Structure−Affinity Relationship Study on *N*-(1,2,3,4-Tetrahydronaphthalen-1-yl)-4-Aryl-1-Piperazinealkylamides, a New Class of 5-Hydroxytryptamine$_7$ Receptor Agents

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A series of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides was prepared and their affinity for serotonin (5-hydroxytryptamine, 5-HT) 5-HT$_7$, 5-HT$_{1A}$, and 5-HT$_{2A}$ receptors was measured by in vitro binding assays. In relation to 5-HT$_7$ receptor affinity, receptor binding studies indicated that (i) the optimal alkyl chain length was five methylenes, (ii) an unsubstituted 1,2,3,4-tetrahydronaphthalenyl nucleus was preferred, and (iii) the substitution pattern of the aryl ring linked to the piperazine ring played a crucial role. Several compound with high affinity for 5-HT$_7$ receptors were identified. Among them, 4-(2-methoxyphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**28**), 4-(2-acetylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**34**), 4-(2-methylthiophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**44**), 4-(2-hydroxyphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**46**), and 4-(2-methylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**49**) were assayed for the 5-HT$_7$ receptor-mediated relaxation of substance P-induced guinea pig ileum contraction. Compounds **28**, **44**, and **49** behaved as full agonists and compound **34** as a partial agonist, whereas derivative **46** acted as an antagonist. Among the compounds presented here, it emerged that **44** was identified as a potent 5-HT$_7$ receptor agonist (K_i = 0.22 nM, EC_{50} = 2.56 μM), endowed with selectivity over 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (200-fold and >1000-fold, respectively).

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) has an array of pharmacological and physiological roles within the central nervous system (CNS) and in the periphery, mediated by its interactions with a total of 14 structurally and pharmacologically distinct receptor subtypes. These receptors have been assigned to one of seven families, 5-HT$_{1-7}$.[1] The 5-HT$_7$ receptor (5-HT$_7$R) is the most recent addition to the 5-HT receptor family, and was cloned for the first time in 1993 from rat[2−5] and mouse.[6] Since then, it has been cloned from other species such as human,[7] guinea pig,[8] and pig.[9] The 5-HT$_7$R was shown to be positively coupled to adenylyl cyclase via Gs proteins; however, it displays a low degree of homology (40%) with other Gs-coupled 5-HT receptors.[10] Four different isoforms have been found, namely, 5-HT$_{7a}$, 5-HT$_{7b}$, 5-HT$_{7c}$, and 5-HT$_{7d}$. Only two isoforms (5-HT$_{7a}$ and 5-HT$_{7b}$) are present in both rat and human, whereas the 5-HT$_{7c}$ receptor is found exclusively in rat and the 5-HT$_{7d}$ is found only in human. Each of the isoforms appears to form a functionally active receptor, with the 5-HT$_{7a}$ being the most abundant (80%) in both rat and human brain.[11] There appear to be no pharmacological differences among the four isoforms.[12] High concentrations of the 5-HT$_7$R have been detected by in situ hybridization and 5-HT$_7$-like immunoreactivity[13,14] in the hypothalamus, entorhinal cortex, septal areas, substantia nigra, amygdala, raphe nuclei, and the trigeminal nucleus. In addition, moderate levels of 5-HT$_7$-like immunoreactivity were found in the thalamus, hippocampus, cingulate and occipital cortex, caudate putamen, and suprachiasmatic nucleus (SCN) of the rat.[15] This distribution correlates well with distribution of mRNA encoding 5-HT$_7$R protein. In fact, the 5-HT$_7$R mRNA has been detected in thalamus, hypothalamus, hippocampus, amygdala, cortex, septum, and suprachiasmatic nucleus.

The potential of therapeutic effects of 5-HT$_7$ agents have been hypothesized on the basis of such anatomical distribution. The link between 5-HT$_7$Rs and the SCN suggests a potential role in circadian rhythms and sleep disorders. Lovenberg et al.[3] demonstrated that phase advances in circadian neuronal activity of the SCN could be elicited by use of serotonergic ligands that display a pharmacological profile consistent with that of the 5-HT$_7$R. Since then, 5-HT$_7$Rs have been shown to be present in postsynaptic areas in the SCN where serotonergic neurons are proposed to play a key role in modulating circadian activity. Mullins et al.[16] have supplied supporting evidence that implicates a possible role for 5-HT$_7$R in depression. They demonstrated that antidepressant-induced expression of the immediate early gene, c-Fos, in the SCN was blocked by ritanserin (a high-affinity, but nonselective, 5-HT$_7$R antagonist) but not by the 5-HT$_{1A}$ antagonist pindolol or the 5-HT$_{1D}$ antagonist Sumatriptan. This suggests that the effect is mediated through 5-HT$_7$Rs, although, with such nonselective antagonists, the involvement of other 5-HT receptors cannot be ruled out.

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Chart 1

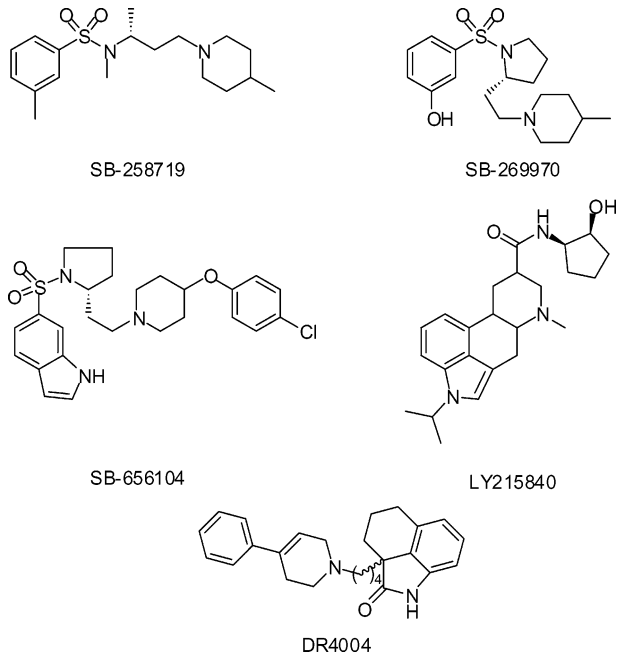
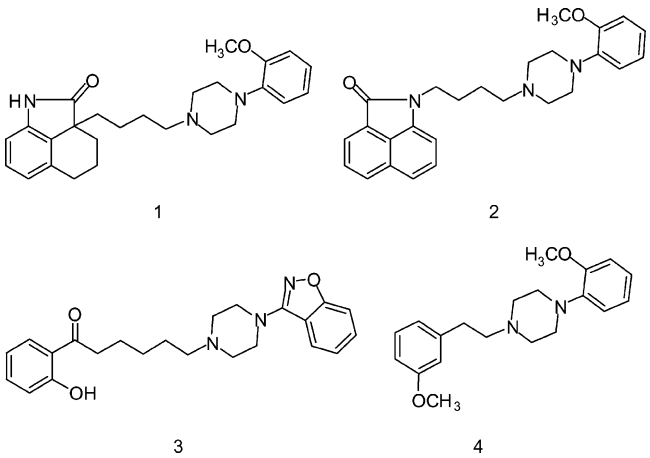
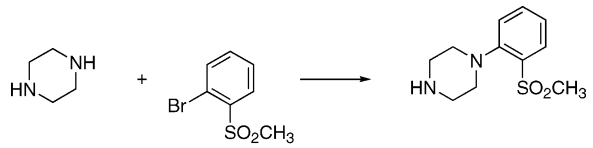


