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ORIGINAL ARTICLE

In vitro and in vivo evaluation of [$^{125/123}$I]-2-[4-(2-iodophenyl) piperidino]cyclopentanol([$^{125/123}$I]-OI5V) as a potential sigma-1 receptor ligand for SPECT

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Abstract

We investigated the characteristics of radio-iodinated 2-[4-(2-iodophenyl)piperidino]cyclopentanol (OI5V) as a single photon emission computed tomography (SPECT) ligand for mapping sigma-1 receptor (σ-1R), which plays an important role in stress remission in many organs.

Methods OI5V was synthesized from o-bromobenzaldehyde in three steps. OI5V was evaluated for its affinity to VAChT, σ-1 and σ-2 receptor by in vitro competitive binding assays using rat tissues and radioligands, [^{3}H]vesamicol, (+)-[^{3}H]pentazocine and [^{3}H]DTG, respectively. [$^{125/123}$I]OI5V was prepared from *o*-trimethylstannyl-cyclopentanevesamicol (OT5V) by the iododestannylation reaction under no-carrier-added conditions. In vivo biodistribution study of [^{125}I]OI5V in blood, brain regions and major organs of rats was performed at 2, 10, 30 and 60 min post-injection. In vivo blocking study and ex vivo autoradiography were performed to assess the binding selectivity of [^{125}I]OI5V for σ-1 receptor. SPECT-CT imaging study was performed using [^{123}I]OI5V.

Results OI5V demonstrated high selective binding affinity for σ-1R in vitro. In the biodistribution study, the blood–brain barrier (BBB) permeability of [^{125}I]OI5V was high and the accumulation of [^{125}I]OI5V in the rat cortex at 2 min post-injection exceeded 2.00%ID/g. In the in vivo blocking study, the accumulation of [^{125}I]OI5V in the brain was significantly blocked by co-administration of 0.5 µmol of SA4503 and 1.0 µmol of pentazocine. Ex vivo autoradiography revealed that the regional brain accumulation of [^{125}I]OI5V was similar to σ-1R-rich regions of the rat brain. SPECT images of [^{123}I]OI5V in the rat brain reflected the distribution of sigma receptors in the brain.

Conclusions This study confirmed that [$^{125/123}$I]OI5V selectively binds σ-1R in the rat brain in vivo. [^{123}I]OI5V was suggested to be useful as a σ-1R ligand for SPECT.

Keywords σ-1 receptor ligand · SPECT · Vesamicol analog · [$^{125/123}$i] radiotracer

Introduction

Sigma receptors are classified into two subtypes, sigma-1 receptor (σ-1R) and sigma-2 receptor (σ-2R) [1, 2]. σ-1R is a 25.3-kD, 233-amino acid transmembrane receptor that has been isolated, purified and cloned in many tissues [3]. σ-1R is an intracellular receptor located mainly on the endoplasmic reticulum (ER) membrane in cells, and is expressed site specifically in neurons and glial cells in the brain. It has been reported that the σ-1R acts as a chaperone molecule on proteins with other physiologically important functions, regulates physiological functions, such as the release of signal transmitters, and is involved in memory and recognition [4]. σ-1R is reduced in the postmortem brain of schizophrenic

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patients [5], and σ-1R antagonists were reported to improve the symptoms in schizophrenia animal models [6]. In addition, σ-1R is closely related to learning and memory mechanisms. A σ-1R agonist was reported to increase the neuronal activity of the glutamate nervous system in the hippocampus, promote the release of acetylcholine in the frontal lobe of the cerebral cortex and the hippocampus [7], and improve learning and memory impairment [8]. The relationship between σ-1R and stress disorders has also been reported, revealing that σ-1R agonists reduce immobility and fear conditioning stress responses by electric foot shock [9]. σ-1R is expressed at a high density in cells under ER stress to reduce cytotoxicity and suppress apoptosis [10, 11]. The relationship with drug dependence has also been reported. Cocaine has a high affinity for σ-1R [12] and a σ-1R antagonist abolishes the reward effects of cocaine [13]. Regarding the relationship between σ-1R and ALS, abnormal dysfunction and motor nerve necrosis are observed in σ-1R knockout mice [14]; moreover, σ-1R gene mutations and σ-1R protein dysfunction in ALS patients have been reported [15, 16]. Thus, σ-1R imaging using PET/CT and SPECT/CT enables the early diagnosis and severity diagnosis of dementia, including Alzheimer's disease, stress-related psychiatric disorders and ALS, and may provide information for deciding treatment policies.

