^{18}F-Labeled Benzamides for Studying the Dopamine D_2 Receptor with Positron Emission Tomography

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Two series of (*N*-benzylpiperidin-4-yl)- and (9-azabicyclo[3.3.1]nonan-3β-yl)benzamides were prepared, and in vitro binding assays were used to measure the affinity of these compounds for dopamine D_2, dopamine D_3, serotonin 5-HT_2, and α_2-adrenergic receptors. The results of these studies indicated compounds **23**, **26b**, and **34** have the selectivity needed for in vivo studies of the D_2 (and possibly D_3) receptors. ^{18}F-Labeled analogues of **23**, **26b** and **34** were prepared by N-alkylation of the corresponding desbenzyl precursors with [^{18}F]-4-fluorobenzyl iodide. Preliminary in vivo studies demonstrated that [^{18}F]-**23** and [^{18}F]-**26b** are suitable candidates for further evaluation in positron emission tomography imaging studies. The slow rate of washout of [^{18}F]-**34** from nondopaminergic regions and its comparatively high lipophilicity indicates that this compound may not be suitable for imaging studies because of a high level of nonspecific binding.

Introduction

Positron emission tomography (PET) is an imaging technique that has the capability of studying neurotransmitter receptor distribution in vivo. Using this technique, it is possible to acquire an index of receptor density in vivo in human brain, assuming that an adequate kinetic model is chosen and the radioligand used displays the appropriate properties. Some of the critical requirements of a PET-based ligand for quantifying CNS receptor density are the ability to cross the blood–brain barrier and a high affinity and selectivity for the neurotransmitter receptor under investigation. Additional features required of a suitable PET ligand include low levels of nonspecific binding and the formation of radiolabeled metabolites that are not capable of entering the brain.

A great deal of attention in the development of PET-based radioligands has focused on agents that can be used to study dopamine receptors. This is primarily due to the identification of a number of potent antipsychotics that bind with high affinity to the D_2 subtype of the dopamine receptor. Additionally, reports of an alteration in D_2 receptor density in schizophrenia,[1,2] Parkinson's disease,[3] Huntington's chorea,[2] and Alzheimer's disease[4,5] have fueled interest in studying these receptors with PET. The antipsychotic that has served as a lead compound for many of the fluorine-18 ([^{18}F]-) and carbon-11 ([^{11}C]-) labeled analogues is the butyrophenone spiperone. A number of human PET imaging studies have been carried out using [^{18}F]spiperone,[6] [^{11}C or ^{18}F]*N*-methylspiperone,[7-11] or [^{18}F]N-(2-fluoroethyl)spiperone.[12,13] However, the high affinity of each of these analogues for serotonin 5-HT_2 receptors may cause problems in receptor quantitation since it is difficult to differentiate the binding of radiotracer to D_2 and 5-HT_2 receptors in regions where they are coexpressed. This difficulty was recently demonstrated by Perlmutter *et al.*,[14] who reported that ~35% of the specific binding of [^{18}F]spiperone in the basal ganglia was to 5-HT_2 receptors in PET imaging studies of the baboon. We recently showed that *N*-(4′-nitrobenzyl)spiperone has a higher D_2:5-HT_2 selectivity than spiperone and *N*-methylspiperone.[15] The low initial brain uptake and oxidative N-dealkylation of the ^{18}F-labeled analogue, resulting in the formation of [^{18}F]spiperone, indicates that this derivative is not suitable for PET studies of the D_2 receptor.[16]

A second class of antipsychotics displaying high affinity and high selectivity for the D_2 receptor are the *N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamides, including sulpiride and raclopride.[17] The only PET-based benzamide analogue used in human imaging studies to date is [^{11}C]raclopride.[18-21] Although raclopride has a higher selectivity for D_2 receptors than spiperone or *N*-methylspiperone, the ability of endogenous dopamine to interfere with the binding of [^{3}H]raclopride to D_2 sites in vitro and in vivo has raised questions regarding the reliability of in vivo estimates of B_{max} in PET studies using [^{11}C]-raclopride.[22-24] This feature of raclopride may be useful, however, in estimating changes in levels of synaptic dopamine under a variety of experimental and pathological conditions.[25] A number of high-affinity radioiodinated benzamide analogues have been developed recently for use in conjunction with single photon emission computed tomography (SPECT).[26-30] Unfortunately, it is not currently possible to conduct quantitative imaging studies of D_2 receptors with SPECT. However, these ligands have served as lead compounds for the development of ^{18}F-labeled benzamide analogues. Examples of this approach include the 5-(2-fluoroethyl) and 5-(3-fluoropropyl) analogues of iodopride,[31,32] epidepride,[33,34] and ioxipride[33,34] (Chart I). A potential drawback of the fluoroalkyl analogues of ioxipride (**4** and **5**) is their relatively high affinity for α_2-adrenergic receptors,[33] which may be as

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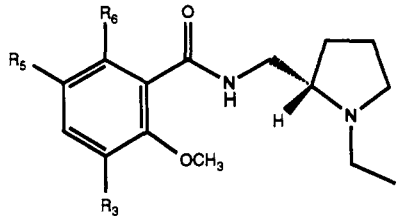
0022-2623/93/1836-3707$04.00/0

Chart I



Compound	R3	R5	R6
Raclopride	Cl	Cl	OH
Iodopride	H	I	H
IBZM	H	I	OH
Epidepride	OCH_3	I	H
NCQ 298 (ioxipride)	OCH_3	I	OH
1	H	$(CH_2)_2F$	H
2	OCH_3	$(CH_2)_2F$	H
3	OCH_3	$(CH_2)_3F$	H
4	OCH_3	$(CH_2)_2F$	OH
5	OCH_3	$(CH_2)_3F$	OH

problematic to quantitative PET studies as the 5-HT_2 binding component is for [^{18}F]spiperone and [^{11}C/^{18}F]-*N*-methylspiperone.

A second class of benzamide analogues that possess a high affinity for D_2 receptors are the *N*-benzyl derivatives including clebopride,[35] YM-09151-2 (emonapride),[36] tropapride,[37] BRL 34778,[38] and (*R*)-flubepride[39] (Chart II). An advantage of these analogues is that they can be labeled readily with ^{18}F via N-alkylation of the corresponding desbenzyl precursor with an ^{18}F-labeled benzyl halide.[40-43] Since most of these analogues contain an unsubstituted *N*-benzyl group, a key requirement in using this appproach is to demonstrate that there is no loss in D_2 affinity on substituting the 4′-position of the *N*-benzyl group with a fluorine atom. In the case of YM 09151-2, it has recently been shown that a marked reduction in affinity for D_2 receptors occurred when making this substitution in the *N*-benzyl group.[40,41] This loss of affinity does not occur with tropapride, since fluorotropapride has an affinity for D_2 receptors similar to that of the parent compound.[44] The occurrence of a 4-fluorobenzyl group in the parent structure of a lead compound avoids this potential problem, and studies using [^{18}F]NCQ 119[45,46] and [^{18}F]FIDA-2,[47] which are analogues of flubepride, were recently reported. The presence of the iodine atom in FIDA-2 is advantageous since cross-comparisons between SPECT and PET can be made using the same ligand for both imaging techniques.[47]

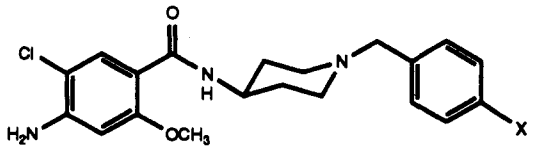
These considerations led us to explore the use of the *N*-benzyl class of benzamides as candidates for developing ^{18}F-labeled radioligands for the D_2 receptor. Our interest in ^{18}F stems from a longer half-life (110 min) in comparison to that of carbon-11 ($t_{1/2}$ = 20.4 min), which is also used in the development of PET-based radiotracers. This increased half-life of ^{18}F is advantageous since fewer time constraints are placed on both radiotracer synthesis and image acquisition. Unlike previous studies, we chose to use clebopride and BRL 34778 as lead compounds for radioligand development. This decision was based on the structural similarity of clebopride and BRL 34778 (Chart II), with the 9-azabicyclo[3.3.1]nonan-3β-yl group of BRL 34778 functioning as a conformationally restricted analogue of clebopride. In addition, the presence of the *N*-(4-fluorobenzyl) group in BRL 34778 suggested that substituting the 4-position of the *N*-benzyl group of clebopride was not likely to result in a loss in affinity for D_2 receptors. We recently confirmed this prediction with our in vitro and in vivo studies of [^{18}F]fluoroclebopride.[43] Although this compound has a relatively high affinity for the D_2 receptor (~1 nM), the rapid rate of washout from dopamine D_2 receptor-rich regions of rat brain indicated that more potent analogues may be more desirable for PET imaging studies. Therefore, we prepared a series of analogues of BRL 34778 and fluoroclebopride by altering the substitution pattern of the aromatic region of the benzamide group. In this report, the synthesis and in vitro binding of a series of structural analogues of BRL 34778 and fluoroclebopride are described. The in vivo behavior of three promising analogues, [^{18}F]-**23**, [^{18}F]-**26b**, and [^{18}F]-**34**, in rats is also reported.

Results

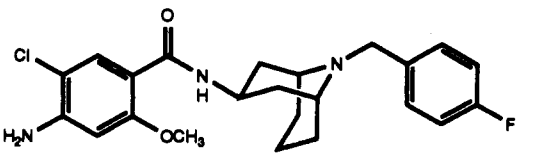
Chemistry. The synthesis of the amine that is required for the preparation of the benzamide analogues containing the 9-benzyl-9-azabicyclo[3.3.1]nonane ring is outlined in Scheme I. Mannich cyclization of 1,3-acetonedicarboxylic acid, glutaric dialdehyde, and either benzylamine or 4-fluorobenzylamine gave the corresponding 9-benzyl-9-azabicyclo[3.3.1]nonan-3-one (**16a,b**) in moderate yield (65%). Conversion to the corresponding methoxime (**17a,b**) and reduction with borane–THF gave the corresponding 3-amino analogues, **18a,b**, as a mixture of α and β isomers (90–98%). Conversion of **18a,b** to the corresponding (*tert*-butyloxycarbonyl)amino derivative, followed by silica gel column chromatography, gave the corresponding α (**19a, 19b**) and β (**20a, 20b**) analogues in approximately a 1:1 mixture. The stereochemistry of the exo and endo isomers of **19** and **20** was assigned by ^{1}H-NMR spectroscopy. With the 3-α (endo) isomer, the (*tert*-butyloxycarbonyl)amino group forces the 9-azabicyclo-[3.3.1]nonane ring into a boat-chair conformation.[48] In the boat-chair conformation, the dihedral angle between the bridgehead protons at C1 and C5 and the equatorial protons of C2 and C4 is 0° and results in a distinctive doublet at 3.05 ppm for the C1 and C5 protons with a coupling constant (J°) of 10.5 Hz. This value of J° is similar to that reported for structurally related analogues.[48] With the 3-β (exo) isomer, the (*tert*-butyloxycarbonyl)-amino group is located in the equatorial position when the 9-azabicyclo[3.3.1]nonane ring is in a chair-chair conformation. In this conformation, the dihedral angle between the bridgehead protons at C1 and C5 and the methylene protons at C2, C4, C6, and C8 is 60° and produces a coupling constant of only ~2 Hz. This small coupling constant results in a broad singlet for the C1 and C5 protons at 2.90 ppm.

