Novel Potent and Selective Central 5-HT$_3$ Receptor Ligands Provided with Different Intrinsic Efficacy. 2. Molecular Basis of the Intrinsic Efficacy of Arylpiperazine Derivatives at the Central 5-HT$_3$ Receptors

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Novel 5-HT$_3$ receptor ligands were designed and synthesized with the aim of obtaining deeper insight into the molecular basis of the intrinsic efficacy of arylpiperazines interacting with the central 5-HT$_3$ receptor. The newly synthesized compounds and some previously published compounds belonging to the same class of heteroarylpiperazines were tested for their potential ability to displace [^{3}H]granisetron from rat cortical membranes. These 5-HT$_3$ receptor binding studies revealed subnanomolar affinity in several of the compounds under study. The most active ligands were quipazine derivatives bearing a phenyl group in the 4-position and various oxygenated alkyl side chains in the 3-position of the quinoline nucleus. Qualitative and theoretical quantitative structure−affinity relationship studies were carried out, and the interaction model for the 5-HT$_3$ ligands related to quipazine with their receptor, proposed in part 1 of the present work, was updated to incorporate the latest data. The potential 5-HT$_3$ agonist/antagonist activity of 12 selected compounds was assessed in vitro on the 5-HT$_3$ receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 cells. Their intrinsic efficacy ranged from the 5-HT$_3$ full agonist properties of compounds **7a** and **8h,i** to those of partial agonists **10a,d** and antagonists **8b,d,e**, and **9c,d,h,i**. The comparison between these functional data and those relative to the previously described compounds suggested that in this class of 5-HT$_3$ ligands the intrinsic efficacy is modulated in a rather subtle manner by the steric features of the heteroaryl moiety.

Introduction

Among the wide variety of serotonin (5-hydroxytryptamine, 5-HT) receptors so far identified, the 5-HT$_3$ receptor subtype is the only one that belongs to the ligand-gated ion channel receptor family.[1] Recently, great interest was developed toward the search for new 5-HT$_3$ receptor antagonists: ondansetron (**1**), granisetron (BRL 43694, **2**), and tropisetron (**3**) (Chart 1) are in use as antiemetic agents associated with anticancer chemotherapy. Furthermore, other possible therapeutic uses in anxiety disorder, Alzheimer's disease, dementia, depression, schizophrenia, psychosis, migraine, drug abuse, and heart arrhythmia are the subjects of intense investigations.[2]

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[3] and that of cholecystokinin from the cortex and nucleus accumbens[4] and inhibits the release of acetylcholine from the entorhinal cortex.[5] However, the potential therapeutic interest of the 5-HT$_3$ agonists still remains to be evaluated because only very recently have potent and selective 5-HT$_3$ agonists became available.

Quipazine (**4a**) was earlier reported to behave as a 5-HT$_3$ antagonist in peripheral models,[6] but subsequent studies revealed its 5-HT$_3$ agonist action in the [^{14}C]-guanidinium accumulation test applied to NG 108-15 cells.[7] It is noteworthy that the 5-HT$_3$ receptor expressed by NG 108-15 cells is very similar to that present in the rat cortex.[8] Thus, quipazine is most probably a 5-HT$_3$ receptor agonist in the CNS. In this respect, we recently reported on a new class of pyrroloquinoxaline derivatives **5** related to quipazine which behaved as 5-HT$_3$ receptor agonists both in NG 108-15 cells and on cortical acetylcholine release in freely moving rats, while they showed partial agonist or antagonist properties in the Bezold−Jarish reflex test.[9]

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Chart 1

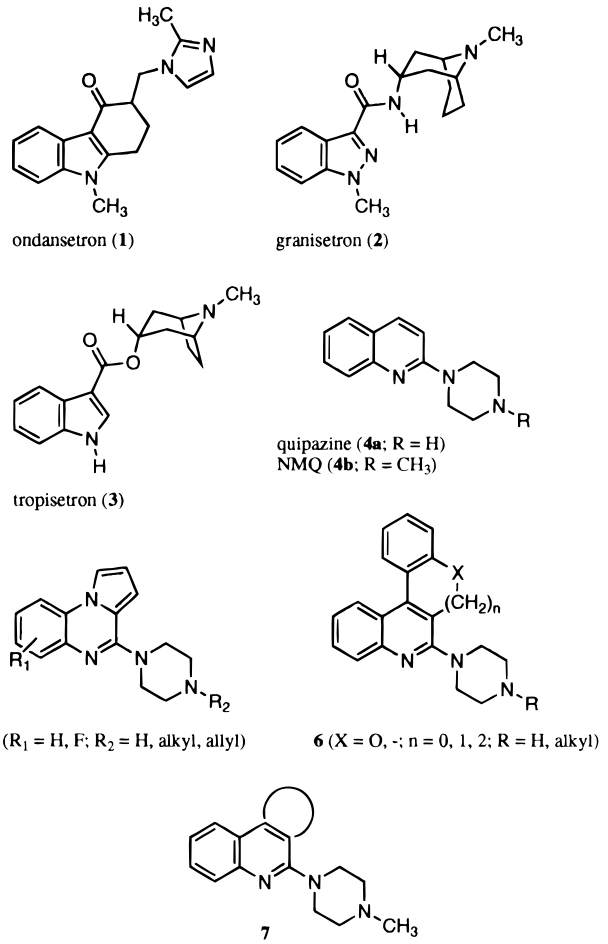
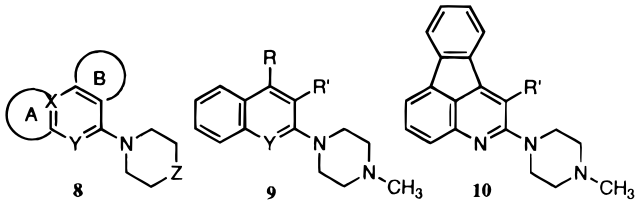


Chart 2



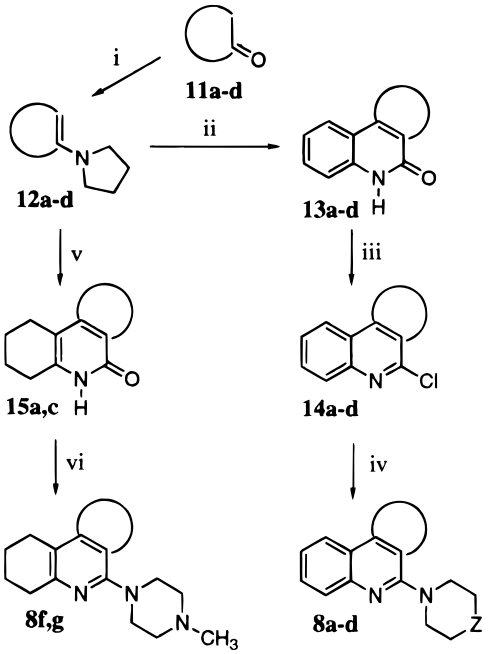
In a previous paper we described the synthesis and the 5-HT$_3$ receptor antagonist properties of a new class of quipazine derivatives **6**,[10] and later we tried to rationalize the differences in the intrinsic efficacy showed by quipazine and antagonists **6**, but some points still remained unclear.[11] Moreover, in part 1 of this work,[12] we described the synthesis and the pharmacological characterization of high-affinity central 5-HT$_3$ receptor ligands **7** related to quipazine provided with different intrinsic efficacy, and we proposed a model for the interaction of these ligands with their receptor.

We thought the fact that the intrinsic efficacy appeared to be modulated by subtle modifications of the substituents at the *c*-edge of the quinoline nucleus of quipazine to be especially intriguing and interesting. Therefore, we have devoted the present work to the understanding of the molecular basis of the intrinsic efficacy of arylpiperazines interacting with the central 5-HT$_3$ receptor.

New 5-HT$_3$ receptor ligands (Chart 2) have been designed starting from the previously described antagonists **6** and have been synthesized and tested for their potential ability to displace [^{3}H]granisetron from the rat cortical membranes. The affinity of some previously published compounds belonging to this class of heteroarylpiperazines was reinvestigated by means of the same test system in order to obtain a more reliable correlation. The intrinsic efficacy of some selected compounds has been assessed in vitro on 5-HT$_3$ receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 cells as a predictive model for the intrinsic efficacy toward the central 5-HT$_3$ receptor.[9]

The results have been quantitatively rationalized by making use of theoretical molecular descriptors. The qualitative and quantitative structure−activity relationships (QSAR) obtained are discussed in term of (a) the structural features responsible for the affinity of the ligands toward the binding site of the central 5-HT$_3$ receptor, on the basis of the interaction model published in part 1 of this work,[12] and (b) the influence of the stereoelectronic parameters of the heteroaryl moiety on receptor activation−deactivation process (intrinsic efficacy toward the central 5-HT$_3$ receptor).

Scheme 1[a]

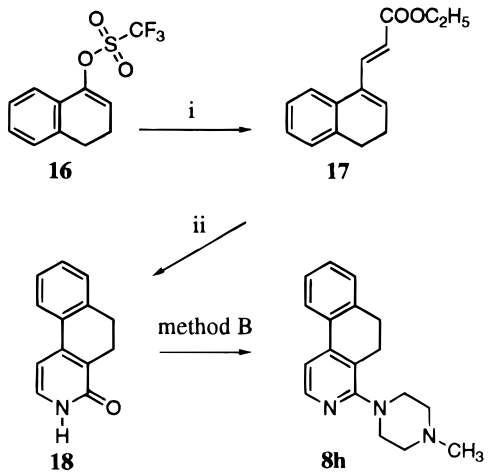


[a] Reagents: (i) pyrrolidine, $C_2H_5OC_2H_5$, $TiCl_4$, C_6H_6; (ii) (a) C_6H_5NCO, $CHCl_3$, (b) PPA; (iii) $POCl_3$; (iv) *N*-methylpiperazine or morpholine (method A); (v) 1-cyclohexene-1-isocyanate, CH_3CN; (vi) (a) $(CF_3SO_2)O$, Na_2CO_3, CH_2Cl_2, (b) *N*-methylpiperazine (method B).

Chemistry

Preparations of target 4-methyl-1-piperazinyl or morpholino derivatives **8**−**10** were carried out starting from the suitable 2(1*H*)-pyridone derivatives via either imidoyl chlorides (method A) or imidoyl triflates (method B) by reaction with the appropriate amine. While imidoyl chlorides were isolated and characterized, imidoyl triflates were reacted with the appropriate amine without further purification. Compounds **8a**−**d**,**f**,**g** were prepared following the procedure summarized in Scheme 1. The required cyclic ketones **11a**−**d** were converted according to the procedure described by White et al.[13]

Scheme 2[a]



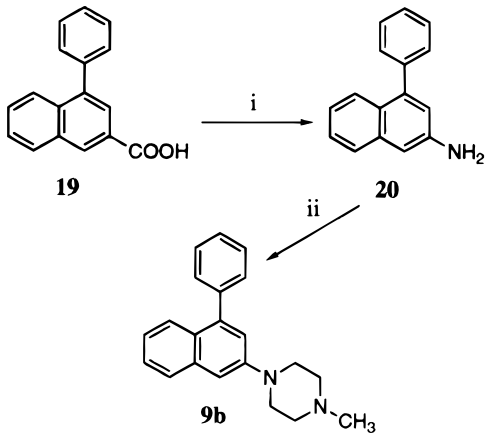
[a] Reagents: (i) $CH_2{=}CH{-}COOC_2H_5$, $(C_2H_5)_3N$, $Pd(PPh_3)_2Cl_2$, DMF; (ii) (a) NaOH, H_2O, C_2H_5OH, (b) $ClCOOCH_2CH(CH_3)_2$, $(C_2H_5)_3N$, NaN_3, CH_3COCH_3, (c) $(CH_3CH_2CH_2CH_2)_3N$, Dowtherm A.

into the corresponding pyrrolidine enamines **12a**−**d** which were reacted with phenyl isocyanate and then cyclized into quinolinones **13a**−**d**. The latter compounds were readily converted into imidoyl chlorides **14a**−**d** by refluxing in phosphorus oxychloride and finally into the target compounds **8a**−**d** by method A. For the synthesis of compound **8d**, commercially available *trans*-1-decalone (**11d**) was used as the starting material, but a diastereomeric mixture (*trans*/*cis* 85:15) of intermediate quinolone **13d** was obtained on the basis of ^{1}H NMR spectroscopy (most probably an isomerization process occurred early in the enamine formation step). Thus, the diastereomeric mixture of **13d** was refluxed in phosphorus oxychloride, and pure *trans*-**14d** was isolated by flash chromatography of its diastereomeric mixture. This compound reacted with *N*-methylpiperazine to give pure *trans*-**8d**, the stereochemistry of which was ascertained by high-field NMR studies (dqfCOSY and NOESY experiments performed at 600 MHz). Further studies concerning the three-dimensional structure of compound **8d** in solution are in progress, and full details will be published elsewhere.

Alternatively, enamines **12a,c** were reacted with 1-cyclohexene-1-isocyanate[14] in acetonitrile to give tetrahydroquinolinones **15a,c** directly which were converted into the corresponding 4-methyl-1-piperazinyl derivatives **8f,g** by method B. The crystal and molecular structure of compound **8g** was determined by single-crystal X-ray diffraction, and the structural data obtained were used as input for molecular modeling studies.

Compounds **8h,i,k** were prepared from the corresponding pyridone derivative available by Curtius rearrangement of the suitable acryloyl azide, followed by a thermal cyclization of the formed isocyanate according to the procedure developed by Eloy et al.[15] The synthesis of **8h** is exemplified in Scheme 2. Thus, vinyl triflate **16**[16] underwent palladium-catalyzed Heck olefination reaction[17] with ethyl acrylate to give α,*β*,*γ*,*δ*-dienoic ester **17**. The latter was saponified, converted into the corresponding α,*β*,*γ*,*δ*-dienoyl azide, and finally heated at 220 °C in Dowtherm A to obtain the cyclized product

Scheme 3[a]



[a] Reagents: (i) (a) DPPA, $(C_2H_5)_3N$, C_6H_6, (b) 50% H_2SO_4; (ii) $(ClCH_2CH_2)_2NCH_3{\cdot}HCl$, K_2CO_3, diglyme.

18 which was converted into target 4-methyl-1-piperazinyl derivative **8h** by method B.

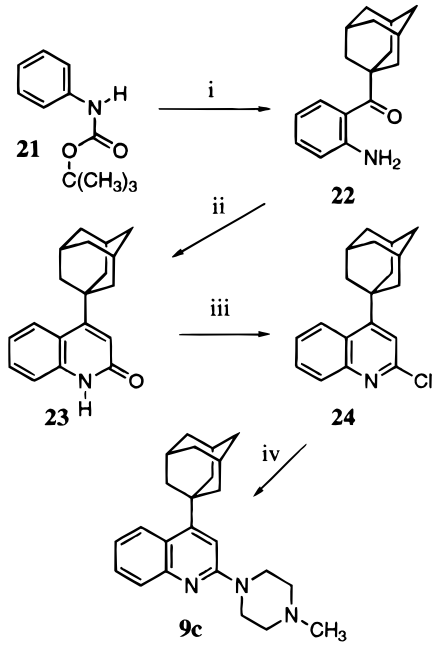
The synthesis of naphthylpiperazine derivative **9b** is shown in Scheme 3. Naphthalenecarboxylic acid **19**[18] reacted with diphenyl phosphorazidate (DPPA), and the resulting acyl azide underwent Curtius rearrangement to give the corresponding isocyanate derivative which was promptly hydrolyzed into naphthylamine **20**. Reaction of the latter with *N*-methylbis(2-chloroethyl)amine (mechlorethamine) hydrochloride in the presence of potassium carbonate in refluxing diglyme gave **9b** in acceptable yield.

Among the quipazine derivatives **9a,c**−**q** reported in Table 2, several compounds were available in our lab as a result of previous studies (see Table 2),[19] while the synthesis of the remaining compounds was carried out as follows. 1-Adamantyl 2-aminophenyl ketone (**22**) was prepared by ortho lithiation of *N*-(*tert*-butoxycarbonyl)-aniline (**21**) with *tert*-butyllithium using the procedure described by Muchowski et al.[20] followed by quenching with methyl 1-adamantanecarboxylate and deprotection. Compound **22** was then acylated with acetic anhydride and cyclized into quinolinone **23**, which was converted into piperazinyl derivative **9c** via imidoyl chloride **24** (Scheme 4).

Moreover, piperazinyl derivative **9q** was prepared by method A from the corresponding imidoyl chloride **26**, which was available from **25**[21] by reaction with phosphorus oxychloride. Lithium aluminum hydride reduction of **9q** gave alcohol **9i** which was transformed into tetrahydrofuranyl derivative **9j** (Scheme 5). Finally, compounds **9f**,**l**,**m** were synthesized in an analogous fashion from alcohol derivatives **9e**,**k**, respectively.

