Synthesis of *N*-Alkyl-Carbazole Derivatives as 5-HT$_7$R Antagonists

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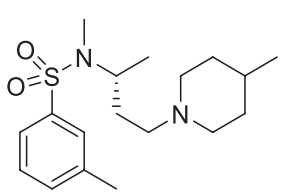
We designed and synthesized a series of *N*-alkyl-carbazoles with different alkyl chains and amine moieties, and biological evaluation was performed to discover novel 5-HT$_7$R antagonists. Among 27 synthesized compounds, **20**, **21**, **23**, and **24** showed excellent binding affinities to 5-HT$_7$R (K_i = 65, 64, 55, and 31 nM, respectively), and good selectivity profiles over other serotonin receptors. In functional assays, those compounds showed weak antagonistic activities against 5-HT$_7$R. In particular, the compound **24**, 2-(4-(5-(9*H*-carbazol-9-yl)pentyl)piperazin-1-yl)phenol, could be considered as a potent and selective 5-HT$_7$R ligand with weak antagonistic effect. From the molecular docking study, the aromatic hydroxyl group in **24** was shown to play an important role in binding to 5-HT$_7$R through a hydrogen bonding interaction with Asp142 in the ligand binding pocket of 5-HT$_7$R.

Keywords: 5-HT$_7$ receptor, Antagonist, *N*-alkyl-carbazole, Serotonin, GPCR

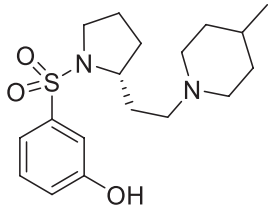
Introduction

Serotonin receptors (5-HTRs) have been identified and divided into seven subfamilies (5-HT$_1$R–5-HT$_7$R). Except 5-HT$_3$R, a ligand-gated ion channel, all other 5-HTRs are G protein-coupled receptors that activate an intracellular second messenger. The 5-HTRs are activated by an endogenous ligand, serotonin (5-hydroxytryptamine, 5-HT).[1] Serotonin is mainly distributed in the central nervous system (CNS) and acts as a neurotransmitter.[2–7] Serotonin activates 5-HTRs to regulate psychological and physiological changes such as mood, anxiety, aggression, pain, and sleep pattern.[8–10] Among 5-HTRs, 5-HT$_7$R is the most recently identified receptor. [11] It is distributed in the several regions of brain such as hypothalamus, thalamus, cortex, and hippocampus, where it is involved in circadian rhythm, thermoregulation, endocrine regulation, sleep, mood regulation, epilepsy, learning, memory, and depression.[12–14] The 5-HT$_7$R activates G_s and G_{12} proteins that engage in the production of cAMP and stimulation of G_{12}-mediated small GTPases (*e.g.*, Rho, Rac, and Cdc42) in living cell.[12–18] Recently, it was also verified that the recruitment of β-arrestin is stimulated when serotonin activates the 5-HT$_7$R.[19]

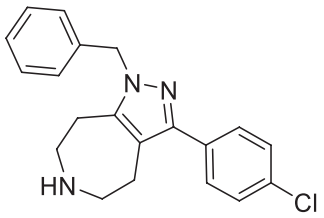
There has been much attention to discover selective 5-HT$_7$R antagonists over the past few decades. Up to now, SB-258719 [(R)-N,3-dimethyl-N-(4-(4-methylpiperidin-1-yl)butan-2-yl)benzenesulfonamide],[20,21] SB-269970 [(R)-3-((2-(2-(4-methylpiperidin-1-yl)ethyl)pyrrolidin-1-yl) sulfonyl)phenol][22–26] and JNJ-18038683 [1-benzyl-3-(4-chlorophenyl)-1,4,5,6,7,8-hexahydropyrazolo[3,4-*d*]azepine][27] have been found as selective and potent 5-HT$_7$R antagonists (Figure 1). SB-269970 and JNJ-18038683 exhibited antidepressant-like effect in the forced swimming test and the tail suspension test.[25,26] Both compounds also changed REM sleep pattern in the electroencephalogram test.[26,27] Especially, JNJ-18038683 is currently in Phase II clinical development for major depressive and bipolar disorder.[28]



SB-258719



SB-269970

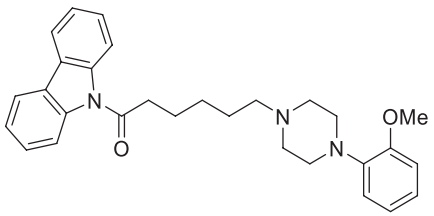


JNJ-18038683

Figure 1. Representative 5-HT_7R antagonists.