The synthesis of benzamides **21** and **23** is outlined in Scheme II. Condensation of 4-amino-1-benzylpiperidine with 2,3-dimethoxybenzoic acid gave **21** in high yield (82%). Catalytic hydrogenation over Pearlman's catalyst gave the desbenzyl analogue, **22** (42%), which was then treated with 4-fluorobenzyl bromide to afford **23** in moderate yield (71%). Condensation of **18b** with 4-amino-5-chloro-2-methoxybenzoic acid[49] (Scheme III) followed by silica gel column chromatography gave benzamides **24a** and **24b** (BRL 34778; total yield 75%). Alternatively, treatment of **20a** with trifluoroacetic acid followed by condensation with 4-amino-5-chloro-2-methoxybenzoic acid gave **25** in excellent yield (88%). A similar approach was used for the synthesis of benzamides **26a-c** (Scheme IV), whereas compounds **27** and **28** were prepared via

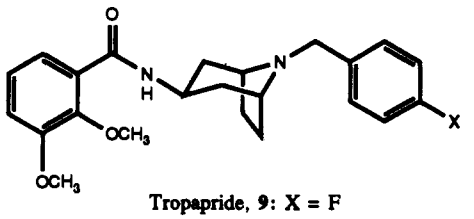
Chart II



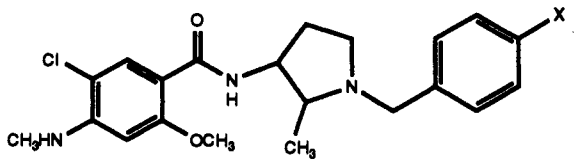
Clebopride, **6**: X = H
Fluoroclebopride, **7**: X = F



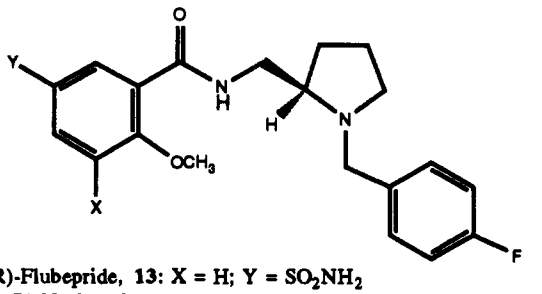
BRL 34778, **8**



Tropapride, **9**: X = F
4-Fluorotropapride, **10**: X = F

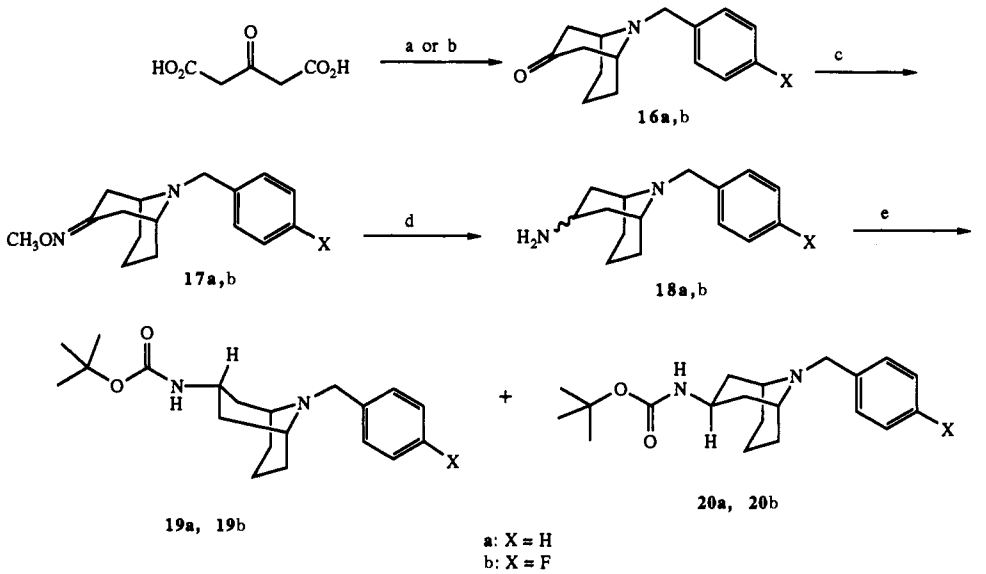


YM 09151-2, **11**: X = H
12: X = F



(R)-Flubepride, **13**: X = H; Y = SO_2NH_2
(R)-NCQ 115, **14**: X = OCH_3; Y = Br
(R)-FIDA-2, **15**: X = OCH_3; Y = I

Scheme I[a]



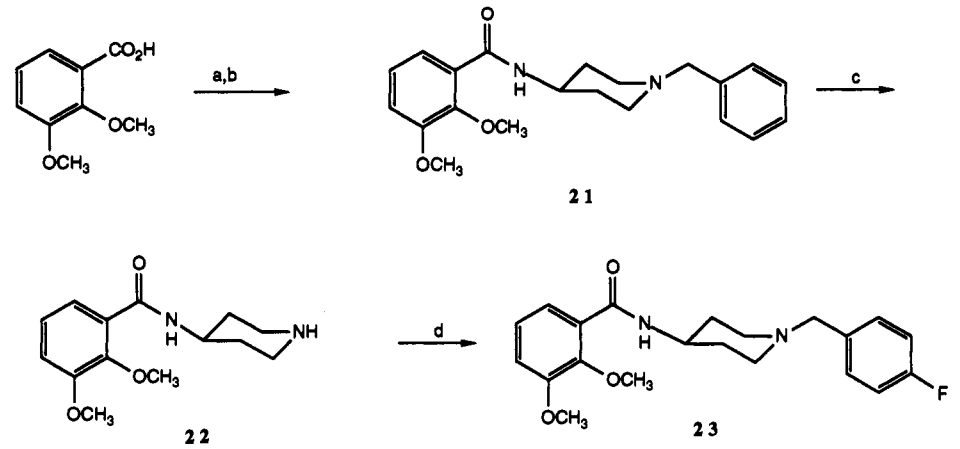
a: X = H
b: X = F

[a]Reagents: (a) $C_6H_5CH_2NH_2/OHC(CH_2)_3CHO/H_2O/12$ h, then pH 2/60 °C/2h; (b) 4-F-$C_6H_4CH_2NH_2/OHC(CH_2)_3CHO/H_2O/12$ h, then pH 2/60 °C/2 h; (c) NH_2OCH_3-HCl/CH_3OH/reflux; (d) BH3/THF/reflux; (e) di-*tert*-butyl dicarbonate/CH_2Cl_2/room temperature.

condensation of the appropriate amine with methyl 5-bromo-2,3-dimethoxybenzoate[50] (Scheme V). The desbenzyl analogue **35** was prepared by hydrogenolysis of **26a** over Pearlman's catalyst (Scheme IV).

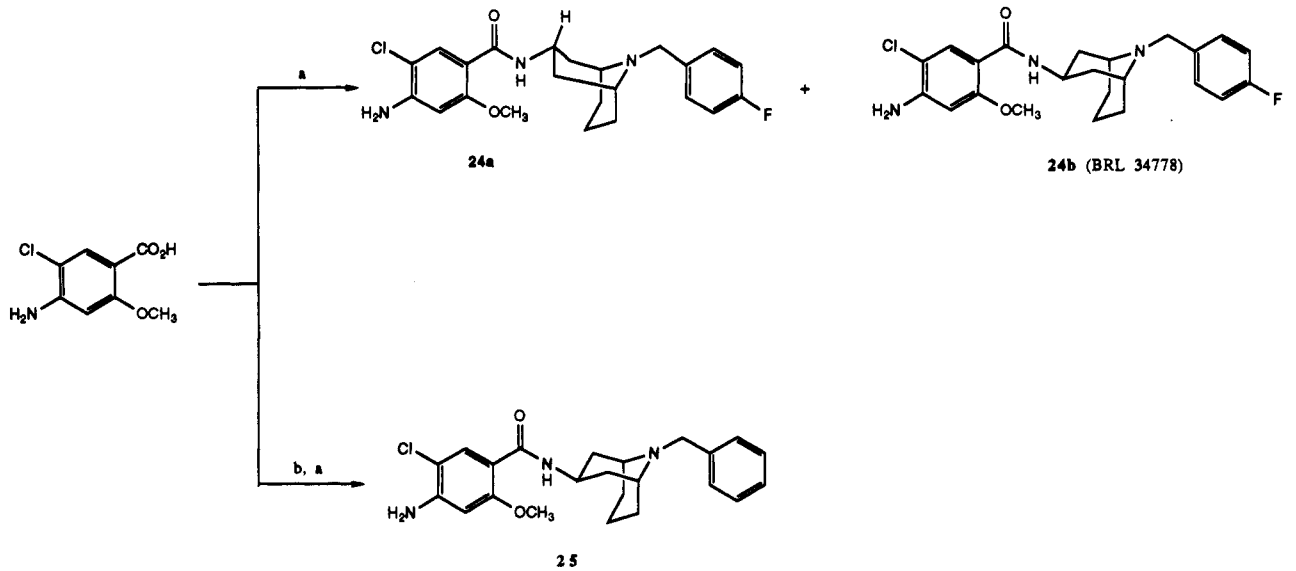
The preparation of **34** required a different approach due to the presence of the 5-bromine atom which is susceptible to hydrogenolysis under the conditions of catalytic hydrogenation. The synthesis was accomplished by using the sequence of reactions outlined in Scheme VI. Treatment of 4-amino-1-benzylpiperidine with trifluoroacetic anhydride gave the trifluoroacetamido derivative, **29**, in high yield (94%). Catalytic hydrogenation over Pearlman's catalyst followed by treatment with di-*tert*-butyl dicarbonate gave the corresponding *tert*-butyloxycarbonyl derivative, **30**, in an overall yield of 82%. Hydrolysis of the trifluoroacetamido group followed by condensation with 5-bromo-2,3-dimethoxybenzoic acid gave the benzamide derivative, **32**. Treatment of **32** with trifluoroacetic acid followed by alkylation with 4-fluorobenzyl bromide gave **34** in an overall yield of 74%.

Scheme II[a]



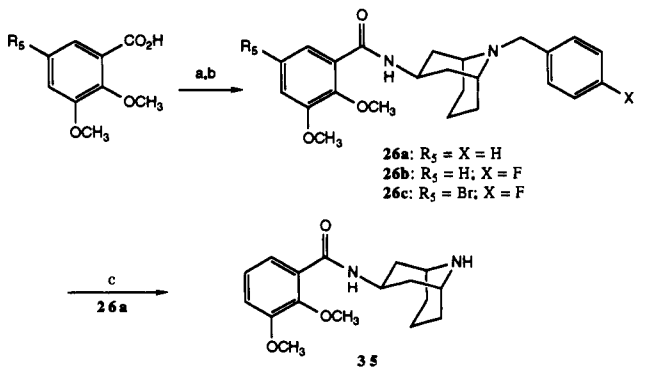
[a]Reagents: (a) $ClCO_2Et/Et_3N/CH_2Cl_2/0$ °C/30 min; (b) 4-amino-1-benzylpiperidine/Et_3N/CH_2Cl_2/1 h; (c) H_2/Pearlman's catalyst/CH_3OH/24 h; (d) 4-fluorobenzyl bromide/CH_3OH/16 h.

Scheme III[a]



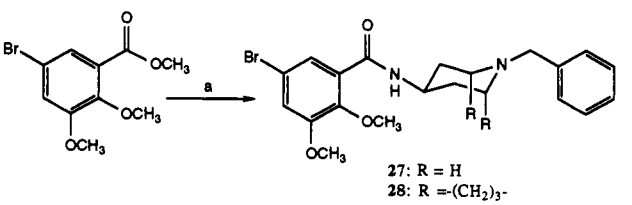
[a]Reagents: (a) $ClCO_2Et/Et_3N/CH_2Cl_2/0$ °C/30 min; then 18a,b or 18a/Et_3N/CH_2Cl2/1 h; (b) 19b/TFA/room temperature/2 h.

Scheme IV[a]



[a]Reagents: (a) $ClCO_2Et/Et_3N/CH_2Cl_2/0$ °C/30 min; (b) amine/Et_3N/CH_2Cl_2/1 h; (c) H_2/Pearlman's catalyst/EtOH.

