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Synthesis and biological evaluation of novel 4-benzylpiperazine ligands for sigma-1 receptor imaging

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ABSTRACT

We report the synthesis and evaluation of 4-benzylpiperazine ligands (BP-CH_3, BP-F, BP-Br, BP-I, and BP-NO_2) as potential σ_1 receptor ligands. The X-ray crystal structure of BP-Br, which crystallized with monoclinic space group $P2_1/c$, has been determined. In vitro competition binding assays showed that all the five ligands exhibit low nanomolar affinity for σ_1 receptors (K_i = 0.43–0.91 nM) and high subtype selectivity (σ_2 receptor: K_i = 40–61 nM; $K_i\sigma_2/K_i\sigma_1$ = 52–94). [^{125}I]BP-I (1-(1,3-benzodioxol-5-ylmethyl)-4-(4-iodobenzyl)piperazine) was prepared in 53 ± 10% isolated radiochemical yield, with radiochemical purity of >99% by HPLC analysis after purification, via iododestannylation of the corresponding tributyltin precursor. The log *D* value of [^{125}I]BP-I was found to be 2.98 ± 0.17, which is within the range expected to give high brain uptake. Biodistribution studies in mice demonstrated relatively high concentration of radiolabeled substances in organs known to contain σ_1 receptors, including the brain, lung, kidney, heart, and spleen. Administration of haloperidol 5 min prior to injection of [^{125}I]BP-I significantly reduced the concentration of radioactivity in the above-mentioned organs. The accumulation of radiolabeled substance in the thyroid was quite low suggesting that [^{125}I]BP-I is relatively stable to in vivo deiodination. These findings suggest that the binding of [^{125}I]BP-I to σ_1 receptors in vivo is specific.

1. Introduction

Sigma receptors represent a distinct class of proteins, with two subtypes (σ_1 and σ_2) 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.[2–4] It is noteworthy that the human σ_1 receptor gene is located on chromosome 9, band p13, which is known to be associated with different psychiatric disorders.[5] Accordingly, the σ_1 receptors are linked to a number of brain disorders, including schizophrenia, depression, Alzheimer's disease, as well as ischemia.[3,4,6–8] They regulate the activity of various ion channels and possess protein chaperone function.[3] Recently, it has been reported that σ-receptor ligands inhibit the cardiac voltage-gated Na^+ channel $Na_v1.5$, which may explain some of the actions of σ-receptor ligands on the cardiovascular system.[9] Moreover, dehydroepiandrosterone sulfate (DHEAS), an agonist of the σ_1 receptors,[10,11] could inhibit the persistent sodium currents, which is involved in the pathological process of brain disease.[12] The effect of DHEAS on persistent sodium currents was also eliminated by σ_1 receptor blockers, and the σ_1 receptor agonist carbetapentane citrate was found to mimic the effect of DHEAS. The main functional consequence of this DHEAS effect is presumably to protect neurons under ischemia.[13] More and more evidence suggests that the σ_1 receptors represent potential therapeutic targets in many human diseases.[14] Development of σ_1 receptor ligands could have wide therapeutic applications, while development of specific radiotracers for in vivo imaging of σ_1 receptors may provide the necessary diagnostic tools in combating the above-mentioned diseases.

It is well known that many compound classes with diverse structures possess high affinity for σ_1 receptors. In the past few years, many potential radiotracers for imaging σ_1 receptor expression with PET and SPECT have also been reported. But until now, only a few tracers such as [^{11}C]SA4503,[15,16] [^{18}F]FPS,[17] and [^{123}I]TPCNE[18] have been evaluated in human studies. Moreover, few of the σ_1 tracers reported to date have ideal properties for imaging. [^{11}C]SA4503 was found to have about 70% specific binding rate,[19] but has high affinity also to the emopamil binding protein (K_i = 1.72 nM)[20] and to the vesicular acetylcholine transporter (K_i = 50.2 nM).[21] On the other hand, [^{18}F]FPS[22] and [^{123}I]TPCNE[18] were found to have irreversible kinetics. Recently, a new promising ^{18}F-labeled spirobenzofuran, named fluspidine,

