thereafter the fluorescence of the drug in the presence and absence of DNA was recorded. The amount of drug bound to DNA (C_b) was determined according to the equation

$$C_b = C_t\,[(I_{obs} - I_f)/I_f(V - 1)]$$

where I_{obs} and I_f represents the fluorescence intensities in the presence and absence of DNA, respectively. The term V, representing the ratio of fluorescence of totally bound drug/fluorescence of free drug, was determined at a ratio of drug to base pairs of 200. Binding constants were calculated according to the neighbor-exclusion model of McGee and Von Hippel.[32]

Registry No. DXR, 23214-92-8; DNR, 20830-81-3; THP, 123639-68-9; N-Ac-DXR, 69299-74-7; N-Ac-DNR, 32385-10-7; ACM, 66676-88-8; RMN I, 1404-52-0; RMN II, 23666-50-4; CTR A, 95599-38-5; CTR D, 100630-83-9; CTR S, 96497-67-5; 1-OH-CTR S, 123639-69-0.

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Synthesis and Characterization of Iodobenzamide Analogues: Potential D-2 Dopamine Receptor Imaging Agents

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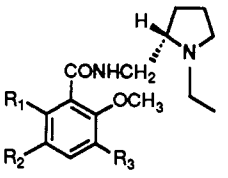
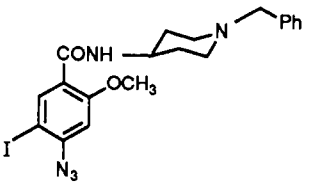
(*S*)-*N*-[(1-Ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide ([^{123}I]IBZM) is a central nervous system (CNS) D-2 dopamine receptor imaging agent. In order to investigate the versatility of this parent structure in specific dopamine receptor localization and the potential for developing new dopamine receptor imaging agents, a series of new iodinated benzamides with fused ring systems, naphthalene (INAP) and benzofuran (IBF), was synthesized and radiolabeled, and the in vivo and in vitro biological properties were characterized. The best analogue of IBZM is IBF (21). The specific binding of [^{125}I]IBF (21) with rat striatal tissue preparation was found to be saturable and displayed a K_d of 0.106 ± 0.015 nM. Competition data of various receptor ligands for [^{125}I]IBF (21) binding show the following rank order of potency: spiperone > IBF (21) > IBZM > (+)-butaclamol > (±)-ADTN,6,7 > ketanserin > SCH-23390 ≫ propanolol. The in vivo biodistribution results confirm that [^{125}I]IBF (21) concentrated in the striatal area after iv injection into rats. The study demonstrates that [^{123}I]IBF (21) is a potential agent for imaging CNS D-2 dopamine receptors.

A variety of substituted benzamide derivatives possessing antipsychotic and antiemetic properties have been reported.[1-5] The pharmacological effects of these agents are assumed to be induced by blocking the central nervous system (CNS) D-2 dopmaine receptor. In this series of benzamide derivatives, agents with an (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidinyl group appear to be the most attractive antagonists—showing the best selectivity and the highest affinity for the CNS D-2 dopamine receptor. Raclopride[6] and eticlopride[7,8] are two excellent examples which show specific D-2 antagonistic activity, with high affinity in rat striatum tissue preparations and low nonspecific binding (Table I). Radioactive iodinated benzamides are not only potentially useful as imaging agents (labeled with ^{123}I, $T_{1/2}$ = 13 h, γ-ray energy = 159 keV) but are also very valuable as pharmacological tools for probing the D-2 dopamine receptor under in vitro and in vivo conditions (labeled with ^{125}I, $T_{1/2}$ = 60 days, γ-energy = 30–65 keV). Several iodinated benzamide derivatives, iodosulpiride,[9] iodoazidoclebopride,[10] iodopride,[11] and IBZM,[12-14] have been reported as showing very high affinity and selectivity to the D-2 dopamine receptor in the same striatal membrane preparation.

Imaging studies of CNS D-2 dopamine receptor in humans with [^{11}C]raclopride (labeled at the *N*-ethyl group), in conjunction with positron emission tomography (PET), have been reported.[15-20] A high ratio of specific striatal to nonspecific cerebellar binding in living human brain was observed. With use of an equilibrium model and Scatchard plots, the affinity constant (K_d = 7.1 nM, B_{max} = 15 pmol/mL) in living human brain was measured by PET.[19,20] The values for the dopamine D-2 receptor density were comparable to those determined earlier using a different imaging agent, *N*-methylspiperone, NMSP (K_d = 0.097 nM, B_{max} = 16.6 pmol/g).[21-23] Planar imaging studies in humans with (*S*)-[^{123}I]IBZM (*S*—the active

Table I. Chemical Structures and in Vitro Binding Constants of Benzamides[a]





iodoazidoclebopride

compound	R_1	R_2	R_3	K_d, nM
iodosulpiride[c]	H	SO_2NH_2	H	1.5
raclopride[d,e]	OH	Cl	Cl	1.1
etichlopride[d,e,f]	OH	C_2H_5	Cl	0.17
IBZM[g]	OH	I	H	0.43
BZM[g]	OH	H	H	31.1
iodopride[h]	H	I	H	3.0[h]
iodoazidoclebopride[i]				14

[a] Unless otherwise stated, all of the benzamides contain a *S*-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidinyl group. [b] IC_{50} against [^{3}H]spiperone binding of rat striatal tissue preparation. [c] See ref 9. [d] See ref 4. [e] See ref 6. [f] See ref 7. [g] See ref 14. [h] See ref 11. [i] See ref 10.