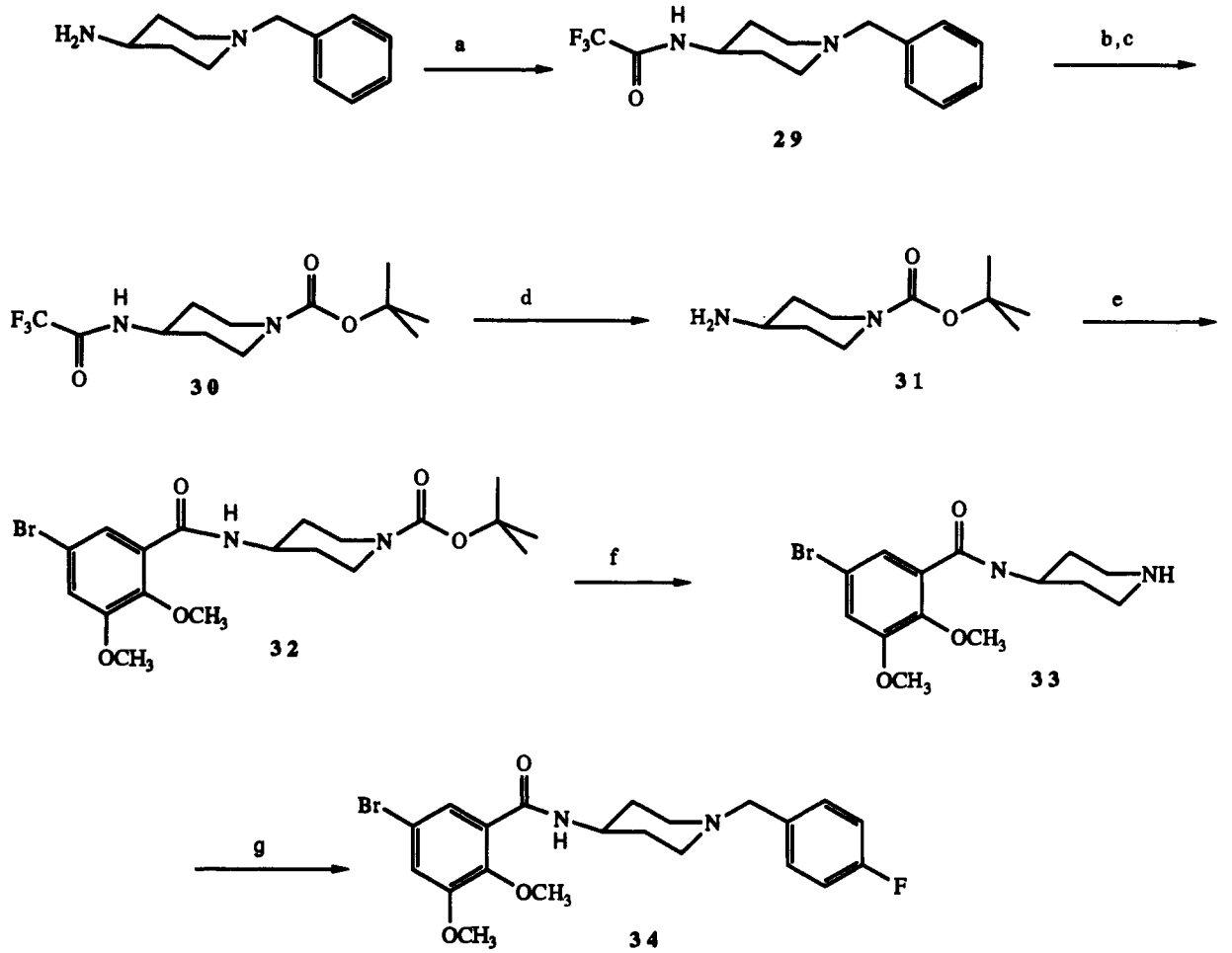
Scheme V[a]



[a]Reagent: (a) $Al(CH_3)_3$/amine/toluene/room temperature, then reflux.

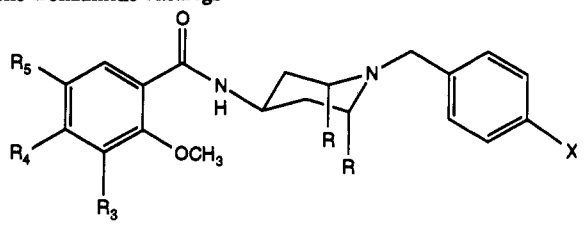
In Vitro Binding Studies. In vitro binding studies were carried out to determine the affinities of the benzamide analogues for dopamine D_2, dopamine D_3, serotonin 5-HT_2, and α_2 -adrenergic receptors. The D_2 receptor assay used tissue homogenates prepared from rat striatum. The 5-HT_2 assays used membrane homogenates prepared from P11 cells derived from pituitary tumor 7315A, which have been shown to express 5-HT_2 receptors in the absence of D_2 receptors or other subtypes of serotonin receptor.[51] The dopamine D_3 assay used membranes prepared from *Spodoptera frugiperda* (Sf9) cells infected with the recombinant baculovirus AcMNPV-rD3. This system has been shown to provide high levels of expression of the D_3 receptor.[52] Radioligands used in the binding assays were [^{125}I]NCQ 298[53] (D_2, D_3), [^{125}I]I-LSD (5-HT_2), and [^{3}H]rauwolscine (α_2). Data from competition experiments were analyzed using a mathematical modeling program to determine the concentration of unlabeled analogue required to inhibit 50% of the binding of the radioligand (IC_{50}). Inhibition constants (K_i) were calculated from IC_{50} values using the method of

Scheme VI[a]



[a]Reagents: (a) $(CF_3CO)_2O$; (b) H_2/Pearlman's catalyst; (c) di-*tert*-butyl dicarbonate/CH_2Cl_2; (d) Et_4NOH/CH_3OH/reflux; (e) 5-bromo-2,3-dimethoxybenzoic acid/$ClCO_2Et/Et_3N/CH_2Cl_2$/0 °C/30 min; (f) CF_3CO_2H/5 min; (g) 4-fluorobenzyl bromide/CH_3OH.

Table I. In Vitro Binding Assays for the Benzamide Analogs



						$K_i \pm$ SEM (nM) [n]				
no.	R_3	R_4	R_5	R	X	D_2[a]	5-HT_2[b]	α_2[c]	log *P*[d]	log K_w[e]
6[f]	H	NH_2	Cl	H	H	1.13 ± 0.30 [3]	89 ± 21 [3]	600 ± 92 [3]	ND[g]	2.72
7[f]	H	NH_2	Cl	H	F	0.95 ± 0.22 [3]	283 ± 53 [3]	1300 ± 460 [3]	2.98	2.95
21	OCH_3	H	H	H	H	0.63 ± 0.05 [3]	ND[g]	ND[g]	ND[g]	ND[g]
23	OCH_3	H	H	H	F	0.38 ± 0.11 [3]	1840 ± 1140 [3]	3000 ± 690 [3]	2.83	2.91
27	OCH_3	H	Br	H	H	0.31 ± 0.24 [3]	ND[g]	ND[g]	ND[g]	ND[g]
34	OCH_3	H	Br	H	F	0.07 ± 0.01 [3]	94 ± 9 [2]	3000 ± 570 [3]	4.00	3.64
25	H	NH_2	Cl	$-(CH_2)_3-$	H	0.036 ± 0.006 [3]	ND[g]	ND[g]	ND[g]	ND[g]
24b	H	NH_2	Cl	$-(CH_2)_3-$	F	0.017 ± 0.002 [3]	3.70 ± 1.60 [3]	>10000 [3]	2.92	2.21
24a	H	NH_2	Cl	$-(CH_2)_3-$	F	>10[h,i]	2200 ± 250 [3]	ND[g]	ND[g]	ND[g]
26a	OCH_3	H	H	$-(CH_2)_3-$	H	0.038 ± 0.001 [3]	ND[g]	ND[g]	ND[g]	ND[g]
26b	OCH_3	H	H	$-(CH_2)_3-$	F	0.028 ± 0.016 [3]	84 ± 18 [3]	1300 ± 390 [3]	2.86	2.36
28	OCH_3	H	Br	$-(CH_2)_3-$	H	0.033 ± 0.019 [3]	ND[g]	ND[g]	ND[g]	ND[g]
26c	OCH_3	H	Br	$-(CH_2)_3-$	F	0.020 ± 0.007 [2]	3.10 ± 1.40 [3]	1900 ± 540 [3]	3.87	3.30

[a] K_i for inhibiting the binding of [^{125}I]NCQ 298 to rat striatal tissue. [b] K_i for inhibiting the binding of [^{125}I]I-LSD to P11 cells. [c] K_i for inhibiting the binding of [^{3}H]rauwolscine to rat cortex. [d] HPLC method of Kraak *et al.*[55] [e] HPLC method of El Tayar *et al.*[56] [f] Reproduced from ref 43. [g] Not determined. [h] Endo isomer of **24b**. [i] IC_{50} was greater than the highest concentration of radioligand used in this assay (10 nM).

Cheng and Prusoff.[54] The results of the binding studies are shown in Tables I and II.

The results of the in vitro binding studies indicate that all the analogues tested possess a high affinity for the D_2 receptor. The only exception to this was **24a**, which is the inactive (endo) isomer of **24b** (BRL 34778). Replacement

Table II. Affinities of Analogues 23, 26b, and 34 for Dopamine D_3 Receptors

compound	K_i (nM)[a]	compound	K_i (nM)[a]
[^{125}I]NCQ 298	0.16 ± 0.02[b]	23	5.91 ± 0.21[d]
spiperone	1.31 ± 0.17[c]	26b	0.21 ± 0.04[e]
clebopride, 6	10.4 ± 1.62[c]	34	0.45 ± 0.09[e]

[a] K_i for inhibiting the binding of [^{125}I]NCQ 298 to Sf9 cells infected with recombinant baculovirus (mean ± SEM). [b] K_d from Scatchard analysis. [c] Reproduced from ref 52. [d] $n = 2$. [e] $n = 3$.

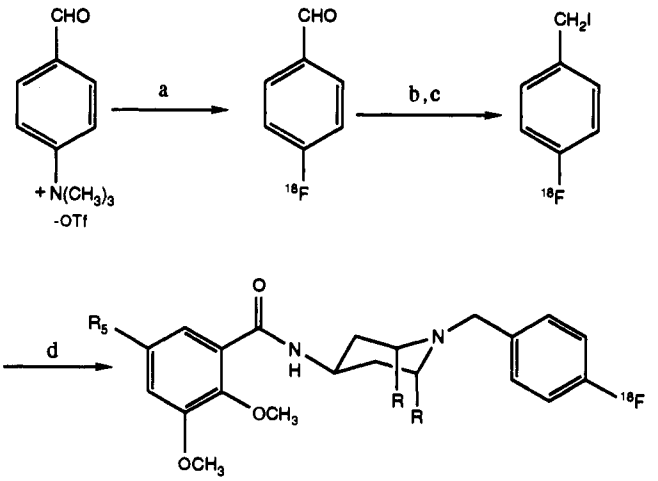
of the 4'-H substituent with a fluorine atom did not reduce the affinity of the compounds for D_2 receptors. The addition of a propylene bridge to give the 9-azabicyclo-[3.3.1]nonane skeleton resulted in a 4–50-fold increase in D_2 affinity (i.e., **6** vs **25**, **7** vs **24b**, and **21** vs **26a**). Replacement of the 4-amino-5-chloro substitution pattern of the clebopride-based analogues (i.e., **6** and **7**) with a 3-methoxy group (to give **21** and **23**) resulted in a 2-fold increase in D_2 affinity. Substitution of the 5-position of **21** and **23** with a bromine atom (to give **27** and **34**) resulted in a further increase in D_2 affinity. Altering the substitution pattern of the analogues containing the 9-azabicyclo-[3.3.1]nonane skeleton did not result in a change in affinity for D_2 receptors since all analogues in this class had picomolar affinity for the D_2 receptor. In vitro binding studies were also carried out to determine the affinities of compounds **23**, **34**, and **26b** for the rat D_3 receptor. The results of this study (Table II) indicate that all three analogues possess a high affinity for the rat dopamine D_3 receptor.

As stated previously, a number of the analogues currently used in PET studies of D_2 receptors possess a high affinity for either serotonin 5-HT_2 or α_2-adrenergic receptors. Although clebopride[39] and BRL 34778[38] are known to have a low affinity for each of these receptors, it was important to determine if the change in substitution pattern in the benzamide ring, as described above, resulted in an analogue possessing an undesired affinity for 5-HT_2 or α_2-adrenergic receptors. Therefore, in vitro binding studies were conducted to determine the affinity of the fluorine-containing compounds for 5-HT_2 and α_2-adrenergic receptors. The results of these studies reveal that the clebopride-based analogues tested (**6**, **7**, **23**, and **34**) have a relatively low affinity for 5-HT_2 receptors ($K_i \sim 100$ nM or higher). In the 9-azabicyclo[3.3.1]nonane series, analogues **24b** and **26c** have a high 5-HT_2 affinity ($K_i \sim 3.70$ and 3.10 nM, respectively). The affinity of **26b** for 5-HT_2 receptors was ~84 nM. None of the analogues were found to have a high affinity for α_2-adrenergic receptors (Table I).

Measurement of Lipophilicity. The lipophilicity of the benzamide analogues was measured by two methods. The first method used the C-18 reversed-phase HPLC procedure of Kraak et al.,[55] which provides a measure of the lipophilicity of a test compound as the log *P*. The second method used the C-18 reversed-phase procedure of El Tayar *et al.*,[56] which gives a relative measurement of lipophilicity, termed log K_w, for protonated basic compounds. This method has been used previously to measure the relative lipophilicities of benzamide analogues.[34,56] The values of log *P* and log K_w for the benzamide analogues are given in Table I.

