Synthesis, Receptor Potency, and Selectivity of Halogenated Diphenylpiperidines as Serotonin 5-HT_{2A} Ligands for PET or SPECT Brain Imaging

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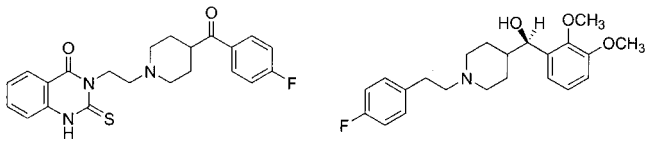
A series of 4′-substituted phenyl-4-piperidinylmethanol and benzoyl-4-piperidine derivatives was synthesized as potential novel serotonin 5-HT_{2A} receptor ligands that can be radiolabeled for in vivo brain imaging. Compounds were prepared by alkylation of 4-substituted benzoyl-4-piperidine with an iodo- or fluoro-substituted phenylalkyl halide followed by reduction with sodium borohydride. Potency of novel compounds was determined by in vitro radioreceptor affinity assays selective for serotonin 5-HT_{2A} receptors. Potent compounds were further evaluated for selectivity at serotonin-2A versus 2C, 6, and 7, as well as dopamine D_2 and adrenergic α_1 and α_2 receptors. The novel compound (4-fluorophenyl)-(1-[2-(4-fluorophenyl)-ethyl]piperidin-4-yl])methanol was particularly promising with high 5-HT_{2A} potency (K_i = 1.63 nM) and >300-fold selectivity over other 5-HT receptor types.

Introduction

Cerebral receptors for serotonin (5-hydroxytryptamine, 5-HT) have been implicated in the pathogenesis of major mood and psychotic disorders such as schizophrenia,[1,2] and 5-HT_{2A} receptors probably contribute to the actions of psychotropic agents used in the treatment of these common disorders.[3,4] Nevertheless, knowledge of the localization and functional roles of the large number of 5-HT receptors, including 5-HT_{2A} receptors, remains incomplete. A powerful method of contributing to such knowledge is the use of clinical anatomical and functional radionuclide brain imaging with PET (positron emission tomography) and SPECT (single photon emission computed tomography). These techniques provide methods of high sensitivity for measuring in vivo neurochemical and pharmacological effects at specific target-receptor proteins. Imaging of the 5-HT_{2A} receptor in vivo can be used to assess receptor occupancy, guide dosing, examine cerebral and plasma pharmacokinetics, and perhaps predict the efficacy of the treatment with antipsychotic and mood-altering drugs that interact with targeted sites.[5] Development of improved radiotracers for PET (labeled with ^{18}F or ^{11}C) and SPECT (^{123}I) with high specific uptake and selectivity for 5-HT_{2A} receptors would, therefore, be very useful.

Among the reported ligands for the 5-HT_{2A} receptor,[6] the two structurally related compounds altanserin and MDL-100 907 (Figure 1) have relatively high affinity and selectivity for the 5-HT_{2A} receptor compared to other 5-HT receptors,[7,8] and [^{18}F]altanserin and [^{11}C]-MDL-100 907 have been developed for in vivo imaging.[9,10] However, neither is fully satisfactory, owing to limitations of potency, selectivity, or biological half-life. Notably, the selectivity of altanserin for 5-HT_{2A} over 5-HT_{2C} is about 20-fold. Although MDL-100 907 has a high affinity and selectivity for the 5-HT_{2A} receptor, its radiolabeling with short-lived ^{11}C (half-life of 20 min) limits its suitability to short-term studies. A more ideal radioligand for clinical in vivo imaging of 5-HT_{2A} receptors would have high 5-HT_{2A} potency ($K_i \leq 1$ nM) and selectivity (>100-fold) toward other, particularly 5-HT, receptors and be radiolabeled with ^{18}F (half-life of 110 min) or ^{123}I (half-life of 13.2 h).



Altanserin MDL 100907

Figure 1. 5-HT_{2A} selective ligands. For altanserin, 5-HT_{2A} K_i = 0.30 nM, and selectivity for serotonin 2A over 2C receptors was 20-fold. For MDL-100 907, 5-HT_{2A} K_i = 0.36 nM, and selectivity was 300-fold.

In our program for the design, synthesis, and neuropharmacological assessment of potent and selective 5-HT_{2A} receptor ligands for PET or SPECT imaging, we developed novel compounds based on common structural features of altanserin and MDL-100 907 to provide a new series of 4′-substituted phenyl-4-piperidinylmethanol and benzoyl-4-piperidine derivatives. We now report on their synthesis and binding affinities to 5-HT_{2A} and other potentially competing receptors.

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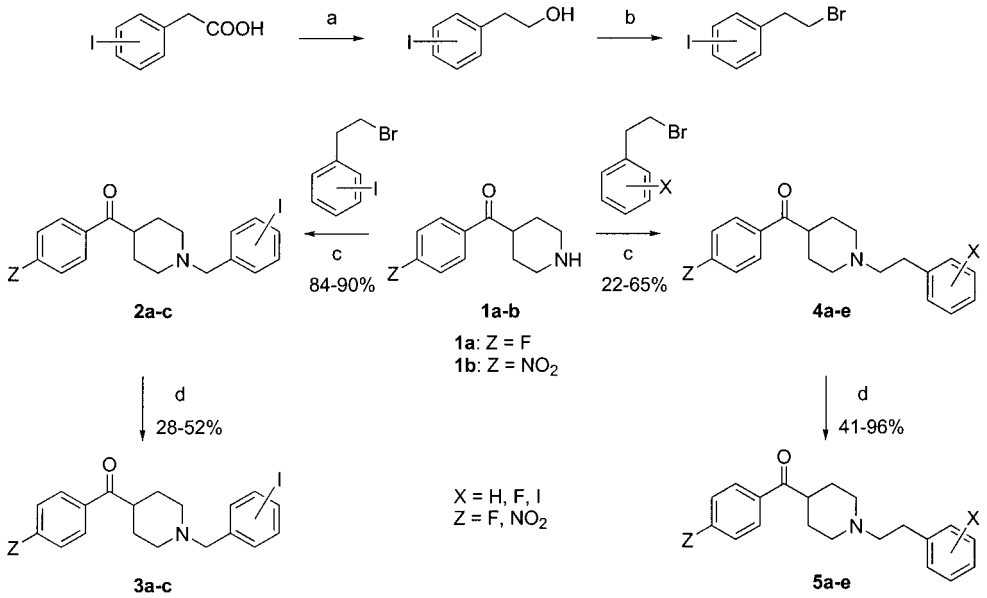
Scheme 1. Synthesis of New Ligands



