Pyrroloquinoxaline Derivatives as High-Affinity and Selective 5-HT$_3$ Receptor Agonists: Synthesis, Further Structure−Activity Relationships, and Biological Studies

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The synthesis, pharmacological evaluation, and structure−activity relationships (SARs) of a series of novel pyrroloquinoxalines and heteroaromatic-related derivatives are described. The new pyrroloquinoxaline-related ligands were tested in rat cortex, a tissue expressing high density of 5-HT$_3$ receptors, and on NG108-15 cells and exhibited IC$_{50}$ values in the low nanomolar or subnanomolar range, as measured by the inhibition of [^{3}H]zacopride binding. The SAR studies detailed herein delineated a number of structural features required for improving affinity. Some of the ligands were employed as "molecular yardsticks" to probe the spatial dimensions of the lipophilic pockets L1, L2, and L3 in the 5-HT$_3$ receptor cleft, while the 7-OH pyrroloquinoxaline analogue was designed to investigate hydrogen bonding with a putative receptor site H1 possibly interacting with the serotonin hydroxy group. The most active pyrroloquinoxaline derivatives showed subnanomolar affinity for the 5-HT$_3$ receptor. In functional studies ([^{14}C]guanidinium accumulation test in NG108-15 hybrid cells, in vitro) most of the tested compounds showed clear-cut 5-HT$_3$ agonist properties, while some others were found to be partial agonists. Several heteroaromatic systems, bearing N-substituted piperazine moieties, have been explored with respect to 5-HT$_3$ affinity, and novel structural leads for the development of potent and selective central 5-HT$_3$ receptor agonists have been identified. Preliminary pharmacokinetic studies indicate that these compounds easily cross the blood−brain barrier (BBB) after systemic administration with a brain/plasma ratio between 2 and 20, unless they bear a highly hydrophilic group on the piperazine ring. None of the tested compounds showed in vivo anxiolytic-like activity, but potential analgesic-like properties have been possibly disclosed for this new class of 5-HT$_3$ receptor agonists.

Introduction

Serotonin (5-HT) is one of the most attractive targets in medicinal chemistry since this neurotransmitter is involved in many biological and pathological processes.[1] 5-HT receptors are classified into several subtypes.[2] Among these, the 5-HT$_3$ receptor has attracted much attention because of the effectiveness of its antagonists in preventing emesis induced by treatment with chemotherapeutic drugs.[3] Furthermore, numerous potential therapeutic applications of 5-HT$_3$ antagonists have been claimed.[4] On the contrary, the information on 5-HT$_3$ receptor agonists and their potential specific therapeutic relevance is scarce, although it has been shown that 5-HT$_3$ receptor agonists may modulate central acetylcholine release[5,6a] making them of interest for the potential treatment of neurodegenerative and neuropsychiatric disorders. The agonist activities of 2-methylserotonin (2-Me-5-HT) (**1**) and quipazine (**2**) are well-documented, but unlike 2-Me-5-HT, quipazine is able to cross the blood−brain barrier (BBB).[7] Based on the structure of quipazine, we have recently designed a new class of high-affinity 5-HT$_3$ receptor ligands.[6a] Along with high affinity for the 5-HT$_3$ receptor, these quipazine-related ligands behaved as agonists in [^{14}C]-guanidinium accumulation test and in cortical acetylcholine release in freely moving rats.[6a] Quinoxalines **3a**,**b**[6a] are the representative compounds of this series. Other high-affinity 5-HT$_3$ receptor ligands, structurally related to quipazine, have been recently described by Castan and co-workers (**4**),[8] while Rault and co-workers[9] reported new 5-HT$_3$ partial agonists (**5**) (Chart 1).

Because of the increasing interest in the development of potent and selective 5-HT$_3$ receptor agonists, we decided to expand our structure−activity relationship (SAR) studies of the quipazine-related class of ligands in order to optimize their 5-HT$_3$ binding affinity and selectivity. The present article details the synthesis of

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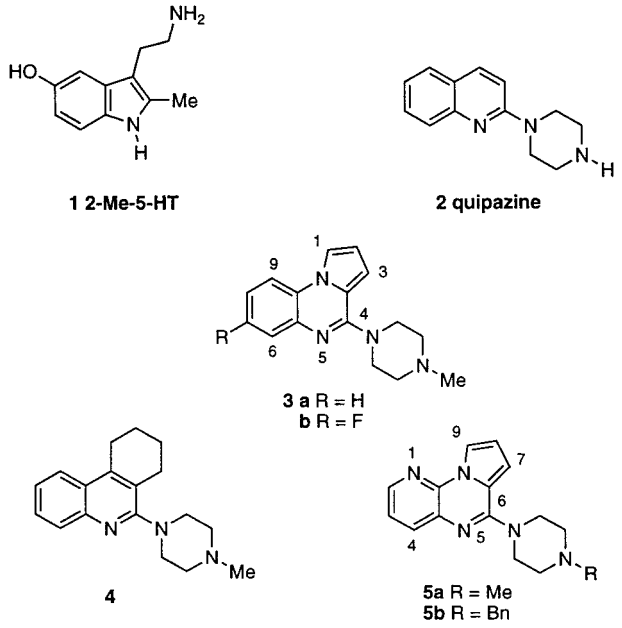
Chart 1

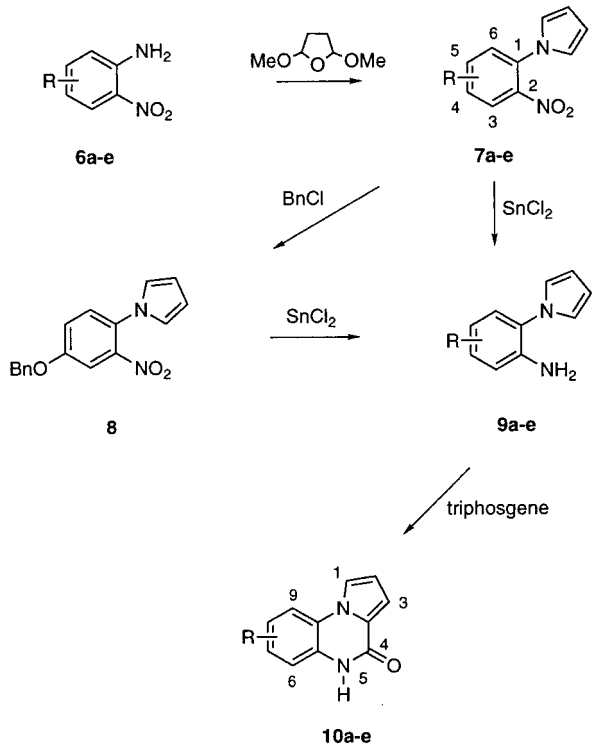


novel pyrroloquinoxalines and pyrroloquinoxaline-related analogues and their SARs for rat cortical 5-HT$_3$ receptor affinity associated with variation of the substituents on the heterocyclic system. A subset of ligands with high affinity in brain was further tested for their ability to inhibit [^{3}H]zacopride binding in NG108-15 hybrid cells, and all were found to have potencies comparable to those in rat cortex. From analysis of the SARs relative to the newly investigated pyrroloquinoxaline analogues, a pharmacophore model, schematically represented in Chart 2, could be defined. Accordingly, a hydrogen bond acceptor/donor function (H1) and three lipophilic pockets (L1, L2, and L3) within the receptor binding site are available to our ligands. The 5-HT$_3$ receptor intrinsic efficacy of selected derivatives was assessed in vitro on 5-HT$_3$ receptor-dependent [^{14}C]-guanidinium uptake in NG108-15 hybrid cells. Additionally, brain and plasma concentrations were simultaneously measured for a subset of ligands after systemic injection, and in vivo studies were performed in order to evaluate potential anxiolytic-like or analgesic-like properties of the tested compounds.

Chemistry

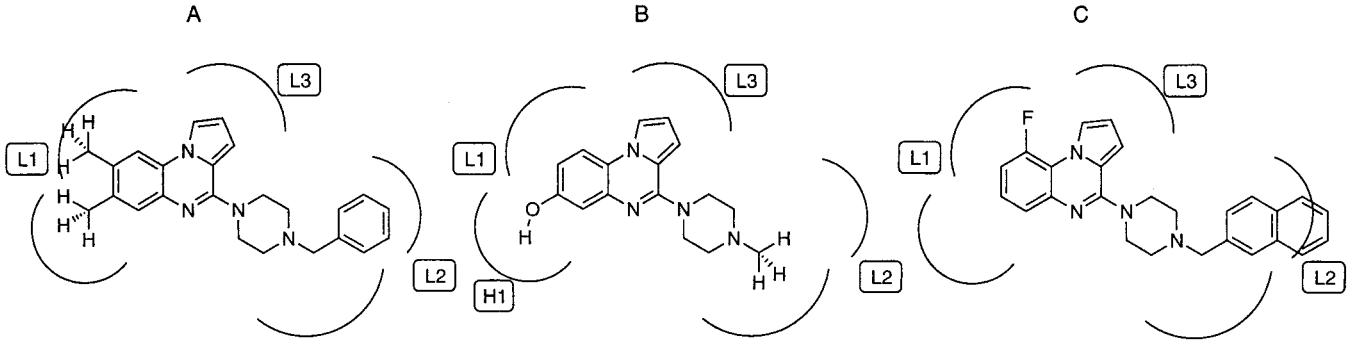
The synthesis of the new pyrroloquinoxalines, imidazoquinoxalines, imidazopyridopyrazines, pyrazoquinazolines, pyrrolopyrazine, imidazolinopyrazine, and pyridopyrrolopyrazines **20a**−**jj** has been accomplished as described in Schemes 1−6. The key lactam intermediates **10a**−**g** have been synthesized following the pathways reported in Schemes 1−3, while the lactams **10h**−**o** were obtained following already known procedures described in refs 6 and 9−11 (see Table 1). As described in Scheme 1, starting from the commercially available 2-nitroanilines **6a**−**e**, by a Clauson−Kaas reaction, the 1-(2-nitroaryl)pyrroles were obtained (**7a**−**e**). Next, the nitro groups were reduced, by exposure to tin(II) chloride, and the 1-(2-aminoaryl)pyrroles **9a**−**c**,**e** were isolated. In the case of **7d**, the free hydroxy function was protected to **8**, and this benzyloxy analogue was treated with tin(II) chloride, obtaining **9d**. Then **10a**−**e** were prepared by reaction of amines **9a**−**e** with triphosgene in high yield.

Scheme 1



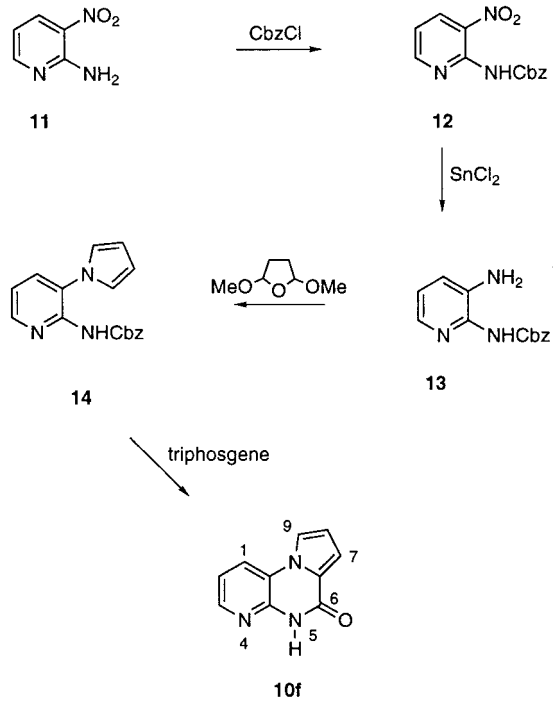
The lactam **10f** was in turn prepared as described in Scheme 2. Starting from **11**, the amino group was protected by a Cbz function by using benzyl chloroformate (Cbz-Cl) (**12**), and the nitro group was treated with tin(II) chloride. The corresponding pyridine **13** was

Chart 2[a]

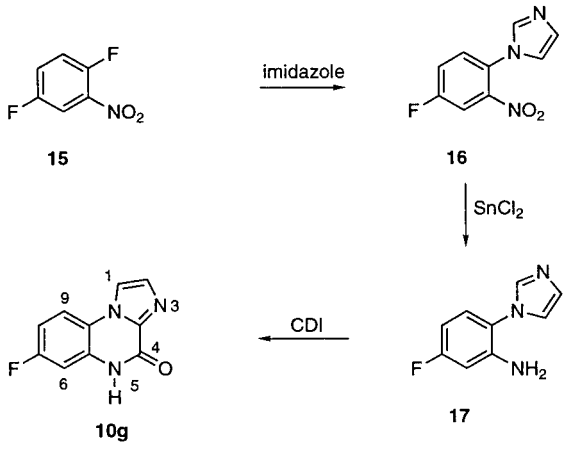


[a] H1 is a hydrogen bond acceptor/donor site on the receptor protein. L1, L2, and L3 are lipophilic pockets in the protein at the binding site.

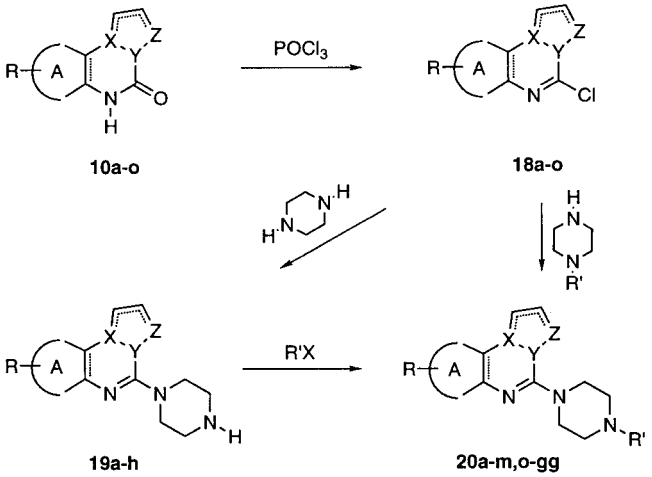
Scheme 2



Scheme 3

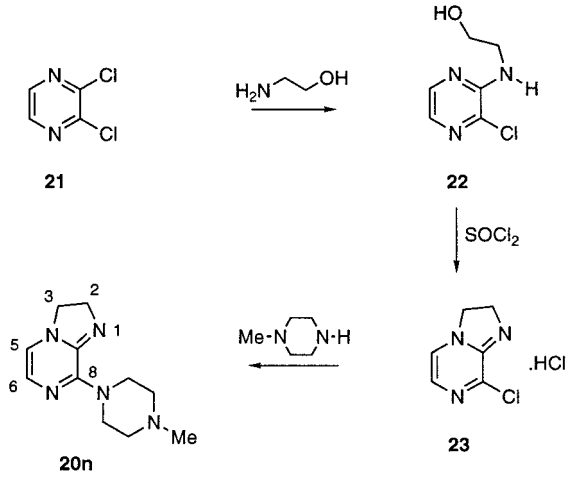


Scheme 4

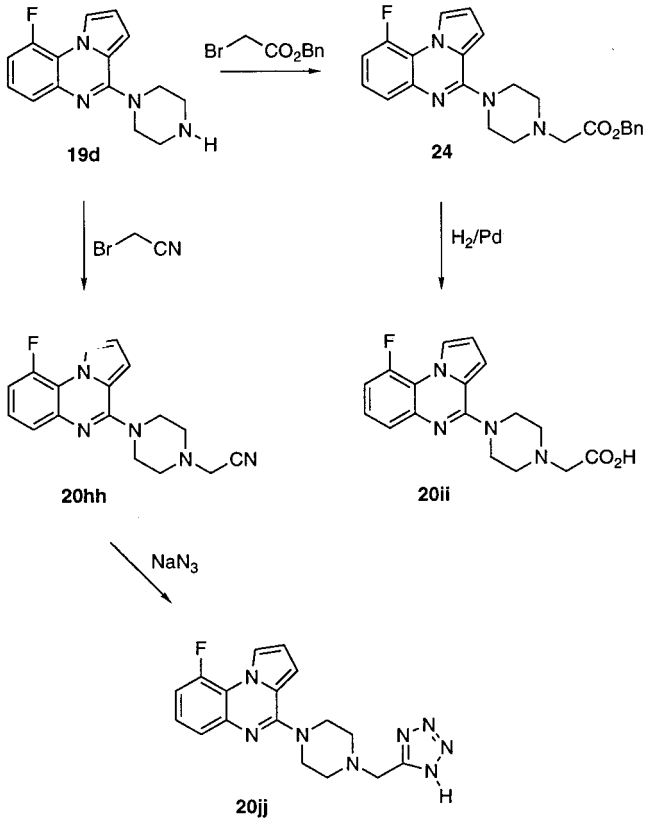


subjected to the Clauson−Kaas reaction (**14**), and the cyclization was performed using triphosgene (**10f**). During this cyclization step the deprotection occurred.

