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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

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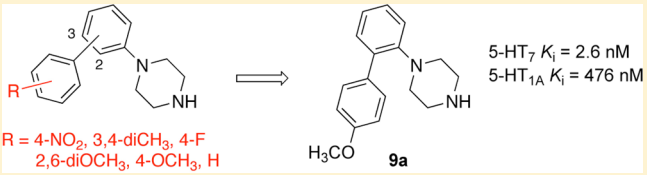
Supporting Information

ABSTRACT: Here we report the design, synthesis, and 5-HT$_7$ receptor affinity of a set of 1-(3-biphenyl)- and 1-(2-biphenyl)piperazines. The effect on 5-HT$_7$ affinity of various substituents on the second (distal) phenyl ring was analyzed. Several compounds showed 5-HT$_7$ affinities in the nanomolar range and >100-fold selectivity over 5-HT$_{1A}$ and adrenergic α_1 receptors. 1-[2-(4-Methoxyphenyl)phenyl]piperazine (**9a**) showed 5-HT$_7$ agonist properties in a guinea pig ileum assay but blocked 5-HT-mediated cAMP accumulation in 5-HT$_7$-expressing HeLa cells.



INTRODUCTION

The serotonin 5-HT$_7$ receptor was identified starting in 1993 by the application of targeted molecular biology techniques. It has been described in various species and remains the last 5-HT receptor to be discovered. The 5-HT$_7$ receptor is localized in discrete areas of the brain and in the periphery. Within the central nervous system (CNS), high levels of this receptor have been detected in the hippocampus, thalamus, and hypothalamus (especially within the suprachiasmatic nucleus).[1] Nearly two decades after its discovery, much information is available on the pathophysiological role of 5-HT$_7$ in the CNS.[2]

Pharmacological blockade of the 5-HT$_7$ receptor by **1** (SB-269970) (Figure 1) or inactivation of the 5-HT$_7$ receptor gene leads to an antidepressant-like behavioral profile in rodent forced swim test and tail suspension test. It has also been suggested that the atypical antipsychotic drug amisulpride exerts its antidepressant action through blockade of 5-HT$_7$ receptors.[3] Recently, a study showed that the pharmacological blockade of 5-HT$_7$ receptors produced a faster antidepressant-like response than the commonly prescribed antidepressant fluoxetine.[4] This is particularly interesting, since antidepressants with a faster onset of action are an unmet need in depression therapy.

Several studies have suggested that 5-HT$_7$ receptors are involved in nociceptive processing. Activation of 5-HT$_7$ receptors exerts antinociceptive effects at the level of the spinal cord and pronociceptive effects in the periphery.[5] Moreover,

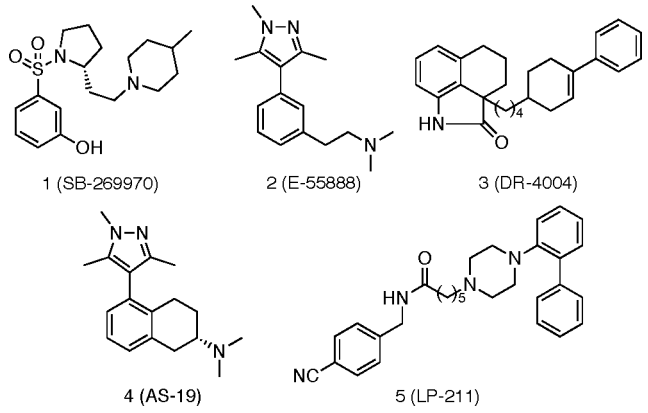


Figure 1. Structures of selective 5-HT$_7$ receptor agents.

since subcutaneous administration of the selective 5-HT$_7$ receptor agonist **2** (E-55888) increased the analgesic potency of oral morphine, it has been proposed that 5-HT$_7$ receptor agonists could be used as adjuvants of opioid analgesia.[6]

Animal studies of learning and memory have suggested a potentially important role for the 5-HT$_7$ receptor in cognitive processes.[7] 5-HT$_7$ drugs such as **1**, **3** (DR-4004), and **4** (AS-19) (Figure 1) are able to reverse amnesia induced by post-

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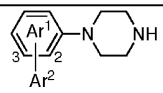
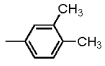
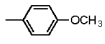
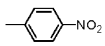
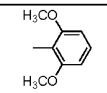
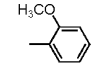
training administration of scopolamine (a cholinergic antagonist). This supports the hypothesis that cholinergic, glutamatergic, and serotonergic systems interact in cognitively impaired animals. Moreover, it shows that the 5-HT$_7$ receptor can significantly influence cognitive dysfunction and therefore represents a potential therapeutic target for the treatment of memory dysfunction in cognitive disorders (schizophrenia, Alzheimer's disease, age-related decline).[8] Collectively, the aforementioned findings underscore the importance of developing new 5-HT$_7$ receptor drugs.

