Synthesis and Structure−Activity Relationships of a New Model of Arylpiperazines. 8.[1] Computational Simulation of Ligand−Receptor Interaction of 5-HT_{1A}R Agonists with Selectivity over α_1-Adrenoceptors

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We have designed and synthesized a new series of arylpiperazines **V** exhibiting high 5-HT_{1A}R affinity and selectivity over α_1-adrenoceptors. The new selective 5-HT_{1A}R ligands contain a hydantoin ($m = 0$) or diketopiperazine ($m = 1$) moiety and an arylpiperazine moiety separated by one methylene unit ($n = 1$). The aryl substituent of the piperazine moiety (Ar) consists of different benzofused rings mimicking the favorable voluminous substituents at ortho and meta positions predicted by 3D-QSAR analysis in the previously reported series **I**. In particular, (*S*)-2-[[4-(naphth-1-yl)piperazin-1-yl]methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [(*S*)-**9**, CSP-2503] (5-HT_{1A}, $K_i = 4.1$ nM; α_1, $K_i > 1000$ nM) has been pharmacologically characterized as a 5-HT_{1A}R agonist at somatodendritic and postsynaptic sites, endowed with anxiolytic properties. Ligand (*S*)-**9** is predicted, in computer simulations, to bind $Asp^{3.32}$ in TMH 3, $Thr^{5.39}$ and $Ser^{5.42}$ in TMH 5, and $Trp^{6.48}$ in TMH 6. We propose that agonists modify, by means of an explicit hydrogen bond, the conformation of $Trp^{6.48}$ from pointing toward TMH 7, in the inactive gauche+ conformation, to pointing toward the ligand binding site, in the active trans conformation.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) represents a major target for neurobiological research because of its involvement in numerous (patho)physiological processes.[2−5] The development of drugs that alter 5-HT neurotransmission is thus an area of intense research because of the potential to find new therapeutic agents. Molecular cloning, pharmacological properties, second messenger coupling, and amino acid sequence have led to the identification of 14 serotonin receptor subtypes that can be classified into seven subfamilies (5-HT_{1-7}).[6,7] The 5-HT_{1A} subtype is one of the best studied, its agonists and partial agonists being effective in the treatment of anxiety and depression.[8−10] Moreover, recent preclinical studies have suggested that 5-HT_{1A}R agonists also have neuroprotective properties.[11−13]

All serotonin receptors, except the 5-HT_3 subtype, belong to the seven transmembrane G-protein-coupled receptor superfamily (GPCR).[14] The GPCR family possesses highly conserved motifs in the transmembrane region, which suggests a common transmembrane helical bundle.[15] In particular, the transmembrane amino acid sequence of the 5-HT_{1A}R presents a high degree of homology with the α_1-adrenergic receptor.[16] As a consequence, many 5-HT_{1A}R ligands are poorly selective over α_1-adrenergic receptors. In this context, some years ago our group participated in a wide research program aimed at developing new high-affinity 5-HT_{1A}R ligands with selectivity over α_1-adrenoceptors.[1,17−26] Using as a starting point a series of arylpiperazines **I** ($n = 3, 4$) (Chart 1) showing affinity at both receptors,[17,18] we have carried out a systematic study based on the nonpharmacophoric and pharmacophoric sites of both receptors to gain insight into the structural factors that are responsible for 5-HT_{1A}/α_1 selectivity.

With respect to the pharmacophore part (arylpiperazine), a training set of 32 compounds of general structure **I** was used to derive classical QSAR and neural networks models for both 5-HT_{1A} and α_1-adrenergic receptors.[24] These models provide a significant correlation of electrostatic, steric, and lipophilic parameters with biological affinities and led us to design and synthesize a new ligand, EF-7412 [X = $-(CH_2)_3-$, $m = 0$, $n = 4$, Ar = *m*-(ethylsulfonamido)phenyl; 5-HT_{1A}, $K_i = 27$ nM; α_1, $K_i > 1000$ nM], with a high selectivity profile for the 5-HT_{1A}R sites.

Regarding the nonpharmacophoric part, the influence of electronic, steric, and lipophilic factors on the stabilization of receptor−ligand complexes has been investigated in different series of derivatives **II**−**IV** (Chart 1). SAR studies of series **II**, focusing on the terminal amide fragment, have revealed a significant influence of electronic factors on the α_1-adrenergic receptor site without modifying the stabilization of the 5-HT_{1A}R−

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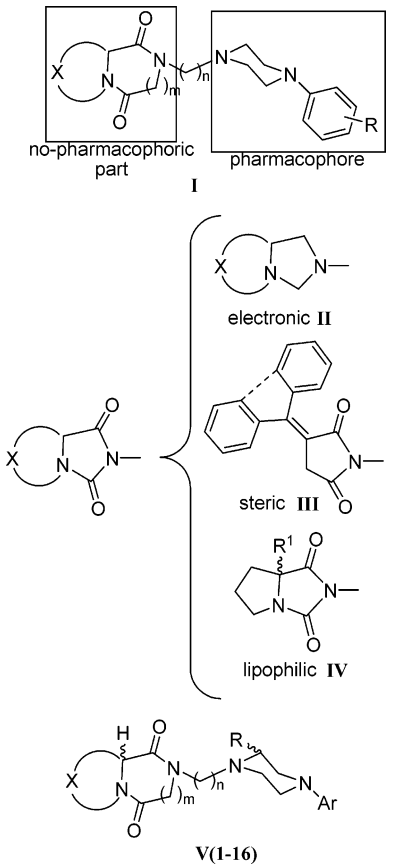
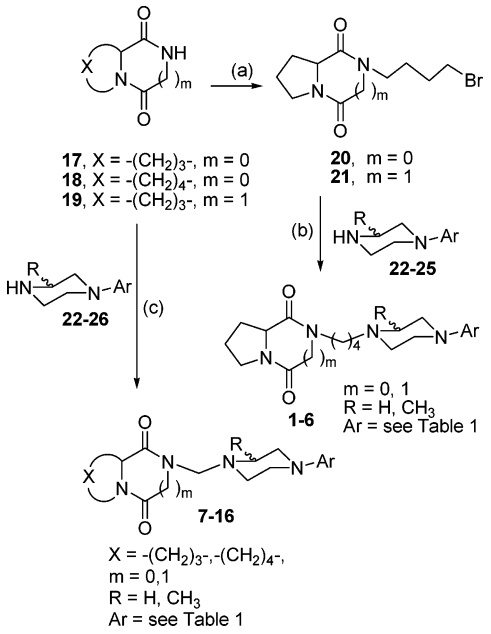
Chart 1. Arylpiperazines of General Structures **I**–**V**



ligand complex.[20] Analysis of series **III**, aiming to study the steric requirements of the terminal amide portion, led us to conclude that the nonpharmacophoric pocket in the 5-HT$_{1A}$R has less restriction than the corresponding pocket in the α_1-adrenergic receptor, suggesting some differences between the nonpharmacophoric sites of both 5-HT$_{1A}$ and α_1-adrenergic receptors.[21] Finally, the influence of lipophilic factors at the amide fragment of a new series **IV** has been studied. Variations of log *P* have been carried out by introducing different hydrocarbonated substituents (R^1) at the 7a position of the bicyclohydantoin. Lipophilicity of the ligands does not exert a crucial influence on the 5-HT$_{1A}$R affinity or on the selectivity over α_1-adrenergic receptors.[1]

We have recently constructed a computer model of the complexes between arylpiperazines of formula **I** [X = $-(CH_2)_3-$, $m = 0$, $n = 4$, Ar = *m*-(ethylsulfonamido)phenyl (EF-7412); X = $-(CH_2)_3-$, $m = 0$, $n = 4$, Ar = *m*-(acetylamino)phenyl] and a rhodopsin-based 3D model of the 5-HT$_{1A}$R transmembrane domain.[24,25] These models have permitted us to identify the molecular determinants of the ligand–receptor interaction that include the ionic interaction between the protonated amine of the ligand and Asp$^{3.32}$, the hydrogen bonds between the *m*-NHSO$_2$Et or *m*-NHCOCH$_3$ groups and Asn$^{7.39}$, and the hydrogen bonds between the hydantoin moiety and Ser$^{5.42}$ and Thr$^{5.43}$. Recently, a kinetic analysis of the agonist-induced conformational changes in the β_2-adrenergic receptor has shown that the interaction of the ligand with Asp$^{3.32}$ and the Ser/Thr residues in transmembrane helix (TMH) 5 is a fast component ($t_{1/2}$ < 5 s) in the process of receptor activation.[27] These interactions of the catechol hydroxyl

Scheme 1[a]



[a] Reagents and conditions: (a) Br(CH$_2$)$_4$Br, NaH, DMF, 110 °C, 1–3 h; (b) NEt$_3$, CH$_3$CN, 60 °C, 20 h; (c) (i) HCHO(aq), EtOH, reflux, 6 h; (ii) for **16**, HCl(g), Et$_2$O, room temp, 20 min.