(a) BH_3, THF; (b) $P(Ph)_3Br_2$, CH_2Cl_2; (c) K_2CO_3, KI, 4-methylpentan-2-one, 90°Cl (d) $NaBH_4$,CH_3OH, 20°C

Results and Discussion

Chemistry. Iodo-substituted phenylethyl bromides were synthesized by the borane reduction of the corresponding substituted phenylacetic acids, followed by a reaction with dibromotriphenylphosphorane in dichloromethane to give alkyl bromides (Scheme 1). 3-Iodophenylacetic acid was prepared by hydrolysis of 3-iodophenylacetonitrile. The target compounds were synthesized by N-alkylation of (4-nitrophenyl)piperidin-4-ylmethanone (**1a**) or (4-fluorophenyl)piperidin-4-ylmethanone (**1b**) with the corresponding chloride, bromide, or iodide, followed by the reduction of the ketone moiety with sodium borohydride. The known compound **4e** and intermediates, such as (4-nitrophenyl)piperidin-4-ylmethanone (**1a**) and 1-(2′-bromoethyl)-4-fluorobenzene, were synthesized according to published methods.[8,11]

Neuropharmacology. Novel compounds were evaluated in vitro for their potency (expressed as an inhibition constant, K_i, nanomolar) in competitive serotonin receptor radioaffinity assays (Table 1). 5-HT_{2A} receptor affinity was initially assessed in homogenates of the rat forebrain with [^{3}H]ketanserin as a radioligand and altanserin as a blank agent to define nonspecific binding. Test compounds with K_i < 500 nM in this 5-HT_{2A} assay were further evaluated in a more specific assay with membranes prepared from genetically transfected cells selectively expressing human 5-HT_{2A} receptor genes and proteins and the same radioligand and blank agent. The two assay methods for 5-HT_{2A} potency agreed very closely (nonparametric rank $r_s = 0.982$, $p < 0.0001$). For compounds with relatively high 5-HT_{2A} receptor potency (K_i < 10 nM), additional tests of selectivity for 5-HT_{2A} receptors were carried out with similar assay methods using transfected cells selectively expressing human 5-HT_{2C}, 5-HT_6, and 5-HT_7 receptors, as well as rat brain tissue to assay for potency at dopamine (D_2) and adrenergic (α_1, α_2) receptors by way of the assay methods summarized in Table 1.

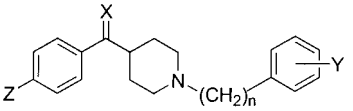
Among the compounds reported here, 5-HT_{2A} potency and selectivity depended significantly on the length of the alkyl link of the piperidine ring to a phenyl moiety. Generally, compounds with an ethylene bridge ($n = 2$) to the second phenyl moiety had higher 5-HT_{2A} potency than those with a methylene bridge ($n = 1$). This contrast can be appreciated by comparing ethylene-bridge compounds (**4a**−**c, 5a**−**c**) with those having a methylene bridge (**2a**−**c, 3a**−**c**). Some of the ethylene-bridge compounds (notably, **4b, 5a,d**) showed high 5-HT_{2A} potency ($K_i = 1.15$ nM) but only limited selectivity (ca. 30-fold), thus, limiting their candidacy for brain-imaging radioligand development. The nature of the linking moiety to a second phenyl ring (carbonyl or methanol) or the *p*-substituent on that ring (F or NO_2) all yielded some compounds with relatively high 5-HT_{2A} affinity (e.g., compare **5a** vs **5d**, and **4a** vs **5a**). Also, in general, for the other phenyl ring, *p*-substituted iodinated derivatives were less potent at the 5-HT_{2A} receptors than the corresponding *o*- and *m*-substituted positional isomers. Nevertheless, compound **5e** showed high affinity for the 5-HT_{2A} receptor ($K_i = 1.63$ nM). This novel compound was approximately 300-, 825-, and >6000-fold less potent at the 5-HT_{2C}, 5-HT_6, and 5-HT_7

Table 1. Serotonin Radioreceptor Affinity Assay Conditions

receptor type	membrane source	[^{3}H]ligand (nM)	blank (μM)
		Serotonin	
5-HT_2r	rat forebrain[a]	ketanserin (1.2)	cinanserin (10)
5-HT_{2A}h	GF-62 cells	ketanserin (1.2)	altanserin (10)
5-HT_{2C}h	PO-1C cells	mesulergine (1.0)	clozapine (10)
5-HT_6h	HD-6 cells	LSD (1.0)[b]	clozapine (10)
5-HT_7h	HD-7 cells	LSD (1.0)[b]	clozapine (10)
		Dopamine	
D_2	rat caudate[a]	nemonapride (0.075)	haloperidol (10)
		Adrenergic	
α_1	rat brain[a]	prazosin (0.20)	phentolamine (2)
α_2	rat brain[a]	prazosin (1.0)	phentolamine (10)

[a] Rat brain tissue was used for initial screening assays, followed by assays with transfected cell membranes expressing human (-h) 5-HT_{2A} receptors when K_i < 500 nM. Rat (-r) brain tissue was forebrain (transected anterior to optic chiasm) for 5-HT_2, caudate-putamen for D_2, and whole brain minus cerebellum for α-adrenergic receptor assays. [b] LSD = lysergic acid diethylamide.