To date, a number of central nervous system (CNS) σ-1R ligands for PET [17–28] or SPECT [29–33] have been reported. Important points in developing a brain receptor radioligand are that it has a high receptor affinity and high selectivity for the receptor, and high accumulation in the brain in vivo. We previously developed radioiodinated *p*-iodovesamicol (pIV, Fig. 1a), a vesamicol analog, as a sigma receptor ligand for SPECT [33]. pIV exhibited high affinity for σ-1R and σ-2R. We hypothesized that the ring size of cyclohexanol of vesamicol affects their affinity for σ-1R and σ-2R. We synthesized four vesamicol analogs in which the ring size of cyclohexanol was changed (5-membered ring to 8-membered ring). Furthermore, eight kinds of vesamicol analogs in which bromine was introduced into the *ortho* or *para* position of the benzene group of these four vesamicol analogs were synthesized to find a σ-1R ligand with high affinity and high selectivity for σ-1R. As a result of investigating the affinities of 12 kinds of vesamicol analogs to σ-1R, σ-2R and acetylcholine transporter (VAChT), *o*-bromocyclopentanevesamicol (OB5V) demonstrated the highest σ-1R affinity and σ-1R selectivity (data not shown). In the present study, we synthesized *o*-iodocyclopentanvesmicol (OI5V), a vesamicol analog in which iodine was introduced into the *ortho* position of the benzene group of cyclopentanevesamicol (5 V), and investigated its affinity for σ-1R. We evaluated the usefulness of *o*-[$^{125/123}$I]-iodocyclopentanevesamicol ([$^{125/123}$I]OI5V, Fig. 1b) as a CNS σ-1R ligand for SPECT.

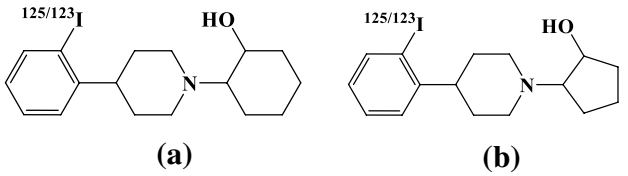


Fig. 1 Chemical structure of [$^{125/123}$I]pIV(**a**) and [$^{125/123}$I]OI5V(**b**)

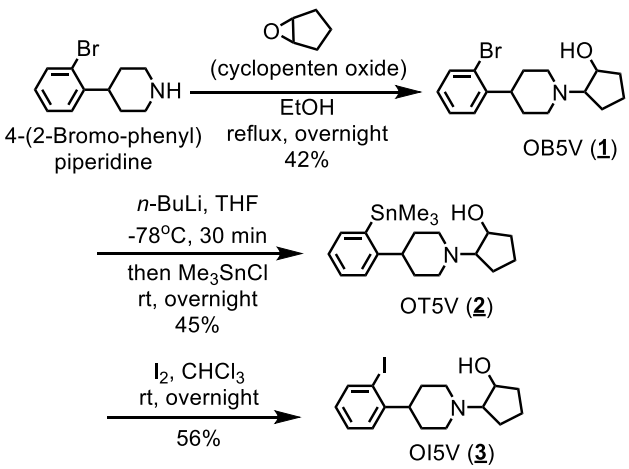
Results

Chemistry

Coupling reaction of 4-(2-bromophenyl)piperidine [34] with cyclopentenoxide produced *o*-bromocyclopentaneves amicol(OB5V)(**1**) in a 41% yield. A bromo substituent of OB5V (**1**) was replaced by a trimethylstannyl group with $(CH_3)_3SnCl$ to obtain the key intermediate *o*-trimethylstannyl-cyclopentanevesamicol(OT5V) (**2**). Treatment of OT5V (**2**) with I_2 in $CHCl_3$ at room temperature introduced an iodo substituent to give non-radioactive OI5V (**3**) in a 56% yield (Scheme 1). 2-(4-piperidino)cyclopentanol (5 V) was prepared according to Roger's protocol [35].

