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Novel Potent and Selective Central 5-HT$_3$ Receptor Ligands Provided with Different Intrinsic Efficacy. 1. Mapping the Central 5-HT$_3$ Receptor Binding Site by Arylpiperazine Derivatives

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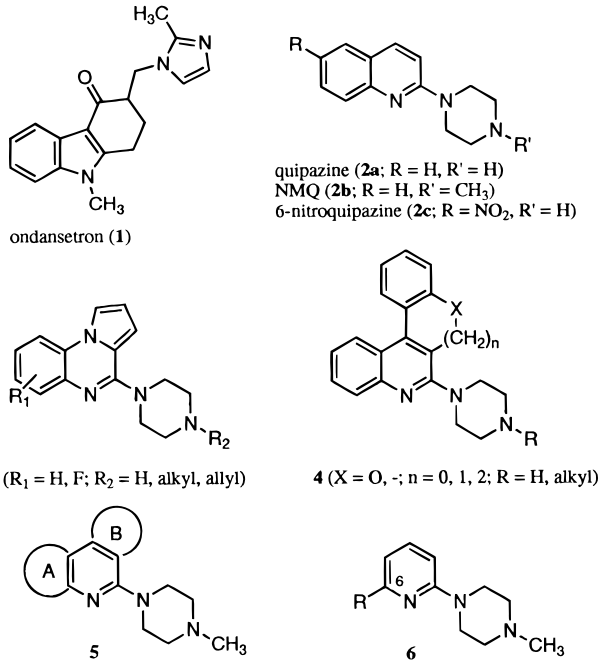
Synthesis and pharmacological evaluation of a series of condensed quinoline and pyridine derivatives bearing a *N*-methylpiperazine moiety attached to the 2-position of the quinoline or pyridine nucleus are described. 5-HT receptor binding studies revealed subnanomolar affinity for the 5-HT$_3$ receptor subtype in some of the compounds under study. The most active compound (**5b**) displayed a K_i value about 1 order of magnitude higher than that of quipazine along with a higher selectivity. The potential 5-HT$_3$ agonist/antagonist activity of four selected compounds was assessed in vitro on 5-HT$_3$ receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 cells. Compound **5j** acted as a 5-HT$_3$ agonist in this assay with an EC_{50} value close to that reported for quipazine, while **5b** was a partial agonist with an EC_{50} value of about 0.25 nM, and compound **5c** possessed antagonist properties with an IC_{50} value (≈8 nM) in the same range as those of previously characterized 5-HT$_3$ receptor antagonists. Qualitative and quantitative structure−affinity relationship studies carried out by making use of theoretical molecular descriptors allowed to elucidate the role of the main pharmacophoric components and to develop a model for the interaction of the 5-HT$_3$ ligands related to quipazine with their receptor.

Introduction

The wide variety of actions exerted by serotonin (5-hydroxytryptamine, 5-HT) has led to the demonstration of the existence of several 5-HT receptors, and at least 14 serotonin receptor subtypes have been so far identified.[1] Among them the 5-HT$_3$ receptor subtype has a special place because it belongs to the ligand-gated ion channel receptor family.[2] Recently, there has been increased interest in the search for new 5-HT$_3$ receptor antagonists: e.g., ondansetron (**1**) (Chart 1), the best known 5-HT$_3$ receptor antagonist, is on the market as an antiemetic agent to prevent the vomiting associated with anticancer chemotherapy. Furthermore, other possible therapeutic uses in anxiety, schizophrenia, drug abuse, and age-associated memory impairment are the subject of intense investigations.[3,4]

It has been suggested that the stimulation of the 5-HT$_3$ receptor in the central nervous system (CNS) enhances the release of dopamine from rat striatal slices[5] and that of cholecystokinin from the cerebral cortex and nucleus accumbens,[6] and it also inhibits the release of acetylcholine from the entorhinal cortex.[7]

Chart 1



However, the potential therapeutic interest of the 5-HT$_3$ agonists still remains to be evaluated because of the lack of potent and selective compounds.

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Quipazine (**2a**) was earlier reported to behave as a 5-HT$_3$ antagonist in peripheral models,[8] but subsequent studies revealed its 5-HT$_3$ agonist action in the [^{14}C]-guanidinium accumulation test applied to NG 108-15 cells.[9] It is noteworthy that the 5-HT$_3$ receptor expressed by NG 108-15 cells is very similar to that present in rat cortex.[10] Thus, quipazine is very probably a 5-HT$_3$ receptor agonist in the CNS.

Very recently, we reported the synthesis and the pharmacological characterization of a new class of pyrroloquinoxaline derivatives (**3**) related to quipazine which behaved as 5-HT$_3$ receptor agonists in both [^{14}C]guanidinium accumulation in NG 108-15 cells and cortical acetylcholine release in freely moving rats, while they showed partial agonist or antagonist properties in the Bezold−Jarish reflex test.[11] Furthermore, in a previous paper we described the synthesis and the 5-HT$_3$ receptor antagonist properties of a new class of quipazine derivatives (**4**).[12] In the context of such work we developed a comparative molecular field analysis (CoMFA) model able to predict nanomolar 5-HT$_3$ receptor affinity for compounds **5a**,[13] **5b**,[14] and **5c** (predicted affinities: **5a**, $K_i = 10$ nM; **5b**, $K_i = 11$ nM; **5c**, $K_i = 9.9$ nM),[15] and we proposed a tentative interaction model which implies different but overlapping binding domains for both antagonists and agonists at the 5-HT$_3$ receptor.[16]

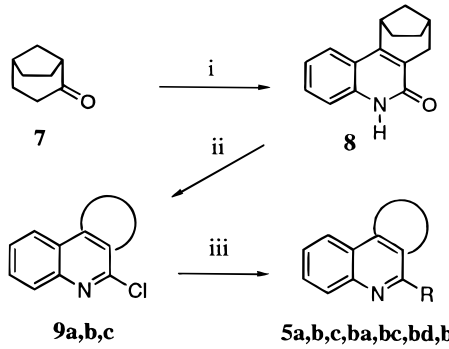
The aim of the present work was two-fold: (a) to obtain new 5-HT$_3$ receptor ligands with improved selectivity versus the main 5-HT receptor subtypes which are known to interact with quipazine derivatives and (b) to map the arylpiperazine interactions at the central 5-HT$_3$ receptor.

Thus, we report in this paper the synthesis and 5-HT$_3$ receptor affinities of several quipazine derivatives in series **5** and **6**. The effects of different heteroaromatic portions on the binding to the 5-HT$_3$ receptor sites are discussed along with the effect obtained by replacing the *N*-methylpiperazine moiety with different N-substituted piperazines or different heterocycles. The 5-HT$_3$ receptor intrinsic efficacy of the selected compounds was assessed in vitro on 5-HT$_3$ receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 hybrid cells.[9] Theoretical descriptors derived by means of the program CODESSA[17] and ad hoc-defined size and shape descriptors[18] have been employed for deciphering, on a quantitative ground, the molecular features responsible for the recognition of the 5-HT$_3$ receptors. Finally, a comprehensive model for the interaction of the 5-HT$_3$ receptor ligands related to quipazine with their receptor is proposed.

Chemistry

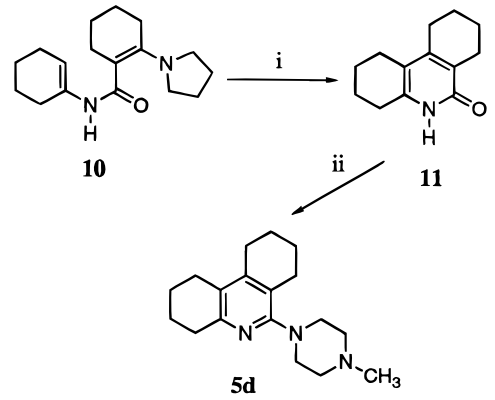
Compounds **5a**−**c**, **5ba,bc,bd,be,bf,** and **6a**[19] were prepared starting from the corresponding imidoyl chlorides (**9a**,[20] **9b**,[21] **9c**, or 2-chloropyridine) by reaction with the appropriate amine. Imidoyl chloride **9c** was prepared following the procedure summarized in Scheme 1. Bicyclo[3.2.1]octan-2-one (**7**) was converted, following the procedure described by White et al.,[22] into the corresponding pyrrolidine enamine which was reacted with phenyl isocyanate and then cyclized into quinolinone **8**. The latter compound was readily converted into imidoyl chloride **9c** by refluxing in phosphorus oxychloride. The quaternary compound **5bb** was obtained by methylation of the terminal piperazine nitrogen of **5b** with iodomethane.

Scheme 1[a]



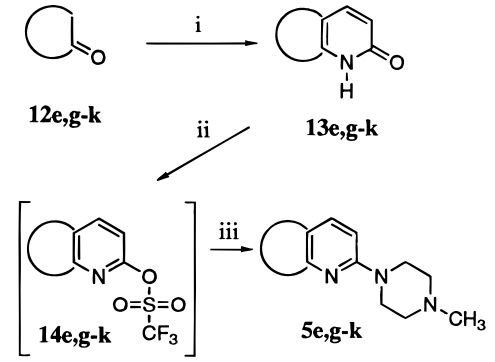
[a] Reagents: (i) (a) pyrrolidine, $C_2H_5OC_2H_5$, $TiCl_4$, C_6H_6, (b) C_6H_5NCO, $CHCl_3$, (c) PPA; (ii) $POCl_3$; (iii) amine (*N*-methylpiperazine, morpholine, etc.).

Scheme 2[a]



[a] Reagents: (i) PPA; (ii) (a) $(CF_3SO_2)_2O$, Na_2CO_3, CH_2Cl_2, (b) *N*-methylpiperazine.

Scheme 3[a]

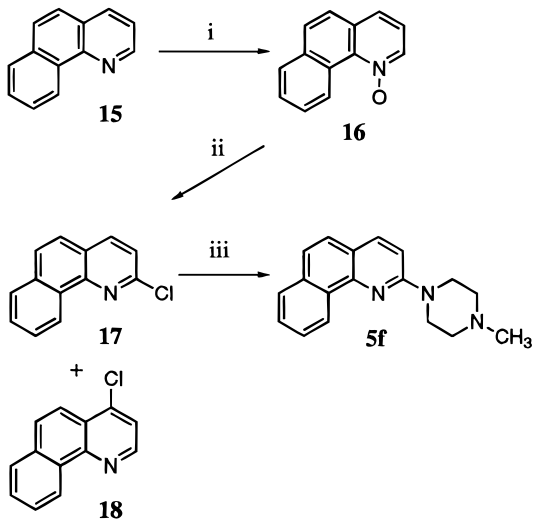


[a] Reagents: (i) HC≡C−$COOCH_3$, NH_3, CH_3OH; (ii) $(CF_3SO_2)_2O$, Na_2CO_3, CH_2Cl_2; (iii) *N*-methylpiperazine.

