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Serotonin 5-HT₇ receptor agents: structure-activity relationships and potential therapeutic applications in central nervous system disorders

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Abstract

Since its discovery in the 1940s in serum, the mammalian intestinal mucosa, and in the central nervous system, serotonin (5-HT) has been shown to be involved in virtually all cognitive and behavioral human functions, and alterations in its neurochemistry have been implicated in the etiology of a plethora of neuropsychiatric disorders. The cloning of 5-HT receptor subtypes has been of importance in enabling them to be classified as specific protein molecules encoded by specific genes. The 5-HT₇ receptor is the most recently classified member of the serotonin receptor family. Since its identification, it has been the subject of intense research efforts driven by its presence in functionally relevant regions of the brain. The availability of some selective antagonists and agonists, in combination with genetically modified mice lacking the 5-HT₇ receptor, has allowed for a better understanding of the pathophysiological role of this receptor. This paper reviews data on localization and pharmacological properties of the 5-HT₇ receptor, and summarizes the results of structure-activity relationship studies aimed at the discovery of selective 5-HT₇ receptor ligands. Additionally, an overview of the potential therapeutic applications of 5-HT₇ receptor agonists and antagonists in central nervous system disorders is presented.

Keywords

Serotonin 5-HT₇ receptor; G-protein-coupled receptors; Structure-Activity relationships; depression; anxiety; circadian rhythm

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is a major neurotransmitter involved in a large number of processes in the central nervous system, including the regulation of feeding behavior, aggression, mood, perception, pain, and anxiety (Bradley et al., 1986). In the

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periphery, 5-HT is involved in the regulation of the autonomic nervous system, cardiovascular function, vascular and nonvascular smooth muscle contraction, platelet aggregation, uterine smooth muscle growth, and gastrointestinal functioning (Hoyer et al., 1994; Ramage, 2006; Ramage and Villalón, 2008). To mediate these functions, a family of receptors, divided into seven main families comprising at least fourteen different receptors, has evolved (Hoyer et al., 1994). The 5-HT₇ receptor was the last addition to the large serotonin subfamily of G-protein coupled receptors. Considering the number of papers published in the serotonin receptor subtypes field, the 5-HT₇ receptor is not the most studied, being surpassed by 5-HT_{1A}, 5-HT_{2A}, 5-HT₃, and 5-HT₄ receptors. This might be explained by the lack for a long time period of selective 5-HT₇ agents that would help researchers to shed light on the pathophysiological role of this receptor. Thus, since antagonists and, to some extent, agonists became available as well as 5-HT₇ receptor-knockout mice, our understanding of the role of the 5-HT₇ receptor in normal and pathological processes has improved.

After a general introduction to the 5-HT₇ receptor (molecular structure, functional expression, distribution), this review will illustrate the medicinal chemistry studies aimed to the identification of selective 5-HT₇ ligands. The structure-activity relationships for each class of compounds will be delineated and the pharmacophore models proposed for agonists and antagonists will be also discussed. A second part of this review will give details on studies that used the identified compounds as well as other techniques to gain insight into the involvement of the 5-HT₇ receptor in normal and pathological processes of the central nervous system

1.1. Characteristics of the 5-HT₇ receptor

1.1.1. Structure—The serotonin (5-HT) 5-HT₇ receptor belongs to the large family of G protein-coupled receptors (GPCR). In contrast to, for example, the 5-HT_{1A} or 5-HT_{2A} receptors that were identified as distinct receptors for 5-HT on the basis of pharmacological and physiological differences, the 5-HT₇ receptor was discovered by targeted analysis of cDNA libraries based on conserved sequences of known receptors. The primary amino acid sequence of the 5-HT₇ receptor of human, rat, mouse, guinea pig, and pig has been elucidated. Comparison of the amino acid sequence of the transmembrane regions of 5-HT₇ receptor from various species indicated the highest homology with the 5-HT_{dro1} receptor. Lower homologies in the transmembrane domains were found with other 5-HT receptors (Table 1).

The 5-HT₇ receptor has been cloned from pig by Bhalla and coworkers (2002). The nucleotide sequence of porcine 5-HT₇ receptor cDNA encoded a protein of 447 amino acids, which showed high homology (92–96%) with the sequence of the 5-HT₇ receptor from the other species.

More recently the 5-HT₇ receptor has been described in several additional species, including the nematode *Caenorhabditis elegans* (Hobson et al., 2003), the yellow fever mosquito *Aedes aegypti* (Lee and Pietrantonio, 2003), the honey bee *Apis mellifera* (Schlenstedt et al., 2006) and the fresh water snail *Helisoma trivolvis* (Mapara et al., 2008).

1.1.2. Splice Variants—Three splice variants of the 5-HT₇ receptor have been identified in humans (designated 5-HT_{7a}, 5-HT_{7b}, and 5-HT_{7d}), which encode proteins that differ in their carboxy terminals (Heidmann et al., 1997). The human 5-HT_{7a} is the full length receptor (445 amino acids), while the human 5-HT_{7b} is truncated at amino acid 432 due to an alternative splice donor site. The human 5-HT_{7d} is a distinct isoform of the receptor: the retention of an exon cassette in the region encoding the carboxyl terminal results a 479-amino acid receptor with a C-terminus markedly different from the human 5-HT_{7a}. A 5-

HT_{7c} splice variant is detectable in rat tissue but is not expressed in humans. Conversely, rats do not express a splice variant homologous to the human 5-HT_{7d}, as the rat 5-HT₇ gene lacks the exon necessary to encode this isoform. Drug binding affinities are similar across the three human splice variants (Krobert et al., 2001). All the three human splice variants constitutively activate adenylyl cyclase but inverse agonist efficacies appear to differ between the splice variants (Krobert and Levy, 2002). Additionally, there appears to be a difference in agonist mediated internalization between the 5-HT_{7d} variant and the 5-HT_{7a} and 5-HT_{7b} variants (Guthrie et al., 2005). All three splice variants have been associated with a protein known as PLAC-24 that might regulate transportation and stabilization of newly synthesized receptor protein (De Martelaere et al., 2007). Also the porcine 5-HT₇ receptor gene contains two introns, but the possibility of alternative splicing has not been investigated (Bhalla et al., 2002).

In humans a pseudogene for the 5-HT₇ receptor has been identified (Lassig et al., 1999; Olsen and Schechter, 1999). It is transcribed, but an in-frame stop codon is present in the fifth trans-membrane region (Olsen and Schechter, 1999). The pseudogene has also been described in rhesus monkeys, but is not found in rats or mice (Olsen and Schechter 1999).

Screening of patients with psychiatric disorders has revealed three base pair exchanges in the 5-HT₇ receptor gene (Erdmann et al., 1996). One of them is silent, but the other two lead to amino acid exchanges. The mutations were equally prevalent (1%) in both the patient and control groups suggesting that they were not relevant for pathogenesis. Nevertheless, the Thr92Lys and Pro279Leu variants are of pharmacological interest. It has been demonstrated that the Thr92Lys exchange leads to decreased agonist, but not antagonist, binding (Brüss et al., 2005). The Pro279Leu variant has been associated with a virtually abolished signal transduction, most likely due to an altered coupling between the third intracellular loop and the G protein (Kiel et al., 2003).

1.1.3. Signaling—5-HT₇ receptors are coupled to stimulation of adenylyl cyclase. The EC₅₀ values for the reference agonists 5-CT, 5-HT, and 8-OH-DPAT are listed in Table 2. As can be noted, the order of agonist potency in assays of adenylyl cyclase activity varies between species. Also, 8-OH-DPAT behaves as full or partial agonist depending on species.

As mentioned above, 5-HT_{7a}, 5-HT_{7b}, and 5-HT_{7c} splice variants constitutively activate adenylyl cyclase. Therefore, 5-HT₇ receptor antagonists display either full or partial inverse agonist activity. In an early study, Thomas et al. (1998) reported potencies of various compounds in antagonizing 5-CT-induced stimulation of adenylyl cyclase activity in comparison with their inverse agonist behavior on 5-HT_{7a} (Table 3). A few years later, Krobert and Levy (2002) published a detailed report of the inverse agonist properties of several antagonists on all three human 5-HT₇ receptor splice variants (Table 4). No differences in inverse agonist potencies (pIC₅₀) were observed between the splice variants. The rank order of potencies was in agreement and highly correlated with antagonist potencies (pK_B) determined by antagonism of 5-HT-stimulated adenylyl cyclase activity (methiothepin > metergoline > mesulergine ≥ clozapine ≥ spiperone ≥ ritanserin > methysergide > ketanserin). It has been demonstrated that the constitutive activity of the 5-HT₇ receptor is regulated by palmitoylation of its C-terminal domain (Kvachnina et al., 2009). Interestingly, it has been found that the inverse agonist properties of 5-HT₇ receptor ligands can lead to heterologous desensitization of other G protein-coupled receptor families (Krobert et al., 2006). In addition to being coupled to G_s, the 5-HT₇ receptor has also been shown to be coupled to the G₁₂-protein which activates small GTPases of the Rho family in turn leading to enhanced neurite outgrowth (Kvachnina et al., 2005). In cell lines, it has been demonstrated that the 5-HT₇ receptor not only activates the adenylyl cyclase AC5 normally linked to G_s, but also cyclases AC1 and AC8 that are activated by intracellular calcium

(Baker et al., 1998). Coexpression of the 5-HT₇ receptor, AC1, and AC8 is present in the hippocampus, and might thus be important for hippocampal function, which is discussed further below. At least when expressed in HEK-293 cells, the receptor is tightly associated with the G protein, regardless of agonist binding (Bruheim et al., 2003). This suggests the existence of a complex between inactive receptor and the G-protein. The 5-HT₇ receptor has been found to activate the extracellular signal-related kinase (ERK) (Errico et al., 2001) through a mechanism dependent on a Ras G protein (Norum et al., 2003). This activation of ERK might be also of importance for hippocampal function and depression. Although cAMP activation is normally believed to be mediated through protein kinase A (PKA), it has been demonstrated that the 5-HT₇ receptor can stimulate ERK through a PKA-independent pathway, possibly by using the cAMP-guanine nucleotide exchange factor Epac (Lin et al., 2003).

1.1.4. Pharmacological properties—Several compounds known to be ligands for other 5-HT receptor subtypes were found to also have high affinity for recombinant 5-HT₇ receptors from various species (human, rat, mouse, guinea-pig), as shown in Table 5. All four species homologues of the 5-HT₇ receptor have high affinity for 5-CT (pK_i 9.0–9.4) and comparable affinities for the endogenous ligand 5-HT (pK_i 8.1–9.0). One of the most important revelations was that 8-OH-DPAT had relatively high affinity (Table 5) since it prior to 1993 was considered a selective agonist for the 5-HT_{1A} receptor. When comparing pK_i values obtained from human cloned receptor with those from rat cloned receptor some remarkable differences in affinity can be noted. In most cases, pK_i values from rat cloned receptor are higher than those from human cloned receptors. Although these differences are not larger than 1 log unit, as in the case of methiothepin, they point to possible discrepancies between binding data obtained from human and rat cloned receptors. In competition assays [³H]5-HT, [³H]5-CT, and [³H]LSD have been used as radioligands to determine binding affinities as shown in Table 6.

1.2. Expression and distribution of the 5-HT₇ receptor

1.2.1. Messenger RNA—The distribution of mRNA encoding the 5-HT₇ receptor protein has been studied by several groups using a variety of techniques. The results of these studies are summarized in Tables 6 and 8. In all species studies, 5-HT₇ receptor mRNA has been identified in the central nervous system. Particularly high levels have been detected in the hypothalamus (notably within the suprachiasmatic nucleus), thalamus and hippocampus. In peripheral tissues, 5-HT₇ receptor mRNA has been described in the ileum, spleen, endocrine glands, and arteries. In blood vessels and the gastrointestinal tract the expression has generally been localized to smooth muscle cells. Some discrepancies have been noted, as in the study by Plassat et al., (1993) where Northern blot analysis was unable to detect 5-HT₇ mRNA in mouse brain, whereas RT-PCR demonstrated high levels in the brainstem. Thus, differences observed, both between species and even within the same species, may depend on the sensitivity of the technique used. Also, low detection of 5-HT₇ receptor mRNA may arise when it is restricted to a small population of cells of a given organ. The distribution of the mRNA encoding the various isoforms of the receptor in the rat has been determined by using either RT-PCR or *in situ* hybridization (Heidmann et al., 1998). The authors ruled out large tissue specific differences in splicing of the 5-HT₇ mRNA.

1.2.2. Protein

1.2.2.1. Radioligand binding studies: An early study attempted to define 5-HT₇ receptor binding sites in rat hypothalamic membranes by labeling with [³H]5-HT in the presence of 100 nM pindolol. Although the level of specific binding detected was low (it represented approximately 70% of the total binding), the pharmacology of the identified binding sites correlated well with that of rat recombinant 5-HT₇ receptors (Sleight et al., 1995). These

results were challenged by a subsequent study (Gobbi et al., 1996) which found that 100 nM pindolol does not completely mask 5-HT_{1A} and 5-HT_{1B} receptors and that the population of pindolol-insensitive [³H]5-HT receptors in rat hypothalamus appeared to be heterogeneous. Also [³H]5-CT failed to define a homogeneous population of 5-HT binding sites in rat hypothalamus homogenates despite the use of a 'cocktail' of drugs ((±)-pindolol, sumatriptan, DOI) (Stowe & Barnes 1998).

To et al. (1995) reported that a single population of receptors isolated from guinea pig cerebral cortex membranes could be labeled by [³H]5-CT in the presence of cyanopindolol and sumatriptan as masking agents. The pharmacology of these binding sites was well correlated to that of the cloned guinea pig 5-HT₇ receptor. Because affinities were consistently lower in the tissue binding assay, the authors hypothesized that cyanopindolol and sumatriptan might occupy at least in part the 5-HT₇ receptors.

[³H]Mesulergine has been used to label 5-HT₇ sites in guinea-pig ileal longitudinal muscle. Also in this case, due to the lack of selectivity of mesulergine over a range of receptors, several masking drugs (cinanserin, RS 102221, raclopride, prazosin, and yohimbine) were used. It was also noted that no binding could be detected in the rat jejunum under the same experimental conditions (Hemedah et al., 1999).

1.2.2.2. Autoradiographic studies: Initial attempts to visualize the distribution of 5-HT₇ receptor binding sites used [³H]5-CT in the presence of various masking agents (To et al., 1995; Mengod et al., 1996; Waeber & Moskowitz, 1995; Gustafson et al., 1996). In guinea pig and rat brain, the distribution of the 5-HT₇ sites was found to be largely consistent with that reported for 5-HT₇ receptor mRNA. The highest densities were in the medial thalamic nuclei and related limbic and cortical regions. However, as densities were very low, the authors concluded that the masking compounds added in these autoradiographic experiments might have sufficient 5-HT₇ affinity to occupy part of the 5-HT₇ binding sites at the concentration used, thus preventing full visualization of the receptor.

In an effort to circumvent these limitations Bonaventure et al. (2002) performed an autoradiographic study using 5-HT_{1A} receptor knockout and 5-HT_{1A/B} receptor double knockout mice. They found that, despite its high affinity for the 5-HT₇ receptor in tissue homogenates, [³H]5-CT was not a good tracer for measuring 5-HT₇ receptor binding sites autoradiographically. Instead they concluded that the lower affinity ligand [³H]8-OH-DPAT was much more suited for autoradiographic studies of 5-HT₇ receptor binding sites. The anatomical distribution of the [³H]8-OH-DPAT binding sites observed in knockout mice matched the distribution of 5-HT₇ receptor mRNA and 5-HT₇ receptor immunoreactivity reported in the literature. Within the hippocampal formation, strong labeling was found in the CA3 region, whereas the densities in CA1 were low. 5-HT₇ receptor binding sites were also found within the dorsal raphe. High densities of 5-HT₇ receptor binding sites were observed throughout the hypothalamus (including the suprachiasmatic nucleus).

By using [³H]8-OH-DPAT in combination with the antagonists pindolol or ritanserin at concentrations selectively blocking either 5-HT_{1A} or 5-HT₇ receptors respectively, Duncan et al. (1999) were able to demonstrate that both of these receptor subtypes are present in important components of the circadian timing system (intergeniculate leaflet, lateral geniculate nucleus, suprachiasmatic nucleus) and dorsal and median raphe nucleus of the Syrian hamster.

Varnäs et al. (2004) have used the selective and high-affinity radioligand [³H]SB-269970 to visualize the distribution of 5-HT₇ receptors in human whole hemisphere brain sections. The data were in good agreement with the autoradiographic studies by Gustafson et al. (1996)

and To et al. (1995). [^3H]SB-269970 binding was mainly found in the thalamus, hypothalamus and hippocampal formation of the human brain. Some important differences were found compared to results obtained with [^3H]mesulergine (Martin-Cora & Pazos, 2004). The authors found that using the selective radioligand [^3H]SB-269970 very low densities were found in the caudate nucleus and the putamen, contrary to the high levels found in the investigation with the non-selective radioligand [^3H]mesulergine. It was suggested that these differences could be due to different brain levels studied, or to the possibility that the concentration of non-labeled masking compounds used in the [^3H]mesulergine autoradiographic study was not sufficiently high to abolish binding to other receptor subtypes. It should also be noted that SB-269970 has affinity for 5-HT_{5A} receptors (Varnäs et al., 2004).

1.2.2.3. Immunocytochemical studies: Immunocytochemistry has been used to localize the distribution of 5-HT₇ receptors in rat forebrain. 5-HT₇ receptors were detected within the cerebral cortex, hippocampal formation, tenia tecta, thalamus, and hypothalamus. These results were in accordance with the reported localization of the 5-HT₇ receptors mRNA. At a microscopic level, both cell bodies and proximal fibers were strongly stained in the suprachiasmatic nucleus, suggesting a somatodentric subcellular distribution (Neumaier et al., 2001). Similar results were obtained by Belenky and Pickard (2001). By using electron microscopic immunocytochemical procedures, they determined the presence of 5-HT₇ receptors in both pre- and postsynaptic GABA, vasoactive intestinal polypeptide, and vasopressin processes in the suprachiasmatic nucleus in mouse. An immunohistochemical study has demonstrated the presence of 5-HT₇ receptors in Purkinje cells of rat cerebellum (Geurts et al., 2002).

An immunocytochemical study of 5-HT₇ receptor distribution at the lumbar level has been performed by Doly et al. (2005). 5-HT₇ immunolabeling was localized mainly in the two superficial laminae of the dorsal horn and in small and medium-sized dorsal root ganglion cells, which is consistent with a predominant role in nociception. In addition, moderate labeling was found in the lumbar dorsolateral nucleus (Onuf's nucleus), suggesting involvement in the control of pelvic floor muscles (Doly et al., 2005). Electron microscopic examination of the dorsal horn revealed a postsynaptic localization on peptidergic cell bodies in laminae I–III and in numerous dendrites, a presynaptic localization on unmyelinated and thin myelinated peptidergic fibers (Doly et al., 2005).

