On the Bioactive Conformation of NAN-190 (1) and MP3022 (2), 5-HT$_{1A}$ Receptor Antagonists†

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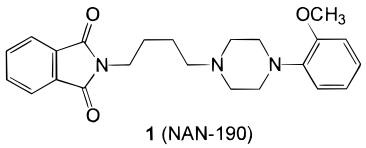
Structural modifications of **1**, a postsynaptic 5-HT$_{1A}$ receptor antagonist, provided its flexible (**8**, **12**) and rigid (**7**, **9**, **11**, **13**) analogues. Compounds **7**, **8**, **9**, and **11** showed high 5-HT$_{1A}$ receptor affinity (K_i = 4−72 nM). They acted as 5-HT$_{1A}$ postsynaptic receptor antagonists, since, like **1**, they inhibited the behavioral syndrome, i.e., flat body posture (FBP) and forepaw treading (FT), in reserpine-pretreated rats as well as the lower lip retraction (LLR) in rats, both induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT), a 5-HT$_{1A}$ receptor agonist. Compound **12**, which demonstrated high 5-HT$_{1A}$ receptor affinity (K_i = 50 nM), revealed properties of a partial 5-HT$_{1A}$ receptor agonist: it induced LLR and, at the same time, inhibited FT in rats. Compound **13** (K_i = 1600 nM) was not tested in a behavioral study. Restriction of the conformational freedom in **2**, a full 5-HT$_{1A}$ receptor antagonist, yielded compound **14** with high 5-HT$_{1A}$ receptor affinity (K_i = 47 nM) and partial agonist properties at postsynaptic 5-HT$_{1A}$ receptors in the above tests in vivo; i.e., it induced LLR and inhibited FBP and FT in rats. New constrained analogues of **1** and **2** (compounds **7** and **14**, respectively) were also synthesized to recognize a bioactive conformation of those 5-HT$_{1A}$ receptor antagonists. On the basis of in vitro and in vivo investigations, binding and functional properties of compound **7** were found to reflect those of **1** at 5-HT$_{1A}$ receptors. On the other hand, compound **14**, a rigid analogue of **2**, showed a different activity in vivo in comparison with the parent compound. PM3 and MM calculations revealed the existence of three low-energy conformers of **7** and six of **14**, all of them belonging to the extended family of conformations. The optimized structures of both analogues had a different angle between aromatic planes of terminal fragments; moreover, the heteroaromatic system of those molecules occupied various space regions. Our present study provides support to the hypothesis that the bioactive conformation of **1**, responsible for its postsynaptic 5-HT$_{1A}$ receptor antagonism, is an extended linear structure represented by **7**.

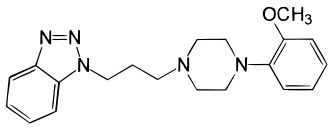
Introduction

The class of arylpiperazines has been of great importance for studies into ligand−serotonin (5-HT) receptor interactions. 1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)-butyl]piperazine (**1**)[1] and 4-[3-(1-benzotriazolyl)propyl]-1-(2-methoxyphenyl)piperazine (**2**)[2] (Chart 1) represent this group of ligands. Both have been shown to bind with high affinity at the 5-HT$_{1A}$ receptor (K_i = 0.6 and 15 nM, respectively). Compound **1** is a mixed agonist/antagonist at central 5-HT$_{1A}$ receptors, since its effect on pre- and postsynaptic 5-HT$_{1A}$ receptors is varied. It behaves like a 5-HT$_{1A}$ receptor agonist at somatodendritic 5-HT$_{1A}$ autoreceptors[3] and like a 5-HT$_{1A}$ receptor antagonist at postsynaptic ones.[3−5] Compound **2** may be regarded as a full potent, 5-HT$_{1A}$ receptor antagonist, as indicated by in vivo,[2] electrophysiological, and biochemical studies.[6,7] Like the majority of 5-HT$_{1A}$ receptor ligands, these compounds are represented by linear molecules which—thanks to a hydrocarbon spacer connecting two terminal fragments—possess high conformational freedom. The fully extended conformations found in crystals which are in an energy minimum as indicated by a conformational analysis are generally believed to be responsible for the biological activity of ligands at the 5-HT$_{1A}$ receptors.[8,9] Several topographic

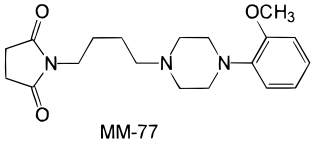
Chart 1



1 (NAN-190)



2 (MP3022)



MM-77

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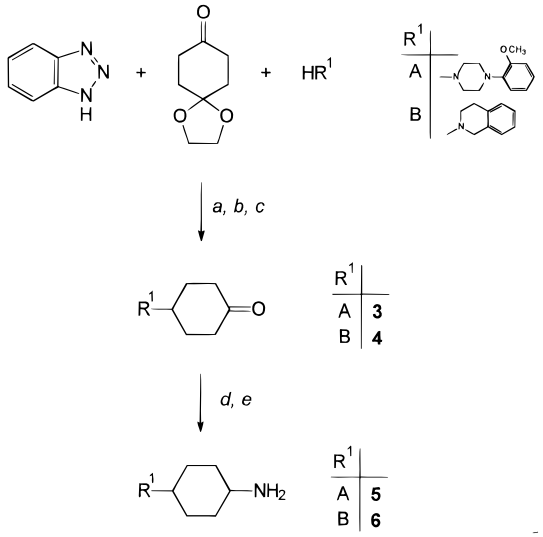
Scheme 1[a]

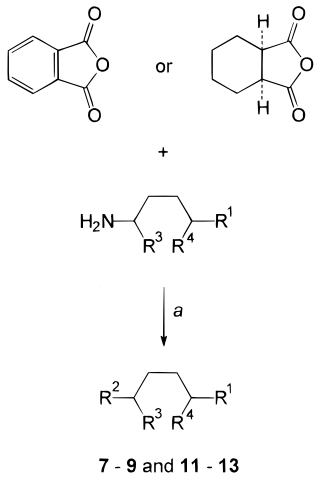


[a] Reagents: (a) benzene, reflux; (b) $NaBH_4$, THF; (c) HCl, H_2O, acetone; (d) $NH_2OH{\cdot}HCl$, Na_2SO_4, MeOH; (e) Na, *n*-BuOH.

models,[9−11] as well as those based on a CoMFA approach,[12,13] have been developed to understand the nature of binding properties of 5-HT_{1A} receptor ligands. In all these models an in vitro activity has been correlated with specific elements of the ligand structures, but since they were based on single chemical classes of ligands, such attempts have permitted only limited general conclusions. The phenyl ring and the basic nitrogen atom, pharmacophores described by Hibert et al.,[14] and at least two additional interactions of, e.g., a lipophilic or $\pi-\pi$ character are needed for the high affinity of 5-HT_{1A} receptor ligands. However, so far no convincing explanation of their functional activity has been offered.