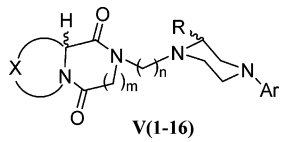
groups of epinephrine with the cluster of Ser/Thr play a critical role in the proper positioning of TMH 5 in the active state of the receptor.[28] In addition, it has recently been observed in the structure of metarhodopsin I, determined by electron crystallography,[29] that Trp$^{6.48}$ undergoes a conformational transition, in the process of receptor activation, from pointing toward TMH 7, in the inactive gauche+ conformation, to pointing toward TMH 5, in the active trans conformation as was previously suggested by computer simulations.[30] The fact that these residues are also present in the serotoninergic receptors led us to suggest that the interaction of agonists of the 5-HT$_{1A}$R with Asp$^{3.32}$, Ser$^{5.42}$, Thr$^{5.43}$, and Trp$^{6.48}$ is probably the key determinant for agonist-induced receptor activation.

In the present work, we have designed and synthesized a new series of arylpiperazines of general structure **V** exhibiting high 5-HT$_{1A}$R affinity and selectivity over α_1-adrenoceptors. The new selective 5-HT$_{1A}$R ligands contain a hydantoin ($m = 0$) or diketopiperazine ($m = 1$) moiety in the nonpharmacophoric part, one methylene unit in the spacer to the pharmacophoric arylpiperazine ($n = 1$), and different benzofused rings as the aryl substituent of the piperazine (Ar). The influence of the stereogenic center present in the nonpharmacophoric part of these derivatives as well as of a substituent in the piperazine ring on 5-HT$_{1A}$R affinity is also discussed. Among these compounds, we report the pharmacological characterization of (*S*)-**9** (CSP-2503) as a potent 5-HT$_{1A}$R agonist.

Results

Chemistry. The synthesis of new compounds of general structure **V** has been performed by using two different strategies, depending on the length of the spacer chain ($n = 4$ or $n = 1$; see Scheme 1). Compounds **1**–**6** ($n = 4$) have been obtained by alkylation of arylpiperazines **22**–**25** with 4-bromobutyl derivatives

Table 1. Binding Data of Compounds **V**[a]



V(1-16)

compd	configuration	X	*m*	*n*	R	Ar	$K_i \pm$ SEM (5-HT$_{1A}$)	$K_i \pm$ SEM (α_1)
1	(7a*RS*)	$(CH_2)_3$	0	4	H	naphth-1-yl	2.4 ± 0.1	64.9 ± 1.5
2	(8a*RS*)	$(CH_2)_3$	1	4	H	naphth-1-yl	0.5 ± 0.2	8.0 ± 1.7
3	(2*R*,8a*RS*)	$(CH_2)_3$	1	4	*R*-Me	naphth-1-yl	4.2 ± 0.9	20.1 ± 0.8
4	(2*S*,8a*RS*)	$(CH_2)_3$	1	4	*S*-Me	naphth-1-yl	15.3 ± 1.8	34.4 ± 1.2
5	(8a*RS*)	$(CH_2)_3$	1	4	H	benzodioxepin-6-yl	3.1 ± 0.9	348 ± 21
6	(7a*RS*)	$(CH_2)_3$	0	4	H	benzimidazol-4-yl	1.2 ± 0.02	19.4 ± 0.6
7	(7a*RS*)	$(CH_2)_3$	0	1	H	naphth-1-yl	10.4 ± 0.8	>1000
8	(8a*RS*)	$(CH_2)_4$	0	1	H	naphth-1-yl	5.6 ± 0.3	>1000
9	(8a*RS*)	$(CH_2)_3$	1	1	H	naphth-1-yl	6.7 ± 3.0	>1000
(*R*)-**9**	(8a*R*)	$(CH_2)_3$	1	1	H	naphth-1-yl	4.6 ± 0.4	>1000
(*S*)-**9**	(8a*S*)	$(CH_2)_3$	1	1	H	naphth-1-yl	4.1 ± 1.2	>1000
(*R,R*)-**10**	(2*R*,8a*R*)	$(CH_2)_3$	1	1	*R*-Me	naphth-1-yl	9.5 ± 2.2	>1000
(*S,S*)-**10**	(2*S*,8a*S*)	$(CH_2)_3$	1	1	*S*-Me	naphth-1-yl	2.5 ± 0.1	>1000
(*R,S*)-**11**	(2*R*,8a*S*)	$(CH_2)_3$	1	1	*R*-Me	naphth-1-yl	6.3 ± 0.2	>1000
(*S,R*)-**11**	(2*S*,8a*R*)	$(CH_2)_3$	1	1	*S*-Me	naphth-1-yl	6.4 ± 0.1	>1000
12	(8a*RS*)	$(CH_2)_4$	0	1	H	benzodioxan-5-yl	9.3 ± 0.4	>1000
13	(8a*RS*)	$(CH_2)_4$	0	1	H	benzodioxepin-6-yl	6.1 ± 0.4	>1000
14	(8a*RS*)	$(CH_2)_3$	1	1	H	benzodioxepin-6-yl	11.6 ± 3.7	>1000
(*S*)-**14**	(8a*S*)	$(CH_2)_3$	1	1	H	benzodioxepin-6-yl	12.3 ± 2.1	>1000
16	(7a*RS*)	$(CH_2)_3$	0	1	H	benzimidazol-4-yl	4.1 ± 0.2	>10000

[a] Values are the mean of two to four experiments performed in triplicate.

20 and **21**, which were obtained as racemic materials by reaction of bicyclohydantoin **17** and bicyclodiketopiperazine **19** with 1,4-dibromobutane, respectively.[23] Piperazines **22**−**24** were prepared according to known procedures.[31−33] Enantiopure (*R*)-**25** and (*S*)-**25** (R = CH_3) were synthesized by Pd(0) amination of naphthyl triflate using commercially available 2(*R*)- and 2(*S*)-methylpiperazines, respectively. Consequently, compounds **3** and **4** were obtained as diastereomeric mixtures. The synthesis of derivative **6** was already published.[33] Compounds **7**−**15** (n = 1) were synthesized from racemic or enantiopure bicyclohydantoins **17**, **18**, and bicyclodiketopiperazine **19**, and arylpiperazines **22**−**26** were synthesized by means of a Mannich reaction in the presence of aqueous formaldehyde. Piperazine **26** was prepared according to a known procedure.[34] **8**, **12**, and **13** were obtained as racemic mixtures from **18**, previously generated from DL-pipecolic acid.[35] **7** and **15** were synthesized from **17** readily available from DL-proline.[36] Selective removal of the trityl protecting group in **15** was easily achieved simultaneous to the transformation into the hydrochloride salt by bubbling HCl(g) through an ethereal solution to afford **16**. To establish the influence of the affinity of a stereogenic center in the no-pharmacophoric fragment of this family of ligands, **9** and **14** were synthesized as racemic and enantiopure materials [(*S*)-**9**, (*R*)-**9**, and (*S*)-**14**] using racemic or enantiopure bicyclodiketopiperazine **19**[37] and arylpiperazines **22** and **23**. An additional stereocenter was introduced into the arylpiperazine fragment by Mannich reaction of (*R*)-**25** or (*S*)-**25** with either of the two enantiomers of **18**, affording the four optically pure diastereoisomers [(*R,R*)- and (*S,S*)-**10**; (*S,R*)- and (*R,S*)-**11**].

Binding Affinities. Target compounds were assessed for in vitro binding affinity at serotoninergic 5-HT$_{1A}$ and α_1-adrenergic receptors by radioligand binding assays, using [^{3}H]-8-OH-DPAT[38] and [^{3}H]prazosin,[39] respectively, in rat cerebral cortex membranes. All the compounds were used in the form of hydrochloride salts and were water- or methanol-soluble. In the experimental binding assays, the compounds were first tested at a fixed dose of 10^{-6} M, and for only those that in the screening process presented a displacement of the radioligand of >55%, the dose−response curves were determined. The inhibition constant K_i was calculated from the IC_{50} by using the Cheng−Prusoff equation.[40] The results of these assays are illustrated in Table 1.

All the synthesized compounds **V** (**1**−**14**, **16**) exhibited high or very high 5-HT$_{1A}$R affinity (K_i = 0.5−15.3 nM). Compounds with four methylene units in the spacer also showed affinity for α_1-adrenergic receptors. In contrast, analogues **7**−**14** and **16**, containing one methylene unit in the spacer (n = 1), are selective over α_1-adrenergic receptors. With respect to the stereogenic center of the no-pharmacophoric part, no influence is observed in 5-HT$_{1A}$R affinity orselectivity. Thus, racemic and both enantiomers of **9** show similar binding constants (6.7, 4.6, and 4.1 nM, respectively), and the same can be seen in the case of racemic and enantiopure **14** (11.6 and 12.3 nM, respectively). When a substituent was introduced in the pharmacophoric arylpiperazine fragment, the noninfluence of an additional stereocenter in the molecule could be assessed if we compare **3** with **4**, (*R,R*)-**10** with (*S,S*)-**10**, and (*S,R*)-**11** with (*R,S*)-**11**.

