New Pyridobenzodiazepine Derivatives as Potential Antipsychotics: Synthesis and Neurochemical Study

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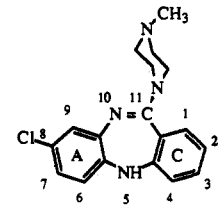
The discovery of a new, safe, atypical antipsychotic remains an important challenge. To achieve this goal, a series of *N*-methylpiperazinopyrido[2,3-*b*][1,4]- and -[1,5]- and -pyrido[4,3-*b*][1,4]- and -[1,5]-benzodiazepines were synthesized. The dopaminergic (D_1, D_2), serotonergic (5-HT_2), and cholinergic (M) affinities, frequently remarked in the action mechanisms of antipsychotic drugs, were determined using their respective *in vitro* receptor binding assays. All affinities were reduced for each compound. Optimal substituents were found to be in the 2- or 8-position for the retention of affinities, while substitution at the 5-position by acyl or alkyl groups dramatically diminished binding affinities. Pyridobenzodiazepine derivatives, such as clozapine, were found to be inactive or only weakly effective against apomorphine-mediated stereotypes in rats. In an original and complex behavioral model developed in dogs and successfully used to differentiate distinct classes of psychotropic drugs and to discriminate between typical and atypical neuroleptic drugs, 8-chloro-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (**9**), 8-methyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (**12**), and 5-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,5]benzodiazepine (**16**) showed most of the behavioral characteristics previously described for neuroleptics. Their neurochemical profiles, particularly their 5-HT_2/D_2 pK_i ratios, were compatible with an atypical antipsychotic effect. These compounds were selected for further investigation. The proposed modulations could lead to new possibilities for the pharmacochemistry of diarylazepines.

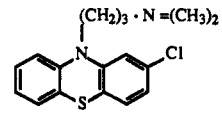
Introduction

Among neuroleptic drugs, several molecules have contradicted the traditional concept defined by Delay and Deniker.[1] One of these, clozapine (**1**), a dibenzodiazepine derivative, was found to be very active against psychotic symptoms with reduced extrapyramidal side effects (EPS)[2-4] and to have an interesting effect on negative symptoms[5] which are poorly treated by classical neuroleptics, such as chlorpromazine (**2**) and haloperidol (**3**).[6] Clozapine was found to be effective in 30% of treatment-resistant schizophrenics whereas chlorpromazine is effective in only 4% of these cases.[5] Clozapine thus represented a great advance in the treatment of psychosis, but its use was hampered by serious side effects like seizures,[7] sialorrhea,[5,8] orthostatic hypotension,[5] and particularly a 1-2% incidence of agranulocytosis.[9-11]

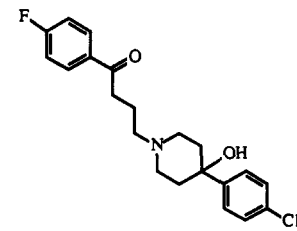
The action mechanisms of clozapine have not been completely elucidated. It has been shown that clozapine presented a great affinity for serotonin (5-HT_2) and acetylcholine (M) receptors while being a weaker antidopaminergic agent.[12,13] For a long time, the dopamine/acetylcholine balance hypothesis was the predominant etiological theory for drug-induced EPS.[12,14-17] Nevertheless, the 5-HT_2 receptor blockade seemed also to be implicated in the atypical antipsychotic activity[18,19] while it counteracted some effects of the D2 receptor blockade.[20] Recently, this 5-HT_2/D_2 ratio concept has been widely developed.[13]



clozapine (**1**)



chlorpromazine (**2**)



haloperidol (**3**)

Other receptors could play a role in determining the atypical profile. Clozapine interacts with other binding sites such as 5-HT_3,[21] 5-HT_{1c},[22] and D_4 receptors;[23] a new dopamine receptor subtype has recently been cloned. Moreover, it must be mentioned that the interactions of antipsychotic drugs with currently undefined receptor systems may play a part in their atypical profile.[24] For example, CI-943, which has an effect on animal behavior and an electrophysiological profile consistent with an antipsychotic drug, has not been shown to bind to any known receptor.[25]

Despite the extensive effort to find a safer drug,[14,15,26-32] no alternative to clozapine has been identified which would have clinical antipsychotic efficacy without EPS and with

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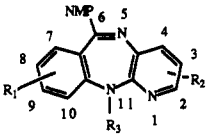
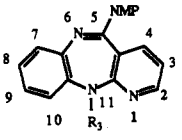
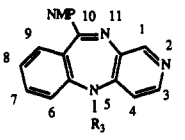
Table I. *N*-Methylpiperazinopyrido[1,4]- and *N*-Methylpiperazinopyrido[1,5]benzodiazepines



8-15



16-17



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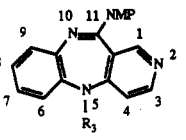


