Articles

New Antipsychotic Agents with Serotonin and Dopamine Antagonist Properties Based on a Pyrrolo[2,1-*b*][1,3]benzothiazepine Structure

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The development of a synthetic approach to the novel pyrrolo[2,1-*b*][1,3]benzothiazepine and its derivatives and their biological evaluation as potential antipsychotic drugs are described. In binding studies these compounds proved to be potent 5-HT_2, D_2, and D_3 receptor ligands. The more potent benzothiazepine (±)-**3b** was resolved into its enantiomers by using HPLC techniques. In vitro testing confirmed that (−)-**3b** is a more potent D_2 receptor ligand, maintaining high affinity for 5-HT_2 receptors. In contrast, the (+)-**3b** enantiomer presents a 35 times higher affinity for 5-HT_2 than for dopamine D_2 receptors with a similar dopamine D_1 receptor affinity to that of (−)-**3b**. Overall, (+)-**3b** shows an "atypical" neuroleptic binding profile, while (−)-**3b** has a more "classical" profile. Furthermore pharmacological and biochemical testing displayed that the novel benzothiazepine (±)-**3b** is able to increase the extracellular levels of dopamine in the rat striatum and causes a dose-related suppression of apomorphine-induced locomotor activity. At low doses (±)-**3b** does not induce catalepsy, showing atypical antipsychotic properties similar to those of olanzapine. These heterocyclic compouds represent new leads for the development of novel antipsychotic drugs with atypical properties.

Introduction

The involvement of dopamine (DA) and dopaminergic neurons in the pathology of a number of psychiatric and neurological disorders has been well-documented,[1] and consequently there is considerable interest in the development of potent DA receptor agonists and antagonists. For schizophrenia, a devastating mental disorder, a dopamine hypothesis[2] has been formulated stating that the classical neuroleptic drugs alleviate the positive symptoms of the disease by blocking the dopamine neurotransmission in certain brain areas. For decades, neuroleptic drugs have been established as the treatment of choice for acute and chronic schizophrenia. Chlorpromazine and haloperidol are representative of these classical (typical) antipsychotics. More recently, several clinical studies have demostrated that 5-HT_2 antagonists could improve the negative symptoms of schizophrenia, and coadministration of 5-HT_2 antagonists to "typical" antipsychotics reduced the incidence of extrapyramidal symptoms compared to treatment with neuroleptics alone.[3,4] The first antipsychotic to show a remarkably different clinical profile was clozapine (**1**) (Chart 1), a drug that antagonizes dopamine at D_2 receptors and serotonin at 5-HT_2 receptors. The intriguing profile of this compound has been called "atypical", and now clozapine represents the standard neuroleptic to which the new antipsychotic agents are compared.[5] Clinical experience suggests that clozapine is more effective than classical neuroleptic drugs in the

Chart 1

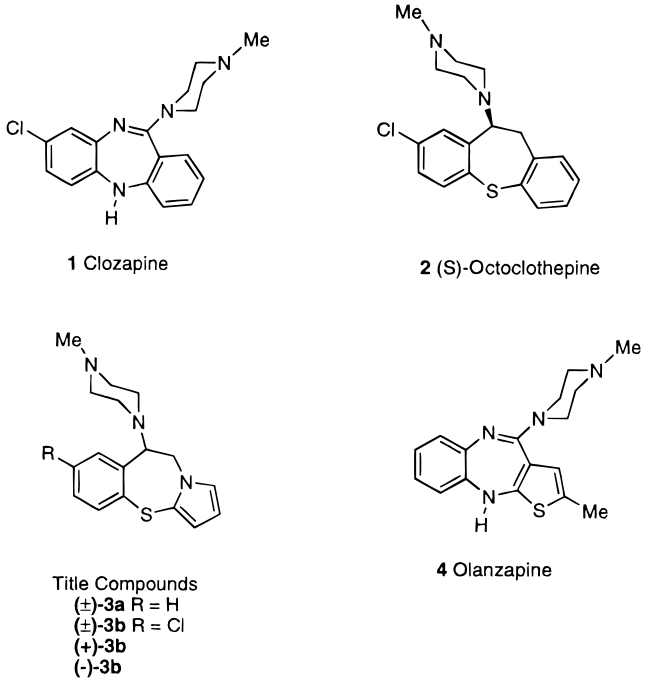

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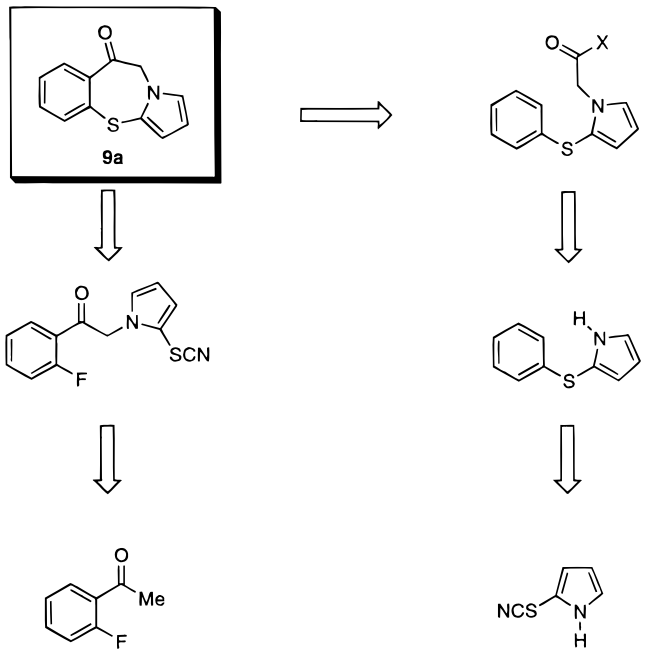
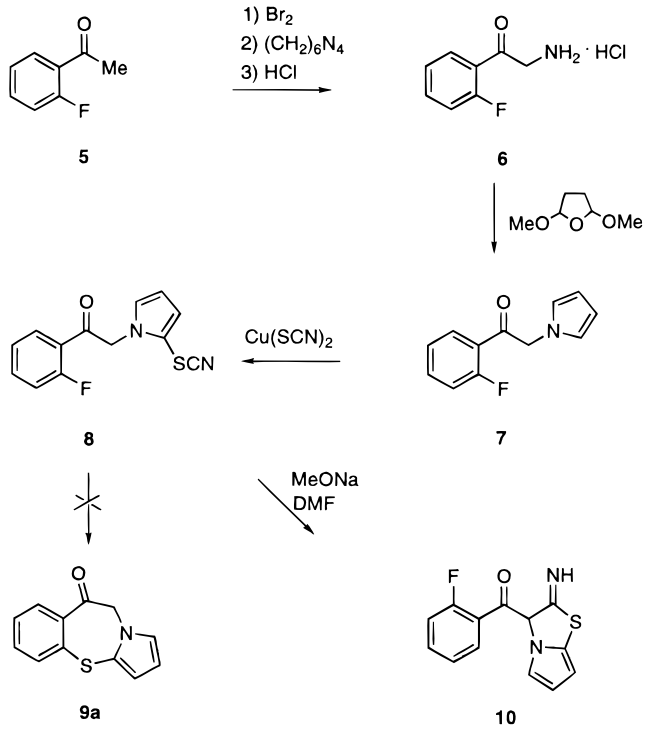
Chart 2. Retrosynthetic Analyses of Compound **9a** Based on an Intramolecular Nucleophilic Displacement of an Aromatic Fluorine Atom and on an Intramolecular Acylation



treatment of schizophrenic patients with positive and negative symptoms. Clozapine does not produce extrapyramidal motor disturbances (EPS), it does not elevate prolactin serum levels, and it has no cataleptic potential. Its "atypical" antipsychotic profile has been explained on the basis of the dopamine/serotonin hypothesis:[3] it has been proposed that the blockade of D_2 and 5-HT_2 receptors, together with the enhancement of dopamine and serotonin release in cortical regions, is a critical element in the action of clozapine. Unfortunately, the occurrence of agranulocytosis in a few percentage of patients during treatment with clozapine has limited its therapeutic usefulness.[6] (*S*)-Octoclothepine (**2**) and (1*S*,3*R*)-tefludazine are neuroleptic drugs with potent antagonist activity on D_2 receptors (higher than that of clozapine) and high affinity for 5-HT_2 receptors. These two compounds have been used to define the spatial relationships of the pharmacophore elements through the superimposition of their active conformations, and these spatial relationships represent a new D_2 receptor interaction model (an aromatic ring, an ionizable nitrogen, and a dummy atom at 2.8 Å from N in the direction of its lone pair).[7a,b] Clozapine matches this model,[8] although the carbon atom containing chlorine is part of the 5-HT_2 pharmacophore proposed by Andersen in 1994.[9] The biochemical and behavioral activity of the two enantiomers of octoclothepine have recently been reinvestigated,[7b] and while the (*S*)-enantiomer was found to possess a classical neuroleptic profile, the (*R*)-enantiomer showed a more "atypical" profile. This fact prompted us to develop a project aimed at identifying novel antipsychotic compounds based on a tricyclic skeleton and related to octoclothepine. In particular, the structure of octoclothepine was modified by replacing a benzo-fused ring with a pyrrole, in an attempt to improve its pharmacological profile.