Our research group has been involved for several years in the study of structure–activity relationships (SARs) of 4-substituted 1-arylpiperazine derivatives, the so-called "long-chain" arylpiperazines.[9−13] Our studies have led to the identification of **5** (LP-211) (Figure 1) which showed high 5-HT$_7$ receptor affinity (K_i = 0.58 nM, rat cloned receptors), high selectivity over 5-HT$_{1A}$ and D$_2$ receptors (324- and 245-fold, respectively), and agonist properties in an ex vivo assay of 5-HT$_7$ receptor activation. Disposition studies in mice evidenced that **5** undergoes N-dealkylation to **6a** (Table 1). This might be of relevance because N-unsubstituted 1-arylpiperazines can reach the brain and may display pharmacological effects opposite to those of their parent drugs. Therefore, the final pharmacological effect of such drugs might result from the interplay between the neurochemical actions of the parent drug and of its active metabolite.[14] For this reason, the affinities for a range of 5-HT receptors of **6a** and **5** were evaluated under the same experimental conditions. Compound **6a** displayed higher 5-HT$_7$ receptor affinity (K_i = 1.4 nM, human cloned receptor) and overall better selectivity profile than **5** (K_i = 15 nM, human cloned receptor).[15] This was unexpected because the majority of the long-chain arylpiperazine derivatives described in the literature display higher 5-HT$_7$ receptor affinity than the N-(4)-unsubstituted counterparts.[11,16,17] Thus, compound **6a** attracted our attention as a low molecular weight lead compound that could deliver a completely new set of selective 5-HT$_7$ receptor ligands.

Table 1. Binding Affinities of Target Compounds



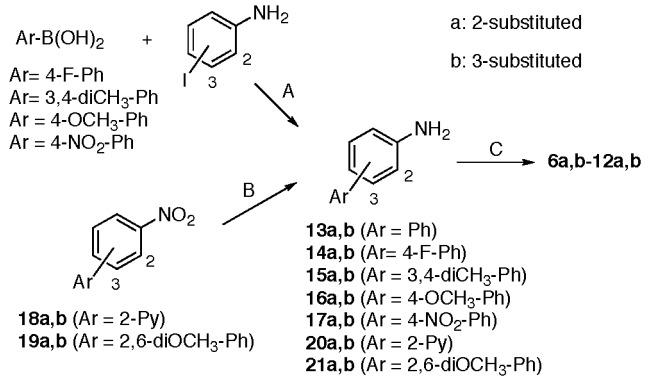
			K_i, nM ± S.E.M.[a]		
compd	pos	Ar^2	5-HT$_7$	5-HT$_{1A}$	α_1
6a	2		1.4[b]	99[b]	59.1 ± 2.5
6b	3		25 ± 1.7	72.1 ± 3.3	(21%)[c]
7a	2		2.7 ± 1.5	(15%)[d]	(14%)[c]
7b	3		109 ± 64	63.2 ± 3.1	(3%)[c]
8a	2		1.9 ± 0.6	(22%)[d]	165 ± 2
8b	3		93 ± 2	(34%)[d]	(7%)[c]
9a	2		2.6 ± 0.1	476 ± 3	156 ± 2
9b	3		> 5000	(42%)[d]	(29%)[c]
10a	2		7.0 ± 1.2	(16%)[d]	105 ± 2
10b	3		13 ± 4	31.4 ± 2.3	(8%)[c]
11a	2		23 ± 6	(23%)[d]	(11%)[c]
11b	3		80 ± 4	14.9 ± 2.3	(27%)[c]
12a	2		96 ± 11	(21%)[d]	(10%)[c]
12b	3		7.1 ± 1.7	149 ± 2	(3%)[c]
22a	2		18.4 ± 6.5	--	--
22b	3		8.2 ± 1.9	--	--
23a	2		6.9 ± 0.3	--	--
23b	3		1.4 ± 0.1	17.4 ± 1.5	--
5-CT			0.3 ± 0.1	--	--
5-HT			--	10 ± 1.5	
Phentolamine			--	--	16 ± 2

[a]Values are the mean ± SEM from three independent experiments. [b]Data taken from ref 15. [c]Displacement of [^{3}H]prazosin from rat cerebral cortex membranes by a single concentration of test compound (100 nM). Data are the mean of two independent experiments. [d]Displacement of [^{3}H]-8-OH-DPAT from human cloned 5-HT$_{1A}$ receptors by a single concentration of test compound (100 nM). Data are the mean of two independent experiments.

CHEMISTRY

The synthesis of the target 1-arylpiperazine derivatives **6a,b**–**12a,b** (Table 1) required the key aniline intermediates **13a,b**–**17a,b**, **20a,b**, and **21a,b** (Scheme 1). Among these, **13a,b** were commercially available, whereas the remaining anilines where prepared according to literature methods by cross-coupling reaction of 2- or 3-iodoaniline and the appropriate benzeneboronic acid under Suzuki conditions (**14a,b**–**17a,b**) or through catalytic reduction of nitroderivatives **18a,b** and **19a,b** (anilines **20a,b** and **21a,b**). The target compounds **6a,b**–**12a,b** were obtained by condensing the intermediate anilines **13a,b**–**17a,b**, **20a,b**, and **21a,b** with bis(2-chloroethyl)amine hydrochloride.