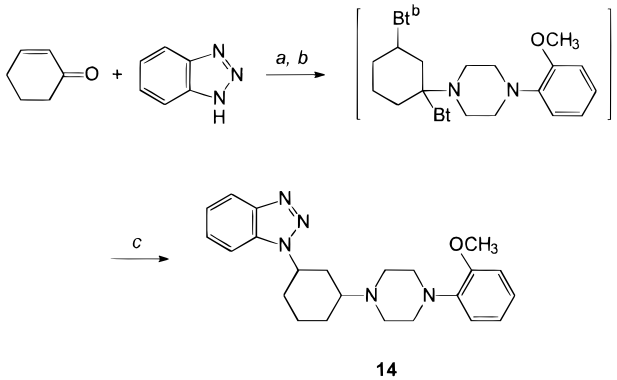
We reported earlier[15] that the removal of a benzene ring from the phthalimide system in **1** provided compound MM-77 (Chart 1) with the same 5-HT_{1A} postsynaptic receptor functional profile. It was also found that the nitrogen atom in 1,2,3,4-tetrahydroquinoline (THIQ) mimicked that in 1-arylpiperazines at 5-HT_{1A} receptors.[16] These findings prompted us to conduct further studies in order to determine how important specific residues of that molecule were to the ligand−receptor complex formation and to its functional activity toward 5-HT_{1A} receptors. For this purpose the effect of saturation of the phthalimide moiety and/or the substitution of a 4-[1-(2-methoxyphenyl)]piperazinyl fragment with a 1,2,3,4-tetrahydroquinolin-2-yl residue in the structure of **1** on the 5-HT_{1A} receptor affinity and in vivo activity was examined and is discussed herein. Evaluation of chemical features that modulate the 5-HT_{1A} receptor in vitro as well as in vivo properties of **2** was discussed previously.[2,17−19] Moreover, on the basis of X-ray studies and a conformational analysis,[20] some efforts were made to determine its bioactive conformation; however, due to the flexibility of the molecule, they did not bring unequivocal results. An insight into ligand−receptor interactions at the molecular level seems to be more attainable when the number of rotatable bonds in a molecule is limited. Replacement of the flexible aliphatic spacer with cyclohexane ring diminishes the possibility of a mutual spatial arrangement of terminal fragments which are responsible for a ligand activity toward, e.g., the 5-HT_{1A} receptor. Thus, in the present paper we would like to report for the first time the synthesis of such conformationally constrained analogues of **1** and **2**. On the basis of their 5-HT_{1A} receptor binding properties and functional activity, we tried to determine the bioactive conformation responsible for the functional behavior of parent compounds **1** and **2**.

Scheme 2[a]



[a] Reagents: (a) xylene, reflux.

Scheme 3[a]



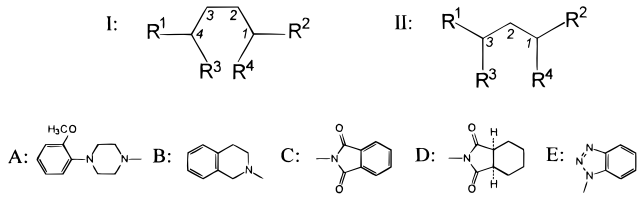
[a] Reagents: (a) Et_2O, room temperature; (b) 4-(2-methoxyphenyl)piperazine, room temperature; (c) $NaBH_4$, 1,4-dioxane, reflux. [b] Bt, benzotriazole.

Chemistry

The structures of the compounds described in this study are shown in Table 1, and the methods of their synthesis are outlined in Schemes 1−3. The key step in the preparation of **7**, **9**, **11**, and **13** was a multistage synthesis of amines **5** and **6** (Scheme 1). Preparation of ketones **3** and **4** involved condensation of benzotriazole, amine [1-(2-methoxyphenyl)piperazine or 1,2,3,4-tetrahydroisoquinoline], and 1,4-cyclohexanedione monoethylene ketal, followed by reduction of the obtained adduct and hydrolysis of the ketal function. Ketones were converted to the corresponding oximes which were directly reduced to yield target amines **5** and **6**.

Analogues of **1** were synthesized from the appropriate anhydrides and amines (Scheme 2) by refluxing in xylene. Benzotriazole readily underwent 1,4-addition to 2-cyclohexen-1-one to afford the respective adduct which

Table 1. Structure and Binding Data on the 5-HT$_{1A}$ Receptors of the Investigated Compounds



compd	structure	R^1	R^2	R^3	R^4	$K_i \pm$ SEM (nM) 5-HT$_{1A}$
1[a]	I	C	A	H	H	0.55 ± 0.14
2[b]	II	E	A	H	H	15 ± 2
7	I	C	A	$-(CH_2)_2-$		8 ± 2
8	I	D	A	H	H	4 ± 2
9	I	D	A	$-(CH_2)_2-$		72 ± 19
10[c]	I	C	B	H	H	355 ± 28
11	I	C	B	$-(CH_2)_2-$		34 ± 4
12	I	D	B	H	H	50 ± 11
13	I	D	B	$-(CH_2)_2-$		1600 ± 270
14	II	E	A	$-(CH_2)_3-$		47 ± 8

[a] K_i value according to Glennon et al. was 0.58 nM, respectively (ref 1). [b] K_i value from ref 2. [c] Compound previous reported as hydrobromide salt (see ref 24).

was directly reacted with 1-(2-methoxyphenyl)piperazine (Scheme 3). Reduction of the obtained intermediate product (not isolated) with sodium borohydride resulted in **14** with a 20% overall yield for a three-step reaction. The structure of the newly synthesized compounds was confirmed by ^{1}H NMR and elemental analysis. In 2D ^{1}H NMR experiments, the observed coupling constants in the cyclohexane ring allowed us to assign a diequatorial conformation for **7**, **9**, **11**, and **14**. For biological assays, the investigated compounds were converted into hydrochloride salts.

Results and Discussion

Radioligand Binding Studies. The affinity of compounds **7**−**14** at 5-HT$_{1A}$ receptors was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT, and the results are summarized in Table 1. The reference compounds **1** and **2** demonstrated very high 5-HT$_{1A}$ (K_i = 0.55 and 15 nM, respectively) and α_1 (K_i = 0.4 and 69 nM, respectively) receptor affinities and pronounced 5-HT$_{2A}$/5-HT$_{1A}$ selectivity. All investigated compounds, except **10** and **13**, showed significant affinity for 5-HT$_{1A}$ receptors (K_i = 4−72 nM), lack of α_1/5-HT$_{1A}$ selectivity, and diverse 5-HT$_{2A}$/5-HT$_{1A}$ selectivity (data not shown). First, we focused our attention on the influence of terminal fragment modifications in **1** on the 5-HT$_{1A}$ receptor affinity. Compound **8** with a hydrophobic, saturated imide fragment demonstrated high affinity for the 5-HT$_{1A}$ receptor (K_i = 4 nM); in contrast, a change of the arylpiperazine residue into THIQ, regardless of the cycloimide moiety (compounds **10** and **12**), caused a decrease in the 5-HT$_{1A}$ receptor affinity (K_i = 355 and 50 nM, respectively). Second, we investigated the 5-HT$_{1A}$ receptor affinity of ring-constrained compounds, i.e., **7**, **9**, **11**, and **13**. Compound **7**, a rigid analogue of **1**, possessed very high 5-HT$_{1A}$ receptor affinity, yet slightly lower than that of the parent compound (K_i = 8 and 0.55 nM, respectively). Additionally replacement of the arylpiperazine moiety with THIQ yielded compound **11**, which also showed significant affinity for 5-HT$_{1A}$ receptors (K_i = 34 nM). Hydrogenation of the phthalimide fragment gave compound **9**, which demonstrated lower 5-HT$_{1A}$ receptor affinity than **1**, but still fairly good (K_i = 72 nM). Modification of both terminal fragments yielded compound **13**, in which a remarkable loss of the 5-HT$_{1A}$ affinity was observed (K_i = 1600 nM). On the other hand, compound **2** and its rigid analogue **14** exhibited comparable 5-HT$_{1A}$ binding properties (K_i = 15 and 47 nM, respectively).