Scheme 1



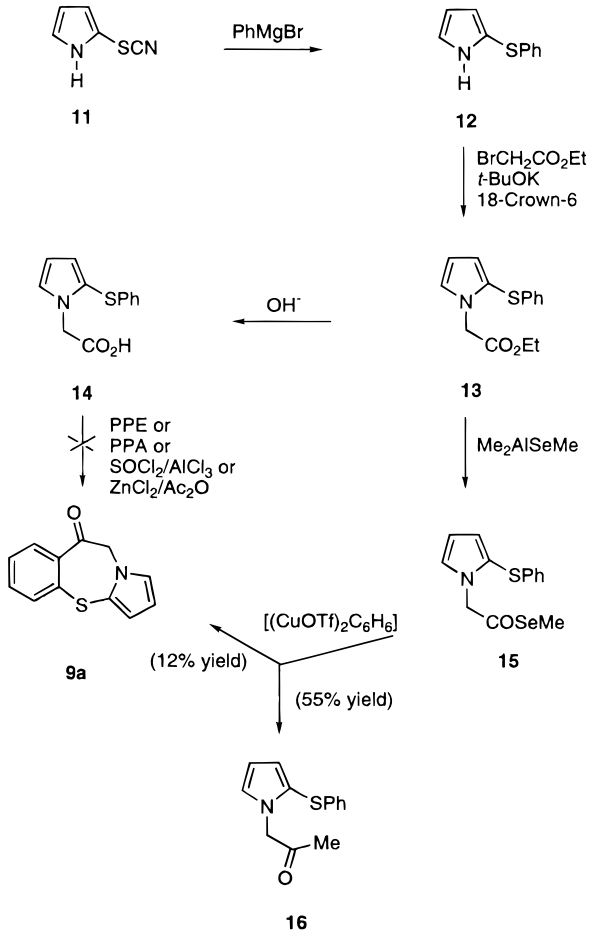
In this work we present the synthesis, the resolution, and the pharmacological characterization of potential antipsychotic agents (**3**) with clozapine- and olanzapine-like properties, based on the novel pyrrolo[2,1-*b*][1,3]-benzothiazepine skeleton. The common features with other tricyclic antipsychotic drugs, such as octoclothepine, clozapine, and olanzapine (**4**), are the butterfly-like conformation of the lipophilic tricyclic system and the presence of the basic side chain.

Chemistry

The synthesis of the novel pyrrolo[2,1-*b*][1,3]benzothiazepine skeleton has been attempted following three retrosynthetic approaches described in Charts 2 and 3 and in Schemes 1−4. The main task to be accomplished in this synthesis is the discovery of a cyclization method to obtain the pyrrolobenzothiazepinone intermediates **9a,b**. Chart 2 reports our retrosynthetic analyses of compound **9a** based on the nucleophilic displacement of an aromatic fluorine atom[10] and on an intramolecular acylation.[11a,b] In the first attempt the 2-(fluorophenyl)-ethanone could be transformed in the thiocyanatopyrrole derivative, the thioenolate of which could displace the fluorine atom, leading to **9a**. As shown in Scheme 1, after bromination and Delepine reaction[12] (**6**), followed by Clauson−Kaas reaction,[13] the 2-fluoroacetophenone **5** was transformed into the pyrrole derivative **7**. This compound was then treated with cupric thiocyanate[14] to give the 2-thiocyanatopyrrole **8**, and the latter, after exposure to sodium methoxide or potassium *tert*-butoxide,[14] did not provide the expected tricyclic compound **9a**, but the "anomalous" novel bicyclic pyrrolo[2,1-*b*]thiazole derivative **10**, resulting from attack at the thiocyanato group by the strong nucleophile generated in situ by removal of hydrogen from the α-position to the keto group of **8**.

Following a different strategy (Scheme 2), when pyrrole was treated with cupric thiocyanate in metha-

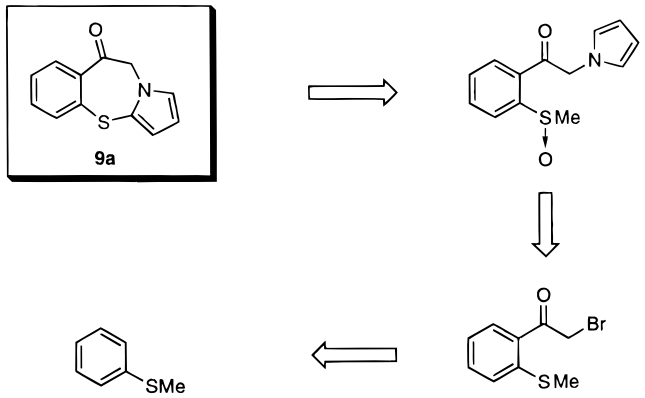
Scheme 2



nol, thiocyanation took place in a few minutes obtaining the 2-thiocyanatopyrrole **11** in good yield.[15a] Grignard reaction with phenylmagnesium bromide (**12**)[15b] followed by alkylation with ethyl bromoacetate provided the ester **13** in high overall yield. Saponification gave the acid **14**, and although different cyclization protocols were attempted (Friedel−Crafts reactions or PPA/PPE-promoted cyclizations), it was impossible to obtain the desired compound **9a**. On the contrary, the ester **13** could serve as starting material to attempt another cyclization method to **9a**, based on a copper(I)-promoted acylation reaction. In fact, dimethylaluminum methylselenolate was used to obtain the acyl-transfer agent **15**.[11a] This selenoester can, in analogy to thioesters, be used in a carbon−carbon bond-forming reaction. Accordingly, by exposure of **15** to the highly reactive crystalline complex of copper(I) triflate and benzene [$(CuOTf)_2PhH$], the tricyclic **9a** in 12% yield[11b] was obtained together with the ketone **16** (55% yield) and other unidentified byproducts.

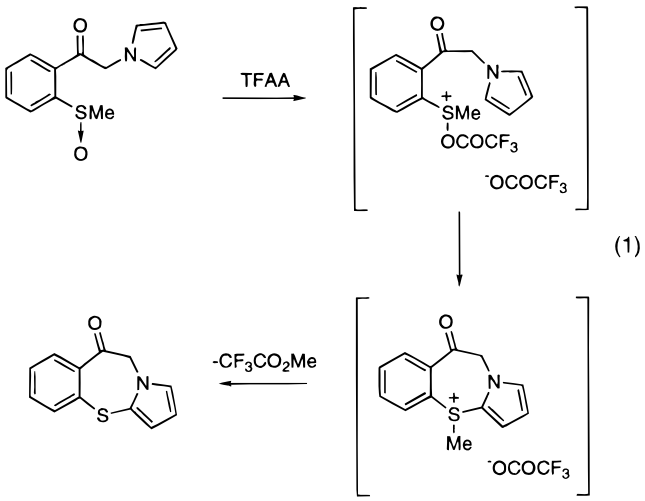
The low yield and the difficulties found in handling the highly reactive copper reagent prompted us to develop another synthetic route to **9a,b**. In Chart 3 is described the synthetic approach to **9a** based on a thionium ion intermediate.[16] Accordingly, the key tricyclic intermediates **9a,b** could be prepared under Pummerer rearrangement conditions, starting from sulfoxides, in turn prepared from 1-[2-(methylthio)-phenyl]ethanones. The key intermediates **21a,b**[17a] were prepared starting by bromination[17b] of the corresponding phenylethanones **18**[18] and **20**, in turn prepared by

Chart 3. Retrosynthetic Analysis of Compound **9a** Based on a Thionium Ion Intermediate



the interaction of methyllithium and the lithium salt of 2-(methylthio)benzoic acid (**17**), or from 1-chloro-4-(methylthio)benzene (**19**), by a Friedel−Crafts reaction with acetic anhydride, respectively (Scheme 3).

