New Benzo[*h*][1,6]naphthyridine and Azepino[3,2-*c*]quinoline Derivatives as Selective Antagonists of 5-HT$_4$ Receptors: Binding Profile and Pharmacological Characterization

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A series of benzo[*h*][1,6]naphthyridine and azepino[3,2-*c*]quinoline derivatives were prepared and evaluated to determine the necessary requirements for high affinity on the 5-HT$_4$ receptors and high selectivity versus other receptors. The compounds were synthesized by substituting the chlorine atom of benzonaphthyridines and azepinoquinolines with various *N*-alkyl-4-piperidinylmethanolates. They were evaluated in binding assays with [^{3}H]GR 113808 as the 5-HT$_4$ receptor radioligand. The affinity values (K_i or inhibition percentages) depended upon the substituent on the aromatic ring on one hand and the substituent on the lateral piperidine chain on the other hand. A chlorine atom produced a marked drop in activity while a *N*-propyl or *N*-butyl group gave compounds with nanomolar affinities ($1 < K_i < 10$ nM). Among the most potent ligands (**3a**, **4a**, **5a)**, **4a** was selected on the basis of its high affinity and selectivity for pharmacological screening and was evaluated in vivo in specific tests. This compound reveals itself as an antagonist/low partial agonist in the COS-7 cells stably expressing the 5-HT$_{4(a)}$ receptor. Derivative **4a** also showed in vivo potent analgesic activity in the writhing test at very low doses.

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) modulates the activity of the central nervous system and peripheral tissues through its actions at numerous receptor subtypes.[1] During the past decades, considerable attention has been centered around the identification of agents which act selectively at each of these receptor subtypes because of the wide range of physiologic systems and pathologic conditions in which 5-HT is known to play a role. One of these receptor subtypes, the 5-HT$_4$ receptor,[2] was first identified in 1988[3] and thereafter has been cloned.[4] Various compounds acting as antagonists on 5-HT$_4$ receptors[5−7] have been described; a generic family of benzamides which act as agonists has been discovered recently.[8,9] Proposed therapeutic applications for agents that bind to the 5-HT$_4$ receptors include the treatment of memory deficits,[10,11] cardiac atrial arrhythmias,[12,13] gastroparesis, urinary incontinence,[14] irritable bowel syndrome,[15] and pain.[16,17]

The availability of the cloned 5-HT$_4$ receptors stimulated more interest in the search of new ligands. Recently, new 5-HT$_4$ antagonists (Chart 1) which possessed a side chain centered around a piperidine ring system were published[5,7] and seemed to afford structure−activity relationships that led to a common pharmacophore.[18,19,20]

In this paper, we report the synthesis, the receptor binding profile, and the in vitro and in vivo pharmacological evaluation of a series of tricyclic benzo[*h*][1,6]-naphthyridine (BN), azepino[3,2-*c*]quinoline (AQ), and tetracyclic dibenzo[*b,h*][1,6]naphthyridine (DBN) derivatives, which are analogues of phenanthridines. These derivatives have the tricyclic skeleton depicted in Chart 2 and the 1-alkylpiperidinylmethyl moiety encountered in carboxylate compounds (GR 113808, SB 204070, SB 207266).

In a manner similar to the one previously conducted for the design of 5-HT$_3$ receptor ligands,[21,22] chemical modifications via substitution of the BN's, AQ's, and DBN's skeletons were systematically carried out to determine the necessary structural requirements for a good affinity on 5-HT$_4$ receptors and a high selectivity toward other receptors.

5-HT$_4$ Receptor antagonists already published (Chart 1) have relatively similar chemical structures. Indeed, they all possess an ester or amide function attached to an aromatic ring and an amine at a variable distance from this ring.[20] In our series, we explored the modification of each of these elements: the aromatic acyl group and the substituent on the basic nitrogen of the piperidine ring system. Because of the expected lability of the ester linkage in compounds such as SB 204070, we focused our synthetic efforts on compounds in which the acyl group was connected to the piperidine ring through

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Chart 1

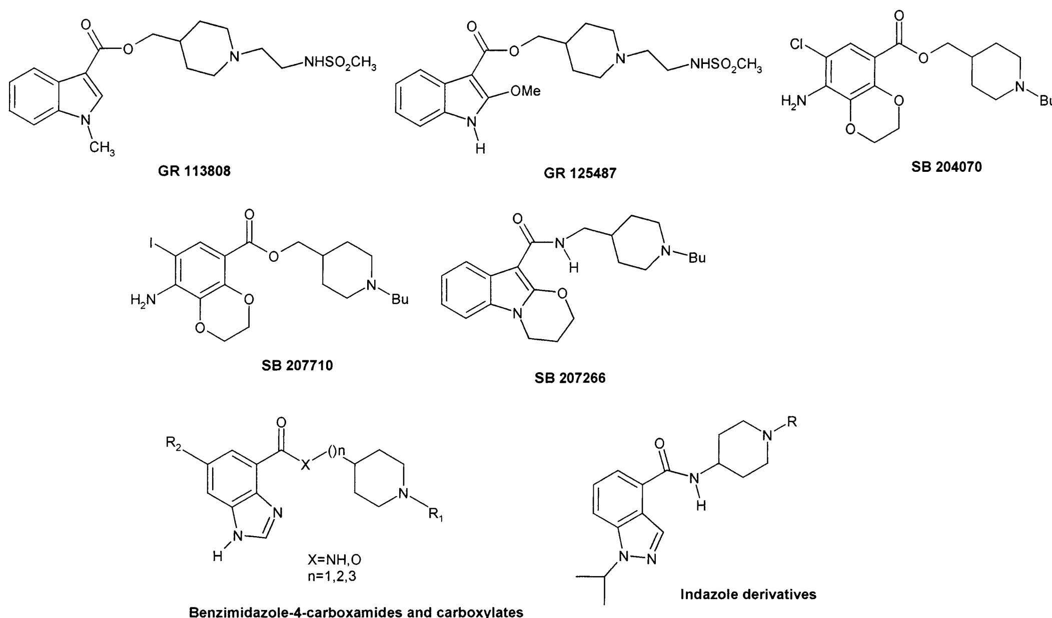
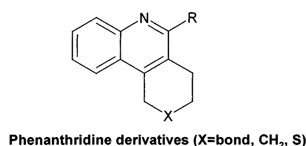


Chart 2



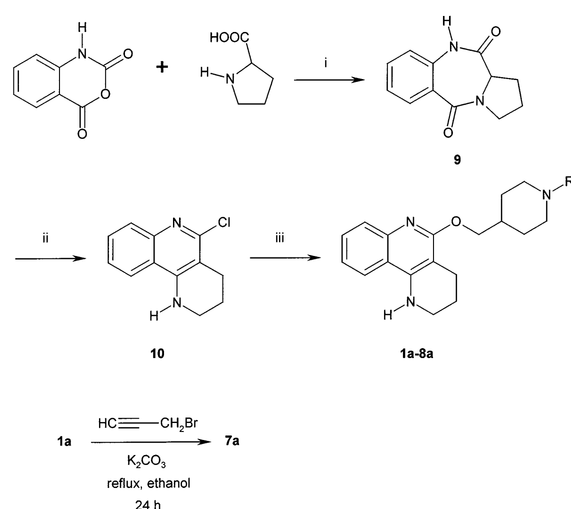
an iminoether linkage. Recent SAR studies that superimpose iminoether derivatives with the pharmacophore of 5-HT$_4$ receptors showed that a cyclic iminoether group could be considered as a bioisoster of the ester function found in 5-HT$_4$ antagonist structures.[20]

Indeed, influence of the substitutions on the pharmacological nature of the interaction with the receptor (partial agonist, antagonist) was also determined for the most active compounds.

