Synthesis and Pharmacological Evaluation of 1-[(1,2-Diphenyl-1*H*-4-imidazolyl)methyl]-4-phenylpiperazines with Clozapine-Like Mixed Activities at Dopamine D_2, Serotonin, and $GABA_A$ Receptors

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A series of 18 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-piperazines (**1a**−**r**) were designed and synthesized as possible ligands with mixed dopamine (DA) D_2/serotonin 5-HT_{1A} affinity, with the aim of identifying novel compounds with neurochemical and pharmacological properties similar to those of clozapine. The binding profile at D_2 like, 5-HT_{1A}, and 5-HT_{2A} receptors of title compounds was determined. Modifications made in the phenyl rings of the parent compound (**1a**) produced congeners endowed with a broad range of binding affinities for DA D_2 like, serotonin 5-HT_{1A}, and 5-HT_{2A} receptors, with IC_{50} values ranging from 25 to >10 000 nM. As for the modification of the piperazine N^4-phenyl ring, the affinities for both D_2 like and 5-HT_{1A} receptors were progressively increased by introduction of ortho-methoxy and ethoxy groups (**1b**,**o**, respectively). Data revealed the presence of a para-chloro substituent in **1g** to be associated with a relatively high affinity and substantial selectivity for D_2 like receptors, whereas the meta-chloro analogue **1f** exhibited preferential affinity for 5-HT_{1A} receptors. A quantitative structure−affinity relationship analysis of the measured binding data resulted in regression equations that highlighted substituent physicochemical properties modulating the binding to subtypes 1A and 2A of serotonin 5-HT receptors but not to D_2 like receptors. Thus, besides an electron-withdrawing field effect and ortho substitution, which both influence binding to serotonin 5-HT receptor subtypes, though to a different extent as revealed by regression coefficients in the multiparametric regression equations, the affinity of congeners **1a**−**r** to 5-HT_{1A} receptors proved to be linearly correlated with volume/polarizability descriptors, whereas their affinity to 5-HT_{2A} receptors correlated with lipophilicity constants through a parabolic relationship. 1-[(1,2-Diphenyl-1*H*-4-imidazolyl)methyl]-4-(2-methoxyphenyl)piperazine (**1b**), with a D_2/5-HT_{1A} IC_{50} ratio of ∼1, was selected for a further pharmacological study. In rats, the intraperitoneal administration of compound **1b**, like that of clozapine, induced an increase in the extracellular concentration of DA measured in the medial prefrontal cortex. Furthermore, **1b** and clozapine each inhibited GABA-evoked Cl^- currents at recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes. These findings suggest that compound **1b** may represent an interesting prototype of a novel class of drugs endowed with a neurochemical profile similar to that of atypical antipsychotics.

Introduction

Schizophrenia is the most common psychotic disorder, with an average worldwide incidence slightly less than 1%.[1] Typical antipsychotic agents, such as chlorpromazine and haloperidol (Chart 1), block the D_2 subtype of dopamine (DA) receptors in a direct relation to their clinical potency.[1−3] This observation supports the so-called "dopamine hypothesis", which asserts to be an increase in dopaminergic activity of the mesolimbic system of the brain that underlies schizophrenia.[4,5] Because the blockade of D_2 receptors by typical antipsychotics results not only in therapeutic effects but also in extrapyramidal symptoms, tardive dyskinesia, and hyperprolactinemia, which strongly limit patient compliance,[1] the development of drugs devoid of such side effects should greatly improve the clinical efficacy and tolerability of the pharmacological treatment of schizophrenia.

Clozapine (Chart 1) is the prototype of a group of "atypical" antipsychotic drugs exhibiting clinical efficacy similar to that of the classical antipsychotics but lacking, or inducing to a lesser extent, most of their motor side effects.[6−8] Clozapine binds with moderate affinity to D_2 receptors but interacts with other dopaminergic (D_1 and D_4), serotonergic (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_6, and 5-HT_7), adrenergic (α_1 and α_2),

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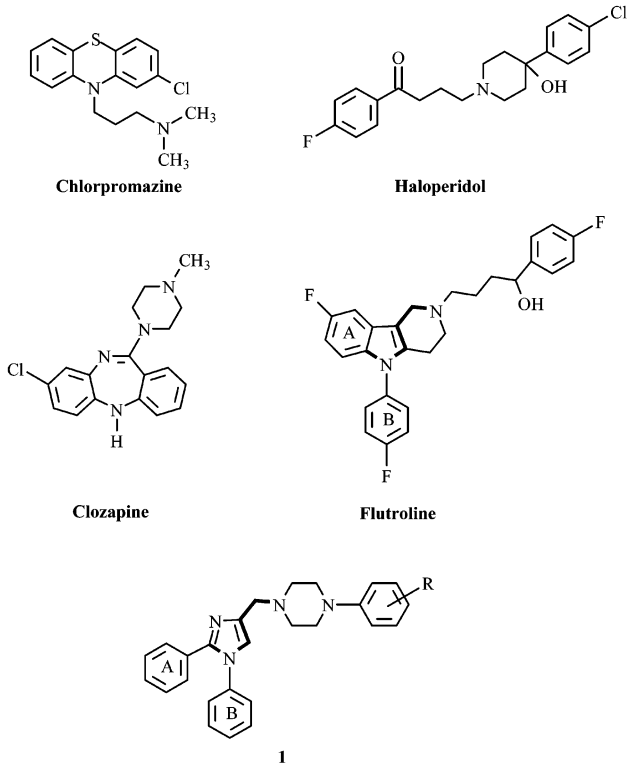
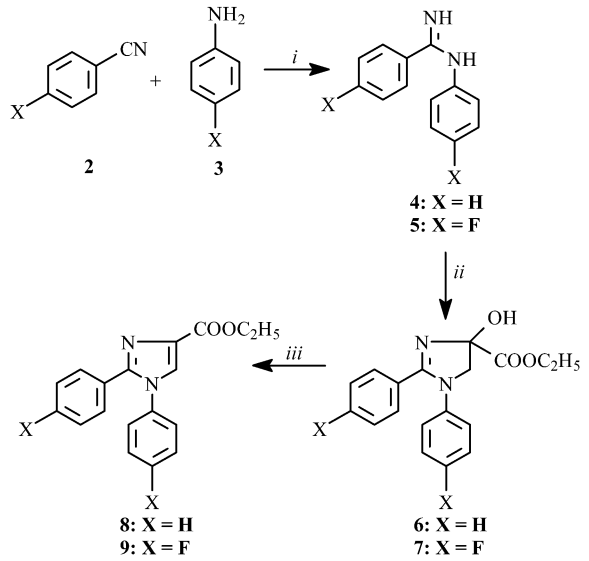
Chart 1



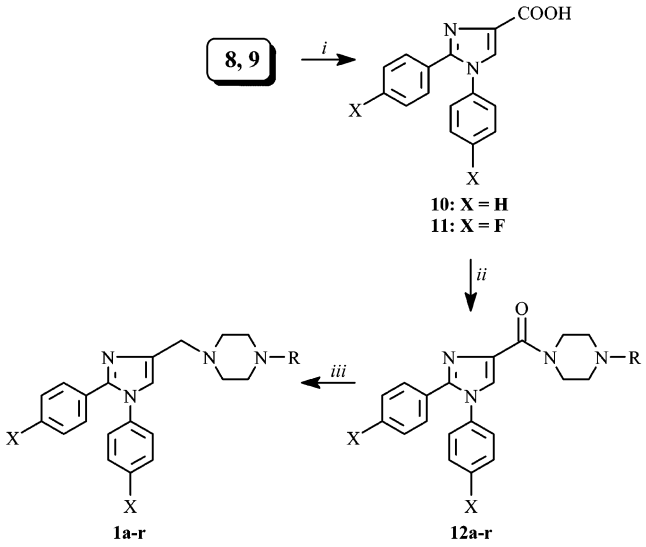
histaminergic (H_1), GABAergic ($GABA_A$), and muscarinic receptors.[9−13] The mixed action at DA and serotonin receptors has been suggested to contribute to the anticataleptic properties of clozapine.[14] The motor side effects of typical antipsychotics have also been associated with the ability of these drugs to impair GABAergic transmission in certain brain regions.[15] Unlike typical antipsychotic drugs, clozapine increases GABA turnover in vivo,[16] and it reversibly inhibits transmission at GABAergic synapses in cultures of ventral tegmental neurons[12] and hypothalamic cells.[17] Induced hematological disorders (e.g., agranulocytosis), possibly correlated to its oxidizability in vivo,[18] are however among the most relevant deleterious effects, which restrict the use of clozapine to some categories of psychotic patients. For these reasons, the development of new drugs to replace clozapine has become an active field of research in the past decade.[7,8,19] Accordingly, on the basis of the mixed DA D_2/serotonin 5-HT_2 hypothesis,[20] a number of atypical antipsychotic compounds have been recently investigated, including risperidone,[21] olanzapine,[22] sertindole,[23] quetiapine,[24] and ziprasidone.[25] An alternative approach to the discovery of atypical antipsychotics has been to target compounds endowed with mixed DA D_2/serotonin 5-HT_{1A} affinity,[26] and several N-arylpiperazine derivatives have emerged as ligands with such a mixed receptor affinity.[26,27] As a part of a project aimed at obtaining compounds potentially endowed with moderate to high affinity for DA D_2 receptors and high affinity for serotonin 5-HT_{1A} receptors, we designed a series of 1,2-diphenyl-4-methylen-imidazole derivatives with the general structure **1**, looking at the structure of flutroline (Chart 1), a 5-aryltetrahydro-γ-carboline previously reported by others,[28] that exhibited high affinity for DA D_2 receptors. In the structure of **1**, we incorporated the S-shaped arrangement of the four atom sequence linking the phenyl ring A to the basic nitrogen and the phenyl ring B of flutroline, whereas we replaced its 4-phenyl-4-hydroxybutyl chain with the N^4-phenylpiperazine moiety.

Scheme 1[a]



[a] Reagents: (i) $Al(CH_3)_3$, toluene. (ii) $BrCH_2COCOOC_2H_5$, $NaHCO_3$, *i*-PrOH. (iii) *p*-TsOH, toluene.

Scheme 2[a]



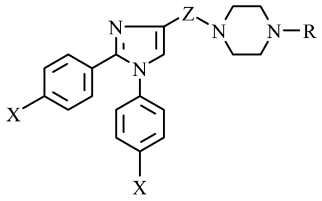
[a] Reagents: (i) NaOH, H_2O/CH_3OH. (ii) Piperazine, DCC/HOBt, CH_2Cl_2 (method A) or $(COCl)_2$/DMF, CH_2Cl_2 (method B). (iii) $BH_3S(CH_3)_2$, THF.

The synthesis of 1-[(1,2-diphenyl-1*H*-4-imidazolyl)-methyl]-4-phenylpiperazines (**1**), their affinities for DA D_2 like, serotonin 5-HT_{1A}, and 5-HT_{2A} receptors, their ability to affect the release of DA in the prefrontal cortex of freely moving rats, and their effects on the function of cloned $GABA_A$ receptors are described in this paper.

Chemistry

The target 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-piperazines (**1a**−**r**) were prepared according to the reaction pathways reported in Schemes 1 and 2. Their structures and physicochemical properties with those of carboxamide intermediates (**12a**−**r**) are summarized

Table 1. Structures and Physical Properties of Compounds **12a**−**r** and **1a**−**r**



		(1,2-diphenyl-1*H*-4-imidazolyl)carboxamides (Z = CO)				1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-piperazines (Z = CH_2)			
R	X	compd	mp (°C)	recryst solv	yield (%)	compd	mp (°C)	recryst solv	yield (%)
C_6H_5	H	**12a**	174−175	$CHCl_3/CH_3OH$	75	**1a**	99−100	ether	88
o-$MeOC_6H_4$	H	**12b**	113−115	$CHCl_3/CH_3OH$	100	**1b**	119−120	AcOEt	60
m-$MeOC_6H_4$	H	**12c**	128−129	ether	73	**1c**	125−126	AcOEt	80
p-$MeOC_6H_4$	H	**12d**	133−134	AcOEt	72	**1d**	106−107	ether	66
o-ClC_6H_4	H	**12e**	132−134	$CHCl_3$/ether	62	**1e**	139−140	*i*-$Pr_2O/CHCl_3$	50
m-ClC_6H_4	H	**12f**	143−144	ether	61	**1f**	158−159	AcOEt	66
p-ClC_6H_4	H	**12g**	175−177	$CHCl_3$/ether	94	**1g**	153−154	$AcOEt/CHCl_3$	78
o-FC_6H_4	H	**12h**	152−153	ether	72	**1h**	130−131	*i*-$Pr_2O/CHCl_3$	60
p-FC_6H_4	H	**12i**	173−175	AcOEt	92	**1i**	116−118	ether	40
m-$CF_3C_6H_4$	H	**12j**	120−123	ether	57	**1j**	133−134	AcOEt	70
p-$CF_3C_6H_4$	H	**12k**	144−145	AcOEt	94	**1k**	131−132	ether	60
2-Pyridyl	H	**12l**	157−158	AcOEt	89	**1l**	145−147	ether	40
$CH_2C_6H_5$	H	**12m**	118−119	$CHCl_3$/ether	70	**1m**	121−122	ether	56
CH_3	H	**12n**	133−134	AcOEt	74	**1n**	112−113	ether	20
o-$EtOC_6H_4$	H	**12o**	146−148	AcOEt	84	**1o**	160−161	$AcOEt/CHCl_3$	90
C_6H_5	F	**12p**	165−166	AcOEt	70	**1p**	110−111	Ether	65
o-$MeOC_6H_4$	F	**12q**	185−186	AcOEt	65	**1q**	150−151	AcOEt	80
o-$EtOC_6H_4$	F	**12r**	168	$CHCl_3$/ether	66	**1r**	132	$AcOEt/CHCl_3$	93

in Table 1. Ethyl 1,2-diphenyl-1*H*-4-imidazolecarboxylate intermediates **8** and **9** (Scheme 1) were synthesized by a three step procedure previously described for the synthesis of substituted 1,2-diphenylimidazoles.[29] In brief, the imidazoline intermediates **6** and **7** were obtained by alkylation−cyclization reaction of appropriate phenylbenzamidines **4** and **5** with ethyl bromopyruvate in 2-propanol and $NaHCO_3$. Dehydration of the resulting carbinol intermediates (**6** and **7**) in the presence of *p*-toluenesulfonic acid afforded the desired esters **8** and **9** in almost quantitative yield. The phenylbenzamidine intermediates **4** and **5** were synthesized in high yield from appropriate benzonitrile **2** and aniline **3** compounds in toluene via trimethylaluminum amide generated in situ. Saponification of **8** and **9** (Scheme 2) with hydro alcoholic NaOH provided carboxylic acids **10** and **11**. The synthesis of carboxamides **12a**−**r** was then performed by direct acid−amine coupling, either by carboxylate activation with dicyclohexylcarbodiimide (DCC) in the presence of 1-hydroxybenzotriazole (HOBt) or by N-acylation of desired N^4-substituted piperazines with acid chlorides. Reduction of the hindered tertiary amides to the corresponding target amines proved to be problematic. Attempts to achieve reduction with the common reducing reagent $LiAlH_4$ resulted in C−N bond breaking, yielding the corresponding aldehyde and alcohol. The mild reducing complex[30] BH_3·tetrahydrofuran (THF) was successfully used in some cases, but the BH_3·$S(CH_3)_2$ complex proved more efficient for the reduction of carboxamides **12a**−**r** to the target piperazines **1a**−**r**. All compounds were fully characterized by IR, ^1H NMR, mass spectra, and elemental analyses (Table 1).

