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Discovery of SMP-304, a Novel Benzylpiperidine Derivative with Serotonin Transporter Inhibitory Activity and 5-HT_{1A} Weak Partial Agonistic Activity showing the Antidepressant-like Effect

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Drug Research Division, Sumitomo Dainippon Pharma Co., Ltd., 33-94 Enoki-cho, Suita, Osaka 564-0053, Japan

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ABSTRACT

We report the discovery of a novel benzylpiperidine derivative with serotonin transporter (SERT) inhibitory activity and 5-HT_{1A} receptor weak partial agonistic activity showing the antidepressant-like effect. The 3-methoxyphenyl group and the phenethyl group of compound **1**, which has weak SERT binding activity, but potent 5-HT_{1A} binding activity, were optimized, leading to compound **35** with potent and balanced dual SERT and 5-HT_{1A} binding activity, but also potent CYP2D6 inhibitory activity. Replacement of the methoxy group in the left part of compound **35** with a larger alkoxy group, such as ethoxy, isopropoxy or methoxy-ethoxy group ameliorated CYP2D6 inhibition, giving **SMP-304** as a candidate. **SMP-304** with serotonin uptake inhibitory activity and 5-HT_{1A} weak partial agonistic activity, which could work as a 5-HT_{1A} antagonist, displayed faster onset of antidepressant-like effect than a representative SSRI paroxetine in an animal model.

1. Introduction

Major depressive disorder (MDD), a major worldwide health issue, is commonly treated with selective serotonin reuptake inhibitors (SSRIs), which are the first line drugs for depression. However, the use of SSRIs has a number of drawbacks, including slow onset and insufficient efficacy. [1-4] Indeed, it takes 2 to 3 weeks of treatment for SSRIs to start producing beneficial antidepressant effect. Furthermore, over one third of patients with MDD do not response to SSRIs. This is because psychiatric disorders, such as depression and schizophrenia have complex etiology. Therefore, it is believed that drugs that can act on a broad range of neuron network would be advantageous for the treatment of psychiatric diseases. [5] The slow onset of SSRIs is due to increased serotonin levels, which in turn induces an initial decrease in the firing activity of serotonin-producing neurons. As this decrease is mediated via activation of somatodendritic 5-HT_{1A} auto-receptor, serotonin levels in the synaptic cleft can be increased only after desensitization of 5-HT_{1A} auto-receptor. [6,7] It has been reported that the use of pindolol, an adjuvant 5-HT_{1A} and β adrenergic receptor antagonist, accelerates the onset of SSRIs therapy. [8,9] It is believed that this acceleration is mediated by the blockage based on 5-HT_{1A} antagonistic activity of negative feedback inhibition via 5-HT_{1A} auto-receptor in response to increased serotonin. [10,11] There is another non-clinical evidence to support the role of 5-HT_{1A} receptors in this response, which comes from the results that a rapid onset of antidepressant effect was observed in combination administration with SSRIs and WAY-100635, a highly selective 5-HT_{1A} receptor antagonist, suggesting the contribution of 5-HT_{1A} antagonistic activity in human. [12] Based on the evidences, it is believed that 5-HT_{1A} auto-receptor antagonists would presumably accelerate SSRIs onset. Accordingly, we launched an exploratory research program aimed at finding antidepressant drug candidates with serotonin transporter (SERT) inhibitory activity and 5-HT_{1A} receptor antagonistic activity.

Morphy and Rankovic propose three approaches to multiple ligands (DMLs) lead generation, i.e. “designing in”, “designing out” and “balancing” activity. [13] The “designing in” approach focuses on incorporating a second activity into a highly selective ligand, which originally has little or no activity for a second target. The “designing out” approach emphasizes the removal or weakening of an undesired activity in a non-selective ligand, which has both the desired and undesired activities. The “balancing” approach entails enhancing the second-activity of a suboptimally selective ligand with weak activity for a second target. In the lead optimization phase, the activity ratio of the lead compound for multiple target proteins is appropriately balanced to achieve maximum therapeutic efficacy and minimum side effects. In the case of our study, it is important that a compound with dual function shows comparable levels of SERT and 5-HT_{1A} receptor occupancy in vivo. This allowed us to set a specific criterion for appropriate balance based on the ratio

between in vitro SERT and 5-HT_{1A} receptor inhibitory activity (within 10-fold of the concentration range). [14,15]

Based on the above, compound **1** with weak SERT binding activity and potent 5-HT_{1A} binding activity (SERT: Ki = 212 nM, 5-HT_{1A}: Ki = 29.3 nM) was identified as a promising candidate. It is reported that binding assay for SERT could be used as a rapid method to find serotonin uptake inhibitors, because binding activity for SERT would be well correlated with serotonin uptake inhibitory activity. [16,17] The throughput of our radio binding assay for SERT is higher than that of our functional assay system for serotonin uptake inhibition. Moreover, 5-HT_{1A} partial agonists could be rather antagonistic for 5-HT_{1A} receptor under an environment where serotonin levels in the synaptic cleft are increased by serotonin uptake inhibition, therefore we thought that it is less reasonable to set a criterion of intrinsic activity for 5-HT_{1A} receptor. [18] Consequently, from the labor efficiency and cost-effectiveness points of view, we selected an exploratory strategy to find a compound with potent and balanced dual SERT and 5-HT_{1A} binding activity followed by characterizing the functional activities for SERT and 5-HT_{1A} receptor and in vivo anti-depressant efficacy in the forced swim model. Optimization of the left substituted phenyl group of compound **1** as well as the right aryl group and finally the alkoxy group led to **SMP-304** (Figure 1) with increased dual binding activity for SERT and 5-HT_{1A} receptor. **SMP-304** with SERT inhibitory activity and 5-HT_{1A} weak partial agonistic activity, which could work as a 5-HT_{1A} antagonist, showed antidepressant-like effect in vivo with faster onset than a known antidepressant. In this report we describe our optimization of compound **1** as well as our pharmacological evaluation of the obtained **SMP-304**.

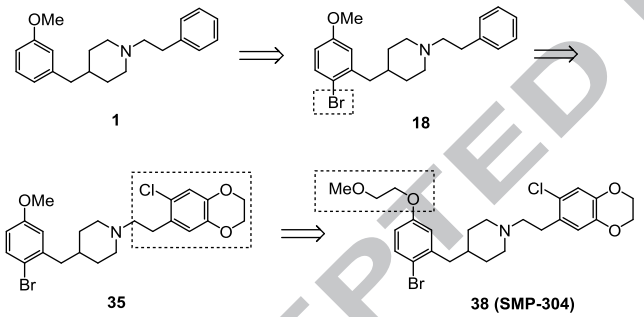
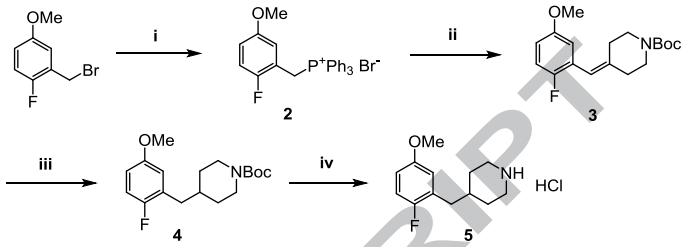


Figure 1.

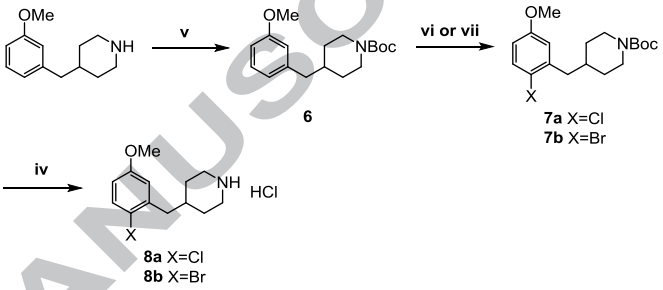
2. Result and Discussion

2.1. Chemistry

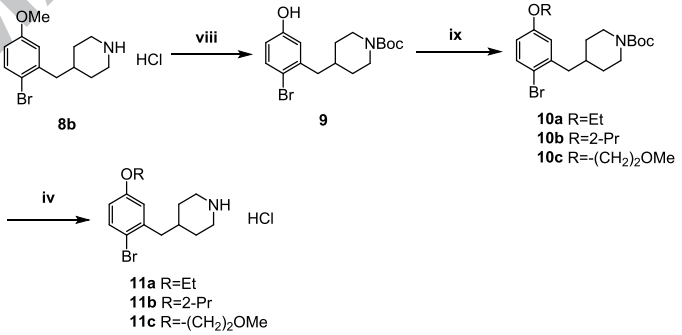
We started our study by optimizing the left methoxyphenyl and the right phenyl groups of compound **1**. The target compounds were efficiently synthesized by coupling various benzyl piperidine intermediates with 2-arylethyl intermediates. The benzyl piperidine **5** was synthesized as shown in Scheme 1. Treatment of the 2-(bromomethyl)-1-fluoro-4-methoxybenzene with triphenylphosphine provided the phosphonium salt **2**. Wittig reaction of the phosphonium salt **2** with tert-butyl 4-oxopiperidine-1-carboxylate afforded the intermediate **3**, which was hydrogenated over palladium on carbon and then deprotected with hydrochloric acid to give the benzyl piperidine **5**. As for the preparation of 2-bromo and 2-chloro-5-methoxybenzyl derivatives (such as **18,** Figure 1), protection of the commercially available 4-(3-methoxybenzyl)piperidine with Boc_2O gave the intermediate **6.** Regiospecific chlorination or bromination of **6** with NCS or NBS followed by deprotection with hydrochloric acid provided the benzyl piperidine **8a** and **8b** (Scheme 2). Substitution of the methoxy group in the benzyl piperidine intermediates was possible by the sequential protocol shown in Scheme 3. Demethylation of **8b** with BBr_3 followed by N-protection with Boc_2O gave the phenol **9**. Alkylation with ethyl iodide, isopropyl iodide or 2-methoxyethyl bromide transformed the intermediate **9** to **10a – 10c**. Finally, deprotection of **10a – 10c** with hydrochloric acid furnished the benzyl piperidine **11a – 11c**.



