streptonigrin molecule. This led us to suggest that nitrogen atoms are involved in the coordination to Au(III). On the other hand, the absorption spectrum of Au–SN exhibits a band at 450 nm (ϵ = 7500) which can be assigned neither to a d–d nor a charge-transfer transition. This strongly suggests that metal complexation occurs at the level of the nitrogen atom or the B ring and either at the nitrogen atom of the C ring or the amino group of the C ring. Such coordination has already been suggested by Hajdu and Armstrong[19] for Cu–SN and Zn–SN complexes. In the present case we can assume that the coordination square is completed by Cl^- and/or OH^- ligands. Such a coordination of the metal ion to streptonigrin can promote π-electronic delocalization over the B and C rings. The band at 450 nm (ϵ = 7500) which appears in the absorption spectrum of Au–SN can thus tentatively be assigned to a $\pi \rightarrow \pi^*$ transition. Such a type of coordination has also been suggested for the complex formed through interaction of streptonigrin with Pd(II).[25]

Glutathione is important in protecting cells against a number of toxic species including H_2O_2 and $^{\bullet}OH$. It accomplishes this protection through a number of independent mechanisms. To accomplish its protective role, it must be maintained in its reduced state. This is accomplished by the enzyme glutathione reductase. In addition to this direct reactivity, glutathione is an important substrate for two enzymes, glutathione peroxidase and glutathione transferase. Glutathione concentration in tissues ranges from 1 to 20 mM, and because of this very high concentration, the direct reactivity of glutathione can be important in modulating the action of commonly used antitumor compounds.

On the other hand, it has been shown that GSH depletion of P-388 leukemia cells markedly enhanced their sensitivity to lysis by a flux of H_2O_2. Such cells could be lysed by stimulated granulocytes and activated macrophages which were ineffective against untreated cells.[9]

In this context Au–SN complex exhibits the following interesting properties: (i) it exhibits antitumor properties which compare with those of the free drug; (ii) it inhibits glutathione reductase, and this can yield glutathione depletion in tumor cells; and (iii) in the presence of GSH, Au(III) is very slowly reduced to Au(I) and removed from its binding site to streptonigrin, releasing free streptonigrin. This strongly suggests that, inside the cell, the complex should be able, in a first step, to modulate the glutathione cycle by inhbiting glutathione reductase and, in a second step, free streptonigrin, which would have been recovered through reduction of Au(III) to Au(I), should be able to exert its cytotoxic action.

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(±)-7-Chloro-8-hydroxy-1-(4′-[^{125}I]iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine: A Potential CNS D-1 Dopamine Receptor Imaging Agent

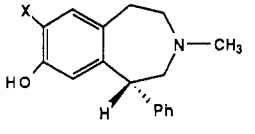
Sumalee Chumpradit, Hank F. Kung,* Jeffrey Billings, Mei-Ping Kung, and Sangren Pan

Division of Nuclear Medicine, Department of Radiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received September 15, 1988*

Synthesis, radiolabeling, and in vitro and in vivo properties of an iodinated benzazepine, (±)-7-chloro-8-hydroxy-1-(4′-[^{125}I]iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine, [^{125}I]FISCH, as a potential imaging agent for evaluation of central nervous system (CNS) D-1 dopamine receptors in humans, were investigated. After an iv injection, this benzazepine derivative showed good brain uptake in rats (2.27, 1.40, 0.55 % dose/whole brain at 2, 15, and 60 min, respectively). The striatum/cerebellum ratio was high (2.47 at 60 min after the injection). The binding affinity of this agent in rat striatum tissue preparation displayed a K_d of 1.43 ± 0.15 nM. Competition data (in vitro) showed the following rank order of potency: SCH-23390 > (±)-FISCH > (±)-IBZP ≫ apomorphine > WB 4010 > ketanserin ≈ spiperone. The preliminary data suggest that the agent is highly selective for the CNS D-1 receptor.