19-20

	R_1	R_2	R_3	formula	anal.	mp, °C	% yield
8	H	H	H	$C_{17}H_{19}N_5$	C, H, N	141	75
9	8-Cl	H	H	$C_{17}H_{18}ClN_5$	C, H, N	180	70
10	9-Cl	H	H	$C_{17}H_{18}ClN_5$	C, H, N	186	80
11	8-F	H	H	$C_{17}H_{18}FN_5$	C, H, N	188	75
12	8-CH_3	H	H	$C_{18}H_{21}N_5$	C, H, N	157	65
13	H	3-CH_3	H	$C_{18}H_{21}N_5$	C, H, N	198	70
14	H	H	CH=O	$C_{18}H_{19}N_5O$	C, H, N	202	85
15	H	H	CH_3	$C_{18}H_{21}N_5$	C, H, N	146	50
16			H	$C_{17}H_{19}N_5$	C, H, N	146	85
17			CH=O	$C_{18}H_{19}N_5O$	C, H, N	194	90
18			H	$C_{17}H_{19}N_5$	C, H, N	193	35
19			H	$C_{17}H_{19}N_5$	C, H, N	216	60
20			CH=O	$C_{18}H_{19}N_5O$	C, H, N	196	80

a low risk of inducing any other serious toxic effect.[24] So there remains a need for improved antipsychotic agents. While clozapine is known to be a weaker dopaminergic agent, most research, in order to find a successor to clozapine with a similar profile, on the basis of *in vitro* binding and classical pharmacological tests, generally retained compounds with a high D_2 antagonism potency. It is therefore probable that the procedures used discarded several molecules or structures which might have presented interesting therapeutical profiles. Thus, much effort has been and is continuing to be expended in the search for ways of attenuating the dopaminergic function and yet retaining or improving clinical efficacy. This paradox led us to synthesize and evaluate a series of *N*-methylpiperazinopyrido[1,4]- and -pyrido[1,5]benzodiazepine derivatives using *in vitro* and *in vivo* models. In our exploratory study, each series of reference, 2- or 8-substituted, dibenzoazepines was modified in order to examine the evolution of their neurochemical and psychopharmacological profiles.

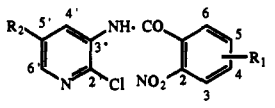
Their ability to interact with dopaminergic (D_2 and D_1), serotonergic (5-HT_2), and muscarinic (M) receptors, frequently remarked in the action mechanisms of antipsychotic drugs, was evaluated. Compounds were studied using the antagonism of apomorphine-mediated stereotypy test to evaluate their *in vivo* dopaminergic potential. An open-field test in rats and a complex operant-conditioning schedule in dogs, successfully developed in previous studies to reveal a neuroleptic profile[33] and to discriminate between acutely[33] or chronically[34] administered typical and atypical antipsychotic drugs, were used to test the new synthesized pyridobenzodiazepine analogues.[35]

Chemistry

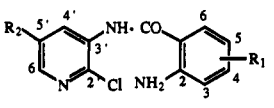
The modifications of the tricyclic structure were deliberately limited. For instance, the *N*-methylpiperazine side chain was retained in all molecules, whereas the lateral benzene rings were alternatively replaced by a pyridine, which determined the preparation of two series of derivatives: *N*-methylpiperazinopyrido[1,4]- and -pyrido[1,5]benzodiazepines (Table I).

The diazepine compounds (**8–13**, **16**, and **19**) were generally prepared from lactams (**23a–23f**, **24**, and **25**) by a modified Fryer amidine synthesis.[36] The diazepinones were obtained by different synthetic pathways which are summarized below.

Table II. Pyridonitrobenzamides and Pyridoanthranilamides



(21a-21f)



(22a-22f)

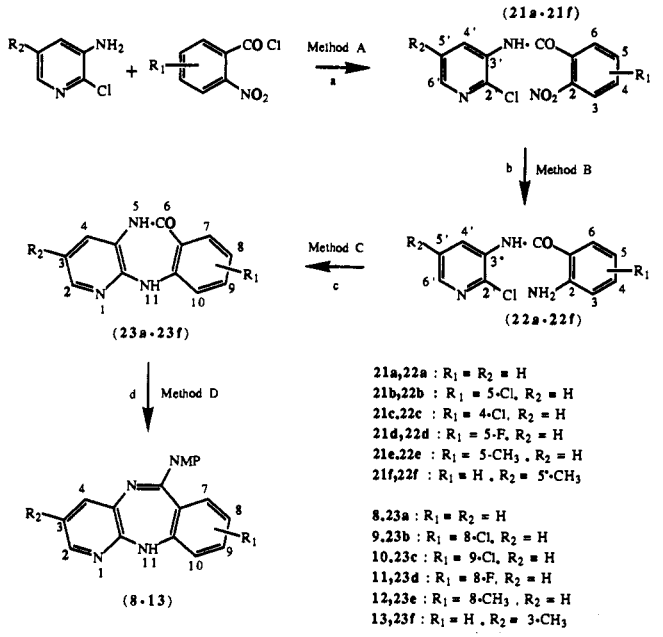
	R_1	R_2	formula	anal	mp, °C	% yield
21a	H	H	$C_{12}H_8ClN_3O_3$	C, H, N	152	85
21b	5-Cl	H	$C_{12}H_7Cl_2N_3O_3$	C, H, N	190	75
21c	4-Cl	H	$C_{12}H_7Cl_2N_3O_3$	C, H, N	193	70
21d	5-F	H	$C_{12}H_7ClFN_3O_3$	C, H, N	149	80
21e	5-CH_3	H	$C_{13}H_{10}ClN_3O_3$	C, H, N	155	77
21f	H	5′-CH_3	$C_{13}H_{10}ClN_3O_3$	C, H, N	169	80
22a	H	H	$C_{12}H_{10}ClN_3O$	C, H, N	175	86
22b	5-Cl	H	$C_{12}H_9Cl_2N_3O$	C, H, N	194	85
22c	4-Cl	H	$C_{12}H_9Cl_2N_3O$	C, H, N	195	75
22d	5-F	H	$C_{12}H_9ClFN_3O$	C, H, N	168	85
22e	5-CH_3	H	$C_{13}H_{12}ClN_3O$	C, H, N	188	75
22f	H	5′-CH_3	$C_{13}H_{12}ClN_3O$	C, H, N	174	80