2. Medicinal chemistry of 5-HT₇ receptor agents

2.1. Nonselective 5-HT₇ receptor agents

As already discussed, immediately after the cloning of the 5-HT₇ receptor, a number of high-affinity nonselective agents were identified (Tables 3–6). From the analysis of their chemical structures (Figure 1) it is apparent that the compounds belong to different classes (tryptamines, ergolines, tricyclic neuroleptics, and other compounds). Because all of these compounds display multi-receptor affinity, none of them has been used as *lead compound* in the discovery of selective 5-HT₇ agents. Thus, no structure-activity relationship studies for those chemical classes have been reported.

This section will focus on studies that have allowed elucidation of structure-activity relationships for the chemical classes of compounds that bind at 5-HT₇ receptors. Comprehensive overviews on patent literature dealing with 5-HT₇ receptor agents can be found elsewhere (Leopoldo 2004, Pittalà et al. 2008, Leopoldo et al. 2010).

2.2. “Long-chain” arylpiperazine derivatives

1-Aryl-4-alkylpiperazines, bearing in the terminal position of the alkyl chain a bulky substituent (the so-called “long-chain” arylpiperazines) are a class of compounds extensively studied for the identification of 5-HT_{1A} ligands. Due to the structural similarity between the 5-HT₇ and 5-HT_{1A} receptors, several research groups have modified “long-chain” arylpiperazine templates, in order to identify selective 5-HT₇ receptor ligands.

Researchers at Meiji Seika Kaisha Ltd. have reported several studies on the development of selective 5-HT₇ antagonists which culminated in the identification of the selective antagonists DR-4004 and DR-4365 (see below). From the screening of a corporate compound library against human cloned 5-HT₇ receptors, Kikuchi et al. (1999) identified 1-aryl piperazine derivatives **1–3** (Table 9). The basic nitrogen-containing substructure was structurally modified because this part of the molecule was responsible for high 5-HT₇ affinity and selectivity over other receptor systems (Kikuchi et al. 2002a). A few simple modifications on the framework of compounds **1–3** gave some key information on structure-activity relationships: optimization of the alkyl chain length indicated that a butyl linker (compound **3**) was preferred; replacement of the phenyl with cyclohexyl removed all affinity (**3** vs **4**); affinities of isomers bearing a OCH₃ group on the phenyl ring (**5–7**) were ranked 3 > 2 > 4, being the 2-OCH₃ substituted compound **5** the most selective over 5-HT_{2A} receptor. On such basis, a variety of 2-substituted derivatives with a butyl spacer were evaluated (compounds **8–14**). The nature of the substituent had significant effect on affinity with pK_i values ranging from 7.13 to 8.82. The most potent compounds were the cyano (**9**) and acetyl (**11**) derivatives, which showed pK_i of 8.42 and 8.10, respectively.

Replacement of the 1-phenylpiperazine group of **3** was accomplished with various basic moieties (Table 10). 4-Phenylpiperidine, 1,2,3,4-tetrahydroisoquinoline, and 4-tetrahydropyridine appeared good substitutes which delivered compounds **16**, **17**, and **18** that showed affinities comparable to **3**. Isosteric replacement on the aromatic ring of tetrahydroisoquinoline **18** was detrimental for 5-HT₇ affinity (compounds **19–22**) (Kikuchi et al., 2002b). It should be noted that the 4-tetrahydropyridine derivative **17** (DR-4004) was the most potent compound identified by this group. The compound **17** behaved as an antagonist because it inhibited 5-HT-induced stimulation of cAMP accumulation. This antagonist was also found to be at least 50-fold selective over serotonergic 5-HT_{1A}, 5-HT₂, 5-HT₄, 5-HT₆ and dopamine D₂ receptors. The structural modification of this class of compounds evolved further toward the identification of a basic moiety alternative to 1-aryl piperazine (Kikuchi et al., 2002a). From the comparison of the affinity values of phenyl and 2,6-diCH₃-phenyl derivatives **3** and **15** (Table 9), the authors hypothesized that the difference in affinity could be due to the conformational difference generated by rotation around the bond between the phenyl ring and the piperazine ring. To rationalize this hypothesis, the energy minimization of 1-phenylpiperazine and 1-(2,6-dimethylphenyl)piperazine was carried out, then lowest energy conformations of the compounds were superimposed. It was found that 1-(2,6-dimethylphenyl)piperazine showed no planar conformation. The authors hypothesized that such a difference could account for the difference in affinity of compounds **3** and **15**. On such basis, the basic moiety of a tetrahydropyridoindole nucleus was tested to replace the arylpiperazine (Table 11). Experimental data confirmed the hypothesis because the phenyl derivative **3** was nearly equipotent to the corresponding tetrahydropyridoindole **23** (pK_i 8.48 vs 8.24). The authors also evaluated various substituents on the nitrogen of the indole ring and found that various substituents were capable of increasing affinity. In particular, compound **30** (DR-4365) had high selectivity over 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, 5-HT₂, 5-HT₃, 5-HT₄, 5-HT₆ receptors and behaved as an antagonist at the 5-HT₇ receptor.

In a series of papers, Perrone and coworkers at Bari University, Italy, have studied the structure-activity relationships of a number of ligands characterized by an 1-arylpiperazine as the basic moiety. The main issue addressed with this framework was selectivity over 5-HT_{1A} receptors. That was indeed achieved in various ligands such as LP-44, LP-12, and LP-211 (see below for structures and details). In the first report, a series of 1-[ω-(4-aryl-1-piperazinyl)alkyl]-1-aryl ketones was disclosed (Perrone et al., 2003). Initially the position of an OH or OCH₃ on the aryl ketone moiety was evaluated together with various intermediate alkyl chains. These modifications led to **31** (R = 2-OH, n = 5) that demonstrated high 5-HT₇ receptor affinity (K_i = 5.8 nM). Modification of **31** on the aromatic ring linked to the piperazine ring gave interesting information (Table 12). Affinity data for compounds **32** and **33** revealed that the absence of an aryl group linked to the piperazine (R = CH₃, cyclohexyl) had a dramatic effect (K_i > 1000 nM), whereas aryl groups other than 2-methoxyphenyl (i.e. 2-Py, Ph, 3-CF₃-Ph, 2-benzoxazolyl, 2-benzimidazolyl) significantly reduced affinity (compounds **34–38**). On the other hand, the 1,2-benzisoxazol-3-yl derivative **40** displayed high 5-HT₇ affinity (K_i = 2.93 nM) and low 5-HT_{1A} affinity (K_i = 189 nM). It was also highlighted that the simple introduction of a methoxy group on the 1,2-benzisoxazolyl moiety (**40** vs **39**) gave a significant loss in 5-HT₇ affinity (K_i = 462 nM), revealing how sensitive to structural manipulation that part of the molecule was. On the other hand, methylation of the hydroxy group of **40** gave derivative **41** which showed subnanomolar 5-HT₇ affinity. Both **40** and **41** showed good selectivity over the 5-HT_{1A} receptor, but they were poorly selective over the 5-HT_{2A} receptor. Derivatives **40** and **41** displayed agonist properties like 5-CT when tested for 5-HT₇ receptor mediated relaxation of substance P-induced guinea-pig ileum contraction.

Leopoldo et al. (2004b) have also evaluated a series of 4-(2-phenylsubstituted-ethyl)-1-(2-methoxyphenyl)piperazines (Table 13). Substitution of the 2-phenylethyl group with one or more methoxy substituents was evaluated and moderated differences in affinity at 5-HT₇ receptor were found (compounds **42–50**). On the other hand, replacement of the 2-methoxyphenyl group linked to the piperazine ring in compound **42** by 1,2-benzisoxazol-3-yl (**51**) or 2-hydroxyphenyl (**52**), or 4-methoxyphenyl (**53**) ring resulted in dramatic effects on affinity, suggesting this part of the molecule was relevant for interaction with the 5-HT₇ receptor. All the compounds studied lacked selectivity over 5-HT_{1A} receptors. Compound **51** behaved as an agonist when tested for 5-HT₇ receptor mediated relaxation of substance P-induced guinea-pig ileum contraction. This result replicated the finding obtained for compound **40** discussed above.

Leopoldo et al. (2004a, 2007) have also reported on a series of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides as 5-HT₇ receptor agents. Initial exploration of structure-affinity relationships indicated that the length of the intermediate alkyl chain had a relevant role, with a five methylene chain being preferred. Also, the presence and the position of one methoxy group on the 1,2,3,4-tetrahydronaphthalen-1-yl ring had a marginal role on affinity. Thus, derivative **54** (Table 14) was selected for further structural modifications. Exploration of various aryl rings linked to the piperazine ring evidenced the critical role played by this portion of the molecule. In particular, the possible isomers of OCH₃, CN, and COCH₃ substituted arylpiperazines were evaluated (compounds **54–62**, Table 14). Affinity data revealed a key role of substitution pattern, being the 4-substituted derivatives nearly devoid of affinity and the 2-substituted derivative the most potent among the isomers. However, none of the above described structural modifications afforded significant selectivity over 5-HT_{1A} receptors. The study of this class of compounds proceeded further by evaluating substituents of different natures in the 2-position of the aryl ring linked to the piperazine ring (Table 15). It was found that the nature of the substituent markedly influenced the affinity: polar substituents were detrimental for affinity, whereas bulky apolar groups gave high affinity ligands. Again, 5-HT_{1A} receptor affinities of these

compounds generally paralleled those for the 5-HT₇ receptor. Relevant exceptions were compounds **63** (R= 2-SCH₃), **74** (R= 2-isopropyl), **76** (2-phenyl), and **77** [2-N(CH₃)₂]: these compounds showed good selectivity over 5-HT_{1A} receptors. Thus, for the first time in this series of compounds a structural feature was identified that was well tolerated by the 5-HT₇ receptor but not by the 5-HT_{1A} receptor. The intrinsic activities at 5-HT₇ receptors of selected compounds were assessed in an isolated guinea-pig ileum assay by measuring 5-HT₇ agonist mediated relaxation of substance P-induced contraction. The intrinsic activity of the phenyl derivative **66** (40% Maximal Activity, EC₅₀= 8 μM) indicated that the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-phenyl-1-piperazinealkylamide framework was able to activate the 5-HT₇ receptor independently from the presence of a substituent in 2-position. The presence of the 2-nitro- or 2-chloro substituent (compounds **67** and **68**, respectively) had little effect on intrinsic activity, both compounds being weak partial agonists. The impact on the ability to activate the 5-HT₇ receptor was more marked in the case of bulky lipophilic groups. In fact, compounds **74** (83% Maximal Activity, EC₅₀= 0.90 μM) and **76** (74% Maximal Activity, EC₅₀= 1.77 μM) acted as partial agonists. Derivatives **63** and **77** were full agonists (100% Maximal Activity, EC₅₀= 1.17 and 2.56 μM, respectively). By contrast, 2-OH and 2-NHCH₃ substituents switched intrinsic activity toward antagonism: compound **78** antagonized the 5-HT₇ receptor (0% Maximal Activity, pA₂= 7.7) to a similar extent as the 2-hydroxy derivative **65**.

In a subsequent development, structural simplification of the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamide was performed in order to obtain compounds endowed with suitable physicochemical properties for rapid and extensive penetration into the brain (Leopoldo et al., 2008). In particular, the structural modifications were targeted on the 1,2,3,4-tetrahydronaphthalene nucleus as suggested by the previous structure-affinity relationship studies. Three structurally related moieties (isoindoline, 1,2,3,4-tetrahydroisoquinoline, benzyl) that would have less impact on the lipophilicity of the target molecules were considered (Tables 16 and 17). Comparing the isoindolines **83–86**, isoquinolines **87–90**, and benzyl derivatives **91–94**, with the corresponding tetrahydronaphthalenyl derivatives **63**, **76**, **74** and **54** a reduction in 5-HT₇ affinity was observed. It was suggested that the apolar nature of the terminal fragment was not the only feature that modulates the interaction of this part of the ligand with the 5-HT₇ receptor, because plotting the p*K*_is against the values of ClogP (logarithm to the base 10 of calculated partition coefficient 1-octanol/water) of compounds **83–94** revealed a low correlation coefficient. Replacement of the benzyl group attached to the amide nitrogen of compounds **91–94** (Table 17) by more polar groups had a limited impact on 5-HT₇ affinity, except in the case of compounds **97** and **101**, which were 42- and 25-fold more potent than **93**. It is interesting to note that the presence of 4-pyridinylmethyl, 4-cyanophenylmethyl, and 4-methanesulfonylphenylmethyl as terminal fragment was also well tolerated in the case of 2-phenyl derivatives **96**, **100**, and **104** that showed high 5-HT₇ affinity (5.7 nM < *K*_i < 0.58 nM). Affinity data for the 5-HT_{1A} receptor of the simplified compounds were quite similar to those of the reference 1,2,3,4-tetrahydronaphthalenyl derivatives **63**, **76**, **74** and **54**. Nonetheless, the 2-phenyl derivatives **88**, **96**, and **100** still retained nanomolar affinity at 5-HT₇ receptors, showed lipophilicity within the target range, and showed good selectivity over 5-HT_{1A} receptors (25- to 324-fold). The authors further characterized the ligands **88**, **96**, and **100** for their intrinsic activity at 5-HT₇ receptor, by measuring the 5-HT₇ agonist mediated relaxation of substance P-induced contraction in an isolated guinea-pig ileum assay. The compounds showed full agonism at the 5-HT₇ receptor. Compounds **96** and **100** demonstrated EC₅₀ values comparable to that of the standard 5-HT₇ agonist 5-CT, and higher potency than the tetrahydronaphthalenyl derivative **76**. It was observed that the modification of the terminal fragment of **76** left unchanged the intrinsic activity of **88**, **96**, and **100**, confirming the predominant role of the arylpiperazine moiety on the intrinsic activity in this class of compounds.

Bojarski et al. (2004) have investigated the role of intermediate alkyl chains of various “long-chain” arylpiperazines with respect to the affinity for 5-HT₇ receptor. Although none of the compounds were significantly selective over the 5-HT_{1A} receptor, the results gave interesting insight into the interaction of the molecules with the 5-HT₇ receptor. The authors developed some constrained analogues of the 5-HT_{1A} receptor antagonist 107 (NAN-190) (Table 18). It was observed an increase in 5-HT₇ affinity in relation to **107** for *trans* derivative **108**. The *cis* isomer **109** and bismethylbenzene derivative **110** was about 10-fold less active than the corresponding *trans* analogue. The rigid compound **111**, containing a cyclohexane moiety, was devoid of 5-HT₇ activity. Since such linearly constrained compound did not bind to the 5-HT₇ receptor, the authors suggested that the bent conformation of flexible “long-chain” arylpiperazines should be regarded as bioactive. Therefore, the partly constrained *trans* derivatives should be able to adopt a bent conformation during the interaction with this receptor. It was finally noted that the flexible butyl derivative could easily adopt a bent bioactive conformation that was close to that of a pharmacophore model for 5-HT₇ antagonism developed by López-Rodríguez et al. (2003).

Researchers at EGIS Pharmaceuticals Plc have developed a series of “long-chain” arylpiperazines bearing as terminal fragment an oxindole group (Table 19) (Volk et al., 2008). The authors formally deduced the framework from the tetrahydrobenzindole derivative previously studied by Kikuchi et al. (1999,2002a,b). Actually, the ring opening of compounds **5** and **8** left the affinity for the 5-HT₇ receptor practically unchanged (compounds **112** and **115**, Table 19). For methoxy substituted isomers **112–114** the affinities were ranked as follows: meta > ortho > para, whereas for chloro derivatives **115–117** the order was para > meta > ortho. The authors noted that the trend observed for compounds **112–117** was different from that found by Leopoldo et al. (2004a,2007) in the case of 1-aryl piperazine derivatives **54–62** (Table 14). Because the methoxy derivatives **112–114** were less selective over the 5-HT_{1A} receptor than the chloro derivative **117**, the authors evaluated derivatives **118** and **119**, in order to assess optimal spacer length for high 5-HT₇ affinity and low 5-HT_{1A} affinity. The highest affinity for 5-HT₇ receptors was obtained with derivative **116** with a four methylene spacer. Inclusion of either Cl, F, or CH₃ in the 4-position of the aryl ring linked to the piperazine ring of **116** was well tolerated (derivatives **120–122**). Removal of the ethyl group on the oxindole ring of **116** was also well tolerated (**123**) whereas replacement by isobutyl (**124**) caused a 4-fold loss in affinity. The authors also evaluated mono and dihalo substitution on the oxindole ring of **116**: compounds **125–128** evidenced a 10-fold loss in affinity except the fluoro derivative **126**.

Paillet-Loilier et al. (2005) have reported the results of a screening at 5-HT₇ receptors of a series of 1-(2-methoxyphenyl)piperazines bearing on the basic nitrogen a phenylpyrrolemethyl substituent. The compounds studied displayed poor affinity for the 5-HT₇ receptor (Table 20). Nonetheless, some information can be deduced. As an example, compounds **131** and **132** showed good affinities for the receptor ($K_i = 4.7$ and 5.4 nM, respectively) whereas the corresponding regioisomers **129** and **130** displayed 63% and 24% of inhibition at 10^{-6} M, respectively. Also, replacement of CN in **132** with OCH₃ (**135**) completely abolished 5-HT₇ affinity. It is noteworthy that 1,2-benzisoxazol-3-yl derivatives **133** and **134** were considerably less potent than the corresponding 2-methoxyphenyl derivatives **131** and **132**, suggesting that the binding mode of these ligands was different from that of similar arylpiperazines described by Leopoldo et al. (2004b).

Na et al. (2008) have reported a new class of “long-chain” arylpiperazine derivatives, bearing as terminal fragment a quinazolinone group (Table 21). The authors evaluated two groups of compounds characterized by a spacer with three or four carbon atoms and varied the nature and the position of the substituent on the aryl group linked to the piperazine ring. The affinity trend shown by this class of compounds generally paralleled that previously

observed for other “long-chain” arylpiperazines. For example, compounds with a four methylene spacer showed binding affinities higher than those with a three methylene chain (**136–140** vs **141–145**). Also, the compounds with a substituent in *ortho* position of the phenyl ring linked to the piperazine ring displayed higher 5-HT₇ affinities, whereas *para*-substituted phenyl derivatives (**137**, **140**, **142**, **145**) were significantly less potent or devoid of affinity for the target receptor. The nature of the substituent in *ortho* position was also relevant for 5-HT₇ affinity: OCH₃ (**143**) and OC₂H₅ (**146**) substituents were preferred over 2-F or Cl (**147** and **148**, respectively). The authors also investigated fluorosubstituted quinazolinone derivatives but found no substantial differences with the unsubstituted counterparts (**143**, **146**, **147**). The selectivity of selected compounds was assessed: the most potent compound **150** (IC₅₀ = 12 nM) was >42-fold selective over 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and D₂ receptors (Na et al., 2008).

