Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

New Pyridobenzoxazepine Derivatives Derived from 5-(4-Methylpiperazin-1-yl)-8-chloro-pyrido[2,3-*b*][1,5]benzoxazepine (JL13): Chemical Synthesis and Pharmacological Evaluation

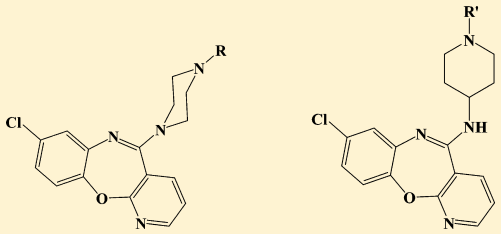
Jean-François Liégeois,*[,†] Marine Deville,[†] Sébastien Dilly,[†] Cédric Lamy,[†] Floriane Mangin,[†] Mélissa Résimont,[†] and Frank I. Tarazi[‡]

[†]Laboratory of Medicinal Chemistry, Drug Research Center, University of Liège, avenue de l'Hôpital 1 (B36), B-4000 Liège 1, Belgium

[‡]Department of Psychiatry and Neuroscience Program, Harvard Medical School and McLean Hospital, Boston, Massachusetts, United States

Supporting Information

ABSTRACT: A series of new pyridobenzoxazepine derivatives with various heterocyclic amine side chains were synthesized to explore two main parameters related to the distal basic nitrogen. These compounds were tested for their affinity for dopamine D_{2L} and D_4, serotonin 5-HT_{1A} and 5-HT_{2A}, and adrenergic α_{2A} receptors in comparison with 5-(4-methylpiperazin-1-yl)-8-chloro-pyrido[2,3-*b*][1,5]benzoxazepine, JL13 (**1**), and other diarylazepine derivatives. In terms of multireceptor target strategy, **2** and **5** present the most promising *in vitro* binding profile. Bulky, polar, and more flexible side chains are not favorable in this context. Compounds **2** and **5** were tested in adult rats to evaluate their long-term effects on dopamine and serotonin receptors density in different brain areas. Similar to **1** and other second-generation antipsychotic drugs, repeated treatment with **2** significantly increased D_1 and D_4 receptors in nucleus accumbens and caudate putamen and D_2 receptors in medial prefrontal cortex and hippocampus, while **5** significantly increased D_2 and D_4 receptors in nucleus accumbens. In addition, **2** increased 5-HT_{1A} and decreased 5-HT_{2A} receptors in cerebral cortex. In contrast, **5** did not alter levels of any 5-HT receptor subtype in any brain region examined. These results encourage further development of **2** as a novel second-generation antipsychotic agent.



INTRODUCTION

The hypothesis of higher affinity for serotonin 5-HT_{2A} receptors versus dopamine D_2 receptors[1] led to the development of the second generation of antipsychotic drugs (SGAs). Other receptors such as dopamine D_4 receptors[2−6] or more recently glutamate receptors[7,8] were targets for extensive pharmaceutical development programs. Importantly, the beneficial combination of several neuronal interactions among different neurotransmitter systems have been raised by in vivo microdialysis and radioligand binding studies.[9] Thus, for instance, serotonin 5-HT_{1A} partial agonism is likely involved in the ability of clozapine and other SGAs to increase dopamine release in several important brain areas such as hippocampus[10] and prefrontal cortex.[11,12] The highest density of 5-HT_{1A} receptors in rat brain is found in the hippocampus followed by the medial prefrontal cortex. The interaction of dissimilar SGAs with 5-HT_{1A} receptors leading to the modulation of external dopamine concentrations has been proposed to be beneficial for improving negative symptoms and cognitive deficits in patients with schizophrenia.[13] Alternatively, α_2-adrenergic receptor antagonism has also been proposed as an explanation for the unique antipsychotic properties of clozapine. Therefore, the combination of a D_2 antagonist such as raclopride and an α_2-antagonist such as idazoxan also produces comparable effect to SGAs on dopamine release in the prefrontal cortex,[14] significantly reduces the conditioned avoidance response in rats,[15] and reverses catalepsy induced by haloperidol in rats.[16] Thus, selective or mixed ligands for these receptors (5-HT_{1A} and D_4 receptors and α_{2A}-adrenoceptors) would be of great interest. Multireceptor target strategy was put forward as an innovative approach for discovering new drug candidates.[9,17,18]

5-(4-Methylpiperazin-1-yl)-8-chloro-pyrido[2,3-*b*][1,5]-benzoxazepine, JL13 (Figure 1), tested as fumarate in the studies reported by our group (see below), is a clozapine-like compound that shares with clozapine its high affinity for 5-HT_{2A} over D_2 receptors.[19] Neurochemical studies reported that JL13 (**1**), like clozapine and other SGAs, selectively increased dopamine (DA) levels in prefrontal cortex in a dose-dependent fashion without altering concentrations of dopamine or its metabolites in striatum.[20] Behavioral studies suggested that **1** might possess antipsychotic activity, evidenced by its ability to reverse amphetamine-induced disruption of prepulse inhibition[21] and antagonize apomorphine-induced climbing. In addition, **1** also appears to exhibit a benign neurological

Received: October 7, 2011
Published: January 23, 2012

dx.doi.org/10.1021/jm2013419 | *J. Med. Chem.* 2012, 55, 1572−1582

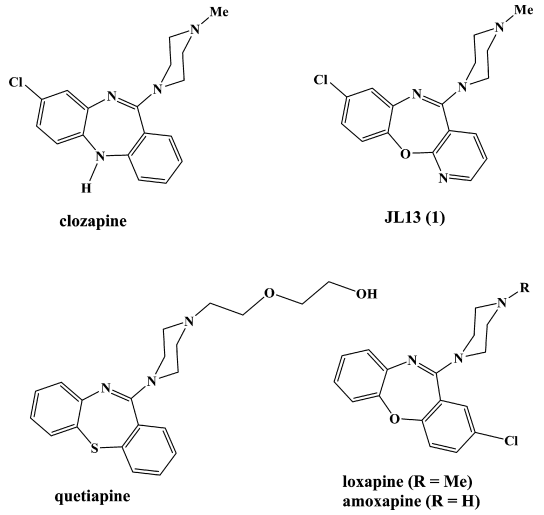


Figure 1. Chemical structure of reference diarylazepine derivatives.

profile, evidenced by its lack of ability to antagonize amphetamine-induced stereotypy or produce catalepsy in rats,[22] as well as its low incidence of adverse side effects in non-human primates.[23] In more recent studies, the long-term effects of **1** on DA (D_1, D_2, D_3, and D_4) receptors and serotonin (5-HT_{1A} and 5-HT_{2A}) receptors subtypes in rat forebrain regions was examined, and it was found that this compound induced regionally selective changes in tissue levels of specific DA and 5-HT receptor subtypes mimicking those observed with clozapine and other SGAs.[24] The long-term effects of **1** on glutamatergic receptors (NMDA, AMPA) were also investigated, and the compound had comparable effects to SGAs.[25]

Interestingly, from a chemical point of view, pyridobenzoxazepine structure allowed maintenance of similar physicochemical properties, such as lipophilicity, to clozapine but also led to less sensitivity to oxidative phenomena.[26] Indeed, clozapine has a low oxidation potential leading to a high sensitivity to oxidative conditions.[26−28] More recently, another consequence of this sensitivity to oxidation has been reported. Unlike oxazepine analogues (loxapine, **1**), the clozapine nitrenium ions generated in oxidative conditions possess a lower affinity for D_2 receptors.[29]

In the present work, we were interested in preparing several compounds related to **1** by changing the nature and the size of the lateral piperazine side chain in order to explore two main parameters related to the pharmacophoric distal nitrogen, namely, the ionization and the spatial position. Bulky, aromatic, more flexible, and also polar groups were selected for this purpose. Otherwise, in the diarylazepine series, *N*-dealkylated analogues are usually produced by metabolism and sometimes used in therapy like amoxapine. Thus, we have synthesized the *N*-dealkylated analogue of **1**. These compounds were tested to evaluate the impact on the affinity for several receptors involved in the mechanism of action of SGAs. Moreover, two compounds showing an interesting *in vitro* binding profile were also tested in long-term studies in rats to evaluate their effects on the density of dopamine and serotonin receptors in different brain areas, and the results were compared with those of **1** from previous studies.[24,25]

