Novel and Highly Potent 5-HT$_3$ Receptor Agonists Based on a Pyrroloquinoxaline Structure†

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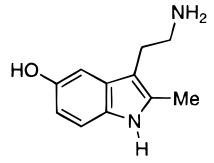
The synthesis and the biological evaluation of a series of novel pyrroloquinoxaline derivatives are described. In binding studies several compounds proved to be potent and selective 5-HT$_3$ receptor ligands. The most active pyrroloquinoxalines, **11d** and **11e**, showed a subnanomolar affinity for 5-HT$_3$ receptor and were able to functionally discriminate the central and peripheral 5-HT$_3$ receptors, being agonists and antagonists, respectively. In functional studies ([^{14}C]-guanidinium accumulation test in NG 108-15 cells, *in vitro*) most of the synthesized compounds showed clear-cut 5-HT$_3$ agonist properties. In *in vivo* studies on the von Bezold−Jarisch reflex test (a peripheral interaction model) the behavior of the tested compounds ranged from agonist to antagonist, while clear agonist properties were obtained with **12a** on cortical acetylcholine release in freely moving rats. Pharmacokinetic studies with **11e** and **12c** indicate that the compounds easily cross the blood−brain barrier (BBB) after systemic administration with a brain/plasma ratio of 17.5 and 37.5, respectively. Thus compounds **11e** and **12c** represent the most potent central 5-HT$_3$ agonists identified to date that are able to cross the blood−brain barrier.

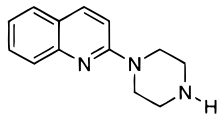
Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter involved in many biological processes at both the central and peripheral level, and its receptors are classified into several subtypes. Among the different subtypes, the 5-HT$_3$ receptor has attracted considerable interest in the last decade, especially with the synthesis of potent and selective antagonists, such as granisetron and ondansetron. On the contrary, few and poorly selective agonists have been developed: among them the 2-methyl-5-HT, **1** (Chart 1), is an agonist rapidly metabolized *in vivo* and (likewise phenylbiguanide derivatives) is unable to cross the blood−brain barrier (BBB).[1] Thus its usefulness is largely restricted to *in vitro* models. Quipazine, **2**, has been reported to behave as a 5-HT$_3$ receptor antagonist in peripheral models,[2] but subsequent studies revealed its 5-HT$_3$ agonism in [^{14}C]guanidinium accumulation in NG 108-15 cells.[3] This pharmacological property, taken together with the fact that the 5-HT$_3$ receptors on NG 108-15 cells and on rat cerebral cortex show common pharmacological and physiochemical properties,[4a] makes quipazine a useful lead structure[4b] for designing more potent and selective central 5-HT$_3$ agonists. The potential therapeutic role of 5-HT$_3$ receptor agonists is based on their modulation of acetylcholine release *in vivo*,[5] which makes these compounds of interest for the treatment of neurodegenerative and neuropsychiatric disorders in which cholinergic neurons are affected. Consequently, there is increasing and considerable interest in the development of potent and selective 5-HT$_3$ receptor agonists. In order to design novel 5-HT$_3$ receptor agonists, the structure of quipazine has been modified by the introduction of the lipophilic pyrrole ring on the quinoline system. However, this structural alteration could lead to compounds interacting with other 5-HT receptor subtypes (e.g. 5-HT$_{1B}$), as in the case of CGS 12066B (**3**).[6] We previously described a novel synthetic approach to pyrroloquinoxaline derivatives[7] (CGS 12066B **3** and related analogues), and later preliminary and unpublished studies revealed that the electron-withdrawing and bulky trifluoromethyl group of **3** was deleterious for 5-HT$_3$ receptor affinity, while its des-CF$_3$ analogue showed a subnanomolar affinity for the same 5-HT receptor subtype. These results stimulated our interest in the development of a series of compounds with potent and selective central 5-HT$_3$

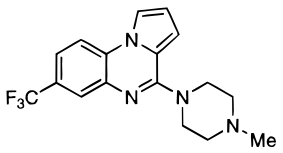
Chart 1



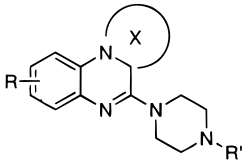
1 2-Me-5-HT



2 quipazine



3 CGS 12066B



4 General Structure for Title Compounds

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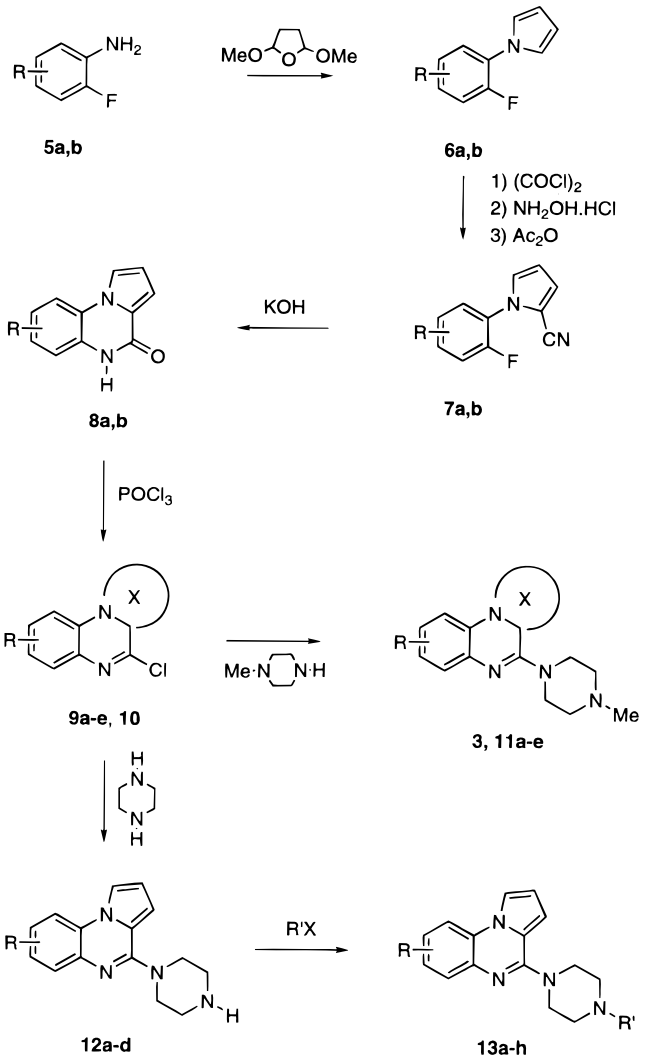
Scheme 1



receptor agonism, based on a pyrroloquinoxaline skeleton (**4**). While this work was in progress, related compounds have been reported in literature,[8] but none of the quinoxalines and thieno derivatives described show central full agonist properties. So, in the present article we report the development of potent central 5-HT$_3$ full agonists. The effect of the introduction of a fluorine atom in the benzo-fused ring with respect to the 5-HT$_3$ selectivity has also been explored, and potent and selective agonists have been developed. The potential 5-HT$_3$ receptor agonist properties were evaluated *in vitro* in [^{14}C]guanidinium accumulation test on NG 108-15 cells,[3] and *in vivo* on the von Bezold-Jarisch reflex test[9] (these data provide further support to functional properties of the 5-HT$_3$ receptors being different in cardiac tissue and in NG 108-15 cells) and on acetylcholine release in frontal cortex of freely moving rats which is modulated by 5-HT$_3$ receptors.[10a] In addition, brain and plasma concentrations of **11e** and **12c** were simultaneously measured, after systemic injection, using a newly developed HPLC procedure with UV detection.