Another series of arylpiperazine derivatives has been reported by the same research group (Yoon et al., 2008). The authors studied several 4-methoxyphenyl- and 2-naphthalenyl sulfonamides (Table 22) that were designed on the basis of the pharmacophoric hypothesis for 5-HT₇ antagonism reported by López-Rodríguez et al. (2000). According to the hypothesis, the compounds presented an aromatic ring, a basic nitrogen atom, an H-bonding acceptor group and a hydrophobic region at 4.9–5.9 Å apart from the basic center. The modifications were for the most part focused on substitutions on the arylpiperazine moiety. Considering the 4-methoxysulfonamides, it was found that the rank order of 5-HT₇ receptor affinity among the methoxy-substituted derivatives was 2 > 3 > 4 (compounds **152–154**), and 3 > 4 for the CF₃ derivatives **155** and **156**. Presence of NO₂ and COCH₃ substituents in the 4-position (compounds **158** and **159**) abolished the 5-HT₇ affinity. The same effect was observed for the phenyl derivative **157**. A similar trend was shown by the corresponding 2-naphthalenesulfonamide derivatives. However, the most potent compounds **160** and **163** lacked selectivity over the 5-HT_{1A} receptor. In general, structure-affinity relationships highlighted in this study paralleled those found by other research groups (Kikuchi et al., 2002a; Leopoldo et al., 2004b; Leopoldo et al., 2007) for ligands belonging to the class of “long-chain” arylpiperazines.

2.3. 4-Methylpiperidine compounds and their structural evolution

One of the first structure-activity relationship studies on 5-HT₇ receptor antagonists was published by researchers at SmithKline Beecham (later GlaxoSmithKline, GSK) in 1998 (Forbes et al., 1998). The studies started with the identification of the sulfonamide **166**, after high throughput screening of the corporate Compound Bank against the human cloned 5-HT₇ receptor (Table 23). The lead compound **166** showed modest 5-HT₇ receptor affinity (pK_i = 7.2) and acceptable selectivity over a range of other receptors. Evaluation of all four possible enantiomers of **166** (compounds **167–170**) indicated that the *R* chirality of the center closer to the sulfonamide function was essential for 5-HT₇ receptor affinity. On such basis, the chiral centre on the piperidine ring was removed by shifting the methyl group from the 3- to the 4- position. Thus, compound **167** originated **171** (Table 24) which showed slightly improved affinity (pK_i = 7.5). Subsequently, the naphthalene was replaced by other aromatic rings (compounds **172–175**) but 5-HT₇ affinity remained basically unchanged. In particular, derivative **172** (SB-258719) showed good 5-HT₇ receptor affinity, 100-fold selectivity over a range of serotonergic receptors, including 5-HT_{1A} and 5-HT_{2A}, and competitive antagonism at human 5-HT₇ receptor.

Starting from the observation that the chiral center in the flexible chain of **171** was critical for 5-HT₇ receptor affinity, the authors performed conformational analysis of this side chain. It was observed that all bonds were relatively free to rotate apart from the S–N and N(Me)–C(Me) bonds. An energy minimum was observed when the two methyl groups were oriented *gauche* with respect to each other, which could represent the binding conformation. This

study suggested that analogues in which both methyl groups were tied together into a ring would possess 5-HT₇ receptor affinities (Lovell et al., 2000). Consequently, the authors designed cyclic analogues of the naphthalene derivative **171** by incorporating both 2-piperidinylethyl and 2-pyrrolidinylethyl side chains (compounds **176** and **177**, respectively, Table 25). In both cases the (*R*)-isomer showed higher affinity than the (*S*)-isomer, and the pyrrolidine ring constrains the side chain in a more optimal conformation. Using the template of (*R*)-**177**, the authors evaluated aromatic rings different from 1-naphthalenyl. This modification was well tolerated (compounds **178–182**, Table 26) and 3-hydroxyphenylsulfonamide **182** showed very high 5-HT₇ receptor affinity ($pK_i = 8.9$). It should be noted that the hydroxyl group of **182** would favour additional interaction of the framework with the 5-HT₇ receptor, but it is not strictly necessary for 5-HT₇ affinity. Compound **182** (SB-269970) was found to be >100-fold selective against a total of 50 receptors, enzymes, or ion channels. It displayed a profile consistent with competitive antagonism when tested for the ability to inhibit agonist induced stimulation of adenylyl cyclase in HEK 293 cells stably expressing the 5-HT₇ receptor (Lovell et al., 2000; Hagan et al., 2000).

Tests *in vivo* in the rat with **182** evidenced extremely high blood clearance, almost certainly due to the presence of the phenolic hydroxyl group. To overcome this limitation, **182** was structurally manipulated by replacing the phenolic moiety with metabolically more stable bioisosters (Forbes et al., 2002). In all cases the proposed changes caused a reduction in 5-HT₇ receptor affinity (compounds **183–188**, Table 26). Only the 6-indolyl derivative **183** was nearly equipotent to **182**, but it showed high blood clearance and zero bioavailability in the rat.

Then, the authors turned their attention on the 4-methylpiperidine moiety of **183**, because metabolism studies on closely related structures indicated that hydroxylation could occur on such moiety. The authors replaced the methyl group because modeling studies indicated the presence of a large lipophilic pocket around this region. This may be considered a turning point of the studies at GSK because they moved from compounds with a single aromatic moiety to a class of compounds with an additional aromatic ring (Forbes et al., 2002). However, it was not commented whether the lipophilic or the aromatic nature of the group was relevant for affinity. This modification significantly enhanced the 5-HT₇ receptor affinity (Table 27). The indole **189**, the benzimidazolone **191**, and the 4-fluorophenoxy **193**, showed the greatest increase in affinity ($pK_i > 9.1$) as compared to **183**. On the other hand, it was found that this modification markedly increased unwanted affinity for the adrenergic α_{1B} receptor subtype. Due to its relatively low affinity for α_{1B} receptor, the 4-chlorophenoxy derivative **196** (SB-656104) progressed for further pharmacological evaluations. **196** showed ≥ 100 fold binding selectivity over most other 5-HT receptor subtypes, except 5-HT_{2A} (30 fold), 5-HT_{2B} (50 fold) and 5-HT_{1D} (12 fold). **196** inhibited 5-CT induced stimulation of cAMP accumulation ($pA_2 = 8.1$), thus behaving as 5-HT₇ antagonist. **196** showed greatly improved pharmacokinetic profile in the rat compared to **182**, possessing a lower blood clearance and a significantly improved half life. The antagonist **196** passed the blood brain barrier in a similar extent of **182**. The oral bioavailability of **196** was 16%, whereas **182** did not show any oral bioavailability. Evaluation of **196** in an *in vivo* functional assay (5-CT induced hypothermia in guinea pigs) resulted in a ED₅₀ profile similar to **182**, but with administration being via the oral route.

2.4. 2-Aminotetralin, 3-aminochroman, and aporphine

The 2-aminotetralin, 3-aminochroman, and aporphine moieties have served as templates for the mapping of the ligand binding sites of certain 5-HT receptors. In 2000, a research group at Uppsala University (Sweden) initiated a research program aimed at the identification of selective 5-HT₇ receptor agents based on the above mentioned chemical classes (Linnanen

et al., 2000). Initially, the novel (R)-1,11-methyleneaporphine ring system was designed (compound **198**, Table 28). This new ring system showed interesting affinity at the 5-HT₇ receptor (K_i = 6.9 nM) as compared to the parent aporphine **197** (K_i = 88 nM). Subsequently, the authors found that selectivity over the 5-HT_{1A} receptor could be significantly modulated by introducing substituents at C12. The most relevant compound was the 12-amino substituted derivative **199** which showed high 5-HT₇ affinity (K_i = 18 nM) and approximately 20-fold selectivity over the 5-HT_{1A} receptor. Also the derivative disubstituted by methyl and methoxy at C12 showed selectivity over 5-HT_{1A} receptors (compounds **200** and **201**). In a subsequent study (Linnanen et al., 2001) the aporphine core was modified further by introducing an aryl group in 11-position (Table 29). This structural modification offered significant advantages in terms of selectivity when the aryl ring was disubstituted in 2' and 6' position. Such substitution pattern generated stable atropisomers that displayed pharmacological differences. In particular, derivative **205** displayed affinity for the 5-HT₇ receptor in the nanomolar range (K_i = 3.79 nM) and 40-fold selectivity over the 5-HT_{1A} receptor. This compound behaved as a 5-HT₇ receptor antagonist. Three years later, the same authors reported on novel 2-aminotetralin and 3-aminochroman derivatives (Table 30) that can be considered formally derived from the above 11-arylporphines (Holmberg et al., 2004). This structural simplification affected neither affinity nor selectivity for the 5-HT₇ receptor: in fact, compounds **208** and **215** showed similar properties as **202**. Subtle changes of the 5-aryl substituent (8-aryl in the chromans) afforded selectivity toward the 5-HT_{1A} receptor. In particular, introduction of a single *ortho* methoxy substituent (**209** and **216**) reduced the affinity for the 5-HT_{1A} receptor 8–13 times, while the affinity for the 5-HT₇ receptor was unchanged. Further introduction of a second *ortho* methoxy substituent (**211** and **218**) substantially reduced the affinity for the 5-HT_{1A} receptor while it only marginally affected binding to the 5-HT₇ receptor. The authors suggested that the *ortho* substituents, by forcing the 5- (or 8-) aryl group out of plane of the tetralin (or chroman), reduced favorable interactions with the 5-HT_{1A} receptor. Decreasing the size of the *N*-alkyl substituents increased the selectivity for the 5-HT₇ receptor, due to a decrease in affinity at 5-HT_{1A} receptors (compounds **212** and **213** for the tetralin series). Comparison of binding affinities of **216** and its enantiomer (not shown in Table 30) revealed that interaction of the ligand with the 5-HT₇ receptor was stereospecific, favoring the (R)-chromans, with an eudismic ratio of approximately 130. The nature of the substituent on the basic nitrogen appeared to influence the signal transduction, because **211** and **218** were full agonists whereas the corresponding dimethylamino derivatives **213** and **219** were antagonists or partial agonists. However, it should be noted that the structurally related dimethylamino derivative **214** (AS-19, Table 30) is a potent and selective 5-HT₇ agonist (Brenchat et al., 2009).

2.5. 2-Arylethylamine derivatives and related analogues

Paillet-Loilier et al. (2007) have reported on a class of aminoethylbiphenyls that were designed by analysing the structural homologies between phenylpyrrole, 11-phenylaporphine, and phenyl-2-aminotetraline derivatives (see Tables 20, 29, and 30). However, as the phenylpyrroles **220** and **221** were found to be inactive (Table 31), the pyrrole ring was replaced by a phenyl ring to better align with both 11-phenylaporphine and 2-aminotetraline compounds. In this series, the 5-HT₇ affinity and selectivity depended on the substitution of the phenyl ring denoted with A (Table 31). Introduction of *ortho* substituents (Me or OMe) into the phenyl ring A increased the 5-HT₇ receptor affinity (**222** and **223**). These *ortho* substituted compounds showed a good selectivity toward 5-HT_{1A} receptor. The presence of a substituent in *ortho* position was essential for the 5-HT₇ receptor affinity. The authors hypothesized that the aryl groups A and B should not be placed in the same plane. Analysis from Cambridge Software Database data (Bruno et al., 2004) of the distributions of the dihedral angle between the two phenyl rings, unsubstituted and *ortho*

substituted, showed a different distribution between the two fragments. The hypothesis was supported by the lack of affinity of the unsubstituted derivative **240**. Introduction of a second substituent in *ortho* position in compounds **222** and **223** did not modify the 5-HT₇ receptor affinity but improved the selectivity over the 5-HT_{1A} receptor: **222** and **223** were about 60-fold selective over 5-HT_{1A} receptor, whereas **225** and **282** showed increased selectivity (360- and 560-fold, respectively). It was also observed that the unsubstituted amino group was tolerated (**228** vs **222**). Compounds **222** and **226** have been evaluated for their pharmacological profile by using a specific test of aldosterone secretion from perfused rat adrenal cortex stimulated by serotonin through 5-HT₇ receptor. Compound **222** behaved as 5-HT₇ antagonist ($pK_b = 7.03$), whereas **226** was found to be a partial agonist.

Thomson et al. (2004) at Merck Sharp & Dohme laboratories have developed a series of 5-HT₇ receptor ligands starting from compound **229** (Table 32). Simple structural modifications of **229** gave interesting information: removal of 4-pyridine ring caused a loss in affinity (**230**), whereas replacement with phenyl or t-butyl was well tolerated (compounds **231** and **232**, respectively). Variation of the core heterocycle was explored on derivative **231** (compounds **233**, **234–237**): when the thiazole of **229** was replaced by a pyridine with the 2,6-substitution pattern very high affinity value at 5-HT₇ receptors was achieved (**237**). The position of the pyridine nitrogen was important, as 2,4-substituted isomer **236** did not show subnanomolar binding affinity. Replacement of the phenyl ring of compound **237** by Br, cyclohexyl, t-butyl afforded high binding affinities at 5-HT₇ receptors. Compound **237**, which was 26-fold selective over the 5-HT_{1A} receptor, was tested in a functional assay and found to behave as a partial agonist, giving 80% of the response of the full agonist 5-CT in 5-HT₇ receptors.

2.6. Aminotriazines and related analogues

Researchers at Bristol-Myers Squibb have identified 5-HT₇ receptor antagonists containing the aminotriazine core as basic moiety (Mattson et al., 2004). The lead compound **241** ($K_i = 60$ nM) was structurally modified to improve affinity and metabolic stability (Table 33). Replacement of the 3-phenylpropyl group with a 2-phenoxyethyl residue (**242**) resulted in an increase in affinity. Interestingly, the (*S*)-enantiomer **243** showed $K_i = 3$ nM whereas its enantiomer **244** was devoid of 5-HT₇ affinity ($K_i > 1000$ nM). Removal of the amino group linked to the triazine ring in the eutomer was well tolerated (compound **245**, $K_i = 3$ nM), whereas substitution by F, CH₃ or N(CH₃)₂ gave a moderate loss in affinity (compounds **246–248**). Introduction of an F substituent on both ends of **242** gave rise to compound **249** with no substantial changes in affinity ($K_i = 5$ nM). Also in the case of **249**, the (*S*)-enantiomer was more potent than the (*R*)- (**250** vs **251**), but the eudismic ratio was considerably lower than that displayed by compounds **243** and **244**. Compounds **243** and **250** displayed > 30-fold selectivity over 5-HT₆, α_1 and 5-HT_{2C} receptors and potent antagonistic activity at the 5-HT₇ receptor.

In a subsequent paper the same authors explored the structure-affinity relationships of the aminotriazine ring (Denhart et al., 2004). Several diaminopyridine and diamino pyrimidine varieties were studied (Table 34). Considering the analogues of compound **245** the most active compounds were pyridines with the nitrogen in position denoted W or Y in the general structure (compounds **252** and **254**, Table 34). A substituted pyridyl ring as in **255** or a phenyl ring (**256**) abolished 5-HT₇ affinity. The addition of a second nitrogen in the pyridyl ring of **255** produced the pyrimidine derivative **257** which showed again nanomolar 5-HT₇ affinity.

2.7. N-N'-Disubstituted guanidine derivatives

Researchers at Pfizer have reported on a series of (4,5-dihydroimidazol-2-yl)biphenylamines as 5-HT₇ receptor agonists (Parikh et al., 2003). The basic fragment employed for the synthesis of the target compounds deserves interest because it is an alternative to the most frequently used piperazine, piperidine, or pyrrolidine scaffolds. However, the compounds displayed significant α_2 adrenergic activity that resulted in marked changes in blood pressure and heart rate after *in vivo* administration in rats, presumably due to the structural similarity of the compounds with the α_2 adrenergic clonidine. The most potent ligand of this series (compound **258**) is shown in Figure 2.

In the search of alternative basic moieties in the design of 5-HT₇ ligands, Zajdel et al. (2009) have studied a series of N-alkyl-N'-dialkylguanidines that showed moderate affinity for the 5-HT₇ receptor. Comparing the 5-HT₇ receptor affinities of the new derivative **259** (Figure 2) with that of its analogue without the guanidine moiety **160** (Table 22), it emerges that the introduction of delocalized positive charge in the ionic center of molecule attenuated affinity for the 5-HT₇ receptor.

2.8. Miscellaneous compounds

Raubo et al. (2006) have reported structure-affinity relationships on a series of alkylphenylsulfones. This fragment was attached to a variety of basic moieties. For this reason, the classification of the compounds reported in Tables 35 and 36 does not fit the criterion used in the present overview. Preliminary modeling studies suggested that a gem-disubstituted cyclobutyl phenyl sulfone could overlay with one of the low energy conformations of **182** (SB-269970, Table 26). Thus, this fragment originated the compounds listed in Table 35. Obtained affinity data suggested that an aromatic moiety near the basic nitrogen was essential for 5-HT₇ receptor affinity, and this points out that the compounds do not bind at 5-HT₇ receptors in the same fashion as **182**. In fact, compound **263** displays 5-HT₇ affinities significantly lower than **182**. The best orientation of the aromatic ring was shown by tetrahydroquinoline derivative **267**, which however showed modest selectivity over 5-HT_{1A} and 5-HT_{2A} receptors (4- to 8-fold). This compound was also evaluated in a functional model of 5-HT₇ receptor activation and found to behave as an antagonist. Replacement of the tetrahydroquinoline ring of **267** with the full hydrogenated counterpart gave **269**, endowed with high 5-HT₇ affinity and 35-fold selectivity over the 5-HT_{1A} receptor. This finding suggested that the hydrophobic nature of the basic moiety was relevant for affinity. Taking this information into account, the authors studied a set of compounds related to **269** (Table 36). Replacement of the spirocycle of **269** by a gem-dimethyl substituent gave **270**, which showed an excellent selectivity profile over 5-HT_{1A} and 5-HT_{2A} receptors (>100-fold) and partial agonist properties. Evaluation of amine structure-activity relationships of compound **270** demonstrated that functional activity can be attenuated by installing a pendant substituent at the 3-position (Table 36, compounds **271–275**). A variety of substituents were tolerated in the 3-position. In particular, sulfones **272** and **274** exhibited high affinity at the 5-HT₇ receptor with minimal or no functional activity and >30-fold selectivity over 5-HT_{1A}, 5-HT_{2A}, 5-HT_{1B}, and D₂ receptors. This study is an interesting example of how modifications of the same fragment can modulate intrinsic activity at the 5-HT₇ receptor.

