2-Phenyl-4-(aminomethyl)imidazoles as Potential Antipsychotic Agents. Synthesis and Dopamine D_2 Receptor Binding

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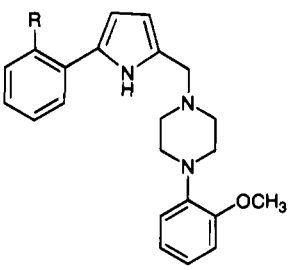
A series of 2-phenyl-4-(aminomethyl)imidazoles were designed as conformationally restricted analogs of the dopamine D_2 selective benzamide antipsychotics. The title compounds were synthesized and tested for blockade of [^{3}H]YM-09151 binding in cloned African green monkey dopamine D_2 receptor preparations. The binding affinity data thus obtained were compared against that of the benzamides and a previously described series of 2-phenyl-5-(aminomethyl)-pyrroles.

Schizophrenia is a complex psychological disorder of unclear etiology. Afflicted individuals may demonstrate a wide range of behavioral patterns characterized at the one extreme by hallucinations, paranoia, and bizarre, disorganized behavior to social withdrawal, catatonia, and affective 'flattening' of the personality at the other extreme.[1–3]

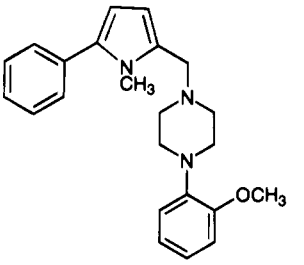
The drugs which have previously been used to treat this disorder have come from many diverse structural classes. Representative examples of some of these classes of compounds are shown in Figure 1 with the phenothiazines represented by chlorpromazine (**1**), the butyrophenones represented by haloperidol (**2**), the dibenzo[*b*,*e*][1,4]diazepines represented by clozapine (**3**), and the benzamides represented by raclopride (**4a**), sulpiride (**4b**), remoxipride (**4c**), and YM-09151 (**5**). A common pharmacological thread running through these diverse structures is the ability to interact with dopamine receptors, in particular D_2.[4] Direct correlation between D_2 receptor binding affinity and antipsychotic activity has been demonstrated.[5,6]

In addition to D_2 receptors, the phenothiazines, butyrophenones, and clozapine bind to a fairly wide array of other receptor systems. The introduction of the benzamides provided a class of compounds with high selectivity for D_2 receptors for examination as potential antipsychotic agents.[7] When compared against chlorpromazine and haloperidol, these compounds appear to show the advantage of a decreased propensity to induce the extrapyramidal side effects characteristic of most of the common antipsychotic agents. A series of papers demonstrated the importance of proper phenyl ring substituents for good binding to the D_2 receptor.[8–11] In particular, the presence of an ortho methoxy group appeared to be optimal. The importance of this group has been accredited to a hydrogen-bonding interaction between a lone electron pair on the oxygen of the ortho methoxy group and the hydrogen of the secondary amide.[12,13]

van Wijngaarden et al. described a series of 2-phenylpyrroles as conformationally restricted benzamide analogs.[14] These compounds, of which **6a** is representative, showed high affinity (1 nM for **6a**) for D_2 receptors in rat striatum. This series of compounds demonstrated that the amide moiety of the benzamides was not essential for D_2 binding. In contrast to the benzamides, an ortho methoxy group lowers D_2 affinity 6.5-fold (compound **6b**, 6.5 nM). Although not critical for binding, the NH of the pyrrole is apparently advantageous since the corresponding *N*-methylphenylpyrrole **7** showed a 20-fold less affinity for the D_2 receptor.



6a R = H
6b R = OCH_3



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As pyrroles are known to be susceptible to oxidation, it would be advantageous to identify compounds of similar geometry without this oxidative liability. The "six–five" carbon backbone of 2-phenylpyrrole allows for a wide variety of analogous series of compounds. When consideration is given to preserving an NH within the five-member ring, the subset is considerably smaller. In this manuscript we describe the preparation and the D_2 receptor binding of a series of 2-phenyl-4-(aminomethyl)imidazoles.