Compound (*S*)-**9** was also evaluated for affinity at serotonin 5-HT$_{2A}$, 5-HT$_3$, 5-HT$_4$, and 5-HT$_7$ receptors, at serotonin transporter (5-HTT), dopamine D_2 receptors, and benzodiazepine receptors, exhibiting high affinity, in the nanomolar range, and at 5-HT$_{2A}$ and 5-HT$_3$ receptors (Table 2). The following specific ligands and tissue sources were used: 5-HT$_{2A}$, [^{3}H]ketanserin, rat cerebral frontal cortex membranes;[41] 5-HT$_3$, [^{3}H]LY278584, rat cerebral cortex membranes;[42] 5-HT$_4$, [^{3}H]GR113808, rat striatum membranes;[43] 5-HT$_7$, [^{3}H]-5-CT, rat hypothalamus membranes;[44] 5-HT trans-

Table 2. Binding Affinity Data of Compound (*S*)-**9**[a]

receptor	$K_i \pm$ SEM (nM)	receptor	$K_i \pm$ SEM (nM)
5-HT$_{1A}$	4.1 ± 1.2	D_2	192 ± 20
5-HT$_{2A}$	13.5 ± 2.5	α_1	>1000
5-HT$_3$	8.9 ± 1.4	5-HTT	976 ± 43
5-HT$_4$	>10000	Bz	>10000
5-HT$_7$	101 ± 1		

[a] Values are the mean of two to four experiments performed in triplicate.

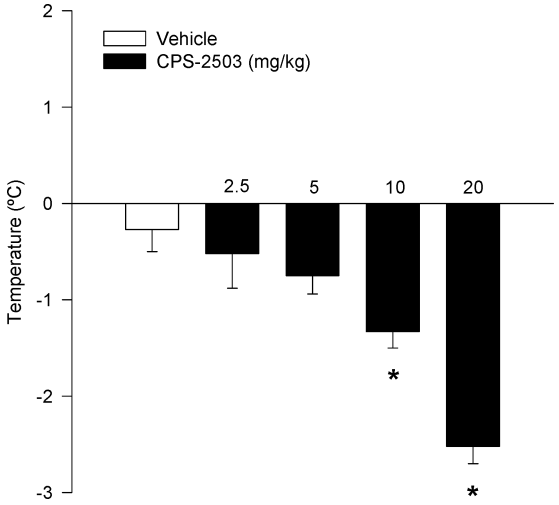


Figure 1. Dose-response effect of (*S*)-**9** on rectal temperature. Values represent the mean ± SEM of rectal temperature in eight to nine mice. The asterisk (*) represents values from (*S*)-**9** that decrease more than 1.1 °C and are significantly different ($p < 0.05$) from their respective basal rectal temperature before drug administration (vehicle alone did not alter rectal temperature). Statistical significance was assessed by ANOVA followed by student Newman Keul's test.

porter, [^{3}H]paroxetine, rat cerebral cortex membranes;[45] D_2, [^{3}H]raclopride, rat striatum membranes;[46] benzodiazepine, [^{3}H]flunitrazepam, rat cerebral cortex membranes.[47]

Pharmacological Characterization of (*S*)-9. Presynaptic 5-HT$_{1A}$R activity was assessed by measuring mouse rectal temperature 60 min after sc administration of either vehicle (water 4 mL/kg) or different doses of (*S*)-**9** (2.5, 5, 10, and 20 mg/kg).[48] The administration of (*S*)-**9** provoked a dose-related decrease in mice rectal temperature (Figure 1). This induced hypothermia suggests that (*S*)-**9** acts on 5-HT$_{1A}$ somatodendritic autoreceptors.

The transduction mechanism of (*S*)-**9** was determined by using HeLa cells expressing human 5-HT$_{1A}$Rs.[49] Assays were done 2 days after plating cells at 75 000 cells/well in 1 mL of DMEM containing glutamax, 10% heat-inactivated calf serum, 5% penicilin/streptomycin, and geneticin (300 μL/mL). The HeLa cells transfected with the human 5-HT$_{1A}$R were pretreated with 1-methyl-3-isobutylxanthine (0.5 mM), forskolin (10 μM), and (*S*)-**9** (10^{-9}–10^{-5} M) for 10 min at 37 °C in a 5% CO_2 incubator. The cAMP content was measured by radioimmunoassay. Adenylate cyclase activity is expressed as the percentage of 5-HT-sensitive forskolin-stimulated adenylate cyclase activity in HeLa cells in the presence of vehicle or different concentrations of (*S*)-**9**. Ligand (*S*)-**9** inhibited in a dose-dependent manner the cAMP increase induced by forskolin (Figure 2). The concentration that produced the half-maximal effect (EC_{50}) was 0.15 μM, and the maximal inhibitory effect was 90.3 ± 1.3%. This negative control of (*S*)-**9** on adenylate cyclase activity indicates a transduction system coupled to 5-HT$_{1A}$R stimulation. As expected for an action mediated through the activation of the 5-HT$_{1A}$R, the inhibition of forskolin-stimulated adenylate cyclase by (*S*)-**9** was significantly reduced by a 10^{-8} M concentration of the 5-HT$_{1A}$R antagonist WAY100,635.

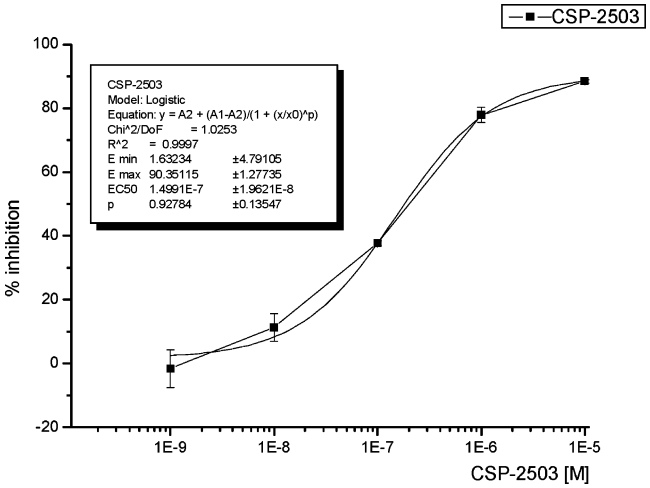


Figure 2. Effect of (*S*)-**9** on cAMP increase induced by forskolin in 5-HT$_{1A}$ transfected HeLa cells. The cAMP content was measured by radioimmunoassay. Adenylate ciclase activity is expressed as the percentage of 5-HT-sensitive forskolin-stimulated adenylate ciclase activity in HeLa cells in the presence of vehicle or different concentrations of (*S*)-**9**. The data shown are the mean values ± SEM of duplicate experiments. In each of them, four wells per concentration were assayed.

Functional activity of (*S*)-**9** on 5-HT$_{1A}$Rs was further assessed by evaluating its ability to decrease hypothalamic 5-HT neuronal activity.[50] Mice were injected sc with vehicle (4 mL/kg water) or different doses of (*S*)-**9** (5, 10, and 20 mg/kg) and killed by decapitation 30 min later. The 5-hydroxyindoleacetic acid (5-HIAA)/5-HT ratio in whole hypothalamus of vehicle or drug-treated mice was measured. The results represented in Figure 3 indicate that the administration of (*S*)-**9** induced a decrease in the 5-HIAA/5-HT ratio, suggesting its behavior as a 5-HT$_{1A}$R agonist acting at the somatodendritic site.

Finally, we evaluated the potential anxiolytic activity of (*S*)-**9** by using a light/dark box test.[51] Thirty minutes after the sc administration of vehicle (4 mL/kg water) or different doses of (*S*)-**9** (0.5, 5, 10, and 20 mg/kg), the time that mice spent in the lit area was measured during a period of 5 min. Indeed, the administration of (*S*)-**9** (5 mg/kg) caused an increase in the time that mice spent in the lit area (116.7 ± 9.3 vs 76.4 ± 7.6 s, $p < 0.05$), as shown in Figure 4. The 5-HT$_{1A}$R agonist 8-OH-DPAT was tested in the same test as for the reference compound, at a dose of 2.5 mg/kg (time spent in the lit area: 188.8 ± 26 vs 105 ± 14.7 s).

Overall, the pharmacological characterization of (*S*)-**9** indicates that this compound is an agonist of the 5-HT$_{1A}$R at the somatodendritic and postsynaptic sites, with anxiolytic potential. The present data suggest that (*S*)-**9** may be therapeutically useful in the treatment of anxiety-related disorders.

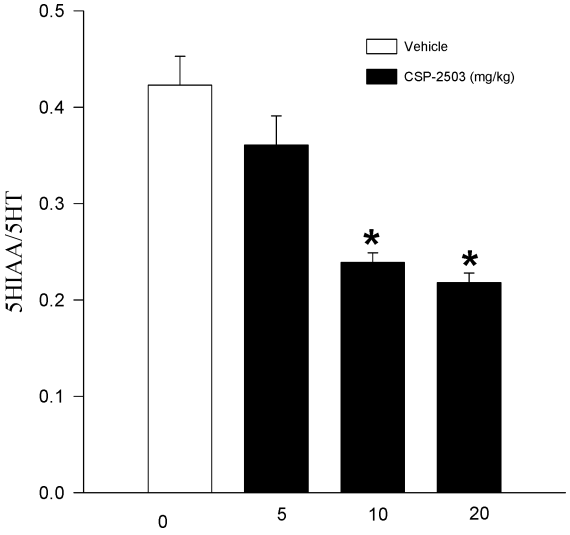


Figure 3. Dose-response effect of (*S*)-**9** on hypothalamic 5-HT activity. 5-HIAA/5-HT ratios in mouse whole hypothalamus are shown. The asterisk (∗) represents values from (*S*)-**9** treated mice that are significantly different from vehicle group ($p < 0.05$, ANOVA followed by student Newman Keul's test).