Chemistry

The general synthetic procedures used in this study are issued of benzonaphthyridine and azepinoquinoline chemistry that we reported previously[23] and are illustrated in Schemes 1−5. Thus, 5-substituted 1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridines **1a**−**8a** (Scheme 1) were obtained in a three-step pathway starting with condensation of isatoic anhydride and l-proline[24−27] to give the pyrrolo[2,1-*c*][1,4]benzodiazepine **9**. Treatment of **9** under drastic conditions with boiling phosphorus oxychloride and a catalytic amount of pyridine gave the rearranged 5-chloro-1,2,3,4-tetrahydrobenzo[*h*][1,6]-naphthyridine **10**. The displacement of the chlorine atom was realized by treatment of **10** with an excess of the appropriate alcoholate in anhydrous dimethylformamide (DMF) at 230−240 °C in a stainless autoclave to give **1a**−**8a**. The synthesis of corresponding alcoholates was described in the literature or could be obtained by well-known processes; thus, 1-alkyl-4-piperidinylmethanol was prepared from ethyl 4-piperidine carboxylate by *N*-alkylation with 1-halogenoalcane followed by lithium aluminum hydride reduction of the intermediate.[28,29]

Scheme 1[a]



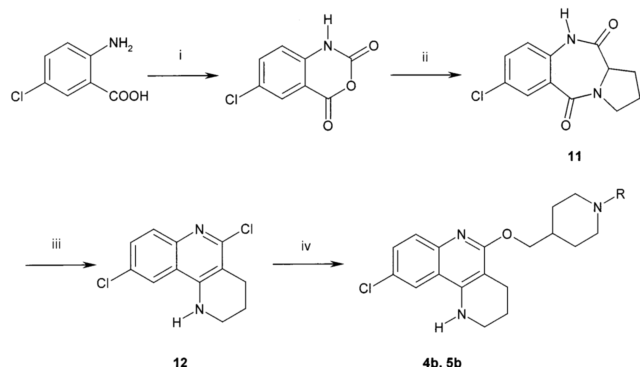
[a] (i) DMF, reflux, 2 h; (ii) $POCl_3$/pyridine, μw (700 W), 1 h 45; (iii) *N*-alkyl-4-piperidinylmethanol, NaH, DMF, or toluene, 230−240 °C, 4−6 bar, 1−2 h.

The (*N*-propargyl-4-piperidinyl) iminoether **7a** was also obtained by treatment of nonsubstituted (4-piperidinyl) iminoether **1a** with propargyl bromide in the presence of K_2CO_3 at reflux of ethanol. No reaction on the naphthyridic NH took place because of its poor reactivity.[23]

Scheme 2 illustrates the synthesis of 9-chloro 5-substituted 1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridines **4b, 5b** which were obtained in a four-step pathway. 6-Chloroisatoic anhydride in the first step was prepared by cyclization of 5-chloroanthranilic acid in the presence of phosgene solution (20% in toluene) in dioxane[30] at room temperature. Then, the following steps of the synthesis were realized in a similar manner as above.

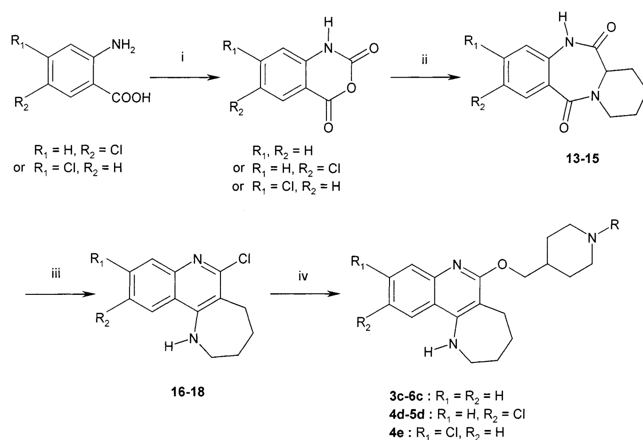
6-Substituted 2,3,4,5-tetrahydro-1*H*-azepino[3,2-*c*]-quinolines **3c**−**6c**, 10-chloro 6-substituted tetrahydroazepino[3,2-*c*]quinolines **4d, 5d** and 9-chloro 6-substituted tetrahydroazepino[3,2-*c*]quinoline **4e** were obtained in a very similar manner as their naphthyridine analogues

Scheme 2[a]



[a] (i) $COCl_2$ (20% in toluene)/dioxane, RT, 18 h; (ii) L-proline, DMF, reflux, 2 h; (iii) $POCl_3$/pyridine, μw (700 W), 1 h 45; (iv) N-alkyl-4-piperidinylmethanol, NaH, toluene, 240 °C, 6 bar, 1.5 h.

Scheme 3[a]



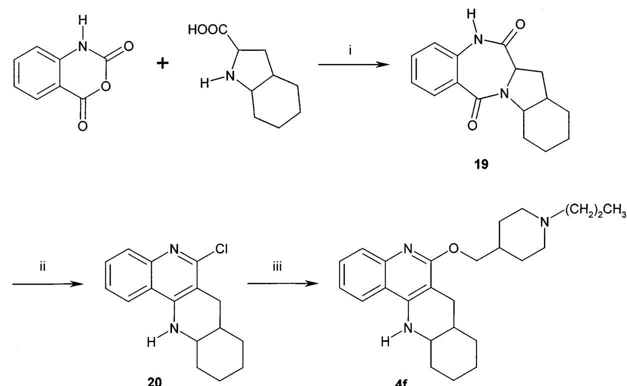
[a] (i) $COCl_2$ (20% in toluene)/dioxane, RT, 18 h; (ii) D,L-pipecolinic acid, DMF, reflux, 2 h; (iii) $POCl_3$/pyridines μw (700W), 1 h 45; (iv) *N*-alkyl-4-piperidinylmethanol, NaH, toluene, 240 °C, 6 bar, 1.5h.

(Scheme 3) starting from D,L-pipecolinic acid which was first condensed with the appropriate isatoic anhydrides to give the pyrido[2,1-*c*][1,4]benzodiazepines **13**–**15**,[31] which then rearranged into chlorotetrahydroazepino-[3,2-*c*]quinolines **16**–**18**.[23,32] Finally, the nucleophilic displacement of the chlorine atom gave **3c**–**6c**, **4d, 5d**, and **4e**.

Scheme 4 illustrates the synthesis of 6-substituted octahydrodibenzo[*b,h*][1,6]naphthyridine **4f** realized in a similar manner as above starting from condensation of isatoic anhydride with *trans*-perhydroindole-2-carboxylic acid to give perhydroindolo[2,1-*c*][1,4]benzodiazepine **19**[33] that rearranged into 6-chloro-octahydrodibenzo[*b,h*][1,6]naphthyridine **20**. Nucleophilic substitution of the chlorine atom with *N*-propyl-4-piperidinylmethanol gave **4f**.

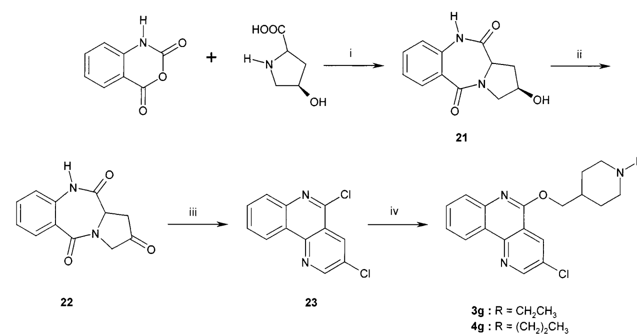
The synthesis of aromatized structures such as 5-substituted 3-chlorobenzo[*h*][1,6]naphthyridines **3g, 4g** was realized in a four-step pathway (Scheme 5) starting with 2-hydroxy-hexahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine **21** prepared from condensation of isatoic anhydride and *trans*-4-hydroxy-L-proline.[34–36] Treatment of alcohol **21** with chromic anhydride and phosphoric acid in acetone gave the ketone **22**.[34,37,38] The 3,5-dichlorobenzo[*h*][1,6]naphthyridine **23** was obtained by rearrangement of **22** in the same condition as described

Scheme 4[a]



a (i) DMF, reflux, 2 h; (ii) $POCl_3$/pyridine, μw (700 W), 1 h 45; (iii) *N*-propyl-4-piperidinylmethanol, NaH, toluene, 240 °C, 6 bar, 1.5h.

Scheme 5[a]



[a] (i) DMF, reflux, 2 h; (ii) CrO_3/H_3PO_4, acetone, 20 h; (iii) $POCl_3$/pyridine, μw (500 W), 50 min; (iv) *N*-alkyl-4-piperidinylmethanol, NaH, toluene, reflux, 4 h.

above.[39] Finally, the rearranged 5-chloro aromatized product **23** was substituted selectively with alcoholates to give the desired iminoethers **3g, 4g**. We did not observe reaction of substitution of the 3-chlorine atom. However, the compound **23** exhibited a better reactivity than the disymmetric rearranged product described above since the reaction of substitution was realized at reflux in toluene.

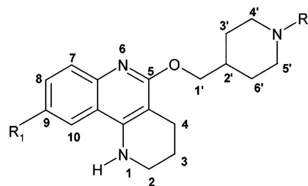
Results and Discussion

Twenty benzo[*h*][1,6]naphthyridines and azepino[3,2-*c*]quinolines derivatives were designed, prepared, and first evaluated in a prescreening procedure for their affinity for 5-HT_4 receptors (Tables 1–2). In addition, selectivity toward other 5-HT receptor subtypes (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_6, and 5-HT_7) and nonserotoninergic receptors as adrenergic, dopamine, histamine, and vasopressin receptors was also evaluated in one representative compound **4a** (Table 3).