Subsequently, the bromophenylethanones **21a,b** were transformed into the pyrrole derivatives **22a,b** by using the methodology described for compoud **7**. Oxidation with sodium periodate (**23a,b**) followed by exposure of these sulfoxides to trifluoroacetic anhydride provided the ketones **9a,b** in 40% yield (Scheme 4).[16]

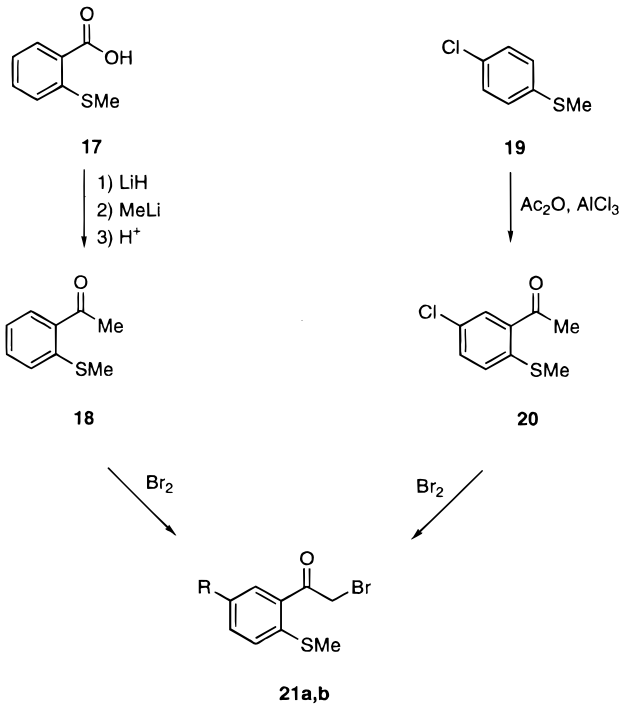


The proposed mechanism of the cyclization step is reported in eq 1. The "interrupted" Pummerer rearrangement begins with the activation of the sulfoxide oxygen followed by the attack of the pyrrole ring on sulfur displacing the trifluoroacetate ion. Then the sulfonium salt undergoes displacement of the methyl group generating the novel heterocyclic system and methyl trifluoroacetate. Starting from ketones **9a,b** the piperazine ring was introduced following a standard method.[19] Accordingly, reduction of ketones **9a,b** provided the alcohols (±)-**24a,b** which were transformed into the bromo derivatives (±)-**25a,b** by means of PBr_3. By treatment of (±)-**25a,b** with *N*-methylpiperazine, the final products (±)-**3a,b** were obtained. The thiazepine (±)-**3b** was resolved by HPLC into the enantiomers (+)-**3b** and (−)-**3b** using a Chiralpak AD Amylose column.

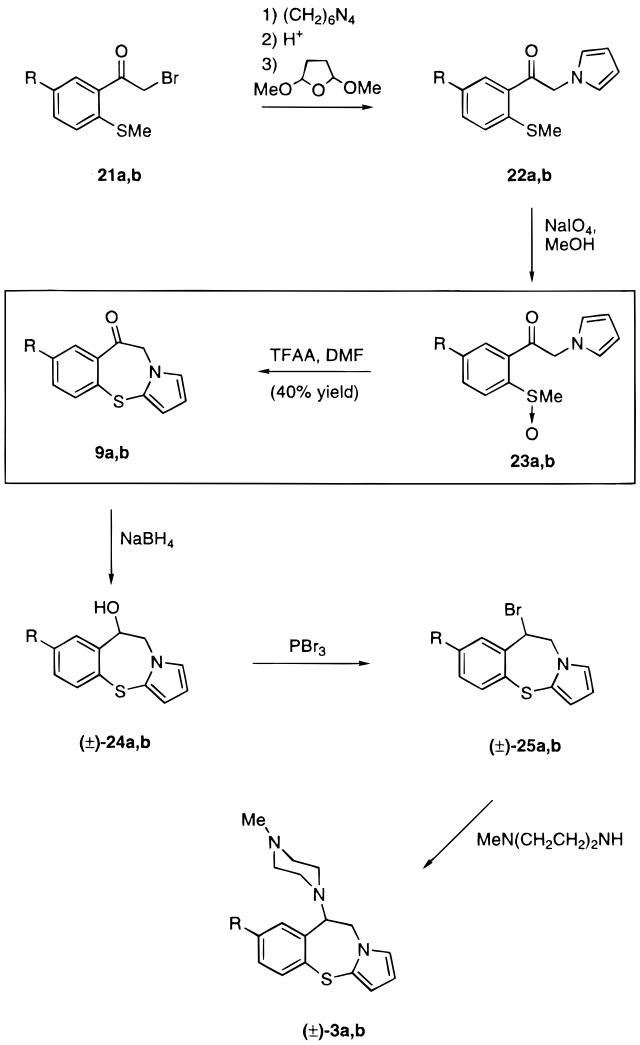
Results and Discussion

Pharmacological Studies. The binding affinities for 5-HT_2, D_1, D_2, and D_3 receptors and the comparison

Scheme 3



Scheme 4



of pK_i's of (±)-**3a,b**, (+)-**3b**, (−)-**3b**, clozapine, olanzapine, tested in the same experimental conditions, and methiothepine are shown in Tables 1 and 2, while Table 3 summarizes the effect of (±)-**3b** on apomorphine-induced locomotor hyperactivity (APO-induced LMA) and catalepsy, compared to the effects of olanzapine, an "atypical" antipsychotic. Figures 1 and 2 display the ability of (±)-**3b**, compared to olanzapine, to increase the release of dopamine and DOPAC from the rat brain striatum.

1. Binding Assays. Binding studies performed on **(±)-3a,b** show that a chlorine atom is critical for the potency of binding to serotonin and dopamine receptors. The chloro-substituted compound (±)-**3b** displays a higher affinity for 5-HT_2, D_2, and D_3 receptors than (±)-**3a** and a weak affinity for D_1 receptors (autoradiography results for D_4 receptors for compounds **3a,b** were negative, see the Experimental Section[20]). Furthermore (±)-**3b** shows a low affinity for the dopamine uptake site, with an IC_{50} of 6500 nM. Compound (±)-**3b**, the most active at D_2 and 5-HT_2 receptors, has been resolved, and the two enantiomers show a different binding profile (Table 1). Despite (+)- and (−)-octoclothepine that present a similar binding profile at 5-HT_2 and D_2 receptors, with (−)-octoclothepine being 5 times less potent at the D_2 receptor,[7b] the (+)-enantiomer of **3b** is 25 times less potent at the D_2 receptor than the (−)-enantiomer, confirming a rather stereoselective interaction of **3b** enantiomers at the D_2 receptor. At D_1 and 5-HT_2 receptors we find that the enantiomers have almost identical affinity, (+)-**3b** being slightly more potent than (−)-**3b**.

Classification of atypical and typical antipsychotic drugs could be done on the basis of D_1, D_2, and 5-HT_2 pK_i values. According to this hypothesis[3] the neuroleptics with a 5-HT_2 versus D_2 affinity ratio (pK_i values) greater than 1 would have the possibility to show atypical antipsychotic properties. In Table 2 the affinity ratios of typical and atypical antipsychotics, such as methiothepine and clozapine and olanzapine, respectively, are compared to those of compounds (±)-**3a,b**, (+)-**3b**, and (−)-**3b**. Accordingly, from the comparison of the pK_i data and of the log Y score[3] of (±)-**3a,b**, (+)-**3b**, and (−)-**3b** with those of reference compounds, we could predict (i) an atypical profile for compound (±)-**3a** (log $Y = 4.98$), which resembles the binding charac-

Table 1. Binding Affinities for 5-HT_2, D_1, D_2, and D_3 Receptors of Compounds (±)-**3a,b**, (+)-**3b**, and (−)-**3b**

	K_i (nM)[a] (±SEM)			
compd	5-HT_2	D_1	D_2	D_3
(±)-**3a**	7.85 ± 2.2	160 ± 77	70 ± 17	57 ± 6.2
(±)-**3b**	1.14 ± 0.12	27 ± 10	3.8 ± 0.5	4.1 ± 1.5
(+)-**3b**	1.48 ± 0.2	16.4 ± 1.0	49.6 ± 6.0	NT[b]
(−)-**3b**	1.72 ± 0.25	22.0 ± 1.3	2.06 ± 0.2	NT[b]
clozapine	11.0 ± 1	353 ± 35	250 ± 57	NT[b]
olanzapine	12.0 ± 1	85 ± 3.5	69 ±17	NT[b]
methysergide	5.3 ± 0.8			
(−)-*cis*-flupentixol		37.8 ± 1.7		
sulpiride			240 ± 58	
dopamine				11 ± 3.4

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximal inhibition of [^{3}H]ketanserin (5-HT_2), [^{3}H]SCH 23390 (D_1), [^{3}H]spiperone (D_2), and [^{3}H]-7-OH-DPAT (D_3) binding to rat tissue homogenate. [b] NT, not tested.

Table 2. pK_i Values of D_1, D_2, and 5-HT_2 Receptor Binding Sites, Ratios for Compounds (±)-**3a,b**, (+)-**3b**, and (−)-**3b**, Clozapine (Atypical), Olanzapine (Atypical), and Methiothepine (Typical), and Their Log *Y* Scores

compd	D_1	D_2	5-HT_2	D_1/D_2	5-HT_2/D_1	5-HT_2/D_2	log Y^a score
(±)-**3a**	6.8	7.15	8.1	0.95	1.19	1.13	4.98
(±)-**3b**	7.6	8.4	8.9	0.90	1.18	1.06	6.50
(+)-**3b**	7.8	7.3	8.8	1.06	1.13	1.20	4.65
(−)-**3b**	7.7	8.7	8.7	0.88	1.14	1.00	7.42
olanzapine	7.1	7.2	7.9	0.98	1.11	1.10	5.55
clozapine	6.5	6.6	7.9	0.98	1.23	1.20	4.00
methiothepine[b]	8.7	9.7	9.4	0.90	1.08	0.97	8.95

[a] Log *Y* score has been calculated according to the equation reported in ref 22. Cutoff point 6.48. [b] Data from ref 22.