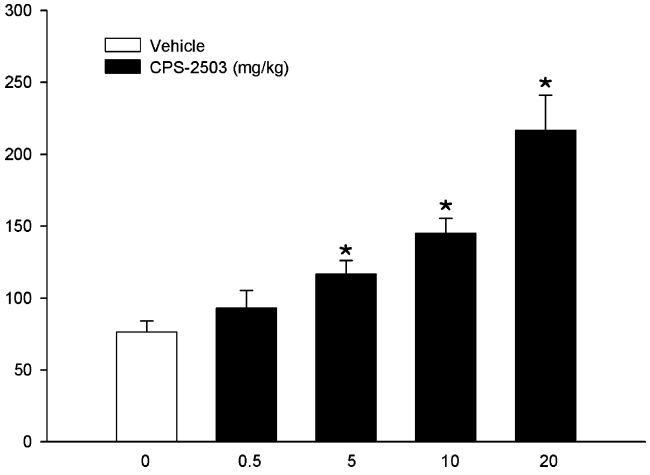


Figure 4. Dose-response effect of (*S*)-**9** in the light/dark box test. Values represent the mean values ± SEM of time spent in the lit area in vehicle or drug treated mice that are significantly different from water vehicle treated mice control group ($p < 0.05$, ANOVA followed by student Newman Keul test).

In agreement with binding data, compound (*S*)-**9** (0.63−5.00 mg/kg, sc) prevented the head-twitch response induced by the 5-$HT_{2A/2C}$R agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) in mice. This would support the activity of (*S*)-**9** at 5-HT_{2A}Rs, since in this animal species such a behavioral pattern induced by this hallucinogenic compound (DOI) is considered as a 5-HT_{2A}R-mediated action.[52] Additionally, ligand (*S*)-**9** reduced the bradycardia reflex due to 2-methyl-5-HT administration in anesthetized rats. This may be taken as an indication of antagonistic activity of (*S*)-**9** at 5-HT_3Rs.[53]

Computational Models of 5-HT_{1A}R−Ligand Interaction. Figure 5a shows compound **2** in the binding pocket of the 5-HT_{1A}R. The protonated piperazine moiety of the ligand is interacting with $Asp^{3.32}$; the oxygen atoms of the diketopiperazine moiety are interacting with $Ser^{5.42}$, $Thr^{5.43}$, and $Trp^{6.48}$; and the naphthalene ring is interacting with $Phe^{3.28}$, $Trp^{7.40}$, and $Tyr^{7.43}$. Importantly, this model considers that agonists bind $Trp^{6.48}$ in the active trans conformation.[29] This mode of binding of compound **2** contrasts with the previously reported models of the complexes between the 5-HT_{1A}R and arylpiperazines of formula **I**.[24,25] The difference resides in the interaction of one of the oxygen atoms of the hydantoin/diketopiperazine group with either $Trp^{6.48}$ or $Thr^{3.37}$. In previous models the conformation of $Trp^{6.48}$ was modeled as observed in the crystal structure of rhodopsin,[54] in the inactive gauche+ conformation and thus far away from the ligand binding site.

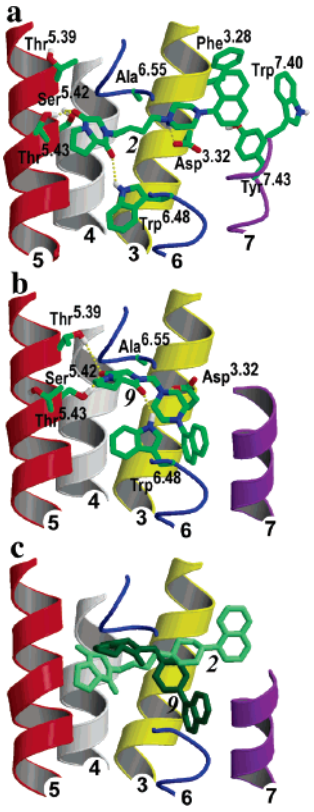


Figure 5. Molecular models of the complexes between compounds **2** (a, c) and (*S*)-**9** (b, c) and the 5-HT_{1A}R in a view parallel to the membrane. The C_α traces of TMHs 3 (yellow), 4 (white), 5 (red), 6 (blue), and 7 (purple) are shown. (a) Compound **2** is predicted to form an ionic interaction between $Asp^{3.32}$ and the protonated piperazine ring; hydrogen bonds are between $Ser^{5.42}$, $Thr^{5.43}$, and $Trp^{6.48}$ and the diketopiperazine moiety; and aromatic−aromatic interactions are between $Phe^{3.28}$, $Trp^{7.40}$, and $Tyr^{7.43}$ and the naphthalene ring. (b) Compound (*S*)-**9** is predicted to form an ionic interaction between $Asp^{3.32}$ and the protonated piperazine ring; hydrogen bonds are between $Thr^{5.39}$, $Ser^{5.42}$, and $Trp^{6.48}$ and the diketopiperazine moiety; and aromatic−aromatic interactions are between $Trp^{6.48}$ and $Phe^{6.51}$ (not shown to clarify) and the naphthalene ring. (c) Comparison of the modes of binding of compounds **2** (light green) and (*S*)-**9** (dark green) to the 5-HT_{1A}R.

Figure 5b shows compound (*S*)-**9** in the binding pocket of the 5-HT_{1A}R. (*S*)-**9** cannot expand deep into the bundle as compound **2** (Figure 5c) because of its shorter side chain. Therefore, the formation of the ionic interaction between the protonated piperazine and $Asp^{3.32}$ allows the oxygen atoms of the diketopiperazine group to interact with $Thr^{5.39}$ (instead of $Thr^{5.43}$ for compound **2**), $Ser^{5.42}$, and $Trp^{6.48}$. The rigidity of (*S*)-**9** also modifies, relative to compound **2**, the binding of the naphthalene

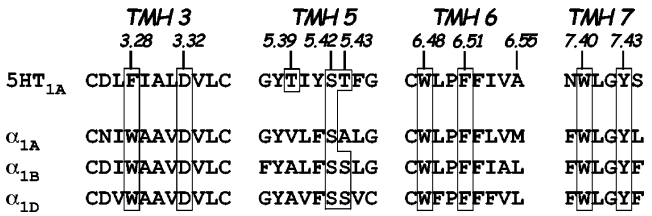


Figure 6. Sequence alignments of TMHs 3, 5, 6, and 7 of 5-HT$_{1A}$ and α_1-adrenergic receptors. Residues referenced in the manuscript are boxed.

ring, which expands between TMHs 6 and 7 (instead of TMHs 3 and 7 for compound **2**), interacting with Trp$^{6.48}$ and Phe$^{6.51}$ (not shown to offer a better view of the ligand) (Figure 5).

Discussion

Structure−Affinity Studies. Using the previously reported computer models of the interaction between the transmembrane domain of the 5-HT$_{1A}$R and compounds **I**[24,25] and 3D-QSAR studies,[19] we designed compound **1** [X = −(CH$_2$)$_3$−, m = 0, n = 4, R = H, Ar = naphth-1-yl]. An increase of the substituent size of the aromatic ring at the ortho and meta positions was predicted to enhance the affinity for the 5-HT$_{1A}$R. Moreover, the presence of a cluster of aromatic rings within TMHs 3 and 7 (Phe$^{3.28}$, Trp$^{7.40}$, and Tyr$^{7.43}$) allows the large naphthalene ring to optimally interact with them in a face-to-edge orientation (T-shaped) (Figure 5a).

The influence of the spacer between the arylpiperazine and hydantoin moieties was explored in series **I**−**IV** (Chart 1). The length of the spacer is of great importance for 5-HT$_{1A}$/α_1 affinity. Ligands with n = 3 or n = 4 (compounds **1**−**6**) possess the maximum binding affinity at both receptors, whereas compounds with n = 2 are inactive or poorly active.[18] Notably, reduction of the spacer to n = 1 [X = −(CH$_2$)$_3$−, m = 0, n = 1, R = H, Ar = naphth-1-yl] leads to compounds **7**−**14** and **16**, which maintain high binding affinity for the 5-HT$_{1A}$R but are inactive at α_1-adrenoceptors (Table 1).

