Structural Modifications of *N*-(1,2,3,4-Tetrahydronaphthalen-1-yl)-4-Aryl-1-piperazinehexanamides: Influence on Lipophilicity and 5-HT7 Receptor Activity. Part III

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Starting from the previously reported 5-HT7 receptor agents **4**−**7** with *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinehexanamide structure, the 1-(2-methylthiophenyl)-, 1-(2-diphenyl)-, 1-(2-isopropyl-phenyl)-, and 1-(2-methoxyphenyl)piperazine derivatives **8**−**31** were designed with the primary aim to obtain new compounds endowed with suitable physicochemical properties for rapid and extensive penetration into the brain. The affinities for 5-HT7, 5-HT1A, and D2 receptors of compounds **8**−**31** were assessed, and several compounds displayed 5-HT7 receptor affinities in the nanomolar range. Among these, *N*-(4-cyanophenyl-methyl)-4-(2-diphenyl)-1-piperazinehexanamide (**25**) showed high 5-HT7 receptor affinity ($K_i = 0.58$ nM), high selectivity over 5-HT1A and D2 receptors (324- and 245-fold, respectively), and agonist properties (maximal effect = 82%, $EC_{50} = 0.60$ μM). After intraperitoneal injection in mice, **25** rapidly reached the systemic circulation and entered the brain. Its brain concentration−time profile paralleled that in plasma, indicating that **25** rapidly and freely distributes across the blood−brain barrier. Compound **25** underwent *N*-dealkylation to the corresponding 1-arylpiperazine metabolite.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) controls a variety of physiological functions in the central and peripheral nervous system by interacting with a multitude of 5-HT receptor subtypes. These subtypes can be divided into seven main classes (5-HT1 to 5-HT7) on the basis of their pharmacological responses to specific ligands, sequence similarities at the gene and amino acid levels, gene organization, and second messenger coupling pathways. Except for the 5-HT3 receptor, which is a ligand-gated ion channel, all serotonin receptors are coupled to G-proteins.[1]

The 5-HT7 receptor has been cloned from human, mouse, rat, guinea pig, and pig cDNA. High sequence homology (90%) has been observed between the 5-HT7 receptors from various species, whereas a low degree of homology (40%) has been evidenced between 5-HT7 receptor and the other G-protein coupled 5-HT receptor subtypes.[2] Studies in rodents on the distribution of the 5-HT7 receptor protein in the central nervous system (CNS)[a] revealed the highest abundance in thalamus, hypothalamus, and hippocampus. Within the suprachiasmatic nucleus of the hypothalamus, the 5-HT7 receptors are located in both dendrites and axon terminals of mostly GABA contain-ing neurons.[3−6] Brain autoradiographic studies in human indicated that these receptors are mainly localized in the anterior thalamus and in the dentate gyrus.[7] Irrespective of the experimental method used, there is general agreement that 5-HT7 receptors are localized in regions of the brain involved in affective behavior and cognition and recent studies support a role for 5-HT7 receptors in the pathogenesis of depression. In vitro binding studies in rat have also suggested that chronic

Chart 1. Structures of 5-HT7 Receptor Agents
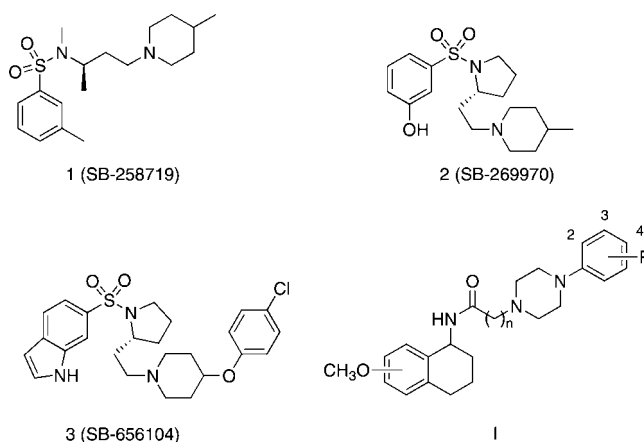
antidepressant treatment results in a functional downregulation of 5-HT7-like binding sites in the hypothalamus.[8] Studies on 5-HT7 receptor knockout mice have displayed "antidepressant-like" phenotype in preclinical assay used to assess antidepressant potential. Also pharmacological 5-HT7 receptor blockade by the antagonists **1** (SB-258719) or **2** (SB-269970) (Chart 1) generated robust antidepressant-like effects.[9−13] It has been also suggested that 5-HT7 receptor function plays a role in the control of learning and memory processes.[14−16] Finally, pharmacological and genetic 5-HT7 blockade has shown to partially modulate glutamatergic and dopaminergic function and therefore to be clinically useful for the treatment of positive symptoms of schizophrenia.[17,18] In view of these outcomes, the 5-HT7 receptor can be considered an interesting target for drug development.
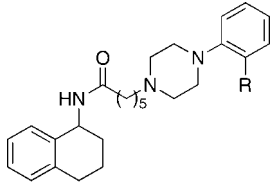
Over the last 10 years, considerable efforts have been focused on the development of novel and selective 5-HT7 receptor agonists and antagonists.[19,20] The first selective antagonist **1** was identified in 1997 by GlaxoSmithKline and displayed an

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[a] Abbreviations: CNS, central nervous system; 5-CT, 5-carboxamido-tryptamine; AUC, area under the curve; 8-OH-DPAT, 8-hydroxy-*N,N*-dipropylaminotetraline.

Table 1. Physicochemical and Binding Affinities of Reference Compounds **4**−**7**[a]

compd	R	ClogP[b]	log k'	K_i, nM		
				5-HT$_7$	5-HT$_{1A}$	D$_{2L}$
4 (LP-44)	SCH$_3$	4.73	1.01	0.22	52.7	7.3
5 (LP-12)	Ph	6.65	1.71	0.13	60.9	224
6	CH(CH$_3$)$_2$	5.60	1.39	1.10	167	15
7	OCH$_3$	4.78	0.63	6.64	8.6	24.6

[a] Data taken from ref 28. [b] Calculated logP values (see ref 51).

arylsulfonamidoalkylamine structure, as well as compound **2**, which is considered to date the standard selective 5-HT$_7$ receptor antagonist.[21] However, further studies with **2** have been hampered by its rapid clearance in vivo in the rat. Years later, GlaxoSmithKline reported on the identification of the antagonist **3** (SB-656104) (Chart 1), which showed an improved pharmacokinetic profile but displayed modest selectivity over 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{1D}$ receptors.[22] 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.[23] Considering the 5-HT$_7$ receptor agonists, a pharmacophore model has been generated from a set of 20 nonselective agonists.[24] This set included also 5-carboxamidotryptamine (5-CT), which is to date used as reference agonist.

More recently, several relatively selective 5-HT$_7$ agonists have been reported,[25,26] including compounds **4** (LP-44) and **5** (LP-12) (Table 1), which were identified during our structure−activity relationship studies on a series of *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamides (structure I, Chart 1).[27,28] Our studies indicated that for 5-HT$_7$ affinity: (i) a methoxy group on the 1,2,3,4-tetrahydronaphthalene ring was not essential, (ii) five methylene units separating the amide moiety and the piperazine ring were preferable, (iii) the position and the nature of the substituent on the phenyl linked to the piperazine ring was critical. In particular, we found that shifting the R substituent from the 2-position to the 3- or 4-position caused a significant loss in 5-HT$_7$ receptor affinity. Examination of a series of substituents in the 2-position, covering a wide range of electronic, steric, and polar properties, revealed a key role on both affinity and intrinsic activity at the 5-HT$_7$ receptor. Certain lipophilic substituents (R = SCH$_3$, CH(CH$_3$)$_2$, N(CH$_3$)$_2$, CH$_3$, Ph) led to high-affinity agonists, while the OH and NHCH$_3$ substituents switched intrinsic activity toward antagonism. The nature of this substituent was also relevant with respect to the selectivity over 5-HT$_{1A}$ and D$_2$ receptors. The most relevant outcome from those studies was the identification of several high-affinity agonists (compounds **4**−**7**, Table 1). In particular, compound **5** showed the highest selectivity among the studied derivatives. However, while studying the structure−activity relationships, we did not optimize the physicochemical properties required for penetration into the brain. It is widely accepted that brain penetration by passive diffusion becomes poor when molecular weight is larger than 450 Da, the logP is greater than 5.0, the number of hydrogen bond donors is greater than five, or when the sum of all nitrogens and oxygens is greater than 10.[29−31] As reported in Table 1, the lipophilicity of compound

5 (ClogP = 6.65) exceeds the guideline value and this could limit its use in vivo. Keeping in mind that compound **5** was the most selective 5-HT$_7$ derivative, we selected also as reference the compounds **4**, **6**, and **7** (Table 1) because they displayed high affinity at 5-HT$_7$ receptor and different selectivity profiles over 5-HT$_{1A}$ and D$_2$ receptors. Therefore, we targeted structural modifications on compounds **4**−**5** to obtain the new derivatives **8**−**31**, which showed ClogP values within the desired range (Tables 2 and 3).

