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5-HT_{2C} receptor selectivity and structure–activity relationship of *N*-methyl-*N*-(1-methylpiperidin-4-yl)benzenesulfonamide analogs

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ABSTRACT

Agonists of the 5-HT_{2C} receptor have attracted much attention as therapeutic agents for the treatment of obesity. Subtype selectivity against other 5-HT_2 receptors is one of the most important prerequisites for reducing side effects. We present the synthesis of *N*-methyl-*N*-(1-methylpiperidin-4-yl)benzenesulfonamide analogs and their structure–activity relationship studies on 5-HT_{2A} and 5-HT_{2C} receptors. Although the compounds showed nanomolar activity to the 5-HT_{2C} receptor, their selectivity against the 5-HT_{2A} receptor was modest to low. Molecular modeling studies using homology modeling and docking simulation revealed that selectivity originated from subtype specific residues. The observed binding modes and receptor–ligand interactions provided us a clue for optimizing the selectivity against the 5-HT_{2A} receptor.

5-Hydroxytryptamine (5-HT) is a neurotransmitter derived from tryptophan that plays a variety of important roles in the human body. Its receptor has various functions related to the central nervous system, such as mood, appetite, and sleep.[1,2] The 5-HT_{2C} receptor has drawn much attention from the pharmaceutical industry because of its involvement in regulating body weight for both humans and rats. 5-HT_{2C} receptor knockout mice were severely obese and defective in food intake; appetite and body weight in humans were reduced by 5-HT_{2C} agonists in clinical studies.[3–5] This receptor belongs to the 5-HT_2 receptor family together with 5-HT_{2A} and 5-HT_{2B}, and is found in high concentrations exclusively in the central nervous system (CNS). Various 5-HT_{2C} agonists have been developed and have shown weight loss in humans by inhibiting food intake. For example, mCPP and PNU-22394, which are non-selective 5-HT_{2C} agonists, have caused considerable weight loss in clinical trials.[6] Selective 5-HT_{2C} agonists, such as BVT933 and APD-356, also have shown significant weight loss.[7]

One of the key requirements for developing more effective and safe 5-HT_{2C} agonists is selectivity against subtypes. The 5-HT_{2A} receptor is included in the same family as the 5-HT_{2C} receptor and is also widely expressed in the brain, especially in the cerebral cortex. It is implicated in hallucinogenic action induced by drugs such as lysergic acid diethylamide (LSD).[8,9] Therefore, there is a need to develop 5-HT_{2C} agonists with high selectivity against 5-HT_{2A}. To this end, we carried out high-throughput screening using a subset of Chemdiv GPCR focused library, and several chemical scaffolds as a 5-HT_{2C} agonist were uncovered. By modifying one of these structures, we designed a novel *N*-methyl-*N*-(1-methylpiperidin-4-yl)benzenesulfonamide as a 5-HT_{2C} agonist. In this study, we report the synthesis and biological evaluation of analogs of this scaffold. Furthermore, to reveal the structural basis of selectivity against 5-HT_{2A}, binding modes to both receptors were investigated through molecular modeling. First, due to the unavailability of crystal structures for these receptors, comparative homology modeling was used to construct the 3D coordinates of the receptors. Then, the synthesized compounds were docked into the active sites of the models. The study of receptor–ligand interactions offered structural insight for selectivity, allowing further optimization of the current scaffold.

The synthesis of the target compounds is shown in Scheme 1. First, methyl-(1-methylpiperidin-4-yl)amine was synthesized from 1-methyl-4-piperidone by reductive amination with methylamine using sodium cyanoborohydride in 45% yield.[10] Then, this secondary amine was reacted with R^1-substituted benzenesulfonyl chlorides in the presence of triethylamine to give sulfonamides in 72–96% yield.[11]

The biological activity of the small focused library of benzenesulfonamide analogs was evaluated for their binding affinities to the serotonin receptors 5-HT_{2A} and 5-HT_{2C}[12] (Table 1). In this series of compounds, studies were carried out to examine the effect of

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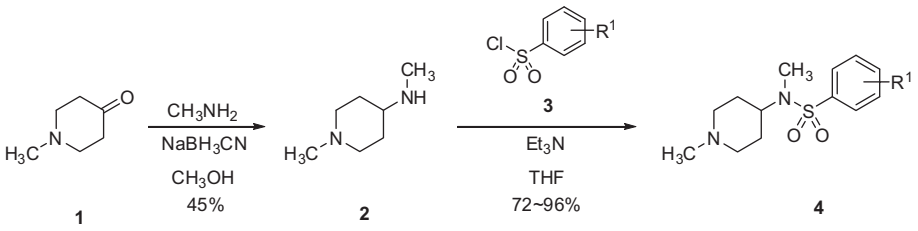
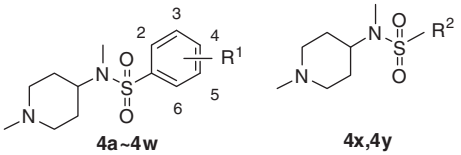


