Novel and Selective Partial Agonists of 5-HT$_3$ Receptors. 2. Synthesis and Biological Evaluation of Piperazinopyridopyrrolopyrazines, Piperazinopyrroloquinoxalines, and Piperazinopyridopyrroloquinoxalines

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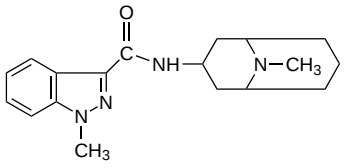
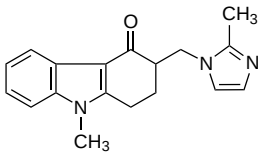
In continuation of our previous work on piperazinopyrrolothienopyrazine derivatives, three series of piperazinopyridopyrrolopyrazines, piperazinopyrroloquinoxalines, and piperazinopyridopyrroloquinoxalines were prepared and evaluated as 5-HT$_3$ receptor ligands. The chemical modifications performed within these new series led to structure−activity relationships regarding both high affinity and selectivity for the 5-HT$_3$ receptors that are in agreement with those established previously for the pyrrolothienopyrazine series. The best compound (**8a**) obtained in these new series is in the picomolar range of affinity for 5-HT$_3$ receptors with a selectivity higher than 10^6. Four of the high-affinity 5-HT$_3$ ligands (**8a**, **15a,b**, and **16d**) were selected in both the pyridopyrrolopyrazine and the pyrroloquinoxaline series and were characterized *in vitro* and *in vivo* as agonists or partial agonists. Compound **8a** was also evaluated in the light/dark test where it showed potential anxiolytic-like activity at very low doses *per os*.

Introduction

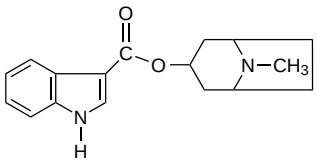
Serotonin (5-hydroxytryptamine, 5-HT) and its receptor subtypes as well as its physiological implications have been the matter of well-documented general reviews.[1−3] Among the 5-HT receptor subtypes, special attention has been paid to the 5-HT$_3$ receptors[4] and the numerous potential therapeutic applications of their antagonists: treatment of emesis associated with anticancer chemotherapy,[5] pain, memory impairment, drug addiction, and psychosis.[6] Highly selective and high-affinity 5-HT$_3$ antagonists have been identified and developed, such as granisetron,[7] ondansetron,[8] and tropisetron.[9]



granisetron

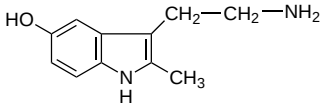


ondansetron

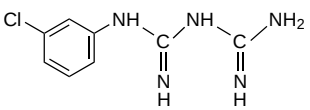


tropisetron

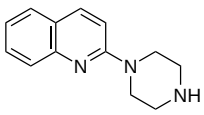
Less is known concerning selective and potent 5-HT$_3$ agonists and their therapeutic potential. The most frequently mentioned agonists are 2-methyl-5-hydroxytryptamine, (*m*-chlorophenyl)biguanide (mCPBG),[10,11] and quipazine.[12−15]



2-methyl-5-hydroxytryptamine

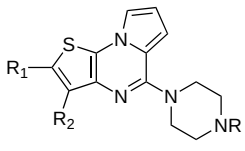


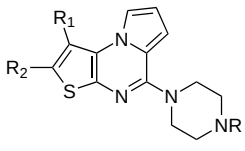
mCPBG



quipazine

Recently we reported the synthesis,[16] the receptor-binding profile, and the *in vitro* and *in vivo* pharmacological evaluation of two series of tricyclic piperazinopyrrolothienopyrazine (PPTP) derivatives which have in common with quipazine a polycyclic aromatic moiety linked to a substituted piperazine via a "pseudo-amidinic" bond.





PPTP

This work on PPTP derivatives led us to obtain, among others, 5-(4-benzylpiperazino)pyrrolo[1,2-*a*]thieno-[3,2-*e*]pyrazine (**2**), which proved to be a very high-affinity and selective 5-HT$_3$ receptor partial agonist, with potent anxiolytic-like activity in the light/dark test.[16]

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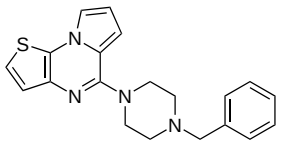
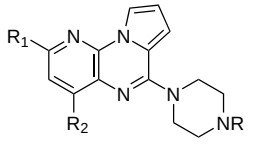
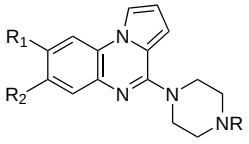


2

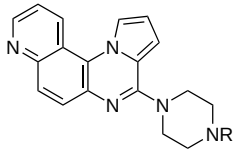
In continuation of our previous studies on PPTP, we now report our work on piperazinopyridopyrrolopyrazines (PPPP), piperazinopyrroloquinoxalines (PPQ), and some piperazinopyridopyrroloquinoxalines (PPPQ).



PPPP



PPQ



PPPQ

Chemical modifications via substitution of the PPPP and PPQ skeletons were systematically carried out in order to compare the structure−activity relationships (SARs) for good affinity and high selectivity for 5-HT$_3$ receptors with those previously established from the PPTP series.[17]

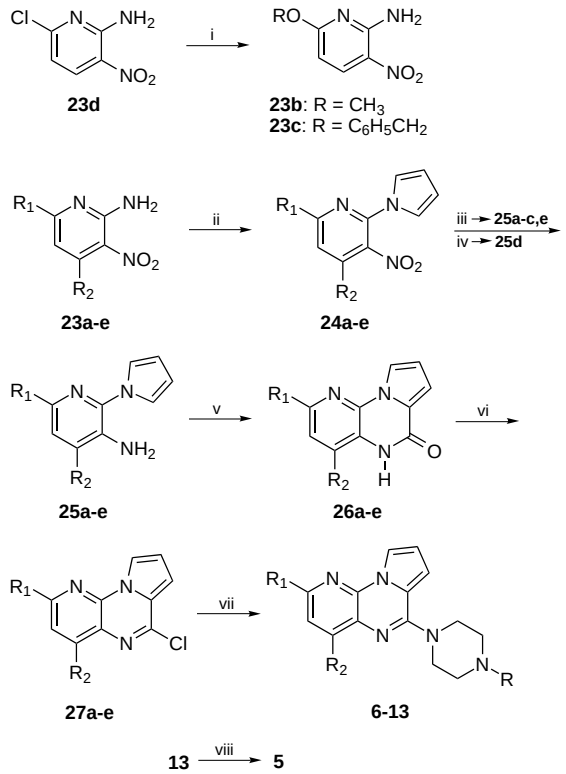
As for the PPTP derivatives, the influence of the substitution on the nature of the interaction with the receptor (agonist, partial agonist, antagonist) was also determined for the most active compounds.

Chemistry

After the chemistry of the pyrrolothienopyrazines,[16,18−25] we now report the syntheses of the PPPP, the PPQ, and the PPPQ series.[26] Some pyrrolo[1,2-*a*]quinoxalines are described in the literature,[27] in particular the 4-(4-methylpiperazino)-7-(trifluoromethyl)pyrrolo[1,2-*a*]quinoxaline.[28]

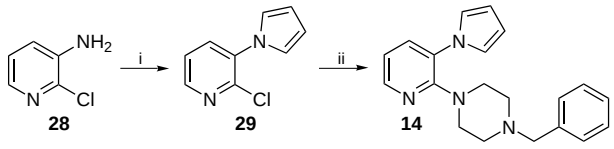
Treatment of 6-chloropyridine **23d** with an alkoxide gave the 6-alkoxypyridines **23b,c** (Scheme 1). The reaction of 2-aminopyridines **23a**−**e** with 2,5-dimethoxytetrahydrofuran (DMTHF) either in 1,4-dioxane with 4-chloropyridine hydrochloride as catalyst or in acetic acid yielded the pyrrolic compounds **24a**−**e**.[29,30] The Béchamp's reduction[31,32] of the nitro group was then carried out to give 3-amino-2-pyrrolopyridine (**25a**), but because a better yield was achieved with Raney nickel (92% instead of 68%), the latter method was chosen for **25a**−**c,e**. For **25d**, the presence of the chlorine atom would not allow the same hydrogenation, so iron(II) sulfate was used because of its good selectivity.[33] The cyclization was then possible between the NH_2 and the Cα of the pyrrole ring by reacting **25a**−**e** with phosgene in a 20% toluenic solution to give the lactams **26a**−**e**, which were subsequently chlorinated with phosphoryl chloride to obtain the chloropyrazines **27a**−**e**.[34] Finally, a nucleophilic substitution with an appropriate piperazine furnished the pyridopyrrolopyrazines **6**−**13**. The deprotection of the carbamate function of **13** by NaOH yielded the monosubstituted piperazine **5**. 3-Amino-2-chloropyridine (**28**) gave compound **14** after formation of the pyrrole ring and subsequent substitution with the *N*-benzylpiperazine (Scheme 2).

Scheme 1[a]



[a] (i) RONa, ROH, Δ; (ii) DMTHF, Δ; (iii) Raney nickel, N_2H_4, H_2O, EtOH, Δ; (iv) $FeSO_4$, $7H_2O$, EtOH, Δ (v) $COCl_2$, toluene, Δ; (vi) $POCl_3$, pyridine, Δ; (vii) *N*-substituted piperazine, K_2CO_3, DMF, Δ; (viii) NaOH, CH_3OH, H_2O, Δ.

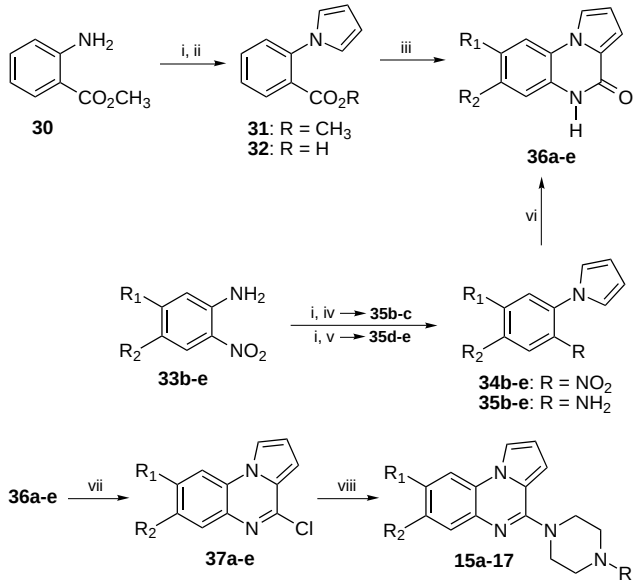
Scheme 2[a]



[a] (i) DMTHF, Δ; (ii) *N*-benzylpiperazine, K_2CO_3, DMF, Δ.

