Structure−Activity Relationship Study on *N*-(1,2,3,4-Tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinehexanamides, a Class of 5-HT$_7$ Receptor Agents. 2

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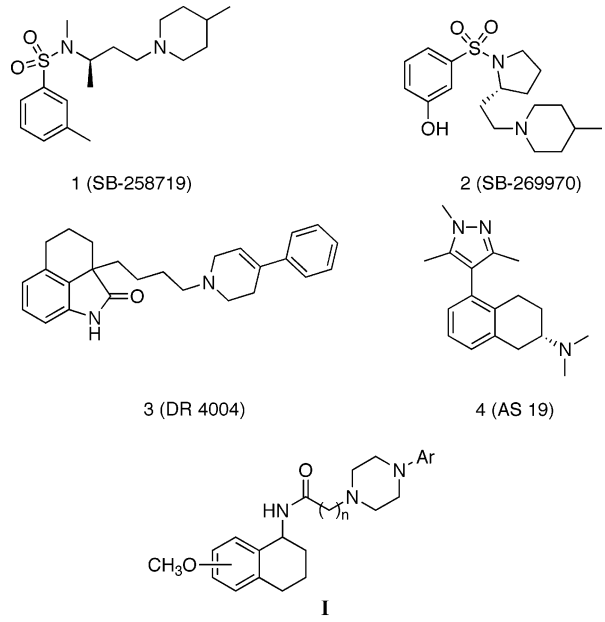
Here we report the synthesis of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides **16**−**29** that were designed to elucidate both structure−affinity and −activity relationships for the 5-HT$_7$ receptor, by targeting the substituent in 2-position of the aryl linked to the piperazine ring. The affinities of **16**−**29** for 5-HT$_7$, 5-HT$_{1A}$, 5-HT$_{2A}$, and D$_2$ receptors were assessed by radioligand binding assays. The intrinsic activities at the 5-HT$_7$ receptor of the most potent compounds were determined. A series of substituents covering a wide range of electronic, steric, and polar properties was evaluated, revealing a key role on 5-HT$_7$ receptor affinity and intrinsic activity. Certain lipophilic substituents (SCH$_3$, CH(CH$_3$)$_2$, N(CH$_3$)$_2$, CH$_3$, Ph) led to high-affinity agonists, whereas OH and NHCH$_3$ substituents switched intrinsic activity toward antagonism. 4-[2-(1-Methylethyl)phenyl]-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**19**), 4-(2-diphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**21**), and 4-(2-dimethylaminophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**22**) were identified as potent 5-HT$_7$ receptor agonists (K_i = 0.13−1.1 nM, EC_{50} = 0.90−1.77 μM), showing selectivity over 5-HT$_{1A}$, 5-HT$_{2A}$, and D$_2$ receptors.

Introduction

The serotonin7 (5-HT$_7$) receptor was discovered in 1993 from the application of molecular biology techniques. This receptor has been identified in several species including human, mouse, rat, guinea pig, and pig. High sequence homology (90%) has been observed between the 5-HT$_7$ receptors from various species, whereas a low degree of homology (40%) has been demonstrated between 5-HT$_7$ receptor and the other Gs-coupled 5-HT receptor subtypes. The 5-HT$_7$ receptor was shown to be positively coupled to adenylyl cyclase via Gs proteins.[1] Distribution studies of the 5-HT$_7$ receptor protein in mice and rats revealed the highest abundance in thalamus, hypothalamus, and hippocampus.[2−5] Within the suprachiasmatic nucleus (SCN[a]) of the hypothalamus, the 5-HT$_7$ receptor is located in both dendrites and axon terminals of mostly GABA-containing neurons.[3] The presence of 5-HT$_7$ receptors in the hypothalamus correlates with their involvement in circadian rhythm, thermoregulation, and endocrine regulation. In addition, thalamic and cortical 5-HT$_7$ receptors might be important for sleep and mood regulation. Finally, 5-HT$_7$ receptors in the hippocampus are of interest in learning and memory.[1] In support of a role for 5-HT$_7$ receptors in depression, *in vitro* radioligand binding studies in rat suggested that chronic antidepressant treatment results in a functional downregulation of 5-HT$_7$-like binding sites in the hypothalamus.[6] New insights concerning the role of 5-HT$_7$ receptors come from studies performed with selective 5-HT$_7$ receptor antagonists or 5-HT$_7$ receptor knockout (KO) mice. 5-HT$_7$ receptor KO mice displayed behavioral and sleep patterns consistent with an antidepressant-like profile.[7] The same robust antidepressant-like effects can be generated in wild type mice by the 5-HT$_7$ receptor antagonists **1** (SB-258719) and **2** (SB-269970) (Chart 1).[8−10] The involvement of 5-HT$_7$ receptors in other pathophysiological mechanisms has been highlighted by various studies. Administration of the selective 5-HT$_7$ receptor antagonist **3** (DR 4004) (Chart 1) significantly inhibited the exploratory behavior of mice, thus suggesting that 5-HT$_7$ receptor blockade might produce changes in some component of emotionality in a novel environment.[11] 5-HT$_7$ receptors are present in microglial cells that represent the resident immune cells of the brain. As such, they are involved in neuroinflammatory processes and can release inflammatory products like interleukin-6 (IL-6).[12] An immunocytochemical study of 5-HT$_7$ receptor distribution at the lumbar level of the spinal cord demonstrated a localization consistent with a predominant role

Chart 1. Structures of 5-HT$_7$ Receptor Agents



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[a] Abbreviations: SCN, suprachiasmatic nucleus; KO, knockout; IL-6, interleukin-6; 5-CT, 5-carboxamidotryptamine; 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropylaminotetralin.

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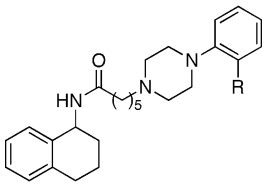
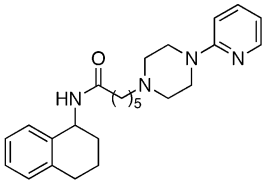
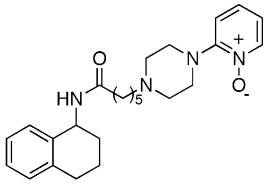
Table 1. Lipophilicity Index and Binding Affinities of Target Compounds **5**−**29**[a]