Chart 2



Scheme 1



The involvement of the 5-HT$_7$R in migraine pathogenesis has been proposed by Terron[17] because the 5-HT$_7$R-mediated vasodilator mechanism operates in vascular structures that have been implicated in migraine, such as the middle cerebral and external carotid arteries. Finally, several compounds possessing high 5-HT$_7$R affinity have therapeutic indications as antipsychotic drugs, and this has suggested that 5-HT$_7$R may mediate therapeutic action of such compounds.[18]

It is therefore clear that the 5-HT$_7$R may be a valuable drug target. During the past decade, considerable research efforts have been directed toward the identification of selective 5-HT$_7$R antagonists,[19] allowing the identification of some interesting compounds such as SB-258719,[20] SB-269970,[21,22] SB-656104,[23] DR4004,[24,25] and LY215840[26] (Chart 1). However, these promising compounds present several limitations because of their low potency (SB-258719), modest selectivity (SB-656104, LY215840), and low metabolic stability (SB-269970, DR4004). Therefore, the search for selectively acting 5-HT$_7$R ligands as useful pharmacological tools or potential drugs is still open. It is noteworthy that most 5-HT$_7$R ligands reported to date act as antagonists, whereas few agonists has been reported.[27]

Of the different chemical classes that bind to 5-HT$_7$Rs, arylpiperazines (Chart 2) have received our attention as well as that of other authors. In particular, Kikuchi and co-workers[24,28] have reported on some 1-arylpiperazines, exemplified by compound **1**, N-substituted by a butyl chain, bearing in the 4-position a tetrahydrobenzindole nucleus. Lopez-Rodriguez et al.[29] studied 1-arylpiperazine derivatives related to **2**. Recently, we have reported structure−affinity relationship studies of two distinct classes of 5-HT$_7$R ligands, based on the structure of 1-arylpiperazine. Examples of these classes are represented by compounds **3**[30] and **4**.[31]

In the present study, we screened the 1-(2-methoxyphenyl)piperazine derivatives **5**−**7**, previously prepared in our laboratory as 5-HT$_{1A}$ ligands,[32,33] against the cloned rat 5-HT$_7$R because they share some structural features with derivatives **1** and **2**. We found that the compounds **6** and **7** possessed moderate affinities for 5-HT$_7$R as well as for 5-HT$_{1A}$ receptor. We here describe the structural modifications of **7** that have led to the identification of a series of high-affinity 5-HT$_7$R ligands based on the *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamide structure. In particular we varied (i) the intermediate alkyl chain length, (ii) the position of the methoxy group on the 1,2,3,4-tetrahydronaphthalene nucleus, and (iii) the aromatic substituent linked to the N-1 piperazine ring.

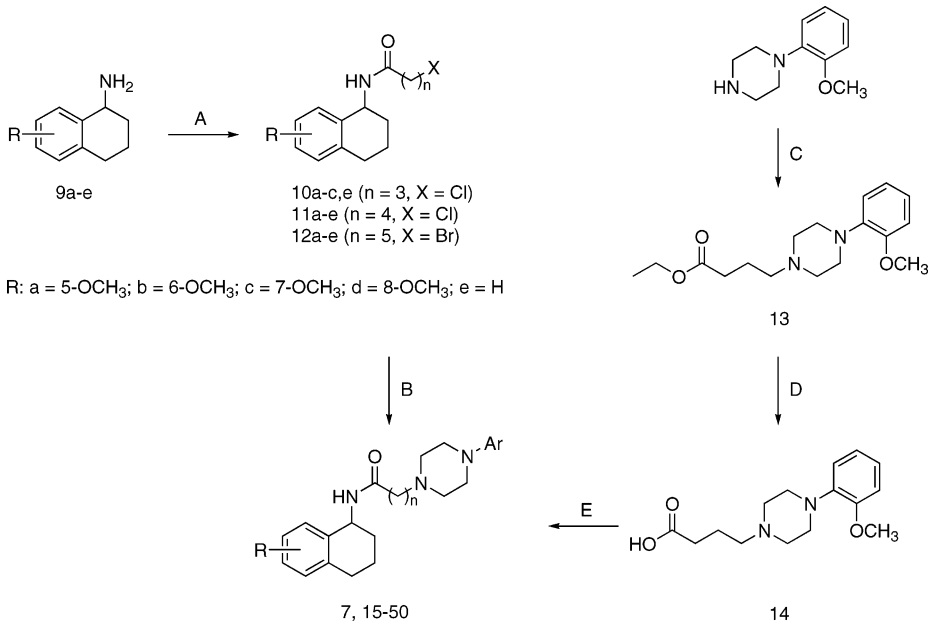
Chemistry

The starting 1-arylpiperazines were obtained from commercial sources or were prepared by literature methods (see Experimental Section), except for 1-[2-(methylsulfonyl)phenyl]piperazine (**8**), which was prepared by reacting anhydrous piperazine with 2-bromo(methylsulfonyl)benzene (Scheme 1). The preparation of the final compounds is depicted in Scheme 2. Acylation of amines **9a**−**e** with the appropriate ω-haloacyl chloride afforded the key intermediates **10a**−**c,e**, **11a**−**e**, and **12a**−**e** that reacted with the appropriate 1-arylpiperazine to give the final compounds **7**, **15**−**22**, and **24**−**50**. This synthetic pathway was not useful to obtain derivative **23** in a pure form, therefore an alternative synthetic route was followed: 1-(2-methoxyphenyl)piperazine was reacted with ethyl 4-bromobutanoate to give ester **13**. Hydrolysis of the latter gave carboxylic acid **14** that reacted with amine **9d** to give the expected final compound. All target compounds were prepared as racemates.

