Journal of Medicinal Chemistry

Perspective

pubs.acs.org/jmc

Structure−Activity Relationships and Therapeutic Potentials of 5-HT$_7$ Receptor Ligands: An Update

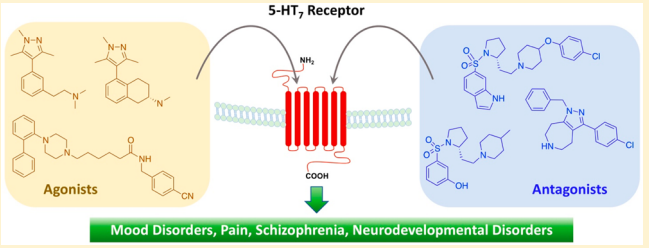
Maria N. Modica,[†] Enza Lacivita,[*,‡] Sebastiano Intagliata,[§] Loredana Salerno,[†] Giuseppe Romeo,[†] Valeria Pittalà,[*,†] and Marcello Leopoldo[‡]

[†]Dipartimento di Scienze del Farmaco, Università di Catania, Viale Andrea Doria 6, 95125 Catania, Italy

[‡]Dipartimento di Farmacia−Scienze del Farmaco, Università degli Studi di Bari Aldo Moro, Via Orabona 4, 70125 Bari, Italy

[§]Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Medical Science Building, 1345 Center Drive, Gainesville, Florida 32610, United States

ABSTRACT: Serotonin 5-HT$_7$ receptor (5-HT$_7$R) has been the subject of intense research efforts because of its presence in brain areas such as the hippocampus, hypothalamus, and cortex. Preclinical data link the 5-HT$_7$R to a variety of central nervous system processes including the regulation of circadian rhythms, mood, cognition, pain processing, and mechanisms of addiction. 5-HT$_7$R blockade has antidepressant effects and may ameliorate cognitive deficits associated with schizophrenia. 5-HT$_7$R has been recently shown to modulate neuronal morphology, excitability, and plasticity, thus contributing to shape brain networks during development and to remodel neuronal wiring in the mature brain. Therefore, the activation of 5-HT$_7$R has been proposed as a therapeutic approach for neurodevelopmental and neuropsychiatric disorders associated with abnormal neuronal connectivity. This Perspective celebrates the silver jubilee of the discovery of 5-HT$_7$R by providing a survey of recent studies on the medicinal chemistry of 5-HT$_7$R ligands and on the neuropharmacology of 5-HT$_7$R.



1. INTRODUCTION

Serotonin is a major neurotransmitter isolated in the late 1940s and soon chemically identified as 5-hydroxytryptamine (5-HT). Over the years, 5-HT has been one of the most widely studied chemical messengers, with more than 200000 scientific papers published until 2017 and approximately 50000 in the past decade, according to the Scifinder database. 5-HT is responsible for multiple physiological functions. In the periphery, it is widely distributed in different organs including the gastrointestinal tract where it regulates peristalsis, the cardiovascular system where it induces vasoconstriction, and the blood where it is involved in the coagulation process. In the central nervous system (CNS), 5-HT is involved in multiple functions, including cognition and memory processes, modulation of circadian rhythms, mood, food intake, and emesis. All of these physiological processes are mediated through a large class of receptors that are classified, according to the IUPHAR, on the basis of structural, functional, and pharmacological criteria into seven subfamilies.[1] The 5-HT receptor subtypes have been classified as follows: 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-ht$_{1e}$, 5-HT$_{1F}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, 5-ht$_{5a}$, (5-ht$_{5a}$ is yet to be confirmed as a receptor because no robust response signal in native tissue has been reported so far), 5-HT$_6$, and 5-HT$_7$.[1] Over the years, the unraveling of such receptors family showed that most of them belong to the rhodopsin-like subfamily (class A) of the G protein-coupled receptors (GPCRs) family, except for 5-HT$_3$, which is a ligand-gated ion channel.[1] The G protein-coupled 5-HT receptors share common seven-transmembrane domains with a conserved architecture, together with the conserved DRY (Asp-Arg-Tyr) and NPxxY motifs that seem to be relevant for the activation of the machinery located at transmembrane (TM) 3 and TM7 domains, respectively.[2] To date, crystal structures have been reported only for the 5-HT$_{1B}$ and 5-HT$_{2B}$ receptors.[3,4] The main functional differences among the 5-HT receptors reside on the stimulatory or inhibitory effects of the coupled intracellular signaling molecules. 5-HT receptors control a wide array of physiopathological functions and 5-HT receptors or 5-HT itself are the target for a high number of the marketed drugs.[2]

While the 5-HT$_{1A}$ receptor (5-HT$_{1A}$R) was the first 5-HT receptor subtype to be cloned and one of the most extensively characterized,[5] the 5-HT$_7$ receptor (5-HT$_7$R) was the last to be discovered in 1993, when at least three different research groups reported the cloning of 5-HT$_7$R from different species.[6−8] The 5-HT$_7$R has been cloned from a variety of species, showing high interspecies homology, ranging from 90% to 96%. Among the 5-HT receptor family, the 5-HT$_7$R shows the highest sequence homology (approximately 40%) with the 5-HT$_{1A}$R that could explain why the majority of 5-HT$_7$R ligands exhibit also 5-HT$_{1A}$R affinity.[9] The gene encoding for 5-HT$_7$R protein is located in humans at the seventh chromosome and has two introns, whereas in rats it is located at the first chromosome and in mice at the 19th.[7,10,11] Three different splice variants. h5-HT$_{7(a)}$, h5-HT$_{7(b)}$, h5-HT$_{7(d)}$, have been described so far in humans and four

Received: December 22, 2017
Published: May 16, 2018

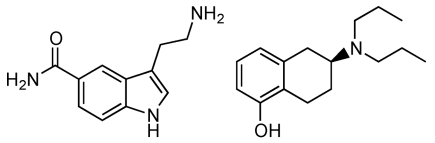
DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

in rats, namely 5-HT$_{7(a)}$, 5-HT$_{7(b)}$, 5-HT$_{7(c)}$, and 5-HT$_{7(e)}$.[12,13] Human variants are structurally closely related, with differences in their carboxyl terminal (C-terminus) tail. The splice variants do not show major differences in their membrane localization nor significant differences in their respective pharmacology and signal transduction or functional coupling to G$_s$ protein (see below), suggesting that the C-terminus is not involved in the binding of serotonergic ligands or the coupling to G protein.[14] h5-HT$_{7(a)}$ and h5-HT$_{7(b)}$ receptors originate from alternative splicing and are composed of 445 and 432 amino acids, respectively. The h5-HT$_{7(d)}$ is composed of 479 amino acids, exhibits the highest differences in the C-terminus, and shows a different internalization pattern compared to the other isoforms, suggesting a different interaction of the C-terminus with the intracellular machinery.[15] h5-HT$_{7(d)}$ is more abundant in the spleen than in the CNS.

Initially, 5-HT$_7$R was pharmacologically characterized by high affinity for compound **1** (5-CT, Figure 1) and compound **2** (8-OH-DPAT, Figure 1). 5-HT$_7$R is positively coupled to adenylate cyclase (AC) through activation of G$_s$, resulting in an intracellular increase of cAMP, and displays a high constitutive AC activity (i.e., a constitutively active conformational state of the 5-HT$_7$R coupled with G$_s$).[6−8,16] 5-HT$_7$R is physically preassociated with G$_s$ in the absence of ligand. Upon agonist activation, the preassociated complex undergoes conformational changes that involve a rapid movement of the Gα subunit relative to the receptor that likely results in GDP release. This event is followed by a slow dissociation of G$_{\beta\gamma}$ from both the receptor and G$_\alpha$.[17,18] The physiological consequence of the preassociation is not completely understood. It has been proposed that preassociation could contribute to biologic function by providing a rapid onset of receptor signaling. In addition, the binding of free G protein can attenuate the signaling of other GPCRs that couple to the same G protein. Consequently, G protein scavenging might serve as a tool by which a given cell could be more sensitive to specific G protein signaling via that specific receptor.[18]

Prolonged stimulation by the agonist 5-HT and by the inverse agonist **5** (SB-269970, Figure 2) induced both homo- and heterologous desensitization of G$_s$ signaling in HEK293 cells, suggesting that the desensitization is independent from the activation of G$_s$.[19] 5-HT$_7$R can also couple to G$_{12}$, a heterotrimeric G protein that modulates the activity of small monomeric GTPases, such as Rho, Rac, and Cdc42, which belong to the Rho family of small GTPases.[20,21] In hippocampal neurons, 5-HT$_7$R/G$_{12}$ signaling regulates serum response element (SRE)-mediated gene transcriptional activity and modulates neuronal morphology through activation of RhoA and Cdc42.[20] In rat cultured hippocampal neurons, stimulation of the 5-HT$_7$R/G$_{12}$ signaling pathway promotes the formation of dendritic spines and accelerates synaptogenesis, leading to enhanced spontaneous synaptic activity.[21] The mechanisms regulating the coupling to G$_s$ or G$_{12}$ are not clear yet. Because the 5-HT$_7$R undergoes post-translational modification through palmitoylation, it has been suggested that agonist-induced palmitoylation of 5-HT$_7$R affects G$_s$-mediated constitutive activity, with no effect on G$_{12}$-mediated activity. Therefore, palmitoylation/depalmitoylation might modify the constitutive activity of the receptor, switch the intracellular coupling, and eventually impact on the physiological effect.[22]

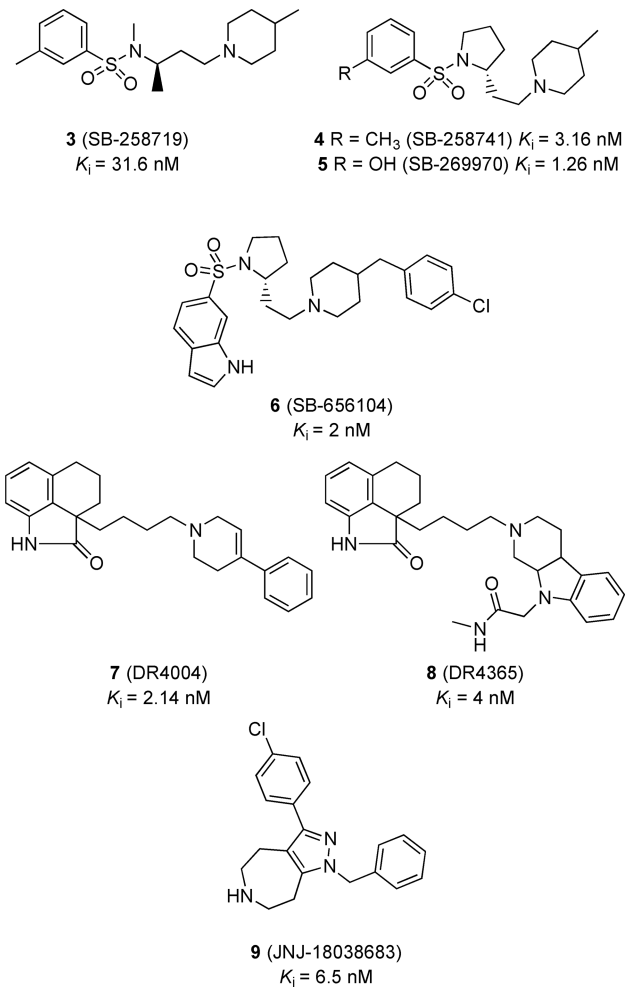
The 5-HT$_7$R is widely distributed in the human body with a prevalence, in the periphery, in the cardiovascular system, including the heart and blood vessels, in the gastrointestinal tract, including the small intestine and colon, in the liver, the ovary, and the testes.[14] Autoradiographic and immunohistochemistry studies in human, guinea pig, and rat CNS showed that 5-HT$_7$R is distributed in discrete areas with high to moderate concentrations in limbic areas, such as the thalamus, hypothalamus, hippocampus, and amygdala, in the putamen, raphe and caudate nuclei, and cortical regions.[9] On the basis of its distribution in the CNS, 5-HT$_7$R was proposed to be involved in important functional roles such as thermoregulation, circadian rhythm, sleep, learning and memory, cognition impairment, stress, and schizophrenia.[9,23,24] Studies consistently suggest a strong implication in mood disorders, including anxiety and depressive disorders.[9,24] Understanding the implication of 5-HT$_7$R in such brain functions/dysfunctions is even more complex in light of the cross-talk between 5-HT$_7$ and 5-HT$_{1A}$ receptors.[25−27] In fact, the two receptor subtypes exert opposite effects on the intracellular level of cAMP and are localized in the



1 (5-CT) K_i = 0.93 nM

2 (8-OH-DPAT) K_i = 466 nM

Figure 1. Structures of nonselective reference 5-HT$_7$R agonists.



3 (SB-258719) K_i = 31.6 nM

4 R = CH$_3$ (SB-258741) K_i = 3.16 nM

5 R = OH (SB-269970) K_i = 1.26 nM

6 (SB-656104) K_i = 2 nM

7 (DR4004) K_i = 2.14 nM

8 (DR4365) K_i = 4 nM

9 (JNJ-18038683) K_i = 6.5 nM

Figure 2. Structures of selective 5-HT$_7$ antagonists.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

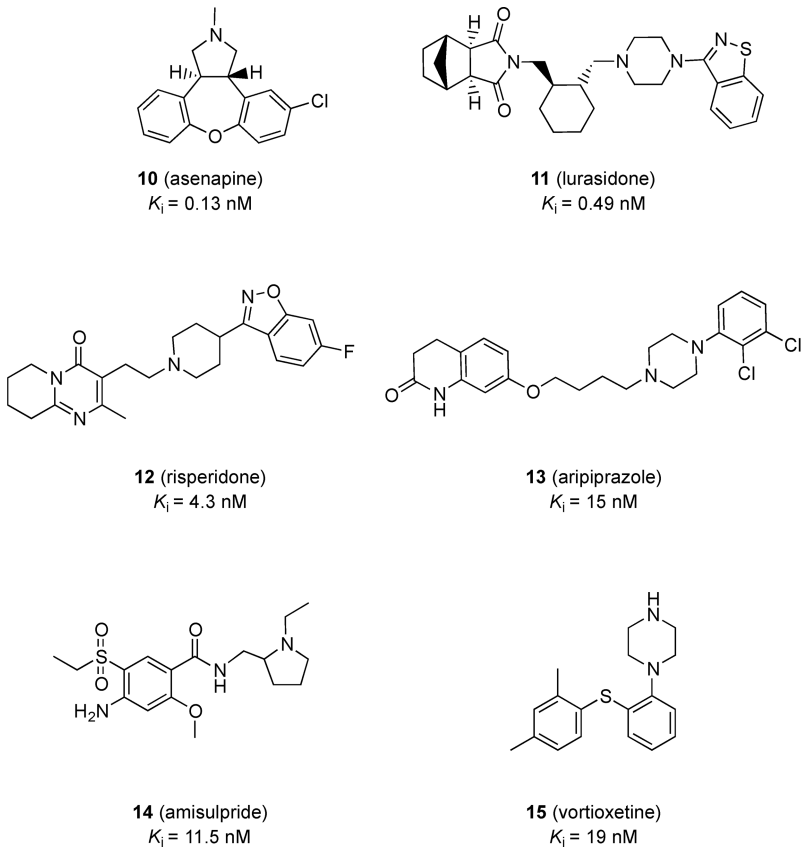


Figure 3. Antipsychotic and antidepressant drugs with antagonist activity at 5-HT$_7$R.

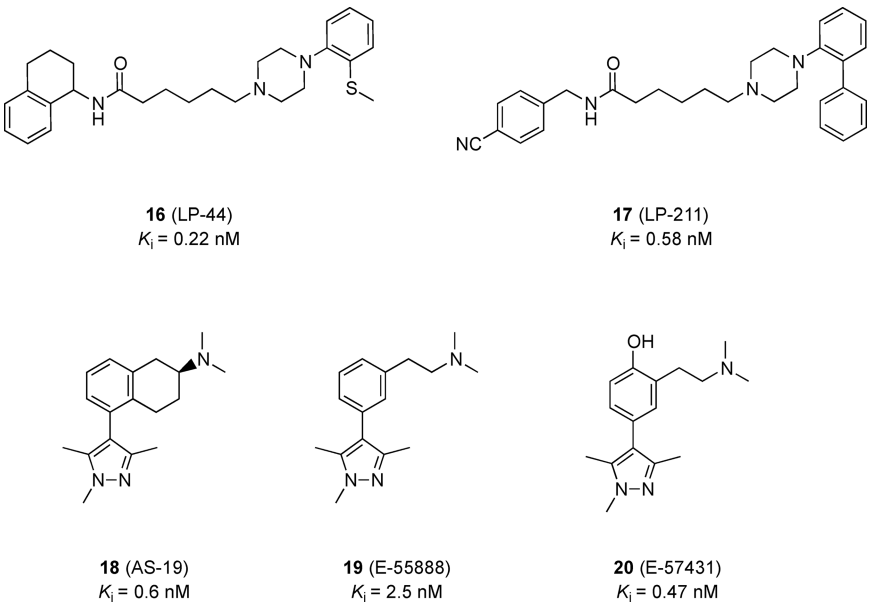


Figure 4. Structures of selective 5-HT$_7$R agonists.

same brain areas. In addition, most of the ligands show high affinity for both receptors. Finally, recent studies evidenced that 5-HT$_7$R and 5-HT$_{1A}$R can form homo- or heterodimers.[28,29]

Soon after the discovery of the 5-HT$_7$R, the lack of selective ligands slowed down the progress in this research area. A number of high-affinity, nonselective 5-HT$_7$R ligands were identified, including ergolines and tricyclic antipsychotic agents.[30,31] As these compounds showed complex pharmacological profiles due to their multireceptorial affinity, they have not been pursued for the development of selective 5-HT$_7$R ligands. The affinity of several psychoactive drugs for the 5-HT$_7$R along with the abundance of the receptor in discrete areas of the CNS stimulated the search for selective 5-HT$_7$R ligands in both academia and pharmaceutical companies. It soon became evident that the high similarity between 5-HT$_7$R and 5-HT$_{1A}$R was a relevant issue that, in some respects, remains unsolved. Starting

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

from 2000, extensive structure–activity relationship (SAR) studies and pharmacokinetic optimization conducted at GlaxoSmithKline led to the identification of the antagonists **3** (SB-258719), **4** (SB-258741), **5**, and **6** (SB-656104) (Figure 2).[32,33] Compounds **3** and **5**, both endowed with a competitive antagonist profile at 5-HT$_7$R, stood out for their potency and selectivity (100-fold selectivity over a range of GPCRs, including 5-HT$_{1A}$R), but their high blood clearance limited their potential application in clinical studies. Subsequent studies led to the identification of the antagonist **6**, which showed high potency and selectivity along with an improved pharmacokinetic profile, e.g., lower blood clearance when compared to **5**, higher oral bioavailability, and blood–brain barrier (BBB) penetration.

Researchers at Meiji Seika Kaisha Ltd. reported on the development of the long chain arylpiperidine derivatives **7** (DR4004)[34] and **8** (DR4365)[35] (Figure 2). Both compounds displayed antagonist properties at the 5-HT$_7$R and good selectivity toward the 5-HT$_{2A}$ receptor. Despite the encouraging in vitro pharmacological profile, derivatives **7** and **8** were not developed further in clinical trials. In 2012, researchers at Janssen Research & Development reported the pyrazolo[3,4-*d*]azepine derivative **9** (JNJ-18038683, Figure 2), as a potent 5-HT$_7$R antagonist with good selectivity over a panel of receptors and ion channels, which was progressed to clinical studies as a potential antidepressant.[36] In addition, a number of atypical antipsychotics, such as compounds **10** (asenapine), **11** (lurasidone), **12** (risperidone), **13** (aripiprazole), and **14** (amisulpride),[24] as well as the multimodal antidepressant drug compound **15** (vortioxetine) were characterized as potent 5-HT$_7$R antagonists (Figure 3).[37]

Thus far, various selective 5-HT$_7$R agonists have been identified. The arylpiperazine derivatives **16** (LP-44)[4,38] and **17** (LP-211)[39,40] (Figure 4) showed high affinity for the 5-HT$_7$R and moderate to low affinity for other 5-HT receptors, including the 5-HT$_{1A}$R. Both compounds were characterized as 5-HT$_7$R agonists as they induced 5-HT$_7$-mediated relaxation of substance P-stimulated guinea pig ileum contraction. The aminotetraline derivative **18** (AS19, Figure 4) was reported as a potent 5-HT$_7$R partial agonist with high selectivity over other 5-HT receptor subtypes.[41] Laboratorios Esteve reported compounds **19** (E-55888) and **20** (E-57431) (Figure 4) as potent and selective full agonists with efficacy and potency similar to that of 5-HT.[41,42] Finally, oleamide has been reported as 5-HT$_7$R negative allosteric modulator.[43]

The availability of 5-HT$_7$R agonists and antagonists as well as of 5-HT$_7$R-knockout mice provided the scientific community with powerful tools to get deeper insights on the role of 5-HT$_7$R in health and disease. However, after 25 years after the discovery of the 5-HT$_7$R, many questions put at the beginning and many others arisen over the years are still open. To celebrate the silver jubilee of the discovery of the 5-HT$_7$R, we report here an overview on the most relevant advances in the understanding of the pathophysiological role of 5-HT$_7$R in CNS disorders and their therapeutic potential. Moreover, this perspective summarizes the medicinal chemistry efforts in the 5-HT$_7$R field from 2011 to date (for a survey of 5-HT$_7$R ligands before 2011 see refs 30,31).

2. PHARMACOPHORE MODELS FOR 5-HT$_7$R LIGANDS

Because the identification of the 5-HT$_7$R, computer-aided techniques (homology models and 3D receptor-based pharmacophore models) have been extensively applied to accelerate the development of selective and potent 5-HT$_7$R ligands.[44−47] Here we briefly report the most consolidated pharmacophore models developed thus far.

The first pharmacophore hypothesis, built on a set of available antagonists using CATALYST software, proposed that five structural elements were essential for the binding to the receptor: a positively charged nitrogen atom (PI), a H-bond acceptor (HBD), and three hydrophobic regions (HYD1−3) (Figure 5a).

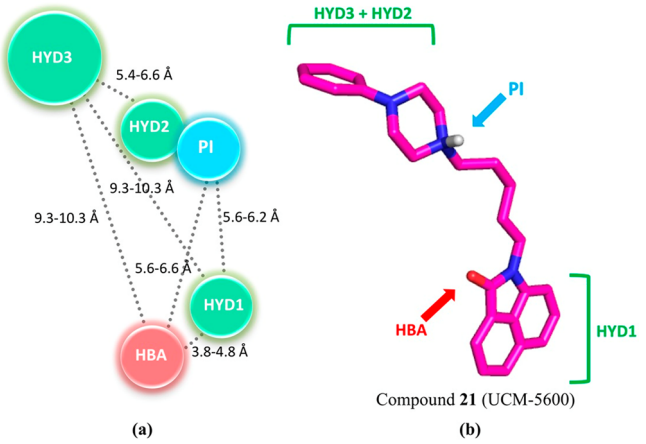


Figure 5. (a) Pharmacophoric features for 5-HT$_7$ antagonism proposed by López-Rodríguez et al. (b) 3D structure of the lead compound **21**.[48]

The hypothesis was validated by designing a set of naphtholactam and naphthosultam derivatives which led to the identification of derivative **21** (UCM-5600, 5-HT$_7$ K_i = 89 nM, Figure 5b) as a new lead compound.[48]

Next, the structural features required for 5-HT$_7$/5-HT$_{1A}$ selectivity were evaluated by identifying specific interactions between pharmacophore features and amino acid residues. These interactions included: ionic interaction with Asp3.32 and the protonated amine (PI) of the ligands, $\pi-\pi$ interaction between aromatic ring (HYD2 + HYD3) and Phe3.28, H-bond interaction of the carbonyl group (HBA) and Ser6.55, hydrophobic interactions between dihydroindolone ring (HYD1) and Phe5.47, or Phe6.52 placed into a small cavity formed between TM5 and TM6 helices (Figure 6). The authors proposed that decreasing the distance between PI and HBA features improves selectivity by forcing the ligand to bind Ser6.55, an amino acid that is present only in 5-HT$_7$R. Polar substitutions at the HYD2+HYD3 region increase selectivity because of a possible

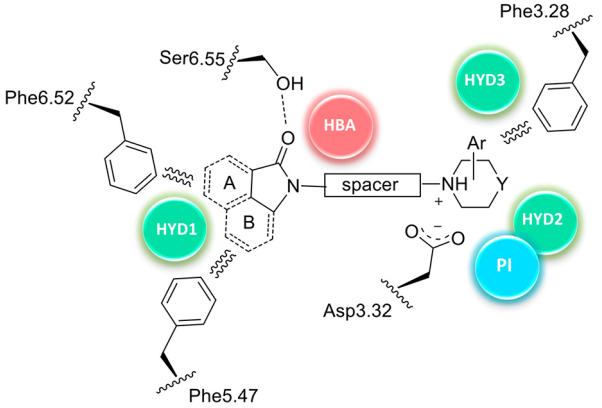


Figure 6. General 2-D binding mode and pharmacophoric features proposed by Medina et al.[49]

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

interaction with Arg7.36, another amino acid that is present only in 5-HT$_7$R. Finally, an increase of the size of HYD2+HYD3 region results in a decrease of selectivity because it induces clashes with Val2.61 in the 5-HT$_7$R and favors the interaction with Tyr2.64 in the 5-HT$_{1A}$R.[49] The hypothesis was confirmed through the synthesis a new series of arylpiperazine derivatives.