Scheme 1. Synthesis of Target Compounds 6a,b–12a,b[a]



[a]Reagents: (A) Pd(dppf)Cl$_2$ and 5 N NaOH or tetrakis-(triphenylphosphino)palladium(0) and 2 M Na$_2$CO$_3$; (B) ammonium formate, 10% Pd/C; (C) bis(2-chloroethyl)amine hydrochloride, K$_2$CO$_3$, KI.

RESULTS AND DISCUSSION

Structure–Activity Relationships for 5-HT$_7$ Receptor. The notion that N-(4)-unsubstituted 1-arylpiperazines can bind to the 5-HT$_7$ receptor was reported by Shen et al. in 1993.[18] In particular, it was shown that 1-(1-naphthalenyl)piperazine, 1-(2-methoxyphenyl)piperazine, and 1-(3-chlorophenyl)-piperazine can bind at 5-HT$_7$ receptors with moderate to good affinities (K_i = 83 nM, 243 nM, and 352 nM, respectively). Over the years, the above-mentioned 1-arylpiperazines were used as starting points for the development of several classes of long-chain arylpiperazine derivatives.

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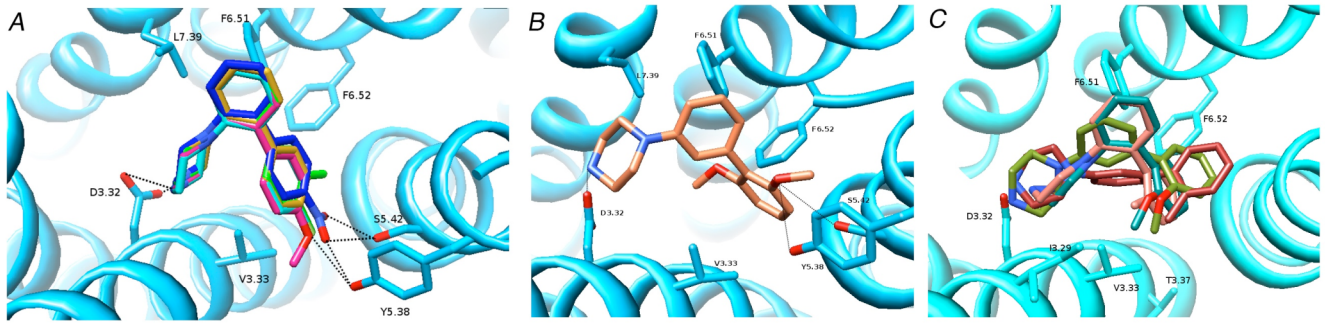

Figure 2. (A) Main interactions of **6a** (yellow), **7a** (cyan), **8a** (green), **9a** (magenta), and **10a** (blue) within the 5-HT$_7$ receptor binding site. The protonated piperazine N(4) nitrogen forms an ionic interaction with D3.32 at the distance of 2.57 Å. The oxygen of methoxy group of **9a** forms hydrogen-bond contacts with S5.42 and Y5.38 at distances of 3.4 and 3.1 Å, respectively. The same pattern of interactions is shown by **7a** and **10a.** (B) Main interactions of **12b** within the 5-HT$_7$ receptor binding site. (C) Main interactions of **22a,b** and **23a,b** within the 5-HT$_7$ receptor binding site.

In many cases the introduction of a large substituent on the basic nitrogen of the piperazine ring had a beneficial effect on the affinity and specificity for the 5-HT$_7$ receptor.[11,16,17] Therefore, it was unexpected that removal of the pendant substituent from the basic nitrogen of **5** afforded the potent and selective 5-HT$_7$ ligand **6a**. Since no studies dealing with N-(4)-unsubstituted 1-arylpiperazines as 5-HT$_7$ receptor ligands can be found in the literature, we decided to explore the SARs of a set of compounds structurally related to **6a**. For our study, we have evaluated the 1-(2-biphenyl)piperazine derivatives **6a**–**12a**, focusing on the aromatic ring denoted as Ar^2 in the general structure reported in Table 1.