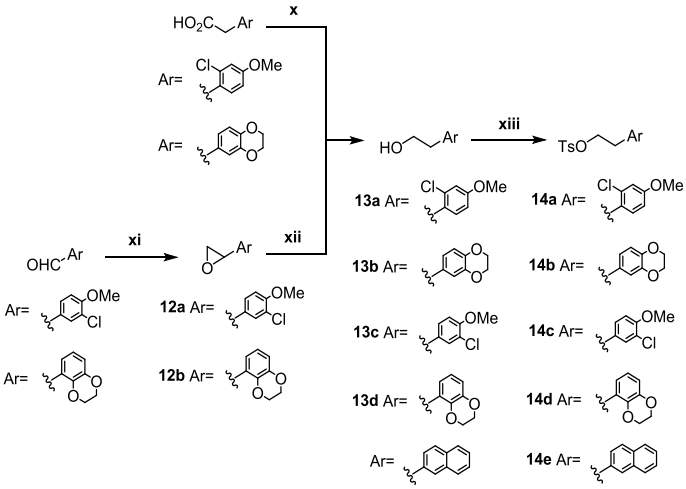
Scheme 1. Reagents and Conditions: (i) PPh_3, toluene, reflux, (ii) tert-butyl 4-oxopiperidine-1-carboxylate, K_2CO_3, 2-PrOH, reflux, (iii) H_2, 10% Pd-C, MeOH, r.t., (iv) 4N HCl/1,4-dioxane, $CHCl_3$, r.t.



Scheme 2. Reagents and Conditions: (v) Boc_2O, THF, r.t., (vi) NCS, DMF, r.t., (vii) NBS, DMF, r.t., (iv) 4N HCl/1,4-dioxane, $CHCl_3$, r.t.



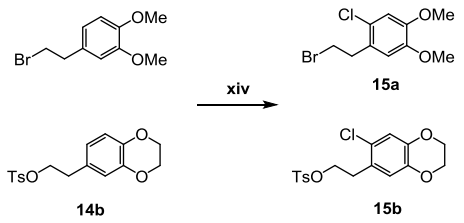
Scheme 3. Reagents and Conditions: (viii) BBr_3, CH_2Cl_2, r.t., then Boc_2O, THF, 20%aq.K_2CO_3, r.t., (ix) R-X (X = Br or I), K_2CO_3, DMF, (iv) 4N HCl/1,4-dioxane, $CHCl_3$, r.t.



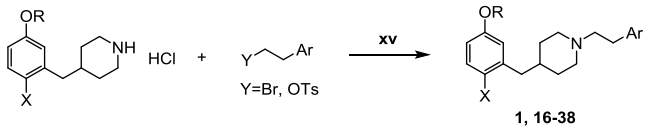
Scheme 4. Reagents and Conditions: (x) BH_3-THF, THF, r.t. (xi) MeS^+I^-, KOH, DMSO, 40°C, (xii) $NaBH_4$, BF_3-OEt_2, THF, r.t., (xiii) TsCl, MeN_3HCl, Et_3N, CH_2Cl_2, 0°C

To optimize the right part of compound **18** (Figure 1), various 2-arylethyl intermediates were synthesized according to the procedure shown in Scheme 4. Reduction of 2-(2-chloro-4-methoxyphenyl)acetic acid or 2-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)acetic acid with borane-THF

complex gave the alcohol **13a** or **13b**, respectively. Corey-Chaykovsky reaction of 3-chloro-4-methoxybenzaldehyde or 2,3-dihydrobenzo[b][1,4]dioxine-5-carbaldehyde followed by ring-opening reaction of the epoxides afforded the alcohol **13c** or **13d**, respectively. The alcohols **13a** – **13d** were transformed to the tosylates **14a** – **14e**. Direct chlorination of 4-(2-bromoethyl)-1,2-dimethoxy-benzene or the tosylate **14b** yielded the intermediate **15a** or **15b**, respectively (Scheme 5). The target compounds were synthesized by coupling reaction of the benzyl piperidine intermediates with the 2-arylethyl intermediates (Scheme 6).



Scheme 5. Reagents and Conditions: (xiv) NCS, DMF, r.t.



Scheme 6. Reagents and Conditions: (xv) K_2CO_3, MeCN, reflux

2.2. Evaluation of SERT and 5-HT_{1A} receptor binding activity

Using the "balancing approach", we started with Compound **1** (X = H), which has weak SERT binding activity, but potent 5-HT_{1A} binding activity. As previous studies have shown that 4-(3-methoxybenzyl)piperidine derivatives with a halogen atom at 6-position of the methoxy phenyl group possess potent SERT affinity with moderate 5-HT_{1A} affinity (Figure 2) [19,20], we introduced a halogen atom (F, Cl, or Br) into the 6-position of the left phenyl group of compound **1** to enhance SERT binding activity while maintaining or improving 5-HT_{1A} binding activity. As a result, introduction of a halogen atom indeed enhanced SERT affinity and, fortunately, improved 5-HT_{1A} affinity. Compound **18** with a bromine atom at the 6-position of the left phenyl group showed the most potent SERT binding activity (Table 1).

As compound **18** showed relatively moderate SERT affinity with potent 5-HT_{1A} affinity (ratio: > 20-fold based on Ki values), it was necessary to further improve its SERT affinity to meet our set criteria for appropriate SERT and 5-HT_{1A} binding activity. First we investigated replacement of the right hand part of compound **18** (Table 2). Introduction of a substituent into the 4-position of the right phenyl group enhanced SERT affinity, while maintaining 5-HT_{1A} affinity. Among the resulting compounds (**20**, **19**, **23** and **24**), **20** with a methoxy group showed the most potent SERT affinity. Next, introduction of a substituent, such as a methoxy or chlorine group into the right 4-methoxy phenyl group of compound **20**, afforded compounds **27**, **28** and **29** with improved SERT affinity. Compound **31** and **32** with a naphtyl group in the right hand part also showed more potent SERT affinity than compound **18.** In particular, the beta-naphtyl group was more appropriate for SERT affinity than the alpha-naphtyl group. As these results pointed to the possible suitability of oxygen-containing bicyclic groups for the right hand part, we introduced the 1,4-benzodioxane group into the right hand part to obtain compound **34**, which exhibited potent dual SERT and 5-HT_{1A} binding activity. Compound **35** with the 1,4-benzodioxane group substituted with a chlorine atom showed dual binding activity for SERT and 5-HT_{1A} receptor in the desired range (< 2-fold).

Although compound **35** showed dual binding activity for SERT and 5-HT_{1A} receptor in the desired range, it also exhibited very potent inhibition of CYP2D6 enzyme, which is a concern for drug-drug interaction (DDI). In fact, antidepressants with minimal potential for DDI are preferable, because patients with depression are often prescribed multiple drugs. [21] With the aim of ameliorating CYP2D6 inhibition, we next turned our attention to substitution of the methoxy group in the left hand part of compound **35.** As shown in Table 3, various alkoxy groups were tolerated at the 3-position of the left phenyl group with no significant influence on the desired potent dual binding activity for SERT and 5-HT_{1A} receptor. Also, introduction of an alkoxy group larger than the methoxy group resulted in improvement in CYP2D6 inhibitory activity. Finally, we selected compound **38** (**SMP-304**) with potent dual binding activity for SERT and 5-HT_{1A} receptor in the desired range and comparably weak CYP2D6 inhibitory activity against CYP2D6 for further evaluation, including onset of antidepressant-like effect.

2.3. Effects of SMP-304 in the rat forced swimming test

We evaluated the antidepressant-like effect of SMP-304, which has potent dual binding activity for SERT and 5-HT_{1A} receptor (SERT: Ki = 32.7 nM, 5-HT_{1A}: Ki = 9.4 nM). **SMP-304** inhibits serotonin reuptake with an IC_{50} value of 306 nM [22] and possesses intrinsic activity for the 5-HT_{1A} receptor estimated at 19.9%, which means **SMP-304** shows weak partial agonistic activity for 5-HT_{1A}. Pindolol, a 5-HT_{1A} and β adrenergic receptor antagonist, accelerated the onset of SSRIs therapy. [8,9] It is believed that the acceleration by pindolol is caused by the mechanism of 5-HT_{1A} autoreceptor antagonistic activity. [10,11] One of the reasons is that propranolol, a widely used blocker for β adrenergic receptor, has no antidepressant efficacy. [6] Pindolol also shows weak partial agonistic activity for 5-HT_{1A} receptor (intrinsic activity: 13.3%). [23] It is reported pindolol works as a 5-HT_{1A} autoreceptor antagonist to prevent the suppression effect of paroxetine, a representative SSRI, on 5-HT firing in the dorsal raphe nucleus. [6] Although further study is needed, it is presumable that **SMP-304** with potent binding activity and weak partial agonistic activity for 5-HT_{1A} as with pindolol could work as a 5-HT_{1A} antagonist under the environment where serotonin levels in the synaptic cleft are increased by serotonin uptake inhibition. The rat forced swimming test is one of the most commonly used behavioral tests as a model to evaluate antidepressant-like effect of drug candidates. [23] In the 2-day administration test (Figure 3), treatment with **SMP-304** at 1 and 3 mg/kg p.o. clearly reduced rats immobility time without increasing locomotor activity, whereas paroxetine had no such effect at 3 or 10 mg/kg p.o.. The total brain concentrations of **SMP-304** (1 and 3 mg/kg, p.o.), as measured following repeated administration of **SMP-304** at 26 hours and 2 hours before concentration measurement, were 6.74 nmol/kg for 1 mg/kg and 30.3 nmol/kg for 3 mg/kg in the wet brain tissue (Figure 4). These findings indicate that sufficient amounts of **SMP-304** migrate to the brain and induce antidepressant-like effect at lower doses and faster onset than paroxetine. In fact, whereas paroxetime requires 3 weeks treatment for any efficacy [23], **SMP-304** produces antidepressant-like effect after the 2-day treatment. It is conceivable that the effect was mediated by SERT inhibitory activity and 5-HT_{1A} antagonistic activity.

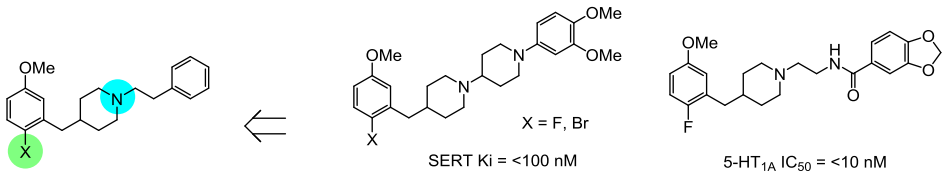
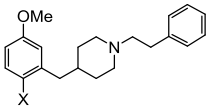


Figure 2.