The first receptor-based pharmacophore for the 5-HT$_7$R was constructed by Kołaczkowski et al., which evaluated, through docking studies, the mode of interaction of selective and nonselective antagonists with the receptor binding site.[50] The authors proposed two hypotheses for antagonists binding to the receptor: an "affinity" pharmacophore and a "selectivity" pharmacophore (Figure 7). The "affinity" pharmacophore was

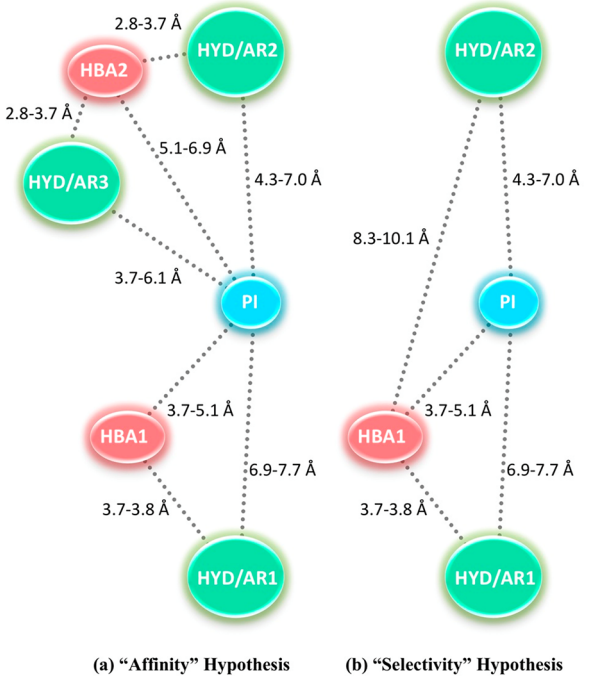


Figure 7. Pharmacophoric features for (a) the "Affinity" hypothesis and (b) the "Selectivity" hypothesis for 5-HT$_7$ antagonists proposed by Kołaczkowski et al.[50]

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 (HBA1,2) (Figure 7). The affinity is determined by the presence of at least three out of six structural features: the basic nitrogen PI and one of ARs (capable of specific CH−π or π−π interaction) are strictly necessary, while the third may be a HBA or another HYD/AR region. Each of the features interacts specifically with the receptor structure. On the other hand, the selectivity is the result of the presence of strong electrostatic (PI) and π−electronic interactions (HYD/AR1), which are common to all selective antagonists, and an additional interaction of HBA1 or HYD/AR2.[50]

The first pharmacophore model for 5-HT$_7$R agonists was proposed by Vermeulen et al.[51] Full conformational analysis of a set of 20 diverse 5-HT$_7$R agonists in their protonated form was performed followed by a pharmacophore-identifying procedure through ligand overlap. 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 8). The residues

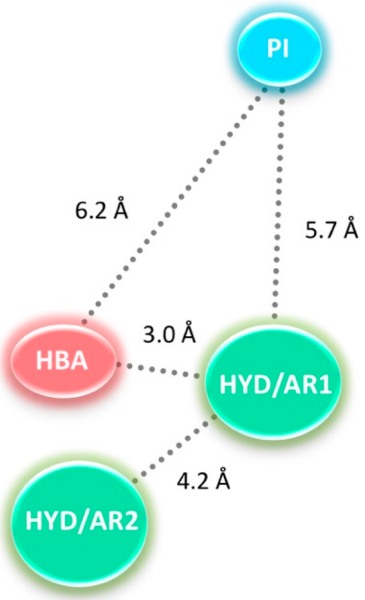


Figure 8. Pharmacophoric features for the 5-HT$_7$R agonism proposed by Vermeulen et al.[51]

Asp3.32 (interaction with a protonated nitrogen) and Thr5.43 (interaction with a substituent at an aromatic moiety) were identified as important for ligand binding. Amino acid residues of the aromatic cluster of TM6 region were hypothesized to play an important role in ligand binding as π−π stacking moieties. It was also proposed that the agonists missing a hydrogen-bond-accepting moiety, but instead possessing an aromatic substituent, could bind to the receptor with high affinity as well by occupying a lipophilic pocket hosted by residues of the TM5 and TM6 helices.[51]

From a comparison of the described pharmacophore models, it is evident that at least four chemical features are necessary for antagonist activity: the positively charged nitrogen atom, the HBA, and two hydrophobic/aromatic centers. The pharmacophore model for agonists is described by the same four chemical features but with different a orientation and intramolecular distances.

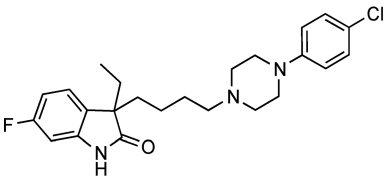
Rague et al. have recently described two pharmacophore models for long-chain arylpiperazine derivatives and sulfonamide-containing compounds using MOE software, which identified pharmacophore features in agreement with those identified in the older models.[52]

3. 5-HT$_7$R LIGANDS

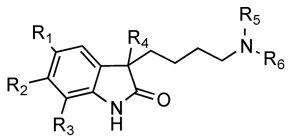
Over the years, a large number of 5-HT$_7$R ligands, agonists, and antagonists have been reported belonging to different chemical classes which are identified by the main scaffold or a functional group present in the molecules (i.e., long chain arylpiperazines, sulfonamides, biphenylmethyl derivatives). As discussed in the previous section, 5-HT$_7$R ligands usually have a protonable nitrogen that serves as an anchoring point to Asp3.32. This interaction is supplemented by additional interactions with aromatic/hydrophobic residues, which modulate affinity and selectivity toward other related receptors.

One of the most thoroughly explored classes of 5-HT$_7$R ligands is the class of long chain arylpiperazine derivatives, which are characterized by an arylpiperazine moiety linked through an alkyl spacer to a terminal fragment. During the years, huge efforts

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX



22 5-HT$_7$ K_i = 0.79 nM;
5-HT$_{1A}$ 9% inhibition at 10^{-7} M;
α_1 K_i = 215 nM



General structure I
R_1, R_2, R_3 = H or halogen, R_4 = H or Et,
R_5, R_6 = Arylpiperazine, Arylpiperidine
Ph-3,6-dihydropyridine

23 R_1 = R_2 = R_3 = H, R_4 = Et,
R_5, R_6 = 1-(4-ClPh)piperazine;
5-HT$_7$ K_i = 0.38 nM;
5-HT$_{1A}$ 11% inhibition at 10^{-7} M;
α_1 K_i = 93 nM

Figure 9. Structures of the long chain arylpiperazine derivatives developed by Egis Pharmaceuticals.[54]

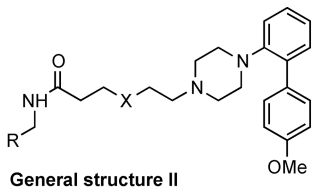
have been made in structural modification of the long chain arylpiperazine template with the aim of identifying and developing selective 5-HT$_7$R ligands. As a result, a large number of compounds showing a wide array of binding properties have been reported.

Egis Pharmaceuticals reported on a series of arylpiperazine derivatives bearing an oxindole nucleus as the terminal fragment exemplified by compound **22**. Although compound **22** exhibited very high binding affinity (5-HT$_7$ K_i = 0.79 nM), good selectivity, and antagonistic profile, the poor pharmacokinetic profile prompted further optimization.[53] Structural modifications were performed by introducing various basic groups, an alkyl residue at the 3-position of the oxindole ring, and inserting one or more halogens on the terminal fragment (general structure **I**, Figure 9).[54] The affinity data of the new derivatives confirmed previous SARs. In fact, the position of the substituent on the phenyl ring linked to the piperazine nucleus was important for 5-HT$_7$R affinity, whereas the introduction of halogens on the oxindole ring did not impact on 5-HT$_7$R affinity. Several ligands having nanomolar affinity for 5-HT$_7$R were identified and, in some cases, good selectivities over the 5-HT$_{1A}$ and α_1 receptors were also observed (i.e., compound **23**, Figure 9). On the other hand, the performed structural modifications did not lead to a substantial improvement of the stability of the compounds toward oxidative metabolism. In addition, despite their high 5-HT$_7$R affinity and antagonistic profile, the compounds did not demonstrate antidepressant activity when tested in forced swimming test (FST) in mice.[54] Therefore, this class of 5-HT$_7$R antagonists was not further developed.

Lacivita and colleagues at the University of Bari have been actively involved for many years in the development of arylpiperazine-based high-affinity 5-HT$_7$R ligands, exemplified by the selective agonist **17** (Figure 4), which has been extensively used as pharmacological tool to study the effect of 5-HT$_7$R activation in vitro and in vivo (see below).[39,55] An in vivo disposition study in mice demonstrated that the agonist **17** rapidly reached the brain after intraperitoneal injection and, like other 1-arylpiperazine derivatives, underwent N-dealkylation of the aliphatic chain attached to the piperazine nitrogen, leading to the formation of the unsubstituted arylpiperazine **24** (RA-7, Figure 10) as the main metabolite. This potentially active metabolite was also able to concentrate in mouse brain, and this might be of relevance because the final pharmacological effect might result from the interplay between the neurochemical action of the parent drug and the active metabolite.[40] Therefore, with the aim to improve the stability of **17** to N-dealkylation, the scaffold of **17** was manipulated with the aim to identifiy new 5-HT$_7$R agonists with pharmacological properties similar to those of **17** and improved metabolic stability. A first attempt to improve metabolic stability was to target compounds with lower lipophilicity than **17**.[56] This was achieved by replacing the cyanophenyl portion of compound **17** with a pyridyl nucleus and the pentamethylene chain with an ethoxyethyl spacer (general structure **II**, Figure 10). The 1-(2-biphenyl)piperazine moiety of **17** was replaced with 1-[2-(4-methoxyphenyl)phenyl]piperazine because this substitution led to an improvement of the selectivity over 5-HT$_{1A}$ and α_1 receptors. The structural modifications were well tolerated because the new derivatives showed nanomolar affinity for 5-HT$_7$R and good selectivities over off-target receptors. In addition, the performed structural modifications translated in improved in vitro microsomal stability as compared to **17**.[56] Compound **26** (Figure 10) was selected for further in vivo pharmacokinetic studies, but it was rapidly metabolized to the unsubstituted 1-[2-(4-methoxyphenyl)phenyl]piperazine and was eliminated more rapidly ($t_{1/2}$ = 44 min) as compared to compound **17** ($t_{1/2}$ = 62 min).[56] A second attempt to obtain compounds with metabolic stability greater than **17** consisted in increasing the "local" polarity in the terminal arylmethyl carboxamide fragment by inserting water-solubilizing groups (general structure **III**, Figure 10).[57] This structural modification did not substantially change the affinity for 5-HT$_7$R but led to a loss of selectivity and had limited beneficial effect on metabolic stability. Next, different substitution patterns of the biphenyl nucleus linked to the piperazine ring were evaluated. The data showed that this structural modification had limited impact on affinity and selectivity but had a beneficial effect on metabolic stability, suggesting that the steric rather than the electronic effect of the substituent on the biphenyl system was responsible for the increased metabolic stability. Among the studied compounds, derivative **27** (Figure 10) exhibited agonist properties and improved metabolic stability in vitro as compared to **17** and was able to stimulate neurite outgrowth in neuronal primary cultures in shorter time and lower concentration than compound **17**. In addition, compound **27** showed a biodistribution profile comparable to that of **17**.[57]

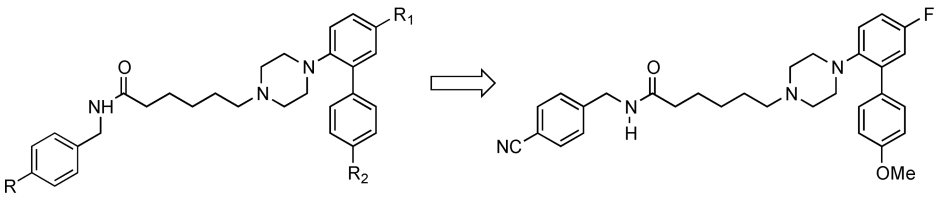
The observation that the unsubstitued piperazine **24** was able to bind to the 5-HT$_7$R with high affinity (K_i = 1.4 nM), despite the lack of N_4-substituent, which is generally considered essential for binding, stimulated interest in studying the SARs of biphenylpiperazines.[58] Therefore, different substituents on the

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX



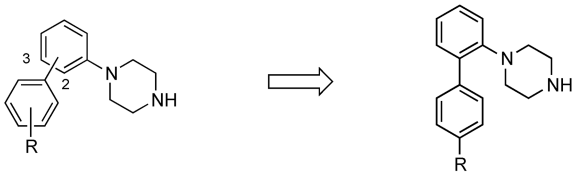
General structure II
R = 2-, 3-, or 4-pyridyl; X = CH_2 or O

26 R = 3-Pyridyl, X = O
5-HT_7 K_i = 23.8 nM;
5-HT_{1A} K_i = 1274 nM;
α_1 K_i = 393 nM



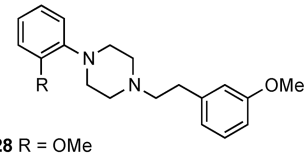
General structure III
R = CN; methoxymethoxy; 2-hydroxyethoxy; 2-(4-morpholino)ethoxy; dimethylaminomethyl
R_1 = F or CN
R_2 = H or OMe

27 5-HT_7 K_i = 25.5 nM;
5-HT_{1A} K_i= 771 nM;
5-HT_6 K_i = 614 nM;
D_2 K_i = 522 nM;
α_1 K_i = 6.6 nM



General Structure IV
R = 4-NO_2, 2,6-OMe, 3,4-Me, 4-F, 4-OMe, H,

24 (RA-7) R = H
5-HT_7 K_i = 1.4 nM; 5-HT_{1A} K_i = 99 nM; α_1 K_i = 59 nM
25 R = OMe
5-HT_7 K_i = 2.6 nM; 5-HT_{1A} K_i = 476 nM; α_1 K_i = 156 nM



28 R = OMe
5-HT_7 K_i = 24.5 nM; 5-HT_{1A} K_i = 2.37 nM
29 R = Ph
5-HT_7 K_i = 7.5 nM; 5-HT_{1A} K_i = 20 nM

Figure 10. Arylpiperazine-based 5-HT_7R ligands developed at the University of Bari.

distal phenyl nucleus at the 2- or 3-position of compound **24** were introduced with the aim to understand the structural requirements for the interaction with 5-HT_7R (general structure **IV**, Figure 10).[58] In general, 1-(2-biphenyl)piperazines exhibited higher affinity as compared to the corresponding 1-(3-biphenyl)piperazine analogues. When substituents such as 2,6-dimethoxy, 2-methoxy, and 2-methyl were introduced on the distal phenyl ring, an opposite trend was observed, with the 1-(3-biphenyl)derivatives more potent than the 1-(2-biphenyl) counterparts. It was proposed that hydrophobic or H-bond interactions might favor a proper orientation of the molecule within the binding pocket. Docking studies supported these results and showed that the 5-HT_7R hydrophobic binding pocket was able to accommodate and tolerate different substituents if the distal phenyl was in the 2-position. Among the studied compounds, derivative **25** (Figure 10) showed the best combination of affinity and selectivity.[58] The functional activity of compounds **24** and **25** were investigated in in vitro and ex vivo assays. Both compounds were able to induce relaxation of guinea pig ileum in the same fashion as 5-CT, thus behaving as agonists in this experimental setup. Instead, both compounds failed to stimulate cAMP accumulation in 5-HT_{7a}-expressing HeLa cells, thus behaving as antagonists. These results were quite interesting because, for the first time, dual agonist/antagonist ligands were reported for the 5-HT_7R, and this might help to explain some inconsistencies in the role of 5-HT_7R in the CNS (see below).

The increase of the metabolic stability and selectivity for the 5-HT_7R over the 5-HT_{1A}R was the main goal of the structural modifications performed on compound **28**, previously reported as dual 5-HT_7/5-HT_{1A} receptor ligand (Figure 10).[59] The

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

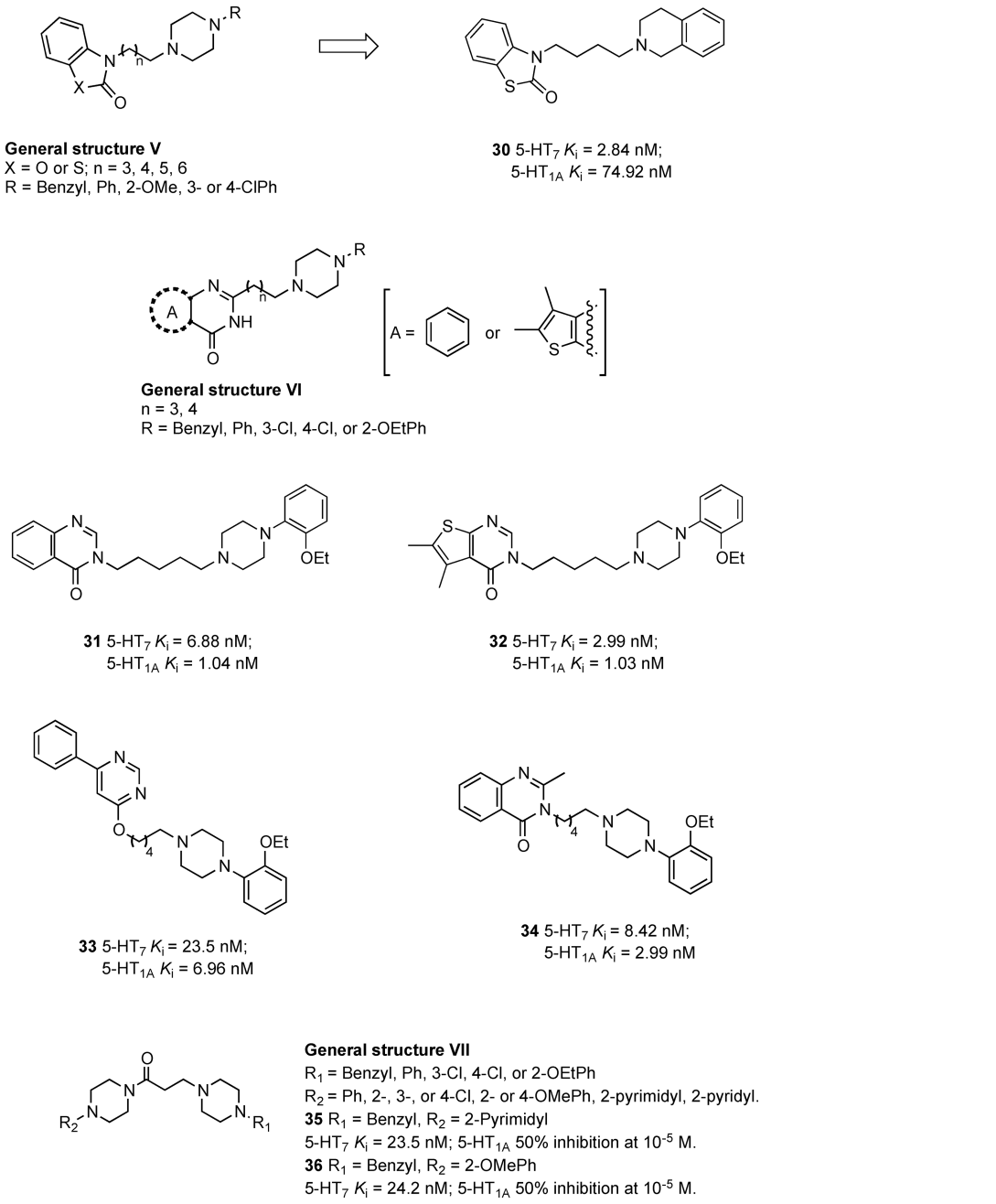


Figure 11. Structures of the arylpiperazine derivatives developed at the University of Catania.[61]

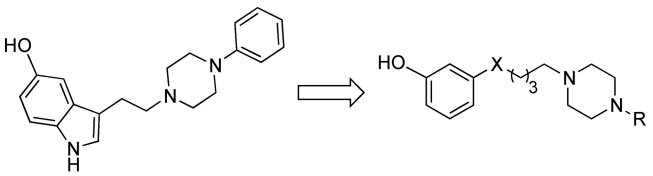
different substituents on the arylpiperazine ring as well as different basic moieties were introduced. The newly synthesized compounds, although showing better in vitro metabolic stability, exhibited poor selectivity toward 5-HT$_{1A}$R. Compound **30** (Figure 10), in which the 2-methoxy group was replaced with a phenyl ring, showed the best combination of affinity, selectivity, and metabolic stability.[59,60]

Researchers at the University of Catania have developed a series of long chain arylpiperazine derivatives bearing a 2-benzoxazolone or 2-benzothiazolone moiety as the terminal fragment which have dual 5-HT$_7$/HT$_{1A}$ ligand properties (general structure **V**, Figure 11).[61] SARs studies evidenced that the substitution of the arylpiperazine moiety influenced the affinity for 5-HT$_7$R, whereas the length of the intermediate alkyl chain was important for the selectivity toward 5-HT$_{1A}$R. The replacement of the arylpiperazine with the isoquinoline nucleus improved the selectivity toward 5-HT$_{1A}$R. Compound **30** (Figure 11) demonstrated nanomolar affinity for the 5-HT$_7$R (K_i = 2.84 nM) and >25-fold selectivity over 5-HT$_{1A}$R. Docking studies using the homology models of 5-HT$_7$ and 5-HT$_{1A}$ receptors were in agreement with the SARs and evidenced that the compounds that bound to 5-HT$_7$R adopted an L-shape conformation, with the terminal fragment pointing toward the extracellular loops. Instead, the compounds adopted an extended conformation when interacting with the 5-HT$_{1A}$R.[61] In a subsequent paper, the 2-benzoxazolone and 2-benzothiazolone was replaced by the 5,6-dimethylthienopyrimidinone or the quinazolinone scaffold.[62] An alkyl chain of four or five methylene units was inserted at the 2- or 3-position of the heterocyclic nucleus, linking differently substituted arylpiperazines (general structures **VI**, Figure 11). SAR studies indicated that the elongation of the alkyl spacer enhanced 5-HT$_7$R affinity and

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

that the 2-ethoxy substituent on the phenyl linked to the piperazine ring was preferred for high 5-HT$_7$R affinity. Compounds **31** and **32** (Figure 11) were potent dual 5-HT$_7$/5-HT$_{1A}$ ligands and behaved as antagonists in a functional test.[62] To extend SAR studies on this class of derivatives, the phenylpyrimidine and 2-methylquinazoline moieties were introduced as the terminal fragment. A 1-arylpiperazine moiety through a five-methylene chain was anchored at the nitrogen or oxygen atom of the heterocyclic scaffolds. The affinity data were in agreement with the previous SAR studies. Although several derivatives showed 5-HT$_7$R affinity in the nanomolar range, poor selectivity toward 5-HT$_{1A}$R was observed. Compounds **33** and **34** (Figure 11) were characterized as 5-HT$_7$R antagonists.[63] The same research group reported new hetero bis-piperazinyl-1-propanone derivatives, in which two arylpiperazine moieties were connected through a butan-2-one spacer (general structure **VII**, Figure 11). Derivatives **35** and **36** (Figure 11) with a benzyl moiety in R_1 and a 2-pyrimidinyl or 2-methoxyphenyl in R_2 exhibited nanomolar affinity for 5-HT$_7$R and selectivity over the 5-HT$_{1A}$R.[64]

Spadoni et al. designed a new series of long chain arylpiperazine derivatives by linking a serotonin-like scaffold with the arylpiperazine moiety through an alkyl spacer (Figure 12).[65] The first group of compounds were prepared by linking



37 5-HT$_7$ K_i = 63.1 nM

38 X = NMe, R = α-Naphthyl
5-HT$_7$ K_i = 2.9 nM

39 X = CH_2, R = Ph
5-HT$_7$ K_i = 3.1 nM

Figure 12. Structures of the 5-HT$_7$R ligands developed by Spadoni et al.[65]

the 5-hydroxyindol-3-ylethylamine with different arylpiperazines. Compound **37** (Figure 12) showed 5-HT$_7$R affinity in the nanomolar range. Next, the 5-hydroxyindole ring was replaced by bioisosteres such as tetraline, aniline, and phenol, while different arylsubstituents were linked to the piperazine ring. Compounds **38** and **39** (Figure 12) showed 5-HT$_7$R affinity in the nanomolar range. No data on selectivity or functional properties were reported for these two compounds. Docking studies using a 5-HT$_7$R homology model contributed to the elucidation of the binding mode of this group of compounds. It was proposed that the 3-hydroxylanilino moiety establishes an H-bond with Ser5.42, while the phenyl ring linked to the piperazine lies in the region of the binding site delimited by TM2, 3, and 7 helices. The authors speculated that this binding pose could be consistent with antagonism at 5-HT$_7$R.[65]

Starting from the dual 5-HT$_{1A}$/5-HT$_7$R ligand **40** (MR25003, Figure 13), Sagnes et al. have developed a series of 1-arylindole-based 5-HT$_7$R antagonists.[66] The pyrrole ring in compound **40** was replaced by an indole,and the impact on 5-HT$_7$R affinity was evaluated. Affinity data showed that this structural modification was well tolerated. In addition, when the N-1 phenyl ring was substituted with small alkyl substituent in the 2-position, nanomolar 5-HT$_7$R affinity values were observed. As regards to the N-substituent of the piperazine, the presence of a phenyl or a substituted phenyl group was crucial to retain the 5-HT$_7$R affinity. As for the selectivity toward 5-HT$_{1A}$R, the substitution pattern on the arylpiperazine moiety was important. Among the studied compounds, derivative **41** (Figure 13) showed nanomolar 5-HT$_7$R affinity and 15-fold selectivity over 5-HT$_{1A}$R.[66]

Continuing their efforts to obtain 5-HT$_7$R ligands,Medina et al. have reported on some indolone derivatives which demonstrated remarkable selectivity toward 5-HT$_{1A}$R (general structure **VIII**, Figure 13).[67] The compounds were designed on the basis of previous computational studies,suggesting that derivatives with short spacers would bind 5-HT$_7$R with the protonated amine and the indolone moiety of the ligand both placed within the orthosteric site and that compounds with long spacers would reverse the binding mode so that the indolone moiety would expand toward the extracellular space. In the latter case, higher selectivity would be observed due sequence differences between 5-HT$_7$R and 5-HT$_{1A}$R. In agreement with this hypothesis, the compounds showed nanomolar affinity for 5-HT$_7$R, whereas their 5-HT$_{1A}$R affinity depended upon the length and the flexibility of the alkyl spacers. Among the studied compounds, compound **42** (Figure 13) showed subnanomolar affinity and remarkable selectivity toward 5-HT$_{1A}$R. Moreover, compound **42** was characterized as a 5-HT$_7$R antagonist and displayed antidepressant-like properties in a mouse tail suspension test (TST) and FST.[67]

