Synthesis and in Vitro and in Vivo Functional Studies of *Ortho*-Substituted Phenylpiperazine and *N*-Substituted 4-*N*-(*o*-Methoxyphenyl)aminopiperidine Analogues of WAY100635

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WAY100635 (**2**), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide, is a silent serotonin 5-HT_{1A} antagonist, which is now widely used to study the 5-HT_{1A} receptor both in vivo and in vitro. In this paper, we describe the synthesis and in vitro (5-HT_{1A} affinity and pA_2 values at guinea pig ileum strips) and in vivo (hypothermia and ultrasonic vocalization) pharmacology at the serotonin 5-HT_{1A} receptor of several closely related analogues of **2**. Test compounds **12** and **14**, in which the arylpiperazine moiety of **2** has been replaced by an arylaminopiperidine moiety, showed no affinity or antagonistic activity at the 5-HT_{1A} receptor. Substitution of the *o*-methoxy group of **2** by larger fluoroalkoxy or sulfonyloxy substituents did not alter the in vitro or in vivo pharmacology to any great extent; in vivo both the fluoropropyl analogue **5** and the triflate analogue **7** are equipotent to WAY100635 itself. The *O*-desmethyl analogue **3** proved to be the most potent antagonist at the serotonin 5-HT_{1A} postsynaptic receptor sites in this series.

Introduction

The serotonin (5-hydroxytryptamine, 5-HT) receptor family consists of seven distinct classes (serotonin 5-HT_{1-7}). All serotonin receptors belong to the G-protein-coupled receptor (GPCR) superfamily, bar the serotonin 5-HT_3 receptor, which is a ligand-gated ion channel.[1] The serotonin 5-HT_1 receptors, based on considerations of structural features, functional pharmacology, and secondary messenger coupling systems, have been further divided into subtypes. They have been classified as serotonin 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} receptors and are all negatively coupled to adenylyl cyclase.[2−5]

The serotonin 5-HT_{1A} receptors are present throughout the whole body, including the central nervous system (CNS). In the CNS, the serotonin 5-HT_{1A} receptors are located both presynaptically on the cell body of the serotonergic neurons in the raphé nucleus − where they control the firing of the serotonin 5-HT neuron via autoinhibition − as well as postsynaptically in the projection areas such as the dentate gyrus of the hippocampus (CA1 region), frontal cortex and neocortex, amygdala, and lateral septum.[6−11] These brain areas have frequently been associated with mood control, and the serotonin 5-HT_{1A} receptor is generally considered to be an important target for the treatment of mood disorders such as anxiety and depression.[12−14] Activation of serotonin 5-HT_{1A} receptors with agonists, like 8-OH-DPAT (**1**) (Chart 1), leads to a number of physiological changes that can easily be quantified in several in vitro and in vivo assays. In rats, the administration of serotonin 5-HT_{1A} agonists results in a rectal temperature decrease,[15] a characteristic set of motor changes collectively known as the serotonin motor syndrome[16] (both models for postsynaptic activation), inhibition of synthesis rate of serotonin 5-HT in the terminal areas,[17] inhibition of ultrasonic vocalization,[18,19] and inhibition of neuronal firing activity[20] (all models for presynaptic activation).

Positron emission tomography (PET), using effective positron-emitting radioligands, provides the means to visualize 5-HT_{1A} receptor densities in living subjects and to monitor inhibition of receptor binding with unlabeled ligands (both endogenous and exogenous).[21−23] Agonists, however, are limited in their use for imaging in vivo, possibly because they bind to the high-affinity agonist state of the receptor only, which quickly reverts to a low-affinity state and a fast dissociation of radioligand.[24−26] Antagonists bind to receptors in both their high- and low-affinity states. WAY100635 (*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridyl)cyclohexanecarboxamide, **2**) (Chart 1) is known to be one of the first potent, silent, and selective antagonists for serotonin 5-HT_{1A} receptors at both somatodendritic and postsynaptic receptor sites.[27−29] The [^{11}C-*carbonyl*]-labeled *O*-desmethyl analogue **3** of WAY100635 has proven to be a successful radioligand for studies of central serotonin 5-HT_{1A} receptors in rat, monkey, and human brain using PET.[30]

We have synthesized several *ortho*-substituted phenylpiperazine and *N*-substituted 4-*N*-(*o*-methoxyphenyl)-aminopiperidine analogues of **2** and have determined their affinity and selectivity at the serotonin 5-HT_{1A}

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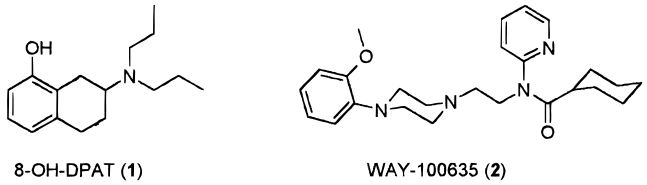
Chart 1



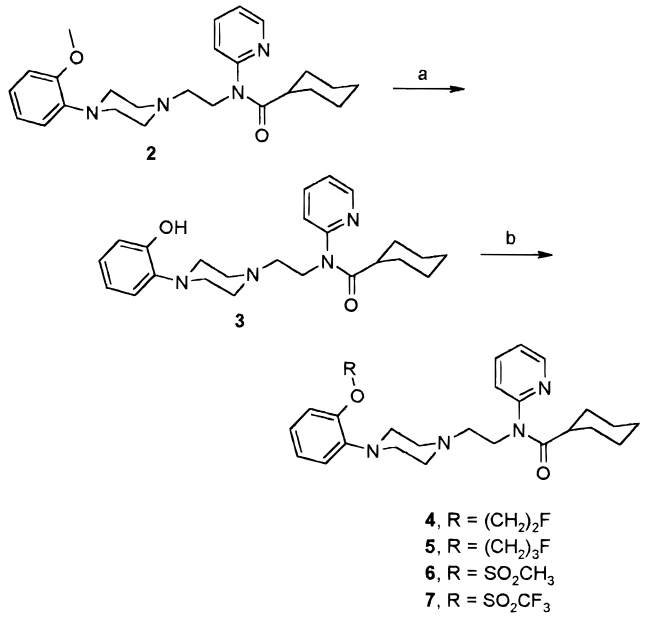
receptor vs the serotonin 5-$HT_{1B/1D}$ receptor, which is also located both presynaptically on the nerve terminals of the serotonergic neurons as well as postsynaptically. In addition, the in vitro antagonistic properties (pA_2 values) and the ability to counteract, in vivo, the 8-OH-DPAT-induced inhibition of ultrasonic vocalization and hypothermia in rats were measured for test compounds displaying a high affinity and selectivity for the serotonin 5-HT_{1A} receptors.

