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1-Aryl-4-(4-succinimidobutyl)piperazines and their conformationally constrained analogues: synthesis, binding to serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_7), α_1-adrenergic, and dopaminergic D_2 receptors, and in vivo 5-HT_{1A} functional characteristics

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Abstract—Starting with the structure of potent 5-HT_{1A} ligands, that is, MM77 [1-(2-methoxyphenyl)-4-(4-succinimidobutyl)piperazine, **4**] and its constrained version **5** (MP349), previously obtained in our laboratory, a series of their direct analogues with differently substituted aromatic ring (R = H, *m*-Cl, *m*-CF_3, *m*-OCH_3, *p*-OCH_3) were synthesized. The flexible and the corresponding 1e,4e-disubstituted cyclohexane derivatives were designed in order to investigate the influence of rigidification on 5-HT_{1A} affinity, selectivity for 5-HT_{2A}, 5-HT_7, D_1, and D_2 binding sites and functional profile at pre- and postsynaptic 5-HT_{1A} receptors. The new compounds **19**–**25** were found to be highly active 5-HT_{1A} receptor ligands (K_i = 4–44 nM) whereas their affinity for other receptors was: either significantly decreased after rigidification (5-HT_7), or controlled by substituents in the aromatic ring (α_1), or influenced by both those structural modifications (5-HT_{2A}), or very low (D_2, K_i = 5.3–31 μM). Since a distinct disfavor towards rigid compounds was observed for 5-HT_7 receptors only, it seems that the bioactive conformation of chain derivatives at those sites should differ from the extended one.

Several in vivo models were used to asses functional activity of **19**–**25** at pre- (hypothermia in mice) and postsynaptic 5-HT_{1A} receptors (lower lip retraction in rats and serotonin syndrome in reserpinized rats). Unlike the parent antagonists **4** and **5**, all the new derivatives tested were classified as partial agonists with different potency, however, similar effects were observed within pairs (flexible and rigid) of the analogues. The obtained results indicated that substitution in the aromatic ring, but not spacer rigidification, controls the 5-HT_{1A} functional activity of the investigated compounds. Moreover, an *o*-methoxy substituent in the structure of **5** seems to be necessary for its full antagonistic properties. Of all the new compounds studied, *trans*-4-(4-succinimidocyclohexyl)-1-(3-trifluoromethylphenyl)piperazine **24** was the most potent 5-HT_{1A} receptor ligand in vitro (K_i = 4 nM) and in vivo, with at least 100-fold selectivity for the other receptors tested.
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1. Introduction

The imposition of conformational restriction on flexible molecules is one of the standard procedures used by medicinal chemists in the search for new agents with high efficacy and selectivity, and in the identification of bioactive ligand conformations.

In regards to long-chain arylpiperazines (LCAPs), this approach has been rarely used[1–17] compared to a vast number of SAR studies with flexible derivatives examined towards various receptor targets. The majority of conformational constraints in LCAPs concern a flexible aliphatic linker, and these compounds have been investigated mainly as 5-HT_{1A} receptor ligands. Semi-rigid analogues were obtained by introducing carbonyl or amide groups[1,3–5,7,9,10] and multiple bonds,[6,12] whereas in more rigid derivatives, a polymethylene chain was incorporated in a cyclic ring.[6,11,13–16]

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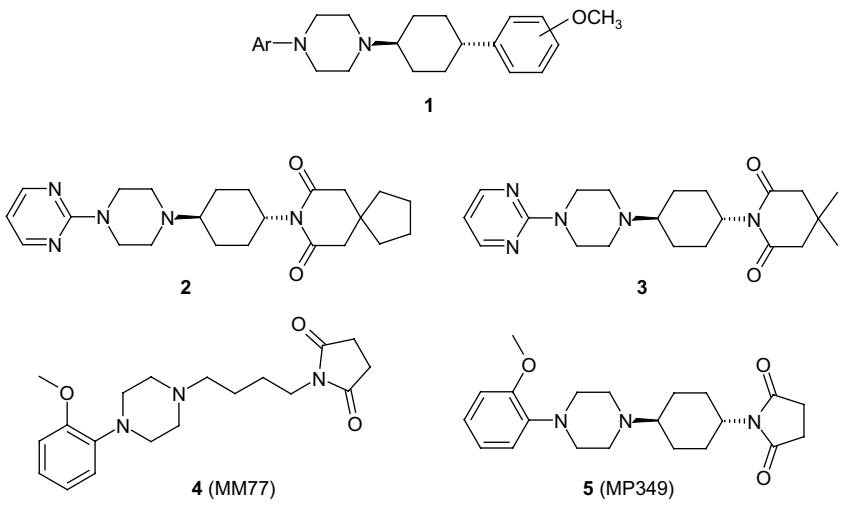


Chart 1.

Recently, Perrone et al. described *trans*-4-[4-(methoxyphenyl)-cyclohexyl]-1-arylpiperazines (**1**; Chart 1) as a new class of 5-HT_{1A} receptor ligands showing high affinity ($K_i \sim 0.02$ nM) and selectivity for the dopaminergic D_2 and α_1-adrenergic receptors.[13] A few selected compounds examined in the [^{35}S]GTPγS binding assay at the human cloned 5-HT_{1A} receptors demonstrated full or partial agonistic properties. On the other hand, similar *trans*-configured, rigid analogues of arylpiperazine-derived drugs buspirone and gepirone caused a significant decrease in the observed 5-HT_{1A} receptor affinity (K_i = 1600 and 492 nM for **2** and **3**, respectively).[15]

In the course of our study, focused on 4-(2-methoxyphenyl)piperazine derivatives, several rigid analogues of well-known 5-HT_{1A} receptor postsynaptic antagonists [e.g., NAN190, MM77 (**4**)] and partial agonists were synthesized.[11,14] All of those compounds were slightly less active than their flexible counterparts and showed features of postsynaptic 5-HT_{1A} receptor antagonists in in vivo experiments. One of them, MP349 (**5**; a constrained analogue of **4** whose anxiolytic-like activity had been described[18,19]), turned out to be a highly potent, full (pre- and postsynaptic) 5-HT_{1A} receptor antagonist.[14,19] Moreover, it revealed pronounced selectivity (at least 150-fold to 5-HT_{2A}, D_1, D_2, and benzodiazepine and 15-fold to α_1 receptors) and—like a parent compound—also demonstrated anxiolytic-like activity in some animal models.[19]

All the above-mentioned rigid derivatives of the highly active flexible 5-HT_{1A} receptor agents, shared a common linear polycyclic structure. Despite the fact that the extended conformation of N1-substituted N4-arylpiperazines is postulated to be bioactive, these compounds presented diverse affinity and various functional profiles.

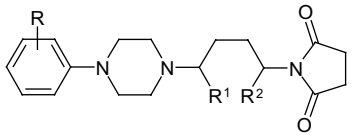
In order to further investigate the consequences of structural rigidification of LACPs, we synthesized new pairs of analogues of both lead compounds **4** and **5**. Since in our previous study we examined such structural transformation in a group of 4-(2-methoxyphenyl)piperazine derivatives only, currently other standard patterns of phenyl substitution were applied. For all the new 5-HT_{1A} receptor ligands functional profile (pre- and postsynaptic) was determined in vivo and selectivity for 5-HT_{2A}, 5-HT_7, D_2, and α_1 receptors was investigated.

2. Chemistry

The structures of the investigated compounds are shown in Table 1, and their syntheses are illustrated in Schemes 1 and 2.

