Pharmacological characterization of a novel potent, selective, and orally active orexin 2 receptor antagonist, SDM-878

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Abstract

Aims: Recently, we identified a novel orexin 2 (OX2) receptor antagonist, SDM-878 (2-(3-(2-(1H-pyrazol-1-yl)nicotinoyl)-3,8-diazabicyclo[3.2.1]octan-8-yl)-3-methoxyisonicotinonitrile). The purpose of the present study is to characterize the in vitro and in vivo pharmacological effects of SDM-878.

Methods: The in vitro potency and selectivity of SDM-878 were examined in CHO cells that exhibit stable expression of human orexin 1 (OX1), human orexin 2 (OX2), rat OX1, and rat OX2 receptors. Then, the plasma half-life, oral bioavailability, and brain penetration of SDM-878 were examined in rats. The in vivo effect of SDM-878 in rats was tested using electroencephalography (EEG). The target engagement of SDM-878 in the rat brain was examined using the antagonistic effect against hyperlocomotion caused by the intracerebroventricular administration of the OX2 receptor agonist, ADL-OXB ([Ala11, d-Leu15]-orexin B).

Results: SDM-878 showed potent inhibitory activities for human and rat OX2 receptors with IC values of 10.6 and 8.8 nM, respectively, and approximately 1000-fold selectivity against the OX1 receptor. In rat studies, SDM-878 exhibited a relatively short half-life in plasma, oral bioavailability, and good brain penetration. These data indicate that SDM-878 is a potent, selective, orally active, and brain-penetrable OX2 receptor antagonist. In behavioral studies using rats, SDM-878 (100 mg/kg) antagonized hyperlocomotion caused by intracerebroventricular administration of ADL-OXB. SDM-878 exhibited a potent sleep-promoting effect at the same dose (100 mg/kg) in a rat EEG study.

Conclusion: Our results suggest that SDM-878 is likely to be a good pharmacological tool for investigating the role of the OX2 receptor and may have therapeutic potential for the treatment of insomnia.

KEYWORDS
electroencephalography, orexin, OX1 receptor, OX2 receptor, sleep

Abbreviations: ADL-OXB, [Ala11, d-Leu15]-orexin-B; EEG, electroencephalography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; NREM, non-rapid eye movement; OX1 receptor, orexin 1 receptor; OX2 receptor, orexin 2 receptor; REM, rapid eye movement; SDM-878, (2-(3-(2-(1H-pyrazol-1-yl)nicotinoyl)-3,8-diazabicyclo[3.2.1]octan-8-yl)-3-methoxyisonicotinonitrile).
INTRODUCTION

Orexin-A and orexin-B, also known as hypocretin 1 and hypocretin 2, are endogenous neuropeptides, which have been identified as ligands of orphan G protein-coupled receptors obtained from rat brain extracts. The physiological functions of orexins are exerted via two receptor subtypes, the orexin 1 (OX₁) receptor and the orexin 2 (OX₂) receptor, which belong to the family of G protein-coupled receptors. Orexin-A is a 33-residue peptide and exhibits potent agonistic activity against both OX₁ and OX₂ receptors. Conversely, orexin-B consists of 28 amino acids and has a 10-fold higher affinity for the OX₂ receptor than for the OX₁ receptor. The expression patterns of the two receptors in the brain partially overlap, and some brain regions express only one type of receptor. Therefore, each receptor is likely to have different pharmacological functions.

Accumulating evidence indicates that orexins are involved in the regulation of multiple functions including feeding, addiction and sleep/wake cycles. The key role of the orexin system in the regulation of sleep/wake cycles has been highlighted in a number of genetic and pharmacological studies. Mice lacking either the OX₂ receptor or both the OX₁ and OX₂ receptors have shown a narcoleptic-like phenotype with cataplexy, as have orexin deficient mice, while OX₂ knockout mice did not display such phenotype. Additionally, it has been reported that familial canine narcolepsy is caused by the disruption of both the OX₁ and OX₂ receptors. Furthermore, a nearly complete loss of orexin neurons was observed in human patients with narcolepsy, as measured by orexin immunoreactivity in postmortem brain slices, resulting in clear clinical proof of the critical role of orexin receptors in humans.

