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Structure–intrinsic activity relationship studies in the group of 1-imido/amido substituted 4-(4-arylpiperazin-1-yl) cyclohexane derivatives; new, potent 5-HT_{1A} receptor agents with anxiolytic-like activity

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Abstract—Introduction of 1,4-disubstituted cyclohexane ring in the structure of flexible long chain arylpiperazines resulted in linearly constrained, potent serotonin (5-HT)$_{1A}$ ligands. In order to trace structure–intrinsic activity relationships in this group, a new series of 1-substituted 4-(4-arylpiperazin-1-yl)cyclohexane derivatives with different cyclic imide/amide termini, and their flexible, tetramethylene analogues were synthesized and pharmacologically evaluated for 5-HT_{1A} receptors. In vitro binding experiments revealed that all the compounds were potent 5-HT_{1A} receptor agents (K_i = 1.9–74 nM). Some derivatives tested additionally showed also high affinity for α_1-adrenergic receptors (K_i = 2.9–101 nM) and for 5-HT_7 receptors. Functional in vivo examination revealed that rigid ligands with *o*-OCH_3 group in the aryl moiety and cyclic imide system in the opposite terminal behaved like postsynaptic 5-HT_{1A} receptor antagonists. On the other hand, unsubstituted, *m*-Cl, or *m*-CF_3 substituted derivatives as well as those with cyclic amide group in the terminal fragment exhibited agonistic or partial agonistic activity. Three out of four derivatives tested, that is, postsynaptic 5-HT_{1A} antagonists **9** and **10**, and partial agonist **16**, showed anxiolytic-like activity in the conflict drinking (Vogel) test in rats.

1. Introduction

An arylpiperazine moiety is one of the most universal templates used for designing CNS-active agents. In the group of the so-called long chain arylpiperazines (LCAPs), it constitutes the main pharmacophoric fragment recognized by, for example, serotonergic, dopaminergic, and adrenergic receptors. Compounds of this class have been extensively studied, especially as 5-HT_{1A} receptor ligands, due to their potential antianxiety and antidepressant properties.[1] A huge number of SAR studies revealed the role of individual fragments, essential for high affinity for 5-HT_{1A} sites and selectivity for other receptors.[1] In contrast, much less is known about relationships between the structure of LCAPs and their functional activity which can be recognized as agonism, partial agonism or antagonism at 5-HT_{1A} receptors located either pre-(somatodendric autoreceptors) and postsynaptically. In fact, typical SAR publications are devoid of comprehensive examination of intrinsic activity, since usually only a few selected compounds from the investigated series were functionally characterized. Moreover, conformational flexibility of LCAPs themselves makes the prediction of their bioactive conformation speculative which hampers determination of structural features responsible for the respective ligands' action.

One of our recent strategies in structural modification of LCAPs consisted in imposing conformational restrictions on the flexible aliphatic spacer by introducing a 1,4-disubstituted cyclohexane ring.[2–5] As a result, a number of highly potent and fairly selective 5-HT_{1A} ligands were obtained, and it was proposed that such

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linearly constrained compounds preserved the bioactive conformation of LCAPs.[5] We reasoned that derivatives of that type were the most suitable to study structure–intrinsic activity relationships at 5-HT_{1A} receptors. Indeed, we previously demonstrated that the rigidification of MM77 (**1**, a potent antagonist of postsynaptic 5-HT_{1A} receptors[6]) resulted in compound MP349 (**2**), which turned out to be the first full antagonist from the arylpiperazine family with a precisely defined 3D structure.[3,7] Further modifications of **1**, **2** and gepirone analogues showed that 5-HT_{1A} intrinsic activity was very sensitive to the mode of substitution in the aromatic pharmacophore.[4,5] With reference to receptor blockade, two structural features can be regarded as crucial to postsynaptic antagonism: the constrained, extended conformation of the compound, and the presence of an *o*-OCH_3 substituent in the phenylpiperazine moiety.[5]

In our current structure–intrinsic activity studies based on 4-(arylpiperazin-1-yl)cyclohexanes, we have focused on the role of terminal imide moiety. Starting with the structure of potent 5-HT_{1A} ligands, that is, **2** and MP245 [**3**, a constrained version of NAN190 (**4**)], a series of their direct derivatives with different imide/amide part, as well as their flexible, tetramethylene analogues were synthesized. For all those new 5-HT_{1A} receptor ligands, a functional profile (pre- and postsynaptic) was determined in vivo, and their selectivity for 5-HT_7 and α_1 receptors was investigated (Table 1). In addition, pyrrolidinone and isoindolinone series were extended using *N*-(phenyl)-, *N*-(*m*-Cl-phenyl)-, and *N*-(*m*-CF_3-phenyl)piperazines (Table 2). Since—like WAY 100635 (a silent 5-HT_{1A} receptor antagonist)—**1** and **2** exhibited anxiolytic-like activity in animal models of anxiety,[7–10] four selected compounds were examined in the Vogel test in rats.

