1. Introduction

5-HT₇ (1, Figure 1) mediates its diverse physiological effects through at least 14 different receptor subtypes, of which 13 belong to the G-protein-coupled or seven transmembrane-spanning receptor families [1]. Defined on the basis of molecular, pharmacological and functional criteria, 5-HT receptors form seven discrete families (5-HT₁–7). The 5-HT₇ receptor was one of the latest identified member of 5-HT receptor family and has been characterized on the basis of structural, operational and transductional characteristics. The 5-HT₇ receptor has been cloned from human, rat, mouse and guinea-pig and is positively linked to adenylyl cyclase [2]. Studies on the localization of the 5-HT₇ receptor mRNA and of 5-HT₇ receptor protein have delivered a distribution map that gave important indications on the pathophysiological role of 5-HT₇ receptors. Various studies on the distribution of mRNA encoding the 5-HT₇ receptor protein from different species have revealed the presence of 5-HT₇ receptor mRNA in the CNS [3]. Particularly high levels have been detected in thalamus, hippocampus and the hypothalamus (especially within the suprachiasmatic nucleus, SCN). In peripheral tissues, 5-HT₇ receptor mRNA has been described in the ileum, spleen, endocrine glands.
5-HT7 receptor modulators: a medicinal chemistry survey of recent patent literature (2004 - 2009)

Article highlights.

- The 5-HT7 receptors have been implicated in depression, disorders related to circadian rhythm, pain and migraine.
- 5-HT7 receptor antagonists have shown ability to reduce or prevent the dural inflammation thought to be symptomatic of migraine.
- 5-HT7 receptor antagonists have displayed anxiolytic activity in the foot electroshock stress-induced cGMP elevation model of anxiety.
- Systemic administration of 5-HT7 receptor agonists exerts antinociceptive effects.
- 5-HT7 receptor agonists seem useful for treating disorders that can be treated by modulating circadian rhythms.

This box summarizes key points contained in the article.

and arteries. In blood vessels and the gastrointestinal tract, the expression has generally been localized to smooth muscle cells. The distribution of the 5-HT7 receptor protein has been studied with the aid of various radioligands [4-7]. The distribution of 5-HT7 receptor binding sites has been studied with the nonselective 5-HT ligand 5-carboxamidotryptamine (5-CT) (2, Figure 1) as tritiated radioligand, in the presence of various masking agents. In guinea-pig and rat brain, the distribution of the 5-HT7 sites was found to be largely consistent with that reported for 5-HT1A receptor mRNA. The highest densities were in the medial thalamic nuclei and related limbic and cortical regions. Another autoradiographic study with the 5-HT1A/5-HT7 agonist 8-hydroxy-N,N-dipropylaminotetrahydroisoquinoline (8-OH-DPAT) (3, Figure 1) in the tritiated form has been conducted on 5-HT1A receptor knockout and 5-HT1A/B receptor double knockout mice. 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, suggesting a 5-HT autoreceptor role for the 5-HT7 receptor. High densities of 5-HT7 receptor binding sites were observed throughout the hypothalamus (including the SCN) [8]. The selective and high-affinity antagonist SB-269970 (4, Figure 1) radiolabeled with [3H] has been used to visualize the distribution of 5-HT7 receptors in human whole hemisphere brain sections [9]. [3H]SB-269970 bound mainly in the thalamus, in hypothalamus and in the hippocampal formation. Immunocytochemistry has been used to localize the distribution of 5-HT7 receptors in rat forebrain. Substantial agreement was found with the reported localization of the 5-HT7 receptors mRNA. 5-HT7 receptors were detected within the cerebral cortex, tenia tecta, hypothalamus thalamus and hippocampal formation. At a microscopic level, both cell bodies and proximal fibers were strongly stained in the SCN, suggesting a somatodendritic subcellular distribution [10]. By using electron microscopic immunocytochemical procedures, the presence of 5-HT7 receptors in both pre- and postsynaptic GABA, vasoactive intestinal polypeptide and vasopressin processes in the SCN in mouse was determined [11]. An immunocytochemical study of 5-HT7 receptor distribution at the lumbar level has shown immunolabeling 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. Moderate labeling was found in the lumbar dorsolateral nucleus (Onuf’s nucleus), suggesting involvement in the control of pelvic floor muscles [12].

Several preclinical studies have evaluated the possible involvement of the 5-HT7 receptor in psychiatric disorders and other pathological processes of the nervous system. Most studies have taken advantage of the use of the selective 5-HT7 antagonist SB-269970 (4) [13] and/or of constitutive knockout mice lacking 5-HT7 receptors in animal behavioral models designed to mimic, at least in part, human disorders [14-17]. In general, there is a significant agreement between the localization of 5-HT7 receptors and the functions they are implicated in [3,18,19]. 5-HT7 receptors in the hypothalamus correlate with involvement in circadian rhythm, thermoregulation and endocrine regulation. Thalamic and cortical 5-HT7 receptors are of relevance for sleep, mood regulation and epilepsy. The presence of 5-HT7 receptors in the hippocampus may be of importance in learning and memory. Finally, 5-HT7 receptors localization in the spinal cord is in agreement with demonstrated functions in nociception and locomotion.