Chemistry

The new 4-chloropyrrolo[1,2-*a*]quinoxalines **9a,b** were synthesized as shown in Scheme 1, according to the procedure utilized to prepare already known **9c**−**e**,[7] while the intermediate 2-chloroquinoxaline (**10**) was prepared according to a literature procedure.[11a] The appropriate anilines (**5a,b**) bearing a fluorine atom at position 2 as a leaving group were chosen as starting materials. These latter were subjected to the Clauson−Kaas reaction to give the corresponding arylpyrroles **6a,b**, which were transformed into 1-aryl-2-cyanopyrroles **7a,b** through a one-pot sequence involving formilation (replacing $POCl_3$ with oxalyl chloride[7]), oximation, and dehydration. The procedure also furnished small amounts of the isomeric 3-nitriles. The nitriles **7a,b** were successively cyclized to the pyrrolo[1,2-*a*]quinoxalinones **8a,b** by treatment with potassium hydroxide in ethylene glycol at 145 °C. This one-pot transformation of nitriles into lactams involves selective hydrolysis to amides, which are able to carry out intramolecular displacement of aromatic fluorine. By treatment with phosphoryl chloride, **8a,b** were transformed into the 4-chloro derivatives **9a,b**. The chloro derivatives **9a**−**e** and **10** were then reacted with excess of *N*-methylpiperazine to give **11a**−**e**, while the piperazine derivatives **13a**−**h** were synthesized by alkylation with the appropriate alkyl halides of **12a**−**d**, in turn prepared by reaction of **9b**−**e** with an excess of piperazine. Phisical and chemical data of the described compounds are reported in Table 1.

Results and Discussion

1. 5-HT$_3$ Binding SAR Study.[11b] The affinities of the new pyrroloquinoxaline derivatives **3**, **11a**−**e**, **12a,c**, and **13a**−**h** for the 5-HT$_3$ receptors in rat cortex homogenate are illustrated in Table 2. The binding data represent the ability of the tested compounds to displace [^{3}H]BRL 43694 from the receptor protein. Binding data of quipazine is also included. In Table 3 the affinity of selected compounds on different 5-HT receptor subtypes is reported. The results show that the introduction of a lipophilic pyrrole moiety on the *c*-edge of the quinoline system of quipazine led to more potent 5-HT$_3$ receptor ligands (e.g. **11d**), maintaining the same selectivity ratios versus the other 5-HT receptor subtypes, as for quipazine, and showing (**11d**) an improved selectivity vs 5-HT$_{1B}$ and the serotonin uptake site. On the contrary, the presence of an extra nitrogen atom on the quipazine structure diminished significantly the receptor affinity (**11c**). These data are consistent with hypothesized favorable lipophilic interactions of the pyrrole portion with the corresponding area in the receptor protein. Since the introduction of the pyrrole moiety was revealed to be crucial for 5-HT$_3$ receptor potency and for selectivity, to further identify structural requirements capable of modulating the affinity and selectivity, the SAR study was carried out as a function of (i) the position of a fluorine atom in the benzo-fused ring and (ii) the nature and hindrance of the alkyl chain at position 4 in the piperazine ring.

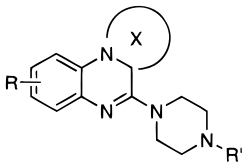
(i) With respect to the unsubstituted pyrroloquinoxaline **11d** (5-HT$_3$ binding IC_{50} = 0.37 nM), the introduction of a bulky trifluoromethyl group at position 7 (CGS 12066B, **3**) plays a key role in the 5-HT receptor subtype selectivity.[12] In fact, compound **3** is devoid of any 5-HT$_3$ receptor affinity, while **11d** shows a subnanomolar 5-HT$_3$ receptor affinity and an affinity for 5-HT$_{1A}$, 5-HT$_{2A}$ receptors in the higher nanomolar range. On the other hand, since the trifluoromethyl group is deleterious for 5-HT$_3$ affinity (**11d** vs CGS 12066B, **3**),

Table 1. Physical and Chemical Data for Compounds **6**−**13**

compd	R	R′	X	% yield[a]	mp (°C)	recryst/solvent	formula	analysis[b]
3[c]	7-CF_3		pyrrolo					
6a	3-F			76			$C_{10}H_7F_2N$	C,H,N
6b	6-F			83	76−77	hexanes	$C_{10}H_7F_2N$	C,H,N
7a	3-F			54	49	hexanes	$C_{11}H_6F_2N_2$	C,H,N
7b	6-F			79	77−78	hexanes	$C_{11}H_6F_2N_2$	C,H,N
8a	6-F			94	265−266	EtOAc	$C_{11}H_7FN_2O$	C,H,N
8b	9-F			80	273−274	EtOAc	$C_{11}H_7FN_2O$	C,H,N
9a	6-F		pyrrolo	95	201−202	hexanes	$C_{11}H_6ClFN_2$	C,H,N
9b	9-F		pyrrolo	72	183−184	hexanes	$C_{11}H_6ClFN_2$	C,H,N
9c[c]	H		pyrrolo					
9d[c]	7-F		pyrrolo					
9e[c]	7-CF_3		pyrrolo					
11a	6-F		pyrrolo	97			$C_{16}H_{17}FN_4$	C,H,N
11b	9-F		pyrrolo	85	108−109	hexanes	$C_{16}H_{17}FN_4$	C,H,N
11c	H			80	107−108	hexanes	$C_{13}H_{16}N_4$	C,H,N
11d[c]	H		pyrrolo					
11e[c]	7-F		pyrrolo					
12a	9-F			91	128−129	EtOAc	$C_{15}H_{15}FN_4$	C,H,N
12b	H			94	147−148	EtOAc	$C_{15}H_{16}N_4$	C,H,N
12c	7-F				130−131	hexanes	$C_{15}H_{15}FN_4$	C,H,N
12d	7-CF_3				154−155	hexanes	$C_{16}H_{15}F_3N_4$	C,H,N
13a	9-F	*n*-propyl		74	48−49	hexanes	$C_{18}H_{21}FN_4$	C,H,N
13b	9-F	allyl		84	44−45	hexanes	$C_{18}H_{19}FN_4$	C,H,N
13c	H	ethyl		87			$C_{17}H_{20}N_4$	C,H,N
13d	H	*n*-propyl		81			$C_{18}H_{22}N_4$	C,H,N
13e	H	allyl		73			$C_{18}H_{20}N_4$	C,H,N
13f	7-F	*n*-propyl		78	69−70	hexanes	$C_{18}H_{21}FN_4$	C,H,N
13g	7-F	allyl		81	53−54	hexanes	$C_{18}H_{19}FN_4$	C,H,N
13h	7-CF_3	allyl		69	97−98	pentane	$C_{19}H_{19}F_3N_4$	C,H,N

[a] Yields refer to isolated and purified materials. [b] All the compounds were analyzed within ±0.4% of the theoretical values. [c] Reference 7.