5-27



28



29

			K_i, nM ± SEM[b]			
compd	R	log k′	5-HT$_7$	5-HT$_{1A}$	5-HT$_{2A}$	D$_{2L}$
5 (LP-44)	SCH_3	1.01	0.22 ± 0.08	52.7 ± 3.2	326 ± 35	7.3 ± 0.50
6	OCH_3	0.63	6.64 ± 0.60	8.60 ± 0.35	1257 ± 165	26.4 ± 1.4
7	CH_3	1.11	15.2 ± 3.2	279 ± 44	262 ± 24	143 ± 10
8	$COCH_3$	0.66	4.14 ± 0.80	3.85 ± 0.10	12200 ± 350	68.0 ± 4.0
9	OH	0.45	11.4 ± 2.3	24.0 ± 6.3	3394 ± 225	987 ± 50
10	H	0.74	65.6 ± 4.7	128 ± 22	77.8 ± 5.7	1000 ± 125
11	NO_2	0.70	63.3 ± 7.5	183 ± 15	282 ± 35	681 ± 15
12	Cl	1.04	40.1 ± 6.7	96.0 ± 8.0	301 ± 12	51.0 ± 8.0
13	CN	0.62	48.7 ± 2.5	16.6 ± 1.4	700 ± 25	265 ± 10
14	$CONH_2$	0.26	229 ± 12	494 ± 35	>4000 (9%)[c]	>1000 (32%)
15	SO_2CH_3	0.41	298 ± 16	3124 ± 260	>4000 (38%)	>1000 (32%)
16	CH_2CH_3	1.29	7.10 ± 0.80	79.2 ± 3.0	3373 ± 320	173 ± 40
17	$(CH_2)_2CH_3$	1.49	49.6 ± 1.5	168 ± 60	3191 ± 270	123 ± 12
18	$(CH_2)_3CH_3$	1.71	2810 ± 490	60.0 ± 9.0	NT[d]	60.0 ± 8.2
19	$CH(CH_3)_2$	1.39	1.10 ± 0.40	167 ± 60	4824 ± 215	15.0 ± 4.1
20	$C(CH_3)_3$	1.60	538 ± 28	1196 ± 630	NT	>1000 (38%)
21	Ph	1.71	0.13 ± 0.05	60.9 ± 2.5	1464 ± 180	224 ± 15
22	$N(CH_3)_2$	1.26	0.90 ± 0.03	112 ± 8	559 ± 240	32.0 ± 5.6
23	$NHCH_3$	0.89	25.4 ± 1.6	133 ± 25	1587 ± 620	107 ± 7.8
24	NH_2	0.45	8178 ± 170	415 ± 20	NT	NT
25	$NHCOCH_3$	0.43	338 ± 29	2500 ± 150	3521 ± 210	>1000 (35%)
26	$NHSO_2CH_3$	0.36	4253 ± 140	NT	NT	>1000 (28%)
27	F	0.82	131 ± 30	29.2 ± 8.0	194 ± 57	292 ± 10
28	--	0.49	278 ± 25	36.4 ± 3.5	535 ± 60	>1000 (15%)
29	--	0.003	8027 ± 740	194 ± 16	NT	NT
5-CT			0.50 ± 0.02			
8-OH-DP AT				2.1 ± 0.5		
ketanserin					3.5 ± 0.2	
haloperidol						2.0 ± 0.4

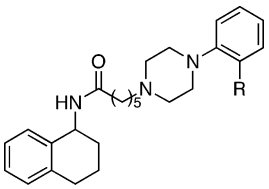
[a] The binding affinities of compounds **5**−**15** at 5-HT$_7$, 5-HT$_{1A}$, and 5-HT$_{2A}$ receptors have been already reported.[21] [b] The values are the means ± SEM. from three independent experiments in triplicate. [c] Full K_i not obtained; percentage inhibition at the concentration shown given in parentheses. [d] Not tested.

in nociception.[13] Finally, analysis of 5-HT$_7$ receptor mRNA expression combined with selective stimulation or blockage of 5-HT$_7$ receptors demonstrated an association between 5-HT$_7$ receptor mRNA expression, memory consolidation, amnesia, or recovery from amnesia, supporting the notion that 5-HT$_7$ receptors play a role in normal and impaired memory.[14,15] In view of these outcomes, the 5-HT$_7$ receptor can be considered an interesting target for drug development.

Soon after the cloning of the 5-HT$_7$ receptor, binding studies revealed that a variety of chemical classes such as ergolines, aporphines, and tricyclic antipsychotic agents were able to bind nonselectively at 5-HT$_7$ receptors. Considerable research efforts directed toward the identification of selective 5-HT$_7$ receptor antagonists have been made by pharmaceutical companies. GlaxoSmithKline identified a class of 5-HT$_7$ receptor antagonists with an arylsulfonamidoalkylamine structure, exemplified by **2** that is considered to date the standard selective 5-HT$_7$ receptor antagonist. Meiji Sieka Kaisha have studied arylpiperidines (and their bioisosteres arylpiperazine and β-carbolines) as 5-HT$_7$ receptor antagonists. The selective antagonist **3** represents the main outcome of those studies.[16] The considerable amount of data on antagonists has allowed the generation of two receptor-based pharmacophores: the first containing features necessary for high 5-HT$_7$ receptor affinity and the other defining selectivity for this receptor subtype.[17] A pharmacophore model has been also generated for 5-HT$_7$ receptor agonists.[18] However, to date a standard selective 5-HT$_7$ receptor agonist has not been identified. In fact, the nonselective 5-carboxamidotryptamine (5-CT) is commonly used as a reference agonist and only two 5-HT$_7$ agonists are commercially available,[19] namely **4** (AS19) (Chart 1)[20] and **5** (LP-44) (Table 1).[21]

As a part of our efforts in identifying selective 5-HT$_7$ receptor ligands with arylpiperazine structure,[22,23] we have discovered a class of 5-HT$_7$ receptor ligands characterized by the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamide structure (Structure I, Chart 1).[21] We demonstrated that for 5-HT$_7$ affinity (i) the presence of a methoxy group on the 1,2,3,4-tetrahydronaphthalen-1-yl group was not essential, (ii) five methylene units separating the amide moiety and the piperazine ring were preferred, and (iii) the position and the nature of the substituent on the phenyl linked to the piperazine ring was critical. In particular, we found that the shifting of the substituent from the 2-position to the 3- or 4-position caused a significant loss in 5-HT$_7$ receptor affinity. Moreover, we found that the nature of the 2-substituent modulated the 5-HT$_7$ receptor affinity (compounds **5**−**15**, Table 1). With this respect, the K_i values at 5-HT$_7$ receptor varied between 298 nM (**15**, R = SO_2CH_3) and 4.14 nM (**8**, R = $COCH_3$). Only compound **5** showed subnanomolar affinity at the 5-HT$_7$ receptor (K_i = 0.22 nM). The most potent compounds **5**−**9** were tested for 5-HT$_7$ intrinsic