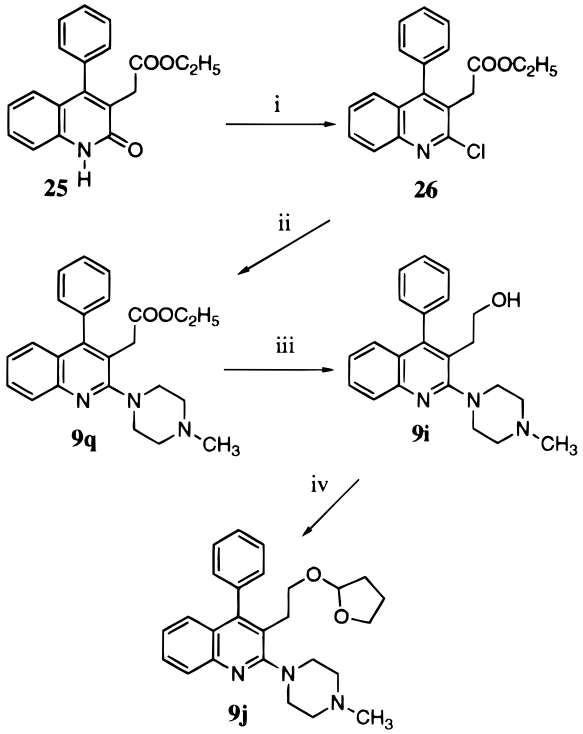
The comparison between the ^{1}H NMR spectra of homologue alcohol derivatives **9e**,**i** revealed interesting differences concerning the OH protons. In fact, in the spectra of higher homologue **9i** the OH proton showed about the same resonance frequency (4.60 ppm) both in $CDCl_3$ and in DMSO-d_6, while in the case of the lower homologue **9e** a signal attributable to its OH proton could be detected only in the spectrum performed in DMSO-d_6. This observation could be explained by assuming that the OH proton of compound **9i** could be engaged in hydrogen bonding (perhaps intramolecular) more stably than that of **9e**. To test this hypothesis the

Scheme 4[a]



[a] Reagents: (i) (a) $(CH_3)_3CLi$, THF, (b) methyl 1-adamantanecarboxylate, THF, (c) HCl, H_2O, C_2H_5OH; (ii) (a) $(CH_3CO)_2O$, (b) CH_3ONa, C_2H_5OH; (iii) $POCl_3$; (iv) *N*-methylpiperazine.

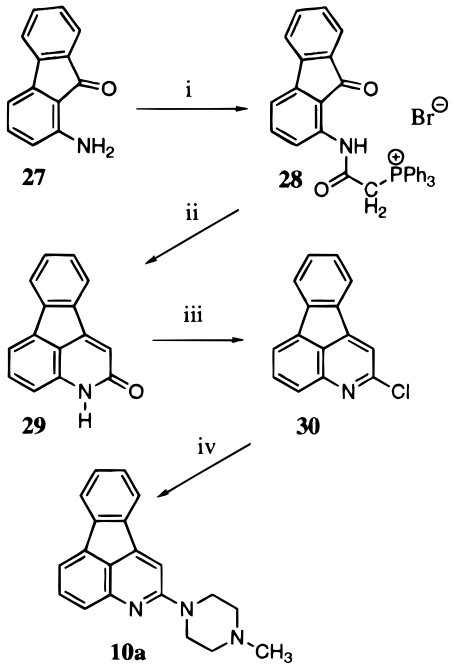
Scheme 5[a]



[a] Reagents: (i) $POCl_3$; (ii) *N*-methylpiperazine; (iii) $LiAlH_4$, THF; (iv) 2,3-dihydrofuran, PTSA, CH_2Cl_2.

crystal and molecular structures of these two homologue alcohol derivatives were determined by single-crystal X-ray diffraction, and in both cases only intermolecular hydrogen bonding was found. However, it is possible for a competition between the engagement of intramolecular and intermolecular hydrogen bonding to exist in the case of compound **9i**, and the intermolecular hydrogen bonding could be preferred in the solid state, while the intramolecular one could be preferred in solution in

Scheme 6[a]



[a] Reagents: (i) (a) $BrCH_2COBr$, CH_2Cl_2, (b) PPh_3, C_6H_6; (ii) NaH, DMF; (iii) $POCl_3$; (iv) *N*-methylpiperazine.

apolar solvents such as $CDCl_3$. The molecular structures of compounds **9e**,**i** as determined by single-crystal X-ray diffraction were used as input for molecular modeling studies.

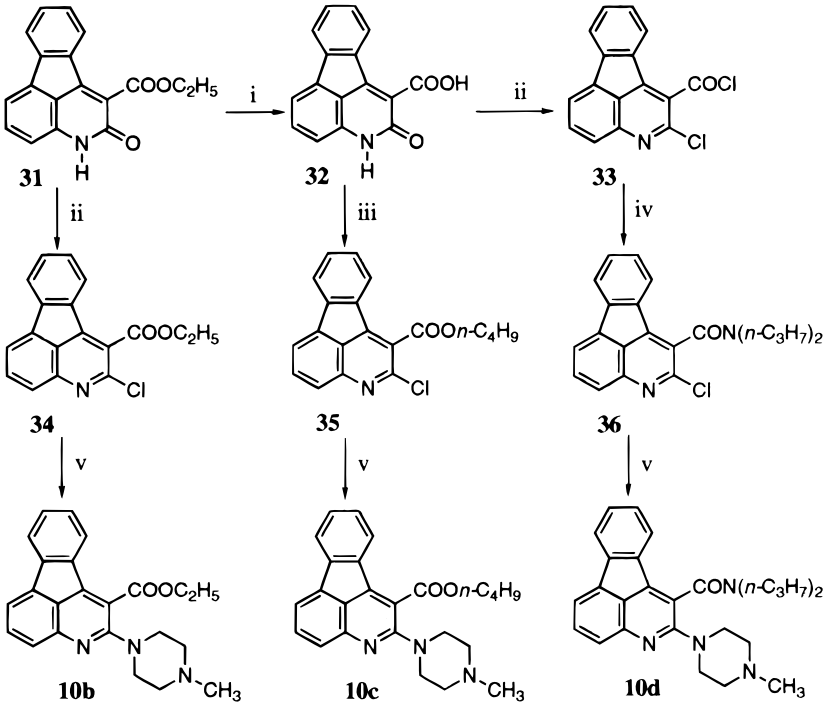
Simple piperazinylindenoquinoline derivative **10a** was obtained via imidoyl chloride **30** from indenoquinolinone **29** (method A), as shown in Scheme 6. Though the synthesis of the latter compound was earlier described to involve decarboxylation of carboxylic acid **32**[22] (see Scheme 7), we found that this compound could be obtained in a more direct manner by intramolecular Wittig reaction of phosphonium salt **28** available from **27** by bromoacetylation followed by reaction of the formed bromoacetamide with triphenylphosphine. Finally, substituted piperazinylindenoquinoline derivatives **10b**−**d** were prepared by standard procedures from ester **31**[22] and acid **32**[22] (Scheme 7).

Computational Methods

Geometry Optimization. Conformational analysis of the N4-protonated form of some simple arylpiperazine derivatives was recently performed,[12] and the resulting AM1[23] absolute minimum structure of quipazine was considered in this study as the starting geometry. The substituents were assembled within the Chemnote module of the program Quanta96.[24] Substituents showing flexible chains were considered in their extended conformations. The three-dimensional structure of ligands **8g** and **9e**,**i** was solved by X-ray crystallography in our laboratory. The protonated structures of the compounds studied were fully optimized by means of molecular orbital calculations (AM1), by means of MOPAC 6.0 (QCPE 455) program.

Molecular Superimposition. Compounds **9e**,**i**−**l**,**n**,**q**, and **10a**, the most structurally different ligands showing high affinity for the 5-HT$_3$ receptor, were chosen for the construction of the reference supermol-

Scheme 7[a]



[a] Reagents: (i) NaOH, H_2O, C_2H_5OH; (ii) $POCl_3$; (iii) *n*-C_4H_9OH, $POCl_3$; (iv) (*n*-$C_3H_7)_2NH$, CH_2Cl_2; (v) *N*-methylpiperazine.

ecule and were superimposed, by a rigid fit procedure, on their common piperazine nucleus. Therefore, the volume of the supermolecule reflects all the original contributions of the three-dimensional shape features permitted by the receptor.

The other compounds were superimposed on the analogous ligand present in the supermolecule or on its structurally closest compound by using the same matching procedure. All the compounds were considered in their protonated form and energy minimum conformation.

The size and shape descriptor V_{in} is the intersection van der Waals volume of the ligands considered with respect to the resultant van der Waals volume of the reference supermolecule. The Quanta96 molecular modeling software was used for molecular comparisons, matching, and computation of van der Waals volumes.

Statistical Treatment of Data. The MOPAC output files were loaded into the CODESSA program[25] along with the experimental binding affinities, and a large number of global as well as fragment descriptors (constitutional, topological, electrostatic, geometrical, and quantum−chemical) were generated for each compound. The molecular size and shape parameters were added as external descriptors.[26]

The search for the best correlation equation was achieved by means of the heuristic method implemented in the CODESSA program.[27] It accomplishes a preselection of descriptors on the basis of their statistical significance. Default values for control parameters and criteria were used:[28] 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 one-parameter correlation, $t_1 = 1.5$; *t*-test value for the descriptor to be considered significant in multiparameter correlation, $t_2 = 3.0$; highest pair correlation coefficient of two descriptor scales, $r_{full} = 0.99$; significant intercorrelation level, $r_{sig} = 0.80$. As a final result, the program lists the 10 correlations found with the highest squared correlation coefficient and the 10 correlations found with the highest *F*-test values. The quantitative structure−affinity relationship models reported in this paper were selected on the basis of the best statistical parameters and the largest diversity in the physical information content of the descriptors involved.

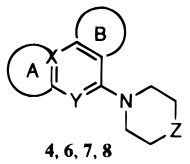
Results

Qualitative Structure−Affinity Relationships. The newly synthesized compounds and some previously published compounds belonging to this class of heteroarylpiperazines were tested for their potential ability to displace [^{3}H]granisetron specifically bound to 5-HT_3 receptor in rat cortical membrane, in comparison with reference compounds quipazine (**2a**) and granisetron, following well-established protocols.[29] The results of the binding studies along with the binding values reported in the literature[30] for *N*-methylquipazine (NMQ, **4b**) (which are included for comparison) are summarized in Tables 1 and 2.

To obtain information on the possible role of the different groups of the molecule of the previously described antagonist **6a**, its structure was modified by using strategies similar to that described in part 1 of the present work.[12] Thus, the deletion of the fused benzene ring of the quinoline nucleus of compounds **6a,b** seems to have little effect (compare **6a** with **8h** and **6b** with **8i**), while its saturation in compounds **6a** and **8c** produces a dramatic decrease in the 5-HT_3 receptor binding affinity (compare **6a** with **8f** and **8c** with **8g**).

It is interesting to note that in the small subseries of compounds without the quinoline benzo ring the shifting of the pyridine nitrogen atom produces a decrease in the affinity of about 1 order of magnitude (compare

Table 1. Effects of Modification of the Heteroaryl Moiety of Quipazine on 5-HT$_3$ Receptor Affinity and Intrinsic Efficacy: Binding Affinities for the Central 5-HT$_3$ Receptor and Effects on [^{14}C]Guanidinium Accumulation in NG 108-15 Cells of Compounds **4** and **6**–**8**



4, 6, 7, 8

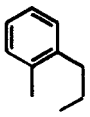
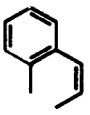
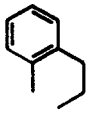
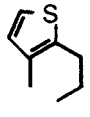
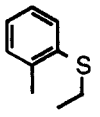
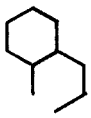
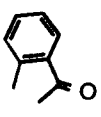
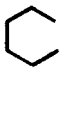
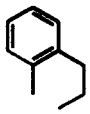
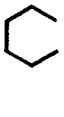
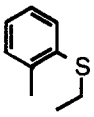
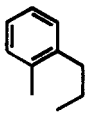
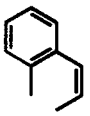
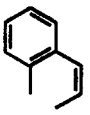
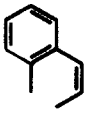
compd	A	B	X	Y	Z	K_i (nM) ±SEM[a]	Intrinsic Efficacy[b]	EC_{50} or IC_{50}[c] (nM)
4a			C	N	N-H	1.8 ± 0.3	A[d]	25[d]
4b			C	N	N-CH$_3$	3[e]		
6a			C	N	N-CH$_3$	4.2 ± 0.7	Ant[f]	4.6[f]
6b			C	N	N-CH$_3$	46 ± 12		
7a			C	N	N-CH$_3$	1.9[g]	A	10
7b			C	N	N-CH$_3$	0.23[g]	PA[g]	0.25[g]
7c			C	N	N-CH$_3$	0.83[g]	Ant[g]	8[g]
8a			C	N	O	> 5300		
8b			C	N	N-CH$_3$	5.7 ± 0.7	Ant	62
8c			C	N	N-CH$_3$	19 ± 5.4		
8d			C	N	N-CH$_3$	29 ± 5.2	Ant	250
8e[h]			C	N	N-CH$_3$	20 ± 3.1	Ant	139

Table 1 (Continued)

compd	A	B	X	Y	Z	K_i (nM) ±SEM[a]	Intrinsic Efficacy[b]	EC_{50} or IC_{50}[c] (nM)
8f			C	N	N-CH_3	2300 ± 230		
8g			C	N	N-CH_3	2700 ± 250		
8h			CH	N	N-CH_3	16 ± 2.4	A	7
8i			CH	N	N-CH_3	29 ± 5.7	A	30
8j			N	CH	N-CH_3	490 ± 110		
8k			CH	N	O	4900 ± 500		
granisetron						0.35 ± 0.06	Ant	2.8
5-HT						120 ± 34	A	298

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximum inhibition of [^{3}H]granisetron specific binding to rat cortical membranes. [b] A, pure agonist; PA, partial agonist; Ant, pure antagonist. [c] The quantitative effects of the compounds on the [^{14}C]guanidinium accumulation in NG 108-15 cells were expressed as EC_{50} values in the case of both pure and partial agonists and as IC_{50} values in the case of pure antagonists. Compounds were tested in triplicate for each concentration. [d] See ref 7. [e] See ref 30. [f] See ref 10. [g] See ref 12. [h] See ref 31.

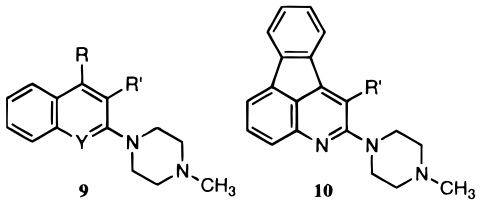
compound **8i** with **8j**), and the replacement of the terminal protonable piperazine nitrogen with an oxygen atom as in compound **8k** leads to a dramatic decrease in the affinity (compare **8i** with **8k**). The latter structure−affinity relationship (SAFIR) trend was observed also in the previously reported antagonist **6a** (compare **6a** with **8a**) and partial agonist **7b** (see part 1 of the present work).[12]

Finally, the modification of the phenyl group in the 4-position of the quinoline nucleus of **6a** reveals the full bioisosterism between the phenyl group and the thienyl group of compound **8b** and the importance of the aromaticity in the interaction with the receptor (compare **6a** with **8d**).

On the other hand, the binding studies performed on the previously published compounds allow some comparisons to be made. The new apparent affinity of compound **6a** is very similar to the previously reported one, while the new K_i values of compounds **6b** and **9a** significantly differ from those previously described.[10] In particular, the apparent affinity of compound **9a** was found to be 6.3-fold higher than that reported earlier and very similar to that reported for NMQ. This finding prompted us to reconsider some earlier observations[10,11,31] and stimulated the design of new derivatives closely related to compound **9a**. It was in this subseries of compounds that the most interesting results were obtained. In fact, it was found that a large variety of substituents was tolerated in the 3-position of the quinoline nucleus of compound **9a**, and these substituents appeared to be capable of operating a fine-tuning of the 5-HT_3 receptor affinity.