In Vivo Experiments. Compounds **7**−**9**, **11**, **12**, and **14**, which showed 5-HT$_{1A}$ receptor affinity up to K_i = 100 nM, were tested in vivo. To determine postsynaptic 5-HT$_{1A}$ agonistic effects of the tested compounds, their ability to induce a behavioral syndrome, i.e., a flat body posture (FBP) and a forepaw treading (FT) in reserpinized rats and a lower lip retraction (LLR) in rats, was tested. The ability of the investigated compounds to inhibit those symptoms produced by 8-OH-DPAT was regarded as a postsynaptic 5-HT$_{1A}$ antagonistic activity. In those models, compounds **1** and **2** acted as 5-HT$_{1A}$ receptor antagonists.[2−5]

In a behavioral syndrome test, compounds **7**−**9**, **11**, and **12**, analogues of **1**, as well as compound **14**, an analogue of **2**, given alone, like **1** and **2**, did not evoke FBP or FT in reserpine-pretreated rats (Table 2, A). On the other hand, like **1** and **2**, they strongly inhibited FBP and/or FT induced by 8-OH-DPAT, showing a profile of postsynaptic 5-HT$_{1A}$ receptor antagonists of diverse potency. The ED$_{50}$ values for **7** and **8** were similar, and those for **9**, **11**, and **12** were higher than for **1**; whereas for **14** and **2** they were practically the same (Table 2, B). In the LLR test, compounds **7**−**9**, given alone, did not induce this symptom in rats, while **12** and **14** did (Table 3, A). The LLR induced by 8-OH-DPAT was inhibited by **7**−**9**, yet at doses higher than in the case of **1**. Compound **11** did not induce LLR, nor did it change the LLR evoked by 8-OH-DPAT (Table 3, B).

The described results of in vivo experiments showed that, like the parent compound **1**, the majority of its analogues, i.e., **7**, **8**, **9**, and **11**, demonstrated 5-HT$_{1A}$ postsynaptic receptor antagonistic properties, while compound **12** could be classified as a partial agonist of these receptors. Thus it may be concluded that neither hydrogenation of the phthalimide moiety (**8** and **9**) nor replacement of the 4-(2-methoxyphenyl)piperazine residue with THIQ (**11**), nor restriction of conformational freedom (**7**, **9**, **11**) affected the functional profile of the investigated compounds in comparison with **1**. Unexpectedly, compound **12**, a flexible analogue of **1** with a saturated cycloimide fragment and a THIQ residue, behaved like a partial 5-HT$_{1A}$ receptor agonist; thus it may be anticipated that in that case the structure of the ligand−receptor complex is of a different mode. It is noteworthy that, in contrast to the postsynaptic 5-HT$_{1A}$ receptor antagonistic properties of **2**, its rigid analogue **14** shows a profile of a partial agonist of these receptors. In this case, like for **12**, a different type of the bioactive complex can be suggested.

Modeling Studies. As we have mentioned elsewhere, inspection of ligand−receptor interactions leading to specific biological effects could be more profound if inflexible model compounds existed. Having synthesized compounds **7** and **14** whose structures were the same as those of **1** and **2** yet constrained, we encountered a unique opportunity to discuss a problem of the bioactive

Table 2. Induction of Behavioral Syndrome by the Investigated Compounds (A) and Their Effect on the 8-OH-DPAT-Induced Behavioral Syndrome (B) in Reserpine-Pretreated Rats

		mean ± SEM behavioral score			
		A		B	
treatment	dose (mg/kg)	FBP	FT	FBP	FT
vehicle		0.2 ± 0.1	0.2 ± 0.2	13.8 ± 0.8	11.5 ± 0.6
1[a]	1	0.1 ± 0.1	0.1 ± 0.1	12.4 ± 0.5	10.8 ± 0.6
	2	0.2 ± 0.1	0.2 ± 0.2	6.0 ± 2.3[b]	5.7 ± 1.2[b]
	4	0.1 ± 0.1	0.2 ± 0.1	2.3 ± 0.8[b]	6.2 ± 1.1[b]
	8	0.2 ± 0.2	0.1 ± 0.1	2.3 ± 0.8[b]	1.8 ± 0.7[b]
				ED_{50} = 2.0 (1.2−3.2)	ED_{50} = 2.5 (1.7−4.3)
vehicle		0.2 ± 0.1	0.2 ± 0.2	14.8 ± 0.2	14.3 ± 0.3
2[c]	4	0.2 ± 0.1	0.2 ± 0.2	11.5 ± 1.2	5.8 ± 1.7[b]
	8	0.2 ± 0.2	0.2 ± 0.1	8.0 ± 2.0[b]	3.7 ± 1.5[b]
	16	0.1 ± 0.1	0.1 ± 0.1	6.1 ± 0.3[b]	2.6 ± 0.9[b]
				ED_{50} = 10.5 (7.0−15.8)	ED_{50} = 5.2 (3.2−8.6)
vehicle		0.2 ± 0.1	0.2 ± 0.2	13.3 ± 0.5	13.0 ± 0.7
7	2	0.1 ± 0.1	0.1 ± 0.1	9.8 ± 1.1[b]	6.2 ± 0.7[b]
	4	0.2 ± 0.1	0.2 ± 0.1	8.8 ± 0.5[b]	4.2 ± 0.3[b]
	8	0.2 ± 0.2	0.2 ± 0.2	5.6 ± 0.2[b]	1.0 ± 0.4[b]
				ED_{50} = 6.3 (3.6−13.9)	ED_{50} = 2.3 (1.4−3.9)
vehicle		0.2 ± 0.1	0.2 ± 0.2	14.6 ± 0.2	13.2 ± 0.4
8	2	0.1 ± 0.1	0.2 ± 0.1	8.3 ± 0.7[b]	3.8 ± 0.3[b]
	4	0.2 ± 0.2	0.2 ± 0.1	6.7 ± 1.0[b]	3.5 ± 0.6[b]
	8	0.2 ± 0.2	0.2 ± 0.2	5.5 ± 0.6[b]	0.5 ± 0.3[b]
				ED_{50} = 3.2 (1.8−5.8)	ED_{50} = 2.6 (1.5−4.4)
vehicle		0.2 ± 0.1	0.2 ± 0.1	13.6 ± 0.7	13.2 ± 0.2
9	4	0.2 ± 0.2	0.2 ± 0.2	11.6 ± 0.6	9.2 ± 0.5[d]
	8	0.1 ± 0.1	0.2 ± 0.1	9.8 ± 0.4[d]	5.4 ± 0.8[b]
	16	0.1 ± 0.1	0.1 ± 0.1	3.2 ± 1.1[b]	2.0 ± 0.4[b]
				ED_{50} = 11.0 (7.6−16.0)	ED_{50} = 7.3 (5.4−9.8)
vehicle		0.2 ± 0.1	0.2 ± 0.1	13.6 ± 0.7	13.2 ± 0.2
11	8	0.3 ± 0.1	0.1 ± 0.1	11.0 ± 0.4	7.5 ± 1.1[b]
	16	0.5 ± 0.2	0.1 ± 0.2	10.3 ± 0.7	5.7 ± 0.7[b]
	32	0.3 ± 0.2	0.1 ± 0.1	11.8 ± 0.6	4.3 ± 0.5[b]
				ED_{50} > 32	ED_{50} = 13.5 (7.9−23.0)
vehicle		0.2 ± 0.1	0.2 ± 0.2	13.7 ± 0.6	13.5 ± 0.8
12	8	0.1 ± 0.1	0.1 ± 0.2	14.6 ± 0.2	9.8 ± 1.2[d]
	16	2.3 ± 0.2	0.2 ± 0.2	13.3 ± 0.6	7.7 ± 0.4[b]
	32	3.7 ± 0.3	0.2 ± 0.1	11.7 ± 0.4	6.0 ± 0.4[b]
				ED_{50} > 32	ED_{50} = 17.0 (11.3−25.5)
vehicle		0.2 ± 0.1	0.2 ± 0.2	13.3 ± 0.4	12.2 ± 1.3
14	4	0.6 ± 0.2	0.1 ± 0.1	12.5 ± 0.4	9.6 ± 0.6
	8	0.5 ± 0.2	0.3 ± 0.3	7.0 ± 0.9[b]	3.5 ± 0.7[b]
	16	1.0 ± 0.4	0.1 ± 0.1	6.2 ± 1.0[b]	0.8 ± 0.5[b]
				ED_{50} = 11.0 (7.6−16.0)	ED_{50} = 7.0 (4.5−10.9)

