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Synthesis and biological evaluation of a radioiodinated spiropiperidine ligand as a potential σ_1 receptor imaging agent

Rui-Qin Chen,[a] Yan Li,[a] Qiu-Yan Zhang,[a] Hong-Mei Jia,[a]* Winnie Deuther-Conrad,[b] Dirk Schepmann,[c] Jörg Steinbach,[b] Peter Brust,[b] Bernhard Wünsch,[c] and Bo-Li Liu[a]

We report the synthesis and evaluation of 1′-(4-[^{125}I]iodobenzyl)-3H-spiro[isobenzofuran-1,4′-piperidine] ([^{125}I]Spiro-I) as a potential SPECT tracer for imaging of σ_1 receptors. [^{125}I]Spiro-I was prepared in 55–65% isolated radiochemical yield, with radiochemical purity of >99%, via iododestannylation of the corresponding tributyltin precursor. In receptor binding studies, Spiro-I displayed low nanomolar affinity for σ_1 receptors (σ_1: $K_i = 2.75 \pm 0.12$ nM; σ_2: $K_i = 340$ nM) and high subtype selectivity ($\sigma_2/\sigma_1 = 124$). Biodistribution in mice demonstrated relatively high concentration of radioactivity in organs known to contain σ_1 receptors, including the lung, kidney, heart, spleen, and brain. Administration of haloperidol 5 min prior to injection of [^{125}I]Spiro-I significantly reduced the concentration of radioactivity in the above-mentioned organs. These findings suggest that the binding of [^{125}I]Spiro-I to σ_1 receptors *in vivo* is specific.

Keywords: σ_1 receptor; spiropiperidine; Iodine-125

Introduction

Sigma receptors represent a distinct class of proteins, of which two subtypes (σ_1 and σ_2) are known.[1] They are expressed in the central nervous system (CNS), endocrine, immune, and certain peripheral tissues, and are believed to play an important role in regulating and integrating nervous, endocrine, and immune responses.[2–3] The σ_1 receptor is believed to regulate glutamate NMDA receptor function and the release of neurotransmitters such as dopamine in the CNS. In the healthy brain, σ_1 receptors have been implicated in learning and memory. Sigma-1 receptors are also linked to a number of brain disorders, including schizophrenia, depression, Alzheimer's disease, as well as ischemia.[4–7] It is noteworthy that the human σ_1 receptor gene is located on chromosome 9, band p13, a region known to be associated with different psychiatric disorders.[8] Development of radiotracers for *in vivo* imaging of σ_1 receptors will allow studies of their involvement in pathological processes in the living brain, and may provide much needed diagnostic tools for the above-mentioned diseases.

Many compound classes with diverse structures, such as arylacetamides, phenylethylamines, benzamides, piperazine, and piperidines, possess affinity for σ_1 receptors. In the past few years, several potential tracers for imaging σ_1 receptor expression with PET and SPECT have been reported. Among them, [^{11}C]SA4503,[9–10] [^{18}F]FPS,[11] and [^{123}I]TPCNE[12] have been evaluated in human studies. For instance, [^{11}C]SA4503 was used to investigate Parkinson's disease (PD) patients with PET and this tracer could be used as an indicator of presynaptic dopaminergic damage in PD.[9] In a recent study using [^{11}C]SA4503, the density of cerebral and cerebellar σ_1 receptors was found to be reduced in patients with early stage Alzheimer's disease as compared with healthy subjects.[10] However, of the σ_1 tracers reported to date none has ideal properties for imaging. [^{11}C]SA4503 was found to have high nonspecific binding,[13] while [^{18}F]FPS and [^{123}I]TPCNE were found to have irreversible kinetics due to their high affinity for σ_1 receptors ([^{18}F]FPS[11,14]: $K_D = 0.5 \pm 0.2$ nM; TPCNE[12,15]: $K_i = 0.67$ nM). In order to overcome the drawback of slow binding kinetics, a lower affinity tracer (in the nanomolar range) needs to be developed. In the context of routine clinical applications, imaging with SPECT have some advantages over PET such as more widespread availability, no need for an on-site cyclotron and lower cost. Considering [^{123}I]TPCNE as the only SPECT radiotracer used for human imaging so far and the drawback related to its use, our interest is focused on the development of σ_1 receptor probes for SPECT with improved pharmacokinetic profiles.

