Synthesis and *in Vitro* Evaluation of Iodinated Derivatives of Piperazine as a New Ligand for Sigma Receptor Imaging by Single Photon Emission Computed Tomography

Masahiko HIRATA, Tetsuya MORI, Seigo SOGA, Takuya UMEDA, and Yoshiro OHMOMO*

Osaka University of Pharmaceutical Sciences; 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan.
Received September 20, 2005; accepted December 15, 2005

A new series of radioiodinated analogues of 1-[2-(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazine (SA4503) was synthesized and evaluated as a potential brain sigma-1 receptor imaging ligands by single photon emission computed tomography (SPECT). Iodinated analogues of SA4503 (4a—c) were prepared from piperazine in a high yield. The *in vitro* competition binding studies using [^{3}H] DTG (sigma-1, 2), [^{3}H] (+)-pentazocine (sigma-1), and [^{3}H] DTG in the presence of carbetapentane (sigma-2) as sigma receptor selective radioligands were revealed that iodinated analogues 4a—c possess high affinities to sigma receptors (IC_{50}: 4a=7.1, 4b=31.0, and 4c=77.3 nM). In particular, the affinity of 4a, bearing iodine at ortho position on the phenyl ring, was 4.4 times greater than SA4503, and 3 times greater than that of haloperidol. The *meta*-iodo analogue 4b was the same to SA4503, the lead compound. The radioiodinated derivatives, [^{125}I] 4a, 4b were synthesized no-carrier-added from the corresponding tributyltin precursors by the iododestannylation reaction with high yields. The binding of [^{125}I] 4a, 4b have been characterized in the rat brain membranes. These compounds were indicated single population binding to sigma receptor with high affinity (4a: K_d=1.86±0.34 nM, B_{max}=205±28.9 fmol/mg protein, 4b: K_d=3.30±0.51 nM, B_{max}=231.5±13.8 fmol/mg protein). *In vitro* blocking studies were confirmed that the high specificity of 4a, 4b. These results suggest that radioiodinated 4a and 4b are promising sigma receptors imaging ligand for pursuing further *in vivo* studies.

Key words sigma receptor; radiopharmaceutical; single photon emission computed tomography (SPECT); SA4503; piperazine; radioiodine

Sigma receptors were suggested as subtypes of the opiate receptors in 1976 by Martin *et al.*,[1] however, the later studies of the pharmacological binding and behavior studies had to be modified that they were non-opiate and non-phencyclidine (PCP) binding site.[2] From binding studies, sigma receptors have been classified into at least two subtypes, termed sigma-1 and sigma-2, for their pharmacological profiles and different molecular weights.[2,3] They are widely distributed in the central nervous system (CNS), endocrine, immune and other peripheral organs.[4—6] Furthermore, they have also been expressed in a variety of human and rodent tumor cell lines.[7,8] Recently, cDNAs of sigma-1 were cloned, and their amino acid sequences were deduced.[9—12] However, sigma-2 has not been cloned yet. It has been demonstrated that sigma receptors are involved in a variety of physiological functions and that sigma ligands have diversified pharmacological effects.[13—18] In addition, sigma receptors may be associated with such as Alzheimer's disease,[19] ischemia,[20] schizophrenia,[21] depression,[22] and certain psychiatric disorders.[23] A wide variety of structurally and pharmacologically diverse compounds such as haloperidol, NE-100, DTG, carbetapentane, (+)-3-PPP, (±)-SKF10047, have been reported to bind toward sigma receptors.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been successfully employed for non-invasive studies of the biochemical transformation and physiological processes in the living human brain, utilizing organic molecules labeled with a positron emitter or a single photon emitter. For imaging sigma receptors by SPECT,[24—26] and PET,[27—29] several radiolabeled ligands have been developed.

Recently, new high selective sigma-1 agonist, 1-[2(3,4-dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazine (SA4503), have been reported that a potential drug candidate with high binding affinity to sigma receptors.[30,31] The binding affinity of SA4503 to sigma receptors indicates over 100 times higher than that of other receptors. Therefore, SA4503 has a potential to become lead compound of useful radiopharmaceutical for sigma receptors studies in the brain. In fact, ^{11}C and ^{18}F labeled SA4503 have been reported and [^{11}C] SA4503 has been successfully used for PET studies of sigma receptors in cat, monkey, and human brain.[32—36] Despite attractive features associated with PET techniques, PET studies are still limited, since they usually require on-site cyclotrons. On the other hand, SPECT studies are more commonly used in nuclear medicine clinics. We have explored the feasibility of ^{123}I radioiodinated sigma ligands as an alternative to [^{11}C] SA4503 for functional sigma receptor studies in the brain with SPECT.

Structure–activity relationships studies of SA4503 to sigma receptor had been reported.[30,31] It is also known that allyl iodides are more stable than alkyl iodides.[37,38] Thus, iodinated analogue of SA4503 were designed on the basis of the structure activity relationships to sigma receptors and *in vivo* stability.

We reported here the synthesis of three iodinated analogues amenable to radiolabeling ^{125}I. *In vitro* studies on the inhibitory potency and selectivity toward sigma receptors were also performed.

Results and Discussion

Chemical Synthesis The synthesis of SA4503 as lead compound has carried out already by Fujimura *et al.*,[39] however, the yield of this procedure was low. Therefore, iodinated derivatives of SA4503 and SA4503 were synthesized by the reaction outlined in Chart 1, using a modified method

* To whom correspondence should be addressed. e-mail: Ohmomo@gly.oups,ac.jp

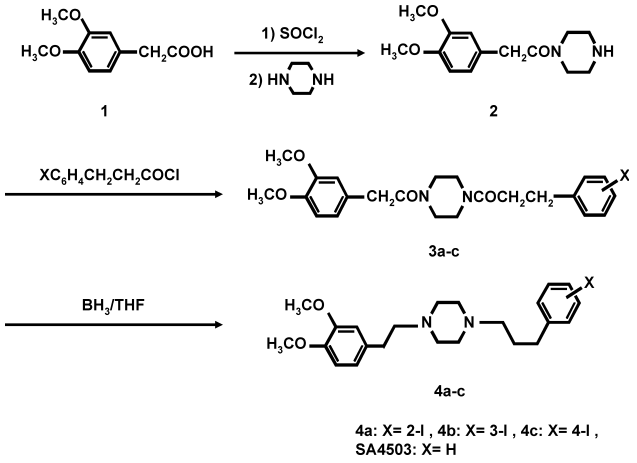

Chart 1

of Moore *et al.* which introduced substituent into piperazine directly.[40]