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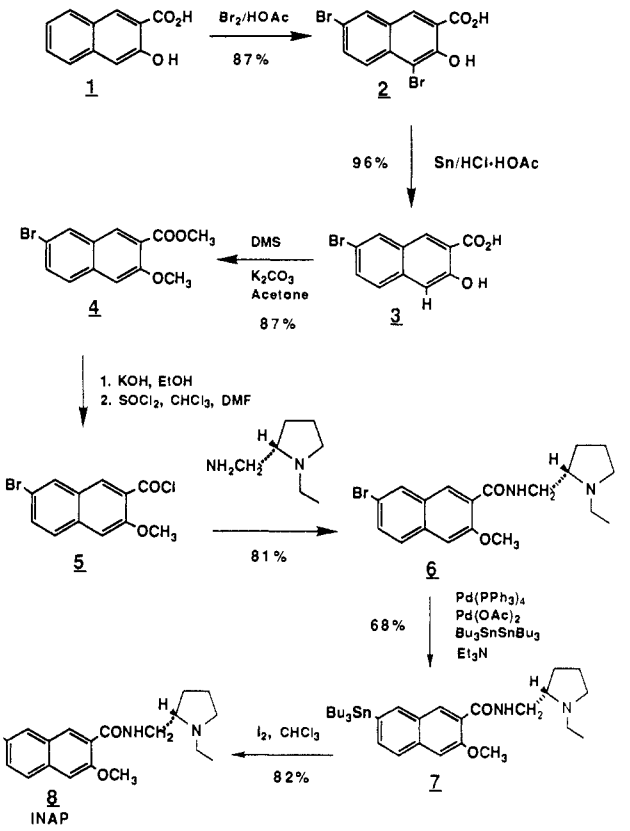
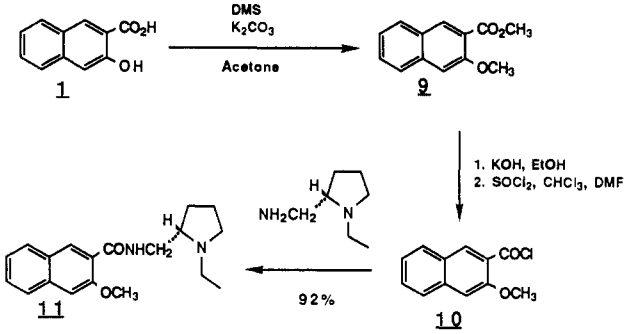
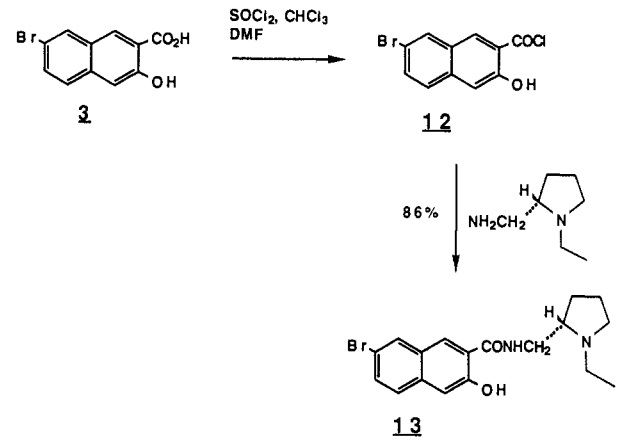
Scheme I. Iodonaphthamide, INAP



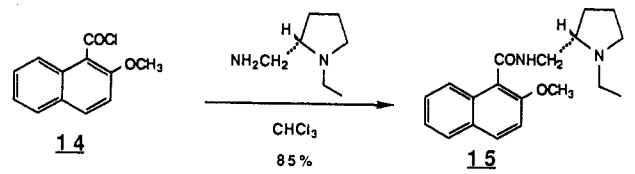
Scheme II. Naphthamide



Scheme III. Bromonaphthamide



Scheme IV. Isonaphthamide



isomer; *R* the inactive isomer), immediately after iv injection demonstrate that this agent, as expected, displayed high concentration in basal ganglia of the brain. Single photon emission computed tomography (SPECT) imaging of a normal human brain at 1 h postinjection displayed a pattern which clearly indicates the highly specific uptake in the basal ganglia of the brain.[24]

Several other potential SPECT and PET dopamine receptor imaging agents, based on radiolabeled spiperone or its derivatives, have been reported.[25-35] Preliminary studies of an iodinated 2′-iodospiperone (2′-ISP) indicate that the spiperone analogue displays excellent D-2 specificity (K_d = 0.25 nM, rat striatum) and in vivo stability as compared to 4-iodospiperone (4-ISP)[36] reported earlier. In vitro binding data for 2′-ISP appears to show a higher nonspecific binding (>40%) than that observed with

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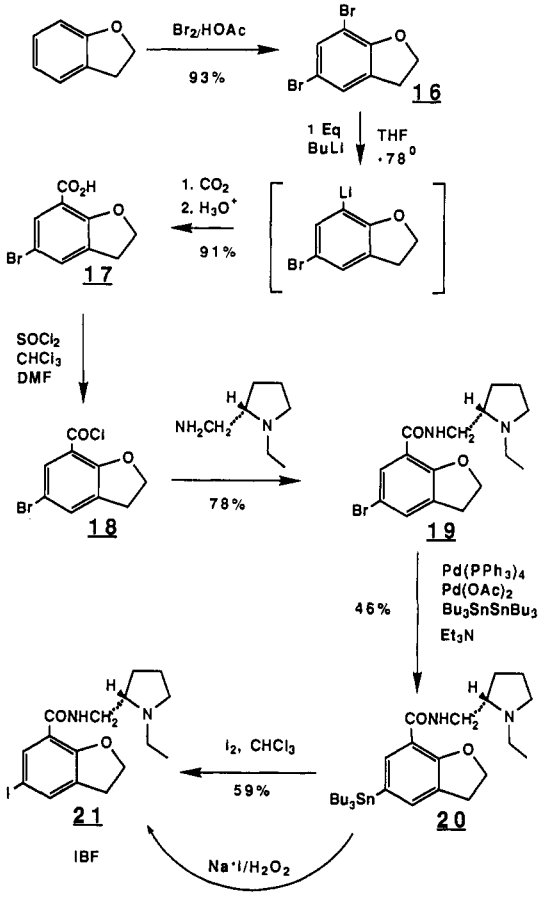
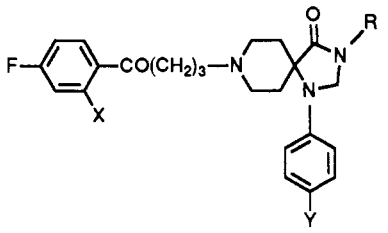
Scheme V. Iododihydrobenzofurancarboxamide



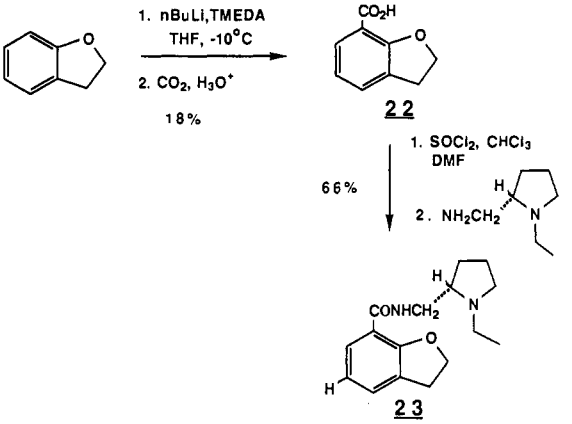
[^{125}I]IBZM (5%).[14] In addition, several new ^{18}F-labeled compounds including spiperone (SP) itself, *N*-methylspiperone (NMSP),[33] *N*-(fluoroalkyl)spiperones (F-Et-SP and F-Pr-SP),[30-33] and [^{77}Br]-4-bromospiperone (4-Br-SP) have been reported.[34,35]



	R	X	Y
SP	H	H	H
NMSP	CH_3	H	H
F-Et-SP	CH_2CH_2F	H	H
F-Pr-SP	$(CH_2)_3F$	H	H
2′-ISP	H	I	H
4-ISP	H	H	I
4-Br-SP	H	H	Br

Recently, several patents describing the synthesis and pharmacological studies of bicyclic benzamide analogues have been reported.[37,38] Of particular interest to the design of new IBZM analogues is the dihydrobenzofuran series: the bromo and chloro derivatives displayed high pharmacological potential and good receptor affinity in in vitro binding assay.[38] In order to investigate the structural versatility of the benzamides for CNS D-2 dopamine receptor binding and develop new ^{123}I-labeled SPECT imaging agents with higher receptor affinity (longer retention time in the brain for data accumulation and less in vivo metabolism), a series of new iodinated benzamides with fused ring systems, naphthalene and benzofuran, was synthesized and radiolabeled with iodine-125, and the in vivo and in vitro biological properties were characterized.