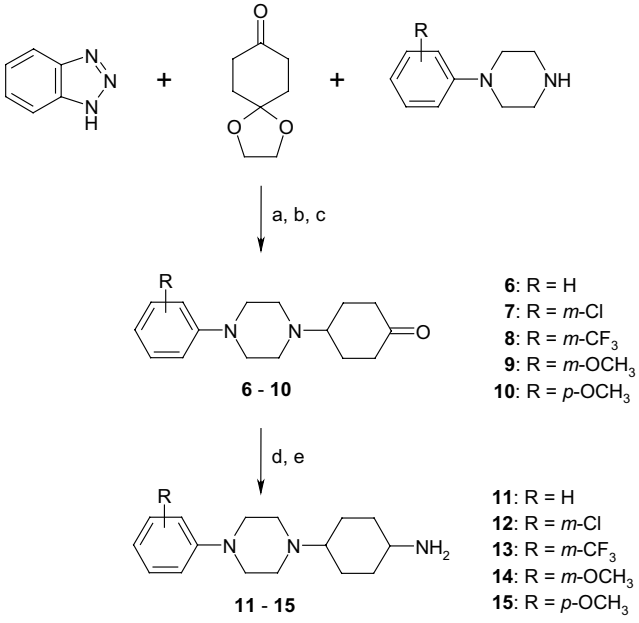
A multi-stage procedure (Scheme 1), published by us earlier[11] for the synthesis of 4-[4-(2-methoxyphenyl)piperazin-1-yl]cyclohexylamine, with modification of the last step was applied, to prepare new 4-(4-arylpiperazin-1-yl)cyclohexylamines (**11**–**15**) necessary for the syntheses of the designed constrained compounds (**20**, **22**, and **24**–**26**). The starting flexible analogous butylamines **16**–**18** were prepared according to Glennon et al.[20] by alkylation of appropriate arylpiperazine with *N*-(4-bromobutyl)phthalimide, followed by a hydrazinolysis of the obtained phthalimides. The constrained target compounds **20**, **22**, **24**–**26** and their flexible analogues **19**, **21**, and **23** were synthesized from the appropriate amines **11**–**15** and **16**–**18**, respectively, and succinic anhydride (Scheme 2) by heating in xylene. In the case of synthesis of compounds **25** and **26**, intermediate noncyclic amido-acids were obtained, which were then closed to target cyclic imides in acetic anhydride according to a modified procedure described to that for the preparation of *N*-phenylmaleimide.[21] The structure of the newly synthesized compounds was confirmed by ^{1}H NMR spectra and an elemental analysis. In the ^{1}H NMR spectra of rigid compounds **20**, **22**, and **24**–**26**, the observed coupling constants in the cyclohexane ring were consistent with those previously assigned by us to the 1e,4e-diequatorial chair conformation of 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)cyclohexyl]piperazine[11] and 1-(2-methoxyphenyl)-4-[4-(2-succinimido)cyclohexyl]piperazine.[14]

Table 1. Structure and binding affinity data on serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_7), α_1-adrenergic, and dopaminergic D_2 receptors of the investigated compounds

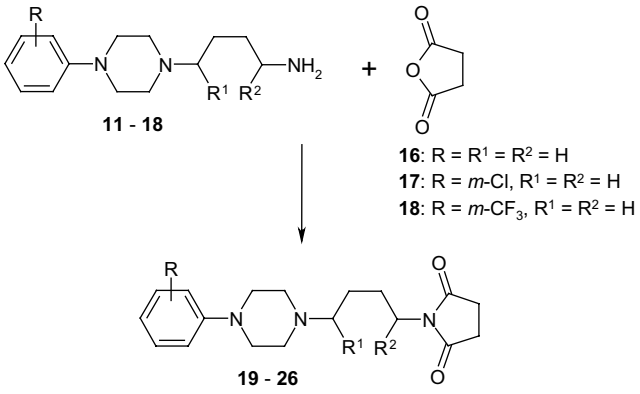


Compd	R	R^1	R^2	K_i [nM] ± SEM				
				5-HT_{1A}	5-HT_{2A}	5-HT_7	α_1	D_2
4 (MM77)	*o*-OMe	H	H	6.4 ± 0.3*	1510 ± 95*	90 ± 5**	11.9 ± 1*	490 ± 50*
5 (MP349)	*o*-OMe	–$(CH_2)_2$–		15.2 ± 3.2***	11,575 ± 20***	11,500 ± 2550**	234 ± 15***	2606 ± 160***
19	H	H	H	7.4 ± 0.3	1100 ± 50	82 ± 7	117 ± 10	15,000 ± 1900
20	H	–$(CH_2)_2$–		43 ± 6	2560 ± 60	6500 ± 50	162 ± 26	14,000 ± 2400
21	*m*-Cl	H	H	32 ± 2	121 ± 14	130 ± 18	60 ± 5	7800 ± 600
22	*m*-Cl	–$(CH_2)_2$–		5.4 ± 0.9	440 ± 20	1700 ± 24	23 ± 8	5300 ± 120
23	*m*-CF_3	H	H	21 ± 1	245 ± 28	128 ± 6	483 ± 36	31,000 ± 2100
24	*m*-CF_3	–$(CH_2)_2$–		4 ± 0.5	462 ± 18	1820 ± 38	505 ± 62	27,000 ± 1900
25	*m*-OMe	–$(CH_2)_2$–		27 ± 2	3450 ± 110	14,780 ± 800	230 ± 18	NT
26	*p*-OMe	–$(CH_2)_2$–		145 ± 15	18,600 ± 200	9500 ± 150	1930 ± 32	NT

NT—not tested.
* Ref. 34.
** Ref. 30.
*** Ref. 14.



Scheme 1. Reagents and conditions: (a) benzene, reflux; (b) $NaBH_4$, THF; (c) HCl, H_2O, acetone; (d) $NH_2OH{\cdot}HCl$, Na_2CO_3, MeOH; (e) Na, *n*-BuOH or H_2/Pd, MeOH, CH_3COOH.



Scheme 2.

3. Pharmacology

The compounds were tested in competition binding experiments for native serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_7, α_1-adrenergic, and dopamine D_2 receptors. The affinity data are collected in Table 1.

The functional activity of the investigated compounds at pre- and postsynaptic 5-HT_{1A} receptors was tested in several commonly used in vivo models. It was previously demonstrated that the hypothermia induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) in mice was connected with activation of presynaptic 5-HT_{1A} receptors[22,23] and was abolished by 5-HT_{1A} receptor antagonists such as, for example, WAY100635 (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide)[24] or MP 3022 (4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)piperazine).[25] Hence the hypothermia produced by the compounds tested in mice (and reduced by WAY 100635) was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity.

To determine a postsynaptic 5-HT_{1A} receptor agonistic effect of the tested 5-HT_{1A} ligands, their ability to induce lower lip retraction (LLR) in rats and behavioral syndrome, that is, flat body posture (FBP) and forepaw treading (FT), in reserpinized rats was tested. The 8-OH-DPAT-induced LLR and behavioral syndrome in rats depended on stimulation of postsynaptic 5-HT_{1A}

receptors;[26–28] moreover, it was shown that those symptoms were sensitive to 5-HT_{1A} receptor antagonists.[24,25] Hence the ability of the investigated compounds to inhibit those symptoms induced by 8-OH-DPAT was regarded as postsynaptic 5-HT_{1A} receptor antagonistic activity.

4. Results and discussion

It is well known that arylpiperazine-containing compounds can bind to at least three populations of neurotransmitter receptors (serotonin, dopaminergic, and adrenergic), and that their selectivity is an important aspect of many investigations. Therefore, in the present work, novel derivatives were evaluated for their in vitro activity at the most relevant receptors.