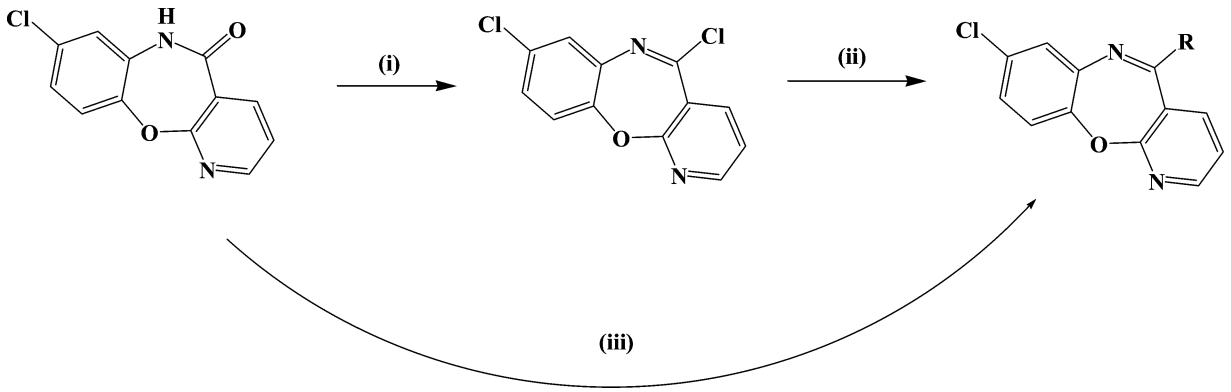
CHEMISTRY

Two chemical pathways were followed for preparing target compounds (Scheme 1). Procedure 1 was used for preparing oxazepine analogues[30] and was the best choice for preparing the new analogues with a hydroxyl side chain (**6**, **7**). In this approach, the lactam reacted with phosphorus oxychloride to give the iminochloride. This reactive entity is immediately used in the next step to give the appropriate amidine by reaction with an excess of the appropriate amine. Procedure 2 was advantageously used for the synthesis of pyridobenzodiazepine analogues[31,32] according to a modified Fryer amidine synthesis[33] and was also followed for preparing other oxazepine analogues (**1**−**5**, **8**−**11**) in this work due to a reduced reaction time. Indeed, the appropriate lactam was reacted with an excess of amine and titanium tetrachloride in refluxing toluene. All target compounds were subjected to ^1H and ^{13}C NMR and elemental analysis before biological testing as criteria for their chemical structure or purity.

RESULTS

In Vitro Binding Experiments. The affinity of these original compounds on D_4, 5-HT_{1A}, 5-HT_{2A}, and α_{2A} receptors is reported in Tables 1 and 2. The screening evaluation of all original compounds indicated that they have low affinity at D_{2L} receptors, evidenced by their inability to show significant

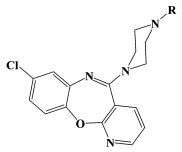
Scheme 1[a]

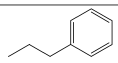
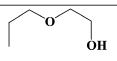


[a]Reagents and conditions: (i) $POCl_3$, reflux; (ii) amine A, toluene, reflux; (iii) amine B, anisole, $TiCl_4$, toluene, reflux. Amine A: R = piperazine, 1-phenylpiperazine, 1-benzylpiperazine, 1-(2-phenylethyl)piperazine, 1-methyl-4-aminopiperidine, 1-benzyl-4-aminopiperidine, 1-(2-aminoethyl)-piperidine, or 4-(2-aminoethyl)morpholine. Amine B: R = 1-(2-hydroxyethyl)piperazine or 1-[2-(2-hydroxyethoxy)ethyl]piperazine.

dx.doi.org/10.1021/jm2013419 | J. Med. Chem. 2012, 55, 1572−1582

Table 1. In Vitro Binding Evaluation of New Pyridobenzoxazepine Analogues with Modified Group on the Distal Basic Nitrogen of the Piperazine in Comparison with Reference Compounds[a]



Compounds	R	D_4	5-HT_{1A}	5–HT_{2A}	α_{2A}
2	**H**	28%	375 ± 32	131 ± 71	729 ± 12
3		-7%	10%	15%	-
4		210 ± 12	266 ± 60	1106 ± 672	50%
5		42 ± 7	19 ± 1.9	180 ± 37	374 ± 0.6
6		798 ± 99	131 ± 6	92 ± 39	412 ± 4
7		32%	37%	47%	51%
8		22%	26%	30%	452 ± 8
1	**CH_3**	109 ± 19	44%	204 ± 31	180 ± 1.7
Clozapine		18 ± 0.4	101 ± 12	4.84 ± 1.89	185 ± 39
Loxapine		9 ± 3	23%	2.42 ± 0.36	48 %
Amoxapine		34 ± 3	221 ± 102	1.77 ± 0.98	43 %
Quetiapine		>1000	125 ± 2.1	101 ± 28	23%

[a]Binding affinity is expressed in K_i, mean ± SD, $n \geq 3$ (except for amoxapine $n = 2$), or in percentage of displacement at 10^{-6} M.

percentage of displacement at 10^{-6} M, and they were not tested further (data not shown).

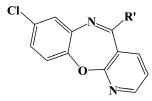
Impact of the Nature of the Side Chain on the Affinity for D_4 Receptors. In comparison with **1**, the affinity for D_4 receptors is reduced when the compound is dealkylated (**2**). Similarly, this is observed with amoxapine versus loxapine. The affinity is increased 2-fold when the methyl group is replaced by a phenethyl side chain (**5**). The benzyl analogue (**4**) is less potent on these receptor sites. The phenyl analogue (**3**) has no affinity, while the cyclohexyl derivative (**6**) presents a weak affinity. All other substitutions gave compounds with low or no significant affinity for these sites. Otherwise, compounds with a polar side chain (**7**, **8**) and quetiapine and compounds with a more flexible side chain (**9**–**12**) possess less affinity in comparison with reference compounds.

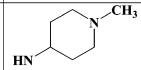
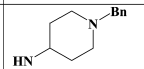
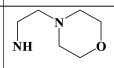
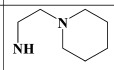
Impact of the Nature of the Side Chain on the Affinity for 5-HT_{1A} Receptors. Compound **2** has a higher affinity for these sites than the parent compound **1**. This is also observed with amoxapine and loxapine. The affinity is also increased when the side chain is a benzyl (**4**), phenethyl (**5**), or cyclohexyl (**6**) group. The phenethyl analogue (**5**) possesses a higher affinity for these sites than **1** and other reference compounds. Compounds with a phenyl ring (**3**), a polar side chain (**7**, **8**), or a more flexible side chain (**11**, **12**) have no significant affinity for these sites.

Impact of the Nature of the Side Chain on the Affinity for 5-HT_{2A} Receptors. Compound **2** has a higher affinity than the parent compound **1**. In the piperazine series, the cyclohexyl (**6**) and the phenethyl (**5**) analogues also possess a similar affinity to **2**. Quetiapine has also an affinity close to these molecules. Other reference compounds possess a higher affinity for these sites. In the piperidine series, *N*-methyl (**9**) and *N*-benzyl (**10**) analogues have an affinity close to that of **1**. Compounds with a phenyl ring (**3**), a polar side chain (**7**, **8**), or a more flexible side chain (**11**, **12**) have no significant affinity for these sites.

Impact of the Nature of the Side Chain on the Affinity for α_{2A} Receptors. Compound **1** has an affinity similar to that of

Table 2. In Vitro Binding Evaluation of New Pyridobenzoxazepine Analogues with Piperidine or More Flexible Side Chains in Comparison with Reference Compounds[a]



Compounds	R'	D_4	5-HT_{1A}	5–HT_{2A}	α_{2A}
9		-5%	8%	352 ± 21	-1%
10		21%	23%	284 ± 28	17%
11		-8%	-8%	> 1000	-
12		2%	0.3%	994 ± 307	-
1		109 ± 19	44%	204 ± 31	180 ± 1.7
Clozapine		18 ± 0.4	101 ± 12	4.84 ± 1.89	185 ± 39
Loxapine		9 ± 3	23%	2.42 ± 0.36	48 %
Amoxapine		34 ± 3	221 ± 102	1.77 ± 0.98	43 %
Quetiapine		>1000	125 ± 2.1	101 ± 28	23%

[a]Binding affinity is expressed in K_i, mean ± SD, $n \geq 3$ (except for amoxapine $n = 2$), or in percentage of displacement at 10^{-6} M.

Table 3. Physicochemical Parameters of Pyridobenzoxazepine Derivatives and Reference Compounds

compounds	pK_a[a]	log k'_{IAM}
2	8.16	2.68
4	6.51	4.16
5	7.03	4.45
6	7.38	4.34
7	6.8	2.46
8	6.24	2.55
9	8.88	2.98
10	8.11	4.63
11	6.24	2.63
12	8.86	3.33
1	7.48	2.95
clozapine	7.51	3.55
loxapine	7.52	3.23
amoxapine	8.36	3.17
quetiapine	6.41	2.84

[a]Predicted by the SPARC online calculator.[58]

dx.doi.org/10.1021/jm2013419 | *J. Med. Chem.* 2012, 55, 1572–1582

Table 4. D1 Receptor Binding in Rats after 4 Weeks of Daily Injections of Compounds 2 and 5[a]

brain region	controls	2	5	1[b]
cerebral cortex				
medial-prefrontal	39.8 ± 2.4 (100)	38.6 ± 1.2 (97)	39.2 ± 2.9 (98)	(107)
dorsolateral frontal	26.3 ± 1.2 (100)	25.1 ± 2.6 (95)	23.7 ± 1.7 (90)	(95)
nucleus accumbens	154.4 ± 7.7 (100)	198.0 ± 6.5 (**128**)*	156.0 ± 1.8 (101)	(**126**)*
caudate putamen				
medial	149.4 ± 8.3 (100)	195.8 ± 6.9 (**131**)*	152.2 ± 1.6 (102)	(**124**)*
lateral	150.4 ± 6.8 (100)	199.1 ± 7.7 (**132**)*	146.0 ± 4.6 (97)	(**122**)*
hippocampus	18.4 ± 3.5 (100)	19.4 ± 1.8 (105)	21.0 ± 2.5 (114)	(106)
entorhinal cortex	28.3 ± 3.1 (100)	27.1 ± 2.5 (96)	25.3 ± 1.1 (89)	(97)

[a]Data are mean ± SEM values (N = 8 rats/group) for binding in fmol/mg tissue and (% of control given in parentheses) following daily ip injections of vehicle, compounds **2** or **5** for 28 days, with significant differences from controls indicated in bold (* indicates $p < 0.001$). [b]Data (% of control) for **1** (10 mg/(kg·d)) in adult animals reported previously[24] and shown for comparison.

