Pyrrolo[1,3]benzothiazepine-Based Serotonin and Dopamine Receptor Antagonists. Molecular Modeling, Further Structure−Activity Relationship Studies, and Identification of Novel Atypical Antipsychotic Agents

Giuseppe Campiani,*[,†,‡] Stefania Butini,[†,‡] Caterina Fattorusso,[‡,§] Bruno Catalanotti,[‡,§] Sandra Gemma,[†,‡] Vito Nacci,[†,‡] Elena Morelli,[‡,§] Alfredo Cagnotto,[∥] Ilario Mereghetti,[∥] Tiziana Mennini,[∥] Miriana Carli,[∥] Patrizia Minetti,[⊥] M. Assunta Di Cesare,[⊥] Domenico Mastroianni,[⊥] Nazzareno Scafetta,[⊥] Bruno Galletti,[⊥] M. Antonietta Stasi,[⊥] Massimo Castorina,[⊥] Licia Pacifici,[⊥] Mario Vertechy,[⊥] Stefano Di Serio,[⊥] Orlando Ghirardi,[⊥] Ornella Tinti,[⊥] and Paolo Carminati[⊥]

Dipartimento Farmaco Chimico Tecnologico, Via Aldo Moro and European Research Centre for Drug Discovery and Development, Universitá degli Studi di Siena, 53100 Siena, Italy, Dipartimento di Chimica delle Sostanze Naturali and Dipartimento di Chimica Farmaceutica e Tossicologica, Universitá degli Studi di Napoli Federico II, via D. Montesano 49, 80131 Napoli, Italy, Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157 Milano, Italy, and Sigma-Tau Industrie Farmaceutiche Riunite spa, Via Pontina Km 30,400, 00040 Pomezia, Italy

Received August 1, 2003

Recently we reported the pharmacological characterization of the 9,10-dihydropyrrolo[1,3]-benzothiazepine derivative (*S*)-(+)-**8** as a novel atypical antipsychotic agent. This compound had an optimum pK_i 5-HT_{2A}/D_2 ratio of 1.21 (pK_i 5-HT_{2A} = 8.83; pK_i D_2 = 7.79). The lower D_2 receptor affinity of (*S*)-(+)-**8** compared to its enantiomer was explained by the difficulty in reaching the conformation required to optimally fulfill the D_2 pharmacophore. With the aim of finding novel atypical antipsychotics we further investigated the core structure of (*S*)-(+)-**8**, synthesizing analogues with specific substituents; the structure−activity relationship (SAR) study was also expanded with the design and synthesis of other analogues characterized by a pyrrolo[2,1-*b*][1,3]benzothiazepine skeleton, substituted on the benzo-fused ring or on the pyrrole system. On the 9,10-dihydro analogues the substituents introduced on the pyrrole ring were detrimental to affinity for dopamine and for 5-HT_{2A} receptors, but the introduction of a double bond at C-9/10 on the structure of (*S*)-(+)-**8** led to a potent D_2/5-HT_{2A} receptor ligand with a typical binding profile (**9f**, pK_i 5-HT_{2A}/D_2 ratio of 1.01, log *Y* = 8.43). Then, to reduce D_2 receptor affinity and restore atypicality on unsaturated analogues, we exploited the effect of specific substitutions on the tricyclic system of **9f**. Through a molecular modeling approach we generated a novel series of potential atypical antipsychotic agents, with optimized $5HT_{2A}$/D_2 receptor affinity ratios and that were easier to synthesize and purify than the reference compound (*S*)-(+)-**8**. A number of SAR trends were identified, and among the analogues synthesized and tested in binding assays, **9d** and **9m** were identified as the most interesting, giving atypical log *Y* scores respectively 4.98 and 3.18 (pK_i 5-HT_{2A}/D_2 ratios of 1.20 and 1.30, respectively). They had a multireceptor affinity profile and could be promising atypical agents. Compound **9d**, whose synthesis is easier and whose binding profile is atypical (log *Y* score similar to that of olanzapine, 3.89), was selected for further biological investigation. Pharmacological and biochemical studies confirmed an atypical antipsychotic profile in vivo. The compound was active on conditioned avoidance response at 1.1 mg/kg, a dose 100-times lower than that required to cause catalepsy (ED_{50} >90 mg/kg), it induced a negligible increase of prolactin serum levels after single and multiple doses, and antagonized the cognitive impairment induced by phencyclidine. In conclusion, the pharmacological profile of **9d** proved better than clozapine and olanzapine, making this compound a potential clinical candidate.

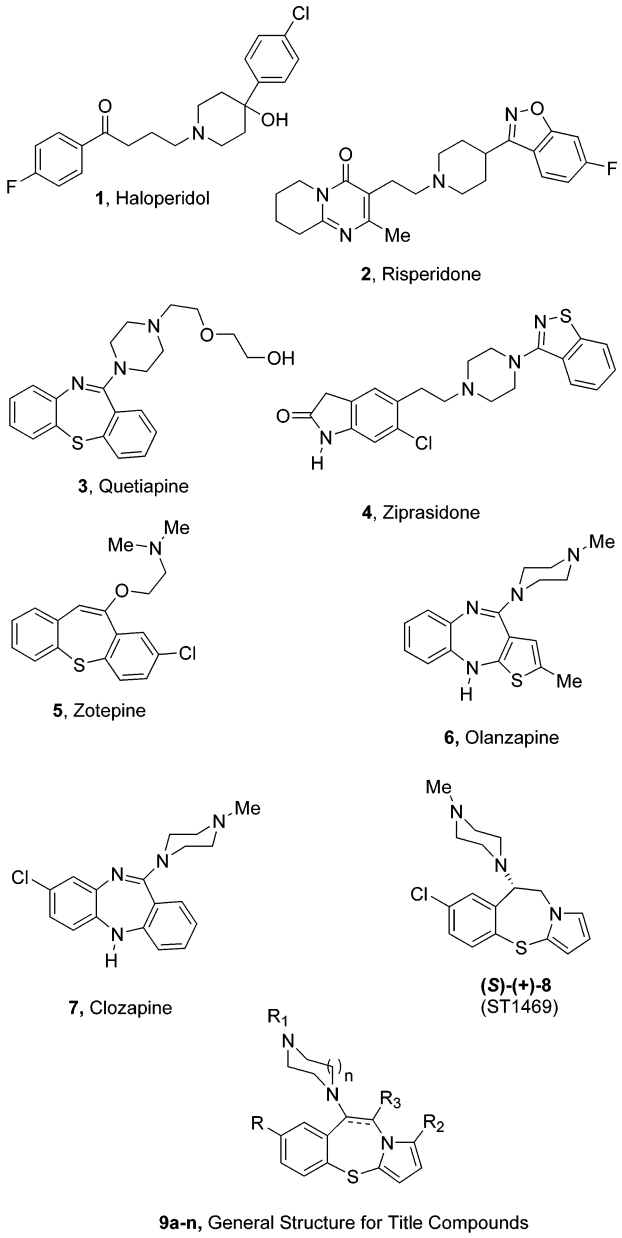
Introduction

Schizophrenia is a chronic, complex neuropsychiatric illness, afflicting approximately 1% of the population.[1,2] There are no specific focal characteristics for the diagnosis of schizophrenia, and no single symptom is consistently present in all patients. Consequently, its diagnosis as a single disorder, or as a variety of different disorders, is still debated.[1,2] In general, schizophrenia involves alterations in cognitive and emotional functioning, and the symptoms can be grouped as positive or negative. Positive symptoms include altered behavior, such as delusions, hallucinations, extreme emotions, excited motor activity, and incoherent speech. Negative symptoms are described as a lack of behavior, such as poverty of speech, social withdrawal, avolition, anhedonia and affective blunting, and are resistant to typical antipsychotics. Cognitive deficits include reduction in working memory, attention, and verbal fluency. For

* To whom correspondence should be addressed: Dipartimento Farmaco Chimico Tecnologico, Universitá degli Studi di Siena, via Aldo Moro, 53100 Siena, Italy. Tel. 0039-0577-234172, fax 0039-0577-234333, e-mail: campiani@unisi.it.
† Universitá degli Studi di Siena.
‡ European Research Centre for Drug Discovery and Development.
§ Universitá degli Studi di Napoli "Federico II".
∥ Istituto di Ricerche Farmacologiche Mario Negri.
⊥ Sigma-Tau Industrie Farmaceutiche Riunite.

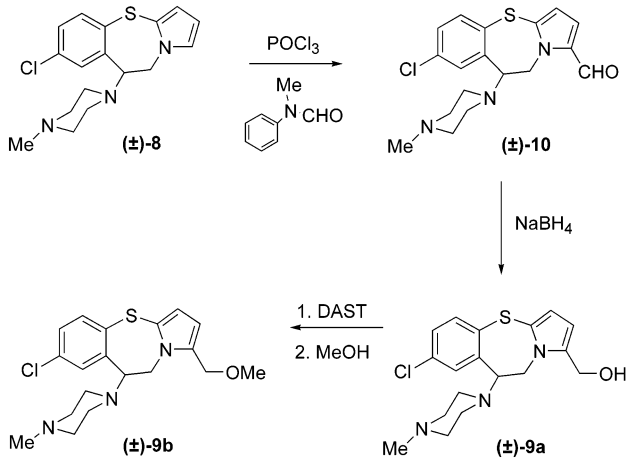
10.1021/jm0309811 CCC: $27.50
Published on Web 11/27/2003

Chart 1



decades dopamine receptor blockers such as haloperidol (**1**) have been the treatment of choice for schizophrenia, but despite the effectiveness of this approach, dopamine antagonism has a number of drawbacks, causing serious side effects such as motor disorders, tardive dyskinesia, and hyperprolactinemia.[3,4] Newer antipsychotic drugs, the so-called atypical antipsychotics,[5] show a wider efficacy against the negative and positive symptoms due to a multireceptor affinity profile. For most atypical drugs, antagonism at D_2 receptors is accompanied by other pharmacological properties, including D_2-like, 5-HT_{2A}, or $\alpha1/\alpha2$ receptor blockades.[6] Although numerous atypical antipsychotic drugs have been recently approved for the treatment of schizophrenia[7] (risperidone (**2**), quetiapine (**3**), ziprasidone (**4**), zotepine (**5**), olanzapine (**6**), and clozapine (**7**)), olanzapine still remains invaluable for psychosis, with clinical efficacy with no cases of agranulocytosis.[8,9] However, olanzapine may precipitate or unmask diabetes in susceptible patients, and its use was associated with a 12% increase in excessive appetite compared with haloperidol (**1**).[10,11]

Scheme 1



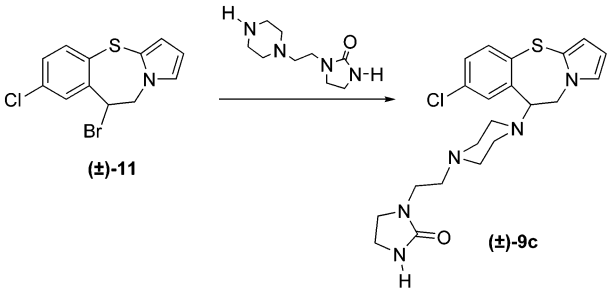
In the past decade the pharmaceutical industry and academia have shown a significant interest in the development of new antipsychotic agents, and several novel strategies for the development of atypical antipsychotics (through interaction with dopamine/serotonin receptors or with less obvious receptors, such as glutamate (mGluRs)[2a] and tachykinin receptors[12]) have started to provide additional tools to relieve the symptoms of schizophrenia, with fewer side effects. Nevertheless, research for an ideal antipsychotic agent has stimulated a continuing search for newer and safer drugs, but the complexity of the disorder, with its wide array of symptoms, hampers the efforts of scientists and clinicians. Recently we developed a new class of atypical antipsychotics with a 9,10-dihydropyrrolo[2,1-*b*][1,3]-benzothiazepine structure.[13] The benzothiazepine (*S*)-(+)-**8** (ST1469) (Chart 1) showed an atypical binding profile, and in vivo pharmacological studies on serotoninergic and dopaminergic systems indicated its pharmacological behavior was similar to that of olanzapine.

In an attempt to provide drug therapies for resistant schizophrenic patients, with prompter therapeutic benefit, and improving the cognitive symptoms, we decided to expand the structure−activity relationships (SAR) of the pyrrolobenzothiazepine class of antipsychotics, with the aim of developing a new atypical drug candidate. Starting from our lead (*S*)-(+)-**8**, the present article describes the design, synthesis, and biological evaluation of novel pyrrolo[1,3]benzothiazepines, with lower structural flexibility, and their SARs for dopamine and serotonin receptor affinity, mainly associated with variations of the substituents on the benzo- and pyrrolo-fused rings. Among the analogues synthesized and tested, compound **9d** (ST1899) was selected for further biological investigation, and its pharmacological profile is discussed, together with a molecular modeling study.