[a] Data from ref 4. [b] $p < 0.01$ vs vehicle + 8-OH-DPAT. [c] Data from ref 2. [d] $p < 0.05$ vs vehicle + 8-OH-DPAT.

conformation of the latter compounds. Taking into account the in vitro and in vivo results, it may be concluded that the constrained structure of **7** represents very well the 5-HT_{1A} receptor binding and functional properties of **1**. In contrast, the functional activity of the rigid molecule **14** differed from that of the parent compound **2**. Such a difference in the characteristics of **7** vs **1** and **14** vs **2** might be attributed to a steric arrangement of terminal fragments in these molecules. Therefore, to explain this phenomenon, we analyzed in detail both those structures. Since the ^{1}H NMR spectra showed that compound **7** existed in a diequatorial 1e,4e chair form with respect to the cyclohexane ring, we performed a conformational analysis to recognize the flexibility of the investigated structure. As generally expected, the above conformation should correspond to a global energy minimum of the molecule, which was confirmed by semiempirical (PM3) and MM (MAXIMIN2, Tripos) calculations. The energy of the other conformations resulting from inversion of the cyclohexane ring, i.e., 1a,4e boat (1-axial, 4-equatorial), 1e,4a boat, and 1a,4a chair, was ca. 4−8 kcal/mol above the global energy minimum according to the first method and up to 12 kcal/mol according to the other. Obviously, only a 1e,4e chair conformer exists, yet some rotations may influence the relative positions of terminal fragments of the molecule. Since the rotation around τ_1 in the 1-(2-methoxyphenyl)piperazine system has already been extensively studied,[21,22] we used a global energy minimum conformation (angle τ_1 = 180°) which is generally accepted as a bioactive conformation of this fragment (Figure 1a). Two other rotations around bonds in positions 1 and 4 of the cyclohexane ring were considered. We calculated a rotation energy profile for two separate piperazine−cyclohexane (τ_2) and cyclohexane−phthalimide (τ_3) subunits. The symmetry of a phthalimide moiety made its two preferred orientations ($\tau_3 \cong 0°$ and 180°) equivalent. On the other hand, in the case of the piperazine subunit we observed an energy minimum for two equivalent conformations (τ_2 = 66.3° or −66.3°). The third possible conformation ($\tau_2 \cong 180°$) was by 0.6 kcal/mol less stable than the two others, the rotation barrier being ca. 2.5 kcal/mol. The above results indicate that the rigid molecule **7** can exist in three

Table 3. Induction of LLR by the Investigated Compounds (A) and Their Effect on the 8-OH-DPAT-Induced LLR (B) in Rats

treatment	dose (mg/kg)	mean ± SEM LLR score A	mean ± SEM LLR score B
vehicle		0.1 ± 0.1	2.8 ± 0.2
1	1	0.1 ± 0.1	1.7 ± 0.3[a]
	2	0.1 ± 0.1	1.4 ± 0.2[b]
	4	0.1 ± 0.2	0.6 ± 0.2[b]
			ED_{50} = 1.7 (0.9−3.1)
vehicle		0.1 ± 0.1	2.6 ± 0.2
2[c]	4	0.0 ± 0.0	1.1 ± 0.2[b]
	8	0.1 ± 0.1	0.7 ± 0.3[b]
	16	0.6 ± 0.2	0.9 ± 0.2[b]
			ED_{50} = 6.5 (4.2−10.1)
vehicle		0.1 ± 0.1	2.8 ± 0.2
7	4	1.3 ± 0.1[d]	2.0 ± 0.2
	8	1.0 ± 0.2	1.5 ± 0.4[a]
	16	1.2 ± 0.1[d]	0.6 ± 0.2[b]
			ED_{50} = 7.5 (4.8−11.6)
vehicle		0.1 ± 0.1	2.8 ± 0.2
8	4	0.5 ± 0.1	1.7 ± 0.2[b]
	8	0.5 ± 0.1	1.3 ± 0.2[b]
	16	0.8 ± 0.1	0.7 ± 0.2[b]
			ED_{50} = 7.5 (4.8−11.6)
vehicle		0.1 ± 0.1	2.8 ± 0.2
9	8	0.2 ± 0.1	2.3 ± 0.2
	16	0.3 ± 0.1	1.6 ± 0.2[b]
	32	0.3 ± 0.1	1.1 ± 0.1[b]
			ED_{50} = 20.0 (12.1−33.0)
vehicle		0.1 ± 0.1	2.8 ± 0.2
11	8	0.1 ± 0.1	2.3 ± 0.3
	16	0.2 ± 0.1	2.5 ± 0.2
	32	0.3 ± 0.1	2.3 ± 0.2
			ED_{50} > 32
vehicle		0.1 ± 0.1	NT
12	2	1.0 ± 0.1[d]	NT
	4	1.9 ± 0.2[e]	NT
	8	2.5 ± 0.3[e]	NT
		ED_{50} = 3.9 (2.9−5.3)	
vehicle		0.1 ± 0.1	NT
14	4	0.9 ± 0.2[d]	NT
	8	1.3 ± 0.1[e]	NT
	16	1.6 ± 0.3[e]	NT
		ED_{50} = 8.0 (4.4−14.4)	

