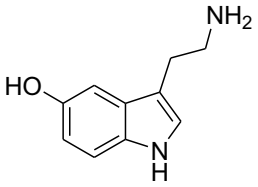
SECTION-II

CHAPTER 1.
INTRODUCTION

1. INTRODUCTION

Serotonin, 5-hydroxytryptamine (5-HT), is one of the members of monoamine neurontransmitters, all of which have a chemical template comprising of a basic amino group separated from an aromatic nucleus by a two carbon aliphatic chain.



Serotonin (5-HT)

In mammals, 5-HT is biosynthetically derived from two enzymatic steps:

1. Ring hydroxylation of the essential amino acid tryptophan by tryptophan hydroxylase, the rate-limiting step[1] followed by
2. Side chain decarboxylation by aromatic amino acid decarboxylase.

A second isoform of tryptophan hydroxylase was identified in 2003 by Walther et. al.[2,3] The original enzyme which is expressed in the gut, is now called *tph1*, and the isoform that is expressed exclusively within the brain is named *tph2*.[4,5] In the brain, serotonin is produced within axon terminals, where it is released in response to an action potential and then diffuses across the synapse to activate postsynaptic receptors. The serotonin receptor family is larger than any other family of G-protein coupled (GPCR) neurotransmitter receptors- 13 distinct genes are encoding for these G-protein coupled seven-transmembrane class of receptors. In addition, there is one ligand-gated ion channel, the 5-HT_3 receptor.

Serotonin mediates a wide range of physiological functions by interacting with multiple receptors, and these receptors have been implicated in certain pathological and psychopathological conditions. In the past 16 years, seven distinct families of 5-

HT receptors have been identified (5-HT_1–5-HT_7), and subpopulations have been described for several of these. At least 15 subpopulations have now been cloned. Among them 5-HT_{2C} receptor is an important class and mediates several neurological and metabolic responses. An account of therapeutic potential of 5-HT_{2C}-receptor modulators is described here.

1.1. Therapeutic potentials of 5-HT_{2C} receptor modulators

The 5-HT_{2C} receptor was one of the first cloned[6] serotonin receptors and was initially named 5-HT_{1C} receptor. After additional serotonin receptors were identified and found to belong to distinct families based upon G-protein coupling and sequence homology, this receptor was reclassified as the 5-HT_{2C} receptor. It shares significant identity with other members of the family, the 5-HT_{2A} and 5-HT_{2B} receptors, and because of that, there are few isoform-selective antagonists and very few selective agonists. This receptor is highly expressed and was first identified in the choroid plexus, where it may be regulating ion exchange between the brain and the cerebrospinal fluid. 5-HT_{2C} receptor mRNA and protein are also found widely distributed throughout the brain, including the cortex, amygdala, basal ganglia, hippocampus, and thalamus.[7] 5-HT_{2C} receptors significantly regulate mood, anxiety, feeding, and reproductive behavior. 5-HT_{2C} receptors regulate dopamine release in the striatum, prefrontal cortex, nucleus accumbens, hippocampus, hypothalamus and amygdala, among others. The therapeutic potentials of 5-HT_{2C} receptor have been established on the basis of number of observations received. Novel chemical agents may offer good therapeutic advantages in different diseases by interacting with 5-HT_{2C} receptor. Some major diseases in which 5-HT_{2C} receptor modulators play an

important role are discussed here. Agonistic effect of 5-HT_{2C} receptors is important for the enhancement of serotonin levels in brain and hence is an important outcome to treat several related disorders. Only 5-HT_{2C} agonist structures and related disorders are discussed herein.

1.1.1. Obesity

Obesity continues to be a burgeoning health problem world-wide and can lead to the development of many disease conditions, including type 2 diabetes, hypertension, stroke, ischemia, osteoarthritis and various forms of cancer including that of the kidney and colon.[8] Several therapeutic strategies have been adopted to tap the market and it is well established that 5-HT neurotransmission in the brain regulates feeding habits by inducing hypophagia. Before their removal from the market, [±]-fenfluramine and the more active enantiomer dexfenfluramine were considered to be the most effective ones among the weight loss agents at that time. Dexfenfluramine was the first anti-obesity drug to be approved for a duration of usage in excess of 3 months in the INDEX study.[9] Norfenfluramine, an active metabolite of fenfluramine, is a potent full 5-HT_{2C} receptor agonist and has similar efficacy as fenfluramine and the results suggest that much of fenfluramine's efficacy results from 5-HT_{2C} receptor activation by norfenfluramine. However, fenfluramine and dexfenfluramine were withdrawn from the market following heart valve abnormalities and psychotrophic effects, possibly related to the non-selectivity with the central 5-HT_{2A} and 5-HT_{2B} receptors.

