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Research paper

5-HT$_7$ receptor modulators: Amino groups attached to biphenyl scaffold determine functional activity

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ABSTRACT

5-HT$_7$ receptor (5-HT$_7$R) agonists and antagonists have been reported to be used for treatment of neuropathic pain and depression, respectively. In this study, as a novel scaffold for 5-HT$_7$R modulators, we designed and prepared a series of biphenyl-3-yl-methanamine derivatives with various amino groups. Evaluation of functional activities as well as binding affinities of the title compounds identified partial agonists (EC_{50} = 0.55–3.2 μM) and full antagonists (IC_{50} = 5.57–23.1 μM) depending on the amino substituents. Molecular docking study suggested that the ligand-based switch in functional activity from agonist to antagonist results from the size of the amino groups and thereby different binding modes to 5-HT$_7$R. In particular, interaction of the ligand with Arg367 of 5-HT$_7$R is shown to differentiate agonists and antagonists. In the pharmacophore model study, two distinct pharmacophore models can tell whether a ligand is an agonist or an antagonist. Taken together, this study provides valuable information for designing novel compounds with selective agonistic or antagonistic properties against 5-HT$_7$R.

1. Introduction

A neurotransmitter serotonin (5-HT, 5-hydroxytryptamine) interacts with seven different subtypes of serotonin receptors (5-HT$_1$R ~ 5-HT$_7$R) [3] to affect central and peripheral nervous systems, and this endogenous molecule is known to be involved in cardiovascular, digestive and psychiatric disorders [1,2]. 5-HT$_7$ receptor (5-HT$_7$R), a G-protein-coupled receptor, is positively associated with adenylyl cyclase [4–6], and its pathophysiological roles are known to be determined by localization [7]. In particular, 5-HT$_7$R mRNA was detected in central nervous systems including thalamus, hippocampus, hypothalamus and suprachiasmatic nucleus. Thus, the potential role of 5-HT$_7$R has been anticipated in control of circadian rhythms, sleep, cognitive processes and neurological disorders such as pain, migraine, depression and anxiety [8,9], which promoted drug discovery research targeting against 5-HT$_7$R modulators. Interestingly, recently discovered 5-HT$_7$R agonist (AS-19, Fig. 1) and antagonist (SB-269970, Fig. 1) showed different pharmacological profiles. While 5-HT$_7$R agonist AS-19 significantly inhibited mechanical hypersensitivity and thermal hyperalgesia in the neuropathic pain animal model [10], 5-HT$_7$R antagonist SB-269970 showed antidepressant-like activity as well as fast onset of depression treatment effects in olfactory bulbectomized rats (OBX) [11]. Therefore, 5-HT$_7$R modulators with high affinity and specific functional characteristics are promising drug candidates for treatment of neurologic and mental disorders. However, due to the limited number of 5-HT$_7$R-selective ligands discovered to date (Fig. 1) and thereby the lack of structure-function relationship of the 5-HT$_7$R ligands, there have been a number of difficulties to rationally design selective agonists or antagonists of 5-HT$_7$R.

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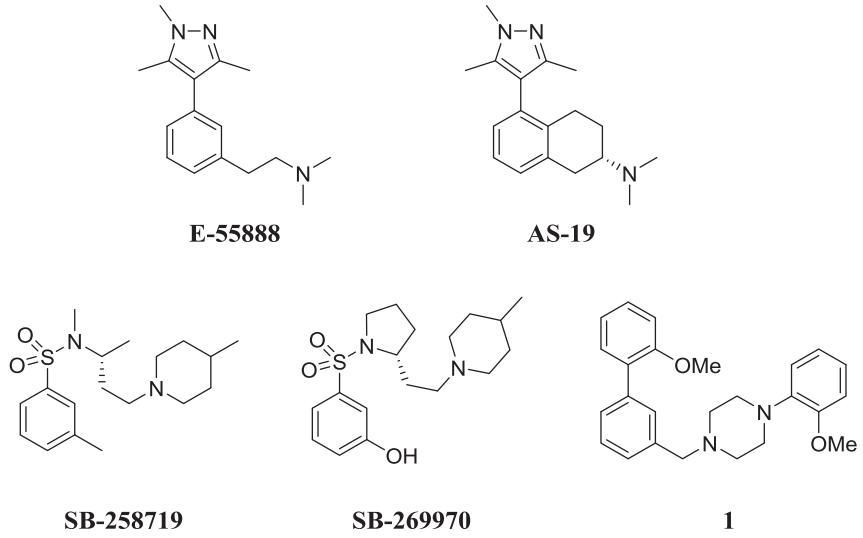


Fig. 1. 5-HT$_7$R agonists and antagonists.

Our group has been in active pursuit of selective 5-HT$_7$R ligands. Recently, we figured out that a combination of a biphenyl moiety and a phenylpiperazine group could provide a selective 5-HT$_7$R antagonist [12–14], which resulted in identification of 1-((2'-methoxy-[1,1'-biphenyl]-3-yl)methyl)-4-(2-methoxyphenyl)piperazine (**1**, Fig. 1) as a potent binder to 5-HT$_7$R with antagonistic property [13]. Structural comparison of the 5-HT$_7$R agonists (E-55888 and AS-19, Fig. 1) and antagonists (SB-258719, SB-269970 and **1**, Fig. 1) shows a common feature with an aromatic ring linked to an amino group. Interestingly, agonists (E-55888 and AS-19, Fig. 1) have terminal dimethylamino groups while cyclic amino groups are usually observed in the antagonists (SB-258719, SB-269970 and **1**, Fig. 1). Thus, we reasoned that the subtle difference at the terminal amino group might differentiate the functional properties of the 5-HT$_7$R ligands. Taken together, we envisaged that the 2′-(methoxy-[1,1'-biphenyl]-3-yl)methyl group would serve as a 5-HT$_7$R-specific scaffold, and appropriate substituted amino groups on the scaffold would modulate 5-HT$_7$R functional activities. Herein, in order to verify a key structural moiety for determining agonistic or antagonistic activity against 5-HT$_7$R, we designed and synthesized a series of biphenyl-3-yl-methanamine derivatives and evaluated their binding affinities as well as functional activities for the 5-HT$_7$R. In addition, the structure-function relationships of those compounds were analyzed by a molecular modeling study, which provided a clue to understanding the distinct structural features of the 5-HT$_7$R agonists and antagonists.

2. Chemistry

Structural modification of the compound **1** was carried out (Fig. 2) to include 4-methylpiperidino, dimethylamino or methylamino group at the terminal position of the structure. Also, the 2'-methoxy-(1,1'-biphenyl) group of **1** was further substituted to give a series of compounds with 2′-methoxy-([1,1'-biphenyl]-2-substitued-3-yl)methanamino (**2**, Fig. 2) and 1-(7-(2-methoxyphenyl)benzo[*d*][1,3]dioxol-5-yl)-methanamino (**3**, Fig. 2) groups.

The target compounds **2** were synthesized from various 3-iodobenzaldehydes **5** (Scheme 1) [15]. Commercially unavailable 3-iodoaldehydes **5** such as 4-chloro-3-iodobenzaldehyde [15a] and 3-iodo-4-methylbenzaldehyde [15b] were synthesized through electrophilic aromatic substitution of aldehydes **4** by treatment with I_2, $NaIO_3$, and H_2SO_4. Suzuki coupling of **5** with 2-methoxyphenylboronic acid **6** was performed in the presence of catalytic amount of $Pd(PPh_3)_4$ and Na_2CO_3 in DMF to afford biphenyl-3-carbaldehydes **7** in 64–98% yields. The synthesis of the compound **7** [16] with $R^1 = H$ was already published [13]. Biphenyl-3-carbaldehydes **7** underwent reductive amination with primary or secondary amines such as 1-(2-methoxyphenyl)piperazine, 4-methylpiperidine, dimethylamine, or methylamine to afford the desired compounds **2** in 8–83% yields. On the other hand, the target compounds **3** with dioxolane moiety were synthesized from commercially available staring material **8** in 4 steps (Scheme 2). Demethylation of 4-hydroxy-3-iodo-5-methoxybenzaldehyde **8** was performed by reaction with $AlCl_3$ and pyridine in dichloromethane (DCM) to afford 3,4-dihydroxy-5-iodobenzaldehyde **9** in 70% yield [17]. The compound **9** was treated with diiodomethane and DBU in DMF to give 7-iodobenzo[*d*][1,3]dioxole-5-carbaldehyde **10** in 38% yield [18]. Suzuki coupling with 2-methoxyphenylboronic acid **6** followed by reductive amination with four different amines afforded the biphenyl-3-yl-methanamines **3** in 40–61% yields.