Chemistry. The syntheses of the title compounds are outlined in Scheme 1. Addition of lithium hexamethyldisilazane to the appropriately substituted benzonitrile provided the corresponding benzamidine **8**.[15] Condensation of hydrochloride salts of the benzamidines with dihydroxyacetone dimer in ammonium hydroxide solution furnished the 2-phenyl-4-(hydroxymethyl)-imidazoles **9**. When the benzamidine was available as the free base, ammonium chloride was added to the condensation mixture to prepare the salt in situ. Attempts to prepare the imidazoles from the amidine free bases lead to dramatically reduced yields. Treatment of the hydroxymethyl compounds **9** with thionyl chloride

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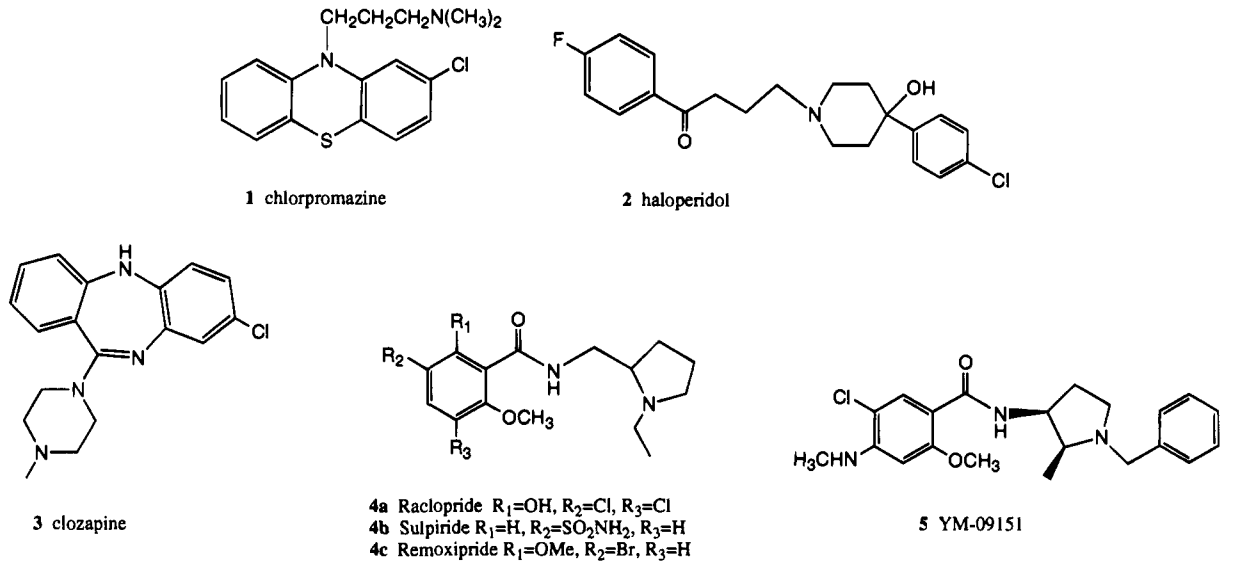
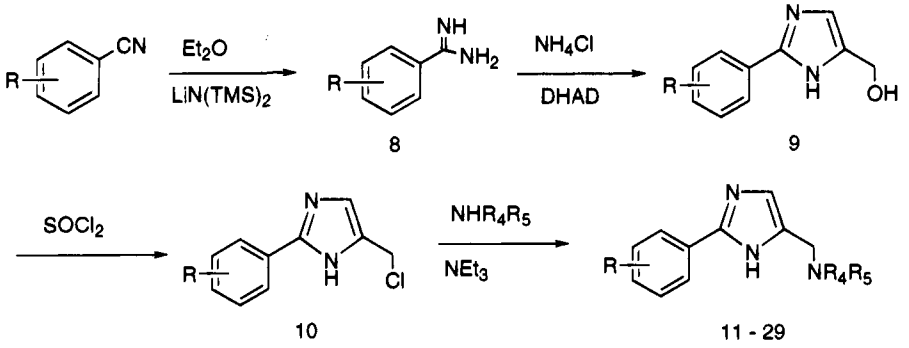


Figure 1. Representative antipsychotic agents.