2.9. Pharmacophore models for 5-HT₇ receptor models

The first pharmacophore model for 5-HT₇ receptor antagonists was proposed by López-Rodríguez et al. (2000). The study was performed using the software package Catalyst to analyze a set of thirty structurally different 5-HT₇ receptor antagonists. This preliminary model was later optimized, with the incorporation in the training set of analogues of **182** (SB-269970, Table 26) and of 11-phenylaporphine derivatives (Table 29). The optimized

model consisted of five features: a positive ionizable atom (PI), an H-bonding acceptor group (HBA), and three hydrophobic regions (HYD1–3). The proposed distances between these chemical features are reported in Figure 3. The proposed hypothesis has been supported by design and synthesis of a series of naphtholactam and naphthosultam derivatives. Considering the compounds in Table 37, the authors noted that the hydrophobic region HYD3 of the model must be aromatic since compounds **283**, **284**, **287** lacked affinity, contrary to the aromatic substituted counterparts **286**, **288**, **289**. The only notable exception to this trend was **280** (R= isopropyl) which showed moderate affinity. Also, it was observed that replacement of the piperazine with a piperidine or tetrahydropyridine ring caused a dramatic loss in affinity (**285** compared to **281** and **282**). The optimum spacer length was four or five methylene units, because they defined the optimum distance between the HBA and the basic center. On the other hand, compounds **276–278** with a shorter spacer were inactive. One exception was **279** (n= 3) but this compound had less affinity than **286**. An increase in the size of the alkyl chain to n= 6 caused a loss in affinity (compounds **290** and **291**). Moreover, it was noted that naphtholactam derivatives were more potent than naphthosultam analogues (**286** vs **292**; **289** vs **293**). However the authors did not give any explanation for this behaviour in terms of ligand geometry. Considering the length of the spacer the only difference with the naphtholactam series was that compound **295** (n= 6) showed moderate affinity. It was suggested that a 6-carbon chain may adopt a folded conformation giving the appropriate distances for interaction with the receptor. It should be noted that although the authors address the model as being for 5-HT₇ receptor antagonists, the intrinsic activity of these ligands was not investigated.

The first pharmacophore for 5-HT₇ receptor agonism was determined by Vermeulen et al. (2003). Full conformational analysis of a set of 20 diverse 5-HT₇ receptor agonists (including 5-HT, 5-CT, 8-OH-DPAT, 1-(1-naphalenyl)piperazine, 1-(2-methoxyphenyl)piperazine, LSD, **214** (AS-19, Table 30), **258**) in their protonated form was performed with the MacroModel molecular modeling software package, and followed by a pharmacophore-identifying procedure through ligand overlap using the Automated Pharmacophore Location through Ligand Overlap (APOLLO) procedure. The obtained model defined the distances between four pharmacophoric elements: a basic nitrogen atom (PI), an H-bonding acceptor group (HBA) and two hydrophobic domains (HYD) (Figure 4). The results of a comparative molecular field analysis (CoMFA) were then used to map the agonist binding site of the model of the 5-HT₇ receptor. Important roles in ligand binding was attributed to Asp^{3.32} (according to Ballesteros & Weinstein 1995) (interaction with a protonated nitrogen), and Thr^{5.43} (interaction with a substituent at an aromatic moiety). Amino acid residues of the aromatic cluster of transmembrane region 6 (TM6) were hypothesized to play an important role in ligand binding as π - π stacking moieties. It was also proposed that agonists missing a hydrogen-bond-accepting moiety, but possessing an aromatic substituent instead, could bind to the receptor with high affinity as well by occupying a lipophilic pocket hosted by residues of TM5 and TM6.

Vermeulen et al. (2004) have also developed a pharmacophore model for 5-HT₇ receptor inverse agonists. A computational study was carried out on a set of 22 inverse agonists, which included various arylsulfonamides, exemplified by **296** and **297** (Figure 5). The model shown in Figure 6 resembles the model proposed by López-Rodríguez for selective antagonists, except for the additional HYD4 region mapping, among others, an aromatic substituent at the sulfonamide group. Slightly shorter distances between HYD3-HBA and HYD2-HYD3 were also hypothesized. Interestingly, the model of inverse agonists revealed a close similarity to that of agonists, the main difference between the models being the presence of both HYD2 and HYD3 regions.

The first receptor-based pharmacophore for the 5-HT₇ receptor was constructed by Kołaczkowski et al. (2006), which evaluated, through docking studies, the mode of interaction of selective and nonselective antagonists with the receptor binding site. It was hypothesized that selective and nonselective antagonists might have different binding modes with the receptor. Thus, the pharmacophore model for antagonists was divided into two distinct submodels: an “affinity” pharmacophore and a “selectivity” pharmacophore.

The “affinity” pharmacophore was characterized by six features representing specific interactions points in the ligand structure: a protonated nitrogen (PI), three hydrophobic/aromatic regions (HYD/AR1-3) and two H-bond acceptors (HB1,2) (Figure 7). For affinity, at least three of the features must be present in a specific spatial arrangement. Among them, PI and one of ARs (capable of specific CH- π or π - π interaction) are strictly necessary, while the third may be HBA or another HYD/AR region. Each of the features interacts specifically with the receptor structure: PI is involved in the salt bridge formation with Asp^{3.32}; AR1 interacts with Phe^{3.28} (CH- π or π - π) and/or Arg^{7.36} (ion- π), AR2 and AR3 have CH- π contact with Phe^{6.52} and Phe^{6.51}, while HBA1 and HBA2 form H-bonds with Tyr^{7.43} and Ser^{5.42}, respectively.

Also the “selectivity” pharmacophore was characterized by three crucial features. Two of them were common for all the selective antagonists: PI and AR1, which form strong specific interactions with the residues in the pocket between transmembrane helices (TMH) 7-3 (especially Phe^{3.28} and Arg^{7.36}). The third feature necessary for selectivity could be either HBA1, an H-bond acceptor situated in the vicinity of Tyr^{7.43} or HYD/AR2, a hydrophobic or an aromatic moiety penetrating the pocket between TMHs 4–6. This latter interaction should not dominate that of the AR1 feature. The authors underlined that the geometry of the terminal moiety containing AR1 (aromatic imide/amide/sulfonamide), which should enable the formation of π - π stacking with Phe^{3.38}, ion- π interaction and/or with Arg^{7.36}, was of crucial importance. Thus, nonselective antagonists such as ergolines, aporphines, tricyclic psychotropes, and aminotetralines were docked to the receptor model in a quite similar way, because they accommodated (one of) the benzene ring by the pocket between TMHs 4–6.

By contrast, the arylsulfonamide part of molecules such as **182** (SB-269970), was situated in the pocket between TMHs 7-3, and the sulfone oxygen formed an H-bond with Tyr^{7.43}, while the aromatic moiety was in an optimal position for the π - π stacking and ion- π interaction with Phe^{3.28} and Arg^{7.36}, respectively.

On the other hand, complex ligands such as those listed in Tables 9–11 consist of two pharmacophoric groups: the main arylpiperidine/arylpiperazine/ β -carboline moiety (containing the crucial basic nitrogen) and the “terminal amide/imide” fragment of different structure (frequently containing another aryl ring), linked together by a flexible alkyl spacer (2–5 methylene units). Compounds of that numerous and diversified class of 5-HT₇ receptor antagonists occupied both pockets of the binding site simultaneously (i.e. one between TMHs 4–6 and the other between TMHs 7-3), having different interactions because of their diversified structure. The aromatic ring of an arylpiperidine/arylpiperazine/ β -carboline moiety penetrated the pocket between TMHs 4–6, forming a specific CH- π interaction or having van der Waal contacts with one or more residues from the aromatic cluster of TMH6 (Phe^{6.52}, Phe^{6.51}, Trp^{6.48}) (compounds **17**, **19**, **23**, **30**, **65**). The terminal amide/imide moiety occupied the pocket between TMHs 7-3 (compounds **17**, **19**, **23**, **30**, **65**). Aromatic rings from terminal groups formed specific interactions with Phe^{3.28} and/or Arg^{7.36} (compounds **17**, **19**, **23**, **30**, **65**). In the case of tetrahydrobenzindole as a terminal imide (compounds **17**, **19**, **23**, **30**), development of π - π stacking with Phe^{3.28} and an ion- π interaction with Arg^{7.36} were possible. That was due to the geometry of the tetrahydrobenzindole group, which positioned its benzene ring in a way that enabled those favorable interactions.

The arylsulfonamidoalkylamines with a bulky aromatic substituent at the 4-position of piperidine (Table 27) shared with complex arylpiperidines-piperazine the binding mode with the receptor. Again, arylsulfonamide part of the molecule was situated in the pocket between TMHs 7-3, and the sulfone oxygen formed an H-bond with Tyr^{7.43}, while the aromatic moiety was in an optimal position for the π - π stacking and ion- π interaction with Phe^{3.28} and Arg^{7.36}, respectively. p-Cl-Phenoxy or indole substituents at the piperidine ring interacted with the hydrophobic/aromatic residues in the pocket created by TMHs 4–6. It was postulated that compounds that fit well the TMHs 7-3 pocket and were therefore independent of the interaction with the TMHs 4–6 cavity, which is highly conserved in monoamine GPCRs, were more likely to show selectivity.

It should be noted that the binding mode for **182** (SB-269970) suggested by Kołaczowski et al. (2006) differed from that proposed by Vermeulen et al. (2004). Apart from the electrostatic interaction between the protonated nitrogen atom with Asp^{3.32}, Vermeulen and coworkers suggested: C-H $\cdots\pi$ interactions between Ph^{7.38} and the piperidine ring; interaction between the sulfonamide oxygen and the hydroxyl group of Thr^{6.45}; interaction between the phenolic-OH and Thr^{6.45}.

Very recently, Medina et al. (2009) have designed a new class of 5-HT₇ ligands on the basis of the pharmacophore model for antagonists (Table 38) proposed by the same research group (López-Rodríguez et al., 2003). From the structure-affinity relationship standpoint the study confirmed previous findings about spacer characteristics and the nature of the aryl linked to the piperazine ring. Computational models of the 5-HT₇ receptor in complex with ligands **299**, **300**, and **309** were also reported. As an example, it was proposed that the lactam carbonyl group of **299** interacts with Ser^{6.55}, and the aromatic ring of this same moiety with Phe^{5.67} and Phe^{6.52} (i.e. all residues present in the TMHs 5 and 6). Most notably, the methoxy group on the aryl ring linked to the piperazine ring is reported to interact with Arg^{7.36}. The authors suggested this interaction to be a “hot spot” of the interaction of the molecule with the receptor, responsible for high selectivity. However, it was not explained why the corresponding derivative **301** which lacked of such feature was still selective. As can be noted, the interaction of these ligands with the 5-HT₇ receptor was quite different from that proposed by Kołaczowski for 1-aryl piperazine derivatives. These findings confirm that the 5-HT₇ binding site can be divided into two pockets: one between TMHs 4–6 and the other between TMHs 7-3. Moreover both pockets have similar interaction points for aromatic moieties and H-bond acceptors, and this makes them fairly symmetrical and, therefore, equivalent upon ligand docking.

3. 5-HT₇ receptor function in normal and pathological processes

3.1. Background

It has consistently been possible to correlate 5-HT₇ receptor distribution with function as there is a significant agreement between the localization of 5-HT₇ receptors and the functions they are implicated in (Hedlund & Sutcliffe, 2004, 2006; Hedlund, 2009). Within the brain, which has attracted the most interest, its presence in the hypothalamus correlates with involvement in circadian rhythm, thermoregulation, and endocrine regulation. Thalamic and cortical 5-HT₇ receptors may be of importance for sleep, mood regulation, and epilepsy. The distribution of 5-HT₇ receptors in the hippocampus is of relevance for its role in learning and memory. Finally, its localization in the spinal cord is in agreement with demonstrated functions in nociception and locomotion.

Interest in the possible involvement of the 5-HT₇ receptor in brain disorders was prompted by the early finding that several antipsychotics (Roth et al., 1994) and antidepressants (Monsma et al., 1993; Mullins et al., 1999) have high affinity for the 5-HT₇ receptor in

combination with its presence in relevant regions of the brain. The result is several preclinical studies that have evaluated the possible involvement of the 5-HT₇ receptor in psychiatric disorders and other pathological processes of the nervous system (Hedlund, 2009). These studies have used pharmacological tools and/or constitutive knockout mice lacking 5-HT₇ receptors in animal behavioral models designed to mimic, at least in part, human disorders. The available pharmacological tools and even more under development to study 5-HT₇ receptors are discussed in detail above. Briefly, most studies have used antagonists that are considered relatively selective, the most widely used being SB-269970 (Hagan et al., 2000) and DR4004 (Kikuchi et al., 1999). Constitutive knockout mouse strains lacking the 5-HT₇ receptor have been created independently by several laboratories (Hedlund et al., 2003; Guscott et al., 2005; Sprouse et al., 2005; Witkin et al., 2007). One strain was developed at The Scripps Research Institute (Hedlund et al., 2003) and has been made available to other academic institutions (Landry et al., 2006; Liu et al., 2009). This strain has been bred on a C57BL/6J background. The other published strains have been made by the pharmaceutical industry. As seen below, most evidence has been collected to support a role for the 5-HT₇ receptor in depression. In fact, a recent study suggests that the clinically established antidepressant effect of amisulpride is due to its action at 5-HT₇ receptors (Abbas et al., 2009). Studies aimed at evaluating the role of 5-HT₇ receptors in disorders such as anxiety and schizophrenia have also generated interesting results.

3.2. Anxiety

Efforts to evaluate a possible link between the 5-HT₇ receptor and anxiety have generated mixed results. Two different strains of mice lacking the 5-HT₇ receptor (5-HT₇^{-/-}) have been evaluated in anxiety models with differing results when compared to 5-HT₇^{+/+} mice. Mice bred on a C57BL/6J background were evaluated in a light-dark transfer test. It was found that both genotypes spent the same amount of time in the light compartment and that they had an equal number of transitions between the light and dark compartments (Roberts et al., 2004). The same strain of mice was also evaluated in the marble burying test. Here 5-HT₇^{-/-} mice buried significantly fewer marbles than the 5-HT₇^{+/+} mice, a response considered anxiolytic (Hedlund & Sutcliffe, 2007). A different strain of 5-HT₇^{-/-} mice bred on a mixed 129SvEv/C57BL/6J background was tested in an elevated plus maze. There was no difference in the time spent exploring the open arms or in the number of entries onto the open arms of the maze between the two genotypes (Guscott et al., 2005). The selective 5-HT₇ receptor antagonist SB-269970 has been evaluated in both rats and mice for potential anxiolytic effect. In rats the drug induced an anxiolytic effect in the Vogel conflict drinking test and the elevated plus maze models of anxiety after both systemic (Wesolowska et al., 2006a) and intra-hippocampal (Wesolowska et al., 2006b) administration. Thus, SB-269970 increased the number of shocks tolerated in the Vogel test. In the elevated plus maze SB-269970 increased both the number of entries onto the open arms and the amount of time spent on those arms. It should be noted that SB-269970 generally had an inverted U-shaped dose-response effect and its anxiolytic effect was not as pronounced as that of diazepam, a reference anxiolytic of the benzodiazepine class (Wesolowska et al., 2006a, 2006b). SB-269970 also had a similar limited anxiolytic effect in the conflict-based four-plate test in mice as the drug increased the number of punished crossings (Wesolowska et al., 2006a). Again the effect was smaller than seen for diazepam. SB-269970 was also effective in the marble burying model (Hedlund & Sutcliffe, 2007).

The clinical relevance of these models for anxiety in humans has been questioned. Traditional benzodiazepine anxiolytics are effective in all the animal models, but only in certain types of human anxiety, whereas chronically administered selective serotonin reuptake inhibitors (SSRI) are effective in most, if not all, forms of human anxiety disorders (Borsini et al., 2002). It appears that inactivation or blockade of the 5-HT₇ receptor more

consistently produce an anxiolytic response in models responding to SSRI than those responding only to benzodiazepines. The latter include light-dark transfer and elevated plus maze (Borsini et al., 2002). Particularly interesting is the observation that the 5-HT₇ receptor appears to modulate the response in the marble burying test, as this procedure has been suggested as a model for the specific anxiety disorder obsessive-compulsive disorder (Njung'e & Handley, 1991). The pharmacological treatment of choice for obsessive-compulsive disorder is antidepressants, specifically SSRIs (Heyman et al., 2006). Taken together with the findings using depression models described below the combined data suggest that the 5-HT₇ receptor might be an alternative or complementary target for disorders currently treated with SSRI. It has also been suggested that modulating the 5-HT₇ receptor might be a new approach to design anxiolytic treatment, based on effects within the bed nucleus of the stria terminalis (Guo et al., 2009).

3.3 CNS Development

The developing cortex is densely innervated by serotonergic neurons with 5-HT acting on several different receptor subtypes. It has been shown that the 5-HT₇ receptor is involved in the postnatal formation of synaptic connectivity in the prefrontal cortex, where early postnatally there is a high expression of 5-HT₇ receptors that later declines and is replaced by an increase in 5-HT_{1A} receptor expression (Beique et al., 2004a,b). The early postnatal appearance of 5-HT₇ receptors in the hypothalamus has also been demonstrated underlining its importance in this region (Russo et al., 2005). Furthermore, serotonergic projections from the dorsal raphe have been shown to modulate cholinergic and noncholinergic neurons within the ventral pallidum through mechanisms that at least partly appear to involve the 5-HT₇ receptor (Bengtson et al., 2004). These studies were made in slices from immature Wistar rats and the findings might be of importance for the etiology of psychiatric disorders (Bengtson et al., 2004). In the developing rat brain 5-HT₇ receptor immunoreactivity has been observed in a cytoplasmic inclusion termed stigmoid body (Muneoka & Takigawa, 2003). In neonatal animals these immuno-positive inclusions were most prominent within the hypothalamus and they have been linked to the development of sexual dimorphism. As discussed further below, the 5-HT₇ receptor is important in regulating spinal locomotion, something that is evident already in neonatal mice (Madriaga et al., 2004).

3.4. Circadian rhythm

Circadian rhythms, sleep and mood are closely linked physiological phenomena that are all regulated by the 5-HT₇ receptor. Soon after the 5-HT₇ receptor was discovered, it was found that 8-OH-DPAT-induced phase resetting within the suprachiasmatic nucleus (SCN) was mediated by this receptor, and not the 5-HT_{1A} receptor as previously thought (Lovenberg et al., 1993). This reevaluation was due to 8-OH-DPAT previously being considered a selective agonist for 5-HT_{1A} receptors (Hjorth et al., 1982). An extensive body of work has confirmed the importance of the 5-HT₇ receptor in SCN function (Antle et al., 2003; Glass et al., 2003; Duncan et al., 2004; Sprouse et al., 2004, 2005). That the phase shifting induced by 8-OH-DPAT is mediated by the 5-HT₇ receptor is further supported by the observation that the shift can be inhibited by the selective antagonists SB-269970 and DR-4004, but not by selective 5-HT_{1A} receptor antagonists (Ehlen et al., 2001; Sprouse et al., 2004). However, in one study the putative 5-HT₇ receptor agonist AS-19 failed to mimic the effect of 8-OH-DPAT after systemic administration (Cuesta et al., 2009). Possible explanations for the inability of AS-19 to induce a phase shift include that only one dose was tested, and/or its lack of selectivity (Bosker et al., 2009; Brenchat et al., 2009). It may also be speculated that AS-19 is metabolically unstable even though a dose-dependent biological effect has been achieved after systemic administration in a study on nociception (Brenchat et al., 2009). Shifting the SCN pacemaker neurons with 8-OH-DPAT is a non-photoc stimulus involving serotonergic input from the dorsal and median raphe nuclei. In these nuclei 5-HT₇

receptors have been shown to modulate SCN phase resetting (Glass et al., 2003). There is also evidence to support a role for the 5-HT₇ receptor in photic regulation of the SCN. It has been demonstrated using pharmacological profiling with unselective drugs (Ying & Rusak, 1997) and with DR-4004 (Ehlen et al., 2001) that 5-HT-mediated reduction of photic stimulation of SCN neurons is most likely mediated by the 5-HT₇ receptor. The inhibitory effect of 8-OH-DPAT on spontaneous SCN activity is also most likely mediated by the 5-HT₇ receptor, as it could be blocked by 5-HT₇ receptor antagonists but not 5-HT_{1A} receptor antagonists (Yu et al., 2001). The above studies were performed in either rats or hamsters. In contrast, one study in the mouse concluded that the effects of 8-OH-DPAT on SCN function was not as pronounced (Antle et al., 2003). Nevertheless, the involvement of the 5-HT₇ receptor in both photic and non-photoc phase resetting has been verified in mice lacking the receptor (Gardani & Biello, 2008).