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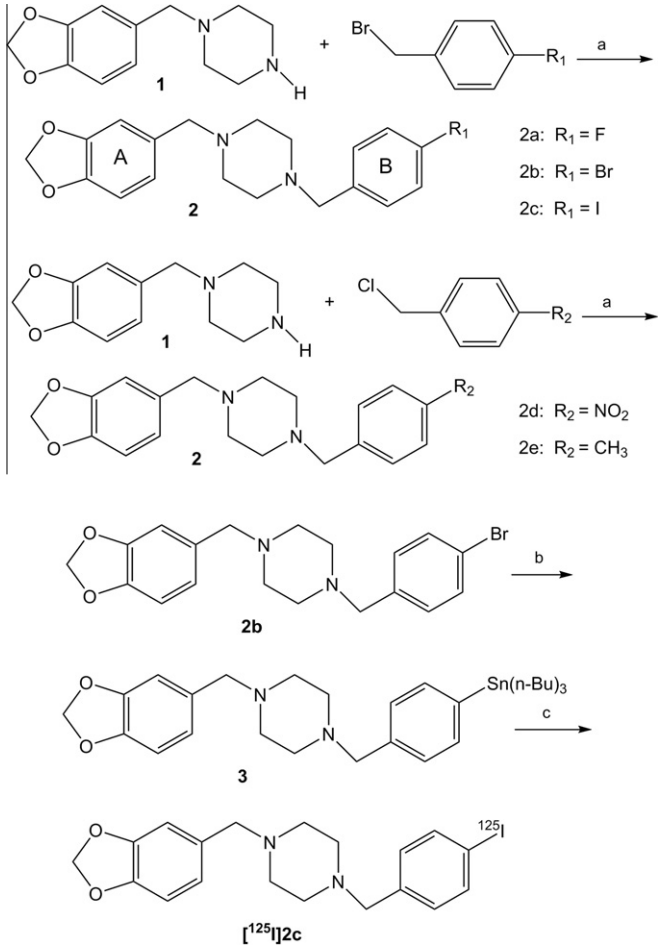
was developed and evaluated in mice. [^{18}F]Fluspidine demonstrated favorable target affinity and specificity as well as metabolic stability both in vitro and in animal experiments. The in vivo properties of [^{18}F]fluspidine offer a high potential of this radiotracer for neuroimaging and quantitation of σ_1 receptors in vivo.[23]

In 2008 1-(1,3-benzodioxol-5-ylmethyl)-4-(4-bromobenzyl)-piperazine (BP-Br) was reported to be used for the treatment of sodium channel mediated disease.[24] Based on the evidence that σ_1 receptors regulate the activity of sodium ion channels,[9,12] the possible mechanisms of the proposed treatment effect of BP-Br could be related to σ_1 receptors. Therefore, we have synthesized five 4-benzylpiperazine ligands (BP-CH_3, BP-F, BP-Br, BP-I, and BP-NO_2), determined the X-ray crystal structure of BP-Br, and measured their affinity to σ_1 receptors by in vitro radioligand binding assays. Moreover, we synthesized [^{125}I]BP-I (1-(1,3-benzodioxol-5-ylmethyl)-4-(4-iodobenzyl)piperazine), determined its log *D* value, and evaluated its potential as a putative SPECT tracer for imaging of σ_1 receptors by biodistribution studies in mice.

2. Results and discussion

2.1. Chemistry

The synthetic route of BP derivatives is shown in Scheme 1. Alkylation of compound **1** with the corresponding benzyl bromide or benzyl chloride provided compound **2a**–**2e**. Compound **2b** reacted with bis(tributyltin) for 6 h in toluene at reflux under nitrogen in the presence of palladium catalyst, followed by purification with chromatography (ethyl acetate/petroleum ether = 3:2 v/v) to afford yellow-light oil **3** in 18% yield. The yields of **2a**–**2e** were ranging from 60.4% to 81.5%. The synthesized compounds were characterized by NMR, MS, and EA.



Scheme 1. Reagents and conditions: (a) CH_2Cl_2, rt, 24 h; (b) $(Bu_3Sn)_2$, $(Ph_3P)_4Pd$, toluene, reflux, 6 h; (c) [^{125}I]NaI, H_2O_2, HCl.

2.2. Crystal data of BP-Br compound

From the general point of view, 4-benzylpiperazine derivatives consist of two amine sites (basic nitrogen atom) flanked by two hydrophobic regions. According to the pharmacophore model proposed by Glennon,[25] a structural feature common to high-affinity σ_1 receptor ligands is C–N(R)–X–Ph. N is a basic nitrogen atom, representing the amine site. Ph and C represent the primary hydrophobic region and secondary hydrophobic region, respectively. Both C and Ph are associated with regions of bulk tolerance. We assume that N1 (Fig. 1) is the amine binding site, and the phenyl ring with dioxol group (aromatic A) is the primary binding site, while the phenyl ring with Br substitute (aromatic B) (Scheme 1) is the secondary binding site. In order to examine the binding model of 4-benzylpiperazine derivatives, the single X-ray crystal structure analysis of BP-Br was performed. The crystal structure with the atomic numbering scheme of the BP-Br compound is shown in Figure 1. Crystal data together with details of the determinations are summarized in the experimental part. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) are listed in Table 1.

Based on the obtained X-ray crystal structure of BP-Br, the distances between N1 and the aromatic centroid (A) as well as the aromatic centroid (B) are 6.410 and 3.787 Å, respectively. The distance between the centroid of the aromatic A and that of B is 10.144 Å. Based on Glennon's pharmacophore model, the distance between N and the primary hydrophobic region (Ph) is 6–10 Å, while the distance between N and the secondary region (C) is 2.5–3.9 Å. Therefore, the above structural features of BP-Br are in good agreement with the general structural features found optimal for σ_1 receptor binding. BP derivatives may show high affinity for σ_1 receptors.

2.3. Radiolabeling

[^{125}I]BP-I was prepared via an iododestannylation reaction using H_2O_2 as the oxidizing agent. The reaction was quenched with saturated $NaHSO_3$ solution, 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]BP-I was eluted with ethanol. The product was further purified by radio-HPLC using a reverse-phase column and mobile phase consisting of acetonitrile with a flow rate of 2 mL/min.