Radiolabeling and in Vivo Studies. The ^{18}F-labeled analogues of **23**, **26b**, and **34** were prepared via N-alkylation of the corresponding desbenzyl precursors with [^{18}F]-4-fluorobenzyl iodide[42,43] as outlined in Scheme VII. The

Scheme VII[a]



[^{18}F]**23**: R_5 = R = H
[^{18}F]**26b**: R_5 = H; R = -$(CH_2)_3$-
[^{18}F]**34**: R_5 = Br; R = H

[a] Reagents: (a) [^{18}F]CsF/aqueous DMSO/120 °C/10 min; (b) $LiAlH_4$/THF/pentane; (c) 57% aqueous HI; (d) desbenzyl precursor/DMF/90 °C/10 min.

product was purified by reversed-phase semipreparative HPLC to give the ^{18}F-labeled analogues in an overall yield ranging from 28 to 57%. The calculated specific activity ranged from 180 to 1250 mCi/μmol decay corrected to end of bombardment (EOB).

In vivo studies were carried out in rats to determine the ability of ^{18}F-labeled **23**, **26b**, and **34** to cross the blood–brain barrier and label D_2 receptors in vivo. Rats were injected with ^{18}F-labeled ligand (20–120 μCi) and sacrificed at predetermined time points (5 min to 5 h). The brain was removed, weighed, and counted in a γ counter to determine radioligand whole-brain uptake. Blood samples were also taken to measure the rate of clearance of radioactivity from blood. The brain was dissected into striatum, frontal cortex, hippocampus, and cerebellum. Each region was weighed and counted to determine the amount of radioactivity in each region of interest as a function of time.

The results of the in vivo studies indicate that all three analogues are capable of crossing the blood–brain barrier (Table III). The 5-min brain uptake (% ID/g brain) of [^{18}F]-**23**, [^{18}F]-**26b**, and [^{18}F]-**34** was 0.81 ± 0.11, 0.39 ± 0.04, and 0.58 ± 0.06, respectively. All three analogues were rapidly cleared from blood. Regional brain dissection revealed that all three analogues have a high accumulation and slow rate of washout of radioactivity from the striatum (Figures 1–3), a region of rat brain expressing a high density of D_2 receptors.[57] There was a rapid rate of washout of radiotracer from the frontal cortex (FC), hippocampus, and cerebellum. However, the rate of washout of [^{18}F]-**34** (Figure 3) from these regions that have a low density of D_2 receptors was much slower than that observed with [^{18}F]-**23** (Figure 1) and [^{18}F]-**26b** (Figure 2). This observation may reflect a higher degree of nonspecific binding of [^{18}F]-**34** and is consistent with its higher lipophilicity relative to [^{18}F]-**23** and [^{18}F]-**26b** (Table I). All three compounds had a high striatum:cerebellum ratio, which is a measure of the specific:nonspecific binding of a dopamine-based radioligand in vivo. The striatum:cerebellum ratio of [^{18}F]-**23** reached a maximum value of

Table III. Brain Uptake of the ^{18}F-Labeled Benzamide Analogues in Rats[a]

t	% ID/brain	% ID/g brain	% ID/g blood	brain:blood ratio[b]
		[^{18}F]-**23**		
5 min	1.28 ± 0.15	0.81 ± 0.11	0.155 ± 0.010	5.32 ± 0.45
30 min	0.61 ± 0.02	0.39 ± 0.01	0.066 ± 0.010	6.34 ± 0.97
1 h	0.57 ± 0.05	0.36 ± 0.04	0.043 ± 0.009	9.12 ± 1.27
2 h	0.50 ± 0.02	0.33 ± 0.02	0.029 ± 0.002	11.26 ± 0.49
3 h	0.38 ± 0.02	0.25 ± 0.02	0.017 ± 0.002	14.91 ± 1.05
4 h	0.17 ± 0.01	0.11 ± 0.01	0.010 ± 0.001	11.44 ± 1.09
5 h	0.19 ± 0.01	0.12 ± 0.01	0.012 ± 0.001	10.04 ± 1.31
		[^{18}F]-**26b**		
5 min	0.64 ± 0.05	0.39 ± 0.04	0.156 ± 0.018	2.63 ± 0.43
30 min	0.35 ± 0.04	0.20 ± 0.02	0.089 ± 0.017	2.63 ± 0.73
1 h	0.31 ± 0.03	0.23 ± 0.03	0.044 ± 0.003	4.55 ± 0.45
2 h	0.44 ± 0.07	0.27 ± 0.03	0.024 ± 0.001	14.77 ± 2.50
3 h	0.37 ± 0.04	0.23 ± 0.03	0.019 ± 0.002	19.37 ± 2.77
4 h	0.36 ± 0.05	0.22 ± 0.03	0.013 ± 0.003	20.25 ± 2.88
5 h	0.23 ± 0.03	0.14 ± 0.02	0.005 ± 0.002	23.50 ± 3.67
		[^{18}F]-**34**		
5 min	1.12 ± 0.10	0.58 ± 0.06	0.088 ± 0.017	7.56 ± 0.85
30 min	0.55 ± 0.05	0.32 ± 0.04	0.036 ± 0.013	7.15 ± 0.80
1 h	0.36 ± 0.01	0.20 ± 0.01	0.025 ± 0.002	7.40 ± 0.29
2 h	0.27 ± 0.02	0.14 ± 0.01	0.016 ± 0.001	8.72 ± 0.48
3 h	0.20 ± 0.01	0.10 ± 0.01	0.013 ± 0.003	10.21 ± 0.80
4 h	0.21 ± 0.01	0.12 ± 0.01	0.012 ± 0.001	10.00 ± 0.64
5 h	0.20 ± 0.06	0.12 ± 0.03	0.012 ± 0.004	10.29 ± 0.55

[a] Mean ± SEM (n = 4–8). [b] (% ID/g of brain)/(% ID/g of blood).

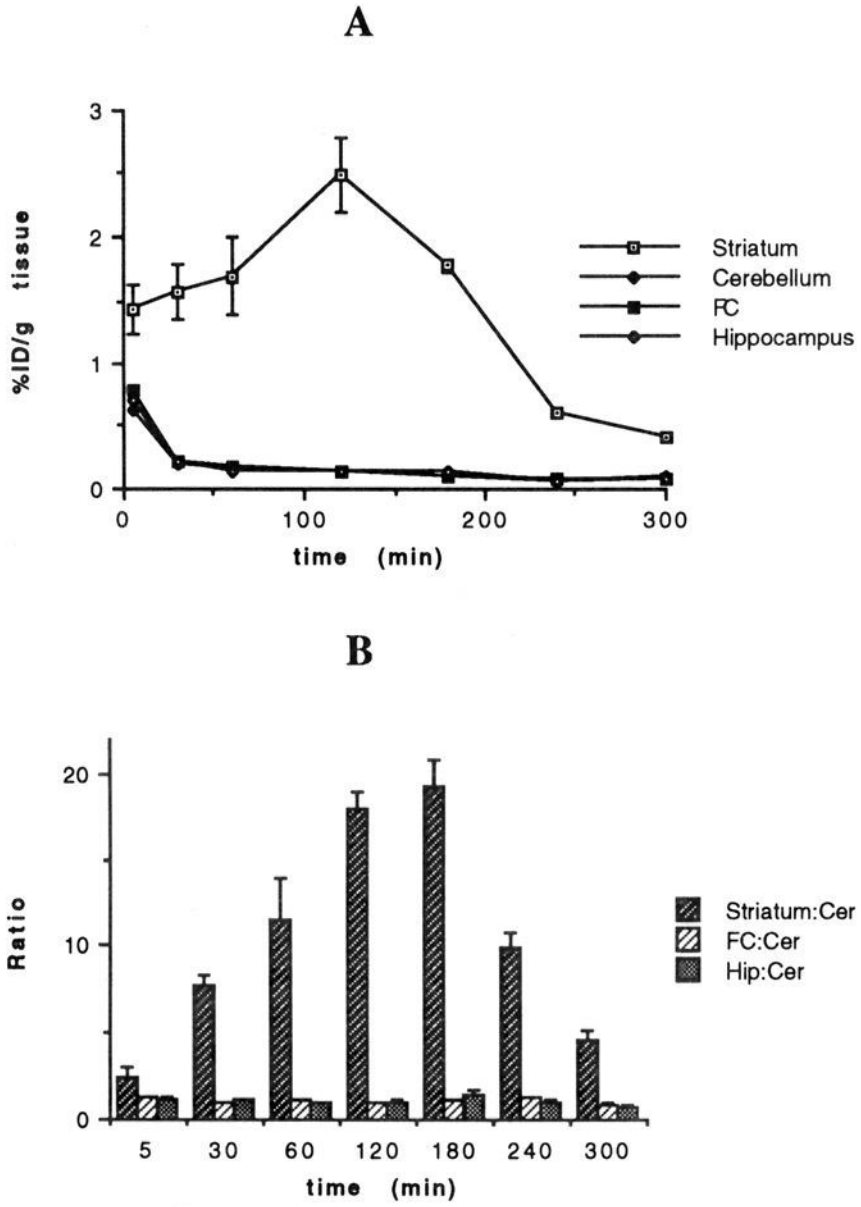


Figure 1. Regional brain uptake (A) and striatum:cerebellum, FC:cerebellum, and hippocampus:cerebellum ratios (B) of [^{18}F]-**23** in rat brain as a function of time. Values are mean + SEM (n = 4–8).

~20:1 and declined to ~5:1 at 3 h post-injection (Figure 1), whereas [^{18}F]-**26b** displayed a progressive increase in striatum:cerebellum ratio over the course of the study (Figure 2). The striatum:cerebellum ratio of [^{18}F]-**34** leveled off at ~30:1 3 h postinjection and remained relatively constant thereafter (Figure 3). The accumulation of all three radiotracers was blocked by coinjection with spiperone (Figure 4), which is consistent with the labeling of D_2 receptors in vivo.

Discussion

The goal of this research was to prepare an ^{18}F-labeled benzamide analogue suitable for conducting quantitative PET studies of the dopamine D_2 receptor. The choice of clebopride and BRL 34778 as lead compounds for these studies was based on their close structural similarity and the presence of the 4-fluorobenzyl group in BRL 34778. Substitution of the 4-position of the benzyl group of clebopride with a fluorine atom was not likely, therefore, to lead to a marked reduction in D_2 receptor affinity and also would provide an analogue that could be radiolabeled using 4-fluorobenzyl iodide.[42,43] These predictions were verified in studies of [^{18}F]fluoroclebopride.[44] Although [^{18}F]fluoroclebopride has a relatively high affinity for D_2 receptors (1 nM), its rapid rate of washout from rat brain indicated that more potent analogues would be required for conducting PET studies of D_2 receptors.

The approach taken to increase the affinity of the lead compounds involved altering the substitution pattern of the aromatic ring of the benzamide moiety. The choice of substituents was based on a series of structure–activity relationship studies within the *N*-benzylpiperidine series which revealed that replacement of the 4-amino-5-chloro-2-methoxy substitution pattern of clebopride with a 2,3-dimethoxy group resulted in an increase in D_2 affinity.[37] These results were confirmed in our studies since the affinity of the 2,3-dimethoxybenzamide analogues **21** and **23** for D_2 receptors (Table I) was higher than that measured for clebopride (**6**) and fluoroclebopride (**7**). Substitution of the 5-position with a bromine atom (**27** and **34**) resulted in an additional increase in D_2 affinity relative to the unsubstituted analogues (**21** and **23**). In a previous study, no difference was observed between **21** and **27** as inhibitors of the binding of [^{3}H]spiperone to rat striatal tissue.[58] The difference in D_2 receptor affinity between **21** and **27** in the current study was not significant due to the large standard error of measurement in the K_i value of **27**. In the 9-azabicyclo[3.3.1]nonane series, the 3α isomer was found to have a D_2 receptor affinity several orders of magnitude lower than that of the corresponding 3β isomer (i.e., **24a** vs **24b**). The D_2 receptor affinity of the 9-azabicyclo[3.3.1]nonan-3β-yl analogues was 4–50-fold higher than that of the corresponding piperidin-4-yl congener. There was, however, no substituent effect with respect to D_2 receptor affinity in the 9-azabicyclo[3.3.1]-nonan-3β-yl series of compounds, since all analogues displayed a picomolar affinity for the D_2 receptor. As expected, substitution of the 4′-benzyl group with a fluorine atom did not result in a reduction in D_2 receptor affinity.