Chemistry

WAY100635 (**2**) was prepared as previously described.[27] The *O*-demethylation of **2** could not be effected with refluxing HBr (48%), with or without acetic acid (partial conversion), or by employing BBr_3 in CH_2Cl_2 (no conversion). The reaction was, however, carried out smoothly and in high yield upon treatment with 6 equiv of $AlCl_3$ refluxing in freshly distilled benzene under a nitrogen atmosphere, yielding *N*-[2-[4-(2-hydroxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**3**).[31] Derivatization of the hydroxyl group of compound **3** was accomplished either with the appropriate alkyl bromides in the presence of Cs_2CO_3, yielding *N*-[2-[4-(2-(2-fluoroethoxy)phenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**4**) and *N*-[2-[4-(2-(3-fluoropropoxy)phenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**5**), or with methanesulfonyl chloride in the presence of triethylamine, yielding *N*-[2-[4-[2-[methanesulfonoxyl]phenyl]-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**6**), or with *N*-phenyltrifluoromethanesulfonimide using phase-transfer conditions with 10% NaOH(aq) and CH_2Cl_2, in the presence of the phase-transfer catalyst tetrabutylammonium iodide, yielding *N*-[2-[4-[2-[(trifluoromethyl)sulfonoxyl]phenyl]-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**7**) (Scheme 1).

For the synthesis of 4-*N*-(*o*-methoxyphenyl)piperidine analogues, 2-methoxyaniline was coupled to 1-benzyl-4-piperidone in refluxing toluene in the presence of *p*-toluenesulfonic acid, hydrogenated using Adam's catalyst, and debenzylated by applying H_2 (60 psi) in the presence of palladium hydroxide on carbon in a mixture of MeOH/THF, yielding 4-*N*-(2-methoxyphenyl)aminopiperidine (**8**). This intermediate was then coupled to 2-chloro-*N*-pyridin-2-ylacetamide[27] in refluxing acetonitrile in the presence of triethylamine, potassium carbonate, and tetrabutylammonium iodide, yielding 2-[4-*N*-(*o*-methoxyphenyl)aminopiperidinyl]-*N*-pyridin-2-ylacetamide (**9**). *N*-Methylation of compound **9** was achieved with reductive amination in a Parr shaker using formaldehyde in EtOH in the presence of palladium hydroxide (20%) on carbon, yielding 2-[4-*N*-(*o*-methoxyphenyl)-*N*-methylaminopiperidinyl]-*N*-pyridin-2-ylacetamide (**10**). The acetamide was reduced in ether by using DIBAL-H, yielding 2-[4-*N*-(*o*-methoxyphenyl)-*N*-methylaminopiperidinyl]-*N*-pyridin-2-ylamine (**11**), which was subsequently coupled to cyclohexanecarboxylic acid chloride in CH_2Cl_2 in the presence of triethylamine yielding 2-[4-*N*-(*o*-methoxyphenyl)-*N*-methylaminopiperidinyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**12**). Reduction of intermediate **9** in ether by using DIBAL-H yielded 2-[4-*N*-(*o*-methoxyphenyl)aminopiperidinyl]-*N*-pyridin-2-ylamine (**13**). Reacting compound **13** with cyclohexanecarboxylic acid chloride in CH_2Cl_2 in the presence of triethylamine yielded [4-*N*-(*o*-methoxyphenyl)-*N*-cyclohexanecarboxamidopiperidinyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (**14**) (Scheme 2).

Scheme 1[a]



[a] Reagents: (a) aluminum chloride, benzene, Δ; (b) fluoroethyl bromide/fluoropropyl bromide, cesium carbonate, acetonitrile, Δ (**4**/**5**); methanesulfonyl chloride, triethylamine, CH_2Cl_2, rt (**6**); *N*-phenyltrifluoromethane sulfonimide, tetrabutylammonium iodide, 10% NaOH(aq)/CH_2Cl_2, rt (**7**).

Pharmacology

In Vitro Binding. The abilities of the new compounds to displace the radioligands [^{3}H]-8-OH-DPAT (5-HT_{1A}) and [^{3}H]-5-HT (5-$HT_{1B/1D}$) were assessed (Table 1).[46−48]

pA_2 Values. The ability of the new compounds to antagonize the 8-OH-DPAT-induced contractions of a strip of guinea pig ileum tissue was measured (Table 1).[38,51]