3.5. Depression

It has been suggested that the action of antidepressants, at least in part, might be mediated directly by the 5-HT₇ receptor (Monsma et al., 1993; Mullins et al., 1999). Thus, several antidepressants, both tricyclics and SSRI, induced *c-fos* expression in a way consistent with 5-HT₇ receptor activation within the SCN (Mullins et al., 1999). The effect on *c-fos* expression was attenuated after chronic treatment with antidepressants. Additionally, chronic antidepressant drug treatment led to a downregulation of 5-HT₇ receptor binding (Mullins et al., 1999).

The forced swim test and the tail suspension test are two of the most common behavioral models for evaluating the antidepressant potential of a drug or to evaluate the phenotype of transgenic animals with respect to depression (Cryan et al., 2002; Cryan & Holmes, 2005). In both of these models, pharmacological blockade of the 5-HT₇ receptor or inactivation of the receptor gene leads to an antidepressant-like behavioral profile; that is, reduced immobility (Guscott et al., 2005; Hedlund et al., 2005; Wesolowska et al., 2006a, 2006b, 2007; Bonaventure et al., 2007; Sarkisyan et al., 2010). More interestingly, it has been shown that there is a synergistic interaction between an individually ineffective dose of the selective antagonist SB-269970 and an individually ineffective dose of one of several antidepressants, leading to reduced immobility in both the forced swim test and the tail suspension test (Wesolowska et al., 2007; Bonaventure et al., 2007; Sarkisyan et al., 2010). Thus, concurrent administration of citalopram, an antidepressant of the SSRI type, and SB-269970 has been shown to reduce immobility in the tail suspension test in C57BL/6J mice (Bonaventure et al., 2007; Sarkisyan et al., 2010). At a higher dose, SB-269970 alone reduces immobility in the mouse tail suspension test (Hedlund et al., 2005; Bonaventure et al., 2007). The higher dose of SB-269970 alone did not alter 5-HT concentration in the rat frontal cortex, but the combination of a low dose of SB-269970 and a low dose of citalopram increased the level of 5-HT in the frontal cortex (Bonaventure et al., 2007). A similar synergistic interaction between individually ineffective doses of SB-269970 and citalopram has also been demonstrated in the mouse forced swim test (Wesolowska et al., 2007; Sarkisyan et al., 2010). These studies further reported that interactions also occur between SB-269970 and other classes of antidepressants. Thus, ineffective doses of imipramine, desipramine, and moclobemide all reduced immobility in the mouse forced swim test when given in combination with an ineffective dose of SB-269970 (Wesolowska et al., 2007; Sarkisyan et al., 2010). The interaction between SB-269970 and imipramine has also been shown in Wistar rats using the forced swim test in which the effect on immobility was accompanied with an increase in 5-HT levels in the prefrontal cortex (Wesolowska & Kowalska, 2008). Besides the prefrontal cortex, the hippocampus has also been implicated in the effects of SB-269970 and imipramine in the rat forced swim test, as direct intra-hippocampal administration reduced immobility (Wesolowska et al., 2006b).

The validity of the tail suspension test and forced swim test as models of depression has been questioned since they are acute, whereas antidepressant treatment in human usually require several days to weeks before clinical effect is seen (Borsini & Meli, 1988; Cryan & Mombereau, 2004). In order to overcome this, models attempting to induce a depression-like state in laboratory animals have been developed. One such model is chronic unpredictable mild stress (Willner et al., 1992). In a recent study it was shown that 5-HT₇ receptor mRNA was upregulated in the hippocampus and hypothalamus, but not cortex, in rats after exposure to such stress (Li et al., 2009). The change in mRNA levels could be inhibited by treatment with fluoxetine and curcumin, an active ingredient in turmeric extracts (Li et al., 2009).

Indirect evidence that targeting the 5-HT₇ receptor for the treatment of depression might be successful was recently presented in a study showing that the antidepressant effect of amisulpride is most likely mediated by the 5-HT₇ receptor. Amisulpride is an atypical antipsychotic that is also a proven antidepressant (Lecrubier et al., 1997; Smeraldi, 1998). Previously it has generally been thought that the antidepressant effect of amisulpride somehow relied on its properties as a dopamine D₂/D₃ receptor antagonist, although the mechanism had never been satisfactorily explained. It has now been demonstrated that amisulpride has high affinity for the 5-HT₇ receptor and that amisulpride reduces immobility in both the tail suspension test and the forced swim test in 5-HT₇^{+/+} mice but not in 5-HT₇^{-/-} mice (Abbas et al., 2009). As such, these findings provide at least indirect evidence that the antidepressant effect of amisulpride is mediated by the 5-HT₇ receptor. It should also be noted that aripiprazole, another atypical antipsychotic that is successfully used to augment the effect of traditional antidepressants (Berman et al., 2009), has high affinity for the 5-HT₇ receptor (Lawler et al., 1999; Shapiro et al., 2003). In fact, aripiprazole has recently been shown to reduce immobility in the tail suspension and forced swim tests in 5-HT₇^{+/+} mice but not in 5-HT₇^{-/-} mice (Sarkisyan et al., 2010). Interestingly, aripiprazole has, like SB-269970, been shown to enhance the effect of an antidepressant, in this case fluoxetine (Kamei et al., 2008).

Hippocampal neurogenesis is a physiological phenomenon that has attracted considerable interest for its potential importance in depression (Sahay & Hen, 2007), especially since depressed patients have been found to have reduced hippocampal volume (Colla et al., 2007) and since neurogenesis is increased by chronic antidepressant treatment (Malberg et al., 2000; Perera et al., 2007). Thus, it has been speculated that the 5-HT₇ receptor might modulate hippocampal neurogenesis (Nandam et al., 2007). This notion is supported by the finding that the 5-HT₇ receptor modulates neurite growth in hippocampal cell cultures (Kvachnina et al., 2005). However, one study that looked directly at hippocampal cell proliferation did not find any difference between 5-HT₇^{+/+} and 5-HT₇^{-/-} mice (Sarkisyan & Hedlund, 2009). This lack of change could possibly be due to compensatory mechanisms in constitutive knockout mice. The lack of selective 5-HT₇ receptor antagonists that are metabolically stable enough to permit long-term treatment has to date made it impossible to study any possible effect of pharmacological inhibition on hippocampal cell proliferation.

Taken together with findings in sleep studies (see below) there is nevertheless a virtually consistent body of evidence using both pharmacological and genetic tools that implicates the 5-HT₇ receptor in depression, which has led to the hypothesis that new antidepressants could be developed that target the 5-HT₇ receptor (Hedlund, 2009; Mnie-Filali et al., 2007, 2009; Nandam et al., 2007).

3.6. Epilepsy

The first study implicating the 5-HT₇ receptor as a modulator of seizure activity was performed before the availability of selective antagonists (Bourson et al., 1997). Instead an affinity correlation analysis was done showing that the relative affinity of seven compounds

for the 5-HT₇ receptor correlated with their ability to protect against audiogenic seizures in DBA/2J mice. The compounds tested had in common that they inhibited 5-HT-stimulated cAMP formation in cells expressing the 5-HT₇ receptor and were thus antagonists at the receptor (Bourson et al., 1997). As outlined above, autoradiographic mapping of 5-HT₇ receptor binding sites has revealed that the highest density of these receptors is found in the thalamus (Bonaventure et al., 2004). It has been hypothesized that the 5-HT₇ receptors in this region might be of importance for epilepsy, especially in the WAG/Rij rat model of absence epilepsy (Graf et al., 2004). These particular rats exhibit spontaneously occurring spike-wave discharges that can be exacerbated by 8-OH-DPAT. With the realization that 8-OH-DPAT is an agonist not only for 5-HT_{1A} receptors, but also for 5-HT₇ receptors, it is likely that at least part of the potentiating effect of 8-OH-DPAT on epileptic activity is mediated by the 5-HT₇ receptor. It was further shown that the selective 5-HT₇ receptor antagonist SB-269970 reduced spontaneous epileptic activity in WAG/Rij rats (Graf et al., 2004).

In a study investigating both electrically- and chemically-induced tonic-clonic seizures it was found that the seizure threshold was lower in 5-HT₇ receptor knockout mice bred on a CD-1 background (Witkin et al., 2007). Using transcorneal electrical stimulation it was found that the 5-HT₇^{-/-} mice had lower tonic but not clonic seizure threshold. For chemically induced seizures it was found that 5-HT₇^{-/-} mice exhibited lower thresholds for seizures induced by the GABA antagonist pentylenetetrazole, as well as for seizures induced by cocaine. Another study found that 5-CT, a potent 5-HT_{1/7} receptor agonist, increased the threshold for seizures chemically induced with picrotoxin (Pericic & Svob Strac, 2007). The effect was present in both previously stressed and unstressed animals. The 5-CT-induced increase of the seizure threshold for picrotoxin in stressed mice could be counteracted with SB-269970, but not with the selective 5-HT_{1A} receptor antagonist WAY-100635. It was thus concluded that the 5-CT effect was mediated by the 5-HT₇ receptor (Pericic & Svob Strac, 2007). Finally, it has been suggested that the 5-HT₇ receptor is involved in regulating tonic-clonic seizure-induced antinociception (Freitas et al., 2009).

In summary, it is evident that further studies are needed to sort out the role of the 5-HT₇ receptor as being either proconvulsant or anticonvulsant depending on the type of epilepsy, species, or 5-HT₇ receptor manipulation studied.

3.7. Learning and memory

An early attempt to assess the role of the 5-HT₇ receptor in various behavioral paradigms used antisense oligonucleotides to inhibit receptor synthesis (Clemett et al., 1998). This report found no effects of the treatment in feeding, locomotor activity or anxiety-like behavior using an elevated plus maze. A more comprehensive study used 5-HT₇ receptor knockout mice to evaluate the role of this receptor in various behavioral and learning tasks (Roberts et al., 2004). It was found that mice lacking the 5-HT₇ receptor had a specific impairment in contextual fear conditioning. Contextual fear conditioning is generally believed to be a hippocampus-dependent learning, as are other types of place learning. It is therefore of interest that a first Barnes maze test included in this study showed no difference between wild-type and knockout mice (Roberts et al., 2004). However, in a later more extensive variant of the Barnes maze task a spatial learning deficit was revealed in the 5-HT₇^{-/-} mice (Sarkisyan & Hedlund, 2009). This later study also found novel location recognition in the knockout mice, another measurement of hippocampus-dependent learning. The conclusion drawn was that the 5-HT₇^{-/-} mice had a specific impairment that reduced their ability to respond to spatial changes in their environment. The two studies did not find any differences in hippocampus-independent learning tasks: cued fear conditioning, operant food conditioning, motor learning (rotarod), and novel object recognition (Roberts et al., 2004; Sarkisyan & Hedlund, 2009). The impairments seen were determined not to be due to

alterations in motor skills, visual acuity or anxiety level. The finding that impaired contextual fear conditioning is an early phenomenon in models of Alzheimer's disease opens up the intriguing possibility for an involvement of the 5-HT₇ receptor in this disorder (Corcoran et al., 2002; Dineley et al., 2002). A possible electrophysiological correlate to the hippocampus-dependent deficits has been established, as there was a reduced ability to induce LTP in the CA1 region of the hippocampus in 5-HT₇^{-/-} mice (Roberts et al., 2004). The 5-HT₇ receptor has also been implicated in memory formation in a Pavlovian learning test as the selective antagonists SB-269970 and DR4004 could reverse amnesia induced by scopolamine and dizocilpine (Meneses, 2004). Also in support of a pro-cognitive effect of the 5-HT₇ receptor it has been shown that the putative agonist AS-19 enhances memory formation and that this effect can be reversed by SB-269970 (Perez-García & Meneses, 2005). Attempts to reconcile the somewhat conflicting results on the 5-HT₇ receptor in learning and memory are ongoing with more work still needed (Perez-Garcia & Meneses, 2009).

3.8. Locomotion

As mentioned above, the 5-HT₇ receptor has been found to be involved in spinal locomotion already in early development (Madriaga et al., 2004). A series of studies have attempted to characterize this involvement in more detail, again using both pharmacological tools and knockout mice. It has been demonstrated that 5-HT has an important role in the central pattern generator for locomotion, parts of which are located within the spinal cord (Jordan et al., 2008). Serotonergic neurons located in the parapyramidal region (PPR) produce locomotion when stimulated. These neurons constitute the first anatomically discrete group of spinally-projecting neurons demonstrated to be involved in the initiation of locomotion in mammals. It has been shown that locomotion evoked from the PPR is mediated by 5-HT₇ and 5-HT_{2A} receptors (Jordan et al., 2008). Furthermore, it has been demonstrated that 5-HT₇ receptor antagonists block locomotion in cat, rat and mouse preparations, but that they have little effect in mice lacking 5-HT₇. In 5-HT₇ receptor knockout mice 5-HT induced rhythmic activity, but coordination among flexor and extensor motor nuclei and left and right sides of the spinal cord is disrupted. In adult 5-HT₇^{+/+} mice, 5-HT₇ receptor antagonists impair locomotion, producing patterns of activity resembling those induced by 5-HT in 5-HT₇^{-/-} mice (Jordan et al., 2008; Liu et al., 2009). Moreover, 5-HT₇^{-/-} mice displayed greater maximal extension at the hip and ankle joints than 5-HT₇^{+/+} mice during voluntary locomotion (Liu et al., 2009). Further evidence that the 5-HT₇ receptor is involved in spinal central pattern generator was obtained in a study examining electrically stimulated locomotion (Dunbar et al., 2010).

Knowledge of the mechanisms involved in spinal locomotion is of extreme importance when trying to find ways to restore function after spinal cord injury. In mice with the spinal cord transected at the thoracic level it has been shown that 8-OH-DPAT administered intraperitoneally acutely induced, within 15 min, hindlimb movements that share some characteristics with normal locomotion (Landry et al., 2006). Paraplegic mice pretreated with the selective 5-HT_{1A} receptor antagonists WAY-100135 or WAY-100635 displayed significantly less 8-OH-DPAT-induced movement. A similar reduction of 8-OH-DPAT-induced movements was found in animals pretreated with SB269970. Moreover, a near complete blockade of 8-OH-DPAT-induced movement was observed in 5-HT₇^{+/+} mice pretreated with 5-HT_{1A} and 5-HT₇ receptor antagonists, and in 5-HT₇^{-/-} mice pretreated with 5-HT_{1A} receptor antagonists (Landry et al., 2006). The overall conclusion of these studies was that 8-OH-DPAT potently induces locomotor-like movement in the previously paralyzed hindlimbs of low-thoracic-transected mice and that both 5-HT_{1A} and 5-HT₇ receptors are involved in spinal locomotor rhythmogenesis *in vivo*. In a related field it has been found that the 5-HT₇ receptor is also involved in modulating phrenic nerve motor

output, a finding that might also be of relevance for spinal cord injury (Macfarlane & Mitchell, 2009).

3.9. Migraine

The ability of 5-HT to induce cranial vasodilation under certain conditions is thought to be one of the mechanisms involved in migraine (Saxena & Ferrari, 1989). Although the effects of 5-HT are most likely not mediated by a single receptor subtype (Ullmer et al., 1995; Schoeffter et al., 1996; Verheggen et al., 2004), available evidence clearly suggest a role for the 5-HT₇ receptor. Initial interest in the 5-HT₇ receptor was driven by the observation that several migraine prophylactic drugs showed moderate to high affinity for the 5-HT₇ receptor (Bard et al., 1993; Ruat et al., 1993; Shen et al., 1993). Thus, it has been proposed that the 5-HT₇ receptor mediates 5-HT-induced dilation of the carotid artery following blockade of 5-HT_{1B/1D} receptors in combination with low sympathetic tone (Villalon et al., 1997). It is, however, not clear how relevant such a mechanism might be for migraine. Before the availability of selective 5-HT₇ receptor antagonists, pharmacological profiling was used to implicate the 5-HT₇ receptor as the most likely mediator of 5-HT induced vasodilation in preparations of the basilar and middle cerebral arteries (Terrón & Falcón-Neri, 1999). Later involvement of the 5-HT₇ receptor was confirmed since such vasodilation could be blocked *in vivo* by SB-269970 (Terrón & Martínez-García, 2007). This has been shown to also be true after 5-HT depletion, suggesting that the 5-HT₇ receptors involved are not sensitized by reduced availability of 5-HT (Martínez-García et al., 2009). The 5-HT₇ receptor has also been shown to mediate relaxation in a pial vein preparation (Ishine et al., 2000). As it is very difficult to model migraine in laboratory animals (Hess, 1996), further studies are needed to determine if the 5-HT₇ receptor can be targeted for the prophylaxis or treatment of migraine.

3.10. Pain

Several studies have investigated a possible role for the 5-HT₇ receptor in pain. Pain mediation and processing occur at many levels of the nervous system. Several of these, including peripheral, spinal, and thalamic mechanisms, have been evaluated with regard to the 5-HT₇ receptor. For peripheral pain, the 5-HT₇ receptor appears to mediate a pronociceptive effect that can be inhibited by SB-269970 (Rocha-González et al., 2005). This study used injections of formalin in the paw to induce flinching behavior. It was found that SB-269970 had a direct local peripheral antinociceptive effect when the drug was injected into the paw prior to formalin. Furthermore, SB-269970 could locally counteract the pronociceptive effect of 5-CT. When given intrathecally, SB-269970 had no direct effect on flinching, but could reverse the pronociceptive effect of 5-CT.

