Effect of N-Alkylation on the Affinities of Analogues of Spiperone for Dopamine D_2 and Serotonin 5-HT_2 Receptors

Robert H. Mach,* Joseph R. Jackson, Robert R. Luedtke, Kathryn J. Ivins, Perry B. Molinoff, and Richard L. Ehrenkaufer

Cerebrovascular Research Center and Department of Pharmacology, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6063. *Received May 7, 1991*

Two series of N-substituted spiperone analogues were prepared and evaluated in vitro to measure their affinities for dopamine D_2 and serotonin 5-HT_2 receptors. Substitution of the amide nitrogen with an alkyl group of five carbon units or less resulted in analogues displaying a low selectivity for D_2 compared to 5-HT_2 receptors. However, a moderate improvement in selectivity for D_2 receptors was observed with *N*-benzylspiperone. Substitution at either the ortho or para position of the benzyl group resulted in a further reduction in affinity for 5-HT_2 receptors and improvement in the selectivity ratio. Examination of N-substituted analogues of spiperone may provide insights into the topography of the antagonist binding region of the 5-HT_2 receptor. The results also suggest that an ^{18}F-labeled analogue of *N*-(4-nitrobenzyl)spiperone (**4p**) may be a suitable tracer for studying D_2 receptors with positron emission tomography since this compound displays a high selectivity for D_2 receptors relative to that of spiperone and *N*-methylspiperone.

The use of positron emission tomography (PET) to evaluate alterations in neurotransmitter receptors permits the longitudinal study of changes associated with neurological and neuropsychiatric disorders antemortem. A primary limitation of this technique is the availability of only a limited number of positron-emitting radiotracers displaying characteristics appropriate for conducting quantitative PET imaging studies. The desired properties of a suitable PET-based radiotracer include the ability to cross the blood–brain barrier, nanomolar affinity and selectivity for the receptor, and low nonspecific binding.[1] The formation of radiolabeled metabolites should be minimal, and those formed should not cross the blood–brain barrier. The neurotransmitter receptor that has been most extensively studied by PET is the dopamine D_2 receptor. This is due to the availability of a number of potent antipsychotics that are high-affinity antagonists for the D_2 receptor. Several of these drugs have been used as "lead" compounds for the development of PET-based radiotracers. A potential problem with many of the radiotracers currently used is that they have nanomolar affinity for more than one class of receptor. Examples of such compounds are [^{18}F]spiperone[2] and [^{18}F]- or [^{11}C]-*N*-methylspiperone,[3] which display a high affinity for both D_2 and serotonin 5-HT_2 receptors;[4] [^{18}F]haloperidol,[2b,c] which binds potently to both D_2 and σ receptors;[5] and [^{11}C]SCH 23390, which has nanomolar affinity for dopamine D_1 and serotonin 5-HT_2 and 5-HT_{1C} receptors.[4c,6]

The lack of dopamine D_2 receptor specificity of spiperone and *N*-methylspiperone is well established.[3a,4] Approximately 15–25% of the saturable in vitro binding of these radiotracers to receptors in rat caudate is to 5-HT_2 sites.[4b,e] The properties of these ligands under in vivo conditions are less clear. In vivo studies using the 5-HT_2 antagonist ketanserin to block binding of the radiotracer indicated that the specific binding of [^3H]spiperone,[7]

(1) Sedvall, S. PET Imaging of Dopamine Receptors in Human Basal Ganglia: Relevance to Mental Illness. *TINS* **1990**, *13*, 302–308.

(2) (a) Kilbourn, M. R.; Welch, M. J.; Dence, C. S.; Tewson, T. J.; Saji, H.; Maeda, M. Carrier-Added and No-Carrier-Added Syntheses of [^{18}F]Spiroperidol and [^{18}F]Haloperidol. *Int. J. Appl. Radiat. Isot.* **1984**, *35*, 591–598. (b) Shiue, C.-Y.; Fowler, J. S.; Wolf, A. P.; Watanabe, M.; Arnett, C. D. Synthesis and Specific Activity Determinations of No-Carrier-Added (NCA) ^{18}F-Labeled Butyrophenone Neuroleptics-Benperidol, Haloperidol, Spiroperidol, and Pipamperone. *J. Nucl. Med.* **1985**, *26*, 181–186. (c) Arnett, C. D.; Shiue, C.-Y.; Wolf, A. P.; Fowler, J. S.; Logan, J.; Watanabe, M. Comparison of Three ^{18}F-Labeled Butyrophenone Neuroleptic Drugs in the Baboon Using Positron Emission Tomography. *J. Neurochem.* **1985**, *44*, 835–844.

(3) (a) Wagner, H. N., Jr.; Burns, H. D.; Dannals, R. F.; Wong, D. F.; Langstrom, B.; Duelfer, T.; Frost, J. J.; Ravert, H. T.; Links, J. M.; Rosenbloom, S. B.; Lukas, S. E.; Kramer, A. V.; Kuhar, M. J. Assessment of Dopamine Receptor Densities in the Human Brain with Carbon-11-Labeled N-Methylspiperone. *Ann. Neurol.* **1984**, *15* (*suppl*), S79–S84. (b) Arnett, C. D.; Fowler, J. S.; Wolf, A. P.; Shiue, C.-Y.; McPherson, D. W. [^{18}F]-N-Methylspiroperidol: The Radioligand of Choice for PETT Studies of the Dopamine Receptor in Human Brain. *Life Sci.* **1985**, *36*, 1359–1366. (c) Shiue, C.-Y.; Fowler, J. S.; Wolf, A. P.; McPherson, D. W.; Arnett, C. D.; Zecca, L. No-Carrier-Added Fluorine-18-Labeled N-Methylspiroperidol: Synthesis and Biodistribution in Mice. *J. Nucl. Med.* **1986**, *27*, 226–234. (d) Fowler, J. S.; Arnett, C. D.; Wolf, A. P.; Shiue, C.-Y.; MacGregor, R. R.; Halldin, C.; Langstrom, B.; Wagner, H. N., Jr. A Direct Comparison of the Brain Uptake and Plasma Clearance of N-[^{11}C]Methylspiroperidol and [^{18}F]N-Methylspiroderidol in Baboon Using PET. *Nucl. Med. Biol.* **1986**, *13*, 281–284. (e) Arnett, C. D.; Wolf, A. P.; Shiue, C.-Y.; Fowler, J. S.; MacGregor, R. R.; Christman, D. R.; Smith, M. R. Improved Delineation of Human Dopamine Receptors Using [^{18}F]-N-Methylspiroperidol and PET. *J. Nucl. Med.* **1986**, *27*, 1878–1882.

(4) (a) Leysen, J. E.; Niemgeers, C. J. E.; Tollenaere, J. P.; Laduron, P. M. Serotonergic Component of Neuroleptic Receptors. *Nature* **1978**, *272*, 168–171. (b) List, S. J.; Seeman, P. Resolution of Dopamine and Serotonin Receptor Components of [^3H]Spiperone Binding to Rat Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2620–2624. (c) Severson, J. A.; de Vellis, J. S.; Finch, C. E. [^3H]Spiperone Binding Sites in Rat Primary Glial Cultures, C6 Glioma, and B104 Neuroblastoma. *J. Neurosci. Res.* **1983**, *9*, 21–26. (d) Hyttel, J.; Arnt, J.; Van Den Bergie, M. Selective Dopamine D_1 and D_2 Receptor Antagonists. In *Clinical Pharmacology in Psychiatry: From Molecular Studies to Clinical Reality*; Dahl, S. G., Gram, L. F., Eds.; Springer-Verlag: New York, 1989; pp 109–122. (e) McGonigle, P. Quantitative Analysis of the Interaction of [^3H]Spiroperidol with Serotonin and Dopamine Receptors. *J. Cardiovas. Pharm.* **1988**, *11* (*Suppl. 1*), S73–S77.

(5) (a) McLean, S.; Weber, E. Autoradiographic Visualization of Haloperidol-Sensitive Sigma Receptors in Guinea-Pig Brain. *Neuroscience* **1988**, *25*, 259–269. (b) Manallack, D. T.; Wong, M. G.; Costa, M.; Andrews, P. R.; Beart, P. M. Receptor Site Topographies for Phencyclidine-Like and σ Drugs: Predictions from Quantitative Conformational, Electrostatic Potential, and Radioreceptor Analyses. *Mol. Pharmacol.* **1988**, *34*, 863–879. (c) Bowen, W. D.; Moses, E. L.; Tolentino, P. J.; Walker, J. M. Metabolites of Haloperidol Display Preferential Activity at σ Receptors Compared to Dopamine D-2 Receptors. *Eur. J. Pharmacol.* **1990**, *177*, 111–118.

0022-2623/92/1835-0423$03.00/0

[^{76}Br]bromospiperone,[8] and [^{11}C]-*N*-methylspiperone[9] in the striatum of rodents occurs predominantly at D_2 sites. However, other investigators using a higher dose of ketanserin observed a reduction in the binding of [^{3}H]spiperone[10] and [^{3}H]-*N*-methylspiperone[11] to 70–80% of controls.

