Synthesis and Pharmacological Testing of 1,2,3,4,10,14b-Hexahydro-6-methoxy-2-methyldibenzo[*c,f*]pyrazino[1,2-*a*]azepin and Its Enantiomers in Comparison with the Two Antidepressants Mianserin and Mirtazapine

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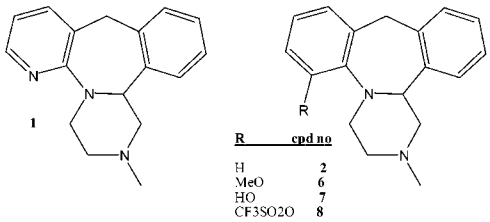
The synthesis and resolution of 1,2,3,4,10,14b-hexahydro-6-methoxy-2-methyldibenzo[*c,f*]-pyrazino[1,2-*a*]azepin (6-methoxymianserin, **6**) are described. Furthermore, the in vitro and in vivo effects of **6** and its enantiomers are presented. **6** displayed high affinity for the 5-HT2A/2C receptors, only moderate affinity for the adrenoceptors, and no affinity for the NA reuptake site. Surprisingly, **6** also showed moderate to high affinity for the dopamine D2 receptor, an effect that resides in the (*R*)-(−)-enantiomer.

Introduction

Depression has long been associated with decreased levels of both noradrenaline (NA) and serotonin (5-HT) in the central nervous system.[1,2] The marketed antidepressants mirtazapine (**1**, azamianserin, Remeron) and mianserin (**2**, Tolvon) possess a tetracyclic structure in which an arylpiperazine moiety is incorporated. The



mechanisms of action of these two antidepressants, however, are believed to be different.

The pharmacological profile of **1** is characterized by α2, 5-HT2, 5-HT3, and histamine H1 receptor antagonist properties. The increase in rat hippocampal levels of NA and 5-HT after administration of **1** are believed to be due to α2-adrenoceptor antagonism.[3−6] The α2-autoreceptor and the 5-HT2 receptor blocking effects of **1** are known to reside predominantly in the (+)-enantiomer, while the (−)-enantiomer is responsible for blockage of the α2-heteroreceptor (located on the nerve terminals of the 5-HT neurons) and the 5-HT3 receptor antagonism.[7,8] The pharmacological profile of **2** is characterized by antagonistic properties at the 5-HT2, 5-HT3, H1 receptor, and the NA reuptake site. The increase in rat hippocampal levels of NA after administration of **2** is believed to be due to blockage of the NA reuptake site. The NA reuptake and 5-HT2 blocking effects of **2** are known to reside in the (+)-enantiomer, while the (−)-enantiomer is responsible for the 5-HT3 receptor antagonism.[9−11] Both antidepressants have negligible affinity for the dopamine D2 receptor.

In this paper, we report the synthesis and resolution of the 6-methoxy analogue of mianserin (**2**) 1,2,3,4,10,14b-hexahydro-6-methoxy-2-methyldibenzo[*c,f*]pyrazino[1,2-*a*]azepin (**6**). It is noteworthy that the *o*-methoxyphenylpiperazine moiety, inherent in the chemical structure of compound **6**, is present in several pharmacologically active compounds that act through the serotonergic system.[12−15]

The in vitro receptor affinities of 6-methoxymianserin (**6**) and both its enantiomers at the different dopaminergic, serotonergic, adrenergic, and histaminergic receptor subtypes, as well as at the NA and the 5-HT reuptake sites, were determined. In a microdialysis study in the ventral hippocampus of awake, freely moving rats, the in vivo effects of 5 mg/kg sc of **6** and 2.5 mg/kg sc of either of its enantiomers on the release of NA and 5-HT and their metabolites dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA) were established. DOPAC is a metabolite formed both in dopaminergic and in noradrenergic neurons. In brain areas with no dopaminergic innervation, like the locus ceruleus and the ventral hippocampus, DOPAC release is believed to reflect noradrenergic activity.[16,17] The result of this microdialysis study is compared with previously reported effects of similar doses of **1** and **2**.[4]

Chemistry

6 was synthesized as follows (Scheme 1). Styrene oxide and 2-methylaminoethanol were reacted at 130 °C, yielding *β*-hydroxy-*N*-methyl-*N*-hydroxyethylphenyl ethylamine (**3**), which was converted to the chlorinated phenylethylamine **4** upon treatment with thionyl chloride in refluxing chloroform. 2-Amino-3-methoxybenzoic acid was reduced to the corresponding alcohol in THF

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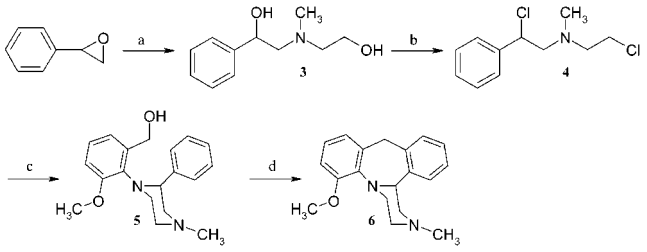
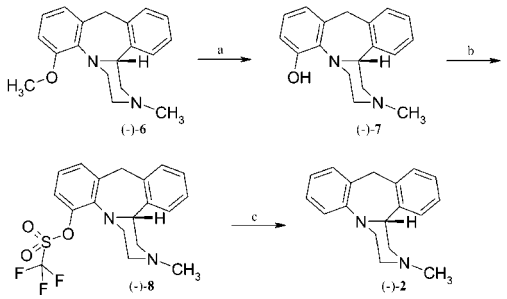
Scheme 1[a]



[a] Reagents: (a) 2-methylaminoethanol, 130 °C, 17 h; (b) $SOCl_2$, CH_2Cl_2, reflux, 3 h; (c) 2-amino-3-methoxybenzyl alcohol, *N*,*N*-diisopropylethylamine, acetonitrile, reflux, 7 h; (d) PPA, 100 °C, 2 h.

