New Pyridobenzodiazepine Derivatives: Modifications of the Basic Side Chain Differentially Modulate Binding to Dopamine (D$_{4.2}$, D$_{2L}$) and Serotonin (5-HT$_{2A}$) Receptors

Jean-François Liégeois,*,[†,‡] Laurence Eyrolles,[†] Bart A. Ellenbroek,[§] Christel Lejeune,[∥] Pascal Carato,[†] Jacques Bruhwyler,[⊥] Joseph Géczy,[⊥] Jacques Damas,[‡] and Jacques Delarge[†]

Natural and Synthetic Drugs Research Center, Laboratory of Medicinal Chemistry, University of Liège, avenue de l'Hôpital 1 (B36), B-4000 Liège 1, Belgium, Laboratory of Physiology, University of Liège, place Delcour 17 (L1), B-4020 Liège, Belgium, Department of Psychoneuropharmacology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands, Faculty of Medicine, University of Namur, rue de Bruxelles 61, B-5000 Namur, Belgium, and Therabel Research, rue Egide Van Ophem 108, B-1180 Brussels, Belgium

Received October 18, 2001

A series of new pyridobenzodiazepines with variation of the basic side chain were synthesized and evaluated for their binding to D$_{4.2}$, D$_{2L}$, and 5-HT$_{2A}$ receptors in comparison with clozapine, haloperidol, and two parent compounds previously described, 8-chloro-6-(4-methyl-1-piperazin-yl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (**8**) and 8-methyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (**9**). In the piperazine series, replacing the *N*-methyl group by a *N*-phenyl moiety (**15–17, 30–32**) provided a dramatic decrease of affinity for all receptors (K_i > 1000 nM). A *N*-cyclohexyl group (**20, 35**) restored some affinity. Compounds with a *N*-benzyl (**18, 33**) or *N*-phenethyl side chain (**19, 34**) had significant affinities at D$_{4.2}$ and 5-HT$_{2A}$ receptors. Homologation of the piperazine nucleus (**29, 44**) led to a significant decrease of the affinity at all receptors investigated. In the 4-aminopiperidine series, *N*-methyl derivatives (**21, 36**) possessed less affinity in comparison with the *N*-methylpiperazine analogues (**8, 9**) while the *N*-benzyl congeners (**22, 37**) showed similar affinities. The rigidification of piperidine nucleus as obtained in azabicyclo[3.2.1]octane derivatives (**23, 38**) involved a slight reduction of the affinity at D$_{4.2}$ and 5-HT$_{2A}$ receptors while the affinity at D$_{2L}$ receptors was dramatically increased. The introduction of N-substituted aminoalkylamines to replace *N*-methylpiperazine generally led to a significant decrease in the affinity for D$_{4.2}$ receptors but some of these molecules (**24, 25, 41**) presented a significant 5-HT$_{2A}$ binding affinity. The presence of a more flexible side chain induced an increased conformational freedom. Consequently, the preferential position of the distal nitrogen or its basicity in piperazine derivatives was greatly modified. **19** with a high D$_{4.2}$ and 5-HT$_{2A}$ affinity (K_i = 40 and 103 nM, respectively) did not induce cataleptic phenomenon in the paw test in rats but significantly reduced the immobility time in Porsolt's test in mice suggesting antidepressant properties.

Introduction

Although numerous substances with antipsychotic potential have been developed and marketed during the past decade, e.g., risperidone (**1**),[1] sertindole (**2**),[2,3] olanzapine (**3**),[4,5] quetiapine (**4**),[6,7] ziprasidone (**5**),[8] and zotepine (**6**)[9] (Figure 1), clozapine (**7**) still remains an invaluable treatment of psychosis thanks to its clinical, economical, or social advantages.[10,11] Nevertheless, its serious adverse effects[10–14] and more particularly the induction of agranulocytosis[15–17] have continuously stimulated the search for newer and safer drugs.

So far, the biochemical mechanisms of action of clozapine have not been completely elucidated. Regarding its in vitro receptor binding profile, a high affinity is observed at serotonin (5-HT$_2$) and acetylcholine (M) receptors but also at α$_1$, D$_4$, 5-HT$_7$, and H$_1$ receptors;

however, its D$_2$ affinity is low.[18–20] This fact was also confirmed using autoradiography techniques in rats.[21]

The dopamine/acetylcholine balance hypothesis was the prior predominant etiological theory to explain the drug-induced extrapyramidal syndrome experienced with conventional antipsychotics.[18,22] Later on, the role of serotonin in schizophrenia was emphasized in different reports.[23,24] Indeed, most of the antipsychotics were found to be 5-HT$_2$ blockers,[23] and this activity was shown to modulate the adverse effects linked to D$_2$ receptor blockade.[25,26] It was further reported that the most discriminant parameter, when examining the binding affinities of a series of conventional and atypical antipsychotics at D$_1$, D$_2$, and 5-HT$_2$ receptors, was the ratio of D$_2$ and 5-HT$_2$ pK_i.[19] The 5-HT$_2$/D$_2$ ratio hypothesis has been widely applied,[27–31] and the majority of recent antipsychotics derive from this concept and constitute the so-called SDA's class (for serotonin–dopamine antagonist) although each of these compounds also binds to additional receptor sites.[32,33] In parallel, the characterization of a new dopamine receptor subtype called D$_4$, in the early 1990s, raised another possible hypothesis to explain the mechanism of action of an-

* Corresponding author: Dr. Jean-François Liégeois. Tel.: 32 4 3664377. Fax: 32 4 3664362. E-mail: JF.Liegeois@ulg.ac.be.
† Laboratory of Medicinal Chemistry, University of Liège.
‡ Laboratory of Physiology, University of Liège.
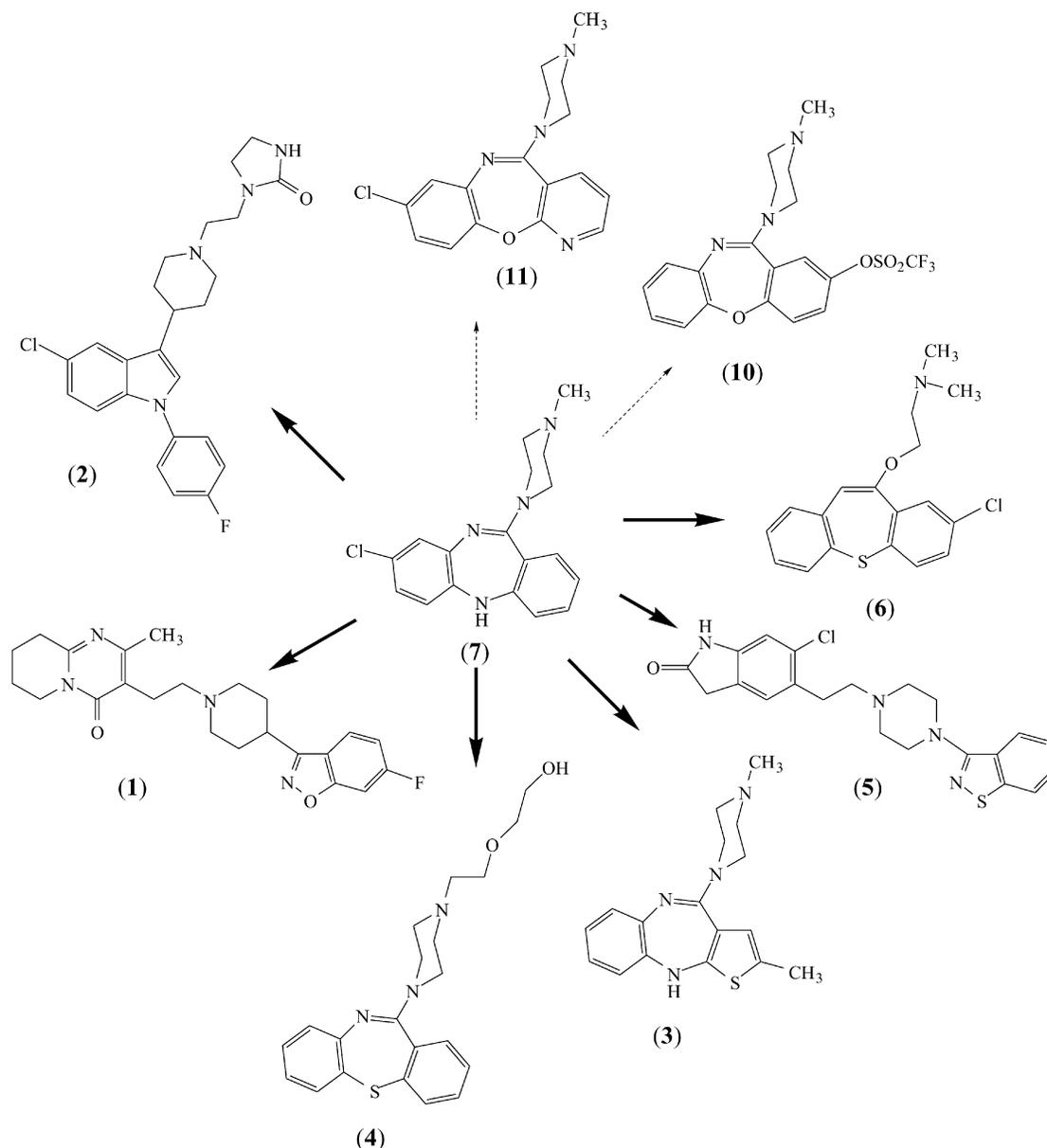§ University of Nijmegen.
∥ University of Namur.
⊥ Therabel Research.

Figure 1. Chemical structures of some atypical antipsychotics derived from the 5-HT$_2$/D$_2$ concept. Bold arrows indicate marketed compounds while dotted arrows are used for drugs in development. **11** is tested as fumarate.

tipsychotic drugs.[34] Indeed, because clozapine (**7**) showed a high affinity for this site, numerous works were undertaken to find more selective compounds.[35–39]

Clozapine-related tricyclics constitute an interesting family of compounds since a small variation of the structure can lead to profound change in the receptor binding profile or clinical activity.[40] Olanzapine, quetiapine, or zotepine with different basic side chains possess multireceptor affinity[41] as well as antipsychotic properties.

In dibenzazepine series, substituent in 2-position of the tricyclic ring, especially a halogen, provided neuroleptic activity.[42] This substituent has been considered an important feature for the drug–receptor recognition. Its favorable influence can be regarded in terms of lipophilicity, sterical hindrance, or electronic effects. In the literature, triflate derivatives were reported and some of them presented promising antipsychotic potential such as GMC 2–83 (**10**).[43,44] The triflate group like fluorine induced less oxidative metabolism.