2. The therapeutic potential of targeting 5-HT7 receptor

Most evidence supports a role for the 5-HT7 receptor in depression. It has been suggested that the action of antidepressants, at least in part, might be mediated directly by the 5-HT7 receptor [20]. Thus, several antidepressants, both tricyclics and selective serotonin reuptake inhibitors (SSRIs), induced c-fos expression in a way consistent with 5-HT7 receptor activation within the SCN. 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 [21]. In both forced swim and the tail suspension tests, pharmacological blockade of the 5-HT7 receptor or inactivation of the receptor gene leads to an antidepressant-like behavioral profile [15,22-26]. More interestingly, it has been shown that there is a synergistic interaction between an individually ineffective dose of SB-269970 (4) and an individually ineffective dose of one of several antidepressants, leading to reduced immobility in both the forced swim and the tail suspension tests [25,26].

Circadian rhythm, sleep and mood are closely linked physiological phenomena that are all regulated by the 5-HT7 receptor. An extensive body of work has confirmed the importance of the 5-HT7 receptor in SCN function [16,27-30]. As an example, 8-OH-DPAT-induced phase resetting within the
SCN is mediated by $5\text{-HT}_7$ receptor and not by the $5\text{-HT}_{1\text{A}}$ receptor as previously thought [31]. Moreover, the shift can be inhibited by SB-269970 (4), but not by selective $5\text{-HT}_{1\text{A}}$ receptor antagonists [30,32]. Shifting the SCN pacemaker neurons with 8-OH-DPAT (3) is a non-photic stimulus involving serotonergic input from the dorsal and median raphe nuclei. There is also evidence to support a role for the $5\text{-HT}_7$ receptor in photic regulation of the SCN. It has been demonstrated that $5\text{-HT}$-mediated reduction of photic stimulation of SCN neurons is most likely mediated by the $5\text{-HT}_7$ receptor [32,33]. The inhibitory effect of 8-OH-DPAT (3) on spontaneous SCN activity is also most likely mediated by the $5\text{-HT}_7$ receptor, as it could be blocked by $5\text{-HT}_7$ receptor antagonists but not $5\text{-HT}_{1\text{A}}$ receptor antagonists [34]. The involvement of the $5\text{-HT}_7$ receptor in both photic and non-photic phase resetting has been verified in mice lacking the receptor [35].

Direct involvement of the $5\text{-HT}_7$ receptor in sleep regulation has been shown using selective antagonists and knockout mice. Both the $5\text{-HT}_7$ antagonists SB-269970 (4) and SB-656104 (5, Figure 1) when administered to Sprague-Dawley rats at the beginning of the light period increased the latency to rapid eye movement (REM) sleep and decreased the amount of time spent in REM sleep [15,36]. Other sleep parameters were not affected. It has further been shown that mice lacking the $5\text{-HT}_7$ receptor exhibit a similar reduction in time spent in REM sleep during the light period, again without affecting other sleep phases [22]. The $5\text{-HT}_7^{-/-}$ mice also had less frequent and longer REM episodes than the $5\text{-HT}_7^{+/+}$ mice. In the $5\text{-HT}_7^{-/-}$ mice, there was no change in latency to REM and citalopram (an SSRI) was equally effective in increasing the latency to REM in both genotypes [22]. Of considerable interest is that, as in the depression models discussed above, an interaction has been observed between individually ineffective doses of SB-269970 (4) 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 [26].

The possible role of the $5\text{-HT}_7$ receptor in spatial memory using $5\text{-HT}_7$ receptor-deficient mice has been investigated. A hippocampus-associated spatial memory deficit in $5\text{-HT}_7$ receptor-deficient mice was demonstrated using a novel location/novel object test. A similar reduction in novel location exploration was observed in wild-type mice treated with SB-269970 (4). An extended analysis using the Barnes maze has demonstrated that $5\text{-HT}_7$ receptor-deficient mice were less efficient in accommodating to changes in spatial arrangement than wild-type mice. $5\text{-HT}_7$ receptor-deficient mice had specific impairments in memory compilation required for resolving spatial tasks, which resulted in impaired allocentric spatial memory. On the other hand, any differences in hippocampus-independent learning tasks have been found: cued fear conditioning, operant food conditioning, motor learning (rotarod) and novel object recognition [37,38]. Also in support of a pro-cognitive effect of the $5\text{-HT}_7$ receptor, it has been shown that the agonist AS-19 (6, Figure 1) enhanced memory formation and that this effect could be reversed by SB-269970 (4) [39].