A second series of compounds that display high affinity and selectivity for the D_2 receptor are the benzamides, sulpiride and raclopride.[12] [^{11}C]Raclopride has been prepared,[13] and a number of quantitative PET imaging studies have been carried out using this tracer.[14] Concerns with the use of [^{11}C]raclopride for in vivo imaging comes from the discrepancy between K_d values observed under in vitro and in vivo conditions[2b,15] and the ability of endogenous dopamine to compete with the radiotracer for the D_2 receptor.[16] These concerns decrease the reliability of estimates of the density of receptors with PET. A number of ^{18}F-labeled benzamide analogues that display a higher affinity than raclopride for the D_2 receptor have recently been described.[17] It is not yet clear if these analogues satisfy all of the criteria necessary for use in quantitative PET imaging studies.

In contrast to raclopride, endogenous dopamine has been shown not to reduce the in vivo binding of [^{3}H]-*N*-methylspiperone to D_2 receptors.[16] As part of our ongoing research on the development of ^{18}F-labeled radiotracers for PET, we explored the possibility of modifying the dopamine D_2 and serotonin 5-HT_2 binding affinities of spiperone-based analogues by substituting the amide nitrogen with alkyl groups of increasing steric demand. An analogue of spiperone possessing a reduced affinity for 5-HT_2 receptors while retaining a high affinity for D_2 re-

(6) (a) Bischoff, S.; Heinrich, M.; Krauss, J.; Sills, M. A.; Williams, M.; Vassout, A. Interaction of the D_1 Receptor Antagonist SCH 23390 with the Central 5-HT System: Radioligand Binding Studies, Measurements of Biochemical Parameters and Effects on L-5-HTP Syndrome. *J. Recep. Res.* **1988**, *8*, 107–120. (b) McQuade, R. D.; Ford, D.; Duffy, R. A.; Chipkin, R. E.; Iorio, L. C.; Barnett, A. Serotonergic Component of SCH 23390: In Vitro and In Vivo Binding Analyses. *Life Sci.* **1988**, *43*, 1861–1869. (c) Meltzer, H. Y. Clinical Studies on the Mechanism of Action of Clozapine: the Dopamine-Serotonin Hypothesis of Schizophrenia. *Psychopharmacology (Berlin)* **1989**, *99*, S18–S27. (d) Nicklaus, K. J.; McGonigle, P.; Molinoff, P. B. [^{3}H]SCH 23390 Labels Both Dopamine-1 and 5-Hydroxytryptamine$_{1C}$ Receptors in the Choroid Plexus. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 343–348.

(7) Chivers, J.; Jenner, P.; Marsden, C. D. Pharmacologic Characterization of Binding Sites Identified in Rat Brain Following In Vivo Administration of [^{3}H]-Spiperone. *Br. J. Pharmacol.* **1987**, *90*, 467–478.

(8) Maziere, B.; Loc'h, C.; Hantraye, P.; Guillon, R.; Duquesnoy, N.; Soussaline, F.; Naquet, R.; Comar, D.; Maziere, M. 76Br-Bromospiroperidol: A New Tool for Quantitative In-Vivo Imaging of Neuroleptic Receptors. *Life Sci.* **1984**, *35*, 1349–1236.

(9) Suehiro, M.; Dannals, R. F.; Scheffel, U.; Stathis, M.; Wilson, A. A.; Ravert, H. T.; Villemagne, V. L.; Sanchez-Roa, P. M.; Wagner, H. N., Jr. In Vivo Labeling of the Dopamine D_2 Receptor with N-^{11}C-Methyl-Benperidol. *J. Nucl. Med.* **1990**, *31*, 2015–2021.

(10) Barone, D.; Luzzani, F.; Assandri, A.; Galliani, G.; Mennini, T.; Garattini, S. In Vivo Stereospecific [^{3}H]Spiperone Binding in Rat Brain: Characteristics, Regional Distribution, Kinetics and Pharmacological Properties. *Eur. J. Pharmacol.* **1985**, *116*, 63–74.

(11) Frost, J. J.; Smith, A. C.; Kuhar, M. J.; Dannals, R. F.; Wagner, H. N., Jr. In Vivo Binding of ^{3}H-N-Methylspiperone to Dopamine and Serotonin Receptors. *Life Sci.* **1987**, *40*, 987–995.

(12) (a) Elliott, P. N. C.; Jenner, P.; Huizing, G.; Marsden, C. D.; Miller, R. Substituted Benzamides as Central Dopamine Antagonists in Rodents. *Neuropharmacology* **1977**, *16*, 333–342. (b) Hogberg, T.; Ramsby, S.; Ogren, S.-O.; Norinder, U. New Selective Dopamine D-2 Antagonists as Antipsychotic Agents: Pharmacological, Chemical, Structural and Theoretical Considerations. *Acta Pharm. Suec.* **1987**, *24*, 289–328. (c) Chivers, J. K.; Gommeren, W.; Leysen, J. E.; Jenner, P.; Marsden, C. D. Comparison of the In-Vitro Receptor Selectivity of Substituted Benzamide Drugs for Brain Neurotransmitter Receptors. *J. Pharm. Pharmacol.* **1988**, *40*, 415–421.

(13) Ehrin, E.; Farde, L.; DePaulis, T.; Eriksson, L.; Greitz, T.; Johnstrom, P.; Litton, J.-E.; Nilsson, J. L. G.; Sedvall, G.; Stone-Elander, S.; Ogren, S.-O. Preparation of ^{11}C-Labelled Raclopride, a New Potent Dopamine Receptor Antagonist: Preliminary PET Studies of Cerebral Dopamine Receptors in the Monkey. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 269–273.

(14) (a) Farde, L.; Hall, H.; Ehrin, E.; Sedvall, G. Quantitative Analysis of D_2 Dopamine Receptor Binding in the Living Human Brain by PET. *Science* **1986**, *231*, 258–261. (b) Farde, L.; Halldin, C.; Stone-Elander, S.; Sedvall, G. PET Analysis of Human Dopamine Receptor Subtypes Using ^{11}C-SCH 23390 and ^{11}C-Raclopride. *Psychopharmacology (Berlin)* **1987**, *92*, 278–284. (c) Sedvall, G.; Farde, L.; Wiesel, F.-A. Quantitative Determination of D_2 Dopamine Receptor Characteristics in Healthy Human Subjects and Psychiatric Patients. *Life Sci.* **1987**, *41*, 813–816. (d) Farde, L.; Wiesel, F.-A.; Hall, H.; Halldin, C.; Stone-Elander, S.; Sedvall, G. No D_2 Receptor Increase in PET Study of Schizophrenia. *Arch. Gen. Psychiatry* **1987**, *44*, 671–672. (e) Farde, L.; Wiesel, F.-A.; Haldin, C.; Sedvall, G. Central D2-Dopamine Receptor Occupancy in Schizophrenic Patients Treated With Antipsychotic Drugs. *Arch. Gen. Psychiatry* **1988**, *45*, 71–76. (f) Farde, L.; Wiesel, F.-A.; Jansson, P.; Uppefeldt, G.; Wahlen, A.; Sedvall, G. An Open Label Trial of Raclopride in Acute Schizophrenia. Confirmation of D_2-Dopamine Receptor Occupancy by PET. *Psychopharmacology (Berlin)* **1988**, *94*, 1–7. (g) Farde, L.; Pauli, S.; Hall, H.; Eriksson, L.; Halldin, C.; Hogberg, T.; Nilsson, L.; Sjogren, L. Stereoselective Binding of ^{11}C-Raclopride in Living Human Brain-A Search for Extrastriatal Central D_2-Dopamine Receptors by PET. *Psychopharmacology (Berlin)* **1988**, *94*, 471–478. (h) Farde, L.; Wiesel, F.-A.; Stone-Elander, S.; Halldin, C.; Nordstrom, A.-L.; Hall, H.; Sedvall, G. D_2 Dopamine Receptors in Neuroleptic-Naive Schizophrenic Patients: A Positron Emission Tomography Study with [^{11}C]Raclopride. *Arch. Gen. Psychiatry* **1990**, *47*, 213–219.

(15) Andreasen, N. C.; Carson, R. C.; Diksic, M.; Evans, A.; Farde, L.; Gjedde, A.; Hakim, A.; Lal, S.; Nair, N.; Sedvall, G.; Tune, L.; Wong, D. Workshop on Schizophrenia, PET, and Dopamine D_2 Receptors in the Human Neostriatum. *Schizo. Bull.* **1988**, *14*, 471–484.

(16) Seeman, P.; Guan, H.-C.; Niznik, H. B. Endogeneous Dopamine Lowers the Dopamine D_2 Receptor Density as Messured by [^{3}H]Raclopride: Implications for Positron Emission Tomography of the Human Brain. *Synapse* **1989**, *3*, 96–97.