[a] $p < 0.05$. [b] $p < 0.01$ vs vehicle + 8-OH-DPAT. [c] Data from ref 2. [d] $p < 0.05$. [e] $p < 0.01$ vs vehicle. NT, not tested.

predominant conformations (**7a** = **7b**, and **7c**) (Table 4) which differ in the angle between the aromatic planes of terminal fragments ($\phi \cong 67°$ or 180°). Conformations of the flexible molecule of **1**, derived from favorable conformers of **7**, were found to be only 1.4 kcal/mol above the global energy minimum which corresponds to its fully extended structure. They were also similar to the crystal structure of **1**[23] (Table 4).

Like in the case of compound **7**, a ^{1}H NMR spectrum analysis of structure **14** indicated its existence in the 1e,3e chair conformation. A conformational analysis was based on the same procedure as previously ($\tau_1 = 180°$), and results for the (1*R*,3*S*)-enantiomer are given in Table 4. Rotation of the benzotriazole−cyclohexane bond (Figure 1b) gave two energy minima at $\tau_3 \cong 0°$ and 180°, which differed by 0.6 kcal/mol in favor of the former. Regarding the three possible values of angle $\tau_2 \cong 60°$, −60°, and 180°, six dominant conformations of **14** (Table 4: **14a**−**14f**) within an energy range of 1.4 kcal/mol should be considered. Those conformers formed three pairs with respect to the angle between the aromatic planes of terminal fragments ($\phi \cong 120°$, 64°, and 92°, respectively). Although each pair was characterized by

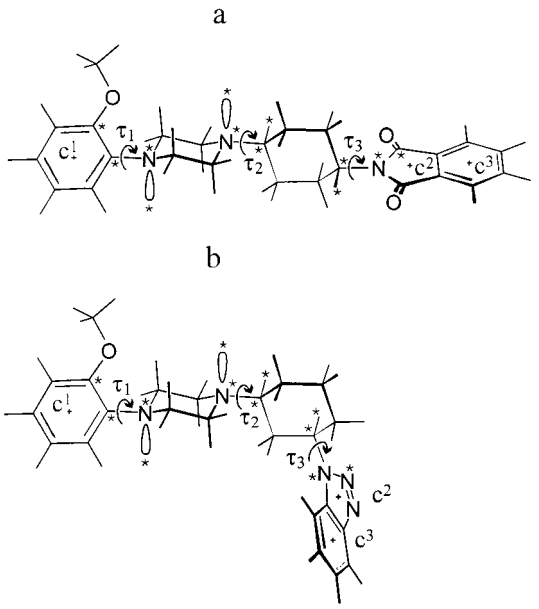


Figure 1. Low-energy conformers of (a) **7** and (b) **14**. Torsional angles τ_1, τ_2, and τ_3 and centers of aromatic rings c^1, c^2, and c^3 are shown.

Table 4. Relative Energy Difference (ΔE; PM3) and Geometry Parameters of Low-Energy Conformers of **7** and **14** and Geometry Parameters from Crystal Structures of **1**[23] and **2**[20]

no.	torsion angle (deg) τ_2	torsion angle (deg) τ_3	distance (Å) c^1-c^2	distance (Å) N4−c^2	angle (deg) c^1−N4−c^2	plane angle ϕ (deg)	ΔE (kcal/mol)
7a	−66.3	−176.5	12.52	6.98	162.2	67.74	0.94
7b	66.3	179.3	12.53	7.00	162.3	67.42	0.89
7c	180.0	−179.9	12.67	6.99	178.4	179.78	0.0
14a	179.2	2.0	11.14	6.01	144.5	121.25	0.82
14b	−66.3	3.4	10.78	6.08	132.9	62.59	0.02
14c	66.9	3.8	11.11	5.81	144.7	93.04	0.00
14d	179.5	177.9	11.09	5.97	144.0	117.75	1.47
14e	−66.3	178.9	10.69	6.02	132.1	66.51	0.44
14f	66.4	178.2	11.03	5.92	143.8	91.79	0.33
1	63.7	−124.5	11.04	5.87	146.5	67.82	
2	179.8	−161.6	12.88	7.33	165.3	2.77	

the same angle ϕ, its components were not equivalent due to an unsymmetrically substituted benzotriazole moiety which resulted in, e.g., different electrostatic potential distribution around the molecule. The six respective conformations of **2**, optimized by an MAXIMIN2 method, were energetically less stable (between 0.86 and 1.85 kcal/mol) than the conformer of the extended family in a global energy minimum, and one of these conformers (derived from **14e**) was very close to the crystal structure of **2**[20] (Table 4).

The conformational analysis has shown that predominant conformers of **7** and **14** belong to an extended family of conformations. A closer insight into these structures reveals that **7** is a linear molecule, whereas compound **14** has an extended yet bent structure, as indicated by the c^1−N4−c^2 angle (Table 4). Consequently, compound **7** appears to be a good model of the fully extended conformation of **1**, and since **7**, like **1**, displayed postsynaptic 5-HT_{1A} antagonistic activity in the tests used, it may be anticipated that the bioactive conformation of **1**, responsible for its postsynaptic antagonism, is represented by the extended linear conformer **7**.

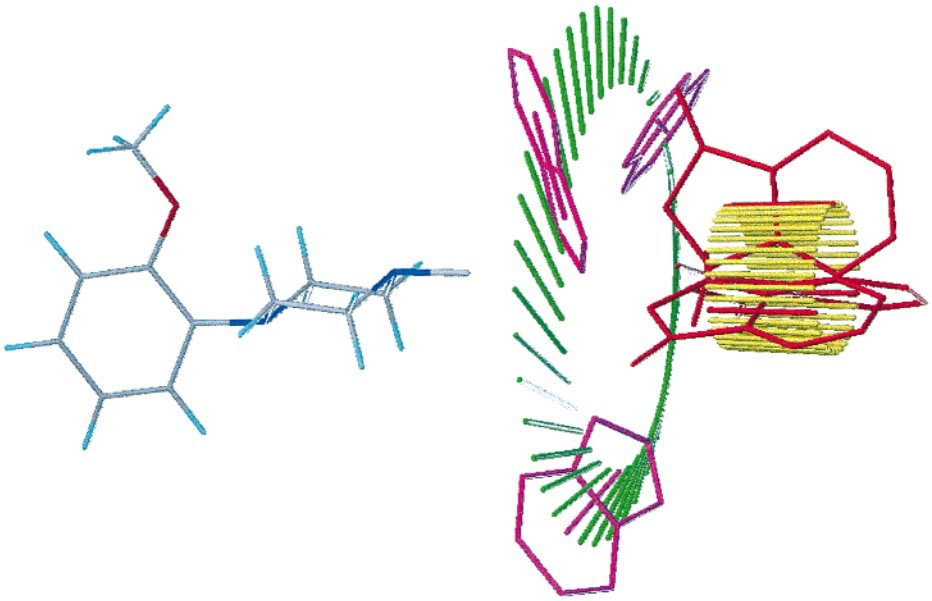


Figure 2. Superimposition of low-energy conformers of **7a**−**c** and **14a**−**c**. For simplification cyclohexane rings and hydrogen atoms in the rotating part of the structure have been omitted. Space explored by phthalimide and benzotriazole moieties are represented by positions of c^2−c^3 segments. Color codes: phthalimide, red; benzotriazole, magenta; c^2−c^3 in phthalimide, yellow; c^2−c^3 in benzotriazole, green.