In vitro competitive binding study

The binding affinity (K_i) of OI5V, OB5V, 5 V, vesamicol and the sigma ligands (pentazocine and DTG) to the sigma receptors (σ-1R and σ-2R) and acetylcholine transporter (VAChT) is shown in Table 1. OI5V (4.7 nM) exhibited a higher binding affinity for σ-1R than (+)-pentazocine (11.1 nM) and SA4503 (6.5 nM). OI5V (173.3 nM) had a lower affinity for σ-2R than SA4503 (39.3 nM) and DTG



Scheme 1 Synthesis of OB5V, OT5V and OI5V

Table 1 In vitro binding assay

Inhibition	Ki (nM)		
	VAChT	σ-1	σ-2
(±)-5 V	112.8	10.3	81.8
(±)-OB5V	91.6	3.5	72.5
(±)-OI5V	165.3	4.7	173.3
SA4503	192.6	6.5	39.3
(±)-Vesamicol	40.4	19.4	59.5
(+)-Pentazocine	–	11.1	–
DTG	–	–	29.9

$K_i = IC_{50} / (1 + C/K_d)$, C = concentration of radioligand
Kd = 7.40 nM (VAChT), 19.9 nM (σ-1R), 22.3 nM (σ-2R)

(29.9 nM). As a result, OI5V demonstrated superior selective affinity for σ-1R compared with SA4503.

Radiolabeling

The radiochemical yield was 70–80%, calculated using the radioactivity of [$^{125/123}$I]NaI, and the radiochemical purity determined by thin layer chromatography (TLC) (Rf value: 0.79) was > 98%

Partition coefficient calculation

The log $P_{o/w}$ value of [^{125}I]OI5V was 2.22 ± 0.07. This suggested that [^{125}I]OI5V has a reasonable degree of lipid solubility to penetrate the blood–brain barrier (BBB).

In vivo biodistribution

The tissue distribution of [^{125}I]OI5V at 2, 10, 30, 60 and 120 min post-injection is shown in Table 2. The high accumulation of [^{125}I]OI5V in the cortex, striatum, cerebellum and other brain areas (2.10, 1.70, 1.49 and 1.69%ID/g, respectively) at 2 min post-injection suggested high BBB permeability of [^{125}I]OI5V. Even at 60 min post-injection, the accumulation of [^{125}I]OI5V in the cortex, striatum, cerebellum and other brain areas remained greater than 1.0%ID/g (1.31, 123, 1.21 and 1.24%ID/g, respectively), although the blood clearance was rapid. Radioactivity in the blood was already low at 2 min post-injection (0.15%ID/g). At 2 min post-injection, the lung accumulation was high (8.31%ID/g), but it decreased to 2.19%ID/g at 60 min post-injection.

In vivo blocking study

The in vivo binding selectivity of [^{125}I]OI5V in each rat brain region is shown in Fig. 2. The accumulation of [^{125}I]OI5V was decreased (42–46% of control) in all brain regions by co-administration with 0.5 μmol of SA4503. (±)-Pentazocine (1.0 μmol) had slightly weaker inhibitory effects on brain accumulation of [^{125}I]OI5V than SA4503 (0.5 μmol) (no significant difference).

In vivo metabolite analysis

The Rf value of [^{125}I]OI5V as a standard on TLC using the mobile phase (ethyl acetate:methanol:triethylamine = 9:1:0.1) was 0.80 (Fig. 3C). From the density of each

Table 2 Biodistribution of [^{125}I]OI5V in rats

Organs	[^{125}I]OI5V				
	%ID/g				
	Time post-injection				
	2 min	10 min	30 min	60 min	120 min
Blood	0.15 ± 0.01	0.09 ± 0.00	0.07 ± 0.00	0.07 ± 0.01	0.07 ± 0.01
Heart	1.43 ± 0.36	0.96 ± 0.06	0.63 ± 0.03	0.45 ± 0.06	0.25 ± 0.03
Lung	8.31 ± 1.09	4.60 ± 0.64	3.17 ± 0.37	2.19 ± 0.43	1.16 ± 0.20
Pancreas	1.92 ± 0.24	3.41 ± 0.48	3.26 ± 0.64	2.91 ± 0.47	2.18 ± 0.29
Spleen	1.48 ± 0.59	1.84 ± 0.67	1.66 ± 0.20	1.71 ± 0.11	1.28 ± 0.09
Kidney	3.97 ± 0.58	3.99 ± 0.27	2.92 ± 0.56	2.36 ± 0.23	1.66 ± 0.17
Small intestines	1.50 ± 0.27	1.66 ± 0.88	2.23 ± 0.84	2.04 ± 0.22	1.52 ± 0.33
Stomach	0.31 ± 0.06	0.89 ± 0.52	1.15 ± 0.38	0.75 ± 0.19	0.63 ± 0.12
Liver	1.28 ± 0.07	1.30 ± 0.26	1.07 ± 0.28	0.95 ± 0.09	0.75 ± 0.11
Cortex	2.10 ± 0.30	1.70 ± 0.10	1.47 ± 0.09	1.31 ± 0.09	0.92 ± 0.07
Striatum	1.70 ± 0.36	1.46 ± 0.26	1.31 ± 0.16	1.23 ± 0.11	0.90 ± 0.09
Cerebellum	1.49 ± 0.18	1.18 ± 0.18	1.15 ± 0.04	1.33 ± 0.25	1.08 ± 0.18
Remaining brain	1.69 ± 0.32	1.42 ± 0.13	1.26 ± 0.06	1.24 ± 0.14	0.92 ± 0.09