The first part of our research program was dedicated to the preparation of pyridine analogues of dibenzazepine derivatives. Chemical modifications of the tricyclic nucleus were made by changing the position of the pyridine nitrogen, the nature of the heteroatom in heteroazepine ring, and also the nature of the substituent in 2- or 8-position while the *N*-methylpiperazine side chain was conserved in all molecules.[45,46] In these series, the affinities at D$_2$, D$_1$, 5-HT$_{2A}$, and muscarinic receptors were found to be dramatically reduced compared to clozapine. Further investigations with respect to the D$_4$ receptor hypothesis (coll. HHM Van Tol, Toronto, CA) revealed that 8-chloro-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (**8**) and 8-methyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (**9**) had a higher D$_4$ selectivity than clozapine.[47] Otherwise, by using a complex procedure in Beagle dogs, some pyridine analogues of dibenzazepines had revealed a potential neuroleptic profile (Figure 2).[48] Following oral acute administration, two pyridobenzodiazepines
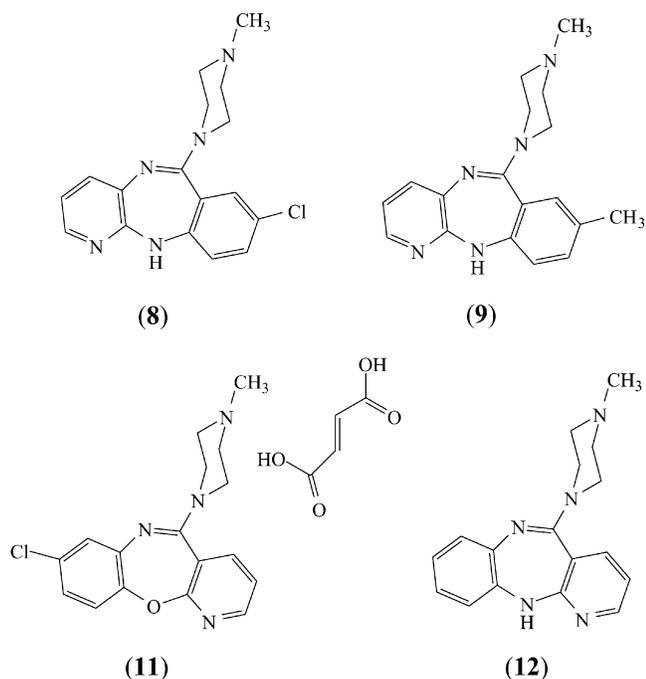
Figure 2. Chemical structures of some pyridobenzoazepine compounds with a neuroleptic/antipsychotic potential detected in a complex operant procedure in dogs.[46] **11** is tested as the fumarate.

(**8**, **9**) showed a neuroleptic profile closer to that of haloperidol than to clozapine. Nevertheless, in the context of chronic studies,[49] **8** resembled clozapine more than haloperidol (unpublished results). Otherwise, the oxazepine, 8-chloro-5-(4-methyl-1-piperazinyl)-pyrido-[2,3-*b*][1,5]benzoxazepine fumarate (**11**), and the diazepine, 5-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,5]-benzodiazepine (**12**), showed profiles similar to clozapine.[48] Extensive investigations with **11** have confirmed the interest of this compound as potential antipsychotic[50,51] with minimal side effects such as extrapyramidal syndrome (EPS)[50,52] and with a low potential for inducing hyperprolactinemia.[53]

Although numerous studies were undertaken on *N*-methylpiperazine derivatives in order to explore the pharmacology of such bioisosteric analogues of clozapine,[43,44,54,55] little has been done regarding the modifications of the basic side chain and the impact on 5-HT$_{2A}$ and D$_4$ selectivity. In this context, some 10-(1,2,3,6-tetrahydro-4-pyridinyl) analogues in dibenzo[*a,d*]cycloheptene and dibenz[*b,f*]oxepin series have been described.[55] Increasing in the effective size of the alkyl substituent (methyl, ethyl, 2-propenyl) at the tertiairy amine nitrogen atom in the 1,2,3,6-tetrahydro-4-pyridinyl moiety in the 5*H*-dibenzo[*a,d*]cycloheptene series reduced the binding to dopamine D$_4$ receptors, but in the dibenz[*b,f*]oxepin series, no significant change in binding affinity to the dopamine D$_4$ receptors was observed. Equal or slightly higher affinity for the serotonin 5-HT$_{2A}$ receptors was measured in both series.[55]

Generally, previous works indicated that replacing the methyl group on distal nitrogen led to reduced antidopaminergic D$_2$ potential. The absence of substituent on the distal nitrogen as in amoxapine, the demethylated analogue of loxapine, or in nor-clozapine led to a decreased affinity for D$_2$ receptors compared to the

N-methyl derivatives.[56] Moreover, in the case of amoxapine, a dramatic change in the clinical profile was also observed since it was and is still considered as an antidepressant drug.[33] The *N*-(2-(2-hydroxyethoxy)ethyl side chain as in quetiapine (**4**) was unfavorable to antidopaminergic potential. Nevertheless, the compound is widely used in clinical practice for treating different psychotic disorders.[6] Other derivatives in this series with different hydroxylated side chains also possessed a weak affinity at D$_2$ receptors.[57] Zotepine (**6**) possessing a dimethylaminoethoxy side chain had high affinity at D$_2$ and 5-HT$_{2A}$ receptors[56] and was also introduced in clinic to treat acute and chronic schizophrenia.[9,58] In a different series, Steiner et al. reported the preparation and the biological evaluation of different 11-cyanomethylene analogues of dibenz[*b,e*]azepin.[59] They examined the effects on sedation and an anticholinergic response in mice. Replacing the methyl group by a hydrogen resulted in substantial loss of activity. The presence of an open-chain aminoalkylamines where the conformational freedom of these chains is greater than that of the rigid piperazine ring was less active. The (piperidin-1-yl-ethyl)amino derivatives showed a sedative effect similar to that of the corresponding methylpiperazine analogue. Increasing the size of the diaminoalkyl bridge by one carbon atom led to a drastic reduction in activity. The alkoxyalkylamino derivatives such as zotepine (**6**) were substantially less active.

In the present paper, we report the preparation and the neurochemical evaluation of a series of pyridobenzodiazepines with various basic side chains (Figure 3). The tricyclic moiety was deliberately limited to one ring with either a chlorine atom or a methyl group in 8-position. The amines were chosen in order to explore two main parameters related to the distal basic nitrogen considered as a pharmacophoric element. First of all, the ionization of this nitrogen seems to be a crucial element for binding recognition. By modifying the group on this nitrogen, it is possible to explore the impact of basicity. Second, the flexibility of the side chain could influence the position of the basic nitrogen. Indeed, we selected some substituted 4-aminopiperidine derivatives and various aminoalkylamines. In the latter, the alkyl group was limited to ethyl or propyl. The impact of such modifications was evaluated for binding to dopamine D$_{4.2}$ and D$_{2L}$, serotonin 5-HT$_{2A}$ receptors, and α_1-adrenoceptors.

Chemistry

In this study, two substituted pyrido[2,3-*b*][1,4]-benzodiazepine rings with R = chlorine or with R = methyl were selected for further chemical modulations (Figure 3). The main modifications concerned the basic side chain. The classical *N*-methylpiperazine side chain was replaced by various amines (Figure 3) such as *N*-phenylpiperazine (Am1a), *N*-(3-chlorophenyl)piperazine (Am1b), *N*-(3-trifluoromethylphenyl)piperazine (Am1c), *N*-benzylpiperazine (Am2), *N*-phenethylpiperazine (Am3), *N*-cyclohexylpiperazine (Am4), 4-amino-1-methylpiperidine (Am5), 4-amino-1-benzylpiperidine (Am6), 3-β-amino-8-benzyl-8-azabicyclo[3.2.1]octane (Am7), *N*-methylhomopiperazine (Am15), and several *N,N*-disubstituted-aminoalkylamines (Am8−14). These amines were either commercially available or prepared
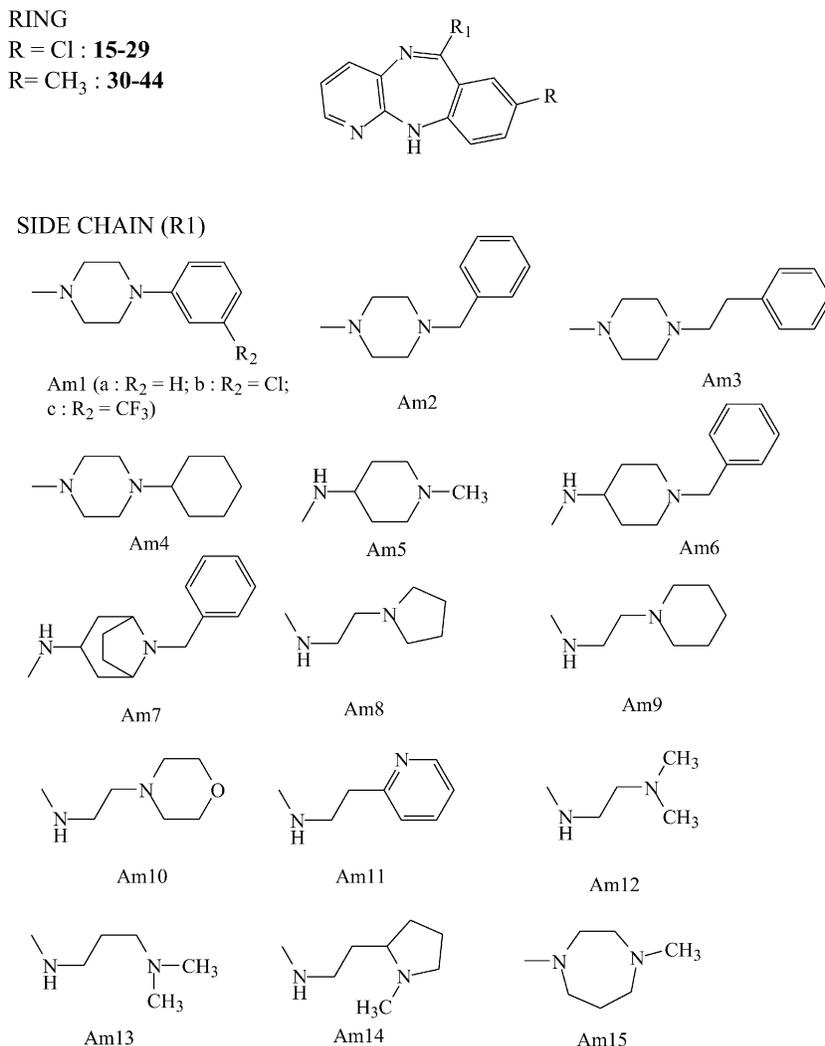
RING
R = Cl : **15-29**
R= CH$_3$: **30-44**

SIDE CHAIN (R1)

Am1 (a : R$_2$ = H; b : R$_2$ = Cl;
c : R$_2$ = CF$_3$)

Am2

Am3

Am4

Am5

Am6

Am7

Am8

Am9

Am10

Am11

Am12

Am13

Am14

Am15

Figure 3. General chemical structures of newly synthesized pyridobenzodiazepine molecules. Tricyclic rings and basic side chain are presented. Corresponding nomenclature for the amines is also reported in Tables 1 and 2.

according to procedures reported in the literature. Some criteria were retained for selecting these amines such as the distance between both nitrogen atoms determined by the presence of two or three carbon atoms. Another parameter to be explored was the basicity of the distal nitrogen. The presence of a phenyl or a cyclohexyl moiety allowed simultaneous exploration of the impact on the basicity and a steric effect. This parameter was also investigated by introducing a benzyl or a phenethyl group. Otherwise, the effect of homologation, a classical tool in medicinal chemistry, was evaluated either by using a homopiperazine side chain or by replacing the piperazine moiety by a 4-aminopiperidine side chain. Flexible side chains were used to explore the spatial position of the nitrogen. They are frequently found in psychotropic drugs.

