DSP-1053, a novel serotonin reuptake inhibitor with 5-HT$_{1A}$ partial agonistic activity, displays fast antidepressant effect with minimal undesirable effects in juvenile rats

Taro Kato$^1$, Yuji Matsumoto$^2$, Masanori Yamamoto$^2$, Kenji Matsumoto$^1$, Satoko Baba$^1$, Keiko Nakamichi$^1$, Harumi Matsuda$^2$, Haruka Nishimuta$^3$ & Kazuki Yabuuchi$^4$

$^1$Drug Development Research Laboratories, Sumitomo Dainippon Pharma Co. Ltd., Osaka, Japan
$^2$Innovative Drug Discovery Laboratories, Sumitomo Dainippon Pharma Co. Ltd., Osaka, Japan
$^3$Preclinical Research Laboratories, Sumitomo Dainippon Pharma Co. Ltd., Osaka, Japan
$^4$Project Management, Sumitomo Dainippon Pharma Co. Ltd., Osaka, Japan

Keywords
5-HT$_{1A}$ receptor, antidepressant, emesis, major depressive disorder, serotonin (5-HT), serotonin reuptake inhibitor, vomiting.

Abstract
Enhancement of serotonergic neurotransmission has been the main stream of treatment for patients with depression. However, delayed therapeutic onset and undesirable side effects are major drawbacks for conventional serotonin reuptake inhibitors. Here, we show that DSP-1053, a novel serotonin reuptake inhibitor with 5-HT$_{1A}$ partial agonistic activity, displays fast antidepressant efficacy with minimal undesirable effects, especially nausea and emesis in animal models. DSP-1053 bound human serotonin transporter and 5-HT$_{1A}$ receptor with the $K_i$ values of 1.02 ± 0.06 and 5.05 ± 1.07 nmol/L, respectively. This compound inhibited the serotonin transporter with an IC$_{50}$ value of 2.74 ± 0.41 nmol/L and had an intrinsic activity for 5-HT$_{1A}$ receptors of 70.0 ± 6.3%. In rat microdialysis, DSP-1053, given once at 3 and 10 mg kg$^{-1}$, dose-dependently increased extracellular 5-HT levels. In the rat forced swimming test, 2-week administration of DSR-1053 (1 mg kg$^{-1}$) significantly reduced rats immobility time after treatment, whereas paroxetine (3 and 10 mg kg$^{-1}$) required 3-week administration to reduce rats immobility time. In olfactory bulbectomy model, 1- and 2-week administration of DSP-1053 reduced both of emotional scores and activity in the open field, whereas paroxetine required 2 weeks to show similar beneficial effects. Although single administration of DSP-1053-induced emesis and vomiting in the rat and Suncus murinus, multiple treatment with this compound, but not with paroxetine, decreased the number of vomiting episodes. These results highlight the important role of 5-HT$_{1A}$ receptors in both the efficacy and tolerability of DSP-1053 as a new therapeutic option for the treatment of depression.

Abbreviations
$K_d$, dissociation constant; $K_i$, inhibition constant; i.v., intravenous; I.A., intrinsic activity; IC$_{50}$, half maximal inhibitory concentration; HPLC, high-performance liquid chromatography; GTP$_{S}$, guanosine 5'-[(γ-thio) triphosphate, $[^{35}S]$-; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin norepinephrine reuptake inhibitor; 5-HT, 5-hydroxytryptamine (serotonin).

Introduction
Major depressive disorder is a chronic, debilitating disease with a lifetime risk of approximately 15% for men and 30% for women (Kessler et al. 2005; Waraich et al. 2005). First line therapy for major depressive disorder relies on the use of selective serotonin reuptake inhibitors (SSRIs) or serotonin norepinephrine reuptake inhibitors (SNRIs)
(Rush et al. 2006; Gelenberg et al. 2010). Although SSRIs and SNRIs have dramatically expanded treatment options for major depressive disorder, there is still a significant unmet medical need for management of this disorder, including therapeutics delayed onset, and treatment resistance (Rush et al. 2006; Warden et al. 2007; Reeves et al. 2008; Gelenberg et al. 2010). It has been reported that pindolol, a 5-HT1A/1B and β adrenergic receptor partial agonist, may accelerate antidepressants onset and enhance SSRIs beneficial effects in treatment-resistant depression. (Artigas et al. 1994; Blier and Bergeron 1995; Pérez et al. 1997). This enhancement would be mediated by blockage of negative feedback inhibition in response to increased serotonin (5-HT) (Bel and Artigas 1993; Rutter et al. 1997). This enhancement would be mediated by blockage of negative feedback inhibition in response to increased serotonin (5-HT). Hence, dual-action antidepressants that can modulate 5-HT1A receptor and inhibit 5-HT reuptake antagonist, may accelerate antidepressants onset and enhance SSRIs beneficial effects in treatment-resistant depression. (Artigas et al. 1994; Blier and Bergeron 1995; Pérez et al. 1997). This enhancement would be mediated by blockage of negative feedback inhibition in response to increased serotonin (5-HT) (Bel and Artigas 1993; Rutter et al. 1997). This enhancement would be mediated by blockage of negative feedback inhibition in response to increased serotonin (5-HT).

Materials and Methods

Animals

All experimental procedures for the use of animals were reviewed and approved by the Institutional Animal Care and Use Committee of Sumitomo Dainippon Pharma, Co., Ltd. Rats were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) or Japan SLC, Inc. (Shizuoka, Japan). Shrews (S. murinus) were purchased from CLEA Japan, Inc. (Tokyo, Japan). All animals were kept in a room with controlled environmental conditions (temperature: 23 ± 3°C, humidity: 55 ± 15%, 12 h light–dark cycle with light on at 07:00 h) and used after a quarantine period of 7 days. The animals were given food (CE-2, Oriental Yeast Co., Ltd., Tokyo, Japan) and filtered tap water ad libitum.

Materials

DSP-1053 (6-[(2-[(4-Bromo-3-(2-methoxyethoxy)benzyl]piperidin-1-yl)ethyl]-2,3-dihydro-4H-chromen-4-one) (Fig. 1) and paroxetine hydrochloride (paroxetine) were synthesized in our laboratories. The route of synthesis of DSP-1053 has been described previously (Nishida et al. 2012). Clomipramine hydrochloride (cloimipramine), serotonin hydrochloride (5-HT), dopamine hydrochloride (dopamine), imipramine hydrochloride (imipramine), WAY-100635, pindolol, and R-(+)-8-hydroxy-DPAT (8-OH-DPAT) were purchased from Sigma Aldrich Japan (Tokyo, Japan). All radioligands were purchased from Perkin Elmer Japan (Kanagawa, Japan). For oral (p.o.) administration in rodent models, DSP-1053 and paroxetine were dissolved in 0.5% methylcellulose. In the S. murinus model, DSP-1053 and paroxetine were dissolved in 40% polyethylene glycol. Dosing volume was determined based on each animal body weight measured in the morning of each administration day (5 mL kg−1). Cell membranes expressing human serotonin transporter and 5-HT1A receptor were purchased from Perkin Elmer Japan. Chinese hamster ovary cells expressing human serotonin transporter used for [3H]-5-HT uptake assay were established in our Pharmacology Research Laboratories at Sumitomo Dainippon Pharma Co., Ltd.