Values are the mean ± standard deviation (SD) of four rats ($n = 4$) at each time point

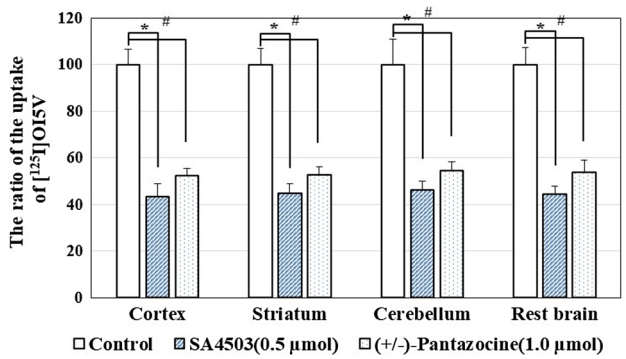


Fig. 2 The effect of inhibitors on the accumulation of [^{125}I]OI5V in rat brain regions

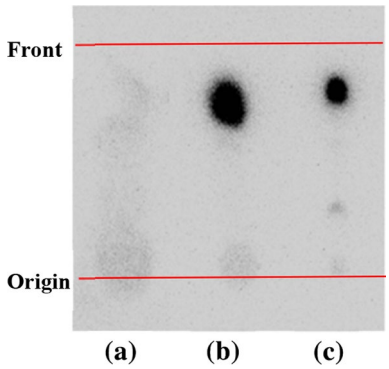


Fig. 3 In vivo metabolites analysis of [^{125}I]OI5V in rat brain 30 min post-injection. (**a**) Blood; (**b**) brain; (**c**) control

spot on the imaging plate analyzed by Multi Gauge V3, image analysis software, most of the radioactivity (> 98%) was observed as an unchanged form in the brain samples (Fig. 3B). In the plasma samples, a highly polar radioactive metabolite around the starting point was mainly observed (> 80%) (Fig. 3A).

Ex vivo autoradiography

Coronal images of rat brains visualized by ex vivo autoradiography with [^{125}I]OI5V at 30 min post-injection are shown in Fig. 4. [^{125}I]OI5V was distributed in characteristically σ-1R-rich regions such as the cortex, striatum, diagonal band, hippocampus, thalamus, hypothalamus, cerebellum, vestibular nucleus and facial nucleus. This accumulation of [^{125}I]OI5V markedly decreased by co-injection of 2.5 µmol of SA4503 or 5.0 µmol of (±)-pentazocine such as σ-1R ligand.

SPECT/CT imaging of [^{123}I]OI5V in the rat brain

SPECT/CT fusion brain images acquired for 60 min starting 30 min after [^{123}I]OI5V administration to rats are shown in Fig. 5. The accumulation of [^{123}I]OI5V in the cortex, striatum, thalamus, hypothalamus and cerebellum was visually observed. The accumulation of [^{123}I]OI5V in the brain was markedly reduced by the co-administration of 2 µmol of SA4503.

Discussion

2-(4-Phenylpiperidino)cyclopentanol (5 V), a core structural component of OI5V, was reported by Rogers et al. [35] as a vesamicol analog with affinity for VAChT. However, the affinity of 5 V for VAChT was low. During the development of σ-1R ligands, we found that 5 V and OB5V have a high affinity for σ-1R in vitro (data not shown). In the present study, we synthesized OI5V with iodine introduced at the *ortho* position of 5 V, and evaluated in vitro and in vivo characteristics of OI5V. We demonstrated that OI5V has a higher affinity for σ-1R than the selective σ-1R ligand (+)-pentazocine, and has a lower affinity for σ-2R than 5 V, OB5V and SA4503, which suggested that OI5V is a superior σ-1R ligand (Table 1). It only took approximately 60 min to complete the radiolabeling and purification of [$^{125/123}$I]OI5V,