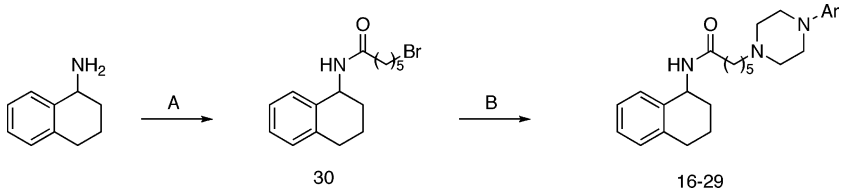
Table 2. Relaxation Effect Induced by Selected Compounds and 5-CT on Substance P-Stimulated Guinea Pig Ileum Contracture



compd	R	% max. activity[a]	EC_{50}, μM	pA_2[b]	pA_2 of **2**[c]
5[d]	SCH_3	100	2.56 ± 0.32		7.70 ± 0.80
6[d]	OCH_3	91	6.32 ± 0.20		8.02 ± 1.40
7[d]	CH_3	98	1.82 ± 0.72		7.80 ± 0.40
8[d]	$COCH_3$	79	2.46 ± 0.70		7.60 ± 0.49
9[d]	OH	0		7.20 ± 0.60	
10	H	40	8.00 ± 0.15		8.1 ± 0.20
11	NO_2	24	10.0 ± 0.7		8.2 ± 0.30
12	Cl	13	17.0 ± 2.3		8.4 ± 0.50
19	$CH(CH_3)_2$	83	0.90 ± 0.04		8.05 ± 0.10
21	Ph	74	1.77 ± 0.08		7.84 ± 0.20
22	$N(CH_3)_2$	100	1.17 ± 0.05		7.90 ± 0.15
23	$NHCH_3$	0		7.70 ± 0.20	
5-CT		100	0.63 ± 0.04		7.48 ± 0.12

[a] Percentage of the relaxation effect mediated by 5-HT_7 receptors. [b] pA_2 values were determined by using the standard 5-HT_7 receptor agonist 5-CT. [c] pA_2 values of the standard antagonist **2** determined by using the corresponding agonist. [d] Data for compounds **5**−**9** have been already reported.[21]

Scheme 1[a]



[a] Reagents: (A) 6-bromohexanoyl chloride, NaOH; (B) 1-arylpiperazine.

activity in an isolated guinea pig ileum assay (Table 2). We found that the 2-hydroxy derivative **9** was an antagonist, whereas the 2-methoxy and 2-acetyl derivatives **6** and **10**, respectively, partially activated the 5-HT_7 receptor, and compounds **5** and **7** (R = SCH_3 and CH_3, respectively) acted as full agonists. Clearly, the nature of the 2-substituent on the phenyl linked to the piperazine ring played a critical role on intrinsic activity. Although that study led to the identification of the potent 5-HT_7 receptor agonist **5**, endowed with good selectivity over 5-HT_{1A} and 5-HT_{2A} receptors (>200-fold), some aspects remained unclear. In particular, we were unable to demonstrate the properties of the substituent in 2-posititon of the phenyl ring responsible for high affinity and for receptor activation, because of the limited number of compounds evaluated. Moreover, the structural features responsible for selectivity over the 5-HT_{1A} receptor were not evident. Therefore, with the aim to clarify those issues, we took under consideration the new derivatives **16**−**29** (Table 1). In particular, from the analysis of intrinsic activities of compounds **5**−**9**, we hypothesized that a lipophilic group could promote the activation of 5-HT_7 receptor whereas an H-bonding donor group would be responsible for the antagonistic property. To test this hypothesis, we prepared the compounds **16**−**22**, bearing in the 2-position of the phenyl linked to the piperazine ring a lipophilic group (R = CH_2CH_3, $(CH_2)_2CH_3$, $(CH_2)_3CH_3$, $CH(CH_3)_2$, $C(CH_3)_3$, Ph, $N(CH_3)_2$) and compounds **23**−**26** showing, instead, an H-bonding donor group (R = $NHCH_3$, NH_2, $NHCOCH_3$, $NHSO_2CH_3$). We also included in the present study the 2-fluorophenyl, pyridyl, pyridyl-*N*-oxide derivatives **27**−**29**. As discussed above, the proposed structural modifications were aimed to the elucidation of the structure−activity relationships. Nonetheless, the newly synthesized compounds would be of great aid to understand the structure−affinity relationships of this class of compounds.

Chemistry. The target compounds were prepared as depicted in Scheme 1. Commercially available 1,2,3,4-tetrahydro-1-naphthalenamine was condensed with 6-bromohexanoyl chloride to afford *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-6-bromohexanamide (**30**)[21] that reacted with the appropriate 1-arylpiperazine to give the final compounds **16**−**29**. The 1-arylpiperazines were obtained from commercial sources or were prepared according to the literature as detailed in the Experimental Section.

Results and Discussion

The target compounds **16**−**29** were evaluated in vitro to determine their affinity for the 5-HT_7 receptors (Table 1). We decided to assess the affinities for 5-HT_{1A}, 5-HT_{2A}, and D_2 receptors (Table 1) because they may interfere with the evaluation of pharmacological actions mediated by 5-HT_7 receptors (i.e., body temperature,[24] sleep patterns[25,26]).

Structure−Affinity Relationships. In the previous study, we noticed that the nature of substituent in 2-position of the *N*-phenyl ring could modulate the affinity for 5-HT_7 receptor, but we were unable to indicate which property could shift affinity values toward the nanomolar range. Comparing the affinities of compounds **7** (R = CH_3), **9** (R = OH), **11** (R = NO_2), **12** (R = Cl), **13** (R = CN), **23** (R = $NHCH_3$), **27** (R = F), bearing substituents with very different electronic properties, one can observe no dramatic variations in 5-HT_7 receptor affinity. These data indicated that the electronic properties of the substituent did not influence the affinity for 5-HT_7 receptor. In fact, compounds **24** (R = NH_2) and **9** (R = OH), which share the same inductive and mesomeric effect, displayed very

