N^4-Unsubstituted N^1-Arylpiperazines as High-Affinity 5-HT_{1A} Receptor Ligands

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In order to explore the structural requirements for high 5-HT_{1A} affinity, a series of aryl-substituted N^1-phenylpiperazines were synthesized and evaluated for their ability to displace [^{3}H]-8-OH-DPAT from its specific binding sites in rat frontal cortex homogenates. We found 2-methoxy substitution to be favorable, while 4-methoxy substitution was detrimental for 5-HT_{1A} affinity. Substitution with annelated rings at the 2,3-positions was highly favorable for all investigated compounds, with the exception of a pyrrole ring. All other substitutions, except fluoro, in this class of heterobicyclic phenylpiperazines decreased affinity in the order: *ortho* > *para* > *meta*. The loss of affinity in the *ortho* and *para* positions is probably due to steric factors: the substituents either cause steric hindrance with the receptor or prevent the compound from adopting the appropriate conformation for binding to the 5-HT_{1A} receptor. Conformational analysis combined with structure–affinity relationships (SAR) indicates that our arylpiperazines may bind at the 5-HT_{1A} receptor in a nearly coplanar conformation. Observed interactions of the compounds in our 5-HT_{1A} receptor model appeared to be in agreement with SAR data. The aromatic part of the arylpiperazine moiety has $\pi-\pi$ interactions with the aromatic residues Trp161 and Phe362 in helices IV and VI, respectively. The positively charged protonated basic nitrogen forms a hydrogen bond with the negatively charged Asp116 in helix III. The ammonium–aspartate complex is surrounded by aromatic residues Trp358 and Phe361 in helix VI. A lipophilic pocket is formed by Phe362, Leu366 (both helix VI), and the methyl group of Thr200 (helix V). In agreement with the model, addition of a methyl substituent to the structure of the benzodioxine analogue **12** in this region, yielding **13**, is favorable for 5-HT_{1A} receptor affinity. Unfavorable positions for substitution with bulky groups, like the 3- and 4-positions in the benzofuran compound **14**, are explained by steric hindrance with the backbone atoms of helix V. Thus, we were able to rationalize the 5-HT_{1A} SAR of existing N^1-phenylpiperazines, as well as a series of newly synthesized bicyclic heteroarylpiperazines, in terms of receptor-ligand interactions. Several of these N^4-unsubstituted compounds had affinities in the low-nanomolar range.

Introduction

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) (**1**, Figure 1) is involved in various physiological (e.g., sleep and thermoregulation) and pathophysiological processes (e.g., migraine and depression).[1] The receptors that are activated by 5-HT have been divided into at least seven classes: 5-HT_1, 5-HT_2, 5HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7.[1-5] The 5-HT_1 receptors have been further subdivided in 5-$HT_{1A,B,D-F,P}$ and 5-HT_1-like.[1,2] Of these receptors, the 5-HT_{1A} receptor subtype is the best studied as a result of the availability of 8-OH-DPAT, the *R* enantiomer being a highly selective, potent 5-HT_{1A} agonist (**2**, Figure 1).[6] This research yielded new potent and selective 5-HT_{1A} ligands, belonging to different chemical classes (for reviews, see Nelson[7] and Glennon[8]). The class of the arylpiperazines, for instance, has yielded many potent 5-HT_{1A} agents such as flesinoxan (**3**), BMY7378 (**4**), and ipsapirone (**5**) (Figure 1).[8] Most of the arylpiperazines contain a long-chain substituent at the basic nitrogen of the piperazine ring, which is important for the high 5-HT_{1A} receptor affinity of these compounds.[7-10] For example, the corresponding *N*-unsubstituted compounds of ipsapirone and BMY7378 are 200 and 27 times less potent, respectively. As a result, structure–affinity relationships (SAR) of N^4-unsubstituted N^1-arylpiperazines reported so far are quite limited, in contrast to the N^4-substituted arylpiperazines.

Therefore, we investigated the effect of variation in the arylpiperazine structure on 5-HT_{1A} receptor affinity. In this paper, we describe the synthesis of a series of aryl-substituted N^1-phenylpiperazines and the affinities for 5-HT_{1A} receptors, obtained by radioligand binding studies. Figure 2 shows the general structures of the compounds investigated (**6–36**). In order to gain insight into the 5-HT_{1A} receptor affinity of the arylpiperazine moiety, we subsequently docked several compounds of the series, possessing high 5-HT_{1A} receptor affinities, into our model for the 5-HT_{1A} receptor. This model was shown to rationalize 5-HT_{1A} receptor affinity and selectivity of several agonist classes such as tryptamines and aminotetralins, as well as (aryloxy)propanolamine antagonists.[11] For the docking studies of the arylpiperazines, the bioactive conformation, as well as the binding region in the model, was established.

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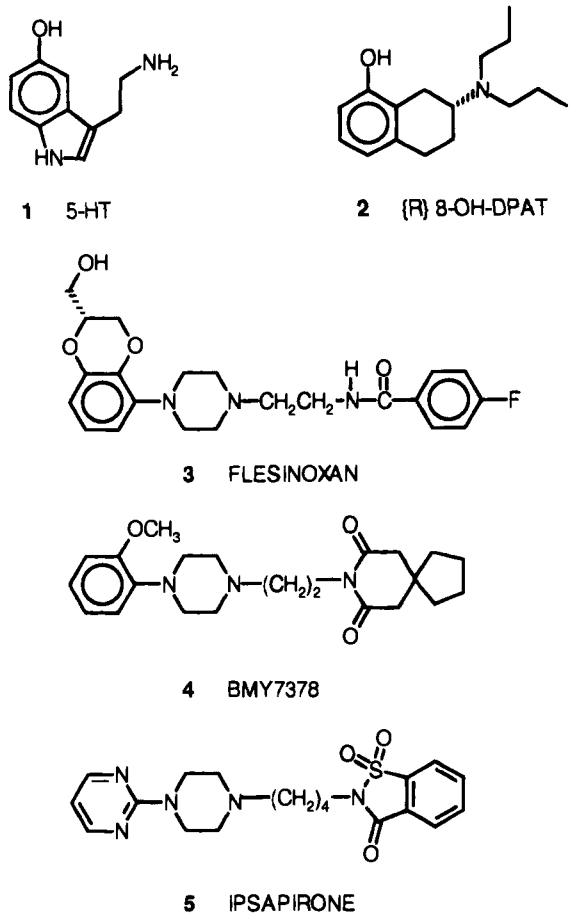


Figure 1. Structures of 5-HT_{1A} receptor ligands.

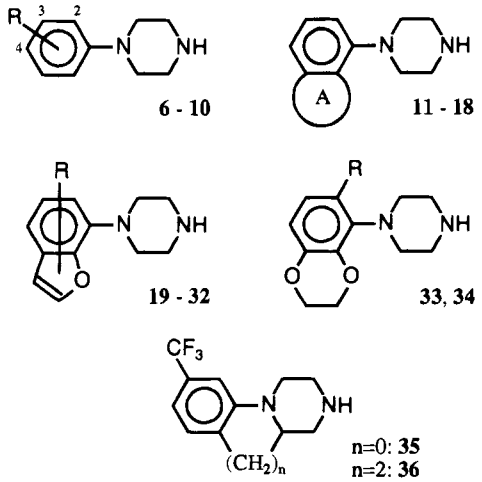


Figure 2. General structures of phenylpiperazine compounds investigated in this study.

Chemistry

Most of the target compounds are either commercially available (**6**–**9** and **17**) or known in the literature (**10**, **11**, **13**–**16**, **18**, **19**, **21**–**24**, **26**–**29**, **35**, and **36**; see the Experimental Section). For the unknown heteroarylpiperazines, two synthetic strategies are feasible (see also Table 1).

The first started with the correctly substituted nitro precursor, which was transformed into the target compounds in two steps (Scheme 1). For the reduction of **37**, which is the nitro precursor of **12**, the Fe/HCl method was chosen, which leaves the dioxine 2,3-double bond untouched. The resulting aniline **38** was converted into **12** by reaction with bis(2-chloroethyl)-amine·HCl in apolar solvent.[12,13] The last step worked equally well with the anilines **40**, **42**, **44**, **45**, and **47**, giving the corresponding piperazines **20**, **25**, **30**, **33**, and **34** in reasonable yields. The unknown anilines were obtained from the nitro precursors **39**, **41**, **43**, and **46**, either by the Fe/HCl or by the equally selective, but cleaner, hydrazine Raney Ni procedure[14] (see Scheme 1).

The unknown nitro compounds were synthesized as presented in Scheme 2. Dehydrogenation of the dioxane moiety in **48** was accomplished in 18% overall yield by a two-step procedure. Radical bromination yielded the corresponding 2,3-dibromo compound, and subsequent elimination with NaI in acetone resulted in the formation of **37**. The 7-nitrobenzofuranyl derivatives **39**, **41**, and **43** were obtained from the corresponding *o*-allylnitrophenols.[15] The unknown **53** and **54** were synthesized from *o*-nitrophenols **49** and **50** by phase-transfer alkylation in toluene to give **51** and **52**, respectively, and a subsequent Claisen rearrangement at 160°. Ozonolysis of **53** at −78° in CH_2Cl–MeOH proceeded smoothly, and the resulting peroxy hemiacetal was reduced by the addition of dimethyl sulfide giving the ring-closed 2-hydroxy-2,3-dihydrobenzofuran derivative (step D). Dehydration was preferably carried out by treatment with concentrated sulfuric acid (step E), giving product **41**. In an analogous procedure, **54** and the known **55** were converted into **43** and **39**, respectively. The 6-methoxy substituent in **46** was introduced by a regioselective nucleophilic aromatic substitution reaction of the known dinitro substrate **56**.

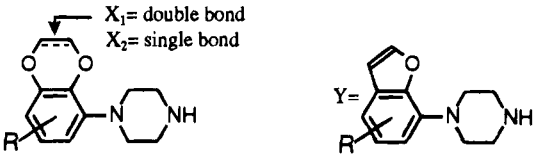
The second strategy started with the unsubstituted arylpiperazine, and the required substituents were introduced by electrophilic aromatic substitution methodology (Scheme 3). This route was chosen for the target products **31** and **32**. Protection of the secondary amine in **14**, by the method of Rosowsky,[16] gave *N*-TeOC derivative **57**, which was nitrated under mild conditions in a two-phase system. The desired 6-nitro derivative **58** could be isolated in 20% yield from a mixture of isomers. Deprotection was possible under standard conditions (TBAF in THF) giving **31**. The introduction of a formyl group in the aromatic ring system of **14** occurred smoothly with $POCl_3$–DMF. The piperazine NH was formylated as well. Although the main product was found to be the 4-substituted derivative, sufficient amounts of **59** were present. After a standard aldehyde into nitrile transformation, the regio isomers could be separated, giving pure **60**, which was deformylated under mild conditions (2 N HCl in THF, 20 °C) to yield **32**.

Modeling

Phenylpiperazines in the 5-HT_{1A} Receptor Model. The 5-HT_{1A} receptor is believed to consist of seven membrane-spanning α-helices connected by three intracellular and three extracellular loops. The N-terminus is located outside the cell, and the C-terminus is located at the cytoplasmic side. Figure 3 shows the sequences of the putative transmembrane parts, which were used for the model construction.[11] For docking studies, the conformation in which the ligands bind to the receptor has to be established. Also the binding site of the ligands in the model has to be identified.