Table 2. 5-HT_3 Receptor Affinity and Functional Behavior of Compounds **3**, **11a**−**e, 12a,c**, and **13a**−**h**



3, 11a-e, 12a,c, 13a-h

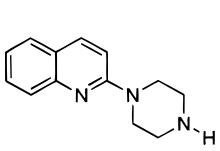
compd	R	R′	X	IC_{50} (nM)[a] (±SEM)	effect on [^{14}C]guanidinium uptake on NG 108-15 cells: efficacy[b]	effect on [^{14}C]guanidinium uptake on NG 108-15 cells: EC_{50} (nM)	von Bezold−Jarisch reflex test: behavior[b]	von Bezold−Jarisch reflex test: ID_{50} (μg/kg iv)
3	7-CF_3	Me	pyrrolo	NA[c]	NA		Ant	300
11a	6-F	Me	pyrrolo	9.5 ± 1	A	4.7 ± 0.8	PA	120
11b	9-F	Me	pyrrolo	0.44 ± 0.05	A	6.5 ± 1	PA	120
11c	H	Me		22 ± 3	A	510 ± 120	A	60−120[d]
11d	H	Me	pyrrolo	0.37 ± 0.06	A	10 ± 3	Ant	300
11e	7-F	Me	pyrrolo	0.81 ± 0.05	A	3.0 ± 0.4	Ant	600
12a	9-F	H	pyrrolo	3.9 ± 0.4	A	98 ± 25	Ant	70
12c	7-F	H	pyrrolo	5.4 ± 0.8	A	3.5 ± 0.6	Ant	10
13a	9-F	*n*-propyl	pyrrolo	82 ± 23	A	73 ± 15	PA	300
13b	9-F	allyl	pyrrolo	28 ± 3	A	30 ± 4	PA	75
13c	H	ethyl	pyrrolo	9 ± 0.5	A	8.0 ± 1	Ant	100
13d	H	*n*-propyl	pyrrolo	51 ± 3	A	8.5 ± 2	PA	240
13e	H	allyl	pyrrolo	11 ± 1	A	8.2 ± 2	PA	180
13f	7-F	*n*-propyl	pyrrolo	88 ± 15	A	580 ± 110	PA	200
13g	7-F	allyl	pyrrolo	22 ± 2	A	76 ± 12	PA	100
13h	7-CF_3	allyl	pyrrolo	NA[c]	NA		Ant	240
quipazine				6.2 ± 0.7	A	25 ± 5		

[a] Each value is the mean ± SEM of three determinations and represent the concentration giving half-maximal inhibition of [^{3}H]BRL43694 binding to rat cortical homogenate. [b] A = pure agonist. PA = partial agonist. Ant = pure antagonist. NA = not active. [c] NA at 10 000 nM. [d] ED_{50}.

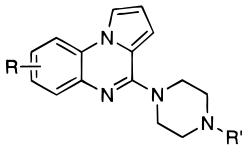
the effect of a smaller fluorine substituent in the benzo-fused ring was evaluated. The introduction of a fluorine atom at positions 7 and 9 led to potent 5-HT_3 receptor ligands, with equal affinity for the unsubstituted **11d** (**11b** and **11e** vs **11d**), while a 6-F substituent was found to decrease the affinity (**11a** vs **11d**). Furthermore, the presence of a fluorine atom at position 7 slightly improve the selectivity: in particular **11e** shows a reduced 5-HT_{2A} affinity (**11e** vs **11d**).

(ii) Among different N-substituents, the methyl group was found to be optimal for 5-HT_3 receptor affinity. Removal of the methyl group led to slightly less potent pyrroloquinoxaline derivatives (**12a,c** vs **11b,e**), while increasing the hindrance with larger lipophilic chains

Table 3. Binding Profile of Compounds **11d,e** and **12a,c** on 5-HT Receptor Subtypes



quipazine



11d,e, 12a,c

			IC$_{50}$ (nM)[a] (±SEM)						
compd	R	R′	5-HT$_3$	5-HT$_{1A}$[d]	5-HT$_{1B}$[e]	5-HT$_{2A}$[f]	5-HT$_{2C}$[g]	5-HT$_4$[h]	5-HT$_{uptake}$[i]
quipazine			6.2 ± 0.7	2700 ± 235 (435)	380 ± 31 (61)	1400 ± 138 (226)	470 ± 42 (76)	>10000 (>1613)	204 ± 27 (33)
11d	H	Me	0.37 ± 0.06[b]	89 ± 7 (240)	232 ± 24 (627)	43 ± 18 (116)	780 ± 320 (2108)	>10000 (>27027)	214 ± 34 (578)
11e	7-F	Me	0.81 ± 0.05[b]	138 ± 14 (170)	371 ± 58 (458)	124 ± 45 (153)	413 ± 134 (510)	>10000 (>12345)	232 ± 20 (286)
12a	9-F	H	3.9 ± 0.4[b]	268 ± 46 (69)	106 ± 13 (27)	761 ± 237 (135)	784 ± 132 (201)	>10000 (>2564)	1129 ± 295 (289)
12c	7-F	H	5.4 ± 0.8[b]	151 ± 24 (28)	167 ± 23 (31)	489 ± 278 (90)	2803 ± 694 (519)	>10000 (>1852)	203 ± 61 (37)
5-HT			727 ± 200[c]	2.6 ± 0.4	4.6 ± 0.5	2350 ± 352	42 ± 1	147 ± 25	369 ± 80
methysergide						3.1 ± 0.4			
mesulergine							2.13 ± 0.38		
GR 38032			11 ± 2[c]						
2-Me-5-HT			779 ± 171[b]						

[a] The concentration of the tested compounds that inhibited the radiolabelled ligand binding to rat cortex homogenate by 50% (IC$_{50}$) was determined by dose−inhibition curves with six concentrations of the displacers, each performed in triplicate. Each value in parentheses is the selectivity ratio calculated as the ratio between the IC$_{50}$ value for the indicated 5-HT receptor subtype over the IC$_{50}$ value for 5-HT$_3$ receptor. Values are the mean ± SE of at least three separate experiments performed in triplicate. [b] [^{3}H]BRL 43694. [c] [^{3}H]Zacopride. [d] [^{3}H]8-OH-DPAT. [e] [^{3}H]5-HT. [f] [^{3}H]Ketanserin. [g] [^{3}H]Mesulergine. [h] [^{3}H]GR 113808. [i] [^{3}H]Citalopram.

such as ethyl, propyl, and allyl gave a progressive drop in 5-HT$_3$ receptor affinity, despite Rault's observations on analogous pyrrolothienopyrazines.[8b]

2. Biological Activity. In addition to binding studies, two functional assays were carried out for assessing the potential agonist/antagonist activity of the whole set of pyrroloquinoxaline derivatives. These two different 5-HT$_3$-dependent responses were the uptake of [^{14}C]guanidinium, which is markedly increased upon the activation of 5-HT$_3$ receptors on the neuroblastoma-glioma hybrid NG 108-15 cells, and the von Bezold−Jarish reflex, which corresponds to a deep but transient bradycardia triggered by the stimulation of cardiac 5-HT$_3$ receptors. The most intriguing result risen up from these pharmacological experiments was that most of the described compounds mimicked the effects of 5-HT on the 5-HT$_3$ receptor-dependent accumulation of [^{14}C]guanidinium in NG 108-15 hybridoma cells[3] up to concentrations in the low nanomolar range, indicating that the novel pyrroloquinoxaline derivatives acted as potent agonists in this assay. Similarly to that observed in binding experiments, the introduction of a pyrrole ring at the *c*-edge of the quinoline system of a quipazine improved the potency (**11d**) in guanidinium uptake test, while the introduction of a nitrogen atom drammatically reduced the potency in this functional test (**11c**). It is noteworthy that the most active members of this series of central 5-HT$_3$ receptor agonists show a fluorine atom at position 7 (**11e** and **12c**), and they appear to be the most potent agonists, in this test, to have been described until to date. Furthermore a good correlation of the binding data (cerebral cortex) and of the EC$_{50}$ values ([^{14}C]guanidinium uptake) was found in this series of central 5-HT$_3$ agonists, whereas the poor correlation shown by **11d** might be due to the complex kinetics of its interaction with 5-HT$_3$ receptors in NG 108-15 cells (data not shown). On the other hand investigations on the 5-HT$_3$ receptor-dependent von Bezold−Jarisch reflex in urethane-anesthetized rats[9] proved a different behavior for the tested compounds ranging from the agonist properties of **11c** to the full antagonist properties of **11d,e**, **12a,c**, and **13c,h** through the partial agonist properties of compounds **11a,b** and **13a,b,d−g**.