The discussion on the SARs are now axed around two moieties: the lateral side chain and the tricyclic platform.

Modification of the Lateral Side Chain. These studies identified a series of *N*-alkyl-4-piperidinylmethoxy derivatives whose affinities ranged between 10^{-8} and 10^{-9} M. We explored the substitution on the piperidine moiety with linear alkyl saturated or nonsaturated groups. The selection of a flexible piperidine system increased 5-HT_4 receptor antagonist activity if we compared with the rigid tropane skeleton encoun-

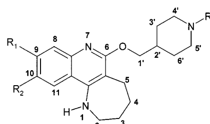
Table 1. Binding Properties of the 1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Derivatives at 5-HT$_4$ Receptors[a]



compd	R	R$_1$	% inhibition 5-HT4 10^{-6} M	% inhibition 5-HT4 10^{-8} M	K_i (nM)
1a	H	H	96	24	
2a	CH_3	H	100	52	79.7
3a	CH_2CH_3	H	100	55	13.1
4a	$(CH_2)_2CH_3$	H	100	70	2.89
5a	$(CH_2)_3CH_3$	H	100	77	10
6a	$(CH_2)_4CH_3$	H	100	51	12.5
7a	CH_2–C≡CH	H	100	15	
8a	CH_2–CH=CH_2	H	100	31	
4b	$(CH_2)_2CH_3$	Cl	100	0	
5b	$(CH_2)_3CH_3$	Cl	100	2	

[a] Percentage of inhibition of 0.6 nM [^{3}H]GR 113808 binding (at fixed concentrations of test compounds) and inhibition constants (K_i, nM) are given.

Table 2. Binding Properties of the 2,3,4,5-tetrahydro-1*H*-azepino[3,2-*c*]quinoline Derivatives at 5-HT$_4$ Receptors[a]



compd	R	R$_1$	R$_2$	% inhibition 5-HT4 10^{-6} M	% inhibition 5-HT4 10^{-8} M	K_i (nM)
3c	CH_2CH_3	H	H	100	51	10.7
4c	$(CH_2)_2CH_3$	H	H	100	55	12.7
5c	$(CH_2)_3CH_3$	H	H	100	44	16.3
6c	$(CH_2)_4CH_3$	H	H	100	0	
4d	$(CH_2)_2CH_3$	H	Cl	91	5	
5d	$(CH_2)_3CH_3$	H	Cl	92	0	
4e	$(CH_2)_2CH_3$	Cl	H	98	0	

[a] Percentage of inhibition of 0.6 nM [^{3}H]GR 113808 binding (at fixed concentrations of test compounds) and inhibition constants (K_i, nM) are given.

tered in Tropisetron that possess a 5-HT$_4$ receptor antagonist activity.[5]

With regard to the basic amino moiety of the molecule, the presence of at least one methylene unit between the acyl group or iminoether group and the 4-substituted piperidine ring is necessary for selective binding at 5-HT$_4$ sites over 5-HT$_3$ receptors.[7] We thus obtained good results of affinities with methyl, ethyl, propyl, and pentyl groups ($2.89 < K_i < 16.3$ nM) for series **a** (**2a**–**6a**) and **c** (**3c**–**5c**). Increasing the length of the linking methylene chain to four and five carbons (**5a, 6a, 5c, 6c**) did not further increase the affinity at 5-HT$_4$ receptors. For compound **6c**, we observed a decrease in affinity with a pentyl group (100% inhibition at 10^{-6} M and 0% inhibition at 10^{-8} M). An increase in the hydrophobic character around the distal nitrogen atom is a favorable parameter since the NH derivative **1a** exhibited a lower affinity for the 5-HT$_4$ receptors (96% inhibition at 10^{-6} M and 24% inhibition at 10^{-8} M) than *N*-alkyl compounds.

Substitution with unsaturated groups (allyl and propargyl) led to a decrease in affinity at the 5-HT$_4$ receptors. It seemed that the loss of affinity could be linked to the unsaturated degree of the alkyl group (allyl compound **8a**, 100% inhibition at 10^{-6} M and 31% inhibition at 10^{-8} M; propargyl compound **7a**, 100% inhibition at 10^{-6} M and 15% inhibition at 10^{-8} M).

Modification of the Aromatic Platform. We also investigated modifications around a quinoline cycle. We thus studied the influence of fused saturated rings (C-6 cycle for the series **a, b**; C-7 cycle for the series **c, d, e**; bicyclic C-6-C-6 for the series **f** and fused aromatic ring for the series **g**). We also studied the influence of substitution of the quinoline cycle with chlorine atoms. In this respect, (a) substitution on the benzene part of the tetrahydrobenzonaphthyridine skeleton with a 9-chloro (**4b, 5b**) and the tetrahydro-1*H*-azepinoquinoline skeleton with a 9-chloro (**4e**) or 10-chloro (**4d, 5d**) clearly decreases the inhibition efficacy at 10^{-8} M; (b) replacement of fused C-6 or C-7 saturated rings by an aromatic ring in the benzonaphthyridine series (ethyl compound **3g**, 81% inhibition at 10^{-6} M and 6% at 10^{-8} M; butyl compound **4g,** 100% inhibition at 10^{-6} M and 8% inhibition at 10^{-8} M) also leads to weaker efficacies; and (c) replacement of fused C-6 or C-7 saturated rings by a bicyclic system in the octahydrodibenzonaphthyridine derivative **4f** results in a marked decrease in affinity for the 5-HT$_4$ receptors (29% inhibition at 10^{-6} M and 0% inhibition at 10^{-8} M).

The above-mentioned results prove the important contribution of the following pharmaphoric sites for high-affinity 5-HT$_4$ ligands: (1) a tricyclic heterocycle, not halogenated on the benzene part and not entirely aromatic; and (2) a voluminous substituent in the basic nitrogen atom of the amino moiety of the lateral side chain and the optimum distance from this nitrogen to the aromatic ring that are important for high-affinity for 5-HT$_4$ receptors. SAR and QSAR studies recently evaluated an ideal distance of around 8 Å between 1 and 2.[18,19,20] Furthermore, the optimum distance from the piperidinic nitrogen to the hydrogen-bond acceptor represented by the "imine" group is 7.4 Å.[20,40]

In conclusion to this preliminary evaluation, the chemical modifications that we carried out led us to the discovery of new compounds having a very high affinity at the 5-HT$_4$ receptor (at least equivalent to the one of SB 204070 or GR 113808) and a high selectivity toward other 5-HT receptor subtypes. Indeed, among these high-affinity compounds, the selectivity of **4a** has been checked versus seven serotonin receptors and reuptake sites (Table 3): the IC$_{50}$ values of this ligand are all representative of low affinities, in the high micromolar range except for the one on 5-HT$_{2B}$ receptors (IC$_{50}$ = 2 × 10^{-7} M). The selectivity of this compound was also extended toward dopaminergic, adrenergic, histaminergic, and vasopressin receptors (Table 3), where **4a** exhibits very limited affinity with IC$_{50}$ values higher than 10^{-6} M for all the receptors tested.

Pharmacology. Compound **4a,** exhibiting the higher affinity, has been selected for characterization of its activity at the 5-HT$_4$ receptor level. The agonist or antagonist properties have been determined in a test evaluating the production of cAMP measured in COS-7 cells expressing the mouse 5-HT$_{4(a)}$ receptor.[41,42] In vivo

Table 3. Binding Selectivity of **4a**

receptor	radioligand	% inhibition 10^{-5} M	% inhibition 10^{-7} M	IC_{50} (M)
5-HT_{1A}	[^{3}H]8-OH-DPAT			9.1×10^{-6}
5-HT_{2A}	[^{3}H]-ketanserin			5×10^{-6}
5-HT_{2B}	[^{3}H]5-HT			2×10^{-7}
5-HT_{2C}	[^{3}H]-mesulergine			3.4×10^{-6}
5-HT_4	[^{3}H]-GR113808			5.6×10^{-10}
5-HT_6	[^{125}I]-LSD	12% at 10^{-6} M	0% at 10^{-8} M	
5-HT_7	[^{125}I]-LSD	36% at 10^{-6} M	20% at 10^{-8} M	
recapture 5-HT	[^{3}H]-paroxetine	79	0	
dopamine D_1	[^{3}H]-SCH23390			$>10^{-5}$
dopamine D_2	[^{3}H]-YM-0915-			3.4×10^{-6}
recapture DA	[^{3}H]-GBR12935	61	13	
histamine H_1	[^{3}H]-mepyramine	91	0	
histamine H_2	[^{3}H]-tiotidine	75	0	
adrenergic β_1	[^{3}H]-CGP12177	39	0	
adrenergic β_2	[^{3}H]-CGP12177	70	0	
recapture NAD	[^{3}H]-nisoxetine	23	17	
vasopressin V_1	[^{3}H]-AVP			3.2×10^{-6}
vasopressin V_2	[^{3}H]-AVP			1.1×10^{-5}