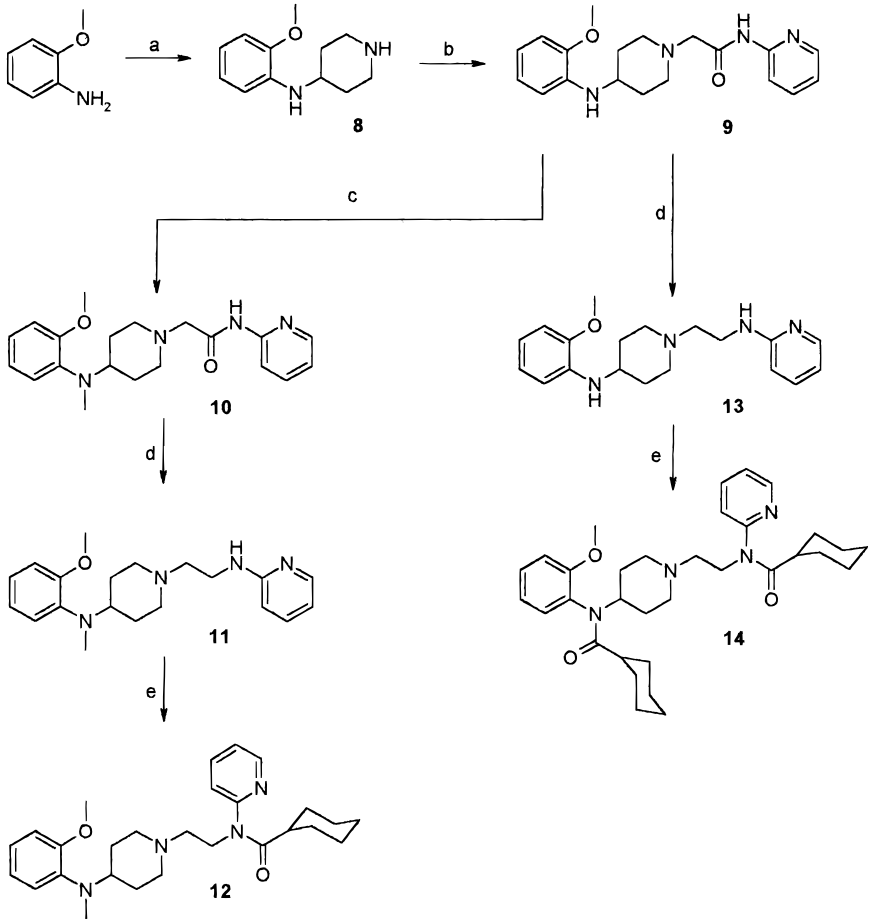
Ultrasonic Vocalization. The ability of the new compounds to antagonize the 8-OH-DPAT-induced inhibition of footshock-evoked ultrasonic vocalization in rats was determined (Table 2).[18,19,45]

Hypothermia. The ability of the new compounds to counteract the 8-OH-DPAT-induced hypothermia in rats was also measured (Table 3).[16]

Results and Discussion

Structure−activity relationships (SARs) were determined from affinity values obtained using radioligand binding for both the 5-HT_{1A} and 5-$HT_{1B/1D}$ autoreceptors (Table 1). The affinity of the phenylpiperazine analogues, in which the methoxy group of WAY100635 (**2**) has been replaced by a hydroxy (**3**), a fluoroalkoxy (**4**, **5**), a methanesulfonoxy (**6**), or the even stronger electron-

Scheme 2[a]



[a] Reagents: (a) i. *p*-TsOH, toluene, Δ, ii. H_2 (50 psi), PtO_2, toluene, 60 °C, iii. H_2 (60 psi), 20% $Pd(OH)_2$/C, MeOH/THF, 60 °C; (b) 2-chloro-*N*-pyridin-2-ylacetamide, K_2CO_3, tetrabutylammonium iodide, acetonitrile, Δ; (c) HCHO, H_2 (60 psi), 20% $Pd(OH)_2$/C, EtOH, 60 °C; (d) DIBAL-H, Et_2O, 0 °C to rt; (e) cyclohexanecarboxylic acid chloride, TEA, CH_2Cl_2, 0 °C to rt.

Table 1. In Vitro Affinities at 5-HT_{1A} and 5-$HT_{1B/1D}$ Receptors (IC_{50} in nM) and pA_2 Values[a]

compd	5-HT_{1A}[b]	5-$HT_{1B/1D}$[c]	pA_2
2	0.37 ± 0.02	>1000	8.7
3	2.10 ± 0.52	>1000	8.0
4	1.55 ± 0.61	>1000	8.5
5	6.05 ± 0.18	>1000	8.3
6	7.25 ± 2.71	>1000	8.6
7	36.0 ± 13.5	>1000	7.7
12	1290 ± 1330	>1000	<7
14	500 ± 266	>1000	<7

[a] vs 8-OH-DPAT (guinea pig ileum). [b] [^{3}H]-8-OH-DPAT (rat hippocampus). [c] [^{3}H]-5-HT (calf striatum).

withdrawing trifluoromethanesulfonoxy group (**7**), for the serotonin 5-HT_{1A} receptor remains in the nanomolar range. None of the test compounds showed affinity for the serotonin 5-HT_{1B} receptor. The aminopiperidines **12** and **14** showed no affinity for the serotonin 5-HT_{1A} receptor (Table 1). These binding results suggest that the serotonin 5-HT_{1A} receptor site can accommodate larger substituents than the methoxy group, also with electron-withdrawing properties, in the *ortho*-position of the phenyl ring. Thus, the fluoroalkyl analogues offer the opportunity to introduce ^{18}F, resulting in radioligands with a much longer half-life than ^{11}C ($t_{1/2}$ of 109 min vs 20 min, respectively).[32,33] The aryl triflate, which is known to make the phenyl ring less prone to metabolic degradation, can serve as an alternative to aromatic methoxy substituents, present in several compounds with CNS activity, improving their oral availability.[34−37] Replacement of the rather flexible piperazine ring with an aminopiperidine leads to a total loss of affinity for the serotonin 5-HT_{1A} receptor. This might be due to the fact that the basic nitrogen of the piperazine has been moved away from the aromatic ring with one extra carbon bond (ca. 1.5 Å).

Table 2. Inhibition of Ultrasonic Vocalization after Administration of 8-OH-DPAT (0.1 mg/kg sc) Alone or in Combination with WAY100635 (**2**) or Test Compounds **3**−**7** and **14** (0.3 mg/kg sc)

treatment (n = 4−8)	vocalization time (s ± SEM)
control	148 ± 11
8-OH-DPAT	4.9 ± 4.1
2	158 ± 11*
3	166 ± 11*
4	164 ± 8*
5	171 ± 4*
6	167 ± 3*
7	146 ± 12*
14	7.4 ± 5.8§

*p < 0.05 vs 8-OH-DPAT. § p < 0.05 vs control.