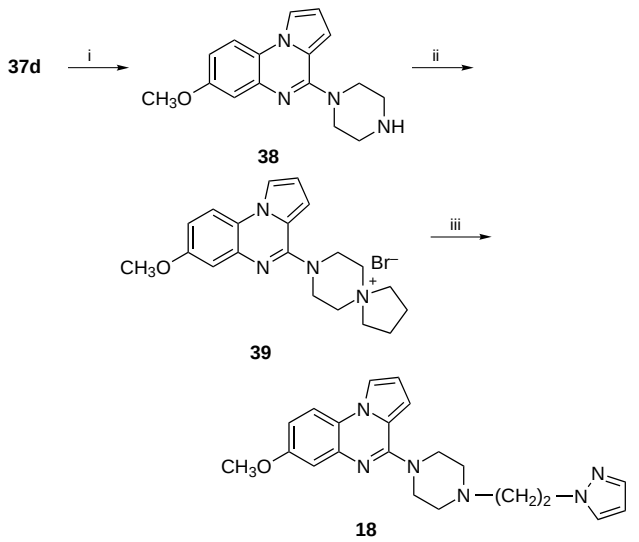
In order to obtain the pyrroloquinoxalines (Scheme 3), methyl anthranylate (**30**) reacted with DMTHF[35,36] and the same catalyst as above for the pyridines. After saponification, the acid was converted to the unstable acyl azide which rearranged and cyclized directly into lactam **36a**. Acid **32** treated by TEA and then by ethyl chloroformate gave *in situ* the carbonic mixed anhydride which reacted with sodium azide. After extraction with Et_2O, the product underwent a spontaneous rearrangement into lactam **36a** during evaporation of the solvent. The last two steps were similar to those of the PPPP series. We studied some substitutions on the 7- or 8-position of the pyrroloquinoxaline. The first part of the synthesis was changed. The raw materials were the substituted 2-nitroanilines **33b**−**e**. The Clauson−Kaas reaction of anilines **33b**−**e** in acetic acid gave the pyrrolic derivatives **34b**−**e**,[29,30] which were reduced either with iron, acetic acid, and hydrochloric acid for **34b,c** or with Raney nickel and hydrazine hydrate for **34d,e** to provide anilines **35b**−**e**. Subsequent cyclization with phosgene gave lactams **36b**−**e**, which were converted to pyrroloquinoxalines **15b**−**17** as previously

Scheme 3[a]



[a] (i) DMTHF, Δ; (ii) NaOH, H_2O, MeOH, Δ; (iii) TEA, $ClCO_2Et$, NaN_3, Me_2CO, MeCN, 0 °C; (iv) Fe, HCl, AcOH, Δ; (v) Raney nickel, N_2H_4, H_2O, EtOH, Δ; (vi) $COCl_2$, toluene, Δ; (vii) $POCl_3$, pyridine, Δ; (viii) *N*-substituted piperazine, K_2CO_3, DMF, Δ.

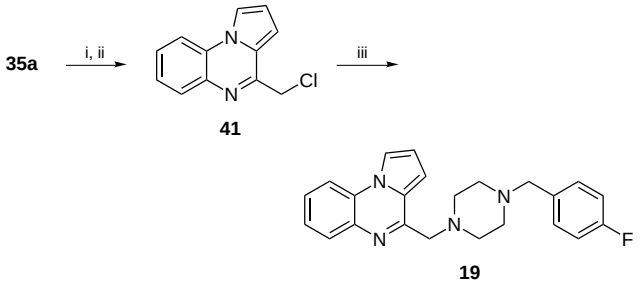
Scheme 4[a]



[a] (i) Piperazine, Δ; (ii) $Br(CH_2)_4Br$, K_2CO_3, EtOH, Δ; (iii) pyrazole, K_2CO_3, toluene, Δ.

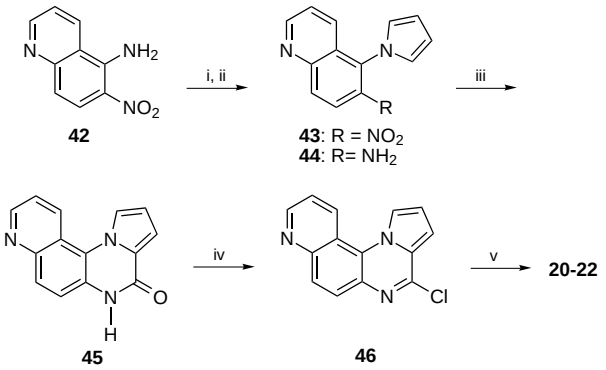
described. From the reaction of excess piperazine with the chloro compound **37d**, it was possible to isolate the monosubstituted piperazine **38** (Scheme 4), which on reaction with 1,4-dibromobutane produced ammonium salt **39**.[37,38] The latter reacted with pyrazole to furnish **18**. Acylation of (*o*-aminophenyl)pyrrole (**35a**) gave chloroacetamide (**40**) which afforded 4-(chloromethyl)-pyrrolo[1,2-*a*]quinoxaline (**41**) on treatment with phosphoryl chloride in toluene (Scheme 5). Subsequent nucleophilic substitution with a piperazine furnished compound **19** with a methylene group between the pyrazine and the piperazine. The synthesis of PPQ derivatives **20**−**22** is very similar to that of the PPPP (Scheme 6).[39] In this case, the formation of the pyrrole ring was done in acetic acid. All the final compounds **5**−**22** were isolated as their stable salt form (hydrochloride, fumarate, or oxalate).

Scheme 5[a]



[a] (i) $ClCOCH_2Cl$, pyridine, dioxane, Δ; (ii) $POCl_3$, toluene, Δ; (iii) *N*-(4-fluorobenzyl)piperazine, K_2CO_3, DMF, Δ.

Scheme 6[a]



[a] (i) DMTHF, AcOH, Δ; (ii) Raney nickel, N_2H_4, H_2O, EtOH, Δ; (iii) $COCl_2$, toluene, Δ; (iv) $POCl_3$, pyridine, Δ; (v) *N*-substituted piperazine, K_2CO_3, DMF, Δ.

Results and Discussion

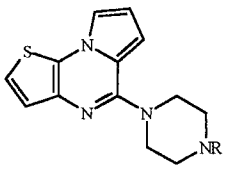
Twenty-eight piperazinopyrido[3,2-*e*]pyrrolo[1,2-*a*]-pyrazines (PPPP), piperazinopyrrolo[1,2-*a*]quinoxalines (PPQ), and piperazinopyrido[2,3-*h*]quinoxalines (PPPQ) were prepared and evaluated in a prescreening procedure for their affinity for the 5-HT_3 receptor and their selectivity compared to other 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, and 5-HT_{2C}) (Tables 1−3). In order to quickly obtain compounds with high affinity and selectivity, we have transposed some of the SARs established in our previous work on tricyclic pyrrolothienopyrazine 5-HT_3 ligands and then synthesized only substituted or nonsubstituted piperazino derivatives.

Strict analogue **5** of quipazine was first synthesized in the PPPP series and proved to be a potent and relatively selective ligand of the 5-HT_3 receptors with $-\log IC_{50}$ of 8.83 for 5-HT_3, 7.19 for 5-HT_{1A}, and 6.82 for 5-HT_{1B}. Both affinity and selectivity were clearly higher than for its counterpart **1** in the PPTP series. Once the potentialities of the PPPP series were established, chemical modifications were systematically performed on the piperazine and the pyridine moieties (Table 1). The following became rapidly apparent:

(a) Substitution of the piperazine with a methyl (**6**, $-\log IC_{50} = 9.01$) results in a slight increase in affinity without change of the selectivity. In contrast, substitution with an allyl (**7**) leads to a very high-affinity and selective ligand for the 5-HT_3 receptors with a $-\log IC_{50}$ of 11.40 and a selectivity greater than 10000−100000, depending on the receptor subtype.

(b) Substitution of the piperazine with an ethoxycarbonyl (**13**, $-\log IC_{50} = 4.55$) or a phenyl (**12**, $-\log IC_{50} = 4.56$) induces a total loss in 5-HT receptor affinity.

Table 1. Binding Properties (−log IC_{50}) of the Pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Derivatives



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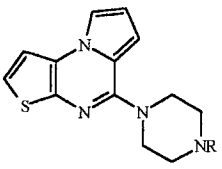

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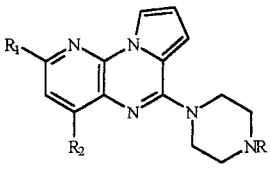
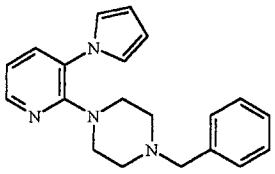
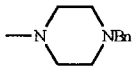