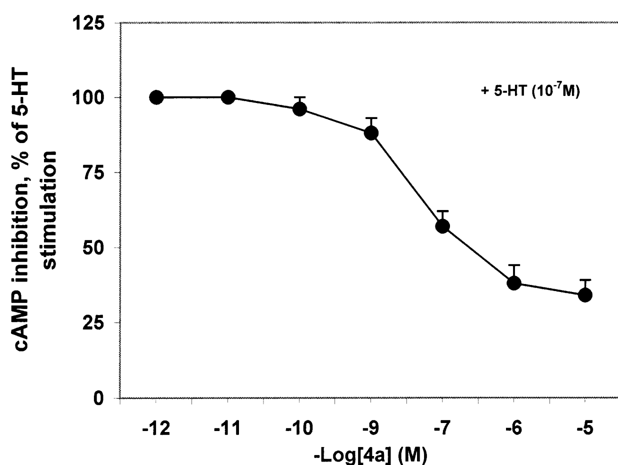


Figure 1. Antagonist profile of **4a**. Concentration-dependent effect of **4a** on adenylyl cyclase activity in COS-7 cells stably transfected with the 5-$HT_{4(a)}$ receptor in the presence of 10^{-7} M of 5-HT.

studies have also been conducted to first determine gross behavioral effects and acute toxicity.[43] Second, considering the demonstrated implication of 5-HT_4 receptors in learning and memory processes,[44−46] the capacity of **4a** to modulate such central action was studied by testing these effects on spontaneous alternation[47] (a working-memory model) in mice treated or not by scopolamine. Finally, the nature of action profile of **4a** was investigated by studying its analgesic potential by simultaneously using the writhing test[48] (peripheral analgesic activity) and the hot plate method[49](central analgesic activity) in the mouse. Recent works have described the potentiality of 5-HT_4 receptor ligands to decrease nociceptive responses in different models of pain in the mouse.[16,17]

Pharmacological Results. The nature of the interaction to the 5-HT_4 receptors was studied in vitro on COS-7 cells. In the presence of 5-HT (10^{-7} M), the compound **4a** decreased the production of cAMP into COS-7 cells. The specific 5-HT_4 antagonist effect of **4a** is ~70% on 5-HT response ($K_i = 5 \pm 1.5$ nM) (Figure 1). In the absence of 5-HT, the compound **4a** produced a slight increase of cAMP which corresponds to ~30% of the 5-HT response that demonstrated an additional partial agonist effect in this case (Figure 2).

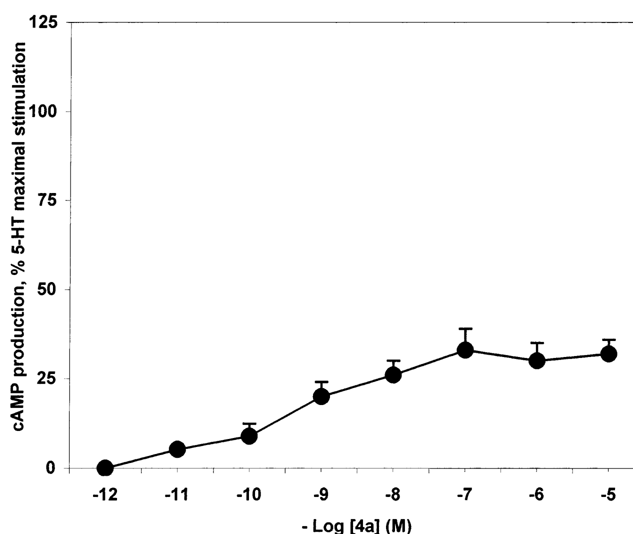


Figure 2. Agonist profile of **4a**. Concentration-dependent effect of **4a** on adenylyl cyclase activity in COS-7 cells stably transfected with the 5-$HT_{4(a)}$ receptor in the absence of 5-HT.

Table 4. Pharmacological and Toxicological Properties of **4a**

compd	doses (mg/kg)	LD_{50} (mg/kg)	symptoms (subtoxic doses)	symptoms (toxic doses)
4a	12.5−25−50	37.5	hypoactivity relaxation passivity	convulsions
methylphenidate	25		hyperactivity irritability stereotypy	
perphenazine	5		hypoactivity passivity ptosis	

The data concerning preliminary toxicological and pharmacological screening of compound **4a** (Table 4) notably showed, at subtoxic doses (at <1/4 approximative LD_{50}), that the major observation was hypoactivity. Considering the LD_{50}, subsequent studies were realized at doses not higher than 1 mg/kg.

At the doses tested (see Experimental Section), compound **4a** had neither a per se effect on spontaneous alternation performance in the mouse, nor an action on the scopolamine-induced deficit in this model (data not shown). In the writhing test (Table 5), **4a** exhibited antinociceptive activities at very weak doses and re-

Table 5. Antinociceptive Activity of Tested Compounds and References in the Mouse Writhing Test after Intraperitoneal Administration

	number of stretches[a]					
	0	0.01 mg/kg	0.1 mg/kg	1 mg/kg	5 mg/kg	30 mg/kg
4a	24.6	19	13.8*	12.5**		
GR 125487	20	16.5	11.5*	11.2*		
GR 113808	21.8	18.2	12.8*	12.3*		
aspirine	25.8					8.7**
piroxicam	24.6				2.4***	

[a] Number of stretches induced by a 0.6% acetic acid solution. Statistical significance between control group and treated group after a combined analysis of variance and a PLSD test.*p < 0.05;**p < 0.01;***p < 0.001.

vealed an activity profile close to the 5-HT$_4$ receptor antagonists GR 125487 and GR 113808. In the same range of doses, no effect could be detected in the hot plate test (data not shown). Taken together, these data suggest a clear peripheral profile for **4a;** considering its high activity in a visceral pain model, this compound could constitute a lead in the search of new peripheral analgesics with molecular targets different than classical nonsteroidal antiinflammatory drugs and thus, potentially devoid of side gastrointestinal effects.

Conclusion

All the compounds we have prepared within this new family of tricyclic benzonaphthyridine and azepinoquinoline derivatives lead us to clearly establish SARs for selective and high-affinity 5-HT$_4$ receptor ligands. Experimental in vivo and in vitro data have revealed the antagonist/partial agonist character of the best 5-HT$_4$ derivatives. Among these, **4a** proved to be of great interest as a peripheral antinociceptive agent. Complementary studies are underway to evaluate the therapeutic potential of this compound. Moreover, these results should be important for the research on the pharmacophores of 5-HT$_4$ receptors.

Experimental Section

Chemistry. Every compound was characterized by elemental analysis, IR spectra, and ^{1}H NMR spectra; data are reported only for the compounds tested in the pharmacological study. IR spectra were recorded on a Genesis Series FTIR infrared spectrometer using KBr pellets; the frequencies are expressed in cm^{-1}. The ^{1}H NMR spectra were obtained on a JEOL Lambda 400 spectrometer, with Me_4Si as the internal standard and DMSO-d_6 as the solvent; the chemical shifts are reported in ppm of Me_4Si in δ units, and the coupling constants are in hertz. The IR and ^{1}H NMR spectra were consistent with assigned structures. Elementary analyses were within ±0.4% of the theoretical values except for compounds **2a**, **7a**, and **3c** for which the hydrogen analysis, respectively, exhibits ±0.42, ±0.53, and ±0.44% errors.

5-Substituted 1,2,3,4-Tetrahydrobenzo[*h*][1,6]naphthyridines 2a–6a. Corresponding alcoholates were prepared from alcohol (9 mmol) dissolved in anhydrous toluene or DMF (25 mL) in the presence of 80% sodium hydride (31.6 mmol). The suspension was stirred under argon and heated at 80–100 °C for 45 min. 5-Chloro-1,2,3,4-tetrahydrobenzo[*h*][1,6]-naphthyridine (**10**) (1.32 g, 6 mmol) diluted in toluene or DMF (10 mL) was added, and the mixture was transferred into a stainless autoclave under pressure (6 bar, 240 °C) for 1.5 h. After cooling, the suspension was filtered and evaporated under reduced pressure. The residue was taken-up with water and extracted with ether (100 mL). The organic layer was washed three times with water, dried ($MgSO_4$), decolorized with vegetal charcoal, and filtered. The solvent was evaporated under reduced pressure. The oil obtained was purified by chromatography on a column of silicagel with $CHCl_3$/MeOH (90:10). The pure oil was dissolved in propan-2-ol; then 1.3 equivalent of fumaric acid was added, and the suspension was refluxed for 10 min. After cooling, the precipitate was filtered and dried first with anhydrous ethyl ether and then in a laboratory oven (50 °C). This gave series **a** (**2a**–**6a**): 11–23% yield.