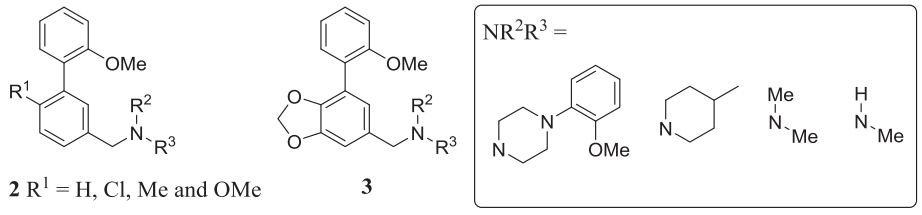
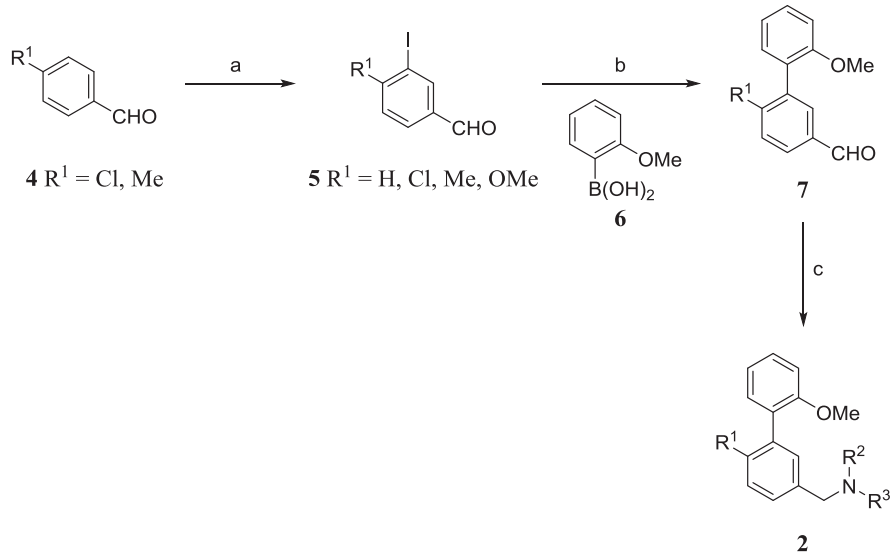
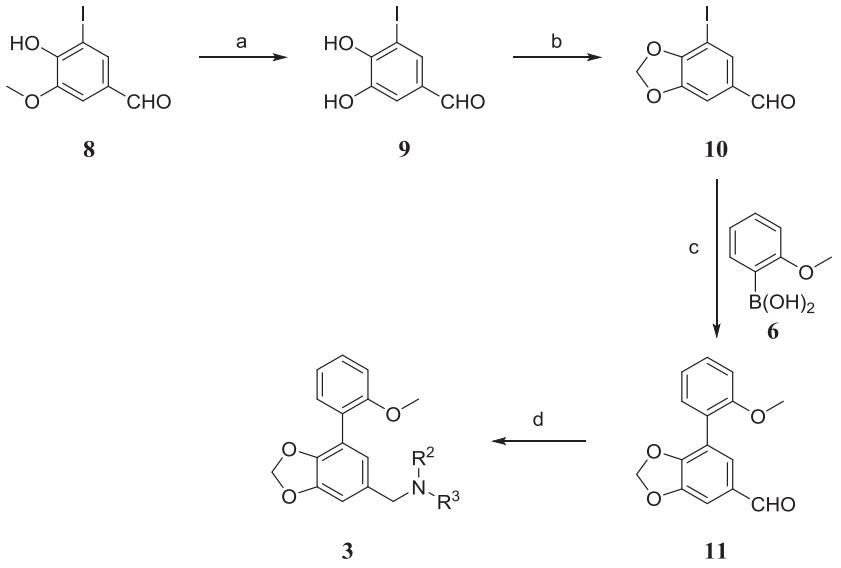


Fig. 2. Designed biphenyl-3-yl-methanamines **2** and **3**.



Reagents and conditions: a) I_2, $NaIO_3$, H_2SO_4, rt, 33-40%; b) 2-methoxyphenylbononic acid **6**, $Pd(PPh_3)_4$, Na_2CO_3, DMF, reflux, 64-98%; c) HNR^2R^3, $NaBH(OAc)_3$, MeOH, rt, 8-83%

Scheme 1. Synthesis of biphenyl-3-yl-methanamines **2**.



Reagents and conditions: a) I_2, $NaIO_3$, H_2SO_4, rt, 70%; b) CH_2I_2, DBU, DMF, rt, 38%; c) 2-methoxyphenylbononic acid **6**, $Pd(PPh_3)_4$, Na_2CO_3, DMF, reflux, 64%; d) HNR^2R^3, $NaBH(OAc)_3$, MeOH, rt, 40-61%

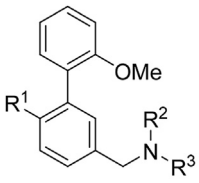
Scheme 2. Synthesis of biphenyl-3-yl-methanamines **3** with dioxolane moiety.

3. Results and discussion

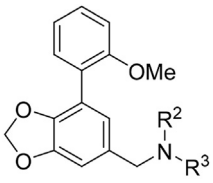
3.1. Binding affinities to 5-HT$_7$R

The synthesized biphenyl-3-yl-methanamines **2** and **3** were evaluated for their binding affinities to 5-HT$_7$R by [^{3}H]LSD radioligand binding assays in transfected HEK293 cells, and the results are summarized in Table 1 [19]. Among the 2′-methoxy-([1,1'-biphenyl]-3-yl)methanamine derivatives (**2a**–**2o**), compounds with 1,1'-biphenyl-2-chloro (**2d**–**2g**) and 1,1'-biphenyl-2-methyl (**2h**–**2k**) substituent showed significant binding affinities to 5-HT$_7$R. Taking into account that a chlorine atom and a methyl group are similar in size, it could be presumed that the 2-substituent on the biphenyl moiety might be involved in favorable binding interaction with the target receptor to increase the binding affinity. In this series (**2d**–**2k**), compounds with acyclic amino groups (**2f**, **2g**, **2j** and **2k**) were identified as high-affinity ligands for 5-HT$_7$R and compound **2h** with 2-methoxyphenylpiperazine also showed high binding affinity. In particular, compound **2g** showed the highest 5-HT$_7$R-binding

Table 1
Binding affinities (K_i) of compounds **2** and **3** against 5-HT$_7$R.



2a ~ 2o



3a ~ 3d

Compound	R_1	-NR_2R_3	K_i[a] in nM
2a	H	4-methylpiperidine	1038.0
2b	H	dimethylamine	126.0
2c	H	methylamine	226.0
2d	Cl	1-(2-methoxyphenyl)piperazine	73.0
2e	Cl	4-methylpiperidine	389.0
2f	Cl	dimethylamine	18.0
2g	Cl	methylamine	5.2
2h	Me	1-(2-methoxyphenyl)piperazine	18.0
2i	Me	4-methylpiperidine	1123.0
2j	Me	dimethylamine	23.0
2k	Me	methylamine	15.0
2l	OMe	1-(2-methoxyphenyl)piperazine	79.0
2m	OMe	4-methylpiperidine	3063.0
2n	OMe	dimethylamine	1019.0
2o	OMe	methylamine	648.0
3a		1-(2-methoxyphenyl)piperazine	34.0
3b		4-methylpiperidine	691.0
3c		dimethylamine	772.0
3d		methylamine	1476.0
1	H	1-(2-methoxyphenyl)piperazine	15.0[b]
SB-269970			0.34
5-HT			6.0

[a] All of values were obtained by triplicate binding experiments at least.
[b] Reference [13].

affinity (K_i = 5.2 nM). In contrast, the 4-methylpiperidine-substitued compounds (**2a**, **2e**, **2i** and **2m**) showed low binding affinities to 5-HT$_7$R regardless of the substituents (R^1) at the biphenyl ring (Table 1). In case of 1-(7-(2-methoxyphenyl)benzo[*d*][1,3]dioxol-5-yl)-methanamine derivatives (**3a**–**3d**), compound **3a** with 2-methoxyphenylpiperazine showed good binding affinity to 5-HT$_7$R (K_i = 34.0 nM), but others (**3b**–**3d**) were found to be only moderate to weak binders (K_i = 691.0 nM–1476.0 nM). Taken together, in terms of the binding affinities of the title compounds to 5-HT$_7$R, a chlorine or a methyl substituent at R^1 gave positive effect, while substitution with a methoxy or a dioxolane group at R^1 was unfavorable. Also, 2-methoxyphenylpiperazine, dimethylamine and methylamine groups were more suitable than 4-methylpiperidine group for binding to 5-HT$_7$R.