different affinity values (K_i = 8178 and 11.4 nM, respectively). By contrast, **11** (R = NO_2) and **7** (R = CH_3) displayed similar affinity values for 5-HT$_7$ receptor (K_i= 63.3 and 15.2 nM, respectively). Moreover, the affinity of the unsubstituted derivative **10** does not differ significantly from that of the above listed compounds. Among the target compounds **5**−**29**, derivatives **5** (R = SCH_3), **19** (R = $CH(CH_3)_2$), **21** (R = Ph), and **22** (R = $N(CH_3)_3$) displayed the highest 5-HT$_7$ affinity values. These data suggested that a lipophilic group could be responsible for high 5-HT$_7$ receptor affinity. In order to verify if a linear relationship existed between 5-HT$_7$ receptor affinity of compounds **5**−**29** and the lipophilicity of the substituent, we have determined the retention factor log k' as the lipophilicity index by using a reversed-phase HPLC method (Table 1).[27] We did not observe a linear relationship between log k' values and pK_i values (data not shown). Therefore, the lipophilicity of the 2-substituent did not seem to be the only requisite for high 5-HT$_7$ affinity. In fact, compounds **17** (R = $(CH_2)_2CH_3$), **18** (R = $(CH_2)_3CH_3$), and **20** (R = $C(CH_3)_3$) possessed significantly lower 5-HT$_7$ receptor affinity values than **19** (R = $CH(CH_3)_2$) and **21** (R = Ph) in spite of comparable log k' values. This may be due to differences in the shape or the volume of the lipophilic substituent. In fact, **19** (R = $CH(CH_3)_2$) proved to be 50-fold more potent than **17** (R = $(CH_2)_2CH_3$). Furthermore, **18** (R = $(CH_2)_3CH_3$) was 50-fold less potent than its analogue **17** (R = $(CH_2)_2CH_3$). Similarly, **5** (R = SCH_3) showed higher affinity than **6** (R = OCH_3) and **12** (R = Cl) was more potent than **27** (R = F). Considering 2-amino derivatives **22**−**24**, it is noteworthy that the simple presence of one CH_3 or two CH_3 substituents on the nitrogen can dramatically change the 5-HT$_7$ affinity (**22**: R = $N(CH_3)_3$, K_i = 0.9 nM; **23**: R = $NHCH_3$, K_i = 25.4 nM; **24**: R = NH_2, K_i = 8178 nM). This trend seemed to be related with the polar nature of the substituents (log k' = 1.26, 0.89, and 0.45, respectively). The negative influence of a polar substituent on 5-HT$_7$ affinity was apparent considering compounds **14**, **15**, **24**−**26**, and **29** (log k' < 0.45). These compounds were the less potent 5-HT$_7$ receptor ligands among those herein reported. However, the lack of 5-HT$_7$ affinity of **24** (R = NH_2) cannot be explained only on the basis of substituent polarity (log k' = 0.45) because derivative **9** (R = OH, log k'= 0.45) displayed good 5-HT$_7$ receptor affinity (K_i = 11.4 nM).

Selectivity. The 5-HT$_{1A}$ receptor affinities of the target compounds generally paralleled those for 5-HT$_7$ receptor. In most cases the K_i ratios 5-HT$_7$/5-HT$_{1A}$ or vice versa were ≤2. The most notably exceptions were represented by compounds **19** (R = $CH(CH_3)_2$), **21** (R = Ph), and **22** (R = $N(CH_3)_2$) which exhibited >100-fold selectivity over 5-HT$_{1A}$ receptor, **21** being even more selective than the previously reported derivative **5** (468- vs 239-fold). Considering the 5-HT$_{2A}$ receptor, all tested compounds, except the unsubstituted derivative **10** (K_i= 77.8 nM), displayed low to very low affinities. This positively reflected on selectivity, especially in the case of the most potent 5-HT$_7$ ligands **5**, **19**, **21**, and **22**. In general, the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamide framework is less prone to bind at 5-HT$_{2A}$ than at 5-HT$_{1A}$ receptor. The dopamine D_2 receptor affinities of the target compounds are lower than those for 5-HT$_7$ receptor (except for **18**). In particular, derivatives **5**, **19**, and **22** displayed acceptable selectivity (33-, 13-, and 35-fold, respectively), whereas exceptional selectivity over D_2 receptor was shown by derivative **21** (R = Ph, D_2/5-HT$_7$ K_i ratio= 1723).

Structure−Activity Relationships. In the previous study for the compounds **5**−**9** we observed that the substituent in the 2-position of the phenyl linked to the piperazine ring played a key role on intrinsic activity. In particular, the 5-HT$_7$ receptor agonists **5**−**8** presented a lipophilic group in the 2-position. To test the hypothesis that lipophilic groups can promote the activation of 5-HT$_7$ receptor, we prepared compounds **16**−**22** as potential 5-HT$_7$ agonists. On the other hand, the antagonistic properties of the 2-hydroxy derivative **9** led us to suppose that an H-bonding donor group could be responsible for 5-HT$_7$ receptor blockade. To elucidate this aspect, we prepared compounds **23**−**26** bearing a 2-substituent with H-bonding donor ability on the phenyl ring. The intrinsic activity at 5-HT$_7$ receptor was assessed in an isolated guinea-pig ileum assay by measuring the 5-HT$_7$ agonist-mediated relaxation of substance P-induced contraction.[28] As discussed in the structure−affinity relationships section, some of the proposed substituents were not tolerated with respect to the 5-HT$_7$ receptor affinity; therefore, compounds showing 5-HT$_7$ affinity values in the proper range could be tested (Table 2). The intrinsic activity of the phenyl derivative **10** (40% maximal activity, EC_{50} = 8 μM) indicated that the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-phenyl-1-piperazinealkylamide framework was able to activate the 5-HT$_7$ receptor independently from the presence of a substituent in 2-position. The insertion of the 2-nitro or 2-chloro substituent (compounds **11** and **12**, respectively) had little effect on intrinsic activity. In fact, both compounds acted as weak partial agonists. The impact on the ability to activate the 5-HT$_7$ receptor is more marked in the case of bulky lipophilic groups. In fact, compounds **19** (83% maximal activity, EC_{50} = 0.90 μM) and **21** (74% maximal activity, EC_{50}= 1.77 μM) acted as partial agonists. Derivative **22** was a full agonist (100% maximal activity, EC_{50} = 1.17 μM). Among the compounds **23**−**26** specifically designed as potential 5-HT$_7$ antagonists, only **23** (R = $NHCH_3$) presented good 5-HT$_7$ affinity. Actually, we found that **23** antagonized the 5-HT$_7$ receptor (0% maximal activity, pA_2 = 7.7) in a similar extent as the 2-hydroxy derivative **9**. Taken together, the intrinsic activities of the new compounds **10**−**12**, **19**, **21**−**23** confirmed our initial hypothesis about the role of the substituent in 2-position of the phenyl ring. Certain apolar substituents (R = $CH(CH_3)_2$, Ph, $N(CH_3)_2$, SCH_3, CH_3) were responsible for 5-HT$_7$ receptor activation, whereas OH and $NHCH_3$ substituents switched intrinsic activity toward antagonism.

Conclusions

In summary, we have described the synthesis of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides **16**−**29**. These compounds were designed to elucidate both structure−affinity and structure−activity relationships for the 5-HT$_7$ receptor by targeting the substituent in 2-position of the aryl linked to the piperazine ring. The affinities of derivatives **16**−**29** for 5-HT$_7$ receptor as well as those for other receptors (serotonin 5-HT$_{1A}$ and 5-HT$_{2A}$, and dopamine D_2) were assessed by radioligand binding assays. The intrinsic activities at 5-HT$_7$ receptor of the most potent compounds were also assessed. Examination of a series of substituents covering a wide range of electronic, steric, and polar properties revealed their key role on both 5-HT$_7$ receptor affinity and intrinsic activity. Certain lipophilic substituents (SCH_3, $CH(CH_3)_2$, $N(CH_3)_2$, CH_3, Ph) led to high affinity agonists, whereas OH and $NHCH_3$ substituents switched intrinsic activity toward antagonism. Among the compounds presented here, derivatives **19**, **21**, and **22** were identified as potent 5-HT$_7$ receptor agonists (K_i = 0.13−1.1 nM, EC_{50} = 0.90−1.77 μM), showing selectivity over 5-HT$_{1A}$, 5-HT$_{2A}$, and D_2 receptors. We believe that these outcomes are relevant in view of the lack of selective 5-HT$_7$ receptor agonists.