[a]*Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing, 100875, China*

[b]*Forschungszentrum Dresden-Rossendorf, Institute of Radiopharmacy/ Neuroradiopharmaceuticals Leipzig, 04318 Leipzig, Germany*

[c]*Institute of Pharmaceutical and Medicinal Chemistry of Westfälische Wilhelms-Universität Münster, 48149 Münster, Germany*

**Correspondence to: Hong-Mei Jia, College of Chemistry, Beijing Normal University, Beijing, 100875, China.*
E-mail: hmjia@bnu.edu.cn

Recently, a new class of spirocyclic piperidines was reported to have low nanomolar affinity for σ_1 receptors and high selectivity over the σ_2 receptor subtype.[16–20] Furthermore, when tested against 15 different receptors and transporters the reported compounds were found to lack activity at submicromolar concentrations. Spirocyclic piperidines are therefore attractive lead compounds for SPECT tracer development. Based on a QSAR model of spirocyclic piperidines recently developed in our group,[21] the affinity of 1′-(4-iodobenzyl)-3*H*-spiro[isobenzofuran-1,4′-piperidine] (Spiro-I) was predicted to be in the low nanomolar range. We have previously reported the crystal structure of this compound.[22] In the present study, we have synthesized [^{125}I]Spiro-I, determined its log *D* value, and evaluated its potential as a putative SPECT tracer for imaging of σ_1 receptors by *in vitro* radioligand binding assays and biodistribution studies in mice.

Results and discussion

Chemistry and radiochemistry

The synthetic route of [^{125}I]Spiro-I is shown in Figure 1. Compound **1** was synthesized according to the method reported in the literature.[23] Alkylation of compound **1** with 4-iodobenzyl bromide provided compound **2** (Spiro-I). Compound **2** reacted with bis(tributyltin) for 6 h in anhydrous triethylamine at reflux under nitrogen in the presence of palladium catalyst, followed by purification to afford yellow-light oil **3** in 87% yield.

[^{125}I]Spiro-I was prepared via an iododestannylation reaction using chloramine-T as the oxidizing agent. The reaction was quenched with sodium metabisulfite, and the resulting mixture was purified using a C18 Sep-Pak cartridge. After removing inorganic salts including [^{125}I]NaI by washing with water, [^{125}I]Spiro-I was eluted with ethanol. The product was further purified by radio-HPLC using a reversed-phase column and mobile phase consisting of acetonitrile and water (90:10 v/v) with a flow rate of 2 mL/min.

In order to identify the radiotracer, the stable Spiro-I was co-injected and co-eluted with the radioactive product. Their HPLC profiles using acetonitrile and water (85:15 v/v) as mobile phase at a flow rate of 1 mL/min are presented in Figure 2. From Figure 2, the retention times of stable Spiro-I and [^{125}I]Spiro-I were observed to be 4.62 and 5.00 min, respectively. The difference in retention time was in good agreement with the time lag which is due to the distance between the UV and radioactivity detector of our HPLC system. After purification by HPLC, no significant chemical impurities were detected by analytical RP-HPLC (UV, 231 nm). The isolated radiochemical yield of [^{125}I]Spiro-I was