The affinity of arylpiperazine analogues, in which an *o*-methoxy group of MM77 and MP349 has been removed (**19**, **20**) or replaced with *m*-Cl (**21**, **22**) or *m*-CF_3 (**23**, **24**) for the 5-HT_{1A} receptor remains at the nanomolar range (K_i = 4–43 nM). Thus the replacement of a tetramethylene chain with a 1e,4e-disubstituted cyclohexane ring resulted in insignificant affinity changes, that is, a 5-fold decrease for unsubstituted and a ca. 5-fold increase for both *m*-substituted phenylpiperazines. Shifting the methoxy group from *ortho* to *meta* and *para* positions in the rigid MP349 analogues **25** and **26** caused a 2-fold and 10-fold reduction of binding constant K_i, respectively. This observation is in agreement with the previously published data that substituents in *para*-position caused unfavorable steric interactions with the 5-HT_{1A} receptor binding site.[29]

Regarding 5-HT_{2A} receptors, new arylpiperazines were at least 60 times less active, except for the two flexible *m*-Cl (**21**) and *m*-CF_3 (**23**) derivatives, which showed only 3- and 11-fold preference for 5-HT_{1A} binding sites. Within pairs of the compounds, cyclohexane derivatives always exhibited lower potency than the respective chain analogues. The same was observed for the compound activity for 5-HT_7 sites; however, the negative effect of rigidification was much more pronounced. All the flexible ligands displayed significant 5-HT_7 receptor affinity (K_i = 90–130 nM), whereas their constrained counterparts, as well as compounds **25** and **26**, were practically inactive. Such consequent preference for the flexible derivatives suggests that, unlike 5-HT_{1A} in the case of 5-HT_7 receptors, other than the extended linear conformations should be considered as bioactive.[30]

The results of the α_1 receptor binding study showed that substitutents in the aromatic part, rather than rigidification, induced affinity changes of the tested compounds. In fact, the unfavorable influence of linker cyclization was found for the lead pair (**4** and **5**) only. Both the *m*-Cl derivatives displayed high affinity for α_1 receptors, and were thus unselective 5-HT_{1A}/α_1 ligands. Improved selectivity was obtained in the case of *m*-CF_3-substituted analogues ($S_{\alpha_1/5\text{-}HT_{1A}}$ = 23 and 126 for **23** and **24**, respectively), since a significant reduction in their α_1 affinity was observed.

Some differences in dopamine D_2 receptor affinity between the respective flexible and rigid counterparts can be seen again for **4** and **5** only. The other investigated derivatives were found to be completely inactive.

As is apparent from these results, rigidification of the investigated group of LCAPs maintaining high 5-HT_{1A} receptor affinity significantly affected the 5-HT_7 binding, had weaker influence on 5-HT_{2A} receptors, and practically did not change the affinity for α_1 and D_2 sites.

As has been mentioned in the introduction, all our previously examined 4-substituted 1-(2-methoxyphenyl)piperazin-4-yl]cyclohexane derivatives (regardless of the functional profile of their flexible analogues) exhibited antagonistic properties at postsynaptic 5-HT_{1A} receptors in in vivo tests. It was then proposed that the rigid extended conformation of compounds of this type is responsible for the blockade of postsynaptic 5-HT_{1A} receptors.[14] However that conclusion cannot be generalized, since the similarly constrained compounds (**1**) reported by Perrone et al., evaluated in in vitro assays, were classified as agonists or partial agonists.[13] Nevertheless, those ligands were devoid of an imide portion in the terminal fragment and contained various arylpiperazine moieties. Interestingly, of all the investigated compounds, *trans*-4-[4-(3-methoxyphenyl)cyclohexyl]-1-(2-methoxyphenyl)piperazine showed the weakest agonistic properties, since it only partly stimulated [^{35}S]GTPγS binding (E_{max} = 26%). Its corresponding flexible analogue has not been synthesized, hence potential intrinsic activity changes after rigidification cannot be analyzed and compared with our previous results. It is worth noting that the above findings are the only available data concerning the functional characteristics of restricted LCAPs containing a 1,4-disubstituted cyclohexane linker.

Taking account of all the above facts, the second part of our present study has focused on determining whether spacer rigidification influences the functional profile, and whether phenyl substitution has any impact the on observed intrinsic activity of the investigated 5-HT_{1A} receptor ligands.

In order to answer those questions, new analogues (**19**–**24**) were tested in vivo in mice and rats to establish their functional activity at pre- and postsynaptic 5-HT_{1A} receptor. As shown in Table 2, those compounds—like 8-OH-DPAT, a 5-HT_{1A} receptor agonist—produced a decrease in mouse body temperature. The hypothermic effect evoked by **19**–**22** was reduced or abolished by WAY 100635 (Table 3), a silent 5-HT_{1A} receptor antagonist,[24] hence those compounds were classified as agonists of presynaptic 5-HT_{1A} receptors. Since the decrease in body temperature in mice induced by both the *m*-CF_3 derivatives **23** and **24** was not sensitive to WAY 100635 (like in the case of **4**[31]), a contribution of 5-HT_{1A} receptors to this effect should be excluded.

In behavioral models used to assess the function at postsynaptic 5-HT_{1A} receptors, compounds **19**–**24**—like 8-

Table 2. The effect of tested compounds on body temperature in mice

Treatment	Dose (mg/kg)	$\Delta t \pm$ SEM °C			
		30 min	60 min	90 min	120 min
Vehicle	—	−0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.1
4[*]	2	−1.2 ± 0.2[b]	−0.8 ± 0.3[b]	0.3 ± 0.2	0.1 ± 0.1
	4	−1.6 ± 0.4[b]	−1.0 ± 0.2[b]	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle	—	−0.3 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1
5[**]	0.25	0.1 ± 0.1	0.1 ± 0.2	−0.1 ± 0.1	0.0 ± 0.2
	0.5	−0.2 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
Vehicle	—	−0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0
19	2.5	−1.2 ± 0.1[b]	−1.3 ± 0.1[b]	−1.3 ± 0.1[b]	−1.2 ± 0.1[b]
	5	−2.1 ± 0.3[b]	−2.0 ± 0.3[b]	−1.4 ± 0.4[b]	−1.2 ± 0.2[b]
20	2.5	−0.3 ± 0.2	−0.3 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1
	5	−2.1 ± 0.3[b]	−1.6 ± 0.2[b]	−1.3 ± 0.2[b]	−1.1 ± 0.2[a]
Vehicle	—	0.1 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.2	−0.1 ± 0.0
21	2.5	−1.7 ± 0.3[b]	−1.1 ± 0.3[b]	−1.0 ± 0.2[a]	−0.5 ± 0.1
	5	−2.0 ± 0.4[b]	−1.8 ± 0.3[b]	−1.5 ± 0.3[b]	−1.7 ± 0.3[b]
22	1.25	−1.5 ± 0.3[b]	−1.3 ± 0.3[b]	−1.0 ± 0.3[b]	−0.9 ± 0.2[b]
	2.5	−1.9 ± 0.3[b]	−1.7 ± 0.3[b]	−1.5 ± 0.4[a]	−1.0 ± 0.3
Vehicle	—	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
23	2.5	−0.1 ± 0.1	−0.1 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1
	5	−1.0 ± 0.2[b]	−0.9 ± 0.2[a]	−0.5 ± 0.3	−0.2 ± 0.2
24	2.5	−0.2 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1
	5	−1.6 ± 0.2[b]	−1.7 ± 0.3[b]	−1.7 ± 0.2[b]	−1.7 ± 0.3[b]
Vehicle	—	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	0.0 ± 0.1
25	1.25	−1.0 ± 0.2[b]	−0.8 ± 0.2[a]	−0.6 ± 0.2	−0.6 ± 0.1
	2.5	−2.2 ± 0.4[b]	−1.7 ± 0.2[b]	−1.6 ± 0.2[b]	−1.4 ± 0.2[b]
WAY 100635	0.1	−0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	−0.1 ± 0.1

The tested compounds were administered 30 min before the test. Absolute initial mean body temperatures were within the range 36.6 ± 0.2 °C; n = 6–8 mice per group.