A role for $5\text{-HT}$ in migraine has been supported by changes in circulating levels of $5\text{-HT}$ and its metabolites during the

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**Figure 1. Reference agonists and antagonists for $5\text{-HT}_7$ receptor.**
phases of a migraine attack, along with the ability of 5-HT-releasing agents to induce migraine-like symptoms [40]. Although the effects of 5-HT are most likely not mediated by a single receptor subtype [41-43], available evidence clearly suggest a role for the 5-HT7 receptor. Before the availability of selective 5-HT7 receptor antagonists, pharmacological profiling was used to implicate the 5-HT7 receptor as a mediator of 5-HT-induced vasodilation in preparations of the basilar and middle cerebral arteries [44]. Also, it was proposed that the 5-HT7 receptor mediates 5-HT-induced dilation of the carotid artery following blockade of 5-HT1B/1D receptors in combination with low sympathetic tone [45]. Involvement of the 5-HT7 receptor was confirmed as SB-269970 (4) blocked in vivo vasodilation of the basilar and middle cerebral arteries [46]. This also happens after 5-HT depletion, suggesting that the 5-HT7 receptors involved are not sensitized by reduced availability of 5-HT [47].

Dogrul and Seyrek [48] highlighted the contribution of spinal 5-HT7 receptors to morphine analgesia. It was found that 5-HT7 receptor blockade by intrathecal administration of SB-269970 (4) inhibited the antinociceptive effect of systemic morphine in the tail flick test. This effect was not reproduced after intrathecal injection of 5-HT1A and 5-HT2 receptor antagonists, supporting the notion that the specific activation of spinal 5-HT7 receptors plays a key role in the inhibitory pathway activated by morphine. It should be noted that the anatomical localization of 5-HT7 receptors within the spinal cord supports such an interpretation [12]. A recent study has shown that the antinociceptive properties of spinal serotoninergic pathways were mediated by the 5-HT7 receptor. It was found that the 5-HT7 receptor modulates capsaicin-induced mechanical hypersensitivity in mice. In particular, the 5-HT7 agonist AS-19 (6) had a dose-dependent antinociceptive effect that could be counteracted by the SB-269970 (4) [49]. It has been also suggested that supraspinal areas, particularly the thalamus, are involved in modulation of nociception by 5-HT, and a role for 5-HT7 receptors has been outlined. In particular, it has been suggested that supraspinal 5-HT7 receptors mediate the antinociceptive effect of S-(-)-ketoprofen because intracerebroventricular administration of the nonselective 5-HT7 receptor antagonist methiothepin significantly inhibited the effect of this non-steroidal anti-inflammatory agent [50]. Furthermore, administration of 8-OH-DPAT (3) into the medial thalamus was shown to raise tailshock intensity thresholds to trigger vocalization, and this antinociceptive effect could be blocked by intrathalamic microinjection of SB-269970 (4) [51].

3. 5-HT7 modulators in 2004 - 2009 patent literature

It is not surprising that, based on the biological proof of concept generated using the potent, selective antagonist SB-269970 (4) and 5-HT7 knockout mice, discovery programs of pharmaceutical companies have been focused on the identification of newer 5-HT7 antagonists and selective agonists. Herein, we attempt to classify all approaches that have appeared in the Patent Cooperation Treaty literature between 2004 and 2009, as two comprehensive reviews cover studies from 1993 to 2004 [52,53]. Each section is categorized according to applicant.

3.1 Mitsubishi

In 2004, Mitsubishi Pharma Corp. filed a patent application describing compounds that combine serotonin 5-HT7 receptor antagonism and muscarinic M4 receptor agonist activity for use in the treatment of schizophrenia and/or bipolar disorders [54]. The rationale for the development of this class of 'mixed' agents stemmed from the evidence that M4 agonists may show antipsychotic activity in some tests and that some of the more effective atypical antipsychotic drugs have significant 5-HT7 affinity as part of their complex pharmacological profile. The invention also described some of the effect of 'serominic' combination (i.e., M4 agonist PTAC + 5-HT7 antagonist SB-258741, 7, Figure 1) in various behavioral models of schizophrenia. One example of the new chemical entities that combined serotonin 5-HT7 receptor antagonist activity and muscarinic M4 receptor agonist activity is compound 8 (Figure 2) which displayed $K_i = 0.4 \mu M$ at 5-HT7 receptor and $K_i = 0.32 \mu M$ at M4 receptors. This compound was devoted with affinity for dopamine D2 receptor ($K_i > 300 \mu M$) that is believed to be responsible of unwanted extrapyramidal side effects of classical antipsychotic drugs. Compound 8 was tested for their inhibitory effect on a standard test for antipsychotic activity (amphetamine-induced hyperactivity in rats) with estimated ED50 of 8.1 mg/kg, intraperitoneal. A detailed report on this class of agents that were termed 'serominic' compounds was published 3 years later [55].