1-(3,4-Dimethoxyphenylacetyl)piperazine (**2**) was synthesized directly without the protection group by condensation of 3,4-dimethoxyphenylacetic chloride. The reaction was controlled acid conditions in the presence of some bromophenol blue as the indicator (pH 3.0—4.6). This method was superior in giving the mono-substitute piperazine analogue in a high yield (78%) and less side reactions. Compounds **3a**—**c** were prepared by condensation of the corresponding iodophenylpropionyl chloride in high yields (77—87%). The desired compounds **4a**—**c** were obtained by reduction of the corresponding derivatives **3a**—**c** using 1.0 M diborane-tetrahydrofuran solution under argon gas in yields of 70—93%. In a synthesis of SA4503, SA4503 was prepared in a high yield (total yield 51.1%) using our method compared with method of Fujimura *et al.* (lit.[39] 26.2%). This yield was about 2 times higher than that of Fujimura's method, and our method was successful in improvement of yield drastically.

***In Vitro* Competitive Binding Assay** The binding affinity of the synthesized compounds **4a**—**c** to sigma receptors in rat brain membranes were evaluated by competitive binding assay using [^{3}H] DTG (sigma-1, 2), [^{3}H] (+)-pentazocine (sigma-1), and [^{3}H] DTG with carbetapentane (sigma-2).[41,42] Several typical sigma ligands, haloperidol (sigma-1, 2 and dopamine), DTG (sigma-1, 2), carbetapentane (sigma-1), (+)-3-PPP (sigma-1), (±)-SKF10047 (sigma-1) and SA4503 (sigma-1), were also assessed under the same conditions. Figure 1 displayed competitive binding curves of these compounds, and 50% inhibition concentration (IC_{50}) values were summarized in Table 1. These results show that the new series of iodinated analogues **4a**—**c** were indicated to possess high affinity to brain sigma receptors (IC_{50}: **4a**=7.1 nM, **4b**=31.0 nM, **4c**=77.3 nM). In particular, the IC_{50} value of **4a** was 4.4 times greater than that of the lead compound, SA4503, and the IC_{50} value of **4b** was the same as the IC_{50} value of SA4503 (IC_{50}=31.0 nM). The IC_{50} value of **4a** was 3-times greater than that of haloperidol (IC_{50}=22.0 nM), known a potent sigma ligand,[43] and the IC_{50} value of **4b** was comparable with that of haloperidol. The compound **4c** was weakest affinity among three new analogues, but it was rank along with (+)-3-PPP which was one of the traditional sigma ligand (IC_{50}=76.3 nM).[44] Among them, compound **4a**, **4b** indicated high binding affinity to sigma receptors. In particular, **4a** indicated good characteristic for binding to sigma receptors. In general, the compound against the receptor as a target was attenuated by the introduction of a heavy heteroatom as

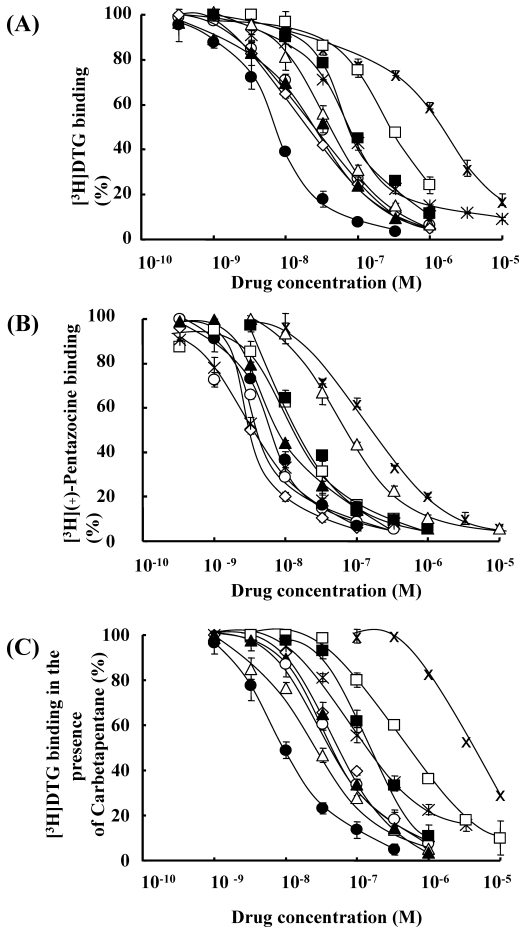

Fig. 1. Inhibition of [^{3}H] DTG Binding to Sigma-1, 2 Receptors (A), [^{3}H] (+)-Pentazocine Binding to Sgma-1 Receptors (B), and [^{3}H] DTG Binding to Sigma-2 Receptors in the Presence of Carbetapentane to Mask Sigma-1 Site (C) in Rat Brain Membranes by, **4a** (●), **4b** (▲), **4c** (■), SA4503 (○), Haloperidol (◇), DTG (△), (+)-3-PPP (*), Carbetapentane (□), and (±)-SKF10047 (×)

Each point is the mean value of triplicate determinations.

Table 1. Inhibitory Effects of Sigma Ligands against Sigma Receptors

Compound	IC_{50} (nM)		
	[^{3}H] DTG (Sigma non-selective)	[^{3}H] (+)-pentazocine (Sigma-1)	[^{3}H] DTG+carbetapentane (Sigma-2)
4a	7.1±1.2	6.4±0.7	10.2±1.9
4b	31.0±6.2	8.9±2.8	54.5±6.6
4c	77.3±11.0	19.2±2.8	160.0±7.8
SA4503	31.0±1.4	4.0±1.9	55.7±4.7
Haloperidol	22.0±4.1	2.4±0.5	65.3±4.1
DTG	45.5±6.4	75.0±7.1	27.7±4.0
(+)-3-PPP	76.3±16.9	29.5±6.4	145.0±7.1
Carbetapentane	307.5±17.7	16.5±0.7	530.0±11.0
(±)-SKF10047	1650±70	151.7±14.4	3700±141

Data are mean value±S.D. of triplicate determinations. DTG: 1,3-di-*o*-tolylguanidine, (+)-3-PPP: (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine, SKF10047: *N*-allylnormetazocine.