Results and Discussion

Design. A molecular graphics analysis performed by HyperChem 5.01 software[31] served as structural basis for the design of 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-piperazines (**1a**−**r**) as potential clozapine-like agents. To provide biologically relevant three-dimensional (3D) models of clozapine, two main structural requirements were taken into account, that is, its protonation state and suitable conformation. Because of its pK_a values,[32,33] clozapine was constructed as a monoprotonated species at the distal piperazine nitrogen (N^4), whereas its suitable conformation was established by looking at the available crystallographic data (the piperazine ring fixed in a chairlike conformation with two equatorial substituents) and geometries of the local minima resulting from a conformational systematic search.[34] Accordingly, a model of clozapine was built and optimized using the HyperChem 5.01 molecular mechanics method and Polak−Ribiere as the minimization algorithm (rms gradient = 0.1 kcal/Å mol). To obtain suitable geometries for compound **1**, which should be protonated only at the proximal piperazine nitrogen (N^1), we roughly examined the conformational space explored by it, starting from an extended conformation, during a molecular dynamic simulation (MMplus force field, simulation temperature of 300 K, run time of 1 ps, and intervals of 0.001 ps), sampled the conformational states fitting the clozapine pharmacophore, and optimized them. An overlay of energetically permitted conformations of clozapine and compound **1** in Figure 1, where the equivalent elements are highlighted by color coding, shows a reasonable degree of similarity between the two molecules. The distances between the protonated nitrogen (color-coded in red), involved in a

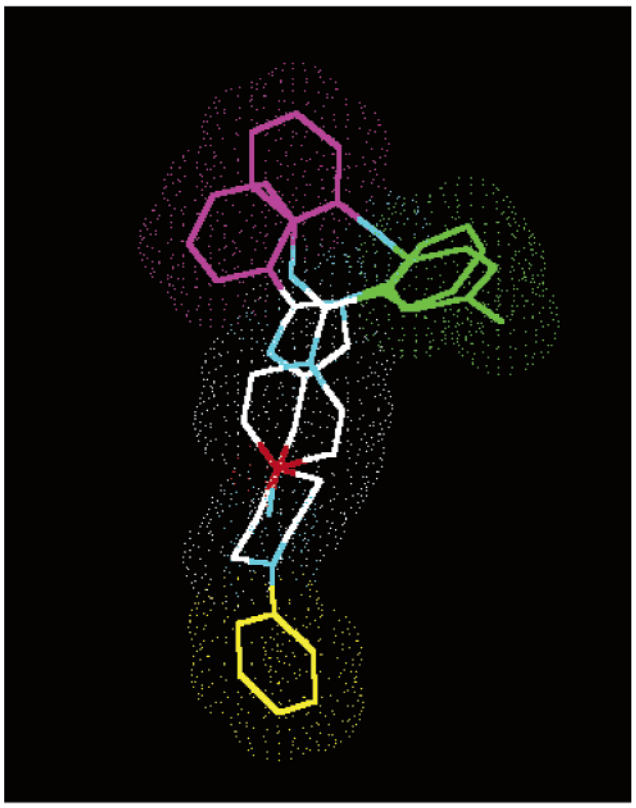


Figure 1. Overlay of energetically permitted conformations of clozapine and structure **1**. Similar partial structure elements are highlighted by color coding: violet = hydrophobic region; green = electron deficient aromatic system; red = protonated nitrogen; cyan = electronegative heteroatoms; and yellow = third phenyl group in structure **1** as a site for aromatic substitutions.

hydrogen bond-enforced interaction with an anionic receptor binding site, and the centroid of the electron deficient aromatic rings (color-coded in green) are 6.24 and 6.21 Å in clozapine and compound **1**, respectively. A region of hydrophobic interactions, colored in violet, could be reached by the phenyl ring B of **1**, whereas the N^4-phenyl (yellow-colored) was the site of aromatic substitutions aimed at possibly modulating the clozapine-like activity of the diphenylimidazolyl-piperazines **1a**−**r** under our investigation.

Drug Binding to DA D_2 Like, Serotonin 5-HT_{1A}, and 5-HT_{2A} Receptors In Vitro. The binding of compounds **1a**−**r** to D_2 like, 5-HT_{1A}, and 5-HT_{2A} receptors was measured by radioligand binding assays in vitro. The median inhibitory concentration (IC_{50}) values determined are listed in Table 2, together with those of the atypical antipsychotic clozapine, the typical antipsychotics haloperidol and raclopride, and the 5-HT_{2A} receptor antagonist cinanserin for comparison. For DA D_2 like receptors, the apparently high IC_{50} value for clozapine (Table 2) is suggestive of a very weak D_2 binding affinity. However, the calculated K_i value (146 nM) is more consistent with previous reported data in the literature.[35]

Compounds **1a**−**r** exhibited a broad range of binding affinities for D_2 like, 5-HT_{1A}, and 5-HT_{2A} receptors (IC_{50} values ranging from 25.3 to >10 000 nM). Compound **1o**, the 2-ethoxy congener of 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-phenylpiperazine, showed the highest binding affinity for the D_2 like receptor, with an IC_{50} value comparable to that of the reference standard haloperidol, and along with the respective di-para-fluorophenyl derivative **1r**, it was among the compounds showing the highest affinity for the 5-HT_{1A} receptor. In contrast, the 2-fluoro congener **1h** displayed high activity toward the 5-HT_{2A} receptor, with an IC_{50} value (25 nM) close to that measured for clozapine. An increase of D_2 like and 5-HT_{1A} (and to a lesser extent 5-HT_{2A}) binding was exhibited by the ortho-methoxy congener **1b**, whose binding data were better than those of clozapine toward D_2 like and 5-HT_{1A} receptors and close to the IC_{50} value of haloperidol on the 5-HT_{2A} receptor.

The D_2 like binding was notably affected by a chloro-substituent at the para position of the phenyl ring at the piperazine N^4 (**1g**), whereas its replacement with fluoro or trifluoromethyl groups did not yield any significant increase in D_2 like activity. An isosteric replacement of the piperazine N^4-phenyl ring in compound **1a** by a 2-pyridyl ring (**1l**) resulted in an increase of affinity for 5-HT_{1A} (and 5-HT_{2A}) receptors but did not improve D_2 like receptor binding.

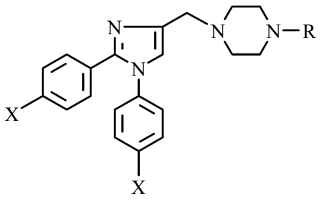
The replacement of the same phenyl group in **1a** with a methyl group (**1n**) abolished the affinity for the three receptors, likely because of an unfavorable effect on binding due to the increasing basicity (i.e., a second protonated site at physiological pH) of the distal piperazine nitrogen. The same unfavorable effect was not exerted by the N^4-benzyl group in compound **1m**, suggesting that the lipophilicity of the benzyl group could compensate for the detrimental effect of a second piperazinium head in the binding to 5-HT_{1A} and 5-HT_{2A} receptors.

Taking into account the structural features of the reference compound flutroline, we examined also the effects of fluorine atoms on the phenyl groups at the positions N^1 and C^2 of the imidazol ring of the parent compound **1a**. The two alkoxy congeners **1b**,**o** proved to be among the most active compounds synthesized and tested so far. A pair wise comparison of binding data (**1a**, **1p**; **1b**, **1q**; and **1o**, **1r**) proved the fluoro-substitution to be nonessential. A marked improvement of affinity was indeed observed only for the fluorinated derivative **1p** over the parent **1a**, whereas compounds **1q**,**r** did not exhibit any increase in receptor affinities over the relative nonfluorinated compound **1b**,**o**, respectively.

For 5-HT_{2A} receptor binding, irrespective of its physicochemical property, any substituent on the piperazine N^4-phenyl group increased affinity. Among the substituents, halogens (Cl and F) elicited the highest activity, and when a comparison between positional isomers was possible (F, Cl, and OCH_3 congeners), ortho substitution appeared the most favorable one. Although the ortho-ethoxyphenyl congeners were the most active ones in binding D_2 and 5-HT_{1A} receptors, the structure−affinity relationship (SAR) trend observed for these two receptors was not exactly the same observed for the 5-HT_{2A} receptor.

In summary, our in vitro binding studies showed that the affinities of 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-phenylpiperazines for DA D_2 like, serotonin 5-HT_{1A}, and 5-HT_{2A} receptors are greatly affected by the pattern

Table 2. Affinities of Compounds **1a**−**r** for DA D_2 Like, 5-HT_{1A}, and 5-HT_{2A} Receptors[a]



			IC_{50} (nM)		
compd	R	X	D_2 like ([^{3}H]YM-09151)	5-HT_{1A} ([^{3}H]8-OH-DPAT)	5-HT_{2A} ([^{3}H]ketanserin)
1a	C_6H_5	H	1017 ± 657	2704 ± 20	>10 000
1b	*o*-$MeOC_6H_4$	H	70.3 ± 7.7	73.9 ± 1.9	131 ± 19
1c	*m*-$MeOC_6H_4$	H	167.5 ± 47.5	270.5 ± 12	2341 ± 221
1d	*p*-$MeOC_6H_4$	H	5796 ± 237	8442 ± 256	1920 ± 150
1e	*o*-ClC_6H_4	H	295.5 ± 60.5	255 ± 40	142 ± 10
1f	*m*-ClC_6H_4	H	2135.5 ± 94.5	165.7 ± 8	717 ± 52
1g	*p*-ClC_6H_4	H	91.8 ± 7.7	1895 ± 351	315 ± 12
1h	*o*-FC_6H_4	H	408.5 ± 17.5	332 ± 22	25.3 ± 0.5
1i	*p*-FC_6H_4	H	1285.5 ± 43.5	806.4 ± 61	501 ± 54.2
1j	*m*-$CF_3C_6H_4$	H	135.5 ± 10.5	206 ± 12	2130 ± 163
1k	*p*-$CF_3C_6H_4$	H	1007.5 ± 14.5	224 ± 20	1510 ± 144
1l	2-Pyridyl	H	2744 ± 1264	197 ± 15	2016 ± 130
1m	$CH_2C_6H_5$	H	4908 ± 542	1127 ± 83	2169 ± 200
1n	CH_3	H	>10 000	>10 000	>10 000
1o	*o*-$EtOC_6H_4$	H	34.7 ± 3.8	56.9 ± 0.3	171.5 ± 9.5
1p	C_6H_5	F	167.2 ± 20.2	525.1 ± 33	783 ± 54
1q	*o*-$MeOC_6H_4$	F	120.2 ± 18.8	171.2 ± 7.3	259.2 ± 10.8
1r	*o*-$EtOC_6H_4$	F	267.5 ± 10.5	51.9 ± 3	273 ± 12
clozapine			2108 ± 154	267.6 ± 16.6	40.7 ± 6.2
haloperidol			17.5 ± 1.1	1993 ± 121	125 ± 9.8
raclopride			3.4 ± 0.5		
cinanserin					14.8 ± 2

[a] The IC_{50} values are means ± SEM of values obtained from at least two experiments, each performed in triplicate and the results of which differed by <15%.

of chemical substitution, both at the phenyl ring linked to the piperazine distal nitrogen and the phenyl groups attached to the imidazole ring. Thus, with regard to modification of the piperazine N^4-phenyl ring, the affinities for both D_2 like and 5-HT_{1A} receptors were progressively increased by introduction of ortho-methoxy and ethoxy groups (**1b**,**o**, respectively), suggesting a possible influence of substituent lipophilicity. Although further suitable derivatives need to be synthesized and tested for better defining clear structure−affinity relationships, our data revealed the presence of a para-chloro substituent in **1g** to be associated with a relatively high affinity and substantial selectivity for D_2 like receptors, whereas the meta-chloro analogue **1f** exhibited preferential affinity for 5-HT_{1A} receptors. Furthermore, the ortho-F congener **1h** was noteworthy for its high affinity for 5-HT_{2A} receptors. A methyl group (**1n**), in place of a phenyl (but also a benzyl) group on piperazine N,[4] led to a complete loss of affinity for all three receptors. With regard to modification of the diphenyl portion of the structure, the introduction of fluorine atoms in **1o**, yielding analogue **1r**, resulted in a decrease in affinity for D_2 like receptors but no change in affinity for 5-HT_{1A} receptors.

Quantitative SAR (QSAR) Studies. To understand the relationships between their binding affinity to D_2, 5-HT_{1A}, and 5-HT_{2A} receptors and physicochemical properties, a Hansch type QSAR study of the 18 congeners of 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-piperazine **1a**−**r** (Table 3) was carried out by a stepwise multiple linear regression (MLR) with cross-validation. To account also for inactive compounds, semiquantitative binding data (i.e., IC_{50} > 10 000 nM) were included in the regression analysis by using a truncated pIC_{50} value of 5. Similar results, sometimes even with better statistics (but likely due to some leverage effects), were obtained when truncated at 4.