Strekowski et al. have reported on a series of 4-mono- or 4,6-disubstituted 2-(4-methylpiperazin-1-yl)pyrimidine derivatives (general structure **IX**, Figure 13) as high 5-HT$_7$R affinity ligands.[68] This scaffold was identified by screening an in-house compound library, previously developed as 5-HT$_{2A}$ receptor antagonists. The SAR studies included structural changes of the pyrimidine core moiety, changes of the 3-furyl group with other heteroaryl substituents, as well as additional substitutions at positions 5 and 6 of the pyrimidine. The affinity data indicated that insertion of an alkyl substituent at position 6 of the pyrimidine resulted in a substantial increase of the 5-HT$_7$R affinity. In addition, the 4-(3-furyl) moiety was crucial for the 5-HT$_7$R affinity of the substituted pyrimidines. The pyrimidine core could be replaced with a pyridine ring without a dramatic loss of the binding affinity. Compounds **43** and **44** (Figure 13) displayed nanomolar affinity (K_i = 7.1 and 1.6 nM, respectively) and selectivity toward 5-HT$_{1A}$, 5-HT$_6$, and D_2 receptors, but not 5-HT$_{2A}$R, and showed antagonistic properties in the cAMP assay.[68]

1-(Biphenyl-2-ylmethyl)arylpiperazine has been proposed as a new scaffold for the development of 5-HT$_7$R ligands (compound **45**, Figure 14).[69] This scaffold was designed by combining the biphenyl group and the arylpiperazine moiety, two structural motifs shared by several 5-HT$_7$R ligands, and evaluated through computational docking studies. The biphenyl-2-methylpiperazinyl moiety of compound **45** showed similar docking mode as the phenylpyrazole group of compound **18** (Figure 14). In particular, the piperazine mimicked the dimethylamine group of **18** interacting with the Asp3.32 and the biphenyl portion occupied a hydrophobic pocket of the receptor, similarly to the phenylpyrazole of **18**. Instead, the phenyl ring linked to the piperazine interacted with an opposite hydrophobic pocket that could not be filled by the relatively small dimethylamino group of **18**. To elucidate the SARs of this new scaffold, a focused library was designed by introducing various aromatic substituents on the biphenyl as well as the piperazinylphenyl moieties. Affinity data indicate that the binding pocket in which the arylpiperazine was located is not large enough to accommodate the aryl-substituted

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

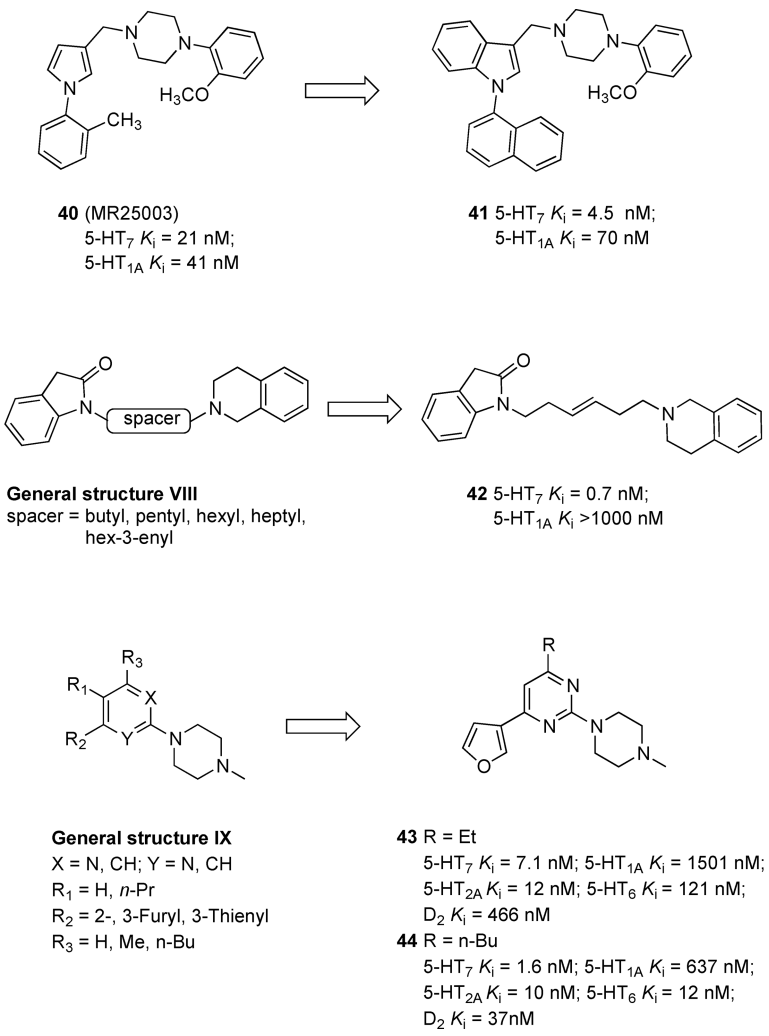
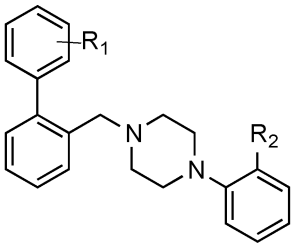


Figure 13. Miscellaneous of 5-HT$_7$R ligands.

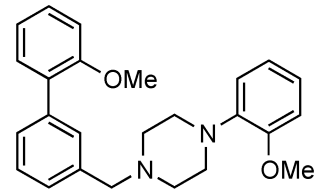
piperazinyl moiety. Only the 2-methoxy derivative **46** possesses 5-HT$_7$R affinity comparable to that of compound **45**. On the other hand, the biphenyl moiety is located in a larger binding pocket which can accommodate different substitution patterns. In particular, compound **47**, which has the 4-methoxy substituent on the biphenyl ring and the 2-methoxyphenylpiperazine, showed nanomolar 5-HT$_7$R affinity (5-HT$_7$ K_i = 46 nM). No data about selectivity and functional activity were reported.[69] In a subsequent paper, the biphenyl-3-ylmethylpiperazine scaffold was studied. Compound **48** (Figure 14) showed a K_i value of 15 nM at the 5-HT$_7$R and antagonistic properties.[70] In molecular docking studies, compound **48** showed a binding mode comparable to that of the antagonist **5**. In fact, the 2-methoxyphenyl moiety of **48** and the 4-methylpiperidine of **5** occupied the same binding pocket, which was left unoccupied by the small dimethylamino group of **18**. On the basis of these results, the authors proposed that the 2-methoxyphenyl moiety may be essential for the antagonistic function. When tested on a panel of 5-HT receptor subtypes, compound **48** exhibited low selectivity, in particular toward 5-HT$_{2A}$ and 5-HT$_{2B}$.[69,71] The introduction of various substituents and basic moieties on the biphenyl portion (general structure **X**, Figure 14) led to the identification of several derivatives endowed with 5-HT$_7$R affinity in the nanomolar range and antagonistic properties (compound **49**, K_i = 5.2 nM, Figure 14). No data on selectivity were reported.[70] In a subsequent paper, the 2-methoxyphenylpiperazine moiety was combined with the biphenylmethyl group through an alkylamide linker similar to that of the reference 5-HT$_7$R ligands **7** (Figure 2), **16**, and **17** (Figure 4).[72] Compound **50** (Figure 14) showed the highest 5-HT$_7$R affinity (K_i = 8.69 nM) and good selectivity over other 5-HT receptor subtypes, except 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{2C}$ receptors. Compound **50** behaved as antagonist in a functional cAMP assay and demonstrated antidepressant-like properties in the FST.[72] In a follow-up study, the biphenylmethyl moiety of compound **50** was replaced by a carbazole. Among the studied compounds, compound **51** (Figure 14) was characterized as a 5-HT$_7$R antagonist with favorable pharmacokinetic properties (good oral bioavailability, long elimination half-life, and brain-to-plasma ratio). Compound **51** exerted antidepressant effect in FST in mice after intraperitoneal injection although at high doses (30 mg/kg).

Arylsulfonamides, which are analogues of the antagonists **5** and **6** (Figure 2), have been thoroughly studied as 5-HT$_7$R ligands by researchers at Jagellonian University. In 2011, Zajdel et al. proposed a new series of arene and quinoline sulfonamides as 5-HT$_7$R ligands (general structures **XI** and **XII**, Figure 15).[73] Structural modifications on these compounds evaluatued different basic moieties (2-methoxyphenylpiperazine (2-MPP), 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrothieno[3,2-*c*]-

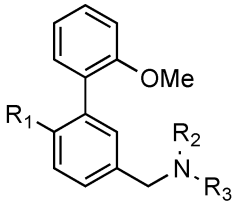
DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX



45 R_1 = H, R_2 = H
5-HT_7 K_i = 537 nM;
46 R_1 = H, R_2 = OMe
5-HT_7 K_i = 432 nM;
47 R_1 = 4-OMe, R_2 = OMe
5-HT_7 K_i = 46 nM

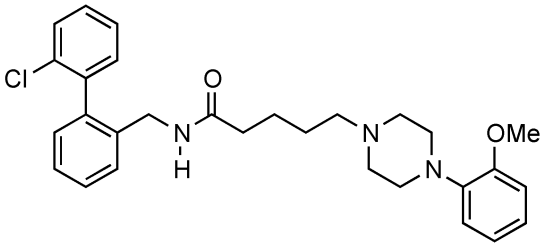


48 5-HT_7 K_i = 15 nM; 5-HT_{1A} K_i = 100 nM;
5-HT_{1B} K_i = 679 nM; 5-HT_{1D} K_i = 225 nM
5-HT_{2A} K_i = 70 nM; 5-HT_{2B} K_i = 43 nM;
5-HT_{2C} K_i = 233 nM; 5-HT_3 K_i = 295 nM;
5-HT_6 K_i = 10000 nM

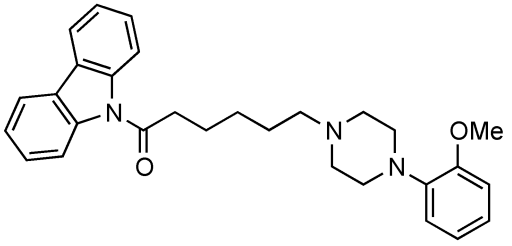


General structure X
R_1 = H, Cl, Me, OMe
R_2, R_3 = 4-Me-piperidine, dimethylamine, methylamine, 1-(2-OMePh)piperazine

49 R_1 = Cl, R_2 = H, R_3 = Me
5-HT_7 K_i = 5.2 nM



50 5-HT_7 K_i = 8.69 nM; 5-HT_{1A} K_i = 20 nM;
5-HT_{1B} K_i = 131 nM; 5-HT_{1D} K_i = 418 nM;
5-HT_{2A} K_i = 478 nM; 5-HT_{2C} K_i = 26 nM;
5-HT_3 K_i = 10000 nM; 5-HT_{5A} K_i = 1178 nM;
5-HT_6 K_i = 1517 nM

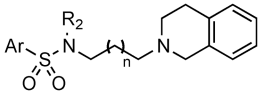


51 5-HT_7 K_i = 74 nM; 5-HT_{1A} K_i = 549 nM;
5-HT_{1B} K_i = 845 nM; 5-HT_{1D} K_i = 1280 nM;
5-HT_{2A} K_i = 1265 nM; 5-HT_{2B} K_i = 419 nM;
5-HT_{2C} K_i = 588 nM;
5-HT_5, 5-HT_6, and 5-HT_3 < 34% of inhibition at 10 μM

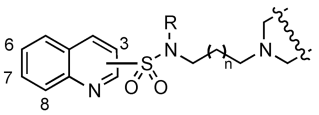
Figure 14. Structures of the biphenylmethyl derivatives developed by Kim et al.[69−72]

pyridine, and perihydroisoquinoline (PHIQ)), the length of the spacer, and the aryl group linked to the sulfonamide residue. Affinity data indicated that the presence of the 2-MPP moiety was beneficial for 5-HT_{1A}R affinity and, to some extent, for 5-HT_6 and 5-HT_{2A} receptors affinity, whereas the PHIQ nucleus was preferable for 5-HT_7R affinity. In addition, increasing the length of the alkyl spacer was beneficial for 5-HT_7R affinity as well as the introduction of a hydrophobic fragment to the nitrogen of the sulfonamide group. In addition, the substitution and localization of the nitrogen atom in the aromatic ring of sulfonamide moiety affect 5-HT_7R affinity and selectivity. Compound **52** (PZ-376, Figure 15) showed nanomolar affinity for 5-HT_7R (K_i = 13 nM) and good selectivity over 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, and α_1 receptors and was characterized as an antagonist in a cAMP functional assay. The antagonist properties of compound **52** was confirmed in vivo as the compound elicited antidepressant-like effects in FST in mice, similarly to the reference antagonist **5**.[73] Subsequent papers reported an extensive exploration on the arylamide and arylsulfonamide scaffolds for 5-HT_7R affinity.[74,75] In particular, using a parallel solid-phase synthesis integrated with virtual combinatorial library design and a multistep virtual screening, privileged molecular

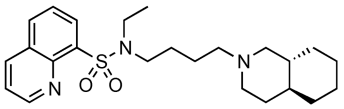
DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX



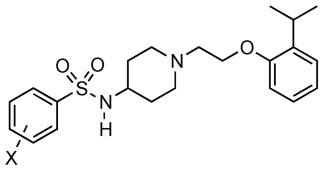
General structure XI
n = 0, 1, 2
Ar = Ph, 2-Naphthyl
R_2 = H, $-(CH_2)_2NH_2$, $-(CH_2)_3NH_2$



General structure XII
n = 1, 2, 3
R = H, Et
Amine = 1-(2-OMePh)-piperazine; 1,2,3,4-tetrahydroisoquinoline; 4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine; perhydroisoquinoline.

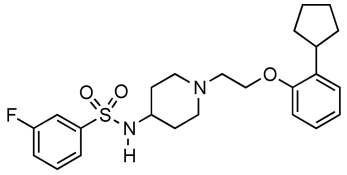


52 (PZ-376)
5-HT_7 K_i = 13 nM; 5-HT_{1A} K_i = 1099 nM; 5-HT_{2A} K_i = 6281 nM; 5-HT_6 K_i = 1950 nM; α_1 K_i = 155 nM

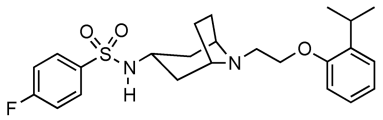


53 (PZ-766) X = 4-F
5-HT_7 K_i = 0.3 nM; 5-HT_{1A} K_i = 436 nM; 5-HT_6 K_i = 240 nM; D_2 K_i = 51 nM; α_1 K_i = 629 nM
54 (PZ-1404) X = 3-F
5-HT_7 K_i = 9 nM; 5-HT_{1A} K_i = 356 nM
5-HT_6 K_i = 471 nM; D_2 K_i = 102 nM; α_1 K_i = 979 nM

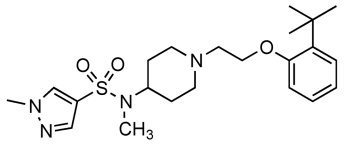
Figure 15. Arylsulfonamide derivatives developed by Zajdel and co-workers.[73]



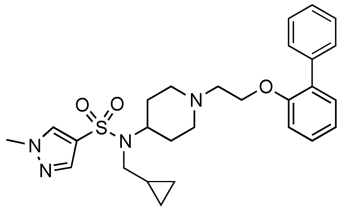
55 (PZ-1417)
5-HT_7 K_i = 19 nM; 5-HT_{1A} K_i = 545 nM; 5-HT_{2A} K_i = 303 nM; 5-HT_6 K_i = 281 nM; D_2 K_i = 322 nM; α_1 K_i = 1525 nM



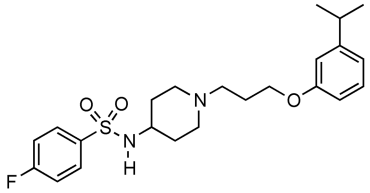
56 (PZ-1150)
5-HT_7 K_i = 1 nM; 5-HT_{1A} K_i = 98 nM; 5-HT_{2A} K_i = 1295 nM; 5-HT_6 K_i = 559 nM; D_2 K_i = 60 nM; α_1 K_i = 339 nM



57 5-HT_7 K_i = 58 nM; 5-HT_{1A} K_i = 9626 nM; 5-HT_{2A} K_i = 557 nM; D_2 K_i = 280 nM



58 5-HT_7 K_i = 10 nM; 5-HT_{1A} K_i = 159 nM; 5-HT_{2A} K_i = 30 nM; D_2 K_i = 16 nM



59 5-HT_7 K_i = 32 nM; 5-HT_{1A} K_i = 2081 nM; 5-HT_{2A} K_i = 1352 nM; 5-HT_6 K_i = 268 nM; D_2 K_i = 328 nM

Figure 16. Arylsulfonamide-based 5-HT_7R ligands developed by Zajdel and co-workers.

substructures were identified as arylpiperazine biomimetics. Thus, the arylpiperazine moiety was replaced with flexible aryloxy-/arylthio-ethyl fragment, while the alkyl spacer of the long chain arylpiperazine derivatives was partially rigidified by including it in cyclic amines such as 4-aminomethylpiperidine, 4-aminopiperidine, or 3-aminopyrrolidine. Affinity data showed that the distance between the basic nitrogen and the amide/sulfonamide moiety was important for 5-HT_7R affinity and that

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

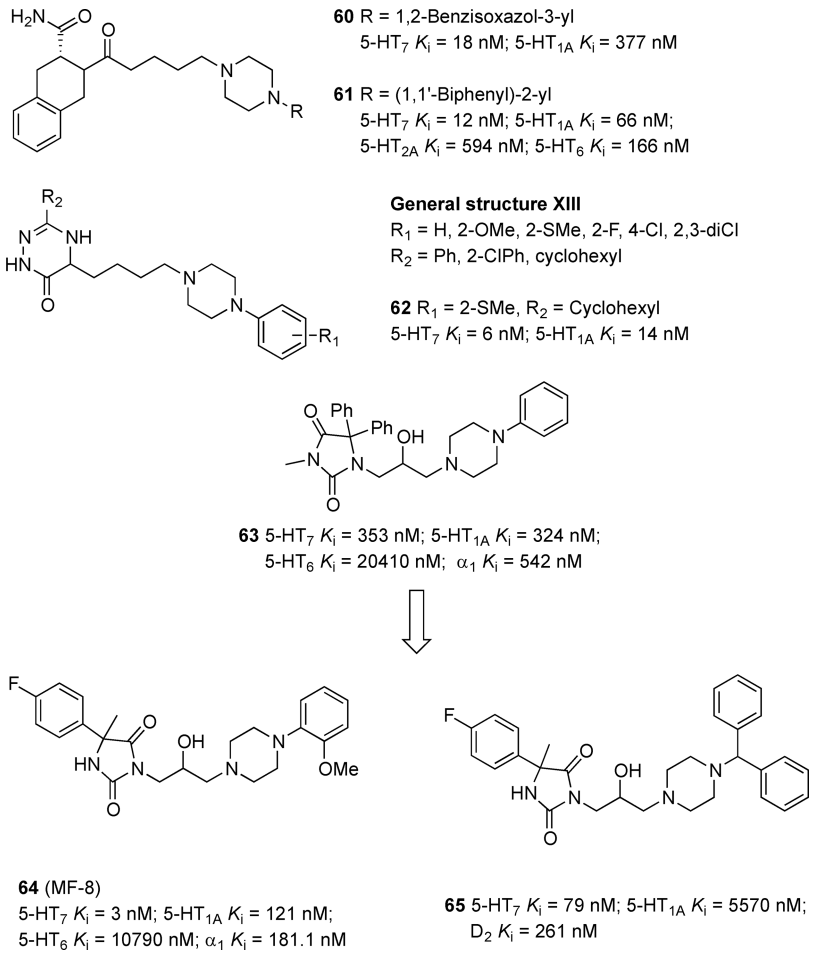


Figure 17. Arylpiperazine-based 5-HT$_7$R ligands developed at Jagellonian University.[79,80]

the sulfonamide fragment was preferred as compared to an amide moiety. The introduction of a substituent at the 2-position of the aryloxy-/arylthio-ethyl fragments was preferred for 5-HT$_7$R high affinity, with the 2-phenyl substituent preferential for selectivity toward 5-HT$_7$R over 5-HT$_{1A}$R. Most of the newly synthesized compounds showed K_i values >200 nM at the 5-HT$_6$R, whereas the majority of them were able to bind D$_2$ receptors with nanomolar affinity. In particular, compound **53** (PZ-766, Figure 15), among others, showed subnanomolar 5-HT$_7$R affinity (K_i = 0.3 nM) good selectivity over 5-HT$_{1A}$, 5-HT$_6$, and D$_2$ receptors, and antagonistic properties in a functional cAMP assay.[74] The binding mode of **53** at the 5-HT$_7$R was studied by molecular docking studies and showed that in addition to the expected interactions with Asp3.32 and Phe6.51, compound **53** formed polar and hydrophobic contacts with Glu7.35 and Arg7.36 and with Leu7.39, respectively.[75] Replacement of the isopropyl group of **53** with methyl, methoxy, and isopropoxy substituents generally afforded derivatives with lower 5-HT$_7$R affinity and decreased selectivity over the 5-HT$_{1A}$R. Compound **53** was further optimized by substituting the isopropyl group in the aryloxy ring with a phenyl group and modifying the aromatic nucleus linked to the sulfonamide moiety. In rats, compounds **53** and **54** (PZ-1404, Figure 15) exerted antidepressant and anxiolytics effect in the FST and in the four-plate test (FPT) and improved cognitive functions when tested in the novel object recognition (NOR) task. Interestingly, compound **54** elicited in vivo effects at lower doses than compound **53** despite lower 5-HT$_7$R affinity and selectivity.[75]

The aryloxy and arylsulfonamide fragments were further modified affording compounds **55** and **56** (PZ-1417 and PZ-1150, respectively, Figure 16), which showed good selectivity over a number of receptors and antagonistic properties at the 5-HT$_7$R. In addition, both compounds exhibited antidepressant- and anxiolytic-like properties assessed in FST, TST, and FPT.[76] In a subsequent paper, the impact of N-alkylation of the sulfonamide moiety on 5-HT$_7$R affinity and selectivity over 5-HT$_{1A}$, 5-HT$_{2A}$, and D$_2$ receptors was evaluated with the aim to extend a polypharmacological approach to the treatment of complex diseases. Therefore, a series of *N*-methyl and *N*-cyclopropylmethyl arylsulfonamide derivatives of aryloxyethylpiperidines was prepared, exemplified by compounds **57** and **58** (Figure 16). SAR studies indicated that *N*-alkylation of the sulfonamide did not affect greatly the affinity for 5-HT$_7$R. On the other hand, this modification had different effect on selectivity depending on the substitution of the aryloxyethyl moiety. In the case of compounds containing isopropyl or phenyl substituents at the 2-position of the phenyl ring, a decrease of 5-HT$_7$R selectivity over 5-HT$_{1A}$R, 5-HT$_{2A}$R, and D$_2$ receptors was observed, whereas compounds bearing a *tert*-butyl group showed improved selectivity toward these receptors. This study allowed the identification of the selective 5-HT$_7$R antagonist **57** (Figure 16) and of the multimodal 5-HT$_{2A}$/5-HT$_7$/D$_2$ receptor antagonist **58** (Figure 16). Both compounds showed acceptable metabolic stability (microsomal CL$_{int}$ = 77.9 and 55.9 μL/mg/min, respectively) and demonstrated antidepressant-like (FST test) and pro-cognitive properties (NOR task) in rats.[77] Next, the

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

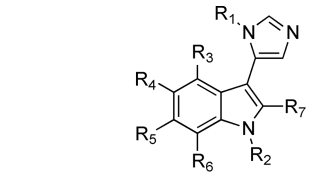
elongation of the alkyl spacer from 2 to 3 methylene units did not reduce affinity and selectivity for the 5-HT$_7$R. In addition, the interaction with the 5-HT$_7$R could be also modulated by the introduction of a small substituent in the propyl spacer. In particular, the introduction a secondary alcohol functional group was preferential for interaction with the 5-HT$_7$R only in compounds with an *ortho*-phenyl substituent at the aryloxy fragment. Compound **59** produced antidepressant-like effects in the FST and TST, potentiated the antidepressant properties of inactive doses of escitalopram and bupropion in the FST, and displayed pro-cognitive properties in rat in NOR task.[78]

The same research group at the Jagiellonian University have also designed and prepared, using solid-phase synthesis, long chain arylpiperazine derivatives that contain cyclic amino acid amides in the terminal fragment (see compound **60** and **61**, Figure 17). The compounds were endowed with good affinity for the 5-HT$_7$R and selectivity over the 5-HT$_{1A}$R. The most interesting results were obtained when 1,2,3,4-tetrahydroisoquinoline-3-carboxamides was introduced as the terminal fragment. Replacing the phenyl ring linked to the piperazine with a benzisoxazole significantly increased 5-HT$_7$R affinity because of an additional H-bond with Ser5.42 or/and Thr3.37 was formed (compound **60**, Figure 17).[79] Subsequently, the length of the alkyl chain and the different substituents at the 2-position of the phenylpiperazine were evaluated.[80] As a general trend, these modifications afforded compounds with increased 5-HT$_7$R affinity and reduced selectivity over the 5-HT$_{1A}$R. Compound **61** (Figure 17) was characterized as a 5-HT$_7$R antagonist and elicited antidepressant-like activity in FST in mice.[80]

The introduction of the 4,5-dihydro-1,2,4-triazine-6(1*H*)-one residue as terminal fragment in the long chain arylpiperazine scaffold provided compounds with dual 5-HT$_{1A}$/5-HT$_7$ receptor properties (general structure **XIV**, Figure 17).[81] The most interesting dual ligand **62** (Figure 17) exhibited K_i = 6 nM and 14 nM at 5-HT$_7$ and 5-HT$_{1A}$ receptors, respectively.