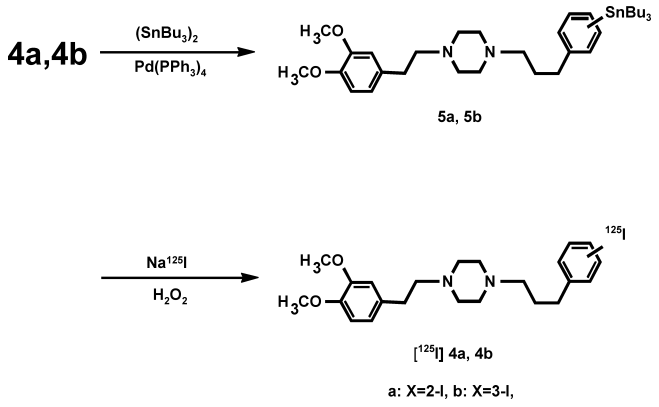

Chart 2

iodine. However, novel compounds **4a** and **4b**, which were introduced iodine to *ortho*- and *meta*-position, increased binding affinity to sigma receptors. These *in vitro* examinations were confirmed the validity of the compound design of new series of iodinated analogues for sigma receptors imaging. The potent compound **4a** and **4b**, among the iodinated derivatives tested *in vitro*, were chosen for further evaluations.

Radioiodination The electrophilic iododestannylation reaction offers several advantages for radioiodination, since it is performed in short times under very mild conditions and with very high regional selectivity, and also affords a high specific radioactivity. Thus, the radiolabeled ligands, [^{125}I] **4a**, and [^{125}I] **4b** were performed using an iododestannylation reaction with the corresponding tributyltin precursor by the reaction outlined in Chart 2.[45] The tri-*n*-butylstannyl precursor **5a**, **5b** was prepared from its corresponding **4a**, **4b** using bis(tri-*n*-butyltin) in the presence of a catalytic amount of tetrakis-(triphenylphosphine)palladium. The radioiodination of **5a**, **5b** was achieved using hydrogen peroxide as an oxidant and sodium [^{125}I] iodide (specific activity 74 GBq/μmol) in 0.1 M HCl/ethanol solution at room temperature, followed by HPLC purification. The radiochemical yields of product, [^{125}I] **4a**, and [^{125}I] **4b**, were 90—94% based on sodium [^{125}I] iodide activity. The radiochemical purity of [^{125}I] **4a**, and [^{125}I] **4b** were higher than 99% as assessed by HPLC analysis, with specific radioactivity of approximately 74 GBq/μmol, respectively. This method should be a applicable for labeling ^{123}I a suitable radioisotope (half-life 13 h and gamma ray energy of 159 keV) for *in vivo* imaging with SPECT. Importantly, the tin precursors **5a**, **5b** were stable over 6 months when stored at −20 °C and [^{125}I] **4a**, and [^{125}I] **4b** can be readily generated.

***In Vitro* Blocking Studies** *In vitro* pharmacological blocking study was performed to examine the degree of specific binding of [^{125}I] **4a** and [^{125}I] **4b** in rat brain membranes. Figure 2 shows that the total amount of [^{125}I] **4a** (top panel) or [^{125}I] **4b** binding (bottom panel) were indicated significant reduction by the pretreatment with sigma ligands, such as SA4503, haloperidol, DTG, (±)-pentazocine, (+)-3-PPP, carbetapentane, rimcazole, and (±)-SKF10047. In addition, the reduction rate of the radioactivity by treatment of these ligands, was correlated with the avidity of each sigma ligand. On the other hand, the non-sigma receptor ligands, such as (+)-SCH23390 (D_1), (−)-sulpiride (D_2), spiperone (D_2, 5-HT_2), naloxone (opiate), atropine (muscarinic), (−)-nicotine (nicotinic), (−)-propranolol (β, 5-HT_1) and phentolamine (α), failed to block the [^{125}I] **4a** or **4b** binding. Haloperidol (sigma-1, -2, dopamine) was observed a significant reduction of radioactivity also binds dopamine receptor. But each of [^{125}I] **4a** and **4b** binding were not reduced by pretreated (+)-SCH23390, D_1 ligand, or (−)-sulpiride, D_2 ligand. These data were indicated the two radioiodinated ligands bound to only sigma receptors.

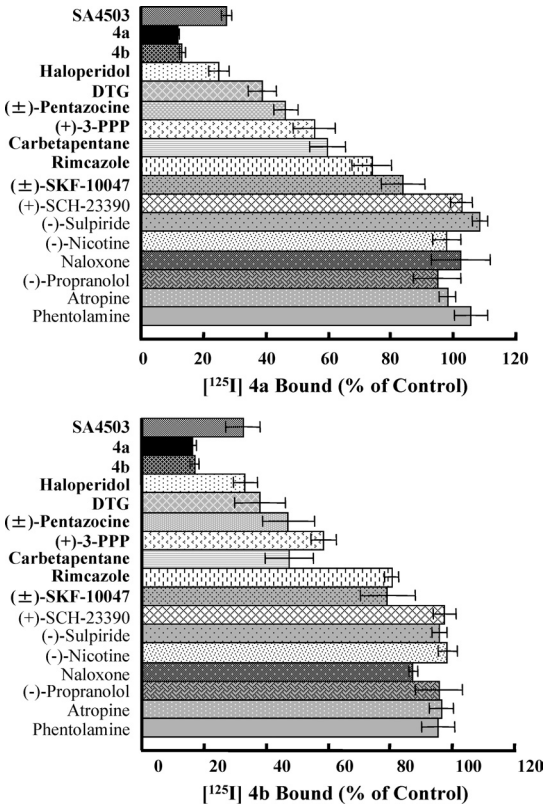

Fig. 2. Blocking Effect of Various Drugs on [^{125}I] **4a** Binding (Top Panel) and [^{125}I] **4b** Binding (Bottom Panel) in Rat Brain Membranes

Saturation Binding of [^{125}I] 4a and [^{125}I] 4b to Rat Brain Membranes For comparison with pharmacological characterization, the crude membrane fraction from rat brain was used for further binding experiments. The saturation binding of [^{125}I] **4a** and [^{125}I] **4b** was studied with the use of Scatchard analysis (Figs. 3, 4).