Therefore, the pharmacological blockade of orexin receptors provides a novel approach for the treatment of insomnia; in fact, an OX₁ and OX₂ receptor antagonists, almorexant, were disclosed in 2007; almorexant induced a physiological sleep architecture characterized by increases in the time spent in both rapid eye movement (REM) and non-REM (NREM) sleep in humans. Almorexant also increased REM and NREM sleep in a dose-dependent manner in mice. However, the sleep induction effect exerted by almorexant was absent in mice lacking both the OX₁ and OX₂ receptors as well as in mice deficient in only the OX₂ receptor, yet no sleep induction effect was observed in OX₁ receptor knockout mice. These findings indicate that the sleep-promoting effect of almorexant was mainly mediated by the blockade of OX₂ receptor, and that antagonism of the OX₂ receptor alone was sufficient to induce sleep in mice. Consequently, the development of a selective OX₂ receptor antagonist would be a meaningful step in the investigation of the contribution of OX₂ receptor to the regulation of sleep/wake cycles.

Recently, we identified SDM-878, a novel OX₂ receptor antagonist. In the present study, we aim to characterize the in vitro and in vivo pharmacological profiles of SDM-878. SDM-878 is a potent, selective, and orally available OX₂ receptor antagonist with relatively short half-life in plasma and good brain penetration. The in vivo effect of SDM-878 in rats was tested using electroencephalography (EEG). The target engagement of SDM-878 in the rat brain was examined using the antagonist effect against hyperlocomotion caused by the intracerebroventricular administration of the OX₂ receptor agonist, ADL-OXB ([Ala¹¹, α-Leu¹⁷]-orexin-B).

METHODS

Animals

Male Sprague-Dawley rats (6 weeks old, Charles River Laboratories Japan, Inc) were purchased and group-housed before surgery. After surgery, each rat was housed individually in an air-conditioned room (room temperature: 23 ± 3°C, humidity: 55 ± 15%), with a 12-hour light-dark cycle (lights on: 05:30‒17:30). Each rat was provided with standard chow (CE-2, CLEA Japan, Inc) and tap water ad libitum. The rats were allowed to acclimatize in the facility for at least 7 days prior to initiation of the experiments.

All experimental procedures were approved by the Institutional Animal Care and Use Committee in compliance with the Japanese law of “Act on Welfare and Management of Animals” and the guidelines of the Ministry of Health, Labor and Welfare of Japan.

Drugs

The structure of SDM-878 is shown in Figure 1. First, based on α-stacked horseshoe-like conformation observed in the crystal structure of human OX₂ receptor bound to suvorexant, we found a lead compound with 3,8-diazabicyclo[3.2.1]octane using ligand-based drug design. Then, SDM-878 was identified in the course of the extensive chemical optimization of the lead compound to find a highly selective OX₂ receptor antagonist with short half-life in plasma.
SDM-878 was synthesized in-house and used as a free base in all experiments. SDM-878 was dissolved in dimethylsulfoxide and stored at −20°C to perform in vitro assays. SDM-878 was suspended in 0.5% methylcellulose solution (Wako Pure Chemical Industries, Ltd.) and orally administered at a volume of 5 mL/kg. For the pharmacokinetic study, SDM-878 was dissolved in 40% (v/v) N, N-dimethylacetamide (Wako Pure Chemical Industries), 40% (v/v) polyethylene glycol 400 (Wako Pure Chemical Industries), and 20% (v/v) distilled water before being administered intravenously at a volume of 1 mL/kg. ADL-OXB ([Ala31, D-Leu15]-orexin-B, Tocris Bioscience), an OX2 receptor agonist, and angiotensin II (Peptide Institute, Inc) were dissolved in physiological saline and administered intracerebroventricularly at a volume of 5 μL/rat. Based on our previous studies, we selected doses of 3 nmol/rat for ADL-OXB and 100 ng/rat for angiotensin II.15

2.3 | In vitro assays

CHO-DXB11 cell lines exhibiting stable expression of human OX1, human OX2, rat OX1, and rat OX2 receptors were generated by transfecting each expression plasmid into cells. CHO cells expressing each receptor were seeded at 4 × 104 cells/well in a 96-well black wall/clear bottom plate (Greiner Bio-One International GmbH) and cultured overnight. The cells were then incubated with calcium-sensitive fluorochrome dye (FLIPR Calcium 5 assay kit; Molecular Devices LLC) in an assay buffer (Hank’s balanced salt solution containing 20 mmol/L HEPES, 0.1% bovine serum albumin, and 1.25 mmol/L probenecid) for 1 hour at 37°C with 5% CO2 in a humidified atmosphere. The intracellular Ca2+ mobilization was measured using a fluorometric imaging plate reader (FDSS, Hamamatsu Photonics KK). The cells were incubated with various concentrations of SDM-878 for 5 minutes at room temperature. Subsequently, the antagonistic activity was evaluated for 3 minutes after the application of orexin-A. The final concentration of orexin-A was determined by calculating the EC50 value after measuring the dose response of orexin-A in each assay. Each assay was performed in duplicate over a 7-point concentration series of SDM-878 (3-10 000 nmol/L). The antagonistic effect of SDM-878 was measured by the change in intracellular Ca2+ concentrations. The IC50 values were calculated using Assay Explorer (Accelrys, Inc).