On the basis of the ability of agonists and antagonists to discriminate between two different distinct dopamine receptors, designated as D-1 and D-2, it is generally accepted that there are two subtypes of dopamine receptors.[1–5] These two subtypes of dopamine receptors exert a synergistic effect on the activity of central nervous system (CNS) dopaminergic neurons in rats.[6,7] More recently, many reports have suggested that D-1 and D-2 agonists invariably exhibit opposite biochemical effects: D-1 agonists stimulate adenyl cyclase activity, while D-2 agonists inhibit the enzyme activity. It is clear that these receptor subtypes influence each other, and yet they display separate and distinct functions on body physiology and biochemistry.[8]

Scheme I. Chemical Structures and in Vitro Binding Constants of Benzazepines



X	Compound	Kd(nM)
H	SKF-83692	197
Cl	SCH-23390	0.36
Br	SKF-83566	2.32
I	IBZP, SKF-103108A (or SCH-23982)	0.7

* Address correspondence to: Hank F. Kung, Ph.D., Division of Nuclear Medicine, Department of Radiology, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104.

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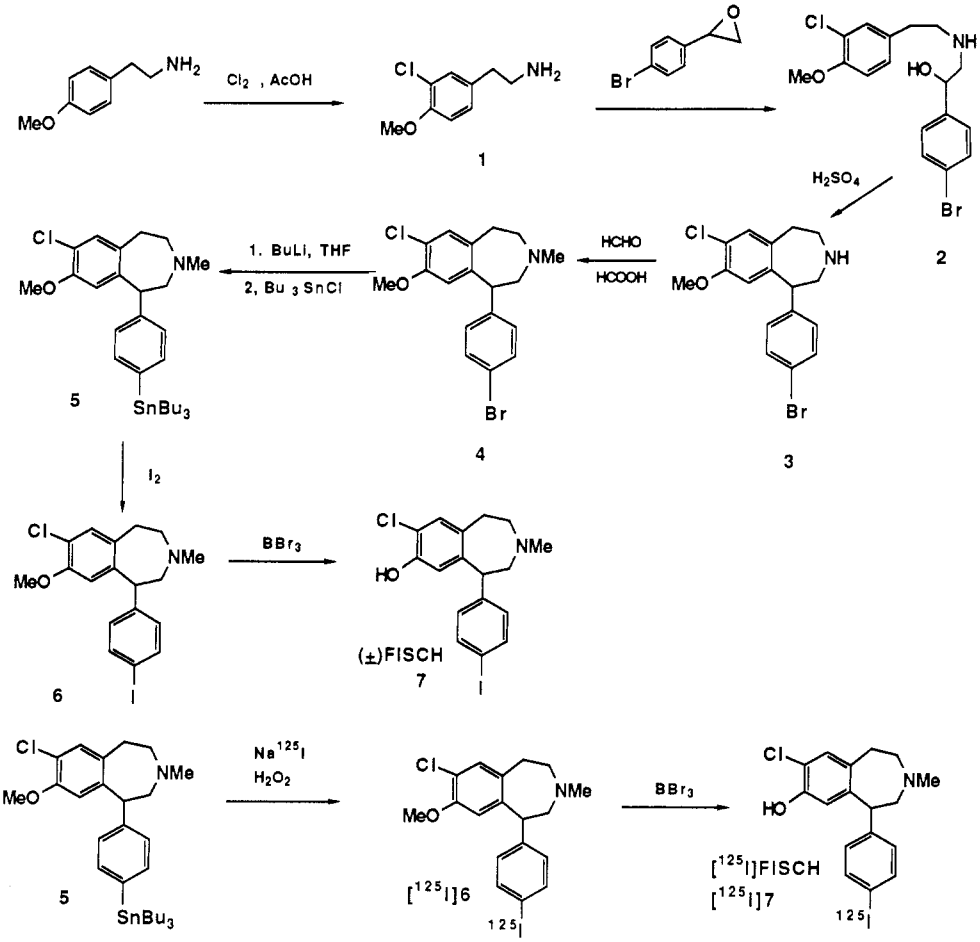
Scheme II. Synthesis of FISCH



SCH-23390 ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine) (Scheme I) is a highly selective central D-1 antagonist.[9-14] The corresponding Br (SKF-83566)[15] and I (SCH-23982 or SKF-103108A)[16-20] compounds have also been shown to have a high specificity for central D-1 dopamine receptors. The in vitro affinity constants for these agents in the rat striatum tissue preparation are 0.36, 2.32, and 0.7 nM for the Cl (SCH-23390), Br (SKF-83566), and I (SCH-23982 or SKF-103108A, which is equivalent to IBZP) derivatives, respectively[9,16-18] (Scheme I).