In order to identify the radiotracer, the non-radioactive BP-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 present in Figure 2. From Figure 2, the retention times of BP-I and [^{125}I]BP-I were observed to be 4.18 and 4.60 min, respectively. The difference in retention time was in good agreement with the time lag which corresponds with the volume and flow rate within 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, 254 nm). The isolated radiochemical yield of [^{125}I]BP-I was 55–65% (n = 5). The radiochemical purity was higher than 99%. The specific activity of the n.c.a. [^{125}I]NaI was >2200 Ci/mmol (>80 GBq/μmol) at time of delivery. The specific activity of the product was not determined. To prepare a suitable solution of [^{125}I]BP-I for in vivo use, the eluted radioactive substance (peak corresponding

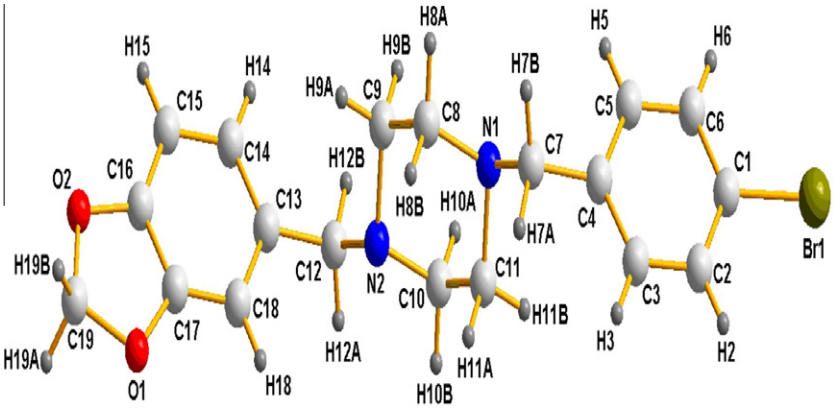


Figure 1. Crystal structure of BP-Br compound.

Table 1
Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($Å^2 \times 10^3$) for BP-Br compound

	x	*y*	*z*	*U* (equiv)
Br(1)	9729(1)	13,543(1)	8232(1)	105(1)
C(1)	9263(2)	11,224(7)	8220(5)	59(1)
C(2)	8940(2)	10,687(8)	7150(4)	63(1)
C(3)	8619(2)	8961(7)	7126(4)	58(1)
C(4)	8616(1)	7737(7)	8140(4)	51(1)
C(5)	8947(2)	8353(7)	9211(4)	61(1)
C(6)	9266(2)	10,087(8)	9262(5)	66(1)
C(7)	8307(2)	5729(8)	8055(4)	67(1)
C(8)	7780(2)	3329(6)	8995(4)	59(1)
C(9)	7422(2)	3068(6)	9917(4)	53(1)
C(10)	7153(2)	6608(6)	9635(4)	54(1)
C(11)	7509(2)	6871(6)	8712(4)	53(1)
C(12)	6615(2)	4214(7)	10,546(4)	57(1)
C(13)	6315(1)	2182(6)	10,469(4)	46(1)
C(14)	6326(2)	943(7)	11,491(4)	49(1)
C(15)	6023(2)	−868(7)	11,481(4)	50(1)
C(16)	5708(1)	−1388(6)	10,408(4)	45(1)
C(17)	5697(1)	−212(6)	9380(4)	45(1)
C(18)	5992(2)	1571(6)	9366(4)	48(1)
C(19)	5146(2)	−2962(7)	8953(4)	56(1)
N(1)	7965(1)	5488(5)	8969(3)	49(1)
N(2)	6964(1)	4453(5)	9651(3)	43(1)
O(1)	5358(1)	−1130(5)	8423(2)	63(1)
O(2)	5376(1)	−3098(4)	10,150(3)	60(1)

to [^{125}I]BP-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 (37 kBq) of radioactive substance per 0.1 mL of solution.

The apparent distribution coefficient of [^{125}I]BP-I was determined between 1-octanol and 0.05 mol L^{-1} sodium phosphate buffer at pH 7.4 as previously reported.[26] At pH 7.4, the log *D* value of [^{125}I]BP-I was determined to be 2.98 ± 0.17 (n = 3), which is within the range expected to give high brain uptake.

2.4. Biological studies

2.4.1. In vitro radioligand competition studies

σ_1 and σ_2 receptor competition binding assays were performed as previously reported.[27] The σ_1 and σ_2 receptor affinities of BP derivatives were determined in competition experiments with the radioligands (+)-[^{3}H]pentazocine and [^{3}H]ditolylguanidine using rat brain and rat liver preparations, respectively. In vitro competition binding assays showed that all five ligands exhibit low nanomolar affinity for σ_1 receptors (K_i = 0.43–0.91 nM, Table 2) and high