Chemistry

The synthesis of the final compounds **8**−**31** required the starting amines **32a,b** and **36a**−**d** (Scheme 1). Among these, only amine **36d** was not commercially available; therefore, it was prepared as follows: 4-hydroxybenzylamine hydrochloride[32] was first *N*-BOC protected, then was reacted with methanesulfonyl chloride to give derivative **35**. Deprotection of the latter gave the benzyl amine **36d**. The amines **32a,b** and **36a**−**d** reacted with 6-bromohexanoyl chloride to give the corresponding bromoalkyl derivatives **33a,b** and **37a**−**d**. Condensation of **33a,b** and **37a**−**d** with the appropriate 1-arylpiperazine gave the desired compounds **8**−**31**.

Results and Discussion

In view of the increasing interest in the development of 5-HT$_7$ receptor agonists, we decided to expand our structure−activity relationship studies on *N*-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinealkylamide derivatives in order to optimize their 5-HT$_7$ affinity and selectivity and to explore their potential for in vivo pharmacological studies. Because a major determinant of the activity of centrally acting drugs is the rate and extent to which they enter the brain, we targeted structural modifications on compounds **4**−**7** to obtain the new derivatives **8**−**31**, which had suitable ClogP values for rapid penetration into the brain. The structural modifications were performed taking into account our previous structure−activity relationship findings. We evidenced in this class of the compounds (Structure I, Chart 1) and also for other arylpiperazine-based 5-HT$_7$ receptor ligands[33,34] that even limited structural changes on the 1-arylpiperazine moiety could reflect in a great variation of 5-HT$_7$ receptor affinity. The highest values of affinity at 5-HT$_7$ receptor were shown by compounds with five methylenes. For these reasons, we did not modify the arylpiperazine moiety and the alkyl chain length of compounds **4**−**7**. Therefore, we reasoned on the possibility to reduce the lipophilicity of the terminal fragment of compounds **4**−**7** by replacing the tetrahydronaphthalene nucleus with three structurally related moieties (isoindoline, tetrahydroisoquinoline, benzyl) that would have less impact on the lipophilicity of the target molecules. As shown in Tables 2 and 3, compounds **8**−**19** displayed lower ClogP values than their corresponding tetrahydronaphthalene counterparts **4**−**7**. However, the benzyl derivative **17** showed a ClogP of 5.28, which is quite above the guideline value. With the aim to obtain a reduction of the lipophilicity, the benzyl group attached to the amide nitrogen was therefore replaced by more polar groups such as 4-pyridinylmethyl, 4-cyanophenylmethyl, and 4-methanesulfonylphenylmethyl. This structural modification was performed on benzyl derivatives **16**−**19**, leading to compounds **20**−**31** (Table 3). The actual reduction of lipophilicity of the new molecules was confirmed by determining the retention factor log k' as lipophilicity index by using a reversed-phase HPLC method.[35] Binding affinities for the rat cloned 5-HT$_7$ receptor were determined by radioligand displacement assay using the condition summarized in the Experimental

Table 2. Physicochemical and Binding Affinities of Compounds **8−15**
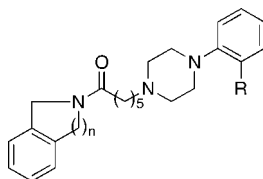
compd	n	R	ClogP	log k'	K_i, nM ± SEM[a]		
					5-HT$_7$	5-HT$_{1A}$	D$_2$
8	1	SCH$_3$	2.89	1.12	42 ± 3.7	1.07 ± 0.05	96 ± 6
9	1	Ph	4.41	1.65	8.3 ± 2.1	124 ± 20	93 ± 2
10	1	CH(CH$_3$)$_2$	3.76	1.55	54 ± 4	22 ± 0.5	65 ± 5
11	1	OCH$_3$	2.35	0.76	179 ± 15	3.2 ± 0.8	730 ± 50
12	2	SCH$_3$	3.45	1.18	32.9 ± 2.1	2.04 ± 0.50	40 ± 8
13	2	Ph	4.97	1.68	3.81 ± 0.5	95 ± 5	362 ± 75
14	2	CH(CH$_3$)$_2$	4.32	1.61	6 ± 0.4	3.7 ± 0.8	1.3 ± 0.3
15	2	OCH$_3$	2.91	0.75	31 ± 3.0	40 ± 4.5	22 ± 4
5-CT					0.50 ± 0.08		
8-OH-DPAT						2.1 ± 0.4	
haloperidol							4.2 ± 0.6

[a] The values are the means ± SEM from three independent experiments with samples in triplicate.

**Table 3.** Physicochemical and Binding Affinities of Compounds **16−31**
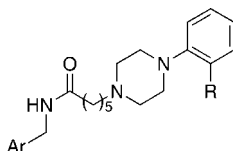
compd	Ar	R	ClogP	log k'	K_i, nM ± SEM.[a]		
					5-HT$_7$	5-HT$_{1A}$	D$_2$
16		SCH$_3$	3.76	0.88	22 ± 1.5	12 ± 2.0	33 ± 5.0
17		Ph	5.28	1.39	ND[b]	161 ± 15	503 ± 70
18		CH(CH$_3$)$_2$	4.63	1.28	215 ± 8	139 ± 18	9.2 ± 0.8
19		OCH$_3$	3.22	0.44	224 ± 15	13 ± 1.2	226 ± 30
20		SCH$_3$	2.26	0.47	34.8 ± 2.1	8.2 ± 0.7	64 ± 4
21		Ph	3.78	0.96	0.98 ± 0.05	70 ± 6	185 ± 30
22		CH(CH$_3$)$_2$	3.13	0.89	5.1 ± 0.4	325 ± 20	21 ± 2
23		OCH$_3$	1.73	0.12	389 ± 10	19 ± 3.5	501 ± 25
24		SCH$_3$	3.19	0.65	9.0 ± 2.0	94 ± 4	5.3 ± 1.5
25		Ph	4.71	1.18	0.58 ± 0.02	188 ± 12	142 ± 10
26		CH(CH$_3$)$_2$	4.06	0.72	8.6 ± 0.5	53 ± 5	20 ± 2.5
27		OCH$_3$	2.66	0.28	296 ± 15	43 ± 2.5	311 ± 18
28		SCH$_3$	2.87	0.71	148 ± 20	10.3 ± 2.5	30 ± 4
29		Ph	4.39	1.08	5.7 ± 0.4	106 ± 10	723 ± 10
30		CH(CH$_3$)$_2$	3.74	0.92	76 ± 4	57 ± 8	7.8 ± 0.4
31		OCH$_3$	2.33	0.11	71 ± 3.5	29 ± 3	91 ± 8

[a] The values are the means ± SEM from three independent experiments with samples in triplicate. [a] Not detectable (see ref 34).

Section. Hill plot slope of all compounds (except **17**) were close to unity, indicating a competitive-type interaction between the tested compounds and [³H]LSD. The analysis of the K_d shift obtained by compound **17** resulted in a linear plot with a slope significantly different from unity, suggesting an allosteric interaction between compound **17** and [³H]LSD under the experimental conditions used here.[36]

The 5-HT$_7$ receptor affinities of target compounds **8−31** are listed in Tables 2 and 3. Considering isoindolines **8−11**, isoquinolines **12−15**, and benzyl derivatives **16−19**, one can

Scheme 1[a]
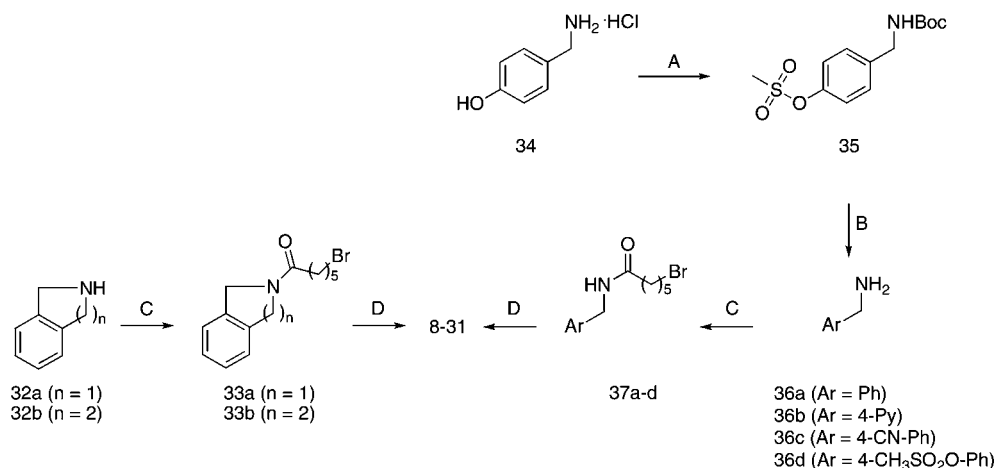
[a] Reagents: (A) (i) (BOC)$_2$O, Et$_3$N; (ii) methanesulfonyl chloride, Et$_3$N. (B) TFA. (C) 6-Bromohexanoyl chloride, 1.2% aqueous NaOH. (D) 1-Arylpiperazine.