Table 2. Structure and Receptor Potency (K_i, nM) of Novel Compounds



					receptor affinity (K_i, nM)							
compound	X	*n*	Y	Z	5-HT_{2A}[a]	5-HT_{2C}	5-HT_6	5-HT_7	ratio 2C/2A	D_2	α_1	α_2
2a	O	1	2-I	F	191	–	–	–	–	–	–	–
2b	O	1	3-I	F	12.7	–	–	–	–	–	–	–
2c	O	1	4-I	F	227	–	–	–	–	–	–	–
3a	H, OH	1	2-I	F	>10000	–	–	–	–	–	–	–
3b	H, OH	1	3-I	F	>10000	–	–	–	–	–	–	–
3c	H, OH	1	4-I	F	>10000	–	–	–	–	–	–	–
4a	O	2	2-I	F	1.95	35.5	138	3.68	18.0	1.3	24.8	140
4b	O	2	3-I	F	0.51	16.6	48.9	8.76	33.0	51.8	4.92	244
4c	O	2	4-I	F	3.62	144	300	7.32	40.0	176	26.1	381
5a	H, OH	2	2-I	F	0.91	28.6	430	101	31.0	>10000	108	813
5b	H, OH	2	3-I	F	13.1	–	–	–	–	–	–	–
5c	H, OH	2	4-I	F	32.1	–	–	–	–	–	–	–
5d	H, OH	2	H	NO_2	1.15	–	–	–	–	–	–	–
4d	O	2	H	NO_2	9.34	–	–	–	–	–	–	–
5e	H, OH	2	4-F	F	1.63	503	>10000	1345	308	>10000	303	1032
4e	O	2	4-F	F	9.54	493	3000	63.0	52.0	453	33.6	614

[a] 5-HT_{2A} data were obtained with the membranes of cells transfected with human 5-HT_{2A} genes. Values obtained in 5-HT_{2A} screening assays for all 16 compounds with rat forebrain tissue agreed closely with the results shown above (Spearman rank $r_s = 0.982$, $p < 0.0001$).

receptors, respectively, and also had a low potency at dopamine D_2 and α-adrenergic receptors (Table 2).

Of the compounds reported here, **5e** seems to satisfy the most important criteria for a radioligand for in vivo imaging of the 5-HT_{2A} receptor with high 5-HT_{2A} potency and high selectivity over serotonin 2C, 6, and 7 receptors. Compared to altanserin (20-fold 2A vs 2C selectivity), compound **5e** has much higher selectivity (300-fold, Table 2). Furthermore, compound **5e** lends itself to ^{18}F labeling by nitro exchange.

All of the alcohol compounds in the present series are racemic by virtue of the asymmetric center introduced in their structure by $NaBH_4$ reduction. One enantiomer may be more potent, as suggested by the previous report that (*R*)-(+)MDL-100 907 had 30-times greater selectivity to 5-HT_{2A} over 5-HT_{2C} receptors than the (*S*)-(-)-enantiomer.[12] We resolved the previously reported analogue 4-fluorophenyl(1-phenethylpiperidin-4-yl)methanol[6] by chiral HPLC and found that the enantiomer eluting earlier ($K_i = 3.07$ nM) was 30-times more potent than the later-eluting enantiomer ($K_i = 94.0$ nM). The absolute configuration of these enantiomers is not known, but it is reasonable to assign the more potent isomer to the (*R*) configuration, based on the analogy to MDL-100 907. Finally, we also resolved compound **5e** by chiral HPLC and found in preliminary experiments that one enantiomer was 30-times more potent in 5-HT_2 radioreceptor assays in the rat brain.

In conclusion, we developed a series of iodo- and fluoro-substituted diphenylpiperidine ligands. In this series of compounds, we found that the potency and selectivity for the 5-HT_{2A} receptors depended on the length of the carbon bridge between the phenyl and piperidinyl ring. The in vitro pharmacological profiles of compound **5e** suggest that this ligand may be a particularly attractive potential candidate for receptor imaging because of the high potency and selectivity for 5-HT_{2A} sites.

Experimental Section

General. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer FT 1600 IR spectrometer, and ^1H-NMR spectra were obtained using a Bruker AM 500 MHz spectrometer. Mass spectra were obtained with a Micromass Q-Tof spectrometer. Elemental analyses were performed by Atlantic Microlab Inc. (Knoxville, TN), and values within 0.4% of the theoretical values were accepted as valid. Flash chromatography[12] was performed with 40 μm mesh silica gel 60 (J. T. Baker) using eluents as indicated. Chiral HPLC was carried out on a Chiralcel OD column (Daicell) using hexane/2-propanol/diethylamine (85:15:0.3, v/v/v) at 1.3 mL/min and 254 nm UV detection. Starting materials for syntheses were purchased either from Sigma-Aldrich Chemicals (St. Louis, MO) or from Acros Corp. (Pittsburgh, PA). Chemicals and drug substances used for binding assays were purchased from Sigma-Research Biochemicals International (Sigma-RBI, Natick, MA) or Sigma Corp. (St. Louis, MO), unless stated otherwise. [^{3}H]Ketanserin (80 Ci/mmol), [^{3}H]LSD (70 Ci/mmol), [^{3}H]MK-912 (70 Ci/mmol), [^{3}H]nemonapride (90 Ci/mmol), and [^{3}H]prazosin (80 Ci/mmol) were obtained from NEN Corp. (Boston, MA); [^{3}H]-mesulergine (80 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). Cell membranes from genetically transfected cells selectively expressing 5-HT_{2A}, 5-HT_{2C}, 5-HT_6, or 5-HT_7 receptor proteins were obtained from the NIMH Screening Program through Bryan Roth, Ph.D., Case Western Reserve University, Cleveland, OH.[13]