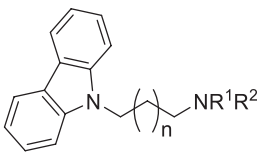
Nevertheless, novel 5-HT_7R antagonists are still required to understand the therapeutic potential of targeting the 5-HT_7R.

Previously, our research group has reported *N*-acyl-carbazole compounds as well as biaryl derivatives as selective 5-HT_7R ligands.[29–34] As a continuation of our previous study, *N*-alkyl-carbazole derivatives were investigated to study structure–activity relationship (SAR) of the carbazole derivatives (Figure 2). Generally, CNS drugs are more hydrophobic than non-CNS drugs.[35] Lipophilicity of the *N*-alkyl-carbazole compounds was increased by removing the carbonyl group in *N*-acyl-carbazoles. Various terminal alkylamino groups were introduced to provide a series of novel *N*-alkyl-carbazole derivatives **1**–**27** to obtain more proper CNS drugs. The title compounds were then biologically evaluated for their 5-HT_7R binding affinities, functional activities and selectivity profiles over other serotonin receptors. In addition, in order to analyze the SAR, a molecular docking study was performed.



K_i (5-HT_7R): 74 nM
Antidepressant effect *in vivo*

1) Remove the acyl group
2) Introduce several amine groups



1~27

Figure 2. Newly designed *N*-alkyl-carbazoles **1**–**27**.

Experimental

Chemistry. 9-(5-bromopentyl)-9*H*-carbazole (29, *n* = 3): To a mixture of 9*H*-carbazole (2 g, 11.96 mmol) and t-BuOK (1.3 g, 11.96 mmol) in THF (15 mL), 1,5-dibromopentane (2.4 mL, 17.94 mmol) was added. The reaction mixture was stirred at 40 °C for 10 h. The mixture was concentrated and the residue diluted with DCM. The solution was washed with water, dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (SiO_2, Hex:DCM = 10:1) to give product **29** (*n* = 3) in 44% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07–8.02 (m, 2H), 7.44–7.38 (m, 2H), 7.30 (d, *J* = 8.2 Hz, 2H), 7.22–7.16 (m, 2H), 4.19 (t, *J* = 7.1 Hz, 2H), 3.23 (t, *J* = 6.8 Hz, 2H), 1.82–1.70 (m, 4H), 1.45–1.36 (m, 2H).

2-(4-(5-(9*H*-Carbazol-9-yl)pentyl)piperazin-1-yl)phenol (24): To a solution of 9-(5-bromopentyl)-9H-carbazole (**29**, *n* = 3) (200 mg, 0.63 mmol) in ACN (5 mL), 1-(2-hydroxyphenyl)piperazine (169 mg, 0.95 mmol) was added. The reaction mixture was stirred under reflux conditions for overnight. The mixture was diluted with DCM, washed with H_2O. The organic phase was dried over Na_2SO_4, concentrated, and purified by column chromatography (SiO_2, DCM:MeOH = 20:1) to give product **24** (172 mg, 0.42 mmol) as a sticky oil in 66% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.7 Hz, 2H), 7.42–7.38 (m, 2H), 7.31 (d, *J* = 8.2 Hz, 2H), 7.19–7.15 (m, 2H), 7.09–7.06 (m, 1H), 7.03–6.99 (m, 1H), 6.92 (dd, *J* = 6.0 Hz, *J* = 1.5 Hz, 1H), 6.80 (td, *J* = 7.6 Hz, *J* = 1.5 Hz, 1H), 4.16 (t, *J* = 7.1 Hz, 2H), 2.79 (t, *J* = 4.7 Hz, 4H), 2.41 (brs, 4H), 2.20 (t, *J* = 7.6 Hz, 2H), 1.79 (quint, *J* = 7.4 Hz, 2H), 1.41 (quint, *J* = 7.5 Hz, 2H), 1.32–1.24 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.16, 140.06, 138.80, 125.96, 125.30, 122.51, 121.02, 120.06, 119.73, 118.48, 113.81, 108.33, 57.98, 53.47, 52.06, 42.52, 28.48, 26.26, 24.82; LC/MS (ESI$^+$): m/z: calcd for $C_{27}H_{31}N_3O$: 413.25, $[M + H]^+$; found: 414.15.