Table 3. Effect of Compound (±)-**3b**·2HCl and Olanzapine on APO-Induced LMA and Catalepsy

compd	dose (μmol/kg, sc)	LMA	catalepsy
control		2263 ± 157	0/4
(±)-**3b**·2HCl[a]	1	877 ± 43	0/4
	3	40 ± 5	0/4
	10	43 ± 3	1/4
	30	39 ± 3	4/4
olanzapine	1	323 ± 42	0/4
	3	ND	ND
	10	47 ± 3	0/4
	30	4 ± 2	0/4

[a] Compound (±)-**3b** has been tested as the dihydrochloride salt (see the Experimental Section). All decreases are significantly different from control values. ND, not determined.

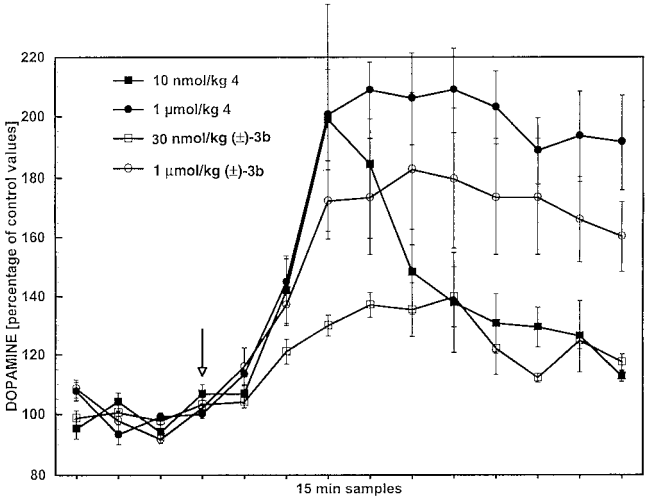


Figure 1. Effect of olanzapine (**4**) and compound (±)-**3b** on extracellular levels of dopamine in striatum expressed as percentages of control values. All data are means ± SEM (*n* = 3). Drug administration (sc) is indicated by an arrow.

teristics of clozapine, and (ii) olanzapine-like properties for the more active thiazepine (±)-**3b** (log *Y* = 6.50). Furthermore the binding data performed on (+)-**3b** and (−)-**3b** confirmed an "atypical" neuroleptic binding profile for (+)-**3b** (log *Y* = 4.65) and a "classical" neuroleptic profile for (−)-**3b** (log *Y* = 7.42), showing a stereoselective interaction at D_2 receptors (Table 2).[3,21,22]

2. Behavioral and Biochemical Effects. Administration of (±)-**3b** prior to 1 mg/kg apomorphine caused a dose-related suppression of apomorphine-induced locomotor activity [$F(4,21) = 8.303$, $p < 0.001$; $n = 4-5$]. At a dose of 1 μmol/kg locomotor activity was suppressed to 39% of control values, whereas the maximal suppression (to 2% of control values) was measured after

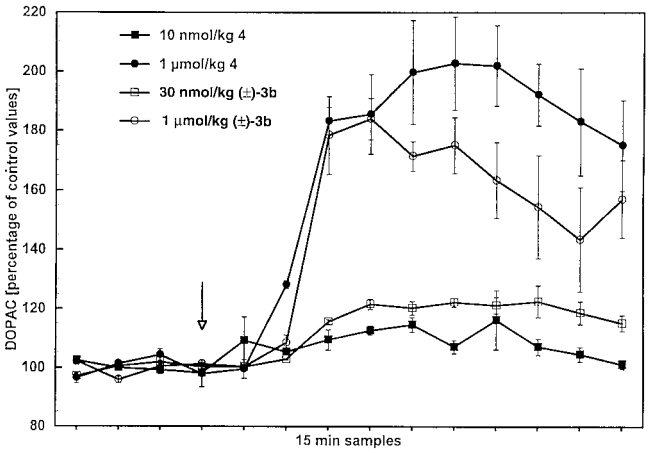


Figure 2. Effect of olanzapine (**4**) and compound (±)-**3b** on extracellular levels of DOPAC in striatum expressed as percentages of control values. All data are means ± SEM (*n* = 3). Drug administration (sc) is indicated by an arrow.

administration of 3 μmol/kg (±)-**3b**. At these doses, (±)-**3b** did not induce catalepsy. However, after administration of 10 and 30 μmol/kg (±)-**3b**, catalepsy was observed in 1/4 and 4/4 rats, respectively. Like for (±)-**3b**, administration of olanzapine caused a dose-related suppression in apomorphine-induced locomotor activity [$F(3,17) = 14.681$, $p < 0.001$; $n = 4-5$]. Locomotor activity was suppressed to 14% of control values after administration of 1 μmol/kg olanzapine. In contrast to (±)-**3b**, olanzapine did not induce catalepsy up to doses of 30 μmol/kg, although at this dose the animals had lower muscle tone (Table 3). Administration of 30 nmol/kg (±)-**3b** elevated extracellular dopamine levels in rat striatum to maximally 140% of control values [$F(10,32) = 4.066$, $p = 0.003$; $n = 3$] (Figures 1 and 2). A smaller, but also significant, increase in extracellular levels of DOPAC to 122% of control levels was observed after administration of (±)-**3b** [$F(10,32) = 7.962$, $p < 0.001$; $n = 3$]. Administration of 1 μmol/kg (±)-**3b** further increased extracellular dopamine levels to 180% of control values [$F(10,32) = 2.345$, $p = 0.046$; $n = 3$] and DOPAC levels to 270% of control values [$F(10,32) = 30.439$, $p < 0.001$; $n = 3$]. The onset of the effect was about 30 min after administration.

Administration of doses ≥ 10 nmol/kg olanzapine also increased extracellular levels of dopamine in striatum. After administration of 10 nmol/kg olanzapine, dopamine levels increased to 200% of control values and returned to baseline levels in about 2 h [$F(10,32) = 2.375$, $p = 0.044$; $n = 3$]. DOPAC levels remained unchanged after administration of 10 nmol/kg olanzapine [$F(10,32) = 1.003$, $p = 0.471$; $n = 3$]. A more profound effect on striatal biochemistry was observed after administration of 1 μmol/kg olanzapine. Both extracellular levels of dopamine [$F(10,32) = 7.744$, $p < 0.001$; $n = 3$] and DOPAC [$F(10,32) = 2.893$, $p = 0.018$; $n = 3$] increased to about 200% of control values. As with (±)-**3b**, the onset of the effect was about 30 min after administration.

Conclusions

The novel compounds (±)-**3b**, (+)-**3b**, and (−)-**3b** are centrally active dopamine receptor antagonists, with high affinity for D_2 and D_3 receptors, together with a

still higher affinity for 5-HT_2 receptors. The novel pyrrolobenzothiazepine (±)-**3b** has been resolved, and although a similar affinity for 5-HT_2 and D_1 receptors has been found for the two enantiomers, the in vitro testing confirmed a stereoselective interaction for (+)- and (−)-**3b** at D_2 receptors. In fact, (+)-**3b** is 25 times less potent at D_2 receptors than (−)-**3b**, maintaining a high affinity for 5-HT_2 receptors. Overall (+)-**3b** has an "atypical" neuroleptic binding profile and represents a new lead compound for the development of novel atypical antipsychotic drugs. Moreover, compound (±)-**3b** is able to increase the extracellular level of dopamine in the rat striatum in a manner similar to that of olanzapine, although it is slightly less potent. At low doses this compound shows atypical antipsychotic properties, but unlike olanzapine, at high doses, it induces catalepsy. The separation between doses that suppress apomorphine-induced locomotor activity and induce catalepsy might indicate a safety margin for the induction of EPS. Further studies are necessary to fully characterize the binding profile of this compound and its enantiomers in order to understand the full spectrum of potential side effects and to define their potential role to treat disorders such as schizophrenia. Furthermore, different-sized π-systems and different fused aromatic rings might possibly lead to further optimization of the atypical properties of these tricyclic compounds.

Experimental Procedures

Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken with Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H NMR spectra were recorded on Bruker 200-MHz and Varian 500-MHz spectrometers with TMS as internal standard; the values of chemical shifts (δ) are given in ppm and coupling constants (J) in hertz. All reactions were carried out in an argon atmosphere. Progress of the reaction was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Merck silica gel (Kieselgel 60) was used for chromatography (70−230 mesh) and flash chromatography (230−400 mesh) columns. Extracts were dried over $MgSO_4$, and solvents were removed under reduced pressure. HPLC separation was performed using a Chiralpak AD Amylose column (097-702-40808) (length × diameter = 250 mm × 10 mm). Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer, and the results are within 0.4% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized.

1-[(2-Fluorophenyl)-2-(pyrrol-1-yl)ethanone (7). A mixture of 1-(2-fluorophenyl)ethanone (**5**) (5.0 g, 36.0 mmol) and anhydrous aluminum chloride (43 mg, 0.32 mmol) in 5 mL of anhydrous ethyl ether was cooled at 0 °C under argon, and a solution of bromine (1.84 mL, 36.0 mmol) in 5 mL of anhydrous ethyl ether was added dropwise. After stirring for 3 h the solvent was removed by filtration, and the solid residue was washed with 3 mL of water and then with 3 mL of light petroleum ether. The residue was recrystallized from methanol to afford yellowish prisms of the 2-bromo derivative (5.3 g, 83%): mp 57−58 °C (hexanes); IR (neat) 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.81 (m, 1 H), 7.50−7.30 (m, 2 H), 7.17 (m, 1 H), 4.40 (s, 2 H). Anal. (C_8H_6BrFO) C, H, N.