(17) (a) Mukherjee, J.; Luh, K. E.; Yasillo, N.; Perry, B. D.; Levy, D.; Chen, C.-T.; Ortega, C.; Beck, R. N.; Cooper, M. Dopamine D-2 Receptors Imaged by PET in Cebus Apella Using [^{18}F]-Benzamide Neuroleptic. *Eur. J. Pharmacol.* **1990**, *175*, 363–364. (b) Mathis, C.; Bishop, J.; Gerdes, J.; Faggin, B.; Mailman, R. High Affinity Aryl-Substituted [F-18]Fluoroalkylbenzamides for PET D-2 Studies. *J. Nucl. Med.* **1990**, *31*, 737 (abstract). (c) de Paulis, T.; Schmidt, D.; Ansari, M. S.; Kessler, R. M. [^{18}F]Fluprepid: The Design and Synthesis of PET Ligands for the Dopamine D2 Receptor. *J. Nucl. Med.* **1990**, *31*, 786 (abstract). (d) Mukherjee, J.; Luh, K. E.; Yasillo, N. J.; Perry, B. D.; Levy, D.; Chen, C.-T.; Chou, J.-S.; Ortega, C.; Cooper, M. Dopamine D-2 Receptors Imaged by PET in Cebus Apella with (S)-N-[(1-Ethyl-2-pyrrolidinyl)methyl]5-(3-[F18]fluoropropyl)-2,3-dimethoxybenzamide. *J. Nucl. Med.* **1990**, *31*, 787 (abstract). (e) Halldin, C.; Hogberg, T.; Bengtsson, S.; Hall, H.; Farde, L. Preparation of [F-18]NCQ 115, A New Selective Reversible D-2 Dopamine Receptor Ligand for PET. *J. Nucl. Med.* **1990**, *31*, 902 (abstract). (f) Halldin, C.; Hogberg, T.; Bengtsson, S.; Hall, H.; Farde, L. Synthesis of [^{18}F]NCQ 115, A New Selective Reversible D-2 Dopamine Receptor Ligand for PET. *J. Label. Compounds Radiopharm.* **1991**, *30*, 355–356. (g) Mathis, C.; Bishop, J.; Gerdes, J.; Faggin, B.; Mailman, R. Synthesis of Aryl-Substituted [F-18]Fluoroalkylbenzamides: High Affinity Ligands for Dopamine D-2 Studies. *J. Label. Compounds Radiopharm.* **1991**, *30*, 357–359.

ceptors may be more suitable for studying D_2 receptors in vivo with PET as compared with radiolabeled analogues of spiperone or *N*-methylspiperone. A number of analogues containing *N*-alkyl and *N*-haloalkyl groups have been reported to exhibit a high affinity for the D_2 receptor.[18] The work reported previously was initiated to address problems resulting from the multistep synthesis and the concomitant low yields associated with the production of [^{18}F]-*N*-methylspiperone and [^{18}F]spiperone for clinical PET imaging studies.[2b,19] Although the affinities of the reported analogues for the 5-HT_2 receptor were not measured, results of recent studies have shown that [^{18}F]-*N*-(fluoroethyl)spiperone labels 5-HT_2 receptors in the frontal cortex of primates with an apparent K_d of 0.5 nM,[18f] indicating that no change in selectivity for D_2 compared to 5-HT_2 receptors was achieved via this substitution. Similarly, both the *E* and *Z* isomers of *N*-([^{125}I]iodoallyl)spiperone have a high affinity for the 5-HT_2 receptor in rat frontal cortex.[20] In the present study, several *N*-alkyl and *N*-benzyl analogues of spiperone were prepared and evaluated to determine their affinities for both D_2 and 5-HT_2 receptors. Structural features resulting in an increase in the selectivity of *N*-alkylspiperone analogues for D_2 receptors were identified.

Chemistry

The synthesis of the target compounds is outlined in Scheme I. Alkylation of 4-phenyl-1,3,8-triazaspiro[4.5]-decan-4-one, **1**, with 4-chloro-1,1-(ethylenedioxy)-1-(4-fluorophenyl)butane, **2**,[21] gave the ethylenedioxy analogue of spiperone, **3**.[18h] Acid hydrolysis of **3** followed by alkylation of spiperone with the appropriate alkyl halide or tosyloxy derivative (method A) afforded the desired *N*-alkyl analogue in moderate yield (50–60%). Alternatively, the desired compounds were prepared by alkylation of **3** followed by acid hydrolysis of the ketal (method B). However, no improvement in overall yield was observed with this modification (Table I). Compound **4p** was synthesized via the sequence of reactions outlined in Scheme II.

Pharmacology

In vitro radioligand binding competition experiments were carried out to determine the affinities of the synthesized compounds for 5-HT_2 and D_2 receptors. The affinities of the compounds for 5-HT_2 receptors were determined using the pituitary tumor cell line, P11,[22] which has been shown to express 5-HT_2 receptors in the absence of D_2 receptors. 5-HT_2 receptors were labeled with [^{125}I]I-LSD. Inhibition of the binding of [^{3}H]spiperone to rat striatal tissue made it possible to determine the affinity of the spiperone analogues for D_2 receptors. 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}). Dissociation constants (K_i) were calculated from IC_{50} values using the method of Cheng and Prusoff.[23] The selectivity of the analogues for D_2 and 5-HT_2 receptors is expressed as the ratio of the K_i values for 5-HT_2 and D_2 receptors ($K_{i_{5\text{-}HT_2}}/K_{i_{D_2}}$). A higher ratio corresponds to a greater selectivity for D_2 receptors.

Results and Discussion

The goal of this study was to determine the structural features required to improve the D_2 vs 5-HT_2 selectivity of spiperone-based analogues. The approach chosen involved the preparation of a series of analogues in which the amide nitrogen of spiperone was substituted with alkyl groups of increasing steric demand. Previous studies have shown this to be a region of bulk tolerance for the binding of spiperone to the D_2 receptor.[18b-f,20,24] The effect of this substitution on the affinity of spiperone analogues for the 5-HT_2 receptor has not been thoroughly investigated. The

(18) (a) Moerlein, S. M.; Laufer, P.; Stocklin, G. Effect of Lipophilicity on the In Vivo Localization of Radiolabelled Spiperone Analogues. *Int. J. Nucl. Med. Biol.* **1985**, *12*, 353–356. (b) Welch, M. J.; Chi, D. Y.; Mathias, C. J.; Kilbourn, M. R.; Brodack, J. W.; Katzenellenbogen, J. A. Biodistribution of N-Alkyl and N-Fluoroalkyl Derivatives of Spiroperidol; Radiopharmaceuticals for PET Studies of Dopamine Receptors. *Nucl. Med. Biol.* **1986**, *13*, 523–526. (c) Chi, D. Y.; Kilbourn, M. R.; Katzenellenbogen, J. A.; Brodack, J. W.; Welch, M. J. Synthesis of N-Carrier-Added N-([^{18}F]Fluoroalkylspiperone Derivatives. *Appl. Radiat. Isot.* **1986**, *37*, 1173–1180. (d) Kiesewetter, D. O.; Eckelman, W. C.; Cohen, R. M.; Finn, R. D.; Larson, S. M. Syntheses and D_2 Receptor Affinities of Derivatives of Spiperone Containing Aliphatic Halogens. *Appl. Radiat. Isot.* **1986**, *37*, 1181–1188. (e) Coenen, H. H.; Laufer, P.; Stocklin, G.; Wienhard, K.; Pawlik, G.; Bocher-Schwarz, H. G.; Heiss, W.-D. 2-N-(2-[^{18}F]-Fluoroethyl)-Spiperone: A Novel Ligand For Cerebral Dopamine Receptor Studies with PET. *Life Sci.* **1987**, *40*, 81–88. (f) Welch, M. J.; Katzenellenbogen, J. A.; Mathias, C. J.; Brodack, J. W.; Carlson, K. E.; Chi, D. Y.; Dence, C. S.; Kilbourn, M. R.; Perlmutter, J. S.; Raichle, M. E.; Ter-Pergossian, M. M. N-(3-[^{18}F]Fluoropropyl)-Spiperone: The Preferred ^{18}F Labeled Spiperone Analog for Positron Emission Tomographic Studies of the Dopamine Receptor. *Nucl. Med. Biol.* **1988**, *15*, 83–97. (g) Huang, S. C.; Bahn, M. M.; Barrio, J. R.; Hoffman, J. M.; Satyamurthy, N.; Hawkins, R. A.; Mazziotta, J. C.; Phelps, M. E. In Vivo Measurement of Serotonin S2 Neuroreceptor Densities in Primates with 3-(2′-[F-18]Fluoroethyl)-Spiperone (FESP) and PET Using a Double-Injection Method. *J. Nucl. Med.* **1988**, *29*, 809. (h) Barrio, J. R.; Satayamurthy, N.; Huang, S.-C.; Keen, R. E.; Nissenson, C. H. K.; Hoffman, J. M.; Ackerman, R. F.; Bahn, M. M.; Mazziotta, J. C.; Phelps, M. E. 3-(2′-[^{18}F]Fluoroethyl)spiperone: In Vivo Biochemical and Kinetic Characterization in Rodents, Nonhuman Primates, and Humans. *J. Cereb. Blood Flow Metab.* **1989**, *9*, 830–839. (i) Satyamurthy, N.; Barrio, J. R.; Bida, G. T.; Huang, S.-C.; Mazziotta, J. C.; Phelps, M. E. 3-(2′-[^{18}F]Fluoroethyl)spiperone, a Potent Dopamine Antagonist: Synthesis, Structural Analysis and In-vivo Utilization in Humans. *Appl. Radiat. Isot.* **1990**, *41*, 113–129.

(19) Kilbourn, M. R.; Welch, M. J. Fluorine-18 Labeled Receptor Based Radiopharmaceuticals. *Appl. Radiat. Isot.* **1986**, *37*, 677–683.

