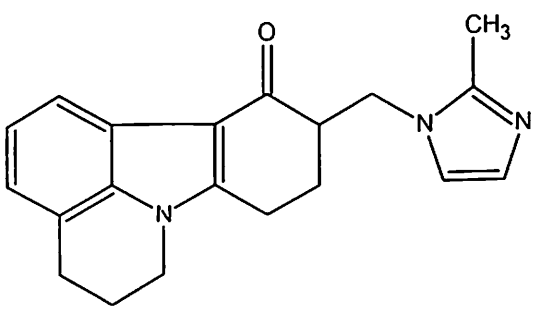
2.16: R = $-CH_2-CH=CH_2$

2.27. Hayashi et al. (1993) designed, synthesized and evaluated a series of 4-hydroxy-3-quinolinecarboxylic acid derivatives as 5-HT_3 RAs. In this series, the compound **2.17** exhibited higher binding affinity (K_i = 1.5 nM) in the [^{3}H]-quipazine labeled 5-HT_3 receptor sites in NG 108-15, whereas ondansetron and granisetron showed less binding affinity (K_i = 7.6 and 2.1 nM, respectively). Comparing the ratios of the ED_{50} value in the BJ reflex test (rat, *i.v.*) with the LD_{50} value in acute lethal toxicity (mouse, *i.v.*), the compound **2.17** was proved to have a 600 fold wider margin of safety than ondansetron. The dose of the compound **2.17** used, dependently attenuated both the incidence and frequency of emetic episodes induced by cisplatin in dogs (ED_{50} = 14 µg/kg, *i.v.*) more potent than ondansetron (ED_{50} = 210 µg/kg, *i.v.*).



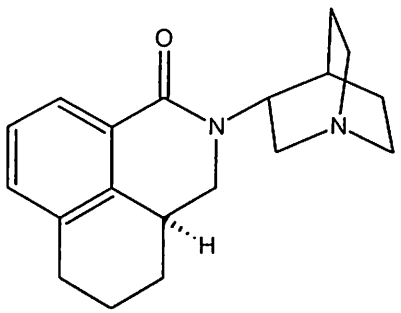
2.17

2.28. Wijngaarden et al. (1993) reported novel 1,7-annelated indole derivatives as potential 5-HT_3 RAs. The new compounds were found to fit the proposed necessary chemical template for binding: a heteroaromatic ring system, a coplanar carbonyl group, and a nitrogen centre at well-defined distances. The receptor binding profile of (-) isomer of **2.18** (cilansetron) showed higher affinity for 5-HT_3 receptors (K_i = 0.19 nM) than ondansetron (K_i = 1.6 nM). The compound (-) **2.18** exhibited a weak affinity for σ-receptors (K_i = 340 nM), muscarine M_1 receptors (K_i = 910 nM) and 5-HT_4 receptors (K_i = 960 nM) and no affinity ($K_i \geq$ 5000 nM) for all the other receptor types tested (n = 37). In the *in vivo* study, cilansetron was found to be more potent with less side-effects than ondansetron. The enhanced potency of the annelated 1,7-indole derivatives indicated that the extra ring provided a favorable hydrophobic area for interaction with the 5-HT_3 receptor site.



2.18 (cilansetron)

2.29. Clark et al. (1993) synthesized and evaluated several series of *N*-(quinuclidin-3-yl)aryl and heteroaryl-fused pyridones for their 5-HT_3 receptor affinity. In the heteroaryl series, the compound (*S*,*S*)-**2.19** was found to possess the highest affinity for 5-HT_3 receptor among the ligands prepared (pK_i = 10.4), which was also more potent than the reference compounds, ondansetron, granisetron and tropisetron (pK_i = 8.5, 8.7 and 9.1 nM, respectively). In the inhibition of BJ reflex in rats, the compound (*S*,*S*)-**2.19** was the most active agent tested (ID_{50} = 0.02 μg/kg, *i.v.*) and this compound was also potent in blocking cisplatin-induced emesis in both, the ferrets and dogs.



2.19

2.30. Tyers et al. (1993) reviewed the anti emetic efficacy of ondansetron against CINV and activity against various CNS disorders like anxiety, cognitive dysfunctions and schizophrenia. In cancer patients, ondansetron (8 or 32 mg) was proven to be more efficacious than high dose metoclopramide against emesis induced by cisplatin (50 – 120 mg/m^2). Ondansetron showed complete protection of emesis in 75 – 80% of patients, whereas metoclopramide was effective in 40 – 60% patients. It was observed that dexamethasome increased the efficacy of ondansetron and metoclopramide treatments.

In CNS, the highest density of 5-HT_3 receptor was found in the hindbrain, which was involved in the physiology of vomiting. However, in other areas like frontal cortex, amygdala and other limbic terminal areas and in the hippocampus, 5-HT_3 receptors were presented in relatively high densities. These observations suggested that 5-HT_3 receptors might have a role in the CNS disorders like anxiety, schizophrenia and cognitive function disorders.

Schizophrenia is believed to be associated with excess mesolimbic dopamine. Stimulation of the mesolimbic system in rats caused behavioral hyperactivity. This response was inhibited not only by the neuroleptic dopamine antagonists, but also by ondansetron. In a preliminary clinical trial in schizophrenic patients, ondansetron (4 mg *b.i.d.* for 4 weeks) produced a significant reduction in BPRS positive and negative symptoms compared with placebo.

Short-term memory losses have been associated with deficiency in cortical acetylcholine. It was observed that administration of 5-HT_3 antagonists removes a potential inhibitory tone on the cholinergic neuron resulting in a net increase in acetylcholine, which enhances cognitive function. Ondansetron was shown to enhance the performance of marmosets in various models. In a clinical trial, ondansetron (0.01, 0.25 and 1 mg *b.i.d.* for 12 weeks), showed a significant improvement in cognitive performance, as assessed using a battery of neuropsychological tests related to learning and memory tasks of daily life.

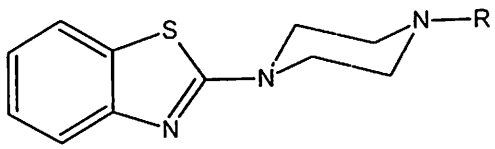
5-HT_3 RAs showed anxiolytic activities in various animals' models like light –dark exploration test, social interaction test and elevated plus-maze in rodents. However, unlike the BDZ, 5-HT_3 RAs were not effective in the water-lick conflict test. In a clinical trial with ondansetron (1 & 4 mg *t.i.d.* for 4 weeks) in generalized anxiety disorder, it was found to have significant anxiolytic activity. Furthermore, at the end of the treatment, there was no rebound anxiety, unlike in diazepam group.

2.31. Kris and Tyson (1993) reviewed the clinical efficacy of tropisetron (ICS 205-930). In a clinical trial, 11 patients (47 courses of chemotherapy) received cisplatin and other agents as the primary emetic stimulus. Nearly half had received chemotherapy previously. Tropisetron was administered as two 15 min. *i.v.* infusions at a dosage of 10 mg before and 10 mg after chemotherapy administration. No vomiting or retching was noted in 31 of the courses. Headache was reported in 9 courses (19%) and mild sedation in 7 courses (15%).

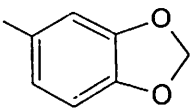
2.32. Martin et al. (1993) carried out a study on LAS 30451 (pancopride). LAS 30451 showed ED_{50} value of 0.56 µg/kg, *i.v.* and 8.7 µg/kg, *p.o.* in the inhibition of BJ reflex in rats. LAS 30451 dose-dependently inhibited a number of vomiting episodes induced by cisplatin (3 mg/kg, *i.v.*) in dogs (ED_{50} = 7.1 µg/kg, *p.o.* and 3.6 µg/kg, *i.v.*). In a clinical trial, 15 patients were given LAS 30451 each by *i.v.* infusion 3 min before and 120 min after chemotherapy at doses ranging from 0.025 – 0.4 mg/kg. Complete protection against acute emesis was observed in 31% and 53% in the emesis induced by cisplatin and other cytotoxic drugs, respectively. No serious adverse effects were observed, at any dose level studied.

2.33. Kamato et al. (1993) studied the involvement of peripheral and central serotonin 5-HT_3 receptors in cisplatin- and *m*-chlorophenylbiguanide-induced emesis in ferrets. Cisplatin (10 mg/kg, *i.v.*) induced emesis was inhibited by a 5-HT_3 RA, YM 060 (0.003 – 0.1 µg/kg, *i.v.*). *m*-Chlorophenylbiguanide (*m*-CPBG), a 5-HT_3 receptor agonist, dose dependently induced emesis, which was inhibited by YM 060 (0.003 – 0.3 µg/kg, *i.v.*). Vagotomy markedly reduced this emesis, and the combination of abdominal vagotomy and greater splanchnicectomy abolished emesis. Intracerebroventricularly (4^{th} ventricle) administered YM 060 inhibited cisplatin- and *m*-CPBG-induced emesis only at higher doses (0.01 – 0.1 and 0.01 – 0.03 µg, respectively). When *m*-CPBG was administered *i.c.v.*, it produced only a weak retching response. These results indicated that the stimulation of abdominal vagal afferent nerves via peripheral 5-HT_3 receptors is important for triggering cisplatin- and *m*-CPBG-induced emesis in ferrets.

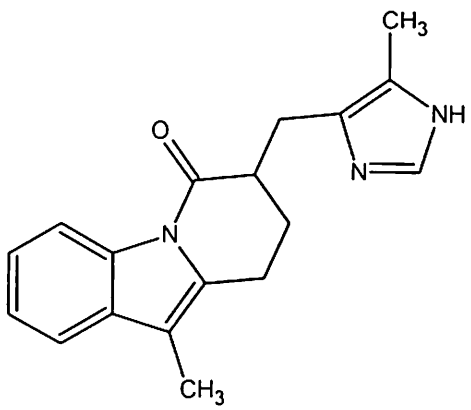
2.34. Monge et al. (1994) synthesized and evaluated 2-piperazinylbenzothiazole and 2-piperazinylbenzoxazole derivatives for 5-HT_3 receptor antagonistic activities. In this series, the compounds **2.20** and **2.21** antagonized (74.4 and 74.5% inhibition, respectively) the effect of 5-HT at the LMMP preparation of guinea-pig ileum, which were more potent than the ondansetron (63.3% inhibition). However, these two compounds (IC_{50} = 3.3 x 10^{-6} and 9.5 x 10^{-7} M, respectively) were much weaker than ondansetron (IC_{50} = 4.6 x 10^{-8} M) as displacers of [^{3}H]-BRL 43694 binding to rat cerebral cortex or as antagonists of the bradycardia response to 5-HT in rats. The compound **2.21** not only enhanced gastric emptying (45% increase at 5 mg/kg, *p.o.*) but was also a potent agonist (pEC_{50} = 6.58) at the rat esophageal tunica muscularis mucosa, a preparation sensitive to 5-HT_4 receptor stimulation.



2.20: R = $-CH_2Ph$

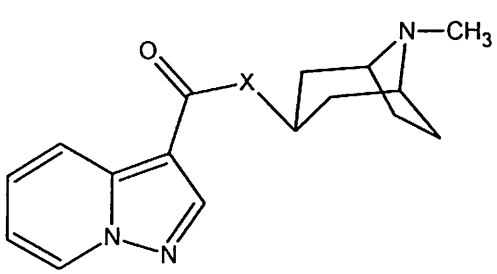
2.21: R = 

2.35. Kato et al. (1994) synthesized and evaluated a series of pyrido[1,2-*a*]indol-6(7*H*)-ones as 5-HT_3 RAs. The (5-methylimidazol-4-yl)methyl group as a basic nitrogen moiety was an important element for high potency. The highest potency was observed for compounds which have 7- and 10-methyl substituents on the pyrido[1,2-*a*]indol-6(7*H*)-one ring. In this series, the compound (+) **2.22** (FK 1052) was a potent 5-HT_3 receptor antagonist in the BJ reflex test in rats (ED_{50} = 0.9 µg/kg, *i.v.*) and a very effective antiemetic agent againt ciplatin-induced emesis in dogs (ED_{50} = 1.1 x 2 µg/kg, *i.v.* and 2.7 x 2 µg/kg, *p.o.*).



2.22 (FK 1052)

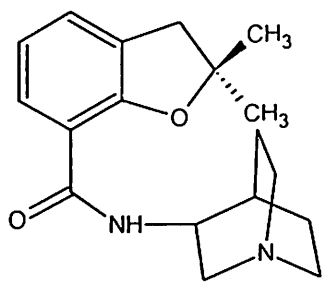
2.36. Hansen et al. (1994) synthesized 3-carboxamide and 3-carboxylates of pyrazolo[1,5-*a*]pyridines and pyrazolo[1,5-b]pyridazines and evaluated for their binding affinities towards 5-HT_3 receptors. In this series, the compounds **2.23** and **2.24** showed IC_{50} value of 28 and 34 nM, respectively in [^{3}H]-GR 65630 labelled 5-HT_3 receptors in rat cortical membranes whereas the reference compound, tropisetron, exhibited IC_{50} of 3.1 nM. Both the compounds (**2.23** & **2.24**) showed low affinities for 5-HT_{1A}, 5-HT_2 or dopamine D_2 receptors (IC_{50} >10 µM). In the inhibition of BJ reflex in rats, **2.23** and **2.24** showed ED_{50} value of 2 and 0.7 µg/kg, respectively; tropisetron exhibited ED_{50} value of 0.5 µg/kg in this test.



2.23: X = O

2.24: X = NH

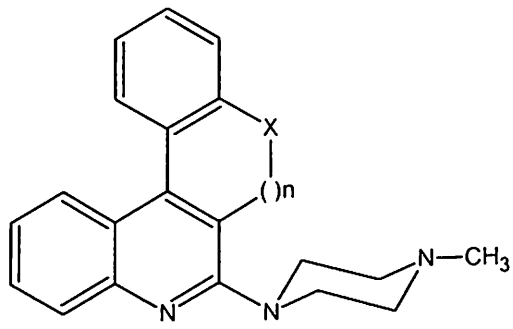
2.37. Kuroita et al. (1994) synthesized a series of *N*-(azabicyclo-3-yl)-2,3-dihydrobenzofuran-7-carboxamide derivatives and evaluated for their 5-HT_3 receptor antagonistic activities. Introduction of methyl group(s) at 2nd position of the dihydrobenzofuran ring increased the pharmacological activities (dimethyl > monomethyl > dihydro). Concerning the basic part, the compounds bearing (*S*)-1-azabicyclo[2.2.2]octan-3-yl moiety were more potent than their counterparts. With respect to the methyl groups at position 2 of the dihydrobenzofuran ring, the rank of potency was dimethyl ≥ (2*S*)-methyl > (2*R*)-methyl > dihydro. These results suggested that the (2*S*)-methyl group of the dihydrobenzofuran part contributed to the enhancement of the pharmacological activity. In this series, the compound (*S*)-**2.25** showed highest affinity for 5-HT_3 receptors (K_i = 0.055 nM) [granisetron K_i = 0.41 nM & zacopride K_i = 0.18 nM] and the most potent antagonistic activity on the BJ reflex (ED_{50} = 0.18 µg/kg, *i.v.*) [granisetron; ED_{50} = 0.74 µg/kg, *i.v.* and zacopride, ED_{50} = 0.50 µg/kg, *i.v.*]



2.25

2.38. Anzini et al. (1995) synthesized and evaluated a series of condensed quinoline derivatives bearing a basic nitrogen on piperazine at the 2nd position of the quinoline nucleus. The most active compound in this series was **2.26,** which displayed a K_i value 1.6 nM very similar to that reported for ondansetron (K_i = 1.2 nM) along with an improved selectivity. Functional and *in vivo* testing carried out on three selected compounds showed that the compounds **2.26**, **2.27** and **2.28** were potent 5-HT_3 RAs with potencies in the same range as the best known 5-HT_3 RAs ondansetron, tropisetron and zacopride.

Comparative molecular field analysis (CoMFA) was applied to binding constants of this new series. The cross-validated r^2 derived from partial least-squares calculations, indicated a good predictive capacity for affinity values in the series of compounds investigated.

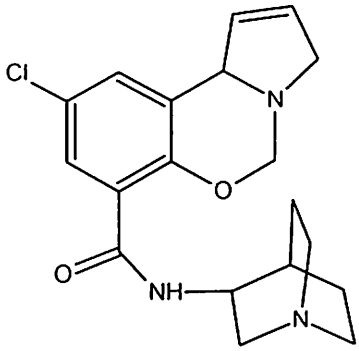


2.26: X = O, n = 1

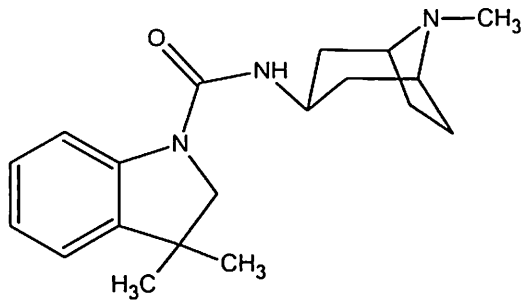
2.27: X = O, n = 2

2.28: X = CH, n = 2

2.39. Kato et al. (1995a) reported a novel pyrrolo [2,1-*c*][1,4]benzoxazine-6-carboxamides as potent 5-HT_3 RAs. Structure activity relationship studies of this series showed that the compounds with small and lipophilic substituents such as chloro and methyl at the 8th position of the aromatic ring portion retained high potency, whereas those with bulky substituents showed essentially no activity. A dimethyl group at the 4th position slightly decreased the potency. 1-Azabicyclo[2.2.2]octan-3-amine as the amine part afforded the most potent activity. From this series, the compound **2.29** was found to be the most potent 5-HT_3 receptor antagonist (ED_{50} = 0.4 µg/kg, *i.v.*), on the BJ reflex in rats, which was approximately 40 fold more potent than ondansetron (ED_{50} = 17.5 µg/kg, *i.v.*) and equipotent with BRL 46470A (ED_{50} = 0.5 µg/kg, *i.v.*).

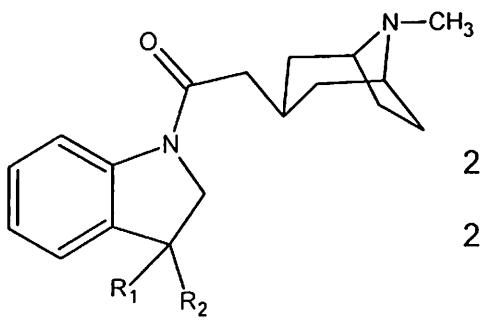


2.29



BRL 46470A

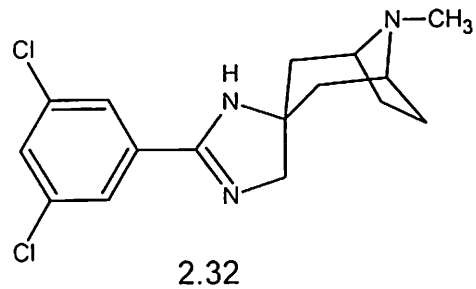
2.40. Kato et al. (1995b) reported the synthesis and SAR of a new series of new azabicyloalkaneacetamides as 5-HT_3 RAs. The study on the azabicycloalkaneacetamide derivatives showed that 2,3-dihydroindole as the aromatic ring moiety afforded potent 5-HT_3 RA activity, as judged by the blockade of bradycardia induced by *i.v.* injection of 2-Me-5-HT in anaesthetized rats. 7-Azaindole as the aromatic moiety afforded weak 5-HT_3 receptor antagonist activity. The best 5-HT_3 RAs in this study were *endo*-3,3-diethyl- (**2.30**) and 3,3-dimethyl-2,3-dihydro-1-(8-methyl-8-azbicyclo[3.2.1]oct-3-yl)acetyl-1*H*-indole (**2.31**) (1.7 and 2.3 µg/kg, *i.v.*, respectively in the inhibition of BJ reflex test), which were approximately 10 fold more potent than ondansetron (ED_{50} = 17.5 µg/kg, *i.v.*). This study showed that the azabicycloalkaneacetyl group was a new pharmacophoric element with a basic nitrogen and a linking carbonyl group.



2.30: $R_1 = R_2 = C_2H_5$

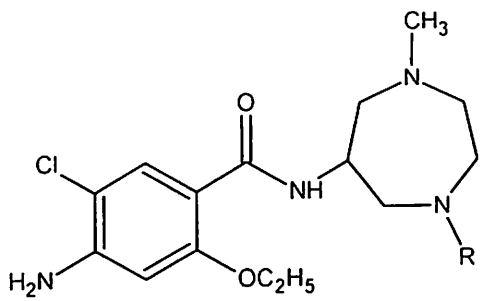
2.31: $R_1 = R_2 = CH_3$

2.41. Whelan et al. (1995) synthesized and evaluated a series of tropane-3-spiro-4'(5')-imidazolines for 5-HT_3 receptor antagonist activities. From binding studies of the synthesized compounds, the compound **2.32** demonstrated the ability to efficiently displace the binding of [^{3}H]-GR 65630 to bovine brain area postrema membranes to an extent comparable to MDL 72222. In the BJ reflex, compound **2.32** was equipotent with metoclopramide. From this study, it was concluded that the imidazoline ring might be useful as a bioisosteric replacement for the carbonyl group in the pharmacophore of 5-HT_3 RAs.



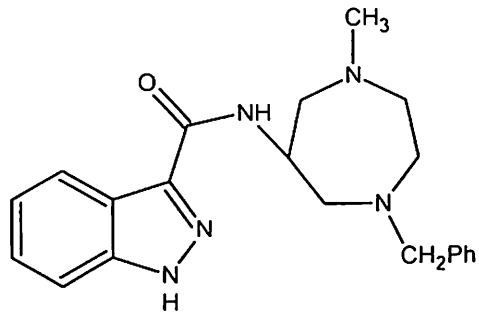
2.32

2.42. Harada et al. (1995) reported the synthesis and SAR studies of *N*-(1-benzyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl) carboxamides as 5-HT_3 RAs. Studies on 4-amino-5-chloro-2-ethoxybenzamides led to the discovery that the *N*-(1,4-dimethylhexahydro-1*H*-1,4-diazepin-6-yl)benzamide (**2.33**) and the 1-benzyl-4-methylhexahydro-1*H*-1-4-diazepin analogue (**2.34**) were potent 5-HT_3 RAs. SAR studies on the influence of the aromatic nucleus of **2.33** and **2.34** upon inhibition of the BJ reflex in rats were described. Within this series, use of the 1*H*-indazole ring as an aromatic moiety led to an increase in the activity; the 1*H*-indazolylcarboxamides showed potent 5-HT_3 RAs activity. In this series compound **2.35** was identified as a potent 5-HT_3 RA, whose effect was superior to that of the corresponding benzamide (**2.34**) and equipotent to that of ondansetron and granisetron.



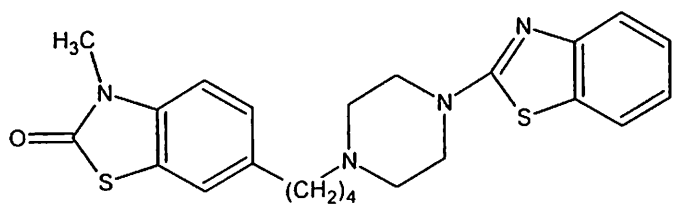
2.33: R = -CH_3

2.34: R = -CH_2Ph

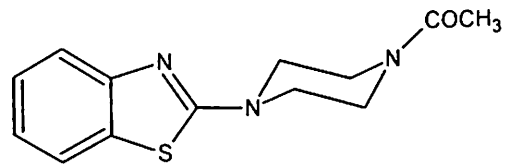


2.35

2.43. Diouf et al. (1995) synthesized 2-piperazinylbenzothiazole derivatives and reported the binding affinities towards both 5-HT_{1A} and 5-HT_3 receptors. In this series, compounds exhibited significant affinities for two serotoninergic receptor subtypes (5-HT_{1A} & 5-HT_3). The pharmacological profile of these ligands was agonist for 5-HT_{1A} receptors and antagonist for 5-HT_3 receptors. It was proposed that compounds with such pharmacological profiles were of clinical relevance in the treatment of psychotropic diseases (e.g. anxiety, depression and schizophrenia). In this series, the compound **2.36** showed binding affinities (IC_{50}) towards various receptors; 5-HT_{1A} (5×10^{-8} M), 5-HT_2 (6×10^{-6} M), 5-HT_3 (4×10^{-8} M), D_2 ($>10^{-4}$ M), α_1 (10^{-7} M), whereas, the compound **2.37** showed good binding affinity towards 5-HT_3 receptors (10^{-8} M), moderate affinity towards 5-HT_2 ($<10^{-6}$ M), less affinity towards 5-HT_{1A} ($>10^{-4}$ M) and no affinity towards D_2 and α_1 receptors.

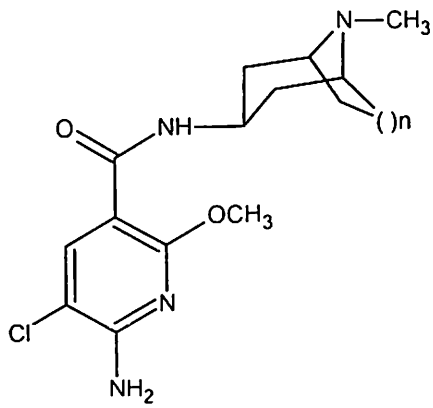


2.36



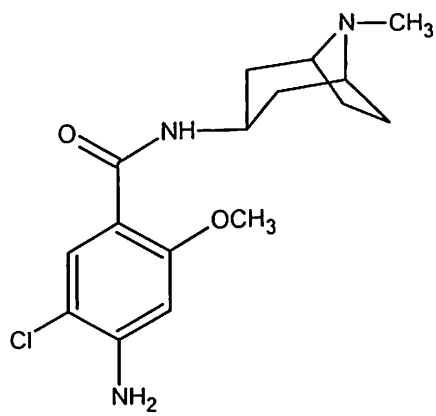
2.37

2.44. Coldwell et al. (1995) reported the synthesis of metabolically blocked 3-aza analogues (pyridyl) of 4-amino-5-chloro-2-methoxybenzamides. In this series, the compound **2.38** showed five fold reduction in 5-HT_3 receptor affinity (pK_i = 8.8) when compared to the corresponding benzamide derivative, BRL 24682 (pK_i = 9.5). However, by changing to the granatane homologue (compound **2.39**) higher 5-HT_3 receptor affinity (pK_i = 9.4) was achieved. The 3rd position of benzamide derivative (BRL 24682) is potentially open to metabolism to the polar 3-hydroxy or sulfated derivatives, which could limit the duration of action of compounds containing aromatic nucleus. The 3-aza derivatives (compounds **2.38** & **2.39**) decreased this potential metabolism.



2.38: n = 1

2.39: n = 2



BRL 24682

2.45. Toral et al. (1995) studied the 5-HT_3 receptor-independent inhibition of the depolarization-induced ^{86}RB efflux from human neuroblastoma cells, TE671, by ondansetron. 5-HT_3 RA, ondansetron, was shown to have positive effects in selected *in-vivo* models of memory impairment and anxiety. In this work, and ^{86}RB efflux bioassay was used to show that ondansetron was unique in blocking voltage-gated potassium channels in TE671 human neuroblastoma cells.

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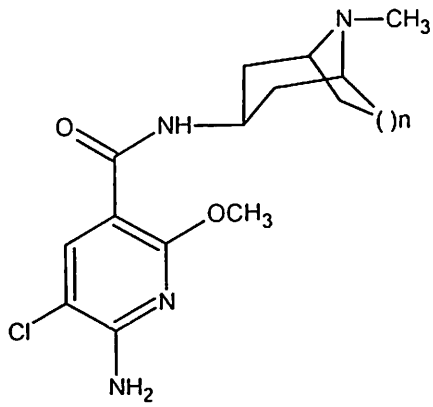
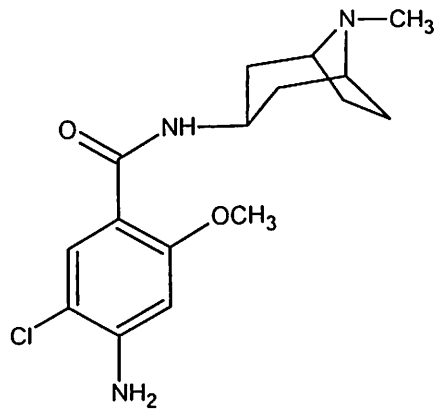


2.38: n = 1

2.39: n = 2



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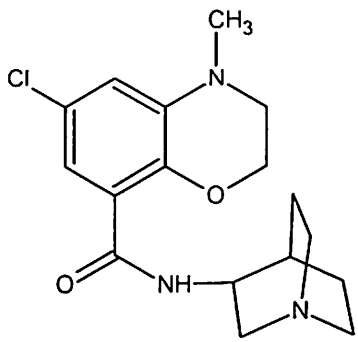
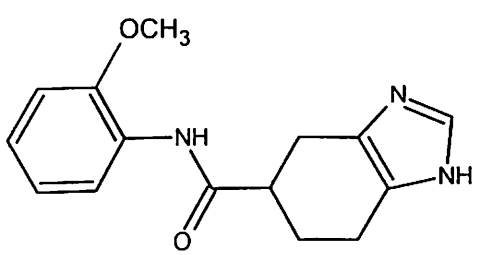
The intrinsic potassium-channel-blocking (KCB) property is relatively weak in ondansetron (IC_{50} = 20 μM), but was not shared by other 5-HT_3 receptor ligands including zatosetron, MDL 72222, LY 278 584, zacopride, 1-phenylbiguanide, and ICS 205-930 (tropisetron). Pre-incubation of the target neuroblastoma cells with several 5-HT-receptor ligands including 5-HT, 2-Me-5-HT, failed to abolish the KCB action of ondansetron. The KCB activity of ondansetron was almost entirely attributable to its structural nucleus, 2,3-dihydro-9-methyl-4(1*H*)-carbazolone. It was hypothesized that the KCB action of ondansetron was mediated through receptors other than 5-HT_3 receptors and it might be a significant factor in the *in-vivo* cognition-enhancing activities of this compound, conceivably due to the depolarization of the hippocampus synaptic membranes and a consequent augmentation of neurotransmission.

2.46. Kuroita et al. (1996a) reported the synthesis and evaluation of 6-chloro-3,4-dihydro-4-methyl-2*H*-1,4-benzoxazine-8-carboxamide derivatives for 5-HT_3 receptor antagonist activity. In this series, *S*-isomer of the compound **2.40** was one fold more potent 5-HT_3 receptor binding affinity (K_i = 0.051 nM) than its *R* isomer (K_i = 0.54 nM), whereas, granisetron, zacopride and azasetron showed K_i = 0.41, 0.18 and 0.54 nM, respectively in the displacement of [^{3}H]-granisetron binding to rat cerebral cortex membranes. The compound (*S*)-**2.40** also showed potent antagonistic activity against the BJ reflex (ED_{50} = 0.089 μg/kg, *i.v.*) in rats, while the compound (*R*)-**2.40** showed ED_{50} = 0.73 μg/kg, *i.v.* whereas, granisetron, zacopride and azasetron exhibited ED_{50} = 0.74, 0.5 and 1.3 μg/kg, *i.v.*, respectively.



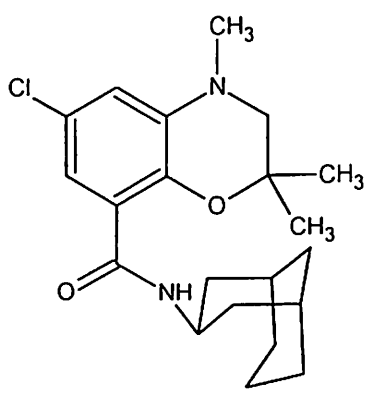
2.40

2.47. Ohta et al. (1996a) synthesized and evaluated a novel series of conformationally restricted fused imidazole derivatives for their 5-HT_3 antagonistic activities. The most potent compound in this series was **2.41** with an ID_{50} value of 0.32 µg/kg, in the BJ reflex in rats and an IC_{50} value of 0.43 µM, in the isolated colonic contraction in guinea-pig (animal model for evaluating IBS), approximately ten and two times potent than ondansetron, respectively. The SAR studies suggested that the high potency of **2.41** may be attributed to the suitable position and direction of the N-C-N centroid in the conformationally restricted imidazole ring against the planar (2-methoxyphenyl)aminocarbonyl part in the binding of **2.41** to the receptor.



2.41

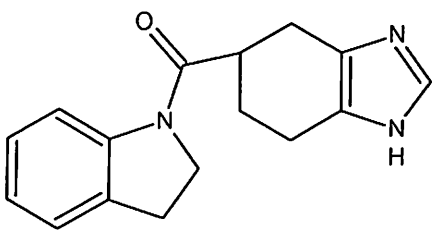
2.48. Kuroita et al. (1996b) synthesized a series of 3,4-dihydro-2*H*-1,4-benxoxazine-8-carboxamide derivatives and evaluated for their 5-HT_3 receptor antagonistic activities. In this series, replacement of the 1,4-benzoxazine ring with a 1,4-benzothiazine ring or seven-membered ring (i.e. 1,5-benzoxepine or 1,5-benzthiepine) resulted in decreased affinity for 5-HT_3 receptors. Introduction of substituents at the 2-position of the 1,4-benzoxazine ring increased the antagonistic activities (dimethyl > methyl > dihydro > phenyl). The compounds bearing 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety as the basic part were equipotent to those bearing 1-azabicycolo[2.2.2]oct-3-yl moiety. In this series, the compound **2.42** showed the highest affinity for 5-HT_3 receptors (K_i = 0.019 nM) and a long-lasting (for about 3 hr) 5-HT_3 receptor antagonistic activity as evidenced by antagonism to the BJ reflex in rats.



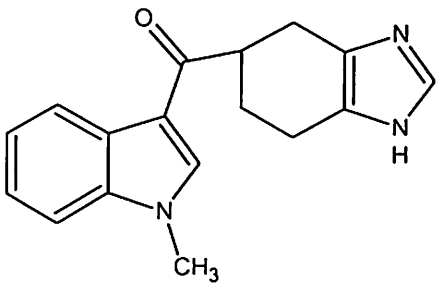
2.42

2.49. Ohta et al. (1996b) reported the synthesis and evaluation of optically active 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives 5-HT_3 antagonism. In this series, most of the *R*-isomers were almost two orders of magnitude more potent than their *S*-isomers as 5-HT_3 RAs, in the BJ reflex test in rats, the contraction of isolated guinea-pig colon and the receptor binding affinity.

(*R*)-Isomers of the compounds **2.43** and **2.44**, when given in *p.o.*, were found to be several hundred times more potent than ondansetron and granisetron in their inhibitory effects on cisplatin-induced emesis in ferrets and restraint stress-induced increased fecal pellet output in rats.

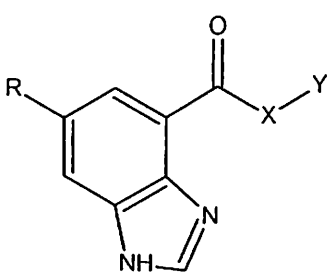


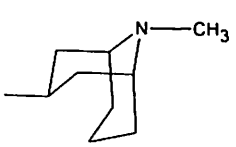
2.43 (YM114 / KAE 393)

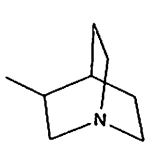


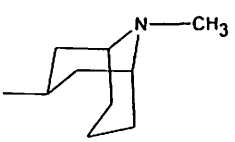
2.44 (ramosetron / YM060)

2.50. Lopez-Rodriguez et al. (1996) synthesized a series of benzimidazole-4-carboxylic acid derivatives and evaluated for affinity at 5-HT_3 and 5-HT_4 receptors. In this series, the compounds **2.45**, **2.46** and **2.47** exhibited high affinity for the 5-HT_3 receptors ([^{3}H]-LY 278584) (K_i = 6.1, 3.7 and 4.9 nM, respectively) and no significant affinity for 5-HT_4 (K_i >1000 nM) and 5-HT_{1A} (K_i > 10,000 nM) receptor sites. Tropisetron and zacopride were found to possess potent affinities for 5-HT_3 receptors (K_i = 1.28 and 0.42 nM, respectively) besides expressing little affinity for 5-HT_4 receptors (K_i = 63 and 158 nM, respectively).

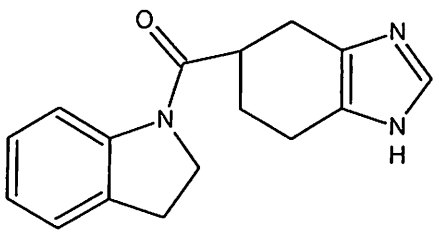


2.45: R = H, X = NH, Y = 

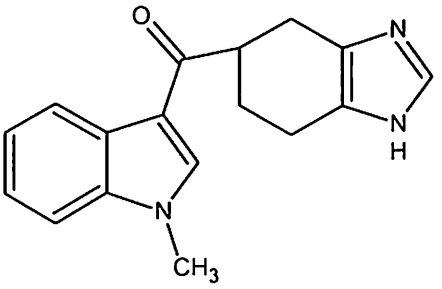
2.46: R = H; X =NH, Y = 

2.47: R = Cl, X = O, Y = 

(*R*)-Isomers of the compounds **2.43** and **2.44**, when given in *p.o.*, were found to be several hundred times more potent than ondansetron and granisetron in their inhibitory effects on cisplatin-induced emesis in ferrets and restraint stress-induced increased fecal pellet output in rats.

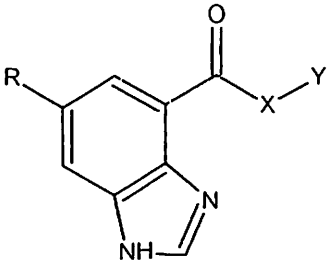


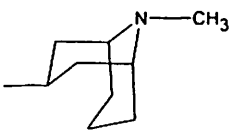
2.43 (YM114 / KAE 393)

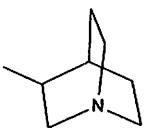


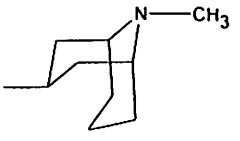
2.44 (ramosetron / YM060)

2.50. Lopez-Rodriguez et al. (1996) synthesized a series of benzimidazole-4-carboxylic acid derivatives and evaluated for affinity at 5-HT_3 and 5-HT_4 receptors. In this series, the compounds **2.45**, **2.46** and **2.47** exhibited high affinity for the 5-HT_3 receptors ([^{3}H]-LY 278584) (K_i = 6.1, 3.7 and 4.9 nM, respectively) and no significant affinity for 5-HT_4 (K_i >1000 nM) and 5-HT_{1A} (K_i > 10,000 nM) receptor sites. Tropisetron and zacopride were found to possess potent affinities for 5-HT_3 receptors (K_i = 1.28 and 0.42 nM, respectively) besides expressing little affinity for 5-HT_4 receptors (K_i = 63 and 158 nM, respectively).

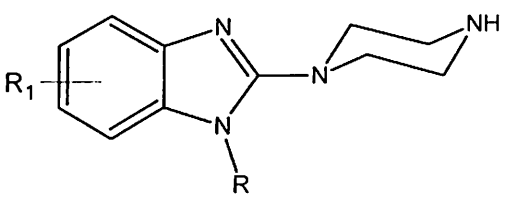


2.45: R = H, X = NH, Y = 

2.46: R = H; X =NH, Y = 

2.47: R = Cl, X = O, Y = 

2.51. Orjales et al. (1997) reported the synthesis and evaluation of a series of 2-piperazinylbenzimidazole derivatives as potential 5-HT_3 receptor antagonists. The compound **2.48** (lerisetron, pK_i = 9.2) exhibited higher affinity for the 5-HT_3 receptor than did tropisetron and granisetron, while compound **2.49** (pK_i = 7.5) had very low affinity and activity. The effects of substitution on the aromatic ring of benzimidazole by several substituents in different positions were also discussed. Thus, while the 4-methoxy derivative **2.50**, showed weak affinity for the 5-HT_3 receptor (pK_i = 6.7), the 7-methoxy derivative, **2.51**, exhibited the highest affinity (pK_i = 9.4).