Chemistry

The synthesis of the pyrrolo[2,1-*b*][1,3]benzothiazepine skeleton was accomplished as previously described,[14] and the synthesis of the new compounds **9a**−**n** is reported in Schemes 1−3. Scheme 1 summarizes the structural modifications of position 1 of the pyrrole ring of our lead 9,10-dihydropyrrolo[1,3]benzothiazepine (±)-**8**. The key aldehyde intermediate (±)-**10** was obtained

Scheme 2



by using the formylating complex originated by reaction between phosphorus oxychloride and *N*-methylformanilide.[15] Successively, reduction of (±)-**10** with sodium borohydride gave the desired product (±)-**9a**, which was in turn transformed into the 1-methoxymethyl derivative (±)-**9b** by treatment with (diethylamino)sulfur trifluoride (DAST) and methanol.[16] The introduction of the ethyl-2-imidazolinone chain at the N-4 of the piperazine ring ((±)-**9c**) was accomplished as reported in Scheme 2, starting from the previously described bromo derivative (±)-**11**. The alkylating agent 1-[2-(1-piperazinyl)ethyl]-2-imidazolinone was synthesized following a literature procedure.[17]

To obtain the pyrrolo[1,3]benzothiazepine analogues **9d**−**n**, previously described ketones (**12a**−**d**), characterized by R = H, F, Cl, Br, were used as starting materials. Scheme 3 summarizes the synthesis of the pyrrolo[2,1-*b*][1,3]benzothiazepine derivatives **9d**−**n** differently functionalized at positions 1, 7, 9, and 10 of the tricyclic system. The synthesis of benzothiazepines **9g**,**n** was reported in ref 13. The enamines **9d**−**f** were prepared starting from **12a**−**c** by reaction with *N*-alkylpiperazines and *N*-methylhomopiperazine in the presence of trimethylsilyltriflate (TMSOTf).[13]

Analogues functionalized at position 1 were synthesized starting from **9d**. By using 1.3 equiv of the above-described formylating complex, the monoformylated analogue **9h** was obtained, while exposure to 2 equiv of the same formylating complex provided the 1,10-diformylated derivative **9i**. The monoformylated derivative **9h** was successively transformed into **9j** by means of sodium borohydride reduction. On the other hand, the 1-isopropoxymethyl derivative **9k** was obtained from **9h** through the synthesis of the corresponding tosylhydrazone (**13**) followed by reduction of this latter with sodium borohydride in 2-propanol.[18]

Then **9h** was easily transformed into the oxyme **9l**[19] with hydroxylamine hydrochloride. Finally the 1-methyl substituted compound **9m** was synthesized by treatment of **9h** with hydrazine monohydrate followed by treatment with potassium *tert*-butoxide.[20]

Results and Discussion

The binding affinities for 5-HT_{2A}, D_1, D_2, and D_3 receptors and the comparison of pK_is and log *Y* scores[21] of compounds **9a**−**n** and (*S*)-(+)-**8** with clozapine, olanzapine, and haloperidol, tested in the same experimental conditions, are given in Table 1. Table 2 compares the binding affinity for rat and human 5-HT_{2A} and D_2 receptors calculated for **9d** in comparison with clozapine and haloperidol. Table 3 summarizes the binding affinity of **9d** and **9m** for a panel of different receptors. Table 4 lists the D_2 receptor affinities of several clozapine analogues. Table 6 shows the effects of oral **9d** on 5-MeO-DMT induced head twitches, apomorphine climbing, spontaneous locomotor activity, MK-801-induced hyperactivity, conditioned avoidance response, and catalepsy compared to olanzapine and clozapine. The effect of **9d**, administered alone or with phencyclidine (PCP), on accuracy and impulsivity of rats in a five-choice serial

Scheme 3

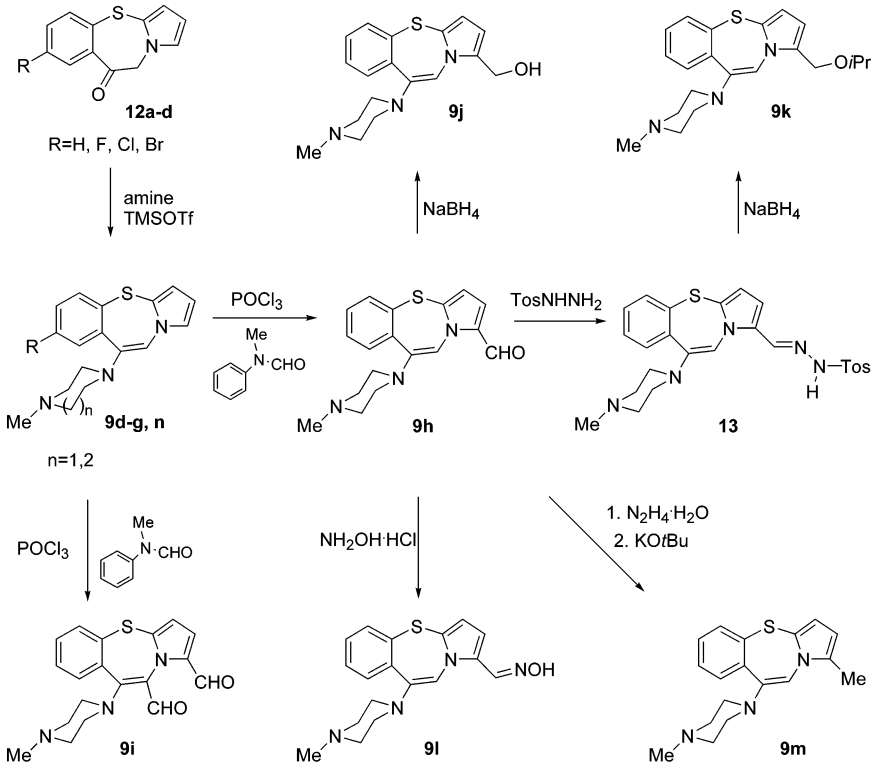
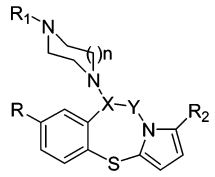
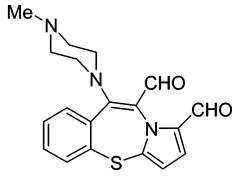


Table 1. Binding Affinities for 5-HT_{2A}, D_1, D_2, and D_3 Receptors of Compounds **9a**−**n**



9a-h, j-n



9i

compd	R	R_1	R_2	X−Y	*n*	K_i (± SD),[a] nM 5-HT_{2A}	D_1	D_2	D_3	pK_i ratio 5-HT_2D_2	log *Y*[b]
(±)-9a	Cl	Me	CH_2OH	CH−CH_2	1	NA[c]	NA[c]	NA[c]	NA[c]	NC[d]	NC[d]
(±)-9b	Cl	Me	CH_2OMe	CH−CH_2	1	405 ± 6.0	>10^5	1430 ± 138	316 ± 42	NC[d]	NC[d]
(±)-9c	Cl	2-(oxoimidazol-idinyl)ethyl	H	CH−CH_2	1	74 ± 4.0	1621 ± 84	125 ± 19.0	66 ± 1.0	1.03	5.48
9d	H	Me	H	C=CH	1	0.65 ± 0.1	19.7 ± 1.3	17.2 ± 4.5	8.3 ± 0.5	1.20	4.98
9e	F	Me	H	C=CH	1	0.35 ± 0.05	7.7 ± 0.8	8.5 ± 0.3	2.7 ± 0.7	1.17	5.37
9f	Cl	Me	H	C=CH	1	0.34 ± 0.06	1.9 ± 0.6	0.43 ± 0.02	2 ± 0.6	1.01	8.20
9g	Br	Me	H	C=CH	1	0.83 ± 0.05	3.4 ± 0.8	0.45 ± 0.05	0.25 ± 0.01	0.97	8.63
9h	H	Me	CHO	C=CH	1	20.0 ± 3.0	4285 ± 232	1342 ± 176	102 ± 28	1.31	2.37
9i	-	-	-	-	-	2300 ± 276	NA[c]	NA[c]	>1000	NC[d]	NC[d]
9j	H	Me	CH_2OH	C=CH	1	19.0 ± 1.1	1741 ± 285	854 ± 110	113 ± 17.0	1.27	2.92
9k	H	Me	CH_2OiPr	C=CH	1	217 ± 57.0	7604 ± 491	3824 ± 780	278 ± 31.0	1.23	2.95
9l	H	Me	CH_2=NOH	C=CH	1	1980±350	NA[c]	NA[c]	4255±270	NC[d]	NC[d]
9m	H	Me	Me	C=CH	1	1.1 ± 0.05	71 ± 8.0	126 ± 15.0	18.0 ± 1.0	1.30	3.18
9n	Cl	Me	H	C=CH	2	4.3 ± 0.75	5.87 ± 0.7	3.5 ± 0.8	4.3 ± 0.13	NC[d]	NC[d]
6						4.0 ± 1.0	85.0 ± 3.5	69.0 ± 17.0	39.0 ± 5.9	1.17	4.69
7						10.0 ± 1.0	353 ± 35.0	250 ± 57.0	319 ± 45.0	1.21	3.89
(*S*)-(+)-*8*						1.48 ± 0.2	16.4 ± 1.0	49.6 ± 6.0	110 ± 7.0	1.21	4.67
R-octoclothepine						0.33 ± 0.03	2.0 ± 0.2	3.6 ± 0.5	21 ± 4.3	1.12	6.35
S-octoclothepine						0.14 ± 0.01	1.9 ± 0.5	0.4 ± 0.04	0.4 ± 0.04	1.05	7.66
haloperidol						164 ±22.0	318 ± 59.0	4.8 ± 1.0	18.0 ± 1.5	0.82	9.14

[a] Each value is the mean ± SD of three determinations and indicates the concentration giving half-maximal inhibition of [^{3}H]ketanserin (5-HT_2), [^{3}H]SCH 23390 (D_1) and [^{3}H]spiperone (D_2) binding to rat tissue homogenate and [^{3}H]-7-OH-DPAT (D_{3r}) binding to Sf9 cell membranes. [b] log *Y* ccore was calculated according to the equation reported in ref 21. Cutoff point 6.48. [c] NA Not active at 10^{-5} M. [d] NC: Not calculated.

Table 2. A Comparison of Binding Affinities for Rat and Human 5-HT_{2A} and D_2 Receptors of Compound **9d**, Haloperidol, and Clozapine and Their pK_i Ratios

	K_i (nM)[a] rat		human		pK_i ratio 5-HT_{2A}/D_2	
compd	5-HT_{2A}	D_2	h5-HT_{2A}	hD_2	rat	human
9d (ST1899)	0.65 ± 0.1	17.0 ± 4.5	1.1 ± 0.1	13.0 ± 0.9	1.18	1.14
haloperidol	164 ± 22.0	4.8 ± 1.0	130 ± 8.0	3.7 ± 0.2	0.82	0.82
clozapine	10.0 ± 1.0	250 ± 57.0	7.8 ± 0.3	260 ± 15.0	1.21	1.23

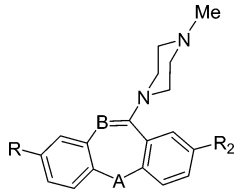
[a] Each value represents the concentration giving half-maximal inhibition of [^{3}H]ketanserin (5-HT_2), and [^{3}H]spiperone (D_2) binding to rat tissue homogenate or to recombinant human receptors.

Table 3. 9d,**m** and Reference Drug Interactions with H_1, M_1, α_1, and α_2 Receptors

compd	K_i (nM)[a] H_1	M_1	α_1	α_2
9d	2.7 ± 0.38	2070 ± 118	0.5 ± 0.02	1020 ± 116
9m	2.7 ± 0.24	2300 ± 112	4.2 ± 0.33	NT
(*S*)-(+)-**8**[b]	21.3 ± 9.3	287 ± 13.3	0.82 ± 0.04	209 ± 21.0
clozapine	2.9 ± 2.0	54.0 ± 2.0	9.0 ± 3.0	128 ± 9.3
olanzapine	4.3 ± 0.44	22.0 ± 13.1	15.0 ± 0.99	2870 ± 384
haloperidol	384 ± 22.0	>10000	12 ± 2.5	2700 ± 242

[a] Each value represents the concentration giving half-maximal inhibition of [^{3}H]pirilamine (H_1), [^{3}H]QNB (M_1), [^{3}H]prazosin (α_1), and [^{3}H]clonidine (α_2) binding to rat frontal cortex homogenate. NT, not tested. [b] From ref 13.

Table 4. Structures and D_2 Receptor Binding Affinities of Clozapine and Clozapine Analogues



compound	R	R_2	A	B	D_2 receptors (K_i, nM)
7 (clozapine)	Cl	H	NH	N	250
14 (isoclozapine)	H	Cl	NH	N	47[a]
15 (isoloxapine)	Cl	H	O	N	150[a]
16 (loxapine)	H	Cl	O	N	21[a]
17	Cl	H	CH_2	CH	520[a]
18	H	Cl	CH_2	CH	1[a]

[a] From ref 25.

reaction time task, and the minimal inhibitory dose after oral **9d** and clozapine on phencyclidine-induced cognitive impairment in rats are reported in Table 7. Figure 4 summarizes the effect of **9d** on the conditioned avoidance response in rats, the results expressed as percentages of avoidances, escapes, and failures before and after different doses, while the ability of **9d**, (*S*)-(+)-**8**, clozapine, olanzapine, and haloperidol to increase prolactin (PRL) serum levels are compared in Figure 5. The effects of **9d** at different doses on MK-801-induced hyperactivity are shown in Figure 6.

Table 5. Compounds **9d**, **9f**, and **9m**: Calculated ΔE from the Lowest Energy Conformer and Torsional Angles[a]

compd	fold[b]	piperazine position	MM ($\epsilon = 80$*r)		PM3	
			ΔE[c]	τ_N[d]	ΔE[c]	τ_N[d]
9d	A/B	MIN1	0	−169	1.73	−139
	A/B	MIN2	1.70	37	0	24
	A	BIO	4.31	−108	1.94	−106
	B	BIO	2.85	140	1.73	146
9f	A/B	MIN1	0	−169	1.76	−141
	A/B	MIN2	1.68	37	0	24
	A	BIO	4.38	−108	2.01	−108
	B	BIO	2.84	140	1.76	147
9m	A/B	MIN1	0	−169	1.72	−140
	A/B	MIN2	1.71	37	0	26
	A	BIO	4.53	−108	1.99	−106
	B	BIO	2.94	140	1.72	145

[a] Torsional angles are defined in Figure 1. [b] A fold indicates a τ value of ∼ −129° (MM results) or ∼ −124° (PM3 results); B fold indicates a τ value of ∼ 129° (MM results) or ∼ 124° (PM3 results). [c] Values in kcal/mol. [d] Values in degrees.

1. Structure−Activity Relationships and Molecular Modeling Studies, Design and Binding Profiles of the Potential Atypical Antipsychotic Agents 9d and 9m. In the 9,10-dihydropyrrolo[1,3]benzothiazepine series the introduction of hydrophilic substituents at position 1 of the pyrrolo-fused ring proved detrimental to activity (Table 1). A hydroxymethyl group led to a compound (**9a**) that was inactive on dopamine and serotonin receptors at 10^{-5} M. The 1-methoxymethyl group was better tolerated by the dopamine and serotonin binding sites, **9b** being more active than **9a**, with affinity in the high nanomolar and micromolar range (Table 1).