(20) Lever, J. R.; Scheffel, U. A.; Stathis, M.; Musachio, J. L.; Wagner, H. N., Jr. In Vitro and In Vivo Binding of (E)- and (Z)-N-(Iodoallyl)spiperone to Dopamine D_2 and Serotonin 5-HT_2 Neuroreceptors. *Life Sci.* **1990**, *46*, 1967–1976.

(21) Moerlein, S. M.; Stocklin, G. L. Synthesis of High Specific Activity [^{75}Br]- and [^{77}Br]Bromperidol and Tissue Distribution Studies in the Rat. *J. Med. Chem.* **1985**, *28*, 1319–1324.

(22) Ivins, K. J.; Molinoff, P. B. Serotonin-2 Receptors Coupled to Phosphoinositide Hydrolysis in a Clonal Cell Line. *Mol. Pharmacol.* **1990**, *37*, 622–630.

(23) Cheng, Y.-C.; Prusoff, W. H. Relationship Between the Inhibition Constant (K_i) and the Concentration of Inhibitor Which Causes 50 Per Cent Inhibition (I_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

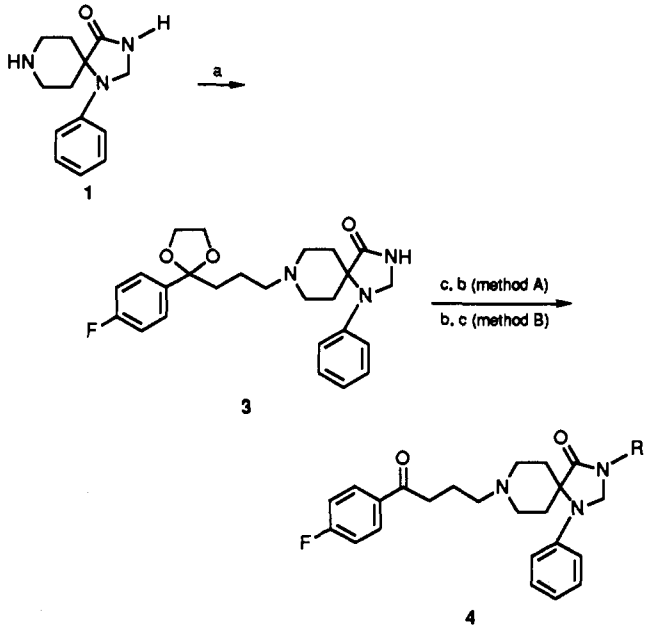
(24) (a) Monsma, F. J., Jr.; Barton, A. C.; Kang, H. C.; Brassard, D. L.; Haugland, R. P.; Sibley, D. R. Characterization of Novel Fluorescent Ligands with High Affinity for D_1 and D_2 Dopaminergic Receptors. *J. Neurochem.* **1989**, *52*, 1641–1644. (b) Barton, A. C.; Kang, H. C.; Rinaudo, M. S.; Monsma, F. J., Jr.; Stewart-Fram, R. M.; Macinko, J. A., Jr.; Haugland, R. P.; Ariano, M. A.; Sibley, D. R. Multiple Fluorescent Ligands for Dopamine Receptors. I. Pharmacological Characterization and Receptor Selectivity. *Brain Res.* **1991**, *547*, 199–207.

Table I. Physical Properties, Yields, and Elemental Analyses

no.	method	% yield	mp (°C)	formula	analytical data	recrystallization solvent
4a	A	10	256–259 dec	$C_{24}H_{29}N_3O_2FCl$	C,H,N	ethanol
4b	B	28	224.5–226.5	$C_{25}H_{31}N_3O_2FCl$	C,H,N[a]	acetone
4c	B	56	210.5–211	$C_{26}H_{33}N_3O_2FCl$	C,H,N	ethanol
4d	B	53	215.5–217	$C_{27}H_{35}N_3O_2FCl$	C,H,N	acetone
4e	B	15	216.5–219	$C_{27}H_{35}N_3O_2FCl$	C,H,N	ethanol
4f	B	67	215–215.5	$C_{28}H_{37}N_3O_2FCl$	C,H,N	ethanol
4g	B	65	226–227	$C_{28}H_{37}N_3O_2FCl$	C,H,N	acetone
4h	B	44	217–219	$C_{30}H_{33}N_3O_2FCl$	C,H,N[b]	ethanol
4i	B	45	175–176	$C_{31}H_{35}N_3O_2FCl$	C,H,N	ethanol–ether
4j	B	40	209–209.5	$C_{30}H_{32}N_3O_2F_2Cl$	C,H,N	acetone
4k	B	26	199.5–200	$C_{30}H_{32}N_3O_2F_2Cl$	C,H,N	acetone
4l	A	40	218–219.5 dec	$C_{30}H_{32}N_3O_2F_2Cl$	C,H,N	ethanol–ether
4m	A	59	230–231.5	$C_{30}H_{32}N_3O_2FICl$	C,H,N	ethanol–acetone
4n	A	51	238–240	$C_{30}H_{32}N_3O_2FICl$	C,H,N	ethanol–acetone
4o	A	46	203–204	$C_{30}H_{32}N_4O_4FCl$	C,H,N	acetone
4p	A	45	246–250 dec	$C_{30}H_{32}N_4O_4FCl$	C,H,N	ethanol–ether
4q	A	28	220–220.5	$C_{31}H_{35}N_3O_2FCl$	C,H,N	acetone
4r	A	43	203–203.5	$C_{31}H_{35}N_3O_2FCl$	C,H,N	acetone
4s	A	33	215–216.5	$C_{31}H_{35}N_3O_3FCl$	C,H,N	ethanol–ether
4t	A	43	207–207.5	$C_{31}H_{35}N_3O_3FCl$	C,H,N	ethanol

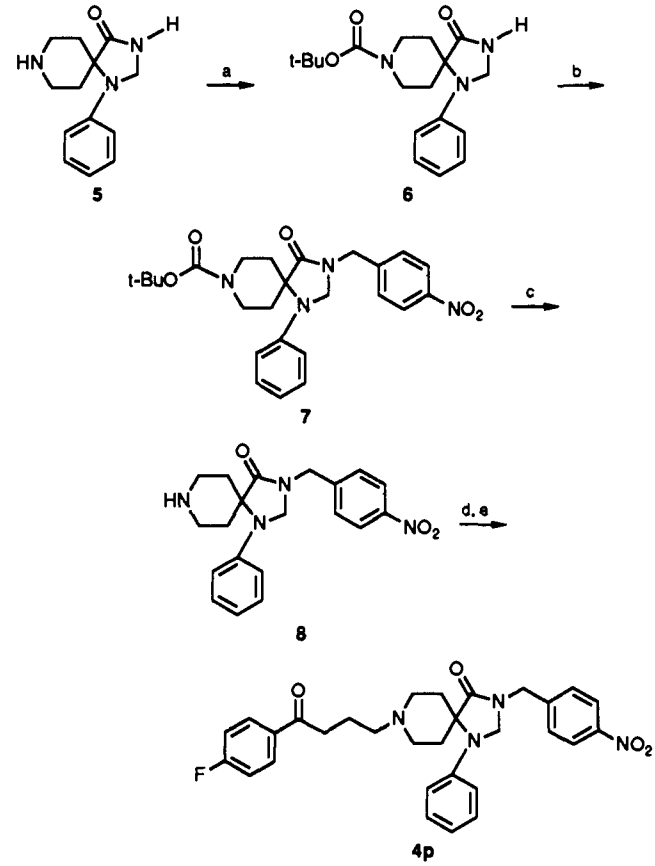
[a] C: calcd, 65.28; found, 63.59. [b] C: calcd, 69.02; found, 68.24.

Scheme I[a]



[a] Reagents: (a) 2/KI/CH_3CN; (b) NaH/RX or ROTs/THF; (c) aqueous HCl/EtOH.

Scheme II[a]



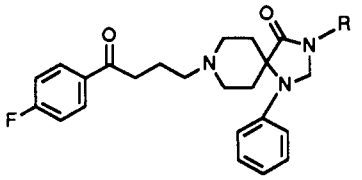
[a] Reagents: (a) $O[CO_2C(CH_3)_3]_2/CH_2Cl_2$; (b) NaH/THF/4-$NO_2C_6H_3CH_2Br$; (c) CF_3COOH; (d) 2/KI/Et_3N/CH_3CN; (e) 3 N HCl/EtOH.

desired analogues were prepared in moderate yield by N-alkylation of spiperone. Alkylation of the ethylenedioxy analogue of spiperone[18h] followed by acid hydrolysis of the protecting group did not result in a significant improvement in overall yield of the *N*-alkyl product.