Recent preclinical data suggested that selective 5-HT_{2C} receptor agonists might be implicated in the regulation of feeding. In 1995, Tecott et. al.[10] demonstrated that

5-HT_{2C} knockout mice are overweight and resistant to the hypophagic effect of meta-chlorophenylpiperazine (*m*-CPP). *m*-CPP is a non-selective 5-HT_{2C} receptor agonist and it reduces feeding both acutely and chronically, and thus produces chronic reduction in body weight. These results implied that a highly selective 5-HT_{2C} agonist with a similar efficacy as fenfluramine in humans, without the side effects related to 5-HT_{2A} or 5-HT_{2B}, could be an attractive target for the discovery of novel treatments for feeding disorders.

1.1.2. Anxiety

5-HT_{2C} receptor has been implicated in mood and anxiety disorders and is a target for development of novel anxiolytic drugs.[11] In several anxiety animal models, 5-HT_{2C} receptor antagonists reduce anxiety-like behaviour at least in an acute fashion.[12,13] For example, SB-242084, a potent and selective 5-HT_{2C} receptor antagonist is anxiolytic in the social interaction test and the Geller-Seifter conflict test of anxiety.[14] Like *m*-CPP, 6-chloro-2-(1-piperazinyl)pyrazine (MK-212), a non-selective 5-HT_{2C} agonist, exerts its anxiogenic actions via activation of 5-HT_{2C} receptor.[15,16] In addition, SB-242084 blocks the anxiogenic effects of *m*-CPP in rats. Activation of 5-HT_{2C} receptors contributes to the anxiogenic effects following acute treatment with SSRIs, which are common antidepressants.[17,18] Taken together, these studies suggest that 5-HT_{2C} receptor activation is an important component of anxiogenesis. A report suggests that 5-HT_{2C} antagonists may be effective anxiolytic agents devoid of many usual side effects associated with benzodiazepines (e.g., tolerance and dependence) and SSRIs (e.g., insomnia, agitation and sexual dysfunction).[19]

1.1.3. Depression

The important role of the serotonergic neurotransmitter system is well established in the pathology of depression. 5-HT_{2C} receptor density and responsiveness are correlated in experimental models of depression and in humans.[20,21] Therefore, the possible role of 5-HT_{2C} receptor warrants consideration in the development of novel antidepressant therapies. Major depressive disorder (MDD) is a significant health problem. Increased mortality and suicidal tendency add to the burden of the MDD. Bakish et. al. reported that selective 5-HT_{2C} receptor antagonists appear to have antidepressant-like activity in some animal models.[22] Eser et. al. reported that antagonism of 5-HT_{2C} receptor by agomelatine results in an increase of dopamine and norepinephrine activity in the frontal cortex.[23]

1.1.4. Schizophrenia

Although a lot of attention has been focussed on 5-HT_{2A} receptor in schizophrenia, there is a growing evidence to suggest that 5-HT_{2C} receptor is also relevant in the disease.[24] There is a close structural relationship between the 5-HT_{2A} and 5-HT_{2C} receptors. Schizophrenia is a chronic mental illness prevalent in nearly 1% of the population. Schizophrenia-treating drugs generally show antagonism of dopaminergic D_2 receptors in the mesolimbic and mesocortical regions of the brain. However, unfortunately, D_2 antagonism in the caudate nucleus and other basal ganglia nuclei produces neurological side effects such as extrapyramidal side effect.[25] Some researchers suggest that 5-HT_{2C} receptor activation inhibits dopaminergic neuronal activity and dopamine release. The opposite effects occur with 5-HT_{2C} antagonists, such as increasing dopaminergic neurotransmission[26] and thus reducing risk of

extrapyramidal side effect. Activation via agonists such as *m*-CPP and MK-212, the non-selective 5-HT_2 agonists, results in feelings of anxiety and panic in humans.[27] In preclinical studies, 5-HT_{2C} receptor antagonists show evidence for antipsychotic efficacy.[28] These findings implicate 5-HT_{2C} receptors in the regulation of neuronal network excitability.

1.1.5. Neurodegenerative disorders

Recently, 5-HT_{2C} agonists were also proposed as potential drugs for anti-Alzheimer's disease. *In vivo* results reported by Wurtman et. al. suggested that dexnorfenfluramine or *m*-CPP with 5-HT_{2C} agonism increases amyloid precursor protein (APP) formation and decreases $A\beta_{1-42}$ in guinea-pig models. In addition, apparently 5-HT_{2C} agonists are localized to the brain, 5-HT_{2C} agonists may not produce peripheral side effects[29] unlike other anti-Alzheimer's disease drugs.