The Br compound (SKF-83566) labeled with ^{76}Br, a positron-emitting radionuclide, has been used for PET (positron-emission tomography) imaging in a rhesus monkey, which showed the highest concentration in the basal ganglia, with more selectivity in the posterior aspect of the caudate nucleus, the region with high D-1 receptor density.[15] Several recent reports have indicated that, in conjunction with PET, [^{11}C]SCH-23390 showed the highest concentration in the basal ganglia area of the human brain.[21,22]

In our previous paper, the potential of a radioiodinated benzazepine derivative, [^{125}I]IBZP, as a specific CNS D-1 dopamine receptor imaging agent for SPECT (single photon emission computed tomography), was reported.[20] This agent exhibited good localization in rat brains after an iv injection, with an uptake of 2.7, 1.2, 0.8, and 0.26 % dose/organ at 2, 15, 30, and 60 min postinjection, respectively.[20] The regional distribution of [^{125}I]IBZP in rat brain, as measured by ex vivo autoradiography, displayed a high uptake in the caudate putamen, accumbens nucleus, and substantia nigra, regions known to have a high concentration of D-1 dopamine receptors. The uptake ratio

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of striatum/cerebellum increased with time; at 30 s and 2 h after injection the ratio was 1.1 and 5.3, respectively. The specific uptake regions (as measured by ex vivo autoradiography), rich in D-1 dopamine receptors, can be blocked by pretreatment with SCH-23390, a selective D-1 dopamine receptor antagonist. However, IBZP suffers two major disadvantages as a potential imaging agent: (1) poor in vivo and in vitro stability and (2) retention time in the brain is too short for imaging. The first disadvantage is based on our observations of the in vivo biodistribution study of [^{125}I]IBZP in rats. The thyroid uptake is high at later time points, which strongly suggests the availability of free iodide in the blood circulation due to in vivo deiodination. Since the radioactive iodine is at an activated position—ortho to the hydroxyl group on the benzene ring—it is not too surprising that in vivo deiodination takes place.[23] The second drawback of [^{125}I]IBZP is the short retention time in the brain. In a normal SPECT study of the brain, it takes about 30–60 min for the data acquisition. It is necessary to use an agent exhibiting a prolonged retention time in the target region (in this case, basal ganglia) for SPECT imaging studies.

In an attempt to develop a better CNS D-1 dopamine receptor imaging agent, a ^{125}I-labeled 4′-iodo analogue of SCH-23390, (±)-7-chloro-8-hydroxy-1-(4′-[^{125}I]iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine ([^{125}I]FISCH), which contains an iodine atom on the other nonactivated benzene ring, was designed. In this paper the synthesis, radiolabeling, and in vivo and in vitro evaluation of [^{125}I]FISCH as a specific CNS D-1 dopamine receptor imaging agent are described.

Chemistry

The synthetic scheme reported by Wyrick et al.[24] for the synthesis of 4′-bromobenzazepine **3** was employed (Scheme II). The overall yield of the three-step synthesis was low (about 16%). The low yield is primarily due to the first step chlorination reaction, which only gives a 47% yield. The second step also displays low yield (40%) for two reasons: (1) the dialkylated amine **2** reacts with another mole of epoxide producing the trialkylated amine side product and (2) the possibility of some molecules of amine attaching at the benzylic carbon instead of at the β carbon. The 4′-bromobenzazepine **4** was prepared by N-methylation with formaldehyde and formic acid. After the silica gel column purification the yield was 80%. Lithiation of **4** with *n*-butyllithium at −78 °C to replace the 4′-bromo group, followed by the addition of tri-*n*-butyltin chloride, afforded the desired tri-*n*-butyltin derivative **5**. The final product, **7**, was prepared by treating **5** with iodine (I_2) and then deprotecting the hydroxy group with boron tribromide to give the final product, **7**, FISCH (yield 33%) (Scheme II). The same reaction sequence, from compound **5** to **7**, was successfully applied to the preparation of [^{125}I]FISCH, described below.