Preparation of rat cell membranes

Five-week-old male rats (Crl:CD(SD)) were killed by decapitation, and their brains were rapidly removed and dissected to obtain the cerebral cortex (for serotonin

![Figure 1. Chemical structure of DSP-1053.](image)
transporter binding) and hippocampus (for 5-HT<sub>1A</sub> binding), which were washed in ice-cold saline and weighed. The tissues were homogenized with a teflon-glass homogenizer in reaction buffer [50 mmol/L Tris-HCl buffer containing 120 mmol/L NaCl and 5 mmol/L KCl (for serotonin transporter) or 50 mmol/L Tris-HCl buffer containing 4 mmol/L CaCl<sub>2</sub> (for 5-HT<sub>1A</sub>)], and the homogenates were centrifuged at 40,000–48,000 g for 10 min at 4°C. The obtained pellets were resuspended in reaction buffer, and the homogenates were centrifuged again at 40,000–48,000 g for 10 min at 4°C. The resulting pellets were resuspended in 8–10 times their volume of reaction buffer, and the protein concentrations were measured by the method of Bradford using the Bio-Rad Protein Assay (Bio-Rad Laboratories Co., Ltd., Hercules, CA, US). The cell membranes were diluted with reaction buffer to a concentration of 4 mg mL<sup>-1</sup>. On the day of the experiment, the stored membranes were diluted with reaction buffer to a concentration of 447 µg mL<sup>-1</sup> (200 µg/L assay).

**Radioligand binding assay**

In a total volume of 500 µL, 2.5 µL of test substance solution, clomipramine solution (2 mmol/L), 8-OH-DPAT (2 mmol/L) or dimethyl sulfoxide, 50 µL of [3H]citalopram or [3H]8-OH-DPAT solution, and 447.5 µL of cell membranes were mixed. Cell membranes expressing human serotonin transporter and 5-HT<sub>1A</sub> receptor were diluted with the reaction buffer to a final concentration of 1 unit/447.5 µL beforehand. All samples were reacted at 25°C for 0.5 (for 5-HT<sub>1A</sub>) or 1 h (for serotonin transporter) in an incubator. The reaction was terminated by addition of 4 mL ice-cold reaction buffer, and the cell membranes were collected by vacuum filtration through GF/B glass filters. The glass filters were then washed with 4 mL of ice-cold reaction buffer and placed in scintillation vials with scintillation fluid. After more than 3 h, the radioactivity in each sample was measured with a liquid scintillation counter for 2 min, and the calculated dpm value was used for data analysis. In the serotonin transporter binding assay, GF/B glass filters were soaked in 0.05% polyethylenimine solution for more than 15 min before use. The inhibition constant (K<sub>i</sub>) was calculated in Microsoft® Office Excel 2003 (Microsoft Corporation) using the Cheng–Prusoff equation [K<sub>i</sub> = IC<sub>50</sub>/(1 + ([L]/K<sub>D</sub>)), where L is the concentration of radioligand in the assay and K<sub>D</sub> is the dissociation constant of the radioligand for the receptor.

**[3H]5-HT uptake assay**

Phosphate-buffered saline containing 0.1 mmol/L CaCl<sub>2</sub> and 1 mmol/L MgCl<sub>2</sub> was used as reaction buffer. One microliter of dimethyl sulfoxide or test substance and 149 µL of human serotonin transporter-expressing cells suspension were added to 96-well assay plates. The plates were preincubated at 37°C for 10 min. During that time, dimethyl sulfoxide or test substance (DSP-1053, paroxetine, or imipramine) was diluted in [3H]5-HT solution in another 96-well plate. After the preincubation, the prepared [3H]5-HT solution containing dimethyl sulfoxide or test substance was added to the cell suspension, and the mixture was incubated at 37°C for 10 min. Ice-cold 3% formamide in 0.9% NaCl was added to each well to stop the reaction. All reaction mixtures in the 96-well plates were then filtered through a glass fiber filter plate prewashed in 200 µL of 0.3% polyethyleneimine and dried under reduced pressure with manifold (Millipore, Billerica, MA, US). To wash the glass fiber filter, 300 µL of phosphate buffered saline was added and filtered twice. Radioactivity in each sample was measured as described in the previous section.

**Guanosine 5’-(γ-thio) Triphosphate, [35S]-GTP(γS) assay for 5-HT<sub>1A</sub> receptor**

To make up a total volume of 500 µL, 2.5 µL of test substance, 2 mmol/L GTP(γS) (to measure nonspecific binding), dimethyl sulfoxide (to measure basal [35S]GTP(γS) binding), or 20 mmol/L 5-HT (to measure maximal [35S]GTP(γS) binding), 50 µL of reaction buffer [2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)-NaOH buffer (20 mmol/L, pH 7.4) containing 100 mmol/L NaCl, 10 mmol/L MgCl<sub>2</sub>, 0.1 mmol/L dithiothreitol, and 1 µmol/L guanosine-5’-diphosphate sodium salt (GDP)] containing 20 mmol/L [35S]GTP(γS), and 447.5 µL of the cell membranes expressing human 5-HT<sub>1A</sub> receptors (1 unit/447.5 µL) were mixed. All samples were allowed to react in an incubator set at 25°C for 20 min. The reaction was terminated by adding 4 mL of ice-cold reaction buffer, and the cell membranes were collected by vacuum filtration using GF/B glass filters. The glass filters were washed twice with 4 mL of ice-cold reaction buffer. Radioactivity in each sample was measured as described in the previous section. Intrinsic activity was expressed as relative value of the activity of 100 µmol/L 5-HT, which was considered to be 100%.

**Off-target radioligand binding assays and enzyme assays**

To determine DSP-1053 interaction with off-target receptors and enzymes, 29 receptor binding assays and 3 enzyme assays (catechol-O-methyltransferase, monoamine oxidase-A and -B) were conducted on our behalf by Sekisui Medical Co., Ltd (Tokyo, Japan). The receptor binding assays were carried out using standard techniques as summarized...
in Table 3. As for the enzyme assays, pig catechol-O-methyltransferase, human monoamine oxidase-A, and -B activity was evaluated using 5-adenosyl-L-[methyl-14C]-methionine, 5-hydroxy[side chain-14C]tryptamine, or beta-[ethyl-1-14C]-, PKI as labeled substrate, and the amount of radioactivity was quantitated.

**Pharmacokinetics of DSP-1053**

Pharmacokinetic study of DSP-1053 was carries out in male rats (Crl:CD(SD)) after intravenous (i.v.) (1 mg kg⁻¹) and p.o. (10 mg kg⁻¹) administration. At appropriate time points after dosing (0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h for i.v., 0.25, 0.5, 1, 2, 4, 6, and 24 h for p.o.), blood was sampled from two (for i.v.) or three (for p.o.) different rats. Each rat was sampled 8 or 7 times in total. Plasma concentrations of DSP-1053 were determined using high-performance liquid chromatography (HPLC)/tandem mass spectrometry and analyzed by non-compartmental analysis using WinNonlin (version 6.3; Pharsight Corporation, Mountain View, CA, US).

**Rat microdialysis**

**Surgery**

This experiment was performed using 5–6-week old male rats (Crlj:WI). A vertical guide cannula (AG-04; EICOM) was implanted in the right side of the frontal cortex (3.7 mm anterior, 3.0 mm lateral, and 1.5 mm ventral from the bregma) of the rat under pentobarbital anesthesia [80 mg kg⁻¹, intraperitoneal (i.p.)]. Microdialysis was conducted on the day after surgery. A dialysis probe (A-I-4-03; EICOM) was inserted into the guide cannula under light anesthesia with isoflurane and continuously perfused by Ringer solution (147 mmol/L NaCl, 4 mmol/L KCl, 2.3 mmol/L CaCl₂) at 2 µL min⁻¹ using a microsyringe pump. Microdialysate samples (10 µL) were continuously collected for 5 min at 20-min intervals and automatically injected into the HPLC system. DSP-1053 or vehicle was orally administered to the rats at least 3 h after the start of perfusion, that is, when stable HPLC baseline values for 5-HT and dopamine were obtained in the dialysate samples. Measurement continued for 3 h after drug or vehicle administration.