Scheme VI. Dihydrobenzofurancarboxamide



Chemistry

The synthesis of two series of bicyclic benzamide analogues, depicted in Schemes I–VI, is described below. All of the amide analogues prepared contain the (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine moiety (only the *S* isomer, the active form, was prepared). They are generally prepared by coupling the pyrrolidine compound with the acid chloride derivatives of dihydrobenzofuran or naphthalene as reported previously for IBZM.[14]

The first series of compounds described in Schemes I–IV has a naphthyl ring as its theme. The synthesis of both 7-Br and 7-I naphthyl amides **5** and **8**, respectively, is outlined in Scheme I. Treatment of 3-hydroxy-2-naphthoic acid with excess bromine in glacial acetic acid gave 4,7-dibromo hydroxy acid **2** in good yield (87%). Dibromo acid **2** was selectively debrominated at the 4-position with tin and acid to afford a high yield (96%) of 7-bromonaphthoic acid **3**. Methoxy ester compound **4** was prepared by refluxing hydroxy acid **3** in acetone with 2 equiv of dimethyl sulfate and excess powdered anhydrous potassium carbonate. Ester **4** was hydrolyzed to the corresponding methoxy acid with ethanolic hydroxide, and the acid was then cleanly converted to the corresponding acid chloride **5** with thionyl chloride in dry chloroform containing a catalytic amount of dimethylformamide. Condensation of 7-bromonaphthoyl chloride **5** with (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine in chloroform gave the desired amide **6** in 81% yield. 7-Iodo compound **8** was then synthesized from this bromo amide **6** via the intermediacy of a stable, versatile tin intermediate. The halide interconversion began with a palladium catalyzed exchange of the aryl bromide with tributyltin in refluxing dry triethylamine with tetrakis(triphenylphosphine)palladium[39] to afford 7-tributylstannyl amide **7** in acceptable yield (68%). Stannyl amide **7** was then converted to iodo derivative **8** in 82% yield by simply stirring with iodine in dry chloroform at room temperature.

The synthesis of naphthamide analogue **11**, which is unsubstituted at the 7-position, is shown in Scheme II and begins with the same starting material, 3-hydroxy-2-

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naphthoic acid (1). Hydroxy acid 1 was exhaustively methylated by refluxing with dimethyl sulfate and powdered anhydrous potassium carbonate in acetone to afford methoxy ester 9. Ester 9 was hydrolyzed to the corresponding acid and then converted directly to acid chloride 10 with thionyl chloride in dry chloroform and a catalytic amount of dimethylformamide. Condensation of acid chloride 10 with (S)-(-)-N-ethyl-2-(aminomethyl)-pyrrolidine in chloroform gave the desired parent naphthamide analogue 11 in 92% yield.

The synthesis of 3-hydroxynaphthamide analogue 13, shown in Scheme III, began with 7-bromo-3-hydroxy-2-naphthoic acid (3), which was treated with excess thionyl chloride in chloroform with enough dimethylformamide to solubilize the hydroxy acid and refluxed to afford the sensitive o-hydroxy acid chloride 12. This acid chloride was condensed immediately with (S)-(-)-N-ethyl-2-(aminomethyl)pyrrolidine in chloroform to afford hydroxynaphthamide 13 in 86% yield.

The synthesis of 2-methoxy-1-naphthyl amide 15, outlined in Scheme IV, is similar to the one employed for its regioisomeric analogue, compound 11 (Scheme II). Acid chloride 14, prepared from the corresponding acid with thionyl chloride, was condensed with (S)-(-)-N-ethyl-2-(aminomethyl)pyrrolidine in chloroform, giving naphthyl amide 15 in good yield (85%).

The second series of compounds was used to investigate the feasibility of a dihydrobenzofuran core. The strategy employed in the synthesis of 5-Br and 5-I dihydrobenzofuranamides 19 and 21, respectively, is shown in Scheme V. 5,7-Dibromo-2,3-dihydrobenzofuran (16) was obtained in 93% yield by treatment of 2,3-dihydrobenzofuran with 2.2 equiv of bromine in cold glacial acetic acid. Taking advantage of the stabilizing effect of the furan oxygen atom, dibromobenzofuran 16 was selectively lithiated at the 7-position with 1 equiv of n-butyllithium in dry tetrahydrofuran at −78 °C and then quenched with CO_2 and acid to afford 5-bromo-7-carboxy-2,3-dihydrobenzofuran (17) in excellent yield (91%). Conversion of bromo acid 17 to the corresponding acid chloride 18 was accomplished with thionyl chloride in refluxing chloroform with a catalytic amount of dimethylformamide. The acid chloride was then condensed with (S)-(-)-N-ethyl-2-(aminomethyl)pyrrolidine in chloroform to give the desired 5-bromobenzofuranamide 19 in 79% yield. Conversion of 5-bromo compound 19 to the 5-iodo compound IBF (21) was accomplished as described for the naphthyl series through the intermediacy of a 5-stannyl derivative, 20. 5-Bromo acid 19 was treated with the zero-valent catalyst tetrakis(triphenylphosphine)palladium and hexabutylditin and refluxed in dry triethylamine for several hours to afford the isolable, purifiable 5-(tributylstannyl)benzofuranamide derivative 20 in moderate yield (46%). 5-Iododihydrobenzofuran derivative IBF (21) was obtained from the tributylstannyl intermediate simply by stirring with iodine in chloroform at room temperature (59% yield).

The synthesis of unsubstituted dihydrobenzofuranamide analogue 23, outlined in Scheme VI, proceeded from 2,3-dihydrobenzofuran. Direct, selective lithiation of dihydrobenzofuran at the 7-position was achieved by treatment with n-butyllithium in TMEDA/tetrahydrofuran at −10 °C. Quenching of the reaction mixture after 20 min with CO_2 and acid afforded the desired 7-carboxyfuran 22. The carboxylic acid was converted to the corresponding acid chloride with thionyl chloride in chloroform–dimethylformamide and then condensed with (S)-(-)-N-ethyl-2-(aminomethyl)pyrrolidine in chloroform to give unsubstituted benzofuranamide 23.

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

compd	K_i, nM (mean ± SEM)	compd	K_i, nM (mean ± SEM)
6	82.0 ± 12	15	507 ± 29
8	168 ± 13	19	3.89 ± 0.62
11	30.0 ± 4.2	21	0.23 ± 0.02
13	280 ± 47	23	135 ± 22

[a] 0.15–0.4 nM [^{125}I]IBZM was incubated in the presence of the indicated compounds in 7–11 concentrations and membrane preparation from rat striatum. Each value represents the mean ± SEM of three to five determinations.