3.2 University of Bari

University of Bari (Italy) has one published patent application identifying 5-HT7 receptor ligands [56]. A series of N-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides were prepared and their affinity for 5-HT7 and 5-HT1A receptors were measured using in vitro binding assays (Table 1). In relation to 5-HT7 receptor affinity, receptor binding studies indicated that: i) the optimal alkyl chain length was four to five methylenes (compounds 9 - 11 vs 12, 13; compounds 14 vs 15, 16); ii) an unsubstituted 1,2,3,4-tetrahydronaphthalen-1-yl nucleus was selected for further substitutions (compounds 12, 13 vs 15, 16) and iii) the presence of a substituent in 2-position of the aryl ring linked to the piperazine ring is preferred over 3- and 4-positions (compounds 16 - 18 vs 19 - 24). The nature of such substituent has also a marked effect on 5-HT7 affinity (compounds 16 - 18, 25 - 32). In particular, compounds 15, 16, 18 and 32 showed 5-HT7 affinity in the nanomolar range. Selected compounds were assayed for the 5-HT7 receptor mediated relaxation of substance P-induced guinea-pig ileum
function. As far as the basic moiety is concerned, the fragment groups or halogen atoms.

nucleus. This nucleus was mono or disubstituted by lower alkyl or piperidines bearing in 2- or 3-position an 8- or 7-quinolinyl receptor ligands in 2004 [61]. In general, the compounds contained a benzenesulfonyl function attached through a butyl chain to various basic fragments. A key feature of these compounds is the presence of a gem-dimethyl group or a 4-membered spirocyclic function in -position of the sulfone function. As far as the basic moiety is concerned, the fragment was selected among those cyclic amines that are most frequently used in the exploration of structure–activity relationships of 5-HT<sub>7</sub> ligands: that is, 1-arylpiperazine, 4-arylpiperidine, tetrahydroisoquinoline, 3-arylpiperidine and 4-methylpiperidine (48, Figure 3). This patent application did not report pharmacological data of the compounds therein described. Later, a detailed report on the development of this group of compounds has been published [62]. The most relevant outcome of the study was compound 49 (already reported in the patent application) which showed high 5-HT<sub>7</sub> affinity (K<sub>i</sub> = 8.0 nM), an excellent selectivity profile over 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors (> 100-fold) and partial agonist properties (Figure 3).

3.3 Pfizer

Pfizer Products, Inc. has filed one patent application describing 5-HT<sub>7</sub> receptor ligands claimed as agonists useful for treating disorder that can be treated by modulating circadian rhythms [59]. Examples of such disorders and conditions are seasonal affective disorders, bipolar disorder, jet lag, sleep deprivation, REM sleep disorders, hypersomnia, parasomnia, mood rhythms are treated with piperidine ring. Other compounds described were structurally related with 33 and 34: the derivatives were pyrrolidines or piperidines bearing in 2- or 3-position an 8- or 7-quinolino nucleus. This nucleus was mono or disubstituted by lower alkyl groups or halogen atoms.

3.4 Ajinomoto

In 2004, Ajinomoto Co., Inc. filed a patent application describing novel piperidine derivatives as 5-HT<sub>7</sub> ligands [60]. Selected examples (compounds 35 – 47) are reported in Table 2. Other compounds included in the patent application presented aryl rings different from 3-Cl-phenyl: these compounds did not show significant difference in affinity as compared to the compounds listed in the Table 2. Derivatives 35, 36 and 38 behaved as agonists (cAMP accumulation assay) with E<sub>C50</sub> of 0.087, 0.099 and 0.023 µM, respectively.

3.5 Merck

Merck has one published patent application describing 5-HT<sub>7</sub> receptor ligands in 2004 [61]. In general, the compounds contain a benzenesulfonyl function attached through a butyl chain to various basic fragments. A key feature of these compounds is the presence of a gem-dimethyl group or a 4-membered spirocyclic function in -position of the sulfone function. In this case one or both the aromatic ends of the molecule presented F or Cl as a substituent. Two representative ligands of this group are compounds 50 and 51 (Figure 4). Another group of compounds still present a basic moiety linked to an oxindole terminal fragment through a butyl chain. This general formula is in common with many other classes of 5-HT<sub>7</sub> ligands reported in the literature. In particular, a first group of compounds was characterized by basic moieties such as tetrahydroisoquinoline and related isosters or 4-arylpiperidines (63). In many cases, one or both aromatic ends of the molecule presented F or Cl or CF<sub>3</sub> as a substituent. Two representative ligands of this group are compounds 50 and 51 (Figure 4). Another group of compounds still present a basic moiety linked to an oxindole terminal fragment through a butyl chain. In this case [64], the basic moiety was a 1-arylpiperazine moiety, which has been widely used in the development of 5-HT<sub>7</sub> ligands. Also in this case one or both the aromatic ends of the molecules were substituted by F or Cl. Details of structure–activity relationships of this class of 5-HT<sub>7</sub> ligands have been reported in an original article [65]. Compound 52 (Figure 4), which was also

Figure 2. Compounds from Mitsubishi (8) and Pfizer (33, 34).
included in the patent application, showed high affinity at 5-HT<sub>7</sub> receptor (K<sub>i</sub> = 7.0 nM), good selectivity over 5-HT<sub>1A</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>6</sub> and dopamine D<sub>2</sub> receptor. This compound behaved as an antagonist in the 5-CT-induced elevation of cAMP level assay. Also, 52 demonstrated high potency in vivo as anxiolytic. However, it was not ruled out that such activity might be due to a multireceptorial action involving not only 5-HT<sub>7</sub> but also 5-HT<sub>2A</sub> receptor and α<sub>1</sub> adrenoceptor.