Serotonin Receptor Binding Affinity Assays. Eleven different concentrations (5X final concentration) of the test and reference compounds were prepared 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) in Standard Binding Buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4). Radioligand appropriately selected for each serotonin subtype receptor was diluted to five times of the assay concentration in the buffer. The selected radioligand (50 μL) was added into the wells of a 96-well plate containing 100 μL of the buffer. Then, prepared 50 μL of the

test and reference compound were treated in triplicate. Lastly, crude membrane 50 μL fractions of cells expressing recombinant receptor are dispensed into each well. The 250 μL solution is protected from light and incubated at room temperature for 1.5 h. Then it is harvested by rapid filtration onto Whatman GF/B glass fiber filters pre-soaked with 0.3% polyethylenimine using a 96-well Brandel harvester. In order to reduce non-specific binding, four rapid washes with chilled buffer (500 μL) are performed. Filters are kept in 6 mL of scintillation tubes and allowed to dry for overnight. The following day, 4 mL of EcoScint scintillation cocktail (National Diagnostics) are treated to each tube. The tubes are capped, labeled, and counted by liquid scintillation counting. The filter mats are dried, then scintillant is melted onto the filters and the radioactivity preserved on the filters is counted in a Microbeta scintillation counter. The IC_{50} values were calculated using Prism 4.0 (GraphPad Software, San Diego, California, US) and converted to K_i values.

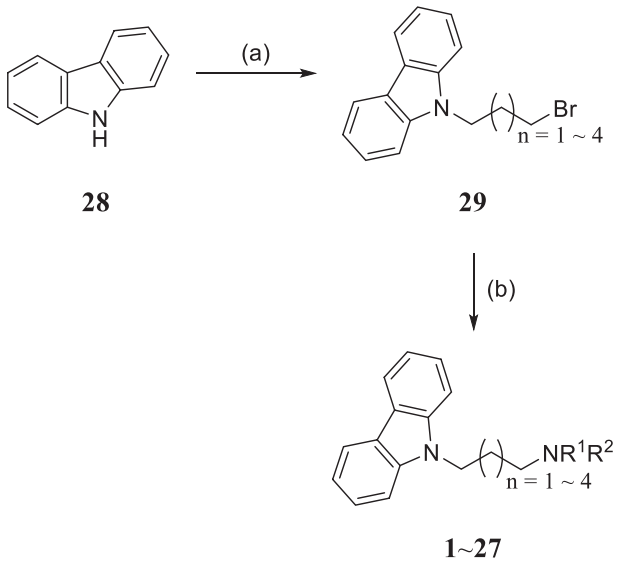
cAMP Accumulation Assay. HEK293T cells co-expressing the cAMP biosensor GloSensor-22F (Promega) and human 5-HT_7R were seeded (30 000–40 000 cells/20 μL/well) into white, clear-bottom, tissue culture 384 well plate in DMEM with 10% dialyzed FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. After 5–6 h recovery, medium are removed from the wells and cells were treated with 3% Glosensor cAMP reagent, luciferin (Promega) 20 μL in filter-sterilized assay buffer including 1X HBSS, 20 mM HEPES and third distilled water, pH 7.4. Then, 10 μL of ×4 of compounds **20**, **21**, **23**, **24**, SB-269970 (10 μM), and serotonin (100 nM) were prepared in assay buffer with 0.1% bovine serum albumin (BSA). After 30 min, cells were treated with 10 μL of compounds prepared above. The plates were incubated at room temperature for 15 min. The luminescence produced by accumulation of cAMP level was quantified by using Flexstation3 microplate reader (Molecular Devices, Downingtown, PA, USA). For conforming antagonism, the plate used above was treated with 10 μL of serotonin (100 nM). After 10 min, the luminescence was measured again. The results were analyzed by using Prism 6.0 program (Graphpad Software).