Scheme 1. Synthesis of *N*-methyl-*N*-(1-methylpiperidin-4-yl)benzenesulfonamide analogs.

the phenyl ring substitution on the binding affinity for serotonin receptors. This structural feature was identified on the basis of the unsubstituted compound **4d**, having IC_{50} values of 1020 and 4260 nM for 5-HT_{2A} and 5-HT_{2C}, respectively. First, the compounds with hydrophobic alkyl or alkoxy substituents at the *para*-position of the phenyl ring, such as compounds **4f–j**, **4l**, and **4x**, were active for both 5-HT_{2A} and 5-HT_{2C} with IC_{50} values within the range of 50–1000 nM. In particular, linear chains at the *para*-position, such as the butoxy group in compound **4l**, showed the best activity in this series, with IC_{50} values of 77 and 55 nM for 5-HT_{2A} and 5-HT_{2C}, respectively. Meanwhile, the bulkier substituents, such as *iso*-propyl or *tert*-butyl in compounds **4f** and **4g**, exhibited reduced affinities for the serotonin receptors (161–1060 nM). When the *para*-substitution was changed to *meta*- or *ortho*-substitution, the affinity values were generally reduced, as shown in compounds **4e** (*para*-methyl, 2130 and 179 nM), **4s** (*meta*-methyl, 3450 and 579 nM) and **4v** (*ortho*-methyl, 1550 and 1820 nM). This trend was also observed for F-substitution in compounds **4a**, **4q**, and **4t**, as well as Cl-substitution in compounds **4b**, **4r**, and **4u**. In this case, when both *para*- and *meta*-positions of the phenyl ring were substituted, as in compound **4w** (3,4-dimethyl), the affinity was further decreased to 5980 and 1940 nM.

Polar substituents were detrimental to both activities. For example, compounds **4n** and **4o**, with cyano and nitro groups as polar substituents, showed very poor affinity (>10,000 nM), as did other polar substituents including fluoro, trifluoromethoxy, and trifluoromethyl groups (compounds **4a**, **4m**, and **4p**; 1680–5900 nM for 5-HT_{2A}, and 2530–>10,000 nM for 5-HT_{2C}). With regard to the selectivity between 5-HT_{2A} and 5-HT_{2C}, compound **4e** (*para*-methyl) showed better affinity for 5-HT_{2C} over 5-HT_{2A} and had the highest selectivity value (15) in this series (2130 vs 179 nM). Compounds **4s** (*meta*-methyl, 3450 vs 579 nM) and **4x** (1-naphthyl, 502 vs 99 nM) were also efficacious, but with lesser degrees of selectivity. However, opposite results were observed in compounds **4f** (*para*-isopropyl, 161 vs 1010 nM), **4m** (*para*-trifluoromethoxy, 2450 vs >10000 nM), **4c** (*para*-iodo, 160 vs 766 nM), and **4d** (unsubstituted, 1020 vs 4260 nM) with better affinity for 5-HT_{2A} over 5-HT_{2C}. Although

Table 1
Binding activity for *N*-methyl-*N*-(1-methylpiperidin-4-yl)benzenesulfonamides at 5-HT_{2A} and 5-HT_{2C}



Compd.	R^1	R^2	Experimental IC_{50} at 5-HT_{2A} (nM)	Experimental IC_{50} at 5-HT_{2C} (nM)	Subtype selectivity[a]
4a	4-F		5900	2534	2.3
4b	4-Cl		537	1208	0.44
4c	4-I		160	766	0.21
4d	H		1019	4260	0.24
4e	4-Methyl		2132	179	11.9
4f	4-Isopropyl		161	1012	0.16
4g	4-*tert*-Butyl		519	1055	0.49
4h	4-Ethyl		452	164	2.8
4i	4-Propyl		329	471	0.70
4j	4-Phenyl		281	523	0.54
4k	4-Methoxy		831	1863	0.45
4l	4-Butoxy		77	55	1.4
4m	4-Trifluoromethoxy		2448	>10000	<0.25
4n	4-Cyano		>10000	>10000	–
4o	4-Nitro		>10000	>10000	–
4p	4-Trifluoromethyl		1683	5733	0.30
4q	3-F		1859	2422	0.77
4r	3-Cl		986	603	1.6
4s	3-Methyl		3451	579	6.0
4t	2-F		>10000	>10000	–
4u	2-Cl		3180	1195	2.7
4v	2-Methyl		1552	1824	0.85
4w	3,4-Dimethyl		5981	1944	3.1
4x		Naphthalene-1-yl	502	99	5.1
4y		Benzo thiophen-7-yl	>10000	>10000	–