Radiolabeling. Radioactive labeling of compound 20 with ^{125}I at a carrier-free level was accomplished by an electrophilic radioiodination reaction using hydrogen peroxide as the oxidant.[40] The radiolabeled compound, [^{125}I]IBF (21), was separated from the starting material, compound 20, by HPLC. The overall yield of carrier-free [^{125}I]IBF (21) was 75% (radiochemical purity >99%) and did not show any UV-detectable material. [^{125}I]IBF (21) was compared with chemically pure, nonradioactive, authentic compound by HPLC using sequential UV and radioactivity detection and was determined to be the desired product on the basis of its identical elution profile (the retention time was 6.5–7.0 min with isocratic elution: 90% acetonitrile–10% 5mM dimethylglutaric acid, pH 7.0, and in a flow rate of 1 mL/min). Since the HPLC separation procedure will effectively separate the starting material, tin compound 20, and the desired product, [^{125}I]IBF (21), and the [^{125}I]sodium iodide was carrier-free, the specific activity of the final product was assumed to be 2 200 Ci/mmol.

In Vitro Binding. The affinity of the analogues of IBZM for D-2 dopamine receptors was studied using in vivo competitive binding assays. Competition binding data using [^{125}I]IBZM are presented in Table II. In the naphthalene series, the rank order of potency is H (11) > Br (6) > I (8); K_i (nM) values are 30.0, 82.0, and 168.0, respectively. The data suggest that the bulk tolerance of the D-2 dopamine receptor binding for this part of the molecule is limited. The results from the same binding study for compounds 13 and 15 indicate that the naphthalene series generally does not maintain the receptor affinity. The benzofuran series, on the other hand, displays high binding affinity. The K_i values for H (23), Br (19) and I (21) are 135, 3.89, and 0.23 nM, respectively. It is important to point out that the addition of an iodine atom appears to enhance the binding affinity significantly. The iodo group is apparently 16 times more effective than the bromine atom for increasing the competitive binding affinity, which has not previously been reported for the dihydrobenzofuran derivatives.[37,38] The data reported in this paper strongly suggest that the bulk tolerance in this part of the molecule, and perhaps the large size of iodine, also enhance the binding. It is surprising that the iodo group has such a strong effect on the affinity of D-2 dopamine receptors. Further structure–activity relationship studies of this series of dihydrobenzofuran derivatives may be warranted.

Similar to the high affinity reported previously for [^{125}I]IBZM,[14] [^{125}I]IBF (21) competitively bound to rat striatal homogenate in vitro. The saturation curve shown in Figure 1 displays an extremely low nonspecific binding (<5% at K_d). The specific binding of [^{125}I]IBF (21) was

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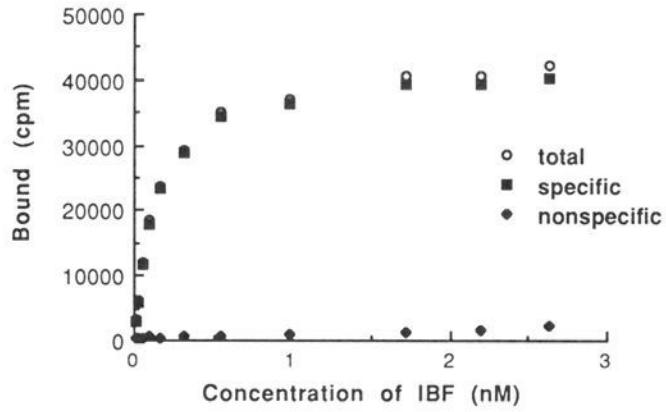

Figure 1. The saturation binding curve of [^{125}I]IBF (**21**) in rat striatum tissue preparation suggests that it binds to rat striatal homogenate with very high affinity (K_d = 0.106 ± 0.015 nM). This curve also indicates a low nonspecific binding (<5% at K_d).

Table III. Inhibition Constants of Various Compounds on [^{125}I]IBF (**21**) Binding to Rat Striatal Membranes[a]

compd	K_i, nM (mean ± SEM)	compd	K_i, nM (mean ± SEM)
spiperone	0.015 ± 0.002	(±)-ADTN,6,7	65.7 ± 13.0
(*S*)-(−)IBZM	0.261 ± 0.018	ketanserin	491 ± 49
(*S*)-(−)IBF (21)	0.085 ± 0.010	dopamine	843 ± 150
(+)-butaclamol	1.190 ± 0.14	SCH-23390	820 ± 164
		propranolol	>10000

[a] 0.05–0.15 nM [^{125}I]IBF (21) was incubated in the presence of the indicated compounds in 7–11 concentrations and membrane preparation from rat striatum. Each value represents the mean ± SEM of three to five determinations.

found to be saturable and displayed a K_d of 0.106 ± 0.015 nM. This value is lower than that of [^{125}I]IBZM (K_d = 0.426 nM) measured under similar conditions.[14] Competition data of various receptor ligands for [^{125}I]IBF (**21**) binding are listed in Table III and show the following rank order of potency: spiperone > IBF (**21**) > IBZM > (+)-butaclamol > (±)-ADTN,**6,7** > ketanserin > SCH-23390 ≫ propranolol. The results confirm that [^{125}I]IBF binds specifically and selectively to the dopamine D-2 receptor.

In Vivo Biodistribution in Rats. Since the in vitro binding data suggest that the naphthalene series was not as promising as the dihydrobenzofuran series, only IBF (**21**) was subjected to a detailed in vivo biodistribution study. After an iv injection, [^{125}I]IBF (**21**) showed good brain uptake in rats (Table IV). The initial uptake (0.98 % dose/organ) at 2 min after injection is lower than that of [^{125}I]IBZM (2.87 % dose/organ). At later time points the brain uptake decreases; at 1 h after injection a large portion of the radioactivity had washed out from the brain (0.17 % dose/organ). The brain washout pattern in rats is similar to that of [^{125}I]IBZM.

High initial uptake in the lungs (6.14 % dose/organ) was also observed, but the radioactivity was rapidly cleared. At 30 and 120 min the lung uptake dropped to 0.82% and 0.37%, respectively. Liver uptake remained high

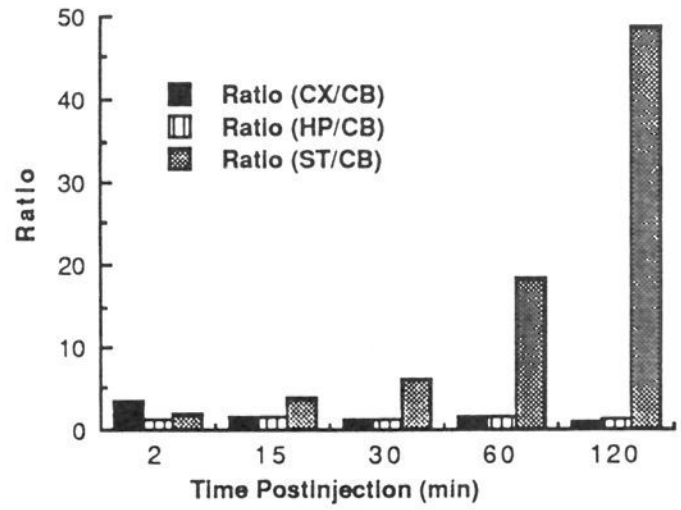

Figure 2. Ratio (based on percent dose/gram) of regional brain uptake of [^{125}I]IBF (**21**) (CB, cerebellum; ST, striatum; HP, hippocampus; CX, cortex). The dramatic increase of ST/CB ratio with time indicates that the agent is specifically concentrated in the target tissue, the striatum (ST), in which D-2 dopamine receptor density is high.

throughout the first hour and gradually declined at 2 h postinjection. The relatively low thyroid uptake at 1 h postinjection (0.02%) suggests that little or no in vivo deiodination of [^{125}I]IBF (**21**) has occurred. As compared with [^{125}I]IBZM, which showed a thyroid uptake of 0.1% at 1 h postinjection, the new iodinated D-2 agent [^{125}I]IBF (**21**), containing an iodine atom at the aromatic ring without a free hydroxy as an activated group, displays better in vivo stability to deiodination.