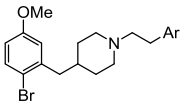
Table 1. SERT and 5-HT_{1A} receptor binding activity in the optimization of X

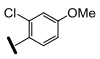
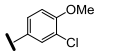
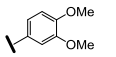
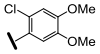


Compound	X	SERT[a]	5-HT_{1A}[a]
1	H	212 ± 46	29.3 ± 4.1
16	F	274 ± 70	6.04 ± 0.0
17	Cl	102 ± 15	2.27 ± 0.38
18	Br	66.4 ± 11.3	2.72 ± 0.81

[a] Ki values (nM) are the means of two independent experiments.

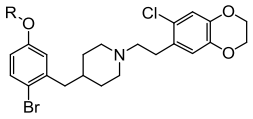
Table 2. SERT and 5-HT_{1A} receptor binding activity in the optimization of Ar group

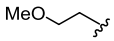


Compound	Ar	SERT[a]	5-HT_{1A}[a]	Compound	Ar	SERT[a]	5-HT_{1A}[a]
18		66.4 ± 11.3	2.72 ± 0.81	**27**		30.5 ± 2.3	1.12 ± 0.48
19		65.3 ± 7.7	1.40 ± 0.66	**28**		18.9 ± 0.2	1.04 ± 0.18
20		37.5 ± 4.1	0.81 ± 0.19	**29**		26.9 ± 1.0	1.56 ± 0.22
21		37.6 ± 3.0	3.03 ± 0.60	**30**		28.0 ± 2.9	1.96 ± 0.45
22		37.5 ± 4.1	0.82 ± 0.17	**31**		38.9 ± 6.8	4.81 ± 0.49
23		51.2 ± 6.5	1.04 ± 0.34	**32**		25.6 ± 5.2	0.89 ± 0.14
24		46.2 ± 1.5	1.34 ± 0.59	**33**		25.6 ± 0.3	2.12 ± 0.09
25		62.4 ± 3.1	4.08 ± 0.75	**34**		14.0 ± 1.4	0.36 ± 0.05
26		42.6 ± 1.2	2.93 ± 0.78	**35**		12.4 ± 1.5	6.46 ± 0.57

[a] Ki values (nM) are the means of two independent experiments.

Table 3. SERT and 5-HT_{1A} receptor binding activity and CYP2D6 inhibitory activity in the optimization of R



Compound	R	SERT[a]	5-HT_{1A}[a]	CYP2D6[b]
35	Me	12.4 ± 1.5	6.46 ± 0.57	<0.4
36	Et	18.7 ± 2.1	23.4 ± 6.1	1.4
37	2-Pr	13.9 ± 0.3	15.9 ± 3.6	1.5
38 (HCl salt: SMP-304)		32.7 ± 3.4	9.4 ± 0.2	3.0

[a] Ki values (nM) are the means of at least two independent experiments.
[b] IC_{50} value (uM).

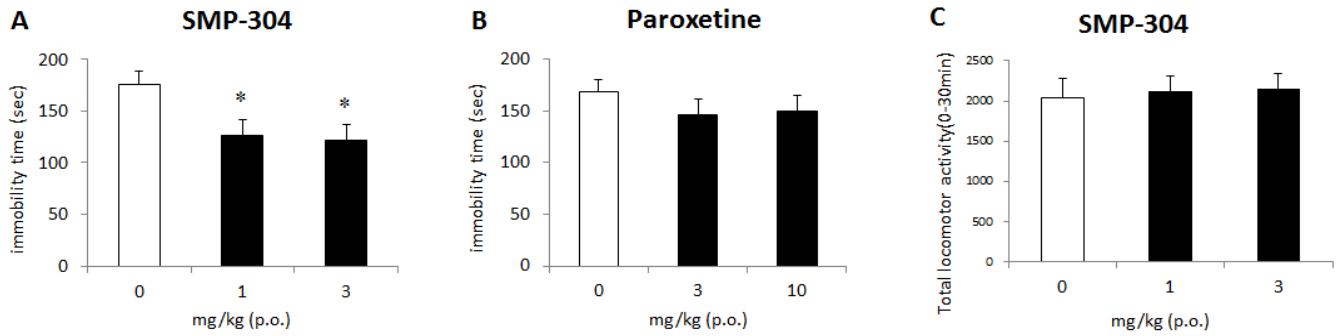


Figure 3. Effects of 2-day of SMP-304 and paroxetine on parameters in the forced swimming test in rats. (A) Effect of SMP-304 on immobility time in the forced swimming test in rats. (B) Effect of paroxetine on immobility time in the forced swimming test in rats. (C) Effect of SMP-304 on spontaneous locomotor activity in rats. Each bar represents the mean ± S.E.M. n = 10 (spontaneous locomotor activity) or 12 (forced swimming test) per group. * P < 0.05, significantly different from the vehicle-treated group (Dunnett's multiple comparison test).

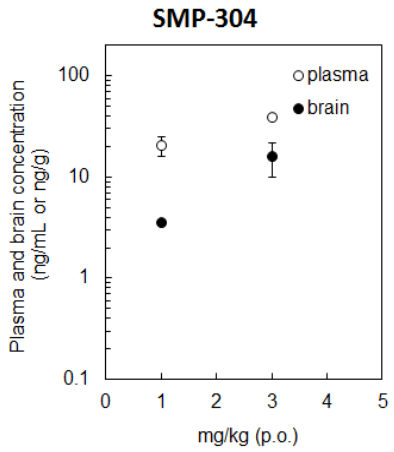


Figure 4. Total plasma and brain concentrations of SMP-304 (1 and 3 mg/kg, p.o.) as measured following repeated administration of SMP-304 at 26 hours and 2 hours before concentration measurement.

3. Conclusion

Starting from compound **1**, focusing on the binding activity for SERT and 5-HT_{1A} receptor from the labor efficiency and cost-effectiveness points of view and using the DMLs "balancing" approach, we found in this study **SMP-304** with potent and balanced dual SERT and 5-HT_{1A} binding activity. **SMP-304** with serotonin uptake inhibition and 5-HT_{1A} weak partial agonistic activity, which could work as a 5-HT_{1A} antagonist, induced antidepressant-like effect in the rat swimming test with faster onset than paroxetine, a representative SSRI. Although further studies with more compounds are needed to validate our hypothesis, these results suggest that a compound with SERT inhibitory activity and 5-HT_{1A} antagonistic activity would likely show antidepressant activities earlier time point than SSRI alone.

4. Experimental section

4.1. Chemistry

Melting points were determined on Stanford Research Systems OptiMelt MPA 100 without correction. NMR spectra were recorded at ambient temperature on a JEOL JMN-LA300 spectrometer. Chemical shifts are expressed in δ values (ppm) relative to a tetramethylsilane as an internal standard, and signals are expressed as s (singlet), d (doublet), t (triplet), m (multiplet) or br (broad). High-resolution mass spectra (HRMS) were recorded on a Thermo Fisher Scientific LTQ orbitrap Discovery MS equipment. Elemental analysis was performed on a CE Instrument EA1110 and a Yokokawa analytical system IC7000. In general, reagents and solvents were used as obtained from commercial suppliers without further purification. Reaction progress was determined by thin layer chromatography (TLC) analysis on a Merck silica gel 60 F254 precoated glass plate. Visualization was done with UV light (254 nm) or iodine. Column chromatography was carried out using a Yamazen W-prep system and performed using prepacked silica-gel columns. All reactions were carried out under a nitrogen atmosphere unless otherwise mentioned.

4.1.1. 4-(3-Methoxybenzyl)-1-(2-phenylethyl)piperidine (1)

To a mixture of 4-(3-methoxy-benzyl)-piperidine (100 mg, 0.487 mmol) and potassium carbonate (101 mg, 0.731 mmol) in MeCN (2.4 mL) was added (2-bromoethyl)benzene (86.5 uL, 0.633 mmol). After reflux for 24 h, EtOAc (7.2 mL) was added to the reaction mixture, and the whole was filtered. The filtrate was evaporated in vacuo and the residue was purified by silica gel chromatography using 25% EtOAc/hexane and 10% MeOH/$CHCl_3$ as eluent to give 100 mg (66%) of the title compound as a pale yellow oil. 1H NMR (300 MHz,$CDCl_3$) δ: 1.26-1.41 (2H, m), 1.46-1.59 (1H, m), 1.63-1.72 (2H, m), 1.95 (2H, td, J = 11.9, 2.3 Hz), 2.50-2.60 (4H, m), 2.76-2.84 (2H, m), 2.95-3.03 (2H, m), 3.80 (3H, s), 6.69-6.77 (3H, m), 7.15-7.23 (4H, m), 7.24-7.31 (2H, m); HRMS (ESI) m/z calcd for $C_{21}H_{28}NO$ [M+H]+ 310.2165; found 310.2164.

4.1.2. 4-(2-Fluoro-5-methoxybenzyl)-1-(2-phenylethyl)piperidine (16)

To a mixture of the benzyl piperidine intermediate **5** (200 mg, 0.770 mmol) and potassium carbonate (266 mg, 1.93 mmol) in MeCN (3.0 mL) was added (2-bromoethyl)benzene (137 uL, 1.00 mmol). After reflux for 24 h, EtOAc (9.0 mL) was added to the reaction mixture and the whole was filtered. The filtrate was evaporated in vacuo and the residue was purified by silica gel chromatography using 0%-1% MeOH/$CHCl_3$ as eluent to give 228 mg (90%) of the title compound as a pale yellow solid. 1H NMR (300 MHz,$CDCl_3$) δ: 1.28-1.46 (2H, m), 1.50-1.74 (3H, m), 1.96 (2H, br t, J = 10.7 Hz), 2.51-2.60 (4H, m), 2.75-2.84 (2H, m), 2.99 (2H, br d, J = 11.4 Hz), 3.77 (3H, s), 6.64-6.71 (2H, m), 6.88-6.97 (1H, m), 7.16-7.23 (3H, m), 7.24-7.32 (2H, m); HRMS (ESI) m/z calcd for $C_{21}H_{27}FNO$ [M+H]+ 328.2071; found 328.2074.