A brief inspection of structures **14** and **2** suggests that both these compounds possess the same spatial arrangements of pharmacophores. However, among the conformers of the flexible compound **2**, one can find such a conformation that fits fairly well the fully extended conformation of **1** or **7**; hence phthalimide or benzotriazole moieties in **1** and **2**, respectively, may interact with the same 5-HT_{1A} receptor site, which results in the same 5-HT_{1A} receptor functional activity. In contrast, the respective fragments of **14** and **7** do not overlap. It is very likely that they interact with different receptor sites, which may be one of the reasons for their distinct functional activity. To visualize differences in the spatial arrangement of **7** and **14**, we superimposed their optimized structures using a common arylpiperazine fragment (Figure 2). Then, the remaining fragments of the molecules were allowed to rotate around τ_2. For simplification, we have presented the condensed five- and six-membered rings as segments connecting their centers. As can be seen, the cycloimide residue of **7** may occupy a very limited space around the c^1−N4 axis, while the benzotriazole moiety in **14** may explore a significantly larger region and, which is even more noteworthy, in a different direction. Although the region penetrated by the benzotriazole residue for both enantiomers of **14** is essentially the same, their configuration may also play a role at the receptor and, alternatively, influence the functional activity of the compound. However, it may be suggested that the bioactive conformation of **2**, responsible for its postsynaptic antagonism at 5-HT_{1A} receptors, is also represented by **7**. Nonetheless, further detailed studies are necessary to solve unequivocally this problem.

Conclusions

Summing up, structure modifications of **1**, a postsynaptic 5-HT_{1A} receptor antagonist, provided its flexible (**8**) and rigid (**7**, **9**, **11**) analogues which demonstrated high affinity for 5-HT_{1A} receptors and postsynaptic 5-HT_{1A} antagonistic activity. Restriction of the conformational freedom of **2** yielded **14** which revealed high affinity and partial agonist properties at those receptors. Conformational investigations of the flexible **1** and **2**, along with their newly synthesized constrained analogues **7** and **14**, allowed us to identify a bioactive conformation of **1**. We showed that the extended linear structure, represented by **7**, was responsible for the postsynaptic 5-HT_{1A} antagonism of the parent compound.

Experimental Section

Chemistry. Melting points were determined on a Boetius apparatus and are uncorrected. ^{1}H NMR spectra were taken with a Bruker AMX 500 (500 MHz) or Varian EM-360 L (60 MHz) spectrometer in $CDCl_3$ solutions with TMS as an internal standard. 2D-NMR (H−H) COSY technique was used to support interpretation of 1D spectra. The spectral data of amines refer to their free bases. Chemical shifts are expressed in δ (ppm) and the coupling constants J in hertz (Hz). All compounds were routinely checked by TLC using Merck Kieselgel 60-F_{254} sheets (detection at 254 nm). Column chromatography separations were carried out on Merck Kieselgel 60 or aluminum oxide 90, neutral (70−230 mesh). Elemental analyses were performed in the Institute of Organic Chemistry, Polish Academy of Sciences (Warsaw, Poland), and were within ±0.4% of the theoretical values. The syntheses of 2-(4-aminobutyl)-1,2,3,4-tetrahydroisoquinoline[24] and 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine[25] have been previously reported.

4-[4-(2-Methoxyphenyl)-1-piperazinyl]cyclohexanone (3). Equimolar amounts (10 mmol) of 1-(2-methoxyphenyl)piperazine, benzotriazole, and 1,4-cyclohexanedione monoethylene ketal were heated under reflux in benzene (100 mL) for 2 h with azeotropic removal of water using a Dean−Stark trap. The reaction mixture was cooled, washed with 15% Na_2SO_4 solution, and dried (K_2CO_3) and the solvent was evaporated under reduced pressure. The residue solidified and without further purification was suspended in THF (50 mL). To this suspension was added portionwise $NaBH_4$ (0.76 g, 20 mmol) and the mixture was stirred under reflux for 3 h. The solvent was evaporated and the residue was treated with ice−water (100 mL) and extracted with benzene. The organic layer

was washed with water and dried over $MgSO_4$. After evaporation of the solvent the resulting oily ketal was dissolved in 100 mL of a 1:1 solution of acetone and 10% HCl and the mixture was left at room temperature overnight. The acetone was removed under reduced pressure and the remaining aqueous solution was made alkaline with concentrated ammonium hydroxide, extracted with $CHCl_3$, and dried ($MgSO_4$). The extract was evaporated and the oily residue was treated with hexane to afford the desired ketone **3** as colorless crystals (1.5 g, 52%): mp 98−100 °C; ^{1}H NMR (60 MHz) δ 7.0 (s, 4H), 3.9 (s, 3H), 3.3−3.0 (m, 4H), 3.0−2.6 (m, 5H), 2.6−2.2 (m, 4H), 2.2−1.5 (m, 4H).

4-[2-(1,2,3,4-Tetrahydroisoquinolinyl)]cyclohexanone (4). The title compound was prepared using 1,2,3,4-tetrahydroisoquinoline according to the procedure described for the preparation of **3**. After evaporation of the final extract 0.6 g (26%) of a colorless oil was obtained: ^{1}H NMR (60 MHz) δ 7.1 (s, 4H), 3.8 (s, 2H), 3.1−2.6 (m, 1H), 2.8 (s, 4H), 2.6−1.6 (m, 8H).