Scheme 1. Preparation of 2-Phenyl-4-(aminomethyl)imidazoles



provided the intermediate (chloromethyl)imidazole hydrochlorides **10** which were reacted with the appropriate secondary amine in the presence of triethylamine to provide the final products **11**.[16]

Receptor Binding. Affinity at D_2 receptors was determined via standard competitive displacement assays using D_2 receptor cloned from the African green monkey with [^{3}H]YM-09151 as the competitive ligand. As the affinity for pyrrole **6a** was originally determined by van Wijngaarden et al. using rat corpus striatum, this compound was prepared and tested against the clones for direct comparison.

Results and Discussion

For our investigation, we wished to explore the major aspects of the structure–activity relationships (SAR) within this series of compounds in two ways. Keeping the 2-phenylimidazole structure inviolate, we first varied the aminomethyl 'tail'. Subsequent to this, the substituent pattern on the 2-phenyl ring of the imidazole was optimized. The optimum aminomethyl tail and phenyl ring substitution pattern determined from these two experiments were then combined, and the resultant compound was compared against its daughters.

Initially, the unsubstituted 2-phenylimidazole system was combined with a series of 4-aminomethyl side chains (Figure 2). In order to limit the number of possible aminomethyl 'tails', most of the amines were selected on the basis of being subunits of known dopamine D_2 blockers. Using the D_2 receptor clones, the model compound **6a** had an affinity of 53 nM. Compound **12**, the imidazole analog of **6a**, displayed a similar affinity of 46 nM. The 2-phenylimidazole analogs having the amine tails found in haloperidol (**13**, 220 nM), pimozide (**14**, 834 nM), and spiperone (**15**, 158 nM) were all found to possess lower affinity for the D_2 receptor clone. Compound **17** having a simple *N*-methylbenzyl tail was also found to have diminished activity (1315 nM) relative to **12**. Compound **16a**, with a 4-phenyl-1,2,3,6-tetrahydropyridine subunit characteristic of the neurotoxin MPTP, was found to have comparable activity (55 nM) to the model compound of van Wijngaarden, **6a**. The relatively poor binding affinity (4200 nM) found for the corresponding unsubstituted 1,2,3,6-tetrahydropyridine (**16b**) derivative demonstrated the importance of the 4-phenyl group toward the activity of **16a**.

In order to examine the effects of various substitution patterns on the 2-phenyl ring, it became neccessary to hold the aminomethyl 'tail' constant. The *N*-methylbenzyl tail was chosen since the binding of its unsubstituted derivative was in the low micromolar range, and thus improvements in binding via changes in the 2-phenyl ring were judged to be easier to discern.

The aromatic SAR of the 2-phenyl ring was carried out as follows (Table 1). Initially, the effect of placing a single electron-withdrawing (**18**–**20**) group, represented by fluorine, or electron-donating (**21**–**23**) group, represented by methoxy, at the ortho, meta, or para