Accordingly, compound **11c** (agonist) (at 60−120 μg/kg, iv) produced a transient bradycardia which was as pronunced as that observed after the iv injection of 40 μg/kg of phenylbiguanide. At the same doses, **11c** only exerted marginal effects on the bradycardia due to the latter drug, indicating that its potential antagonist properties at cardiac 5-HT$_3$ receptor are most negligible. On the contrary, a slight bradycardia was observed in rats which had been treated with 10−100 μg/kg iv of compound **12c** alone. However, this effect did not exhibit a clear dependence on the dose. In contrast, the inhibition by **12c**, representative of the antagonists, of phenylbiguanide-induced bradicardia was clearly dose-dependent with an ID$_{50}$ value close to 10 μg/kg iv. These data led to the conclusion that **12c** is a potent antagonist at cardiac 5-HT$_3$ receptors. In addition, it had a rather long duration of action since a reduction in the cardiac response to phenylbiguanide was still observed 1 h after administration of 100 μg/kg iv of **12c**.

Quinoxaline **11a**, representative of the partial agonist compounds, at the dose of 60 μg/kg iv, induces a transient bradycardia which is about 65% of that due to 40 μg/kg iv of the potent 5-HT$_3$ receptor agonist phenylbiguanide. In addition to this agonist effect, **11a** also exerts an antagonist action since it can prevent phenylbiguanide-induced bradycardia. In this case the effect of **11a** is clearly dose-dependent, with half-blockade at 0.12 mg/kg iv, and maximal blockade at 0.4 mg/kg iv when this drug is injected 5 min before

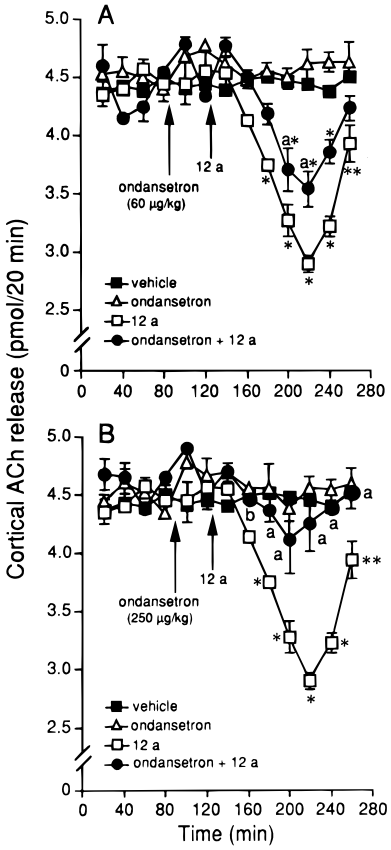


Figure 1. Effect of **12a** and ondansetron, alone or together, on ACh release in rat frontal cortex. Ondansetron at the dose of 60 μg sc (A) or 250 μg sc (B) was administered after collection of four base-line fractions, 20-min each fraction. **12a** (30 nmol icv per side) was given 20 min after ondansetron. For each time point, data are means ± SEM (vertical bars) (n = 4), expressed as picomoles of ACh released/20 min. Split-plot ANOVA followed by Turkey's test for unconfounded means revealed a significant difference in ACh release between **12a** (in A and B) group, and the [ondansetron + **12a**] group (in A) with the veichle group (*P < 0.05; *P < 0.01), and a significant interaction between [**12a**] vs [ondansetron + **12a**] groups.

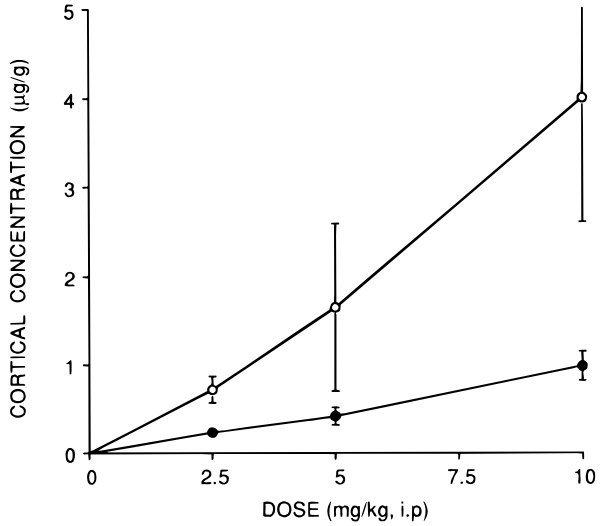


Figure 2. Relationship between the **11e** and **12c** dose and mean cortical concentrations at 60 min after dosing. Each point is the mean (±SD) of four animals for **11e** (●) and **12c** (○).

phenylbiguanide. So, we concluded that **11a** is a mixed agonist−antagonist at peripheral 5-HT_3 receptors.

The different behavior observed in the functional studies suggested that the tested compounds are able to functionally discriminate between the central and the peripheral 5-HT_3 receptors (e.g. **12c** behaved as a potent full agonist on [^{14}C]guanidinium accumulation test and as a potent full antagonist in von Bezold−Jarisch reflex test).

***In Vivo* Studies. 1. Acetylcholine Release.** In previous studies we have shown that 2-Me-5-HT, given icv, reduces acetylcholine release in rat cortex by a mechanism which is blocked by ondansetron.[10a] Thus, for comparison, we have studied the effect of **12a** using the same treatment schedule as for 2-Me-5-HT. The 5-HT_3 receptor agonist **12a** (as hydrochloride) reduced the cortical extracellular ACh content of freely moving rats (Figure 1A,B). Its inhibitory effect reached its nadir of 34% below the basal values at 100 min after its administration. This effect was quantitatively similar to the effect obtained with 1−2 μmol icv of 2-Me-5-HT.[10a] However, for a direct comparison of the *in vivo* potencies of the two 5-HT_3 agonists, a full response−dose study is in progress with **12a**. Ondansetron, a selective 5-HT_3 serotoninergic antagonist, at 60 μg sc or 250 μg sc did not affect by itself the ACh content of frontal cortex perfusates during the entire 200-min collection period, but at 250 μg icv, it completely prevented the lowering effect of compound **12a**. At the lower dose 30 μg icv, ondansetron elicited only a partial antagonism.