Scheme 5



Scheme 6

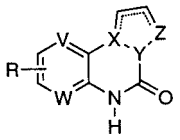


Following a similar approach, the lactam intermediate **10g** was synthesized. Starting from **15**, after introduction of the imidazole ring[10a] (**16**) and reduction of the nitro group with tin(II) chloride (**17**), the cyclization step was perfomed by using *N*,*N*-carbonyldiimidazole (CDI) (**10g**).

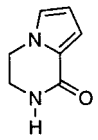
Successively, the N-4-substituted piperazine ring was introduced following a standard method[6a] as described in Scheme 4. Lactams **10a**−**o** were transformed into the 4-chloro derivatives,[6a,11a] and the latter were treated with piperazine to obtain **19a**−**c**,**g** or with *N*-alkylpiperazines to obtain **20a**−**t**,**v**,**w**,**ee**−**gg**. Some of the desired compounds were synthesized by alkylation of the corresponding **19a**−**h** (**20u**,**x**−**dd**).[11b]

The imidazopyrazine derivative **20n** was prepared as reported in Scheme 5. Commercially available dichlo-

Table 1. Physical and Chemical Data for Compounds **7**−**10** and **12**−**17**



10a-i,k-o



10j

compd	V	W	R	X	Y	Z	yield[a] (%)	mp (°C)	recryst solvent	formula	anal.[b]
7a			4,5-diMe				76	60−61	hexanes	$C_{12}H_{12}N_2O_2$	C,H,N
7b			6-Me				68	103−104	hexanes	$C_{11}H_{10}N_2O_2$	C,H,N
7c			4-CN				57	91−92	EtOAc	$C_{11}H_7N_3O_2$	C,H,N
7d			4-OH				89	157−158	EtOH	$C_{10}H_8N_2O_3$	C,H,N
7e			4,5-diCl				72	68−69	hexanes	$C_{10}H_6Cl_2N_2O_2$	C,H,N
8							98	69−70	EtOAc	$C_{17}H_{14}N_2O_3$	C,H,N
9a			4,5-diMe				95	83−85	EtOAc	$C_{12}H_{14}N_2$	C,H,N
9b			6-Me				76	46−47	hexanes	$C_{11}H_{12}N_2$	C,H,N
9c			4-CN				71	101−103	hexanes	$C_{11}H_9N_3$	C,H,N
9d			4-OBn				87	135−136	EtOAc	$C_{17}H_{16}N_2O$	C,H,N
9e			4,5-diCl				81	145−146	hexanes	$C_{10}H_8Cl_2N_2$	C,H,N
10a	CH	CH	7,8-diMe	N	C	CH	86	>300	EtOAc	$C_{13}H_{12}N_2O$	C,H,N
10b	CH	CH	9-Me	N	C	CH	79	280 dec	EtOAc	$C_{12}H_{10}N_2O$	C,H,N
10c	CH	CH	7-CN	N	C	CH	75	280 dec	EtOAc	$C_{12}H_7N_3O$	C,H,N
10d	CH	CH	7-OBn	N	C	CH	79	272−273	EtOAc	$C_{18}H_{14}N_2O_2$	C,H,N
10e	CH	CH	7,8-diCl	N	C	CH	82	>300	EtOAc	$C_{11}H_6Cl_2N_2O$	C,H,N
10f	CH	N	H	N	C	CH	73	>250	EtOAc	$C_{10}H_7N_3O$	C,H,N
10g	CH	CH	7-F	N	C	N	51	>250	EtOAc	$C_{10}H_6FN_3O$	C,H,N
10h[c]	CH	CH	7-Me	N	C	CH					
10i[c]	N	CH	H	N	C	CH					
10j[d]											
10k[e]	CH	CH	H	N	C	N					
10l[f]	CH	CH	H	C	N	N					
10m[e]	N	CH	H	N	C	N					
10n[g]	CH	CH	7-F	N	C	CH					
10o[g]	CH	CH	9-F	N	C	CH					
12							78			$C_{13}H_{11}N_3O_4$	C,H,N
13							94	148−149	benzene	$C_{13}H_{13}N_3O_2$	C,H,N
14							42	96−98	hexanes	$C_{17}H_{15}N_3O_2$	C,H,N
16							48	83−84	hexanes	$C_9H_6FN_3O_2$	C,H,N
17							77	127−128	cyclohexane	$C_9H_8FN_3$	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 9. [d] Reference 10c. [e] Reference 10a. [f] Reference 10b. [g] Reference 6.

ropyrazine **21** was reacted with 2-aminoethanol to give **22**,[12a] and this was treated with thionyl chloride to obtain **23** as hydrochloride salt. The 4-chloro derivative **23** was then heated with *N*-methylpiperazine to obtain **20n**.

Compounds bearing highly hydrophilic moieties at N-4 of the piperazine ring were obtained following Scheme 6. Starting from **19d** the benzyloxycarbonylmethyl function at N-4 was introduced by using benzyl bromoacetate (**24**). Next, the benzyl group was removed by catalytic hydrogenation (**20ii**). The reaction performed by using bromoacetonitrile led to **20hh**, which was transformed into the 5-tetrazolylmethyl analogue **20jj** by using sodium azide.[12b]

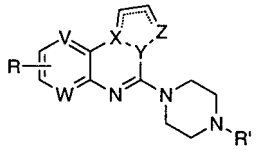
Results and Discussion

1. 5-HT$_3$ Binding SAR Studies.[13a] The affinities of the new pyrroloquinoxaline-related analogues **19a**−**c**,**g** and **20a**−**jj** for 5-HT$_3$ receptors in rat cortex homogenate are illustrated in Tables 3−5, while Table 6 reports the ability of selected compounds to inhibit [^{3}H]zacopride binding in rat cortex and NG108-15 cells. Binding data of quipazine and **3b** are also included. Table 7 lists the affinities of selected compounds for different 5-HT receptor subtypes. The results reported in these tables are summarized as follows.

(i) Key Role of the Benzo-Fused System on 5-HT$_3$ Receptor Affinity (L1 Site). Compounds **20h**,**n** (Tables 3 and 4), analogues in which the fused aromatic system is missing, were investigated to evaluate the role of the benzo-fused system in the binding to the 5-HT$_3$ receptor. Both compounds were found to be at least 4 orders of magnitude less active than **3b** or **20b**, indicating that the aromatic ring plays a key role in the interaction with the receptor protein.

(ii) Substitutions on the Benzo-Fused Ring of Quinoxalines. The 7,8-dimethyl (**19a**, **20a**), 7-methyl (**19c**, **20g**), and 9-methyl (**19b**, **20b**) substituted pyrroloquinoxalines have been synthesized and tested to probe the spatial dimension of the lipophilic pocket L1 within the receptor cleft. As shown in Table 3, the drop in affinity of analogues **19a** and **20a** with respect to the 7-monoalkylated analogues **19b** and **20g** indicates that the steric hindrance of a methyl group at C-8 is detrimental to affinity (Chart 2). In contrast, the introduction of a methyl group at C-9 leads to a high-affinity ligand (**20b**) with subnanomolar affinity (IC_{50} = 0.79 nM), similar to that of **3a**.[6a] On NG108-15 cells **20b** proves to be extremely efficacious in displacing the [^{3}H]zacopride from the 5-HT$_3$ receptor. Replacement of the methyl groups at C-7 and C-8 of **20a** with chlorine atoms (**20e**) or introduction at position 7 of a cyano group (**20c**) caused a large drop in affinity, the IC_{50}s being equal to 97 and 104 nM, respectively. The introduction of a hydroxy group at C-7 (**20f**), intended to mimic the hydroxy group of serotonin (5-HT), confers

Table 2. Physical and Chemical Data for Compounds **18a**−**o**, **19a**−**h**, **20a**−**jj**, and **22**−**24**



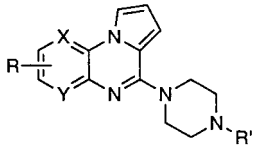
19a-h, 20a-g,i-m,o-jj

compd	V	W	R	X	Y	Z	R′	yield[a] (%)	mp (°C)	recryst solvent[b]	formula	anal.[c]
18a	CH	CH	7,8-diMe	N	C	CH		91	139−140	A/B	$C_{13}H_{11}ClN_2$	C,H,N
18b	CH	CH	9-Me	N	C	CH		83	145−146	A/B	$C_{12}H_9ClN_2$	C,H,N
18c	CH	CH	7-CN	N	C	CH		72	140−141	A/B	$C_{12}H_6ClN_2$	C,H,N
18d	CH	CH	7-OBn	N	C	CH		79	113−114	B/C	$C_{18}H_{13}ClN_2O$	C,H,N
18e	CH	CH	7,8-diCl	N	C	CH		80	206−207	C	$C_{11}H_5Cl_3N_2$	C,H,N
18f	CH	N	H	N	C	CH		82	163−164	A/B	$C_{10}H_6ClN_3$	C,H,N
18g	CH	CH	7-F	N	C	N		84	178−179	A/B	$C_{10}H_5FClN_3$	C,H,N
18h[d]	CH	CH	7-Me	N	C	CH						
18i[d]	N	CH	H	N	C	CH						
18j				N	C	CH		69	112−113	B	$C_7H_7ClN_2$	C,H,N
18k	CH	CH	H	N	C	N		72	188−189	A/B	$C_{10}H_6ClN_3$	C,H,N
18l	CH	CH	H	C	N	N		77	122−123	A/B	$C_{10}H_6ClN_3$	C,H,N
18m	N	CH	H	N	C	N		81	214−215	A/B	$C_9H_5ClN_4$	C,H,N
18n[e]	CH	CH	7-F	N	C	CH						
18o[e]	CH	CH	9-F	N	C	CH						
19a	CH	CH	7,8-diMe	N	C	CH	H	80	127−129	C	$C_{17}H_{20}N_4$	C,H,N
19b	CH	CH	9-Me	N	C	CH	H	84	124−125	A/B	$C_{16}H_{18}N_4$	C,H,N
19c	CH	CH	7-Me	N	C	CH	H	82	128−130	A/B	$C_{16}H_{18}N_4$	C,H,N
19d[e]	CH	CH	9-F	N	C	CH	H					
19e[e]	CH	CH	7-F	N	C	CH	H					
19f[e]	CH	CH	H	N	C	CH	H					
19g	CH	N	H	N	C	CH	H	73	107−108	A	$C_{14}H_{15}N_5$	C,H,N
19h[d]	N	CH	H	N	C	CH	H					
20a	CH	CH	7,8-diMe	N	C	CH	Me	87	133−134	A/B	$C_{18}H_{22}N_4$	C,H,N
20b	CH	CH	9-Me	N	C	CH	Me	72	146−147	A/B	$C_{17}H_{20}N_4$	C,H,N
20c	CH	CH	7-CN	N	C	CH	Me	67	159−161	A/B	$C_{17}H_{17}N_5$	C,H,N
20d	CH	CH	7-OBn	N	C	CH	Me	84	150−152	A/B	$C_{23}H_{24}N_4O$	C,H,N
20e	CH	CH	7,8-diCl	N	C	CH	Me	65	148−149	A/B	$C_{16}H_{16}Cl_2N_4$	C,H,N
20f	CH	CH	7-OH	N	C	CH	Me	78	164−165	A	$C_{16}H_{18}N_4O$	C,H,N
20g	CH	CH	7-Me	N	C	CH	Me	83	151−153	A/B	$C_{17}H_{20}N_4$	C,H,N
20h				N	C	CH	Me	71	103−105	A	$C_{12}H_{18}N_4$	C,H,N
20i	CH	CH	H	N	C	N	Me	68	97−98	A/B	$C_{15}H_{17}N_5$	C,H,N
20j	CH	CH	7-F	N	C	N	Me	87	126−127	A/B	$C_{15}H_{16}FN_5$	C,H,N
20k	CH	CH	H	C	N	N	Me	56	91−92	A/B	$C_{15}H_{17}N_5$	C,H,N
20l	CH	N	H	N	C	CH	Me	83	89−91	A	$C_{15}H_{17}N_5$	C,H,N
20m	N	CH	H	N	C	N	Me	60	108−109	A	$C_{14}H_{16}N_6$	C,H,N
20n				N	C	N	Me	48	93−95	A	$C_{11}H_{17}N_5$	
20o	CH	CH	7-Me	N	C	CH	CH_2Ph	85	132−134	A/B	$C_{23}H_{24}N_4$	C,H,N
20p	CH	CH	7,8-diMe	N	C	CH	CH_2Ph	89	122−123	A/B	$C_{24}H_{26}N_4$	C,H,N
20q	CH	CH	9-Me	N	C	CH	CH_2Ph	75	119−120	A/B	$C_{23}H_{24}N_4$	C,H,N
20r	CH	CH	7,8-diCl	N	C	CH	CH_2Ph	67	147−148	A/B	$C_{22}H_{20}Cl_2N_4$	C,H,N
20s	CH	CH	7-F	N	C	CH	CH_2Ph	78	153−154	A/B	$C_{22}H_{21}FN_4$	C,H,N
20t	CH	CH	9-F	N	C	CH	CH_2Ph	83	149−151	A/B	$C_{22}H_{21}FN_4$	C,H,N
20u	CH	CH	9-F	N	C	CH	2-naphthylmethyl	58	128−130	A/B	$C_{26}H_{23}FN_4$	C,H,N
20v	CH	N	H	N	C	CH	CH_2Ph	87	118−119	A/B	$C_{21}H_{21}N_5$	C,H,N
20w	CH	N	H	N	C	CH	Ph	82	163−165	C	$C_{20}H_{19}N_5$	C,H,N
20x	CH	CH	9-F	N	C	CH	2-thienylmethyl	39	113−114	A/B	$C_{20}H_{19}FN_4S$	C,H,N
20y	CH	CH	H	N	C	CH	2-thienylmethyl	44	117−118	A/B	$C_{20}H_{20}N_4S$	C,H,N
20z	CH	CH	7-F	N	C	CH	2-thienylmethyl	46	122−123	A/B	$C_{20}H_{19}FN_4S$	C,H,N
20aa	N	CH	H	N	C	CH	2-thienylmethyl	43	144−146	A/B	$C_{19}H_{19}N_5S$	C,H,N
20bb	CH	CH	9-Me	N	C	CH	2-Br-5-thienylmethyl	36	108−110	A/B	$C_{21}H_{21}BrN_4S$	C,H,N
20cc	CH	CH	7-F	N	C	CH	2-furylmethyl	46	133−134	A/B	$C_{20}H_{19}FN_4O$	C,H,N
20dd	CH	CH	7-F	N	C	CH	2-Br-5-furylmethyl	35	99−100	A/B	$C_{18}H_{18}FBrN_4O$	C,H,N
20ee	CH	CH	9-F	N	C	CH	CH_2CH_2OH	85	123−124	A	$C_{17}H_{19}FN_4O$	C,H,N
20ff	CH	CH	7-F	N	C	CH	CH_2CH_2OH	88	166−168	A	$C_{17}H_{19}FN_4O$	C,H,N
20gg	N	CH	H	N	C	N	CH_2CH_2OH	82	149−151	A	$C_{15}H_{18}N_6O$	C,H,N
20hh	CH	CH	9-F	N	C	CH	CH_2CN	70	133−134	A	$C_{17}H_{16}FN_5$	C,H,N
20ii	CH	CH	9-F	N	C	CH	CH_2CO_2H	88	217−218	A	$C_{17}H_{17}FN_4O_2$	C,H,N
20jj	CH	CH	9-F	N	C	CH	5-tetrazolylmethyl	70	233−234	A	$C_{17}H_{17}FN_8$	C,H,N
22[f]												
23								62	274−275	A/B	$C_6H_7Cl_2N_3$	C,H,N
24								75	74−75	A/B	$C_{24}H_{23}FN_4O_2$	C,H,N