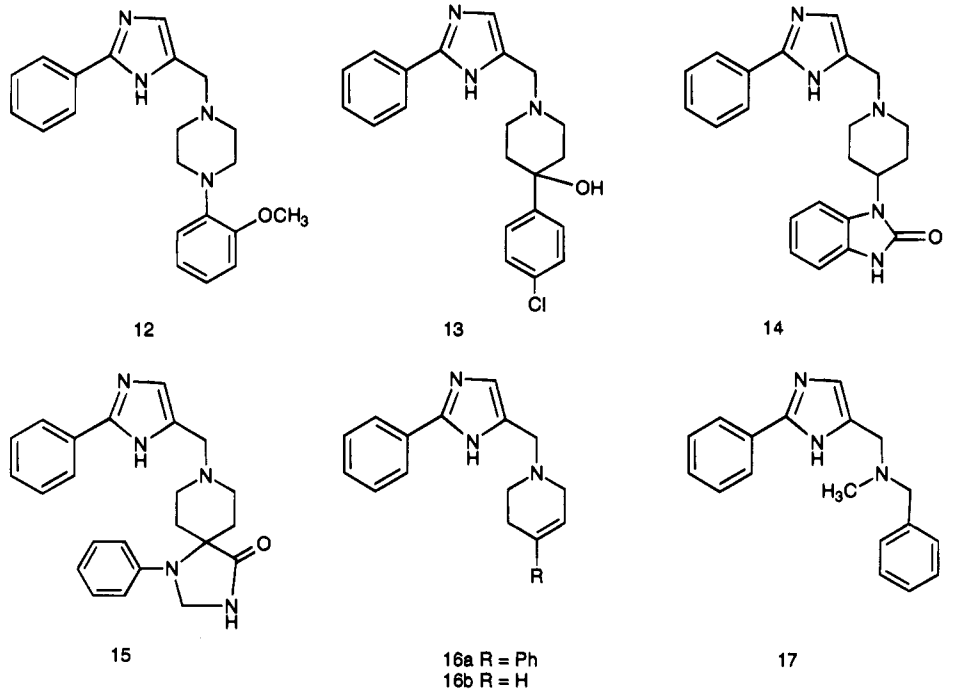
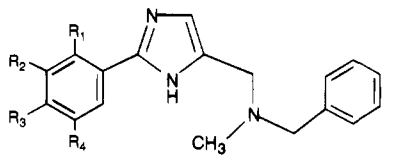


Figure 2. 2-Phenyl-4-(aminomethyl)imidazoles.

Table 1. Blockade of [^{3}H]YM-09151 Binding to African Green Monkey D_2 Receptor Clone by Substituted 2-Phenyl-4-[(*N*-methyl-*N*-benzylamino)methyl]imidazoles

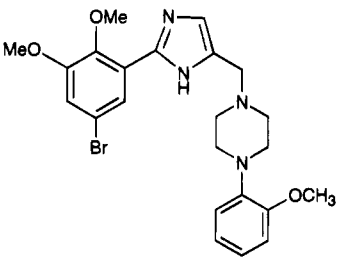


compd	R_1	R_2	R_3	R_4	mp (°C)[a]	K_i vs [^{3}H]YM-01915 (nM)[b]
17	H	H	H	H	245–246	1320
18	F	H	H	H	229–234 dec	560
19	H	F	H	H	139–141	460
20	H	H	F	H	238–240	2140
21	OMe	H	H	H	245–247	74
22	H	OMe	H	H	187–190	2230
23	H	H	OMe	H	242–244	3470
24	OMe	H	H	Cl	216–217	45
25	OMe	H	H	Br	179–180	32
26	OMe	OMe	H	H	201–203	5.1
27	H	OMe	OMe	H	256–258	3750
28	OMe	OMe	H	Br	204–205	0.7
haloperidol						4.8
clozapine						254

[a] All compounds were dihydrochloride salts except compound **19** was a dimaleate. [b] The K_i for binding is the average of three experiments. The SEM for all values was <10%.

position was examined. Both the 4-fluoro and 4-methoxy substitution displayed lower affinity than the parent compound **17**. Among the remaining ortho and meta substituents, only 2-methoxy displayed a significant improvement in binding versus the unsubstituted phenyl compound (74 versus 1324 nM). In an effort to improve overall binding in this test series, more complicated aromatic patterns which had been previously found by Hogberg et al.[11] to be advantageous to D_2 binding in the benzamide series were prepared and tested against the cloned D_2 receptors. Although the aromatic SAR data of van Wijngaarden were not elaborated along the same lines as those of the benzamide researchers, the pattern of aromatic substituent data within this study (Table 1, compounds **24**–**28**) most surely follows that of the benzamides. To wit, Hogberg demonstrated that a (2-methoxy-5-halophenyl)benzamide displayed improved D_2 binding over the simple 2-(methoxyphenyl)benzamide. A similar finding occurred within the 2-phenylimidazole series where the binding of the 2-phenylimidazole was surpassed by both the 5-chloro (**24**) and 5-bromo (**25**) derivatives. A further improvement in D_2 binding in the benzamide series is found in the 2,3-dimethoxy-5-bromo pattern. Compound **28** in the 2-phenylimidazoles shows a similar increase.