**Chromatography**

The collected microdialysate samples (10 µL) were separated by HPLC using a PP-ODS column (EICOM) and a mobile phase containing 0.1 mol/L phosphate buffer (pH 6.0), 1% methanol, 50 mg L⁻¹ ethylenediamine tetraacetic acid disodium, and 500 mg L⁻¹ sodium 1-decanesulfonate at a flow rate of 0.5 mL min⁻¹. The peaks corresponding to 5-HT and dopamine were amperometrically detected using a graphite electrode set at 400 mV with an Ag/AgCl reference electrode (RE-100; EICOM). Online data acquisition was performed using PowerChrom software (Version 2.2; AD Instruments Pty Ltd., Nagoya, Aichi, Japan). Before performing the microdialysis, the retention time of the HPLC peak for 5-HT and dopamine was determined using a standard solution. The peak height (mV) of 5-HT and dopamine at each measurement was converted into a percentage of the average of the last 4 pre-drug baseline values (percentage of baseline).

**Rat forced swimming test**

Test compounds antidepressant-like activity was assessed using a previously described (Porsolt et al. 1978) with slight modifications. In the training session (Day 1), each animal (5–6-week old male rats (Crlj:WI)) was gently placed into a plastic cylinder (40 cm in height, 19 cm in diameter) containing 5.8 L of water set at 25 ± 1°C. Fifteen min after the beginning of the training session, the animal was removed from the water and returned to its home cage. Test compounds dosing suspensions were administered to animals in a blind manner 15 min after the end of the training session. From Day 2 to Day 13 or Day 20, the animals were administered the dosing suspensions once a day between 7:00AM and 7:00PM. In the test session (Day 14 or Day 21), the animals were treated with the dosing suspensions as described in the training session, and the swimming test was performed for 5 min in the same manner as in the training session. In the swimming test, the behavior of each animal was horizontally recorded onto a DVD recorder using a video camera. An animal was judged to be immobile whenever it remained floating on the water without moving its body or forepaws, except for slight movements to maintain posture. The total time the animal remained immobile was defined as immobility time. An observer blinded to test compounds doses measured the immobility time.

**Olfactory bulbectomy**

**Surgery**

The test was performed using 6–7-week-old male rats (Crlj:WI). Bilateral olfactory bulbectomy was performed on rats anesthetized with pentobarbital (50 mg kg⁻¹; i.p.), essentially as previously described (Cryan et al. 1999). The head was shaved and a midline sagittal incision was made extending at 1 cm rostral to bregma. A burr hole was drilled at points 7 mm anterior to bregma and 2 mm either side of the midline at a point corre-
sponding to the posterior margin of the orbit of the eye. The olfactory bulbs were removed by suction, and the burr holes filled with a hemostatic sponge (Spongell; Astellas Pharma Inc., Tokyo, Japan). Tetracycline powder was applied to the wound prior to closure. Sham-operated rats underwent the same procedure with the dura above the bulbs punctured, but the bulbs left intact. Following surgery, the animals were allowed 7 days recovery prior to drug administration. During recovery, the general condition of each animal was monitored.

**Emotional scoring**

Emotional scoring was conducted, essentially as previously reported (Cairncross et al. 1978; Gomita et al. 1983). Emotional scoring consisted of the following five tests; (1) response to a stick presented just in front of the nose, (2) response to a puff of air blown sharply onto the rat’s back, (3) response to grasping the animal, (4) response to tail pinching by a forceps, (5) vocalization during scoring. In each of the tests numbered 1–4, responses were graded as follows: 0 (no response), 1 (slight response), 2 (moderate response), 3 (marked response), or 4 (extreme response). In test 5), vocalization was graded as follows: 0 (no vocalization), 1 (occasional vocalization), or 2 (extensive vocalization). Emotional scoring was performed in animals’ home cages. The scores in each scoring system were added to give a single emotional score for each individual animal. The maximum emotional score was 18. Animals emotional scoring was performed just before the first drug administration (pre), and on the day after the open-field test (post).

**Open-field test**

The open-field test was carried out, essentially as described elsewhere (Cryan et al. 1999). Each rat was placed onto the center of the open-field apparatus, and the number of line crosses over a 5-min period was recorded.

**Conditioned taste aversion test in rats**

The test was performed using 10-week-old male rats (Slc: SD). Drinking water was removed overnight (from 18:00 until 09:00 h) prior to the start of the experiment. On the following 2 days, water was first given for 60 min, and then ad libitum from 12:00 until 18:00 h. On day 3, all rats had access to a 0.5% saccharin solution for 60 min instead of water. At 10:20 h of day 3, the animals were dosed with DSP-1053 or vehicle. Water was then made available ad libitum for the rest of the day and removed overnight (from 18:00 until 09:00 h). On the test day (day 4), the animals had again access to 0.5% saccharin solution for 60 min in the absence of test compound. Rats saccharin consumption was measured for each 60-min period on day 3 and 4, and saccharin intake ratio was calculated as follows.

\[
\text{(Saccharin intake ratio)} = \frac{\text{saccharin intake on day } 4}{\text{saccharin intake on day } 3}.
\]

**Evaluation of emesis in Suncus murinus**

Male Jic:SUN-Her, 5 weeks (body weight: 40–63 g) (experiment 1), or 10 weeks (body weight: 53–73 g) (experiment 2) of age at the initiation of dosing were used in this study.

**Experiment 1**

DSP-1053 (10, 30, 60, or 100 mg kg\(^{-1}\)) or 40% polyethylene glycol was orally administered to the animals, and the number of animals that vomited as well as the number of vomiting episodes were counted for 60 min. Paroxetine-induced emesis, as evaluated under the same protocol (Mine et al. 2013), was used as reference.

**Experiment 2**

On the first day of the experiment, DSP-1053 (60 mg kg\(^{-1}\)), paroxetine (60 mg kg\(^{-1}\)), or 40% polyethylene glycol was orally administered to the animals and the number of vomiting episodes was counted. Animals that showed emesis on the first day in each group (9 of 19 in DSP-1053 group, 12 of 19 in paroxetine group), and three animals in 40% polyethylene glycol group were subsequently dosed once a day for 7 days with DSP-1053 (60 mg kg\(^{-1}\)), paroxetine (60 mg kg\(^{-1}\)), or 40% polyethylene glycol, respectively, and the number of animals that vomited as well as the number of vomiting episodes was counted for 60 min after administration on each day.

**Data analysis**

Scatchard plots approximated by regression line with Microsoft\textsuperscript{®} Office Excel 2003 (Microsoft Corporation) were used to calculate \(K_d\) value in each binding assay. The IC\(_{50}\) value in each binding assay and the maximal specific binding of each test substance [intrinsic activity (I.A.) of the test substance] as well as EC\(_{50}\) values in GTP\(_{y}\)S assay were determined by fitting logistic curve using “Dx calculation (logistic curve fitting) with measured value input function” method in Stat Prelinica Version 1.0.3 (Takumi Information Technology Inc., Tokyo, Japan).