5-13



14

compd	R[a]	R_1[a]	R_2	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{2A}	5-HT_{2C}	5-HT_3
1	H			7.23 ± 0.04	7.86 ± 0.13	6.22 ± 0.05	5.58 ± 0.08	7.46 ± 0.13	7.92 ± 0.06
2	Bn			6.40 ± 0.04	5.99 ± 0.09	4.85 ± 0.08	5.36 ± 0.10	5.73 ± 0.12	8.85 ± 0.07
3	allyl			7.17 ± 0.02	6.38 ± 0.04	6.33 ± 0.05	5.63 ± 0.18	6.12 ± 0.01	9.04 ± 0.02
4	4F-Bn			4.84 ± 0.11	< 4	< 4	4.42 ± 0.39	< 4	8.93 ± 0.08
5	H	H	H	7.19 ± 0.03	6.82 ± 0.06	5.77 ± 0.07	4.84 ± 0.10	6.57 ± 0.09	8.83 ± 0.02
6	CH_3	H	H	7.01 ± 0.13	6.61 ± 0.04	6.02 ± 0.09	5.10 ± 0.05	6.56 ± 0.05	9.01 ± 0.08
7	allyl	H	H	6.93 ± 0.02	6.69 ± 0.05	6.02 ± 0.16	4.60 ± 0.12	6.43 ± 0.08	11.40 ± 0.10
8a	Bn	H	H	5.53 ± 0.04	4.88 ± 0.15	4.53 ± 0.15	4.82 ± 0.05	5.76 ± 0.10	12.09 ± 0.27
8b	Bn	OCH_3	H	4.39 ± 0.17	< 4	< 4	4.15 ± 0.06	< 4	7.85 ± 0.04
8c	Bn	OBn	H	4.26 ± 0.14	< 4	< 4	< 4	< 4	4.43 ± 0.08
8d	Bn		H	< 4	< 4	< 4	< 4	< 4	7.06 ± 0.25
8e	Bn	H	CH_3	4.30 ± 0.03	4.36 ± 0.04	< 4	4.29 ± 0.04	4.24 ± 0.06	5.52 ± 0.05
9a	4F-Bn	H	H	5.17 ± 0.02	4.25 ± 0.11	4.08 ± 0.06	4.36 ± 0.02	5.28 ± 0.16	8.58 ± 0.08
9b	4F-Bn	OCH_3	H	4.25 ± 0.18	< 4	< 4	< 4	< 4	6.93 ± 0.04
9e	4F-Bn	H	CH_3	4.01 ± 0.09	4.15 ± 0.02	< 4	< 4	< 4	< 4
10	piperonyl	H	H	4.96 ± 0.05	4.31 ± 0.09	< 4	4.34 ± 0.05	4.87 ± 0.05	9.10 ± 0.02
11	2,4-Cl-Bn	H	H	4.21 ± 0.06	4.11 ± 0.01	< 4	< 4	< 4	5.89 ± 0.31
12	phényl	H	H	< 4	< 4	< 4	< 4	< 4	4.56± 0.39
13	CO_2Et	H	H	< 4	< 4	< 4	< 4	< 4	4.55 ± 0.19
14				5.64 ± 0.01	4.58 ± 0.07	4.96 ± 0.04	5.13 ± 0.07	4.86 ± 0.10	7.34 ± 0.08
Quipazine				5.84 ± 0.11	5.15 ± 0.10	5.43 ± 0.12	5.46 ± 0.05	7.21 ± 0.07	9.35 ± 0.03
CGS 12066B				7.33 ± 0.03	6.47 ± 0.07	6.91 ± 0.10	4.73 ± 0.06	4.01 ± 0.05	5.88 ± 0.08

[a] Bn = benzyl, 4F-Bn = 4-fluorobenzyl, 2,4-Cl-Bn = 2,4-dichlorobenzyl

(c) As with the allyl, substitution of the piperazine with a benzyl results in a very high-affinity 5-HT_3 ligand (**8**, −log IC_{50} = 12.09). Affinity for other 5-HT receptor subtypes is clearly lowered with a selectivity greater than 1000000, depending on the receptor subtype.

(d) The various substitutions performed on the benzylpiperazine moiety show that substitution with a 3,4-methylenedioxy (**10**) or with a 4-fluoro (**9a**) results in a good selectivity but approximately a 1000-fold decrease in affinity with respect to **8a**. However, affinities remain relatively high with −log IC_{50} values of 9.10 and 8.58 for **10** and **9a**, respectively. In contrast, substitution with 2,4-dichloro results in a dramatic loss in affinity (**11**, −log IC_{50} = 5.89).

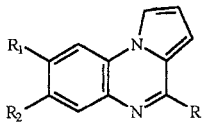
(e) All of the substitutions performed on the pyridine ring of the PPPP skeleton result, regardless of the site of substitution and the substituent (2-methoxy, **8b** and **9b**; 2-benzyloxy, **8c**; 2-benzylpiperazino, **8d**; 4-methyl, **8e** and **9e**), in a marked decrease of affinity compared to the nonsubstituted derivatives **8a** and **9a**.

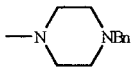
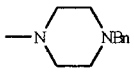
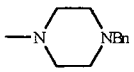
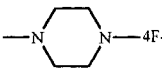
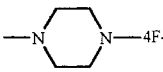
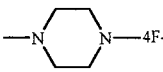
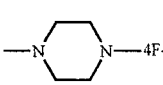
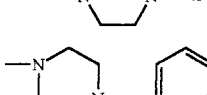
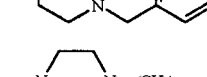
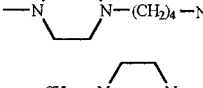
(f) Compound **14**, which, to some extent, can be considered as an open monocyclic analogue of **8a**, was found to be 100000 times less active.

In a general manner, these results correlate well with those previously established with the PPTP series, but with sometimes much higher affinity and selectivity as is the case for the compounds **8a** (−log IC_{50} = 12.09) and **7** (−log IC_{50} = 11.40) which are between 100 and 1000 times more potent than their PPTP analogues **2** and **3**.

Except for a few differences, results are the same with the PPQ derivatives. There again, substitution of the piperazine with a benzyl leads to a good affinity for the 5-HT_3 receptor (**15a**, −log IC_{50} = 9.45) with a very high

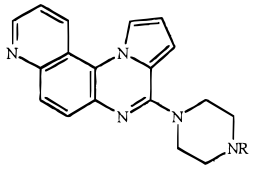
Table 2. Binding Properties ($-\log IC_{50}$) of the Pyrrolo[1,2-*a*]quinoxaline Derivatives



cpd	R[a]	R_1	R_2	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{2A}	5-HT_{2C}	5-HT_3
15a		H	H	5.59 ± 0.04	4.89 ± 0.05	4.62 ± 0.10	4.98 ± 0.03	5.61 ± 0.15	9.45 ± 0.02
15b		H	Cl	< 4	4.72 ± 0.03	< 4	< 4	5.28 ± 0.16	9.54 ± 0.09
15c		Cl	H	4.05 ± 0.03	< 4	< 4	< 4	4.35 ± 0.34	7.69 ± 0.03
16a		H	H	5.27 ± 0.03	4.42 ± 0.09	< 4	4.33 ± 0.07	4.86 ± 0.13	8.62 ± 0.05
16b		H	Cl	4.39 ± 0.03	< 4	< 4	< 4	< 4	7.71 ± 0.04
16d		H	OCH_3	4.38 ± 0.09	4.16 ± 0.22	< 4	< 4	< 4	8.18 ± 0.06
16e		H	CH_3	4.89 ± 0.09	5.00 ± 0.04	< 4	< 4	< 4	7.87 ± 0.04
17		H	Cl	5.02 ± 0.07	4.84 ± 0.14	4.33 ± 0.01	< 4	< 4	10.09 ± 0.10
18		H	OCH_3	6.31 ± 0.04	5.45 ± 0.07	< 4	5.79 ± 0.11	5.13 ± 0.07	< 4
19		H	H	4.94 ± 0.56	4.50 ± 0.06	4.07 ± 0.09	4.77 ± 0.11	4.97 ± 0.08	5.59 ± 0.10

[a] Bn = benzyl ; 4F-Bn = 4-fluorobenzyl

Table 3. Binding Properties ($-\log IC_{50}$) of the Pyrido[2,3-*h*]Pyrrolo[1,2-*a*]quinoxaline Derivatives



compd	R[a]	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{2A}	5-HT_{2C}	5-HT_3
20	Bn	4.20 ± 0.03	4.01 ± 0.09	<4	<4	<4	7.51 ± 0.03
21	piperonyl	4.64 ± 0.07	4.23 ± 0.03	4.40 ± 0.12	4.70 ± 0.15	<4	7.55 ± 0.02
22	allyl	5.34 ± 0.06	4.71 ± 0.01	5.24 ± 0.09	4.67 ± 0.02	4.44 ± 0.07	7.71 ± 0.02

[a] Bn = benzyl.

selectivity (around 10000). This compound is however 430 times less potent than its PPPP direct analogue **8a**. Substitution of the benzylpiperazine with a 4-fluoro (**16a**, $-\log IC_{50} = 8.62$) results in a slight decrease in affinity with respect to **15a** while substitution with a 3,4-methylenedioxy (**17**, $-\log IC_{50} = 10.09$) increases it.

In agreement with the SARs previously established in the PPTP series, substitution of the piperazine with a 4-(pyrazolobutyl) (**18**) results in an inactive compound perhaps because of the π-electron density located too far from the basic nitrogen atom of the piperazine. Substitution on the benzene part of the pyrroloquinoxaline skeleton with an 8-chloro (**15c**) clearly decreases the affinity. Introduction of a 7-chloro, 7-methyl, or 7-methoxy substituent has no effect (**15b**) or only moderate deleterious incidence (**16b** and **16d**,**e**).

Three compounds (**20**−**22**) have also been prepared and evaluated in the PPPQ series; these show $-\log IC_{50}$ values of 7.51, 7.55, 7.71, respectively. All three proved to be less active than their strict analogues in the PPQ and PPPP series.

In conclusion to this preliminary evaluation, the chemical modifications achieved within these novel three series led us to confirm the SARs previously

Table 4. *In Vitro* 5-HT$_3$ Agonist Activity on the [^{14}C]Guanidinium Accumulation in NG 108-15 Cells

compd	EC$_{50}$ (nM)[a]
15a	18 ± 3
15b	52 ± 3
16d	104 ± 14
8a	32 ± 4
2	11 ± 3
5-HT	293 ± 26

[a] Compounds were tested in triplicate for each concentration. Each value is the mean ± SEM of three separate determinations.

established with the PPTP derivatives. Extremely potent and selective 5-HT$_3$ ligands in the picomolar range of affinity were obtained in the PPPP series (**8a** and **7**) which, of all the heteropolycyclic skeletons investigated, gives the best results.

Pharmacology. Among the high-affinity and selective 5-HT$_3$ ligands described above, four compounds, **8a**, **15a,b**, and **16d**, have been selected for characterization of their activity at 5-HT$_3$ receptors. Their agonist and/or antagonist properties have been determined in two different models generally used for 5-HT$_3$ receptor ligands: *in vitro* on the [^{14}C]guanidinium influx into NG 108-15 cells in the presence of substance P[15] and *in vivo* on the Von Bezold−Jarisch reflex[7,9] in anesthetized rats. The most potent 5-HT$_3$ compound, **8a**, has been chosen for behavioral studies in the mouse light/dark test in order to detect possible anxiolytic-like activity.[40,41] In this test, 5-HT$_3$ antagonists[42] and partial agonists[16] are active at very low doses.