With a brain regional-dissection technique, the striatum/cerebellum (ST/CB) ratio (target to nontarget ratio) displayed a dramatic increase with time: 2.0, 3.8, 6.2, 18, and 48 at 2, 15, 30, 60, and 120 min, respectively (Figure 2). This type of profound increase in target to nontarget ratio vs time was not observed for the other two regions (hippocampus and cortex) (Figure 2). These results suggest that in regions with nonspecific association, i.e. regions low in dopamine receptors, the agent is washed out rapidly, whereas the striatum (rich in dopamine receptors) shows prolonged retention. A preliminary study (data not shown) on ex vivo autoradiography of this compound also confirms the high striatal uptake and low cerebellar activity. The data are further confirmed by an in vivo imaging study of a monkey (data not shown). The planar images displayed a high uptake and retention in the basal ganglia, where the CNS D-2 dopamine receptors are located.

In conclusion, on the basis of the preliminary data presented in this paper, the best analogue of IBZM is IBF (**21**). When labeled with ^{125}I, IBF (**21**) displayed in vivo and in vitro properties comparable to those of [^{125}I]IBZM. The in vivo stability and high target to nontarget ratio, coupled with the extremely low nonspecific binding in vitro, suggest that it is superior to IBZM as a D-2 binding

Table IV. Biodistribution of [^{125}I]IBF (**21**) in Rats after an Intraveneous Injection[a]

organ	2 min	15 min	30 min	60 min	120 min
blood	3.50 ± 0.22	1.80 ± 0.31	1.26 ± 0.10	0.98 ± 0.11	0.23 ± 0.02
heart	0.81 ± 0.25	0.20 ± 0.03	0.11 ± 0.01	0.05 ± 0.002	0.02 ± 0.001
muscle	11.25 ± 2.62	14.36 ± 1.29	9.00 ± 1.66	4.97 ± 0.56	2.30 ± 0.22
lung	6.14 ± 1.19	1.85 ± 0.33	0.82 ± 0.08	0.37 ± 0.10	0.12 ± 0.02
kidney	7.58 ± 0.66	2.92 ± 0.30	1.58 ± 0.46	1.01 ± 0.01	0.68 ± 0.32
spleen	0.96 ± 0.08	0.58 ± 0.08	0.31 ± 0.03	0.09 ± 0.01	0.03 ± 0.007
liver	16.32 ± 4.00	16.16 ± 7.54	11.59 ± 0.93	9.84 ± 1.50	3.95 ± 0.31
skin	8.54 ± 1.21	8.46 ± 0.56	5.34 ± 0.17	2.38 ± 0.17	1.18 ± 0.18
thyroid	0.06 ± 0.02	0.03 ± 0.004	0.02 ± 0.004	0.02 ± 0.01	0.08 ± 0.03
brain	0.98 ± 0.15	0.54 ± 0.06	0.30 ± 0.05	0.17 ± 0.02	0.07 ± 0.006
brain/blood[b]	2.64 ± 0.32	3.01 ± 0.91	2.26 ± 0.33	1.90 ± 0.29	1.53 ± 0.12
striatum[b]	0.743 ± 0.14	0.842 ± 0.15	0.66 ± 0.015	0.705 ± 0.023	0.444 ± 0.102

[a] Percent dose/organ, an average of three rats ± SD.

agent for pharmacological evaluations. When labeled with ^{123}I ($T_{1/2}$ = 13 h, 159 keV), it is potentially useful as a CNS D-2 dopamine receptor imaging agent for 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., Norcross, GA. Mass spectra were obtained with a VG mass spectrometer (Model ZAB-E).

4,7-Dibromo-3-hydroxy-2-naphthoic Acid (2). 3-Hydroxy-2-naphthoic acid (1, 10 g, 0.053 mol) was suspended in glacial acetic acid (100 mL) and cooled to 0 °C. To this mechanically stirred mixture was added dropwise a solution of bromine (21 g, 0.133 mol) in glacial acetic acid (50 mL) so as to maintain the reaction temperature below 5 °C. Following the addition, the reaction mixture was refluxed for 2 h, cooled, and poured into ice water (1000 mL). The solid dibrominated product was filtered, washed with water (3 × 100 mL) and ether (100 mL), and air-dried to afford a bright yellow solid (16 g, 87%): ^1H NMR ($CDCl_3$) δ 8.45 (1 H, s), 8.3 (phenol OH, br s), 8.05 (1 H, d), 7.81 (1 H, s), 7.76 (1 H, d).

7-Bromo-3-hydroxy-2-naphthoic Acid (3). Dibromo acid (2, 7 g 0.02 mol) was suspended in glacial acetic acid (100 mL) and 12 N HCl (25 mL) and mossy tin (2.3 g, 0.02 mol) was added. The reaction mixture was heated at reflux for 3 h, cooled, and diluted with water (100 mL). The yellow solid product was filtered, washed with water (3 × 100 mL), and air-dried to afford 7-bromo compound 3 (5.1 g, 96%): ^1H NMR ($CDCl_3$) δ 8.38 (1 H, s), 7.95 (1 H, s), 7.5 (2 H, d), 7.16 (1 H, s). The low-resolution mass spectrum showed an M^+ peak at m/e 280. Anal. ($C_{11}H_7O_3Br$) C, H.

Methyl 7-Bromo-3-methoxy-2-naphthoate (4). Bromo acid 3 (5.0 g, 0.018 mol), powdered, anhydrous potassium carbonate (12 g), and dimethyl sulfate (4.5 g, 0.041 mol) in dry acetone were refluxed for 4 h. The reaction mixture was cooled, water (5 mL) was added, and the mixture was stirred for 2 h to destroy any remaining dimethyl sulfate. The inorganic material was filtered and the acetone was removed under reduced pressure. The residue was taken up in methylene chloride, washed several times with water, dried over anhydrous magnesium sulfate, filtered, and concentrated to afford methoxy ester 4 (4.6 g, 87%): ^1H NMR ($CDCl_3$) δ 8.21 (1 H, s), 7.79 (1 H, s), 7.38 (2 H, s), 7.1 (1 H, s), 3.92 (3 H, s), 3.87 (3 H, s).