Compound **5d** was synthesized following the procedure described in Scheme 2. Cyclization of amide **10** under the conditions described by Rigby et al.[23] appeared to be slow even in refluxing toluene, while it proceeded rapidly when it was run in polyphosphoric acid (PPA) at 140 °C, and 2(1*H*)-pyridone **11** was obtained in high yield. The latter was converted in two steps into the corresponding piperazinyl derivative **5d**.

Compounds **5e,g**−**k** were prepared according to the procedure described in Scheme 3. The procedure for 2(1*H*)-pyridone synthesis originally developed by

Scheme 4[a]



[a] Reagents: (i) MCPBA, $CHCl_3$; (ii) $POCl_3$; (iii) *N*-methylpiperazine.

Kozikowski et al.[24] was slightly modified[25] and applied to the required cyclic ketones **12e,g**−**k** (see Experimental Section) to obtain pyridones **13e,g**−**k**. These compounds were transformed into the piperazinyl derivatives **5e,g**−**k** in two steps involving the reaction of the pyridones **13e,g**−**k** with trifluoromethanesulfonic anhydride to give the corresponding imidoyl triflates **14e,g**−**k** which, without further purification,[26] reacted with *N*-methylpiperazine to give **5e,g**−**k**. Compound **6b** was prepared following the same reaction sequence as that described above for compounds **5e,g**−**k** starting from 1-adamantyl methyl ketone.

The procedure for the synthesis of compound **5f** is shown in Scheme 4. Commercial benzo[*h*]quinoline (**15**) was transformed into the corresponding *N*-oxide derivative **16**[27] which reacted with phosphorus oxychloride to give the 2-chloro derivative **17**[27] along with a minor amount of its 4-chloro isomer **18** which was separated by flash chromatography. Reaction of **17** with *N*-methylpiperazine gave the corresponding piperazinyl derivative **5f**.

Computational Methods

Geometry Optimization. Conformational analysis of the N4-protonated form of some simple arylpiperazine derivatives was recently performed,[28] and the resulting absolute minimum structure of quipazine was considered in this study as the starting geometry. The substituents were assembled within the Editor module of the QUANTA 4.1 program.[29] Then, the protonated structures were fully optimized by means of molecular orbital calculations (AM1),[30] using the MOPAC 6.0 (QCPE 455) program.

Molecular Superimposition. The most structurally different ligands which show the highest affinity for the 5-HT_3 receptor (compounds **5b**,**j**) were chosen for the construction of the reference supermolecule and were superimposed by a rigid fit procedure over the piperazine moiety. The QUANTA 4.1 molecular modeling software was used for molecular comparisons, matching, and computation of van der Waals volumes.

Molecular Descriptors and Statistical Treatment of Data. A large number of global and fragment descriptors (constitutional, topological, electrostatic, geometrical, and quantum-chemical) were generated for each compound within the framework of the CODESSA[17] program. The ad hoc-defined molecular size and shape parameters[18a] described above were added as external descriptors.[18b] The program was used in this work to accomplish a preselection of descriptors on the basis of their statistical significance and, owing to the limited number of compounds considered, to obtain simple regression models.

The search for the best correlation equation was achieved by means of a heuristic method, which accomplished a preselection of descriptors on the basis of their statistical significance.[17,31] Default values for control parameters and criteria were used: minimum squared correlation coefficient for the one-parameter correlation to be considered significant, $R^2_{min} = 0.1$; *t*-test value for the descriptor to be considered significant in the one-parameter correlation, $t_1 = 1.5$; significant intercorrelation level, $r_{sig} = 0.8$. As a final result, the program lists the 10 correlations found with the highest *F*-test values. The quantitative structure−affinity relationship (QSAR) models reported in this paper were selected on the basis of the best statistical parameters and the largest diversity of the descriptors involved.

Definitions of the Descriptors Used.[32] (1) $E_L - E_H$ is the energy difference between the lowest unoccupied (LUMO) and the highest occupied (HOMO) molecular orbitals. A decrease in the energy gap usually leads to easier polarizability of the molecule. (2) $S^L(N)$ is the nucleophilic superdelocalizability on the protonated terminal piperazine nitrogen atom. It characterizes the propensity to behave as a hydrogen bond donor. (3) $S^H(N_h)$ is the electrophilic superdelocalizability on the nitrogen atom of the heteroaryl moiety. It characterizes the propensity to behave as a hydrogen bond acceptor. For this purpose, the relevant orbital for **5a** is the HOMO-1, whereas the HOMO molecular orbital is localized on the carbon atoms of the conjugate system. The energy gap between the two orbitals is only 0.2 eV. (4) $\sum f_a - S^H$ is the sum of the electrophilic superdelocalizability on the atoms of the pyridine ring of the heteroaryl moiety. (5) $\sum f_{a'} - S^H$ is the sum of the electrophilic superdelocalizability on the atoms of the benzene ring of either the quinoline nucleus or the saturated ring in an analogous position, or even of the substituent of ligands **6b**,**c**. (6) $\sum f_c - S^H$ is the sum of the electrophilic superdelocalizability on the atoms of the additional ring fused to the bicyclic system. (7) $\sum f - S^H$ is the sum of the electrophilic superdelocalizability on the atoms of the entire heteroaryl moiety. These indices are used to parametrize $\pi-\pi$ drug−receptor interactions at specific atoms in unsaturated molecules.

(8) *HASA* is the area-weighted surface charge of the hydrogen-bonding acceptor atoms in the molecule.[31] (9) $f_{aa'} - WNSA\text{-}3$ is the surface-weighted charged partial surface area of the heteroaryl moiety.[33] *WNSA-3* is calculated according to the following formula: $WNSA\text{-}3 = [\sum(-SA_i)(Q^-_i)]TMSA/1000$, where $-SA_i$ is the surface area contribution of the *i*th negative atom in the molecule, Q^-_i is the partial atomic charge for the *i*th negative atom, and *TMSA* is the total molecular surface

area. (10) V_{in} and V_{out} are ad hoc-defined size and shape descriptors[18] and represent, respectively, the intersection and the outer van der Waals volume of the ligands considered with respect to a supermolecule constituted by the most structurally different ligands showing the highest binding affinity for the 5-HT$_3$ receptor (compounds **5b**,**j**). (11) V_{dif} is computed according to the following formula: $V_{dif} = V_{in} - V_{out}/V^{sup}$, where V^{sup} is the resulting van der Waals volume of the reference supermolecule. According to the ligand−receptor complementarity paradigm, this approach assumes that the volume obtained by superimposing the most structurally different ligands which show the highest affinities for the same receptor might reflect the overall shape and the conformational flexibility of the high-affinity receptor binding site.

Results and Discussion

Qualitative Structure−Affinity Relationships. The arylpiperazine structure can be formally divided into two substructures: (a) the piperazine moiety which incorporates a nitrogen atom protonated at physiological pH and (b) the aryl moiety which, in the case of most of the 5-HT$_3$ ligands, is a nitrogen heterocycle linked to the piperazine moiety by a "pseudoamidinic" bond.

As the first step of this work we selected the optimized *N*-methylpiperazine substructure,[11,12] and the heteroaryl moiety was modified with the aim of exploring the role of this substructure in the interaction with 5-HT$_3$ receptor. Subsequently, the optimized heteroaryl moiety was retained, and the effects of replacement of the *N*-methylpiperazine moiety with either different N-substituted piperazines or related heterocycles were assessed.

The newly synthesized compounds were tested for their potential ability to displace [^{3}H]granisetron specifically bound to 5-HT$_3$ receptors in rat cortical membranes (in comparison with the reference compounds quipazine (**2a**) and unlabeled granisetron) following well-established protocols.[34] The results of the binding studies along with the K_i values reported in the literature[35] for *N*-methylquipazine (NMQ, **2b**) and pyridine derivatives **6c,d** (which are included for comparison) are summarized in Tables 1 and 2. A detailed picture of the reactivity determinants of some of these ligands is reported in Table 3. The descriptors have been computed, within the framework of the AM1 Hamiltonian,[30] on the protonated ligands, and their definition is given in the Computational Methods section.

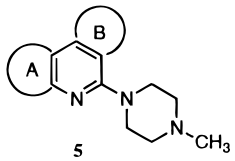
Effects of the Modification of the Heteroaryl Substructure. (a) Introduction of Additional Rings at the *c*-Edge of the Quinoline Nucleus of NMQ. The most intriguing result emerging from these studies is that the fusion of a benzo ring, a cyclohexane ring, or a saturated bicyclic system on the *c*-edge of the quinoline nucleus of NMQ (leading to compounds **5a**−**c**, respectively) enhances the affinity for 5-HT$_3$ receptors to a variable extent. In fact, benzo derivative **5a** shows a 5-HT$_3$ receptor affinity slightly higher than that reported for NMQ, while **5c** is found to be slightly more potent than **5a,** and compound **5b** shows an affinity about 1 order of magnitude higher than NMQ. Therefore, the incorporation of a cyclohexane ring into the structure of NMQ (as in compound **5b**) seems to confer the best stereoelectronic features for the interaction of these quipazine derivatives with the 5-HT$_3$ receptor. A comparative inspection of the electronic indices listed in Table 3 shows an average reduction of 5.6 kcal/mol in the energy gap ($E_L - E_H$) of these ligands with respect to NMQ. This effect can be interpreted as an increase in their polarizability and, hence, in their potentiality of establishing attractive short-range interactions with the receptor area approaching the *c*-edge of the quinoline nucleus of NMQ. Moreover, a slightly higher propensity to behave as a hydrogen-bonding acceptor ($S^H(N_h)$) is also observed for the quinoline nitrogen atom of compounds **5a**−**c** with respect to NMQ through a perturbation of the electronic cloud localized on the pyridine ring of the heteroaryl moiety ($\sum f_a - S^H$).

(b) Modification of the Benzene Ring of the Quinoline Nucleus of NMQ. It is interesting to observe that both the deletion of the fused benzene ring of NMQ (compare NMQ with **6a**) and its saturation (compare NMQ with **5e** and **5b** with **5d**) lead to a significant decrease of the 5-HT$_3$ affinity suggesting that this ring plays a key role in the interaction of this class of compounds with the receptor. In fact, the trend of the dynamic reactivity indices seems to suggest a dual role of the fused benzene ring for (1) a specific interaction ($\pi-\pi$ or π-charge) with suitable amino acid residues of the receptor (compare $\sum f_{a'} - S^H$ of NMQ and **5b** with that of **5e** and **5d**) and (2) an indirect but substantial effect on the capability of establishing hydrogen-bonding interactions of the nitrogen atom of the quinoline moiety (compare $S^H(N_h)$ of NMQ and **5b** with that of **5e** and **5d**).

It is noteworthy that the halogen atom of compounds **6c,d** is able to mimic the condensed benzo ring of NMQ only to a limited extent. Although an underestimation of the halogen effects might be expected since d-orbitals on the halogen atoms are neglected in the AM1 calculation, the trend in the binding affinity of these ligands is well accounted for by $\sum f - S^H$ and $S^H(N_h)$. These indices show that the introduction of the halogen atoms on the pyridine ring increases both the ability to donate electron density through orbitals and the propensity of the pyridine nitrogen atom to behave as a hydrogen-bonding acceptor.