1.1.6. Other clinical indications

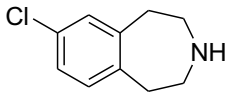
Recent studies showed that central 5-HT_{2C} receptor increases urethral muscle tone and inhibits micturition reflexes, with control of parasympathetic outflow to the bladder and somatic out-flow to the external urethral sphincter in the rat. In animal study, 5-HT_{2C} agonists such as MK-212 and *m*-CPP have potent effects for urinary incontinence.[30-32] A compound (4-benzyl-2-methyl-7,8,9,10-tetrahydro-*6H*-1,3,3a,5,8-pentaazacyclohepta[*e*]indene) from Brennan et. al. at Pfizer was shown to be a potent, selective, metabolically stable *in vitro* and efficacious in *in vivo* model for stress-caused urinary incontinence.[33] 5-HT_{2C} receptor is also involved in hot flushes and bouts of perspiration in women. [34] There are also available some experimental results

for a role of 5-HT_{2C} receptor in premature ejaculation. There is a report stating that administration of (±)-2,5-dimthyoxy-4-isoamphetamine hydrochloride (DOI), a 5-$HT_{2A/2C}$ agonist, suppressed premature ejaculation in animal models and the condition was restored with pre-treatment with a 5-HT_{2C} antagonist.[35]

5-HT_{2C} receptor has been established as an important target for development of new chemical ligands to pretence associated disorders. There are different chemical scaffolds which have been reported as potent agonists of 5-HT_{2C} receptor activities. Among them 3-benzazepine derivatives are of much importance. Number of patents has been filed for these derivatives by different pharmaceutical organizations. Recently lorcaserin, the first 5-HT_{2C} receptor agonist based on 3-benzazepine scaffold has been approved by US-FDA for the treatment of obesity. Accounts of 3-benzazepine derivatives, which are claimed to be selective 5-HT_{2C} agonists, are described in next section of this review.

1.2. Benzazepine based 5-HT_{2C} receptor agonists

The first 3-benzazepine structure based compound, claimed for its 5-HT_{2C} agonist activity was reported in a patent document by Ciba-Geigy in the 1970s.[36] The compound, a 7-chloro substituted benzazepine (**1**) was claimed for its anorectic activity to be useful in the treatment of weight management. The compound was



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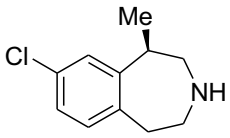
shown to decrease food intake in rats after oral administration. However, no accounts regarding the mechanism of its *in vivo* efficacy were disclosed.

In the year 2003, Arena Pharmaceuticals published a PCT patent regarding a benzazepine derivative lorcaserin (**2**) as a potent 5-HT_{2C} receptor agonist.[37] In Arena's related papers, Smith et. al.[38,39] reported the methodology for designing and synthesis of 3-benzazepine scaffold. They have incorporated the basic structure of phenethylamine and the structure of nordexfenfluramine to construct a constrained ring system. Using structural features from known 5-HT_{2C} agonists and incorporating these into a rigid framework, a series of 3-benzazepines (e. g. **3**) was designed. These compounds were amongst those with the greatest potency at 5-HT_{2C} receptors. Lorcaserin (**2**) was identified as one of the multiple compounds that were potent and selective *in vitro* and were also potent in an acute *in vivo* rat food intake model on oral



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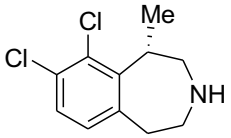

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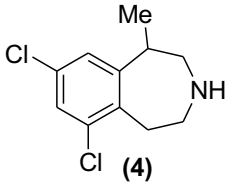

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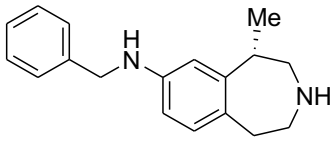
administration. Later on in subsequent years Arena has filed multiple patent applications for compounds based on benzazepine structural scaffold. As Arena Pharmaceutical's related applications, Arena's benzazepine derivatives also appear to be useful for the treatment of 5-HT_{2C} receptor-associated diseases.[40] For example, benzazepine (**4**) showed good *in vitro* activity in inositol phosphate (IP) accumulation assay. Arena also published benzazepine derivatives of structure (**5**) for prophylaxis



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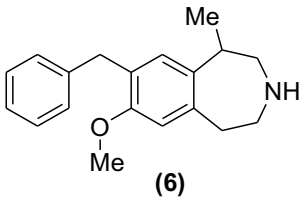