In particular, we wanted to explore if introduction of substituent(s) that could modify the electronic properties or the steric hindrance of the Ar^2 group had an effect on 5-HT$_7$ receptor affinity. We also evaluated the affinity for the 5-HT$_7$ receptor of the corresponding 1-(3-biphenyl)piperazine counterparts **6b**–**12b**. The 1-(4-biphenyl)piperazine framework was not taken into consideration at this stage because a previous study showed that 1-(4-substituted-phenyl)piperazines were nearly devoid of 5-HT$_7$ receptor affinity.[11] The binding affinity values of the target compounds at the 5-HT$_7$ receptor are shown in Table 1. In comparison of the 5-HT$_7$ affinity values of **6a** (Ar^2 = phenyl), **7a** (Ar^2 = 4-fluorophenyl), **8a** (Ar^2 = 3,4-dimethylphenyl), **9a** (Ar^2 = 4-methoxyphenyl), and **10a** (Ar^2 = 4-nitrophenyl), no great differences can be noted. This indicates that there is no preference between electron-rich and electron-poor aromatic rings in that position. Introduction of an aza group in **6a** afforded compound **11a** (Ar^2 = 2-pyridyl), causing a >10-fold loss in affinity. The presence of substituents in different positions of the Ar^2 ring had different effects on 5-HT$_7$ receptor affinity. In particular, **8a** (Ar^2 = 3,4-dimethylphenyl) and **9a** (Ar^2 = 4-methoxyphenyl) displayed affinities in the same range as **6a**, suggesting that the binding pocket of the receptor is large enough to accommodate such substituents. By contrast, a marked decrease in affinity was observed in the case of **12a** (Ar^2 = 2,6-dimethoxyphenyl). Next, we evaluated the effect on 5-HT$_7$ receptor affinity of shifting the Ar^2 ring from 2- to 3-position (derivatives **6b**–**12b**). This modification reduced the 5-HT$_7$ receptor affinity (except for **12b**) to various extents: compound **10b** showed only half the affinity of its isomer **10a**, whereas **9b** was devoid of 5-HT$_7$ affinity (K_i > 5000 nM), contrary to the isomer **9a** (K_i = 2.6 nM). A notable exception to this trend was derivative **12b** (K_i = 7.1 nM), which has higher affinity than its isomer **12a** (K_i = 96 nM).

To rationalize the SARs, we developed a pharmacophore model (see Supporting Information). Matrix distances between the pharmacophoric features (i.e., a positively charged nitrogen atom and two aromatic rings that corresponded to Ar^1 and Ar^2) were in general agreement with those reported in the pharmacophore models by Kolaczkowski et al.[19] (the general "affinity" hypothesis) and by Badarau et al.[20] (the "second hypothesis"). The analysis of aligned target compounds clearly indicated that the pharmacophoric groups of compounds **6a**–**12a** matched the features of the pharmacophore model more closely than those of compounds **6b**–**12b** (Figure SI3, Supporting Information). Next, docking simulations were conducted by use of the Autodock4 program[21] to analyze the binding mode of **6a,b**–**12a,b.** Kolaczkowski et al.[19] defined the 5-HT$_7$ receptor binding site as constituted by the interaction point of the positively charged nitrogen atom and two pockets: one localized between transmembrane helices (TMHs) 4–6 in a deep cavity and the other one between TMHs 7–3 in the extracellular exposed area. According to our docking studies, the compounds reported in Table 1 preserve the crucial ionic interaction between protonated piperazine N(4) nitrogen with D3.32 (according to Ballesteros and Weinstein)[22] and the CH–π interaction between the phenyl ring attached to the piperazine ring (Ar^1) with F6.51 (Figure 2A). Moreover, the compounds with the highest affinities also showed CH–π interaction between Ar^2 and F6.52. The binding mode analysis suggested that the TMH 4–6 pocket is not large enough to accommodate the Ar^2 group when it is in the 3-position of Ar^1, thus explaining the lower affinity of **6b**–**11b** as compared to **6a**–**11a**. In particular, the conformations proposed for **9b** do not allow the crucial ionic interaction with D3.32. On the other hand, a different binding scenario could be hypothesized for compound **12b**: the methoxy group formed H-bonding interactions with Y5.38 and S5.42 that drove Ar^2 into a hydrophobic pocket formed by I3.29, V3.33, and F6.52 (Figure 2B). Consequently, the structure-based conformation of **12b** showed that the orientation and distances of the essential triplet features were comparable with those observed for the compounds with highest affinity. To check this further, we have studied an additional small set of compounds having a 2-methoxy- or a 2-methyl group on Ar^2, with Ar^2 in either 2- or 3-position on Ar^1 (compounds **22a,b** and **23a,b**, Table 1). These compounds were prepared following the synthetic routes

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depicted in Scheme 1 (see Supporting Information). As in the case of compounds **12a,b**, 1-(3-biphenyl)piperazine derivatives **22b** and **23b** showed higher 5-HT$_7$ receptor affinity than the 1-(2-biphenyl) counterparts **22a** and **23a**, but in this case, a small difference was shown. Docking analysis suggested that the methoxy or methyl substituent in 2-position of compounds **22a,b** and **23a,b** interacts with the pocket created by I3.29, V3.33, M3.34, T3.37, and I4.37 residues and allows optimal interaction of Ar^1 and Ar^2 with F6.51 and F6.52, respectively (Figure 2C). Therefore, affinity data and docking analysis of compounds **12a,b**, **22a,b**, and **23a,b** indicate that the introduction of small substituents in one or both ortho-positions of Ar^2 enables the molecules to adopt the optimal conformation for interaction through hydrophobic or hydrogen-bonding interactions. All in all, the binding mode analysis evidenced that the interaction of biphenyl piperazines with the 5-HT$_7$ receptor is driven essentially by steric and hydrophobic requirements. In the case of compound **12b**, the hydrogen-bond formation with Y5.38 and S5.42 is fundamental for the optimal orientation of Ar^2.