2.4 | Off-target activity

The selectivity of SDM-878 was assessed at a concentration of 10 μmol/L against 55 molecular targets, including neurotransmitter receptors, ion channels, and transporters, using the Cerep ExpresSProfile In Vitro Pharmacology Profiling from Cerep SA (l’Evescault).

2.5 | Pharmacokinetic study

SDM-878 was administered intravenously at a dose of 1 mg/kg to the fed rats or orally at a dose of 30 mg/kg to the rats fasted overnight. Blood samples were collected at appropriate time intervals after the administration of SDM-878 and then centrifuged at 2150 g for 15 minutes at 4°C to obtain plasma. Each plasma sample was centrifuged at 540 g for 5 minutes at 4°C to yield supernatants before being filtrated using MultiScreen HTS HV (Millipore Corp) and analyzed by liquid chromatography with tandem mass spectrometry (LC-MS/MS). To assess brain penetration, SDM-878 was administered to the fed rats orally at doses of 30 and 100 mg/kg. Blood samples were collected 30 minutes after the administration, after which the rats were immediately euthanized, and their brains were collected. The collected brain samples were homogenized in 5 times the brain volume of H2O using a Precellys24 (Bertin Technologies SAS). An internal standard was added, and the homogenates were centrifuged at 3000 rpm for 5 minutes at 4°C to yield supernatants for analysis by LC-MS/MS. Pharmacokinetic parameters were calculated via non-compartmental analysis using WinNolin, version 6.2 (Pharsight).

2.6 | ADL-OXB-induced hyperlocomotion

Locomotor activity was measured using a digital counter system with an infrared sensor (SUPERMEX, Muromachi Kikai Co, Ltd.) in rats implanted with a guide cannula as previously described. Each rat was placed on a stereotaxic frame (NARISHIGE Group) under anesthesia. For intracerebroventricular administration of ADL-OXB, a guide cannula (21 G, 10 mm) was implanted at 0.9 mm posterior, 1.2 mm lateral, and 2.3 mm ventral to the bregma and was secured with screws and dental cement; all rats were individually housed after surgery.

After a recovery period of at least 7 days after surgery, the placement of the guide cannula was tested by injection of angiotensin II (100 ng/rat, i.c.v.). Only rats displaying consumption of at least 5 g of water within a 30 minutes period were selected and used for the subsequent experiments. An injection cannula (25 G, 13 mm) was connected to a microsyringe (Hamilton Company,) through polyethylene tubing and inserted into the guide cannula. Depending on treatment group, each rat received an infusion of ADL-OXB or vehicle via the injection cannula. After infusion, the injection cannula was left in place for 30 seconds before removal to avoid liquid backflow.

Each rat was placed into a novel plastic cage (width, 270 mm × depth, 440 mm × height, 187 mm) with clean paper chips and subsequently habituated to the plastic cage for 2 hours before administration of the test compound. SDM-878 was orally administered to each rat at 30 minutes before administration of ADL-OXB (3 nmol/rat, i.c.v.), after which each rat was returned to the plastic cage. Immediately after administration of ADL-OXB or vehicle, locomotor activity was measured for 2 hours.

2.7 | Electroencephalography (EEG)

Each rat was placed on a stereotaxic frame (NARISHIGE Group) under anesthesia. Stainless steel screw electrodes (TF209-038,
Unique Medical Co., Ltd.) were implanted in the frontal and occipital cortex for the EEG. Two stainless steel wire electrodes (TF209-045, Unique Medical Co., Ltd.) were implanted in the trapezius muscles of neck region for the electromyogram (EMG). These electrodes were socketed into an electrode connector body (TF209-046d, Unique Medical Co., Ltd.) and secured with dental cement and instant glue.