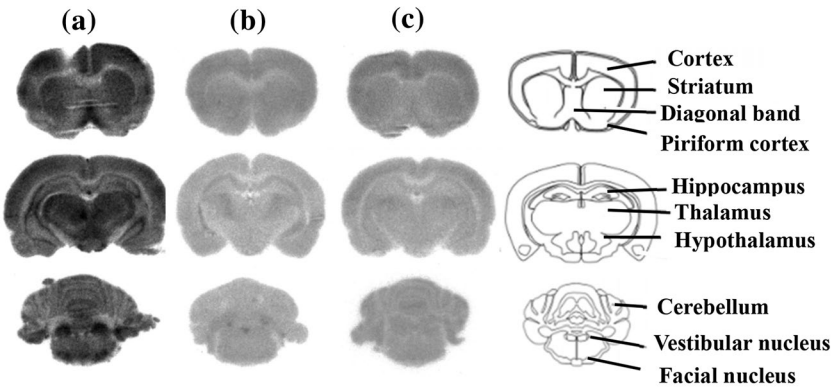


Fig. 4 Ex vivo autoradiography of [^{125}I]OI5V in the rat brain 30 min post-injection, (**a**) control; (**b**) with SA4503 (2.50 µmol); (**c**) with (±)-pentazocine (5.0 µmol)

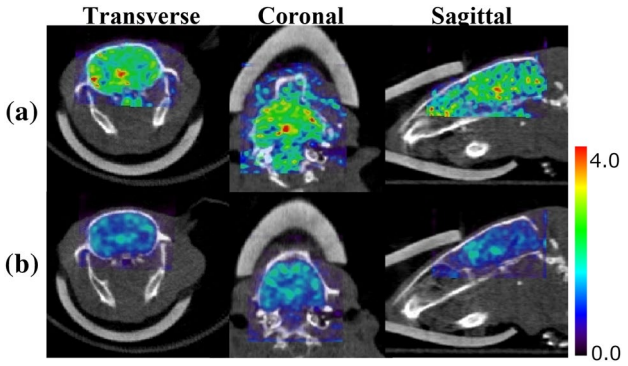


Fig. 5 SPECT/CT fusion brain images with [^{123}I]OI5V administered alone (**a**) or with 2.0 μmol of SA4503 as an inhibitor (**b**)

which is sufficiently short for the synthesis and purification of short half-life ^{123}I-labeled ligands ($T_{1/2}$ = 12.3 h). [^{125}I]OI5V with moderate lipophilicity (log $P_{o/w}$ = 2.22 ± 0.07) was considered to be suitable as a brain σ-1R imaging agent. Indeed, the accumulation of [^{125}I]OI5V in the cortex at 2 min post-injection was high (2.10 ± 0.30%ID/g). The retention of [^{125}I]OI5V in the cortex (2.10, 1.70, 1.47, 1.31 and 0.92%ID/g at 2, 10, 30, 60 and 120 min post-injection, respectively) was sufficiently long to visualize σ-1R in the brain by SPECT/CT (Table 2). Furthermore, the low level of radioactivity detected in the blood and non-existence of [^{125}I]OI5V metabolite in the brain may be advantageous for σ-1R imaging for SPECT/CT (Fig. 3). Indeed, SPECT imaging of [^{123}I]OI5V visualized σ-1R-rich areas in the brain. The inhibitory effects of the accumulation of [^{125}I]OI5V in the cortex by σ-1R ligand SA4503 (42% of control) suggested that [^{125}I]OI5V bound selectively to σ-1R in vivo (Fig. 2). Ex vivo autoradiography (Fig. 4) revealed that the characteristic regional brain distribution of [^{125}I]OI5V, especially in the cortex, striatum, hippocampus, diagonal band nuclei, piriform cortex, thalamus, hypothalamus, Purkinje cell layer in the cerebellum and cranial nerve nucleus, was similar to σ-1R rich regions of the brain determined by in vitro autoradiography [36, 37] and immunostaining of σ-1R [38]. We obtained SPECT images of σ-1R in the rat brain using [^{123}I]OI5V (Fig. 5).