(c) Introduction of Additional Rings at *h*- and *f*-Edges of the Quinoline Nucleus. The benzofusion on the *h*-edge of the quinoline nucleus of NMQ and **5e** (leading to compounds **5f**,**g**, respectively) significantly decreases the affinity for the 5-HT$_3$ receptor suggesting a limited tolerance to large substituents in the area of the receptor protein surrounding the *h*-edge of the quinoline nucleus of NMQ. This consideration appears to be also supported by the low affinity shown by compounds **5h**,**i** and **6b**. However, the comparison between compounds **5g** and **5h** suggests that the phenyl ring in the 6-position of the pyridine ring plays a role in the interaction with the receptor similar to that of the fused benzo ring of NMQ. A comparison of the $\sum f_c - S^H$ index of **5g** with $\sum f_{a'} - S^H$ of NMQ reveals similarity in the reactivity of their π-electron. Moreover, the unsaturation of the ring makes the basicity of the quinoline nucleus comparable with that of NMQ (compare $S^H(N_h)$ for compounds **5h**,**i** and NMQ).

Table 1. Preparative, Analytical, and 5-HT_3 Receptor Binding Affinity Data of Compounds **5a**−**k** and **6a,b**: Effects of Modification of the Heteroaryl Substructure



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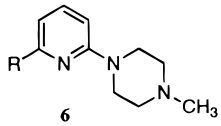


6

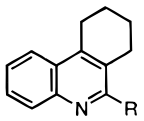
compd	A	B	R	yield, %[a]	mp, °C (recr. solv.)[b]	formula	anal.[c]	K_i (nM) ±SEM[d]
5a				86	64-65	$C_{18}H_{19}N_3 \cdot 0.25H_2O$	CHN	1.9±0.4
5b				89	94-95	$C_{18}H_{23}N_3$	CHN	0.23±0.002
5c				98	93-94	$C_{20}H_{25}N_3$	CHN	0.83±0.03
5d				85	63-64	$C_{18}H_{27}N_3$	CHN	110±11
5e				79	oil	$C_{14}H_{21}N_3 \cdot 0.25H_2O$	CHN	59±5.5
5f				94 (81)	177-178 (E-M)	$C_{18}H_{19}N_3 \cdot C_4H_4O_4$	CHN	200±44
5g				68 (53)	144-145 (E-D)	$C_{18}H_{21}N_3 \cdot C_4H_4O_4$	CHN	200±24
5h				61	oil	$C_{18}H_{27}N_3 \cdot 0.33H_2O$	CHN	660±66
5i				58	73-75	$C_{19}H_{23}N_3$	CHN	> 550
5j				63	83-85	$C_{17}H_{19}N_3 \cdot 0.125H_2O$	CHN	14±1.1
5k				65	93-94	$C_{18}H_{21}N_3$	CHN	830
6a			H	95 (66)	115-116 (E-M)	$C_{10}H_{15}N_3 \cdot 2C_4H_4O_4$	CHN	81±21
6b			1-adamantyl	33	62-63	$C_{20}H_{29}N_3$	CHN	> 550
6c			Cl					38[e]
6d			I					48[e]
2a (quipazine)								1.8±0.3
2b (NMQ)								3.0[e]
granisetron								0.35±0.06
5-HT								120±34
2c (6-nitroquipazine)								58±3.2

[a] Yields are not optimized. Values in parentheses are the yields as salt. [b] Recrystallization solvents: E, ethyl acetate; M, methanol; D, diethyl ether. [c] Analyses for the elements indicated were within ±0.4% of theoretical values. [d] Each value is the mean ± SEM of three determinations. [e] See ref 35.

In this respect, the high affinity shown by compound **5j** appears to be particularly interesting. This ligand is nearly equipotent to NMQ, is about 1 order of magnitude more potent than its higher homologue **5g**, and seems to combine the best electronic features for the interaction together with a geometry which forces the phenyl ring into a position in space favorable for interaction with the 5-HT_3 receptor.

On the contrary, the low affinity shown by compound **5k** (compare **5k** with **5e**) suggests that in the area of the receptor protein interacting with the *f*-edge of the quinoline nucleus of NMQ there is a limited tolerance

Table 2. 5-HT$_3$ Receptor Binding Affinities and Nucleophilic Superdelocalizabilities on the Terminal Piperazine Nitrogen Atom of Compounds **5b**,**ba**−**bf**: Effects of Modification of the Piperazinyl Substructure



5b,ba-bf

compd	R	K_i (nM) ± SEM[a]	S^L(N) (eV^{-1})
5b	4-methyl-1-piperazinyl	0.23 ± 0.002	0.0417
5ba	morpholino	>17000	
5bb	4,4-dimethylpiperazinium-1-yl	13 ± 1.1	0.0401
5bc	1-piperazinyl	1.7 ± 0.6	0.0433
5bd	4-ethyl-1-piperazinyl	1.9 ± 0.2	0.0404
5be	4-benzyl-1-piperazinyl	11 ± 2.1	0.0297
5bf	4-methyl-1-homopiperazinyl	4.8 ± 0.7	0.0405
2a		1.8 ± 0.3	0.0436
granisetron		0.35 ± 0.06	

[a] Each value is the mean ± SEM of three determinations.

Table 3. 5-HT$_3$ Binding Affinities and Theoretical Descriptors of the Protonated Form of the Ligands Reported in Table 1

compd	pK_i	$E_L - E_H$ (eV^{-1})	S^L(N) (eV^{-1})	S^H(N$_h$) (eV^{-1})	$\Sigma f_a - S^H$ (eV^{-1})	$\Sigma f_{a'} - S^H$ (eV^{-1})	$\Sigma f_c - S^H$ (eV^{-1})	$\Sigma f - S^H$ (eV^{-1})	*HASA* (Å^2)	$f_{aa'} - WNSA\text{-}3$ (Å^2)	V_{in} (Å^3)	V_{out} (Å^3)	V_{dif}
2a	8.74	7.6071	0.0436	0.00639	0.0220	0.0560		0.0780	41.6916	−5.722	193.13	2.25	0.5689
2b	8.52	7.6302	0.0419	0.00648	0.0240	0.0550		0.0790	22.4493	−5.941	208.63	2.87	0.6133
2c	7.24	8.1125	0.0435	0.00718	0.0520	0.0240		0.0760	23.2511	−4.8064	213.63	16.5	0.5876
5a	8.72	7.3386	0.0419	0.00670	0.0240	0.0280	0.0290	0.0810	24.8546	−4.5851	247.75	4.13	0.7261
5b	9.64	7.4383	0.0417	0.00762	0.0190	0.0470	0.0120	0.0780	26.4581	−4.8512	264.5	0	0.7884
5c	9.08	7.4074	0.0417	0.00770	0.0195	0.0470	0.0135	0.0800	23.2511	−4.2586	287.5	0	0.8569
5d	6.96	7.8286	0.0414	0.000305	0.0410	0.0210	0.00800	0.0690	16.0352	−1.1223	264.75	15.13	0.744
5e	7.23	8.039	0.0417	0.00000695	0.0460	0.0210		0.0670	17.6387	−2.5778	209.75	12.63	0.5875
5f	6.7	7.061	0.0418	0.000437	0.0145	0.0310	0.0365	0.0820	19.8511	−4.1577	231.13	20	0.6293
5g	6.7	7.2663	0.0417	0.00186	0.0205	0.0225	0.0370	0.0800	18.4405	−2.5246	230.25	28.5	0.6013
5h	6.18	8.0012	0.0416	0.0000239	0.0460	0.0215	0.00250	0.0700	9.4282	−2.0343	232	44.88	0.5577
5i	<6.26	7.2498	0.0416	0.00155	0.0140	0.0200	0.0460	0.0800	11.8335	−2.3992	212.25	62.13	0.4475
5j	7.85	7.4124	0.0416	0.00283	0.0195	0.0225	0.0400	0.0820	21.6475	−2.6689	241.75	0	0.7206
5k	6.08	7.1557	0.0416	0.0000723	0.0195	0.0230	0.0385	0.0810	20.8458	−2.4163	213.88	44.12	0.506
6a	7.09	8.5464	0.0422	0.0000251	0.0670			0.0670	26.4581	−3.5771	168.63	1.37	0.4985
6b	<6.26	8.122	0.0418	0.000470	0.0560	0.0140		0.0710	10.4229	−3.6804	222.63	76.37	0.4359
6c	7.42	8.3273	0.0426	0.000681	0.0620	0.0130		0.0750	17.6387	−3.1531	182.5	4.63	0.5302
6d	7.32	8.3327	0.0425	0.000493	0.0490	0.0240		0.0730	22.4493	−3.432	183.75	4	0.5358

to large substituents. The superimposition among the active ligands NMQ and **5j**, the less active **5g**,**k**, and the inactive **5i** accounts for these observations (Figure 1).

In conclusion, the strict geometric prerequisites for the interaction of the phenyl ring with the suitable amino acid residue(s) in the receptor strongly support the assumption that an aromatic specific interaction is involved in this area of the receptor binding site. Moreover, the differences between the structure−affinity relationships (SAFIR) concerning the phenyl ring in the 4-position of the quinoline nucleus of 5-HT$_3$ receptor antagonists **4** (where compounds with the phenyl group restrained with different geometries showed very similar 5-HT$_3$ receptor affinities)[12] and those concerning the phenyl ring in the 6-position of the pyridine nucleus of compounds **5f**−**j** suggest that the phenyl group in these two series of compounds binds different receptor areas, thereby reflecting their differences in functional properties (compounds **4** are antagonists[12] and **5j** is an agonist at the central 5-HT$_3$ receptor).

Effects of Modifications of the Piperazinyl Substructure. To establish whether the nitrogen atom of **5b** binds the receptor in its protonated form, the terminal piperazine tertiary nitrogen of **5b** was replaced by an isosteric oxygen atom as in compound **5ba** (oxygen retains the hydrogen-bonding acceptor properties of nitrogen, but it is not protonated at physiological pH). Since **5ba** was found to be at least 5 orders of magnitude less active than **5b** (see Table 2), the crucial role of the protonated terminal piperazine nitrogen in the interaction with the receptor became rapidly apparent. Similar results have been reported in the literature about the replacement of the terminal piperazine NH of quipazine[35] and related arylpiperazines[36] either by an isosteric methylene group or by an oxygen atom. It is noteworthy that the estimated value of the free energy contribution (ΔG_{bind})[37] of the protonated nitrogen atom of **5b** to the binding of 5-HT$_3$ receptors (>6.6 kcal/mol, as an absolute value) is in the range of the assumed charge-assisted hydrogen bond interactions.[38]

Quaternization (i.e., N-methylation) of the terminal piperazine nitrogen of compound **5b** leads to a 56-fold decrease of 5-HT$_3$ receptor affinity, in agreement with previous observations on other arylpiperazine derivatives.[35,36,39] This compound is likely to retain the electrostatic component in its binding to the receptor, but it loses the hydrogen-bonding component (ΔG_{bind} = 2.4 kcal/mol, as an absolute value) because its terminal piperazine nitrogen cannot be protonated.