Table 3. Physicochemical Parameters[a] Used in the QSAR Study of 1-[(1,2-Diphenyl-1*H*-4-imidazolyl)methyl]-4-piperazines (**1a**−**r**)

compd	π	ws	mr	mv[b]	σ	F	R	I_{ortho}
1a	0	0	0	0	0	0	0	0
1b	0.02	0.21	0.62	0.22	0	0.32	−0.44	0.21
1c	0.02	0.21	0.62	0.22	0.12	0.25	−0.18	0.21
1d	0.02	0.22	0.62	0.22	−0.27	0.26	−0.51	0.22
1e	0.88	0.55	0.49	0.09	0.67	0.51	−0.19	0.55
1f	0.88	0.53	0.49	0.09	0.37	0.40	−0.05	0.53
1g	0.88	0.52	0.49	0.09	0.23	0.41	−0.15	0.52
1h	0.31	0.73	0.02	0.03	0.47	0.54	−0.29	0.73
1i	0.31	0.66	0.02	0.03	0.06	0.43	−0.34	0.66
1j	1.18	2.09	0.51	0.30	0.43	0.37	0.07	2.09
1k	1.18	2.05	0.51	0.30	0.54	0.38	0.19	2.05
1l	−0.95	−0.45	−0.21	−0.11	0.56	0.38	−0.18	−0.45
1m	0.86	0.12	0.46	0.16	0	−0.08	−0.01	0.12
1n	−0.85	−0.93	−2.05	−0.53	0	0	0	−0.93
1o	0.55	0.33	1.08	0.38	0.02	0.27	−0.38	0.33
1p	0.30	1.19	0.03	0.06	0	0	0	1.19
1q	0.32	1.44	0.65	0.27	0	0.32	−0.44	1.44
1r	0.85	1.57	1.11	0.43	0.02	0.27	−0.38	1.57

[a] The physicochemical descriptors (see text for definition and softwares used for calculation) reported in the table represent the substituent contribution to the molecular parameter, i.e., the difference between the parameter calculated for each molecule and the reference compound **1a**. [b] Calculated molar volumes (cm^3) were normalized multiplying them by a factor of 1×10^{-2}.

Each substituent was described by eight parameters. Classical Hammett (σ) and Swain−Lupton (F and R) constants were used as electronic parameters.[36] While

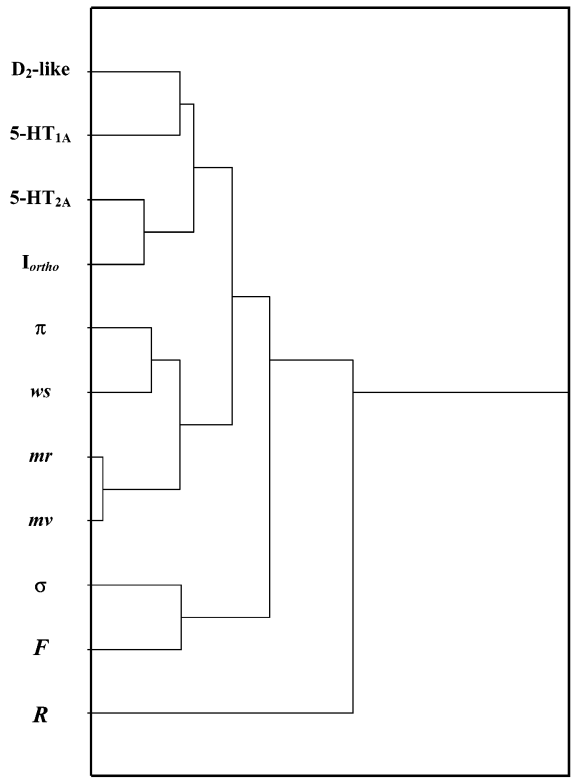


Figure 2. Dendrogram of similarity among variables obtained using a hierarchical cluster analysis based on correlation coefficients.

special σ constants for anilines were taken from standard compilations,[37] the F and R parameters for the substituents at the meta and ortho positions were corrected according to Williams and Norrington.[38] The Hansch constant (π), calculated from CLOGP values,[39] was used as a descriptor of lipophilicity, whereas water solubility (ws) was calculated using an online available program.[40] Bulkiness was parametrized by molar volume (mv/100), calculated with the ACDLab program,[41] and molar refractivity (mr).[39] An indicator variable (I_{ortho}) was also used to account for ortho substitution effect, regularly favorable for 5-HT_{2A} affinity, on the receptor binding ($I_{ortho} = 1$ for ortho-substituted derivatives and 0 for the others).

A selection of the pertinent variables was carried out by considering the intercorrelation among predictors (physicochemical parameters) and their influence (explained variance of the biological data) on the regression model. A cluster analysis based on correlation coefficients among variables (Figure 2) showed which parameters contain comparable information, namely, mr and mv or π and ws. Cluster analysis proved ortho effects, not completely accounted for by electronic parameters, to be somehow related to receptor binding, especially to 5-HT_{2A} receptors. Thus, we performed a stepwise multiparametric regression analysis (MRA) using only six predictors (I_{ortho}, π, mv, σ, F, and R), retaining only QSARs with cross-validated (leave-one-out procedure, loo) $q^2 > 0.5$. MRA carried out on the whole set of congeners retained four variables (lipophilicity constant π in quadratic term) to generate eq 1, explaining more than 80% of the variance in the 5-HT_{2A} binding data

$$\mathrm{pIC_{50}}\ (5\text{-}HT_{2A}) = 0.28\ (\pm 0.15)\ \pi - 0.37\ (\pm 0.19)\ \pi^2 + 1.61\ (\pm 0.48)\ F + 0.75\ (\pm 0.19)\ I_{ortho} + 5.52\ (\pm 0.16) \quad (1)$$

$$n = 18,\ r^2 = 0.8317,\ q^2 = 0.7147,\ \text{and}\ s = 0.3748$$

where n represents the number of data points, r^2 represents the squared correlation coefficient, q^2 represents the loo cross-validation coefficient (an assessment of the "internal: predictive ability of the QSAR model), and s represents the standard deviation of the regression equation; 95% confidence intervals of the regression coefficients are given in parentheses.

Equation 1 relates 5-HT_{2A} affinity mainly to electronic field effects (electron poor phenyl ring favorable) and lipophilic properties of the substituent. The significant contribution to the QSAR model of I_{ortho} suggests the importance for binding 5-HT_{2A} receptors of effect not already accounted for by electronic and lipophilic constants. Actually, the importance of lipophilicity of the ligands binding 5-HT_{2A} receptors had been elucidated by a number of QSAR studies, one recent example being represented by the GRID/GOLPE-derived 3D QSAR of (aminoalkyl)benzo and heterocycloalkanones.[42] The regression eq 1 incorporates a field electronic effect of phenyl substituents as a relevant one for increasing 5-HT_{2A} affinity, in good agreement with QSARs published by others, which included highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies as descriptors of interaction between 5-HT_{2A} receptor antagonists and a biomimetic donor in charge−transfer complexation.[43]

With the same independent variables in eq 1, no significant result was obtained for both 5-HT_{1A} and D_2 like receptor binding data, whereas a three parameter equation having a loo q^2 of 0.65 was obtained, which related 5-HT_{1A} binding data to the Hammett σ constant, mv, and I_{ortho}. The following eq 2, explaining about 74% of the variance in the biological data, was derived by omitting from regression compound **1d** (the para-methoxy congener) as the strongest outlier

$$\mathrm{pIC_{50}}\ (5\text{-}HT_{1A}) = 1.70\ (\pm 0.43)\ \mathrm{mv} + 0.73\ (\pm 0.50)\ F + 0.33\ (\pm 0.20)\ I_{ortho} + 5.92\ (\pm 0.16) \quad (2)$$

$$n = 17,\ r^2 = 0.7386,\ q^2 = 0.6482,\ \text{and}\ s = 0.3488$$

Equation 2, statistically poorer than eq 1, suggests an increasing 5-HT_{1A} binding to be related to favorable effects of bulky and electron-withdrawing substituents and favorable ortho substitution effects as well, as already observed for 5-HT_{2A} binding. The three parameter eq 2 is in good agreement with the comparative molecular field analysis results of Testa and co-workers.[44] Studying more than 100 arylpiperazines, indeed they found high electron density in the ortho position (compare with I_{ortho} and F in eq 2), steric bulk (compare with mv in eq 2), and electron deficiency (compare with F in eq 2) of the N-phenyl group as factors increasing 5-HT_{1A} affinity. The positive and high coefficient multiplying the substituent molar volume would indicate that the piperazine N^4-(X)phenyl could interact with a

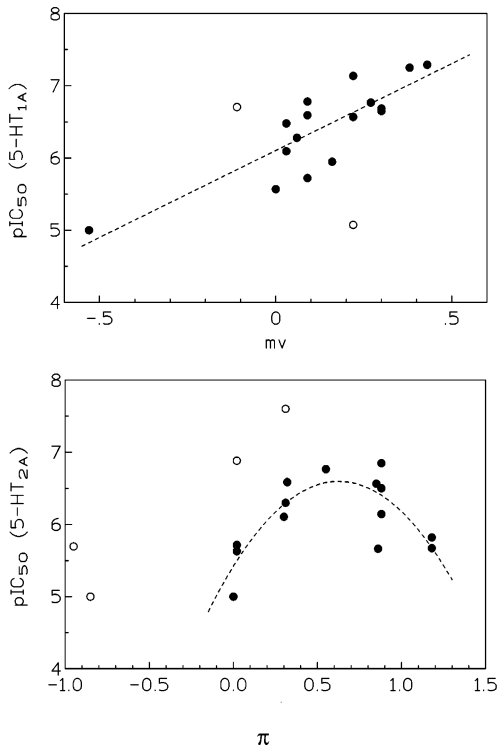


Figure 3. Plots of pIC_{50} values against substituent molar volume (5-HT_{1A}) and hydrophobicity constant (5-HT_{2A}). Filled circles represent the congeners whose binding and physicochemical data are reasonably correlated.

semipolar surface of the 5-HT_{1A} rather than with a hydrophobic pocket, as instead one could infer for 5-HT_{2A} receptor from the parabolic model accounted for by eq 1. This is somehow evident from the plots in Figure 3, which suggest a trend of linear correlation between 5-HT_{1A} binding data and substituent's molar volumes and a trend of nonlinear (likely parabolic) relationship between 5-HT_{2A} binding data and lipophilicity constants.

Within the property space defined by the eight physicochemical descriptors in Table 3, no significant QSAR was obtained for D_2 like receptor binding data, which were however poorly correlated with both 5-HT_{1A} ($r^2 = 0.40$) and 5-HT_{2A} ($r^2 = 0.34$) pIC_{50} values.

While the above QSARs highlighted the main physicochemical factors eliciting the binding of 1-[(1,2-diphenyl-1*H*-4-imidazolyl)methyl]-4-(2-methoxyphenyl)-piperazines to serotonergic 5-HT receptors and could have value for molecular design, we further explored their pharmacological profile by measuring effects on the release of DA and on $GABA_A$ receptor function. As a representative compound for these studies, we chose the 2-methoxyphenyl congener **1b**, which exhibited a D_2/5-HT_{1A} IC_{50} ratio of ~1. Its affinity for D_2 like receptors was moderately lower than those of typical antipsychotics (e.g., haloperidol and raclopride) but higher than that of clozapine, whereas it showed a binding to 5-HT_{1A} receptors higher than that of clozapine.

Drug Effects on DA Release in the Rat Prefrontal Cortex In Vivo. The blockade of hyperactive DA function in the subcortical areas, especially in the nucleus accumbens, has been implicated in the positive symptom efficacy of antipsychotics.[45] However, increasing evidence supports the crucial role of the prefrontal cortex in schizophrenia.[46] Moreover, it has been shown that atypical antipsychotics preferentially increase DA output in the prefrontal cortex as compared with the nucleus accumbens or striatum, a feature that was not observed with the typical antipsychotic haloperidol.[47,48] In light of these differences, with the use of microdialysis, we compared the effects of acute administration of compound **1b** on the extracellular concentrations of DA in the medial prefrontal cortex of freely moving rats with those of clozapine.

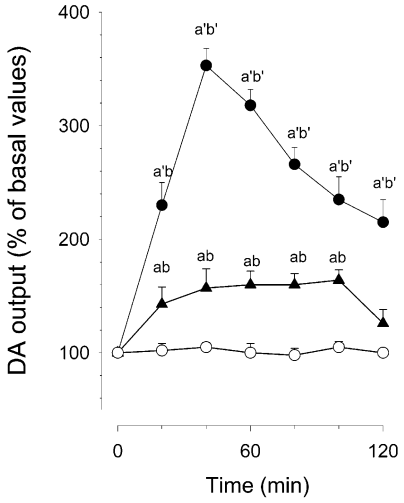


Figure 4. Effects of acute administration of clozapine [2.5 (open circles), 5 (triangles), 10 (closed circles) mg/kg, ip] on the extracellular concentrations of DA in the rat prefrontal cortex. Data are means ± SEM of values from at least five rats per dose and are expressed as a percentage of basal values. $^{a}P < 0.05$, $^{a'}P < 0.01$ vs basal values; $^{b}P < 0.05$, $^{b'}P < 0.01$ vs previous dose.

The basal extracellular concentrations of DA in this brain region were 14.75 ± 2.03 fmol per 40 μL sample. Intraperitoneal administration of the respective vehicles for treatment with clozapine and **1b** had no effect on the output of DA in the prefrontal cortex (data not shown; $P = 0.8254$).

The acute administration of clozapine (2.5−10 mg/kg, ip) induced a dose-dependent increase in DA release in the medial prefrontal cortex (Figure 4). The maximal increase (+250%) was observed at the dose of 10 mg/kg 40 min after drug injection; DA release remained significantly increased for ~160 min and returned to basal levels 3 h after drug administration (data not shown). The increase in DA release induced by clozapine at 5 mg/kg was also maximal (+60%) 40 min after drug injection and persisted for ~100 min. The lowest dose of clozapine tested (2.5 mg/kg) had no significant effect on DA output. Analysis of variance (ANOVA) confirmed that the acute administration of clozapine resulted in a significant dose-related increase in DA release ($F = 42.423$; $P < 0.001$).