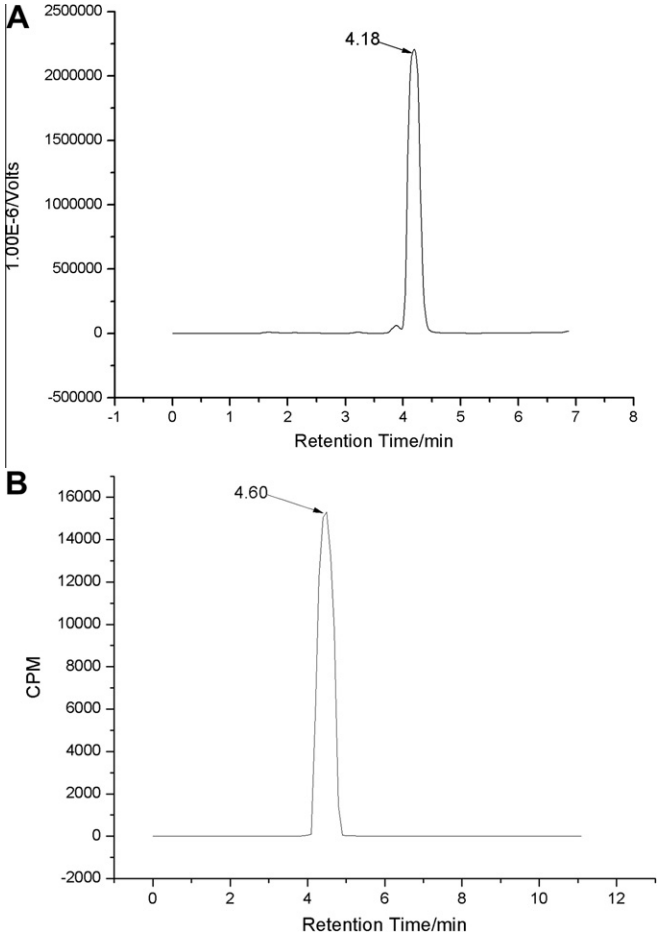


Figure 2. HPLC co-elution profiles of BP-I and [^{125}I]BP-I, with retention times of 4.18 and 4.60 min, respectively.

Table 2
Binding affinities of the 4-benzylpiperazine compounds towards σ_1 and σ_2 receptors

	K_i (σ_1) (nM)	K_i (σ_2) (nM)	$K_i(\sigma_2)/K_i(\sigma_1)$
BP-I	0.80 ± 0.14	61.1 ± 20.1	76.4
BP-F	0.85 ± 0.91	52.2 ± 13.3	61.4
BP-CH_3	0.91 ± 0.81	49.8 ± 8.89	54.7
BP-NO_2	0.43 ± 0.19	40.5 ± 11.7	94.2
BP-Br	0.78 ± 0.26	40.5 ± 9.23	51.9

Values are means ± SD of three experiments performed in triplicate.

subtype selectivity (σ_2 receptors: K_i = 40–61 nM; $K_i\sigma_2/K_i\sigma_1$ = 52–94, Table 2). Substitution with electron-withdrawing group, such as NO_2, in *p*-position of the benzyl moiety led to a slight increase of σ_1 receptor affinity. The high affinities of the BP derivatives are in good agreement with the structural features of BP-Br found optimal for the σ_1 receptors binding.

2.4.2. Biodistribution and blocking studies in mice

In vivo biodistribution studies of [^{125}I]BP-I were performed in female ICR mice. The uptake of radiolabeled substance in the organ of interest at 15, 30, 60, 120, and 240 min after intravenous administration of (0.10 mL) 1 μCi [^{125}I]BP-I is summarized in Table 3. [^{125}I]BP-I showed a high initial brain uptake, with slow clearance and high brain-to-blood ratios. The radioactivity concentration determined in the brain was highest at 15 min post-injection (p.i.) with 5.03 ± 0.60 %ID/g, and slowly cleared thereafter with 4.96 ± 0.16 %ID/g at 30 min, 4.63 ± 0.41 %ID/g at 60 min, 3.75 ± 0.20 %ID/g at 120 min, and 2.09 ± 0.21 %ID/g at 240 min p.i. The values are higher than that of [^{11}C]SA4503[19] and comparable to those determined for [^{18}F]fluspidine.[23] In contrast to the high uptake and retention in the brain, the blood radioactivity levels were low with 1.34 ± 0.37 %ID/g at 15 min and 0.72 ± 0.03 %ID/g at 240 min p.i., resulting in high brain-to-blood ratios (3.75, 5.64, 5.72, and 4.87 at 15, 30, 60 and 120 min p.i., respectively). Similar to what was found for [^{18}F]fluspidine high uptakes (4.0–12.7 %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]BP-I is relatively stable to in vivo deiodination.

In order to verify the specific binding of [^{125}I]BP-I to σ receptors in vivo, the effects of preinjection of haloperidol (0.1 mL, 1.0 mg kg^{-1}) 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 4. Of particular interest was a significant reduction (63%, p <0.001) in the brain accumulation of radioactivity at 60 min p.i. which corresponds to values obtained with [^{18}F]fluspidine.[23] Moreover, the concentration of radioactivity in organs known to possess σ receptors was significantly reduced with heart by 32%, spleen by 47%, and lung by 38%. These data suggest that the binding of [^{125}I]BP-I to σ receptors was specific in vivo.