2. Brain Distribution Studies. The previously known 5-HT3 agonists 2-Me-5-HT and phenylbiguanide derivatives do not cross the BBB, thus making difficult their use by systemic route administration. In order to demonstrate the brain accumulation of our compounds, we studied the brain and plasma concentrations of two representative compounds of our series, **11e** and **12c**. Previous kinetic studies with arylpiperazines indicated that in the rat most of these compounds are rapidly adsorbed and eliminated with half-lives of about 1 h. Therefore in these preliminary studies the brain-to-plasma partition ratio was investigated 1 h after dosing, when presumably pseudoequilibrium will be achieved for both the investigated compounds. As quipazine and structurally related compounds,[13] these new pyrroloquinoxaline derivatives rapidly enter the rat brain, achieving concentrations higher than in the plasma. Figure 2 illustrates the relationships between the dose and the mean cortical concentrations of **11e** and **12c** measured 60 min after dosing. The mean brain concentration of the two compounds increased from the lowest to the highest dose, although the proportion was better for **11e** (r^2 = 0.91) than for **12c** (r^2 = 0.73). The mean brain concentrations of **12c** were approximately 4 times those of **11e**, although there was variability in brain (and plasma) concentrations of both compounds, but particularly with **12c** at the higher doses. At 10 mg/kg, for instance, the coefficient of variation (CV) was 36% compared to a 16% CV for **11e** brain concentrations (0.98 ± 0.16 μg/g) at the same dose. With both compounds the brain drug concentrations were a linear function of plasma drug concentrations (Figure 3), with **12c** having a larger brain to plasma ratio (brain concentration = 38.5−0.01 plasma concentration; r^2 = 0.95) than **11e** (brain concentration = 18.5−0.04 plasma concentration; r^2 = 0.89). There was no evidence of preferential concentration of the two compounds in any

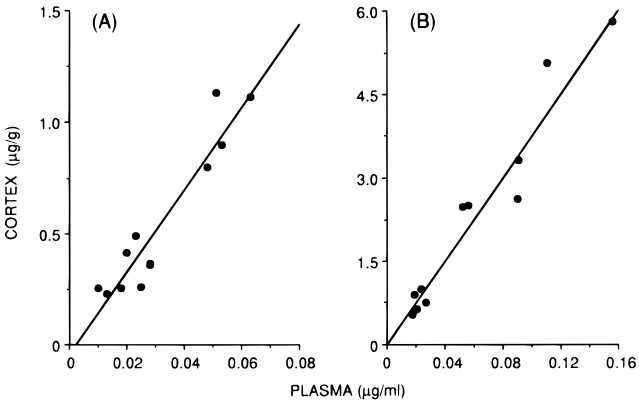


Figure 3. Relationship between plasma and brain (cortex) concentrations. Each point represents the plasma and brain concentrations of **11e** (A) and **12c** (B) for an individual rat.

of the brain region examined. One hour after dosing, the concentrations in hippocampus, striatum, and hypothalamus were comparable to those in cortex (data not shown).

Conclusions

By appropriate structural modification of the quinoline system of quipazine, this work has resulted in the discovery of more potent and selective 5-HT$_3$ receptor ligands. By virtue of their agonist potencies displayed in [^{14}C]guanidinium accumulation test, on *in vivo* acetylcholine release, and of their ability to cross the blood−brain barrier, these pyrroloquinoxalines represent potent pharmacological tools to explore the central 5-HT$_3$ receptor-mediated functions.

Experimental Procedures

Melting points were determined using an Electrothermal 8103 apparatus and are uncorrected. IR spectra were taken with Perkin-Elmer 398 and FT 1600 spectrophotometers. ^{1}H-NMR spectra were recorded on a Bruker 200 MHz spectrometer with TMS as internal standard; the values of chemical shifts (δ) are given in ppm and coupling constants (J) in hertz. All reactions were carried out in an argon atmosphere. Progress of the reaction was monitored by TLC on silica gel plates (Riedel-de-Haen, Art. 37341). Merck silica gel (Kieselgel 60) was used for chromatography (70−230 mesh) and flash chromatography (230−400 mesh) columns. Extracts were dried over $MgSO_4$, and solvents were removed under reduced pressure. Elemental analyses were performed on a Perkin-Elmer 240C elemental analyzer, and the results are within ±0.4% of the theoretical values, unless otherwise noted. Yields refer to purified products and are not optimized. Physical data for compounds **3** and **6**−**13** are reported in Table 1.

1-(2,3-Difluorophenyl)-1*H*-pyrrole (6a). To a solution of 2,3-difluoroaniline **5a** (1.0 g, 7.7 mmol) in glacial acetic acid (5 mL) was slowly added 2,5-dimethoxytetrahydrofuran (1.0 mL, 7.8 mmol). The reaction mixture was refluxed for 1 h, cooled, and evaporated to afford an oily residue which was chromatographed (EtOAc) to give 1.05 g of **6a** as a colorless oil: IR ($CHCl_3$) 3420 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.37 (m, 1 H), 7.00−7.24 (m, 5 H). Anal. ($C_{10}H_7F_2N$) C, H, N.

1-(2,6-Difluorophenyl)-1*H*-pyrrole (6b). Similarly to **6a**, the pyrrole derivative **6b** was prepared starting from 2,6-difluoroaniline **5b**. **6b** was obtained as a colorless oil: bp 132 °C/0.2 mmHg; IR (neat) 3390 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.36 (m, 2 H), 6.89−7.08 (m, 4 H), 7.20 (m, 1 H). Anal. ($C_{10}H_7F_2N$) C, H, N.

1-(2,3-Difluorophenyl)-1*H*-pyrrole-2-carbonitrile (7a). To a cooled (−5 °C) mixture of anhydrous *N*,*N*-dimethylformamide (0.48 mL, 6.2 mmol) and anhydrous 1,2-dichloroethane (3 mL) was slowly added within 20 min a solution of oxalyl chloride (0.54 mL, 6.2 mmol) in 1,2-dichloroethane (3 mL). After the mixture was stirred for 15 min at room temperature and cooled in a ice−water bath, a solution of **6a** (1.05 g, 5.9 mmol) in anhydrous 1,2-dichloroethane (10 mL) was slowly added. The reaction mixture was allowed to stir at room temperature for 4 h, and then a warm solution of hydroxylamine hydrochloride (0.43 g, 6.2 mmol) and dry sodium acetate (0.51 g, 6.2 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) was slowly added. After the mixture was refluxed for 20 h, acetic anhydride (2.3 mL, 24.4 mmol) was added and the solution was refluxed for 4 h. The cooled mixture was treated with 10% sodium carbonate solution until pH 8, diluted with water (50 mL), and extracted with ethyl acetate. The organic layer was washed with brine, dried, and concentrated. The residue was chromatographed (EtOAc) to give 0.65 g of **7a** as colorless prisms: IR ($CHCl_3$) 2221 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.42 (m, 1 H), 7.04−7.09 (m, 2 H), 7.17−7.37 (m, 3 H). Anal. ($C_{11}H_6F_2N_2$) C, H, N.

1-(2,6-Difluorophenyl)-1*H*-pyrrole-2-carbonitrile (7b). Starting from **6b**, the title compound was obtained as colorless prisms: IR ($CHCl_3$) 2220 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.41 (m, 1 H), 7.10 (m, 4 H), 7.45 (m, 1 H). Anal. ($C_{11}H_6F_2N_2$) C, H, N.

6-Fluoropyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (8a). A suspension of **7a** (0.2 g, 1.0 mmol) and 85% potassium hydroxide (0.2 g, 4.0 mmol) in 8 mL of *tert*-butyl alcohol was heated at 80 °C for 2 h under argon. The mixture was then cooled, poured into crushed ice, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and concentrated. The residue was chromatographed (ethyl acetate) to afford 0.18 g of **8a** as a white solid: IR (Nujol) 1670 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.71 (t, 1 H, J = 3.4 Hz), 7.01−7.22 (m, 2 H), 7.31 (d, 1 H, J = 3.9 Hz), 7.44 (d, 1 H, J = 8.8 Hz), 7.66 (d, 1 H, J = 2.9 Hz), 8.31 (br s, 1 H). Anal. ($C_{11}H_7FN_2O$) C, H, N.