The affinities of compounds for the 5-HT_2 receptor were determined from competition experiments using membrane homogenates prepared from P11 cells. P11 cells are derived from a pituitary tumor that has been shown to express 5-HT_2 receptors, while no detectable binding to D_2 receptors has been observed. [^{125}I]I-LSD was used to selectively label 5-HT_2 receptors since P11 cells do not express 5-HT_{1c} receptors.[22] In vitro binding assays for the D_2 receptor were conducted using rat striatal tissue homogenates and [^{3}H]spiperone. Based on results of previous studies on the binding of [^{3}H]spiperone to membranes from rat striatum, the ratio of D_2 to 5-HT_2 receptors in the striatum is 73:27 (D_2:5-HT_2).[4e] Considering the relative affinities of D_2 and 5-HT_2 receptors for [^{3}H]spiperone and the conditions of our assay system, less than 15% of the binding of [^{3}H]spiperone to striatal membranes was due to binding to 5-HT_2 receptors.[4e] This estimate appears to be validated by the fact that the K_i value calculated for the competition of [^{3}H]spiperone (Table II: 0.058 nM) is in good agreement with the K_d values (0.02–0.05 nM) obtained from Scatchard transformations of direct radioligand binding experiments.

The structures of the *N*-alkyl analogues of spiperone, their physicochemical parameters, and the results of in

Table II. Structures, Physicochemical Properties, and in Vitro Binding Data of Spiperone and Analogues 4a–i



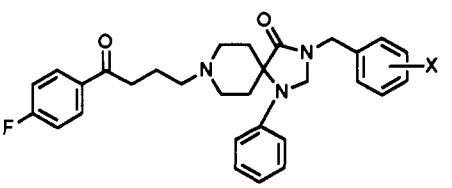
no.	R	π	MR	log P^a	K_i D_2 (nM)^b [n]	K_i 5-HT_2 (nM)^c [n]	5-HT_2/D_2^{d}
-	H	0.00	1.03	2.67^e	0.058 ± 0.022 [4]	0.45 ± 0.08 [5]	7.8
4a	CH_3	0.56	5.70	3.23	0.118 ± 0.045 [4]	0.55 ± 0.16 [5]	4.7
4b	CH_2CH_3	1.02	10.3	3.69	0.057 ± 0.014 [4]	0.31 ± 0.04 [4]	5.4
4c	$(CH_2)_2CH_3$	1.55	14.9	4.22	0.029 ± 0.013 [4]	0.096 ± 0.039 [4]	3.3
4d	$(CH_2)_3CH_3$	2.05	19.5	4.72	0.036 ± 0.017 [4]	0.41 ± 0.16 [4]	11
4e	$CH_2CH(CH_3)_2$	2.05	19.5	4.72	0.039 ± 0.015 [4]	0.13 ± 0.02 [4]	3.3
4f	$(CH_2)_4CH_3$	2.55	24.2	5.22	0.062 ± 0.032 [4]	1.1 ± 0.1 [4]	18
4g	$CH_2CH_2CH(CH_3)_2$	2.55	24.2	5.22	0.063 ± 0.031 [4]	1.3 ± 0.5 [4]	21
4h	CH_2Ph	2.01	30.0	4.68	0.023 ± 0.008 [4]	3.2 ± 1.1 [4]	140^f
4i	CH_2CH_2Ph	2.66	34.6	5.33	0.035 ± 0.012 [4]	1.0 ± 0.5 [4]	29

^{a} Calculated value. ^{b} Mean K_i value for inhibiting [^{3}H]spiperone binding to D_2 sites ± SE. ^{c} Mean K_i value for inhibiting [^{125}I]I-LSD binding to 5-HT_2 sites ± SE. ^{d} Ratio of K_i values.' ^{e} Octanol–water partition coefficient reported in ref 18a. ^{f} Indicates *N*-benzylspiperone has a 140-fold higher selectivity for the D_2 receptor vs the 5-HT_2 receptor.

vitro binding studies for each compound are given in Table II. All of the *N*-alkyl analogues displayed a high affinity for the D_2 receptor, a result that is consistent with those of other studies in which the in vitro affinities of a series of *N*-alkyl and *N*-haloalkyl analogues of spiperone for D_2 receptors were reported.[18a,b,d,h,20] There is no clear trend with respect to the effect of increasing steric demand (MR) or lipophilicity (π) on either the affinity of 5-HT_2 receptors or D_2 vs 5-HT_2 selectivity for this series of compounds. Substitution of the amide nitrogen with an alkyl group with up to four carbon units resulted in analogues that possessed subnanomolar affinity for the 5-HT_2 receptor and low D_2 selectivity (5-HT_2/D_2 ratio ≤ 11). Further extension of the alkyl group resulted in an analogue (**4f**) that had a lower affinity for the 5-HT_2 receptor ($K_i \sim 1$ nM). Branching of the alkyl group appears to have little effect on binding to 5-HT_2 receptors since compounds **4f** and **4g** had similar affinities for 5-HT_2 receptors. This is further exemplified by the isobutyl analogue, **4e**, which had a K_i value similar to that of the *n*-propyl derivative, **4c**, as opposed to the corresponding *n*-butyl analogue, **4d**. ANOVA and Dunnett tests were used to compare the affinities of compounds **4a–i** for 5-HT_2 receptors. The only analogue found to differ significantly ($p < 0.01$) with respect to its affinity for 5-HT_2 receptors was *N*-benzylspiperone, **4h**. This analogue displayed the lowest affinity for 5-HT_2 receptors (K_i = 3.2 nM) and a relatively high selectivity for D_2 receptors (5-HT_2/D_2 ratio = 140). No significant differences in affinity for D_2 receptors were found for the compounds listed in Table II. An unexpected result was the relatively high affinity (K_i = 1.0 nM) and low D_2 selectivity (5-HT_2/D_2 ratio = 29) of the *N*-phenylethyl analogue, **4i**. This suggests that the selectivity of this series of compounds for D_2 and 5-HT_2 receptors is not determined by the values of MR or π for the substituent since the phenylethyl group possessed the highest value for these two parameters.[25] The differences in the affinities of **4h** and **4i** for 5-HT_2 receptors may be attributed to the increased conformational flexibility of **4i** due to the presence of the additional methylene group.

The results of the initial study indicate that an improvement in the dopaminergic/serotonergic selectivity of spiperone could be achieved by substitution of the amide nitrogen with a benzyl group. To determine whether there are stereoelectronic effects with respect to D_2 or 5-HT_2 binding, a series of substituted *N*-benzylspiperone analogues was prepared. The structures of the analogues and the results of in vitro binding studies are shown in Table III. Substituents were chosen on the basis of physicochemical parameters (σ, *F*, *R*, MR, π) as well as the availability of suitable starting materials. The binding of substituted *N*-benzyl derivatives to D_2 receptors was unaffected by the nature of the substituent since all representatives of this series of compounds had a subnanomolar affinity for the D_2 receptor. There was, however, a pronounced substituent effect with respect to binding to 5-HT_2 receptors. A comparison of fluorine-substituted analogues revealed that para substitution was preferred over the corresponding ortho and meta positions with respect to increasing the K_i value for the inhibition of the binding of [^{125}I]I-LSD [4′-F (21 nM) > 3′-F (5.3 nM) ~ 2′-F (4.4 nM)]. Para-substituted analogues consistently exhibited higher K_i values for the inhibition of the binding

Table III. In Vitro Binding Data for the Substituted *N*-Benzylspiperone Analogues



no.	X	K_i D_2 (nM)^a [n]	K_i 5-HT_2 (nM)^b [n]	5-HT_2/D_2^{c}
4h	H	0.023 ± 0.008 [3]	3.2 ± 1.1 [3]	140
4j	2′-F	0.043 ± 0.008 [3]	4.4 ± 0.7 [3]	100
4k	3′-F	0.023 ± 0.013 [3]	5.3 ± 2.5 [3]	230
4l	4′-F	0.069 ± 0.032 [4]	21 ± 6 [6]	300
4m	2′-I	0.063 ± 0.028 [3]	19 ± 4 [3]	300
4n	4′-I	0.213 ± 0.099 [3]	89 ± 19 [3]	420
4o	2′-NO_2	0.120 ± 0.097 [3]	0.68 ± 0.29 [3]	5.7
4p	4′-NO_2	0.119 ± 0.054 [3]	14 ± 3 [6]	120
4q	2′-CH_3	0.033 ± 0.013 [3]	9.6 ± 4.7 [3]	290
4r	4′-CH_3	0.067 ± 0.035 [3]	37 ± 12 [3]	550
4s	2′-OCH_3	0.047 ± 0.027 [3]	1.9 ± 0.5 [3]	40
4t	4′-OCH_3	0.087 ± 0.059 [3]	12 ± 2 [3]	140

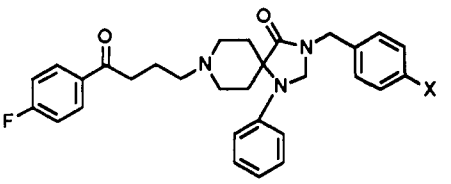
$^{a-c}$ Refer to Table II.