Selectivity over 5-HT$_{1A}$ and Adrenergic α_1 Receptors. Target compounds were counterscreened for their affinity against the serotonin 5-HT$_{1A}$ receptor because it is colocalized with the 5-HT$_7$ receptor. Also, target compounds were evaluated for their adrenergic α_1 receptor affinity because it has been reported that the 1-(2-biphenyl)piperazinyl scaffold might have affinity for this receptor.[23] As far as the affinity for 5-HT$_{1A}$ receptors is concerned, all the 2-biphenyl derivatives displayed poor affinities except **9a** (Ar = 4-methoxyphenyl, K_i = 476 nM). On the other hand, the 3-biphenyl derivatives showed a different trend. Compounds **6b**, **7b**, **10b**, and **11b** displayed good 5-HT$_{1A}$ receptor affinity (72.1 nM < K_i < 14.9 nM), differently from **8b** and **9b**. With regard to the adrenergic α_1 receptor, all compounds showed weak affinities, except **6a** and **10a** (K_i = 59.1 nM and 105 nM, respectively). Taken together, these data indicated that the 1-(2-biphenyl)piperazine motif can deliver high-affinity 5-HT$_7$ ligands and that an appropriate substitution pattern around the Ar^2 ring can lead to selective 5-HT$_7$ receptor ligands.

Activation of 5-HT$_7$ Receptors in Guinea Pig Ileum. 5-HT$_7$ receptor activation is known to mediate relaxation of gastrointestinal smooth muscle. Previous studies have demonstrated that activation of 5-HT$_7$ receptors inhibits contractions induced by substance P in the guinea pig ileum.[24] We investigated the effect of compounds **6a**–**9a**, which showed the highest 5-HT$_7$ receptor affinity and selectivity values among the newly reported compounds, on substance P-induced contractions in the guinea pig ileum. The assays were conducted in the presence of a cocktail of non-5-HT$_7$ receptor antagonists (see Supporting Information) in order to elucidate whether or not the relaxation response in this particular tissue is due to direct activation of the 5-HT$_7$ receptor. For comparative purposes, we also tested **4**, a selective 5-HT$_7$ ligand that behaves as a potent (EC_{50} = 9 nM) partial 5-HT$_7$ receptor agonist (77% maximal effect compared to 5-HT) toward cAMP accumulation in HEK-293F/h5-HT$_7$ cells.[25] As already reported,[11] the 5-HT$_7$ agonist 5-CT was able to reduce 40% of the contraction induced by substance P, an effect that was competitively reverted by the antagonist **1**. Compounds **6a**–**9a** were able to induce relaxation of substance P-mediated contraction (Figure 3). However, the effects elicited by **6a** (0.5 μM and 1 μM) and **8a** (2 μM) were not 5-HT$_7$ receptor-mediated because they were not reverted by **1** (3 μM). On the other hand, **7a** and **9a** were able to induce 5-HT$_7$ receptor-mediated relaxation at concentrations of 0.5 μM and 1 μM, because the effects were abolished by **1** (3 μM). Finally, **4** induced relaxation at concentrations of 1 μM and 2 μM but not at 0.5 μM (data not shown), the effects being reverted by **1** (3 μM). All in all, these data indicate that compounds **7a** and **9a** behave as 5-HT$_7$ competitive agonists in this assay with specificity as good as **4**, contrary to **6a** and **8b**.

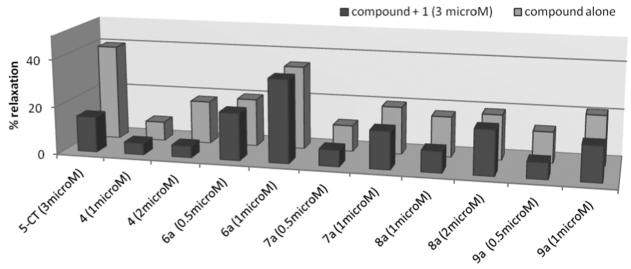

Figure 3. Percent relaxation of substance P-mediated contraction induced by 5-HT$_7$ agonists in isolated guinea pig ileum.