Currently, in vivo [^{11}C]SA4503 imaging studies have shown that the density of σ_1 receptors was decreased in Parkinson's disease (PD) and Alzheimer's disease (AD) patients.[15,16] This tracer could be used as a probe in clinical studies of σ_1 receptors imaging in the above brain diseases. However, [^{11}C]SA4503 needs an on-site cyclotron. Longer half-life ^{18}F- and ^{123}I- labeled compounds are more suitable for σ_1 receptors imaging. Unfortunately, there is lack of suitable ^{18}F-labeled PET radiotracer and ^{123}I-labeled SPECT radiotracer for the neuroimaging of σ_1 receptors until now. The major problems of the currently available σ_1 receptor radiotracers such as [^{18}F]FPS[22] and [^{123}I]TPCNE,[18] are the irreversible kinetics and lack of a suitable reference region. Since σ_1 receptors regulate the activity of sodium ion channels,[9,12] we proposed that the possible mechanisms of the treatment effect of sodium channel mediated disease of BP-Br could be related to σ_1 receptors. BP-Br could possess affinity for σ_1 receptors. Based on the obtained X-ray crystal structure of BP-Br, the structural features of BP-Br are in good agreement with the Glennon's pharmacophore model for σ_1 receptor binding. Moreover, the five synthesized 4-benzylpiperazine ligands exhibit low nanomolar affinity for σ_1 receptors and high subtype selectivity. It is reasonable to synthesize [^{18}F]BP-F and [^{123}I]BP-I radioligands for further evaluation. For the 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. So [^{125}I]BP-I was synthesized first for in vivo evaluation. In biodistribution studies, [^{125}I]BP-I was found to have higher brain uptake than that of [^{11}C]SA4503, high specific binding to σ_1 receptors comparable to that determined for [^{11}C]SA4503, as well as limited in vivo deiodination. It is noteworthy that [^{125}I]BP-I showed clearance from the brain after the peak uptake at 15 min. This is an advantage compared to [^{123}I]TPCNE. The brain uptake of [^{123}I]TPCNE remained constant after initial uptake over the whole investigation time of 180 min.[18] Our results encourage the synthesis and evaluation of iodine-123 labeled BP-I as a putative tracer for imaging σ_1 receptors with SPECT.

Table 4
Effects of preinjection of haloperidol (0.1 mL, 1.0 mg kg^{-1}) 5 min prior to the injection of [^{125}I]BP-I on the biodistribution of radioactivity in female ICR mice[a]

Organ	60 min (control)	60 min (blocking)	% Blocking	p[b]
Blood	1.32 ± 0.41	1.08 ± 0.28	−18	0.311
Brain	3.64 ± 0.66	1.36 ± 0.19	−63	<0.001
Heart	1.72 ± 0.31	1.17 ± 0.15	−32	0.007
Liver	5.73 ± 0.95	6.85 ± 1.17	20	0.136
Spleen	4.35 ± 0.66	2.30 ± 0.39	−47	<0.001
Lung	3.84 ± 0.69	2.37 ± 0.44	−38	0.011
Kidney	8.18 ± 0.13	5.48 ± 0.31	−33	<0.001

[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).

Table 3
Biodistribution of [^{125}I]BP-I in female ICR mice[a]

Organ	15 min	30 min	60 min	120 min	240 min
Blood	1.34 ± 0.37	0.88 ± 0.16	0.81 ± 0.12	0.77 ± 0.19	0.72 ± 0.03
Brain	5.03 ± 0.60	4.96 ± 0.16	4.63 ± 0.41	3.75 ± 0.20	2.09 ± 0.21
Heart	3.98 ± 0.33	2.79 ± 0.29	1.71 ± 0.17	1.24 ± 0.18	0.75 ± 0.03
Liver	9.00 ± 0.90	6.39 ± 0.20	5.68 ± 0.42	5.08 ± 0.88	3.95 ± 0.27
Spleen	6.68 ± 0.67	6.53 ± 0.21	5.70 ± 0.37	4.79 ± 0.60	2.83 ± 0.24
Lung	12.49 ± 2.18	8.70 ± 0.23	6.29 ± 0.83	4.91 ± 1.36	2.69 ± 0.34
Kidney	12.72 ± 1.43	9.65 ± 0.72	8.73 ± 1.37	7.82 ± 0.76	6.89 ± 0.51
Thyroid	6.55 ± 0.66	2.30 ± 0.54	1.53 ± 0.16	1.40 ± 0.16	1.22 ± 0.20
Stomach[b]	2.32 ± 0.11	2.12 ± 0.14	1.78 ± 0.26	2.54 ± 0.17	1.68 ± 0.35
Muscle	2.35 ± 0.11	1.78 ± 0.29	1.50 ± 0.32	1.06 ± 0.29	0.72 ± 0.09
Small intestine	5.77 ± 1.33	10.21 ± 1.29	7.45 ± 1.32	10.73 ± 4.12	10.95 ± 1.31

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

3. Conclusions

4-Benzylpiperazine derivatives have been synthesized and evaluated as high-affinity σ_1 receptor ligands with relatively high subtype selectivity. [^{125}I]BP-I has been prepared in good radiochemical yield and high radiochemical purity. The log *D* value of [^{125}I]BP-I was within the range expected to give excellent brain uptake. In biodistribution studies [^{125}I]BP-I was found to possess very high initial brain uptake followed by slow clearance. The in vivo binding pattern of the tracer was in good agreement with the known distribution of σ_1 receptors, and blocking studies confirmed high specific binding. These findings suggest further synthesis and evaluation of [^{123}I]BP-I as a suitable radiotracer for imaging σ_1 receptors with SPECT in vivo.