(25) Hansch, C.; Leo, A.; Unger, S. H.; Kim, K. H.; Nikaitani, D.; Lien, E. J. "Aromatic" Substituent Constants for Structure–Activity Correlations. *J. Med. Chem.* **1973**, *16*, 1207–1216.

of [^{125}I]I-LSD compared to the corresponding ortho-substituted derivatives. No correlation was found between the electronic substituent parameters (σ, F, or R) or the molar refractivity (MR) and the affinity for 5-HT$_2$ receptors or the selectivity ratio. Analogues possessing a lipophilic substituent ($+\pi$ value) generally possessed a lower affinity for the 5-HT$_2$ receptor relative to the N-benzyl analogue, **4h**. A linear relationship was observed between the Hansch lipophilicity constant π and the K_i value for inhibiting the binding of [^{125}I]I-LSD to 5-HT$_2$ receptors for both the ortho- and para-substituted analogues (data not shown). However, the limited number of analogues evaluated precludes a detailed structure–activity relationship analysis. The possible correlation between π and K_i for inhibiting the binding of [^{125}I]I-LSD suggests the presence of polar or charged amino acid residues in the spirodecanone binding region of the 5-HT$_2$ receptor. As with other members of the G protein-coupled receptor family, the 5-HT$_2$ receptor is thought to contain seven transmembrane regions that together form the ligand-binding domain of the receptor.[26] The results of our binding studies in concert with receptor model-building studies may provide insights into the orientation and mechanism of binding of spiperone to the ligand-binding region of the 5-HT$_2$ receptor.

The primary goal of the present work was to identify spiperone analogues that displayed an improved selectivity for D$_2$ vs 5-HT$_2$ receptors. The results of this study suggest that several 4′-substituted N-benzylspiperone analogues may possess this property. A potential problem with using these analogues for PET studies is the relatively high lipophilicity of the compounds, a feature that may interfere with the ability of an ^{18}F-labeled analogue to cross the blood–brain barrier. Previous studies with ^{18}F-labeled N-alkyl- and N-(fluoroalkyl)spiperone analogues revealed an optimal log P range of 2.67–4.00 for high initial brain extraction of this series of compounds.[18a,b] The initial brain uptake of the N-substituted spiperone analogues began to decrease when the log P exceeded a value of 4.20.[18b] However, a suitable brain uptake and striatum:cerebellum ratio was observed with analogues possessing a log P as high as 4.54.[18b] A comparison of the calculated log P values of the 4′-substituted N-benzyl analogues (Table IV)[27] indicates that compound **4p** may be appropriate for further investigation. This analogue displays a high D$_2$:5-HT$_2$ selectivity (5-HT$_2$/D$_2$ = 120) as compared with spiperone (5-HT$_2$/D$_2$ = 7.8) and N-methylspiperone (5-HT$_2$/D$_2$ = 4.7). It also has a calculated log P value (4.40) that is reasonably close to 4.20, the value at which a decline in brain uptake was observed with the N-alkylspiperone analogues.[18a,b] Although **4n** displays a high D$_2$:5-HT$_2$ selectivity (5-HT$_2$/D$_2$ = 420), the relatively high log P value of this analogue may preclude the use of the corresponding ^{123}I-labeled analogue as a single-photon emission computed tomography (SPECT) tracer. However, a ^{125}I-labeled analogue could prove to be useful as an in vitro ligand for studies of D$_2$ receptors.

Table IV. Structures and Physicochemical Parameters of the N-(4′-Substituted-benzyl)spiperone Analogues



no.	X	π_x	log P^a	σ_p	F_x	R_x	MR$_x$
4h	H	0.00	4.68	0.00	0.00	0.00	1.03
4l	F	0.14	4.82	0.06	0.43	−0.34	0.92
4n	I	1.12	5.80	0.18	0.40	−0.19	13.9
4p	NO_2	−0.28	4.40	0.78	0.67	0.16	7.36
4r	CH_3	0.56	5.24	−0.17	−0.04	−0.13	5.65
4t	OCH_3	−0.02	4.66	−0.27	0.26	−0.51	7.87

[a] Calculated value.[27]

In conclusion, two series of N-alkylspiperone and substituted N-benzylspiperone analogues were prepared, and their affinities for both D$_2$ and 5-HT$_2$ receptors were determined. Substitution of the amide nitrogen with a benzyl group resulted in an analogue displaying a moderate selectivity for the dopamine D$_2$ receptor. A further improvement in D$_2$:5-HT$_2$ selectivity was observed with compounds containing substitutions on the 4′-position of the benzyl group. The results indicate that ^{18}F-labeled **4p** may be a suitable analogue for measuring D$_2$ receptor density in vivo with 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 a Varian EM-360L NMR spectrometer. Microanalyses were performed by Atlantic Microlab, Inc., Norcross, GA, and were within ±0.4% of the theoretical value unless otherwise noted (Table I). Tetrahydrofuran was distilled from sodium metal immediately prior to use. All other reagents and solvents were used without further purification. The sample of spiperone used in the in vitro binding assay was purchased from Janssen Life Sciences Products, 40 Kingsbridge Rd, Piscataway, NJ 08854.

1-Phenyl-8-(*tert*-butyloxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (6). A solution of di-*tert*-butyl dicarbonate (10.21 g, 45.36 mmol) in dichloromethane (25 mL) was added in portions to a stirred suspension of 1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (**5**; 10 g, 43.2 mmol) in dichloromethane (50 mL), and the reaction mixture was stirred at ambient temperature for 20 h. Volatile components were removed in vacuo, and the residue was suspended in pentane and filtered to yield the crude product as a tan solid. Purification by silica gel column chromatography (dichloromethane–acetone, 3:1) gave **6** as fine white needles (12.17 g, 85%): mp 209–212 °C; NMR ($CDCl_3$/TMS) δ 1.45 (s, 9 H), 1.90–2.90 (complex m, 4 H), 3.10–4.20 (complex m, 4 H), 4.70 (s, 2 H), and 6.50–7.70 (complex m, 6 H); IR (film) 3200, 3120, 3080, 2990, 2950, 1720, 1700, 1610, 1520, 1470, 1430, 1390, 1370, 1290, 1250, 1190, 1155, 1110, 1100, 1050, 1040, 1000, 975, 950, 925, 870, 785, 775, and 750 cm^{-1}. Anal. ($C_{18}H_{25}N_3O_3$) C, H, N.

1-Phenyl-3-(*p*-nitrobenzyl)-8-(*tert*-butyloxycarbonyl)-1,3,8-triazaspiro[4.5]decan-4-one (7). Solid sodium hydride (60% dispersion in mineral oil; 0.46 g, 11.5 mmol) was added in portions to a stirred solution of **6** (4 g, 12.1 mmol) in tetrahydrofuran (15 mL), and the reaction mixture was stirred at ambient temperature for 5 min. A solution of *p*-nitrobenzyl bromide (2.61 g, 12.1 mmol) in tetrahydrofuran (5 mL) was added dropwise, and the reaction mixture stirred at ambient temperature for 4 h. Volatile components were removed in vacuo, the resultant residue was dissolved in saturated aqueous sodium bicarbonate (30 mL), and the mixture extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give a yellow oil. Purification by

(26) (a) Dohlman, H. G.; Caron, M. G.; Lefkowitz, R. J. A Family of Receptors Coupled to Guanine Nucleotide Regulatory Proteins. *Biochemistry* **1987**, *26*, 2657–2664. (b) Pritchett, D. B.; Bach, A. W. J.; Wozny, M.; Taleb, O.; Dal Toso, R.; Shih, J. C.; Seeburg, P. H. Structure and Functional Expression of Cloned Rat Serotonin 5HT-2 Receptor. *EMBO J.* **1988**, *7*, 4135–4140. (c) Julius, D.; Huang, K. N.; Livelli, T. J.; Axel, R.; Jessel, T. M. The 5HT2 Receptor Defines a Family of Structurally Distinct but Functionally Conserved Serotonin Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 928–932.

(27) Leo, A.; Hansch, C.; Elkins, D. Partition Coefficients and Their Uses. *Chem. Rev.* **1971**, *71*, 525–554.

silica gel column chromatography (dichloromethane–acetone, 9.5:0.5) yielded the product as a yellow foam (4.42 g, 78.4%): mp softens at 56–59 °C; NMR ($CDCl_3$/TMS) δ 1.50 (s, 9 H), 2.00–2.90 (complex m, 4 H), 3.10–4.25 (complex m, 4 H), 4.55 (s, 2 H), 4.65 (s, 2 H), 6.50–7.55 (complex m, 7 H), and 8.20 (d, J = 9 Hz, 2 H); IR (film) 2960, 2910, 2850, 1700, 1610, 1535, 1510, 1475, 1425, 1390, 1375, 1350, 1290, 1250, 1180, 1160, 1100, 1070, 1010, 1000, 970, 910, 850, 800, 750, and 740 cm^{-1}.

N-(4′-Nitrobenzyl)spiperone (4p). A solution of 7 (4.42 g, 9.48 mmol) in trifluoroacetic acid (20 mL) was stirred at ambient temperature for 2 h. Volatile components were removed in vacuo to give a dark oil that was dissolved in saturated aqueous sodium bicarbonate (25 mL) adjusted to pH 10 by addition of 10% aqueous sodium hydroxide. The aqueous mixture was extracted with dichloromethane (3 × 75 mL), and the combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give 8 as a dark yellow foam (3.16 g, 91%).