Scheme 2[a]



[a] Reagents: (a) $AlCl_3$, benzene, reflux, 17 h; (b) trifluoromethanesulfonyl anhydride, CH_2Cl_2, TEA, −78 °C to room temperature, 17 h; (c) $Pd(OAc)_2$, PPh_3, TEA, MeOH, reflux, 48 h.

using lithium aluminum hydride, and the resulting compound was subsequently coupled to **4** in refluxing acetonitrile in the presence of diisopropylethylamine, yielding 1-methyl-3-phenyl-4-(2-methoxy-6-hydroxyethyl)-phenylpiperazine·HCl (**5**). Ring closure of **5** was accomplished upon treatment with PPA at 100 °C, affording **6**.

The direct separation of the enantiomers of **6** was achieved by means of HPLC using a semipreparative Chiralcel OD column.[18] Determination of the absolute configuration was carried out indirectly by chemically linking the levorotatory isomer (−)-**6** to (*R*)-(−)-**2** (Scheme 2).[19]

Pharmacology

The ability of **1**, **2**, **6**, and its enantiomers to displace the respective radioligands at the dopamine D2, D3, D4, serotonin 5-HT1A, 5-HT1D, 5-HT2A, 5-HT2C, 5-HT3, and α1- and α2-adrenergic, histamine H1 and H2 receptors, and the 5-HT and NA reuptake sites were determined.[20−29] The extracellular levels of NA, DOPAC, 5-HT, and 5-HIAA in the ventral hippocampus were measured by in vivo microdialysis after administration of 5 mg/kg sc of the racemate of 6-methoxymianserin or 2.5 mg/kg sc of either of the enantiomers. Levels were measured every 15 min for a period of 2.5 h after administration of the test compound. Before administration of test compounds, four consecutive samples were collected (baseline).[30]

Results and Discussion

The binding study (Table 1) shows that **6** has high affinity for the 5-HT2A and 5-HT2C receptors, which is found in both enantiomers. While both **1** and **2** have high affinity for the 5-HT3 receptor, **6** is devoid of affinity for this receptor subtype. **6** displays only moderate affinity and no selectivity for the α1- and α2-adrenoceptors and displays no affinity for the NA reuptake site. The (*S*)-(+)-enantiomer of **6** shows remarkable selectivity for the 5-HT2 receptor subtypes. Surprisingly, the (*R*)-(−)-enantiomer showed a moderate affinity for the dopamine D2 receptor. In antipsychotic research, the ratio 5-HT2A/D2 affinity, the "Meltzer ratio", is used as a marker to classify atypical neuroleptics such as clozapine.[31] The "Meltzer ratio" of (−)-**6** to clozapine is highly favorable.[32] The moderate affinity of the (−)-enantiomer for the α2-adrenoceptor may add to a possible positive antipsychotic profile, since it was recently shown that α2-antagonists potentiate the cortical output of dopamine.[33] The two other 6-substituted analogues **7** and **8** were only tested on a few receptors (Table 1), and these data are not further discussed.

In a previous microdialysis study in the rat ventral hippocampus, it was shown that **1** (5 mg/kg sc) causes an increase in DOPAC levels up to 200% above baseline (90−150 min) and a transient increase in 5-HT levels up to 180% after 60 min.[4] A similar dose of **2** induced an increase in DOPAC levels up to 160% above baseline (90−150 min) but had no effect on the 5-HT release.[4] Administration of **1** had no effect on the 5-HIAA release.

Administration of **6** (5.0 mg/kg sc) causes an increase in NA levels up to 200% (45−150 min) and in DOPAC levels up to 160% above baseline (60−150 min). Fur-

Table 1. In Vitro Binding Profiles of (±)-, (+)-, and (−)-**6**, (±)-**7**, (±)-**8**, (±)-**2**, and (±)-**1** (K_i in nM)[a]

	affinity K_i (nM)					affinity IC_{50} (nM)	
receptor	(±)-**6**	(+)-**6**	(−)-**6**	(±)-**2**	(±)-**1**	(±)-**7**	(±)-**8**
D_2	28 ± 9	460 ± 120	20 ± 5	1000 ± 5	1460 ± 430	nt	nt
D_3	260 ± 45	3570 ± 410	100 ± 6	nt	>20	nt	nt
$D_{4.2}$	92 ± 6	160 ± 11	47 ± 2	nt	>25	nt	nt
5-HT_{1A}	345 ± 20	800 ± 130	255 ± 61	>500	>18	nt	nt
5-HT_{1D}	nt	1470 ± 150	80 ± 20	nt	nt	nt	nt
5-HT_{2A}	0.70 ± 0.1	0.55 ± 0.09	0.75 ± 0.18	1.5 ± 0.6	2.0 ± 0.4	12	277
5-HT_{2C}	0.21 ± 0.03	0.16 ± 0.01	0.39 ± 0.11	1.4 ± 0.1	5.5 ± 0.8	2.0	nt
5-HT_3	2900 ± 35	>5000	1650 ± 80	70 ± 3	nt	nt	nt
$alpha_1$	84 ± 15	205 ± 31	74 ± 12	28 ± 5	1050 ± 130	1000	nt
$alpha_2$	nt	73 ± 24	37 ± 8	nt	nt	1000	1000
H_1	nt	13 ± 6	14 ± 4	4.2 ± 0.3	5.1 ± 0.9	21	nt
H_2	56	60	43	nt	nt	nt	nt
			IC_{50} (nM)				
NA-upt	1780 ± 100	1240 ± 70	2860 ± 630	22 ± 6	260 ± 99	nt	nt
5-HT-upt	2900 ± 100	1650 ± 80	>10000	2900 ± 340	>100	nt	nt

[a] Affinities of compounds were determined using competition binding assays to determine IC_{50} values at the various receptors. See text.