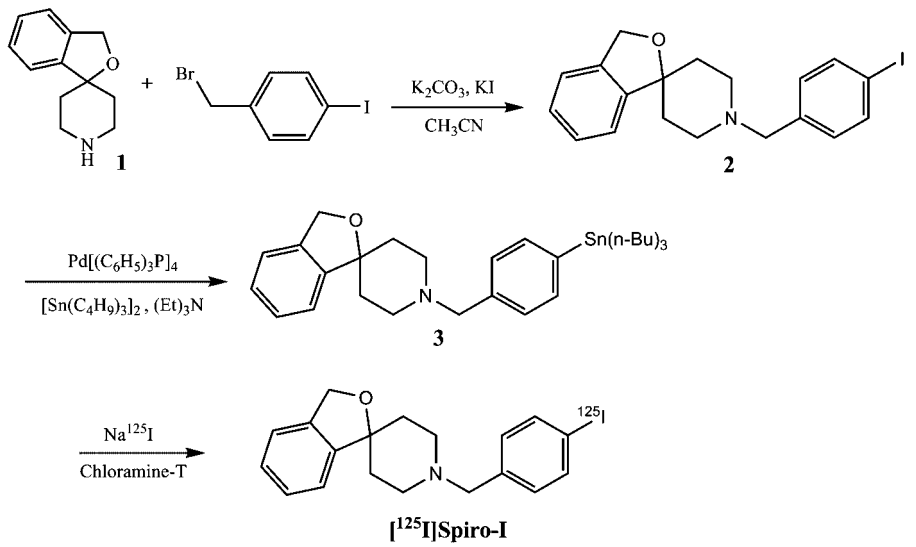

Figure 1. Synthetic route to [^{125}I]Spiro-I.

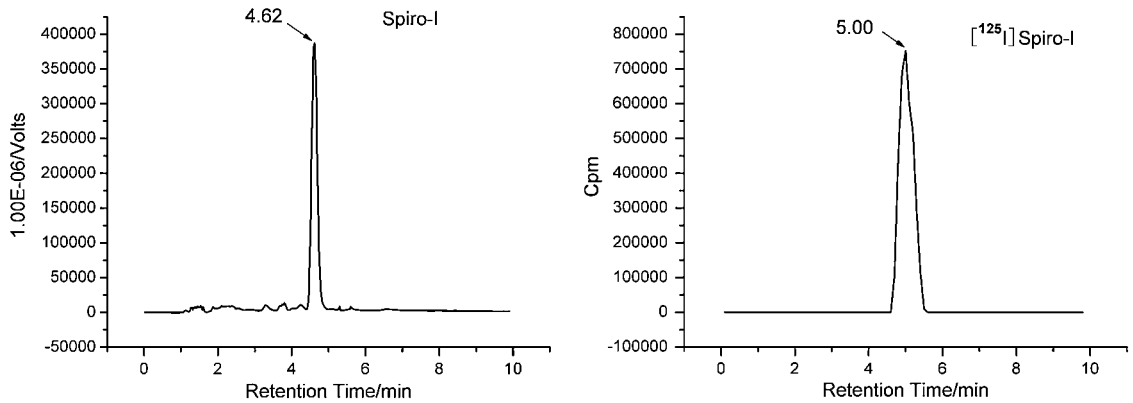

Figure 2. HPLC co-elution profiles of Spiro-I and [^{125}I]Spiro-I, with retention time of 4.62 and 5.00 min, respectively.

55–65% ($n = 5$). The radiochemical purity was higher than 99%. The specific activity of the n.c.a. Na[^{125}I] was >2200 Ci/mmol at the time of delivery. No attempt was made to measure the specific activity of [^{125}I]Spiro-I. To prepare a suitable solution of [^{125}I]Spiro-I for *in vivo* use, the eluted radioactive peak corresponding to [^{125}I]Spiro-I was collected. After the solvent was removed *in vacuo*, the product was re-dissolved in less than 0.1 mL ethanol and diluted with sterile saline to provide approximately 1 μCi of radioactivity per 0.1 mL of solution.

The *in vitro* stability of [^{125}I]Spiro-I was evaluated by measuring the radiochemical purity (RCP) at different time points. The results showed that [^{125}I]Spiro-I was chemically stable in 0.9% NaCl solution at room temperature. After 6 h of incubation with mouse plasma at 37°C, the RCP of [^{125}I]Spiro-I was still higher than 99%, which suggests that [^{125}I]Spiro-I possesses high stability *in vitro*.