The diazepine compounds (R = Cl: **15**−**29**; R = CH$_3$: **30**−**44**) were synthesized from the corresponding lactams (**13**, **14**) by using a modified Fryer amidine synthesis (Scheme 1).[60] The lactam intermediates were made by following the previously described methods.[45] The crude products were purified either directly by means of recrystallization from dichloromethane/hexane mixture or by means of flash chromatography (acetone as the solvent) followed by recrystallization of the residue from dichloromethane/hexane mixture (Tables

1 and 2). In some case, fumaric acid salts were isolated and further recrystallized from a methanol/ethyl acetate mixture (Table 2).

Results

New pyridobenzodiazepine derivatives were obtained by the reaction of various amine side chains with two lactams by using a previously described procedure.[45] The yield of the reaction varied greatly depending on the nature of the amine. Generally, it was lower than that obtained in *N*-methylpiperazine series. Newly synthesized molecules (Tables 1 and 2) were characterized by classical procedures (^{1}H NMR, IR, and elemental analysis). In some cases, X-ray crystallography was used to confirm the chemical structure in parallel to the determination of the 3D-structure.[61]

The new drugs were tested in vitro for their ability to interact at D$_{4.2}$, D$_{2L}$ and 5-HT$_{2A}$ receptors in comparison with the parent *N*-methylpiperazine analogues (**8**, **9**), haloperidol (**45**), and clozapine (**7**). Affinities were expressed as K_i values in nM. A preliminary screening at 10^{-6} and 10^{-7} M was performed to select the most interesting drug for further evaluation. Generally, compounds with low D$_4$ potential were not tested for their binding at D$_{2L}$ receptors. Some experiments were carried out on rat cortical α$_1$-adrenoceptors. Moreover,

Scheme 1[a] Chemical Pathways for the Preparation of 6-Substituted Pyridobenzodiazepine Derivatives (**15−29**, **30−44**)

R = Cl (**13**); R = CH$_3$ (**14**)   R = Cl (**15-29**); R = CH$_3$ (**30-44**)
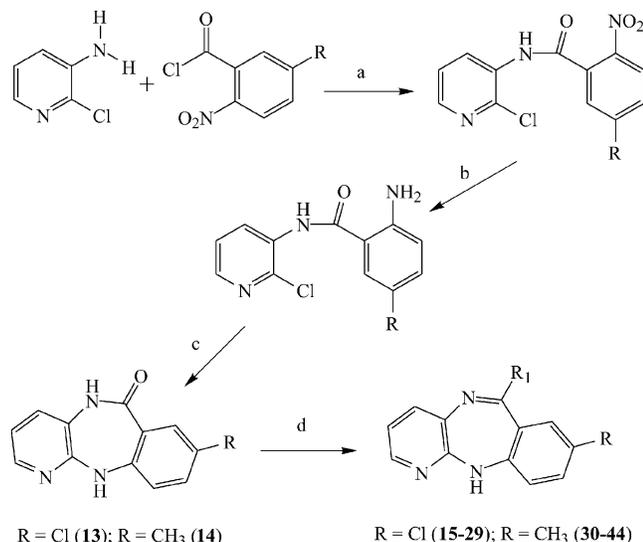
[a] Reagents and conditions: (a) dioxane, pyridine, RT; (b) SnCl$_2$, HCl; (c) DEGMME, Δt; (d) amine, TiCl$_4$, toluene, anisole, Δt (for significance of R$_1$ see Figure 3).

Table 1. 8-Chloro-6-substituted-11H-pyrido[2,3-b][1,4]-benzodiazepine Derivatives

compd	R	R1	R2	formula	analysis	mp (°C)	yield (%)
15	Cl	Am1a	H	C$_{22}$H$_{20}$N$_5$Cl	CHN	185−186	30
16	Cl	Am1b	Cl	C$_{22}$H$_{19}$N$_5$Cl$_2$	CHN	189−192	40
17	Cl	Am1c	CF$_3$	C$_{23}$H$_{19}$N$_5$ClF$_3$	CHN	157−159	20
18	Cl	Am2	−	C$_{23}$H$_{22}$N$_5$Cl	CHN	235−237	20
19	Cl	Am3	−	C$_{24}$H$_{24}$N$_5$Cl	CHN	190−192	25
20	Cl	Am4	−	C$_{22}$H$_{26}$N$_5$Cl	CHN	192−194	10
21	Cl	Am5	−	C$_{18}$H$_{20}$N$_5$Cl	CHN	193−195	25
22	Cl	Am6	−	C$_{24}$H$_{24}$N$_5$Cl	CHN	163−164	30
23	Cl	Am7	−	C$_{26}$H$_{26}$N$_5$Cl	CHN	185−186	20
24	Cl	Am8	−	C$_{18}$H$_{20}$N$_5$Cl	CHN	142−143	25
25	Cl	Am9	−	C$_{19}$H$_{22}$N$_5$Cl	CHN	172−174	65
26	Cl	Am10	−	C$_{18}$H$_{20}$N$_5$OCl	CHN	155−156	50
27	Cl	Am11	−	C$_{19}$H$_{16}$N$_5$Cl	CHN	161−162	45
28	Cl	Am13	−	C$_{17}$H$_{20}$N$_5$Cl	CHN	138−139	10
29	Cl	Am15	−	C$_{18}$H$_{20}$N$_5$Cl	CHN	139−141	60

Table 2. 8-Methyl-6-substituted-11H-pyrido[2,3-b][1,4]-benzodiazepine Derivatives

compd	R	R1	R2	formula	anal.	mp (°C)	yield (%)
30	CH$_3$	Am1a	H	C$_{23}$H$_{23}$N$_5$	CHN	155−157	45
31	CH$_3$	Am1b	Cl	C$_{23}$H$_{22}$N$_5$Cl	CHN	159−161	15
32	CH$_3$	Am1c	CF$_3$	C$_{24}$H$_{22}$N$_5$F$_3$	CHN	144−145	10
33	CH$_3$	Am2	−	C$_{24}$H$_{25}$N$_5$	CHN	223−224	15
34	CH$_3$	Am3	−	C$_{25}$H$_{27}$N$_5$	CHN	158−159	40
35	CH$_3$	Am4	−	C$_{23}$H$_{29}$N$_5$	CHN	173−174	35
36	CH$_3$	Am5	−	C$_{19}$H$_{23}$N$_5$	CHN	181−182	50
37	CH$_3$	Am6	−	C$_{25}$H$_{27}$N$_5$	CHN	174−176	40
38	CH$_3$	Am7	−	C$_{27}$H$_{29}$N$_5$	CHN	146−147	25
39	CH$_3$	Am10	−	C$_{19}$H$_{23}$N$_5$O	CHN	158−159	15
40	CH$_3$	Am11	−	C$_{20}$H$_{19}$N$_5$·C$_4$H$_4$O$_4$	CHN	190−193	45
41	CH$_3$	Am12	−	C$_{17}$H$_{21}$N$_5$	CHN	188−189	15
42	CH$_3$	Am13	−	C$_{18}$H$_{23}$N$_5$	CHN	122−124	35
43	CH$_3$	Am14	−	C$_{20}$H$_{25}$N$_5$	CHN	147−148	25
44	CH$_3$	Am15	−	C$_{19}$H$_{23}$N$_5$	CHN	141−143	15

we also estimated some binding ratios (5-HT$_2$/D$_2$, D$_2$/D$_4$) which represent the selectivity of these molecules.

The binding data for haloperidol (**45**) and clozapine (**7**) were similar to those reported in the literature.[20,41]

Influence of the Nature of the Basic Side Chain on the Affinity at D$_{4.2}$ Receptors. Piperazine Derivatives (Table 3). *N*-Methylpiperazine derivatives (**8**, **9**) possessed affinities close to clozapine (K_i = 24, 36, and 18 nM for **8**, **9**, and clozapine, respectively). *N*-Phenyl analogues (**15−17**, **30−32**) had no affinity for this receptor (K_i > 1000 nM). Homologation of the *N*-substituted radical showed that this modulation was favorable not only in the case of the *N*-benzyl analogues (K_i = 109 and 183 nM for **18** and **33**, respectively) but mainly in the presence of a *N*-phenethyl group (K_i = 40 and 37 nM for **19** and **34**, respectively). In the latter cases, compounds possessed affinities similar to **8** and **9**. *N*-Cyclohexyl derivatives (**20**, **35**) also possessed significant D$_{4.2}$ affinity (K_i = 48 and 84 nM for **20** and **35**, respectively).

Homologation of the piperazine ring led to a decreased affinity (K_i = 163 and 356 nM for **29** and **44** respectively).

4-Aminopiperidine Analogues (Table 4). In both series, *N*-methyl analogues possessed about 10-fold less affinity at this site (K_i = 393 and 510 nM for **21** and **36**, respectively) in comparison with the *N*-methylpiperazine congeners (**8**, **9**). Otherwise, the *N*-benzyl derivatives had affinities in the same range as the parent drugs (K_i = 89 and 46 nM for **22** and **37**, respectively). Rigidification of the piperidine nucleus in azabicyclo-[3.2.1]octane derivatives led to a decrease in affinity for D$_{4.2}$ sites (K_i = 115 and 131 nM for **23** and **38**, respectively).

N-Aminoalkylamine Derivatives (Table 5). Generally, these molecules (**25−28**, **39−43**) bearing a more flexible side chain, e.g., 1-piperidinylethylamine, 4-morpholinylethylamine, 2-pyridylethylamine, 2-dimethylaminoethylamine, showed a very low D$_{4.2}$ affinity (K_i > 1000 nM). **24** with a 1-pyrrolidinylethylamine showed a weak affinity for this site (K_i = 341 nM).

Influence of the Nature of the Basic Side Chain on the Affinity at 5-HT$_{2A}$ Receptors. Piperazine Derivatives (Table 3). The *N*-phenyl analogues (**15−17**, **30−32**) did not show any affinity for this site (K_i > 1000 nM). The presence and the nature of a substituent on the phenyl ring had no detectable influence. In the homologous series (benzyl to phenethyl analogues), it was found that only the phenethyl derivatives possessed significant 5-HT$_{2A}$ affinity (K_i = 103 and 36 nM for **19** and **34**, respectively). The *N*-cyclohexyl substituent was well tolerated, and these analogues showed similar affinity than the lead compounds (K_i = 34 and 33 nM for **20** and **35**, respectively). Homologation of the piperazine nucleus led to reductions in the affinity which was more pronounced in the case of the 8-methyl derivative (K_i = 164 and 303 nM for **29** and **44**, respectively).