In vitro binding assays were also conducted to determine the affinity of the fluorine-containing benzamide analogues for serotonin 5-HT_2 and α_2-adrenergic receptors. Since many PET-based radioligands have been shown to have a high affinity for these receptors, it was necessary to carry out these studies to determine if any of the compounds described above are more selective than the analogues currently in use. The results of assays of 5-HT_2 receptors (Table I) indicate that all of the piperidin-4-yl analogues

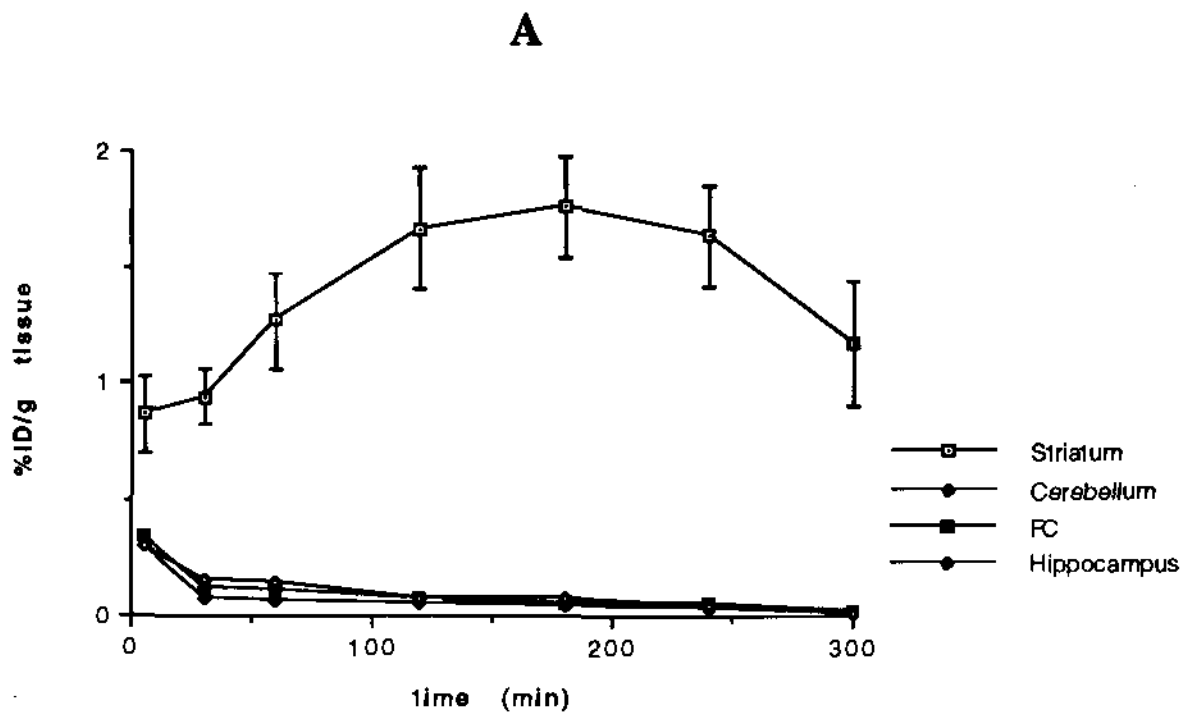


Figure 2. Regional brain uptake (A) and striatum:cerebellum, FC:cerebellum, and hippocampus:cerebellum ratios (B) of [^{18}F]-**26c** in rat brain as a function of time. Values are mean + SEM (n = 4–8).

tested have a relatively low affinity for the 5-HT_2 receptor. In the 9-azabicyclo[3.3.1]nonan-3β-yl series, only **26b** was found to have the low affinity for 5-HT_2 receptors that is required for in vivo studies of D_2 receptors. The high 5-HT_2 affinity of **21b** (BRL 34778; 3.70 ± 1.60 nM) and **23c** (3.10 ± 1.40) would potentially lead to the same set of complications that has been observed with in vivo studies using radiolabeled spiperone and N-methylspiperone.[14] None of the analogues tested had a high affinity for α_2-adrenergic receptors (Table I).[59]

The results of the in vitro binding assays indicated that compounds **23**, **26b**, and **34** were the most suitable candidates for in vivo evaluation. The ^{18}F-labeled analogues were prepared by N-alkylation of the corresponding desbenzyl precursors with [^{18}F]-4-fluorobenzyl iodide (Scheme VII), and in vivo studies were carried out in rats. The results of these studies indicate that all three analogues are capable of crossing the blood–brain barrier and show a high initial brain uptake (Table III). The % ID/g brain at 5 min postinjection displayed the following rank order: [^{18}F]-**23** > [^{18}F]-**34** > [^{18}F]-**26b**. All three compounds displayed rapid clearance from blood and an increasing brain:blood ratio over the course of the study (Table III). Regional brain dissection revealed a high accumulation of radioactivity of all three radiotracers in the striatum, a region of rat brain expressing a high density of dopamine D_2 receptors.[57] The rate of washout from the striatum was inversely proportional to D_2 receptor affinity, with [^{18}F]-**23** (Figure 1) displaying the fastest rate of striatal washout. There was very little washout of radioactivity of [^{18}F]-**26b** (Figure 2) and [^{18}F]-**34** (Figure 3) from the striatum over the 5-h course of the study. The washout of radioactivity from the frontal cortex (FC), hippocampus, and cerebellum was faster for [^{18}F]-**23** and [^{18}F]-**26b** than for [^{18}F]-**34**. This may be attributed to the higher lipophilicity of [^{18}F]-**34** (log P = 4.00; log K_w = 3.64) vs [^{18}F]-**23** (log P = 2.83; log K_w = 2.91) and [^{18}F]-**26b** (log P = 2.86; log K_w = 2.36), which results in a higher nonspecific binding and slower rate of washout from regions not expressing D_2 receptors. The ratio of specific: nonspecific binding was approximated by calculating the ratio of the % ID/g of radioactivity in the striatum to that of the cerebellum (i.e., the striatum:cerebellum ratio). The striatum:cerebellum ratio of [^{18}F]-**23** reached a maximum value of ~20:1 at 3 h postinjection and then gradually decreased (Figure 1). However, [^{18}F]-**26b** displayed a progressive increase in striatum:cerebellum ratio over the course of the study (Figure 2). The striatum:cerebellum ratio of [^{18}F]-**34** leveled off and remained at ~30:1 3 h post-injection (Figure 3). The accumulation of radioactivity in the striatum for all three analogues was blocked by co-injection with spiperone (~60 μg/animal), which is also consistent with the labeling of D_2 receptors in vivo (Figure 4). Spiperone had no effect on the accumulation of [^{18}F]-**23**, [^{18}F]-**26b**, or [^{18}F]-**34** in the frontal cortex, hippocampus, and cerebellum (data not shown). The

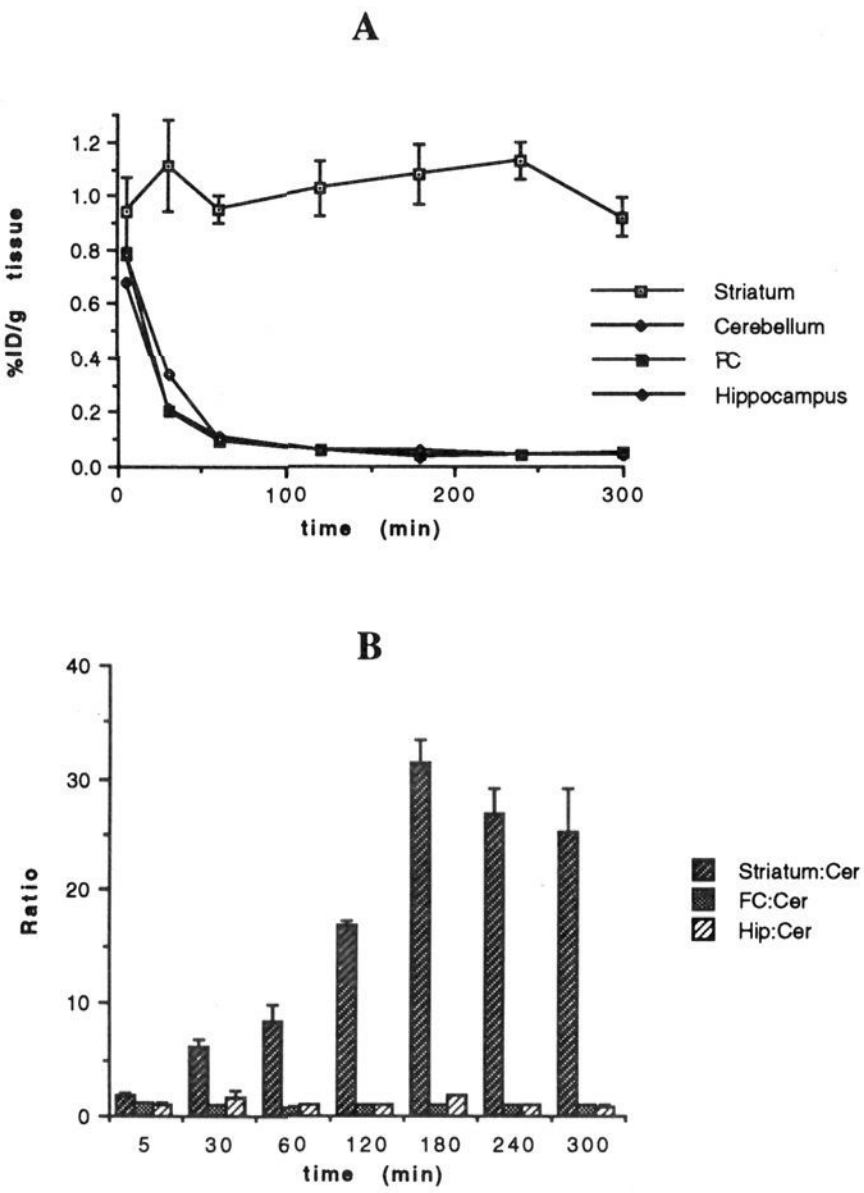


Figure 3. Regional brain uptake (A) and striatum:cerebellum, FC:cerebellum, and hippocampus:cerebellum ratios (B) of [^{18}F]-**34** in rat brain as a function of time. Values are mean + SEM (n = 4–6).

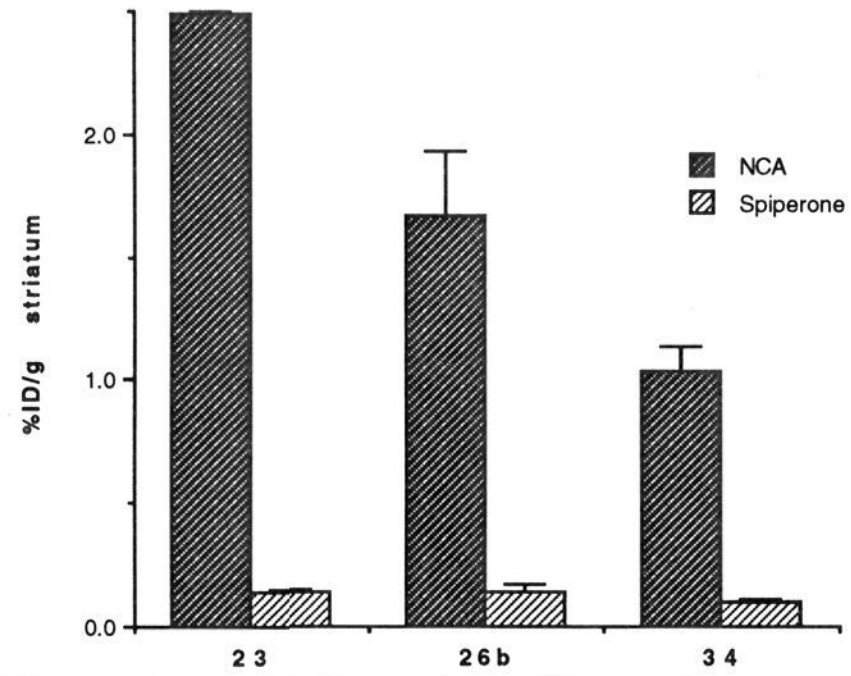


Figure 4. In vivo blocking studies of [^{18}F]-**23**, [^{18}F]-**26c**, and [^{18}F]-**34**. NCA refers to no-carrier-added study; blocking studies were conducted by co-injection of the ^{18}F-labeled radiotracer with ~60 μg of spiperone. Values are mean + SEM (n = 4–6).

above data indicate that [^{18}F]-**23** and [^{18}F]-**26b** are suitable candidates for further evaluation as PET ligands for studies of the D_2 receptor. The relatively high nonspecific binding of [^{18}F]-**34** makes this compound less attractive for PET imaging studies.