In a previous study[13] we observed that modifications of the alkyl chain at the N-4 of the piperazine ring provided analogues with nanomolar affinity, with the following order of potency on 5-HT_{2A}, D_2, and D_3 receptors: Me > Et > $(CH_2)_2OH$. Since in the indole series of antipsychotics an imidazolidinone group influenced the 5-HT_{2A}/D_2 affinity ratio,[22] we synthesized and tested an analogue with a (2-oxoimidazolidin-1-yl)ethyl substituent at the distal nitrogen of the piperazine ring (**9c**). Although this N-4 piperazine substituent is hindered and hydrophilic, it was well tolerated at the 5-HT_{2A}, D_2, and D_3 binding sites, showing a log *Y* score of 5.48.

To develop (*S*)-(+)-**8** analogues, still with an atypical pharmacological profile but easier to scale-up (i.e. without a chiral carbon), we investigated the possibility of specifically influencing D_2 receptor affinity by modifying the 9,10-dihydropyrrolo[1,3]benzothiazepine tricyclic system of our lead, through the introduction of a double bond at C9−10 position, and evaluated specific substituents in the tricyclic system.

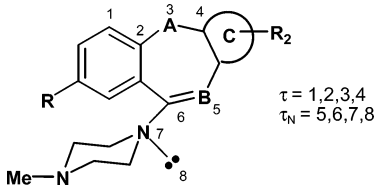


Figure 1. Schematic displaying dihedral angles τ and τ_N for compounds **7**, **9d**−**m**, **14**−**18**.

The D_2 proposed pharmacophore[23,24] requires the protonatable nitrogen within 3.0 Å above the plane of the "relevant aromatic ring", so this pharmacophoric requirement is not compatible with an axial conformation of the piperazine ring, which represents the global minimum conformer of the saturated analogues (i.e. (*S*)-(+)-**8**).[13] As expected, introduction of a double bond in the thiazepine ring (**9f**), which constrains the piperazine in a 'pseudoequatorial' position, led to increased potency at D_2 receptors (**9f**, $K_i = 0.43$ nM vs (*S*)-(+)-**8**, $K_i = 49.6$ nM) with no change in 5-HT_{2A} receptor affinity. Consequently, **9f** has a typical log *Y* score, similar to haloperidol (Table 1).

The C9/C10 double bond also influenced the rotation of the piperazine ring (τ_N, Figure 1) by means of conjugation and steric effects, favoring the right orientation of the protonatable nitrogen lone pair for an optimal D_2 receptor interaction. The proposed D_2 bioactive conformation of **9f** (A-fold, BIO; Table 5) was therefore expected to be energetically favored with respect to the parent compound (*S*)-(+)-**8**; semiempirical (MOPAC, PM3, Insight2000) calculations did indicate a smaller energy difference between the D_2 bioactive conformation and the global minimum conformer of **9f** with respect to (*S*)-(+)-**8** ($\Delta E_{\mathbf{9f}} = 2.01$ kcal/mol vs $\Delta E_{(S)-(+)-\mathbf{8}} = 5.06$ kcal/mol).[13]

To confer atypicality on the pyrrolo[1,3]benzothiazepine analogues, represented by **9f**, we explored the possibility of specifically lowering D_2 receptor affinity by exploiting the effect of different substituents on the tricyclic skeleton. Our strategy was based on the hypothesis that tricyclic antipsychotics may interact with the D_2 receptor active site by adopting both folds of the tricyclic system (positive or negative values of τ, as defined in Figure 1), and that the chemical/physical properties of the tricyclic skeleton can drive the binding mode determining which aromatic ring is preferentially recognized as the "relevant" one (see Figures 2 and 3). This hypothesis is supported by the different D_2 receptor

Table 6. In Vivo Pharmacological Profile of **9d**. Inhibition of Different Behavioral Responses after Oral Administration of the Test and Reference Compounds

compd	5-MeO-DMT induced head twitches		apomorphine climbing		spontaneous locomotor activity		MK-801 induced hyperactivity (ID_{min})		CAR		CAT		CAT/CAR ratio
	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	mg/kg	μmol/kg[a]	
clozapine	5.35	16.4	5.72	17.5	5.50	16.8			4.89	15	>100	>306	>20.5
olanzapine	1.45	4.65	2.69	8.61	2.81	8.99	1.92	6.15	1.46	4.67	21.2	67.9	15
9d	1.99	6.7	1.64	5.5	0.51	1.71	1.1	3.69	1.1	3.7	>100	>336.2	>90.9
(*S*)-(+)-**8**[c]		14.3		19.5								114.8	

[a] Results are expressed as ED_{50} or minimal effective dose (MED). [b] **9d** and reference drugs were used as a free base. [c] From ref 13, used as hydrochloride salt. CAT: catalepsy. CAR: conditioned avoidance response. CAT/CAR ratio: atypicality index in vivo. 5-MeO-DMT: 5-methoxy-*N*,*N*-dimethyltryptamine; MK-801: (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate (dizocilpine).

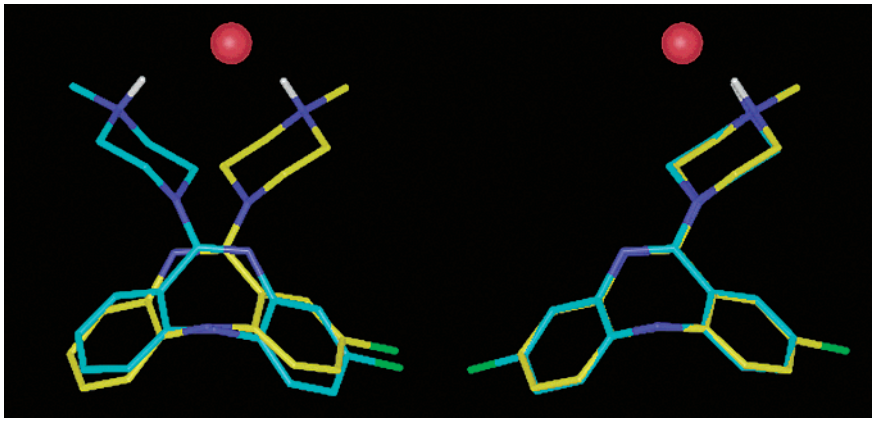


Figure 2. Superimposition of the two hypothetical binding modes of clozapine (**7**; cyan) at D_2 receptors on the D_2 bioactive conformation of isoclozapine (**14**; yellow); fitting points: the centroids of the aromatic rings and a point positioned at 2.8 Å along the distal nitrogen lone pair vector, simulating the receptor site interaction point (red ball). Nitrogens are in blue, chlorine atoms in green. Hydrogens are omitted, for clarity, with the exception of the one protonating the distal nitrogen (white).

Table 7. Effect of **9d** (0.3 mg/kg, ip) Administered Alone or with Phencyclidine (PCP, 2 mg/kg, sc) on Accurancy (% correct) and Impulsivity (no. premature responses) of Rats ($n = 9$) Performing a Five-Choice Serial Reaction Time Task[a,b]

treatment and compd	%correct responses	no. of premature responses	min ID (μmol/kg, ip)
vehicle + saline	87.4 ± 1.6	6.4 ± 1.2	
9d + saline	80.1 ± 1.8	5.2 ± 1.9	
vehicle + PCP	72.5 ± 3.5*	24.1 ± 5.4*	
9d + saline	81.5 ± 2.4**	11.6 ± 5.2	
9d			1.0
clozapine			6.9

[a] Minimal inhibitory dose (min ID) after intraperitoneal administration of **9d** and clozapine on phencyclidine-induced cognitive impairment in rats. [b] Tukey's test: $^*p \leq 0.05$ vs vehicle + saline. $^{**}p \leq 0.05$ vs vehicle + PCP.

affinity of clozapine analogues substituted at position 2 or 8,[25] as reported in Table 4.

The distance between the centroid of the substituted aromatic ring and the distal nitrogen atom was an essential parameter for D_2 receptor affinity,[23] and the lower D_2 receptor affinity of clozapine (**7**) is responsible for its atypical pharmacological profile, whereas isoclozapine (**14**) is typical.[26] In the most potent D_2 receptor ligands reported in Table 4, i.e., **14**, loxapine (**16**), and **18**, and in **9f**, the distance between the centroid of the substituted aromatic ring and the protonatable nitrogen is ~6.0 Å, and ~3.0 Å between the protonatable nitrogen and the plane of the substituted aromatic ring. When the chlorine substituent is at position 2, as in **7**, isoloxapine (**15**) and **17** (weak D_2 receptor ligands), the distance between the centroid of the substituted aromatic ring and the protonatable nitrogen rises to ~8.0 Å, with ~0.5 Å between the protonatable nitrogen and the plane of the substituted aromatic ring. Nevertheless, for both series of clozapine and isoclozapine analogues it is possible to direct the distal nitrogen lone pair toward the same point at 2.8 Å along the lone pair vector, which simulates the receptor site interaction point,[24] through rotation of τ_N; however, for the two series of compounds, the piperazine ring occupies different regions from the substituted aromatic ring. If the substituted aromatic ring of clozapine (D_2, $K_i = 250$ nM) is still the "relevant" one (Figure 2, left), the piperazine ring is positioned differently from the isoclozapine one (D_2, $K_i = 47$ nM; Table 4). On the other hand, if clozapine adopts the opposite fold of the tricyclic system (Figure 2, right), the piperazine ring is correctly oriented but the substituted aromatic ring occupies a different area of the receptor. Consequently, in both cases clozapine analogues would be less effective in binding D_2 receptors than isoclozapine-related compounds.

To test the two possible binding modes at the D_2 receptor for our compounds, we initially calculated (Discover, Insight2000, Accelrys, San Diego) the conformational energy inversion barrier for the pyrrolo[1,3]-benzothiazepine system, which was 13 kcal/mol (Figure 7, Supporting Information), in accordance with that calculated for dibenzo[*b*,*e*]azepine derivatives.[27] This suggested that the pyrrolo[1,3]benzothiazepine system can undergo a ring inversion under physiological conditions, as happens with similar tricyclic systems (e.g. dibenzodiazepines, dibenzothiepines, dibenzoxazepines),[23,24a] resulting in A-fold and B-fold types (negative and positive values of τ, respectively; Table 5).

Second, we made a conformational search on the rotation of the piperazine ring, systematically varying τ_N, to determine the corresponding energy minima. Since, as for other tricyclic systems[28] the A- and B-fold behaved as conformational enantiomers, a single conformational energy value is reported in Table 5 for the conformers of both folds. The τ_N value required for positioning the lone pair of the distal piperazine nitrogen to optimally fulfill the D_2 pharmacophore was established for both folds and the calculated energies of the resulting conformations are reported in Table 5 (BIO piperazine positions). Compounds bearing a hydrogen (**9d**) or a chlorine atom (**9f**) at C-7, or a methyl group at C-1 (**9m**), were subjected to a thorough analysis using their molecular mechanic (MM) resulting structures as starting points for full semiempirical PM3 geometry optimization.

Our calculations, done in both an aqueous environment (MM, $\epsilon = 80$*r) and in the gas phase (PM3) confirmed that the correct orientation of the distal piperazine nitrogen lone pair is energetically accessible for both folds of the pyrrolo[1,3]benzothiazepine system. When the chlorine atom of **9f** is replaced by a hydrogen (**9d**), if the binding mode is driven by the dipole of the electron rich pyrrole ring, favorably interacting with the same receptor area occupied by the chloro-substituted

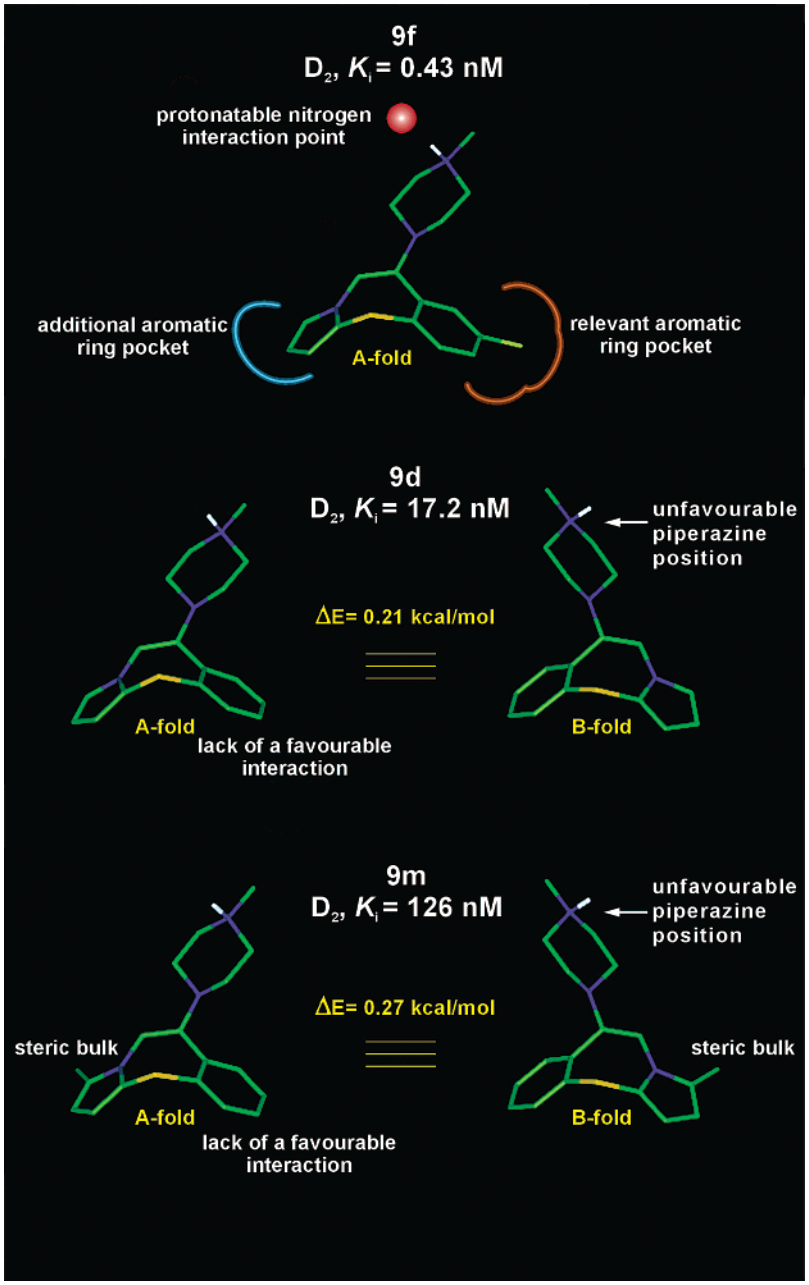


Figure 3. Proposed D_2 bioactive conformation of **9f** and hypothetical D_2 receptor binding modes for compounds **9d** and **9m**. All structures are superimposed on the centroids of the aromatic rings and a point positioned at 2.8 Å along the distal nitrogen lone pair vector, simulating the receptor site interaction point. Nitrogens are in blue, carbons green, chlorine atoms light green. Hydrogens were omitted for clarity with the exception of the one protonating the distal nitrogen (white). ΔE values ($E_{A-fold} - E_{B-fold}$) refer to PM3 conformational energies.

aromatic ring of **9f**, then the piperazine ring would be unfavorably positioned (i.e. pyrrole recognized as the "relevant" ring; Figure 3, **9d**, B-fold). Optimal orientation of the piperazine ring can only be achieved by positioning the benzo-fused system in the "relevant ring receptor pocket", thus lacking a favorable interaction (Figure 3, **9d**, A-fold). In both cases the D_2 receptor pharmacophore would not be optimally fulfilled and, accordingly, the resulting compound **9d** was assumed to have lower D_2 receptor affinity (in binding assays: **9d**, $K_i = 17.2$ nM, log $Y = 4.98$; **9f**, $K_i = 0.43$ nM, log $Y = 8.20$).