Results and Discussion

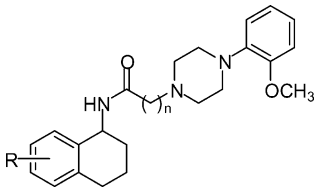
The first modification performed on compound **7** was the optimization of the intermediate alkyl chain length. Therefore, we evaluated compounds **15** and **16** (Table 1) having a four- or five-methylene alkyl chain, respectively. 5-HT$_7$R affinity values indicated that alkyl chain

Scheme 2[a]



[a] Reagents: (A) ω-haloacyl chloride, NaOH; (B) 1-arylpiperazine; (C) ethyl 4-bromobutanoate; (D) NaOH; (E) **9d**, 1,1′-carbonyl diimidazole.

Table 1. Physical Properties and Binding Affinities of Compounds **5**−**7** and **15**−**28**



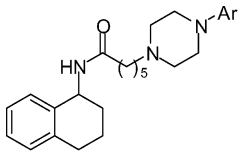
					K_i, nM	
compd	R	n	formula[a]	mp, °C	5-HT_7	5-HT_{1A}
5[b]	5-CH_3O	1			>1000	NT
6[c]	5-CH_3O	2			269 ± 18	253 ± 25
7	5-CH_3O	3	$C_{26}H_{35}N_3O_3$·HCl·0.2H_2O	215 decomp	35 ± 3.2	254 ± 65
15	5-CH_3O	4	$C_{27}H_{37}N_3O_3$·HCl·H_2O	187−190	28.2 ± 4.20	54.4 ± 6.5
16	5-CH_3O	5	$C_{28}H_{39}N_3O_3$·2HCl·0.5H_2O	194−197	20 ± 2.5	24.5 ± 1.8
17	6-CH_3O	3	$C_{26}H_{35}N_3O_3$·2HCl	128−129	186 ± 40	257 ± 25
18	6-CH_3O	4	$C_{27}H_{37}N_3O_3$·2HCl	149−152	43.1 ± 4.8	23.2 ± 2.3
19	6-CH_3O	5	$C_{28}H_{39}N_3O_3$·$(COOH)_2$	102−105	30 ± 3.15	55 ± 8.0
20	7-CH_3O	3	$C_{26}H_{35}N_3O_3$·2HCl·H_2O	118−120	129 ± 5.0	160 ± 12
21	7-CH_3O	4	$C_{27}H_{37}N_3O_3$·2HCl·0.4H_2O	187−188	38.4 ± 4.6	78.9 ± 6.30
22	7-CH_3O	5	$C_{28}H_{39}N_3O_3$·2HCl	175−178	41 ± 11	39 ± 6.5
23	8-CH_3O	3	$C_{26}H_{35}N_3O_3$·2HCl	131−133	154 ± 35	441 ± 20
24	8-CH_3O	4	$C_{27}H_{37}N_3O_3$·2HCl·0.2H_2O	136−137	64.0 ± 12	72.0 ± 18
25	8-CH_3O	5	$C_{28}H_{39}N_3O_3$·2HCl	125−127	31.4 ± 3.5	30.0 ± 2.6
26	H	3	$C_{25}H_{33}N_3O_2$·2HCl	171−173	92.0 ± 12	245 ± 20
27	H	4	$C_{26}H_{35}N_3O_2$·2HCl·0.8H_2O	199−200	6.05 ± 0.25	9 ± 0.70
28	H	5	$C_{27}H_{37}N_3O_2$·2HCl·0.5H_2O	150−151	6.64 ± 0.60	8.6 ± 0.35
5-CT					0.51 ± 0.01	
8-OH-DPAT						1.2 ± 0.2

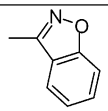
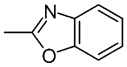
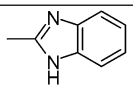
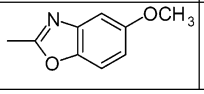
[a] All compounds were recrystallized from CH_3OH/Et_2O. Analysis for C, H, N; results were within ±0.4% of the theoretical values for the formulas given. [b] See ref 33. [c] See ref 32.

elongation resulted in increasing affinity. Second, we shifted the methoxy group from the 5-position to the 6-, 7-, and 8-position of the tetrahydronaphthalenyl ring, because previous studies indicated that the position of the methoxy group on the terminal aromatic nucleus influenced the 5-HT_7R affinity of compounds **3** and **4**.[30,31] This modification was performed on the compounds **7**, **15**, and **16** that displayed good 5-HT_7R affinities. Upon consideration of each group of isomers (i.e., compounds having the same alkyl chain length), no significant difference in 5-HT_7R affinity was observed. Moreover, within each group of homologues (i.e., compounds bearing the methoxy group in the same position), affinity values replicate the affinity rank already noted for the 5-methoxy-substituted derivatives **7**, **15**, and **16**. Because the position of the methoxy group at the tetrahydronaphthalenyl ring did not exert a significant role on 5-HT_7R affinity of compounds **7** and **15**−**25**, we evaluated the unsubstituted derivatives **26**, **27**, and **28**. This modification improved the 5-HT_7R affinity.

The results in Table 1 indicate that the modifications of either the 1,2,3,4-tetrahydronaphthalenyl nucleus or of the linker between this group and the *N*-(2-methoxy-

Table 2. Physical Properties and Binding Affinities of Compounds **29**−**32**



				K_i, nM	
compd	**Ar**	**formula**[a]	**mp, °C**	**5-HT$_7$**	**5-HT$_{1A}$**
29		$C_{27}H_{34}N_4O_2$•2HCl•H_2O	164 dec	125 ± 30	3900 ± 120
30		$C_{27}H_{34}N_4O_2$	148 dec	820 ± 90	NT[b]
31		$C_{27}H_{35}N_5O$•3HCl	300 dec	356 ± 55	2600 ± 280
32		$C_{28}H_{36}N_4O_3$•2HCl•H_2O	196-198	704 ± 30	NT

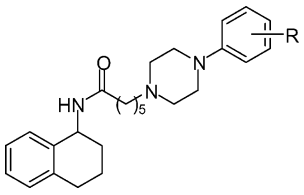
[a] All compounds were recrystallized from CH_3OH/Et_2O except **30** (from $CHCl_3$/*n*-hexane). Analysis for C, H, N; results were within ±0.4% of the theoretical values for the formulas given. [b] Not tested.

phenyl)piperazine moiety of compound **7** influenced the 5-HT$_7$R affinity only and not the selectivity over 5-HT$_{1A}$ receptor.