2. Chemistry

The synthetic methodology employed to develop target compounds is summarized in Schemes 1 and 2. The starting 4-(4-arylpiperazin-1-yl)butylamines were prepared according to Glennon et al.,[11] whereas their rigid counterparts, that is, 4-(4-arylpiperazin-1-yl)cyclohexylamines, were obtained by the method worked out in our laboratory.[2,5] The new flexible target compounds **5**, **7**, **9**, and **13** as well as their constrained analogues **6**, **8**, **10**, and **14** were synthesized from the appropriate amines and anhydrides by heating in xylene. However, in the case of preparation of compounds **10** and **14**, non-cyclic amidoacids were obtained, which were then closed to the required cyclic imides according to the procedure described for the synthesis of *N*-phenylmaleimide.[12]

Five-membered lactams **15**, **16**, and **19**–**24** were prepared in a two-step sequence by coupling of 4-chlorobutyryl chloride to the respective amines, followed by the reductive cyclization of intermediate amides using NaH in dry THF.[13] Compounds **17**, **18**, and **25**–**30** were received from the appropriate 4-aryl-1-[4-(2-phthalimido)butyl]piperazines[11] or 4-aryl-1-[4-(2-phthalimido)-cyclohexyl]piperazines, the latter being prepared according to the procedure previously described by us for the *o*-OCH_3-phenyl derivative.[2] Afterward phthalimide derivatives were reduced with zinc in acetic acid according to the procedure described by Brewster et al.[14] to afford the required final compounds **17**, **18**, and **25**–**30**. The structure of the newly synthesized compounds was confirmed by ^1H NMR spectra and an elemental analysis of their salts.

3. Pharmacology

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

The functional activity of the investigated compounds at pre- and postsynaptic 5-HT_{1A} receptors was tested in 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 depended primarily on stimulation of presynaptic 5-HT_{1A} receptors.[15,16] and was abolished by 5-HT_{1A} receptor antagonists such as, for example, WAY 100635.[17] Thus, the hypothermia produced by the tested compounds and reduced by WAY 100635 was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity.

To determine the postsynaptic 5-HT_{1A} receptor agonistic effect of the tested 5-HT_{1A} ligands, their ability to induce lower lip retraction (LLR) in rats was tested. The 8-OH-DPAT-induced LLR was found to be related to the activation of postsynaptic 5-HT_{1A} receptors;[18,19] moreover, it was shown that the latter symptom was sensitive to 5-HT_{1A} receptor antagonists.[17,20]

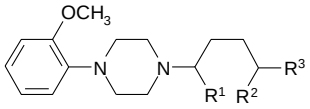
The potential anxiolytic activity of selected compounds was measured in the conflict drinking (Vogel)[21] test in rats.

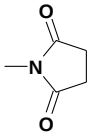
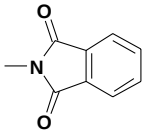
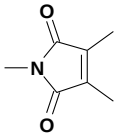
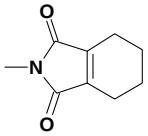
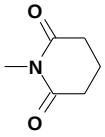
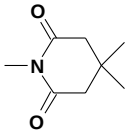
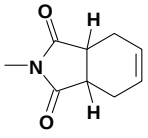
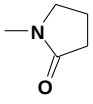
4. Results and Discussion

Based on the results obtained so far,[2,3] we designed new 1-(*o*-methoxyphenyl)piperazine (oMPP) derivatives with anticipated high affinity for 5-HT_{1A} receptors. Indeed, the applied modifications of the terminal imide moiety did not significantly influence the level of the 5-HT_{1A} binding of the new ligands compared to either pair (**1**, **2** and **3**, **4**) of the parent LCAPs (Table 1). Consistent with previous studies, the rigid analogues turned out to be potent agents, which again pointed to the extended conformation as bioactive, and enabled further structure–intrinsic activity investigations.

In addition, the target compounds were evaluated for their in vitro affinity for 5-HT_7 and α_1-adrenergic receptors, since it was established that pharmacophoric oMPP group can strongly interact with those binding sites. In the case of the former receptors, all the flexible

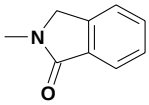
Table 1. Structure, 5-HT$_{1A}$, 5-HT$_7$, and α_1-adrenergic receptor affinities and functional in vivo activity of the compounds **1**–**18**