4-Aminopiperidine Derivatives (Table 4). *N*-Methyl derivatives had four times less affinity (K_i = 123 and 132 nM for **21** and **36**) than *N*-methylpiperazine analogues (K_i = 35 and 37 nM for **8** and **9**, respectively). *N*-Benzyl compounds possessed intermediate affinities (K_i = 81 and 48 nM for **22** and **37**, respectively). They were quite similar to that of the lead compound (**9**). Azabicyclo[3.2.1]octane analogues had similar 5-HT$_{2A}$ affinity than *N*-methylpiperazine congeners (K_i = 36

Table 3. In Vitro Affinities and Binding Ratios of 8-Chloro- and 8-Methylpyridobenzodiazepine Analogues with a N-Substituted Piperazine Side Chain on $D_{4.2}$, D_{2L}, and 5-HT_{2A} Receptors, and α_1-Adrenoceptors[a]

Compounds	Ring	Amine	Substituent (R)	$D_{4.2}$	D_{2L}	5-HT_{2A}	α_1	$5\text{-HT}_2/D_2$	D_2/D_4
15	8-Cl	—N(piperazine)N—R	phenyl	>1,000	-	>1,000	>1,000		
30	8-CH$_3$			>1,000	-	>1,000	>1,000		
16	8-Cl	idem	(Cl-phenyl)	>1,000	-	>1,000	-		
31	8-CH$_3$			>1,000	-	>1,000	>1,000		
17	8-Cl	idem	(CF$_3$-phenyl)	>1,000	-	>1,000	>1,000		
32	8-CH$_3$			>1,000	-	>1,000	>1,000		
20	8-Cl	idem	(cyclohexyl)	48 ± 2	571 ± 37	34 ± 3	-	1.24	19
35	8-CH$_3$			84 ± 6	-	33 ± 10	-		
18	8-Cl	idem	(benzyl)	109 ± 30	9,943 (1)	>1,000	>1,000		91
33	8-CH$_3$			183 ± 97	-	>1,000	>1,000		
19	8-Cl	idem	(phenethyl)	40 ± 3	892 ± 52	103 ± 42	367 ± 71	1.16	22
34	8-CH$_3$			37 ± 1	635 ± 8	36 ± 2	172 ± 43	1.20	17
29	8-Cl	—N(homopiperazine)N—CH$_3$		163 ± 1	19,117±1,490	164 ± 57	>1,000	1.44	117
44	8-CH$_3$			356 ± 9	-	303 ± 77	-		
Clozapine (**7**)				18 ± 0.4	372 ± 100	6.7 ± 0.7	14 ± 4	1.27	21
Haloperidol (**45**)				2.2 ± 1	8.5 ± 0.5	49 ± 19	13 ± 4	0.91	4
8				24 ± 6	2,235 ± 1,413	35 ± 10	79 ± 11	1.32	93
9				36 ± 3	5,940 ± 240	37 ± 6	108 ± 30	1.42	165

[a] K_i in nM; mean ± SD; $n = 2$ if unspecified. $5\text{-HT}_2/D_2$ ratios were calculated by using 5-HT_{2A} and D_{2L} pK_i ($-\log K_i$) values according to Meltzer's paper.[19] D_2/D_4 ratios were estimated from K_i values.

Table 4. In Vitro Affinities and Binding Ratios of 8-Chloro- and 8-Methylpyridobenzodiazepine Analogues with a N-Substituted 4-Aminopiperidine Side Chain on $D_{4.2}$, D_{2L}, and 5-HT_{2A} Receptors, and α_1-Adrenoceptors[a]

Compounds	Ring	Amine	$D_{4.2}$	D_{2L}	5-HT_{2A}	α_1	$5\text{-HT}_2/D_2$	D_2/D_4
21	8-Cl	HN—(piperidine)—N—CH$_3$	393 ± 31	1,099 ± 18	123 ± 38	-	1.20	4.5
36	8-CH$_3$		510 ± 54	2,596 ± 200	132 ± 45	18 ± 4	1.23	5
22	8-Cl	HN—(piperidine)—N—benzyl	89 ± 6	183 ± 35	81 ± 14	163 ± 7	1.05	2.4
37	8-CH$_3$		46 ± 19	183 ± 0.7	48 ± 10	100 ± 7	1.09	4
23	8-Cl	HN—(bicyclic)—N—benzyl	115 ± 24	136 ± 6	36 ± 10	-	1.11	1.9
38	8-CH$_3$		131 ± 11	149 ± 12	34 ± 10	-	1.13	1.8
Clozapine (**7**)			18 ± 0.4	372 ± 100	6.7 ± 0.7	14 ± 4	1.27	21
Haloperidol (**45**)			2.2 ± 1	8.5 ± 0.5	49 ± 19	13 ± 4	0.91	4
8			24 ± 6	2,235 ± 1,413	35 ± 10	79 ± 11	1.32	93
9			36 ± 3	5,940 ± 240	37 ± 6	108 ± 30	1.42	165

[a] K_i in nM; mean ± SD; $n = 2$ if unspecified. $5\text{-HT}_2/D_2$ ratios were calculated by using 5-HT_{2A} and D_{2L} pK_i ($-\log K_i$) values according to Meltzer's paper.[19] D_2/D_4 ratios were estimated with K_i values.
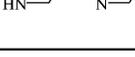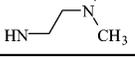
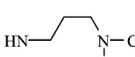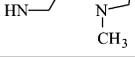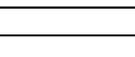and 34 nM, respectively, for **23** and **38** compared to 35 and 37 nM for **8** and **9**).

N-Aminoalkylamine Derivatives (Table 5). Some derivatives showed significant affinity at these receptors. The presence of dimethylaminoethylamine ($K_i = 150$ nM for **41**), 1-pyrrolidinylethylamine ($K_i = 250$ nM for **24**), and 1-piperidinylethylamine ($K_i = 357$ for

25) side chains seems most favorable for interaction with the 5-HT_{2A} receptors.

Influence of the Nature of the Basic Side Chain on the Affinity at D_{2L} Receptors. Piperazine Derivatives (Table 3). N-Methylpiperazine compounds did not show significant affinity for this site ($K_i = 2235$ and 5940 nM for **8** and **9**, respectively). Due to the

Table 5. In Vitro Affinities and Binding Ratios of 8-Chloro- and 8-Methylpyridobenzodiazepine Analogues with a Flexible Substituted Alkylamine Side Chain on $D_{4.2}$, D_{2L}, and 5-HT$_{2A}$ Receptors, and α_1-Adrenoceptors[a]

Compounds	Ring	Amine	$D_{4.2}$	D_{2L}	5-HT$_{2A}$	α_1	5-HT$_2$/D$_2$	D$_2$/D$_4$
24	8-Cl	[HN–CH₂CH₂–pyrrolidine]	341	-	250 ± 45	-		
25	8-Cl	[HN–CH₂CH₂–piperidine]	±1,000	-	357 ± 80	63 ± 8		
26	8-Cl	[HN–CH₂CH₂–morpholine]	>1,000	-	-	>1,000		
39	8-CH$_3$		>1,000	-	>1,000	-		
27	8-Cl	[HN–CH₂CH₂–pyridyl]	>1,000	-	-	>1,000		
40	8-CH$_3$		>1,000	-	-	-		
41	8-CH$_3$	[HN–CH₂CH₂–N(CH₃)₂]	>1,000	-	150 ± 23	>1,000		
28	8-Cl	[HN–CH₂CH₂–N(CH₃)–CH₃]	>1,000	-	>1,000	-		
42	8-CH$_3$		>1,000	-	>1,000	>1,000		
43	8-CH$_3$	[HN–CH₂–pyrrolidine–N–CH₃]	>1,000	-	>1,000	-		
Clozapine (7)			18 ± 0.4	372 ± 100	6.7 ± 0.7	14 ± 4	1.27	21
Haloperidol (45)			2.2 ± 1	8.5 ± 0.5	49 ± 19	13 ± 4	0.91	4
8			24 ± 6	2,235 ± 1,413	35 ± 10	79 ± 11	1.32	93
9			36 ± 3	5,940 ± 240	37 ± 6	108 ± 30	1.42	165

[a] K_i in nM; mean ± SD; $n = 2$ if unspecified. 5-HT$_2$/D$_2$ ratios were calculated by using 5-HT$_{2A}$ and D$_{2L}$ pK_i ($-\log K_i$) values according to Meltzer's paper.[19] D$_2$/D$_4$ ratios were estimated with K_i values.

inactivity of *N*-substituted aromatic analogues at D$_{4.2}$ receptors, their affinity at D$_{2L}$ site was not determined.

N-Cyclohexyl compound had a weak affinity ($K_i = 571$ nM for **20**) but approximately four times higher than the corresponding *N*-methyl derivative ($K_i = 2235$ nM for **8**). The *N*-benzyl derivative possessed lower affinity ($K_i = 9,943$ nM for **18**) while the *N*-phenethyl analogue had higher affinity for D$_{2L}$ receptors than *N*-methyl compounds ($K_i = 892$ and 635 nM for **19** and **34**, respectively).

Homologation of the piperazine was not favorable as found with **29** ($K_i > 10000$ nM).

4-Aminopiperidine Derivatives (Table 4). *N*-Methyl analogues presented low affinity at this site ($K_i = 1099$ and 2596 nM for **21** and **36**, respectively) but approximately two times higher than *N*-methylpiperazine analogues ($K_i = 2235$ and 5940 nM for **8** and **9**, respectively). Especially, the *N*-benzyl substitution strongly increased binding ($K_i = 183$ nM for **22** and **37**). These molecules had 10–20 times higher affinity in comparison with the *N*-methylpiperazine compounds ($K_i = 2235$ and 5940 nM for **8** and **9**, respectively). Azabicyclo[3.2.1]octane analogues possessed affinities ($K_i = 136$ and 149 nM for **23** and **38**, respectively) close to those of *N*-benzylpiperidine derivatives ($K_i = 183$ nM for **22** and **37**).

Influence of the Nature of the Basic Side Chain on the Affinity at α_1-Adrenoceptors. Piperazine Derivatives (Table 3). *N*-Methyl congeners ($K_i = 79$ and 108 nM for **8** and **9**, respectively) possessed less

adrenergic affinity than clozapine ($K_i = 14$ nM) and haloperidol (**45**) ($K_i = 13$ nM). *N*-Substitution of the piperazine by an aromatic ring appeared to be unfavorable for α_1 affinity; these analogues (**15**, **17**, **30**–**32**) had very weak affinity ($K_i > 1000$ nM). Homologation of the side chain was favorable as found with a phenethyl side chain ($K_i = 367$ and 172 nM for **19** and **34**, respectively).

4-Aminopiperidine Derivatives (Table 4). *N*-Benzylpiperidine analogues possessed similar affinities ($K_i = 163$ and 100 nM for **22** and **37**, respectively) to *N*-methylpiperazine compounds ($K_i = 79$ and 108 nM for **8** and **9**, respectively). The *N*-methylpiperidine derivative in the 8-methyl series (**36**) had affinity close to clozapine and haloperidol ($K_i = 18$ nM).