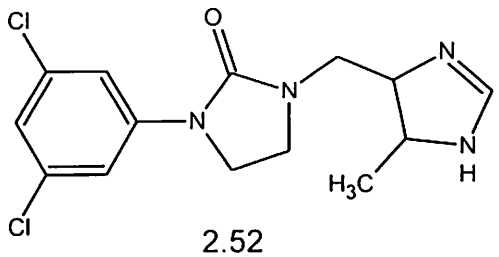
2.48: R = CH_2Ph, R_1 = H

2.49: R = H; R_1 = H

2.50: R = CH_2Ph, R_1 = (4)CH_3O

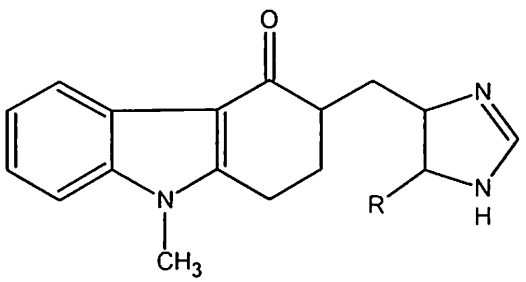
2.51: R = CH_2Ph, R_1 = (7)CH_3O

2.52. Heidempergher et al. (1997) synthesized and evaluated a series of phenylimidazolidin-2-one derivatives bearing a basic azabicycloalkyl or an imidazolylalkyl moiety for 5-HT_3 receptor antagonism. A possible bioisosterism between the benzamido and the phenylimidazolidin-2-one moieties had been suggested on the basis of the similarity between the molecular electrostatic potential (MEP) of metoclopramide, a D_2 receptor antagonist with weak 5-HT_3 . In general, imidazoalkyl derivatives were found to be more active 5-HT_3 RAs than azabicycloalkyls. In particular, the compound **2.52** displayed very high affinity (K_i = 0.038 nM) in [^{3}H]-GR 43694 binding to 5-HT_3 receptors in the rat entorhinal cortex, with a K_b of 5.62 nM in the *in-vitro* guinea-pig ileum assay, being more potent than the reference compounds (ondansetron, tropisetron, granisetron and BRL 46470) tested. The compound **2.52** showed an ID_{50} comparable to that of ondansetron (2.2 µg/kg, *i.v.*) in the BJ reflex test in rats.



2.52

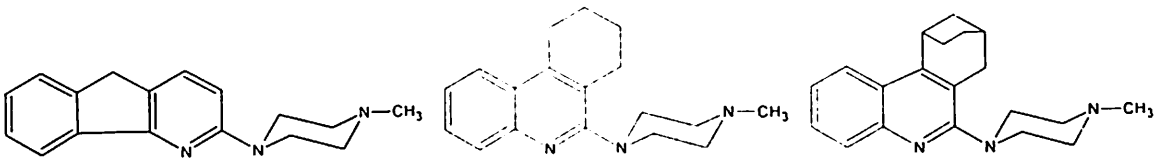
2.53. Suzuki et al. (1997) reported the synthesis and evaluation of 9-methyl-2,3,4,9-tetrahydropyrano[2,3-*b*]indol-4-one derivatives for their 5-HT_3 antagonistic activities. In this series, the compound **2.53** was found to be 79 times more potent than ondansetron in the BJ reflex in rats (ID_{50} = 0.048 μg/kg, *i.v.*) and the compound **2.54** was found to be 126 times more potent than ondansetron in the colonic contraction of guinea-pig (IC_{50} = 0.0062 μM).



2.53: R = Et

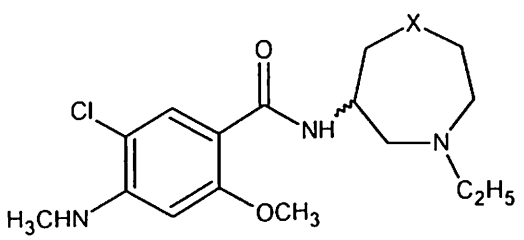
2.54: R = Me

2.54. Cappelli et al. (1998) reported the synthesis and pharmacological evaluation of a series of condensed quinoline and pyridine derivatives bearing *N*-methylpiperazine moiety attached to the 2-position of the quinoline or pyridine nucleus. The compounds were studied for 5-HT_3 agonist / antagonist activity *in-vitro* on 5-HT_3 receptor-dependent [^{14}C]-guanidinium uptake in NG108-15 cells. Compound **2.55** acted as a 5-HT_3 agonist in this assay with an EC_{50} value close to that reported for quipazine, while the compound **2.56** was a partial agonist with an EC_{50} value of about 0.25 nM, and the compound **2.57** possessed antagonist properties with an IC_{50} value (≈ 8 nM) in the same range as those of reference 5-HT_3 RAs.



2.55 2.56 2.57

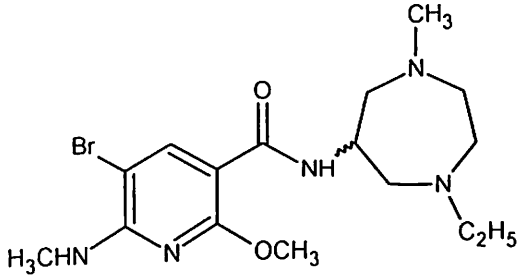
2.55. Hirokawa et al. (1998a & 2002) reported the synthesis of a novel series of benzamides with hexahydro-1,4-diazepine or hexahydroazepine ring in the amine moiety and their binding affinities for 5-HT_3 and dopamine D_2 receptors. The *R* isomer of the compound **2.58** had potent affinity for both 5-HT_3 (determined in rat cortical membranes using [^{3}H]-GR 65630) and dopamine D_2 receptors (determined in rat brain synaptic membranes using [^{3}H]-spiperone) (IC_{50} = 2.86 Vs 34.6 nM). The corresponding (*S*)-enantiomer had a potent and selective 5-HT_3 receptor binding affinity over the D_2 receptors (IC_{50} = 1.49 Vs 320 nM). The *R*-enantiomer of the compound **2.59** showed potent affinity for D_2 receptors with reduced affinity for 5-HT_3 receptors (IC_{50} = 4.5 Vs 97 nM), while the *S*-isomer was found to be a potent and selective 5-HT_3 receptor antagonist with less affinity for D_2 receptors (IC_{50} = 4.5 Vs 367 nM). Metoclopramide exhibited less affinity towards both D_2 and 5-HT_3 receptors and domperidone showed potent affinity for D_2 and less affinity for 5-HT_3 receptors (IC_{50} = 480 Vs 880 nM and 2.5 Vs >100 nM, respectively). Ondansetron was found to have potent affinity for 5-HT_3 receptors and no affinity for D_2 receptors (IC_{50} = 1.4 Vs > 1000 nM).



2.58: X = N-CH_3

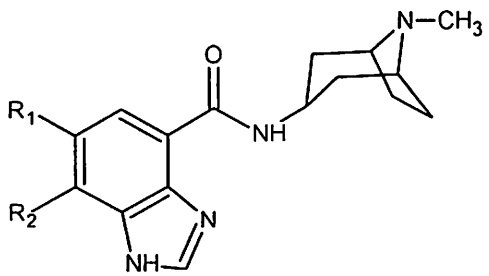
2.59: X = CH_2

2.56. Hirokawa et al. (1998a and 2003) synthesized a series of *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)nicotinamide derivatives and evaluated for their binding to 5-HT_3 and dopamine D_2 receptors. In this series, the compound **2.60** and its *R*-isomer were found to have potent affinities for both 5-HT_3 (determined in rat cortical membranes using [^{3}H]-GR 65630) and dopamine D_2 receptors (determined in rat brain synaptic membranes using [^{3}H]-spiperone). (IC_{50} = 0.97 Vs 1.20 nM and 23.3 Vs 6.88 nM, respectively). (*S*)-**2.60** had a potent 5-HT_3 receptor binding affinity and a moderate D_2 receptor binding affinity (IC_{50} = 1.28 Vs 122 nM). The affinities of (*R*)-**2.60** for 5-HT_3 and D_2 receptors were approximately 3 to 5 fold higher than those of the corresponding benzamide (*R*)-**2.58** (IC_{50} = 1.20 Vs 2.86 nM and 6.88 Vs 34.6 nM, respectively). Ondansetron showed potent and selective affinity for the 5-HT_3 receptor over the D_2 receptor site while metoclopramide exhibited moderate affinities for both receptors (IC_{50} = 1.54 Vs 228 nM and >1000 Vs 444 nM, respectively). Pharmacologically, (*R*)-**2.60** showed a potent antagonistic activity for both the serotonin 5-HT_3 and dopamine D_2 receptors in *in-vivo* tests and dose-dependently inhibited both the incidence and frequency of emetic episodes induced by Cisplatin (ferrets, 10 mg/kg, *i.v.*) and morphine (dogs, 0.3 mg/kg, *s.c.*) with ID_{50} values of 27.1 µg/kg, *p.o.* (17.6 µg/kg, *i.v.*) and 136 µg/kg, *p.o.* (14.2 µg/kg, *i.v.*), respectively.



2.60

2.57. Lopez-Rodriguez et al. (1999) reported the synthesis of a new series of azabicylic benzimidazole-4-carboxamides and azabicylic benzimidazole-4-carboxylates and evaluation for their binding at serotoninergic 5-HT_3 and 5-HT_4 receptors in the CNS. Most of the synthesized compounds exhibited high or very high affinity for the 5-HT_3 binding sites and low to no significant activity for the 5-HT_4 receptor. SAR observations indicated that a halogen atom at the 6-position and a nitro group at the 7-position of the benzimidazole ring is the best substitution pattern for 5-HT_3 affinities and 5-HT_3/5-HT_4 selectivity. The compounds (*S*)-**2.61**, (*S*)-**2.62** and (*S*)-**2.63** bound at central 5-HT_3 sites with high affinity (K_i = 2.6, 0.13 and 1.7 nM, respectively) and excellent selectivity over serotonin 5-HT_4 and 5-HT_{1A} receptors (K_i >1000 – 10,000nM). Furthermore, these new 5-HT receptor ligands were exhibited as potent and selective 5-HT_3 RAs in the isolated guinea-pig ileum (pA_2 = 9.6, 9.9 and 9.1, respectively).



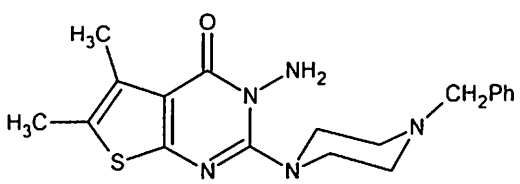
2.61: R_1 = H, R_2 = H

2.62: R_1 = Cl, R_2 = H

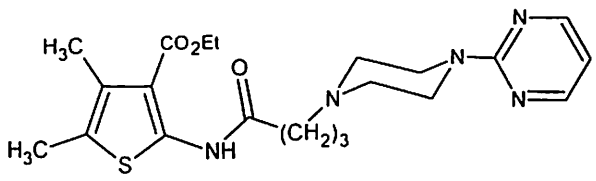
2.63: R_1 = Cl, R_2 = NO_2

2.58. Fauser et al. (1999) reported the guidelines for acute anti-emetic therapy. 5-HT_3 RAs and corticosteroids are the two categories of anti-emetics that are most effective, with fewest side-effects and are convenient to use. These agents are recommended in combination for highly emetogenic chemotherapy regimes and as a single agent or in combination for moderate to high emetogenic chemotherapy.

2.59. Modica et al. (2000) reported the synthesis and the binding tests on the 5-HT_3 and 5-HT_4 receptors of new thienopyrimidopiperazine and piperazinylacylamidomethylthiophene derivatives. The compound with higher affinity and selectivity for the 5-HT_3 over the 5-HT_4 receptor was 3-amino-2(4-benzy-1-piperazinyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4(3*H*)-one, **2.64,** (5-HT_3 K_i = 3.92 nM, not active on 5-HT_4); the compound with higher affinity and selectivity for the 5-HT_4 over the 5-HT_3 receptor was the 2{4-[4-(2-pyrimidinyl)-1-piperzinyl]butanoylamino}-4,5-dimethyl-3-thiophene carboxylic acid ethyl ester, **2.65,** (5-HT_4 K_i = 81.3 nM, not active on 5-HT_3).



2.64



2.65

2.60. Oliver et al. (2000) reviewed the anxiolytic activity of 5-HT_3 RAs in animal models of anxiety and in clinical trials in human volunteers. 5-HT_3 RAs displayed a different anxiolytic profile when compared to the established anxiolytics (benzodiazepine receptor agonists). They were anxiolytic in a limited number of animal anxiety models. If active, they often were very potent and displayed bell-shaped dose-response curves. 5-HT_3 RAs remained active after chronic dosing and no indications for tolerance, dependence, or rebound effects were found, which make these drugs an attractive alternative to the benzodiazepines.

2.61. Yoshikawa et al. (2001a) investigated the anti-emetic and pharmacological profile of AS-8112. In guinea-pig isolated colon, AS 8112 was found to antagonize the effect of 2-Me-5-HT, a 5-HT_3 receptor agonist (pA_2 = 7.04). Other 5-HT_3 RAs also produced antagonistic potency in the following order: granisetron > ondansetron = AS 8112 >> metoclopramide. In mice, AS 8112 (1.0 – 3.0 mg/kg, *s.c.*) strongly inhibited hypothermia induced by the dopamine D_3 agonist, *R*-(+)-7-OH-DPAT. Domperidone and haloperidol, which have affinity for D_3 receptors, also inhibited *R*-(+)-7-OH-DPAT-induced hypothermia. In ferrets and dogs, AS 8112 dose-dependently inhibited the emesis induced by *R*-(+)-7-OH-DPAT, apomorphine, morphine or cisplatin with ED_{50} values of 2.22 µg/kg, *s.c.*, 10.5 µg/kg, *s.c.*, 14.2 µg/kg, *i.v.*, and 17.6 µg/kg, *i.v.*, respectively. Moreover, oral administration of AS 8112 significantly inhibited the emesis induced by these emetogens. AS 8112 (0.3 mg/kg, *i.v.*) significantly inhibited the emesis induced by cyclophosphamide and doxorubicin.

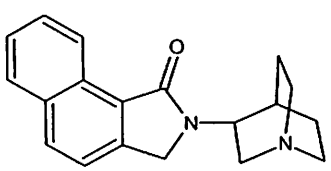
2.62. Yoshikawa et al. (2001b) studied the involvement of a central mechanism in the anti-emetic effect of AS 8112, a potent dopamine D_2, D_3 and 5-HT_3 receptor antagonist in ferrets. AS 8112 dose dependently inhibited *R*-(+)-7-OH-DPAT-induced emesis (ED_{50} = 0.11 µg/kg, *i.c.v.*). In addition, AS 8112 (10 µg/kg, *i.c.v.*) significantly inhibited the emesis induced by cisplatin. Ondansetron (10 µg/kg, *i.c.v.*) also inhibited cisplatin-induced emesis, but did not inhibit *R*-(+)-7-OH-DPAT-induced emesis. *S*-(-)-Eticlopride (10 µg/kg, *i.c.v.*) did not inhibit the emesis induced by cisplatin. CP-99,994 (10 µg/kg, *i.c.v.*) inhibited cisplatin- and *R*-(+)-7-OH-DPAT-induced emesis. These results suggested that the anti-emetic effects of AS 8112 were centrally mediated via dopamine D_3 and 5-HT_3 receptors in ferrets.

2.63. Eguchi et al. (2001) studied the effects of MCI 225, a selective noradrenaline reuptake inhibitor with 5-HT_3 receptor antagonism, in anxiety models. In social interaction test (SI) in rats, MCI 225 (10 and 30 mg/kg, *p.o.*) diazepam (1 – 10 mg/kg, *p.o.*), and ondansetron (1 mg/kg, *p.o.*) significantly increased SI to an unfamiliar partner under high light conditions without changes in ambulation. The increase in SI induced by MCI 225 and ondansetron was blocked by a 5-HT_3 agonist, *m*-CPBG (1 mg/kg, *i.p.*), which didn't change SI when administered alone. MCI 225 (10 mg/kg, *p.o.*) showed comparable anxiolytic like effect between single and 5-day repeated administration. On the other hand, maprotiline, trazodone, and imipramine didn't affect SI at doses of 3 – 30 mg/kg, *p.o.* In the elevated plus-maze test in rats, MCI 225 (10 – 100 mg/kg, *p.o.*) increased the number of entries into the open arms only, while diazepam increased not only the number of open-arm entries (30 mg/kg, *p.o.*), but also the total number of entries (10 mg/kg, *p.o.*). Ondansetron (0.001 – 1 mg/kg, *p.o.*) was less effective. Maprotline, imipramine, and trazodine didn't affect the number of open-arm entries, while trazodone and imipramine (100 mg/kg, *p.o.*) decreased the total number of entries. These results showed that MCI 225 had anxiolytic like effect without causing sedation and suggested that the 5-HT_3 receptor antagonism of MCI 225 probably contributes to its anxiolytic-like property.

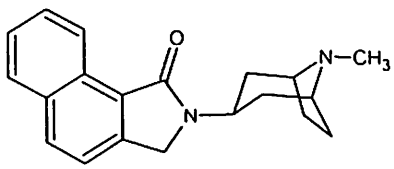
2.64. Zhang et al. (2001) evaluated the anxiolytic effects of desamino-3-iodozacopride (DAIZAC), a selective 5-HT_3 RA (K_d = 0.14 nM) in the mice elevated plus-maze model. DAIZAC treated animals produced a significant dose-related increase in the number of entries and the time spent in the open arm. The minimum dose of DAIZAC associated with a statistically significant increase in entries and the time spent in the open arm was 0.05 mg/kg, *i.p.* The anxiolytic effects of DAIZAC reached maximum by 20 – 30 min, which returned to baseline level within 90 min.

2.65. Watters et al. (2001) evaluated the effectiveness of three 5-HT_3 RAs (ondansetron, granisetron, tropisetron) in routine clinical practice. Each of the three 5-HT_3 RAs were studied for a 4 month period and data was collected from patients on nausea, vomiting (both acute and delayed) and side-effects by means of a diary card. A total of 274 patients were used in this study. Success rate for acute emesis seen over the study period was more than 90%. There was no statistically significant difference between any of the three drugs investigated with respect to both acute and delayed nausea and vomiting. Similarly, there was no difference between the three groups for the incidence of constipation, diarrhoea, and headache.

2.66. Cappelli et al. (2002) designed and synthesized novel, potent 5-HT_3 receptor ligands on the pyrrolidone structure with the aim of probing the central 5-HT_3 receptor recognition sites in a systematic way. The most potent ligand in this series was found to be (*S*)-**2.66**, which showed an affinity for 5-HT_3 receptors comparable to the reference ligand, granisetron (K_i = 0.34 and 0.35 nM, respectively) in the inhibition of [^{3}H]-granisetron specific binding to 5-HT_3 receptor in rat cortical membranes. Investigations on the 5-HT_3 receptor-dependent BJ reflex in urethane-anaesthetized rats confirmed the 5-HT_3 receptor antagonist properties of the compounds (*S*)-**2.66** and **2.67**, which expressed ID_{50} values of 81 and 2.8 μg/kg, respectively. Moreover, the compounds (*S*)-**2.66**, **2.67** and **2.68** (at the doses of 1.0, 0.01 and 0.01 mg/kg, *i.p.*, respectively) prevented scopolamine-induced amnesia in the mouse passive avoidance test, suggesting their potential use in cognitive function disorders.



2.66



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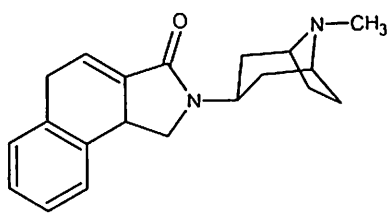
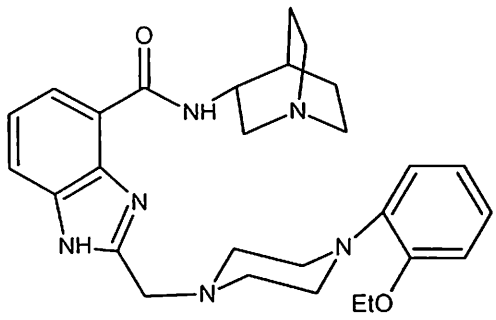


2.68

2.67. Sridhar et al. (2002) studied the role of 5-HT_3 receptors in cholinergic hypo-functional models of cognitive impairment in the elevated plus maze model and a passive avoidance model. Cognitive impairment was induced by scopolamine (1 mg/kg, *i.p.*) in mice and 5-HT_3 ligands *m*-CPBG (1 and 5 mg/kg, *i.p.*). Ondansetron (0.5 and 5 mg/kg, *i.p.*) was administered before the pre-learning phase to study the effects of acquisition, while post-learning administration was used to determine the effects on consolidation. Ondansetron improved acquisition and retention in cholinergic hypo-functional models while *m*-CPBG potentiated selected impaired cognitive indices. These results indicated the role of 5-HT_3 receptors in cognition. Ideal evaluation of 5-HT_3 ligands in cognition should distinguish true cognitive effects from locomotor, motivational and emotional effects.

2.68. Lopez-Rodriguez et al. (2004) reported the synthesis of a series of new mixed benzimidazole-arylpiperazine derivatives and evaluation for their binding affinity for 5-HT_{1A} and 5-HT_3 receptors. All the synthesized compounds in this series exhibited high 5-HT_3 receptor affinity (K_i = 10 – 62 nM) and derivatives with *o*-alkoxy group in the arylpiperazine ring showed high affinity for 5-HT_{1A} receptors (K_i = 18 – 150 nM). Additionally all the synthesized compounds were selective over α_1-adrenergic and dopamine D_2 receptors (K_i > 1000 nM). The compound **2.69** showed high affinity for both receptors (5-HT_{1A}: K_i = 18.0 nM, 5-HT_3: K_i = 27.2 nM). *In-vitro* and *in-vivo* findings suggested that this compound acted as a partial agonist at 5-HT_{1A} receptors and as a 5-HT_3 RA. This novel mixed 5-HT_{1A}/5-HT_3 ligand was also effective in preventing the cognitive deficits induced by muscarinic receptors blockade in a passive avoidance learning test, suggesting a potential interest in the treatment of cognitive dysfunction.



2.69

The aforementioned literature reports gave an insight into the design and development of new class of chemical derivatives as potential 5-HT_3 receptor antagonists.

Chapter III

Objective & Plan of Work

CHAPTER III

OBJECTIVE & PLAN OF WORK

3.1. Objective

As mentioned earlier chemotherapy regimens for the treatment of cancer produce more toxicity than efficacy, which may sometimes be life threatening and patients suffer from the discomforts of the nausea and vomiting associated with chemotherapy. Prevention and control of nausea and vomiting are significant important in the treatment of cancer patients. Nausea and vomiting can result in serious metabolic derangements, nutritional depletion and anorexia, esophageal tears, fractures, wound dehiscence, deterioration of patients' physical and mental status which prompts the patient to discontinue the potentially useful and curative anti-neoplastic treatment. It has been observed that patients receiving chemotherapy are prone to central nervous system disorders like anxiety and cognitive dysfunction.

The recent developments of serotonin 5-HT_3 antagonists have dramatically improved the treatment of emesis induced by anticancer therapy. However, presently available 5-HT_3 antagonists such as ondansetron and granisetron, tropisetron that possess asymmetric centres increase the synthetic costs. In order to overcome this problem, the search for a novel series of 5-HT_3 antagonists was initiated in this present study.

It was thought worthwhile to develop novel series of molecules as 5-HT_3 receptor antagonists keeping in mind the following:

- the basic properties of the pharmacophore, as seen in most 5-HT_3 receptor antagonists without much deviation
- the cost effectiveness
- simplicity in approach.

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It was thought worthwhile to develop novel series of molecules as 5-HT_3 receptor antagonists keeping in mind the following:

- the basic properties of the pharmacophore, as seen in most 5-HT_3 receptor antagonists without much deviation
- the cost effectiveness
- simplicity in approach.

The main objectives of this research work were to:

1. Design novel 5-HT_3 receptor antagonists by combining molecular modeling and traditional medicinal chemistry approach.

2. Synthesize the designed molecules using conventional methods and an environmental benign technique, Microwave Assisted Organic Synthesis (MAOS).

3. Evaluate the new chemical entities for the 5-HT_3 receptor antagonist activities and against central nervous system disorders such as anxiety, which could have beneficial effect in patients receiving cancer chemotherapy.

3.2. Plan of work

The plan of work is broadly classified into 3 headings.

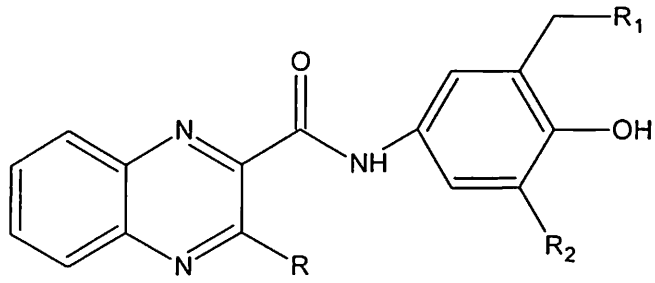
3.2.1. Design

The pharmacophore of 5-HT_3 RAs consists of three components: an aromatic ring, a carbonyl group containing linking moiety and a basic centre preferably a nitrogen in a specific spatial arrangement. Various studies revealed some refinements in the above pharmacophoric model, which formed the basis for the synthesis of novel molecules, as described in the current work using Tripos – Alchemy 2000 software.

3.2.2. Synthesis

The following series of compounds have been synthesized by conventional method and also by microwave assisted organic synthesis.

a) 3-Substituted-2-quinoxalinecarboxamides (**QC-1 – QC-36**)

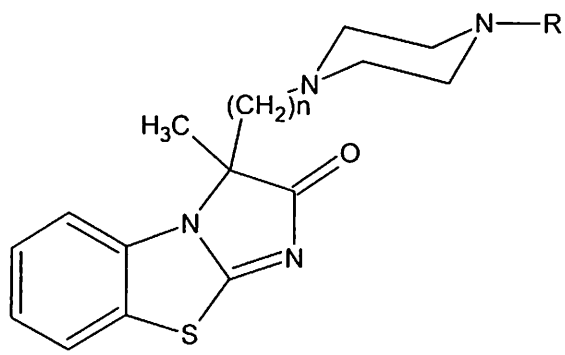


R = Cl, OCH_3, OC_2H_5

R_1 = dialkylamino

R_2 = H, CH_2-R_1

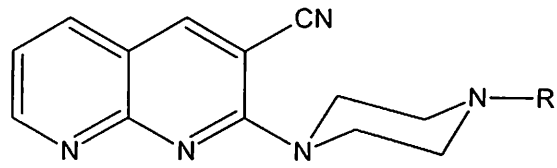
b) 3-Methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-ones (**IP-1 – IP-24**)



n = 2, 3

R = alkyl, aryl

c) 2-(4-Substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles (**NA-1 – NA-12**)



R = alkyl, aryl

3.2.3. Pharmacological Evaluation

a) 5-HT_3 receptor antagonism studies

All the synthesized compounds were evaluated for 5-HT_3 receptor antagonisms in the longitudinal muscle myenteric plexus preparation of guinea-pig ileum.

b) Anxiolytic studies

Compounds which showed potent 5-HT_3 receptor antagonism in the above method were selected for studies pertaining to anxiolytic activity in mice by the following models.

i. Elevated plus-maze
ii. Light-Dark exploration
iii. Hole board test.

Chapter IV
Ligand Design

CHAPTER IV

LIGAND DESIGN

Many pharmacophore models for the 5-HT_3 receptor antagonisms have already been reported in the literature. However, substantial discrepancies between these pharmacophore models can be noted because of the different 5-HT_3 compounds chosen and the different models used to build them. Bull et al. (1993) proposed three necessary components for high 5-HT_3 receptor affinity on the basis of a critical examination of five pharmacophore models: (Schmidt and Peroutka 1989; Hibert et al. 1990; Rosen et al. 1990; Evans et al. 1991; Swain et al. 1992) an aromatic ring, a carbonyl group directly attached to the aromatic ring, and a basic nitrogen. The aromatic ring may be the benzene of a benzoate or benzamide, or the five-atom ring of an indole or indazole compound. The carbonyl group may be substituted by a bioisosteric equivalent function, such a 1,2,4-oxadiazole ring (Swain et al. 1991). Clark et al. (1993) proposed a pharmacophore model where the alignment of the receptor element that interacts with basic nitrogen was judged to be a more critical factor than the alignment of the nitrogen itself. In this pharmacophore model, it is the lone pair of the nitrogen atom and not the nitrogen itself that is used for the superposition of the molecules. In this case the lone pair would point to a specific atom on the receptor, but their direction is not necessarily the same for all the compounds. Hibert et al. (1990) proposed the distance between the centroid of the aromatic ring and the oxygen of the carbonyl group to be ~ 3.3 Å, centroid of the aromatic ring and the basic nitrogen to be ~6.7 Å, and the oxygen of the carbonyl group and the basic nitrogen to be ~5.2 Å.

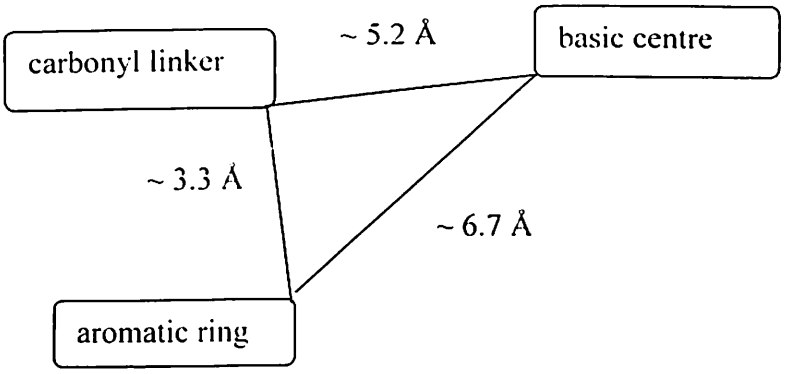
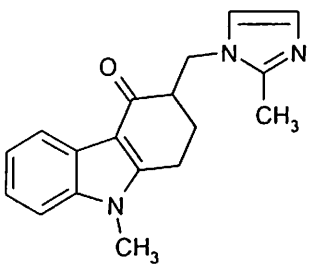
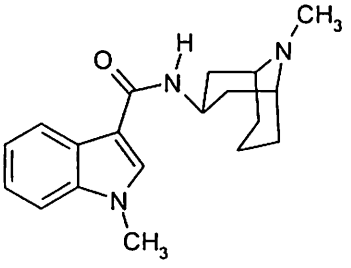


Fig. 4.1. Pharmacophore of 5-HT_3 receptor antagonists

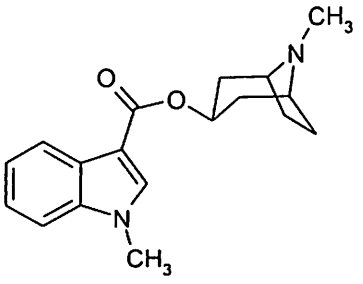
In the present study, the standard and test compounds were submitted to a systemic conformational analysis, and the distances between the pharmacophore features described previously were measured using Tripos-Alchemy 2000 software. The distance between the three pharmacophoric points were calculated for five different conformations and represented as mean ± standard deviation and the values are presented in Table 4.1.