Bioactive Conformation of Phenylpiperazines. Phenylpiperazines appear to prefer a conformation in

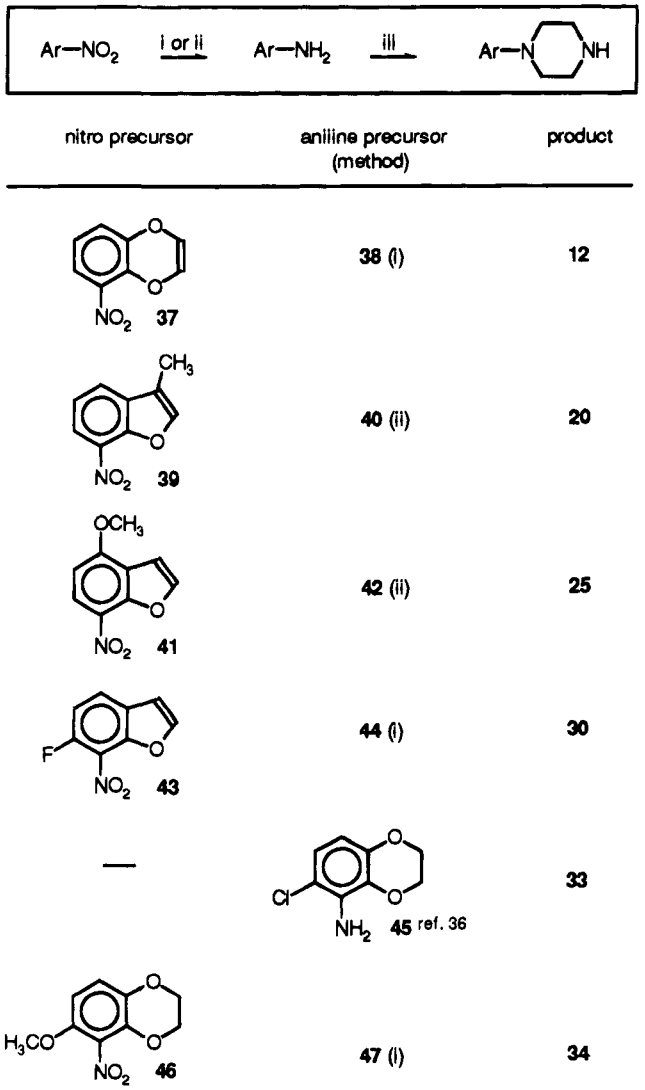
Table 1. Physicochemical Properties and Synthesis Methods of Compounds **12**, **20**, **25**, and **30–34**



compd	structure	R	formula	prep method scheme (route)	mp (°C)	C, H, N anal.[a]
12	X_1		$C_{12}H_{14}N_2O_2 \cdot C_4H_4O_4$	2 (A); 1 (i, iii)	202–3	C, H, N
20	Y	3-CH_3	$C_{13}H_{16}N_2O \cdot HCl \cdot 0.50H_2O$	2 (D,E); 1 (ii, iii)	206–9.5	C, H, N
25	Y	4-OCH_3	$C_{13}H_{16}N_2O_2 \cdot HCl$	2 (B–E);1 (ii, iii)	198–200	C, H, N
30	Y	6-F	$C_{12}H_{13}FN_2O \cdot 2HCl$	2 (B–E); 1 (i, iii)	157–63	C, H, N
31	Y	6-NO_2	$C_{12}H_{13}N_3O_3 \cdot HCl \cdot 0.10H_2O$	3 (A–C)	238–43	C, H, N
32	Y	6-CN	$C_{13}H_{13}N_3O \cdot HCl \cdot 0.25H_2O$	3 (D–F)	271–5 dec	C, H, N
33	X_2	6-Cl	$C_{13}H_{15}ClN_2O_2 \cdot C_7H_8O_3S$	1 (iii)	224–6	C, H, N
34	X_2	6-OCH_3	$C_{13}H_{18}N_2O_3 \cdot C_4H_4O_4$	2 (F); 1 (i, iii)	193–5	C, H, N

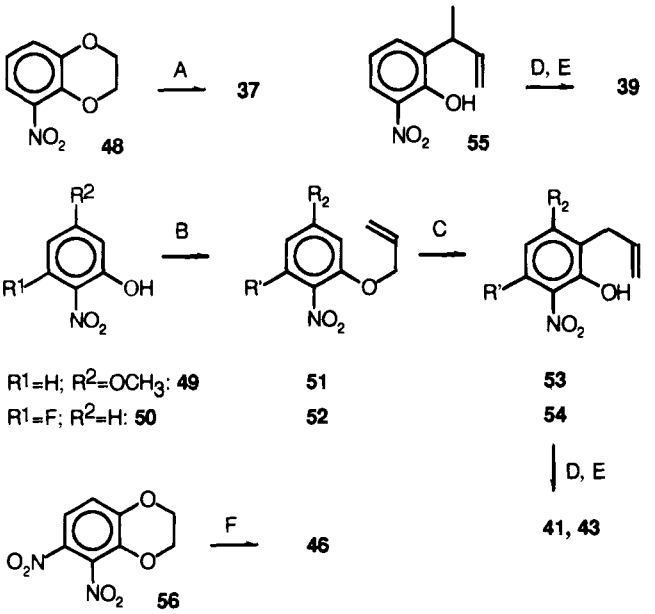
[a] All values are within 0.40% of the calculated theoretical values.

Scheme 1[a]



[a] (i) Fe, HCl, EtOH–H_2O; (ii) H_2NNH_2/Raney Ni, MeOH; (iii) $HN(CH_2CH_2Cl)_2 \cdot HCl$, *p*-TsOH, C_6H_5Cl.

Scheme 2[a]

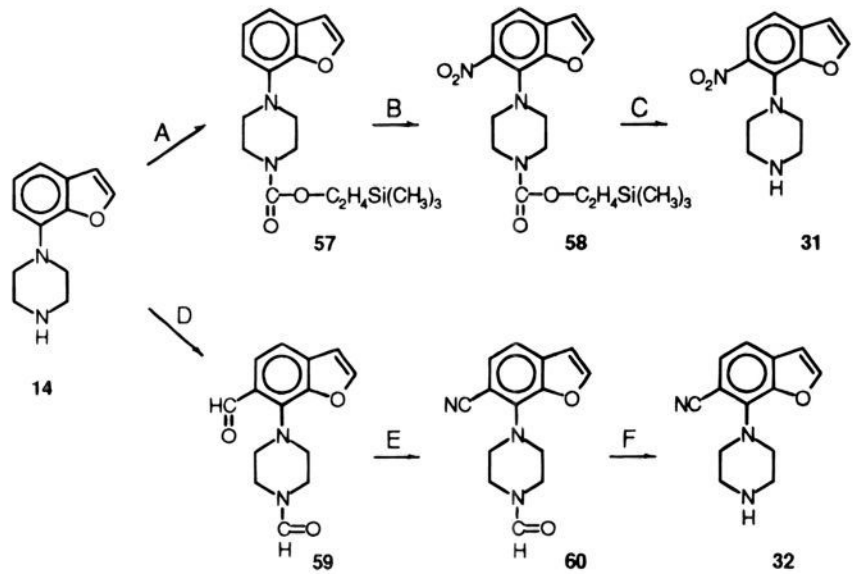


[a] (A) 1. *N,N*-Dibromodimethylhydantoin, dibenzoyl peroxide, CCl_4, 2. NaI, acetone; (B) allyl bromide, K_2CO_3, TBAB, toluene; (C) heat, Δ; (D) O_3, CH_2Cl_2–MeOH followed by Me_2S; (E) H_2SO_4, CH_2Cl_2; (F) NaOMe, DMF.

which the nitrogen lone pair is almost perpendicular to the plane of the aromatic ring and can participate in the π-system.[17–19] This interaction, which is schematically shown in Figure 4, requires a relatively coplanar orientation of the piperazine ring with respect to the benzene ring. In the fully coplanar conformations (plane angle ≈ 0°), the torsion angle amounts to τ ≈ 30° or −150° (τ is defined by C6′–C1′–N1–C6; see Figure 4). We use the term 'coplanar' for these fully coplanar conformations as well as for conformations with τ ≈ 0°, 60°, −180°, or −120°, in which the piperazine ring is somewhat tilted with respect to the plane of the benzene ring (plane angle ≈ 30°). This is in agreement with definitions used in previous arylpiperazine conformational studies.[18,20] As an example, in these studies the AM1-minimized structure of compound **36** is called coplanar but has torsion angle τ = −7° (a deviation of almost 40° from a fully coplanar conformation). Where necessary in this paper, we specify the conformations. In the fully twisted conformations (plane angle ≈ 90°), in which conjugation between the nitrogen lone pair and the benzene ring is not possible, the torsion angle is τ ≈ 120° or −60°.

In this study we tried to establish whether the bioactive conformation is fully twisted or relatively coplanar. For this purpose, we studied the effects of substituents in the *ortho* position (with respect to the piperazine ring) of two heterobicyclic arylpiperazine analogues on 5-HT_{1A} receptor affinity. Bulky substituents in this position will cause steric hindrance with

Scheme 3[a]



[a] (A) $Me_3Si(CH_2)_2OCOO$-4-NO_2-C_6H_4, Et(*i*-Pr)$_2$N, CH_3CN; (B) $NaNO_3$, HCl, $NaNO_2$ (cat.), $CHCl_3-H_2O$; (C) TBAF, THF; (D) $POCl_3$, DMF; (E) H_2NOH·HCl, HCO_2Na, HCO_2H; (F) 2 N HCl.

Helix	residue number																							
I	38	I	T	S	L	L	L	G	T	L	I	F	C	A	V	L	G	N	A	C	V	V	A	A
II	74	L	I	G	S	L	A	V	T	D	L	M	V	S	V	L	V	L	P	M	A	A	L	Y
III	109	C	D	L	F	I	A	L	**D**	V	L	C	**C**	T	S	S	I	L	H	L	C	A	I	
IV	153	A	A	A	L	I	S	L	**T**	**W**	L	I	G	F	L	I	S	I	P	P	M	L		
V	195	Y	T	I	Y	**S**	**T**	F	G	A	F	Y	I	P	L	L	I	M	L	V	L	Y		
VI	347	L	G	I	I	M	G	T	F	I	L	C	**W**	L	P	**F**	**F**	I	V	**A**	**L**	V	L	P	F
VII	381	L	G	A	I	I	N	W	L	G	Y	S	N	S	L	L	N	P	V	I	Y	A	Y	F	N

Figure 3. Sequence of the putative transmembrane domains of the rat 5-HT_{1A} receptor, which was used for construction of the receptor model. Marked residues were found to be part of the binding site (residues within a sphere of 4 Å) of compound **12**.

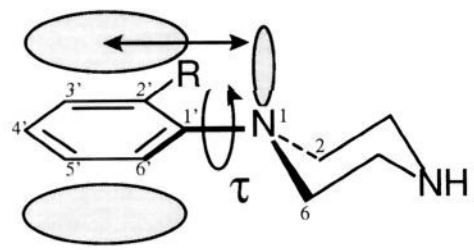


Figure 4. Schematic model of the conjugation of the nitrogen lone pair with the aromatic π-system, which stabilizes coplanar conformations in phenylpiperazines. The torsion angle τ is defined by C6′−C1′−N1−C6.