The replacement of the methyl group on the terminal piperazine nitrogen of **5b** by a hydrogen atom (as in **5bc**), by an ethyl group (as in **5bd**), or by a bulkier benzyl group (as in **5be**) invariably leads to a drop in

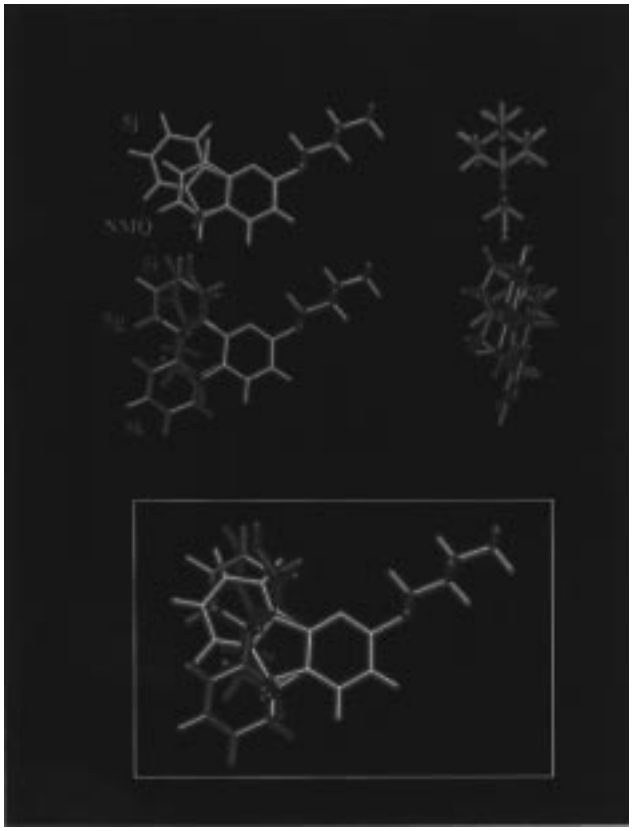


Figure 1. Mapping of the aromatic interactions within the 5-HT_3 receptor binding site by superimposition of active ligands NMQ and **5j** (red) to the less active **5g**,**k** and inactive **5i** (blue). At the top of the figure the ligands are split according to their affinity, and front and side views are reported.

the 5-HT_3 receptor affinity. Thus, the methyl group appears to be optimal (in the limited series of substituents considered) for the interaction of these central 5-HT_3 partial agonists (see Biological Activity section) with their receptor. This SAFIR trend appears to be in good agreement with that observed for analogous 5-HT_3 agonists[11] and antagonists;[12,39] thus, though a common mode of binding for arylpiperazine derivatives showing different intrinsic efficacy at the central 5-HT_3 receptor may be assumed, ad hoc molecules need to be designed to test this hypothesis. On the contrary, the discrepancy between the above-mentioned SAFIR trend and that reported for a series of piperazinylpyrrolothienopyrazine derivatives[36] may be related to the different receptor sources used in the respective studies.

The reactivity determinants reported in Table 2 only roughly account for the variation in the binding affinity of these ligands. According to these indices, substitution of the protonated nitrogen of quipazine results in a decrease of the N-H^+ acidity, the most significant reduction being registered for **5be**. Therefore, to explain the positive contribution of the methyl group linked to the terminal piperazine nitrogen on the binding affinity, other effects have to be invoked, such as lipophilic interactions with minimal steric demand.

Finally, the replacement of the *N*-methylpiperazine moiety of **5b** by the higher homologue *N*-methylhomopiperazine leads to a 21-fold decrease in 5-HT_3 receptor affinity (compare **5b** with **5bf**). The ring distortion seems to prevent the protonated nitrogen from reaching the optimal distance and/or directionality for the interaction with the suitable amino acid residue within the receptor binding site.

Table 4. Regression Models for the Correlation between Theoretical Molecular Structure Descriptors and 5-HT_3 Receptor Binding Affinity

descriptor	regression coeff	SD of regression coeff
(1) $R = 0.839$, $n = 16$, $F = 33.2$, $s = 0.596$		
intercept	6.763	0.207
$S^H(N_h)$	271.648	47.128
$R = 0.942$, $n = 13$, $F = 86.5$, $s = 0.411$; Omitted **6c**,**d**, 6-Nitroquipazine		
intercept	6.612	0.159
$S^H(N_h)$	334.620	35.974
(2) $R = 0.824$, $n = 11$, $F = 19.1$, $s = 0.644$; Omitted **5k**, **6a**,**c**,**d**, Quipazine		
intercept	3.776	0.923
HASA	0.194	0.044
(3) $R = 0.823$, $n = 13$, $F = 22.9$, $s = 0.646$; Omitted **5d**,**f**, 6-Nitroquipazine		
intercept	5.105	0.580
$f_{aa'} - WNSA\text{-}3$	−0.716	0.149
(4) $R = 0.795$, $n = 11$, $F = 15.5$, $s = 0.761$; Omitted **6c**,**d**, NMQ, Quipazine		
intercept	4.742	0.735
$\sum f_{a'} - S^H$	98.884	25.144
(5) $R = 0.841$, $n = 13$, $F = 26.61$, $s = 0.611$; Omitted **5d**, NMQ, Quipazine		
intercept	2.469	0.986
V_{dif}	8.017	1.554

Quantitative Structure−Affinity Relationship Analysis. The ability of the CODESSA[17] program to provide a fast and adequate search for the best correlation equations for a given property has been exploited to obtain linear QSAR models. All theoretical descriptors derived within CODESSA were considered with this goal in view, together with ad hoc-defined size and shape descriptors.[18]

A limited set of descriptors can provide a quantitative rationalization of the binding properties of these ligands (Table 3). The QSAR models obtained are reported in Table 4. The descriptors involved emphasize (a) the role of the quinoline nitrogen atom as hydrogen-bonding acceptor with respect to a suitable amino acid residue of the receptor (eqs 1 and 2), (b) the electrostatic component (eq 3) and the perturbation effect (eq 4) on the electron density localized on the heteroaryl moiety, and (c) the dispersive and steric interaction realized by the heteroaryl moiety (eq 5).

The crucial role of the piperazine protonated nitrogen is missing from this scenario. In fact, the values of the nucleophilic index $S^L(N)$ (reported in Table 3) being almost constant in this series of ligands are unable to describe the variation of the ligand binding affinities quantitatively. Therefore, in conformity to a general mode of interaction of bioamine ligands with their receptors, a long-range electrostatic interaction has to be assumed as a preliminary recognition step.[18a] Once the main docking has been accomplished, the binding affinity may be modulated by the optimization of the short-range intermolecular interactions and dispersion contributions of the heteroaryl moiety.

The data in Table 4 show that the best correlation is obtained by the $S^H(N_h)$ descriptor. This index explains 70% of the variation in the binding affinity, when the

Table 5. 5-HT Receptor Binding Profile of Compounds **5b,c,j** (K_i (nM) ± SEM)[a]

compd	5-HT$_3$	5-HT$_{1A}$	5-HT$_{1B}$	5-HT$_{2A}$	5-HT$_{2C}$	5-HT$_{transporter}$
5b	0.23 ± 0.002	2800 ± 88 (12000)	>4000 (>17000)	3000 ± 140 (13000)	4400 ± 470 (19000)	12000 ± 3700 (52000)
5c	0.83 ± 0.03	2400 ± 410 (2900)	2300 ± 890 (2800)	3700 ± 240 (4500)	2800 ± 910 (3400)	24000 ± 3200 (29000)
5j	14 ± 1.1	5700 ± 180 (410)	3800 ± 420 (270)	2600 ± 130 (190)	16000 ± 5200 (1100)	59 ± 1.9 (4.2)
2a (quipazine)	1.8 ± 0.3	3600 ± 800 (2000)	310[b] (170)	1800 ± 480 (1000)	190[b] (110)	31 ± 2.9 (17)
granisetron	0.35 ± 0.06					
5-HT	120 ± 34	7.3 ± 1.2	1.1 ± 0.098			740 ± 120
8-OH-DPAT		1.2 ± 0.13				
mesulergine					0.73 ± 0.083	
2c (6-nitroquipazine)	58 ± 3.2					0.12 ± 0.01

[a] Each value is the mean ± SEM of three determinations. Each value in parentheses is the selectivity ratio calculated as the ratio of the K_i value for the indicated site over that for 5-HT$_3$ receptors. [b] See: Schoeffter, P.; Hoyer, D. Interaction of Arylpiperazines with 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1C}$, and 5-HT$_{1D}$ Receptors: Do Discriminatory 5-HT$_{1B}$ Receptor Ligands Exist? *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1989**, *339*, 675−683.

16 ligands with significant affinities listed in Table 3 are considered. A notable improvement in the correlation statistics is obtained by omitting 6-nitroquipazine and compounds **6c**,**d**, for which the neglect of d-orbitals in the computation might result in an underestimation of the effects of the halogen atoms on the pyridine ring. A satisfactory correlation is also obtained by using *HASA* (eq 2). Since the molecular descriptor is computed on the whole ligand, it takes into account all the possible hydrogen-bonding acceptor centers of the molecule simultaneously.

The surface-weighted charged partial surface area of the heteroaryl moiety, $f_{aa'} - WNSA\text{-}3$, might be considered to rationalize the electrostatic component of the binding adjustments which modulate the binding affinity after the main docking has been accomplished. A good correlation (eq 3) is obtained by omitting compounds **5f**,**d**, outliers with overpredicted binding affinity, and 6-nitroquipazine, which is, on the contrary, underpredicted.

Correlation 4, involving the sum of either the electrophilic superdelocalizability on the atoms of the fused benzene ring of the heteroaryl moiety or the saturated ring in an analogous position, $\sum f_{a'} - S^H$, corroborates the qualitative conclusions reported in the previous section on the important role of this portion of the ligands in the binding to the 5-HT$_3$ receptor. It explains 60% of the variation in the binding affinity, by omitting the halogen derivatives **6c**,**d**, quipazine, and NMQ which were found to have been overestimated.

Finally, eq 5 involves the ad hoc-derived size and shape descriptor V_{dif}, which, by taking into account in its formulation both the inner and outer molecular volumes of the ligands considered with respect to the reference volume of the supermolecule, indicates that, in these series of compounds, the binding affinity is modulated by the molecular shape of the heteroaryl moiety through the optimization of dispersive and steric interactions.

Selectivity. Three representative compounds were selected among the most potent inhibitors of [^{3}H]-granisetron specific binding. These compounds were tested for their possible binding to several 5-HT receptor subtypes interacting with quipazine derivatives (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2C}$) and the 5-HT transporter in comparison with reference compounds using well-established protocols.[40−43] The results of these binding studies (summarized in Table 5) show that, while **5j** displays a binding profile very similar to that of quipazine, the highly potent compounds **5b**,**c** are also very selective, indeed showing selectivity ratios higher than those of quipazine. These results clearly demonstrate that among the 5-HT receptor subtypes interacting with quipazine (with medium to high affinity) only 5-HT$_3$ tolerates additional steric hindrance in the receptor area corresponding to the *c*-edge of quipazine.