When additional studies performed on spinal and thalamic nociception are considered, the interpretation of 5-HT₇ receptor function becomes more complex. One study using mice lacking 5-HT₇ receptors did not see a difference in spinal pain mediation using the tail-flick test (Roberts et al., 2004). In another study also using the tail-flick model in mice, it was found that intrathecally administered SB-269970 blocked the analgesic effect of morphine given subcutaneously, thus suggesting that the antinociceptive activity of descending serotonergic pathways is mediated by 5-HT₇ receptors (Dogrul & Seyrek, 2009). Similar conclusions were drawn in a study assessing the effects of spinal microinjections of morphine and SB-269970 using a paw-flick model (Dogrul et al., 2009). It should be noted, that the anatomical localization of 5-HT₇ receptors within the spinal cord supports such an interpretation (Doly et al., 2005). Another study also showed that the antinociceptive properties of spinal serotonergic pathways were mediated by the 5-HT₇ receptor (Brenchat et al., 2009). Using both the putative agonist AS-19 and SB-269970, it was found that the 5-HT₇ receptor modulates capsaicin-induced mechanical hypersensitivity in mice. Thus, the agonist had a dose-dependent antinociceptive effect that could be counteracted by the

antagonist (Brenchat et al., 2009). Capsaicin was injected in the plantar surface of a paw, which was then mechanically stimulated. The serotonergic compounds evaluated were administered systemically by subcutaneous injections. In a different study, rats were exposed to an electric shock to the tail. Here, it was found that 8-OH-DPAT had an antinociceptive effect when injected into the medial thalamus, specifically the nucleus parafascicularis and the central lateral thalamic nucleus (Harte et al., 2005). This action could be counteracted by selective antagonists for both the 5-HT_{1A} and the 5-HT₇ receptor. The effect was observed for pain behaviors organized at the medullary and forebrain levels, but not at the spinal level, of the neuraxis (Harte et al., 2005). As noted above, in a possible link between 5-HT₇ receptors, epilepsy, and pain modulation it has been suggested that 5-HT₇ receptors are involved in regulating tonic-clonic seizure-induced antinociception (Freitas et al., 2009). Taken together, available data appear to suggest differing roles for the 5-HT₇ receptor in peripheral versus central pain mediation. Future studies will in all likelihood help to clarify fully the relationship between the 5-HT₇ receptor and pain.

3.11. Schizophrenia

Soon after the 5-HT₇ receptor was discovered a screen of several drugs of different classes was made to determine their affinity for the new receptor. Among the findings was the discovery that several antipsychotics had high affinity for the 5-HT₇ receptor (Roth et al., 1994), thus opening up the possibility that some of their effects may be mediated by this receptor. Even though certain typical antipsychotics were included in this group, particularly high affinities were seen for atypical antipsychotics such as clozapine and risperidone. It was thus speculated that some of the unique properties (e.g. the lack of extrapyramidal side effects) of these drugs could be attributed to an action at the 5-HT₇ receptor (Roth et al., 1994). More recently another drug, amisulpride, with both antipsychotic and antidepressant properties has also been shown to have high affinity for the 5-HT₇ receptor (Abbas et al., 2009). As discussed above, action at the 5-HT₇ receptor is most likely mediating the antidepressant effect of amisulpride, but might also play a role in its antipsychotic action. Several studies have attempted to test the possible relevance of 5-HT₇ receptors in schizophrenia using prepulse inhibition (PPI). This is a particularly attractive model as it can be used in both humans and laboratory animals. Even though PPI does not reflect all modalities of schizophrenia, it provides a model of the sensorimotor gating deficits seen in schizophrenia patients (Geyer et al., 2001). Of particular interest for the evaluation of antipsychotics, possible new treatments, and new treatment mechanisms is the ability to pharmacologically disrupt PPI. Thus, it has been possible to identify dopaminergic, serotonergic, and glutamatergic components of PPI. Of these it seems that the dopaminergic mechanisms are most relevant for the action of typical antipsychotics and the glutamatergic mechanisms are most relevant for atypical antipsychotics (Geyer et al., 2001). Using the selective 5-HT₇ receptor antagonist SB-258741 (Lovell et al., 2000), it has been shown that the 5-HT₇ receptor can modulate behavior in the PPI model in Wistar rats (Pouzet et al., 2002). Specifically, it was found that SB-258741 did not affect amphetamine-induced disruption of PPI, but that phencyclidine (PCP)-induced disruption was counteracted by SB-258741. Thus, the conclusion was that the 5-HT₇ receptor could influence the glutamatergic, but not the dopaminergic, component of PPI (Pouzet et al., 2002). Results in support of that notion were for the most part obtained in a study using 5-HT₇^{-/-} mice (Semenova et al., 2008). Thus, the ability of the dopaminergic drugs apomorphine and amphetamine to disrupt PPI was unaltered in 5-HT₇^{-/-} mice compared to 5-HT₇^{+/+} mice, whereas the ability of PCP to disrupt PPI was diminished in the 5-HT₇^{-/-} mice. However, this study did not find any influence of SB-269970 on PCP-disrupted PPI in either C57BL/6J mice (the background strain of the 5-HT₇^{-/-} mice) or Wistar rats (Semenova et al., 2008). This and another study using a different strain of 5-HT₇^{-/-} mice did not observe any inherent differences in PPI in mice lacking the 5-HT₇ receptor (Guscott et al., 2005;

Semenova et al., 2008). A recently published study made somewhat conflicting observations using C57BL/6J mice (Galici et al., 2008). This report described an effect also on the dopaminergic component of PPI. Thus, SB-269970 was able to reverse amphetamine-induced disruption of PPI in C57BL/6J mice. Again, no effect of SB-269970 on the glutamatergic component of PPI was observed as evaluated using ketamine-induced disruption (Galici et al., 2008).

In a study of humans, it has been shown that 5-HT₇ receptor mRNA is downregulated in the dorsolateral prefrontal cortex, but not the hippocampus, of individuals with schizophrenia (East et al., 2002). A study analyzing a possible correlation between single nucleotide polymorphisms in the 5-HT₇ receptor gene and schizophrenia found a positive association for two of four evaluated polymorphisms within cohorts of 383 Japanese schizophrenia patients and 351 controls (Ikeda et al., 2006). It is unlikely, however, that the polymorphisms in question cause any differences in 5-HT₇ receptor function. In another population study no correlation was found between single nucleotide polymorphisms in the 5-HT₇ receptor gene and the clinical efficacy of risperidone (Wei et al., 2009), an antipsychotic with high affinity for the 5-HT₇ receptor (Roth et al., 1994).

3.12. Sleep

Among the clinical manifestations of depression is often a dysregulated circadian rhythm and sleep disturbances (Belmaker & Agam, 2008). Specifically, a decreased latency to and increased amount of rapid eye movement (REM) sleep can often be detected in EEG recordings (Yadid et al., 2000). Direct involvement of the 5-HT₇ receptor in sleep regulation has been shown using selective antagonists and knockout mice. Both SB-269970 and SB-656104, a structurally related selective antagonist (Thomas et al., 2003), when administered to Sprague-Dawley rats at the beginning of the light period, increased the latency to REM sleep and decreased the amount of time spent in REM sleep (Hagan et al., 2000; Thomas et al., 2003). Other sleep parameters were not affected. It has further been shown that mice lacking the 5-HT₇ receptor exhibit a similar reduction in time spent in REM sleep during the light period, again without affecting other sleep phases (Hedlund et al., 2005). The 5-HT₇^{-/-} mice also had less frequent and longer REM episodes than the 5-HT₇^{+/+} mice. In the 5-HT₇^{-/-} mice there was no change in latency to REM and citalopram was equally effective in increasing the latency to REM in both genotypes (Hedlund et al., 2005). Of considerable interest is that, as in the depression models discussed above, an interaction has been observed between individually ineffective doses of SB-269970 and citalopram also for sleep where such a combination of drugs resulted in an increase in latency to REM and a decrease in the amount of REM sleep in rats (Bonaventure et al., 2007). A more detailed analysis revealed that the decrease in the amount of REM sleep was due to a reduced number of REM episodes. Citalopram alone caused an increased fragmentation of sleep as seen from an increase in the number of microarousals. This fragmentation could be reversed by SB-269970 (Bonaventure et al., 2007). Furthermore, three antidepressants of the SSRI class (citalopram, fluoxetine, and paroxetine), but not a tricyclic antidepressant (desipramine), have been demonstrated to augment the effects on latency to REM sleep and REM sleep duration seen in 5-HT₇^{-/-} mice (Shelton et al., 2009). Again the reduction in the amount of REM sleep was caused by a reduced number of REM episodes. A recent study found that microinjections of either the putative 5-HT₇ receptor agonist LP-44 or SB-269970 into the dorsal raphe nucleus both reduced REM sleep and the number of REM episodes when injected during the light cycle (Monti et al., 2008). Interestingly, the LP-44 effect could be counteracted by pretreatment with SB-269970. Further studies are clearly needed to fully clarify the interplay between 5-HT₇ receptor agonism and antagonism on sleep. In view of the importance of the 5-HT₇ receptor in the

hippocampus and its effect on REM sleep it is of interest to note that the levels of 5-HT are influenced by the different sleep phases in this region of the brain (Peñalva et al., 2003).

Sleep is also often disturbed in schizophrenia. It is interesting to note that atypical antipsychotics with high affinity for the 5-HT₇ receptor, such as clozapine and risperidone, show the greatest improvements on sleep parameters (Cohrs, 2008; Roth et al., 1994). This is another area that warrants further study.

3.13. Substance abuse

A possible link between the 5-HT₇ receptor and substance abuse is an area that remains to be explored. Nevertheless, a phenomenon that is closely linked with drug addiction is novelty-seeking behavior and it has recently been hypothesized that the 5-HT₇ receptor influences such behavior (Ballaz et al., 2007a, 2007b). In a first study Sprague-Dawley rats were classified as high or low responders when measuring the amount of locomotor activity after the animals were placed in an enclosed open arena (Ballaz et al., 2007a). There were differences in 5-HT₇ receptor mRNA expression in several brain regions between low and high responders. Notably, there was higher expression in the hippocampus in the low responding rats. This finding was interpreted to indicate that low levels of 5-HT₇ mRNA expression correlated with decreased aversion to forced exposure to novelty (Ballaz et al., 2007a). In a follow-up study it was found that the low responding rats showed increased exploration of a new object in a novel object discrimination task and that this increase could be diminished by SB-269970 (Ballaz et al., 2007b). It should, however, be noted that mice lacking the 5-HT₇ receptor did not differ from 5-HT₇^{+/+} mice in novel object recognition, but instead exhibited reduced novel location recognition which is a more hippocampus-dependent behavior (Sarkisyan & Hedlund, 2009).

In a more direct model of impulsivity, delayed reinforcement, with possible relevance for drug abuse and attention deficit hyperactivity disorder, it was found that rats treated with methylphenidate during adolescence showed reduced impulse behavior as adults (Leo et al., 2009). This behavior could be counteracted by SB-269970 administered at the time of testing. The observed behavioral changes are possibly related to changes in gene expression since methylphenidate has been shown to upregulate 5-HT₇ mRNA expression in the striatum (Adriani et al., 2006) and nucleus accumbens (Leo et al., 2009). For now it is unclear if and how these results relate to each other. Thus, any possible relevance for the 5-HT₇ receptor in novelty seeking, impulsivity, and substance abuse remains to be fully determined.

3.14. Thermoregulation

That 5-HT is involved in the regulation of body temperature is a well-known phenomenon. Injection of 5-CT or 8-OH-DPAT will induce hypothermia. It has been established that the effect is similar regardless of whether the drug is administered systemically or directly into the brain. Thus, there is in all likelihood a central mechanism of action. As 5-CT and 8-OH-DPAT are both 5-HT_{1A} receptor agonists, this receptor was generally considered the main mediator of the hypothermia, although some reports had suggested involvement of other receptor subtypes. The first evidence that the 5-HT₇ receptor is of importance for 5-HT-induced hypothermia came when it was reported that the effect of 5-CT on body temperature in guinea pigs could be blocked by SB-269970 (Hagan et al., 2000). Such an antagonistic effect has also been observed for SB-656104, another selective 5-HT₇ receptor antagonist (Thomas et al., 2003). It was not possible to block the hypothermic effect of 5-CT in mice with the 5-HT_{1A} receptor antagonist WAY-100635 or the 5-HT_{1B/D} antagonist GR127935 (Guscott et al., 2003). In contrast, the hypothermic effect of 8-OH-DPAT in rats could be inhibited by a 5-HT_{1A} antagonist (WAY-100135), but only partially by SB-269970

(Hedlund et al., 2004). The importance of the 5-HT₇ receptor was further emphasized when it was observed that 5-HT and 5-CT failed to induce hypothermia in 5-HT₇ receptor knockout mice (Guscott et al., 2003; Hedlund et al., 2003). When including 8-OH-DPAT in the analysis in combination with selective antagonists and knockout mice to discriminate between 5-HT_{1A} and 5-HT₇ receptors it was revealed that both receptor subtypes are involved in 5-HT-mediated hypothermia (Hedlund et al., 2004). These findings provide an explanation for the results obtained with antagonists and also why 8-OH-DPAT fails to induce hypothermia in 5-HT_{1A} receptor knockout mice (Heisler et al., 1998). LP-211 has been found to induce hypothermia in mice. The hypothermic effect could be inhibited by SB-269970, but not WAY-100135, and was absent in 5-HT₇ receptor knockout animals (Hedlund et al., 2010). Interestingly, the 5-HT₇ receptor seems to be most important at low agonist concentrations, thus contributing to the fine-tuning of temperature homeostasis, whereas the 5-HT_{1A} receptor comes into play at higher agonist concentrations possibly providing a defense against hyperthermia (Hedlund et al., 2004).

4. Conclusion

Through the combined use of modern molecular biology, knockout animal models, and other more traditional research methods such as medicinal chemistry and classical pharmacology, a clearer picture of the pathophysiological role of the 5-HT₇ receptor is emerging. The availability of selective antagonists and knockout mice strains has significantly increased our knowledge about this receptor. Together with nonselective agonists, these tools have helped to reveal the 5-HT₇ receptor distribution and function in more detail. Important functional roles for the 5-HT₇ receptor in thermoregulation, circadian rhythm, hippocampal signaling and sleep have been established. Hypotheses driving current research indicate that 5-HT₇ receptor might be involved in mood regulation, suggesting that the 5-HT₇ receptor is a target in the treatment of depression. The recent availability of more selective 5-HT₇ receptor agonists is opening new perspectives about the involvement of 5-HT₇ receptors in learning and memory, and in the control of nociception. Hopefully, these early results will be substantiated by future studies, especially *in vivo*, that will use newer selective antagonists and agonists endowed with optimal druglike properties. While the identification of selective antagonists would take advantage from a large body of medicinal chemistry literature about the design of selective antagonists, the development of selective agonists will require more extensive studies because the structural features that differentiate a selective agonist versus a nonselective one are yet to be identified.

Abbreviations

AC	adenylyl cyclase
CoMFA	comparative molecular field analysis
GPCR	G protein-coupled receptor
PPI	prepulse inhibition
REM	rapid eye movement
SCN	suprachiasmatic nucleus
SSRI	selective serotonin reuptake inhibitor
TMH	transmembrane helix

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Figure 1.
Chemical structures of nonselective 5-HT₇ agents

Figure 2.
Examples of N-N'-Disubstituted guanidine derivatives

Figure 3.

Optimized pharmacophore model for 5-HT₇ antagonists proposed by López-Rodríguez et al. (2003)^a

^aDistances between pharmacophoric features: PI-HYD3 = 5.4–6.4 Å; PI-HYD1 = 5.2–6.2 Å; PI-HBA = 5.6–6.6 Å; HYD1-HBA = 3.6–4.8 Å; HYD1-HYD3 = 9.3–10.3 Å

Figure 4.

Pharmacophore model for 5-HT₇ agonists proposed by Vermeulen et al. (2003)^a

^aDistances between pharmacophoric features: PI-HYD1 = 5.7 Å; PI-HBA = 6.2 Å; HYD1-HBA = 3.0 Å; HYD1-HYD2 = 4.2 Å

296 $K_i = 19$ nM

297 $K_i = 19$ nM

Figure 5.
5-HT₇ inverse agonists developed by Vermeulen et al. (2004)

Figure 6.

Pharmacophore model for 5-HT₇ inverse agonists proposed by Vermeulen et al. (2004)^a

^aDistances between pharmacophoric features: PI-HYD1 = 4.4 Å; PI-HBA = 5.8 Å; HYD1-HBA = 3.3 Å; HYD1-HYD4 = 4.4 Å; HYD4-HBA = 3.8 Å; HY3-HBA = 8.7 Å;

Figure 7.

Receptor based pharmacophore model for 5-HT₇ antagonists proposed by Kołaczkowski et al. (2006)^a

^aDistances between pharmacophoric groups: PI-HYD/AR1= 6.9–9.4 Å; PI-HBA1 = 3.7–5.1 Å; PI-HYD/AR3= 3.7–6.1 Å; PI-HYD/AR2= 4.3–7.8 Å; PI-HBA2= 5.1–6.9 Å

Table 1

Percentages of amino acid homologies in the transmembrane domains between the 5-HT₇ receptor and other cloned 5-HT receptors.