The resulting linear Scatchard plot was consistent with the theory that [^{125}I] **4a** and [^{125}I] **4b** bind to single population of binding sites with high affinity and kinetic parameter, **4a**: $K_d = 1.86 \pm 0.34$ nM and $B_{max} = 205 \pm 28.9$ fmol/mg protein, **4b**: $K_d = 3.30 \pm 0.51$ nM and $B_{max} = 231.5 \pm 13.8$ fmol/mg protein, respectively. These data confirm the possibility that similar parameter with [^{3}H] (+) pentazocine and [^{3}H] DTG as well known sigma ligand, has indicated single class of binding site in rat brain membranes, ([^{3}H] (+) pentazocine: K_d= 6.90 nM, B_{max}=280 fmol/mg protein, [^{3}H] DTG: K_d=15.8 nM, B_{max}=291 fmol/mg protein).[43,44] These results suggested that [^{125}I] **4a** and [^{125}I] **4b** bound to same binding site of [^{3}H] (+) pentazocine and [^{3}H] DTG, in rat brain membranes with high affinity.

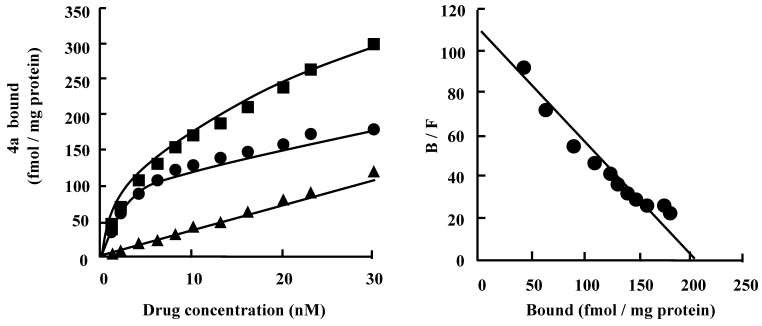

Fig. 3. Binding of [^{125}I] **4a** in Rat Brain Membranes

Left: binding curves of [^{125}I] **4a**; total binding (■), specific binding (●), non-specific binding (▲). Right: scatchard plot of the binding. Data represent mean±S.D. of triplicate determinations.

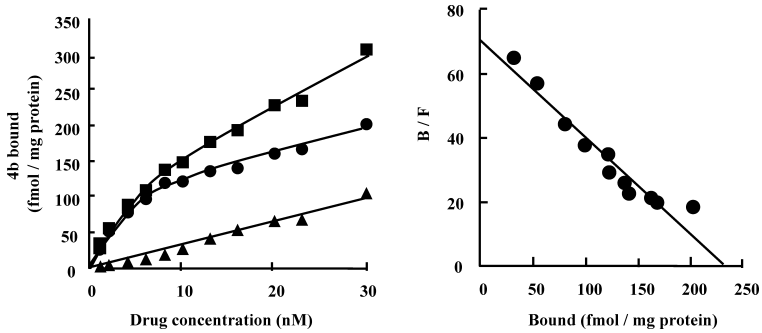

Fig. 4. Binding of [^{125}I] **4b** in Rat Brain Membranes

Left: binding curves of [^{125}I] **4b**; total binding (■), specific binding (●), non-specific binding (▲). Right: scatchard plot of the binding. Data represent mean±S.D. of triplicate determinations.

Conclusion

This work demonstrated that a new series of iodinated compound have been synthesized and characterized *in vitro* receptor binding assay. Among the prepared compounds, **4a** and **4b** were found to possess high inhibitory potency against sigma receptors. Each of [^{125}I] **4a** and [^{125}I] **4b** were synthesized by iododestannylation reaction with no-carrier-conditions which can apply to label with ^{123}I using for SPECT. The binding and the blocking study data suggest that [^{125}I] **4a** and [^{125}I] **4b** were considered to be a candidate for further studies as a SPECT radiopharmaceutical for imaging sigma receptors. Further studies of these new agents are now in progress.

Experimental

All melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer and the chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard. Infrared (IR) spectra were recorded on a JASCO IR-700 spectrometer. High resolution mass spectra (HR-MS) were obtained on a Hitachi M-80 instrument. The radio HPLC system used included a Waters M-600 pump, a Lambda-Max 481 UV detector (254 nm), a Beckman 170 NaI radioactivity detector, and a Cosmosil 5C18-AR column (10×250 mm, Nacalai Tesque). The ^{3}H radioactivity was determined by a Packard TRI-CARB1600CA liquid scintillation spectrometer. The ^{125}I radioactivity was measured using an Aloka ARC-300 NaI (Tl) gamma scintillation counter.

[^{125}I] NaI, [^{3}H] DTG, and [^{3}H] pentazocine were purchased from Amersham Japan. All other chemicals used were of reagent grade and were purchased commercially. Wistar rats were obtained from Japan SLC Co., Ltd. These animals were kept at least one week before the experiments. Animals chow and drinking water were allowed *ad libitum*. Animals were housed and experiments were performed according to guidelines stipulated by the Osaka University of Pharmaceutical Sciences Animal Care and Use Committee.

Chemical Synthesis. 1-(3,4-Dimethoxyphenylacetyl)piperazine (2) To the solution of 3,4-dimethoxyphenylacetic acid (2.00 g, 10.2 mmol) in 20 ml tetrahydrofuran was added thionyl chloride (20 ml) and heated at reflux for 2 h. The volatiles were removed *in vacuo* to give yellow oil as acid chloride. Then, 4 M HCl was added to a solution of piperazine·6H_2O (7.92 g, 40.8 mmol) in water (10 ml) containing some bromophenol blue until the neutral tint of the indicator appeared. The acid chlorides were dissolved in dry $CHCl_3$ (2 ml), and 1 M NaOH were added in the piperazine solution at intervals with vigorous stirring at 0 °C. The reaction mixture was stirred at room temperature for 30 min, followed by, 0.5 N NaOH was added until pH 9. The mixture was extracted with $CHCl_3$ (30 ml×3). The combined organic extracts were washed with water (30 ml), dried over sodium sulfate and the solvent was removed *in vacuo* to give an oil. This oil was purified by silica gel column chromatography (MeOH/$CHCl_3$=5/1) to provide the pure product.