7-Bromo-3-methoxy-2-naphthoic Acid. Ester 4 (4.0 g, 0.0136 mol) was dissolved in ethanolic sodium hydroxide and heated to reflux for 2 h. The reaction mixture was cooled and the ethanol was removed under reduced pressure. The sodium salt of the acid was treated with 2 N HCl, stirred for 20 min, filtered, washed with water (3 × 50 mL), and air-dried to afford the acid product as a white solid (3.5 g, 92%): ^1H NMR ($CDCl_3$) δ 9.1 (OH, br s), 8.55 (1 H, s), 7.92 (1 H, s), 7.6 (2 H, s), 7.21 (1 H, s), 4.11 (3 H, s).

7-Bromo-3-methoxy-2-naphthoic Acid Chloride (5). The acid prepared above (1 g, 0.0036 mol) was dissolved in dry chloroform (25 mL) and treated with thionyl chloride (1.27 g, 0.01 mol) and a catalytic amount of *N,N*-dimethylformamide. The reaction mixture was refluxed for 3 h and the solvent was removed under reduced pressure. The residue was dissolved in toluene (25 mL) and concentrated again to remove any remaining thionyl chloride: ^1H NMR ($CDCl_3$) δ 8.36 (1 H, s), 7.9 (1 H, s), 7.51 (2 H, s), 7.05 (1 H, s), 3.95 (3 H, s).

(*S*)-(−)-7-Bromo-3-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]naphthalene-2-carboxamide (6). Acid chloride 5 prepared as described above was dissolved in chloroform (25 mL) and added slowly, dropwise, to a solution of excess (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine. The reaction was stirred at room temperature for 1 h and then concentrated in vacuo to afford a yellow, oily product. The oil was dissolved in 2 N HCl and the pH of the solution was then carefully adjusted to 7. The product was extracted into chloroform (3 × 30 mL), dried over anhydrous magnesium sulfate, filtered, and rotary evaporated to afford a yellowish, solid product. The product was chromatographed on silica gel [methylene chloride–methanol (8:2)] to afford a whitish solid (1.15 g, 81%): ^1H NMR ($CDCl_3$) δ 8.59 (1 H, s), 8.5 (NH, br s), 7.9 (1 H, s), 7.5 (2 H, s), 7.1 (1 H, s), 4.0 (3 H, s), 2.5–3.8 (11 H, m), 1.25 (3 H, t); IR (film) 3390 (NH), 1650 cm^{-1} (CO). Anal. Calcd (CI high-resolution mass spectrum) for $C_{19}H_{23}N_2O_2Br$ (M + H) 391.1024, found 391.1021. Anal. ($C_{19}H_{23}N_2O_2Br$) H; C: calcd, 58.32; found 57.55.

7-(Tributylstannyl)-3-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]naphthalene-2-carboxamide (7). Bromo compound 6 (0.8 g, 0.002 mol), hexabutylditin (1.4 g, 0.0024 mol), palladium(II) acetate (0.05 g, 2×10^{-4} mol, 0.1 equiv), and tetrakis(triphenylphosphine)palladium(0) (0.120 g, 1×10^{-4} mol, 0.05 equiv) were placed in a flame-dried flask equipped for reflux under argon and dissolved in dry triethylamine (distilled from calcium hydride and stored over potassium hydroxide) (30 mL). The reaction mixture was heated at 85 °C under argon for 2.5 h and cooled, and the solvent was removed in vacuo to afford a black oil. The crude product was dissolved in methylene chloride (100 mL) and filtered through Celite to remove precipitated palladium metal. The filtrate was concentrated and the yellow, oily residue was purified on silica gel. The column was eluted initially with chloroform to remove excess hexabutylditin and phosphines and then 10% methanolic chloroform was added to elute the pure product, which upon removal of solvent appeared as a yellow oil (0.4 g, 68%): ^1H NMR ($CDCl_3$) δ 8.55 (NH), 7.8 (1 H, s), 7.45 (2 H, s), 7.0 (1 H, s), 4.0 (3 H, s), 3.8–1.8 (11 H, m), 1.7–0.8 (30 H, m). The low-resolution mass spectrum showed the M^+ peak at m/e 614.

(*S*)-(−)-7-Iodo-3-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]naphthalene-2-carboxamide, INAP (8). Tin compound 7 (0.3 g, 5×10^{-4} mol) was dissolved in dry chloroform (30 mL), treated with iodine (0.8 g, 0.003 mol), and stirred at room temperature overnight under argon. The excess iodine was quenched with a 10% aqueous sodium thiosulfate solution; the organic layer was washed with water (2 × 25 mL) and dried over anhydrous sodium sulfate. The solvent was removed to afford the crude product as a yellow oil, which was purified on silica gel to afford the iodo product (0.180 g, 82%). ^1H NMR ($CDCl_3$) δ 8.6 (1 H, s), 7.81 (1 H, s), 7.45 (2 H, s) 7.05 (1 H, s) 4.04 (3 H, s) 3.8–1.7 (11 H, m), 1.7–0.7 (30 H, m). Anal. Calcd (CI high-resolution mass spectrum) for $C_{19}H_{23}N_2O_2I$ (M + H) 439.0886, found 439.0882.

3-Methoxy-2-naphthoic Acid Chloride (10). The acid (3 g, 0.016 mol), prepared by hydrolysis of the methyl ester, was dissolved in dry chloroform (100 mL) and treated with thionyl chloride (3.7 g, 0.031 mol) and a catalytic amount of *N,N*-dimethylformamide. The reaction mixture was refluxed for 3 h and the solvent was removed under reduced pressure. The residue was dissolved in toluene (25 mL) and concentrated again to remove any remaining thionyl chloride.

(*S*)-(−)-3-Methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]naphthalene-2-carboxamide (11). Acid chloride 10, prepared as described above, was dissolved in chloroform (30 mL) and added slowly, dropwise, to a solution of excess (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine. The reaction was stirred at room temperature for 1 h and then concentrated in vacuo to afford a yellow, oily product. The oil was dissolved in 2 N HCl and the pH of the solution was then carefully adjusted to 7. The product was extracted into chloroform (3 × 30 mL), dried over anhydrous magnesium sulfate, filtered, and rotary evaporated to afford a yellowish, solid product. The product was chromatographed on silica gel [methylene chloride–methanol (8:2)] to afford a brown oil (4.6 g, 92%): ^1H NMR ($CDCl_3$) δ 8.55 (1 H, s), 8.3 (NH), 7.8–7.1 (4 H, m), 7.03 (1 H, s), 3.91 (3 H, s), 3.8–1.6 (11 H, m), 1.08 (3 H, t); IR (film) 3350 (NH), 1660 cm^{-1} (CO). Anal. Calcd (CI high-resolution mass spectrum) for $C_{19}H_{24}N_2O_2$ (M + H) 313.1919, found 313.1916.

7-Bromo-3-hydroxynaphthoic Acid Chloride (12). Hydroxy acid 3 (2.0 g, 0.0075 mol) was suspended in dry chloroform (50 mL) and treated with thionyl chloride (2.6 g, 0.023 mol) and sufficient *N,N*-dimethylformamide to solubilize the hydroxy acid. The reaction mixture was refluxed for 3 h and then the chloroform was removed under reduced pressure. The residue was dissolved in toluene (25 mL) and concentrated again to remove all thionyl chloride. The gummy, solid acid chloride was used directly in the condensation reaction without purification.