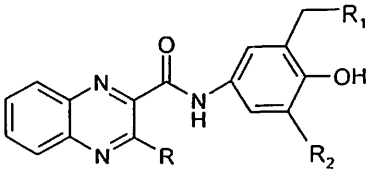
ondansetron



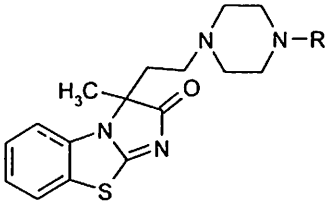
granisetron



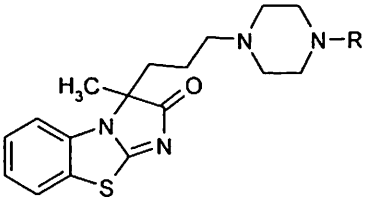
tropisetron



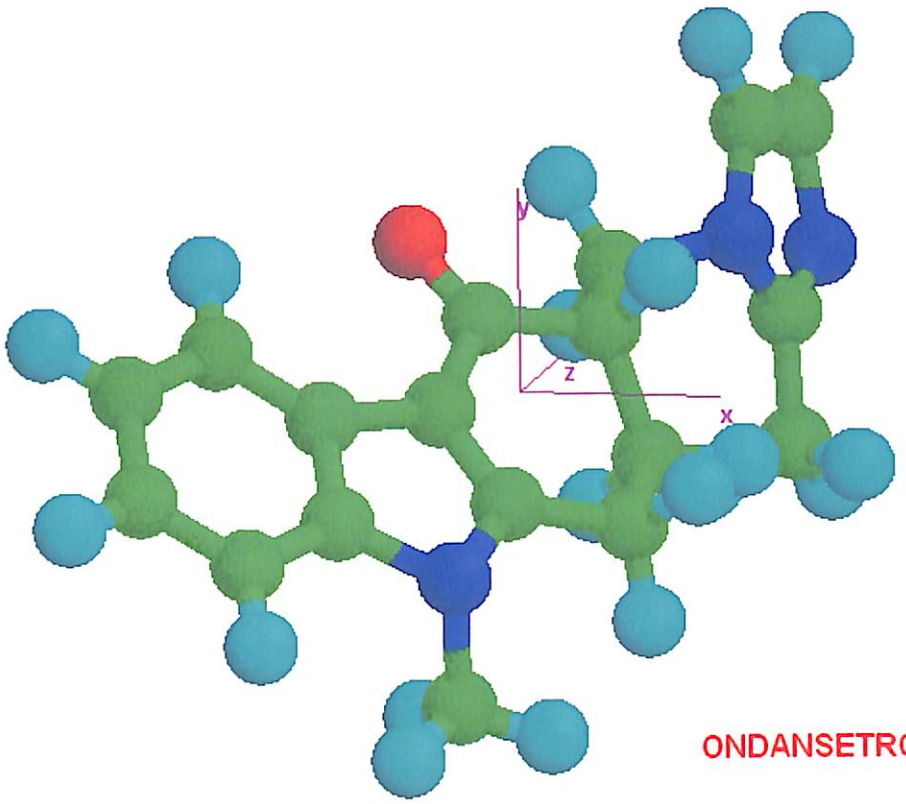
QC



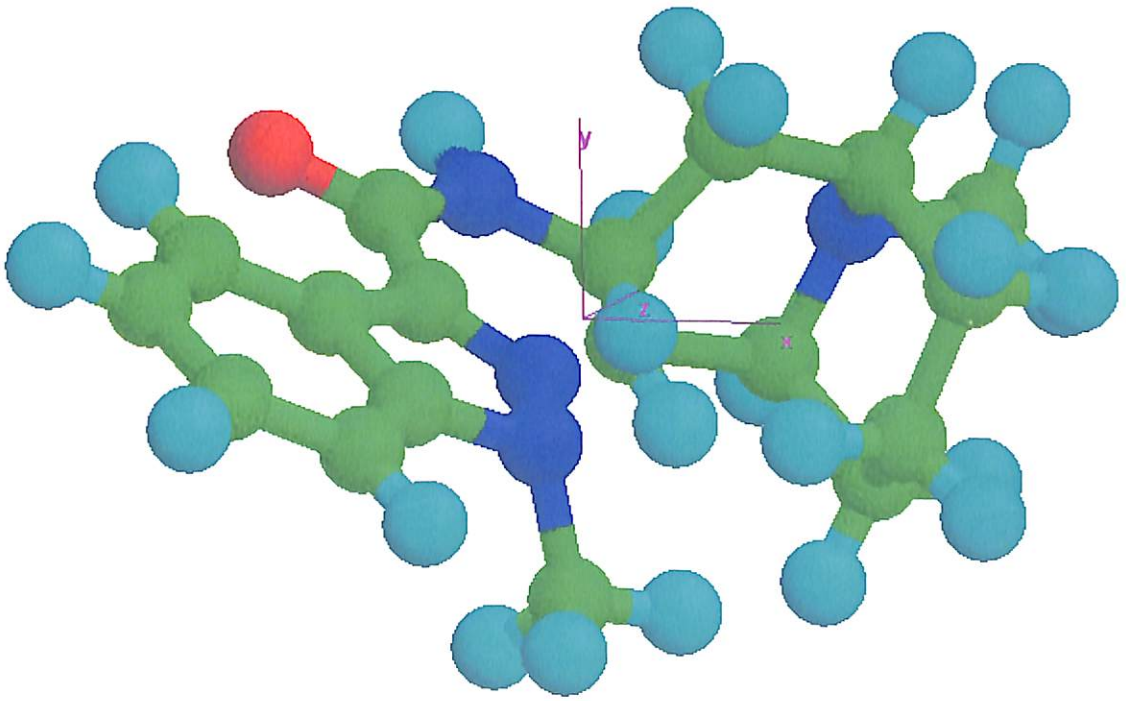
IPa



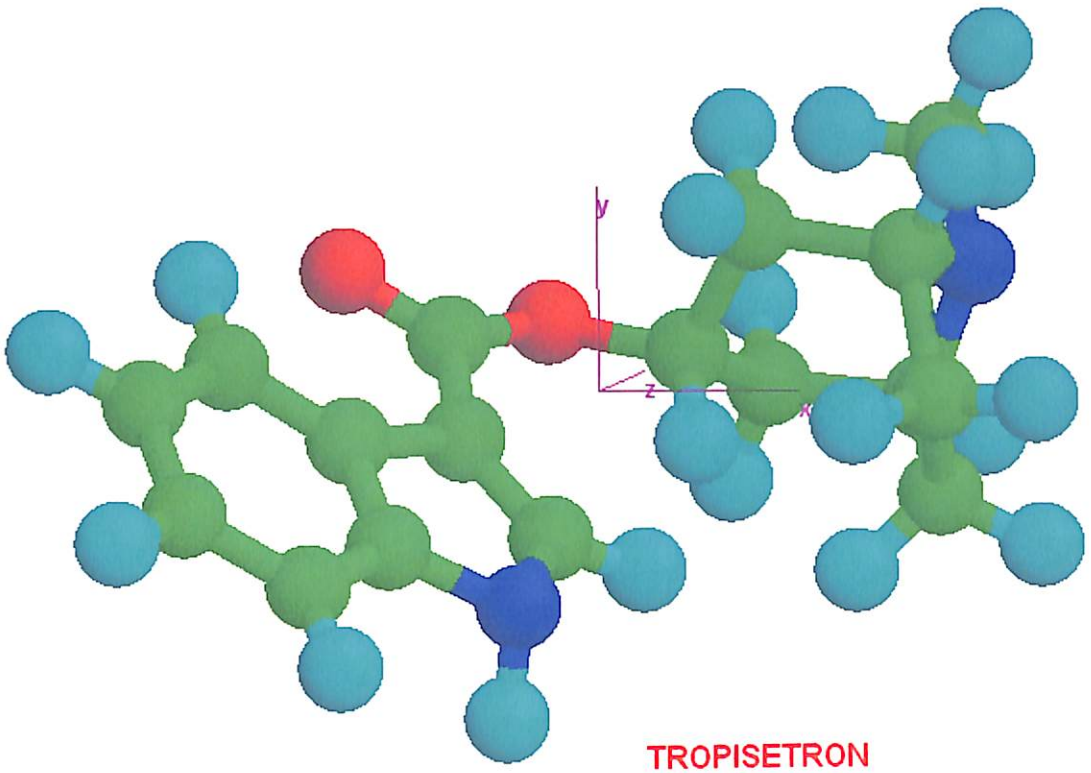
IPb



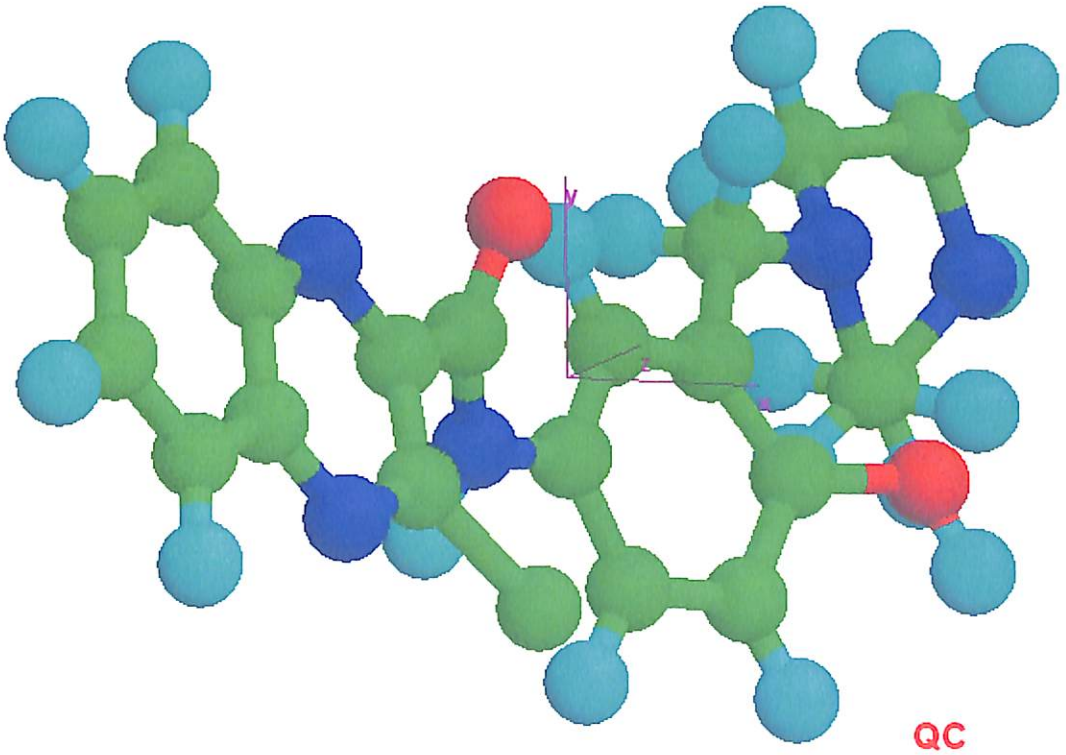
ONDANSETRON



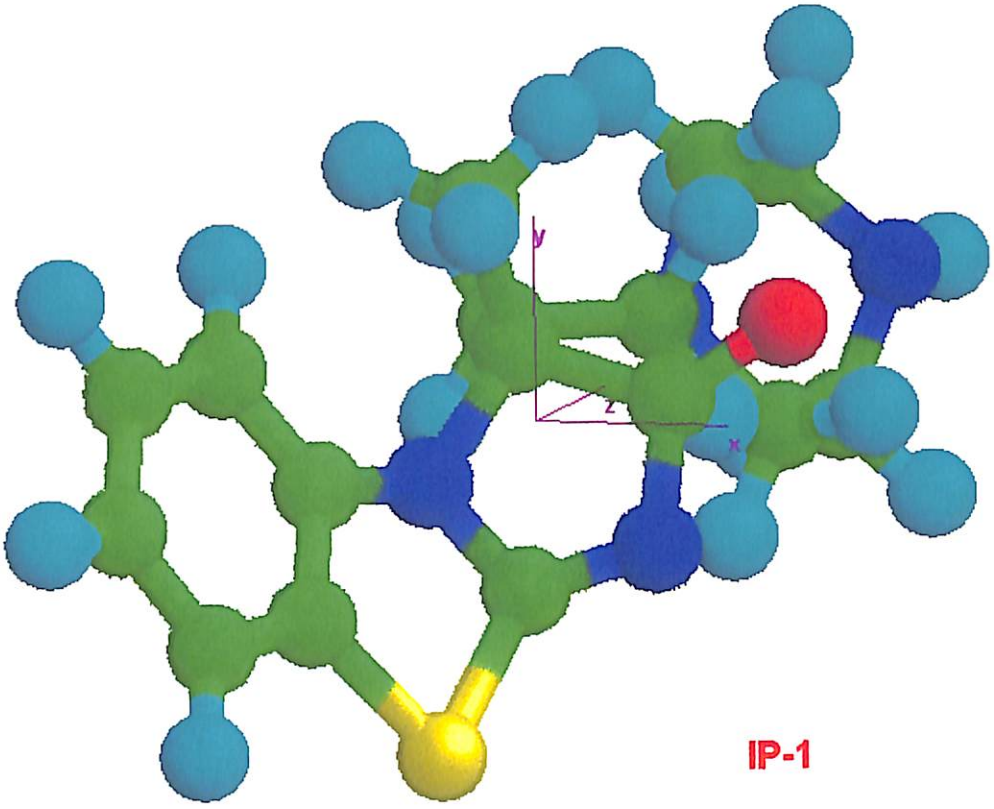
GRANISETRON



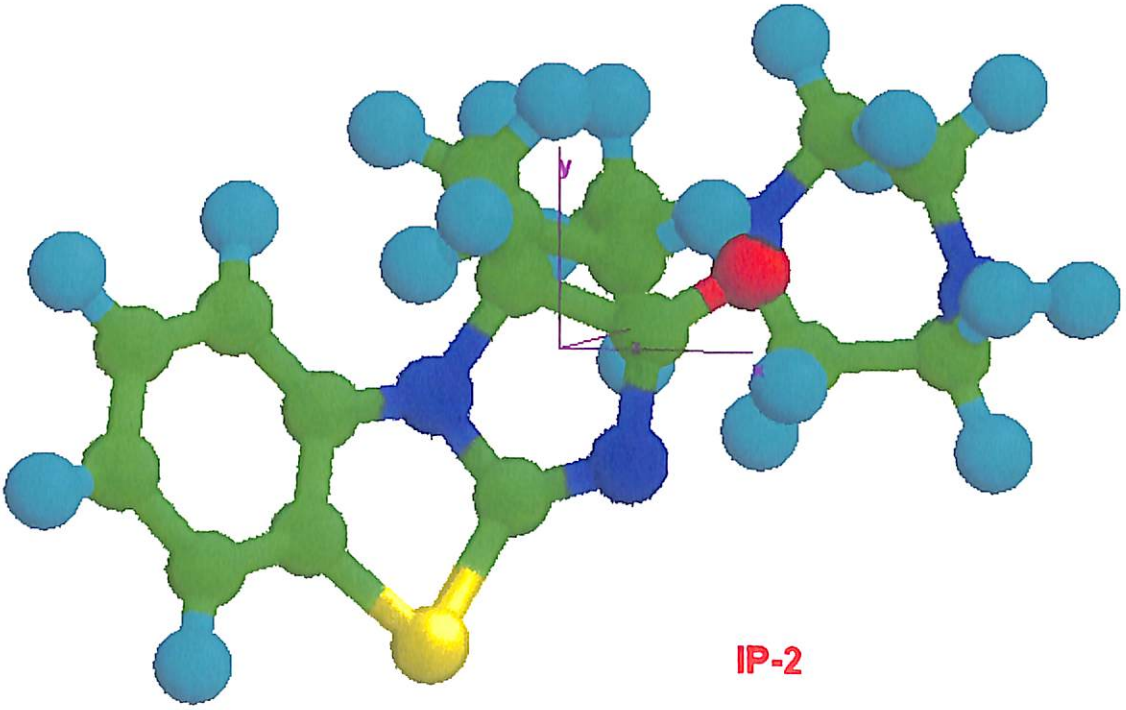
TROPISETRON



QC



IP-1



IP-2

Table 4.1. Distance range between pharmacophoric elements

Compound	Interatomic distances (Å)		
	Ar – N	Ar – O	O - N
Ondansetron	6.90 ± 0.06	3.51 ± 0.07	5.55 ± 0.11
Granisetron	6.88 ± 0.10	3.83 ± 0.10	5.38 ± 0.09
Tropisetron	6.67 ± 0.12	3.39 ± 0.06	5.41 ± 0.07
QC	7.12 - 7.38	3.31–3.44	5.61-5.70
IPa	7.24 - 7.35	3.50-3.62	5.66-5.75
IPb	7.41 - 7.49	3.51-3.64	5.81-5.89

It has been hypothesized that the carbonyl moiety is not essential in the pharmacophore of 5-HT_3 ligands for the high affinity. This component could serve as a hydrogen-bonding region (Rosen et al. 1990). One of the 5-HT_3 ligands that proved this concept is quipazine, which exhibits 5-HT_3 antagonist properties (Round and Wallis 1987) as well as agonist character in some preparations (Emerit et al. 1993). Although quipazine lacks a carbonyl group, the negative electrostatic potential energy field generated by the quinoline nitrogen may resemble that generated by a carbonyl group (Laguerre et al. 1994). Indeed, it has been anticipated that the lone pair electrons of the quipazine nitrogen may play a role equivalent to the carbonyl oxygen (Hibert et al. 1990). Based on this hypothesis, different heteroaryl piperazines viz., piperazinylquinoxaline (**4.2.1**) (Monge et al. 1993; Lasheras et al. 1996), piperazinylbenzothiazole (**4.2.2**) (Monge et al.1994), piperazinylbenzoxazole (**4.2.3**) (Monge et al. 1994), piperazinylbenzimidazole (**4.2.4**) (Orjales et al. 1997) have been studied for their 5-HT_3 antagonistic activities. Among these derivatives piperazinylquinoxaline (**4.2.1**) showed good 5-HT_3 antagonistic activities in the isolated guinea-pig ileum as well as in radioligand binding studies (Monge et al. 1993; Lasheras et al. 1996). The structures of these heteroaryl piperazines are given in Fig. 4.2.

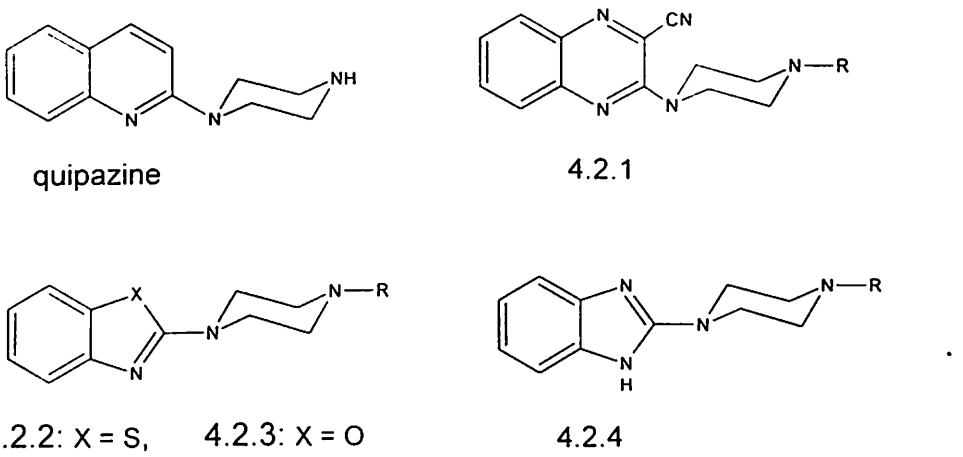


Fig. 4.2. Structure of heteroaryl piperazines as 5-HT_3 receptor antagonists

In the present study, the standard 5-HT_3 ligand, quipazine, and the test molecule, **NA-4**, were subjected to energy minimization by molecular mechanics method using Tripos-Alchemy 2000 software. Molecular superposition was performed by root mean square (RMS) fit after selecting identical atoms in both molecules. The RMS fit routine is provided to determine how closely molecules resemble each other. The value of the RMS distance is shown in Fig. 4.3.

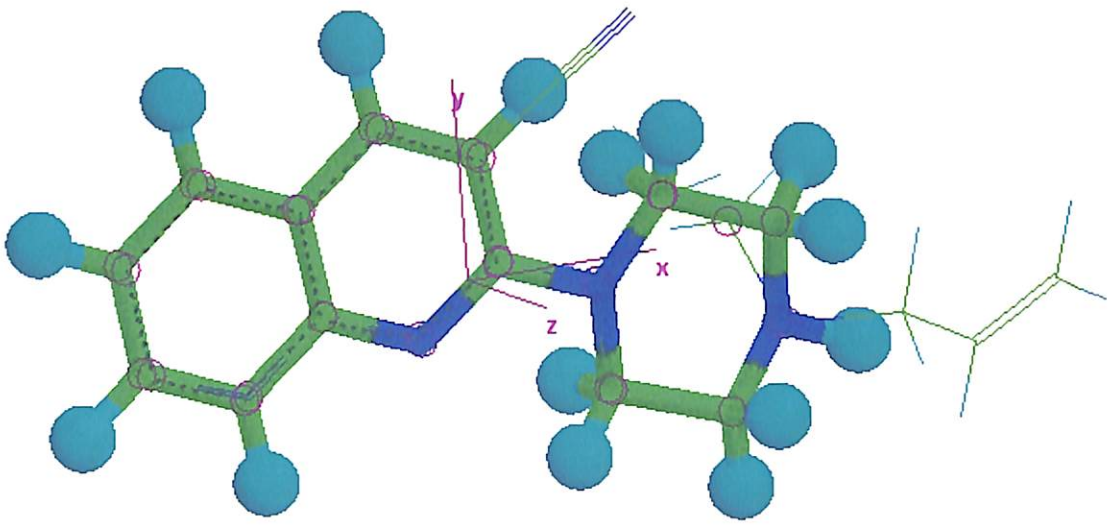


Fig. 4.3. Superposition of Quipazine (ball and stick) and NA-4 (stick) (RMS = 0.097).

Chapter V
Experimental Work

CHAPTER V

EXPERIMENTAL WORK

5.1. MATERIALS AND METHODS

Melting points were determined in open capillaries using Büchi 530 melting point apparatus without correction. The reactions were monitored and the purity of the compounds was checked by ascending thin layer chromatography (TLC) using silica gel coated aluminium plates (Merck 60 F254, 0.25mm) and the spots were visualized under ultra violet light at 254 and 366 nm. Microwave irradiations were carried out in domestic microwave oven (LG Electronics, model MG-605AP, 2450MHz, 900W). Infra red (IR) spectra were recorded in KBr pellets on Jasco IR Report-100 or Schimadzu IR Prestige-21 FT-IR spectrophotometer (cm^{-1}). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained from Bruker DRX300 spectrometer using tetramethylsilane (TMS) as internal standard [chemical shifts in δ, parts per million (ppm)], mass spectra on a VG-70-S mass spectrometer and elemental analysis (C, H, N) on a Perkin Elmer 2400 CHN elemental analyzer. Unless otherwise indicated, all reagents were purchased from commercial suppliers and were used without further purification.

5.2. SYNTHESIS

Synthesis of the present work is discussed under the following heads.

Series I : 3-Substituted-2-quinoxalinecarboxamides (**QC-1 – QC-36**)

Series II : 3-Methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo-[2,1-*b*][1,3]benzothiazol- 2(3*H*)-ones (**IP-1 – IP-24**)

Series III : 2-(4-Substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles (**NA-1 - NA-12**)

5.2.1. Series I: 3-Substituted-2-quinoxalinecarboxamides (QC-1 – QC-36)

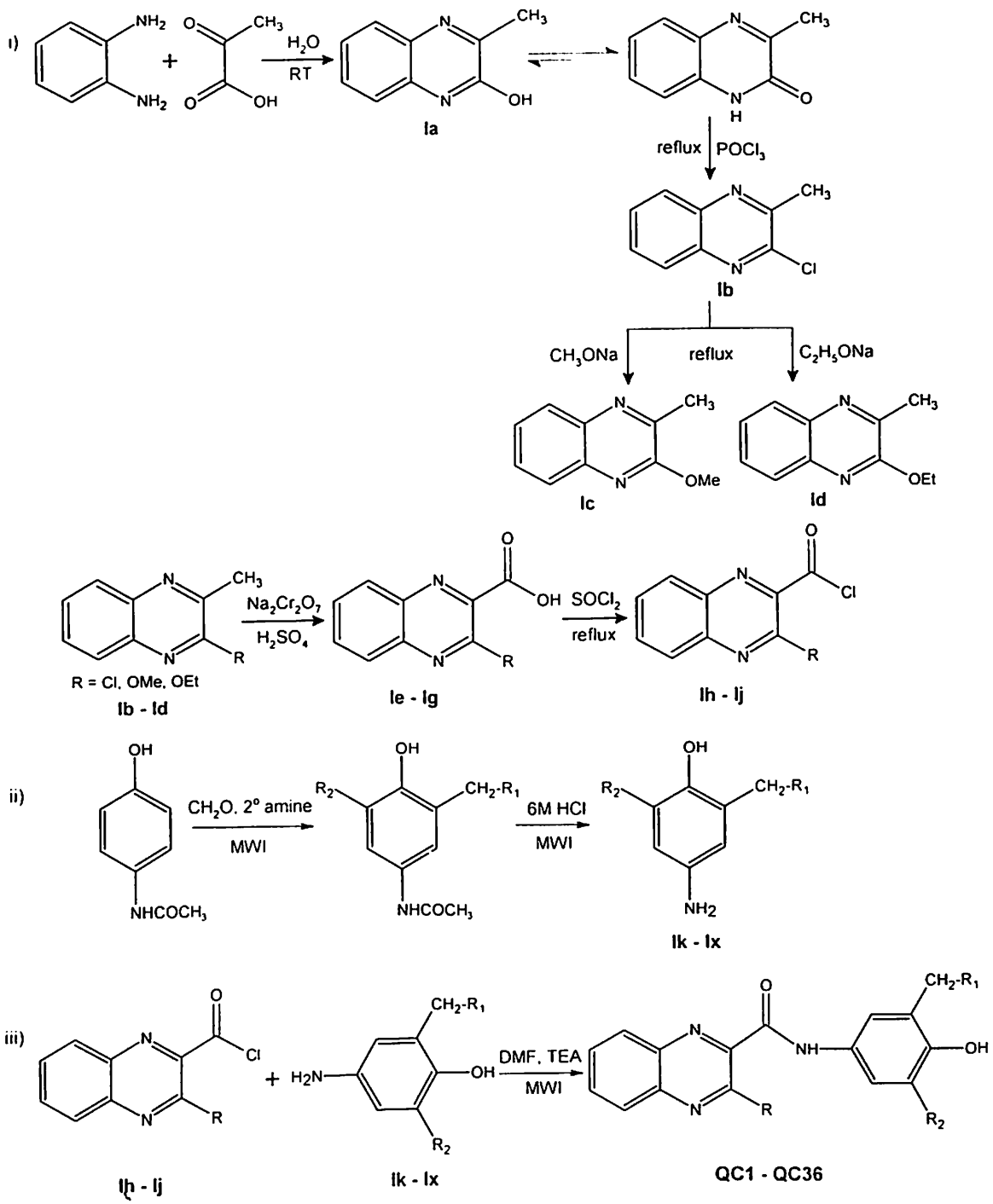


Fig. 5.1. Synthesis of 3-substituted-2-quinoxalinecarboxamides

2-Hydroxy-3-methylquinoxaline (Ia):

To the solution of *o*-phenylenediamine (5.4 gm, 0.05 mol) in water (60 ml), a solution of pyruvic acid (3.5 ml, 0.05 mol) in water (20 ml) was added. The reaction mixture was stirred at room temperature for 10 min. A yellow coloured precipitate got separated out, which was filtered, washed with water (2 x 20 ml) and dried. The product was purified by dissolving in 5% sodium hydroxide (30 ml), digested with charcoal for 5 min and filtered. The clear filtrate was cooled and neutralized with glacial acetic acid to pH 6.0. The separated product was filtered, washed with water (2 x 20 ml), dried and recrystallized from butan-2-one to afford **Ia** in 64% (5.15 gm) yield. It showed a m.p. of 249-250 °C which was comparable to literature data (Krishnan et al. 2001: 247-249 °C).

IR (KBr) cm^{-1} : 3341 (N-H str.), 3045, 3017 (aromatic C-H str.), 1667 (C=O str.), 1595, 1487 (C=C & C=N ring str.).

2-Chloro-3-methylquinoxaline: (Ib):

Phosphorus oxychloride (80 ml) was added slowly to 2-hydroxy-3-methylquinoxaline, **Ia**, (8.0 gm, 0.05 mol) in ice-cold condition. A few drops of dimethyl formamide was added to the reaction mixture and heated to reflux for 45 min. Excess phosphorous oxychloride was removed under reduced pressure and the obtained viscous mass was poured into crushed ice and then neutralized with 10% sodium hydroxide solution in ice-cold condition. The separated solid was filtered, washed with water (2 x 20 ml), dried and recrystallized from acetone to afford **Ib** in 67% (6.0 gm) yield. It showed a m.p.of 84-85 °C which was comparable to literature data (Krishnan et al. 2001: 85.8 °C).

IR (KBr) cm^{-1} : 3041, 3007 (aromatic C-H str.), 1601, 1499 (C=C & C=N ring str.).

2-Methoxy-3-methylquinoxaline (Ic):

A solution of sodium methoxide (prepared from sodium (1gm) and 50 ml dry methanol) was added drop-wise to a solution of 2-chloro-3-methylquinoxaline, **Ib**, (8.0 gm, 0.05 mol) in boiling methanol. The mixture was refluxed for 2 hr in an oil bath and then concentrated under reduced pressure. Water was added and the precipitated material was filtered out and recrystallized from methanol-water (80:20) mixture to afford **Ic** in 92% (7.2 gm) yield. It showed a m.p. of 69-70 °C which was comparable to literature data (Cheeseman 1945: 68-69 °C).

IR (KBr) cm^{-1} : 3050, 3018 (aromatic C-H str.), 1589, 1487 (C=C & C=N ring str.).

2-Ethoxy-3-methylquinoxaline (Id):

A solution of sodium ethoxide (prepared from sodium (1gm) and 50 ml dry ethanol) was added drop-wise to a solution of 2-chloro-3-methylquinoxaline, **Ib**, (8.0 gm, 0.05 mol) in boiling ethanol. The mixture was refluxed for 1 hr in an oil bath. The mixture was concentrated under reduced pressure. Water was added to the residue and the precipitated material was filtered out and recrystallized from *n*-hexane to afford **Id** in 73% (8.7 gm) yield. It showed a m.p. of 56-57 °C (Newbold and Spring 1948: 55-57 °C).

IR (KBr) cm^{-1} : 3039, 3010 (aromatic C-H str.), 1584, 1491 (C=C & C=N ring str.).

3-Chloroquinoxaline-2-carboxylic acid (Ie):

To a solution of sodium dichromate (8.9 gm, 0.03 mol) in H_2O (15 ml), 2-chloro-3-methylquinoxaline, **Ib**, (3.6 gm, 0.02 mol) was added with stirring. To this H_2SO_4 (11.5 ml) was added drop by drop with occasional cooling. After the addition of the acid, the reaction mixture was heated on water bath for 30 min, followed by cooling and filtration. To the obtained residue, 5% sulphuric acid (12 ml) was added and digested for 5 min. After cooling, the product was filtered and dissolved in 5% sodium hydroxide solution and refiltered to remove any chromium hydroxide. The filtrate was acidified with 5% H_2SO_4 and the resultant product was filtered, washed with water, dried and recrystallized from ethanol-acetone (90:10) mixture to afford **Ie** in 65% (2.7 gm) yield. It showed a m.p. of 146-147 °C (decomp.).

IR (KBr) cm^{-1} : 3317 (broad O-H str. COOH), 3046, 3017 (aromatic C-H str.), 1688 (C=O str.), 1603,1484 (C=C & C=N ring str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 7.58 (d, 2H, H_6 and H_7 quinoxaline), 8.06 (d, 2H, H_5 and H_8 quinoxaline), 12.78 (s, 1H, CO_2H).

3-Methoxyquinoxaline-2-carboxylic acid (If):

The compound was prepared from 2-methoxy-3-methylquinoxaline, **Ic**, (3.5 gm, 0.02 mol) by a similar method as described for **Ie** and the resultant product was recrystallized from methanol-water (80:20) mixture to afford **If** in 76% (3.0 gm) yield. It showed a m.p. of 131-132 °C.

IR (KBr) cm^{-1} : 3303 (broad O-H str. COOH), 3037, 3005 (aromatic C-H str.), 1701 (C=O str.), 1594, 1488 (C=C & C=N ring str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 3.71 (s, 3H, OCH_3), 7.71 (d, 2H, H_6 & H_7 quinoxaline), 8.17 (d, 2H, H_5 & H_8 quinoxaline), 12.60 (s, 1H, CO_2H).

3-Ethoxyquinoxaline-2-carboxylic acid (Ig):

The compound was prepared from 2-ethoxy-3-methylquinoxaline, **Id**, (3.8 gm, 0.02 mol) by a similar method as described for **Ie** and was recrystallized from methanol-water (80:20) mixture to afford **Ig** in 73% (3.2 gm) yield. It showed a m.p. of 120-121 °C.

IR (KBr) cm^{-1} : 3310 (broad O-H str. COOH), 3043, 3010 (aromatic C-H str.), 1706 (C=O str.), 1585, 1491 (C=C & C=N ring str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.31 (t, 3H, CH_3), 3.97 (q, 2H, CH_2), 7.67 (d, 2H, H_6 & H_7 quinoxaline), 8.10 (d, 2H, H_5 & H_8 quinoxaline), 12.67 (s, 1H, CO_2H).

4-Amino-2-[(dimethylamino)methyl]phenol (Ik):

A mixture of 4-acetamidophenol (3.02 gm, 0.02 mol), 37% formaldehyde (2.4 ml) and *N,N*-dimethylamine (2.5 ml, 0.02 mol) in ethanol (3 ml) were taken in a 100 ml Erlenmeyer flask covered with a small funnel. This system was then placed in an alumina bath inside the microwave oven and heated the contents for 3 min at 60% power output (540 watt). Upon completion of the reaction (monitored by TLC), the solvent was removed using a rotary flash evaporator. The residue was dissolved in 16 ml of 6 M HCl and again heated in the microwave oven for 5 min at 80% power output (720 watt). The mixture was cooled, the pH made alkaline with ammonia solution, extracted with dichloromethane (4 x 30 ml), washed with water, dried ($MgSO_4$) and evaporated to give the free amine which was converted to a salt by dissolving the free amine in ether and treating with alcoholic hydrogen chloride, by which the solid separated out. The solvent was removed and recrystallized from ethanol-ethylacetate (75:25) mixture to afford **Ik** in 74% (3.52 gm) yield. It showed a m.p. of 239-240 °C.

IR (KBr) cm^{-1} : 3412-3156 (broad, OH & NH_2), 3063, 3040 (aromatic C-H str.), 2958, 2901 (aliphatic C-H str.), 1591, 1484 (C=C ring str.), 1130 (aliphatic C-N str.).

4-Amino-2-[(diethylamino)methyl]phenol (Il):

The compound was prepared in a similar manner as **Ik** by using *N,N*-diethylamine (2.0 ml, 0.02 mol) in place of *N,N*-dimethylamine and the product was recrystallized from ethanol to afford **Il** in 96% (5.1 gm) yield. It showed a m.p. of 217-220 °C (Burckhalter et al. 1948: 218-220 °C).

IR (KBr) cm^{-1} : 3400-3184 (broad, OH & NH_2), 3045, 3010 (aromatic C-H str.), 2960, 2896 (aliphatic C-H str.), 1585, 1497 (C=C ring str.), 1174 (aliphatic C-N str.).

4-Amino-2-(pyrrolidin-1-ylmethyl)phenol (Im):

The compound was prepared in a similar manner as **Ik** by using pyrrolidine (1.7 ml, 0.02 mol) in place of *N,N*-dimethylamine and the resultant product was recrystallized from ethanol to afford **Im** in 67% (3.53 gm) yield. It showed a m.p. of 159-160 °C.

IR (KBr) cm^{-1} : 3422-3155 (broad, OH & NH_2), 3054, 3017 (aromatic C-H str.), 2960, 2896, 2875 (aliphatic C-H str.), 1591, 1456 (C=C ring str.), 1154 (aliphatic C-N str.).

4-Amino-2-(piperidin-1-ylmethyl)phenol (In):

The compound was prepared in a similar manner as **Ik** by using piperidine (1.9 ml, 0.02 mol) in place of *N,N*-dimethylamine and the resultant product was recrystallized from ethanol to afford **In** in 92% (5.11 gm) yield. It showed a m.p. of 153-155 °C (decomp.) (Burckhalter et al. 1948: 153-155 °C).

IR (KBr) cm^{-1} : 3408-3139 (broad, OH & NH_2), 3071, 3011 (aromatic C-H str.), 2981, 2904, 2891 (aliphatic C-H str.), 1587, 1471 (C=C ring str.), 1139 (aliphatic C-N str.).

4-Amino-2-(morpholin-4-ylmethyl)phenol (Io):

The compound was prepared in a similar manner as **Ik** by using morpholine (1.7 ml, 0.02 mol) in place of *N,N*-dimethylamine and the resultant product was recrystallized from ethanol to afford **Io** in 52% (2.91 gm) yield. It showed a m.p. of 260-261 °C.

IR (KBr) cm^{-1} : 3416-3155 (broad, OH & NH_2), 3034, 3010 (aromatic C-H str.), 2971, 2896, 2889 (aliphatic C-H str.), 1591, 1456 (C=C ring str.), 1150 (aliphatic C-N str.).

4-Amino-2-(piperazin-1-ylmethyl)phenol (Ip):

The compound was prepared in a similar manner as **Ik** by using piperazine (1.7 gm, 0.02 mol) in place of *N,N*-dimethylamine and the resultant product was recrystallized from ethanol-acetone (75:25) mixture to afford **Ip** in 63% (3.51 gm) yield. It showed a m.p. of 201-202 °C.

IR (KBr) cm^{-1} : 3466-3195 (broad, OH & NH_2), 3037, 3010 (aromatic C-H str.), 2984, 2927, 2894 (aliphatic C-H str.), 1589, 1491 (C=C ring str.), 1155 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.0 (s, 1H, NH), 2.65-2.67 (t, 4H, $N^4(CH_2)_2$), 2.48-2.50 (t, 4H, $N^1(CH_2)_2$), 5.1 (s, 2H, NH_2), 6.0 (s, 1H, OH), 6.18 (s, 1H, C_3-H), 6.27-6.28 (d, 1H, C_5-H), 6.46-6.47 (d, 1H, C_6-H).

4-Amino-2-[(4-methylpiperazin-1-yl)methyl]phenol (Iq):

The compound was prepared in a similar manner as **Ik** by using 1-methylpiperazine (2.2 ml, 0.02 mol) in place of *N,N*-dimethylamine and the resultant product was recrystallized from ethanol-ethylacetate (80:20) mixture to afford **Iq** in 62% (3.63 gm) yield. It showed a m.p. of 160-162 °C.

IR (KBr) cm^{-1} : 3443-3177 (broad, OH & NH_2 str.), 3041, 3004 (aromatic C-H str.), 2977, 2931, 2897 (aliphatic C-H str.), 1577, 1475 (C=C ring str.), 1094 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.27 (s, 1H, N^4CH_3), 2.46-2.48 (t, 4H, $N^4(CH_2)_2$), 2.55-2.57 (t, 4H, $N^1(CH_2)_2$), 5.1 (s, 2H, NH_2), 6.0 (s, 1H, OH), 6.18 (s,1H, C_3-H), 6.27-6.28 (d, 1H, C_5-H), 6.46-6.47 (d, 1H, C_6-H).

4-Amino-2-[(4-ethylpiperazin-1-yl)methyl]phenol (Ir):

The compound was prepared in a similar manner as **Ik** by using 1-ethylpiperazine (2.5 ml, 0.02 mol) in place of *N,N*-dimethylamine and the resultant product was recrystallized from ethanol:ethylacetate (80:20) mixture to afford **Ir** in 61% (3.74 gm) yield. It showed a m.p. of 179-180 °C.

IR (KBr) cm^{-1} : 3439-3186 (broad, OH & NH_2 str.), 3049, 3028 (aromatic C-H str.), 2993, 2926, 2986 (aliphatic C-H str.), 1596, 1459 (C=C ring str.), 1186 (aliphatic C-N str.).

4-Amino-2,6-bis[(diethylamino)methyl]phenol (Is):

The compound was prepared in a similar manner as **Ik** by using a mixture a 4-acetamidophenol (2.95 gm, 0.0195 mol), 37% formaldehyde (6.5 ml) and *N,N*-diethylamine (5.8 ml, 0.054 mol) in ethanol (3 ml) and the resultant product was recrystallized from ethanol to afford **Is** in 71% (4.35 gm) yield. It showed a m.p. of 132-134 °C.

IR (KBr) cm^{-1} : 3424-3193 (broad, OH & NH_2 str.), 3038, 3021 (aromatic C-H str.), 2986, 2957 (aliphatic C-H str.), 1589, 1474 (C=C ring str.), 1167 (aliphatic C-N str.).

4-Amino-2,6-bis(pyrrolidin-1-ylmethyl)phenol (It):

The compound was prepared in a similar manner as **Ik** by using the same reactants as that for **Is** but using pyrrolidine (4.5 ml, 0.054 mol) instead of *N,N*-diethylamine and the resultant product was recrystallized from ethanol to afford **It** in 54% (3.26 gm) yield. It showed a m.p. of 218-219 °C (Stout et al., 1983: 219-221 °C).

IR (KBr) cm^{-1} : 3411-3145 (broad, OH & NH_2), 3057, 3017 (aromatic C-H str.), 2969, 2896, 2875 (aliphatic C-H str.), 1582, 1450 (C=C ring str.), 1146 (aliphatic C-N str.).

4-Amino-2,6-bis(morpholin-4-ylmethyl)phenol (Iu):

The compound was prepared in a similar manner as **Ik** by using the same reactants as that for **Is** but using morpholine (4.7 ml, 0.054 mol) instead of *N,N*-diethylamine and the resultant product was recrystallized from ethanol-acetone (90:10) mixture to afford **Iu** in 82% (5.47 gm) yield. It showed a m.p. of 209-211 °C.

IR (KBr) cm^{-1} : 3430-3147 (broad, OH & NH_2), 3035, 3021 (aromatic C-H str.), 2961, 2890, 2875 (aliphatic C-H str.), 1585, 1466 (C=C ring str.), 1144 (aliphatic C-N str.).

4-Amino-2,6-bis(piperazin-1-ylmethyl)phenol (Iv):

The compound was prepared in a similar manner as **Ik** by using the same reactants as that for **Is** but using piperazine (4.6 gm, 0.054 mol) instead of *N,N*-diethylamine and the resultant product was recrystallized from ethanol to afford **Iv** in 67% (4.45 gm) yield. It showed a m.p. of 243-245 °C.

IR (KBr) cm^{-1} : 3438-3192 (broad, OH & NH_2 str.), 3044, 3012 (aromatic C-H str.), 2989, 2936, 2885 (aliphatic C-H str.), 1586, 1461 (C=C ring str.), 1176 (aliphatic C-N str.).

4-Amino-2,6-bis[(4-methylpiperazin-1-yl)methyl]phenol (Iw):

The compound was prepared in a similar manner as **Ik** by using the same reactants as that for **Is** but using 1-methylpiperazine (6.0 ml, 0.054 mol) instead of *N,N*-diethylamine and the resultant product was recrystallized from ethanol-ethylacetate (90:10) mixture to afford **Iw** in 67% (4.81 gm) yield. It showed a m.p. of 206-208 °C.

IR (KBr) cm^{-1} : 3441-3187 (broad, OH & NH_2 str.), 3037, 3006 (aromatic C-H str.), 2991, 2957, 2894 (aliphatic C-H str.), 1580, 1494 (C=C ring str.), 1161 (aliphatic C-N str.).

4-Amino-2,6-bis[(4-ethylpiperazin-1-yl)methyl]phenol (Ix):

The compound was prepared in a similar manner as **Ik** by using the same reactants as that for **Is** but using 1-ethylpiperazine (6.9 ml, 0.054 mol) instead of *N,N*-diethylamine and the resultant product was recrystallized from ethanol-ethylacetate (90:10) mixture to afford **Ix** in 67% (5.18 gm) yield. It showed a m.p. of 226-228 °C.

IR (KBr) cm^{-1} : 3452-3165 (broad, OH & NH_2), 3044, 3019 (aromatic C-H str.), 2981, 2856, 2875 (aliphatic C-H str.), 1587, 1459 (C=C ring str.), 1144 (aliphatic C-N str.).

3-Chloro-*N*-{3-[(dimethylamino)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide (QC-1):

A mixture of 3-chloroquinoxaline-2-carboxylic acid, **Ie,** (0.62 gm, 0.003 mol), thionyl chloride (5 ml) and 2 drops of dimethyl formamide (DMF) was refluxed for 1 hr. Excess thionyl chloride was removed under reduced pressure to obtain the crude acid chloride (**Ih**). To the acid chloride, DMF (1 ml) was added and this solution was added slowly to a solution of hydrochloride salt of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik**, (0.71 gm, 0.003 mol) in DMF (2 ml) and triethylamine (1.5 ml, 0.01 mol). This reaction mixture was then irradiated with microwaves for 2 min at 80% power output (720 watt). DMF was removed using rotary flash evaporator and to the residue, water (10 ml) was added. The obtained product was collected by filtration, dried and recrystallized from ethanol-acetone (75:25) mixture to afford **QC-1** in 57% (1.02 gm) yield. It showed a m.p. of 225-226 °C.

IR (KBr) cm^{-1} : 3406-3217 (broad, OH & NH str.), 3037, 3018 (aromatic C-H str.), 2989, 2955, (aliphatic C-H str.), 1670 (C=O str.), 1621 (N-H bend.), 1601, 1491 (C=C & C=N ring str.), 1184 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.28 (s, 6H, $N(CH_3)_2$), 3.74 (s, 2H, $\underline{CH_2}$-$N(CH_3)_2$), 7.12-8.34 (m, 7H, ArH), 9.86 (br s, 1H, CONH), 11.47 (br s,1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	60.67	04.77	15.73
Found :	60.93	04.56	16.03

3-Chloro-*N*-{3-[(diethylamino)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide (QC-2):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-[(diethylamino)methyl]phenol, **II**, (0.8 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-acetone (75:25) mixture to afford **QC-2** in 63% (0.72 gm) yield. It showed a m.p. of 210-212 °C.

IR (KBr) cm^{-1} : 3417-3201 (broad, OH & NH str.), 3046, 3011 (aromatic C-H str.), 2991, 2960, (aliphatic C-H str.), 1653 (C=O str.), 1617 (N-H bend.), 1592, 1479 (C=C & C=N ring str.), 1166 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.02-1.04 (t, 6H, $(CH_3)_2$) 2.42-2.45 (q, 4H, $N(CH_2)_2$), 3.64 (s, 2H, CH_2-N), 7.27-8.17 (m, 7H, ArH), 9.90 (br s, 1H, CONH), 11.51 (br s, 1H, OH).

Elemental analysis (%) :

		C	H	N
Calculated	:	62.50	05.46	14.58
Found	:	62.34	05.59	14.37

3-Chloro-*N*-[4-hydroxy-3-(pyrrolidin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-3):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-(pyrrolidin-1-ylmethyl)phenol, **Im**, (0.8 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-chloroform (75:25) mixture to afford **QC-3** in 56% (0.64 gm) yield. It showed a m.p. of 218-219 °C.

IR (KBr) cm^{-1} : 3401-3187 (broad, OH & NH str.), 3051, 3019 (aromatic C-H str.), 2987, 2948, 2892 (aliphatic C-H str.), 1664 (C=O str.), 1627 (N-H bend.), 1586, 1490 (C=C & C=N ring str.), 1185 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.62-1.64 (t, 4H, $(CH_2)_2$ pyrrolidinyl) 2.31-2.33 (t, 4H, $N(CH_2)_2$ pyrrolidinyl), 3.62 (s, 2H, CH_2-N), 7.12-8.34 (m, 7H, ArH), 9.86 (br s, 1H, CONH), 11.47 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	62.83	04.97	14.66
Found :	62.95	04.66	14.35

3-Chloro-*N*-[4-hydroxy-3-(piperidin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-4):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-(piperidin-1-ylmethyl)phenol, **In**, (0.83 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-chloroform (75:25) mixture to afford **QC-4** in 56% (0.66 gm) yield. It showed a m.p. of 236-239 °C.

IR (KBr) (cm^{-1}) : 3409-3205 (broad, OH & NH str.), 3043, 3011 (aromatic C-H str.), 2990, 2964, 2887 (aliphatic C-H str.), 1655 (C=O str.), 1615 (N-H bend.), 1593, 1476 (C=C & C=N ring str.), 1157 (aliphatic C-N str.).

3-Chloro-*N*-[4-hydroxy-3-(morpholin-4-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-5):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-(morpholin-4-ylmethyl)phenol, **Io**, (0.84 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-acetone (75:25) mixture to afford **QC-5** in 64% (0.76 gm) yield. It showed a m.p. of 188-190 °C.

IR (KBr) cm^{-1} : 3412-3195 (broad, OH & NH str.), 3039, 3017 (aromatic C-H str.), 2987, 2964, 2886 (aliphatic C-H str.), 1661 (C=O str.), 1627 (N-H bend.), 1581, 1494 (C=C & C=N ring str.), 1175 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 3.67-3.70 (t, 4H, $O(CH_2)_2$ morpholinyl) 2.37-2.40 (t, 4H, $N(CH_2)_2$ morpholinyl), 3.74 (s, 2H, CH_2-N), 7.12-8.34 (m, 7H, ArH), 9.89 (br s, 1H, CONH), 11.39 (br s, 1H, OH).

Elemental analysis (%) :

		C	H	N
Calculated	:	60.30	04.77	14.07
Found	:	60.03	04.96	14.01

3-Chloro-*N*-[4-hydroxy-3-(piperazin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-6):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-(piperazin-1-ylmethyl)phenol, **Ip**, (0.84 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-ethylacetate (75:25) mixture to afford **QC-6** in 66% (0.78 gm) yield. It showed a m.p. of 221-223 °C.