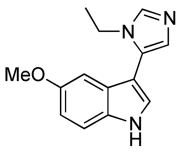
Starting from previously studied hydantoin-based α-adrenergic ligands, Handzlik et al. performed an extensive study on a new class of phenylpiperazine derivatives as 5-HT$_7$R ligands, starting from the lead compound **63** (Figure 17).[82] The exploration of the SARs indicated that introducing a 2-methoxy substituent on the phenylpiperazine ring was beneficial for 5-HT$_7$R affinity and that shifting the insertion of the alkyl chain from the 1- to 3-position of the hydantoin nucleus as well as removing one of the phenyl ring from the 5-position of the hydantoin led to optimal affinity and selectivity for 5-HT$_7$R. On the other hand, unbranched alkyl linkers promoted 5-HT$_{1A}$R affinity as well as a pentyl linker coupled with a 5-methyl-5-phenyl hydantoin residue enhanced α_1 receptor affinity. Compound **64** (MF-8, Figure 17) exhibited high 5-HT$_7$R affinity (K_i = 3 nM) and >40-fold selectivity toward 5-HT$_{1A}$, 5-HT$_6$, 5-HT$_3$, and α_1 receptors.[83] Further optimization of compound **64** was focused on the N_4-substituent of the piperazine nucleus. Compound **65** (Figure 17), bearing a diphenyl methyl group, showed good 5-HT$_7$R affinity (K_i = 79 nM) and 70-fold selectivity over 5-HT$_{1A}$ and was characterized as an antagonist.[84]

All the 5-HT$_7$R ligands described so far fit well into the pharmacophore hypotheses described in the previous paragraphs, as they share common pharmacophoric features such as a basic center (often represented by a piperazine ring), one or two hydrophobic domains (generally represented by aryl residues), and a H-bonding acceptor group. The importance of the basic center is in agreement with mutagenesis and crystallographic studies, which have shown that the interaction between the basic amine of the ligand and the conserved Asp3.32 is crucial for the binding of both agonists and antagonists of aminergic GPCRs. On the other hand, over the last several years, nonbasic ligands of aminergic GPCRs have emerged as rule breakers. In fact, some low-basicity ligands with nanomolar and subnanomolar affinities for 5-HT$_{2A}$, 5-HT$_6$, and 5-HT$_{1B}$ receptors have been identified.[85,86] These ligands lack the capability of forming a strong, charge-assisted hydrogen bond with Asp3.32 of the receptor protein. Recently, low-basicity 5-HT$_7$R ligands have also been described. Starting from the hypothesis that an aromatic basic moiety can mimic the amine functionality of a 5-HT$_7$R ligand, Hogendorf et al. have recently described a new series of 5-aryl-1-alkylimidazoles as 5-HT$_7$R ligands in which the indole ring represents the basic fragment (general formula **XIV**, Figure 18).[87] The SARs indicated that the indole NH group was crucial

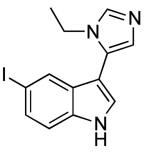


General structure XIV

R_1 = Me, Et, *n*-Pr, *n*-Bu, cyclopropyl, allyl
R_2 = H, Me; R_3 = H, Br; R_4 = H, OMe, F, Cl, Br, I, CN, Me, OBn, $CONH_2$; R_5 = H, Br; R_6 = H, F; R_7 = H, Me



66 5-HT$_7$ K_i = 30 nM



67 5-HT$_7$ K_i = 6 nM

Figure 18. Low-basicity 5-HT$_7$R ligands.

for the interaction of this set of compounds with the receptor because *N*-methylated indoles were inactive. The presence of bulky and lipophilic substituents at the 5-position of the indole ring was beneficial. The presence of small alkyl substituents, specifically an ethyl residue, are preferred on the imidazole ring. Compounds **66** and **67** (Figure 18) showed nanomolar affinity along with remarkable selectivity toward 5-HT$_{1A}$R and behaved as full 5-HT$_7$R agonists. The binding mode of this class of compounds was studied by docking the compounds in the active site of a 5-HT$_7$R homology model. It was proposed that the indole nucleus formed a hydrogen bond with Asp3.32 and aromatic interactions (CH−π or π−π stacking) with the Phe6.51 or Phe6.52 residues, whereas the imidazole ring formed an H-bond with Arg6.58. Because Arg6.58 is a unique feature of the 5-HT$_7$R, this could explain the high selectivity of compounds **66** and **67**. Because the introduction a halogen atom in the 5-position of the indole ring increased 5-HT$_7$R affinity proportionally to the size of the halogen atom, a halogen bond between the ligand and the carbonyl oxygen of the backbone of the TM5 helix was hypothesized. Compounds **66** and **67** were water-soluble, stable in liver microsomes (human CL_{int} = 3.69 and 6.3 mL/min/kg), and showed very low toxicity in HEK-293 and HepG2 cells. In addition, an in vivo disposition study revealed that compound **66** rapidly accumulated in the brain with a good brain-to-plasma ratio (6.34). Moreover, compound **66** improved

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

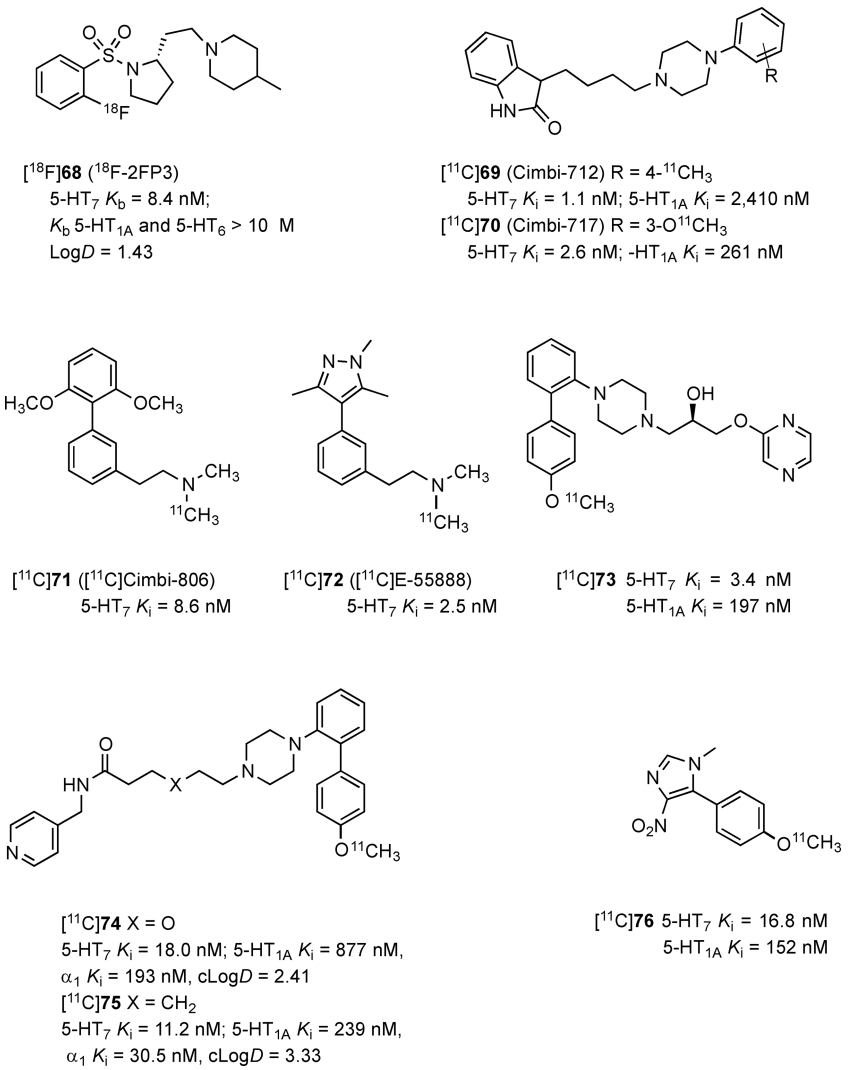


Figure 19. Potential PET radioligands for 5-HT$_7$R visualization.

cognitive dysfunctions in mice because it dose-dependently reversed MK-801-induced disruption in NOR.[87]

4. PET RADIOLIGANDS

Positron emission tomography (PET) is a molecular imaging technique that is increasingly being used in drug development because PET radioligands allow quantification of molecular processes and interactions between a candidate drug and its molecular target.[88] Accordingly, imaging of the5-HT$_7$R using PET might be useful in the quantification of receptor distribution and expression in the brain as well as to elucidate the involvement of this receptor in physiological and pathological conditions.

In the past few years, considerable effort has been devoted to the development of potential PET radioligands for the visualization of the 5-HT$_7$R in the brain. An adequate PET radiotracer for neuroreceptor visualization must fulfill many criteria: the candidate compound should have high affinity and specificity for the target receptor, low toxicity, low metabolic clearance, high brain penetrance, low nonspecific binding, absence of brain penetrant radiolabeled metabolites, and rapid kinetics.[88]

Lemoine et al. have reported a series of fluorinated 5-HT$_7$R PET radioligands, which were developed on the scaffold of the antagonist **5**.[89] The compounds were designed by inserting the ^{18}F radionuclide in the 2-position of the phenylsulfonamide moiety. Autoradiography studies in rat brain were encouraging because the observed binding pattern was consistent with 5-HT$_7$R distribution, and the specific binding was confirmed by pretreatment with the antagonist **5**. Instead, modifications, such as (i) shifting of the fluorine from the 2- to the 4-position of the phenylsulfonamide moiety, (ii) introduction of the 2-methoxyphenylpiperazine instead of the 4-methylpiperidine, and (iii) elongation of the intermediate alkyl chain gave unsatisfactory results.[89] Compound [^{18}F]**68** (^{18}F-2FP3, Figure 19) was selected for in vivo study in cats because of its promising properties (5-HT$_7$ K_b = 8.4 nM; 5-HT$_{1A}$ and 5-HT$_6$ K_b > 10 μM; log D = 1.43). [^{18}F]**68** showed high brain uptake, but in the absence of a valid reference region or an arterial input function, the full validity of this PET radioligand was not assessed. In a subsequent study, new 18-fluorinated analogues of [^{18}F]**68**, which exhibited an improved affinity for the 5-HT$_7$R and selectivity over the 5-HT$_{1A}$R, have been studied. However, the new radiolabeled compounds showed unsatisfactory brain uptake.[90]

Researchers at the Center for Integrated Molecular Brain Imaging (Cimbi) in Copenhagen (Denmark) have been actively involved in the development of PET radioligands for in vivo imaging of the 5-HT$_7$R. Several long chain arylpiperazine derivatives bearing an oxindole nucleus as the terminal fragment

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

have been studied as potential PET radioligands. Compounds [^{11}C]**69** and [^{11}C]**70** ([^{11}C]Cimbi-712 and [^{11}C]Cimbi-717, respectively, Figure 19), which showed a selectivity profile adequate for the development of a PET radiotracer, were labeled with ^{11}C in high yields and studied in pig brain. Time−activity curves of [^{11}C]**69** and [^{11}C]**70** showed high brain uptake and distribution consistent with 5-HT$_7$R brain distribution. Both radioligands were specific for the 5-HT$_7$R, as the binding could be blocked by pretreatment with the antagonist **5** in a dose-dependent manner. In addition, [^{11}C]**70** showed a more reversible tracer kinetic profile compared to [^{11}C]**69**.[91] In subsequent work, an ethyl moiety at the 3-position of the oxindole nucleus of [^{11}C]**69** and [^{11}C]**70** was introduced to prevent the rapid racemization of the compounds. However, the new derivatives showed high nonspecific binding in vivo.[92] Further efforts to obtain useful PET radioligands for the 5-HT$_7$R afforded compounds [^{11}C]**71** and [^{11}C]**72** ([^{11}C]Cimbi-806 and [^{11}C]E-55888, respectively Figure 19). These compounds showed high pig brain uptake which was not, however, blocked by the antagonist **5**, suggesting a high degree of nonspecific binding (Figure 19).[93,94] Therefore, compound [^{11}C]**70** is currently the most promising radioligand for in vivo investigation of the 5-HT$_7$R.

[^{11}C]-(*R*)-**73** (Figure 19) was identified by following a medicinal chemistry campaign at the University of Bari aimed at the identification of a potential radioligand for 5-HT$_7$R visualization. [^{11}C]-(*R*)-**73** showed high 5-HT$_7$R affinity (K_i = 3.4 nM) and good selectivity toward the 5-HT$_{1A}$R (K_i = 197 nM). After intravenous injection in pigs, [^{11}C]-(*R*)-**73** easily crossed the BBB and had a high brain uptake. However, displacement studies with the antagonist **5** indicated that the binding of [^{11}C]-(*R*)-**73** could be only partially blocked, suggesting that [^{11}C]-(*R*)-**73** cannot be used as a radioligand for PET imaging of the 5-HT$_7$R.[95]

In another attempt to develop a 5-HT$_7$R PET radioligand, the structure of the agonist **17** was manipulated with the aim to obtain potent and selective 5-HT$_7$R ligands with suitable properties for a PET radioligand, including ease of labeling with ^{11}C or ^{18}F. Compounds **74** and compound **75** (Figure 19) were then labeled with ^{11}C. The PET scan in vivo revealed that both compounds were very rapidly metabolized and that they were substrates of the efflux pumps present on the BBB. For these reasons, the two radioligands were not investigated further.[96]

Tiwari et al. have reported the synthesis and biological evaluation of the imidazole derivative **76** (Figure 19) as a potential PET radioligand for 5-HT$_7$R visualization.[97] This compound showed good in vitro binding and selectivity properties for 5-HT$_7$R (K_i = 16.8 nM, 5-HT$_{1A}$R K_i = 152 nM) and adequate lipophilicity for BBB permeation. A preliminary PET study in rat brain with [^{11}C]**76** revealed rapid accumulation of radioactivity in the brain. However, in this case, displacement studies with the unlabeled **76** or with the antagonist **5** also revealed only a minimal decrease in brain radioactivity, suggesting a high level of nonspecific binding.[97]

All of these studies clearly indicate that no valid selective 5-HT$_7$R PET radioligand has been identified so far and that more efforts will be needed to fulfill this aim.

5. 5-HT$_7$R IN CNS FUNCTIONS AND DISORDERS

The developments in 5-HT$_7$R pharmacology and the availability of 5-HT$_7$R knockout mice have contributed to an understanding of the role of 5-HT$_7$R in CNS functions as well as in the pathophysiology of several neuropsychiatric disorders. Preclinical data link the 5-HT$_7$R to a variety of CNS processes, including regulation of circadian rhythms, body temperature, mood, cognition, seizure threshold, and pain processing as well as mechanisms of addiction (for extensive reviews see refs 9,24). In recent years, increasing evidence suggests that 5-HT$_7$R is part of the molecular cascade required for the establishment and maintenance of connectivity within neuronal networks and that 5-HT$_7$R-mediated structural reorganization during early and postnatal brain development might have a crucial role for the development and plasticity of forebrain areas and, consequently, can be implicated in the regulation of higher cognitive functions.[98] In addition, central 5-HT$_7$Rs modulate neuronal excitability and synaptic function and are important modulators in learning and memory.[23] Here we discuss first the role of 5-HT$_7$R in synaptic and structural plasticity of neuronal circuits in order to support the subsequent discussion about the role of 5-HT$_7$R in neuropsychiatric disorders.

5.1. Role of 5-HT$_7$R in Structural Plasticity of Neuronal Circuits and Synaptic Plasticity. It is well-known that 5-HT plays a crucial role in brain development through modulation of neural cell proliferation, migration, and differentiation as well as neurite outgrowth, dendritic spine shape, and synaptogenesis.[99] Alterations of 5-HT brain levels during development produce severe abnormalities in the serotonergic signaling which affect the functional organization of neuronal circuits and may underlie the pathogenesis of several neurodevelopmental disorders.[100] The role of 5-HT$_7$R in the development of postnatal neurons has been studied in hippocampal neurons, and it was demonstrated that 5-HT$_7$R activation promotes neurite outgrowth, dendritic spines formation, and elevation of synaptic transmission and reduces synaptically evoked Ca^{2+} entry and long-term potentiation (LTP).[20,21]

As discussed above, 5-HT$_7$Rs are coupled to G_s or G_{12} protein. The expression levels of 5-HT$_7$R and G_{12} protein in mice hippocampus progressively decrease during postnatal development. As a result, the stimulatory effects of 5-HT$_7$R/G_{12} signaling on spinogenesis, synaptogenesis, and synaptic plasticity are restricted to early postnatal development stages and abolished in adult mice, suggesting that regulated expression of the 5-HT$_7$R/G_{12} signaling pathway may represent a mechanism by which 5-HT specifically modulates formation of basal neuronal connections during the early postnatal development.[21] The morphogenic effect of the 5-HT$_7$R activation has been studied using different agonists, such as 5-HT and **1**, the mixed 5-HT$_{1A/7}$ agonist **2**, or the selective agonist **18**.[101,102] The specific involvement of 5-HT$_7$R has been confirmed because the selective antagonist **5** was able to abolish the effect of the above agonists. In line with these findings, the selective 5-HT$_7$R agonist **17** was able to stimulate neurite outgrowth in embryonic primary cultures from hippocampus, cortex, and striatum through multiple signal transduction pathways, such as the Rho GTPase, Cdc42 and Cdk5, ERK, and mTOR, which are known to converge on reorganization of cytoskeletal proteins.[103,104]

Rojas et al. have studied the roles of the 5-HT$_{1A}$R and the 5-HT$_7$R on dendritic growth in cultured hippocampal neurons. They found that, at early neuronal stages, both 5-HT$_{1A}$R and 5-HT$_7$R promote the growth of secondary neurites, with no effect on neuritogenesis.[102] Considering that the levels of these receptors change during development, the role of both receptor subtypes on neural morphology was studied at a more mature neuronal stage. The results indicated that 5-HT$_{1A}$R restricts dendritogenesis and outgrowth of primary dendrites, whereas

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

both 5-HT_{1A}R and 5-HT_7R promote short secondary dendrites through Akt and ERK activation.[105] These observations are of particular interest because 5-HT_{1A}R and 5-HT_7R can form homo- and heterodimers. Functionally, heterodimerization decreases 5-HT_{1A}R-mediated activation of G_i protein without affecting 5-HT_7R-mediated signaling. In addition, 5-HT_{1A}/5-HT_7 heterodimerization alters the profile of 5-HT_{1A}R internalization, whereas the proportion of receptor heterodimers can vary during development because the production of 5-HT_7R in the hippocampus decreases during postnatal development.[29] Therefore, changes in the functionality of these receptors on neuronal membranes along with variations in the ratio of 5-HT_{1A}R and 5-HT_7R expression during neuronal maturity could explain the complex role of 5-HT on morphology during development.

Considering that the expression of 5-HT_7R in the cortex and striatum remains stable during the whole postnatal development period, the effect of acute and prolonged activation of 5-HT_7R in postnatal cortical and striatal neurons has been studied.[106] Pronounced neurite elongation and increased formation of dendritic spines and synaptogenesis were observed after treatment of cultured postnatal murine cortical neurons with the agonist **17**. These data are in agreement with the previous observation that administration of **17** to adolescent rats leads to increased neural dendritic arborization in the nucleus accumbens.[107] In addition, neuronal cultures treated with the antagonist **5** as well as those obtained from 5-HT_7R knockout mice showed reduced levels of dendritic protrusions, similar to those observed in untreated or wild-type cultures, suggesting that the constitutive activity of 5-HT_7R may have an effect on synapse morphogenesis and may contribute to structural plasticity in adult cortex and striatum.[106]

Formation of new dendritic protrusions and their conversion into functional synapses requires multiple and coordinated changes in the extracellular and intracellular environment that can involve extracellular proteases and proteolytic remodeling of cell-to-cell and cell-to-extracellular matrix (ECM) interactions. However, the mechanisms underlying the molecular interactions between ECM and receptor-mediated signaling in neurons have been poorly explored. Bijata et al. have addressed this aspect by focusing on 5-HT receptors and uncovering a signaling pathway that involve 5-HT_7R and the matrix metalloproteinase 9 (MMP-9). They showed in neuronal cultures and organotypic slices from mice hippocampus that stimulation of 5-HT_7R increases local MMP-9 activity by triggering spine remodeling, synaptic pruning, and impairment of LTP. In particular, the 5-HT_7R-mediated activation of MMP-9 modulates the activity of the hyaluronan receptor CD44, a component of ECM, through the proteolytic cleavage of its extracellular domain. This leads to disinhibition of the small GTPase Cdc42, which becomes more accessible to 5-HT_7R-mediated activation.[108]

In agreement with the morphogenic role and the role in dendritic spines formation and synaptic contacts observed in hippocampus, 5-HT_7R is involved in the modulation of synaptic plasticity. Hippocampal LTP and long-term depression (LTD) are the most studied paradigms of synaptic plasticity that participate in strengthening or weakening of synapses, paralleled by an increase or decrease of dendritic spine volume. In mouse hippocampal neurons, Kobe et al. have reported that the activation of 5-HT_7R enhanced basal synaptic transmission by increasing the number of AMPA receptors. The enhancement of basal glutamatergic transmission might prevent further potentiation and, thus, a reduction of LTP was observed.[21] In contrast to this report, 5-HT_7R knockout mice have been reported to show decreased LTP in the CA3-CA1 hippocampal synapses, suggesting that 5-HT_7R are required for LTP.[109] Because LTP is mainly dependent on *N*-methyl-D-aspartic acid (NMDA) receptors activation, such contrasting results can be explained by considering the complex role of 5-HT_7R activation on NMDA receptors activity. In fact, in hippocampal neurons, acute activation of 5-HT_7R promotes NMDA receptor activity, whereas long-term activation of 5-HT_7R reduces the expression of NMDA receptors on the cell membrane, thus inhibiting glutamate receptor signaling.[110]

Activation of 5-HT_7R modulates LTD mediated by metabotropic glutamate receptors (mGluR-LTD), a form of synaptic plasticity related to the removal of the AMPA receptor from synaptic membranes by endocytosis. 5-HT_7R activation prevented mGluR-induced endocytosis of AMPA receptors and reversed mGluR-LTD in the CA3−CA1 synapse in mouse hippocampal slices.[111]

5-HT_7R activation by the agonist **18** improves synaptic dysfunction in streptozotocin (STZ) treated rats, a model of sporadic Alzheimer's disease (AD), which is characterized by impairments of LTP and neuronal apoptosis. In STZ-treated rats, one month of treatment with **18** restored hippocampal LTP and reduced neuronal apoptosis, suggesting that 5-HT_7R activation may open new strategies to slow down AD progression.[112]

5-HT_7R can modulate synaptic plasticity also in other brain areas. 5-HT_7R activation using 5-HT or compound **2** increases the NMDA receptor-mediated component of mEPSCs in layer II/III pyramidal neurons of the rat visual cortex.[113,114] It has been recently reported that in juvenile rats 5-HT_7R activation reverses NMDA receptor-dependent LTD in medial vestibular nucleus, a major output area for neurons that project to vestibulo-spinal pathway involved in postural control and higher cognitive functions, thus contributing to the maturation of the vestibulo-spinal circuit.[123] 5-HT_7Rs are also critically involved in synaptic plasticity of the parallel fiber-Purkinje cell (PF−PC) synapse in the cerebellum that has been proposed as a mechanism for motor learning. Activation of the 5-HT_7R by the selective agonist **17** in cerebellar slices from adult mice causes LTD of the PF−PC synapse through a postsynaptic mechanism that involves PKC-MAPK signaling pathway and culminates in AMPA receptor internalization. In addition, 5-HT_7R exerts a fine regulation of synaptic plasticity at PF−PC synapses by favoring the emergence of LTD vs LTP. In fact, pharmacological blockade of 5-HT_7R reduces the expression of postsynaptic PF-LTD, whereas activation impairs postsynaptic LTP. This type of synaptic control may enable the serotonergic pathways to prevent the simultaneous occurrence of conflicting forms of plasticity at PF−PC.[115]

5.2. Mood Disorders. Early studies evidenced that several antipsychotics and antidepressants can bind 5-HT_7R with high affinity. Next, it was reported that 5-HT_7R knockout mice display antidepressant-like behaviors in commonly used preclinical animal models of depression such as the TST and the FST, and similar results were obtained after pharmacological blockade of 5-HT_7R (for extensive reviews see refs 9,24). Moreover, the antagonist **5** produced a faster antidepressant-like behavior as compared to selective serotonin reuptake inhibitors (SSRIs) when tested in the olfactory bulbectomy paradigm, which is considered a model of agitated depression.[116] 5-HT_7R seems to be implicated in several physiological functions linked to mood disorders such as sleep disorders. Similar to SSRIs, the blockade or inactivation of 5-HT_7R can counteract the reduction in sleep

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

time and rapid eye movement (REM) sleep latency in depressed patients.[117] In addition to exerting an antidepressant-like effect, selective 5-HT$_7$R blockade may also augment the behavioral effects of antidepressant drugs. In fact, an individually ineffective dose of compound **5** and an individually ineffective dose of one of several antidepressants, including SSRIs, are synergistic in reducing immobility in both FST and TST.[118,119]

Thus, 5-HT$_7$R represents a useful target for the development of new antidepressant therapeutic strategies. Preclinical studies in mice suggest that the clinically established antidepressant effect of the atypical antipsychotics **11**, **13**, and **14** (Figure 3) is most likely due to 5-HT$_7$R blockade. In fact, all of these drugs behave as potent 5-HT$_7$R antagonists[120−123] and are able to reduce immobility in both the TST and the FST in wild-type mice but not in 5-HT$_7$R knockout mice.[119,121,123] In addition, the combination of compound **11** and citalopram reduced immobility in wild-type mice but not in 5-HT$_7$R knockout mice.[123] In line with these studies, the selective 5-HT$_7$R antagonist, compound **9** (JNJ-18038683, Figure 2), was able to reduce immobility in mice in the TST and showed a synergistic antidepressant effect when administered with subeffective doses of citalopram.[36] However, compound **9**, when tested in patients with major depressive disorder (MDD), produced no statistically significant improvement over placebo on the Montgomery−Åsberg depression rating scale (MADRS). It should be noted that in that same study escitalopram was inactive as well. Nonetheless, a posthoc analysis using an enrichment window strategy showed a clinically meaningful difference between compound **9** and placebo, suggesting that compound **9** still deserved further investigations.[36]