In rat microdialysis, data are presented as time-course changes in peak height and in cumulative values of percentage of baseline over 3\,h after drug administration.
analyzed with one-way ANOVA followed post hoc parametric Dunnett’s multiple comparison test. Differences in immobility time in the rat forced swimming test between test compounds-treated groups and the vehicle-treated group were analyzed with one-way ANOVA followed post hoc parametric Dunnett’s multiple comparison test.

In olfactory bulbectomy, the statistical significance of differences in emotional scores and the number of line crosses between each group was assessed with three-way ANOVA, and post hoc individual group comparison were made with t-test and parametric Dunnett’s multiple comparison test.

In rat conditioned taste aversion model, differences in water or saccharin and saccharin intake ratio between DSP-1053-treated group and the vehicle-treated group were analyzed with one-way ANOVA followed post hoc parametric Tukey’s multiple comparison test.

In fast acting antidepressant with 5-HTergic actions (Mine et al. 2013). In emesis test using S. murinus, data analysis was conducted as previously reported (Mine et al. 2013). In experiment 1, differences in incidence of vomiting between the vehicle-treated group and compounds-treated groups were analyzed by Fisher’s exact test. The number of emetic episodes was determined as average for all tested animals that vomited. In experiment 2, the number of emetic episodes was determined as average for all tested animals. Differences in the number of emetic episodes between Day 1 and Day 2 to Day 7 of treatment were analyzed with one-way ANOVA followed post hoc parametric Tukey’s multiple comparison test.

In all animal experiments, Stat Prelinica Version 1.2 was used as analysis software.

**Results**

**DSP-1053 in vitro binding to the serotonin transporter**

DSP-1053 inhibited the binding of $[^3H]$citalopram to human and rat serotonin transporter, with $K_i$ values of 1.02 ± 0.06 and 0.489 ± 0.039 nmol/L (mean ± SEM duplicate, 3 independent experiments ($n = 3$), respectively. In addition, in 5-HT uptake assay using Chinese hamster ovary cell membrane expressing human serotonin transporter, DSP-1053 inhibited $[^3H]$5-HT uptake with an IC$_{50}$ value of 2.47 ± 0.41 (n = 3). In both assays, DSP-1053 binding affinity for human serotonin transporter was lower than that of paroxetine, but higher than that of imipramine (Table 1).

**DSP-1053 in vitro binding to the 5-HT$_{1A}$ receptor**

DSP-1053 inhibited the binding of $[^3H]$ 8-OH-DPAT to human and rat 5-HT$_{1A}$ receptor with $K_i$ values of 5.05 ± 1.07 and 5.09 ± 1.03 nmol/L (mean ± SEM n = 3), respectively. In GTP$_7$S assay using Chinese hamster ovary cell membrane expressing human 5-HT$_{1A}$ receptor, DSP-1053 displayed I.A. of 70.0 ± 6.3% [EC$_{50}$ 98.0 ± 34.9 nmol/L] (n = 3). DSP-1053 binding affinity for human 5-HT$_{1A}$ receptor was comparable to that of pindolol or 8-OH-DPAT, but lower than that of WAY-106655. On the other hand, DSP-1053 I.A. for 5-HT$_{1A}$ receptor was higher than that of WAY-106655 or pindolol, but lower than that of 8-OH-DPAT (Table 2).

**DSP-1053 binding to off-target receptors and enzymes**

As shown in Table 3, DSP-1053 (1 μmol/L) showed affinity for histamine H$_1$ receptor with $K_i$ value of 7.46 ± 1.37 nmol/L (mean ± SEM n = 3). DSP binding affinity for the other 28 tested receptors was weak ($K_i$ values > 100 nmol/L). Moreover, DSP-1053 (1 μmol/L) did not inhibit pig catechol-O-methyltransferase, human monoamine oxidase-A, and -B (percent inhibition; 0.00, 5.28 and 0.19%, respectively).

**Pharmacokinetics of DSP-1053**

DSP-1053 reached maximum plasma levels within 1 h after p.o. administration with 7.3% bioavailability (Fig. 2). DSP-1053 clearance (CL) and volume of distribution at steady state (Vdss) after injection were 57.6 mL min$^{-1}$ kg$^{-1}$ and 5.1 L kg$^{-1}$, respectively. In all DSP-1053 in vivo studies, dosing time was selected based on the above pharmacokinetic parameters.

**Effects of DSP-1053 on extracellular 5-HT and dopamine levels in the frontal cortex of rats**

Basal microdialysate levels of 5-HT and DA in the rat frontal cortex were 0.355 ± 0.025 and 0.305 ± 0.019 pg/10 μL (n = 17), respectively. DSP-1053 increased 5-HT

| Drug       | $IC_{50}$ (nmol/L) | $K_i$ (nmol/L) | $K_i$ (nmol/L) |
|------------|-------------------|----------------|----------------|
| Human SERT binding |                   |                |                |
| DSP-1053   | 1.56 ± 0.09       | 3.45 ± 0.19    | 1.02 ± 0.06    |
| Paroxetine | 0.279 ± 0.014     | 0.183 ± 0.008  |                |
| Imipramine | 3.60 ± 0.07       | 2.36 ± 0.04    |                |
| Rat SERT binding |           |                |                |
| DSP-1053   | 8.84 ± 0.07       | 1.14 ± 0.02    | 0.489 ± 0.039  |
| 5-HT uptake inhibition |             |                |                |
| DSP-1053   | 2.47 ± 0.41       | –              | –              |
| Paroxetine | 0.181 ± 0.020     | –              | –              |
| Imipramine | 3.73 ± 0.27       | –              | –              |
extracellular levels in the rat frontal cortex. This increase reached a maximum of $180 \pm 25.9\%$ and $264 \pm 58.0\%$ (mean ± SEM) of baseline value 100 min after DSP-1053 administration at 3 and 10 mg kg$^{-1}$, respectively (Fig. 3A). In addition, DSP-1053 (3 and 10 mg kg$^{-1}$) significantly increased cortical 5-HT cumulative value over 3 h after administration, $F(3, 13) = 30.90, P < 0.05$ (Fig. 3B). On the other hand, DSP-1053 did not affect dopamine extracellular levels in the rat frontal cortex at any dose, $F(3, 13) = 0.13, P > 0.05$ (Fig. 3C and D).

DSP-1053 antidepressant-like effect in the rat forced swimming test

As shown in Figure 4A, DSP-1053 significantly decreased immobility time following a 2-week consecutive administration.
with paroxetine (3 and 10 mg kg⁻¹) reduced immobility time compared to the animals treated with the vehicle, F(3, 60) = 5.01, P < 0.05. On the other hand, animals treated with paroxetine (3 and 10 mg kg⁻¹) for 3-week had reduced immobility time compared to the animals treated with the vehicle, F(2, 51) = 4.64, P < 0.05 (Fig. 4B). Treatment with paroxetine for 2-week had no effect on rats immobility time, F(3, 60) = 1.59, P > 0.05 (Fig. 4C).

DSP-1053 antidepressant-like effect in the rat olfactory bulbectomy test

Effects on emotional scores

Figure 5A and B show the effects of 1- and 2-week administration of DSP-1053 on emotional scores of sham-operated and olfactory bulbectomized animals. Three-way (drug, surgery, and dosing period) ANOVA revealed no main effect for drug × surgery × dosing period, drug × dosing period and surgery × dosing period interaction and a significant main effect for drug × surgery interaction (drug × surgery × dosing period interaction, F(15, 144) = 1.16, P > 0.05; drug × dosing period interaction, F(15, 144) = 0.17, P > 0.05; surgery × dosing period interaction, F(15, 144) = 1.84, P > 0.05; drug × surgery interaction, F(15, 144) = 22.55, P < 0.05). Olfactory bulbectomy significantly increased emotional scores, F(15, 144) = 598.83, P < 0.05. Post hoc test analysis showed that in olfactory bulbectomized animals, paroxetine (3 and 10 mg/kg) produced a significant decrease in emotional scores, F(3, 35) = 7.38, P < 0.05, and no effect in the sham-operated animals, F(3, 36) = 1.26, P > 0.05.