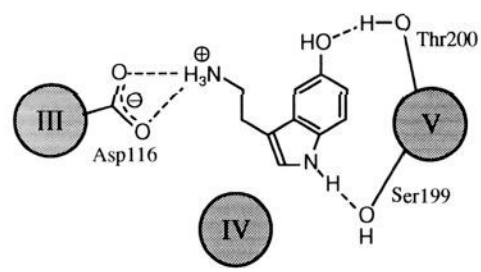


Figure 5. Schematic view of 5-HT docked into the 5-HT_{1A} receptor model.[11] The dashed lines represent hydrogen-bonding interactions with the essential residues Asp116 (helix III), Ser199, and Thr200 (both helix V).

one of the C_α protons of the piperazine ring. Gilli and Bertolasi[17] showed that severe steric hindrance forces the piperazine ring out of the plane of the aromatic ring and causes the decoupling of the N-aromatic system interaction. Also, the effect of fixation of the coplanar conformation in a tricyclic framework on 5-HT_{1A} receptor affinity was taken into consideration.

Position of Phenylpiperazines in the Receptor Model. The previously reported position of 5-HT in the 5-HT_{1A} receptor model (schematically shown in Figure 5) was hypothesized on the basis of site-directed mutagenesis data.[11] Such data are not available for phenylpiperazines. Therefore, we docked the phenylpiperazines into the receptor model, by fitting them on docked 5-HT.

In the literature, however, two possibilities for the fit of the phenylpiperazine part on 5-HT were presented.[21] In the first orientation, used by Hibert[22] for definition of a 5-HT_{1A} receptor map, the benzene ring of phenylpiperazines coincides with that of 5-HT. In the second orientation, which is shown in Figure 6, this benzene ring is fitted on the aromatic pyrrole part of 5-HT.[23] We used the latter orientation to dock several of the high-affinity bicyclic piperazines into the 5-HT_{1A} receptor model.

Results and Discussion

Structure−Affinity Relationships. Substitution of the Phenylpiperazine Part. The results in Table

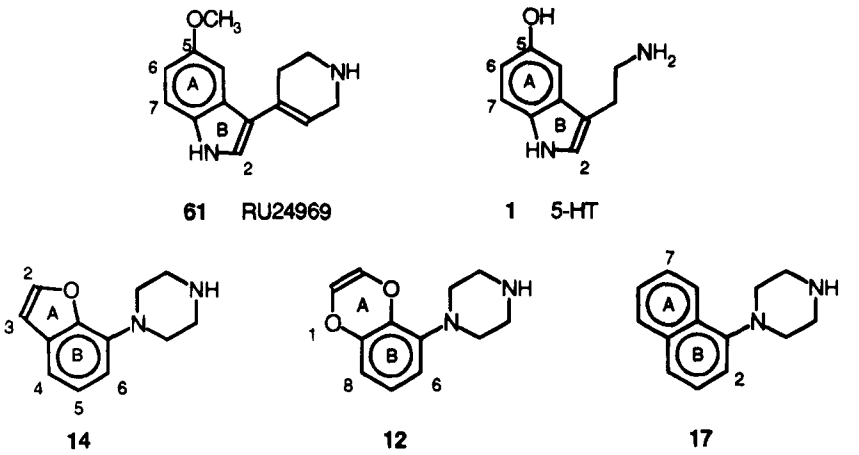
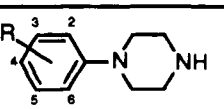


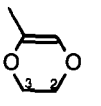
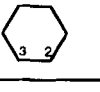
Figure 6. Relative orientations of **12**, **14**, and **17** with respect to 5-HT and the "key" compound RU24969 (**61**). The substitution pattern at the indole ring of 5-HT equals that of the indole part of RU24969. The piperidine ring of RU24969 resembles the piperazine ring of **12**, **14**, and **17**. Like the nitrogen in the piperazine ring of **12**, **14**, and **17**, the double bond of the piperidine ring of RU24969 may participate in the aromatic π-system, stabilizing a coplanar conformation.

2 show that 2- or 3-methoxy substitution of the phenylpiperazine structure increases 5-HT_{1A} receptor affinity by a factor 3 and 1.5, respectively (compare compounds **7** and **8** with **6**). Addition of a methoxy group at the 4-position (**9**) is detrimental for 5-HT_{1A} receptor affinity, indicating severe steric hindrance in this position. The 2,3-dimethoxy analogue **10** is 6- and 3-fold less active than the corresponding monomethoxy compounds **7** and **8**, respectively. This decrease can be explained by steric hindrance of one of the CH_3 groups with either the second methoxy substituent or the receptor. It is very unlikely that the 2-methoxy group can be directed toward the piperazine ring because of steric repulsion (the only accessible local minimum with C1′−C2′−O−C $\approx 0°$ is a twisted conformation with $\tau \approx -60°$ and the energy is 2.9 kcal/mol above the AM1-calculated absolute minimum). As a result, the 3-methoxy group must be directed away from the 2-methoxy group to avoid steric hindrance. In the resulting conformation, the methyl group of the 3-substituent in compound **10** may cause excess bulk in the same region as the 3-substituents in compounds **20** and **21**, respectively, which is unfavorable for affinity. This explanation is further substantiated by the higher affinity of compound **11** (eltoprazine), in which the 2- and 3-methoxy groups are incorporated into a fused benzodioxane ring.

Introduction of a double bond in compound **11**, yielding compound **12**, increases 5-HT_{1A} receptor affinity by a factor 3.3. Addition of an extra methyl group in the structure of compound **12** (yielding **13**) increases affinity even further, possibly via a lipophilic interaction with the receptor. This compound (**13**) is 100 times more potent than 1-phenylpiperazine (**6**) itself. Replacement of the dioxine ring in **12** with other aromatic rings like furan (**14**) or thiophene (**15**) has little influence on 5-HT_{1A} receptor affinity. In contrast to this, replacement with an indole ring yields the only moderately active compound, **16**. Replacement of the oxygen atoms in the annelated hetero ring with CH_2 groups seems to have a negative effect (2-fold decrease) on 5-HT_{1A} receptor affinity (compare the naphthyl analogue **17** with the benzodioxine **12** and the tetrahydronaphthyl analogue **18** with the benzodioxane **11**). The corresponding tetrahydronaphthyl analogue **18** is 5 times less potent than **17**.

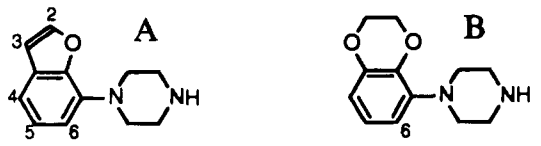
Table 2. K_i Values for the Displacement of [^{3}H]-2-(Di-*n*-propylamino)-8-hydroxytetralin (**2**) from Central 5-HT_{1A} Recognition Sites in Rat Frontal Cortex Homogenates with N^4-Unsubstituted 2-, 3-, and **4**-Substituted N^1-Phenylpiperazines



R	no.	K_i ± SEM (nM)[a]	(n=)
H	6	500 ± 20	(3)
2-OCH_3	7	170 ± 20	(3)
3-OCH_3	8	320 ± 40	(4)
4-OCH_3	9	20,000 ± 5,000	(2)
2,3-OCH_3	10	1,000 ± 100	(4)
	11	40 ± 5	(5)
	12	12 ± 3	(3)
	13	5.2 ± 1.2	(3)
	14	13 ± 2	(3)
	15	9.9	(1)
	16	200 ± 10	(3)
	17	18 ± 2	(3)
	18	84 ± 11	(3)

[a] K_i Values are based on n determinations, each using four to six concentrations in triplicate.

Table 3. K_i Values for the Displacement of [^{3}H]-2-(Di-*n*-propylamino)-8-hydroxytetralin (**2**) from Central 5-HT_{1A} Recognition Sites in Rat Frontal Cortex Homogenates with N^4-Unsubstituted 2-, 3-, and 4-Substituted N^1-Arylpiperazines



structure		no.	$K_i \pm$ SEM (nM)[a]	(n =)
H	A	**14**	13 ± 2	(3)
2-Me	A	**19**	13 ± 2	(3)
3-Me	A	**20**	73 ± 20	(3)
3-Br	A	**21**	130 ± 10	(3)
4-Me	A	**22**	220	(1)
4-Br	A	**23**	300	(1)
4-F	A	**24**	18 ± 0	(3)
4-OCH_3	A	**25**	430 ± 10	(3)
5-Me	A	**26**	120 ± 0	(3)
5-Br	A	**27**	35 ± 5	(3)
5-F	A	**28**	105 ± 30	(3)
5-OCH_3	A	**29**	28 ± 1	(3)
6-F	A	**30**	5.8 ± 1.1	(4)
6-NO_2	A	**31**	140 ± 30	(3)
6-CN	A	**32**	220 ± 110	(2)
H	B	**11**	40 ± 5	(5)
6-Cl	B	**33**	200 ± 20	(3)
6-OCH_3	B	**34**	>10 000	(3)

[a] K_i Values are based on n determinations, each using four to six concentrations in triplicate.

Substitution of Bicyclic Arylpiperazines. Results in Table 3 show that substitution of the benzofuran ring of compound **14** with a methyl group at the 2-position (**19**) has no influence on 5-HT_{1A} receptor affinity. Introduction of a methyl group (**20**) or a bromine atom (**21**) in the 3-position of compound **14** decreases affinity 5.5- and 10-fold, respectively. In the 4-position, only a small atom like fluoro is tolerated (**24**). As with the unsubstituted phenylpiperazine **6**, the 4-methoxy substitution markedly decreases 5-HT_{1A} receptor affinity (compare compounds **9** and **25** with compounds **6** and **14**, respectively). A methyl group (**22**) or a bromine atom (**23**) is unfavorable in the 4-position, lowering 5-HT_{1A} receptor affinity by a factor of 17 and 23, respectively. At the 5-position, all substituents act negatively on 5-HT_{1A} receptor affinity, but the rank order is different from observations for either 3- or 4-substitution. Methyl or fluoro substitution of the 5-position (**26** and **28**, respectively) decreases affinity 10-fold, but the presence of either bromine or methoxy (**27** and **29**, respectively) causes only a 2–2.5-fold decrease. As the most bulky substituents only moderately affect affinity, while the affinity decrease with a small substituent like fluoro is considerable, steric effects seem to play only a minor role in this 5-position. A fluoro atom in the 6-position of **14** (**30**) increases affinity by a factor of 2, while the presence of the more bulky NO_2 (**31**) and CN (**32**) decreases affinity 10- and 17-fold, respectively. A (5-fold) decrease was also observed by 6-substitution of compound **11** with chlorine (**33**). A methoxy group in this position completely abolished 5-HT_{1A} receptor affinity (**34**).

Arylpiperazine Conformation. Thus, bulky substituents in the *ortho* position of the benzene ring (with respect to the piperazine ring) of compounds **11** and **14** destroy 5-HT_{1A} receptor affinity. These results indicate that either these substituents prevent a good fit of the compounds on the receptor or the 5-HT_{1A} receptor prefers phenylpiperazines in a more or less coplanar conformation. This last hypothesis is substantiated by the fact that 5-HT_{1A} receptor affinity is increased by fixation of *m*-CF_3-phenylpiperazine (**35**) into a relatively coplanar conformation ($\tau = -7°$ in the AM1-minimized structure) in compound **36** (unpublished results: K_i values amount to 250 ± 60 and 44 ± 5 nM for compounds **35** and **36**, respectively; n = 3). This increase in receptor affinity may be explained by a contribution of the lipophilic bridging substituent[20] or a loss of conformational freedom. Because both **35** and **36** display in-vivo serotonergic agonist activity, Huff et al. concluded that the bioactive conformation of these compounds must be coplanar.[18] This hypothesis is also supported by a pharmacophore model derived from superimposition of potent 5-HT_{1A} agonists from different structural classes by Hibert et al.[22] Only relatively coplanar conformations of the arylpiperazine moiety can be fitted into this model in which the nitrogen lone pair is almost perpendicular with respect to the aromatic plane. Compounds **11** (eltoprazine) and **14** possess 5-HT_{1A} agonistic properties (decrease of 5-HTP accumulation and inhibition of adenylate cyclase[24,25]), suggesting a relatively coplanar binding mode at 5-HT_{1A} receptors. Therefore we docked these compounds and the eltoprazine analogue **12** into the 5-HT_{1A} receptor model in a coplanar conformation. For this purpose we investigated the accessibility of such conformations with MOPAC/AM1 calculations and evaluated the results with the use of crystallographic data.