Central nervous system (CNS) σ-1R imaging agents for PET [17, 22–28] or SPECT [29, 30, 33], which exhibited high affinity and selectivity for σ-1R in vitro, high BBB permeability and/or bound selectively to σ-1R in vivo, were previously reported. However, several σ-1R imaging agents demonstrated low accumulation in the mouse brain (< 10%ID/g) or rat brain (< 1.0%ID/g), reflecting relatively low BBB permeability. Mouse brain accumulation of [^{125}I]OI5V was greater than 15%ID/g at 10 min post-injection (data not shown). Other σ-1R imaging agents exhibited high brain accumulation, but low binding to σ-1R because the accumulation of these σ-1R imaging agents was only slightly blocked by σ-1R inhibitors in vivo. [^{123}I]OI5V, in addition to [^{11}C]SA4503 [20, 23], [^{18}F]FPS [24], [^{18}F]SFE [22], [^{18}F]tetrahydrofuranyl-piperazine analog [27], [^{123}I]TPCNE [29] and [^{123}I]CNBN [30], demonstrated high affinity and selectivity for σ-1R in vitro, and high brain accumulation and high selective binding affinity to σ-1R in the brain in vivo. When comparing [$^{125/123}$I]OI5V with other [^{123}I]labeled CNS σ-1R ligands for SPECT [29–32], [$^{125/123}$I]OI5V was considered superior to other [^{123}I]-labeled CNS σ-1R ligands in terms of high brain accumulation and brain kinetics in vivo.

Conclusion

[^{123}I]OI5V can be radiosynthesized within a short duration (approximately 60 min) with a high radiochemical yield (70–80%) and high radiochemical purity (> 99%), making it suitable as an imaging agent for SPECT/CT. [^{123}I]OI5V has the potential to be a prospective σ-1R imaging agent for SPECT/CT because of its high BBB permeability and regional brain distribution reflected by σ-1R-rich regions in the brain.

Experiments

General

(±)-Vesamicol, (±)-pentazocine and 1,3-di-*o*-tolylguanidine (DTG) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Radioisotopes were purchased from PerkinElmer, Inc. (Waltham, MA, USA), unless otherwise noted. The reversed-phase HPLC column (Zorbax-ODS RX-18, 9.6 mm × 250 mm) was purchased from Agilent Technologies Inc. (Santa Clara, CA, USA). [^{125}I]NaI was purchased from PerkinElmer Inc. [^{123}I]NaI was purchased from FUJIFILM Toyama Chemical Co., Ltd. (Japan).

Animal experiments were performed in compliance with the Guidelines for the Care and Use of Laboratory Animals at the Takara-machi Campus of Kanazawa University.

Synthesis

2-[4-(2-Bromophenyl)piperidino]cyclopentanol (OB5V)(1)

A mixture of 4-(2-bromophenyl)piperidine (5.00 g, 20.7 mmol) [34] and cyclopentenoxide (5.5 mL, 63.0 mmol) in EtOH (21 mL) was refluxed for 20 h. The solvent was evaporated off. The residue was chromatographed on silica gel with hexane-AcOEt (1:1) to give **1** (2.82 g, 42%) as a

white solid: ^{1}H NMR δ 7.55–7.53 (m, 1H), 7.30–7.26 (m, 2H), 7.07–7.03 (m, 1H), 3.28–3.24 (m, 1H), 3.10–3.06 (m, 1H), 3.00 (tt, 1H, J = 3.4, 12.0 Hz), 2.60–2.55 (m, 1H), 2.31 (dt, 1H, J = 2.4, 11.7 Hz), 2.21 (dt, ^{1}H, J = 2.4, 11.7 Hz), 2.02–1.87 (m, 4H), 1.79–1.49 (m, 8H); MS m/z 324 (M^{+} + 1, 100), 326 (M^{+} + 1, 97.3).

2-[4-(2-Trimethylstannylphenyl)piperidino] cyclopentanol (OT5V)(2)

A solution of *n*-BuLi (0.37 mmol) in hexane (0.14 mL) was added dropwise under Ar to a stirring solution of **1** (100 mg, 0.31 mmol) in dry THF (3.1 mL) cooled to -70 °C (dry ice – acetone). After the mixture was stirred at -78 °C for 30 min, a solution of $(CH_3)_3SnCl$ (184 mg, 0.92 mmol) in dry THF (3.1 mL) was added over 10 min. The dry ice–acetone bath was removed and the mixture was allowed to warm to room temperature. After 20 h, the reaction was quenched with 5% aqueous NH_4Cl and the mixture was extracted with AcOEt. The solvent was evaporated off. The residue was chromatographed on silica gel with hexane-AcOEt (1:1) to give **2** (56.9 mg, 45%) as a white solid: ^{1}H NMR δ 7.50–7.26 (m, 3H), 7.23–7.17 (m, 1H), 3.38–3.17 (m, 3H), 2.71–2.16 (m, 2H), 2.07–1.55 (m, 13H), 0.31 (s, 9H); MS m/z 410 (M^{+} + 1, 100).