Therefore, we focused on the aromatic ring attached to the piperazine nitrogen, bearing in mind that minimal changes in this part of the molecule might result in major changes in 5-HT$_7$R affinity as well as in 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity, as documented.[28,30] Because the derivatives with a five-methylene linker displayed the higher 5-HT$_7$R affinity values, we have further modified compound **28**. Initially, on the basis of literature data, we substituted the 2-methoxyphenyl group with a bicyclic aromatic system[34] or a 2-acetylphenyl or a 2-cyanophenyl group.[28] The replacement of the 2-methoxyphenyl group with a bicyclic aromatic system (Table 2, compounds **29**−**32**) reduced the 5-HT$_7$R affinity. In particular, it can be noted that the presence of the benzisoxazolyl group was detrimental for 5-HT$_7$ affinity (compound **29**), whereas in previous studies we found that this particular replacement resulted in the opposite effect.[30] In contrast, compounds **33** and **34** (Table 3) retained reasonably good 5-HT$_7$R affinity but were unselective over 5-HT$_{1A}$ receptors. Moreover, we prepared compounds **35** and **36** that present an additional substituent in the 4- or 3-position of the aromatic ring, because this substitution pattern has been reported to be detrimental for 5-HT$_{1A}$ receptor affinity.[35,36] This modification determined a loss in 5-HT$_7$R affinity and no significant improvement in selectivity over 5-HT$_{1A}$ receptors. Additionally, we shifted the substituent from the 2-position of compounds **28**, **33**, and **34** to the 3- and 4-positions (Table 3, derivatives **37**−**42**). Binding data of derivatives **37**−**42** indicate that affinity for 5-HT$_7$R strongly depends on the position of the substituent. In fact, the 3-substitued derivatives **37**, **39**, and **41** are less potent at 5-HT$_7$R than the 2-substitued isomers **28**, **33**, and **34**. The 4-substituted derivatives **38**, **40**, and **42** are nearly devoid of 5-HT$_7$R affinity. Taken together, these data confirm that this part of the molecule is quite sensitive to minimal structural changes. Subsequently, we evaluated analogues of **28** having a substituent in the 2-position other than methoxy, as well as the unsubstituted derivative (Table 3, derivatives **43**−**50**). For this purpose we selected several substituents with different electronic properties. Considering the unsubstituted derivative **50** as reference compound, it can be noted that the cyano, chloro, and nitro substituents (compounds **33**, **47**, and **48**, respectively) did not change the 5-HT$_7$R affinity. In contrast, carboxamido and methylsulfonyl substituents (derivatives **43** and **45**, respectively) caused a drop in 5-HT$_7$R affinity. Substitution of the 2-position by a methoxy, acetyl, methylthio, hydroxy, or methyl groups resulted in high-affinity 5-HT$_7$R ligands (derivatives **28**, **34**, **44**, **46**, and **49**, respectively). These data indicate that the presence of a substituent in the 2-position modulates the affinity of this class of compounds for 5-HT$_7$R. The affinity values seem not to be related to electronic, steric, or H-bonding properties of these substituents. As a result, clear structure−affinity relationships are not evident. Moreover, the 5-HT$_{1A}$ receptor affinities of compounds **33**−**50** parallel the 5-HT$_7$R affinities, whereas 5-HT$_{2A}$ receptor affinities are negligible. Notably, only compound **44** showed considerable selectivity over 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors (200-fold and >1000-fold, respectively).

We tested the structurally related compounds **28**, **34**, **44**, **46**, and **49** for 5-HT$_7$ intrinsic activity in an isolated guinea pig ileum assay (Table 4). It has been reported that 5-HT$_7$ agonists produce a dose-dependent guinea pig ileum relaxation of substance P-induced contraction.[37] Compounds **28**, **44**, and **49** behaved as full agonists and compound **34** as a partial agonist, whereas derivative **46** acted as an antagonist. These results indicate that the nature of the 2-substituent is critical for the intrinsic activity. In particular, the difference in intrinsic activity between hydroxy derivative **46** and the corresponding methoxy derivative **28** might indicate

Table 3. Physical Properties and Binding Affinities of Compounds **33**−**50**



				K_i, nM		
compd	R	formula[a]	mp, °C	5-HT_7	5-HT_{1A}	5-HT_{2A}
33	2-CN	$C_{27}H_{34}N_4O\cdot HCl\cdot 0.5H_2O$	175−178	48.7 ± 2.5	16.6 ± 1.4	700 ± 25
34	2-$COCH_3$	$C_{28}H_{37}N_3O_2\cdot HCl\cdot H_2O$	136−139	4.14 ± 0.80	3.8 ± 0.10	12200 ± 350
35	2-OCH_3-4-Cl	$C_{27}H_{36}N_3O_2Cl$	145−147	122 ± 14	332 ± 27	7168 ± 150
36	2,5-di-OCH_3	$C_{28}H_{39}N_3O_3\cdot 2HCl\cdot 0.6H_2O$	124−126	70.3 ± 5.2	911 ± 23	259 ± 20
37	3-OCH_3	$C_{27}H_{37}N_3O_2\cdot 2HCl\cdot 0.5H_2O$	156−159	119 ± 20	105 ± 12	142 ± 20
38	4-OCH_3	$C_{27}H_{37}N_3O_2$	129−130	2100 ± 150	NT[b]	NT
39	3-CN	$C_{27}H_{34}N_4O\cdot HCl$	170−172	97.8 ± 5.6	291 ± 15	909 ± 85
40	4-CN	$C_{27}H_{34}N_4O\cdot HCl\cdot 1.5H_2O$	102−104	1400 ± 120	NT	NT
41	3-$COCH_3$	$C_{28}H_{37}N_3O_2\cdot 2HCl\cdot H_2O$	146−148	496 ± 24	676 ± 32	1127 ± 200
42	4-$COCH_3$	$C_{28}H_{37}N_3O_2\cdot 2HCl$	112−114	2639 ± 130	NT	NT
43	2-$CONH_2$	$C_{27}H_{36}N_4O_2\cdot 2HCl\cdot 0.5H_2O$	184−187	229 ± 12	494 ± 35	>4000 (9%)
44	2-SCH_3	$C_{27}H_{37}N_3OS\cdot HCl\cdot H_2O$	181−182	0.22 ± 0.08	52.7 ± 3.2	326 ± 35
45	2-SO_2CH_3	$C_{27}H_{37}N_3O_3S\cdot HCl\cdot H_2O$	120−122	298 ± 16	3124 ± 260	>4000 (38%)
46	2-OH	$C_{26}H_{35}N_3O_2\cdot 2HCl\cdot 0.3H_2O$	162−164	11.4 ± 2.3	24 ± 6.3	3394 ± 225
47	2-Cl	$C_{26}H_{34}ClN_3O\cdot HCl\cdot 0.3H_2O$	168−169	40.1 ± 6.7	96 ± 8.0	301 ± 12
48	2-NO_2	$C_{26}H_{34}N_4O_3\cdot HCl\cdot 0.5H_2O$	152−155	63.3 ± 7.5	183 ± 15	282 ± 35
49	2-CH_3	$C_{27}H_{37}N_3O\cdot 2HCl\cdot 0.5H_2O$	212−214	15.2 ±3.2	279 ± 44	262 ± 24
50	H	$C_{26}H_{35}N_3O\cdot 2HCl\cdot 0.5H_2O$	172−174	65.6 ± 4.7	128 ± 22	77.8 ± 5.7