observe a reduction in 5-HT$_7$ affinity as compared to the corresponding tetrahydronaphthalenyl derivatives **4**−**7**. In particular, the isoquinoline derivatives showed less marked lowering than the isoindoline and benzyl derivatives. The reduction was particularly evident for 2-methylthio derivatives **8**, **12**, and **16** and for derivative **18** (≥100-fold), whereas compounds **9**, **13**, and **14** retained affinity values in the nanomolar range. Plotting the pK_is against ClogP values of compounds **4**−**19** revealed a low correlation coefficient (data not shown), suggesting that the apolar nature of the terminal fragment is not the only feature that modulates the interaction of this part of the ligand with the 5-HT$_7$ receptor. To some extent, this observation was confirmed by comparing 5-HT$_7$ receptor affinity data of derivatives **16**, **18**, and **19** with those of their less lipophilic analogues **20**, **22**−**24**, **26**−**28**, **30**, **31** (Table 3). In fact, replacement of the benzyl group attached to the amide nitrogen by more polar groups had a limited impact on 5-HT$_7$ affinity, except for **22** and **26**, which were 42- and 25-fold more potent than **18**. It is interesting to note that the presence of 4-pyridinylmethyl, 4-cyanophenylmethyl, and 4-methanesulfonylphenylmethyl as a terminal fragment was also well tolerated in the case of 2-phenyl derivatives **21**, **25**, and **29**, which showed high 5-HT$_7$ affinity (5.7 nM < K_i < 0.58 nM).

The affinities for 5-HT$_{1A}$ and D$_2$ receptors of the target compounds were also assessed because they may interfere with the evaluation of pharmacological actions mediated by 5-HT$_7$ receptors (i.e., body temperature, sleep patterns).[37,38] The structural simplification of the tetrahydronaphthalene nucleus of **4**−**7** did not cause great variations in 5-HT$_{1A}$ affinity. The isoquinoline, isoindoline, and benzyl derivatives **8**−**19** showed K_i values that differed at least 10-fold from the parents **4**−**7**, except **12**, which was about 26-fold more potent than **4**. These data indicated that, within this class of arylpiperazine-based ligands, the affinity at 5-HT$_{1A}$ receptor is marginally affected by the nature of the terminal fragment. A similar trend was shown by affinity data at dopamine D$_2$ receptor: K_i values of isoindolines **8**−**11**, isoquinolines **12**−**15**, and benzyl derivatives **16**−**19** were within 1 order of magnitude as compared to tetrahydronaphthalenyl counterparts **4**−**7**. This binding profile was replicated by derivatives **20**−**31**, which showed even smaller variation of affinity at both 5-HT$_{1A}$ and D$_2$ receptors. As a result of the above-discussed trend of affinity at 5-HT$_7$, 5-HT$_{1A}$, and D$_2$ receptors, the specificity for 5-HT$_7$ receptor of the new compounds **8**−**31** was, at best, unchanged as compared to the reference compounds **4**−**7**. From these data, it became

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

compd	% max activity[a]	EC$_{50}$, μM	pA$_2$ of compd **2**[b]
5[c]	74	1.77 ± 0.08	7.84 ± 0.20
13	89	0.49 ± 0.03	7.80 ± 0.20
21	87	0.31 ± 0.08	7.96 ± 0.20
25	82	0.60 ± 0.04	7.69 ± 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 of the standard antagonist **2** determined by using the corresponding agonist. [c] Data taken from ref 28.

apparent that maintaining potency and selectivity for the biochemical target while optimizing the physicochemical properties is a complex challenge. Nonetheless, the 2-phenyl derivatives **13**, **21**, and **25** showed appreciable selectivity (25- to 324-fold over 5-HT$_{1A}$ and 95- to 245-fold over D$_2$ receptors), still retaining nanomolar affinity at 5-HT$_7$ receptor and showing lipophilicity within the target range. These ligands were further characterized for their intrinsic activity at 5-HT$_7$ receptor by measuring the 5-HT$_7$ agonist mediated relaxation of substance P-induced contraction in an isolated guinea pig ileum assay (Table 4). They showed full competitive agonism at 5-HT$_7$ receptor, being that their effect abolished dose-dependently by the selective 5-HT$_7$ antagonist **2**. In particular, compounds **21** and **25** demonstrated EC$_{50}$ values comparable to that of the standard 5-HT$_7$ agonist 5-CT and higher potency than the tetrahydronaphthalenyl derivative **5**. It can be noted that the modification of the terminal fragment of **5** left unchanged the intrinsic activity of **13**, **21**, and **25**, confirming the predominant role of the arylpiperazine moiety on the intrinsic activity in this class of compounds.

On the basis of its affinity for the 5-HT$_7$ receptor (K_i = 0.58 nM), selectivity over 5-HT$_{1A}$ and D$_2$ receptors (324- and 245-fold, respectively), and intrinsic activity profile (maximal effect = 82%, EC$_{50}$ = 0.60 μM), compound **25** (LP-211) was injected in male mice (10 mg/kg) to preliminarily determine its distribution between blood and brain and thus find out to what extent it passes the blood−brain barrier and the concentrations reached at the site of action. Moreover, the potential formation of 1-(2-diphenyl)piperazine and its brain-to-plasma concentration ratio were also examined because arylpiperazine derivatives generally undergo N-dealkylation of the aliphatic chain attached to the piperazine nitrogen.[39] Similar studies were performed on compound **4**, which has a lipophilicity comparable with **25**. As shown in Figure 1, compound **25** rapidly reached the systemic
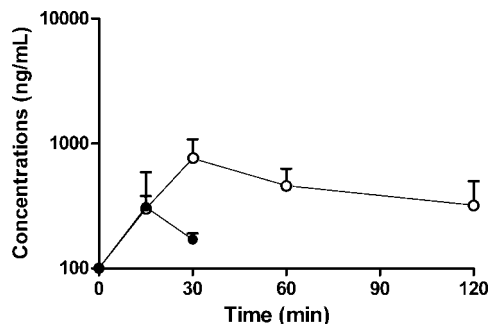
Figure 1. Plasma concentration−time curves of compounds **25** (open symbols) and **4** (closed symbols) after intraperitoneal injection in mice (10 mg/kg; $N = 3$).
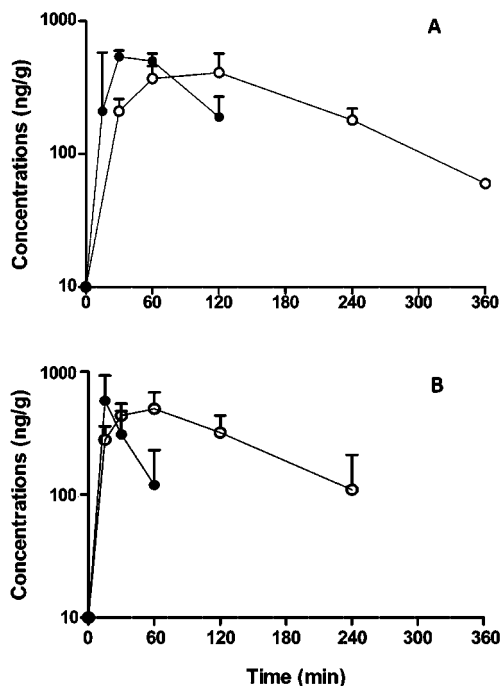

Figure 2. Brain concentration−time curves of compounds **25** (A) and **4** (B) after intraperitoneal injection in mice (10 mg/kg). Each value is the mean of 3 mice for the parent compound (closed symbols) and their 1-aryl-piperazine metabolite (open symbols).

circulation in the mouse, achieving mean C_{max} of 0.76 ± 0.32 μg/mL at 30 min, with quantifiable levels consistently evident for up to 2 h. Under the assumption of complete absorption of this derivative from the injection site into the systemic circulation, the mean total body clearance approached liver blood flow. In conjunction with a relatively high volume of distribution of about 11 L/kg, this resulted in a mean elimination half-life of 65 min. In the same experimental conditions, compound **4** reached plasma C_{max} (0.31 ± 0.07 μg/mL) at the first sampling time (15 min). It was then cleared rapidly, with plasma concentrations approximately halving after 30 min, and the compound was no longer measurable in mouse plasma after 1 h (<0.1 μg/mL, using 0.1 mL of plasma). In both cases, the plasma concentrations of their 1-arylpiperazine metabolite were always below the detection limit of the analytical procedure, at this dose of **25** and **4**.