Effects on the number of line crosses

Figure 6A and B show the effects of 1- and 2-week administration of DSP-1053 on the number of line crosses of sham-operated and olfactory bulbectomized animals. Three-way ANOVA revealed no main effect for drug × surgery × dosing period, drug × dosing period and surgery × dosing period interaction and a significant main effect for drug × surgery interaction [drug × surgery × dosing period interaction, F(15, 144) = 0.87, P > 0.05; drug × dosing period interaction, F(15, 144) = 0.80, P > 0.05; surgery × dosing period interaction, F(15, 144) = 0.58, P > 0.05; drug × surgery interaction, F(15, 144) = 7.25, P < 0.05]. Olfactory bulbectomy significantly increased the number of line crosses, F(15, 144) = 41.65, P < 0.05. Post hoc test analysis showed that in olfactory bulbectomized animals, DSP-1053 (0.3, 1 and 3 mg/kg) produced a significant decrease in the number of line crosses, F(3, 76) = 7.85, P < 0.05, and no effect in the sham-operated animals, F(3, 76) = 1.55, P > 0.05. Figure 6C and D show the effects of 1- and 2-week administration of paroxetine on the number of line crosses of sham-operated and olfactory bulbectomized animals. Three-way ANOVA revealed a significant main effect for drug × surgery × dosing period interaction, F(13, 125) = 5.18, P < 0.05. In 1-week administration group, subeffect two-way (drug and surgery) ANOVA revealed that paroxetine did not alter emotional scores compared to the vehicle, F(5, 54) = 0.95, P > 0.05. On the other hand, olfactory bulbectomy significantly increased emotional scores, F(5, 54) = 453.49, P < 0.05, and it did not significantly affect the drug effect – drug × surgery interaction, F(5, 54) = 1.94, P > 0.05. In the 2-week administration group, subeffect two-way (drug and surgery) ANOVA revealed a significant main effect for drug × surgery interaction, F(7, 71) = 4.16, P < 0.05. Post hoc test analysis showed that in olfactory bulbectomized animals, paroxetine (3 and 10 mg/kg) produced a significant decrease in emotional scores, F(3, 35) = 7.38, P < 0.05, and no effect in the sham-operated animals, F(3, 36) = 1.26, P > 0.05.
significant main effect for drug × surgery interaction, $F(7, 71) = 2.97, P < 0.05$. Post hoc test analysis showed that in olfactory bulbectomized animals, paroxetine (10 mg/kg) produced a significant decrease in the number of line crosses, $F(3, 35) = 2.92, P < 0.05$, and no effect in the sham-operated animals, $F(3, 36) = 2.58, P > 0.05$. 

Figure 3. Effects of DSP-1053 on 5-HT and dopamine levels in rat frontal cortex. (A and C) Time-course changes in 5-HT and dopamine (DA) levels in the rat frontal cortex after DSP-1053 p.o. administration. Each point with a vertical bar represents the mean ± SEM of percentage baseline value. (B and D) Effects of DSP-1053 on extracellular 5-HT and DA levels in the rat frontal cortex. Each column with vertical bar represents the mean ± SEM of AUC of 5-HT or DA percent over 3 h. **$P < 0.01$, compared to the vehicle-treated group using parametric Dunnett’s multiple comparison test. Vehicle group, $n = 6$; DSP-1053 1 and 3 mg kg$^{-1}$ groups, $n = 4$; and DSP-1053 10 mg kg$^{-1}$, $n = 3$.

Figure 4. Effects of DSP-1053(A) and paroxetine (B and C) on immobility time in the forced swimming test in rat. Each bar represents the mean ± SEM of immobility time during a 5 min test session ($n = 16–18$ per group). *$P < 0.05$, **$P < 0.01$, compared to the vehicle-treated group using parametric Dunnett’s multiple comparison test. 

© 2015 The Authors. Pharmacology Research & Perspectives published by John Wiley & Sons Ltd, British Pharmacological Society and American Society for Pharmacology and Experimental Therapeutics.
Figure 5. Effects of DSP-1053 (A and B) and paroxetine (C and D) on emotional score in sham-operated and olfactory bulbectomized (OB) rats. Each bar represents the mean ± SEM (n = 9–10 per group). $^{##}P < 0.01$ OB group versus sham-operated group (t-test with two-sided significance of 5%). $^*P < 0.05$, $^{**}P < 0.01$ versus vehicle-treated subgroup in OB group (parametric Dunnett’s multiple comparison test with two-sided significance of 5%).
Figure 6. Effects of DSP-1053 (A and B) and paroxetine (C and D) on the number of line crosses in sham-operated and OB rats. Each bar represents the mean ± SEM (n = 9–10 per group). **P < 0.01 OB group versus sham-operated group with vehicle treatment (t-test with two-sided significance of 5%). *P < 0.05, **P < 0.01 versus vehicle-treated subgroup in OB group (parametric Dunnett’s multiple comparison test with two-sided significance of 5%).
DSP-1053 potential to induce emesis in rats conditioned taste aversion test

On Day 4, DSP-1053 (100 mg kg\(^{-1}\)) significantly inhibited saccharin consumption 24 h after administration compared to the vehicle, \(F(2, 15) = 4.73, \ P < 0.05\) (Fig. 7A). Saccharine intake ratio was significantly inhibited at the doses of 60 and 100 mg kg\(^{-1}\), \(F(2, 15) = 22.77, \ P < 0.05\) (Fig. 7B).

DSP-1053 potential to induce emesis in *Suncus murinus*

In experiment 1, DSP-1053 at the dose of 10, 30, or 60 mg kg\(^{-1}\) induced emesis in 0, 1, or 3 of 6 animals, respectively, whereas, as shown in Table 4, paroxetine at 10, 30, or 60 mg kg\(^{-1}\) induced emesis in 0, 2, or 6 of 6 animals, respectively (Mine et al. 2013). In experiment 2, both DSP-1053 (60 mg kg\(^{-1}\)) and paroxetine (60 mg kg\(^{-1}\)) induced emesis in 9 and 12 of 19 animals, respectively. Figure 8 shows the incidence of vomiting and the number of vomiting episodes during a 60-min period in each day of a 7-day consecutive administration of DSP-1053 or paroxetine. A significant reduction in the number of vomiting episodes was observed from Day 2 in DSP-1053-treated group, \(F(6, 56) = 5.60, \ P < 0.05\). On the other hand, repeated administration of paroxetine did not have significant effect on the number of vomiting episodes throughout the 7-day administration period, \(F(6, 77) = 1.64, \ P > 0.05\).

Discussion

In this study, we evaluated the in vitro and in vivo profile of DSP-1053, a structurally novel 5-HT reuptake inhibitor with 5-HT\(_{1A}\) partial agonistic activity. Our results show that DSP-1053 exhibits a potent fast antidepressant-like effect with minimal undesirable effects in animal models.