When the compound derived from the best results of the aminomethyl side chain variants and the 2-phenyl aromatic SAR experiments was prepared, it showed that the results of the two optimizations were not additive. The hybrid compound **29** showed an affinity for the D_2 receptor clone of 204 nM.



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In conclusion, extension of the ideas of van Wijngaarden for conformational restriction of the benzamide antipsychotics through the use of five-member ring heterocycles has been expanded upon via the preparation of 2-phenyl-4-(aminomethyl)imidazoles. Structural modifications of the 2-phenylimidazole series resulted in 2-(5-bromo-2,3-dimethoxyphenyl)-4-[(*N*-methyl-*N*-benzylamino)methyl]imidazole (**28**), a compound with high binding affinity for the dopamine D_2 receptor.

Experimental Section

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were obtained for all compounds tested for D_2 binding. ^1H NMR and/or mass spectral analysis were carried out on all isolated intermediates and are available upon request. Elemental analyses were performed at Robertson Microlabs, Madison, NJ, and are within 0.4% of the theoretical C, H, and N with the exception of **20** where carbon analysis was 0.68% below the calculated value. Electron ionization mass spectra were obtain using a Hewlett-Packard 5890 mass spectrometer. ^1H NMR spectra were recorded from $CDCl_3$ solutions using a Varian Gemini 300 spectrometer unless otherwise indicated; results are recorded as ppm downfield from the TMS signal. Spectral data for all amines are reported in the free base form. [^{3}H]YM-09151 was purchased from NEN-DuPont (Boston, MA).

General Procedure for the Preparation of Compounds 3–15. The following four-step preparation of **24** represents a generalized experimental procedure which was utilized to prepare all of the new compounds described in this report. The starting nitriles were all commercially available with the exceptions of 5-chloro-2-methoxybenzonitrile and 5-bromo-2,3-dimethoxybenzonitrile whose preparations are described herein.

5-Chloro-2-methoxybenzonitrile. 5-Chlorosalicylaldehyde (10 g) and dimethyl sulfate (9.65 g, 1.2 equiv) were added to a suspension of potassium carbonate (35 g) in 125 mL of dimethylformamide. The reaction mixture was heated to 60 °C for 12 h. The mixture was then treated concentrated NH_4OH (10 mL) and stirred for 20 min before being diluted with water (200 mL) and extracted with ether (200 mL). The organic extract was washed with water, dried ($MgSO_4$), and concentrated to give 10 g of the methyl ether as a white crystalline solid (mp 74–75 °C, R_f = 0.75 in 10% EtOAc/hexane).

The solid was dissolved in 75 mL of formic acid. To this were added 4.7 g of hydroxylamine hydrochloride and 7 g of sodium acetate. The resulting mixture was refluxed for 14 h, diluted with water (200 mL) and extracted with chloroform (3 × 50 mL). The organic extracts were washed with dilute ammonium hydroxide, dried, and concentrated to give 9.7 g of crystalline 5-chloro-2-methoxybenzonitrile (mp 89–91 °C).

5-Chloro-2-methoxybenzamidine (8). A solution of lithium hexamethyldisilazane was prepared by the dropwise addition of 48 mL of 2.5 N *n*-butyllithium in hexanes to a solution of 25.3 mL of hexamethyldisilazane in 200 mL of ether at 0 °C. 5-Chloro-2-methoxybenzonitrile (9.7 g) was then added and the solution allowed to stand at room temperature for 2 h. The reaction flask was then cooled in an ice bath, and the reaction was quenched by the careful addition of 80 mL of 3 N HCl solution. After stirring for 30 min, 200 mL of water was added and the ether layer was discarded. Chloroform (200 mL) was added, and the aqueous layer was made basic with 3 N NaOH solution. The aqueous layer was washed twice with chloroform (2 × 50 mL), and the combined organic extracts were dried with K_2CO_3 and concentrated to provide 5.1 g (48%) of the amidine as a tan solid (mp 100 °C): ^1H NMR (DMSO-d_6) 7.48 (d, J = 3 Hz, 1H), 7.40 (dd, J = 3, 3 Hz, 1H), 7.09 (d, J = 3 Hz, 1H), 6.25–6.4 (b, 3H, NH), 3.81 (s, 3H, OMe).