In vitro functional data were obtained using the guinea pig ileum assay, in which the ability of the test compounds (**2**−**7**, **12,** and **14**) to antagonize the 8-OH-DPAT-induced contractions of a strip of ileum tissue was measured (Table 1).[38] The data obtained with this model

Table 3. Effects of WAY100635 (**2**) and Test Compounds **3**−**7** on 8-OH-DPAT (1 mg/kg sc)-Induced Hypothermia ($n = 4$)

	decrease in body temperature (°C)		
compd	0.1 (μmol/kg)	1.0 (μmol/kg)	10 (μmol/kg)
2	1.5 ± 0.2	0.9 ± 0.1*	0.9 ± 0.0*
3	0.6 ± 0.0*	0.2 ± 0.1*	0.3 ± 0.1*
4	1.8 ± 0.1	1.1 ± 0.1	0.4 ± 0.2*
5	2.4 ± 0.1	0.6 ± 0.1*	0.5 ± 0.2*
6	1.1 ± 0.3	0.5 ± 0.2*	0.4 ± 0.0*
7	1.5 ± 0.2	0.0 ± 0.1*	0.3 ± 0.1*
control	0.0 ± 0.1		
8-OH-DPAT	2.0 ± 0.1		

*$p < 0.05$ vs 8-OH-DPAT.

show that compounds **4**−**6** are potent antagonists, while the aminopiperidine analogues **12** and **14** failed to antagonize the 8-OH-DPAT-induced contractions.

In vivo functional data of the test compounds were obtained using the rat ultrasonic vocalization model (Table 2) and the rat hypothermia model (Table 3). Inhibition of ultrasonic vocalization by serotonin 5-HT_{1A} receptor agonists results from activation of presynaptic (somatodendritic) receptors, because local (as well as systemic) injections inhibit firing of raphé nuclei neurons and reduce ultrasonic vocalization even after hippocampal lesions.[39−41] WAY100635 (**2**), as well as compounds **3**−**7**, did antagonize the 8-OH-DPAT-induced inhibition of ultrasonic vocalization at 0.3 mg/kg sc test compound vs 0.1 mg/kg agonist, demonstrating their ability to act as autoreceptor antagonists in vivo. This suggests that the arylpiperazine analogues are all able to cross the blood−brain barrier. The aminopiperidine **14** was not able to counteract the agonist-induced inhibition at the doses tested, which is in line with in vitro data. The decrease in body temperature, as observed in rats treated with serotonin 5-HT_{1A} receptor agonists, is postsynaptically mediated, because hypothermia is still present following depletion of endogenous 5-HT.[16,42−45] The *O*-desmethyl arylpiperazine analogue **3** proved to be the most potent functional postsynaptic antagonist in this model. It significantly antagonized the 8-OH-DPAT-induced hypothermia (3 μmol/kg sc) at the lowest dose tested (0.1 μmol/kg). WAY100635 (**2**) and the fluoropropyl (**5**), the mesylate (**6**), and the triflate (**7**) analogues proved to be equipotent in this assay. They were all able to significantly block the decrease in body temperature at 1.0 μmol/kg sc. The fluoroethyl analogue **4** was the weakest antagonist in this postsynaptic serotonin 5-HT_{1A} activation model. It significantly blocked the decrease in body temperature at the highest dose tested (10 μmol/kg sc). The in vivo results show that the in vitro data obtained are not able to discriminate the most potent serotonin 5-HT_{1A} receptor antagonist. The subnanomolar affinity of WAY100635 (**2**) for the serotonin 5-HT_{1A} receptor binding site combined with the highest pA_2 value do not ensure that it is indeed the best serotonin 5-HT_{1A} antagonist. In fact, in vivo in the postsynaptic 8-OH-DPAT-induced hypothermia assay, WAY100635 (**2**) showed to be equipotent to the triflate analogue **7**, which is, in vitro, the weakest arylpiperazine. This discrepancy between the in vitro and in vivo data might be due to differences in pharmacokinetic, pharmacodynamic, and/or metabolic profiles of the different test compounds and WAY100635 (**2**).

To summarize, a small series of structural analogues of WAY100635 (**2**) have been synthesized and their in vitro and in vivo pharmacology has been evaluated. From the pharmacological results, it may be concluded that the arylaminopiperidine moiety is not able to act as a bioisostere for the arylpiperazine that is present in WAY100635 (**2**). Compounds **12** and **14** showed no serotonin 5-HT_{1A} receptor affinity or activity, neither in vitro nor in vivo. The substituent in the *ortho*-position of the phenylpiperazine, on the other hand, can be both smaller and larger than the original methoxy substituent of WAY100635 (**2**) and have strong electron-withdrawing properties. In the 8-OH-DPAT-induced hypothermia assay, WAY100635 (**2**) is equipotent to the fluoropropyl analogue **5** (a possible [^{18}F]PET ligand)[32] and the triflate analogue **7**. However, the *O*-desmethyl analogue **3**, which has shown to be a very promising radioligand in PET studies,[30] is the functionally most potent serotonin 5-HT_{1A} antagonist at the postsynaptic receptor sites.

Experimental Section

Chemistry. Melting points were determined with an Electrothermal digital capillary melting point apparatus and are uncorrected. NMR spectra were acquired on a Varian Gemini-200 spectrometer at 200 MHz (proton) and 50.3 MHz (carbon); J values are given in hertz (Hz). $CDCl_3$ was employed as the solvent unless otherwise stated. Chemical shifts are given in δ units (ppm) and relative to tetramethylsilane or deuterated solvent. Infrared spectra were recorded with an ATI-Mattson spectrometer. Elemental analyses were performed in the labs of Merck KGaA Darmstadt (D) or in the Microanalytical Department of the University of Groningen (NL) and were within 0.4% of theoretical values unless otherwise indicated. GC/MS (EI and CI) mass spectra were recorded on a Unicam610/Automass 150 GC/MS system (70 mV). HRMS spectra were performed in the Microanalytical Department of the University of Groningen (NL) and were recorded on a JEOL MS Route JMS 600H. For column chromatography silica gel 60 (70−230 mesh) (Merck) was used. Reactions were carried out under a nitrogen atmosphere, solvents used were distilled and/or dried by standard techniques immediately prior to use, and in the case of an aqueous workup, magnesium sulfate monohydrate was used as the drying agent. WAY100635 (**2**) was prepared as previously described.[27]