Following the theory that modification of the chemical/physical properties of the tricyclic system might drive the D_2 receptor binding mode, and taking into account 3D-SAR studies on an indole series of antipsychotics,[24c] we introduced a methyl substituent at C-1 of the pyrrole ring. As reported in Figure 3, the resulting compound **9m** presents further unfavorable features, assuming either an A- or a B-fold. We therefore assumed a further decrease in D_2 receptor affinity in comparison with **9d** and **9f**, maintaining an optimum 5-HT_{2A} receptor affinity. The expected binding profile of **9m** was confirmed by pharmacological experiments (Table 1) (D_2, $K_i = 126$ nM, 5-HT_{2A}, $K_i = 1.1$ nM, log $Y = 3.18$, rank order of D_2 receptor potency: **9f** > **9d** > **9m**).

The resulting pharmacological profile of compounds **9f**, **9d**, and **9m** indicated that small changes in the

chemical structure of the tricyclic skeleton could be rationally used to drive the D_2 receptor binding mode, with the aim of obtaining an antipsychotic with an atypical binding profile. These data suggested the methyl group at C-1 and a hydrogen at C-7 would provide a balanced interaction between serotonin and dopamine receptors.

We expanded our SAR studies by introducing different substituents at the C-7 (**9e**, **9g**), and C-1 (**9h-l**) positions of the pyrrolo[1,3]benzothiazepine system. In particular we evaluated the influence on the pharmacological profile of: 1) different halogens at C-7, 2) substitution of the methyl group at C-1 of **9d** with substituents with either weak electron-donating (CH_2-OH) or electron-withdrawing (CHO, CH_2=NOH) effects on the pyrrole ring, 3) a large lipophilic substituent at C-1 (CH_2O*i*Pr, **9k**).

The substitution of the chlorine atom of **9f** with a bromine (**9g**) had negligible effect on dopamine and $5HT_{2A}$ receptor affinity (**9g**, log $Y = 8.63$); however, a fluorine atom in the same position led to a compound (**9e**) 20 times less potent toward D_2 receptors compared to the other halogenated analogues (D_2, $K_i = 8.5$ nM, 5-HT_{2A}, $K_i = 0.35$ nM, log $Y = 5.37$, Table 1). These results indicated that the balance between electronic and lipophilic properties of the halogen at C-7 is crucial for an optimal interaction with the D_2 binding site, but it does not affect $5HT_{2A}$ receptor affinity.

Substitutions of the methyl group at C-1 of the pyrrole ring of **9m** led to compounds (**9h**−**l**) showing weak-to-negligible affinity toward dopamine (D_1, D_2, D_3) receptors; small hydrophilic substituents at this position were still tolerated by $5HT_{2A}$ receptors (**9h** $K_i = 20$ nM, **9j** $K_i = 19$ nM, Table 1). A conformational search on the analogues **9h**,**j**−**l**, followed by MM energy minimizations, indicated that none of the structural modifications affected the compounds' ability to reach the D_2 bioactive conformation (Table 8, Supporting Information); this suggested that the loss of affinity toward this receptor was due to specific unfavorable interactions between the substituents introduced at C-1 and the protein binding site. In the case of **9i**, the additional formyl substituent at C-10 unfavorably affected the rotation of the piperazine ring, preventing the correct orientation of the protonatable nitrogen lone pair with respect to the tricyclic system.

In our series, compounds **9d** and **9m** showed potent 5-HT_{2A} receptor affinity accompanied by lower affinity for D_2 and D_1 receptors, maintaining D_3 receptor affinity in the nanomolar range. Their favorable 5-HT_{2A}/D_2 affinity ratio may greatly contribute to the atypical profile and efficacy in vivo of these new potential antipsychotic agents. Indeed, serotonin, through its interaction with the 5-HT_{2A} receptor, inhibits neuronal activity in the substantia nigra (SN) and ventral tegmental area (VTA).[29,30] 5-HT_{2A} antagonists may increase the firing rate of midbrain dopaminergic neurons in a state-dependent manner and potentiate the increase in the activity of nigrostriatal DA-containing neurons in response to moderate D_2 receptor blockade by antipsychotic drugs.[31] 5-HT_{2A} antagonism may therefore prevent or alleviate extrapyramidal symptoms (EPS) induced by acute or long-term treatment with typical neuroleptics (haloperidol), through their influence on nigrostriatal dopaminergic transmission.[32−36]

On the basis of their atypical binding profile, **9d** and **9m** were therefore tested against a panel of other receptors including H_1, M_1, and adrenergic receptors, and compared to reference drugs (Table 3). Both analogues showed similar binding affinities for H_1 and M_1 receptors, but differed at the α_1 receptors, the unsubstituted **9d** being 8 times more potent. The affinity for α receptors is a critical aspect of the clozapine/olanzapine binding profile[37] and the α_2 receptor occupancy might explain the marked increase in dopamine output in the prefrontal cortex induced by clozapine, which is beneficial to cognitive functions. As reported in Table 3, compound **9d** showed significant affinity for the α_2 receptor, although lower than that of clozapine and of (*S*)-(+)-**8**, and potent α_1 receptor affinity. The α_2 blocking activity might enhance the clinical efficacy of **9d**, and its α_1 blocking activity may protect against dopamine deficits.

Taking into account the greater potency of **9d** than **9m** on serotonin, dopamine and α receptors ($5HT_{2A}/D_2$ affinity ratio 1.20 and calculated log Y 4.98, similar to olanzapine), this compound was considered a promising atypical antipsychotic agent and subjected to in vivo pharmacological characterization.

2. Behavioral and Biochemical Effects. In Vivo Characterization of the Promising Atypical Antipsychotic Agent 9d. We have previously reported the pharmacological characterization of (*S*)-(+)-**8**, a prototype of the pyrrolobenzothiazepine class of atypical antipsychotic agents.[13] This compound was as active as clozapine and olanzapine, serving as an important lead for developing atypical antipsychotics with an optimized pharmacological profile in terms of potency with limited adverse effects. In a comprehensive study involving molecular modeling, synthesis, and biology, we identified **9d** as a potential atypical antipsychotic agent, and its pharmacological profile was confirmed in vivo. Tests were done using the compound as a free base, and haloperidol, clozapine, and olanzapine were tested in the same experimental conditions.

1. Antipsychotic Activity and Atypicality of 9d. Blockade of spontaneous locomotor activity, antagonism of climbing elicited by the direct dopamine agonist apomorphine in mice, and reduction of conditioned avoidance response (CAR) in rats are robust and reproducible models, sensitive to D_2 receptor antagonists, for predicting therapeutic efficacy against positive symptoms. These behavioral assays have been employed in vivo to demonstrate **9d** dopamine antagonist activity and, indirectly, its own ability to interact with the mesolimbic dopaminergic system, as predictive of **9d** antipsychotic potential.

Rat spontaneous locomotor activity was significantly reduced by **9d**, with an ID_{50} of 0.51 mg/kg (Table 6). The ability to antagonize apomorphine-induced climbing behavior in mice was also evaluated. Oral **9d**, prior to 1.3 mg/kg apomorphine challenge, caused dose-related suppression of apomorphine-induced climbing behavior (Table 6). The compound showed an ED_{50} (5.5 μmol/kg) lower than olanzapine (ED_{50} 8.61 μmol/kg). This pyrrolobenzothiazepine also proved more potent than clozapine and (*S*)-(+)-**8**. These latter compounds caused dose-

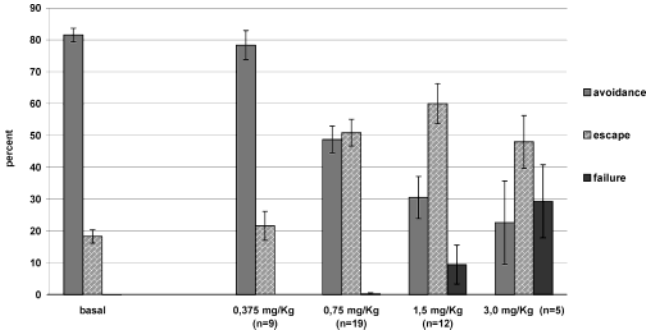


Figure 4. Effect of **9d** (0.37 mg/kg to 3 mg/kg, po) on conditioned avoidance response in rat. The results are expressed as percentages of avoidance, escapes and failures before and after different doses (number of rats for each dose are shown in parentheses).

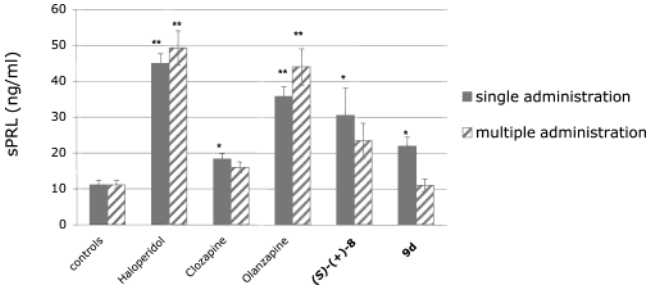


Figure 5. Serum prolactin (PRL) 180 min after single or multiple (3 days) administration of **9d**, (*S*)-(+)-**8** (as hydrochloride salts), clozapine, and olanzapine (12.6 μmol/kg corresponding to 5 mg/kg/day, sc), and haloperidol (2.66 μmol/kg corresponding to 1 mg/kg/day, sc), in rats. Each value is the mean ± SE of eight rats per group. Student's *t*-test: *: $p < 0.05$; and **: $p < 0.001$ vs corresponding control group.

related suppression of apomorphine-induced climbing up to the dose of 15 μmol/kg (ED_{50} 17.5 and 19.5 μmol/kg, respectively). In light of these results, **9d** was selected for further studies.

The antipsychotic potential of **9d** was measured by evaluation of the conditioned avoidance response (CAR) in rats. In the shuttle-box test, oral **9d** suppressed CAR with no significant effects on escape responses (Figure 4). As shown in Table 6, compound **9d** was more potent than clozapine ($ED_{50\mathbf{9d}}$ 3.7 μmol/kg; clozapine 15 μmol/kg) and as potent as olanzapine.

Furthermore, **9d** antagonizes 5-HT_{2A} receptors in vivo. After sc injection (6 min) of 5-methoxy-*N*,*N*-dimethyltriptamine (5-MeO-DMT, 10 mg/kg), we measured the number of 5-MeO-DMT-induced head twitches for 15 min. **9d** was administered orally 60 min before 5-MeO-DMT, and data were compared with clozapine, olanzapine, and (*S*)-(+)-**8**. **9d** antagonized 5-MeO-DMT-induced head twitches at doses similar to olanzapine (ED_{50} 6.7 and 4.65 μmol/kg, respectively), while higher doses of clozapine and (*S*)-(+)-**8** were necessary to reach the same effect ($ED_{50(S)-(+)-\mathbf{8}}$ 14.3 μmol/kg; **7** 16.4 μmol/kg).

Blockade of limbic dopamine D_2 receptors has been proposed as a critical component in the positive antipsychotic effects though excessive blockade of dopamine D_2 receptors in striatum and pituitary gland is believed to be associated with the frequently reported EPS and hyperprolactinemia, respectively.

The development of new potential antipsychotic agents with a multireceptor affinity profile, causing minimal D_2 receptor antagonism, raises the possibility of treating positive symptoms without the adverse effects resulting from D_2 receptor blockade. In particular, blockade of 5-HT_{2A} receptors may potentiate the increase in the activity of nigrostriatal dopamine containing neurons in response to the blockade of D_2 dopamine receptors by antipsychotic drugs. Stimulation of 5-HT_{2A} receptors inhibits neuronal activity in the SN and VTA.[29,30] Several studies have shown that 5-HT_{2A} antagonists increase the firing rate of midbrain dopaminergic neurons in a state-dependent manner. As long as the D_2 receptor blockade is not complete, such potentiation of dopaminergic function might be expected at least partially to overcome the effects of the moderate D_2 receptor antagonism.

The degree of catalepsy is often used as measure for predicting the incidence of extrapyramidal motor disorders. Compound **9d** was administered orally to rats at doses of 3.75, 7.0, 15.0, 30.0, 60.0, and 100 mg/kg, and the catalepsy response was evaluated after 60, 120, 180, 240, and 300 min. **9d** did not induce catalepsy, with behavior similar to clozapine (100 mg/kg, p.o.). As shown in Table 6, **9d** had low cataleptogenic potential (ED_{50} > 100 mg/kg, > 336.2 μmol/kg), like clozapine but differently from olanzapine, which, in the same experimental conditions, induced catalepsy in 50% of animals 240 min after oral administration of 21 mg/kg (ED_{50} 67.9 μmol/kg). Thus, compared to olanzapine, **9d** has higher threshold for inducing catalepsy and may, by analogy, translate into lower clinical EPS liability.

Thus, this limited propensity to elicit catalepsy results in a large spread between doses possibly inducing catalepsy and blocking the avoidance response (CAT/CAR ratio >90.9, Table 6). These data suggest a preferential ability of **9d** to modulate mesolimbic instead of nigrostriatal dopaminergic neurotransmission, highlighting its atypicality together with a low propensity to induce unwanted extrapyramidal motor disturbances at therapeutically useful doses.