Compound	R^1	R^2	R^3	K_i (nM) ± SEM			5-HT$_{1A}$ receptor functional profile	
				5-HT$_{1A}$	5-HT$_7$	α_1	Presynaptic	Postsynaptic
1 (MM77)	H	H		6.4 ± 0.3[a]	90 ± 5[b]	11.9 ± 1[a]	—	Antagonist[a]
2 (MP349)	–(CH$_2$)$_2$–			15.2 ± 3.2[c]	11,500 ± 2550[b]	234 ± 15[d]	Antagonist[d]	Antagonist[c]
3 (NAN190)	H	H		0.6 ± 0.1[e]	87 ± 2[b]	0.8 ± 0.04	Agonist[f]	Antagonist[g]
4 (MP245)	–(CH$_2$)$_2$–			8 ± 2[h]	2045 ± 120[b]	29 ± 3[f]	Agonist[f]	Antagonist[h]
5	H	H		27 ± 3	155 ± 18	2.9 ± 0.2	Agonist	Antagonist
6	–(CH$_2$)$_2$–			19 ± 2	ND	31 ± 4	Agonist	Antagonist
7	H	H		7 ± 0.5	65 ± 8	8.8 ± 2	—	Antagonist
8	–(CH$_2$)$_2$–			21 ± 4	ND	15.7 ± 2	—	Antagonist
9	H	H		6 ± 0.8	100 ± 9	6.7 ± 0.5	—	Antagonist
10	–(CH$_2$)$_2$–			3.4 ± 0.3	ND	67 ± 4	—	Antagonist
11	H	H		8 ± 2[i]	114 ± 21	ND	Agonist[i]	Partial agonist[i]
12	–(CH$_2$)$_2$–			22 ± 4[i]	ND	ND	Agonist[i]	Antagonist[i]
13	H	H		3.5 ± 0.4	82 ± 4	7.9 ± 1.3	—	Partial agonist
14	–(CH$_2$)$_2$–			30 ± 5	ND	57 ± 8	Agonist	Antagonist
15	H	H		22 ± 1	166 ± 12	67 ± 5	—	Partial agonist
16	–(CH$_2$)$_2$–			4 ± 0.6	13,000 ± 540	101 ± 14	Agonist	Partial agonist

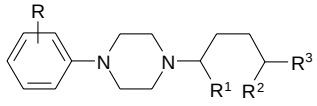
(*continued on next page*)

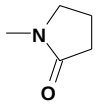
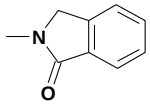
Table 1 (*continued*)

Compound	R^1	R^2	R^3	K_i (nM) ± SEM			5-HT_{1A} receptor functional profile	
				5-HT_{1A}	5-HT_7	α_1	Presynaptic	Postsynaptic
17	H	H		11 ± 1	475 ± 28	6.3 ± 1	Agonist	Partial agonist
18	–$(CH_2)_2$–			28 ± 3	1600 ± 120	5.3 ± 0.9	Agonist	Partial agonist

[a] Data from Ref. 6.
[b] Data from Ref. 32.
[c] Data from Ref. 3.
[d] Data from Ref. 7.
[e] Data from Ref. 11.
[f] Data from Ref. 33.
[g] Data from Ref. 34.
[h] Data from Ref. 2.
[i] Data from Ref. 4.

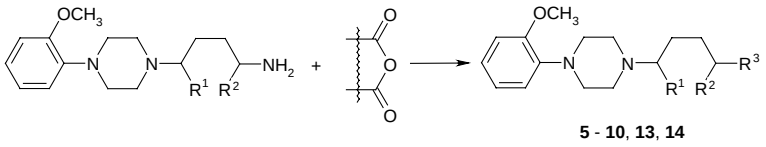
Table 2. Structure, 5-HT_{1A} receptor affinity, and functional in vivo activity of the compounds **15**–**30**



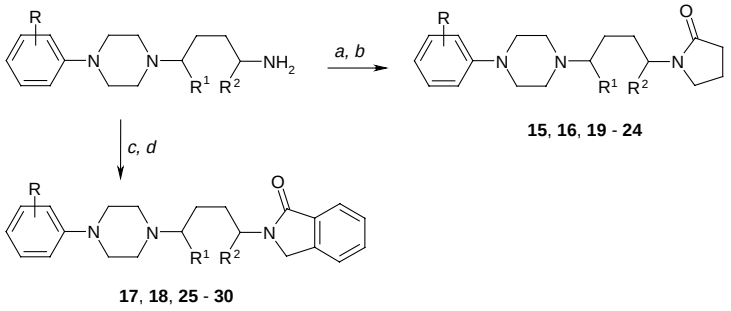
Compound	R	R^1	R^2	R^3	K_i (nM) ± SEM (5-HT_{1A})	5-HT_{1A} receptor functional profile	
						Presynaptic	Postsynaptic
15	*o*-OCH_3	H	H		22 ± 5	—	Partial agonist
16	*o*-OCH_3	–$(CH_2)_2$–			4 ± 0.7	Agonist	Partial agonist
19	H	H	H		74 ± 9	Agonist	Agonist
20	H	–$(CH_2)_2$–			36 ± 6	Agonist	Partial agonist
21	*m*-Cl	H	H		26 ± 5	Agonist	Agonist
22	*m*-Cl	–$(CH_2)_2$–			0.9 ± 0.1	Agonist	Agonist
23	*m*-CF_3	H	H		16 ± 4	Agonist	Agonist
24	*m*-CF_3	–$(CH_2)_2$–			1.9 ± 0.2	—	Agonist
17	*o*-OCH_3	H	H		11 ± 1.7	Agonist	Partial agonist
18	*o*-OCH_3	–$(CH_2)_2$–			28 ± 5	Agonist	Partial agonist
25	H	H	H		72 ± 5	Agonist	Partial agonist
26	H	–$(CH_2)_2$–			30 ± 4	Agonist	Partial agonist
27	*m*-Cl	H	H		27 ± 6	Agonist	Partial agonist
28	*m*-Cl	–$(CH_2)_2$–			6.7 ± 1.2	Agonist	Partial agonist
29	*m*-CF_3	H	H		13 ± 2	Agonist	Partial agonist
30	*m*-CF_3	–$(CH_2)_2$–			4.4 ± 1.2	Agonist	Agonist