N-Aminoalkylamine Derivatives (Table 5). Introduction of dimethylaminoethyl- (**41**) and dimethyl-(aminopropyl)amino (**42**) side chain led to a complete loss of affinity for this site ($K_i > 1000$ nM). Likewise, 4-morpholinoethyl- (**26**) and 2-pyridylethylamine (**27**) analogues also did not possess affinity for the α_1-adrenoceptor ($K_i > 1000$ nM). The 1-piperidinylethyl derivative (**25**), on the other hand, had a significant affinity ($K_i = 63$ nM).

Discussion

The present paper is, to our knowledge, the first examining the impact of a large modulation of the basic side chain for the binding affinity to D$_{4.2}$, 5-HT$_{2A}$, and D$_{2L}$ receptors. The work of Phillips et al. reported the influence of the replacement of the methyl group by

either an ethyl or 2-propenyl moiety. Other manuscripts as mentioned in the Introduction detailed the consequence of a modulation on the distal nitrogen in the context of an antidopaminergic D_2 or anticholinergic potential.[59,62–66] For a long time, the distal nitrogen of the piperazine and the proximal benzene ring have been considered as pharmacophoric elements.[42] Generally, such modifications led to reduced interaction with dopamine D_2 receptors.

In our previous work, we detailed the evolution of the binding profile of various pyridobenzazepine derivatives in comparison with their benzene analogues.[45,46] These original molecules presented lower affinity at four receptors investigated (D_2, D_1, 5-HT_{2A}, M) but some of them presented a promising neuroleptic profile in behavioral models[48] while two pyridobenzothiazepine derivatives showed a disinhibitory/antidepressant profile in mice and rats.[67] Electrophysiological studies confirmed such antidepressant potential.[68,69]

Keeping the piperazine template, we observed that aromatic substitution of the distal nitrogen (phenyl, 3-chlorophenyl, or 3-trifluoromethylphenyl) (**15**–**17**, **30**–**32**) totally suppressed the affinity at the four receptors while the substitution by a cyclohexyl ring retained some affinity (Table 3). As mentioned above, the basicity of this nitrogen is frequently emphasized, and in the case of N-phenylpiperazine analogues this basicity is highly reduced by the electron-withdrawing potential of a phenyl ring. Sterical hindrance does not seem the most critical aspect since the N-cyclohexyl derivatives (**20**, **35**) showed similar affinity for $D_{4.2}$ and 5-HT_{2A} but even higher affinity for D_{2L} receptors compared to the N-methylpiperazine compound (**8**, **9**). Classical homologation from the phenyl to the phenethyl substituent (**19**, **34**) through a benzyl moiety (**18**, **33**) indicated that the latter provided molecules with significant affinities for the $D_{4.2}$ and 5-HT_{2A} receptor close to those of the N-methyl analogues but with significantly increased D_{2L} receptor affinity even though this affinity remained low in comparison with haloperidol. The phenethyl group could interact in some hydrophobic pocket of the receptor, stabilizing the drug–receptor complex and thus increasing the affinity. A similar observation can be made when we observe the increased affinity of the N-benzylpiperidine analogues (**22**, **37**) for D_{2L} receptor sites (Table 4). Indeed, the N-methylpiperidine derivatives (**21**, **36**) showed reduced affinity for $D_{4.2}$ and 5-HT_{2A} receptors compared to the N-methylpiperazine analogues (**8**, **9**). However such a basic side chain bearing a N-benzyl group seems to be more favorable for D_{2L} interaction because an important increase in affinity for this site was observed. Regarding the structural requirements mentioned above, the impact of the higher distance of the distal nitrogen to the adjacent benzene ring (by adding one carbon atom compared to piperazine analogues) could be counterbalanced by the benzyl substituent which can interact in another part of the receptor and so increase the stability of the whole complex. Lipophilic parameter seems to have a lesser impact because N-methylpiperidine analogues (**21**, **36**) had only a 2-fold higher affinity for D_{2L} receptors compared to the N-methylpiperazine derivatives (**8**, **9**). Moreover, regarding the interaction with

$D_{4.2}$ and 5-HT_{2A} receptors, these molecules showed lower affinity compared to the N-methylpiperazine analogues.

Rigidification of the piperidine side chain in azabicyclo-[3.2.1]octane derivatives (**23**, **38**) led to a small reduction in $D_{4.2}$ affinity while the compounds presented higher affinity at 5-HT_{2A} and D_{2L} receptors compared to the piperidine derivatives (Table 4).

More flexible chains such as in dimethylaminoethylamine (**41**) or 1-piperidinylethylamine (**25**), in which conformational freedom is higher, were found to be unfavorable for D_{2L}, $D_{4.2}$, and 5-HT_{2A} receptor binding (Table 5). Finally, homologation of the piperazine nucleus (**29**, **44**) led to reduced affinity for all tested receptors (Table 3).

When examining binding to α_1-adrenoceptors, similar conclusion can be drawn. Nevertheless, the 4-amino-1-methylpiperidine (**21**, **36**) (Table 4) and the 1-piperidinylethylamine (**25**) (Table 5) analogues presented higher affinity compared to the N-methylpiperazine derivatives.

The HT_2 and D_4 selectivity of these new compounds was estimated when possible (Tables 3–5) to evaluate the impact of such chemical modulations in comparison with the N-methyl analogues (**8**, **9**). Regarding the 5-HT_2/D_2 ratios, some remarks should be made since methodologies are quite different. Indeed, in Meltzer's paper[19] and in our previous manuscript,[45,46] native D_2 receptors were used while cloned D_{2L} receptors are used in the present one. Nevertheless, for reference substances, similar tendencies were found. Clozapine (**7**) and haloperidol (**45**) presented a ratio of 1.27 and 0.91, respectively, while the original paper reported values of 1.19 and 0.86.[19] In our previous manuscript, we reported similar ratios of 1.15 and 0.84.[45] Variations in binding data reported in the literature are frequently observed but the 5-HT_2/D_2 ratio remains in the same range. Although D_{2L} binding affinities of new pyridobenzodiazepines were increased in some cases, the 5-HT_2/D_2 ratio was generally closer to that of clozapine rather than haloperidol. However, the 4-amino-1-benzylpiperidine derivatives (**22**, **37**) or the 8-benzyl-8-azabicyclo[3.2.1]octane analogues (**23**, **38**) due to a higher D_{2L} binding had a lower ratio near to that of haloperidol (**45**). Thus, the impact of these modulations on 5-HT_{2A} selectivity was relatively limited. This ratio has been widely used during the past decade to develop new atypical antipsychotic. In fact, the original idea was to detect putative antipsychotic drugs with low potential to induce EPS. Effectively, in our previous work although several molecules possessed low affinity for receptors tested, their 5-HT_2/D_2 ratios were superior to the limit of 1.12 originally defined and effectively they showed reduced potential to induce motor disturbances. Those with a ratio near 1.12 such as **8** and **9** had some antagonist properties against apomorphine-induced stereotypies.[45] Otherwise, two pyridobenzothiazepine derivatives possessing a high 5-HT_2/D_2 ratio did not induce motor side effects and did not antagonize apomorphine-induced stereotypies[46] but further evaluation revealed an antidepressant potential.[68,69] Therefore, this ratio should be used as an indication of a potential to induce EPS rather than as a prediction of an atypical antipsychotic profile. This point was raised by Meltzer et al. when they mentioned: "It may also be the 5-HT_2/D_2

ratios is relevant only to EPS and TD liability and not to increased antipsychotic efficacy".[19]

When examining the D_2/D_4 ratio, *N*-methylpiperazine analogues (**8**, **9**) showed higher D_4 selectivity in comparison with clozapine. This is also previously reported using rat native D_2 receptors.[47] Consequently, **9** was selected as a pharmacological tool for exploring the role of D_4 receptors.[70–72] Despite extensive investigations, the role of these receptor sites remains virtually unclear.[38,39] By modulating the basic side chain, the selectivity of newer compounds for D_4 over D_2 receptors decreased because D_4 affinities was lower than those of *N*-methylpiperazine derivatives (**8**, **9**). The impact observed on this ratio was mainly due to an increased affinity at D_{2L} receptors.

The neurochemical definition of the atypicality remains unclear. Seeman et al. reported that "Atypical neuroleptics have low affinity for dopamine D_2 receptors or are selective for D_4 receptors".[73] The low D_2 affinity of atypical compounds is clearly a discriminant feature as it appeared in the original paper of Meltzer's team.[19] Clinically, compounds possessing a high 5-HT$_{2A}$ potential and a high antidopaminergic D_2 potential, such as risperidone (**1**) or olanzapine (**3**), have an interesting profile to treat schizophrenia, but increasing the dose generally leads to the classical dopaminergic syndrome (extrapyramidal side effects, hyperprolactinemia). At low doses, risperidone or olanzapine occupy minimal percentage of central D_2 sites (50–75%) but sufficiently to improve positive manifestations. Higher doses lead to higher occupancy (>80%) and are responsible for extrapyramidal manifestations. The question of receptor occupancies remain a hot topic so far.

To evaluate the impact of the present chemical modifications on the binding profile, crystallographic studies were carried out (Table 6). It is clearly shown that structural features proposed as crucial elements in drug–receptor interaction (basic nitrogen, distance between this atom and the adjacent benzene ring, d_1 and d_2) may be influenced by the nature of the lateral side chain. When examining the 3D-structure of compounds **25** (R1 = 1-piperidinylethylamine) and **28** (R1 = dimethylaminopropylamine) it is observed that the higher flexibility of the side chain led to a reduction of the distance between the distal nitrogen and the adjacent benzene ring in comparison with isoclozapine (**46**), a *N*-methylpiperazine derivative (5.803, 4.754, and 5.948 Å for **25**, **28**, and **46**, respectively). Increasing the size of the carbon chain between the two nitrogens (ethyl to propyl) leads to a more folded structure as found in the crystal.[61] A significant difference between *N*-methylpiperazine compounds and the newly synthesized compounds is the value of the dihedral angle between the planes of the benzene rings (defined as the obtuse angle sustended by the plane normals). For *N*-methylpiperazine derivatives such as clozapine (**7**),[74] loxapine (**47**),[74] clothiapine (**48**),[75] this angle is inferior to 120° with a sequence as followed: NH > O > S. In pyridine isosters such as 5-(4-methyl-1-piperazinyl)-pyrido[2,3-*b*][1,5]benzothiazepine (**49**)[76] and 6-(4-methyl-1-piperazinyl)-11*H*-pyrido[4,3-*b*][1,4]benzothiazepine (**50**),[77] or the pyridobenzoxazepine (**11**) (as free base), the sequence is approximately similar. In the presence of longer and more flexible side chain, a higher value of

Table 6. Structural Features of Different Diarylazepine Analogues Determined from X-ray Crystallography Measurement
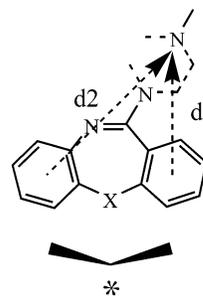
compounds	central atom (X)	dihedral angle (deg)*	d_1 (Å)	d_2 (Å)
clozapine (**7**)[a]	NH	115.0	5.972	7.716
isoclozapine (**46**)[a]	NH	117.6	5.948	7.775
loxapine (**47**)[a]	O	114.0	6.188	7.731
clothiapine (**48**)[b]	S	104.6	6.098	7.737
49[c]	S	110.6	5.988	7.770
50[d]	S	110.5	6.056	7.726
11[e]	O	114.0	6.154	7.758
11[f]	O	122.4	5.958	7.544
17	NH	126.9	6.128	7.640
20	NH	123.3	5.990	7.760
25	NH	127.9	5.803	7.692
28[g]	NH	134.6	4.754	7.669