2. Prolactin Secretion. In the anterior pituitary gland dopamine, interacting with D_2 receptors, inhibits prolactin (PRL) release. The induction of PRL secretion by antipsychotic agents may reflect a negative intrinsic activity at pituitary D_2 sites and may be considered an indicator of typicality.[38]

Clozapine produces a transient increase in plasma PRL levels in rodents. This has been attributed to the drug's low D_2 receptor affinity and to its ability to increase dopamine release from the tuberoinfundibular dopaminergic neurons, which displaces clozapine from pituitary D_2 receptors. A dose of 12.6 μmol/kg (sc) of **9d** increased PRL serum levels to the same extent as clozapine but less than olanzapine and (*S*)-(+)-**8**, at the same dose, and the typical neuroleptic haloperidol at the dose of 2.66 μmol/kg (Figure 5).[38] However, while the olanzapine-induced increase after a single dose was lower than after multiple doses, PRL rose less after multiple doses of **9d**, than after a single dose. These results indicate that **9d** has a weak influence on the tuberoinfundibular dopaminergic system and suggest indirectly that its antipsychotic activity may be associated with fewer effects on prolactin secretion than olanzapine.

3. Therapeutic Potential of 9d against Cognitive and Negative Symptoms. DA blocking agents are

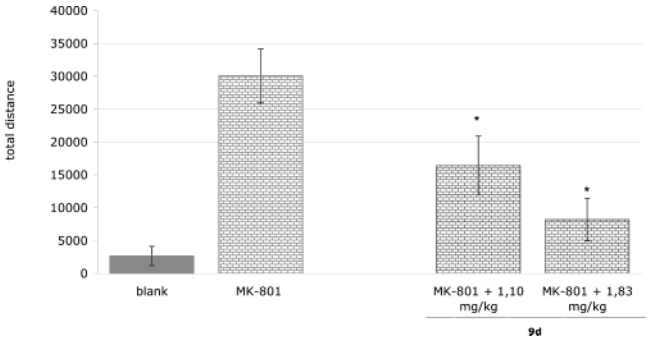


Figure 6. Effect of **9d** (1.1 mg/kg and 1.83 mg/kg, po) on MK-801 (0.3 mg/kg, ip)-induced hyperactivity in rats. The means of eight rats for each treatment group are shown. Student's *t*-test: *: $p \leq 0.05$ vs MK-801 group.

highly effective in treating positive symptoms, but are not an ideal approach to schizophrenia. A more effective strategy would be to increase tonic DA receptor stimulation in the prefrontal cortex to prevent dopaminergic mesolimbic hyperactivity.

Atypical antipsychotics may attenuate negative and cognitive symptoms through this mechanism. There are currently no straightforward experimental approaches for predicting the activity of new antipsychotic drugs against negative and cognitive symptoms. However, selective antagonism of the effects of the noncompetitive *N*-methyl-D-aspartate (NMDA) antagonists phencyclidine (PCP) or dizocilpine (MK-801) has been proposed as a robust animal model for the negative and cognitive symptoms of schizophrenia.

The MK-801-induced rat hyperactivity model has been used to indirectly evaluate the ability of **9d** to oppose cortical dopaminergic hypofunction induced by NMDA receptor blockade, and the subsequent dopaminergic mesolimbic hyperfunction. In this test **9d** caused dose dependent inhibition of MK-801-induced hyperactivity and was more potent than olanzapine (**9d** ID_{min} 3.69 μmol/kg vs olanzapine 6.15 μmol/kg) (Table 6 and Figure 6). The ability of **9d** to attenuate attentional dysfunctions in schizophrenia was also investigated in rats after systemic administration of PCP, which produces attentional and cognitive deficits. A five-choice serial reaction time (5-CSRT) task was used, in which hungry rats were required to locate brief visual targets presented randomly in one of five locations in a specially designed chamber. PCP reduced the percentage of correct responses and increased the number of premature responses (Table 7). **9d** (0.3 mg/kg ip) reversed the decrease in correct responses and the increase in premature responses induced by PCP. Compared to clozapine, **9d** was also more potent in inhibiting PCP-induced impairment of cognitive functions (6.9 μmol/kg and 1.0 μmol/kg, respectively).

Conclusion

In summary, we expanded the SAR studies of the class of pyrrolo[1,3]benzothiazepine dopamine and serotonin receptor antagonists, represented by the atypical antipsychotic (*S*)-(+)-**8**. New tricyclic analogues showing an atypical binding profile have been identified. The analogues **9d** and **9m**, which have unique receptor affinity properties, 5-HT_{2A}/D_2 ratios, and log *Y* scores were identified as potential atypical drugs. Although these two compounds showed similar atypical binding properties, the choice of **9d** for further pharmacological investigation was mainly dictated by its more convenient synthesis, in terms of number of synthetic steps and overall yield. Consequently, we pharmacologically characterized **9d** as a new drug candidate. Its receptor affinity profile suggested a complex interaction on the cortical receptors involved in the regulation of the activity of prefrontal cortical cells innervated by the VTA neurons, like 5-HT_{2A}, dopaminergic and α-adrenergic receptor subtypes. The 5-HT_{2A}/D_2 ratio (expressed by the log *Y* score, 4.98) is particularly favorable and preliminary in vivo studies confirmed pharmacological effects superior than olanzapine and clozapine on 5-OMe-DMT-induced head twitches, apomorphine-induced climbing, and spontaneous locomotor activity in animals. The benzothiazepine **9d** also showed significant inhibitory properties against MK-801-induced hyperactivity and PCP-induced cognitive impairment, two animal models in which most of the potential antipsychotics fail. This compound displayed atypical antipsychotic activity at 1.1 mg/kg, a dose 100 times lower than that required to generate catalepsy; it therefore has very low cataleptogenic potential (ED_{50} >100 mg/kg, CAT/CAR ratio >90), and does not significantly elevate serum PRL in comparison with olanzapine, clozapine, and haloperidol. These findings indirectly illustrate the high, selective activity of compound **9d** toward the mesolimbic and mesocortical dopaminergic system and describe a pharmacological profile superior to olanzapine and (*S*)-(+)-**8**. Consequently, the benzothiazepine **9d** was selected for thorough pharmacological investigation. Molecular modeling studies on this compound and the design of its 1-methyl analogue **9m** led to the identification of several structural and conformational features responsible for their atypicality, and they will pave the way for the design of novel atypical antipsychotics with a tricyclic core system.

Experimental Procedure

Melting points were determined using an Electrothermal 8103 apparatus. 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 value of chemical shifts (δ) are given in ppm and coupling constants (*J*) in hertz (Hz). All reactions were carried out in an argon atmosphere. Flash chromatography purification were performed by using Merck silica gel 230−400 mesh. GC-MS were performed on a Saturn 3 (Varian) or Saturn 2000 (Varian) GC-MS System using a Chrompack DB5 capillary column (30 m × 0.25 mm i.d.; 0.25 μm film thickness). Mass spectra were recorded using a VG 70−250S spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 °C elemental analyzer and the results were within 0.4% of the theoretical values. Yields refer to purified products and are not optimized.

(±)-7-Chloro-9-(4-methylpiperazin-1-yl)-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepin-1-carbaldehyde (10). A mixture of phosphorus oxychloride (17.1 μL, 30.0 mg, 0.18 mmol) and *N*-methylformanilide (22.7 μL, 25.0 mg, 0.18 mmol) was stirred for 30 min at room temperature. Then (±)−(8) (30.0 mg, 0.09 mmol) was added, and the resulting mixture was stirred overnight at room temperature. Then water (1 mL) was added, and the mixture was extracted with dichloromethane (3 × 2.5 mL). Combined organic layers were dried over sodium sulfate, filtered, and evaporated. The crude product was purified by means of flash chromatography (5% methanol in dichloromethane as eluant) and afforded 20.0 mg of **10** as a yellowish amorphous solid (59% yield): IR ($CHCl_3$) 1653, 1509 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.48 (s, 1H), 7.64 (s, 1H),

7.29 (d, 1H, J = 8.4 Hz), 7.10 (m, 1H), 6.80 (d, 1H, J = 4.0 Hz), 6.36 (d, 1H, J = 3.9 Hz), 5.40 (dd, 1H, J = 13.8, 3.9 Hz), 5.10−5.03 (m, 1H), 4.01 (dd, 1H, J = 10.7, 3.9 Hz), 2.70−2.24 (m, 11H). Anal. ($C_{18}H_{20}ClN_3OS$) C, H, N.

(±)-7-Chloro-1-hydroxymethyl-9-(4-methylpiperazin-1-yl)-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepine (9a). To a solution of **10** (40.0 mg, 0.11 mmol) in ethanol (5 mL) was added portionwise sodium borohydride (15.0 mg, 0.40 mmol). The resulting mixture was stirred at room temperature for 5 h. Then the solvent was removed, and the residue was treated with water. After extraction with dichloromethane (3 × 2.5 mL), combined organic layers were dried over sodium sulfate, filtered, and evaporated. The crude product was purified by means of flash chromatography (8% methanol and 8% triethylamine in ethyl acetate as eluant) to give 40.0 mg of **9a** as a white amorphous solid (99% yield): IR (Nujol) 3330, 3300 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.31−7.20 (m, 2H), 7.15−7.14 (m, 1H), 6.22 (d, 1H, J = 3.6 Hz), 6.04 (d, 1H, J = 3.6 Hz), 4.84−4.50 (m, 4H), 4.03 (d, 1H, J = 6.1 Hz), 2.29−2.21 (m, 8H), 2.17 (s, 3H). Anal. ($C_{18}H_{22}ClN_3OS$) C, H, N.

(±)-7-Chloro-1-methoxymethyl-9-(4-methylpiperazin-1-yl)-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepine (9b). To a cooled solution (0 °C) of **9a** (40.0 mg, 0.11 mmol) in dichloromethane (1.5 mL) was added dropwise a solution of (*N*,*N*-dimethylamino)sulfurtrifluoride (DAST) (20.0 μL, 20.0 mg, 0.15 mmol) in dichloromethane (1.5 mL). The resulting mixture was stirred at room temperature for 1 h. Then methanol was added, and, after stirring for 10 min, the solvent was removed and the crude product was chromatographed (8% methanol and 8% triethylamine in ethyl acetate as eluant) to give 20 mg of **9b** (46% yield) as a yellowish amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.56 (m, 1H), 7.28 (d, 1H, J = 8.6 Hz), 7.07−7.02 (m, 1H), 6.22 (d, 1H, J = 3.7 Hz), 6.02 (d, 1H, J = 3.5 Hz), 4.84 (dd, 1H, J = 14.0, 10.1 Hz), 4.62−4.25 (m, 3H), 3.93 (dd, 1H, J = 10.1, 3.4 Hz), 3.29 (s, 3H), 2.63−2.26 (m, 11H); MS m/z 377 (M^+), 346, 332, 292, 277, 267, 246, 214, 113 (100), 100. Anal. ($C_{19}H_{24}ClN_3OS$) C, H, N.

(±)-7-Chloro-9-[4-[2-(imidazolidin-2-on-1-yl)ethyl]piperazin-1-yl]-9,10-dihydro pyrrolo[2,1-*b*][1,3]benzothiazepine (9c). To a solution of 1-[2-(1-piperazinyl)ethyl]-2-imidazolinone (60.0 mg, 0.29 mmol) in 2-butanone (3 mL), **(±)**-11 (40.0 mg, 0.13 mmol) was added. The resulting mixture was stirred at reflux overnight. Then the solvent was removed in vacuo, and the crude brown oily product was chromatographed (0.1% methanol and 0.1% triethylamine in ethyl acetate) to afford the desired product **9c** as a brown oil (37% yield): IR (Nujol) 3410, 1653 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.50−7.49 (d, 1H, J = 2.1 Hz), 7.29−7.26 (m, 1H), 7.05 (dd, 1H, J = 8.3, 2.3 Hz), 6.85−6.82 (m, 1H), 6.34−6.26 (m, 1H), 6.05 (m, 1H), 4.67 (dd, 1H, J = 14.1, 8.9 Hz), 4.44 (dd, 1H, J = 14.2, 3.5 Hz), 3.92 (dd, 1H, J = 8.9, 3.5 Hz), 3.53−3.24 (m, 6H), 2.56−2.20 (m, 10H). Anal. ($C_{21}H_{26}ClN_5OS$) C, H, N.

9-(4-Methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepine (9d). A mixture of 9,10-dihydropyrrolo[2,1-b][1,3]-benzothiazepin-9-one **12a** (0.24 g, 1.11 mmol), *N*-methylpiperazine (0.55 mL, 0.50 g, 4.99 mmol), and trimethylsilyl triflate (0.55 mL, 0.68 g, 3.05 mmol) was stirred at 120 °C under argon for 20 min. Then more *N*-methylpiperazine (0.55 mL) was added, and stirring was continued for 3 h at 120 °C. Water (5 mL) was added, and the mixture was extracted with dichloromethane (3 × 10 mL). The combined organic layers were dried over sodium sulfate, filtered, and evaporated to give a crude oily product that was purified by means of a flash chromatography (20% methanol in ethyl acetate) to afford 0.114 g of pure **9d** as a yellowish amorphous solid (84% yield): ^{1}H NMR ($CDCl_3$) δ 7.65 (m, 1H), 7.50 (m, 1H), 7.34−7.22 (m, 2H), 6.75 (m, 1H), 6.20 (m, 1H), 6.12 (m, 1H), 2.89 (m, 4H), 2.53 (m, 4H), 2.34 (s, 3H). Anal. ($C_{17}H_{19}N_3S$) C, H, N.

7-Fluoro-9-(4-methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]-benzothiazepine (9e). The title compound was obtained following the procedure as described for **9d**, starting from 7-fluoro-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepin-9-one **12b** (0.10 g, 0.429 mmol). After purification, **9e** was obtained as a white amorphous solid (0.13 g, 96% yield): ^{1}H NMR (DMSO-d_6) δ 7.60−7.50 (m, 1H), 7.43−7.32 (m, 1H), 7.23 (m, 1H), 7.0 (m, 1H), 6.85 (s, 1H), 6.21 (m, 1H), 6.10 (m, 1H), 2.87 (m, 4H), 2.45 (m, 4H), 2.24 (s, 3H). Anal. ($C_{17}H_{18}FN_3S$) C, H, N.