compounds, except for **17**, displayed marked 5-HT_7 affinity (K_i = 65–166 nM), whereas two constrained derivatives tested (**16** and **18**) were practically inactive. The negative effect of rigidification was less pronounced for compounds' activity at α_1-adrenoreceptors where ligands generally showed high affinity—comparable to that at 5-HT_{1A} sites.

According to the main goal of our work, functional profile of ligands was determined in vivo, to find out whether slight changes in the structure of the terminal imide fragment influenced their 5-HT_{1A} intrinsic activity. A decrease of body temperature in mice, an effect linked with stimulation of presynaptic 5-HT_{1A} receptors, was observed after administration of all the investigated compounds. Since in the case of six derivatives (**7**–**10**, **13**, and **15**) the evoked hypothermia was neither reduced nor abolished by WAY 100635, a silent 5-HT_{1A} receptor antagonist, the contribution of other than 5-HT_{1A} receptors to this effect should be considered. Therefore, merely **5**, **6**, **14**, and **16**–**18**, like the previously described **11** and **12**,[4] could be classified as presynaptic 5-HT_{1A} receptor agonists. In light of these results, MP349 remains the only rigid derivative for



Scheme 1.



Scheme 2. Reagents and conditions: (a) $Cl(CH_2)_3COCl$, $CHCl_3$/20%K_2CO_3, rt; (b) NaH, THF, reflux, 6 h; (c) phthalic anhydride, xylene, reflux, 5 h; (d) Zn, AcOH, reflux, 1 h.

which antagonistic properties at somatodendric autoreceptors have been determined.[7]

Our previous studies revealed that rigidification of oMPP derivatives (regardless of the functional profile presented by flexible analogues) always yielded ligands with an antagonistic function at postsynaptic 5-HT_{1A} receptors.[3] Consistent data were obtained for compounds **5**–**14**, where the first six derivatives as well as the constrained analogues of partial agonists **11** and **13** showed features of postsynaptic antagonists in the LLR test. However, in the case of compounds with a reduced carbonyl group, rigid derivatives (**16** and **18**) exhibited the same partial agonistic profile as their flexible counterparts (**15** and **17**). It has to be stressed that the effect of postsynaptic receptor activation by rigid oMPP derivatives was observed by us for the first time but had also been shown by Perrone et al. for *trans*-4-[4-(3-methoxyphenyl)cyclohexyl]-1-(2-methoxyphenyl)piperazine.[22] A comparison of all these results indicates that, besides linearly frozen conformation and oMPP pharmacophore, the presence of both carbonyl groups may play a role in the blockade of postsynaptic sites.

Since some effects of postsynaptic 5-HT_{1A} receptor activation were observed for ligands containing other-than-oMPP fragments,[5] as well as for those with cyclic terminal amide moiety, both features, potentially engaged in the receptor activation, were then combined in the structure of new compounds (Table 2). As expected, they exhibited pronounced 5-HT_{1A} receptor affinity which ranged from very high (K_i = 0.9–6.7 nM) for rigid derivatives with *m*-substituted phenyl moiety (**22**, **24**, **28**, and **30**) to moderate (K_i = 72–74 nM) for both flexible phenylpiperazines (**19** and **25**). All the tested compounds induced effects characteristic of presynaptic 5-HT_{1A} receptor agonists in a model of hypothermia in mice; only in the case of **24**, the decrease in body temperature was not abolished by WAY 100635. Of the derivatives evaluated in the LLR test, **20** and almost all isoindolinones (**25**–**29**) behaved like partial agonists, that is, they maintained the functional profile of their oMPP analogues. The remaining compounds, that is, pyrrolidones **19** and **21**–**24**, as well as the rigid isoindolinone derivative with *m*-CF_3 group (**30**), showed greater intrinsic activity, and only symptoms of postsynaptic 5-HT_{1A} receptor activation were observed in the behavioral model used (Table 2).