[a] $p < 0.05$.

[b] $p < 0.01$ versus respective vehicle group.

[*] Ref. 31.

[**] Ref. 19.

OH-DPAT—given alone induced LLR in rats (Table 4); moreover, **21**–**24** produced flat body posture (FBP) and forepaw treading (FT) in reserpinized rats (Table 5).

On the other hand—like partial agonists of postsynaptic 5-HT_{1A} receptors, for example, buspirone[32,33]—compounds **19**–**22** attenuated both symptoms of behavioral syndrome, and **19**, **21**, and **22** inhibited LLR induced by 8-OH-DPAT (Tables 4 and 5). The *m*-CF_3 derivatives **23** and **24** only weakly reduced FT, but failed to inhibit FBP. In the same models, compound **5** and WAY 100635 completely blocked the effects induced by the 5-HT_{1A} agonist. The results of the present behavioral study suggest that **19**–**24** can be classified as partial agonists of postsynaptic 5-HT_{1A} receptors, and that the intrinsic activity of **23** and **24** is higher than that of **19**–**22**.

As can be inferred from our functional in vivo study, the effects induced by **19**–**24** were not identical, however, similar within pairs of the tested ligands, that is, flexible and constrained analogues showed the same functional activity at pre- and postsynaptic sites (Table 6). Therefore the applied spacer rigidification did not influence 5-HT_{1A} intrinsic activity, which is in contrast to our previous suggestion, but again, indicates that the extended conformation of flexible LCAPs can be regarded as bioactive.

On the other hand, compounds **19**–**24** had a different functional profile than did the parent 1-(2-methoxyphenyl)piperazine derivatives **4** and **5**: the latter did not produce any agonistic effect at 5-HT_{1A} receptors.[14,19,31,34] This data suggest that the mode of phenyl substitution plays a pivotal role in controlling the intrinsic activity of the investigated compounds, and that the presence of an *o*-methoxy substituent is a prerequisite for the 5-HT_{1A} receptor full antagonistic activity of compound **5**. In connection with the above conclusion, another question arises concerning the role of a position of a methoxy group in the aromatic ring in functional activity of **5**. For that reason, the *meta*-isomer (**25**) was additionally evaluated. In vivo experiments demonstrated that **25** (1.25–10 mg/kg) behaved like an agonist of presynaptic (in the hypothermia model in mice), and a partial agonist of postsynaptic 5-HT_{1A} receptors (in the LLR model in rats). The affinity of *p*-methoxy derivative **26** (K_i = 145 nM) was insufficient for its functional characterization at 5-HT_{1A} receptors. Therefore the *o*-methoxy substituent of our lead compound **5** is a primary structural feature determining its antagonistic properties at pre- and postsynaptic 5-HT_{1A} receptor binding sites.

Table 3. The effect of WAY 100635 on the hypothermia induced by the tested compounds in mice

Treatment and dose (mg/kg)	$\Delta t \pm$ SEM °C	
	30 min	60 min
Vehicle + vehicle	-0.1 ± 0.1	0.1 ± 0.1
Vehicle + **4** (4)*	-1.6 ± 0.1^{b}	-1.2 ± 0.1^{b}
WAY 100635 (0.1) + **4** (4)	-1.9 ± 0.2^{b}	-1.4 ± 0.2^{b}
Vehicle + vehicle	-0.0 ± 0.1	-0.0 ± 0.1
Vehicle + **19** (2.5)	-2.0 ± 0.2^{b}	-1.9 ± 0.2^{b}
WAY 100635 (0.1) + **19** (2.5)	-0.7 ± 0.2^{aB}	-0.5 ± 0.2^{B}
Vehicle + vehicle	-0.1 ± 0.1	-0.1 ± 0.1
Vehicle + **20** (5)	-1.9 ± 0.2^{b}	-1.4 ± 0.1^{b}
WAY 100635 (0.1) + **20** (5)	-1.5 ± 0.3^{b}	-0.7 ± 0.2^{bB}
Vehicle + vehicle	-0.2 ± 0.1	-0.1 ± 0.1
Vehicle + **21** (2.5)	-1.7 ± 0.3^{b}	-1.1 ± 0.3^{b}
WAY 100635 (0.1) + **21** (2.5)	-0.6 ± 0.1^{B}	-0.4 ± 0.1^{A}
Vehicle + vehicle	-0.1 ± 0.1	0.0 ± 0.1
Vehicle + **22** (1.25)	-1.5 ± 0.3^{b}	-1.3 ± 0.3^{b}
WAY 100635 (0.1) + **22** (1.25)	-0.3 ± 0.1^{B}	-0.1 ± 0.1^{B}
Vehicle + vehicle	0.0 ± 0.1	0.0 ± 0.1
Vehicle + **23** (5)	-1.0 ± 0.2^{b}	-0.9 ± 0.3^{a}
WAY 100635 (0.1) + **23** (5)	-1.4 ± 0.3^{b}	-0.8 ± 0.2^{a}
Vehicle + vehicle	0.0 ± 0.1	0.0 ± 0.1
Vehicle + **24** (5)	-1.8 ± 0.3^{b}	-1.9 ± 0.3^{b}
WAY 100635 (0.1) + **24** (5)	-2.4 ± 0.3^{b}	-2.0 ± 0.3^{b}
Vehicle + vehicle	-1.0 ± 0.1	0.0 ± 0.1
Vehicle + **25** (1.25)	-1.4 ± 0.2^{b}	-1.4 ± 0.2^{b}
WAY 100635 (0.1) + **25** (1.25)	-0.6 ± 0.2^{aB}	-0.6 ± 0.2^{aB}
Vehicle + vehicle	0.1 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT (5)	-1.0 ± 0.1^{b}	-0.2 ± 0.1
WAY 100635 (0.1) + 8-OH-DPAT (5)	-0.1 ± 0.1^{B}	0.2 ± 0.1

WAY 100635 was administered 15 min before the compounds studied. Body temperature was recorded 30 and 60 min after injection of the tested compounds. Absolute initial mean body temperatures were within the range 36.5 ± 0.3 °C; $n = 8$ mice per group.
[A] $p < 0.05$.
[B] $p < 0.01$ versus respective vehicle + tested compound group.
[a] $p < 0.05$.
[b] $p < 0.01$ versus respective vehicle + vehicle group.
* Ref. 31.