Pharmacology Results. Compounds **8a**, **15a,b**, and **16d** were studied *in vitro* on NG 108-15 cells. In the presence of substance P (10 μM), the four compounds increased the uptake of [^{14}C]guanidinium into the cells. The EC$_{50}$ values ranged from 18 to 104 nM (Table 4), indicating that these compounds are more potent than 5-HT (EC$_{50}$ = 293 nM). However, the maximum increase of uptake (≃+150%) due to **8a**, **15a,b**, and **16d** was similar to that observed with 5-HT. Compound **16d** was also able to reduce the stimulatory effect of 5-HT (1 μM) on the uptake of [^{14}C]guanidinium, with a maximal effect of −25% at 3 nM. In this model, compounds **8a** and **15a,b** act as 5-HT$_3$ agonists like **2**, and **16d** is a partial agonist.

The activity of these compounds was also tested *in vivo* on the Von Bezold−Jarisch reflex. In this model, 5-HT administration (30 μg/kg iv) induces a rapid and transient decrease in heart rate which represents the Von Bezold−Jarisch reflex. Of the four compounds studied in this model, only two of them, **8a** and **15a**, were able to trigger this reflex. The bradycardic effects (≃−55% reduction in heart rate) of **8a** at 120 μg/kg iv or **15a** at 60 μg/kg iv were similar to those of 5-HT at 30 μg/kg iv. In order to determine whether these compounds were able to antagonize the Von Bezold−Jarisch reflex induced by 5-HT (30 μg/kg iv), rats were pretreated with these compounds 5 min before 5-HT injection. All of them have presented some antagonist activity (Table 5) with a total prevention of the 5-HT-induced bradycardia at 120 μg/kg iv for **15b** and **16d**. Nevertheless, this antagonist effect is very transient. For example, the reduction in 5-HT evoked bradycardia shifts from −100% to only −60% when the time interval between the pretreatment with **15b** (120 μg/kg iv) and the subsequent injection of 5-HT (30 μg/kg iv) increased from 5 min to 15 min. In the Von Bezold−Jarisch reflex, **15b** and **16d** behave like antagonists whereas **15a** and **8a** are partial agonists like compound **2**.

Table 5. *In Vivo* 5-HT$_3$ Activity in the Von Bezold−Jarisch Reflex in Rats

	agonist activity[a]		antagonist activity[b]	
compd	dose (μg/kg iv)	bradycardia (%)[a]	dose (μg/kg iv)	inhibition of bradycardia (%)[b]
15a	60	54 ± 4.5	60	80 ± 3.5
15b	120	0		IC$_{50}$: 31.2 μg/kg
16d	120	0		IC$_{50}$: 75.4 μg/kg
8a	120	59 ± 7	120	40 ± 4
2	120	40 ± 6	60	42 ± 3

[a] Decrease in heart rate, as a percentage of the baseline control value (prior to any treatment). [b] Percent of inhibition or IC$_{50}$ of bradycardia induced by 5-HT (30 μg/kg iv). The reported values are the means ± SEM of four to seven independent determinations.

Table 6. Active Doses (μg/kg) in the Mouse Light/Dark Test after *per os* Administration

	time in the lit box[a]					transitions[b]				
compd	0.1	1	10	100	1000	0.1	1	10	100	1000
8a	+++	+++	+	++	0	+++	+	++	++	0
2	++	+++	+++	+++	+++	+	++	+	+	++

[a] Significant increase in time spent in the lit compartment. [b] Significant increase in the number of transitions between the lit and dark compartments. Statistical significance between control group and treated group after a combined analysis of variance and a Bonferroni's posteriori *t*-test: +++, $p < 0.0001$; ++, $p < 0.01$; + $p < 0.05$; 0, $p \geq 0.05$.

The differences in binding affinities between compounds **8a**, **15a,b**, or **16d** are not reflected in either the [^{14}C]guanidinium influx model or the Von Bezold−Jarisch reflex. These differences could be explained either by the existence of variations in the pharmacological properties of 5-HT$_3$ receptors from one model to another[43] or by different affinity states of the 5-HT$_3$ receptor.[44−46]

The most potent 5-HT$_3$ compound, **8a**, was studied in the light/dark test. Mice were treated *per os* with **8a** in a range of doses from 0.1 μg/kg to 1 mg/kg. Compound **8a** increased the time spent in the aversive compartment and the transitions between the two compartments (Table 6). This effect was significant for both parameters at 0.1, 1, 10, and 100 μg/kg. In comparison, compound **2** which is a partial 5-HT$_3$ agonist[16] has a similar effect. Both compounds **8a** and **2** which were characterized as agonists *in vitro* in the [^{14}C]guanidinium uptake test and partial agonists *in vivo* in the Von Bezold−Jarisch reflex assay presented an anxiolytic-like activity in the light/dark test.

The anxiolytic-like activity of the partial agonists can be explained either by a long-lasting receptor desensitization[46] or by an antagonist activity on 5-HT$_3$ receptors in some tissues.

Conclusion

As previously achieved with the tricyclic piperazinopyrrolothienopyrazine derivatives,[16] the chemical modifications made in the piperazinopyridopyrrolopyrazine and piperazinopyrroloquinoxaline series have enabled us to obtain very potent and selective ligands for the 5-HT$_3$ receptors. The SARs established with these new compounds are in agreement with those previously inferred from the pyrrolothienopyrazine series. There

again, *in vivo* and *in vitro* data have shown the agonist or partial agonist character of the best 5-HT_3 derivatives. Among these, **8a** proved to be of great interest as a potential anxiolytic agent.

Experimental Section

Chemistry. Every compound was characterized by elemental analysis, IR spectra, and ^{1}H-NMR spectra; these data are reported only for the compounds tested in the pharmacological study. IR spectra were recorded on a Philips PU 9716 infrared spectrometer using KBr pellets; the frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained on a Varian EM 90 spectrometer, with Me_4Si as the internal standard and DMSO-d_6 as the solvent; the chemical shifts are reported in ppm of Me_4Si in δ units, and the coupling constants are in hertz. The IR and ^{1}H-NMR spectra were consistent with assigned structures. Elementary analyses were within ±0.4% of the theoretical values.

6-Piperazinopyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Dihydrochloride (5). 13 (2.0 g, 9.9 mmol) was dissolved in CH_3OH (50 mL) with aqueous sodium hydroxide at 40% (50 mL). The solution was heated at 60 °C for 4 h. The methanol was eliminated under reduced pressure. The precipitate in water was extracted with Et_2O. The organic layer was washed and concentrated under vacuum to furnish the base of **5**. The latter was dissolved in *i*-PrOH (50 mL) and then salified with 37% HCl (3 mL). After stirring, the precipitate was filtered, washed with *i*-PrOH, and dried. This gave **5** as a white powder (0.54 g, 32% yield). Mp: 265 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.33 (m, 4H, H piperazine), 4.17 (m, 4H, H piperazine), 4.63 (m, 2H, NH_2^+), 6.93 (q, 1H, H_8, $J_{7,8} = 4.2$ Hz, $J_{8,9} = 3.0$ Hz), 7.37 (q, 1H, H_7, $J_{7,9} = 1.2$ Hz), 7,43 (q, 1H, H_3, $J_{2,3} = 4.5$ Hz, $J_{3,4} = 7.5$ Hz), 8.27, 8.38 (m, 3H, H_2, H_4, H_9), 9.73 (m, 1H, NH^+). IR: 3440 (m, NH_2^+), 2760 (s, NH^+), 1630 (s, C=N) cm^{-1}. Anal. ($C_{14}H_{17}Cl_2N_5$) C, H, Cl, N.

6-(4-Methylpiperazino)pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (6). 27a (2.0 g, 9.8 mmol) was added in DMF (30 mL) with *N*-methylpiperazine (0.99 g, 9.8 mmol) and K_2CO_3 (1.63 g, 11.8 mmol). The solution was heated at 130 °C for 3 h and, after cooling, was added to 100 mL of stirred water. The suspension was extracted with Et_2O. After usual treatments, the oil obtained was converted into its hydrochloride salt. The base was dissolved in *i*-PrOH with heating, and an excess of HCl (3.5 equiv) was added. After 30 min of stirring at 20 °C, the precipitate was filtered. This gave **6** as a white powder (1.02 g, 27% yield). Mp: >265 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 2.81 (s, 3H, CH_3), 3.47 (m, 4H, H piperazine), 3.83 (m, 2H, H piperazine), 4.66 (m, 2H, H piperazine), 6.10 (m, 3H, NH^+), 6.93 (q, 1H, H_8, $J_{7,8} = 4.2$ Hz, $J_{8,9} = 3.0$ Hz), 7.37 (q, 1H, H_7, $J_{7,9} = 1.5$ Hz), 7.43 (q, 1H, H_3, $J_{2,3} = 4.8$ Hz, $J_{3,4} = 8.1$ Hz), 8.27, 8.37 (m, 3H, H_2, H_4, H_9, $J_{2,4} = 1.5$ Hz). IR: 3380 (s, NH^+), 3120, 2970 (m, CH), 1625 (s, C=N) cm^{-1}. Anal. ($C_{15}H_{20}Cl_3N_5$) C, H, Cl, N.

6-(4-Allylpiperazino)pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (7). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those described to obtain **6**. This gave **7** as a beige powder (0.91 g, 23% yield). Mp: 250 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.43 (m, 4H, H piperazine), 3.90 (d, 2H, N-CH_2), 4.20 (m, 7H, H piperazine, NH^+), 5.56 (d, 2H, CH_2), 6.00 (m, 1H, CH), 6.96 (t, 1H, H_8), 7.50 (m, 2H, H_3, H_7), 8.43 (m, 3H, H_2, H_4, H_9). IR: 2340, 2530 (s, NH^+), 1620 (s, C=N) cm^{-1}. Anal. ($C_{17}H_{22}Cl_3N_5$) C, H, Cl, N.