To date, the nonselective 5-carboxamidotryptamine (5-CT) is commonly used as reference agonist and only two 5-HT_7 agonists are commercially available, namely **4** and **5**. However, the selectivity of **4** is poorly documented and **5** displays significant dopamine D_2 affinity.

Experimental Section

Chemistry. Column chromatography was performed with 1:30 Merck silica gel 60A (63−200 μm) as the stationary phase. Melting points were determined in open capillaries on a Gallenkamp electrothermal apparatus. Elemental analyses (C, H, N) were performed on Eurovector Euro EA 3000 analyzer; the analytical results were within ±0.4% of the theoretical values for the formula given. ^{1}H NMR spectra were recorded at 300 MHz on a Varian Mercury-VX spectrometer. All spectra were recorded on free bases. All chemical shift values are reported in ppm (δ). Recording of mass spectra was done on an HP6890-5973 MSD gas chromatograph/mass spectrometer; only significant *m*/*z* peaks, with their percentage of relative intensity in parentheses, are reported. ESI$^+$/MS/MS analysis were performed with an Agilent 1100 Series LC-MSD trap System VL workstation. All spectra were in accordance with the assigned structures. The purity of new compounds that were essential to the conclusions drawn in the text were determined by HPLC on a Perkin-Elmer series 200 LC instrument using a Phenomenex Prodigy ODS-3 RP-18 column (250 × 4.6 mm, 5 μm particle size) and equipped with a Perkin-Elmer 785A UV/vis detector setting λ = 254 nm. Compounds **16**−**21** were eluted at a the same flow rate with $CH_3CN/H_2O/Et_3N$, 9:1:0.01, whereas compounds **22**−**29** were eluted with $CH_3OH/H_2O/Et_3N$, 4:1:0.01, v/v at a flow rate of 1 mL/min. A standard procedure was used to transform final compounds into their hydrochloride or oxalate salts. The following compounds were synthesized according to published procedures: 1-(2-ethylphenyl)piperazine,[29] 1-(2-propylphenyl)piperazine,[29] 1-(2-(1-methylethyl)phenyl)piperazine,[29] 1-(2-aminophenyl)piperazine,[30] 1-(2-acetamidophenyl)piperazine,[30] 1-(2-methanesulfonylphenyl)piperazine,[31] 1-(2-methylaminophenyl)piperazine,[32] 1-(2-dimethylaminophenyl)piperazine,[32] 1-(1-oxido-2-pyridinyl)piperazine.[33]

General Procedure for Preparation of Final Compounds 16−**29.** A stirred mixture of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-6-bromohexanamide (**30**) (2.0 mmol), 1-arylpiperazine (2.0 mmol), and K_2CO_3 (2.0 mmol) in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H_2O (20 mL) was added to the residue. The aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL). The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed ($CHCl_3/CH_3OH$, 19:1 as eluent) to yield pure compounds as pale yellow oils.

4-(2-Ethylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (16). 87% Yield. GC-MS *m*/*z* 434 (M^+ + 1, 3), 433 (M^+, 9), 273 (100), 203 (94). ^{1}H NMR: δ 1.24 (t, 3H, *J* = 7.6 Hz), 1.34−1.44 (m, 2H), 1.53−1.63 (m, 2H), 1.67−1.74 (m, 2H), 1.77−1.87 (m, 3H), 2.01−2.07 (m, 1H), 2.22 (t, 2H, *J* = 7.4 Hz), 2.43 (t, 2H, *J* = 7.7 Hz), 2.61 (br s, 4H), 2.69 (q, 2H, *J* = 7.5 Hz), 2.75−2.79 (m, 2H), 2.93 (app t, 4H), 5.18−5.23 (m, 1H), 5.68 (br d, 1H), 7.02−7.11 (m, 3H), 7.14−7.21 (m, 3H), 7.23−7.32 (m, 2H). The hydrochloride salt melted at 207−209 °C (from CH_3OH/Et_2O). Anal. ($C_{28}H_{39}N_3O$·HCl) C, H, N.

4-(2-Propylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (17). 89% Yield. GC-MS *m*/*z* 448 (M^+ + 1, 2), 447 (M^+, 7), 273 (100), 217 (84). ^{1}H NMR: δ 0.97 (t, 3H, *J* = 7.3 Hz), 1.34−1.44 (m, 2H), 1.53−1.74 (m, 6H), 1.77−1.87 (m, 3H), 2.01−2.07 (m, 1H), 2.22 (t, 2H, *J* = 7.4 Hz), 2.42 (t, 2H, *J* = 7.7 Hz), 2.60−2.65 (m, 6H), 2.76−2.80 (m, 2H), 2.92 (app t, 4H), 5.18−5.23 (m, 1H), 5.68 (br d, 1H), 7.00−7.21 (m, 6H), 7.24−7.28 (m, 2H). The hydrochloride salt melted at 208−209 °C (from CH_3OH/Et_2O). Anal. ($C_{29}H_{41}N_3O$·HCl·0.8H_2O) C, H, N.

4-(2-Butylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (18). 43% Yield. GC-MS *m*/*z* 462 (M^+ + 1, 1), 461 (M^+, 2), 273 (100), 231 (87). ^{1}H NMR: δ 0.95 (t, 3H, *J* = 7.3 Hz), 1.34−1.44 (m, 4H), 1.54−1.62 (m, 4H), 1.63−1.74 (m, 2H), 1.77−1.87 (m, 3H), 2.00−2.09 (m, 1H), 2.22 (t, 2H, *J* = 7.6 Hz), 2.43 (t, 2H, *J* = 7.7 Hz), 2.62−2.67 (m, 6H), 2.75−2.81 (m, 2H), 2.93 (app t, 4H), 5.18−5.23 (m, 1H), 5.69 (br d, 1H), 7.00−7.19 (m, 6H), 7.21−7.27 (m, 2H). The hydrochloride salt melted at 206−208 °C (from CH_3OH/Et_2O). Anal. ($C_{30}H_{43}N_3O$·HCl·0.3H_2O) C, H, N.