4.1.3. 4-(2-Chloro-5-methoxybenzyl)-1-(2-phenylethyl)piperidine (17)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8a** and (2-bromoethyl)benzene. (94%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.32-1.49 (2H, m), 1.55-1.65 (2H, m), 1.68-1.74 (1H, m), 1.89-2.04 (2H, m), 2.49-2.61 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.76-2.86 (2H, m), 2.95-3.04 (2H, m), 3.78 (3H, s), 6.65-6.74 (2H, m), 7.16-7.24 (4H, m), 7.24-7.31 (2H, m); HRMS (ESI) m/z calcd for $C_{21}H_{27}ClNO$ $[M+H]^+$ 344.1776; found 344.1773.

4.1.4. 4-(2-Bromo-5-methoxybenzyl)-1-(2-phenylethyl)piperidine (18)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and (2-bromoethyl)benzene. (72%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.31-1.49 (2H, m), 1.61-1.74 (3H, m), 1.90-2.03 (2H, m), 2.52-2.61 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.76-2.85 (2H, m), 2.93-3.04 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.8, 2.9 Hz), 6.72 (1H, d, J = 3.1 Hz), 7.14-7.23 (3H, m), 7.24-7.31 (2H, m), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{27}BrNO$ $[M+H]^+$ 388.1271; found 388.1270.

4.1.5. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-methylphenyl)ethyl]piperidine (19)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 4-methylphenethyl bromide. (98%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.33-1.50 (2H, m), 1.62-1.73 (3H, m), 1.89-2.03 (2H, m), 2.31 (3H, s), 2.50-2.59 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.72-2.82 (2H, m), 2.94-3.05 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 7.09 (4H, s), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO$ $[M+H]^+$ 402.1427; found 402.1426.

4.1.6. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-methoxyphenyl)ethyl]piperidine (20)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate 8b and 4-methoxyphenethyl bromide. (98%) pale yellow oil. 1H NMR (300 MHz,$CDCl_3$) δ: 1.37-1.59 (2H, m), 1.62-1.76 (3H, m), 1.93-2.13 (2H, m), 2.52-2.71 (4H, m), 2.74-2.88 (2H, m), 2.98-3.13 (2H, m), 3.78 (6H, s), 6.64 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 2.9 Hz), 6.82 (2H, d, J = 8.8 Hz), 7.12 (2H, d, J = 8.6 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO_2$ [M+H]+ 418.1376; found 418.1377.

4.1.7. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-methoxyphenyl)ethyl]piperidine (21)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 2-methoxyphenethyl bromide. (86%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.36-1.54 (2H, m), 1.58-1.74 (3H, m), 1.93-2.07 (2H, m), 2.50-2.60 (2H, m), 2.65 (2H, d, J = 6.4 Hz), 2.79-2.89 (2H, m), 2.98-3.09 (2H, m), 3.78 (3H, s), 3.80 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.72 (1H, d, J = 3.1 Hz), 6.80-6.91 (2H, m), 7.11-7.22 (2H, m), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO_2$ $[M+H]^+$ 418.1376; found 418.1371.

4.1.8. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3-methoxyphenyl)ethyl]piperidine (22)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 3-methoxyphenethyl bromide. (89%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.31-1.51 (2H, m), 1.63-1.75 (3H, m), 1.89-2.03 (2H, m), 2.52-2.61 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.74-2.83 (2H, m), 2.94-3.04 (2H, m), 3.78 (3H, s), 3.79 (3H, s), 6.63 (1H, dd, J = 8.8, 2.9 Hz), 6.70-6.82 (4H, m), 7.19 (1H, dd, J = 8.1, 8.1 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{29}BrNO_2$ $[M+H]^+$ 418.1376; found 418.1376.

4.1.9. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-fluorophenyl)ethyl]piperidine (23)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate

8b and 4-fluorophenethyl bromide. (98%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.30-1.49 (2H, m), 1.63-1.74 (3H, m), 1.88-2.03 (2H, m), 2.48-2.57 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.73-2.82 (2H, m), 2.93-3.02 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 6.95 (2H, dd, J = 8.8, 8.8 Hz), 7.14 (2H, dd, J = 8.6, 5.5 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrFNO$ $[M+H]^+$ 406.1176; found 406.1188.

4.1.10. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(4-chlorophenyl)ethyl]piperidine (24)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 4-chlorophenethyl bromide. (98%) white solid. 1H NMR (300 MHz,$CDCl_3$) δ: 1.30-1.48 (2H, m), 1.61-1.74 (3H, m), 1.88-2.01 (2H, m), 2.49-2.56 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.73-2.80 (2H, m), 2.92-3.00 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 7.12 (2H, d, J = 8.4 Hz), 7.24 (2H, d, J = 8.4 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrClNO$ [M+H]+ 422.0881; found 422.0881.

4.1.11. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-chlorophenyl)ethyl]piperidine (25)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 2-chlorophenethyl bromide. (54%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.33-1.51 (2H, m), 1.63-1.75 (3H, m), 1.95-2.08 (2H, m), 2.52-2.61 (2H, m), 2.65 (2H, d, J = 6.4 Hz), 2.90-2.97 (2H, m), 2.98-3.06 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.6, 3.1 Hz), 6.72 (1H, d, J = 3.1 Hz), 7.09-7.21 (2H, m), 7.24 (1H, dd, J = 7.2, 2.3 Hz), 7.33 (1H, dd, J = 7.4, 1.7 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrClNO$ $[M+H]^+$ 422.0881; found 422.0882.

4.1.12. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3-chlorophenyl)ethyl]piperidine (26)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 3-chlorophenethyl bromide. (86%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.31-1.47 (2H, m), 1.62-1.73 (3H, m), 1.90-2.01 (2H, m), 2.50-2.58 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.74-2.81 (2H, m), 2.92-3.00 (2H, m), 3.78 (3H, s), 6.63 (1H, dd, J = 8.6, 3.1 Hz), 6.71 (1H, d, J = 2.9 Hz), 7.05-7.10 (1H, m), 7.14-7.23 (3H, m), 7.42 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{21}H_{26}BrClNO$ $[M+H]^+$ 422.0881; found 422.0882.

4.1.13. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-chloro-4-methoxyphenyl)ethyl]piperidine (27)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the tosylate intermediate **14a**. (99%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.36-1.53 (2H, m), 1.58-1.74 (3H, m), 1.96-2.08 (2H, m), 2.49-2.59 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.83-2.93 (2H, m), 2.98-3.07 (2H, m), 3.77 (3H, s), 3.78 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.70-6.77 (2H, m), 6.89 (1H, d, J = 2.6 Hz), 7.13 (1H, d, J = 8.4 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{28}BrClNO_2$ $[M+H]^+$ 452.0986; found 452.0986.

4.1.14. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3-chloro-4-methoxyphenyl)ethyl]piperidine (28)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the tosylate intermediate **14c**. (98%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.32-1.50 (2H, m), 1.55-1.74 (3H, m), 1.89-2.02 (2H, m), 2.48-2.57 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.69-2.77 (2H, m), 2.93-3.01 (2H, m), 3.78 (3H, s), 3.87 (3H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 6.84 (1H, d, J = 8.4 Hz), 7.05 (1H, dd, J = 8.3, 2.1 Hz), 7.20 (1H, d, J = 2.0 Hz), 7.41 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{22}H_{28}BrClNO_2$ $[M+H]^+$ 452.0986; found 452.0982.

4.1.15. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(3,4-dimethoxyphenyl)ethyl]piperidine (29)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 3,4-dimethoxyphenethyl bromide. (98%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.45-1.76 (5H, m), 1.95-2.15 (2H, m), 2.58-2.70 (4H, m), 2.77-2.87 (2H, m), 3.01-3.15 (2H, m), 3.78 (3H, s), 3.85 (3H, s), 3.87 (3H, s), 6.64 (1H, dd, J = 8.6, 3.1 Hz), 6.70-6.81 (4H, m), 7.42 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{31}BrNO_3$ $[M+H]^+$ 448.1482; found 448.1478.

4.1.16. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2-chloro-4,5-dimethoxyphenyl)ethyl]piperidine (30)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the bromide intermediate **15b**. (97%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.41-1.75 (5H, m), 1.99-2.15 (2H, m), 2.53-2.62 (2H, m), 2.65 (2H, d, J = 6.4 Hz), 2.85-2.96 (2H, m), 3.00-3.11 (2H, m), 3.78 (3H, s), 3.84 (3H, s), 3.85 (3H, s), 6.64 (1H, dd, J = 8.6, 3.1 Hz), 6.72 (1H, d, J = 3.1 Hz), 6.76 (1H, s), 6.83 (1H, s), 7.42 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{30}BrClNO_3$ $[M+H]^+$ 482.1092; found 482.1091.

4.1.17. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(5,8-dihydronaphthalen-1-yl)ethyl]piperidine (31)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and 2-(1-naphthyl)ethyl bromide. (99%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.39-1.55 (2H, m), 1.67-1.77 (3H, m), 2.00-2.14 (2H, m), 2.64-2.76 (4H, m), 3.05-3.16 (2H, m), 3.26-3.36 (2H, m), 3.79 (3H, s), 6.64 (1H, dd, J = 8.8, 2.9 Hz), 6.73 (1H, d, J = 3.1 Hz), 7.32-7.54 (5H, m), 7.71 (1H, d, J = 7.5 Hz), 7.82-7.87 (1H, m), 8.06 (1H, d, J = 7.7 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{29}BrNO$ $[M+H]^+$ 438.1427; found 438.1427.

4.1.18. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(naphthalen-2-yl)ethyl]piperidine (32)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the tosylate intermediate **14e**. (86%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.34-1.52 (2H, m), 1.63-1.75 (3H, m), 1.95-2.06 (2H, m), 2.61-2.71 (4H, m), 2.93-3.08 (4H, m), 3.78 (3H, s), 6.64 (1H, dd, J = 8.8, 3.1 Hz), 6.72 (1H, d, J = 2.9 Hz), 7.34 (1H, dd, J = 8.3, 1.7 Hz), 7.38-7.48 (3H, m), 7.63 (1H, br s), 7.74-7.82 (3H, m); HRMS (ESI) m/z calcd for $C_{25}H_{29}BrNO$ $[M+H]^+$ 438.1427; found 438.1429.