Reflecting the time profile in the systemic circulation, compound **25** achieved mean brain C_{max} at approximately the same time as in plasma, i.e., 30 min after dosing (Figure 2A). Mean C_{max} averaged 0.54 ± 0.06 μg/g. These concentrations then declined almost parallel with plasma concentrations,

yielding a mean brain-to-plasma distribution ratio (plasma/brain AUC) of about 0.7. Compound **4** too peaked in brain at approximately the same time as in plasma (Figure 2B), but mean C_{max} (0.58 ± 0.35 μg/g) was about twice that in plasma, looking at the whole brain concentrations. However, the low and short-lasting compound levels did not allow an accurate determination of the terminal $t_{1/2}$, although a rough estimate suggested that, at least at this dose, compound **4** was less persistent in the brain than compound **25** (elimination $t_{1/2}$ about 20 and 69 min, respectively). Unlike plasma concentrations, brain concentrations of 1-(2-diphenyl)piperazine (Figure 2A) rose rapidly and reached C_{max} of 0.41 ± 0.06 μg/g within 120 min for compound **25**, then declined with an elimination $t_{1/2}$ of 87 min. Mean AUC was 94 μg/mL·min, yielding a metabolite-to-parent compound ratio of about 1.6. Similarly, the metabolite of compound **4**, 1-(2-methylthiophenyl)piperazine, appeared rapidly in mouse brain ($C_{max} = 0.50 \pm 0.11$ μg/g, at 60 min), disappearing thereafter at a slower rate (elimination $t_{1/2}$ about 82 min) than the parent compound (Figure 2B). The AUC of 1-(2-methyl-tiophenyl)piperazine (85 μg/mL·min) exceeded that of its parent compound by about 4.1 times. This was not surprising, as previous studies have shown that 1-arylpiperazines concentrate in brain tissue, achieving concentrations several times those in the blood.[39] This is probably related to their lipophilicity, which leads to the extensive uptake into lipid-containing tissue.[40]

Conclusions

In summary, we have described the synthesis of the new 1-arylpiperazine-based derivatives **8**−**31**. These compounds originated from the structural simplification of the previously reported 5-HT$_7$ receptor agents **4**−**7** with N-(1,2,3,4-tetrahydronaphthalen-1-yl)-4-aryl-1-piperazinehexanamide structure. The affinities of derivatives **8**−**31** for 5-HT$_7$ receptor as well as those for serotonin 5-HT$_{1A}$ and dopamine D$_2$ were assessed by radioligand binding assays. The proposed structural modifications on the tetrahydronaphthalenyl ring of compounds **4**−**7** were, in most cases, detrimental for 5-HT$_7$ receptor, whereas they had limited impact on the affinity for 5-HT$_{1A}$ and D$_2$ receptors. Nonetheless, the pursued strategy led to the identification of the 1-(2-diphenyl)piperazine derivatives **13**, **21**, and **25**, which retained nanomolar affinity at 5-HT$_7$ receptor, appreciable selectivity (25- to 324-fold over 5-HT$_{1A}$ and 95- to 245-fold over D$_2$ receptors), still showing lipophilicity within the target range. Compounds **13**, **21**, and **25** showed full competitive agonism at 5-HT$_7$ receptor in an isolated guinea pig ileum assay, as well as their corresponding tetrahydronaphthalenyl derivative **5**. Compound **25** rapidly crossed the blood−brain barrier and equilibrated with plasma, achieving concentrations in the low micromolar range after intraperitoneal doses in mice. Compound **25** undergoes N-dealkylation of the aliphatic chain attached to the piperazine nitrogen, resulting in the formation of 1-(2-diphenyl)piperazine, whose pharmacokinetic behavior is different from the parent compound. Future studies will focus on characterization of the neuropharmacological profile of this metabolite compared with compound **25**.

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 mm × 4.6 mm, 5 μm particle size) and equipped with a Perkin-Elmer 785A UV/vis detector setting λ = 254 nm. Compounds **8**–**31** were eluted with CH$_3$OH/H$_2$O/Et$_3$N, 4:1:0.01, v/v/v, at a flow rate of 1 mL/min. When necessary, a standard procedure was used to transform final compounds into their hydrochloride salts. The following compounds were synthesized according to published procedures: 4-hydroxybenzylamine,[32] 1-(2-isopropylphenyl)piperazine,[41] 1-(2-diphenyl)piperazine.[42]

N-t-Butoxycarbonyl-4-(methanesulfonyloxyphenyl)-methanamine (35). 4-Hydroxybenzylamine hydrochloride (3.18 g, 14.7 mmol) was solubilized in a mixture of H$_2$O (25 mL) and THF (60 mL). Et$_3$N (2.16 mL, 14.7 mmol) and di-t-butylcarbonate (3.4 g, 14.7 mmol) was added to the solution. The mixture was stirred at room temperature overnight. Then THF was evaporated in vacuo and the aqueous residue was extracted with AcOEt (3 × 20 mL). The organic phases were collected, dried over Na$_2$SO$_4$, and concentrated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/ AcOEt 9:1 as eluent) to give the Boc protected amine as a yellow solid. Triethylamine (2.5 mL, 17.9 mmol) and methanesulfonyl chloride (1.5 mL, 19.4 mmol) were added to a solution of Boc protected amine (2.7 g, 12.1 mmol) in CH$_2$Cl$_2$ cooled at −10 °C. The mixture was stirred at room temperature for 6 h. Then, the reaction mixture was first washed with a saturated aqueous solution of NaHCO$_3$ and then with 3 N HCl. The separated organic phase was dried over Na$_2$SO$_4$ and concentrated under reduced pressure to yield pure **35** as a white semisolid (2.8 g, 77% yield). ^{1}H NMR (CDCl$_3$): δ 1.45 (s, 9H), 3.13 (s, 3H), 4.31 (br d, 2H), 7.21−7.25 (m, 2H), 7.33 (d, 2H, *J*= 8.5 Hz).

4-Methanesulfonyloxyphenylmethanamine (36d). A mixture of **35** (1.4 g, 4.65 mmol) and trifluoroacetic acid (1.04 mL, 14 mmol) in CH$_2$Cl$_2$ (30 mL) was stirred at room temperature overnight. Then the reaction mixture was washed twice with aqueous 20% Na$_2$CO$_3$ (2 × 20 mL). The organic phase was separated, dried over Na$_2$SO$_4$, and evaporated under reduced pressure, giving pure **36d** as a yellow oil (0.78 g, 83% yield). ^{1}H NMR (CDCl$_3$): δ 2.12 (s, 2H, D$_2$O exchanged), 3.07 (s, 3H), 3.82 (s, 2H), 7.15−7.19 (m, 2H), 7.25−7.33 (m, 2H).

General Procedure for Preparation of Alkylating Agents 33a,b and 37a−d. A cooled solution of amine **32a,b** and **36a−d** (4.0 mmol) in CH$_2$Cl$_2$ was stirred vigorously with 2% aqueous NaOH (9.6 mL, 4.8 mmol) while 6-bromohexanoyl chloride (4.8 mmol) in CH$_2$Cl$_2$ 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, with H$_2$O, and then dried over Na$_2$SO$_4$ and evaporated under reduced pressure. The crude residue was chromatographed as detailed below to give compounds **33a,b** and **37a−d** as white semisolids.

N-(6-Bromo-1-oxohexyl)isoindoline (33a). Eluted with CHCl$_3$/AcOEt, 9:1; 80% yield. ^{1}H NMR (CDCl$_3$): δ 1.48−1.59 (m, 2H), 1.71−1.81 (m, 2H), 1.87−1.97 (m, 2H), 2.40 (app t, 2H), 3.43 (t, 2H, *J* = 6.8 Hz), 4.80 (s, 4H), 7.24−7.31 (m, 4H). GC/MS: *m/z* 297 (M$^+$ +2, 32), 295 (M$^+$, 32), 216 (32), 161 (68), 160 (64), 146 (43), 118 (100).

N-(6-Bromo-1-oxohexyl)-1,2,3,4-tetrahydroisoquinoline (33b). Eluted with CHCl$_3$/AcOEt 9:1; 90% yield. ^{1}H NMR (CDCl$_3$): δ 1.46−1.56 (m, 2H), 1.66−1.76 (m, 2H), 1.85−1.95 (m, 2H), 2.42 (app t, 2H), 2.83−2.92 (m, 2H), 3.39−3.44 (m, 2H), 3.38 (t, 1H, *J* = 5.8 Hz), 3.83 (app t, 1H), 4.62 (s, 1H), 4.73 (s, 1H), 7.09−7.23 (m, 4H). GC/MS: *m/z* 311 (M$^+$ +2, 91), 309 (M$^+$, 93), 203 (54), 188 (43), 174 (93), 132 (100), 117 (51), 104 (42).