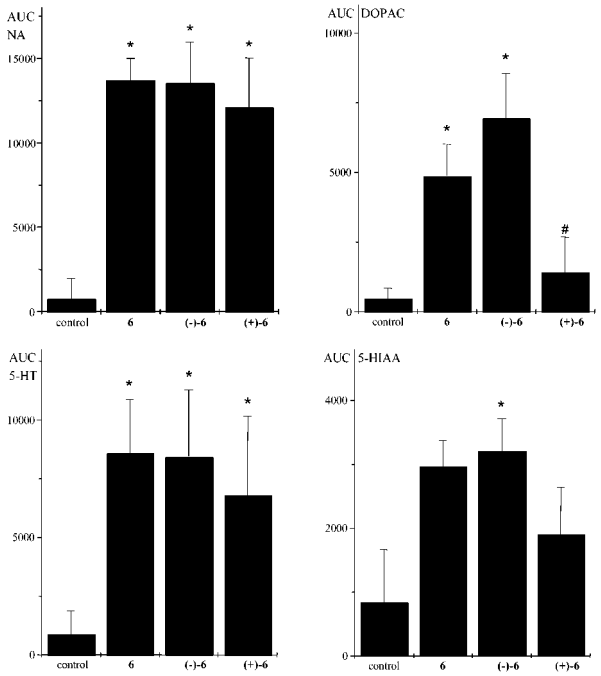


Figure 1. Effects of 6-methoxymianserin and its enantiomers on dopaminergic, noradrenergic, and serotonergic transmission. Effects were measured by on-line microdialysis of the racemic mixture **6** (5.0 mg/kg sc, $n = 4$) and the (*R*)-(−)-**6** and (*S*)-(+)-**6** enantiomers (each 2.5 mg/kg sc, $n = 6$) on NA, DOPAC, 5-HT, and 5-HIAA release in the rat ventral hippocampus. Results are expressed as the area under the curve and refer to cumulative percentage above baseline. Statistics are from Mann−Whitney rank sum test: (∗) $p < 0.05$ compared to control; (#) $p < 0.05$ compared to **2**.

thermore, it induces a concurrent, transient increase of 5-HT levels up to 200% above baseline at 60 min after administration of **6**, with stabilization at 140−150% further during the course of the experiment. Administration of (−)- or (+)-**6** (2.5 mg/kg sc) also resulted in an increase in NA levels up to 200% (45−150 min). The DOPAC levels were elevated to 160% and 130% above baseline for (−)- and (+)-**6**, respectively (60−150 min). Both enantiomers induced a transient, concurrent increase of the 5-HT release up to 200% above baseline at 60 min after administration of the test compound. The 5-HIAA release was elevated up to 120% after the administration of the racemic mixture or of either of the enantiomers (significant for the (−)-enantiomer). For a graphical representation of the microdialysis data, see Figure 1.

In summary, **6**, a new tetracyclic azepine, showed high affinity for the 5-HT2A/2C receptors, moderate affinity for the α1- and α2-adrenoceptors, and no affinity for the 5-HT3 receptor and for the NA reuptake sites. In vivo, it increases the levels of NA and 5-HT to the same extent as **1**. The (*R*)-(−)-enantiomer of **6** appears to be more potent in the microdialysis study than the (*S*)-(+)-enantiomer (significant only for the DOPAC release). This implies that at least the neurochemical effects of **6** are similar to those of the antidepressant **1**. Future studies in animal models of depression will have to prove whether **6** has prospective antidepressant properties. The unexpected finding that (*R*)-(−)-**6**, in vitro, shows moderate affinity for the D2 receptor subtype and a favorable "Meltzer ratio" compared to the atypical antipsychotic clozapine justifies future studies of this enantiomer for its potential as an antipsychotic agent.

Experimental Section

General. Melting points were determined with an electrothermal digital capillary melting point apparatus and are uncorrected. NMR spectra were recorded at 200 and 300 MHz on Varian Gemini and Varian VXR spectrometers, respectively. $CDCl_3$ was employed as the solvent unless otherwise stated. Chemical shifts are given in δ units (ppm) and relative to 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) data were recorded on a Unicam610 Automass 150 system (70 mV). HRMS spectra were performed in the Microanalytical Department of the University of Groningen (NL), and were recorded on a JEOL The 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 case of an aqueous workup, magnesium sulfate monohydrate was used as the drying agent. HPLC experiments were carried out using a semipreparative LC system consisting of a Chiralcel OD column: Daicel Chemical Industry Ltd. (Tokyo, Japan) 25 cm × 1.0 cm i.d., 10 μm particle size silica gel; ISCO 2300 Waters HPLC pump equipped with a Rheodyne injection valve and a 20 or 100 μL injection loop; ISCO V4 absorbance detector with a 5 μL flow cell ($\lambda = 250$ nm, pathway 5 mm); Kipp and Zonen BD 40 two channel recorder. The mobile phase used was *n*-hexane with 5% ethanol (both HPLC grade) and 0.1% triethylamine (analytical grade). Flow rate was set at 2.5−4.0 mL/min (ca. 0.15 psi). Optical rotations were measured in methanol ($c = 2.0$) at 21 °C on a Perklin-Elmer 241 polarimeter.