[a] After Petcher et al. (1976).[74] [b] After Sbit et al. (1987).[75] [c] After Sbit et al. (1988).[76] [d] After Dupont et al. (1991).[77] [e] As fumarate. [f] As free base. [g] After Dupont et al. (2002).[61]

Table 7. Effects of Compound (**19**) in the Paw Test in Rats and Porsolt's Test in Mice

compounds	paw test (FRT–HRT)[a]	Porsolt's test[b]
saline	1.00–2.50	–
19	1.00–2.50	52% (4)[d]
		36% (8)[d]
		49% (16)[d]
clozapine (**7**)	1.10–30[c]	95% (4)
		103% (8)
		139% (16)[d]
haloperidol (**45**)	22.5[c]–30[c]	–
imipramine	–	98% (7.5)
		45% (15)[d]
		70% (30)[d]

[a] HRT: hindlimb retraction time, FRT: forelimb retraction time, at the highest effective dose, in seconds. [b] Percentages of the total immobility time measured with the vehicle; the dose in mg/kg, i.p., is presented in parentheses. [c] Significant differences from saline using Mann–Whitney *U* test. [d] Significant differences from vehicle determined by analysis of variance followed by appropriate post hoc tests when significant.

this angle is observed. This is particularly marked with the dimethylaminopropyl derivative (**28**) in which the dihedral angle is 134.6° (Table 6). Accordingly, the *N*-piperonyl analogue of clozapine reported in the literature also presented an angle of 120.73° superior to the 115° observed in clozapine.[65]

To explore the in vivo impact of the present chemical modifications, **19** presenting a high affinity for D_4 and 5-HT$_2$ receptors (K_i = 40 and 103 nM, respectively) was tested in behavioral models. In the paw test in rats,[78] **19** did not significantly modify both hindlimb and forelimb retraction time up to 20 mg/kg (Table 7). Clozapine was found to selectively increase hindlimb retraction time at doses of 5 mg/kg i.p. and higher.[51] For quetiapine, similar effects appeared at higher doses.[79] In this test, haloperidol produced significant

increase of both hindlimb and forelimb retraction time. It appeared that this drug might induce few extrapyramidal side effects. Otherwise, we were interested in testing this drug in another screening procedure. In our previous work, using the Porsolt's test,[80] two pyridobenzothiazepine derivatives showed an antidepressant potential. Indeed, in the forced swimming test in mice, **19** at 4−8−16 mg/kg i.p. induced a significant decrease in the immobility time (Table 7). Clozapine (4−8−16 mg/kg) significantly increased the immobility time at the higher dose while imipramine (7.5−15−30 mg/kg) produced a significant decrease in the immobility time (Table 7).[67] The profile of **19** is interesting and suggest further investigations. As mentioned above, tricyclic compounds constitute a pharmacological crossroad between several therapeutic drug families (antipsychotic, antidepressant). Such profile can be questionable but when examining the paper defining the 5-HT$_2$/D$_2$ ratio one can see that different tricyclic compounds were misclassified.[19] This was particularly true with loxapine and amoxapine which were classified as atypical drugs. Although it was used as an antidepressant drug for a very long time, amoxapine can be identified as a 5-HT$_2$/D$_2$ receptor antagonist.[81] Recently, the drug showed antipsychotic-like effects without catalepsy in animal models[82] and a PET study indicated that it possessed a 5-HT$_2$ over D$_2$ receptor occupancy profile very similar to established atypical antipsychotics.[83]

It thus appears that further behavioral testing of **19** or others with significant affinity will be particularly interesting since schizophrenia or some other psychiatric illnesses are complex pathologies with many possible causes and etiologies. Otherwise, the present structure−activity relationship studies will aid the development of new drug candidates, as subtle variations in the binding profile for the different receptors have been detected. Further studies should be directed toward improving the selectivity of such molecules for different receptors.

Experimental Section

All compounds were characterized by physical methods using FTIR (Perkin-Elmer model 297 spectrophotometer) and ^{1}H-NMR (Bruker AW 250 spectrometer with HMDS as the internal standard). Flash chromatography was carried out using Merck silica gel (Kieselgel 60, 230−400 mesh). Elemental analyses were performed in our laboratory on a Carlo Erba CHNS-O EA1108 Elemental Analyzer and were within 0.4% of theoretical values. Melting points were determined with a Tottoli (Buchi) melting point apparatus in open capillary tubes and are uncorrected.

The different amines were commercially available except three molecules. Indeed, 4-amino-1-methylpiperidine was prepared from 1-methyl-4-piperidone converted to oxime and subsequently reduced with sodium in pentanol according to a procedure reported in the literature.[84] 3-β-Amino-8-benzyl-8-azabicyclo[3.2.1]octane was synthesized following a Robinson−Schöpf cyclization giving 8-benzyl-8-azabicyclo[3.2.1]octan-3-one which is converted to oxime and finally reduced with sodium in pentanol to give the 3-β-amino analogue.[85] *N*-Phenethylpiperazine was synthesized from *N*-ethoxycarbonylpiperazine and 2-phenethyl bromide followed by decarbamoylation.[62] After extraction, these crude amines were purified by distillation under reduced pressure.

The syntheses of 8-chloro-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-benzodiazepin-6-one (**13**) and 8-methyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (**14**) were described in a previous paper.[45]

General Procedure for Amidine Synthesis: 8-Chloro-6-(4-phenyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (15). To a mixture of 0.01 mol of 8-chloro-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one, *N*-phenylpiperazine (10 mL, 0.09 mol), and anhydrous toluene (20 mL) was added dropwise a solution of titanium tetrachloride (1.2 mL) in anisole (5 mL). The mixture was heated to 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 10 min and filtered. The solid was washed with CHCl$_3$. The combined organic layers were extracted with 2 N HCl (4 × 200 mL), which was made basic with 30% aqueous ammonia solution and extracted with CHCl$_3$ (4 × 150 mL). The organic layer, dried over MgSO$_4$, was evaporated under reduced pressure. The residue was recrystallized from CH$_2$Cl$_2$/hexane mixture to afford compound **15** as yellow crystalline solid. When necessary a second crystallization was performed: yield 30%; mp 185−186 °C; ^{1}H NMR (CDCl$_3$) δ 3.30 (br s, 4H), 3.62 (br s, 4H), 6.37 (s, 1H), 6.65−6.99 (m, 5H), 7.26−7.39 (m, 5H), 7.83−7.85 (d, 1H). Anal. (C$_{22}$H$_{20}$N$_5$Cl) C, H, N.

8-Chloro-6-(4-(3-chlorophenyl)-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (16). ^{1}H NMR (CDCl$_3$) δ 3.43 (s, 4H), 3.73 (s, 4H), 6.11 (s, 1H), 6.96−7.01 (m, 3H), 7.06 (s, 1H), 7.07−7.11 (dd, 1H), 7.10−7.35 (t, 1H), 7.40−7.44 (m, 2H), 7.49−7.51 (d, 1H), 7.97−7.98 (d, 1H); yield 40% (hexane); mp 189−192 °C; Anal. (C$_{22}$H$_{19}$N$_5$Cl$_2$) C, H, N.

8-Chloro-6-(4-(3-trifluoromethylphenyl)-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (17). ^{1}H NMR (CDCl$_3$) δ 3.34 (br s, 4H), 3.62 (br s, 4H), 6.12 (s, 1H), 6.86 (d, 1H), 6.95 (dd, 1H), 7.11−7.15 (m, 3H), 7.26−7.40 (m, 4H), 7.84 (dd, 1H); yield 20% (CH$_2$Cl$_2$/hexane); mp 157−159 °C; Anal. (C$_{23}$H$_{19}$N$_5$ClF$_3$) C, H, N.

8-Chloro-6-(4-benzyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (18). ^{1}H NMR (CDCl$_3$) δ 2.55 (br s, 4H), 3.47 (br s, 4H), 3.58 (s, 2H), 5.90 (s, 1H), 6.82−6.85 (d, 1H), 6.90−6.95 (dd, 1H), 7.24−7.34 (m, 8H), 7.78−7.81 (dd, 1H); yield 20% (CH$_2$Cl$_2$); mp 235−237 °C; Anal. (C$_{23}$H$_{22}$N$_5$Cl) C, H, N.

8-Chloro-6-(4-phenethyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (19). ^{1}H NMR (CDCl$_3$) δ 2.64−2.71 (m, 6H), 2.82−2.88 (m, 2H), 3.51 (br s, 4H), 6.26 (s, 1H), 6.83−6.85 (d, 1H), 6.90−6.95 (dd, 1H), 7.20−7.36 (m, 8H), 7.81−7.83 (dd, 1H); yield 25% (CH$_2$Cl$_2$/hexane); mp 190−192 °C; Anal. (C$_{24}$H$_{24}$N$_5$Cl) C, H, N.

8-Chloro-6-(4-cyclohexyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (20). ^{1}H NMR (CDCl$_3$) δ 1.17−1.24 (m, 4H), 1.62−1.98 (m, 6H), 2.31 (br m, 1H), 2.66 (br s, 4H), 3.46 (br s, 4H), 6.01 (s, 1H), 6.82−6.85 (dd, 1H), 6.89−6.94 (dd, 1H), 7.24−7.28 (m, 2H), 7.30−7.34 (dd, 1H), 7.78−7.81 (dd, 1H); yield 10% (CH$_2$Cl$_2$/hexane); mp 192−194 °C; Anal. (C$_{22}$H$_{26}$N$_5$Cl) C, H, N.

8-Chloro-6-((1-methyl-4-piperidinyl)amino)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (21). ^{1}H NMR (CDCl$_3$) δ 2.07−2.10 (d, 2H), 2.67−2.70 (m, 4H), 2.80 (s, 3H), 3.30−3.33 (d, 2H), 4.54 (m, 1H), 5.09−5.11 (d, 1H), 6.69 (s, 1H), 7.25−7.28 (d, 1H), 7.38−7.43 (dd, 1H), 7.70−7.81 (m, 3H), 8.25−8.27 (dd, 1H); yield 25% (CH$_2$Cl$_2$/hexane); mp 193−195 °C; Anal. (C$_{18}$H$_{20}$N$_5$Cl) C, H, N.

8-Chloro-6-((1-benzyl-4-piperidinyl)amino)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (22). ^{1}H NMR (CDCl$_3$) δ 1.52−1.64 (m, 2H), 2.16−2.26 (m, 4H), 2.84−2.88 (m, 2H), 3.54 (s, 2H), 4.04−4.08 (m, 1H), 4.58−4.61 (d, 1H), 6.25 (s, 1H), 6.75−6.78 (d, 1H), 6.68−6.93 (dd, 1H), 7.21−7.34 (m, 8H), 7.76−7.77 (dd, 1H); yield 30% (CH$_2$Cl$_2$/hexane); mp 163−164 °C; Anal. (C$_{24}$H$_{24}$N$_5$Cl) C, H, N.