N-Benzyl-6-bromohexanamide (37a). Eluted with CHCl$_3$; 81% yield. ^{1}H NMR (CDCl$_3$): δ 1.42−1.53 (m, 2H), 1.64−1.74 (m, 2H), 1.82−1.92 (m, 2H), 2.25 (app. t, 2H), 3.40 (t, 2H, *J* = 6.8 Hz), 4.44 (d, 2H, *J* = 5.8 Hz), 5.76 (br t, 1H, D$_2$O exchanged), 7.23−7.37 (m, 5H). GC/MS: *m/z* 285 (M$^+$ +2, 28), 283 (M$^+$, 28), 149 (99), 106 (54), 91 (100).

6-Bromo-N-(4-pyridinylmethyl)hexanamide (37b). Eluted with CHCl$_3$/MeOH 19:1; 84% yield. ^{1}H NMR (CDCl$_3$): δ 1.34−1.43 (m, 2H), 1.53−1.61 (m, 2H), 1.72−1.80 (m, 2H), 2.17 (app. t, 2H), 3.30 (t, 2H, *J* = 6.8 Hz), 4.30 (d, 2H, *J* = 5.8 Hz), 5.76 (br t, 1H, D$_2$O exchanged), 7.09−7.11 (m, 2H), 8.40−8.42 (m, 2H). GC/MS: *m/z* 286 (M$^+$ +2, 2), 284 (M$^+$, 3), 150 (100), 92 (58).

6-Bromo-N-(4-Cyanophenylmethyl)hexanamide (37c). Eluted with CHCl$_3$/AcOEt 1:1; 78% yield. ^{1}H NMR (CDCl$_3$): δ 1.43−1.53 (m, 2H), 1.65−1.73 (m, 2H), 1.83−1.92 (m, 2H), 2.26 (app. t, 2H), 3.41 (t, 2H, *J* = 6.6 Hz), 4.49 (d, 2H, *J* = 6.05 Hz), 5.93 (br t, 1H, D$_2$O exchanged), 7.37 (d, 2H, *J* = 8.5 Hz), 7.59−7.63 (m, 2H). GC/MS: *m/z* 310 (M$^+$ +2, 13), 308 (M$^+$, 13), 174 (100), 131 (27), 116 (82).

6-Bromo-N-(4-Methanesulfonyloxyphenylmethyl)hexanamide (37d). Eluted with CHCl$_3$/AcOEt 1:1; 30% yield. ^{1}H NMR (CDCl$_3$): δ 1.43−1.53 (m, 2H), 1.62−1.75 (m, 2H), 1.83−1.92 (m, 2H), 2.24 (t, 2H, *J* = 7.4 Hz), 3.14 (s, 3H), 3.40 (t, 2H, *J* = 6.6 Hz), 4.44 (d, 2H, *J* = 5.8 Hz), 5.82 (br t, 1H, D$_2$O exchanged), 7.22−7.26 (m, 2H), 7.31−7.35 (m, 2H).

General Procedure for Preparation of Final Compounds 8−31. A stirred mixture of alkylating agent **33a,b** and **37a−d** (8.0 mmol), 1-substituted piperazine (9.6 mmol), and K$_2$CO$_3$ (8.0 mmol) in acetonitrile was refluxed overnight. After cooling, the mixture was evaporated to dryness and H$_2$O (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$_2$SO$_4$ and evaporated under reduced pressure. The crude residue was chromatographed (CHCl$_3$/MeOH, 19:1, as eluent) to afford pure compounds **8−31**.

2-[6-[4-(2-Methylthiophenyl)-1-piperazinyl]-1-oxohexyl]isoindoline (8). Yield 73%. ^{1}H NMR (CDCl$_3$): δ 1.39−1.48 (m, 2H), 1.57−1.68 (m, 2H), 1.77 (quintet, 2H, *J*= 7.7 Hz), 2.37−2.40 (m, 2H), 2.40 (s, 3H), 2.47 (app. t, 2H), 2.68 (br s, 4H), 3.06 (br s, 4H), 4.81 (s, 4H), 7.05−7.11 (m, 4H), 7.26−7.31 (m, 4H). GC/MS: *m/z* 424 (M$^+$ +1, 3), 423 (M$^+$, 15), 245 (94), 221 (100), 136 (33). The hydrochloride salt melted at 187−189 °C (from MeOH/Et$_2$O). Anal. (C$_{25}$H$_{33}$N$_3$OS·HCl·0.3H$_2$O) C, H, N.

2-[6-[4-(2-Diphenyl)-1-piperazinyl]-1-oxohexyl]isoindoline (9). Yield 82%. ^{1}H NMR (CDCl$_3$): δ 1.33−1.43 (m, 2H), 1.49−1.59 (m, 2H), 1.72 (quintet, 2H, *J*= 7.4 Hz), 2.32−2.39 (m, 8H), 2.88 (app t, 4H), 4.79 (s, 4H), 7.02−7.09 (m, 2H), 7.23−7.30 (m, 7H), 7.36−7.41 (m, 2H), 7.61−7.64 (m, 2H). GC/MS: *m/z* 454 (M$^+$ +1, 5), 453 (M$^+$, 7), 251 (71), 245 (100), 180 (48), 118 (30); mp 134−136 °C (from CHCl$_3$/n-hexane). Anal. (C$_{30}$H$_{35}$N$_3$O) C, H, N.

2-[6-[4-(2-(1-Methylethyl)phenyl)-1-piperazinyl]-1-oxohexyl]isoindoline (10). Yield 56%. ^{1}H NMR (CDCl$_3$): δ 1.18 (s, 3H), 1.21 (s, 3H), 1.42−1.49 (m, 2H), 1.61−1.67 (m, 2H), 1.77 (quintet, 2H, *J* = 7.4 Hz), 2.40 (t, 2H, *J* = 7.4 Hz), 2.48 (app. t, 2H), 2.66 (br s, 4H), 2.93 (app t, 4H), 3.45−3.51 (m, 1H), 4.81 (s, 4H), 7.08−7.16 (m, 3H), 7.25−7.31 (m, 5H). GC/MS: *m/z* 420 (M$^+$ +1, 3), 419 (M$^+$, 7), 245 (100), 217 (45), 118 (28); mp 114−116 °C (from CHCl$_3$/n-hexane). Anal. (C$_{27}$H$_{37}$N$_3$O) C, H, N.

2-[6-[4-(2-Methoxyphenyl)-1-piperazinyl]-1-oxohexyl]isoindoline (11). Yield 74%. ^{1}H NMR (CDCl$_3$): δ 1.37−1.47 (m, 2H), 1.55−1.65 (m, 2H), 1.76 (quintet, 2H, *J* = 7.4 Hz), 2.39 (t, 2H, *J* = 7.4 Hz), 2.42−2.46 (m, 2H), 2.66 (br s, 4H), 3.10 (br s, 4H), 3.86 (s, 3H), 4.80 (s, 4H), 6.84−7.02 (m, 4H), 7.25−7.33 (m, 4H). GC/MS: *m/z* 408 (M$^+$ +1, 5), 407 (M$^+$, 18), 392 (39), 245 (74), 205 (100), 118 (50). The hydrochloride salt melted at 198−200 °C (from MeOH/Et$_2$O). Anal. (C$_{25}$H$_{33}$N$_3$O$_2$·2HCl·0.2H$_2$O) C, H, N.

2-[6-[4-(2-Methylthiophenyl)-1-piperazinyl]-1-oxohexyl]-1,2,3,4-tetrahydroisoquinoline (12). Yield 60%. ^{1}H NMR (CDCl$_3$): δ 1.38−1.46 (m, 2H), 1.58−1.61 (m, 2H), 1.66−1.76 (m, 2H), 2.41 (s, 3H), 2.42−2.47 (m, 4H), 2.64 (br s, 4H), 2.85 (app t, 1H), 2.90 (t, 1H *J* = 5.8 Hz), 3.06 (br s, 4H), 3.68 (app t, 1H), 3.83 (t, 1H, *J* = 6.05 Hz), 4.62 (s, 1H), 4.73 (s, 1H), 7.04−7.10 (m, 4H),

7.12−7.22 (m, 4H). GC/MS: *m/z* 438 (M$^+$ +1, 4), 437 (M$^+$, 8), 259 (83), 221 (100), 206 (30), 132 (54). The hydrochloride salt melted at 112−114 °C dec (from MeOH/Et$_2$O). Anal. (C$_{26}$H$_{35}$N$_3$OS·HCl·H$_2$O) C, H, N.