Table 4. Induction of lower lip retraction (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	B
Vehicle	—	0.1 ± 0.1	2.7 ± 0.1
4*	4	0.1 ± 0.1	1.6 ± 0.1^{b}
	8	0.1 ± 0.1	1.1 ± 0.2^{b}
5**	0.25	0.0 ± 0.0	1.2 ± 0.2^{b}
	0.5	0.1 ± 0.1	0.7 ± 0.1^{a}
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
19	5	1.4 ± 0.1^{b}	1.4 ± 0.2^{b}
	10	2.5 ± 0.3^{b}	NT
20	5	2.1 ± 0.3^{b}	NT
	10	2.2 ± 0.3^{b}	NT
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
21	10	1.7 ± 0.2^{b}	1.9 ± 0.3^{b}
	20	1.9 ± 0.2^{b}	1.7 ± 0.2^{b}
22	5	1.8 ± 0.3^{b}	2.0 ± 0.4
	10	1.9 ± 0.4^{b}	1.6 ± 0.4^{a}
Vehicle	—	0.1 ± 0.1	—
23	1.25	2.1 ± 0.3^{b}	NT
	2.5	2.4 ± 0.3^{b}	NT
	5	2.5 ± 0.2^{b}	NT
	10	2.7 ± 0.2^{b}	NT
Vehicle	—	0.1 ± 0.1	—
24	2.5	2.0 ± 0.2^{b}	NT
	5	2.2 ± 0.2^{b}	NT
	10	2.5 ± 0.3^{b}	NT
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
25	5	0.8 ± 0.2^{b}	1.3 ± 0.3^{a}
	10	1.7 ± 0.1^{a}	1.2 ± 0.2^{a}
WAY 100635	0.1	0.0	0.2 ± 0.2^{b}

The investigated compounds were administered 15 min before test (A) or 45 min before 8-OH-DPAT (1 mg/kg); $n = 6$ rats per group.
NT—not tested.
[a] $p < 0.01$.
[b] $p < 0.05$ versus vehicle (A) or versus vehicle + 8-OH-DPAT (B).
* Ref. 34.
** Ref. 14.

5. Conclusions

A series of 1-aryl-4-(4-succinimidobutyl)piperazine derivatives and their constrained 1e,4e-disubstituted cyclohexane analogues were synthesized and evaluated in in vitro binding assays for serotonin (5-HT_{1A}, 5-HT_{2A}, 5-HT_7): α_1-adrenergic, and dopaminergic D_2 receptors.

The new compounds **19**–**25** exhibited nanomolar 5-HT_{1A} receptor affinity, which indicates that the extended linear arrangement, frozen in a cyclohexane ring, reflects the most probable bioactive conformation of flexible molecules. Of all the other target receptors analyzed, only 5-HT_7 receptors showed clear disfavor towards rigid compounds, hence it seems that in this case the bioactive conformation of chain derivatives may be different from the extended one. Since similarities in both receptor binding sites were described,[35] and since a number of 5-HT_{1A} ligands possess simultaneous affinity for 5-HT_7 receptors, our data provide an important clue to the modeling of both receptors and the design of new ligands.

On the basis of our in vivo study—unlike the parent antagonists **4** and **5**—all the new compounds were classified as partial 5-HT_{1A} receptor agonists, of which *m*-CF_3 derivatives (**23** and **24**) exhibited pronounced intrinsic activity. Moreover, in contrast to our earlier observations, the pre- and postsynaptic 5-HT_{1A} receptor functional properties of the respective pairs of compounds did not change after rigidification. It seems that in the group of arylpiperazines under study, 5-HT_{1A} intrinsic activity is very sensitive to modifications in aromatic phamacophore. Additionally, the obtained data directly indicate, that the full antagonistic profile observed for the lead compound **5** should be correlated with the engagement of *o*-methoxy group in ligand–receptor interactions.

Table 5. 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

Treatment	Dose (mg/kg)	Mean ± SEM behavioral score			
		A		B	
		Flat body posture	Forepaw treading	Flat body posture	Forepaw treading
Vehicle	—	0.2 ± 0.1	0.2 ± 0.1	15.0 ± 0.0	12.3 ± 1.0
4[*]	4	0.2 ± 0.2	0.1 ± 0.1	11.8 ± 1.3[a]	8.3 ± 1.5[a]
	8	0.1 ± 0.1	0.1 ± 0.1	2.3 ± 1.0[b]	6.8 ± 1.6[a]
Vehicle	—	0.2 ± 0.1	0.2 ± 0.1	14.8 ± 0.2	13.0 ± 0.9
5[**]	0.25	0.1 ± 0.2	0.1 ± 0.1	11.7 ± 0.7[a]	4.8 ± 0.7[b]
	0.5	0.1 ± 0.1	0.1 ± 0.1	2.0 ± 0.8[b]	1.8 ± 0.8[b]
Vehicle	—	0.0 ± 0.0	0.0 ± 0.0	14.5 ± 0.3	13.2 ± 0.2
19	5	0.0 ± 0.0	0.5 ± 0.2	4.7 ± 1.0[b]	4.8 ± 0.9[b]
	10	1.2 ± 0.1	0.0 ± 0.0	1.7 ± 0.9[b]	3.0 ± 0.9[b]
20	5	0.0 ± 0.0	1.0 ± 0.5	2.7 ± 0.8[b]	2.3 ± 0.7[b]
	10	1.5 ± 0.4	2.7 ± 0.7[a]	2.0 ± 1.1[b]	0.7 ± 0.5[b]
Vehicle	—	0.0 ± 0.0	0.0 ± 0.0	14.5 ± 0.2	13.0 ± 0.4
21	20	4.0 ± 0.9[b]	2.5 ± 0.9	2.2 ± 0.7[b]	1.3 ± 0.8[b]
22	10	10.7 ± 0.8[b]	7.8 ± 1.1[b]	6.0 ± 0.7[b]	7.0 ± 0.9[b]
Vehicle	—	0.0 ± 0.0	0.0 ± 0.0	14.5 ± 0.2	13.0 ± 0.4
23	10	11.3 ± 1.3[b]	5.7 ± 0.8[b]	13.3 ± 0.7	8.8 ± 0.8[b]
24	10	11.8 ± 1.2[b]	8.3 ± 0.6[b]	15.0 ± 0.0	10.2 ± 0.7[a]
WAY 100635	0.1	0.0 ± 0.0	0.0 ± 0.0	0.8 ± 0.4[b]	1.2 ± 0.7[b]

Reserpine (1 mg/kg) was administered 18 h before the test. The investigated compounds were administered 3 min before the test (A) or 60 min before 8-OH-DPAT (5 mg/kg) (B); $n = 6$ rats per group.

[a] $p < 0.05$.

[b] $p < 0.01$ versus vehicle (A) or versus vehicle + 8-OH-DPAT (B).

[*] Ref. 34.

[**] Ref. 14.

Table 6. 5-HT_{1A} receptor functional profile of the tested compounds

Compound	Presynaptic	Postsynaptic
4	—	Antagonist
5	Antagonist	Antagonist
19	Agonist	Partial agonist
20	Agonist	Partial agonist
21	Agonist	Partial agonist
22	Agonist	Partial agonist
23	—	Partial agonist
24	—	Partial agonist

6. Experimental

6.1. Chemistry

Melting points were determined in a Boetius apparatus and are uncorrected. ^{1}H NMR spectra were taken with a Varian EM-360L (60 MHz) or a Varian Mercury-VX (300 MHz) spectrophotometer in $CDCl_3$ solutions with TMS as an internal standard. The spectral data of new compounds refer to their free bases. Chemical shifts were expressed in δ (ppm) and the coupling constants J in hertz (Hz). All compounds were routinely checked by TLC using Merck Kieselgel or Aluminum oxide neutral 60-F_{254} aluminum 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 within ±0.4% of the theoretical values.

The starting 4-(1-phenylpiperazin-4-yl)butylamine (**16**) was synthesized by published procedure.[20] 4-[1-(3-Chlorophenyl)piperazin-4-yl]butylamine (**17**) and 4-[1-(3-trifluoromethylphenyl)piperazin-4-yl]butylamine (**18**) obtained in the same manner are characterized below:

6.1.1. 4-[1-(3-Chlorophenyl)piperazin-4-yl]butylamine (17). Oil, yield 46%, $R_f = 0.17$ (Al_2O_3, $CHCl_3$/MeOH = 9/1); ^{1}H NMR (60 MHz) δ 7.4–6.6 (m, 4H), 3.5–3.0 (m, 4H), 2.9–2.2 (m, 6H), 2.1–1.3 (m, 8H).