***β*-Hydroxy-*N*-methyl-*N*-hydroxyethylphenylethylamine (3).** 2-Methylaminoethanol (4.90 g, 65.3 mmol) was heated to 100 °C. To this, styrene oxide (5.30 g, 44.2 mmol) was added dropwise. The temperature was raised to 130 °C, and the mixture was stirred for 24 h, after which it was purified by flash chromatography (CH_2Cl_2 with 10% EtOH) yielding 7.5 g (57.5 mmol, 88%) of **3** as a light-yellow oil: ^{1}H NMR δ 7.40−7.16 (m, 5H), 4.80−0.4.73 (dd, $J_1 = 9.64$, $J_2 = 3.79$, 1H), 3.69−3.60 (m, 2H), 3.50 (broad peak, OH), 2.79−2.41 (m, 4H), 2.38 (s, 3H); ^{13}C NMR δ 142.2, 128.3, 127.5, 125.9, 70.4, 61.4, 59.3, 54.9, 42.3; MS (EIPI) *m/z* 195 (M^+).

***β*-Chloro-*N*-methyl-*N*-chloroethylphenylethylamine (4).** To an ice-cooled solution of **3** (1.30 g, 6.70 mmol) in $CHCl_3$ (20 mL) a solution of thionyl chloride (10 mL) in $CHCl_3$ (20 mL) was slowly added. The reaction mixture was refluxed for 2 h, after which it was quenched with H_2O (10 mL). The aqueous layer was basified with a 2 N aqueous NaOH solution and extracted with $CHCl_3$. The combined organic layers were washed with brine, dried, and concentrated in vacuo, yielding 1.00 g (4.49 mmol, 67%) of **4** as a brown oil. The intermediate was used without further purification: ^{1}H NMR δ 7.43−7.32 (m, 5H), 4.93 (t, $J = 7.08$, 1H), 3.48 (t, $J = 7.10$, 2H), 3.18−2.94 (m, 2H), 2.83 (t, $J = 7.05$, 2H), 2.38 (s, 3H); ^{13}C NMR δ 140.2, 128.6, 128.4, 127.3, 65.6, 60.8, 59.3, 42.6, 41.3; MS (EIPI) *m/z* 231 (M^+).

2-Amino-3-methoxybenzyl Alcohol. To an ice-cooled solution of 2-amino-3-methoxybenzoic acid (2.50 g, 15.0 mmol) in THF (150 mL) lithium aluminum hydride (2.30 g, 60.5 mmol) was added in small portions. The reaction mixture was stirred at room temperature for 17 h. The reaction was quenched subsequently with H_2O (2.3 mL), a solution of 10% aqueous NaOH (2.3 mL), and three more portions of H_2O (2.3 mL). The mixture was stirred for 2 h at room temperature after which the solvent was removed in vacuo. The residue was purified using flash chromatography (CH_2Cl_2 with 2.5% methanol), yielding 1.90 g (12.3 mmol, 82%) of 2-amino-3-methoxy-

benzyl alcohol (dark-brown solid): mp 38 °C; ^{1}H NMR δ 6.81−6.68 (m, 3H), 4.60 (s, 2H), 3.86 (s, 3H); ^{13}C NMR δ 147.5, 135.5, 121.2, 117.4, 110.2, 63.7, 55.7; IR (NaCl) 3367 cm^{-1} (OH); MS (EIPI) *m*/*z* 153 (M^+).

1-Methyl-3-phenyl-4-(2-methoxy-6-hydroxyethyl)phenylpiperazine (5). To a solution of 2-amino-3-methoxybenzyl alcohol (2.70 g, 11.6 mmol) in acetonitrile (20 mL) was added a solution of **4** (1.20 g, 7.80 mmol) in acetonitrile (10 mL). The reaction mixture was stirred at room temperature for 30 min. *N*,*N*-Diisopropylethylamine (1.00 g, 7.80 mmol) was added, and the reaction mixture was refluxed for 7 h, after which it was stirred at room temperature for 16 h. The formed hydrochloride salt was filtered and recrystallized from acetonitrile to give 1.80 g (5.77 mmol, 74%) of **5** as white crystals (HCl salt): mp 222 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 6.71−6.32 (m, 7H), 4.47 (s, 3H), 4.42−4.38 (m, 1H), 3.96−3.91 (m, 1H), 3.48 (m, 1H), 3.35 (s, 2H), 2.92−2.85 (m, 1H), 2.72−2.68 (m, 1H), 2.50−2.45 (m, 1H), 2.35−2.32 (m, 1H); ^{13}C NMR (CD_3OD) δ 157.8, 140.7, 137.0, 133.2, 128.0, 127.8, 127.5, 126.9, 119.6, 110.0, 60.8, 60.5, 59.2, 53.9, 53.8, 48.5, 42.2; MS (EIPI) *m*/*z* 312 (M^+).