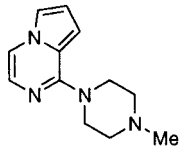
[a] Yields refer to isolated and purified materials. [b] A = EtOAc; B = hexanes; C = benzene. [c] All the compounds were analyzed within ±0.4% of the theoretical values. [d] Reference 9. [e] Reference 6. [f] Reference 12a.

subnanomolar affinity for the central 5-HT_3 receptor, similar to that of **3b** and **20b** (IC_{50}s = 0.81 and 0.79 nM, respectively) which lack the hydroxy function. The nature of the interaction involving the substituent at

Table 3. 5-HT$_3$ Receptor Binding Affinities for Compounds **19a**−**c**,**g** and **20a**−**h**,**l**,**o**−**dd** and Functional Behavior of Selected Compounds



19a-c,g, 20a-g,l,o-dd



20h

compd	X	Y	R	R′	IC$_{50}$ (nM)[a] (±SEM)	effect on [^{14}C]guanidinium uptake on NG108-15 cells	
						efficacy[b]	EC$_{50}$ (nM)[c]
19a	CH	CH	7,8-diMe	H	113 ± 28		
19b	CH	CH	9-Me	H	1.13 ± 0.3	PA	9.5
19c	CH	CH	7-Me	H	16.4 ± 4.1		
19g	CH	N	H	H	3.1 ± 0.3		
20a	CH	CH	7,8-diMe	Me	30 ± 8		
20b	CH	CH	9-Me	Me	0.79 ± 0.1	A	3.8
20c	CH	CH	7-CN	Me	104 ± 17		
20d	CH	CH	7-OBn	Me	225 ± 52		
20e	CH	CH	7,8-diCl	Me	97 ± 22		
20f	CH	CH	7-OH	Me	0.67 ± 0.09	A	1.6
20g	CH	CH	7-Me	Me	18 ± 7	A	14.0
20h					1930 ± 315		
20l	CH	N	H	Me	2.4 ± 0.5	PA	1.7
20o	CH	CH	7-Me	CH_2Ph	24 ± 9		
20p	CH	CH	7,8-diMe	CH_2Ph	44 ± 9		
20q	CH	CH	9-Me	CH_2Ph	8.8 ± 0.9	A	12.0
20r	CH	CH	7,8-diCl	CH_2Ph	118 ± 19		
20s	CH	CH	7-F	CH_2Ph	13 ± 3	PA	4.2
20t	CH	CH	9-F	CH_2Ph	61 ± 18		
20u	CH	CH	9-F	2-naphthylmethyl	780 ± 148		
20v	CH	N	H	CH_2Ph	2.2 ± 0.5	PA	4.2
20w	CH	N	H	Ph	NA		
20x	CH	CH	9-F	2-thienylmethyl	60 ± 13		
20y	CH	CH	H	2-thienylmethyl	22 ± 5		
20z	CH	CH	7-F	2-thienylmethyl	12 ± 2		
20aa	N	CH	H	2-thienylmethyl	3.6 ± 0.5		
20bb	CH	CH	9-Me	2-Br-5-thienylmethyl	104 ± 24		
20cc	CH	CH	7-F	2-furylmethyl	11 ± 3	PA	6.6
20dd	CH	CH	7-F	2-Br-5-furylmethyl	148 ± 43		
2					3.8 ± 0.4	PA	1.0
3b[d]					0.81 ± 0.05	A	3.0
5a[e]					1.0 ± 0.3		
5b[e]					2.3 ± 0.8	PA	32[f]
GR38032					6.80 ± 1.4		
MDL72222					97 ± 30		

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximal inhibition of [^{3}H]zacopride binding to rat cortical homogenate. [b] A = pure agonist; PA = partial agonist. [c] EC$_{50}$ values are the mean of two independent experiments. [d] Reference 6. [e] **5a**,**b** have been previously reported by Rault et al.[9] They have been resynthesized and used as reference compounds. Binding data are those obtained following our protocol. [f] Reference 9. NA = not active.

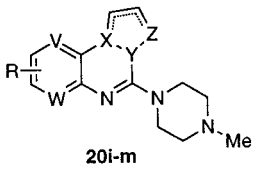
the 7-position remains elusive since a ligand/receptor neutral−neutral hydrogen bond contributes to binding affinity from 15-fold to 0.[13b] In our pharmacophore model, the role of hydrogen bond acceptor/donor assigned to the 7-OH group of **20f** relied on the hypothesis that this hydroxy group mimics the one of 5-HT at the binding site. Overall, analogues **20b**,**f** represent two of the most potent and selective 5-HT$_3$ receptor ligands reported to date. Transformation of the hydroxy group at C-7 of **20f** into a benzyloxy function (**20d**) caused a significant decrease of affinity, due to unfavorable steric interactions.

(iii) Replacement of the Methyl Group at N-4 by *π*-Electron-Rich Systems (L2 Site). In general, affinity is owered by replacement of the methyl group at N-4 of the piperazine ring with a benzyl group. This trend can be seen in Table 3 by comparison of the affinity values of several pair of analogues: **3b**/**20s**, **20a**/**20p**, **20b**/**20q**, and **20g**/**20o**. Conversely, for the pyrido-fused pair **20l**/**20v** (see the following section) a similar affinity has been found. Substitution of the benzyl by isosteric heteroaryl groups leads to less active analogues (**20x**−**z**,**bb**,**cc**,**dd** vs **20q**,**s**,**t**). Among the 2-thienylmethyl derivatives synthesized, **20aa** shows the better binding profile (Table 3).

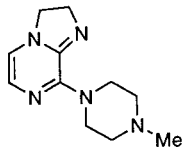
Replacement of the benzyl group of **20t** with a bulkier 2-naphthylmethyl group gave an inactive analogue (**20u**) due to unfavorable steric interactions (Chart 2). Furthermore, a phenyl substituent directly linked to the N-4 of the piperazine ring leads to an inactive analogue (**20w**), probably due to the reduced basicity of the N-4 piperazine nitrogen and to unfavorable shape effects.

(iv) Pyrido-Fused versus Benzo-Fused Analogues: Effect on 5-HT$_3$ Receptor Affinity. The presence of an extra nitrogen (pyridopyrrolopyrazines) on the benzo-fused ring of our quinoxalines is slightly unfavorable for affinity (Table 3). In fact, while **3a** showed an IC$_{50}$ equal to 0.37 nM, the pyrido-fused analogue **20l** is 8-fold less potent at the 5-HT$_3$ receptor. Though in the series of pyrido-fused analogues (**19g**,

Table 4. Comparison of the 5-HT_3 Receptor Binding Affinity of Compounds **20i**−**n** Characterized by Different Fused Five-Membered Rings and Functional Behavior of Compounds **20i**−**l**



20i-m

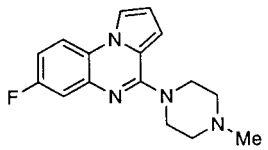


20n

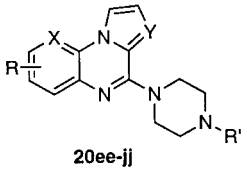
compd	R	V	W	X	Y	Z	IC_{50} (nM)[a] (±SEM)	effect on [^{14}C]guanidinium uptake on NG108-15 cells: efficacy[b]	effect on [^{14}C]guanidinium uptake on NG108-15 cells: EC_{50} (nM)[c]
3b[d]							0.81 ± 0.05	A	3.0
5a[e]							1.0 ± 0.3		
20i	H	CH	CH	N	C	N	3.8 ± 0.5	A	15.0
20j	7-F	CH	CH	N	C	N	5.2 ± 0.4	A	7.2
20k	H	CH	CH	C	N	N	1.6 ± 0.1	A	2.7
20l	H	CH	N	N	C	CH	2.4 ± 0.5	PA	1.7
20m	H	N	CH	N	C	N	6.6 ± 1.0		
20n							3650 ± 320		

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximal inhibition of [^{3}H]zacopride binding to rat cortical homogenate. [b] A = pure agonist; PA = partial agonist. [c] EC_{50} values are the mean of two independent experiments. [d] Reference 6. [e] **5a** has been previously reported by Rault et al.[9] This analogue has been resynthesized and used as a reference compound. Binding data is that obtained following our protocol.

Table 5. Comparison of the 5-HT_3 Receptor Binding Affinity of Compounds **20ee**−**jj** Bearing Hydrophilic Substituents on the Piperazine Ring and Functional Behavior for Compounds **20ee,ff**



3b



20ee-jj

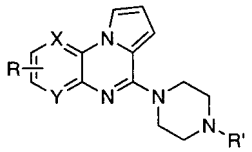
compd	R	X	Y	R′	IC_{50} (nM)[a] (±SEM)	effect on [^{14}C]guanidinium uptake on NG108-15 cells: efficacy[b]	effect on [^{14}C]guanidinium uptake on NG108-15 cells: EC_{50} (nM)[c]
3b[d]					0.81 ± 0.05	A	3.0
20ee	9-F	CH	CH	CH_2CH_2OH	8.2 ± 2.4	PA	54.0
20ff	7-F	CH	CH	CH_2CH_2OH	2.6 ± 0.3	A	2.4
20gg	H	N	N	CH_2CH_2OH	38 ± 7		
20hh	9-F	CH	CH	CH_2CN	3800 ± 300		
20ii	9-F	CH	CH	CH_2CO_2H	920 ± 170		
20jj	9-F	CH	CH	5-tetrazolylmethyl	690 ± 115		

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximal inhibition of [^{3}H]zacopride binding to rat cortical homogenate. [b] A = pure agonist; PA = partial agonist. [c] EC_{50} values are the mean of two independent experiments. [d] Reference 6.

20l,**v**,**aa**) the 5-HT_3 binding data are virtually identical, in the low nanomolar range, a different selectivity profile is shown by compounds **20l**,**v** versus the different 5-HT receptor subtypes (see Selectivity section and Table 7). The new 5-HT_3 receptor ligands **19g** and **20l**,**v** represent three novel pyridopyrrolopyrazines, bearing a hydrogen, a methyl group, and a benzyl group, respectively, on the piperazine ring and are isomers of those pyridopyrrolopyrazines recently described by Rault and co-workers (**5a**,**b**).[9b] To compare, in the same experimental conditions, compounds **20l**,**v** with those described by Rault and co-workers we decided to resynthesize **5a**,**b**, for which −log IC_{50}s of 9.01 and 12.09, respectively, were reported. In our hands (Table 3) the affinity of **20l**,**v** overlaps the values of their isosters **5a**,**b**. It is to be noticed that our 5-HT_3 binding affinity for compound **5b** (rat cortex and NG108-15 cells, [^{3}H]-zacopride) does not confirm the one (IC_{50} = 0.81 pM) reported by Rault's group (NG108-15 cells, [^{3}H]granisetron[9b]). We have no explanation for this apparent discrepancy, since binding values for 5-HT_3 receptors obtained in rat cortex with our compounds and **5b** are similar to those obtained in NG108-15 cells (Table 6). We also replaced the benzyl group of the Rault compound **5b** by a 2-thienylmethyl moiety (**20aa**). This derivative showed an affinity (IC_{50} = 3.6 nM) comparable to those of **20v** and **5b** (5-HT_3 binding IC_{50} = 2.2 and 2.3 nM, respectively), with a selectivity profile similar to that of **20v**.

(v) Effect of Different Fused Five-Membered Rings (L3 Site). With respect to unsubstituted pyrroloquinoxalines (**3a**, IC_{50} = 0.37 nM)[6a] the introduction of an extra basic nitrogen on the pyrrole ring (**20i**,**j**) caused a 6-fold decrease of affinity, although it remains in the low nanomolar range (see Table 4). Replacement of the imidazole ring of **20i** with a pyrazole (**20k**) led to a more potent 5-HT_3 receptor ligand, whose affinity overlaps those of unsubstituted pyrroloquinoxalines, suggesting that the basicity of the nitrogen could be slightly unfavorable for receptor interaction (**20k** vs **20i**). Combination of a pyrido-fused ring with an imidazole system, in place of the pyrrole ring of **5a**, leads to **20m** whose affinity appears slightly lower than those of **5a** and **20i**.

Table 6. Comparison of Inhibition of [^{3}H]Zacopride Binding in Rat Brain and NG108-15 Hybrid Cells



5a, 20b,l,aa

					IC$_{50}$ (nM)[a] (±SEM)	
compd	R	X	Y	R′	rat cortex	NG108-15 cells
5a[b]	H	N	CH	Me	1.0 ± 0.3	5.4 ± 0.4
20b	9-Me	CH	CH	Me	0.79 ± 0.1	0.26 ± 0.02
20l	H	CH	N	Me	2.4 ± 0.5	9.3 ± 0.9
20aa	H	N	CH	2-thienyl-methyl	3.6 ± 0.5	6.4 ± 0.5

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximal inhibition of [^{3}H]zacopride binding to rat cortical homogenate or NG108-15 hybrid cells. [b] Compound **5a** has been resynthesized and tested following our binding protocols. **5a** has been reported by Rault et al.[9b]

(vi) Effect of Hydrophilic Substituents on the Piperazine Ring. In our previous publication[6a] we discussed the effect of different alkyl chains at the N-4 of the piperazine ring. In particular, the ethyl chain derivative of unsubstituted pyrroloquinoxaline showed an IC$_{50}$ of 8 nM. As shown in Table 5 we explore herein the effect of hydrophilic functions at the piperazine ring on 5-HT$_3$ affinity. Introduction of a hydroxy group on the β-carbon of the ethyl chain leads to high-affinity analogues (**20ee**,**ff**). In particular, the β-hydroxyethyl-substituted 7-fluoroquinoxaline **20ff** shows an IC$_{50}$ value of 2.57 nM, indicating that a hydroxy group is well-tolerated in the receptor cleft. On the other hand, if the β-hydroxyethyl chain is combined with a basic nitrogen on the heteroaromatic system (**20gg**), affinity decreases. Replacement of the ethyl chain with a cyanomethyl group (**20ii**) leads to a compound devoid of any 5-HT$_3$ receptor affinity, probably due to unfavorable steric interactions. In contrast, the replacement of the cyano group with a carboxylic function or with a tetrazolyl moiety leads to analogues **20hh**,**jj** with still significant affinity for the 5-HT$_3$ receptor. By virtue of the residual affinity of **20hh** and especially of **20jj** (IC$_{50}$ = 690 nM) for the 5-HT$_3$ receptor and taking into account that the presence of highly hydrophilic functions (**20hh**) do not allow the brain penetration of these compounds (see In Vivo Studies), we can consider **20hh**,**jj** lead compounds for the development of specific peripheral 5-HT$_3$ receptor agonists.

2. Selectivity Studies on a Panel of 5-HT Receptor Subtypes. A subset of compounds, chosen among the most potent 5-HT$_3$ receptor ligands, were further examined for their selectivity toward 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$, and 5-HT$_4$ receptors. Table 7 summarizes the results of the binding data for compounds **19b** and **20b**,**c**,**v**,**aa**,**ee**. Among these compounds, **20b**,**v**,**aa** and, to a lesser extent, **19b** reveal to be extremely selective for the 5-HT$_3$ receptor and, in this regard, much more performant than quipazine.