2-(5-Chloro-2-methoxyphenyl)-4-(hydroxymethyl)-imidazole (9). A suspension of **8** (2.5 g, 13.6 mmol), dihydroxyacetone dimer (2.5 g), and ammonium chloride (3 g) in 20 mL of NH_4OH was heated at 80 °C for 30 min. The cooled mixture was extracted with ethyl acetate (300 mL), dried (Na_2SO_4), and concentrated. The crude material was triturated with ether, and the resulting tan solid was filtered to provide 1.65 g (51%) of the desired imidazole (mp 173–175 °C): ^1H NMR ($CDCl_3$) 8.32 (d, J = 3 Hz, 1H), 7.27 (dd, J = 3, 7 Hz, 1H), 7.08 (s, 1H, imidazole C5), 6.95 (d, J = 7 Hz, 1H), 4.74 (s, 2H, CH_2OH), 4.05 (s, 3H, OMe).

2-(5-Chloro-2-methoxyphenyl)-4-[(*N*-benzyl-*N*-methylamino)methyl]imidazole (24). (Hydroxymethyl)imidazole **9** (250 mg, 1.05 mmol) was dissolved in 4 mL of thionyl chloride, heated to 80 °C for 10 min, and concentrated. The residue was dissoved in 10 mL of $CHCl_3$ and reconcentrated. The resulting oil was redissolved in $CHCl_3$ (10 mL), and *N*-benzylmethylamine (127 mg, 1.05 mmol) and triethylamine (3 mL) were added. After 30 min the solution was washed with 1 N NaOH, dried (Na_2SO_4), and concentrated to provide **24** (325 mg, 91%) as an oil: ^1H NMR ($CDCl_3$) 8.32 (s, 1H), 7.2–7.4 (m, 6H), 7.05 (s, 1H, imidazole C5), 6.92 (d, J = 7 Hz, 1H), 4.02 (s, 3H, OMe), 3.65 (s, 2H), 3.51 (s, 2H), 2.28 (s, 3H, NMe); MS (CI mode) *m/z* 342. A sample of the fumarate salt was prepared in ether and recrystallized from ethyl acetate (mp 88–91 °C).

5-Bromo-2,3-dimethoxybenzonitrile. To a solution of 3-methoxysalicylaldehyde (15.2 g, 0.1 mol) in 125 mL of acetone containing 10 mL of water was added 18.5 g of *N*-bromosuccinimide. An exothermic reaction was observed. After 20 min the reaction was quenched with 0.5 N sodium bisulfite solution and the mixture extracted with ether. The ether extracts were washed with water, dried ($MgSO_4$), and concentrated. The resulting solid was recrystallized from ether to give 12 g of the bromoaldehyde (55%, mp 116–117 °C).

This material was then dissolved in DMF containing 8 g of dimethyl sulfate and 28 g of K_2CO_3. The reaction mixture was stirred at room temperature for 4 h, diluted with 1.6 N NH_4-OH solution (100 mL), and extracted with ether. The ether extracts were washed with water, dried, and concentrated. The resultant material was recrystallized from hexane to give 8 g (62%) of the desired bromodimethoxybenzaldehyde (mp 78-80 °C).

The solid was dissolved in 75 mL of formic acid. To this were added 6.2 g of hydroxylamine hydrochloride and 8 g of sodium acetate. The resulting mixture was refluxed for 14 h, diluted with water (200 mL), and extracted with chloroform (3 x 50 mL). The organic extracts were washed with dilute ammonium hydroxide, dried, and concentrated to give 7.4 g of crystalline 5-bromo-2,3-dimethoxybenzonitrile (mp 85–87 °C).