The apparent distribution coefficient of [^{125}I]Spiro-I was determined by measuring the distribution of the radiotracer between 1-octanol and 0.05 mol/L sodium phosphate buffer at pH = 7.4. Considering the significant influence of small quantities of radioactive impurity on the apparent log *p* value of the radiotracer using the counting method,[24] pre-washing of the 1-octanol layer containing [^{125}I]Spiro-I with aqueous buffer was performed. It was found that the log *D* value was <2.0 without pre-washing, while it increased to nearly 3.0 after pre-washing for one time. With two successive washing procedures, the log *D* value of [^{125}I]Spiro-I was finally determined to be 3.06 ± 0.11 ($n = 4$) at pH 7.4, from which one can expect a good blood-brain barrier penetration of this radiotracer.

In vitro radioligand competition studies

The σ_1 and σ_2 receptor affinities of Spiro-I were determined in competition experiments with the radioligands (+)-[^{3}H]pentazocine and [^{3}H]ditolylguanidine using guinea pig brain and rat liver preparations, respectively. In the σ_1 assay Spiro-I showed high affinity ($K_i = 2.75 \pm 0.12$ nM) and the corresponding binding curve is displayed in Figure 3. The low σ_2 affinity of Spiro-I ($K_i = 340$ nM) indicates a high subtype selectivity ($\sigma_2/\sigma_1 = 124$), which suggests that it is a promising σ_1 receptor ligand for neuroimaging.

Biodistribution and blocking studies in mice

In vivo biodistribution studies of [^{125}I]Spiro-I were performed in female ICR mice. The uptake of radioactivity in the organ of interest at 15, 30, 60, 120, and 240 min after intravenous administration of 1 μCi [^{125}I]Spiro-I is summarized in Table 1. [^{125}I]Spiro-I showed a high initial brain uptake, with slow clearance and high brain-to-blood ratios. The radioactivity concentration in the brain peaked at 15 min post-injection (p.i.) with 5.28 ± 0.41 %ID/g, and slowly cleared thereafter with 4.66 ± 0.38 %ID/g at 30 min, 4.10 ± 0.33 %ID/g at 60 min, 2.70 ± 0.23 %ID/g at 120 min and 1.52 ± 0.16 %ID/g at 240 min p.i.. In contrast to the high uptake and retention in the brain, the blood radioactivity levels were low with 1.26 ± 0.25 %ID/g at 15 min and 0.58 ± 0.10 %ID/g at 240 min p.i., resulting in high brain-to-blood ratios (4.19, 5.18, 5.06, and 3.70 at 15, 30, 60, and 120 min p.i., respectively). Compared with its ^{18}F-labelled analog, [^{125}I]Spiro-I showed higher uptake in the brain and a similar clearance rate.[20] In terms of clearance rate in the brain, [^{125}I]Spiro-I may be superior to [^{123}I]TPCNE, the most promising SPECT imaging agent of σ_1 receptor until now. [^{125}I]Spiro-I showed slow washout from the brain, while nearly no brain radioactivity of [^{123}I]TPCNE was decreased at 4 h p.i. (1.06 ± 0.08 and 1.07 ± 0.20 %ID/g at 0.33 and 4 h p.i., respectively).[15] Second, high uptakes (3.41–12.13 %ID/g at 15 min p.i.) were observed in organs known to contain σ receptors, including the lungs, kidneys, heart, spleen, and liver, followed by slow clearance over time. Third, the accumulation of radioactivity in the thyroid at 240 min p.i. was quite low suggesting that [^{125}I]Spiro-I is relatively stable to *in vivo* deiodination.

In order to verify the specific binding of [^{125}I]Spiro-I to σ receptors *in vivo*, the effects of preinjection of haloperidol

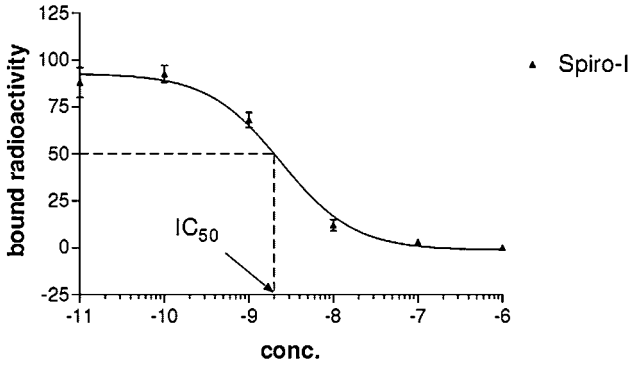

Figure 3. Binding curve of Spiro-I in competitive binding assay.