4. Experimental section

4.1. General information

[^{125}I]NaI (specific activity as I: 17.4 Ci/mg, >80 GBq/μmol, solvent: 1.0 E–05 mol/L, pH 8–11) was bought from Perkin–Elmer Life and Analytical Sciences. All other reagents and chemicals were purchased from commercial sources and used without further purification unless otherwise indicated. ^{1}H NMR spectra were recorded on 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 Quattro micro API ESI/MS (Waters, USA). Elemental analyses were obtained on an Elementar 240C (Perkin–Elmer, 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 (SHIMAZU Corporation, Japan) which consisted of a binary pump with on-line degasser, a model SPD-10AVP UV–vis detector operating at a wavelength of 254 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 × 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 a Shimadzu LC-20AT HPLC system with a UV–vis detector operating at a wavelength of 254 nm, and a SPD-M20A flow scintillation analyzer. The sample was separated on an Alltech Alltima RPC-18 column (250 × 4.6 mm, 5 μm) using acetonitrile as mobile phase at a flow rate of 2 mL/min.

4.2. General procedure for synthesis of BP derivatives

To produce compounds **2a**, **2b**, **2c**, **2d**, and **2e**, 1-(1,3-benzodioxol-5-ylmethyl)piperazine (220 mg, 1.0 mmol) was dissolved in anhydrous dichloromethane (5 mL), the corresponding benzyl bromide or benzyl chloride (1.0 mmol) were added. The mixture was stirred at room temperature for 24 h. After filtration, the solvent was removed under reduced pressure. The residue was purified by chromatography (ethyl acetate/petroleum ether = 1:1 v/v) to afford white solid. The solid was crystallized from ethyl acetate as colorless crystal.

4.3. 1-(1,3-Benzodioxol-5-ylmethyl)-4-(4-fluorobenzyl)piperazine (2a)

Mp 101–103 °C. Yield 71%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.26 (t, *J* = 8.6 Hz, 2H), 6.98 (t, *J* = 8.7 Hz, 2H), 6.84 (s, 1H), 6.73 (s, 2H), 5.92 (s, 2H), 3.46 (s, 2H), 3.41 (s, 2H), 2.45 (br s, 8H). ESI$^+$-MS([M+H]$^+$): 329.4. Anal. Calcd for $C_{19}H_{21}FN_2O_2$: C, 69.49; N, 8.53; H, 6.45; F, 5.79. Found: C, 69.61; N, 8.48; H, 6.48.

4.4. 1-(1,3-Benzodioxol-5-ylmethyl)-4-(4-bromobenzyl)piperazine (2b)

Mp 106–108 °C. Yield 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.42 (d, *J* = 8.3 Hz, 2H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.84 (s, 1H), 6.73 (s, 2H), 5.93 (s, 2H), 3.44 (s, 2H), 3.41 (s, 2H), 2.45 (br s, 8H). ESI$^+$-MS([M+H]$^+$): 389.4. Anal. Calcd for $C_{19}H_{21}BrN_2O_2$: C, 58.62; N, 7.20; H, 5.44; O, 8.22; Br, 20.53. Found: C, 58.57; N, 6.89; H, 5.99.

4.5. 1-(1,3-Benzodioxol-5-ylmethyl)-4-(4-iodobenzyl)piperazine (2c)

Mp 104–106 °C. Yield 59%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.62 (d, *J* = 7.4 Hz, 2H), 7.06 (d, *J* = 7.5 Hz, 2H), 6.84 (s, 1H), 6.73 (s, 2H), 5.93 (s, 2H), 3.42 (d, *J* = 9.4 Hz, 4H), 2.44 (br s, 8H). ESI$^+$-MS([M+H]$^+$): 437.1. Anal. Calcd for $C_{19}H_{21}IN_2O_2$: C, 52.31; N, 6.42; H, 4.85; I, 29.09; O, 7.33. Found: C, 52.39; N, 6.37; H, 4.85.

4.6. 1-(1,3-Benzodioxol-5-ylmethyl)-4-(4-nitrobenzyl)piperazine (2d)

Mp 104–105 °C. Yield 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.16 (d, *J* = 8.6 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 2H), 6.84 (s, 1H), 6.73 (s, 2H), 5.93 (s, 2H), 3.59 (s, 2H), 3.43 (s, 2H), 2.47 (br s, 8H). ESI$^+$-MS([M+H]$^+$): 356.4. Anal. Calcd for $C_{19}H_{21}N_3O_4$: C, 64.21; N, 11.82; H, 5.96; O, 18.01. Found: C, 64.46; N, 11.65; H, 6.08.