The third patent application was filed in 2008 [66], describing 5-HT<sub>7</sub> selective inhibitors with affinity values (K<sub>i</sub>) lower than 30 nM, endowed with selectivity over 5-HT<sub>1A</sub> receptor (K<sub>i</sub> > 200 nM). The general formula overlaps that of the previous patent applications from the same company. In this case, the terminal fragment was a 2,2-dimethylbenzofuran linked to an oxypropyl chain to the basic structure. The basic portions were: a piperidine with a heterobicyclic system in 4-position or a 4-arylpiperazine. Also for this group of compounds, one or both the aromatic ends of the molecule presented F or Cl or Br as a substituent. A general formula of the compounds disclosed in the patent application (53) and two selected examples (54, 55) are shown in Figure 4.

### Table 1. Compounds 9 – 32 from University of Bari.

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### 3.7 Eli Lilly

Eli Lilly has three published patent applications describing 5-HT<sub>7</sub> receptor ligands with 1-arylpiperazine structure. In 2008, a patent application included about 350 derivatives belonging to the general formula of 2-[4-(pyrazol-4-yl-alkyl)piperazin-1-yl]-3-phenyl-pyrazines or -pyridines and 3-[4-(pyrazol-4-yl-alkyl)piperazin-1-yl]-2-phenylpyridines [67]. The compounds belonging to these classes were claimed as 5-HT<sub>7</sub> antagonists. Pharmacological data of compounds 56 -- 60 (Figure 5) were reported in detail. The binding panel of compound 56 was as follows: 5-HT<sub>7</sub>: K<sub>i</sub> = 44 nM;
5-HT1A: $K_i = 1250 \text{ nM}$; 5-HT1B: $K_i > 3580 \text{ nM}$; 5-HT1D: $K_i > 2400 \text{ nM}$; 5-HT2A: $K_i > 7470 \text{ nM}$; 5-HT2B: $K_i > 3160 \text{ nM}$; 5-HT2C: $K_i > 8200 \text{ nM}$; 5-HT4: $K_i > 6310 \text{ nM}$; 5-HT5: $K_i > 7020 \text{ nM}$; 5-HT6: $K_i > 1790 \text{ nM}$. Compound 57 was found to be a full antagonist showing a $K_b$ of about 44 nM (inhibition about 96% of 5-HT activity in cAMP accumulation assay). The compounds described in the filing were able to significantly reduce or prevent dural plasma protein extravasation in male rats. This behavior is considered indicative of the compound’s ability to reduce or prevent the dural inflammation thought to be symptomatic of migraine [40]. In brief, the extravasation...
induced by the electrical stimulation of the trigeminal ganglion is an ipsilateral effect. This allows the use of the other unstimulated half of the dura as a control. The ratio of the amount of extravasation in the dura from the stimulated side, over the amount of extravasation in the unstimulated side, is calculated. A compound which effectively prevents the extravasation in the dura from the stimulated side would yield a ratio of ~1, whereas control animals yield a ratio of ~2. Compound 58 was found to have a ratio of about 1.16. Compound 59 was reported to be active in the 'formalin model' of persistent pain which is used to characterize pain blocking and/or analgesic activity [68,69]. The compounds disclosed in this patent application were also tested in the L5/L6 nerve ligation model (Chung model) [70] for persistent pain, especially neuropathic pain. In particular, compound 60 was found to be active. Compound 60 also displayed anxiolytic activity in the foot electroshock stress-induced cGMP elevation model for anxiety, wherein stress from an unavoidable electroshock to the feet induces an elevation in cerebellar cGMP levels, which elevation is reduced or blocked by preadministration of anxiolytic compounds [71].

Another patent application from Eli Lilly described a series of 5-HT7 antagonists structurally related to that described above [72]. From the structural standpoint, the main difference was the presence of a 1-piperidinyl group in 2-position of the pyridine or pyrazine ring linked to the piperazine ring. The majority of the compounds filed are comprised in general formula 61 depicted in Figure 5, together with one example (62). The binding panel of 62 was as follows: 5-HT7: \( K_i = 16.2 \text{nM} \); 5-HT1A: \( K_i = 213 \text{nM} \); 5-HT1B: \( K_i > 3580 \text{nM} \); 5-HT1D: \( K_i = 1840 \text{nM} \); 5-HT2A: \( K_i > 7470 \text{nM} \); 5-HT2B: \( K_i > 6810 \text{nM} \); 5-HT2C: \( K_i > 8360 \text{nM} \); 5-HT5: \( K_i = 4550 \text{nM} \); 5-HT6: \( K_i > 5830 \text{nM} \); \( \alpha_1 \) adrenergic \( K_i = 1380 \text{nM} \); \( \alpha_2 \) adrenergic \( K_i > 2670 \text{nM} \). Compound 62 was found to be a full antagonist showing a \( K_i \) of about 2.97 nM (inhibition about 108% of 5-HT activity in cAMP.