IR (KBr) cm^{-1} : 3407-3206 (broad, OH & NH str.), 3044, 3020 (aromatic C-H str.), 2991, 2967, 2885 (aliphatic C-H str.), 1655 (C=O str.), 1634 (N-H bend.), 1595, 1487 (C=C & C=N ring tr.), 1179 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.20 (s, 1H, NH), 2.48-2.51 (t, 4H, $N^1(CH_2)_2$), 2.65-2.68 (t, 4H, $N^4(CH_2)_2$), 3.70 (s, 2H, CH_2-N^1), 7.12-8.14 (m, 7H, ArH), 9.81 (br s, 1H, CONH), 11.53 (br s, 1H, OH).

Elemental analysis (%) :

		C	H	N
Calculated	:	60.45	05.04	14.11
Found	:	60.73	05.26	14.01

3-Chloro-*N*-{4-hydroxy-3-[(4-methylpiperazin-1-yl)methyl]phenyl}-quinoxaline-2-carboxamide (QC-7):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-[(4-methylpiperazin-1-yl)methyl]phenol, **Iq**, (0.88 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-ethylacetate (75:25) mixture to afford **QC-7** in 65% (0.79 gm) yield. It showed a m.p. of 211-213 °C.

IR (KBr) cm^{-1} : 3397-3187 (broad, OH & NH str.), 3036, 3012 (aromatic C-H str.), 2984, 2946, (aliphatic C-H str.), 1664 (C=O str.), 1619 (N-H bend.), 1603, 1500 (C=C & C=N ring str.), 1160 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.28 (s, 1H, N^4CH_3), 2.47-2.49 (t, 4H, $N^4(CH_2)_2$), 2.58-2.60 (t, 4H, $N^1(CH_2)_2$), 3.74 (s, 2H, CH_2-N^1), 7.01-8.14 (m, 7H, ArH), 9.90 (br s, 1H, CONH), 11.47 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated	: 61.31	05.35	17.03
Found	: 61.54	05.66	16.84

3-Chloro-*N*-{3-[(4-ethylpiperazin-1-yl)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide (QC-8):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2-[(4-ethylpiperazin-1-yl)methyl]phenol, **Ir**, (0.92 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-ethylacetate (75:25) mixture to afford **QC-8** in 59% (0.75 gm) yield. It showed a m.p. of 202-205 °C.

IR (KBr) cm^{-1} : 3413-3204 (broad, OH & NH str.), 3042, 3008 (aromatic C-H str.), 2991, 2937, (aliphatic C-H str.), 1657 (C=O str.), 1630 (N-H bend.), 1583,1475 (C=C & C=N ring str.), 1176 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 1.01-1.03 (t, 3H, $N^4CH_2\underline{CH_3}$), 2.42-2.44 (q, 1H, $N^4\underline{CH_2}CH_3$), 2.47-2.50 (t, 4H, $N^4(CH_2)_2$), 2.60-2.63 (t, 4H, $N^1(CH_2)_2$), 3.74 (s, 2H, CH_2-N^1), 7.01-8.14 (m, 7H, ArH), 9.89 (br s, 1H, CONH), 11.52 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	62.12	05.65	16.47
Found :	61.96	05.51	16.23

N-{3,5-bis[(diethylamino)methyl]-4-hydroxyphenyl}-3-chloroquinoxaline-2-carboxamide (QC-9):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2,6-bis[(diethylamino)methyl]phenol, **Is**, (0.95 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from acetone to afford **QC-9** in 60% (0.84 gm) yield. It showed a m.p. of 222-224 °C.

IR (KBr) cm^{-1} : 3404-3195 (broad, OH & NH str.), 3051, 3023 (aromatic C-H str.), 2981, 2945, (aliphatic C-H str.), 1666 (C=O str.), 1626 (N-H bend.), 1583, 1494 (C=C & C=N ring str.), 1184 (aliphatic C-N str.).

3-Chloro-N-[4-hydroxy-3,5-bis(pyrrolidin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-10):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2,6-bis(pyrrolidin-1-ylmethyl)phenol, **It**, (0.93 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from chloroform to afford **QC-10** in 55% (0.77 gm) yield. It showed a m.p. of 229-231 °C.

IR (KBr) cm^{-1} : 3421-3206 (broad, OH & NH str.), 3035, 3007 (aromatic C-H str.), 2997, 2947, 2903 (aliphatic C-H str.), 1656 (C=O str.),1631 (N-H bend.), 1586, 1477 (C=C & C=N ring str.), 1175 (aliphatic C-N str.).

3-Chloro-*N*-[4-hydroxy-3,5-bis(morpholin-4-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-11):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2,6-bis(morpholin-4-ylmethyl)phenol, **Iu**, (1.0 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from chloroform to afford **QC-11** in 62% (0.93 gm) yield. It showed a m.p. of 205-208 °C.

IR (KBr) cm^{-1} : 3407-3190 (broad, OH & NH str.), 3043, 3016 (aromatic C-H str.), 2984, 2931, 2891 (aliphatic C-H str.), 1659 (C=O str.),1619 (N-H bend.), 1575, 1488 (C=C & C=N ring str.), 1156 (aliphatic C-N str.).

3-Chloro-*N*-[4-hydroxy-3,5-bis(piperazin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-12):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2,6-bis(piperazin-1-ylmethyl)phenol, **Iv**, (1.0 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-acetone (75:25) mixture to afford **QC-12** in 65% (0.97 gm) yield. It showed a m.p. of 240-243 °C.

IR (KBr) cm^{-1} : 3414-3203 (broad, OH & NH str.), 3037, 3013 (aromatic C-H str.), 2992, 2943, 2910 (aliphatic C-H str.), 1663 (C=O str.), 1610 (N-H bend.), 1591, 1473 (C=C & C=N ring str.), 1185 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.02 (s, 2H, 2 x NH), 2.51-2.54 (t, 8H, 2 x $N^1(CH_2)_2$), 2.67-2.70 (t, 8H, 2 x $N^4(CH_2)_2$), 3.70 (s, 4H, 2 x CH_2-N^1), 7.39-8.19 (m, 6H, ArH), 9.87 (br s, 1H, CONH), 11.57 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	60.48	06.05	19.76
Found :	60.20	06.39	19.84

3-Chloro-*N*-{4-hydroxy-3,5-bis[(4-methylpiperazin-1-yl)methyl]phenyl}-quinoxaline-2-carboxamide (QC-13):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2,6-bis[(methylpiperazin-1-yl)methyl]phenol, **Iw**, (1.11 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-ethylacetate (75:25) mixture to afford **QC-13** in 63% (0.98 gm) yield. It showed a m.p. of 232-234 °C.

IR (KBr) cm^{-1} : 3397-3186 (broad, OH & NH str.), 3043, 3019 (aromatic C-H str.), 2983, 2957, 2895 (aliphatic C-H str.), 1659 (C=O str.), 1633 (N-H bend.), 1584, 1491 (C=C & C=N ring str.), 1197 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.27 (s, 6H, 2 x N^4-CH_3), 2.48-2.50 (t, 8H, 2 x $N^4(CH_2)_2$), 2.56-2.58 (t, 8H, 2 x $N^1(CH_2)_2$), 3.74 (s, 2H, CH_2-N^1), 7.11-8.94 (m, 6H, ArH), 9.94 (br s, 1H, CONH), 11.50 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	61.83	06.49	18.70
Found :	62.22	06.16	18.97

N-{3,5-bis[(4-ethylpiperazin-1-yl)methyl]-4-hydroxyphenyl}-3-chloro-quinoxaline-2-carboxamide (QC-14):

The compound was prepared in a similar procedure as **QC-1** by using 4-amino-2,6-bis[(ethylpiperazin-1-yl)methyl]phenol, **Ix**, (1.19 gm, 0.003 mol) in place of 4-amino-2-[(dimethylamino)methyl]phenol, **Ik** and the resultant product was recrystallized from ethanol-ethylacetate (75:25) mixture to afford **QC-14** in 59% (0.97 gm) yield. It showed a m.p. of 218-220 °C.

IR (KBr) cm^{-1} : 3417-3187 (broad, OH & NH str.), 3040, 3016 (aromatic C-H str.), 2987, 2931, 2891 (aliphatic C-H str.), 1660 (C=O str.), 1627 (N-H bend.), 1587, 1481 (C=C & C=N ring str.), 1174 (aliphatic C-N str.).

N-[4-hydroxy-3-(pyrrolidin-1-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-15):

The compound was prepared in a similar procedure as **QC-1** by using 3-methoxyquinoxaline-2-carboxylic acid, **If**, (0.61 gm, 0.003 mol) and 4-amino-2-(pyrrrolidin-1-ylmethyl)phenol, **Im**, (0.8 gm, 0.003 mol) instead of 3-chloro-*N*-{3-[(dimethylamino)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide, **QC1**, and 4-amino-2-[(dimethylamino)methyl]phenol, **Ik**, respectively. The obtained product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **QC-15** in 61% (0.69 gm) yield. It showed a m.p. of 156-158 °C.

IR (KBr) cm^{-1} : 3401-3194 (broad, OH & NH str.), 3031, 3017 (aromatic C-H str.), 2993, 2952, 2907 (aliphatic C-H str.), 1667 (C=O str.), 1618 (N-H bend.), 1595, 1477 (C=C & C=N ring str.), 1187 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.59-1.62 (t, 4H, $(CH_2)_2$ pyrrolidinyl) 2.27-2.30 (t, 4H, $N(CH_2)_2$ pyrrolidinyl), 3.62 (s, 2H, CH_2-N), 3.73 (s, 3H, OCH_3), 7.02-8.24 (m, 7H, ArH), 9.80 (br s, 1H, CONH), 11.45 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	66.67	05.82	14.81
Found :	66.37	05.53	14.52

_N_-[4-hydroxy-3-(piperidin-1-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-16):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2-(piperidin-1-ylmethyl)phenol, **In**, (0.83 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **QC-16** in 65% (0.76 gm) yield. It showed a m.p. of 170-171 °C.

IR (KBr) cm^{-1} : 3412-3187 (broad, OH & NH str.), 3031, 3011 (aromatic C-H str.), 2989, 2952, 2911 (aliphatic C-H str.), 1648 (C=O str.), 1610 (N-H bend.), 1585, 1501 (C=C & C=N ring str.), 1163 (aliphatic C-N str.).

_N_-[4-hydroxy-3-(morpholin-4-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-17):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2-(morpholin-4-ylmethyl)phenol, **Io**, (0.84 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **QC-17** in 62% (0.73 gm) yield. It showed a m.p. of 124-126 °C.

IR (KBr) cm^{-1} : 3402-3210 (broad, OH & NH str.), 3044, 3019 (aromatic C-H str.), 2995, 2952, 2917 (aliphatic C-H str.),1661 (C=O str.), 1622 (N-H bend.), 1591, 1505 (C=C & C=N ring str.), 1174 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.35-2.37 (t, 4H, $N(CH_2)_2$ morpholinyl), 3.67-3.69 (t, 4H, $O(CH_2)_2$ morpholinyl), 3.62 (s, 2H, CH_2-N), 3.73 (s,3H, OCH_3), 7.07-8.24 (m, 7H, ArH), 9.89 (br s, 1H, CONH), 11.47 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	63.96	05.58	14.21
Found :	64.30	05.39	14.58

N-[4-hydroxy-3-(piperazin-1-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-18):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2-(piperazin-1-ylmethyl)phenol, **Ip**, (0.84 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol to afford **QC-18** in 67% (0.79 gm) yield. It showed a m.p. of 161-163 °C.

IR (KBr) cm^{-1} : 3413-3196 (broad, OH & NH str.), 3035, 3021 (aromatic C-H str.), 2984, 2953, 2890 (aliphatic C-H str.), 1659 (C=O str.), 1619 (N-H bend.), 1589, 1495 (C=C & C=N ring str.), 1186 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.10 (s, 1H, NH), 2.45-2.48 (t, 4H, $N^1(CH_2)_2$), 2.67-2.70 (t, 4H, $N^4(CH_2)_2$), 3.63 (s, 2H, CH_2-N^1), 3.75 (s, 3H, OCH_3), 7.05-8.11 (m, 7H, ArH), 9.85 (br s, 1H, CONH), 11.50 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	64.12	05.85	17.81
Found :	63.87	05.55	17.61

N-{4-hydroxy-3-[(4-methylpiperazin-1-yl)methyl]phenyl}-3-methoxy-quinoxaline-2-carboxamide (QC-19):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2-[(4-methylpiperazin-1-yl)methyl]phenol, **Iq**, (0.88 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-ethylacetate (80:20) mixture to afford **QC-19** in 69% (0.84 gm) yield. It showed a m.p. of 149-150 °C.

IR (KBr) cm^{-1} : 3401-3187 (broad, OH & NH str.), 3041, 3011 (aromatic C-H str.), 2994, 2960, 2914 (aliphatic C-H str.), 1664 (C=O str.), 1630 (N-H bend.), 1603, 1498 (C=C & C=N ring str.), 1374 (C-H bend. CH_3), 1170 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 2.26 (s, 1H, N^4-CH_3), 2.45-2.48 (t, 4H, $N^4(CH_2)_2$), 2.55-2.58 (t, 4H, $N^1(CH_2)_2$), 3.66 (s, 2H, CH_2N^1), 3.73 (s, 3H, OCH_3), 7.12-8.14 (m, 7H, ArH), 9.85 (br s, 1H, CONH), 11.40 (br s,1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated	: 64.86	06.14	17.19
Found	: 64.47	06.50	16.83

N-{3-[(4-ethylpiperazin-1-yl)methyl]-4-hydroxyphenyl}-3-methoxy-quinoxaline-2-carboxamide (QC-20):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2-[(4-ethylpiperazin-1-yl)methyl]phenol, **Ir**, (0.92 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-acetone (80:20) mixture to afford **QC-20** in 65% (0.81 gm) yield. It showed a m.p. of 137-138 °C.

IR (KBr) cm^{-1} : 3391-3202 (broad, OH & NH str.), 3050, 3023 (aromatic C-H str.), 2988, 2964, 2894 (aliphatic C-H str.), 1660 (C=O str.), 1633 (N-H bend.), 1586, 1506 (C=C & C=N ring str.), 1177 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 1.10-1.12 (t, 3H, $N^4CH_2\underline{CH_3}$), 2.45-2.48 (q, 1H, $N^4\underline{CH_2}CH_3$), 2.48-2.51 (t, 4H, $N^4(CH_2)_2$), 2.61-2.64 (t, 4H, $N^1(CH_2)_2$), 3.68 (s, 2H, CH_2N^1), 3.73 (s, 3H, OCH_3), 7.07-8.29 (m, 7H, ArH), 9.78 (br s, 1H, CONH), 11.45 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	66.99	06.55	16.99
Found :	66.93	06.91	16.73

***N*-[4-hydroxy-3,5-bis(pyrrolidin-1-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-21):**

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2,6-bis(pyrrolidin-1-ylmethyl)phenol, **It,** (0.93 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The resultant product was purified by recrystallization using acetone to afford **QC-21** in 56% (0.77 gm) yield. It showed a m.p. of 177-178 °C.

IR (KBr) cm^{-1} : 3403-3205 (broad, OH & NH str.), 3040, 3007 (aromatic C-H str.), 2991, 2942, 2895 (aliphatic C-H str.), 1662 (C=O str.), 1632 (N-H bend.), 1605, 1509 (C=C & C=N ring str.), 1175 (aliphatic C-N str.).

***N*-[4-hydroxy-3,5-bis(morpholin-4-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-22):**

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2,6-bis(morpholin-4-ylmethyl)phenol, **Iu**, (1.0 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The resultant product was purified by recrystallization using acetone to afford **QC-22** in 55% (0.82 gm) yield. It showed a m.p. of 143-145 °C.

IR (KBr) cm^{-1} : 3412-3197 (broad, OH & NH str.), 3033, 3013 (aromatic C-H str.), 2987, 2963, 2886 (aliphatic C-H str.), 1658 (C=O str.), 1627 (N-H bend.), 1595, 1487 (C=C & C=N ring str.), 1180 (aliphatic C-N str.).

N-[4-hydroxy-3,5-bis(piperazin-1-ylmethyl)phenyl]-3-methoxyquinoxaline-2-carboxamide (QC-23):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2,6-bis(piperazin-1-ylmethyl)phenol, **Iv**, (1.0 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethylacetate to afford **QC-23** in 59% (0.88 gm) yield. It showed a m.p. of 181-183 °C.

IR (KBr) cm^{-1} : 3400-3193 (broad, OH & NH str.), 3042, 3020 (aromatic C-H str.), 2994, 2955, 2905 (aliphatic C-H str.), 1654 (C=O str.), 1631 (N-H bend.), 1586, 1495 (C=C & C=N ring str.), 1179 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.31 (s, 2H, 2 x NH), 2.48-2.51 (t, 8H, 2 x $N^1(CH_2)_2$), 2.71-2.74 (t, 8H, 2 x $N^4(CH_2)_2$), 3.67 (s, 4H, 2 x CH_2-N^1), 3.75 (s, 3H, OCH_3), 7.40-8.21 (m, 6H, ArH), 9.77 (br s,1H, CONH), 11.55 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	63.41	06.30	19.92
Found :	63.65	06.67	20.32

***N*-{4-hydroxy-3,5-bis[(4-methylpiperazin-1-yl)methyl]phenyl}-3-methoxy-quinoxaline-2-carboxamide (QC-24):**

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2,6-bis[(4-methylpiperazin-1-yl)methyl]phenol, **Iw**, (1.11 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethylacetate to afford **QC-24** in 60% (0.93 gm) yield. It showed a m.p. of 170-172 °C.

IR (KBr) cm^{-1} : 3396-3201 (broad, OH & NH str.), 3036, 3012 (aromatic C-H str.), 2986, 2943, 2890 (aliphatic C-H str.), 1662 (C=O str.), 1624 (N-H bend.), 1599, 1487 (C=C & C=N ring str.), 1380 (C-H bend. CH_3), 1165 (aliphatic C-N str.)

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.24 (s, 6H, 2 x N^4CH_3), 2.44-2.47 (t, 8H, 2 x $N^4(CH_2)_2$), 2.51-2.54 (t, 8H, 2 x $N^1(CH_2)_2$), 3.64 (s, 2H, CH_2N^1), 3.73 (s, 3H, OCH_3), 7.26-8.40 (m, 6H, ArH), 9.97 (br s, 1H, CONH), 11.53 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	64.62	07.12	18.85
Found :	64.30	07.46	19.12

N-{3,5-bis[(4-ethylpiperazin-1-yl)methyl]-4-hydroxyphenyl}-3-methoxyquinoxaline-2-carboxamide (QC-25):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-15**; using 4-amino-2,6-bis[(4-ethylpiperazin-1-yl)methyl]phenol, **Ix**, (1.19 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The resultant product was purified by recrystallization using ethylacetate to afford **QC-25** in 58% (0.95 gm) yield. It showed a m.p. of 155-156 °C.

IR (KBr) cm^{-1} : 3403-3213 (broad, OH & NH str.), 3042, 3021 (aromatic C-H str.), 2992, 2956, 2913 (aliphatic C-H str.), 1664 (C=O str.), 1633 (N-H bend.), 1604, 1484 (C=C & C=N ring str.), 1180 (aliphatic C-N str.).

3-Ethoxy-N-[4-hydroxy-3-(pyrrolidin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-26):

The compound was prepared in a similar procedure as **QC-1** by using 3-ethoxyquinoxaline-2-carboxylic acid, **Ig**, (0.65 gm, 0.003 mol) and 4-amino-2-(pyrrolidin-1-ylmethyl)phenol, **Im**, (0.8 gm, 0.003 mol) instead of 3-chloro-*N*-{3-[(dimethylamino)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide, **QC1**, and 4-amino-2-[(dimethylamino)methyl]phenol, **Ik**, respectively. The obtained product was purified by recrystallization using ethanol-chloroform (75:25) mixture to afford **QC-26** in 63% (0.74 gm) yield. It showed a m.p. of 194-196 °C.

IR (KBr) cm^{-1} : 3412-3197 (broad, OH & NH str.), 3037, 3014 (aromatic C-H str.), 2987, 2966, 2895 (aliphatic C-H str.), 1660 (C=O str.), 1625 (N-H bend.), 1586, 1491 (C=C &C=N ring str.), 1187 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.33-1.35 (t, 3H, $OCH_2\underline{CH_3}$), 1.60-1.63 (t, 4H, $(CH_2)_2$ pyrrolidinyl) 2.25-2.28 (t, 4H, $N(CH_2)_2$ pyrrolidinyl), 3.64 (s, 2H, CH_2-N), 3.99-4.02 (q, 2H, $O\underline{CH_2}CH_3$), 7.09-8.44 (m, 7H, ArH), 9.83 (br s, 1H, CONH), 11.47 (br s, 1H, OH).

Elemental analysis (%) :

		C	H	N
Calculated	:	67.35	06.12	14.29
Found	:	67.67	06.45	14.55

3-Ethoxy-*N*-[4-hydroxy-3-(piperidin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-27):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2-(piperidin-1-ylmethyl)phenol, **In**, (0.83 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-chloroform (75:25) mixture to afford **QC-27** in 65% (0.79 gm) yield. It showed a m.p. of 207-208 °C.

IR (KBr) cm^{-1} : 3388-3185 (broad, OH & NH str.), 3037, 3019 (aromatic C-H str.), 2979, 2943, 2901 (aliphatic C-H str.), 1658 (C=O str.), 1627 (N-H bend.), 1596, 1504 (C=C & C=N ring str.), 1167 (aliphatic C-N str.).

3-Ethoxy-*N*-[4-hydroxy-3-(morpholin-4-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-28):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2-(morpholin-4-ylmethyl)phenol, **Io**, (0.84 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-ethylacetate (75:25) mixture to afford **QC-28** in 63% (0.77 gm) yield. It showed a m.p. of 159-160 °C.

IR (KBr) cm^{-1} : 3408-3205 (broad, OH & NH str.), 3051, 3020 (aromatic C-H str.), 2995, 2934, 2896 (aliphatic C-H str.), 1664 (C=O str.), 1632 (N-H bend.), 1600, 1494 (C=C & C=N ring str.), 1155 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.35-1.37 (t, 3H, $OCH_2\underline{CH_3}$), 2.35-2.38 (t, 4H, $N(CH_2)_2$ morpholinyl), 3.64-3.67 (t, 4H, $O(CH_2)_2$ morpholinyl), 3.62 (s, 2H, CH_2N), 3.98-4.02 (q, 2H, $O\underline{CH_2}CH_3$), 7.10-8.22 (m, 7H, ArH), 9.80 (br s, 1H, CONH), 11.41 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	64.70	05.88	13.73
Found :	64.97	05.50	14.03

3-Ethoxy-*N*-[4-hydroxy-3-(piperazin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-29):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**, but using 4-amino-2-(piperazin-1-ylmethyl)phenol, **Ip**, (0.84 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **QC-29** in 66% (0.80 gm) yield. It showed a m.p. of 196-197 °C.

IR (KBr) cm^{-1} : 3410-3215 (broad, OH & NH str.), 3046, 3013 (aromatic C-H str.), 2987, 2950, 2898 (aliphatic C-H str.), 1666 (C=O str.), 1624 (N-H bend.), 1587, 1503 (C=C & C=N ring str.), 1168 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 1.37-1.40 (t, 3H, $OCH_2\underline{CH_3}$), 2.11 (s, 1H, NH), 2.44-2.47 (t, 4H, $N^1(CH_2)_2$), 2.69-2.72 (t, 4H, $N^4(CH_2)_2$), 3.67 (s, 2H, CH_2N^1), 4.01-4.05 (q, 2H, $O\underline{CH_2}CH_3$), 7.15-8.23 (m, 7H, ArH), 9.88 (br s, 1H, CONH), 11.56 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	64.86	06.14	17.19
Found :	65.11	05.87	17.42

3-Ethoxy-*N*-{4-hydroxy-3-[(4-methylpiperazin-1-yl)methyl]phenyl}-quinoxaline-2-carboxamide (QC-30):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**, but using 4-amino-2-[(4-methylpiperazin-1-yl)methyl]phenol, **Iq**, (0.88 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethanol-ethylacetate (75:25) mixture to afford **QC-30** in 67% (0.84 gm) yield. It showed a m.p. of 182-183 °C.

IR (KBr) cm^{-1} : 3397-3205 (broad, OH & NH str.), 3039, 3007 (aromatic C-H str.), 2994, 2949, 2887 (aliphatic C-H str.),1655 (C=O str.), 1631 (N-H bend.), 1596, 1486 (C=C & C=N ring str.), 1164 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 1.35-1.37 (t, 3H, $OCH_2\underline{CH_3}$), 2.20 (s, 1H, N^4CH_3), 2.48-2.51 (t, 4H, $N^4(CH_2)_2$), 2.57-2.60 (t, 4H, $N^1(CH_2)_2$), 3.67 (s, 2H, CH_2N^1), 4.00-4.04 (q, 2H, $O\underline{CH_2}CH_3$), 7.10-8.15 (m, 7H, ArH), 9.89 (br s, 1H, CONH), 11.47 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	65.56	06.41	16.62
Found :	65.19	06.73	16.27

3-Ethoxy-*N*-{3-[(4-ethylpiperazin-1-yl)methyl]-4-hydroxyphenyl}quinoxaline-2-carboxamide (QC-31):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2-[(4-ethylpiperazin-1-ylmethyl]phenol, **Ir**, (0.92 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The resultant product was purified by recrystallization using ethanol-ethylacetate (75:25) mixture to afford **QC-31** in 65% (0.84 gm) yield. It showed a m.p. of 169-170 °C.

IR (KBr) cm^{-1} : 3411-3185 (broad, OH & NH str.), 3047, 3018 (aromatic C-H str.), 2984, 2932, 2895 (aliphatic C-H str.), 1660 (C=O str.), 1635 (N-H bend.), 604, 1497 (C=C & C=N ring str.), 1147 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 1.03-1.05 (t, 3H, $N^4CH_2\underline{CH_3}$), 1.33-1.35 (t, 3H, $OCH_2\underline{CH_3}$), 2.43-2.47 (q, 1H, $N^4\underline{CH_2}CH_3$), 2.49-2.52 (t, 4H, $N^4(CH_2)_2$), 2.63-2.66 (t, 4H, $N^1(CH_2)_2$), 3.68 (s, 2H, CH_2N^1), 3.98-4.02 (q, 2H, $O\underline{CH_2}CH_3$), 7.17-8.30 (m, 7H, ArH), 9.75 (br s, 1H, CONH), 11.43 (br s, 1H, OH).

Elemental analysis (%) :

	C	H	N
Calculated :	66.21	06.67	16.09
Found :	66.31	06.33	15.82

3-Ethoxy-*N*-[4-hydroxy-3,5-bis(pyrrolidin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-32):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2,6-bis(pyrrolidin-1-ylmethyl)phenol, **It,** (0.93 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using chloroform to afford **QC-32** in 55% (0.77 gm) yield. It showed a m.p. of 219-220 °C.

IR (KBr) cm^{-1} : 3391-3185 (broad, OH & NH str.), 3041, 3021 (aromatic C-H str.), 2979, 2935, 2901 (aliphatic C-H str.), 1658 (C=O str.), 1632 (N-H bend.), 1591, 1501 (C=C & C=N ring str.), 1174 (aliphatic C-N str.).

3-Ethoxy-*N*-[4-hydroxy-3,5-bis(morpholin-4-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-33):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2,6-bis(morpholin-4-ylmethyl)phenol, **Iu**, (1.0 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using chloroform to afford **QC-33** in 56% (0.84 gm) yield. It showed a m.p. of 177-179 °C.

IR (KBr) cm^{-1} : 3403-3211 (broad, OH & NH str.), 3050, 3012 (aromatic C-H str.), 2986, 2943, 2897 (aliphatic C-H str.), 1663 (C=O str.), 1630 (N-H bend.), 1596, 1495 (C=C & C=N ring str.), 1187 (aliphatic C-N str.).

3-Ethoxy-*N*-[4-hydroxy-3,5-bis(piperazin-1-ylmethyl)phenyl]quinoxaline-2-carboxamide (QC-34):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2,6-bis(piperazin-1-ylmethyl)phenol, **Iv**, (1.0 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using acetone to afford **QC-34** in 58% (0.87 gm) yield. It showed a m.p. of 211-212 °C.

IR (KBr) cm^{-1} : 3391-3194 (broad, OH & NH str.), 3043, 3022 (aromatic C-H str.), 2980, 2932, 2893 (aliphatic C-H str.), 1667 (C=O str.), 1625 (N-H bend.), 1594, 1486 (C=C & C=N ring str.), 1184 (aliphatic C-N str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 1.31-1.33 (t, 3H, $OCH_2\underline{CH_3}$), 2.15 (s, 2H, 2 x NH), 2.46-2.49 (t, 8H, 2 x $N^1(CH_2)_2$), 2.69-2.72 (t, 8H, 2 x $N^4(CH_2)_2$), 3.62 (s, 4H, 2 x CH_2-N^1), 3.99-4.03 (q, 2H, $O\underline{CH_2}CH_3$), 7.41-8.29 (m, 6H, ArH), 9.73 (br s, 1H, CONH), 11.50 (br s, 1H, OH).

Elemental analysis :

	C	H	N
Calculated :	64.03	06.52	19.37
Found :	64.31	06.40	19.09

3-Ethoxy-*N*-{4-hydroxy-3,5-bis[(4-methylpiperazin-1-yl)methyl]phenyl}-quinoxaline-2-carboxamide (QC-35):

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2,6-bis[(4-methylpiperazin-1-yl)methyl]phenol, **Iw**, (1.11 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The obtained product was purified by recrystallization using ethylacetate to afford **QC-35** in 54% (0.86 gm) yield. It showed a m.p. of 202-203 °C.

IR (KBr) cm^{-1} : 3403-3211 (broad, OH & NH str.), 3051, 3014 (aromatic C-H str.), 2989, 2933, 2887 (aliphatic C-H str.), 1658 (C=O str.), 1627 (N-H bend.), 1585, 1495 (C=C & C=N ring str.), 1181 (aliphatic C-N str.).

***N*-{3,5-bis[(4-ethylpiperazin-1-yl)methyl]-4-hydroxyphenyl}-3-ethoxy-quinoxaline-2-carboxamide (QC-36):**

The compound was prepared in a similar procedure as **QC-1** by using the same reactants as that for **QC-26**; using 4-amino-2,6-bis[(4-ethylpiperazin-1-yl)methyl]phenol, **Ix**, (1.19 gm, 0.003 mol) in place of 4-amino-2-(piperidin-1-ylmethyl)phenol, **Im**. The resultant product was purified by recrystallization using ethylacetate to afford **QC-36** in 57% (0.96 gm) yield. It showed a m.p. of 189-190 °C.

IR (KBr) cm^{-1} : 3394-3197 (broad, OH & NH str.), 3030, 3009 (aromatic C-H str.), 2991, 2945, 2897 (aliphatic C-H str.), 1660 (C=O str.), 1633 (N-H bend.), 1598, 1490 (C=C & C=N ring str.), 1175 (aliphatic C-N str.).

5.2.2. Series II:

3-Methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo[2,1-b][1,3]-benzothiazol-2(3H)-ones (IP-1 – IP-24)

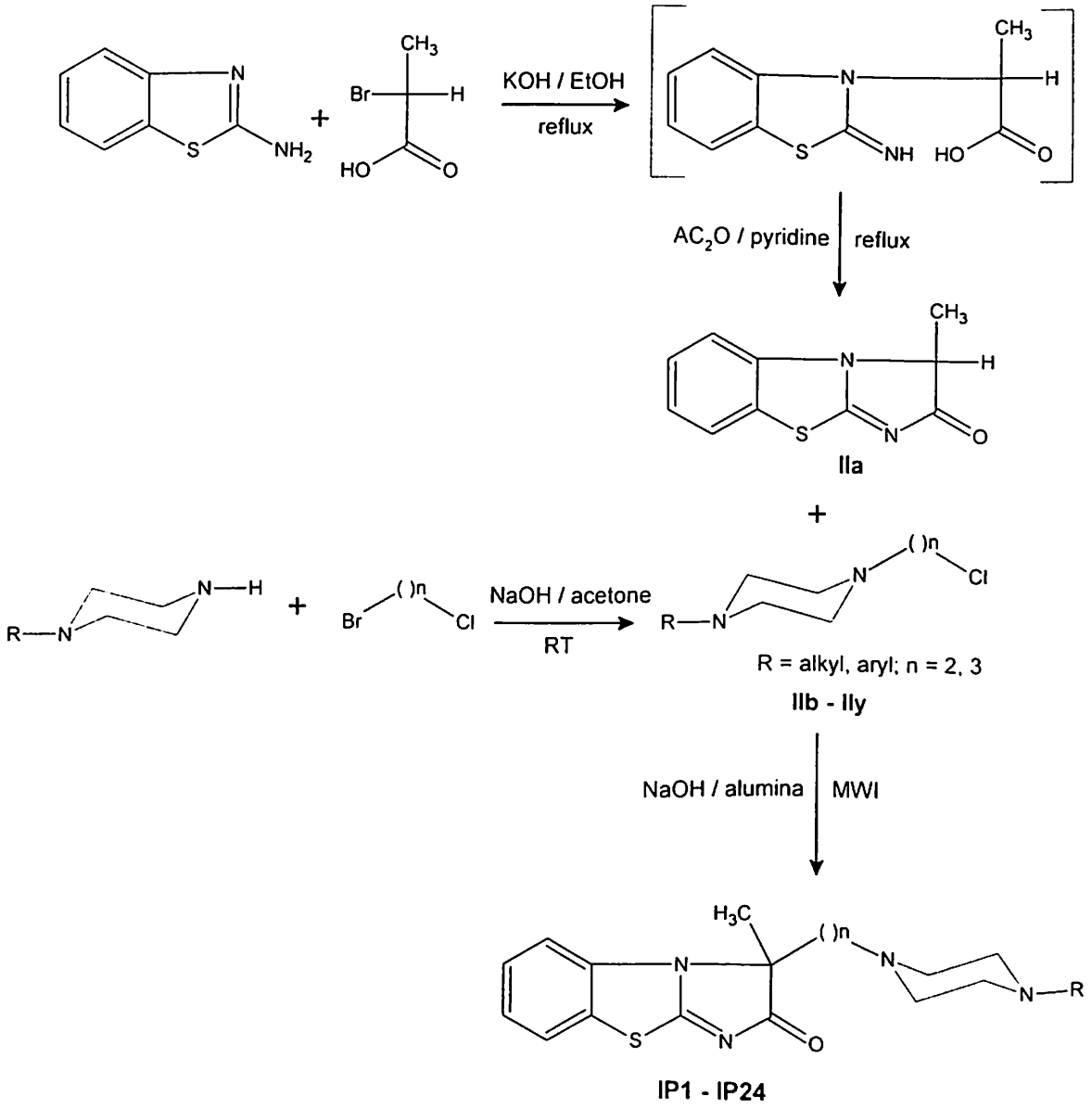


Fig. 5.2. Synthesis of 3-methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-ones

3-Methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IIa):

A mixture of 2-aminobenzothiazole (7.5 gm, 0.05 mol), potassium hydroxide (2.8 gm, 0.05 mol) and 2-bromopropionic acid (4.3 ml, 0.05 mol) in ethanol (50 ml) were heated under reflux for 3 hr. The solvent was removed under reduced pressure and the resulting yellow syrupy product was heated with acetic anhydride (15 ml) in pyridine (30 ml) for 1 hr. The reaction mixture was cooled and poured into ice water. The precipitated material was collected by filtration, dried and recrystallized using ethanol to afford **IIa** in 44% (4.5 gm) yield. It showed a m.p. of 184-185 °C (Ogura and Itoh 1981: 185 °C).

IR (KBr) cm^{-1} : 3035, 3017 (aromatic C-H str.), 1675 (C=O str.), 1603, 1498 (C=C ring str.).

1-(2-Chloroethyl)piperazine (IIb):

To a stirred solution of piperazine (0.86 gm, 0.01 mol) in acetone (20 ml) and 25% NaOH (2 ml), 1-bromo-2-chloroethane (0.8 ml, 0.01) was added slowly in ice-cooled condition. Once the addition was complete, the reaction mixture was stirred at room temperature for 8 hr. The solvent was removed under reduced pressure and water was added to the residue. The resulting solution was extracted with CH_2Cl_2 (3 x 10 ml), dried (Na_2SO_4) and evaporated to yield the product which was converted to its hydrochloride salt and recrystallized from ethanol-water (80:20) mixture to afford **IIb** in 86% (1.58 gm) yield. It showed a m.p. of 291-293 °C.

IR (KBr) cm^{-1} : 3310 (N-H str.), 2985, 2954, 2910 (aliphatic C-H str.), 1633 (N-H bend.), 1153 (aliphatic C-N str.).

1-(2-Chloroethyl)-4-methylpiperazine (IIc):

The compound was prepared in a same manner as **IIb** by using 1-methylpiperazine (1.1 ml, 0.0096 mol) instead of piperazine and the resultant product was purified by recrystallization using ethanol-water (80:20) mixture to afford **IIc** in 78% (1.82 gm) yield. It showed a m.p. of 227-230 °C.

IR (KBr) cm^{-1} : 2985, 2954, 2910 (aliphatic C-H str.), 1153 (aliphatic C-N str.).

1-(2-Chloroethyl)-4-ethylpiperazine (IId):

The compound was prepared in a same manner as **IIb** by using 1-ethylpiperazine (1.2 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-water (80:20) mixture to afford **IId** in 82% (2.03 gm) yield. It showed a m.p. of 207-210 °C.

IR (KBr) cm^{-1} : 2993, 2960, 2907 (aliphatic C-H str.), 1153 (aliphatic C-N str.).

1-(2-Chloroethyl)-4-phenylpiperazine (IIe):

The compound was prepared in a same manner as **IIb** by using 1-phenylpiperazine (1.5 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol to afford **IIe** in 76% (1.97 gm) yield. It showed a m.p. of 244-247 °C.

IR (KBr) cm^{-1} : 3043, 3010 (aromatic C-H str.), 2993, 2960, 2907 (aliphatic C-H str.), 1153 (aliphatic C-N str.).

1-Benzyl-4-(2-chloroethyl)piperazine (IIf):

The compound was prepared in a same manner as **IIb** by using 1-benzylpiperazine (1.7 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (90:10) mixture to afford **IIf** in 79% (2.16 gm) yield. It showed a m.p. of 231-233 °C.

IR (KBr) cm^{-1} : 3031, 3019 (aromatic C-H str.), 2975, 2955, 2911 (aliphatic C-H str.), 1145 (aliphatic C-N str.).

1-(2-Chloroethyl)-4-(2-methoxyphenyl)piperazine (IIg):

The compound was prepared in a same manner as **IIb** by using 1-(2-methoxyphenyl)piperazine hydrochloride (2.2 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (75:25) mixture to afford **IIg** in 85% (2.46 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3044, 3009 (aromatic C-H str.), 2967, 2955 (aliphatic C-H str.), 1156 (aliphatic C-N str.), 1026 (C-O str.).

1-(2-Chloroethyl)-4-(3-methoxyphenyl)piperazine (IIh):

The compound was prepared in a same manner as **IIb** by using 1-(3-methoxyphenyl)piperazine (1.6 ml, 0.0096 mol) instead of piperazine and the resultant product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **IIh** in 81% (2.64 gm) yield. It showed a m.p. of 236-239 °C.

IR (KBr) cm^{-1} : 3031, 3019 (aromatic C-H str.), 2959, 2940 (aliphatic C-H str.), 1137 (aliphatic C-N str.), 1031 (C-O str.).

1-(2-Chloroethyl)-4-(4-methoxyphenyl)piperazine (IIi):

The compound was prepared in a same manner as **IIb** by using 1-(4-methoxyphenyl)piperazine dihydrochloride (2.5 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (75:25) mixture to afford **IIi** in 79% (2.57 gm) yield. It showed a m.p. of 229-232 °C.

IR (KBr) cm^{-1} : 3037, 3006 (aromatic C-H str.), 2985, 2938, (aliphatic C-H str.), 1142 (aliphatic C-N str.), 1040 (C-O str.).

^{1}H-NMR ($CDCl_3$, free base) (δ) ppm : 2.35–2.38 (t, 2H, N^1CH_2), 2.56–2.59 (t, 4H, $N^1(CH_2)_2$), 3.01–3.06 (t, 4H, $N^4(CH_2)_2$), 3.62–3.66 (t, 2H, CH_2Cl), 3.76 (s, 3H, OCH_3), 6.70–6.73 (d, 2H, *m*-OCH_3),6.83–6.85 (d, 2H, *o*-CH_3).