Receptor	Human ^a	Rat ^b	Guinea Pig ^c	Mouse ^d
5-HT _{dro1}	57	56	48	42
5-HT _{1A}		45	40	
5-HT _{1B}		50	43	36
5-HT _{1D}		47	43	36
5-HT _{1E}		45	42	36
5-HT _{1F}		48	42	
5-HT _{2A}		39	35	
5-HT _{2C}			34	
5-HT ₅		46		32
5-HT ₆		44		

^a Bard et al. (1993)

^b Shen et al. (1993)

^c Tsou et al. (1994)

^d Plassat et al. (1993)

Table 2Stimulation of cAMP accumulation by cloned 5-HT₇ receptor from various species

Agonist	Species	Cell Line	EC ₅₀ (nM)	Antagonists
5-HT ^a	Rat	HEK-293	63	methiothepin, clozapine
5-HT ^b	Rat	CHO	1.2	mianserin
5-HT ^c	Rat	HeLa	13	--
5-HT ^d	Human	Cos-7	992	methiothepin
5-HT ^e	Mouse	Cos-7	45	methiothepin, methysergide, ergotamine
5-CT ^c	Rat	HeLa	156	mesulergine, ritanserin, NAN-190
5-CT ^f	Guinea Pig	CHO-K1	3.2	methiothepin, spiperone
5-CT ^e	Mouse	Cos-7	5.0	methiothepin, methysergide, mesulergine

^aShen et al. (1993)^bRuat et al. (1993)^cLovenberg et al. (1993)^dBard et al. (1993)^ePlassat et al. (1993)^fTsou et al. (1994)

Table 3Drug effects on basal and 5-CT-stimulated adenylyl cyclase activity^a

Antagonist	p <i>K_B</i> ^b	pIC ₅₀ ^c	% Inhibition of Basal ^d
Risperidone	8.3 (0.1)	7.6	86
Methiothepin	8.1 (1)	7.3	82
Mesulergine	7.5 (1)	7.1	49
Clozapine	7.2 (1)	6.6	79
Olanzapine	6.3 (10)	6.3	88
Ketanserin	6.1 (10)	6.0	77

^aThomas et al. (1998)^bp*K_B* (−log antagonist equilibrium dissociation constant) calculated from the antagonism of 5-CT-stimulated adenylyl cyclase activity. The concentration of drug tested (μM) is shown in parentheses^cpIC₅₀ (−log IC₅₀) for inhibition of basal adenylyl cyclase activity^d% inhibition of basal adenylyl cyclase activity for antagonists, measured in the absence of added agonist

Table 4

Inverse agonist potency (pIC_{50}) and efficacy at human 5-HT₇ receptor splice variants determined from inhibition of constitutive adenylyl cyclase activity.^a

Antagonist	pIC_{50} ^b					Efficacy (% of methiothepin)		
	5-HT _{7a}	5-HT _{7b}	5-HT _{7c}	5-HT _{7a}	5-HT _{7b}	5-HT _{7c}	5-HT _{7b}	5-HT _{7c}
Methiothepin	9.04	9.07	9.18	100	100	1000	1000	100
Metergoline	7.96	8.22	8.49	26	36	20	36	20
Mesulergine	NA ^c	7.73	NA	0	47	-10	47	-10
Clozapine	7.47	7.69	7.68	96	91	96	91	96
Spiperone	7.75	7.73	7.79	103	101	97	101	97
Rianserin	7.62	7.37	7.64	110	100	93	100	93
Methysergide	7.36	7.35	7.21	18	19	-2	19	-2
Ketanserin	6.59	6.42	6.61	106	95	93	95	93

^aKrobert & Levy (2002)

^b Adenylyl cyclase activity measured in the presence of increasing concentrations of antagonist in membranes of HEK-293 cells stably expressing either human 5-HT_{7a}, 5-HT_{7b} or 5-HT_{7c} receptors, and potency and efficacy of antagonist inhibition of constitutive adenylyl cyclase activity was determined. Inverse agonist efficacy is reported as a percentage of the inhibition of basal adenylyl cyclase activity produced by 10 μ M methiothepin

^cNot applicable

Table 5

pK_i values of selected ligands at recombinant 5-HT₇ receptor from human, rat, mouse, and guinea-pig expressed in Cos-7 cells using [³H]5-HT as radioligand

Ligand	Human ^a	Rat ^b	Mouse ^c	Guinea pig ^d
5-CT	9.0	9.8	9.0	9.4
Methiothepin	8.4	9.4	8.2	8.0
5-MeOT	8.3	9.2	8.2	9.0
Metergoline	8.2	8.2	7.5	
5-HT	8.1	8.8	8.3	9.0
Mesulergine	7.7	7.7	7.6	
2-Br-LSD	7.5		8.0	
Methysergide	7.1	7.9	7.9	7.4
Sipiperone	7.0		7.2	7.0
Cyproheptadine	6.9	7.5		
Tryptamine	6.8	7.8		
8-OH-DPAT	6.3	7.5	6.6	7.3
Sumatriptan	6.0	6.6	5.7	6.6
Ketanserin	5.9	6.6	6.4	

^aBard et al. (1993)

^bShen et al. (1993)

^cPlassat et al. (1993)

^dTsou et al. (1994)

Table 6

K_i values [nM] of selected ligands at human cloned 5-HT₇ receptor obtained from competition assays with different radioligands.

Ligand	[³ H]5-HT	[³ H]5-CT	[³ H]LSD
5-CT	0.93 ^a	0.79 ^b	
5-HT	8.1 ^a	6.3 ^b	
8-OH-DPAT	466 ^a	251 ^b	
Clozapine	48 ^c	63.1 ^b	17.9 ^d
Ketanserin	1134 ^a	794 ^b	
Mesulergine	18 ^a	31.6 ^b	
Risperidone	4.3 ^c		6.6 ^d
Amisulpride		135.5 ^e	11.5 ^e

^aBard et al. (1993)

^bThomas et al. (1998)

^cFernández et al. (2005)

^dKroeze et al. (2003)

^eAbbas et al. (2009)

Table 7

Localization and abundance of 5-HT receptor mRNA in the rat

Technique	Localization (Relative Abundance)
Northern Blot ^a	hypothalamus (+++), hippocampus (++), mesencephalon (++), cortex (++), olfactory bulb (+), olfactory tubercle (+), spleen (+) (not detected in retina, pituitary, testis, stomach, prostate, ovary, skeletal muscle, lung, liver, kidney, gut)
Northern Blot ^b	hypothalamus (+++), brainstem (+++), hippocampus (+++), stomach (+), ileum (+)
<i>In situ</i> hybridization ^b	retrosplenial cortex (+++), hippocampus (+++), tenia tecta (+++), indusium griseum (+++), posterior hypothalamus (++) (+), medial amygdala nucleus (+++), thalamus (+++), cerebellum-Purkinje cell layer- (++++), pontine nuclei (+++), superior colliculus (+), raphe nucleus (+)
Northern Blot ^c	hypothalamus (+++), thalamus (+++), hippocampus (+), cortex (+) (not detected in urinary bladder, testis, liver, spleen, adrenal gland, kidney, lung, heart, pituitary)
<i>In situ</i> hybridization ^c	hippocampus (+++), thalamus (+), hypothalamus (+)
Northern Blot ^d	hypothalamus (+++), thalamus (+++), hippocampus (++), cortex (++), medulla (++) (not detected in cerebellum, striatum, heart, liver, kidney, adrenal glands, testis, ovaries, spleen)
<i>In situ</i> hybridization ^e	hippocampus, thalamus, entorhinal and piriform cortices, tenia tecta
<i>In situ</i> hybridization ^d	thalamus (+++), hippocampus (++), retrosplenial cortex (++) (+), neocortex (++) (+), hypothalamus (+) (not detected in suprachiasmatic nucleus)
RT-PCR ^f	lumbar dorsal root ganglia, superior cervical ganglia, lumbar sympathetic ganglia
RT-PCR ^g	vena cava (+++), femoral vein (++) (+), aorta (+), renal artery (+), portal vein (+) (not detected in jugular vein)
<i>In situ</i> hybridization ^h	outer layer of the cortex, thalamus, hippocampus
<i>In situ</i> hybridization ⁱ	thalamus (+++), hippocampus (+++), retrosplenial cortex (+++), mammillary region (+++), posterior thalamic nucleus (+), suprachiasmatic nucleus (+)
RT-PCR ^j	frontocortical astrocytes, hypothalamus
RT-PCR ^k	adrenal gland
RT-PCR ^l	submandibular gland
RT-PCR ^m	thymus, peripheral blood lymphocytes, spleen, mitogen activate spleen cells
RT-PCR ⁿ	adrenal cortex
<i>In situ</i> hybridization ^o	forebrain (+++), olfactory complex (+++), thalamus (+++), hippocampal formation (+++), hypothalamus (+++), suprachiasmatic nucleus (+++), septal region (++) (+), amygdala (++) (+), parvocellular (++)

^aShen et al. (1993);^bRuat et al. (1993);^cMeyerhof et al. (1993);^dLovenberg et al. (1993);^eKinsey et al. (2001);^fPierce et al. (1997);^gUlmer et al. (1995);^hMengod et al. (1996);ⁱVenero et al. (1997);^jShimizu et al. (1996);^kContesse et al. (1999);

^lBourdon et al. (2000);

^mStefulj et al. (2000);

ⁿLenglet et al. (2000);

^oNeumaier et al. (2001)

Table 8Localization and abundance of 5-HT₇ receptor mRNA in guinea-pig, human, mouse, and pig

Species	Technique	Localization (Relative Abundance)
Guinea pig ^a	Northern Blot	parietal cortex (+++), hippocampus (++), frontal cortex (++), cerebellum (+), ileum (+), spleen (+)
Guinea pig ^a	<i>In situ</i> hybridization	hippocampus (+++), periventricular thalamus (+++), superficial cortex (+++), cerebellar granule cells (+)
Guinea pig ^b	<i>In situ</i> hybridization	medial thalamic nucleus (+++), hippocampal formation (+++), superficial layer cortex (++), medial geniculate nucleus (++), amygdala (++), hypothalamus (++), midbrain (+), hindbrain (+)
Guinea pig ^c	<i>In situ</i> hybridization	outer layer of the cortex, thalamus, hippocampus
Guinea pig ^d	Northern Blot ^b	thalamus (+++), brainstem (+++), hypothalamus (+++), substantia nigra (+++), olfactory bulb (+++), olfactory tubercle (+++) (not detected in peripheral organs)
Human ^e	RT-PCR	artery smooth cells (+++), pulmonary artery smooth cells (++) (not detected in coronary artery, pulmonary artery, aortic endothelial)
Human ^f	RT-PCR	dorsal root ganglia
Human ^g	RT-PCR	granulosa-lutein cells
Human ^g	Northern Blot	granulosa-lutein cells
Human ^h	RT-PCR	brain (+++), kidney (+), liver (+), pancreas (+), spleen (+), coronary artery (++), stomach (++), descending colon (++), ileum (++)
Mouse ⁱ	Northern Blot	not detected in brain, heart, kidney, lung, liver
Mouse ⁱ	RT-PCR	forebrain (+++), brain stem (+++), cerebellum (++), colliculi (++), intestine (+), heart (+), not detected in spleen, kidney, lung, liver
Pig ^e	RT-PCR	pulmonary artery (++), coronary artery (++), cerebral artery (++), cerebral vein (++)
Pig ^j	RT-PCR	myometrium
Pig ^k	RT-PCR	brain cortex, trigeminal ganglion, cerebellum, pulmonary artery, coronary artery, superior vena cava, saphenous vein, not detected in heart

^aTsou et al. (1994);^bTo et al. (1994);^cMengod et al. (1996);^dRuat et al. (1993);^eUlmer et al. (1995);^fPierce et al. (1997);^gGraveleau et al. (2000);^hBard et al. (1993);ⁱPlassat et al. (1993);^jKitazawa et al. (2000);^kBhalla et al. (2002)

Table 9

Binding affinities at 5-HT₇ and 5-HT_{2A} receptors of “long-chain” arylpiperazine derivatives with a tetrahydrobenzindole nucleus in the terminal position.

Compound	Ar	n	p <i>K</i> _i	
			5-HT ₇ ^a	5-HT _{2A} ^b
1^c	Ph	2	6.99	8.27
2^c	Ph	3	8.29	7.79
3^c	Ph	4	8.48	7.37
4^c	cyclohexyl	4	<6	<6
5^c	2-MeO-Ph	4	8.29	6.95
6^d	3-MeO-Ph	4	8.63	7.19
7^d	4-MeO-Ph	4	7.76	6.69
8^d	2-Cl-Ph	4	7.91	7.01
9^c	2-CN-Ph	4	8.42	6.98
10^d	2-CONH ₂ -Ph	4	7.76	6.05
11^d	2-COCH ₃ -Ph	4	8.10	6.45
12^d	2-CF ₃ -Ph	4	7.13	5.86
13^d	2-NO ₂ -Ph	4	7.62	7.34
14^d	2-CH ₃ -Ph	4	7.98	7.35
15^d	2,6-diCH ₃ -Ph	4	6.83	5.85

^a Determined at human 5-HT₇ receptors in COS-7 cells using [³H]5-CT.

^b Determined at 5-HT_{2A} receptors in rat cerebral cortex membranes using [³H]ketanserin.

^c Kikuchi et al. (1999)

^d Kikuchi et al. (2002a)

Table 10

Structure-activity relationships of tetrahydrobenzindole derivatives

Compound	X	p <i>K_i</i>	
		5-HT ₇ ^a	5-HT _{2A} ^b
16 ^c		8.40	6.84
17 (DR4004) ^d		8.67	7.01
18 ^e		8.35	6.21
19 ^e		8.19	6.08

Compound	X	p <i>K_i</i>	
		5-HT ₇ ^a	5-HT _{2A} ^b
20 ^e		7.80	<6
21 ^e		6.25	<6
22 ^e		6.09	<6

^aDetermined at human 5-HT₇ receptors in COS-7 cells using [³H]5-CT.

^bDetermined at 5-HT_{2A} receptors in rat cerebral cortex membranes using [³H]ketanserin.

^cKikuchi et al. (1999)

^dKikuchi et al. (2002a)

^eKikuchi et al. (2002b)

Table 11

Binding affinities at 5-HT₇ and 5-HT_{2A} receptors of 2a-[4-(tetrahydropyridoindol-2-yl)butyl]tetrahydrobenzindole derivatives^a

Compound	R	p <i>K</i> _i	
		5-HT ₇ ^b	5-HT _{2A} ^c
23	H-	8.24	6.62
24	CH ₃ -	7.72	6.98
25	CH ₃ OCH ₂ -	7.77	6.24
26	CH ₃ CO-	8.19	6.27
27	CH ₂ =CHCH ₂ -	8.22	6.55
28	H ₂ NCOCH ₂ -	8.06	6.09
29	(CH ₃) ₂ NCOCH ₂ -	7.72	<6
30 (DR-4365)	(CH ₃)HNCCH ₂ -	8.45	<6

^a Kikuchi et al. (2002a)

^b Determined at human 5-HT₇ receptors in COS-7 cells using [³H]5-CT.

^c Determined at 5-HT_{2A} receptors in rat cerebral cortex membranes using [³H]ketanserin.

Table 12Structure-activity relationships of 1-[ω -(4-aryl-1-piperazinyl)alkyl]-1-arylketone derivatives^a

Compound	R ₁	R ₂	K _i (nM)	
			5-HT ₇	5-HT _{1A}
31	2-OH	2-CH ₃ O-Ph	5.8	5.8
32	2-OH	CH ₃	>1000	>850
33	2-OH	cyclohexyl	>800	>850
34	2-OH	2-Py	105	56
35	2-OH	Ph	43	137
36	2-OH	3-CF ₃ -Ph	384	282
37	2-OH		148	1459
38	2-OH		682	>850
39	2-OH		462	389

Compound	R ₁	R ₂	K _i (nM)	
			5-HT ₇	5-HT _{1A}
40	2-OH		2.93	189
41	2-OCH ₃		0.90	175

^aPerrone et al. (2003)

^bDetermined at rat 5-HT₇ receptors in HEK-293 cells using [³H]LSD.

^cDetermined at 5-HT_{1A} receptors in rat cerebral hippocampus membranes using [³H]8-OH-DPAT.

Table 13Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of 4-arylethyl-1-arylpiperazine derivatives^a

Compound	Ar	Ar'	<i>K_i</i> (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
42	3-OCH ₃ -Ph	2-OCH ₃ -Ph	24.5	2.37
43	3-OH-Ph	2-OCH ₃ -Ph	35.4	8.12
44	2-OCH ₃ -Ph	2-OCH ₃ -Ph	160	not tested
45	4-OCH ₃ -Ph	2-OCH ₃ -Ph	126	not tested
46	2,6-di-OCH ₃ -Ph	2-OCH ₃ -Ph	747	not tested
47	2,5-di-OCH ₃ -Ph	2-OCH ₃ -Ph	31.9	21.9
48	3,4-di-OCH ₃ -Ph	2-OCH ₃ -Ph	48.3	8.36
49	2,3,4-tri-OCH ₃ -Ph	2-OCH ₃ -Ph	71	4.50
50	2,4,6-tri-OCH ₃ -Ph	2-OCH ₃ -Ph	300	not tested
51	3-OCH ₃ -Ph	3-(1,2-benzisoxazolyl)-	8.2	3.63
52	3-OCH ₃ -Ph	2-OH-Ph	51.5	3.50
53^d	3-OCH ₃ -Ph	4-OCH ₃ -Ph	> 1000	not tested

^aLeopoldo et al. (2004b)^bDetermined at rat 5-HT₇ receptors in HEK-293 cells using [³H]LSD.^cDetermined at 5-HT_{1A} receptors in rat cerebral hippocampus membranes using [³H]8-OH-DPAT.^dLeopoldo et al. (unpublished)

Table 14

K_i values at 5-HT₇ and 5-HT_{1A} receptors of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides^a

Compound	R	K_i (nM)	
		5-HT ₇ ^b	5-HT _{1A} ^c
54	2-OCH ₃	6.64	8.6
55	3-OCH ₃	119	105
56	4-OCH ₃	2100	not tested
57	2-CN	48.7	16.6
58	3-CN	97.8	291
59	4-CN	1400	not tested
60	2-COCH ₃	4.14	3.8
61	3-COCH ₃	496	676
62	4-COCH ₃	2639	not tested

^a Leopoldo et al. (2004a)

^b Determined at rat cloned 5-HT₇ receptors in HEK-293 cells using [³H]LSD.

^c Determined at human cloned 5-HT_{1A} receptors in HEK-293 using [³H]8-OH-DPAT.

Table 15 K_i values at 5-HT₇ and 5-HT_{1A} receptors of 1-arylpiperazine derivatives^a

Compound	R	K_i (nM)	
		5-HT ₇ ^b	5-HT _{1A} ^c
63 (LP-44)	SCH ₃	0.22	52.7
64	CH ₃	15.2	279
65	OH	11.4	24.0
66	H	65.6	128
67	NO ₂	63.3	183
68	Cl	40.1	96.0
69	CONH ₂	229	494
70	SO ₂ CH ₃	298	3124
71	CH ₂ CH ₃	7.10	79.2
72	(CH ₂) ₂ CH ₃	49.6	168
73	(CH ₂) ₃ CH ₃	2810	60.0
74	CH(CH ₃) ₂	1.10	167
75	C(CH ₃) ₃	538	1196
76 (LP-12)	Ph	0.13	60.9
77	N(CH ₃) ₂	0.90	112
78	NHCH ₃	25.4	133
79	NH ₂	8178	415
80	NHCOCH ₃	338	2500
81	NHSO ₂ CH ₃	4253	not tested
82	F	131	29.2

^aLeopoldo et al. (2004a, 2007)^bDetermined at rat cloned 5-HT₇ receptors in HEK-293 cells using [³H]LSD.^cDetermined at human cloned 5-HT_{1A} receptors in HEK-293 using [³H]8-OH-DPAT.

Table 16

Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of some 1-arylpiperazine derivatives reported by Leopoldo et al. (2008)

Compound	n	R	<i>K_i</i> (nM)	
			5-HT ₇ ^a	5-HT _{1A} ^b
83	1	SCH ₃	42	1.07
84	1	Ph	8.3	124
85	1	CH(CH ₃) ₂	54	22
86	1	OCH ₃	179	3.2
87	2	SCH ₃	32.9	2.04
88	2	Ph	3.81	95
89	2	CH(CH ₃) ₂	6	3.7
90	2	OCH ₃	31	40

^aDetermined at rat 5-HT₇ receptors in HEK-293 cells using [³H]LSD.

^cDetermined at human cloned 5-HT_{1A} receptors in HEK-293 using [³H]8-OH-DPAT.