Table 5. D2 Receptor Binding in Rats after 4 Weeks of Daily Injections of Compounds 2 and 5[a]

brain region	controls	2	5	1[b]
cerebral cortex				
medial-prefrontal	25.1 ± 1.8 (100)	35.4 ± 0.6 (**141**)*	25.2 ± 0.7 (100)	(**132**)*
dorsolateral frontal	20.2 ± 1.7 (100)	20.9 ± 0.8 (103)	21.6 ± 0.9 (107)	(93)
nucleus accumbens	100.7 ± 7.2 (100)	141.0 ± 8.9 (**140**)*	153.5 ± 4.0 (152)*	(108)
caudate putamen				
medial	143.3 ± 8.1 (100)	157.9 ± 6.2 (110)	156.4 ± 4.8 (109)	(106)
lateral	198.8 ± 8.5 (100)	195.3 ± 7.0 (98)	202.9 ± 3.9 (102)	(103)
hippocampus	44.9 ± 2.9 (100)	60.0 ± 1.5 (**134**)*	46.3 ± 2.7 (103)	(**119**)*
entorhinal cortex	22.5 ± 4.3 (100)	21.5 ± 3.6 (96)	23.8 ± 2.5 (106)	(110)

[a]Data are mean ± SEM values (N = 8 rats/group) for binding in fmol/mg tissue (% of control is given in parentheses) following daily ip injections of vehicle or compounds **2** or **5** for 28 days, with significant differences from controls indicated in bold (* indicates $p < 0.05$). [b]Data (% of control) for **1** (10 mg/(kg·d)) in adult animals reported previously[24] and shown for comparison.

Table 6. D_4 Receptor Binding in Rats after 4 Weeks of Daily Injections of Compounds 2 and 5[a]

brain region	controls	2	5	1[b]
cerebral cortex				
medial-prefrontal	18.4 ± 0.7 (100)	20.3 ± 0.7 (110)	17.1 ± 0.8 (93)	(111)
dorsolateral frontal	19.7 ± 0.9 (100)	21.3 ± 0.6 (108)	20.6 ± 1.5 (105)	(108)
nucleus accumbens	23.2 ± 3.7 (100)	41.2 ± 3.3 (**178**)*	52.4 ± 1.2 (**225**)*	(**161**)*
caudate putamen				
medial	24.1 ± 1.7 (100)	39.9 ± 1.3 (**166**)*	21.6 ± 0.7 (89)	(**152**)*
lateral	33.0 ± 1.6 (100)	50.1 ± 1.8 (**152**)*	28.7 ± 0.5 (87)	(**140**)*
hippocampus	22.7 ± 0.5 (100)	26.6 ± 2.8 (117)	23.5 ± 1.5 (104)	(**157**)*
entorhinal cortex	12.2 ± 1.0 (100)	12.6 ± 1.3 (103)	13.2 ± 1.2 (108)	(92)

[a]Data are mean ± SEM values (N = 8 rats/group) for binding in fmol/mg tissue (% of control is given in parentheses) following daily ip injections of vehicle or compounds **2** or **5** for 28 days, with significant differences from controls indicated in bold (* indicates $p < 0.001$). [b]Data (% of control) for 1 (10 mg/(kg·d)) in adult animals reported previously[24] and shown for comparison.

clozapine. The dealkylated analogue (**2**) has a 4-fold lower affinity than the parent compound, while phenethyl (**5**), cyclohexyl (**6**), and hydroxyethoxyethyl (**8**) analogues have a 2-fold lower affinity. Compounds with a polar side chain (**7**, **8**) have a low affinity for these sites. 4-Aminopiperidine analogues (**9**, **10**) have no significant affinity for these sites.

Long-Term Effects of *N*-Dealkylated Analogue 2 and *N*-Phenethyl Analogue 5 on the Density of Dopaminergic and Serotonergic Receptors in Rat Brain. *Effects on Dopaminergic Receptors.* Repeated treatment with **2** increased D_1 receptor levels in nucleus accumbens (by 28%), medial caudate putamen (31%), and lateral caudate putamen (32%) [all $p < 0.001$, Table 4]. In contrast, repeated treatment with **5** did not alter levels of D_1 receptors in all brain regions examined (Table 4). Treatment with **2** also increased abundance of D_2 receptors in medial prefrontal cortex (41%), nucleus accumbens (40%), and hippocampus (34%) [$p < 0.05$, Table 5]. Treatment with **5** selectively increased levels of D_2 receptors in nucleus accumbens (52%) but not in other brain regions (Table 5). DA D_4 receptors were also altered after repeated treatment with both agents. Compound **2** increased D_4 receptors in nucleus accumbens (78%), medial caudate putamen (66%), and lateral caudate putamen (52%), whereas **5** profoundly increased D_4 receptors in nucleus accumbens (by 125%) [all $p < 0.05$, Table 6].

Effects on Serotonergic Receptors. Long-term administration of **2** increased 5-HT_{1A} receptors in medial prefrontal cortex (37%) and dorsolateral frontal cortex (41%) [$p < 0.001$, Table 7]. In addition, **2** selectively decreased 5-HT_{2A} receptors in medial prefrontal cortex (by 44%) and dorsolateral frontal cortex (47%) [$p < 0.001$, Table 8]. Long-term administration of

dx.doi.org/10.1021/jm2013419 | J. Med. Chem. 2012, 55, 1572–1582

Table 7. 5-HT_{1A} Receptor Binding in Rats after 4 Weeks of Daily Injections of Compounds 2 and 5[a]

brain region	controls	2	5	1[b]
cerebral cortex				
medial-prefrontal	42.2 ± 1.2 (100)	57.9 ± 1.9 (**137**)*	44.4 ± 1.4 (105)	(**128**)*
dorsolateral frontal	38.2 ± 1.5 (100)	53.7 ± 1.3 (**141**)*	42.6 ± 0.9 (112)	(**125**)*
nucleus accumbens	4.0 ± 0.7 (100)	3.5 ± 0.8 (88)	3.6 ± 0.5 (90)	(97)
caudate putamen				
medial	4.5 ± 0.9 (100)	3.9 ± 0.7 (87)	4.0 ± 0.5 (89)	(97)
lateral	4.3 ± 1.0 (100)	4.1 ± 0.6 (95)	4.2 ± 0.4 (98)	(98)
hippocampus				
CA1 region	84.5 ± 1.9 (100)	80.6 ± 3.1 (95)	81.0 ± 3.0 (96)	(109)
CA3 region	50.5 ± 1.7 (100)	52.1 ± 1.2 (105)	49.4 ± 2.1 (98)	(105)
entorhinal cortex	55.8 ± 1.6 (100)	52.6 ± 1.9 (103)	55.6 ± 1.8 (100)	(108)

[a]Data are mean ± SEM values (N = 8 rats/group) for binding in fmol/mg tissue (% of control is given in parentheses) following daily ip injections of vehicle or compounds **2** or **5** for 28 days, with significant differences from controls indicated in bold (* indicates $p < 0.001$). [b]Data (% of control) for 1 (10 mg/(kg·d)) in adult animals reported previously[24] and shown for comparison.

Table 8. 5-HT_{2A} Receptor Binding in Rats after 4 Weeks of Daily Injections of Compounds 2 and 5[a]

brain region	controls	2	5	1[b]
cerebral cortex				
medial-prefrontal	67.8 ± 3.0 (100)	38.3 ± 2.6 (**56**)*	64.8 ± 3.8 (96)	(**62**)*
dorsolateral frontal	66.7 ± 5.2 (100)	35.6 ± 3.1 (**53**)*	67.1 ± 2.2 (101)	(**65**)*
nucleus accumbens	20.1 ± 2.7 (100)	19.7 ± 2.5 (98)	22.2 ± 3.0 (110)	(102)
caudate putamen				
medial	24.7 ± 3.1 (100)	21.5 ± 2.8 (87)	23.3 ± 2.0 (94)	(94)
lateral	25.5 ± 2.9 (100)	27.2 ± 2.5 (107)	26.0 ± 3.6 (102)	(106)
hippocampus				
CA1 region	44.5 ± 1.6 (100)	42.7 ± 1.1 (96)	44.2 ± 1.9 (99)	(94)
CA3 region	27.0 ± 1.5 (100)	24.8 ± 1.3 (92)	25.8 ± 1.6 (96)	(96)
entorhinal cortex	32.8 ± 2.5 (100)	29.5 ± 1.0 (90)	27.9 ± 1.3 (85)	(94)

[a]Data are mean ± SEM values (N = 8 rats/group) for binding in fmol/mg tissue (% of control is given in parentheses) following daily ip injections of vehicle or compounds **2** or **5** for 28 days, with significant differences from controls indicated in bold (* indicates $p < 0.001$). [b]Data (% of control) for 1 (10 mg/(kg·d)) in adult animals reported previously[24] and shown for comparison.