5-[(*N*-Methylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Monofumarate (2a). Obtained as a white powder, 0.50 g, 19% yield. MP: 125°C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.53 (m, 2H, CH_2), 1.84 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.47 (s, 3H, N–CH_3), 2.50 (m, 2H, $H_{4'}$, $H_{5'}$), 2.62 (m, 2H, CH_2), 3.14 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'}$ = 11.10 Hz), 3.34 (m, 2H, CH_2), 4.21 (d, 2H, O–CH_2, $J_{1',2'}$ = 5.40 Hz), 6;53 (s, 2H, CH=CH, fumarate), 7.06 (s, 1H, NH), 7.22 (t, 1H, H_9), 7.45 (t, 1H, H_8), 7.51 (d, 1H, H_7), 7.90 (d, 1H, H_{10}). IR: 3358 (m, NH), 1690 (s, C=O fumarate), 1636 (s, C=N) cm^{-1}. Anal. ($C_{23}H_{29}N_3O_5$) C, H, N.

5-[(*N*-Ethylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Monofumarate (3a). Obtained as a white powder, 0.28 g, 14% yield. MP: 220 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.12 (t, 3H, CH_3, J = 7.20 Hz), 1.56 (m, 2H, CH_2), 1.86 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.47 (m, 2H, N–CH_2), 2.62 (m, 2H, CH_2), 2.74 (m, 2H, $H_{4'}$, $H_{5'}$), 3.23 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5'5'}$ = 11.60 Hz), 3.34 (m, 2H, CH_2), 4.21 (d, 2H, O–CH_2, $J_{1',2'}$ = 5.90 Hz), 6;51 (s, 2H, CH=CH, fumarate), 7.07 (s, 1H, NH), 7.22 (t, 1H, H_9), 7.45 (t, 1H, H_8), 7.51 (d, 1H, H_7), 7.90 (d, 1H, H_{10}). IR: 3378 (m, NH), 1690 (s, C=O, fumarate), 1637 (s, C=N) cm^{-1}. Anal. ($C_{24}H_{31}N_3O_5$) C, H, N.

5-[(*N*-Propylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Monofumarate (4a). Obtained as a yellow powder, 1.10 g, 11% yield. MP: 200 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.86 (t, 3H, CH_3, J = 7.30 Hz), 1.51 (m, 4H, 2CH_2), 1.82 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.31 (m, 2H, N–CH_2), 2.50 (m, 2H, $H_{4'}$, $H_{5'}$), 2.62 (m, 2H, CH_2), 3.12 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'}$ = 11.10 Hz), 3.34 (m, 2H, CH_2), 4.19 (d, 2H, O–CH_2, $J_{1',2'}$ = 5.70 Hz), 6;53 (s, 2H, CH=CH, fumarate), 7.05 (s, 1H, NH), 7.22 (t, 1H, H_9), 7.45 (t, 1H, H_8), 7.51 (d, 1H, H_7), 7.90 (d, 1H, H_{10}). IR: 3379 (m, NH), 1705 (s, C=O, fumarate), 1595 (s, C=N) cm^{-1}. Anal. ($C_{25}H_{33}N_3O_5$) C, H, N.

5-[(*N*-Butylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Monofumarate (5a). Obtained as a white powder, 0.30 g, 14% yield. MP: 210 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.88 (t, 3H, CH_3, J = 7.30 Hz), 1.29 (sext, 2H, CH_2, $J_{9',8'} = J_{9',10'}$ = 7.30 Hz), 1.51 (m, 4H, 2CH_2), 1.83 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.39 (m, 2H, N–CH_2), 2.61 (m, 4H, CH_2, $H_{4'}$, $H_{5'}$), 3.18 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'}$ = $J_{5',5'}$ = 11.50 Hz), 3.34 (m, 2H, CH_2), 4.20 (d, 2H, O–CH_2, $J_{1',2'}$ = 5.80 Hz), 6;53 (s, 2H, CH=CH, fumarate), 7.06 (s, 1H, NH), 7.22 (t, 1H, H_9), 7.45 (t, 1H, H_8), 7.51 (d, 1H, H_7), 7.90 (d, 1H, H_{10}). IR: 3302 (m, NH), 1698 (s, C=O, fumarate), 1639 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{35}N_3O_5$) C, H, N.

5-[(*N*-Pentylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Monofumarate (6a). Obtained as a white powder, 0.50 g, 23% yield. MP: 212 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.87 (t, 3H, CH_3, J = 6.90 Hz), 1.27 (m, 4H, 2CH_2), 1.46 (m, 4H, 2CH_2), 1.83 (m, 2H, $H_{4'}$, $H_{5'}$), 2.45 (t, 2H, N–CH_2), 2.62 (m, 2H, CH_2), 3.05 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'}$ = 10.30 Hz), 3.34 (m, 2H, CH_2), 4.19 (d, 2H, O–CH_2, $J_{1',2'}$ = 5.50 Hz), 6;50 (s, 2H, CH=CH, fumarate), 7.03 (s, 1H, NH), 7.21 (t, 1H, H_9), 7.44 (t, 1H, H_8), 7.51 (d, 1H, H_7), 7.89 (d, 1H, H_{10}). IR: 3300 (m, NH), 1694 (s, C=O, fumarate), 1618 (s, C=N) cm^{-1}. Anal. ($C_{27}H_{37}N_3O_5$) C, H, N.

5-[(Piperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo-[*h*][1,6]naphthyridine (1a). A suspension of 4-hydroxymethylpiperidine (1.5 g, 12.8 mmol) and sodium hydride (1,17 g, 39 mmol) in anhydrous toluene (40 mL) was stirred under argon and heated at 80–100 °C for 45 min. 5-Chloro-1,2,3,4-

tetrahydrobenzo[*h*][1,6]naphthyridine (**10**) (2 g, 9.1 mmol) was added and the mixture was heated under reflux for 24 h. Then, the mixture was transferred into a stainless autoclave under pressure (4 bar, 230 °C) for 1.5 h. After cooling, the suspension was filtered and the solvent evaporated to give a residual oil that was crystallized by adding Et_2O. This gave **1a** as a yellow powder: 0.72 g, 26% yield. MP: 140 °C (Et_2O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.15 (m, 2H, $H_{3'}$, $H_{6'}$), 1.67 (m, 2H, $H_{3'}$, $H_{6'}$), 1.84 (m, 3H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.43 (m, 2H, $H_{4'}$, $H_{5'}$), 2.60 (m, 2H, CH_2), 2.92 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 12.20$ Hz), 3.33 (m, 2H, CH_2), 4.12 (d, 2H, O$-CH_2$, $J_{1',2'} = 6.10$ Hz), 7.02 (s, 1H, NH), 7.20 (t, 1H, H_9), 7.43 (t, 1H, H_8), 7.50 (d, 1H, H_7), 7.88 (d, 1H, H_{10}). IR: 3274 (m, NH), 1620 (s, C=N) cm^{-1}. Anal. ($C_{18}H_{23}N_3O$) C, H, N.

5-[(*N*-Propargylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine (7a). Procedure A. A suspension of *N*-propargyl-4-hydroxymethylpiperidine (1 g, 6.8 mmol) and sodium hydride (0.72 g, 24 mmol) in anhydrous toluene (20 mL) was stirred under argon and heated at 80−100 °C for 45 min. 5-Chloro-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine (**10**) (1 g, 4.5 mmol) was added and the mixture was heated under reflux for 72 h. Then, the mixture was transferred in a stainless autoclave under pressure (4 bar, 228 °C) for 1 h 10 min. After cooling, the suspension was filtered and evaporated to give a residue that was purified by chromatography on a column of silica gel with $CHCl_3$/EtOAc/ Et_3N (49.3:49.3:1.4). This gave **7a** as a yellow powder: 0.08 g, 5% yield.

Procedure B. 5-[(Piperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine (**1a**) (0.40 g, 1.30 mmol) was dissolved in ethanol (6 mL). Potassium carbonate (0.28 g, 1.90 mmol) and then propargyl bromide (0.17 mL, 1.40 mmol) were added. The mixture was refluxed for 24 h, then filtered. The solvent was evaporated to give a residue that was extracted by Et_2O. We obtained **7a** as a yellow powder washed with petroleum ether: 0.33 g, 73% yield. MP: 50 °C (Et_2O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.33 (m, 2H, $H_{3'}$, $H_{6'}$), 1.69−1.84 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$, CH_2), 2.10 (t, 2H, $H_{4'}$, $H_{5'}$), 2.60 (m, 2H, CH_2), 2.80 (d, 2H, $H_{4'}$, $H_{5'}$), 3.12 (s, 1H, $H_{9'}$), 3.23 (s, 2H, N$-CH_2$), 3,34 (m, 2H, CH_2), 4.15 (d, 2H, O$-CH_2$, $J_{1',2'} = 5.80$ Hz), 7.03 (s, 1H, NH), 7.20 (t, 1H, H_9), 7.43 (t, 1H H_8), 7.50 (d, 1H, H_7), 7.88 (d, 1H, H_{10}). IR: 3287 (m, NH), 1618 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{25}N_3O$) C, H, N.