The acute administration of **1b** (5−10 mg/kg, ip) also increased the extracellular concentration of DA in the prefrontal cortex in a dose-dependent manner (Figure 5). The highest dose (10 mg/kg) elicited the greatest effect (+100%), whereas the dose of 7.5 mg/kg induced a maximal increase in DA release of +80%, whereas a 5 mg/kg dose did not show any significant effect. The maximal effects of the two highest doses were each apparent 40 min after drug injection, and DA release

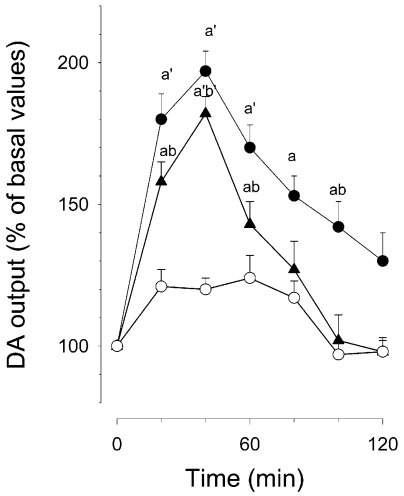


Figure 5. Effects of acute administration of compound **1b** [5 (open circles), 7.5 (triangles), 10 (closed circles) mg/kg, ip] on the extracellular concentrations of DA in the rat prefrontal cortex. Data are means ± SEM of values from at least five rats per dose and are expressed as a percentage of basal values. $^{a}P < 0.05$, $^{a'}P < 0.01$ vs basal values; $^{b}P < 0.05$, $^{b'}P < 0.01$ vs previous dose.

remained significantly increased for ~60−100 min. ANOVA confirmed that the acute administration of **1b** induced a significant dose-dependent increase in the extracellular concentration of DA ($F = 11.2788$; $P < 0.001$).

Finally, preliminary pharmacological results indicate that in rats, the administration of **1b** (5−10 mg/kg, ip), similarly to that of clozapine (5−10 mg/kg, ip), fails to induce cataleptic rigidity, as assessed by the inclined (60°) grid method.[49] Thus, in this respect, compound **1b** resembles clozapine and markedly differs from haloperidol, which, as expected at the dose of 2 mg/kg ip, elicits a cataleptic effect.

Our demonstration that **1b** stimulates DA output in the prefrontal cortex is consistent with a putative antagonistic action of this compound at presynaptic DA D_2 receptors. Activation of such receptors by released DA is thought to be one of the main mechanisms for regulating the dopaminergic neuronal activity.[50] Stimulation of autoreceptors located on nerve terminals thus results in inhibition of DA synthesis or release, whereas stimulation of autoreceptors located in the somatodendritic region reduces the firing rate of dopaminergic neurons.[50] Blockade of these receptors by **1b**, as well as by clozapine and other antipsychotic drugs, would therefore be expected to increase the extracellular concentration of DA. However, the observed changes in DA output in the medial prefrontal cortex induced by these drugs cannot be explained solely on the basis of blockade of D_2 receptors. In fact, the acute administration of haloperidol, a selective D_2 receptor antagonist, does not affect the extracellular concentration of DA in this brain region. Other receptor interactions have been suggested to contribute to the neurochemical changes induced by atypical antipsychotics in the medial prefrontal cortex; in particular, 5-HT_2 receptors have been demonstrated to be involved in the effects of these drugs.[51]

Drug Effects on $GABA_A$ Receptor Function. Clozapine and other atypical antipsychotic drugs as well have been shown to interact with $GABA_A$ receptors and to affect their function at pharmacologically relevant concentrations.[11,12,16,52,53] With an electrophysiological approach, Michel and Trudeau[12] have also shown that clozapine inhibits synaptic transmission at GABAergic synapses in cultured ventral tegmental area neurons. This inhibitory effect of clozapine was apparent at concentrations between 1 and 50 μM.

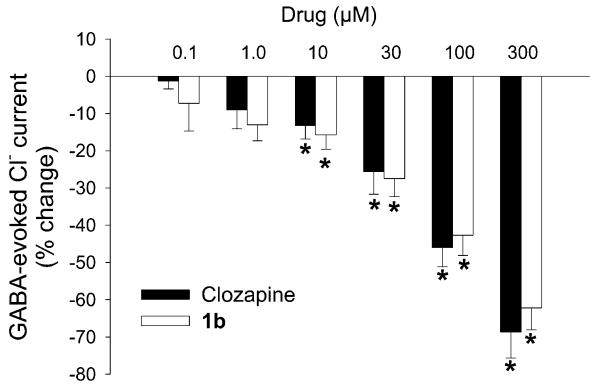


Figure 6. Effects of clozapine and compound **1b** on GABA-evoked Cl^- currents at human $GABA_A$ receptors. *Xenopus* oocytes expressing α_1, β_2, and γ_{2L} subunits of human $GABA_A$ receptors were voltage-clamped at −70 mV in order to measure Cl^- currents evoked by GABA (EC_{30}) in the absence or presence of clozapine or **1b** at the indicated concentrations. Data are expressed as percentage change relative to the control response elicited by GABA alone and are means ± SEM of values from 12 to 14 different oocytes. *$P < 0.05$ vs the control response.

We thus investigated whether compound **1b** also shared this property. We adopted an electrophysiological approach to evaluate the effects of **1b** and clozapine on GABA-evoked Cl^- currents at recombinant human $GABA_A$ receptors expressed in *Xenopus* oocytes. Our results revealed that compound **1b**, like clozapine, both at doses ranging from 0.1 to 300 μM, inhibited in a concentration-dependent manner the Cl^- currents evoked by GABA at $GABA_A$ receptors comprising α_1, β_2, and γ_{2L} subunits (Figure 6). The inhibitory effects of both compounds were statistically significant at concentrations as low as 10 μM and maximal (60−70% inhibition) at the highest concentration tested (300 μM).

Evidence suggests that $GABA_A$ receptors contribute to modulation of the activity of mesocortical and mesolimbic dopaminergic neurons. Selective, partial, and full agonists of benzodiazepine receptors reduce both the basal and the stress-induced output of DA in the cerebral cortex and nucleus accumbens;[54−58] conversely, acute treatment with anxiogenic inverse agonists of these receptors, such as FG 7142, induces an increase in the extracellular concentration of DA.[59−61] Our observation that **1b**, like clozapine, is able to inhibit $GABA_A$ receptor activity suggests that the observed increase in DA output induced by the acute administration of these drugs also might result from a decrease in the extent of GABAergic inhibitory control of dopaminergic neurons.

Conclusion

Combination of a 1,2-diphenyl-4-methylen-imidazole framework with substituted N^4-phenylpiperazines yielded a new class of compounds (**1**) with mixed DA D_2/serotonin 5-HT_{1A} and 5-HT_{2A} affinity. In good agree-

ment with previous results obtained by others, a QSAR analysis allowed us to develop regression models highlighting factors modulating the binding to subtypes 1A and 2A of serotonin 5-HT receptors. After the initial screening of the neurochemical profiles of compounds **1a**−**r**, we selected compound **1b**, exhibiting high affinity for DA D_2 like, 5-HT_{1A}, and 5-HT_{2A} receptors, as a representative one for further pharmacological studies. The results of our in vitro and in vivo studies demonstrated that compound **1b** possesses pharmacological properties similar to those of clozapine, the prototype of atypical antipsychotic drugs. Thus, the acute intraperitoneal administration of **1b**, like that of clozapine, induced a selective increase in the extracellular concentration of DA in the medial prefrontal cortex of rats. Moreover, compound **1b** and clozapine each inhibited GABA-evoked Cl^- currents at recombinant $GABA_A$ receptors.

The important role of serotonergic neurotransmission, in addition to those of the dopaminergic and GABAergic systems, in mediating the clinical action of atypical antipsychotics such as clozapine is well-established.[10] Although the effects of these compounds on the function of these receptors remain to be determined and their full safety profile remains to be established, our neurochemical data suggested that an activity at 5-HT receptors might be relevant to their pharmacological actions. Finally, preliminary results indicate that **1b**, like clozapine, does not induce cataleptic effects in rats.

Experimental Section

Chemistry. Reactions were monitored by thin-layer chromatography (TLC) using Polygram SIL and ALOX N/UV_{254} precoated plastic sheets (0.2 mm) and iodine vapor and/or UV light for detection. Pure compounds showed a single spot on TLC. Flash chromatography was performed using Merck silica gel type 60 (size 230−240 mesh ASTM). Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 500 FT IR spectrophotometer (KBr pellets for solid samples). ^{1}H NMR spectra were recorded on a VARIAN XL 200 FT NMR spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane, and coupling constants (J) are expressed in Hz. Electron ionization mass spectra (70 eV) were recorded on a Hewlett-Packard 5790-5970 MSD gas chromatograph/mass spectrometer. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer, and results were within ±0.40% of the theoretical values. All moisture sensitive reactions were performed under nitrogen atmosphere, using oven-dried glassware and syringes to transfer solutions. The organic extracts were dried over anhydrous $MgSO_4$ prior to solvent evaporation. Anhydrous THF was obtained from Aldrich in sure-seal bottles. All starting materials and reagents were commercially available from Aldrich.

***N*-Phenylbenzenecarboximidamide (4).** To a cooled (0 °C) solution of freshly distilled aniline (4.9 mL, 54 mmol) in toluene (285 mL), trimethylaluminum (38 mL, 2 M solution in toluene, 76 mmol) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 3.5 h. A solution of benzonitrile (7.2 mL, 70 mmol) in toluene (210 mL) was then added, and the resulting mixture was warmed to 70 °C for 17 h. Next, the solution was cooled at room temperature and poured into a slurry of silica gel (200 g) in 600 mL of $CHCl_3$/CH_3OH (2:1). The silica gel was filtered off and washed with a mixture of CH_2Cl_2/CH_3OH (2:1). The combined filtrates were evaporated in vacuo to leave a crude residue, which was triturated with a mixture of hexane/Et_2O (2:1). The resulting solid was collected by filtration to give **4** as a white powder (8.4 g, 80%). Crystallization from petroleum ether/Et_2O gave pure crystals of **4**; mp 116−118 °C.[62] TLC (AcOEt/petroleum ether, 7:3) R_f = 0.45. IR (KBr): 3467, 3346, 1616, 1591 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.85 (br s, 2H, NH and C═NH, 2H D_2O exchanged), 6.95−7.08 (m, 3H, ArH), 7.30−7.44 (m, 5H, ArH), 7.83−7.86 (m, 2H, ArH). MS m/z 196 (M^+, base). Anal. ($C_{13}H_{12}N_2$) C, H, N.

Ethyl 4-Hydroxy-1,2-diphenyl-4,5-dihydro-1*H*-imidazole-4-carboxylate (6). A mixture of amidine **4** (6 g, 30.6 mmol) and $NaHCO_3$ (5 g, 60 mmol) in 2-propanol (450 mL) was warmed at 40 °C, and then, ethyl bromopyruvate (5.2 mL, 42 mmol) was added dropwise. The reaction mixture was heated at 70 °C for 1.5 h, cooled, and concentrated in vacuo. The residue was solubilized in CH_2Cl_2 and washed with water. The dried organic layer was evaporated in vacuo yielding an orange oil. The crude oil was purified by flash silica gel chromatography using a gradient of 70−80% AcOEt/petroleum ether as eluent to give **6** (6 g, 63%) as a pale yellow solid. Crystallization from $CHCl_3$ gave pure crystals of **6**; mp 144−145 °C. TLC (AcOEt/petroleum ether, 7:3) R_f = 0.38. IR (KBr): 3400, 1749, 1588 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 7.0 Hz, 3H, CH_3), 4.05 (d, J = 10.8 Hz, 1H, CH-5), 4.27 (q, J = 7.0 Hz, 2H, CH_2O), 4.57 (d, J = 11.0 Hz, 1H, CH-5), 5.21 (br s, 1H, OH, 1H D_2O exchanged), 6.86−7.56 (m, 10H, ArH). MS m/z 292 (M^+ − 18, 95), 220 (base). Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

Ethyl 1,2-Diphenyl-1*H*-imidazole-4-carboxylate (8). A mixture of carbinol **6** (4.5 g, 14.5 mmol) and 0.56 g of *p*-toluenesulfonic acid in 450 mL of toluene was refluxed in a Dean−Stark apparatus until removal of water was completed (1.5 h). After it was cooled at room temperature and the solvent was evaporated in vacuo, the crude residue was solubilized in CH_2Cl_2 and washed with water and 5% aqueous $NaHCO_3$. The dried organic layer, after evaporation, gave an oily residue, which was triturated with ether to afford **8** as a white solid (4 g, 94%). Crystallization from petroleum ether/CH_2Cl_2 gave pure crystals of **8**; mp 132−134 °C. TLC (AcOEt/petroleum ether, 7:3) R_f = 0.69. IR (KBr): 1702, 1552 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.41 (t, J = 8.0 Hz, 3H, CH_3), 4.42 (q, J = 8.0 Hz, 2H, CH_2O), 7.20−7.44 (m, 10H, ArH), 7.84 (s, 1H, CH-5). MS m/z 292 (M^+, 79), 220 (base). Anal. ($C_{18}H_{16}N_2O_2$) C, H, N.

Compounds **5**, **7**, and **9** were prepared following the procedures reported for compounds **4**, **6**, and **8**, respectively.

4-Fluoro-*N*-(4-fluorophenyl)benzenecarboximidamide (5). Yield 75%; mp 120−121 °C ($CHCl_3$). TLC (AcOEt/petroleum ether, 3:7) R_f = 0.50. IR (KBr): 3455, 3317, 1639, 1612, 1574 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 6.73−7.41 (m, 8H, ArH), 10.10−10.61 (br s, 2H, NH and C═NH, 2H D_2O exchanged). MS m/z 232 (M^+, base). Anal. ($C_{13}H_{10}F_2N_2$) C, H, N.

Ethyl 1,2-Bis(4-fluorophenyl)-4-hydroxy-4,5-dihydro-1*H*-imidazole-4-carboxylate (7). Yield 57%; mp 149−150 °C (ether/CH_2Cl_2). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.41. IR (KBr): 3450, 1748, 1686, 1607 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, J = 6.0 Hz, 3H, CH_3), 3.99 (d, J = 11.0 Hz, 1H, CH-5), 4.28 (q, J = 6.0 Hz, 3H, CH_2O and OH, 1H D_2O exchanged), 4.48 (d, J = 10.8 Hz, 1H, CH-5), 6.90−7.57 (m, 8H, ArH). MS m/z 328 (M^+ − 18, 93), 256 (base). Anal. ($C_{18}H_{16}F_2N_2O_3$) C, H, N.