Functional Activity. The measurements of [^{14}C]-guanidinium uptake in the presence of substance P (SP) appear to be a rapid and reliable method for assessing functional properties of 5-HT$_3$ receptor ligands in NG 108-15 hybridoma cells.[9] Owing to the similarities existing between 5-HT$_3$ receptors present on NG 108-15 cells and those of rat cortex,[10] relevant inferences can be drawn from this pharmacological model regarding the functional properties of ligands of the central 5-HT$_3$ receptor.[11] Accordingly, the potential 5-HT$_3$ receptor agonist/antagonist activity of three selected compounds was assessed on the 5-HT$_3$ receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 cells. In the presence of SP (10 μM), compound **5j** increased in a concentration-dependent manner the uptake of [^{14}C]-guanidinium into NG 108-15 cells with an EC$_{50}$ value (≈20 nM) close to that reported for quipazine,[9] indicating that it acted as a 5-HT$_3$ agonist in this assay. The maximum increase of the [^{14}C]guanidinium uptake was similar to that due to 5-HT, and the effect of **5j** could be completely antagonized by ondansetron (**1**) but was unaltered in the presence of 5-HT (1 μM). On the other hand, compound **5b** appeared to act as a partial agonist with an EC$_{50}$ value of about 0.25 nM, since it stimulated [^{14}C]guanidinium uptake in the presence of SP (10 μM), up to a level equal to about two-thirds of that reached with 5-HT (1 μM) plus SP (10 μM). Indeed, in the presence of the latter two agents, **5b** produced a decrease in the accumulation of [^{14}C]guanidinium (Figure 2). Finally, compound **5c** was revealed to possess antagonist properties in this test as it reversed in a concentration-dependent manner the stimulation effect of 5-HT (in the presence of SP) on [^{14}C]guanidinium uptake with an IC$_{50}$ value (≈8 nM) in the same range as those of well-characterized 5-HT$_3$ receptor antagonists.[12]

Arylpiperazine Interactions at the Central 5-HT$_3$ Receptor. In conclusion, our work provides evidence

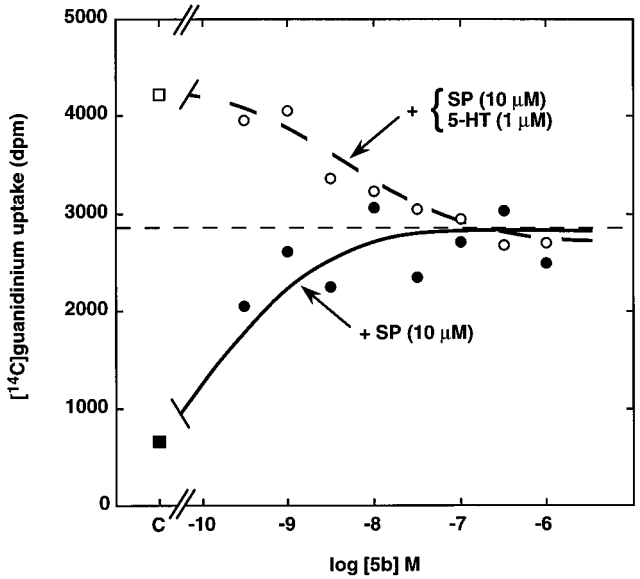


Figure 2. Effects of compound **5b** on the 5-HT_3 receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 cells: ■, with 10 μM SP alone; □, with SP (10 μM) and 5-HT (1 μM); ●, **5b** and SP (10 μM); ○, **5b**, SP (10 μM), and 5-HT (1 μM). Assays were carried out as described in the Experimental Section, and the accumulation of [^{14}C]guanidinium is expressed as dpm/culture dish. Each point is the mean of triplicate determinations with less than 5% variation among them.

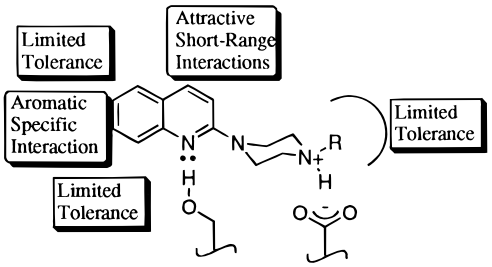


Figure 3. Arylpiperazine interactions at the central 5-HT_3 receptors.

for the key role played by the protonated piperazine nitrogen atom, the role of the quinoline nitrogen atom as a hydrogen-bonding acceptor, and the dual role of the fused benzene ring of quipazine derivatives in the interaction with the 5-HT_3 receptor.

Accordingly, a three-component model for the interaction of central 5-HT_3 ligands related to quipazine with their receptor is schematically represented in Figure 3. High-affinity arylpiperazine interaction with the central 5-HT_3 receptor probably requires (a) a charge-assisted hydrogen bond between the protonated terminal piperazine nitrogen atom and a negatively charged carboxylic amino acid residue in the receptor, (b) a hydrogen-bonding interaction between the heterocyclic nitrogen atom and a suitable H-bond donor in the receptor, and (c) an aromatic specific interaction between an aromatic ring and a suitable amino acid residue in the receptor. Furthermore, attractive short-range (e.g., van der Waals) interactions in the receptor area corresponding to the *c*-edge of the quinoline nucleus of quipazine may be assumed to take place. These interactions seem to be of crucial importance in modulating the intrinsic efficacy at the central 5-HT_3 receptor since quipazine (agonist), **5b** (partial agonist), and **5c** (antagonist) mainly differ in the presence and the type of substituent on the *c*-edge of the quinoline nucleus. Finally, the existence of limited tolerance to large substituents in the areas surrounding both the fused benzene ring and the terminal piperazine nitrogen atom of quipazine completes this model.

Conclusions

Starting from the high affinity and low selectivity shown by quipazine for 5-HT_3 receptors, our work has resulted in the discovery of novel central 5-HT_3 receptor ligands provided with a full range of intrinsic efficacies which, by virtue of their selectivity and potency, are interesting candidates as pharmacological tools to evaluate the role(s) of 5-HT_3 receptors in the CNS. Moreover, qualitative and quantitative structure−affinity relationship studies carried out by means of descriptors capable of capturing the strict ligand−receptor complementarity criteria (which determine the binding affinity of those ligands) allowed the role of the main pharmacophoric components to be elucidated and a model for the interaction of the 5-HT_3 ligands related to quipazine with their receptor to be developed. The essential nature of the arylpiperazine interaction mode toward the receptor can be summarized as follows: (a) a charge-assisted hydrogen bond, (b) a hydrogen-bonding interaction, and (c) an aromatic specific interaction. Furthermore, short-range interactions in the receptor area corresponding to the *c*-edge of the quinoline nucleus of quipazine seem to be able to modulate the intrinsic efficacy of these quipazine derivatives toward the central 5-HT_3 receptor. Finally, the existence of limited bulk tolerance in the areas surrounding the fused benzene ring and the terminal piperazine nitrogen atom of quipazine completes this model.

Experimental Section

Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Microanalyses were carried out using a Perkin-Elmer 240C elemental analyzer. Merck silica gel 60 (70−230 or 230−400 mesh) and Merck aluminum oxide 90 II−III, 70−230 mesh, were used for column chromatography. Merck TLC plates, silica gel 60 F_{254}, were used for TLC. ^{1}H NMR spectra were recorded with a Bruker AC 200 spectrometer in the indicated solvents (TMS as internal standard); the values of chemical shifts are expressed in ppm and coupling constants (*J*) in Hz. Mass spectra (EI, 70 eV) were recorded on either a VG 70−250S spectrometer (Centro di Analisi e Determinazioni Strutturali, Università di Siena) or a Varian Saturn 3 (Dipartimento Farmaco Chimico Tecnologico, Università di Siena). NMR spectra and elemental analyses were performed in the Dipartimento Farmaco Chimico Tecnologico, Università di Siena.

6-(4-Methyl-1-piperazinyl)phenanthridine (5a). A mixture of 6-chlorophenanthridine (**9a**)[20] (0.214 g, 0.1 mmol) with *N*-methylpiperazine (5 mL) was heated at 130−140 °C under argon for 24 h. The reaction mixture was then poured into ice−water (250 mL) and extracted with chloroform (4 × 15 mL). The organic layer was washed with water (2 × 30 mL), dried over sodium sulfate, and evaporated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate−triethylamine (8:2) as the eluent gave pure **5a** as a pale brown oil (0.24 g) which crystallized on standing. ^{1}H NMR ($CDCl_3$): 2.42 (s, 3H), 2.72 (t, J = 4.8, 4H), 3.55 (t, J = 4.7, 4H), 7.47 (t, J = 7.3, 1H), 7.56−7.65 (m, 2H), 7.76 (t, J = 7.6, 1H), 7.92 (d, J = 8.6, 1H), 8.19 (d, J = 8.0, 1H), 8.41 (d, J = 7.8, 1H), 8.54 (d, J = 8.2, 1H).

6-(4-Methyl-1-piperazinyl)-7,8,9,10-tetrahydrophenanthridine (5b). This compound was prepared in a similar way as **5a** starting from 6-chloro-7,8,9,10-tetrahydrophenanthridine (**9b**)[21] (0.2 g, 0.92 mmol) and 6 mL of *N*-methylpiperazine (reaction time 24 h at 130−140 °C). Compound **5b** (0.23 g) was obtained as a pale-yellow oil which slowly crystallized on standing. ^{1}H NMR ($CDCl_3$): 1.71−1.82 (m, 2H), 1.91−2.03 (m, 2H), 2.38 (s, 3H), 2.62 (t, J = 4.7, 4H), 2.77 (t, J = 5.6, 2H), 3.10 (t, J = 6.4, 2H), 3.34 (t, J = 4.8, 4H), 7.31−7.39 (m, 1H), 7.50−7.58 (m, 1H), 7.79−7.86 (m, 2H).

6-Morpholino-7,8,9,10-tetrahydrophenanthridine (5ba). This compound was prepared in a similar way as **5a** starting from **9b**[21] (0.22 g, 1.01 mmol) and 5 mL of morpholine (reaction time 17 h at 130−150 °C) and purified by flash chromatography with *n*-hexane−ethyl acetate (8:2) as the eluent. Compound **5ba** (0.26 g, yield 96%) was obtained as a colorless oil which slowly crystallized on standing (mp 76−77 °C). ^{1}H NMR ($CDCl_3$): 1.74−1.85 (m, 2H), 1.92−2.05 (m, 2H), 2.79 (t, J = 5.6, 2H), 3.12 (t, J = 6.4, 2H), 3.30 (t, J = 4.7, 4H), 3.90 (t, J = 4.5, 4H), 7.38 (t, J = 7.8, 1H), 7.56 (t, J = 7.3, 1H), 7.82−7.88 (m, 2H). Anal. ($C_{17}H_{20}N_2O$) C, H, N.