11*H*-Pyrido[2,3-*b*][1,4]benzodiazepine Derivatives. The preparation of these rings has been widely investigated in pirenzepine chemistry.[37–40]

Nitrobenzamide derivatives **21a–21f** (Table II) were prepared by the reaction of the appropriate 2-nitrobenzoic acid chloride with 3-amino-2-chloropyridine (method A) (Scheme I). The different nitrobenzoic acids were commercially available except for the 5-fluoro-2-nitro analogue, which was synthesized according to the Slothouwer method.[41] The nitro group was reduced using an acidic stannous chloride mixture (method B) to give anthranilamide analogues **22a–22f** (Table II). The closure achieved by nucleophilic substitution at the 2′-position upon heating in diethylene glycol monomethyl ether (DEGMME) (method C) produced the lactam derivatives **23a–23f** in appreciable yields (Table III). The diazepinones were heated with an excess of *N*-methylpiperazine and titanium tetrachloride (method D), in refluxing toluene, to give the *N*-methylpiperazinopyrido[2,3-*b*][1,4]benzodiazepines **8–13** (Table I).

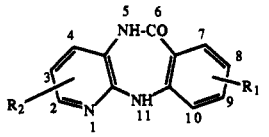
11*H*-Pyrido[2,3-*b*]- and 5*H*-Pyrido[4,3-*b*][1,5]benzodiazepine Derivatives. Two compounds of this series were prepared (Scheme II) from the benzodiazepinones **24** and **25**, synthesized according to the method of Hoffmann and Faure.[42] Excess of *N*-methylpiperazine, titanium tetrachloride, and **24** or **25** in refluxing toluene

Scheme I[a]



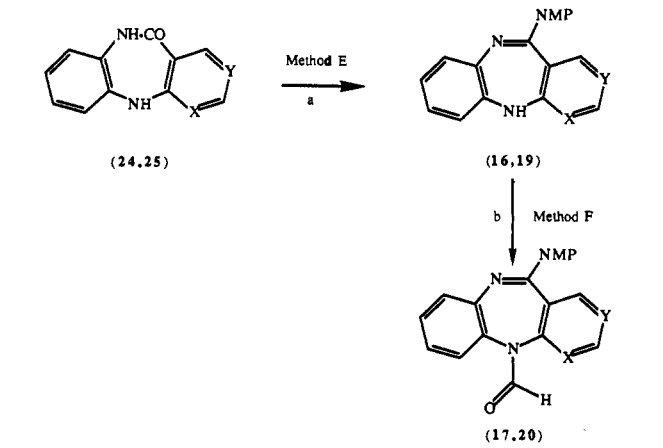
[a] Key: (a) dioxane, pyridine; (b) $SnCl_2$, HCl; (c) DEGMME, Δt; (d) NMP, $TiCl_4$, toluene. NMP: *N*-methylpiperazine.

Table III. Pyrido[2,3-*b*][1,4]benzodiazepinones



	R_1	R_2	formula	anal	mp, °C	% yield
23a	H	H	$C_{12}H_9N_3O$	C, H, N	283	60
23b	8-Cl	H	$C_{12}H_8ClN_3O$	C, H, N	296	84
23c	9-Cl	H	$C_{12}H_8ClN_3O$	C, H, N	>350	68
23d	8-F	H	$C_{12}H_8FN_3O$	C, H, N	270	80
23e	8-CH_3	H	$C_{18}H_{11}N_3O$	C, H, N	258	65
23f	H	3-CH_3	$C_{18}H_{11}N_3O$	C, H, N	264	55

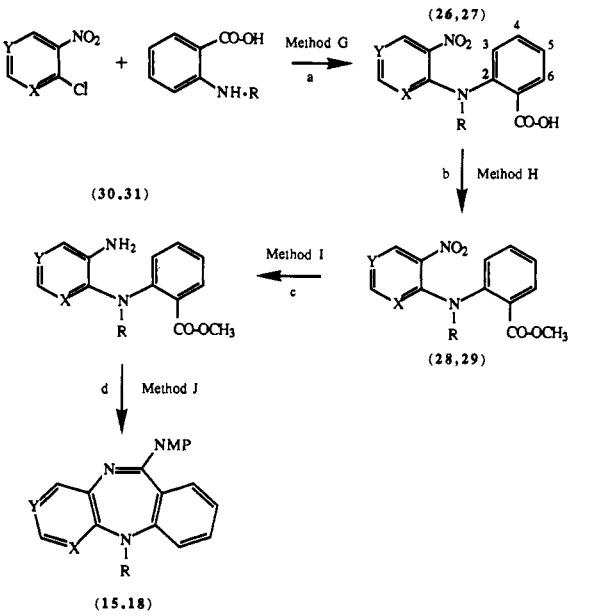
Scheme II[a]



[a] Key: (a) NMP, $TiCl_4$, toluene; (b) HCOOH, $(CH_3CO)_2O$. **24, 16, 17**: X = N, Y = CH. **25, 19, 20**: X = CH, Y = N.