2-[6-[4-(2-Diphenyl)-1-piperazinyl]-1-oxohexyl]-1,2,3,4-tetrahydroisoquinoline (13). Yield 48%. ^{1}H NMR (CDCl$_3$): δ 1.30−1.43 (m, 2H), 1.47−1.52 (m, 2H), 1.61−1.73 (m, 2H), 2.36−2.42 (m, 8H), 2.82−2.88 (m, 6H), 3.66 (app t, 1H), 3.82 (app t, 1H), 4.60 (s, 1H), 4.72 (s, 1H), 7.02−7.10 (m, 2H), 7.12−7.31 (m, 7H), 7.36−7.41 (m, 2H), 7.61−7.64 (m, 2H). GC/MS: *m/z* 468 (M$^+$ +1, 4), 467 (M$^+$, 10), 259 (100), 251 (78), 180 (65). The hydrochloride salt melted at 70−72 °C dec (from MeOH/Et$_2$O). Anal. (C$_{31}$H$_{37}$N$_3$O·HCl·H$_2$O) C, H, N.

2-[6-[4-(2-(1-Methylethyl)phenyl]-1-piperazinyl]-1-oxohexyl]-1,2,3,4-tetrahydroisoquinoline (14). Yield 95%. ^{1}H NMR (CDCl$_3$): δ 1.19 (s, 3H), 1.21 (s, 3H), 1.38−1.46 (m, 2H), 1.55−1.62 (m, 2H), 1.66−1.77 (m, 2H), 2.40−2.47 (m, 4H), 2.67 (br s, 4H), 2.85 (app t, 1H), 2.88−2.94 (m, 5H), 3.44−3.53 (m, 1H), 3.67 (app t, 1H), 3.83 (t, 1H, J = 6.05 Hz), 4.62 (s, 1H), 4.73 (s, 1H), 7.06−7.20 (m, 6H), 7.21−7.26 (m, 2H). GC/MS: *m/z* 434 (M$^+$ +1, 2), 433 (M$^+$, 5), 259 (100), 217 (38). The oxalate salt melted at 210−212 °C (from MeOH/Et$_2$O). Anal. (C$_{28}$H$_{39}$N$_3$O·(COOH)$_2$) C, H, N.

2-[6-[4-(2-Methoxyphenyl)-1-piperazinyl]-1-oxohexyl]-1,2,3,4-tetrahydroisoquinoline (15). Yield 90%. ^{1}H NMR (CDCl$_3$): δ 1.35−1.45 (m, 2H), 1.55−1.61 (m, 2H), 1.65−1.77 (m, 2H), 2.39−2.46 (m, 4H), 2.67 (br s, 4H), 2.85 (t, 1H, J = 6.05 Hz), 2.90 (app. t, 1H), 3.11 (br s, 4H), 3.68 (app t, 1H), 3.83 (t, 1H, J = 6.05 Hz), 3.86 (s, 3H), 4.62 (s, 1H), 4.73 (s, 1H), 6.84−7.03 (m, 4H), 7.10−7.22 (m, 4H). GC/MS: *m/z* 422 (M$^+$ +1, 5), 421 (M$^+$, 16), 406 (46), 259 (83), 205 (100), 190 (32). The hydrochloride salt melted at 164−166 °C (from MeOH/Et$_2$O). Anal. (C$_{26}$H$_{35}$N$_3$O$_2$·2HCl·0.7H$_2$O) C, H, N.

***N*-Benzyl-4-(2-methylthiophenyl)-1-piperazinehexanamide (16).** Yield 90%. ^{1}H NMR (CDCl$_3$): δ 1.34−1.42 (m, 2H), 1.52−1.62 (m, 2H), 1.71 (quintet, 2H, J = 7.4 Hz), 2.23 (t, 2H, J = 7.4 Hz), 2.41 (s, 3H), 2.42−2.45 (m, 2H), 2.64 (br s, 4H), 3.03 (br t, 4H), 4.44 (d, 2H, J = 5.5 Hz), 5.80 (br t, 1H, D$_2$O exchanged), 7.04−7.11 (m, 4H), 7.26−7.36 (m, 5H). GC/MS: *m/z* 442 (M$^+$ +1, 3), 441 (M$^+$, 10), 251 (100), 233 (38), 180 (61). The hydrochloride salt melted at 150−152 °C (from MeOH/Et$_2$O). Anal. (C$_{24}$H$_{33}$N$_3$OS·HCl·H$_2$O) C, H, N.

***N*-Benzyl-4-(2-diphenyl)-1-piperazinehexanamide (17).** Yield 90%. ^{1}H NMR (CDCl$_3$): δ 1.27−1.37 (m, 2H), 1.44−1.54 (m, 2H), 1.66 (quintet, 2H, J = 7.4 Hz), 2.20 (t, 2H, J = 7.4 Hz), 2.30 (app. t, 2H), 2.35 (br s, 4H), 2.85 (app t, 4H), 4.43 (d, 2H, J = 5.8 Hz), 5.79 (br t, 1H, D$_2$O exchanged), 7.02−7.09 (m, 2H), 7.23−7.41 (m, 10H), 7.61−7.64 (m, 2H). GC/MS: *m/z* 412 (M$^+$ +1, 3), 411 (M$^+$, 6), 233 (42), 221 (100), 206 (27). The hydrochloride salt melted at 212−214 °C (from MeOH/Et$_2$O). Anal. (C$_{29}$H$_{35}$N$_3$O·HCl) C, H, N.

***N*-Benzyl-4-[2-(1-methylethyl)phenyl]-1-piperazinehexanamide (18).** Yield 90%. ^{1}H NMR (CDCl$_3$): δ 1.19 (s, 3H), 1.21 (s, 3H), 1.33−1.43 (m, 2H), 1.53−1.63 (m, 2H), 1.71 (quintet, 2H, J = 7.4 Hz), 2.24 (app t, 2H), 2.43 (app. t, 2H), 2.62 (br s, 4H), 2.92 (app t, 4H), 3.41−3.55 (m, 1H), 4.45 (d, 2H, J = 5.8 Hz), 5.80 (br t, 1H, D$_2$O exchanged), 7.06−7.18 (m, 4H), 7.24−7.37 (m, 5H). GC/MS: *m/z* 408 (M$^+$ +1, 2), 407 (M$^+$, 4), 233 (100), 217 (73), 160 (21). The hydrochloride salt melted at 116−119 °C (from MeOH/Et$_2$O). Anal. (C$_{26}$H$_{37}$N$_3$O·HCl·1.2H$_2$O) C, H, N.

***N*-Benzyl-4-(2-methoxyphenyl)-1-piperazinehexanamide (19).** Yield 82%. ^{1}H NMR (CDCl$_3$): δ 1.32−1.42 (m, 2H), 1.52−1.62 (m, 2H), 1.71 (quintet, 2H, J = 7.4 Hz), 2.23 (t, 2H, J = 7.4 Hz), 2.42 (app. t, 2H), 2.66 (br s, 4H), 3.10 (br s, 4H), 3.86 (s, 3H), 4.44 (d, 2H, J = 5.5 Hz), 5.79 (br t, 1H, D$_2$O exchanged), 6.84−7.03 (m, 4H), 7.27−7.37 (m, 5H). GC/MS: *m/z* 396 (M$^+$ +1, 4), 395 (M$^+$, 15), 233 (27), 205 (100), 190 (29). The hydrochloride salt melted at 174−176 °C (from MeOH/Et$_2$O). Anal. (C$_{24}$H$_{33}$N$_3$O$_2$·2HCl·0.5H$_2$O) C, H, N.

4-(2-Methylthiophenyl)-*N*-(4-pyridinylmethyl)-1-piperazinehexanamide (20). Yield 87%. ^{1}H NMR (CDCl$_3$): δ 1.36−1.46 (m, 2H), 1.61−1.69 (m, 2H), 1.71−1.79 (m, 2H), 2.31 (t, 2H, J = 7.4 Hz),

2.40 (s, 3H), 2.53 (app. t, 2H), 2.75 (br s, 4H), 3.10 (br t, 4H), 4.46 (d, 2H, J = 6.1 Hz), 6.23 (br t, 1H, D$_2$O exchanged), 7.03−7.12 (m, 4H), 7.20 (dd, 2H, J = 1.65, 4.4 Hz), 8.55 (dd, 2H, J = 1.65, 4.4 Hz). GC/MS: *m/z* 413 (M$^+$ +1, 3), 412 (M$^+$, 16), 397 (39), 234 (72), 221 (100), 206 (59); mp 50−52 °C (from *n*-hexane). Anal. (C$_{23}$H$_{32}$N$_4$OS) C, H, N.