Thus, the introduction of a methyl group, a hydroxymethyl group, or a hydroxyethyl group in the 3-position of the quinoline nucleus of compound **9a** (leading to compounds **9d**,**e**,**i**, respectively) increases the

Table 2. Effects of Modification of the Heteroaryl Moiety of Quipazine Derivatives on 5-HT$_3$ Receptor Affinity and Intrinsic Efficacy: Binding Affinities for the Central 5-HT$_3$ Receptor and Effects on [^{14}C]Guanidinium Accumulation in NG 108-15 Cells of Compounds **9** and **10**



compd	Y	R	R′	K_i (nM) ± SEM[a]	intrinsic efficacy[b]	EC_{50} or IC_{50}[c] (nM)
9a[d]	N	C_6H_5	H	5.4 ± 0.3		
9b	CH	C_6H_5	H	1100 ± 110		
9c	N	1-adamantyl	H	80 ± 12	Ant	80
9d	N	C_6H_5	CH_3	2.4 ± 0.3	Ant	67
9e[d]	N	C_6H_5	CH_2OH	0.98 ± 0.07		
9f	N	C_6H_5	CH_2OTHF[e]	3.6 ± 0.2		
9g[d]	N	C_6H_5	CH_2O*n*-C_4H_9	29 ± 4.2		
9h[d]	N	C_6H_5	$CH_2OCOC_6H_5$	8.8 ± 1.3	Ant	9
9i	N	C_6H_5	CH_2CH_2OH	0.4 ± 0.03	Ant	10
9j	N	C_6H_5	CH_2CH_2OTHF[e]	0.8 ± 0.04		
9k[f]	N	2-F-C_6H_4	CH_2CH_2OH	0.6 ± 0.03		
9l	N	2-F-C_6H_4	CH_2CH_2OTHF[e]	0.7 ± 0.1		
9m	N	2-F-C_6H_4	CH_2CH_2OTHP[g]	1.9 ± 0.04		
9n[f]	N	2-OH-C_6H_4	$CH{=}CH_2$	0.9 ± 0.03		
9o[d]	N	C_6H_5	$COOC_2H_5$	4.1 ± 1.3		
9p[d]	N	C_6H_5	COO*n*-C_4H_9	12 ± 0.2		
9q	N	C_6H_5	$CH_2COOC_2H_5$	0.6 ± 0.04		
10a			H	1.8 ± 0.2	PA	12
10b			$COOC_2H_5$	3.9 ± 0.04		
10c			COO*n*-C_4H_9	78 ± 4.4		
10d			$CON(n\text{-}C_3H_7)_2$	3.0 ± 0.2	PA	≈300
granisetron				0.35 ± 0.06		
5-HT				120 ± 34		

[a] Each value is the mean ± SEM of three determinations and represents the concentration giving half-maximum inhibition of [^{3}H]granisetron specific binding to rat cortical membranes. [b] A, pure agonist; PA, partial agonist; Ant, pure antagonist. [c] The quantitative effects of the compounds on the [^{14}C]guanidinium accumulation in NG 108-15 cells expressed as EC_{50} values in the case of both pure and partial agonists and as IC_{50} values in the case of pure antagonists. Compounds were tested in triplicate for each concentration. [d] See ref 19a. [e] THF, 2-tetrahydrofuranyl. [f] See ref 19b. [g] THP, 2-tetrahydropyranyl.

affinity to a variable extent. In fact, methyl derivative **9d** shows an affinity slightly higher than that shown by **9a**, while hydroxymethyl derivative **9e** is more potent than **9d**, and hydroxyethyl derivative **9i** showing subnanomolar affinity is about 1 order of magnitude more potent than **9a**.

Additional steric hindrance appears to be tolerated depending on the nature of the group involved. Linear alkyl groups such as *n*-butyl in compound **9g** are less well-tolerated than cyclic substituents such as those present in compounds **9h**,**f**,**j**,**l**,**m**. It is noteworthy that the replacement of the hydroxyl hydrogen atoms of compounds **9e**,**i**,**k** with the bulkier tetrahydrofuranyl group to give **9f,j,l** produces negligible change in the affinity. Similarly, the presence of the fluorine atom in the 2-position of the phenyl group in the 4-position of the quinoline nucleus has negligible effects (compare **9i** with **9k** and **9j** with **9l**).

The introduction of a carboxyethyl group in **9a** leading to compound **9o** has negligible effect on the 5-HT$_3$ receptor affinity, while the corresponding acetic ester derivative **9q** is about 1 order of magnitude more potent than **9a**. The elongation of the ethyl ester side chain of compound **9o** to *n*-butyl as in compound **9p** leads to a decrease in the affinity similar to that observed in ether **9g**. This SAFIR trend is paralleled also in the conformationally constrained compounds **10b**,**c**. The SAFIR analysis in this subseries of compounds reveals the bioisosterism between the ethyl ester group of compound **10b** and the *N*,*N*-dipropylcarboxamido group of **10d**.

The replacement of the phenyl group of compound **9a** with a 1-adamantyl group as in compound **9c** leads to a 15-fold decrease in the affinity, while the replacement of its quinoline nitrogen atom with a sp^2 carbon atom as in compound **9b** appears to be detrimental.

Quantitative Structure−Affinity Relationship Analysis. A quantitative rationalization of this SAFIR study can be obtained by means of theoretical molecular descriptors. The molecular determinants which better rationalize the variation in the 5-HT$_3$ binding affinity data values are reported in Table 3, together with the cologarithmic form of the experimental data values of the ligands considered. A quantitative overview of the collinearities existing between the theoretical descriptors of Table 3 is shown in Table 4, where the respective intercorrelation coefficients are given.

More than 50% variation in the 5-HT$_3$ binding affinity is captured by the average information content molecular descriptor (AIC(1)),[32] as shown by the following equation:

$$pK_i = -5.14(\pm 2.14) + 3.75(\pm 0.61)\mathrm{AIC}(1) \quad (1)$$

$$R^2 = 0.559;\ n = 32;\ F = 37.98;\ s^2 = 0.493;\ R^2_{cv} = 0.501$$

where R is the correlation coefficient, n is the number of compounds, F is the value of the Fisher ratio, s is the standard deviation, and R_{cv} is the cross-validated correlation coefficient; the numbers in parentheses are

Table 3. 5-HT_3 Binding Affinities and Theoretical Descriptors of the Protonated Form of the Ligands Reported in Tables 1 and 2

compd	pK_i	V_{in}/V_{mol}	AIC(1)	ABIC(1)	f_a−FNSA-1	$\Sigma f_{a'}-S^H$ (eV^{-1})	$\Delta E_{HOMO-LUMO}$ (eV^{-1})	$P(N)$	$P\sigma-\pi(N-H)^+$
6a	8.38	0.9467	3.5616	0.6218	0.461	0.022	7.0732	1.2313	0.000076
6b	7.34	0.9358	3.4793	0.6134	0.5022	0.0125	6.963	1.2118	0.000059
8b	8.24	0.9571	3.6515	0.647	0.4669	0.018	6.9197	1.2325	0.000061
8c	7.72	0.9561	3.68	0.6488	0.5057	0.0	6.5274	1.2298	0.000071
8d	7.54	0.9375	3.1322	0.5324	0.5057	0.046	7.3523	1.2284	0.000068
8e	8.52	0.9263	3.6684	0.6534	0.4594	0.029	7.6263	1.235	0.000059
8f	5.64	0.9047	3.1611	0.5419	0.7142	0.0015	7.3036	1.355	0.000086
8g	5.57	0.9072	3.354	0.5801	0.7872	0.0	6.3321	1.3481	0.000089
8h	7.8	0.9455	3.3771	0.6114	0.8893	0.0	7.3342	1.3562	0.000038
8i	7.54	0.9526	3.5026	0.6416	0.8867	0.0	7.1295	1.3283	0.000071
8j	6.3	0.9406	3.3194	0.608	0.8422	0.0	6.847	1.2732	0.000050
9a	8.27	0.9652	3.4722	0.6217	0.5867	0.0355	7.3404	1.24	0.000046
9b	5.96	0.9402	2.8679	0.5108	0.9474	0.034	6.9641		0.000039
9c	7.1	0.8877	3.1354	0.5226	0.644	0.054	7.4769	1.2396	0.000066
9d	8.62	0.997	3.4716	0.612	0.5254	0.049	7.4005	1.2382	0.000028
9e	9	1	3.6505	0.6404	0.5708	0.048	7.6242	1.2328	0.000045
9f	8.4	0.9834	3.5973	0.5995	0.487	0.048	7.7154	1.2331	0.000049
9g	7.54	0.9032	3.5005	0.5834	0.436	0.05	7.5415	1.2376	0.000804
9h	8.05	0.9066	3.5598	0.5911	0.5347	0.052	7.7826	1.2363	0.000813
9i	9.4	1	3.6477	0.6309	0.4675	0.046	7.53	1.2377	0.000033
9j	9.01	1	3.5607	0.587	0.4443	0.051	7.4378	1.2378	0.000037
9k	9.22	1	3.8042	0.658	0.4194	0.04	7.5707	1.2384	0.000027
9l	9.15	1	3.7329	0.6222	0.5514	0.053	7.7654	1.2338	0.000053
9m	8.72	0.9385	3.6384	0.5936	0.486	0.045	7.4611	1.2384	0.000167
9n	9.08	1	3.7617	0.6567	0.4848	0.011	7.3374	1.2362	0.000054
9o	8.39	0.9783	3.6412	0.6243	0.5274	0.046	7.6969	1.2344	0.000840
9p	7.92	0.927	3.5973	0.6018	0.5784	0.046	7.6763	1.2348	0.000849
9q	9.22	1	3.6715	0.6216	0.4897	0.054	7.6649	1.2343	0.000043
10a	8.74	1	3.4829	0.627	0.6014	0.032	7.1143	1.2416	0.000059
10b	8.41	0.9674	3.6396	0.6267	0.4779	0.029	7.2873	1.2353	0.000060
10c	7.1	0.902	3.589	0.6028	0.5303	0.03	7.2827	1.2349	0.000061
10d	8.52	0.922	3.3876	0.5546	0.4365	0.031	7.2984	1.2372	0.000051

the 95% confidence intervals of the regression coefficient and the intercept. The average information content, defined on the basis of Shannon's information theory,[32] encodes the main structural characteristics of the ligands and explains the portion of the biological response elicited by nonspecific interactions (dispersion forces).

The branching ratio and the constitutional diversity of the different substituents introduced in the 3-position of the quinoline nucleus are very well-explained by this index when the subset of 17 more congeneric compounds (**9a**−**q**) is considered:

$$pK_i = -4.08(\pm 1.56) + 3.52(\pm 0.48)\mathrm{AIC}(1) \quad (2)$$

$$R^2 = 0.811;\ n = 17;\ F = 64.56;\ s^2 = 0.416;\ R^2_{cv} = 0.782$$

Moreover, the inclusion of the molecular orbital index $\sigma-\pi$ bond order for the $(N-H)^+$ pair[33] in this correlation leads to:

$$pK_i = -4.22(\pm 0.98) + 3.62(\pm 0.27)\mathrm{AIC}(1) - 918.8(\pm 187.0)P\sigma-\pi\,(N-H)^+ \quad (3)$$

$$R^2 = 0.931;\ n = 17;\ F = 94.12;\ s^2 = 0.06;\ R^2_{cv} = 0.898$$

Therefore the propensity of the protonated nitrogen atom to act as a proton donor toward a nucleophilic site of the receptor is explicitly considered in this correlation.

Interestingly, the role of specific interactions ($\pi-\pi$ or π charge) between the fused benzene ring of the heteroaryl moiety and the suitable amino acids of the receptor, combined with the averaged bonding information content (ABIC(1)) index,[32] furnishes a very good QSAR model explaining 75% of the variation in the 5-HT_3 binding affinity of the 32 ligands considered:

$$pK_i = -4.57(\pm 1.55) + 18.94(\pm 2.49)\mathrm{ABIC}(1) + 34.97(\pm 5.01)\Sigma f_{a'}-S^H \quad (4)$$

$$R^2 = 0.757;\ n = 32;\ F = 45.23;\ s^2 = 0.281;\ R^2_{cv} = 0.705$$

where $\Sigma f_{a'}-S^H$ is the sum of the electrophilic superdelocalizability[33] on the atoms of either the benzene ring of the quinoline nucleus or the saturated ring in the analogous position.

Omission of compound **10d** significantly improves the equation stability, as underlined by the cross-validated correlation coefficient:

$$pK_i = -5.79(\pm 1.36) + 20.82(\pm 2.18)\mathrm{ABIC}(1) + 35.74(\pm 4.26)\Sigma f_{a'}-S^H \quad (5)$$

$$R^2 = 0.83;\ n = 31;\ F = 68.31;\ s^2 = 0.202;\ R^2_{cv} = 0.793;\ \text{omitted } \mathbf{10d}$$

Other significant QSAR models are obtained by means of the molecular descriptor V_{in}/V_{mol}:

$$pK_i = -12.47(3.44) + 21.47(\pm 3.60)V_{in}/V_{mol} \quad (6)$$

$$R^2 = 0.542;\ n = 32;\ F = 35.57;\ s^2 = 0.511;\ R^2_{cv} = 0.482$$

Table 4. Correlation Matrix of the Experimental Binding Affinities and Molecular Descriptors of Table 3

	pK_i	V_{in}/V_{mol}	AIC(1)	ABIC(1)	f_a−FNSA-1	$\sum f_{a'}-S^H$ (eV^{-1})	$\Delta E_{HOMO-LUMO}$ (eV^{-1})	P(N)	$P\sigma-\pi(N-H)^+$
pK_i	1.00								
V_{in}/V_{mol}	0.73	1.00							
AIC(1)	0.75	0.52	1.00						
ABIC(1)	0.59	0.57	0.86	1.00					
f_a−FNSA-1	−0.67	−0.26	−0.63	−0.32	1.00				
$\sum f_{a'}-S^H$	0.52	0.24	0.16	−0.18	−0.50	1.00			
$\Delta E_{HOMO-LUMO}$	0.58	0.24	0.32	0.05	−0.40	0.76	1.00		
P(N)	−0.60	−0.29	−0.45	−0.20	0.83	−0.63	−0.35	1.00	
$P\sigma-\pi(N-H)^+$	−0.03	−0.30	0.12	−0.07	−0.13	0.32	0.37	−0.13	1.00

This index describes the size and shape similarity with respect to a reference supermolecule.[12,26,34] It is assumed that the volume obtained by superimposing the most structurally different ligands which show the highest affinities might reflect the overall shape and the conformational freedom of the 5-HT$_3$ receptor binding site. The sign of the regression coefficient in the equation indicates that the higher the molecular volume shared by a generic ligand (V_{in}) and the supermolecule the higher the binding affinity. Thus, in this series of compounds the binding affinity is modulated by the molecular shape of the heteroaryl moiety through the optimization of dispersive interactions.

According to the rationale behind these molecular descriptors, which have been devised to account for the strict complementary requirements toward receptor binding, a significant improvement in the statistical parameters is obtained by considering only the ligands which show high affinity (pK_i = 7 ÷ 9.4) toward the 5-HT$_3$ receptor:

$$pK_i = -6.47(\pm 2.09) + 15.42(\pm 2.18)V_{in}/V_{mol} \quad (7)$$

$R^2 = 0.658$; $n = 28$; $F = 49.95$; $s^2 = 0.160$; $R^2_{cv} = 0.608$; **8d**,**f**,**j**, and **9b** omitted

The addition of the valency-related bond order descriptor for the nitrogen atom of the quinoline ring (P(N))[33] as a second parameter improves the predictivity of the model for the whole set of compounds carrying a nitrogen atom in position 1 of the quinoline ring:

$$pK_i = 3.97(\pm 4.26) + 17.14(\pm 2.49)V_{in}/V_{mol} - 9.77(\pm 2.32)P(N) \quad (8)$$

$R^2 = 0.771$; $n = 30$; $F = 45.44$; $s^2 = 0.219$; $R^2_{cv} = 0.683$; **8j** and **9b** omitted

This index renders the model fit to describe the propensity of the quinoline nitrogen to give hydrogen-bonding interactions with the receptor, as shown by the negative value of the regression coefficient.