Radiolabeling. Radioactive labeling of compound **5** with I-125 at a carrier-free level was accomplished by an electrophilic radioiodination reaction using hydrogen peroxide as the oxidant. The radiolabeled compound **6** was separated from the starting material, compound **5**, by HPLC. The carrier-free [^{125}I]-**6** was O-demethylated by boron tribromide and the desired final product [^{125}I]FISCH, **7**, was separated again from the impurity by HPLC resulting in an overall radiolabeling yield of 56% (radiochemical purity of >99%). The [^{125}I]FISCH was compared with chemically pure, nonradioactive FISCH by HPLC with simultaneous UV and radioactivity detection, and was determined to be the desired product on the basis of its identical elution profile.

Table I. Biodistribution of [^{125}I]FISCH in Rats after an IV Injection[a]

	2 min	15 min[c]	60 min
blood	3.46 ± 1.69	2.28 ± 0.55	1.83 ± 0.27
heart	1.97 ± 0.52	0.38 ± 0.16	0.18 ± 0.09
muscle	35.68 ± 19.59	34.22 ± 12.95	19.07 ± 7.00
lung	8.55 ± 4.64	2.73 ± 1.07	1.47 ± 0.85
kidney	5.98 ± 1.58	2.09 ± 0.27	1.31 ± 0.07
spleen	2.79 ± 1.77	0.56 ± 0.15	0.67 ± 0.94
liver	17.10 ± 1.66	14.72 ± 1.47	12.32 ± 0.96
skin	7.43 ± 2.11	9.62 ± 1.82	9.25 ± 3.05
thyroid	0.11 ± 0.02	0.06 ± 0.02	0.04 ± 0.01
brain	2.27 ± 0.80	1.40 ± 0.13	0.55 ± 0.09
brain[b]/blood	7.35 ± 3.33	6.19 ± 3.10	3.08 ± 1.06

[a] Percent dose/organ, average of six rats ± SD. [b] Percent dose/gram ratio. [c] Average of five rats.

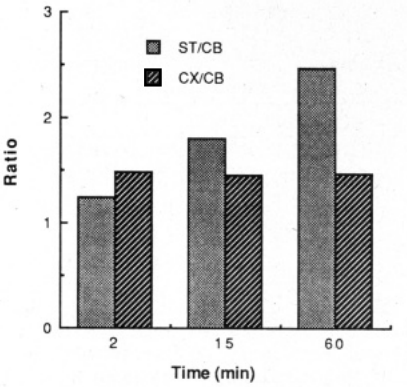

Figure 1. Ratios (based on the percent dose/gram) of regional cerebral uptake of [^{125}I]FISCH (CX = cortex, ST = striatum, CB = cerebellum). Only the ST/CB ratio shows the dramatic increase with time, suggesting that the agent is concentrated in the target tissue, in which the concentration of D-1 dopamine receptors is high.

In Vivo Biodistribution in Rats. After an iv injection, [^{125}I]FISCH showed good brain uptake in rats (Table I). The high initial uptake (2.27 % dose/organ) at 2 min after injection indicates that the compound passes through the blood–brain barrier with ease. The maximum brain uptake for rats, i.e. 100% first-pass extraction, is between 2.5 and 4.0% of the injected dose.[25] At later time points, brain uptake decreases; at 1 h after injection most of the [^{125}I]FISCH activity had washed out from the brain (0.55 % dose/organ). The brain retention at 1 h postinjection is much better than that of [^{125}I]IBZP (0.26 % dose/organ).[20]

High initial uptake in the lungs was also observed with rapid clearance at 15 and 60 min. Liver uptake remains high throughout the first hour. The relatively low thyroid uptake at 1 h postinjection (0.04%) suggests that little in vivo deiodination of [^{125}I]FISCH has occurred. As compared with [^{125}I]IBZP, which showed a thyroid uptake of 0.1% at 1 h postinjection, the new iodinated D-1 agent, [^{125}I]FISCH, containing an iodine atom at the 4′-position, displays better in vivo stability.