The above observation indicates that a combination of structural features, found to be connected with the agonistic properties of ligands, may indeed lead to an increase in efficacy (as is the case in pyrrolidine series), but their overall effect also depends on the terminal imide/amide fragment. Summing up all the in vivo data, it is clear that even for rigid arylpiperazines small structural changes strongly influence the functional profile, which points to high sensitivity of 5-HT_{1A} receptor binding site and shows that both active and inactive receptor states can accept compounds in the linearly extended conformation. This is well illustrated by, for example, the transformation of pre- and postsynaptic 5-HT_{1A} receptor antagonist MP349 into the very close analogue **22** with an entirely opposite intrinsic activity (i.e., agonistic) after removal of one carbonyl group from the terminal imide moiety, and the replacement of *o*-OCH_3 substituent with *m*-Cl in the arylpiperazine fragment.

It has been well documented that partial agonists of 5-HT_{1A} receptors, like buspirone[23] and homologous

compounds,[24] and agonists (e.g., flesinoxan[25] and F11440[26]) produce anxiolytic-like effects. Interestingly, such activity has also been described for some 5-HT_{1A} receptor antagonists such as, for example, WAY 100635[9,10,27] as well as for a pair of our model compounds **1**[8,10] and **2**.[7] Therefore, we decided to examine four of the new derivatives (**9**, **10**, **16**, and **24**) in the conflict drinking (Vogel) test in rats. The obtained results showed that both postsynaptic antagonists **9** and **10**, and partial agonist **16** induced anxiolytic-like activity (Table 3), and that their effects seemed to be specific, since in the doses effective in the Vogel test they affected neither the response to threshold current nor water intake (data not shown). The postsynaptic 5-HT_{1A} receptor agonist **24** (0.1–0.3 mg/kg) did not evoke effects characteristic of anxiolytics in the conflict drinking test. With increasing doses of **24** (>0.3 mg/kg), only a 5-HT-like behavioral syndrome, that is, flat body posture, forepaw treading, and tremor, was observed (data not shown).

The anticonflict effect produced by compounds **9** and **10**, similar to that observed for parent compound **1**,[7] appeared exclusively after significantly lower doses (at least 20-fold) than those counteracting the effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT in functional in vivo tests. On the other hand, our model derivative **2** as well as new, partial agonist **16** induced anticonflict activity at the same doses in which they exhibited 5-HT_{1A} receptor functional activity, whereas WAY 100635 was active at doses 5–10 times higher than those which produced full 5-HT_{1A} receptor antagonistic effects.[7] The anxiolytic-like activity of postsynaptic antagonists **9** and **10** was comparable, in terms of their potency and active doses, with the effect of **2**, but weaker than that of **1** or commonly used reference drug diazepam (given in high dose of 10 mg/kg).[7] The maximal anticonflict effect of partial agonist **16** was similar to those of **9** and **10**, and only slightly weaker than that previously found for buspirone;[28] however, it was observed after higher doses (Table 3).

Based on the investigations with 1-substituted 4-(4-arylpiperazin-1-yl)cyclohexane derivatives, that have been done so far, some general conclusions can be drawn. First, as originally proposed by Perrone et al.,[22] they indeed constitute structurally distinct class of pharmacologically active 5-HT_{1A} receptor ligands with confirmed anxiolytic-like properties. Next, unlike for flexible arylpiperazines, structure–intrinsic activity studies with rigid analogues give more coherent results, indicating important fragments that control functional profile of a compound. It was found that ligands with the *o*-OCH_3 group in the aryl moiety and cyclic imide system in the opposite terminal have a tendency to block postsynaptic 5-HT_{1A} receptors, whereas unsubstituted, *m*-Cl or *m*-CF_3 substituted derivatives and those with cyclic amide moiety show agonistic or partial agonistic properties. Finally, the currently obtained new representatives of rigid arylpiperazines with different profiles of intrinsic activity represent optimal tools in docking studies simulating interactions with both active and inactive 5-HT_{1A} receptor states.

Table 3. Effects of compounds **9**, **10**, **16**, and **24** in the conflict drinking test in rats[a]

Treatment	Dose (mg/kg)	*n*	Number of shocks accepted/5 min mean ± SEM
Vehicle **9**	—	7	8.3 ± 0.9
	0.1	7	15.0 ± 3.8
	0.3	8	30.0 ± 5.6*
			$F(2,19) = 7.478$ $p < 0.01$
Vehicle **9**	—	6	10.8 ± 2.4
	5	6	8.8 ± 1.4
	10	6	11.2 ± 1.2
			$F(2,15) = 0.515$ ns
Vehicle **10**	—	7	8.3 ± 0.9
	0.1	7	25.4 ± 4.7**
	0.3	8	31.0 ± 6.1*
			$F(2,19) = 6.206$ $p < 0.01$
Vehicle **10**	—	6	9.2 ± 1.5
	5	6	8.2 ± 1.6
	10	6	9.2 ± 2.8
			$F(2,15) = 0.080$ ns
Vehicle **16**	—	7	11.1 ± 2.5
	2.5	8	19.5 ± 3.3
	5	8	32.1 ± 4.4*
			$F(2,20) = 8.680$ $P < 0.01$
Vehicle **24**	—	6	9.8 ± 1.2
	0.1	7	10.9 ± 1.4
	0.3	7	9.4 ± 1.5
			$F(2,17) = 0.282$ ns

[a] All compounds were administered (ip) 60 min before the test.
* $p < 0.01$.
** $p < 0.05$ versus respective vehicle group (Dunnett test).