The involvement of the fractional negative partial surface area[35] (f_a −FNSA-1) of the pyridine ring of the heteroayl moiety in the following equation leads to a QSAR model with analogous statistical parameters:

$$pK_i = -6.45(\pm 2.66) + 17.30(\pm 2.65)V_{in}/V_{mol} - 3.59(\pm 0.43)f_a-FNSA\text{-}1 \quad (9)$$

$R^2 = 0.779$; $n = 32$; $F = 51.27$; $s^2 = 0.255$; $R^2_{cv} = 0.714$

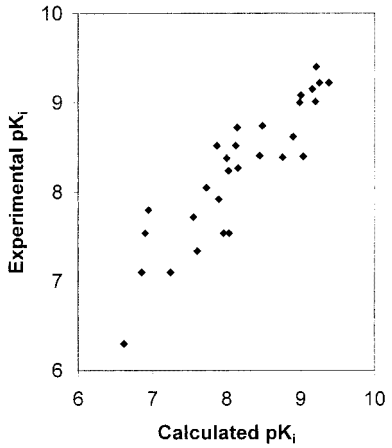


Figure 1. Calculated versus experimental 5-HT$_3$ binding affinities according to eq 10.

f_a−FNSA-1 is calculated according to the following formula: PNSA-1/TFSA, where PNSA-1 is the partial negative surface area of the pyridine ring and TFSA is the total fragment surface area of the pyridine ring. This index may be thought to rationalize the electrostatic component of the binding adjustments which modulates the binding affinity after the main docking has been accomplished and which helps to overcome the main drawback of the V_{in}/V_{mol} descriptor which considers equally active all the ligands whose V_{in} coincides with V_{mol}, irrespective of their size.[34]

Finally, the predictivity of the model is increased by considering as third parameter, the energy difference between the lowest unoccupied (LUMO) and the highest occupied (HOMO) molecular orbitals ($\Delta E_{HOMO-LUMO}$):[33]

$$pK_i = -11.59(\pm 2.79) + 16.02(\pm 2.33)V_{in}/V_{mol} - 2.91(\pm 0.59)f_a-FNSA\text{-}1 + 0.81(\pm 0.25)\Delta E_{HOMO-LUMO} \quad (10)$$

$R^2 = 0.840$; $n = 32$; $F = 49.07$; $s^2 = 0.191$; $R^2_{cv} = 0.784$

This molecular descriptor, being related to the polarizability of the molecules, expresses the potential ability to establish attractive short-range interactions with the receptor. The comparison between the experimental binding affinities and those calculated by eq 10 is shown in Figure 1. The latter equation can be considered, on the basis of its statistical indices and predictive power, the best QSAR equation to describe the variation in the 5-HT$_3$ binding affinity in the whole set of ligands considered.

Selectivity. Three compounds, representative of the various structural subclasses, were tested for their

Table 5. 5-HT Receptor Binding Profile of Compounds **8b**, **9h, and 10a** (K_i (nM) ±SEM)[a]

compd	5-HT_3	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2C}	5-$HT_{transporter}$
8b	5.7 ± 0.7	12000 ± 1200 (2100)	1500 ± 510 (260)	3100 ± 31 (540)	420000 ± 51000 (74000)	4600 ± 1300 (810)
9h	8.8 ± 1.3	4100 ± 1200 (470)	7600 ± 1200 (860)	>10000 (>1100)	31 ± 2.1 (3.5)	1100 ± 340 (130)
10a	1.8 ± 0.2	24000 ± 5100 (13000)	640 ± 82 (360)	13000 ± 3400 (7200)	>10000 (>5600)	1600 ± 370 (890)
4a (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 ref 46.

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 to the 5-HT transporter in comparison with reference compounds by means of well-established protocols.[36] From the results of these binding studies, summarized in Table 5, it appeared that compounds **10a** and **8b** are very selective, indeed showing selectivity ratios higher than those of quipazine, while the binding profile of **9h** appears to be characterized by the residual affinity for 5-HT_{2C} receptor subtype, so that the latter compound can be considered a mixed 5-$HT_{3/2C}$ receptor ligand.

Functional Activity. The measurements of [^{14}C]-guanidinium uptake in the presence of substance P (SP) appears to be a rapid and reliable method to assess the functional properties of 5-HT_3 agents in NG 108-15 hybridoma cells.[7] Owing to the similarities existing between 5-HT_3 receptors present in NG 108-15 cells and those of rat cortex,[8] relevant inferences can be drawn from this pharmacological model about the functional properties of the ligands of the central 5-HT_3 receptor.[9] Accordingly, the potential 5-HT_3 agonist/antagonist activity of some selected compounds was assessed in vitro on the 5-HT_3 receptor-dependent [^{14}C]guanidinium uptake in NG 108-15 cells. The results of these functional studies are summarized in Tables 1 and 2. The previously reported intrinsic efficacy data of compounds **6a** and **7b,c** and new data for the previously described compounds **7a** and **8e** are included for comparative purposes.

In the presence of substance P (10 μM) compounds **7a** and **8h,i** increased in a concentration-dependent manner the uptake of [^{14}C]guanidinium into NG 108-15 cells with EC_{50} values of about 10, 7, and 30 nM, respectively, showing themselves to act as 5-HT_3 agonists. The maximum uptake increase was similar to that of 5-HT and was antagonized by ondansetron (**1**). Moreover, these compounds were unable to inhibit the effects of 5-HT (1 μM).

On the other hand, compound **10a** appeared to act as a partial agonist with an EC_{50} value of about 12 nM since it stimulated [^{14}C]guanidinium uptake in the presence of substance P (10 μM), up to a level equal to about two-thirds of that reached with 5-HT (1 μM) plus substance P (10 μM), while in the presence of the latter two agents **10a** produced a decrease in the accumulation of [^{14}C]guanidinium. Similarly, the closely related compound **10d** behaved as a partial agonist, but it displayed a potency and intrinsic efficacy lower than that of **10a**.

Finally, compounds **8b**,**d**,**e** and **9c**,**d**,**h**,**i** showed antagonist properties with IC_{50} values ranging from 9 to 250 nM.

Discussion

Arylpiperazine Interactions at the Central 5-HT_3 Receptor. In the already published part 1 of this work,[12] a three-component model for the interaction of central 5-HT_3 ligands related to quipazine with their receptor was proposed, which involves (1) a charge-assisted hydrogen bond between the protonated terminal piperazine nitrogen atom and a negatively charged carboxylic amino acid residue in the receptor, (2) a hydrogen-bonding interaction between the heterocyclic nitrogen atom and a suitable H-bond donor in the receptor, and (3) a specific interaction between an aromatic ring and a suitable amino acid residue in the receptor.

In the development of a comprehensive model for the interaction of central 5-HT_3 receptor ligands related to quipazine showing different intrinsic efficacy with their receptor, it is of central importance to ascertain the existence of a common mode of binding of these arylpiperazines to their receptor. This task has been accomplished through the comparative analysis of the SAFIR trends involving the key pharmacophoric points of the different subseries of compounds belonging to this class of heteroarylpiperazines,[37] as well as through the derivation of predictive and interpretative QSAR models.[12,38]

For example, in the case of our heteroarylpiperazine antagonists, the differences in the affinity observed between **6a** and **8a** and the one shown by the couple of closely related compounds **9a**,**b** support the key role played by the terminal piperazine protonatable nitrogen (first pharmacophoric point) and that of the quinoline nitrogen atom (second pharmacophoric point), respectively. Moreover, it is worth noting that the quinoline benzo ring plays a key role in the interaction with the receptor (third pharmacophoric point), as shown by the difference in affinity between **6a** and **8f** and between **8c** and **8g**.

On the other hand, compounds **8i**,**h** apparently lack the third pharmacophoric point and show relatively high affinity and agonist properties at the central 5-HT_3 receptor. In this compound subseries, the low affinity shown by compounds **8j**,**k** shows the importance of the two remaining pharmacophoric points and suggests that

the assumed pharmacophoric model probably works also in these central 5-HT$_3$ receptor agonists. On the whole, the SAFIR analysis clearly suggests a common mode of binding at the central 5-HT$_3$ receptor of these heteroarylpiperazine derivatives. Further support to this conclusion is provided by the results obtained by a quantitative rationalization of the binding affinity data values for these ligands.

The molecular descriptors involved in the QSAR models obtained codified for the three-component pharmacophore previously presented. In fact, the long-range interaction component is explicitly considered in eq 3 by the $\sigma-\pi$ bond order for the $(N-H)^+$ pair showing the propensity of the protonated nitrogen to act as a proton donor and form charge-reinforced hydrogen-bonding interactions. The modulation in the binding affinity data values supposed to be due to a hydrogen-bonding interaction between the heterocyclic nitrogen atom and a suitable H-bond donor of the receptor is accounted for by the valency-related bond order descriptor P(N) (eq 7). Moreover, the partial negative surface area of the heteroaryl moiety pyridine ring (f_a-FNSA-1) rationalizes the electrostatic component of this H-bonding interaction (eqs 9 and 10). Finally, the aromatic specific interaction with the receptor is represented by the sum of the electrophilic superdelocalizability on the atoms of the benzene ring of either the quinoline nucleus or the saturated ring in analogous position (eqs 4 and 5). It is worth noting that the most appreciable contribution to the rationalization of the binding affinity in this set of ligands is given by theoretical descriptors (AIC(1), ABIC(1), V_{in}/V_{mol}, and $\Delta E_{HOMO-LUMO}$) which encode the potential for dispersion-type interactions with nonspecific receptive surfaces. In particular, eqs 6−10, which involve the size and shape descriptor V_{in}/V_{mol}, suggest that optimization of the binding affinity is obtained by maximizing the amount of bulk in one ligand in the region (delimited by V_{sup}) assumed to represent a map of the target 5-HT$_3$ binding site (see QSAR section).

In the refinement of the original interaction model, our attention was particularly focused on the receptor area interacting with the *c*-edge (positions 3 and 4) of the quinoline nucleus of quipazine since interactions of crucial importance in modulating the intrinsic efficacy at the central 5-HT$_3$ receptor appear to be involved in this area. The effects of the occupancy of this receptor area are indeed very complex. In fact, the fusion of a monocyclic system on the *c*-edge enhances the affinity to a variable extent depending on the nature of the system involved, and in this first stage the saturated ring, as it occurs in the case of compound **7b**, is better recognized than the aromatic one of compound **7a**. On the other hand, the presence of an additional ring decreases the affinity (compare **7b** with **6a**, **8b**,**d** and **7a** with **6b**). Also in this second stage the nature of the system involved affects the affinity, but in this case an aromatic ring is better tolerated than a saturated one (compare **6a** with **8d**). This preference for aromaticity is also supported by the comparison between **9a**,**c**.

As far the spatial requirements of this aromatic group are concerned, the new data imply a revision of some earlier conclusions.[10,11,31] The comparison of compounds **10a** (phenyl ring coplanar with quinoline), **6a** (35° deviation from the coplanarity), and **9a**,**d** (phenyl ring perpendicular to quinoline) showing very close 5-HT$_3$ receptor affinities fails to show a clear preference of the receptor for a particular conformation of the phenyl. Therefore, if an aromatic specific interaction involves this aromatic moiety in the interaction with the receptor, it appears to lack any evident directionality.

The high affinity shown by the derivative **9a** allowed the substitution on the 3-position of the quinoline nucleus of **9a** to be explored. The SAFIR trends analyzed above suggest that in the area of the receptor binding site interacting with the groups present in the 3-position of the quinoline nucleus of quipazine or of compound **9a** there might exist a pocket mainly composed of lipophilic amino acid residues in which a hydrogen-bonding interaction may be involved with the ligand oxygen atoms. The limited dimensions of this pocket can be inferred from the reduced affinity of the *n*-butyl derivatives **9g**,**p** and **10c**.

Very good QSAR models rationalizing the main structural characteristics of this subset of ligands are obtained (eqs 2 and 3) by means of the average information content index (AIC(1)). This index, which reflects the branching ratio and constitutional diversity of the various substituents introduced in the 3-position of the quinoline nucleus, accounts for more than 80% of the variation in the binding affinity of the subset considered.

Molecular Basis of the Intrinsic Efficacy of Arylpiperazine Derivatives at the Central 5-HT$_3$ Receptors. In the already published part 1 of this work,[12] the interactions in the area of the receptor binding site interacting with the *c*-edge of the quinoline nucleus of quipazine were assumed to be of crucial importance in modulating the intrinsic efficacy at the central 5-HT$_3$ receptor. The comparison between the functional data available for the newly synthesized compounds (reported in Tables 1 and 2 and described in the relative section) with those relative to the previously described ones allows the structural determinants responsible for the intrinsic efficacy of the quipazine derivatives to be more comprehensively defined.

On the whole, the intrinsic efficacy appears to be rather subtly modulated by the steric features of the heterocyclic moiety, as shown in Figures 2 and 3.

In particular, given the plane *xy* coincident with the quinoline nucleus, the intrinsic efficacy appears to be modulated by the steric hindrance on the *z*-axis of the substituents on the *c*-edge of the quinoline nucleus. In fact, among the compounds showing almost the same projection in the *xy*-plane, compound **7a** possesses a benzo ring (low occupancy on the *z*-axis) on the *c*-edge and is a full agonist, while **7c** possesses a rigid bicyclic system (higher occupancy on the *z*-axis) and shows antagonist properties. Finally, **7b** possessing a cyclohexane ring condensed on the *c*-edge (intermediate occupancy joined to a certain degree of mobility) shows partial agonist properties (Figure 2). Furthermore, the intrinsic efficacy appears to be modulated also by the molecular geometry of the substituent on faces *c*, *d*, and *e* of the quinoline nucleus. In fact, compounds **7a**, **10a**, and **8e**[31] show planar heteroaryl moieties and quite similar 5-HT$_3$ receptor affinities, but the differences in their heteroaryl moieties (Figure 3) are reflected in the intrinsic efficacy, the compounds being full agonist,

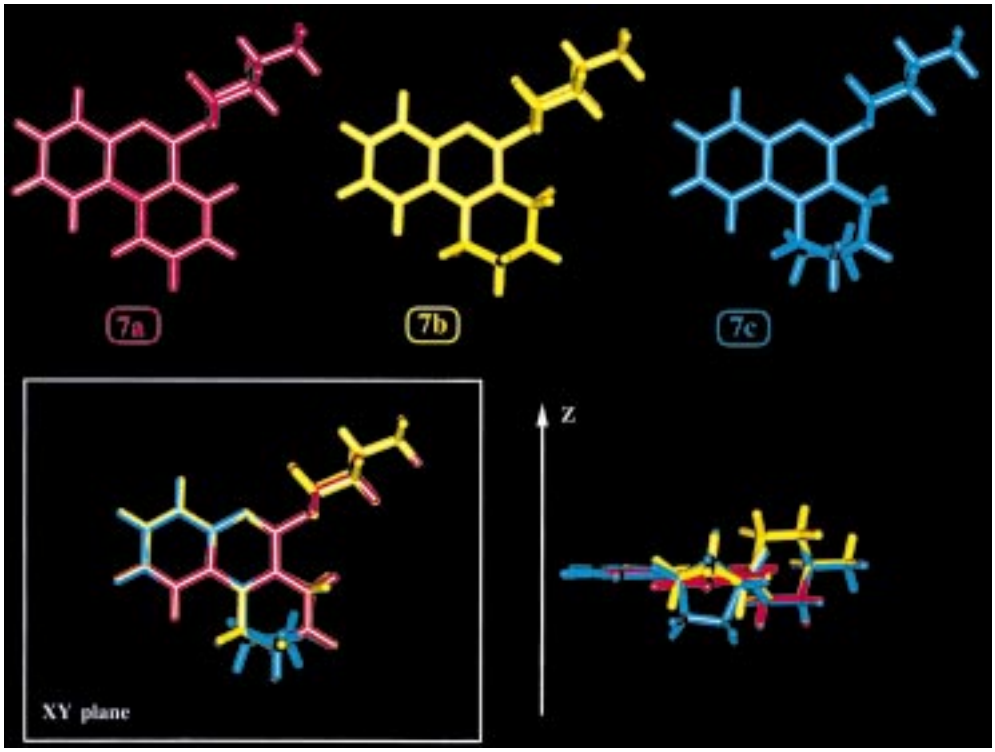


Figure 2. Superposition of agonist **7a** (red), partial agonist **7b** (yellow), and antagonist **7c** (blue) showing the occupancy in planes *xy* and *xz*.

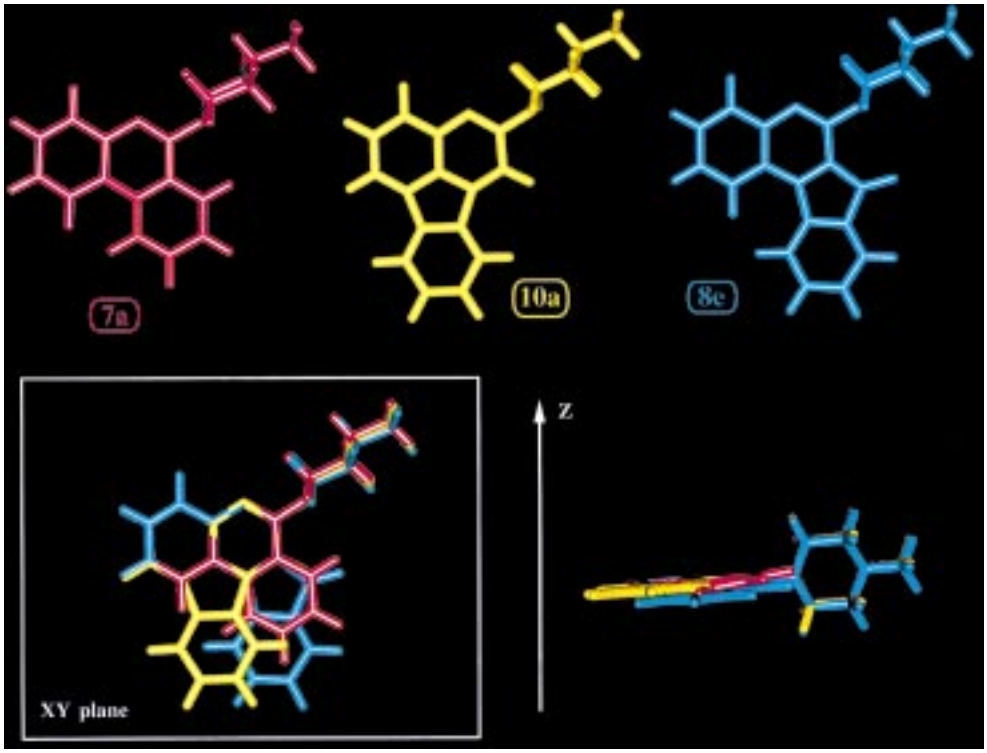


Figure 3. Superposition of agonist **7a** (red), partial agonist **10a** (yellow), and antagonist **8e** (blue) showing the occupancy in planes *xy* and *xz*.

partial agonist, and antagonist, respectively. Finally, the intrinsic efficacy appears to be related to the presence of the quinoline benzo ring as the ring deletion in antagonist **6a** yields the full agonist **8h**.