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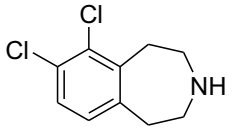
or treatment of 5-HT_{2C} receptor associated diseases.[41] Simultaneously, Arena published another benzazepine derivative (**6**) for 5-HT_{2C} agonist activity and selectivity over 5-HT_{2A} and 5-HT_{2B}.[42]

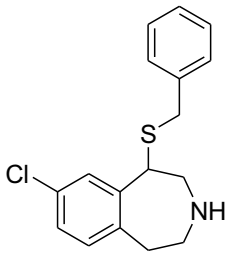
Yamanouchi et. al.[43] have filed a patent application claiming a series of benzazepine derivatives in the year 2002. A representative compound (**7**) exhibited a



(7)

good affinity and selectivity for 5-HT_{2C} receptors over 5-HT_{2A} and 5-HT_{2B} receptors. This compound (**7**) was also found to be active in rat penile erection model, which was a reflection of 5-HT_{2C} receptor activation *in vivo*.

In the year 2005, Eli Lilly filed a patent application for benzazepine structure based compound (**8**). This compound was a chemical modification of previously published compound (**1**), which was the first 3-benzazepine based patented structure.[44] Compound (**8**) was derived by introduction of a 6-benzylthio substituent in compound (**6**) and was claimed to be more selective for 5-HT_{2C} agonist activity. Eli Lilly have filed several other patent applications for 5-HT_{2C} activity of benzazepine derivatives.



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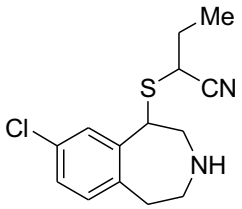

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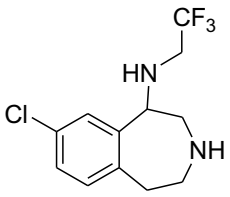
As claimed by Eli Lilly, compound (**8**) also blocked food intake in rat. In a 14-day rat study, **8** caused a 20% reduction in weight, due to fat loss. Replacement of S-benzyl

by a cyanopropyl group (**9**) gave a lead compound possessing decent binding affinity for 5-HT_{2C} with full efficacy and a good selectivity over 5-HT_{2A} and 5-HT_{2B}.[45] In the same year, Lilly disclosed related 6-(2,2,2-trifluoroethylamino)-7-chloro-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (**10**) as a 5-HT_{2C} receptor agonist.[46] Later on, Lilly



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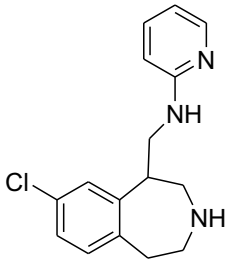

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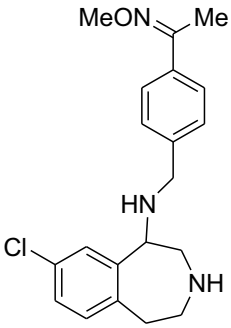

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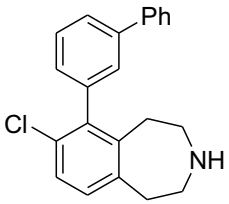

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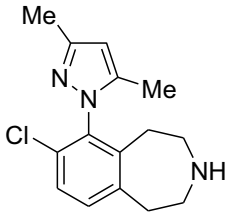

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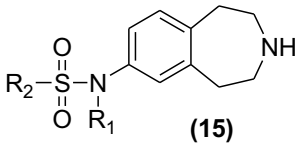
filed a series of different benzazepine analogs in patent applications exemplified by structures (**11-14**). However, no specific pharmacological data was provided for the publications.[47-50]

Fish and group[51] from Pfizer laboratories have reported 7-sulfonamido-3-benza-



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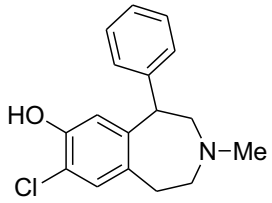
-zepine derivatives (**15**) as 5-HT_{2C} agonists. Different substituents at R_1 and/or R_2 positions gave compounds that were potent 5-HT_{2C} agonists with minimal activation

of the 5-HT_{2A} and 5-HT_{2B} receptors. Representative compounds also demonstrated excellent *in vitro* ADME properties and good selectivity over ion channel activity.

SCH-23390 (**16**) a benzazepine-based well-known dopamine D_1 receptor antagonist has also demonstrated a highly potent agonistic activity for human 5-HT_{2C}



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receptor.[52] However, another study indicates considerably lower efficacy for SCH-23390 at human 5-HT_{2C} receptor.[53]