Compound **15** (vortioxetine, Figure 3) is a multimodal antidepressant recently approved by the Food and Drug Administration (FDA) for the treatment of MDD.[124] Compound **15** behaves as an antagonist at 5-HT$_3$ and 5-HT$_7$ receptors, a partial agonist at the 5-HT$_{1B}$ receptor, a full agonist at the 5-HT$_{1A}$R, and inhibits 5-HT reuptake. At the molecular level, compound **15** increases extracellular levels of 5-HT through a combination of inhibition of 5-HT reuptake and modulation of an inhibitory feedback system involving various 5-HT receptor subtypes, including the 5-HT$_7$R.[124] Compound **15** showed antidepressant efficacy in several preclinical animal models as well as in clinical studies where a significant improvement on all items of MADRS was reported.[124]

While the role of 5-HT$_7$R in depression is well-established, the involvement in the regulation of anxiety-like behaviors is less consistent. No differences in assays sensitive to anxiety states have been observed between 5-HT$_7$R knockout mice and wild-type controls.[109] On the other hand, the antagonist **5** showed anxiolytic-like effects in several animal models such as the elevated plus maze and the marble burying test. However, it has been speculated that these effects could be more likely related to the antidepressant activity of **5**.[125] Instead, administration of the selective agonist **17** reduced anxiety-like behavior in the black and white box test and the dark/light test in mice.[126] In addition, subchronic stimulation of the 5-HT$_7$R with **17** during the prepuberal period reduced anxiety-related behavior in a rat model of attention-deficit hyperactivity disorder.[127]

5.3. Schizophrenia. A possible role for 5-HT$_7$R in schizophrenia was suggested by post-mortem studies that evidenced a marked reduction of 5-HT$_7$R mRNA expression levels in brain tissue from schizophrenic patients.[128] In addition, considerable preclinical and clinical evidence suggests that action on certain 5-HT receptor subtypes, especially partial agonism at 5-HT$_{1A}$, and antagonism at 5-HT$_{2A}$, 5-HT$_6$, and 5-HT$_7$, contributes to a reduce the risk of extrapyramidal side effects and to improve specific cognition domains of atypical antipsychotic drugs.[129] A number of atypical antipsychotic drugs, such as compounds **10**−**14** (Figure 3), which have been proved to improve cognition domains, have high affinity for 5-HT$_7$Rs.[129]

Thus, 5-HT$_7$R antagonists have been evaluated in animal models of schizophrenia based on the administration of antagonists of the NMDA receptors, such as phencyclidine (PCP), ketamine, or dizocilpine, that evoke not only behaviors reflecting positive symptoms (e.g., hyperlocomotor activity) but also negative symptoms (e.g., social withdrawal) and cognitive impairments (e.g., deficits in working memory and attention).[130]

The selective 5-HT$_7$R antagonist **4** reverses hyperactivity induced by PCP in rats,[131] whereas the antagonist **5** partially but significantly blocked hyperactivity induced by ketamine in mice.[132] However, the available data suggest that the antipsychotic-like activity elicited by selective 5-HT$_7$R blockade is weaker than that obtained with clinically proven antipsychotic drugs.[133]

It has been proposed that the antimanic properties of compound **10** (asenapine, Figure 3), a new atypical antipsychotic prescribed for the treatment of psychosis and bipolar disorders, are related to the antagonistic activity at 5-HT$_7$Rs. Compound **10** is a multimodal drug displaying antagonist activity, beside 5-HT$_7$R, also at several 5-HT receptor subtypes, including adrenergic and dopamine receptors. Compound **10** showed a potent antimanic effect in the sleep deprivation (SD) rat model of mania by significantly reducing hyperlocomotion. Electrophysiological studies suggested that this effect was mediated by 5-HT$_7$R antagonism because acute administration of **10** in the dorsal raphe nucleus of SD rats was able to reduce the suppressant effect on 5-HT neuronal firing activity induced by the selective 5-HT$_7$R agonist **16** or the mixed 5-HT$_{1A/7}$ agonist **2**.[134]

Cognitive impairments are a core feature of schizophrenia and involve multiple domains of cognition. The serotonergic action of the atypical antipsychotics, in particular activation of 5-HT$_{1A}$R and blockade of 5-HT$_6$ and 5-HT$_7$ receptors, may contribute to the beneficial effect of these drugs on cognition.[129] Indeed, there is evidence that blockade of 5-HT$_7$R may have a pro-cognitive effect in animal models predictive of schizophrenia.

Subchronic PCP administration in rats is believed to mimic cognitive deficits in schizophrenia by selectively impairing performance in a reversal learning test (a paradigm of executive functioning), attentional set-shifting test (a measure of cognitive flexibility), and NOR test (a paradigm of declarative memory).[135]

The antipsychotic drugs **11** and **14** are able to ameliorate NOR deficits induced by subchronic administration of PCP, and this effect is abolished by the selective 5-HT$_7$R agonist **18**.[136] The antagonist **5** potentiates subeffective doses of **11** or **14** to restore NOR in PCP-treated rats.[136] Acute administration of **5** as well as pretreatment with **5** or **11** was able to reverse the subchronic PCP-induced deficit in reversal learning in mice.[26,137] This effect was not elicited by the 5-HT$_7$R agonist **18**, confirming that antagonism at 5-HT$_7$R, but not agonism, is able to restore function in principal cortical neurons impaired by NMDA receptor blockade.

The antagonist **5** was able to revert the dizocilpine-induced cognitive deficit in the delayed nonmatching to position task, a translational behavioral model of working memory, and in an autoshaping Pavlovian instrumental learning task in rats.[138,139]

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

Acute administration of the antagonist **5** in rats ameliorates deficits in the attentional set-shifting task and in NOR paradigm after ketamine administration, a model of transient neurocognitive impairments.[140]

Pharmacological blockade of the 5-HT$_7$R may also have therapeutic implications for the treatment of negative symptoms in schizophrenia. In fact, compounds **5** and **14** ameliorate ketamine-induced social withdrawal in rats and this effect was abolished by the 5-HT$_7$R agonist **18**.[141]

5.4. Drug Addiction. On the basis of neuroanatomical, biochemical, physiological, and behavioral observations, 5-HT$_7$R may play a key role in the mechanisms underlying addiction.[142] This hypothesis has been formulated starting from the observation that the tendency for novelty-seeking is inversely correlated with the gene expression of the 5-HT$_7$R in brain areas crucial for addiction such as thalamo-cortical projection areas and the dorsal hippocampus.[143] Novelty seeking is one of the defining characteristics of a sensation-seeking personality in humans, which is defined by the seeking of novel sensations and the willingness to take physical, social, legal, and financial risks for the sake of such experience and has been correlated with vulnerability to psychopathological disorders and drug addiction.[144] In rodents, novelty seeking has been defined as a preference for novel objects or environments. High novelty-seeking rodents display enhanced exploratory behavior toward novel situations, objects, or stimuli.[145] In high responder (HR) rats, which express a high level of novelty seeking and drug-taking behavior, mRNA expression levels of 5-HT$_7$R were significantly lower than those in low responder (LR) rats, which express the opposite phenotype. It was suggested that low levels of 5-HT$_7$R mRNA correlated with decreased aversion to forced exposure to novelty.[143] When tested in the NOR task, LR rats showed increased exploration of novel object and an ability to discriminate visual stimuli better than HR rats. The administration of the antagonist **5** decreased exploration of novel object in LR rats but not in HR rats, suggesting that 5-HT$_7$R activity may play a role in the cognitive processes that regulate the emotional adaptation to changes in the environment.[146]

Another study proposed that 5-HT$_7$R stimulation increases novelty-seeking and promotes risk-prone behavior by boosting motivation or behavioral disinhibition.[147] In a novelty-preference test, acute stimulation of 5-HT$_7$R by the selective agonist **17** (Figure 4) induced a clear novelty preference and novelty-induced hyperactivity. In the "Probabilistic-Delivery Task" (rPDT) operant paradigm, which is used to evaluate risk proneness of rats, subchronic 5-HT$_7$R activation increased risk-seeking behavior by shifting the choice of the rats toward a larger yet unlikely reward. Although further studies are needed to understand the role of the 5-HT system in modulating the pathways related to vulnerability to various addictive features, this study opens new perspectives for the treatment of addictive behavior, including those related to gambling.[147]

While a high level of sensation seeking may be a powerful incentive to start experimenting with recreational drugs or alcohol at an early age, impulsive traits may be more involved in the subsequent loss of behavioral control and the development of abuse or dependence.[148] Leo et al. have provided evidence for a direct relationship between tonic 5-HT$_7$R function and the modulation of impulsive behavior and self-control capacity.[149] In intolerance-to-delay task, an animal model of impulsivity with possible relevance for drug abuse, rats treated with methylphenidate during adolescence showed reduced impulsive behavior in adulthood.[149] This behavior could be counteracted by administration of the antagonist **5**. Instead, stimulation of 5-HT$_7$R by the agonist **2** (Figure 1) reduced impulsive behavior in naïve adolescent and adult rats. These behavioral changes may be related to changes in gene expression because methylphenidate has been shown to upregulate 5-HT$_7$R mRNA expression in different brain areas.[149]

5.5. Pain. It is known that 5-HT plays complex modulatory roles in pain signaling mechanisms exerting pro-nociceptive effects at the periphery and antinociceptive effects at the spinal cord level. This complex role could be related to the existence of multiple 5-HT receptor subtypes expressed in both the periphery and CNS.[150] Convergent anatomical studies showed that 5-HT$_7$Rs are expressed at critical synaptic relays along nociceptive neuronal pathways.[151,152] Early studies suggested a peripheral pro-nociceptive action of 5-HT through 5-HT$_7$R activation because the selective antagonist **5** prevented the pain-promoting effect of 5-HT or of the agonist **1** (Figure 1) injected into a hindpaw in formalin-induced local nociceptive responses in rat.[151,153] Furthermore, in rat models of neuropathic pain (i.e., chronic constriction injury to the sciatic nerve or spinal nerve ligation), systemic administration of the antagonist **5** reduced hyperalgesia and tactile allodynia.[154,155] However, such findings appear in contradiction with the studies of Brenchat et al., who reported that systemic administration of the antagonists **4** or **5** enhanced mechanical hypersensitivity associated with capsaicin-induced hyperalgesia or nerve injury in mice.[41,156] These discrepancies could be explained in terms of possible species differences and/or model of neuropathic pain selected for the study.

The antinociceptive potential of 5-HT$_7$R antagonists would suggest a pro-nociceptive effect of 5-HT$_7$R agonists. Accordingly, intra-articular administration of the agonist **2** to rats increased c-Fos expression in the dorsal horn of the spinal cord through activation of 5-HT$_7$R located on peripheral terminals of primary afferent nociceptive fibers.[151] Similarly, after an intracisternal injection of capsaicin, systemic administration of the selective agonist **17** strongly increased Fos-like immunoreactivity in superficial laminae of the caudal nucleus of the trigeminal nerve in rats.[157] In contrast to the above finding, several studies have reported that the 5-HT$_7$R mediates antinociceptive effects at the CNS level. Blockade of spinal 5-HT$_7$R by intrathecal injection of the antagonist **5** prevented the antinociceptive effects of systemic administration of analgesic drugs.[158,159] Furthermore, systemic treatment with different 5-HT$_7$R agonists, such as compounds **19** and **20** (Figure 4), produced marked reductions in mechanical and thermal hypersensitivity in various chronic pain models with central and/or peripheral sensitization.[41,42,150,155,156] These contradictory results could be explained, at least in part, by the rapid desensitization of the 5-HT$_7$R triggered by agonists which reflects on 5-HT neuronal firing. Moreover, considering that 5-HT$_7$R are expressed on GABAergic interneurons within the dorsal horn of the spinal cord, a 5-HT$_7$R-mediated activation of inhibitory interneurons cannot be ruled out.[150]

Santello and Nevian have shown that activation of 5-HT$_7$R alleviates pain by reversing dendritic dysfunctions in the anterior cingulate cortex (ACC), a cortical area essential for the conscious experience of pain.[160] Sciatic nerve injury in mice induced an activity-dependent dysfunction of hyperpolarization-activated cyclic nucleotide-regulated (HCN) channels in the dendrites of layer 5 pyramidal neurons, the principal output neurons of the ACC, resulting in enhanced temporal summation of synaptic inputs and increased neuronal firing. Activation of 5-HT$_7$R by the agonist **1** (Figure 1) increases HCN channel function, via

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

adenylate cyclase, restores normal dendritic integration and reduces in vivo allodynia-like behavior. In addition, in vivo activation of 5-HT$_7$R in mice with the agonist **17** completely abolished the pain-induced shift in place preference in a place escape/avoidance paradigm, which depends on the ACC, and it is thought to be related to the emotional/affective component of pain. Therefore, 5-HT$_7$R activation could be useful to reduce the emotional distress associated with chronic pain.[161]

5.6. Cognitive Functions. It is well-known that 5-HT neurotransmission modulates cognitive functions in brain areas involved in learning and memory, such as prefrontal cortex, striatum, and hippocampus, which receive serotonergic projection.[162] A possible role for 5-HT$_7$R in learning and memory processes is suggested by the high expression levels within the hippocampus, a brain area crucially involved in learning, and by its involvement in the modulation of hippocampal synaptic transmission and plasticity.

Behavioral studies using 5-HT$_7$R knockout and wild-type mice have evaluated the role of 5-HT$_7$R in place-based learning such as spatial and contextual memory and navigation ability (see ref 24). 5-HT$_7$ R knockout mice show impaired contextual but not cued fear conditioning, suggesting that 5-HT$_7$R are needed for integrative learning mechanisms involved in contextual experiments.[109] In the NOR test, a type of visual episodic memory that depends on brain cortex, 5-HT$_7$R knockout mice displayed the same ability to discriminate a novel object as wild-type mice. Pharmacological blockade of 5-HT$_7$R by **5** exerted no effect or even improved recognition memory when administered before the acquisition trial, whereas a deficit in the recognition memory is observed when **5** is administered during the consolidation phase.[163] Instead, the administration of the agonist **1** during the consolidation phase improved memory, suggesting that 5-HT$_7$R activation can affect also cortex-dependent memory.[164] Interestingly, it has been hypothesized that the decrease in hippocampal expression of the 5-HT$_7$R may underlie age-related deficits in allocentric spatial navigation.[165]

Behavioral studies in rodents have demonstrated that activation of 5-HT$_7$R exerts a pro-cognitive effect on hippocampus-dependent contextual learning. In the passive avoidance task for emotional learning, 5-HT$_7$R stimulation counteracts the learning impairment induced by 5-HT$_{1A}$R receptor activation.[166] In adult rats trained in an autoshaping Pavlovian/instrumental learning task, administration of the 5-HT$_7$R agonist **18** impaired short-term memory but improved long-term memory,[167] whereas the 5-HT$_7$R agonist **17** had no effect on short-term memory but improved long-term memory.[168,169] At the molecular level, these pro-cognitive effects seem to be related to increased levels of cAMP and were blocked by the administration of **5**.

These studies indicate that 5-HT$_7$R agonists and antagonists may have both promnesic or amnesic effects. This is not surprising considering the different models and the different type of memory that have been tested, which involve different brain areas and neuronal circuits. Moreover, learning and memory are related to stress, therefore, the observed effects might also be influenced by 5-HT$_7$R-mediated effects on mood.

Cognitive dysfunctions are prevalent in patients with MDD and can persist even in remitted patients.[170] In preclinical studies, the antidepressant drug **15** (Figure 3) enhances hippocampal LTP and memory in various cognitive tasks.[171] In addition, compound **15** induces dendritic spine enlargement and increases the number of spines in contact with presynaptic terminals.[172] As dendritic spines are a major locus of synaptic plasticity, these results suggest that the beneficial effect of **15** on cognitive dysfunctions in MDD could be related to the effect on remodeling neuronal circuitry and morphological plasticity. To date, it is not known which of 5-HT receptors is involved in modulation of the neuronal plasticity shown by compound **15**. It is likely that 5-HT$_7$R may contribute to such an effect.

5.7. Neurodevelopmental Disorders and Autism Spectrum Disorder (ASD). A large body of evidence suggests that abnormalities in the brain 5-HT system can be a casual mechanism in ASD.[173] Numerous clinical studies have evidenced abnormal synthesis and increased uptake of 5-HT, morphological alteration of serotonergic fibers, and reduced expression of 5-HT receptor subtypes, such as 5-HT$_{1A}$ and 5-HT$_2$ receptors, in the brain of ASD patients.[174,175] In addition, the lack of 5-HT during early stages of development is likely to disrupt the wiring architecture of the brain.[175]

The involvement of 5-HT$_7$R in brain development has recently emerged, and several studies have contributed to illuminating its role in the reorganization of neuronal networks and the modulation of neural plasticity during later developmental stages and in adulthood.[21,22,109] Recent studies have shown that activation of 5-HT$_7$Rs corrects molecular, electrophysiological, and behavioral abnormalities in animal models of fragile X syndrome (FXS) and Rett syndrome (RTT).[113,176−178] FXS and RTT are genetic forms of intellectual disabilities associated with autistic behavior. FXS, the most common inherited intellectual disability, is caused by silencing of the Fmr1 gene coding for the fragile X mental retardation protein (FMRP), an mRNA binding protein that functions as a regulator of protein translation. The absence of FMRP results in a dysregulation of protein synthesis, leading to altered synapse morphology and synaptic dysfunction.[179] Fmr1 knockout mice exhibit altered dendritic spine density and morphology, abnormally enhanced mGluR-LTD, autistic features, and a reduced behavioral flexibility. Activation of the 5-HT$_7$R by the agonists **2** or **17** is able to rescue mGluR-LTD in Fmr1 knockout mice, restoring LTD levels comparable to that of WT mice,[113,176] and this effect is mediated by an increase of the cellular levels of cAMP (unpublished results). This might have important functional consequences because long-term synaptic plasticity plays a fundamental role in shaping the structure and function of brain circuits, is crucially involved in learning and memory, and is believed to underlie behavioral flexibility.[180] In addition, in vivo activation of 5-HT$_7$R by agonist **17** is able to improve cognitive functions in the mouse model of FXS (unpublished results).

RTT is a rare neurodevelopmental disorder due to loss-of-function mutations in the MECP2 gene, which encodes a multifunctional protein that binds to methylated DNA and acts as a key transcriptional regulator. RTT is characterized by severe behavioral symptoms, including autistic-like behaviors, anxiety, motor disturbances, stereotypic hand movements, and severe cognitive dysfunction.[181] It has been demonstrated that the administration of the selective 5-HT$_7$ agonist **17** is able to rescue the behavioral impairments in MeCP2-308 mice, a mouse model of RTT, improving anxiety-related profiles in a light/dark test, motor abilities in a dowel test, exploratory behavior in the marble burying test, as well as memory in the novelty preference task. At a molecular level, administration of **17** to MeCP2-308 mice restored control levels of the Rho GTPases effector molecules PAK and cofilin, key regulators of actin cytoskeleton dynamics and thus is crucially involved in neuronal plasticity.[177,178] In addition, targeting the 5-HT$_7$R can rescue brain mitochondrial dysfunction in heterozygous female MeCP2-308 and MeCP2-

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

Bird mice (a more severely affected RTT model). Administration of **17** was able to rescue brain mitochondrial respiratory chain impairment, oxidative phosphorylation deficiency, and enhance a reduced energy status. Moreover, treatment with **17** completely restored the overproduction of radical species by brain mitochondria in the MeCP2-308 model and partially recovered the oxidative imbalance in MeCP2-Bird mice.[182]

A recent study has suggested that partial agonism at both 5-HT_{1A}R and 5-HT_7R can be useful to improve repetitive and stereotypic behavior, which is a core symptom in ASD. The systemic administration of the mixed 5-HT_{1A}/5-HT_7 partial agonist (+)-5-(2′-fluorophenyl)-*N*,*N*-dimethyl-1,2,3,4-tetrahydronaphthalen-2-amine ((+)-5-FPT) is able to reduce or eliminate stereotypy in three different mouse models without altering locomotor activity on its own and to enhance social interactions.[183]

Finally, it has been proposed that administration of 5-HT_7R antagonists, such as compound **14** (Figure 3), can contribute to reduced behavioral inflexibility, and this might be of relevance in ASD because reduced behavioral flexibility (i.e., a reduced ability to replace a previously acquired rule with a new one in adaptation to a new environmental context) is considered a typical feature of ASD.[23,141,184]

Further studies are necessary to clarify the relationship between altered synaptic plasticity and behavioral flexibility in ASD and thus the therapeutic potential of 5-HT_7 receptor agonists or antagonists in the treatment of behavioral deficits related to ASD.

6. 5-HT_7R INVESTIGATIONAL DRUGS AND CLINICAL TRIALS

Over the past few years, several multimodal drugs acting also on 5-HT_7Rs have been approved for clinical use and have entered the market mainly for schizophrenia and depression treatment. As discussed in paragraph 5.2, compound **9** (Figure 2) was the first relatively selective 5-HT_7R antagonist with suitable drug-like properties that entered a phase II clinical trial for the treatment of MDD (NCT00566202).[36] The lack of assay sensitivity contributed to the failure of this study and did not allow a definitive conclusion regarding the antidepressant efficacy of compound **9**. Currently, **9** is under evaluation in a phase II clinical trial as adjunctive treatment to standard pharmacologic therapy to ameliorate cognition deficits and reduce residual depressive symptoms in stable bipolar patients (NCT02466685).

The dopamine/serotonin stabilizer **77** (RP5063, also known as oxaripiprazole, Figure 20) is being developed by Reviva Pharmaceuticals for the treatment of schizophrenia and schizoaffective disorders. Compound **77** exhibits high binding affinity with partial agonism at the dopamine D_2, D_3, and D_4 and serotonin 5-HT_{1A} and 5-HT_{2A} receptors and antagonism at 5-HT_{2B}, 5-HT_{2C}, 5-HT_6, and 5-HT_7 receptors. The balance of agonism and antagonism at dopaminergic and serotonergic receptors is believed to be responsible for the overall stabilizing effect and the improved side effect profile as compared to classic antipsychotic drugs. In a multidose study in schizophrenic patients, compound **77** induced improvements of both positive and negative symptoms and showed a favorable safety profile that may result in good patients compliance (NCT01490086).[185] Breaburn Pharmaceuticals is currently developing compound ATI-9242 as an atypical antipsychotic. This compound is purpoted to be a modulator of acetylcholine, dopamine, GABA, NMDA, and 5-HT_7 receptor; unfortunately, the structure has not been disclosed.

Behavioral and psychological symptoms of dementia represent a substantial medical challenge among elderly patients because antypsychotic drugs may worsen cognitive functioning in patients who already suffer from cognitive deficits. It has been proposed that a combination of potent antagonism at 5-HT_6, 5-HT_7, and 5-HT_{2A} receptors with moderate antagonism at dopamine D_2 and D_3 receptors can be useful for the treatment of psychotic symptoms and mood deficits without affecting cognition. The investigational drug **78** (ADN-1184, Figure 20) possesses a preclinical profile that corresponds to these criteria, being able to improve measures of antipsychotic-like and antidepressant-like efficacy without affecting motor control and memory performance.[186]

Compound **79** (AVN-101, Figure 20), a potent 5-HT_7R antagonist (K_i = 153 pM) with slightly lesser potency toward 5-HT_6, 5-HT_{2A}, and 5-HT_{2C} receptors (K_i = 1.2−2.0 nM), has been proposed as a multitarget drug candidate for the treatment of CNS disorders with cognitive impairment, including AD. In fact, compound **79** had positive effects in animal models of impaired and innate cognition along with anxyolitic and antidepressive activity. In addition, a phase I clinical study indicated that **79** was well tolerated after oral administration.[187]

7. CONCLUSION AND FUTURE PERSPECTIVES

Twenty-five years after the discovery of the 5-HT_7R, much has been learned about expression, signaling, and function in vitro and in vivo of this GPCR. Starting from 2000, the availability of selective antagonists and agonists as well as of 5-HT_7R knockout mice drove significant progress in the understanding of the physiopathological role of the 5-HT_7R. Around the year 2000, there was a burst of medicinal chemistry effort centered on the development of selective 5-HT_7R antagonists and agonists. Various chemical classes, especially arylsulfonamides and arylpiperazines, were thoroughly investigated, leading to the identification of the molecules that are currently considered as gold standard pharmacological tools for 5-HT_7R research. Later medicinal chemistry efforts were focused on the identification of 5-HT_7R antagonists and agonists characterized by metabolic stability greater than that of the available pharmacological tools. These efforts were strongly supported by computational protocols. One successful example is represented by the study of Zajdel and co-workers,[74] in which basic moieties as an alternative to the arylpiperazine element were identified within a virtual combinatorial library by combining a virtual screening protocol with the potential of solid phase synthesis. This study led to the identification of a new class of potent 5-HT_7R

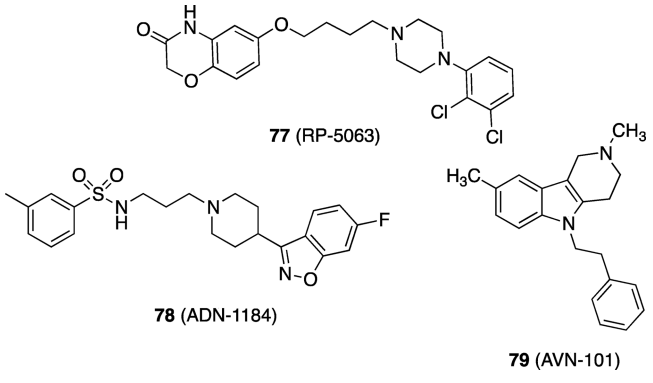


Figure 20. Structure of investigational drugs acting on 5-HT_7R.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

antagonists with different degrees of selectivity and in some cases good metabolic stability. SAR studies have been also devoted to the identification of a 5-HT$_7$R PET radioligand candidatse. However, these efforts have not led to to the identification of a suitable 5-HT$_7$R radioligand, and this is still an unmet need. As underlined above, in silico methodologies gave support to the identification of relatively new scaffolds capable of binding to the 5-HT$_7$R. To this end, the most relevant progress is represented by the recent identification of low-basicity 5-HT$_7$R ligands. These ligands showed a better pharmacokinetic profile when compared to basic ligands. Future studies will show how valuable these molecules are in 5-HT$_7$R research. Additional progress is expected from a combination of in silico and in vitro approaches such as molecular dynamics simulation and site-directed mutagenesis, as in the case of the study by Impellizzeri et al.,[188] who identified the essential residues for binding and activation to the 5-HT$_7$R, paving the way for the design of new agonist chemotypes. In silico approaches would also take great advantages from the crystallization of 5-HT$_7$R, which is yet to come.