4-[2-(1-Methylethyl)phenyl]-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (19). 88% Yield. GC-MS *m*/*z* 448 (M^+ + 1, 2), 447 (M^+, 5), 273 (100), 217 (63). ^{1}H NMR: δ 1.20 (d, 6H, *J* = 6.9 Hz), 1.34−1.44 (m, 2H), 1.52−1.62 (m, 2H), 1.67−1.77 (m, 2H), 1.79−1.87 (m, 3H), 2.01−2.06 (m, 1H), 2.22 (t, 2H, *J* = 7.6 Hz), 2.42 (t, 2H, *J* = 7.7 Hz), 2.60 (br s, 4H), 2.76−2.82 (m, 2H), 2.91 (app t, 4H), 3.49 (quintet, 1H, *J* = 6.9 Hz), 5.18−5.23 (m, 1H), 5.70 (br d, 1H), 7.06−7.21 (m, 6H), 7.24−7.27 (m, 2H). The hydrochloride salt melted at 192−195 °C (from CH_3OH/Et_2O). Anal. ($C_{29}H_{41}N_3O$·HCl·0.2H_2O) C, H, N.

4-[2-(1,1-Dimethylethyl)phenyl]-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (20). 46% Yield. GC-MS *m*/*z* 462 (M^+ + 1, 3), 461 (M^+, 7), 273 (100), 231 (91). ^{1}H NMR: δ 1.37−1.44 (s + m, 11H), 1.54−1.63 (m, 2H), 1.70−1.78 (m, 2H), 1.79−1.87 (m, 3H), 2.03−2.07 (m, 1H), 2.23 (t, 2H, *J* = 7.4 Hz), 2.31 (app t, 2H), 2.43 (t, 2H, *J* = 7.4 Hz), 2.77−2.81 (m, 4H), 2.90 (app d, 2H), 3.03 (app t, 2H), 5.18−5.21 (m, 1H), 5.70 (br d, 1H), 7.08−7.28 (m, 6H), 7.36 (app d, 2H). The hydrochloride salt melted at 194−197 °C (from CH_3OH/Et_2O). Anal. ($C_{30}H_{43}N_3O$·$(COOH)_2$) C, H, N.

4-(2-Diphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (21). 64% Yield. GC-MS *m*/*z* 482 (M^+ + 1, 4), 481 (M^+, 12), 273 (75), 251 (100). ^{1}H NMR: δ 1.30−1.38 (m, 2H), 1.44−1.54 (m, 2H), 1.62−1.72 (m, 2H), 1.76−1.84 (m, 3H), 1.99−2.06 (m, 1H), 2.18 (t, 2H, *J* = 7.6 Hz), 2.28−2.33 (m, 6H), 2.75−2.79 (m, 2H), 2.86 (br t app t, 4H), 5.16−5.30 (m, 1H), 5.65 (br d, 1H), 7.02−7.11(m, 3H), 7.14−7.18 (m, 2H), 7.22−7.31 (m, 4H), 7.36−7.41 (m, 2H), 7.62 (dd, 2H, *J* = 1.2, 8.1 Hz). The hydrochloride salt melted at 213−215 °C (from CH_3OH/Et_2O). Anal. ($C_{32}H_{39}N_3O$·2HCl) C, H, N.

4-(2-Dimethylaminophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (22). 77% Yield. GC-MS *m*/*z* 449 (M^+ + 1, 2), 448 (M^+, 7), 204 (22), 162 (65), 149 (100). ^{1}H NMR: δ 1.39−1.44 (m, 2H), 1.49−1.62 (m, 2H), 1.69−1.76 (m, 2H), 1.78−1.86 (m, 3H), 1.97−2.06 (m, 1H), 2.22 (t, 2H, *J* = 7.6 Hz), 2.40 (t, 2H, *J* = 7.6 Hz), 2.59 (br s, 4H), 2.69−2.77 (m, 2H), 2.81 (s, 6H), 3.15 (br s, 4H), 5.16−5.23 (m, 1H), 5.68 (br d, 1H), 6.86−6.98 (m, 4H), 7.01−7.14 (m, 1H), 7.16−7.19 (m, 2H), 7.20−7.31 (m, 1H). The hydrochloride salt melted at 77−79 °C (from CH_3OH/Et_2O). Anal. ($C_{28}H_{40}N_4O$·3HCl·H_2O) C, H, N.

4-(2-Methylaminophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (23). 33% Yield. GC-MS *m*/*z* 435 (M^+ + 1, 11), 434 (M^+, 29), 419 (68), 273 (62), 147 (67), 133 (100). ^{1}H NMR: δ 1.34−1.44 (m, 2H), 1.53−1.63 (m, 2H), 1.67−1.77 (m, 2H), 1.78−1.87 (m, 3H), 1.96−2.07 (m, 1H), 2.22 (t, 2H, *J* = 7.6 Hz), 2.43 (t, 2H, *J* = 7.6 Hz), 2.61 (br s, 4H), 2.76−2.79 (m, 2H), 2.84 (d, 3H, *J* = 2.1 Hz), 2.92 (app t, 4H), 4.65 (br s, 1H, D_2O exchanged), 5.18−5.23 (m, 1H), 5.68 (br d, 1H), 6.59−6.71 (m, 2H), 6.99−7.11 (m, 2H), 7.16−7.19 (m, 2H), 7.23−7.27 (m, 2H). The hydrochloride salt melted at 111−115 °C (from CH_3OH/Et_2O). Anal. ($C_{27}H_{38}N_4O$·3HCl) C, H, N.

4-(2-Aminophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (24). 50% Yield. GC-MS *m*/*z* 421 (M^+ + 1, 7), 420 (M^+, 23), 405 (76), 273 (99), 190 (35), 176 (52), 131 (100). ^{1}H NMR: δ 1.34−1.44 (m, 2H), 1.53−1.63 (m, 2H), 1.67−1.77 (m, 2H), 1.78−1.87 (m, 3H), 2.00−2.07 (m, 1H), 2.22 (t, 2H, *J* = 7.6 Hz), 2.43 (t, 2H, *J* = 7.6 Hz), 2.61 (br s, 4H), 2.75−2.79 (m, 2H), 2.96 (app t, 4H), 3.95 (br s, 2H, D_2O exchanged), 5.18−5.28 (m, 1H), 5.68 (br d, 1H), 6.71−6.77 (m, 2H), 6.90−7.02 (m, 2H), 7.08−7.19 (m, 2H), 7.21−7.27 (m, 2H). The hydrochloride salt melted at 159−163 °C (from CH_3OH/Et_2O). Anal. ($C_{26}H_{36}N_4O$·2HCl·H_2O) C, H, N.