7-Chloro-9-(4-methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]-benzothiazepine (9f). The title compound was obtained following the procedure as described for **9d**, starting from 7-chloro-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepin-9-one **12c** (0.10 g, 0.40 mmol). After purification, **9f** was obtained as a white amorphous solid (0.14 g, 98% yield): ^{1}H NMR ($CDCl_3$) δ 7.62 (d, 1H, J = 2.1 Hz), 7.41 (d, 1H, J = 8.4 Hz), 7.20 (d, 1H, J = 2.6 Hz), 6.75−6.72 (m, 1H), 6.58 (s, 1H), 6.22−6.19 (m, 1H), 6.10 (m, 1H), 2.90−2.85 (m, 4H), 2.55−2.48 (m, 4H), 2.34 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 143.8, 140.5, 137.9, 134.8, 133.2, 129.8, 129.6, 127.9, 123.2, 112.7, 111.6, 111.2, 55.2, 50.1, 46.2. Anal. ($C_{17}H_{18}ClN_3S$) C, H, N.

7-Bromo-9-(4-methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]-benzothiazepine (9g). The title compound was obtained following the procedure described for **9d**, starting from 7-bromo-9,10-dihydropyrrolo[2,1-*b*][1,3]benzothiazepin-9-one **12d** (0.10 g, 0.34 mmol). After purification by means of flash chromatography (20% methanol in ethyl acetate), the title compound **9g** was obtained as a yellowish amorphous solid (0.114 g, 84% yield): ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H),7.37 (m, 2H), 6.73 (m, 1H),6.57 (m, 1H), 6.20 (m, 1H), 6.10 (m, 1H), 2.87 (m, 4H), 2.53 (m, 4H), 2.35 (s, 3H). Anal. ($C_{17}H_{18}BrN_3S$) C, H, N.

9-(4-Methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepine-1-carbaldehyde (9h). A mixture of phosphorus oxychloride (50.7 μL, 80.0 mg, 0.54 mmol) and *N*-methylformanilide (67.15 μL, 70.0 mg, 0.54 mmol) was stirred for 30 min at room temperature. Then **9d** (120.0 mg, 0.42 mmol) was added, and the resulting mixture was stirred overnight at room temperature. Then water (5 mL) was added, and the reaction mixture was extracted with dichloromethane (3 × 5 mL). Combined organic layers were dried over sodium sulfate, filtered, and concentrated. Purification was accomplished by means of flash chromatography (5% methanol in dichloromethane) and afforded 50.0 mg of **9h** as a yellowish amorphous solid (37% yield); IR (KBr) 1660, 1510 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.45 (s, 1H), 7.65 (m, 1H), 7.46 (m, 1H), 7.32 (m, 2H), 7.04 (s, 1H), 6.93 (d, 1H, J = 3.9 Hz), 6.24 (d, 1H, J = 4.3 Hz), 3.15−2.95 (m, 4H), 2.57 (m, 4H), 2.35 (s, 3H); MS m/z 325 (M^+), 256, 81, 69 (100), 41. Anal. ($C_{18}H_{19}N_3OS$) C, H, N.

9-(4-Methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepine-1,10-dicarbaldehyde (9i). A mixture of phosphorus oxychloride (18.0 μL, 30.0 mg, 0.20 mmol) and *N*-methylformanilide (24 μL, 26 mg, 0.20 mmol) was stirred for 30 min at room temperature. Then **9d** (30.0 mg, 0.10 mmol) was added, and the resulting mixture was stirred overnight at room temperature. Then water was added, and the mixture was extracted with dichloromethane (3 × 5 mL). Combined organic layers were dried over sodium sulfate, filtered, and concentrated. Purification was accomplished by means of flash chromatography (5% methanol in dichloromethane) and afforded 11.3 mg of **9i** as a yellowish amorphous solid (35% yield): IR (KBr) 1664, 1505 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.68 (s, 1H), 9.42 (s, 1H), 7.59 (m, 2H), 7.42 (m, 2H), 6.87 (m, 1H), 6.31 (m, 1H), 3.70−3.62 (m, 4H), 2.59 (m, 4H), 2.38 (s, 3H); MS m/z 353 (M^+), 324, 295, 83, 70 (100), 57, 43. Anal. ($C_{19}H_{19}N_3O_2S$) C, H, N.

1-Hydroxymethyl-9-(4-methylpiperazin-1-yl)pyrrolo-[2,1-*b*][1,3]benzothiazepine (9j). To a solution of **9h** (17.0 mg, 0.05 mmol) in absolute ethanol (2.5 mL) was added sodium borohydride (7.13 mg, 0.19 mmol). The resulting mixture was stirred overnight at room temperature. The solvent was removed, water (5 mL) was added, and the solution was extracted with dichloromethane; combined organic layers were dried over sodium sulfate, filtered, and concentrated. The crude product was chromatographed (10% methanol and 10% triethylamine in ethyl acetate) to afford 9.5 mg of **9j** as a yellowish amorphous solid (59% yield): IR (KBr) 3342−3308 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.63 (m, 1H), 7.49 (m, 1H), 7.29 (m, 2H), 6.76 (s, 1H), 6.14 (d, 1H, J = 3.7 Hz), 6.05 (d, 1H, J = 3.8 Hz), 4.51 (m, 2H), 3.05 (m, 4H), 2.47 (m, 4H), 2.32 (s, 3H); MS

m/z 327 (M^+), 296, 225, 198, 87, 70 (100), 58. Anal. ($C_{18}H_{21}N_3OS$) C, H, N.

Tosylhydrazone of 9-(4-Methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepin-1-carbaldehyde (13). To a solution of **9h** (20.0 mg, 0.07 mmol) in anhydrous methanol (1.7 mL) was added tosyl hydrazide (20.0 mg, 0.10 mmol). The resulting mixture was stirred at room-temperature overnight and then 2 h at reflux. After cooling, the solvent was removed in vacuo, and the crude product was directly purified by means of a flash chromatography (10% methanol in ethyl acetate). Pure **13** was obtained (67% yield) as a white amorphous solid: IR (KBr) 3285 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.76−7.72 (d, 2H, J = 8.2 Hz), 7.67 (d, 1H, J = 9.3 Hz), 7.55 (s, 1H), 7.50−7.46 (m, 1H), 7.39−7.28 (m, 4H), 6.90 (s, 1H), 6.38−6.36 (d, 1H, J = 3.9 Hz), 6.13 (d, 1H, J = 3.9 Hz), 3.26−3.09 (m, 2H), 3.02−2.88 (m, 2H), 2.71−2.50 (m, 4H), 2.38 (s, 3H), 2.35 (s, 3H); MS *m/z* 465 ($M^+ - N_2$), 310 (100), 278, 239, 156, 91, 70, 43. Anal. ($C_{25}H_{27}N_5O_2S_2$) C, H, N.

1-Isopropoxymethyl-9-(4-methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepine (9k). To a solution of **13** (37.0 mg, 0.075 mmol) in 2-propanol (4.0 mL) was added sodium borohydride (13.0 mg, 0.45 mmol) in portions while stirring at 0 °C. The resulting mixture was stirred for 24 h at reflux and then for 48 h at room temperature. The solvent was removed in vacuo, water (5 mL) was added, and the aqueous mixture was extracted with dichloromethane (3 × 5 mL); combined organic layers were dried over sodium sulfate, filtered, and concentrated to give the crude product which was chromatographed (0.8% methanol in ethyl acetate) to afford **9k** (14.0 mg) as a yellowish amorphous solid (51% yield): ^1H NMR ($CDCl_3$) δ 7.63 (m, 1H), 7.48 (m, 1H), 7.27 (m, 2H), 6.76 (s, 1H), 6.14 (m, 1H), 6.05 (m, 1H), 4.37 (br s, 2H), 3.60 (m, 1H), 2.52 (m, 4H), 2.92 (m, 4H), 2.34 (s, 3H), 1.17 (s, 3H), 1.14 (s, 3H); MS *m/z* 369 (M^+) (100), 326, 310, 296, 97, 70. Anal. ($C_{21}H_{27}N_3OS$) C, H, N.

1-Methylenoxime-9-(4-methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepine (9l). To a solution of **9h** (10.0 mg, 0.031 mmol) in dichloromethane (1.0 mL) were added hydroxylamine hydrochloride (43.0 mg, 0.062 mmol) and pyridine (5.0 μL, 49.0 mg, 0.062 mmol). The reaction mixture was stirred 1 h at room temperature; then dry potassium carbonate (8.0 mg, 0.062 mmol) was added, and the mixture was stirred for further 72 h at room tempertaure. Then further hydroxylamine hydrochloride (43.0 mg, 0.062 mmol) and dry potassium carbonate (17.0 mg, 0.124 mmol) were added, and the solution was stirred at room-temperature overnight. Then water (3 mL) was added, the organic phase was separated, and the aqueous phase was extracted with dichloromethane; combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The crude product was chromatographed (10% methanol in ethyl ether) to afford 2.5 mg of the pure **9l** as a yellowish oil (17% yield); IR (KBr) 3285 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.80 (s, 1H);7.68 (m, 1H), 7.48 (m, 1H), 7.30 (m, 2H), 6.94 (s, 1H), 6.38 (d, 1H, J = 3.9 Hz), 6.15 (d, 1H, J = 3.8 Hz), 3.02 (m, 4H), 2.62 (m, 4H), 2.40 (s, 3H); MS *m/z* 340 (M^+), 323, 297, 225, 99, 70 (100), 56, 43. Anal. ($C_{18}H_{20}N_4OS$) C, H, N.

1-Methyl-9-(4-methylpiperazin-1-yl)pyrrolo[2,1-*b*][1,3]benzothiazepine (9m). To a solution of **9h** (35.0 mg, 0.107 mmol) in absolute ethanol (1 mL), hydrazine monohydrate (182.0 μL, 19.0 mg, 3.74 mmol) was added. The resulting mixture was stirred at reflux for 1 h. Then, the solvent was removed in vacuo and the resulting yellow solid was dissolved in anhydrous toluene (1.5 mL), and potassium *tert*-butoxyde (36.0 mg, 0.321 mmol) was added. The reaction mixture was heated at reflux for 8 h under argon. Then water was added (3 mL), the organic phase was separated, and the aqueous phase was extracted with dichloromethane; the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated. The crude product was chromatographed (20% methanol in ethyl acetate), and **9m** (20.0 mg) was obtained as a white amorphous solid (60% yield): ^1H NMR ($CDCl_3$) δ 7.62 (m, 1H), 7.48 (m, 1H), 7.26 (m, 2H), 6.32 (s, 1H), 6.03 (m, 1H), 5.90 (m, 1H), 2.89 (m, 4H), 2.53 (m, 4H), 2.34 (s, 3H), 2.20 (s, 3H); MS *m/z* 311 (M^+), 256, 213, 98, 69, 55 (100). Anal. ($C_{18}H_{21}N_3S$) C, H, N.

7-Chloro-9-(4-methylhexahydro-1*H*-1,4-diazepin-1-yl)pyrrolo[2,1-*b*][1,3] benzothiazepine (9n). The title compound was obtained following the above-described procedure as for **9d**, starting from **12c** (30.0 mg, 0.12 mmol) and 1-methylhomopiperazine (60.0 μL, 5.4 mmol). After purification, **9n** was obtained as a white amorphous solid (17.0 mg, 41% yield): ^1H NMR ($CDCl_3$) δ 7.53 (d, 1H, J = 2.4 Hz), 7.43 (d, 1H, J = 8.8 Hz), 7.22 (dd, 1H, J = 8.4, 2.4 Hz), 6.75 (m, 1H), 6.55 (s, 1H), 6.19 (m, 1H), 6.11 (m, 1H), 3.20 (m, 4H), 3.15−2.61 (m, 4H), 2.40 (s, 3H), 1.95 (m, 2H); MS *m/z* 345 (M^+) (100), 205, 140, 97. Anal. ($C_{18}H_{20}ClN_3S$) C, H, N.

Olanzapine. A sample of this antipsychotic agent has been obtained following the procedure as described in ref 13. The structure was confirmed by ^1H NMR and MS.

Pharmacology. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U. suppl.40, 18 Febbraio 1992, Circolare no. 8, G.U. 14 Luglio 1994) and international laws and policies (EEC Council Directives 86/609, OJL 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, U.S. National Research Council, 1996).

In Vitro Binding Assays.[13,14a−d] 1. Serotonin and Dopamine Receptors. 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 assay. Tissues were homogenized in about 50 volumes of ice-cold Tris HCl, 50 mM, pH 7.4 (for D_1, D_2, and 5-HT_2 receptors), or 50 mM Hepes Na, pH 7.5 (for D_3 receptors) using an Ultra-Turrax TP-1810 homogenizer (2 × 20 s) and centrifuged at 48000*g* for 10 min (Beckman Avanti J-25 centrifuge). Each pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again at 48000*g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The resulting pellets were resuspended just before the binding assay in the appropriate incubation buffer (50 mM Tris HCl, pH 7.4, 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; 50 mM Hepes Na, pH 7.5, containing 1 mM EDTA, 0.005% ascorbic acid, 0.1% albumin, 200 nM eliprodil for D_3 receptors and 50 mM Tris HCl, pH 7.7 for 5-HT_2 receptors).

[^{3}H]-SCH 23390 (specific activity, 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 μM (−)-*cis*-flupentixol.

[^{3}H]-Spiperone (specific activity, 16.5 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 μM (−)-sulpiride.

[^{3}H]-7-OH-DPAT (specific activity, 159 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 (specific activity, 63.3 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 20 μL of displacing agent or solvent. Nonspecific binding was obtained in the presence of 1 μM methisergide.