Table 1. Biodistribution of [^{125}I]Spiro-I in female ICR mice[a]

Organ	15 min	30 min	60 min	120 min	240 min
Blood	1.26 ± 0.25	0.90 ± 0.06	0.81 ± 0.13	0.73 ± 0.27	0.58 ± 0.10
Brain	5.28 ± 0.41	4.66 ± 0.38	4.10 ± 0.33	2.70 ± 0.23	1.52 ± 0.16
Heart	3.41 ± 0.40	2.52 ± 0.25	2.03 ± 0.27	1.37 ± 0.15	0.76 ± 0.07
Liver	12.13 ± 1.46	11.70 ± 1.89	8.92 ± 1.11	7.48 ± 1.10	4.22 ± 0.49
Spleen	6.17 ± 0.23	6.68 ± 0.70	6.66 ± 0.47	4.40 ± 0.42	2.73 ± 0.56
Lung	8.20 ± 0.88	6.81 ± 0.32	5.63 ± 0.78	3.86 ± 0.49	2.60 ± 0.27
Kidney	11.24 ± 1.68	9.16 ± 0.47	7.64 ± 0.94	5.56 ± 1.31	3.49 ± 0.80
Thyroid	3.22 ± 0.82	4.06 ± 1.28	3.80 ± 0.91	2.18 ± 0.83	1.39 ± 0.52
Stomach[b]	2.80 ± 0.49	4.14 ± 0.84	4.31 ± 0.99	5.01 ± 1.22	1.79 ± 0.25
Muscle	1.94 ± 0.27	1.42 ± 0.18	1.43 ± 0.22	1.35 ± 0.25	0.89 ± 0.54
Small intestine	16.93 ± 1.62	26.51 ± 4.85	16.29 ± 3.31	12.94 ± 0.43	9.38 ± 3.75

[a]Data are means of %ID/g of tissue $\pm$ SD, $n = 5$.
[b]%ID/organ.

(0.1 mL, 1.0 mg/kg) on the biodistribution of radioactivity in various organs of female ICR mice were examined. Either saline or haloperidol was injected 5 min prior to the radiotracer injection. The results of blocking studies at 60 min p.i. are given in Table 2. Of particular interest was a significant reduction (84%, $p < 0.001$) in the brain accumulation of radioactivity at 60 min p.i.. Moreover, the concentration of radioactivity in organs known to possess σ receptors was significantly reduced with heart by 55%, spleen by 60%, and lung by 60%. These data suggest that the binding of [^{125}I]Spiro-I to σ receptors was specific *in vivo*.

Currently, there are no SPECT tracers for imaging of σ_1 receptors in clinic use, and only one putative tracer, [^{123}I]TPCNE, has been evaluated in human studies. Unfortunately, [^{123}I]TPCNE suffers from irreversible binding in the human brain suggesting that a tracer with less avid binding to σ_1 receptors will be better suited for clinical applications.[12] With a K_i value of 2.75 nM, Spiro-I may therefore possess more suitable binding affinity to σ_1 receptors than TPCNE ($K_i = 0.67$ nM).[12,15] Moreover, Spiro-I has lower σ_2 receptor affinity ($K_i = 340$ nM vs $K_i = 38.8$ nM, respectively), and better subtype selectivity ($\sigma_2/\sigma_1 = 124$ vs $\sigma_2/\sigma_1 = 58$). In biodistribution studies, [^{125}I]Spiro-I was found to have high brain uptake, high specific binding to σ_1 receptors, as well as limited *in vivo* deiodination. It is noteworthy that [^{125}I]Spiro-I showed clearance from the brain after the peak uptake at 15 min. The results warrants further evaluation of [^{125}I]Spiro-I as a putative tracer for imaging σ_1 receptors with SPECT.