4.1.19. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2,3-dihydro-1,4-benzodioxin-5-yl)ethyl]piperidine (33)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the tosylate intermediate **14d**. (99%) pale yellow oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.35-1.52 (2H, m), 1.63-1.74 (3H, m), 1.92-2.05 (2H, m), 2.50-2.60 (2H, m), 2.64 (2H, d, J = 6.4 Hz), 2.74-2.84 (2H, m), 2.96-3.07 (2H, m), 3.78 (3H, s), 4.24 (4H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.68-6.77 (4H, m), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{29}BrNO_3$ $[M+H]^+$ 446.1325; found 446.1324.

4.1.20. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]piperidine (34)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the tosylate intermediate **14b**. (72%) colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.31-1.47 (2H, m), 1.61-1.72 (3H, m), 1.88-2.00 (2H, m), 2.47-2.56 (2H, m), 2.60-2.74 (4H, m), 2.92-3.01 (2H, m), 3.78 (3H, s), 4.23 (4H, s), 6.60-6.68 (2H, m), 6.70-6.72 (2H, m), 6.77 (1H, d, J = 8.3 Hz), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{29}BrNO_3$ $[M+H]^+$ 446.1325; found 446.1330.

4.1.21. 4-(2-Bromo-5-methoxybenzyl)-1-[2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]-piperidine (35)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **8b** and the tosylate intermediate **15b**. (83%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.33-1.50 (2H, m), 1.63-1.73 (3H, m), 1.93-2.05 (2H, m), 2.46-2.55 (2H, m), 2.64 (2H, d, J = 6.6 Hz), 2.76-2.85 (2H, m), 2.94-3.04 (2H, m), 3.78 (3H, s), 4.22 (4H, s), 6.63 (1H, dd, J = 8.7, 3.0 Hz), 6.71 (1H, d, J = 3.1 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.41 (1H, d, J = 8.6 Hz); HRMS (ESI) m/z calcd for $C_{23}H_{28}BrClNO_3$ $[M+H]^+$ 480.0936; found 480.0927.

4.1.22. 4-(2-Bromo-5-ethoxybenzyl)-1-[2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]-piperidine (36)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **11a** and the tosylate intermediate **15b**. (84%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.32-1.50 (5H, m), 1.63-1.73 (3H, m), 1.92-2.06 (2H, m), 2.46-2.57 (2H, m), 2.63 (2H, d, J = 6.4 Hz), 2.76-2.85 (2H, m), 2.94-3.04 (2H, m), 3.99 (2H, q, J = 7.0 Hz), 4.22 (4H, s), 6.61 (1H, dd, J = 8.8, 2.9 Hz), 6.71 (1H, d, J = 2.9 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.39 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{24}H_{30}BrClNO_3$ $[M+H]^+$ 494.1092; found 494.1099.

4.1.23. 4-[2-Bromo-5-(propan-2-yloxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]piperidine (37)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **11b** and the tosylate intermediate **15b**. (81%) colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.32 (6H, d, J = 6.1 Hz), 1.35-1.50 (2H, m), 1.63-1.73 (3H, m), 1.93-2.07 (2H, m), 2.47-2.56 (2H, m), 2.62 (2H, d, J = 6.4 Hz), 2.76-2.86 (2H, m), 2.94-3.05 (2H, m), 4.22 (4H, s), 4.42-4.56 (1H, m), 6.61 (1H, dd, J = 8.8, 2.9 Hz), 6.70 (1H, d, J = 2.9 Hz), 6.73 (1H, s), 6.85 (1H, s), 7.39 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{32}BrClNO_3$ $[M+H]^+$ 508.1248; found 508.1251.

4.1.24. 4-[2-Bromo-5-(2-methoxyethoxy)benzyl]-1-[2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]piperidine (38)

The title compound was prepared in a manner similar to that for the preparation of **16** using the benzyl piperidine intermediate **11c** and the tosylate intermediate **15b**. (81%) colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.30-1.50 (2H, m), 1.62-1.73 (3H, m), 1.92-2.07 (2H, m), 2.46-2.57 (2H, m), 2.63 (2H, d, J = 6.2 Hz), 2.76-2.86 (2H, m), 2.94-3.05 (2H, m), 3.45 (3H, s), 3.71-3.77 (2H, m), 4.05-4.11 (2H, m), 4.22 (4H, s), 6.65 (1H, dd, J = 8.8, 3.1 Hz), 6.73 (1H, s), 6.76 (1H, d, J = 2.9 Hz), 6.85 (1H, s), 7.40 (1H, d, J = 8.8 Hz); HRMS (ESI) m/z calcd for $C_{25}H_{32}BrClNO_4$ $[M+H]^+$ 524.1197; found 524.1199. The product was converted into the corresponding hydrochloride salt. Mp: 155-158°C. Anal. Calcd for $C_{25}H_{31}BrClNO_4HCl$: C, 53.49; H, 5.75; N, 2.50; Cl, 12.63; Br, 14.23. Found: C, 53.69; H, 5.76; N, 2.57; Cl, 12.69; Br, 14.22.

4.1.25. (2-Fluoro-5-methoxybenzyl)(triphenyl)phosphonium bromide (2)

To a solution of 2-fluoro-5-methoxybenzyl bromide (12.5 g, 57.1 mmol) in toluene (150 mL) was added triphenylphosphine (16.5 g, 62.8 mmol). After reflux for 4 h, the resulting solid was collected and washed with toluene (20 mL x 3) to give 19.8 g (64%) of the title compound as a white solid. ^{1}H NMR (300 MHz,DMSO-D_6) δ: 3.48 (3H, s), 5.10 (2H, d, J = 15.4 Hz), 6.47-6.54 (1H, m), 6.87-6.95 (1H, m), 7.06 (1H, dd, J = 9.2, 9.2 Hz), 7.66-7.80 (12H, m), 7.87-7.97 (3H, m); HRMS (ESI) m/z calcd for $C_{26}H_{23}FOP$ $[M+H]^+$ 401.1465; found 401.1472.

4.1.26. *tert*-Butyl 4-(2-fluoro-5-methoxybenzylidene)piperidine-1-carboxylate (3)

To a mixture of the phosphonium salt **2** (15 g, 27.7 mmol) and potassium carbonate (5.74 g, 41.6 mmol) in 2-PrOH was added 1-(*tert*-Butoxycarbonyl)-4-oxopiperidine (6.06 g, 30.4 mmol). After reflux for 5 h, the reaction mixture was filtered, and the filtrate was evaporated in vacuo. Et_2O (100 mL) was then added to the residue and stirred for 30 min at room temperature. The resulting solid was filtered out, and the filtrate was concentrated. The residue was purified by silica gel chromatography using 0-13% EtOAc/hexane as eluent to give 7.32 g (82%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.47 (9H, s), 2.30-2.39 (4H, m), 3.41 (2H, t, J = 5.7 Hz), 3.52 (2H, t, J = 5.9 Hz), 3.77 (3H, s), 6.23 (1H, s), 6.65-6.76 (2H, m), 6.96 (1H, dd, J = 9.1, 9.1 Hz).

4.1.27. *tert*-Butyl 4-(2-fluoro-5-methoxybenzyl)piperidine-1-carboxylate (4)

The olefin intermediate **3** (5.30 g, 16.5 mmol) was dissolved in MeOH (30 mL) and hydrogenated over 10% Pd on carbon (water ~50%, 1.00 g) at room temperature for 5 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by silica gel chromatography using 3-23% EtOAc/hexane as eluent to give 4.95 g (93%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.08-1.24 (2H, m), 1.45 (9H, s), 1.58-1.66 (2H, m), 1.66-1.78 (1H, m), 2.54 (2H, dd, J = 7.1, 1.0 Hz), 2.57-2.71 (2H, m), 3.77 (3H, s), 3.99-4.15 (2H, m), 6.62-6.71 (2H, m), 6.93 (1H, dd, J = 9.1, 9.1 Hz).

4.1.28. 4-(2-Fluoro-5-methoxybenzyl)piperidine hydrochloride (5)

To a solution of intermediate **4** (4.00 g, 12.4 mmol) in $CHCl_3$ (15 mL) was added 4 N HCl/1,4-dioxane (30 mL). After stirring at room temperature for 21 h, the reaction mixture was evaporated in vacuo. Et_2O (50 mL) was then added to the residue and stirred at room temperature for 30 min. The resulting solid was collected to obtain 3.16 g (98%) of the title compound as a white solid. ^{1}H NMR (300 MHz,DMSO-D_6) δ: 1.27-1.46 (2H, m), 1.62-1.75 (2H, m), 1.75-1.89 (1H, m), 2.53 (2H, d, J = 6.1 Hz), 2.71-2.85 (2H, m), 3.16-3.26 (2H, m), 3.72 (3H, s), 6.76-6.86 (2H, m), 7.08 (1H, dd, J = 9.2, 9.2 Hz), 8.72 (2H, br s); HRMS (ESI) m/z calcd for $C_{13}H_{19}FNO$ $[M+H]^+$ 224.1445; found 224.1451.

4.1.29. *tert*-Butyl 4-(3-methoxybenzyl)piperidine-1-carboxylate (6)

To a solution of 4-(3-methoxybenzyl)piperidine (1.70 g, 8.28mmol) in THF (15 mL) was added a solution of di-*tert*-butyl dicarbonate (1.90 g, 8.69 mmol) in THF (5 mL). After stirring at room temperature for 3h, the reaction mixture was concentrated. The residue was purified by silica gel chromatography using 1-

22% EtOAc/hexane as eluent to give 2.57 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.05-1.22 (2H, m), 1.45 (9H, s), 1.52-1.74 (3H, m), 2.51 (2H, d, J = 6.8 Hz), 2.63 (2H, t, J = 12.4 Hz), 3.80 (3H, s), 3.97-4.16 (2H, m), 6.66-6.71 (1H, m), 6.71-6.78 (2H, m), 7.20 (1H, dd, J = 7.8, 7.8 Hz).

4.1.30. *tert*-Butyl 4-(2-chloro-5-methoxybenzyl)piperidine-1-carboxylate (7a)

To a solution of intermediate **6** (300 mg, 0.982 mmol) in DMF (3 mL) was added N-chlorosuccinimide (138 mg, 1.03 mmol). After stirring at 60°C for 12h, H_2O (30 mL) was added to the reaction mixture and the whole was extracted with EtOAc (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 1-22% EtOAc/hexane as eluent to give 290 mg (87%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.11-1.28 (2H, m), 1.45 (9H, s), 1.58-1.67 (2H, m), 1.69-1.85 (1H, m), 2.56-2.72 (4H, m), 3.78 (3H, s), 3.99-4.17 (2H, m), 6.67-6.72 (2H, m), 7.21-7.26 (1H, m).