1-(2-Chloroethyl)-4-pyridin-2-ylpiperazine (IIj):

The compound was prepared in a same manner as **IIb** by using 1-(2-pyridyl)piperazine (1.5 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (75:25) mixture to afford **IIj** in 83% (2.46 gm) yield. It showed a m.p. of 255-257 °C.

IR (KBr) cm^{-1} : 3051, 3019 (aromatic C-H str.), 2975, 2960, 2921 (aliphatic C-H str.), 1155 (aliphatic C-N str.).

1-(2-Chloroethyl)-4-(4-chlorophenyl)piperazine (IIk):

The compound was prepared in a same manner as **IIb** by using 1-(4-chlorophenyl)piperazine dihydrochloride (2.6 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (75:25) mixture to afford **IIk** in 77% (2.54 gm) yield. It showed a m.p. of 254-256 °C.

IR (KBr) cm^{-1} : 3037, 3006 (aromatic C-H str.), 2985, 2938, (aliphatic C-H str.), 1142 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$, free base) (δ) ppm : 2.36–2.39 (t, 2H, N^1CH_2), 2.59–2.62 (t, 4H, $N^1(CH_2)_2$), 3.38–3.41 (t, 4H, $N^4(CH_2)_2$), 3.50–3.53 (t, 2H, CH_2Cl), 6.59–6.61 (d, 2H, *m*-Cl), 7.15–7.17 (d, 2H, *o*-Cl).

1-(2-Chloroethyl)-4-(4-nitrophenyl)piperazine (IIl):

The compound was prepared in a same manner as **IIb** by using 1-(4-nitrophenyl)piperazine (2.0 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (75:25) mixture to afford **IIl** in 89% (2.71 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3075, 3021 (aromatic C-H str.), 2990, 2953, (aliphatic C-H str.), 1077 (aliphatic C-N str.), 861 (C-N str. $ArNO_2$).

^{1}H-NMR ($CDCl_3$, freee base) (δ) ppm : 2.44–2.47 (t, 2H, N^1CH_2), 2.63–2.66 (t, 4H, $N^1(CH_2)_2$), 3.45–3.48 (t, 4H, $N^4(CH_2)_2$), 3.57–3.60 (t, 2H, CH_2Cl), 6.85–6.87(d, 2H, *m*-NO_2), 8.05–8.07 (d, 2H, *o*-NO_2).

1-(2-Chloroethyl)-4-[3-(trifluoromethyl)phenyl]piperazine (IIm):

The compound was prepared in a same manner as **IIb** by using 1-(3-trifluoromethyl)phenylpiperazine (1.8 ml, 0.0096 mol) instead of piperazine and the resultant product recrystallized using ethanol-acetone (75:25) mixture to afford **IIm** in 79% (2.59 gm) yield. It showed a m.p. of 224-227 °C.

IR (KBr) cm^{-1} : 3047, 3011 (aromatic C-H str.), 2986, 2942 (aliphatic C-H str.), 1138 (aliphatic C-N str.).

1-(3-Chloropropyl)piperazine (IIn):

To a stirred solution of piperazine (0.86 gm, 0.01 mol) in acetone (20 ml) and 25% NaOH (2 ml), 1-bromo-3-chloropropane (1.0 ml, 0.01) was added slowly in ice-cooled condition. Once the addition was complete, the reaction mixture was stirred at room temperature for 8 hr. The solvent was removed under reduced pressure and water was added to the residue. The resulting solution was extracted with CH_2Cl_2 (3 x 10 ml), dried (Na_2SO_4) and evaporated to yield the product, which was converted to its hydrochloride salt and recrystallized from ethanol to afford **IIn** in 82% (1.62 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3235 (N-H str.), 2996, 2960, 2921 (aliphatic C-H str.), 1630 (N-H bend.), 1153 (aliphatic C-N str.).

1-(3-Chloropropyl)-4-methylpiperazine (IIo):

The compound was prepared in a same manner as **IIn** by using 1-methylpiperazine (1.1 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol to afford **IIo** in 80% (1.98 gm) yield. It showed a m.p. of 242-245 °C.

IR (KBr) cm^{-1} : 2990, 2947, 2905 (aliphatic C-H str.), 1373 (C-H bend. CH_3), 1167 (aliphatic C-N str.).

1-(3-Chloropropyl)-4-ethylpiperazine (IIp):

The compound was prepared in a same manner as **IIn** by using 1-ethylpiperazine (1.2 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol to afford **IIp** in 78% (2.04 gm) yield. It showed a m.p. of 221-223 °C.

IR (KBr) cm^{-1} : 2984, 2934, 2987 (aliphatic C-H str.), 1383 (C-H bend. CH_3), 1176 (aliphatic C-N str.).

1-(3-Chloropropyl-4-phenylpiperazine (IIq):

The compound was prepared in a same manner as **IIn** by using 1-phenylpiperazine (1.5 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (75:25) mixture to afford **IIq** in 75% (2.05 gm) yield. It showed a m.p. of 263-265 °C.

IR (KBr) cm^{-1} : 3044, 3011 (aromatic C-H str.), 2975, 2967, 2915 (aliphatic C-H str.), 1132 (aliphatic C-N str.).

1-Benzyl-4-(3-chloropropyl)piperazine (IIr):

The compound was prepared in a same manner as **IIn** by using 1-benzylpiperazine (1.7 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-acetone (75:25) mixture to afford **IIr** in 83% (2.4 gm) yield. It showed a m.p. of 245-247 °C.

IR (KBr) cm^{-1} : 3029, 3007 (aromatic C-H str.), 2980, 2949, 2922 (aliphatic C-H str.), 1136 (aliphatic C-N str.).

1-(3-Chloropropyl)-4-(2-methoxyphenyl)piperazine (IIs):

The compound was prepared in a same manner as **IIn** by using 1-(2-methoxyphenyl)piperazine hydrochloride (2.2 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (80:20) mixture to afford **IIs** in 79% (2.4 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3037, 3011 (aromatic C-H str.), 2990, 2947, (aliphatic C-H str.), 1147 (aliphatic C-N str.), 1034 (C-O str.).

1-(3-Chloropropyl)-4-(3-methoxyphenyl)piperazine (IIt):

The compound was prepared in a same manner as **IIn** by using 1-(3-methoxyphenyl)piperazine (1.6 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (80:20) mixture to afford **IIt** in 81% (2.75 gm) yield. It showed a m.p. of 247-250 °C.

IR (KBr) cm^{-1} : 3054, 3007 (aromatic C-H str.), 2974, 2951, (aliphatic C-H str.), 1139 (aliphatic C-N str.), 1029 (C-O str.).

1-(3-Chloropropyl)-4-(4-methoxyphenyl)piperazine (IIu):

The compound was prepared in a same manner as **IIn** by using 1-(4-methoxyphenyl)piperazine dihydrochloride (2.5 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (80:20) mixture to afford **IIu** in 83% (2.82 gm) yield. It showed a m.p. of 252-255 °C.

IR (KBr) cm^{-1} : 3044, 3012 (aromatic C-H str.), 2991, 2944, (aliphatic C-H str.), 1140 (aliphatic C-N str.), 1044 (C-O str.).

^{1}H-NMR ($CDCl_3$, free base) (δ) ppm : 1.90–1.95 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.35–2.38 (t, 2H, N^1CH_2), 2.56–2.59 (t, 4H, $N^1(CH_2)_2$), 3.01–3.05 (t, 4H, $N^4(CH_2)_2$), 3.62–3.66 (t, 2H, CH_2Cl), 3.76 (s, 3H, OCH_3), 6.70–6.73 (d. 2H, *m*-OCH_3), 6.83–6.85 (d, 2H, *o*-OCH_3).

1-(3-Chloropropyl)-4-pyridin-2-ylpiperazine (IIv):

The compound was prepared in a same manner as **IIn** by using 1-(2-pyridyl)piperazine (1.5 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (80:20) mixture to afford **IIv** in 80% (2.48 gm) yield. It showed a m.p. of 277-280 °C.

IR (KBr) cm^{-1} : 3041, 3019 (aromatic C-H str.), 2984, 2953, 2920 (aliphatic C-H str.), 1129 (aliphatic C-N str.).

1-(4-Chlorophenyl)-4-(3-chloropropyl)piperazine (IIw):

The compound was prepared in a same manner as **IIn** by using 1-(4-chlorophenyl)piperazine dihydrochloride (2.6 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (80:20) mixture to afford **IIw** in 75% (2.58 gm) yield. It showed a m.p. of 271-273 °C.

IR (KBr) cm^{-1} : 3037, 3013 (aromatic C-H str.), 2993, 2954, 2924 (aliphatic C-H str.), 1142 (aliphatic C-N str.).

1-(3-Chloropropyl)-4-(4-nitrophenyl)piperazine (IIx):

The compound was prepared in a same manner as **IIn** by using 1-(4-nitrophenyl)piperazine (2.0 gm, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-chloroform (80:20) mixture to afford **IIx** in 84% (2.68 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3063, 3015 (aromatic C-H str.), 2994, 2967, (aliphatic C-H str.), 1070 (aliphatic C-N str.), 854 (C-N str. $ArNO_2$).

^{1}H-NMR ($CDCl_3$, free base) (δ) ppm : 1.95–2.01 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.40–2.43 (t, 2H, N^1CH_2), 2.61–2.65 (t, 4H, $N^1(CH_2)_2$), 3.41–3.45 (t, 4H, $N^4(CH_2)_2$), 3.63–3.67 (t, 2H, CH_2Cl), 6.85–6.87 (d, 2H, *m*-NO_2), 8.11–8.14 (d, 2H, *o*-NO_2).

1-(3-Chloropropyl)-4-[3-(trifluoromethyl)phenyl]piperazine (IIy):

The compound was prepared in a same manner as **IIn** by using 1-(3-trifluoromethyl)phenylpiperazine (1.8 ml, 0.0096 mol) instead of piperazine and the resultant product was recrystallized using ethanol-ethylacetate (80:20) mixture to afford **IIy** in 76% (2.6 gm) yield. It showed a m.p. of 242-244 °C.

IR (KBr) cm^{-1} : 3042, 3014 (aromatic C-H str.), 2979, 2954, 2927 (aliphatic C-H str.), 1121 (aliphatic C-N str.).

3-Methyl-3-(2-piperazin-1-ylethyl)imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-1):

To the solution of 3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one, **IIa**, (1.02 gm, 0.005 mol) in ethanol (10 ml) and sodium hydroxide (0.4 gm, 0.01mol), 1-(2-chloroethyl)piperazine, **IIb**, (0.92 gm, 0.005 mol) were added. To this mixture neutral alumina (0.5 gm) was added. The reaction mixture was thoroughly mixed and dried in air. The solid mixture was then placed in the microwave oven and irradiated at 100% power output (900 watt) for about 5 min. Upon the completion of reaction as monitored by TLC, the reaction mixture was cooled and the product was extracted with dichloromethane (3 x 10 ml) and dried (sodium sulphate). Removal of the solvent under reduced pressure and recrystallization from ethanol afforded **IP-1** in 65% (1.02 gm) yield. It showed a m.p. of 254-257 °C.

IR (KBr) cm^{-1} : 3318 (N-H str.), 3041, 3011 (aromatic C-H str.), 2987, 2944, (aliphatic C-H str.), 1675 (C=O str.), 1585, 1495 (C=C ring str.), 1159 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.55 (s, 3H, CH_3), 1.83-1.85 (t, 2H, $\underline{CH_2}CH_2$-N^1), 2.11 (s, 1H, NH), 2.31-2.33 (t, 2H, $CH_2\underline{CH_2}$-N^1) 2.45-2.48 (t, 4H, $N^1(CH_2)_2$), 2.66-2.68 (t, 4H, $N^4(CH_2)_2$), 6.35–6.36 (d, 1H, C_5-H), 6.47–6.50 (t, 1H, C_7-H), 6.78–6.81 (t, 1H, C_6-H), 6.97–6.98 (d, 1H, C_8-H).

Elemental analysis (%) :

	C	H	N
Calculated :	60.76	06.33	17.72
Found :	60.39	05.96	18.01

3-Methyl-3-[2-(4-methylpiperazin-1-yl)ethyl]imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-2):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-methylpiperazine, **IIc**, (1.17 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **IP-2** in 76% (1.25 gm) yield. It showed a m.p. of 176-178 °C.

IR (KBr) cm^{-1} : 3032, 3015 (aromatic C-H str.), 2993, 2967, (aliphatic C-H str.), 1680 (C=O str.), 1604, 1487 (C=C ring str.), 1175 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.58 (s, 3H, CH_3), 1.87-1.90 (t, 2H, $\underline{CH_2}CH_2$-N^1), 2.26 (s, 1H, N^4CH_3), 2.36-2.38 (t, 2H, $CH_2\underline{CH_2}$-N^1), 2.47-2.50 (t, 4H, $N^4(CH_2)_2$), 2.58-2.61 (t, 4H, $N^1(CH_2)_2$), 6.36–6.37 (d, 1H, C_5-H), 6.47–6.50 (t, 1H, C_7-H), 6.78–6.81 (t, 1H, C_6-H), 7.01–7.02 (d, 1H, C_8-H).

Elemental analysis (%) :

	C	H	N
Calculated :	61.82	06.67	16.97
Found :	61.53	06.35	17.24

3-[2-(4-Ethylpiperazin-1-yl)ethyl]-3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-3):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-ethylpiperazine, **IId**, (1.24 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The resultant product was purified by recrystallization using ethanol-acetone (75:25) mixture to afford **IP-3** in 69% (1.18 gm) yield. It showed a m.p. of 155-157 °C.

IR (KBr) cm^{-1} : 3047, 3021 (aromatic C-H str.), 2985, 2944, (aliphatic C-H str.), 1667 (C=O str.), 1596, 1490 (C=C ring str.), 1164 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.02-1.04 (t, 3H, $N^4CH_2\underline{CH_3}$)1.58 (s, 3H, CH_3), 1.87-1.90 (t, 2H, $\underline{CH_2}CH_2$-N^1), 2.36-2.39 (t, 2H, $CH_2\underline{CH_2}$-N^1), 2.41-2.44 (q, 1H, $N^4\underline{CH_2}CH_3$), 2.47-2.50 (t, 4H, $N^4(CH_2)_2$), 2.58-2.61 (t, 4H, $N^1(CH_2)_2$), 6.40–6.41 (d, 1H, C_5-H), 6.47–6.50 (t, 1H, C_7-H), 6.81–6.84 (t, 1H, C_6-H), 7.10–7.11 (d, 1H, C_8-H).

Elemental analysis (%) :

	C	H	N
Calculated :	62.80	06.98	16.28
Found :	63.10	06.77	15.91

3-Methyl-3-[2-(4-phenylpiperazin-1-yl)ethyl]imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-4):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-phenylpiperazine, **IIe**, (1.3 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using acetone to afford **IP-4** in 67% (1.31 gm) yield. It showed a m.p. of 193-196 °C.

IR (KBr) cm^{-1} : 3035, 3017 (aromatic C-H str.), 2994, 2892, (aliphatic C-H str.), 1671 (C=O str.), 1608, 1487 (C=C ring str.), 1158 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.58 (s, 3H, CH_3), 1.87-1.90 (t, 2H, $\underline{CH_2}CH_2$-N^1), 2.36-2.39 (t, 2H, $CH_2\underline{CH_2}$-N^1), 2.59-2.62 (t, 4H, $N^1(CH_2)_2$), 3.37-3.40 (t, 4H, $N^4(CH_2)_2$), 6.45-7.07 (m, 9H, ArH).

Elemental analysis (%) :

	C	H	N
Calculated :	67.35	06.12	14.29
Found :	67.62	06.32	14.57

3-[2-(4-Benzylpiperazin-1-yl)ethyl]-3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-5):

The compound was prepared in a similar procedure as **IP-1** by using 1-benzyl-4-(2-chloroethyl)piperazine, **IIf**, (1.37 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using acetone to afford **IP-5** in 71% (1.44 gm) yield. It showed a m.p. of 184-185 °C.

IR (KBr) cm^{-1} : 3049, 3012 (aromatic C-H str.), 2987, 2885, (aliphatic C-H str.), 1677 (C=O str.), 1587, 1494 (C=C ring str.), 1166 (aliphatic C-N str.).

3-{2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-6):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine, **IIg**, (1.45 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using methanol-acetone (75:25) mixture to afford **IP-6** in 74% (1.56 gm) yield. It showed a m.p. of 249-252 °C.

IR (KBr) cm^{-1} : 3036, 3006 (aromatic C-H str.), 2990, 2894, (aliphatic C-H str.), 1668 (C=O str.), 1607, 1490 (C=C ring str.), 1175 (aliphatic C-N str.).

3-{2-[4-(3-Methoxyphenyl)piperazin-1-yl]ethyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-7):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-(3-methoxyphenyl)piperazine, **IIh**, (1.63 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The resultant product was purified by recrystallization using acetone to afford **IP-7** in 67% (1.41 gm) yield. It showed a m.p. of 181-183 °C.

IR (KBr) cm^{-1} : 3044, 3015 (aromatic C-H str.), 2985, 2912, (aliphatic C-H str.), 1680 (C=O str.), 1600, 1503 (C=C ring str.), 1178 (aliphatic C-N str.).

3-{2-[4-(4-Methoxyphenyl)piperazin-1-yl]ethyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-8):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-(4-methoxyphenyl)piperazine, **IIi**, (1.63 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using methanol-acetone (75:25) mixture to afford **IP-8** in 69% (1.45 gm) yield. It showed a m.p. of 175-177 °C.

IR (KBr) cm^{-1} : 3038, 3017 (aromatic C-H str.), 2980, 2924, (aliphatic C-H str.), 1671 (C=O str.), 1585, 1493 (C=C ring str.), 1167 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.56 (s, 3H, CH_3), 1.87-1.90 (t, 2H, $\underline{CH_2}CH_2$-N^1), 2.36-2.38 (t, 2H, $CH_2\underline{CH_2}$-N^1), 2.56–2.59 (t, 4H, $N^1(CH_2)_2$), 3.11–3.14 (t, 4H, $N^4(CH_2)_2$), 3.77 (s, 3H, OCH_3), 6.41-6.99 (m, 8H, ArH).

Elemental analysis (%) :

		C	H	N
Calculated	:	65.40	06.16	13.27
Found	:	65.72	06.07	12.98

3-Methyl-3-[2-(4-pyridin-2-ylpiperazin-1-yl)ethyl]imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-9):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-pyridin-2-ylpiperazine, **IIj**, (1.49 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using acetone to afford **IP-9** in 66% (1.30 gm) yield. It showed a m.p. of 203-206 °C.

IR (KBr) cm^{-1} : 3040, 3015 (aromatic C-H str.), 2981, 2921, (aliphatic C-H str.), 1670 (C=O str.), 1607, 1501 (C=C & C=N ring str.), 1182 (aliphatic C-N str.).

3-{2-[4-(4-Chlorophenyl)piperazin-1-yl]ethyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-10):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-(4-chlorophenyl)piperazine, **IIk**, (1.66 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The obtained product was purified by recrystallization using acetone to afford **IP-10** in 70% (1.49 gm) yield. It showed a m.p. of 195-197 °C.

IR (KBr) cm^{-1} : 3036, 3020 (aromatic C-H str.), 2994, 2952, (aliphatic C-H str.), 1677 (C=O str.), 1604, 1487 (C=C ring str.), 1165 (aliphatic C-N str.).

3-Methyl-3-{2-[4-(4-nitrophenyl)piperazin-1-yl]ethyl}imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-11):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-(4-nitrophenyl)piperazine, **III**, (1.53 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The resultant product was purified by recrystallization using chloroform to afford **IP-11** in 77% (1.68 gm) yield. It showed a m.p. of 264-266 °C.

IR (KBr) cm^{-1} : 3051, 3017 (aromatic C-H str.), 2984, 2975, (aliphatic C-H str.), 1674 (C=O str.), 1595, 1484 (C=C ring str.), 1152 (aliphatic C-N str.), 855 (C-N str. $ArNO_2$).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.59 (s, 3H, CH_3), 1.84-1.86 (t, 2H, $\underline{CH_2}CH_2$-N^1), 2.40-2.42 (t, 2H, $CH_2\underline{CH_2}$-N^1), 2.63–2.67 (t, 4H, $N^1(CH_2)_2$), 3.45–3.49 (t, 4H, $N^4(CH_2)_2$), 6.41–6.42 (d, 1H, C_5-H), 6.53–6.56 (t, 1H, C_7-H), 6.81–6.84 (t, 1H, C_6-H), 6.93-6.95 (t, 2H, *m*-NO_2), 7.14–7.16 (d, 1H, C_8-H). 8.07-8.09 (t, 2H, *o*-NO2).

Elemental analysis (%) :

	C	H	N
Calculated :	60.41	05.26	16.02
Found :	60.77	05.57	15.97

3-Methyl-3-(2-{4-[3-(trifluromethyl)phenyl]piperazin-1-yl}ethyl)imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-12):

The compound was prepared in a similar procedure as **IP-1** by using 1-(2-chloroethyl)-4-[3-(trifluoromethyl)phenyl]piperazine, **IIm**, (1.64 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-12** in 69% (1.58 gm) yield. It showed a m.p. of 169-172 °C.

IR (KBr) cm^{-1} : 3036, 3009 (aromatic C-H str.), 2994, 2946, (aliphatic C-H str.), 1683 (C=O str.), 1587, 1503 (C=C ring str.), 1180 (aliphatic C-N str.).

3-Methyl-3-(3-piperazin-1-ylpropyl)imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-13):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)piperazine, **IIn**, (0.99 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using ethanol to afford **IP-13** in 70% (1.15 gm) yield. It showed a m.p. of 269-271 °C.

IR (KBr) cm^{-1} : 3325 (N-H str.), 3040, 3014 (aromatic C-H str.), 2986, 2966, (aliphatic C-H str.), 1681 (C=O str.), 1578, 1510 (C=C ring str.), 1165 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.54 (s, 3H, CH_3), 1.61–1.66 (m, 2H, $CH_2\underline{CH_2}CH_2$-N^1), 2.05–2.08 (t, 2H, $\underline{CH_2}CH_2CH_2$-N^1), 2.15 (s, 1H, NH), 2.45-2.47 (t, 4H, $N^1(CH_2)_2$), 2.51–2.54 (t, 2H, $CH_2CH_2\underline{CH_2}$-N^1), 2.66-2.68 (t, 4H, $N^4(CH_2)_2$), 6.40–6.42 (d, 1H, C_5-H), 6.47–6.50 (t, 1H, C_7-H), 6.81–6.84 (t, 1H, C_6-H), 7.10–7.12 (d, 1H, C_8-H).

Elemental analysis (%) :

	C	H	N
Calculated :	61.82	06.67	16.97
Found :	61.53	06.45	17.19

3-Methyl-3-[3-(4-methylpiperazin-1-yl)propyl]imidazo[2,1-b][1,3]-benzothiazol-2(3*H*)-one (IP-14):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-methylpiperazine, **IIo**, (1.24 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using ethanol-acetone (80:20) mixture to afford **IP-14** in 72% (1.23 gm) yield. It showed a m.p. of 191-194 °C.

IR (KBr) cm^{-1} : 3037, 3019 (aromatic C-H str.), 2995, 2955, (aliphatic C-H str.), 1674 (C=O str.), 1580, 1489 (C=C ring str.), 1174 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.55 (s, 3H, CH_3), 1.69–1.74 (m, 2H, $CH_2\underline{CH_2}CH_2$-N^1), 2.03–2.07 (t, 2H, $\underline{CH_2}CH_2CH_2$-N^1), 2.29 (s, 3H. N^4CH_3), 2.51–2.55 (t, 2H, $CH_2CH_2\underline{CH_2}$-N^1), 2.60–2.65 (t, 4H, $N^4(CH_2)_2$), 2.72–2.76 (t, 4H, $N^1(CH_2)_2$), 6.40–6.42 (d,1H, C_5-H), 6.47–6.51 (t, 1H, C_7-H), 6.81–6.85 (t, 1H, C_6-H), 7.01–7.03 (d, 1H, C_8-H).

Elemental analysis (%) :

	C	H	N
Calculated :	62.80	06.98	16.28
Found :	63.10	07.22	15.81

3-[3-(4-Ethylpiperazin-1-yl)propyl]-3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-15):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-ethylpiperazine, **IIp**, (1.31 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-15** in 69% (1.23 gm) yield. It showed a m.p. of 172-175 °C.

IR (KBr) cm^{-1} : 3042, 3017 (aromatic C-H str.), 2985, 2966, (aliphatic C-H str.), 1672 (C=O str.), 1590, 1505 (C=C ring str.), 1173 (aliphatic C-N str.).

3-Methyl-3-[2-(4-phenylpiperazin-1-yl)ethyl]imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-16):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl-4-phenylpiperazine, **IIq**, (1.37 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-16** in 71% (1.44 gm) yield. It showed a m.p. of 209-211 °C.

IR (KBr) cm^{-1} : 3050, 3023 (aromatic C-H str.), 2991, 2940, (aliphatic C-H str.), 1677 (C=O str.), 1582, 1496 (C=C ring str.), 1184 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.58 (s, 3H, CH_3), 1.61–1.66 (m, 2H, $CH_2\underline{CH_2}CH_2$-N^1), 2.05–2.08 (t, 2H, $\underline{CH_2}CH_2CH_2$-N^1), 2.51–2.54 (t, 2H, $CH_2CH_2\underline{CH_2}$-N^1), 2.59-2.61 (t, 4H, $N^1(CH_2)_2$), 3.36-3.38 (t, 4H, $N^4(CH_2)_2$), 6.44-7.11 (m, 9H, ArH).

Elemental analysis (%) :

	C	H	N
Calculated :	67.98	06.40	13.79
Found :	68.13	06.72	14.00

3-[3-(4-Benzylpiperazin-1-yl)propyl]-3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-17):

The compound was prepared in a similar procedure as **IP-1** by using 1-benzyl-4-(3-chloropropyl)piperazine, **IIr**, (1.44 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-17** in 73% (1.53 gm) yield. It showed a m.p. of 199-202 °C.

IR (KBr) cm^{-1} : 3037, 3016 (aromatic C-H str.), 2985, 2951, (aliphatic C-H str.), 1670 (C=O str.), 1583, 1492 (C=C ring str.), 1165 (aliphatic C-N str.).

3-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl}-3-methylimidazo [2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-18):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine, **IIs**, (1.52 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using methanol-acetone (75:25) mixture to afford **IP-18** in 69% (1.50 gm) yield. It showed a m.p. of 266-267 °C.

IR (KBr) cm^{-1} : 3040, 3014 (aromatic C-H str.), 2989, 2963, (aliphatic C-H str.), 1674 (C=O str.), 1595, 1497 (C=C ring str.), 1171 (aliphatic C-N str.).

3-{3-[4-(3-Methoxyphenyl)piperazin-1-yl]propyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-19):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-(3-methoxyphenyl)piperazine, **IIt**, (1.7 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-19** in 74% (1.61 gm) yield. It showed a m.p. of 200-202 °C.

IR (KBr) cm^{-1} : 3047, 3017 (aromatic C-H str.), 2994, 2960, (aliphatic C-H str.), 1678 (C=O str.), 1590, 1489 (C=C ring str.), 1159 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 1.55 (s, 3H, CH_3), 1.60–1.64 (m, 2H, $CH_2\underline{CH_2}CH_2$-N^1), 2.01–2.03 (t, 2H, $\underline{CH_2}CH_2CH_2$-N^1), 2.51–2.53 (t, 2H $CH_2CH_2\underline{CH_2}$-N^1), 2.59–2.62 (t, 4H, $N^1(CH_2)_2$), 3.16–3.19 (t, 4H, $N^4(CH_2)_2$), 3.79 (s, 3H, OCH_3), 6.43-7.09 (m, 8H, ArH).

Elemental analysis (%) :

	C	H	N
Calculated :	66.06	06.42	12.84
Found :	66.27	06.65	13.13

3-{3-[4-(4-Methoxyphenyl)piperazin-1-yl]propyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-20):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-(4-methoxyphenyl)piperazine, **IIu**, (1.7 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using methanol-acetone (75:25) mixture to afford **IP-20** in 68% (1.48 gm) yield. It showed a m.p. of 195-197 °C.

IR (KBr) cm^{-1} : 3034, 3004 (aromatic C-H str.), 2990, 2942, (aliphatic C-H str.), 1679 (C=O str.), 1590, 1491 (C=C ring str.), 1164 (aliphatic C-N str.).

3-Methyl-3-[3-(4-pyridin-2-ylpiperazin-1-yl)propyl]imidazo[2,1-*b*][1,3]-benzothiazol-2(3*H*)-one (IP-21):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-pyridin-2-ylpiperazine, **IIv**, (1.56 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-21** in 65% (1.32 gm) yield. It showed a m.p. of 219-222 °C.

IR (KBr) cm^{-1} : 3031, 3014 (aromatic C-H str.), 2981, 2963, (aliphatic C-H str.), 1674 (C=O str.), 1594, 1497 (C=C ring str.), 1173 (aliphatic C-N str.).

3-{3-[4-(4-Chlorophenyl)piperazin-1-yl]propyl}-3-methylimidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-22):

The compound was prepared in a similar procedure as **IP-1** by using 1-(4-chlorophenyl)-4-(3-chloropropyl)piperazine, **IIw**, (1.73 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone to afford **IP-22** in 68% (1.49 gm) yield. It showed a m.p. of 213-215 °C.

IR (KBr) cm^{-1} : 3037, 3010 (aromatic C-H str.), 2992, 2941, (aliphatic C-H str.), 1682 (C=O str.), 1595, 1490 (C=C ring str.), 1158 (aliphatic C-N str.).

3-Methyl-3-{3-[4-(4-nitrophenyl)piperazin-1-yl]propyl}imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-23):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-(4-nitrophenyl)piperazine, **IIx**, (1.6 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using chloroform to afford **IP-23** in 75% (1.69 gm) yield. It showed a m.p. of 277-280 °C.

IR (KBr) cm^{-1} : 3033, 3017 (aromatic C-H str.), 2984, 2950, (aliphatic C-H str.), 1678 (C=O str.), 1600, 1493 (C=C ring str.), 1174 (aliphatic C-N str.).

3-Methyl-3-(3-{4-[3-(trifluromethyl)phenyl]piperazin-1-yl}propyl)imidazo-[2,1-*b*][1,3]benzothiazol-2(3*H*)-one (IP-24):

The compound was prepared in a similar procedure as **IP-1** by using 1-(3-chloropropyl)-4-[3-(trifluoromethyl)phenyl]piperazine, **IIy**, (1.71 gm, 0.005 mol) instead of 1-(2-chloroethyl)piperazine, **IIb**. The product was purified by recrystallization using acetone-chloroform (80:20) mixture to afford **IP-24** in 66% (1.58 gm) yield. It showed a m.p. of 183-185 °C.

IR (KBr) cm^{-1} : 3041, 3010 (aromatic C-H str.), 2987, 2958, (aliphatic C-H str.), 1681 (C=O str.), 1582, 1497 (C=C ring str.), 1180 (aliphatic C-N str.).

5.2.3. Series III:

2-(4-Substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles (NA-1 – NA-12)

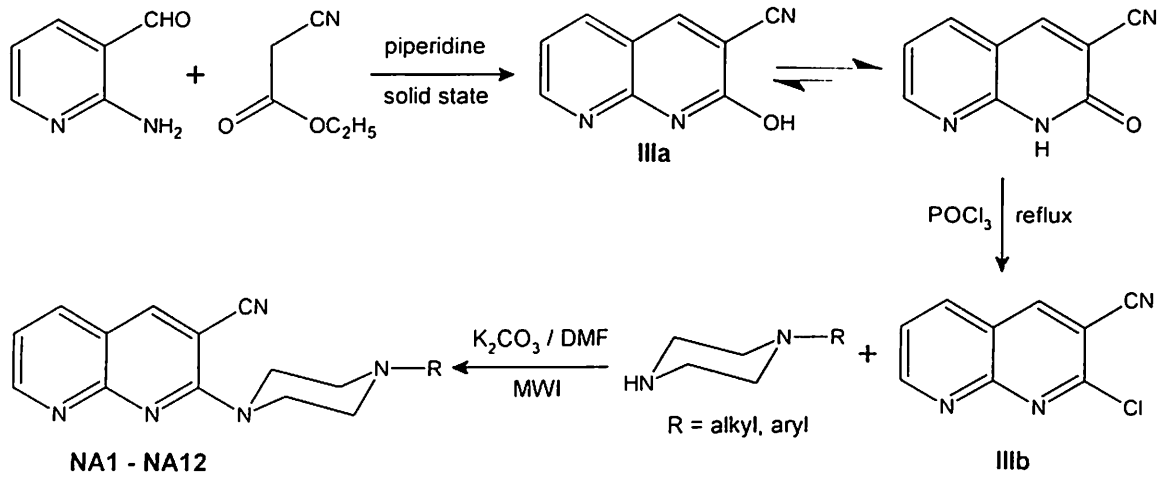


Fig. 5.3. Synthesis of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles

2-Hydroxy-1,8-naphthyridine-3-carbonitrile (IIIa):

A mixture of 2-aminonicotinaldehyde (1.24 gm, 0.01 mol), ethyl cyanoacetate (1.1 ml, 0.01 mol) and piperidine (1.0 ml, 0.01 mol) was triturated in a mortar and pestle at room temperature for about 10 min. Completion of reaction was marked by the change of state from solid to semi-solid and reconversion to solid, which was also confirmed by TLC. The solid thus obtained was treated with water, filtered, dried, and recrystallized from DMF-water (80:20) mixture to afford **IIIa** in 90% (1.54 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3335 (N-H str.), 3043, 3012 (aromatic C-H str.), 2216 (C≡N str.), 1671 (C=O str.), 1593, 1488 (C=C & C=N ring str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 7.29-7.33 (dd, 1H, C_6-H), 7.98-8.01 (d, 1H, C_5-H), 8.39 (s, 1H, C_4-H), 8.95-8.98 (d, 1H, C_7-H), 12.01 (s, 1H, NH).

Elemental analysis :

	C	H	N
Calculated :	63.16	02.92	24.56
Found :	63.08	02.76	24.68

2-Chloro-1,8-naphthyridine-3-carbonitrile (IIIb):

A mixture of 2-hydroxy-1,8-naphthyridine-3-carbonitrile (1.71 gm, 0.01 mol), phosphorus oxychloride (15 ml) and 2 drops of DMF was refluxed (105 °C) for 1 hr. It was then cooled to room temperature and treated with ice water. The resulting solution was basified slowly under cooling with aqueous NaOH (40%). The separated product was filtered, washed with water, dried and recrystallized from DMF-water (80:20) mixture to afford **IIIb** in 79% (1.5 gm) yield. Its m.p. was not recordable (>300 °C).

IR (KBr) cm^{-1} : 3038, 3010 (aromatic C-H str.), 2222 (C≡N str.), 1601, 1493 (C=C & C=N ring str.).

^{1}H-NMR (DMSO-d_6) (δ) ppm : 7.31-7.36 (dd, 1H, C_6-H), 7.80-7.82 (d, 1H, C_5-H), 8.36 (s, 1H, C_4-H), 9.01-9.03 (d, 1H, C_7-H).

Elemental analysis (%) :

		C	H	N
Calculated	:	56.99	02.11	22.16
Found	:	56.63	01.91	22.41

2-Piperazin-1-yl-1,8-naphthyridine-3-carbonitrile (NA-1):

A mixture of 2-chloro-1,8-naphthyridine-3-carbonitrile, **IIIb**, (0.95 gm, 0.005mol), piperazine (0.5 gm, 0.006 mol), K_2CO_3 (1.38 gm, 0.01 mmol) and DMF (15 ml) were taken in a 100 ml Erlenmeyer flask and subjected to microwave irradiation at 80% power output (720 watt) for about 5 min. After completion of the reaction (monitored by TLC), the reaction mixture was poured into ice water and the precipitated material was filtered, dried and recrystallized from ethanol to afford **NA-1** in 74% (0.9 gm) yield. It showed a m.p. of 226-227 °C.

IR (KBr) cm^{-1} : 3360 (N-H str.), 3040, 3017 (aromatic C-H str.), 2987, 2953 (aliphatic C-H str.), 2220 (C≡N str.), 1606, 1494 (C=C & C=N ring str.). 1155 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.07 (s, 1H, NH), 2.69-2.72 (t, 4H, $N^4(CH_2)_2$), 3.18-3.21 (t, 4H, $N^1(CH_2)_2$), 7.35-7.30 (dd, 1H, C_6-H), 7.83-7.86 (d, 1H, C_5-H), 8.36 (s, 1H, C_4-H), 9.07-9.10 (d, 1H, C_7-H).

Elemental analysis (%) :

	C	H	N
Calculated :	65.27	05.44	29.29
Found :	64.92	05.15	29.51

2-(4-Methylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-2):

The compound was prepared in a similar manner as that of **NA-1** by using 1-methylpiperazine (0.6 ml, 0.005 mol) in place of piperazine and the resultant product was recrystallized from ethanol to afford **NA-2** in 82% (1.04 gm) yield. It showed a m.p. of 152-154 °C.

IR (KBr) cm^{-1} : 3034, 3011 (aromatic C-H str.), 2990, 2962 (aliphatic C-H str.), 2218 (C≡N str.), 1614, 1504 (C=C & C=N ring str.), 1146 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.27 (s, 1H, N^4CH_3), 2.57-2.60 (t, 4H, $N^4(CH_2)_2$), 3.13-3.16 (t, 4H, $N^1(CH_2)_2$), 7.31-7.36 (dd, 1H, C_6-H), 7.80-7.82 (d, 1H, C_5-H), 8.35 (s, 1H, C_4-H), 9.10-9.12 (d, 1H, C_7-H).

MS (*m/z*) : 253 [M^+] (8.2%), 238 (0.9%), 128 (15.2%), 154 (7.7%), 99 (3.3%), 84 (100%)

Elemental analysis (%) :

	C	H	N
Calculated :	66.40	05.93	27.67
Found :	66.62	05.63	27.32

2-(4-Ethylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-3):

The compound was prepared in a similar manner as that of **NA-1** by using 1-ethylpiperazine (0.6 ml, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol to afford **NA-3** in 76% (1.01 gm) yield. It showed a m.p. of 130-132 °C.

IR (KBr) cm^{-1} : 3035, 3017 (aromatic C-H str.), 2986, 2950 (aliphatic C-H str.), 2227 (C≡N str.), 1614, 1506 (C=C & C=N ring str.), 1173 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm :1.03-1.05 (t, 3H, $N^4CH_2\underline{CH_3}$), 2.41-2.44 (q, 2H, $N^4\underline{CH_2}CH_3$), 2.59-2.62 (t, 4H, $N^4(CH_2)_2$), 3.16-3.19 (t, 4H, $N^1(CH_2)_2$), 7.39-7.43 (dd, 1H, C_6-H), 7.81- 7.84 (d, 1H, C_5-H), 8.33 (s, 1H, C_4-H), 9.07-9.10 (d,1H, C_7-H).

MS (*m/z*) : 267 [M^+] (7.4%), 252 (1.5%), 238 (0.6%), 128 (13.5%), 154 (6.4%), 113 (3.0%), 84 (100%)

Elemental analysis (%) :

	C	H	N
Calculated :	67.42	06.37	26.22
Found :	67.12	06.65	25.89

2-(4-Allylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-4):

The compound was prepared in a similar manner as that of **NA-1** by using 1-allylpiperazine (0.8 ml, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol to afford **NA-4** in 75% (1.05 gm) yield. It showed a m.p. of 176-178 °C.

IR (KBr) cm^{-1} : 3051, 3024 (aromatic C-H str.), 2990, 2963 (aliphatic C-H str.), 2216 (C≡N str.), 1610, 1497 (C=C & C=N ring str.), 1163 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 2.57-2.60 (t, 4H, $N^4(CH_2)_2$), 3.01-3.02 (d, 2H, $N^4\underline{CH_2}CH=CH_2$), 3.13-3.16 (t, 4H, $N^1(CH_2)_2$), 5.15-5.17 (d, 2H, $N^4CH_2CH=\underline{CH_2}$), 5.80-5.85 (m, 1H, $N^4CH_2\underline{CH}=CH_2$), 7.35-7.39 (dd, 1H, C_6-H), 7.76-7.77 (d, 1H, C_5-H), 8.37 (s, 1H, C_4-H), 9.07-9.09 (d, 1H, C_7-H).