Conclusions

Novel central 5-HT_3 receptor ligands provided with nanomolar or subnanomolar affinity and a full range of intrinsic efficacies were discovered starting from quipazine and previously published 5-HT_3 receptor antagonists **6**. The interaction model for the quipazine-related 5-HT_3 ligands with their receptor, proposed in the already published part 1 of this work,[12] has been revised and refined to incorporate the latest data on the basis of qualitative and quantitative structure−affinity relationship studies.

The physical information carried by the theoretical molecular descriptors involved in the QSAR models obtained confirms the fundamental role of the protonated terminal piperazine nitrogen for the recognition step (electrostatic interaction) and the key role of the heterocyclic nitrogen atom for a correct docking (hydrogen-bonding interaction). Moreover, a fine-tuning of the binding affinity is accomplished by the optimization of short-range intermolecular interactions and dispersion contributions of the various molecular regions. In fact, both strength and specificity arise from the cumulation and interplay of many weak interactions established by the receptor with the quinoline benzo ring, the cyclic system on the *c*-edge, and/or the substituent in the 3-position of the quinoline nucleus of the ligands.

Finally, the comparison between the functional data available for the new ligands and those relative to the previously described compounds has allowed a comprehensive definition of the structural determinants responsible for the intrinsic efficacy of the quipazine derivatives in terms of steric hindrance and molecular geometry of the heteroaryl moiety of these arylpiperazines.

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 either on a VG 70-250S spectrometer (Centro di Analisi e Determinazioni Strutturali, Università di Siena) or on 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.

Preparations of Target 4-Methyl-1-piperazinyl or Morpholino Derivatives 8−10. Method A. A mixture of the appropriate imidoyl chloride (1.0 mmol) with *N*-methylpiperazine or morpholine (5 mL) was heated at 130−140 °C under argon for a suitable time (2−44 h), and the reaction progress was monitored by TLC. When the chloro derivative disappeared from the chromatogram, the reaction mixture was 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 (or *n*-hexane−ethyl acetate (8:2) in the case of morpholino derivatives) gave the pure target compounds.

Method B. To a mixture of the appropriate pyridones (1.0 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 1 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 for a suitable time (the reaction progress was monitored by TLC). 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 the elution with *n*-hexane−ethyl acetate (65:35) gave the pure target 4-methyl-1-piperazinyl derivatives.

7,8-Dihydro-6-morpholinobenzo[*k*]phenanthridine (8a). The title compound was prepared by method A from **14a** and morpholine (reaction time 17 h) to obtain a white crystalline solid (0.29 g, 92%): mp 165−166 °C. ^{1}H NMR ($CDCl_3$): 2.80 (m, 4H), 3.35 (t, *J* = 4.5, 4H), 3.92 (t, *J* = 4.4, 4H), 7.38 (m, 4H), 7.59 (t, *J* = 7.1, 1H), 7.92 (m, 2H), 8.34 (d, *J* = 8.7, 1H). Anal. ($C_{21}H_{20}N_2O$) C, H, N.

7,8-Dihydro-6-(4-methyl-1-piperazinyl)thieno[2,3-*k*]-phenanthridine (8b). The title compound was prepared by method A from **14b** and *N*-methylpiperazine (reaction time 22 h) to obtain a pale-yellow oil which crystallized on standing (0.31 g, 92%): mp 147−149 °C. ^{1}H NMR ($CDCl_3$): 2.39 (s, 3H), 2.64 (t, *J* = 4.4, 4H), 2.88−3.02 (m, 4H), 3.35 (t, *J* = 4.7, 4H), 7.27 (d, *J* = 5.1, 1H), 7.38 (t, *J* = 7.8, 1H), 7.59 (t, *J* = 7.3, 1H), 7.71 (d, *J* = 5.3, 1H), 7.92 (d, *J* = 8.2, 1H), 8.31 (d, *J* = 8.4, 1H). Anal. ($C_{20}H_{21}N_3S$) C, H, N.

7-(4-Methyl-1-piperazinyl)-6*H*-[1]benzothiopyrano[3,4-*c*]quinoline (8c). The title compound was prepared by method A from **14c** and *N*-methylpiperazine (reaction time 21 h) to obtain a pale-yellow oil which crystallized on standing (0.33 g, 95%): mp 140−141 °C. ^{1}H NMR ($CDCl_3$): 2.40 (s, 3H), 2.65 (t, *J* = 4.4, 4H), 3.35 (t, *J* = 4.7, 4H), 3.88 (s, 2H), 7.36 (m, 3H), 7.61 (m, 2H), 7.84 (m, 1H), 7.95 (d, *J* = 8.7, 1H), 8.13 (d, *J* = 8.6, 1H). Anal. ($C_{21}H_{21}N_3S\cdot0.25H_2O$) C, H, N

***trans*-6-(4-Methyl-1-piperazinyl)-7,8,8a,9,10,11,12,12a-octahydrobenzo[*k*]phenanthridine (8d).** The title compound was prepared by method A from **14d** and *N*-methylpiperazine (reaction time 23 h) to obtain a pale-yellow oil which crystallized on standing (0.30 g, 89%): mp 156−157 °C. ^{1}H NMR ($CDCl_3$): 1.21 (m, 2H), 1.52 (m, 3H), 1.75−2.04 (m, 5H), 2.37 (s, 3H), 2.49−2.72 (m, 6H), 2.90 (m, 2H), 3.25 (m, 2H), 3.53 (m, 2H), 7.30 (m, 1H), 7.50 (t, *J* = 7.9, 1H), 7.83 (m, 2H). Anal. ($C_{22}H_{29}N_3$) C, H, N.

1,2,3,4,7,8-Hexahydro-6-(4-methyl-1-piperazinyl)benzo-[*k*]phenanthridine (8f). The title compound was prepared by method B from **15a** and *N*-methylpiperazine (reaction time 4 h) to obtain a colorless oil which crystallized on standing (0.22 g, 66%): mp 150−151 °C. ^{1}H NMR ($CDCl_3$): 1.72 (m, 2H), 1.92 (m, 2H), 2.35 (s, 3H), 2.57 (t, *J* = 4.7, 4H), 2.66 (s, 4H), 2.91 (q, *J* = 6.1, 4H), 3.18 (t, *J* = 4.7, 4H), 7.27 (m, 3H), 7.59 (m, 1H). Anal. ($C_{22}H_{27}N_3$) C, H, N.

7-(4-Methyl-1-piperazinyl)-9,10,11,12-tetrahydro-6*H*-[1]-benzothiopyrano[3,4-*c*]quinoline (8g). The title compound was prepared by method B from **15c** and *N*-methylpiperazine (reaction time 2 h) to obtain a white crystalline solid (0.29 g, 82%): mp 184−185 °C. ^{1}H NMR ($CDCl_3$): 1.63 (m, 2H), 1.90 (m, 2H), 2.36 (s, 3H), 2.58 (t, *J* = 4.2, 4H), 2.81 (t, *J* = 5.9, 2H), 2.91 (t, *J* = 6.4, 2H), 3.14 (t, *J* = 4.7, 4H), 3.74 (s, 2H), 7.24 (m, 2H), 7.50−7.62 (m, 2H). Anal. ($C_{21}H_{25}N_3S$) C, H, N.

5,6-Dihydro-4-(4-methyl-1-piperazinyl)benz[*f*]isoquinoline (8h). The title compound was prepared by method B from **18** and *N*-methylpiperazine (reaction time 3 h) to obtain an oil which crystallized on standing (0.21 g, 75%): mp 116−118 °C. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 2.60 (t, *J* = 4.5, 4H), 2.81 (s, 4H), 3.20 (t, *J* = 4.7, 4H), 7.30 (m, 4H), 7.70 (m, 1H), 8.26 (d, *J* = 5.4, 1H). Anal. ($C_{18}H_{21}N_3$) C, H, N.

4-(4-Methyl-1-piperazinyl)benz[*f*]isoquinoline (8i). The title compound was prepared by method A from 4-chlorobenz-[*f*]isoquinoline[15b] and *N*-methylpiperazine (reaction time 9 h) to obtain an oil which crystallized on standing (0.26 g, 94%). An analytical sample was obtained by recrystallization from *n*-hexane: mp 92−94 °C. ^{1}H NMR ($CDCl_3$): 2.44 (s, 3H), 2.74 (t, *J* = 4.7, 4H), 3.48 (t, *J* = 4.8, 4H), 7.68 (m, 2H), 7.78 (d, *J* = 9.0, 1H), 7.88−8.06 (m, 3H), 8.40 (d, *J* = 5.8, 1H), 8.62 (m, 1H). Anal. ($C_{18}H_{19}N_3$) C, H, N.

4-(4-Methyl-1-piperazinyl)benzo[*h*]quinoline (8j). The title compound was prepared by method A starting from 4-chlorobenzo[*h*]quinoline[12] and *N*-methylpiperazine (reaction time 44 h) to obtain an oil which crystallized on standing (0.24 g, 86%). An analytical sample was obtained by recrystallization from *n*-hexane: mp 154−156 °C. ^{1}H NMR ($CDCl_3$): 2.43 (s,

3H), 2.74 (t, J = 4.6, 4H), 3.28 (t, J = 4.7, 4H), 7.04 (d, J = 5.0, 1H), 7.63−7.78 (m, 3H), 7.86−7.96 (m, 2H), 8.83 (d, J = 5.0, 1H), 9.26 (m, 1H). Anal. ($C_{18}H_{19}N_3$) C, H, N.

4-Morpholinobenz[*f*]isoquinoline (8k). The title compound was prepared by method A from 4-chlorobenz[*f*]-isoquinoline[15b] and morpholine (reaction time 22 h) to obtain a white crystalline solid (0.23 g, 87%): mp 143−145 °C. ^{1}H NMR ($CDCl_3$): 3.42 (t, J = 4.7, 4H), 4.00 (t, J = 4.7, 4H), 7.68 (m, 2H), 7.79 (d, J = 9.0, 1H), 7.90−8.09 (m, 3H), 8.41 (d, J = 5.9, 1H), 8.62 (m, 1H). Anal. ($C_{17}H_{16}N_2O$) C, H, N.

4-(1-Adamantyl)-2-(4-methyl-1-piperazinyl)quinoline (9c). The title compound was prepared by method A starting from **24** and *N*-methylpiperazine (reaction time 3 h) to obtain an oil which crystallized on standing (0.31 g, 86%): mp 133−135 °C. ^{1}H NMR ($CDCl_3$): 1.86 (s, 6H), 2.18 (s, 3H), 2.25 (s, 6H), 2.36 (s, 3H), 2.55 (t, J = 4.9, 4H), 3.75 (t, J = 5.0, 4H), 6.90 (s, 1H), 7.18 (m, 1H), 7.46 (m, 1H), 7.73 (m, 1H), 8.37 (d, J = 7.9, 1H). Anal. ($C_{24}H_{31}N_3$) C, H, N.

3-Methyl-2-(4-methyl-1-piperazinyl)-4-phenylquinoline (9d). The title compound was prepared by method A starting from 2-chloro-3-methyl-4-phenylquinoline[39] and *N*-methylpiperazine (reaction time 5 h) to obtain an oil which crystallized on standing (0.23 g, 72%): mp 133−134 °C. ^{1}H NMR ($CDCl_3$): 2.13 (s, 3H), 2.38 (s, 3H), 2.63 (t, J = 4.8, 4H), 3.39 (t, J = 4.7, 4H), 7.21 (m, 4H), 7.48 (m, 4H), 7.89 (d, J = 8.4, 1H). Anal. ($C_{21}H_{23}N_3 \cdot 0.25\ H_2O$) C, H, N.

Ethyl 2-(4-Methyl-1-piperazinyl)-4-phenyl-3-quinolineacetate (9q). The title compound was prepared by method A starting from **26** and *N*-methylpiperazine (reaction time 8 h) to obtain an oil which crystallized on standing (0.31 g, 79%): mp 109−110 °C. ^{1}H NMR ($CDCl_3$): 1.14 (t, J = 7.0, 3H), 2.36 (s, 3H), 2.59 (t, J = 4.6, 4H), 3.29 (t, J = 4.8, 4H), 3.61 (s, 2H), 4.01 (q, J = 7.0, 2H), 7.23 (m, 4H), 7.49 (m, 3H), 7.58 (m, 1H), 7.94 (d, J = 8.2, 1H). Anal. ($C_{24}H_{27}N_3O_2$) C, H, N.

2-(4-Methyl-1-piperazinyl)indeno[1,2,3-*de*]quinoline (10a). The title compound was prepared by method A starting from **30** and *N*-methylpiperazine (reaction time 2 h) to obtain an oil which crystallized on standing (0.29 g, 96%): mp 94−96 °C.^{1}H NMR ($CDCl_3$): 2.39 (s, 3H), 2.61 (t, J = 5.1, 4H), 3.89 (t, J = 5.0, 4H), 7.30−7.48 (m, 3H), 7.58 (m, 3H), 7.83 (m, 2H). Anal. ($C_{20}H_{19}N_3 \cdot 0.25H_2O$) C, H, N.

Ethyl 2-(4-Methyl-1-piperazinyl)indeno[1,2,3-*de*]quinoline-1-carboxylate (10b). The title compound was prepared by method A starting from **34** and *N*-methylpiperazine (reaction time 2 h and 30 min) to obtain a yellow oil which crystallized on standing (0.33 g, 88%): mp 139−140 °C.^{1}H NMR ($CDCl_3$): 1.46 (t, J = 7.0, 3H), 2.36 (s, 3H), 2.57 (t, J = 4.7, 4H), 3.61 (t, J = 4.7, 4H), 4.53 (q, J = 7.1, 2H), 7.31 (t, J = 7.5, 1H), 7.44 (t, J = 7.4, 1H), 7.62 (m, 3H), 7.79 (d, J = 7.3, 1H), 7.97 (d, J = 7.6, 1H). Anal. ($C_{23}H_{23}N_3O_2$) C, H, N.

***n*-Butyl 2-(4-Methyl-1-piperazinyl)indeno[1,2,3-*de*]quinoline-1-carboxylate (10c).** The title compound was prepared by method A starting from **35** and *N*-methylpiperazine (reaction time 1 h) to obtain a yellow oil which crystallized on standing (0.37 g, 92%): mp 95−97 °C.^{1}H NMR ($CDCl_3$): 0.97 (t, J = 7.2, 3H), 1.48 (m, 2H), 1.80 (m, 2H), 2.37 (s, 3H), 2.58 (t, J = 4.8, 4H), 3.61 (t, J = 4.8, 4H), 4.47 (t, J = 6.8, 2H), 7.32 (t, J = 7.2, 1H), 7.44 (t, J = 7.3, 1H), 7.63 (m, 3H), 7.79 (d, J = 7.4, 1H), 7.96 (d, J = 7.6, 1H). Anal. ($C_{25}H_{27}N_3O_2$) C, H, N.

***N*,*N*-Dipropyl-2-(4-methyl-1-piperazinyl)indeno[1,2,3-*de*]quinoline-1-carboxamide (10d)**. The title compound was prepared by method A starting from **36** and *N*-methylpiperazine (reaction time 2 h and 30 min) to obtain a yellow oil (0.39 g, 91%). ^{1}H NMR ($CDCl_3$): 0.45 (t, J = 7.3, 3H), 1.02−1.55 (m, 5H), 1.82 (m, 2H), 2.34 (s, 3H), 2.54 (m, 4H), 2.99 (m, 2H), 3.38 (m, 3H), 3.76 (m, 3H), 7.28 (t, J = 7.4, 1H), 7.40 (t, J = 7.3, 1H), 7.63 (m, 3H), 7.77 (d, J = 7.4, 1H), 7.84 (d, J = 7.6, 1H). Anal. ($C_{27}H_{32}N_4O$) C, H, N.