5. Experimental

5.1. Chemistry

Melting points (mp) were determined with 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 (parts per million) and the coupling constants J in hertz (Hz). All compounds were routinely checked by TLC using Merck silica gel 60 F_{254} plates (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 found within ±0.4% of the theoretical values.

The starting 4-(4-arylpiperazin-1-yl)butylamines[5,11] and 4-(4-arylpiperazin-1-yl)cyclohexylamines.[2,5] were synthesized by published procedures. The preparation of compounds **11** and **12** had been previously published.[4]

5.1.1. General procedure for the preparation of compounds 5–9 and 13. Equimolar amounts (2 mmol) of appropriate 4-(4-arylpiperazin-1-yl)butylamine or 4-(4-arylpiperazin-1-yl)cyclohexylamine and proper 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. For pharmacological assays free bases were converted into the hydrochloride salts in acetone solutions by the treatment with excess Et_2O saturated with gaseous HCl.

5.1.2. 1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-3,4-dimethyl-3-pyrroline-2,5-dione (5). Compound **5** was prepared by the general procedure from 4-[4-(2-methoxyphenyl)piperazin-1-yl]butylamine.[11] and 2,3-dimethylmaleic anhydride in 75% yield as a pale yellow oil, R_f = 0.50 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^{1}H NMR (60 MHz) δ 7.0 (s, 4H, Ar–H), 3.9 (s, 3H, OCH_3), 3.7–3.4 (m, 2H, –CH_2–imide), 3.2–2.8 (m, 4H, piperazine 2CH_2), 2.8–2.2 (m, 6H, piperazine 2CH_2 and –$(CH_2)_3$–CH_2–piperazine), 1.9 (s, 6H, 2CH_3), 1.8–1.4 (m, 4H, –CH_2–$(CH_2)_2$–CH_2–). **5**·2HCl: colorless crystals, mp 235–237 °C. Anal. ($C_{21}H_{29}N_3O_3$·2HCl) C, H, N.

5.1.3. *trans*-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]cyclohexyl}-3,4-dimethyl-3-pyrroline-2,5-dione (6). Compound **6** was prepared by the general procedure from 4-[4-(2-methoxyphenyl)piperazin-1-yl]cyclohexylamine.[2] and 2,3-dimethylmaleic anhydride in 73% yield as colorless crystals, mp 202–204 °C, R_f = 0.55 (SiO_2, ethyl acetate); ^{1}H NMR (300 MHz) δ 7.06–6.92 (m, 3H, phenyl H-3, H-4 and H-5), 6.88 (dd, J = 7.8, 1.3 Hz, 1H, phenyl H-6), 3.98–3.85 (m, 4H, cyclohexane axial H-1 and OCH_3), 3.20–3.10 (m, 4H, piperazine 2CH_2), 2.90–2.80 (m, 4H, piperazine 2CH_2), 2.58–2.46 (m, 1H, cyclohexane axial H-4), 2.20 (qd, J = 12.9, 3.0 Hz, 2H, cyclohexane axial H's), 2.14–2.02 (m, 2H, cyclohexane equatorial H's), 1.96 (s, 6H, 2CH_3), 1.83–1.71 (m, 2H, cyclohexane equatorial H's), 1.45 (qd, J = 12.8, 2.8 Hz, 2H, cyclohexane axial H's). **6**·2HCl: colorless crystals, mp 259–261 °C. Anal. ($C_{23}H_{31}N_3O_3$·2HCl) C, H, N.

5.1.4. 2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-2,3,4,5,6,7-hexahydro-1*H*-isoindole-1,3-dione (7). Compound **7** was prepared by the general procedure from 4-[4-(2-methoxyphenyl)piperazin-1-yl]butylamine[11] and 3,4,5,6-tetrahydrophthalic anhydride in 68% yield as a yellow oil, R_f = 0.70 (SiO_2, $CHCl_3/CH_3OH$ = 49/1); ^{1}H NMR (60 MHz) δ 7.0 (s, 4H, Ar–H), 3.9 (s, 3H, OCH_3), 3.7–3.3 (m, 2H, –CH_2–imide), 3.3–2.9 (m, 4H, piperazine 2CH_2), 2.9–2.0 (cluster, 10H), 2.0–1.3 (cluster, 8H). **7**·2HCl: colorless crystals, mp 250–252 °C. Anal. ($C_{23}H_{31}N_3O_3$·2HCl) C, H, N.