1,2,3,4,10,14b-Hexahydro-6-methoxy-2-methyldibenzo[*c*,*f*]pyrazino[1,2-*a*]azepin (6). Compound **5** (1.30 g, 4.20 mmol) was suspended in polyphosphoric acid (14 g), and the mixture was stirred at a 100 °C for 2 h. The reaction was quenched with ice (140 mL), after which CH_2Cl_2 (140 mL) was added. The mixture was basified with 2 N aqueous NaOH. The organic layer was separated from the aqueous layer, which was subsequently extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated in a vacuum. The residue was purified by flash chromatography (CH_2Cl_2 with 5% EtOH), yielding 0.89 g (3.07 mmol, 73%) of **6** as a pinkish solid. Of the obtained product, 0.29 g was converted to the oxalate salt and recrystallized from ethanol, yielding 0.31 g (2.45 mmol, 80%) of **6** as off-white crystals; mp 205 °C (monooxalate salt); ^{1}H NMR (300 MHz, CD_3OD) δ 6.77−6.68 (m, 4H), 6.36−6.21 (m, 3H), 4.47 (s, 3H), 4.44−4.39 (m, 1H), 4.20−4.10 (m, 1H), 3.75−3.65 (m, 1H), 3.02−2.68 (m, 4H), 2.91 (s, 3H), 2.43 (s, 2H); ^{13}C NMR (CD3OD) δ 154.0, 143.3, 140.4, 137.0, 128.7, 127.8, 126.6, 126.1, 123.3, 118.6, 111.2, 63.4, 62.9, 55.8, 54.5, 50.9, 48.2, 47.8, 44.5, 38.2; MS (EIPI) *m*/*z* 294 (M^+); HRMS calc (obsd) for $C_{19}H_{22}N_2O$, 294.173 (294.172).

1,2,3,4,10,14b-Hexahydro-6-hydroxy-2-methyldibenzo[*c*,*f*]pyrazino[1,2-*a*]azepin (7). To a solution of **6** (300 mg, 1.02 mmol) in freshly distilled benzene (30 mL) aluminum trichloride (1.00 g, 7.59 mmol) was added. The reaction was refluxed for 48 h, after which the solvent was removed in a vacuum. The residue was purified by flash chromatography (CH_2Cl_2 with 5% ethanol basified with 25% NH_4OH(aq)), yielding 150 mg (0.53 mmol, 53%) of **7** as a white foam and 100 mg (33%) of starting material: ^{1}H NMR (300 MHz, CD_3OD) δ 6.89−6.28 (m, 7H), 4.69−4.23 (m, 3H), 3.44 (s, 3H), 3.32−2.84 (m, 4H), 2.58 (s, 2H); ^{13}C NMR δ 129.1, 127.9, 125.6, 118.9, 116.6, 63.0, 61.6, 56.3, 49.5, 44.4, 39.7; MS (EIPI) *m*/*z* 280 (M^+); HRMS calc (obsd) for $C_{18}H_{20}N_2O$, 280.158 (280.158).

1,2,3,4,10,14b-Hexahydro-2-methyl-6-trifluoromethanesulfonoxydibenzo[*c*,*f*]pyrazino[1,2-*a*]azepin (8). A solution of **7** (150 mg, 0.53 mmol) and triethylamine (1 mL) in CH_2Cl_2 (15 mL) was cooled to −78 °C, after which trifluoromethanesulfonic anhydride (90 μL) was added. The reaction mixture was subsequently stirred at room temperature for 4 h. The solvent was removed in a vacuum, and the residue was purified by flash chromatography (CH_2Cl_2 with 5% ethanol), yielding 220 mg (0.53 mmol, 100%) of **8** as a brown oil: ^{1}H NMR δ 7.28−6.82 (m, 7H), 4.72−4.48 (dd, $J_1 = 0.18$, $J_2 = 0.06$, 2H), 3.74 (t, $J = 0.05$, 1H), 3.49 (d, $J = 0.07$, 1H), 3.15 (d, $J = 0.06$, 1H), 2.94−2.73 (dd, $J_1 = 0.15$, $J_2 = 0.06$, 2H), 2.55−2.25 (m, 2H), 2.36 (s, 3H); ^{13}C NMR δ 128.2, 126.5, 126.1, 125.7, 125.2, 121.1, 119.6, 57.9, 54.5, 50.1, 44.5, 37.7; MS (EIPI) *m*/*z* 412 (M^+); HRMS calc (obsd) for $C_{19}H_{19}N_2O_3F_3S$, 412.107 (412.108).

Chromatographic (HPLC) Resolution of Racemic 6 on a Chiral Stationary Phase. The semipreparative LC system consisted of a Chiralcel OD column: Daicel Chemical Industry Ltd. (Tokyo, Japan) 25 cm × 1.0 cm i.d., 10 μm particle size silica gel; ISCO 2300 HPLC pump equipped with a Rheodyne injection valve and 1 mL injection loop; ISCO V4 absorbance detector with a 5 μL flow cell (λ = 250 nm, pathway 5 mm); Kipp and Zonen BD 40 two-channel recorder; mobile phase, *n*-hexane with 5% ethanol (HPLC grade) and 0.1% triethylamine (analytical grade); flow rate of 4.0 mL/min at ca. 0.5 psi. The favorable separation of the two enantiomers allowed for injecting 5 mg of racemic **6** per run. The direct separation by means of HPLC using a Chiralcel OD column afforded 50 mg each of the base form of the enantiomers (−)-**6** ([α]$^{21}_D$ −403.0°) and (+)-**6** ([α]$^{21}_D$ +407.6°), both rotations measured at *c* = 2.0 mg/mL MeOH.