Preparation of Enamines 12a−d. To a 100-mL, three-necked flask fitted with a condenser, thermometer, and dropping funnel (under argon) were added the appropriate cyclic ketone (α-tetralone (**11a**), 4-keto-4,5,6,7-tetrahydrothianaphthene (**11b**), thiochroman-4-one (**11c**), *trans*-1-decalone (**11d**)) obtained from Aldrich (10 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 10 mL of dry benzene. The temperature was kept between 0 and 10 °C during the addition. When the $TiCl_4$ addition was complete, the mixture was allowed to stir at room temperature for 19 h. The reaction mixture was then filtered, the solvent was removed under reduced pressure, and the corresponding enamine was used immediately without further purification.

General Procedure for the Preparation of Quinolinones 13a−d. The suitable enamine **12a−d** (10 mmol) 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, the solvent was removed under reduced pressure, and the residue was refluxed for 1 h in ethanol (40 mL). The solvent was then removed, and the residue was partitioned between dichloromethane and 1 N HCl. The organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification by chromatography of the residue (*n*-hexane−ethyl acetate (65:35) as the eluent) gave an oil which crystallized on standing or by treatment with *n*-hexane−ethyl ether. To the material so obtained was added PPA (20 g), and the resulting mixture was heated at 150 °C with stirring for 10−50 min. Then, the cooled brown mass was decomposed with ice−water, and the precipitate was extracted with $CHCl_3$ (3 × 40 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 or by flash chromatography gave the pure title compounds as white solids.

7,8-Dihydrobenzo[*k*]phenanthridin-6(5*H*)-one (13a). This compound was prepared in 21% yield starting from enamine **12a** and was purified by flash chromatography (ethyl acetate as the eluent): mp 246−248 °C (lit.[21] mp 250−252 °C).^{1}H NMR ($CDCl_3$): 2.86 (m, 4H), 7.19−7.50 (m, 6H), 7.86 (m, 1H), 8.17 (d, J = 8.3, 1H), 11.56 (br s, 1H).

7,8-Dihydrothieno[2,3-*k*]phenanthridin-6(5*H*)-one (13b). This compound was prepared in 11% yield starting from enamine **12b** and was purified by flash chromatography (ethyl acetate as the eluent): mp 227−229 °C. ^{1}H NMR ($CDCl_3$): 3.02 (m, 4H), 7.27 (m, 2H), 7.38 (d, J = 7.5, 1H), 7.51 (t, J = 7.9, 1H), 7.63 (d, J = 5.5, 1H), 8.19 (d, J = 7.9, 1H), 11.02 (br s, 1H). HR-MS: *m*/*z* calcd for ($C_{15}H_{11}NOS$) 253.0561, found 253.0552.

6,8-Dihydro-7*H*-[1]benzothiopyrano[3,4-*c*]quinolin-7-one (13c). This compound was prepared in 53% yield starting from enamine **12c** and was purified by washing with ethyl acetate: mp 264−265 °C. ^{1}H NMR ($CDCl_3$): 3.92 (s, 2H), 7.23 (m, 1H), 7.36 (m, 2H), 7.50 (m, 2H), 7.64 (m, 1H), 7.80 (m, 1H), 7.98 (d, J = 8.3, 1H), 11.98 (br s, 1H).

7,8,8a,9,10,11,12,12a-Octahydrobenzo[*k*]phenanthridin-6(5*H*)-one (13d). This compound was obtained from enamine **12d** in 78% yield as a white solid. Recrystallization from ethyl acetate−methanol gave a diastereomeric mixture (*trans*/*cis* 88:12) of **13d**: mp 295−297 °C (lit.[40] mp 285−288 °C). ^{1}H NMR ($CDCl_3$) (only the resonances of the major diasteromer are given): 1.07−1.92 (m, 10H), 2.58 (m, 3H), 2.88 (m, 1H), 7.17 (t, J = 7.4, 1H), 7.26 (d, J = 7.5, 1H), 7.40 (t, J = 7.5, 1H), 7.62 (d, J = 8.1, 1H), 10.57 (br s, 1H). Anal. ($C_{17}H_{19}NO$) C, H, N.

General Procedure for the Preparation of Imidoyl Chlorides 14a−d. A mixture of the appropriate quinolinone derivative (1.0 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 the title compounds which were purified by flash chromatography when necessary.

6-Chloro-7,8-dihydrobenzo[*k*]phenanthridine (14a). The title compound was prepared from **13a** and was purified by flash chromatography with *n*-hexane−ethyl acetate (8:2) as the eluent to obtain a colorless oil which crystallized on standing to give a white crystalline solid (0.23 g, 86%): mp 96−97 °C, (lit.[21] mp 80−81 °C; lit.[41] mp 95−96 °C).^{1}H NMR ($CDCl_3$): 2.89 (m, 2H), 3.06 (m, 2H), 7.37−7.42 (m, 3H), 7.56 (m, 1H), 7.69 (m, 1H), 7.91 (m, 1H), 8.07 (d, J = 8.0, 1H), 8.42 (d, J = 8.5, 1H).

6-Chloro-7,8-dihydrothieno[2,3-*k*]phenanthridine (14b). This compound was prepared in 98% yield starting from **13b** and was revealed pure by GC−MS, so it was used in the subsequent step without further purification.^{1}H NMR ($CDCl_3$): 3.05 (m, 2H), 3.23 (m, 2H), 7.31 (d, J = 5.1, 1H), 7.56 (m, 1H), 7.69 (m, 2H), 8.03 (d, J = 8.8, 1H), 8.41 (d, J = 8.5, 1H). MS: m/z 271 (M^+, 100).

7-Chloro-6*H*-[1]benzothiopyrano[3,4-*c*]quinoline (14c). This compound was prepared starting from **13c** and was purified by flash chromatography with *n*-hexane−ethyl acetate (8:2) as the eluent to obtain a colorless oil which crystallized on standing (0.26 g, 92%): mp 139−140 °C. ^{1}H NMR ($CDCl_3$): 4.08 (s, 2H), 7.35−7.45 (m, 2H), 7.55 (m, 1H), 7.64−7.76 (m, 2H), 7.85 (m, 1H), 8.08 (d, J = 8.9, 1H), 8.23 (d, J = 8.0, 1H). MS: m/z 283 (M^+, 100).

***trans*-6-Chloro-7,8,8a,9,10,11,12,12a-octahydrobenzo-[*k*]phenanthridine (14d).** This compound was prepared starting from a diastereomeric mixture (*trans*/*cis* 85:15) of **13d** and was isolated by flash chromatography with *n*-hexane−dichloromethane (6:4) as a colorless oil which crystallized on standing (0.076 g, 28%): mp 86−87 °C. ^{1}H NMR ($CDCl_3$): 1.04−1.95 (m, 10H), 2.63 (br d, J = 13.3, 1H), 2.76−2.91 (m, 3H), 7.48 (m, 1H), 7.62 (m, 1H), 7.89 (d, J = 8.5, 1H), 7.98 (d, J = 8.9, 1H). MS: m/z 271 (M^+, 100). Anal. ($C_{17}H_{18}ClN$) C, H, N.

1,2,3,4,7,8-Hexahydrobenzo[*k*]phenanthridin-6(5*H*)-one (15a). To a solution of 1-cyclohexene-1-isocyanate[14] (1.0 g, 8.1 mmol) in dry acetonitrile (10 mL) cooled at 0 °C was added enamine **12a** (2.0 g, 10 mmol) dissolved into 10 mL of dry acetonirile, and the resulting solution was stirred for 48 h at room temperature. The solvent was then removed, and the residue was partitioned between chloroform and 1 N HCl. The organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Treatment of the residue with methanol−ethyl acetate gave **15a** as white crystals (0.51 g, 25%). An analytical sample was obtained from methanol as colorless prisms: mp 268−270 °C. ^{1}H NMR ($CDCl_3$): 1.70 (m, 2H), 1.87 (m, 2H), 2.75 (m, 8H), 7.29 (m, 3H), 7.65 (m, 1H), 11.96 (br s, 1H). Anal. ($C_{17}H_{17}NO$) C, H, N.

6,8,9,10,11,12-Hexahydro-7*H*-[1]benzothiopyrano[3,4-*c*]quinolin-7-one (15c). This compound was prepared in 22% yield from enamine **12c** employing the same procedure described for **15a** and was purified by treatment with ethyl acetate−methanol: mp 288 °C dec. ^{1}H NMR ($CDCl_3$): 1.64 (m, 2H), 1.87 (m, 2H), 2.62 (t, J = 5.6, 2H), 2.78 (t, J = 6.5, 2H), 3.80 (s, 2H), 7.24 (m, 2H), 7.55 (m, 1H), 7.65 (m, 1H), 12.01 (br s, 1H).

Ethyl (*E*)-3-(3,4-Dihydronaphth-1-yl)propenoate (17). To a slurry of $Pd(PPh_3)_2Cl_2$ (0.12 g, 0.17 mmol) in dry DMF (15 mL) was added a solution of 3,4-dihydro-1-[[(trifluoromethyl)sulfonyl]oxy]naphthalene[16] (**16**) (2.1 g, 7.5 mmol), ethyl acrylate (1.95 mL, 18.0 mmol), and triethylamine (3.6 mL, 26 mmol) in dry DMF (5 mL). The resulting mixture was heated at 75−80 °C in an argon atmosphere for 3 h, cooled to room temperature, and poured into ice−water. The precipitate was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with water (2 × 100 mL), dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with *n*-hexane−ethyl acetate (9:1) as the eluent gave **17** as a pale-yellow oil (0.47 g, 27%). ^{1}H NMR ($CDCl_3$): 1.30 (t, J = 7.1, 3H), 2.33 (m, 2H), 2.72 (t, J = 7.9, 2H), 4.22 (q, J = 7.1, 2H), 6.24 (d, J = 15.3, 1H), 6.43 (t, J = 4.5, 1H), 7.18 (m, 3H), 7.35 (m, 1H), 7.61 (d, J = 15.4, 1H).

5,6-Dihydrobenz[*f*]isoquinolin-4(3*H*)-one (18). A mixture of **17** (0.46 g, 2.0 mmol) in ethanol (30 mL) with 3 N NaOH solution (10 mL) was stirred at room temperature for 2 h. The organic solvent was then removed under reduced pressure; the residue was poured into ice−water and acidified with 2 N HCl solution. The precipitate was extracted with ethyl acetate (3 × 30 mL), and the organic layer was washed with brine (2 × 100 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was dissolved into acetone (10 mL), and triethylamine (0.33 mL, 2.38 mmol) was added. To the resulting mixture cooled at 0 °C was added dropwise a solution of isobutyl chloroformate (0.31 mL, 2.38 mmol) in acetone (8 mL). The reaction mixture was stirred at 0 °C for 30 min, and a solution of sodium azide (0.18 g, 2.76 mmol) in 5 mL of water was slowly added keeping the temperature below 10 °C. When the addition was complete, the reaction mixture was stirred for 1 h at room temperature and then poured into ice−water, and the precipitate was extracted with dichloromethane (4 × 10 mL). The combined organic extracts were dried over sodium sulfate and slowly added to a mixture of tributylamine (1.0 mL, 4.2 mmol) in Dowtherm A (20 mL) preheated at 200−220 °C into a distillation apparatus which allowed the distillation of the dichloromethane during the addition. When the addition was complete, the solvent was removed under reduced pressure and the residue was purified by flash chromatography. Elution with ethyl acetate−methanol (85:15) gave pure **18** (46 mg, 12%). ^{1}H NMR ($CDCl_3$): 2.88 (s, 4H), 6.72 (d, J = 6.9, 1H), 7.31 (m, 4H), 7.64 (m, 1H), 12.19 (br s, 1H). HR-MS: m/z calcd for ($C_{13}H_{11}NO$) 197.0841, found 197.0840.

3-Amino-1-phenylnaphthalene (20). To a mixture of **19**[18] (0.25 g, 1.0 mmol) in dry benzene (10 mL) with triethylamine (0.14 mL, 1.0 mmol) cooled at 0−5 °C was added DPPA (0.22 mL, 1.0 mmol). The resulting mixture was stirred at room temperature for 3 h, heated to reflux for 4 h, and cooled, and 20 mL of 50% sulfuric acid was added. After overnight stirring at room temperature, the reaction mixture was poured into ice−water, made alkaline with concentrated NH_4OH, and extracted with chloroform (3 × 20 mL). The combined extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with *n*-hexane−ethyl acetate (65:35) gave pure **20** (0.18 g, 82%) as a pale-yellow oil which was used immediately in the subsequent step. ^{1}H NMR ($CDCl_3$): 3.86 (br s, 2H), 6.90 (d, J = 2.0, 1H), 7.01 (d, J = 2.2, 1H), 7.16 (t, J = 7.1, 1H), 7.32−7.48 (m, 6H), 7.64 (d, J = 8.1, 1H), 7.70 (d, J = 8.4, 1H).

3-(4-Methyl-1-piperazinyl)-1-phenylnaphthalene (9b). A mixture of **20** (0.14 g, 0.64 mmol), *N*-methylbis(2-chloroethyl)amine hydrochloride (0.18 g, 0.93 mmol), K_2CO_3 (0.26 g, 1.88 mmol), and diglyme (15 mL) was heated to reflux for 21 h under argon atmosphere. The cooled reaction mixture was poured into ice−water, and the solution was made basic with 50% NaOH solution and extracted with chloroform (4 × 20 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure, and the residue obtained was purified by flash chromatography with ethyl acetate-triethylamine (8:2) to obtain **9b** (43 mg, 22%) as a brownwish oil. ^{1}H NMR ($CDCl_3$): 2.37 (s, 3H), 2.63 (t, J = 4.9, 4H), 3.35 (t, J = 4.8, 4H), 7.14 (d, J = 2.0, 1H), 7.21 (d, J = 2.1, 1H), 7.35−7.47 (m, 7H), 7.72 (m, 2H). HR-MS: m/z calcd for ($C_{21}H_{22}N_2$) 302.1783, found 302.1787. Anal. ($C_{21}H_{22}N_2$) C, H, N.

1-Adamantyl 2-Aminophenyl Ketone (**22**). A 1.7 M solution of *tert*-butyllithium in pentane (11.3 mL, 19.2 mmol) was added in a dropwise manner to a solution of *N*-(*tert*-butoxycarbonyl)aniline (**21**)[20] (1.5 g, 7.8 mmol) in anhydrous THF (10 mL, argon atmosphere) maintained at −65 °C. After 15 min at −65 °C, the solution was allowed to warm to −20 °C where it was maintained for 2 h. A solution of methyl 1-adamantanecarboxylate (1.5 g, 7.7 mmol) in anhydrous THF (10 mL) was then added, and the resulting mixture was stirred at −15 °C for 1 h and at room temperature for 30 min. The reaction mixture was poured into ice−water and extracted

with chloroform (3 × 30 mL). The combined extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography with *n*-hexane−ethyl acetate (8:2) as the eluent to give an oil which crystallized on standing. This product was dissolved into ethanol (60 mL), and 3 N HCl (30 mL) was added under stirring. The resulting mixture was heated to reflux for 1 h, and the organic solvent was removed under reduced pressure. The residue was diluted with water, made alkaline with solid Na_2CO_3, and extracted with dichloromethane (3 × 30 mL). The combined extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with *n*-hexane−ethyl acetate (8:2) as the eluent gave **22** (0.57 g, 29%) as an oil which crystallized on standing. An analytical sample recrystallized from *n*-hexane melted at 143−144 °C. ^{1}H NMR ($CDCl_3$): 1.76 (s, 6H), 2.07 (s, 9H), 5.08 (br s, 2H), 6.66 (m, 2H), 7.17 (t, J = 8.0, 1H), 7.68 (d, J = 7.9, 1H). Anal. ($C_{17}H_{21}NO$) C, H, N.

4-(1-Adamantyl)quinolin-2(1*H*)-one (23). A mixture of **22** (0.22 g, 0.86 mmol) in acetic anhydride (4.0 mL) was stirred at room temperature for 4 h and then poured into ice−water. The precipitate was extracted with chloroform (3 × 25 mL), and the organic layer was washed with water, dried over sodium sulfate, and concentrated under reduced pressure to give an oil which crystallized on standing. This product was dissolved into absolute ethanol (10 mL), and a 30% solution of sodium methoxide in methanol (0.8 mL, 4.3 mmol) was added under stirring. The resulting mixture was heated to reflux for 8 h under an argon atmosphere, cooled to room temperature, and poured into ice−water. The aqueous mixture was acidified with 6 N HCl, and the precipitate was collected by filtration, washed in sequence with water and *n*-hexane−diethyl ether, and finally dried to give **23** (0.17 g, 71%) as a white crystalline solid. An analytical sample was obtained by recrystallization from methanol−chloroform: mp 276 °C dec. ^{1}H NMR ($CDCl_3$): 1.86 (s, 6 H), 2.21 (s, 9H), 6.67 (s, 1H), 7.19 (m, 1H), 7.40 (m, 2H), 8.35 (d, J = 8.4, 1H), 11.44 (br s, 1H). Anal. ($C_{19}H_{21}NO$) C, H, N.