5 did not alter concentrations of 5-HT_{1A} and 5-HT_{2A} receptors in all brain regions examined (Tables 7 and 8).

DISCUSSION

In the present study, we have synthesized and tested different analogues of **1** by changing the characteristics of the *N*-methyl piperazine side chain. Compound **1** has been extensively studied by our group and by others.[34,35] Recently, different chemical developments in related series have also been reported.[36−39] Accordingly, we show that the *N*-dealkylated analogue of **1**, a compound that could originate by biotransformation, possesses significant affinity for two important receptors, namely, serotonin 5-HT_{1A} and 5-HT_{2A} receptors. This molecule like amoxapine has higher affinity for these sites than its parent compound. The interest of **2** is reported for other compounds, for example, clozapine/norclozapine,[40,41] loxapine/amoxapine,[42] quetiapine/desalkylquetiapine,[43] and zotepine/nor-zotepine.[44]

The low D_2 affinity in this series was also previously reported[30,31] except for some pyridobenzodiazepine analogues.[32] This low dopaminergic D_2 potential was also found in apomorphine-induced climbing in mice[22] and follows this sequence in a more recent study. Clozapine and **1** inhibit the climbing at 10 and 30 mg/kg, while **2** reduced the climbing value by ∼20% at 30 mg/kg.[45]

The affinity for 5-HT_{1A} and α_{2A} receptors, combined with limited D_2 antagonism, may be beneficial for modulating neuronal activity in frontal cortex or limbic brain regions, which are involved in the pathophysiology of neuropsychiatric diseases such as depression, schizophrenia, and other idiopathic psychotic disorders.

The data obtained with different compounds raise the importance of the basicity of the distal nitrogen more than a steric hindrance in its vicinity, but this remark depends on the receptor examined. Indeed, the phenyl analogue (**3**) has no affinity for any receptors, while the compound with a bulky cyclohexyl group (**6**) has significant affinity for the receptors examined. Flexible (**9**−**12**) and polar (**7**, **8**) side chains are not favorable for receptor binding. Nevertheless, the higher distance of the distal nitrogen in **9** and **10** is tolerated in terms of interaction with 5-HT_{2A} receptors. In this *in vitro* binding study, two compounds, **2** and **5**, emerged in the context of multireceptor target strategy.

Indeed, **5**, with a phenethyl side chain, displays an interesting multireceptor affinity profile with a significant affinity for D_4 and particularly 5-HT_{1A} receptors, while its affinity for 5-HT_{2A} receptors is reduced compared with those of reference compounds but remained in the same range as that of **1** and its *N*-dealkylated analogue **2**. This phenethyl side chain is more hydrophobic because an increase in lipophilicity is observed (Table 3). Nevertheless, it seems difficult to correlate such increase of lipophilicity and the higher affinity for some receptors because **10** has also a higher hydrophobicity but exhibits lower affinity for D_4, 5-HT_{1A}, and α_{2A} receptors.

dx.doi.org/10.1021/jm2013419 | J. Med. Chem. 2012, 55, 1572−1582

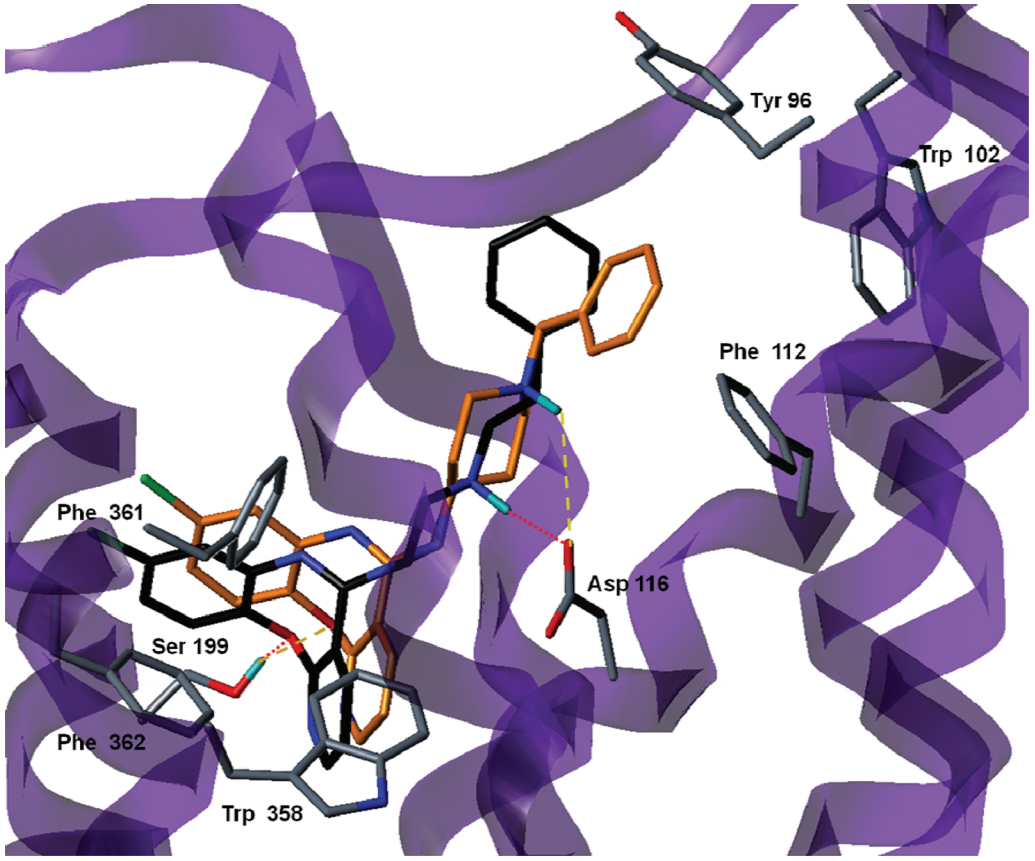


Figure 2. Binding mode of **5** (C, N, O, Cl, and H atoms in black, blue, red, green, and cyan, respectively) and **10** (C, N, O, Cl, and H atoms in orange, blue, red, green and cyan, respectively) in a human 5-HT_{1A} receptor model built by homology modeling from the crystal structure of the turkey β1 adrenergic GPCR (Protein Data Bank entry 2Y03).[57] The hydrogen and ionic bonds are indicated by red dots for **5** and yellow dashed lines for **10**.

Another parameter is the position of the distal nitrogen and the higher degree of freedom in **10** that could not be favorable in terms of interactions with the receptor site. To evaluate the impact of this parameter on the binding affinity, molecular docking studies of **5** and **10** on the 5-HT_{1A} receptor were carried out (Figure 2). It is clearly shown that the position of the protonated nitrogen greatly affects interactions with Ser199 and Asp116, two residues known to be important in the binding process.[48,49] Indeed, the hydrogen bond with the residue Ser199 and the ionic bond with the residue Asp116 appears to be weaker for **10** with a distance of 3.3 and 3.4 Å, respectively (versus 1.9 and 2.5 Å for **5**).

However, in some cases the low affinity observed for some compounds (**7**, **8**) could be related to a lower pK_a (Table 3) since the protonated nitrogen is a well-known pharmacophore in several GPCRs establishing an electrostatic interaction with an acidic residue like an aspartate residue.[50,51] Moreover, the increase of polar character of **7** and **8** (Table 3) can also be involved.

Following the *in vitro* binding evaluation, **2** and **5** were tested in a long-term study to assess its effect on the density of several neurotransmitter receptors in rat brain. This approach is quite original because it explores the impact on several targets in one animal and after a few weeks of administration. Most of the experimental procedures are frequently made following acute administration.

Thus, the effects of **2** on DA receptors were very similar to the superior profile of the effective SGA clozapine. Compound **2** increased the abundance of cortical and hippocampal D_2 receptors as well as striatolimbic D_4 receptors, while sparing D_2 receptors in extrapyramidal brain regions. Compound **2** displayed directional effects on DA receptors similar to those of **1** (Tables 4−6), except the effects of **2** were more profound than those of **1** on the same targets. For example, repeated treatment with **2** increased D_2 receptors in medial prefrontal cortex (by 41%) and hippocampus (by 34%). An equivalent dose of **1** increased D_2 receptors in medial prefrontal cortex by 32% and in hippocampus by 19%. Greater effects of **2** versus **1** were also detected on D_1 and D_4 receptors. In addition, **2** selectively increased levels of D_2 receptors in nucleus accumbens (by 40%); an effect not shared by **1** (Table 5).