3.2. Functional activities against 5-HT$_7$R

The biphenyl-3-yl-methanamines **2** and **3** were then subjected to functional assays by measuring cAMP levels from HEK293 cells stably transfected with cloned human 5-HT$_7$R gene. The endogenous 5-HT$_7$R agonist serotonin (5-HT) or the well-known selective 5-HT$_7$R antagonist SB-269970 was used as reference compound, and the results of the functional assays are shown in Table 2.

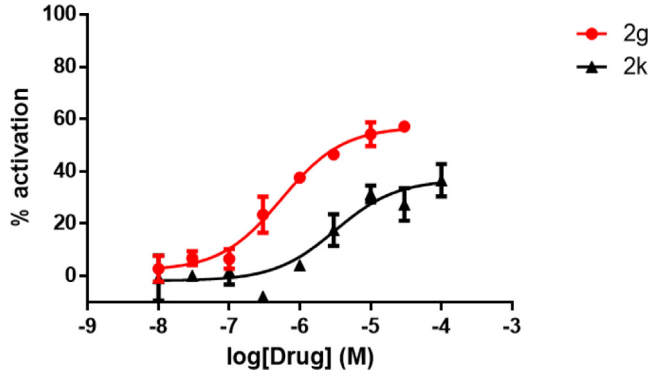
For functional assay to determine agonists, the 5-HT$_7$R-trasfected HEK293 cells were treated with the title compounds (10 μM), and induction of cAMP level was measured. The cAMP levels in the cells treated with the title compounds were compared with those obtained from 5-HT-treated cells. Among the tested compounds, the methylamine-substituted **2g** and **2k** increased cAMP levels by 66.82% and 55.50%, respectively. However, treatment with other compounds failed to show significant increase in intracellular cAMP level (Table 2). Next, we also carried out functional antagonist assay of compounds **2** and **3** (Table 2). The 5-HT$_7$-transfected HEK293 cells were treated with the title compounds (10 μM) for 10 min followed by stimulation by treatment with 5-HT (100 nM). Interestingly, in this case, compounds **2d, 2h, 2l** and **3a** with 2-methoxyphenylpiperazine showed good antagonistic activities comparable to the control compound SB-269970 with maximum inhibition values of 91.03%, 69.34%, 91.58% and 93.70%, respectively, while compounds **2a** and **2e** with 4-methylpiperindine showed only partial inhibition (39.02% and 52.32% inhibition, respectively) of cAMP levels stimulated by treatment with 100 nM of serotonin 5-HT. The compound **2b** with dimethylamino group also showed partial inhibitory activity with a maximum inhibition value of 40.84%. According to both functional assays, the compounds **2g** and **2k** are identified as agonists and the compounds **2d, 2h, 2l** and **3a** are as antagonists.

We chose two compounds **2g** and **2k** to measure EC_{50} values that represent the concentration of agonists which gives half-

Table 2
Functional assays at 10 μM of the compounds **2** and **3**.

Compound	Maximum %activation (± SEM %)	Maximum %inhibition (± SEM %)	agonist or antagonist
2a	8.59 ± 0.08	39.02 ± 3.14	–[a]
2b	15.39 ± 0.94	40.84 ± 2.28	–[a]
2c	24.54 ± 1.42	−0.87 ± 0.77	–[a]
2d	−7.72 ± 0.18	**91.03 ± 4.33**	**antagonist**
2e	−14.39 ± 1.79	52.32 ± 2.38	–[a]
2f	27.62 ± 0.44	10.32 ± 1.33	–[a]
2g	**66.82 ± 1.13**	4.66 ± 0.98	**agonist**
2h	14.04 ± 1.16	**69.34 ± 3.36**	**antagonist**
2i	2.53 ± 0.45	29.00 ± 2.12	–[a]
2j	33.26 ± 0.77	6.61 ± 0.50	–[a]
2k	**55.50 ± 0.46**	2.49 ± 0.57	**agonist**
2l	−2.42 ± 0.90	**91.58 ± 5.24**	**antagonist**
2m	−1.14 ± 2.66	7.74 ± 0.59	–[a]
2n	28.75 ± 1.42	4.54 ± 0.98	–[a]
2o	11.01 ± 2.20	2.25 ± 1.40	–[a]
3a	−1.56 ± 0.65	**93.70 ± 4.72**	**antagonist**
3b	7.60 ± 2.48	15.60 ± 1.49	–[a]
3c	3.97 ± 1.20	13.52 ± 0.49	–[a]
3d	13.58 ± 0.74	8.40 ± 0.88	–[a]
serotonin (5-HT)	100.00	–[a]	agonist
SB269970	–[a]	100.00	antagonist

[a] Not determined.

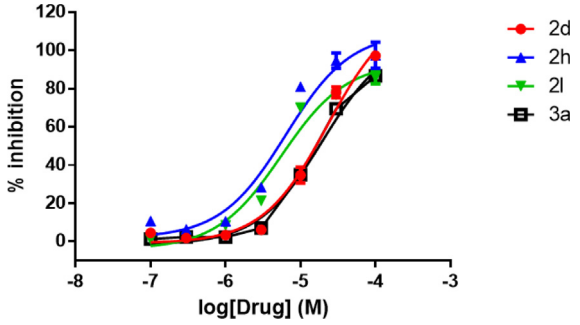


Compound	pEC$_{50}$ (±SEM)	EC$_{50}$ (μM)[a]
2g	6.26 ± 0.08	0.55
2k	5.50 ± 0.17	3.2
serotonin (5-HT)	8.24 ± 0.05	5.8 x 10^{-3}

[a]EC$_{50}$ is the concentration of an agonist which gives half-maximal response

Fig. 3. EC$_{50}$ values of the compounds **2g** and **2k** against 5-HT$_7$R.

maximal response (Fig. 3). Compound **2g** had agonistic potency with an EC$_{50}$ value of 0.55 μM, and **2k** also showed good agonistic effect (EC$_{50}$ = 3.2 μM). Both **2g** and **2k** could be defined as partial 5-HT$_7$R agonists because maximum activation did not reach to 100% activation. And also, we chose the four compounds to measure IC$_{50}$ values (Fig. 4) where IC$_{50}$ is the concentration of an antagonist required to produce 50% reduction of maximal response of an agonist (in this case, serotonin). The four compounds **2d, 2h, 2l** and **3a** with 2-methoxyphenylpiperazine group were analyzed to show good antagonistic potency with IC$_{50}$ values of 23.1 μM, 6.35 μM, 5.57 μM, and 19.8 μM, respectively. Those four compounds were defined as full antagonists due to high maximum inhibition values



Compound	pIC$_{50}$ (± SEM)	IC$_{50}$ (μM) [a]
2d	4.640 ± 0.072	23.1
2h	5.200 ± 0.097	6.35
2l	5.250 ± 0.083	5.57
3a	4.700 ± 0.058	19.8
SB269970	8.430 ± 0.045	3.71 x 10^{-3}

[a]IC$_{50}$ is the concentration of an antagonist required to produce 50% reduction of maximal response of an agonist (in this case, serotonin)

Fig. 4. IC$_{50}$ values of the compounds **2d**, **2h**, **2l**, and **3a** against 5-HT$_7$R.

in 100 μM. According to these results, it can be suggested that the methylamino group plays an important role to provide agonistic effect to the corresponding biphenyl-3-yl-methanamine derivatives, while 2-methoxyphenylpiperazine group make the corresponding compounds antagonistic against 5-HT$_7$R.