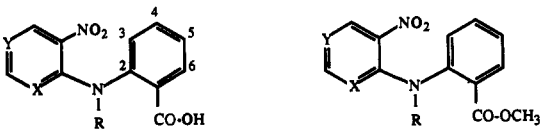
(method E) gave the *N*-methylpiperazino-11*H*-pyrido[2,3-*b*]- (**16**) and -5*H*-pyrido[4,3-*b*][1,5]benzodiazepines (**19**) (Table I). (**16**) was previously described by Chakrabarti et al.[14] as an inactive compound mainly on account of its weak D_2 affinity. 4-Chloronicotinic acid was synthesized according to the method of Taylor and Crovetti,[43] as modified by Delarge.[44]

Scheme III[a]



[a] Key: (a) K_2CO_3, 2-propanol, Δt; (b) CH_2N_2; (c) 10% Pd/C, H_2, EtOAc, 50 psi; (d) NMP, anisole, $TiCl_4$. **26, 28, 30, 15**: X = N, Y = CH, R = CH_3. **27, 29, 31, 18**: X = CH, Y = N, R = H.

Table IV. Pyridonitro Acids and Pyridonitro Esters



	X	Y	R	formula	anal	mp, °C	% yield
26	N	CH	CH_3	$C_{13}H_{11}N_3O_4$	C, H, N	174	70
27	CH	N	H	$C_{12}H_9N_3O_4$	C, H, N	288	65
28	N	CH	CH_3	$C_{14}H_{13}N_3O_4$	C, H, N	71	95
29	CH	N	H	$C_{13}H_{11}N_3O_4$	C, H, N	180	70

11-Formylpyrido[2,3-*b*][1,4]benzodiazepines, 11-Formylpyrido[2,3-*b*][1,5]benzodiazepines, and 5-Formylpyrido[4,3-*b*][1,5]benzodiazepines. Surprisingly, it was not possible to obtain the 11-N-alkyl analogues using a reductive acylation procedure.[45] However, the acylation of some diazepines (**8, 16, 19**) using a formic acid/acetic anhydride mixture (Scheme II, method F) provided *N*-formyl analogues **14, 17,** and **20** (Table I). The structure of **17** was confirmed by X-ray crystallography.[46]

11-Methylpyrido[2,3-*b*][1,4]benzodiazepines and 5*H*-pyrido[4,3-*b*][1,4]benzodiazepines. The 11-methyl derivative **15** was prepared following the method illustrated in Scheme III. The appropriate orthohalogenonitropyridine reacted with *N*-methylanthranilic acid in the presence of potassium carbonate, in refluxing 2-propanol (method G), to provide the *N*-methyl-*N*-(3-nitro-2-pyridinyl)anthranilic acid (**26**) (Table IV). Esterification by diazomethane provided **28** (method H). The nitro group was then hydrogenated by using 10% Pd/C as catalyst in ethyl acetate to give methyl *N*-methyl-*N*-(3-amino-2-pyridinyl)anthranilate (**30**) (method I). The reaction of the crude amino ester with an excess of *N*-methylpiperazine and titanium tetrachloride in a refluxing toluene–anisole mixture furnished the corresponding diazepine **15** (method J). **18** was prepared following the same synthetic pathway.

Table V. Neurochemical Data: Binding Affinities and Ratio Values and Pharmacological Data of (*N*-Methylpiperazino)pyridobenzodiazepines and Reference Compounds

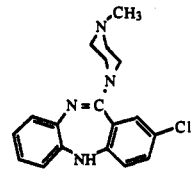
	D_2[a]	D_1[a]	5-HT_2[a]	M[a]	5-HT_2/D_2[b]	D_2/M[b]	D_2/D_1[b]	apomorphine antagonism[c]	
8	36.52	24.00	1.24	6.65	1.270	0.880	0.968	0	
9	1.18	21.4	0.062	0.65	1.183	0.964	1.039	75	
10	10.20	2.14	0.48	3.66	1.222	0.930	1.056	70	
11	15.50	6.77	1.24	4.15	1.189	0.911	0.942	55	
12	5.30	3.98	0.94	0.48	1.120	0.857	0.980	75	
13	2.97	11.35	1.03	5.23	1.070	1.040	1.098	NT	
14	1000	479	18.7	3.11	1.432	0.614	1.432	0	
15	271	60.2	29	1.75	1.212	0.676	0.875	0	
16	19.66	7.26	1.03	3.03	1.224	0.876	0.930	0	
17	1000	302	71.2	34.15	1.287	0.731	0.885	0	
18	39.7	135	8.37	4.30	1.126	0.848	1.109	NT	
19	4.42	3.02	0.29	32.6	1.186	1.157	0.974	45	
20	239	30.2	20.53	34.5	1.232	0.846	0.837	0	
clozapine (1)	0.45	1.15	0.038	0.25	1.146	0.966	1.058	0	I*
isoclozapine (4)	0.13	0.28	0.018	0.21	1.108	1.029	1.046	NT	1.7*
clothiapine (5)	0.044	0.14	0.006	1.36	1.105	1.215	1.064	100	0.72*
isoclothiapine (6)	0.30	2.40	0.041	0.056	1.114	0.905	1.136	NT	I*
fluperlapine (7)	1.33	1.41	0.038	0.25	1.222	0.961	1.004	NT	
chlorpromazine (2)	0.0201	0.35	0.033	0.607	0.975	1.205	1.168	NT	2.6*
haloperidol (3)	0.0088	0.76	0.234	74.66	0.842	1.766	1.272	100	0.14*

[a] K_i [10^{-7} M]. [b] From -log K_i. [c] % inhibition, by 20 mg/kg (s.c.) of drug except for compound 9 (10 mg/kg, s.c.), 1 h after apomorphine administration (2.5 mg/kg, s.c.). NT = not tested. * from ref 12, ED_{50} in mg/kg, s.c., I = inactive.

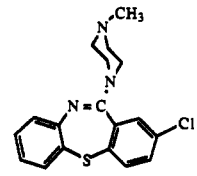
Results and Discussion[47]

According to the literature, the known pyridine analogues of clozapine appear to be weaker antidopaminergic agents.[14,48-49] Nevertheless, this characteristic may constitute an interesting possibility for the development of new atypical antipsychotics[24,50] since a high D_2 affinity also appears to be responsible for EPS.