MS (*m/z*) : 279 [M^+] (5.9%), 265 (0.4%), 252 (1.2%), 238 (0.5%), 154 (5.0%), 128 (11.4%), 125 (2.7%), 84 (100%)

Elemental analysis (%) :

	C	H	N
Calculated :	68.82	06.10	25.09
Found :	68.55	05.82	25.32

2-(4-Phenylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-5):

The compound was prepared in a similar manner as that of **NA-1** by using 1-phenylpiperazine (0.8 ml, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol-acetone (75:25) mixture to afford **NA-5** in 75% (1.18 gm) yield. It showed a m.p. of 158-160 °C.

IR (KBr) cm^{-1} : 3044, 3012 (aromatic C-H str.), 2993, 2942 (aliphatic C-H str.), 2214 (C≡N str.), 1600, 1484 (C=C & C=N ring str.), 1157 (aliphatic C-N str.).

2-(4-Benzylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-6):

The compound was prepared in a similar manner as that of **NA-1** by using 1-benzylpiperazine (0.8 ml, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol to afford **NA-6** in 81% (1.34 gm) yield. It showed a m.p. of 151-152 °C.

IR (KBr) cm^{-1} : 3049, 3017 (aromatic C-H str.), 2988, 2964 (aliphatic C-H str.), 2225 (C≡N str.), 1612, 1496 (C=C & C=N ring str.), 1146 (aliphatic C-N str.).

2-[4-(2-Methoxyphenyl)piperazin-1-yl]-1,8-naphthyridine-3-carbonitrile (NA-7):

The compound was prepared in a similar manner as that of **NA-1** by using 1-(2-methoxyphenyl)piperazine hydrochloride (1.14 gm, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol-acetone (75:25) mixture to afford **NA-7** in 74% (1.28 gm) yield. It showed a m.p. of 223-225 °C.

IR (KBr) cm^{-1} : 3038, 3021 (aromatic C-H str.), 2994, 2955 (aliphatic C-H str.), 2220 (C≡N str.), 1606, 1502 (C=C & C=N ring str.), 1139 (aliphatic C-N str.).

2-[4-(3-Methoxyphenyl)piperazin-1-yl]-1,8-naphthyridine-3-carbonitrile (NA-8):

The compound was prepared in a similar manner as that of **NA-1** by using 1-(3-methoxyphenyl)piperazine (0.9 ml, 0.005 mol) in place of piperazine and the obtained product was recrystallized from acetone-water (80:20) mixture to afford **NA-8** in 82% (1.42 gm) yield. It showed a m.p. of 150-152 °C.

IR (KBr) cm^{-1} : 3049, 3017 (aromatic C-H str.), 2987, 2964 (aliphatic C-H str.), 2218 (C≡N str.), 1606, 1508 (C=C & C=N ring str.), 1159 (aliphatic C-N str.).

2-[4-(4-Methoxyphenyl)piperazin-1-yl]-1,8-naphthyridine-3-carbonitrile (NA-9):

The compound was prepared in a similar manner as that of **NA-1** by using 1-(4-methoxyphenyl)piperazine dihydrochloride (1.32 gm, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol to afford **NA-9** in 77% (1.33 gm) yield. It showed a m.p. of 142-143 °C.

IR (KBr) cm^{-1} : 3039, 3011 (aromatic C-H str.), 2991, 2955 (aliphatic C-H str.), 2220 (C≡N str.), 1606, 1497 (C=C & C=N ring str.), 1168 (aliphatic C-N str.).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 3.16-3.19 (t, 4H, $N^1(CH_2)_2$), 3.41-3.44 (t, 4H, $N^4(CH_2)_2$), 3.73 (s, 1H, OCH_3), 6.48-6.50 (t, 2H, *m*-OCH_3), 6.61-6.63 (t, 2H, *o*-OCH_3), 7.39-7.43 (dd, 1H, C_6-H), 7.81-7.82 (d, 1H, C_5-H), 8.33 (s, 1H, C_4-H), 9.07-9.09 (d, 1H, C_7-H).

Elemental analysis (%) :		C	H	N
	Calculated :	69.57	05.51	20.29
	Found :	69.69	05.81	19.96

2-[4-(4-Nitrophenyl)piperazin-1-yl]-1,8-naphthyridine-3-carbonitrile (NA-10):

The compound was prepared in a similar manner as that of **NA-1** by using 1-(4-nitrophenyl)piperazine (1.1 gm, 0.005 mol) in place of piperazine and the obtained product was recrystallized from acetone-chloroform (75:25) mixture to afford **NA-10** in 84% (1.51 gm) yield. It showed a m.p. of 239-240 °C.

IR (KBr) cm^{-1} : 3030, 3017 (aromatic C-H str.), 2984, 2949 (aliphatic C-H str.), 2216 (C≡N str.), 1593, 1501 (C=C & C=N ring str.), 1157 (aliphatic C-N str.), 862 (C-N str. $ArNO_2$).

^{1}H-NMR ($CDCl_3$) (δ) ppm : 3.23-3.26 (t, 4H, $N^1(CH_2)_2$), 3.58-3.61 (t, 4H, $N^4(CH_2)_2$), 7.03-9.11 (m, 8H, ArH).

Elemental analysis (%) :		C	H	N
	Calculated :	63.33	04.44	23.33
	Found :	63.10	04.21	23.64

2-[4-(4-Chlorophenyl)piperazin-1-yl]-1,8-naphthyridine-3-carbonitrile (NA-11):

The compound was prepared in a similar manner as that of **NA-1** by using 1-(4-chlorophenyl)piperazine dihydrochloride (1.35 gm, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol-acetone (75:25) mixture to afford **NA-11** in 76% (1.33 gm) yield. It showed a m.p. of 169-171 °C.

IR (KBr) cm^{-1} : 3033, 3010 (aromatic C-H str.), 2991, 2942 (aliphatic C-H str.), 2222 (C≡N str.), 1593, 1588 (C=C & C=N ring str.), 1175 (aliphatic C-N str.).

2-(4-Pyridin-2-ylpiperazin-1-yl)-1,8-naphthyridine-3-carbonitrile (NA-12):

The compound was prepared in a similar manner as **NA-1** by using 1-(2-pyridyl)piperazine (0.8 ml, 0.005 mol) in place of piperazine and the obtained product was recrystallized from ethanol to afford **NA-12** in 80% (1.26 gm) yield. It showed a m.p. of 172-174 °C.

IR (KBr) cm^{-1} : 3041, 3018 (aromatic C-H str.), 2984, 2966 (aliphatic C-H str.), 2216 (C≡N str.), 1600, 1593 (C=C & C=N ring str.), 1146 (aliphatic C-N str.).

5.3. Pharmacological Studies

5.3.1. 5-HT_3 Receptor Antagonism Studies

5.3.1.1. Animals:

Experimentation on guinea-pigs was approved by Institutional Animal Ethics Committee of the Birla Institute of Technology & Science, Pilani (Protocol No. IAEC/RES/6, dated 21.04.2003).

Dunkin Hartley guinea-pigs (250-300 gm) of either sex were obtained from Hissar Agricultural University, Hisar, Haryana, India. The guinea-pigs were housed under ideal laboratory conditions in a 12 hr light-dark cycle with free access to food and water.

5.3.1.2. Longitudinal muscle myenteric plexus (LMMP) preparation of the guinea-pig ileum:

Guinea-pigs weighing 250-300 gm were fasted overnight with free access to water. The animals were sacrificed by stunning followed by exsanguination. The abdomen was cut open, the caecum lifted and the ileo-caecal junction was located. The ileum was excised approximately 2 cm from the ileo-caecal junction, and the longitudinal muscle strips with the myenteric muscles attached (LMMP) were prepared and mounted as per the literature method (Paton and Zar 1968). The tissue was equilibrated for 30 min under a resting tension of 500 mg and constant aeration, in a 40 ml organ bath, containing Tyrode solution maintained at 37 °C.

5.3.1.3. Evaluation of 5-HT_3 antagonisms in the guinea-pig LMMP preparation:

After a 30 min equilibration period, non-cumulative concentrations (10^{-8} - 10^{-4} M) of 2-methyl-5-HT (Tocris, UK) were added with a 15 min dosing cycle (to prevent desensitization) and left in contact with the tissue until the maximal contraction had developed. A fixed 2-Me-5-HT concentration (10^{-5} M), approximately ED_{80}, was used for antagonism studies. To study the antagonist effect of the standard/test compounds on the response evoked by 2-Me-5-HT, the compounds were added to the organ bath and left in contact with the tissue for atleast 10 min prior to the addition of 2-Me-5-HT. The tissue contractions were recorded using a T-305 Force transducer coupled to a Student's physiograph (Bio Devices, Ambala, India). Antagonisms were expressed in the form of pA_2 values, which were graphically determined (Mackay 1978). *The pA_2 value is defined as the negative logarithm of molar concentration of antagonist required to reduce the effect of standard agonist by 50%.* The pA_2 values of the test compounds were compared with a standard antagonist ondansetron (Natco Pharma, Hyderabad, India).

5.3.2. Anxiolytic Studies:

Experimentation on mice was approved by Institutional Animal Ethics Committee of the Birla Institute of Technology & Science, Pilani (Protocol No. IAEC/RES/4/1, dated 08.09.2004).

5.3.2.1. Animals:

Swiss albino mice (20-25 gm) of either sex obtained from Hissar Agricultural University, Hissar, Haryana, India were housed under normal laboratory conditions (12 hr light-dark cycle) with free access to food and water. The animals were housed in groups of six in Perspex cages in the laboratory one day prior to experimentation. The experimental sessions were conducted during the light phase of the cycle between 8 a.m. and 12.00 noon.

5.3.2.2. Drugs:

The following drugs were used for the study.

Diazepam (0.2 mg/kg)

Ondansetron (0.1, 1 & 10 mg/kg)

NA-4 (0.1, 1 & 10 mg/kg).

The drugs were dissolved in normal saline and were injected intraperitoneally (*i.p.*), 1 hr before testing.

5.3.2.3. Statistical analysis:

Statistical analysis were done using GraphPad InStat (version 3.01) software and Microsoft Excel (MS Office 2000) software. The results of the anxiolytic studies are presented as the mean ± SEM. The results of the study were sufficiently close to normal distribution so as to allow the application of parametric statistics. Experimental data were analyzed by one way analysis of variance (ANOVA) test followed by Tukey-Kramer multiple comparisons test. Statistical significance was set at $p < 0.05$.

5.3.2.4. Elevated plus maze:

The elevated plus maze (EPM) was similar to that of reported method (Pellow et al. 1985; Sharma and Kulkarni 1991). The apparatus consisted of two opposed open arms (30 x 5 cm) at right angles, with two opposed closed arms of same size. The later was enclosed by walls on all sides (10 cm) but were open-topped; there was no lip on the open arms. The four arms were delimited at the central area (5 x 5 cm). The whole apparatus was elevated (30 cm) above the floor. The mice were placed individually in the centre of the maze and the behavior during the 5-min test period was observed. The time spent in open arms of maze (expressed as % time spent in open arms), the entries into the open arms (expressed as % open arms entries), and the total number of entries were recorded; time spent in the centre of maze was disregarded. The results obtained were compared with vehicle treated control group.

5.3.2.5. Light-Dark Box:

The light-dark box test adopted was similar to that of the reported method (Hascoet and Bourin 1998). The apparatus was an open-topped rectangular box (46 x 27 x 30 cm high) made of plexi- glass. It was divided into a small (18 x 27 cm) compartment and a large (27 x 27 cm) compartment with an opening (7.5 x 7.5 cm) located in the centre of the partition at floor level. The small compartment was painted black and illuminated by a dim red light (60 watt), whereas the large compartment was painted white and brightly illuminated with a 60 watt light source.

The mice were placed individually in the light compartment and the following parameters were recorded over a 5 min period.

The latency to first entry to the dark compartment;

Shuttle crossings between both compartments;

Time spent in each compartment.

A mouse with all four paws in either of the compartments was considered as having changed compartment. After each trial, the base was cleaned with 20% v/v ethyl alcohol and dried.

5.3.2.6. Hole board test:

The hole board apparatus used for the study was similar to that of reported method (Agarwal 1995). It consisted of a square board (50 x 50 cm) made of plexi-glass (painted black) with 16 equally spaced holes of 3 cm diameter each. The board was held 20 cm above the ground with the help of a wooden frame. The board was divided into 25 squares to measure the locomotor activity.

The mice were placed individually in the centre of the board and allowed to explore the hole board apparatus for 5 min.

The number of head dips, into the holes was taken as the exploration score and the number of squares crossed by the hind legs was taken as the locomotion score of the animal.

Chapter VI
Results & Discussion

CHAPTER VI

RESULTS AND DISCUSSION

6.1. Ligand Design

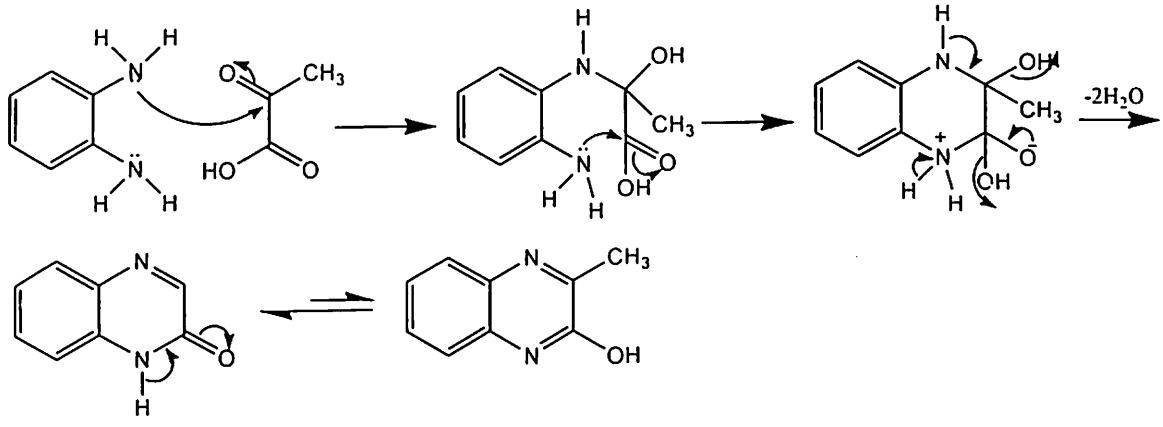
In the present study, a preliminary molecular modeling study was performed in order to verify whether the proposed series of molecules comply with the pharmacophoric model as suggested by various researchers for 5-HT_3 receptor antagonists. Various studies proposed the pharmacophore for 5-HT_3 receptor antagonists (Schmidt and Peroutka 1989; Hibert et al. 1990; Rosen et al. 1990; Evans et al. 1991; Swain et al. 1992; Bull et al. 1993). Perhaps the most well-known 5-HT_3 pharmacophore is the one proposed by Hibert et al. (1990). It consists of three components: an aromatic ring, a carbonyl group containing linking moiety and an out-of-plane basic centre. The proposed distance between the centroid of the aromatic ring and carbonyl oxygen is ~3.3 Å, between the centroid of the aromatic ring and basic nitrogen is ~6.7 Å and the distance between carbonyl oxygen and basic nitrogen is ~5.2 Å. In the present study, the standard 5-HT_3 RAs, ondansetron, granisetron and tropisetron, almost complied with the above pharmacophoric distances. On the other hand, the pharmacophoric distances of the test compounds deviated slightly (3.5 to 11%) from that of the standard drugs. In few studies (Heidempergher et al. 1997; Kuroita et al. 1996a), compounds exhibited potent 5-HT_3 RAs even though they deviated from the Hibert (1990) model.

Various heteroaryl piperazines (Monge et al. 1993; 1994; Lasheras et al. 1996; Orjales et al. 1997) showed 5-HT_3 receptor antagonism in the isolated guinea-pig ileum and in radio-ligand binding assays. In the present study, 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles were developed for the 5-HT_3 receptor antagonism studies. In this series, few compounds were superimposed with quipazine, a standard 5-HT_3 ligand. The Compounds **NA-1**, **NA-2**, **NA-3** and **NA-4** showed RMS distance of 0.038, 0.099, 0.87 and 0.97 Å, respectively.

6.2. Synthesis

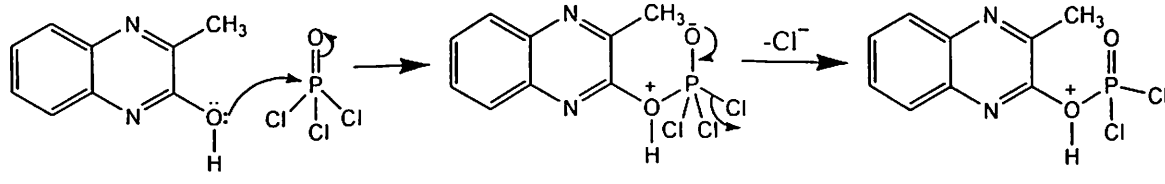
6.2.1. Series I: 3-Substituted-2-quinoxalinecarboxamides (QC-1 – QC-36)

2-Hydroxy-3-methylquinoxaline was prepared by the reaction of o-phenylenediamine and pyruvic acid as per method already reported (Krishnan et al. 2001). The reaction occurred at room temperature and the product was obtained in excellent yield. The reaction mechanism is indicated below.

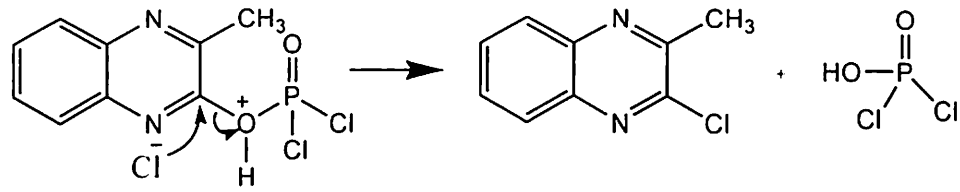


Chlorination of 2-hydroxy-3-methylquinoxaline was achieved by treatment with phosphorus oxychloride as per method cited in literature i.e., refluxing the contents for 1 hr and after removal of the excess $POCl_3$, the remaining unreacted $POCl_3$ was decomposed by pouring into ice-cold water. Initially the reaction mixture was basified with solid Na_2CO_3. It consumed excess of Na_2CO_3 and excess frothing occurred due to the liberation of CO_2. Hence, 10% NaOH solution was used in order to overcome the aforementioned setbacks. The reaction mechanism is indicated below.

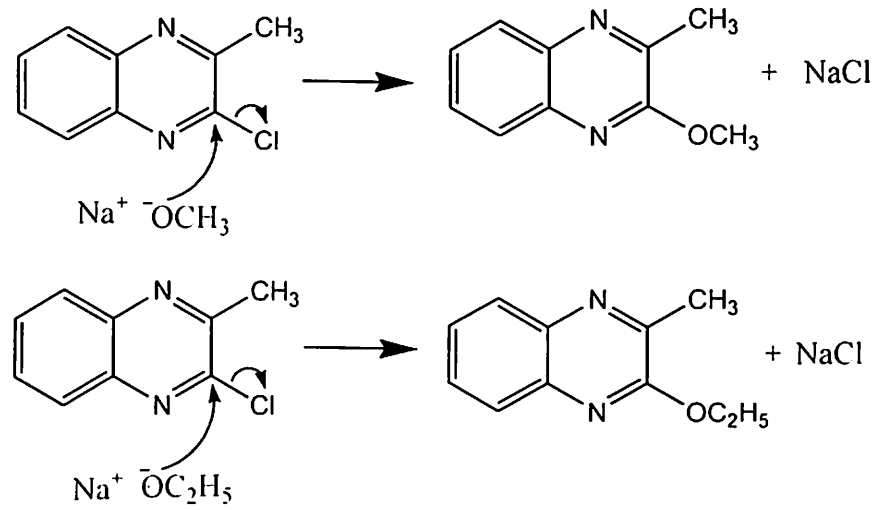
Displacement of chlorine ion from phosphorus oxychloride forms a leaving group (Cl^-).



The leaving group (Cl^-) then attacks the electrophilic carbon atom.

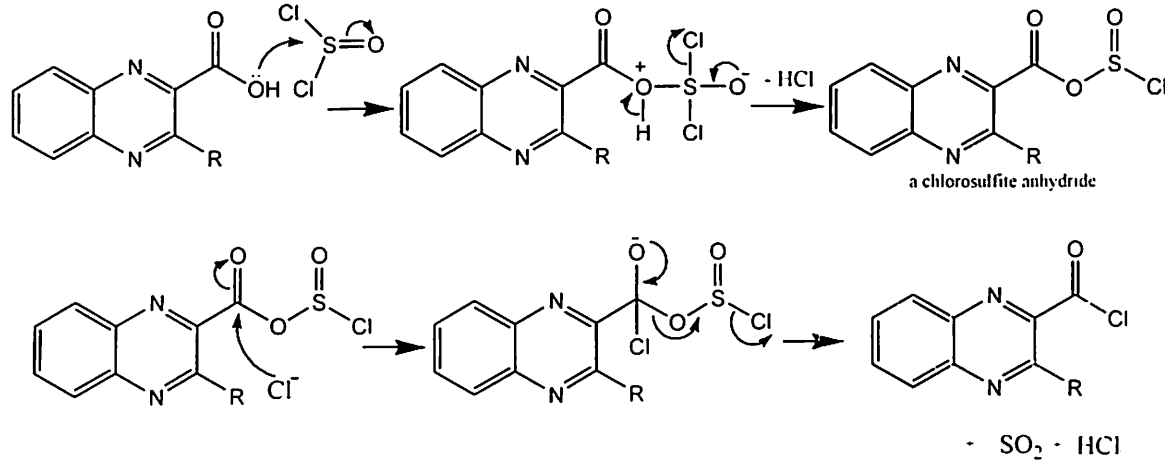


The chlorine atom in 2-chloro-3-methylquinoxaline was substituted by alkoxide ion to prepare 2-substituted-3-methylquinoxaline. 2-methoxy-3-methylquinoxaline was synthesized by the literature method (Newbold and Spring 1948). The reaction of 2-chloro-3-methylquinoxaline with sodium methoxide afforded 2-methoxy-3-methylquinoxaline. In a similar way 2-ethoxy-3-methylquinoxaline was prepared by the reaction of 2-chloro-3-methylquinoxaline with sodium ethoxide. The mechanism involved in this reaction is as follows.



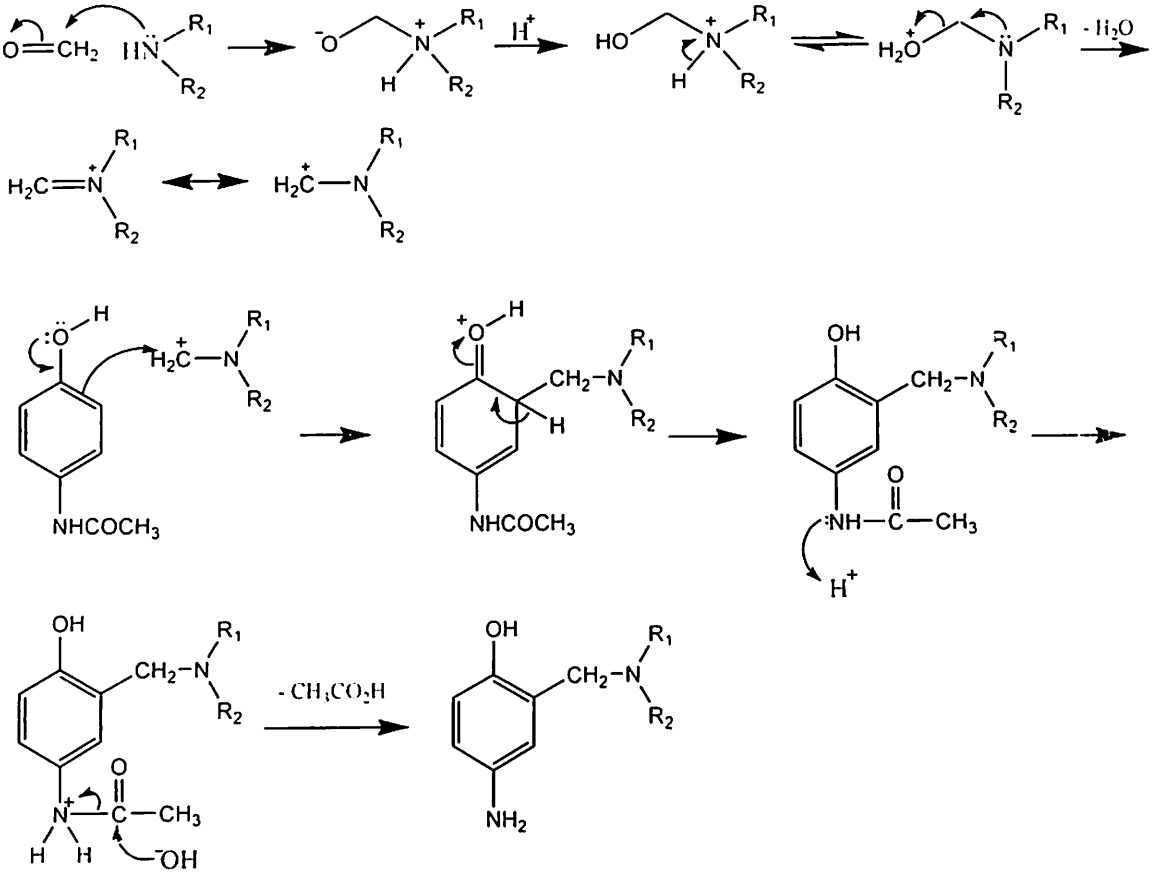
3-substituted quinoxaline-2-carboxylic acid was prepared by the oxidation of 2-substituted-3-methylquinoxaline using sodium dichromate and sulphuric acid mixture adopting the method cited in the literature (Kamm and Matthews 1946).

3-substituted quinoxaline-2-carboxylic acid was converted to the corresponding quinoxaloyl chloride by acylation using thionyl chloride, adopting the procedure cited in the literature (Usifoh et al. 2001). The reaction mechanism is represented below.



Mannich reaction of *p*-acetamidophenol with formaldehyde and selected secondary amines in ethanol was carried out in microwave environment. The reaction was completed (monitored by TLC) in 3 min. The solvent was removed using rotary flash evaporator and without isolating the compound, 6M HCl was added to the viscous residue and subjected to microwave irradiation for 5 min to deacetylate the acetamido group to primary amine. The reaction was fairly general, facile and was observed that this reaction was more effective when compared to conventional heating (Burchalter et al. 1948; Stout et al. 1983). The reaction time was brought down from hours to minutes using microwaves.

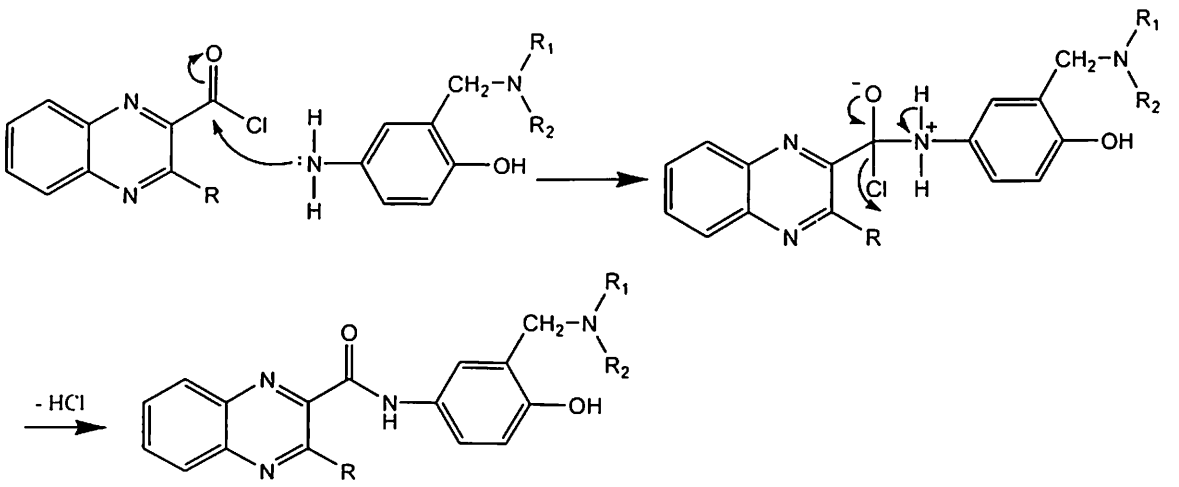
In the conventional method (Burchalter et al. 1948; Stout et al. 1983), *p*-acetamidophenol with formaldehyde and selected secondary amines in ethanol were refluxed for 3 hours. After isolating Mannich derivates of *p*-aminophenol, it was refluxed with 6M HCl for 1 hr to deacetylate the acetamindo group to primary amino group. The mechanism of the reaction is given in the following scheme.



Since the 3-substituted-2-quinoxaloylchloride was found to be hygroscopic and unstable, it was condensed with the amino group of the mono & bis Mannich derivatives of *p*-aminophenol without isolating the compound. Triethylamine was used as an acidic scavenger. This reaction was carried out in the microwave environment for 2 minutes. When this reaction was carried out by conventional method adopting the method cited in literature (Usifoh et al. 2001), the reaction took about 6 hours. The product obtained by both the methods was identical in all aspects (m.p., m.m.p., co-TLC and superimposable IR spectra). Almost similar yields were obtained by both the methods.

The compounds were converted into hydrochloride salts [the compound was dissolved in a suitable solvent and dry alcoholic HCl was added (Blackburn et al. 1987)] for their pharmacological evaluation. Some of the compounds were found to be slightly hygroscopic with change in colour on standing, which was assumed to be due to the oxidation of the aminophenol moiety.

The possible reaction mechanism for the condensation of acid chloride of quinoxaline with primary amines of Mannich derivatives of *p*-aminophenol is as given below.



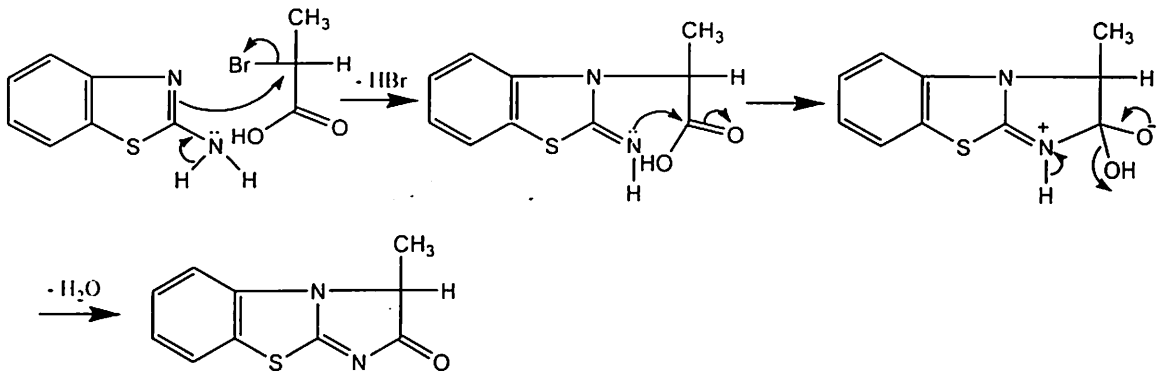
IR spectral analysis of the final compounds (**QC-1 – QC-36**) showed broad peak at ~3400 cm^{-1} - 3200 cm^{-1} due to OH & NH and ~1670 cm^{-1} due to C=O functions. In ^{1}H-NMR spectra, OH showed singlet at δ ~ 11.5, NH showed singlet at δ ~9.8 and aromatic protons showed multiplet in the range of δ 7.1-8.4. Elemental analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%).

6.2.2. Series II:

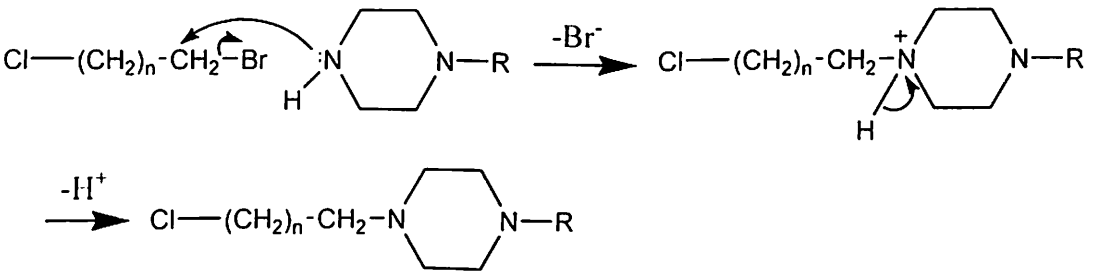
*3-Methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo[2,1-b][1,3]benzothiazol-2(3H)-ones (**IP-1 – IP-24**)*

Condensation of 2-bromo fatty acids with 2-aminobenzothiazole in the presence of alkali, followed by the reaction of acetic anhydride and pyridine, produced 3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one. The method described in the literature (Ogura and Itoh 1970) was chosen, as the procedure is a one-pot reaction. When this procedure was adopted as such, poor yields were obtained with incompleteness in reaction. Various modifications were incorporated in the procedure to obtain yields as comparable to literature yield.

The solvent used in the reaction was absolute alcohol, which had to be freshly distilled (Furniss et al. 1989). 2-Aminobenzothiazole was added slowly with stirring to the alcohol containing sodium hydroxide in the flask. 2-bromopropionic acid was added dropwise using pressure equalizing dropping funnel in cold condition with stirring, as they react vigorously. On completion of the addition of 2-bromopropionic acid, the mixture was heated to reflux for 1.5 hour. Upon completion of the reaction, the solvent was removed from the reaction mixture, which resulted in a syrupy yellow-brown liquid containing the intermediate. It was then refluxed for 1 hr with a mixture of dry pyridine and acetic anhydride taken in 1:1 ratio. The results were good when these were taken in equal proportions; the same had to be confirmed by trial reactions performed with different proportions of acetic anhydride and dry pyridine. Pyridine acted as the dehydrating agent; bringing about cyclization of the intermediate, whereas acetic anhydride acted as a solvent for the reaction. The excess of pyridine and acetic anhydride were removed from the final product by transferring the reaction mixture into ice-cold water whereby the product precipitated. The mechanism involved in the reaction is explained hereunder.



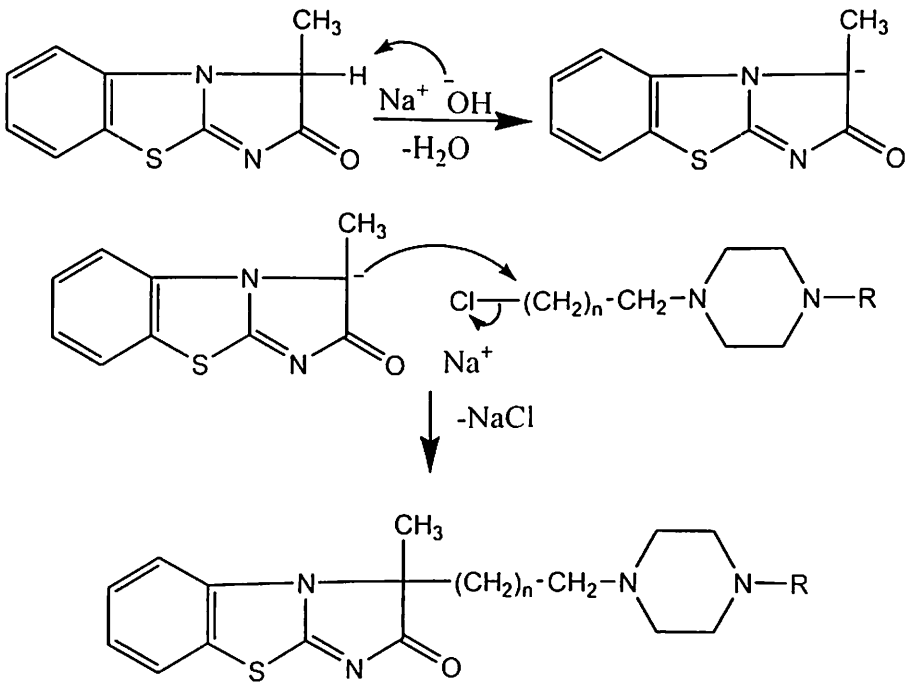
The N^1 of various piperazines were substituted with the chloroalkyl groups. 1,2-dichloro/1,3-dichloropropane was not used for the preparation of 1-(chloroalkyl)-4-substituted piperazines, since piperazines might get attached to both the ends of the alkyl groups. 1-Bromo-2-chloroethane/1-bromo-3-chloropropane was used for the preparation of 1-(chloroalkyl)-4-substituted piperazine, since the bromo group is a better leaving group than chloro group. Since most of the products were obtained in oil/liquid form, they were converted to the corresponding hydrochloride salts (Blackburn et al. 1987). The mechanism involved in the reaction is as follows.



The final compounds (**IP1 – IP24**) were prepared by the reaction of 3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one and selected 1-(chloroalkyl)-4-substituted piperazines in the presence of sodium hydroxide. This reaction was carried out by a novel technique, microwave assisted solvent free synthesis. In this reaction, the reactants were adsorbed on the surface of alumina (solid support) and subjected to microwave irradiation.

The solid support facilitated the reactants to absorb the microwaves by increasing the surface area, without absorbing or restricting the microwave transmission. The bulk temperature was observed to be relatively low in such solvent-free reactions although high-localized temperatures may be reached during microwave irradiation (Varma 1999). Solvent free microwave assisted reactions provided an opportunity to work with open vessels with suitable set up thus avoiding the risk of high pressure development (due to organic solvents in closed vessels) and increasing the potential of such reactions to be upscaled. When this reaction was carried out by conventional heating using ethanol as solvent, the reaction took 8 hours. The products obtained by both the methods were identical in all aspects (m.p., m.m.p., co-TLC and superimposable IR spectra). Almost similar yields were obtained by both the methods.

In this reaction, the base abstracts a proton to form a carbanion, which attacks the electrophilic carbon of 1-(chloroalkyl)-4-substituted piperazines, as shown below.



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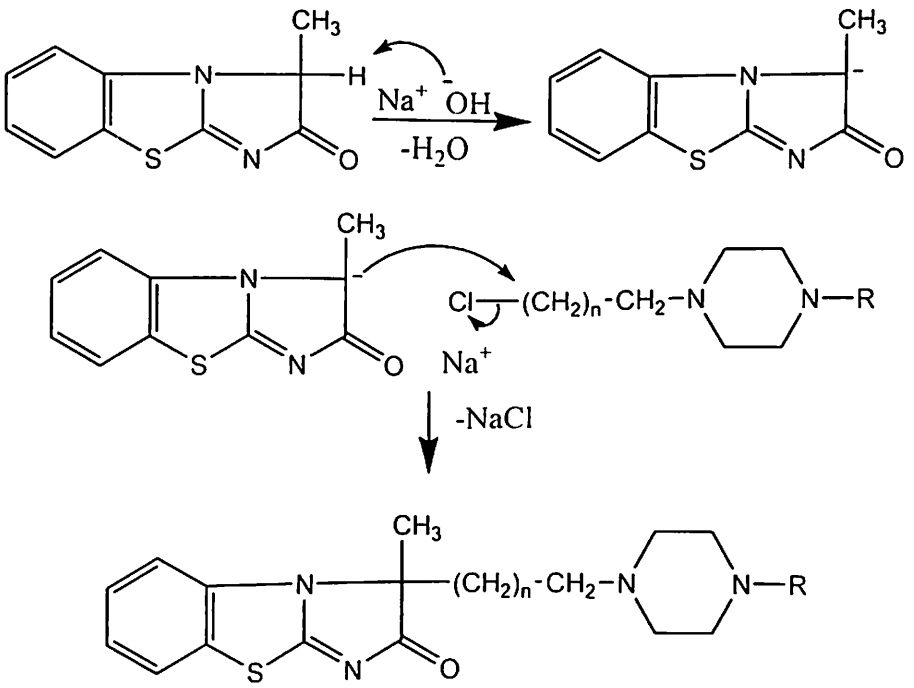


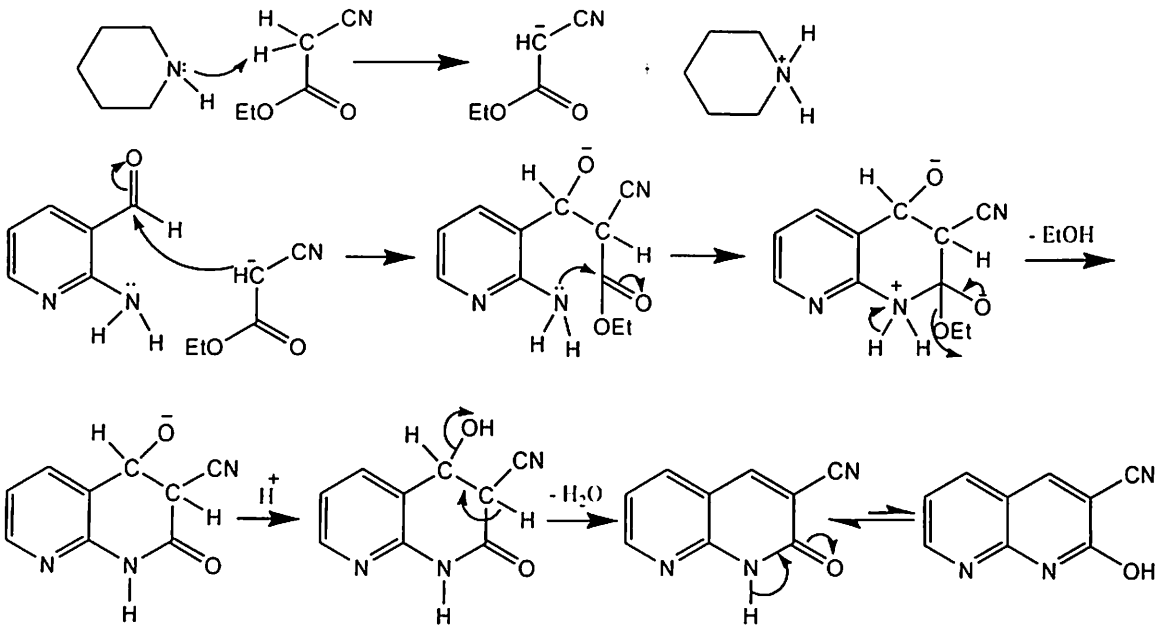
IR spectral analysis of the final compounds (**IP-1 – IP-24**) showed a characteristic peak at ~1670 cm^{-1} for C=O stretching. In ^{1}H-NMR spectra, methylene protons (cyclic) adjacent to N^1 nitrogen of piperazine showed triplet in the range of δ 2.36-2.76 whereas the methylene protons (cyclic) adjacent to N^4 nitrogen of piperazine showed triplet in the range of δ 2.41-3.49. The final compounds (**IP-1 – IP-24**) showed the following ^{1}H-NMR signals for 3-methylimidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-one moiety; C_5-H: δ ~6.4 (d), C_6-H: δ ~6.8 (t), C_7-H: δ ~6.5 (t), C_8-H: δ ~7.1 (d). Elemental analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%).