Modeling: Phenylpiperazines in the 5-HT_{1A} Receptor Model. Arylpiperazine Conformation. Results of rotational barrier calculations for 2-methoxyphenylpiperazine (**7**) and the benzodioxine analogue **12** are shown in Figure 7a. AM1 predicts the coplanar conformations ($\tau \approx 60°$, 160°, −10°, and −120°) as local energy minima which are only 0.5–1 kcal/mol higher than the absolute minimum at $\tau \approx 120°$. For compound **14** (Figure 7b), a slightly different curve is obtained, as the coplanar conformations $\tau \approx 60°$, 170°, −10°, and −120° are calculated as absolute minima and the twisted conformation $\tau \approx 120°$ is predicted as slightly less stable. In the $\tau \approx 120°$ minimum, the nitrogen lone pair is in the direction of the C6′ atom. Apparently, the other twisted conformation ($\tau \approx -60°$) is less favored by compounds **7**, **12**, and **14**. In this conformation the N1 lone pair is directed toward the C2′ substituent, which may cause repulsion between the nitrogen and oxygen lone pairs. In contrast, for compounds **16** and **17**, the $\tau \approx -60°$ twisted conformation seems better accessible (Figure 7c). These compounds possess a CH or NH group at the C2′ position instead of an oxygen atom as in compounds **7**, **12**, and **14**. For compounds **16** and **17** all conformations between $\tau \approx -120°$ and −10° seem to be favorable.

For all compounds investigated, the truly coplanar conformations ($\tau \approx 30°$ and −150°) are unfavorable as a result of steric hindrance with the *ortho* substituent. This is in agreement with CNDO/2 calculations by Mokrosz et al.[26] However, for the compounds investigated, relatively coplanar conformations with plane angle ≈ 30° ($\tau \approx 0°$ and −120°) seem to be easily accessible. This hypothesis is supported by the fact that all 10 structures of singly *ortho*-substituted phenylpip-

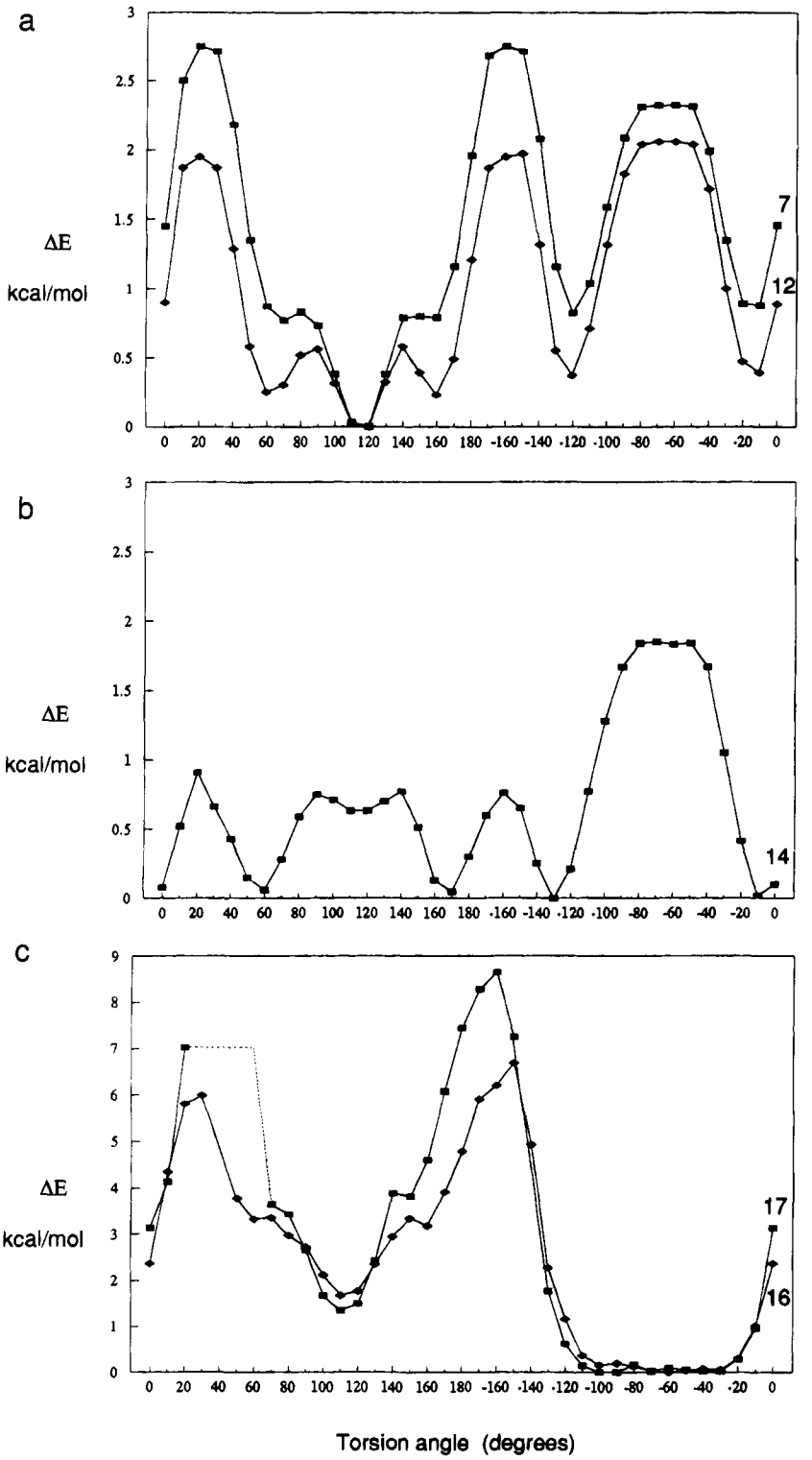


Figure 7. Plot of energy against the torsion angle τ (C6′−C1′−N1−C6) of (a) compounds **7** (■) and **12** (◆), (b) compound **14** (■), and (c) compounds **16** (◆) and **17** (■), calculated with MOPAC AM1. Energy is relative to the calculated absolute minimum. In the fully coplanar conformations (plane angle ≈ 0°), $\tau \approx 30°$ or −150°. In conformations with $\tau \approx 0°$, 60°, 180°, or −120°, the piperazine ring is somewhat tilted with respect to the plane of the benzene ring (plane angle ≈ 30°). In the fully twisted conformations (plane angle ≈ 90°), in which conjugation between the N-aromatic system is not possible, $\tau \approx 120°$ or −60°. For compound **17** conformations with τ values between 30° and 70° are highly unfavorable as a result of steric hindrance of the aryl substituent (NH or CH at the *ortho* position) with the C_α protons of the piperazine ring. As a result, AM1 may invert the piperazine N1 atom which obscures the analysis. Conformations that could not be calculated because of inversion of the piperazine N1 are represented with a dashed line in plot c.

erazines that we derived from the Cambridge Crystallographic Database (CSD) were in conformations with $\tau \approx 0°$ or −120° (substituents were methoxy, fluoro, -CH_2OH, and -C=N-R). The apparent strong preference for these conformations is, however, not predicted by AM1. This may be due to crystal packing effects which favor a coplanar conformation. It is also possible that AM1 underestimates stabilization by N-aromatic conjugation. The fact that calculated C1′−N1 bond distances in coplanar conformations are longer than those observed in crystal structures supports the second hypothesis.[19]

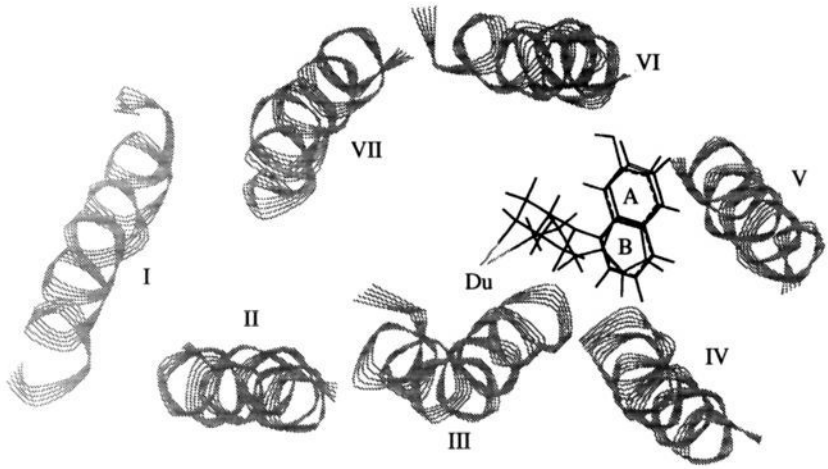


Figure 8. Fit of compound **12** on 5-HT (**1**), which was used for docking **12** into the 5-HT_{1A} receptor model. Fitting points were the centers of rings A and B and the dummy atom "Du". This dummy atom represents an oxygen atom (of the negatively charged Asp116) on the receptor, interacting with the ligands' basic nitrogens (distance N−Du was chosen as 2.8 Å).

Results from AM1 calculations in Figure 7c indicate that the rather low affinity of the indole derivative **16**, when compared to the more potent naphthyl **17**, is probably not due to conformational differences. Probably, the electron distribution in **16** is unfavorable in its direct interaction with the receptor. A coplanar conformation may be stabilized by the addition of electron-withdrawing substituents at the benzene ring.[19] This effect may be the cause of the slight increase in affinity in 6-fluoro substitution of **14**, yielding **30**.

Position of Phenylpiperazines in the Receptor Model. The relative orientation of the docked arylpiperazines (**11**, **12**, and **14**) with respect to 5-HT (**1**) is schematically illustrated in Figure 6. In this orientation, which was previously proposed by Glennon,[23] the phenyl part of the arylpiperazine ring (B) coincides with the pyrrole ring of 5-HT (**1**) and the A-ring coincides with the phenyl part of 5-HT. RU24969 (**61**) was included as a hybrid compound between the class of arylpiperazines and 5-HT. The basic nitrogen of RU24969 (**61**) is part of an unsaturated piperidine ring, which resembles a piperazine ring. Like the nitrogen lone pair in arylpiperazines, the double bond in the unsaturated piperidine ring may participate in the indole aromatic system, stabilizing a coplanar conformation.