The most recent studies are opening new perspectives for the study of the 5-HT$_7$R at the cell and organism level. At the cellular level, the physiological relevance of post-translational events, such as the formation of 5-HT$_7$R homodimers or 5-HT$_7$R/5-HT$_{1A}$R heterodimers, is almost completely unexplored. 5-HT$_7$R/5-HT$_{1A}$R heterodimer formation can influence signaling of each receptor of the dimer, and this is likely to play a role in the pathophysiology of 5-HT$_7$R and 5-HT$_{1A}$R receptors in the CNS. In this respect, it has been proposed that different amounts of heterodimers in pre- versus postsynaptic neurons may be critically involved in the onset of psychiatric disorders such as depression and anxiety and in the response to drug treatment.[189] One additional element of complexity is brought by the different roles played by the 5-HT$_7$R and the 5-HT$_{1A}$R on dendritic growth. Is there any role 5-HT$_7$R/5-HT$_{1A}$R heterodimers in these events? From a medicinal chemistry viewpoint, 5-HT$_7$R/5-HT$_{1A}$R heterodimers will likely represent a new area of exploration in order to identify powerful tools for studying 5-HT$_7$R/5-HT$_{1A}$R dimerization, as it is happening for other GPCR heterodimers.[190]

As for the clinical relevance of the 5-HT$_7$R, it is widely accepted that 5-HT$_7$R blockade produces antidepressant effects. The failure of the first clinical trial with the selective 5-HT$_7$R antagonist **9** has not allowed the drawing of a definitive conclusion regarding selective 5-HT$_7$R blockade as a therapeutic strategy that would be an alternative to selective serotonin reuptake inhibition. Instead, studies on the multimodal antidepressant drug **15** indicates that 5-HT$_7$R blockade along with the modulation of multiple 5-HT receptors can deliver new antidepressants drugs with the potential to treat cognitive dysfunction associated with major depression. The notion that 5-HT$_7$R blockade is an ancillary but yet favorable activity to improve mood and/or cognition in multitargeted drugs has been confirmed by several studies on antipsychotic drugs acting at multiple monoaminergic GPCRs. Preclinical data are showing that the new investigational drugs capable of blocking the 5-HT$_7$R ameliorate cognitive deficits associated with schizophrenia or dementia. These findings confirm that the "magic bullet" concept in drug discovery is coming to an end and can explain the modest interest of pharmaceutical companies in developing selective 5-HT$_7$R antagonists and, on the contrary, the increasing interest in searching modulators of multiple 5-HTRs.

In recent years, various studies have shown that 5-HT$_7$R signaling is implicated in neuronal plasticity, excitability, and morphology, hence contributing to the establishment of brain connectivity during embryonic and early postnatal life and even in the mature brain. Various recent studies have clearly demonstrated that 5-HT$_7$R activation is able to rescue molecular and behavioral phenotypes in animal models of neurodevelopmental disorders like FXS and RTT, which are conditions with no current therapy. Further translational research is necessary to establish the therapeutic potential of 5-HT$_7$R agonists in these rare diseases. In this respect, the huge potential offered by human induced pluripotent stem cells from FXS and RTT individuals waits to be exploited.[191] ASD is a common comorbid condition in FXS patients, and this led to the assumption that ASD symptoms may reflect the same underlying neurobiological impairments in both FXS and nonsyndromic ASD. Thus, the study of neurodevelopmental disorders such as FXS and RTT, which are a syndromic form of ASD, has yielded information at the molecular level on pathways critical for cognitive and social development and also in nonsyndromic (idiopathic) ASD patients. Because new research is revealing common features between syndromic and nonsyndromic forms of ASD, shared therapeutic approaches seem possible for this class of conditions. It will therefore be of interest to examine the effect of 5-HT$_7$R modulators in animal models of nonsyndromic ASD.

On the basis of the involvement of the 5-HT$_7$R subtype in hippocampal neuronal function and dendritic rearrangement, a recent study has focused on the effects of 5-HT$_7$R activation on hippocampal synaptic plasticity and apoptosis in a rat model of AD, leading to promising results. Further investigations will reveal the therapeutic potential of targeting 5-HT$_7$R activation in AD, particularly in view of the proposed link between 5-HT$_7$R signaling and tau pathology.[192]

In conclusion, after 25 years of research, much information are known about the 5-HT$_7$R has been developed but further research is required to dissect the role of the 5-HT$_7$R in physiology and pathology.

AUTHOR INFORMATION

Corresponding Authors

*For E.L.: phone, +39 080 5442750; fax, +39 080 5442231; E-mail, enza.lacivita@uniba.it.

*For V.P.: phone, +390957384269; E-mail, vpittala@unict.it.

ORCID

Maria N. Modica: 0000-0002-6350-931X

Enza Lacivita: 0000-0003-2443-1174

Sebastiano Intagliata: 0000-0002-0201-1745

Loredana Salerno: 0000-0001-6458-3717

Giuseppe Romeo: 0000-0003-2160-4164

Valeria Pittalà: 0000-0003-1856-0308

Marcello Leopoldo: 0000-0001-8401-2815

Author Contributions

The manuscript was written through contribution of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Biographies

Maria N. Modica graduated in pharmacy, in 1989, at the University of Catania, discussing a thesis in medicinal chemistry. In 1994, she received

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

the Ph.D. in pharmaceutical sciences. Since 1997, she is a researcher at the Department of Drugs Science at the University of Catania. Her research topics include the design and synthesis of ligands for the 5-HT_{1A} and 5-HT_7 serotonin, α1-adrenergic, and endothelin receptors, and of HO-1 and NOS inhibitors. She has published 57 papers, one contribution of a volume for a book, five proceedings, and 49 congress communications.

Enza Lacivita obtained her Ph.D. in medicinal chemistry in 2001, working on the development of 5-HT_{1A} receptor ligands at the University of Bari Aldo Moro. She was appointed as Assistant Professor in Medicinal Chemistry (2005) and as Associate Professor in Medicinal Chemistry in 2017 (Department of Pharmacy−Drug Science) at the same University. She has published more than 70 papers in peer-reviewed journals, focused on the development of selective ligands for GPCRs involved in neuropsychiatric disorders. She has contributed to the identification of the selective 5-HT_7 receptor agonist, LP-211.

Sebastiano Intagliata received his PhD. in Pharmaceutical Sciences at the University of Catania working on synthesis, structure−activity relationships, and molecular modeling studies of new alkylpiperazines as 5-HT_7 receptor ligands. In 2015, he spent a few months in the Department of Biomolecular Sciences at the University of Mississippi, working on lead optimization of highly selective σ-1 receptor antagonists. From January 2017, he has been working as a postdoctoral associate in the Dr. McCurdy's Lab at the University of Florida College of Pharmacy's Department of Medicinal Chemistry. Currently, his research is focused on developing novel σ-1 and σ-2 receptor ligands as potential treatments for pain and drug abuse.

Loredana Salerno obtained the degree in pharmacy in 1986 at the University of Catania, and in 1993, the Ph.D. in pharmaceutical sciences. At the Department of Pharmaceutical Sciences of the same university, she was appointed as Researcher (1996) and as Associate Professor in Medicinal Chemistry (2006). She has focused her research activity on the development of ligands for serotonergic and adrenergic receptor ligands, enzymatic inhibitors of nitric oxide synthase and heme oxygenase-1 as antitumor drugs, and modified natural compounds as inducers of heme oxygenase-1 useful in stress-induced diseases. She is the author of more than 70 scientific publications in peer-reviewed journals.

Giuseppe Romeo got the degree in pharmacy (honors) at the University of Catania in 1985. At the same University, he was appointed Research Associate in Medicinal Chemistry (1991) and Associate Professor in Medicinal Chemistry in 1998 (Department of Drug Sciences). In his research activity, he has been mainly involved in the design, synthesis, and characterization of novel heterocyclic compounds endowed with potential pharmacological activity. The main topics of his research include the development of selective ligands for the αa1-adrenoceptor subtypes and for the serotonin 5-HT_{1A} and 5-HT_7 receptors.

Valeria Pittalà attained an M.Sc. in chemistry and pharmaceutical technologies and completed a Ph.D. in pharmaceutical sciences at the University of Catania (Italy). She joined Pharmacia Corporation, where she worked as member of the Combinatorial Chemistry Group. She contributed to the discovery of the Aurora kinase inhibitor danusertib, which underwent clinical investigation, by being a co-inventor of the bicyclopyrazoles class. Subsequently, she returned to the University of Catania as a Medicinal Chemistry Assistant Professor. Valeria Pittalà has published over 50 peer-reviewed papers in peer-reviewed journals focused on structure−activity relationships for biological targets including GPCRs. She is a co-inventor of seven patents.

Marcello Leopoldo obtained the degree in chemistry and pharmaceutical technologies (honors) at the University of Bari in 1991. At the same University, he was appointed Research Associate in Medicinal Chemistry (1992) and then Associate Professor in 2001. He has received the Habilitation as Full Professor in Medicinal Chemistry in 2015. He is member of the International Society for Serotonin Research. He has published 110 papers in peer-reviewed journals focused on the development of selective ligands for dopamine, serotonin, and formyl peptide receptors. Various selective 5-HT_7 receptor agonists have been identified in his lab, including LP-44, LP-12, LP-211, and LP-20. LP-211 is being used to explore the therapeutic potential of 5-HT_7 receptor activation in neurodevelopmental disorders. He is an inventor of eight patent applications.

DEDICATION

This article is dedicated to Prof. Roberto Perrone, Dipartimento di Farmacia-Scienze del Farmaco, Università degli Studi di Bari Aldo Moro, on the occasion of his retirement.

ABBREVIATIONS USED

ASD, autism spectrum disorder; BBB, blood−brain barrier; CNS, central nervous system; mEPSCs, miniature excitatory postsynaptic currents; FXS, fragile X syndrome; FPT, four plate test; FST, forced swimming test; GPCRs, G-protein coupled receptors; 5-HT, 5-hydrotryptamine; NMDA, *N*-methyl-D-aspartate; NOR, novel object recognition; PCP, phencyclidine; RTT, Rett syndrome; SAR, structure−activity relationship; TST, tail suspension test

REFERENCES

(1) Hoyer, D.; Hannon, J. P.; Martin, G. R. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol., Biochem. Behav.* **2002**, *71*, 533−554.

(2) McCorvy, J. D.; Roth, B. L. Structure and function of serotonin G protein-coupled receptors. *Pharmacol. Ther.* **2015**, *150*, 129−142.

(3) Wang, C.; Jiang, Y.; Ma, J.; Wu, H.; Wacker, D.; Katritch, V.; Han, G. W.; Liu, W.; Huang, X. P.; Vardy, E.; McCorvy, J. D.; Gao, X.; Zhou, X. E.; Melcher, K.; Zhang, C.; Bai, F.; Yang, H.; Yang, L.; Jiang, H.; Roth, B. L.; Cherezov, V.; Stevens, R. C.; Xu, H. E. Structural basis for molecular recognition at serotonin receptors. *Science* **2013**, *340*, 610−614.

(4) Wacker, D.; Wang, S.; McCorvy, J. D.; Betz, R. M.; Venkatakrishnan, A. J.; Levit, A.; Lansu, K.; Schools, Z. L.; Che, T.; Nichols, D. E.; Shoichet, B. K.; Dror, R. O.; Roth, B. L. Crystal structure of an LSD-bound human serotonin receptor. *Cell* **2017**, *168*, 377−389.

(5) Lacivita, E.; Leopoldo, M.; Berardi, F.; Perrone, R. 5-HT_{1A} receptor, an old target for new therapeutic agents. *Curr. Top. Med. Chem.* **2008**, *8*, 1024−1034.

(6) Bard, J. A.; Zgombick, J.; Adham, N.; Vaysse, P.; Branchek, T. A.; Weinshank, R. L. Cloning of a novel human serotonin receptor (5-HT_7) positively linked to adenylate cyclase. *J. Biol. Chem.* **1993**, *268*, 23422−23426.

(7) Lovenberg, T. W.; Baron, B. M.; de Lecea, L.; Miller, J. D.; Prosser, R. A.; Rea, M. A.; Foye, P. E.; Racke, M.; Slone, A. L.; Siegel, B. W.; Danielson, P. E.; Sutcliffe, J. G.; Erlander, M. G. A novel adenylyl cyclase-activating serotonin receptor (5-HT_7) implicated in the regulation of mammalian circadian rhythms. *Neuron* **1993**, *11*, 449−458.

(8) Ruat, M.; Traiffort, E.; Leurs, R.; Tardivel-Lacombe, J.; Diaz, J.; Arrang, J. M.; Schwartz, J. C. Molecular cloning, characterization, and localization of a high-affinity serotonin receptor (5-HT_7) activating cAMP formation. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 8547−8551.

(9) Leopoldo, M.; Lacivita, E.; Berardi, F.; Perrone, R.; Hedlund, P. B. Serotonin 5-HT_7 receptor agents: Structure-activity relationships and potential therapeutic applications in central nervous system disorders. *Pharmacol. Ther.* **2011**, *129*, 120−148.

(10) Plassat, J. L.; Amlaiky, N.; Hen, R. Molecular cloning of a mammalian serotonin receptor that activates adenylate cyclase. *Mol. Pharmacol.* **1993**, *44*, 229−236.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

(11) Gellynck, E.; Laenen, K.; Andressen, K. W.; Lintermans, B.; De Martelaere, K.; Matthys, A.; Levy, F. O.; Haegeman, G.; Vanhoenacker, P.; Van Craenenbroeck, K. Cloning, genomic organization and functionality of 5-HT_7 receptor splice variants from mouse brain. *Gene* **2008**, *426*, 23−31.
(12) Heidmann, D. E.; Metcalf, M. A.; Kohen, R.; Hamblin, M. W. Four 5-hydroxytryptamine7 (5-HT_7) receptor isoforms in human and rat produced by alternative splicing: species differences due to altered intron-exon organization. *J. Neurochem.* **1997**, *68*, 1372−1381.
(13) Heidmann, D. E.; Szot, P.; Kohen, R.; Hamblin, M. W. Function and distribution of three rat 5-hydroxytryptamine$_7$ (5-HT_7) receptor isoforms produced by alternative splicing. *Neuropharmacology* **1998**, *37*, 1621−1632.
(14) Krobert, K. A.; Bach, T.; Syversveen, T.; Kvingedal, A. M.; Levy, F. O. The cloned human 5-HT_7 receptor splice variants: a comparative characterization of their pharmacology, function and distribution. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2001**, *363*, 620−632.
(15) Guthrie, C. R.; Murray, A. T.; Franklin, A. A.; Hamblin, M. W. Differential agonist-mediated internalization of the human 5-hydroxytryptamine 7 receptor isoforms. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1003−1010.
(16) Krobert, K. A.; Levy, F. O. The human 5-HT_7 serotonin receptor splice variants: constitutive activity and inverse agonist effects. *Br. J. Pharmacol.* **2002**, *135*, 1563−1571.
(17) Andressen, K. W.; Norum, J. H.; Levy, F. O.; Krobert, K. A. Activation of adenylyl cyclase by endogenous G_s-coupled receptors in human embryonic kidney 293 cells is attenuated by 5-HT_7 receptor expression. *Mol. Pharmacol.* **2006**, *69*, 207−215.
(18) Andressen, K. W.; Ulsund, A. H.; Krobert, K. A.; Lohse, M. J.; Bunemann, M.; Levy, F. O. Related GPCRs couple differently to G_s: preassociation between G protein and 5-HT_7 serotonin receptor reveals movement of Galphas upon receptor activation. *FASEB J.* **2018**, *32*, 1059−1069.
(19) Krobert, K. A.; Andressen, K. W.; Levy, F. O. Heterologous desensitization is evoked by both agonist and antagonist stimulation of the human 5-HT_7 serotonin receptor. *Eur. J. Pharmacol.* **2006**, *532*, 1−10.
(20) Kvachnina, E.; Liu, G.; Dityatev, A.; Renner, U.; Dumuis, A.; Richter, D. W.; Dityateva, G.; Schachner, M.; Voyno-Yasenetskaya, T. A.; Ponimaskin, E. G. 5-HT_7 receptor is coupled to G alpha subunits of heterotrimeric G_{12}-protein to regulate gene transcription and neuronal morphology. *J. Neurosci.* **2005**, *25*, 7821−7830.
(21) Kobe, F.; Guseva, D.; Jensen, T. P.; Wirth, A.; Renner, U.; Hess, D.; Muller, M.; Medrihan, L.; Zhang, W.; Zhang, M.; Braun, K.; Westerholz, S.; Herzog, A.; Radyushkin, K.; El-Kordi, A.; Ehrenreich, H.; Richter, D. W.; Rusakov, D. A.; Ponimaskin, E. 5-HT_7R/G_{12} signaling regulates neuronal morphology and function in an age-dependent manner. *J. Neurosci.* **2012**, *32*, 2915−2930.
(22) Kvachnina, E.; Dumuis, A.; Wlodarczyk, J.; Renner, U.; Cochet, M.; Richter, D. W.; Ponimaskin, E. Constitutive G_s-mediated, but not G_{12}-mediated, activity of the 5-hydroxytryptamine 5-$HT_{7(a)}$ receptor is modulated by the palmitoylation of its C-terminal domain. *Biochim. Biophys. Acta, Mol. Cell Res.* **2009**, *1793*, 1646−1655.
(23) Ciranna, L.; Catania, M. V. 5-HT_7 receptors as modulators of neuronal excitability, synaptic transmission and plasticity: physiological role and possible implications in autism spectrum disorders. *Front. Cell. Neurosci.* **2014**, *8*, 250.
(24) Nikiforuk, A. Targeting the serotonin 5-HT_7 receptor in the search for treatments for CNS disorders: Rationale and progress to date. *CNS Drugs* **2015**, *29*, 265−275.
(25) Naumenko, V. S.; Popova, N. K.; Lacivita, E.; Leopoldo, M.; Ponimaskin, E. G. Interplay between serotonin 5-HT_{1A} and 5-HT_7 receptors in depressive disorders. *CNS Neurosci. Ther.* **2014**, *20*, 582−290.
(26) Rajagopal, L.; Massey, B. W.; Michael, E.; Meltzer, H. Y. Serotonin (5-HT)$_{1A}$ receptor agonism and 5-HT_7 receptor antagonism ameliorate the subchronic phencyclidine-induced deficit in executive functioning in mice. *Psychopharmacology (Berl)* **2016**, *233*, 649−660.
(27) Eriksson, T. M.; Holst, S.; Stan, T. L.; Hager, T.; Sjogren, B.; Ogren, S. O.; Svenningsson, P.; Stiedl, O. 5-HT_{1A} and 5-HT_7 receptor crosstalk in the regulation of emotional memory: implications for effects of selective serotonin reuptake inhibitors. *Neuropharmacology* **2012**, *63*, 1150−1160.
(28) Teitler, M.; Toohey, N.; Knight, J. A.; Klein, M. T.; Smith, C. Clozapine and other competitive antagonists reactivate risperidone-inactivated h5-HT_7 receptors: radioligand binding and functional evidence for GPCR homodimer protomer interactions. *Psychopharmacology (Berl)* **2010**, *212*, 687−697.
(29) Renner, U.; Zeug, A.; Woehler, A.; Niebert, M.; Dityatev, A.; Dityateva, G.; Gorinski, N.; Guseva, D.; Abdel-Galil, D.; Frohlich, M.; Doring, F.; Wischmeyer, E.; Richter, D. W.; Neher, E.; Ponimaskin, E. G. Heterodimerization of serotonin receptors 5-HT_{1A} and 5-HT_7 differentially regulates receptor signalling and trafficking. *J. Cell Sci.* **2012**, *125*, 2486−2499.
(30) Leopoldo, M. Serotonin7 receptors (5-HT_7Rs) and their ligands. *Curr. Med. Chem.* **2004**, *11*, 629−261.
(31) Pittalà, V.; Pittalà, D. Latest advances towards the discovery of 5-HT_7 receptor ligands. *Mini-Rev. Med. Chem.* **2011**, *11*, 1108−1121.
(32) Lovell, P. J.; Bromidge, S. M.; Dabbs, S.; Duckworth, D. M.; Forbes, I. T.; Jennings, A. J.; King, F. D.; Middlemiss, D. N.; Rahman, S. K.; Saunders, D. V.; Collin, L. L.; Hagan, J. J.; Riley, G. J.; Thomas, D. R. A novel, potent, and selective 5-HT_7 antagonist: (R)-3-(2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidine-1-sulfonyl) phenol (SB-269970). *J. Med. Chem.* **2000**, *43*, 342−345.
(33) Forbes, I. T.; Douglas, S.; Gribble, A. D.; Ife, R. J.; Lightfoot, A. P.; Garner, A. E.; Riley, G. J.; Jeffrey, P.; Stevens, A. J.; Stean, T. O.; Thomas, D. R. SB-656104-A: a novel 5-HT_7 receptor antagonist with improved in vivo properties. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3341−3344.
(34) Kikuchi, C.; Nagaso, H.; Hiranuma, T.; Koyama, M. Tetrahydrobenzindoles: selective antagonists of the 5-HT_7 receptor. *J. Med. Chem.* **1999**, *42*, 533−535.
(35) Kikuchi, C.; Ando, T.; Watanabe, T.; Nagaso, H.; Okuno, M.; Hiranuma, T.; Koyama, M. 2*a*-[4-(Tetrahydropyridoindol-2-yl)butyl]-tetrahydrobenzindole derivatives: new selective antagonists of the 5-hydroxytryptamine$_7$ receptor. *J. Med. Chem.* **2002**, *45*, 2197−2206.
(36) Bonaventure, P.; Dugovic, C.; Kramer, M.; De Boer, P.; Singh, J.; Wilson, S.; Bertelsen, K.; Di, J.; Shelton, J.; Aluisio, L.; Dvorak, L.; Fraser, I.; Lord, B.; Nepomuceno, D.; Ahnaou, A.; Drinkenburg, W.; Chai, W.; Dvorak, C.; Sands, S.; Carruthers, N.; Lovenberg, T. W. Translational evaluation of JNJ-18038683, a 5-hydroxytryptamine type 7 receptor antagonist, on rapid eye movement sleep and in major depressive disorder. *J. Pharmacol. Exp. Ther.* **2012**, *342*, 429−440.
(37) Bang-Andersen, B.; Ruhland, T.; Jorgensen, M.; Smith, G.; Frederiksen, K.; Jensen, K. G.; Zhong, H.; Nielsen, S. M.; Hogg, S.; Mork, A.; Stensbol, T. B. Discovery of 1-[2-(2,4-dimethylphenylsulfanyl)phenyl]piperazine (Lu AA21004): a novel multimodal compound for the treatment of major depressive disorder. *J. Med. Chem.* **2011**, *54*, 3206−3221.
(38) Leopoldo, M.; Berardi, F.; Colabufo, N. A.; Contino, M.; Lacivita, E.; Niso, M.; Perrone, R.; Tortorella, V. Structure-affinity relationship study on *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides, a new class of 5-HT_7 receptor agents. *J. Med. Chem.* **2004**, *47*, 6616−6624.
(39) Leopoldo, M.; Lacivita, E.; De Giorgio, P.; Fracasso, C.; Guzzetti, S.; Caccia, S.; Contino, M.; Colabufo, N. A.; Berardi, F.; Perrone, R. Structural modifications of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinehexanamides: influence on lipophilicity and 5-HT_7 receptor activity. Part III. *J. Med. Chem.* **2008**, *51*, 5813−5822.
(40) Hedlund, P. B.; Leopoldo, M.; Caccia, S.; Sarkisyan, G.; Fracasso, C.; Martelli, G.; Lacivita, E.; Berardi, F.; Perrone, R. LP-211 is a brain penetrant selective agonist for the serotonin 5-HT_7 receptor. *Neurosci. Lett.* **2010**, *481*, 12−16.
(41) Brenchat, A.; Romero, L.; Garcia, M.; Pujol, M.; Burgueno, J.; Torrens, A.; Hamon, M.; Baeyens, J. M.; Buschmann, H.; Zamanillo, D.; Vela, J. M. 5-HT_7 receptor activation inhibits mechanical hypersensitivity secondary to capsaicin sensitization in mice. *Pain* **2009**, *141*, 239−247.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