4-(2-Diphenyl)-*N*-(4-pyridinylmethyl)-1-piperazinehexanamide (21). Yield 64%. ^{1}H NMR (CDCl$_3$): δ 1.28−1.38 (m, 2H), 1.43−1.54 (m, 2H), 1.68 (q, 2H, J = 7.4 Hz), 2.25 (t, 2H, J = 7.4 Hz), 2.31−2.33 (m, 2H), 2.35 (br s, 4H), 2.86 (app t, 4H), 4.44 (d, 2H, J = 6.1 Hz), 6.00 (br t, 1H, D$_2$O exchanged), 7.01−7.09 (m, 2H), 7.16−7.18 (m, 2H), 7.22−7.31 (m, 3H), 7.36−7.41 (m, 2H), 7.60−7.64 (m, 2H), 8.54 (dd, 2H, J = 1.7, 4.4 Hz). GC/MS: *m/z* 443 (M$^+$ +1, 1), 442 (M$^+$, 10), 251 (82), 234 (78), 180 (100). The hydrochloride salt melted at 50 °C dec (from MeOH/Et$_2$O). Anal. (C$_{28}$H$_{34}$N$_4$O·2HCl·1.5H$_2$O) C, H, N.

4-[2-(1-Methylethyl)phenyl]-*N*-(4-pyridinylmethyl)-1-piperazinehexanamide (22). Yield 95%. ^{1}H NMR (CDCl$_3$): δ 1.18 (s, 3H), 1.21 (s, 3H), 1.35−1.45 (m, 2H), 1.55−1.65 (m, 2H), 1.73 (quintet, 2H, J = 7.4 Hz), 2.29 (t, 2H, J = 7.4 Hz), 2.44 (app. t, 2H), 2.64 (br s, 4H), 2.94 (app t, 4H), 3.43−3.52 (m, 1H), 4.46 (d, 2H, J = 6.1 Hz), 6.10 (br t, 1H, D$_2$O exchanged), 7.06−7.16 (m, 4H), 7.18−7.20 (m, 1H), 7.24−7.25 (m, 1H), 8.55 (dd, 2H, J = 1.4, 4.4 Hz). GC/MS: *m/z* 409 (M$^+$ +1, 1), 408 (M$^+$, 6), 234 (100), 217 (58), 160 (30). The hydrochloride salt melted at 163−166 °C dec (from MeOH/Et$_2$O). Anal. (C$_{25}$H$_{36}$N$_4$O·3HCl·H$_2$O) C, H, N.

4-(2-Methoxyphenyl)-*N*-(4-pyridinylmethyl)-1-piperazinehexanamide (23). Yield 70%. ^{1}H NMR (CDCl$_3$): δ 1.34−1.43 (m, 2H), 1.53−1.63 (m, 2H), 1.67−1.77 (m, 2H), 2.28 (t, 2H, J = 7.4 Hz), 2.42 (app. t, 2H), 2.66 (br s, 4H), 3.10 (br s, 4H), 3.85 (s, 3H), 4.45 (d, 2H, J = 6.1 Hz), 6.10 (br t, 1H, D$_2$O exchanged), 6.84−7.03 (m, 4H), 7.18 (dd, 2H, J = 1.6, 4.4 Hz), 8.54 (dd, 2H, J = 1.6, 4.4 Hz). GC/MS: *m/z* 397 (M$^+$ +1, 2), 396 (M$^+$, 10), 234 (36), 205 (100), 190 (38); mp 93−94 °C (from *n*-hexane). Anal. (C$_{23}$H$_{32}$N$_4$O$_2$) C, H, N.

***N*-(4-Cyanophenylmethyl)-4-(2-methylthiophenyl)-1-piperazinehexanamide (24).** Yield 66%. ^{1}H NMR (CDCl$_3$): δ 1.33−1.43 (m, 2H), 1.52−1.62 (m, 2H), 1.66−1.76 (m, 2H), 2.27 (t, 2H, J = 7.4 Hz), 2.40 (s, 3H), 2.42−2.45 (m, 2H), 2.63 (br s, 4H), 3.03 (app t, 4H), 4.50 (d, 2H, J = 4.5 Hz), 6.00 (br t, 1H, D$_2$O exchanged), 7.02−7.13 (m, 4H), 7.36−7.39 (m, 2H), 7.60−7.63 (m, 2H). GC/MS: *m/z* 437 (M$^+$ +1, 5), 436 (M$^+$, 16), 221 (100), 206 (19), 116 (23). The hydrochloride salt melted at 100−103 °C dec (from MeOH/Et$_2$O). Anal. (C$_{25}$H$_{32}$N$_4$OS·HCl·H$_2$O) C, H, N.

***N*-(4-Cyanophenylmethyl)-4-(2-diphenyl)-1-piperazinehexanamide (25).** Yield 90%. ^{1}H NMR (CDCl$_3$): δ 1.27−1.37 (m, 2H), 1.44−1.54 (m, 2H), 1.66 (quintet, 2H, J = 7.4 Hz), 2.23 (t, 2H, J = 7.4 Hz), 2.28−2.32 (m, 2H), 2.35 (br s, 4H), 2.85 (app t, 4H), 4.48 (d, 2H, J = 6.1 Hz), 5.97 (br t, 1H, D$_2$O exchanged), 7.00−7.10 (m, 2H), 7.22−7.31 (m, 3H), 7.33−7.41 (m, 4H), 7.58−7.64 (m, 4H). GC/MS: *m/z* 467 (M$^+$ +1, 9), 466 (M$^+$, 20), 251 (100), 180 (51), 116 (26); mp 50 °C dec (from *n*-hexane). Anal. (C$_{30}$H$_{34}$N$_4$O) C, H, N.

***N*-(4-Cyanophenylmethyl)-4-[2-(1-methylethyl)phenyl]-1-piperazinehexanamide (26).** Yield 83%. ^{1}H NMR (CDCl$_3$): δ 1.18 (s, 3H), 1.21 (s, 3H), 1.34−1.43 (m, 2H), 1.53−1.64 (m, 2H), 1.72 (quintet, 2H, J = 7.4 Hz), 2.28 (t, 2H, J = 7.4 Hz), 2.43 (app. t, 2H), 2.62 (br s, 4H), 2.92 (app t, 4H), 3.43−3.52 (m, 1H), 4.50 (d, 2H, J = 6.1 Hz), 6.03 (br t, 1H, D$_2$O exchanged), 7.07−7.18 (m, 3H), 7.24−7.27 (m, 1H), 7.38 (d, 2H, J = 8.3 Hz), 7.60−7.63 (m, 2H). GC/MS: *m/z* 433 (M$^+$ +1, 3), 432 (M$^+$, 8), 258 (100), 217 (99), 160 (34), 132 (31). The hydrochloride salt melted at 83−85 °C dec (from MeOH/Et$_2$O). Anal. (C$_{27}$H$_{36}$N$_4$O·2HCl) C, H, N.

***N*-(4-Cyanophenylmethyl)-4-(2-methoxyphenyl)-1-piperazinehexanamide (27).** Yield 95%. ^{1}H NMR (CDCl$_3$): δ 1.33−1.43 (m, 2H), 1.53−1.63 (m, 2H), 1.71 (quintet, 2H, J = 7.4 Hz), 2.27 (t, 2H, J = 7.4 Hz), 2.43 (app. t, 2H), 2.67 (br s, 4H), 3.10 (app t, 4H), 3.86 (s, 3H), 4.49 (d, 2H, J = 6.1 Hz), 6.03 (br t, 1H, D$_2$O exchanged), 6.84−6.89 (m, 1H), 6.916.96 (m, 2H), 6.97−7.03 (m, 1H), 7.37 (d, 2H, J = 8.5 Hz), 7.59−7.62 (m, 2H). GC/MS: *m/z*

421 (M[+] +1, 7), 420 (M[+], 24), 205 (100), 190 (20); mp 123−125 °C dec (from CHCl$_3$/n-hexane). Anal. (C$_{25}$H$_{32}$N$_4$O$_2$) C, H, N.

N-(4-Methanesulfonyloxyphenylmethyl)-4-(2-methylthiophenyl)-1-piperazinehexanamide (28). Yield 95%. ^{1}H NMR (CDCl$_3$): δ 1.32−1.42 (m, 2H), 1.53−1.63 (m, 2H), 1.70 (quintet, 2H, J = 7.4 Hz), 2.24 (t, 2H, J = 7.4 Hz), 2.41 (s, 3H), 2.43−2.46 (m, 2H), 2.65 (br s, 4H), 3.04 (app t, 4H), 3.13 (s, 3H), 4.45 (d, 2H, J = 5.8 Hz), 5.94 (br t, 1H, D$_2$O exchanged), 7.03−7.16 (m, 4H), 7.23−7.25 (m, 2H), 7.32−7.35 (m, 2H). ESI[+]/MS m/z 506 (MH+). ESI[+]/MS/MS m/z 341 (42), 298 (100), 249 (49), 185 (53). The hydrochloride salt melted at 118−120 °C (from MeOH/Et$_2$O). Anal. (C$_{25}$H$_{35}$N$_3$O$_4$S$_2$·HCl·0.7H$_2$O) C, H, N.