Table 17 K_i values at 5-HT₇ and 5-HT_{1A} receptors of 1-arylpiperazine derivatives^a

Compound	Ar	R	K_i (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
91		SCH ₃	22	12
92		Ph	not determined	161
93		CH(CH ₃) ₂	215	139
94		OCH ₃	224	13
95		SCH ₃	34.8	8.2
96		Ph	0.98	70
97		CH(CH ₃) ₂	5.1	325
98		OCH ₃	389	19

Compound	Ar	R	K_i (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
99		SCH ₃	9.0	94
100 (LP-211)		Ph	0.58	188
101		CH(CH ₃) ₂	8.6	53
102		OCH ₃	296	43
103		SCH ₃	148	10.3
104		Ph	5.7	106
105		CH(CH ₃) ₂	76	57
106		OCH ₃	71	29

^a Leopoldo et al. (2008)

^b Determined at rat cloned 5-HT₇ receptors in HEK-293 cells using [³H]LSD.

^c Determined at human cloned 5-HT_{1A} receptors in HEK-29 using [³H]8-OH-DPAT.

Table 18Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of 1-(2-methoxyphenyl)piperazine derivatives^a

Compound	Linker	<i>K_i</i> (nM)	
		5-HT ₇ ^b	5-HT _{1A} ^c
107 (NAN-190)		87	0.6
108		36	5
109		353	77
110		367	405
111		2045	8

^a Bojarski et al. 2004^b Determined at 5-HT₇ receptors in rat hypothalamus membranes using [³H]5-CT.^c Determined at 5-HT_{1A} receptors in rat cerebral hippocampus membranes using [³H]8-OH-DPAT.

Table 19

Binding affinities at 5-HT₇ receptors of a series 1-aryl piperazine derivatives^a

Compound	R ₁	R ₂	n	R ₃	K _i (mM) ^b
112	H	Et	4	2-OCH ₃	5.38
113	H	Et	4	3-OCH ₃	2.55
114	H	Et	4	4-OCH ₃	20.40
115	H	Et	4	2-Cl	5.11
116	H	Et	4	3-Cl	0.41
117	H	Et	4	4-Cl	0.38
118	H	Et	3	3-Cl	21
119	H	Et	5	3-Cl	1.45
120	H	Et	4	3-Cl, 4-F	0.60
121	H	Et	4	3-Cl, 4-CH ₃	0.66
122	H	Et	4	3,4-diCl	0.63
123	H	H	4	3-Cl	0.49
124	H	<i>i</i> -But	4	3-Cl	1.80
125	5-Cl	Et	4	3-Cl	8.27
126	5-F	Et	4	3-Cl	0.67
127	5-F, 7-Cl	Et	4	3-Cl	4.75
128	5,7-diCl	Et	4	3-Cl	9.46

^aVolk et al. (2008)

^b Determined at human cloned 5-HT₇ receptors in CHO cells using [³H]LSD.

Table 20

K_i values at 5-HT₇ receptor of phenylpyrrole derivatives reported by Paillet-Loilier et al. (2005)

Compound	Substitution	R	Ar	K_i (nM) ^a
129	2	CH ₃	2-MeO-Ph	(63% @ 10 ⁻⁶ M)
130	2	CN	2-MeO-Ph	(24% @ 10 ⁻⁶ M)
131	3	CH ₃	2-MeO-Ph	4.7
132	3	CN	2-MeO-Ph	5.4
133	3	CH ₃	1,2-benzisoxazol-3-yl	(42% @ 10 ⁻⁶ M)
134	3	CN	1,2-benzisoxazol-3-yl	(34% @ 10 ⁻⁶ M)
135	3	OCH ₃	2-MeO-Ph	(6% @ 10 ⁻⁶ M)

^a Determined at human cloned 5-HT₇ receptors in *sf9* cells using [³H]LSD.

Table 21IC₅₀ values at 5-HT₇ receptor of quinazolinone derivatives^a

Compound	X	n	R	IC ₅₀ (nM)
136	H	3	3-Cl	510
137	H	3	4-Cl	370
138	H	3	2-OCH ₃	80
139	H	3	3-OCH ₃	1400
140	H	3	4-OCH ₃	710
141	H	4	3-Cl	110
142	H	4	4-Cl	450
143	H	4	2-OCH ₃	21
144	H	4	3-OCH ₃	>10000
145	H	4	4-OCH ₃	2000
146	H	4	2-OC ₂ H ₅	26
147	H	4	2-F	400
148	H	4	2-Cl	130
149	F	4	2-OCH ₃	120
150	F	4	2-OC ₂ H ₅	12
151	F	4	2-Cl	200

^aNa et al. (2008)^bDetermined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]LSD.

Table 22Binding affinities at 5-HT₇ receptor of some 1-arylpiperazine derivatives reported by Yoon et al. (2008)

Compound	Ar ₁	Ar ₂	IC ₅₀ (nM)
152	4-OCH ₃ -Ph	2-OCH ₃ -Ph	37
153	4-OCH ₃ -Ph	3-OCH ₃ -Ph	339
154	4-OCH ₃ -Ph	4-OCH ₃ -Ph	9314
155	4-OCH ₃ -Ph	3-CF ₃ -Ph	89
156	4-OCH ₃ -Ph	4-CF ₃ -Ph	384
157	4-OCH ₃ -Ph	Ph	>10000
158	4-OCH ₃ -Ph	4-NO ₂ -Ph	>10000
159	4-OCH ₃ -Ph	4-COCH ₃ -Ph	>10000
160	2-naphthalenyl	2-OCH ₃ -Ph	20
161	2-naphthalenyl	3-OCH ₃ -Ph	223
162	2-naphthalenyl	4-OCH ₃ -Ph	3241
163	2-naphthalenyl	3-CF ₃ -Ph	12
164	2-naphthalenyl	4-CF ₃ -Ph	567
165	2-naphthalenyl	Ph	681

^aDetermined at human cloned 5-HT₇ receptors in CHO cells using [³H]LSD.

Table 23Binding affinities at 5-HT₇ receptor of naphthalenesulfonamide derivatives^a

Compound	Stereochemistry 1	Stereochemistry 2	p <i>K</i> _i ^b
166	<i>R,S</i>	<i>R,S</i>	7.2
167	<i>R</i>	<i>R</i>	6.9
168	<i>R</i>	<i>S</i>	6.2
168	<i>S</i>	<i>R</i>	5.8
170	<i>S</i>	<i>S</i>	<5.0

^aForbes et al. (1998)^bDetermined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]5-CT.

Table 24Binding affinities at 5-HT₇ receptors of a series of 4-methylpiperidines.^a

Compound	Ar	p <i>K</i> _i ^b
171	1-naphthalenyl	7.5
172 (SB-258719)	3-methylphenyl	7.5
173	3,4-dichlorophenyl	7.5
174	3,4-dibromophenyl	7.7
175	3,4-dibromo-2-thienyl	7.8

^aForbes et al. (1998)^bDetermined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]5-CT.

Table 25

pK_i values at 5-HT₇ and 5-HT_{1A} receptors of 4-methylpiperidine derivatives^a

Compound	pK_i	
	5-HT ₇ ^b	5-HT _{1A} ^c
R-176	7.8	5.8
S-176	6.4	--
R-177	8.0	6.0
S-177	6.4	--

^a Lovell et al. (2000)

^b Determined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]5-CT.

^c Determined at human cloned 5-HT_{1A} receptors in HEK-293 cells using [³H]8-OH-DPAT.

Table 26Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of 4-methylpiperidine derivatives

Compound	Ar	p <i>K_i</i>	
		5-HT ₇ ^a	5-HT _{1A} ^b
178 ^c	3,4-diCl-Ph	8.4	6.5
179 ^c	3-Br-Ph	8.7	6.4
180 ^c	3-CH ₃ -Ph	8.5	6.0
181 ^c	3-OCH ₃ -Ph	8.0	-
182 (SB-269970) ^c	3-OH-Ph	8.9	<5
183 ^d		8.62	-

184^d

7.64

-

Compound	Ar	p <i>K_i</i>	
		5-HT ₇ ^a	5-HT _{1A} ^b
185 ^d		8.19	-
186 ^d		8.19	-
187 ^d		7.59	-
188 ^d		7.24	-

^a Determined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]5-CT.

^b Determined at human cloned 5-HT_{1A} receptors in HEK-293 cells using [³H]8-OH-DPAT.

^c Lovell et al. (2000)

^d Forbes et al. (2002)

Table 27Binding affinities at 5-HT₇ and α_{1B} receptors of 4-arylpiperidine derivatives^a

Compound	R	p <i>K</i> _i	
		5-HT ₇ ^b	α_{1B} ^b
189	indol-3-yl	9.17	8.89
190	indol-2-yl	8.20	7.05
191	benzimidazol-2-on-3-yl	9.15	7.36
192	benzoxazol-2-on-3-yl	8.74	6.51
193	4-F-phenoxy	9.30	7.52
194	4-Cl-benzoyl	9.10	7.03
195	4-F-benzoyl	9.08	7.72
196 (SB-656104)	4-Cl-phenoxy	8.70	6.66

^a Forbes et al. (2002)^b Determined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]5-CT.^c Experimental details not specified.

Table 28Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of 1,11-methyleneaporphine derivatives^a

Compound	R ₁	R ₂	K _i [nM]	
			5-HT ₇ ^b	5-HT _{1A} ^c
197	--	--	88.0	80.0
198	H	H	6.9	40.7
199	NH ₂	H	18.0	355
200	CH ₃	OCH ₃	1.1	17.3
201	OCH ₃	CH ₃	1.1	16.9

^a Linnanen et al. (2000)^b Determined at rat cloned 5-HT₇ receptors in CHO cells using [³H]5-HT.^c Determined at human cloned 5-HT_{1A} receptors in CHO cells using [³H]8-OH-DPAT.

Table 29Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of 11-phenylaporphine derivatives^a

Compound	R	R ₁	K _i (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
202	H	H	9.78	1.8
203	OCH ₃	OCH ₃	13.0	554
204	CN	CH ₃	20.8	778
205	CH ₃	CN	3.79	142
206	OH	CH ₃	42.1	319
207	CH ₃	OH	23.0	48.8

^aLinnanen et al. (2001)^bDetermined at rat cloned 5-HT₇ receptors in CHO cells using [³H]5-HT.^cDetermined at human cloned 5-HT_{1A} receptors in CHO cells using [³H]8-OH-DPAT.

Table 30 K_i values at 5-HT₇ and 5-HT_{1A} receptors of 2-aminotetralin and 3-aminochroman derivatives

264-270	271-275

Compound	Ar	R	<i>K</i> _i (nM)	
			5-HT ₇ ^a	5-HT _{1A} ^b
208 ^c	Ph	C ₃ H ₇	3.38	0.87
209 ^c	2-MeO-Ph	C ₃ H ₇	1.73	11.7
210 ^c	4-MeO-Ph	C ₃ H ₇	12.4	1.49
211 ^c	2,6-diMeO-Ph	C ₃ H ₇	7.9	347
212 ^c	2,6-diMeO-Ph	H	78.7	>1000
213 ^c	2,6-diMeO-Ph	CH ₃	2.55	1420
214 (AS19) ^d	1,3,5-Trimethylpyrazol-4-yl	CH ₃	0.6	89.7
215 ^c	Ph	C ₃ H ₇	2.92	1.13
216 ^c	2-MeO-Ph	C ₃ H ₇	2.7	10.3
217 ^c	4-MeO-Ph	C ₃ H ₇	12.6	1.31
218 ^c	2,6-diMeO-Ph	C ₃ H ₇	6.44	174
219 ^c	2,6-diMeO-Ph	CH ₃	5.29	>1000

^a Determined at rat cloned 5-HT₇ receptors in CHO cells using [³H]5-HT.^b Determined at human cloned 5-HT_{1A} receptors in CHO cells using [³H]8-OH-DPAT.^c Holmberg et al. (2004)^d Brenchat et al. (2009)

Table 31Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of aminoethylbiphenyl derivatives^a

Compound	X	R	<i>K_i</i> (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
220	--	CH ₃	(5% @ 10 ⁻⁸ M) ^d	(0% @ 10 ⁻⁸ M) ^d
221	--	H	(16% @ 10 ⁻⁸ M) ^d	(14% @ 10 ⁻⁸ M) ^d
222	2-CH ₃	CH ₃	7.6	443
223	2-OCH ₃	CH ₃	8.4	531
224	H	CH ₃	(18% @ 10 ⁻⁸ M) ^d	(2% @ 10 ⁻⁸ M) ^d
225	2,6-di-CH ₃	CH ₃	6.2	2250
226	2,6-di-OCH ₃	CH ₃	8.6	4826
227	4-OCH ₃	CH ₃	(9% @ 10 ⁻⁸ M) ^d	(0% @ 10 ⁻⁸ M) ^d
228	2-CH ₃	H	7.5	1470

^a Paillet-Loilier et al. (2007)^b Determined at human cloned 5-HT₇ receptors in *sf9* cells using [³H]LSD.^c Determined at 5-HT_{1A} receptors in rat hippocampus membranes using [³H]8-OH-DPAT.^d Percentage of displacement at the concentration given.

Table 32Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of thiazoles and thiopyridine derivatives^a

Compound	R	Cy	<i>K_i</i> (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
229	4-Py		27	90
230	H		6800	--
231	Ph		12	210

Compound	R	Cy	K_i (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
232	<i>t</i> -Butyl		22	2200
233	Ph		3200	--
234	Ph		1200	--
235	Ph		>400	--

Compound	R	Cy	K_i (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
236	Ph		100	1200

237	Ph		0.6	16
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Compound	R	Cy	K_i (nM)	
			5-HT ₇ ^b	5-HT _{1A} ^c
238	Br		4.1	37
239	Cyclohexyl		0.7	8.7
240	<i>t</i> -Butyl		1.3	17

^aThomson et al. (2004)

^bDetermined at cloned 5-HT₇ receptors in CHO cells using [³H]5-HT (species not specified).

^cDetermined at cloned 5-HT_{1A} receptors in HeLa cells using [³H]5-HT (species not specified).

Table 33

Binding affinities at 5-HT₇ receptor of aminotriazine derivatives^a

Compound	Chirality	R ₁	R ₂	X	R ₃	K _i (nM) ^b
241	±	H	NH ₂	CH ₂	H	60
242	±	H	NH ₂	O	H	8
243	S	H	NH ₂	O	H	3
244	R	H	NH ₂	O	H	>1000
245	S	H	H	O	H	3
246	S	H	F	O	H	10
247	S	H	CH ₃	O	H	23
248	S	H	N(CH ₃) ₂	O	H	54
249	±	F	NH ₂	O	F	5
250	S	F	NH ₂	O	F	2
251	R	F	NH ₂	O	F	36

^aMattson et al. (2004a)
^bDetermined at human cloned 5-HT₇ receptors in CHO cells using [³H]5-CT.

Table 34
Binding affinities at 5-HT₇ receptor of ligands reported by Denhart et al. (2004)

Compound	W	X	Y	Z	K _i (nM) ^a
252	N	CH	CH	CH	0.4
253	CH	N	CH	CH	4
254	CH	CH	N	CH	0.4
255	CH	CH	CH	N	> 1000
256	CH	CH	CH	CH	>1000
257	CH	CH	N	N	2

^aDetermined at human cloned 5-HT₇ receptors in CHO cells using [³H]5-CT.

Table 35 K_i values at 5-HT₇ receptor of ligands reported by Raubo et al. (2006)

Compound	NR ₁ R ₂	K_i (nM) ^a
260		>7000
261		2700
262		>7000

Compound	NR ₁ R ₂	K _i (nM) ^a
263		550
264		5800
265		22
266		26

Compound	NR_1R_2	K_i (nM) ^a
267		8
268		9
269		6

^a Determined at human cloned 5-HT₇ receptors in CHO cells using [³H]5-HT.

Table 36Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of phenylsulfone derivatives^a

Compound	NR ₁ R ₂	K _i (nM)	
		5-HT ₇ ^a	5-HT _{1A} ^b
270		8	1500
271		54	>7000
272		24	2400
273		455	NT
274		15	>7000

Compound	NR ₁ R ₂	K _i (nM)	
		5-HT ₇ ^a	5-HT _{1A} ^b
275		7	400

^aRaubo et al. (2006)

^bDetermined at human cloned 5-HT₇ receptors in CHO cells using [³H]5-HT.

^cDetermined at human cloned 5-HT_{1A} receptors in HeLa cells using [³H]5-HT.

Table 37

Binding affinities at 5-HT₇ receptor of naphtholactam derivatives^a

Compound	X	n	Y	R	pK _i ^b
276	CO	1	N	Ph	<5
277	CO	1	N	2-MeO-Ph	<5
278	CO	3	N	Ph	<5
279	CO	3	N	2-MeO-Ph	6.4
280	CO	4	CH	<i>i</i> -Pr	6.7
281	CO	4	CH	Ph	<6
282	CO	4	C	Ph	<6
283	CO	4	N	Me	<5
284	CO	4	N	cyclohexyl	<5
285	CO	4	N	Ph	6.2
286	CO	4	N	2-MeO-Ph	7.2
287	CO	5	N	cyclohexyl	<5
288	CO	5	N	Ph	7.1
289	CO	5	N	2-MeO-Ph	7.0
290	CO	6	N	Ph	<6
291	CO	6	N	2-MeO-Ph	<6

Compound	X	n	Y	R	p <i>K_i</i> ^b
292	SO ₂	4	N	2-MeO-Ph	6.7
293	SO ₂	5	N	2-MeO-Ph	6.6
294	SO ₂	6	N	Ph	<6
295	SO ₂	6	N	2-MeO-Ph	6.7

^aLópez-Rodríguez et al. (2003)

^bDetermined at 5-HT₇ receptors in rat hypothalamus membranes using [³H]5-CT.

Table 38Binding affinities at 5-HT₇ and 5-HT_{1A} receptors of 1-arylpiiperazine derivatives^a

Compound	Spacer	Ar	<i>K_i</i> (nM)	
			5-HT ₇ ^a	5-HT _{1A} ^b
298	(CH ₂) ₄	Ph	74	124
299	(CH ₂) ₄	2-OCH ₃ -Ph	32	>1000
300	(CH ₂) ₄	1-naphthalenyl	47	22
301	(CH ₂) ₅	Ph	63	>1000
302	(CH ₂) ₅	2-OCH ₃ -Ph	63	>1000
303	(CH ₂) ₅	1-naphthalenyl	62	16
304		1-naphthalenyl	250	11.5
305		1-naphthalenyl	69	39
306		1-naphthalenyl	>1000	26
307		1-naphthalenyl	>1000	>1000
308		1-naphthalenyl	>1000	181
309	(CH ₂) ₄	<i>d</i>	7	219
310	(CH ₂) ₃	<i>d</i>	105	>1000
311	(CH ₂) ₂	<i>d</i>	350	>1000

^aMedina et al. (2009)^bDetermined at human cloned 5-HT₇ receptors in HEK-293 cells using [³H]LSD.

^cDetermined at human cloned 5-HT_{1A} receptors in CHO cells using [³H]8-OH-DPAT.

^dThe basic moiety is 1,2,3,4-tetrahydroisoquinoline.