3.3. Molecular modeling studies on 5-HT$_7$R

It was of our interest that a single amino group such as methylamine and 2-methoxyphenylpiperazine substituted to the same biphenyl scaffold could differentiate 5-HT$_7$R agonists and antagonists. In order to elaborate the role of the methylamine and 2-methoxyphenylpiperazine on the functional activity of the biphenyl-3-yl-methanamines, we performed induced-fit molecular docking study. We constructed a comparative model structure of 5-HT$_7$R by the SwissModel server using the β_2-adrenergic receptor structure (PDB: 2RH1) as a template [12,13,20]. Structures of the biphenyl-3-yl-methanamines (**2g** and **2h**) were built in protonated forms on their amino group, energy-minimized and docked to the ligand-binding site of 5-HT$_7$R according to the standard docking protocol of the Glide program implemented in Maestro 9.1 (Schrödinger Inc.) [20]. The docked poses of both **2g** and **2h** showed a salt bridge between the protonated amine and the acid side chain of Asp162 (dotted lines in Fig. 5a and b) as common binding interaction, which is known to be critical for high binding affinity of a ligand to 5-HT$_7$R [13,22]. Interestingly, however, the agonist (**2g**, Fig. 5a) and antagonist (**2h**, Fig. 5b) showed distinct binding modes to 5-HT$_7$R; the relatively small agonist (**2g**) occupied only a half of the cavity (Fig. 5a), while the antagonist (**2h**) were bound to 5-HT$_7$R in an extended conformation covering the full cavity of the ligand binding site (Fig. 5b). These binding modes for **2g** and **2h** are well-matched with previously reported ones [22] where there is one ionic interaction point with ASP162 in the middle of the 5-HT$_7$R binding site which has two hydrophobic pockets which have one hydrogen-bonding interaction depending on ligands. The docked poses of **2g** and **2h** to 5-HT$_7$R were then compared and the superimposed structures clearly showed that Arg367 side chain swings from pointing outside to the solvent (**2g**) to pointing inside toward the ligand in the active site (**2h**) (Fig. 6). As Arg367 is located outside of the binding site, it has no significant contribution to binding of the relatively small agonist **2g**. However, upon binding to the full antagonist **2h**, the side chain of Arg367 is located in the vicinity of the ligand bearing extended 2-methoxyphenylpiperazine, which suggests its involvement in the binding events [21]. The contribution of Arg367 and the size of the amino groups in determining the function of 5-HT$_7$R was further supported by docking the known agonist (AS-19) and antagonist (SB-269970) to 5-HT$_7$R (shown in supporting information). Thus, it can be presumed that Arg367 is the key to the agonist-induced or antagonist-induced conformational changes and the switch in functional activity from agonist to antagonist occurred due to the size of the amino groups attached to the biphenyl scaffold.

Based on the docking poses, pharmacophore models of AS-19 and SB-269970 were built and the compounds **2g** and **2h**, respectively, were mapped onto the pharmacophore model of AS-19 or SB-269970 (Fig. 7). Each compound has kept the docking conformation to the 5-HT$_7$R binding site. The pharmacophore model by the agonists AS-19 and **2g** have three hydrophobic groups (HYDs) and one positively ionizable feature (PI) (Fig. 7a). The PI pharmacophore could make an ionic interaction with Asp 162 and all three HYDs are located in one side of the PI where distances between PI and HYDs are 7.11, 7.89, and 9.12 Å, respectively. On the other hand, the pharmacophore model of antagonists SB-269970 and **2h** also included three HYDs and one PI. However, two HYPs are located in

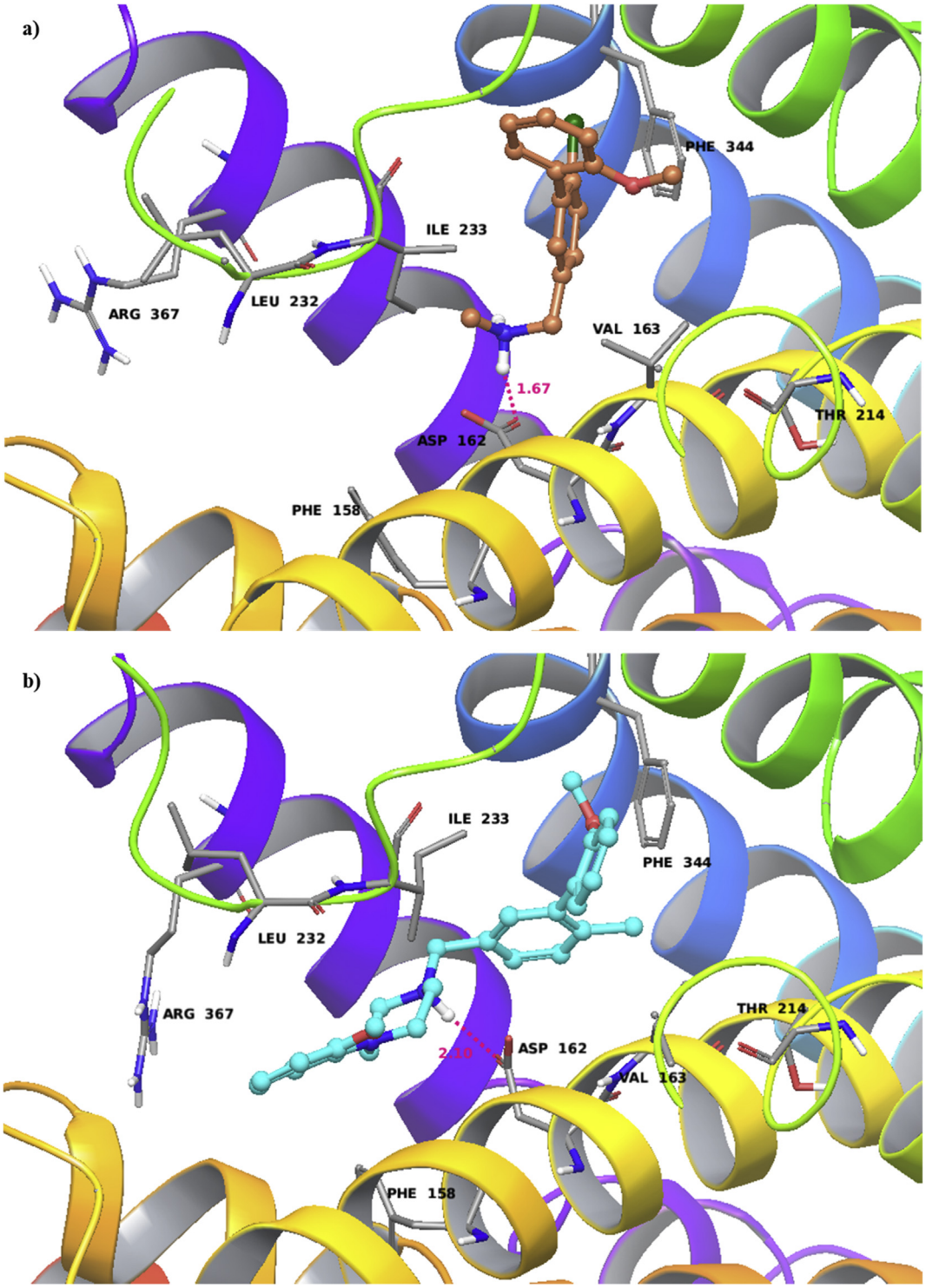


Fig. 5. Induced-fit molecular docking mode of a) the compound **2g** and b) the compound **2h** in the binding site of $5HT_7$ receptor.

one side of the PI and the third HYD is at the other side of the PI with a distance of 4.30 Å. (Fig. 7b). These pharmacophoric features in two pharmacophore models are mostly similar with the reported ones for agonists [20c] and antagonists [20d]. However, there are no hydrogen-bonding acceptor groups (HBAs) in both pharmacophore models due to our selected ligands. By virtual screening with these distinct pharmacophore models, novel agonists and antagonists are expected to be easily distinguished and obtained.