2-Phenyl-4-[[4-(2-methoxyphenyl)-1-piperazinyl]methyl]imidazole dihydrochloride (12): mp 245–246 °C; ^1H NMR (DMSO) 7.90 (m, 2H), 7.40 (dd, J = 7.3, 7.9 Hz, 2H), 7.29 (dd, J = 6.7, 7.9 Hz, 1H), 6.82–6.92 (m, 4H), 3.73 (s, 3H), 3.4–3.5 (m, 2H) 3.3 (bs, 2H, H_2O), 2.95 (m, 4H), 2.58 (m, 4H); MS (CI mode) *m/z* 349.

2-Phenyl-4-[[4-hydroxy-4-(4-chlorophenyl)-1-piperidyl]-methyl]imidazole dihydrochloride (13): mp 239–240 °C; ^1H NMR (DMSO) 8.18 (m, 2H), 7.85 (s, 1H), 7.58–7.64 (m, 3H), 7.43 (m, 4H), 4.51 (s, 2H), 3.45 (d, J = 12 Hz, 2H), 3.32 (m, 2H), 2.39 (dd, J = 14, 12 Hz, 2H), 1.80 (d, J = 14 Hz, 2H); MS (CI mode) *m/z* 368.

2-Phenyl-4-[[4-(2-keto-1-benzimidazolinyl)-1-piperidyl]-methyl]imidazole dimaleate (14): mp 197–199 °C; ^1H NMR (DMSO) 7.96 (d, J = 7 Hz, 2H), 7.48 (m, 3H), 7.40 (q, J = 7 Hz, 2H), 7.32 (m, 1H), 6.99 (s, 3H), 6.17 (s, 4H, maleate), 4.5 (m, 1H), 4.27 (s, 2H), 3.62 (d, J = 11 Hz, 2H), 3.22 (dd, J = 11, 12 Hz, 2H), 2.6 (m, 2H), 1.85 (m, 2H); MS (CI mode) *m/z* 374.

2-Phenyl-4-[[8-(1-phenyl-1,3,8-triazaspiro[4.5]decan-4-onyl)]methyl]imidazole dihydrochloride (15): mp 254–256 °C; ^1H NMR (DMSO) 9.0 (s, 1H), 8.0 (d, J = 7.3 Hz, 2H), 7.36–7.55 (m, 4H), 7.20 (dd, J = 7.3, 7.9 Hz, 2H), 7.03 (d, J = 7.9 Hz, 2H), 6.77 (dd, J = 7.3, 6.7 Hz, 1H), 4.60 (s, 2H), 4.28 (s, 2H), 3.70 (m, 2H), 3.7 (m, 2H), 3.5 (m, 2H), 2.92 (s, 2H), 1.82 (d, J = 14 Hz, 2H); MS (CI mode) *m/z* 388.

2-Phenyl-4-[(4-phenyl-1,2,3,6-tetrahydro-1-pyridinyl)-methyl]imidazole dihydrochloride (16a): mp 246–248 °C; ^1H NMR (DMSO) 8.19 (d, J = 6 Hz, 2H), 7.91 (s, 1H), 7.62 (m, 3H), 7.35 (d, J = 7 Hz, 2H), 7.37 (dd, J = 8, 8 Hz, 2H), 7.30 (m, 1H), 6.2 (s, 1H), 4.58 (s, 2H), 3.96 (m, 2H), 3.76 (m, 2H), 2.84 (m, 2H); MS (CI mode) *m/z* 316.

2-Phenyl-4-[(1,2,3,6-tetrahydro-1-pyridinyl)methyl]-imidazole dihydrochloride (16b): mp 259–261 °C; ^1H NMR (DMSO) 8.21 (m, 2H), 7.90 (s, 1H), 7.62 (dd, J = 6, 2 Hz, 3H), 5.89 (d, J = 10.4 Hz, 1H), 5.70 (d, J = 10.4 Hz, 1H), 4.52 (s, 2H), 3.78 (s, 2H), 3.6 (m, 2H), 2.4 (m, 2H); MS spectra (CI mode) *m/z* 240.