Figure 4. Compounds from Egis.
Figure 5. 5-HT7 antagonists from Eli Lilly.
accumulation assay). The compounds described in the filing were able to significantly reduce or prevent dural plasma protein extravasation in male rats. The ratio of the amount of extravasation in the dura from the stimulated side over the amount of extravasation in the unstimulated side for 62 was 1.15.

The third patent application by Eli Lilly reported another group of 1-arylpiperazine derivatives [73]. In particular, the basic nitrogen of the piperazine of either 2-((piperazin-1-yl)-3-phenylpiperazine or 3-((piperazin-1-yl)-2-phenylpyridine core was substituted by an ethyl chain bearing in 2-position an arylsulfonamide fragment. Selected examples are compounds 63 – 65 (Figure 5). Pharmacological data of these compounds were given: compounds 63 and 64 displayed $K_i$ values at 5-HT7 receptor of 33.5 and 19.5 nM, respectively. Both compounds were described to be at least 50-fold selective over 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT4, 5-HT6, 5-HT6, G1, and G2 adrenoceptors. Compounds 64 and 65 behaved as full antagonists showing a $K_i$ of 8.5 and 24 nM, respectively. Also in this case, the antagonists described in the filing were able to significantly reduce or prevent dural plasma protein extravasation in male rats. The ratio of the amount of extravasation in the dura from the stimulated side over the amount of extravasation in the unstimulated side for 64 was 1.1.

### 3.8 Esteve

Laboratorios del Dr Esteve SA Chemical-Pharmaceutical Group has been particularly active in the search of 5-HT7 receptor ligands, by filing ~10 patent applications dealing with this target. The ligands described in these patent applications can be grouped on the basis of the common basic framework: 2-aminoindan derivatives, indane amine derivatives, 2-phenylethylamino derivatives and azacenaphthylene derivatives.

The structure of heterocycl-substituted 2-aminoindan derivatives disclosed by Esteve [74,75] was closely related to the structure of one of the very few selective 5-HT7 agonists available to date: that is, AS-19 (6) that was initially reported by researchers at University of Uppsala, Sweden [76]. Affinity data of AS-19 (6) reported in the patent application was $IC_{50} = 18.4$ nM, whereas a $K_i$ value of 0.6 nM was reported in the literature [49]. The patent application reported affinity data of some analogues of 6, which are listed in Table 3. In particular, removal of one or both methyl groups on the basic nitrogen was well tolerated with respect to 5-HT7 affinity (compounds 66 and 67). By contrast, the presence of at least one bulky substituent (benzyl, isopropyl, propyl) was detrimental for 5-HT7 affinity (compounds 68 and 71). Compound 72, that is the racemic form of AS-19 (6), displayed higher affinity of both enantiomers, being the $S$ enantiomer (6) more potent than the $R$ (73). Affinity data of racemic derivatives 74 – 77 related to 72 confirmed that increasing the size of the substituents around the basic nitrogen was detrimental for affinity. Replacement of the (1,3,5-trimethyl)pyrazol-4-yl nucleus of 72 with other aromatic nuclei afforded compounds with 5-HT7 affinity (compounds 78 – 80). Many other five-membered heterocyclic ring systems were described as possible substituted of the (1,3,5-trimethyl)pyrazol-4-yl ring. It is important to note that the role of the aromatic ring in 5-position of 2-aminoindan nucleus has been explored by the research group that described first AS-19 (6) [77]. Esteve filed another patent application describing a structurally distinct chemical series of 5-HT7 ligands with indanamine structure [78] that can be considered formally derived from the 2-aminoindan series by ring constriction. Affinity data of indanamine congeners of AS-19 (6) were reported: compounds 81 and 82 (Figure 6) displayed $IC_{50}$ values at 5-HT7 of 15.5 and 18.5 nM. This patent application describes about 35 derivatives structurally related with 81, bearing a variety of aromatic rings in place of (1,3,5-trimethyl)pyrazol-4-yl ring, all showing the 2,6-disubstitution pattern. The compounds were claimed to be 5-HT7 agonists.