6.3. Series III:

2-(4-Substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles ***(NA-1 – NA-12)***

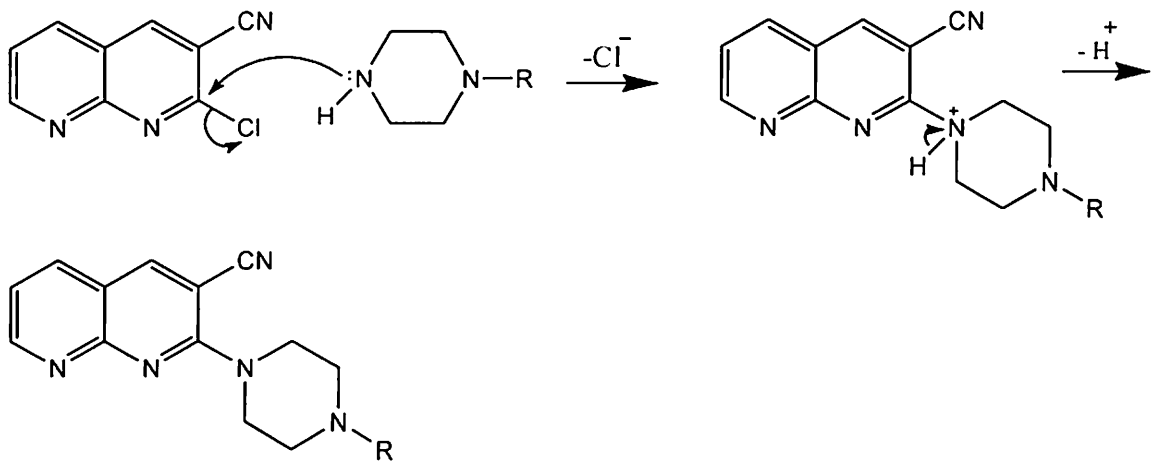
Equimolar concentrations of 2-aminonicotinaldehyde, ethylcyanoacetate and piperidine were triturated in a mortar and pestle for 10 min at room temperature in solid state condition to afford 2-hydroxy-1,8-naphthyridine-3-carbonitrile in 90% yield. When this reaction was carried out by conventional method i.e., by dissolving the reactants in ethanol and stirring the contents at room temperature for 10 min, the product was obtained in 11% yield only. In solid-state, reaction was found to occur more efficiently and much faster than in solution, since molecules in a solid are arranged tightly and regularly (Tanaka and Toda 2000). Besides, absence of a solvent has a high concentration of reagents so that the reaction goes faster (Mogilaiah et al. 2001). Furthermore, the solid-state reaction has many advantages: reduced pollution, low costs and simplicity in process and handling, but it may not be applicable for all types of reactions, as it depends on the reactivity of the interacting molecules.

The synthesis of 2-hydroxy-1,8-naphthyridine-3-carbonitrile is an example of Friedlander synthesis which involves the base catalyzed condensation of 2-aminonicotinaldehyde with ethylcyanoacetate. The mechanism of the reaction is represented below.



2-Hydroxy-1,8-naphthyridine-3-carbonitrile was chlorinated with excess $POCl_3$ by which hydroxyl group at 2-position was substituted by chloro group. The excess of $POCl_3$ was removed and the reaction mixture was decomposed by pouring into ice-cold water. It was basified with 10% NaOH solution. The separated product was filtered and recrystallised. The reaction mechanism is similar to the chlorination of 2-hydroxy-3-methylquinoxaline with $POCl_3$ (page no.180-181).

Microwave irradiation of 2-chloro-1,8-naphthyridine-3-carbonitrile with appropriate piperazines in the presence of K_2CO_3 in DMF for about 5 min gave the corresponding nucleophilic substituted product, 2-(4-substituted piperazine-1-yl)-1,8-naphthyridine-3-carbonitrile. When this reaction was carried out by conventional heating (oil bath), the reaction took (monitored by TLC) 8-10 hr for completion. The products obtained by both the methods were identical (m.p., m.m.p., co-TLC and superimposable IR). Almost similar yields were obtained by both the methods. It was observed that the reaction was simple and accelerated many fold when carried out in microwave environment. The reaction mechanism is as follows.



IR spectral analysis of the final compounds (**NA-1 – NA-12**) showed strong peaks at ~2200 cm^{-1} and ~1600 cm^{-1} due to C≡N and C=N functions, respectively. In ^{1}H-NMR spectra, methylene protons (cyclic) adjacent to N^1 nitrogen of piperazine showed triplet in the range of δ 3.13-3.26 whereas methylene protons (cyclic) adjacent to N^4 nitrogen of piperazine showed triplet in the range of δ 2.57-3.61. The final compounds (**NA-1 – NA-12**) showed the following ^{1}H-NMR signals for 1,8-naphthyridinyl moiety; C_4-H: δ ~8.4 (s), C_5-H: δ ~8.00 (d), C_6-H: δ ~7.3 (dd), C_7-H: δ ~9.00 (d). Elemental (CHN) analysis indicated that the calculated and observed values were within the acceptable limits (± 0.4%).

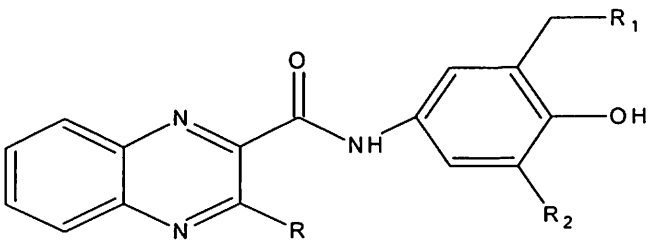
6.3. Pharmacological Studies

6.3.1. 5-HT_3 Receptor Antagonistic Studies

The longitudinal muscle myenteric plexus (LMMP) preparation of the guinea-pig ileum is the most common *in vitro* model for the evaluation of 5-HT_3 receptor modulators. The ileum comprises of the mucosa followed by the circular muscle and the longitudinal muscle. The intestinal muscle is innervated with two layers of neurons, the myenteric plexus and the submucosal plexus that comprise the enteric nervous system (Paton and Zar 1968). The terminal ileum was excised 2 cm from the ileocecal junction, due to the presence of excitatory alpha adrenoreceptors near the ileocecal junction. 2-Me-5-HT was used as it is a selective 5-HT_3 receptor agonist. Pharmacological data (pA_2) of new chemical entities are given in Table 6.1, 6.2 & 6.3.

6.3.1.1. Series I: 3-Substituted-2-quinoxalinecarboxamides (QC-1 – QC-36)

Table 6.1. Pharmacological data of compounds QC-1 – QC-36



Compd.	R	R_1	R_2	pA_2
QC-1	Cl	Dimethylamino	H	< 3.0
QC-2	Cl	Diethylamino	H	< 3.0
QC-3	Cl	Pyrrolidinyl	H	4.8
QC-4	Cl	Piperidinyl	H	4.6
QC-5	Cl	Morpholinyl	H	4.0
QC-6	Cl	Piperazinyl	H	5.4
QC-7	Cl	1-Methylpiperazinyl	H	6.4
QC-8	Cl	1-Ethylpiperazinyl	H	5.9
QC-9	Cl	Diethylamino	$-CH_2$-diethylamino	< 3.0
QC-10	Cl	Pyrrolidinyl	$-CH_2$-pyrrolidinyl	4.0
QC-11	Cl	Morpholinyl	$-CH_2$-morpholinyl	3.3
QC-12	Cl	Piperazinyl	$-CH_2$-piperazinyl	5.0
QC-13	Cl	1-Methylpiperazinyl	$-CH_2$-1-methylpiperazinyl	6.0
QC-14	Cl	1-Ethylpiperazinyl	$-CH_2$-1-ethylpiperazinyl	5.6
QC-15	OCH_3	Pyrrolidinyl	H	3.9
QC-16	OCH_3	Piperidinyl	H	3.5
QC-17	OCH_3	Morpholinyl	H	3.0
QC-18	OCH_3	Piperazinyl	H	4.5
QC-19	OCH_3	1-Methylpiperazinyl	H	5.7

(Continued)

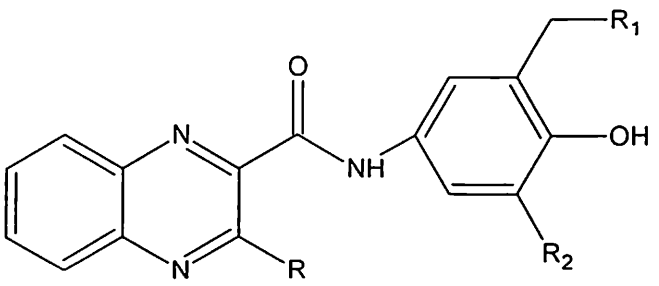
Table 6.1. (Continued)

Compd.	R	R_1	R_2	pA_2
QC-20	OCH_3	1-Ethylpiperazinyl	H	4.9
QC-21	OCH_3	Pyrrolidinyl	$-CH_2$-pyrrolidinyl	< 3.0
QC-22	OCH_3	Morpholinyl	$-CH_2$-morpholinyl	< 3.0
QC-23	OCH_3	Piperazinyl	$-CH_2$-piperazinyl	4.0
QC-24	OCH_3	1-Methylpiperazinyl	$-CH_2$-1-methylpiperazinyl	4.8
QC-25	OCH_3	1-Ethylpiperazinyl	$-CH_2$-1-ethylpiperazinyl	4.1
QC-26	OC_2H_5	Pyrrolidinyl	H	3.5
QC-27	OC_2H_5	Piperidinyl	H	3.1
QC-28	OC_2H_5	Morpholinyl	H	< 3.0
QC-29	OC_2H_5	Piperazinyl	H	3.4
QC-30	OC_2H_5	1-Methylpiperazinyl	H	4.6
QC-31	OC_2H_5	1-Ethylpiperazinyl	H	3.9
QC-32	OC_2H_5	Pyrrolidinyl	$-CH_2$-pyrrolidinyl	< 3.0
QC-33	OC_2H_5	Morpholinyl	$-CH_2$-morpholinyl	< 3.0
QC-34	OC_2H_5	Piperazinyl	$-CH_2$-piperazinyl	< 3.0
QC-35	OC_2H_5	1-Methylpiperazinyl	$-CH_2$-1-methylpiperazinyl	< 3.0
QC-36	OC_2H_5	1-Ethylpiperazinyl	$-CH_2$-1-ethylpiperazinyl	< 3.0
Ondansetron				6.9

Results are the mean from three separate experiments except for those pA_2 values < 3.0 which are the mean of two separate experiments. SE was less than 10% of the mean.

Among the 36 compounds in this series, **QC-7** showed most potent 5-HT_3 antagonism with a pA_2 value of 6.4, followed by **QC-13**, **QC-8** and **QC-14** which showed pA_2 value of 6.0, 5.9 and 5.6, respectively. Whereas the standard 5-HT_3 RA, ondansetron showed pA_2 value of 6.9. The compounds, which showed pA_2 values < 3.0 were considered as inactive.

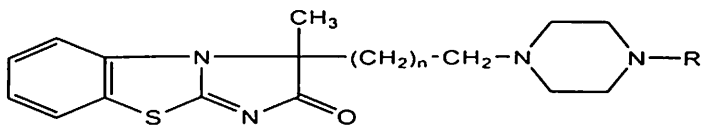
Structure Activity Relationships



3-Substituted quinoxaline-2-carboxamides exhibited varying degrees of 5-HT_3 receptor antagonisms in the isolated guinea-pig ileum. Chloro group (**QC1 - QC14**) in the third position of the quinoxaline moiety showed higher 5-HT_3 receptor antagonism followed by methoxy (**QC-15 – QC-25**) and ethoxy groups (**QC-26 – QC-36**). Piperazines attached with methylene group of *p*-aminophenol exhibited higher antagonisms followed by cyclic secondary amines and acyclic secondary amines. These results clearly indicate that N^4 piperazine is essential for 5-HT_3 antagonistic activity. Compounds **QC-6**, **QC-8** & **QC-29** (with no substitution at N^4 piperazine) showed pA_2 values of 5.4, 4.5 & 3.4, respectively. With increased lipophilicity (i.e., methyl group, **QC-7**, **QC-19** & **QC-30**), 5-HT_3 antagonistic activity increased (pA_2 = 6.4, 5.7 & 4.6, respectively). Further increase in lipophilicity (i.e., ethyl group, **QC-8**, **QC-20** & **QC-31**), decreased the activity (pA_2 = 5.9, 4.9 & 3.9, respectively) among the tested compounds. It has been observed that when the side-chain of the 3-substituted quinoxaline-2-carboxamides is attached with bis Mannich derivatives of *p*-aminophenol (**QC-9 – QC-14**, **QC-21 – QC-25** & **QC-32 – QC-36**), the antagonism decreased as compared to mono Mannich derivates of *p*-aminophenol (**QC-1 – QC-8**, **QC-15 – QC-20** & **QC-26 – QC-31**).

6.3.1.2. Series II: 3-Methy-l[(4-substituted piperazin-1-yl)alkyl]imidazo-[2,1-b][1,3]benzothiazol-2(3H)-ones (IP-1 – IP-24)

Table 6.2. Pharmacological data of compounds IP-1 – IP-24



Compd.	R	n	pA_2
IP-1	-H	1	6.1
IP-2	-CH_3	1	6.7
IP-3	-C_2H_5	1	6.4
IP-4	-C_6H_5	1	4.0
IP-5	-CH_2-C_6H_5	1	< 3.0
IP-6	*o*-OCH_3-C_6H_4	1	3.7
IP-7	*m*-OCH_3-C_6H_4	1	5.9
IP-8	*p*-OCH_3-C_6H_4	1	< 3.0
IP-9	2-pyridyl	1	< 3.0
IP-10	*p*-Cl-C_6H_4	1	< 3.0
IP-11	*p*-NO_2-C_6H_4	1	< 3.0
IP-12	*m*-CF_3-C_6H_4	1	3.5
IP-13	-H	2	5.0
IP-14	-CH_3	2	5.5
IP-15	-C_2H_5	2	5.2
IP-16	-C_6H_5	2	< 3.0
IP-17	-CH_2-C_6H_5	2	< 3.0
IP-18	*o*-OCH_3-C_6H_4	2	< 3.0
IP-19	*m*-OCH_3-C_6H_4	2	4.5
IP-20	*p*-OCH_3-C_6H_4	2	< 3.0

(Continued)

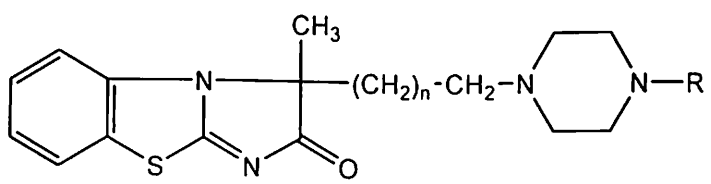
Table 6.2 (Continued)

Compd.	R	n	pA_2
IP-21	2-pyridyl	2	< 3.0
IP-22	*p*-Cl-C_6H_4	2	< 3.0
IP-23	*p*-NO_2-C_6H_4	2	< 3.0
IP-24	*m*-CF_3-C_6H_4	2	< 3.0
Ondansetron			6.9

Results are the mean from three separate experiments except for those pA_2 values < 3.0 which are the mean of two separate experiments. SE was less than 10% of the mean.

Among the 24 compounds in this series, **IP-2** showed most potent 5-HT_3 antagonism with a pA_2 value of 6.7, followed by **IP-3** and **IP-1**, which showed pA_2 values of 6.4 and 6.1, respectively. Whereas the standard 5-HT_3 receptor antagonist, ondansetron showed pA_2 value of 6.9. The compounds, which showed pA_2 values < 3.0, were considered as inactive.

Structure Activity Relationships

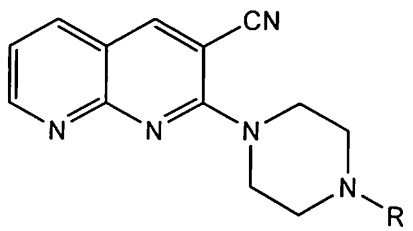


Compound **IP-1** & **IP-13** (with no substitution at N^4 piperazine) showed pA_2 values of 6.1 & 5.0, respectively. With increased lipophilicity (methyl group, **IP-2** & **IP-14**) activity was found to increase (pA_2 = 6.7 & 5.5, respectively). Further increase in lipophilicity (ethyl group, **IP-3** & **IP-15**) decreased the activity (pA_2 = 6.4 & 5.2, respectively). Placement of bulkier groups like aryl / substituted aryl at N^4 piperazine (compounds **IP-4** – **IP-12** & **IP-16** – **IP-24**) decreased the activity. Compounds with ethyl side-chain (**IP-1** – **IP-12**) showed higher 5-HT_3 antagonism, compared to those with propyl side chain (**IP-13** – **IP-24**).

6.3.1.3. Series III:

*2-(4-Substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles (**NA-1 – NA-12**)*

Table 6.3. Pharmacological data of compounds NA-1 – NA-12

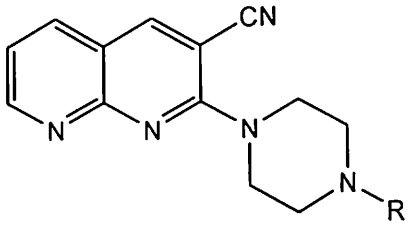


Compd.	R	pA_2
NA-1	-H	6.5
NA-2	$-CH_3$	7.4
NA-3	$-C_2H_5$	7.1
NA-4	$-CH_2-CH=CH_2$	8.2
NA-5	$-C_6H_5$	5.7
NA-6	$-CH_2-C_6H_5$	5.1
NA-7	*o*-$OCH_3-C_6H_4$	5.4
NA-8	*m*-$OCH_3-C_6H_4$	6.0
NA-9	*p*-$OCH_3-C_6H_4$	4.9
NA-10	*p*-$NO_2-C_6H_4$	3.8
NA-11	*p*-Cl-C_6H_4	< 3.0
NA-12	2-pyridyl	3.9
Ondansetron		6.9

Results are the mean from three separate experiments except for those pA_2 values < 3.0 which are the mean of two separate experiments. SE was less than 10% of the mean.

Among the 12 compounds in this series, **NA-4** showed most potent 5-HT_3 antagonism with a pA_2 value of 8.2, followed by **NA-2**, **NA-3** and **NA-1**, which showed pA_2 values of 7.4, 7.1 and 6.5, respectively. Three compounds in this series, **NA-4**, **NA-2** and **NA-3** exhibited more potent 5-HT_3 receptor antagonistic activities than the standard 5-HT_3 RA, ondansetron, which showed pA_2 value of 6.9.

Structure Activity Relationships



Compound **NA-1** (with no substitution at N^4 piperazine) showed good antagonism (pA_2 = 6.5); with increased liphophilicity (i.e., methyl group, **NA-2**) activity increased (pA_2 = 7.4). Further increase in liphophilicity (i.e., ethyl group, **NA-3**) decreased the activity (pA_2 = 7.1), whereas substitution with allyl group (**NA-4**) showed most favourable antagonism (pA_2 = 8.2). Placement of bulkier groups like aryl/substituted aryl at N^4 piperazine (**NA-5 – NA-12**) decreased the activity. In addition, electron withdrawing substituents at aryl group of N^4 piperazine (compounds **NA-10 – NA-12**) showed the least activity among the aryl derivatives.

The heteroarylpiperazine ligands, 2-(4-substituted piperazine-1-yl)-1,8-naphthyridine-3-carbonitriles, are to be considered as atypical, since a heterocyclic nitrogen atom replaces the carbonyl group in the three component pharmacophoric model (i.e., aromatic ring, carbonyl group and a basic nitrogen).

6.3.2. Anxiolytic Studies

6.3.2.1. Elevated Plus Maze

The effect of drugs on % entries in open arms is depicted in Fig. 6.1. ANOVA showed extremely significant difference among the different treatments [$F(7, 40) = 7.01$, $p < 0.0001$]. Tukey's test revealed that diazepam (0.2 mg/kg, *i.p.*) ($p < 0.05$), ondansetron (10 mg/kg, *i.p.*) and **NA-4** (10 mg/kg, *i.p.*) ($p < 0.01$), significantly increased % entries in open arms. Ondansetron (0.1 & 1.0 mg/kg, *i.p.*) and **NA-4** (0.1 & 1.0 mg/kg, *i.p.*) did not alter the measure significantly at $p < 0.05$ in comparison to saline control. The compound **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in % entries in open arms compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The effect of drugs on % time spent in open arms is given in Fig. 6.2. ANOVA showed significant difference among the different treatments [$F(7, 40) = 3.94$, $p = 0.0023$]. Tukey's test revealed that diazepam (0.2 mg/kg, *i.p.*), ondansetron (10 mg/kg, *i.p.*) and **NA-4** (10 mg/kg, *i.p.*) significantly ($p < 0.05$) increased the % time spent in open arms. Ondansetron (0.1 & 1.0 mg/kg, *i.p.*) and **NA-4** (0.1 & 1.0 mg/kg, *i.p.*) did not alter the measure significantly at $p < 0.05$ in comparison to saline control. **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in % time spent in open arms compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The effect of drugs on total number of entries is shown in Fig. 6.3. ANOVA showed significant difference among the different treatments [$F(7, 40) = 2.29$, $p = 0.0463$]. Tukey's test revealed that none of the tested drugs alter the measure significantly at $p < 0.05$ in comparison to saline control. Moreover, **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in total number of entries compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The elevated plus-maze is undoubtedly one of the most widely used animal models in contemporary pre-clinical research on anxiety (Rodgers and Cole 1994; Hogg 1996), which is based on the natural fear of rodents for open space and height. The primary indices of anxiety in the elevated plus-maze have been spatio-temporal in nature (i.e., the number of open arm entries expressed as a percentage or ratio of total arm entries, and time spent in the open arms expressed as a percentage or ratio of total arm time), while total arm entries have frequently been employed as a measure of general activity.

The advantages of the conventional elevated plus-maze are economy, speed, simplicity and bi-directional sensitivity coupled with the fact that the procedure does not require lengthy procedures involving the use of food / water deprivation and electric shock (Pellow et al. 1985). While there is no doubt that the conventional elevated plus-maze model is highly sensitive to the influence of benzodiazepine/$GABA_A$ receptor-related manipulations, effects obtained with other anxiety-modulating agents (e.g. Buspirone) have been very much more variable (Handley and McBlane 1993; Rodgers and Cole 1994).

Major inter-laboratory differences exist in the use of the elevated plus-maze paradigm and the pharmacological response is heavily influenced by the choice of strain, pre-test manipulation of subjects and the aversiveness of the test conditions (Hogg 1996). It appears that sensitivity to potential (particularly non-benzodiazepine) anxiolytics is enhanced by stressing animals prior to testing (e.g. by moving from holding to test room) and/or by using more aversive test conditions (e.g. high light), thereby suggesting the fundamental importance of endogenous tone in key neurochemical systems. The utility/reliability/sensitivity of this model might also be improved by adopting a more ethological approach to data collection (Moser 1989; Rodgers et al. 1992; Falter et al. 1992; Shepherd 1992; Adamec and Shallow 1993).

There are many reports which studied the anxiolytic activities of 5-HT_3 RAs at lower doses (0.01 – 1.0 mg/kg) (Rodgers et al. 1995; Eguchi et al. 2001; Andrews and File 1993b; Artaiz et al. 1995; Blackburn et al. 1993; Costall and Naylor 1991; Costall et al. 1989, 1993; Dunn et al. 1991; Filip et al. 1992; Piper et al. 1988; File and Johnson 1989; Griebel et al. 1997; Piper et al. 1988; Wright et al. 1992), but there is no report which studied the anxiolytic activity of 5-HT_3 RAs at higher doses. Hence it was thought that it will be fruitful to study the anxiolytic activity at three different dose levels (i.e., 0.1, 1.0 & 10 mg/kg, *i.p.*).

In the present study, the standard 5-HT_3 RA, ondansetron, and the test compound, **NA-4** did not show significant anxiolytic activity at lower doses (0.1 & 1.0 mg/kg, *i.p.*). These results correlate with the reported methods (Rodgers et al. 1995; Eguchi et al. 2001). At higher doses (i.e., 10 mg/kg, *i.p.*), both ondansetron and **NA-4** showed significant anxiolytic activity (increase in % open arm entries and % time spent in open arms) in elevated plus-maze model. The parameters observed in this animal model revealed that the standard 5-HT_3 RA, ondansetron and the test molecule, **NA-4**, exhibited equipotent anxiolytic activity.

6.3.2.2. Light/Dark exploration test:

The effect of drugs on latency to the first transition (light area to dark area) is shown in Fig. 6.4. ANOVA showed insignificant difference among the different treatments [$F(7, 40) = 1.034$, $p = 0.423$]. Tukey's test revealed that none of the tested drugs alter the latency significantly at $p < 0.05$ in comparison to saline control. Moreover, the compound **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in latency to the first transition compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The effect of drugs on % time spent in light area is given in Fig. 6.5. ANOVA showed very significant difference among the different treatments [F (7, 40) = 3.19, p = 0.0088]. Tukey's test revealed that diazepam (0.2 mg/kg, *i.p.*) ($p < 0.05$), ondansetron (10 mg/kg, *i.p.*) ($p < 0.05$) and **NA-4** (10 mg/kg, *i.p.*) ($p < 0.01$) significantly increased the % time spent in light area. Ondansetron (0.1 & 1.0 mg/kg, *i.p.*) and **NA-4** (0.1 & 1.0 mg/kg, *i.p.*) did not alter the measure significantly at $p < 0.05$ in comparison to saline control. **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in % time spent in light area compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The effect of drugs on transitions between two compartments is shown in Fig. 6.6. ANOVA showed very significant difference among the different treatments [F (7, 40) = 3.21, p = 0.0085]. Tukey's test revealed that diazepam (0.2 mg/kg, *i.p.*), ondansetron (10 mg/kg, *i.p.* and **NA-4** (10 mg/kg, *i.p.*) significantly ($p < 0.05$) increased the number of transitions between the two compartments. Ondansetron (0.1 & 1.0 mg/kg, *i.p.*) and **NA-4** (0.1 & 1.0 mg/kg, *i.p.*) did not alter the measure significantly at $p < 0.05$ in comparison to saline control. The compound **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in transitions between two compartments compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The light dark box was originally described by Crawley and Goodwin (1980) and is based on the natural aversion of rodents for brightly lit places. In a two compartment box, one dark and one brightly lit, the total activity, the time spent in the light compartment and the crossings between the light and dark compartments provide information about the preference of the animal for the dark compartment. As anxiolytics should reduce the natural aversion to light, the essential feature of this model is that anxiolytic drugs increase the number of crossings and / or the time spent in the light compartment. The latter parameter is generally considered to be the most relevant.

The effect of drugs on % time spent in light area is given in Fig. 6.5. ANOVA showed very significant difference among the different treatments [F (7, 40) = 3.19, p = 0.0088]. Tukey's test revealed that diazepam (0.2 mg/kg, *i.p.*) ($p < 0.05$), ondansetron (10 mg/kg, *i.p.*) ($p < 0.05$) and **NA-4** (10 mg/kg, *i.p.*) ($p < 0.01$) significantly increased the % time spent in light area. Ondansetron (0.1 & 1.0 mg/kg, *i.p.*) and **NA-4** (0.1 & 1.0 mg/kg, *i.p.*) did not alter the measure significantly at $p < 0.05$ in comparison to saline control. **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in % time spent in light area compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

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The light dark box was originally described by Crawley and Goodwin (1980) and is based on the natural aversion of rodents for brightly lit places. In a two compartment box, one dark and one brightly lit, the total activity, the time spent in the light compartment and the crossings between the light and dark compartments provide information about the preference of the animal for the dark compartment. As anxiolytics should reduce the natural aversion to light, the essential feature of this model is that anxiolytic drugs increase the number of crossings and / or the time spent in the light compartment. The latter parameter is generally considered to be the most relevant.

Benzodiazepines are reliably detected in this paradigm using mice (Crawley 1981; Jones et al. 1988; Kilfoil et al. 1989; Costall et al. 1989). Also the 5-HT_{1A} receptor agonist, buspirone has been reported to show an anxiolytic profile in this test (Costall et al. 1988b; Carli et al. 1989; Kilfoil et al. 1989). The 5-HT_3 RAs ondansetron, bemesetron, tropisetron, zacopride, itasetron (DAU 6215), WAY 100289, RS-42385-197, and WAY-SEC-579 have been reported to produce similar effects as 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). There are few studies which did not find anxiolytic activity in a number of 5-HT_3 RAs (Morinan 1989; Kshama et al. 1990).

Costall and Naylor (1991) tested ondansetron for 14 days (10 µg/kg, *i.p.*, *b.i.d.*) and showed that it retained its anxiolytic activity in a white / dark box in mice and in a social interaction test in rats. Moreover, no evidence for tolerance or sensitization occurred. After stopping the treatment, the anxiolytic effects slowly waned until control levels were reached after about 96 hours. No rebound anxiety was observed. A similar pattern was observed with buspirone (1.0 mg/kg, *i.p.*, *b.i.d.*), but not with diazepam, which, upon cessation of treatment, led to anxiogenic effects. When diazepam (7 days treatment, 10 mg/kg, *i.p.*, *b.i.d.*) was withdrawn, a clear anxiogenic effect was noted in mice tested at 8, 24, 48 and 96 hrs after withdrawal in the black-white exploration test (Costall and Naylor 1991). This withdrawal anxiety could not be antagonized by buspirone, whereas ondansetron (10 µg/kg, *i.p.*, *b.i.d.*) was able to restore control levels besides gain in the anxiolytic level, previously obtained by diazepam.

In the present study, the standard 5-HT_3 RA, ondansetron, and the test compound, **NA-4** did not show significant anxiolytic activity at lower doses (0.1 & 1.0 mg/kg, *i.p.*). At a high dose (i.e., 10 mg/kg, *i.p.*) both ondansetron and **NA-4** showed significant anxiolytic activity (increase in % time spent in light area and the number of transition between two compartments) in light/dark box model. The parameters observed in this animal model revealed that the standard 5-HT_3 RA, ondansetron and the test molecule, **NA-4**, exhibited equipotent anxiolytic activity.

6.3.2.3. Hole board test:

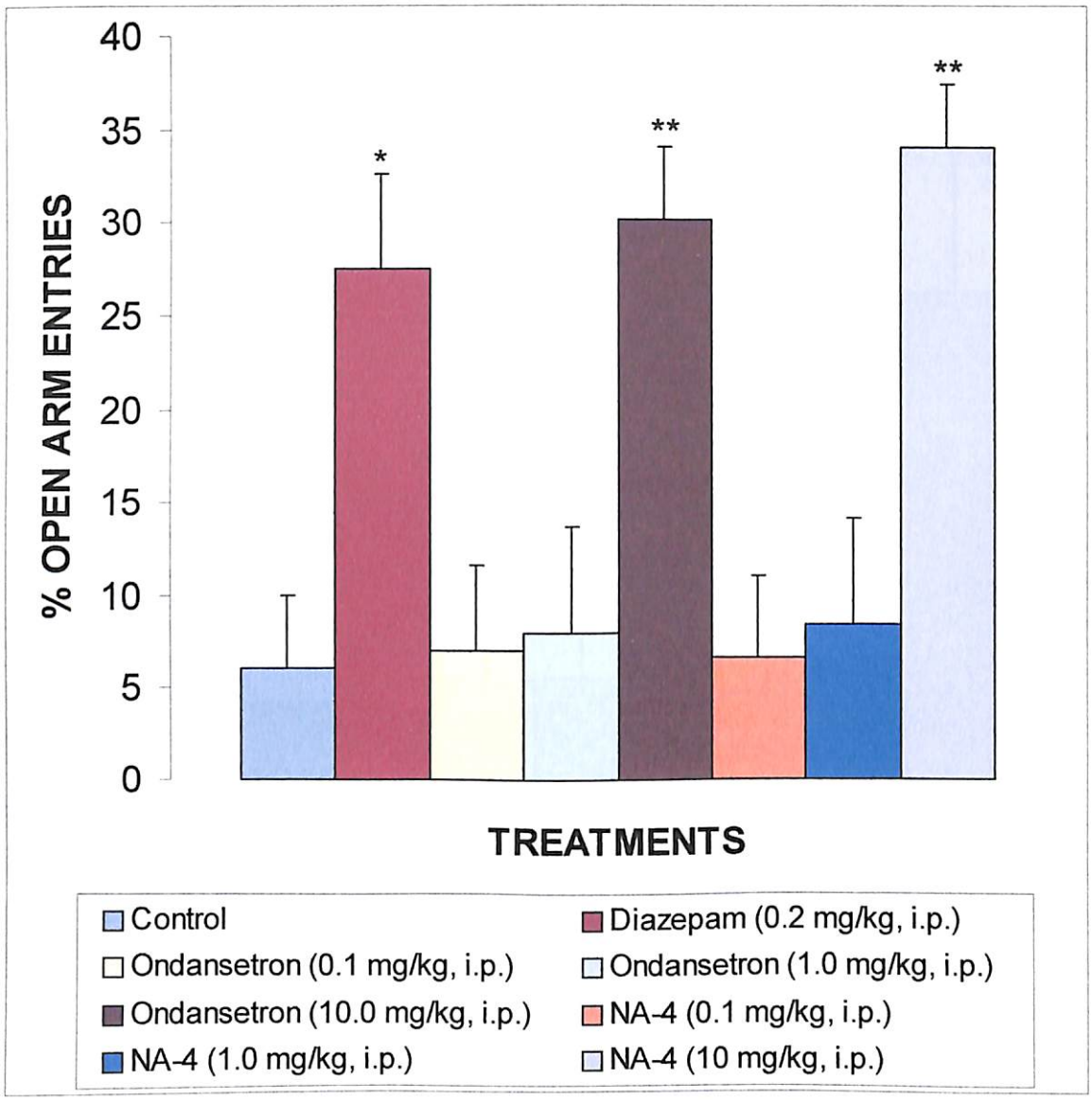
The effect of drugs on number of head dips into holes in the hole board test is given in Fig 6.7. ANOVA showed significant difference among the different treatments [F (7, 40) = 2.37, p = 0.0403]. Tukey's test revealed that diazepam (0.2 mg/kg, *i.p.*), ondansetron (10 mg/kg, *i.p.* and **NA-4** (10 mg/kg, *i.p.*) significantly ($p < 0.05$) increased the number of head dips. Ondansetron (0.1 & 1.0 mg/kg, *i.p.*) and **NA-4** (0.1 & 1.0 mg/kg, *i.p.*) did not alter the measure significantly at $p < 0.05$ in comparison to saline control. The compound **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in number of head dips compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The effect of drugs on number of line crossings is given in Fig. 6.8. ANOVA showed insignificant difference among the different treatments [F (7, 40) = 0.4666, p = 0.853]. Tukey's test revealed that none of the tested drugs altered the number of line crossings significantly at $p < 0.05$ in comparison to saline control. Moreover, **NA-4** at 0.1, 1.0 and 10 mg/kg, *i.p.* did not show significant difference in number of line crossings compared to ondansetron at 0.1, 1.0 and 10 mg/kg, *i.p.*, respectively.

The hole board test is a measure of exploratory behavior in rodents (File and Wardill 1975). In addition, head dips of mice observed in the hole-board experiments have been accepted as a parameter for the evaluation of anxiety conditions in animals. In this model, non-sedative doses of benzodiazepines and other anxiolytic drugs, have been reported to increase the number of head dips in mice (Crawley 1985). The effect of the anxiolytic drugs on locomotion can be assessed by the number of squares crossed by the animal during the experimentation.

In the present study, the standard 5-HT_3 RA, ondansetron, and the test compound, **NA-4** did not show significant anxiolytic activity at lower doses (0.1 & 1.0 mg/kg, *i.p.*). At a high dose (i.e., 10 mg/kg, *i.p.*), both ondansetron and **NA-4** showed significant anxiolytic activity (increase in the number of head dips) in the hole board test. The parameters observed in this animal model revealed that the standard 5-HT_3 RA, ondansetron and the test molecule, **NA-4**, exhibited equipotent anxiolytic activity. None of the tested compounds showed significant change in the locomotion (no. of line crossings) compared to the control group.