4.1.31. 4-(2-Chloro-5-methoxybenzyl)piperidine hydrochloride (8a)

The title compound was prepared in a manner similar to that for the preparation of **5** using intermediate **7a**. (89%) white solid. ^{1}H NMR (300 MHz,DMSO-D_6) δ: 1.33-1.50 (2H, m), 1.64-1.75 (2H, m), 1.77-1.95 (1H, m), 2.62 (2H, d, J = 7.0 Hz), 2.73-2.86 (2H, m), 3.17-3.26 (2H, m), 3.75 (3H, s), 6.84 (1H, dd, J = 8.8, 3.0 Hz), 6.91 (1H, d, J = 3.0 Hz), 7.33 (1H, d, J = 8.8 Hz), 8.69 (2H, br s).

4.1.32. *tert*-butyl 4-(2-bromo-5-methoxybenzyl)piperidine-1-carboxylate (7b)

To a solution of intermediate **6** (150 mg, 0.491 mmol) in DMF (2.0 mL) was added N-bromosuccinimide (96.0 mg, 0.540 mmol) at 0°C. After stirring at room temperature for 12h, H_2O (20 mL) was added to the reaction mixture, and the whole was extracted with EtOAc (20 mL x 2). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 1-22% EtOAc/hexane as eluent to give 169 mg (90%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.12-1.29 (2H, m), 1.46 (9H, s), 1.52-1.68 (2H, m), 1.70-1.86 (1H, m), 2.56-2.71 (4H, m), 3.78 (3H, s), 3.98-4.16 (2H, m), 6.64 (1H, dd, J = 8.7, 3.1 Hz), 6.70 (1H, d, J = 3.1 Hz), 7.42 (1H, d, J = 8.7 Hz).

4.1.33. 4-(2-bromo-5-methoxybenzyl)piperidine hydrochloride (8b)

The title compound was prepared in a manner similar to that for the preparation of **5** using intermediate **7b**. (91%) white solid. ^{1}H NMR (300 MHz,DMSO-D_6) δ: 1.33-1.52 (2H, m), 1.63-1.75 (2H, m), 1.78-1.95 (1H, m), 2.62 (2H, d, J = 7.2 Hz), 2.72-2.86 (2H, m), 3.16-3.28 (2H, m), 3.74 (3H, s), 6.78 (1H, dd, J = 8.6, 3.0 Hz), 6.91 (1H, d, J = 3.0 Hz), 7.48 (1H, d, J = 8.6 Hz), 8.66 (2H, br s).

4.1.34. *tert*-butyl 4-(2-bromo-5-hydroxybenzyl)piperidine-1-carboxylate (9)

To a suspension of intermediate **8b** (1.50 g, 4.68 mmol) in CH_2Cl_2 (10 mL) was added dropwise 1N BBr_3 in CH_2Cl_2 solution (5.61 mL, 5.61 mmol) at 0°C for 10min. After stirring at room temperature for 12h, MeOH (10 mL) was added to the reaction mixture, and the whole was stirred at room temperature for 30min and concentrated. To a mixture of the residue, THF (10 mL) and 20% aqueous K_2CO_3 (50 g) was added di-*tert*-butyl dicarbonate (1.12 g, 5.15 mmol). After stirring at room temperature for 12h, the reaction mixture was extracted with EtOAc (50 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was crystallized using EtOAc (4 mL) and stirred at room temperature for 10min. Hexane (8 mL) was then added to the mixture, and the whole was stirred at room temperature for 20min. The resulting solid was filtered, rinsed with EtOAc/hexane = 1/2 (1 mL) and collected to give 1.38 g (80%) of the title compound as a white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.11-1.27 (2H, m), 1.46 (9H, s), 1.60-1.69 (2H, m), 1.70-1.87 (1H, m), 2.49-2.75 (4H, m), 3.99-4.15 (2H, m), 5.93 (1H, s), 6.58 (1H, dd, J = 8.6, 2.9 Hz), 6.68 (1H, d, J = 3.1 Hz), 7.35 (1H, d, J = 8.6 Hz).

4.1.35. *tert*-butyl 4-(2-bromo-5-ethoxybenzyl)piperidine-1-carboxylate (10a)

To a mixture of intermediate **9** (1.00 g, 2.70 mmol) and potassium carbonate (1.16 g, 8.37 mmol) in DMF (5 mL) was added iodoethane (0.648 mL, 8.10 mmol). After stirring at 70°C for 8 h, the reaction mixture was treated with H_2O (40 mL) and extracted with EtOAc (40 mL). To the organic layer was added toluene (40 mL), and the mixture was washed with H_2O (40 mL). The organic layer was dried over MgSO4, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 0~16% EtOAc/hexane as eluent to give 1.12 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.12-1.28 (2H, m), 1.40 (3H, t, J = 7.0 Hz), 1.45 (9H, s), 1.59-1.67 (2H, m), 1.69-1.86 (1H, m), 2.56-2.71 (4H, m), 3.94-4.15 (4H, m), 6.62 (1H, dd, J = 8.7, 3.0 Hz), 6.69 (1H, d, J = 3.1 Hz), 7.40 (1H, d, J = 8.8 Hz).

4.1.36. 4-(2-bromo-5-ethoxybenzyl)piperidine hydrochloride (11a)

The title compound was prepared in a manner similar to that for the preparation of **5** using intermediate **10a**. (94%) white solid. ^{1}H NMR (300 MHz,DMSO-D_6) δ: 1.31 (3H, t, J = 7.0 Hz), 1.34-1.51 (2H, m), 1.63-1.74 (2H, m), 1.78-1.94 (1H, m), 2.61 (2H, d, J = 7.0 Hz), 2.71-2.85 (2H, m), 3.17-3.26 (2H, m), 4.00 (2H, q, J = 7.0 Hz), 6.76 (1H, dd, J = 8.6, 2.9 Hz), 6.90 (1H, d, J = 3.1 Hz), 7.46 (1H, d, J = 8.8 Hz), 8.73 (2H, br s); HRMS (ESI) m/z calcd for $C_{14}H_{21}BrClNO$ $[M+H]^+$ 298.0801; found 298.0814.

4.1.37. *tert*-butyl 4-[2-bromo-5-(propan-2-yloxy)benzyl]piperidine-1-carboxylate (10b)

The title compound was prepared in a manner similar to that for the preparation of **10a** using intermediate **9**. (quant.) white solid. ^{1}H-NMR (300 MHz,$CDCl_3$) δ: 1.11-1.28 (2H, m), 1.32 (6H, d, J = 6.1 Hz), 1.46 (9H, s), 1.61-1.65 (2H, m), 1.69-1.87 (1H, m), 2.55-2.73 (4H, m), 3.95-4.19 (2H, m), 4.42-4.56 (1H, m), 6.62 (1H, dd, J = 8.6, 3.0 Hz), 6.68 (1H, d, J = 3.0 Hz), 7.39 (1H, d, J = 8.6 Hz).

4.1.38. 4-[2-bromo-5-(propan-2-yloxy)benzyl]piperidine hydrochloride (11b)

The title compound was prepared in a manner similar to that for the preparation of **5** using intermediate **10b**. (99%) white solid. ^{1}H-NMR (300 MHz,DMSO-D_6) δ: 1.24 (6H, d, J = 6.1 Hz), 1.30-1.48 (2H, m), 1.61-1.74 (2H, m), 1.75-1.92 (1H, m), 2.59 (2H, d, J = 7.0 Hz), 2.70-2.84 (2H, m), 3.14-3.25 (2H, m), 4.52-4.64 (1H, m), 6.74 (1H, dd, J = 8.8, 3.1 Hz), 6.87 (1H, d, J = 3.1 Hz), 7.43 (1H, d, J = 8.8 Hz), 8.50 (1H, br s) ; HRMS (ESI) m/z calcd for $C_{15}H_{23}BrClNO$ $[M+H]^+$ 312.0957; found 312.0971.

4.1.39. *tert*-butyl 4-[2-bromo-5-(2-methoxyethoxy)benzyl]piperidine-1-carboxylate (10c)

To a mixture of intermediate **9** (1.5 g, 4.05 mmol) and potassium carbonate (1.12 g, 8.10 mmol) in DMF (10 mL) was added 2-bromoethylmethyl ether (0.571 mL, 6.08 mmol). After stirring at 100°C for 3h, the reaction mixture was treated with

H_2O (50 mL) and extracted with EtOAc (50 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 8~29% EtOAc/hexane as eluent to give 1.70 g (98%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.11-1.28 (2H, m), 1.46 (9H, s), 1.57-1.68 (2H, m), 1.69-1.85 (1H, m), 2.55-2.71 (4H, m), 3.45 (3H, s), 3.74 (2H, t, J = 4.6 Hz), 4.00-4.16 (4H, m), 6.66 (1H, dd, J = 8.7, 3.0 Hz), 6.74 (1H, d, J = 3.0 Hz), 7.41 (1H, d, J = 8.7 Hz).

4.1.40. 4-[2-bromo-5-(2-methoxyethoxy)benzyl]piperidine hydrochloride (11c)

The title compound was prepared in a manner similar to that for the preparation of **5** using intermediate **10c**. (97%) white solid. ^{1}H NMR (300 MHz,DMSO-D_6) δ: 1.33-1.51 (2H, m), 1.64-1.75 (2H, m), 1.79-1.95 (1H, m), 2.62 (2H, d, J = 7.2 Hz), 2.72-2.86 (2H, m), 3.16-3.27 (2H, m), 3.30 (3H, s), 3.64 (2H, t, J = 4.6 Hz), 4.07 (2H, t, J = 4.6 Hz), 6.78 (1H, dd, J = 8.8, 3.1 Hz), 6.93 (1H, d, J = 3.1 Hz), 7.47 (1H, d, J = 8.8 Hz), 8.68 (2H, br s).