***N*-[2-[4-(2-Hydroxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (3).** A suspension of **2** (50.0 mg, 118 μmol) and $AlCl_3$ (95.0 mg, 700 μmol) in benzene (5 mL) was refluxed for 2 h. The reaction was quenched with H_2O (5 mL) and neutralized with solid $NaHCO_3$. The mixture was extracted with CH_2Cl_2, (3 × 20 mL) and the combined organic layers were washed with brine, dried, and concentrated in vacuo, to yield 45 mg (93%) of a colorless oil, which was converted to the oxalate salt and recrystallized from *i*-PrOH yielding 43 mg (73%) of **3** as white crystals: mp 204−207 °C; ^{1}H NMR δ 8.56−8.53 (dd, $J_1 = 4.96$, $J_2 = 1.78$, 1 H), 7.84−7.75 (dt, $J_1 = 7.75$, $J_2 = 2.01$, 1 H), 7.31−7.23 (m, 2 H), 7.14−7.03 (m, 2 H), 6.96−6.81 (m, 2 H), 4.03−3.96 (t, $J = 6.93$, 2 H), 2.82−2.78 (m, 4 H), 2.67−2.60 (m, 6 H), 2.31−2.18 (m, 1 H), 1.79−1.57 (m, 7 H), 1.26−0.98 (m, 3 H); ^{13}C NMR δ 176.1, 151.5, 149.3, 138.9, 138.0, 126.4, 122.1, 119.9, 114.0, 100.0. 56.1, 53.8, 52.5, 45.1, 42.4, 29.5, 25.6; IR (NaCl) 3299, 1659 cm^{-1}; MS (CI with NH_3) m/z 409 (M^+); HRMS calcd (obsd) for $C_{24}H_{32}N_4O_2$: 408.253 (408.253). Anal. ($C_{24}H_{32}N_4O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[4-(2-(2-Fluoroethoxy)phenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (4).** To a suspension of **3** (0.10 g, 0.25 mmol) and Cs_2CO_3 (0.60 g, 1.84 mmol) in acetonitrile (30 mL) was added fluoroethyl bromide (0.05 g, 0.40 mmol). After refluxing for 3 h, the solvent was removed in vacuo. The residue was redissolved in H_2O and extracted

with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated in vacuo. The oil obtained was converted to the oxalate salt and recrystallized from EtOH, yielding 60 mg (45%) of **4** as white crystals: mp 207−209 °C; ^{1}H NMR δ 8.55−8.52 (dd, J_1 = 5.25, J_2 = 1.59, 1 H), 7.81−7.72 (dt, J_1 = 7.70, J_2 = 2.06, 1 H), 7.32−7.21 (m, 2 H), 6.99−6.82 (m, 4 H), 4.88 (t, J = 4.11, 1 H), 4.64 (t, J = 4.13, 1 H), 4.31 (t, J = 4.09, 1 H), 4.17 (t, J = 4.13, 1 H), 3.99 (t, J = 6.96, 2 H), 3.10−2.95 (m, 4 H), 2.65−2.58 (m, 6 H), 2.24−2.18 (m, 1 H), 1.89−0.83 (m, 10 H); IR (KBr) 1658; MS (CI with NH_3) m/z 455 (M^+); HRMS calcd (obsd) for $C_{26}H_{35}N_4O_2F_1$: 454.274 (454.274). Anal. ($C_{26}H_{35}N_4O_2F_1 \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N.

***N*-[2-[4-(2-(2-Fluoropropoxy)phenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (5).** To a suspension of **3** (0.02 g, 0.05 mmol) and Cs_2CO_3 (0.08 g, 0.25 mmol) in acetonitrile (4 mL) was added fluoropropyl bromide (0.08 g, 0.06 mmol). After refluxing for 2 h, the solvent was removed in vacuo. The residue was redissolved in H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated in vacuo. The oil obtained was converted to the oxalate salt and recrystallized from EtOH yielding 17 mg (62%) of **5** as white crystals (monooxalate salt): mp 181−183 °C; ^{1}H NMR δ 8.55−8.52 (dd, J_1 = 5.22, J_2 = 1.61, 1 H), 7.81−7.72 (dt, J_1 = 7.70, J_2 = 1.93, 1 H), 7.32−7.21 (m, 2 H), 7.02−6.84 (m, 4 H), 4.78 (t, J = 5.82, 1 H), 4.55 (t, J = 5.80, 1 H), 4.12 (t, J = 6.63, 1 H), 4.00 (t, J = 6.96, 1 H), 3.00 (m, 4 H), 2.71−2.58 (m, 6 H), 2.27 (t, J = 5.96, 1 H), 2.14 (t, J = 5.96, 1 H), 1.93 (m, 1 H), 1.79−1.52 (m, 7 H), 1.26−0.97 (m, 3 H); ^{13}C NMR δ 176.2, 156.0, 149.2, 141.5, 138.0, 131.0, 122.7, 122.3, 122.2, 121.3, 118.2, 82.5, 79.3, 63.8, 63.7, 56.2, 53.4, 50.6, 45.1, 42.4, 30.9, 30.5, 29.5, 25.6; IR (KBr) 1656; MS (CI with NH_3) m/z 469 (M^+); HRMS calcd (obsd) for $C_{26}H_{35}N_4O_2F_1$: 468.290 (468.290). Anal. ($C_{26}H_{35}N_4O_2F_1 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[4-[2-[Methanesulfonoxyl]phenyl]-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (6).** To a cooled solution (−78 °C) of **3** (0.05 g, 0.12 mmol) and triethylamine (10 μL) in CH_2Cl_2 (2 mL) was slowly added methanesulfonyl chloride (0.02 g, 0.18 mmol). The reaction was allowed to warm to room temperature, after which it was stirred for another 6 h. The mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated in vacuo. The residue was purified by chromatography (CH_2Cl_2 with 3% MeOH), converted to the oxalate salt, and recrystallized from EtOH to yield 30 mg (43%) of **6** as white crystals: mp 234−237 °C; ^{1}H NMR δ 8.55−8.52 (dd, J_1 = 5.49, J_2 = 1.93, 1 H), 7.82−7.73 (dt, J_1 = 7.71, J_2 = 2.00, 1 H), 7.32−7.20 (m, 4 H), 7.08−7.00 (m, 2 H), 3.98 (t, J = 6.89, 2 H), 3.15 (s, 3 H), 3.02−2.98 (m, 4 H) 2.65−2.58 (m, 6 H), 2.32−2.15 (m, 1 H), 1.77−0.96 (m, 10 H); ^{13}C NMR δ 176.3, 155.8, 149.3, 144.7, 142.9, 138.1, 128.2, 124.6, 123.3, 122.2, 122.1, 119.8, 56.1, 53.4, 51.0, 45.0, 42.3, 38.3, 29.5, 25.5; IR (KBr) 1657, 1366, 1157; HRMS calcd (obsd) for $C_{25}H_{34}N_4O_4S_1$: 486.230 (486.230). Anal. ($C_{25}H_{34}N_4O_4S_1 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