Experimental

General

[^{125}I]NaI (specific activity as I: 17.4 Ci/mg, solvent: 1.0E−05 mol/L NaOH, pH = 8–11) was bought from PerkinElmer Life and Analytical Sciences. All other reagents and chemicals were purchased from commercial sources and used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance-500 (500 MHz) or a Bruker Avance III (400 MHz) NMR spectrometer. Chemical shift (δ) are reported in ppm downfield from tetramethylsilane and coupling constants (*J*) are reported in Hertz (Hz). MS spectra were obtained by GC2000/TRACE^{TM} EI/MS (Finigan Co., USA) or Quattro micro API ESI/MS (Waters, USA). Melting point was recorded on an X-6 micro melting point apparatus (Beijing Taike Co., LTD, China) and was uncorrected.

HPLC analyses were performed on a Shimadzu SCL-10AVP system (SHIMADZU Corporation, Japan) which consisted of a binary pump with on-line degasser, a model SPD-10AVP UV-VIS detector operating at a wavelength of 231 nm, and a Packard 500TR series flow scintillation analyzer (Packard BioScience Co., USA). The samples were analyzed on a Agilent TC-C18(2) column (150 mm × 4.6 mm, 5 μm) using acetonitrile and water (85:15 v/v) as mobile phase at a flow rate of 1 mL/min. HPLC separation was carried out on an Alltech HPLC system with Alltech HPLC pump model 626, a linear UVIS-201 detector operating at a wavelength of 231 nm, and a Bioscan flow count. The sample was separated on an Alltech Alltima RPC-18 column (250 mm × 4.6 mm, 5 μm) using acetonitrile and water (90:10 v/v) as mobile phase at a flow rate of 2 mL/min.

Chemistry

*1′-(4-iodobenzyl)-3H-spiro[isobenzofuran-1,4′-piperidine](**2**)*

Compound **1** (178 mg, 0.94 mmol) was dissolved in anhydrous acetonitrile (50 mL). K_2CO_3 (1.3 g, 94 mmol), KI (120 mg, 0.72 mmol), and 4-iodobenzyl bromide (280 mg, 0.94 mmol) were added. The mixture was refluxed for 8 h. After cooling the inorganic salts were filtered off and the solvent was removed under reduced pressure. The residue was purified by chromotography (ethyl acetate: petroleum ether = 1:4 v/v) to afford white solid: yield 30%. The solid was crystallized from ether/petroleum ether as colorless crystal. Melting point: 118.7–119.6°. IR (KBr, cm^{-1}): 2940, 1049, 758. ^{1}H-NMR(400 MHz, $CDCl_3$)δ(ppm): 7.64–7.66 (d, $J = 8.2$ Hz, 2H), 7.12–7.28 (m, 6H), 5.06 (s, 2H), 3.55 (s, 2H), 2.83 (d, $J = 10.4$ Hz, 2H), 2.45 (t, $J = 11.6$ Hz, 2H), 1.97–2.04 (m, 2H), 1.76 (d, $J = 12.2$ Hz, 2H). ^{13}C-NMR (400 MHz, $CDCl_3$)δ: 145.61, 138.95, 137.39, 131.29, 127.81, 127.61, 127.38, 121.08, 120.83, 92.47, 84.58, 70.76, 62.67, 50.09, 36.53. EI-MS (*m/z*): 405(M^+).

*1′-(4-(tributylstannyl)benzyl)-3H-spiro[isobenzofuran-1,4′-piperidine] (**3**)*

Compound **2** (98 mg, 0.24 mmol) was dissolved in anhydrous triethylamine (10 mL) under nitrogen. Bis(tributyltin) (0.36 mL,

Table 2. Effects of preinjection of haloperidol (0.1 mL, 1.0 mg/kg) 5 min prior to the injection of [^{125}I]Spiro-I on the biodistribution of radioactivity in female ICR mice[a]