4.7. 1-(1,3-Benzodioxol-5-ylmethyl)-4-(4-methylbenzyl)-piperazine (2e)

Mp 102–103 °C. Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.18 (d, *J* = 7.8 Hz, 2H), 7.10 (d, *J* = 7.8 Hz, 2H), 6.83 (s, 1H), 6.72 (s, 2H), 5.92 (s, 2H), 3.46 (s, 2H), 3.40 (s, 2H) 2.45 (br s, 8H), 2.32 (s, 3H). ESI$^+$-MS([M+H]$^+$): 325.5. Anal. Calcd for $C_{20}H_{24}N_2O_2$: C, 74.04; N, 8.64; H, 7.46; O, 9.86. Found: C, 74.32; N, 8.69; H, 6.92.

4.8. 1-(1,3-Benzodioxol-5-ylmethyl)-4-(4-(tributylstannyl)-benzyl)piperazine (3)

Compound **2b** (100 mg, 0.24 mmol) was dissolved in anhydrous toluene (5 mL) under nitrogen. Bis(tributyltin) (0.36 mL, 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 chromatography (ethyl acetate/petroleum ether = 3:2 v/v) to afford 25 mg white solid, yield 18%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.39 (d, *J* = 8.5 Hz, 2H), 7.25 (d, *J* = 8.5 Hz, 2H), 6.84 (s, 1H), 6.73 (s, 2H), 5.92 (s, 2H), 3.49 (s, 2H), 3.41 (s, 2H), 2.46 (br s, 8H), 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 146.57, 145.51, 139.23, 136.79, 135.30, 131.17, 127.84, 121.21, 108.54, 106.78, 99.81, 62.10, 61.78, 52.04, 29.62, 28.07, 26.46, 26.35, 25.76, 16.30, 12.60, 8.97, 8.55. ESI$^+$-MS([M+H]$^+$): 600.9.

4.9. Crystal data for BP-Br compound

Data were collected for a colorless crystal on a Bruker Smart APEX II diffractometer (Bruker Co., Germany). Crystal data together with details of the determinations are summarized as follows. $C_{19}H_{21}BrN_2O_2$, M_r = 389.29, monocyclic, space group $P2_1/c$, a = 25.48 50(19) Å, b = 6.3928(5) Å, c = 11.1585(9) Å, α = 90°, β = 100.247(2)°, γ = 90°, V = 1789.0(2) Å^3; Z = 4; calculated density = 1.445 Mg m^{-3}; Absorption coefficient 2.311 mm^{-1}; F(0 0 0) = 800; crystal size:

0.24 × 0.20 × 0.04 mm. Reflections collected/unique: 6493/3135 [R(int) = 0.0275]; absorption correction: semi-empirical from equivalents. Max. and min. transmission: 0.9132 and 0.6070; refinement method: full-matrix least-squares on F^2; data/restraints/parameters: 3135/0/217; Goodness-of-fit on F^2: 1.026; final R indices [$I > 2\sigma(I)$]: R_1 = 0.0499, wR_2 = 0.1178; R indices (all data): R_1 = 0.0950, wR_2 = 0.1399; largest diff. peak and hole: 0.839 and −0.826 e.A^{-3}.

4.10. Preparation of [^{125}I]BP-I

To a solution of the tributyltin precursor (BP-Sn, **3**) in ethanol (1 mg/mL, 100 μL), 2 μL Na^{125}I (795 μCi, carrier free, specific activity >2200 Ci/mmol), 100 μL H_2O_2 (3%, freshly prepared) and 100 μL HCl (1 mol L^{-1}) were added to a sealed vial. The reaction mixture was kept at room temperature for 15 min. The reaction was quenched with 50 μL saturated $NaHSO_3$ solution. The pH value of the mixture was adjusted to 7.5 with NaOH solution (1 mol L^{-1}). 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]BP-I was 55–65% (n = 5). In order to identify the radioactive product, the stable BP-I was co-injected and co-eluted with the radioactive product.

4.11. Determination of log D value

The log D value of [^{125}I]BP-I was determined by measuring the distribution of the radiotracer between 1-octanol and 0.05 mol L^{-1} 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. Two microliters of a solution of HPLC-purified [^{125}I]BP-I (2 μCi) in ethanol was then added. The tube was vortexed for 5 min, followed by centrifugation for 15 min (3500 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 redistributed until consistent distribution coefficient values were obtained. The measurement was carried out in triplicate and repeated three times.

4.12. In vitro radioligand competition studies

σ_1 and σ_2 receptor competition binding assays were performed as previously reported.[27] Briefly, the σ_1 receptor assay was performed using a rat brain membrane preparation as receptor material and (+)-[^{3}H]pentazocine as radioligand. The σ_2 receptor affinity was determined using rat liver membrane preparation with the radioligand [^{3}H]ditolylguanidine in the presence of 10 μM dextrallorphan to mask σ_1 binding sites. Non-specific binding was determined with 10 μM haloperidol. 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 deviation (SD).

4.13. 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]BP-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^{-1}) 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 $p \leqslant 0.05$.

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Supplementary data

Supplementary data (CIF file of BP-Br) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.03.037.