4-(1-Adamantyl)-2-chloroquinoline (24). This compound was prepared in 89% yield starting from **23** using the same procedure described for the preparation of imidoyl chlorides **14a**−**d** and was used in the subsequent step without further purification. ^{1}H NMR ($CDCl_3$): 1.89 (s, 6H), 2.23 (s, 3H), 2.30 (s, 6H), 7.30 (s, 1H), 7.51 (m, 1H), 7.68 (m, 1H), 8.06 (m, 1H), 8.60 (d, J = 8.5, 1H).

Ethyl 2-Chloro-4-phenyl-3-quinolineacetate (26). The title compound was prepared in 94% yield starting from ethyl 1,2-dihydro-2-oxo-4-phenyl-3-quinolineacetate[21] (**25**) using the same procedure described for the preparation of imidoyl chlorides **14a**−**d** and was used in the subsequent step without further purification: mp 110−111 °C. ^{1}H NMR ($CDCl_3$): 1.21 (t, J = 7.0, 3H), 3.70 (s, 2H), 4.14 (q, J = 7.0, 2H), 7.26−7.52 (s, 7H), 7.69 (m, 1H), 8.05 (d, J = 8.4, 1H).

3-(2-Hydroxyethyl)-2-(4-methyl-1-piperazinyl)-4-phenylquinoline (9i). To a suspension of lithium aluminum hydride (0.5 g, 13.2 mmol) in anhydrous THF (10 mL) cooled at 0−5 °C was added a solution of **9q** (0.86 g, 2.2 mmol) in anhydrous THF (10 mL). After stirring for 30 min at 0−5 °C and 30 min at room temperature, the hydride was hydrolyzed by addition of water and the inorganic material was filtered off and washed with tetrahydrofuran. The filtrate was washed with brine, dried over sodium sulfate, and evaporated to give pure **9i** as a white solid (0.75 g, yield 98%). Crystallization from ethyl acetate gave an analytical sample: mp 165−166 °C. ^{1}H NMR ($CDCl_3$): 2.38 (s, 3H), 2.68 (t, J = 4.4, 4H), 2.91 (t, J = 5.7, 2H), 3.35 (t, J = 4.8, 4H), 3.64 (m, 2H), 4.60 (br s, 1H), 7.24 (m, 4H), 7.52 (m, 4H), 7.95 (d, J = 8.4, 1H). Anal. ($C_{22}H_{25}N_3O$) C, H, N.

2-(4-Methyl-1-piperazinyl)-4-phenyl-3-[2-[(2-tetrahydrofuranyl)oxy]ethyl]quinoline (9j). To a stirred solution of **9i** (0.17 g, 0.49 mmol) in dichloromethane (10 mL) with 2,3-dihydrofuran (0.15 mL, 2.0 mmol) was added 0.11 g (0.58 mmol) of *p*-toluenesulfonic acid monohydrate, and the mixture was maintained at room temperature for 6 h. The solution was then diluted with 30 mL of dichloromethane, washed with a 1% solution of Na_2CO_3, dried over sodium sulfate, and concentrated under reduced pressure. Purification of the residue by flash chromatography with ethyl acetate−triethylamine (8:2) as the eluent gave **9j** (0.18 g, 88%) as an oil which crystallized on standing: mp 102−103 °C. ^{1}H NMR ($CDCl_3$): 1.78 (m, 4H), 2.38 (s, 3H), 2.64 (t, J = 4.5, 4H), 2.89 (t, J = 7.6, 2H), 3.37 (m, 5H), 3.68 (m, 3H), 4.90 (d, J = 3.1, 1H), 7.23 (m, 4H), 7.49 (m, 4H), 7.89 (d, J = 8.4, 1H). Anal. ($C_{26}H_{31}N_3O_2$) C, H, N.

2-(4-Methyl-1-piperazinyl)-4-phenyl-3-[[(2-tetrahydrofuranyl)oxy]methyl]quinoline (9f). The title compound was prepared in 94% yield starting from 3-hydroxymethyl-2-(4-methyl-1-piperazinyl)-4-phenylquinoline[19a] (**9e**) using the same procedure described for the preparation of **9j** (reaction time 24 h at room temperature). Compound **9f** was obtained as an oil which crystallized on standing: mp 140−141 °C. ^{1}H NMR ($CDCl_3$): 1.83 (m, 4H), 2.38 (s, 3H), 2.64 (m, 4H), 3.44−3.85 (m, 6H), 4.03 (d, J = 9.5, 1H), 4.61 (d, J = 9.8, 1H), 4.74 (br s, 1H), 7.25 (m, 4H), 7.51 (m, 4H), 7.86 (d, J = 8.5, 1H). Anal. ($C_{25}H_{29}N_3O_2$) C, H, N.

4-(2-Fluorophenyl)-2-(4-methyl-1-piperazinyl)-3-[2-[(2-tetrahydrofuranyl)oxy]ethyl]quinoline (9l). The title compound was prepared in 92% yield starting from 4-(2-fluorophenyl)-3-(2-hydroxyethyl)-2-(4-methyl-1-piperazinyl)quinoline[19b] (**9k**) using the same procedure described for the preparation of **9j** (reaction time 6 h at room temperature). Compound **9l** was obtained as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): 1.76 (m, 4H), 2.38 (s, 3H), 2.64 (t, J = 4.7, 4H), 2.91 (m, 2H), 3.36 (m, 5H), 3.69 (m, 3H), 4.92 (t, J = 3.2, 1H), 7.20 (m, 5H), 7.52 (m, 2H), 7.90 (d, J = 8.3, 1H). MS: m/z 435 (M^+, 12). Anal. ($C_{26}H_{30}FN_3O_2$) C, H, N.

4-(2-Fluorophenyl)-2-(4-methyl-1-piperazinyl)-3-[2-[(2-tetrahydropyranyl)oxy]ethyl]quinoline (9m). The title compound was prepared in 91% yield by reaction of 4-(2-fluorophenyl)-3-(2-hydroxyethyl)-2-(4-methyl-1-piperazinyl)-quinoline[19b] (**9k**) with 3,4-dihydro-2*H*-pyran following the same procedure described for the preparation of **9j** (reaction time 19 h at room temperature). Compound **9m** was obtained as a pale-yellow oil. ^{1}H NMR ($CDCl_3$): 1.42−1.66 (m, 6H), 2.38 (s, 3H), 2.64 (t, J = 4.7, 4H), 2.95 (m, 2H), 3.44 (m, 6H), 3.68 (m, 2H), 4.41 (t, J = 5.1, 1H), 7.22 (m, 5H), 7.50 (m, 2H), 7.91 (d, J = 8.3, 1H). MS: m/z 450 (M + 1, 29). Anal. ($C_{27}H_{32}FN_3O_2$) C, H, N.

[[(9-Oxofluoren-1-yl)aminocarbonyl]methyl]triphenylphosphonium Bromide (28). To a solution of 1-amino-9-fluorenone (**27**) (0.57 g, 2.9 mmol) in dichloromethane (20 mL) cooled at 0−5 °C was added a solution of bromoacetyl bromide (0.31 mL, 3.5 mmol) in 10 mL of dichloromethane. After the addition was over, the resulting mixture was stirred overnight at room temperature, and 50 mL of a staturated solution of $NaHCO_3$ was added with vigorous stirring and cooling in ice−water. The organic layer was separated, washed with water, dried over sodium sulfate, and evaporated under reduced pressure. The residue was dissolved into 50 mL of dry benzene, and 0.94 g (3.6 mmol) of triphenylphosphine was added. The resulting mixture was heated to reflux under argon atmosphere for 9 h. The precipitate formed was collected by filtration, washed with dry benzene and *n*-hexane in sequence, and dried under reduced pressure to give pure **28** (1.42 g, 85%) as a yellow solid: mp 229 °C dec. ^{1}H NMR ($CDCl_3$): 5.70 (d, J = 13.8, 2H), 7.26−7.80 (m, 16H), 7.88−7.99 (m, 6H), 10.93 (br s, 1H). Anal. ($C_{33}H_{25}BrNO_2P$) C, H, N.

Indeno[1,2,3-*de*]quinolin-2(3*H*)-one (29). A mixture of **28** (0.72 g, 1.2 mmol) in dry DMF (10 mL) with NaH (0.089 g, 3.7 mmol) was stirred at room temperature for 3 h under argon atmosphere. The reaction mixture was then poured into ice−water and acidified with 3 N HCl. The precipitate was collected by filtration, washed in sequence with water, ethyl acetate, and *n*-hexane, and dried under reduced pressure to give pure **29** (0.25 g, 95%) as a yellow solid: mp 288−289 °C (lit.[22] mp 277−279 °C). ^{1}H NMR ($CDCl_3$): 7.07 (s, 1H), 7.13 (d, J = 8.4,

1H), 7.35−7.58 (m, 4H), 7.79 (d, J = 7.3, 1H), 7.86 (d, J = 7.2, 1H), 9.85 (br s, 1H).

2-Chloroindeno[1,2,3-*de*]quinoline (30). This compound was prepared in 82% yield starting from **30** using the same procedure described for the preparation of imidoyl chlorides **14a**−**d** and was purified by flash chromatography with dichloromethane as the eluent to obtain a colorless oil which crystallized on standing: mp 124−125 °C (lit.[22] mp 121−122 °C). ^{1}H NMR ($CDCl_3$): 7.36−7.53 (m, 2H), 7.70−7.90 (m, 6H).

2-Chloroindeno[1,2,3-*de*]quinoline-1-carboxylic Acid Chloride (33). This compound was prepared in 66% yield starting from ethyl 2,3-dihydro-2-oxoindeno[1,2,3-*de*]quinoline-1-carboxylic acid[22] (**32**) using the same procedure described for the preparation of imidoyl chlorides **14a**−**d** and was purified by flash chromatography with dichloromethane as the eluent. ^{1}H NMR ($CDCl_3$): 7.39 (t, J = 7.8, 1H), 7.53 (t, J = 7.6, 1H), 7.82 (m, 5H). MS: m/z 299 (M^+, 24).

Ethyl 2-Chloroindeno[1,2,3-*de*]quinoline-1-carboxylate (34). This compound was prepared in 83% yield starting from ethyl 2,3-dihydro-2-oxoindeno[1,2,3-*de*]quinoline-1-carboxylate[22] (**31**) using the same procedure described for the preparation of imidoyl chlorides **14a**−**d** and was purified by flash chromatography with dichloromethane as the eluent to obtain a yellow solid: mp 122−123 °C. ^{1}H NMR ($CDCl_3$): 1.50 (t, J = 6.9, 3H), 4.62 (q, J = 7.2, 2H), 7.36 (t, J = 7.2, 1H), 7.49 (m, 1H), 7.71−7.88 (m, 5H). Anal. ($C_{18}H_{12}ClNO_2$) C, H, N.

***n*-Butyl 2-Chloroindeno[1,2,3-*de*]quinoline-1-carboxylate (35).** To a mixture of 2,3-dihydro-2-oxoindeno[1,2,3-*de*]-quinoline-1-carboxylic acid[22] (**32**) (0.36 g, 1.4 mmol) in 1-butanol (20 mL) was added $POCl_3$ (1.5 mL). The resulting mixture was refluxed for 1 h and then concentrated under reduced pressure. The residue was treated with ice−water, and the precipitate was collected by filtration, washed with water, and dried under reduced pressure to give a yellow solid. A mixture of this solid in $POCl_3$ (5 mL) was refluxed for 1 h, then cooled, and poured into ice−water. The precipitate was extracted with dichloromethane (3 × 30 mL), and the combined extracts were washed with water, dried over sodium sulfate, and concentrated under reduced pressure. The oil residue was purified by flash chromatography with dichloromethane as the eluent to obtain **35** (0.29 g, 61%) as a yellow crystalline solid: mp 104−105 °C. ^{1}H NMR ($CDCl_3$): 0.99 (t, J = 7.3, 3H), 1.52 (m, 2H), 1.85 (m, 2H), 4.57 (t, J = 6.8, 2H), 7.36 (t, J = 7.3, 1H), 7.49 (t, J = 7.2, 1H), 7.72−7.88 (m, 5H). Anal. ($C_{20}H_{16}ClNO_2$) C, H, N.

2-Chloro-*N*,*N*-dipropylindeno[1,2,3-*de*]quinoline-1-carboxamide (36). To a solution of **33** (0.16 g, 0.53 mmol) in dichloromethane (20 mL) cooled at 0−5 °C was added dipropylamine (0.3 mL, 2.2 mmol). The resulting mixture was stirred at room temperature for 2 h, then washed with water, dried over sodium sulfate, and concentrated under reduced pressure to give pure **36** (0.19 g, 98%) as yellow crystals: mp 152−153 °C. ^{1}H NMR ($CDCl_3$): 0.61 (t, J = 7.4, 3H), 1.09 (t, J = 7.4, 3H), 1.25−1.57 (m, 2H), 1.89 (m, 2H), 3.16 (m, 2H), 3.41 (m, 1H), 3.92 (m, 1H), 7.34 (t, J = 8.0, 1H), 7.47 (t, J = 7.2, 1H), 7.71−7.89 (m, 5H). Anal. ($C_{22}H_{21}ClN_2O$) C, H, N.

X-ray Crystallography. Single crystals of **8g** and **9e,i** were submitted to X-ray data collection on a Siemens P4 four-circle diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71069 Å). The $\omega/2\theta$ scan technique was used. The structures were solved by direct methods, and the refinements were carried out by full-matrix anisotropic least-squares of F^2 against all reflections. The hydrogen atoms were located on Fourier difference maps and included in the structure-factor calculations with a common isotropic temperature factor. Atomic scattering factors including f' and f'' were taken from ref 42. Structure solution and refinement were carried out using the SHELX-97 package,[42] while molecular graphics was performed by the SHELXTL PC program.[43]

8g: $C_{21}H_{25}N_3S$ (MW 351.5), a single crystal, dimensions 0.3 × 0.2 × 0.1 mm, was used for data collection; triclinic; space group $P\bar{1}$ (No. 2); a = 10.3890(10), b = 13.613(2), c = 14.1350(10) Å; α = 75.39(1), β = 78.78(1), γ = 79.10(1)°; V = 1876.8(5) Å^3, Z = 4, D_c = 1.24 g/cm^3. A total of 6567 unique reflections (R_{int} = 0.018) were collected at 22 °C of which 4549 were observed with $F > 4\sigma(F)$. The final refinement converged to R = 0.048 and $wR2$ = 0.113 for $F^2 > 2\sigma(I)$. Minimun and maximum heights in the last $\Delta\rho$ map were −0.25 and 0.26 eÅ^{-3}.

9e: $C_{21}H_{23}N_3O$ (MW 333.42), a single crystal, dimensions 0.2 × 0.2 × 0.1 mm, was used for data collection; monoclinic; space group $P2_1/c$ (No. 14); a = 6.470(2), b = 9.328(3), c = 30.739(7) Å; β = 94.75(2)°; V = 1848.8(9) Å^3; Z = 4, D_c = 1.20 g/cm^3. A total of 2395 unique reflections (R_{int} = 0.062) were collected at 22 °C. The final refinement converged to R = 0.067 and $wR2$ = 0.092 for $F^2 > 2\sigma(I)$. Minimum and maximum heights in the last $\Delta\rho$ map were −0.20 and 0.17 eÅ^{-3}.

9i: $C_{22}H_{25}N_3O$ (MW 347.4), a single crystal, dimensions 0.2 × 0.3 × 0.2 mm, was used for data collection; triclinic; space group $P\bar{1}$ (No. 2); a = 7.734(2), b = 12.364(2), c = 21.504(3) Å; α = 103.25(1), β = 94.08(1), γ = 105.70(1)°; V = 1907.3(6) Å^3, Z = 4, D_c = 1.21 g/cm^3. A total of 4933 unique reflections (R_{int} = 0.043) were collected at 22 °C of which 4549 were observed with $F > 4\sigma(F)$. The final refinement converged to R = 0.062 and $wR2$ = 0.102 for $F^2 > 2\sigma(I)$. Minimun and maximum heights in the last $\Delta\rho$ map were −0.20 and 0.32 eÅ^{-3}.