4-[4-(2-Methoxyphenyl)-1-piperazinyl]cyclohexylamine (5). Hydroxylamine hydrochloride (0.43 g, 6.25 mmol) was slurred in MeOH (10 mL), cooled to 0 °C, and treated with Na_2CO_3 (0.33 g, 3.12 mmol). The mixture was stirred for 5 min and a solution of ketone **3** (1.44 g, 5 mmol) in 20 mL of MeOH was added. The reaction mixture was allowed to come to room temperature, after stirring for 4 h water (30 mL) was added, and stirring was continued for 30 min. Oxime of ketone **3** was obtained as colorless crystals (1.5 g, 100%): mp 161−162 °C. The resulting crude oxime was reduced in boiling *n*-BuOH (50 mL) with sodium (1.6 g, 70 mmol). The solvent was removed under reduced pressure and the residue was treated with water (100 mL) and extracted with $CHCl_3$ (3 × 30 mL). Combined extracts were washed with water and dried (K_2CO_3). After evaporation of the solvent the product was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 9/1) to afford the desired amine (1.0 g, 69%) as a pale yellow oil, which was immediately used in the next step of synthesis: ^{1}H NMR (60 MHz) δ 7.0 (s, 4H), 3.9 (s, 3H), 3.3−3.0 (m, 4H), 2.9−2.6 (m, 4H), 2.5−0.8 (cluster, 10H), 2.0 (s, 2H).

4-[2-(1,2,3,4-Tetrahydroisoquinolinyl)]cyclohexylamine (6). Starting with ketone **4** (0.69 g, 3 mmol) and following the method described for the preparation of amine **5**, the appropriate oxime was obtained (0.70 g, 95%) as colorless crystals: mp 109−111 °C. Reduction of the crude oxime (0. 64 g, 2.6 mmol) gave the amine **6**, which was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 4/1). Compound **6** was obtained as a pale yellow oil (0.48 g, 80%): ^{1}H NMR (60 MHz) δ 7.1 (s, 4H), 3.8 (s, 2H), 2.8 (s, 4H), 2.8−0.8 (cluster, 12H).

General Method for the Preparation of Compounds 7−9 and 11−13. Equimolar amounts (1 mmol) of phthalimide or *cis*-1,2-cyclohexanedicarboxylic anhydride and 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine or 2-(4-aminobutyl)-1,2,3,4-tetrahydroisoquinoline or amines **5** and **6** were refluxed in xylene (20 mL) for 3 h. After the mixture was allowed to cool to room temperature, xylene was evaporated under reduced pressure and the products were isolated on a column with silica gel using eluents as follows: **7**, $CHCl_3$/MeOH = 19/1; **8** and **11**, $CHCl_3$; **9**, **12**, and **13**, $CHCl_3$/MeOH = 49/1. Free bases were converted into the hydrochloride salts in $CHCl_3$ or acetone solution by treatment with excess of Et_2O saturated with gaseous HCl.

***trans*-1-(2-Methoxyphenyl)-4-[4-(2-phthalimido)cyclohexyl]piperazine (7).** This compound was prepared by the general procedure from phthalimide anhydride and amine **5** in 62% yield as colorless crystals: mp 210−212 °C (benzene); ^{1}H NMR (500 MHz) δ 7.85−7.79 (m, 2H, phthalimide H-4 and H-7), 7.72−7.68 (m, 2H, phthalimide H-5 and H-6), 7.03−6.90 (m, 3H, aromatic H) 6.87 (dd, J = 8.0, 2.0, 1H, aromatic H), 4.12 (dt, J = 12.4, 4.0, 1H, cyclohexane axial H-4), 3.88 (s, 3H, OCH_3), 3.12 (app br s, 4H, piperazine $2CH_2$), 2.82 (app br t, 4H, piperazine $2CH_2$), 2.54 (dt, J = 11.7, 3.3, 1H, cyclohexane axial H-1), 2.34 (dq, J = 12.9, 3.2, 2H, cyclohexane axial H-3 and H-3′), 2.08 (app br d, 2H, cyclohexane equatorial H-2 and H-2′), 1.84 (app br d, 2H, cyclohexane equatorial H-3 and H-3′), 1.47 (dq, J = 12.8, 3.2, 2H, cyclohexane axial H-2 and H-2′). **7·2HCl**: mp 256−258 °C (MeOH/acetone). Anal. ($C_{25}H_{29}N_3O_3$·2HCl) C, H, N.

4-{4-[2-(*cis*-1,2-Cyclohexanedicarboxyimido)]butyl}-1-(2-methoxyphenyl)piperazine (8). This compound was prepared by the general procedure from *cis*-1,2-cyclohexanedicarboxylic anhydride and 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine in 86% yield as a pale yellow oil: ^{1}H NMR (60 MHz) δ 7.0 (s, 4H), 3.85 (s, 3H), 3.8−3.3 (m, 2H), 3.3−3.0 (m, 4H), 3.0−2.2 (cluster, 8H), 2.1−1.1 (cluster, 12H). **8·2HCl**: mp 178−180 °C (EtOH). Anal. ($C_{23}H_{33}N_3O_3$·2HCl) C, H, N.

***trans*-4-{4-[2-(*cis*-1,2-Cyclohexanedicarboxyimido)]cyclohexyl}-1-(2-methoxyphenyl)piperazine (9).** This compound was prepared by the general procedure from *cis*-1,2-cyclohexanedicarboxylic anhydride and amine **5** in 43% yield as colorless crystals: mp 180−181 °C (EtOH); ^{1}H NMR (60 MHz) δ 7.0 (s, 4H), 3.9 (s, 3H), 4.3−3.6 (m, 1H), 3.3−3.0 (m, 4H) 3.0−1.1 (cluster, 23H). **9·2HCl**: mp 249−251 °C (MeOH/acetone). Anal. ($C_{25}H_{35}N_3O_3$·2HCl) C, H, N.

***trans*-2-[4-(2-Phthalimido)cyclohexyl]-1,2,3,4-tetrahydroisoquinoline (11).** This compound was prepared by the general procedure from phthalimide anhydride and amine **6** in 65% yield as colorless crystals: mp 188−189 °C ($CHCl_3$); ^{1}H NMR (500 MHz) δ 7.85−7.77 (m, 2H, phthalimide H-4 and H-7), 7.74−7.66 (m, 2H, phthalimide H-5 and H-6), 7.16−7.07 (m, 3H, THIQ), 7.06−7.00 (m, 1H, THIQ), 4.15 (dt, J = 12.4, 4.0, 1H, cyclohexane axial H-4), 3.82 (s, 2H, THIQ H's-1), 2.92−2.82 (m, 4H, THIQ H's-3 and H's-4), 2.69 (dt, J = 11.7, 3.3, 1H, cyclohexane axial H-1), 2.36 (dq, J = 12.9, 3.3, 2H, cyclohexane axial H-3 and H-3′), 2.11 (app br d, 2H, cyclohexane equatorial H-2 and H-2′), 1.85 (app br d, 2H, cyclohexane equatorial H-3 and H-3′), 1.54 (dq, J = 12.8, 3.1, 2H, cyclohexane axial H-2 and H-2′). **11·HCl·0.4H$_2$O**: mp 230 °C dec (MeOH/acetone). Anal. ($C_{23}H_{24}N_2O_2$·HCl·0.4H_2O) C, H, N.