Recent advances in molecular genetics have shown that there is a family of D_2-like receptors, which includes the D_2 (both long and short isoforms), D_3, and D_4 receptors.[60] In situ hybridization techniques have revealed a high expression of D_2 mRNA in caudate, putamen, nucleus accumbens, and olfactory tubercle, which correspond to dopaminergic projection regions.[61,62] In contrast, D_3 receptor mRNA is primarily expressed in limbic regions of rat brain including the olfactory tubercle, nucleus accumbens, hypothalamus, islands of Calleja, and the ventral striatum.[61,62] The relative expression of D_3 mRNA in rat striatum was significantly less than that found for D_2 receptor mRNA.[62] This is consistent with in vitro autoradiographic studies demonstrating low levels of binding of [^{3}H]quinpirole to D_3 sites in rat ventral striatum.[64] However, using [^{125}I]epidepride, Murray *et al.*[65] recently demonstrated islands of dense labeling of D_3 receptors in the nucleus accumbens and ventral putamen of human brain. Since these correspond to regions of interest in human CNS PET studies, a knowledge of the D_2/D_3 selectivity of a D_2-based ligand may prove to be important for properly interpreting PET studies using radiotracers that label the D_2 receptor in vivo. The availability of a baculovirus expression system providing a homogeneous source of D_3 receptors[52] permitted measurement of the affinities of **23**, **26b**, and **34** for D_3 receptors. The results of this study, shown in Table II, indicate that all three analogues have a high affinity for D_3 receptors. Analogues **26b** (K_i = 0.21 ± 0.04 nM) and **34** (K_i = 0.45 ± 0.09 nM) are of additional interest since only a limited number of compounds have been shown to possess a subnanomolar affinity for dopamine D_3 receptors.[52,61]

In conclusion, two series of piperidin-4-yl- and (9-azabicyclo[3.3.1]nonan-3β-yl)benzamide analogues were prepared, and their affinities for D_2, D_3, 5-HT$_2$, and α_2-adrenergic receptors were determined in vitro. The results reveal that **23**, **26b**, and **34** have a high affinity for both D_2 and D_3 dopamine receptors and a low affinity for 5-HT$_2$ and α_2-adrenergic receptors. In vivo studies with the corresponding ^{18}F-labeled analogues indicate that [^{18}F]-**23** and [^{18}F]-**26b** may be suitable as PET radioligands. The high lipophilicity and slow rate of washout from nondopaminergic regions indicate [^{18}F]-**34** may not be suitable for PET studies because of its high degree of nonspecific binding. Imaging studies in nonhuman primates are currently being conducted with [^{18}F]-**23** and [^{18}F]-**26b** in order to determine if these analogues have the proper kinetics for conducting quantitative studies of D_2 receptors with PET. Additional studies are also being conducted in order to determine if [^{18}F]-**23** and [^{18}F]-**26b** label dopamine D_3 receptors under the conditions of PET.

Experimental Section

Melting points were determined in an open capillary tube with a Mel-Temp melting point apparatus and are uncorrected. IR spectra were determined on a Perkin Elmer 1600 Series FT-IR. ^{1}H NMR spectra were recorded on either a Varian EM-360L (60 MHz) or a Varian XL-200 (200 MHz) NMR spectrometer. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA, and were within ±0.4% of the theoretical value unless otherwise noted. Tetrahydrofuran was distilled from sodium metal immediately prior to use. All other reagents and solvents were used without further purification. Spiperone was purchased from Janssen Life Sciences Products, 40 Kingsbridge Rd, Piscataway, NJ 08854. Radioligands for the receptor binding assays were either purchased from or provided by Dupont NEN Research Products, Boston, MA.

9-Benzyl-9-azabicyclo[3.3.1]nonan-3-one (16a). Acetone-1,3-dicarboxylic acid (90%, 10.14 g, 62.4 mmol) and glutaraldehyde (25% w/w in H_2O, 35 mL, 93 mmol) were dissolved in water (100 mL). Benzylamine (6.00 mL, 4.9 mmol) was added to the vigorously stirred solution in one portion. After about 2

min the reaction mixture turned orange and gas evolution commenced, resulting in a thick gummy foam. The reaction mixture was stirred for an additional 12 h and then adjusted to pH < 2 with aqueous 12 N HCl. The now-clear solution was then heated at 60 °C for 1 h to drive the decarboxylation to completion. After cooling the solution was extracted with dichloromethane (3 × 50 mL) to remove excess glutaraldehyde. An aqueous solution of NaOH (12 N) was added to adjust the solution to pH > 10, and the free amine was extracted with CH_2Cl_2 (4 × 30 mL). The organic extracts were dried (sodium sulfate), filtered, and concentrated to yield a pale orange solid (8.15 g, 65%). This material was pure enough for subsequent reactions, although further purification by silica gel chromatography with 1:3 EtOAc/hexanes as the eluent gave a white waxy solid: mp 63–66 °C; NMR ($CDCl_3$/TMS) δ 1.1–1.9 (m, 6H), 2.2 (br d, J = 16 Hz, 2H), 2.7 (dd, J = 7, 16 Hz, 2H), 3.25 (br m, 2H), 3.85 (br s, 2H), 7.25 (br s, 5H); IR (KBr) 1704 (C=O) cm^{-1}. Anal. ($C_{15}H_{19}NO$) C, H, N.

9-(4-Fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3-one (16b): yield 65%; mp 63–64 °C; NMR ($CDCl_3$/TMS) δ 1.0–3.0 (m, 10H), 3.0–3.4 (m, 2H), 3.80 (s, 2H), and 6.6–7.4 (m, 4H); IR (KBr) 1705 (C=O) cm^{-1}.

9-Benzyl-9-azabicyclo[3.3.1]nonan-3-one Methoxime (17a). 9-Benzyl-9-azabicyclo[3.3.1]non-2-one (11.785 g, 51.4 mmol) and methoxylamine hydrochloride (11.73 g, 140 mmol) were dissolved in methanol (100 mL), and the clear solution was stirred at reflux for 2 h. The reaction mixture was concentrated and the residue dissolved in 50 mL of water. Aqueous NaOH (2 N) was added to adjust the solution to pH > 12 and the methoxime extracted with dichloromethane (3 × 25 mL). The organic extracts were combined, dried (sodium sulfate), filtered, and concentrated to leave a viscous oil. Chromatography of this residue on silica gel with 6:1 hexanes/EtOAc gave the product as an amorphous tan solid (13.139 g, 99%): mp 40–45 °C; NMR ($CDCl_3$/TMS) δ 1.2–2.0 (br m, 6), 2.1–2.8 (m, 4), 3.0 (br m, 2), 3.75 (s, 5), and 7.25 (br s, 5); IR (KBr) 1585 (C=N) cm^{-1}. Anal. ($C_{16}H_{22}N_2O$) C, H, N.

9-(4-Fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3-one methoxime (17b): yield 97%; mp 37–39 °C; NMR ($CDCl_3$/TMS) δ 1.10–3.30 (m, 12 H), 3.80 (s, 5H), and 6.70–7.50 (m, 4H); IR (film) 1604 (C=N) cm^{-1}.

3α/β-Amino-9-benzyl-9-azabicyclo[3.3.1]nonane (18a). A 1 M solution of borane–THF complex in tetrahydrofuran (54 mL, 54 mmol) was added slowly to a stirred solution of 17a (7.0 g, 27 mmol) in dry tetrahydrofuran (20 mL), and the reaction mixture was stirred at reflux for 20 h. Volatile components were removed in vacuo to give a residue that was dissolved in absolute ethanol (25 mL) and treated with a saturated solution of HCl in ethanol (100 mL). The reaction mixture was stirred at reflux for 3 h, cooled to ambient temperature, and concentrated in vacuo to give a viscous yellow oil. The residue was dissolved in saturated aqueous sodium bicarbonate (50 mL), and an aqueous solution of sodium hydroxide (1 M) was added to adjust the solution to a pH of 12. The aqueous solution was extracted with dichloromethane (3 × 100 mL), and the combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give a dark yellow oil. Purification by silica gel column chromatography (dichloromethane/acetone/ammonium hydroxide, 4:1:0.1) afforded 18a as a light yellow oil of sufficient purity for the next step (5.63 g, 90%): NMR ($CDCl_3$/TMS) δ 0.95–2.35 (m, 12H), 2.90 (br s, 2H), 3.10- 3.75 (m, 2H), 3.80 (s, 2H), and 7.30 (br s, 5H); IR (film) 3350, 3084, 3061, 3026, and 1584 cm^{-1}.

3α/β-Amino-9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonane (18b): yield 98%; NMR ($CDCl_3$/TMS) δ 0.80–2.20 (m, 12H), 2.70–3.30 (m, 3H), 3.70 (s, 2H), and 6.70–7.45 (m, 4H); IR (film): 2924, 2868, and 1604 cm^{-1}.

3α- and 3β-[(*tert*-Butyloxycarbonyl)amino]-9-benzyl-9-azabicyclo[3.3.1]nonane (19a, 20a). A solution of di-*tert*-butyl dicarbonate (15.2 mmol) in dichloromethane (10 mL) was added to a stirred solution of 18a (4.63 g, 20 mmol) in dichloromethane (10 mL) and the reaction mixture was stirred at ambient temperature for 2 h. Volatile components were removed in vacuo to give a white solid that was purified by florisil column chromatography (dichloromethane/acetone/ammomium hydroxide, 9:1:0.1) to give the endo isomer, 19a, as a fluffy white solid (2.03 g, 40.4%): mp 169–170 °C; NMR ($CDCl_3$/TMS) δ 0.80–2.00 (complex m, 17H), 2.35 (dt, J = 11, 6 Hz, 2H), 3.05 (d, J = 11 Hz, 2H), 3.80 (s, 2H), 3.90–4.40 (m, 2H), and 7.10–7.40 (m, 5H); IR (film) 3323 (NH) and 1680 (C=O) cm^{-1}. Anal. ($C_{20}H_{30}N_2O_2$) C, H, N.

Further elution of the column afforded the exo isomer, **20a**, as a fluffy white solid (2.19 g, 43.6%): mp 161–162 °C; NMR ($CDCl_3$/TMS) δ 1.10–2.10 (complex m, 19H), 2.90 (br s, 2H), 3.80 (s, 2H), 4.15–4.45 (m, 2H), and 7.20–7.45 (m, 5H); IR (film) 3325 (NH) and 1681 (C=O) cm^{-1}. Anal. ($C_{20}H_{30}N_2O_2$) H, N; C: calcd, 72.69; found, 71.74.

3α-[(*tert*-Butyloxycarbonyl)amino]-9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonane (19b): yield 21.2%; mp 166–168 °C; NMR ($CDCl_3$/TMS) δ 0.9–2.0 (complex m, 17H), 2.30 (dt, J = 12, 6 Hz, 2H), 3.00 (d, J = 12 Hz, 2H), 3.70 (s, 2H), 3.90–4.40 (m, 2H), 6.85–7.00 (m, 2H), and 7.20–7.35 (m, 2H); IR (film) 3324 (NH) and 1674 (C=O) cm^{-1}. Anal. ($C_{20}H_{28}N_2O_2F$) H, N; C: calcd, 68.94; found, 68.20.

3β-[(*tert*-Butyloxycarbonyl)amino]-9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonane (20b): yield: 26.9%; mp 140–145 °C; NMR ($CDCl_3$/TMS) δ 1.30–2.10 (complex m, 19H), 2.85 (s, 2H), 3.85 (s, 2H), 4.10–4.50 (m, 2H), 6.90–7.05 (m, 2H), and 7.20-7.40 (m, 2H); IR (film) 3330 (NH) and 1684 (C=O) cm^{-1}. Anal. ($C_{20}H_{29}N_2O_2F$) H, N; C: calcd, 68.94; found, 68.48.

2,3-Dimethoxy-*N*-(1-benzylpiperidin-4-yl)benzamide (21). Triethylamine (1.60 mL, 11.5 mmol) was added to a stirred solution of 2,3-dimethoxybenzoic acid (2.00 g, 11 mmol) in dichloromethane (10 mL), and the reaction mixture was cooled to 0 °C (ice bath). Ethyl chloroformate (1.06 mL, 11.1 mmol) was added, and the reaction mixture was stirred at 0 °C for 45 min. A solution of *N*-benzyl-4-aminopiperidine (1.90 g, 10 mmol) in dichloromethane (10 mL) was added, and the reaction mixture was stirred at ambient temperature for 2 h. Dichloromethane (25 mL) was added, and the mixture was washed with an aqueous mixture of sodium bicarbonate/sodium hydroxide, pH 10 (2 × 25 mL). The organic layer was dried (sodium sulfate) and concentrated in vacuo to give a yellow oil that precipitated from ether/hexanes (1:1) to give the product as a fluffy white solid (2.90 g, 82%): mp (free base) 119–121 °C; NMR ($CDCl_3$/TMS) δ 1.30–2.10 (m, 4H), 2.10–2.63 (m, 2H), 2.80–3.14 (m, 2H), 3.61 (s, 2H), 3.82 (s, 3H), 3.85 (s, 3H), 3.95–4.30 (m, 1H), 6.90–7.18 (m, 2H), 7.27 (br S, 5 H), 7.55 (dd, J = 7, 3 Hz, 1H), and 7.90 (br d, J = 8 Hz, 1H); IR (KBr) 3375 (NH) and 1640 (C=O) cm^{-1}. Anal. ($C_{21}H_{28}N_2O_3 \cdot H_2O$) C, H, N.