After a recovery period of at least 7 days after surgery, each rat was habituated to the cage for at least 30 minutes before the initiation of the EEG and EMG recordings. For each rat, the EEG and EMG recordings began 10–20 minutes before the administration of either SDM-878 or the vehicle (recordings began at 17:10 and the test compounds were administered between 17:20 and 17:30). The EEG and EMG signals were recorded for up to 13 hours after the administration of the test compounds; recordings were amplified and passed through bandpass filters using a biophysical amplifier (Nihon Kohden Corp.). The signals were digitized at a sampling rate of 128 Hz with an analog-to-digital converter (Contec) and were recorded using the computer program VitalRecorder_ver. 1.3 (Kissei Comtec). Recording results were analyzed by the computer software SleepSign version 3.0 (Kissei Comtec). Sleep-wake states were classified into 10-sec epochs as either wakefulness, NREM sleep, REM sleep, and total sleep (NREM sleep plus REM sleep) during a 2-hour period were calculated.

2.8 | Statistical analysis

Student’s t test was used to analyze the effect of ADL-OXB on locomotor activity. The effects of the test compound were analyzed using one-way analysis of variance, and post hoc comparisons were performed using Dunnett’s test. A probability level of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | In vitro and pharmacokinetic profiles of SDM-878

The antagonistic activity of SDM-878 was examined using cloned human OX₁, human OX₂, rat OX₁, and rat OX₂ receptors. In CHO cells that exhibit stable expression of human and rat OX₂ receptors, SDM-878 inhibited orexin-A-induced increases in intracellular Ca²⁺ concentrations in a concentration-dependent manner and completely blocked these responses at 1000 nmol/L. The IC₅₀ values of SDM-878 were 10.6 nmol/L against human OX₂ receptors and 8.8 nmol/L against rat OX₂ receptors, respectively. On the other hand, in CHO cells that exhibit stable expression of human and rat OX₁ receptors, SDM-878 partially inhibited orexin-A-induced increases in intracellular Ca²⁺ concentrations even at 10 000 nmol/L. The inhibition rate was 49% against human OX₁ receptors and 52% against rat OX₁ receptors, respectively. We found that SDM-878 had the IC₅₀ values of 9643 nmol/L against human OX₁ receptors and 9682 nmol/L against rat OX₁ receptors, showing that the activity of SDM-878 is about 1000-fold weaker against human and rat OX₁ receptors than against human and rat OX₂ receptors. The selectivity of SDM-878 was also examined against 55 other molecular targets (Table S1). SDM-878 exhibited no inhibitory effects of greater than 50% at a concentration of 10 μmol/L (Table S1).

SDM-878 exhibited a plasma half-life of 0.37 hours at an intravenous dose of 1 mg/kg and oral bioavailability of 8.5% at a dose of 30 mg/kg (Table 1). SDM-878 also exhibited good brain penetration 1 hour after the oral administration at doses of 30–100 mg/kg in rats (Table 2).

3.2 | Effect of SDM-878 on ADL-OXB-induced hyperlocomotion in rats

We examined the effect of SDM-878 on ADL-OXB-induced hyperlocomotion in rats. SDM-878 significantly antagonized ADL-OXB-induced hyperlocomotion at a dose of 100 mg/kg (Figure 2).

3.3 | Effect of SDM-878 on EEG in rats

In our rat EEG study on the sleep-promoting effects of SDM-878, we found that a dose of 100 mg/kg completely antagonized ADL-OXB-induced hyperlocomotion in rats. Administration of SDM-878 significantly decreased time spent in wakefulness (Figure 3A) and significantly increased total sleep (Figure 3D). The duration of NREM sleep was significantly increased (Figure 3B), while we did not observe a significant effect on the duration of REM sleep at a dose of 100 mg/kg (Figure 3C). The administration of SDM-878 did not appear to affect sleep architecture, as expressed as the REM/total sleep ratio, which was 10.2 ± 1.7% in the SDM-878 treatment group and 9.5 ± 1.1% in the vehicle-treated group.

| Dose (mg/kg) | Route | t₁/₂ (h) | T_max (h) | C_max (ng/mL) | F (%) |
|-------------|-------|----------|-----------|--------------|-------|
| 1           | iv    | 0.37     |           |              |       |
| 30          | p.o.  | 0.43     | 0.25      | 1179         | 8.5   |

Note: Data are expressed as mean of 2 animals.

| TABLE 1 | Pharmacokinetic profile of SDM-878 in rats |
TABLE 2 Plasma and brain concentrations of SDM-878 in rats

| Dose (mg/kg) | Plasma (ng/mL) | Brain (ng/g) | Brain/plasma ratio | n  |
|-------------|----------------|--------------|--------------------|----|
| 30          | 110 ± 37       | 28 ± 16      | 0.25               | 3  |
| 100         | 601            | 198          | 0.33               | 2  |

Note: Data are expressed as mean ± SD of 2-3 animals.