Molecular Docking Studies. For molecular docking study, the homology model protein structure of the 5-HT_7R was constructed by the SwissModel server using the co-crystal structure of 5-HT_{1B}R with ergotamine (PDB: 4IAR).[36] The Prepare Protein module (CHARMm force field) was used to prepare the homology model structure of 5-HT_7R. The binding site was determined by the center of co-crystal ligand, ergotamine. Ligands were prepared with protonation at pH 7.4 and energy minimization. The docking modes of ligands were procured using CDOCKER of Discovery Studio 2016 (v16.1.0.15350) program (Accelrys, Inc., San Diego, California, US) based on the CHARMm docking algorithm. For analysis, the docking poses of ligands were obtained up to 10 poses.

Results and Discussion

The *N*-alkyl-carbazoles containing a variety of the terminal alkylamino groups were synthesized straightforwardly in two steps (Scheme 1). Carbazole **28** underwent nucleophilic substitution with several dibromoalkanes ($BrCH_2(CH_2)_nCH_2Br$, n = 1–4) in the presence of *t*-BuOK at 40 °C to give *N*-bromoalkyl-carbazoles **29** in 17–53% yields. The *N*-bromoalkyl-carbazoles **29**, thus obtained, were treated with various secondary amines under reflux conditions to afford the title compounds **1**–**27** in 40–99% yields. The structures of final compounds **1–27** were verified by ^1H and ^{13}C nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS), which can be found in Appendix S1 (Supporting information).

The synthesized *N*-alkyl-carbazoles **1**–**27** were assessed for their binding affinities to 5-HT_7R through $^{[3H]}$LSD radioligand binding assays in transfected HEK293 cells including SB-269970 as a positive control. According to the previous results of *N*-acyl carbazoles,[34] we first fixed the carbon tether with n = 4. In order to investigate the role of the terminal amine functionalities, the title compounds with n = 4 and 15 different alkylamino groups (**1–15**) were evaluated for their binding affinities to 5-HT_7R, and the results are summarized in Table 1. Compounds with a terminal acyclic amino (**1** and **2**) or pyrrolidine (**3**) group failed to show potent binding affinity to 5-HT_7R with K_i values of 511, 1980, and 1546 nM, respectively. The piperidinyl carbozoles (**4**–**8**), however, showed substituent-dependent binding affinities: the alkylpiperidine derivatives (**4**–**6**) were found to bind to the target with only moderate to marginal binding affinities (K_i = 597–1598 nM) while the piperidines with an aromatic substituent, **7** and **8**, showed good 5-HT_7R binding affinities with K_i values of

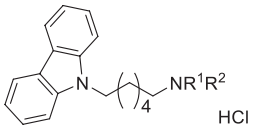


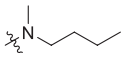
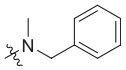
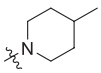
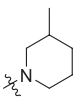
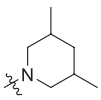
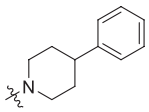
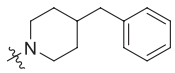
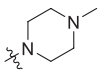
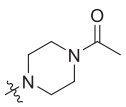
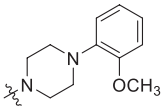
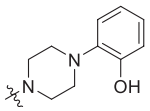
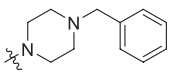
Scheme 1. Synthesis of the *N*-alkyl-carbazoles **1**–**27**: (a) 1,3-dibromopropane (n = 1), 1,4-dibromobutane (n = 2), 1,5-dibromopentane (n = 3), or 1,6-dibromohexane (n = 4), *t*-BuOK, THF, 40 °C, 17–53%; (b) amines, CH_3CN, reflux, 40–99%.

212 and 232 nM, respectively. Other amino functionalities such as azepane (**9**), morpholine (**10**), *N*-methylpieprazine (**11**) and *N*-acetylpiperazine (**12**) did not provide significant target binding affinities to the corresponding carbazole derivatives. However, *N*-arylpiperazine group made compounds **13** and **14** potent with *Ki* values of 145 and 89 nM, respectively. In contrast, compound **15** with an *N*-benzylpiperazine group showed only moderate binding affinity with a K_i value of 602 nM.

Next, we selected the most active compounds **7**, **13**, and **14**, and a moderately active compound **15** for further SARs study, and the effect of alkyl chain length on 5-HT$_7$R binding affinity was examined.[32,34] Thus, the chain length was sequentially reduced from $n = 4$ to $n = 1$, and the 5-HT$_7$R binding affinities of the corresponding compounds (**16**–**27**) were evaluated (Table 2). All of the compounds **16**–**27** showed good to potent binding affinities with K_i values

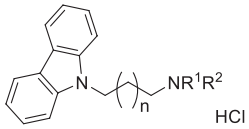
Table 1. Binding affinities (K_i) of the compounds **1**–**15** against 5-HT$_7$R.