(S)-(−)-7-Bromo-3-hydroxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]naphthalene-2-carboxamide (13). Acid chloride **12**, prepared as described above, was dissolved in chloroform (25 mL) and added slowly, dropwise, to excess 2-(aminomethyl)-*N*-ethylpyrrolidine. The reaction mixture was stirred at room temperature for 2 h and concentrated in vacuo. The residue was dissolved in 2 N HCl and then the pH was carefully adjusted to 7. The product was extracted into chloroform (3 × 50 mL), dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to afford a yellow oil. The oil was purified by silica gel chromatography [methylene chloride–methanol (9:1)] to afford a golden solid (2.43 g, 86%): ^{1}H NMR ($CDCl_3$) δ 9.3 (OH, br s), 8.21 (1 H, s), 7.9 (1 H, s), 7.42 (2 H, s), 7.18 (1 H, s), 2.6–3.7 (11 H, m), 1.38 (3 H, t); IR (film) 3270 (NH), 1660 cm^{-1} (CO). Anal. Calcd (CI high-resolution mass spectrum) for $C_{18}H_{21}N_2O_2Br$ (M + H) 378.0868, found 378.0864. Anal. ($C_{18}H_{21}N_2O_2Br$) H; C: calcd, 57.30; found, 54.79.

2-Methoxy-1-naphthoic Acid Chloride (14). 2-Methoxy-1-naphthoic acid (3 g, 0.016 mol) was dissolved in dry chloroform (100 mL) and treated with thionyl chloride (3.7 g, 0.031 mol) and a catalytic amount of *N,N*-dimethylformamide. The reaction mixture was refluxed for 3 h and the solvent removed was under reduced pressure. The residue was dissolved in toluene (25 mL) and concentrated again to remove any remaining thionyl chloride.

2-Methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]naphthalene-1-carboxamide (15). Acid chloride **14**, prepared as described above, was dissolved in chloroform (30 mL) and added slowly, dropwise, to a solution of excess (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine. The reaction was stirred at room temperature for 1 h and then concentrated in vacuo to afford a yellow, oily product. The oil was dissolved in 2 N HCl and then the pH of the solution was carefully adjusted to 7. The product was extracted into chloroform (3 × 30 mL), dried over anhydrous magnesium sulfate, filtered, and rotary evaporated to afford a yellowish, solid product. The product was chromatographed on silica gel [methylene chloride–methanol (8:2)] to afford a brown oil (4.25 g, 85%): ^{1}H NMR ($CDCl_3$) δ 7.8 (1 H, d), 7.15 (1 H, d), 7.9–7.3 (4 H, m), 6.5 (NH), 3.89 (3 H, s), 3.8–1.6 (11 H, m), 1.1 (3 H, t); IR (film) 3350 (NH), 1660 cm^{-1} (CO). Anal. Calcd (CI high-resolution spectrum) for $C_{19}H_{24}N_2O_2$ (M + H) 313.1919, found 313.1916.

5,7-Dibromo-2,3-dihydrobenzofuran (16). To a solution of 2,3-dihydrobenzofuran (10 g, 0.083 mol) in glacial acetic acid (100 mL) at 0 °C was added dropwise over a period of 30 min bromine (40 g, 0.25 mol) in glacial acetic acid (50 mL). Following the addition, the reaction mixture was stirred overnight at room temperature. Excess bromine was destroyed by addition of 10% sodium thiosulfate solution and the acetic acid was removed under reduced pressure. The oily residue was taken up in methylene chloride (150 mL), washed with a saturated sodium bicarbonate solution (3 × 50 mL) and water (50 mL), and dried over anhydrous sodium sulfate. The solvent was removed in vacuo to afford a yellow oil (21.5 g, 93%): ^{1}H NMR ($CDCl_3$) δ 7.28 (1 H, s), 7.18 (1 H, s), 4.6 (2 H, t), 3.21 (2 H, t).

5-Bromo-7-carboxy-2,3-dihydrobenzofuran (17). Dibromobenzofuran **16** (4 g, 0.014 mol) in dry tetrahydrofuran (distilled from sodium and benzophenone) was placed in a flame-dried flask under argon and cooled to −78 °C. *n*-Butyllithium (8.9 mL, 0.014 mol in a 1.6 M hexane solution) was added to the reaction mixture dropwise via a syringe. Immediately following the addition, carbon dioxide was bubbled through the reaction mixture and the solution was allowed to come to room temperature. The reaction was quenched with water and the solvent was removed in vacuo. To the residue was added 3 N HCl (100 mL) and the mixture was stirred for 10 min. The solid product was filtered, washed with water, and air-dried to afford carboxylic acid **17** (3.1 g, 91%): ^{1}H NMR ($CDCl_3$) δ 7.65 (1 H, s), 7.45 (1 H, s), 5.62 (2 H, t), 3.25 (2 H, t). The low-resolution mass spectrum showed the M$^+$ peak at *m/e* 242. Anal. ($C_9H_7O_3Br$) C, H.

5-Bromo-7-carboxy-2,3-dihydrobenzofuran Chloride (18). Carboxylic acid **17** (2.0 g, 0.0083 mol) was suspended in dry chloroform, treated with thionyl chloride (2.9 g, 0.0248 mol) and a catalytic amount of *N,N*-dimethylformamide, and refluxed for 2 h. The solvent was removed under reduced pressure, and the semisolid residue was dissolved in toluene (30 mL) and concentrated again to remove any remaining thionyl chloride.

5-Bromo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dihydrobenzofuran-7-carboxamide (19). Crude acid chloride **18** was dissolved in dry chloroform (25 mL) and added dropwise to an excess of (*S*)-*N*-ethyl-2-(aminomethyl)pyrrolidine in chloroform (100 mL), and stirred at room temperature for 2 h. The chloroform was concentrated in vacuo and the residue was dissolved in 2 N HCl. The solution was carefully brought to pH 7 with 10% NaOH and the product was extracted with chloroform (3 × 50 mL). The organic extracts were combined, dried, and concentrated to afford a yellow oil, which after purification on silica gel [methylene chloride–methanol (9:1)] gave a white, solid product (**19**, 2.3 g, 79%): ^{1}H NMR ($CDCl_3$) δ 7.85 (1 H, s), 7.7 (NH, br s), 7.15 (1 H, s), 4.62 (2 H, t), 3.5 (2 H, m), 3.2 (2 H, t), 2.4–3.5 (9 H, m), 1.05 (3 H, t); IR (film) 3410, 3320 (NH), 1655 cm^{-1} (CO). Anal. Calcd (CI high-resolution mass spectrum) for $C_{16}H_{21}N_2O_2Br$ (M + H) 353.0868, found 353.0864. Anal. ($C_{16}H_{21}N_2O_2Br$) C, H.