(42) Brenchat, A.; Rocasalbas, M.; Zamanillo, D.; Hamon, M.; Vela, J. M.; Romero, L. Assessment of 5-HT_7 receptor agonists selectivity using nociceptive and thermoregulation tests in knockout versus wild-type mice. *Adv. Pharmacol. Sci.* **2012**, *2012*, 312041.
(43) Hedlund, P. B.; Carson, M. J.; Sutcliffe, J. G.; Thomas, E. A. Allosteric regulation by oleamide of the binding properties of 5-hydroxytryptamine$_7$ receptors. *Biochem. Pharmacol.* **1999**, *58*, 1807−1813.
(44) Bielenica, A.; Koziol, A. E.; Struga, M. Binding modes of chain arylpiperazines to 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors. *Mini-Rev. Med. Chem.* **2013**, *13*, 1516−1539.
(45) Kurczab, R.; Bojarski, A. J. New strategy for receptor-based pharmacophore query construction: a case study for 5-HT_7 receptor ligands. *J. Chem. Inf. Model.* **2013**, *53*, 3233−3243.
(46) Rataj, K.; Witek, J.; Mordalski, S.; Kosciolek, T.; Bojarski, A. J. Impact of template choice on homology model efficiency in virtual screening. *J. Chem. Inf. Model.* **2014**, *54*, 1661−1668.
(47) Podlewska, S.; Kafel, R.; Lacivita, E.; Satała, G.; Kooistra, A. J.; Vass, M.; de Graaf, C.; Leopoldo, M.; Bojarski, A. J.; Mordalski, S. Structural insights into serotonin receptor ligands polypharmacology. *Eur. J. Med. Chem.* **2018**, *151*, 797−814.
(48) Lopez-Rodriguez, M. L.; Porras, E.; Morcillo, M. J.; Benhamu, B.; Soto, L. J.; Lavandera, J. L.; Ramos, J. A.; Olivella, M.; Campillo, M.; Pardo, L. Optimization of the pharmacophore model for 5-HT_7R antagonism. Design and synthesis of new naphtholactam and naphthosultam derivatives. *J. Med. Chem.* **2003**, *46*, 5638−5650.
(49) Medina, R. A.; Sallander, J.; Benhamu, B.; Porras, E.; Campillo, M.; Pardo, L.; Lopez-Rodriguez, M. L. Synthesis of new serotonin 5-HT_7 receptor ligands. Determinants of 5-HT_7/5-HT_{1A} receptor selectivity. *J. Med. Chem.* **2009**, *52*, 2384−2392.
(50) Kolaczkowski, M.; Nowak, M.; Pawlowski, M.; Bojarski, A. J. Receptor-based pharmacophores for serotonin 5-HT_7R antagonists-implications to selectivity. *J. Med. Chem.* **2006**, *49*, 6732−6741.
(51) Vermeulen, E. S.; Schmidt, A. W.; Sprouse, J. S.; Wikstrom, H. V.; Grol, C. J. Characterization of the 5-HT_7 receptor. Determination of the pharmacophore for 5-HT_7 receptor agonism and CoMFA-based modeling of the agonist binding site. *J. Med. Chem.* **2003**, *46*, 5365−5374.
(52) Rague, A.; Tidgewell, K. J. Pharmacophore comparison and development of recently discovered long chain arylpiperazine and sulfonamide based 5-HT_7 Ligands. *Mini-Rev. Med. Chem.* **2018**, *18*, 552−560.
(53) Volk, B.; Barkoczy, J.; Hegedus, E.; Udvari, S.; Gacsalyi, I.; Mezei, T.; Pallagi, K.; Kompagne, H.; Levay, G.; Egyed, A.; Hársing, L. G., Jr.; Spedding, M.; Simig, G. (Phenylpiperazinyl-butyl)oxindoles as selective 5-HT_7 receptor antagonists. *J. Med. Chem.* **2008**, *51*, 2522−2532.
(54) Volk, B.; Gacsalyi, I.; Pallagi, K.; Poszavacz, L.; Gyonos, I.; Szabo, E.; Bako, T.; Spedding, M.; Simig, G.; Szenasi, G. Optimization of (arylpiperazinylbutyl)oxindoles exhibiting selective 5-HT_7 receptor antagonist activity. *J. Med. Chem.* **2011**, *54*, 6657−6669.
(55) Di Pilato, P.; Niso, M.; Adriani, W.; Romano, E.; Travaglini, D.; Berardi, F.; Colabufo, N. A.; Perrone, R.; Laviola, G.; Lacivita, E.; Leopoldo, M. Selective agonists for serotonin 7 (5-HT_7) receptor and their applications in preclinical models: an overview. *Rev. Neurosci.* **2014**, *25*, 401−415.
(56) Lacivita, E.; Di Pilato, P.; Stama, M. L.; Colabufo, N. A.; Berardi, F.; Perrone, R.; De Filippis, B.; Laviola, G.; Adriani, W.; Niso, M.; Leopoldo, M. Novel highly potent serotonin 5-HT_7 receptor ligands: structural modifications to improve pharmacokinetic properties. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6083−6086.
(57) Lacivita, E.; Podlewska, S.; Speranza, L.; Niso, M.; Satala, G.; Perrone, R.; Perrone-Capano, C.; Bojarski, A. J.; Leopoldo, M. Structural modifications of the serotonin 5-HT_7 receptor agonist *N*-(4-cyanophenylmethyl)-4-(2-biphenyl)-1-piperazinehexanamide (LP-211) to improve in vitro microsomal stability: a case study. *Eur. J. Med. Chem.* **2016**, *120*, 363−379.
(58) Lacivita, E.; Patarnello, D.; Stroth, N.; Caroli, A.; Niso, M.; Contino, M.; De Giorgio, P.; Di Pilato, P.; Colabufo, N. A.; Berardi, F.; Perrone, R.; Svenningsson, P.; Hedlund, P. B.; Leopoldo, M. Investigations on the 1-(2-biphenyl)piperazine motif: identification of new potent and selective ligands for the serotonin$_7$ (5-HT_7) receptor with agonist or antagonist action in vitro or ex vivo. *J. Med. Chem.* **2012**, *55*, 6375−6380.
(59) Leopoldo, M.; Berardi, F.; Colabufo, N. A.; Contino, M.; Lacivita, E.; Perrone, R.; Tortorella, V. Studies on 1-arylpiperazine derivatives with affinity for rat 5-HT_7 and 5-HT_{1A} receptors. *J. Pharm. Pharmacol.* **2004**, *56*, 247−255.
(60) Lacivita, E.; De Giorgio, P.; Patarnello, D.; Niso, M.; Colabufo, N. A.; Berardi, F.; Perrone, R.; Satala, G.; Duszynska, B.; Bojarski, A. J.; Leopoldo, M. Towards metabolically stable 5-HT_7 receptor ligands: a study on 1-arylpiperazine derivatives and related isosters. *Exp. Brain Res.* **2013**, *230*, 569−582.
(61) Salerno, L.; Pittalà, V.; Modica, M. N.; Siracusa, M. A.; Intagliata, S.; Cagnotto, A.; Salmona, M.; Kurczab, R.; Bojarski, A. J.; Romeo, G. Structure-activity relationships and molecular modeling studies of novel arylpiperazinylalkyl 2-benzoxazolones and 2-benzothiazolones as 5-HT_7 and 5-HT_{1A} receptor ligands. *Eur. J. Med. Chem.* **2014**, *85*, 716−726.
(62) Modica, M. N.; Intagliata, S.; Pittalà, V.; Salerno, L.; Siracusa, M. A.; Cagnotto, A.; Salmona, M.; Romeo, G. Synthesis and binding properties of new long-chain 4-substituted piperazine derivatives as 5-HT_{1A} and 5-HT_7 receptor ligands. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1427−1430.
(63) Intagliata, S.; Modica, M. N.; Pittalà, V.; Salerno, L.; Siracusa, M. A.; Cagnotto, A.; Salmona, M.; Kurczab, R.; Romeo, G. New *N*- and *O*-arylpiperazinylalkyl pyrimidines and 2-methylquinazolines derivatives as 5-HT_7 and 5-HT_{1A} receptor ligands: synthesis, structure-activity relationships, and molecular modeling studies. *Bioorg. Med. Chem.* **2017**, *25*, 1250−1259.
(64) Intagliata, S.; Modica, M. N.; Pittalà, V.; Salerno, L.; Siracusa, M. A.; Cagnotto, A.; Salmona, M.; Romeo, G. Design and synthesis of new homo and hetero bis-piperazinyl-1-propanone derivatives as 5-HT_7R selective ligands over 5-HT_{1A}R. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 4052−4056.
(65) Spadoni, G.; Bedini, A.; Bartolucci, S.; Pala, D.; Mor, M.; Riccioni, T.; Borsini, F.; Cabri, W.; Celona, D.; Marzi, M.; Tarzia, G.; Rivara, S.; Minetti, P. Towards the development of 5-HT_7 ligands combining serotonin-like and arylpiperazine moieties. *Eur. J. Med. Chem.* **2014**, *80*, 8−35.
(66) Sagnes, C.; Fournet, G.; Satala, G.; Bojarski, A. J.; Joseph, B. New 1-arylindoles based serotonin 5-HT_7 antagonists. Synthesis and binding evaluation studies. *Eur. J. Med. Chem.* **2014**, *75*, 159−168.
(67) Medina, R. A.; Vazquez-Villa, H.; Gomez-Tamayo, J. C.; Benhamu, B.; Martin-Fontecha, M.; de la Fuente, T.; Caltabiano, G.; Hedlund, P. B.; Pardo, L.; Lopez-Rodriguez, M. L. The extracellular entrance provides selectivity to serotonin 5-HT_7 receptor antagonists with antidepressant-like behavior in vivo. *J. Med. Chem.* **2014**, *57*, 6879−6884.
(68) Strekowski, L.; Saczewski, J.; Raux, E. A.; Fernando, N. T.; Klenc, J.; Paranjpe, S.; Raszkiewicz, A.; Blake, A. L.; Ehalt, A. J.; Barnes, S.; Baranowski, T. C.; Sullivan, S. M.; Satala, G.; Bojarski, A. J. Synthesis and structure-activity relationship analysis of 5-HT_7 receptor antagonists: piperazin-1-yl substituted unfused heterobiaryls. *Molecules* **2016**, *21*, 433.
(69) Kim, Y.; Kim, J.; Tae, J.; Roth, B. L.; Rhim, H.; Keum, G.; Nam, G.; Choo, H. Discovery of aryl-biphenyl-2-ylmethylpiperazines as novel scaffolds for 5-HT_7 ligands and role of the aromatic substituents in binding to the target receptor. *Bioorg. Med. Chem.* **2013**, *21*, 2568−2576.
(70) Kim, Y.; Park, H.; Lee, J.; Tae, J.; Kim, H. J.; Min, S. J.; Rhim, H.; Choo, H. 5-HT_7 receptor modulators: amino groups attached to biphenyl scaffold determine functional activity. *Eur. J. Med. Chem.* **2016**, *123*, 180−190.
(71) Kim, J.; Kim, Y.; Tae, J.; Yeom, M.; Moon, B.; Huang, X. P.; Roth, B. L.; Lee, K.; Rhim, H.; Choo, I. H.; Chong, Y.; Keum, G.; Nam, G.; Choo, H. Aryl biphenyl-3-ylmethylpiperazines as 5-HT_7 receptor antagonists. *ChemMedChem* **2013**, *8*, 1855−1864.
(72) Kim, Y.; Tae, J.; Lee, K.; Rhim, H.; Choo, I. H.; Cho, H.; Park, W. K.; Keum, G.; Choo, H. Novel *N*-biphenyl-2-ylmethyl 2-methoxyphe-

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

nylpiperazinylalkanamides as 5-HT$_7$R antagonists for the treatment of depression. *Bioorg. Med. Chem.* **2014**, *22*, 4587−4596.
(73) Zajdel, P.; Marciniec, K.; Maslankiewicz, A.; Paluchowska, M. H.; Satala, G.; Partyka, A.; Jastrzebska-Wiesek, M.; Wrobel, D.; Wesolowska, A.; Duszynska, B.; Bojarski, A. J.; Pawlowski, M. Arene- and quinoline-sulfonamides as novel 5-HT$_7$ receptor ligands. *Bioorg. Med. Chem.* **2011**, *19*, 6750−6759.
(74) Zajdel, P.; Kurczab, R.; Grychowska, K.; Satala, G.; Pawlowski, M.; Bojarski, A. J. The multiobjective based design, synthesis and evaluation of the arylsulfonamide/amide derivatives of aryloxyethyl- and arylthioethyl- piperidines and pyrrolidines as a novel class of potent 5-HT$_7$ receptor antagonists. *Eur. J. Med. Chem.* **2012**, *56*, 348−360.
(75) Zajdel, P.; Canale, V.; Partyka, A.; Marciniec, K.; Kurczab, R.; Satala, G.; Siwek, A.; Jastrzebska-Wiesek, M.; Wesolowska, A.; Kos, T.; Popik, P.; Bojarski, A. J. Arylsulfonamide derivatives of (aryloxy) ethylpiperidines as selective 5-HT$_7$ receptor antagonists and their psychotropic properties. *MedChemComm* **2015**, *6*, 1272−1277.
(76) Canale, V.; Kurczab, R.; Partyka, A.; Satala, G.; Lenda, T.; Jastrzebska-Wiesek, M.; Wesolowska, A.; Bojarski, A. J.; Zajdel, P. Towards new 5-HT$_7$ antagonists among arylsulfonamide derivatives of (aryloxy)ethyl-alkyl amines: multiobjective based design, synthesis, and antidepressant and anxiolytic properties. *Eur. J. Med. Chem.* **2016**, *108*, 334−346.
(77) Canale, V.; Kurczab, R.; Partyka, A.; Satala, G.; Sloczynska, K.; Kos, T.; Jastrzebska-Wiesek, M.; Siwek, A.; Pekala, E.; Bojarski, A. J.; Wesolowska, A.; Popik, P.; Zajdel, P. *N*-Alkylated arylsulfonamides of (aryloxy)ethyl piperidines: 5-HT$_7$ receptor selectivity versus multi-receptor profile. *Bioorg. Med. Chem.* **2016**, *24*, 130−139.
(78) Canale, V.; Partyka, A.; Kurczab, R.; Krawczyk, M.; Kos, T.; Satala, G.; Kubica, B.; Jastrzebska-Wiesek, M.; Wesolowska, A.; Bojarski, A. J.; Popik, P.; Zajdel, P. Novel 5-HT$_7$R antagonists, arylsulfonamide derivatives of (aryloxy)propyl piperidines: add-on effect to the antidepressant activity of SSRI and DRI, and pro-cognitive profile. *Bioorg. Med. Chem.* **2017**, *25*, 2789−2799.
(79) Canale, V.; Guzik, P.; Kurczab, R.; Verdie, P.; Satala, G.; Kubica, B.; Pawlowski, M.; Martinez, J.; Subra, G.; Bojarski, A. J.; Zajdel, P. Solid-supported synthesis, molecular modeling, and biological activity of long-chain arylpiperazine derivatives with cyclic amino acid amide fragments as 5-HT$_7$ and 5-HT$_{1A}$ receptor ligands. *Eur. J. Med. Chem.* **2014**, *78*, 10−22.
(80) Canale, V.; Kurczab, R.; Partyka, A.; Satala, G.; Witek, J.; Jastrzebska-Wiesek, M.; Pawlowski, M.; Bojarski, A. J.; Wesolowska, A.; Zajdel, P. Towards novel 5-HT$_7$ versus 5-HT$_{1A}$ receptor ligands among LCAPs with cyclic amino acid amide fragments: design, synthesis, and antidepressant properties. Part II. *Eur. J. Med. Chem.* **2015**, *92*, 202−211.
(81) Grychowska, K.; Masurier, N.; Verdie, P.; Satala, G.; Bojarski, A. J.; Martinez, J.; Pawlowski, M.; Subra, G.; Zajdel, P. Solid-supported synthesis and 5-HT$_7$/5-HT$_{1A}$ receptor affinity of arylpiperazinylbutyl derivatives of 4,5-dihydro-1,2,4-triazine-6-(1*H*)-one. *Chem. Biol. Drug Des.* **2015**, *86*, 697−703.
(82) Handzlik, J.; Bajda, M.; Zygmunt, M.; Maciag, D.; Dybala, M.; Bednarski, M.; Filipek, B.; Malawska, B.; Kiec-Kononowicz, K. Antiarrhythmic properties of phenylpiperazine derivatives of phenytoin with alpha$_1$-adrenoceptor affinities. *Bioorg. Med. Chem.* **2012**, *20*, 2290−2303.
(83) Handzlik, J.; Bojarski, A. J.; Satala, G.; Kubacka, M.; Sadek, B.; Ashoor, A.; Siwek, A.; Wiecek, M.; Kucwaj, K.; Filipek, B.; Kiec-Kononowicz, K. SAR-studies on the importance of aromatic ring topologies in search for selective 5-HT$_7$ receptor ligands among phenylpiperazine hydantoin derivatives. *Eur. J. Med. Chem.* **2014**, *78*, 324−339.
(84) Kucwaj-Brysz, K.; Warszycki, D.; Podlewska, S.; Witek, J.; Witek, K.; Gonzalez Izquierdo, A.; Satala, G.; Loza, M. I.; Lubelska, A.; Latacz, G.; Bojarski, A. J.; Castro, M.; Kiec-Kononowicz, K.; Handzlik, J. Rational design in search for 5-phenylhydantoin selective 5-HT$_7$R antagonists. Molecular modeling, synthesis and biological evaluation. *Eur. J. Med. Chem.* **2016**, *112*, 258−269.
(85) Ladduwahetty, T.; Gilligan, M.; Humphries, A.; Merchant, K. J.; Fish, R.; McAlister, G.; Ivarsson, M.; Dominguez, M.; O'Connor, D.; MacLeod, A. M. Non-basic ligands for aminergic GPCRs: the discovery and development diaryl sulfones as selective, orally bioavailable 5-HT$_{2A}$ receptor antagonists for the treatment of sleep disorders. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3708−3712.
(86) Nugiel, D. A.; Krumrine, J. R.; Hill, D. C.; Damewood, J. R., Jr.; Bernstein, P. R.; Sobotka-Briner, C. D.; Liu, J.; Zacco, A.; Pierson, M. E. De novo design of a picomolar nonbasic 5-HT$_{1B}$ receptor antagonist. *J. Med. Chem.* **2010**, *53*, 1876−1880.
(87) Hogendorf, A. S.; Hogendorf, A.; Kurczab, R.; Satala, G.; Lenda, T.; Walczak, M.; Latacz, G.; Handzlik, J.; Kiec-Kononowicz, K.; Wieronska, J. M.; Wozniak, M.; Cieslik, P.; Bugno, R.; Staron, J.; Bojarski, A. J. Low-basicity 5-HT$_7$ receptor agonists synthesized using the van Leusen multicomponent protocol. *Sci. Rep.* **2017**, *7*, 1444.
(88) Pike, V. W. Considerations in the development of reversibly binding PET radioligands for brain imaging. *Curr. Med. Chem.* **2016**, *23*, 1818−1869.
(89) Lemoine, L.; Andries, J.; Le Bars, D.; Billard, T.; Zimmer, L. Comparison of 4 radiolabeled antagonists for serotonin 5-HT$_7$ receptor neuroimaging: toward the first PET radiotracer. *J. Nucl. Med.* **2011**, *52*, 1811−1818.
(90) Colomb, J.; Becker, G.; Forcellini, E.; Meyer, S.; Buisson, L.; Zimmer, L.; Billard, T. Synthesis and pharmacological evaluation of a new series of radiolabeled ligands for 5-HT$_7$ receptor PET neuro-imaging. *Nucl. Med. Biol.* **2014**, *41*, 330−337.
(91) Hansen, H. D.; Herth, M. M.; Ettrup, A.; Andersen, V. L.; Lehel, S.; Dyssegaard, A.; Kristensen, J. L.; Knudsen, G. M. Radiosynthesis and in vivo evaluation of novel radioligands for PET imaging of cerebral 5-HT$_7$ receptors. *J. Nucl. Med.* **2014**, *55*, 640−646.
(92) Herth, M. M.; Andersen, V. L.; Hansen, H. D.; Stroth, N.; Volk, B.; Lehel, S.; Dyssegaard, A.; Ettrup, A.; Svenningsson, P.; Knudsen, G. M.; Kristensen, J. L. Evaluation of 3-ethyl-3-(phenylpiperazinylbutyl)-oxindoles as PET ligands for the serotonin 5-HT$_7$ receptor: synthesis, pharmacology, radiolabeling, and in vivo brain imaging in pigs. *J. Med. Chem.* **2015**, *58*, 3631−3636.
(93) Herth, M. M.; Hansen, H. D.; Ettrup, A.; Dyssegaard, A.; Lehel, S.; Kristensen, J.; Knudsen, G. M. Synthesis and evaluation of [^{11}C]Cimbi-806 as a potential PET ligand for 5-HT$_7$ receptor imaging. *Bioorg. Med. Chem.* **2012**, *20*, 4574−4581.
(94) Hansen, H. D.; Andersen, V. L.; Lehel, S.; Magnussen, J. H.; Dyssegaard, A.; Stroth, N.; Kristensen, J. L.; Knudsen, G. M.; Herth, M. M. Labeling and preliminary in vivo evaluation of the 5-HT$_7$ receptor selective agonist [^{11}C]E-55888. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1901−1904.
(95) Hansen, H. D.; Lacivita, E.; Di Pilato, P.; Herth, M. M.; Lehel, S.; Ettrup, A.; Andersen, V. L.; Dyssegaard, A.; De Giorgio, P.; Perrone, R.; Berardi, F.; Colabufo, N. A.; Niso, M.; Knudsen, G. M.; Leopoldo, M. Synthesis, radiolabeling and in vivo evaluation of [^{11}C](R)-1-[4-[2-(4-methoxyphenyl)phenyl]piperazin-1-yl]-3-(2-pyrazinyloxy)-2-propanol, a potential PET radioligand for the 5-HT$_7$ receptor. *Eur. J. Med. Chem.* **2014**, *79*, 152−163.
(96) Lacivita, E.; Niso, M.; Hansen, H. D.; Di Pilato, P.; Herth, M. M.; Lehel, S.; Ettrup, A.; Montenegro, L.; Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Knudsen, G. M. Design, synthesis, radiolabeling and in vivo evaluation of potential positron emission tomography (PET) radioligands for brain imaging of the 5-HT$_7$ receptor. *Bioorg. Med. Chem.* **2014**, *22*, 1736−1750.
(97) Tiwari, A. K.; Yui, J.; Pooja; Aggarwal, S.; Yamasaki, T.; Xie, L.; Chadha, N.; Zhang, Y.; Fujinaga, M.; Shimoda, Y.; Kumata, K.; Mishra, A. K.; Ogawa, M.; Zhang, M. R. Design, synthesis and biological evaluation of small molecule-based PET radioligands for the 5-hydroxytryptamine 7 receptor. *RSC Adv.* **2015**, *5*, 19752−19759.
(98) Volpicelli, F.; Speranza, L.; di Porzio, U.; Crispino, M.; Perrone-Capano, C. The serotonin receptor 7 and the structural plasticity of brain circuits. *Front. Behav. Neurosci.* **2014**, *8*, 318.
(99) Gaspar, P.; Cases, O.; Maroteaux, L. The developmental role of serotonin: news from mouse molecular genetics. *Nat. Rev. Neurosci.* **2003**, *4*, 1002−1012.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