Ethyl 1,2-Bis(4-fluorophenyl)-1*H*-imidazole-4-carboxylate (9). Yield 85%; mp 123−124 °C (ether/$CHCl_3$). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.75. IR (KBr): 1701, 1548 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.39 (t, J = 8.0 Hz, 3H, CH_3), 4.40 (q, J = 8.0 Hz, 2H, CH_2O), 6.92−7.42 (m, 8H, ArH), 7.80 (s, 1H, CH-5). MS m/z 328 (M^+, 92), 256 (base). Anal. ($C_{18}H_{14}F_2N_2O_2$) C, H, N.

1,2-Diphenyl-1*H*-imidazole-4-carboxylic Acid (10). A mixture of ester **8** (1.8 g, 6.1 mmol) in CH_3OH (43 mL) and NaOH 1 N (43 mL) was refluxed for 1.5 h, cooled, and concentrated in vacuo. The residue was solubilized in water and then acidified with concentrated HCl. The mixture was extracted with CH_2Cl_2, and the combined extracts were dried and evaporated in vacuo. The residue was triturated with ether to give compound **10** as a yellow solid (1.3 g, 80%). Crystal-

lization from CCl_4/CH_2Cl_2 gave pure, white crystals of **10**; mp 208−212 °C. TLC ($CHCl_3/CH_3OH$, 8:2) R_f = 0.2. IR (KBr): 1700, 1555 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.23−7.46 (m, 10H, ArH), 7.92 (s, 1H, CH-5), 9.14 (br s, 1H, OH, 1H D_2O exchanged). MS *m/z* 264 (M^+, 15), 219 (base). Anal. ($C_{16}H_{12}N_2O_2$) C, H, N.

1,2-Bis(4-Fluorophenyl)-1*H*-imidazole-4-carboxylic Acid (11). This compound was obtained following the procedure to prepare compound **10**; yield 94%; mp 197−198 °C (ether). TLC ($CHCl_3/CH_3OH$, 8:2) R_f = 0.25. IR (KBr): 1702, 1511 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.64 (br s, 1H, OH, 1H D_2O exchanged) 6.90−7.42 (m, 8H, ArH), 7.79 (s, 1H, CH-5). MS *m/z* 300 (M^+, 20), 256 (base). Anal. ($C_{16}H_{10}F_2N_2O_2$) C, H, N.

General Procedure for Preparation of Intermediate Carboxamides 12a−r. Method A for Carboxamides 12a−d,h−n,p,q. The synthesis of 1-[(1,2-diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-phenylpiperazine (**12a**) is described as a general procedure. A mixture of **10** (0.5 g, 1.9 mmol), 1-phenylpiperazine (0.29 mL, 1.9 mmol), and HOBt (0.25 g, 1.9 mmol) in anhydrous THF (12 mL) was stirred at room temperature for 15 min. The reaction mixture was cooled at 0 °C, and an equimolar amount of DCC was added. Stirring was prolonged for 1 h, and then, the reaction mixture was allowed to warm at room temperature and left overnight. The precipitated dicyclohexylurea (DCU) was removed by filtration and washed with THF. The filtrate was washed with 5% aqueous $NaHCO_3$, dried, and evaporated in vacuo. The oily residue was purified by flash silica gel chromatography, eluting with AcOEt/petroleum ether 1:1 to give **12a** (0.58 g, 75%) as a white powder. Recrystallization from $CHCl_3/CH_3OH$ afforded pure crystals of **12a**; mp 174−175 °C. TLC (AcOEt/petroleum ether, 7:3) R_f = 0.52. IR (KBr): 1601, 1549 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.27−3.32 (m, 4H, 2×CH_2), 3.98 (m, 2H, CH_2), 4.62 (m, 2H, CH_2), 6.86−6.99 (m, 3H, ArH), 7.22−7.44 (m, 12H, ArH), 7.81 (s, 1H, CH-5). MS *m/z* 408 (M^+, 19), 247 (base). Anal. ($C_{26}H_{24}N_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(2-methoxyphenyl)piperazine (12b). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.39. IR (KBr): 1603, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.15−3.20 (m, 4H, 2 × CH_2), 3.90 (s, 3H, OCH_3), 3.97−4.00 (m, 2H, CH_2), 4.63 (m, 2H, CH_2), 6.91−7.00 (m, 4H, ArH), 7.22−7.44 (m, 10H, ArH), 7.80 (s, 1H, CH-5). MS *m/z* 438 (M^+, 34), 247 (base). Anal. ($C_{27}H_{26}N_4O_2$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(3-methoxyphenyl)piperazine (12c). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.54. IR (KBr): 1606, 1537 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.27−3.32 (m, 4H, 2 × CH_2), 3.80 (s, 3H, OCH_3), 3.96 (m, 2H, CH_2), 4.60 (m, 2H, CH_2), 6.44−6.60 (m, 4H, ArH), 7.16−7.42 (m, 10H, ArH), 7.81 (s, 1H, CH-5). MS *m/z* 438 (M^+, 8), 247 (base). Anal. ($C_{27}H_{26}N_4O_2$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(4-methoxyphenyl)piperazine (12d). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.42. IR (KBr): 1610, 1545 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.15−3.20 (m, 4H, 2 × CH_2), 3.77 (s, 3H, OCH_3), 3.94−3.97 (m, 2H, CH_2), 4.59 (m, 2H, CH_2), 6.85 (d, J = 8.8 Hz, 2H, ArH), 6.95 (d, J = 9.2 Hz, 2H, ArH), 7.26−7.40 (m, 10H, ArH), 7.80 (s, 1H, CH-5). MS *m/z* 438 (M^+, 49), 247 (base). Anal. ($C_{27}H_{26}N_4O_2$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(2-fluorophenyl)piperazine (12h). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.66. IR (KBr): 1623, 1543 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.18−3.21 (m, 4H, 2 × CH_2), 3.99 (m, 2H, CH_2), 4.63 (m, 2H, CH_2), 6.96−7.05 (m, 4H, ArH), 7.27−7.41 (m, 10H, ArH), 7.81 (s, 1H, CH-5). MS *m/z* 426 (M^+, 9), 247 (base). Anal. ($C_{26}H_{23}FN_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(4-fluorophenyl)piperazine (12i). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.52. IR (KBr): 1604, 1552 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.18−3.23 (m, 4H, 2 × CH_2), 3.95−3.96 (m, 2H, CH_2), 4.59−4.61 (m, 2H, CH_2), 6.87−7.03 (m, 4H, ArH), 7.21−7.43 (m, 10H, ArH), 7.80 (s, 1H, CH-5). MS *m/z* 426 (M^+, 29), 247 (base). Anal. ($C_{26}H_{23}FN_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(3-trifluoromethylphenyl)piperazine (12j). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.68. IR (KBr): 1608, 1545 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.33−3.38 (m, 4H, 2 × CH_2), 4.00 (m, 2H, CH_2), 4.60−4.65 (m, 2H, CH_2), 7.10−7.16 (m, 4H, ArH), 7.27−7.41 (m, 10H, ArH), 7.82 (s, 1H, CH-5). MS *m/z* 476 (M^+, 11). 247 (base). Anal. ($C_{27}H_{23}F_3N_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(4-trifluoromethylphenyl)piperazine (12k). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.53. IR (KBr): 1618, 1546 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.33−3.38 (m, 4H, 2 × CH_2), 3.97−4.00 (m, 2H, CH_2), 4.62−4.64 (m, 2H, CH_2), 7.09−7.23 (m, 4H, ArH), 7.24−7.44 (m, 10H, ArH), 7.82 (s, 1H, CH-5). MS *m/z* 476 (M^+, 17), 247 (base). Anal. ($C_{27}H_{23}F_3N_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(2-pyridyl)piperazine (12l). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.32. IR (KBr): 1600, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.66−3.71 (m, 4H, 2 × CH_2), 3.93 (m, 2H, CH_2), 4.56−4.57 (m, 2H, CH_2), 6.67−6.71 (m, 2H, ArH), 7.23−7.56 (m, 11H, ArH), 7.81 (s, 1H, CH-5), 8.22 (m, 1H, ArH). MS *m/z* 409 (M^+, 28), 247 (base). Anal. ($C_{25}H_{23}N_5O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-benzylpiperazine (12m). TLC (AcOEt/petroleum ether, 8:2) R_f = 0.34. IR (KBr): 1601, 1534 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.53−2.58 (m, 4H, 2 × CH_2), 3.56 (s, 2H, CH_2Ar), 3.81 (m, 2H, CH_2), 4.43 (m, 2H, CH_2), 7.20−7.43 (m, 15H, ArH), 7.75 (s, 1H, CH-5). MS *m/z* 422 (M^+, 22), 331 (M^+ − 91, 11), 247 (base). Anal. ($C_{27}H_{26}N_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-methylpiperazine (12n). TLC ($CHCl_3/CH_3OH$, 8:2) R_f = 0.73. IR (KBr): 1609, 1524 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.34 (s, 3H, CH_3), 2.49−2.54 (m, 4H, 2 × CH_2), 3.83 (m, 2H, CH_2), 4.45 (m, 2H, CH_2), 7.22−7.41 (m, 10H, ArH), 7.76 (s, 1H, CH-5). MS *m/z* 346 (M^+, 8), 247 (base). Anal. ($C_{21}H_{22}N_4O$) C, H, N.

1-{[1,2-Bis(4-fluorophenyl)-1*H*-imidazol-4-yl]carbonyl}-4-phenylpiperazine (12p). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.71. IR (KBr): 1607, 1531 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.26−3.31 (m, 4H, 2 × CH_2), 3.96 (m, 2H, CH_2), 4.57 (m, 2H, CH_2), 6.90−7.03 (m, 5H, ArH), 7.13−7.37 (m, 8H, ArH), 7.76 (s, 1H, CH-5). MS *m/z* 444 (M^+, 19), 283 (base). Anal. ($C_{26}H_{22}F_2N_4O$) C, H, N.

1-{[1,2-Bis(4-fluorophenyl)-1*H*-imidazol-4-yl]carbonyl}-4-(2-methoxyphenyl)piperazine (12q). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.50. IR (KBr): 1629, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.15−3.20 (m, 4H, 2 × CH_2), 3.90 (s, 3H, OCH_3), 3.99 (m, 2H, CH_2), 4.59 (m, 2H, CH_2), 6.88−7.03 (m, 4H, ArH), 7.09−7.40 (m, 8H, ArH), 7.76 (s, 1H, CH-5). MS *m/z* 474 (M^+, 32), 283 (base). Anal. ($C_{27}H_{24}F_2N_4O_2$) C, H, N.

Method B for Carboxamides 12e−g,o,r. The synthesis of 1-[(1,2-diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(2-chlorophenyl)piperazine (**12e**) is described as a general procedure. To a solution of acid **10** (0.5 g 1.9 mmol) in CH_2Cl_2 (15 mL), two drops of dimethyl formamide (DMF) were added. Oxalyl chloride (0.33 mL, 5.67 mmol) was then added, and the solution was stirred at room temperature for 30 min and then evaporated in vacuo. The acid chloride was solubilized in CH_2Cl_2 (15 mL) and cooled at 0 °C (ice bath). At this temperature, a mixture of 1-(2-chlorophenyl)piperazine monohydrochloride (0.44 g, 1.9 mmol), K_2CO_3 (0.78 g, 5.67 mmol) in H_2O (1 mL), and CH_2Cl_2 (1 mL) was added. The reaction mixture was stirred at 0 °C for 4 h, and then, it was allowed to warm at room temperature and washed with 5% aqueous $NaHCO_3$. The dried organic layer, evaporated in vacuo, gave a crude residue, which was triturated with ether to afford **12e** (0.52 g, 62%) as a white solid. Recrystallization from $CHCl_3$/ether gave pure crystals of **12e**; mp 132−134 °C. TLC (AcOEt/petroleum ether, 7:3) R_f = 0.71. IR (KBr): 1611, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.14−3.20 (m, 4H, 2 × CH_2), 3.99 (m, 2H, CH_2), 4.61 (m, 2H, CH_2), 6.97−7.07 (m, 4H, ArH), 7.21−7.41 (m, 10H, ArH), 7.80 (s, 1H, CH-5). MS *m/z* 442 (M^+, 11), 247 (base). Anal. ($C_{26}H_{23}ClN_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(3-chlorophenyl)piperazine (12f). TLC (AcOEt/petroleum ether, 7:3) R_f = 0.71. IR (KBr): 1602, 1540 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.27−3.30 (m, 4H, 2 × CH_2), 3.95 (m, 2H, CH_2), 4.61 (m, 2H, CH_2), 6.60−6.91 (m, 3H, ArH), 7.14−7.40 (m, 11H, ArH),

7.81 (s, 1H, CH-5). MS *m*/*z* 442 (M^+, 12), 247 (base). Anal. ($C_{26}H_{23}ClN_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(4-chlorophenyl)piperazine (12g). TLC (AcOEt/petroleum ether, 7:3) $R_f = 0.69$. IR 1625, 1547 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.23−3.28 (m, 4H, 2 × CH_2), 3.96 (m, 2H, CH_2), 4.61 (m, 2H, CH_2), 6.87 (d, $J = 8.8$ Hz, 2H, ArH), 7.20−7.41 (m, 12H, ArH), 7.81 (s, 1H, CH-5). MS *m*/*z* 442 (M^+, 15), 247 (base). Anal. ($C_{26}H_{23}ClN_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)carbonyl]-4-(2-ethoxyphenyl)piperazine (12o). TLC (AcOEt/petroleum ether, 7:3) $R_f = 0.63$. IR (KBr): 1612, 1542 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.49 (t, $J = 6.6$ Hz, 3H, CH_3), 3.20 (m, 4H, 2 × CH_2), 4.0 (m, 2H, CH_2), 4.13 (q, $J = 6.6$ Hz, 2H, CH_2O), 4.62 (m, 2H, CH_2), 6.86−6.96 (m, 4H, ArH), 7.26−7.42 (m, 10H, ArH), 7.80 (s, 1H, CH-5). MS *m*/*z* 452 (M^+, 29), 247 (base). Anal. ($C_{28}H_{28}N_4O_2$) C, H, N.