6.1.2. 4-[1-(3-Trifluoromethylphenyl)piperazin-4-yl]butylamine (18). Oil, yield 42%, $R_f = 0.27$ (SiO_2, $CHCl_3$/MeOH = 4/1); ^{1}H NMR (60 MHz) δ 7.5–6.8 (m, 4H), 3.5–3.0 (m, 4H), 2.9–2.2 (m, 8H), 2.0–1.2 (m, 6H).

6.1.3. General procedure for the preparation of intermediate ketones 6–10. These compounds and their oximes were prepared according to our previously published procedure.[11]

6.1.4. 4-(1-Phenylpiperazin-4-yl)cyclohexanone (6). This was prepared in 84% yield: mp 134–136 °C; $R_f = 0.27$ (SiO_2, $CHCl_3$); ^{1}H NMR (60 MHz) δ 7.6–6.7 (m, 5H), 3.4–3.0 (m, 4H), 3.0–1.6 (cluster, 13H); oxime: mp 157–158 °C.

6.1.5. 4-[1-(3-Chlorophenyl)piperazin-4-yl]cyclohexanone (7). This was prepared in 69% yield: mp 80–82 °C; $R_f = 0.40$ (Al_2O_3, AcOEt/hexane = 1/3); ^1H NMR (60 MHz) δ 7.4–6.5 (m, 4H), 3.4–3.0 (m, 4H), 2.9–2.2 (cluster, 9H), 2.2–1.6 (m, 4H); oxime: mp 154–155 °C.

6.1.6. 4-[1-(3-Trifluoromethylphenyl)piperazin-4-yl]cyclohexanone (8). This was prepared in 61% yield as a pale yellow oil; $R_f = 0.30$ (Al_2O_3, AcOEt/hexane = 1/3); ^1H NMR (60 MHz) δ 7.6–6.9 (m, 4H), 3.4–3.0 (m, 4H), 3.0–2.6 (m, 5H), 2.6–2.2 (m, 4H), 2.2–1.5 (m, 4H); oxime: mp 57–59 °C.

6.1.7. 4-[1-(3-Methoxyphenyl)piperazin-4-yl]cyclohexanone (9). This was prepared in 52% yield: mp 131–132 °C; $R_f = 0.40$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (60 MHz) δ 7.4–7.0 (m, 1H), 6.7–6.3 (m, 3H), 3.8 (s, 3H), 3.4–3.0 (m, 4H), 3.0–2.5 (m, 5H), 2.5–2.2 (m, 4H), 2.2–1.7 (m, 4H); oxime: mp 178–180 °C.

6.1.8. 4-[1-(4-Methoxyphenyl)piperazin-4-yl]cyclohexanone (10). This was prepared in 62% yield: mp 214–216 °C; $R_f = 0.37$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (60 MHz) δ 7.0 (s, 4H), 3.8 (s, 3H), 3.3–2.9 (m, 5H) 2.9–2.6 (m, 4H), 2.6–1.6 (cluster, 8H); oxime: mp 188–189 °C.

6.1.9. General procedure for the preparation of amines 11–15. Starting with ketones **6**–**10** and following the method published for the preparation of 4-[1-(2-methoxyphenyl)piperazin-4-yl]cyclohexylamine[11] the appropriate oximes were obtained. The oximes of ketones **6**, **9**, and **10** were then reduced in boiling *n*-BuOH with sodium, and the amines **11**, **14**, and **15** were isolated by column chromatography.

The oximes of ketones **7** and **8** (2 mmol) were reduced in an autoclave, in MeOH (40 mL) and glacial acetic acid (4 mL) with hydrogen (2.5 atm) in the presence of platinum(IV) oxide (140 mg) at 50 °C for 6 h. The catalyst was filtered off, washed with water, and the filtrate was evaporated. The residue was made alkaline with NH_3 (25% aqueous solution) and extracted with $CHCl_3$. After evaporation the residue was purified by column chromatography to afford amines **12** and **13**.

6.1.10. 4-(1-Phenylpiperazin-4-yl)cyclohexylamine (11). This was prepared from oxime of **6** in 65% yield: mp 158–161 °C, $R_f = 0.23$ (SiO_2, $CHCl_3$/MeOH = 9/1); ^1H NMR (60 MHz) δ 7.6–6.7 (m, 5H), 3.5–3.0 (m, 4H), 3.0–2.4 (m, 5H), 2.4–0.7 (cluster, 9H), 1.5 (br s, 2H).

6.1.11. 4-[1-(3-Chlorophenyl)piperazin-4-yl]cyclohexylamine (12). This was prepared from oxime of **7** as an oil in 57% yield, $R_f = 0.20$ (Al_2O_3, $CHCl_3$/MeOH = 19/1); ^1H NMR (60 MHz) δ 7.4–6.6 (m, 4H), 3.4–3.0 (m, 4H), 3.0–2.5 (m, 5H), 2.5–0.9 (cluster, 9H), 1.6 (br s, 2H).

6.1.12. 4-[1-(3-Trifluoromethylphenyl)piperazin-4-yl]cyclohexylamine (13). This was prepared from oxime of **8** as an oil in 78% yield, $R_f = 0.31$ (Al_2O_3, $CHCl_3$/MeOH = 9/1); ^1H NMR (60 MHz) δ 7.6–6.9 (m, 4H), 3.4–3.0 (m, 4H), 3.0–2.5 (m, 5H), 2.5–0.8 (cluster, 9H), 1.2 (br s, 2H).

6.1.13. 4-[1-(3-Methoxyphenyl)piperazin-4-yl]cyclohexylamine (14). This was prepared from oxime of **9** as an oil in 64% yield, $R_f = 0.14$ (SiO_2, $CHCl_3$/MeOH = 4/1); ^1H NMR (60 MHz) δ 7.5–7.0 (m, 1H), 6.8–6.3 (m, 3H), 3.8 (s, 3H), 3.5–3.0 (m, 4H), 3.0–2.5 (m, 5H), 2.5–0.9 (cluster, 9H), 1.4 (br s, 2H).

6.1.14. 4-[1-(4-Methoxyphenyl)piperazin-4-yl]cyclohexylamine (15). This was prepared from oxime of **10** in 49% yield, mp 274–276 °C, $R_f = 0.17$ (Al_2O_3, $CHCl_3$/MeOH = 9/1); ^1H NMR (60 MHz) δ 6.9 (s, 4H), 3.8 (s, 3H), 3.3–3.0 (m, 4H), 3.0–2.6 (m, 5H), 2.6–1.0 (cluster, 9H), 1.7 (br s, 2H).

6.1.15. General procedure for the preparation of compounds 19–24. Equimolar amounts (2 mmol) of appropriate 4-(1-arylpiperazin-4-yl)cyclohexylamine or 4-(1-arylpiperazin-4-yl)butylamine and succinic anhydride were refluxed in xylene (20 mL) for 5 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2, $CHCl_3$/MeOH = 49/1). For pharmacological assays free bases were converted into the hydrochloride salts in acetone, MeOH, or $CHCl_3$/MeOH solutions by the treatment with excess of Et_2O saturated with gaseous HCl.

6.1.16. 1-Phenyl-4-(4-succinimidobutyl)piperazine (19). The title compound was prepared by the general procedure from succinic anhydride and amine **16** in 68% yield as colorless crystals: mp 97–99 °C, $R_f = 0.36$ (SiO_2, $CHCl_3$/MeOH = 49/1); ^1H NMR (60 MHz) δ 7.5–6.7 (m, 5H), 3.8–3.4 (m, 2H), 3.3–3.0 (m, 4H), 2.8–2.2 (m, 6H), 2.6 (s, 4H), 1.8–1.3 (m, 4H). **19·2HCl**: 170–172 °C. Anal. ($C_{18}H_{25}N_3O_2 \cdot 2HCl$) C, H, N.