[a] All compounds were recrystallized from CH_3OH/Et_2O except **35** and **38** (from $CHCl_3$/*n*-hexane). Analysis for C, H, N; results were within ±0.4% of the theoretical values for the formulas given. [b] Not tested.

Table 4. Relaxation Effect Induced by Selected Compounds and 5-CT on Substance P-Stimulated Guinea Pig Ileum Contracture with 5-HT_7 Receptor Desensitization

compd	maximal effect, %	EC_{50}, μM	pA_2 (vs SB-269970)	Schild plot	N
28	91	6.32 ± 0.20	8.02 ±1.40	1.0 ($p < 0.0001$)	15
34	79	2.46 ± 0.70	7.60 ± 0.49	1.3 ($p < 0.0001$)	14
44	100	2.56 ± 0.32	7.70 ± 0.80	0.9 ($p < 0.0001$)	15
46	0		7.20 ± 0.60	1.6 ($p < 0.005$)	16
49	98	1.82 ± 0.72	7.80 ± 0.40	0.9 ($p < 0.0001$)	15
5-CT	100	0.63 ± 0.04	7.48 ± 0.12	1.2 ($p < 0.0001$)	12

that the H-bonding donor property of the hydroxy is responsible for the antagonistic property of **46**. In contrast, an apolar group seems to promote the activation of 5-HT_7R. However, due to the limited number of compounds tested, a general trend cannot be drawn. A complete structure−activity relationship study will be carried out on a wider set of compounds, by evaluation of the capacity to stimulate cAMP production in cell lines expressing the cloned 5-HT_7R.

In conclusion, starting from *N*-(5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-(2-methoxyphenyl)-1-piperazinebutanamide (**7**), we have identified a new class of 5-HT_7R ligands. The structural modification introduced on **7** allowed the elucidation of the structural requirements for high 5-HT_7R affinity of this class of compounds. In particular, all structural modifications introduced on either the 1,2,3,4-tetrahydronaphthalenyl nucleus or on the linker between this particular group and the *N*-(2-methoxyphenyl)piperazine moiety influenced only the 5-HT_7R affinity and not the selectivity over 5-HT_{1A} receptor. In contrast, modifications of the aryl group linked to the piperazine ring resulted in major changes in 5-HT_7R affinity. Therefore, the 4-aryl-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide structure was identified as a promising framework to obtain high-affinity 5-HT_7R ligands. Among the compounds displaying the highest 5-HT_7R affinity, derivatives **28**, **34**, **44**, **46**, and **49** were submitted to a functional assay to establish their intrinsic activity. Compounds **28**, **44**, and **49** behaved as full agonists and compound **34** as a partial agonist, whereas derivative **46** acted as an antagonist. Among the compounds presented here, 4-(2-methylthiophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (**44**) was identified as a potent 5-HT_7R full agonist ($K_i = 0.22$ nM, $EC_{50} = 2.56$ μM), with selectivity over 5-HT_{1A} and 5-HT_{2A} receptors (200-fold and >1000-fold, respectively).

Experimental Section

Chemistry. Column chromatography was performed with 1:30 ICN 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 a 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 Bruker AM 300 WB spectrometer or on a Varian Mercury-VX spectrometer. All chemical shift values are reported in parts per million, ppm (δ). Recording of mass spectra was done on an HP68905973 MSD gas chromatograph/mass spectrometer; only significant *m/z* peaks, with their percentage of relative intensity in parentheses, are reported. Compounds **42**, **43**, and **45** were characterized by ESI^+/MS/MS 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 was determined by HPLC on a Perkin-Elmer series 200 LC instrument with 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. All compounds were eluted with $CH_3OH/H_2O/EtN_3$, 4:1:0.01 v/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 that were recrystallized as detailed in Tables 1−3.

The following compounds were synthesized according to published procedures: 1-(2-acetylphenyl)piperazine,[28] 1-(3-

acetylphenyl)piperazine,[38] 2-bromo(methylsulfonyl)benzene,[39] 1-(2-carboxamidophenyl)piperazine,[40] 1-(4-chloro-2-methoxyphenyl)piperazine,[41] 1-(2-cyanophenyl)piperazine,[40] 1-(3-cyanophenyl)piperazine,[42] 1-(2,5-dimethoxyphenyl)piperazine,[43] 5-methoxy-1,2,3,4-tetrahydro-1-naphthalenamine,[44] 6-methoxy-1,2,3,4-tetrahydro-1-naphthalenamine,[45] 7-methoxy-1,2,3,4-tetrahydro-1-naphthalenamine,[45] 8-methoxy-1,2,3,4-tetrahydro-1-naphthalenamine,[45] 1-(2-methylthiophenyl)piperazine,[46] 1-(2-nitrophenyl)piperazine,[47] 2-(1-piperazinyl)-1*H*-benzimidazole,[48] 3-(1-piperazinyl)-1,2-benzisoxazole,[49] and 2-(1-piperazinyl)-benzoxazole.[50]

1-[2-(Methylsulfonyl)phenyl]piperazine (8). A mixture of 2-bromo(methylsulfonyl)benzene (1.10 g, 4.7 mmol) and anhydrous piperazine (2.02 g, 23.5 mmol) was heated at 110 °C overnight. Then, the mixture was cooled and partitioned between 2 N NaOH and CH_2Cl_2. The separated organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude residue was chromatographed ($CHCl_3$/CH_3OH, 9:1, as eluent) to give **8** as a white semisolid (0.36 g, 34% yield). ^{1}H NMR δ 2.58 (s, 1H, NH, D_2O exchanged), 2.84 (s, 4H, piperazinic), 3.14 (s, 3H, CH_3), 7.10−7.83 (m, 4H, aromatic).