Yield 77.9%, IR ($CHCl_3$): 2938, 1631, 1516, 1463, 1262 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 1.94 (1H, br, NH), 2.67 (2H, t, *J*=5.1 Hz, piperazine), 2.81 (2H, t, *J*=5.1 Hz, piperazine), 3.43 (2H, t, *J*=5.1 Hz, piperazine), 3.62 (2H, t, *J*=5.1 Hz, piperazine), 3.68 (2H, s, phCH_2CO), 3.87 (6H, s, OCH_3×2), 6.74—6.83 (3H, m, aromatics). CI-HR-MS Calcd for $C_{14}H_{20}N_2O_3$ (MH^+) *m/z*: 264.1474, Found: 264.1470.

Substituted 1-(3,4-Dimethoxyphenylacetyl)-4-(3-phenylpropionyl)-piperazine (3a—c) 3-(Iodophenyl)propionyl acid (0.79 g, 3.0 mmol) was added to thionyl chloride (5 ml) and the solution was refluxed for 2 h. The volatiles were removed *in vacuo* to give a light yellow oil. This acid chloride was dissolved in $CHCl_3$ (2 ml) and added dropwise to another flask containing compound **2** (0.50 g, 3.0 mmol) and triethylamine (0.30 g, 3.0 mmol) and $CHCl_3$ (20 ml). The reaction mixture was stirred for 15 h at room temperature and then washed with water (25 ml). The organic layer was dried with K_2CO_3 and the solvent was evaporated *in vacuo* to afford the crude product as yellow oil. This oil was purified by silica gel column chromatography (MeOH/$CHCl_3$=5/1) to provide the pure product.

1-(3,4-Dimethoxyphenylacetyl)-4-[3-(2-iodophenyl)propionyl]piperazine (**3a**): Yield 87.6%, IR ($CHCl_3$): 2962, 1639, 1515, 1459, 1261 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.59 (2H, t, *J*=7.5 Hz, ph$\underline{CH_2}CH_2$), 2.90 (2H, t, *J*=7.5 Hz, ph$CH_2\underline{CH_2}$), 3.29—3.58 (8H, m, piperazine), 3.68 (2H, s, ph$\underline{CH_2}$CO), 3.86 (6H, s, O$\underline{CH_3}$×2), 6.76—6.83 (3H, m, aromatics), 6.99—7.55 (4H, m, aromatics). CI-HR-MS Calcd for $C_{23}H_{27}IN_2O_4$ (MH^+) *m/z*: 522.1017. Found:

522.1010.

1-(3,4-Dimethoxyphenylacetyl)-4-[3-(3-iodophenyl)propionyl]piperazine (**3b**): Yield 90.5%, IR ($CHCl_3$): 2962, 1639, 1515, 1459, 1261 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.59 (2H, t, J=7.5 Hz, ph$\underline{CH_2}CH_2$), 2.90 (2H, t, J=7.5 Hz, ph$CH_2\underline{CH_2}$), 3.29—3.58 (8H, m, piperazine), 3.68 (2H, s, ph$\underline{CH_2}$CO), 3.86 (6H, s, O$\underline{CH_3}\times2$), 6.76—6.83 (3H, m, aromatics), 6.99—7.55 (4H, m, aromatics). CI-HR-MS Calcd for $C_{23}H_{27}IN_2O_4$ (MH^+) m/z: 522.1017. Found: 522.1014.

1-(3,4-Dimethoxyphenylacetyl)-4-[3-(4-iodophenyl)propionyl]piperazine (**3c**): Yield 86.7%, IR ($CHCl_3$): 2930, 1640, 1515, 1431, 1261 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 2.59 (2H, t, J=7.8 Hz, ph$\underline{CH_2}CH_2$), 2.90 (2H, t, J=7.8 Hz, ph$CH_2\underline{CH_2}$), 3.33—3.58 (8H, m, piperazine), 3.69 (2H, s, ph$\underline{CH_2}$CO), 3.87 (6H, s, O$\underline{CH_3}\times2$), 6.76—6.83 (3H, m, aromatics), 6.96 (2H, d, J=8.4 Hz, aromatics), 7.60 (2H, d, J=8.4 Hz, aromatics). CI-HR-MS Calcd for $C_{23}H_{27}IN_2O_4$ (MH^+) m/z: 522.1017. Found: 522.1007.

Substitute 1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-(3-phenylpropyl)piperazine (4a—c) The compounds **3a**—**c** (0.91 g, 2.28 mmol) were dissolved in anhydrous tetrahydrofuran (5 ml), followed by, 1.0 M diborane-tetrahydrofuran solution were dropwised (17.0 ml) at room temperature under argon gas. The reaction mixture was heated at reflux for 18 h. The resulting solution was added 1 M HCl (25 ml) at 0 °C, and stirred for 15 min at room temperature. After refluxed for 1 h, the solution was cooled, and evaporated *in vacuo* to reduce tetrahydrofran. The residue was added 4 M NaOH until pH 11 and the mixture was extracted with $CHCl_3$ (20 ml×3). The organic layer was dried over K_2CO_3 and the solvent was evaporated *in vacuo* to afford the crude product as yellow oil. This oil was purified by silica gel column chromatography ($MeOH/CHCl_3$=1/20), the oil product was converted to its hydrobromide salt. Recrystallized from methanol afforded **4a**—**c**.