Treatment with **2** produced different and opposite effects on 5-HT receptor subtypes in cerebral cortex. The tested dose (13.75 mg/kg) of **2** significantly increased concentrations of 5-HT_{1A} receptors and decreased 5-HT_{2A} receptors in medial prefrontal and dorsolateral frontal cortex (Tables 7, 8). The mechanism for modulation of receptor level by **2** remains unclear and requires further investigation. However, the pattern of 5-HT_{1A} and 5-HT_{2A} receptor changes after repeated treatment with **2** is similar to that observed after repeated treatment with **1** and several other established SGAs, including clozapine, olanzapine, risperidone, and quetiapine, and not typical neuroleptics such as haloperidol.[46,47] However, the effects of **2** were more profound than those of **1** on both 5-HT_{1A} and 5-HT_{2A} receptors in cerebral cortex (Tables 7, 8).

dx.doi.org/10.1021/jm2013419 | J. Med. Chem. 2012, 55, 1572−1582

Overall, **2**-induced changes in DA and 5HT receptor further encourage the development of this compound as a novel effective antipsychotic agent with atypical properties, perhaps superior to **1**, for improved treatment of positive symptoms, negative symptoms, and cognitive deficits reported to occur in patients diagnosed with schizophrenia. Nonetheless, the link between the modulation of receptor level by SGAs and their antipsychotic efficacy requires further study.

After long-term administration, **5** had more distinct effects on DA and 5-HT receptor subtypes. Repeated treatment with **5** profoundly and selectively increased abundance of D_2 and D_4 receptors in nucleus accumbens but did not alter DA or 5-HT receptor subtypes in any other cortical, extrapyramidal, or limbic brain regions. These findings suggest that **5** may be less likely to be an effective antipsychotic drug. However, the unique effects of **5** on D_2 and D_4 receptors in nucleus accumbens, a key brain region involved in mediating the rewarding actions of drugs of abuse,[52] suggest that this agent may play a role in mechanisms of drug addiction and may be useful to attenuate drug-induced rewarding behaviors. However, this hypothesis requires further investigation.

In conclusion, chemical modulation of **1** led us to show that the *N*-dealkylated analogue **2** possesses a biological activity that could contribute to the neurochemical effects of **1** in vivo. Among other chemical modifications, the presence of an *N*-phenethyl moiety in the piperazine series is favorable as **5** has high affinity for D_4, 5-HT_{1A}, and 5-HT_{2A} receptors. Moreover, the profiles of **2** on DA and 5-HT receptors after long-term administration, which are similar to that of clozapine and **1**, encourage its development as a novel antipsychotic agent for improved treatment of schizophrenia and other idiopathic psychotic disorders.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined on a Büchi-Tottoli capillary melting point apparatus in open capillary and are uncorrected. NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 MHz using compounds as free base dissolved in $CDCl_3$. IR spectra were performed on a Perkin-Elmer FTIR-1750 spectrometer using KBr discs. Only significant bands from IR are reported. Elemental analyses were performed using a Carlo-Erba elemental analyzer CHNS-O model EA1108, and the results are within 0.4% of the theoretical values. Based on these evaluations, the purity of key compounds is assumed to be >95%. All starting materials and reagents were obtained from Aldrich Chemical Co. or Acros Chemical Co. and were used without further purification. When necessary separations by column chromatography were carried out using Merck Kieselgel 60 (230−400 mesh). In the case of **3**, the compound crystallized during the separation process. Concentration and evaporation refer to removal of volatile materials under reduced pressure (10−15 mmHg at 30−50 °C) on a Büchi-Rotavapor.

General Procedure for the Synthesis of Oxazepine Derivatives (2−6, 9−12). To a mixture of 0.01 mol of 8-chloro-6*H*-pyrido[2,3-*b*][1,5]benzoxazepin-5-one, 0.1 mol of the appropriate amine A, and anhydrous toluene (20 mL), a solution of titanium tetrachloride (1.2 mL) in anisole (5 mL) was added dropwise. The mixture was heated at reflux for 2−3 h and cooled. 2-Propanol (10 mL), ammonia (3 mL), and Kieselgel 60 (5 g) were added. The suspension was stirred for 10 min and filtered. The solid was washed with chloroform. The combined organic layers were extracted with 2 N aqueous HCl (4 × 200 mL). The acidic phase was then basified with 30% aqueous ammonia solution and extracted with chloroform (4 × 150 mL). The organic layer, dried over anhydrous $MgSO_4$, was concentrated under reduced pressure. The solvent for recrystallization is mentioned in brackets after the melting point. For free base, the residue is solubilized in a limited volume of dichloromethane (or ethyl acetate), and hexane is added. The mixture is carefully concentrated on a Büchi-Rotavapor until crystallization. For salt, an equimolar quantity of the acid is solubilized separately in a minimal volume of the polar solvent as the base. Both solutions are mixed and diethylether is added carefully until crystallization. The mixture can be also maintained at 0−4 °C to get crystallization.

General Procedure for the Synthesis of Oxazepine Derivatives with Hydroxyl Group on Side Chain (7, 8). 8-Chloro-6*H*-pyrido[2,3-*b*][1,5]benzoxazepin-5-one (0.01 mol) was heated to reflux in the presence of an excess of phosphorus oxychloride (20 mL) for 12 h. After that, the excess phosphorus oxychloride was removed under reduced pressure. Toluene (20 mL) was added to the brown sticky residue and evaporated under reduced pressure. The crude iminochloride was used without further purification. It was dissolved in toluene (20 mL), and an excess of the amine B (∼0.1 mol) was added. The mixture was heated to reflux for 2−4 h. The solvent was then evaporated under reduced pressure, and the residue was dissolved in chloroform (100 mL) and washed with water (2 × 50 mL). The organic layer was clarified with activated charcoal, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue can be purified by crystallization directly or after liquid/solid chromatography on Kieselgel 60 using the appropriate solvent.

Lipophilicity Measurement (log k'_{IAM}). The column was an IAM-PC-DD2 HPLC column (30 × 4.6 mm^2 I.D.; particle size 12 μm; pore diameter 300 Å) from Regis Technologies (Morton Grove, IL, USA). The LC system consisted of a Merck-Hitachi L-6000 pump, a Merck-Hitachi D-2000 chromato-integrator, and a Rheodyne 7125 injector module equipped with a 20 μL loop (Rohnert Park, CA, USA). Column was maintained in a thermostatted water-bath, Tectron 473-100 from Selecta (Barcelona, Spain), and detection was made with a Merck-Hitachi L-4000 UV detector. Prior to use, mobile phases were degassed for 15 min in an ultrasonic bath, Branson 5510 (Branson Ultrasonics Corporation, Danbury, CT, USA). Buffer was adjusted to the expected pH values with a Radiometer Analytical pH meter, model ION check 10 (Villeurbanne, France). Each drug was dissolved in 10.0 mL of methanol at a concentration of 200 μg/mL. One milliliter of this stock solution was then diluted with methanol in a 10 mL flask in order to obtain a daughter solution of each compound (20 μg/mL). Twenty microliters of these diluted solutions was injected. The flow rate was maintained at 1 mL/min. Temperature of the column was fixed at 25 °C, and the ultraviolet absorption wavelength was set at 250 nm. The mobile phases were phosphate buffer (50 mM, pH 7.4) containing 10−60% methanol, according to the lipophilicity of the compounds. The increment of methanol between two mobile phases was set to 10%. All retention factors given represent the mean of three determinations of each sample solution. Retention times of the test compounds were transformed into capacity factors (k'_{IAM}) according to the following equation: $k'_{IAM} = (t_r - t_0)/t_0$ where t_r and t_0 are the retention time of the test compound and a compound that is not retained by the stationary phase (e.g., methanol) to indicate the dead time/void volume. Capacity factors were expressed as logarithm (log k'_{IAM}). The values reported in the text correspond to the extrapolation at 100% aqueous medium.

***In Vitro* Binding Procedures.** *5-HT_{1A} Receptor Binding Assays.* CHO cells expressing recombinant human serotonin receptors subtype 1A were used as membrane preparations (Perkin-Elmer 6110501). Briefly, incubations were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgSO_4$, 0.5 mM EDTA, and 0.1% ascorbic acid. Binding assays were performed in 540 μL total volume consisting of 500 μL of diluted membranes, 20 μL of radioligand ([^{3}H]-8-OH-DPAT at ∼0.25 nM), and 20 μL of buffer, unlabeled ligand, or tested drugs. After 60 min at 27 °C, incubations were terminated by rapid filtration on Whatman GF/C filters presoaked in 0.3% polyethylenimine followed by washing two times with ice cold 50 mM Tris-HCl (pH 7.4). Filters were placed in a vial containing 7.5 mL of Ecoscint A. Radioactivity remaining on the filter was evaluated by liquid scintillation using a TRI-CARB 1600TR liquid scintillation analyzer. Nonspecific binding was estimated in the presence of 10 μM metergoline.

Affinities were determined at least in duplicate with eight concentrations in duplicate. A preliminary screening was made at 10^{-6} M. Compounds displacing more than 60% specific radioactivity were further tested for K_i determinations (K_d of the radioligand is 0.32 nM).