[a] Subtype selectivity: IC_{50} at 5-HT_{2A}/IC_{50} at 5-HT_{2C}.

compound **4l** showed the best activity to the 5-HT_{2C} receptor, compounds **4e** and **4x** were more promising candidates for 5-HT_{2C} agonists when selectivity was considered. To find the structural basis for further optimization of selectivity, we set out to use molecular modeling methods.

Structure-based molecular modeling methods were adopted for the purpose of investigating receptor–ligand interactions. In the absence of crystal structures for our target proteins, homology modeling is a useful way of building the three-dimensional protein structures. The active form of human β_2-adrenergic receptor was used as a template crystal structure (PDB code: 3P0G). The sequences of the human 5-HT_{2A} (P28223) and 5HT_{2C} (P28335) receptors were retrieved from the Swiss-Prot database as a target. Automated sequence alignment of the template with the targets was performed using the ClustalW method. The obtained results were manually adjusted to properly align the highly conserved residues[13] of the second extracellular loop (EL) and the fifth TM region. Full sequence alignments of the targets and template generated by the methods above are shown in Figure 1. The sequence identity and similarity between the targets and template (5-HT_{2A}: 21.2%/36.7%, 5-HT_{2C}: 24.4%/41.5%) appeared to be too close to the border line (20% identity) to rely on the homology model. However, the statistics in the TM region were almost two-fold higher than those of the proteins as a whole, indicating good reliability for application to homology modeling (5-HT_{2A}: 39.1%/62.8%, 5-HT_{2C}: 41.4%/64.7%).

The sequence alignment also revealed the possible origin of selectivity—regions in which residues were different among the subtypes. Most of the residues in the proximity of the putative ligand binding pocket were well conserved across the subtypes and even with the template. However, two positions differed in their residue type: position 4.56 (numbering scheme by Ballesteros and Weinstein[14]), where isoleucine in 5-HT_{2A} was substituted by the smaller valine in 5-HT_{2C} and position 5.46, where the serine in 5-HT_{2A} was replaced by non-polar alanine in 5-HT_{2C}. Therefore, these different residues might have different interactions with the ligand. On the other hand, loop regions in protein structures mutate in high probability throughout the same subtype family. Furthermore, extracellular loop 2 (EL2) region in the GPCR family has been considered important because they affect the protein–ligand interactions, thus influencing the docking results and virtual screening performance.[15] We thus carefully considered this region for subtype-specific ligand binding, but both subtypes displayed identical residues (leucine at ×l2.52 and aspartate at ×l2.54). Therefore, the EL2 region does not play any role in ligand selectivity.

The final alignment and templates were submitted as input in MODELLER 9v4[16] implemented in Discovery Studio 2.5 (http://accelrys.com). We chose the best final homology models for each subtype of receptor by the following method. In total, 100 candidate structures for 5-HT_{2A} and for 5-HT_{2C} were initially generated. Among them, 10 models having the lowest total probability density function (PDF) value of MODELLER were selected based on the assumption that the lowest energy conformation is near the native structure.[17] The side chains of these models were refined using local rotamer libraries.[18] Then, minimization of these structures was performed with the CHARMm forcefield.[19] During the minimization, the protein backbones were held fixed to prevent collapse of the small GPCR receptor binding site. The optimized structures were evaluated by their ability to accommodate the known ligand structures from docking simulation. The interactions between the ligands and receptors were considered