The orientation as depicted in Figure 6 is in good agreement with the 5-HT_{1A} SAR of our series, as well as with previously reported SAR of 5-HT, naphthylpiperazine, and RU24969. For instance, the affinity decrease by substitution in the 3- and 4-positions of compound **14** is in agreement with observed negative effects of substitution of the corresponding 7- and 1-positions of 5-HT.[22,27] Also, the effect of bulky substituents in the 6-position of compounds **11** and **14**, like that of bulky substituents in the 2-position of 5-HT (**1**), RU24969 (**61**), and naphthylpiperazine **17**, are unfavorable for 5-HT_{1A} receptor affinity.[23,28] On the other hand, the respective hydroxy and methoxy substituents of 5-HT and RU24969 are essential for the high affinity of these compounds. This is in agreement with the fact that methoxy substitution of the corresponding 7-position of naphthylpiperazine increases 5-HT_{1A} receptor affinity, although the effect is less pronounced than in other classes.[23]

The alternative orientation in which the benzene rings of 5-HT and the arylpiperazines coincide, as proposed by Hibert et al.,[22] is less substantiated by SAR results. In this orientation, for instance, the proposed sterically hindered 2-position of 5-HT and RU24969 is located in the same region as the 2-position of **12** and the 7-position of naphthylpiperazine **17**, which were shown to be favorable for methyl and methoxy substitution, respectively.[23]

We docked three compounds with high 5-HT_{1A} receptor affinity, **11**, **12**, and **14**, into our previously constructed model for the 5-HT_{1A} receptor, by fitting the compounds in the coplanar conformation on docked 5-HT (**1**) in the preferred orientation (thus $\tau \approx 0°$). In Figure 8, this is illustrated for compound **12**. The resulting position and environment of **12** in the receptor, after minimization, are shown in Figure 9, top. The compound is located between the helices III, IV, V and VI, close to the extracellular side of the receptor. Interactions are shown in detail in Figure 9, bottom.

The protonated basic nitrogen atom forms a hydrogen bond with the negatively charged Asp116 in helix III. This complex is surrounded by the aromatic residues Trp358 and Phe361 in helix VI. Such a surrounding of the ammonium−aspartate interaction has also been observed in other models for GPCR−ligand complexes.[29,30] Recently, Verdonk et al.[31] showed that in crystal structures R-$N(CH_3)_3^+$ complexes have a specific spatial preference for close contacts with aromatic residues and postulated that such contacts may play a role in ligand−receptor recognition and activation.

The benzene ring of the ligand is near the aromatic Trp161 residue in helix IV. The unsaturated heterocyclic ring of **12** may form an antiparallel $\pi-\pi$ stacking interaction with Phe362. The more polar nature of the binding site in the vicinity of helix V, because of the presence of Thr160, Ser199, and Thr200, is in agreement with the observed preference for polar heterocyclic rings (compare compounds **11** and **12** with **18** and **17**, respectively). As CH_n is more bulky than an oxygen atom, this preference may also be caused by steric factors (e.g., conformational effects). Thr200 has a dualistic character, as it also contains a methyl group. Thus it may form a hydrophobic pocket with Leu366 and Phe362 (helix VI), in which the methyl substituent of **13** can be located. This is in agreement with SAR observations, that the effect in the 6-position of 5-HT, coinciding with the 2-position of **12**, seems to be determined by the lipophilicity of the substituent.

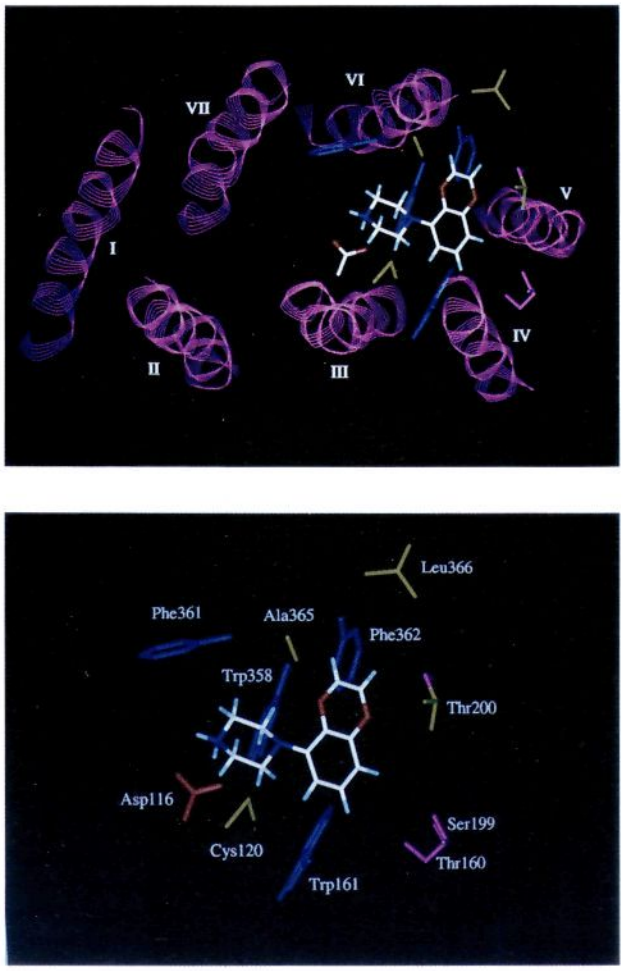


Figure 9. Resulting position of compound **12** in the 5-HT_{1A} receptor model after docking and minimization, viewed upon from the extracellular side (top). In the bottom panel, the environment of the ligand is shown in more detail. Aromatic residues, colored blue, surrounded the aspartate−ammonium complex and interact with the aryl part of **12**. The backbone of helix V, shown in white, is located close to the positions at the right side of the docked molecule. This part of the binding site is rather polar (pink) as a result of the presence of Thr160, Ser199, and Thr200. The methyl group of Thr200 forms a hydrophobic region (green) with Ala365, Leu366, and the aromatic Phe362, in which the methyl substituent of **13** may be located.

Hydroxy substitution of tryptamine in this position (Figure 6) is detrimental for 5-HT_{1A} receptor affinity,[22] while methyl substitution in the corresponding position of **12** slightly increases affinity (**13**). The methyl group in **19** is more distant from Thr200 than the methyl substituent in **13**. In an atom-to-atom fit of the phenylpiperazine parts of **19** and **13**, the respective methyl carbons are located 1.8 Å apart.

The 1- and 8-positions of molecule **12** in Figure 9, bottom, coinciding with the 3- and 4-positions of **14**, seem to be sterically hindered by the presence of the backbone of helix V, which is colored white. Indeed, a small atom like fluoro does not alter affinity, but the addition of bulky substituents in these positions decreases affinity (Table 3). According to the model, it is expected that very large substituents in the 5-position of **14** will not be tolerated because it is directed toward the space between helices IV and V (which contains side chains of both helices).

In the model, the 6-position of the docked compounds points between the backbones of helices III and IV. Therefore, it is expected that large substituents in this position will not be tolerated. Thus, although it was shown that the -C_2H_5-bridge in **36** has no adverse effect on 5-HT_{1A} receptor affinity, the adverse effect on 5-HT_{1A} receptor affinity of larger, bulkier *ortho* substituents may, besides conformational effects, also be caused by steric hindrance with the receptor. This may play a role in the total loss of 5-HT_{1A} receptor affinity in the *o*-methoxy-substituted compound **34**. It is not very likely that the methoxy group of **34** can be directed toward the piperazine ring because of steric hindrance (see also the first paragraph of Results and Discussion). However, other conformations of the methoxy group have less influence on the arylpiperazine conformation than a chlorine atom like in compound **33**. Therefore, the more than 100-fold decrease in 5-HT_{1A} receptor affinity by replacement of the chlorine atom of **33** into a methoxy group in **34** can not be attributed to effects on the arylpiperazine conformation. Thus it is likely that the methoxy group of compound **34** also causes steric hindrance with the receptor.

Conclusions

We synthesized a series of new N^1-heterobicyclic arylpiperazines with high affinity for the 5-HT_{1A} receptor. The most potent compound, **13**, has an affinity comparable to the selective $5HT_{1A}$ reference compound 8-OH-DPAT (**2**) and the neurotransmitter 5-HT (**1**) itself.

In addition, we were able to rationalize affinities with a computer modeling study, in which we investigated the bioactive conformation and studied the interactions of these ligands in our 5-HT_{1A} receptor model. Our data agree with a preference of the 5-HT_{1A} receptor for a coplanar conformation of the arylpiperazine moiety of compounds **11**, **12**, and **14**. SAR observations favor the relative orientation of the arylpiperazine moiety with respect to other classes with potential 5-HT_{1A} affinity in which the benzene ring of arylpiperazines coincides with the pyrrole ring of 5-HT. Three of the high-affinity arylpiperazine compounds were fitted on 5-HT (**1**) and RU24969 (**61**) in this orientation. The position of these compounds in the receptor model was in accordance with the observed SAR.

Experimental Section

Chemistry. Melting points are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker WP-200 or AM400 instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (J) are in hertz. Elemental analyses were performed at the TNO Laboratory of Organic Chemistry, Utrecht, The Netherlands. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. 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 used were high-grade commercial products. All reactions were performed under a nitrogen atmosphere.

Compounds **6**−**9**, **17**, and **35** are commercially available. Compound **10** was synthesized as described in patent application Brit 850,663.[32] The synthesis of compound **14** is described in ref 12 and of compound **36** in ref 18. Compounds **16** and **18** were obtained by the method described in Scheme 1 using commercially available 7-nitro-(1*H*)-indole and 1-amino-5,6,7,8-tetrahydronaphthalene. The physical data are in agreement with those given in patent application NL 8201708[33] and ref 34.

Compounds **11**, **13**, **15**, **19**, **21**−**24**, and **26**−**29** were synthesized as described in patent application JP 61,152,655.[13]

All compounds, except **31** and **32**, were obtained by the method in Scheme 1; compounds **31** and **32** were obtained by the method in Scheme 3. The physical data of previously reported compounds matched those given in the corresponding references.

5-Nitro-1,4-benzodioxin (37) (Scheme 2, A) (1). To a solution of **48**[35] (2.5 g, 13.8 mmol) and 1,3-dibromo-5,5-dimethyl-2,4-imidazolidinedione (DDH) (7.9 g, 27.6 mmol) in CCl_4 (30 mL) was added a catalytic amount of dibenzoyl peroxide. The mixture was refluxed for 15 h and irradiated (visible light, 150 W), after which the remaining sticky suspension was diluted with ether (25 mL). The resulting solid, *N,N*-dimethylhydantoin, was filtered off. The filtrate was concentrated in vacuo, and the resulting crude oil was purified by flash chromatography (ether–petroleum ether (40–60 °C), 1:3): yield 0.95 g (20%) of an oil, which crystallized on standing; R_f 0.3 (ether–petroleum ether (40–60 °C), 1:3).

(**2**) The obtained 2,3-dibromo-5-nitro-2,3-dihydro-1,4-benzodioxin (0.95 g, 2.8 mmol) and NaI (2.1 g, 14.0 mmol) were dissolved in dry acetone (25 mL) and heated to reflux for 3 h. The solution was concentrated in vacuo and the residue taken up in ether (25 mL). After filtration, the solution was washed with H_2O (10 mL), 20% aqueous Na_2SO_3 (2 × 10 mL), 5% aqueous $NaHCO_3$ (10 mL), and H_2O (10 mL), dried ($MgSO_4$), and concentrated. The obtained brown oil was purified by flash chromatography (ether–petroleum ether (40–60 °C), 1:3): yield 0.44 g (18%, based on 5-nitro-2,3-dihydro-1,4-benzodioxin) of an orange solid; R_f 0.5 (toluene); ^{1}H-NMR ($CDCl_3$) δ 5.98 (d, 1H, Bzd H-2 or H-3, J = 4), 6.04 (d, 1H, Bzd H-2 or H-3, J = 4), 6.84 (dd, 1H, Bzd H-8, J = 2 and 8), 6.90 (t, 1H, Bzd H-7, J = 8), 7.44 (dd, 1H, Bzd H-6, J = 8 and 2).