General Procedure for Preparation of Alkylating Agents 10a−c,e, 11a−e, and 12a−e. A cooled solution of amine **9a−e** (4.0 mmol) in CH_2Cl_2 was stirred vigorously with 2% aqueous NaOH (9.6 mL, 4.8 mmol) while the appropriate ω-haloalkyl chloride (4.8 mmol) in CH_2Cl_2 was added dropwise. The same NaOH solution was then used to maintain pH at 9, and at constant pH the layers were separated. The organic phase was washed with 3 N HCl and with H_2O and then dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed as detailed below to give compounds **10a−c,e**, **11a−e**, and **12a−e** as white semisolids.

***N*-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-chlorobutanamide (10a).** Eluted with $CHCl_3$/AcOEt, 1:1; 39% yield. ^{1}H NMR δ 1.72−1.86, 1.92−2.04 (m, 4H, *endo*-CH_2CH_2), 2.10−2.19 (m, 2H, $CH_2CH_2CH_2$) 2.37 (t, 2H, J = 7.2 Hz, $COCH_2$), 2.53−2.79 (m, 2H, benzylic CH_2), 3.63 (t, 2H, J = 6.0 Hz, CH_2Cl), 3.82 (s, 3H, CH_3), 5.15−5.20 (m, 1H, CH), 5.75 (br d, 1H, NH), 6.72−7.18 (m, 3H, aromatic). GC-MS *m/z* 283 (M^+ + 2, 1), 281 (M^+, 2), 161 (26), 160 (100), 159 (27).

***N*-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-5-chloropentanamide (11a).** Eluted with CH_2Cl_2; 33% yield. ^{1}H NMR δ 1.75−1.85, 1.93−2.01 (m, 8H, $CH_2(CH_2)_2CH_2$, *endo*-CH_2CH_2), 2.19−2.26 (m, 2H, $COCH_2$), 2.55−2.73 (m, 2H, benzylic CH_2), 3.52−3.58 (m, 2H, CH_2Cl), 3.81 (s, 3H, CH_3), 5.14−5.19 (m, 1H, CH), 5.73 (br d, 1H, NH), 6.71−7.17 (m, 3H, aromatic). GC-MS *m/z* 297 (M^+ + 2, 2), 295 (M^+, 5), 161 (28), 160 (100), 159 (31), 145 (20).

***N*-(5-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-6-bromohexanamide (12a).** Eluted with CH_2Cl_2; 35% yield. ^{1}H NMR: δ 1.43−1.53 [m, 2H, $(CH_2)_2CH_2(CH_2)_2$], 1.61−1.99 [m, 8H, CH_2CH_2Br, $COCH_2CH_2$, *endo*-CH_2CH_2], 2.20 (t, 2H, J = 7.4 Hz, $COCH_2$), 2.53−2.75 (m, 2H, benzylic CH_2), 3.40 (t, 2H, J = 6.7 Hz, CH_2Br), 3.81 (s, 3H, CH_3), 5.17 (br t, 1H, CH), 5.69 (br d, 1H, NH), 6.74−7.17 (m, 3H, aromatic). GC-MS *m/z* 355 (M^+ + 2, 1), 353 (M^+, 1), 160 (100).

Ethyl 4-[4-(2-Methoxyphenyl)piperazin-1-yl]butanoate (13). A stirred mixture of 1-(2-methoxyphenyl)piperazine (1.50 g, 7.8 mmol), ethyl 4-bromobutanoate (0.9 mL, 6.3 mmol), and K_2CO_3 (0.87 g, 6.3 mmol) in acetonitrile was refluxed overnight. After the mixture was cooled, 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 ($CHCl_3$/AcOEt, 1:1, as eluent) to afford pure **13** as a pale yellow oil (1.32 g, 68% yield). ^{1}H NMR δ 1.24 (t, 3H, J = 7.1 Hz, CH_2CH_3), 1.79−1.89 (m, 2H, CH_2CH_2CO), 2.34 (t, 2H, J = 7.3 Hz, $COCH_2$), 2.41 [t, 2H, J = 7.4 Hz, $(CH_2)_2NCH_2$], 2.63 [br s, 4H, $(CH_2)_2NCH_2$], 3.06 [br s, 4H, $ArN(CH_2)_2$], 3.83 (s, 3H, OCH_3), 4.11 (q, 2H, J = 7.1 Hz, CH_2CH_3), 6.82−7.00 (m, 3H, aromatic). GC-MS *m/z* 307 (M^+ + 1, 18), 306 (M^+, 77), 261 (32), 205 (100), 190 (37).

4-[4-(2-Methoxyphenyl)piperazin-1-yl)]butanoic acid (14). Ester **13** (1.20 g, 3.9 mmol) was refluxed for 4 h in 20 mL of 4% aqueous NaOH. Then the mixture was cooled and washed with $CHCl_3$. The separated aqueous phase was neutralized with 3 N HCl and extracted with AcOEt (3 × 30 mL). The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure to give 0.58 g of acid **14** as a white solid (51% yield). ^{1}H NMR δ 1.84−1.89 (m, 2H, CH_2CH_2CO), 2.58−2.62 (m, 2H, $COCH_2$), 2.77 (br t, 2H, $(CH_2)_2NCH_2$], 2.2.96 [br s, 4H, $(CH_2)_2NCH_2$], 3.20 [br s, 4H, $ArN(CH_2)_2$], 3.87 (s, 3H, CH_3), 6.87−7.06 (m, 3H, aromatic), 9.52 (br s, 1H, OH, D_2O exchanged). GC-MS *m/z* 279 (M^+ + 1, 20), 278 (M^+, 96), 219 (25), 205 (100), 190 (39).

General Procedure for Preparation of Final Compounds. A stirred mixture of alkylating agent **10a−c,e, 11a−e,** or **12a−e** (8.0 mmol), 1-substituted piperazine (9.6 mmol), and K_2CO_3 (8.0 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 AcOEt (2 × 30 mL). The collected organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was chromatographed (CH_2Cl_2/CH_3OH, 19:1, as eluent) to yield pure compounds **7**, **15−22**, **24−43**, and **45−50** as pale yellow oils. Yields were 20−30% for butanamide derivatives, 35−44% for pentanamide derivatives, and 65−75% for the other compounds.