(4-Fluorophenyl)-[1-(2-iodobenzyl)piperidin-4-yl]methanone (2a). To a solution of 200 mg (0.97 mmol) of (4-fluorophenyl)-piperidin-4-ylmethanone (1a) in 10 mL of dry 4-methylpentan-2-one under a nitrogen atmosphere was added 244 mg (0.97 mmol) of *o*-iodobenzyl chloride, followed by 199 mg (1.45 mmol) of K_2CO_3 and 20 mg (0.12 mmol) of KI. The mixture was heated at reflux for 22 h. After being cooled to room temperature, the insoluble material was removed by filtration. The filtrate was evaporated to dryness on a rotary evaporator, and the residue was purified by flash chromatography with 10:50 EtOAc/hexane containing 10% Et_3N to give 346 mg (85% yield) of **2a** as a white solid. Mp: 89.5−91 ×b0C. ^1H-NMR ($CDCl_3$, **δ**): 7.97 (q, 2H, F−ArH), 7.83 (dd, 1H, I−ArH, $J = 0.9$ Hz, $J = 7.8$ Hz), 7.44 (dd, 1H, I−ArH, $J = 1.3$ Hz, $J = 7.7$ Hz), 7.31 (dt, 1H, $J = 7.6$ Hz), 7.13 (t, 2H, F−ArH), 6.94 (dt, 1H, I−ArH, $J = 1.6$ Hz, $J = 7.6$ Hz), 3.54 (s, 2H, CH_2Ar), 3.23 (m, 1H), 3.00 (dt, 2H), 2.24 (dt, 2H), 1.83 (m, 4H). *m*/*z* (ES+): 424 (m + 1). Anal. ($C_{19}H_{19}FINO$) C, H, N.

4-(Fluorophenyl)-[1-(3-iodobenzyl)piperidin-4-yl]methanone Hydrochloride (2b). Compound **2b** was prepared as described for **2a** using 200 mg (0.97 mmol) of **1a** in 10 mL of dry 4-methylpentan-2-one, 244 mg (0.97 mmol) of *m*-iodobenzyl

bromide, 199 mg (1.45 mmol) of K_2CO_3, and 20 mg (0.12 mmol) of KI. Flash chromatography (10:30 EtOAc/hexane, 10% Et_3N) afforded 366 mg (90%) of **2b**. The HCl salt was made from HCl in ether. Mp: 202−204 °C. ^{1}H-NMR (CD_3OD, δ): 8.09 (q, 2H, F−ArH), 7.97 (m, 1H, I−ArH), 7.89 (mm, 1H, I−ArH), 7.54 (mm, 1H, I−ArH), 7.25 (m, 3H, ArH), 4.33 (s, 2H, CH_2Ar), 3.72 (tt, 1H), 3.59 (d, 2H), 3.19 (t, 2H), 2.15 (d, 2H), 1.94 (dq, 4H). *m*/*z* (ES+): 424 (m + 1). Anal. ($C_{19}H_{20}ClFINO$) C, H, N.

(4-Fluorophenyl)-[1-(4-iodobenzyl)piperidin-4-yl]methanone (2c). Compound **2c** was prepared as described for **2a** above using 70 mg (0.34 mmol) of **1a** in 10 mL of dry 4-methylpentan-2-one, 100 mg (0.34 mmol) of 1-bromomethyl-4-iodobenzene, 70 mg (0.55 mmol) of K_2CO_3, and 7 mg of KI. Flash chromatography (50:50 EtOAc/hexane, 10% Et_3N) afforded 120 mg (84%) of **2c**. Mp: 118.5−120.5 °C. ^{1}H-NMR ($CDCl_3$, δ): 7.96 (q, 2H, F−ArH), 7.65 (d, 2H, I−ArH), 7.13 (t, 2H, F−ArH), 7.09 (d, 2H, I−ArH), 3.47 (s, 2H, CH_2Ar), 3.19 (m, 1H), 2.93 (dt, 2H), 2.12 (dt, 2H), 1.83 (m, 4H). *m*/*z* (ES+): 424 (m + 1). Anal. ($C_{19}H_{19}FINO$) C, H, N.

(4-Fluorophenyl)-[1-(2-iodobenzyl)piperidin-4-yl]methanol Hydrochloride (3a). To a solution of 150 mg (0.355 mmol) of 4-fluorophenyl[1-(2-iodobenzyl)piperidin-4-yl]methanone (**2a**) in 25 mL MeOH was added $NaBH_4$ (40 mg, 1.1 mmol) at 0 °C. The mixture was stirred at room temperature overnight. Excess $NaBH_4$ was decomposed with 0.6 N HCl; the acidic solution was then neutralized with NH_4OH (28%) to pH >10, and the solution was extracted with ether (5 × 20 mL). The pooled ether extract was dried with K_2CO_3, filtered, and evaporated on a rotary evaporator. Flash chromatography (10:30 EtOAc/hexane, 5% Et_3N) gave 66 mg (44%) of 4-fluorophenyl[1-(3-iodobenzyl)piperidin-4-yl]methanol (**3a**), which was converted to the HCl salt by a reaction with HCl in ether to give 72 mg. Mp: 123 °C dec. ^{1}H-NMR (CD_3OD, δ): 8.03 (d, 1H, I−ArH), 7.63 (m, 1H, I−ArH), 7.51 (t, 1H, I−ArH), 7.34 (q, 2H, F−ArH), 7.22 (m, 1H, I−ArH), 7.07 (t, 2H, F−ArH), 4.45 (s, 2H, CH_2Ar), 4.42 (d, 1H, CHOH), 3.62 (d, 1H), 3.54 (d, 1H), 3.16 (q, 2H), 2.13 (d, 1H), 1.93 (m, 1H), 1.62 (m, 3H). *m*/*z* (ES+): 426 (m + 1). Anal. ($C_{19}H_{22}ClFNO\cdot0.5H_2O$) C, H, N.