Activity at 5-HT$_7$ Receptors Stably Expressed in HeLa Cells. Compounds **6a** and **9a** were active in the guinea pig ileum assay, but the effect of **6a** appeared not to be specific to 5-HT$_7$ (no blockade with **1**). To further characterize their function, we therefore tested both compounds against 5-HT$_{7(a)}$ receptors stably expressed in HeLa cells. Neither **6a** nor **9a** stimulated cAMP accumulation (data not shown), and both compounds behaved as antagonists instead. Thus, cAMP accumulation induced by 1 μM 5-HT was inhibited by **6a** and **9a** with IC_{50} values of 531 and 438 nM, respectively (Figure 4A). To confirm their inhibitory action at recombinant 5-HT$_{7(a)}$, the compounds were compared with established receptor antagonists regarding their capacity to block forskolin-induced cAMP. Similar to **1**, clozapine, and ketanserin, **6a** and **9a** significantly inhibited cAMP accumulation, thus providing further evidence for antagonist effects, at least in the context of a heterologous system (Figure 4B).

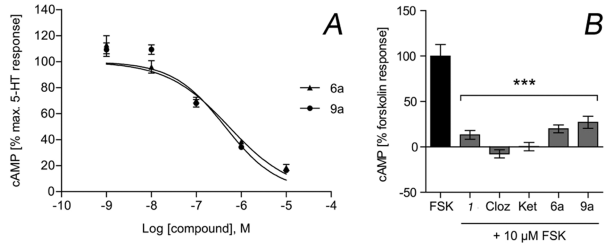

Figure 4. Effects of compounds **6a** and **9a** on (A) 5-HT- and (B) forskolin-induced cAMP accumulation in vitro.

CONCLUSIONS

The main aim of the present study was exploration of the SARs of a set of 1-(2-biphenyl)- and 1-(3-biphenyl)piperazines, toward the goal of identifying new high-affinity 5-HT$_7$ ligands. This goal was achieved, as several compounds with K_is in the low nanomolar range were identified. The SARs showed that various substituents can be introduced in the distal phenyl ring of the 1-(2-biphenyl)piperazine scaffold without affecting 5-HT$_7$ receptor affinity. On the other hand, the 1-(3-biphenyl)-piperazine scaffold appears to be more sensitive to the presence and position of substituents. Considering the selectivity over 5-

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HT_{1A} and α_1 receptors, it is noteworthy that, despite their low molecular weight, several ligands herein reported showed affinities 2 orders of magnitude lower than those at 5-HT_7 receptors. All in all, affinity and computational data indicate that both 1-(2-biphenyl)- and 1-(3-biphenyl)piperazine scaffolds can deliver high-affinity 5-HT_7 ligands or can be starting points for development of newer long-chain arylpiperazines capable of binding at 5-HT_7 receptors.

Investigation of signaling through the 5-HT_7 receptor yielded interesting results. While compounds **9a** and **10a** induced relaxation of guinea pig ileum in the same fashion as 5-CT and the selective 5-HT_7 receptor agonist **4**, compounds **6a** and **9a** failed to stimulate cAMP accumulation in 5-HT_{7a}-expressing HeLa cells. Instead, inhibition was observed when cells were cotreated with 5-HT and **6a** or **9a**. Moreover, similar to established antagonists of 5-HT_7,[26] both compounds inhibited forskolin-stimulated cAMP. Thus, at least in the context of our heterologous system, **6a** and **9a** displayed antagonist properties.

It has previously been shown that a given 5-HT receptor ligand can act as agonist in one and antagonist in another receptor-expressing system. For example, the 5-HT_4 ligand 4-amino-5-chloro-2-methoxybenzoic acid 2-(1-piperidinyl)ethyl ester (ML 10302) is a partial agonist in guinea pig ileum but an antagonist of cAMP accumulation in guinea pig hippocampus.[27] Similarly, partial agonist as well as antagonist properties at central 5-HT_{1A} receptors have been described for 8-{2-[4-(methoxyphenyl)-1-piperazinyl]ethyl}-8-azaspiro[4.5]decane-7,9-dione (BMY 7378).[28] Our present results demonstrate that dual agonist/antagonist ligands also exist for the 5-HT_7 receptor, exemplified by compound **9a**. In light of this finding, we believe that the reinvestigation of agonists and antagonists previously published by our laboratory and others will tell us if the dual agonist/antagonist feature of **9a** is shared by other 5-HT_7 ligands. This might ultimately help to explain the observed inconsistencies in the role of 5-HT_7 receptors in the CNS.[8]

EXPERIMENTAL SECTION

General Procedure for the Preparation of Piperazines 6a,b−12a,b, 22a,b, and 23a,b. A mixture of the appropriate aniline (4.0 mmol), bis(2-chloroethyl)amine hydrochloride (0.71 g, 4.0 mmol), K_2CO_3 (0.55 g, 4.0 mmol), and KI (0.66 g, 4.0 mmol) in xylene (30 mL) was refluxed for 48 h. After cooling, the solvent was distilled off in vacuo and the residue was taken up with AcOEt (30 mL) and 5% aqueous NaOH solution (30 mL). The organic phase was separated and then washed with brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was chromatographed with $CHCl_3$/MeOH, 9:1, to afford the desired compounds as pale yellow oil. The purity of the tested compounds **6a,b−12a,b**, **22a,b**, and **23a,b** was assessed by RP-HPLC and combustion analysis. All compounds showed ≥95% purity.