8-Chloro-6-((8-benzyl-3β-tropanyl)amino)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (23). ^{1}H NMR (CDCl$_3$) δ 1.59−1.67 (m, 2H), 1.77−1.83 (m, 2H), 2.08 (br s, 4H), 3.28 (s, 2H), 3.58 (s, 2H), 4.40−4.49 (m, 1H), 4.55−4.58 (d, 1H), 6.52 (s, 1H), 6.74−6.77 (d, 1H), 6.88−6.93 (dd, 1H), 7.18−7.42 (m, 8H), 7.75−7.78 (dd, 1H); yield 20% (CH$_2$Cl$_2$/hexane); mp 185−186 °C; Anal. (C$_{26}$H$_{26}$N$_5$Cl) C, H, N.

8-Chloro-6-(2-(1-pyrrolidinyl)ethylamino)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (24). ^{1}H NMR (CDCl$_3$) δ 1.79−

1.81 (m, 4H), 2.57 (m, 4H), 2.74–2.79 (t, 2H), 3.59 (m, 2H), 5.56 (br m, 1H), 6.17 (s, 1H), 6.74–6.77 (d, 1H), 6.87–6.92 (dd, 1H), 7.20–7.24 (dd, 1H), 7.26–7.33 (m, 2H), 7.74–7.77 (dd, 1H); yield 25% (CH$_2$Cl$_2$/hexane/MeOH); mp 142–143 °C; Anal. (C$_{18}$H$_{20}$N$_5$Cl) C, H, N.

8-Chloro-6-(2-(1-piperidinyl)ethylamino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (25). ^{1}H NMR (CDCl$_3$) δ 1.59 (br s, 2H), 1.87–1.89 (m, 5H), 2.97 (br s, 4H), 3.14 (m, 2H), 3.84–3.86 (m, 2H), 5.98 (br s, 1H), 6.74–6.77 (d, 1H), 6.87–6.92 (dd, 1H), 7.24–7.26 (m, 2H), 7.48 (s, 1H), 7.76–7.78 (d, 1H); yield 65% (CH$_2$Cl$_2$/hexane/MeOH); mp 172–174 °C; Anal. (C$_{19}$H$_{22}$N$_5$-Cl) C, H, N.

8-Chloro-6-(2-(4-morpholinyl)ethylamino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (26). ^{1}H NMR (CDCl$_3$) δ 2.69–2.71 (t, 4H), 2.83–2.86 (t, 2H), 3.76–3.80 (m, 2H), 3.90–3.92 (m, 4H), 5.56 (br s, 1H), 6.07 (s, 1H), 6.94–6.96 (d, 1H), 7.07–7.10 (dd, 1H), 7.43–7.50 (m, 3H), 7.94–7.95 (dd, 1H); yield 50% (CH$_2$Cl$_2$/hexane/MeOH); mp 155–156 °C; Anal. (C$_{18}$H$_{20}$N$_5$-OCl) C, H, N.

8-Chloro-6-(2-(2-pyridyl)ethylamino)-11H-pyrido[2,3-b][1,4]benzodiazepine (27). ^{1}H NMR (CDCl$_3$) δ 3.33–3.36 (t, 2H), 4.05–4.09 (m, 2H), 6.02 (s, 1H), 6.20 (s, 1H), 6.91–6.93 (d, 1H), 7.06–7.09 (dd, 1H), 7.35–7.50 (m, 5H), 7.80–7.82 (dd, 1H), 7.92–7.94 (dd, 1H), 8.74–8.75 (d, 1H); yield 45% (ethyl acetate/hexane/MeOH); mp 161–162 °C; Anal. (C$_{19}$H$_{16}$N$_5$-Cl) C, H, N.

8-Chloro-6-(3-dimethyl(aminopropyl)amino)-11H-pyrido[2,3-b][1,4]benzodiazepine (28). ^{1}H NMR (CDCl$_3$) δ 1.78–1.81 (m, 2H), 2.25 (s, 6H), 2.47–2.50 (m, 2H), 3.56–3.60 (m, 2H), 5.89 (s, 1H), 6.71–6.74 (d, 1H), 6.85–6.89 (dd, 1H), 7.19–7.21 (dd, 1H), 7.24–7.29 (m, 3H), 7.71–7.73 (dd, 1H); yield 10% (CH$_2$Cl$_2$/hexane); mp 138–139 °C; Anal. (C$_{17}$H$_{20}$N$_5$-Cl) C, H, N.

8-Chloro-6-(4-methyl-1-homopiperazinyl)-11H-pyrido-[2,3-b][1,4]benzodiazepine (29). ^{1}H NMR (CDCl$_3$) δ 1.91 (br s, 2H), 2.33 (s, 3H), 2.58–2.62 (m, 4H), 3.62 (br s, 4H), 6.83–6.88 (m, 3H), 7.15–7.18 (m, 2H), 7.25–7.28 (dd, 1H), 7.72–7.74 (dd, 1H); yield 60% (CH$_2$Cl$_2$/hexane); mp 139–141 °C; Anal. (C$_{18}$H$_{20}$N$_5$Cl) C, H, N.

8-Methyl-6-(4-phenyl-1-piperazinyl)-11H-pyrido[2,3-b]-[1,4]benzodiazepine (30). ^{1}H NMR (CDCl$_3$) δ 2.28 (s, 3H), 3.30 (br s, 4H), 3.65 (br s, 4H), 6.09 (s, 1H), 6.82–7.00 (m, 5H), 7.15 (mm, 2H), 7.27–7.38 (m, 3H), 7.82 (dd, 1H); yield 45% (CH$_2$Cl$_2$/hexane); mp 155–157 °C; Anal. (C$_{23}$H$_{23}$N$_5$) C, H, N.

8-Methyl-6-(4-(3-chlorophenyl)-1-piperazinyl)-11H-pyrido[2,3-b][1,4]benzodiazepine (31). ^{1}H NMR (CDCl$_3$) δ 2.27 (s, 3H), 3.28 (br s, 4H), 3.61 (br s, 4H), 6.06 (s, 1H), 6.80–6.93 (m, 5H), 7.11–7.21 (m, 3H), 7.34 (dd, 1H), 7.81 (dd, 1H); yield 15% (CH$_2$Cl$_2$/hexane); mp 159–161 °C; Anal. (C$_{23}$H$_{22}$N$_5$Cl) C, H, N.

8-Methyl-6-(4-(3-trifluoromethylphenyl)-1-piperazinyl)-11H-pyrido[2,3-b][1,4]benzodiazepine (32). ^{1}H NMR (CDCl$_3$) δ 2.28 (s, 3H), 3.33 (br s, 4H), 3.64 (br s, 4H), 5.92 (s, 1H), 6.80–6.84 (d, 1H), 6.89–6.94 (dd, 1H), 7.10–7.13 (m, 5H), 7.33–7.40 (m, 2H), 7.80–7.82 (dd, 1H); yield 10% (hexane); mp 144–145 °C; Anal. (C$_{24}$H$_{22}$N$_5$F$_3$) C, H, N.

8-Methyl-6-(4-benzyl-1-piperazinyl)-11H-pyrido[2,3-b]-[1,4]benzodiazepine (33). ^{1}H NMR (CDCl$_3$) δ 2.26 (s, 3H), 2.55 (br s, 4H), 3.50 (br s, 4H), 3.57 (s, 2H), 5.84 (s, 1H), 6.78–6.81 (d, 1H), 6.86–6.91 (dd, 1H), 7.07–7.12 (m, 2H), 7.26–7.35 (m, 6H), 7.77–7.79 (dd, 1H); yield 15% (CH$_2$Cl$_2$/hexane); mp 223–224 °C; Anal. (C$_{24}$H$_{25}$N$_5$) C, H, N.

8-Methyl-6-(4-phenethyl-1-piperazinyl)-11H-pyrido-[2,3-b][1,4]benzodiazepine (34). ^{1}H NMR (CDCl$_3$) δ 2.28 (s, 3H), 2.64–2.70 (m, 6H), 2.82–2.88 (m, 2H), 3.53 (br s, 4H), 5.96 (s, 1H), 6.79–6.83 (d, 1H), 6.87–6.92 (dd, 1H), 7.09–7.13 (m, 2H), 7.20–7.33 (m, 6H), 7.78–7.80 (dd, 1H); yield 40% (CH$_2$Cl$_2$/hexane); mp 158–159 °C; Anal. (C$_{25}$H$_{27}$N$_5$) C, H, N.

8-Methyl-6-(4-cyclohexyl-1-piperazinyl)-11H-pyrido-[2,3-b][1,4]benzodiazepine (35). ^{1}H NMR (CDCl$_3$) δ 1.15–1.22 (m, 4H), 1.60–1.89 (m, 6H), 2.24 (m, 4H), 2.64 (br s, 4H), 3.47 (br s, 4H), 6.10 (s, 1H), 6.77–6.88 (d, 1H), 6.85–6.88 (dd, 1H), 7.05–7.09 (m, 2H), 7.27–7.30 (dd, 1H), 7.75–7.77 (dd,

1H); yield 35% (CH$_2$Cl$_2$/hexane); mp 173–174 °C; Anal. (C$_{23}$H$_{29}$N$_5$) C, H, N.

8-Methyl-6-((1-methyl-4-piperidinyl)amino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (36). ^{1}H NMR (CDCl$_3$) δ 1.61 (m, 2H), 2.16 (br s, 4H), 2.25 (s, 3H), 2.30 (s, 3H), 2.82 (d, 2H), 4.05 (m, 1H), 4.61 (d, 1H), 6.01 (s, 1H), 6.71 (d, 1H), 6.85 (dd, 1H), 7.06 (bs, 2H), 7.26 (dd, 1H), 7.81 (dd, 1H); yield 50% (CH$_2$-Cl$_2$/hexane); mp 181–182 °C; Anal. (C$_{19}$H$_{23}$N$_5$) C, H, N.

8-Methyl-6-((1-benzyl-4-piperidinyl)amino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (37). ^{1}H NMR (CDCl$_3$) δ 1.58–1.62 (m, 2H), 2.19–2.23 (m, 4H), 2.25 (s, 3H), 2.84–2.87 (m, 2H), 3.55 (s, 2H), 4.10–4.13 (s, 1H), 4.68–4.70 (d, 1H), 6.34 (s, 1H), 6.73–6.76 (d, 1H), 6.85–6.90 (dd, 1H), 7.06–7.09 (m, 2H), 7.27–7.35 (m, 6H), 7.75–7.77 (d, 1H); yield 40% (CH$_2$-Cl$_2$/hexane); mp 174–176 °C; Anal. (C$_{25}$H$_{27}$N$_5$) C, H, N.