Utilizing a brain regional dissection technique, the striatum/cerebellum (ST/CB) ratio (target to nontarget

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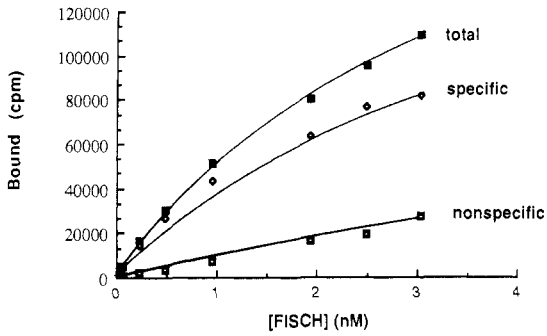

Figure 2. Saturation binding curve of [^{125}I]FISCH in rat striatum. The radiolabeled [^{125}I]FISCH binds to rat striatal homogenate with high affinity. The saturation curve indicates that this ligand demonstrates a low nonspecific binding (approximately 15% at K_d).

Table II. Inhibition Constants of Compounds on [^{125}I]FISCH Binding to Rat Striatal Membranes[a]

compound	K_i, nM
(±)-FISCH	1.71 ± 0.17
SCH-23390	0.39 ± 0.04
(±)-IBZP	13.40 ± 0.54
(−)-apomorphine	888 ± 115
WB 4010	1270 ± 203
ketanserin	>3000
spiperone	>3000

[a] 0.15–0.30 nM [^{125}I]FISCH was incubated in the presence of the indicated compounds in 7–11 concentrations and of membrane preparation from rat striatum. Each value represents the mean ± SEM of three to five determinations.

ratio) displayed a significant increase with time: 1.24, 1.80, and 2.47 at 2, 15, and 60 min, respectively (Figure 1). The data are consistent with the distribution pattern obtained with in vivo autoradiography (data not shown). It is important to note that this agent is a racemic mixture; therefore, the ratio would have been much higher if the optically resolved active form were used for this study. Currently, the resolved compound is under investigation.

In Vitro Binding. Similarly to that reported earlier for [^{125}I]IBZP,[16-19] [^{125}I]FISCH bound with high affinity to rat striatal homogenate in vitro. The saturation curve shown in Figure 2 indicates that this ligand has a fairly low nonspecific binding (approximately 15% at K_d). The specific binding of [^{125}I]FISCH (racemic mixture) was found to be saturable and displayed a K_d of 1.43 ± 0.15 nM. These values were comparable to those for [^{125}I]IBZP (*R*-(+), active form), measured under similar conditions.[16-19] Competition data of various compounds for [^{125}I]FISCH binding are listed in Table II with the following rank order of potency: SCH-23390 > (±) IBZP ≫ apomorphine > WB 4010 > ketanserin, spiperone. The results indicate that [^{125}I]FISCH binds specifically to the dopamine D-1 receptor with high selectivity.

In conclusion, on the basis of the preliminary data presented in this paper, [^{125}I]FISCH displayed superior in vivo and in vitro properties to [^{125}I]IBZP. The corresponding resolved active isomer, *R*-(+), labeled with I-123 ($T_{1/2}$ = 13 h, 159 keV) may be a useful ligand for imaging D-1 dopamine receptors in the living human brain with SPECT.

Experimental Section

Proton NMR was recorded on a Varian EM 360A spectrometer. The chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with a Mattson Polaris FT-IR spectrometer. Melting points were determined on a Meltemp apparatus and are reported uncorrected. Elemental analyses were performed by Atlantic Microlabs Inc., of Norcross, GA, and were within 0.4% of the theoretical values.

3-Chloro-4-methoxyphenethylamine (1).[26] Methoxyphenethylamine (39.4 g, 0.261 mol) was dissolved in water (300 mL) and concentrated HCl (22 mL). To this solution was added chlorine gas (20.3 g, 0.287 mol) in glacial acetic acid (300 mL) over a 15-min period while the temperature was maintained below 35 °C. After standing for 10 min, the volatiles were removed in vacuo and the dark, solid residue was dissolved in absolute ethanol (100 mL) and allowed to crystallize at −10 °C. The collected precipitate was dissolved in a mixture of saturated sodium bicarbonate (400 mL) and dichloromethane (400 mL). The organic layer was separated and dried over anhydrous sodium sulfate. It was condensed on a rotorevaporator to afford a dark oil which was distilled at 115–116 °C (1.75 mmHg) (lit.[26] bp 115 °C/0.5 mmHg) to give 22.2 g (47.4%) of a clear oil: IR (neat) λ 3600–3310 (br, NH_2), 1600, 1500, 1250, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$ δ 7.36–6.71 (m, 3 H, ArH), 3.85 (s, 3 H, OCH_3), 3.15–2.48 (m, 4 H, $(CH_2)_2$), 1.12 (s, 2 H, NH_2).