(4-Fluorophenyl)-[1-(3-iodobenzyl)piperidin-4-yl]methanol Hydrochloride (3b). Compound **3b** was prepared as described for **3a** above using 150 mg (0.355 mmol) of 4-fluorophenyl[1-(3-iodobenzyl)piperidin-4-yl]methanone (**2b**) in 25 mL of MeOH, 40 mg (1.1 mmol) of $NaBH_4$, flash chromatography (10:20 ethyl acetate/hexane, 5% Et_3N), and HCl in ether to give 46 mg of **3b** (28%). Mp: 125 °C dec. ^{1}H-NMR (DMSO-d_6, δ): 8.00 (s, 1H, I−ArH), 7.78 (d, 1H, I−ArH), 7.62 (d, 1H, I−ArH), 7.29 (q, 2H, F−ArH), 7.21 (t, 1H, I−ArH), 7.12 (t, 2H, F−ArH), 4.27 (d, 1H, CHOH), 4.17 (s, 2H, CH_2Ar), 3.28 (d, 1H), 3.22 (d, 1H), 2.81 (m, 2H), 1.89 (d, 1H), 1.64−1.58 (m, 2H), 1.37 (m, 1H). *m*/*z* (ES+): 426 (m + 1). Anal. ($C_{19}H_{22}ClFNO\cdot0.5H_2O$) C, H, N.

(4-Fluorophenyl)-[1-(4-iodobenzyl)piperidin-4-yl]methanol Hydrochloride (3c). Compound **3c** was prepared as described for **3a** above using 70 mg (0.16 mmol) of 4-fluorophenyl[1-(4-iodobenzyl)piperidin-4-yl]methanone (**2c**) in 20 mL of MeOH, 20 mg (0.5 mmol) of $NaBH_4$, flash chromatography with 50:50 EtOAc/hexane (10% Et_3N), and HCl in ether to give 40 mg of **3c** (52%). Mp: 175 °C dec. ^{1}H-NMR (DMSO-d_6, δ): 7.81 (d, 2H, I−ArH), 7.35 (d, 2H, I−ArH), 7.30 (q, 2H, F−ArH), 7.13 (t, 2H, F−ArH), 4.29 (d, 1H, CHOH), 4.16 (s, 2H, CH_2-Ar), 3.26 (m, 2H), 2.80 (m, 2H), 1.87 (d, 1H), 1.67 (m, 1H), 1.54 (m, 2H), 1.42 (m, 1H). *m*/*z* (ES+): 426 (m + 1). Anal. ($C_{19}H_{22}ClFNO\cdot0.5H_2O$) C, H, N.

(4-Fluorophenyl)-[1-[2-(2-iodophenyl)ethyl]piperidin-4-yl]methanone Hydrochloride (4a). Compound **4a** was prepared as described for **2a** above using 79 mg (0.39 mmol) of **1a** in 5 mL of dry 4-methylpentan-2-one, 120 mg (0.385 mmol) of 1-(2′-bromoethyl)-2-iodobenzene, 79 mg (0.58 mmol) of K_2CO_3, and 8 mg of KI. Flash chromatography with 50:50 EtOAc/hexane (5% Et_3N) afforded 110 mg (65% yield) of (4-fluorophenyl)-[1-[2-(2-iodophenyl)ethyl]piperidin-4-yl]methanone **4a**. The HCl salt was made from HCl in ether. Mp: 203−205 °C. IR (KBr, cm^{-1}): 1676. ^{1}H-NMR (DMSO-d_6, δ): 8.11 (q, 2H, F−ArH), 7.88 (d, 1H, I−ArH), 7.41−7.37 (m, 4H, I−ArH or F−ArH), 7.04 (m, 1H, I−ArH), 3.73 (t, 1H), 3.67 (d, 2H), 3.20−3.16 (m, 6H), 2.04−1.95 (m, 4H). *m*/*z* (ES+): 438 (m + 1). Anal. ($C_{20}H_{22}ClFINO$) C, H, N.

(4-Fluorophenyl)-[1-[2-(3-iodophenyl)ethyl]piperidin-4-yl]methanone (4b). Compound **4b** was prepared as described for **2a** above using 132 mg (0.643 mmol) of **1a** in 10 mL of dry 4-methylpentan-2-one, 200 mg (0.643 mmol) of 1-(2′-bromoethyl)-3-iodobenzene, 133 mg (0.964 mmol) of K_2CO_3, 13 mg of KI, and flash chromatography (20:10 EtOAc/hexane, 5% Et_3N) to give 90 mg (32%) of **4b**. Mp: 94−95 °C. ^{1}H-NMR ($CDCl_3$, δ): 7.97 (q, 2H, F−ArH), 7.57 (s, 1H, I−ArH), 7.54 (dt, 1H, I−ArH), 7.18 (d, 1H, I−ArH), 7.14 (t, 2H, F−ArH), 7.02 (t, 1H, I−ArH), 3.19 (m, 1H), 3.07 (dt, 2H), 2.76 (t, 2H), 2.59 (t, 2H), 2.17 (m, 2H), 1.86 (m, 4H). *m*/*z* (ES+): 438 (m + 1). Anal. ($C_{20}H_{21}FINO$) C, H, N.