5-Amino-1,4-benzodioxin (38) (Scheme 1, i). To a suspension of **37** (7.0 g, 39.0 mmol) in EtOH (165 mL), H_2O (165 mL), and 2 N HCl (2.7 mL) was added Fe powder (27.4 g, 0.49 mol). After being stirred at 60°C for 45 min, the suspension was cooled to 20 °C and diluted with CH_2Cl_2. The solid was filtered off over Hyflo. The organic layer of the filtrate was separated, washed with H_2O (30 mL), and dried on $MgSO_4$. After filtration and removal of the solvent, the remaining crude oil was purified by flash chromatography (toluene): yield 3.81 g (66%) of an oil; R_f 0.3 (toluene); ^{1}H-NMR ($CDCl_3$) δ 3.52 (br s, 2H, NH_2), 5.84 (d, 1H, Bzd H-2 or H-3, J = 4), 5.91 (d, 1H, Bzd H-2 or H-3, J = 4), 6.05 (dd, 1H, Bzd H-6, J = 8 and 2), 6.28 (dd, 1H, Bzd H-8, J = 8 and 2), 6.61 (t, 1H, Bzd H-7, J = 8).

1-(1,4-Benzodioxin-5-yl)piperazine, (*E*)-2-Butenedioate (12) (Scheme 1, iii). *p*-TsOH·H_2O (2.64 g, 13.9 mmol) in chlorobenzene (470 mL) was heated in Dean–Stark equipment until 20 mL of chlorobenzene (and water) was distilled off. After the solution was cooled to 20 °C, **38**·HCl (2.86 g, 15.4 mmol) and bis(2-chloroethyl)amine hydrochloride (3.02 g, 16.9 mmol) were added. This mixture was stirred at reflux temperature for 72 h. After this was concentrated in vacuo, toluene (20 mL), H_2O (20 mL), and 2 N NaOH (16 mL) were added and the resulting mixture was stirred for 30 min. The organic layer was separated. The water layer was extracted with toluene (15 mL). The combined organic layers were washed with H_2O (15 mL), dried ($MgSO_4$), and concentrated. The obtained oil was purified by flash chromatography (THF–MeOH–NH_4OH, 89:10:1). The resulting pure product was taken up in absolute EtOH (25 mL), and after addition of 1 equiv of fumaric acid in EtOH, **12** crystallized from the solution: yield 2.09 g (41%); mp 202–203 °C; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 3.0–3.15 (cluster, 8H, CH_2 pip), 6.13 (d, 1H, Bzd H-2 or H-3, J = 4), 6.16 (d, 1H, Bzd H-2 or H-3, J = 4), 6.35 (dd, 1H, Bzd H-6, J = 2 and 8), 6.52 (s, 2H, fumaric acid), 6.55 (dd, 1H, Bzd H-8, J = 2 and 8), 6.78 (t, 1H, Bzd H-7, J = 8), 4–7 (br, H_2O, COOH and NH). Anal. ($C_{12}H_{14}N_2O_2$·$C_4H_4O_4$) C, H, N.

3-Methyl-7-nitro-benzofuran (39) (Scheme 2, D, E) (D). A solution of **55**[36] (21.18 g, 109.7 mmol) in CH_2Cl_2 (150 mL) and MeOH (150 mL) was ozonized and subsequently treated with dimethyl sulfide (10 mL, 136.5 mmol) by the method described for **43** (D): yield 22.66 g (100%) of a crude mixture of *cis*- and *trans*-2-hydroxy-3-methyl-7-nitro-2,3-dihydrobenzofuran, oil; R_f 0.18 (ether–petroleum ether (40–60 °C), 1:3); ^{1}H-NMR (DMSO–$CDCl_3$, 4:1, TFA) *trans* compound δ 1.30 (d, 3H, CH_3, J = 7), 3.24 (m, 1H, Bzf H-3), 5.86 (d, 1H, Bzf H-2, J = 3), 7.02 (t, 1H, Bzf H-5, J = 8), 7.56 (m, 1H, Bzf H-4), 7.91 (m, 1H, Bzf H-6); *cis* compound δ 1.30 (d, 3H, CH_3, J = 7), 3.49 (m, 1H, Bzf H-3), 6.23 (d, 1H, Bzf H-2, J = 6), 7.02 (t, 1H, Bzf H-5, J = 8), 7.52 (m, 1H, Bzf H-4), 7.88 (m, 1H, Bzf H-6).

(**E**) The compound obtained by step D was treated with H_2SO_4 (96%) (15.6 mL) by the method described for **43** (E). The crude product was purified by column chromatography (CH_2Cl_2 and toluene): yield 9.81 g (51%); mp 118.5–120 °C; ^{1}H-NMR ($CDCl_3$) δ 2.31 (d, 3H, CH_3, J = 1), 7.37 (t, 1H, Bzf H-5, J = 8), 7.63 (q, 1H, Bzf H-2, J = 1), 7.85 (dd, 1H, Bzf H-4, J = 8 and 1), 8.15 (dd, 1H, Bzf H-6, J = 8 and 1).

7-Amino-3-methylbenzofuran, Hydrochloride (40) (Scheme 1, ii). A stirred suspension of **39** (3.54 g, 20.0 mmol) and Raney nickel catalyst (50 mg) in MeOH (35 mL) was heated at 50–60 °C. Hydrazine hydrate (3.0 mL, 62.2 mmol) in MeOH (10 mL) was added dropwise, giving an exothermic reaction. The temperature was maintained at 55–60 °C by external cooling. When the addition was complete (0.5 h), the mixture was stirred at reflux for 0.5 h and then cooled to 20 °C. The catalyst was filtered off over Hyflo, and the solvent was evaporated. The residue was dissolved in ether (100 mL), and 1 equiv of HCl in absolute EtOH (20 mL) was added. Product **40** crystallized and was collected by filtration and dried in vacuo: yield 3.37 g (92%); mp 196.5–202 °C.

1-(3-Methyl-7-benzofuranyl)piperazine, Hydrochloride (20) (Scheme 1, iii). Compound **20** was prepared from **40** (3.35 g, 18.3 mmol) and bis(2-chloroethyl)amine hydrochloride (3.26 g, 18.3 mmol) by the method described for **12**. Purification was performed by column chromatography (THF–MeOH–NH_4OH, 85:15:2). The free base (3.30 g) was taken up in EtOAc (100 mL), and after addition of 1 equiv of HCl in absolute EtOH (5 mL), **20** crystallized from the solution: yield 3.43 g (74%); mp 206–209.5 °C; ^{1}H-NMR ($CDCl_3$) δ 2.23 (d, 3H, CH_3, J = 1), 3.6 (m, 8H, CH_2 pip), 6.76 (m, 1H, Bzf H-6), 7.16 (t, 1H, Bzf H-5, J = 8), 7.21 (m, 1H, Bzf H-4), 7.39 (d, 1H, Bzf H-2, J = 1), 9.95 (br, 2H, NH_2^+). Anal. ($C_{13}H_{16}N_2O$·HCl·0.50H_2O) C, H, N.

5-Methoxy-2-nitro-1-(2-propenyloxy)benzene (51) (Scheme 2, B). Compound **51** was prepared from **49**[37] (30.0 g, 0.178 mol), tetrabutylammonium bromide (5.7 g, 0.018 mol), K_2CO_3 (38.0 g, 0.275 mol), and allyl bromide (19.35 g, 0.223 mol) in toluene (300 mL) by the method described for **52**. Reflux time was 48 h: yield 38.16 g (103%) of an oil; R_f 0.26 (toluene); ^{1}H-NMR ($CDCl_3$) δ 3.88 (s, 3H, $PhOCH_3$), 4.66 (dt, 2H, $-OCH_2CH{=}CH_2$, J = 5, 2, and 2), 5.33 (dq, 1H, $C{=}CH_{trans\text{-}H}$, J = 10, 2, 2, and 2), 5.53 (dq, 1H, $C{=}CH_{cis\text{-}H}$, J = 17, 2, 2, and 2), 6.05 (m, 1H, $-CH{=}$), 6.44–6.56 (cluster, 2H, H-4 and H-6), 7.98 (m, 1H, H-3).

3-Methoxy-6-nitro-2-(2-propenyl)phenol (53) (Scheme 2, C). Compound **53** was prepared from **51** (37.50 g, 0.178 mol) by the method described for **54** and was obtained pure and crystalline after column chromatography (ether–petroleum ether (40–60 °C), 1:9): yield 21.5 g (57%); mp 52–53 °C; ^{1}H-NMR ($CDCl_3$) δ 3.46 (dt, 2H, $CH_2CH{=}CH_2$, J = 6, 2, and 2), 3.94 (s, 3H, $PhOCH_3$), 4.94–5.10 (cluster, 2H, H-1 and H-2), 5.92 (m, 1H, H-3), 6.56 (d, 1H, H-4, J = 9), 8.05 (d, 1H, H-5, J = 9), 11.12 (br band, 1H, PhOH).

4-Methoxy-7-nitrobenzofuran (41) (Scheme 2, D, E) (D). A solution of **53** (21.0 g, 100.5 mmol) in CH_2Cl_2 (70 mL) and MeOH (70 mL) was ozonized and subsequently treated with dimethyl sulfide (8.05 mL, 109.6 mmol) by the method described for **43** (D): yield 15.1 g (71%) of crude product; mp 116–118 °C, containing 39% of 2-hydroxy-4-methoxy-7-nitro-2,3-dihydrobenzofuran; ^{1}H-NMR ($CDCl_3$) δ 2.83 (dd, 1H, Bzf H-3_{eq}, J = 16 and 3), 3.11 (dd, 1H, Bzf H-3_{ax}, J = 16 and 7), 3.80 (s, 3H, $PhOCH_3$), 6.20 (m, 1H, Bzf H-2), 6.37 (d, 1H, Bzf H-4, J = 9), 7.44 (d, 1H, CHOH, J = 5), 7.84 (d, 1H, Bzf H-5, J = 9).

(**E**) The compound obtained by step D (12.75 g, 60.4 mmol) was treated with 96% H_2SO_4 (7.5 mL) by the method described for **43** (E). The crude product was purified by column chromatography (CH_2Cl_2): yield 4.0 g (34%) of **41**; mp 168–169 °C; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 3.97 (s, 3H, $PhOCH_3$), 6.98 (d, 1H, Bzf H-5, J = 9), 7.08 (d, 1H, Bzf H-3, J = 2), 8.06 (d, 1H, Bzf H-2, J = 2), 8.22 (d, 1H, Bzf H-6, J = 9).

7-Amino-4-methoxybenzofuran, Hydrochloride (42) (Scheme 1, ii). Compound **42** was prepared from **41** (3.92 g, 20.3 mmol), Raney nickel catalyst (50 mg), and hydrazine hydrate (3.64 mL, 75.5 mmol) in MeOH (60 mL) by the method described for **40**. The crude product was purified by column chromatography (CH_2Cl_2). The free base was taken up in EtOAc (50 mL), and after addition of 1 equiv of HCl in absolute EtOH (5 mL), **42** crystallized from the solution: yield 4.26 g (100%); mp 215–225 °C.