***N*-[2-(4′-Bromophenyl)-2-hydroxyethyl]-3-chloro-4-methoxyphenethylamine (2).**[24] Compound 1 (6.40 g, 0.034 mol) and 4-bromostyrene oxide (6.90 g, 0.034 mol) were dissolved in acetonitrile (50 mL) and refluxed overnight. The solvent was evaporated under reduced pressure and a gummy residue was triturated with ether. The white powder was filtered to afford 6.5 g (40%): mp 83–84 °C (lit.[27] mp 181–183 °C); IR (KBr) λ 3400 (br, NH), 3140 (br, OH), 1500, 1400, 1250, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.60–6.70 (m, 7 H, ArH), 4.80–4.48 (m, 1 H, CH), 3.85 (s, 3 H, OCH_3) 3.00–2.40 (m, 6 H, $(CH_2)_3$), 2.55 (s, 1 H, NH).

7-Chloro-8-methoxy-1-(4′-bromophenyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (3).[24] The hydroxy amine 2 (7.4 g, 0.019 mol) was added in portions to concentrated sulfuric acid (60 mL) with stirring, and the temperature was kept below 12 °C. The reaction mixture was then stirred at 8 °C for 30 min and then at room temperature for 90 min. The mixture was poured into ice (500 g); concentrated ammonium hydroxide (100 mL) was added followed by solid sodium hydroxide (40 g) while the temperature was maintained below 30 °C. The precipitate was extracted into dichloromethane and the organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to afford 6.5 g (93%) of a light yellow solid, which was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH, 95:5:1) to obtain 3, 5.9 g (84%): mp 139–140 °C (lit.[24] mp 136–137 °C); FT-IR (KBr) λ 3430 (br, NH), 1500, 1480, 1400, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.65–7.31 and 7.10–6.89 (AA′BB′, 4 H, ArH′), 7.13 (s, 1 H, ArH-6), 6.50 (s, 1 H, ArH-9), 4.29–4.05 (m, 1 H, CH), 3.72 (s, 3 H, OCH_3), 3.45–2.53 (m, 6 H, $(CH_2)_3$), 1.92 (s, 1 H, NH).

7-Chloro-8-methoxy-1-(4′-bromophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (4). To a solution of benzazepine 3 (7.20 g, 19.6 mmol) in formic acid (2.3 g) was added 37% formaldehyde (1.8 g). The mixture was heated to 90–100 °C for 4 h. After cooling of the reaction mixture to room temperature, 4 N hydrochloric acid solution (5.16 mL) was added. The mixture was concentrated to dryness under reduced pressure. The residue was dissolved in water and then made basic with 25% sodium hydroxide solution. The mixture was extracted three times with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate. The solvent was evaporated to obtain a solid, which was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH, 95:5:1) to give 4, 6.0 g (80%): mp 116–118 °C; FT-IR (KBr) λ 1500, 1380, 1270, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.65–7.35 and 7.21–6.90 (AA′BB′, 4 H, ArH′), 7.13 (s, 1 H, ArH-6), 6.38 (s, 1 H, ArH-9), 4.40–4.10 (m, 1 H, CH) 3.70 (s, 3 H, OCH_3), 3.10–2.45 (m, 6 H, $(CH_2)_3$), 2.37 (s, 3 H, NCH_3). Anal. $C_{18}H_{19}BrClNO$: C, H, N.

7-Chloro-8-methoxy-1-[4′-(tri-*n*-butyltin)phenyl]-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (5). The *N*-methyl benzazepine 4 (2.0 g, 5.2 mmol) in dried THF (50 mL) was cooled to −78 °C in a dry ice/acetone bath. To this solution,

(26) Wyrick, S. D.; Mailman, R. B. *J. Labelled Compd. Radiopharm.* **1985**, *22*, 189.