Fig. 6.1. Effect of drugs on % entries in open arms (EPM)

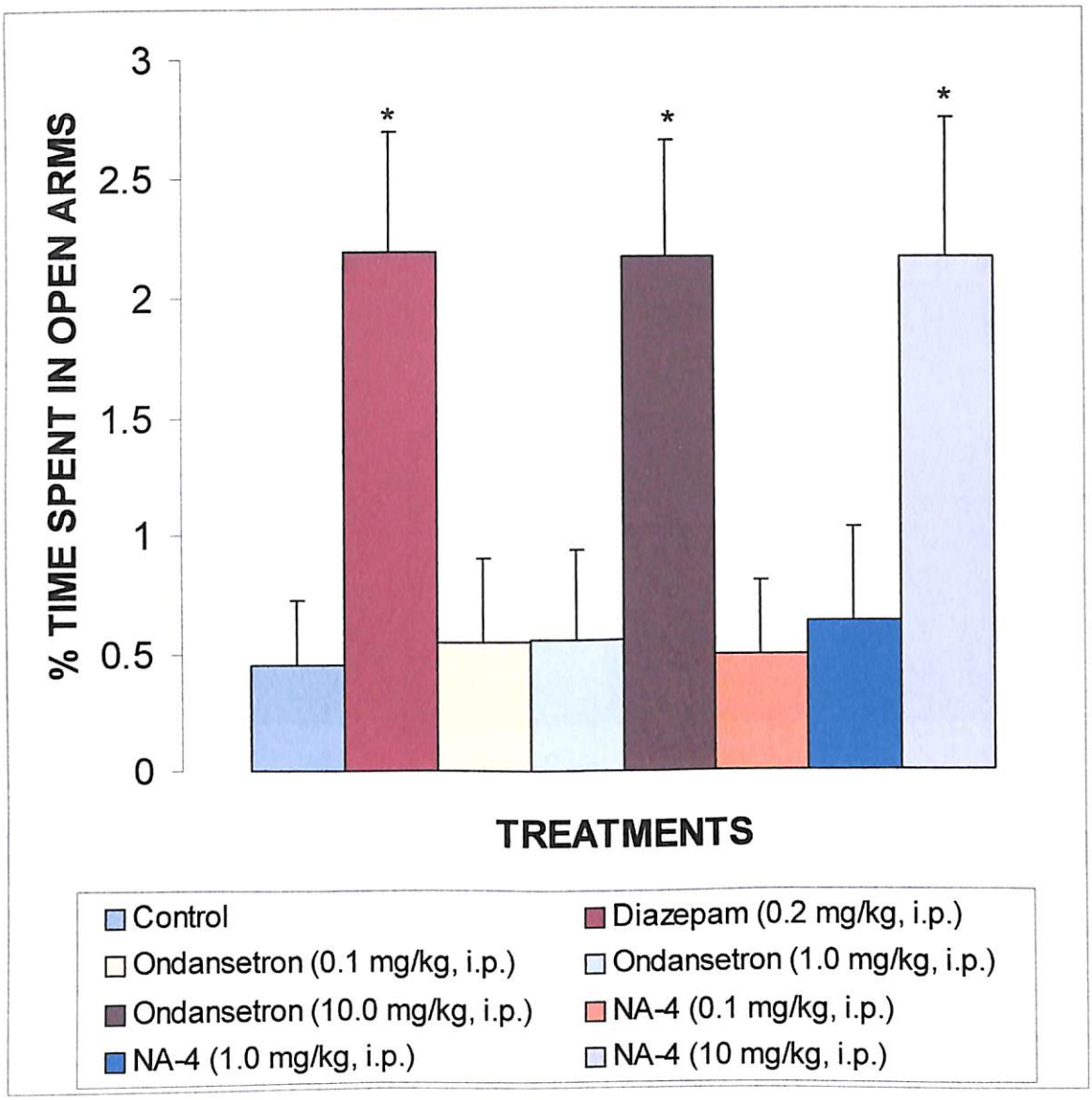


n = 6, values are mean ± SEM

** indicates significant difference from respective controls, p < 0.05*

*** indicates significant difference from respective controls, p < 0.01*

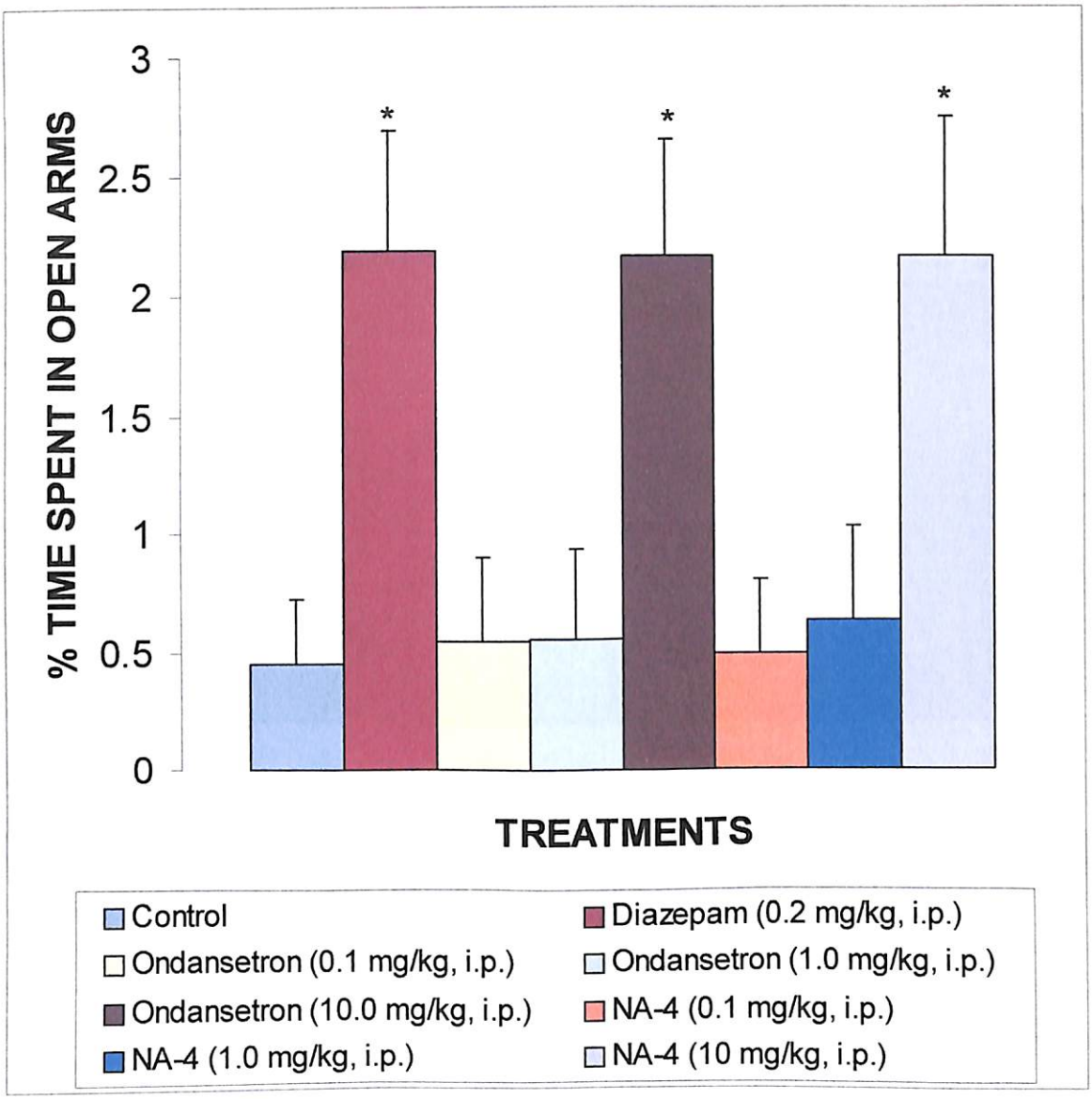
Fig. 6.2. Effect of drugs on % time spent in open arms (EPM)



n = 6, values are mean ± SEM

** indicates significant difference from respective controls, p < 0.05*

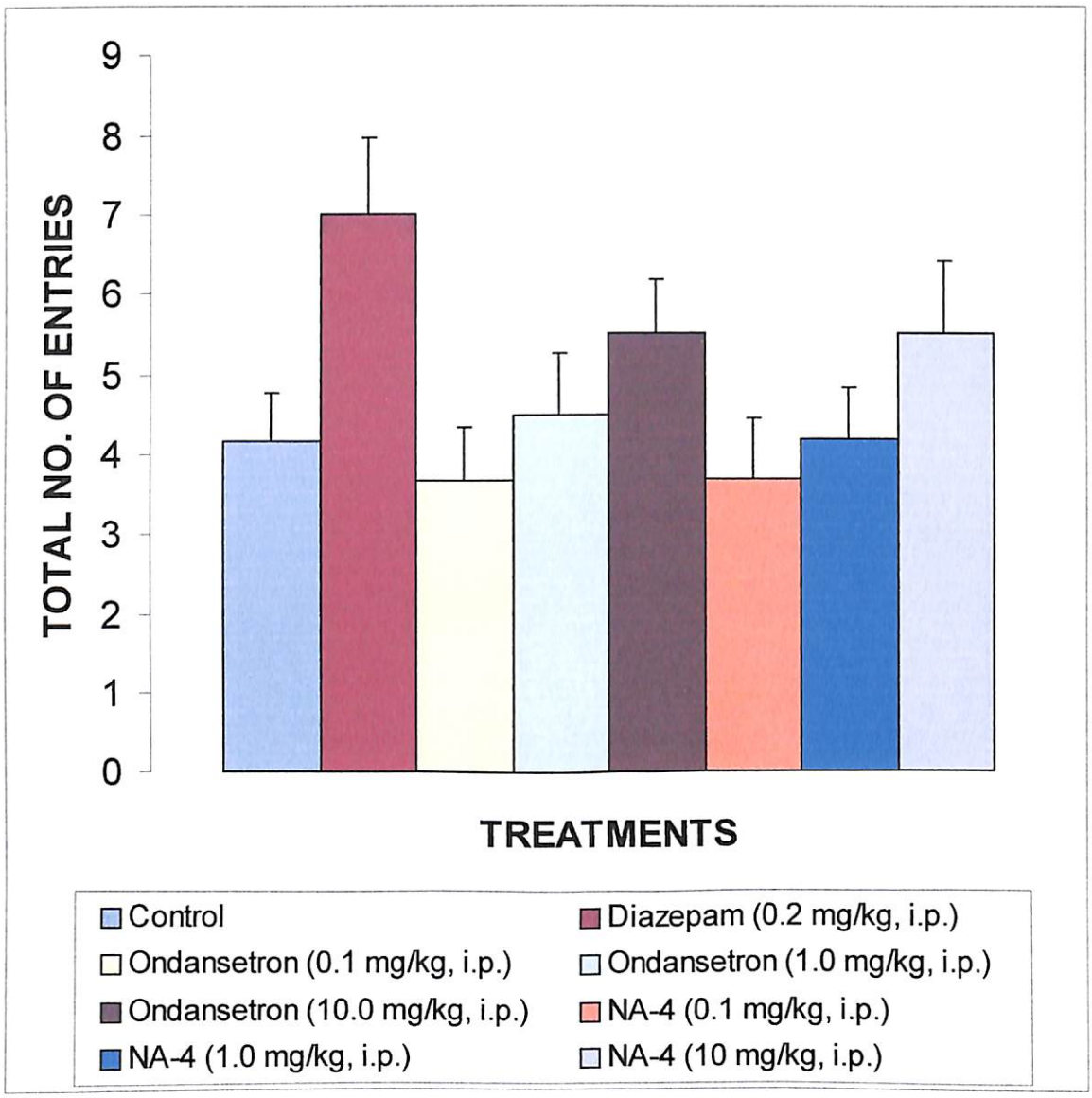
Fig. 6.2. Effect of drugs on % time spent in open arms (EPM)



n = 6, values are mean ± SEM

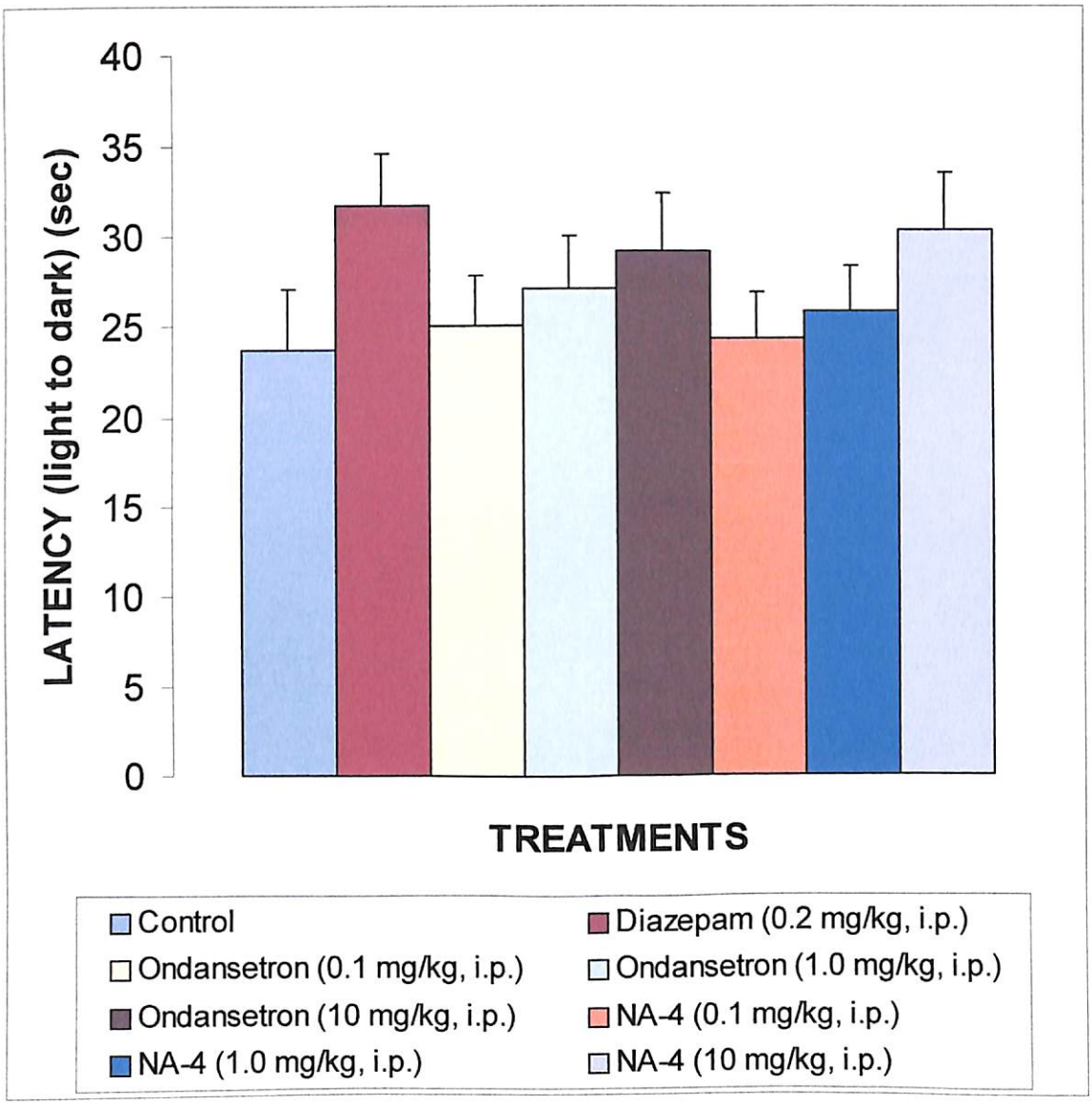
** indicates significant difference from respective controls, p < 0.05*

Fig. 6.3. Effect of drugs on total number of entries (EPM)



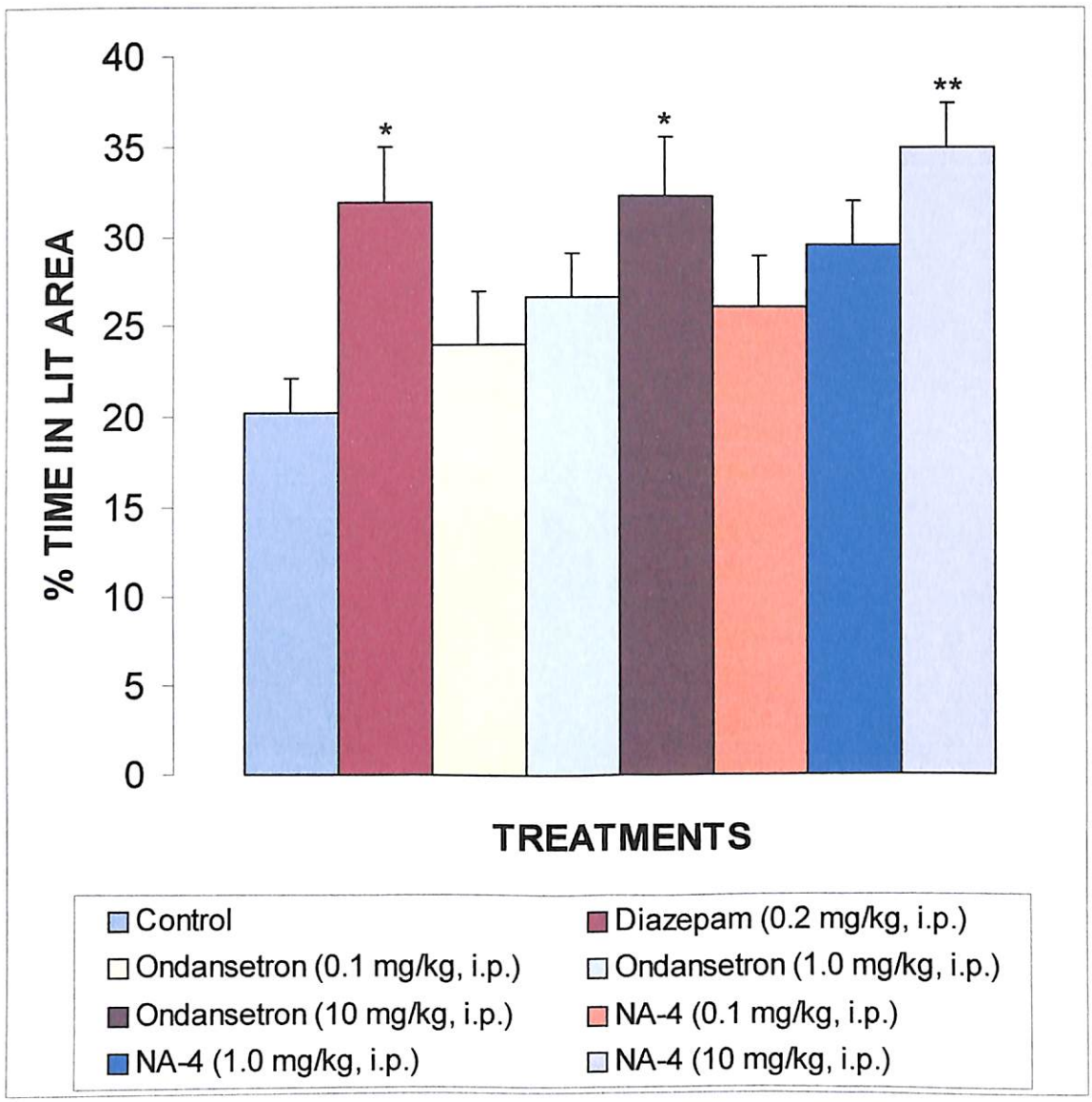
n = 6, values are mean ± SEM

Fig. 6.4. Effect of drugs on latency to the first transition (Light/dark test)



n = 6, values are mean ± SEM

Fig. 6.5. Effect of drugs on % time spent in light area (Light/dark test)

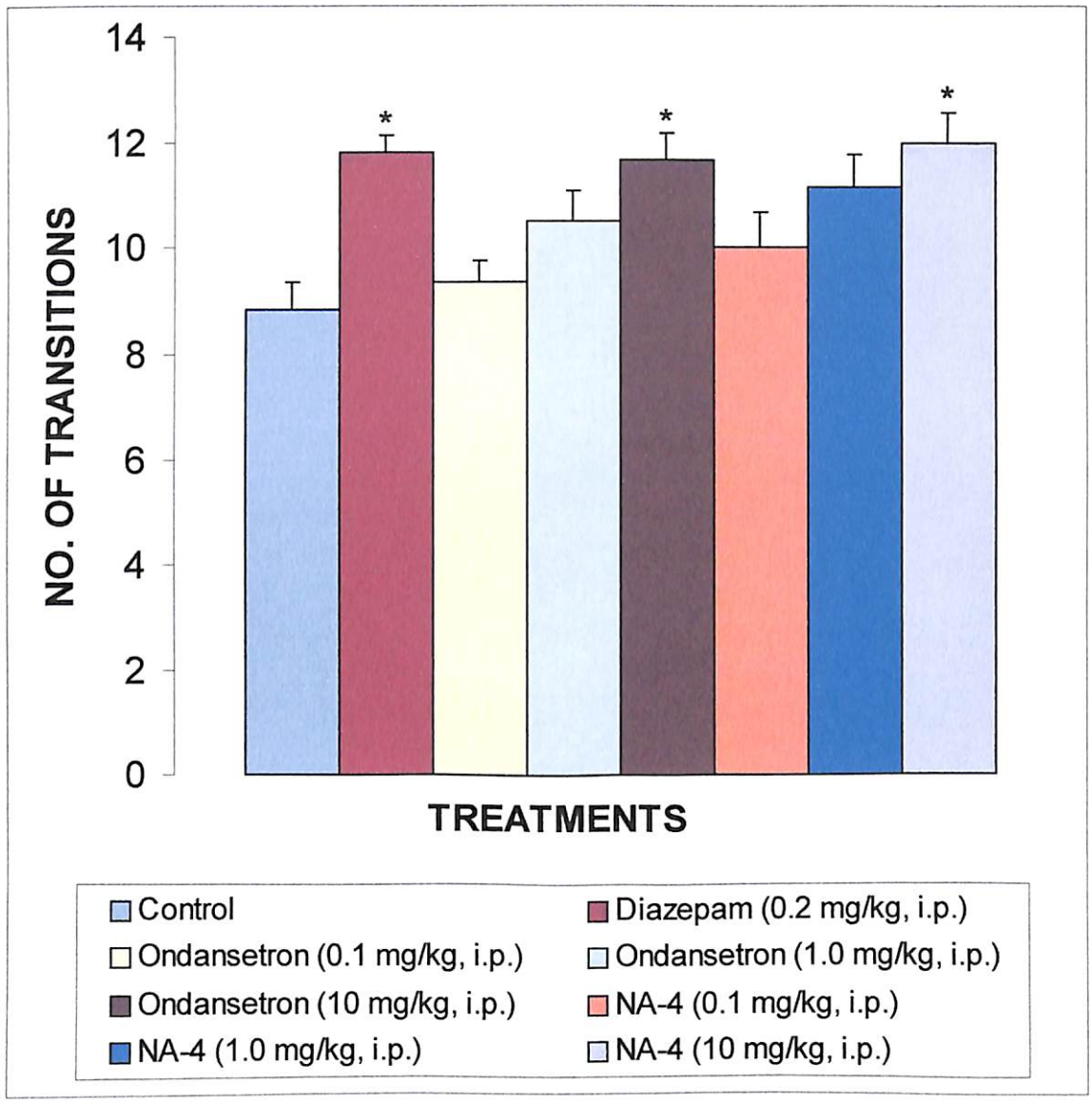


n = 6, values are mean ± SEM

** indicates significant difference from respective controls, p < 0.05*

*** indicates significant difference from respective controls, p < 0.01*

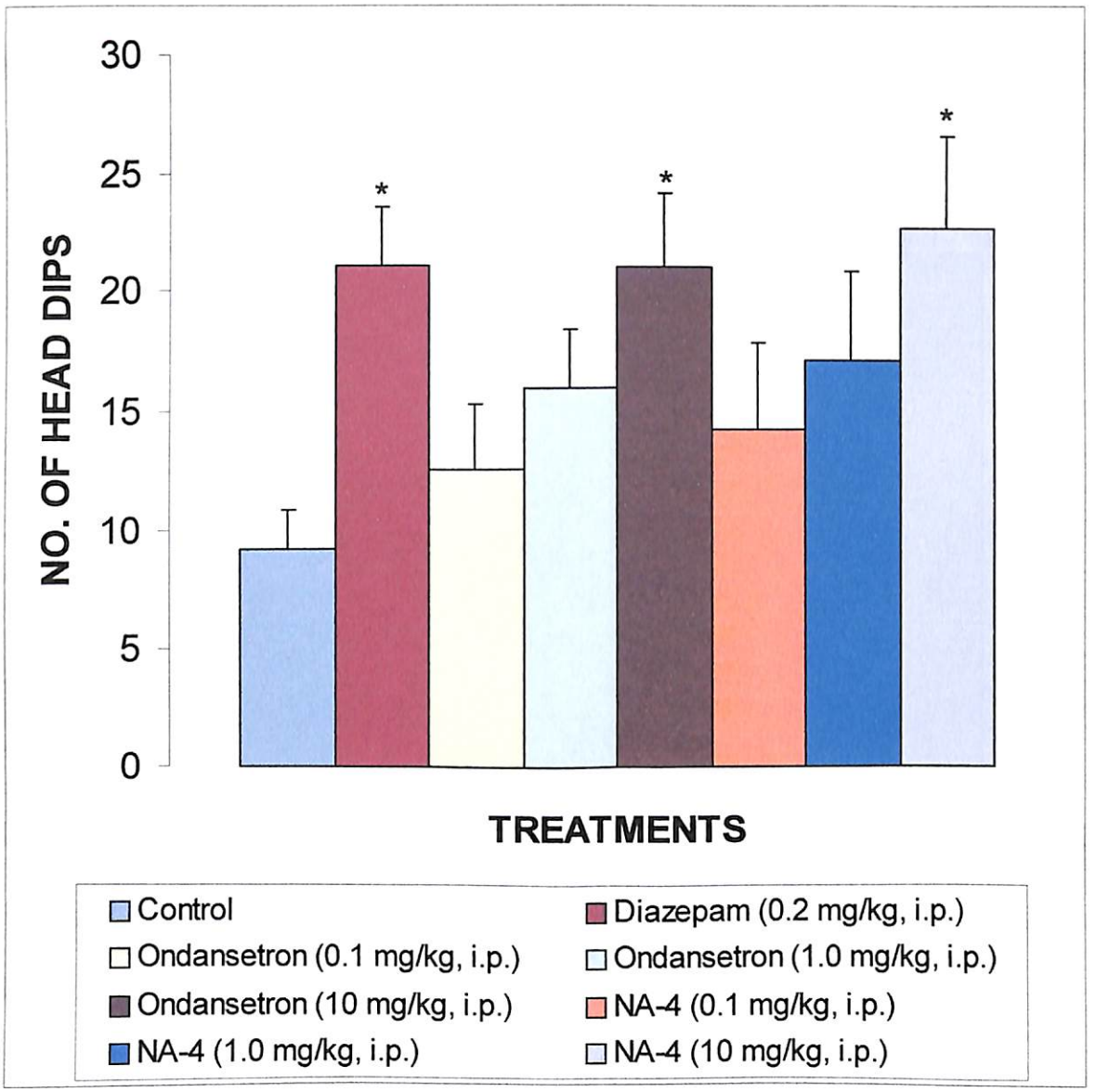
Fig. 6.6. Effect of drugs on transitions between light and dark compartments (Light/dark test)



n = 6, values are mean ± SEM

** indicates significant difference from respective controls, $p < 0.05$*

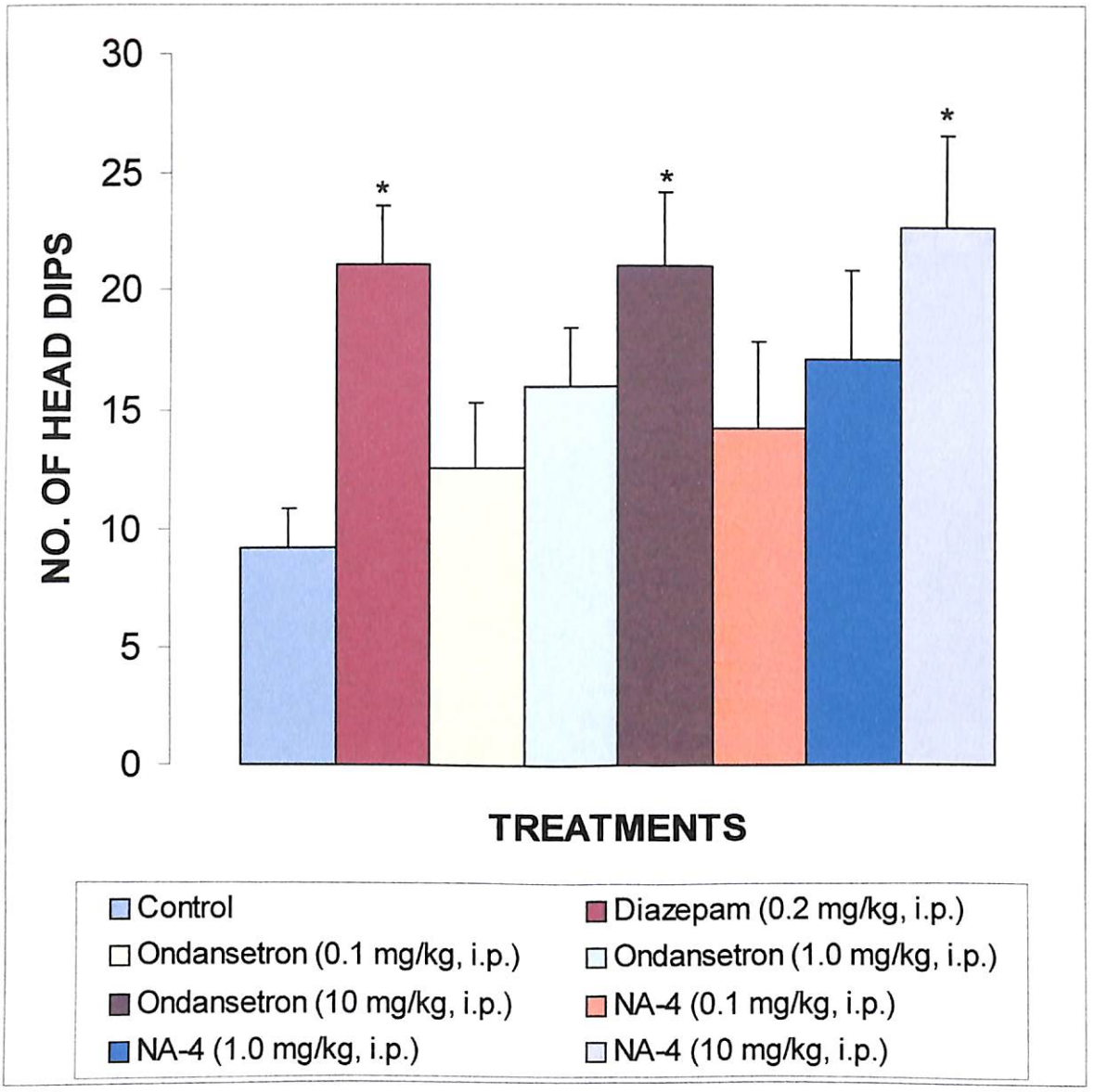
Fig. 6.7. Effect of drugs on number of head dips into holes (hole board test)



n = 6, values are mean ± SEM

** indicates significant difference from respective controls, $p < 0.05$*

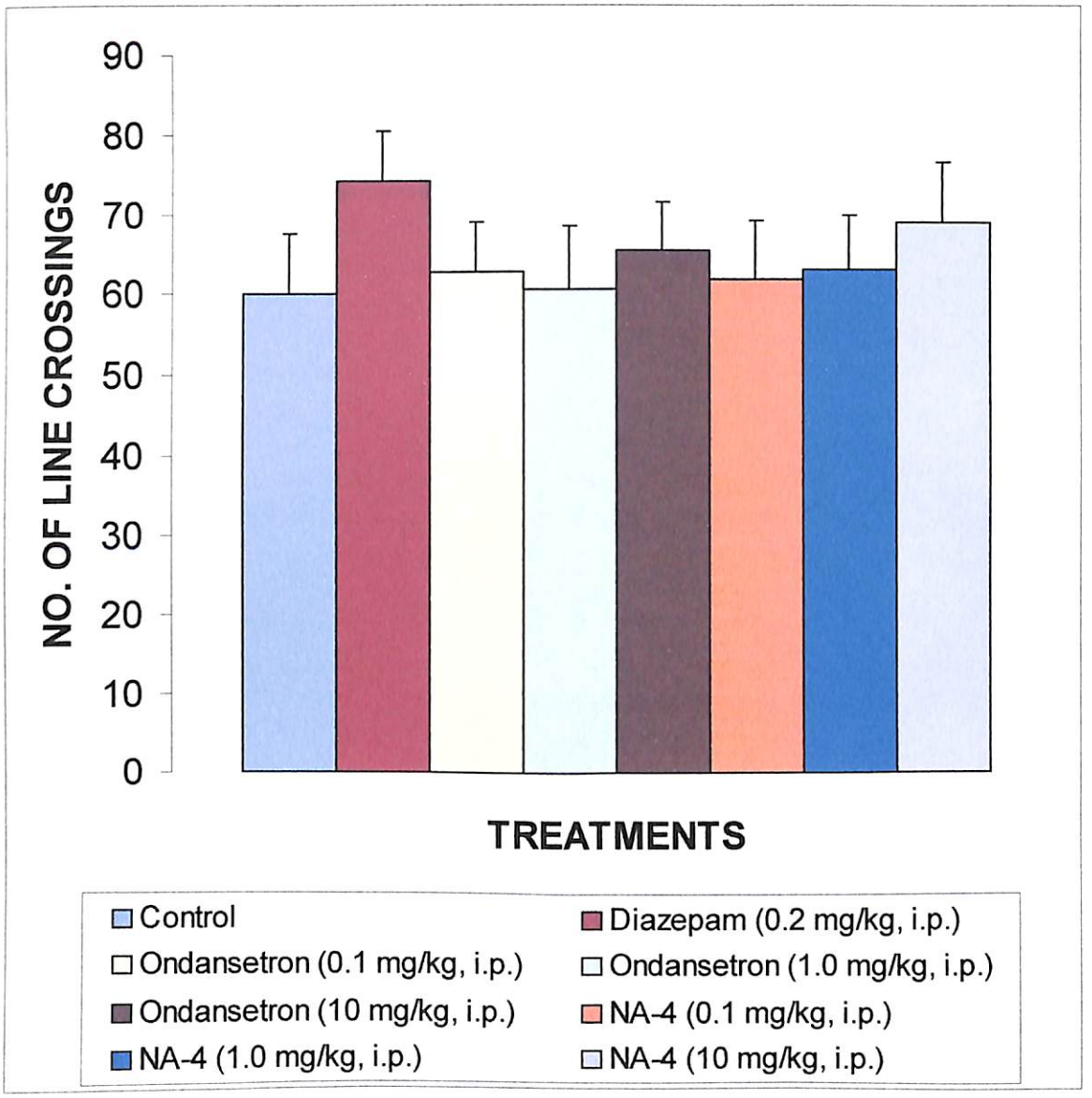
Fig. 6.7. Effect of drugs on number of head dips into holes (hole board test)



n = 6, values are mean ± SEM

** indicates significant difference from respective controls, p < 0.05*

Fig. 6.8. Effect of drugs on number of line crossings (hole board test)



n = 6, values are mean ± SEM

Chapter VII
Summary & Conclusions

CHAPTER VII

SUMMARY AND CONCLUSIONS

- Three series of compounds, 3-substituted-2-quinoxalinecarboxamides, 3-methyl-[(4-substituted piperazin-1-yl)alkyl]imidazo-[2,1-*b*][1,3]benzo-thiazol-2(3*H*)-ones and 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitriles, were designed in accordance with the phamacophoric requirements for 5-HT_3 receptor antagonists.

- The designed molecules were synthesized by conventional methods and wherever possible, reactions were simultaneously carried out using novel techniques such as microwave assisted organic synthesis in liquid medium or by using solid support and by solid state organic reactions at room temperature.

- The newly synthesized molecules were charaterized by spectral data and the purity was ascertained by TLC and elemental analysis.

- The new chemical entities so synthesized were evaluated for their 5-HT_3 receptor antagonistic activites in isolated guinea-pig ileum against a reference 5-HT_3 agonist, 2-Me-5-HT.

    - In series I, the compound **QC-7** showed favourable 5-HT_3 antagonistic acitivity with a pA_2 value of 6.4.

    - In series II, the compounds **IP-2** and **IP-3** exhibited favourable antagonism with pA_2 values of 6.7 and 6.4, respectively.

    - In series III, the compounds **NA-2**, **NA-3** and **NA-4** showed most potent 5-HT_3 receptor antagonistic acitivities with pA_2 values of 7.4, 7.1 and 8.2, respectively. On the other hand, the standard 5-HT_3 receptor antagonist, ondansetron showed a pA_2 value of 6.9.

- The most active compound in the isolated guinea-pig ileum, **NA-4**, was selected for anxiolytic studies in mice. In all the three animal models (elevated plus-maze, light / dark test and hole board test), standard 5-HT_3 receptor antagonist, ondansetron, and the test compound, **NA-4**, showed significant anxiolytic activities at higher dose (i.e., 10 mg/kg, *i.p.*) but failed to show significant activities at lower doses (0.1 & 1.0 mg/kg, *i.p.*). Ondansetron and the test molecule, **NA-4**, exhibited equipotent anxiolytic activity in all the three animal models of anxiety.

Future Scope of the Work

- Different substituents in the 1,8-naphthyridine ring and its effect on the 5-HT_3 receptors may be studied.
- *In-vivo* studies on higher animal models to correlate the activity with the *in-vitro* method may be performed.
- Radio-ligand binding assays to establish the selectivity towards 5-HT_3 receptors may be carried out.
- Sub-acute and acute toxicological screening of these compounds has to be carried out.
- Extensive pharmacodynamic and pharmacokinetic studies of the safer compounds (from toxicological screening) have to be undertaken in various animal models.
- Further, the feasibility, cost effectiveness and reproducibility of synthesizing these compounds in bulk have to be tried, so as to convert these compounds into clinically useful agents.

Chapter VIII
References

CHAPTER VIII

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Appendix

List of Publications

1. Mahesh, R., **Venkatesha Perumal, R.**, Vijaya Pandi, P., Cancer Chemotherapy-Induced Nausea and Vomiting: Role of Mediators, Development of Drugs and Treatment Methods, ***Pharmazie***, 2005, 60(2), 83-96.

2. Mahesh, R., **Venkatesha Perumal, R.**, Vijaya Pandi, P., Microwave Assisted Synthesis of 2-(4-substituted piperazin-1-yl)-1,8-naphthyridine-3-carbonitrile as a New Class of Serotonin 5-HT_3 Receptor Antagonists, ***Bioorg. Med. Chem. Lett.***, 2004, 14(20), 5179-5181.

3. Mahesh, R., **Venkatesha Perumal, R.**, Vijaya Pandi, P., Pharmacophore Based Synthesis of 3-chloroquinoxaline-2-carboxamides as Serotonin$_3$ (5-HT_3) Receptor Antagonist, ***Biol. Pharm. Bull.***, 2004, 27(9), 1403-1405.

4. Mahesh, R., **Venkatesha Perumal, R.**, *Microwave-Induced Mannich* Reaction – Synthesis of Some Mannich Derivatives of *p*-Aminophenol, ***Indian J. Chem.***, 2004, 43B, 1012-1014.

5. Mahesh, R., **Venkatesha Perumal, R.**, Vijaya Pandi, P., Microwave–Assisted Solvent–Free Synthesis of 3-[(4-substituted piperazin-1-yl)alkyl]imidazo[2,1-*b*][1,3]benzothiazol-2(3*H*)-ones as Serotonin$_3$ (5-HT_3) Receptor Antagonists, ***Pharmazie***, 2005, (In Press).

(Continued)

Papers in Conference / Symposium

1. Mahesh, R., **Venkatesha Perumal, R.**, Vijaya Pandi, P., Microwave assisted synthesis of piperazinyl-1,8-naphthyridine derivates as a new class of serotonin 5-HT_3 receptor antagonists for the treatment of cancer chemotherapy-induced nausea and vomiting, 56th Indian Pharmaceutical Congress, Kolkata, 3-5 December 2004, p. 29.

2. Mahesh, R., **Venkatesha Perumal, R.**, Vijaya Pandi, P., Microwave assisted synthesis of quinoxaline carboxamides as 5-hydroxytryptamine$_3$ (5-HT_3) antagonists for the treatment of cancer chemotherapy induced nausea and vomiting, 55th Indian Pharmaceutical Congress, Chennai, 19–21 December 2003, p. 41.

3. Mahesh, R., **Venkatesha Perumal, R.**, Atom economy – A Novel approach in synthetic/medicinal chemistry, 55th Indian Pharmaceutical Congress, Chennai, India, 19–21 December 2003, p.121

4. **Venkatesha Perumal, R.,** Mahesh, R., Design and Synthesis of Some Novel 5-Hydroxytrptamine$_3$ (5-HT_3) Antagonist, International Symposium on Emerging Trends in Genomics and Proteomics, BITS, Pilani, 12 & 13 January 2003, p. 29.

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Dr. R. Mahesh is presently working as an Associate Professor & Group Leader (Head) of the Pharmacy Group, Birla Institute of Technology, Pilani. He received his Ph.D. degree in 1997 from Birla Institute of Technology & Science, Pilani. He has been involved in teaching for 16 years and in research for 15 years. His areas of interest are, Medicinal Chemistry and Clinical Pharmacy & Therapeutics. Dr. R. Mahesh has published various research articles and review articles in peer reviewed international/national journals. He has successfully completed a major research project funded by University Grants Commission (UGC) New Delhi. He has got a best paper award at 55th Indian Pharmaceutical Congress, Chennai, held on 19-21 December 2003. He is a life member of Association of Pharmacy Teachers of India (APTI).

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Mr. Venkatesha Perumal R., completed his B. Pharmacy in 1997 and M. Pharmacy (Pharmaceutical Chemistry) in 1999 from Tamil Nadu Dr. M.G.R. Medical University, Chennai. He had been working as a Lecturer in S. B. College of Pharmacy, Sivakasi, Tamil Nadu for about 2 years. His areas of interest are, Synthetic Medicinal Chemistry and Drug Design. Mr. Venkatesha Perumal R. has been a Research Fellow working for his Ph.D., at BITS, Pilani from 2001-2005 during which he was a recipient of the Senior Research Fellowship (SRF) award, granted by the Council of Scientific and Industrial Research (CSIR), New Delhi. He has published research articles in well renowned international/national journals and presented paper in various conferences/symposiums.