6.1.17. *trans*-1-Phenyl-4-(4-succinimidocyclohexyl)piperazine (20). The title compound was prepared by the general procedure in 59% yield as colorless crystals: mp 257–258 °C, $R_f = 0.46$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (300 MHz) δ 7.30 (dd, $J = 8.5$, 7.4, 2H, aryl H-3 and H-5), 6.96 (d, $J = 8.0$, 2H, aryl H-2 and H-6), 6.88 (dd, $J = 7.4$, 7.2, 1H, aryl H-4), 4.02 (tt, $J = 12.4$, 4.0, 1H, cyclohexane axial H-4), 3.24 (app br t, 4H, piperazine $2CH_2$), 2.78 (app br t, 4H, piperazine $2CH_2$), 2.68 (s, 4H, CH_2CH_2 in succinimide), 2.50 (tt, $J = 11.7$, 3.3, 1H, cyclohexane axial H-1), 2.31 (qd, $J = 12.9$, 3.3, 2H, cyclohexane axial H's), 2.06 (app br d, 2H, cyclohexane equatorial H's), 1.72 (app br d, 2H, cyclohexane equatorial H's), 1.42 (qd, $J = 12.9$, 3.3, 2H, cyclohexane axial H's). **20·2HCl·0.25H$_2$O**: mp 307–308 °C. Anal. ($C_{20}H_{27}N_3O_2 \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

6.1.18. 1-(3-Chlorophenyl)-4-(4-succinimidobutyl)piperazine (21). The title compound was prepared by the general procedure in 40% yield as an oil, $R_f = 0.35$ (SiO_2, $CHCl_3$/MeOH = 97/3); ^1H NMR (60 MHz) δ 7.4–6.6 (m, 4H), 3.7–3.3 (m, 2H), 3.3–3.0 (m, 4H), 2.7–2.2 (m, 6H), 2.7 (s, 4H), 1.8–1.4 (m, 4H). **21·2HCl**: mp 178–180 °C. Anal. ($C_{18}H_{24}ClN_3O_2 \cdot 2HCl$) C, H, N.

6.1.19. *trans*-1-(3-Chlorophenyl)-4-(4-succinimidocyclohexyl)piperazine (22). The title compound was prepared by the general procedure in 36% yield as colorless crystals: mp 218–220 °C, $R_f = 0.74$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (300 MHz) δ 7.19 (dd, $J = 8.2, 8.0$, 1H, aryl H-5), 6.90 (dd, $J = 2.2, 1.9$, 1H, aryl H-2), 6.84–6.80 (m, 2H, aryl H-4 and H-6), 4.05 (tt, $J = 12.4, 4.0$, 1H, cyclohexane axial H-4), 3.22 (app br t, 4H, piperazine $2CH_2$), 2.74 (app br t, 4H, piperazine $2CH_2$), 2.69 (s, 4H, CH_2CH_2 in succinimide), 2.49 (tt, $J = 11.7$, 3.3, 1H, cyclohexane axial H-1), 2.31 (qd, $J = 12.9$, 3.3, 2H, cyclohexane axial H's), 2.04 (app br d, 2H, cyclohexane equatorial H's), 1.72 (app br d, 2H, cyclohexane equatorial H's), 1.41 (qd, $J = 12.9$, 3.3, 2H, cyclohexane axial H's). **22·2HCl·H$_2$O**: mp 252–254 °C. Anal. ($C_{20}H_{26}ClN_3O_2 \cdot 2HCl\ H_2O$) C, H, N.

6.1.20. 4-(4-Succinimidobutyl)-1-(3-trifluoromethylphenyl)piperazine (23). The title compound was prepared by the general procedure in 75% yield as an oil, $R_f = 0.35$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (60 MHz) δ 7.6–6.8 (m, 4H), 3.8–3.0 (m, 6H), 2.9–2.2 (m, 6H), 2.7 (s, 4H), 1.9–1.4 (m, 4H). **23·2HCl**: mp 167–168 °C. Anal. ($C_{19}H_{24}F_3N_3O_2 \cdot 2HCl$) C, H, N.

6.1.21. *trans*-4-(4-Succinimidocyclohexyl)-1-(3-trifluoromethylphenyl)piperazine (24). The title compound was prepared by the general procedure in 50% yield as colorless crystals: mp 181–183 °C, $R_f = 0.60$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (300 MHz) δ 7.37 (dd, $J = 8.0, 7.7$, 1H, aryl H-5), 7.14–7.07 (m, 3H, aryl H-2, H-4 and H-6), 4.02 (dddd, $J = 12.4, 12.1, 4.1, 3.8$, 1H, cyclohexane axial H-4), 3.28 (app br t, 4H, piperazine $2CH_2$), 2.77 (app br t, 4H, piperazine $2CH_2$), 2.69 (s, 4H, CH_2CH_2 in succinimide), 2.51 (app br t, 1H, cyclohexane axial H-1), 2.32 (dddd, $J = 12.9, 12.6, 3.3, 3.0$, 2H, cyclohexane axial H's), 2.06 (app br d, 2H, cyclohexane equatorial H's), 1.72 (app br d, 2H, cyclohexane equatorial H's), 1.42 (qd, $J = 12.9, 3.0$, 2H, cyclohexane axial H's). **24·2HCl**: mp 255–256 °C. Anal. ($C_{21}H_{26}F_3N_3O_2 \cdot 2HCl$) C, H, N.

6.1.22. General procedure for the preparation of compounds 25 and 26. Equimolar amounts (2 mmol) of **14** or **15** and succinic anhydride were refluxed in xylene (20 mL) for 5 h. The resulting precipitate of noncyclic amidoacid was filtered off and then was heated in acetic anhydride (20 mL) in the presence of anhydrous sodium acetate (30% excess) for 5 h. After cooling the reaction mixture was poured into ice-water, neutralized with 10% NaOH and extracted with $CHCl_3$ (3×30 mL). The combined extracts were dried (K_2CO_3) and evaporated, to give the oily residue, which was purified by silica gel column chromatography. Free bases were then converted into the hydrochloride salts in $CHCl_3$/MeOH solution by the treatment with excess of Et_2O saturated with gaseous HCl.

6.1.23. *trans*-1-(3-Methoxyphenyl)-4-(4-succinimidocyclohexyl)piperazine (25). The title compound was prepared by the general procedure in 60% yield as colorless crystals: mp 162–164 °C, $R_f = 0.41$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (300 MHz) δ 7.20 (dd, $J = 8.2, 8.0$, 1H, aryl H-5), 6.58 (dd, $J = 8.2, 2.2$, 1H, aryl H-6), 6.50 (t, $J = 2.2$, 1H, aryl H-2), 6.44 (dd, $J = 8.0, 1.9$, 1H, aryl H-4), 4.02 (dddd, $J = 12.4, 12.1, 4.1, 3.8$, 1H, cyclohexane axial H-4), 3.82 (s, 3H, OCH_3), 3.23 (app br t, 4H, piperazine $2CH_2$), 2.75 (app br t, 4H, piperazine $2CH_2$), 2.68 (s, 4H, CH_2CH_2 in succinimide), 2.48 (tt, $J = 11.6, 3.3$, 1H, cyclohexane axial H-1), 2.31 (dddd, $J = 12.9, 12.6, 3.6, 3.3$, 2H, cyclohexane axial H's), 2.04 (app br d, 2H, cyclohexane equatorial H's), 1.72 (app br d, 2H, cyclohexane equatorial H's), 1.42 (dddd, $J = 12.9, 12.6, 3.6, 3.3$, 2H, cyclohexane axial H's). **25·2HCl**: mp 284–286 °C. Anal. ($C_{21}H_{29}N_3O_3 \cdot 2HCl$) C, H, N.