1-(4-Methoxy-7-benzofuranyl)piperazine, Hydrochloride (25) (Scheme 1, iii). Compound **25** was prepared from **42** (3.26 g, 16.3 mmol) and bis(2-chloroethyl)amine hydrochloride (2.92 g, 16.3 mmol) by the method described for **12**. Purification was done by column chromatography (THF–MeOH–NH_4OH, 94.5:5:0.5). The free base (2.37 g) was taken up in EtOAc (50 mL) and converted into its HCl salt by addition of 1 equiv of HCl in absolute EtOH (10 mL). **25** crystallized from the solution after addition of ether (25 mL): yield 2.06 g (47%); mp 198–200 °C; ^{1}H-NMR ($CDCl_3$) δ 3.4–3.7 (cluster, 8H, CH_2 pip), 3.90 (s, 3H, $PhOCH_3$), 6.55 (d, 1H, Bzf H-5, J = 9), 6.72 (d, 1H, Bzf H-6, J = 9), 6.87 (d, 1H, Bzf H-3, J = 2), 7.55 (d, 1H, Bzf H-2, J = 2), 8.5–10 (br, 2H, NH_2^+). Anal. ($C_{13}H_{16}N_2O_2$·HCl) C, H, N.

3-Fluoro-2-nitro-1-(2-propenyloxy)benzene (52) (Scheme 2, B). A mixture of **50**[38] (37.7 g, 0.24 mol), anhydrous finely powdered K_2CO_3 (49.5 g, 0.36 mol), and tetrabutylammonium bromide (7.7 g, 0.024 mol) in toluene (480 mL) was azeotropically dried by heating in a water trap apparatus at 80 °C. Allyl bromide (265 mL, 0.31 mol) was added at 20 °C, and the mixture was refluxed for 1 h. The mixture was washed with H_2O (150 mL), and the water layer was extracted with EtOAc (2 × 150 mL). The combined organic layers were washed with H_2O (2 × 150 mL), 2 N NaOH (2 × 150 mL), and brine (150 mL), respectively. After drying ($MgSO_4$) and concentrating, product **52** was obtained in a nearly pure form: yield 49.22 g of an oil; R_f 0.3 (ether–petroleum ether (40–60 °C), 1:3); ^{1}H-NMR ($CDCl_3$) δ 4.65 (dt, 2H, OCH_2-$CH{=}CH_2$, J = 5, 2, and 2), 5.32 (dq, 1H, $-C{=}CH_{trans\text{-}H}$, J = 10, 2, 2,, and 2), 5.41 (dq, 1H, $-C{=}CH_{cis\text{-}H}$, J = 17, 2, 2, and 2), 5.98 (m, 1H, $-CH{=}$), 6.76–6.9 (m, 2H, Bz H-4 and H-6), 7.37 (td, 1H, Bz H-5, $^3J_{HH}$ = 9 and 9, $^4J_{HF}$ = 6).

3-Fluoro-2-nitro-6-(2-propenyl)phenol (54) (Scheme 2, C). **52** (49.22 g, 0.24 mol) was heated at 160 °C under dry nitrogen for 6.5 h. The oily product was taken up in ether (100 mL) and washed with H_2O (2 × 25 mL). The organic layer was dried ($MgSO_4$) and concentrated. The obtained crude **54** was purified by column chromatography (ether–light petroleum ether, 1:9): yield 17.89 g (38%) of an liquid; n_D^{20} = 1.5583.

6-Fluoro-7-nitrobenzofuran (43) (Scheme 2, D, E) (D). A solution of **54** (17.78 g, 90.2 mmol) in CH_2Cl_2 (200 mL) and MeOH (150 mL) was ozonized (100 L/h, capacity ≈ 4 g of O_3/100 L) at −70 °C. After 1 h the addition was complete, and N_2 was passed through the solution to remove the excess of ozone. Dimethyl sulfide (10 mL, 136.5 mmol) was added, and the temperature was slowly raised to 20 °C. The solution was stirred overnight at 20 °C while N_2 was passed through. The solvent was evaporated, and the residue was taken up in EtOAc (100 mL). The organic layer was washed with brine (6 × 50 mL), dried, and concentrated: yield 19.33 g (100%) of crude 6-fluoro-2-hydroxy-7-nitro-2,3-dihydrobenzofuran, light yellow solid; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 2.82–3.08 (cluster, 2H, Bzf H-3), 6.33 (br, 1H, Bzf H-2), 6.85 (dd, 1H, Bzf H-5, J_{HH} = 9, J_{HF} = 11), 7.44 (dd, 1H, Bzf H-4, J_{HH} = 9, J_{HF} = 5), 7.98 (br band, 1H, OH).

(E) The compound obtained by step D (19.33 g, 90.2 mmol) was dissolved in CH_2Cl_2 (150 mL). H_2SO_4 (96%) (6 mL) was added dropwise at −30 °C. The solution was stirred for 30 min at −20 °C and for 2 h at 20 °C and then poured into ice. The organic layer was separated. The water layer was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layers were washed with 5% aqueous $NaHCO_3$ (2 × 100 mL) and 2 N NaOH (4 × 100 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by flash chromatography (toluene–petroleum ether (40–60 °C), 1:3): yield 7.23 g of **43** (40%, based on **54**), light yellow solid; mp 113–116.5 °C; ^{1}H-NMR ($CDCl_3$) δ 6.90 (d, 1H, Bzf H-3, J = 2), 7.21 (dd, 1H, Bzf H-5, J_{HH} = 9, J_{HF} = 11), 7.79 (dd, 1H, Bzf H-4, J_{HH} = 9, J_{HF} = 5), 7.82 (d, 1H, Bzf H-2, J = 2).

7-Amino-6-fluorobenzofuran, Hydrochloride (44) (Scheme 1, i). Compound **44** was prepared from **43** (1.81 g, 10.0 mmol) and Fe (5.6 g, 100 mmol) by the method described for **38**. Column chromatography was not applied; the crude product was converted into its hydrochloride salt in ether, by the addition of 1 equiv of HCl in absolute EtOH (5 mL), and recrystallized from EtOH: yield 1.48 g (79%); mp 177–179 °C; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 6.87 (d, 1H, Bzf H-3, J = 2), 6.96–7.10 (m, 2H, Bzf H-4 and H-5), 7.30 (br, 3H, NH_3^+), 7.92 (d, 1H, Bzf H-2, J = 2).

1-(6-Fluoro-7-benzofuranyl)piperazine, Dihydrochloride (30) (Scheme 1, iii). Compound **30** was prepared from **44** (2.96 g, 15.8 mmol) and bis(2-chloroethyl)amine hydrochloride (2.82 g, 15.8 mmol) by the method described for **12**. Purification was by column chromatography (CH_2Cl_2–MeOH–NH_4OH, 97.8:2:0.2). The resulting pure product was converted into its dihydrochloride salt in ether, by the addition of 2 equiv of HCl in absolute EtOH (5 mL): yield 2.31 g (50%); mp 157–163 °C; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 3.27 (m, 4H, CH_2 pip), 3.57 (m, 4H, CH_2 pip), 6.89 (d, 1H, Bzf H-3, J = 2), 7.06 (dd, 1H, Bzf H-5, J = 9 and 13 (HF)), 7.30 (dd, 1H, Bzf H-4, J = 9 and 5 (HF)), 7.93 (d, 1H, Bzf H-2, J = 2), 9.5 (br, 2H, NH_2^+). Anal. ($C_{12}H_{13}FN_2O$·2HCl) C, H, N.

1-(7-Benzofuranyl)-4-[[2-(trimethylsilyl)ethoxy]carbonyl]piperazine (57) (Scheme 3, A). To a suspension of **14**[12] (8.29 g, 34.8 mmol) in CH_3CN (50 mL) were added diisopropylethylamine (6.2 mL, 35.6 mmol) and 2-(trimethylsilyl)ethyl *p*-nitrophenyl carbonate (10.0 g, 35.3 mmol) in CH_3CN (15 mL). The resulting clear solution was stirred at 20 °C for 2 h. The solvent was evaporated, and the residue was taken up in EtOAc (100 mL). The organic layer was washed with 1 N NaOH (4 × 50 mL) and H_2O (2 × 50 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by column chromatography (ether–light petroleum ether, 1:9): yield 9.99 g (83%) of an oil; R_f 0.18 (ether–petroleum ether (40–60 °C), 1:3).

1-(6-Nitro-7-benzofuranyl)-4-[[2-(trimethylsilyl)ethoxy]carbonyl]piperazine (58) (Scheme 3, B). To a solution of $NaNO_3$ (2.34 g, 27.5 mmol) in H_2O (20 mL) were added at 0 °C respectively concentrated HCl (21 mL) and **57** (9.0 g, 26.0 mmol) in $CHCl_3$ (55 mL). The mixture was vigorously stirred. A few drops of 5% aqueous $NaNO_2$ were then added to start the nitration. The resulting brown solution was vigorously stirred for 0.5 h at 0 °C and subsequently for 0.5 h at 20 °C. The organic layer was separated. The water layer was extracted with CH_2Cl_2 (50 mL). The combined organic layers were washed with H_2O (2 × 50 mL) and 5% aqueous $NaHCO_3$ (50 mL), dried ($MgSO_4$), and then concentrated. The residue was a mixture of the 4- and 6-nitro isomers. The two isomers were separated by flash chromatography (ether–petroleum ether (40–60 °C), 1:2) to yield 1.99 g (20%) of the 6-nitro compound **58**, waxy solid, R_f 0.29 (ether–petroleum ether (40–60 °C), 1:1), and 3.30 g (33%) of the 4-nitro compound, solid, R_f 0.16 (ether–petroleum ether (40–60 °C), 1:1).

1-(6-Nitro-7-benzofuranyl)piperazine, Hydrochloride (31) (Scheme 3, C). To a solution of **58** (1.99 g, 5.09 mmol) in dry THF (20 mL) was added tetrabutylammonium fluoride (4.92 mL, 1 mol) in dry THF (10 mL). The solution was allowed to stand overnight. The solvent was evaporated and the residue taken up in EtOAc (25 mL). The organic layer was washed with 1 N NaOH (5 × 25 mL) and brine (2 × 25 mL), dried ($MgSO_4$), and concentrated. Conversion into the HCl salt was carried out in EtOAc (25 mL) by the addition of 1 equiv of HCl in absolute ethanol (5 mL). Product **24** slowly crystallized and was filtered off and dried in vacuo: yield 0.59 g (41%); mp 238–243 °C, ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 3.25 (m, 4H, CH_2 pip), 3.54 (m, 4H, CH_2 pip), 7.09 (d, 1H, Bzf H-3, J = 2), 7.51 (d, 1H, Bzf H-4, J = 9), 7.75 (d, 1H, Bzf H-5, J = 9), 8.26 (d, 1H, Bzf H-2, J = 2), 9.5 (br, 2H, NH_2^+). Anal. ($C_{12}H_{13}N_3O_3$·HCl·0.10H_2O) C, H, N.