In 2008, Esteve filed a patent application describing heterocyclic substituted 2-phenylethylamino derivatives targeting 5-HT7 receptors [79]. This framework can be considered a further structural simplification of 2-aminoindan and indane amine derivatives discussed above. The compounds showed high affinity for 5-HT7 receptor as well as high selectivity for this receptor in comparison to the 5-HT6 or the G1, the G2 and the 5-HT1 receptors. In addition, some of these compounds showed agonistic activity at 5-HT1 receptor. Affinity data of selected compounds (83 – 105) reported in the patent application are shown in Table 4. Analysis of the data indicated that structure-activity relationship for this class of compounds parallel those described for 2-aminoindan derivatives. Also in this case, increasing the size of the substituent on the basic nitrogen was detrimental for affinity. Replacement of (1,3,5-trimethyl)pyrazol-4-yl ring by 2,6-disubstituted phenyl system was well tolerated. A full paper on the pharmacological characterization of compound 83 (i.e., E-55888) appeared recently in the literature [49]. The effects of the 5-HT7 receptor agonists AS-19 (6) and 83 were assessed on capsaicin-induced mechanical hypersensitivity, a pain behavior involving hypersensitivity of dorsal horn neurons (central sensitization). In vivo results revealed that systemic administration of 5-HT7 receptor agonists exerted a clear-cut dose-dependent antinociceptive effect that was prevented by the 5-HT7 receptor antagonist SB-269970 (4), but not by the 5-HT1A receptor antagonist. Contrary to agonists, a dose-dependent promotion of mechanical hypersensitivity was observed after administration of 5-HT7 receptor antagonists, substantiating the involvement of the 5-HT7 receptor in the control of capsaicin-induced mechanical hypersensitivity. These findings suggested that 5-HT exerts an inhibitory role in the control of nociception through activation of 5-HT7 receptors, suggesting a role of the 5-HT7 receptor in nociception secondary to a sensitizing stimulus in mice.

Esteve has also disclosed a series of tetrahydroisoquinoline derivatives as 5-HT7 receptor ligands. In a first patent
application, compounds showing the general formulas 106 and 107 (Figure 6) were described [80]. These derivatives presented the tetrahydroisoquinoline moiety, linked through a straight alkylene chain (propyl or butyl in most cases) with an arylsulfonamide moiety. It was found that the compounds with a secondary arylsulfonamide moiety and a linker of 4, 5 or 6 methylene units displayed IC₅₀ values in the nanomolar range (> 10 nM) at human 5-HT₇ receptors and exhibited at least 30-fold selectivity over 5-HT₁A, 5-HT₂A, 5-HT₂B, 5-HT₂C, 5-HT₃, 5-HT₄, 5-HT₅A, dopaminergic D₁, D₂, D₃, D₄, adrenergic α₁A, α₁B, β₁ and β₂ receptors. These compounds were described as 5-HT₇ antagonists. It can be noted that these compounds share with other well-known 5-HT₇ antagonists the arylsulfonamide motif. Another group of tetrahydroisoquinolines can be considered as formally related to sulfonamide derivatives 107, where the sulfonamide nitrogen and one carbon atom of the alkyl chain are included into a six-membered ring (general formula 108, Figure 6). Selected examples showed affinity values at 5-HT₇ receptors in the same range as the compounds with a straight alkylene chain 106 (17 nM < IC₅₀ < 83 nM).

In 2006, Esteve also filed a patent application describing 2,2a,4,5-tetrahydro-1H-3-azaacenethylene derivatives as 5-HT₇ agents [81] (general formula 109, Figure 6). These derivatives closely resembled the sulfonamide derivatives 107 described above, apart from the basic framework, which can be considered a rigid congener of the tetrahydroisoquinoline nucleus. Selected compounds are reported to have IC₅₀ values of about 150 nM, thus, showing slightly lower affinity values than 107.

4. Expert opinion

Four years after the cloning of 5-HT₇ receptor, SmithKline Beecham (now GlaxoSmithKline) identified the first selective 5-HT₇ antagonist SB-278519 (7). Soon after, another antagonist (namely SB-269970, 4) was discovered. The identification of a valuable pharmacological tool to study the 5-HT₇ receptor function has allowed significant progress in the study of the pathophysiological role of this receptor. In parallel, availability of genetically modified mice lacking the 5-HT₇ receptors was a further support in this area. Many studies have pointed to a role of 5-HT₇ antagonists in depression. In particular, 5-HT₇ antagonists display antidepressant properties. However, at the moment, no 5-HT₇ receptor antagonist has entered clinical trials as a new antidepressant. Is 5-HT₇ antagonism relevant in depression? The presence in the market of well established drugs to treat depression leaves little room for newer antidepressants that must indeed demonstrate an overall profile significantly better than that of currently available drugs. An example in this regard are dual-acting serotonergic antidepressants. Fifteen years ago, it was...
demonstrated that the treatment of patients with major depression using an SSRI and a 5-HT1A antagonist markedly reduced the latency of the antidepressant response [82]. In 2009, Clinical Data, Inc. announced that vilazodone, which combines the effects of a SSRI with 5-HT1A receptor partial agonist activity, has completed Phase III trials for the treatment of major depressive disorder [83]. Vilazodone, if approved, would represent a first-in-class drug for the treatment of depression, due to its novel dual mechanism of action.