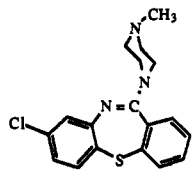
The newly synthesized drugs were tested *in vitro* for their ability to interact with the aforementioned receptors (D_1, D_2, M, 5-HT_2) (Table V) and were compared with relevant reference compounds (chlorpromazine, haloperidol, clozapine, isoclozapine (4), clothiapine (5), isoclothiapine (6), fluperlapine (7)). Moreover, different



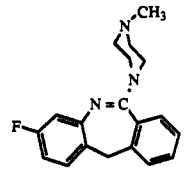
isoclozapine (4)



clothiapine (5)



isoclothiapine (6)



fluperlapine (7)

binding ratios, such as the 5-HT_2/D_2 binding ratio which seemed critical for the atypical profile,[13] were calculated.

(1) Evolution of Binding Affinities in the Dibenzoazepine Series. Results obtained for reference compounds including chlorpromazine and haloperidol were consistent with literature data.[13] Clozapine, fluperlapine, and isoclothiapine were less dopaminergic than their 2-substituted analogues. Clothiapine combined a high affinity for both 5-HT_2 and D_2 sites and presented a classical neuroleptic profile. For clozapine, isoclozapine, fluperlapine, and isoclothiapine, 5-HT_2 receptor affinities were similar. Tricyclic derivatives generally displayed strong muscarinic affinities, but, while clozapine and isoclozapine presented a similar muscarinic affinity, isoclothiapine presented a higher one than clothiapine.

(2) Influence of the Pyridine Ring on the Neurochemical Profile of the Pyrido[1,4]benzodiazepine Analogues. It appeared that the introduction of a pyridine ring reduced all the affinities. The presence of substituents at the 2- or 8-position seemed crucial for retaining at least some of these affinities. A chloro group (9) was more favorable than a methyl (12) or a fluoro (11) group. For these compounds, D_1 and D_2 affinities were reduced in a similar fashion. 13, with a methyl group at the 8-position, presented affinities similar to 12, but a lower muscarinic affinity was observed.

In this series, a 5-substitution by an acyl or an alkyl group (14, 15) dramatically decreased the affinity for each receptor type. A steric hindrance rather than an electronic effect appeared to be the cause since the influence of an alkyl (15) or an acyl (14) group was similar. However, 15 retained a muscarinic potential in the same range as other pyridobenzodiazepine analogues. This could be explained by the fact that this compound possesses some structural similarities with pirenzepine, a known anticholinergic drug. The replacement of the benzenic C–Cl moiety of clozapine by a pyridine nitrogen (18) reduced dopaminergic and serotonergic affinities more than muscarinic binding. The influence of a substituent at this position on the muscarinic potential has previously been noted.[51] The introduction of a pyridine nitrogen at the 6-position (8) or at the 8-position (18) produced very few differences. 18 was less active on D_1 and 5-HT_2 receptors.

(3) Influence of the Pyridine Ring on the Neurochemical Profile of the Pyrido[1,5]benzodiazepine Analogues. In this series, pyridinic analogues also presented fewer affinities for the receptors. 19, when compared with 16, displayed more affinity for dopaminergic and serotonergic receptors but had a lower muscarinic potential. The substitution at the 5-position by an acyl group (17, 20) was less favorable for dopaminergic and serotonergic affinities in the case of 17. 19 and its 5-formyl analogue 20 presented the same muscarinic potential. 16 and 19, when compared with their nonsubstituted ana-

logues in the [1,4] series 8 and 18, presented greater dopaminergic and serotonergic affinities. For muscarinic affinities a different evolution was observed. Pyrido[2,3-*b*] analogues 8 and 16 showed a similar activity while 19 was less potent than its isomer 18.

Binding affinities presented a similar evolution for at least three receptors (D_1, D_2, and 5-HT_2). The same structures could be implicated in ligand/receptor recognition. Our observation could be related to the similarity recently demonstrated between the interaction sites of D_1, D_2, and 5-HT_2 receptors.[52] A parameter such as lipophilicity could influence the binding interaction. In these series the reduction of certain affinities might be related to a lower lipophilicity, since pyridine markedly enhances hydrophilicity and polarity as seen with pirenzepine.[53]

(4) The Binding-Ratio Hypothesis and the Atypical Neuroleptic Profile. Although the 5-HT_2 system appears to be implicated in psychosis, there is no evidence that antipsychotic action can be mediated solely by a 5-HT_2 receptor blockade. Nevertheless, 5-HT_2 receptor antagonists reduce the catalepsy generated by neuroleptic treatment.[54,55] The recent hypothesis[13] which considers the 5-HT_2/D_2 ratio as a discriminant parameter for the classification of typical and atypical neuroleptics could constitute a criterion for the selection of new antipsychotic drugs. The appropriate combination of a 5-HT_2 and a D_2 antagonism apparently results in promising clinical properties which could not be achieved by existing neuroleptic medications in monotherapy.[56-58]

In our study the different reference compounds showed 5-HT_2/D_2 ratio values which agreed with those found by Meltzer *et al.*[13] (Table V). For chlorpromazine, haloperidol, isoclozapine, and clothiapine, considered as typical neuroleptics, the values were inferior to 1.12. For clozapine and fluperlapine, the ratio was superior to 1.12. Isoclothiapine was borderline. According to this critical ratio value most of our derivatives could be considered as atypical drugs.