5.1.5. *trans*-2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]-cyclohexyl}-2,3,4,5,6,7-hexahydro-1*H*-isoindole-1,3-dione (8). Compound **8** was prepared by the general procedure from 4-[4-(2-methoxyphenyl)piperazin-1-yl]cyclohexylamine.[2] and 3,4,5,6-tetrahydrophthalic anhydride in 48% yield as colorless crystals, mp 186–188 °C, R_f = 0.58 (SiO_2, ethyl acetate); ^{1}H NMR (300 MHz) δ 7.07–6.91 (m, 3H, phenyl H-3, H-4 and H-5), 6.88 (dd, J = 7.8, 1.2 Hz, 1H, phenyl H-6), 3.97–3.84 (m, 4H, cyclohexane axial H-1 and OCH_3), 3.20–3.10 (m, 4H, piperazine 2CH_2), 2.90–2.77 (m, 4H, piperazine 2CH_2), 2.58–2.44 (m, 1H, cyclohexane axial H-4), 2.40–2.28 (m, 4H, 2CH_2 in tetrahydroisoindole-1,3-dione), 2.20 (qd, J = 12.9, 3.0 Hz, 2H, cyclohexane axial H's), 2.12–2.02 (m, 2H, cyclohexane equatorial H's), 1.84–1.70 (m, 6H, 2CH_2 in tetrahydroisoindole-1,3-dione and cyclohexane equatorial H's), 1.44 (qd, J = 12.2, 2.3 Hz, 2H, cyclohexane axial H's). **8**·2HCl: colorless crystals, mp 235–237 °C. Anal. ($C_{25}H_{33}N_3O_3$·2HCl) C, H, N.

5.1.6. 1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}piperidine-2,6-dione (9). Compound **9** was prepared by the general procedure from 4-[4-(2-methoxyphenyl)piperazin-1-yl]-butylamine[11] and glutaric anhydride in 66% yield as a pale yellow oil, R_f = 0.55 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^{1}H NMR (60 MHz) δ 7.0 (m, 4H, Ar-H), 3.85 (s, 3H, OCH_3), 4.0–3.6 (m, 2H, CH_2–imide), 3.3–2.9 (m, 4H, piperazine 2CH_2), 2.9–2.2 (cluster, 10H), 2.2–1.3 (cluster, 6H). **9**·2HCl: colorless crystals, mp 226–228 °C. Anal. ($C_{20}H_{29}N_3O_3$·2HCl) C, H, N.

5.1.7. 2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-*cis*-2,3,3a,4,7,7a-hexahydro-1*H*-isoindole-1,3-dione (13). Compound **13** was prepared by the general procedure from 4-[4-(2-methoxyphenyl)piperazin-1-yl]butylamine[11] and *cis*-1,2,3,6-tetrahydrophthalic anhydride in 79% yield as a pale yellow oil, R_f = 0.40 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^{1}H NMR (60 MHz) δ 6.9 (m, 4H, Ar–H); 5.9–5.7 (m, 2H, –CH=CH– in tetrahydroisoindole-1,3-dione), 3.8 (s, 3H, OCH_3), 3.6–3.3 (m, 2H, –CH_2–imide), 3.3–2.8 (m, 6H, piperazine 2CH_2 and –$(CH_2)_3$–CH_2–piperazine), 2.8–1.9 (cluster, 10H), 1.9–1.1 (m, 4H, –CH_2–$(CH_2)_2$–CH_2–). **13**·2HCl: colorless crystals, mp 203–205 °C. Anal. ($C_{23}H_{31}N_3O_3$·2HCl) C, H, N.

5.1.8. General procedure for the preparation of compounds 10 and 14. Equimolar amounts (2 mmol) of 4-[4-(2-methoxyphenyl)piperazin-1-yl]cyclohexylamine[2] and

glutaric or *cis*-1,2,3,6-tetrahydrophthalic anhydride were refluxed in xylene (20 ml) for 5 h. The resulting precipitate of non-cyclic 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 column chromatography. For pharmacological assays free bases were converted into the hydrochloride salts in acetone solutions by the treatment with excess of Et_2O saturated with gaseous HCl.

5.1.9. *trans*-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]-cyclohexyl}piperidine-2,6-dione (10). Compound **10** was prepared by the general procedure in 67% yield as colorless crystals, mp 203–204 °C, $R_f = 0.30$ (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^1H NMR (300 MHz) δ 7.06–6.91 (m, 3H, phenyl H-3, H-4 and H-5), 6.88 (dd, $J = 7.7$, 1.1 Hz, 1H, phenyl H-6), 4.57 (tt, $J = 12.2$, 3.8 Hz, 1H, cyclohexane axial H-1), 3.90 (s, 3H, OCH_3), 3.20–3.06 (m, 4H, piperazine $2CH_2$), 2.88–2.76 (m, 4H, piperazine $2CH_2$), 2.65 (t, $J = 6.6$ Hz, 4H, $2CH_2CO$ in piperidine-2,6-dione), 2.58–2.45 (m, 1H, cyclohexane axial H-4), 2.40 (qd, $J = 12.9$, 3.1 Hz, 2H, cyclohexane axial H's), 2.1–1.98 (m, 2H, cyclohexane equatorial H's), 1.97–1.88 (m, 2H, $–CH_2–CH_2–CH_2–$ in piperidine-2,6-dione), 1.72–1.60 (m, 2H, cyclohexane equatorial H's), 1.45 (qd, $J = 12.5$, 3.0 Hz, 2H, cyclohexane axial H's). **10**·2HCl: colorless crystals, mp 258–260 °C. Anal. ($C_{22}H_{31}N_3O_3$·2HCl) C, H, N.