To a solution of 2-bromo-1-(2-fluorophenyl)ethanone (7.8 g, 36 mmol) in 70 mL of dry chloroform was added hexamethylenetetramine (5.04 g, 36 mmol) in portions. The reaction mixture was allowed to stir at room temperature for 15 h. The solid formed was removed by filtration, washed with 5 mL of chloroform, and dried. The hexamethylenetetrammonium salt obtained (9.9 g, 27.7 mmol) was added to a solution of concentrated hydrochloric acid (7.94 mL) in 63.5 mL of ethanol. The mixture was stirred for 96 h in the dark at room temperature. The white solid (ammonium chloride) was removed by filtration, and the filtrate was evaporated. The residue was recrystallized from ethanol to give 2-amino-1-(2-fluorophenyl)ethanone hydrochloride (**6**) (4.9 g) as colorless prisms, which was used in the next step without further purification (ref 13).

To a solution of 2-amino-1-(2-fluorophenyl)ethanone hydrochloride (**6**) (2.0 g, 10.5 mmol) in 17 mL of water, heated at 90 °C, were added sodium acetate (1.44 g), glacial acetic acid (9.5 mL), and 2,5-dimethoxytetrahydrofuran (1.11 mL). After stirring for 15 min at 100 °C the mixture was cooled and extracted with ethyl acetate. The organic layers were washed with 20% $NaHCO_3$ and brine, dried, and evaporated. The residue was chromatographed (chloroform) to afford 1.83 g (86% yield) of **7** as white crystals: mp 49−50 °C (hexanes); IR ($CHCl_3$) 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.98−7.89 (m, 1 H), 7.62 (m, 1 H), 7.31−7.19 (m, 2 H), 6.66 (m, 2 H), 6.23 (m, 2 H), 5,27 (d, 2 H, J = 3.4 Hz). Anal. ($C_{12}H_{10}FNO$) C, H, N.

1-(2-Fluorophenyl)-2-(2-thiocyanatopyrrol-1-yl)ethanone (8). To a stirred solution of **7** (0.46 g, 2.0 mmol) in dry methanol (12 mL) cooled at 0 °C and kept under argon, was added freshly prepared cupric thiocyanate (prepared from sodium thiocyanate and cupric sulfate) (0.78 g, 4.0 mmol) in portions. The reaction mixture was left to stir at 0 °C until the black suspension turned white (12 h). The white cuprous thiocyanate was filtered off and washed with methanol, and the filtrate was evaporated. The residue was partitioned between cold water and EtOAc. The organic layers were dried and evaporated, and the crude product was chromatographed (30% hexanes in chloroform) to give 0.32 g (62% yield) of **8** as colorless prisms: mp 62−63 °C (cyclohexane); IR (Nujol) 2156, 1676 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.96 (m, 1 H), 7.60 (m, 1 H), 7.32 (m, 2 H), 6.93 (m, 1 H), 6.75 (m, 1 H), 6.32 (m, 1 H), 5.46 (d, 2 H, J = 3.5 Hz). Anal. ($C_{13}H_9FN_2OS$) C, H, N.

2,3-Dihydro-2-imino-3-(2-fluorobenzoyl)pyrrolo[2,1-*b*]thiazole (10). To a stirred and cooled (−20 °C) solution of the thiocyanatopyrrole **8** (160 mg, 0.6 mmol) in freshly distilled *N*,*N*-dimethylformamide (DMF) (3.6 mL) was added sodium methoxide (82 mg, 1.51 mmol) in portions. After 2 h at −20 °C, the suspension was warmed to room temperature and allowed to stir for 3 h. The solvent was evaporated, and the residue was taken up in 10 mL of water. The aqueous suspension was neutralized with 1 N HCl and then extracted with EtOAc. The organic layers were washed with brine, dried, and evaporated. The residue was chromatographed (ethyl acetate) to afford the thiazole **10** (126 mg, 81%) as brown prisms: mp 93−94 °C (EtOAc); IR (Nujol) 3300 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.47 (m, 2 H), 7.35−7.20 (m, 3 H), 7.13 (br s, 1 H), 6.10 (m, 3 H); MS *m*/*z* 260 (50, M^+), 240, 172, 123 (100), 110, 95. Anal. ($C_{13}H_9FN_2OS$) C, H, N.

2-(Phenylthio)pyrrole (12). To a solution of phenylmagnesium bromide (prepared from bromobenzene (0.75 mL, 6 mmol) and magnesium turnings (0.19 g, 7.8 mmol)) in anhydrous THF (20 mL), cooled at 0 °C, was slowly added a solution of 2-thiocyanatopyrrole (**11**) (0.5 g, 4.0 mmol) in anhydrous THF (20 mL). After stirring at 0 °C for 30 min, the mixture was poured into crushed ice and extracted with ethyl acetate. The organic phase was washed with 20% NH_4Cl, dried, and evaporated. The residue was purified by chromatography (35% hexanes in chloroform) to afford 0.6 g (93% yield) of **12** as colorless prisms: mp 86−87 °C (hexanes); IR ($CHCl_3$) 3420 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.20 (br s, 1 H), 7.25−6.95 (m, 5 H), 6.92 (m, 1 H), 6.55 (m, 1 H), 6.31 (m, 1 H). Anal. (C_9H_9NS) C, H, N.

2-(Phenylthio)pyrrole-1-acetic Acid Ethyl Ester (13). To a mixture of 18-crown-6 (20 mg, 0.074 mmol) and potassium *tert*-butoxide (0.166 mg, 1.48 mmol) in anhydrous THF (5 mL) was added a solution of **12** (0.2 g, 1.14 mmol) in anhydrous THF (5 mL), under argon. After the mixture was left for 2 h at room temperature, a solution of ethyl bromoacetate (0.254 mL, 2.28 mmol) in anhydrous THF (1 mL) was added dropwise. After stirring for 30 min at room temperature and addition of 5 mL of water, the solvent was removed under reduced pressure and the residue extracted with EtOAc. The organic

layers were washed with brine, dried, and evaporated. The residue was chromatographed (30% hexanes in chloroform) to give 0.28 g (96%) of **13** as colorless prisms: mp 101−102 °C (cyclohexane); IR ($CHCl_3$) 1760 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.25−6.90 (m, 6 H), 6.61 (m, 1 H), 6.31 (m, 1 H), 4.68 (s, 2 H), 4.05 (q, 2 H, J = 7.0 Hz), 1.14 (t, 3 H, J = 7.1 Hz). Anal. ($C_{14}H_{15}NO_2S$) C, H, N.

2-(Phenylthio)pyrrole-1-acetic Acid (14). Ester **13** (0.25 g, 0.95 mmol) was dissolved in 4 mL of ethanol/THF (1:1), and 5% NaOH (3.5 mL) was added. The mixture was stirred at room temperature for 30 min, the solution was adjusted to pH 5−6 with 1 N HCl, and ethanol and THF were evaporated. The aqueous residue was extracted with EtOAc. The organic layers were washed with brine, dried, and concentrated. The crude product was purified by chromatography (EtOAc) to give 0.2 g (89%) of **14** as white crystals: mp 126−127 °C (EtOAc); IR (Nujol) 2491 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.88 (br s, 1 H), 7.31−6.98 (m, 6 H), 6.55 (m, 1 H), 6.23 (m, 1 H), 4.69 (s, 2 H). Anal. ($C_{12}H_{11}NO_2S$) C, H, N.

2-(Phenylthio)pyrrole-1-selenoacetic Acid Methyl Ester (15). A solution of dimethylaluminum methylselenolate (2.2 mmol) (prepared by heating a toluene solution of trimethylaluminum with powdered selenium for 2 h at reflux under argon) in dry toluene (1.1 mL) was added dropwise to a solution of **13** (0.57 g, 2.2 mmol) in dry dichloromethane (5 mL) cooled at 0 °C, under argon. The mixture was stirred at 0 °C for 45 min, warmed to room temperature, and stirred for an additional 45 min. Water was added (2 mL), and the mixture was extracted with EtOAc. The organic layers were washed with brine, dried, and evaporated. The crude product was purified by distillation (85 °C/0.1 mmHg) to give 0.6 g (97%) of **15** as a colorless oil: IR (neat) 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.24−6.95 (m, 6 H), 6.69 (m, 1 H), 6.37 (m, 1 H), 4.69 (s, 2 H), 2.11 (s, 3 H); MS m/z 311 (40, M^+), 188, 155, 109, 91 (100). Anal. ($C_{13}H_{13}SeNOS$) C, H, N.

1-[2-(Methylthio)phenyl]ethanone (18). To a suspension of lithium hydride (0.57 g, 6.7 mmol) in anhydrous 1,2-dimethoxyethane (5 mL), vigorously stirred, was added dropwise a solution of acid **17** (1.0 g, 5.9 mmol) in anhydrous 1,2-dimethoxyethane (5 mL). The suspension was refluxed for 2.5 h and cooled at −10 °C, and methyllithium (4.2 mL, 6.7 mmol, 1.6 M) was added within 30 min. The reaction mixture was stirred for 2 h at room temperature; 1 N HCl (15 mL) was added, and the mixture was extracted with ethyl ether. The organic layers were washed with brine, dried, and concentrated. Chromatography of the crude product (5% benzene in dichloromethane) gave 0.78 g (79%) of **18** as colorless prisms, whose spectroscopic data were identical with those reported in ref 19.