***N*-[2-[4-[2-[(Trifluoromethyl)sulfonoxyl]phenyl]-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (7).** To a suspension of **3** (0.10 g, 0.20 mmol) and tetrabutylammonium iodide (10 mol %) in CH_2Cl_2 (3.0 mL) and a 10% solution of NaOH(aq) (1.5 mL) was added *N*-phenyltrifluoromethanesulfonimide (0.36 g, 1.0 mmol). The reaction was stirred at room temperature for 36 h, after which it was quenched with H_2O (10 mL) and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated in vacuo. The residue was purified by flash chromatography (CH_2Cl_2 with 5% MeOH), converted to the oxalate salt, and recrystallized from EtOH to yield 46 mg (36%) of **7** as white crystals: mp 182−184 °C; ^{1}H NMR δ 8.55−8.52 (dd, J_1 = 5.55, J_2 = 2.04, 1 H), 7.82−7.73 (dt, J_1 = 7.72, J_2 = 1.91, 1 H), 7.36−7.25 (m, 4 H), 7.22−7.03 (m, 2 H), 4.04−3.97 (t, J = 6.89, 2 H), 3.04−2.94 (m, 4 H), 2.76−2.64 (m, 6 H), 2.31−2.20 (m, 1 H), 1.88−1.46 (m, 7 H), 1.27−0.96 (m, 3 H); IR (KBr) 1665, 1414, 1207 cm^{-1}; MS (EI) m/z 540 (M^+); HRMS calcd (obsd) for $C_{25}H_{31}N_4O_4S_1F_3$: 540.201 (540.201). Anal. ($C_{25}H_{31}N_4O_4S_1F_3 \cdot C_2H_2O_4$) C, H, N.

4-*N*-(2-Methoxyphenyl)aminopiperidine (8). A solution of *o*-anisidin (3.00 g, 25.0 mmol) and *N*-benzyl-4-piperidone (3.80 g, 20.0 mmol) and *p*-toluenesulfonic acid (0.10 g) in toluene (50 mL) was refluxed under Dean−Stark conditions for 3 h. After cooling to room temperature, PtO_2 (0.10 g) was added and the mixture was hydrogenated in a Parr shaker with H_2 (60 psi) for 2 h at 60 °C. A solution of 25% ammonia(aq) (5 mL) was added and the mixture was hydrogenated further for another 1 h (50 psi) at room temperature. The reaction mixture was filtered over Celite and the solvent removed in vacuo. The residue was purified by gradient flash chromatography (hexane to EtOAc), yielding 2.7 g (46%) of the intermediate as a brown-yellow oil. For the debenzylation, this intermediate (2.7 g, 9.1 mmol) was dissolved in THF (10 mL) and MeOH (190 mL), 20% $Pd(OH)_2$/C (200 mg) was added, and the mixture was hydrogenated in the Parr shaker with H_2 (60 psi) for 2 h at 60 °C. The reaction mixture was filtered over Celite and the solvent removed in vacuo. The residue was recrystallized from hexane, yielding 1.7 g (91%) of **8** as white crystals: mp 107−109 °C; ^{1}H NMR δ 6.89−6.60 (m, 4 H), 3.83 (s, 3 H), 3.39−2.70 (m, 4 H), 2.3−2.08 (m, 2 H), 1.51−1.10 (m, 4 H), 0.88 (m, 1 H); ^{13}C NMR δ 145.3, 135.2, 119.7, 114.8, 108.7, 108.1, 53.9, 48.1, 43.7, 31.9; MS (EIPI) m/z 206 (M^+); HRMS calcd (obsd) for $C_{12}H_{18}N_2O_1$: 206.14 (206.14).

2-[4-*N*-(*o*-Methoxyphenyl)aminopiperidinyl]-*N*-pyridin-2-ylacetamide (9). A suspension of compound **8** (0.25 g, 1.20 mmol), 2-chloro-*N*-pyridin-2-ylacetamide (0.20 g, 1.17 mmol), triethylamine (2 mL), K_2CO_3 (0.10 g, 0.72 mmol), and tetrabutylammonium iodide (0.05 g) was refluxed for 18 h, after which the solvent was removed in vacuo. The residue was purified by flash chromatography (hexane with 20% EtOAc to pure EtOAc), yielding 270 mg (66%) of a brown-yellow oil. The oil was redissolved in CH_2Cl_2, converted to the HCl salt, and recrystallized from MeOH/Et_2O: mp 156 °C; ^{1}H NMR δ 9.65 (b, 1 H), 8.32−8.22 (m, 2 H), 7.73−7.63 (m, 1 H), 7.06 (m, 1 H), 6.85−6.59 (m, 4 H), 4.15 (b, 1 H), 3.83 (s, 3 H), 3.35 (m, 1 H), 3.16 (s, 2 H), 2.87 (m, 2 H)2.40 (m, 2 H), 2.10 (m, 2 H), 1.60 (m, 2 H); ^{13}C NMR δ 169.0, 105.9, 147.9, 146.8, 132.2, 136.6, 121.0, 119.7, 116.3, 113.6, 110.2, 109.5, 62.0, 55.2, 52.8, 48.5, 32.3; MS (EI) m/z 340 (M^+); HRMS calcd (obsd) for $C_{19}H_{24}N_3O_2$: 340.19 (340.19).