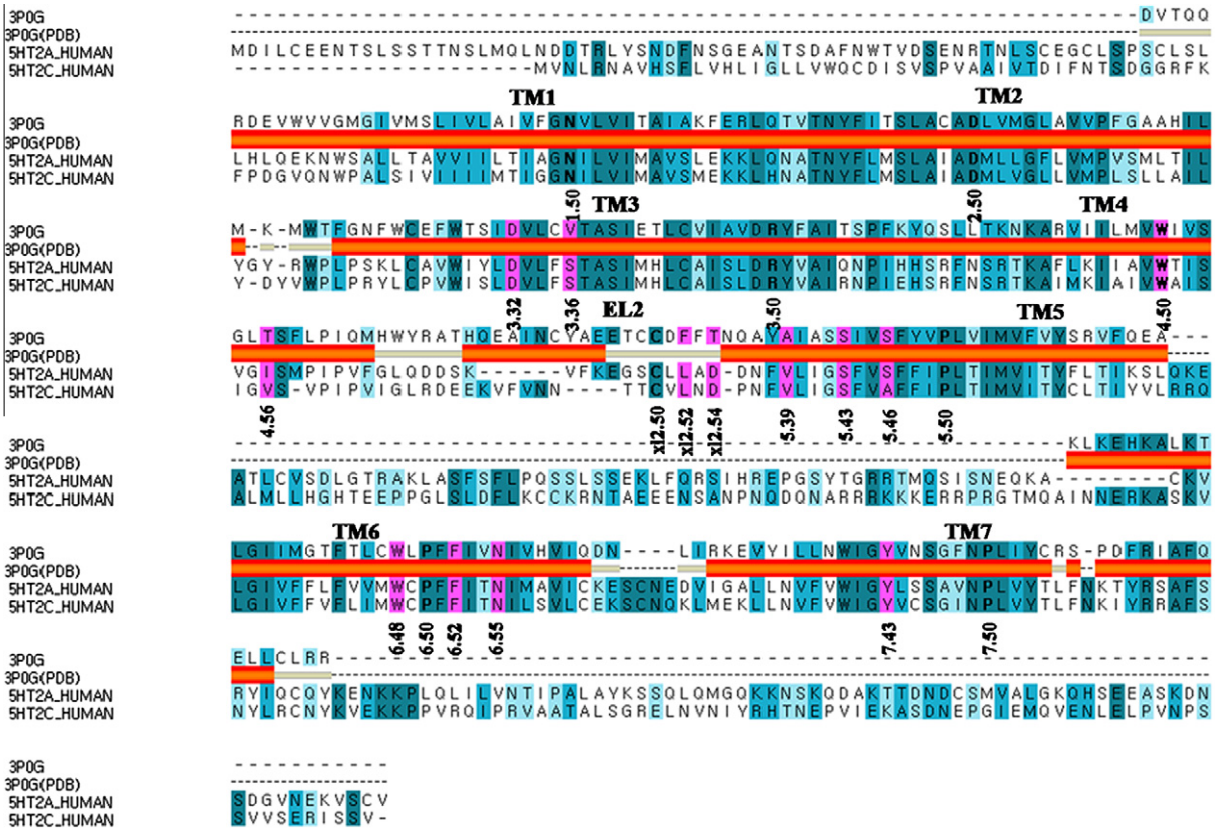


Figure 1. Sequence alignment of human 5-HT_{2A} and 5-HT_{2C} relative to β_2 adrenergic receptor. Identical residues are dark blue, and closely related residues are light blue. The most conserved residues among the GPCR super-family, cysteine residues of the disulfide bridge between the second extracellular loop region and the third transmembrane region, are shown in bold. Residues involved in ligand binding are pink and are numbered according to the Ballesteros–Weinstein scheme.

reliable if they were consistent with site-directed mutagenesis data[20–22] (Supplementary data). After discarding the models that missed interactions, the models were submitted to PROCHECK[23] and QPACK[24] analysis to validate the geometry of the residues and assess the quality of the final structures. The Ramachandran plot of PROCHECK showed that 91.7% and 92.8% of residues were in the most favored regions for the 5-H_{2A} and 5-HT_{2C} homology models, respectively. This result indicated the reasonable accuracy of the obtained structures (Supplementary data).

Molecular docking was carried out using the final homology models to study the receptor–ligand interaction. GOLD5.0[25] molecular docking software was used to produce a protein-ligand complex. The active site was defined using all the residues within a radius of 10 Å around the highly conserved Asp3.32, which has an important role in charge-assisted hydrogen bond interaction. In addition, soft potential was applied to the residues in the active site to make an induced fit effect during docking. As shown in Figure 2, all of the subtype-specific residues are exposed to the ligand binding pocket, implying their importance for interacting with ligands. Furthermore, the derivatizing groups were located near these residues, improving the chances for optimizing selectivity. It was noted that the selectivity determinant regions were mostly hydrophobic with residues such as Gly5.42, Thr3.37, and Val3.33, suggesting the necessity of hydrophobic substituents for activity. This observation was consistent with our observations from SAR studies that all of the compounds with polar substituents in the R^1 phenyl ring had no activity to either receptor. All other residues had similar or identical conformations.