Structure−Selectivity Studies. Figure 6 shows the sequence alignments of TMHs 3, 5, 6, and 7 of 5-HT$_{1A}$ and α_1-adrenergic receptors. The presence of aromatic residues at positions 3.28, 7.40, and 7.43, the acidic Asp$^{3.32}$ residue, and the hydrogen bond donor Ser$^{5.42}$, Thr/Ser$^{5.43}$, and Trp$^{6.48}$ residues, which are the predicted anchoring points of arylpiperazine derivatives with n = 4 (Figure 5a), in both 5-HT$_{1A}$ and α_1-adrenergic receptors (see boxed residues in Figure 6) explains their lack of selectivity. In contrast, the predicted anchoring points of arylpiperazine derivatives with n = 1 (Figure 5b) are the aromatic Trp$^{6.48}$ and Phe$^{6.51}$ residues, the acidic Asp$^{3.32}$ residue, and the hydrogen bond donor Thr$^{5.39}$, Ser$^{5.42}$, and Trp$^{6.48}$ residues. The fact that Thr$^{5.39}$ is present only in the 5-HT$_{1A}$R (Figure 6) explains the observed selectivity of compounds **7**−**14** and **16** over α_1-adrenergic receptors. The side chain at position 6.55, close to the ligand binding site (Figure 5b), might also influence the observed selectivity of these compounds. While the 5-HT$_{1A}$R contains the small Ala residue, the α_1-adrenergic receptors contain the bulky Met or Leu residue. Thus, the cavity present at this locus in the α_1-adrenergic receptors is significantly smaller than in the 5-HT$_{1A}$R, impeding the binding of arylpiperazine derivatives with n = 1 at this site.

Structure−Activity Studies. Molecular Mechanism of Receptor Activation. The structural basis of 5-HT$_{1A}$R activation by agonists is poorly understood. It has recently been described that Trp$^{6.48}$, a partially conserved side chain among family A of GPCRs (71% of the receptors),[15] undergoes a conformational transition during receptor activation, from pointing toward TMH 7, in the inactive gauche+ conformation, to pointing toward TMH 5, in the active trans conformation.[29,30] We suggest that agonists studied herein, by means of an explicit hydrogen bond between one oxygen atom of the diketopiperazine group and Trp$^{6.48}$, trigger the experimentally observed active conformation of Trp$^{6.48}$.

Conclusion

We have designed and synthesized a new series of arylpiperazines **V** that exhibit high 5-HT$_{1A}$R affinity and selectivity over α_1-adrenoceptors. The new selective 5-HT$_{1A}$R ligands contain a hydantoin (m = 0) or diketopiperazine (m = 1) moiety in the no-pharmacophoric part, one methylene unit in the spacer to the pharmacophoric arylpiperazine (n = 1), and different benzofused rings as the aryl substituent of the piperazine (Ar). Also, there is not a notable influence on 5-HT$_{1A}$R affinity of the stereogenic center present in the no-pharmacophoric part of these derivatives or of an additional stereocenter introduced with a substituent in the piperazine ring. In particular, (*S*)-2-[[4-(napht-1-yl)piperazin-1-yl]methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [(*S*)-**9**] (5-HT$_{1A}$, K_i = 4.1 nM; α_1, K_i > 1000 nM) has been pharmacologically characterized as a 5-HT$_{1A}$R agonist at somatodendritic and postsynaptic sites and may be therapeutically useful in the treatment of anxiety-related disorders. Ligand (*S*)-**9** is predicted, in computer simulations, to bind Asp$^{3.32}$ in TMH 3, Thr$^{5.39}$ and Ser$^{5.42}$ in TMH 5, and Trp$^{6.48}$ in TMH 6. We propose that agonists modify, by means of an explicit hydrogen bond, the conformation of Trp$^{6.48}$ from pointing toward TMH 7, in the inactive gauche+ conformation, to pointing toward the ligand binding site, in the active trans conformation.

Experimental Section

Chemistry. Melting points (uncorrected) were determined on a Gallenkamp electrothermal apparatus. Infrared (IR) spectra were obtained on a Perkin-Elmer 781 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian VXR-300S, Bruker Avance 300, or Bruker AC-200 instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane used as internal reference. Coupling constants (J) are in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), qt (quintet), m (multiplet), br (broad). Elemental analyses (C, H, N) were carried out on a Perkin-Elmer 2400 apparatus at the UCM's analysis services and were within 0.4% of the theoretical values. Optical rotation [α] was measured using a Perkin-Elmer 781 polarimeter. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254) with detection by UV light, iodine, acidic vanillin solution, or 10% phosphomolybdic acid solution in ethanol. For normal pressure and flash chromatography, Merck silica gel type 60 (size 70−230 and 230−400 mesh,

respectively) was used. Unless stated otherwise, starting materials and reagents used were high-grade commercial products purchased from Aldrich, Fluka, or Merck. All solvents were distilled prior to use.

The following compounds were synthesized according to described procedures: 2-[4-[4-(benzimidazol-4(7)-yl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (**6**),[33] 1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (**17**),[36] 1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (**18**),[35] 1,4-dioxoperhydropyrrolo-[1,2-*a*]pyrazine (**19**)[37] as racemic and as enantiopure materials, 2-(4-bromobutyl)-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (**20**),[23] 2-(4-bromobutyl)-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (**21**),[23] 1-(naphth-1-yl)piperazine (**22**),[31] 1-(3,4-dihydro-2*H*-1,5-benzodioxepin-6-yl)piperazine (**23**),[32] 1-(1-tritylbenzimidazol-4-yl)-piperazine (**24**),[33] 1-(2,3-dihydro-1,4-benzodioxan-5-yl)piperazine (**26**).[34] Collected data for compounds **1**−**15** refer to free bases, and then hydrochloride salts were prepared prior to melting point determination, elemental analyses, and biological assays. Spectroscopic data of all described compounds were consistent with the proposed structures. For series **1**−**6** and **7**−**16** we include the data of compounds **1**, **5**, **12**, **15**, and **16**.

Synthesis of (*R*)- and (*S*)-3-Methyl-1-(naphth-1-yl)-piperazine (*R*)-25 and (*S*)-25. To a solution of 100 mg (0.35 mmol) of 1-naphthyl trifluoromethanesulfonate in toluene (10 mL × mmo), under an argon atmosphere, (*R*)- or (*S*)-2-methylpiperazine (2.11 equiv), NaO^{t}Bu (0.51 equiv), Pd(OAc)$_2$ (0.007 mmol), and (±)-BINAP (0.021 mmol) were added. The mixture was heated at 100 °C (5 h). Then, the mixture was cooled and filtered through a short column of Celite. The resulting solution was evaporated to dryness under vacuum. The crude mixture was purified by column chromatography on silica gel using dichloromethane/methanol mixtures as eluent. (*R*)-**25** was obtained in 72% yield ($[\alpha]^{25}_D$ +10.9 (*c* 1.3, $CHCl_3$)), and (*S*)-**25** was obtained in 78% yield ($[\alpha]^{25}_D$ −12.4 (*c* 1.2, $CHCl_3$)). IR ($CHCl_3$) 3700−3200, 3020, 1595, 1575, 1510, 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.12 (d, J = 6.3, 3H), 1.89 (br s, 1H), 2.47 (ap t, J = 10.1, 1H), 2.81 (dt, J = 10.1, 2.7, 1H), 3.10−3.30 (m, 5H), 7,06 (dd, J = 7.3, 1.0, 1H), 7.39 (t, J = 7.3, 1H), 7.42−7.48 (m, 2H), 7.53 (d, J = 8.0, 1H), 7.77−7.83 (m, 1H), 8.17−8.22 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 19.7, 46.4, 51.0, 53.5, 61.0, 114.7, 123.4, 123.5, 125,3, 125.7, 125.8, 128.3, 128.9, 134.7, 149.8.

General Procedure for the Synthesis of Arylpiperazines 1−6 (*n* = 4). To a suspension of the bromoalkyl derivative **20** or **21** (4.5 mmol) and the appropriate arylpiperazine **22**−**25** (7.5 mmol) in dry acetonitrile (10 mL) was added triethylamine (1.0 mL, 7.5 mmol), and the mixture was refluxed for 20−24 h. After cooling, the solvent was evaporated under reduced pressure and the residue was resuspended in water and extracted with dichloromethane (3 × 50 mL). The combined organic layers were washed with water and dried over anhydrous Na_2SO_4. After evaporation of the solvent the crude oil was purified by column chromatography in silica gel using the appropriate eluent.

2-[4-[4-(Naphth-1-yl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (1). From **20** and **22** was obtained **1** in 60% yield. Chromatography: chloroform/methanol, from 9.5:0.5 to 9:1; IR ($CHCl_3$) 1770, 1705, 1610, 1590, 1520, 1485 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50−1.71 (m, 5H), 2.01−2.07 (m, 2H), 2.19−2.24 (m, 1H), 2.44 (t, J = 7.4, 2H), 2.70 (br s, 4H), 3.18−3.24 (m, 1H), 3.30−3.47 (m, 6H), 3.65−3.75 (m, 1H), 4.07−4.24 (m, 1H), 7.50 (d, J = 7.2, 1H), 7.38−7.46 (m, 3H), 7.53 (d, J = 7.8, 1H), 7.78−7.82 (m 1H), 8.11−8.15 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 23.3, 25.8, 27.1, 27.9, 38.5, 45.6, 51.0 (2 C), 53.0 (2 C), 57.5, 63.1, 114.7, 123.6 (2 C), 125.4, 125.9 (2 C), 128.5, 128.8, 134.8, 149.6, 161.5, 175.1. Anal. ($C_{26}H_{34}N_4O_2$·2HCl) C, H, N.