1-(6-Formyl-7-benzofuranyl)-4-formylpiperazine (59) (Scheme 3, D). $POCl_3$ (4 mL, 42.9 mmol) was added dropwise to DMF (12 mL) at 0–10 °C. Stirring was continued for 0.5 h at 20 °C. The free base of **14**[12] (3.96 g, 19.6 mmol) in DMF (7 mL) was added dropwise at 10–15 °C. The solution was

heated at 90 °C for 1 h. After being cooled to 20 °C, the reaction mixture was poured into ice and the solution was made alkaline with 50% aqueous NaOH. After being extracted with CH_2Cl_2 (4 × 50 mL), the organic layer was washed with H_2O (100 mL), dried ($MgSO_4$), and concentrated. The residue was taken up in EtOAc (100 mL) and washed with brine (5 × 50 mL). Evaporation of the solvent gave a dark oil which was purified by flash chromatography (CH_2Cl_2–MeOH, 99:1): yield 3.35 g (66.2%) of an oil which was a mixture of compounds, containing approximately 90% 1-(4-formyl-7-benzofuranyl)-4-formylpiperazine and 10% **59**; ^{1}H-NMR ($CDCl_3$) δ 3.32 (m, 2H, CH_2 pip), 3.36 (m, 2H, CH_2 pip), 3.54 (m, 4H, CH_2 pip), 6.75 (d, 1H, Bzf H-3, J = 2), 7.33 (d, 1H, Bzf H-4, J = 8), 7.66 (d, 1H, Bzf H-5, J = 8), 7.70 (d, 1H, Bzf H-2, J = 2), 8.07 (s, 1H, NCHO), 10.5 (s, 1H, PhCHO).

1-(6-Cyano-7-benzofuranyl)-4-formylpiperazine (60) (Scheme 3, E). A mixture of the aldehydes (3.31 g, 12.8 mmol), NH_2OH·HCl (1.0 g, 14.4 mmol), and natrium formate (1.65 g, 24.2 mmol) in formic acid (20 mL) was heated at reflux for 1.5 h. After being cooled to 20 °C, the mixture was poured into ice and made alkaline with 50% aqueous NaOH (28 mL). After being extracted with CH_2Cl_2 (3 × 50 mL), the organic layer was washed with H_2O (50 mL), dried, and concentrated. The residue was purified by flash chromatography (CH_2Cl_2–MeOH, 99:1). The two isomers (4- and 6-cyano compound) were separated by flash chromatography (acetone–hexane, 1:1) to yield 1.73 g (53.1%) of the crystalline 6-cyano compound, mp 159–162 °C, and 0.25 g (7.7%) of crystalline **60**: mp 119–126 °C; ^{1}H-NMR ($CDCl_3$) δ 3.5–3.68 (cluster, 6H, CH_2 pip), 3.80 (m, 2H, CH_2 pip), 6.82 (d, 1H, Bzf H-3, J = 2), 7.31 (d, 1H, Bzf H-4, J = 8), 7.41 (d, 1H, Bzf H-5, J = 8), 7.75 (d, 1H, Bzf H-2, J = 2), 8.15 (s, 1H, CHO).

1-(6-Cyano-7-benzofuranyl)piperazine, Hydrochloride (32) (Scheme 3, F). A solution of **60** (0.25 g, 1.0 mmol) in THF (7.5 mL) and 2 N HCl (1.5 mL) was allowed to stand for 10 days. The solvent was evaporated, and the residue was taken up in H_2O (10 mL) and washed with EtOAc (2 × 15 mL). The water layer was made alkaline with 50% aqueous NaOH at 5 °C and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried ($MgSO_4$) and concentrated giving the free base of **32** (0.16 g). Conversion into the HCl salt was carried out in EtOAc (25 mL) by the addition of 1 equiv of HCl in absolute ethanol (2 mL). Product **32** crystallized and was filtered off and dried in vacuo: yield 0.16 g (61%) of a white solid; mp 271–275 °C dec; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 3.32 (m, 4H, CH_2 pip), 3.72 (m, 4H, CH_2 pip), 7.05 (d, 1H, Bzf H-3, J = 2), 7.44 (d, 1H, Bzf H-5, J = 8), 7.50 (d, 1H, Bzf H-4, J = 8), 8.17 (d, 1H, Bzf H-2, J = 2), 9.5 (br, 2H, NH_2^+). Anal. ($C_{13}H_{13}N_3O$·HCl·0.25H_2O) C, H, N.

1-(6-Chloro-2,3-dihydro-1,4-benzodioxin-5-yl)piperazine, p-Toluenesulfonate (33) (Scheme 1, iii). Compound **33** was prepared from **45**[39] (2.20 g, 9.9 mmol), p-TsOH·H_2O (1.88 g, 9.9 mmol), and bis(2-chloroethyl)amine hydrochloride (1.77 g, 9.9 mmol) by the method described for **12**. Purification was performed by column chromatography (CH_2Cl_2–MeOH–NH_4OH, 89:10:1); crystallization from EtOH yielded pure **33**: 1.40 g (33%); mp 224–226 °C; ^{1}H-NMR (DMSO–$CDCl_3$, 4:1) δ 2.31 (s, 3H, CH_3 tosylate), 3.0–3.3 (br, 8H, CH_2 pip), 4.28 (m, 4H, Bzd H-2 and H-3), 6.68 (d, 1H, Bzd H-8, J = 9), 6.86 (d, 1H, Bzd H-7, J = 9), 7.12 (d, 2H, arom H tosylate, J = 8), 7.56 (d, 2H, arom H tosylate, J = 8), 8.7 (br, 2H, NH_2^+). Anal. ($C_{12}H_{15}ClN_2O_2$·$C_7H_8O_3S$) C, H, N.

6-Methoxy-5-nitro-2,3-dihydro-1,4-benzodioxin (46) (Scheme 2, F). NaOMe (2.59 g, 48.0 mmol) was added to **56**[39] (10.0 g, 44.0 mmol) in DMF (100 mL). The solution was stirred for 20 h at 110 °C. After the solution was cooled to 20 °C, H_2O (100 mL) was added, and the solution was acidified with 2 N HCl to pH = 5. The solution was extracted with CH_2Cl_2 (75 mL), and the organic layer was washed with H_2O (4 × 20 mL). The organic layer was concentrated in vacuo, and the obtained product was purified by column chromatography (CH_2Cl_2): yield 5.00 g (54%) of **46** and 6-hydroxy-5-nitro-2,3-dihydro-1,4-benzodioxin (ratio 2:1); ^{1}H-NMR ($CDCl_3$) δ 4.00 (s, 3H, $PhOCH_3$), 4.55 (s, 4H, Bzd H-2 and H-3), 6.86 (d, 1H, Bzd H-7, J = 9), 7.44 (d, 1H, Bzd H-8, J = 9).

5-Amino-6-methoxy-2,3-dihydro-1,4-benzodioxin, Hydrochloride (47) (Scheme 1, i). Compound **47** was prepared from **46** (5.00 g, 23.7 mmol) and Fe (10.5 g, 190.0 mmol) by the method described for **38**. Column chromatography was not applied; the crude product was converted into its hydrochloride salt: yield 3.00 g (60%).

1-(6-Methoxy-2,3-dihydro-1,4-benzodioxin-5-yl)piperazine, (E)-2-Butenedioate (34) (Scheme 1, iii). Compound **34** was prepared from **47** (3.00 g, 13.8 mmol), p-TsOH·H_2O (2.65 g, 13.8 mmol), and bis(2-chloroethyl)amine hydrochloride (2.46 g, 13.8 mmol) by the method described for **12**. Purification was done by column chromatography (CH_2Cl_2–MeOH–NH_4OH, 89:10:1). The resulting pure product was taken up in absolute EtOH (25 mL), and after addition of 1 equiv of fumaric acid in EtOH, **34** crystallized from the solution: yield 2.83 g (56%); mp 193–195 °C; ^{1}H-NMR (D_2O) δ 3.24 (m, 4H, CH_2 pip), 3.47 (m, 4H, CH_2 pip), 3.91 (s, 3H, $PhOCH_3$), 4.29 (m, 4H, Bzd H-2 and H-3), 6.62–6.68 (cluster, 4H, Bzd H-7 and H-8 and fumaric acid). Anal. ($C_{13}H_{18}N_2O_3$·$C_4H_4O_4$) C, H, N.

Modeling. Software and Hardware. Small-ligand building and computation (MAXIMIN, MOPAC) were performed with the SYBYL package, version 6.1 (Tripos Assoc., Inc., St. Louis, MO), running on a Silicon Graphics Iris Indigo Elan 4000 workstation. For MAXIMIN calculations (TRIPOS force field), the Powell method was chosen (default values).

The BIOGRAF package, version 2.2 (Molecular Simulations, Sunnyvale, CA), was used to build the receptor and perform ligand docking and energy minimizations. This was done on a Silicon Graphics Iris Indigo XZ 4000 workstation. The conjugate gradient method was used for minimizations (default values). File transfer between the two packages was via the PDB (Brookhaven Protein Data Bank) format.

Ligand Building and Minimization. Ten crystal structures, containing various *ortho* substituents like F (2×), OCH_3 (5×), CH_2OH, and C=N-R (2×), were derived from the CSD. The $\tau \approx 0°$ conformations varied between −5.5° and −24.8°, and $\tau \approx -120°$ structures varied between −104.9° and −123.7°. References of singly *o*-methoxy-substituted phenylpiperazines were CONJUK, FUYKIT, CAFMUR, COKTAX, and FUCVOO. The initial conformation of the phenylpiperazine moiety was derived from the reference FUCVOO (measured torsion angle $\tau = -12.7°$). All phenylpiperazines were built by modification of this structure with the SKECTCH option in SYBYL. Then the structures were roughly minimized with MAXIMIN2 (TRIPOS force field). During the calculation, the conformation of the phenylpiperazine part had to be constrained because most molecular mechanics packages (including MM2 and the TRIPOS force field) contain no parameters for the N(sp^3)-aromatic system. Therefore they underestimate the tendency for a coplanar conformation of the two rings. As the semiempirical program MOPAC (AM1 Hamiltonian)[40] was shown to be suitable for the calculation of arylpiperazine conformations,[19] we used this method to further optimize the full geometry. For all MOPAC (AM1) calculations the default settings were used. Also, the rotational barrier between the piperazine ring and the aromatic part of the ligands was studied with MOPAC (AM1). For this purpose, the structures of compounds **7** and **12** (Figure 7a), **14** (Figure 7b), and **16** and **17** (Figure 7b) were minimized over all bonds and angles, except the torsion angle τ, which was constrained at values between 0° and 360° (intervals of 10°).

Model Building and Docking. A model of serotonin (1) docked in the 5-HT_{1A} receptor was built according to Kuipers et al.[11] As described in the concerning paper, serotonin (1) was docked into the receptor model on the basis of site-directed mutagenesis data.[41] A schematic view of this is shown in Figure 5. Phenylpiperazine **12** was docked into the receptor model, by fitting it on (docked) serotonin (1). The relative orientation with respect to serotonin (1) was derived by fitting compound **12** on 5-HT (1) in the orientation derived from comparison with the "key" compound RU24969 (**61**). The aromatic centers and a dummy atom in the direction of the nitrogen lone pair (distance N–dummy = 2.8 Å), representing an interacting oxygen atom of the receptor, were used as fitting points. Subsequently, 5-HT (1) was removed from the model, and the complex of compound **12** and the receptor was energy-

minimized using molecular mechanics calculations. For this purpose, an "active site" was created, containing all side chains of residues within a distance of 4 Å from the ligand (see Figure 9, top). These side chains, and the ligand itself, were allowed to optimize their position and conformation; the backbone atoms and all other side chains were kept fixed. The Dreiding force field tends to underestimate the conjugation of the nitrogen lone pair with the aromatic ring system. Therefore, the torsion angle τ was constrained at 0°.

Biochemistry. Receptor Binding Assay. Radioligand binding studies were carried out on rat frontal cortex using [^{3}H]-2-(di-*n*-propylamino)-8-hydroxytetralin (8-OH-DPAT) as radioligand.[42]

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