Our in vitro experiments demonstrate that DSP-1053 works as a serotonin reuptake inhibitor with relatively high partial agonistic activity for 5-HT\(_{1A}\) receptor (70.0 ± 6.3%). It is well reported that SSRIs enhancement of 5-HT neurotransmission is dampened by activation of somatodendritic or postsynaptic 5-HT\(_{1A}\) receptors (Artigas et al. 1996; Casanovas et al. 1999). However, acute increase in 5-HT levels in the rat frontal cortex has also been achieved when antidepressants are used in combination with pindolol, a weak 5-HT\(_{1A}\) receptor partial agonist or WAY-100635, a 5-HT\(_{1A}\) receptor antagonist (Pauwels et al. 1997; Watson et al. 2000; Jerning et al. 2002; Papp et al. 2006; Hughes et al. 2007). On the other hand, acute treatment with DSP-1053, which has a relatively high 5-HT\(_{1A}\) partial agonistic activity, enhanced 5-HT neurotransmission in the rat prefrontal cortex without affecting dopamine neurotransmission. It has been suggested that stimulation of 5-HT\(_{1A}\) receptors enhances dopamine neurotransmission via activation of presynaptic 5-HT\(_{1A}\) receptors expressed in the ventral tegmental area (Lejeune et al. 1997; Millan et al. 1997). This indicates that DSP-1053 may act as antagonist for presynaptic 5-HT\(_{1A}\) receptors. On the other hand, the prolonged stimulation of 5-HT neurotransmission that occurs during chronic SSRI treatment has been reported to desensitize raphe 5-HT\(_{1A}\) autoreceptors, as assessed by single unit recordings and brain microdialysis (Blier and de Montigny 1994; Invernizzi et al. 1994; Arborelius et al. 1995; Le Poul et al. 1995). These findings suggest that DSP-1053 acute enhancement of 5-HT release may be attributed to early desensitization of 5-HT\(_{1A}\) receptors.

![Figure 7](image-url)
Like DSP-1053, vilazodone, and vortioxetine, which inhibit the serotonin transporter with 5-HT1A partial or full agonistic activity, have been reported to enhance 5-HT neurotransmission by acute treatment (Hughes et al. 2005; Pehrson et al. 2013). Although further studies are needed to clarify the exact contribution of 5-HT1A partial activation in DSP-1053 beneficial effects, evidence highlighting the involvement of this receptor in antidepressants early onset suggests that both inhibition and activation of 5-HT1A receptors would fasten SSRI efficacy.

In the rat forced swimming test, which is the most commonly used behavioral test to assess potential antidepressants efficacy (see reviews, e.g., Cryan and Momberreu 2004; Pollak et al. 2004), DSP-1053 decreased rats immobility time after 2 weeks treatment, whereas treatment with paroxetine, a representative SSRI, required 3 weeks for comparable efficacy. These results indicate that DSP-1053 would have earlier onset of efficacy than SSRIs. Next, we evaluated the efficacy and onset of DSP-1053 in the rat olfactory bulbectomy model. The rat olfactory bulbectomy model is a suitable tool for investigating antidepressants onset, because olfactory bulbectomy-induced behavioral abnormalities can be reversed by chronic, but not acute, antidepressants treatment (Kelly et al. 1997; Roche et al. 2007, 2008). As shown in Figures 5, 6, DSP-1053 exerted significant antidepressant-like effects after 1-week administration, whereas paroxetine required 2-week treatment to show similar efficacy. This indicates that DSP-1053 has early onset of action compared to paroxetine. On the other hand, DSP-1053 improvement of hyperemotional behavior may reflect an anxiolytic-like effect as rats muricidal behavior has been reported to be improved by anxiolytics (Takaoka et al. 1988). In the rat forced swimming test and olfactory bulbectomy model, DSP-1053 showed efficacy at a lower dose range than the doses that increased 5-HT level in the rat frontal cortex. The following two reasons might explain this discrepancy. One major difference between the olfactory bulbectomy test and rat microdialysis is the duration of drug administration. That is, chronic treatment with DSP-1053 is considered to induce further desensitization of 5-HT1A receptors and enhancement of 5-HT neurotransmission compared to single administration. As for the second reason, it is believed that activation of postsynaptic 5-HT1A receptors contributes to DSP-1053 low dose antidepressant-like effect. This

| Drugs       | Dose (mg kg⁻¹, po) | Number of animals vomited/tested | Total emetic episode |
|-------------|--------------------|----------------------------------|----------------------|
| Vehicle     | –                  | 0/6                              | –                    |
| DSP-1053    | 10                 | 0/6                              | –                    |
|             | 30                 | 1/6                              | 2                    |
|             | 60                 | 3/6                              | 5.3 ± 1.0            |
| Paroxetine¹| 10                 | 0/6                              | –                    |
|             | 30                 | 2/6                              | 6.8                  |
|             | 60                 | 6/6*                             | 6.7 ± 0.5            |

Incidence of vomiting is shown as the number of shrews that vomited/the number of shrews tested. Total emetic episodes during the 1 h observation period were calculated for animals that vomited and expressed as mean ± SEM.

¹Mine et al. 2013.

*P < 0.01 versus vehicle treatment, as analyzed by Fisher’s exact test.

Figure 8. Effects of 7-day treatment with DSP-1053 or paroxetine on the number of vomiting episodes in Suncus murinus. Each column represents the mean ± SEM of the number of emetic episodes. The number above each column represents the incidence of vomiting as the number of animals that vomited/the number of animals tested. *P < 0.05, **P < 0.01, significantly different from the number of vomiting episodes observed in Day 1 using Dunnett’s multiple comparison test.
hypothesis may be supported by evidence showing that postsynaptic 5-HT_{1A} receptors are particularly important in antidepressant response (Blier and Ward 2003). However, further investigation of changes in neurotransmission after chronic administration of DSP-1053 as well as evaluation of DSP-1053 effects at the efficacy pre and postsynaptic regions would be necessary to confirm the above hypothesis.

Next, we investigated DSP-1053 potential for inducing emesis in experimental animals, because emesis is one of the common side effects of SSRIs (Brambilla et al. 2005; Gelenberg et al. 2010). As emesis is uncommon in rodents, we used the conditioned taste aversion test, which is recognized as a highly reliable tool for evaluation of behavioral alterations induced by radiation or other environmental agents that cause emesis and nausea (Rabin and Hunt 1986). DSP-1053 significantly inhibited saccharin consumption compared to vehicle treatment, indicating this compound potential for nausea or feeling of vomiting. On Day 3, the lower saccharine consumption was observed in vehicle-treated rats than Day 2. This phenomenon was also reported in previous study (Hatcher et al. 1998). Although the reason of this lower saccharine intake is not clear, the first experiment for new taste might defeat the appetite for water. In addition, we used shrews (S. marinus) which can positively respond to various emetic stimuli including motion, X-radiation, and emetogenic substances such as cisplatin and SSRI (Matsuki et al. 1992; Okada et al. 1995; Mine et al. 2013) to further evaluate DSP-1053 potential for emesis. As a result, single administration of DSP-1053-induced emesis in a dose-dependent manner, suggesting that acute treatment with DSP-1053 can produce emesis. This undesirable effect may be due to acute increase in 5-HT level following inhibition of serotonin transporter. On the other hand, the number of vomiting episodes decreased following multiple dosing with DSP-1053, but not paroxetine. This finding indicates that repeated treatment with DSP-1053 results in a fast adaptation to the feeling of nausea and therefore reduction in emetic episodes. It has been reported that enhancement of 5-HT_{1A} receptors activation can reduce vomiting and nausea in animals (Wolff and Leander 1997; Rock et al. 2014). This beneficial effect is believed to be triggered by attenuation of 5-HT neurotransmission following somatodendritic 5-HT_{1A} receptors activation. On the other hand, DSP-1053 enhanced 5-HT neurotransmission in rat microdialysis, indicating fast desensitization of the serotonergic system play an important role in the fast adaptation to the feeling of nausea and therefore emesis. It is reported that SSRI-induced nausea typically ceases as treatment continues (Peretti et al., 2000). Although the precise mechanism of SSRI-induced nausea is still unclear, 5-HT_{3} receptors in the chemoreceptor trigger zone are considered to have an important role, as 5-HT_{3} antagonists, including cisapride and ondansetron, are reported to reduce SSRI-induced gastrointestinal side effects (Bergeron and Blier 1994). In addition, HTR3B gene polymorphisms are considered as significant predictors of paroxetine-induced nausea (Sugai et al. 2006). Moreover, chronic activation of ionotropic 5-HT_{3} receptors is believed to produce significant desensitization of 5-HT_{3} receptors (see review, e.g., Jackson and Yakel 1995). Taken together, these findings indicate that DSP-1053 fast onset enhancement of 5-HT neurotransmission induces fast desensitization of 5-HT_{3} receptors, resulting in fast adaptation to the feeling of nausea and emesis. However, further work is needed to confirm this hypothesis.