4-(2-Acetylaminophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (25). 47% Yield. ESI$^+$/MS *m*/*z* 463

(MH$^+$). ESI$^+$/MS/MS *m/z* 333 (100). ^{1}H NMR: δ 1.38−1.46 (m, 2H), 1.68−1.78 (m, 4H), 1.80−1.88 (m, 4H), 2.00−2.07 (m, 1H), 2.20 (s, 3H), 2.24 (t, 2H, J = 7.5 Hz), 2.58 (br s, 2H), 2.72−2.85 (m, 6H), 3.00 (br s, 3H), 5.17−5.23 (m, 1H), 5.78 (br d, 1H), 7.03−7.20 (m, 6H), 7.22−7.24 (m, 2H), 8.33 (br d, 0.5 H), 8.39 (br s, 0.5H). The hydrochloride salt melted at 127−130 °C (from CH_3OH/Et_2O). Anal. ($C_{28}H_{38}N_4O_2$·2HCl·0.5H_2O) C, H, N.

4-(2-Methylsulfonamidophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (26). 63% Yield. ESI$^+$/MS *m/z* 499 (MH$^+$). ESI$^+$/MS/MS *m/z* 369 (100). ^{1}H NMR: δ 1.36−1.45 (m, 2H), 1.63 (app t, 2H), 1.67−1.77 (m, 2H), 1.79−1.87 (m, 3H), 2.00−2.07 (m, 1H), 2.22 (t, 2H, J = 7.4 Hz), 2.51 (br s, 2H), 2.71−2.87 (m, 6H), 2.95 (br s, 4H), 3.06 (s, 3H), 5.18−5.23 (m, 1H), 5.73 (br d, 1H), 7.06−7.27 (m, 7H), 7.49 (dd, 1H, J = 1.4, 8.0 Hz), 7.79 (br s, 1H, D_2O exchanged). The hydrochloride salt melted at 84−88 °C (from CH_3OH/Et_2O). Anal. ($C_{27}H_{38}N_4O_3S$·2HCl·H_2O) C, H, N.

4-(2-Fluorophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (27). 90% Yield. GC-MS *m/z* 424 (M$^+$ + 1, 2), 423 (M$^+$, 5), 273 (55), 193 (100). ^{1}H NMR: δ 1.34−1.44 (m, 2H), 1.51−1.61 (m, 2H), 1.66−1.76 (m, 2H), 1.77−1.86 (m, 3H), 1.98−2.07 (m, 1H), 2.21 (t, 2H, J = 7.4 Hz), 2.41 (t, 2H, J = 7.7 Hz), 2.62 (app t, 4H), 2.71−2.85 (m, 2H), 3.11 (app t, 4H), 5.21 (app t, 1H), 5.67 (br d, 1H), 6.89−6.98(m, 2H), 7.01−7.11 (m, 2H), 7.14−7.20 (m, 2H), 7.24−7.27 (m, 2H). The hydrochloride salt melted at 187−191 °C (from CH_3OH/Et_2O). Anal. ($C_{26}H_{34}FN_3O$·HCl) C, H, N.

4-(2-Pyridinyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (28). 70% Yield. GC-MS *m/z* 407 (M$^+$ + 1, 8), 406 (M$^+$, 29), 312 (53), 299 (37), 287 (42), 131 (49), 107 (100). ^{1}H NMR: δ 1.35−1.43 (m, 2H), 1.51−1.61 (m, 2H), 1.65−1.76 (m, 2H), 1.77−1.86 (m, 3H), 1.99−2.07 (m, 1H), 2.21 (t, 2H, J = 7.6 Hz), 2.38 (t, 2H, J = 7.7 Hz), 2.54 (app t, 4H), 2.74−2.78 (m, 2H), 3.53 (app t, 4H), 5.17−5.22 (m, 1H), 5.69 (br d, 1H), 6.59−6.65 (m, 2H), 7.07−7.10 (m, 1H), 7.15−7.18 (m, 2H), 7.19−7.24 (m, 1H), 7.44−7.49 (m, 1H), 8.17−8.19 (m, 1H). The hydrochloride salt melted at 116−121 °C (from CH_3OH/Et_2O). Anal. ($C_{25}H_{34}N_4O$·2HCl·H_2O) C, H, N.

4-(2-Pyridinyl-N-oxide)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (29). 77% Yield. ESI$^+$/MS *m/z* 573 (MH$^+$). ESI$^+$/MS/MS *m/z* 137 (100). ^{1}H NMR: δ 1.32−1.42 (m, 2H), 1.51−1.61 (m, 2H), 1.65−1.75 (m, 2H), 1.77−1.85 (m, 3H), 1.98−2.05 (m, 1H), 2.21 (t, 2H, J = 7.6 Hz), 2.42 (t, 2H, J = 7.7 Hz), 2.67 (br s, 4H), 2.73−2.77 (m, 2H), 3.45 (br s, 4H), 5.16−5.21 (m, 1H), 5.79 (br d, 1H), 6.82−6.87 (m, 2H), 7.06−7.09 (m, 1H), 7.14−7.17 (m, 2H), 7.19−7.26 (m, 1H), 8.11 (dd, 2H, J = 1.1, 6.6 Hz). The hydrochloride salt melted at 135−141 °C (from CH_3OH/Et_2O). Anal. ($C_{24}H_{34}N_4O_2$·2HCl·0.5H_2O) C, H, N.

Lipophilicity. Lipophilicity indices were measured by a reversed-phase HPLC method consisting in a Perkin-Elmer series 200 LC apparatus equipped with a Perkin-Elmer 785A UV/vis detector set at 254 nm. UV signals were monitored and obtained peaks integrated using a personal computer running Perkin-Elmer Turbochrom Software. The capacity factors were measured with a Phenomenex Prodigy ODS-3 RP-18 (250 × 4.6 mm, 5 μm particle size) as nonpolar stationary phase and with MeOH/0.01 M phosphate buffer pH 5 (70:30 v/v) as mobile phase. This ratio was found to be the best compromise for a large number of derivatives. All compounds were dissolved in methanol, and the measurements were made at a flow rate of 1 mL/min. Capacity factors were calculated as: $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the solute and t_0 is the column dead time, measured as the solvent front.

Biological Methods. 1. General. Male Wistar Hannover rats (200−250 g) and male albino Dunkin-Hartley guinea-pigs (300−350 g) were from Harlan (S. Pietro al Natisone, Italy). The animals were handled according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, O.J. No. L358, Dec 18, 1986). Rat recombinant serotonin 5-HT$_7$ receptors and human recombinant serotonin 5-HT$_{1A}$ receptors expressed in HEK-293 cells were purchased from Perkin-Elmer (Zaventem, Belgium). Human recombinant D_{2L} dopamine receptors expressed in rat C6 glioma cells were gifted by Prof. Roberto Maggio (Università di L'Aquila, Italy). [^{3}H]-LSD, [^{3}H]-8-OH-DPAT, [^{3}H]-ketanserin, and [^{3}H]-spiroperidol were obtained from Perkin-Elmer-NEN (Zaventem, Belgium). 5-CT, substance P, and ketanserin were purchased from Tocris Bioscience (Bristol, UK). 8-OH-DPAT hydrobromide was from RBI. Compound **2** and haloperidol were purchased from Sigma-Aldrich (Milan, Italy).