N-(4-Methanesulfonyloxyphenylmethyl)-4-(2-diphenyl)-1-piperazinehexanamide (29). Yield 95%. ^{1}H NMR (CDCl$_3$): δ 1.27−1.36 (m, 2H), 1.44−1.54 (m, 2H), 1.66 (quintet, 2H, J = 7.7 Hz), 2.21 (t, 2H, J = 7.7 Hz), 2.28−2.33 (m, 2H), 2.36 (br s, 4H), 2.86 (app t, 4H), 3.13 (s, 3H), 4.43 (d, 2H, J = 6.1 Hz), 5.89 (br t, 1H, D$_2$O exchanged), 7.01−7.10 (m, 2H), 7.22−7.41 (m, 9H), 7.61−7.64 (m, 2H). ESI[+]/MS m/z 536 (MH[+]). ESI[+]/MS/MS m/z 298 (100), 251 (32), 185 (56). The hydrochloride salt melted at 160−163 °C (from MeOH/Et$_2$O). Anal. (C$_{30}$H$_{37}$N$_3$O$_4$S·HCl·H$_2$O) C, H, N.

N-(4-Methanesulfonyloxyphenylmethyl)-4-[2-(1-methylethyl)phenyl]-1-piperazinehexanamide (30). Yield 88%. ^{1}H NMR (CDCl$_3$): δ 1.18 (s, 3H), 1.21 (s, 3H), 1.33−1.43 (m, 2H), 1.53−1.63 (m, 2H), 1.71 (quintet, 2H, J = 7.7 Hz), 2.25 (t, 2H, J = 7.7 Hz), 2.42 (app. t, 2H), 2.61 (br s, 4H), 2.92 (app t, 4H), 3.13 (s, 3H), 3.43−3.53 (m, 1H), 4.45 (d, 2H, J = 4.5 Hz), 5.94 (br t, 1H, D$_2$O exchanged), 7.06−7.18 (m, 4H), 7.22−7.26 (m, 2H), 7.32−7.35 (m, 2H). ESI[+]/MS m/z 502 (MH[+]). ESI[+]/MS/MS m/z 298 (100), 185 (56). The hydrochloride salt melted at 83−85 °C dec (from MeOH/Et$_2$O). Anal. (C$_{27}$H$_{39}$N$_3$O$_4$S·HCl·1.5H$_2$O) C, H, N.

N-(4-Methanesulfonyloxyphenylmethyl)-4-(2-methoxyphenyl)-1-piperazinehexanamide (31). Yield 80%. ^{1}H NMR (CDCl$_3$): δ 1.32−1.42 (m, 2H), 1.52−1.62 (m, 2H), 1.70 (quintet, 2H, J = 7.4 Hz), 2.24 (t, 2H, J = 7.4 Hz), 2.40 (app. t, 2H), 2.65 (br s, 4H), 3.09 (br s, 4H), 3.13 (s, 3H), 3.86 (s, 3H), 4.44 (d, 2H, J = 6.1 Hz), 5.89 (br t, 1H, D$_2$O exchanged), 6.84−7.03 (m, 4H), 7.21−7.27 (m, 2H), 7.31−7.36 (m, 2H). ESI[+]/MS m/z 490 (MH[+]). ESI[+]/MS/MS m/z 396 (65), 298 (100), 185 (56). The hydrochloride salt melted at 146−149 °C dec (from MeOH/Et$_2$O). Anal. (C$_{25}$H$_{35}$N$_3$O$_5$S·2HCl·H$_2$O) 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 mm × 4.6 mm, 5 μm particle size) as nonpolar stationary phase and with MeOH/0.01 M phosphate buffer pH 6 (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 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 (EEC 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, and [^{3}H]-spiroperidol were obtained from Perkin-Elmer-NEN (Zaventem, Belgium). Compound **2**, 5-CT, and substance P were purchased from Tocris Bioscience (Bristol, UK). 8-OH-DPAT hydrobromide was from RBI. 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.[43] 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 (6−9 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 × 53 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.[44] 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 Human Cloned D$_{2L}$ Dopaminergic Receptors. Binding of [^{3}H]-spiroperidol at human cloned receptors was performed according to Scarselli et al.[45] 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 6−9 concentrations of drug solution in a final volume of 500 μL. The samples were incubated for 120 min at 25 °C, 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. Non specific binding was determined in the presence of 10 μM haloperidol. The radioactivity bound to the filters was measured by liquid scintillation using LS6500 multipurpose scintillation Counter, Beckman.

5. 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$_2$PO$_4$, 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.[46] 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.[47] 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). 100 nM substance P 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). We have determined that 5-CT induced relaxation with maximal response (39%) at 3 μM concentration. Analogously, each compound tested (0.001−10 μM) was added 5 min before substance P addition and noncumulative concentration−response curve was constructed. Relaxant responses to full agonists 5-CT **13**, **21**, and **25** were surmountably antagonized by **2** (0.1−3 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 4. 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.

6. Disposition Studies. Male CD1 albino mice, 25−30 g (Charles River, Italy) were used. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L., no. 116, G.U., suppl. 40, 18 Febbraio 1992, Circolare no. 8, G.U., 14 Luglio 1994) and international laws and policies (EEC Council Directive 86/609, O. J. L358, 1, Dec 12, 1987; Guide for the Care and Use of Laboratory, U.S. National Research Council, 1996).

Mice were given the test compounds intraperitoneally (10 mg/kg) and were killed by decapitation at various times thereafter. Mixed arteriovenous trunk blood was collected in heparinized tubes, centrifuged at 3000g for 10 min, and the plasma was stored at −20 °C. Brain was removed immediately, blotted with paper to remove surface blood, and quickly frozen in dry ice. Compounds and their 1-arylpiperazine metabolites were extracted from plasma and brain homogenate and quantified by reversed-phase HPLC with UV detection (230 nm). Briefly: to 0.1 mL of plasma, 0.2 mL of 20 mM of ammonium bicarbonate and 0.02 mL of a methanolic solution of the internal standard (100 μg/mL) were added; samples were then extracted twice with 1.5 mL of hexane containing 1% of isoamyl alcohol, and the combined extracts were evaporated to dryness and reconstituted in 0.15 mL of the mobile phase, which was injected into the chromatographic column. Brain tissue was homogenized in distilled water (1 g/10 mL), and 1 mL of the homogenate was extracted twice with 1.5 mL of hexane/isoamyl alcohol as described for plasma. Then, the organic phase was shaken with 0.2 mL of the mobile phase (compound **25** only); after centrifugation, 0.1 mL of the aqueous phase was injected into the chromatographic column.

Separation was done on a Brownlee Laboratory Spheri 5 RP 8 (4.6 mm × 250 mm; 5 μm), with 0.01 M KH$_2$PO$_4$/CH$_3$CN/CH$_3$OH (50:45:5 v/v, compound **25**; 50:40:10 v/v, compound **4**), at pH 3.5, as the mobile phase. A derivative of the present series was used as internal standard.

Standard curves were prepared by spiking blank mouse plasma and brain homogenate. The lower limit for quantification was about 0.1 μg/mL in plasma and 0.1 μg/g in brain tissue, using respectively 0.1 mL of plasma and 1 mL of brain homogenate (or approximately 100 mg of brain tissue). At these concentrations the coefficient of variation for the precision of the assay was 10−20%. Higher concentrations generally gave coefficients of variation less than 10%, regardless of the compound and tissue analyzed. Brain concentrations were not corrected for compounds **4** and **25** contributed by the residual blood, because this generally amounted to only 1−3% of the measured brain concentrations, assuming a mean value of 20 μL per gram of brain.[48]

7. Statistical and Pharmacokinetic Analysis. The inhibition curves on the different binding sites of the compounds reported in Tables 2 and 3 were analyzed by nonlinear curve fitting utilizing the GraphPad Prism program.[49] The value for the inhibition constant, K_i, was calculated by using the Cheng−Prusoff equation.[50] Agonist potencies, expressed as EC$_{50}$, were obtained from nonlinear iterative curve fitting by GraphPad Prism.

Mean plasma and brain and plasma area under the concentration-time curve (AUCt) were determined using the linear trapezoidal rule and extrapolated to infinity (AUC), when possible, using the elimination rate constant. The maximum concentration (C_{max}) and the time of its occurrence were read directly from the plasma and brain concentration−time data. Standard noncompartmental analysis, using the Win Nonlin software Pro Node 4.1 (Pharsight Co, Mountain View, CA) was done to calculate total body clearance, volume of distribution, and elimination half-life ($t_{1/2}$).

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