4. Conclusions

As a novel scaffold with tunable functional properties for modulating the activity of 5-HT_7R, we designed and prepared a series of biphenyl-3-yl-methanamine derivatives with various amino groups. Evaluation of the binding affinities as well as 5-HT_7R-modulating activities of the title compounds identified partial agonists **2g** (EC_{50} = 0.55 μM) and **2k** (EC_{50} = 3.2 μM) and full

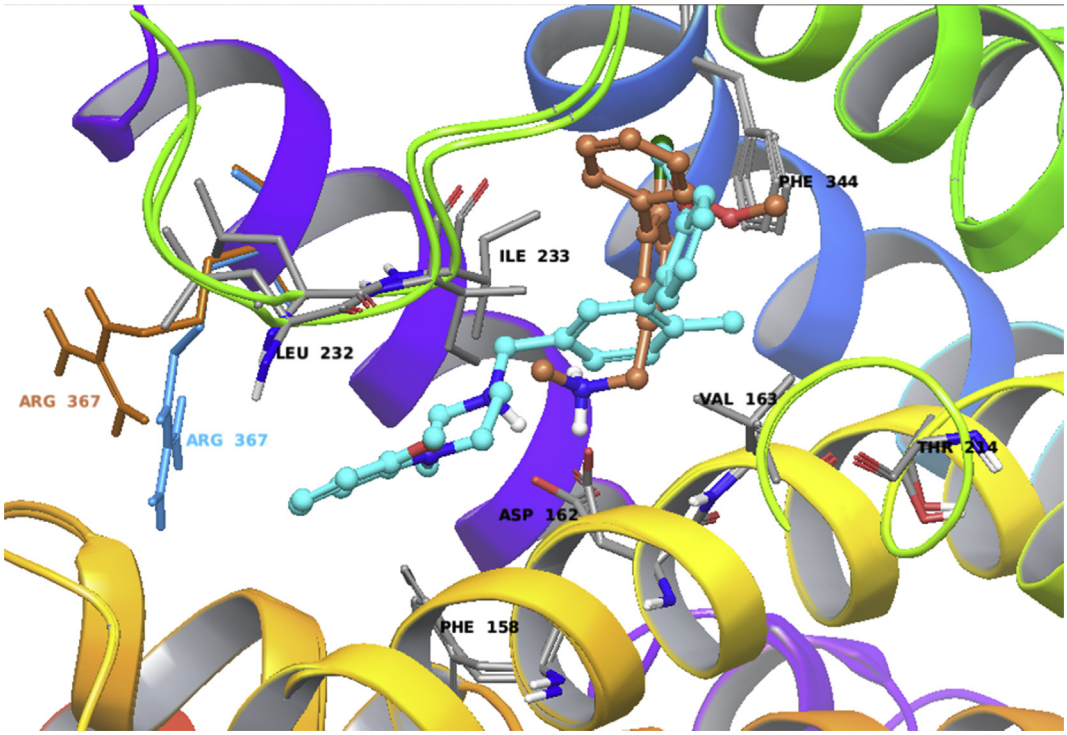


Fig. 6. Superimposed structures of the compounds **2g** in brown color and **2h** in cyan color: conformational change of Arg367 was presented by distinct colors such as brown and cyan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

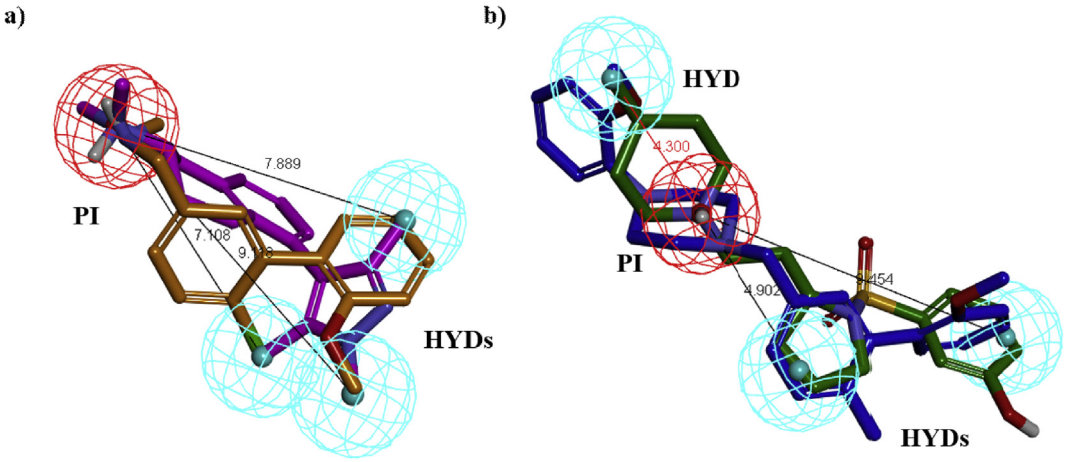


Fig. 7. The pharmacophore models of a) agonists **2g** in brown and AS-19 in pink, and b) antagonists **2h** in blue and SB-269970 in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

antagonists (**2d, 2h, 2l** and **3a**; IC_{50} = 23.1 μM, 6.35 μM, 5.57 μM, and 19.8 μM, respectively) depending on the amino substituents. Interestingly, a single amino group such as methylamine and 2-methoxyphenylpiperazine substituted to the same scaffold (biphenyl-3-yl-methyl) was found to differentiate 5-HT_7R agonists (**2g** and **2k**) and antagonists (**2d**, **2h**, **2l** and **3a**). Molecular docking study suggested that the ligand-based switch in functional activity from agonist to antagonist results from the size of the amino groups and thereby different binding modes to 5-HT_7R. In particular, interaction of the ligand with Arg367 of 5-HT_7R is shown to differentiate agonist and antagonist. In the pharmacophore model study, two distinct pharmacophore models can tell whether a ligand is an agonist or an antagonist. Taken together, this study provides valuable information for designing novel compounds with selective agonistic or antagonistic properties against 5-HT_7R.

5. Experimental section

5.1. Chemistry

General: All reactions were carried out under dry nitrogen unless otherwise indicated. Commercially available reagents were used without further purification. Solvents and gases were dried according to standard procedures. Organic solvents were evaporated with reduced pressure using a rotary evaporator. Analytical thin layer chromatography (TLC) was performed using glass plates

precoated with silica gel (0.25 mm). TLC plates were visualized by exposure to UV light (UV), and then were visualized with a *p*-anisaldehyde stain followed by brief heating on hot plate. Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck) with the indicated solvents. ^{1}H and ^{13}C spectra were recorded on Bruker 300, Bruker 400 NMR spectrometers. ^{1}H NMR spectra are represented as follows: chemical shift, multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), integration, and coupling constant (*J*) in Hertz (Hz). ^{1}H NMR chemical shifts are reported relative to $CDCl_3$ (7.26 ppm). ^{13}C NMR was recorded relative to the central line of $CDCl_3$ (77.0 ppm). Melting points were determined on an OptiMelt melting point apparatus (Stanford Research System, Inc.). HRMS analyses were performed on Bruker Compact ESI$^+$ positive mode.

*5.1.1. Synthesis of biphenyl-3-carbaldehyde derivatives (**7**)*

General: 2-Methoxyphenylboronic acid **6** (1.2 mmol), $Pd(PPh_3)_4$ (0.1 mmol) and Na_2CO_3 (1.5 mmol) were added to a solution of benzaldehyde **5** (1.0 mmol) in DMF (20 ml). The mixture was stirred for overnight at 150 °C. After cooling down to room temperature, the reaction mixture was quenched with saturated $NaHCO_3$ and then extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel (Hexane:Ether = 10:1) to obtain desired product **7** in 64–98% yields.

*5.1.1.1. 6-Chloro-2'-methoxy-[1,1'-biphenyl]-3-carbaldehyde (**7b**).* 64% Yield (as a white solid): mp 82–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.99 (s, C**H**O), 7.81–7.78 (m, 2Ar**H**), 7.61 (d, J = 8.8 Hz, Ar**H**), 7.43–7.39 (m, Ar**H**), 7.20 (dd, J = 7.6, 2.0 Hz, Ar**H**), 7.04 (td, J = 7.6, 0.8 Hz, Ar**H**), 7.00 (d, J = 8.4 Hz, Ar**H**), 3.78 (s, OC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.09, 156.64, 140.80, 138.90, 134.75, 133.30, 130.79, 130.25, 130.01, 129.01, 127.21, 120.54, 111.04, 55.58; HRMS (ESI$^+$) calcd for $C_{14}H_{11}ClNaO_2^+$ $[M + Na]^+$ 269.0340, found 269.0343.

*5.1.1.2. 2'-methoxy-6-methyl-[1,1'-biphenyl]-3-carbaldehyde (**7c**).* 89% Yield (as a white solid): mp 146–148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.98 (s, C**H**O), 7.77 (dd, J = 7.6, 1.6 Hz, Ar**H**), 7.69 (d, J = 1.6 Hz, Ar**H**), 7.41–7.35 (m, 2Ar**H**), 7.14 (dd, J = 7.6, 2.0 Hz, Ar**H**), 7.03 (td, J = 7.2, 0.8 Hz, Ar**H**), 6.98 (d, J = 8.4 Hz, Ar**H**), 3.76 (s, OC**H$_3$**), 2.21 (s, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 192.14, 156.50, 144.78, 139.66, 134.36, 131.93, 130.83, 130.41, 129.35, 129.28, 128.33, 120.69, 110.78, 55.41, 20.49; HRMS (ESI$^+$) calcd for $C_{15}H_{14}NaO_2^+$ $[M + Na]^+$ 249.0886, found 249.0884.