2-[4-[4-(Naphth-1-yl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (2). From **21** and **22** was obtained **2** in 43% yield. Chromatography: chloroform/methanol, from 9.5:0.5 to 9:1; mp 277−280 °C (dec) (methanol/ethyl ether). Anal. ($C_{25}H_{32}N_4O_2$·HCl·$^1/_2H_2O$) C, H, N.

(2*R*,8a*RS*)- and (2*S*,8a*RS*)-2-[4-[4-(Naphth-1-yl)-2-methylpiperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]-pyrazine (3 and 4). From **21** and (*R*)-**25** was obtained **3** as an equimolecular mixture of diasteroisomers in 57% yield (oil, $[\alpha]^{25}_D$ −20.0 (*c* 1.1, $CHCl_3$)). From **21** and (*S*)-**25** was obtained **4** as an equimolecular mixture of diasteroisomers in 35% yield (oil, $[\alpha]^{25}_D$ +21.0 (*c* 1.1, $CHCl_3$)). Chromatography: chloroform/ethanol, from 20:1 to 12:1.

2-[4-[4-(3,4-Dihydro-2*H*-1,5-benzodioxepin-6-yl)piperazin-1-yl]butyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (5). From **21** and **23** was obtained **5** in 52% yield. Chromatography: chloroform/methanol, from 9.5:0.5 to 9:1; mp 212−213 °C (dec) (methanol/ethyl ether); IR ($CHCl_3$) 1670, 1590, 1485, 1460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43−1.55 (m, 4H), 1.82−2.05 (m, 3H), 2.13 (qt, J = 5.7, 2H), 2.27−2.31 (m, 1H), 2.35 (t, J = 7.2, 2H), 2.55 (br s, 4H), 3.00 (br s, 4H), 3.27−3.34 (m, 1H), 3.41−3.57 (m, 3H), 3.72 (d, J = 16.2, 1H), 4.01 (t, J = 7.5, 1H), 4.07 (d, J = 16.5, 1H), 4.14−4.21 (m, 4H), 6.54 (dd, J = 7.8, 1.5, 1H), 6.59 (dd, J = 8.2, 1.4, 1H), 6.76 (t, J = 7.9, 1H); ^{13}C NMR ($CDCl_3$) δ 22.6, 23.9, 25.1, 28.8, 31.5, 45.2, 46.0, 51.0, 51.6, 53.4, 58.0, 59.0, 70.2, 70.3, 112.9, 115.5, 122.5, 144.6, 145.0, 152.1, 163.1, 167.1. Anal. ($C_{24}H_{34}N_4O_4$·2HCl·2H_2O) C, H, N.

General Procedure for the Synthesis of Arylpiperazines 7−15 (*n* = 1). To a suspension of **17**−**19** (7 mmol) and formaldehyde (7 mmol from a 35% aqueous solution) in methanol (15 mL) was added the corresponding arylpiperazine **22**−**26** (7 mmol). The resultant suspension was refluxed for 2−6 h after complete disappearance of the starting materials (TLC). The mixture was then cooled to room temperature, and the solvent was evaporated at reduced pressure. The crude mixture was diluted in chloroform (75 mL) and washed with water (3 × 75 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated at reduced pressure. The obtained crude was purified by column chromatography on silica gel using the appropriate eluent.

2-[[4-(Naphth-1-yl)piperazin-1-yl]methyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (7). From **17** and **22** was obtained **7** in 68% yield. Chromatography: ethyl acetate/hexane, 1:1; mp 168−170 °C (methanol/ethyl ether). Anal. ($C_{21}H_{24}N_4O_2$·2HCl) C, H, N.

2-[[4-(Naphth-1-yl)piperazin-1-yl]methyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (8). From **18** and **22** was obtained **8** in 77% yield. Chromatography: hexane/ethyl acetate, 2:8; mp 171−174 °C (dec) (methanol/ethyl ether). Anal. ($C_{22}H_{26}N_4O_2$·HCl·$^3/_2H_2O$) C, H, N.

(*RS*)-, (*R*)-, and (*S*)-2-[[4-(Naphth-1-yl)piperazin-1-yl]-methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [9, (*R*)-9, and (*S*)-9]. From **19** and **22** was obtained **9** in 62% yield. From (*R*)-**19** and **22** was obtained (*R*)-**9** in 53% yield ($[\alpha]^{25}_D$ +68.2 (*c* 1.3, $CHCl_3$)). From (*S*)-**19** and **7** was obtained (*S*)-**9** in 59% yield ($[\alpha]^{25}_D$ −68.1 (*c* 1.4, $CHCl_3$)). Chromatography: from ethyl acetate to ethyl acetate/ethanol, 9:1; mp [(*S*)-**9**] 256−259 °C (dec) (acetone). **9**: Anal. ($C_{22}H_{26}N_4O_2$·HCl·$^3/_2H_2O$). (*R*)-**9**: Anal. ($C_{22}H_{26}N_4O_2$·HCl·2H_2O) C, H, N. (*S*)-**9**: Anal. ($C_{22}H_{26}N_4O_2$·HCl·$^3/_2H_2O$) C, H, N.

(2*R*,8a*R*)-2-[[4-(Naphth-1-yl)-2-methylpiperazin-1-yl]-methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [(*R*,*R*)-10]. From (*R*)-**19** and (*R*)-**25** was obtained (*R*,*R*)-**10** in 23% yield ($[\alpha]^{25}_D$ +36.6 (*c* 1.9, $CHCl_3$)). Chromatography: from ethyl acetate to ethyl acetate/ethanol, 9:1. Anal. ($C_{23}H_{28}N_4O_2$·HCl·$^5/_2H_2O$) C, H, N.

(2*S*,8a*S*)-2-[[4-(Naphth-1-yl)-2-methylpiperazin-1-yl]-methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [(*S*,*S*)-10]. From (*S*)-**19** and (*S*)-**25** was obtained (*S*,*S*)-**10** in 36% yield ($[\alpha]^{25}_D$ −38.0 (*c* 1.2, $CHCl_3$)). Spectroscopic data are identical to those of (*R*,*R*)-**10** (see Supporting Information). Anal. ($C_{23}H_{28}N_4O_2$·HCl·$^2/_3H_2O$) C, H, N.

(2*R*,8a*S*)-2-[[4-(Naphth-1-yl)-2-methylpiperazin-1-yl]-methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [(*R*,*S*)-11]. From (*S*)-**19** and (*R*)-**25** was obtained (*R*,*S*)-**11** in 43% yield ($[\alpha]^{25}_D$ −53.2 (*c* 1.4, $CHCl_3$)). Chromatography: from ethyl acetate to ethyl acetate/ethanol, 9:1. Anal. ($C_{23}H_{28}N_4O_2$·HCl·H_2O) C, H, N.

(2*S*,8a*R*)-2-[[4-(Naphth-1-yl)-2-methylpiperazin-1-yl]-methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine [(*S*,*R*)-

11]. From (*R*)-**19** and (*S*)-**25** was obtained (*S,R*)-**11** in 40% yield ($[\alpha]^{25}_D$ +53.7 (*c* 0.9, $CHCl_3$)). Spectroscopic data are identical to those of (*R,S*)-**11** (see Supporting Information). Anal. ($C_{23}H_{28}N_4O_2 \cdot HCl \cdot {}^3/_2H_2O$) C, H, N.

2-[[4-(2,3-Dihydro-1,4-benzodioxan-5-yl)piperazin-1-yl]-methyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (12). From **18** and **26** was obtained **12** in 88% yield. Chromatography: hexane/ethyl acetate, 1:9; mp 172−174 °C (chloroform/ethyl ether); IR (KBr) 1770, 1710, 1600, 1500, 1450 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30−1.51 (m, 3H), 1.74 (d, J = 13.2, 1H), 1.99 (d, J = 13.5, 1H), 2.20 (d, J = 13.2, 1H), 2.76−2.84 (m, 5H), 2.97−3.08 (m, 4H), 3.77 (dd, J = 11.7, 4.2, 1H), 4.15 (dd, J = 13.5, 3.6, 1H), 4.20−4.30 (m, 4H), 4.53 (s, 2H), 6.50 (dd, J = 7.8, 1.5, 1H), 6.56 (dd, J = 8.2, 1.6, 1H), 6.75 (t, J = 8.1, 1H); ^{13}C NMR ($CDCl_3$) δ 22.8, 25.0, 27.9, 39.4, 50.6 (2 C), 50.8 (2 C), 57.5, 60.1, 64.0, 64.4, 110.8, 112.1, 120.7, 136.4, 141.7, 144.1, 155.3, 174.5. Anal. ($C_{20}H_{26}N_4O_4 \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

2-[[4-(3,4-Dihydro-2*H*-1,5-benzodioxepin-6-yl)piperazin-1-yl]methyl]-1,3-dioxoperhydroimidazo[1,5-*a*]pyridine (13). From **18** and **23** was obtained **13** in 63% yield. Chromatography: hexane/ethyl acetate, 1:9; mp 183−185 °C (dec) (methanol/ethyl ether). Anal. ($C_{21}H_{28}N_4O_4 \cdot 2HCl \cdot H_2O$) C, H, N.