3. Biological Activity.[7] In addition to binding studies a functional assay was carried out for assessing the potential agonist/antagonist activity of selected pyrroloquinoxaline, pyridopyrrolopyrazine, and imidazoquinoxaline derivatives (Tables 3−5). This 5-HT$_3$-dependent response was the uptake of [^{14}C]guanidinium in the presence of substance P (SP), which is markedly increased upon the activation of 5-HT$_3$ receptors on the neuroblastoma-glioma hybrid NG108-15 cells. The most intriguing result arising from these pharmacological experiments was that all the tested compounds mimicked the effects of 5-HT on the 5-HT$_3$ receptor-dependent accumulation of [^{14}C]guanidinium in NG108-15 hybridoma cells in the low nanomolar concentration range, indicating that the novel pyrroloquinoxalines and pyrroloquinoxaline-related derivatives act as potent agonists in this assay. Similarly to that observed in binding experiments, the introduction of a methyl group at position 9 (**20b**) or a C-7 hydroxy substituent (**20f**), coupled with a methyl at N-4, improves the potency (**20b**,**f** vs **19b**, **20q**,**g**) in the guanidinium uptake test. Agonist properties are also dependent upon the nature of the fused five-membered ring. Accordingly, the pyrrole confers maximum efficacy (**3b**), followed by the pyrazole (**20k**) and the imidazole (**20i**,**j**). In the pyrido-fused series of analogues, the same rank order of magnitude is seen, with **20l** 3 times more active than the benzyl analogue **20v**. Intrinsic efficacy of substituted pyrroloquinoxalines and related analogues in guanidinium uptake is further influenced by the nature of the π-electron-rich system at N-4 of the piperazine ring. In fact, maximum potency is achieved with the benzyl analogue **20s**, followed by the 2-furylmethyl-substituted **20cc** and the 2-thienylmethyl derivative **20z** (EC$_{50}$s =

Table 7. Binding Profile of Selected Compounds on 5-HT Receptor Subtypes

	pK_i[a] ± SEM					
compd	5-HT$_3$[b]	5-HT$_{1A}$[d]	5-HT$_{1B}$[e]	5-HT$_{2A}$[f]	5-HT$_{2C}$[g]	5-HT$_4$[h]
quipazine	8.2 ± 0.9	5.8 ± 0.5	6.7 ± 0.5	6.1 ± 0.6	6.5 ± 0.6	<5
19b	9.0 ± 2.1	<5	6.6 ± 1.4	6.0 ± 1.8	6.0 ± 1.7	<5
20b	9.1 ± 1.6	<5	6.4 ± 0.6	6.3 ± 1.2	<5	<5
20l	8.6 ± 1.8	7.6 ± 1.0	7.6 ± 1.4	<5	6.1 ± 1.2	<5
20v	8.7 ± 3.1	<5	<5	6.1 ± 2.3	<5	<5
20aa	8.5 ± 1.3	<5	5.4 ± 0.5	5.8 ± 0.8	<5	<5
20ee	8.1 ± 2.4	6.3 ± 0.8	5.8 ± 1.4	6.3 ± 0.3	5.7 ± 1.8	<5
5-HT	6.4 ± 1.8[b]	8.8 ± 1.4	8.6 ± 0.9	5.9 ± 0.9	7.5 ± 0.2	7.0 ± 1.2
methysergide				8.7 ± 1.1		
mesulergine					8.8 ± 1.6	
GR38032	8.0 ± 1.4[b]					
2-Me-5-HT	6.7 ± 1.5[c]					

[a] Data are pK_i values ± SEM of at least three separate experiments performed in triplicate. K_i values were previously derived from the IC$_{50}$s according to the method of Cheng and Prusoff.[24] [b] [^{3}H]Zacopride. [c] [^{3}H]BRL 43694. [d] [^{3}H]8-OH-DPAT. [e] [^{3}H]5-HT. [f] [^{3}H]Ketanserin. [g] [^{3}H]Mesulergine. [h] [^{3}H]GR113808.

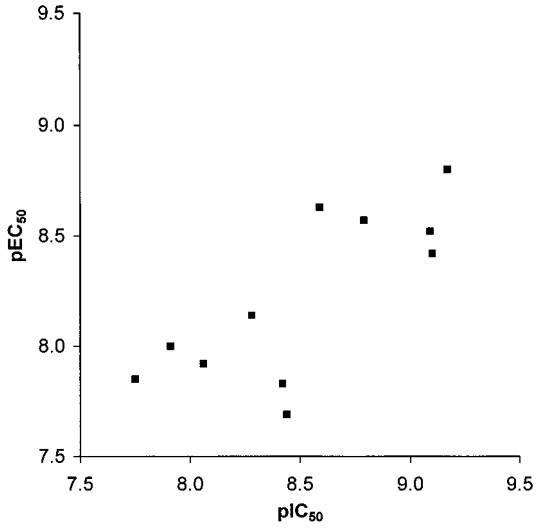


Figure 1. Scatter plot of pEC_{50} vs pIC_{50} values relative to the functional and binding data of the 11 pure agonists described.

4.2, 6.58, and 10, respectively). Overall, a good correlation of the binding data (cerebral cortex and NG108-15 cells) and of the EC_{50} values ([^{14}C]guanidinium uptake) of the tested compounds is achieved in the series of 5-HT_3 full agonists. Accordingly, a highly statistically linear correlation is found between the functional and 5-HT_3 binding data expressed as pEC_{50} and pIC_{50} values, respectively, of the 11 pure agonists described (correlation coefficient 0.77, standard deviation 0.26). A scatter plot of pEC_{50} vs pIC_{50} for these 11 compounds is shown in Figure 1. Interestingly, functional and 5-HT_3 binding data were not correlated at all when the full agonists were merged with the remaining compounds classified as partial agonists. Since common pharmacological and physicochemical properties of 5-HT_3 receptors in cerebral rat cortex and NG108-15 cells have been recently described,[14] the [^{14}C]guanidinium uptake test on NG108-15 cells is considered a reliable pharmacological model to infer central functional properties of ligands of the 5-HT_3 receptor. Accordingly, the most active members of this series of 5-HT_3 receptor ligands, which show a methyl atom at position 9 (**20b**) or a C-7 hydroxy group (**20f**), which mimicked that one of serotonin, or an unsubstituted pyrido-fused ring (**20l**), could be considered among the most potent central 5-HT_3 receptor agonists described until to date.

4. In Vivo Studies. i. Assessment of Potential Anxiolytic-like and Analgesic-like Properties. The compound **5b**, with chemical structure, affinity, and full agonist property on NG108-15 cells[9b] similar to our compounds, has been described to have anxiolytic properties in the light/dark exploratory test in mice.[9b]

We therefore evaluated the potential anxiolytic-like properties of comparable oral doses of **3b**, previously characterized as a potent 5-HT_3 agonist able to cross the blood−brain barrier after systemic administration in rats,[6] and of **20b**, a newly described central 5-HT_3 receptor agonist, sharing with **3b** similar affinity and efficacy for 5-HT_3 receptors (IC_{50} = 0.79 and 0.81 nM, respectively) and a good brain-to-plasma distribution ratio. In the 5 min of light/dark exploratory test, chlordiazepoxide, given subcutaneously 30 min before the test at a dose of 10 mg kg^{-1} (but not 1 and 3 mg kg^{-1}), significantly increased the time spent by the animals in the white compartment [from 132 ± 9 to 189 ± 11 s, $F(3,36) = 5.1$, $P < 0.01$, Dunnett's test] as well as the number of transitions between the black and white compartments [from 26 ± 2.4 to 38 ± 2, $F(3,36) = 3.0$, $P < 0.05$, Dunnett's test], in agreement with previous findings.[15,16]

Compounds **3b** and **20b**, given orally 45 min before the test at doses ranging from 0.1 to 100 μg kg^{-1}, did not modify the mice's behavior in the light/dark exploratory test.

The apparent discrepancy between our negative results and those reported by Prunier et al.[9b] using compounds with very similar structural and in vitro properties on the 5-HT_3 receptor could be due either to the fact that, after oral administration, the compounds could be differently absorbed and metabolized, thus giving rise to different brain levels of active product, or to the fact that the compounds used by Prunier et al.[9b] behaved as partial agonists in the Von Bezold−Jarisch reflex assay in vivo, while compound **3b** is a full agonist in this test.[6] This latter possibility seems more plausible, since anxiolytic-like activity has been reported for 5-HT_3 antagonists in mice, rats, and cynomologus monkey.[17,18]

We also evaluated the possible analgesic-like activity of compounds **3b** and **20b**, since 5-HT as well as 5-HT_3 agonists, like 2-methylserotonin, have been reported to produce analgesia after intratecal administration in rats, and this effect was completely abolished by 5-HT_3 antagonists such as ICS 205-930, MDL 72222, and zacopride.[19−21]

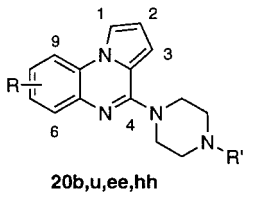
We tested the effect of 10 mg kg^{-1} i.p. of **3b**, a dose reported to give, 60 min after dosing, brain levels high enough to fully saturate the 5-HT_3 receptor,[6] and comparable dose of **20b** on the radiant heat tail flick test. In this test morphine, 3 mg kg^{-1} s.c., induced a clear analgesic effect [$F_{\text{Treatment}\times\text{Time}}(4,60) = 42.7$, $P < 0.01$, two-way ANOVA for repeated measures] at 30 and 60 min posttreatment (11.9 ± 1.1 and 8.6 ± 1.1 s, respectively, compared to control group: 2.1 ± 0.2 and 2.0 ± 0.2 s, $P < 0.01$, Tukey's test).

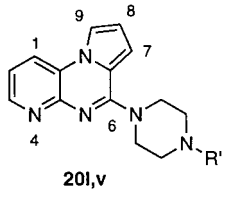
Analysis of variance for repeated measures found a significant interaction between treatment and time [$F_{\text{Treatment}\times\text{Time}}(4,84) = 2.2$, $P < 0.05$, two-way ANOVA for repeated measures]. Post-hoc comparison by Tukey's test revealed that **3b** but not **20b** slightly but significantly increased the latency of rats' tail flick 60 min posttreatment (4.6 ± 0.9 s, compared to control group: 2.2 ± 0.2 s, $P < 0.05$, Tukey's test).

However, in a second experiment, we failed to show a clear dose−response effect of **3b** [from 3 to 20 mg kg^{-1} i.p.; $F_{\text{Treatment}\times\text{Time}}(4,112) = 1.5$, $P > 0.05$, two-way ANOVA for repeated measures]. Therefore further experiments will clarify whether compound **3b** exerts analgesic-like activity only in a very narrow dose range, and by using selective 5-HT_3 antagonists it will be studied whether it is related to 5-HT_3 receptor activation.

ii. Brain-to-Plasma Distribution Studies. Preliminary brain-to-plasma partition studies were performed with compounds **20b**,**l**,**u**,**v**,**ee**,**hh**, given intravenously in rats (5 mg/kg). These compounds cover a wide range of structural characteristics, including different heteroaromatic-fused rings, halogens, and acidic

Table 8. Brain-to-Plasma Partition of Representative Compounds in the Rat





compd[a]	R	X	R′	brain/plasma[b]
20b	9-Me	CH	Me	9.5
20l	H	N	Me	4.9
20u	9-F	CH	2-naphthylmethyl	2.8
20v	H	N	CH_2Ph	2.1
20ee	9-F	CH	CH_2CH_2OH	20.3
20hh	9-F	CH	CH_2CO_2H	0.1

[a] Compounds were tested as hydrochloride salts. [b] Calculated as brain-to-plasma AUC_t ratio.

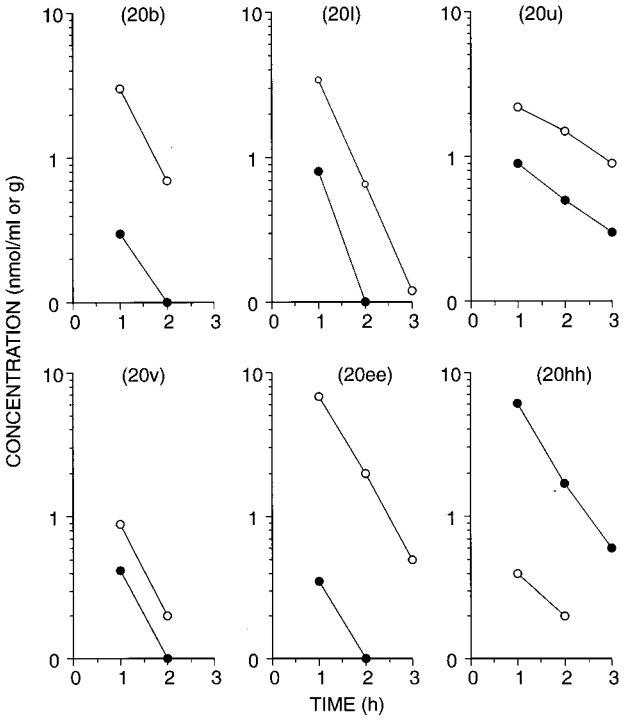


Figure 2. Mean plasma and brain concentration−time curves of selected compounds after intravenous injection in rats. The dose was 5 mg/kg. Each value is the mean of 2−3 rats.

or hindered fuctions on the piperazine ring. The mean brain-to-plasma distribution ratios (brain-to-plasma AUC_t ratio) of these compounds are listed in Table 8. With the exception of compound **20hh** (carboxylic function on the piperazine ring) mean plasma concentrations were generally low, hardly amounting to about 1 μM at 1 h after dosing (see Figure 2). These concentrations rapidly declined to levels close to the limits of analytical procedure within 2 h after injection, except for compound **20u** which eliminated more slowly than the other compounds, although the elimination $t_{1/2}$ could not be determined because of the shortage of experimental points. The different pharmacokinetic behavior of these derivatives may be the result of differences in clearance and/or distribution, which could not be evaluated in these preliminary studies. For all compounds the mean brain concentrations paralleled those in plasma, within the time after dosing examined. This suggests that these compounds rapidly enter the brain and attain equilibrium between the brain and peripheral blood. With the exception of compound **20hh**, again mean brain concentrations exceeded those in plasma. This was not surprising as previous studies have shown that lipophilic structurally related compounds do concentrate in brain tissue, achieving concentrations several times higher than those in blood.[6,22a] This is probably explained by their high lipophilicity which leads to the extensive uptake into lipid-containing tissues. However, there were differences in the brain-to-plasma ratios of the various derivatives. The alcoholic derivative **20ee** had the higher brain-to-plasma partition ratio (about 20) followed by compounds **20b** (9.5) and **20l** (4.9). These compounds possess calculated log P values[22b] between 2.8 (**20l**) and 4.3 (**20b**). The ratio for the most lipophilic compounds studied, **20v** (log $P = 4.3$) and **20u** (log $P =$ 7), was 2−3. This is consistent with the observation that within a series of molecules there is an optimal octanol/water partition for the brain-to-plasma distribution ratio. However, for the present series of centrally acting compounds, the observed brain-to-plasma ratios might not reflect the true extent of brain uptake since plasma protein binding has not yet been evaluated.

Conclusions

This work led to the discovery of high-affinity and selective 5-HT$_3$ receptor ligands. Most of the newly described compounds show full agonist properties in functional studies. Binding affinity, selectivity, and efficacy are dependent upon several structural features: 1. the nature of the fused six-membered ring, 2. the nature of the fused five-membered ring, and 3. the substituents on the benzo-fused ring and at N-4 of the piperazine ring. Starting from our lead **3b**, we identified high-affinity ligands endowed with a greatly improved selectivity for the 5-HT$_3$ receptor, compared to quipazine (**19b**, **20b**,**c**,**v**,**aa**). The compounds with pure agonists properties in functional studies present efficacies well-correlated to their affinity for the 5-HT$_3$ receptor. Compounds **20b**,**f** could be considered two of the most potent 5-HT$_3$ receptor agonists described to date. We also determined the ability of the new agonists to cross the blood−brain barrier. The compounds tested differ in their physicochemical properties, but most are lipophilic compounds. Thus, they rapidly enter the brain and equilibrate between brain and plasma. With the exception of the polar derivative **20hh**, brain concentrations of **20b**,**l**,**u**,**v**,**ee** are generally higher than plasma concentrations, although with differences in the brain-to-blood partition. By virtue of the agonist potencies displayed in the [^{14}C]guanidinium accumulation test and of the ability to cross the blood−brain barrier, pyrroloquinoxalines and pyridopyrrolopyrazines represent potent pharmacological tools to explore the central 5-HT$_3$ receptor-mediated functions. Accordingly, the potent agonists **3b** and **20b** were tested in vivo to assess anxiolytic-like or analgesic-like properties. No anxiolytic-like effect could be detected in the light/dark exploratory test in mice. However, compound **20b** induces analgesia in rats. Further studies are required to assess if this effect is related to central 5-HT$_3$ receptor activation. Furthermore, we have also demostrated that highly hydrophilic groups at N-4 of the piperazine ring could be tolerated at the receptor level. In fact, pyrroloquinoxalines bearing a carboxylic or tetrazolyl group

(**20hh**,**jj**) still bind the receptor with a significant affinity. By virtue of their affinity and of the fact that **20hh** and, presumibly, **20jj** do not cross the blood−brain barrier, these analogues represent interesting lead compounds to develop new peripheral 5-HT_3 receptor agonists.