In conclusion, this study shows that DSP-1053, a novel serotonin reuptake inhibitor with 5-HT_{1A} receptor partial agonistic activity, shows fast antidepressant-like effect and fast adaptation to the feeling of nausea and emesis in rodent and shrew models. These results highlight the important role of 5-HT_{1A} receptors in both the efficacy and tolerability of DSP-1053 as a new therapeutic option for the treatment of depression.

Acknowledgements

We thank Tomohiro Toyoda, Hidehumi Yoshinaga, Tomaoki Nishida, and Izumi Sasaki for drugs chemical synthesis. We also thank Hitomi Oki and Tadanori Sugimoto for their technical support.

Author Contribution

T. K. and K. Y. participated in research design and wrote or contributed to the writing of the manuscript. T. K., Y. M., M. Y., K. M., S. B, K. N, H. M., and H. N. conducted the experiments. T. K., M. Y., K. M., and S. B. performed data analysis.

Disclosures

All authors are employees of Sumitomo Dainippon Pharma Co., Ltd.

References

Arborelius L, Nomikos GG, Grillner P, Hertel P, Höök BB, Hacksell U, et al. (1995). 5-HT_{1A} receptor antagonists increase the activity of serotonergic cells in the dorsal raphe nucleus in rats treated acutely or chronically with citalopram. Naunyn Schmiedebergs Arch Pharmacol 352: 157–165.
Arborelius L, Nomikos GG, Hertel P, Salmi P, Grillner P, Höök BB, et al. (1996). The 5-HT_{1A} receptor antagonist
(S)-UH-301 augments the increase in extracellular concentrations of 5-HT in the frontal cortex produced by both acute and chronic treatment with citalopram. Naunyn Schmiedebergs Arch Pharmacol 353: 630–640.

Artigas F, Pérez V, Alvarez E (1994). Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors. Arch Gen Psychiatry 51: 248–251.

Artigas F, Romero L, de Montigny C, Blier P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. Trends Neurosci 19: 378–383.

Bel N, Artigas F (1993). Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. Synapse 15: 243–245.

Bergeron R, Blier P (1994). Cisapride for the treatment of nausea produced by selective serotonin reuptake inhibitors. Am J Psychiatry 151: 1084–1086.

Blier P, Bergeron R (1995). Effectiveness of pindolol with selected antidepressant drugs in the treatment of major depression. J Clin Psychopharmacol 15: 217–222.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. Trends Pharmacol Sci 15: 220–226.

Blier P, Ward NM (2003). Is there a role for 5-HT_{1A} agonists in the treatment of depression? Biol Psychiatry 53: 193–203.

Brambilla P, Cipriani A, Hotopf M, Barbui C (2005). Side-effect profile of fluoxetine in comparison with other SSRIs, and in combination with paroxetine, on olfactory bulbectomy model for the detection of antidepressant drugs: Olfactory bulbectomy in the rat compared with existing models. J Pharmacol Sci 2013: 143.

Bull SA, Hunkeler EM, Lee JY, Rowland CR, Williamson TE, Schwab JR, et al. (2002). Discontinuing or switching selective serotonin reuptake inhibitors. Ann Pharmacother 36: 578–584.

Cairncross KD, Cox B, Forster C, Wren AF (1978). A new rationale and current status of research. CNS Drugs 2013: 703–716.

Cryan JF, Mombereau C (2004). In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. Mol Psychiatry 9: 326–357.

Cryan JF, McGraith C, Leonard BE, Norman TR (1999). Onset of the effects of the 5-HT_{1A} antagonist, WAY-100635, alone, and in combination with paroxetine, on olfactory bulbectomy and 8-OH-DPAT-induced changes in the rat. Pharmacol Biochem Behav 63: 333–338.

Dawson LA, Nguyen HQ (1998). Effects of 5-HT_{1A} receptor antagonists on fluoxetine-induced changes in extracellular serotonin concentrations in rat frontal cortex. Eur J Pharmacol 345: 41–46.

Dawson LA, Watson JM (2009). Vilazodone: a 5-HT_{1A} receptor agonist/serotonin transporter inhibitor for the treatment of affective disorders. CNS Neurosci Ther 15: 107–117.

Gartside SE, Umbers V, Hajós M, Sharp T (1995). Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRI in vivo: effects on 5-HT cell firing and extracellular 5-HT. Br J Pharmacol 115: 1064–1070.

Gelenberg A, Freeman M, Markowitz J (2010). Practice guideline for the treatment of patients with major depressive disorder. 3rd ed U.S. Department of Health & Human Services, Washington, D.C.

Gomita Y, Morii M, Ichimaru Y, Moriyama M, Ueki S (1983). Behavioral and electroencephalographic study of 7-chloro-1-methyl-5-phenyl-1 H-1,5-benzodiazepine-2,4-(3H,5H)-dione (clobazam). Nihon Yakurigaku Zasshi 82: 267–292.

Hatcher JP, Loudon JM, Hagan JJ, Clark MS (1998). Sibutramine (SR-20206), a functionally selective M1 receptor partial agonist, reverses delay-induced deficits in the T-maze. Psychopharmacology 138: 275–282.

Hughes ZA, Starr KR, Langmead CJ, Hill M, Bartoszyk GD, Hagan JJ, et al. (2005). Neurochemical evaluation of the novel 5-HT_{1A} receptor partial agonist/serotonin reuptake inhibitor, vilazodone. Eur J Pharmacol 510: 49–57.

Hughes ZA, Starr KR, Scott CM, Newson MJ, Sharp T, Watson JM, et al. (2007). Simultaneous blockade of 5-HT_{1A/B} receptors and 5-HT transporters results in acute increases in extracellular 5-HT in both rats and guinea pigs: in vivo characterization of the novel 5-HT_{1A/B} Receptor antagonist/5-HT transport inhibitor SB-649915-B. Psychopharmacology 192: 121–133.

Invernizzi R, Bramante M, Samanin R (1994). Chronic treatment with citalopram facilitates the effect of a challenge dose on cortical serotonin output: role of presynaptic 5-HT_{1A} receptors. Eur J Pharmacol 260: 246–251.

Jackson MB, Yakel JL (1995). The 5-HT_{3} receptor channel. Annu Rev Physiol 57: 447–468.