2-Phenyl-4-[(*N*-methyl-*N*-benzylamino)methyl]imidazole dihydrochloride (17): mp 245–246 °C; ^1H NMR (DMSO) 8.11 (d, J = 6.7 Hz, 2H), 7.81 (s, 1H), 7.62 (dd, J = 8,

4 Hz, 2H), 7.59 (d, J = 8 Hz, 2H), 7.45 (m, 2H), 4.4–4.5 (b, 2H), 4.25 (b, 2H), 2.62 (s, 3H, NMe); MS (CI mode) m/z 276.

Recombinant Expression of D_2 Receptor. The recombinant monkey D_2 receptor (Genbank Accession Number U18547) was cloned from substantia nigra enriched midbrain cDNA of African green monkey (*Cercopithecus aethiops*). The sequence of the clone corresponds to the longer variant (D_{2a}) of the two alternative human splice variants.[17–20]

The sequenced cDNA was introduced into the expression vector pcDNA I/Neo (Stratagene). This vector was transfected into the Chinese hamster ovary cell CHO-K1 by a modification of the calcium phosphate method of Graham and Van der Eb.[21] Colonies resistant to the antibiotic G418 were isolated and expanded. Membrane preparations from the transfectant clones were tested for their ability to bind [^{3}H]YM-09151. A clone showing high levels of binding, D2-12, was grown in roller bottles and harvested, and the cell pellets were stored at −80 °C until needed, up to 4 months.

Membrane Preparation. Pellets containing D_2 membrane were thawed on ice and resuspended in ice-cooled 50 mM Tris buffer (pH 7.4 at 25 °C) containing 120 mM NaCl, 1 mM EDTA, and 5 mM $MgCl_2$. All subsequent work was performed on ice. The membranes were homogenized on a Brinkmann Polytron homogenizer (10 s, setting 5). The homogenate was centrifuged at 48000g and 4 °C for 10 min (DuPont Sorvall RC5B). The pellet was resuspended in fresh buffer, and centrifugation was repeated. The pellet was again resuspended in fresh buffer and centrifuged a final time at 48000g and 4 °C for 10 min. The pellet was resuspended to a final concentration of 100 μg of protein/mL with 50 mM Tris buffer (pH 7.4 at 25 °C) containing 120 mM NaCl, just prior to addition to the assay tubes. The protein content was determined using the Bio-Rad assay (Hercules, CA), with bovine plasma γ-globulin as a standard.

Binding Assay. Each sample was tested in triplicate in a final volume of 1.0 mL in 12 × 75 mm poly(propylene) test tubes containing 0.1 nM [^{3}H]YM-09151 (81.4 Ci/mmol, NEN DuPont) and tissue homogenate (40 μg of protein) in 50 mM Tris buffer (pH 7.4 at 25 °C) containing 120 mM NaCl. After a 90 min incubation at 25 °C in a shaking water bath (Precision Scientific), the samples were rapidly filtered through a 48-well Brandel cell harvester (Gaithersburg, MD), using Whatman GF/C filters. The filters were rinsed with two 5 mL washes of assay buffer. Filters were equilibrated in 3 mL of Ultima Gold scintillation fluid. Bound radioactivity was then quantitated via liquid scintillation spectroscopy (Packard Instruments, Meriden, CT) at an efficiency of 65%. Nonspecific binding was defined with 1 μM spiperone.

Data Analysis. Binding data were analyzed with the nonlinear curve-fitting program RS/1 (BBN Software Products, Cambridge, MA). Calculated IC_{50} values were then converted to K_i values using the Cheng–Prusoff correction[22] with the following equation: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the radioligand concentration and K_d is the previously determined dissociation constant for [^{3}H]YM-09151 at the cloned primate D_2 receptor (0.070 nM).

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