4-(2-Methoxyphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinebutanamide (26). ^{1}H NMR δ 1.75−1.93, 1.98−2.10 (m, 6H, $COCH_2CH_2$, *endo*-CH_2CH_2), 2.34 (t, 2H, J = 7.0 Hz, $COCH_2CH_2$), 2.42−2.58 [m, 6H, $CH_2N(CH_2)_2$], 2.76−2.78 (m, 2H, benzylic CH_2), 2.90 [br s, 4H, $(CH_2)_2NAr$], 3.84 (s, 3H, CH_3), 5.19−5.29 (m, 1H, CH), 6.80−7.29 (m, 9H, aromatic, NH). GC-MS *m/z* 408 (M^+ + 1, 7), 407 (M^+, 27), 392 (88), 245 (52), 205 (100).

4-(2-Methoxyphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinepentanamide (27). ^{1}H NMR δ 1.56−1.85, 2.01−2.07 [m, 8H, $CH_2(CH_2)_2CH_2$, *endo*-CH_2CH_2], 2.25 (t, 2H, J = 7.3 Hz, $COCH_2CH_2$), 2.43 [t, 2H, J = 7.3 Hz, $CH_2N(CH_2)_2$], 2.62 [br s, 4H, $CH_2N(CH_2)_2$], 2.71−2.79 (m, 2H, benzylic CH_2), 3.06 [br s, 4H, $(CH_2)_2NAr$], 3.86 (s, 3H, CH_3), 5.19−5.23 (m, 1H, CH), 5.79 (br d, 1H, NH), 6.84−7.25 (m, 8H, aromatic). GC-MS *m/z* 422 (M^+ + 1, 4), 421 (M^+, 14), 406 (41), 259 (45), 205 (100), 131 (36).

4-(2-Methoxyphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (28). ^{1}H NMR δ 1.36−1.43 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.51−1.59, 1.61−1.86, 2.00−2.06 (m, 8H, $CH_2CH_2CH_2CH_2CH_2$, *endo*-CH_2CH_2), 2.21 (t, 2H, J = 7.6 Hz, $COCH_2$), 2.40 [br t, 2H, $CH_2N(CH_2)_2$], 2.64 [br s, 4H, $CH_2N(CH_2)_2$], 2.71−2.80 (m, 2H, benzylic CH_2), 3.09 [br s, 4H, $(CH_2)_2NAr$], 3.86 (s, 3H, CH_3), 5.17−5.23 (m, 1H, CH), 5.67 (br d, 1H, NH), 6.83−7.25 (m, 8H, aromatic). GC-MS *m/z* 436 (M^+ + 1, 4), 435 (M^+, 13), 420 (27), 273 (41), 205 (100).

4-(2-Acetylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (34). ^{1}H NMR δ 1.33−1.43 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.51−1.86, 1.98−2.06 (m, 8H, $CH_2CH_2CH_2CH_2CH_2$, *endo*-CH_2CH_2), 2.21 (t, 2H, J = 7.4 Hz, $COCH_2$), 2.43 [t, 2H, J = 7.6 Hz, $CH_2N(CH_2)_2$], 2.62 [br s, 4H, $CH_2N(CH_2)_2$], 2.65 (s, 3H, CH_3), 2.71−2.79 (m, 2H, benzylic CH_2), 3.04 [br t, 4H, $(CH_2)_2NAr$], 5.17−5.29 (m, 1H, CH), 5.69 (br d, 1H, NH), 7.02−7.40 (m, 7H, aromatic). GC-MS *m/z* 448 (M^+ + 1, 8), 447 (M^+, 26), 299 (60), 287 (65), 273 (100), 217 (90).

4-(2-Methylthiophenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (44). ^{1}H NMR δ 1.33−1.43 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.53−1.63, 1.66−1.86, 2.00−2.06 (m, 8H, $CH_2CH_2CH_2CH_2CH_2$, *endo*-CH_2CH_2), 2.22 (t, 2H, J = 7.4 Hz, $COCH_2$), 2.40 (s, 3H, CH_3), 2.43 [t, 2H, J = 7.4 Hz, $CH_2N(CH_2)_2$], 2.63 [br s, 4H, $CH_2N(CH_2)_2$], 2.74−2.79 (m, 2H, benzylic CH_2), 3.03 [br s, 4H, $(CH_2)_2NAr$], 5.18−5.29 (m, 1H, CH), 5.70 (br d, 1H, NH), 7.03−7.26 (m, 8H, aromatic). GC-MS *m/z* 452 (M^+ + 1, 2), 451 (M^+, 8), 273 (61), 221 (100).

4-(2-Methylphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (49). ^{1}H NMR δ 1.34−1.44 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.53−1.63, 1.66−1.86, 2.00−

2.08 (m, 8H, $CH_2CH_2CH_2CH_2CH_2$, *endo*-CH_2CH_2), 2.19 (t, 2H, J = 7.4 Hz, $COCH_2$), 2.30 (s, 3H, CH_3), 2.42 [br t, 2H, $CH_2N(CH_2)_2$], 2.60 [br s, 4H, $CH_2N(CH_2)_2$], 2.70−2.79 (m, 2H, benzylic CH_2), 2.95 [br t, 4H, $(CH_2)_2NAr$], 5.18−5.23 (m, 1H, CH), 5.69 (br d, 1H, NH), 6.94−7.26 (m, 8H, aromatic). GC-MS *m/z* 420 (M^+ + 1, 2), 419 (M^+, 7), 273 (99), 189 (100).