Indirect Determination of the Absolute Configuration of (−)-6. O-Demethylation of an analytical amount of (−)-**6** (20 mg) was effected, as descibed for the preparation of racemic **7** above, upon treatment with 6 equiv of $AlCl_3$ in freshly distilled, refluxing benzene, providing (−)-6-hydroxymianserin ((−)-**7**), which was triflated without further purification with trifluoromethanesulfonyl anhydride in CH_2Cl_2 and in the presence of triethylamine. This yielded 6-trifluoromethanesulfonyloxymianserin ((−)-**8**), which was detriflated, without further purification, with palladium acetate in refluxing methanol in the presence of triphenylphospine and triethylamine to afford, after purification, 10 mg of (−)-**2** (Scheme 2), as determined by measuring the *k*′, which under the present condition was measured to be 0.77, which is the same as that measured for an authentic sample of (−)-**2** ((+)-**2** shows a *k*′ = 1.00).[19]

Biological Assays. For the microdialysis experiments male Wistar rats (280−350 g) were used. 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 6:30−18:30) until surgery. Food and water were available ad libitum at all times. Testing was done between 10:00 and 18:00 during the light phase of the day−night cycle. Procedures were conducted in accordance with guidelines published in the NIH Guide for the Care and Use of Laboratory Animals, and protocols were approved by the Groningen University Institutional Animal Care and Use Committee.

Affinities of compounds were determined using competition binding assays to determine IC_{50} values at the various receptors: dopamine D2 receptors, displacement of 0.50 nM [3H]-spiperone at membranes from rat corpus striatum;[20] h-dopamine D3 receptor, displacement of 0.30 nM [3H]-spiperone at human-cloned D3 receptors expressed in CHO-cells;[21] h-dopamine D4.2 receptor, displacement of 0.06 nM [3H]-YM-09151-2 at human-cloned D4.2 receptors expressed in CHO-cells (method modified from NEN Life Science Products, Inc., technical data certificate PC2533-10/96); h-serotonin 5-HT1A receptor, displacement of 2 nM [3H]-5-CT at human-cloned 5-HT1A receptors expressed in HeLa cells.[22] Additional parameters are as follows: serotonin 5-HT1D receptor, displacement of 2 nM [3H]-5-CT at membranes from bovine caudate tissue;[23] serotonin 5-HT2A receptor, displacement of 0.50 nM [3H]-ketanserin at membranes from rat cortex tissue;[24] serotonin 5-HT2C receptor, displacement of 0.50 nM [3H]-mesulergine from rat cloned 5-HT2C receptors expressed in NIH/3T3 cells (method available from H. Lundbeck A/S, Copenhagen); serotonin 5-HT3 receptor, displacement of [3H]-LY-278584 at membranes of rat brain tissue;[25] α1-adrenoceptor, displacement of 0.25 nM [3H]-prazosin at membranes of rat brain tissue;[20] α2-adrenoceptor, displacement of 0.50 nM [3H]-RX-821002 at membranes of rat brain tissue;[26] histamine H1 receptor, displacement of 1.0 nM [3H]-pyrilamine at membranes of guinea pig lung tissue;[27] histamine H2 receptor, displacement of 0.10 nM [3H]-APT at membranes of guinea pig striatum tissue.[28] Inhibition of 5-HT and NA uptake into rat brain synaptasomes was described by K. P. Bøgesø.[29] Data calculations are as follows: Results on receptor binding studies are given as K_i values (nM). Two complete concentration−response curves were determined by using five concentrations of the test drugs in triplicate (covering 3 decades). IC_{50} values

were estimated from hand-drawn log−logit analysis. In a series of *n* determinations, the variance of the log ratio (VARR) between the double determinations is determined according to the formula VARR = Σ(log R1)2/(2*n*), where R1 is the ratio and *n* is the number of observations. The VARR is equivalent to the square of the standard deviation of the log ratio (SDR2). In case the ratio is greater than that corresponding to 2 × SDR (95% confidence interval), further determinations were performed and outliers discarded. For the binding analysis, the following SDRs were obtained: D2, 1.5 ($n = 100$); D3, 1.4 ($n = 26$); D4.2, 1.4 ($n = 26$); 5-HT1A, 1.5 ($n = 24$); 5-HT1D, see ref 23; 5-HT2A, 1.4 ($n = 100$); 5-HT2C, 1.3 ($n = 24$); α1, 1.5 ($n = 100$), see ref 26; H1, see ref 27; H2, see ref 28; 5-HT and NA uptake, 95% confidence ratios of 2.04 and 2.68, respectively.[3]