1-(7,8,9,10-Tetrahydrophenanthridin-6-yl)-4,4-dimethylpiperazinium Iodide (5bb). A mixture of **5b** (0.282 g, 1.0 mmol) in 20 mL of diethyl ether with CH_3I (0.062 mL, 1.0 mmol) was stirred at room temperature for 3 days. The precipitate was collected by filtration, washed with diethyl ether (10 mL), and dried under reduced pressure to yield 0.17 g (40%) of white powder (mp 252−253 °C). ^{1}H NMR ($CDCl_3$): 1.73−1.85 (m, 2H), 1.92−2.05 (m, 2H), 2.76 (t, J = 5.5, 2H), 3.13 (t, J = 6.1, 2H), 3.71 (s, 10H), 3.93 (t, J = 4.7, 4H), 7.40−7.48 (m, 1H), 7.56−7.64 (m, 1H), 7.80−7.89 (m, 2H). Anal. ($C_{19}H_{26}N_3I$) C, H, N.

6-(1-Piperazinyl)-7,8,9,10-tetrahydrophenanthridine (5bc). This compound was prepared in a similar way as **5a** starting from **9b**[21] (0.22 g, 1.01 mmol) and 1.0 g (11.6 mmol) of anhydrous piperazine with ethylene glycol (10 mL) as the solvent (reaction time 17 h at 130−140 °C) and purified by flash chromatography with ethyl acetate−triethylamine−MeOH (6:2:2) as the eluent. Compound **5bc** (0.21 g, yield 78%) was obtained as a colorless oil which crystallized on standing (mp 98−100 °C). ^{1}H NMR ($CDCl_3$): 1.72−1.83 (m, 2H), 1.91−2.04 (m, 2H), 2.19 (br s, 1H), 2.78 (t, J = 5.4, 2H), 3.07−3.14 (m, 6H), 3.24−3.29 (m, 4H), 7.36 (t, J = 8.0, 1H), 7.55 (t, J = 7.3, 1H), 7.80−7.87 (m, 2H). Anal. ($C_{17}H_{21}N_3$) C, H, N.

6-(4-Ethyl-1-piperazinyl)-7,8,9,10-tetrahydrophenanthridine (5bd). This compound was prepared in a similar way as **5a** starting from **9b**[21] (0.22 g, 1.01 mmol) and 4 mL of *N*-ethylpiperazine (reaction time 24 h at 130−140 °C). Compound **5bd** (0.25 g, yield 84%) was obtained as a pale-yellow oil which slowly crystallized on standing (mp 66−67 °C). ^{1}H NMR ($CDCl_3$): 1.15 (t, J = 7.1, 3H), 1.71−1.82 (m, 2H), 1.92−2.04 (m, 2H), 2.51 (q, J = 7.2, 2H), 2.64 (t, J = 4.6, 4H), 2.77 (t, J = 5.6, 2H), 3.10 (t, J = 6.4, 2H), 3.34 (t, J = 4.8, 4H), 7.35 (t, J = 7.4, 1H), 7.54 (t, J = 7.3, 1H), 7.79−7.86 (m, 2H). Anal. ($C_{19}H_{25}N_3$) C, H, N.

6-(4-Benzyl-1-piperazinyl)-7,8,9,10-tetrahydrophenanthridine (5be). This compound was prepared in a similar way as **5a** starting from **9b**[21] (0.22 g, 1.01 mmol) and 0.20 mL (1.1 mmol) of *N*-benzylpiperazine with ethylene glycol (3 mL) as the solvent and 0.12 g (1.1 mmol) of Na_2CO_3 (reaction time 18 h at 130−140 °C) and purified by flash chromatography with *n*-hexane−ethyl acetate (1:1) as the eluent. Compound **5be** (0.13 g, yield 36%) was obtained as a pale-yellow oil which crystallized on standing (mp 107−109 °C). ^{1}H NMR ($CDCl_3$): 1.71−1.82 (m, 2H), 1.92−2.03 (m, 2H), 2.65 (t, J = 4.6, 4H), 2.76 (t, J = 5.5, 2H), 3.10 (t, J = 6.3, 2H), 3.32 (t, J = 4.7, 4H), 3.61 (s, 2H), 7.23−7.40 (m, 6H), 7.54 (t, J = 7.2, 1H), 7.79−7.86 (m, 2H). Anal. ($C_{24}H_{27}N_3$) C, H, N.

6-(4-Methyl-1-homopiperazinyl)-7,8,9,10-tetrahydrophenanthridine (5bf). This compound was prepared in a similar way as **5a** starting from **9b**[21] (0.22 g, 1.01 mmol) and 2 mL of *N*-methylhomopiperazine (reaction time 24 h at 130−140 °C). Compound **5bf** (0.21 g, yield 70%) was obtained as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): 1.70−1.81 (m, 2H), 1.90−2.08 (m, 4H), 2.43 (s, 3H), 2.72−2.82 (m, 6H), 3.09 (t, J = 6.4, 2H), 3.59 (t, J = 6.1, 2H), 3.67 (t, J = 4.8, 2H), 7.31 (t, J = 7.3, 1H), 7.52 (t, J = 7.6, 1H), 7.78 (d, J = 8.7, 2H). Anal. ($C_{19}H_{25}N_3$) C, H, N.

2-(4-Methyl-1-piperazinyl)pyridine Dimaleate (6a). This compound was prepared in a similar way as **5a** starting from 2-chloropyridine (0.15 mL, 1.6 mmol) and 4 mL of *N*-methylpiperazine (reaction time 21 h at 130−140 °C). The free base (0.27 g, 1.52 mmol) was dissolved in methanol−$CHCl_3$ (10 mL), and maleic acid (0.38 g, 3.3 mmol) was added. The resulting mixture was stirred at room temperature for 15 min and concentrated under reduced pressure, and the residue was recrystallized to give 0.43 g of **6a**. ^{1}H NMR ($CDCl_3$): 2.87 (s, 3H), 3.19−3.33 (m, 4H), 3.83−3.98 (m, 4H), 6.32 (s, 4H), 6.69−6.81 (m, 2H), 7.54−7.64 (m, 1H), 8.21−8.24 (m, 1H).

6-(4-Methyl-1-piperazinyl)-7,8,9,10-tetrahydro-8,10-ethanophenanthridine (5c). This compound was prepared in a similar way as **5a** starting from **9c** (0.098 g, 0.40 mmol) and 5 mL of *N*-methylpiperazine (reaction time 20 h at 130−140 °C). Compound **5c** (0.12 g) was obtained as a pale-yellow oil which slowly crystallized on standing. ^{1}H NMR ($CDCl_3$): 1.37−1.45 (m, 1H), 1.75−2.05 (m, 5H), 2.37 (s, 3H), 2.51−2.69 (m, 6H), 3.02−3.44 (m, 5H), 3.83 (br s, 1H), 7.33−7.41 (m, 1H), 7.50−7.58 (m, 1H), 7.86 (d, J = 8.0, 1H), 7.96 (d, J = 8.2, 1H).

7,8,9,10-Tetrahydro-8,10-ethano-6(5*H*)-phenanthridinone (8). To a 100-mL, three-necked flask fitted with a condenser, thermometer, and dropping funnel (under argon) were added **7** (1.24 g, 9.98 mmol), freshly distilled anhydrous ethyl ether (40 mL), and pyrrolidine (3.3 mL, 39.5 mmol). To the resulting solution was added, over a 20−30-min period, $TiCl_4$ (0.55 mL, 5.0 mmol) in an additional 10 mL of dry benzene. The temperature was kept between 0 and 10 °C during the addition. Once the $TiCl_4$ addition was complete, the mixture was allowed to stir at room temperature for 19 h. The reaction mixture was then filtered, and the solvent was removed under reduced pressure. The residue was dissolved into 10 mL of $CHCl_3$, and the resulting solution was cooled at 0 °C. To this was added a solution of phenyl isocyanate (1.1 mL, 10.1 mmol) in $CHCl_3$ (10 mL) dropwise. When the addition was complete, the reaction mixture was stirred for 2 h at room temperature and the solvent was removed under reduced pressure. Purification of the residue by chromatography (*n*-hexane−ethyl acetate, 65:35, as eluent) gave an oil which crystallized on standing. To this material was added PPA (20 g), and the resulting mixture was heated at 150 °C with stirring for 15 min. Then the cooled brown mass was decomposed with ice−water, and the precipitate was extracted with $CHCl_3$ (3 × 20 mL). The combined organic extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by washing with ethyl acetate gave pure **8** (0.48 g, yield 21%) as a white solid. Recrystallization from ethyl acetate−methanol gave an analytical sample melting at 248−249 °C. ^{1}H NMR ($CDCl_3$): 1.49−1.63 (m, 1H), 1.76−2.05 (m, 5H), 2.52−2.67 (m, 2H), 2.86−2.97 (m, 1H), 3.58 (br s, 1H), 7.18−7.29 (m, 2H), 7.44 (t, J = 7.3, 1H), 7.80 (d, J = 8.1, 1H), 10.64 (br s, 1H). Anal. ($C_{15}H_{15}NO$) C, H, N.

6-Chloro-7,8,9,10-tetrahydro-8,10-ethanophenanthridine (9c). A mixture of **8** (0.25 g, 1.11 mmol) and $POCl_3$ (5 mL) was refluxed for 1 h. Then the cooled reaction mixture was poured into ice−water, and the precipitate was extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure to obtain pure **9c** (0.26 g, yield 96%) as a white solid. Recrystallization from *n*-hexane gave an analytical sample melting at 127−128 °C. ^{1}H NMR ($CDCl_3$): 1.49−1.64 (m, 1H), 1.79−2.14 (m, 5H), 2.70−2.79 (m, 2H), 3.06−3.18 (m, 1H), 3.87 (br s, 1H), 7.50−7.69 (m, 2H), 7.97 (d, J = 8.3, 1H), 8.05 (d, J = 8.2, 1H). MS: m/z 243 (M^+, 100). Anal. ($C_{15}H_{14}NCl$) C, H, N.

1,2,3,4,7,8,9,10-Octahydro-6(5*H*)-phenanthridinone (11). A mixture of **10**[23] (0.25 g, 0.91 mmol) in 14 g of PPA was heated at 140 °C with stirring for 30 min. The cooled reaction mixture was then decomposed with ice−water, and the resulting

solution was neutralized with solid Na_2CO_3. The precipitate was extracted with $CHCl_3$ (4 × 30 mL), and the combined extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure to give pure **11** as a white solid (0.154 g, yield 83%) melting at 295−297 °C (lit.[23] mp 295−297 °C). ^{1}H NMR ($CDCl_3$): 1.68−1.82 (m, 8H), 2.30−2.67 (m, 8H), 11.33 (br s, 1H).