2,3-Dimethoxy-*N*-(piperidin-4-yl)benzamide (22). Solid palladium hydroxide on carbon (0.120 g) was added to a solution of 21 (2.50 g, 7.05 mmol) in ethanol (100 mL), and the mixture was hydrogenated (Parr hydrogenation apparatus) for 72 h over 60 psig of hydrogen. The mixture was filtered through a pad of neutral alumina, the solid was washed with several portions of hot ethanol, and the combined filtrate was concentrated in vacuo to give a yellow oil. The oil was dissolved in ethyl acetate (10 mL) and treated with a concentrated solution of HCl in ethyl acetate (5 mL) to give a white solid. The solid was filtered and recrystallized from ethanol to give the product as a fluffy white solid (0.882 g, 42%); mp 218–220 °C; NMR (free base, $CDCl_3$/TMS) δ 0.70–3.10 (m, 10 H), 3.30 (s, 6H), 6.60–7.00 (m, 4H); IR (free base, KBr) 3361, 3299, and 1649 (C=O) cm^{-1}. Anal. ($C_{14}H_{20}N_2O_3 \cdot HCl$) H, N; C: calcd, 55.91; found, 55.29.

2,3-Dimethoxy-*N*-[1-(4-fluorobenzyl)piperidin-4-yl]benzamide (23). 4-Fluorobenzyl bromide (0.400 mL, 3.21 mmol) was added to a solution of **22** (0.823 g, 3.11 mmol) in 1:1 methanol/tetrahydrofuran (20 mL), and the reaction mixture was stirred at reflux for 1 h. The reaction mixture was concentrated in vacuo and partitioned between dichloromethane (10 mL) and 1 N aqueous sodium hydroxide (20 mL). The organic layer was separated, and the aqueous layer was extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried (sodium sulfate), filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/triethylamine, 98:2) to give **23** as a fluffy white solid (0.818 g, 71%: mp 83–86 °C; NMR ($CDCl_3$/TMS) δ 0.6–3.0 (m, 9H), 3.40 (s, 2H), 3.85 (s, 6H), 6.40–7.90 (m, 8H); IR (KBr) 3377, 3358, 3246, and 1639 (C=O) cm^{-1}. Anal. ($C_{21}H_{25}N_2O_3F \cdot H_2O$) C, H, N.

2,3-Dimethoxy-*N*-(9-benzyl-9-azabicyclo[3.3.1]nonan-3β-yl)benzamide (26a). Solid **20a** (1 g, 3.03 mmol) was added to trifluoroacetic acid (7 mL), and the mixture was stirred at ambient temperature for 2 h. Volatile components were removed in vacuo

to give a colorless oil that was dissolved in an aqueous mixture of sodium bicarbonate/sodium hydroxide, pH 10 (10 mL), and extracted with dichloromethane (2 × 25 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give 2β-amino-9-benzyl-9-azabicyclo[3.3.1]nonane as a colorless oil (0.68 g, 98%).

Triethylamine (0.46 mL, 3.3 mmol) was added to a solution of 2,3-dimethoxybenzoic acid (0.55 g, 3.03 mmol) in dichloromethane (5 mL), and the reaction mixture was cooled to 0 °C in an ice bath. Ethyl chloroformate (0.32 mL, 3.3 mmol) was added and the reaction mixture stirred at 0 °C for 45 min. A solution of 2β-amino-9-benzyl-9-azabicyclo[3.3.1]nonane (0.68 g, 3.3 mmol) and triethylamine (0.46 mL, 3.3 mmol) in dichloromethane (5 mL) was added dropwise to the stirred suspension during which time carbon dioxide evolution commenced. The reaction mixture was stirred at ambient temperature for an additional 2 h. Dichloromethane (25 mL) was added and the mixture washed with aqueous sodium bicarbonate/NaOH (pH = 10). Volatile components were removed in vacuo to give a colorless solid that was purified by silica gel column chromatography (dichloromethane/ acetone/ammonium hydroxide, 9:1:0.1) to give **26a** as a fluffy white solid (0.78 g, 72.6%). The free base was converted to the corresponding hydrochloride salt by treatment with an ethanolic solution of HCl and recrystallization from ethanol: mp 223–225 °C; NMR (free base; $CDCl_3$/TMS) δ 1.30–2.35 (m, 10H), 2.95 (br s, 2H), 3.85–4.15 (m, 8H), 4.60–5.05 (m, 1H), and 6.90–7.85 (m, 9H); IR (film, free base) 3376 (NH) and 1656 (C=O) cm^{-1}. Anal. ($C_{24}H_{31}N_2O_3Cl$) C, H, N.

2,3-Dimethoxy-*N*-[9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3β-yl]benzamide (26b): yield: 72.2%. The product was characterized as the free base: mp 132–133.5 °C; NMR ($CDCl_3$/TMS) δ 1.30–2.30 (m, 10H), 2.95 (br s, 2H), 3.70–4.10 (m, 8H), 4.50–5.10 (m, 1H), 6.80–7.90 (m, 8H); IR (film) 3371 (NH) and 1654 (C=O) cm^{-1}. Anal. ($C_{24}H_{29}N_2O_3F$) C, H, N.

5-Bromo-2,3-dimethoxy-*N*-[9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3β-yl]]benzamide (26c): yield 45%; mp (HCl salt) 172–174 °C; NMR (free base, $CDCl_3$/TMS) δ 1.10–2.50 (m, 10H), 2.70–3.10 (m, 2H), 3.70–4.10 (m, 8H), 4.50–5.20 (m, 1H), 6.70–7.90 and (m, 7H); IR (KBr) 3416 (NH) and 1656 (C=O) cm^{-1}. Anal. ($C_{24}H_{28}N_2O_3FBr \cdot HCl \cdot 0.5H_2O$) C, H, N.

5-Amino-3-chloro-2-methoxy-*N*-(9-benzyl-9-azabicyclo-[3.3.1]nonan-3β-yl)benzamide (25): yield 88%. The product was characterized as the free base: mp 211–213 °C; NMR ($CDCl_3$/TMS) δ 1.10–2.30 (m, 10H), 2.75 (br s, 2H), 3.50–3.80 (m, 5H), 4.15 (s, 2H), 4.20–4.80 (m, 1H), 5.90 (s, 1H), 6.90 (br s, 5H), and 7.60 (s, 1H); IR (KBr) 3482, 3391, 3301, 3187, and 1627 (C=O) cm^{-1}. Anal. ($C_{23}H_{29}N_3O_2Cl$) C, H, N.

5-Amino-3-chloro-2-methoxy-*N*-[9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonan-3α- and -3β-yl]benzamide (24a,b). Triethylamine (0.80 mL, 5.74 mmol) was added to a stirred solution of 2,3-dimethoxybenzoic acid (1.07 g, 5.31 mmol) in dichloromethane (5 mL), and the reaction mixture was cooled to 0 °C (ice bath). Ethyl chloroformate (0.52 mL, 5.44 mmol) was added, and the reaction mixture was stirred at 0 °C for 45 min. A solution of 2α,β-9-(4-fluorobenzyl)-9-azabicyclo[3.3.1]nonane (**18b**: 1.18 g, 4.74 mmol) in dichloromethane (5 mL) was added, the ice bath was removed, and the reaction mixture was stirred at ambient temperature for 16 h. Dichloromethane (25 mL) was added, and the mixture was washed with a 3 N solution of sodium hydroxide (25 mL). The organic layer was dried (sodium sulfate) and concentrated in vacuo to give a yellow solid that was purified by silica gel column chromatography to give a white solid that was recrystallized from toluene to give **24a** as a fluffy white solid (0.76 g, 37%): mp 206–207 °C; NMR ($CDCl_3$/TMS) δ 0.55–2.70 (m, 10H), 3.05 (br d, J = 11 Hz, 2H), 3.5–3.95 (m, 5H), 4.2–4.8 (m, 3H), 6.15 (s, 1H), 6.55–6.70 (m, 5H), and 7.90 (s, 1H); IR (KBr) 3478, 3385, 3323, 3195, and 1638 (C=O) cm^{-1}. Anal. ($C_{23}H_{27}N_3O_2ClF$) C, H, N.

Further elution of the column afforded the exo isomer (BRL 34778) as a fluffy white solid that was recrystallized from toluene to give **24b** as fine white needles (0.36 g, 18%): mp 221–222 °C; NMR ($CDCl_3$/TMS) δ 0.60–2.30 (m, 10H), 2.65–3.10 (m, 2H), 3.60–4.00 (m, 5H), 4.30–4.90 (m, 3H), 6.15 (s, 1H), 6.50–7.50 (m, 5H), 7.90 (s, 1H); IR (KBr) 3444, 3403, 3314, 3196, and 1637 (C=O) cm^{-1}. ($C_{23}H_{27}N_3O_2ClF$) C, H, N.

5-Bromo-2,3-dimethoxy-*N*-(1-benzylpiperidin-4-yl)benzamide (27). A 2 M solution of trimethylaluminum in toluene (2.95 mL, 5.90 mmol) was added to a soultion of 4-amino-1-benzylpiperidine (1.20 mL, 5.88 mmol) in toluene (4 mL) and the reaction mixture stirred at ambient temperature for 2 h. Methyl 5-bromo-2,3-dimethoxybenzoate 1.194 g, 4.34 mmol) was added and the reaction mixture stirred at reflux for 1 h. The reaction was quenched by addition of an aqueous solution of 3 N sodium hydroxide (3 mL), and the mixture was extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried (sodium sulfate), volatile components were removed in vacuo, and the residue was purified by silica gel column chromatography to afford **27** as a fluffy white solid (1.03 g, 55%): mp 71.5–73.5 °C; NMR ($CDCl_3$/TMS) δ 1.15–3.00 (m, 8H), 3.50 (s, 2H), 3.80-4.20 (m, 7H), 7.00 (d, J = 3 Hz, 1H), 7.20 (s, 5H), 7.80 (d, J = 3 Hz, 1H), and 7.90 (s, 1H); IR (KBr) 3377 (NH) and 1630 (C=O) cm^{-1}. Anal. ($C_{21}H_{25}N_2O_3Br$) C, H, N.

5-Bromo-2,3-dimethoxy-*N*-(9-benzyl-9-azabicyclo[3.3.1]-nonan-3β-yl)benzamide (28): yield 23%; mp 149–150 °C; NMR ($CDCl_3$/TMS) δ 1.30–2.10 (complex m, 10H), 2.90 (br s, 2H), 3.70–3.90 (m, 8H), 4.70–5.00 (m, 1H), 7.10 (d, J = 3 Hz, 1H), 7.20–7.40 (m, 5H), 7.75 (d, J = 8 Hz, 1H), and 7.75 (d, J = 3 Hz, 1H); IR (KBr) 3374 (NH) and 1652 (C=O) cm^{-1}. Anal. ($C_{24}H_{29}N_2O_3Br$) C, H, N.

4-(Trifluoroacetamido)-1-benzylpiperidine (29). Trifluoroacetic anhydride (3.50 mL, 24.8 mmol) was added dropwise over 15 min to a stirred solution of 4-amino-1-benzylpiperidine (5.00 mL, 24.5 mmol) and triethylamine (4.00 mL, 28.7 mmol) in dichloromethane (25 mL), and the reaction mixture was stirred at 0 °C for an additional 15 min. Volatile components were removed in vacuo, and the residue was purified by silica gel column chromatography (ethyl acetate/hexanes, 1:1) to give **29** as white crystals (6.63 g, 94%): mp 124.5–126 °C; NMR ($CDCl_3$/TMS) δ 1.10–3.10 (m, 8H), 3.50 (s, 2H), 3.60–4.10 (m, 1H), 6.50 (br d, J = 7 Hz, 1H), 7.20 (s, 5H); IR (KBr) 3310 (NH) and 1700 (C=O) cm^{-1}. Anal. ($C_{14}H_{17}N_2OF_3$) C, H, N.