4 | DISCUSSION

We identified a novel potent, selective, orally active, and brain-penetrable OX2 receptor antagonist, SDM-878. SDM-878 showed potent inhibitory activities for the human and rat OX2 receptors, with IC50 values of 10.6 and 8.8 nmol/L, respectively. SDM-878 did not exhibit significant off-target activity against the OX1 receptor or other pharmacological targets, including neurotransmitter receptors, ion channels, and transporters. Furthermore, SDM-878 exhibited favorable pharmacokinetic profiles such as a relatively short half-life in plasma, oral bioavailability, and good brain penetration in rats. These results indicate that SDM-878 is a promising pharmacological tool for the investigation of the role of the OX2 receptor in vitro and in vivo.

Suvorexant, the first approved drug for the treatment of primary insomnia, improved the quality of sleep in humans. However, suvorexant exhibits an extended half-life in plasma, prompting the US Food and Drug Administration to express concerns about adverse next-day residual effects associated with high doses. Therefore, orexin receptor antagonists with short half-life are desirable, as they are more likely to avoid next-day residual effects. Accordingly, ACT-541468, a dual orexin receptor antagonist, and JNJ-42847922, an OX2 receptor selective antagonist, were developed; these compounds exhibit pharmacokinetic profiles that include short half-life in plasma in rats and dogs. Consequently, ACT-541468 and JNJ-42847922 showed half-lives of 5.9–8.8 hours and 2.02–2.44 hours, respectively, much shorter than the 9–13 hours half-life of suvorexant in healthy subjects. ACT-541468 and JNJ-42847922 are currently under clinical trials to clarify whether these compounds cause next-day residual effects in patients with insomnia. In the present study, SDM-878 was rapidly absorbed, with a Tmax of 0.25 hours and a relatively short plasma half-life of 0.43 hours in rats at an oral dose of 30 mg/kg. These pharmacokinetic profiles of SDM-878 were similar to those of ACT-541468 and JNJ-42847922, indicating that SDM-878 has an optimal pharmacokinetic profile for the treatment of insomnia. However, further clinical studies are needed to demonstrate whether SDM-878 exhibits these suitable pharmacokinetic profiles in humans. Ultimately, we suggest that SDM-878 should be a useful agent for the treatment of insomnia that is likely to exhibit both a rapid onset of action and a low risk of next-day residual effects in humans.

ADL-OXB is a selective OX2 receptor agonist that exhibits a 400-fold selectivity for the OX2 receptor over the OX1 receptor and causes the increase in locomotor activity in rats. To examine the sleep-promoting effect of SDM-878, we investigated the effect of SDM-878 in rat EEG study. First, we determined an effective dose at which SDM-878 fully blocks the OX2 receptor in rats by using SDM-878 to antagonize ADL-OXB-induced hyperlocomotion in rats. SDM-878 antagonized ADL-OXB-induced hyperlocomotion in a dose-dependent manner and completely attenuated the hyperlocomotion at a dose of 100 mg/kg. Because SDM-878 did not affect the spontaneous locomotor activity up to 100 mg/kg (data not shown), we suggest that the observed SDM-878-mediated antagonism of ADL-OXB-induced hyperlocomotion is a specific pharmacological effect by the blockade of OX2 receptor in vivo. These results indicate that SDM-878 sufficiently blocks the OX2 receptor at a dose of 100 mg/kg in rats. Consequently, we selected 100 mg/kg to examine the effect of SDM-878 in rat EEG study.