Jerning E, Rosqvist S, Mohell N (2002). Nad-299 antagonises 5-HT-stimulated and spiperone-inhibited [125I]GTPgammaS binding in cloned 5-HT_{1A} receptors. J Recept Signal Transduct Res 22: 483–495.

Kelly JP, Wrynn AS, Leonard BE (1997). The olfactory bulbectomized rat as a model depression: an update. Pharmacol Ther 74: 299–316.

Kessler RC, Chiu WC, Demler O, Walters F (2005). Prevalence, severity and co-morbidity of 12 month DSM-IV...
disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 62: 617–627.

Kreiss DS, Lucki I (1995). Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. J Pharmacol Exp Ther 274: 866–876.

Le Poul E, Laaris N, Doucet E, Laporte AM, Hamon M, Lanfumey L (1995). Early desensitization of somato-dendritic 5-HT1A autoreceptors in rats treated with fluoxetine or paroxetine. Naunyn Schmiedebergs Arch Pharmacol 352: 141–148.

Lejeune F, Newman-Tancredi A, Audinot V, Millan MJ (1997). Interactions of (+)- and (-)-8- and 7-hydroxy-2-(di-n-propylamino)tetralin at human (h)D3, hD3 and h serotonin1A receptors and their modulation of the activity of serotoninergic and dopaminergic neurones in rats. J Pharmacol Exp Ther 280: 1241–1249.

Matsuki N, Torii Y, Ueno S, Saito H (1992). Suncus murinus as an experimental animal model for emesis and motion sickness. Pp. 323–329 in A. L. Bianchi, L. Grelot, A. D. Miller and G. L. King, eds. Mechanisms and control of emesis. John Libbey Eurotext Ltd, Montrouge.

Millan MJ, Newman-Tancredi A, Rivet JM, Brocco M, Lacroix P, Audinot V, et al. (1997). S 15535, a novel benzodioxopiperazine ligand of serotonin (5-HT)1A receptors: I. Interaction with cloned human (h)3-HT1A, dopamine hD3/ hD3 and h serotonin1A receptors and h2A-adrenergic receptors in relation to modulation of cortical monoamine release and activity in models of potential antidepressant activity. J Pharmacol Exp Ther 282: 132–147.

Mine Y, Oku S, Yoshida N (2013). Anti-emetic effect of mosapride citrate hydrate, a 5-HT4 receptor agonist, on selective serotonin reuptake inhibitors (SSRIs)-induced emesis in experimental animals. J Pharmacol Sci 121: 58–66.

Nishida T, Yoshinaga H, Toyoda T (2012). First- and second-generation practical syntheses of chroman-4-one derivative: a key intermediate for the preparation of SERT/5-HT1A dual inhibitors. Org Process Res Dev 16: 625–634.

Okada F, Saito H, Matsuki N (1995). Blockade of motion- and cisplatin-induced emesis by a 5-HT4 receptor agonist in Suncus murinus. Br J Pharmacol 114: 931–934.

Olsson M, Marcus SC, Tedeschi M, Wan GJ (2006). Continuity of antidepressant treatment for adults with depression in the US. Am J Psychiatry 63: 101–108.

Papp M, Grupa P, Litwa E, Lason M, Przegalinski E (2006). Antidepressant-like activity of WF-516, an antagonist of 5-HT1A receptors and inhibitor of 5-HT reuptake, in a chronic mild stress model of depression in rat. Eur Neuropsychopharmacol 16: S305.

Pauwels PJ, Tardif S, Wurth T, Colpaert FC (1997). Stimulated [35S]GTP gamma S binding by 5-HT1A receptor agonists in recombinant cell lines. Modulation of apparent efficacy by G-protein activation state. Naunyn Schmiedebergs Arch Pharmacol 356: 551–561.

Pehrson AL, Cremers T, Betry C, van der Hart MG, Jørgensen L, Madsen A, et al. (2013). Lu AA21004, a novel multimodal antidepressant, produces regionally selective increases of multiple neurotransmitters—a rat microdialysis and electrophysiology study. Eur Neuropsychopharmacol 23: 133–145.

Peretti S, Judge R, Hindmarch I (2000). Safety and tolerability considerations: tricyclic antidepressant vs. selective reuptake inhibitors. Acta Psychiatr Scand Suppl 403: 17–25.

Pérez V, Gilaberte I, Faries D, Alvarez E, Artigas F (1997). Randomised, double-blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment. Lancet 31: 1594–1597.

Pollak DD, Monje FJ, Zuckerman L, Denny CA, Drew MR, Kandel ER (2004). An animal model of a behavioral intervention for depression. Neuron 60: 149–161.

Porsolt RD, Anton G, Blavet N, Jalfre M (1978). Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47: 379–391.

Rabin BM, Hunt WA (1986). Mechanisms of radiation-induced conditioned taste aversion learning. Neurosci Biobehav Rev 10: 55–65.

Reeves H, Batra S, May RS, Zhang R, Dahl DC, Li X (2008). Efficacy of risperidone augmentation to antidepressants in the management of suicidality in major depressive disorder: a randomized, double-blind, placebo-controlled pilot study. J Clin Psychiatry 69: 1228–1236.

Roche M, Harkin A, Kelly JP (2007). Chronic fluoxetine treatment attenuates stressor-induced changes in temperature, heart rate, and neuronal activation in the olfactory bulbectomized rat. Neuropsychopharmacology 32: 1312–1320.

Roche M, Shanahan E, Harkin A, Kelly JP (2008). Trans-species assessment of antidepressant activity in a rodent model of depression. Pharmacol Rep 60: 404–408.

Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ, et al. (2014). Cannabidiol, a non-psychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT1A somatodendritic autoreceptors in the dorsal raphe nucleus. Br J Pharmacol 165: 2620–2634.

Rush AJ, Trivedi MH, Wisniewski SR, Stewart JW, Nierenberg AA, Thase ME, et al. (2006). Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. N Eng J Med 354: 1231–1242.

Rutter JJ, Gundlah C, Auerbach SB (1994). Increase in extracellular serotonin produced by uptake inhibitors is enhanced after chronic treatment with fluoxetine. Neurosci Lett 171: 183–186.
Sugai T, Suzuki Y, Sawamura K, Fukui N, Inoue Y, Someya T (2006). The effect of 5-hydroxytryptamine 3A and 3B receptor genes on nausea induced by paroxetine. Pharmacogenomics J 6: 351–356.

Takaoka N, Yoshimura H, Ogawa N (1988). Comparison of the effects of benzodiazepine and nonbenzodiazepine anxiolytics on mouse-killing behavior in rats. Jpn J Pharmacol 46: 315–318.

Waraich P, Goldner EM, Somers JM, Hsu L (2005). Prevalence and incidence studies of mood disorders: a systematic review of the literature. Can J Psychiatry 50: 569–570.

Warden D, Rush AJ, Trivedi MH, Fava M, Wisniewsk SR (2007). The STAR*D Project results: a comprehensive review of findings. Curr Psychiatry Rep 9: 449–459.

Watson J, Collin L, Ho M, Riley G, Scott G, Selkirk JV, et al. (2000). 5-HT_{1A} receptor agonist-antagonist binding affinity difference as a measure of intrinsic activity in recombinant and native tissue systems. Br J Pharmacol 130: 1108–1114.

Wolff MC, Leander JD (1997). Effects of a 5-HT_{1A} receptor agonist on acute and delayed cyclophosphamide-induced vomiting. Eur J Pharmac 340: 217–220.