References and notes

1. Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T.-P.; Tam, S. W.; Taylor, D. P. *Trends Pharmacol. Sci.* **1992**, *13*, 85.
2. Hayashi, T.; Su, T. P. *Curr. Neuropharmacol.* **2005**, *3*, 267.
3. Maurice, T.; Su, T. P. *Pharmacol. Ther.* **2009**, *124*, 195.
4. Collier, T. L.; Waterhouse, R. N.; Kassiou, M. *Curr. Pharm. Des.* **2007**, *13*, 51.
5. Prasad, P. D.; Li, H. W.; Fei, Y.-J.; Ganapathy, M. E.; Fujita, T.; Plumley, L. H.; Yang-Feng, T. L.; Leibach, F. H.; Ganapathy, V. *J. Neurochem.* **1998**, *70*, 443.
6. Guitart, X.; Codony, X.; Monroy, X. *Psychopharmacology* **2004**, *174*, 301.
7. Hashimoto, K.; Ishiwata, K. *Curr. Pharm. Des.* **2006**, *12*, 3857.
8. Hayashi, T.; Su, T.-P. *Expert Opin. Ther. Targets* **2008**, *12*, 45.
9. Johannessen, M.; Ramachandran, S.; Riemer, L.; Ramos-Serrano, A.; Ruoho, A. E.; Jackson, M. B. *Am. J. Physiol. Cell Physiol.* **2009**, *296*, C1049.
10. Maurice, T.; Gregoire, C.; Espallergue, J. *Pharmacol. Biochem. Behav.* **2006**, *84*, 581.
11. Ueda, H.; Yoshida, A.; Tokuyama, S.; Mizuno, K.; Maruo, J.; Matsuno, K.; Mita, S. *Neurosci. Res.* **2001**, *41*, 33.
12. Cheng, Z.-X.; Lan, D.-M.; Wu, P.-Y.; Zhu, Y.-H.; Dong, Y.; Ma, L.; Zheng, P. *Exp. Neurol.* **2008**, *210*, 128.
13. Kaasik, A.; Kalda, K.; Jaako, K.; Zharkovsky, A. *Neuroscience* **2001**, *102*, 427.
14. Cobos, E. J.; Entrena, J. M.; Nieto, F. R.; Cendan, C. M.; Del Pozo, E. *Curr. Neuropharmacol.* **2008**, *6*, 344.
15. Mishina, M.; Ishiwata, K.; Ishii, K.; Kitamura, S.; Kimura, Y.; Kawamura, K.; Oda, K.; Sasaki, T.; Sakayori, O.; Hamamoto, M.; Kobayashi, S.; Katayama, Y. *Acta Neurol. Scand.* **2005**, *112*, 103.
16. Mishina, M.; Ohyama, M.; Ishii, K.; Kitamura, S.; Kimura, Y.; Oda, K.; Kawamura, K.; Sasaki, T.; Kobayashi, S.; Katayama, Y.; Ishiwata, K. *Ann. Nucl. Med.* **2008**, *22*, 151.
17. Waterhouse, R. N.; Nobler, M. S.; Zhou, Y.; Chang, R. C.; Morales, O.; Kuwabara, H.; Kumar, A.; VanHeertum, R. L.; Wong, D. F.; Sackeim, H. A. *Neuroimage* **2004**, *22*, T29.
18. Stone, J. M.; Arstad, E.; Erlandsson, K.; Waterhouse, R. N.; Ell, P. J.; Pilowsky, L. S. *Synapse* **2006**, *60*, 109.
19. Kawamura, K.; Ishiwata, K.; Shimada, Y.; Kimura, Y.; Kobayashi, T.; Matsuno, K.; Homma, Y.; Senda, M. *Ann. Nucl. Med.* **2000**, *14*, 285.

20. Berardi, F.; Ferorelli, S.; Colabufo, N. A.; Leopoldo, M.; Perrone, R.; Tortorella, V. *Bioorg. Med. Chem.* **2001**, *9*, 1325.
21. Shiba, K.; Ogawa, K.; Ishiwata, K.; Yajima, K.; Mori, H. *Bioorg. Med. Chem.* **2006**, *14*, 2620.
22. Waterhouse, R. N.; Chang, R. C.; Atuehene, N.; Collier, T. L. *Synapse* **2007**, *61*, 540.
23. Fischer, S.; Wiese, C.; Grosse Maestrup, E.; Hiller, A.; Deuther-Conrad, W.; Scheunemann, M.; Schepmann, D.; Steinbach, J.; Wünsch, B.; Brust, P. *Eur. J. Nucl. Med. Mol. Imaging* **2010**, *38*, 540.
24. Fraser, R.; Fu, J.; Kamboj, R.; Kodumuru, V.; Sadalapure. K. WO 2008147864 A2 20081204.
25. Glennon, R. A. *Mini-Rev. Med. Chem.* **2005**, *5*, 927.
26. Chen, R.-Q.; Li, Y.; Zhang, Q.-Y.; Jia, H.-M.; Deuther-Conrad, W.; Schepmann, D.; Steinbach, J.; Brust, P.; Wünsch, B.; Liu, B.-L. *J. Labelled Compd. Radiopharm.* **2010**, *53*, 569.
27. Fan, C.; Jia, H.; Deuther-Conrad, W.; Brust, P.; Steinbach, J.; Liu, B. *Sci. China, Ser. B Chem.* **2006**, *49*, 169.