5-[(*N*-Allylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine (8a). This compound was synthesized with the procedure A used for **7a**. **8a** was purified by chromatography on a column of silicagel with $CHCl_3$/MeOH/ EtOAc (85.7:9.3:5) to give a brown powder: 0.66 g, 21% yield. MP < 50 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.17 (m, 2H, $H_{3'}$, $H_{6'}$), 1.73−1.92 (m, 7H, $H_{2'}$, $H_{3'}$, $H_{4'}$, $H_{5'}$, $H_{6'}$, CH_2), 2.61 (t, 2H, CH_2), 2.87 (d, 2H, $H_{4'}$, $H_{5'}$), 2.93 (d, 2H, N$-CH_2$, $J_{7',8'} = 5.80$ Hz) 3.35 (m, 2H, CH_2), 4.16 (d, 2H, O$-CH_2$, $J_{1',2'} = 5.50$ Hz), 5.10 (d, 1H, $H_{9'}$, $J_{cis} = 10.10$ Hz), 5.16 (d, 1H, $H_{9'}$, $J_{trans} = 17.30$ Hz), 5.82 (m, 1H, $H_{8'}$), 7.04 (s, 1H, NH), 7.21 (t, 1H, H_9), 7.44 (t, 1H H_8), 7.51 (d, 1H, H_7), 7.90 (d, 1H, H_{10}). IR: 3339 (m, NH), 1618 (s, C=N) cm^{-1}. Anal. ($C_{21}H_{27}N_3O$) C, H, N.

5-Substituted9-Chloro-1,2,3,4-tetrahydrobenzo[*h*][1,6]-naphthyridines 4b,5b. The compounds **b** (**4b, 5b**) were prepared from **12** by the same way described above for the derivatives **a** (**2a**−**6a**). This gave series **b** (**4b, 5b**): 8−12% yield.

9-Chloro-5-[(*N*-propylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1,6]naphthyridine Monofumarate (4b). Obtained as a white powder, 0.23 g, 12% yield. MP: 230 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.86 (t, 3H, CH_3, $J = 7.20$ Hz), 1.49 (m, 4H, $2CH_2$), 1.80 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.18 (m, 2H, N$-CH_2$), 2.43 (m, 2H, $H_{4'}$, $H_{5'}$), 2.61 (t, 2H, CH_2), 3.05 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 10.70$ Hz), 3.33 (m, 2H, CH_2), 4.18 (d, 2H, O$-CH_2$, $J_{1',2'} = 5.50$ Hz), 6;51 (s, 2H, CH=CH, fumarate), 7.14 (s, 1H, NH), 7.44 (d, 1H, H_8), 7.52 (d, 1H, H_7), 8.03 (s, 1H, H_{10}). IR: 3271 (m, NH), 1695 (s, C=O, fumarate), 1594 (s, C=N) cm^{-1}. Anal. ($C_{25}H_{32}ClN_3O_5$) C, H, Cl, N.

9-Chloro-5-[(*N*-butylpiperidin-4-yl)methoxy]-1,2,3,4-tetrahydrobenzo[*h*][1, 6]naphthyridine Monofumarate (5b). Obtained as a yellow powder, 0.15 g, 8% yield. MP: 218 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.88 (t, 3H, CH_3, $J = 7.20$ Hz), 1.28 (m, 2H, CH_2), 1.45 (m, 4H, $2CH_2$), 1.80 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.18 (m, 2H, N$-CH_2$), 2.48 (m, 2H, $H_{4'}$, $H_{5'}$), 2.61 (t, 2H, CH_2), 3.05 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 9.60$ Hz), 3.33 (m, 2H, CH_2), 4.18 (d, 2H, O$-CH_2$, $J_{1',2'} = 5.50$ Hz), 6;51 (s, 2H, CH=CH, fumarate), 7.15 (s, 1H, NH), 7.44 (d, 1H, H_8), 7.52 (d, 1H, H_7), 8.04 (s, 1H, H_{10}). IR: 3279 (m, NH), 1695 (s, C=O, fumarate), 1594 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{34}ClN_3O_5$) C, H, Cl, N.

6-Substituted 2,3,4,5-Tetrahydro-1*H*-azepino[3,2-c]quinolines 3c−6c. The compounds **c** (**3c**−**6c**) were prepared from **16** by the same way described above for the derivatives **a** (**2a**−**6a**). This gave series **c** (**3c**−**6c**): 3−13% yield.

6-[(*N*-Ethylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1H-azepino[3,2-c]quinoline Monofumarate (3c). Obtained as a yellow powder, 0.39 g, 13% yield. MP: 126 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.10 (t, 3H, CH_3, $J = 7.10$ Hz), 1.51 (m, 2H, CH_2), 1.83 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$, CH_2), 2.35 (m, 2H, N$-CH_2$), 2.66 (m, 2H, $H_{4'}$, $H_{5'}$), 2.88 (m, 2H, CH_2), 3.16 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 11.10$ Hz), 3.42 (m, 2H, CH_2), 4.19 (d, 2H, O$-CH_2$, $J_{1',2'} = 5.60$ Hz), 6;50 (s, 2H, CH=CH, fumarate), 6.61 (s, 1H, NH), 7.24 (t, 1H, H_{10}), 7.46 (t, 1H, H_9), 7.54 (d, 1H, H_8), 8.02 (d, 1H, H_{11}). IR: 3417 (m, NH), 1692 (s, C=O, fumarate), 1591 (s, C=N) cm^{-1}. Anal. ($C_{25}H_{33}N_3O_5$) C, H, N.

6-[(*N*-Propylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1*H*-azepino[3,2-c]quinoline Monofumarate (4c). Obtained as a beige powder, 0.17 g, 4% yield. MP: 194 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.93 (t, 3H, CH_3, $J = 7.40$ Hz), 1.58 (m, 4H, $2CH_2$), 1.89 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$ CH_2), 2.44 (m, 2H, N$-CH_2$), 2.63 (m, 2H, $H_{4'}$, $H_{5'}$), 2.95 (m, 2H, CH_2), 3.23 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 9.15$ Hz), 3.48 (m, 2H, CH_2), 4.26 (d, 2H, O$-CH_2$, $J_{1',2'} = 3.80$ Hz), 6;60 (s, 2H, CH=CH, fumarate), 6.67 (s, 1H, NH), 7.31 (t, 1H, H_{10}), 7.53 (t, 1H, H_9), 7.61 (d, 1H, H_8), 8.09 (d, 1H, H_{11}). IR: 3394 (m, NH), 1704 (s, C=O, fumarate), 1593 (s, C=N) cm^{-1}. Anal. ($C_{26}H_{35}N_3O_5$) C, H, N.

6-[(*N*-Butylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1*H*-azepino[3,2-c]quinoline Monofumarate (5c). Obtained as a beige powder, 0.23 g, 5.5% yield. MP: 202 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.88 (t, 3H, CH_3, $J = 7.30$ Hz), 1.29 (sext, 2H, $H_{9'}$ CH_2, $J_{9',8'} = J_{9',10'} = 6.80$ Hz), 1.51 (m, 4H, $2CH_2$), 1.83 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$ CH_2), 2.41 (t, 2H, N$-CH_2$), 2.61 (m, 2H, $H_{4'}$, $H_{5'}$), 2.88 (m, 2H, CH_2), 3.17 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 11.30$ Hz), 3.42 (m, 2H, CH_2), 4.19 (d, 2H, O$-CH_2$, $J_{1',2'} = 5.70$ Hz), 6;53 (s, 2H, CH=CH, fumarate), 6.61 (s, 1H, NH), 7.25 (t, 1H, H_{10}), 7.46 (t, 1H, H_9), 7.54 (d, 1H, H_8), 8.02 (d, 1H, H_{11}). IR: 3340 (m, NH), 1696 (s, C=O, fumarate), 1641 (s, C=N) cm^{-1}. Anal. ($C_{27}H_{37}N_3O_5$) C, H, N.