5-HT$_{2A}$ Receptor Binding Assays. CHO cells expressing recombinant human serotonin receptor subtype 1A were used as membrane preparations (Perkin-Elmer 6110501). Briefly, incubations were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 4 mM $CaCl_2$ and 0.1% ascorbic acid. Binding assays were performed in 540 μL total volume consisting of 500 μL of diluted membranes, 20 μL of radioligand ([^{3}H]-ketanserin at ~0.6 nM), and 20 μL of buffer, unlabeled ligand, or tested drugs. After 60 min at 27 °C, incubations were terminated by rapid filtration on Whatman GF/C filters presoaked in 0.3% polyethylenimine followed by washing two times with ice cold 50 mM Tris-HCl (pH 7.4). Filters were placed in a vial containing 7.5 mL of Ecoscint A. Radioactivity remaining on the filter was evaluated by liquid scintillation using a TRI-CARB 1600TR liquid scintillation analyzer. Nonspecific binding was estimated in the presence of 10 μM mianserin. Affinities were determined at least in duplicate with eight concentrations in duplicate. A preliminary screening was made at 10^{-6} M. Compounds displacing more than 60% specific radioactivity were further tested for K_i determinations (K_d of the radioligand is 0.95 nM).

α_{2A}-Adrenoceptor Binding Assays. Sf9 cells expressing human cloned α_{2A}-adrenoceptors were used as membrane preparations (Perkin-Elmer 6110113). Briefly, incubations were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 12.5 mM $MgCl_2$ and 2 mM EDTA. Binding assays were performed in 540 μL total volume consisting of 500 μL of diluted membranes, 20 μL of radioligand ([^{3}H]-MK912 at ~0.7 nM), and 20 μL of buffer, unlabeled ligand, or tested drugs. After 60 min at 27 °C, incubations were terminated by rapid filtration on Whatman GF/C filters followed by washing two times with ice cold 50 mM Tris-HCl (pH 7.4). Filters were placed in a vial containing 7.5 mL of Ecoscint A. Radioactivity remaining on the filter was evaluated by liquid scintillation using a TRI-CARB 1600TR liquid scintillation analyzer. Nonspecific binding was estimated in the presence of 10 μM WB4101. Affinities were determined at least in duplicate with eight concentrations in duplicate. A preliminary screening was made at 10^{-6} M. Compounds displacing more than 60% specific radioactivity were further tested for K_i determinations (K_d of the radioligand is 0.75 nM).

D$_4$ Receptor Binding Assays. Sf9 cells expressing human cloned D_4 receptors were used as membrane preparations (Sigma D2439). Briefly, incubations were carried out in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$, 5 mM KCl, 1.5 mM $CaCl_2$, and 5 mM EDTA. Binding assays were performed in 540 μL total volume consisting of 500 μL of diluted membranes, 20 μL of radioligand ([^{3}H]-YM-09151-2 at ~0.2 nM), and 20 μL of buffer, unlabeled ligand, or tested drugs. After 60 min at 27 °C, incubations were terminated by rapid filtration on Whatman GF/C filters followed by washing two times with ice cold 50 mM Tris-HCl (pH 7.4). Filters were placed in a vial containing 7.5 mL of Ecoscint A. Radioactivity remaining on the filter was evaluated by liquid scintillation using a TRI-CARB 1600TR liquid scintillation analyzer. Nonspecific binding was estimated in the presence of 10 μM clozapine. Affinities were determined at least in duplicate with eight concentrations in duplicate. A preliminary screening was made at 10^{-6} M. Compounds displacing more than 60% specific radioactivity were further tested for K_i determinations (K_d of the radioligand is 0.06 nM).

Evaluation of Long-Term Effects of 2 and 5 on DA and 5-HT Receptor Levels Using Receptor Autoradiography Procedure. *Radioligands.* The radiochemicals [*N*-methyl-^{3}H]*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SCH-23390, 81 Ci/mmol), *R*,*S*(±)-[*N*-methyl-^{3}H]nemonapride (86 Ci/mmol), and [ethylene-^{3}H]-ketanserin hydrochloride ([^{3}H]ketanserin; 63.8 Ci/mmol) were obtained from New England Nuclear-PerkinElmer Inc. (Boston, MA, USA). Kodak Biomax MR films and D-19 photographic developer and fixative were obtained from Eastman-Kodak (Rochester, NY).

Drug Treatment and Tissue Preparation. Male Sprague–Dawley rats (Charles River), initially weighing 200–225 g, were maintained under controlled artificial daylight (on, 07:00–19:00 h), temperature, and humidity with free access to standard food and tapwater in a USDA-inspected, veterinarian-supervised, small-animal research facility of the Mailman Research Center, with approval by the Institutional Animal Care and Use Committee (IACUC) of McLean Hospital. Three groups (N = 8/group) of rats received control vehicle, **2** (13.75 mg/(kg·d)) or **5** (3.87 mg/(kg·d)) by daily (ip) injections for 28 days. All injections occurred between 9:00 and 10:00 a.m. At the end of treatment, rats were decapitated; brains were removed, quick-frozen in isopentane on dry ice, and stored at −80 °C until autoradiographic analysis. Frozen coronal sections (10 μm) were cut in a cryostat at −20 °C, mounted on gelatin-coated microscopic slides, and stored at −80 °C until use. Tissue sections were obtained from caudate putamen, nucleus accumbens, hippocampal CA1 and CA3 regions, areas of cerebral cortex including dorsolateral frontal and medial prefrontal, and entorhinal cortex.

Receptor Autoradiography. Brain sections from all drug- and vehicle-treated rats were evaluated at the same time in each receptor assay to minimize experimental variability. Assays were performed under saturating conditions to allow detection of changes in maximal binding. Sections were first preincubated for 1 h at room temperature in assay buffer to minimize the effects of endogenous DA and 5-HT.

D$_1$ Receptors. Rat forebrain sections were incubated for 1 h at room temperature in the incubating buffer [50 mM Tris-HCl buffer (pH 7.4), 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$] containing 1 nM [^{3}H]SCH-23390. The incubating buffer also contained 100 nM ketanserin to block 5-HT$_{2A/2C}$ receptors. Nonspecific binding was determined with an excess (1 μM) of *cis*-flupenthixol. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and dried under a stream of air.[24,53]

D$_2$ Receptors. Sections were incubated for 1 h at room temperature in the same incubating buffer described above. For D_2 binding, this buffer also contained 1 nM [^{3}H]nemonapride with 0.5 μM DTG and 0.1 μM pindolol to block sigma (σ1,2) and 5HT$_{1A}$ sites, respectively. Nonspecific binding was determined with 10 μM *S*(−)-sulpiride. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and air-dried.[24,53]

D$_4$ Receptors. Tissue sections were preincubated for 1 h at room temperature in the D_2 assay buffer, and then for 1 h with 1 nM [^{3}H]nemonapride, 300 nM *S*(−)-raclopride to occupy D_2/D_3 sites, and the other blocking agents (0.5 μM DTG and 0.1 μM pindolol) used in the D_2 assay. Nonspecific binding was determined with 10 μM *S*(−)-sulpiride. After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and air-dried.[24,53]

5-HT$_{1A}$ Receptors. Sections were preincubated for 1 h at room temperature in 50 mM Tris-HCl buffer (pH 7.6) containing ascorbic acid (0.1%, w/v), $CaCl_2$ (4 mM), and the monoamine oxidase inhibitor pargyline-HCl (10 μM). Sections were then incubated for another 1 h at room temperature in buffer containing 2.0 nM [^{3}H]8-OH-DPAT. Nonspecific binding was defined by 5-HT (1 μM). After incubation, slides were washed twice for 5 min in ice-cold buffer, dipped in ice-cold water, and air-dried.[47,54]

5-HT$_{2A}$ Receptors. Brain sections were preincubated for 1 h at room temperature in 50 mM Tris-HCl buffer (pH 7.7), then incubated for another 1 h at room temperature in fresh buffer containing 3.0 nM [^{3}H]ketanserin. Assays included 1 μM prazosin (to block α_1-adrenoceptors) and 100 nM tetrabenazine (to block a site associated with monoaminergic nerve terminals). Nonspecific binding was defined by methysergide (1 μM). After incubation, slides were washed twice for 30 min in ice-cold buffer, dipped in ice-cold water, and air-dried.[24,47]

Autoradiography and Image Analysis. Radiolabeled sections and calibrated [^{3}H]standards (Amersham, Arlington Heights, IL, USA) were exposed to Kodak Biomax MR films for 3–6 weeks at 4 °C in light-tight cassettes, developed, and then fixed. Optical density in brain

dx.doi.org/10.1021/jm2013419 | J. Med. Chem. 2012, 55, 1572–1582

regions of interest was measured with a computerized densitometric image analyzer (MCID-M4, Imaging Research, St. Catharines, ON, Canada). Brain regions of interest were outlined, and their optical density was measured. Optical density was converted to nCi/mg of tissue with calibrated [^{3}H]standards, and after subtracting nonspecific from total binding, specific binding was expressed as fmol/mg tissue.[24,47,53]

Statistical Analysis. DA and 5-HT receptor binding data were analyzed first for overall effects of drug versus vehicle for all receptor types and brain regions using multiple regression modeling methods. Density measures were logarithmically transformed to achieve more Gaussian-like distributions before regression modeling. Model goodness-of-fit was checked using partial residual plot methods. Since individual brain specimens provided receptor-binding data for several brain regions, resulting in incomplete independence across observations, we used robust standard error estimates to adjust for this clustering effect. This estimation method permits relaxation of the assumption of independence of all observations and requires only that the observations be independent across specimens.[55] Estimates of interaction effects were employed for post hoc tests of drug effects for specific receptors and brain regions, with adjustment of p-values obtained from the regression analyses estimating these multiple comparisons by the standard method of Sidák.[56] Data are presented as means ± SEM. Comparisons were considered significant at two-tailed $p < 0.05$ based on 8 rats per treatment group.