(4-Fluorophenyl)-[1-[2-(4-iodophenyl)ethyl]piperidin-4-yl]methanone (4c). Compound **4c** was prepared as described for **2a** above using 250 mg (1.21 mmol) of **1a** in 10 mL of dry 4-methylpentan-2-one, 276 mg (1.21 mmol) of 1-(2′-bromoethyl)-4-iodobenzene, 250 mg (2.21 mmol) of K_2CO_3, 25 mg of KI, and flash chromatography (20:20 EtOAc/hexane, 10% Et_3N) to give 240 mg (45%) of **4c**. Mp: 280−282 °C. ^{1}H-NMR ($CDCl_3$, δ): 7.97 (q, 2H, F−ArH), 7.61 (d, 2H, I−ArH), 7.14 (t, 2H, F−ArH), 6.97 (d, 2H, I−ArH), 3.21 (m, 1H), 3.06 (dt, 2H), 2.76 (t, 2H), 2.58 (t, 2H), 2.17 (m, 2H), 1.86 (m, 4H). *m*/*z* (ES+): 438 (m + 1). Anal. ($C_{20}H_{21}FINO$) C, H, N.

(4-Nitrophenyl)-(1-phenethylpiperidin-4-yl)methanone (4d). Compound **4d** was prepared as described for **2a** above using 100 mg (0.427 mmol) of **1b** in 5 mL of dry 4-methylpentan-2-one, 93 mg (0.70 mmol) of 2-bromoethylbenzene, 100 mg (0.70 mmol) of K_2CO_3, 10 mg of KI, and flash chromatography (50:50 EtOAc/hexane, 2% Et_3N) to give 37 mg (22%) of **4d** as a yellow powder. Mp: 155.5−156.5 °C. IR (KBr, cm^{-1}): 1678. ^{1}H-NMR ($CDCl_3$, δ): 8.32 (d, 2H, ArH, J = 8.8 Hz), 8.08 (d, 2H, ArH, J = 8.8 Hz), 7.31−7.21 (m, 5H, ArH), 3.25 (tt, 1H, J = 5.0, 5.0 Hz), 3.09 (d, 2H, J = 11.8 Hz), 2.83 (d, 2H, J = 8.2 Hz, CH_2N), 2.64 (d, 2H, J = 8.2 Hz, CH_2Ar), 2.05 (dt, 2H, J = 3.8, 10.9 Hz), 1.90 (m, 4H). *m*/*z* (ES+): 339 (m + 1). Anal. ($C_{20}H_{22}N_2O_3$) C, H, N.

(4-Fluorophenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanone (4e). Compound **4e** was prepared as described for **2a** using 273 mg (1.32 mmol) of **1a** in 10 mL of dry 4-methylpentan-2-one, 268 mg (1.32 mmol) of 1-(2′-bromoethyl)-4-fluorobenzene, 273 mg (1.98 mmol) of K_2CO_3, 27 mg of KI, and flash chromatography (50:20 EtOAc/hexane, 5% Et_3N) to give 260 mg (60%) of **4e** as a white powder. Mp: 106−108 °C. ^{1}H-NMR (DMSO-d_6, δ): 8.11 (q, 2H, ArH), 7.39 (t, 2H, ArH), 7.34 (q, 2H, ArH), 7.18 (t, 2H, ArH), 3.72 (t, 1H), 3.64 (d, 2H), 3.29 (m, 2H), 3.10−3.05 (m, 4H), 2.03−1.88 (m, 4H). *m*/*z* (ES+): 330 (m + 1). Anal. ($C_{20}H_{21}F_2NOH_2O\cdot0.25H_2O$) C, H, N.

(4-Fluorophenyl)-[1-[2-(2-iodophenyl)ethyl]piperidin-4-yl]methanol Hydrochloride (5a). Compound **5a** was prepared as described for **3a** above using 60 mg (0.13 mmol) of 4-fluorophenyl[1-[2-(2-iodophenyl)ethyl]piperidin-4-yl]methanone hydrochloride **4a** in 10 mL of MeOH, 15 mg (0.38 mmol) of $NaBH_4$, flash chromatography (EtOAc, 3.5% Et_3N), and HCl in ether to give 25 mg (41%) of **5a**. Mp: 174 °C dec. ^{1}H-NMR (DMSO-d_6, δ): 7.86 (d, 1H, I−ArH), 7.40−7.32 (m, 4H, I−ArH or F−ArH), 7.16 (t, 3H, F−ArH), 7.06 (m, 1H, I−ArH), 4.34 (t, 1H, CHOH), 3.56 (d, 1H), 3.50 (d, 1H), 2.91 (q, 2H), 1.89 (d, 1H), 1.75−1.48 (m, 4H). *m*/*z* (ES+): 440 (m + 1). Anal. ($C_{20}H_{24}$-ClFINO) C, H, N.

(4-Fluorophenyl)-[1-[2-(3-iodophenyl)ethyl]piperidin-4-yl]methanol Hydrochloride (5b). Compound **5b** was prepared as described for **3a** above using 60 mg (0.14 mmol) of (4-fluorophenyl)-[1-[2-(3-iodophenyl)ethyl]piperidin-4-yl]-methanone (**4b)** in 10 mL of MeOH, 15 mg (0.38 mmol) of $NaBH_4$, flash chromatography (EtOAc, 3.5%Et_3N), and HCl in ether to give 63 mg (96% yield) of **5b**. Mp: 228 °C dec. ^{1}H-NMR (DMSO-d_6, δ): 7.66 (s, 1H, I−ArH), 7.62 (d, 1H, I−ArH), 7.33 (q, 2H, F−ArH), 7.27 (d, 1H, I−ArH), 7.15 (t, 2H, F−ArH), 7.13 (t, 1H, I−ArH), 4.34 (d, 1H, CHOH), 3.54 (dd, 2H), 3.19

(t, 2H), 2.99 (t, 2H), 2.84 (q, 2H), 1.88 (d, 1H), 1.71−1.43 (m, 4H). *m*/*z* (ES+): 440 (m + 1). Anal. ($C_{20}H_{24}ClFINO$) C, H, N.