*5.1.1.3. 2',6-dimethoxy-[1,1'-biphenyl]-3-carbaldehyde (**7d**).* 98% Yield (as a white solid): mp 100–101 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.96 (s, C**H**O), 7.93 (dd, J = 8.4, 2.1 Hz, Ar**H**), 7.83 (d, J = 2.4 Hz, Ar**H**), 7.44–7.38 (m, Ar**H**), 7.28 (dd, J = 7.5, 1.8 Hz, Ar**H**), 7.13–7.07 (m, 3Ar**H**), 3.91 (s, OC**H$_3$**), 3.82 (s, OC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.04, 162.22, 157.01, 133.36, 131.36, 131.29, 129.58, 129.29, 128.66, 126.38, 120.50, 111.13, 110.95, 55.98, 55.69; HRMS (ESI$^+$) calcd for $C_{15}H_{14}NaO_3^+$ $[M + Na]^+$ 265.0835, found 265.0834.

*5.1.1.4. 7-(2-methoxyphenyl)benzo[d][1,3]dioxole-5-carbaldehyde (**11**).* 64% Yield (as a white solid): mp 80–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.82 (s, C**H**O), 7.52 (d, J = 1.2 Hz, Ar**H**), 7.40–7.36 (m, 2Ar**H**), 7.30 (d, J = 1.6 Hz, Ar**H**), 7.06–7.00 (m, 2Ar**H**), 6.04 (s, OC**H$_2$**O), 3.82 (s, OC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 190.61, 156.72, 150.74, 148.70, 131.47, 131.02, 130.89, 129.97, 123.42, 120.72, 120.30, 111.42, 105.39, 102.01, 55.69; HRMS (ESI$^+$) calcd for $C_{15}H_{12}NaO_4^+$ $[M + Na]^+$ 279.0628, found 279.0630.

*5.1.2. Synthesis of biphenyl-3-yl-methanamine derivatives (**2** and **3**)*

The mixture of amines (2.0 mmol) and aldehydes **7** or **11** (1.0 mmol) in MeOH (5 ml) were stirred at room temperature for 2h and then $NaBH(OAc)_3$ (3.0 mmol) was added. The mixture was stirred for overnight at room temperature. The reaction mixture was quenched with $NaHCO_3$ and then extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by column chromatography on silica gel (Hexane:Ether = 5:1) to obtain desired product **2** or **3** in 8–83% yields.

*5.1.2.1. 1-((2'-methoxy-[1,1'-biphenyl]-3-yl)methyl)-4-methylpiperidine (**2a**).* 79% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54–7.30 (m, 6Ar**H**), 7.11–7.03 (m, 2Ar**H**), 3.87 (s, OC**H$_3$**), 3.61 (s, C**H$_2$**), 2.97 (d, J = 11.4 Hz, **2H** piperidine), 2.04 (t, J = 11.4 Hz, **2H** piperidine), 1.67 (d, J = 11.1 Hz, **2H** piperidine), 1.44–1.30 (m, **3H** piperidine), 0.99 (d, J = 6.0 Hz, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.51, 138.27, 138.14, 130.96, 130.79, 130.51, 128.55, 128.12, 127.91, 127.79, 120.83, 111.25, 63.60, 55.57, 53.93, 34.41, 30.83, 21.99; HRMS (ESI$^+$) calcd for $C_{20}H_{26}NO^+$ $[M+H]^+$ 296.2009, found 296.2006.

*5.1.2.2. 1-(2'-methoxy-[1,1'-biphenyl]-3-yl)-N,N-dimethylmethanamine (**2b**).* 74% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.30 (m, 6Ar**H**), 7.10–7.01 (m, 2Ar**H**), 3.85 (s, OC**H$_3$**), 3.61 (s, C**H$_2$**), 2.37 (s, N(C**H$_3$**)**$_2$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.50, 138.60, 137.03, 130.94, 130.66, 130.56, 128.68, 128.04, 128.01, 120.88, 111.33, 63.96, 55.60, 44.85; HRMS (ESI$^+$) calcd for $C_{16}H_{20}NO^+$ $[M+H]^+$ 242.1539, found 242.1537.

*5.1.2.3. 1-(2'-methoxy-[1,1'-biphenyl]-3-yl)-N-methylmethanamine (**2c**).* 37% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.30 (m, 6Ar**H**), 7.09–7.01 (m, 2Ar**H**), 3.86 (s, C**H$_2$**), 3.85 (s, OC**H$_3$**), 2.53 (s, NC**H$_3$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.49, 139.19, 138.72, 130.93, 130.64, 127.48, 128.65, 128.41, 128.10, 126.88, 120.85, 111.29, 55.88, 55.60, 35.71; HRMS (ESI$^+$) calcd for $C_{15}H_{18}NO^+$ $[M+H]^+$ 228.1383, found 228.1384.

*5.1.2.4. 1-((6-Chloro-2'-methoxy-[1,1'-biphenyl]-3-yl)methyl)-4-(2-methoxyphenyl)piperazine (**2d**).* 62% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.35 (m, 2Ar**H**), 7.29–7.27 (m, 2Ar**H**), 7.20 (dd, J = 7.6, 1.6 Hz, Ar**H**), 7.04–6.88 (m, 5Ar**H**), 6.85 (d, J = 8.0 Hz, Ar**H**), 3.85 (s, OC**H$_3$**), 3.78 (s, OC**H$_3$**), 3.58 (s, C**H$_2$**), 3.09 (brs, **4H** piperazine), 2.67 (brs, **4H** piperazine); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.78, 152.29, 141.42, 137.39, 136.51, 132.47, 132.42, 131.09, 129.33, 129.30, 129.11, 128.60, 122.86, 120.99, 120.33, 118.23, 111.17, 111.04, 62.38, 55.63, 55.35, 53.29, 50.69; HRMS (ESI$^+$) calcd for $C_{25}H_{28}ClN_2O_2^+$ $[M+H]^+$ 423.1834, found 423.1831.

*5.1.2.5. 1-((6-Chloro-2'-methoxy-[1,1'-biphenyl]-3-yl)methyl)-4-methylpiperidine (**2e**).* 80% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.33 (m, 2Ar**H**), 7.24–7.21 (m, 2Ar**H**), 7.18 (dd, J = 7.6, 1.6 Hz, Ar**H**), 7.00 (td, J = 7.6, 1.2 Hz, Ar**H**), 6.96 (d, J = 8.0 Hz, Ar**H**), 3.76 (s, OC**H$_3$**), 3.45 (s, C**H$_2$**), 2.84 (d, J = 11.6 Hz, **2H** piperidine), 1.92 (td, J = 12.0, 2.4 Hz, **2H** piperidine), 1.59–1.56 (m, **2H** piperidine), 1.39–1.17 (m, **3H** piperidine), 0.89 (d, J = 6.4 Hz, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.79, 137.23, 137.16, 132.37, 132.13, 131.10, 129.26, 129.20, 128.97, 128.69, 120.30, 111.03, 62.72, 55.63, 53.91, 34.37, 30.76, 21.92; HRMS (ESI$^+$) calcd for $C_{20}H_{25}ClNO^+$ $[M+H]^+$ 330.1619, found 330.1617.

*5.1.2.6. 1-(6-Chloro-2'-methoxy-[1,1'-biphenyl]-3-yl)-N,N-dimethylmethanamine (**2f**).* 63% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.35 (m, 2Ar**H**), 7.24–7.22 (m, 2Ar**H**), 7.20 (dd, J = 7.6, 2.0 Hz, Ar**H**), 7.02 (td, J = 7.6, 1.2 Hz, Ar**H**), 6.98 (dd, J = 8.4, 0.8 Hz,

Ar**H**), 3.78 (s, OC**H$_3$**), 3.42 (s, C**H$_2$**), 2.26 (s, N(C**H$_3$**)**$_2$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.77, 137.47, 137.20, 132.42, 132.31, 131.05, 129.30, 129.11, 129.09, 128.59, 120.31, 110.99, 63.60, 55.63, 45.35; HRMS (ESI^+) calcd for $C_{16}H_{19}ClNO^+$ $[M+H]^+$ 276.1150, found 276.1151.