1-[5-Chloro-2-(methylthio)phenyl]ethanone (20). A mixture of **19** (1.0 g, 6.3 mmol), anhydrous aluminum chloride (1.88 g, 13.5 mmol), and carbon disulfide (20 mL) was heated at reflux under argon, and acetic anhydride (0.46 mL, 6.3 mmol) was added dropwise. After refluxing for 4 h, the solution was poured into crushed ice, and 20 mL of 6 N HCl was added. The mixture was extracted with EtOAc, and the organic layers were washed with brine, dried, and concentrated. The oily residue was chromatographed (30% hexanes in chloroform) to give 0.5 g (40%) of **20** as a waxy solid: IR (neat) 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.83 (d, 1 H, J = 2.1 Hz), 7.44 (dd, 1 H, J = 8.1, 2.1 Hz), 7.29 (d, 1 H, J = 8.1 Hz), 2.63 (s, 3 H), 2.41 (s, 3 H). Anal. (C_9H_9ClOS) C, H.

2-Bromo-1-[2-(methylthio)phenyl]ethanone (21a). The title compound was prepared starting from **18** (2.09 g, 12.6 mmol) and following the procedure as described to obtain **6**. **21a** was obtained as colorless prisms (69% yield): mp 81−82 °C (hexanes); IR (Nujol) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.00−7.80 (m, 2 H), 7.35 (m, 2 H), 5.53 (s, 2 H), 2.27 (s, 3 H). Anal. (C_9H_9BrOS) C, H.

2-Bromo-1-[5-chloro-2-(methylthio)phenyl]ethanone (21b). The title compound was obtained starting from **20** (1.26 g, 6.3 mmol) and following the procedure as described for **6**. **21b** was obtained as colorless prisms (62% yield): mp 97−98 °C (hexanes); IR ($CHCl_3$) 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.98−7.73 (m, 3 H), 5.57 (s, 2 H), 2.31 (s, 3 H). Anal. ($C_9H_8BrClOS$) C, H.

1-[2-(Methylthio)phenyl]-2-(pyrrol-1-yl)ethanone (22a). Starting from **21a** (2.1 g, 8.6) the 2-amino-1-[2-(methylthio)-phenyl]ethanone hydrochloride was obtained according to the procedure described for **6**: 78% yield; ^1H NMR (DMSO-d_6) δ 8.43 (br s, 2 H), 8.11−7.31 (m, 5 H), 4.59 (d, 2 H, J = 3.2 Hz), 2.46 (s, 3 H). Anal. ($C_9H_{12}ClNOS$) C, H, N.

Starting from 2-amino-1-[2-(methylthio)phenyl]ethanone hydrochloride the title compound was obtained as colorless prisms following the procedure described for **7**: 50% yield; mp 113−114 °C (hexanes); IR (Nujol) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.74−7.23 (m, 4 H), 6.66 (m, 2 H), 6.21 (m, 2 H), 5.25 (d, 2 H, J = 3.4 Hz), 2.44 (s, 3 H). Anal. ($C_{13}H_{13}NOS$) C, H, N.

1-[5-Chloro-2-(methylthio)phenyl]-2-(pyrrol-1-yl)ethanone (22b). Starting from **21b** (5.58 g, 20.0 mmol) the 2-amino-1-[5-chloro-2-(methylthio)phenyl]ethanone hydrochloride was obtained according to the procedure described for **6**: 75% yield; ^1H NMR (DMSO-d_6) δ 8.41 (br s, 2 H), 8.10−7.28 (m, 3 H), 4.48 (d, 2 H, J = 3.2 Hz), 2.42 (s, 3 H). Anal. ($C_9H_{11}Cl_2NOS$) C, H, N.

Starting from 2-amino-1-[5-chloro-2-(methylthio)phenyl]ethanone hydrochloride the title compound was obtained as colorless prisms following the procedure described for **7**: 51% yield; mp 124−125 °C (hexanes); IR (Nujol) 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (d, 1 H, J = 2.3 Hz), 7.45 (dd, 1 H, J = 8.2, 2.3 Hz), 7.29 (d, 1 H, J = 8.2 Hz), 6.65 (m, 2 H), 6.22 (m, 2 H), 5.21 (s, 2 H), 2.43 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 194.2, 140.6, 134.5, 132.4, 130.1, 128.9, 127.7, 121.8, 109.2, 56.6, 16.6. Anal. ($C_{13}H_{12}ClNOS$) C, H, N.

1-[2-(Methylsulfinyl)phenyl]-2-(pyrrol-1-yl)ethanone (23a). To a suspension of sodium periodate (0.55 g, 2.6 mmol) in methanol (7 mL) and water (1.4 mL) was added a solution of **22a** (0.6 g, 2.6 mmol) in methanol (2 mL). After the mixture stirred for 20 h at room temperature, sodium iodate was removed by filtration, and the filtrate was evaporated. The residue was chromatographed (5% EtOAc in dichloromethane) to give 0.59 g (92%) of **23a** as colorless prisms: mp 174−175 °C (ethanol); IR (Nujol) 1710, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.42−7.64 (m, 4 H), 6.66 (m, 2 H), 6.27 (m, 2 H), 5.44 (0.5 ABq, 1 H, J = 18.0 Hz), 5.27 (0.5 ABq, 1 H, J = 18.0 Hz), 2.80 (s, 3 H). Anal. ($C_{13}H_{13}NO_2S$) C, H, N.

1-[5-Chloro-2-(methylsulfinyl)phenyl]-2-(pyrrol-1-yl)-ethanone (23b). Starting from **22b** (1.1 g, 4.45 mmol) the title compound was prepared following the above-described procedure: colorless prisms (89% yield); mp 218−219 °C (ethanol); IR (Nujol) 1710, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.37 (d, 1 H, J = 8.0 Hz), 7.85 (m, 2 H), 6.66 (m, 2 H), 6.28 (m, 2 H), 5.40 (0.5 ABq, 1 H, J = 17.7 Hz), 5.25 (0.5 ABq, 1 H, J = 17.8 Hz), 2.79 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 193.2, 149.6, 136.9, 134.7, 132.5, 128.8, 127.0, 121.8, 109.8, 55.7, 44.3. Anal. ($C_{13}H_{12}ClNO_2S$) C, H, N.

9,10-Dihydropyrrolo[2,1-*b*][1,3]benzothiazepin-9-one (9a). Method A: The following reaction must be performed in inert atmosphere, and the copper reagent has been prepared according to ref 12b. To a solution of the highly reactive crystalline complex of copper(I) triflate and benzene (0.81 g, 1.6 mmol) in dry benzene (20 mL), cooled at 0 °C, was added a solution of selenoester **15** (0.5 g, 1.6 mmol) in dry benzene (11 mL), and the mixture was allowed to stir at room temperature for 16 h. Ethyl ether (10 mL) was added; the organic phase was washed with 6 N ammonia, dried, and concentrated. The crude product was chromatographed (30% hexanes in dichloromethane) to give 51 mg (12%) of **9a** as colorless prisms. The ketone **16** (55%) was also recovered from the reaction mixture as a tick oil. Compound **9a**: mp 94−95 °C (hexanes); IR ($CHCl_3$) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14−7.30 (m, 4 H), 6.88 (m, 1 H), 6.42 (m, 1 H), 6.12 (m, 1 H), 5.15 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 190.9, 140.0, 136.1, 133.3, 132.3, 130.8, 127.6, 123.9, 120.2, 114.4, 109.2, 57.6; MS m/z 265 (10, M^+), 215 (100), 187, 154, 115, 97. Anal. ($C_{12}H_9NOS$) C, H, N. Compound **16**: IR (neat) 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22−

6.81 (m, 6 H), 6.70 (m, 1 H), 6.37 (m, 1 H), 4.61 (s, 2 H), 2.20 (s, 3 H); MS *m*/*z* 231 (100, M^+). Anal. ($C_{13}H_{13}NOS$) C, H, N.

Method B: Trifluoroacetic anhydride (1.0 mL, 7.4 mmol) was added dropwise to freshly distilled *N*,*N*-dimethylformamide (8 mL) cooled at 0 °C. After the mixture stirred for 20 min at 0 °C, a solution of **23a** (1.0 g, 4.0 mmol) in freshly distilled *N*,*N*-dimethylformamide (24 mL) was added. After 15 min at 0 °C and 1 h at room temperature, the pH of the dark-red solution was adjusted to 7 with 1 N NaOH, and the mixture was stirred for an additional 30 min. Extraction with dichloromethane, drying of the extracts, and evaporation of the solvent gave an oily residue which was chromatographed (30% hexanes in chloroform). Compound **9a** was obtained in 45% yield.