Organ	60 min(control)	60 min(blocking)	% blocking	p[b]
Blood	1.35 ± 0.23	1.54 ± 0.09	+14	0.176
Brain	4.03 ± 0.51	0.63 ± 0.11	−84	< 0.001
Heart	2.71 ± 0.22	1.23 ± 0.21	−55	< 0.001
Liver	7.94 ± 0.84	5.42 ± 0.43	−32	< 0.001
Spleen	5.35 ± 0.57	2.14 ± 0.27	−60	< 0.001
Lung	6.62 ± 0.13	2.63 ± 0.34	−60	< 0.001
Kidney	5.82 ± 0.27	4.48 ± 0.52	−23	0.002
Thyroid	2.46 ± 0.66	1.45 ± 0.17	−41	0.052
Stomach[c]	5.93 ± 0.79	5.56 ± 0.69	−6	0.494
Muscle	1.49 ± 0.21	0.87 ± 0.14	−42	0.004
Small intestine	10.34 ± 2.45	8.32 ± 1.69	−20	0.261

[a]Data are means of %ID/g of tissue ± SD, $n = 4$–5;
[b]p values for the control vs blocking group at 60 min p.i. calculated by Student's t test (independent, two-tailed).
[c]%ID/organ.

0.71 mmol), tetrakis(triphenylphosphine) Palladium(0) (11 mg, 0.01 mmol) were added. The mixture was refluxed for 6 h. After cooling the inorganic salts were filtered off and the solvent was removed under reduced pressure. The residue was purified by chromotography (ethyl acetate:petroleum ether = 1:4 v/v) to afford yellow-light oil, yield 87%. IR(KBr, cm^{-1}): 2922, 1464, 1049. ^{1}H-NMR(500 MHz, $CDCl_3$)δ(ppm): 7.47−7.17(m, 8H), 5.10 (s, 2H), 3.61(s, 2H), 2.90(d, J = 10.2 Hz, 2H), 2.47(t, J = 11.5 Hz, 2H), 2.05 (t, J = 12.2 Hz, 2H), 1.80 (d, J = 13.2 Hz, 2H), 1.60−1.54 (m, 6H), 1.39−1.34 (m, 6H), 1.10−1.06 (m, 6H), 0.93−0.90 (m, 9H). ^{13}C-NMR (400 MHz, $CDCl_3$)δ: 145.78, 140.36, 138.95, 136.40, 129.00, 127.52, 127.32, 126.61, 121.02, 120.85, 84.76, 70.72, 63.49, 50.20, 36.60, 29.10, 27.39, 13.68, 9.56. ESI (*m/z*): 570.5 ($[M+H]^+$).

Radiochemistry

To a solution of the tributyltin precursor (**3**) in ethanol (1 mg/mL), 100 μL solution of the precursor, 2 μL $Na^{125}I$ (795 μCi, carrier free, specific activity > 2200 Ci/mmol), 100 μL chloramine-T (2.0 mg/mL), and 100 μL HCl (1 mol/L) were added to a sealed vial. The reaction mixture was kept at room temperature for 10 min. The reaction was quenched with 100 μL $Na_2S_2O_5$ solution (50 mg/mL). The pH value of the mixture was adjusted to 7.0–7.2 with NaOH solution (1 mol/L). The mixture was then passed across a C18 Sep-Pak cartridge. After elution with water, inorganic salts including [^{125}I]NaI was separated from the product. The crude product was recovered into a sample vial by slowly flushing the cartridge with 10 mL of absolute ethanol. The solvent was evaporated under reduced pressure and the residue was purified by radio-HPLC. As analyzed by HPLC, the final product was obtained with a radiochemical purity of > 99%. The isolated radiochemical yield of [^{125}I]Spiro-I was 55–65% (n = 5). In order to identify the radioactive product, the stable Spiro-I was co-injected and co-eluted with the radioactive product.

In vitro stability

The *in vitro* stability of [^{125}I]Spiro-I was evaluated by monitoring the RCP at different time points. [^{125}I]Spiro-I in 0.9% NaCl was kept at room temperature for up to 6 h. The RCP of [^{125}I]Spiro-I was determined by radio-HPLC chromatography at 1, 2, 3, 4, 5, and 6 h.

To 1.0 mL of fresh mouse plasma, 0.1 mL of [^{125}I]Spiro-I solution (188 μCi) was added and incubated at 37°C. At 1, 2, 3, 4, 5, and 6 h, 0.15 mL aliquots were withdrawn and treated with 0.15 mL methanol to precipitate the proteins. Samples were then centrifuged at 3000 rpm for 10 min. The supernatants of each sample were analyzed by radio-HPLC.