(4-Fluorophenyl)-[1-[2-(4-iodophenyl)-ethyl]piperidin-4-yl]methanol (5c). Compound **5c** was prepared as described for **3a** using 80 mg (0.18 mmol) of (4-fluorophenyl)-[1-[2-(4-iodophenyl)ethyl]piperidin-4-yl]methanone (**4c**) in 10 mL of MeOH, 20 mg (0.55 mmol) of $NaBH_4$, and flash chromatography (EtOAc, 3.5% Et_3N) which gave 50 mg (64%) of **5c**. Mp: 117−119 °C. ^{1}H-NMR ($CDCl_3$, δ): 7.58 (d, 2H, I−ArH), 7.26 (q, 2H, F−ArH), 7.02 (t, 2H, F−ArH), 6.93 (d, 2H, I−ArH), 4.36 (d, 1H, CHOH), 3.01 (dd, 2H), 2.71 (t, 2H), 2.49 (t, 2H), 1.98 (m, 2H), 1.94 (m, 1H), 1.58 (m, 1H), 1.42 (m, 1H), 1.25 (m, 2H). *m*/*z* (ES+): 440 (m + 1). Anal. ($C_{20}H_{23}FINO$) C, H, N.

(4-Nitrophenyl)-(1-phenethylpiperidin-4-yl)methanol (5d). Compound **5d** was prepared as described for **3a** above using 130 mg (0.385 mmol) of (4-nitrophenyl)-(1-phenethylpiperidin-4-yl)methanone (**4d**) in 20 mL THF, 30 mg (0.77 mmol) of $NaBH_4$, and flash chromatography (EtOAc, 4% Et_3N) to give 60 mg (46%) of **5d** as a white solid powder. Mp: 154−155 °C. IR (KBr, cm^{-1}): 3420, 3083, 2929, 2813, 1602, 1515, 1340, 852. ^{1}H-NMR (CD_2Cl_2, δ): 8.19 (d, 2H, ArH, J = 9.0 Hz), 7.51 (d, 2H, ArH, J = 9 Hz), 7.27−7.17 (m, 5H, ArH), 4.58 (d, 1H, J = 6.6 Hz, CHOH), 2.99 (m, 2H), 2.75 (t, 2H, CH_2N), 2.54 (t, 2H, CH_2Ar), 1.96 (m, 4H), 1.80 (m, 2H), 1.41 (m, 1H). *m*/*z* (ES+): 341 (m + 1). Anal. ($C_{20}H_{24}N_2O_3$) C, H, N.

(4-Fluorophenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (5e). Compound **5e** was prepared as described for **3a** using 90 mg (0.27 mmol) of (4-fluorophenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanone (**4e**) in 20 mL of THF, 33 mg (0.82 mmol) of $NaBH_4$, and flash chromatography (EtOAc, 3.5% Et_3N) to give 45 mg (50%) of **5e** white powder. Mp: 109−110 °C. ^{1}H-NMR (DMSO-d_6, δ): 7.33 (q, 2H, ArH), 7.28 (q, 2H, ArH), 7.15 (m, 4H, ArH), 4.34 (d, 1H, CHOH), 3.46 (q, 2H), 3.12 (t, 2H, CH_2N), 2.93 (t, 2H, CH_2Ar), 2.79 (m, 2H), 1.90 (d, 1H), 1.69 (m, 1H), 1.53−1.40 (m, 3H). *m*/*z* (ES+): 332 (m + 1). Anal. ($C_{20}H_{23}F_2NO$) C, H, N.

Radioreceptor Assays. Serotonin 5-HT$_{2A}$. The affinity and selectivity were tested at 5-HT$_2$ receptors initially using rat brain homogenates incubated with 0.4 nM [^{3}H]ketanserin for 15 min at 37 °C and using 10 μM altanserin as a blank agent with which to determine nonspecific binding. Compounds were further tested on genetically transfected COS-7 cell membranes selectively expressing human 5-HT$_{2A}$ receptors. Cell membranes and test drugs were incubated with 1.0 nM [^{3}H]ketanserin at room temperature for 60 min, again using 10 μM altanserin as the blank. Cell membranes were harvested on glass fiber filter sheets (ISC Bioexpress; Kaysville, UT) in a Brandel cell harvester (Gaithersburg, MD), and tritium radioactivity was counted in mini-vials containing Emulsifier-Safe (Packard Instruments; Meriden, CT) in a Beckman-Coulter β-scintillation spectrometer (Fullerton, CA) at 50% efficiency. Similar competition assay, harvesting, and radioactivity-counting procedures were used in the other radioreceptor assays described below.

Typically, each test agent was initially screened at 1 and 10 μM. If inhibition of radioligand binding was ≥30% at 1 μM, at least 6 concentrations were tested further to bracket the approximate IC_{50}. Resulting concentration−inhibition data were used to compute $IC_{50} \pm$ SE with the ALLFIT program[14] adapted for the Macintosh microcomputer, following the initial determination of the affinity (apparent K_d) of each radioligand in each assay system using the LIGAND program.[15] Both programs were generously provided by Drs. P. J. Munson and D. Rodbard of the NIH Biostatistical and Computing Center. Values of IC_{50} were then converted to K_i by the Chang and Prusoff relationship,[16] $K_i = IC_{50}/(1 +$ radioligand concentration/radioligand K_d), all as described in detail previously.[17]

Selected compounds showing relatively high affinity (K_i = 10 nM) at 5-HT$_{2A}$ receptors in the initial screening with transfected cell membranes were tested further for receptor selectivity by comparing their affinities at other 5-HT receptor types. Selectivity is defined as the ratio of K_i values for each test site versus the 5-HT$_{2A}$ receptor. The following radioreceptor assays were used to test agents with relatively high 5-HT$_{2A}$ affinity for receptor selectivity, thus, to determine K_i values as already described.