(27) The melting point of 2 is 100 °C lower than that reported in the literature, but the spectral data are identical with those in the literature.

n-butyllithium (4.0 mL, 6.4 mmol) was added with stirring. The reaction solution turned dark red immediately. Tri-*n*-butyltin chloride (1.5 mL) was added to the reddish solution. After stirring at −78 °C for 5 min, the reaction mixture was quenched with ammonium chloride solution (3 mL, saturated). The mixture was allowed to warm to room temperature and THF was evaporated under reduced pressure. The residue was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate. After evaporation of the solvent, the desired product, **5**, was separated by column chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH, 95:5:1) to obtain 1.8 g (58%): FT-IR (neat) λ 2960–2800 (strong and broad band of *n*-butyl group), 1600, 1500, 1405, 1270, 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65–7.38 and 7.25–7.03 (AA′BB′, 4 H, ArH′), 7.13 (s, 1 H, ArH-6), 6.30 (s, 1 H, ArH-9), 4.54–4.17 (m, 1 H, CH), 3.60 (s, 3 H, OCH_3), 3.10–2.59 (m, 6 H, $(CH_2)_3$), 2.40 (s, 3 H, NCH_3), 1.70–0.65 (m, 27 H, Sn-$(C_4H_9)_3$). Anal. $C_{30}H_{46}ClNOSn$: C, H, N.

7-Chloro-8-methoxy-1-(4′-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (6). A 0.1 M solution of iodine in chloroform was added to a solution of tri-*n*-butyltin derivative of benzazepine **5** (500 mg, 0.85 mmol) in chloroform at room temperature until the color of iodine persisted. The mixture was stirred overnight at room temperature. Then a solution of potassium fluoride (1 M, 1 mL, 1 mmol) in methanol and a 5% aqueous sodium bisulfite solution (1 mL) were added, respectively. After 5 min of stirring, water (2 mL) was added. The organic layer was separated and the aqueous layer was extracted with chloroform twice. The combined organic layers were dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain a yellow solid, which was purified by column chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH, 95:5:1) to yield the product **6**: 342 mg (94%); mp 130–132 °C; FT-IR (KBr) λ 1500, 1460, 1400, 1260, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85-7.48 and 7.10–6.75 (AA′BB′, 4 H, ArH′), 7.13 (s, 1 H, ArH-6), 6.35 (s, 1 H, ArH-9), 4.41–4.01 (m, 1 H, CH), 3.70 (s, 3 H, OCH_3), 3.15–2.50 (m, 6 H, $(CH_2)_3$), 2.38 (s, 3 H, NCH_3). Anal. $C_{18}H_{19}ClNO$: C, H, N.

7-Chloro-8-hydroxy-1-(4′-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (7). A solution of iodobenzazepine **6** (342 mg, 0.80 mmol), in dried dichloromethane, was cooled in a dry ice/2-propanol bath. To this stirred solution was added a BBr_3 solution (1.6 mL, 1.6 mmol), dropwise. The reaction mixture was then allowed to warm to room temperature. The stirring was continued for 2 h. The reaction mixture was partly concentrated and chilled in an ice bath. Methanol was added to the mixture and it was stirred for several hours at room temperature. After the methanol had been evaporated under reduced pressure, the residue was stirred with water. The mixture was made strongly basic with 10% sodium hydroxide. The precipitate was filtered. The pH of the filtrate was adjusted to 7–8 with dilute hydrochloric acid. The cloudy mixture was extracted several times with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to obtain 110 mg (33%): mp 216–218 °C; FT-IR (KBr) λ 3450 (br, OH), 1500, 1460, 1400, 1100, 1060, 1000 cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 7.80–7.52 and 7.10–6.85 (AA′BB′, 4 H, ArH′), 7.10 (s, 1 H, ArH-6), 6.32 (s, 1 H, ArH′-9), 4.31–3.92 (m, 1 H, CH), 3.10–2.65 (m, 6 H, $(CH_2)_3$), 2.35 (s, 3 H, NCH_3). Anal. $C_{17}H_{17}ClINO$: C, H, N.