(*RS*)- and (*S*)-2-[[4-(3,4-Dihydro-2*H*-1,5-benzodioxepin-6-yl)piperazin-1-yl]methyl]-1,4-dioxoperhydropyrrolo-[1,2-*a*]pyrazine [14 and (*S*)-14]. From **19** and **23** was obtained **14** in 56% yield. From (*S*)-**19** and **23** was obtained (*S*)-**14** in 41% yield ($[\alpha]^{25}_D$ −22.3 (*c* 0.6, MeOH)); mp [(*S*)-**14**] 144−145 °C (methanol/ethyl ether). Chromatography: ethyl acetate/ethanol, 9:1. (*S*)-**14**: Anal. ($C_{21}H_{28}N_4O_4 \cdot 2HCl \cdot H_2O$) C, H, N.

2-[[4-(1-Tritylbenzimidazol)piperazin-1-yl]methyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (15). From **17** and **24** was obtained **15** in 86% yield. Chromatography: from chloroform to chloroform/methanol, 9:1; mp 120−123 °C (hexane/chloroform); IR ($CHCl_3$) 1770, 1710, 1495, 1445, 1430 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.62−1.83 (m, 1H), 2.00−2.31 (m, 3H), 2.92 (br s, 4H), 3.20−3.33 (m, 1H), 3.51 (br s, 4H), 3.62−3.78 (m, 1H), 4.12 (m, 1H), 4.53 (s, 2H), 6.11 (d, J = 8.1, 1H), 6.53 (d, J = 7.8, 1H), 6.77 (t, J = 8.1, 1H), 7.11−7.19 (m, 5H), 7.27−7.35 (m, 10H), 7.83 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.5, 27.3, 45.2, 49.7 (2 C), 50.1 (2 C), 59.9, 62.8, 74,9, 107.2, 108.4, 118.4, 122.4, 127.5, 127.6, 129.6, 135.5, 136.4, 140.9, 143.2, 160.9, 174.5. Anal. ($C_{37}H_{36}N_6O_2$) C, H, N.

2-[[4-(Benzimidazol-4(7)-yl)piperazin-1-yl]methyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (16). Removal of the trityl group in **15** was carried out simultaneous to the transformation into the hydrochloride salt. HCl (g) (generated from H_2SO_4 and anhydrous $CaCl_2$) was bubbled through a solution of 54 mg of **15** in Et_2O (3 mL) for 1 h. Solid **16** as the hydrochloride salt was separated by filtration, washed with Et_2O, and dried under vacuum (yield 90%). All data of compound **16** are measured as the hydrochloride salt: mp >300 °C (dec). Anal. ($C_{18}H_{22}N_6O_2 \cdot 2HCl \cdot H_2O$) C, H, N.

Radioligand Binding Assays. For all receptor binding assays, male Sprague-Dawley rats (*Rattus norvegicus albinus*) weighing 180−200 g were killed by decapitation, and the brains were rapidly removed and dissected. Tissues were stored at −80 °C for subsequent use and homogenized on a Polytron PT-10 homogenizer. Membrane suspensions were centrifuged on a Beckman J2-HS instrument.

5-HT$_{1A}$ Receptor. Binding assays were performed by a modification of the procedure previously described by Clark et al.[38] The cerebral cortex was homogenized in 10 volumes of ice-cold Tris buffer (50 mM Tris-HCl, pH 7.7 at 25 °C) and centrifuged at 28000*g* for 15 min. The membrane pellet was washed twice by resuspension and centrifugation. After the second wash the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation, and the final pellet was resuspended in 50 mM Tris-HCl, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer (pH 7.4 at 37 °C). Fractions of 100 μL of the final membrane suspension (about 1 mg of protein) were incubated at 37 °C for 15 min with 0.6 nM [^{3}H]-8-OH-DPAT (133 Ci/mmol), in the presence or absence of the competing drug, in a final volume of 1.1 mL of assay buffer (50 mM Tris-HCl, 10 nM clonidine, 30 nM prazosin, pH 7.4 at 37 °C). Nonspecific binding was determined with 10 μM 5-HT.

5-HT$_{2A}$ Receptor. Binding assays were performed by a modification of the procedure previously described by Titeler et al.[41] The frontal cortex was homogenized in 60 volumes of ice-cold buffer (50 mM Tris-HCl, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$, pH 7.4 at 25 °C) and centrifuged at 30000*g* for 15 min at 4 °C. The membrane pellet was washed by resuspension and centrifugation. After the second wash the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation, and the final pellet was resuspended in 10 volumes of assay buffer (50 mM Tris-HCl, 0.5 mM Na_2EDTA, 10 mM $MgSO_4$, 0.1% ascorbic acid, 10 μM pargyline, pH 7.4 at 25 °C). Fractions of 100 μL of the final membrane suspension (about 5 mg/mL of protein) were incubated at 37 °C for 15 min with 0.4 nM [^{3}H]ketanserin, in the presence or absence of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 1 μM cinanserin.

5-HT$_3$ Receptor. Binding assays were performed by a modification of the procedure previously described by Wong et al.[42] The cerebral cortex was homogenized in 9 volumes of ice-cold 0.32 M sucrose and centrifuged at 1000*g* for 10 min at 4 °C. The supernatant was centrifuged at 17000*g* for 20 min at 4 °C. The membrane pellet was washed twice by resuspension in 60 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) and centrifugation at 48000*g* for 10 min at 4 °C. After the second wash the resuspended pellet was incubated at 37 °C for 10 min and centrifuged at 48000*g* for 10 min at 4 °C. Membranes were resuspended in 2.75 volumes of assay buffer (50 mM Tris-HCl, 10 μM pargyline, 0.6 mM ascorbic acid, and 5 mM $CaCl_2$, pH 7.4 at 25 °C). Fractions of 100 μL of the final membrane suspension (about 2 mg/mL of protein) were incubated at 25 °C for 30 min with 0.7 nM [^{3}H]LY 278584, in the presence or absence of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 10 μM 5-HT.

5-HT$_4$ Receptor. Binding assays were performed by a modification of the procedure previously described by Grossman et al.[43] The striatum was homogenized in 15 volumes of ice-cold 50 mM HEPES buffer (pH 7.4 at 4 °C) and centrifuged at 48000*g* for 10 min. The pellet was resuspended in 20 volumes of assay buffer (50 mM HEPES, pH 7.4 at 25 °C). Fractions of 100 μL (about 5 mg/mL of protein) of the final membrane suspension were incubated at 37 °C for 30 min with 0.1 nM [^{3}H]GR 113808, in the presence or absence of the competing drug, in a final volume of 1 mL of assay buffer. Nonspecific binding was determined with 30 μM 5-HT.

5-HT$_7$ Receptor. Binding assays were performed by a modification of the procedure previously described by Aguirre et al.[44] The hypothalamus was homogenized in 5 mL of ice-cold Tris buffer (50 mM Tris-HCl, pH 7.4 at 25 °C) and centrifuged at 48000*g* for 10 min. The membrane pellet was washed by resuspension and centrifugation, and then the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation, and the final pellet was resuspended in 100 volumes of ice-cold 50 mM Tris-HCl, 4 mM $CaCl_2$, 1 mg/mL ascorbic acid, 0.01 mM pargyline, and 3 μM pindolol[55] buffer (pH 7.4 at 25 °C). Fractions of 400 μL of the final membrane suspension were incubated at 23 °C for 120 min with 0.5 nM [^{3}H]-5-CT (88 Ci/mmol), in the presence or absence of several concentrations of the competing drug, in a final volume of 0.5 mL of assay buffer (50 mM Tris-HCl, 4 mM $CaCl_2$, 1 mg/mL ascorbic acid, 0.01 mM pargyline, and 3 μM pindolol buffer (pH 7.4 at 25 °C)). Nonspecific binding was determined with 10 μM 5-HT.

5-HT Transporter. Binding assays were performed by a modification of the procedure previously described by Habert et al.[45] The cerebral cortex was homogenized in 50 volumes of ice-cold Tris buffer (50 mM Tris-HCl containing 20 mM NaCl and 5 mM KCl, pH 7.4 at 25 °C) and centrifuged at 20000 rpm for 10 min at 4 °C. The membrane pellet was

washed twice by resuspension and centrifugation. The final pellet was resuspended in assay buffer, and fractions of 1 mL of the final membrane suspension (about 120 μg of protein) were incubated at 25 °C for 60 min with 0.2 nM [^{3}H]paroxetine, in the presence or absence of the competing drug, in a final volume of 1.2 mL of assay buffer. Nonspecific binding was determined with 10 μM fluoxetine.