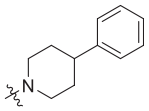
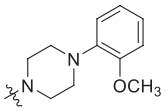
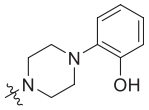
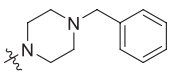


Compd	–NR^{1}R^2	pK_i[a]	K_i[b] in nM
1		6.29 ± 0.09	511
2		5.7 ± 0.08	1980
3		5.81 ± 0.08	1546
4		6.22 ± 0.08	597
5		6.04 ± 0.09	909
6		5.80 ± 0.08	1598
7		6.67 ± 0.08	212
8		6.63 ± 0.09	232
9		6.02 ± 0.08	952
10		45.3%[c]	—[d]
11		49.4%[c]	—[d]
12		5.83 ± 0.09	1485
13		6.84 ± 0.08	145
14		7.00 ± 0.10	89
15		6.22 ± 0.08	602
SB-269970		9.47 ± 0.05	0.34

[a] Values are the mean ± SEM of triplicate binding experiments.
[b] Values are calculated from pK_i.
[c] %-Inhibitions of the compound at 10 μM.
[d] Not determined.

Table 2. Binding affinities (K_i) of the compounds **16**–**27** against 5-HT$_7$R.



Compd	*n*	–NR^{1}R^2	pK_i[a]	K_i[b] in nM
16	1		6.45 ± 0.09	353
17	2		6.69 ± 0.08	205
18	3		6.75 ± 0.08	178
7	4		6.67 ± 0.08	212
19	1		6.81 ± 0.09	155
20	2		7.19 ± 0.09	64
21	3		7.19 ± 0.09	65
13	4		6.84 ± 0.08	145
22	1		7.02 ± 0.09	96
23	2		7.26 ± 0.09	55
24	3		7.51 ± 0.09	31
14	4		7.00 ± 0.10	89
25	1		6.39 ± 0.09	404
26	2		6.66 ± 0.08	218
27	3		6.63 ± 0.09	233
15	4		6.22 ± 0.08	602
SB-269970			9.47 ± 0.05	0.34

[a] Values are the mean ± SEM of triplicate binding experiments.
[b] Values are calculated from pK_i.

between 31 and 404 nM. Overall, the binding affinities of compounds with $n = 2$ or $n = 3$ were better than those with $n = 1$ or $n = 4$ and, among the series, compounds **20**, **21**, **23**, and **24** with $n = 2$ or $n = 3$ showed the most potent binding affinities to 5-HT$_7$R (K_i = 31–65 nM).

The most active compounds **20**, **21**, **23**, and **24** were then tested for their binding affinities to other 5-HTR subtypes, and the selectivity to 5-HT$_7$R were evaluated as selectivity indexes (SIs) (Table 3). The 5-HTR subtypes investigated in this study include 5-HT$_{1A}$R, 5-HT$_{1B}$R, 5-HT$_{1D}$R, 5-HT$_{1E}$R, 5-HT$_{2A}$R, 5-HT$_{2B}$R, 5-HT$_{2C}$R, 5-HT$_3$R, 5-HT$_5$R, and 5-HT$_6$R. All of the selected compounds **20**, **21**, **23**, and **24** showed 5-HT$_7$R-selective binding affinities. In particular, they were very selective over 5-HT$_{1B}$R, 5-HT$_{1E}$R, and 5-HT$_3$R, while moderate (5-HT$_{1D}$R, 5-HT$_{2C}$R, 5-HT$_5$R, and 5-HT$_6$R) and relatively low (5-HT$_{1A}$R, 5-HT$_{2A}$R and 5-HT$_{2B}$R) SI values were observed for other 5-HTR subtypes. Among the selected four compounds, **24** demonstrated the most potent (K_i = 31 nM) and selective 5-HT$_7$R binding affinity (Tables 2 and 3).