{[1,2-Bis(4-fluorophenyl)-1*H*-imidazol-4-yl]carbonyl}-4-(2-ethoxyphenyl)piperazine (12r). TLC (AcOEt/petroleum ether, 7:3) $R_f = 0.61$. IR (KBr): 1615, 1540 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.49 (t, $J = 6.6$ Hz, 3H, CH_3), 3.19 (m, 4H, 2 × CH_2), 3.99 (m, 2H, CH_2), 4.12 (q, $J = 6.6$ Hz, 2H, CH_2O), 4.57 (m, 2H, CH_2), 6.66−7.40 (m, 12H, ArH), 7.75 (s, 1H, CH-5). MS *m*/*z* 488 (M^+, 48), 283 (base). Anal. ($C_{28}H_{26}F_2N_4O_2$) C, H, N.

General Procedure for Preparation of 1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-piperazines 1a−r. The synthesis of 1-[(1,2-diphenyl-1*H*-imidazol-4-yl)methyl]-4-phenylpiperazine (**1a)** is described as a general procedure. To a solution of carboxamide **12a** (0.71 g, 1.74 mmol) in anhydrous THF (30 mL) at room temperature, 2 M borane−methyl sulfide complex (2.55 mL, 5.1 mmol) was slowly added. The resulting mixture was refluxed for 1.5 h and then allowed to cool at room temperature. The solution was acidified with 1 N HCl and refluxed for 15 min. The cooled reaction mixture was neutralized with a saturated aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with water, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography, eluting with $CHCl_3/CH_3OH$ (9.5:0.5) to give a colorless oil, which was dried under vacuum to give **1a** (0.6 g, 88%). This compound crystallized on standing as white crystals of **1a**; mp 99−100 °C (ether). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.47$. IR (KBr): 1599 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.79−2.84 (m, 4H, 2 × CH_2), 3.23−3.28 (m, 4H, 2 × CH_2), 3.69 (s, 2H, CH_2), 6.61−6.96 (m, 3H, ArH), 7.11 (s, 1H, CH-5), 7.19−7.41 (m, 12H, ArH). MS *m*/*z* 394 (M^+, 1), 233 (base). Anal. ($C_{26}H_{26}N_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(2-methoxyphenyl)piperazine (1b). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.26$. IR (KBr): 1591 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.88−2.90 (m, 4H, 2 × CH_2), 3.14−3.16 (m, 4H, 2 × CH_2), 3.75 (s, 2H, CH_2), 3.86 (s, 3H, OCH_3), 6.95−6.98 (m, 4H, ArH), 7.15 (s, 1H, CH-5), 7.23−7.40 (m, 10H, ArH). MS *m*/*z* 424 (M^+, 3), 233 (base). Anal. ($C_{27}H_{28}N_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(3-methoxyphenyl)piperazine (1c). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.31$. IR (KBr): 1598 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.77−2.82 (m, 4H, 2 × CH_2), 3.23−3.26 (m, 4H, 2 × CH_2), 3.72 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 6.39−6.57 (m, 4H, ArH), 7.11 (s, 1H, CH-5), 7.16−7.36 (m, 10H, ArH). MS *m*/*z* 424 (M^+, 1), 233 (base). Anal. ($C_{27}H_{28}N_4O$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(4-methoxyphenyl)piperazine (1d). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.30$. IR (KBr): 1598 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.80−2.84 (m, 4H, 2 × CH_2), 3.13−3.16 (m, 4H, 2 × CH_2), 3.70 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2), 6.87 (d, $J = 9.2$ Hz, 2H, ArH), 6.92 (d, $J = 9.6$ Hz, 2H, ArH), 7.12 (s, 1H, CH-5), 7.19−7.40 (m, 10H, ArH). MS *m*/*z* 424 (M^+, 8), 233 (base). Anal. ($C_{27}H_{28}N_4O$) C, H, N.

1-(2-Chlorophenyl)-4-[(1,2-diphenyl-1*H*-imidazol-4-yl)-methyl]piperazine (1e). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.46$. IR (KBr): 1598 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.81−2.86 (m, 4H, 2 × CH_2), 3.15−3.19 (m, 4H, 2 × CH_2), 3.71 (s, 2H, CH_2), 6.94−7.06 (m, 4H, ArH), 7.11 (s, 1H, CH-5), 7.19−7.36 (m, 10H, ArH). MS *m*/*z* 428 (M^+, 2), 233 (base). Anal. ($C_{26}H_{25}ClN_4$) C, H, N.

1-(3-Chlorophenyl)-4-[(1,2-diphenyl-1*H*-imidazol-4-yl)-methyl]piperazine (1f). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.46$. IR (KBr): 1594 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.76−2.81 (m, 4H, 2 × CH_2), 3.23−3.27 (m, 4H, 2 × CH_2), 3.68 (s, 2H, CH_2), 6.76−6.88 (m, 4H, ArH), 7.11 (s, 1H, CH-5), 7.15−7.36 (m, 10H, ArH). MS *m*/*z* 428 (M^+, 3), 233 (base). Anal. ($C_{26}H_{25}ClN_4$) C, H, N.

1-(4-Chlorophenyl)-4-[(1,2-diphenyl-1*H*-imidazol-4-yl)-methyl]piperazine (1g). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.40$. IR (KBr): 1596 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.80 (m, 4H, 2 × CH_2), 3.21 (m, 4H, 2 × CH_2), 3.68 (s, 2H, CH_2), 6.82−6.86 (m, 3H, ArH), 7.11 (s, 1H, CH-5), 7.17−7.36 (m, 11H, ArH). MS *m*/*z* 428 (M^+, 3), 233 (base). Anal. ($C_{26}H_{25}ClN_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(2-fluorophenyl)piperazine (1h). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.43$. IR (KBr): 1598 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.84 (m, 4H, 2 × CH_2), 3.12−3.15 (m, 4H, 2 × CH_2), 3.71 (s, 2H, CH_2), 6.95−7.07 (m, 4H, ArH), 7.11 (s, 1H, CH-5), 7.17−7.39 (m, 10H, ArH). MS *m*/*z* 412 (M^+, 3), 233 (base). Anal. ($C_{26}H_{25}FN_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(4-fluorophenyl)piperazine (1i). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.33$. IR (KBr): 1595 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.79−2.83 (m, 4H, 2 × CH_2), 3.15−3.20 (m, 4H, 2 × CH_2), 3.69 (s, 2H, CH_2), 6.89−6.95 (m, 4H, ArH), 7.11 (s, 1H, CH-5), 7.23−7.37-(m, 10H, ArH). MS *m*/*z* 412 (M^+, 1), 233 (base). Anal. ($C_{26}H_{25}FN_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-[3-(trifluoromethyl)phenyl]piperazine (1j). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.33$. IR (KBr): 1603 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.79−2.84 (m, 4H, 2 × CH_2), 3.27−3.31 (m, 4H, 2 × CH_2), 3.69 (s, 2H, CH_2), 7.04−7.08 (m, 3H, ArH), 7.11 (s, 1H, CH-5), 7.20−7.40 (m, 11H, ArH). MS *m*/*z* 462 (M^+, 2), 233 (base). Anal. ($C_{27}H_{25}F_3N_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-[4-(trifluoromethyl)phenyl]piperazine (1k). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.37$. IR (KBr): 1607 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.80−2.84 (m, 4H, 2 × CH_2), 3.28−3.33 (m, 4H, 2 × CH_2), 3.70 (s, 2H, CH_2), 7.05−7.40 (m, 14H, ArH and CH-5). MS *m*/*z* 462 (M^+,1), 233 (base). Anal. ($C_{27}H_{25}F_3N_4$) C, H, N.

[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(2-pyridyl)-piperazine (1l). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.32$. IR (KBr): 1591 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.74−2.79 (m, 4H, 2 × CH_2), 3.58−3.63 (m, 4H, 2 × CH_2), 3.69 (s, 2H, CH_2), 6.58−6.67 (m, 3H, ArH), 7.12 (s, 1H, CH-5), 7.20−7.50 (m, 10H, ArH), 8.19 (d, $J = 4.0$ Hz, 1H, ArH). MS *m*/*z* 395 (M^+, 3), 233 (base). Anal. ($C_{25}H_{25}N_5$) C, H, N.

1-Benzyl-4-[(1,2-diphenyl-1*H*-imidazol-4-yl)methyl]piperazine (1m). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.21$. IR (KBr): 1596 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.55 (m, 4H, 2 × CH_2), 2.68 (m, 4H, 2 × CH_2), 3.52 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 7.06 (s, 1H, CH-5), 7.16−7.33 (m, 15H, ArH). MS *m*/*z* 408 (M^+, 3), 233 (base). Anal. ($C_{27}H_{28}N_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-methylpiperazine (1n). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.32$. IR (KBr): 1598 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.32 (s, 3H, N−CH_3), 2.56 (m, 4H, 2 × CH_2), 2.71 (m, 4H, 2 × CH_2), 3.66 (s, 2H, CH_2), 7.10 (s, 1H, CH-5), 7.17−7.39 (m, 10H, ArH). MS *m*/*z* 332 (M^+, 6), 233 (base). Anal. ($C_{21}H_{24}N_4$) C, H, N.

1-[(1,2-Diphenyl-1*H*-imidazol-4-yl)methyl]-4-(2-ethoxyphenyl)piperazine (1o). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.44$. IR (KBr): 1596 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.45 (t, $J = 6.8$ Hz, 3H, CH_3), 2.86 (m, 4H, 2 × CH_2), 3.18 (m, 4H, 2 × CH_2), 3.71 (s, 2H, CH_2), 4.06 (q, $J = 7.0$ Hz, 2H, CH_2O), 6.62−6.96 (m, 4H, ArH), 7.12 (s, 1H, CH-5), 7.19−7.41 (m, 10H, ArH). MS *m*/*z* 438 (M^+, 3), 233 (base). Anal. ($C_{28}H_{30}N_4O$) C, H, N.

1-{[1,2-Bis(4-fluorophenyl)-1*H*-imidazol-4-yl]methyl}-4-phenylpiperazine (1p). TLC ($CHCl_3/CH_3OH$, 9.5:0.5) $R_f = 0.26$. IR (KBr): 1598 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.77−2.82 (m, 4H, 2 × CH_2), 3.23−3.28 (m, 4H, 2 × CH_2), 3.67 (s, 2H, CH_2),

6.84−7.39 (m, 14H, ArH and CH-5). MS *m*/*z* 430 (M^+, 3), 269 (base). Anal. ($C_{26}H_{24}F_2N_4$) C, H, N.

1-{[1,2-Bis(4-fluorophenyl)-1*H*-imidazol-4-yl]methyl}-4-(2-methoxyphenyl)piperazine (1q). TLC ($CHCl_3$/CH_3OH, 9.5:0.5) R_f = 0.20. IR (KBr): 1595 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.85 (m, 4H, 2 × CH_2), 3.15 (m, 4H, 2 × CH_2), 3.70 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 6.83−7.39 (m, 13H, ArH and CH-5). MS *m*/*z* 460 (M^+, 3), 269 (base). Anal. ($C_{27}H_{26}F_2N_4O$) C, H, N.

1-{[1,2-Bis(4-fluorophenyl)-1*H*-imidazol-4-yl]methyl}-4-(2-ethoxyphenyl)piperazine (1r). TLC ($CHCl_3$/CH_3OH, 9.5:0.5) R_f = 0.20. IR (KBr): 1596 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.45 (t, J = 7.0 Hz, 3H, CH_3), 2.84 (m, 4H, 2 × CH_2), 3.18 (m, 4H, 2 × CH_2), 3.68 (s, 2H, CH_2), 4.06 (q, J = 7.0 Hz, 2H, CH_2O), 6.85−7.39 (m, 13H, ArH and CH-5). MS *m*/*z* 474 (M^+, 2), 269 (base). Anal. ($C_{28}H_{28}F_2N_4O$) C, H, N.

Animals. Male Sprague−Dawley CD rats (Charles River, Como, Italy), with initial body masses of 200−220 g, were maintained under an artificial 12 h light, 12 h dark cycle (light on 0800 to 2000 hours) at a temperature of 22 ± 2 °C and 65% humidity. Food and water were freely available, and the rats were acclimatized to the animal facility for at least 8 days before experiments. Animal care and handling throughout the experimental procedures were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The experimental protocols were approved by the Animal Ethics Committee of the University of Cagliari.

DA D_2 Receptor Binding Assay. Rats were killed by decapitation, and the striatum was immediately dissected on ice. The tissue was rapidly homogenized in 200 vol (200 mL per gram of wet tissue) of ice-cold 50 mM Tris-HCl (pH 7.7 at 25 °C), designated buffer A, with the use of a Teflon pestle and glass homogenizer (two series of 15 strokes with a 1 min interval between series). The homogenate was centrifuged for 10 min at 43 000*g*, and the resulting pellet was resuspended and homogenized in 200 vol of buffer B (buffer A containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM EDTA, and 5.7 mM ascorbic acid) before use in the binding assay. The binding of [^{3}H]YM-09151 was determined in a final volume of 1 mL consisting of 400 μL of membrane suspension (1.5−2.0 mg of protein), 100 μL of 0.4 nM [^{3}H]YM-09151 (84 Ci/mmol; New England Nuclear), 5 μL of drug [10^{-11} to 10^{-4} M, dissolved and diluted in dimethyl sulfoxide (DMSO)] or solvent, and buffer B to volume. Nonspecific binding was determined in the presence of 50 μM L-sulpiride (Knoll S.p.A., Milan, Italy). After they were incubated for 60 min at 25 °C in the dark, the binding reaction mixtures were passed through glass-fiber (Whatman GF/B) filters with the use of a Cell-Harvester filtration manifold (M24; Brandel, Gaithersburg, MD). Filters were then rinsed twice with 4 mL portions of ice-cold buffer A and transferred to vials containing 3.5 mL of scintillation cocktail (Ultima Gold, Camberra Packard). Radioactivity associated with the filters was quantified with a scintillation spectrometer.