Second, we performed a rat EEG study to examine the sleep-promoting effect of SDM-878. SDM-878 significantly decreased the time spent in wakefulness and increased the total sleep at a dose of 100 mg/kg. SDM-878 significantly increased the duration of NREM sleep but did not affect the duration of REM sleep. These results were mostly consistent with previous reports that an OX2 receptor selective antagonist, JNJ-42847922, prolonged NREM sleep time in the first 2 hours of sleep, but did not exert a significant effect on REM sleep in rats. Recently, a study of individuals with insomnia without psychiatric comorbidity treated with JNJ-42847922 found increased sleep efficiency, prolonged total sleep time, and decreased latency to persistent sleep and wake after sleep onset compared with a placebo treatment; furthermore, there were no expected safety findings. These findings...
indicate that the sleep-promoting effect of JNJ-42847922 occurs in both rats and humans and that blockade of the OX₂ receptor exerts a potent sleep-promoting effect without adverse effects in humans. In combination with the data from the JNJ-42847922 studies, our data suggest that SDM-878 should exhibit a sleep-promoting effect in humans similar to JNJ-42847922. Furthermore, Minerva Neurosciences Inc recently reported that, in patients with insomnia, JNJ-42847922 at doses of 10 and 20 mg exhibited superior latency to persistent sleep as primary efficacy endpoint compared with zolpidem, an active comparator, as well as to a placebo (ClinicalTrials.gov: NCT03375203). These results indicate that the OX₂ receptor selective antagonist may cause patients to fall asleep rapidly and is likely to be a promising alternative approach for the treatment of insomnia.

In the present study, the sleep architecture of SDM-878, expressed as the REM/total sleep ratio, was preserved at a dose of 100 mg/kg. These results were mostly consistent with the previous report of the effects of JNJ-42847922⁹ and serve to further confirm that the blockade of the OX₂ receptor primarily promotes NREM sleep.¹⁹,²⁵,²⁶ However, various dual orexin receptor antagonists have been shown to promote both NREM and REM sleep in animals and humans¹⁰⁻¹¹,²⁷ and to predominantly promote REM sleep.²⁵,²⁶ Therefore, high selectivity for OX₂ receptor antagonism may preserve sleep architecture as expressed as maintenance of the REM/total sleep ratio, indicating that OX₂ receptor selective antagonists induce physiological sleep. Because OX₁ receptor selective antagonists did not exert sleep-promoting effects in rats,²⁸,²⁹ the complement of the blockade of OX₂ receptor may lead to the difference between OX₂ receptor selective antagonists and dual orexin receptor antagonists in their relative impact on REM vs NREM. It has been postulated that the different role of each orexin receptor is responsible for each receptor’s distinct distribution in the brain,¹³⁰ that is, the presence of OX₁ receptors in the locus coeruleus may promote the regulation of REM sleep,²⁸,³¹ and the presence of OX₂ receptors in the hypothalamus may promote the regulation of NREM sleep.³² Additionally, dual orexin receptor knockout mice have been reported to show more pronounced sleep than do OX₂ receptor knockout mice, while OX₁ receptor knockout mice exhibited only minor sleep alterations.³³ These findings indicate that the blockade of the OX₁ receptor may influence the sleep-promoting effect of OX₂ receptor antagonism. However, whether an OX₂ receptor selective antagonist or a dual orexin receptor antagonist is more appropriate for the treatment of insomnia is yet to be determined. Further studies with wide dose ranges that provide direct head-to-head comparison between OX₂ receptor selective antagonists and dual orexin receptor antagonists will be worthwhile in assessing multiple sleep parameters and determining the occurrence of any adverse effects, including sleep onset and next-day residual effects.

In summary, SDM-878 was confirmed to be a highly potent, selective, orally active, and brain-penetrable OX₂ receptor antagonist. SDM-878 exhibited a favorable pharmacokinetic profile for the treatment of insomnia, including a rapid onset of action and a relatively short half-life in plasma, which reduces the likelihood of next-day residual effects. We demonstrated that SDM-878 showed sleep-promoting effects at the same dose that completely antagonized ADL-OXB-induced hyperlocomotion in rats. We also demonstrated that SDM-878 significantly increased the duration of NREM sleep and had no significant effect on REM sleep, indicating that SDM-878 induces physiological sleep. These findings suggest that SDM-878 is a good pharmacological tool for the investigation of the role of OX₂ receptor in sleep and in the brain and may have therapeutic potential for the treatment of insomnia.
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Conflict of Interest
All authors are employees of Mochida Pharmaceutical Co., Ltd.

Author Contributions
SM, NY, CH, TO, JF, and TT participated in research design. NY, CH, TO, JF, and TT conducted experiments and performed data analysis. SM wrote or contributed to the writing of the manuscript.

Animal Studies
All experimental procedures were approved by the Institutional Animal Care and Use Committee of Mochida Pharmaceutical Co., Ltd.

Data Availability Statement
The data that supports the findings of this study are available in the supplementary material of this article.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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