A solution of 8 (1 g, 2.73 mmol), 2 (0.634 g, 2.59 mmol), KI (0.453 g, 2.73 mmol), and triethylamine (0.304 g, 3.00 mmol) in acetonitrile (5 mL) was stirred at reflux for 20 h. Volatile components were removed in vacuo to give a dark yellow residue that was dissolved in saturated aqueous sodium bicarbonate (20 mL) and extracted with dichloromethane (2 × 25 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give a yellow foam that was dissolved in absolute ethanol (20 mL). A 3 N solution of aqueous HCl (4 mL) was added, and the reaction mixture was stirred at reflux for 15 min. Volatile components were removed in vacuo to give a yellow oil that was dissolved in saturated aqueous sodium bicarbonate (25 mL) and extracted with dichloromethane (2 × 20 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give a dark yellow oil that was purified by silica gel column chromatography (dichloromethane–acetone–NH_4OH, 8:2:0.1) to afford **4p** as a yellow foam. The product was converted to the hydrochloride salt by treatment with a saturated HCl–ethyl acetate solution; recrystallization from ethanol–ether afforded **4p** as yellow prisms (0.83 g, 60%): NMR (free base, $CDCl_3$/TMS) δ 1.10 to 3.25 (complex m, 14 H), 4.50 (s, 2 H), 4.60 (s, 2 H), 6.50–7.55 (complex m, 9 H), and 7.65–8.30 (m, 4 H); IR (free base, neat) 3050, 3400, 3300, 1700, 1680, 1600, 1520, 1500, 1470, 1420, 1375, 1350, 1300, 1270, 1230, 1150, 1110, 1060, 1000, 980, 850, 810, 750, 740, 700, and 660 cm^{-1}.

General Methods for the Synthesis of the N-Alkyl- and N-Benzylspiperone Analogues. Method A. N-(4′-Fluorobenzyl)spiperone (4l). Solid sodium hydride (0.096 g of 60% NaH in oil, 2.40 mmol) was added to a stirred suspension of spiperone (1 g, 2.53 mmol) in tetrahydrofuran (5 mL), and the reaction mixture was stirred at ambient temperature for 5 min. A solution of *p*-fluorobenzyl bromide (0.454 g, 2.40 mmol) in tetrahydrofuran (1 mL) was added dropwise, and the reaction mixture was stirred at ambient temperature for 2 h. Volatile components were removed in vacuo to give a yellow oil that was dissolved in dichloromethane (25 mL) and washed with saturated aqueous sodium bicarbonate (2 × 20 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 (dichloromethane–acetone–NH_4OH, 85:15:0.1) to afford **4l** as a colorless foam (0.484 g, 40%), which was then treated with an HCl-saturated solution of ethyl acetate to give an analytical sample of **4l** (HCl salt) as a fluffy white solid: NMR (free base, $CDCl_3$/TMS) δ 1.40–3.25 (complex m, 14 H), 4.50 (s, 4 H), 6.50–7.35 (complex m, 11 H), and 7.95 (dd, J = 5 Hz, 2 H); IR (free base, film) 3100, 3080, 2950, 2850, 2280, 1700, 1600, 1500, 1475, 1430, 1380, 1300, 1275, 1230, 1160, 1125, 1100, 1075, 1000, 925, 850, 780, 750, 740, 710, and 660 cm^{-1}.

N-Methylspiperone (4a): NMR (HCl salt, CF_3COOH/TMS) δ 1.90–4.30 (complex m, 19 H) and 6.85–8.30 (complex m, 9 H); IR (HCl salt, KBr) 3072, 2964, 2936, 2663, 2568, 2491, 2415, 1702, 1678, 1599, 1506, 1485, 1458, 1443, 1405, 1379, 1326, 1288, 1227, 1156, 1098, 1084, 1054, 1002, 957, 891, 854, 752, 699, and 584 cm^{-1}.

N-(2′-Iodobenzyl)spiperone (4m): NMR (HCl salt, $CDCl_3$/TMS) δ 1.35–2.45 (complex m, 5 H), 2.70–4.00 (complex m, 10 H), 4.38 (d, J = 7 Hz, 4 H), 6.10–7.15 (complex m, 10 H), and 7.20–7.90 (complex m, 3 H); IR (HCl salt, KBr) 3448, 3059, 2951, 2374, 2346, 1702, 1696, 1599, 1508, 1474, 1466, 1458, 1438, 1391, 1376, 1296, 1282, 1259, 1226, 1209, 1157, 1095, 826, 746, and 695 cm^{-1}.

N-(4′-Iodobenzyl)spiperone (4n): NMR (HCl salt, CF_3COOH/TMS) δ 1.90–2.85 (complex m, 6 H), 2.90–3.45 (complex m, 4 H), 3.50–4.15 (complex m, 4 H), 4.45 (s, 2 H), 5.05 (s, 2 H), and 6.40–7.90 (complex m, 13 H); IR (HCl salt, KBr) 3448, 3059, 2966, 2799, 2363, 1702, 1688, 1598, 1508, 1560, 1542, 1508, 1499, 1475, 1451, 1400, 1389, 1375, 1345, 1292, 1221, 1211, 1160, 1098, 1007, 990, 816, 790, 746, 695, 670, and 600 cm^{-1}.

N-(2′-Nitrobenzyl)spiperone (4o): NMR (HCl salt, $CDCl_3$/TMS) δ 1.30–2.50 (complex m, 6 H), 2.65–4.10 (complex m, 9 H), 4.35 (s, 2 H), 4.60 (s, 2 H), and 6.20–7.70 (complex m, 13 H); IR (HCl salt, KBr) 3448, 2472, 2378, 2346, 1713, 1692, 1599, 1535, 1508, 1473, 1391, 1369, 1346, 1295, 1280, 1253, 1211, 1158, 826, 748, 696, and 602 cm^{-1}.

N-(2′-Methylbenzyl)spiperone (4q): NMR (HCl salt, $CDCl_3$/TMS) δ 1.10–2.50 (complex m, 8 H), 2.65–3.85 (complex m, 10 H), 4.28 (d, J = 6 Hz, 4 H), 6.20–7.10 (complex m, 11 H), and 7.25–7.75 (complex m, 2 H); IR (HCl salt, KBr) 3448, 3062, 2951, 2371, 2346, 1701, 1694, 1599, 1508, 1474, 1390, 1375, 1298, 1282, 1265, 1209, 1157, 826, 748, 696, 668, and 600 cm^{-1}.

N-(4′-Methylbenzyl)spiperone (4r): NMR (HCl salt, $CDCl_3$/TMS) δ 1.20–2.60 (complex m, 8 H), 2.65–3.90 (complex m, 10 H), 4.33 (d, J = 2 Hz, 4 H), 6.35–7.10 (complex m, 11 H), and 7.25–7.80 (complex m, 2 H); IR (HCl salt, KBr) 3448, 2906, 2530, 2365, 2346, 1704, 1687, 1654, 1599, 1560, 1508, 1500, 1468, 1458, 1389, 1375, 1291, 1223, 1160, 1099, 1004, 990, 818, and 745 cm^{-1}.

N-(2′-Methoxybenzyl)spiperone (4s): NMR (HCl salt, $CDCl_3$/TMS) δ 1.20–2.55 (complex m, 6 H), 2.60–3.90 (complex m, 12 H), 4.25 (s, 4 H), 6.15–7.00 (m, 11 H), and 7.10–7.60 (complex m, 2 H); IR (HCl salt, KBr) 3449, 3063, 2951, 2373, 2346, 1702, 1694, 1599, 1508, 1496, 1475, 1391, 1376, 1295, 1282, 1247, 1209, 1158, 1121, 1097, 1051, 1028, 826, 748, 696, 669, and 600 cm^{-1}.

N-(4′-Methoxybenzyl)spiperone (4t): NMR (HCl salt, $CDCl_3$/TMS) δ 1.25–2.55 (complex m, 5 H), 2.60–3.95 (complex m, 13 H), 4.25 (s, 4 H), 6.15–7.05 (m, 11 H), and 7.10–7.80 (complex m, 2 H); IR (HCl salt, KBr) 3449, 3062, 2962, 2372, 2346, 1700, 1685, 1598, 1512, 1467, 1390, 1375, 1297, 1282, 1247, 1213, 1180, 1159, 1034, 1003, 990, 824, 745, 696, 656, and 601 cm^{-1}.

Method B. N-Ethylspiperone (4b). Solid sodium hydride (0.091 g of 60% suspension in oil, 2.27 mmol) was added to a stirred suspension of **3** (1 g, 2.27 mmol) in tetrahydrofuran (5 mL), and the reaction mixture was stirred at ambient temperature for 5 min. Iodoethane (0.551 g, 3.41 mmol) was added, and the reaction mixture was stirred at reflux for 18 h. Volatile components were removed in vacuo, and the residue was dissolved in absolute ethanol (10 mL) and treated with a 3 M aqueous solution of HCl (2 mL). The mixture was stirred at reflux for 15 min, volatile components were removed in vacuo, and the mixture was dissolved in saturated aqueous sodium bicarbonate (35 mL) and extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give an oil that was purified by silica gel column chromatography (dichloromethane–acetone–NH_4OH, 4:2:0.1) to afford **4b** as a viscous oil (0.612 g, 63.7%), which was then treated with an HCl-saturated solution of ethanol to give an analytical sample of **4b** (HCl salt) as an amorphous, white solid: NMR (HCl salt, CF_3COOH/TMS) δ 1.34 (t, J = 7 Hz, 3 H), 1.8–4.15 (complex m, 18 H), and 6.35–7.75 (complex m, 9 H); IR (HCl salt, KBr) 3104, 3068, 2966, 2938, 2884, 2661, 2475, 2405, 1695, 1680, 1599, 1576, 1506, 1472, 1446, 1393, 1380, 1299, 1227, 1157, and 1098 cm^{-1}.