Serotonin 5-HT_{1A} Receptor Binding Assay. The cerebral cortex of rats was homogenized in 20 vol of ice-cold 50 mM Tris-HCl (pH 7.7 at 22 °C) with a Polytron disrupter (setting 5 for 5 s), and the resulting homogenate was centrifuged at 50 000*g* for 10 min. The pellet was resuspended in 20 vol of the same buffer, incubated for 10 min at 37 °C, and centrifuged again at 50 000*g* for 10 min. The final pellet was resuspended in 80 vol of homogenization buffer containing 10 μM pargyline (Sigma Chemical), 4 mM $CaCl_2$, and 0.1% ascorbic acid. The binding of [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) was determined in a final volume of 1 mL consisting of 0.8 mL of membrane suspension (0.6−0.8 mg of protein), 0.1 mL of drug (10^{-11} to 10^{-4} M) or solvent, and 0.1 mL of 0.5 nM [^{3}H]8-OH-DPAT (135 Ci/mmol; NEN Life Science, Milan, Italy) in membrane buffer. After it was incubated for 30 min at 37 °C, the binding reaction was terminated by vacuum filtration through Whatman GF/B filters. The filters were washed twice with 5 mL portions of ice-cold 50 mM Tris-HCl (pH, 7.7 at 22 °C) buffer, and the radioactivity bound to the filters was measured by liquid scintillation spectrometry. Specific [^{3}H]8-OH-DPAT binding was defined as the difference between binding in the absence of serotonin (10 μM) and that in its presence.

Serotonin 5-HT_{2A} Receptor Binding Assay. Rat cerebral cortex was dissected on ice, frozen on dry ice, and stored at −70 °C until use. The tissue was thawed on ice and homogenized in 10 vol of 0.32 M sucrose with a Polytron disrupter (setting 5 for 15 s), and the homogenate was centrifuged twice at 4 °C (900*g* for 10 min followed by 40 000*g* for 30 min). The final supernatant was discarded, and the pellet was resuspended in 10 vol of 50 mM Tris-HCl (pH 8.07 at 22 °C) with a Teflon pestle and glass homogenizer (10 manual strokes). After it was incubated at 37 °C for 15 min to deplete endogenous serotonin, the suspension was centrifuged for 30 min at 40 000*g*. The resulting pellet was resuspended in 20 vol of the same buffer containing 4 mM $CaCl_2$ and 0.1% ascorbic acid. The binding of [^{3}H]ketanserin was assayed in a reaction mixture (1 mL) consisting of 750 μL of membrane suspension (0.2−0.4 mg of protein), 50 μL of 0.4 nM [^{3}H]ketanserin (63.3 Ci/mmol; NEN Life Science, Milan, Italy), 5 μL of drug (10^{-11} to 10^{-4} M) or solvent, and membrane buffer to volume. After it was incubated for 30 min at 37 °C, the assay was terminated by rapid filtration through Whatman GF/C filter strips [presoaked in 3% poly(ethylenimine)] in a filtration manifold. The filters were washed with ice-cold 50 mM Tris-HCl (pH 6.6) to remove unbound radioligand, and the radioactivity retained on the filters was determined by liquid scintillation spectrometry. Nonspecific binding was determined in the presence of 1 μM cinanserin (Tocris, U.K.).

Evaluation of DA Release in the Rat Medial Prefrontal Cortex. Clozapine was dissolved in distilled water with one drop of HCl per 5 mL, after which the pH was adjusted to 7.3 with NaOH; compound **1b** was dissolved in distilled water with one drop of Tween 80 per 5 mL. Drugs were injected intraperitoneally in a volume of 3 mL per kilogram of body mass; control animals received an identical volume of the respective vehicle.

Rats were anesthetized with chloral hydrate (0.4 g/kg, ip), and a concentric dialysis probe was inserted at the level of the medial prefrontal cortex (A +3.2, ML +0.8, V −5.3 relative to the bregma) according to the Paxinos atlas.[63] The active length of the dialysis membrane (Hospal Dasco, Bologna, Italy) was restricted to 2 mm.

Experiments were performed ∼24 h after probe implantation, between 0900 and 1800 h. Ringer's solution [125 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 23 mM $NaHCO_3$, and 1.5 mM potassium phosphate (pH 7.3)] was pumped through the dialysis probe at a constant rate of 2 μL/min. Samples of dialysate were collected every 20 min and immediately analyzed for DA by high-performance liquid chromatography with coulometric detection as described.[64] The average neurotransmitter concentration in the last three samples before drug treatment was taken as 100%, and all posttreatment values were expressed relative to the basal value. The mean in vitro recovery of the probes was 24 ± 3%. All probes were tested before implantation, and those with a recovery value outside this range were not used. The absolute concentration of DA was not corrected for recovery values. At the end of each experiment, the placement of the probe was verified histologically; those rats in which the probe was detected outside of the prefrontal cortex were excluded from the analysis.

Electrophysiological Recording from *Xenopus* Oocytes. Complementary DNAs encoding human $GABA_A$ receptor α_1, β_2, and γ_{2L} subunits were purified with a Wizard Plus Miniprep DNA Purification System (Promega, Madison, WI), resuspended in sterile distilled water, divided into portions, and stored at −20 °C until use. Stage V and VI oocytes were manually isolated from sections of *Xenopus laevis* ovary, placed in modified Barth's saline [MBS: 88 mM NaCl, 1 mM KCl, 10 mM Hepes-NaOH (pH 7.5), 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 0.91 mM $CaCl_2$, and 0.33 mM $Ca(NO_3)_2$], and treated with collagenase type IA (0.5 mg/mL; Sigma) in collagenase buffer [83 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM Hepes-NaOH (pH 7.5)] for 10 min at room temperature in order to remove the follicular layer. A mixture of $GABA_A$ receptor α_1,

β_2, and γ_{2L} subunit cDNAs (1.5 ng in 30 nL) was injected into the oocyte nucleus with a 10 μL glass micropipet (tip diameter, 10−15 μm).[65] The injected oocytes were cultured at 19 °C in sterile MBS supplemented with streptomycin (10 μg/mL), penicillin (10 U/mL), gentamicin (50 μg/mL), 0.5 mM theophylline, and 2 mM sodium pyruvate.

Electrophysiological recording was begun 12−24 h after cDNA injection, as previously described.[65] Briefly, oocytes were placed in a 100 μL rectangular recording chamber and continuously perfused with MBS at room temperature and a flow rate of 2 mL/min. The animal pole of each oocyte was impaled with two glass electrodes (0.5−3 MΩ) filled with filtered 3 M KCl, and the voltage was clamped at −70 mV with an Axoclamp 2-B amplifier (Axon Instruments, Burlingame, CA). Chloride currents were continuously recorded on a strip-chart recorder. The resting membrane potential of most oocytes varied between −30 and −50 mV. Clozapine and compound **1b** were dissolved in DMSO and diluted to the final concentration with MBS. In control experiments, DMSO at concentrations of 1−3% did not significantly affect GABA-evoked Cl^- currents. Oocytes were exposed to drugs for 20 s (7−10 s was required to achieve equilibrium in the recording chamber). Intervals of 5−10 min were allowed between drug applications. GABA at the EC_{30} (concentration that induces a Cl^- current with an amplitude of 30 ± 5% of the maximal response evoked by 1 mM GABA and which was determined for each oocyte at the beginning of the experiment) was used to produce the control response.

Statistical Analysis. Data from microdialysis experiments are expressed as a percentage of basal values and are presented as means ± SEM. Comparisons among groups were performed by two way ANOVA for repeated measures, with factors being treatment (three levels = three doses of drug) and time points (seven levels = seven time points between 0 and 120 min). Given that a "percentage of baseline" measure of neurotransmitter release is confounded when basal concentrations vary among groups, we used raw baseline values for statistical analysis. Post hoc comparisons were performed by the Neuman−Keuls test. Data from electrophysiological experiments are expressed as percentage variation from the control response and are presented as means ± SEM. Data were analyzed by ANOVA followed by Scheffe's post hoc test. For all statistical comparisons, a P value of <0.05 was considered statistically significant.

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References

(1) Reynolds, G. P. Developments in the Drug Treatment of Schizophrenia. *Trends Pharmacol. Sci.* **1992**, *13*, 116−121.
(2) Creese, I.; Burt, D. R.; Snyder, S. H. Dopamine Receptor Binding Predicts Clinical and Pharmacological Potencies of Antischizophrenic Drugs. *Science* **1976**, *192*, 481−483.
(3) Seeman, P.; Van Tol, H. H. M. Dopamine Receptor Pharmacology. *Trends Pharmacol. Sci.* **1994**, *15*, 264−270.
(4) Davis, K. L.; Kahn, R. S.; Ko, G.; Davidson, M. Dopamine in Schizophrenia: A Review and Reconceptualization. *Am. J. Psychiatry* **1991**, *148*, 1474−1486.
(5) Seeman, P. Dopamine Receptors and the Dopamine Hypothesis of Schizophrenia. *Synapse* **1987**, *1*, 133−152.
(6) Hippius, H. A Historical Perspective of Clozapine. *J. Clin. Psychiatry* **1999**, *60* (Suppl. 12), 22−23.
(7) Worrel, J. A.; Marken, P. A.; Beckman, S. E.; Ruehter, V. L. Atypical Antipsychotic Agents: A Critical Review. *Am. J. Health Syst. Pharm.* **2000**, *57*, 238−255.
(8) Remington, G.; Kapur, S. Atypical Antipsychotics: Are Some More Atypical Than Others? *Psychopharmacology* **2000**, *148*, 3−15.
(9) Ashby, C. R., Jr.; Wang, R. Y. Pharmacological Actions of the Atypical Antipsychotic Drug Clozapine: A Review. *Synapse* **1996**, *24*, 349−394.
(10) Millan, M. J. Improving the Treatment of Schizophrenia: Focus on Serotonin $(5\text{-HT})_{1A}$ Receptors. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 853−861.
(11) Squires, R. F.; Saederup, E. Clozapine and Several Other Antipsychotic/Antidepressant Drugs Preferentially Block the Same 'Core' Fraction of $GABA_A$ Receptors. *Neurochem. Res.* **1998**, *23*, 1283−1290.
(12) Michel, F. J.; Trudeau, L.-E. Clozapine Inhibits Synaptic Transmission at GABAergic Synapses Established by Ventral Tegmental Area Neurones in Culture. *Neuropharmacology* **2000**, *39*, 1536−1543.
(13) Carlsson, A.; Waters, N.; Holm-Waters, S.; Tedroff, J.; Nilsson, M.; Carlsson, M. L. Interaction Between Monoamines, Glutamate, and GABA in Schizophrenia: New Evidence. *Annu. Rev. Toxicol.* **2001**, *41*, 237−260.
(14) Kalkman, H. O.; Neumann, V.; Hoyer, D.; Tricklebank, M. D. The Role of α_2-Adrenoceptor Antagonism in the Anticataleptic Properties of the Atypical Neuroleptic Agent, Clozapine, in the Rat. *Br. J. Pharmacol.* **1998**, *124*, 1550−1556.
(15) Ossowska, K.; Wedzony, K.; Wolfarth, D. The Role of GABA Mechanisms in the Globus Pallidus in Mediating Catalepsy, Stereotypy and Locomotor Activity. *Pharmacol. Biochem. Behav.* **1984**, *21*, 825−831.
(16) Mao, C. C.; Marco, E.; Revuelta, A.; Bertilsson, L.; Costa, E. The Turnover of γ-Aminobutyric Acid in the Nuclei of Telencephalon: Implications in the Pharmacology of Antipsychotics and of a Minor Tranquillizer. *Biol. Psychiatry* **1977**, *12*, 359−371.
(17) Korpi, E. R.; Wong, G.; Luddens, H. Subtype Specificity of γ-Aminobutyric Acid Type A Receptor Antagonism by Clozapine. *Naunyn Schmiedeberg's Arch. Pharmacol.* **1995**, *352*, 365−373.
(18) Mouithys-Mickalad, A.; Kauffmann, J. M.; Petit, C.; Bruhwyler, J.; Liao, Y.; Wikstrom, H.; Damas, J.; Delarge, J.; Deby-Dupont, G.; Geczy, J.; Liegeois, J. F. Electrooxidation Potential as a Tool in the Early Screening for New Safer Clozapine-like Analogues. *J. Med. Chem.* **2001**, *44*, 769−776.
(19) (a) Kapur, S.; Remington, G. Atypical Antipsychotics: New Directions and New Challenges in the Treatment of Schizophrenia. *Annu. Rev. Med.* **2001**, *52*, 503−517. (b) Stip, E. Novel Antipsychotics: Issues and Controversies. Typicality of Atypical Antipsychotics. *J. Psychiatry Neurosci.* **2000**, *25*, 137−153. (c) Tallman, J. F. Development of Novel Antipsychotic Drugs. *Brain Res. Brain Res. Rev.* **2000**, *31*, 385−390. (d) Moore, N. A. Behavioural Pharmacology of the New Generation of Antipsychotic Agents. *Br. J. Psychiatry Suppl.* **1999**, *38*, 5−11.
(20) (a) 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 $Serotonin_2$ p*Ki* Values. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 238−246. (b) Stockmeier, C. A.; DiCarlo, J. J.; Zhang, Y.; Thompson, P.; Meltzer, H. Y. Characterization of Typical and Atypical Antipsychotic Drugs Based on In Vivo Occupancy of $Serotonin_2$ and Dopamine 2 Receptors. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 1374−1384.
(21) (a) Love, R. C.; Nelson, M. W. Pharmacology and Clinical Experience with Risperidone. *Exp. Opin. Pharmacother.* **2000**, *1*, 1441−1453. (b) Nyberg, S.; Farde, L.; Eriksson, L.; Halldin, C.; Eriksson, B. 5-HT_2 and D_2 Dopamine Receptor Occupancy in the Living Human Brain: A PET Study with Risperidone. *Psychopharmacology* (*Berlin*) **1993**, *110*, 265−272.
(22) (a) Bhana, N.; Foster, R. H.; Olney, R.; Plosker, G. L. Olanzapine: An Update Review of Its Use in the Management of Schizophrenia. *Drugs* **2001**, *61*, 111−161. (b) Nyberg, S.; Farde, L.; Halldin, C. A PET Study of 5-HT_2 and D_2 Dopamine Receptor Occupancy Induced by Olanzapine in Healthy Subjects. *Neuropsychopharmacology* **1997**, *16*, 1−7.
(23) (a) Brown, L. A.; Levin, G. M. Sertindole, A New Atypical Antipsychotic for the Treatment of Schizophrenia. *Pharmacotherapy* **1998**, *18*, 69−83. (b) Kane, J. M. Sertindole: A Review of Clinical Efficacy. *Int. Clin. Psychopharmacol.* **1998**, *13*, S59−S63.
(24) (a) Kasper, S.; Tauscher, J.; Heiden, A. Quetiapine: Efficacy and Tolerability in Schizophrenia. *Eur. Neuropsychopharmacol.* **2001**, *Suppl. 4*, S405−413. (b) Kasper, S.; Muller-Spahn, F. Review of Quetiapine and Its Clinical Applications in Schizophrenia. *Exp. Opin. Pharmacother.* **2000**, *1*, 783−801.
(25) Gunasekara, N. S.; Spencer, C. M.; Keating, G. M. Ziprasidone: A Review of Its Use in Schizophrenia and Schizoaffective Disorder. *Drugs* **2002**, *62*, 1217−1251.
(26) (a) Birch, A. M.; Bradley, P. A.; Gill, J. C.; Kerrigan, F.; Needham, P. L. N-Substituted (2,3-Dihydro-1,4-benzodioxin-2-yl)methylamine Derivatives as D_2 Antagonists/5-HT_{1A} Partial Agonists with Potential as Atypical Antipsychotic Agents. *J. Med. Chem.* **1999**, *42*, 3342−3355. (b) Reitz, A. B.; Baxter, E. W.; Codd, E. E.; Davis, C. B.; Jordan, A. D.; Maryanoff, B. E.; Maryanoff, C. A.; McDonnel, M. E.; Powell, E. T.; Renzi, M. J.; Schott, M. R.; Scott, M. K.; Shank, R. P.; Vaught, J. L. Orally Active Benzamide Antipsychotic Agents with Affinity for Dopamine D_2, Serotonin 5-HT_{1A}, and Adrenergic α_1 Receptors. *J. Med. Chem.* **1998**, *41*, 1997−2009. (c) Taverne, T.; Diouf, O.; Depreux,