Serotonin 5-HT$_{2C}$. Transfected cell membranes selectively expressing human 5-HT$_{2C}$ receptors were assayed with 0.80 nM [^{3}H]mesulergine at room temperature for 60 min using 10 μM clozapine as the blank agent.

Serotonin 5-HT$_6$ and 5-HT$_7$. Cell membranes (1−10 μg of protein) from transfected cells selectively expressing human 5-HT$_6$ or 5-HT$_7$ receptors were incubated with 1 nM [^{3}H]LSD at room temperature for 90 min in the dark using 10 μM clozapine as the blank agent.[18]

Dopamine D$_2$. Homogenates of rat caudate-putamen tissue were incubated with 75 pM [^{3}H]nemonapride at 30 °C for 90 min, using 10 μM haloperidol as the blank agent.[19]

Alpha-1 (α1) Adrenergic. Homogenates of whole rat brain were incubated with 0.20 nM [^{3}H]prazosin at 30 °C for 30 min, using 2 μM phentolamine as the blank agent.[20]

Alpha-2 (α_2) Adrenergic. Homogenates of whole rat brain were incubated with 1.0 nM [^{3}H]MK-912 for 80 min at room temperature, using 10 μM phentolamine as the blank agent.[20]

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References

(1) Sandler, M.; Coppen, A.; Harnett, S. *5-Hydroxytryptamine in Psychiatry*; Oxford University Press: Oxford, U.K., 1991.
(2) Mann, J. J.; Underwood, M. D.; Baird, F.; McBride, P. A. Neurochemical correlates of suicidal behavior: involvement of serotonergic and nonserotonergic systems. *Pharmacol. Toxicol.* **1990**, *66*, 37−60.
(3) Baldessarini, R. J. Drugs and the treatment of psychiatric disorders: Antidepressant and antianxiety agents. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th ed.; McGraw-Hill Press: New York, 2001; Chapter 19.
(4) Baldessarini, R. J.; Tarazi, F. I. Drugs and the treatment of psychiatric disorders: Antipsychotic and antimanic agents. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 10th ed.; McGraw-Hill Press: New York, 2001; Chapter 20.
(5) van Dyck, C. H.; Tan, P.-Z.; Baldwin, R. M.; Amici, L. A.; Garg, P. K.; et al. PET quantification of 5-HT$_{2A}$ receptors in the human brain: a constant infusion paradigm with [^{18}F]altanserin. *J. Nucl. Med.* **2000**, *41*, 234−241.
(6) Ismaiel, A. M.; Arruda, K.; Teitler, M.; Glennon, R. A. Ketanserin analogues: Effect of structural modification on 5-HT$_2$ serotonin receptor binding. *J. Med. Chem.* **1995**, *38*, 1196−1202.
(7) Janssen, P. A. Pharmacology of potent and selective S$_2$-serotonergic antagonists. *J. Cardiovasc. Pharmacol.* **1985**, *7*, S2−S11.
(8) Lundkvist, C.; Halldin, C.; Ginovart, N.; Nyberg, S.; Swahn, C. G.; et al. [^{11}C]MDL 100 907, a radioligand for selective imaging of 5-HT$_{2A}$ receptors with positron emission tomography. *Life Sci.* **1996**, *58*, 187−192.
(9) Lemaire, C.; Cantineau, R.; Guillaume, M.; Plenevaux, A.; Christiaens, L. Fluorine-18 altanserin: a radioligand for the study of serotonin receptors with PET: radiolabeling and *in vivo* biologic behavior in rats. *J. Nucl. Med.* **1991**, *32*, 2266−2272.
(10) Mathis, C. A.; Huang, Y.; Simpson, N. R.; Mahmood, K.; Gerdes, J. M.; et al. [^{11}C]MDL 100 907: a potent and selective antagonist of 5-HT$_{2A}$ receptors labeled with ^{11}C at two different positions. *J. Labelled Compd. Radiopharm.* **1996**, *37*, 316−317.
(11) Sugimaoto, H. N.; Takahiro; Ueno, M.; Yamatsu, K. Preparation of N-containing heterocycles for treatment of cerebral disorders. PCT Int. Appl. JP WO8802365, 1988.
(12) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(13) Tan, P.-Z.; Baldwin, R. M.; Fu, T.; Charney, D. S.; Innis, R. B. Rapid synthesis of ^{18}F and ^{2}H dual-labeled altanserin, a metabolically resistant PET ligand for 5-HT$_{2A}$ receptors. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 457−467.
(14) DeLean, A. M.; Rodbard, D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose−response curves. *Am. J. Physiol.* **1978**, *235*, 97−102.

(15) Munson, P.; Rodbard, D. LIGAND: A versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.* **1980**, *107*, 220−239.
(16) Cheng, Y.; Prusoff, W. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(17) Baldessarini, R. J.; Marsh, E. R.; Kula, N. S. Interactions of fluoxetine with metabolism of dopamine and serotonin in rat brain regions. *Brain Res. Bull.* **1992**, *579*, 152−156.
(18) Roth, B. L.; Craigo, S. C.; Choudhary, M. S.; Uluer, A.; Monsma, F. J., Jr.; et al. Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1403−1410.
(19) Baldessarini, R. J.; Kula, N. S.; Campbell, A.; Bakthavachalam, V.; Yuan, J.; et al. Prolonged D2 antidopaminergic activity of alkylating and nonalkylating derivatives of spiperone in rat brain. *Mol. Pharmacol.* **1992**, *42*, 856−863.
(20) Kula, N. S.; Baldessarini, R. J.; Kebabian, J. W.; Bakthavachalam, V.; Xu, L. X. RBI-257−a potent dopamine D4 receptor-selective ligand. *Eur. J. Pharmacol.* **1997**, *331*, 333−336.

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