Determination of log *D* value

The log *D* value of [^{125}I]Spiro-I was determined by measuring the distribution of the radiotracer between 1-octanol and 0.05 mol/L sodium phosphate buffer at pH = 7.4. The two phases were pre-saturated with each other. 1-octanol (3 mL) and phosphate buffer (3 mL) were pipetted into a 10 mL plastic centrifuge tube. 1.5 μL of a solution of HPLC-purified [^{125}I]Spiro-I (1 μCi) in ethanol was then added. The tube was vortexed for 5 min, followed by centrifugation for 10 min (3000 rpm, Anke TDL80-2B, China). About 50 μL of the 1-octanol layer was weighed in a tared tube. The 1-octanol layer was removed and about 500 μL of the buffer layer was weighed in a second tared tube. After adding 0.50 mL buffer to the octanol fraction and 0.05 mL of 1-octanol to the aqueous fraction, activity in both tubes was measured in an automatic γ-counter (Wallac 1470 Wizard, USA). Accurate volumes of each counted phase were determined by weight and known densities. The distribution coefficient was determined by calculating the ratio of cpm/mL of 1-octanol layer to that of buffer layer and expressed as log *D*. Samples from the 1-octanol layer were re-distributed until consistent distribution coefficient values were obtained. The measurement was carried out in triplicate and repeated four times.

In vitro radioligand competition studies

σ_1 and σ_2 receptor competition binding assays were performed as previously reported.[16] The σ_1 receptor asssay was performed using a guinea pig brain membrane preparation as receptor material and (+)-[^{3}H]pentazocine as radioligand. Nonspecific binding was determined with 10 μM unlabeled (+)-pentazocine. The σ_2 receptor affinity was determined using rat liver membrane preparation with the radioligand [^{3}H]ditolylguanidine in the presence of 2 μM (+)-pentazocine to mask σ_1-binding sites. Nonspecific binding was determined with 10 μM ditolylguanidine. K_i values were calculated according to Cheng and Prusoff and represent data from at least three independent experiments, each performed in triplicate. The results are given as mean ± standard error of the mean

Biodistribution and blocking studies in mice

Experiments in female ICR mice (n = 5, 18–22 g) were carried out in compliance with the national laws related to the care and experiments on laboratory animals. The HPLC-purified [^{125}I]Spiro-I was injected via tail vein (0.1 mL, 1 μCi). The mice were sacrificed by cervical fracture at various time points. Samples of blood and organs of interest were removed, weighed and counted in an automatic γ-counter (Wallac 1470 Wizard, USA). The results were expressed in terms of the percentage of the injected dose per gram (%ID/g) of blood or organs.

For blocking studies, mice were injected via tail vein with either saline (0.1 mL) or haloperidol (0.1 mL, 1.0 mg/kg) 5 min prior to radiotracer injection. The animals were sacrificed by cervical fracture at 60 min p.i. Blood or organs were isolated and analyzed as described above for the biodistribution study. Significant differences between control and test groups were determined by Student's t test (independent, two-tailed). The criterion for significance was $p \leqslant 0.05$.

Conclusion

[^{125}I]Spiro-I has been synthesized and evaluated as a potential tracer for imaging of σ_1 receptors with SPECT. The radiotracer has been prepared in good radiochemical yield and high radiochemical purity. *In vitro* competition binding assays showed that Spiro-I exhibit low nanomolar affinity for σ_1 receptors and high subtype selectivity. The log *D* value of [^{125}I]Spiro-I was found to be 3.06 ± 0.11, which is within the range expected to give high brain uptake. In biodistribution studies [^{125}I]Spiro-I was found to have high initial brain uptake followed by slow clearance. The binding pattern of the tracers *in vivo* was found to be in good agreement with the known distribution of σ_1 receptors, and blocking studies confirmed

high specific binding. The results warrants further evaluation of [^{125}I]Spiro-I as a putative tracer for imaging σ_1 receptors with SPECT.

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