4.1.41. 2-(2-chloro-4-methoxyphenyl)ethanol (13a)

To a solution of (2-chloro-4-methoxyphenyl)acetic acid (1.50 g, 7.48 mmol) in THF (20 mL) was added 0.9 M BH_3-THF complex in THF solution (10.8 mL, 9.72 mmol). After stirring at room temperature for 3 h, MeOH (10 mL) was added, and the reaction mixture was stirred at room temperature for 30 min and concentrated in vacuo. The residue was purified by silica gel chromatography using 26~47% EtOAc/hexane as eluent to give 1.44 g (quant.) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.36 (1H, t, J = 5.9 Hz), 2.95 (2H, t, J = 6.7 Hz), 3.79 (3H, s), 3.85 (2H, td, J = 6.4, 6.4 Hz), 6.77 (1H, dd, J = 8.4, 2.6 Hz), 6.93 (1H, d, J = 2.8 Hz), 7.17 (1H, d, J = 8.4 Hz).

4.1.42. 2-(2-chloro-4-methoxyphenyl)ethyl 4-methylbenzenesulfonate (14a)

To a mixture of intermediate **13a** (1.20 g, 6.43 mmol), triethylamine (1.08 mL, 7.72 mmol) and trimethylamine hydrochloride (61.5 mg, 0.643 mmol) in CH_2Cl_2 (12 mL) was added p-toluenesulfonyl chloride (1.35 g, 7.07 mmol) at 0°C. After stirring at 0°C for 1 h, the reaction mixture was treated with H_2O (50 mL) and extracted with $CHCl_3$ (30 mL x 2). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 4~25% EtOAc/hexane as eluent to give 2.08 g (95%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.43 (3H, s), 3.01 (2H, t, J = 6.9 Hz), 3.77 (3H, s), 4.20 (2H, t, J = 6.9 Hz), 6.71 (1H, dd, J = 8.4, 2.6 Hz), 6.82 (1H, d, J = 2.6 Hz), 7.08 (1H, d, J = 8.4 Hz), 7.27 (2H, d, J = 7.9 Hz), 7.68 (2H, d, J = 8.3 Hz).

4.1.43. 2-(2,3-dihydro-1,4-benzodioxin-6-yl)ethanol (13b)

The title compound was prepared in a manner similar to that for the preparation of **13a** using (2,3-dihydro-benzo[1,4]dioxin-6-yl)-acetic acid. (88%) 1.59 g. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.39 (1H, t, J = 5.9 Hz), 2.76 (2H, t, J = 6.5 Hz), 3.82 (2H, td, J = 6.1, 6.1 Hz), 4.25 (4H, s), 6.70 (1H, dd, J = 8.3, 2.0 Hz), 6.74 (1H, d, J = 2.0 Hz), 6.81 (1H, d, J = 8.3 Hz).

4.1.44. 2-(2,3-dihydro-1,4-benzodioxin-6-yl)ethyl 4-methylbenzenesulfonate (14b)

The title compound was prepared in a manner similar to that for the preparation of **14a** using intermediate **13b**. (quant.) 1.94 g. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.44 (3H, s), 2.84 (2H, t, J = 7.2 Hz), 4.15 (2H, t, J = 7.2 Hz), 4.23 (4H, s), 6.57 (1H, dd, J = 8.1, 2.2 Hz), 6.61 (1H, d, J = 2.0 Hz), 6.74 (1H, d, J = 8.1 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.72 (2H, d, J = 8.3 Hz).

4.1.45. 2-(3-chloro-4-methoxyphenyl)oxirane (12a)

To a mixture of 3-chloro-4-methoxybenzaldehyde (2.00 g, 11.7 mmol) and trimethylsulfonium iodide (3.35 g, 16.4 mmol) in DMSO (12 mL) was added potassium hydroxide (0.920 g, 16.4 mmol). After stirring at 40°C for 7 h, the reaction mixture was diluted with EtOAc (50 mL) and washed with H_2O (100 mL). The aqueous layer was extracted with EtOAc (50 mL). To the combined organic layer was added toluene (100 mL), and the whole was washed with H_2O (100 mL), dried over Na_2SO_4, filtered and concentrated in vacuo to give 2.25 g (quant.) of the title compound as a pale yellow oil. The obtained compound **12a** was used in the next reaction without further purification. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.77 (1H, dd, J = 5.3, 2.6 Hz), 3.13 (1H, dd, J = 5.3, 4.0 Hz), 3.80 (1H, dd, J = 4.0, 2.6 Hz), 3.90 (3H, s), 6.90 (1H, d, J = 8.4 Hz), 7.16 (1H, dd, J = 8.5, 2.1 Hz), 7.28 (1H, d, J = 2.2 Hz).

4.1.46. 2-(3-chloro-4-methoxyphenyl)ethanol (13c)

To a suspension of sodium borohydride (168 mg, 4.44 mmol) in THF (12 mL) was added BF_3-Et_2O complex (0.903 mL, 7.13 mmol). After stirring at room temperature for 15 min, a solution of intermediate **12a** (2.00 g, 10.8 mmol) in THF (12 mL) was added dropwise for 10 min to the reaction mixture at 0°C. After stirring at room temperature for 2 h, H_2O (50 mL) was added to the reaction mixture, and the whole was extracted with EtOAc (40 mL). The organic layer was washed with brine (20 mL), dried over $MgSO_4$, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography using 30~51% EtOAc/hexane as eluent to give 1.65 g (81%) of the title compound as a colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 1.37 (1H, t, J = 5.9 Hz), 2.79 (2H, t, J = 6.5 Hz), 3.83 (2H, td, J = 6.2, 6.2 Hz), 3.89 (3H, s), 6.88 (1H, d, J = 8.3 Hz), 7.09 (1H, dd, J = 8.4, 2.2 Hz), 7.25 (1H, d, J = 2.2 Hz).

4.1.47. 2-(3-chloro-4-methoxyphenyl)ethyl 4-methylbenzenesulfonate (14c)

The title compound was prepared in a manner similar to that for the preparation of **14a** using intermediate **13c**. (89%) colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.44 (3H, s), 2.86 (2H, t, J = 6.8 Hz), 3.88 (3H, s), 4.17 (2H, t, J = 6.8 Hz), 6.80 (1H, d, J = 8.4 Hz), 6.99 (1H, dd, J = 8.3, 2.2 Hz), 7.04 (1H, d, J = 2.0 Hz), 7.28 (2H, d, J = 8.4 Hz), 7.66 (2H, d, J = 8.4 Hz).

4.1.48. 5-(oxiran-2-yl)-2,3-dihydro-1,4-benzodioxine (12b)

The title compound was prepared in a manner similar to that for the preparation of **12a** using 2,3-dihydro-1,4-benzodioxine-5-carbaldehyde. (quant.) colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.75 (1H, dd, J = 5.7, 2.6 Hz), 3.14 (1H, dd, J = 5.7, 4.0 Hz), 4.14 (1H, dd, J = 4.0, 2.6 Hz), 4.26-4.30 (2H, m), 4.30-4.34 (2H, m), 6.69-6.73 (1H, m), 6.79-6.82 (2H, m).

4.1.49. 2-(2,3-dihydro-1,4-benzodioxin-5-yl)ethanol (13d)

The title compound was prepared in a manner similar to that for the preparation of **13c** using intermediate **12b**. (72%) colorless oil. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.88 (2H, t, J = 6.5 Hz), 3.84 (2H, td, J = 6.2, 6.2 Hz), 4.23-4.31 (4H, m), 6.72-6.80 (3H, m).

4.1.50. 2-(2,3-dihydro-1,4-benzodioxin-5-yl)ethyl 4-methylbenzenesulfonate (14d)

The title compound was prepared in a manner similar to that for the preparation of as **14a** using intermediate **13d**. (89%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.43 (3H, s), 2.92 (2H, t, J = 7.0 Hz), 4.11-4.18 (4H, m), 4.21 (2H, t, J = 7.0 Hz), 6.64 (1H, dd, J = 6.6, 2.6 Hz), 6.68-6.78 (2H, m), 7.26 (2H, d, J = 8.1 Hz), 7.65 (2H, d, J = 8.3 Hz).

4.1.51. 2-(naphthalen-2-yl)ethyl 4-methylbenzenesulfonate (14e)

The title compound was prepared in a manner similar to that for the preparation of **14a** using 2-naphthalene ethanol. (91%) white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 2.34 (3H, s), 3.11 (2H, t, J = 6.9 Hz), 4.31 (2H, t, J = 6.9 Hz), 7.08-7.14 (2H, m), 7.21 (1H, dd, J = 8.4, 1.7 Hz), 7.41-7.50 (2H, m), 7.52 (1H, br s), 7.60 (2H, d, J = 8.5 Hz), 7.71 (2H, d, J = 8.5 Hz), 7.76-7.83 (1H, m).

4.1.52. 1-(2-bromoethyl)-2-chloro-4,5-dimethoxybenzene (15a)

To a solution of 3,4-dimethoxyphenethyl bromide (300 mg, 1.22 mmol) in DMF (6.0 mL) was added N-chlorosuccinimide (179 mg, 1.34 mmol). After stirring at room temperature for 20h, saturated aqueous $NaHCO_3$ (4 mL) and H_2O (20 mL) were added to the reaction mixture, and the whole was extracted with EtOAc (30 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 0-15% EtOAc/hexane as eluent to give 324 mg (95%) of the title compound as a white solid. ^{1}H NMR (300 MHz,$CDCl_3$) δ: 3.21 (2H, t, J = 7.6 Hz), 3.56 (2H, t, J = 7.5 Hz), 3.86 (3H, s), 3.88 (3H, s), 6.74 (1H, s), 6.86 (1H, s).

4.1.53. 2-(7-chloro-2,3-dihydro-1,4-benzodioxin-6-yl)ethyl 4-methylbenzenesulfonate (15b)

To a solution of **14b** (500 mg, 1.50 mmol) in DMF (7.0 mL) was added N-chlorosuccinimide (179 mg, 1.34 mmol). After stirring at 50°C for 4h, H_2O (40 mL) was added to the reaction mixture, and the whole was extracted with EtOAc (40 mL x 2). The combined organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purified by silica gel chromatography using 10-31% EtOAc/hexane as eluent to give 481 mg (87%) of the title compound as a white solid. ^{1}H-NMR (300 MHz,$CDCl_3$) δ: 2.44 (3H, s), 2.94 (2H, t, J = 7.0 Hz), 4.18 (2H, t, J = 7.0 Hz), 4.22 (4H, s), 6.67 (1H, s), 6.79 (1H, s), 7.29 (2H, d, J = 8.3 Hz), 7.71 (2H, d, J = 8.3 Hz).