*5.1.2.7. 1-(6-Chloro-2′-methoxy-[1,1'-biphenyl]-3-yl)-N-methylmethanamine (**2g**).* 72% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.33 (m, 2Ar**H**), 7.23–7.21 (m, 2Ar**H**), 7.16 (dd, *J* = 7.2, 1.6 Hz, Ar**H**), 6.99 (td, *J* = 7.2, 0.8 Hz, Ar**H**), 6.95 (d, *J* = 8.4 Hz, Ar**H**), 3.76 (s, OC**H$_3$**), 3.73 (s, C**H$_2$**), 2.44 (s, NC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.75, 138.41, 137.64, 132.41, 131.39, 130.98, 129.34, 129.22, 128.57, 128.26, 120.34, 110.98, 55.63, 55.29, 35.99; HRMS (ESI^+) calcd for $C_{15}H_{17}ClNO^+$ $[M+H]^+$ 262.0993, found 262.0993.

*5.1.2.8. 1-((2′-methoxy-6-methyl-[1,1'-biphenyl]-3-yl)methyl)-4-(2-methoxyphenyl)piperazine (**2h**).* 83% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.30 (m, Ar**H**), 7.23–7.12 (m, 4Ar**H**), 7.02–6.87 (m, 5Ar**H**), 6.83 (dd, *J* = 8.0, 1.6 Hz, Ar**H**), 3.84 (s, OC**H$_3$**), 3.75 (s, OC**H$_3$**), 3.57 (s, C**H$_2$**), 3.08 (brs, **4H** piperazine), 2.67 (brs, **4H** piperazine), 2.12 (s, ArC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.64, 152.30, 141.53, 138.39, 135.55, 134.93, 131.15, 131.12, 129.50, 128.68, 128.52, 128.31, 126.11, 122.77, 120.96, 120.41, 118.23, 111.15, 110.69, 62.89, 55.39, 55.33, 53.27, 50.71, 19.60; HRMS (ESI^+) calcd for $C_{26}H_{31}N_2O_2^+$ $[M+H]^+$ 403.2380, found 403.2377.

*5.1.2.9. 1-((2′-methoxy-6-methyl-[1,1'-biphenyl]-3-yl)methyl)-4-methylpiperidine (**2i**).* 64% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.30 (m, Ar**H**), 7.22–7.11 (m, 4Ar**H**), 6.99 (t, *J* = 7.2 Hz, Ar**H**), 6.94 (d, *J* = 8.0 Hz, Ar**H**), 3.75 (s, OC**H$_3$**), 3.48 (s, C**H$_2$**), 2.88 (d, *J* = 11.2 Hz, **2H** piperidine), 2.12 (s, ArC**H$_3$**), 1.93 (t, *J* = 10.8 Hz, **2H** piperidine), 1.58 (d, *J* = 12.4 Hz, **2H** piperidine), 1.38–1.18 (m, **3H** piperidine), 0.90 (d, *J* = 6.0 Hz, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.65, 138.27, 135.43, 135.31, 131.16, 131.10, 130.93, 129.40, 128.48, 128.26, 120.41, 110.71, 63.23, 55.41, 53.88, 34.36, 30.80, 21.95, 19.60; HRMS (ESI^+) calcd for $C_{21}H_{28}NO^+$ $[M+H]^+$ 310.2165, found 310.2166.

*5.1.2.10. 1-(2′-methoxy-6-methyl-[1,1'-biphenyl]-3-yl)-N,N-dimethylmethanamine (**2j**).* 42% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33–7.29 (m, Ar**H**), 7.21–7.16 (m, 2Ar**H**), 7.14 (dd, *J* = 7.2, 1.6 Hz, Ar**H**), 7.10 (brs, Ar**H**), 6.98 (td, *J* = 7.6, 1.2 Hz, Ar**H**), 6.96 (d, *J* = 8.0 Hz, Ar**H**), 3.74 (s, OC**H$_3$**), 3.41 (s, C**H$_2$**), 2.14 (s, N(C**H$_3$**)**$_2$**), 2.11 (s, ArC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.62, 138.47, 135.65, 135.55, 131.13, 130.92, 130.85, 129.49, 128.51, 128.08, 120.40, 110.67, 64.05, 55.40, 45.32, 19.60; HRMS (ESI^+) calcd for $C_{17}H_{22}NO^+$ $[M+H]^+$ 256.1696, found 256.1694.

*5.1.2.11. 1-(2′-methoxy-6-methyl-[1,1'-biphenyl]-3-yl)-N-methylmethanamine (**2k**).* 75% Yield (as an oil): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34–7.30 (m, Ar**H**), 7.22–7.08 (m, 4Ar**H**), 6.98 (td, *J* = 7.6, 12 Hz, Ar**H**), 6.94 (d, *J* = 8.0 Hz, Ar**H**), 3.75 (s, C**H$_2$**), 3.74 (s, OC**H$_3$**), 2.45 (s, NC**H$_3$**), 2.10 (s, ArC**H$_3$**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.57, 138.74, 135.92, 135.86, 131.05, 130.66, 130.14, 129.78, 128.61, 127.34, 120.46, 110.63, 55.38, 55.30, 35.44, 19.59; HRMS (ESI^+) calcd for $C_{16}H_{20}NO^+$ $[M+H]^+$ 242.1539, found 242.1542.

*5.1.2.12. 1-((2′,6-dimethoxy-[1,1′-biphenyl]-3-yl)methyl)-4-(2-methoxyphenyl)piperazine (**2l**).* 26% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.29 (m, 4Ar**H**), 7.09–6.96 (m, 6Ar**H**), 6.90 (d, *J* = 7.5 Hz, Ar**H**), 3.91 (s, OC**H$_3$**), 3.83 (s, OC**H$_3$**, OC**H$_3$**), 3.63 (s, C**H$_2$**), 3.15 (brs, **4H** piperazine), 2.74 (brs, **4H** piperazine); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.09, 156.26, 152.36, 141.58, 132.70, 131.60, 129.58, 128.60, 127.89, 127.48, 122.79, 121.02, 120.37, 118.26, 111.23, 110.97, 62.59, 55.86, 55.74, 55.37, 53.22, 50.73; HRMS (ESI^+) calcd for $C_{26}H_{31}N_2O_3^+$ $[M+H]^+$ 419.2329, found 419.2330.

*5.1.2.13. 1-((2′,6-dimethoxy-[1,1′-biphenyl]-3-yl)methyl)-4-methylpiperidine (**2m**).* 8% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.30 (m, 3Ar**H**), 7.24 (d, *J* = 1.8 Hz, Ar**H**), 7.08–6.97 (m, 3Ar**H**), 3.82 (s, OC**H$_3$**, OC**H$_3$**), 3.63 (s, C**H$_2$**), 3.03 (d, *J* = 11.1 Hz, **2H** piperidine), 2.15–2.07 (**2H** piperidine), 1.67 (d, *J* = 9.3 Hz, **2H** piperidine), 1.42–1.32 (m, **3H** piperidine), 0.98 (d, *J* = 4.8 Hz, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.04, 156.42, 133.07, 131.59, 129.94, 128.65, 128.29, 127.69, 127.38, 120.37, 111.22, 110.94, 62.25, 55.80, 55.73, 53.25, 33.68, 30.53, 21.77; HRMS (ESI^+) calcd for $C_{21}H_{28}NO_2^+$ $[M+H]^+$ 326.2115, found 326.2117.

*5.1.2.14. 1-(2′,6-dimethoxy-[1,1′-biphenyl]-3-yl)-N,N-dimethylmethanamine (**2n**).* 13% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39–7.21 (m, 4Ar**H**), 7.06–6.96 (m, 3Ar**H**), 3.81 (s, OC**H$_3$**), 3.80 (s, OC**H$_3$**), 3.50 (s, C**H$_2$**), 2.32 (s, N(C**H$_3$**)**$_2$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.07, 156.37, 132.66, 131.54, 129.55, 129.49, 128.61, 127.78, 127.56, 120.36, 111.19, 110.99, 63.36, 55.82, 55.73, 44.82; HRMS (ESI^+) calcd for $C_{17}H_{22}NO_2^+$ $[M+H]^+$ 272.1645, found 272.1645.