8-Methyl-6-((8-benzyl-3β-tropanyl)amino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (38). ^{1}H NMR (CDCl$_3$) δ 1.59–1.68 (t, 2H), 1.79–1.84 (m, 2H), 2.09–2.16 (m, 4H), 2.25 (s, 3H), 3.29 (s, 2H), 3.58 (s, 2H), 4.46–4.48 (m, 1H), 4.58–4.61 (d, 1H), 6.14 (s, 1H), 6.71–6.74 (d, 1H), 6.85–6.90 (dd, 1H), 7.08 (m, 2H), 7.25–7.42 (m, 6H), 7.73–7.76 (dd, 1H); yield 25% (CH$_2$Cl$_2$/hexane); mp 146–147 °C; Anal. (C$_{27}$H$_{29}$N$_5$) C, H, N.

8-Methyl-6-(2-(4-morpholinyl)ethylamino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (39). ^{1}H NMR (CDCl$_3$) δ 2.26 (s, 3H), 2.50–2.52 (m, 4H), 2.64–2.67 (m, 2H), 3.58–3.62 (m, 2H), 3.69–3.72 (m, 4H), 5.40 (br s, 1H), 5.77 (s, 1H), 6.71–6.73 (m, 1H), 6.84–6.87 (m, 1H), 7.10–7.11 (m, 2H), 7.26–7.28 (m, 1H), 7.72–7.73 (dd, 1H); yield 15% (ethyl acetate/hexane); mp 158–159 °C; Anal. (C$_{19}$H$_{23}$N$_5$O) C, H, N.

8-Methyl-6-(2-(2-pyridyl)ethylamino)-11H-pyrido[2,3-b][1,4]benzodiazepine fumarate (40). ^{1}H NMR (CDCl$_3$/DMSO-d_6) δ 2.19 (s, 3H), 3.10–3.16 (t, 2H), 3.71–3.76 (t, 2H), 6.62 (s, 2H), 6.77–6.82 (dd, 1H), 6.89–6.92 (m, 1H), 7.04–7.06 (m, 2H), 7.14–7.17 (m, 2H), 7.26–7.29 (d, 1H), 7.36 (s, 1H), 7.61–7.68 (m, 2H), 8.47–8.49 (dd, 1H), 9.75 (br s, 2H); yield 45% (ethyl acetate/MeOH); mp 190–193 °C; Anal. (C$_{24}$H$_{23}$N$_5$O$_4$) C, H, N.

8-Methyl-6-(2-dimethylaminoethylamino)-11H-pyrido-[2,3-b][1,4]benzodiazepine (41). ^{1}H NMR (CDCl$_3$) δ 2.23 (s, 3H), 2.27 (s, 6H), 2.58 (t, 2H), 3.58 (m, 2H), 5.53 (br s, 1H), 6.06 (s, 1H), 6.70–6.74 (d, 1H), 6.83–6.88 (dd, 1H), 7.06–7.09 (d, 1H), 7.12 (s, 1H), 7.29–7.31 (d, 1H), 7.72–7.73 (dd, 1H); yield 15% (ethyl acetate); mp 188–189 °C; Anal. (C$_{17}$H$_{21}$N$_5$) C, H, N.

8-Methyl-6-(3-dimethyl(aminopropyl)amino)-11H-pyrido[2,3-b][1,4]benzodiazepine (42). ^{1}H NMR (CDCl$_3$) δ 1.78–1.82 (m, 2H), 2.22 (br s, 9H), 2.43–2.45 (m, 2H), 3.57 (m, 2H), 6.24 (br s, 1H), 6.69–6.72 (d, 1H), 6.81–6.86 (m, 2H), 7.02–7.09 (m, 2H), 7.26–7.29 (dd, 1H), 7.70–7.72 (dd, 1H); yield 35% (CH$_2$Cl$_2$/hexane); mp 122–124 °C; Anal. (C$_{18}$H$_{23}$N$_5$) C, H, N.

8-Methyl-6-(2-(1-methyl-2-pyrrolidinyl)ethylamino)-11H-pyrido[2,3-b][1,4]benzodiazepine (43). ^{1}H NMR (CDCl$_3$) δ 1.70–1.98 (m, 6H), 2.11–2.22 (m, 1H), 2.25 (s, 3H), 2.35 (m, 4H), 2.99–3.05 (m, 1H), 3.48–3.56 (m, 1H), 3.64–3.74 (m, 1H), 5.84 (s, 1H), 6.56 (br s, 1H), 6.70–6.73 (d, 1H), 6.84–6.89 (dd, 1H), 7.07–7.09 (m, 2H), 7.26–7.31 (dd, 1H), 7.71–7.74 (dd, 1H); yield 25% (CH$_2$Cl$_2$/hexane); mp 147–148 °C; Anal. (C$_{20}$H$_{25}$N$_5$) C, H, N.

8-Methyl-6-(4-methyl-1-homopiperazinyl)-11H-pyrido-[2,3-b][1,4]benzodiazepine (44). ^{1}H NMR (CDCl$_3$) δ 1.95 (br s, 2H), 2.24 (s, 3H), 2.38 (s, 3H), 2.66 (m, 4H), 3.68 (br s, 4H), 5.93 (s, 1H), 6.80–6.88 (m, 2H), 7.03–7.09 (m, 2H), 7.25–7.28 (dd, 1H), 7.71–7.73 (dd, 1H); yield 15% (CH$_2$Cl$_2$/hexane); mp 141–143 °C; Anal. (C$_{19}$H$_{23}$N$_5$) C, H, N.

In Vitro Receptor Binding Studies. Dopamine D$_{4.2}$ Receptors Binding. Sf9 cell membrane preparations expressing the human cloned receptors were purchased from NEN Life Science Products (CRM-016). Briefly, incubations were carried out in 50 mM Tris-HCl buffer at pH 7.4 containing 5 mM MgCl$_2$, 5 mM EDTA, 5 mM KCl, and 1.5 mM CaCl$_2$.

Binding assays were performed in 540 μL total volume divided into 500 μL of diluted membranes, 20 μL of radioligand [^{3}H]nemonapride (0.06 nM), and 20 μL of buffer or unlabeled

ligand. Incubations were run for 60 min at 27 °C and terminated by dilution with 1 mL ice-cold 50 mM Tris-HCl buffer, pH 7.4 followed by rapid filtration on Whatman GF/C filters and two washes with the same buffer. Non specific binding was estimated in the presence of 10 μM clozapine. Results obtained with reference compounds such as clozapine or haloperidol were in the same range as those mentioned by the manufacturer. Generally, affinities were determined in duplicate (six to nine concentrations in duplicate). A preliminary screening was performed at 10^{-6} and 10^{-7} M to select the most active compounds for further evaluation.

Dopamine D_{2L} Receptors Binding. Sf9 cell membrane preparations containing the human cloned receptor were purchased from NEN Life Science Products (CRM-002). Briefly, incubations were carried out in 50 mM Tris-HCl buffer at pH 7.4 containing 10 mM $MgCl_2$, and 1 mM EDTA.

Binding assays were performed in 540 μL total volume divided in 500 μL of diluted membranes, 20 μL of radioligand ([^{3}H]spiperone (0.2 nM)), and 20 μL of buffer or unlabeled ligand. Incubations were run for 60 min at 27 °C and terminated by dilution with 1 mL ice-cold 50 mM Tris-HCl buffer, pH 7.4, followed by rapid filtration on Whatman GF/C filters and two washes with the same buffer. Nonspecific binding was estimated in the presence of 10 μM haloperidol. Results obtained with reference compounds such as clozapine or haloperidol were in the same range as those mentioned by the manufacturer. Generally, affinities were determined in duplicate for compounds which present significant affinity determined in a preliminary screening at 10^{-6} and 10^{-7} M.

Serotonin $5-HT_{2A}$ Receptors Binding. The binding assay was performed as previously described[86] with slight modifications.[45,46] The main change was the preparation of batches of rat cortical membrane kept at -80 °C before use. Generally, such preparation is used within two weeks. Briefly, female Wistar rats were used throughout this study. After decapitation, brains ($n = 20-25$) were quickly removed and kept on ice during dissection. Frontal cortical tissues were isolated, weighed, and homogenized in 10 volumes 0.25 M sucrose. After centrifugation at 1000g for 10 min at 4 °C, the supernatant is kept and the pellet is rehomogenized in the same volume of sucrose. After centrifugation, both supernatants were mixed and diluted with 40 volumes 50 mM Tris buffer, pH 7.7. The suspension is centrifugated at 30000g for 10 min at 4 °C. The pellet is rehomogenized and centrifugated again. Finally, the pellet is resuspended in 5 volumes of Tris buffer, aliquoted and frozen at -80 °C before use. Final protein concentration in incubation medium is 2 mg/mL. Incubation volume is 540 μL corresponding to 500 μL of membrane preparation, 20 μL of radioligand ([^{3}H]ketanserin at 1 nM), and 20 μL of buffer, nonspecific ligand (methysergide at 10 μM) or drugs. After 30 min at 37 °C, suspension were diluted with 1 mL of ice-cold buffer and quickly filtered on Whatman GF/C presoaked with 0.3% aqueous polyethyleneimine. Filters were washed by 2 $\times$ 1 mL ice-cold buffer and immediately placed in a vial containing scintillation liquid. Generally, affinities were determined at least in duplicate for compounds which present significant affinity determined in a preliminary screening at 10^{-6} and 10^{-7} M. For each curve, nine concentrations in triplicate were used.

α_1-Adrenoceptors Binding. The procedure followed is taken after Skarsfeldt and Hyttel.[87] Briefly, female Wistar rats were used throughout this study. After decapitation, brains were quickly removed and kept on ice during dissection. Cortical tissues were isolated and homogenized in 25 volumes 50 mM Tris-HCl, pH 7.7. After centrifugation at 20000g for 10 min at 4 °C, the pellet is homogenized in 25 volume buffer. After centrifugation, the final pellet is diluted in 85 volumes buffer. Incubation volume is 2 mL corresponding to 200 μL of buffer containing drugs or nonspecific ligand, 100 μL of radioligand ([^{3}H]prazosine at 0.25 nM), and finally 1700 μL of membrane preparation. Incubation is performed at 25 °C for 20 min. Nonspecific binding was determined in the presence of chlorpromazine (1 μM). After period of incubation, the suspension was filtrated on Whatman GF/C filter and washed with 2 $\times$ 5 mL ice-cold buffer. Each drug presenting a

significant affinity (determined after a preliminary screening at 10^{-6} and 10^{-7} M) is tested at least in duplicate. For a curve, eight concentrations in triplicate were used.

Evaluation of the Radioactivity. For each binding procedures, immediately after washing, filters were placed in vial containing 5 mL of Ecoscint A to evaluate radioactivity after one night. Specific binding was defined as the difference between total and nonspecific binding (with and without [^{3}H]-drug). K_i values were calculated according to the Cheng−Prusoff equation:[88] $K_i = IC_{50}/(1 + L/K_d)$ with L the concentration and K_d the apparent dissociation constant of the [^{3}H] ligand obtained from Scatchard analysis of saturation experiments or mentioned by the manucfacturer for the cloned receptor preparation. Each K_i value was determined at least in duplicate. The pK_i ($-\log K_i$) values were used to calculate $5-HT_2/D_2$ binding ratios.

Psychopharmacological Evaluation. Paw Test in Rats. Experiments were performed following the classical method previously described.[75]

Forced Swimming Test in Mice. Experiments were performed following the previously described procedure.[79]

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