It was suggested by the docking results of compound **4e** and **4f** that the bulkier substituent would be harmful to 5-HT_{2C} selectivity over 5-HT_{2A}. Figure 2 shows that compounds **4e** and **4f**, which displayed the highest selectivity for each receptor, have different binding conformations and interactions with each active site. While highly conserved ionic interactions between the protonated nitrogen of piperidine and residue Asp3.32 occurred in both cases, the sulfone groups of the ligands interacted with different residues: Asn6.55 in 5-HT_{2A} and Ser3.36 in 5-HT_{2C}. This result was probably due to different residues located below the R^1 phenyl ring. In the case of 5-HT_{2C}, Ala5.46 is positioned to be directly facing the R^1 phenyl ring. Therefore, the ligand adopted a binding mode where the R^1 phenyl ring was located deeper in the hydrophobic cleft (surrounded by Val3.33, Thr3.37, Val5.45, and Ala5.46). As a result, the methyl substituent of compound **4e** was well positioned without any steric hindrance, whereas the bulkier isopropyl group of compound **4f** had a poorer fit in the hydrophobic cleft due to repulsion of surrounding residues. As for 5-HT_{2A}, the mutation from Ala5.46 to the polar Ser5.46 caused the hydrophobic cleft to become less hydrophobic, thereby forcing the R^1 phenyl away from position 5.46 due to unfavorable polar–non-polar interactions. As a result, the sulfone group formed hydrogen bonds with Asn6.55 instead of Ser3.36. The resulting binding orientation caused the *para*-substituent to come into closer contact with Ile4.56 located in front of the substituent. Therefore, the bulkier isopropyl substituent of compound **4f** would be more stabilized than the smaller methyl group of compound **4e**, probably because of well-established van der Waals interactions.

The binding mode suggested above also makes it possible to explain activities and selectivities of other compounds between two subtypes. The ethyl substituent of compound **4h**, which is not bulky, but linear, showed high activity to 5-HT_{2C} and lower activity to 5-HT_{2A}, although the selectivity was not significant. This result is in line with the explanation that the ligands exhibit different binding conformation to each receptor, and 5-HT_{2C} hydrophobic pocket can accommodate smaller and more linear component of *para*-substituent of phenyl ring than that of 5-$HT2_A$. Compound **4d**, which has no substituent at the *para*-position, showed the reversal selectivity compared to **4e**, but both activities were decreased simultaneously, which supports the existence of hydrophobic cavity near the *para*-position of R^1 phenyl ring where filling the substituents with appropriate size and volume in the space is needed for a stabilization of binding energy. On the other hand, the activities of compounds with halogen substituents such as **4a**, **4b**, and **4c** can be accounted for by the binding mode above. The smallest electron size, F-substituent, has better affinity to 5-HT_{2C}, whereas the largest electron size, I-substituent, was more stabilized in the active site of 5-HT_{2A}. This is consistent with the result that the smaller *para*-methyl substituent of compound **4e** has higher affinity to 5-HT_{2C} than that of **4f**, whereas bulkier isopropyl group of **4f** binds the pocket of 5-HT_{2A} more favorably than that of 5-HT_{2C}. It was noticed that the activities of compound **4c** to