7-Chloro-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepin-9-one (9b). The title compound was obtained in 42% yield, as colorless prisms, starting from **23b** and following the procedure as described for **9a** (method B): mp 106−107 °C (hexanes); IR ($CHCl_3$) 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14 (d, 1 H, J = 2.2 Hz), 7.49 (d, 1 H, J = 8.1 Hz), 7.38 (dd, 1 H, J = 8.0, 2.3 Hz), 6.88 (m, 1 H), 6.43 (m, 1 H), 6.12 (m, 1 H), 5.14 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 189.7, 138.3, 137.3, 134.1, 133.1, 132.2, 131.9, 124.1, 119.5, 114.7, 109.5, 57.3; MS *m*/*z* 250 (20, M^+), 249 (100), 221, 216, 188, 158, 110. Anal. ($C_{12}H_8ClNOS$) C, H, N.

(±)-9,10-Dihydro-9-hydroxypyrrolo[2,1-*b*][1,3]benzothiazepine (24a). To a solution of **9a** (61 mg, 0.23 mmol) in dry methanol (1 mL), cooled at 0 °C and under argon, was added sodium borohydride (80 mg, 0.23 mmol) in portions. After stirring for 1 h at 0 °C, the reaction was quenched with water (1 mL) and the mixture was extracted with EtOAc. The organic layers were washed with brine, dried, and concentrated. The residue was chromatographed (15% EtOAc in dichloromethane) to give **24a** (64 mg, 92% yield) as colorless prisms: mp 101−102 °C (ethanol); IR (Nujol) 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.47−7.12 (m, 4 H), 6.86 (m, 1 H), 6.33 (m, 1 H), 6.02 (m, 1 H), 5.08 (m, 1 H), 4.89 (dd, 1 H, J = 13.9, 1.7 Hz), 4.28 (dd, 1 H, J = 13.9, 6.0 Hz), 2.05 (d, 1 H, J = 9.6 Hz). Anal. ($C_{12}H_{11}NOS$) C, H, N.

(±)-7-Chloro-9,10-dihydro-9-hydroxypyrrolo[2,1-*b*][1,3]-benzothiazepine (24b). Starting from **9b** (112 mg, 0.45 mmol) the title compound was obtained following the above-described procedure: 88% yield; mp 118−119 °C (ethanol); IR ($CHCl_3$) 3300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.48 (d, 1 H, J = 2.1 Hz), 7.32 (d, 1 H, J = 8.0 Hz), 7.15 (dd, 1 H, J = 8.1, 2.1 Hz), 6.88 (m, 1 H), 6.34 (m, 1 H), 6.11 (m, 1 H), 5.02 (m, 1 H), 4.85 (dd, 1 H, J = 13.9, 1.9 Hz), 4.29 (dd, 1 H, J = 13.9, 6.6 Hz), 2.10 (d, 1 H, J = 9.6 Hz). Anal. ($C_{12}H_{10}ClNOS$) C, H, N.

(±)-9-Bromo-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepine (25a). To a solution of **24a** (0.26 g, 1.0 mmol) in anhydrous ethyl ether (4 mL) was added dropwise a solution of PBr_3 (0.13 g, 0.5 mmol) in anhydrous ethyl ether (1 mL), and the reaction mixture was refluxed for 2 h, under argon. Anhydrous ethanol (0.2 mL) was added, and the resulting solution was heated under reflux for an additional hour. Then 5 mL of 10% aqueous Na_2CO_3 was added; the organic phase was separated, dried, and evaporated. The crude product was chromatographed (hexanes and chloroform, 1/1) to give **25a** (0.2 g, 64%) as colorless prisms: mp 115−116 °C (cyclohexane); ^1H NMR ($CDCl_3$) δ 7.46−7.09 (m, 4 H), 6.93 (m, 1 H), 6.39 (m, 1 H), 6.12 (m, 1 H), 5.75 (dd, 1 H, J = 6.9, 2.6 Hz), 5.07 (dd, 1 H, J = 14.7, 2.6 Hz), 4.61 (dd, 1 H, J = 14.7, 6.9 Hz). Anal. ($C_{12}H_{10}BrNS$) C, H, N.

(±)-9-Bromo-7-chloro-9,10-dihydropyrrolo[2,1-*b*][1,3]-benzothiazepine (25b). Starting from **24b** (0.31 g, 1.6 mmol) the title compound was obtained following the above-described procedure: 51% yield; mp 106−107 °C (cyclohexane); ^1H NMR ($CDCl_3$) δ 7.45 (d, 1 H, J = 2.1 Hz), 7.27 (d, 1 H, J = 8.6 Hz), 7.11 (dd, 1 H, J = 8.6, 2.1 Hz), 6.92 (m, 1 H), 6.39 (m, 1 H), 6.12 (m, 1 H), 5.63 (dd, 1 H, J = 7.0, 2.3 Hz), 5.06 (dd, 1 H, J = 14.4, 2.3 Hz), 4.61 (dd, 1 H, J = 14.0, 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 139.9, 134.1, 133.2, 131.7, 131.5, 128.9, 125.6, 119.6, 114.6, 108.1, 51.2, 51.0. Anal. ($C_{12}H_9BrClNS$) C, H, N.

(±)-9-(4-Methylpiperazin-1-yl)-9,10-dihydropyrrolo-[2,1-*b*][1,3]benzothiazepine (3a). A mixture of **25a** (0.65 g, 2.0 mmol) and *N*-methylpiperazine (1.1 mL, 10.0 mmol) was heated at 130 °C for 2 h under argon, cooled, poured into crushed ice, and extracted with ethyl ether. The combined organic extracts were washed with brine, dried, and concentrated. The residue was chromatographed (EtOAc) to give 0.45 g (75%) of **3a** as colorless prisms: 206−207 °C (hexanes); ^1H NMR ($CDCl_3$) δ 7.49−7.09 (m, 4 H), 6.87 (m, 1 H), 6.29 (m, 1 H), 6.06 (m, 1 H), 4.68 (dd, 1 H, J = 14.4, 8.6 Hz), 4.51 (dd, 1 H, J = 14.4, 3.7 Hz), 4.04 (dd, 1 H, J = 8.6, 3.7 Hz), 2.56−2.34 (m, 8 H), 2.23 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 138.1, 134.6, 132.9, 130.4, 127.3, 126.9, 123.9, 121.7, 113.3, 107.7, 66.1, 55.9, 48.8, 46.6, 46.1; MS *m*/*z* 299 (100, M^+), 219, 200, 167, 149, 113. Anal. ($C_{17}H_{21}N_3S$) C, H, N.

(±)-7-Chloro-9-(4-methylpiperazin-1-yl)-9,10-dihydropyrrolo[2,1-*b*][1,3] benzothiazepine (3b). Starting from **25b** (0.3 g, 0.95 mmol) the title compound was obtained following the above-described procedure. **3b** was obtained as colorless prisms (68%): mp 210−211 °C (hexanes); ^1H NMR ($CDCl_3$) δ 7.51 (d, 1 H, J = 2.4 Hz), 7.30 (d, 1 H, J = 8.5 Hz), 7.06 (dd, 1 H, J = 8.5, 2.4 Hz), 6.86 (m, 1 H), 6.29 (m, 1 H), 6.05 (m, 1 H), 4.71 (dd, 1 H, J = 14.0, 8.6 Hz), 4.45 (dd, 1 H, J = 14.0, 3.4 Hz), 3.95 (dd, 1 H, J = 8.6, 3.4 Hz), 2.61−2.25 (m, 8 H), 1.42 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 140.1, 133.2, 133.0, 132.4, 131.6, 127.3, 123.9, 121.1, 113.6, 107.9, 65.9, 55.8, 55.7, 47.7, 45.9, 44.9, 26.8; MS *m*/*z* 333 (10, M^+), 250, 233 (100), 201, 166, 139. Anal. ($C_{17}H_{20}ClN_3S$) C, H, N. The dihydrochloride salt was obtained dissolving an analytical sample in 1 N methanolic HCl. The solvent was evaporated, and the residue was recrystallized (methanol and ethyl ether, 1/1). Anal. ($C_{17}H_{22}Cl_3N_3S$) C, H, N.

Semipreparative Chiral Separation of (±)-3b. At first the dihydrochloride salt of (±)-**3b** was purified over a short column filled with silica gel using dichloromethane and methanol (9/1) as eluant. The purified compound was converted to the free base. Evaporation of the solvent afforded an oily residue which was dissolved in 2-propanol, and the solution was diluted with hexanes until a ratio of 95/5 was reached. For separation of enantiomers a concentration of 10−15 mg/mL racemate was made. A mixture of hexanes (plus 0.1% triethylamine) and 2-propanol was used as the mobile phase. A gradient mixer kept the ratio of both solvents (hexanes and 2-propanol) at 95:5. The injections were 100 μL per injection. The enantiomers were collected using a fraction collector. Only fractions with a signal above 10% (10 mv) of the whole scale were collected. The amount below the 10% was collected apart and used for a second purification. The purity of both enantiomers was determined by weighing of the plotted peaks separately.