9-Fluoropyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (8b). Similarly to **8a**, the lactam **8b** was prepared starting from **7b**, and using ethylene glycol as solvent. **8b** was obtained as a white solid: IR (Nujol) 1665 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.73 (m, 1 H), 7.21 (m, 4 H), 7.33 (m, 1 H), 8.00 (s, 1 H). Anal. ($C_{11}H_7FN_2O$) C, H, N.

4-Chloro-6-fluoropyrrolo[1,2-*a*]quinoxaline (9a). A mixture of **8a** (0.3 g, 1.5 mmol) and phosphorus oxychloride (4 mL) was refluxed for 4 h under argon, cooled, poured into crushed ice, and extracted with dichloromethane. The organic layers were washed with brine, dried, and concentrated. The residue was chromatographed (dichloromethane) and recrystallized to give 0.31 g of **9a** as colorless prisms: IR ($CHCl_3$) 3320 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.93 (m, 1 H), 7.10−7.25 (m, 2 H), 7.43−7.65 (m, 2 H), 7.95 (d, 1 H, J = 3.1 Hz). Anal. ($C_{11}H_6ClFN_2$) C, H, N.

4-Chloro-9-fluoropyrrolo[1,2-*a*]quinoxaline (9b). Similarly to **9a**, the chloro derivative **9b** was prepared starting from **8b**. **9b** was obtained as a white solid: IR (Nujol) 3300 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.83 (m, 1 H), 7.21−7.33 (m, 2 H), 7.65 (m, 3 H). Anal. ($C_{11}H_6ClFN_2$) C, H, N.

6-Fluoro-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (11a). A mixture of **9a** (0.2 g, 0.91 mmol) and *N*-methylpiperazine (2 mL) was heated at 130 °C for 2 h, under argon, cooled, poured into crushed ice, and extracted with ethyl ether. The combined organic extracts were washed with brine, dried, and concentrated. The residue was chromatographed (EtOAc) to give 0.25 g of **11a** as a yellow oil: IR ($CHCl_3$) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3 H), 2.62 (m, 4 H), 3.90 (m, 4 H), 6.77 (t, 1 H, J = 3.4 Hz), 6.83 (d, 1 H, J = 3.7 Hz), 7.02−7.22 (m, 2 H), 7.50 (d, 1 H, J = 7.8 Hz), 7.78 (d, 1 H, J = 2.7 Hz). Anal. ($C_{16}H_{17}FN_4$) C, H, N.

9-Fluoro-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (11b). Similarly to the procedure as described for **11a**, the title compound was prepared starting from **9b**. After recrystallization **11b** was obtained as a white solid: IR ($CHCl_3$): 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3 H), 2.61 (m, 4 H), 3.85 (m, 4 H), 7.07 (m, 5 H), 8.17 (m, 1 H). Anal. ($C_{16}H_{17}FN_4$) C, H, N.

2-(4-Methylpiperazin-1-yl)quinoxaline (11c). Starting from **10** the title compound was obtained as for **11a**. After recrystallization **11c** was obtained as red-orange prisms. IR ($CHCl_3$) 1609 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3 H), 2.57 (m,

4 H), 3.82 (m, 4 H), 7.39 (t, 1 H, J = 7.3 Hz), 7.57 (t, 1 H, J = 7.3 Hz), 7.68 (d, 1 H, J = 8.4 Hz), 7.87 (d, 1 H, J = 8.3 Hz), 8.58 (s, 1 H). Anal. ($C_{13}H_{16}N_4$) C, H, N.

General Procedure for Preparation of Compounds 12a−d. This procedure is illustrated for the preparation of 9-fluoro-4-(piperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (**12a**). A mixture of **9b** (1.0 g, 4.5 mmol) and dry piperazine (4.0 g, 46.0 mmol) in ethylene glycol (40 mL) was heated at 140 °C for 2 h under argon. After cooling the mixture was poured into crushed ice and extracted with chloroform. The organic layers were washed with brine, dried, and concentrated. The residue was chromatographed (EtOAc) to give 1.12 g of **12a** as colorless prisms: IR ($CHCl_3$) 3320 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.08 (t, 4 H, J = 4.9 Hz), 3.78 (t, 4 H, J = 4.9 Hz), 6.76 (t, 1 H, J = 3.8 Hz), 6.83 (d, 1 H, J = 3.9 Hz), 6.96−7.07 (m, 1 H), 7.15−7.26 (m, 1 H), 7.44 (d, 1 H, J = 7.8 Hz), 8.18 (d, 1 H, J = 2.3 Hz). Anal. ($C_{15}H_{15}FN_4$) C, H, N.

4-(Piperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (12b). Starting from **9c** the title compound was obtained following a procedure as for **12a**. After recrystallization **12b** was obtained as colorless prisms: IR ($CHCl_3$) 1580 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.13 (t, 4 H, J = 5.0 Hz), 3.81 (t, 4 H, J = 5.1 Hz), 6.77 (m, 2 H), 7.26−7.36 (m, 2 H), 7.65−7.75 (m, 2 H), 7.82 (m, 1 H), 8.20 (d, 1 H, J = 2.3 Hz). Anal. ($C_{15}H_{16}N_4$) C, H, N.

7-Fluoro-4-(piperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (12c). Similarly to the procedure as described for **12a**, the title compound was prepared starting from **9d**. After recrystallization **12c** was obtained as a white solid: IR ($CHCl_3$) 1609 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.06 (m, 4 H), 3.80 (m, 4 H), 6.75 (m, 2 H), 6.95 (dt, 1 H, J = 2.4, 8.5 Hz), 7.32 (dd, 1 H, J = 2.4, 10.6 Hz), 7.64 (m, 2 H), 7.76 (d, 1 H, J = 3.2 Hz). Anal. ($C_{15}H_{15}FN_4$) C, H, N.

4-(Piperazin-1-yl)-7-(trifluoromethyl)pyrrolo[2,1-*a*]quinoxaline (12d). Similarly to the procedure as described for **12a**, the title compound was prepared starting from **9e**. After recrystallization **12d** was obtained as a yellowish solid: IR (Nujol) 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.07 (m, 4 H), 3.87 (m, 4 H), 6.80 (m, 2 H), 7.52 (dd, 1 H, J = 1.5, 8.7 Hz), 7.68 (d, 1 H, J = 8.4 Hz), 7.84 (d, 1 H, J = 2.7 Hz), 7.93 (m, 1 H). Anal. ($C_{16}H_{15}F_3N_4$) C, H, N.

General Procedure for Preparation of Compounds 13a−h. This procedure is illustrated for the preparation of 9-fluoro-4-(4-*n*-propylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (**13a**). A mixture of **12a** (0.26 g, 0.96 mmol), anhydrous potassium carbonate (0.13 g, 0.96 mmol), and *n*-propyl iodide (93.5 μL, 0.96 mmol) in ethyl methyl ketone (40 mL) was heated at reflux for 3 h under argon. The solvent was evaporated, and the residue was partitioned between water and dichloromethane. The organic layer was washed with brine, dried, and concentrated. The residue was chromatographed (EtOAc) to give 0.23 g of **13a** as a white solid: IR (Nujol) 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3 H, J = 7.3 Hz), 1.65 (m, 2 H), 2.45 (m, 2 H), 2.71 (t, 4 H, J = 4.7 Hz), 3.91 (t, 4 H, J = 4.9 Hz), 6.76 (t, 1 H, J = 3.4 Hz), 6.83 (d, 1 H, J = 3.9 Hz), 7.00 (m, 1 H), 7.20 (m, 1 H), 7.44 (d, 1 H, J = 8.3 Hz), 8.18 (d, 1 H, J = 2.1 Hz). Anal. ($C_{18}H_{21}FN_4$) C, H, N.