Microdialysis[30] rats were anaesthetized with ketamine/xylazine (50/8 mg/kg ip) and atropine (0.1 mg/kg sc) after a premedication of midazolam (5 mg/kg sc). Preceding insertion of the microdialysis probe, rats were placed in a stereotaxic frame (Kopf). Following local lidocaine application (10% m/v), a homemade I-shaped probe, made of polyacrylonitrile/sodium methyl sulfonate copolymer dialysis fiber (i.d. 0.22 mm, o.d. 0.31 mm, AN 69, Hospal, Italy) was implanted in the ventral hippocampus (coordinates: (IA) +3.7 mm; (lateral) +4.8 mm; (ventral) −8.0 mm from the Dura Mater, Paxinos and Watson, 1982) and secured with stainless steel screws and dental cement. The exposed length of the membrane was 4 mm. After surgery, rats were allowed a recovery period of at least 24 h before starting the experiments. Probes were perfused with artificial CSF containing 147 mM NaCl, 3.0 mM KCl, 1.2 mM $CaCl_2$, and 1.2 mM $MgCl_2$, at a speed of 1.5 μL/min (Harvard apparatus, South Natick, MA). Rats were housed individually in Plexiglas cages (25 cm × 25 cm × 35 cm) with food and water ad libitum. Samples were collected on-line in a 20 μL loop and injected automatically onto the column every 15 min. 5-HT was analyzed using an HPLC pump (Shimadzu LC-10 AD liquid chromatograph) in combination with a reversed-phase column (Phenomenex hypersil 3 C18, 100 mm × 2.0 mm, 3 μm, Bester, Amstelveen, The Netherlands) and an EC detector with a carbon electrode (Intro, Antec Leyden, Leiden, The Netherlands) working at a potential of 500 mV vs Ag/AgCl reference electrode. The mobile phase consisted of 5.0 g/L $(NH_4)_2SO_4$, 0.5 g/L EDTA, 50.0 mg/L heptanesulfonic acid, and 30 μL/L triethylamine in 4.5% methanol (v/v) at pH 4.65 and 30 °C. The flow of the mobile phase was set at 400 μL/min. The detection limit of 5-HT was 0.5 fmol per 20 μL of sample (signal-to-noise ratio of 2). NA, DOPAC, and HIAA were analyzed using an HPLC pump (Shimadzu LC-10 AD liquid chromatograph) in combination with a reversed-phase column (C18, 15 mm × 0.46 mm, 3 m, Supelco, Zwijndrecht, The Netherlands). Quantification was performed using an electrochemical detector (ESA, Bedford, MA). Upon oxidation at 175 mV, molecules were reduced at −250 mV. The mobile phase consisted of 4.1 g/L sodiumacetate, 140.0 mg/L octanesulfonic acid, 50.0 mg/L EDTA, and 7% methanol (v/v (pH = 4.4.). At a flow of 1 mL/min, the detection limit of the assay was 2 fmol/sample (signal-to-noise ratio of 2). Four consecutive microdialysis samples with less than 20% difference were considered as control and set at 100%. Data are presented as percentages of control level (mean + SEM). Statistical analysis was performed using Sigmastat for Windows (Jandel Corporation). Treatment values were compared using two-way ANOVA for repeated measurements, followed by Student Newman−Keuls posthoc analysis. For the area under the curves, a Mann−Whitney rank sum test was performed. Significance levels were set at $p < 0.05$.

References

(1) Schildkraut, J. J. The catecholamine hypothesis of affective disorders. A review of supporting evidence. *Am. J. Psychiatry* **1965**, *122*, 509−522.

(2) Blier, P.; De Montigny, C. D.; Chaput, Y. A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J. Clin. Psychopharmacol.* **1990**, *10*, S77−S85.

(3) De Boer, T.; Nefkens, F.; Van Helvoirt, A. The α2-antagonist Org 3770 enhances serotonin transmission in vivo. *Eur. J. Pharmacol.* **1994**, *253*, R5−R6.

(4) De Boer, T.; Nefkens, F.; Van Helvoirt, A.; Van Delft, A. M. L. Differences in modulation of noradrenergic and serotonergic transmission by the alpha-2 adrenoceptor antagonists, mirtazapine, mianserin and idazoxan. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 852−860.

(5) Haddjeri, N.; Blier, P.; De Montigny, C. Effect of the alpha-2 adrenoceptor antagonist mirtazapine on the 5-hydroxytryptamine system in the rat brain. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 861−871.

(6) De Boer, Th. The pharmacologic profile of mirtazapine. *J. Clin. Psychiatry* **1996**, *57*, 19−25.

(7) De Boer, T.; Maura, G.; Raiteri, M.; De Vos, C. J.; Wieringa, J.; Pinder, R. M. Neurochemical and autonomic profiles of the 6-aza-analogue of mianserin, Org 3770 and its enantiomers. *Neuropharmacology* **1988**, *27*, 399−408.

(8) Kooyman, A. R.; Zwart, R.; Vanderheyden, P. M. L. Interaction between enantiomers of mianserin and Org 3770 at 5-HT3 receptors in cultured mouse neuroblastoma cells. *Neuropharmacology* **1994**, *33*, 501−510.

(9) Nickolson, V. J.; Wieringa, J. H. Presynaptic α-block and inhibition of noradrenaline and 5-hydroxytryptamine reuptake by a series of compounds related to mianserin. *J. Pharm. Pharmacol.* **1981**, *33*, 760−766.

(10) Wood, M. D.; Thomas, D. R. Watkins, C. J.; Newberry, N. R. Stereoselective interaction of mianserin with 5-HT3 receptors. *J. Pharm. Pharmacol.* **1993**, *45*, 711−714.

(11) Pinder, R. M.; Van Delft, A. M. L. The potential therapeutic role of the enantiomers and metabolites of mianserin. *Br. J. Clin. Pharmacol.* **1983**, *15*, 269S−276S.

(12) Fletcher, A.; Bill, D. J.; Cliffe, I. A.; Foster, E. A.; Jones, D.; Reilly, Y. A pharmacological profile of WAY-100635, a potent and selective 5-HT-1A receptor antagonist. *Br. J. Pharmacol.* **1993**, *112*, 91P.

(13) Clitherow, J. W.; Scopes, D. I. C.; Skingle, M.; Jordan, C. C.; Feniuk, W.; Campbell, I. B.; Carter, M. C.; Collington, E. W.; Conner, H. E.; Higgins, G. A.; Beattie, D.; Kelly, H. A.; Mitchell, W. L.; Oxford, A. W.; Wadsworth, A. H.; Tyers, M. B. Evolution of a novel series of [(*N*,*N*-dimethylamino)propyl]- and piperazinylbenzanilides as the first selective 5-HT1D antagonists. *J. Med. Chem.* **1994**, *37*, 2252−2257.