The D_1 affinity also seems relevant for the achievement of the atypical profile.[59,60] It is conceivable that with clozapine and other mixed D_1/D_2 antagonists, the synergy known to exist between D_1 and D_2 receptors[61] might allow an antipsychotic response to be achieved below the threshold for extrapyramidal effects; however, the hypothesis of a D_2/D_1 binding ratio explaining the atypical neuroleptic profile has been contradicted.[13,62] D_2/D_1 ratios were presented as being less discriminant than 5-HT_2/D_2 values.[13] Our results (Table V) tended to confirm this conclusion since the calculated D_2/D_1 pK_i values occured in the same range for both typical and atypical reference compounds, while they were more markedly differentiated by the 5-HT_2/D_2 and D_2/M values.

In agreement with the dopamine/acetylcholine balance, although no significant correlation between these interactions was reported, typical antipsychotic drugs such as chlorpromazine and haloperidol presented a higher D_2/M ratio than atypical compounds and many pyridobenzodiazepine derivatives. Some compounds such as 9, 12, and 19, which showed a weak activity in the apomorphine-antagonism test, were characterized by a value more related to those of typical neuroleptic drugs. This fact could reinforce the role of the dopamine/acetylcholine hypothesis in the atypical neuroleptic profile, but, as mentioned above, 5-HT_2 antagonism might also reduce the effects of the D_2 blockade.

(5) *In Vivo* Pharmacological Studies. It is well known that one of the biggest problems in studying neuroleptics is the lack of a simple and adequate animal model capable of revealing the antipsychotic profile of drugs as well as discriminating between typical and atypical properties. In our study we used different *in vivo* models to test the newly synthesized pyridobenzodiazepine derivatives: apomorphine-mediated stereotypy antagonism (rat), open-field (rat), and a temporal conditioning schedule (dog). While the first two models are well-known pharmacological tests, the third is a newly-developed procedure which has been shown to be very effective not only in differentiating several classes of psychotropic drugs (barbiturates, benzodiazepines, neuroleptics, etc.) but also in discriminating between typical and atypical neuroleptic drugs.[33,34]

As with clozapine,[12] very few of tested, mainly substituted, compounds inhibited apomorphine-mediated stereotypy in rats (Table V). We found that 9 (10 mg/kg, sc) and 10–12 and 19 (20 mg/kg, sc) decreased apomorphine-induced stereotypy by a maximum of 75% of the saline-group value while haloperidol (0.6 mg/kg, sc) completely eliminated it. Logically, as is the case for clozapine, these analogues should present a low tendency to induce EPS.

Some of our compounds, tested in behavioral models such as the open-field test in rats[63] or a temporal conditioning schedule in dogs,[33-35] revealed an interesting clozapine-like profile while retaining most of the behavioral characteristics previously described for antipsychotic drugs.[33,34] In this procedure our molecules, like neuroleptic drugs, decreased both total and correct response rates, produced incomplete responses, and disturbed the temporal distribution of response durations by inducing both shortened and delayed responses.

In the open-field test, 9 (2 mg/kg, ip) and 12 (8 mg/kg, ip), like classical neuroleptics at very low doses, significantly increased the total ambulation score[35] probably due to a predominant presynaptic D_2 antagonism.[64] At higher doses (16 and 24 mg/kg, ip), 9 and 12 decreased the ambulation score while haloperidol completely suppressed locomotion. However, 16, like clozapine (up to 24 mg/kg, ip), did not significantly modify the total ambulation score.[63]

In the operant conditioning schedule in dogs, 16 showed a high degree of similarity with clozapine. Like clozapine, it did not induce catalepsy and stereotypy/hyperkinesia.[35] Moreover, other motor effects observed with clozapine were reduced (ataxia, akinesia, dystony), and tremor and sialorrhea were completely absent. Although 9 and 12 presented a typical neuroleptic profile in acute treatment,[35] a pilot study with 9, using a repeated-administration design, revealed a clozapine-like profile.

Conclusion

Clozapine and derivatives possess many receptor binding sites, and the clinical or pharmacological profile could be the result of several interactions such as 5-HT_2/D_2, D_2/M, etc. acting in synergy.

The proposed modulations of dibenzodiazepine analogues lead to very promising new compounds. Although the binding affinities D_2, D_1, 5-HT_2, and M were reduced for all compounds, many pyridobenzodiazepine derivatives

could be classified with atypical antipsychotic drugs according to the 5-HT_2/D_2 ratio hypothesis.[13] Moreover, some compounds (9, 12, 16, etc.) tested in behavioral models[35,63] presented, even in acute treatment, an antipsychotic potential with a high degree of similarity with clozapine. For comparable doses some side effects, well-known for clozapine (sialorrhea, tremor, and sedation), were either completely absent or strongly reduced with these derivatives. From this exploratory study, and taking into account a recent pharmacological hypothesis widely developed by Meltzer *et al.*,[13] we conclude that, despite decreasing dopaminergic affinity even in the 2-substituted dibenzoazepine series, an interesting neuroleptic profile could be retained.

Experimental Section

Melting points were determined with a Tottoli (Buchi) melting point apparatus in open capillary tubes and are uncorrected. All compounds were characterized by physical methods using IR (Perkin-Elmer model 297 spectrophotometer) and ^{1}H-NMR (Bruker AW 80 spectrometer with Me_4Si as the internal standard). Column chromatography was carried out using Kieselgel 60, 230–400 mesh (Merck). Microanalyses were performed in house (Carlo Erba CHNS-O EA1108 elemental analyzer) and were within ±0.4% of the theoretical values.