6-(4-Benzylpiperazino)pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (8a). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those described to obtain **6**. This gave **8a** as a white powder (3.65 g, 81% yield). Mp: 186 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.43 (m, 4H, H piperazine), 4.16, 4.43 (m, 6H, CH_2, H piperazine), 5.03 (m, 3H, NH^+), 6.96 (t, 1H, H_8), 7.43, 7.70 (m, 7H, C_6H_5, H_3, H_7), 8.40 (m, 3H, H_2, H_4, H_9). IR: 2800, 2540, 2470, 2300 (m, NH^+), 1600 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{24}Cl_3N_5$) C, H, Cl, N.

6-(4-Benzylpiperazino)-2-methoxypyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (8b). 27b (0.7 g, 4 mmol) reacted under the same conditions as those described to obtain **6**. This gave **8b** as a white powder (0.70 g, 40% yield). Mp: >264 °C (60% MeCN, 40% *i*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 3.42 (m, 4H, H piperazine), 3.98 (s, 3H, CH_3), 4.43 (m, 9H, CH_2, H piperazine, NH^+), 6.93 (m, 2H, H_7, H_8), 7.43, 7.63 (m, 6H, C_6H_5, H_3), 8.30 (m, 2H, H_4, H_9). IR: 3360 (m, NH^+), 1610 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{26}Cl_3N_5O$) C, H, Cl, N.

2-(Benzyloxy)-6-(4-benzylpiperazino)pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Monofumarate (8c). 27c (1.0 g, 3 mmol) reacted with the method described for **6** until obtainment of the base of **8c**. The latter was dissolved in acetone (60 mL). Fumaric acid (1.1 equiv) was added, and the mixture was heated at 40 °C for 15 min. After cooling, the precipitate was filtered and dried. This gave **8c** as a white powder (0.88 g, 48% yield). Mp: 208 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 2.17 (m, 2H, H piperazine), 2.63 (m, 2H, H piperazine), 3.40 (m, 4H, H piperazine), 4.20 (s, 2H, CH_2), 5.27 (s, 2H, CH_2O), 6.27 (s, 2H, CH=CH), 6.47, 6.60 (m, 3H, H_3, H_7, H_8), 7.00 (m, 10H, C_6H_5, C_6H_5), 7.40 (m, 2H, NH^+, OH), 7.47 (d, 1H, H_4), 7.80 (q, 1H, H_9). IR: 3080 (s, NH^+), 1670 (s, C=O), 1575 (m, C=N) cm^{-1}. Anal. ($C_{32}H_{31}N_5O_5$) C, H, N.

2,6-Bis(4-benzylpiperazino)pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Tetrahydrochloride (8d). 27d (1.3 g, 5.5 mmol) was dissolved in DMF (30 mL) in the presence of *N*-benzylpiperazine (2.12 g, 12 mmol) and K_2CO_3 (1.89 g, 13 mmol). The mixture was then treated as described for the synthesis of **6**. This gave **8d** as a yellow powder (1.37 g, 37% yield). Mp: 210 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.26 (m, 20H, H piperazine, NH^+), 4.14 (s, 2H, CH_2), 4.36 (s, 2H, CH_2), 6.90 (t, 1H, H_8), 7.15 (t, 1H, H_7), 7.40, 7.68 (m, 11H, C_6H_5, C_6H_5, H_3), 7.95 (d, 1H, H_4), 8.23 (t, 1H, H_9). IR: 3350 (s, NH^+), 1570 (m, C=N) cm^{-1}. Anal. ($C_{32}H_{39}Cl_4N_7$) C, H, Cl, N.

6-(4-Benzylpiperazino)-4-methylpyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Dihydrochloride (8e). 27e (2.0 g, 9.2 mmol) reacted under the same conditions as those described to obtain **6**. This gave **8e** as a yellow powder (3.00 g, 75% yield). Mp: 240 °C (60% MeCN, 40% *i*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 2.60 (s, 3H, CH_3), 3.40 (m, 4H, H piperazine), 4.28 (m, 6H, CH_2, H piperazine), 6.83 (q, 1H, H_8), 7.10 (q, 1H, H_7), 7.30 (d, 1H, H_3), 7.46, 7.67 (m, 5H, C_6H_5), 8.18 (d, 1H, H_2), 8.30 (q, 1H, H_9), 11.93 (m, 2H, NH^+). IR: 3400 (s, NH^+), 1610 (m, C=N) cm^{-1}. Anal. ($C_{22}H_{25}Cl_2N_5$) C, H, Cl, N.

6-[4-(4-Fluorobenzyl)piperazino]pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (9a). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those used to obtain **6**. This gave **9a** as a yellow powder (2.93 g, 63% yield). Mp: 202 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.40 (m, 4H, H piperazine), 4.43 (m, 6H, CH_2, H piperazine), 5.10 (m, 3H, NH^+), 6.93 (t, 1H, H_8), 7.20, 7.43, 7.76 (m, 6H, C_6H_4, H_3, H_7), 8.40 (m, 3H, H_2, H_4, H_9). IR: 2580, 2560 (s, NH^+), 1620 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{23}Cl_3FN_5$) C, H, Cl, N.

6-[4-(4-Fluorobenzyl)piperazino]-2-methoxypyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Dihydrochloride (9b). 27b (2.0 g, 8 mmol) reacted under the same conditions as those used to obtain **6**. This gave **9b** as a yellow powder (1.25 g, 31% yield). Mp: 210 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.45 (m, 4H, H piperazine), 3.95 (s, 3H, CH_3), 4.47 (m, 4H, H piperazine), 4.85 (m, 4H, CH_2, NH^+), 6.78, 6.91 (m, 2H, H_7, H_8), 7.25, 7.37, 7.75 (m, 5H, C_6H_4, H_3), 8.40 (m, 2H, H_4, H_9). IR: 3380 (s, NH^+), 1610 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{24}Cl_2FN_5O$) C, H, Cl, N.

6-[4-(4-Fluorobenzyl)piperazino]-4-methylpyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Dihydrochloride (9e). 27e (2.0 g, 9.2 mmol) reacted under the same conditions as those used to obtain **6**. This gave **9e** as a yellow powder (3.34 g, 81% yield). Mp: 228 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 2.47 (s, 3H, CH_3), 3.21, 3.35, 3.65 (m, 6H, H piperazine), 4.36 (s, 2H, CH_2), 4.50 (m, 2H, H piperazine), 5.46 (m, 2H, NH^+), 6.80 (q, 1H, H_8), 7.07 (q, 1H, H_7), 7.27 (m, 3H, H_3, C_6H_2), 7.73 (m, 2H, C_6H_2), 8.13 (d, 1H, H_2), 8.27 (q, 1H, H_9). IR: 3440 (s, NH^+), 1590 (m, C=N) cm^{-1}. Anal. ($C_{22}H_{24}Cl_2FN_5$) C, H, Cl, N.

6-[4-[3,4-(Methylenedioxy)benzyl]piperazino]pyrido-[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (10). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those described to obtain **6**. This gave **10** as a white powder (2.26 g, 46% yield). Mp: 220 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.43 (m, 4H, H piperazine), 3.98 (m, 2H, H piperazine), 4.33 (s, 2H, CH_2-Ar), 4.65 (m, 2H, H piperazine), 5.27 (m, 3H, NH^+),

6.03 (s, 2H, CH_2O), 6.97, 7.33 (m, 6H, C_6H_3, H_3, H_7, H_8), 8,40 (m, 3H, H_2, H_4, H_9). IR: 3400 (s, NH^+), 1605 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{24}Cl_3N_5O_2$) C, H, Cl, N.

6-[4-(2,4-Dichlorobenzyl)piperazino]pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Dihydrochloride (11). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those described to obtain **6**. This gave **11** as a white powder (2.80 g, 60% yield). Mp: 220 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.45 (m, 8H, H piperazine), 3.93, 4.25 (m, 2H, NH^+), 4.51 (s, 2H, CH_2), 6.89 (q, 1H, H_8, $J_{8,9} = 3.9$ Hz), 7.22 (d, 1H, H_7, $J_{7,8} = 3.0$ Hz), 7.44 (q, 1H, H_3, $J_{2,3} = 4.7$ Hz, $J_{3,4} = 7.9$ Hz), 7.54 (q, 1H, $H_{5'}$, $J_{5',6'} = 8.4$ Hz), 7.70 (d, 1H, $H_{3'}$, $J_{3',5'} = 2.2$ Hz), 8.11 (m, 2H, H_4, $H_{6'}$), 8.34 (m, 2H, H_2, H_9). IR: 3440 (s, NH^+), 1605 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{21}Cl_4N_5$) C, H, Cl, N.

6-(4-Phenylpiperazino)pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine Trihydrochloride (12). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those described to obtain **6**. This gave **12** as a white powder (0.90 g, 20% yield). Mp: 180 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.61 (m, 4H, H piperazine), 4.40 (m, 4H, H piperazine), 6.97 (q, 1H, H_8, $J_{7,8} = 3.0$ Hz, $J_{8,9} = 4.2$ Hz), 7.23 (m, 10H, C_6H_5, H_3, H_7, NH^+), 8.37 (q, 1H, H_9, $J_{7,9} = 1.2$ Hz), 8.43 (q, 1H, H_2, $J_{2,3} = 2.7$ Hz, $J_{2,4} = 1.2$ Hz), 8.63 (q, 1H, H_4, $J_{3,4} = 8.4$ Hz). IR: 3510 (s, NH^+), 1620 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{22}Cl_3N_5$) C, H, Cl, N.

6-[4-(Ethoxycarbonyl)piperazino]pyrido[3,2-*e*]pyrrolo-[1,2-*a*]pyrazine Dihydrochloride (13). 27a (2.0 g, 9.8 mmol) reacted under the same conditions as those described to obtain **6**. This gave **13** as a white powder (2.60 g, 66% yield). Mp: 196 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 1.25 (t, 3H, CH_3, $J_{Et} = 7.5$ Hz), 3.40 (m, 4H, H piperazine), 4.14 (m, 6H, CH_2, H piperazine), 4.80 (m, 2H, NH^+), 7.00 (q, 1H, H_8), 7.55 (m, 2H, H_3, H_7), 8.47 (m, 3H, H_2, H_4, H_9). IR: 3500, 3440 (s, NH^+), 1690 (s, CO), 1620 (s, C=N) cm^{-1}. Anal. ($C_{17}H_{21}Cl_2N_5O_2$) C, H, Cl, N.