*5.1.2.15. 1-(2′,6-dimethoxy-[1,1′-biphenyl]-3-yl)-N-methylmethanamine (**2o**).* 24% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1H NMR (300 MHz, CDCl3) δ 7.66 (d, *J* = 7.8 Hz, Ar**H**), 7.30–7.16 (m, 3Ar**H**), 6.95–6.88 (m, 3Ar**H**), 4.04 (s, C**H$_2$**), 3.66 (s, OC**H$_3$**), 3.61 (s, OC**H$_3$**), 2.47 (s, NC**H$_3$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.41, 156.90, 133.58, 131.48, 130.87, 128.79, 127.69, 127.01, 123.05, 120.41, 111.76, 111.09, 55.60, 55.52, 51.17, 31.07; HRMS (ESI^+) calcd for $C_{16}H_{20}NO_2^+$ $[M+H]^+$ 258.1489, found 258.1491.

*5.1.2.16. 1-(2-methoxyphenyl)-4-((7-(2-methoxyphenyl)benzo[d][1,3]dioxol-5-yl)methyl)piperazine (**3a**).* 54% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.35 (m, 2Ar**H**), 7.09–6.88 (m, 8Ar**H**), 6.00 (s, OC**H$_2$**O), 3.90 (s, OC**H$_3$**), 3.87 (s, OC**H$_3$**), 3.60 (s, C**H$_2$**), 3.14 (brs, **4H** piperazine), 2.73 (brs, **4H** piperazine); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.77, 152.33, 147.59, 144.30, 141.52, 131.09, 129.21, 125.10, 124.52, 122.81, 121.00, 120.61, 119.57, 118.25, 111.44, 111.22, 108.50, 100.82, 62.96, 55.73, 55.35, 53.15, 50.71; HRMS (ESI^+) calcd for $C_{26}H_{29}N_2O_4^+$ $[M+H]^+$ 433.2122, found 433.2120.

*5.1.2.17. 1-((7-(2-methoxyphenyl)benzo[d][1,3]dioxol-5-yl)methyl)-4-methylpiperidine (**3b**).* 61% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.35 (m, 2Ar**H**), 7.08–7.01 (m, 3Ar**H**), 6.93 (brs, Ar**H**), 5.99 (s, OC**H$_2$**O), 3.88 (brs, C**H$_2$**), 3.85 (s, OC**H$_3$**), 3.20 (d, *J* = 9.6 Hz, **2H** piperidine), 2.31 (brs, **2H** piperidine), 1.73–1.45 (m, **5H** piperidine), 0.95 (d, *J* = 11.1 Hz, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 162.33, 156.69, 147.81, 145.12, 131.04, 129.37, 125.99, 124.60, 120.64, 119.63, 111.47, 109.63, 101.03, 61.66, 55.72, 52.48, 32.56, 30.00, 21.37; HRMS (ESI^+) calcd for $C_{21}H_{26}NO_3^+$ $[M+H]^+$ 340.1907, found 340.1909.

*5.1.2.18. 1-(7-(2-methoxyphenyl)benzo[d][1,3]dioxol-5-yl)-N,N-dimethylmethanamine (**3c**).* 40% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43–7.35 (m, 2Ar**H**), 7.08–7.02 (m, 2Ar**H**), 6.90–6.87 (m, 2Ar**H**), 6.00 (s, OC**H$_2$**O), 3.86 (s, OC**H$_3$**), 3.53 (s, C**H$_2$**), 2.36 (s, N(C**H$_3$**)**$_2$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.75, 147.69, 144.64, 131.06, 130.25, 129.29, 124.94, 120.62, 119.68, 111.45, 108.64, 100.93, 63.39, 55.73, 44.35; HRMS (ESI^+) calcd for $C_{17}H_{20}NO_3^+$ $[M+H]^+$ 286.1438, found 286.1436.

*5.1.2.19. 1-(7-(2-methoxyphenyl)benzo[d][1,3]dioxol-5-yl)-N-methylmethanamine (**3d**).* 41% Yield (as an oil): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.35 (m, 2Ar**H**), 7.08–7.01 (m, 2Ar**H**), 6.93 (s, Ar**H**), 6.89 (s, Ar**H**), 5.98 (s, OC**H$_2$**O), 3.86 (s, OC**H$_3$**), 3.77 (s, C**H$_2$**), 2.50 (s, C**H$_3$**); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.73, 147.75, 144.54, 131.93, 131.05, 129.32, 124.88, 123.82, 120.64, 119.91, 111.40, 107.78, 100.93, 55.73, 55.27, 35.03; HRMS (ESI^+) calcd for $C_{16}H_{18}NO_3^+$ $[M+H]^+$ 272.1281, found 272.1284.

5.2. Biological assays

5.2.1. Serotonin receptor binding affinity assays [1]

Eleven dilutions (5× assay concentration) of the test and reference compounds were prepared in standard binding buffer (50 mm tris(hydroxymethyl)-aminomethane–HCl (Tris-HCl), 10 mM $MgCl_2$, 1 mm ethylenediaminetetraacetate (EDTA), pH7.4) by serial dilution: 0.05 nM, 0.5 nM, 1.5 nM, 5 nM, 15 nM, 50 nM, 150 nM, 500 nM, 1.5 μM, 5 μM, and 50 μM. The [^{3}H]D-lysergic acid diethylamide ([^{3}H] LSD) radioligand was diluted to five times the assay concentration in standard binding buffer. Aliquots (50 mL) of the radioligand were dispensed into the wells of a 96-well plate containing 100 ml of standard binding buffer. Triplicate aliquots (50 mL) of the test and reference compound dilutions were then added. Finally, crude membrane fractions (50 mL) of cells expressing recombinant target were dispensed into each well. Total 250 ml of the reaction mixtures were incubated at room temperature and shielded from light for 1.5 h, then harvested by rapid filtration onto Whatman GF/B glass fiber filters presoaked with 0.3% polyethyleneimine, by using a 96-well Brandel harvester.

Four rapid washes were performed with chilled standard binding buffer (500 mL) to decrease nonspecific binding. Filters were placed in 6 mL scintillation tubes and allowed to dry overnight. The next day, 4-ml of EcoScint scintillation cocktail (National Diagnostics) was added to each tube. The tubes were capped, labeled, and counted by liquid scintillation counting. The filter mats were dried, and the scintillant was melted onto the filters, then the radioactivity retained on the filters was counted in a Microbeta scintillation counter. The IC_{50} values were obtained by using the Prism 4.0 program (GraphPad Software) and converted into *Ki* values. Each compound was tested in triplicate at least.

5.2.2. cAMP accumulation assay

All of the synthesized compounds were evaluated for adenylate cyclase activity by using HEK293 stable cell line that transfected with the human 5-HT_7 receptor [2,3]. To analyze cAMP levels, cAMP dynamic 2HTRF kits (Cisbio, USA), which provide homogeneous high throughput, were used. Transfected HEK293 cells were suspended in phosphate-buffered saline (PBS) containing 2 mM 3-isobutyl-1-methylxanthine (IBMX), which blocks the phosphodiesterase enzyme degradation of cAMP. Cells were stimulated by 5-HT for 30 min, with or without pretreatment with the compounds for 10 min. After 30 min, cAMP labeled with the dye d2 and anti-cAMP antibodies labeled with cryptate were added into the cell plates. The plates were incubated at room temperature for 1h. The fluorescence intensity of the accumulated cAMP level was measured by using a Flexstation3 microplate reader (Molecular Devices, Downingtown, PA). The EC_{50} and IC_{50} values were obtained by using the Prism 6.0 program (GraphPad Software). Each compound was tested in triplicate at least.

5.3. Molecular docking

The previously constructed model structure of the human 5-HT_7 receptor was used [4]. Three dimensional structures of compounds **2g** and **2h** were sketched by the "Build" module of the Maestro 9.1 software (Schrçdinger Inc.). Energy minimization was performed by using a conjugate gradient minimization (0.05 convergence criteria), the OPLS-AA force field, and the GB/SA continuum water model. A torsional scan along every rotatable bond was then performed for the minimized structures. The "Conformational search" module implemented in the Maestro 9.1 software was used with the automatic setup. With the modeled structure, docking of the both compounds was carried out. The protein preparation utilities in Maestro 9.1 were used to assign the charge state of ionizable residues, add hydrogen atoms, and carry out energy minimization. The ligands were then docked into the comparative model structure of 5-HT_7 receptor by using induced-fit docking. The default setting of the extreme precision mode of the induced-fit docking was employed for the docking, and up to ten poses was saved for analysis. The pharmacophore generation and mapping of compounds onto the pharmacophore were performed using Discovery Studio 2016 (Accelrys, Inc.) according to the standard pharmacophore generation protocol.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2016.07.029.

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