(14) Blier, P.; Pineyro, G.; el Mansari, M.; Bergeron, R.; De Montigny, C. Role of somatodendritic 5-HT autoreceptors in modulating 5-HT neurotransmission (review). *Ann.* N.Y. *Acad. Sci.* **1998**, *861*, 204−216.

(15) Hartig, P. R.; Hoyer, D.; Humphrey, P. P. A.; Martin, G. R. Alignment of receptor nomenclature with the human genome: classification of 5-HT1B and 5-HT1D receptor subtypes (review). *Trends Pharmacol. Sci.* **1996**, *17*, 103−105. Pauwels, P. J. 5-HT1B/1D antagonists. *Gen. Pharmacol.* **1997**, *29*, 293−303.

(16) Vahabzadeh, A.; Fillenz, M. Studies on the origin of rat hippocampal dihydroxyphenylacetic acid using microdialysis. *Neurosci. Lett.* **1992**, *136*, 51−55.

(17) Ortemann, C.; Robert, F.; Renaud, B.; Lambás-Señas, L. In vivo microdialysis study of the extracellular 3,4-dihydroxyphenylacetic acid in the rat locus ceruleus: topographical and pharmacological aspects. *J. Neurochem.* **1993**, *61*, 594−601.

(18) Selditz, U.; Liao, Y.; Franke, J. P.; De Zeeuw, R. A.; Wikström, H. Direct enantiomeric separation of mianserin and 6-azamianserin derivatives using chiral stationary phases. *J. Chromatogr. A* **1998**, *803* (1−2), 169−177.

(19) Van Rij, C.; Feil, D. The molecular and crystal structure of (+)-mianserin hydrobromide. *Tetrahedron Lett.* **1973**, *29*, 1891−1893.

(20) Hyttel, J.; Larsen, J. J. Neurochemical profile of Lu 19-005, a potent inhibitor of uptake of dopamine, noradrenaline and serotonin. *J. Neurochem.* **1985**, *4*, 1615−1622.

(21) MacKenzie, R. G.; VanLeeuwen, D.; Pugsley, T. A.; Shih, Y. H.; Demattos, S.; Todd, R. D.; O'Malley, K. L. Characterization of the human dopamine D3 receptor expressed in transfected cell lines. *Eur. J. Pharmacol.* **1994**, *266*, 79−85.

(22) Harrington, M. A.; Shaw, K.; Zong, P.; Ciaranello, R. D. Agonist-induced desensitization and loss of high-affinity binding sites of stably expressed human 5-HT1A receptors. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 1098−1106.

(23) Heuring, R. E.; Peroutka, S. J. Characterization of a novel 3H-5-hydrosytrytamine binding site subtype in bovine brain membranes. *J. Neurosci.* **1987**, *7*, 894−903.

(24) Sánchez, C.; Arnt, J.; Dragsted, N.; Hyttel, J.; Love-Lembol, H.; Meier, E.; Perregaard, J.; Skarsfelt, T. Neurochemical and in vivo pharmacological profile of sertindole, a limbic selective neuroleptic compound. *Drug Dev. Res.* **1991**, *22*, 239−250.

(25) Wong, D. T.; Robertson, D. W.; Reid, L. R. Specific [3H]LY278584 binding to 5-HT3 recognition sites in rat cerebral cortex. *Eur. J. Pharmacol.* **1989**, *166*, 107−110.

(26) Uhlen, S.; Wikberg, J. E. Rat spinal cord alpha 2-adrenoceptors are of the alpha 2A-subtype: comparison with alpha 2A- and alpha 2B-adrenoceptors in rat spleen, cerebral cortex and kidney using 3H-RX821002 ligand binding. *Pharmacol. Toxicol*. **1991**, *69*, 341−350.
(27) Dini, S.; Caselli, G. F.; Ferrari, M. P.; Giani, R.; Clavenna, G. Heterogeneity of [3H]-mepyramine binding sites in guinea pig cerebellum and lung. *Agents Actions* **1991**, *33*, 181−184.
(28) Ruat, M.; Traiffort, E.; Bouthenet, M. L.; Schwartz, J. C.; Hirschfeld, J.; Buschauer, A.; Schunack, W. Reversible and irreversible labeling and autoradiographic localization of the cerebral histamine H2 receptor using [^{125}I]iodinated probes. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1658−1662.
(29) Bøgesø, K. P. Neuroleptic activity and dopamine-uptake inhibition in 1-piperazino-3-phenylindans. *J. Med. Chem*. **1983**, *26*, 935−947.
(30) Santiago, M.; Westerink, B. H. C. Characterization of the in vivo release of dopamine as recorded by different types of intracerebral microdialysis probes. *Naunyn-Schmiedeberg's Arch. Pharmacol*. **1990**, *342*, 407−414.
(31) Meltzer, H.; Matsubara, S.; Lee, J. C. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin-2 p*K*i values. *J. Pharmacol. Exp. Ther*. **1989**, *251*, 238−246.
(32) Liao, Y.; Venhuis, B. J.; Rodenhuis, N.; Timmerman, W.; Wikström, H.; Meier, E.; Bartoszyk, G. D.; Böttcher, H.; Seyfried, C. A.; Sundell, S. New (sulfonyloxy)piperazinyl dibenzazepines as potential atypical antipsychotics: chemistry and pharmacological evaluation. *J. Med. Chem*. **1999**, *42*, 2235−2244.
(33) Hertel, P.; Fagerquist, M. V.; Svensson, T. H. Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by α2 adrenoceptor blockade. *Science* **1999**, *286*, 105−107.

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