5-(Tributylstannyl)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dihydrobenzofuran-7-carboxamide (20). Bromo compound **19** (0.96 g, 0.0027 mol), hexabutylditin (1.87 g, 0.0032 mol), palladium(II) acetate (0.06 g, 3 × 10^{-4} mol, 0.1 equiv), and tetrakis(triphenylphosphine) palladium[39] (0.150 g, 1 × 10^{-4} mol, 0.05 equiv) were placed in a flame-dried flask equipped for reflux under argon and dissolved in dry triethylamine (distilled from calcium hydride and stored over potassium hydroxide) (30 mL). The reaction mixture was heated at 85 °C under argon for 2.5 h and cooled, and the solvent was removed in vacuo to afford a black oil. The crude product was dissolved in methylene chloride (100 mL) and filtered through Celite to remove precipitated palladium metal. The filtrate was concentrated and the yellow, oily residue purified on silica gel. The column was eluted initially with chloroform to remove excess hexabutylditin and phosphines and then 10% methanolic chloroform was added to elute the pure product, which upon removal of the solvent appeared as a yellow oil (0.710 g, 46%): ^{1}H NMR ($CDCl_3$) δ 8.2 (NH), 7.82 (1 H, s), 7.29 (1 H, s), 4.62 (2 H, t), 3.2 (2 H, t), 4.2–1.6 (11 H, m), 1.5–0.8 (30 H, m). The low-resolution mass spectrum showed the M$^+$ peak at *m/e* 563.

5-Iodo-N-[(1-ethyl-2-pyrrolidinyl)methyl]-2,3-dihydrobenzofurancarboxamide, IBF (21). Tin compound **20** (0.5 g, 9 × 10^{-4} mol) was dissolved in dry chloroform (30 mL), treated with iodine (0.91 g, 0.0036 mol), and stirred at room temperature overnight under argon. The excess iodine was quenched with a 10% aqueous sodium thiosulfate solution; the organic layer washed with water (2 × 25 mL) and dried over anhydrous sodium sulfate. The solvent was removed to afford the crude product as a yellow oil, which was purified on silica gel to afford iodo product **21** (0.210 g, 59%): ^{1}H NMR ($CDCl_3$) δ 8.15 (NH), 7.91 (1 H, s), 7.32 (1 H, s), 4.61 (2 H, t), 3.1 (2 H, t), 3.8–1.6 (11 H, m), 1.27 (3 H, t). Anal. Calcd (CI high-resolution mass spectrum) for $C_{16}H_{21}O_2I$ (M + H) 401.0730, found 401.0726. The HPLC profile of IBF (**21**) showed a single peak (>98% pure) with a retention time of 6.83 min (isocratic elution: 90% CH_3CN–10% dimethylglutaric acid, pH 7.0, and a flow rate of 1 mL/min).

7-Carboxy-2,3-dihydrobenzofuran (22). To a stirred solution of 2,3-dihydrobenzofuran (1 g, 0.0083 mol) and dry TMEDA (1 equiv) in dry tetrahydrofuran (30 mL) at −10 °C under argon was added *n*-BuLi (1.6 M hexane solution, 5 mL, 0.008 mol). The reaction mixture was maintained at this temperature for 20 min and then carbon dioxide was bubbled through the reaction mixture as it warmed to room temperature. The reaction mixture was concentrated in vacuo to afford a yellow oil. The oil was dissolved in 10% sodium hydroxide (100 mL), washed with methylene chloride (2 × 25 mL), and acidified with HCl. The white, solid product was filtered, washed with water, and air-dried to afford carboxylic acid **22** (0.22 g, 18%): ^{1}H NMR ($CDCl_3$) δ 7.35 (1 H, d), 7.11 (1 H, d), 6.59 (1 H, t), 4.37 (2 H, t), 2.99 (2 H, t).

N-[(1-Ethyl-2-pyrrolidinyl)methyl]-2,3-dihydrobenzofurancarboxamide (23). Carboxylic acid **22** was suspended in dry chloroform and treated with thionyl chloride (0.25 g, 0.002 mol) and several drops of dry DMF and the reaction mixture was refluxed for 2 h. The solvent was removed under vacuum to afford the corresponding acid chloride as a yellow oil, which was used directly in the condensation with the pyrrolidyl amine without purification.

The crude acid chloride was dissolved in dry chloroform (5 mL) and added dropwise to an excess of (*S*)-*N*-ethyl-2-(aminomethyl)pyrrolidine in chloroform (25 mL) and stirred at room

temperature for 2 h. The chloroform was concentrated in vacuo and the residue was dissolved in 2 N HCl. The solution was carefully brought to pH 7 with 10% NaOH and the product was extracted with chloroform (3 × 25 mL). The organic extracts were combined, dried, and concentrated to afford a yellow oil, which after purification on silica gel [methylene chloride–methanol (9:1)] gave a thick clear oil (0.26 g, 66%): ^{1}H NMR ($CDCl_3$) δ 8.05 (NH), 7.72 (1 H, d), 7.15 (1 H, d), 6.78 (1 H, d), 4.65 (2 H, t), 3.15 (2 H, t), 3.7–1.6 (11 H, m), 1.14 (3 H, t); IR (film) 3400 (NH), 1650 cm^{-1} (CO). Anal. Calcd (CI high-resolution mass spectrum) for $C_{16}H_{22}N_2O_2$ (M + H) 275.1763, found 275.1760. The HPLC profile of **23** showed a single peak with a retention time of 3.7 min (isocratic elution: 90% CH_3CN–10% dimethylglutaric acid, pH 7.0, and a flow rate of 1 mL/min).

Radiolabeling. Aqueous hydrogen peroxide (50 μL, 3% w/v) was added to a mixture of 50 μL of compound **20** (1 mg/mL of EtOH), 50 μL of 0.1 N HCl, and 5 μL of [^{125}I]sodium 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 30 min, after which it was terminated by the addition of 0.1 mL of sodium bisulfite (300 mg/mL). The reaction mixture was neutralized via the addition of saturated $NaHCO_3$ solution and then 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]IBF (**21**), was isolated from unreacted compound **20** and a small amount of unknown radioactive impurities by HPLC using 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%). After dilution with saline, this agent was used for the in vivo and in vitro studies.

Biodistribution in Rats. Male Sprague–Dawley rats (225–300 g), which were allowed free access to food and water, were used for in vivo biodistribution study. While under halothane anesthesia, 0.2 mL of a saline solution containing [^{125}I]IBF (**21**, 8–10 μCi) was injected directly into the femoral vein of rats, and they 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 gamma automatic counter (Model 4000). The percent dose per 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 under the assumption that they were 7% and 40% of the total body weight, respectively.

Regional brain distribution in rats was obtained after an iv injection of [^{125}I]IBF (**21**). By dissecting, weighing, and counting samples from different brain regions (cortex, striatum, hippocampus, and cerebellum), the percent dose/gram of sample 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 by 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 $MgCl_2$ and kept at −20 °C for the following binding assay.

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-labeled ligand and competitors in a total volume of 0.2 mL of the assay buffer. After an incubation period of 15 min at 37 °C (with stirring), the samples were rapidly filtered in a cell harvester (Brandel M-24R) under vacuum through Whatman GF/B glass-fiber filters pretreated with 0.2% protamine base 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 spiperone. The filters were counted in a γ-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.[41]

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