2-[4-*N*-(*o*-Methoxyphenyl)-*N*-methylaminopiperidinyl]-*N*-pyridin-2-ylacetamide (10). A mixture of compound **9** (0.50 g, 1.47 mmol), formaldehyde (10 mL), and 20% $Pd(OH)_2$/C (0.10 g) in EtOH (100 mL) was hydrogenated with H_2 (60 psi) for 18 h at 60 °C. The residue was purified by gradient flash chromatography (CH_2Cl_2 with 10−20% EtOH), yielding 400 mg (77%) of a viscous brown oil. The oil was redissolved in Et_2O and converted to the HCl salt: mp 180 °C; ^{1}H NMR δ 9.60 (b, 1 H), 8.32−8.21 (m, 2 H), 7.69−7.65 (m, 1 H), 7.06−6.85 (m, 5 H), 3.84 (s, 3 H), 3.22 (m, 1 H), 3.11 (s, 2 H), 2.97 (m, 2 H), 2.74 (s, 3 H), 2.24 (m, 2 H), 1.82 (m, 2 H), 1.76 (m, 2 H); ^{13}C NMR δ 169.5, 153.1, 150.9, 147.8, 140.9, 138.2, 122.8, 121.3, 120.4, 119.7, 113.8, 111.1, 62.1, 58.4, 55.2, 53.9, 34.2, 28.2; MS (EIPI) m/z 354 (M^+); HRMS calcd (obsd) for $C_{20}H_{26}N_3O_2$: 354.21 (354.21).

2-[4-*N*-(*o*-Methoxyphenyl)-*N*-methylaminopiperidinyl]-*N*-pyridin-2-ylamine (11). To an ice-cooled solution of compound **10** (0.30 g, 0.85 mmol) in Et_2O (15 mL) was added dropwise a 1.0 M solution of DIBAL-H in toluene (6 mL). The mixture was stirred for 18 h at room temperature after which H_2O (3 drops) and acetone (1 mL) were added. The solvent was removed in vacuo. The residue was purified by gradient flash chromatography (EtOAc, THF, and then CH_2Cl_2 with 10−25% EtOH and 2% aq ammonia), yielding 260 mg (90%) of **11** as a yellow oil. The oil was redissolved in Et_2O and converted to the HCl salt: mp 165 °C; ^{1}H NMR δ 8.09 (m, 1 H), 7.41 (m, 1 H), 7.02−6.81 (m, 4 H), 6.57−6.37 (m, 2 H), 5.13 (b, 1 H), 3.84 (s, 3 H), 3.32 (m, 1 H), 3.18−2.99 (m, 2 H), 2.73 (s, 3 H), 2.57 (m, 2 H), 1.97−1.73 (m, 8 H); ^{13}C NMR δ 153.5, 148.0, 137.2, 137.1, 122.6, 121.3, 120.4, 120.3, 112.5, 111.1,

106.8, 59.2, 56.4, 55.2, 53.1, 38.6, 33.9, 28.1; MS (EIPI) *m/z* 340 (M^+); HRMS calcd (obsd) for $C_{20}H_{28}N_3O_1$: 340.23 (340.23).

2-[4-*N*-(*o*-Methoxyphenyl)-*N*-methylaminopiperidinyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (12). To an ice-cooled solution of **11** (0.17 g, 0.50 mmol) and triethylamine (2 mL) in CH_2Cl_2 (10 mL) was added dropwise a solution of cyclohexanecarboxylic acid chloride (1 mL, 7.5 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 2 h, after which the solvent was removed in vacuo. The residue was purified by gradient flash chromatography (CH_2Cl_2 with 5−15% EtOH), yielding 140 mg (62%) of **12** as a yellow oil. The oil was redissolved in Et_2O and converted to the HCl salt: mp 130−135 °C; ^{1}H NMR δ 8.50 (m, 1 H), 7.78 (m, 1 H), 7.26 (m, 2 H), 7.00−6.82 (m, 4 H), 4.11 (m, 2 H), 3.84 (s, 3 H), 3.28 (m, 3 H), 2.90 (m, 2 H), 2.68 (s, 3 H), 2.15−0.93 (m, 17 H). Anal. ($C_{27}H_{38}N_4O_2$·2.4HCl·4.6H_2O) C, H, N, Cl.

2-[4-*N*-(*o*-Methoxyphenyl)aminopiperidinyl]-*N*-pyridin-2-ylamine (13). To an ice-cooled solution of **9** (0.27 g, 0.80 mmol) in Et_2O (15 mL) was added a 1.0 M solution of DIBAL-H in toluene (6 mL). The reaction was stirred for 18 h at room temperature. The excess of DIBAL-H was destroyed with H_2O (3 drops) after which the solvent was removed in vacuo. The residue was purified by gradient flash chromatography (EtOAc to THF to CH_2Cl_2 with 10−25% EtOH and 2% aq ammonia), yielding 200 mg (77%) of **13** as a yellow oil. The oil was redissolved in Et_2O and converted to the HCl salt: mp 122 °C; ^{1}H NMR δ 8.09 (m, 1 H), 7.26 (m, 1 H), 6.99−6.38 (m, 6 H), 5.16 (b, 1 H), 4.18 (b, 1 H), 3.84 (s, 3 H), 3.33 (m, 3 H), 2.88 (m, 2 H), 2.66 (s, 3 H), 2.33−2.17 (m, 4 H), 1.43 (m, 2 H); ^{13}C NMR δ 158.7, 148.0, 146.7, 137.2, 136.8, 121.1, 116.0, 112.5, 110.1, 109.4, 106.9, 56.2, 55.2, 52.0, 49.3, 38.6, 33.2; MS (EIPI) *m/z* 326 (M^+); HRMS calcd (obsd) for $C_{20}H_{26}N_3O_2$: 326.23 (326.22).

[4-*N*-(*o*-Methoxyphenyl)-*N*-cyclohexanecarboxamidopiperidinyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (14). To an ice-cooled solution of **13** (0.20 g, 0.62 mmol) and triethylamine (2 mL) in CH_2Cl_2 (10 mL) was added dropwise a solution of cyclohexanecarboxylic acid chloride (2 mL, 15.0 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 2 h, after which the solvent was removed in vacuo. The residue was purified by gradient flash chromatography (EtOAc to CH_2Cl_2 with 10% EtOH), yielding 140 mg (62%) of a yellow oil. The oil was redissolved in Et_2O and converted to the HCl salt: mp 137−140 °C; ^{1}H NMR δ 8.40 (m, 1 H), 7.39 (m, 1 H), 7.26−6.88 (m, 6 H), 4.48 (m, 1 H), 3.81 (m, 2 H), 3.72 (s, 3 H), 2.88 (m, 2 H), 2.41 (m, 2 H), 2.20−1.96 (m, 4 H), 1.74−1.40 (m, 18 H), 1.06−0.81 (6 H); ^{13}C NMR δ 176.7, 176.1, 156.4, 155.8, 148.9, 137.8, 131.1, 129.3, 127.7, 122.0, 120.3, 116.0, 57.9, 55.7, 54.9, 52.3, 45.1, 42.1, 42.0, 30.7, 29.6, 29.2, 28.6, 28.5, 25.4, 25.3. Anal. ($C_{33}H_{46}N_4O_3$·1.9HCl·3.3H_2O) C, H, N, Cl.