2. Radioligand Binding Assay at Rat Cloned 5-HT$_7$Rs. Binding of [^{3}H]-LSD at rat cloned 5-HT$_7$ receptor was performed according to Jasper et al.[34] with minor modifications. In 1 mL of incubation buffer (50 mM Tris, 10 mM $MgCl_2$ and 0.5 mM EDTA, pH 7,4) were suspended 30 μg of membranes, 2.5 nM [^{3}H]-LSD, the drugs or reference compound (six to nine concentrations). The samples were incubated for 60 min at 37 °C. The incubation was stopped by rapid filtration on GF/A glass fiber filters (presoaked in 0.5% polyethylenimine for 30 min). The filters were washed with 3 × 3 mL of ice-cold buffer (50 mM Tris, pH 7.4). Nonspecific binding was determined in the presence of 10 μM 5-CT. Approximately 90% of specific binding was determined under these conditions.

3. Radioligand Binding Assay at Human Cloned 5-HT$_{1A}$ Receptor. Human 5-HT$_{1A}$ serotonin receptors stably expressed in HEK-293 cells were radiolabeled with 1.0 nM [^{3}H]-8-OH-DPAT.[35] Samples containing 40 μg of membrane protein and different concentrations of each compound ranging from 0.1 nM to 10 μM were incubated in a final volume of 500 μL of 50 mM Tris-HCl pH 7.4, 5 mM $MgSO_4$ for 120 min at 37 °C. After this incubation time, samples were filtered through GF/C presoaked in polyethylenimine 0.5% for at least 30 min prior to use. The filters were washed twice with 1 mL of ice-cold buffer (50 mM Tris-HCl, pH 7.4). Nonspecific binding was determined in the presence of 10 μM 5-HT.

4. Radioligand Binding Assay at Rat Cortex Membranes 5-HT$_{2A}$ Receptors. Binding experiments were performed according to Leysen et al.[36] with minor modifications. Rats were killed by decapitation, the brain was quickly removed, and the cortex was dissected. The cortex (1.0 g) was homogenized with a Brinkman polytron (setting 5 for 3 × 15 s) in 25 mL of 0.25 M sucrose. The homogenate was centrifuged at 2000*g* for 10 min at 4 °C. The supernatant was saved, and the pellet was resuspended in 25 mL of buffer. The supernatants were collected and diluted 1:10 w/w with 10 mM Tris pH 7.4. The homogenate was centrifugated at 35000*g* for 15 min at 4 °C. The supernatant was discarded, and the final pellet was stored at −80 °C until used. Each tube received, in a final volume of 2 mL of 50 mM Tris (pH 7.7), cortex membranes suspension and 2.5 nM [^{3}H]-ketanserin. For competitive inhibition experiments, various concentrations of drugs studied were incubated. Nonspecific binding was defined using 10 μM ketanserin. Samples were incubated at 37 °C for 15 min and then filtered on Whatman GF/B glass microfiber filters. The K_d value determined for ketanserin was 0.42 nM.

5. Radioligand Binding Assay at Human Cloned D_{2L} Dopaminergic Receptors. Binding of [^{3}H]-spiroperidol at human cloned receptors was performed according to Scarselli et al.[37] with minor modifications. The incubation buffer (120 mM NaCl, 5.0 mM KCl, 5.0 mM $MgCl_2$, 1 mM EDTA, 50 mM Tris, pH 7.4) contained 100 μg of dopamine dilute membranes, 0.30−0.50 nM [^{3}H]-spiroperidol (K_d = 0.093 nM), and six to nine concentrations of drug solution in a final volume of 500 μL. The samples were incubated for 120 min at 25 °C, and then the incubation was stopped by rapid filtration through Whatman GF/C glass fiber filters (presoaked in 0.5% polyethylenimine for 60 min). The filters were washed 3 × 1 mL of ice-cold 50 mM Tris, 0.9% NaCl, pH 7.4. Nonspecific binding was determined in the presence of 10 μM haloperidol. The radioactivity bound to the filters was measured by liquid scintillation using LS6500 Multi-Purpose scintillation Counter, Beckman.

6. Isolated Guinea-Pig Ileum Assay. Guinea-pigs were anesthetized and then decapitated and the proximal ileum removed. The intestine was carefully flushed several times with warm Krebs−Henseleit solution (118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl,

0.6 mM $MgSO_4$, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 11.2 mM glucose, pH 7.4). Whole ileal segments, of about 3 cm in length, were suspended under 1.0 g tension in Krebs solution gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C. According to Carter et al.[28] with minor modification, the bathing medium contained 1 μM atropine to antagonize cholinergically mediated contractions due to activation of 5-HT_3 and 5-HT_4 receptors, 1 μM ketanserin to block 5-HT_{2A} receptors, and 1 μM pyrilamine to block H_1 receptors. Changes in tension of the tissue were recorded by Fort 10 Original WPI isometric transducer (2Biological Instruments, Italy) connected to a PowerLab/400 workstation.[38] Tissue responses were recorded as gram changes in isometric tension and expressed as percentage of reduction in the height of the contraction. Tissue was contracted by 100 nM substance P. This value was preliminary determined by concentration−response curves (1 nM to 200 nM). Substance P (100 nM) elicited 80% of maximum contraction. The reference agonist 5-CT was added 5 min before substance P addition and noncumulative concentration−response curves were constructed (0.001 μM to 10 μM). We have determined that 5-CT induced relaxation with maximal response (39%) at 3 μM concentration. Analogously, each compound tested (0.001 μM to 10 μM) was added 5 min before substance P addition and noncumulative concentration−response curve was constructed. Relaxant responses to full agonists 5-CT and **22** and partial agonists **10**−**12**, **19**, and **21** were surmountably antagonized by **2** (0.1 μM to 3 μM). The isolated guinea pig ileum was equilibrated for 15 min with **2** before constructing concentration−response curves of tested compounds. The corresponding pA_2 values of **2** versus each studied agonist are listed in Table 2. Desensitization was then used to discriminate if the studied compounds were acting as 5-HT_7 receptor agonists or nonspecific smooth muscle relaxant. The agonist studies were consequently repeated in the presence of 3 μM 5-CT, following equilibration with 5-CT for 60 min, during which time the bathing solution was changed at 15 min intervals. This concentration of 5-CT abolished relaxant responses emanating from 5-HT_7 receptor stimulation.

7. Statistical Analysis. The inhibition curves on the different binding sites of the compounds reported in Table 1 were analyzed by nonlinear curve fitting utilizing the GraphPad Prism program.[39] The value for the inhibition constant, K_i, was calculated by using the Cheng−Prusoff equation.[40] Agonist potencies, expressed as EC_{50}, were obtained from nonlinear iterative curve fitting by GraphPad Prism.

Supporting Information Available: Elemental analysis data of target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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