2-{4-[2-(*cis*-1,2-Cyclohexanedicarboxyimido)]butyl}-1,2,3,4-tetrahydroisoquinoline (12). This compound was prepared by the general procedure from *cis*-1,2-cyclohexanedicarboxylic anhydride and 2-(4-aminobutyl)-1,2,3,4-tetrahydroisoquinoline in 89% yield as a pale yellow oil: ^{1}H NMR (60 MHz) δ 7.1 (s, 4H), 3.6 (s, 2H), 3.7−3.3 (m, 2H), 3.1−2.3 (cluster, 8H), 2.0−1.2 (cluster, 12H). **12·HCl·0.5H$_2$O**: mp 178−180 °C (EtOH/acetone). Anal. ($C_{21}H_{28}N_2O_2$·HCl·0.5H_2O) C, H, N.

2-{4-[2-(*cis*-1,2-Cyclohexanedicarboxyimido)]cyclohexyl}-1,2,3,4-tetrahydroisoquinoline (13). This compound was prepared by the general procedure from *cis*-1,2-cyclohexanedicarboxylic anhydride and amine **6** in 50% yield as colorless crystals: mp 159−161 °C (EtOH/hexane); ^{1}H NMR (60 MHz) δ 7.0 (s, 4H), 4.3−3.8 (m, 1H), 3.85 (s, 2H), 3.0−1.2 (cluster, 19H), 2.9 (s, 4H). **13·HCl**: mp 235 °C dec (MeOH/acetone). Anal. ($C_{23}H_{30}N_2O_2$·HCl) C, H, N.

2-[4-(2-Phthalimido)butyl]-1,2,3,4-tetrahydroisoquinoline (10). This compound was obtained according to Mokrosz et al.[24] **10·HCl**: mp 244−246 °C (acetone). Anal. ($C_{21}H_{22}N_2O_2$·HCl) C, H, N.

***cis*-4-[3-(1-Benzotriazolyl)cyclohexyl]-1-(2-methoxyphenyl)piperazine (14).** A mixture of benzotriazole (1.19 g, 10 mmol) and 2-cyclohexen-1-one (0.48 g, 5 mmol) in Et_2O (30 mL) was stirred at room temperature for 6 h and left overnight. The reaction mixture was cooled in an ice−water bath, a solution of 1-(2-methoxyphenyl)piperazine (1.06 g, 5.5 mmol) in Et_2O (10 mL) was added, and the stirring was continued for 20 h. Afterward the intermediate product was filtered off, washed with Et_2O, and suspended in dioxane (40 mL). This suspension was treated with $NaBH_4$ (0.1 g, 2.5 mmol) on stirring, refluxed for 4 h, and left overnight at room temperature. The reaction mixture was poured into 10% NaOH (40 mL) and extracted with Et_2O (3 × 30 mL). The combined organic layers were washed with water and dried ($MgSO_4$). Then the solvent was evaporated and the residue was purified by column chromatography (Al_2O_3 neutral, AcOEt/hexane = 1/2) to afford **14** (0.31 g, 16%) as a pale yellow oil: ^{1}H NMR (500 MHz) δ 8.07 (d, J = 8.4, 1H, benzotriazole H-4), 7.59 (d,

J = 8.4, 1H, benzotriazole H-7), 7.48 (t, J = 7.4, 1H, benzotriazole), 7.37 (t, J = 7.6, 1H, benzotriazole), 7.02−6.99 (m, 1H, aromatic H), 6.95−6.90 (m, 2H, aromatic H), 6.86 (d, J = 7.9, 1H, aromatic H), 4.76 (dddd, J = 12.0, 11.9, 4.0, 3.8, 1H, cyclohexane H-3), 3.86 (s, 3H, OCH_3), 3.14 (app br s, 4H, $2CH_2$), 2.89 (app br s, 4H, $2CH_2$), 2.76 (app br t, $J \cong$ 11.3, 1H, cyclohexane H-1), 2.50 (app br d, 1H, cyclohexane H), 2.21 (br s, 1H, cyclohexane H), 2.18−2.11 (m, 4H, cyclohexane H), 1.61−1.46 (m, 2H, cyclohexane H). **14·2HCl**: mp 186−188 °C (MeOH/acetone). Anal. ($C_{23}H_{29}N_5O$·2HCl) C, H, N.

Molecular Modeling. All of the compounds investigated in molecular modeling experiments were first built with the sketch option in the Sybyl package, version 6.4 (Tripos Associates, Inc., St. Louis, MO). Geometry optimizations were performed using the semiempirical PM3 (MOPAC) method as well as molecular mechanics MAXIMIN2. The rotation barriers between separate piperazine−cyclohexane (τ_2) and cyclohexane−phthalimide (τ_3) subunits were studied with PM3 method. The fragments were minimized over all bonds and angles except the respective torsion angle, which was constrained at values between 0° and 360° with a 10° increment. Global energy minima of **1** and **2** were found by RANDOMSEARCH procedure (energy cutoff: 5 kcal/mol) in which only internal C−C bonds of the spacer were allowed to rotate.

Radioligand Binding Studies. The affinity of the investigated compounds for 5-HT_{1A} receptors was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT (220 Ci/mmol; Amersham), and experiments were carried out in the hippocampus of rat brain, according to published procedures.[26] K_i values were determined from at least three competition binding experiments in which 10−14 drug concentrations, run in triplicate, were used. The Cheng and Prusoff[27] equation was used for K_i calculations.

In Vivo Studies. The experiments were carried out on male Wistar rats (260−300 g). The animals were kept at an ambient temperature of 20 ± 1 °C throughout the experiment and had free access to food (standard laboratory pellets, LSM) and tap water. All experiments were conducted in the light phase on a natural light−dark cycle (from May to August), between 9 a.m. and 2 p.m. 8-OH-DPAT (Research Biochemical, Inc.) and reserpine (Ciba; ampules) were dissolved in saline. The investigated salts of tested compounds were used in the form of freshly prepared suspensions in 1% Tween 80. 8-OH-DPAT and reserpine were injected subcutaneously (sc) and tested compounds intraperitoneally (ip) in a volume of 2 mL/kg. Each group consisted of 6 animals. The obtained data were analyzed by the Newman−Keuls test.

Behavioral Syndrome in Reserpinized Rats. The rats were individually placed in cages (30 × 25 × 25 cm) 5 min before injection of tested compounds or 8-OH-DPAT. Observation sessions, lasting 45 s each, began 3 min after drug administration and were repeated every 3 min. Reciprocal FT and FBP were scored using a ranked intensity scale where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. The maximum score, summed up over 5 observation periods, amounted to 15 for each symptom/animal.[28] The effect of tested compounds on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg) was tested in a separate experiment. The investigated compounds were administered 60 min before 8-OH-DPAT, and the animals were scored of 3, 6, 9, 12, and 15 min after 8-OH-DPAT treatment. Reserpine (1 mg/kg) was administered 18 h before the test.

Lower Lip Retraction in Rats. LLR was assessed according to the method described by Berendsen et al.[29] The rats were individually placed in cages (30 × 25 × 25 cm) and were scored three times (at 15, 30, and 45 min after tested compound or 8-OH-DPAT administration) as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The maximum score, summed, amounted to 3 for each rat. The effect of investigated compounds on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested in a separate experiment. The investigated compounds were administered 45 min before 8-OH-DPAT, and the animals were scored at 15, 30, and 45 min after 8-OH-DPAT administration.

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