6-(4-Methyl-1-piperazinyl)-1,2,3,4,7,8,9,10-octahydrophenanthridine (5d). To a mixture of **11** (0.21 g, 1.03 mmol) and anhydrous Na_2CO_3 (0.26 g, 2.45 mmol) in CH_2Cl_2 (20 mL) cooled to − 60 °C was added trifluoromethanesulfonic anhydride (0.5 mL, 2.97 mmol). The resulting mixture was allowed to stir at the same temperature for 10 min and at 0 °C for 3 h and then filtered, and the filtrate was concentrated under reduced pressure. The residue was diluted with 5 mL of *N*-methylpiperazine, and the resulting mixture was heated at 130−140 °C under argon overnight. The reaction mixture was then poured into ice−water, and the precipitate was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography (Al_2O_3), and elution with *n*-hexane−ethyl acetate (65:35) gave analytically pure **5d** (0.25 g) as a colorless oil which slowly crystallized on standing. ^{1}H NMR ($CDCl_3$): 1.62−1.87 (m, 8H), 2.34 (s, 3H), 2.49−2.64 (m, 10H), 2.78 (br s, 2H), 3.12 (t, J = 4.8, 4H).

General Procedure for the Synthesis of 2(1*H*)-Pyridones (13e,g−k). Commercially available cyclohexanone (**12e**), α-tetralone (**12g**), *trans*-1-decalone (**12h**), 1-benzosuberone (**12i**), 1-indanone (**12j**), and β-tetralone (**12k**) (obtained from Aldrich) were used as starting material for the synthesis of the title compounds without further purification. The appropriate ketone (10 mmol) and methyl propiolate (1.7 mL, 19.1 mmol) were mixed in an ammonia-saturated methanol solution (50 mL) in a 125-mL stainless steel vessel (Parr model 4754), and the reaction mixture was heated at 150 °C for 10 h. After cooling, the reaction mixture was concentrated under reduced pressure and the residue flash-chromatographed on silica gel. Elution with ethyl acetate−methanol (85:15) gave the title compounds.

5,6,7,8-Tetrahydro-2(1*H*)-quinolinone (13e). This compound was prepared in 55% yield starting from cyclohexanone (**12e**). An analytical sample was obtained from ethyl acetate as colorless prisms melting at 205−206 °C (lit.[44] mp 205−206 °C). ^{1}H NMR ($CDCl_3$): 1.68−1.78 (m, 4H), 2.47 (t, J = 5.0, 2H), 2.67 (t, J = 5.2, 2H), 6.37 (d, J = 9.0, 1H), 7.18 (d, J = 9.0, 1H), 12.71 (br s, 1H). Anal. ($C_9H_{11}NO$) C, H, N.

5,6-Dihydrobenzo[*h*]quinolin-2(1*H*)-one (13g). This compound was prepared in 61% yield starting from α-tetralone (**12g**). An analytical sample was obtained from ethyl acetate−methanol as pale-green prisms melting at 204−206 °C. ^{1}H NMR ($CDCl_3$): 2.64−2.71 (m, 2H), 2.83−2.92 (m, 2H), 6.52 (d, J = 9.5, 1H), 7.24−7.44 (m, 4H), 7.86−7.90 (m, 1H), 11.58 (br s, 1H). Anal. ($C_{13}H_{11}NO$) C, H, N.

5,6,6a,7,8,9,10,10a-Octahydrobenzo[*h*]quinolin-2(1*H*)-one (13h). This compound was prepared in 56% yield starting from *trans*-1-decalone (**12h**). An analytical sample was obtained from ethyl acetate as pale-yellow prisms melting at 200−204 °C. ^{1}H NMR ($CDCl_3$): 1.11−2.04 (m, 11H), 2.16−2.31 (m, 1H), 2.44−2.63 (m, 2H), 6.36 (d, J = 9.6, 1H), 7.13−7.18 (m, 1H), 10.15 (br s, 1H). Anal. ($C_{13}H_{17}NO$) C, H, N.

6,7-Dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*b*]pyridin-2(1*H*)-one (13i). This compound was prepared in 65% yield starting from 1-benzosuberone (**12i**). An analytical sample was obtained from ethyl acetate−methanol as colorless prisms melting at 204−205 °C. ^{1}H NMR ($CDCl_3$): 2.16−2.28 (m, 4H), 2.57 (t, J = 6.3, 2H), 6.48 (d, J = 8.9, 1H), 7.30−7.55 (m, 5H), 11.43 (br s, 1H). Anal. ($C_{14}H_{13}NO$) C, H, N.

5*H*-Indeno[1,2-*b*]pyridin-2(1*H*)-one (13j). This compound was prepared starting from 1-indanone (**12j**) and further purified by recrystallization from methanol−$CHCl_3$ (12% yield). An analytical sample melted at 298 °C dec. ^{1}H NMR ($CDCl_3$): 3.70 (s, 2H), 6.61 (d, J = 8.9, 1H), 7.35−7.56 (m, 3H), 7.67 (d, J = 9.2, 1H), 8.21 (d, J = 7.36, 1H), 14.15 (br s, 1H). MS: m/z 183 (M^+, 100). Anal. ($C_{12}H_9NO\cdot 0.33H_2O$) C, H, N.

5,6-Dihydrobenzo[*f*]quinolin-3(4*H*)-one (13k). This compound was prepared in 68% yield starting from β-tetralone (**12k**). An analytical sample was obtained from ethyl acetate−methanol as pale-green plates melting at 254−255 °C. ^{1}H NMR ($CDCl_3$): 2.97 (s, 4H), 6.59 (d, J = 9.3, 1H), 7.14−7.33 (m, 3H), 7.49 (d, J = 7.6, 1H), 7.93 (d, J = 9.7, 1H), 13.66 (br s, 1H). Anal. ($C_{13}H_{11}NO$) C, H, N.

General Procedure for the Synthesis of 4-Methyl-1-piperazinyl Derivatives 5e,g−k. A mixture of the appropriate 2(1*H*)-pyridone **13e,g−k** (1.0 mmol) in CH_2Cl_2 (20 mL) with anhydrous Na_2CO_3 (0.26 g, 2.45 mmol) was cooled at − 60 °C, and trifluoromethanesulfonic anhydride (0.5 mL, 2.97 mmol) was added under an argon atmosphere. The resulting mixture was stirred at − 60 °C for 10 min and then at 0 °C for 1 h. The solid was removed by filtration and washed with $CHCl_3$, and the filtrate was concentrated under reduced pressure. The residue was diluted with *N*-methylpiperazine (5 mL), and the resulting mixture was heated at 130−140 °C under argon for a suitable time (1−2 h). The cooled reaction mixture was poured into ice−water, the precipitate was extracted with CH_2Cl_2 (5 × 15 mL), and the combined extracts were washed with water (2 × 20 mL), dried over sodium sulfate, and evaporated under reduced pressure. Chromatographic purification (Al_2O_3) of the residue with *n*-hexane−ethyl acetate (65:35) as the eluent gave analytically pure title compounds (as free bases).

2-(4-Methyl-1-piperazinyl)-5,6,7,8-tetrahydroquinoline (5e). ^{1}H NMR ($CDCl_3$): 1.69−1.89 (m, 4H), 2.34 (s, 3H), 2.51 (t, J = 5.2, 4H), 2.62 (t, J = 5.9, 2H), 2.74 (t, J = 6.0, 2H), 3.50 (t, J = 5.1, 4H), 6.43 (d, J = 8.3, 1H), 7.17 (d, J = 8.4, 1H).

5,6-Dihydro-2-(4-methyl-1-piperazinyl)benzo[*h*]quinoline Maleate (5g). The free base (0.19 g, 0.68 mmol) was dissolved into methanol (10 mL), and maleic acid (0.079 g, 0.68 mmol) was added. The resulting mixture was stirred at room temperature for 15 min and concentrated under reduced pressure, and the residue was recrystallized to give crystalline maleate salt **5g** (0.21 g).^{1}H NMR ($CDCl_3$): 2.80−3.09 (m, 9H), 3.38−3.72 (m, 4H), 4.39−4.58 (m, 2H), 6.32 (s, 2H), 6.60 (d, J = 8.5, 1H), 7.19−7.38 (m, 3H), 7.42 (d, J = 8.4, 1H), 8.15−8.20 (m, 1H).

2-(4-Methyl-1-piperazinyl)-5,6,6a,7,8,9,10,10a-octahydrobenzo[*h*]quinoline (5h). ^{1}H NMR ($CDCl_3$): 0.98−1.60 (m, 6H), 1.73−1.94 (m, 4H), 2.14−2.28 (m, 1H), 2.32 (s, 3H), 2.50 (t, J = 5.0, 4H), 2.64−2.81 (m, 3H), 3.40−3.60 (m, 4H), 6.41 (d, J = 8.4, 1H), 7.15 (d, J = 8.3, 1H).

6,7-Dihydro-2-(4-methyl-1-piperazinyl)-5*H*-benzo[6,7]-cyclohepta[1,2-*b*]pyridine (5i). ^{1}H NMR ($CDCl_3$): 2.14−2.24 (m, 2H), 2.35−2.41 (m, 5H), 2.52−2.59 (m, 6H), 3.61 (t, J = 5.0, 4H), 6.57 (d, J = 8.3, 1H), 7.19−7.38 (m, 4H), 7.70−7.75 (m, 1H).

2-(4-Methyl-1-piperazinyl)-5*H*-indeno[1,2-*b*]pyridine (5j). ^{1}H NMR ($CDCl_3$): 2.38 (s, 3H), 2.59 (t, J = 4.9, 4H), 3.68 (t, J = 4.9, 4H), 3.73 (s, 2H), 6.58 (d, J = 8.2, 1H), 7.30−7.43 (m, 2H), 7.52 (d, J = 6.8, 1H), 7.62 (d, J = 8.6, 1H), 7.97−8.01 (m, 1H).

5,6-Dihydro-3-(4-methyl-1-piperazinyl)benzo[*f*]quinoline (5k). ^{1}H NMR ($CDCl_3$): 2.36 (s, 3H), 2.54 (t, J = 5.0, 4H), 2.94 (s, 4H), 3.62 (t, J = 5.0, 4H), 6.59 (d, J = 8.5, 1H), 7.10−7.29 (m, 3H), 7.56 (d, J = 7.5, 1H), 7.83 (d, J = 8.6, 1H).

5,6-Dihydro-3-[[(trifluoromethyl)sulfonyl]oxy]benzo-[*f*]quinoline (14k). This compound was isolated as an intermediate of the synthesis of **5k** and purified by flash chromatography with *n*-hexane−ethyl acetate (8:2) as the eluent (colorless thick oil). ^{1}H NMR ($CDCl_3$): 2.96−3.11 (m, 4H), 7.10 (d, J = 8.4, 1H), 7.28−7.38 (m, 3H), 7.65−7.69 (m, 1H), 8.15 (d, J = 8.3, 1H). Anal. ($C_{14}H_{10}NO_3SF_3$) C, H, N.