We also carried out functional assays to identify whether the four compounds **20**, **21**, **23**, and **24** act as agonist or antagonist against 5-HT$_7$R (Figure 3). The functional assays were performed by using HEK293T cells co-expressing the cAMP biosensor and human 5-HT$_7$R, and intracellular accumulation of cAMP was monitored. Upon treatment with the carbazole derivatives, no appreciable increase in cAMP level was observed, which demonstrates their lack of agonistic activity to 5-HT$_7$R. In contrast, serotonin-induced increase in cAMP in the HEK293T cells was attenuated upon co-treatment with **20**, **21**, **23**, or **24**. The inhibitory activity of the title carbazoles (10 μM) against 5-HT$_7$R was estimated to be between 46% and 56%. Under the same conditions, the well-known 5-HT$_7$R antagonist SB-269970 showed complete inhibition of serotonin-induced increase in cAMP. Thus, it indicates that the *N*-alkyl-carbazoles discovered in this study act as weak antagonists against 5-HT$_7$R.

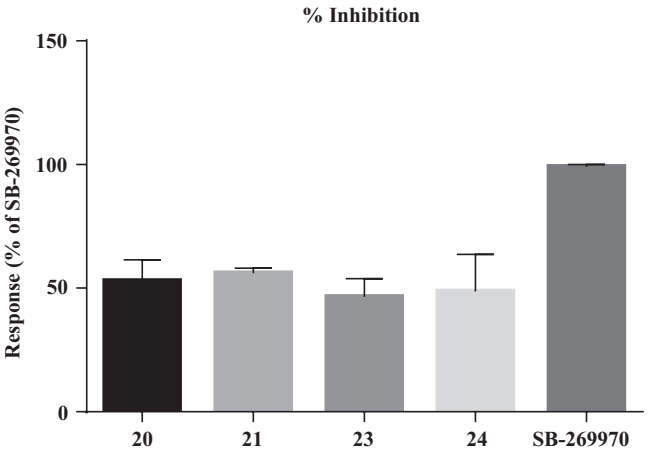


Figure 3. Percentage inhibitions of **20**, **21**, **23**, and **24** against 5-HT7R at 10 μM. The results are the mean of triplicate binding experiments.

In order to elaborate the difference in binding affinities of the *N*-alkyl-carbazoles against 5-HT$_7$R, we performed molecular docking study on **21** and **24**. We constructed a homology model of 5-HT$_7$R through the SwissModel server using the co-crystal structure of 5-HT$_{1B}$ with ergotamine (PDB: 4IAR) as a template.[36] Structures of **21** and **24** were prepared by protonation of their amino groups and energy-minimization. Both compounds docked to the ligand-binding site of the 5-HT$_7$R homology model structure by using the CDOCKER docking module in Discovery Studio. We have already found that there is a large and extended hydrophobic binding pocket in 5-HT$_7$R and the antagonists fully occupy the hydrophobic binding site.[29–34] Both of **21** and **24** were smoothly bound to the 5-HT$_7$R

Table 3. Selectivity of **20**, **21**, **23**, and **24** over other 5-HTR subtypes.

	20		**21**		**23**		**24**	
5-HTR subtypes	K_i[a] (nM)	SI[b]	K_i[a] (nM)	SI[b]	K_i[a] (nM)	SI[b]	K_i[a] (nM)	SI[b]
5-HT$_7$R	64	1.0	65	1.0	55	1.0	31	1.0
5-HT$_{1A}$R	104	1.6	172	2.7	111	2.0	180	5.8
5-HT$_{1B}$R	>10,000[c]	—[d]	>10,000[c]	—[d]	>10,000[c]	—[d]	>10,000[c]	—[d]
5-HT$_{1D}$R	545	8.5	671	10.3	1125	20.5	1342	43.3
5-HT$_{1E}$R	>10,000[c]	—[d]	>10,000[c]	—[d]	>10,000[c]	—[d]	>10,000[c]	—[d]
5-HT$_{2A}$R	78	1.2	121	1.9	138	2.5	168	5.4
5-HT$_{2B}$R	73	1.1	93	1.4	175	3.2	131	4.2
5-HT$_{2C}$R	449	7.0	490	7.5	739	13.4	531	17.1
5-HT$_3$R	>10,000[c]	—[d]	>10,000[c]	—[d]	>10,000[c]	—[d]	>10,000[c]	—[d]
5-HT$_5$R	596	9.3	588	9.1	608	11.1	335	10.8
5-HT$_6$R	1512	23.6	863	13.3	683	12.4	161	5.2