A recent study on amisulpride may offer a new perspective to consider 5-HT7 antagonists potential drugs for treatment of depression [84]. Amisulpride is approved for clinical use in treating schizophrenia in a number of European countries and also for treating dysthymia, a mild form of depression, in Italy. Amisulpride has also been demonstrated to be an antidepressant for patients with major depression in many clinical trials. It has been widely assumed that modulation of dopaminergic D2 and D3 receptors was responsible for mediating its antidepressant and antipsychotic properties. It has been discovered that amisulpride is a potent competitive antagonist at 5-HT7 receptors and it does not interact with other molecular targets that could explain its antidepressant actions in vivo. Moreover, it has been demonstrated that 5-HT7 receptor knockout mice did not respond to amisulpride in two widely used rodent models of depression, in contrast to their wild-type counterparts. It has been, therefore, suggested that 5-HT7 receptor antagonism, and not D2/D3 receptor antagonism, underlies the antidepressant actions of amisulpride.

The potential involvement of 5-HT7 receptor in the pathogenesis of migraine has been hypothesized soon after the cloning of the receptor. Now, pharmacological studies are reported by Eli Lilly on the ability of selective 5-HT7 antagonists to significantly reduce or prevent dural plasma protein extravasation (that is thought to be symptomatic of migraine) open to new therapeutic potential for 5-HT7 antagonists.

The identification of selective agonists has revealed a more complex task. A large part of the work originated from the
Interestingly, a significant improvement in selectivity was achieved with less structurally constrained compounds as 83 (E-55888). Esteve has been particularly active in this field, especially considering that its studies have suggested a therapeutic utility for 5-HT7 agonists. It has been demonstrated that selective 5-HT7 agonist can be used for treatment of pain and symptoms of pain, especially neuropathic pain and inflammatory pain and symptoms involving allodynia and hyperalgesia.

From a medicinal chemist point of view, the compounds disclosed in the patent applications examined in the present review generally belong to chemical classes of known 5-HT7 receptor agents. According to previous reviews covering 5-HT7 receptor ligands from both patent and research tables, the compounds 83, 84, and 85 show moderate to high selectivity for 5-HT7 receptors.

**Table 4. 5-HT7 receptor affinities of selected ligands from Esteve.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>R1</th>
<th>R2</th>
<th>IC50, nM</th>
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<tbody>
<tr>
<td>83</td>
<td>Methyl</td>
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<td>Ethyl</td>
<td>Ethyl</td>
<td></td>
<td>76.2</td>
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<tr>
<td>85</td>
<td>Propyl</td>
<td>Propyl</td>
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</tr>
<tr>
<td>86</td>
<td>H</td>
<td>H</td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>87</td>
<td>-CH2CH2CH2CH2-</td>
<td>Methyl</td>
<td>Methyl</td>
<td>139</td>
</tr>
<tr>
<td>88</td>
<td>2,6-diCH3Ph</td>
<td>Methyl</td>
<td>Methyl</td>
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<tr>
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<tr>
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<td>Propyl</td>
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<td>Methyl</td>
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<tr>
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<tr>
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<tr>
<td>96</td>
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<tr>
<td>101</td>
<td>2,6-diF-Ph</td>
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<td>Methyl</td>
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<tr>
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<td>2-Cl,6-OCH3-Ph</td>
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<td>24</td>
</tr>
<tr>
<td>103</td>
<td>Methyl</td>
<td>Methyl</td>
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<tr>
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<td>Methyl</td>
<td>Methyl</td>
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<tr>
<td>105</td>
<td>Methyl</td>
<td>Methyl</td>
<td></td>
<td>753</td>
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</table>

literature [52,53], the 5-HT7 ligands can be grouped on the basis of a common basic framework. The only new frameworks disclosed by the patent applications examined here were those reported by Esteve (2,2a,4,5-tetrahydro-1H-3-azaacenylene, 109) and by Ajinomoto (Table 2). However, this decorated piperidine system is reminiscent of the structure of cyproheptadine, which also binds at 5-HT7 receptors [85]. It is important to note that a large part, if not all, of the ligands disclosed have been designed to achieve good pharmaceutical properties related to administration, distribution, metabolism and excretion. In fact, it is apparent that the ligands present chemical features that would facilitate fulfilling these requirements for druggability (electron-withdrawing groups to prevent metabolic degradation, optimal lipophilicity for use in vivo). The in vivo data described in the patent applications examined here confirm that the need of agents with a better pharmacokinetic profile has been largely met.

It might be surprising that nearly 20 years after the cloning of the 5-HT7 receptor, no 5-HT7 agents have entered Phase II clinical trials. How can this be explained? It is likely that the lack of an apparent therapeutic potential for 5-HT7 agents has decreased the interest in the development of newer agents. Therefore, one might expect a renewed interest in this area, as the new agonists and antagonists presented herein will allow deeper studies on 5-HT7 receptor function and, hopefully, unveil new therapeutic options.

**Declaration of interest**

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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