***N*-(8-Methoxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-(2-methoxyphenyl)-1-piperazinebutanamide (23).** A mixture of carboxylic acid **14** (0.50 g, 1.8 mmol) and 1,1′-carbonyldiimidazole (0.29 g, 1.8 mmol) in 10 mL of anhydrous THF was stirred for 8 h. A solution of amine **9d** (0.32 g, 1.8 mmol) in 10 mL of anhydrous THF was added and the resulting mixture was stirred for 1 h. The reaction mixture was partitioned between AcOEt and H_2O. The organic layer was washed with aqueous Na_2CO_3 solution, dried (Na_2SO_4), and concentrated in vacuo. The crude residue was chromatographed (CH_2Cl_2/CH_3OH, 19:1, as eluent) to afford pure amide **23** (0.33 g, 42% yield). ^{1}H NMR δ 1.61−1.90, 2.10−2.19 (m, 6H, $COCH_2CH_2$, *endo*-CH_2CH_2), 2.24 (t, 2H, J = 7.4 Hz, $COCH_2CH_2$), 2.28−2.47, 2.55−2.58 [m, 6H, $CH_2N(CH_2)_2$], 2.68−2.77 (m, 2H, benzylic CH_2), 2.90 [br s, 4H, $(CH_2)_2N$], 3.79, 3.84 (2 s, 6H, 2 CH_3), 5.27−5.29 (m, 1H, CH), 6.46 (br d, 1H, NH), 6.67−7.18 (m, 7H, aromatic). GC-MS *m/z* 438 (M^+ + 1, 1), 437 (M^+, 4), 422 (27), 205 (24), 161 (100).

4-(2-Hydroxyphenyl)-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-1-piperazinehexanamide (46). A stirred mixture of alkyl bromide **12e** (0.36 g, 1.1 mmol) and 1-(2-hydroxyphenyl)-piperazine (0.29 g, 1.6 mmol) in acetonitrile was refluxed overnight. After the mixture was cooled, the solvent was evaporated in vacuo and a saturated aqueous solution of $NaHCO_3$ (20 mL) was added to the residue. The aqueous phase was extracted with AcOEt (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 **46** as a pale yellow oil (0.30 g, 65% yield). ^{1}H NMR δ 1.34−1.44 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.53−1.63, 1.66−1.86, 2.00−2.07 (m, 8H, $CH_2CH_2CH_2CH_2CH_2$, *endo*-CH_2CH_2), 2.22 (t, 2H, J = 7.6 Hz, $COCH_2$), 2.43 [t, 2H, J = 7.6 Hz, $CH_2N(CH_2)_2$], 2.63 [br s, 4H, $CH_2N(CH_2)_2$], 2.75−2.79 (m, 2H, benzylic CH_2), 2.90 [br s, 4H, $(CH_2)_2NAr$], 5.18−5.29 (m, 1H, CH), 5.69 (br d, 1H, NH), 6.83−7.27 (m, 9H, aromatic, OH, 1H, D_2O exchanged). GC-MS *m/z* 422 (M^+ + 1, 2), 421 (M^+, 5), 273 (100), 191 (28).

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, December 18, 1986).

Rat recombinant serotonin 5-HT_7R expressed in HEK-293 cells were purchased from PerkinElmer−NEN (Betsville, MD).

[^{3}H]LSD, [^{3}H]-8-OH-DPAT, and [^{3}H]ketanserin were obtained from PerkinElmer−NEN (Zaventem, Belgium). 5-CT, substance P, and ketanserin were purchased from Tocris Cookson Ltd. (Bristol, U.K.). 8-OH-DPAT hydrobromide was from RBI. SB-269970 was purchesed from Sigma−Aldrich (Milan, Italy).

For receptor binding studies, compounds **5**−**7** and **15**−**50** were dissolved in absolute ethanol. For isolated guinea pig ileum assay, compounds **28**, **34**, **44**, **46**, and **49** were dissolved in Krebs−Henseleit solution, pH 7.4.

2. Radioligand Binding Assay at Rat Cloned 5-HT_7Rs. Binding of [^{3}H]LSD at rat cloned 5-HT_7 receptor was performed according to Jasper et al.[51] 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, and 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 Rat Hippocampal Membranes 5-HT_{1A} Receptors. Binding experiments were performed according to Borsini et al.[52] with minor modifications. Rats were killed by decapitation, the brain was quickly removed, and the hippocampus was dissected. The hippocampus (1.0 g) was homogenized with a Brinkman Polytron (setting 5 for 3 × 15 s) in 25 mL of 50 mM Tris buffer, pH 7.6. The homogenate was centrifuged at 48000*g* for 15 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in 25 mL of buffer and then preincubated for 10 min at 37 °C. The homogenate was centrifuged at 48000*g* for 15 min at 4 °C. The supernatant was discarded, and the final pellet was stored at −80 °C until use. Each tube received, in a final volume of 1 mL of 50 mM Tris (pH 7.6), hippocampus membranes suspension and 1 nM [^{3}H]-8-OH-DPAT. For competitive inhibition experiments, various concentrations of drugs studied were incubated. Nonspecific binding was defined relative to 1 μM 8-OH-DPAT. Samples were incubated at 37 °C for 20 min and then filtered on Whatman GF/B glass microfiber filters. The K_d value determined for 8-OH-DPAT was 8.8 nM.

4. Radioligand Binding Assay at Rat Cortex Membranes 5-HT_{2A} Receptors. Binding experiment was performed according to Leysen et al.[53] 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 relative to 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. Isolated Guinea Pig Ileum Assay. Guinea pigs were anesthetized and then decapitated and the proximal ileum was 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 $CaCl_2$, and 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 Eglen and co-workers[37] 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.[54] Tissue was contracted by 100 nM substance P. This value was preliminarily determined by concentration−response curves (1−200 nM). A 100 nM concentration of substance P elicited 80% of maximum contraction. The reference agonist 5-CT or tested compound was added 3 min before substance P addition, and noncumulative concentration−response curves were constructed (0.001−10 μM). Because we determined that 5-CT induced relaxation with maximal response (39%) at 3 μM concentration, 5-HT_7 desensitization was achieved by equilibrating for 1 h in the presence of 3 μM 5-CT, changing the bathing solution every 15 min. Tested compounds were added 3 min before substance P addition.

Full agonists 5-CT, **28**, **44**, and **49** and partial agonist **34** were also tested in the presence of the antagonist SB-269970 (0.1−3 μM). The isolated guinea pig ileum was equilibrated

for 75 min with antagonist before concentration−response curves of tested compounds were constructed.

Tissue responses were recorded as gram changes in isometric tension and expressed as percentage of reduction in the height of the contraction.

6. 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.[55] The value for the inhibition constant, K_i, was calculated from the Cheng−Prusoff equation.[56] Agonist potencies, expressed as EC_{50}, were obtained from nonlinear iterative curve-fitting by GraphPad Prism.

Supporting Information Available: Spectral data for compounds **7**, **10b−e**, **11b−e**, **12b−e**, **15−22**, **24**, **25**, **29−33**, **35−43**, **45**, **47**, **48**, and **50**. This material is available free of charge via the Internet at http://pubs.acs.org.

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