2-(4-Benzylpiperazino)-3-pyrrolopyridine Trihydrochloride (14). 29 (2.0 g, 11 mmol) reacted under the same conditions as those described to obtain **6**. This gave **14** as a white powder (0.70 g, 21% yield). Mp: 232 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.00 (m, 8H, H piperazine), 4.17 (s, 2H, CH_2), 5.07 (m, 3H, NH^+), 6.07 (t, 2H, $H_{3'}$, $H_{4'}$), 6.90 (m, 3H, H_5, $H_{2'}$, $H_{5'}$), 7.23, 7.50 (m, 6H, C_6H_5, H_4), 8.02 (dd, 1H, H_6). IR: 2580 (s, NH^+), 1610 (s, C=N) cm^{-1}. Anal. ($C_{20}H_{25}Cl_3N_4$) C, H, Cl, N.

4-(4-Benzylpiperazino)pyrrolo[1,2-*a*]quinoxaline Trihydrochloride (15a). 37a (2.0 g, 10 mmol) reacted under the same conditions as those described to obtain **6**. This gave **15a** as a white powder (2.52 g, 56% yield). Mp: 182 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.43 (m, 4H, H piperazine), 4.20, 4.67 (m, 4H, H piperazine), 4.43 (s, 2H, CH_2), 4.70 (m, 3H, NH^+), 6.98 (q, 1H, H_2), 7.47, 7.63 (m, 8H, C_6H_5, H_3, H_7, H_8), 8.17 (m, 2H, H_6, H_9), 8.61 (q, 1H, H_1). IR: 2880, 2650, 2550 (m, NH^+), 1590 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{25}Cl_3N_4$) C, H, Cl, N.

4-(4-Benzylpiperazino)-7-chloropyrrolo[1,2-*a*]quinoxaline Dihydrochloride (15b). 37b (2.0 g, 8.4 mmol) reacted under the same conditions as those described for the production of **6**. This gave **15b** as a white powder (2.67 g, 70% yield). Mp: 228 °C (50% MeCN, 50% *i*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 3.36 (m, 4H, H piperazine), 4.06 (m, 2H, H piperazine), 4.43 (s, 2H, CH_2), 4.83 (m, 4H, H piperazine, NH^+), 6.93 (q, 1H, H_2), 7.33, 7.60, 8.13 (m, 9H, C_6H_5, H_3, H_6, H_8, H_9), 8.53 (q, 1H, H_1). IR: 3400, 2700, 2600 (s, NH^+), 1600 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{23}Cl_3N_4$) C, H, Cl, N.

4-(4-Benzylpiperazino)-8-chloropyrrolo[1,2-*a*]quinoxaline Trihydrochloride (15c). 37c (2.0 g, 8.4 mmol) reacted under the same conditions as those described to synthesize **6**. This gave **15c** as a white powder (1.52 g, 37% yield). Mp: 210 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.44 (m, 4H, H piperazine), 4.05, 4.69 (m, 7H, H piperazine, NH^+), 4.45 (s, 2H, CH_2), 6.92 (q, 1H, H_2), 7.34, 7.42 (m, 5H, C_6H_3, H_3, H_7), 7.66 (m, 2H, C_6H_2), 8.03 (d, 1H, H_6), 8.26 (s, 1H, H_9), 8.51 (q, 1H, H_1). IR: 2700, 2600 (s, NH^+), 1595 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{24}Cl_4N_4$) C, H, Cl, N.

4-[4-(4-Fluorobenzyl)piperazino]pyrrolo[1,2-*a*]quinoxaline Trihydrochloride (16a). 37a (2.0 g, 10 mmol) reacted under the same conditions as those described to obtain **6**. This gave **16a** as a white powder (2.28 g, 49% yield). Mp: 182 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.43 (m, 4H, H piperazine), 4.20 (m, 4H, H piperazine), 4.40 (s, 2H, CH_2), 4.46 (m, 3H, NH^+), 7.00 (q, 1H, H_2), 7.36, 7.73 (m, 7H, C_6H_4, H_3, H_7, H_8), 8.20 (m, 2H, H_6, H_9), 8.60 (q, 1H, H_1). IR: 2880, 2660, 2560 (m, NH^+), 1580 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{24}Cl_3FN_4$) C, H, Cl, N.

7-Chloro-4-[4-(4-fluorobenzyl)piperazino]pyrrolo[1,2-*a*]quinoxaline Trihydrochloride (16b). 37b (2.0 g, 8.4 mmol) reacted under the same conditions as those used to obtain **6**. This gave **16b** as a white powder (3.04 g, 71% yield). Mp: 212 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.33 (m, 4H, H piperazine), 4.00 (m, 2H, H piperazine), 4.40 (s, 2H, CH_2), 4.65 (m, 2H, H piperazine), 5.00 (m, 3H, NH^+), 6.93 (q, 1H, H_2), 7.33 (m, 1H, H_3), 7.33, 7.76 (m, 4H, C_6H_4), 7.33, 7.76, 8.13 (m, 3H, H_6, H_8, H_9), 8.56 (q, 1H, H_1). IR: 2720, 2620 (m, NH^+), 1600 (s, C=N) cm^{-1}. Anal. ($C_{22}H_{23}Cl_4FN_4$) C, H, Cl, N.

4-[4-(4-Fluorobenzyl)piperazino]-7-methoxypyrrolo-[1,2-*a*]quinoxaline Dihydrochloride (16d). 37d (2.0 g, 8.6 mmol) reacted under the same conditions as those used to obtain **6**. This gave **16d** as a white powder (2.80 g, 70% yield). Mp: 220 °C (70% MeCN, 30% *n*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 3.43 (m, 4H, H piperazine), 3.81 (s, 3H, CH_3), 4.12, 4.43, 4.69 (m, 8H, H piperazine, CH_2, NH^+), 6.93 (t, 1H, H_2), 7.02 (d, 1H, H_8), 7.27 (t, 2H, C_6H_2), 7.40 (t, 1H, H_3), 7.78 (m, 3H, C_6H_2, H_6), 8.10 (d, 1H, H_9), 8.47 (t, 1H, H_1). IR: 2700, 2600 (s, NH^+), 1600 (s, C=N) cm^{-1}. Anal. ($C_{23}H_{25}Cl_2FN_4O$) C, H, Cl, N.

4-[4-(4-Fluorobenzyl)piperazino]-7-methylpyrrolo[1,2-*a*]quinoxaline Trihydrochloride (16e). 37e (2.0 g, 9.2 mmol) reacted under the same conditions as those described to obtain **6**. This gave **16e** as a white powder (2.55 g, 57% yield). Mp: 206 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 2.40 (s, 3H, CH_3), 3.40, 4.10, 4.40 (m, 10H, CH_2), 4.66 (m, 3H, NH^+), 7.00 (q, 1H, H_2), 7.20 (q, 1H, H_3), 7.33, 7.73 (m, 4H, C_6H_4), 7.33, 7.96, 8.13 (m, 3H, H_6, H_8, H_9), 8.55 (q, 1H, H_1). IR: 3340, 2600, 2540 (s, NH^+), 1605 (s, C=N) cm^{-1}. Anal. ($C_{23}H_{26}Cl_3FN_4$) C, H, Cl, N.

4-[4-[3,4-(Methylenedioxy)benzyl]piperazino]-7-chloropyrrolo[1,2-*a*]quinoxaline Trihydrochloride (17). 37b (2.0 g, 8.4 mmol) reacted under the same conditions as those described to obtain **6**. This gave **17** as a white powder (3.0 g, 67% yield). Mp: 208 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.36 (m, 4H, H piperazine), 4.00, 4.30 (m, 6H, H piperazine, CH_2), 4.90 (m, 3H, NH^+), 6.00 (s, 2H, CH_2O), 6.96, 7.36 (m, 5H, H_2, H_3, C_6H_3), 7.36, 8.13 (m, 3H, H_6, H_8, H_9), 8.50 (q, 1H, H_1). IR: 2700, 2600 (m, NH^+), 1600 (s, C=N) cm^{-1}. Anal. ($C_{23}H_{24}Cl_4N_4O_2$) C, H, Cl, N.

7-Methoxy-4-[4-(4-pyrazolobut-1-yl)piperazino]pyrrolo-[1,2-*a*]quinoxaline Oxalate (18). 39 (1.0 g, 2.3 mmol), pyrazole (0.16 g, 2.3 mmol), K_2CO_3 (0.4 g, 2.9 mmol), and dibenzo-18-crown-6 (10 mg) in toluene (20 mL) were heated at 100 °C for 22 h. The hot suspension was filtered and then concentrated under reduced pressure. The residue was dissolved in *i*-PrOH (50 mL), oxalic acid (0.17 g) was added, and the mixture was heated at 50 °C for 15 min. After cooling, the precipitate was filtered and dried. This gave **18** as a white powder (0.7 g, 59% yield). Mp: 178 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 1.80 (m, 4H, $(CH_2)2,3$), 3.10, 3.30 (m, 6H, CH_2), 3.80 (s, 3H, CH_3), 3.90, 4.10 (m, 6H, CH_2), 6.20 (m, 1H, H_4 pyrazole), 6.90 (m, 4H, H_2, H_3, H_6, H_8), 7.40 (d, 1H, H pyrazole), 7.70 (d, 1H, H pyrazole), 8.00 (d, 1H, H_9), 8.20 (t, 1H, H_1), 10.20 (m, 2H, NH^+, OH). IR: 3360 (s, OH), 1710 (s, C=O), 1600 (s, C=N) cm^{-1}. Anal. ($C_{25}H_{30}N_6O_5$) C, H, N.

4-[[4-(4-Fluorobenzyl)piperazino]methyl]pyrrolo[1,2-*a*]quinoxaline Trihydrochloride Monohydrate (19). 41 (2.0 g, 9.2 mmol) reacted under the same conditions as those described to obtain **6**. This gave **19** as a yellow powder (1.90 g, 41% yield). Mp: 196 °C (MeCN). ^{1}H-NMR (DMSO-d_6): δ 3.44 (m, 8H, H piperazine), 3.57 (m, 5H, NH^+, H_2O), 4.42 (s, 2H, CH_2-Ar), 4.73 (s, 2H, CH_2), 7.09 (t, 1H, H_2), 7.26 (t, 2H, C_6H_2), 7.56 (m, 1H, H_3), 7.56, 7.68 (m, 2H, H_7, H_8), 7.75 (q, 2H, C_6H_2), 8.12, 8.38 (m, 2H, H_6, H_9), 8.72 (t, 1H, H_1). IR: 3380 (s, NH^+), 3070, 2900 (m, CH), 1610 (s, C=N) cm^{-1}. Anal. ($C_{23}H_{28}Cl_3FN_4O$) C, H, Cl, N.