(100) Lesch, K. P.; Waider, J. Serotonin in the modulation of neural plasticity and networks: implications for neurodevelopmental disorders. *Neuron* **2012**, *76*, 175−191.
(101) Tajiri, M.; Hayata-Takano, A.; Seiriki, K.; Ogata, K.; Hazama, K.; Shintani, N.; Baba, A.; Hashimoto, H. Serotonin 5-HT$_7$ receptor blockade reverses behavioral abnormalities in PACAP-deficient mice and receptor activation promotes neurite extension in primary embryonic hippocampal neurons: therapeutic implications for psychiatric disorders. *J. Mol. Neurosci.* **2012**, *48*, 473−481.
(102) Rojas, P. S.; Neira, D.; Munoz, M.; Lavandero, S.; Fiedler, J. L. Serotonin (5-HT) regulates neurite outgrowth through 5-HT$_{1A}$ and 5-HT$_7$ receptors in cultured hippocampal neurons. *J. Neurosci. Res.* **2014**, *92*, 1000−1009.
(103) Speranza, L.; Chambery, A.; Di Domenico, M.; Crispino, M.; Severino, V.; Volpicelli, F.; Leopoldo, M.; Bellenchi, G. C.; di Porzio, U.; Perrone-Capano, C. The serotonin receptor 7 promotes neurite outgrowth via ERK and Cdk5 signaling pathways. *Neuropharmacology* **2013**, *67*, 155−167.
(104) Speranza, L.; Giuliano, T.; Volpicelli, F.; De Stefano, M. E.; Lombardi, L.; Chambery, A.; Lacivita, E.; Leopoldo, M.; Bellenchi, G. C.; di Porzio, U.; Crispino, M.; Perrone-Capano, C. Activation of 5-HT$_7$ receptor stimulates neurite elongation through mTOR, Cdc42 and actin filaments dynamics. *Front. Behav. Neurosci.* **2015**, *9*, 62.
(105) Rojas, P. S.; Aguayo, F.; Neira, D.; Tejos, M.; Aliaga, E.; Munoz, J. P.; Parra, C. S.; Fiedler, J. L. Dual effect of serotonin on the dendritic growth of cultured hippocampal neurons: involvement of 5-HT$_{1A}$ and 5-HT$_7$ receptors. *Mol. Cell. Neurosci.* **2017**, *85*, 148−161.
(106) Speranza, L.; Labus, J.; Volpicelli, F.; Guseva, D.; Lacivita, E.; Leopoldo, M.; Bellenchi, G. C.; di Porzio, U.; Bijata, M.; Perrone-Capano, C.; Ponimaskin, E. Serotonin 5-HT$_7$ receptor increases the density of dendritic spines and facilitates synaptogenesis in forebrain neurons. *J. Neurochem.* **2017**, *141*, 647−661.
(107) Canese, R.; Zoratto, F.; Altabella, L.; Porcari, P.; Mercurio, L.; de Pasquale, F.; Butti, E.; Martino, G.; Lacivita, E.; Leopoldo, M.; Laviola, G.; Adriani, W. Persistent modification of forebrain networks and metabolism in rats following adolescent exposure to a 5-HT$_7$ receptor agonist. *Psychopharmacology (Berl)* **2015**, *232*, 75−89.
(108) Bijata, M.; Labus, J.; Guseva, D.; Stawarski, M.; Butzlaff, M.; Dzwonek, J.; Schneeberg, J.; Bohm, K.; Michaluk, P.; Rusakov, D. A.; Dityatev, A.; Wilczynski, G.; Wlodarczyk, J.; Ponimaskin, E. Synaptic remodeling depends on signaling between serotonin receptors and the extracellular matrix. *Cell Rep.* **2017**, *19*, 1767−1782.
(109) Roberts, A. J.; Krucker, T.; Levy, C. L.; Slanina, K. A.; Sutcliffe, J. G.; Hedlund, P. B. Mice lacking 5-HT receptors show specific impairments in contextual learning. *Eur. J. Neurosci.* **2004**, *19*, 1913−1922.
(110) Vasefi, M. S.; Kruk, J. S.; Heikkila, J. J.; Beazely, M. A. 5-Hydroxytryptamine type 7 receptor neuroprotection against NMDA-induced excitotoxicity is PDGFbeta receptor dependent. *J. Neurochem.* **2013**, *125*, 26−36.
(111) Costa, L.; Spatuzza, M.; D'Antoni, S.; Bonaccorso, C. M.; Trovato, C.; Musumeci, S. A.; Leopoldo, M.; Lacivita, E.; Catania, M. V.; Ciranna, L. Activation of 5-HT$_7$ serotonin receptors reverses metabotropic glutamate receptor-mediated synaptic plasticity in wild-type and Fmr1 knockout mice, a model of Fragile X syndrome. *Biol. Psychiatry* **2012**, *72*, 924−933.
(112) Hashemi-Firouzi, N.; Komaki, A.; Soleimani Asl, S.; Shahidi, S. The effects of the 5-HT$_7$ receptor on hippocampal long-term potentiation and apoptosis in a rat model of Alzheimer's disease. *Brain Res. Bull.* **2017**, *135*, 85−91.
(113) Xiang, K.; Zhao, X.; Li, Y.; Zheng, L.; Wang, J.; Li, Y. H. Selective 5-HT$_7$ receptor activation may enhance synaptic plasticity through N-methyl-D-aspartate (NMDA) receptor activity in the visual cortex. *Curr. Neurovasc. Res.* **2016**, *13*, 321−328.
(114) Li, Y. H.; Han, L.; Wu, K. L. K.; Chan, Y. S. Activation of 5-HT$_7$ receptors reverses NMDA-R-dependent LTD by activating PKA in medial vestibular neurons. *Neuropharmacology* **2017**, *123*, 242−248.
(115) Lippiello, P.; Hoxha, E.; Speranza, L.; Volpicelli, F.; Ferraro, A.; Leopoldo, M.; Lacivita, E.; Perrone-Capano, C.; Tempia, F.; Miniaci, M. C. The 5-HT$_7$ receptor triggers cerebellar long-term synaptic depression via PKC-MAPK. *Neuropharmacology* **2016**, *101*, 426−438.
(116) Mnie-Filali, O.; Faure, C.; Lambas-Senas, L.; El Mansari, M.; Belblidia, H.; Gondard, E.; Etievant, A.; Scarna, H.; Didier, A.; Berod, A.; Blier, P.; Haddjeri, N. Pharmacological blockade of 5-HT$_7$ receptors as a putative fast acting antidepressant strategy. *Neuropsychopharmacology* **2011**, *36*, 1275−1288.
(117) Thomas, D. R.; Melotto, S.; Massagrande, M.; Gribble, A. D.; Jeffrey, P.; Stevens, A. J.; Deeks, N. J.; Eddershaw, P. J.; Fenwick, S. H.; Riley, G.; Stean, T.; Scott, C. M.; Hill, M. J.; Middlemiss, D. N.; Hagan, J. J.; Price, G. W.; Forbes, I. T. SB-656104-A, a novel selective 5-HT$_7$ receptor antagonist, modulates REM sleep in rats. *Br. J. Pharmacol.* **2003**, *139*, 705−714.
(118) Bonaventure, P.; Kelly, L.; Aluisio, L.; Shelton, J.; Lord, B.; Galici, R.; Miller, K.; Atack, J.; Lovenberg, T. W.; Dugovic, C. Selective blockade of 5-hydroxytryptamine (5-HT)$_7$ receptors enhances 5-HT transmission, antidepressant-like behavior, and rapid eye movement sleep suppression induced by citalopram in rodents. *J. Pharmacol. Exp. Ther.* **2007**, *321*, 690−698.
(119) Sarkisyan, G.; Roberts, A. J.; Hedlund, P. B. The 5-HT$_7$ receptor as a mediator and modulator of antidepressant-like behavior. *Behav. Brain Res.* **2010**, *209*, 99−108.
(120) Ishibashi, T.; Horisawa, T.; Tokuda, K.; Ishiyama, T.; Ogasa, M.; Tagashira, R.; Matsumoto, K.; Nishikawa, H.; Ueda, Y.; Toma, S.; Oki, H.; Tanno, N.; Saji, I.; Ito, A.; Ohno, Y.; Nakamura, M. Pharmacological profile of lurasidone, a novel antipsychotic agent with potent 5-hydroxytryptamine 7 (5-HT$_7$) and 5-HT$_{1A}$ receptor activity. *J. Pharmacol. Exp. Ther.* **2010**, *334*, 171−181.
(121) Abbas, A. I.; Hedlund, P. B.; Huang, X. P.; Tran, T. B.; Meltzer, H. Y.; Roth, B. L. Amisulpride is a potent 5-HT$_7$ antagonist: relevance for antidepressant actions in vivo. *Psychopharmacology (Berl)* **2009**, *205*, 119−128.
(122) Shapiro, D. A.; Renock, S.; Arrington, E.; Chiodo, L. A.; Liu, L. X.; Sibley, D. R.; Roth, B. L.; Mailman, R. Aripiprazole, a novel atypical antipsychotic drug with a unique and robust pharmacology. *Neuropsychopharmacology* **2003**, *28*, 1400−1411.
(123) Cates, L. N.; Roberts, A. J.; Huitron-Resendiz, S.; Hedlund, P. B. Effects of lurasidone in behavioral models of depression. Role of the 5-HT$_7$ receptor subtype. *Neuropharmacology* **2013**, *70*, 211−217.
(124) Sanchez, C.; Asin, K. E.; Artigas, F. Vortioxetine, a novel antidepressant with multimodal activity: review of preclinical and clinical data. *Pharmacol. Ther.* **2015**, *145*, 43−57.
(125) Holuj, M.; Popik, P.; Nikiforuk, A. Improvement of ketamine-induced social withdrawal in rats: the role of 5-HT$_7$ receptors. *Behav. Pharmacol.* **2015**, *26*, 766−775.
(126) Adriani, W.; Travaglini, D.; Lacivita, E.; Saso, L.; Leopoldo, M.; Laviola, G. Modulatory effects of two novel agonists for serotonin receptor 7 on emotion, motivation and circadian rhythm profiles in mice. *Neuropharmacology* **2012**, *62*, 833−842.
(127) Ruocco, L. A.; Treno, C.; Gironi Carnevale, U. A.; Arra, C.; Boatto, G.; Nieddu, M.; Pagano, C.; Illiano, P.; Barbato, F.; Tino, A.; Carboni, E.; Laviola, G.; Lacivita, E.; Leopoldo, M.; Adriani, W.; Sadile, A. G. Prepuberal stimulation of 5-HT$_7$-R by LP-211 in a rat model of hyper-activity and attention-deficit: permanent effects on attention, brain amino acids and synaptic markers in the fronto-striatal interface. *PLoS One* **2014**, *9*, e83003.
(128) Dean, B.; Pavey, G.; Thomas, D.; Scarr, E. Cortical serotonin 7, 1D and 1F receptors: effects of schizophrenia, suicide and antipsychotic drug treatment. *Schizophr. Res.* **2006**, *88*, 265−274.
(129) Meltzer, H. Y.; Massey, B. W. The role of serotonin receptors in the action of atypical antipsychotic drugs. *Curr. Opin. Pharmacol.* **2011**, *11*, 59−67.
(130) Neill, J. C.; Barnes, S.; Cook, S.; Grayson, B.; Idris, N. F.; McLean, S. L.; Snigdha, S.; Rajagopal, L.; Harte, M. K. Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism. *Pharmacol. Ther.* **2010**, *128*, 419−432.
(131) Pouzet, B.; Didriksen, M.; Arnt, J. Effects of the 5-HT$_7$ receptor antagonist SB-258741 in animal models for schizophrenia. *Pharmacol., Biochem. Behav.* **2002**, *71*, 655−665.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

(132) Galici, R.; Boggs, J. D.; Miller, K. L.; Bonaventure, P.; Atack, J. R. Effects of SB-269970, a 5-HT$_7$ receptor antagonist, in mouse models predictive of antipsychotic-like activity. *Behav. Pharmacol.* **2008**, *19*, 153−159.
(133) Thomas, D. R.; Hagan, J. J. 5-HT$_7$ receptors. *Curr. Drug Targets: CNS Neurol. Disord.* **2004**, *3*, 81−90.
(134) Delcourte, S.; Abrial, E.; Etievant, A.; Rovera, R.; Arnt, J.; Didriksen, M.; Haddjeri, N. Asenapine modulates mood-related behaviors and 5-HT$_{1A/7}$ receptors-mediated neurotransmission. *CNS Neurosci. Ther.* **2017**, *23*, 518−525.
(135) Meltzer, H. Y.; Rajagopal, L.; Huang, M.; Oyamada, Y.; Kwon, S.; Horiguchi, M. Translating the N-methyl-D-aspartate receptor antagonist model of schizophrenia to treatments for cognitive impairment in schizophrenia. *Int. J. Neuropsychopharmacol.* **2013**, *16*, 2181−2194.
(136) Horiguchi, M.; Huang, M.; Meltzer, H. Y. The role of 5-hydroxytryptamine 7 receptors in the phencyclidine-induced novel object recognition deficit in rats. *J. Pharmacol. Exp. Ther.* **2011**, *338*, 605−614.
(137) McLean, S. L.; Woolley, M. L.; Thomas, D.; Neill, J. C. Role of 5-HT receptor mechanisms in sub-chronic PCP-induced reversal learning deficits in the rat. *Psychopharmacology (Berl)* **2009**, *206*, 403−414.
(138) Bonaventure, P.; Aluisio, L.; Shoblock, J.; Boggs, J. D.; Fraser, I. C.; Lord, B.; Lovenberg, T. W.; Galici, R. Pharmacological blockade of serotonin 5-HT$_7$ receptor reverses working memory deficits in rats by normalizing cortical glutamate neurotransmission. *PLoS One* **2011**, *6*, e20210.
(139) Meneses, A. Effects of the 5-HT$_7$ receptor antagonists SB-269970 and DR 4004 in autoshaping Pavlovian/instrumental learning task. *Behav. Brain Res.* **2004**, *155*, 275−282.
(140) Nikiforuk, A.; Kos, T.; Fijal, K.; Holuj, M.; Rafa, D.; Popik, P. Effects of the selective 5-HT$_7$ receptor antagonist SB-269970 and amisulpride on ketamine-induced schizophrenia-like deficits in rats. *PLoS One* **2013**, *8*, e66695.
(141) Nikiforuk, A.; Popik, P. Amisulpride promotes cognitive flexibility in rats: the role of 5-HT$_7$ receptors. *Behav. Brain Res.* **2013**, *248*, 136−140.
(142) Hauser, S. R.; Hedlund, P. B.; Roberts, A. J.; Sari, Y.; Bell, R. L.; Engleman, E. A. The 5-HT$_7$ receptor as a potential target for treating drug and alcohol abuse. *Front. Neurosci.* **2015**, *8*, 448.
(143) Ballaz, S. J.; Akil, H.; Watson, S. J. The 5-HT$_7$ receptor: role in novel object discrimination and relation to novelty-seeking behavior. *Neuroscience* **2007**, *149*, 192−202.
(144) Zuckerman, M.; Kuhlman, D. M.; Joireman, J.; Teta, P.; Kraft, M. A Comparison of 3 structural models for personality - the Big 3, the Big 5, and the Alternative 5. *Journal of Personality and Social Psychology* **1993**, *65*, 757−768.
(145) Piazza, P. V.; Deminiere, J. M.; Le Moal, M.; Simon, H. Factors that predict individual vulnerability to amphetamine self-administration. *Science* **1989**, *245*, 1511−1513.
(146) Ballaz, S. J.; Akil, H.; Watson, S. J. Analysis of 5-HT$_6$ and 5-HT$_7$ receptor gene expression in rats showing differences in novelty-seeking behavior. *Neuroscience* **2007**, *147*, 428−438.
(147) Beaudet, G.; Paizanis, E.; Zoratto, F.; Lacivita, E.; Leopoldo, M.; Freret, T.; Laviola, G.; Boulouard, M.; Adriani, W. LP-211, a selective 5-HT$_7$ receptor agonist, increases novelty-preference and promotes risk-prone behavior in rats. *Synapse* **2017**, *71*, e21995.
(148) Dom, G.; Hulstijn, W.; Sabbe, B. Differences in impulsivity and sensation seeking between early- and late-onset alcoholics. *Addict Behav.* **2006**, *31*, 298−308.
(149) Leo, D.; Adriani, W.; Cavaliere, C.; Cirillo, G.; Marco, E. M.; Romano, E.; di Porzio, U.; Papa, M.; Perrone-Capano, C.; Laviola, G. Methylphenidate to adolescent rats drives enduring changes of accumbal Htr7 expression: implications for impulsive behavior and neuronal morphology. *Genes, Brain Behav.* **2009**, *8*, 356−368.
(150) Viguier, F.; Michot, B.; Hamon, M.; Bourgoin, S. Multiple roles of serotonin in pain control mechanisms-implications of 5-HT$_7$ and other 5-HT receptor types. *Eur. J. Pharmacol.* **2013**, *716*, 8−16.
(151) Meuser, T.; Pietruck, C.; Gabriel, A.; Xie, G. X.; Lim, K. J.; Pierce Palmer, P. 5-HT$_7$ receptors are involved in mediating 5-HT-induced activation of rat primary afferent neurons. *Life Sci.* **2002**, *71*, 2279−2289.
(152) Doly, S.; Fischer, J.; Brisorgueil, M. J.; Verge, D.; Conrath, M. Pre- and postsynaptic localization of the 5-HT$_7$ receptor in rat dorsal spinal cord: immunocytochemical evidence. *J. Comp. Neurol.* **2005**, *490*, 256−269.
(153) Rocha-Gonzalez, H. I.; Meneses, A.; Carlton, S. M.; Granados-Soto, V. Pronociceptive role of peripheral and spinal 5-HT$_7$ receptors in the formalin test. *Pain* **2005**, *117*, 182−192.
(154) Amaya-Castellanos, E.; Pineda-Farias, J. B.; Castaneda-Corral, G.; Vidal-Cantu, G. C.; Murbartian, J.; Rocha-Gonzalez, H. I.; Granados-Soto, V. Blockade of 5-HT$_7$ receptors reduces tactile allodynia in the rat. *Pharmacol., Biochem. Behav.* **2011**, *99*, 591−597.
(155) Viguier, F.; Michot, B.; Kayser, V.; Bernard, J. F.; Vela, J. M.; Hamon, M.; Bourgoin, S. GABA, but not opioids, mediates the anti-hyperalgesic effects of 5-HT$_7$ receptor activation in rats suffering from neuropathic pain. *Neuropharmacology* **2012**, *63*, 1093−1106.
(156) Brenchat, A.; Nadal, X.; Romero, L.; Ovalle, S.; Muro, A.; Sanchez-Arroyos, R.; Portillo-Salido, E.; Pujol, M.; Montero, A.; Codony, X.; Burgueno, J.; Zamanillo, D.; Hamon, M.; Maldonado, R.; Vela, J. M. Pharmacological activation of 5-HT$_7$ receptors reduces nerve injury-induced mechanical and thermal hypersensitivity. *Pain* **2010**, *149*, 483−494.
(157) Martinez-Garcia, E.; Sanchez-Maldonado, C.; Terron, J. A. 5-HT$_7$ receptor-mediated meningeal dilatation induced by 5-carboxamidotryptamine in rats is not altered by 5-HT depletion and chronic corticosterone treatment. *Proc. West Pharmacol. Soc.* **2011**, *54*, 57−61.
(158) Dogrul, A.; Seyrek, M. Systemic morphine produce antinociception mediated by spinal 5-HT$_7$, but not 5-HT$_{1A}$ and 5-HT$_2$ receptors in the spinal cord. *Br. J. Pharmacol.* **2006**, *149*, 498−505.
(159) Yanarates, O.; Dogrul, A.; Yildirim, V.; Sahin, A.; Sizlan, A.; Seyrek, M.; Akgul, O.; Kozak, O.; Kurt, E.; Aypar, U. Spinal 5-HT$_7$ receptors play an important role in the antinociceptive and antihyperalgesic effects of tramadol and its metabolite, O-Desmethyl-tramadol, via activation of descending serotonergic pathways. *Anesthesiology* **2010**, *112*, 696−710.
(160) Santello, M.; Nevian, T. Dysfunction of cortical dendritic integration in neuropathic pain reversed by serotoninergic neuromodulation. *Neuron* **2015**, *86*, 233−246.
(161) Santello, M.; Bisco, A.; Nevian, N. E.; Lacivita, E.; Leopoldo, M.; Nevian, T. The brain-penetrant 5-HT$_7$ receptor agonist LP-211 reduces the sensory and affective components of neuropathic pain. *Neurobiol. Dis.* **2017**, *106*, 214−221.
(162) Meneses, A. Neural activity, memory, and dementias: serotonergic markers. *Behav. Pharmacol.* **2017**, *28*, 132−141.
(163) Waters, K. A.; Stean, T. O.; Hammond, B.; Virley, D. J.; Upton, N.; Kew, J. N.; Hussain, I. Effects of the selective 5-HT$_7$ receptor antagonist SB-269970 in animal models of psychosis and cognition. *Behav. Brain Res.* **2012**, *228*, 211−218.
(164) Freret, T.; Paizanis, E.; Beaudet, G.; Gusmao-Montaigne, A.; Nee, G.; Dauphin, F.; Bouet, V.; Boulouard, M. Modulation of 5-HT$_7$ receptor: effect on object recognition performances in mice. *Psychopharmacology (Berl)* **2014**, *231*, 393−400.
(165) Beaudet, G.; Bouet, V.; Jozet-Alves, C.; Schumann-Bard, P.; Dauphin, F.; Paizanis, E.; Boulouard, M.; Freret, T. Spatial memory deficit across aging: current insights of the role of 5-HT$_7$ receptors. *Front. Behav. Neurosci.* **2014**, *8*, 448.
(166) Eriksson, T. M.; Golkar, A.; Ekstrom, J. C.; Svenningsson, P.; Ogren, S. O. 5-HT$_7$ receptor stimulation by 8-OH-DPAT counteracts the impairing effect of 5-HT$_{1A}$ receptor stimulation on contextual learning in mice. *Eur. J. Pharmacol.* **2008**, *596*, 107−110.
(167) Meneses, A.; Perez-Garcia, G.; Liy-Salmeron, G.; Flores-Galvez, D.; Castillo, C.; Castillo, E. The effects of the 5-HT$_6$ receptor agonist EMD and the 5-HT$_7$ receptor agonist AS19 on memory formation. *Behav. Brain Res.* **2008**, *195*, 112−119.
(168) Perez-Garcia, G. S.; Meneses, A. Effects of the potential 5-HT$_7$ receptor agonist AS 19 in an autoshaping learning task. *Behav. Brain Res.* **2005**, *163*, 136−140.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX

(169) Meneses, A.; Perez-Garcia, G.; Liy-Salmeron, G.; Ponce-Lopez, T.; Lacivita, E.; Leopoldo, M. 5-HT$_7$ receptor activation: procognitive and antiamnesic effects. *Psychopharmacology (Berl)* **2015**, *232*, 595−603.
(170) Lam, R. W.; Kennedy, S. H.; McIntyre, R. S.; Khullar, A. Cognitive dysfunction in major depressive disorder: effects on psychosocial functioning and implications for treatment. *Can. J. Psychiatry* **2014**, *59*, 649−654.
(171) Dale, E.; Zhang, H.; Leiser, S. C.; Xiao, Y.; Lu, D.; Yang, C. R.; Plath, N.; Sanchez, C. Vortioxetine disinhibits pyramidal cell function and enhances synaptic plasticity in the rat hippocampus. *J. Psychopharmacol.* **2014**, *28*, 891−902.
(172) Waller, J. A.; Chen, F.; Sanchez, C. Vortioxetine promotes maturation of dendritic spines in vitro: a comparative study in hippocampal cultures. *Neuropharmacology* **2016**, *103*, 143−154.
(173) Lacivita, E.; Perrone, R.; Margari, L.; Leopoldo, M. Targets for drug therapy for autism spectrum disorder: challenges and future directions. *J. Med. Chem.* **2017**, *60*, 9114−9141.
(174) Marazziti, D.; Baroni, S.; Pirone, A.; Giannaccini, G.; Betti, L.; Testa, G.; Schmid, L.; Palego, L.; Borsini, F.; Bordi, F.; Piano, I.; Gargini, C.; Castagna, M.; Catena-Dell'Osso, M.; Lucacchini, A. Serotonin receptor of type 6 (5-HT$_6$) in human prefrontal cortex and hippocampus post-mortem: an immunohistochemical and immunofluorescence study. *Neurochem. Int.* **2013**, *62*, 182−188.
(175) Azmitia, E. C.; Singh, J. S.; Whitaker-Azmitia, P. M. Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology* **2011**, *60*, 1347−1354.
(176) Costa, L.; Sardone, L. M.; Lacivita, E.; Leopoldo, M.; Ciranna, L. Novel agonists for serotonin 5-HT$_7$ receptors reverse metabotropic glutamate receptor-mediated long-term depression in the hippocampus of wild-type and Fmr1 KO mice, a model of Fragile X syndrome. *Front. Behav. Neurosci.* **2015**, *9*, 65.
(177) De Filippis, B.; Nativio, P.; Fabbri, A.; Ricceri, L.; Adriani, W.; Lacivita, E.; Leopoldo, M.; Passarelli, F.; Fuso, A.; Laviola, G. Pharmacological stimulation of the brain serotonin receptor 7 as a novel therapeutic approach for Rett syndrome. *Neuropsychopharmacology* **2014**, *39*, 2506−2518.
(178) De Filippis, B.; Chiodi, V.; Adriani, W.; Lacivita, E.; Mallozzi, C.; Leopoldo, M.; Domenici, M. R.; Fuso, A.; Laviola, G. Long-lasting beneficial effects of central serotonin receptor 7 stimulation in female mice modeling Rett syndrome. *Front. Behav. Neurosci.* **2015**, *9*, 86.
(179) Bhakar, A. L.; Dolen, G.; Bear, M. F. The pathophysiology of fragile X (and what it teaches us about synapses). *Annu. Rev. Neurosci.* **2012**, *35*, 417−443.
(180) Collingridge, G. L.; Peineau, S.; Howland, J. G.; Wang, Y. T. Long-term depression in the CNS. *Nat. Rev. Neurosci.* **2010**, *11*, 459−473.
(181) Ricceri, L.; De Filippis, B.; Laviola, G. Rett syndrome treatment in mouse models: searching for effective targets and strategies. *Neuropharmacology* **2013**, *68*, 106−115.
(182) Valenti, D.; de Bari, L.; Vigli, D.; Lacivita, E.; Leopoldo, M.; Laviola, G.; Vacca, R. A.; De Filippis, B. Stimulation of the brain serotonin receptor 7 rescues mitochondrial dysfunction in female mice from two models of Rett syndrome. *Neuropharmacology* **2017**, *121*, 79−88.
(183) Canal, C. E.; Felsing, D. E.; Liu, Y.; Zhu, W.; Wood, J. T.; Perry, C. K.; Vemula, R.; Booth, R. G. An orally active phenylaminotetralin-chemotype serotonin 5-HT$_7$ and 5-HT$_{1A}$ receptor partial agonist that corrects motor stereotypy in mouse models. *ACS Chem. Neurosci.* **2015**, *6*, 1259−1270.
(184) Nikiforuk, A. Selective blockade of 5-HT$_7$ receptors facilitates attentional set-shifting in stressed and control rats. *Behav. Brain Res.* **2012**, *226*, 118−123.
(185) Cantillon, M.; Prakash, A.; Alexander, A.; Ings, R.; Sweitzer, D.; Bhat, L. Dopamine serotonin stabilizer RP5063: a randomized, double-blind, placebo-controlled multicenter trial of safety and efficacy in exacerbation of schizophrenia or schizoaffective disorder. *Schizophr. Res.* **2017**, *189*, 126−133.
(186) Kołaczkowski, M.; Mierzejewski, P.; Bieńkowski, P.; Wesołowska, A.; Newman-Tancredi, A. ADN-1184 a monoaminergic ligand with 5-HT$_{6/7}$ receptor antagonist activity: pharmacological profile and potential therapeutic utility. *Br. J. Pharmacol.* **2014**, *171*, 973−984.
(187) Ivachtchenko, A. V.; Lavrovsky, Y.; Okun, I. AVN-101: A multi-target drug candidate for the treatment of CNS disorders. *J. Alzheimer's Dis.* **2016**, *53*, 583−620.
(188) Impellizzeri, A. A.; Pappalardo, M.; Basile, L.; Manfra, O.; Andressen, K. W.; Krobert, K. A.; Messina, A.; Levy, F. O.; Guccione, S. Identification of essential residues for binding and activation in the human 5-HT$_{7(a)}$ serotonin receptor by molecular modeling and site-directed mutagenesis. *Front. Behav. Neurosci.* **2015**, *9*, 92.
(189) Borroto-Escuela, D. O.; Carlsson, J.; Ambrogini, P.; Narváez, M.; Wydra, K.; Tarakanov, A. O.; Li, X.; Millon, C.; Ferraro, L.; Cuppini, R.; Tanganelli, S.; Liu, F.; Filip, M.; Diaz-Cabiale, Z.; Fuxe, K. Understanding the role of GPCR heteroreceptor complexes in modulating the brain networks in health and disease. *Front. Cell. Neurosci.* **2017**, *11*, 37.
(190) Hubner, H.; Schellhorn, T.; Gienger, M.; Schaab, C.; Kaindl, J.; Leeb, L.; Clark, T.; Möller, D.; Gmeiner, P. Structure-guided development of heterodimer-selective GPCR ligands. *Nat. Commun.* **2016**, *7*, 12298.
(191) Linda, K.; Fiuza, C.; Nadif Kasri, N. The promise of induced pluripotent stem cells for neurodevelopmental disorders. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2018**, *84*, 382−391.
(192) Butzlaff, M.; Ponimaskin, E. The role of serotonin receptors in Alzheimer's disease. *Opera Medica Physiologica* **2016**, *2*, 77−86.

DOI: 10.1021/acs.jmedchem.7b01898
J. Med. Chem. XXXX, XXX, XXX−XXX