(+)-3b: ^1H NMR (500 MHz, $CDCl_3$) δ 7.52 (d, 1 H, J = 2.4 Hz), 7.32 (d, 1 H, J = 8.3 Hz), 7.09 (dd, 1 H, J = 2.4, 8.3 Hz), 6.88 (m, 1 H), 6.30 (m, 1 H), 6.07 (m, 1 H), 4.71 (dd, 1 H, J = 8.8, 14.2 Hz), 4.50 (dd, 1 H, J = 3.9, 14.7 Hz), 3.97 (dd, 1 H, J = 3.4, 8.8 Hz), 2.55 (m, 4 H), 2.40 (m, 4 H), 2.27 (s, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 139.9, 133.0, 132.9, 132.3, 131.6, 127.3, 123.8, 121.0, 113.5, 107.9, 88.2, 65.8, 55.6, 48.6, 45.9, 45.8; purity (ee) 94.6%; $[\alpha]_D$ = +46.0° (*c* 0.48, MeOH).

(−)-3b: ^1H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, 1 H, J = 2.3 Hz), 7.32 (d, 1 H, J = 8.3 Hz), 7.09 (d, 1 H, J = 6.8 Hz), 6.88 (m, 1 H), 6.30 (m, 1 H), 6.07 (m, 1 H), 4.71 (dd, 1 H, J = 9.3, 14.5 Hz), 4.48 (dd, 1 H, J = 3.4, 14.2 Hz), 3.98 (dd, 1 H, J = 2.9, 8.8 Hz), 2.49 (m, 8 H), 2.27 (s, 3 H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 140.0, 133.0, 132.9, 132.3, 131.6, 127.3, 123.8, 121.0, 113.5, 107.9, 88.2, 65.8, 55.6, 48.6, 45.9, 45.8; purity (ee) 98.0%; $[\alpha]_D$ = −47.9° (*c* 0.54, MeOH).

Materials and Methods. 1. In Vitro Binding Assay. Male CRL:CD(SD)BR-COBS rats (Charles River, Italy) were killed by decapitation; their brains were rapidly dissected into the various areas (striatum for D_1 and D_2 receptors, olfactory tubercle for D_3 receptors, and cortex for 5-HT_2 receptors) and stored at −80 °C until the day of assay. Tissues were homogenized in about 50 volumes of Tris HCl, 50 mM, pH 7.4 (for D_1, D_2, and 5-HT_2 receptors) or Hepes Na 50 mM, pH 7.5

(for D_3 receptors), using an Ultra-Turrax TP-1810 (2 × 20 s), and centrifuged at 50000*g* for 10 min. The pellets were then washed once by resuspension in fresh buffer and centrifugation as before. The pellets obtained were resuspended in the appropriate incubation buffer (Tris HCl, 50 mM, pH 7.1, containing 10 μM pargyline, 0.1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ for D_1 and D_2 receptors; Hepes Na, 50 mM, pH 7.5, containing 1 mM EDTA, 0.005% ascorbic acid, 0.1% albumine, 200 nM eliprodil for D_3 receptors) just before the binding assay. [^{3}H]SCH 23390 (specific activity (sa) 71.1 Ci/mmol; NEN) binding to D_1 receptors was assayed in a final incubation volume of 0.5 mL, consisting of 0.25 mL of membrane suspension (2 mg of tissue/sample), 0.25 mL of [^{3}H]ligand (0.4 nM), and 10 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 10 mM (−)-*cis*-flupentixol. [^{3}H]Spiperone (sa 19.0 Ci/mmol; NEN) binding to D_2 receptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (1 mg of tissue/sample), 0.5 mL of [^{3}H]ligand (0.2 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 100 μL of (−)-sulpiride. [^{3}H]-7-OH-DPAT (sa 139 Ci/mmol; Amersham) binding to D_3 receptors was assayed in a final incubation volume of 1 mL, consisting of 0.5 mL of membrane suspension (10 mg of tissue/sample), 0.5 mL of [^{3}H]ligand (0.7 nM), and 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM dopamine. [^{3}H]Ketanserin (sa 60 Ci/mmol; Amersham) binding to 5-HT_2 receptors was assayed in a final incubation volume of 1 mL consisting of 0.5 mL of membrane suspension (5 mg of tissue/sample), 0.5 mL of [^{3}H]ligand (0.7 nM), and 0.02 mL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM methysergide. Incubations (15 min at 37 °C for D_1, D_2, and 5-HT_2 receptors, 60 min at 25 °C for D_3 receptors) were stopped by rapid filtration under vacuum through GF/B (for D_1, D_2, and 5-HT_2 receptors) or GF/C (for D_3 receptors) filters which were then washed with 12 mL of ice-cold buffer, using a Brandel M-48R. The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a LKB 1214 rack beta liquid scintillation spectrometer with a counting efficiency of 60%.

[^{3}H]Spiperone binding to D_4 receptors was performed using autoradiography as follows: the rats were killed by decapitation, and the brains were rapidly removed, frozen in liquid nitrogen, and stored at −80 °C until use. Consecutive coronal sections (14 μm) were cut at −16° at the level of anterior caudate putamen, including nucleus accumbens and islands of Calleja (plate 12−13 of the Paxinos and Watson rat brain atlas, 1982) using a cryostat (microm HM 500 OM), thaw-monted onto gelatin-coated glass slides, and stored at −20 °C until the binding experiments. After a 5-min preincubation in 50 mM Tris HCl, pH 7.4, containing 5 mM EDTA, 1.5 mM $CaCl_2$, and 5 mM KCl, the slides were incubated for 30 min at room temperature in the same buffer with the addition of 0.4 nM [^{3}H]spiperone. Nonspecific binding was determined in the presence of 1 μM spiperone. Incubations were stopped with two 5-min rinses in ice-cold 50 mM Tris HCl, pH 7.4, and one rapid dipping in ice-cold distilled water. After washing, the slides were dried overnight under a stream of cold air, apposed to Hyperfilm (Amersham), and placed in X-ray biox. After appropriate exposure (6 weeks), the films were developed in Kodak D19 at 18 °C for 10 min, and optical densities of autoradiograms were determined with a RAS 3000 image analyzer (Loats Associate). Drugs were tested in triplicate at 10^{-5} M.

2. [^{3}H]Dopamine Uptake. Crude mitochondrial pellets (P2) were obtained from fresh rat (Male CRL:CD(SD)BR-COBS rats, Charles River, Italy) striata as previously described.[23] The final pellet was diluted (about 100 vol initial weight) with Krebs-Henseleit buffer having the following composition (mmol/L): NaCl (116); $NaHCO_3$ (25); NaH_2PO_4 (1); KCl (6); $MgSO_4$ (1); $CaCl_2$ (2); glucose (10); pargyline (1); EDTA (0.07); ascorbic acid (0.3); pH 7.2−7.4. Samples of 0.6 mL were incubated for 5 min at 30 °C in a water bath. Uptake was started by the addition of 20 nM [^{3}H]dopamine (sa 11.8 Ci/mmol; Amersham). The reaction was stopped 5 min later by adding 2 mL of ice-cold Krebs-Henseleit buffer and rapid filtration under vacuum on cellulose nitrate filters (0.65-μm pore size; Millipore), which were washed twice with 2 mL of the same buffer. The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a LKB 1214 rack beta liquid scintillation spectrometer with a counting efficiency of 60%.

3. Calculations. Drugs were tested in triplicate at different concentrations, from 10^{-5} to 10^{-10} M. IC_{50}'s, the concentration of drug that caused 50% inhibition of [^{3}H]-dopamine uptake or [^{3}H]ligand binding, were obtained using "Allfit" program running on an IBM XT personal computer.

4. Behavioral Assay and Dialysis Experiments. For behavioral testing male rats (Harlan, Zeist, The Netherlands) weiging 200−250 g were used. Until the experiment, animals were group-housed (6/cage) with food and water available ad libitum and on a 12:12-h day−night schedule (lights on 7.30 a.m.; lights off 7.30 p.m.). Catalepsy was tested on a grid (grid size: 1.5 × 1.5 cm) that was vertically placed. The time to leave the grid was measured in three consecutive sessions 30 min after injection of vehicle or drug. Rats that remained for >30 s on the grid (average of three sessions) were considered cataleptic. After testing for catalepsy, rats were injected with 1 mg/kg apomorphine (40 min after drug administration), and locomotor activity was measured for 30 min using AUTOMEX II (Columbus Instruments, Columbus, OH) activity monitors. Dialysis experiments were performed in male rats (Harlan, Zeist, The Netherlands) weighing 275−350 g. Until surgery for microdialysis probe implantation, rats were treated as described for behavioral experiments. Twenty-four hours before the dialysis experiments, rats were anesthetized with 400 mg/kg chloral hydrate and microdialysis probes inserted in the left and right striatum. After probe implantation rats were housed individually. At the start of the dialysis experiments, probes were perfused with CSF (mmol/L: NaCl, 147; KCl, 4; $CaCl_2$, 1.2; and $MgCl_2$, 1.0). Probe outlets were connected to an injection valve of an HPLC system, and dialysates were automatically injected every 15 min. After stabilization of the baseline output, drugs were injected sc, and effects were recorded for 150 min. Levels of both dopamine and DOPAC were measured using HPLC with electrochemical detection.

Behavioral data are given as counts per 30 min and dialysis data as percentage of control values (i.e., the average output value 60 min preceding drug administration). Data were analyzed with ANOVA.

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