4-(*N*-substituted piperazino)pyrido[2,3-*h*]pyrrolo[1,2-*a*]quinoxalines (20−22). 46 (1.5 g, 6 mmol) reacted with the wanted amine (6.5 mmol) and K_2CO_3 (0.98 g, 7 mmol) with the method described for **6**. This gave **20**−**22**: 35−56% yield.

4-(4-Benzylpiperazino)pyrido[2,3-*h*]pyrrolo[1,2-*a*]-quinoxaline trihydrochloride (20) was obtained as an orange powder (1.50 g, 50% yield). Mp: 205 °C (60% MeCN, 40% *i*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 3.40 (m, 4H, H piperazine), 4.56 (m, 9H, H piperazine, CH_2, NH^+), 7.00 (t, 1H, H_2), 7.16 (t, 1H, H_3), 7.43, 7.63 (m, 5H, C_6H_5), 7.93 (q, 1H, H_{10}), 8.16 (m, 2H, H_6, H_7), 8.73 (t, 1H, H_1), 9.06 (d, 1H, H_{11}), 9.63 (d, 1H, H_9). IR: 3400 (s, NH^+), 1610 (s, C=N) cm^{-1}. Anal. ($C_{25}H_{26}Cl_3N_5$) C, H, Cl, N.

4-[4-[3,4-(Methylenedioxy)benzyl]piperazino]pyrido-[2,3-*h*]pyrrolo[1,2-*a*]quinoxaline trihydrochloride (21) was obtained as an orange powder (1.15 g, 35% yield). Mp: 245 °C (50% MeCN, 50% *i*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 3.40 (m, 4H, H piperazine), 4.33 (s, 2H, CH_2), 4.73 (m, 7H, H piperazine, NH^+), 6.04 (s, 2H, CH_2O), 7.00, 7.16, 7.33 (m, 5H, C_6H_3, H_2, H_3), 7.90 (q, 1H, H_{10}), 8.16 (d, 2H, H_6, H_7), 8.76 (q, 1H, H_1), 9.04 (d, 1H, H_{11}), 9.60 (d, 1H, H_9). IR: 3400 (s, NH^+), 1615 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{26}Cl_3N_5O_2$) C, H, Cl, N.

4-(4-Allylpiperazino)pyrido[2,3-*h*]pyrrolo[1,2-*a*]-quinoxaline trihydrochloride (22) was obtained as an orange powder (1.50 g, 56% yield). Mp: 225 °C (60% MeCN, 40%*i*-PrOH). ^{1}H-NMR (DMSO-d_6): δ 3.50 (m, 6H, H piperazine),3.83 (d, 2H, CH_2), 4.56 (m, 2H, H piperazine), 5.43, 5.53 (m, 6H, CH=CH_2, NH^+), 7.00 (q, 1H, H_2), 7.13 (q, 1H, H_3), 7.90 (q, 1H, H_{10}), 8.13 (d, 2H, H_6, H_7), 8.66 (q, 1H, H_1), 9.00 (d, 1H, H_{11}), 9.47 (d, 1H, H_9). IR: 3420 (s, NH^+), 1620 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{24}Cl_3N_5$) C, H, Cl, N.

2-Amino-6-methoxy-3-nitropyridine (23b). 2-Amino-6-chloro-3-nitropyridine (**23d**) (6.2 g, 35 mmol) was added to MeOH (100mL) in which Na (1.65 g, 71 mmol) was previously dissolved. The solution was heated at 70 °C for 8 h. MeOH was eliminated under reduced pressure. The resulting product was extracted with Et_2O and EtOAc. Usual treatments of the organic layers gave **23b** as a yellow powder (4.7 g, 77% yield). Mp: 172 °C.

2-Amino-6-(benzyloxy)-3-nitropyridine (23c). Benzyl alcohol (7.73 g, 71 mmol) was added to toluene (150 mL) with Na (1.65 g, 71 mmol). After dissolution, **23d** (10.34 g, 59 mmol) was added and the mixture was heated first carefully for 30 min and then at reflux for 3 h. After cooling, water (50 mL) was added before elimination of toluene under vacuum. Extraction of the residual solution with Et_2O allowed the isolation of the product. This gave **23c** as an orange powder (6.73 g, 46% yield). Mp: 134 °C (Et_2O).

6-Methoxy-3-nitro-2-pyrrolopyridine (24b). DMTHF (10.45 g, 79 mmol) and 4-chloropyridine hydrochloride (12.0 g, 79 mmol) were stirred for 10 min in dioxane (300 mL). **23b** (11.15 g, 65 mmol) was added, and the suspension was heated for 4 h. The dioxane was evaporated under reduced pressure, and the residue was extracted with Et_2O. Usual treatments gave **24b** as an orange powder (11.12 g, 76% yield). Mp: 70 °C.

6-(Benzyloxy)-3-nitro-2-pyrrolopyridine (24c). DMTHF (8.2 g, 62 mmol) was stirred for 10 min in glacial AcOH (200 mL). After addition of **24b** (12.68 g, 51 mmol), the solution was heated under reflux for 4 h. The acid was eliminated under reduced pressure. The residue was alcalinized with a saturated $NaHCO_3$ solution and was extracted with Et_2O. This gave **24c** as a red powder (8.48 g, 55% yield). Mp: 130 °C (Et_2O).

6-Chloro-3-nitro-2-pyrrolopyridine (24d). 23d (15.4 g, 88 mmol) reacted with DMTHF (12.9 g, 97 mmol) and 4-chloropyridine hydrochloride (14.64 g, 97 mmol) in dioxane (300 mL) with the same method as that used to obtain **24b**. This gave **24d** as a red powder (10.27 g, 51% yield). Mp: 110 °C.

3-Amino-6-methoxy-2-pyrrolopyridine (25b). Raney nickel (about 8 g) and $N_2H_4{\cdot}H_2O$ (2 mL) were added to a solution of **24b** (11.12 g, 50 mmol) in EtOH (300 mL). The resulting suspension was heated for 5 h while $N_2H_4{\cdot}H_2O$ (28 mL) was added drop by drop. After cooling, the suspension was filtered and the filtrate was concentrated under reduced pressure. This gave **25b** as a brown oil (8.21 g, 85% yield).

3-Amino-6-(benzyloxy)-2-pyrrolopyridine (25c). 24c (7.8 g, 26 mmol) reacted according to the method described for **25b** with Raney nickel (about 7 g) and $N_2H_4{\cdot}H_2O$ (10 mL, 206 mmol). This gave **25c** as a brown oil (6.41 g, 91% yield).

3-Amino-6-chloro-2-pyrrolopyridine (25d). 24d (9.27 g, 41 mmol) was dissolved in EtOH (300 mL). $FeSO_4{\cdot}7H_2O$ (115.25 g, 414 mmol), 10 N HCl (0.5 mL), and water (5 mL) were successively added. The mixture was heated at 80 °C for 90 min while the ammonia solution at 30% was added by little fractions in order to maintain a basic pH. After cooling, EtOH was evaporated under vacuum. The residue was poured in water (100 mL) and extracted with Et_2O; after the usual treatments, a first amount of the product was obtained. The aqueous phase was alcalinized with ammonia solution and extracted with EtOAc. Finally, this gave **25d** as a beige powder (4.59 g, 57% yield). Mp: 89 °C (80% Et_2O, 20% *n*-hexane).

5,6-Dihydro-2-methoxy-6-oxopyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine (26b). 25b (8.21 g, 43 mmol) in toluene (400 mL) was heated at 100 °C with $COCl_2$ in toluenic solution at 20% (30 mL, 57 mmol) for 3 h. After elimination of $COCl_2$ in excess and cooling, the precipitate was filtered and neutralized with a saturated $NaHCO_3$ solution to give a first amount of **26b**. The toluenic filtrate was concentrated under vacuum, the residue was triturated with Et_2O, and the resulting precipitate was treated as before. This gave **26b** as a whitish powder (6.96 g, 74% yield). Mp: 250 °C.

5,6-Dihydro-2-(benzyloxy)-6-oxopyrido[3,2-*e*]pyrrolo-[1,2-*a*]pyrazine (26c). 25c (6.4 g, 24 mmol) reacted under the same conditions as those described to obtain **26b**. This gave **26c** as a beige powder (2.73 g, 38% yield). Mp: 264 °C.

5,6-Dihydro-2-chloro-6-oxopyrido[3,2-*e*]pyrrolo[1,2-*a*]-pyrazine (26d). 25d (4.59 g, 23 mmol) reacted under the same conditions as those described to obtain **26b**. This gave **26d** as a gray powder (2.60 g, 49% yield). Mp: 270 °C.

6-Chloropyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine (27a). 5,6-Dihydro-6-oxopyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine (**26a**) (9.25 g, 50 mmol) was refluxed in $POCl_3$ (200 mL) with pyridine (10 mL) for 4 h. After cooling, the reactives were eliminated under vacuum. The residue was carefully dissolved in water at 0 °C, and the resulting solution was alcalinized with 30% ammonium hydroxide. The precipitate was filtered and extracted with Et_2O and then EtOAc. The organic layers were washed, treated with animal charcoal and $MgSO_4$, and concentrated under reduced pressure to give **27a** as a yellow powder (8.35 g, 82% yield). Mp: 146 °C (MeCN).

6-Chloro-2-methoxypyrido[3,2-*e*]pyrrolo[1,2-*a*]-pyrazine (27b). 26b (2.6 g, 12 mmol) reacted under the same conditions as those described to obtain for **27a**. This gave **27b** as a yellow powder (0.94 g, 33% yield). Mp: 163 °C.

2-(Benzyloxy)-6-chloropyrido[3,2-*e*]pyrrolo[1,2-*a*]-pyrazine (27c). 26c (2.65 g, 9 mmol) reacted under the same conditions as those described to obtain **27a**. This gave **27c** as a brown powder (1.47 g, 52% yield). Mp: 106 °C (EtOAc).