Full crystallographic details will be given elsewhere.

In Vitro Binding Assays. Binding assays were performed as described in refs 36a (5-HT$_{1A}$ receptors), 36b (5-HT$_{1B}$ receptors), 36c (5-HT$_{2A}$ receptors), 36b (5-HT$_{2C}$ receptors), 29 (5-HT$_3$ receptors), and 36d (5-HT transporter).

Male Wistar rats (Charles River, Calco, Italy) were killed by decapitation, and their brains were rapidly dissected into various areas (hippocampus for 5-HT$_{1A}$; striatum for 5-HT$_{1B}$; pre-frontal cortex for 5-HT$_{2A}$; cortex for 5-HT$_{2C}$; cortex and hippocampus for 5-HT$_3$; forebrain for 5-HT transporter). Tissues were homogenized (Polytron PTA 10TS) in ice-cold Tris HCl, 50 mM, at the appropriate pH value (or 50 mM Hepes buffer, 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 (s.a. 137 Ci/mmol, for 5-HT$_{1A}$; NEN) and [^{3}H]ketanserin (s.a. 80.9 Ci/mmol, for 5-HT$_{2A}$; NEN) binding were assayed in a final incubation volume of 0.5 mL. Tissue concentrations and [^{3}H]ligand final concentrations were 4 mg of tissue/sample and 0.5 nM and 8 mg of tissue/sample and 0.5 nM, respectively.

[^{3}H]Mesulergine (s.a. 86 Ci/mmol, for 5-HT$_{2C}$; Amersham) and [^{3}H]5-HT (s.a. 30 Ci/mmol, for 5-HT$_{1B}$; NEN) binding were 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 (s.a. 81 Ci/mmol, for 5-HT$_3$; NEN) and [^{3}H]-paroxetine (s.a. 29.7 Ci/mmol, for 5-HT transporter; NEN) binding were 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 into 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[44] to obtain the concentration of unlabeled drug that caused 50% inhibition of ligand binding (IC_{50}). Apparent affinity costants (K_i) were derived from the IC_{50} values according to the Cheng and Prusoff equation.[45] 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; 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.[7] 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[7] 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 μM 5-HT, 10 μM substance P, and/or eight different concentrations of each drug to be tested. After 10 min at 37 °C, the assay was stopped by aspiration of the medium, and the cell layer was washed three times with 1.5 mL of ice-cold buffer C (same composition as buffer A except that NaCl was replaced by choline chloride). The cells were then dissolved into 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 substance P than in their absence (basal uptake). 5-HT_3 receptor antagonists (zacopride, ondansetron, tropisetron, etc.) completely prevented the stimulatory effect of 5-HT (with substance P) (see ref 7 for details).

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References

(1) (a) Hartig, P. R. Molecular Pharmacology of Serotonin Receptors. *Experientia, Suppl.* **1994**, 71P, 93−102. (b) Kilpatrick, G. J.; Bunce, K. T.; Tyers, M. B. 5-HT_3 Receptors. *Med. Res. Rev.* **1990**, *10*, 441−475 and references therein.

(2) Silverstone, P. H.; Greenshaw, A J. 5- HT_3 Receptor Antagonists. *Exp. Opin. Ther. Patents* **1996**, *6*, 471−481.

(3) Blandina, P.; Goldfarb, J.; Green, J. P. Activation of a 5-HT_3 Receptor Releases Dopamine from Rat Striatal Slices. *Eur. J. Pharmacol.* **1988**, *155*, 349−350.

(4) Paudice, P.; Raiteri, M. Cholecystokinin Release Mediated by 5-HT_3 Receptors in Rat Cerebral Cortex and Nucleus Accumbens. *Br. J. Pharmacol.* **1991**, *103*, 1790−1794.

(5) Barnes, J. M.; Barnes, N. M.; Costall, B.; Naylor, R. J.; Tyers, M. B. 5-HT_3 Receptors Mediate Inhibition of Acetylcholine Release in Cortical Tissue. *Nature* **1989**, *338*, 762−763.

(6) (a) Ireland, S. J.; Tyers, M. B. Pharmacological Characterization of 5-Hydroxytryptamine-Induced Depolarization of the Rat Isolated Vagus Nerve. *Br. J. Pharmacol.* **1987**, *90*, 229−238. (b) Round, A.; Wallis, D. I. Further Studies on the Blockade of 5-HT Depolarizations of Rabbit Vagal Afferent and Sympathetic Ganglion Cells by MDL 72222 and Other Antagonists. *Neuropharmacology* **1987**, *26*, 39−48.

(7) Emerit, M. B.; Riad, M.; Fattaccini, C. M.; Hamon, M. Characteristics of [^{14}C]Guanidinium Accumulation in NG 108-15 Cells Exposed to Serotonin 5-HT_3 Receptor Ligands and Substance P. *J. Neurochem.* **1993**, *60*, 2059−2067.

(8) Bolanos, F. J.; Schechter, L. E.; Miquel, M. C.; Emerit, M. B.; Rumigny, J. F.; Hamon, M.; Gozlan, H. Common Pharmacological and Physicochemical Properties of 5-HT_3 Binding Sites in the Rat Cerebral Cortex and NG 108-15 Clonal Cells. *Biochem. Pharmacol.* **1990**, *40*, 1541−1550.

(9) Campiani, G.; Cappelli, A.; Nacci, V.; Anzini, M.; Vomero, S.; Hamon, M.; Cagnotto, A.; Fracasso, C.; Uboldi, C.; Caccia, S.; Consolo, S.; Mennini, T. Novel and Highly Potent 5-HT_3 Receptor Agonists Based on a Pyrroloquinoxaline Structure. *J. Med. Chem.* **1997**, *40*, 3670−3678.

(10) Anzini, M.; Cappelli, A.; Vomero, S.; Giorgi, G.; Langer, T.; Hamon, M.; Merahi, N.; Emerit, B. M.; Cagnotto, A.; Skorupska, M.; Mennini, T.; Pinto, J. C. Novel, Potent, and Selective 5-HT_3 Receptor Antagonists Based on the Arylpiperazine Skeleton: Synthesis, Structure, Biological Activity, and Comparative Molecular Field Analysis Studies. *J. Med. Chem.* **1995**, *38*, 2692−2704.

(11) Cappelli, A.; Donati, A.; Anzini, M.; Vomero, S.; De Benedetti, P. G.; Menziani, M. C.; Langer, T. Molecular Structure and Dynamics of Some Potent 5-HT_3 Receptor Antagonists. Insight into the Interaction with the Receptor. *Bioorg. Med. Chem.* **1996**, *4*, 1255−1269.

(12) Cappelli, A.; Anzini, M.; Vomero, S.; Mennuni, L.; Makovec, F.; Doucet, E.; Hamon, M.; Bruni, G.; Romeo, M. R.; Menziani, M. C.; De Benedetti, P. G.; Langer, T. 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. *J. Med. Chem.* **1998**, *41*, 728−741.

(13) White, W. A.; Weingarten, J. J. A Versatile New Enammine Synthesis. *J. Org. Chem.* **1967**, *32*, 213−214.

(14) Rigby, J. H.; Balasubramanian, N. Preparation of Highly Substituted 2-Pyridones by Reaction of Vinyl Isocyanates and Enamines. *J. Org. Chem.* **1989**, *54*, 224−228.

(15) (a) Eloy, F.; Deryckere, A. Novel Synthesis of Pyridine Derivatives. *J. Heterocycl. Chem.* **1970**, *7*, 1191−1193 and references therein. (b) Eloy, F.; Deryckere, A. Sur une Méthode Nouvelle de Synthèse des Aza-2 Phénanthrènes (Benzo[*f*]isoquinoléines). (New Method of Synthesizing 2-Azaphenanthrenes (Benzo[*f*]-isoquinolines)). *Chim. Ther.* **1971**, *6*, 48−49.

(16) Pal, K. An Improved Synthesis of 5-Ethenyl-4a-methyl-2-oxo-2,3,4,4a,7,8-hexahydronaphthalene and Similar 1,3-Dienes Using Palladium Catalyzed Cross-Coupling Methology. *Synthesis* **1995**, 1485−1487.

(17) Scott, W. J.; Peña, M. R.; Swärd, K.; Stoessel, S. J.; Stille, J. K. Palladium-Catalyzed Olefination of Vinyl Triflates. *J. Org. Chem.* **1985**, *50*, 2302−2308.

(18) Filler, R.; Leipold, H. A. Lactones. IX. Reactions of Highly Conjugated Butenolides under Friedel−Crafts Conditions. Synthesis of Polynuclear and Aryl-substituted Butadiene Carboxylic Acids. *J. Org. Chem.* **1962**, *27*, 4440−4443.

(19) (a) Anzini, M.; Cappelli, A.; Vomero, S.; Campiani, G. Synthesis and 5-HT Receptor Binding Studies of some 3-Substituted-2-(4-methyl-1-piperazinyl)-4-phenylquinolines. *Farmaco* **1991**, *46*, 1435−1447. (b) Anzini, M.; Cappelli, A.; Vomero, S. Synthesis of 6,7-Dihydro-8-(4-methyl-1-piperazinyl)-[1]benzoxepino[4,5-*c*]-quinoline as Potential 5-HT_3 Receptor Ligand. *Heterocycles* **1993**, *36*, 1065−1074.

(20) Muchowski, J. M.; Venuti, M. C. Ortho Functionalization of *N*-(*tert*-Butoxycarbonyl)aniline. *J. Org. Chem.* **1980**, *45*, 4798−4801.

(21) Paramasivam, K.; Ramasamy, K.; Shanmugam, P. Furoquinolines; Part XI, A Novel $AlCl_3$−Catalysed Rearrangement of 4-Phenyl-2,3-dihydrofuro[2,3-*b*]quinolines. A New Route to the 5,6-Benzophenanthridine System. *Synthesis* **1977**, 768−770.

(22) Koelsch, C. F.; Steinhauer, A. F. Synthesis, Nitration and Oxidation of 3-Azafluoranthene. *J. Org. Chem.* **1953**, *18*, 1516−1522.

(23) Dewar, M. J. S.; Zoebisch, E. G.; Healey, E. F.; Stewart, J. J. P. AM1: A New General Purpose Quantum Mechanical Molecular Model. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

(24) QUANTA/CHARMm, 1996 Molecular Simulations Inc., 16 New England Executive Park, Burlington, MA 01803-5297.

(25) Katritzky, A. R.; Lobanov, V. S.; Karelson, M. QSPR: The Correlation and Quantitative Prediction of Chemical and Physical Properties from Structure. *Chem. Soc. Rev.* **1995**, 279−287.
(26) Menziani, M. C.; De Benedetti, P. G.; Karelson, M. Theoretical Descriptors in Quantitative Structure-Affinity and Selectivity Relationship Study of potent N4-substituted arylpiperazine 5-HT$_{1A}$ receptor antagonists. *Bioorg. Med. Chem.* **1998**, *6*, 535−550.
(27) Katritzky, A. R.; Mu, L.; Lobanov, V. S.; Karelson, M. Correlation of Boiling Points with Molecular Structure. 1. A Training Set of 298 Diverse Organics and a Test Set of 9 Simple Inorganics. *J. Phys. Chem.* **1996**, *100*, 10400−10407.
(28) Katritzky, A. R.; Lobanov, V. S.; Karelson, M. CODESSA, training manual; CODESSA: Gainesville, FL, 1995.
(29) Nelson, D. R.; Thomas, D. R. [^{3}H]-BRL 43694 (Granisetron) a Specific Ligand for 5-HT$_3$ Binding Sites in Rat Brain Cortical Membranes. *Biochem. Pharmacol.* **1989**, *38*, 1693−1695.
(30) Dukat, M.; Abdel-Rahman, A. A.; Ismaiel, A. M.; Ingher, S.; Teitler, M.; Gyermek, L.; Glennon, R. A. Structure−Activity Relationships for the Binding of Arylpiperazines and Arylbiguanides at 5-HT$_3$ Serotonin Receptors. *J. Med. Chem.* **1996**, *39*, 4017−4026.
(31) Anzini, M.; Cappelli, A.; Vomero, S.; Cagnotto, A.; Skorupska, M. 6-(1-Piperazinyl)7H-indeno[2,1-c]quinoline Derivatives with High Affinity and Selectivity for 5-HT$_3$ Serotonin Sites. *Med. Chem. Res.* **1993**, *3*, 44−51.
(32) Kier, L. B. Use of Molecular Negentropy to Encode Structure Governing Biological Activity. *J. Pharm. Sci.* **1980**, *69*, 807−810.
(33) Karelson, M.; Lobanov, V. S.; Katritzky, A. R. Quantum-Chemical Descriptors in QSAR/QSPR Studies. *Chem. Rev.* **1996**, *96*, 1027−1043 and references cited therein.
(34) Cocchi, M.; De Benedetti, P. G. Use of the Supermolecule Approach to Derive Molecular Similarity Descriptors for QSAR Analysis. *J. Mol. Model.* **1998**, *4*, 113−131.
(35) Stanton, D. T.; Jurs, P. C. Development and Use of Charged Partial Surface Area Structural Descriptors in Computer-Assisted Quantitative Structure-Propertiy Relationship Studies. *Anal. Chem.* **1990**, *62*, 2323−2329.
(36) (a) Hall, M. D.; El Mestikawy, S.; Emerit, M. B.; Pichat, L.; Hamon, M.; Gozlan, M. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino)-tetralin Binding to Pre- and Postsynaptic 5-Hydroxytryptamine Sites in Various Region of the Rat Brain. *J. Neurochem.* **1985**, *44*, 1685−1696. (b) Peroutka, S. J. Pharmacological Differentiation and Characterization of 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ Binding Sites in Rat Frontal Cortex. *J. Neurochem.* **1986**, *47*, 529−540. (c) Leysen, J. E.; Niemegeers, C. J. E.; Van Neuten, J. M.; Laduron, P. M. [^{3}H]Ketanserin (R41468), a Selective 3H−Ligand for Serotonin$_2$ Receptor Sites. *Mol. Pharmcol.* **1982**, *21*, 301−314. (d) Plenge, P.; Mellerup, E. T.; Nielsen, M. Inhibitory and Regulatory Binding Sites on the Rat Brain Serotonin Transporter: Molecular Weight of the [^{3}H]Paroxetine and [^{3}H]Citalopram Binding Proteins. *Eur. J. Pharmacol.* **1990**, *189*, 129−134.
(37) (a) Hibert, M. F.; Hoffmann, R.; Miller, R. C.; Carr, A. A. Conformation-Activity Relationship Study of 5-HT$_3$ Receptor Antagonists and a Definition of a Model for This Receptor Site. *J. Med. Chem.* **1990**, *33*, 1594−1600. (b) Rival, Y.; Hoffmann, R.; Didier, B.; Rybaltchenko, V.; Bourguignon, J.-J.; Wermuth, C. G. *J. Med. Chem.* **1998**, *41*, 311−317.
(38) Morreale, A.; Gàlvez-Ruano, E.; Iriepa-Canalda, I.; Boyd, D. B. Arylpiperazines with Serotonin-3 Antagonist Activity: A Comparative Molecular Field Analysis. *J. Med. Chem.* **1998**, *41*, 2029−2039.
(39) Marsili, A. Trasformazione di Indoni in Derivati della Chinolina e della Isochinolina. Nota I. (Conversion of Indones to Quinoline and Isoquinoline Derivatives. I.) *Ann. Chim.* **1962**, *52*, 3−16.
(40) Kaneko, C.; Naito, T.; Ito, M. Cycloadditions of 1,2-Dihydrocyclobuta[*c*]quinolin-3(4H)ones to Olefins. New Aza-Analogues of *ortho*-Quinodimethanes. *Tetrahedron Lett.* **1980**, *21*, 1645−1648.
(41) Veeramani, K.; Paramasivam, K.; Ramakrishnasubramanian, S.; Shanmugam, P. Photolysis of 4-Phenyl-3-vinylquinolines; A Facile New Route to the Benzo[*k*]phenanthridine System. *Synthesis* **1978**, 855−857.
(42) Sheldrick, G. M. SHELX-97, Rel. 97-2, program for X-ray data diffraction; Gottingen University Gottingen, Germany, 1997.
(43) SHELXTL PC, version 5; Siemens Industrial Automation Inc.: Madison, WI, 1994.
(44) De Lean, K. W.; Munson, P. J.; Rodbard, D. Simultaneous Analysis of Families of Sigmoidal Curves: Application to Bioassay, Radioligand Assay and Physiological Dose−Response Curves. *Am. J. Physiol.* **1978**, *235*, E97−E102.
(45) Cheng, Y.; Prusoff, W. H. Relationship between the Inhibition Constant (Ki) and the Concentration of Inhibitor Which Causes 50 Per Cent Inhibition (IC$_{50}$) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(46) 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.

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