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 Hz. 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. 2-(Bromomethyl)thiophene, 2-(bromomethyl)furan, 2-bromo-5-(bromomethyl)thiophene, and 2-bromo-5-(bromomethyl)furan were prepared starting from 2-methylthiophene and 2-methylfuran by means of *N*-bromosuccinimide using catalytic amounts of azobis(isobutyronitrile) (AIBN).[11b] Physical data for compounds **7**−**17** and **18**−**24** are reported in Tables 1 and 2.

General Procedure for Preparation of Compounds 7a−**e.** This procedure is illustrated for the preparation of 1-(4,5-dimethyl-2-nitrophenyl)-1*H*-pyrrole (**7a**). To a solution of 4,5-dimethyl-2-nitroaniline (**6a**) (1.0 g, 6.0 mmol) in glacial acetic acid (3.9 mL) was slowly added 2,5-dimethoxytetrahydrofuran (0.78 mL, 6.0 mmol). The reaction mixture was refluxed for 1 h, cooled, and evaporated to afford an oily residue which was chromatographed (20% ethyl acetate in hexanes) to give 0.97 g of **7a** as pale yellow prisms: IR (Nujol) 1599, 1520 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 6H), 6.37 (m, 2H), 6.78 (m, 2H), 7.22 (s,1H), 7.71 (s, 1H). Anal. ($C_{12}H_{12}N_2O_2$) C, H, N.

1-(6-Methyl-2-nitrophenyl)-1*H*-pyrrole (7b). Similarly to the procedure as described for **7a**, the title compound was prepared starting from 6-methyl-2-nitroaniline (**6b**). **7b** was obtained as yellow prisms after recrystallization: IR ($CHCl_3$) 1515 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 3 H), 6.35 (m, 2 H), 6.66 (m, 2 H), 7.39−7.56 (m, 2 H), 7.67 (d, 1 H, J = 7.7 Hz). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

1-(4-Cyano-2-nitrophenyl)-1*H*-pyrrole (7c). Starting from 4-cyano-2-nitroaniline (**6c**) the title compound was obtained as for **7a**. After recrystallization **7c** was obtained as red-orange prisms: IR ($CHCl_3$) 2230, 1340 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.39 (m, 2 H), 6.77 (m 2 H), 7.74 (m, 2 H), 7.86 (m, 1 H). Anal. ($C_{11}H_7N_3O_2$) C, H, N.

1-(4-Hydroxy-2-nitrophenyl)-1*H*-pyrrole (7d). Similarly to the procedure as described for **7a**, the title compound was prepared starting from 4-hydroxy-2-nitroaniline (**6d**). **7d** was obtained as a dark brown solid: IR ($CHCl_3$) 3620, 1580, 1335 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.90 (br s, 1 H), 6.30 (m, 2 H), 6.72 (m, 2 H), 7.08 (dd, 1 H, J = 2.6, 8.3 Hz), 7.30 (m, 2 H). Anal. ($C_{10}H_8N_2O_3$) C, H, N.

1-(4,5-Dichloro-2-nitrophenyl)-1*H*-pyrrole (7e). Starting from 4,5-dichloro-2-nitroaniline (**6e**) the title compound was obtained following a procedure as described for **7a**. After recrystallization, **7e** was obtained as colorless prisms: IR ($CHCl_3$) 1510, 1340 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.37 (m, 2 H), 6.75 (m 2 H), 7.59 (s, 1 H), 7.99 (s, 1 H). Anal. ($C_{10}H_6Cl_2N_2O_2$) C, H, N.

1-(4-Benzyloxy-2-nitrophenyl)-1*H*-pyrrole (8). A solution of **7d** (0.5 g, 2.45 mmol) and benzyl chloride (0.42 mL, 3.67 mmol) in DMF (4.28 mL) was treated with K_2CO_3 (0.67 g, 4.9 mmol), and the resulting mixture was heated at 60 °C overnight. After cooling, the solvent was evaporated and the residue was dissolved in dichloromethane. The organic phase was washed with 1 N HCl and brine, dried, and concentrated. The residue was chromathographed (dichloromethane and hexanes, 1/1) to give, after recrystallization, 0.70 g of **8** as yellow-orange prisms: IR (Nujol) 1530, 1315 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.13 (s, 2 H), 6.31 (m, 2 H), 6.71 (m, 2 H), 7.18 (d, 1 H, J = 2.6 Hz), 7.22 (t, 1 H, J = 2.37 Hz), 7.33−7.43 (m, 6 H). Anal. ($C_{17}H_{14}N_2O_3$) C, H, N.

General Procedure for Preparation of Compounds 9a−**e.** This procedure is illustrated for the preparation of 1-(2-amino-4,5-dimethylphenyl)-1*H*-pyrrole (**9a**). A solution of **7a** (1.0 g, 4.6 mmol) and tin(II) chloride dihydrate (5.36 g, 23.8 mmol) in ethanol (63 mL) was heated to reflux under argon for 30 min. The mixture was cooled, the pH was adjusted to 8 with saturated sodium bicarbonate solution, and the mixture was filtered through Celite. The filter cake was washed with ethanol, and the filtrates were evaporated. The residue was chromatographed (0.5% methanol in dichloromethane) to give 0.81 g of **9a** as red-orange prisms: IR (Nujol) 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.18 (s, 6 H), 3.58 (br s, 2 H), 6.36 (m, 2 H), 6.58 (s, 1 H), 6.75 (m, 2 H), 7.03 (m, 1 H). Anal. ($C_{12}H_{14}N_2$) C, H, N.

1-(2-Amino-6-methylphenyl)-1*H*-pyrrole (9b). Similarly to the procedure described for **9a**, the title compound was prepared starting from **7b**. After recrystallization **9b** was obtained as a yellowish solid: IR ($CHCl_3$) 3410 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.88 (s, 3 H), 4.48 (br s, 2 H), 6.27 (m, 2 H), 6.52 (d, 1 H, J = 7.4 Hz), 6.68 (m, 3 H), 7.03 (t, 1 H, J = 7.6 Hz). Anal. ($C_{11}H_{12}N_2$) C, H, N.

1-(2-Amino-4-cyanophenyl)-1*H*-pyrrole (9c). Starting from **7c** the title compound was obtained following an identical procedure as for **9a**. After recrystallization **9c** was obtained as deep red prisms: IR ($CHCl_3$) 3400, 2240 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.98 (br s, 2 H), 6.36 (m, 2 H), 6.82 (m, 2 H), 7.04 (m, 2 H), 7.18 (m, 1 H). Anal. ($C_{11}H_9N_3$) C, H, N.

1-(2-Amino-4-benzyloxyphenyl)-1*H*-pyrrole (9d). Similarly to the procedure as described for **9a**, the title compound was prepared starting from **8**. **9d** was obtained as colorless prisms after recrystallization: IR ($CHCl_3$) 3405 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.64 (br s, 2 H), 5.03 (s, 2 H), 6.29 (m, 2 H), 6.38 (m, 2 H), 6.75 (m, 2 H), 7.04 (m, 1 H), 7.28−7.40 (m, 5 H). Anal. ($C_{17}H_{16}N_2O$) C, H, N.

1-(2-Amino-4,5-dichlorophenyl)-1*H*-pyrrole (9e). Starting from **7e** the title compound was obtained as described for **9a**. After recrystallization **9e** was obtained as colorless prisms: IR ($CHCl_3$) 3400 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.81 (br s, 2 H), 6.35 (m, 2 H), 6.78 (m, 2 H), 6.89 (s, 1 H), 7.23 (s, 1 H). Anal. ($C_{10}H_8Cl_2N_2$) C, H, N.

(3-Nitropyridin-2-yl)carbamic Acid Benzyl Ester (12). To a mixture of 2-amino-3-nitropyridine (5.0 g, 36.0 mmol) in dichloromethane (18 mL) was added a solution of bis(trimethylsilyl)acetamide (10.5 mL, 43.2 mmol) in dichloromethane (18 mL), and the resulting solution was stirred under argon at room temperature for 1 h. The reaction mixture was cooled to 0 °C, and a solution of benzyl chloroformate (13.4 mL, 93.6 mmol) in dichloromethane (39 mL) was added dropwise. The reaction was stirred at room temperature for 25 h. The mixture was cooled again to 0 °C, quenched with a pH 7 buffer, and extracted with diethyl ether. The ether extract was washed with brine, dried, and concentrated. The residue was chromatographed (10% ethyl acetate in dichloromethane) to give 7.65 g of **12** as a colorless oil: IR (neat) 3350, 1670, 1559 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.25 (s, 2 H), 7.17 (dd, 1 H, J = 4.7, 7.7 Hz), 7.34−7.40 (m, 5 H), 8.46 (dd, 1 H, J = 1.8, 7.6 Hz), 8.71 (dd, 1 H, J = 1.7, 4.9 Hz), 9.81 (br s, 1 H). Anal. ($C_{13}H_{11}N_3O_4$) C, H, N.

(3-Aminopyridin-2-yl)carbamic Acid Benzyl Ester (13). Similarly to the procedure as described for **9a**, the title compound was prepared starting from **12**. After recrystallization **13** was obtained as colorless prisms: IR ($CHCl_3$) 3410, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.26 (br s, 2 H), 5.19 (s, 2 H), 6.94 (dd, 1 H, J = 4.8, 7.6 Hz), 7.05 (dd, 1 H, J = 1.6, 7.8 Hz), 7.33−7.42 (m, 6 H), 7.76 (d, 1 H, J = 3.88 Hz). Anal. ($C_{13}H_{13}N_3O_2$) C, H, N.

1-[2-(Benzyloxycarbonylamino)pyridin-3-yl]-1*H*-pyrrole (14). Starting from **13** the title compound was obtained following an identical procedure as for **7a**, except that the oily residue was partitioned between saturated $NaHCO_3$ solution and ethyl acetate, and the organic layer was washed with brine, dried, and concentrated. After recrystallization, **14** was obtained as colorless prisms: IR (Nujol) 3360, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.15 (s, 2 H), 6.38 (m, 2 H), 6.80 (m, 2 H), 6.95 (br s, 1 H), 7.13 (dd, 1 H, J = 4.7, 7.6 Hz), 7.26−7.34 (m, 5 H), 7.58 (dd, 1 H, J = 1.6, 7.8 Hz), 8.47 (dd, 1 H, J = 1.7, 4.8 Hz). Anal. ($C_{17}H_{15}N_3O_2$) C, H, N.

1-(4-Fluoro-2-nitrophenyl)-1*H*-imidazole (16). A solution of 2,5-difluoronitrobenzene **15** (2.0 g, 12.6 mmol) and imidazole (2.56 g, 37.8 mmol) in dimethyl sulfoxide (23 mL) was heated to 50 °C under an argon atmosphere for 24 h. The mixture was then cooled to room temperature, poured into water, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and concentrated. The residue was flash-chromatographed (5% methanol in dichloromethane) to afford 1.25 g of **16** that after recrystallization (hexanes) was obtained as yellow-orange prisms: IR (Nujol): 1620, 1549, 1335 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.03 (s, 1 H), 7.20 (s, 1 H), 7.44 (m, 2 H), 7.59 (s, 1 H), 7.73 (dd, 1 H, J = 2.8, 8.8 Hz). Anal. ($C_9H_6FN_3O_2$) C, H, N.

1-(2-Amino-4-fluorophenyl)-1*H*-imidazole (17). Similarly to the procedure as described for **9a**, the title compound was prepared starting from **16**. After recrystallization **17** was obtained as yellow prisms: IR ($CHCl_3$) 3400, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.78 (br s, 2 H), 6.48 (m, 2 H), 7.05 (m, 2 H), 7.21 (s, 1 H), 7.56 (s, 1 H). Anal. ($C_9H_8FN_3$) C, H, N.

General Procedure for Preparation of Compounds 10a−f. This procedure is illustrated for the preparation of 7,8-dimethylpyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (**10a**). To a solution of **9a** (0.81 g, 4.36 mmol) in toluene (9.5 mL) was added triphosgene (0.43 g, 1.45 mmol). The reaction mixture was refluxed for 30 min, and nitrogen was bubbled in to drive off excess of phosgene. The solution was then set aside for 18 h. The heavy crystalline precipitate was filtered off and washed with hexanes to give, after recrystallization (EtOAc), 0.79 g of **10a** as colorless prisms: IR (Nujol) 3280, 1693 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.22 (s, 3 H), 2.27 (s, 3 H), 6.62 (t, 1 H, J = 3.3 Hz), 6.96 (d, 1 H, J = 3.8 Hz), 7.03 (s, 1 H), 7.82 (s, 1 H), 8.07 (m, 1H), 11.06 (br s, 1 H). Anal. ($C_{13}H_{12}N_2O$) C, H, N.

9-Methylpyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (10b). Similarly to the procedure as described for **10a**, the title compound was prepared starting from **9b**. After recrystallization, **10b** was obtained as colorless prisms: IR (Nujol) 3220, 1660 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.80 (s, 3 H), 6.70 (t, 1 H, J = 3.4 Hz), 7.04−7.21 (m, 4 H), 8.19 (d, 1 H, J = 2.7 Hz), 11.26 (br s, 1 H). Anal. ($C_{12}H_{10}N_2O$) C, H, N.

7-Cyanopyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (10c). Starting from **9c** the title compound was obtained as for **10a**. After recrystallization **10c** was obtained as colorless prisms: IR (Nujol) 2230, 1655 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.79 (t, 1 H, J = 3.4 Hz), 7.13 (d, 1 H, J = 3.9 Hz), 7.61 (d, 1 H, J = 1.8 Hz), 7.70 (dd, 1 H, J = 1.6, 7.8 Hz), 8.30 (m, 2 H), 11.53 (br s, 1 H). Anal. ($C_{12}H_7N_3O$) C, H, N.

7-(Benzyloxy)pyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (10d). Similarly to the procedure as described for **10a**, the title compound was prepared starting from **9d**. After recrystallization, **10d** was obtained as colorless prisms: IR (Nujol) 1665 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.14 (s, 2 H), 6.63 (t, 1 H, J = 2.9 Hz), 6.93 (m, 3 H), 7.32−7.50 (m, 5 H), 7.96 (d, 1 H, J = 8.5 Hz), 8.09 (m, 1 H), 11.38 (br s, 1 H). Anal. ($C_{18}H_{14}N_2O_2$) C, H, N.

7,8-Dichloropyrrolo[1,2-*a*]quinoxalin-4(5*H*)-one (10e). Starting from **9e** the title compound was obtained as for **10a**. After recrystallization, **10e** was obtained as colorless prisms: IR (Nujol) 1685 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.34 (t, 1 H, J = 3.4 Hz), 6.86 (d, 1 H, J = 3.8 Hz), 6.93 (s, 1 H), 7.72 (s, 1 H), 8.02 (m, 1 H), 11.02 (br s, 1 H). Anal. ($C_{11}H_6Cl_2N_2O$) C, H, N.

5,6-Dihydro-6-oxopyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrazine (10f). Starting from **14** the title compound was obtained as for **10a**, except that the reaction was refluxed for 1.5 h. After recrystallization **10f** was obtained as colorless prisms: IR (Nujol) 3200, 1670 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.32 (m, 1 H), 6.74 (m, 1 H), 7.02 (m, 1 H), 7.22 (dd, 1 H, J = 4.6, 7.9 Hz), 8.23 (dd, 1 H, J = 1.8, 7.6 Hz), 8.50 (dd, 1 H, J = 1.7, 4.7 Hz), 11.71 (br s, 1 H). Anal. ($C_{10}H_7N_3O$) C, H, N.