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 (4 × 3 times) of ice-cold buffer (50 mM Tris HCl, pH 7.7) using a Brandel M-48R cell harvester. The radioactivity trapped on the filters was counted in 4 mL of Ultima Gold MV (Packard) in a LKB 1214 rack beta liquid scintillation spectometer with a counting efficiency of 50%. Binding affinity to human serotonin $5HT_{2A}$ subtype and human dopamine D_2 subtype was tested by CEREP (Le Bois l'Eveque, 86600 Celle L'Evescault, France). 5-HT_{2A} affinity was determined on human recombinant 5-HT_{2A} receptors stably expressed in CHO (human ovarian cells) membranes using [^{3}H]ketanserine (0.5 nM) as radioligand. Binding assays were done according to the procedure of Bonhaus et al.[14b] For human dopamine D_2 subtype affinity, the binding assay employed human recombinant D_2 receptors stably expressed in CHO cell membranes and [^{3}H]spiperone (0.3 nM) as radioligand, according to the procedure of Grandy et al.[14c]

2. Histamine H_1 Receptor. Binding affinity was tested by CEREP according to procedure of Dini et al.[14d] Binding was determined using membranes prepared from guinea pig cerebellum with [^{3}H]pyrilamine (0.5 nM) as radioligand.

3. Muscarinic M_1 Receptors. Binding was determined using membranes prepared from rat cerebral cortex homogenized in phosphate buffer. The homogenate was centrifuged at 48000*g* for 10 min, and the pellet was suspended in phosphate buffer and washed two times more. One milliliter of this homogenate was incubated with 1 mL of 0.16 nM [^{3}H]-QNB (quinuclidinyl benzylate, L-[benzylic-4,4′-3*H*]-), and 40 μL of test compound for 60 min at 37 °C, and then filtered through a Whatman GF/B filter (Whatman International Ltd). Nonspecific binding was determined in the presence of 1 μM atropine.

4. Adrenergic α_1 Receptors. Binding was determined using membranes prepared from rat cerebral cortex homogenized in phosphate buffer. The homogenate was centrifuged at 44000*g* for 10 min and the pellet was suspended in phosphate buffer and washed two times more; 500 μL of this homogenate was incubated with 500 μL of 0.2 nM [^{3}H]prazosin and 20 μL of test compound for 30 min at 25 °C, then filtered through a Whatman GF/B filter (Whatman International Ltd). Radioactivity on the filter was measured with a liquid scintillation counter. Complete (100%) inhibition of [^{3}H]prazosin binding was determined in the presence of 10 μM prazosin. Nonspecific binding was determined in the presence of 10 μM prazosin.

5. Adrenergic α_2 Receptors. Binding was determined using membranes prepared from rat cerebral cortex homogenized in phosphate buffer. The homogenate was centrifuged at 44000*g* for 10 min, and the pellet was suspended in phosphate buffer and washed two times more; 500 μL of this homogenate was incubated with 500 μL of 1 nM [^{3}H]clonidine and 20 μL of test compound for 30 min at 25 °C, then filtered through a Whatman GF/B filter (Whatman International Ltd). Nonspecific binding was determined in the presence of 10 μM clonidine.

For all binding assays, 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 50%. Dose inhibition curves were analyzed by the "Allfit"[39] program to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding. The K_i values were derived from the IC_{50} values according to the method of Cheng and Prusoff.[40]

Behavioral Tests. 1. Antagonism of Apomorphine-Induced Climbing in Mice. Male albino mice CD1, weighing 20−25 g at the beginning of the studies were used. Mice presented wall-climbing behavior after subcutaneous (sc) injection of apomorphine (1.3 mg/kg). To quantify this behavior, the animals were placed individually in upturned steel cylinders (h.18 cm; diameter14 cm) with walls of vertical bars (diameter 2 mm; 1 cm apart).

Animals given control saline injections remained on the floor of their cylinders, with normal exploratory behavior, while animals given apomorphine climbed up the walls of the cylinders holding onto the wire mesh with their paws. During the climbing period animals did not remain in one position, but moved constantly around the sides or tops of the cages, clinging to the mesh with all four paws.[41]

Test compound or vehicle was given subcutaneously or orally respectively 30 or 60 min before apomorphine. Climbing behavior was assessed at 5-min intervals for 30 min, starting 5 min after apomorphine.

2. Antagonism of 5-MeO-DMT-Induced Head-Twitches in Mouse. Groups of 10 male CD1 mice were used to evaluate head twitches induced by 5 methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT) 10 mg/kg, sc. Observation started 6 min after 5-MeO-DMT treatment and lasted 15 min (counting the number of head twitches). The test substances were given orally 60 min before 5-MeO-DMT.[42]

3. Catalepsy. Male Wistar rats (7−8 per group) were given either **9d** (3.75−100 mg/kg) or clozapine (100 mg/kg). The behavioral test was run as described by Moore[43] with minor changes. Catalepsy was evaluated on a metal bar 0.6 cm in diameter positioned 10 cm above the tabletop. The test consisted in positioning the animal with its forepaws on the bar and recording how long it remained hanging onto the bar; the end-point was 60 s and an all-or-none criterion was used.

The test substances were administered orally 60 min before the first evaluation and subsequent observations were made 60, 120, 180, 240, 300 min later.

4. Spontaneous locomotor Activity in Rats. The effect of **9d** on motor performance was examined in Fisher 344 male rats. The animals were given either **9d** (0.125 mg/kg, 2 mg/kg, p.o.) or vehicle (2-hydroxypropyl-β-cyclodextrin, 10%) 60 min before the test. To assess the inhibition of spontaneous motor activity, rats were individually placed in Plexiglas activity cages (40 × 40 cm) with photocells on the walls. The photocells were connected through an interface to a computer. The consecutive interruption of photocell beams was taken as a locomotion count. Spontaneous activity was recorded for 30 min after administration of the test compound.

5. MK-801-Induced Hyperactivity. Fisher 344 male rats were orally dosed with either vehicle or the test compounds, and placed in Plexiglas cages for evaluation of locomotor activity. After 30 min, the animals were challenged with 0.3 mg/kg (sc) of MK-801 and the locomotor activity of each animal was recorded for 90 min.

6. Conditioned Avoidance Response (CAR). Rats were trained daily and tested in a computer-assisted two-way active avoidance apparatus (shuttle box) equipped with a tilting grid floor with microswitch detection and connected to a high-resistant power supply. These boxes are divided into two compartments of equal size by a partition with one opening. Upon presentation of the light-conditioned stimulus (CS), the animal had 3 s to move from one compartment of the shuttle box into the other. If the rat remained in the same compartment for more than 3 s, the unconditioned stimulus (UCS) was presented as an electric shock in the grid floor, until the rat escaped. If it did not respond within 7 s, including the first 3 s, the trial was terminated (failure). The interval between trials was 45 s. The following variables were recorded: avoidance (response to CS within 3 s); escape (response to CS + UCS); failure (failure to respond to CS and CS + UCS); intertrial crossing.

The animals were trained on consecutive days until they achieved about 70% conditioned avoidance. The selected animals were given the different doses of either **9d** (0.37−3 mg/kg, po) or vehicle. CAR was then tested 60 min later. All experimental sessions were run for 15 min resulting in 20 trials in any session. The number of trials in which an avoidance response occurred was divided by the total number of trials per session to give the percentage avoidance response.

7. PCP-Induced Cognitive Impairment. The test apparatus has been described in detail.[44] Briefly, attentional functions were evaluated using a five-choice serial reaction time (5-CSRT) task, in which hungry rats were trained daily to locate brief (0.5 s) visual targets presented randomly in one of five locations in a specially designed chamber. When the animals reached a stable performance with a mean of 80%

correct responses they were allocated to different treatment schedules. Attentional deficit was induced by intraperitonal phencyclidine (PCP: 2 mg/kg, ip) in the selected rats.

The animals (nine rats per group) received **9d** (0.3 mg/kg, ip) or vehicle [(10%) 2-hydroxypropyl-β-cyclodextrin/NaCl (0.9%)] 20 min before the PCP. Ten min after PCP, the rats were put in the box and the session started. On each test day the different drug doses were administered according to a Latin square design. Drug test session were 30 min long and at least 48 h apart. To investigate whether the test and reference compounds antagonized the PCP-induced attentional deficit the following variables were recorded: percentage of correct responses, number of premature responses, percentage of omissions. Data were analyzed by Tukey's test ($p < 0.05$).

Statistics. To estimate the potency of test and reference compounds, doses inhibiting the different behavioral responses by 50% were calculated by sigmoidal dose−response curve analysis using the program PRISM (Graphpad Software, San Diego, CA), and reported as ED_{50}.

Serum Prolactin. Fisher 344 male rats (275−300 g) were assigned to six groups of eight animals each and treated as follows: vehicle (2 mL/kg NaCl 0.9%, sc); haloperidol (2.7 μmol/kg, sc, MW 375.88); olanzapine (12.3 μmol/kg, sc, MW 312.44); clozapine (12.3 μmol/kg, sc, MW 326.83), (*S*)-(+)-**8** (12.3 μmol/kg, sc, MW 406.81), and **9d** (12.3 μmol/kg, sc, MW 297.42).

The animals received a single or multiple doses (3 day) of each compound. The rats were killed by decapitation 180 min after the last treatment. Blood samples (2 mL) were collected and centrifuged (300*g* for 30 min) and the resulting serum samples were stored at −20 °C until analyzed for prolactin (PRL). Serum PRL was determined by an EIA-kit from Amersham. Data were analyzed by Student's *t*-test ($p < 0.05$).

Molecular Modeling. All molecular modeling was run on a Silicon Graphics Indigo2 R10000 workstation. Structure of (*S*)-(+)-octoclothepin and compounds **7**, **14**, **15**, **16** were extracted from the Cambridge Crystallographic Structural Data Bank. Molecules **17, 18, 9d**−**9m** were built by using the Builder module in Insight2000 (Accelrys, San Diego). Atomic potentials and charges were assigned using the cff91 force field.[45] All the compounds were considered protonated on the piperazine distal nitrogen. The starting conformations were geometrically optimized (Discover module, Accelrys, San Diego) using a distance dependent dielectric constant mimicking an aqueous environment ($\epsilon = 80$*r). Energy minimizations were performed with conjugate gradient[46] as minimization algorithm, until the maximum RMS derivative was less than 0.001 kcal/mol. The above-described energy minimization protocol has been applied to all the molecular mechanic (MM) calculations.

To find the global minimum conformer, compounds **9d**−**9m** were subjected to a Systematic Conformational Search (SCS), using the SEARCH routine within Sybyl 6.6 (Tripos, St. Louis, MO). The piperazine ring was assumed in a chair conformation, since, as already reported, it is energetically preferred, as compared to the boat conformation.[13] Torsional angle τ was analyzed using the Ring Search module by increment of 10° using 0−359° as interval of variation. The permissible variance on the distance between the ring closure atoms was set to 1 Å, while the permissible variance on the valence angles about the ring closure atoms was set to 15°. Rotatable single bonds were analyzed with an angle increment of 20° within a 0−359° range. To generate all theoretically possible conformations, a 0.05 van der Waals Radii Scaling Factors was used in the rigid rotamers, and no conformational energy evaluation was included in all the searches. Resulting structure files were transferred in Insight2000 package (Accelrys, San Diego) to be subjected to the above-mentioned MM energy minimization protocol.

Potential energy curves were calculated for each molecule by scanning of τ_N and τ values, followed by a full MM energy minimization, except for the dihedral angle used as driving angle. For each molecule, obtained torsional angles values were plotted against their conformational energies using the Table function in Insight2000.

The value of τ_N required for the optimum positioning of the lone pair of the distal piperazine nitrogen with respect to the tricyclic system has been established for both folds (A,B) of **7**, **14**, **9d**−**m** using the following procedure. The structures were superimposed (fitting points: the centroids of the aromatic rings) on the bioactive conformation of (*S*)-(+)-octoclothepin and τ_N was rotated to optimize the superimposition of the points positioned at 2.8 Å along the direction of the lone pair, according to the D_2 pharmacophore proposed by Liljefors et al.[24b] A final three-point superimposition yielded the following RMS values: (i) < 0.3 Å for both folds of compounds **9d**, **9f**, and **9m**, (ii) 0.77 Å and 0.49 Å for the A-fold and B-fold of **7** respectively, and (iii) 0.43 Å for A-fold of **14**. Conformational energies of the resulting conformations have been evaluated through a constrained MM energy minimization followed by a 1 iteration of unconstrained MM energy minimization.

All MM resulting conformations of compounds **7, 14, 9d**, **9f**, and **9m** were subjected to a full geometry optimization by semiempirical calculations using the quantum mechanical method PM3 in the Mopac[47] 6.0 package in Ampac/Mopac module of Insight2000. GNORM value was set to 0.5. To reach a full geometry optimization the criteria for terminating all optimizations was increased by a factor of 100, using the keyword PRECISE.

Acknowledgment. We thank MURST − Rome, Italy (PRIN 03) and Sigma-Tau, Italy for financial support. The authors thank Mrs. J. Baggot for English editing.

Supporting Information Available: Figure 7 and Table 8. This information is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Work Group on Schizophrenia. Practice guidelines for the treatment of patients with schizophrenia. *Am. J. Psychiatry* **1997**, *153*, 1−63

(2) Wickelgren, I.A route to treating schizophrenia? *Science* **1998**, *281*, 1264−1265. (b) Harrison, P. J. The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* **1999**, *122*, 593−624.

(3) Carlsson, A. The current status of the dopamine hypothesis of schizophrenia. *Neuropsychopharmacology* **1988**, *1*, 179−186.

(4) Meltzer, H. Y. Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. *Psychopharmacology* **1989**, *99*, S18−S27.

(5) Campbell, M.; Young, P. I.; Bateman, D. N. The use of atypical antipsychotics in the menagement of schizophrenia. *Br. J. Pharmacol.* **1999**, *47*, 13−22.

(6) Richelson, E. Receptor pharmacology of neuroleptics: relation to clinical effects. *J. Clin. Psychiatry* **1999**, *60*, 5−14.

(7) Kelleher, J. P.; Centorrino, F.; Albert, M. J.; Baldessarrini, R. J. Advances in atypical antipsychotics for the treatment of schizophrenia. *CNS Drugs* **2002**, *16*, 249−261.

(8) Tollefson, G. D.; Kuntz, A. J. Review of recent clinical studies with olanzapine. *Br. J. Psychiatry* **1999**, *174*, 30−35. (b) Bhana, N.; Perry, M. C. Olanzapine. A review of its use in the treatment of bipolar I disorder. *CNS Drugs* **2001**, *15*, 871−904.