4.2. Biology

4.2.1. Radio assay

Radio assays were conducted according to the methods of Owens et al. for serotonin transporter [24] and Yabuuchi et al. for 5-HT_{1A} [25]. Briefly, the following were used: for serotonin transporter assays, cell membranes expressing human serotonin transporter and [3H]citalopram; for 5-HT_{1A} assays, cell membranes expressing human 5-HT_{1A} and [3H]8-OH-DPAT. The inhibition constant (Ki) was calculated in Microsoft Office Excel 2003 (Microsoft Corporation) using the Cheng–Prusoff equation [Ki = IC50/(1 + ([L]/Kd)], where L is the concentration of radioligand in the assay and Kd is the dissociation constant of the radioligand for the receptor.

4.2.2. [^{3}H]5-HT uptake assay

Phosphate-buffered saline containing 0.1 mmol/L $CaCl_2$ and 1 mmol/L $MgCl_2$ was used as reaction buffer. One microliter of dimethyl sulfoxide or test substance and 199 μL of the reaction buffer were mixed, and 50 μL of the mixed solution was added to human serotonin transporter-expressing CHO cells cultured in 96-well assay plates. The plates were pre-incubated at 37°C for 10 min. During that time, dimethyl sulfoxide or test substance (**SMP-304**, paroxetine, milnaciplan or imipramine) was diluted with [^{3}H]5-HT solution in another 96-well plate. After cells pre-incubation, 50 μL of the prepared [^{3}H]5-HT solution containing dimethyl sulfoxide or test substance was added to the wells, and the mixture was incubated at 37°C for 20 min. After the incubation, the liquid layer was discarded, and the cells were rinsed twice with 200 μL reaction buffer before being lysed with 100 μL of the Solvable solution. Radioactivity in each lysate sample was measured as described in the previous section.

4.2.3. Guanosine 5'-(γ-thio) Triphosphate, [^{35}S]-(GTPγS) assay for 5-HT1A receptor

To make up a total volume of 500 μL, 2.5 μL of test compound, GTPγS (2 mM, to measure nonspecific binding), DMSO (to measure basal [^{35}S]GTPγS binding) or serotonin (20 mM, to measure maximal [^{35}S]GTPγS binding); 50 μL of reaction buffer [HEPES-NaOH buffer (20 mM, pH 7.4) containing 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM DTT, and 1 μM GDP] containing 0.5 nM [^{35}S]GTPγS; and 447.5 μL of cell membranes expressing human 5-HT_{1A} receptors were mixed. The following manipulation was carried out as described in the above 5-HT transporter binding assay. Intrinsic activity was expressed as relative value of the activity of 100 μM serotonin, which was considered to be 100%.

The 5-HT_{1A} agonistic activity of SMP-304 was evaluated three times. The means of the results at each concentration are as follows:

1 nM: -0.87 ± 5.70%, 10 nM: 0.54 ± 4.28%, 100 nM: 6.66 ± 4.03%, 1000 nM: 16.2 ± 4.37%, 10000 nM: 19.6 ± 5.41%

4.2.3.1. Data analyses

The following formulae were used:

1) Basal [35S]GTPγS binding

Basal [35S]GTPγS binding (dpm) = Binding activity of the DMSO group (dpm) − Binding activity of the GTPγS group (dpm)

2) Maximal [35S]GTPγS binding

Maximal [35S]GTPγS binding (dpm) = Binding activity of the serotonin group (dpm) − Binding activity of the GTPγS group (dpm)

3) Specific binding of the test substance

Specific binding of the test substance (dpm) = Binding activity of test substance group (dpm) − Binding activity of GTPγS group (dpm)

4) Maximal specific binding

The maximal specific binding of the test substance was calculated using the Dx calculation (logistic curve fitting) with the "measurement value input" function in Stat Preclinica Client Version 1.0. The direct estimation method was used. The maximal specific binding was calculated using the logistic curve of the concentrations of the test substance and the specific binding values.

5) Intrinsic activity of the test substance

When the increment in maximal [35S]GTPγS binding (Maximal [35S]GTPγS binding − Basal [35S]GTPγS binding) was considered as 100%, intrinsic activity of the test substance, which is the percentage of the increment in maximal specific binding of the test substance (Maximal specific binding of test substance − Basal [35S]GTPγS binding), was calculated using the following formula:

Intrinsic activity of the test substance (%) = 100 ×{ [Maximal specific binding of test substance (dpm) − Basal [35S]GTPγS binding (dpm)] / [Maximal [35S]GTPγS binding (dpm) − Basal [35S]GTPγS binding (dpm)]}

4.2.4. CYP2D6 inhibition assay

4.2.4.1. Materials

Bufuralol was purchased from Sigma-Aldrich Corp., and Pooled Human Liver Microsomes were purchased from Xenotech, LLC.

4.2.4.1.1. Preparation of 0.5 M Potassium Phosphate Buffer (pH 7.4)

Monopotassium phosphate solution (150 mL, 0.5 M) and dipotassium phosphate solution (700 mL, 0.5 M) were mixed, giving a solution with pH 7.4.

4.2.4.1.2. Preparation of Magnesium Chloride Solution (165 mM)

Magnesium chloride hexahydrate (3.35 g) was dissolved in distilled water (100 ml) to a final concentration of 165 mM ($MgCl_2.6H_2O$).

4.2.4.1.3. Preparation of Human Liver Microsome Solution

Human Liver Microsome Solution was prepared by mixing Pooled Human Liver Microsomes (150 μL, 20 mg/mL), potassium phosphate buffer (12 mL, 0.5 M), magnesium chloride solution (1.2 mL, 165 mM), and distilled water (34.65 mL).

4.2.4.1.4. Preparation of β-NADPH Solution (13 mM)

β-NADPH was dissolved in distilled water to a final concentration of 11.75 mg/mL.

4.2.4.1.5. Preparation of Substrate Solution

Bufuralol was dissolved in DMSO to a final concentration of 2.0 mM and then diluted 200-fold with distilled water.

4.2.4.2. Experimental Procedures

Step 1: The test drug in DMSO solution (10 mM) was serially diluted 5-fold with DMSO to prepare 10, 2, 0.4 and 0.08 mM test drug solutions.

Step 2: Each test drug solution and DMSO were separately diluted 96-fold with the human liver microsome solution, and 80 uL of each dilution was dispensed into 96-well microplates.

Step 3: The substrate solution (10 uL) and β-NADPH solution (10 uL) were added to each well, and the plate was incubated at 37 °C for 10 min.

Step 4: The reaction was terminated by addition of 300 uL of methanol.

Step 5: The reaction mixture was filtered, and the filtrate was loaded onto an LC-MSMS system.

4.2.4.3. Quantification and Calculation

The amount of 1'-hydroxybufuralol produced was quantified by LC-MSMS and used as CYP2D6 metabolic activity. The remaining activity of each sample was determined by comparing the activity in DMSO to that in the presence of the test drug. IC_{50} value for CYP2D6 inhibition was determined from test drug concentration and the remaining activity. The IC_{50} value was calculated by linear interpolation between two points that span the remaining activity (50%). A larger IC_{50} value for CYP2D6 inhibition indicates weak CYP2D6 inhibition.

4.2.5. Rat forced swimming test

On the first day of the experiment, male Wistar rats weighing 121.3 – 178.1 g were placed in plastic cylinders (40 cm in height, 19 cm in diameter) containing water (25 ± 1°C) to a depth of 19 cm. After spending 15 min in the water, the rats were removed from the cylinders and wiped with paper towels (training session). SMP-304 (1, 3 mg/kg), paroxetine (3, 10 mg/kg), or vehicle (0.5% methylcellulose) was orally administered to each rats 30 min after the beginning of the training session. The test session was carried out the following day with each rat treated with one of the test-drugs or vehicle 2 h before the start of the test session. During the test session each animal behavior was videotaped for 6 min, and a rat was judged to be immobile whenever it remained floating in water without movement, except for slight movement to keep posture. An observer blind to test-drugs measured twice the immobility time for each animal. When the difference between the first and second measured immobility time was within 30 sec, the first recorded immobility time was used for data analysis. When the difference between the two measured immobility times was more than 30 sec, another measurement was conducted, and the obtained immobility time was used for data analysis. After all measurements were completed, animals' assignment to test drugs was disclosed.

4.2.6. Spontaneous locomotion

This experiment was performed using male Wistar rats weighing 121.3 – 178.1 g. **SMP-304** (1, 3 mg/kg), or vehicle (0.5% methylcellulose) was orally administered to the rats 26hr and 2hr before the test. The animals were placed in plastic cages, and their spontaneous locomotion was measured for 30 min using an automatic behavior analyzing system (Supermex; Muromachi Kikai Co. Ltd., Tokyo, Japan).

4.2.7. Pharmacokinetics of SMP-304

Pharmacokinetic study of **SMP-304** was carried out in male Wistar rats weighing 121.3 – 178.1 g. **SMP-304** (1, 3 mg/kg) was orally administered to the rats 26hr and 2hr before blood and brain sampling (3 rats). **SMP-304** plasma and brain concentrations were determined using high-performance liquid chromatography (HPLC)/tandem mass spectrometry.

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∗ Corresponding author. Tel.: +81-6-6337-5885; fax: +81-6-6337-6010; e-mail: hidefumi-yoshinaga@ds-pharma.co.jp

Graphical Abstract

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Discovery of SMP-304, a Novel Benzylpiperidine Derivative with Serotonin Transporter Inhibitory Activity and 5-HT$_{1A}$ Weak Partial Agonistic Activity showing the Antidepressant-like Effect

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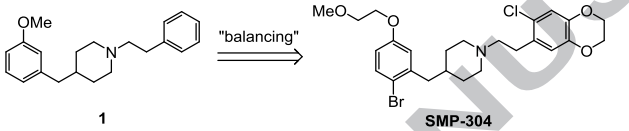
Hidefumi Yoshinaga, Shuji Masumoto, Koji Koyama, Naoya Kinomura, Yuji Matsumoto, Taro Kato, Satoko Baba, Kenji Matsumoto, Tomoko Horisawa, Hitomi Oki, Kazuki Yabuuchi and Toru Kodo
Drug Research Division, Sumitomo Dainippon Pharma Co., Ltd., 33-94 Enoki-cho, Suita, Osaka 564-0053, Japan



1
SERT: Ki = 212 nM
5-HT$_{1A}$: Ki = 29.3 nM

SMP-304
SERT: Ki = 32.7 nM
5-HT$_{1A}$: Ki = 9.4 nM, I.A. = 19.9%