7-Fluoroimidazo[1,2-*a*]quinoxalin-4(5*H*)-one (10g). A mixture of the amino derivative **17** (0.74 g, 4.18 mmol) and *N*,*N*-carbonyldiimidazole (0.73 g, 4.57 mmol) in 1,2-dichlorobenzene (31.7 mL) was heated at reflux under argon for 1.5 h. After cooling to room temperature, the solid was filtered off and washed with acetone to give, after recrystallization, 0.42 g of **10g** as brown prisms: IR ($CHCl_3$) 1660 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.14 (m, 2 H), 7.59 (s, 1 H), 8.15 (m, 1 H), 8.51 (s, 1 H), 11.90 (br s, 1 H). Anal. ($C_{10}H_6FN_3O$) C, H, N.

General Procedure for Preparation of Compounds 18a−g,j−m. This procedure is illustrated for the preparation of 4-chloro-7,8-dimethylpyrrolo[1,2-*a*]quinoxaline (**18a**). A mixture of **10a** (0.30 g, 1.4 mmol), *N*,*N*-dimethylaniline (0.35 mL), and phosphorus oxychloride (4.7 mL) was heated at reflux under argon for 5 h. The excess $POCl_3$ was removed under vacuum, and the residue was dissolved in dichloromethane, washed with brine, dried, and concentrated. The residue was chromatographed (10% EtOAc in dichloromethane) and recrystallized to give 0.28 g of **18a** as colorless prisms: IR ($CHCl_3$) 3320, 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3 H), 2.45 (s, 3 H), 6.85 (t, 1 H, J = 3.4 Hz), 7.01 (d, 1 H, J = 3.8 Hz), 7.59 (s, 1 H), 7.65 (s, 1 H), 7.90 (d, 1 H, J = 2.9 Hz). Anal. ($C_{13}H_{11}ClN_2$) C, H, N.

4-Chloro-9-methylpyrrolo[1,2-*a*]quinoxaline (18b). Starting from **10b** the title compound was obtained following the synthetic procedure described for **18a**. After recrystallization **18b** was obtained as pale yellow prisms: IR ($CHCl_3$) 3300, 1601 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.87 (s, 3 H), 6.82 (t, 1 H, J = 3.4 Hz), 7.04 (d, 1 H, J = 3.4 Hz), 7.27 (m, 2 H), 7.72 (m, 1 H), 8.27 (t, 1 H, J = 2.2 Hz). Anal. ($C_{12}H_9ClN_2$) C, H, N.

4-Chloro-7-cyanopyrrolo[1,2-*a*]quinoxaline (18c). Similarly to the procedure described for **18a**, the chloro derivate **18c** was prepared starting from **10c**. After recrystallization **18c** was obtained as colorless prisms: IR (Nujol) 3315, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.99 (m, 1 H), 7.15 (d, 1 H, J = 3.7 Hz), 7.75 (dd, 1 H, J = 1.6, 7.8 Hz), 7.91 (d, 1 H, J = 8.7 Hz), 8.01 (d, 1 H, J = 3.0 Hz), 8.19 (d, 1 H, J = 1.8 Hz). Anal. ($C_{12}H_6ClN_2$) C, H, N.

7-Benzyloxy-4-chloropyrrolo[1,2-*a*]quinoxaline (18d). Starting from **10d** the title compound was obtained as for **18a**. After recrystallization **18d** was obtained as colorless prisms: IR ($CHCl_3$) 2990, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.14 (s, 2 H), 6.84 (t, 1 H, J = 3.3 Hz), 7.01 (d, 1 H, J = 3.8 Hz), 7.17−7.46 (m, 7 H), 7.73 (d, 1 H, J = 9.0 Hz), 7.87 (m, 1 H). Anal. ($C_{18}H_{13}ClN_2O$) C, H, N.

4,7,8-Trichloropyrrolo[1,2-*a*]quinoxaline (18e). Similarly to the procedure described for **18a**, the chloro derivate **18e** was prepared starting from **10e**. After recrystallization **18e** was obtained as colorless prisms: IR (Nujol) 3120, 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.91 (t, 1 H, J = 3.4 Hz), 7.06 (d, 1 H, J = 3.7 Hz), 7.87 (d, 1 H, J = 2.9 Hz), 7.90 (s, 1 H), 7.96 (s, 1 H). Anal. ($C_{11}H_5Cl_3N_2$) C, H, N.

6-Chloropyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrazine (18f). Similarly to the procedure described for **18a**, the chloro derivate **18f** was prepared starting from **10f**. After recrystallization **18f** was obtained as colorless prisms: IR ($CHCl_3$) 1685, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.92 (dd, 1 H, J = 2.3, 4.1 Hz), 7.08 (t, 1 H, J = 2.8 Hz), 7.45 (dd, 1 H, J = 4.8, 7.8 Hz), 7.99 (d, 1 H, J = 3.2 Hz), 8.16 (dd, 1 H, J = 1.7, 7.6 Hz), 8.71 (dd, 1 H, J = 1.8, 4.8 Hz). Anal. ($C_{10}H_6ClN_3$) C, H, N.

4-Chloro-7-fluoroimidazo[1,2-*a*]quinoxaline (18g). Starting from **10g** the title compound was obtained as for **18a**. After recrystallization **18g** was obtained as colorless prisms: IR ($CHCl_3$) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.44 (m, 1 H), 7.74 (dd, 1 H, J = 2.8, 8.8 Hz), 7.85 (m, 2 H), 8.14 (s, 1 H). Anal. ($C_{10}H_5FClN_3$) C, H, N.

4-Chloro-6,7-dihydropyrrolo[1,2-*a*]pyrazine (18j). Starting from **10j**, following an identical procedure as for **18a**, the title compound was obtained as a colorless thick oil: IR (Nujol)

1599 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.59 (m, 2 H), 4.15 (m, 2 H), 6.88 (m, 2 H), 6.74 (m, 1 H). Anal. ($C_7H_7ClN_2$) C, H, N.

4-Chloroimidazo[1,2-*a*]quinoxaline (18k). Similarly to the procedure described for **18a**, the chloro derivate **18k** was prepared starting from **10k**. **18k** was obtained as colorless prisms: IR ($CHCl_3$) 3250, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.23 (s, 1 H), 7.72−7.84 (m, 3 H), 8.06 (m, 1 H), 8.16 (s, 1 H). Anal. ($C_{10}H_6ClN_3$) C, H, N.

6-Chloropyrazolo[1,5-*c*]quinazoline (18l). Similarly to the procedure described for **18a**, the chloro derivate **18l** was prepared starting from **10l**. **18l** was obtained as colorless prisms after recrystallization: IR ($CHCl_3$) 1599 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.08 (d, 1 H, J = 1.9 Hz), 7.65 (m, 2 H), 7.90 (d, 1 H, J = 8.4 Hz), 8.03 (m, 1 H), 8.13 (d, 1 H, J = 1.9 Hz). Anal. ($C_{10}H_6ClN_3$) C, H, N.

6-Chloroimidazo[1,2-*a*]pyrido[2,3-*e*]pyrazine (18m). Starting from **10m** the title compound was obtained as for **18a**. After recrystallization **18m** was obtained as colorless prisms: IR ($CHCl_3$) 1630, 1599 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (dd, 1 H, J = 4.7, 7.7 Hz), 7.86 (s, 1 H), 8.37 (dd, 1 H, J = 1.7, 7.7 Hz), 8.57 (s, 1 H), 8.71 (dd, 1 H J = 1.5, 7.9 Hz). Anal. ($C_9H_5ClN_4$) C, H, N.

General Procedure for Preparation of Compounds 19a−c,g. This procedure is illustrated for the preparation of 7,8-dimethyl-4-(piperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (**19a**). A mixture of **18a** (1.0 g, 4.34 mmol) and dry piperazine (3.86 g, 44.4 mmol) in ethylene glycol (38 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 (50% dichlorometane in methanol) to give 1.11 g of **19a** as colorless prisms: IR (neat) 3340, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 2.38 (s, 3 H), 2.64 (m, 4 H), 3.78 (m, 4 H), 6.75 (m, 2 H), 7.47 (s, 1 H), 7.48 (s, 1 H), 7.75 (t, 1 H, J = 1.8 Hz). Anal. ($C_{17}H_{20}N_4$) C, H, N.

9-Methyl-4-(piperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (19b). Starting from **18b** the title compound was obtained as for **19a**. After recrystallization **19b** was obtained as colorless prisms: IR ($CHCl_3$) 3340, 1601 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.83 (s, 3 H), 3.08 (m, 4 H), 3.71 (m, 4 H), 6.73 (t, 1 H, J = 3.4 Hz), 6.79 (d, 1 H, J = 3.7 Hz), 7.08 (m, 1 H), 7.21 (m, 1 H), 7.57 (m, 1 H), 8.13 (d, 1 H, J = 2.8 Hz); MS m/z 266 (M^+), 210, 198 (100), 183, 154. Anal. ($C_{16}H_{18}N_4$) C, H, N.

7-Methyl-4-(piperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (19c). Similarly to the procedure described for **19a**, the title compound was prepared starting from **18h**. After recrystallization **19c** was obtained as a white solid: IR ($CHCl_3$) 3325, 1599 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.45 (s, 3 H), 2.68 (m, 4 H), 3.84 (m, 4 H), 6.75 (m, 2 H), 7.08 (m, 1 H), 7.45 (m, 1 H), 7.56 (d, 1 H, J = 8.3 Hz), 7.71 (m, 1 H). Anal. ($C_{16}H_{18}N_4$) C, H, N.

6-(Piperazin-1-yl)pyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrazine (19g). Similarly to the procedure described for **19a**, the title compound was prepared starting from **18f**. After recrystallization **19g** was obtained as colorless prisms: IR ($CHCl_3$) 3330, 1685, 1601 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.14 (m, 4 H), 3.84 (m, 4 H), 6.75 (t, 1 H, J = 3.4 Hz), 6.83 (t, 1 H, J = 1.9 Hz), 7.26 (dd, 1 H, J = 4.6, 7.9 Hz), 7.87 (dd, 1 H, J = 1.8, 7.6 Hz), 8.26 (m, 2 H). Anal. ($C_{14}H_{15}N_5$) C, H, N.

8-Chloro-2,3-dihydroimidazo[1,2-*a*]pyrazine Hydrochloride (23). A solution of **22** (0.93 g, 5.36 mmol) in thionyl chloride (2.3 mL) and chloroform (2.9 mL) was heated under reflux for 2 h. After cooling, the solid was collected and washed with ethyl ether to give 0.64 g of **23** as a pale yellow solid: IR ($CHCl_3$) 3380 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.03 (t, 2 H, J = 10.8 Hz), 4.78 (t, 2 H, J = 10.3 Hz), 7.86 (d, 1 H, J = 4.0 Hz), 8.39 (d, 1 H, J = 3.9 Hz), 10.71 (br s, 1 H). Anal. ($C_6H_7Cl_2N_3$) C, H, N.

α-[4-(9-Fluoropyrrolo[1,2-*a*]quinoxalin-1-yl)piperazin-1-yl]acetic Acid Benzyl Ester (24). A suspension of **19d** (0.2 g, 0.74 mmol), anhydrous potassium carbonate (96 mg, 0.74 mmol), and benzyl bromoacetate (0.17 g, 0.74 mmol) in ethyl methyl ketone (33 mL) was heated at reflux for 1 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 flash chromatographed (20% EtOAc in dicloromethane) to give 0.23 g of **24** as colorless prisms: IR (Nujol) 1680, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.79 (m, 4 H), 3.35 (s, 2 H), 3.87 (m, 4 H), 5.17 (s, 2 H), 6.78 (m, 2 H), 7.01 (m, 1 H), 7.18−7.45 (m, 7 H), 8.16 (m, 1 H). Anal. ($C_{24}H_{23}FN_4O_2$) C, H, N.

General Procedure for Preparation of Compounds 20a−t,v,w. This procedure is illustrated for the preparation of 7,8-dimethyl-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (**20a**). A mixture of **18a** (0.2 g, 0.87 mmol) and *N*-methylpiperazine (2 mL) was heated at 130 °C for 6 h, under argon. Then the reaction mixture was cooled, poured into crushed ice, and extracted with EtOAc. The combined organic extracts were washed with brine, dried, and concentrated. The residue was flash chromatographed (EtOAc) to give, after recrystallization, 0.22 g of **20a** as colorless prisms: IR ($CHCl_3$) 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (s, 3 H), 2.37 (s, 3 H), 2.38 (s, 3 H), 2.62 (m, 4 H), 3.77 (m, 4 H), 6.72 (m, 2 H), 7.46 (s, 1 H), 7.48 (s, 1 H), 7.75 (t, 1 H, J = 1.7 Hz). Anal. ($C_{18}H_{22}N_4$) C, H, N.

9-Methyl-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20b). Starting from **18b** the title compound was obtained following a similar procedure as described for **20a**. After recrystallization, **20b** was obtained as colorless prisms: IR ($CHCl_3$) 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35 (s, 3 H), 2.59 (m, 4 H), 2.83 (s, 3 H), 3.76 (m, 4 H), 6.70 (t, 1 H, J = 2.3 Hz), 6.77 (d, 1 H, J = 3.8 Hz), 7.04 (d, 1 H, J = 7.0 Hz), 7.19 (m, 1 H), 7.54 (dd, 1 H, J = 1.1, 7.8 Hz), 8.23 (m, 1 H). Anal. ($C_{17}H_{20}$-N4) C, H, N.

7-Cyano-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20c). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18c**. After recrystallization, **20c** was obtained as colorless prisms: IR (Nujol) 2225, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3 H), 2.58 (m, 4 H), 3.89 (m, 4 H), 6.81 (m, 2 H), 7.42 (dd, 1 H, J = 1.6, 7.8 Hz), 7.70 (d, 1 H, J = 8.6 Hz), 7.78 (d, 1 H, J = 2.5 Hz), 7.88 (m, 1 H). Anal. ($C_{17}H_{17}N_5$) C, H, N.

7-Benzyloxy-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20d). Starting from **18d** the title compound was obtained following the procedure as described for **20a**. After recrystallization, **20d** was obtained as colorless prisms: IR (Nujol) 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3 H), 2.88 (m, 4 H), 4.02 (m, 4 H), 5.13 (s, 2 H), 6.72 (m, 2 H), 6.96 (dd, 1 H, J = 2.6, 8.3 Hz), 7.31−7.47 (m, 6 H), 7.63 (d, 1 H, J = 8.9 Hz), 7.74 (d, 1 H, J = 1.5 Hz). Anal. ($C_{23}H_{24}N_4O$) C, H, N.

7,8-Dichloro-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20e). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18e**. After recrystallization, **20e** was obtained as colorless prisms: IR (Nujol) 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35 (s, 3 H), 2.57 (m, 4 H), 3.85 (m, 4 H), 6.76 (m, 2 H), 7.70 (m, 3 H). Anal. ($C_{16}H_{16}$-Cl_2N_4) C, H, N.

7-Hydroxy-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20f). A solution of **20d** (0.1 g, 0.27 mmol) in THF (1 mL) was hydrogenated at atmospheric pressure over 10% Pd−C (1.62 mg) overnight. The catalyst was removed by filtration, the solvent was evaporated, and the residue was recrystallized to give 74 mg of **20f** as colorless prisms: IR (Nujol) 3610, 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 2.48 (br s, 1 H), 2.59 (m, 4 H), 3.75 (m, 4 H), 6.51 (m, 1 H), 6.70 (m, 2 H), 6.93 (m, 1 H), 7.20 (m, 1 H), 7.48 (m, 1 H). Anal. ($C_{16}H_{18}N_4O$) C, H, N.

7-Methyl-4-(4-methylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20g). Starting from **18h** the title compound was obtained following the procedure described for **20a**. After recrystallization, **20g** was obtained as colorless prisms: IR ($CHCl_3$) 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.37 (s, 3 H), 2.42 (s, 3 H), 2.61 (m, 4 H), 3.82 (m, 4 H), 6.69−6.75 (m, 2 H), 7.07 (m, 1 H), 7.49 (m, 1 H), 7.58 (d, 1 H, J = 8.5 Hz), 7.75 (d, 1 H, J = 3.2 Hz). Anal. ($C_{17}H_{20}N_4$) C, H, N.