6-(1-Adamantyl)-2-(4-methyl-1-piperazinyl)pyridine (6b). 1-Adamantyl methyl ketone (0.45 g, 2.5 mmol) and methyl propiolate (0.45 mL, 5.0 mmol) were mixed in an ammonia-saturated methanol solution (30 mL) in a 125-mL stainless steel vessel, and the reaction mixture was heated at

150 °C for 11 h. After cooling, the reaction mixture was concentrated under reduced pressure and the residue flash-chromatographed with ethyl acetate as the eluent to give a solid. To a mixture of this solid in CH_2Cl_2 (30 mL) and anhydrous Na_2CO_3 (0.4 g, 3.8 mmol) cooled at −60 °C was added trifluoromethanesulfonic anhydride (0.75 mL, 4.5 mmol) under an argon atmosphere. The resulting mixture was stirred at −60 °C for 10 min and then at 0 °C for 1 h. The salt was removed by filtration and washed with CH_2Cl_2, and the filtrate was concentrated under reduced pressure. The residue was diluted with *N*-methylpiperazine (10 mL), and the resulting mixture was heated at 110−120 °C under argon for 1 h. The cooled reaction mixture was poured into ice−water, the precipitate was extracted with $CHCl_3$ (4 × 15 mL), and the combined extracts were washed with brine (2 × 40 mL), dried over sodium sulfate, and evaporated under reduced pressure. Chromatographic purification (Al_2O_3) of the residue with *n*-hexane−ethyl acetate (65:35) as the eluent gave analytically pure **6b** (0.26 g). ^{1}H NMR ($CDCl_3$): 1.77 (br s, 6H), 1.96 (br s, 6H), 2.07 (br s, 3H), 2.35 (s, 3H), 2.53 (t, J = 5.1, 4H), 3.58 (t, J = 5.0, 4H), 6.44 (d, J = 8.5, 1H), 6.59 (d, J = 7.3, 1H), 7.38−7.46 (m, 1H).

Benzo[*h*]quinoline 1-Oxide (16). To a solution of benzo[*h*]quinoline (1.8 g, 10.0 mmol) in 40 mL of $CHCl_3$ cooled at 0−5 °C was added a solution of MCPBA (3.0 g, 15.0 mmol) in 60 mL of $CHCl_3$. The resulting mixture was stirred at room temperature for 4 days, then washed with a 5% solution of K_2CO_3 (6 × 60 mL), dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by washing with diethyl ether gave pure **16** (1.7 g, yield 87%) as a crystalline solid melting at 120 °C (lit.[27] mp 123 °C).

2-Chlorobenzo[*h*]quinoline (17). A mixture of **16** (0.8 g, 4.1 mmol) in 5 mL of $POCl_3$ was refluxed for 30 min. The cooled reaction mixture was poured into crushed ice, neutralized with concentrated NH_4OH solution, and extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were washed with water, dried over sodium sulfate, and evaporated under reduced pressure. The residue was flash-chromatographed with CH_2Cl_2 as the eluent to give **17** (0.49 g, yield 56%) as the less-polar fraction. A sample recrystallized from *n*-hexane melted at 109 °C (lit.[27] mp 111 °C). ^{1}H NMR ($CDCl_3$): 7.51 (d, J = 8.6, 1H), 7.65−7.93 (m, 5H), 8.12 (d, J = 8.2, 1H), 9.20−9.25 (m, 1H). Anal. ($C_{13}H_8NCl$) C, H, N.

4-Chlorobenzo[*h*]quinoline (18). Compound **18** was isolated from the above flash chromatography as the more-polar fraction (0.11 g, yield 12%) (white crystalline powder melting at 83−84 °C). ^{1}H NMR ($CDCl_3$): 7.59 (d, J = 4.7, 1H), 7.71−7.80 (m, 2H), 7.88−7.95 (m, 2H), 8.12 (d, J = 9.5, 1H), 8.85 (d, J = 4.8, 1H), 9.25−9.30 (m, 1H). Anal. ($C_{13}H_8NCl$) C, H, N.

2-(4-Methyl-1-piperazinyl)benzo[*h*]quinoline Maleate (5f). The title compound was prepared in a similar way as **5a** starting from 2-chlorobenzo[*h*]quinoline (**17**) (0.22 g, 1.0 mmol) and 8 mL of *N*-methylpiperazine (reaction time 7 h at 120−130 °C). The free base (0.26 g, 0.94 mmol) was dissolved in methanol (10 mL), and maleic acid (0.12 g, 1.0 mmol) was added. The resulting mixture was stirred at room temperature for 15 min and concentrated under reduced pressure, and the residue was recrystallized to give **5f** (0.32 g) as colorless prisms. ^{1}H NMR ($CDCl_3$): 2.87 (s, 3H), 2.93−3.21 (m, 2H), 3.43−3.95 (m, 4H), 4.45−4.96 (m, 2H), 6.32 (s, 2H), 7.06 (d, J = 8.9, 1H), 7.56−7.69 (m, 4H), 7.83−7.89 (m, 1H), 8.06 (d, J = 8.9, 1H), 9.03−9.08 (m, 1H).

In Vitro Binding Assays. Binding assays were performed as described in refs 40 (5-HT$_{1A}$ receptors), 41 (5-HT$_{1B}$ receptors), 42 (5-HT$_{2A}$ receptors), 41 (5-HT$_{2C}$ receptors), 34 (5-HT$_3$ receptors), and 43 (5-HT transporter).

Male Wistar rats (Charles River, Calco, Italy) were killed by decapitation, and their brains were rapidly dissected into various areas (rat hippocampus for 5-HT$_{1A}$, rat striatum for 5-HT$_{1B}$, rat pre-frontal cortex for 5-HT$_{2A}$, rat cortex for 5-HT$_{2C}$, rat cortex and hippocampus for 5-HT$_3$, rat forebrain for 5-HT transporter). Tissues were homogenized (Polytron PTA 10TS) in ice-cold Tris HCl, 50 mM, at appropriate pH value (or Hepes buffer, 50 mM, pH 7.4, for 5-HT$_3$ receptors) and centrifuged according to the procedures indicated in the above-cited references. The pellet obtained was finally resuspended in the appropriate incubation buffer (Tris HCl, 50 mM, pH 7.4, for 5-HT$_{1A}$ receptors; Tris HCl, 50 mM, pH 7.7, containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbic acid for 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors; Tris HCl, 50 mM, pH 7.7, for 5-HT$_{2A}$ receptors; Hepes HCl, 50 mM, pH 7.4, for 5-HT$_3$ receptors; Tris HCl, 50 mM, pH 7.4, containing 120 mM NaCl and 5 mM KCl for 5-HT transporter) just before the binding assay. Membrane preparations were used the day of the experiment or stored at −80 °C (for 5-HT transporter binding assay).

[^{3}H]-8-OH-DPAT (sa 137 Ci/mmol, for 5-HT$_{1A}$; NEN) and [^{3}H]ketanserin (sa 80.9 Ci/mmol, for 5-HT$_{2A}$; NEN) binding was assayed in a final incubation volume of 0.5 mL. Tissue concentrations and [^{3}H]ligand final concentrations were respectively 4 mg of tissue/sample and 0.5 nM, and 8 mg of tissue/sample and 0.5 nM.

[^{3}H]Mesulergine (sa 86 Ci/mmol, for 5-HT$_{2C}$; Amersham) and [^{3}H]-5-HT (sa 30 Ci/mmol, for 5-HT$_{1B}$; NEN) binding was assayed in a final incubation volume of 1.0 mL. Tissue concentration and [^{3}H]ligand final concentration were 23 mg of tissue/sample and 1.3 nM ([^{3}H]mesulergine binding) and 14 mg of tissue/sample and 2.3 nM ([^{3}H]-5-HT binding).

[^{3}H]Granisetron (sa 81 Ci/mmol, for 5-HT$_3$; NEN) and [^{3}H]-paroxetine (sa 29.7 Ci/mmol, for 5-HT transporter; NEN) binding was assayed in final incubation volumes of 1.0 and 2.0 mL, respectively. Tissue concentration and [^{3}H]ligand final concentration were 20 mg of tissue/sample and 0.5 nM ([^{3}H]-granisetron binding) and 2 mg of tissue/sample and 0.1 nM ([^{3}H]paroxetine binding).

The specific binding of the tritiated ligands was defined as the difference between total binding and nonspecific binding in the presence of 10 μM 5-HT (for 5-HT$_{1A}$ and 5-HT$_{1B}$), 3 μM ketanserin (for 5-HT$_{2A}$), 1 μM mianserine (for 5-HT$_{2C}$), 100 μM 5-HT (for 5-HT$_3$), and 1 μM 6-nitroquipazine (for 5-HT transporter); it represented 80%, 62%, 90%, 70%, 70%, and 75%, respectively, of the total binding.

Incubations were stopped by rapid filtration under vacuum through Whatman GF/B filters presoaked in assay buffer or poly(ethylenimine) at 0.1% (for 5-HT$_3$) or 0.5% (for 5-HT transporter). Filters were immediately rinsed with 12 mL (3 × 4 mL) of ice-cold buffer using a Brandel M-24R cell harvester, dried, and immersed in vials containing 8 mL of Ultima Gold MV (or Filter Count) (Packard) for the measurement of trapped radioactivity with a TRI-CARB 1900TR (or 300C) (Packard) liquid scintillation spectrometer, at a counting efficiency of about 60%. Competition experiments were analyzed by the "Allfit" program[45] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}). Apparent affinity constants (K_i) were derived from the IC_{50} values according to the Cheng and Prusoff equation.[46] The K_d (nM) values of the radiolabeled ligands obtained in saturation isotherms were 2.3 for [^{3}H]-8-OH-DPAT binding, 2.5 for [^{3}H]-5-HT binding, 0.5 for [^{3}H]ketanserin binding, 2.0 for [^{3}H]mesulergine binding, 0.6 for [^{3}H]granisetron binding, and 0.09 for [^{3}H]paroxetine binding.

Measurement of [^{14}C]Guanidinium Uptake in NG 108-15 Cells. This procedure has been described by Emerit et al.[9] Briefly, mouse neuroblastoma × rat glioma hybrid cells of the NG 108-15 clone were grown in Dulbecco's modified Eagle's medium supplemented with the appropriate nutrients[9] 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 (sa 59 mCi/mmol; Service des Molécules Marquées at CEA, 91191 Gif-sur-Yvette, France) and, where indicated, 1 μM 5-HT, 10 μM SP, and/or eight different concentrations of each drug to be tested. After 10 min at 37 °C, the assay was stopped by aspiration of the medium, and

the cell layer was washed three times with 1.5 mL of ice-cold buffer C (same composition as buffer A except that NaCl was replaced by choline chloride). The cells were then dissolved in 0.5 mL of 0.4 M NaOH, and the resulting extracts were transferred to scintillation vials. The culture dishes were further rinsed with 0.5 mL of 1 M HCl and then 0.5 mL of 0.4 M NaOH, which were added to the vials. Each mixture (1.5 mL) was supplemented with 10 mL of the scintillation fluid Aquasol (New England Nuclear, Les Ulis, France) for radioactivity counting at 50% efficiency. All assays were performed in triplicate.

Under these conditions, [^{14}C]guanidinium accumulation in NG 108-15 cells was 4−5 times higher in the presence of both 1 μM 5-HT and 10 μM 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 9 for details).

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