N-Propylspiperone (4c): NMR (HCl salt, CF_3COOH/TMS) δ 0.95 (t, J = 6, 7 Hz, 3 H), 1.30–2.75 (complex m, 10 H), 2.80–4.00 (complex m, 11 H), 6.30–6.90 (complex m, 3 H), and 6.95–7.80 (complex m, 6 H); IR (HCl salt, KBr) 3066, 2960, 2934, 2876, 2470, 2417, 1699, 1684, 1599, 1507, 1476, 1392, 1372, 1301, 1273, 1225, 1191, 1158, 1061, 1012, 974, 888, 841, 824, 746, and 697 cm^{-1}.

N-Butylspiperone (4d): NMR (HCl salt, DMSO-$CDCl_3$/TMS) δ 0.70–2.55 (complex m, 11 H), 2.60–4.20 (complex m, 14 H), 4.45 (s, 1 H), 6.10–7.05 (complex m, 7 H), 7.10–7.70 (complex m, 2 H); IR (HCl salt, KBr) 3102, 3074, 2960, 2932, 2653, 2478, 2418, 1693, 1687, 1598, 1507, 1479, 1371, 1303, 1278, 1228, 1215, 1158, and 1101 cm^{-1}.

N-(2-Methylpropyl)spiperone (4e): NMR (HCl salt, CF_3COOH/TMS) δ 1.10 (d, J = 6 Hz, 6 H), 1.50–4.20 (complex m, 19 H), 6.50–7.00 (m, 2 H), 7.10–7.45 (m, 5 H), and 7.50–7.90

(complex m, 2 H); IR (HCl salt, KBr) 3070, 2962, 2874, 2468, 2413, 1698, 1682, 1599, 1507, 1476, 1394, 1373, 1296, 1222, 1158, 974, 840, 748, and 698 cm^{-1}.

***N*-Pentylspiperone (4f)**: NMR (HCl salt, CF_3COOH/TMS) δ 0.80–1.20 (m, 3 H), 1.25–3.15 (complex m, 13 H), 3.20–4.20 (complex m, 11 H), 6.50–7.10 (m, 2 H), 7.15–7.40 (m, 5 H), and 7.45–8.10 (m, 2 H); IR (HCl salt, KBr) 3067, 2958, 2930, 2862, 2813, 2655, 2476, 2407, 1691, 1599, 1508, 1480, 1444, 1372, 1302, 1276, 1229, 1215, 1159, 1101, 1056, 974, 749, and 697 cm^{-1}.

***N*-(3-Methylbutyl)spiperone (4g)**: NMR (HCl salt, CF_3COOH/TMS) δ 1.10 (d, J = 5 Hz, 6 H), 1.35–3.10 (complex m, 10 H), 3.15–4.20 (complex m, 11 H), 6.60–7.05 (m, 2 H), 7.10–7.40 (m, 5 H), and 7.45–8.00 (m, 2 H); IR (HCl salt, KBr) 3103, 3065, 2961, 2873, 2811, 2652, 2478, 2418, 1691, 1599, 1507, 1479, 1371, 1303, 1280, 1228, 1214, 1158, 1101, 1000, 973, 888, 845, 820, 750, 698, 587, and 567 cm^{-1}.

***N*-Benzylspiperone (4h)**: NMR (HCl salt, $CDCl_3$/TMS) δ 1.30–3.90 (complex m, 15 H), 4.30 (s, 4 H), and 5.95–7.60 (complex m, 14 H); IR (HCl salt, KBr) 3063, 3032, 2956, 2883, 2642, 2470, 2364, 2335, 1701, 1694, 1599, 1506, 1473, 1390, 1374, 1295, 1281, 1267, 1210, 1158, 1002, 985, 826, 748, and 704 cm^{-1}.

***N*-Phenethylspiperone (4i)**: NMR (HCl salt, $CDCl_3$/TMS) δ 1.10–3.70 (complex m, 19 H), 4.20 (s, 2 H), 5.80–7.00 (complex m, 12 H), and 7.10–7.60 (complex m, 2 H); IR (HCl salt, KBr) 3063, 3026, 2952, 2647, 2488, 2418, 1697, 1689, 1600, 1506, 1472, 1378, 1300, 1274, 1230, 1157, 972, 827, 745, and 702 cm^{-1}.

***N*-(2′-Fluorobenzyl)spiperone (4j)**: NMR (HCl salt, $CDCl_3$/TMS) δ 1.40–2.55 (complex m, 5 H), 2.65–4.20 (complex m, 10 H), 4.40 (s, 4 H), and 6.00–7.70 (complex m, 13 H); IR (HCl salt, KBr) 3455, 3067, 2957, 2471, 2374, 2472, 2375, 2339, 1710, 1692, 1600, 1506, 1494, 1474, 1454, 1391, 1375, 1293, 1279, 1231, 1210, 1159, 1109, 1098, 1003, 984, 826, 761, and 748 cm^{-1}.

***N*-(3′-Fluorobenzyl)spiperone (4k)**: NMR (HCl salt, $CDCl_3$/TMS) δ 1.20–2.55 (complex m, 5 H), 2.60–4.00 (complex m, 10 H), 4.35 (s, 4 H), and 5.80–7.65 (complex m, 13 H); IR (HCl salt, KBr) 3457, 2362, 1706, 1693, 1599, 1507, 1474, 1457, 1391, 1374, 1293, 1210, 1158, 1136, 826, 795, 749, and 696 cm^{-1}.

In Vitro Binding Assays. In vitro binding assays for rat D_2 receptors were performed using rat striatal homogenates suspended in 50 mM Tris-HCl/150 mM NaCl/10 mM EDTA buffer (pH 7.4). The assay volume was 1 mL, and the concentration of [^{3}H]spiperone was approximately 1.0 nM. Assays were incubated at 37 °C for 45 min, and samples were then filtered through Whatman GF/B filters. The filters were washed with 10 mL of 10 mM Tris-HCl/150 mM NaCl (pH 7.4). (+)-Butaclamol (2 μM) was used to define nonspecific binding. Filters were incubated overnight in 3.0 mL of Econolite scintillation fluid, and radioactivity was determined by scintillation spectroscopy at an efficiency for tritium of 30%.

The affinities of compounds for the 5-HT_2 receptor were determined in competition experiments with 0.5 nM [^{125}I]I-LSD in 50 mM Tris-HCl (pH 7.4) using membranes from P11 cells.[22] The assay volume was 100 μL, and 1 mM ketanserin was used to define nonspecific binding. Samples were incubated at 37 °C for 60 min and were filtered through Scheicher and Schuell filters coated with 3% polyethylenimine. Filters were washed with 10 mL of 50 mM Tris-HCl (pH 7.4), and radioactivity was determined using a Beckman 4000 gamma counter.

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:[23] $K_i = IC_{50}/(1 - [L]/K_d)$, 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 [^{3}H]spiperone, D_2 sites, and 1.6 nM for [^{125}I]I-LSD, 5-HT_2 sites). The D_2:5-HT_2 selectivity (5-HT_2/D_2 ratio) of each analogue is expressed as the ratio of the K_i values at 5-HT_2 and D_2 receptors ($K_{i_{5\text{-}HT_2}}/K_{i_{D_2}}$).

Acknowledgment. This project was funded by Grants NS 14867, GM 34781, and NS 18591 awarded by the National Institutes of Health and Grants MH 43880 and MH 48125 awarded by the National Institute of Mental Health.

Registry No. **1**, 1021-25-6; **2**, 3308-94-9; **3**, 54080-21-6; **4a**, 3725-68-6; **4a** base, 87539-19-3; **4b**, 102504-73-4; **4b** base, 104066-91-3; **4c**, 138091-55-1; **4c** base, 104066-92-4; **4d**, 138091-56-2; **4d** base, 114115-90-1; **4e**, 138091-57-3; **4e** base, 138091-58-4; **4f**, 138091-59-5; **4f** base, 138091-60-8; **4g**, 138091-61-9; **4g** base, 138091-62-0; **4h**, 138091-63-1; **4h** base, 138091-64-2; **4i**, 138091-65-3; **4i** base, 138091-66-4; **4j**, 138091-67-5; **4j** base, 138091-68-6; **4k**, 138091-69-7; **4k** base, 138091-70-0; **4l**, 138091-71-1; **4l** base, 138091-72-2; **4m**, 138091-73-3; **4m** base, 138091-74-4; **4n**, 138091-75-5; **4n** base, 138091-76-6; **4o**, 138091-77-7; **4o** base, 138091-78-8; **4p**, 138091-79-9; **4p** base, 138091-80-2; **4q**, 138091-81-3; **4q** base, 138091-82-4; **4r**, 138091-83-5; **4r** base, 138091-84-6; **4s**, 138091-85-7; **4s** base, 138091-86-8; **4t**, 138091-87-9; **4t** base, 138091-88-0; **6**, 138091-52-8; **7**, 138091-53-9; **8**, 138091-54-0; di-*tert*-butyl dicarbonate, 24424-99-5; *p*-nitrobenzyl bromide, 100-11-8; *p*-fluorobenzyl bromide, 459-46-1.