6-[(*N*-Pentylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1*H*-azepino[3,2-c]quinoline Monofumarate (6c). Obtained as a beige powder, 0.06 g, 3% yield. MP: 174 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.87 (t, 3H, CH_3, $J = 6.0$ Hz), 1.27 (m, 4H, $2CH_2$), 1.50 (m, 4H, $2CH_2$), 1.83 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$ CH_2), 2.33 (m, 2H, N$-CH_2$), 2.56 (m, 2H, $H_{4'}$, H_5), 2.88 (m, 2H, CH_2), 3.14 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 11.10$ Hz), 3.42 (m, 2H, CH_2), 4.19 (d, 2H, O$-CH_2$, $J_{1',2'} = 4.50$ Hz), 6;53 (s, 2H, CH=CH, fumarate), 6.60 (s, 1H, NH), 7.24 (t, 1H, H_{10}), 7.46 (t, 1H, H_9), 7.54 (d, 1H, H_8), 8.02 (d, 1H, H_{11}). IR: 3406 (m, NH), 1701 (s, C=O, fumarate), 1639 (s, C=N) cm^{-1}. Anal. ($C_{28}H_{39}N_3O_5$) C, H, N.

6-Substituted 10-Chloro-2,3,4,5-tetrahydro-1*H*-azepino-[3,2-c]quinolines 4d, 5d. The compounds **d** (**4d**−**5d**) were prepared from **17** by the same way described above for the derivatives **a** (**2a**−**6a**). This gave series **d** (**4d**−**5d**): 5% yield.

10-Chloro-6-[(*N*-Propylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1*H*-azepino[3,2-c]quinoline Monofumarate (4d). Obtained as a white powder, 0.08 g, 5% yield. MP: 190 °C (*i*-PrOH). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.87 (t, 3H, CH_3, $J = 7.30$ Hz), 1.55 (m, 4H, $2CH_2$), 1.83 (m, 5H, CH_2, $H_{2'}$, $H_{3'}$, $H_{6'}$), 2.40 (t, 2H, N$-CH_2$), 2.59 (m, 2H, $H_{4'}$, $H_{5'}$), 2.88 (t,

2H, CH_2), 3.18 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 11{,}70$ Hz), 3.42 (m, 2H, CH_2), 4.19 (d, 2H, O−CH_2, $J_{1',2'} = 5.80$ Hz), 6;53 (s, 2H, CH═CH, fumarate), 6.68 (s, 1H, NH), 7.46 (d, 1H, H_9), 7.54 (d, 1H, H_8), 8.17 (s, 1H, H_{11}). IR: 3361 (m, NH), 1702 (s, C═O, fumarate), 1638 (s, C═N) cm^{-1}. Anal. ($C_{26}H_{34}ClN_3O_5$) C, H, Cl, N.

10-Chloro-6-[(*N*-butylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1*H*-azepino[3,2-c]quinoline Monofumarate (5d). Obtained as a white powder, 0.07 g, 5% yield. MP: 186 °C (*i*-PrOH). ^1H NMR (DMSO-d_6, 400 MHz): δ 0.89 (t, 3H, CH_3, $J = 7.30$ Hz), 1.29 (m, 2H, CH_2), 1.50 (m, 4H, 2CH_2), 1.83 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$ CH_2), 2.34 (t, 2H, N−CH_2), 2.57 (m, 2H, $H_{4'}$, $H_{5'}$), 2.88 (m, 2H, CH_2), 3.14 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 10.30$ Hz), 3.42 (m, 2H, CH_2), 4.18 (d, 2H, O−CH_2, $J_{1',2'} = 5.20$ Hz), 6;54 (s, 2H, CH═CH, fumarate), 6.67 (s, 1H, NH), 7.47 (d, 1H, H_9), 7.54 (d, 1H, H_8), 8.17 (d, 1H, H_{11}). IR: 3346 (m, NH), 1710 (s, C═O, fumarate), 1594 (s, C═N) cm^{-1}. Anal. ($C_{27}H_{36}ClN_3O_5$) C, H, Cl, N.

9-Chloro-6-[(*N*-Propylpiperidin-4-yl)methoxy]-2,3,4,5-tetrahydro-1*H*-azepino[3,2-c]quinoline Monofumarate (4e). This compound was prepared from **18** by the same way described above for the derivatives **a** (**2a**−**6a**). This gave **4e** as a yellow powder, 0.2 g, 26% yield. MP: 248 °C (*i*-PrOH). ^1H NMR (DMSO-d_6, 400 MHz): δ 0.87 (t, 3H, CH_3, $J = 7.30$ Hz), 1.55 (m, 4H, 2CH_2), 1.82 (m, 5H, $H_{2'}$, $H_{3'}$, $H_{6'}$ CH_2), 2.41 (t, 2H, N−CH_2), 2.57 (m, 2H, $H_{4'}$, $H_{5'}$), 2.87 (t, 2H, CH_2), 3.18 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 10{,}70$ Hz), 3.43 (m, 2H, CH_2), 4.19 (d, 2H, O−CH_2, $J_{1',2'} = 5.80$ Hz), 6;53 (s, 2H, CH═CH, fumarate), 6.74 (s, 1H, NH), 7.28 (d, 1H, H_{10}), 7.54 (d, 1H, H_8), 8.06 (s, 1H, H_{11}). IR: 3328 (m, NH), 1699 (s, C═O, fumarate), 1641 (s, C═N) cm^{-1}. Anal. ($C_{26}H_{34}ClN_3O_5$) C, H, Cl, N.

6-[(*N*-Propylpiperidin-4-yl)methoxy]-7,7a,8,9,10,11,11a,-12-octahydrodibenzo[*b,h*][1,6]naphthyridine Monofumarate (4f). The synthesis of this compound followed the same pathway described above for the iminoethers **a** (**2a**−**6a**). This gave **4f** as a white powder, 0.20 g, 17% yield. MP: 184 °C (*i*-PrOH). ^1H NMR (DMSO-d_6, 400 MHz): δ 0.87 (t, 3H, CH_3, $J = 7.20$ Hz), 1.38−1.57 (m, 13H, H piperidine and cyclohexane, CH_2), 1.83−2.00 (m, 3H, H piperidine, $H_{7,7a}$), 2.50 (m, 2H, $H_{4'}$, $H_{5'}$), 2.65 (m, 2H, N−CH_2), 3.24 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 11.10$ Hz), 3.51 (m, 1H, H_{11a}), 4.21 (d, 2H, O−CH_2), 6;54 (s, 2H, CH═CH, fumarate), 6.76 (s, 1H, NH), 7.22 (t, 1H, H_2), 7.45 (d, 1H, H_4), 7.51 (t, 1H, H_3), 8.06 (d, 1H, H_1). IR: 3355 (m, NH), 1705 (s, C═O, fumarate), 1641 (s, C═N) cm^{-1}. Anal. ($C_{29}H_{39}N_3O_5$) C, H, N.

5-Substituted 3-Chlorobenzo[*h*][1,6]naphthyridines 3g, 4g. Corresponding alcoholates was prepared from alcohol (1.3 mmol) dissolved in anhydrous toluene (5 mL) in the presence of 80% sodium hydride (4.7 mmol). The suspension was stirred under argon and heated at 80−100 °C for 45 min. 3,5-Dichlorobenzo[*h*][1,6]naphthyridine (**23**) (0.21 g, 0.8 mmol) was added, and the mixture was heated under reflux for 4 h. After cooling, the solvent was evaporated to give a residue that was extracted with Et_2O. After the usual treatments, the obtained solid was salified by fumaric acid in propan-2-ol. This gave series **g** (**3g, 4 g**): 51−70% yield.

3-Chloro-5-[(*N*-Ethylpiperidin-4-yl)methoxy]benzo[*h*]-[1,6]naphthyridine Monofumarate (3g). Obtained as a white powder, 0.28 g, 70% yield. MP: 196 °C (*i*-PrOH). ^1H NMR (DMSO-d_6, 400 MHz): δ 1.13 (t, 3H, CH_3, $J = 7.30$ Hz), 1.62 (m, 2H, H piperidine), 1.99 (m, 3H, H piperidine), 2.43 (m, 2H, N−CH_2), 2.74 (m, 2H, $H_{4'}$, $H_{5'}$), 3.25 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 11.60$ Hz), 4.46 (d, 2H, O−CH_2, $J_{1',2'} = 5.90$ Hz), 6;53 (s, 2H, CH═CH, fumarate), 7.61 (t, 1H, H_9), 7.79 (t, 1H, H_8), 7.84 (d, 1H, H_7), 8.66 (s, 1H, H_4), 8.82 (d, 1H, H_{10}), 9.21 (s, 1H, H_2). IR: 1687 (s, C═O, fumarate), 1602 (s, C═N) cm^{-1}. Anal. ($C_{24}H_{26}ClN_3O_5$) C, H, Cl, N.