α_1 Adrenoceptor. Binding assays were performed by a modification of the procedure previously described by Ambrosio et al.[39] The cerebral cortex was homogenized in 20 volumes of ice-cold buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4 at 25 °C) and centrifuged at 30000*g* for 15 min. Pellets were washed twice by resuspension and centrifugation. Final pellets were resuspended in the same buffer. Fractions of the final membrane suspension (about 250 μg of protein) were incubated at 25 °C for 30 min with 0.2 nM [^{3}H]prazosin (23 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of buffer. Nonspecific binding was determined with 10 μM phentolamine.

D_2 Receptor. Binding assays were performed by a modification of the procedure previously described by Hall et al.[46] The striatum was homogenized in 50 mM Tris-HCl (pH 7.7 at 25 °C) and centrifuged at 48000*g* for 10 min. The pellet was resuspended and centrifuged as before. The final pellet was resuspended in 50 mM Tris-HCl (pH 7.7 at 25 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid. Fractions of the final membrane suspension (125−150 μg of protein) were incubated at 25 °C for 60 min with 0.8 nM [^{3}H]raclopride (77 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 1.1 mL of the assay buffer (pH 7.4 at 25 °C). Nonspecific binding was determined with 1 μM (+)-butaclamol.

Benzodiazepine Receptor. Binding assays were performed by a modification of the procedure previously described by Orensanz et al.[47] The tissue was homogenized in 25 mM potassium phosphate (KPi) buffer (pH 7.4). Homogenized fractions of 100 μL of the membrane suspension (about 100 μg of protein) were incubated at 0−4 °C for 90 min with 0.25 nM [^{3}H]flunitrazepam (37 Ci/mmol), in the presence or absence of the competing drug, in a final volume of 1 mL of assay buffer. Nonspecific binding was determined with 2 μM diazepam.

For all binding assays, competing drug, nonspecific, total, and radioligand bindings were defined in triplicate. Incubation was terminated by rapid vacuum filtration through Whatman GF/B filters, presoaked in 0.05% poly(ethylenimine), using a Brandel cell harvester. The filters were then washed with the assay buffer, dried, and placed in poly(ethylene) vials to which were added 4 mL of a scintillation cocktail (Aquasol). The radioactivity bound to the filters was measured by liquid scintillation spectrometry. The data were analyzed by an iterative curve-fitting procedure (program Prism, Graph Pad), which provided IC_{50}, K_i, and r^2 values for test compounds, K_i values being calculated from the Cheng and Prusoff equation.[40] The protein concentrations of the rat cerebral cortex and the rat striatum were determined by the method of Lowry,[56] using bovine serum albumin as the standard.

Rectal Temperature. Male Swiss albino mice (24−30 g) were obtained from Interfauna Ibérica and maintained in a temperature- and light-controlled environment (25 ± 1 °C, light on between 8.00 a.m. and 8.00 p.m.). Food and tap water were provided ad libitum. All experiments were performed between 9.00 a.m. and 2.00 p.m. After removal of mice from their home cages, basal rectal temperature was measured with a lubricated digital thermistoprobe that was inserted into the rectum 1.5 cm for 40 s. Rectal temperature was determined again after the appropriate treatments with (*S*)-**9** or vehicle. The difference between the temperatures measured before and 60 min after the administration represents an index of hypothermia. A decrease of more than 1.1 °C from basal rectal temperature was considered to be a hypothermic response.

Cell Culture and Determination of cAMP Levels after Stimulation of the Adenylate Cyclase Enzyme with Forskolin. HeLa cells transfected with the human 5-HT_{1A} receptor (HA 6 cells) were kindly provided by Dr. J. del Río (Departamento Farmacología, Universidad de Navarra) and grown in 75 mL flasks containing 20 mL of Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 500 units of penicillin, 500 μg of streptomycin/mL (P/S), and 0.3 mg/mL geneticin. Forty-eight hours before the experiment, cells were plated at a density of 75 × 10^3 cells in 1.5 mL of DMEM−P/S−fetal calf serum−geneticin medium in 12 multiwell plates. On the day of the experiment, cells were treated with 0.5 mM 1-methyl-3-isobutylxanthine (IBMX), 10 μM forskolin, and vehicle or different concentrations of (*S*)-**9** in a 37 °C and 5% CO_2 incubator. Ten minutes later, treatment was stopped and cells were lysed with a 65% ethanol solution for 2 h, and then the ethanol was collected and evaporated at 55 °C, leaving a pellet with cAMP. For the study of (*S*)-**9** receptor specificity, cells were preincubated for 20 min with 10^{-8} M WAY 100,635 prior to adding (*S*)-**9**. Samples were stored at −20 °C and later analyzed using a commercial radioimmunoassay (RIA) kit ([^{3}H]cAMP assay system, model TRK 432; Amersham). Protein was measured by Bradford's method. Competition binding isotherms were analyzed by using an iterative curve-fitting procedure (program Origin 7.0), which provided EC_{50} values for test compounds.

Neurochemical Activity. 5-HIAA/ 5-HT Ratios. Following appropriate treatments, mice were decapitated and brains were removed from the skull. Hypothalamus were dissected on ice and immediately frozen over dry ice. Tissue samples were placed in 200 μL of 0.1 M phosphate−citrate buffer (pH 2.5) containing 15% methanol and stored at −80 °C until assayed. On the day of assay, tissue samples were thawed, sonicated for 3 s (Vibra Cell, model VC-501, Sonics and Materials, Danbury, CT), and centrifuged for 60 s in a Microfuge (IEC, Centra-MP4R, Needham, MA). 5-Hydroxyindolacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), and dopamine concentrations in hypothalamus tissue extracts were measured by high-performance liquid chromatography (HPLC) with electrochemical detection. A total of 20 μL of the supernatant was injected onto a Nucleosil 120 5 C18 reverse-phase analytical column. The HPLC column was coupled to a single coulometric electrode conditioning cell in series with dual electrode analytical cells (Coulochem II, ESA, Bedford, MA). The conditioning electrode potential was set at 100 nA, and the analytical electrodes were set at +0.12 and −0.31 V relative to internal Ag reference electrodes. The HPLC mobile phase consisted of 0.1 M phosphate/citrate buffer (pH 2.8) containing 0.1 mM ethylenediaminetetracetic acid (EDTA), 0.05% sodium octylsulfate, and 25% methanol. The 5-HIAA and 5-HT content of each sample was quantified by comparing peak heights with those peaks of standards assayed the same day as determined by the Shimadzu integrator. The lower limit of sensitivity of this assay for these compounds was 2−8 pg per sample. Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein.[56]

Light/Dark Box Test. The light/dark test uses the rodent natural aversion to bright areas compared with darker ones.[57] In a two-compartment box, rodents will prefer the dark areas, whereas anxiolytics should increase the time spent in the lit compartment. The apparatus consisted of two methacrylate boxes (20 cm × 20 cm × 14 cm), one transparent and one black and opaque separated by an opaque tunnel (5 cm × 7 cm × 10 cm). A light from a 60 W desk lamp placed 25 cm above the light box provided the room illumination. Male Swiss albino mice were housed 5 days before the experiment. On the day of the experiment drugs were dissolved in distilled water and sc injected. After 30 min of absorption time mice were individually tested in 5 min sessions in the apparatus described above. The floor of each box was cleaned between sessions. At the beginning of the session, mice were placed in the tunnel facing the dark box. The amount of time spent by mice in the lit area was recorded over 5 min periods. A mouse whose four paws were in the new box was considered as having changed boxes.

Statistical Analyses. Statistical analyses were performed using analysis of variance (ANOVA) followed by the Student−Newman−Keul's test. Differences were considered significant if the probability of error was less than 5%.

Models of 5-HT$_{1A}$R−Ligand Interaction. The previously reported 3D model of the 5-HT$_{1A}$R complexed with arylpiperazines of formula **I**[25] was employed in the manuscript. The conformation of Trp$^{6.48}$ was modeled in the observed active trans conformation,[29] and thus, Phe$^{6.52}$ was also modeled in trans to avoid the steric clash.[30] These models were placed in a rectangular box (∼80 Å × 93 Å × 75 Å in size) containing a lipid bilayer (∼95 molecules of palmitoyloleoylphosphatidylcholine and ∼13000 molecules of water), resulting in a final density of ∼1.0 g cm^{-3}. The ligand-receptor−lipid bilayer systems were subjected to 500 iterations of energy minimization and then heated to 300 K in 15 ps. This was followed by an equilibration period (15−250ps) and a production run (250−500ps) at constant pressure with anisotropic scaling, using the particle mesh Ewald method to evaluate electrostatic interaction. During the processes of minimization, heating, and equilibration a positional restraint of 10 kcal mol^{-1} Å^{-2} was applied to the C$_\alpha$ atoms of the receptor structure. Structures were collected for analysis every 10 ps during the production run. Parameters for the system were obtained from the ff99 force field[58] and the "general Amber force field" for compounds **2** and **9** using RESP point charges.[59] The molecular dynamics simulations were run with the Sander module of AMBER 8.[60]

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Supporting Information Available: Spectroscopic data of final compounds **2**−**4**, **7**−**11**, **13,** and **14**, and results from elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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