2,6-Dichloropyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine (27d). 26d (2.6 g, 11 mmol) reacted under the same conditions as those described to obtain **27a**. This gave **27d** as a yellow powder (1.27 g, 45% yield). Mp: 211 °C (EtOAc).

4-Methyl-6-chloropyrido[3,2-*e*]pyrrolo[1,2-*a*]-pyrazine (27e). 5,6-dihydro-4-methyl-6-oxopyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine (**26e**) (7.8 g, 39 mmol) reacted using the same procedure as for **27a** . This gave **27e** as a yellow powder (5.83 g, 68% yield). Mp: 139 °C (MeCN).

7- or 8-Substituted 4,5-Dihydro-4-oxopyrrolo[1,2-*a*]-quinoxalines (36b−e). These derivatives were prepared with the same method as that described above to obtain **26b**. This gave **36b**−**e** (64−91% yield).

4,5-Dihydro-7-chloro-4-oxopyrrolo[1,2-*a*]quinoxaline (36b). 35b (11.9 g, 62 mmol) gave **36b** as a gray powder (12.15 g, 90% yield). Mp: >270 °C (MeCN).

4,5-Dihydro-8-chloro-4-oxopyrrolo[1,2-*a*]quinoxaline (36c). 35c (20.0 g, 104 mmol) gave **36c** as a beige powder (14.5 g, 64% yield). Mp: >270 °C (MeCN).

4,5-Dihydro-7-methoxy-4-oxopyrrolo[1,2-*a*]quinoxaline (36d). 35d (20.6 g, 109 mmol) gave **36d** as a beige powder (21.33 g, 91% yield). Mp: 252 °C (MeCN).

4,5-Dihydro-7-methyl-4-oxopyrrolo[1,2-*a*]quinoxaline (36e). 35e (29.4 g, 170 mmol) gave **36e** as a beige powder (25.9 g, 76% yield). Mp: 250 °C (MeCN).

7- or 8-Substituted 4-Chloropyrrolo[1,2-*a*]quinoxalines (37b−e). These compounds were synthesized with the same method as that described to obtain **27a**. This gave **37b−e** (53−71% yield).

4,7-Dichloropyrrolo[1,2-*a*]quinoxaline (37b). 36b (11.0 g, 46 mmol) gave **37b** as a white powder (8.50 g, 71% yield). Mp: 204 °C (MeCN).

4,8-Dichloropyrrolo[1,2-*a*]quinoxaline (37c). 36c (13.0 g, 59 mmol) gave **37c** as a beige powder (8.10 g, 57% yield). Mp: 188 °C (MeCN).

4-Chloro-7-methoxypyrrolo[1,2-*a*]quinoxaline (37d). 36d (10 g, 46 mmol) gave **37d** as a beige powder (7.06 g, 65% yield). Mp: 132 °C (EtOAc).

4-Chloro-7-methylpyrrolo[1,2-*a*]quinoxaline (37e). 36e (10.0 g, 50 mmol) gave **37e** as a beige powder (5.85 g, 53% yield). Mp: 158 °C (EtOAc).

7-Methoxy-4-piperazinopyrrolo[1,2-*a*]quinoxaline (38). 37d (3.0 g, 13 mmol) was heated with piperazine (14.64 g, 170 mmol) at 160 °C for 4 h. Water (200 mL) was gradually added, and the mixture was cooled in a crushed ice bath. Extraction of the suspension with a Et_2O/EtOAc mixture (25/75, v/v) allowed the purification of **38** as a yellow powder (2.36 g, 64% yield). Mp: 95 °C.

7-Methoxy-4-(8-aza-5-azoniaspiro[4.5]decan-8-yl)pyrrolo[1,2-*a*]quinoxaline Bromide (39). 38 (2.0 g, 7 mmol), 1,4-dibromobutane (1.68 g, 8 mmol), K_2CO_3 (2.34 g, 17 mmol), and dibenzo-18-crown-6 (30 mg) were dissolved in EtOH (30 mL), and the mixture was heated at 80 °C for 24 h. The hot mixture was filtered and then concentrated to about one-third. The precipitate was filtered and washed with anhydrous Et_2O. In the filtrate appeared a new precipitate which was filtered 1 day later. This gave **39** as a white powder (1.46 g, 49% yield). Mp: >270 °C.

4-Chloropyrido[2,3-*h*]pyrrolo[1,2-*a*]quinoxaline (46). 45 (9,5 g, 40 mmol) reacted with the same method as that described to obtain **27a**. This gave **46** as a yellow powder (5.3 g, 51% yield). Mp: 210 °C (MeCN).

Pharmacological Methods: Binding Experiments. 5-HT$_{1A}$ receptor binding to bovine frontal cortex and hippocampus membranes was determined using a slight modification of the method of Hoyer et al.[47] Membranes (0.5 mg of protein/mL) were incubated at 23° C for 40 min with 0.5 nM [^{3}H]-8-OH-DPAT in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM buspirone.

5-HT$_{1B}$ receptor binding to rat frontal cortex and striatum membranes was determined using the method of Peroutka[48] with slight modifications. Membranes (0.8 mg of protein/mL) were incubated at 25 °C for 30 min with 2 nM [^{3}H]-5-OH-tryptamine, 1 μM spiperone, and 50 nM mesulergine in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM propranolol.

5-HT$_{1D}$ receptor binding to pig frontal cortex and striatum membranes was determined following the pocedure of Waeber et al.[49] with slight modifications. Membranes (0.8 mg of protein/mL) were incubated at 25 °C for 30 min with 2 nM [^{3}H]-5-OH-tryptamine, 1 μM spiperone, and 50 nM mesulergine in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM 5-HT.

5-HT$_{2A}$ receptor binding to bovine frontal cortex membranes was determined as described by Leysen et al.[50] Membranes (0.6 mg of protein/mL) were incubated at 37 °C for 30 min with 0.8 nM [^{3}H]ketanserin and 100 nM WB4101 in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 5 mM $MgCl_2$, 10 mM NaCl, 0.5 mM EDTA, and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM spiperone.

5-HT$_{2C}$ receptor binding to pig choroid plexus membranes was determined following a slight adaptation of the protocol of Sanders-Bush and Breeding.[51] Membranes (0.2 mg of protein/mL) were incubated at 25 °C for 60 min with 1.2 nM [^{3}H]-*N*-methylmesulergine and 1 μM spiperone in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 4 mM $CaCl_2$ and 10 μM pargyline. Nonspecific binding was determined in the presence of 10 μM mianserin.

5-HT$_3$ receptor binding to NG 108-15 cell membranes was determined following a slight modification of the procedure of Hoyer and Neijt.[52] Membranes (0.5 mg of protein/mL) were incubated at 25 °C for 60 min with 1 nM [^{3}H]granisetron in 50 mM Tris-HCl buffer, pH 7.4, supplemented with 25 mM NaCl. Nonspecific binding was determined in the presence of 10^{-5} M tropisetron.

The affinity of the ligands tested for these receptors was calculated using LUNDON2 Software and expressed as log $IC_{50} \pm$ SEM (IC_{50} = concentration inhibiting 50% of the specific binding). The results obtained are reported in Tables 1−3.

[^{14}C]Guanidinium Influx into NG 108-15 Cells.[15] Cells were grown for 2 days in 35-mm culture dishes with 3 mL of growth medium.[15] Before the experiment was started, the cell layer was washed twice with 1.5 mL of buffer A (145 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose, and 20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfanic acid (HEPES), the pH being adjusted to 7.4 with NaOH). The incubation (10 min at 37 °C) was then performed in 1 mL of buffer B (135 mM NaCl, 4.5 mM KCI, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Na_2HPO_4, 20 mM glucose, 20 mM HEPES, pH 7.4 with NaOH), supplemented with 10 mM guanidinium chloride, 200−250 nCi (7.40−9.25 kBq) of [^{14}C]guanidinium, 10 μM substance P, and the appropriate drugs.

The incubation was stopped by aspiration of the medium, and the cell layer was washed three times with 1.5 mL of ice-cold buffer C (same composition as buffer A except that NaCl was replaced by choline chloride). The cells were then dissolved in 0.5 mL of 0.4 N NaOH, and extracts were transferred to scintillation vials. The culture dishes were rinsed with 0.5 mL of 1 N HCl and 0.5 mL of 0.4 N NaOH, which were mixed with the first extract for the quantification of radioactivity in the presence of 10 mL of Aquasol (NEN). For each experiment, the protein content of a control dish was determined.[15]

Bezold−Jarisch Reflex in Rats. Male Crl:CD(SD)BR rats (Charles River) weighing 280−320 g were fasted for 24 h and then anesthetized with urethane (1.25 g/kg ip). In order to monitor the Bezold−Jarisch reflex (an abrupt dose-related reduction in heart rate following a rapid iv bolus injection of 5-HT, 30 μg/kg), the carotid artery was cannulated and connected to a Statham transducer, as described by Richardson et al.[9] Heart rate and blood pressure were monitored by using the pressure transducer signal and a cardiotachymeter coupler and recorded onto a Gemini polygraph (Ugo Basile, Italy). Test compounds were dissolved in saline and administered intravenously (0.5 mL/kg) via a cannula inserted into the jugular vein. To test antagonist activity, compounds were usually administered iv 5 min before 5-HT (30 μg/kg).

Light/Dark Test. The anxiolytic-like activity of the compounds was tested using an unconditioned conflict test, the light/dark test, behaviorally validated for detecting antianxiety agents in mice.[40,42]

In brief, the apparatus consisted of two poly(vinyl chloride) tools covered by plexiglass. One of these boxes was darkened, and the other was lightened by a lamp. Mice were placed in the lit box at the beginning of the test session. The amount of time spent by mice in the lit box (TLB) and the number of transitions through the tunnel between the two boxes were recorded over a 5-min period, after the first entry into the dark box. A mouse with all four paws in the new box was considered as having changed box. Tested compounds were administered *per os* from 0.1 μg/kg to 1 mg/kg. The lack of sedative or excitatory effects of the compounds at the tested doses was previously assessed in a free exploratory test. The statistical significance of differences between control and treated groups was ascertained by a combined analysis of variance and a Bonferroni's posteriori *t*-test.

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