Radiolabeling. Aqueous hydrogen peroxide (10 μL, 30% w/v) was added to a mixture of 10 μL of compound **5** (1 mg/mL), 100 μL of 50% EtOH/H_2O, 10 μL of 1 N HCl, and 5 μL of sodium [^{125}I]iodide (2–3 mCi, carrier-free, sp act. 2200 Ci/mmol) in a sealed vial. The reaction was allowed to proceed at 23 °C for 2 h, after which it was terminated by the addition of 0.5 mL of sodium bisulfite (100 mg/mL). The reaction mixture was made basic via the addition of 100 mg of $NaHCO_3$ and extracted with ethyl acetate (3 × 1 mL). The combined organic layers were passed through an anhydrous sodium sulfate column (0.2 cm × 5 cm) and evaporated to dryness by a stream of nitrogen. The residue was dissolved in 100% ethanol (50–100 μL), and the desired product, [^{125}I]-**6**, was isolated from the unreacted compound **5** and a small amount of unknown radioactive impurities by HPLC with a reverse-phase column (PRP-1, Hamilton Inc.) and an isocratic solvent of 90% acetonitrile/10% pH 7.0 buffer (5 mM, 3,3-dimethylglutaric acid). The appropriate fractions were collected, condensed, and reextracted with ethyl acetate (1 × 3 mL). The solution containing the no-carrier-added product was condensed to dryness and redissolved into 100% ethanol (purity >99%, overall yield 75%).

To an anhydrous CH_2Cl_2 solution of [^{125}I]-**6** under an argon atmosphere was added BBr_3 (40 μL, 1 M in CH_2Cl_2). The reaction was terminated after 1 h at 23 °C. The mixture was condensed to dryness, and the residue was dissolved into 100% EtOH (100 μL). The desired product, [^{125}I]FISCH, was again separated from a small amount of unknown radioactive impurities by the same HPLC system but using 80%/20% acetonitrile/buffer. The appropriate fractions were collected, condensed, and reextracted with ethyl acetate (1 × 3 mL). The solution containing the no-carrier-added product was condensed to dryness and redissolved into 100% ethanol (purity >99%, overall yield 75%). After dilution with saline, this agent was used in the in vivo and in vitro studies.

Biodistribution in Rats. Biodistribution of [^{125}I]FISCH was studied in male Sprague–Dawley rats (225–300 g) which were allowed free access to food and water. While under halothane anesthesia, 0.2 mL of a saline solution containing [^{125}I]FISCH was injected directly into the femoral vein, and the rats were sacrificed at various time points postinjection by cardiac excision under halothane anesthesia. The organs of interest were removed and weighed and the radioactivity was counted with a Beckman automatic gamma counter (Model 4000). The percent dose/organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activities of blood and muscle were calculated assuming that they are 7% and 40% of the total body weight, respectively.

Regional brain distribution in rats was obtained after an iv injection of [^{125}I]FISCH. By dissecting, weighing, and counting samples from different brain regions (cortex, striatum, hippocampus, and cerebellum), the percent dose/gram of samples was calculated by comparing the sample counts with the counts of the diluted initial dose. The uptake ratio of each region was obtained by dividing the percent dose/gram of each region with that of the cerebellum.

Tissue Preparation. Male Sprague–Dawley rats (200–250 g) were decapitated, and the brains were removed and placed in ice. Striatal tissues were excised, pooled, and homogenized in 100 volumes (w/v) of ice-cold Tris·HCl buffer (50 mM), pH 7.4. The homogenates were centrifuged at 20000*g* for 20 min. The resultant pellets were rehomogenized in the same buffer and centrifuged again. The final pellets were resuspended in assay buffer containing 50 mM Tris buffer, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$.

Binding Assays. The binding assays were performed by incubating 50 μL of tissue preparations containing 40–60 μg of protein with appropriate amounts of [^{125}I]FISCH ligand and competitors in a total volume of 0.2 mL of the assay buffer. After an incubation period of 20 min at 37 °C (with stirring), the samples were rapidly filtered in the cell harvester (Brandel M-24R) under vacuum through Whatman GF/B glass-fiber filters pretreated with 0.2% protamine sulfate and washed with 3 × 5 mL of cold (4 °C) 50 mM Tris·HCl buffer, pH 7.4. The nonspecific binding was obtained in the presence of 10 μM SCH-23390. The filters were counted in a gamma counter (Beckman 5500) at an efficiency of 70%.

Data Analysis. Both Scatchard and competition experiments were analyzed with the iterative nonlinear least-squares curve-fitting program LIGAND.[28]

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