(9) Gardner, I.; Zahid, N.; McCrimmon, D.; Uetrecht J. P. A comparison of the oxidation of clozapine and olanzapine to reactive metabolites and the toxicity of these metabolites to human leukocytes.*Mol. Pharmacol.* **1998**, *53*, 991−998. (b) Gardner, I.; Leeder, J. S.; Chin, T.; Zahid, N.; Uetrecht, J. P. A comparison of the covalent binding of clozapine and olanzapine to human neutrophils in vitro and in vivo. *Mol. Pharmacol.* **1998**, *53*, 999−1008.

(10) Koller, E. A.; Doraiswamy, P. M. Olanzapine-associated diabetes mellitus. *Pharmacotherapy* **2002**, *22*, 841−852.

(11) Geddes, J.; Freemantle, N.; Harrison, P.; Bebbington, P. Atypical antipsychotics in the treatment of schizophrenia: systematic overview and meta-regression analysis. *Br. Med. J.* **2000**, *321*, 1371−1376.

(12) http://www.abpi.org.uk/publications/publication_details/targetSchizophrenia/section5_asp.

(13) Campiani, G.; Butini, S.; Gemma, S.; Nacci, V.; Fattorusso, C.; Catalanotti, B.; Giorgi, G.; Cagnotto, A.; Goegan, M.; Mennini, T.; Minetti, P.; Di Cesare, M. A.; Mastroianni, D.; Scafetta, N.; Galletti, B.; Stasi, M. A.; Castorina, M.; Pacifici, L.; Ghirardi, O.; Tinti, O.; Carminati, P. Pyrrolo[1,3]benzothiazepine-based atypical antipsychotic agents. Synthesis, structure−activity relationship, molecular modeling, and biological studies. *J. Med. Chem.* **2002**, 45, 344−359.

(14) Campiani, G.; Nacci, V.; Bechelli, S.; Ciani, S. M.; Garofalo, A.; Fiorini, I.; Wikstrom, H.; de Boer, P.; Liao, Y.; Tepper, P. G.; Cagnotto, A.; Mennini, T. New antipsychotic agents with serotonin and dopamine antagonist properties based on a pyrrolo-[2,1-*b*][1,3]benzothiazepine structure. *J. Med. Chem.* **1998**, *41*, 3763−3772. (b) Bonhaus, D. W.; Bach, C.; De Souza, A.; Salazar, F. H. R.; Matsuooka, B. D.; Zuppan, P.; Chan, H. W.; Eglen, R. M. The pharmacology and distribution of human 5-hydroxytryptamine$_{2b}$ (5-HT$_{2b}$) receptor gene products: comparison with 5-HT$_{2a}$ and 5-HT$_{2c}$ receptors. *Br. J. Pharmacol.* **1995**, *115*, 622−628. (c) Grandy, D.K.; Marchionni, M. A.; Makam, H.; Stofko, R. E.; Alfano, M.; Frothingham, L.; Fischer, J. B.; Burke-howie, K. J.; Bunzow, J. R.; Server, A. C.; Civelli, O. Cloning of the cDNA and gene for a human D$_2$ dopamine receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9762−9766. (d) Dini S.; Caselli, G. F.; Ferrari, M. P.; Giani, R.; Clavenna, G. Heterogenity of [^{3}H]-mepyramine binding sites in guinea pig cerebellum and lung. *Agents Actions* **1991**, *33*, 181−184.

(15) Stjernölf, P.; Gullme, M.; Elebring, T.; Andersson, B.; Wikström, H.; Lagerquist, S.; Svensson, K.; Ekman, A.; Clarsson, A.; Sundell, S. (S)- and (R)-8-(di-*n*-propylamino)-6,7,8,9-tetrahydro-3H-benz[*e*]indole-1-carbaldehyde: a new class of orally active 5-HT$_{1A}$-receptor agonist. *J. Med. Chem.* **1993**, *36*, 2059−2065.

(16) Haigh, D.; Jelfcott, L. J.; Magee, K.; McNab, H.; J.*Chem. Soc. Perk. Trans. 1* **1996**, *52*, 2895−2900; b) A. J. Phillips, Y. Uto, P. Wipf, M. J. Reno, D. R. Williams, *Org. Lett.* **2000**, *2*, 1165−1168.

(17) Perregaard, J.; Arnt, J.; Bøgesø, K. P.; Hittel, J.; Sanchez K. Noncataleptogenic, centrally acting dopamine D$_2$ and serotonin 5-HT$_2$ antagonists within a series of 3-substituted 1-(4-fluorophenyl)-1*H*-indoles. *J. Med. Chem.* **1992**, *35*, 1092−1101. (b) Bøgesø, K. P.; Arnt, J.; Boeck, V.; Christiensen, A. V.; Hyttel, J.; Jensen K. G. Antihypertensive activity in a series of 1-piperazino-3-phenylindans with potent 5-HT$_2$-antagonistic activity. *J. Med. Chem.* **1988**, *31*, 2247−2256.

(18) Caglioti, L. *Org. Synth.* **1972**, *52*, 122−124.

(19) Ornstein, P. L.; Schoepp, D. D.; Arnold, M. B.; Leander, J. D.; Lodge, D.; Paschal, J. W.; Elzey, T. 4-(Tetrazolylalkyl)piperidine-2-carboxylic acids. Potent and selective *N*-methyl-D-aspartic acid receptor antagonists with a short duration of action. *J. Med. Chem.* **1991**, *34*, 90−97.

(20) Coffen, D. L.; Fryer, R. I.; Katonak, D. A.; Wong, F. Quinazolines and 1,4-benzodiazepines. LXX. *v*-Triazolo[1,5-*a*][1,4]benzodiazepines. *J. Org. Chem.* **1975**, *40*, 894−897.

(21) Meltzer, H. Y.; Matsubara, S.; Lee, J.-C. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pki values. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238−246.

(22) Andersen, K.; Liljefors, T.; Hyttel, J.; Perregaard, J. Serotonin 5-HT$_2$ receptors dopamine D$_2$ receptor and α_1 adrenoceptor antagonists. Conformationally flexible analogues of the atypical antipsychotic sertindole. *J. Med. Chem.* **1996**, *39*, 3723−3738. (b) Perregaard, J.; Arnt, J.; Bøgesø, K. P.; Hyttel, J.; Sanchez, C. Noncataleptogenic, centrally acting dopamine D$_2$ and serotonin 5-HT$_2$ antagonists within a series of 3-substituted 1-(4-fluorophenyl)-1*H*-indoles. *J. Med. Chem.* **1992**, *35*, 1092−1101.

(23) Froimowitz, M.; Rämsby, S. Conformational properties of semirigid antipsychotic drugs: the pharmacophore for dopamine D-2 antagonist activity. *J. Med. Chem.* **1991**, *34*, 1707−1714.

(24) Liljefors, T.; Bøgesø, K. P. Conformational analysis and structural comparisons of (1*R*,3*S*)-(+)- and (1*S*,3*R*)-(−)-tefludazine, (*S*)-(+)- and (*R*)-(−)-octoclothepin, and (+)-dexclamol in relation to dopamine receptor antagonism and amine-uptake inhibition. *J. Med. Chem.* **1988**, *31*, 306−312. (b) Bøgesø, K. P.; Liljefors, T.; Arnt, J.; Hyttel, J.; Pedersen, H. Octoclothepin enantiomers. A reinvestigation of their biochemical and pharmacological activity in relation to a new receptor-interaction model for dopamine D$_2$ receptor antagonists. *J. Med. Chem.* **1991**, *34*, 2023−2030. (c) Andersen, K.; Liljefors, T.; Gundertofte, K.; Perregaard, J.; Bøgesø, K. P. Development of a receptor interaction model for serotonin 5-HT$_2$ receptor antagonist. Predicting selectivity with respect to dopamine D$_2$ receptors. *J. Med. Chem.* **1994**, *37*, 950−962.

(25) Phillips, S. T.; Paulis, T.; Neergaard, J. R.; Baron, B. M.; Seigel, B. W.; Seeman, P.; Van Tol, H. H. M.; Guan, H.; and Smith, H. E. Binding of 5*H*-Dibenzo[*a*,*d*]cycloheptene and 5*H*-Dibenz[*b*,*f*]-oxepine analogues of clozapine to dopamine and serotonin receptors. *J. Med. Chem.* **1995**, *38*, 708−714.

(26) Kapur, S.; McClelland, R. A.; VanderSpek, S. C.; Wadenberg, M. L.; Baker, G.; Nobrega, J.; Zipursky, R. B.; Seeman, P. Increasing D$_2$ affinity results in the loss of Clozapine's atypical antipsychotic action. *Neuroreport* **2002**, *13*, 831−835.

(27) Irrure, J.; Narbon, E.; Serra, B.; Teixido, J. Conformational barrier calculation of dibenzo[*b*,*e*]azepine derivatives by semiempirical method AM1. In *QSAR and Molecular Modeling: Concepts, Computational Tools and Biological Applications;* Prous Science Publisher: Barcelona, 1995; pp 329−331.

(28) Randall, W. C., Anderson, P. S.,. Cresson, E. L, Hunt, C. A., Thomas F. Lyon, Rittle, K. E., Remy, D. C., Springer, J. P., Hirshfield, J. M., Hoogsteen, K., Williams, M., Risley, E. A., Totaro J. A. Synthesis, assignment of absolute configuration, and receptor binding studies relevant to the neuroleptic activities of a series of chiral 3-substituted Cyproheptadine atropisomers. *J. Med. Chem.* **1979**, *22*, 1222−1230.

(29) Lieberman, J. A.; Mailman, R. B.; Buncan, G.; Sikich, L.; Chakos, M.; Nichols, D. E.; Kraus, J. E. A decade of serotonin research: role of serotonin in treatment of psychosis. Serotoninergic basis of antipsychotic drug effects in schizophrenia. *Biol. Psychiatry* **1998**, *44*, 1099−1117.

(30) Abi-dargham, A.; Laruelle, M.; Charney, D.; Krystal, J.The role of serotonin in pathophisiology and treatment of schizophrenia. *J. Neuropsychiatry* **1997**, 9, 1−17.

(31) Saller, C. F.; Czupryna, M. J.; Salama, A. I. 5-HT$_2$ receptor blockade by ICI 169, 369 and other 5-HT$_2$ antagonists modulate the effects of D$_2$ dopamine receptor blockade. *J. Pharm. Exp. Ther.* **1991**, *253*, 1162−1170.

(32) Lucas, G.; De Deurwardére, P.; Caccia, S.; Spampinato, U. The effect of serotonergic agents on haloperidol-induced striatal dopamine release in vivo: opposite role of 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor subtypes and significance of the haloperidol dose used. *Neuropharmacology* **2000**, *39*, 1053−1063.

(33) Lucas, G.; De Deurwardére, P.; Porras, G.; Spampinato, U. Endogenous serotonin enhances the release of dopamine in the striatum only when nigro-striatal dopaminergic transmission is activated. *Neuropharmacology* **2000**, *39*, 1984−1995.

(34) De Deurwardére, P.; Spampinato, U. Role of serotonin$_{2B\backslash 2C}$ receptor subtypes in the control of accumbal and striatal dopamine release elicited in vivo by dorsal raphe nucleus electrical stimulation. *J. Neurochem.* **1999**, *73 (2)*, 1033−1042.

(35) Di Giovanni, G.; De Deurwardére, P.; Di Mascio, M.; Di Matteo, V.; Esposito, E.; Spampinato, U. Selective blockade of serotonin-2C\2B receptors enhances mesolimbic and mesostriatal dopaminergic function: a combined in vivo electrophysiological and microdialysis study. *Neurosci.* **1999**, *91 (2)*, 587−597.

(36) Lucas, G.; Spampinato, U. Role of striatal serotonin 2A and serotonin 2C receptor subtypes in the control of in vivo dopamine outflow in the rat striatum. *J. Neurochem.* **2000**, *74*, 693−701.

(37) Hertel, P.; Fagerquist, M. V.; Svensson, T. H. Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by α_2 adrenoceptor blockade. *Science* **1999**, *286*, 105−107. (b) Lindström, L. H. Schizophrenia, the dopamine hypothesis and α2-adrenoceptor antagonists. *Trends Pharmacol. Sci.* **2000**, *21*, 198−199.

(38) Gudelsky, G. A.; Koening, J. I.; Simonovic, M.; Koiama, T.; Ohmori, T.; Meltzer, H. Y. Differential effects of haloperidol, clozapine and fluperlapine on tuberoinfundibular dopamine neurons and prolactin secretion in the rat. *J. Neurol. Transm.* **1987**, *68*, 227−240.

(39) De Lean, K. W.; Munson, P. J.; Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and phisiological dose−response curves. *Am. J. Physiol.* **1978**, *235*, E97-E102.

(40) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition costant (K_i) and the concentration of inhibition which causes 50% inhibition (IC$_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(41) Costall, B.; Naylor, R. J.; Nohria, V. Climbing behavior induced by apomorphine in mice: a potential model for detection of neuroleptic activity. *Eur. J. Pharmacol.* **1978**, *50*, 39−50.

(42) Rigdon, G. C.; Norman, M. H.; Cooper, B.B.; Howard, J. L.; Boncek, V. M.; Faison, W. L.; Nanry, K. P.; Pollard, G. T. 1192U90 in animal tests that predict antipsychotic efficacy, anxiolysis, and extrapyramidal side effects. *Neuropsychopharmacology* **1996**, *15*, 231−242.

(43) Moore, N. A.; Tye, N. C.; Axton, M. S.; Risius, F. C. The behavioral pharmacology of olanzapine, a novel "atypical" antipsychotic agent. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 545−551.

(44) Carli, M.; Robbins, T. W.; Evenden, J. L.; Everitt, B. J. Effects of lesions to ascendine noradrenergic neurons on performance of a 5-choice serial reaction task in rats; implications for theories of dorsal noradrenergic bundle function based on selective attention and arousal. *Behav. Brain Res.* **1983**, *9*, 361−380.

(45) Maple, J. R., Diniur, U., Hagler, A. T. Derivation of force fields for molecular mechanics and dynamics from ab initio energy surfaces. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 5350−5354. (b) Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. Structure and energetics of ligand binding to proteins. *Proteins: Struct., Function Genet.* **1988**, *4*, 31−47.

(46) Fletcher, R. Unconstrained Optimization. In *Pratical Methods of Optimization*; John Wiley & Sons: New York, 1980; Vol. 1.

(47) Stewart, J. J. P. MOPAC: A Semiempirical Molecular Orbital Program *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1−105.

JM0309811