■ ASSOCIATED CONTENT

Supporting Information

Spectroscopic data, elemental analysis results, and melting points for compounds **2**−**12**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +32 (0)43 66 43 77. Fax: +32 (0)43 66 43 62. E-mail:JF.Liegeois@ulg.ac.be.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The technical assistance of Y. Abrassart, P. Fraikin, S. Counerotte, and J. Widart for IR spectra, ^{13}C NMR measurements, elemental analyses, and mass spectra, respectively, is gratefully acknowledged. Supported in part by grants of the F.R.S.-FNRS and the Fonds Spéciaux pour la Recherche of the University of Liège (Belgium). J.-F.L. is Research Director of the F.R.S.-FNRS.

■ ABBREVIATIONS

SGA, second generation of antipsychotic; DA, dopamine; 5-HT, 5-hydroxytryptamine; NMDA, *N*-methyl-D-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GPCR, G-protein coupled receptor; IAM-PC-DD2, immobilized artificial membrane phosphatidylcholine drug discovery; k'_{IAM}, capacity factor on immobilized artificial membrane; CHO cells, Chinese hamster ovary cells; Sf9 cells, *Spodoptera frugiperda* cloned cells; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; DTG, 1,3-di(2-tolyl)guanidine; 8-OH-DPAT, 8-hydroxy-2-(dipropylamino)tetralin; K_d, apparent equilibrium dissociation constant; K_i, apparent equilibrium inhibition constant

■ REFERENCES

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

(2) Liégeois, J.-F.; Eyrolles, L.; Bruhwyler, J.; Delarge, J. Dopamine D4 receptors: A new opportunity for research on schizophrenia. *Curr. Med. Chem.* **1998**, *5*, 77−100.

(3) Tarazi, F. I.; Zhang, K.; Baldessarini, R. J. Dopamine D4 receptors: Beyond schizophrenia. *J. Recept. Signal Transduction Res.* **2004**, *24*, 131−147.

(4) Enguehard-Gueiffier, C.; Gueiffier, A. Recent progress in medicinal chemistry of D4 agonists. *Curr. Med. Chem.* **2006**, *13*, 2981−2993.

(5) Rondou, P.; Haegeman, G.; Van Craenenbroeck, K. The dopamine D4 receptor: Biochemical and signalling properties. *Cell. Mol. Life Sci.* **2010**, *67*, 1971−1986.

(6) Löber, S.; Hübner, H.; Tschammer, N.; Gmeiner, P. Recent advances in the search for D3- and D4-selective drugs: Probes, models and candidates. *Trends Pharmacol. Sci.* **2011**, *32*, 148−157.

(7) Niswender, C. M.; Conn, P. J. Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 295−322.

(8) Krystal, J. H.; Mathew, S. J.; D'Souza, D. C.; Garakani, A.; Gunduz-Bruce, H.; Charney, D. S. Potential psychiatric applications of metabotropic glutamate receptor agonists and antagonists. *CNS Drugs* **2010**, *24*, 669−693.

(9) Roth, B. L.; Sheffler, D. L.; Kroeze, W. K. Magic shotguns versus magic bullets: Selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discovery* **2004**, *3*, 353−259.

(10) Chung, Y.-C.; Li, Z.; Dai, J.; Meltzer, H. Y.; Ichikawa, J. Clozapine increases both acetylcholine and dopamine release in rat ventral hippocampus: Role of 5-HT1A receptor agonism. *Brain Res.* **2004**, *1023*, 54−63.

(11) Ichikawa, J.; Ishii, H.; Bonaccorso, S.; Fowler, W. L.; O'Laughlin, I. A.; Meltzer, H. Y. 5-HT$_{2A}$ and D$_2$ receptor blockade increases cortical DA release via 5-HT$_{1A}$ receptor activation: A possible mechanism of atypical antipsychotic-induced cortical dopamine release. *J. Neurochem.* **2001**, *76*, 1521−1531.

(12) Ichikawa, J.; Li, Z.; Dai, J.; Meltzer, H. Y. Atypical antipsychotic drugs, quetiapine, iloperidone, and melperone, preferentially increase dopamine and acetylcholine release in rat medial prefrontal cortex: Role of 5-HT$_{1A}$ receptor agonism. *Brain Res.* **2002**, *956*, 349−357.

(13) Roth, B. L.; Hanizavareh, S. M.; Blum, A. E. Serotonin receptors represent highly favourable molecular targets for cognitive enhancement in schizophrenia and other disorders. *Psychopharmacology (Berlin)* **2004**, *174*, 17−24.

(14) Hertel, P.; Fagerquist, M. V.; Svensson, T. H. Enhanced cortical dopamine output and antipsychotic-like effects of raclopride by alpha2 adrenoceptor blockade. *Science* **1999**, *286*, 105−107.

(15) Jacobson, S. M.; Prus, A. J. Evaluation of the effects of a2-adrenoceptor antagonism with the D2 receptor antagonist raclopride on conditioned avoidance responding in rats. *Behav. Pharmacol.* **2010**, *21*, 654−659.

(16) Invernizzi, R. W.; Garavaglia, C.; Samanin, R. The alpha 2-adrenoceptor antagonist idazoxan reverses catalepsy induced by haloperidol in rats independent of striatal dopamine release: role of serotonergic mechanisms. *Neuropsychopharmacology* **2003**, *28*, 872−879.

(17) Morphy, R.; Rankovic, Z. Designed multiple ligands. An emerging drug discovery paradigm. *J. Med. Chem.* **2005**, *48*, 6523−6543.

(18) Marino, M. J.; Knutsen, L. J. S.; Williams, M. Emerging opportunities for antipsychotic drug discovery in the postgenomic era. *J. Med. Chem.* **2008**, *51*, 1077−1107.

(19) Ellenbroek, B. A.; Liégeois, J.-F. JL13, an atypical antipsychotic: A preclinical review. *CNS Drug Rev.* **2003**, *9*, 41−57.

(20) Invernizzi, R. W.; Garavaglia, C.; Samanin, R. JL13, a pyridobenzoxazepine compound with potential atypical antipsychotic activity, increases extracellular dopamine in the prefrontal cortex, but not in the striatum and the nucleus accumbens of rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2000**, *361*, 298−302.