Method A. *N*-(2-Chloro-3-pyridinyl)-5-methyl-2-nitrobenzamide (21e). A mixture of 5-methyl-2-nitrobenzoic acid (0.01 mol) and $SOCl_2$ (25 mL) containing 2 drops of DMF was heated to reflux to obtain a pale yellow solution. Excess $SOCl_2$ was removed under reduced pressure and the residue dissolved in dioxane (30 mL). This solution was added dropwise to a well-stirred solution of 3-amino-2-chloropyridine (0.01 mol) and pyridine (0.01 mol) in dioxane (50 mL). After 30 min, the mixture was diluted to 400 mL with water. The product was then collected by filtration, washed with water, and dried at room temperature under reduced pressure. The product could be recrystallized from 2-propanol: yield 77%; mp 155 °C; IR (KBr) 1667, 1587, 1517, 1454, 1413, 1352, 1316, 850 cm^{-1}; [^{1}H] NMR ($CDCl_3$) δ 8.75 (dd, HC(6′)), 8.15 (dd, HC(4′)), 7.9–7.4 (m, benzene), 7.3 (dd, HC(5′), 2.5 (s, CH_3–). Anal. ($C_{13}H_{10}ClN_3O_3$) C, H, N.

Method B. *N*-(2-Chloro-3-pyridinyl)-2-amino-5-methylbenzamide (22e). To a solution of (21e) (0.01 mol) in HCl (25 mL) was added stannous chloride (0.05 mol) in HCl (20 mL) dropwise at 50–60 °C. The solution was then heated to 100 °C for 15 min, cooled, and filtered to give a pale yellow crystalline solid, which was dissolved in water (300 mL). The solution was made basic with 2 N NaOH and extracted with $CHCl_3$ (5 × 150 mL). The $CHCl_3$ extract was dried over anhydrous magnesium sulfate and concentrated at room temperature under reduced pressure in the presence of petroleum ether (100–140 °C) (100 mL) until crystallization. The product was collected by filtration, washed with petroleum ether (40–60 °C), and dried at room temperature under reduced pressure: yield 75%; mp 188 °C; IR (KBr) 1650, 1595, 1577, 1510, 1397, 1298, 1258, 1212, 805 cm^{-1}; [^{1}H] NMR ($CDCl_3$) δ 8.3 (dd, HC(6′)), 8.1 (dd, HC(4′)), 7.35 (d, HC(6)), 7.3 (dd, HC(5′)), 7 (dd, HC(4)), 6.65 (d, HC(3)), 5.95 (s, NH_2), 2.15 (s, CH_3–). Anal. ($C_{13}H_{12}ClN_3O$) C, H, N.

Method C. 5,11-Dihydro-8-methyl-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (23e). 22e (0.01 mol) in DEGMME (25 mL) was stirred for 2–3 h at 130 °C until the completion of the reaction (TLC monitoring, EtOAc). The mixture was then cooled at 0 °C, and the crystals were collected by filtration, washed with MeOH, and dried at room temperature under reduced pressure: yield 65%; mp 250 °C; IR (KBr) 1664, 1574, 1503, 1438, 1377, 815, 735 cm^{-1}; [^{1}H] NMR δ 9.9 (broad s, HNCO), 8.02–7.9 (m, 3H), 7.59 (s, HC(7)), 7.3–6.9 (m, 3H), 2.26 (s, CH_3–). Anal. ($C_{13}H_{11}N_3O$) C, H, N.

Method D. 8-Methyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (12). To a mixture of 0.01 mol of 23e, *N*-methylpiperazine (10 mL, 0.09 mol), and anhydrous toluene (20 mL) was added a solution of titanium tetrachloride (1.2 mL) in anisole (5 mL) dropwise. The mixture was heated to reflux for 2–3 h, cooled, treated with 2-propanol (10 mL), ammonia (3 mL), and Kieselgel 60 (5 g), stirred, 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 magnesium sulfate, was evaporated under reduced pressure. The residue was recrystallized from 15% CH_2Cl_2/hexane mixture to give yellow crystals: yield 65%; mp 157 °C; IR (KBr) 1612, 1585, 1561, 1496, 1424, 1307 cm^{-1}; [^{1}H] NMR ($CDCl_3$) δ 7.68 (dd, HC(2)), 7.22 (d, HC(10)), 6.98 (s and dd, HC(7) and HC(4)), 6.75 (dd, HC(3), 6.70 (d, HC(9)), 5.90 (s, HN), 3.42 (t, $-CH_2CH_2-$), 2.41 (t, $-CH_2CH_2-$), 2.20 (s, CH_3–), 2.15 (s, CH_3–). Anal. ($C_{13}H_{21}N_5$) C, H, N.

Method E. 5-(4-Methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,5]benzodiazepine (16). The method described above or in the literature[14] was applied to each isomer (16 and 19).

Method F. General Method for the Preparation of *N*-Formyl Derivatives. 11-Formyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (14). Formic acid (99%, 7 mL) was added dropwise to cooled acetic anhydride (15 mL). The mixture was heated at 50 °C for 15 min and then cooled to 0 °C. 8 (0.02 mol) was added and the solution stirred at room temperature overnight. The mixture was then poured onto ice–water (100 g), made basic with 30% aqueous ammonia solution, and extracted with CH_2Cl_2 (4 × 150 mL). The organic layers were dried over magnesium sulfate and evaporated under reduced pressure. The residue recrystallized from hexane gave pale yellow crystals: yield 85%; mp 202 °C; IR (KBr) 1688, 1593, 1557, 1423, 1298, 818 cm^{-1}; [^{1}H] NMR ($CDCl_3$) δ 8.67 (s, HC=O), 7.95 (dd, HC(2)), 7.5–7.15 (m, HC(4) and benzene), 7.09 (dd, HC(3)), 3.5 (m, $-CH_2CH_2-$), 2.38 (m, $-CH_2CH_2-$), 2.22 (s, CH_3–). Anal. ($C_{18}H_{19}N_5O$) C, H, N.