Biological Assays. 1. Animals. For the guinea pig ileum assay male Dunkin Hartley guinea pigs (300−400 g) were used. The hypothermia experiments were performed using male Wistar rats (200−300 g). The animals were housed in groups (four rats per cage) in a temperature- and humidity-controlled colony room (20 ± 2 °C; 50−60%) on a natural day−night cycle (light period 6:30−17:00 h). Food and water were available ad libitum except during the actual experiments. Testing was done between 10 and 16 h during the light phase of the day−night cycle. For the ultrasonic vocalization experiments Sprague−Dawley rats (290−390 g) were used. Animals were housed in groups (two rats per cage) in a temperature- and humidity-controlled colony room (29 ± 2 °C; 50−60%) under a natural day−night cycle (light period 6:00−18:00 h) with food and water ad libitum except during the actual experiments. Procedures were conducted in accordance with guidelines published in the *NIH Guide for the Care and Use of Laboratory Animals*.

2. Receptor Binding. Affinities of compounds were determined using competition binding assays to determine IC_{50} values at the various receptors. Affinities at the 5-HT_{1A} receptors were measured as the displacement of [^{3}H]-8-OH-DPAT (rat frontal cortex).[46,47] For the 5-$HT_{1B/D}$ receptor binding assays, membranes prepared from calf striatum were used with 1.7 nM [^{3}H]-5-HT in the presence of 100 nM 8-OH-DPAT and 100 nM mesulergine to mask the 5-HT_{1A} and 5-HT_{2C} binding sites.[48]

3. Guinea Pig Ileum Assay for Determination of pA_2 Value.[38] The animals were stunned and exsanguinated. After removal of the ileum, segments of about 1 cm were dissected 10 cm from the ileoceacal junction. After clearance of the mesenteric connective tissue and removal of the luminal contents, each ileal segment was mounted between platinum stimulation electrodes in a 50-mL, two-chambered organ bath with an internal circulation. The segments were bathed in a modified Krebs Henseleit solution (in mmol: NaCl 118; $NaHCO_3$ 25; KH_2PO_4 1.19; $CaCl_2$ 1.25; KCl 4.7; $MgSO_4$ 0.6; glucose 10). Ketanserin (0.3 μmol) and tropisetron (3 μmol) were added to block 5-HT_2, 5-HT_3, and 5-HT_4 receptors. The solution, maintained at 35 °C, was gassed and continuously mixed by bubbling with 95% O_2/5% CO_2. Contractions were recorded isometrically under a load of 1 g. After an equilibration for 20 min the preparations were stimulated continuously with square-wave pulses (0.05 Hz, 1 ms, 40 V) until the contractions reached a constant level. The preparations were washed and then stimulated with trains of 6 stimulations at intervals of 7 min, until the mean responses were comparable. Cumulative concentration−response curves were constructed with an exposure period of 7 min with 8-OH-DPAT, followed by 6 electrical stimulations, after which the next higher concentration would be applied. Controls received the corresponding volume of the vehicle only. For examining antagonistic activity, the compounds were applied at fixed concentrations 60 min before starting the cumulative concentration−response curve of the agonist. Responses were measured as an increase in the isometric tension and expressed as a percentage of the mean effect of the last 6 stimulations before the start of the experiment. Because the 5-HT_{1A} agonist 8-OH-DPAT decreases the electrically induced contractions maximally by about 40%, EC_{20} values were determined graphically form the mean effect of 4−6 experiments. Apparent pA_2 values were calculated according to MacKay: $pA_2 = -\log[\text{antagonist}] + (DR - 1)$, where DR is the ratio of EC_{20} values in the presence and absence of the antagonist.[51]

4. 8-OH-DPAT-Induced Hypothermia.[16] Animals were treated with 1 mg/kg 8-OH-DPAT 30 min prior to measurement; test compounds/saline were administered 45 min prior to measurement. The core temperature was determined by insertion of a digital temperature probe (CMA/150 temperature controller, CMA/Microdialysis Sweden) 4−5 cm into the rectum for 60 s until a stable reading was obtained, to the nearest 1/10 °C. For each independent experiment new control and 8-OH-DPAT studies were conducted and changes in temperature due to test compounds were assessed using a one-way analysis of variance (ANOVA) with post hoc analysis carried out using Dunnett's *t*-tests against their own control data.

5. Ultrasonic Vocalization.[18,19,45] Ultrasonic vocalization was measured in a sound-attenuated test chamber (W 24 cm, L 22 cm, H 22 cm) with a grid floor for delivery of footshocks (scrambled chock of 1.8 mA for 0.3 s; shocker Getra BN 2002). Ultrasonic vocalization was recorded (microphone 4004, Bruel and Kjaer) and processed by an interface (developed at Merck KGaA) to select 22 ± 4 kHz signals and to digitize the resulting signals for automatic processing in a personal computer. In a priming phase, each rat was placed in the test chamber. After 2 min, a series of at most 10 shocks (trials), 1.8 mA for 0.3 s, separated by 20-s shock-free intervals, was delivered via the grid floor of the test chamber. In the shock-free intervals the occurrence of ultrasonic vocalization was automatically recorded, and the duration of ultrasonic vocalization was calculated immediately. The priming session was terminated either when the rat constantly vocalized at least for 10 s on 3 consecutive trials or after the tenth trial. Rats that did not respond with ultrasonic vocalization were excluded from the test. In the actual test performed the next day, each rat received 5 initial shocks (1.8 mA for 0.3 s, separated by 20-s

shock-free intervals) in the test chamber, and the duration of ultrasonic vocalization was recorded after the last shock for 3 min. Antagonists or vehicle were given 40 min and 8-OH-DPAT or vehicle 30 min before the start of the ultrasonic vocalization test. Means and SEM values were calculated for each group and compared to the data for the respective placebo group by one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test.

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