2-[4-(2-Iodophenyl)piperidino] cyclopentanol (OI5V)(3)

Iodine (187 mg, 0.74 mmol) was added to a reaction mixture of **2** (115 mg, 0.28 mmol) in $CHCl_3$ (0.49 mL). The reaction mixture was stirred for 24 h at the same temperature, quenched by the addition of saturated aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$, extracted with $CHCl_3$, washed with water and brine, dried and concentrated to dryness. The residue was chromatographed on silica gel with hexane-AcOEt (1:1) to give **3** (50.4 mg, 56%) as a white solid: ^{1}H NMR δ 7.84–7.82 (dd, 1H), 7.32–7.29 (t, 1H), 7.25–7.23 (dd, 1H), 6.91–6.87 (ddd, 1H), 4.23–4.20 (m, 1H), 3.28–3.26 (d, 1H), 3.10–3.08 (d, 1H), 2.86–2.78 (m, 1H), 2.61–2.56 (m, 1H), 2.35–2.28 (ddd, 1H), 2.25–2.18 (ddd, 1H), 2.01–0.88 (m, 11H). MS m/z 371 (M^{+} + 1, 84.4).

Tissue preparation

Rat brain and liver tissue preparations were prepared from dissected brains (not including the cerebellum) and livers from male Sprague–Dawley rats (250–300 g), as previously described [39].

In vitro competitive binding study

The following binding assays were performed using methods reported previously [39] and are described briefly here.

VAChT binding

As a radioligand, (-)-[^{3}H]vesamicol (K_d = 7.40 nM) was used. Varying concentrations of 5 V, OB5V, OI5V, SA4503 or vesamicol (from 10^{-10} to 10^{-5} M) were used as subject compounds. After addition of the compounds to the rat brain tissue preparation (300–400 µg protein), samples were incubated in quadruplicate at 37 °C for 60 min in the presence of 200 nM DTG to mask the sigma receptors (σ-1R and σ-2R). Radioactivity retained on the filters after filtration of the samples was measured using a liquid scintillation counter (Aloka, LSC-5100).

σ-1 receptor binding

Samples (0.5 mL in 50 mM Tris–HCl (pH 7.8) including rat brain tissue preparation (400–500 µg protein), 5 nM (+)-[^{3}H]pentazocine (Kd = 19.9 nM) and varying concentrations of 5 V, OB5V, OI5V, SA4503, vesamicol or (+)-pentazocine (from 10^{-10} to 10^{-5} M) were incubated in quadruplicate for 90 min at 37 °C. Nonspecific binding was determined in the presence of 10 µM (+)-pentazocine. After incubation, radioactivity retained on the filters after filtration of the samples was measured using a liquid scintillation counter (Aloka, LSC-5100).

σ-2 receptor binding

Samples of 0.5 mL of 50 mM Tris–HCl (pH 7.8) including rat liver tissue preparation (approximately 100 µg protein), 5 nM [^{3}H]DTG (Kd = 22.3 nM), varying concentrations of 5 V, OB5V, OI5V, SA4503, vesamicol or DTG (from 10^{-10} to 10^{-5} M) and 1 µM (+)-pentazocine to mask the σ-1 sites were incubated in quadruplicate for 90 min at 37 °C. Nonspecific binding was determined in the presence of 10 µM DTG and 1 µM (+)-pentazocine. The incubated samples were treated in the same manner as described for the σ-1 receptor binding assay.

Data analysis

K_i values were calculated using GraphPad Prism Version 7 software (GraphPad Software, Inc. San Diego, CA, USA).

Radiolabeling

[^{125}I]OI5V was prepared from OT5V(50 µg/50 µL) and [^{125}I]NaI (37 MBq) using the iodo-destannylation reaction under no-carrier-added conditions [33]. After incubation for 10–20 min at room temperature, the prepared [^{125}I]OI5V was purified by HPLC (reversed-phase HPLC column (Zorbax-ODS RX-C18, 9.6 mm × 250 mm), 40 °C, 2.0 mL/min flow rate, 80:20:0.2 v/v/v acetonitrile/H_2O/monoethanolamine). Radiolabeling of [^{123}I]OI5V was performed using the same method as for [^{125}I]OI5V except for the use of [^{123}I]NaI (111 MBq) instead of [^{125}I]NaI (37 MBq). The radiochemical purity was analyzed by TLC (SiO_2) using mobile phase (ethyl acetate:methanol:triethylamine = 9:1:0.1).