4-(4-Methylpiperazin-1-yl)-6,7-dihydropyrrolo[1,2-*a*]pyrazine (20h). Starting from **18j** the title compound was obtained following the procedure described for **20a**. After

recrystallization, **20h** was obtained as a white solid: IR ($CHCl_3$) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3 H), 2.45 (m, 4 H), 3.50 (m, 4 H), 3.63 (m, 2 H), 4.12 (m, 2 H), 6.73 (m, 1 H), 6.94 (m, 2 H). Anal. ($C_{12}H_{18}N_4$) C, H, N.

4-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]quinoxaline (20i). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18k**. After recrystallization **20i** was obtained as colorless prisms: IR (Nujol) 1625, 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3 H), 2.63 (m, 4 H), 4.45 (m, 4 H), 6.61 (d, 1 H, J = 1.7 Hz), 7.23−7.45 (m, 2 H), 7.70 (dd, 2 H, J = 2.8, 7.8 Hz), 7.96 (s, 1 H). Anal. ($C_{15}H_{17}N_5$) C, H, N.

7-Fluoro-4-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]quinoxaline (20j). Starting from **18g** the title compound was obtained following the procedure as for **20a**. After recrystallization, **20j** was obtained as colorless prisms: IR ($CHCl_3$) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3 H), 2.57 (m, 4 H), 4.44 (m, 4 H), 6.95 (m, 1 H), 7.32 (dd, 1 H, J = 2.8, 8.8 Hz), 7.58 (m, 2 H), 7.89 (s, 1 H). Anal. ($C_{15}H_{16}FN_5$) C, H, N.

6-(4-Methylpiperazin-1-yl)pyrazo[1,5-*c*]quinazoline (20k). Starting from **18l** the title compound was obtained following a procedure described for **20a**. After recrystallization **20k** was obtained as colorless prisms: IR ($CHCl_3$) 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3 H), 2.67 (m, 4 H), 4.02 (m, 4 H), 6.90 (d, 1 H, J = 1.9 Hz), 7.33 (m, 1 H), 7.53 (m 1 H), 7.69 (d, 1 H, J = 8.0 Hz), 7.90 (dd, 1 H, J = 1.4, 7.8 Hz), 7.97 (d, 1 H, J = 1.9 Hz). Anal. ($C_{15}H_{17}N_5$) C, H, N.

6-(4-Methylpiperazin-1-yl)pyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrazine (20l). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18f**. After recrystallization **20l** was obtained as colorless prisms: IR (Nujol) 1601 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3 H), 2.54 (m, 4 H), 4.02 (m, 4 H), 6.76 (m, 1 H), 6.83 (m, 1 H), 7.12 (dd, 1 H, J = 4.7, 7.6 Hz), 7.79 (m, 1 H), 7.95 (dd, 1 H, J = 1.7, 7.3 Hz), 8.50 (dd, 1 H, J = 1.9, 4.5 Hz). Anal. ($C_{15}H_{17}N_5$) C, H, N.

6-(4-Methylpiperazin-1-yl)imidazo[1,2-*a*]pyrido[2,3-*e*]pyrazine (20m). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18m**. After recrystallization **20m** was obtained as colorless prisms: IR (Nujol) 1680, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.34 (s, 3 H), 2.57 (m, 4 H), 4.45 (m, 4 H), 7.34 (dd, 1 H, J = 4.2, 7.8 Hz), 7.58 (s, 1 H), 7.91 (dd, 1 H, J = 1.7, 7.5 Hz), 8.26 (dd, 1 H, J = 1.4, 4.6 Hz), 8.36 (s, 1 H). Anal. ($C_{14}H_{16}N_6$) C, H, N.

2,3-Dihydro-8-(4-methylpiperazin-1-yl)imidazo[1,2-*a*]pyrazine (20n). Similarly to the procedure described for **20a**, the title compound was prepared starting from **23**. After recrystallization **20n** was obtained as colorless prisms: IR (Nujol) 1630, 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.28 (s, 3 H), 2.46 (m, 4 H), 3.89 (m, 6 H), 6.45 (m, 2 H). Anal. ($C_{11}H_{17}N_5$) C, H, N.

7-Methyl-4-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20o). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18h** and *N*-benzylpiperazine. After recrystallization, **20o** was obtained as colorless prisms: IR ($CHCl_3$) 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3 H), 2.65 (m, 4 H), 3.60 (s, 2 H), 3.82 (m, 4 H), 6.73 (m, 2 H), 7.06 (m, 1 H), 7.27−7.37 (m, 5 H), 7.49 (m, 1 H), 7.59 (d, 1 H, J = 8.2 Hz), 7.74 (d, 1 H, J = 2.0 Hz). Anal. ($C_{23}H_{24}N_4$) C, H, N.

7,8-Dimethyl-4-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20p). Starting from **18a** and *N*-benzylpiperazine the title compound was obtained following the procedure described for **20a**. After recrystallization, **20p** was obtained as colorless prisms: IR ($CHCl_3$) 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.32 (s, 3 H), 2.37 (s, 3 H), 2.65 (m, 4 H), 3.59 (s, 2 H), 3.76 (m, 4 H), 6.70 (m, 2 H), 7.25−7.36 (m, 5 H), 7.45 (s, 1 H), 7.47 (s, 1 H), 7.73 (t, 1 H, J = 1.6 Hz). Anal. ($C_{24}H_{26}N_4$) C, H, N.

9-Methyl-4-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20q). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18b** and *N*-benzylpiperazine. After recrystallization **20q** was obtained as colorless prisms: IR ($CHCl_3$) 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.66 (m, 4 H), 2.85 (s, 3 H), 3.59 (s, 2 H), 3.77 (m, 4 H), 6.70 (t, 1 H, J = 3.4 Hz), 6.77 (d, 1 H, J = 3.7 Hz), 7.06 (d, 1 H, J = 7.4 Hz), 7.16−7.39 (m, 6 H), 7.55 (dd, 1 H, J = 1.1, 7.8 Hz), 8.11 (d, 1 H, J = 2.7). Anal. ($C_{23}H_{24}N_4$) C, H, N.

7,8-Dichloro-4-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20r). Starting from **18e** and *N*-benzylpiperazine the title compound was obtained following the procedure described for **20a**. After recrystallization, **20r** was obtained as colorless prisms: IR (Nujol) 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.61 (m, 4 H), 3.57 (s, 2 H), 3.84 (m, 4 H), 6.76 (m, 2 H), 7.22−7.37 (m, 5 H), 7.71 (m, 3 H). Anal. ($C_{22}H_{20}Cl_2N_4$) C, H, N.

7-Fluoro-4-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20s). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18n** and *N*-benzylpiperazine. After recrystallization, **20s** was obtained as colorless prisms: IR (Nujol) 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.59 (m, 4 H), 3.63 (s, 2 H), 3.85 (m, 4 H), 6.76 (m, 2 H), 6.98 (m, 1 H), 7.18−7.29 (m, 5 H), 7.32 (m, 1 H), 7.65 (m, 1 H), 7.78 (m, 1 H). Anal. ($C_{22}H_{21}FN_4$) C, H, N.

9-Fluoro-4-(4-benzylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20t). Starting from **18o** and *N*-benzylpiperazine the title compound was obtained following the procedure as for **20a**. After recrystallization, **20t** was obtained as colorlesss prisms: IR (Nujol) 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.63 (m, 4 H), 3.58 (s, 2 H), 3.82 (m, 4 H), 6.73 (t, 1 H, J = 3.4 Hz), 6.80 (d, 1 H, J = 3.8 Hz), 6.98 (m, 1 H), 7.13−7.44 (m, 7 H), 8.16 (d, 1 H, J = 2.6 Hz). Anal. ($C_{22}H_{21}FN_4$) C, H, N.

9-Fluoro-4-[4-(2-naphthylmethyl)piperazin-1-yl]pyrrolo-[1,2-*a*]quinoxaline (20u). Similarly to the procedure as described for **20a**, the title compound was prepared starting from **18o** and 2-(bromomethyl)naphthalene. After recrystallization, **20u** was obtained as colorless prisms: IR (Nujol) 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.70 (m, 4 H), 3.73 (s, 2 H), 3.88 (m, 4 H), 6.75 (m, 2 H), 6.98 (m, 1 H), 7.35 (m, 1 H), 7.43−7.68 (m, 4 H), 7.73−7.86 (m, 5 H). Anal. ($C_{26}H_{23}FN_4$) C, H, N.

6-(4-Benzylpiperazin-1-yl)pyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrazine (20v). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18f** and *N*-benzylpiperazine. After recrystallization **20v** was obtained as colorless prisms: IR ($CHCl_3$) 1670, 1600 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.62 (m, 4 H), 3.56 (s, 2 H), 4.01 (m, 4 H), 6.73 (m, 1 H), 6.81 (m, 1 H), 7.11 (dd, 1 H, J = 4.5, 7.6 Hz), 7.27−7.34 (m, 5 H), 7.77 (m, 1 H), 7.93 (dd, 1 H, J = 1.8, 7.4 Hz), 8.48 (dd, 1 H, J = 1.7, 4.7 Hz). Anal. ($C_{21}H_{21}N_5$) C, H, N.

6-(4-Phenylpiperazin-1-yl)pyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrazine (20w). Similarly to the procedure described for **20a**, the title compound was prepared starting from **18f** and *N*-phenylpiperazine. After recrystallization, **20w** was obtained as colorless prisms: IR (Nujol) 1599 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.58 (m, 4 H), 4.02 (m, 4 H), 6.76 (t, 1 H, J = 3.4 Hz), 6.83 (d, 1 H, J = 3.7 Hz); 7.12 (dd, 1 H, J = 4.5, 7.9 Hz), 7.32 (m, 5 H), 7.79 (m, 1 H), 7.95 (dd, 1 H, J = 1.6, 7.8 Hz), 8.50 (dd, 1 H, J = 1.7, 4.7 Hz). Anal. ($C_{20}H_{19}N_5$) C, H, N.

General Procedure for Preparation of Compounds 20x−dd. This procedure is illustrated for the preparation of 9-fluoro-4-[4-(2-thienylmethyl)piperazin-1-yl]pyrrolo[1,2-*a*]-quinoxaline (**20x**). A mixture of **19d** (0.2 g, 0.74 mmol), anhydrous potassium carbonate (96 mg, 0.74 mmol), and 2-(bromomethyl)thiophene (0.13 g, 0.74 mmol) in ethyl methyl ketone (33 mL) was heated at reflux for 1.5 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 flash-chromatographed (30% EtOAc in chloroform) to give 0.1 g of **20x** as colorless prisms: IR (Nujol) 1590 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.69 (m, 4 H), 3.85 (m, 6 H), 6.74 (t, 1 H, J = 3.4 Hz), 6.81 (d, 1 H, J = 3.8 Hz), 6.95−7.05 (m, 3 H), 7.14−7.27 (m, 2 H), 7.43 (d, 1 H, J = 8.1 Hz), 8.16 (d, 1 H, J = 2.4 Hz). Anal. ($C_{20}H_{19}FN_4S$) C, H, N.

4-[4-(2-Thienylmethyl)piperazin-1-yl]pyrrolo[1,2-*a*]quinoxaline (20y). Similarly to the procedure described for **20x**, the title compound was prepared starting from **19f**. After recrystallization, **20y** was obtained as colorless prisms: IR (Nujol) 1585 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.70 (m, 4 H), 3.84 (m, 6 H), 6.75 (m, 2 H), 6.96 (m, 2 H), 7.28 (m, 3 H), 7.64−7.80 (m, 3 H). Anal. ($C_{20}H_{20}N_4S$) C, H, N.

7-Fluoro-4-[4-(2-thienylmethyl)piperazin-1-yl]pyrrolo-[1,2-*a*]quinoxaline (20z). Starting from **19e** the title compound was obtained following a procedure as for **20x**. After recrystallization, **20z** was obtained as yellowish prisms: IR ($CHCl_3$) 1595 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.68 (m, 4 H), 3.81 (s, 2 H), 3.87 (m, 4 H), 6.76 (m, 2 H), 6.95 (m, 3 H), 7.27 (t, 1 H, J = 3.8 Hz), 7.34 (d, 1 H, J = 2.4 Hz), 7.63 (m, 1 H), 7.73 (m, 1 H). Anal. ($C_{20}H_{19}FN_4S$) C, H, N.

6-[4-(2-Thienylmethyl)piperazin-1-yl]pyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrazine (20aa). Similarly to the procedure as described for **20x**, the title compound was prepared starting from **19g**. After recrystallization, **20aa** was obtained as colorless prisms: IR (Nujol) 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.67 (m, 4 H), 3.80 (s, 2 H), 3.88 (m, 4 H), 6.73 (t, 1 H, J = 3.4 Hz), 6.80 (d, 1 H, J = 3.8 Hz), 6.94 (m, 2 H), 7.22−7.28 (m, 2 H), 7.86 (dd, 1 H, J = 1.7, 7.8 Hz), 8.24 (m, 2 H). Anal. ($C_{19}H_{19}N_5S$) C, H, N.

9-Methyl-4-[4-(2-bromo-5-thienylmethyl)piperazin-1-yl]pyrrolo[1,2-*a*]quinoxaline (20bb). Starting from **19b** and 2-bromo-5-(bromomethyl)thiophene the title compound was obtained following a procedure as described for **20x**. After recrystallization, **20bb** was obtained as brown prisms: IR (Nujol) 1615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (m, 4 H), 2.87 (s, 3 H), 3.60 (s, 2 H), 3.77 (m, 4 H), 6.69 (t, 1 H, J = 3.4 Hz), 6.74 (d, 1 H, J = 3.7 Hz), 6.93 (m, 2 H), 7.08 (d, 1 H, J = 7.4 Hz), 7.28 (m, 1 H), 7.55 (dd, 1 H, J = 1.1, 7.8 Hz), 8.14 (d, 1 H, J = 2.7). Anal. ($C_{21}H_{21}BrN_4S$) C, H, N.

7-Fluoro-4-[4-(2-furylmethyl)piperazin-1-yl]pyrrolo-[1,2-*a*]quinoxaline (20cc). Similarly to the procedure as described for **20x**, the title compound was prepared starting from **19e** and 2-(bromomethyl)furan. After recrystallization, **20cc** was obtained as colorless prisms: IR (Nujol) 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (m, 4 H), 3.61 (s, 2 H), 3.86 (m, 4 H), 6.23 (m, 1 H), 6.32 (m, 1 H), 6.69−6.76 (m, 2 H), 6.95 (m, 1 H), 7.29 (dd, 1 H, J = 2.8, 9.8 Hz), 7.38 (m, 1 H), 7.63 (m, 1 H), 7.72 (m, 1 H). Anal. ($C_{20}H_{19}FN_4O$) C, H, N.

7-Fluoro-4-[4-(2-bromo-5-furylmethyl)piperazin-1-yl]-pyrrolo[1,2-*a*]quinoxaline (20dd). Starting from **19e** and 2-bromo-5-(bromomethyl)furan the title compound was obtained following the procedure as for **20x**. After recrystallization, **20dd** was obtained as a white solid: IR ($CHCl_3$) 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.68 (m, 4 H), 3.63 (s, 2 H), 3.88 (m, 4 H), 6.23 (m, 2 H), 6.73 (m, 2 H), 6.98 (m, 1 H), 7.31 (dd, 1 H, J = 2.6, 9.9 Hz), 7.63 (dd, 1 H, J = 5.2, 8.9 Hz), 7.72 (d, 1 H, J = 2.5 Hz). Anal. ($C_{18}H_{18}FBrN_4O$) C, H, N.

General Procedure for Preparation of Compounds 20ee−gg. This procedure is illustrated for the preparation of 9-fluoro-4-[4-(2-hydroxyethyl)piperazin-1-yl]pyrrolo[1,2-*a*]-quinoxaline (**20ee**). To a solution of **18o** (0.15 g, 0.68 mmol) in ethyl methyl ketone (2 mL) was added 1-(2-hydroxyethyl)-piperazine (0.18 mL, 1.50 mmol). The reaction mixture was refluxed for 21 h under argon, cooled, and evaporated to afford a residue which was partitioned between water and ethyl acetate. The organic layers were washed with brine, dried, and concentrated. The residue was flash-chromatographed (10% methanol in dichloromethane) to give, after recrystallization, 0.18 g of **20ee** as colorless prisms: IR ($CHCl_3$) 3590, 1600 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (br s, 1 H), 2.62 (m, 2 H), 2.69 (m, 4 H), 3.56 (m, 2 H), 3.81 (m, 4 H), 6.73 (t, 1 H, J = 3.4 Hz), 6.80 (d, 1 H, J = 3.8 Hz), 7.03 (m, 1 H), 7.19 (m, 1 H), 7.43 (d, 1 H, J = 7.9 Hz), 8.16 (m, 1 H). Anal. ($C_{17}H_{19}FN_4O$) C, H, N.