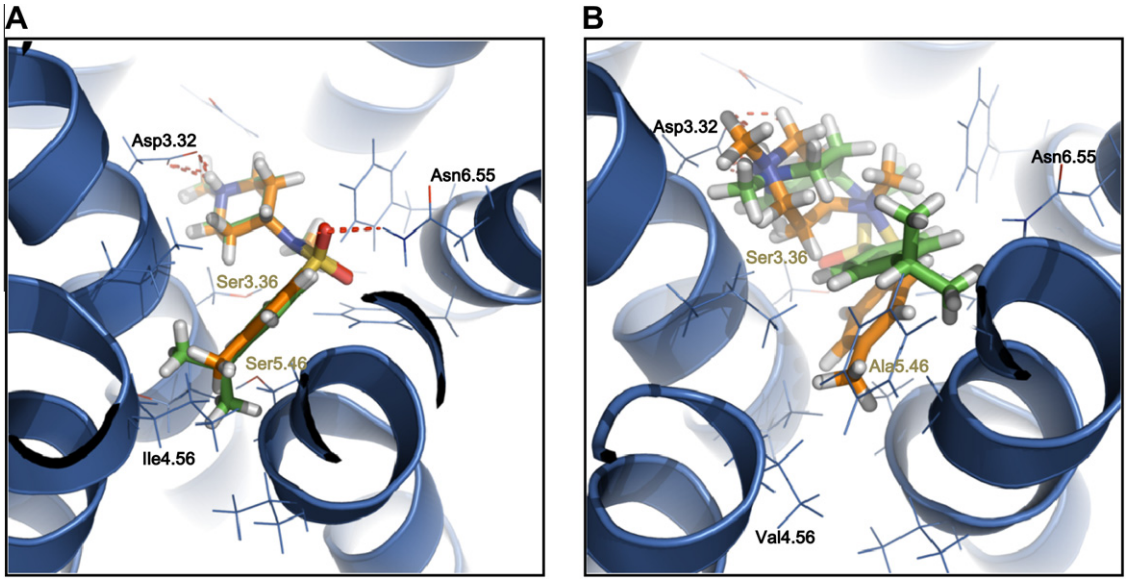


Figure 2. Results from docking of compounds **4e** and **4f** in each binding site of the homology models. (A) Homology model of 5-HT_{2A} and docking result to its active site. (B) Homology model of 5-HT_{2C} and docking result to its active site. The ligand structures are displayed in the stick form and colored orange and green for **4e** and **4f**, respectively. The receptor residues are represented in line form and information is listed for residues involved in ligand interactions.

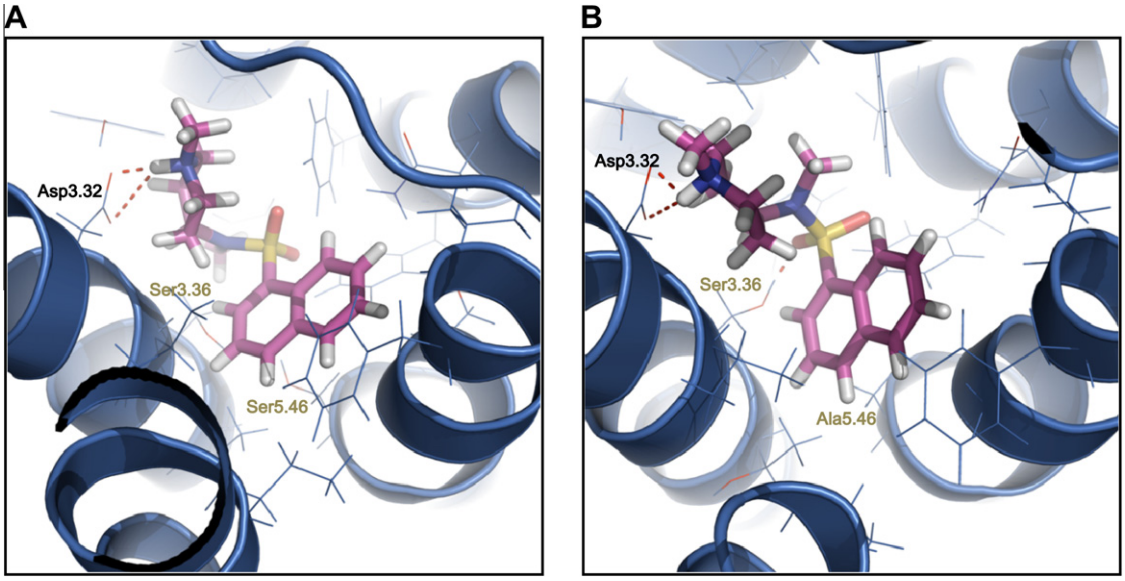


Figure 3. Results from docking compound **4x** in each binding pocket of the homology model. (A) Homology model of 5-HT_{2A} and docking result to its active site. (B) Homology model of 5-HT_{2C} and docking result to its active site. The ligand structures are displayed in stick form and the receptor residues are in line form.