(21) Ellenbroek, B. A.; Liégeois, J.-F.; Bruhwyler, J.; Cools, A. R. The effects of JL13, a pyridobenzoxazepine with potential atypical antipsychotic activity, in animal models for schizophrenia. *J. Pharmacol. Exp. Ther.* **2001**, *298*, 386−391.
(22) Bruhwyler, J.; Liégeois, J.-F.; Bergman, J.; Carey, G.; Goudie, A.; Taylor, A.; Meltzer, H. Y.; Delarge, J.; Géczy, J. JL13, a pyridobenzoxazepine compound with potential atypical antipsychotic activity: A review of its behavioural properties. *Pharmacol. Res.* **1997**, *36*, 255−264.
(23) Casey, D. E.; Bruhwyler, J.; Delarge, J.; Géczy, J.; Liégeois, J.-F. The behavioral effects of acute and chronic JL13, a putative antipsychotic, in Cebus non-human primates. *Psychopharmacology* **2001**, *157*, 228−235.
(24) Moran-Gates, T.; Massari, C.; Graulich, A.; Liégeois, J.-F.; Tarazi, F. I. Long-term effects of novel atypical antipsychotic JL13 on dopamine and serotonin receptor subtypes. *J. Neurosci. Res.* **2006**, *84*, 675−682.
(25) Tarazi, F. I.; Moran-Gates, T.; Gardner, M.; Graulich, A.; Lamy, C.; Liégeois, J.-F. Long-term effects of JL13, a potential atypical antipsychotic, on ionotropic glutamate receptors. *J. Mol. Neurosci.* **2007**, *32*, 192−198.
(26) Liégeois, J.-F.; Mouithys-Mickalad, A.; Bruhwyler, J.; Delarge, J.; Petit, C.; Kauffmann, J.-M.; Lamy, M. JL13, a potential successor to clozapine is less sensitive to oxidative phenomenons. *Biochem. Biophys. Res. Commun.* **1997**, *238*, 252−255.
(27) Liégeois, J.-F.; Rogister, F.; Delarge, J.; Pincemail, J. Peroxidase-catalysed oxidation of different dibenzazepine derivatives. *Arch. Pharm. (Weinheim)* **1995**, *328*, 109−112.
(28) Liégeois, J.-F.; Bruhwyler, J.; Petit, C.; Damas, J.; Delarge, J.; Géczy, J.; Kauffmann, J.-M.; Lamy, M.; Meltzer, H. Y.; Mouithys-Mickalad, A. Oxidation sensitivity may be a useful tool for the detection of the hematotoxic potential of newly developed molecules: Application to antipsychotic drugs. *Arch. Biochem. Biophys.* **1999**, *370*, 126−137.
(29) Dilly, S.; Liégeois, J.-F. The formation of nitrenium ion also influences binding of clozapine on rat D2 dopamine receptors: docking studies and *in vitro* binding assays. *J. Comput.-Aided Mol. Des.* **2011**, *25*, 163−169.
(30) Liégeois, J.-F.; Rogister, F.; Bruhwyler, J.; Damas, J.; Nguyen, T. P.; Inarejos, M. O.; Chleide, E.; Mercier, M.; Delarge, J. Pyridobenzoxazepine and pyridobenzothiazepine derivatives as potential central nervous system agents: Synthesis and neurochemical study. *J. Med. Chem.* **1994**, *37*, 519−525.
(31) Liégeois, J.-F.; Bruhwyler, J.; Damas, J.; Nguyen, T. P.; Chleide, E.; Mercier, M.; Rogister, F.; Delarge, J. New pyridobenzodiazepine derivatives as potential antipsychotics: synthesis and neurochemical study. *J. Med. Chem.* **1993**, *36*, 2107−2114.
(32) Liégeois, J.-F.; Eyrolles, L.; Carato, P.; Bruhwyler, J.; Géczy, J.; Damas, J.; Delarge, J. New pyridobenzodiazepine derivatives as potentially centrally acting drugs. Modifications on the basic side chain differentially modulate dopamine (D4, D_{2L}) and serotonin (5-HT_{2A}) binding. *J. Med. Chem.* **2002**, *45*, 5136−5149.
(33) Fryer, R. I.; Earley, J. V.; Field, G. F.; Zally, W.; Sternbach, L. H. A synthesis of amidines from cyclic amides. *J. Org. Chem.* **1969**, *34*, 1143−1145.
(34) Goudie, A. J.; Cole, J. C.; Sumnall, H. R. Olanzapine and JL13 induce cross-tolerance to the clozapine discriminative stimulus in rats. *Behav. Pharmacol.* **2007**, *18*, 9−17.
(35) Capuano, B.; Crosby, I. T.; MacRobb, F. M.; Taylor, D. A.; Vom, A.; Blessing, W. W. JL13 has clozapine-like actions on thermoregulatory cutaneous blood flow in rats: involvement of serotonin 5-HT1A and 5-HT2A receptor mechanisms. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2010**, *34*, 136−142.
(36) Capuano, B.; Crosby, I. T.; Lloyd, E. J.; Neve, J. E.; Taylor, D. A. Aminimides as potential CNS acting agents. I. Design, synthesis, and receptor binding of 4′-aryl aminimide analogues of clozapine as prospective novel antipsychotics. *Aust. J. Chem.* **2007**, *60*, 673−684.
(37) Capuano, B.; Crosby, I. T.; Lloyd, E. J.; Taylor, D. A. Synthesis and preliminary pharmacological evaluation of 4′-arylalkyl analogues of clozapine. III. Replacement of the tricyclic nucleus with a bicyclic template. *Aust. J. Chem.* **2007**, *60*, 928−933.
(38) Capuano, B.; Crosby, I. T.; Lloyd, E. J.; Neve, J. E.; Taylor, D. A. Aminimides as potential CNS acting agents. II. Design, synthesis, and receptor binding of 4′-arylalkyl aminimide analogues of clozapine as prospective novel antipsychotics. *Aust. J. Chem.* **2008**, *60*, 5−10.
(39) Capuano, B.; Crosby, I. T.; Lloyd, E. J.; Podloucka, A.; Taylor, D. A. Synthesis and preliminary pharmacological evaluation of 4′-arylalkyl analogues of clozapine. IV. The effects of aromaticity and isosteric replacement. *Aust. J. Chem.* **2008**, *60*, 930−940.
(40) Li, Z.; Huang, M.; Ichikawa, J.; Dai, J.; Meltzer, H. Y. N-Desmethylclozapine, a major metabolite of clozapine, increases cortical acetylcholine and dopamine release in vivo via stimulation of M1 muscarinic receptors. *Neuropsychopharmacology* **2005**, *30*, 1986−1995.
(41) Prus, A. J.; Pehrson, A. L.; Philibin, S. D.; Wood, J. T.; Vunck, S. A.; Porter, J. H. The role of M1 muscarinic cholinergic receptors in the discriminative stimulus properties of N-desmethylclozapine and the atypical antipsychotic drug clozapine in rats. *Psychopharmacology (Berlin)* **2009**, *203*, 295−301.
(42) Apiquian, R.; Ulloa, E.; Fresan, A.; Loyzaga, C.; Nicolini, H.; Kapur, S. Amoxapine shows atypical antipsychotic effects in paptients with schizophrenia: results from a prospective open-label study. *Schizophr. Res.* **2003**, *59*, 35−39.
(43) Jensen, N. H.; Rodriguiz, R. M.; Caron, M. G.; Wetsel, W. C.; Rothman, R. B.; Roth, B. L. N-desalkylquetiapine, a potent norepinephrine reuptake inhibitor and partial 5-HT1A agonist, as a putative mediator of quetiapine's antidepressant activity. *Neuropsychopharmacology* **2008**, *33*, 2303−2312.
(44) Shobo, M.; Kondo, Y.; Yamada, H.; Mihara, T.; Yamamoto, N.; Katsuoka, M.; Harada, K.; Ni, K.; Matsuoka, N. Norzotepine, a major metabolite of zotepine, exerts atypical antipsychotic-like and antidepressant-like actions through its potent inhibition of norepinephrine reuptake. *J. Pharmacol. Exp. Ther.* **2010**, *333*, 772−781.
(45) Lamy, C. Synthesis, evaluation and psychopharmacological evaluation of potential antipsychotics. Master Thesis in Pharmaceutical Sciences, University of Liège, 2005
(46) Florijn, W. J.; Tarazi, F. I.; Creese, I. Dopamine receptor subtypes: Differential regulation after 8 months treatment with antipsychotic drugs. *J. Pharmacol. Exp. Ther.* **2007**, *280*, 561−569.
(47) Tarazi, F. I.; Zhang, K.; Baldessarini, R. J. Long-term effects of olanzapine, risperidone, and quetiapine on serotonin 1A, 2A and 2C receptors in rat forebrain regions. *Psychopharmacology* **2002**, *161*, 263−270.
(48) Ho, B. Y.; Karschin, A.; Branchek, T.; Davidson, N.; Lester, H. A. The role of conserved aspartate and serine residues in ligand binding and in function of the 5-HT1A receptor: A site-directed mutation study. *Febs Lett.* **1992**, *312*, 259−262.
(49) Strader, C. D.; Candelore, M. R.; Hill, W. S.; Sigal, I. S.; Dixon, R. A. F. Identification of two serine residues involved in agonist activation of the beta-adrenergic receptor. *J. Biol. Chem.* **1989**, *264*, 13572−13578.
(50) Hibert, M. F.; Trumpp-Kallmeyer, S.; Bruinvels, A.; Hoflack, J. Three-dimensional models of neurotransmitter G-binding protein-coupled receptors. *Mol. Pharmacol.* **1991**, *40*, 8−15.
(51) Trumpp-Kallmeyer, S.; Hoflack, J.; Bruinvels, A.; Hibert, M. F. Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. *J. Med. Chem.* **1992**, *35*, 3448−3462.
(52) Carelli, R. M. The nucleus accumbens and reward: Neurophysiological investigations in behaving animals. *Behav. Cogn. Neurosci. Rev.* **2002**, *1*, 281−296.
(53) Tarazi, F. I.; Zhang, K.; Baldessarini, R. J. Long-term effects of olanzapine, risperidone, and quetiapine on dopamine receptor types in regions of rat brain: implications for antipsychotic drug treatment. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 711−717.
(54) Simpson, M. D.; Lubman, D. I.; Slater, P.; Deakin, J. F. Autoradiography with [^{3}H]8-OH-DPAT reveals increases in 5-HT_{1A} receptors in ventral prefrontal cortex in schizophrenia. *Biol. Psychiatry* **1996**, *39*, 919−928.

(55) Greene, W. H. *Econometric Analysis*, 4th ed.; Prentice Hall: Upper Saddle River, NJ, 2000; pp 462−465.
(56) Winer, B. J.; Brown, D. R.; Michels, K. M. *Statistical Principles in Experimental Drug Design*; 3rd ed.; McGraw Hill: New York, 1991, pp 165−166.
(57) Dilly, S.; Scuvée-Moreau, J.; Wouters, J.; Liégeois, J.-F. The 5-HT_{1A} agonism potential of substituted-piperazine-ethyl-amide derivatives is conserved in the hexyl homologues: Molecular modelling and pharmacological evaluation. *J. Chem. Inf. Model.* **2011**, *51*, 2961−2966.
(58) Hilal, S. H.; El-Shabrawy, Y.; Carreira, L. A.; Karickhoff, S. W.; Toubar, S. S.; Rizk, M. Estimation of the ionization pK(a) of pharmaceutical substances using the computer program Sparc. *Talanta* **1996**, *43*, 607−619.