7-Fluoro-4-[4-(2-hydroxyethyl)piperazin-1-yl]pyrrolo-[1,2-*a*]quinoxaline (20ff). Similarly to the procedure described for **20ee**, the title compound was prepared starting from **18n**. After recrystallization, **20ff** was obtained as colorless prisms: IR ($CHCl_3$) 3615 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.04 (br s, 1 H), 2.66 (m, 2 H), 2.76 (m, 4 H), 3.70 (m, 2 H), 3.88 (m, 4 H), 6.74 (m, 2 H), 6.95 (m, 1 H), 7.31 (dd, 1 H, J = 2.8, 9.8 Hz), 7.63 (m, 1 H), 7.75 (d, 1 H, J = 2.6 Hz). Anal. ($C_{17}H_{19}FN_4O$) C, H, N.

6-[4-(2-Hydroxyethyl)piperazin-1-yl]imidazo[1,2-*a*]-pyrido[2,3-*e*]pyrazine (20gg). Similarly to the procedure described for **20ee**, the title compound was prepared starting from **18m**. After recrystallization, **20gg** was obtained as colorless prisms: IR (Nujol) 3595, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.25 (br s, 1 H), 2.65 (m, 2 H), 2.72 (m, 4 H), 3.65 (m, 2 H), 4.47 (m, 4 H), 7.35 (dd, 1 H, J = 4.8, 7.9 Hz), 7.60 (s, 1 H), 7.93 (dd, 1 H, J = 1.8, 7.8 Hz), 8.29 (dd, 1 H, J = 1.8, 4.9 Hz), 8.38 (s, 1 H). Anal. ($C_{15}H_{18}N_6O$) C, H, N.

9-Fluoro-4-(4-cyanomethylpiperazin-1-yl)pyrrolo[1,2-*a*]quinoxaline (20hh). A mixture of **19e** (0,22 g, 0.83 mmol), anhydrous potassium carbonate (0.11 g, 0.83 mmol), and bromoacetonitrile (57.8 μL, 0.83 mmoL) in ethyl methyl ketone (35 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 flash-chromatographed (30% EtOAc in dichloromethane) to give 0.22 g of **20hh** as colorless prisms: IR (Nujol) 2245 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.80 (m, 4 H), 3.60 (s, 2 H), 3.84 (m, 4 H), 6.78 (m, 2 H), 7.04 (dd, 1 H, J = 8.1, 11.9 Hz), 7.21 (m, 1 H), 7.45 (d, 1 H, J = 7.9 Hz), 8.19 (m, 1 H). Anal. ($C_{17}H_{16}FN_5$) C, H, N.

α-[4-(9-Fluoropyrrolo[1,2-*a*]quinoxalin-4-yl)piperazin-1-yl]acetic Acid (20ii). A solution of **24** (0.23 g, 0.55 mmol) in methanol (5 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (56 mg) for 1 h. The catalyst was removed by filtration, the solution was evaporated, and the residue was recrystallized from ethyl acetate to provide 0.16 g of **20ii** as colorless prisms: IR (Nujol) 3360, 1710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.73 (m, 4 H), 3.13 (s, 2 H), 3.20 (m, 4 H), 6.81 (m, 1 H), 7.01 (m, 1 H), 7.27 (m, 3 H), 8.11 (m, 1 H). Anal. ($C_{17}H_{17}FN_4O_2$) C, H, N.

9-Fluoro-4-[4-(1*H*-tetrazol-5-yl)methylpiperazin-1-yl]-pyrrolo[1,2-*a*]quinoxaline (20jj). A mixture of **20hh** (0.1 g, 0.32 mmol), piperidine hydrochloride (97 mg, 0.8 mmol), and sodium azide (52 mg, 0.8 mmol) in anhydrous *N*,*N*-dimethylformamide (1 mL) was heated at 115 °C for 16 h, under argon. The reaction mixture was diluted with aqueous NH_4Cl and extracted with EtOAc. The organic layers were washed with brine, dried, and concentrated. The residue was flash-chromatographed (30% methanol in dicloromethane) to give 78 mg of **20jj** as pale yellow prisms: IR (Nujol) 3200−2400, 1625 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.72 (m, 4 H), 3.78 (m, 4 H), 4.02 (s, 2 H), 6.86 (t, 1 H, J = 3.3 Hz), 7.05 (d, 1 H, J = 4.0 Hz), 7.28 (m, 3 H), 8.17 (m, 1 H). Anal. ($C_{17}H_{17}FN_8$) C, H, N.

In Vitro Binding Assays. Binding assays have been performed as described in refs 6 and 23.

1. [^{3}H]Zacopride Binding to Rat Cortical Membranes. Male CRL:CD(SD)BR-COBS rats were killed by decapitation; their cortices were rapidly removed and stored at −80 °C until the day of assay. The frozen tissues were homogenized in about 50 vol of ice-cold Tris HCl, 25 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 refrigerated centrifuge). 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 Tris HCl, 25 mM, pH 7.4, containing 10 μM pargyline.

[^{3}H]Zacopride binding was done as described previously[7] in a final incubation volume of 0.5 mL consisting of 0.25 mL of membrane suspension (10 mg of tissue/sample), 0.25 mL of [^{3}H]zacopride (s.a. 85 Ci/mmol, final concentration 0.4 nM), and 10 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 pretreated with 0.5% poly-(ethylenimmine) 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[6,13] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding.

2. [^{3}H]Zacopride Binding to NG108-15 Cells.[14] Neuroblastoma-glioma cells (NG108-15) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% inactivated fetal calf serum and HAT (10 μM hypoxanthine, 5 μM aminopterine, and 16 μM thymine). Cells were cultured

at 37 °C under CO_2/air (10/90%) atmosphere and the medium was replaced every 2 days. The cells were harvested by shaking and pelleted by centrifugation at 900*g* for 10 min.

Binding assays with NG108-15 cell membranes were performed as described for cortical membranes with little modification: a final incubation volume of 260 μL consisting of 150 μL of cells suspension (about 420 000 cells/sample in Tris HCl, 50 mM, pH 7.5, containing 25 mM NaCl), 100 μL of [^{3}H]-zacopride (final concentration 0.4 nM), and 10 μL of displacing agents or solvent. In addition the cell membranes sample were incubated for 60 min at 25 °C.

3. Binding Assays for 5-HT Receptor Subtypes. Binding assays have been performed as described in ref 6. Briefly, male CRL:CD(SD)BR-COBS rats and male albino guinea pigs were killed by decapitation; their brains (rat hippocampus for 5-HT$_{1A}$, rat striatum for 5-HT$_{1B}$, rat cortex for for 5-HT$_{2A}$ and 5-HT$_{2C}$, guinea pig striatum for 5-HT$_4$) were rapidly dissected into the various areas and stored at −80 °C until the day of assay.

Tissues were homogenized in about 50 vol of ice-cold appropriate buffer using an Ultra Turrax TP-1810 homogenizer (2 × 20 s), and homogenates were centrifuged at 50000*g* for 10 min (Beckman Avanti J-25 centrifuge). Each 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 the appropriate incubation buffer just before the binding assay.

The following incubation conditions were used. 5-HT$_{1A}$: [^{3}H]8-OH-DPAT (s.a. 127 Ci/mmol; NEN), final concentration 1 nM, 30 min at 25 °C (nonspecific binding: 5-HT 10 μM). 5-HT$_{1B}$: [^{3}H]5-HT (s.a. 30.0 Ci/mmol; NEN), final concentration 2 nM, 30 min at 25 °C (nonspecific binding: 5-HT 10 μM). 5-HT$_{2A}$: [^{3}H]ketanserin (s.a. 80.9 Ci/mmol; NEN), final concentration 0.7 nM, 15 min at 37 °C (nonspecific binding: methysergide 1 μM). 5-HT$_{2C}$: [^{3}H]mesulergine (s.a. 82.0 Ci/mmol; Amersham), final concentration 1 nM, 30 min at 37 °C (nonspecific binding: meulergine 10 μM). 5-HT$_4$: [^{3}H]GR 113808 (s.a. 84.0 Ci/mmol; Amersham), final concentration 0.1 nM, 30 min at 37 °C (nonspecific binding: 5-HT 10 μM). Incubations were stopped by rapid filtration under vacuum through GF/B filters.

Measurement of [^{14}C]Guanidinium Uptake in NG108-15 Cells. This procedure has been described by Emerit et al.[7] 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 NG108-15 clone were grown in DMEM 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 was 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 SP, and/or 8 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 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 NG108-15 cells was 4−5 times higher in the presence of both 1 mM 5-HT and 10 mM SP than in their absence (basal uptake). 5-HT$_3$ receptor antagonists (zacopride, ondansetron, tropisetron, etc.) completely prevented the stimulatory effect of 5-HT (with SP) (see ref 7 for details).

In Vivo Studies: Potential Anxiolytic-like and Analgesic-like Properties and Brain-to-Plasma Distribution Studies. Procedure involving animals and their care was conducted in conformity with the institutional guidelines that are in compliance with national laws and policies (D.L. no. 116, G.U., Suppl. 40, Feb 18, 1992; Circolare No. 8, G.U., 1994) and international laws and policies (EEC Council Directive 86/609, OJL 358,1, Dec 12, 1987; *Guide for the Care and Use of Laboratory Animals*, U.S. National Research Council, 1996).

1. Light/Dark Exploratory Test in Mice. Male CD-1 mice (Charles River, Italy), weighing 20−25 g at the beginning of the experiments were used. They were housed five per cage at constant room temperature (21 ± 1 °C) and relative humidity (60%) under a regular, inverted, light/dark schedule (light: 10:00 p.m.−10:00 a.m.) with food and water available ad libitum. Animals were allowed to adapt to laboratory conditions for at least 3 weeks. Testing was done in the dark phase between 2:00 and 5:00 p.m. Ten animals per group were used in the various experiments.

The procedure described by Jones et al.[17] is based on the aversion of the animals to wide brightly lit areas. The apparatus consisted of a poly(vinyl chloride) open-topped box (42.5 × 25.5 × 30 cm) divided into a small (17.0 × 25.5 × 30 cm) and a large (25.5 × 25.5 × 30 cm) area by a partition which had a hole at floor level. The small area was painted black, while the large compartment was white. The floor of each compartment was marked in 9-cm squares. The white compartment was illuminated with one 60-W incandescent bulb and the black compartment with a red dark-room incandescent bulb. Compounds **3b** and **20b**, as hydrochloride salts, were dissolved in 0.9% NaCl and given orally 45 min before testing. Chlordiazepoxide hydrochloride was dissolved in 0.9% NaCl and administered subcutaneously 30 min before mice were gently put in the central square of the white compartment and their behavior videorecorded for 5 min. The time the animals spent in the white compartment and the number of transitions between the two compartments were later determinated by one observer unaware of animal treatments. For "transition", it was considered a mouse moving from one compartment to the other, with all four paws in the new compartment. The statistical significance of differences between control and treated groups was analyzed by a one-way ANOVA followed by a Dunnett's test.

2. Radiant Heat Tail Flick Test. Male Sprague−Dawley rats (CD-COBS, Charles River, Italy) weighing 175−200 g at the beginning of the study were used. They were housed two per cage at costant room temperature (21 ± 1 °C) and relative humidity (60%) under a regular light/dark schedule (light: 7:00 a.m.−7:00 p.m.) with food and water ad libitum. Animals were allowed to adapt to laboratory conditions for at least 1 week before the experiments and were handled daily during this period. Testing was done between 2:00 and 5:00 p.m. Eight rats per group were used.

Compounds **3b** and **20b**, as hydrochloride salts, were dissolved in 0.9% NaCl and administered i.p. Morphine hydrochloride, dissolved in 0.9% NaCl, was administered subcutaneously. Nociceptive threshold was determined using an automated tail flick unit (Basile, Comerio, Italy) with the radiant heat adjusted to attain a mean baseline between 2 and 3 s, and a 15-s cutoff was imposed to avoid tissue damage.

Thirty minutes before drug administration, rats were taken from their home cage and gently handled on the tail flick unit to measure the baseline latency. The antinociceptive activity of the compounds was measured 30, 60, 90, and 120 min after drug administration. The statistical significance of differences between control and treated groups was ascertained by a two-way ANOVA for repeated measures with treatment as between-

subjects factor and time as within-subjects factor. Post-hoc comparisons were made with Tukey's test.

3. Drug Administration and Plasma and Brain Sampling. Male CD-COBS rats weighing 175−200 g (Charles River, Italy) were used. Rats received the test compounds (dissolved in saline) intravenously at the dose of 5 mg/kg (compounds have been used as hydrochloride salts) and were killed by decapitation under deep anesthesia 60, 120, and 180 min after dosing. Blood samples were collected in heparinized tubes and centrifuged and the plasma was stored at −20 °C. Brains were rapidly removed, blotted with paper to remove excess surface blood, and stored at −20 °C until analysis.

4. Drug Analysis. Plasma and brain concentrations of the test compounds were determined by high-performance liquid chromatography (HPLC) after a liquid−liquid or solid−liquid extraction procedure. A chromatographically suitable analogue with similar extraction characteristics was used as an internal standard (I.S.) in each assay. The acidic derivative **20hh** was extracted from 1 mL of plasma (buffered to pH 3.5) or 1 mL of brain homogenate (0.1 M buffer phosphate, pH 3.5/CH_3CN, 80:30 v/v, 10 mL/g) with 4 mL of ethyl actetate. The very lipophilic compound **20u** was extracted from plasma (1 mL) and brain homogenate (0.1 M buffer phosphate/CH_3CN, 80: 20, v/v, 10 mL/g) with 4 mL of hexane/ethyl acetate (80:20, v/v), after adding 1 N NaOH to a final pH of about 10. After centrifugation the organic extracts were evaporated to dryness and the residues were dissolved in the mobile phase and injected in the HPLC column.

Supelchem LC-18 SPE cartridges (Supelco, Milano, Italy) were used to clean up plasma and brain samples in the case of compounds **20b**,**l**,**v**,**ee**. Briefly, the cartridges were prewetted with 2 mL of methanol, 2 mL of distilled water, and 2 mL of 0.1 N KH_2PO_4, pH 5. Then, after adding the I.S., 1−2 mL of plasma (diluted to 2 mL with 0.1 M KH_2PO_4) was added and the cartridges were washed with 3 mL of KH_2PO_4, 4 mL of distilled water, and 2 mL of CH_3CN−water (30:70 v/v). The compound was removed by eluting the cartridges with 3 mL of 1 M NH_4OH in methanol and evaporated to dryness in vacuo. Brain tissue was homogenized as described for compound **20u** and 1−3 mL were centrifuged at 15000*g* for 15 min. The supernatant was processed as described for plasma. The residue was dissolved in the mobile phase and analyzed by HPLC with UV detection (254 nm).

Separation was done on a Hypersil ODS column (25-cm × 4.6-mm i.d., 5-μm particle size) (C.P.S. Analitica, Milano, Italy) at 30 °C, protected by a MPLC New Guard precolumn (Applied Biosystem, Inc., CA). The mobile phase was 0.01 M KH_2PO_4: CH_3CN:CH_3OH in a ratio (v/v) of 57:40:3 for the analysis of compound **20u** and 70:27:3 for all other compounds. The retention times were 20 min for **20u** and 5.4, 9.8, 12, 13, and 14.5 for **20l**, **20v**, **20hh**, **20ee**, and **20b** respectively.

The methods had sensitivity limits of 0.03−0.1 and 0.1−0.3 nmol/mL, depending on the compound and volume of plasma and brain homogenized analyzed. At these concentrations the coefficients of variation (CV) were between 13% and 18%, and all higher concentrations gave CV below 15% in both tissues.

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