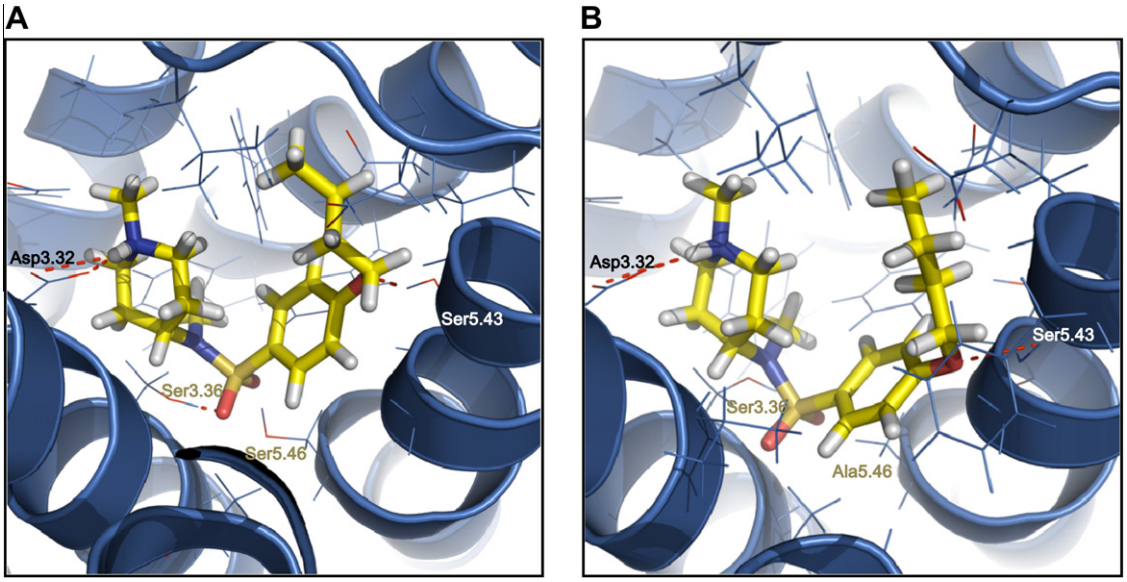


Figure 4. Results from the docking of compound **4l** into each binding pocket of the homology models. (A) Homology model of 5-HT_{2A} and docking result to its active site. (B) Homology model of 5-HT_{2C} and docking result to its active site. The ligand structure is displayed in stick form and the receptor residues are in line form.

both subtypes are similar to that of **4f**, although properties of iodo and isopropyl substituents are quite different each other. It seems that similar activities are attributable to a similar steric effect to the hydrophobic pocket of both moieties, which contributes more dominantly than other electronic effects that are different between these two substituents.

Compound **4x** also seemed to be a promising candidate for further optimization. Although the compound displayed high activity to the 5-HT_{2C} receptor (99 nM for 5-HT_{2C}), the selectivity was determined to be moderate (IC_{50} at 5-HT_{2A}/IC_{50} at 5-HT_{2C} = 5.1). Contrary to the compounds with a R^1 phenyl ring, this compound showed similar binding modes for both active sites (Figure 3). The naphthyl substituent was leaning against TM5 and faced the subtype specific residues (Ser for 5-HT_{2A} and Ala for 5-HT_{2C}). It was therefore clear that the activity difference of the compound was attributable to the changed hydrophobic interaction that originated from the mutation of Ala to Ser at position 5.46. The binding mode analysis of compounds **4e**, **4f**, and **4x** prompted us to modify the naphthyl substituent such that it had an additional methyl or ethyl group to better fit the hydrophobic active site.

Compound **4l** showed the highest activity for the 5-HT_{2C} receptor, but with no selectivity against the 5-HT_{2A} receptor. Our homology model and docking experiment also provided structural insight for the underlying mechanism of such biological activity. As shown in Figure 4, while hydrogen bond interactions with Asp3.32 and Ser3.35 were similar to previous compounds, there were additional hydrogen bond interactions between the butoxy oxygen and Ser5.43 in both cases. These extra hydrogen bond interactions would explain why the compound is equipotent on 5-HT_{2A} and 5-HT_{2C}. Therefore, the strategy of attaching an alkoxy substituent

or other substituents that can act as hydrogen bond acceptors is unsuitable for ligand selectivity, which is an essential requirement for the development of a 5-HT_{2C} agonist.

In summary, thorough investigation of R^1 and R^2 substituents of *N*-methyl-*N*-(1-methylpiperidin-4-yl)benzenesulfonamide analogs revealed a set of compounds with nanomolar activity to the 5-HT_{2C} receptor. In an effort to improve the selectivity against the 5-HT_{2A} receptor, three-dimensional structures of both subtypes were constructed using homology modeling. Molecular docking of synthesized compounds into receptor models and the resulting binding modes guided us in understanding the structural determinants for selectivity. These models also provided us a clue for modifying the structures to improve selectivity. Further modification and optimization of the current scaffold as 5-HT_{2C} agonists will be published in the near future.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.001.