P.; Poupaert, J. H.; Lesieur, D.; Guardiola-Lemaître, B.; Renard, P.; Rettori, M. C.; Caignard, D. H.; Pfeiffer, B. Novel Benzothiazolin-2-one and Benzoxazin-3-one Arylpiperazine Derivatives with Mixed $5HT_{1A}/D_2$ Affinity as Potential Atypical Antipsychotics. *J. Med. Chem.* **1998**, *41*, 2010−2018. (d) Perrone, R.; Berardi, F.; Colabufo, N. A.; Tortorella, V.; Fiorentini, F.; Olgiati, V.; Vanotti, E.; Govoni, S. Mixed $5HT_{1A}$/D-2 Activity of a New Model of Arylpiperazines: 1-Aryl-4-[3-(1,2-dihydronaphthalen-4-yl)-*n*-propyl]piperazines. 1. Synthesis and Structure−Activity Relationships. *J. Med. Chem.* **1994**, *37*, 99−104.

(27) (a) Kuipers, W.; van Wijngaarden, I.; Kruse, C. G.; ter Horst-van Amstel, M.; Tulp, M. Th. M.; IJzerman, A. P. N^4-Unsubstituted N^1-Arylpiperazines As High-Affinity $5\text{-}HT_{1A}$ Receptor Ligands. *J. Med. Chem.* **1995**, *38*, 1942−1954. (b) Lyon, R. A.; Titeler, M.; McKenney, J. D.; Magee, P. S.; Glennon, R. A. Synthesis and Evaluation of Phenyl- and Benzoylpiperazines as Potential Serotonergic Agents. *J. Med. Chem.* **1986**, *29*, 630−634.

(28) (a) Harbert, C. A.; Plattner, J. J.; Welch, W. M.; Weissman, A.; Koe, B. K. Neuroleptic Activity in 5-Aryltetrahydro-γ-carbolines. *J. Med. Chem.* **1980**, *23*, 635−643. (b) Abou-Gharbia, M. Biological Activity of Substituted γ-Carbolines. *Drugs Future* **1989**, *14*, 453−459.

(29) Khanna, I. K.; Weier, R. M.; Yu, Y.; Xu, X. D.; Koszyk, F. J.; Collins, P. W.; Koboldt, C. M.; Veenhuizen, A. W.; Perkins, W. E.; Casler, J. J.; Mesferrer, J. L.; Zhang, Y. Y.; Gregory, S. A.; Seibert, K.; Isakson, P. C. 1,2-Diarylimidazoles as Potent, Cyclooxygenase-2 Selective, and Orally Active Antiinflammatory Agents. *J. Med. Chem.* **1997**, *40*, 1634−1647.

(30) Brown, H. C.; Heim, P. Selective Reductions. XVIII. The Fast Reaction of Primary, Secondary, and Tertiary Amides with Diborane. A Simple, Convenient Procedure for the Conversion of Amides to the Corresponding Amines. *J. Org. Chem.* **1973**, *38*, 912−916.

(31) *HyperChem Program release 5.01 for Windows*; Hypercube Inc., 1996).

(32) Schmutz, J. Neuroleptic Pyperazinyl-dibenzo-azepines. Chemistry and Structure−Activity Relationships. *Arzneim. Forsch.* (*Drug Res.*) **1975**, *25*, 712−720.

(33) Chakrabarti, J. K.; Hotten, T. M.; Morgan, S. E.; Pullar, I. A.; Rackham, D. M.; Risius, F. C.; Wedley, S.; Chaney, M. O.; Jones, N. D. Effects of Conformationally Restricted 4-Piperazinyl-10*H*-thienobenzodiazepine Neuroleptics on Central Dopaminergic and Cholinergic Systems. *J. Med. Chem.* **1982**, *25*, 1133−1140.

(34) Lanig, H.; Utz, W.; Gmeiner, P. Comparative Molecular Field Analysis of Dopamine D4 Receptor Antagonists Including 3-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]pyrazolo[1,5-*a*]pyridine (FAUC 113), 3-[4-(4-Chlorophenyl)piperazin-1-ylmethyl]-1*H*-pyrrolo-[2,3-*b*]pyridine (L-745,870), and clozapine. *J. Med. Chem.* **2001**, *44*, 1151−1157.

(35) Assié, M.-B.; Sleight, A. J.; Koek, W. Biphasic Displacement of [^{3}H]YM-09151-2 Binding in the Rat Brain by Thioridazine, Risperidone and Clozapine, but Not by other Antipsychotics. *Eur. J. Pharmacol.* **1993**, *237*, 183−189.

(36) (a) Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979. (b) Hansch, C.; Leo, A. *Exploring QSAR*: *Fundamentals and Applications in Chemistry and Biology*; ACS Professional Reference Book; American Chemical Society: Washington, DC, 1995. (c) van de Waterbeemd, H.; Testa, B. The Parametrization of Lipophilicity and Other Structural Properties in Drug Design. In *Advances in Drug Research*; Testa, B., Ed.; Academic Press: London, 1987; Vol. 16, pp 87−227.

(37) Perrin, D. D.; Dempsey, B.; Serijeant, E. P. *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: London and New York, 1981; pp 137−138.

(38) Williams, S. G.; Norrington, F. E. Determination of Positional Weighting Factors for the Swain and Lupton Substituent Constants F and R. *J. Am. Chem. Soc.* **1976**, *98*, 508−516.

(39) *CLOG P for Windows, Version 4.0*; BioByte Corp.: Claremont, CA.

(40) Tetko, I. V.; Tanchuk, V. Yu.; Kasheva, T. N.; Villa, A. E. P. Internet Software for Calculation of Lipophilicity and Aqueous Solubility of Chemical Compounds. *J. Chem. Inf. Sci.* **2001**, *41*, 246−252.

(41) *ACD/Labs package, release 5.0*; Advanced Chemistry Development Inc.: Toronto, Ontario, Canada.

(42) Brea, J.; Rodrigo, J.; Carrieri, A.; Sanz, F.; Cadavid, M. I.; Enguix, M. J.; Villazon, M.; Mengod, G.; Caro, Y.; Masaguer, C. F.; Raviña, E.; Centeno, N. B.; Carotti, A.; Loza, M. I. New Serotonin $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, $5\text{-}HT_{2C}$ Receptor Antagonists: Synthesis, Pharmacology, 3D-QSAR, and Molecular Modeling of (Aminoalkyl)benzo and Heterocycloalkanones. *J. Med. Chem.* **2002**, *45*, 54−71.

(43) Höltje, H. D. Pharmacophore Identification and Receptor Mapping. In *The Practice of the Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press Limited: New York, 1996; pp 437−457.

(44) Gaillard, P.; Carrupt, P. A.; Testa, B.; Schambel, P. Binding of Arylpiperazines, (Aryloxy)propanolamines, and Tetrahydropyridylindoles to the $5\text{-}HT_{1A}$ Receptor: Contribution of the Molecular Lipophilicity Potential to Three-Dimensional Quantitative Structure-Affinity Relationship Models. *J. Med. Chem.* **1996**, *39*, 126−134.

(45) Deutch, A. Y. The Regulation of Subcortical Dopamine Systems by the Prefrontal Cortex: Interactions of Central Dopamine Systems and the Pathogenesis of Schizophrenia. *J. Neural Transm.* (*Gen. Sect.*) (*Suppl.*) **1992**, *36*, 61−89.

(46) Goldman-Rakic, P. S. Cerebral Cortical Mechanisms in Schizophrenia. *Neuropsychopharmacology* **1994**, *10*, 22S−27S.

(47) Moghaddam, B.; Bunney, B. S. Utilization of Microdialysis for Assessing the Release of Mesotelencephalic Dopamine Following Clozapine and Other Antipsychotic Drugs. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **1990**, *14*, S51−S57.

(48) Li, X. M.; Perry, K. W.; Wong, D. T.; Bymaster, F. P. Olanzapine Increases In Vivo Dopamine and Norepinephrine Release in Rat Prefrontal Cortex, Nucleus Accumbens and Striatum. *Psychopharmacology* **1998**, *136*, 153−161.

(49) Ahlenius, S.; Hillegaart, V. Involvement of Extrapyramidal Motor Mechanisms in the Suppression of Locomotor Activity by Antipsychotic Drugs: A Comparison Between the Effects Produced by Pre- and Postsynaptic Inhibition of Dopaminergic Neurotransmission. *Pharmacol. Biochem. Behav.* **1986**, *24*, 1409−1415.

(50) Elsworth, J. D.; Roth, R. H. Dopamine Synthesis, Uptake, Metabolism, and Receptors: Relevance to Gene Therapy of Parkinson's Disease. *Exp. Neurol.* **1997**, *144*, 4−9.

(51) Westerink, B. H.; Kawahara, Y.; De Boer, P.; Geels, C.; De Vries, J. B.; Wik Van Kalkeren, A.; Van Vliet, B.; Kruse, C. G.; Long, S. K. Antipsychotic Drugs Classified by Their Effects on the Release of Dopamine and Noradrenaline in the Prefrontal Cortex and Striatum. *Eur. J. Pharmacol.* **2001**, *412*, 127−138.

(52) Squires, R. F.; Saederup, E. A Review of Evidence for GABAergic Predominance/Glutamatergic Deficit as a Common Etiological Factor in Both Schizophrenia and Affective Psychoses: More Support for a Continuum Hypothesis of "Functional" Psychosis. *Neurochem. Res.* **1991**, *16*, 1099−1111.

(53) Squires, R. F.; Saederup, E. Clozapine and Some Other Antipsychotic Drugs May Preferentially Block the Same Subset of $GABA_A$ Receptors. *Neurochem. Res.* **1997**, *22*, 151−162.

(54) Ross, R. J.; Waszczak, B. L.; Lee, E. K.; Walters, J. R. Effects of Benzodiazepines on Single-Unit Activity in the Substantia Nigra Pars Reticulata. *Life Sci.* **1982**, *31*, 1025−1035.

(55) Zetterstrom, T.; Fillenz, M. Local Administration of Flurazepam Has Different Effects on Dopamine Release in Striatum and Nucleus Accumbens: A Microdialysis Study. *Neuropharmacology* **1990**, *29*, 129−134.

(56) Invernizzi, R.; Pozzi, L.; Samanin, R. Release of Dopamine Is Reduced by Diazepam More in the Nucleus Accumbens Than in the Caudate Nucleus of Conscious Rats. *Neuropharmacology* **1991**, *30*, 575−578.

(57) Finlay, J. M.; Damsma, G.; Fibiger, H. C. Benzodiazepine-Induced Decreases in the Extracellular Concentration of Dopamine in the Nucleus Accumbens after Acute and Repeated Administration. *Psychopharmacology* **1992**, *106*, 202−208.

(58) Dazzi, L.; Motzo, C.; Imperato, A.; Serra, M.; Gessa, G. L.; Biggio, G. Modulation of Basal and Stress-Induced Release of Acetylcholine and Dopamine in the Rat Brain by Abecarnil and Imidazenil, Two Anxioselective, γ-Aminobutyric $Acid_A$ Receptor Modulators. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 241−247.

(59) Tam, S. Y.; Roth, R. H. Selective Increases in Dopamine Metabolism in the Prefrontal Cortex by the Anxiogenic β-Carboline FG 7142. *Biochem. Pharmacol.* **1985**, *34*, 1595−1598.

(60) Dazzi, L.; Spiga, F.; Pira, L.; Ladu, S.; Vacca, G.; Rivano, A.; Jentsch, J. D.; Biggio, G. Inhibition of Stress- or Anxiogenic-Drug-Induced Increases in Dopamine Release in the Rat Prefrontal Cortex by Long-Term Treatment with Antidepressant Drugs. *J. Neurochem.* **2001**, *76*, 1212−1220.

(61) Hutson, P. H.; Barton, C. L. L-701,324, a Glycine/NMDA Receptor Antagonist, Blocks the Increase of Cortical Dopamine Metabolism by Stress and DMCM. *Eur. J. Pharmacol.* **1997**, *326*, 127−132.

(62) Zhou, L.; Zhang, Y. Samarium(II) Iodide Induced Reductive Coupling of Nitriles with Nitro-Compounds. *J. Chem. Soc., Perkin Trans.* **1998**, *1*, 2899−2902.

(63) Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*; Academic Press: London, 1982.

(64) Dazzi, L.; Serra, M.; Porceddu, M. L.; Sanna, A.; Chessa, M. F.; Biggio, G. Enhancement of Basal and Pentylenetetrazol (PTZ)-Stimulated Dopamine Release in the Brain of Freely Moving Rats by PTZ-Induced Kindling. *Synapse* **1997**, *26*, 351−358.

(65) Sanna, E.; Murgia, A.; Casula, A.; Usala, M.; Maciocco, E.; Tuligi, G.; Biggio, G. Direct Activation of $GABA_A$ Receptor by Loreclezole, An Anticonvulsant Drug with Selectivity for the β-Subunit. *Neuropharmacology* **1997**, *35*, 1753−1760.

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