Partition coefficient calculation

The partition coefficient (log $P_{o/w}$) of [^{125}I]OI5V was calculated by the standard method with *n*-octanol and 0.1 M phosphate buffer [40]. The partition coefficient was calculated by the following formula: log $P_{o/w} = \log_{10} C_o/C_w$ (radioactivity in n-octanol layer/radioactivity in aqueous layer). The log $P_{o/w}$ value of [^{125}I]OI5V was 2.22 ± 0.07.

In vivo biodistribution

Five groups of male Sprague–Dawley (SD) rats (n = 4 in each group), weighing 250–300 g, were anesthetized with isoflurane and administered an intravenous (i.v.) injection of [^{125}I]OI5V (0.4 mL, 185 kBq). At 2, 10, 30, 60 and 120 min post-injection, the animals were killed by decapitation under isoflurane anesthesia. The organs of interest were dissected, weighed and the radioactivity levels were measured in a gamma scintillation counter (AccuFLEX γ7010, Aloka, Tokyo, Japan). The degree of accumulation of radiotracer was expressed as a percentage of the injected dose per gram of tissue (%ID/g).

In vivo blocking study

To estimate the in vivo binding characteristics of [^{125}I]OI5V to σ-1R, three groups of male SD rats (250–300 g, n = 4 in each group) received an intravenous injection of [^{125}I]OI5V (0.4 mL, 185 kBq) with 0.5 µmol of SA4503 or 1.0 µmol of (±)-pentazocine, or were not injected as a control. The rats were killed by decapitation under isoflurane anesthesia at 30 min post-injection and four brain regions (the cortex, striatum, cerebellum and remainder) were collected and weighted. The radioactivity of each part was measured using an auto well gamma system (Aloka, AccuFLEX γ7010).

In vivo metabolite analysis

In vivo metabolite analysis with [^{125}I]OI5V was performed by autoradiographic analysis of thin layer chromatography (TLC) [41]. Male SD rats (250–300 g, n = 3) received an intravenous injection of [^{125}I]OI5V (0.4 mL, 1.85 MBq) via the tail vein. At 30 min post-injection, the rats were killed by decapitation under isoflurane anesthesia and blood samples were collected in a heparin-coated tube. The brain without the cerebellum was immediately collected. The plasma, which was separated from the blood by centrifugation (3,000 × g for 5 min at 4 °C), was adjusted to a twofold volume solution (acetonitrile:H_2O = 2:1), and then centrifuged at 3,000 × g for 5 min at 4 °C. The brain tissues, which were adjusted to a tenfold volume solution (acetonitrile: H_2O = 2:1), were homogenized by a glass homogenizer. The brain homogenate suspensions were centrifuged at 20,000 × g for 20 min at 4 °C. Each of the two kinds of resulting supernatants were analyzed by TLC (SiO_2) using the mobile phase (ethyl acetate:methanol:triethylamine = 9:1:0.1). The TLC plates were exposed to an imaging plate (Fujifilm, BAS-IP SR 2025) for 1 week, and then the imaging plates were scanned by an FLA7000 (GE Healthcare). The density of each spot on the imaging plate was analyzed by Multi Gauge V3, image analysis software.

Ex vivo autoradiography

SD rats were injected intravenously with [^{125}I]OI5V (0.4 mL, 1.85 MBq) either alone as a control or with 2.5 µmol of SA4503 or 5.0 µmol of (±)-pentazocine via the tail vein. At 30 min post-injection, the rats were killed by decapitation under isoflurane anesthesia. Whole brains were removed, frozen in embedding medium at -78 °C and cut into 20-µm sections at -25 °C using a cryostat microtome. The sections

were exposed to an imaging plate (BAS-IP SR 2025) for eight days. The imaging plates were scanned by a FLA7000 phosphor image reader.

SPECT/CT imaging of [^{123}I]OI5V in the rat brain

SD rats were immobilized on a SPECT-CT camera, (VECTor/CT, MILabs), and injected intravenously with [^{123}I]OI5V (0.4 mL, 37 MBq) either alone or with 2.0 µmol of SA4503. SPECT scanning was performed for 60 min (from 30 to 90 min after injection). The rats were anesthetized under isoflurane (1.5%, 1 mL/min) and the body temperature was maintained at 35—37 °C using a heated animal bed during the scans. Following a reconstruction process by OSEM (iteration, 6; subset, 16), the obtained image data were analyzed using Amide image analysis software.

Statistical analysis

In the in vivo blocking study, statistical comparisons were performed using one-way ANOVA (non-parametric) and Tukey's multiple comparison test in GraphPad Prism Version 7 software.

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