4-(4-Allylpiperazin-1-yl)-9-fluoropyrrolo[1,2-*a*]quinoxaline (13b). Starting from **12a** the title compound was obtained as a colorless oil following a procedure as described for **13a**: IR (Nujol) 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.71 (t, 4 H, J = 4.9 Hz), 3.14 (d, 2 H, J = 6.4 Hz), 3.90 (t, 4 H, J = 4.9 Hz), 5.28 (m, 2 H), 5.90 (m, 1 H), 6.76 (t, 1 H, J = 3.4 Hz), 6.82 (d, 1 H, J = 4.0 Hz), 7.00 (m, 1 H), 7.25 (m, 1 H), 7.44 (d, 1 H, J = 8.4 Hz), 8.18 (d, 1 H, J = 2.7 Hz). Anal. ($C_{18}H_{19}FN_4$) C, H, N.

4-(4-Ethylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (13c). Starting from **12b** the title compound was obtained as a pale yellow oil following a procedure as described for **13a**: IR (Nujol) 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3 H, J = 7.1 Hz), 2.51 (q, 2 H, J = 7.2 Hz), 2.65 (t, 4 H, J = 5.0 Hz), 3.85 (t, 4 H, J = 4.9 Hz), 6.77 (m, 2 H), 7.25 (m, 2 H), 7.68 (m, 2 H), 7.81 (d, 1 H, J = 2.4 Hz). Anal. ($C_{17}H_{20}N_4$) C, H, N.

4-(4-*n*-Propylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (13d). Starting from **12b** the title compound was obtained as a pale yellow oil following a procedure as described for **13a**: IR (Nujol) 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3 H, J = 7.3 Hz), 1.48−1.67 (m, 2 H), 2.40 (m, 2 H), 2.64 (t, 4 H, J = 5.0 Hz), 3.84 (t, 4 H, J = 5.0 Hz), 6.77 (m, 2 H), 7.22−7.36 (m, 2 H), 7.64−7.74 (m, 2 H), 7.80 (d, 1 H, J = 2.8 Hz). Anal. ($C_{18}H_{22}N_4$) C, H, N.

4-(4-Allylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (13e). Starting from **12b** the title compound was obtained as a pale yellow oil following a procedure as described for **13a**: IR (Nujol) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.66 (t, 4 H, J = 5.0 Hz), 3.09 (d, 2 H, J = 6.8 Hz), 3.84 (t, 4 H, J = 4.9 Hz), 5.17−5.28 (m, 2 H), 5.84−6.03 (m, 1 H), 6.77 (m, 2 H), 7.28 (m, 2 H), 7.70 (m, 2 H), 7.80 (m, 1 H). Anal. ($C_{18}H_{20}N_4$) C, H, N.

7-Fluoro-4-(4-*n*-propylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (13f). Similarly to the procedure as described for **13a**, the title compound was prepared starting from **12c** and *n*-propyl bromide. After recrystallization **13f** was obtained as colorless prisms: IR ($CHCl_3$) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3 H, J = 7.4 Hz), 1.55 (m, 2 H), 2.37 (m, 2 H), 2.61 (m, 4 H), 3.85 (m, 4 H), 6.74 (m, 2 H), 6.93 (dt, 1 H, J = 2.8, 8.5 Hz), 7.30 (dd, 1 H, J = 2.5, 9.4 Hz), 7.61 (m, 1 H), 7.72 (m, 1 H). Anal. ($C_{18}H_{21}FN_4$) C, H, N.

4-(4-Allylpiperazin-1-yl)-7-fluoropyrrolo[1,2-*a*]quinoxaline (13g). Similarly to the procedure as described for **13a**, the title compound was prepared starting from **12c** and allyl bromide. After recrystallization **13g** was obtained as colorless prisms: IR ($CHCl_3$) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.61 (m, 4 H), 3.08 (d, 2 H, J = 6.8 Hz), 3.87 (m, 4 H), 5.22 (m, 2 H), 5.91 (m, 1 H), 6.77 (m, 2 H), 6.94 (m, 1 H), 7.31 (dd, 1 H, J = 2.9, 9.9 Hz), 7.63 (m, 1 H), 7.73 (d, 1 H, J = 2.7 Hz). Anal. ($C_{18}H_{19}FN_4$) C, H, N.

4-(4-Allylpiperazin-1-yl)-7-(trifluoromethyl)pyrrolo[1,2-*a*]quinoxaline (13h). Similarly to the procedure as described for **13a**, the title compound was prepared starting from **12d** and allyl bromide. After recrystallization **13h** was obtained as yellow prisms: IR ($CHCl_3$) 1602 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.64 (m, 4 H), 3.08 (d, 2 H, J = 6.5 Hz), 3.93 (m, 4 H), 5.23 (m, 2 H), 5.95 (m, 1 H), 6.80 (m, 2 H), 7.52 (d, 1 H, J = 8.1 Hz), 7.68 (d, 1 H, J = 8.4 Hz), 7.84 (m, 1 H), 7.93 (m, 1 H). Anal. ($C_{19}H_{19}F_3N_4$) C, H, N.

Microdialysis and Acetylcholine Assay. Under Equithesin anesthesia (1% pentobarbital, 4% chloral hydrate; 3 mL/kg ip), male CD/COBS rats (Charles River, Calco, Italy) weighing 200−250 g were implanted with AN 69 dialysis fibers (310 μm o.d.; Dasco Bologna), inserted transversally through both frontal cortices according to the following coordinates: A, +0.5 mm from bregma and V, −2.8 mm from the occipital bone.[10b] The procedure used to prepare and implant the dialysis tube was previously described.[10c,d] At the end of the release experiments the placement of the dialysis probe was verified histologically. A polyethylene cannula, 4 mm long, was inserted into each lateral ventricle for intracerebroventricular (icv) injections of the drug. The coordinates used were as follows: AP, −1.5 mm; L, ±1.5 mm from bregma. The average *in vitro* recovery of ACh through three dialysis tubes was 67.4 ± 1.7% for a probe 8 mm long. On the day after the implantation, the dialysis tube was perfused at a constant rate of 2 μL/min with Ringer's solution (147 mM NaCl, 2.2 mM $CaCl_2$, and 4.0 mM KCl), containing 5 μM physostigmine, and adjusted to pH 7.0 with NaOH. The perfusate was discarded during the first 30-min equilibration period and then collected at 20-min intervals in small ice-cooled polyethylene test tubes containing 10 μL of 0.05 mM HCl to prevent ACh hydrolysis. After an 80-min period of perfusion to allow the ACh output to reach a steady baseline, saline or drugs were administered systemically or through chronically implanted cannulae. At the end of the collection, the perfusate samples were immediately frozen on dry ice and lyophilized. ACh content of the dialysate was quantified by a specific and sensitive radioenzymatic method as previously described.[10c,d]

Drugs. All reagents were of analytical grade. Drugs and reagents used in this study and their sources were as follows: [^{3}H]acetylcoenzyme-A (2.5−4.3 Ci/mmol) from Amersham (Bucks, U.K.); physostigmine sulfate (M.W. 324.4), acetylcholinesterase (EC 3.1.1.7, type V-S), and choline kinase (EC 2.7.1.32) were purchased from Sigma Chemical Co. (St. Louis, MO). None of the drugs used interfere with the ACh assay.