1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-[3-(2-iodophenyl)propyl]piperazine (**4a**): Yield 80.8%, mp 246—248 °C, IR (KBr): 2910, 2439, 1521, 1462, 1267 cm^{-1}. ^{1}H-NMR (Free Base, $CDCl_3$): 1.80 (2H, q, J=7.8 Hz, ph$CH_2\underline{CH_2}CH_2$), 2.37 (4H, t, J=7.8 Hz, $\underline{CH_2}$N), 2.56—2.62 (10H, m, ph$\underline{CH_2}CH_2CH_2$ and piperazine), 2.77 (2H, t, J=7.8 Hz, ph$\underline{CH_2}CH_2$N), 3.87 (6H, s, O$\underline{CH_3}\times2$), 6.73—6.81 (3H, m, aromatics), 6.98—7.56 (4H, m, aromatics). CI-HR-MS (Free Base) Calcd for $C_{23}H_{31}IN_2O_2$ (MH^+) m/z: 494.1432. Found: 494.1423. *Anal.* Calcd for $C_{23}H_{31}IN_2O_2\cdot2HBr$: C, 42.10; H, 5.07; N, 4.27. Found: C, 41.93; H, 5.26; N, 4.24.

1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-[3-(3-iodophenyl)propyl]piperazine (**4b**): Yield 92.6%, mp 247—249 °C, IR (KBr): 2910, 2439, 1521, 1462, 1267 cm^{-1}. ^{1}H-NMR (Free Base, $CDCl_3$): 1.80 (2H, q, J=7.8 Hz, ph$CH_2\underline{CH_2}CH_2$), 2.37 (4H, t, J=7.8 Hz, $\underline{CH_2}$N), 2.56—2.62 (10H, m, ph$\underline{CH_2}CH_2CH_2$ and piperazine), 2.77 (2H, t, J=7.8 Hz, ph$\underline{CH_2}CH_2$N), 3.87 (6H, s, O$\underline{CH_3}\times2$), 6.73—6.81 (3H, m, aromatics), 6.98—7.56 (4H, m, aromatics). CI-HR-MS (Free Base) Calcd for $C_{23}H_{31}IN_2O_2$ (MH^+) m/z: 494.1432. Found: 494.1414. *Anal.* Calcd for $C_{23}H_{31}IN_2O_2\cdot2HBr$: C, 42.10; H, 5.07; N, 4.27. Found: C, 42.16; H, 5.04; N, 4.09.

1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-[3-(4-iodophenyl)propyl]piperazine (**4c**): Yield 91.4%, mp 247—249 °C. IR (KBr): 2938, 2434, 1517, 1451, 1264 cm^{-1}. ^{1}H-NMR (Free Base, $CDCl_3$): 1.75 (2H, q, J=7.8 Hz, ph$CH_2\underline{CH_2}CH_2$), 2.33 (4H, t, J=7.8 Hz, $\underline{CH_2}$N), 2.49—2.58 (10H, m, ph$\underline{CH_2}CH_2CH_2$ and piperazine), 2.71 (2H, t, J=7.8 Hz, ph$\underline{CH_2}CH_2$N), 3.80 (6H, s, O$\underline{CH_3}\times2$), 6.67—6.74 (3H, m, aromatics), 6.87 (2H, d, J=8.4 Hz, aromatics), 7.52 (2H, d, J=8.4 Hz, aromatics). CI-HR-MS (free base) Calcd for $C_{23}H_{31}IN_2O_2$ (MH^+) m/z: 494.1432. Found: 494.1424. *Anal.* Calcd for $C_{23}H_{31}IN_2O_2\cdot2HBr$: C, 42.10; H, 5.07; N, 4.27. Found: C, 42.00; H, 5.00; N, 4.05.

Preparation of Rat Brain Membranes Receptor binding assays were performed using the crude synaptosomal (P_2) membrane fraction of rat brain, according to a modified the method of Leitner *et al.*[44] Male Wistar rats (250—300 g) were decapitated and the brain, minus cerebellum, were homogenized in 10 volumes of ice-cold 10 mM Tris–HCl/0.32 M sucrose buffer (pH 7.4), using a Polytorone homogenizer at 24000 rpm for 20 s. The homogenates were centrifuged at 1000 ***g*** for 10 min at 4 °C to remove cell debris. The supernatants were centrifuged at 45000 ***g*** for 15 min at 4 °C. The final pellets were taken and adjusted to a protein concentration of 2 mg/ml in ice-cold 10 mM Tris–HCl buffer (pH 7.4). Protein concentration was determined according to the Lowly method.[46]

***In Vitro* Competitive Binding Assays** Sigma receptor binding assay was carried out under the follow conditions. Crude P_2 membrane fractions of rat brain (2.0 mg of protein) were incubated with 0.1 nmol [^{3}H] DTG (0.46 kBq) and the test compound (3.3×10^{-9}—1.0×10^{-5} M) in 50 mM Tris–HCl (pH 8.0) at 25 °C for 90 min. The total incubation volume was 1.0 ml. Nonspecific binding were determined in the presence of haloperidol (10 μM). Assays were terminated by rapid vacuum filtration through Whatman GF/B glass fiber filters presoaked in 0.5% polyethylenimine for at least 30 min at room temperature prior to use, followed by a rapid washing 3 times with 4 ml of ice-cold 10 mM Tris–HCl buffer (pH 8.0). The radioactivity trapped on the filters was counted by scintillation spectrometer after an overnight extraction of counts in 10 ml scintillation fluid. These IC_{50} values were determined from displacement curves of the percent inhibition of [^{3}H] DTG binding.

Sigma-1 and sigma-2 receptor binding assays were examined the same manner described above except using [^{3}H]-(+)-pentazocine (0.1 nmol, 0.46 kBq) and [^{3}H] DTG (0.1 nmol, 0.46 kBq) in the presence of carbetapentane (0.1 μM) to mask sigma-1 binding site, respectively.

Synthesis of Precursors. Substitute 1-[2-(3,4-Dimethoxyphenyl)-ethyl]-4-[3-(tributylstannylphenyl)propyl]piperazine (5a—c) The mixture of compound **4a**—**c** (0.20 g, 0.40 mmol) and bistributyltin (0.64 g, 1.12 mmol) and tetrakis(triphenylphosphine)palladium (30 mg, 26 μmol) in anhydrous toluene (7 ml) was refluxed under argon for 17 h. After cooling, the reaction mixture was filtered through celite. The filtrate was concentrated *in vacuo*. The oily residue was purified by silica gel column chromatography ($MeOH/CHCl_3$=1/20) affording **4a**—**c**.