1-[2-(4-Methoxyphenyl)phenyl]piperazine (9a). Yield 30%. ^{1}H NMR ($CDCl_3$) δ 1.71 (br s, 1H, D_2O exchanged), 3.12 (br s, 8H), 3.84 (s, 3H), 6.94 (d, 2H, J = 8.8 Hz), 7.00 (d, 1H, J = 8.0 Hz), 7.10−7.15 (m, 1H), 7.22−7.30 (m, 2H), 7.46 (d, 2H, J = 8.8 Hz). GC-MS m/z 269 (M^+ + 1, 11), 268 (M^+, 56), 226 (100), 210 (43), 167 (24). The hydrochloride salt melted at 198−200 °C (from MeOH/Et_2O). Anal. ($C_{17}H_{20}N_2O$·HCl·0.5 H_2O) C, H, N.

Radioligand Binding Assays. Binding of [^{3}H]-5-CT at human cloned 5-HT_7 receptor was performed according to Jasper et al.[29] Binding of [^{3}H]-8-OH-DPAT at human cloned 5-HT_{1A} receptors was performed as previously described.[30] Binding of [^{3}H]prazosin at α_1-adrenoceptors was performed according to Glossmann and Hornung.[31]

Isolated Guinea Pig Ileum Assay. Inhibition of substance P-induced contraction in guinea pig ileum by 5-HT_7 receptor agonists was performed as previously described.[11]

Measurement of Intracellular cAMP Accumulation. HeLa cells stably expressing human 5-$HT_{7(a)}$ (kindly provided by Dr. Mark Hamblin) were cultured as described[32] and seeded into 96-well plates before treatment. Intracellular cAMP was measured by a commercial enzyme-linked immunosorbent assay (ELISA; Abnova, Taipei City, Taiwan).

ASSOCIATED CONTENT

Supporting Information

Additional text, three figures, and two tables with synthetic details and spectral data for **6a,b−12a,b**, **15a,b**, **17a,b**, and **20a,b−23a,b**; elemental analysis of **6a,b−12a,b**, **22a,b**, and **23a,b**; matrix distances; molecular modeling and molecular docking methods; PHASE alignment analysis; training set for pharmacophore model identification; list of inactive compounds; and biological methods and statistical analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Hedlund, P. B.; Sutcliffe, J. G. Functional, molecular and pharmacological advances in 5-HT_7 receptor research. *Trends Pharmacol. Sci.* **2004**, *25*, 481−486.

(2) 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.

(3) 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 (Berlin)* **2009**, *205*, 119−128.

(4) Mnie-Filali, O.; Faure, C.; Lambás-Señas, L.; El Mansari, M.; Belblidia, H.; Gondard, E.; Etiévant, 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.

(5) Brenchat, A.; Zamanillo, D.; Hamon, M.; Romero, L.; Vela, J. M. Role of peripheral versus spinal 5-HT_7 receptors in the modulation of pain undersensitizing conditions. *Eur. J. Pain* **2012**, *16*, 72−81.

(6) Brenchat, A.; Ejarque, M.; Zamanillo, D.; Vela, J. M.; Romero, L. Potentiation of morphine analgesia by adjuvant activation of 5-HT_7 receptors. *J. Pharmacol. Sci.* **2011**, *116*, 388−391.

(7) Roberts, A. J.; Hedlund, P. B. The 5-HT_7 receptor in learning and memory. *Hippocampus* **2012**, *22*, 762−771.

(8) Matthys, A.; Haegeman, G.; Van Craenenbroeck, K.; Vanhoenacker, P. Role of the 5-HT_7 receptor in the central nervous system: from current status to future perspectives. *Mol. Neurobiol.* **2011**, *43*, 228−253.