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11. General procedure for preparation of **4a**–**y**, *N*-methyl-*N*-(1-methylpiperidin-4-yl)-*p*-butoxybenzenesulfonamide (**4d**): To a solution of methylamine hydrochloride (17.7 g, 262 mmol) in methanol (150 mL), potassium hydroxide (4.00 g, 71.3 mmol) was added and the mixture was stirred until the potassium hydroxide was completely dissolved. 1-Methyl-4-piperidone (22.6 g, 200 mmol) was then added and the reaction mixture was stirred for 15 min. Next, a solution of sodium cyanoborohydride (4.74 g, 75.4 mmol) in methanol (30 mL) was added dropwise over 30 min and the reaction mixture was stirred for an additional 30 min. Potassium hydroxide (15.0 g, 267 mmol) was added, the mixture was stirred for 30 min, and the resulting precipitate was filtered off. The filtrate was concentrated to 80 mL in vacuo at 30 °C and water (10 mL) and brine solution (25 mL) were added. The separated organic layer was saved and the aqueous layer was extracted twice with diethyl ether (50 mL each). The combined organic layer was extracted three times with cold 6 M hydrochloric acid (20 mL each), and then potassium hydroxide was slowly added to the acidic aqueous layer at 0 °C until the solution reached pH 12. This solution was extracted three times with diethyl ether (40 mL each). The organic layer was dried with anhydrous magnesium sulfate, filtered, and concentrated in vacuo at 30 °C. The resulting oil was purified by Kugelrohr distillation at 53 °C at 10 mmHg to give 11.5 g (45%) of methyl-(1-methylpiperidin-4-yl)amine as a clear oil. To a solution of obtained methyl-(1-methylpiperidin-4-yl)amine (0.200 g, 1.56 mmol) in THF (2 mL), triethylamine (0.118 g, 1.17 mmol) was added, and the mixture was cooled to 0 °C. *p*-Butoxybenzenesulfonyl chloride (0.200 g, 0.780 mmol) was added and the reaction mixture was stirred for 3 h at room temperature. Brine solution (1 mL) and water (1 mL) were added to the mixture, and the mixture was extracted three times with methylene chloride (6 mL each). The organic layer was dried with anhydrous magnesium sulfate, filtered, and concentrated in vacuo. The resulting oil was purified by column chromatography (methanol:ethyl acetate = 3:1) to give 0.212 g (80%) of the title compound: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.70 (2H, dt, J = 8.9, 2.9 Hz), 6.93 (2H, dt, J = 8.9, 2.8 Hz), 4.00 (2H, t, J = 6.5 Hz), 3.77 (1H, m), 2.85 (2H, t, J = 11.7 Hz), 2.72 (3H, s), 2.25 (3H, s), 2.00 (2H, t, J = 10.8 Hz), 1.73 (4H, m), 1.46 (4H, m), 0.97 (3H, t, J = 7.3 Hz). HRMS (ESI, M+H); 340.1821 calculated for $C_{17}H_{28}N_2O_3S$, found 341.1906.
12. Biological assays: Radioligands [3*H*]ketanserin and [3*H*]imipramine were purchased from PerkinElmer (PerkinElmer Life and Analytical Sciences, Boston, USA), and [3*H*] mesulergine was obtained from Amersham Biosciences (Buckinghamshire, UK). Cloned human recombinant serotonin 5-HT_{2A} and 5-HT_{2C} receptors were obtained from Euroscreen (Brussels, Belgium). Competition binding assays at the serotonin 5-HT_{2A} receptor were performed using 1 nM [3H]ketanserin by the protocol provided by the supplier of CHO-K1 membranes (Euroscreen, Brussels, Belgium) with minor modifications. Briefly, receptor membranes (15 μg/well) were incubated at 25 °C for 60 min in a final volume of 0.25 mL reaction mixture containing [3*H*]ketanserin and various concentrations of the drug in 50 mM Tris–HCl (pH 7.4*)* buffer containing 5 mM $CaCl_2$, 0.1% ascorbic acid and 10 μg/mL saponin. Then, the incubations were terminated by rapid filtration using an Innotech cell harvester (Innotech Biosystems, Switzerland) through Whatman GF/C glass fiber filter presoaked in 0.05% Brij. The filter was covered with MeltiLex, sealed in a sample bag followed by drying in a microwave oven, and counted using MicroBeta Plus (Wallac, Finland). Non-specific binding was determined in the presence of mianserin (0.5 μM). Competition binding studies were carried out with 7–8 varied concentrations of the test compounds run in duplicate tubes, and isotherms from three assays were calculated by computerized non-linear regression analysis (GraphPad Prism Program, San Diego, USA) to yield IC_{50} values. Frozen membranes from stable CHO-K1 cell line expressing the human recombinant 5-HT_{2C} receptor were used. For the binding assay, [3*H*]mesulergine (1 nM), receptor membrane (4 μg/well) and test compounds were added into 50 mM Tris–HCl (pH 7.7) buffer containing 0.1% ascorbic acid and 10 μM pargyline. Non-specific binding was determined using 0.5 μM mianserin. The incubations were performed for 30 min at 37 °C, then terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked in 1% BSA.
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