6.1.24. *trans*-1-(4-Methoxyphenyl)-4-(4-succinimidocyclohexyl)piperazine (26). The title compound was prepared by the general procedure in 67% yield as colorless crystals: mp 237–234 °C, $R_f = 0.70$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (300 MHz) δ 6.90 (dddd, $J = 9.4, 9.1, 3.0, 2.5$, 4H, aryl), 4.02 (dddd, $J = 12.4, 12.1, 4.1, 3.8$, 1H, cyclohexane axial H-4), 3.80 (s, 3H, OCH_3), 3.13 (app br t, 4H, piperazine $2CH_2$), 2.77 (app br t, 4H, piperazine $2CH_2$), 2.69 (s, 4H, CH_2CH_2, in succinimide), 2.49 (tt, $J = 11.6, 3.3$, 1H, cyclohexane axial H-1), 2.31 (dddd, $J = 12.9, 12.6, 3.3, 3.0$, 2H, cyclohexane axial H's), 2.06 (app br d, 2H, cyclohexane equatorial H's), 1.72 (app br d, 2H, cyclohexane equatorial H's), 1.42 (dddd, $J = 12.9, 12.7, 3.3, 3.0$, 2H, cyclohexane axial H's). **26·2HCl·0.5H$_2$O**: mp 264–266 °C. Anal. ($C_{21}H_{29}N_3O_3 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

6.2. In vitro radioligand binding assays

All the assays were carried out on rat brain tissues; inhibition constants (K_i) were determined from at least three separate experiments in which 8–10 drug concentrations, run in triplicate, were used. The binding reaction was terminated by rapid filtration through Whatman GF/B filters followed by three 4-mL washes with ice-cold incubation buffer.

The radioactivity retained on the filters was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4 mL scintillation fluid (Akwascynt, BioCare). Binding isotherms of the tested compounds were analyzed by nonlinear regression (Prism, GrafPad Software Inc., San Diego, USA), using the Cheng–Prusoff equation[36] to calculate K_i values.

6.2.1. Serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, and α_1-adrenergic binding assays. Radioligand studies with native 5-HT_{1A}, 5-HT_{2A}, and α_1-adrenergic receptors were conducted according to the methods previously described by us. Briefly: 5-HT_{1A} assays used rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals) and 5-HT for nonspecific binding; 5-HT_{2A} assays used rat cortical membranes, [^{3}H]-ketanserin (88.0 Ci/mmol, NEN Chemicals) and methysergide for nonspecific binding; α_1 assays used rat cortical membranes, [^{3}H]-prazosine (25.0 Ci/mmol, Amersham) and phentolamine for nonspecific binding.

6.2.2. Serotonin 5-HT$_7$ binding assays. The serotonin 5-HT receptor binding assay was performed using rat hypothalamic membranes, according to the method described by Aguirre et al.[37] with minor modifications. In brief, hypothalami dissected from male Wistar rats (200–250 g) were frozen at −80 °C prior to the preparation of radioligand binding homogenate. On the day of experiment hypothalami were allowed to defrost, then immediately homogenized in 20 volumes of 50 mM Tris–HCl buffer (pH 7.4 at 23 °C) and centrifuged at 48,000*g* for 10 min at 4 °C. The supernatant was removed, resulting pellet rehomogenized and incubated at 37 °C for 15 min, to remove endogenous serotonin. After incubation, the homogenate was centrifuged twice under the same conditions as before. The final pellet was resuspended in assay buffer (50 mM) Tris–HCl containing 0.01 mM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbate. Aliquots of membranes (10 mg original wet tissue weight) were incubated in the presence of 3 μM (±)-pindolol (to eliminate binding to 5-HT receptors) with 0.5 nM [^{3}H]-5-CT (specific activity, 34.5 Ci/mmol; NEN) and eight concentrations of the displacing drug. Nonspecific binding was determined using 10 μM of serotonin. After incubation at 23 °C for 120 min, the reaction was terminated by rapid filtration through a Whatman GF/B filter.

6.2.3. Dopamine D$_2$ binding assays. The preparation of rat striatal membranes was conducted as described previously.[38] The final tissue concentration for D_2 receptor binding was 3 mg original wet weight mL^{-1}. All the assays were carried out in 50 mM potassium phosphate buffer (pH 7.4). The radioligand used was [^{3}H]-spiperone (15.70 Ci/mmol, NEN Chemicals) in the presence of 50 nM ketanserin to prevent radioligand binding to 5-HT$_{2A}$ receptors. Displacement experiments were performed in a total volume of 1.2 mL. Assay tubes (in triplicate) containing: 0.1 mL of 1 nM [^{3}H]-spiperone, 0.1 mL competing drug, or 0.1 mL of vehicle (total binding) and 1 mL of tissue were incubated at 37 °C for 30 min. Nonspecific binding was determined using 0.1 mL of 5 μM butaclamol.

6.3. In vivo experiments

The experiments were performed on male Wistar rats (250–300 g) or male Albino Swiss mice (24–28 g). The animals were kept at a room temperature (20 ± 1 °C) on a natural day–night cycle (September–December) and housed under standard laboratory conditions. They had free access to food and tap water before the experiment. Each experimental group consisted of 6–8 animals/dose, and all the animals were used only once. All experiments were done between 9.00 a.m. and 2.00 p.m. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemical Inc.), reserpine (ampoules, Ciba), and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were used as aqueous solutions. Compounds **19**–**25** were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT, reserpine, and WAY 100635 were injected subcutaneously (sc), compounds **19**–**25** were given intraperitoneally (ip) in a volume of 2 mL/kg (rats) and 10 mL/kg (mice). The experimental procedures were approved by the Local Animal Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków. The obtained data were analyzed by Dunnett's test (when only one drug was given) or by the Newman–Keuls test (when two drugs were administered).

6.3.1. Body temperature in mice. The effect of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In an independent experiment, the effect of WAY 100635 (0.1 mg/kg, sc) on the hypothermia induced by compounds **19**–**24** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before compounds **19**–**24** or 8-OH-DPAT and the rectal body temperature was recorded 30 and 60 min after injection of the tested compounds. The results were expressed as a change in body temperature (Δt) with respect to the basal body temperature as measured at the beginning of the experiment.

6.3.2. Lower lip retraction (LLR) in rats. The LLR was assessed according to the method described by Berendsen et al.[26] The rats were individually placed in cages (30 × 25 × 25 cm), and they were scored three times (at 15, 30, and 45 min after the administration of the tested compounds) as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The total maximum score amounted to 3/rat. In a separate experiment, the effect of the studied compounds on LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds were administered 45 min before 8-OH-DPAT, and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

6.3.3. Behavioral syndrome in reserpinized rats. Reserpine (1 mg/kg) was administered 18 h before the test. The rats were individually placed in the experimental cages (30 × 25 × 25 cm) 5 min before injection of the tested compounds. Observation sessions, lasting 45 s each, began 3 min after the injection and were repeated every 3 min. Reciprocal forepaw treading and flat body posture were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. The total maximum score of five observation periods amounted to 15/for each symptom/animal.[28] The effect of the tested compounds on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg) in reserpinized rats was estimated in an independent experiment. The investigated compounds were administered 60 min before 8-OH-DPAT. Observations began 3 min after 8-OH-DPAT administration and were repeated every 3 min for a period of 15 min.

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