Statistical Analysis. Statistical analysis was done on the values calculated as pmol/20 min, not corrected for the *in vitro* recovery of the mycrodialysis probe. Analysis of variance (split-plot design) followed by a multiple comparison test (Turkey's test for unconfounded means) was used.

Drug Administration and Plasma and Brain Sampling. Male Sprague−Dawley rats (Charles River, Italy) weighing 175−200 g were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (ECC Concil Directive 86/609, OJ L 358, 1, Dec; 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH publication No. 85-23, 1985).

Animals were injected intraperitoneally with **11e** or **12c** (as hydrochloride salts, 2.5, 5, and 10 mg/kg) dissolved in saline and were killed 1 h thereafter. Blood samples were collected in heparinized tubes and were centrifuged, and the plasma was stored at −20 °C. Brains were removed immediately after exanguination, and brain areas were blotted with paper to remove excess surface blood and stored at −20 °C until analysis.

Drug Analysis. Plasma and brain concentrations of the two piperazine derivatives **11e** and **12c** were determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 254 nm.

Briefly, 1 mL of heparin-treated plasma or brain homogenate (0.1 N HCl, 10 mL/g) were basified (pH 9−10) by using 1 N NaOH and extracted twice with 3 mL of *n*-hexane/isoamylic alcohol (98:2 v/v), after adding one of the two compounds as an internal standard. After centrifugation the organic extracts were transferred to test tubes containing 0.2 mL of 0.01 M phosphoric acid and were shaken for 15 min. After centrifugation the organic phase was discarded and the acidic aqueous phase was injected into the chromatographic column. Separation was done on a μBondapack C18 column (30 cm × 3.9 mm i.d., 10 μM particle size), at room temperature. The mobile phase was 0.01 M KH_2PO_4:CH_3CN:CH_3OH (69:28:3, v/v) delivered isocratically at a flow rate of 1.1 mL/min. The retention times were 7.8 min for **12c** and 9.8 min for **11e**.

Daily standard curves containing known concentrations of the analytes (from 0.01 to 0.25 μg/mL for plasma and from 0.025 to 0.75 μg/mL for brain tissue) were analyzed concurrently with each of the unknown samples and quality control samples. The relationships between the peak height of the compounds to the internal standard and of the amount of the compounds added were always linear, with coefficients of correlation exceeding 0.99. Replicate analysis at two different concentrations yielded mean coefficients of variation of 5−8% and 10−15% in plasma and brain tissue for both compounds.

Measurement of [^{14}C]Guanidinium Uptake in NG 108-15 Cells. This procedure has been described by Emerit et al.[3] as a convenient assay for assessing the agonist/antagonist activity of drugs acting at 5-HT$_3$ receptors. Thus, 5-HT$_3$ receptor agonists markedly enhance [^{14}C]guanidinium uptake by these cells, and this response is selectively blocked by 5-HT$_3$ receptor antagonists.[3] Briefly, mouse neuroblastoma × rat glioma hybrid cells of the NG 108-15 clone were grown in Dulbecco's modified Eagle's medium supplemented with the appropriate nutrients[3] for 2 days. The cell layer in each culture dish (35 mm) was then 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, 20 mM HEPES, pH adjusted to 7.4 with NaOH), and covered with 1 mL of buffer B (same composition as buffer A except that [NaCl] was reduced to 135 mM and 10 mM guanidinium were added) containing 0.20−0.25 mCi of [^{14}C]guanidinium (s.a. 59 mCi/mmol, Service des Molécules Marquées at CEA, 91191 Gif-surYvette, France) and, where indicated, 1 mM 5-HT, 10 mM substance P, and/or eight different concentrations of each drug to be tested. After 10 min at 37 °C, the assay 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 M NaOH, and the resulting extracts were transferred to scintillation vials. The culture dishes were further rinsed with 0.5 mL of 1 M HCl and then 0.5 mL of 0.4 M NaOH, which were added to the vials. Each mixture (1.5 mL) was supplemented with 10 mL of the scintillation fluid Aquasol (New England Nuclear, Les Ulis, France) for radioactivity counting at 50% efficiency. All assays were performed in triplicate.

Under these conditions, [^{14}C]guanidinium accumulation in NG 108-15 cells was 4−5 times higher in the presence of both 1 mM 5-HT and 10 mM substance P than in their absence (basal uptake). 5-HT$_3$ receptor antagonists (zacopride, ondansetron, tropisetron, etc.) completely prevented the stimulatory effect of 5-HT (with substance P) (see ref 3 for details).

von Bezold−Jarisch Reflex. The stimulation of cardiac 5-HT$_3$ receptors is well-known to trigger a transient bradycardia known as the von Bezold−Jarisch reflex[9] in urethane-anesthetized rats. The ability of drugs to either induce or prevent this reflex has been used to further assess the 5-HT$_3$ receptor agonist or antagonist properties of the newly synthesized arylpiperazine derivatives. Briefly, adult male Sprague−Dawley rats (250−300 g body weight, Centre d'Elevage R. Janvier, Le Genest, France) were anesthetized with urethane (1.4 g/kg ip), and a tracheotomy was performed to insert an endotracheal tube. A catheter (0.3 mm internal diameter) was inserted into the abdominal aorta via the femoral artery in order to record the arterial pressure and heart rate. A femoral vein was exposed and cannulated for iv injection of drugs. The von Bezold−Jarisch reflex (which consists of a 60% drop in heart rate within 10−15 s following the injection of 30 mg/kg iv of serotonin) was assessed 5, 15, and 30 min after the iv administration of various doses of each arylpiperazine derivative. Under these conditions, 10 mg/kg iv of either zacopride, ondansetron, or tropisetron injected 5 min before 5-HT completely prevented the bradycardia normally evoked by the indoleamine.[9]

***In Vitro* Binding Assays.** Binding assays have been performed as described in ref 4b.

[^{3}H]BRL 43694 Binding to Rat Cortical Membranes. Male CRL:CD(SD)BR-COBS rats were killed by decapitation; their cortex were rapidly removed and stored at −80 °C until the day of assay. The frozen tissues were homogenized in about 50 volumes of ice-cold Hepes HCl, 50 mM, pH 7.4 using an Ultra Turrax TP 1810 homogenizer (2 × 20 s) and centrifuged at 50000*g* for 10 min (Beckman model J-21 B refrigerate centrifuged). The pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again at 50000*g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet obtained was finally resuspended in Hepes HCl, 50 mM, pH 7.4 containing 10 μM pargyline.

[^{3}H]BRL 43694 binding was done as previously described[5] in a final incubation volume of 1 mL consisting of 0.5 mL of membrane suspension (20 mg tissue/sample), 0.5 mL of [^{3}H]-BRL 43694 (s.a. 83.5 Ci/mmol, final concentration 1 nM), and 20 μL of displacing agents or solvent. Incubation (30 min at 25 °C) was stopped by rapid filtration *in vacuo* (Brandell MR 48R) through GF/B filters which were then washed with 12 mL of cold buffer and counted in a Wallac 1204 betaplate BS liquid scintillation counter with a counting efficiency of 45%. Dose−inhibition curves were analyzed by the "Allifit" program[11b] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding.

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