1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-[3-(2-tributylstannylphenyl)propyl]-piperazine (**5a**): Yield 47.5%, IR ($CHCl_3$): 3018, 2398, 1518, 1422, 1225 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 0.86—1.57 (27H, m, Bu_3), 1.83 (2H, q, J=7.5 Hz, ph$CH_2\underline{CH_2}CH_2$), 2.41 (4H, t, J=7.5 Hz, $\underline{CH_2}$N), 2.56—2.64 (10H, m, ph$\underline{CH_2}CH_2CH_2$ and piperazine), 2.76 (2H, t, J=7.5 Hz, ph$\underline{CH_2}CH_2$N), 3.87 (6H, s, O$\underline{CH_3}\times2$), 6.73—6.81 (3H, m, aromatics), 7.10—7.35 (4H, m, aromatics). CI-HR-MS Calcd for $C_{35}H_{58}N_2O_2Sn$ (MH^+) m/z: 658.3520. Found: 658.3514.

1-[2-(3,4-Dimethoxyphenyl)ethyl]-4-[3-(3-tributylstannylphenyl)propyl]-piperazine (**5b**): Yield 45.0%, IR ($CHCl_3$): 3018, 2398, 1518, 1422, 1225 cm^{-1}. ^{1}H-NMR ($CDCl_3$): 0.86—1.57 (27H, m, Bu_3), 1.83 (2H, q, J=7.5 Hz, ph$CH_2\underline{CH_2}CH_2$), 2.41 (4H, t, J=7.5 Hz, $\underline{CH_2}$N), 2.56—2.64 (10H, m, ph$\underline{CH_2}CH_2CH_2$ and piperazine), 2.76 (2H, t, J=7.5 Hz, ph$\underline{CH_2}CH_2$N), 3.87 (6H, s, O$\underline{CH_3}\times2$), 6.73—6.81 (3H, m, aromatics), 7.10—7.35 (4H, m, aromatics). CI-HR-MS Calcd for $C_{35}H_{58}N_2O_2Sn$ (MH^+) m/z: 658.3520. Found: 658.3517.

Radiolabeling Aqueous hydrogen peroxide (10 μl, 30%, w/v) was added to a mixture of [^{125}I] NaI (10 μl, 37.0 MBq, 74 TBq/mmol), 0.1 N HCl (25 μl) and tributylstannyl precursor **5a**, **b** (0.01 mg in 10 μl ethanol) in a sealed vial. After stirring for 10 min at room temperature, the reaction mixture was quenched with aqueous sodium bisulfite (0.1 mg in 10 μl). The mixture was isolated by HPLC using NaH_2PO_4 solution (10 mM)/MeOH (15/85) as an eluent at a flow rate of 3.0 ml/min. The fraction corresponding of **4a**, **b** was collected and the solvent was removed *in vacuo*. The retention times were 13.8 min for [^{125}I] **4a** and 14.5 min for [^{125}I] **4b**. The radiochemical yields based on [^{125}I] NaI were 90.0 and 94.0%, respectively. The radiochemical purity and the specific activities of all three tracers were greater than 99% and approximately 74 TBq/mmol.

***In Vitro* Binding Studies** Crude P_2 membrane fractions of rat brain (2.0 mg of protein) in Tris–HCl (50 mM, pH 8.0) were incubated for 90 min at 25 °C with [^{125}I] **4a** or [^{125}I] **4b** (1—300 nM, 0.74 kBq, respectively). The total incubation volume was 1.0 ml. Nonspecific binding was determined in the presence of haloperidol (10 μM). Specific binding was defined as the difference between the total and the nonspecific binding. Assays were terminated by rapid vacuum filtration through Whatman GF/B glass fiber filters presoaked in 0.5% polyethylenimine for at least 30 min at room temperature prior to use, and assayed filters were washed 6 times with 4 ml ice-cold 10 mM Tris–HCl buffer (pH 7.4). The filter-bound radioactivity was counted in an auto-γ-counter. The saturation binding data were analyzed by Scatchard plot analysis for determination of the equilibrium dissociation constant (K_d), and the maximal number of binding site (B_{max}).

***In Vitro* Blocking Studies** Crude P_2 membrane fractions of rat brain (2.0 mg of protein) were incubated in Tris–HCl (50 mM, pH 8.0) for 90 min at 25 °C with [^{125}I] **4a** or [^{125}I] **4b** (0.74 kBq) and various receptor ligand (1 μM, 100 μl), **4a**—**c**, SA4503, haloperidol, rimcazole, carbetapentane, DTG, (+)-3-PPP, (±)-SKF10047, (+)-SCH23390, (−)-sulpiride, (−)-nicotine, phentolamine, atropine, and naloxone. The total incubation volume was 1.0 ml. Assays were terminated by rapid vacuum filtration through Whatman GF/B glass fiber filters, presoaked in 0.5% polyethylenimine for at least 30 min at room temperature prior to use. These filters were washed 6 times with 4 ml ice-cold Tris–HCl buffer (10 mM, pH 7.4). The filter-bound radioactivity was counted in an auto-γ-counter.

Acknowledgements This work was supported by Grant-in-Aid for Scientific Research (C) No. 13670972, 17591298 and High Technology Re-