Method G. *N*-Methyl-*N*-(3-nitro-2-pyridinyl)anthranilic Acid (26). 2-Chloro-3-nitropyridine (0.02 mol) and *N*-methylanthranilic acid (0.01 mol) were dissolved in anhydrous 2-propanol (50 mL). Anhydrous potassium carbonate (0.02 mol) was added and the mixture heated to reflux for 24 h. The solvant was removed under reduced pressure, and the residue, dissolved in water (250 mL), was clarified with Norit. After cooling, the pH was adjusted and the separated product was collected by filtration, washed with water, and dried at room temperature: yield 70%; mp 174 °C; IR (KBr) 1687, 1593, 1516, 1408, 1338, 848 cm^{-1}; [^{1}H] NMR ($CDCl_3$) δ 12.5 (s, H(OC=O)), 8.38 (dd, HC(6′)), 7.95 (dd, HC(4′)), 7.75–7.1 (m, benzene), 6.85 (dd, HC(5′)), 3.45 (s, CH_3–). Anal. ($C_{13}H_{11}N_3O_4$) C, H, N.

Method H. Methyl *N*-Methyl-*N*-(3-nitro-2-pyridinyl)-anthranilate (28). To a solution of 26 (0.05 mol) in anhydrous diethyl ether (300 mL) was added a solution of diazomethane (decomposition of nitrosomethylurea (4 g) in 10% ice-cold aqueous NaOH solution and extraction with diethyl ether) dropwise until the completion of the reaction (TLC monitoring, EtOAc–Pet (7:3)). The yellow solution of nitro ester was then washed with a 10% aqueous $NaHCO_3$ solution, dried over magnesium sulfate, filtered, and evaporated under reduced pressure to give a yellow solid which could be recrystallized from petroleum ether (100–140 °C): yield 95%; mp 71 °C; IR (KBr) 1720, 1592, 1555, 1404, 1328, 848 cm^{-1}; [^{1}H] NMR ($CDCl_3$) δ 8.4 (dd, HC(6′)), 7.95 (dd, HC(4′)), 7.7–7.1 (m, benzene), 6.85 (dd, HC(5′)), 3.45 (s, CH_3–), 3.35 (s, CH_3–). Anal. ($C_{14}H_{13}N_3O_4$) C, H, N.

Method I. Methyl *N*-Methyl-*N*-(3-amino-2-pyridinyl)-anthranilate (30). To a solution of 28 (0.01 mol) in ethanol (100 mL) was added a slurry of 10% palladium on charcoal catalyst (0.75 g) in ethanol (20 mL). The suspension was hydrogenated on a Parr apparatus at room temperature to a pressure of 50 psi until the required amount of hydrogen was taken up. The suspension was filtered and evaporated under reduced pressure to give a pale brown oil, which was used without any purification in the next step.

Method J. 11-Methyl-6-(4-methyl-1-piperazinyl)-11*H*-pyrido[2,3-*b*][1,4]benzodiazepine (15). Crude 30 was dissolved in anisole (50 mL) and *N*-methylpiperazine (20 mL, 0.18 mol). A solution of titanium tetrachloride (5 mL) in anisole (10 mL) was added dropwise. The mixture was heated at reflux under nitrogen overnight and cooled, 2-propanol (10 mL), 30% aqueous ammonia solution (10 mL), and silica gel (2 g) were added, and the resulting mixture was stirred and then filtered. The solid

was washed with hot $CHCl_3$ (250 mL), and the combined organic layers were extracted by 2 N HCl (4 × 200 mL). Clarified with Norit, the aqueous solution was then made basic with 30% aqueous ammonia solution and extracted with $CHCl_3$ (4 × 200 mL), which was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by column chromatography on Kieselgel 60 using 15% petroleum ether (40–60 °C)/acetone as eluent to give a pale yellow solid. The product was recrystallized from 10% CH_2Cl_2/hexane: yield 50%; mp 146 °C; IR (KBr) 1600, 1558, 1494, 1427, 1298, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.75 (dd, HC(2)), 8.15 (dd, HC(4)), 7.25–6.85 (m, HC(3)) and benzene), 3.45 (t, $-CH_2CH_2-$), 3.2 (s, CH_3-), 2.43 (m, $-CH_2CH_2-$), 2.25 (s, CH_3-). Anal. ($C_{13}H_{21}N_5$) C, H, N.

Radioligand Binding Study. Experiments on receptor preparations were performed following classical methods previously described: D_1,[65,66] D_2,[67] 5-HT_2,[68] and M.[69] 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:[70] $K_i = IC_{50}/(1 + L/K_d)$ with L the concentration and K_d the apparent dissociation constant of the [^{3}H] ligand obtained from Scatchard analysis of saturation experiments. Each K_i value was determined at least in duplicate with nine concentrations of the drug in triplicate. The pK_i (−log K_i) values were used in the calculation of binding ratios.

Apomorphine Antagonism Test in the Rat. Experiments were performed following the classical method previously described.[71]

Open Field in the Rat. Experiments were performed following the classical method previously described.[63,64]

Temporal Regulation Schedule in the Dog. Experiments were performed following the previously described method.[33–35]

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