4-(Trifluoroacetamido)-1-(*tert*-butyloxycarbonyl)piperidine (30). Pearlman's catalyst (0.32 g) was added to a solution of **29** (7.50 g, 26.2 mmol) in methanol (100 mL), and the mixture was hydrogenated (Parr hydrogenation apparatus) for 48 h over 60 psig of hydrogen. The mixture was filtered through a pad of neutral alumina, the solid was washed with several portions of hot ethanol, and the combined filtrate was concentrated in vacuo to give a colorless oil that was dissolved in dichloromethane (10 mL) containing triethylamine (4.8 mL, 35 mmol). A solution of di-*tert*-butyl dicarbonate (8.68 g, 39.8 mmol) in dichloromethane (10 mL) was added, and the reaction mixture was stirred at ambient temperature for 2 h. Volatile components were removed in vacuo to give an oil that was purified by silica gel column chromatography to afford **30** as a colorless oil (7.09 g, 82%): NMR ($CDCl_3$/TMS) δ 0.90–2.10 (m, 13H), 2.30–3.10 (m, 2H), 3.60–4.30 (m, 3H), and 7.10 (br d, J = 7 Hz, 1H); IR (film) 3291 (NH), 1700 (carbamate C=O), and 1673 (amide C=O) cm^{-1}.

4-Amino-1-(*tert*-butyloxycarbonyl)piperidine (31). A solution of **30** (1.22 g, 4.11 mmol) and 37% aqueous ammonium hydroxide (2 mL) in methanol (10 mL) was stirred at reflux for 2 h. Volatile components were removed in vacuo, and the residue was purified by silica gel column chromatography (ethyl acetate/methanol/ ammonium hydroxide, 2:1:0.1) to give **31** as a viscous oil (0.667 g, 81%): NMR ($CDCl_3$/TMS) δ 1.00–2.10 (m, 15H), 2.30–3.10 (m, 3H), and 3.70–4.20 (m, 2H); IR (film) 3351 (NH) and 1686 (C=O) cm^{-1}.

5-Bromo-2,3-dimethoxy-*N*-[1-(*tert*-butyloxycarbonyl)piperidin-4-yl]benzamide (32). This benzamide was prepared as described above for **26**: yield 71%; mp 108–109 °C; NMR ($CDCl_3$/TMS) δ 1.45 (s, 9H), 1.50–2.25 (m, 4H), 2.60–3.35 (m, 4H), 3.60–4.30 (m, 7H), 7.10 (d, J = 2 Hz, 1H), 7.60 (d, J = 2 Hz, 1H), and 7.90 (s, 1H); IR (KBr) 3357 (NH) 1694 (carbamate C=O), and 1657 (amide C=O) cm^{-1}. Anal. ($C_{19}H_{27}N_2O_3Br$) C, H, N.

5-Bromo-2,3-dimethoxy-*N*-(piperidin-4-yl)benzamide (33). Solid **32** (0.50 g, 1.27 mmol) was added to trifluoroacetic acid (5.00 mL), and the reaction mixture was stirred at ambient temperature for 30 min. Volatile components were removed in vacuo and the residue dissolved in saturated aqueous bicarbonate (30 mL). An aqueous solution of 1 N sodium hydroxide was added to adjust the pH of the solution to 12, and the mixture was

extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give a tan solid that was dissolved in absolute ethyl acetate and treated with a solution of HCl-saturated ethyl acetate (10 mL). Volatile components were removed in vacuo, and the residue was recrystallized from absolute ethanol to afford **33** as an amorphous white solid (0.35 g, 91%): mp 210–212 °C; NMR (free base, $CDCl_3$/TMS) δ 0.90–2.20 (m, 5H), 2.30–3.30 (m, 4H), 3.50–4.40 (m, 7H), 7.00 (d, J = 2 Hz, 1H), 7.70 (d, J = 2 Hz, 1H), and 7.90 (s, 1H); IR (KBr) 3548, 3446, 3370, 3329, and 1648 (C=O) cm^{-1}.

5-Bromo-2,3-dimethoxy-*N*-[1-(4-fluorobenzyl)piperidin-4-yl]benzamide (34). A solution of **33** (0.36 g, 0.82 mmol) in trifluoroacetic acid (3 mL) was stirred at ambient temperature for 5 min. Volatile components were removed in vacuo, and the residue was dissolved in methanol (12 mL). Solid potassium carbonate (0.625 g, 4.88 mmol) and 4-fluorobenzyl bromide (0.12 mL, 1.00 mmol) were added, and the reaction mixture was stirred at ambient temperature for 30 min. The mixture was filtered, and volatile components were removed in vacuo to give a tan solid that was purified by silica gel column chromatography to afford **34** as a white solid (0.30 g, 81%). An analytical sample was prepared by dissolving the free base in HCl-saturated methanol, volatile components were removed in vacuo, and the residue was recrystallized from methanol/2-propanol (2:5) to afford the hydrochloride salt as white needles: mp 220.5–221 °C; NMR (free base, $CDCl_3$/TMS) δ 1.50–3.90 (complex m, 17H), 6.70–8.10 (complex m, 7H); IR (KBr) 3373 (NH) and 1656 (C=O) cm^{-1}. Anal. ($C_{21}H_{24}N_2O_3FBr$·HCl) C, H, N.

2,3-Dimethoxy-*N*-(9-azabicyclo[3.3.1]nonan-3β-yl)benzamide (35). Solid palladium hydroxide on carbon (0.06 g) was added to a solution of **26a** (0.60 g, 1.52 mmol) in ethanol (50 mL) and the mixture hydrogenated (Parr hydrogenation apparatus) for 96 h over 45 psig of hydrogen. The mixture was filtered through a pad of neutral alumina, the solid was washed with several portions of hot ethanol, and the combined filtrate was concentrated in vacuo to give a tan solid. The solid was dissolved in ethyl acetate (10 mL) and treated with a concentrated solution of HCl in ethyl acetate (5 mL) to give a white solid. The solid was filtered and recrystallized from ethanol to give the product as a fluffy white solid (0.244 g, 47%): mp 244–246 °C; NMR (free base, $CDCl_3$/TMS) δ 0.50–2.50 (m, 11 H), 2.65-4.25 (m, 8H), 4.30–5.10 (m, 1H), and 6.70–8.10 (m, 4H); IR (free base, KBr) 3378 (NH) and 1646 (C=O) cm^{-1}. Anal. ($C_{17}H_{25}N_2O_3$·HCl) C, H, N.

[^{18}F]-2,3-Dimethoxy-*N*-[1-(4-fluorobenzyl)piperidin-4-yl]-benzamide ([^{18}F]-23). A solution of **22** (0.5 mg) and [^{18}F]-4-fluorobenzyl iodide (6.20–12.6 mCi) in dimethylformamide (0.5 mL) was stirred at 90 °C for 10 min. The mixture was dissolved in methanol/0.1 M aqueous ammonium formate, 55:45 (1.50 mL) and purified by C-18 reversed-phase semipreparative HPLC (methanol/0.1 M aqueous ammonium formate, 55:45, flow rate = 4.5 mL/min). The product was collected into a rotary evaporator (Buchi) and concentrated in vacuo to approximately 10% of its original volume. Saline (~2 mL) was added, and the product was transferred into a sterile dose vial to give 2.10–4.10 mCi of [^{18}F]-**23** (58–62% yield, specific activity = 180–860 mCi/μmol decay corrected to EOB). The radiolabeled product was found to coelute with authentic **23** using analytical C-18 reversed-phase HPLC (methanol/0.1 M aqueous ammonium formate, 75:25).

[^{18}F]-2,3-Dimethoxy-*N*-[9-(4-fluorobenzyl)-9-azabicyclo-[3.3.1]nonan-3β-yl]benzamide ([^{18}F]-26b). The product was purified by C-18 reversed-phase semipreparative HPLC (methanol/0.1 M aqueous ammonium formate, 55:45, flow rate = 4.5 mL/min: yield 28–57%;, specific activity = 180–1250 mCi/μmol decay corrected to EOB. The radiolabeled product was found to coelute with authentic **26b** using analytical C-18 reversed-phase HPLC (methanol/0.1 M aqueous ammonium formate, 75:25).

[^{18}F]-5-Bromo-2,3-dimethoxy-*N*-[1-(4-fluorobenzyl)piperidin-4-yl]benzamide ([^{18}F]-34). The product was purified by C-18 reversed-phase semipreparative HPLC (methanol/0.1 M aqueous ammonium formate, 60:40, flow rate = 4.5 mL/min): yield 56–60%; specific activity = 650–800 mCi/μmol decay corrected to EOB. The radiolabeled product was found to coelute with authentic **34** using analytical C-18 reversed-phase HPLC (methanol/0.1 M aqueous ammonium formate, 75:25).

Animal Tissue Distribution. Sprague–Dawley rats (200–300 g) were injected intravenously (femoral vein) under anesthesia (ketamine/xylazine) with 0.5–1.0 mL of a 0.9% saline solution of ^{18}F-labeled compound (20–150 μCi). At selected intervals rats were killed by decapitation. A blood sample was obtained and the brain removed, weighed, and counted in a Packard Autogamma 5000 Series γ counter to determine whole-brain uptake. Samples from the striatum, frontal cortex, hippocampus, and cerebellum were then removed. The striatum:cerebellum ratio was calculated by dividing the number of background-corrected counts/gram of striatum by the number of background-corrected counts/gram of cerebellum; the same method was used to calculate the frontal cortex:cerebellum ratio and hippocampus:cerebellum ratio. Blocking studies were carried out by co-injecting spiperone with the no-carrier-added dose (~60 μg/animal).

In Vitro Binding Assays. a. Assay of D_2 Receptors. The in vitro binding assays for D_2 receptors were performed in competition experiments with 0.5 nM [^{125}I]NCQ 298 in 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and 10 mM EDTA using rat striatal membranes. The assay volume was 150 μL, and 1 μM (+)-butaclamol was used to define nonspecific binding. Samples were incubated at 37 °C for 60 min and were filtered through Schleicher and Schuell filters (no. 30). Filters were washed with 15 mL of 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl, and radioactivity was determined using a Beckman 4000 γ counter.

b. Assay of D_3 Receptors. In vitro binding assays for the D_3 receptor were performed using membranes prepared from Sf9 cells infected with recombinant baculovirus and expressing a high density of D_3 receptors. Membranes (50 μL) were incubated with [^{125}I]NCQ 298 (0.3 nM) in 50 nM Tris, pH 7.4 (150 μL), containing 150 mM NaCl, 10 mM EDTA, 0.1% BSA, and inhibitor (0.001–30 nM). Samples were incubated at 30 °C for 45 min, and assays were terminated by the addition of 5 mL of cold wash buffer (10 mM Tris, pH 7.4 containing 150 mM NaCl). Samples were filtered through Schleicher and Schuell filters (no. 30). Filters were washed with 10 mL of wash buffer and dried under vacuum. Radioactivity remaining on the filters was determined using a Beckman 4000 γ counter. Nonspecific binding was defined using 2 μM (+)-butaclamol.

c. Assay of 5-HT_2 Receptors. The affinities of compounds for the 5-HT_2 receptor were determined with 0.4–0.5 nM [^{125}I]I-LSD in 50 mM Tris-HCl (pH 7.4) using membranes from P11 cells. The assay volume was 100 μL, and 1 μM ketanserin was used to define nonspecific binding. Samples were incubated at 37 °C for 60 min and were filtered through Schleicher and Schuell filters (no. 30). Filters were washed with 10 mL of 50 mM Tris-HCl (pH 7.4), and radioactivity was determined using a Beckman 4000 γ counter.

d. Assay of α_2 Receptors. In vitro binding assays for α_2-adrenergic receptors were performed in triplicate using 2 nM [^{3}H]rauwolscine in 20 mM HEPES buffer (pH 7.4) and membranes from rat cerebral cortex.[66] The assay volume was 500 μL, and 10 μM phentolamine was used to define nonspecific binding. Samples were incubated at 20 °C for 30 min and were filtered through Whatman GF/B glass fiber filters. Filters were washed with 3 × 5 mL of 20 mM HEPES buffer (pH 7.4), and retained radioactivity was measured by liquid scintillation spectrometry using a Beckman 6001 spectrometer at an efficiency for tritium of 50%.

Data were analyzed using the mathematical modeling program FITCOMP available through the National Institutes of Health-sponsored PROPHET computer system. The K_i of each compound was calculated using the following equation as described by Cheng and Prusoff (1973): $K_i = IC_{50}/(1 - [L]/K_d)$, where IC_{50} = the concentration of the unlabeled analogue required to inhibit 50% of radioligand binding, [L] = the concentration of the radioligand used in the assay, and K_d = the dissociation constant of the radioligand (0.05 nM for binding of [^{125}I]NCQ 298 to D_2 receptors; 0.15 nM for binding of [^{125}I]NCQ 298 to D_3 receptors; 0.85 nM for binding of [^{125}I]I-LSD to 5-HT_2 receptors; 1.8 nM for binding of [^{3}H]rauwolscine to α_2 receptors).

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