3-Chloro-5-[(*N*-Propylpiperidin-4-yl)methoxy]benzo-[*h*][1,6]naphthyridine Monofumarate (4g). Obtained as a white powder, 1 g, 51% yield. MP: 190 °C (*i*-PrOH). ^1H NMR (DMSO-d_6, 400 MHz): δ 0.88 (t, 3H, CH_3, $J = 5.80$ Hz), 1.59 (m, 4H, CH_2, H piperidine), 1.98 (m, 3H, H piperidine), 2.50 (m, 2H, N−CH_2), 2.62 (m, 2H, $H_{4'}$, $H_{5'}$), 3.22 (d, 2H, $H_{4'}$, $H_{5'}$, $J_{4',4'} = J_{5',5'} = 9.80$ Hz), 4.46 (d, 2H, O−CH_2, $J_{1',2'} = 4.20$ Hz), 6;55 (s, 2H, CH═CH, fumarate), 7.62 (t, 1H, H_9), 7.81 (m, 2H, H_7, H_8), 8.66 (s, 1H, H_4), 8.82 (d, 1H, H_{10}), 9.21 (s, 1H, H_2). IR: 3539 (m, NH^+), 1683 (s, C═O, fumarate), 1603 (s, C═N) cm^{-1}. Anal. ($C_{25}H_{28}ClN_3O_5$) C, H, Cl, N.

Pharmacological Methods: Membrane Preparation and Radioligand Binding Assays. Briefly, male guinea pigs (220−224 g, Iffa Credo, France) were subjected to euthanasia and decapitated. Brains were rapidly removed at 4 °C and striatal regions carefully dissected and pooled. The tissues were then suspended in 10 volumes of HEPES buffer (50 mM, pH 7.4) at 4 °C. After homogenization at 4 °C (Ultra-Turrax, maximal speed, 15 s) and ultracentrifugation (23000 x g, 60 min, 4 °C), the pellet was resuspended in HEPES buffer (50 mM, pH 7.4) at 4 °C to obtain a tissue concentration of about 15 mg protein/ml. The protein concentration was determined by the method of Lowry[50] using bovine serum albumin as the standard.

For radioligand binding studies, membrane preparations were incubated in duplicate (HEPES buffer: 50 mM, pH 7.4) at 37 °C for 30 min with 0.6 nM [^{3}H]-GR 113808 (Amersham, France) and fixed concentrations of compounds under study.[51] Incubation was terminated by rapid filtration through 0.5% polyethylenimine-presoaked Whatman GF/B filters using a Brandel cell harvester. Filters were subsequently washed three times with 4 mL of HEPES buffer (50 mM, pH 7.4) at 4 °C. Nonspecific binding of [^{3}H]-GR 113808 was defined in the presence of 10 μM 5-HT. Results were expressed as the percentage of inhibition of the [^{3}H]-GR 113808 binding (at 10^{-6} and 10^{-8} M of compounds under study, concentrations chosen to first screen for intermediate and high affinity compounds) and as inhibition constants (K_i) for drugs that exhibit inhibition higher than 40% at 10^{-8}M.

Cell Culture and Transfection. cDNA, subcloned into pRK5, was introduced into COS-7 cells by electroporation.[52] Briefly, cells were trypsinized, centrifuged, and resuspended in electroporated buffer (50 mM K_2HPO_4, 20 mM CH_3CO_2K, 20 mM KOH, 26.7 mM $MgSO_4$, pH 7.4) with 25−2000 ng of receptor cDNA. The total amount of DNA was kept constant at 15 μg/transfection with wild-type pRK5 vector. After 15 min at room temperature (RT), 300 μl of cell suspension (10^7 cells) was transferred to a 0.4-cm electroporation cuvette (Bio-Rad, Heidemannstrabe, Munchem) and pulsed with a Gene pulser apparatus (setting 1000 μf, 280 V). Cells were diluted in Dulbecco's modified Eagle's medium (DMEM; 10^6 cells/ml) containing 10% dialyzed fetal bovine serum (dFBS) and plated on 15-cm Falcon Petri dishes or into 12-well clusters at the desired density.

cAMP Formation. Intracellular cAMP levels were determined by measuring the conversion of the [^{3}H]adenine nucleotide precursor [^{3}H]ATP to [^{3}H]cAMP, as described previously.[3] On the sixth day of culture and before each experiment, neurons were incubated at 37 °C for 2 h with culture medium containing 2 μCi/ml [^{3}H]adenine (24 Ci/mmol) (Amersham, UK). After 2 h, the cultures were washed and incubated with 0.75 mM IBMX, 0.1 μM FK, and test agents (agonists or antagonists prepared in culture medium), in a volume of 1 mL, for 5 min at 37 °C. The reaction was stopped by aspiration of the medium and addition of 1 mL of ice-cold 5% trichloracetic acid. Cells were loosened with the aid of a rubber scraper and 100 μl of 5 mM ATP/5 mM cAMP were added to the mixture. Cellular protein was centrifuged at 500 x g and the supernatant was eluted through sequential chromatography on Dowex and alumina columns, which separated [^{3}H]ATP from [^{3}H]-cAMP. We have previously shown that, in neuronal cultures, 0.1 μM FK does not modify basal cAMP concentrations but increases neurotransmitter efficacy in cAMP production; potency remains unaffected.[53]

Behavioral Studies. Animals and Drug Administration. In all studies, male OF1 mice (20−24 g, Iffa Credo, France) were used. All compounds tested were dissolved in saline solution and administered intraperitoneally (10 mL /kg).

CNS−Activity and Acute Toxicity Test. Behavioral and neurological changes induced by graded doses (12.5, 25, 50 mg/kg) of the tested derivatives were evaluated in mice, in groups

of four, by a standardized observation technique[43] at different times (30 min, 3 and 24 h) after intraperitoneal administration. Major changes of behavioral data (for example, hypo- or hyperactivity, ataxia, tremors, convulsion, etc.) were noted in comparison to the control group. The approximate LD_{50} of the compounds were also calculated through the quantification of deaths after 24 h.

Spatial Working Memory. Promnesiant activity of tested compounds was evaluated by reversal of scopolamine-induced deficit on spontaneous alternation behavior in the Y maze test.[47] The black wooden maze consisted of three equally spaced arms (22-cm long, 6.5-cm wide with walls 10-cm high). The mouse was placed at the end of one of the arms and allowed to move freely through the maze during a 5 min session while the sequence of arm entries was recorded by an observer. An arm entry was scored when all four feet crossed into the arm. An alternation was defined as entries into all three arms on a consecutive occasion. The number of possible alternation is thus the total number of arm entries *minus* two; the percentage of alternation was calculated as (actual alternation/possible alternation) × 100. The percentage of alternation of scopolamine-treated mice (1 mg/kg) was significantly reduced in comparison with control mice (52% vs 66%, respectively, $p = 0.0049$ (PLSD of Fisher)). Compound **4a** was tested at 0.25, 0.5, and 1 mg/kg. The administration was realized 30 min before testing. For each dose, four groups were constituted: control (saline + saline), scopolamine (saline + scopolamine), tested compound (compound + saline), and association (compound + scopolamine). In this condition, arecoline (1 mg/kg), used as pharmacological reference, significantly reversed the scopolamine-induced deficit (48% for scopolamine group vs 64% for arecoline + scopolamine group, $p < 0.0001$ (PLSD of Fisher).

Writhing Test. The test employed was essentially that described by Hendershot and Forsaith;[54] however, acetic acid[48] rather than phenylquinone was used to elicit stretching. Groups of eight mice (20−24 g) were injected ip with 10 mL/kg of 0.6% aqueous acetic acid. The mice were placed in an observation beaker, and the number of stretches per animal was counted during a 10-min period starting 10 min after acetic acid treatment. A stretch was defined as a sequence of arching of the back, pelvic rotation, and hind limb extension. Tested and reference compounds were administered 15 min before acetic acid solution.

Hot Plate Test. The method employed for measuring central analgesic effect was first described by Woolfe and McDonald.[49] Briefly, every mouse was individually placed on a plate heated to 55 °C and the time until forepaws licking occurs was recorded by a stop-watch. We measured the reaction times of groups of 10 mice twice before injections (mice must react between 4 and 12 s). The compounds were tested at 0.01, 0.1, and 1 mg/kg ip and reaction times were determined at 15 and 30 min after injection. If an animal did not respond by 30 s (cutoff time), it was removed from the plate to avoid tissue damage. Morphine used as reference at 8 mg/kg induced abolition of avoidance behavior (mean reaction times, 30 s and 26 s at 15 and 30 min, respectively; $p < 0.0001$ vs control at the two times, PLSD of Fisher).

Statistical Analyses. All quantitative data were expressed as mean ±SEM and were analyzed using analysis of variance (ANOVA) followed, in case of significant effects, post-hoc multiple comparison tests (PLSD of Fisher). *p*-Values less than 0.05 were considered to be significant.

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