search from Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

1) Martin W. R., Eades C. E., Thompson J. A., Huppler R. E., Gilbert P. E., *J. Pharmacol. Exp. Ther.*, **197**, 517—532 (1976).
2) Walker J. M., Bowen W. D., Walker F. O., Matsumoto R. R., De Costa B. R., Rice K. C., *Pharmacol. Rev.*, **42**, 355—402 (1990).
3) Quirion R., Bowen W. D., Itzhak Y., Junien J. L., Rothman R. B., Su T. P., Tam S. W., Taylor D. P., *Trends Neurosci.*, **10**, 444—446 (1992).
4) Hellewell S. B., Bruce A., Feinstein G., Orringer J., Williams W., Bowen W. D., *Eur. J. Pharmcol.*, **268**, 9—18 (1994).
5) Wolfe S. A., Jr., Clup S. G., De Souza E. B., *Endocrinology*, **124**, 1160—1172 (1988).
6) Su T. P., London E. D., Jaffe J. H., *Science*, **240**, 219—221 (1988).
7) Brent P. J., Pang G. T., *Eur. J. Pharmcol.*, **278**, 151—160 (1995).
8) Vilner B. J., John C. S., Bowen W. D., *Cancer Res.*, **55**, 408—413 (1995).
9) Honner M., Moebius F. F., Flandorfer A., Knaus H.-G., Striessnig J., Kempner E., Glossmann H., *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 8072—8077 (1996).
10) Pan Y.-X., Mei J., Wan B.-L., Zukermann A., Pasternak G. W., *J. Neurochem.*, **70**, 2279—2285 (1998).
11) Prasad P. D., Li H. W., Fey Y.-J., Ganpathy M. E., Fujita T., Plumley L. H., Yang-Feng T. L., Leibach F. H., Ganapathy V., *J. Neurochem.*, **70**, 443—451 (1998).
12) Seth P., Fei Y.-J., Li H. W., Huang W., Leibach F. H., Ganapathy V., *J. Neurochem.*, **78**, 922—931 (1998).
13) Su T. P., *Eur. J. Biochem.*, **200**, 6336—6342 (1991).
14) Junien J. L., Gue M., Bueno L., *Neurophamacol.*, **30**, 1119—1124 (1991).
15) Kamei J., Iwamoto Y., Kawashima N., Hitosugi H., Misawa M., Kasuya Y., *Eur. J. Pharmacol.*, **224**, 39—43 (1992).
16) Riviere P. J. M., Rao R. K., Pascaud X., Junien J. L., Porreca F., *J. Pharmacol. Exp. Ther.*, **264**, 1268—1274 (1993).
17) Maurice T., Su T. P., Privat A., *Neuroscience*, **83**, 739—748 (1998).
18) Nakazawa M., Matsuno K., Mita S., *Neurochem. Int.*, **32**, 337—343 (1998).
19) Matsuno K., Kobayashi T., Tanaka M. K., Mita S., *Eur. J. Pharmacol.*, **312**, 267—271 (1996).
20) Maurice T., Lockhart A. A., *Prog. Neuro-Psycopharmacol. Biol. Psychiat.*, **21**, 69—102 (1997).
21) Nabeshima T., Okuyama S., *Jpn. J. Psychopharmacol.*, **14**, 51—76 (1994).
22) Amano M., Yamada K., Matsuno K., Nabeshima T., *Jpn. J. Psychopharmacol.*, **16**, 73—84 (1996).
23) Hayashi T., Su T. P., *CNS Drugs*, **18**, 269—284 (2004).
24) John C. S., Gulden M. E., Vilner B. J., Bowen W. D., *Nucl. Med. Biol.*, **23**, 761—766 (1996).
25) Waterhouse R. N., Chapman J., Izard B., Donald A., Belbin K., O'Brien J. C., Collier T. L., *Nucl. Med. Biol.*, **24**, 587—593 (1997).
26) Waterhouse R. N., Mardon K., Brien J. C. O., *Nucl. Med. Biol.*, **24**, 45—51 (1997).
27) Waterhouse R. N., Collier T. L., *Nucl. Med. Biol.*, **24**, 127—134 (1997).
28) Dence C. S., John C. S., Bowen W. D., Welch M. J., *Nucl. Med. Biol.*, **24**, 333—340 (1997).
29) Ishiwata K., Noguchi J., Ishii S., Hatano K., Ito K., Nabeshima T., Senda M., *Nucl. Med. Biol.*, **25**, 195—202 (1998).
30) Kobayashi T., Matsuno K., Nakata K., Mita S., *J. Pharmacol. Exp. Ther.*, **279**, 106—113 (1996).
31) Matsuno K., Nakazawa M., Okamoto K., Kawashima Y., Mita S., *Eur. J. Pharmacol.*, **306**, 271—279 (1996).
32) Kawamura K., Ishiwata K., Tajima H., Ishii S., Matsuno K., Homma Y., Senda M., *Nucl. Med. Biol.*, **27**, 255—261 (2000).
33) Kawamura K., Ishiwata K., Shimada Y., Kimura Y., Kobayashi T., Matsuno K., *Ann. Nucl. Med.*, **14**, 285—292 (2000).
34) Kawamura K., Elsinga P. H., Kobayashi T., Ishii S., Wang W. F., Matsuno K., Vaalburg W., Ishiwata K., *Nucl. Med. Biol.*, **30**, 273—284 (2003).
35) Ishiwata K., Tsukada H., Kawamura K., Kimura Y., Nishiyama S., Kobayashi T., *Synapse*, **40**, 235—237 (2001).
36) Kawamura K., Kimura Y., Tsukada H., Kobayashi T., Nishiyama S., Kakiuchi T., Ohba H., Harada N., Matsuno K., Ishii K., Ishiwata K., *Neurobiol. Aging*, **24**, 745—752 (2003).
37) Magata Y., Saji H., Horiuchi K., Torizuka K., Yokoyama A., *Nucl. Med. Biol.*, **14**, 7—13 (1987).
38) Eckelman W. C., "Radiopharmaceuticals," ed. by Nunn A. D., Marcel Dekker, New York, 1992, pp. 167—219.
39) Fujimura K., Matsumoto J., Niwa M., Kobayashi T., Kawashima Y., *Bioorg. Med. Chem.*, **5**, 1675—1683 (1997).
40) Moore T. S., Boyle M., Thorn V. M., *J. Chem. Soc.*, Part 1, 39—51 (1929).
41) De Costa B. R., Radesaca L., Paolo L. D., Bowen W. D., *J. Med. Chem.*, **35**, 38—47 (1992).
42) Weber W., Sonders M., Quarum M., Mclean S., Pou S., Keana J. F. W., *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8784—8788 (1986).
43) Cagnotto A., Bastone A., Mennini T., *Eur. J. Pharmacol.*, **266**, 131—138 (1994).
44) Leitner M. L., Hohmann A. G., Patrick S. L., Walker J. M., *Eur. J. Pharmacol.*, **259**, 65—69 (1994).
45) Blaszczac L. C., Halligan N. G., Seitz D. E., *J. Labelled. Compd.*, **27**, 401—406 (1989).
46) Gornal A. G., Bradwill C. J., David M. M., *J. Biol. Chem.*, **177**, 751—766 (1948).