(9) Perrone, R.; Berardi, F.; Colabufo, N. A.; Lacivita, E.; Leopoldo, M.; Tortorella, V. Synthesis and structure−affinity relationships of 1-[omega-(4-aryl-1-piperazinyl)alkyl]-1-aryl ketones as 5-HT_7 receptor ligands. *J. Med. Chem.* **2003**, *46*, 646−649.
(10) 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.
(11) 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-hydroxytryptamine$_7$ receptor agents. *J. Med. Chem.* **2004**, *47*, 6616−6624.
(12) Leopoldo, M.; Lacivita, E.; Contino, M.; Colabufo, N. A.; Berardi, F.; Perrone, R. Structure−activity relationship study on *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinehexanamides, a class of 5-HT_7 receptor agents. 2. *J. Med. Chem.* **2007**, *50*, 4214−4221.
(13) 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.
(14) Caccia, S. N-dealkylation of arylpiperazine derivatives: disposition and metabolism of the 1-aryl-piperazines formed. *Curr. Drug Metab.* **2007**, *8*, 612−622.
(15) 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.
(16) Medina, R. A.; Sallander, J.; Benhamú, B.; Porras, E.; Campillo, M.; Pardo, L.; López-Rodríguez, 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.
(17) Volk, B.; Barkóczy, J.; Hegedus, E.; Udvari, S.; Gacsályi, I.; Mezei, T.; Pallagi, K.; Kompagne, H.; Lévay, G.; Egyed, A.; Hársing, L. G.; Spedding, M.; Simig, G. (Phenylpiperazinyl-butyl)oxindoles as selective 5-HT_7 receptor antagonists. *J. Med. Chem.* **2008**, *51*, 2522−2532.
(18) Shen, Y.; Monsma, F. J., Jr.; Metcalf, M. A.; Jose, P. A.; Hamblin, M. W.; Sibley, D. R. Molecular cloning and expression of a 5-hydroxytryptamine$_7$ serotonin receptor subtype. *J. Biol. Chem.* **1993**, *268*, 18200−18204.
(19) Kołaczkowski, M.; Nowak, M.; Pawłowski, M.; Bojarski, A. J. Receptor-based pharmacophores for serotonin 5-HT_7R antagonists—implications to selectivity. *J. Med. Chem.* **2006**, *49*, 6732−6741.
(20) Badarau, E.; Bugno, R.; Suzenet, F.; Bojarski, A. J.; Finaru, A. L.; Guillaumet, G. SAR studies on new bis-aryls 5-HT7 ligands: Synthesis and molecular modeling. *Bioorg. Med. Chem.* **2010**, *18*, 1958−1967.
(21) Goodsell, D. S.; Morris, G. M.; Olson, A. J. Automated docking of flexible ligands: applications of AutoDock. *J. Mol. Recognit.* **1996**, *9*, 1−5.
(22) Ballesteros, J. A.; Weinstein, H. Integrated methods for modeling G-protein coupled receptors. *Methods Neurosci.* **1995**, *25*, 366−428.
(23) Elworthy, T. R.; Ford, A. P.; Bantle, G. W.; Morgans, D. J.; Ozer, R. S.; Palmer, W. S.; Repke, D. B.; Romero, M.; Sandoval, L.; Sjogren, E. B.; Talamás, F. X.; Vazquez, A.; Wu, H.; Arredondo, N. F.; Blue, D. R.; De Sousa, A.; Gross, L. M.; Kava, M. S.; Lesnick, J. D.; Vimont, R. L.; Williams, T. J.; Zhu, Q. M.; Pfister, J. R.; Clarke, D. E. *N*-arylpiperazinyl-*N*′-propylamino derivatives of heteroaryl amides as functional uroselective alpha 1-adrenoceptor antagonists. *J. Med. Chem.* **1997**, *40*, 2674−2687.
(24) Carter, D.; Champney, M.; Hwang, B.; Eglen, R. M. Characterization of a postjunctional 5-HT receptor mediating relaxation of guinea-pig isolated ileum. *Eur. J. Pharmacol.* **1995**, *280*, 243−250.
(25) Brenchat, A.; Romero, L.; García, M.; Pujol, M.; Burgueño, 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.
(26) Klein, M. T.; Teitler, M. Antagonist interaction with the human 5-HT_7 receptor mediates the rapid and potent inhibition of non-G-protein-stimulated adenylate cyclase activity: a novel GPCR effect. *Br. J. Pharmacol.* **2011**, *162*, 1843−1854.
(27) Hegde, S. S.; Eglen, R. M. Peripheral 5-HT_4 receptors. *FASEB J.* **1996**, *10*, 1398−1407.
(28) Sharp, T.; Backus, L. I.; Hjorth, S.; Bramwell, S. R.; Grahame-Smith, D. G. Further investigation of the in vivo pharmacological properties of the putative 5-HT_{1A} antagonist, BMY 7378. *Eur. J. Pharmacol.* **1990**, *176*, 331−340.
(29) Jasper, J. R.; Kosaka, A.; To, Z. P.; Chang, D. J.; Eglen, R. M. Cloning, expression and pharmacology of a truncated splice variant of the human 5-HT_7 receptor (h5-HT_{7b}). *Br. J. Pharmacol.* **1997**, *122*, 126−132.
(30) Fargin, A.; Raymond, J. R.; Regan, J. W.; Cotecchia, S.; Lefkowitz, R. J.; Caron, M. G. Effector coupling mechanisms of the cloned 5-HT_{1A} receptor. *J. Biol. Chem.* **1989**, *264*, 14848−14852.
(31) Glossmann, H.; Hornung, R. alpha-Adrenoceptors in rat brain: sodium changes the affinity of agonists for prazosin sites. *Eur. J. Pharmacol.* **1980**, *61*, 407−408.
(32) Sjögren, B.; Hamblin, M. W.; Svenningsson, P. Cholesterol depletion reduces serotonin binding and signaling via human 5-HT_{7a} receptors. *Eur. J. Pharmacol.* **2006**, *552*, 1−10.

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