[a] Values are the mean of triplicate binding experiments.
[b] Selectivity Index (SI) = K_i for other 5-HTR subtype/K_i for 5-HT$_7$R.
[c] K_i values against 5-HT$_7$R were set as >10 000 nM due to lower than 50%-inhibition at 10 μM.
[d] Not calculated.

binding site sufficiently covering the hydrophobic pocket, and the docking poses of the two compounds were similar (Figure 4(a) and (b)). From the molecular docking study, 2D schematic molecule–protein interactions were deduced and shown in Figure 4(c) and (d). Like previously reported molecular docking studies, the key interaction was an ionic interaction between the protonated amino group of each compound and Asp162 in the 5-HT$_7$R binding site.[31,37–41] Besides, both **21** and **24** showed additional interactions such as ionic interactions with ASP142, π–sulfur interactions with Cys166, carbon hydrogen bond interactions with Cys231, π–π interactions with Phe343, and π–alkyl interactions with Val 163, Leu232, Ile233 and Arg367. However, comparison of the binding modes of **21** and **24** to 5-HT$_7$R reveals a significant difference. While **24** forms additional hydrogen bond with ASP142 by using its aromatic hydroxyl group as a hydrogen bonding donor (shown as an ionic interaction in Figure 4(d)), the methoxy substituent of 21 is not capable of forming this hydrogen bond. Thus, it could be presumed that **24** with an aromatic hydroxy group is more potent 5-HT$_7$R binder than **21** with a methoxy group due to the additional hydrogen bonding interaction between the hydroxy group and Asp 142.

Conclusion

We designed and synthesized a series of *N*-alkyl-carbazole derivatives. All of compounds **1**–**27** were biologically evaluated to obtain binding affinities, selectivity profiles, and functional activities against 5-HT$_7$R. Among those compounds, **20**, **21**, **23**, and **24** with *N*-(2-methoxyphenyl)piperazine or *N*-(2-hydroxyphenyl)piperazine moiety showed the best binding affinities with K_i values of 64, 65, 55, and 31 nM, respectively. In terms of the selectivity over other 5-HTR subtypes, **24** showed the best profiles compared with others. In the functional assays, selected four compounds **20**, **21**, **23**, and **24** showed weak antagonistic activities against 5-HT$_7$R. Overall, the compounds discovered in this study could be considered as potent and selective 5-HT$_7$R ligand with weak antagonistic effect. Among those, **24** showed the best properties. From the molecular docking study, it was shown that the aromatic hydroxyl group in **24** might play an important role to show potent 5-HT$_7$R-binding affinity by hydrogen bonding interaction with Asp142 in the ligand binding pocket. Based on these results, further modifications and evaluations such as pharmacokinetics and *in vivo* test for depression and sleep disorder will be done in due course.

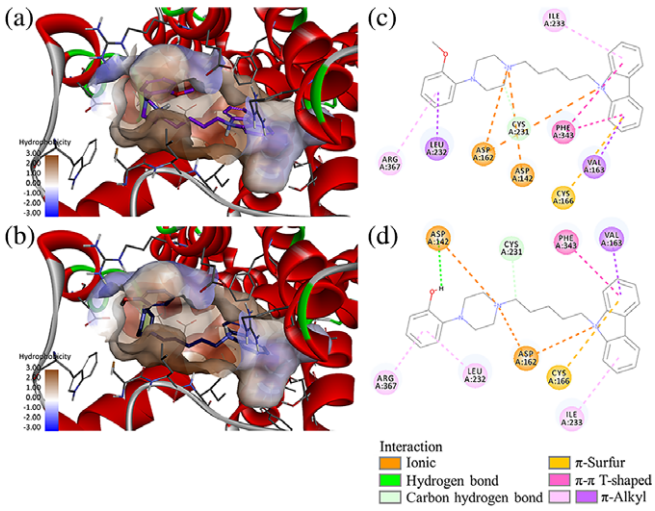


Figure 4. Molecular docking modes of (a) **21** (violet) and (b) **24** (dark blue) and 2D schematic molecule–protein interactions of (c) **21** and (d) **24**.

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Supporting Information. Additional supporting information may be found online in the Appendix S1 (Supporting Information) section at the end of the article.

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