5.1.10. *trans*-2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]cyclohexyl}-*cis*-2,3,3a,4,7,7a-hexahydro-1*H*-isoindole-1,3-dione (14). Compound **14** was prepared by the general procedure in 76% yield as colorless crystals, mp 179–180 °C, $R_f = 0.50$ (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^1H NMR (300 MHz) δ 7.06–6.91 (m, 3H, phenyl H-3, H-4 and H-5), 6.88 (dd, $J = 7.7$, 1.4 Hz, 1H, phenyl H-6), 5.96–5.86 (m, 2H, –CH=CH– in tetrahydroisoindole-1,3-dione), 3.96 (tt, $J = 12.2$, 4.0 Hz, 1H, cyclohexane axial H-1), 3.89 (s, 3H, OCH_3), 3.20–3.08 (m, 4H, piperazine $2CH_2$), 3.08–2.98 (m, 2H, CH–CH in tetrahydroisoindole-1,3-dione), 2.86–2.73 (m, 4H, piperazine $2CH_2$), 2.68–2.55 (m, 2H, methylene H's in tetrahydroisoindole-1,3-dione), 2.55–2.40 (m, 1H, cyclohexane axial H-4), 2.35–2.15 (m, 4H, methylene H's in tetrahydroisoindole-1,3-dione and cyclohexane axial H's), 2.10–1.97 (m, 2H, cyclohexane equatorial H's), 1.72–1.58 (m, 2H, cyclohexane equatorial H's), 1.41 (qd, $J = 12.4$, 2.7 Hz, 2H, cyclohexane axial H's). **14**·2HCl: colorless crystals, mp 242–244 °C. Anal. ($C_{25}H_{33}N_3O_3$·2HCl) C, H, N.

5.1.11. General procedure for the preparation of compounds 15, 16, and 19–24. To a vigorously stirred mixture of appropriate 4-(4-arylpiperazin-1-yl)butylamine or 4-(4-arylpiperazin-1-yl)cyclohexylamine (1mmol) in $CHCl_3$ (9 ml) and 20% aqueous solution of K_2CO_3 (9 ml) 4-chlorobutyryl chloride (2 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 3 h and then the organic layer was separated and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by column chromatography. The obtained 4-chloro-*N*-{4-[4-arylpiperazin-1-yl]butyl}- or 4-chloro-*N*-{4-[4-arylpiperazin-1-yl]cyclohexyl}butyramide (1 mmol) was dissolved in dry THF (80 ml) and the solution was added dropwise to a suspension of sodium hydride (0.37 g) in dry THF (10 ml). The reaction mixture was heated at 65–75 °C for 6 h and after cooling to room temperature was slowly quenched in an ice bath with methanol (10 ml). Then the solvent was evaporated and the residue was diluted with chloroform, washed with brine, and dried ($MgSO_4$). Finally, after evaporation of the solvent the residue was purified by column chromatography. For pharmacological assays free bases were converted into the fumarate salts in ethanol solution by the treatment with an equimolar amount of fumaric acid.

5.1.12. 1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}pyrrolidin-2-one (15). Compound **15** was prepared by the general procedure in 38% yield as a yellow oil, $R_f = 0.34$ (SiO_2, $CHCl_3$/CH_3OH = 9/1); ^1H NMR (60 MHz) δ 7.2–6.7 (m, 4H, Ar-H), 3.8 (s, 3H, OCH_3), 3.6–2.8 (cluster, 8H), 2.8–1.8 (cluster, 10H), 1.7–1.1 (m, 4H, $–CH_2–(CH_2)_2–CH_2–$). **15**·$1.25C_4H_4O_4·H_2O$: colorless crystals, mp 131–133 °C. Anal. ($C_{19}H_{29}N_3O_2$·$1.25C_4H_4O_4·H_2O$) C, H, N.

5.1.13. *trans*-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]cyclohexyl}pyrrolidin-2-one (16). Compound **16** was prepared by the general procedure in 70% yield as colorless crystals, mp 180–182 °C, $R_f = 0.23$ (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^1H NMR (300 MHz) δ 7.03–6.87 (m, 3H, phenyl H-3, H-4 and H-5), 6.85 (dd, $J = 7.7$, 1.3 Hz, 1H, phenyl H-6), 4.00–3.84 (m, 1H, cyclohexane axial H-1), 3.86 (s, 3H, OCH_3), 3.33 (t, $J = 6.9$ Hz, 2H, pyrrolidin-2-one H-3 and H-3′), 3.18–3.02 (m, 4H, piperazine $2CH_2$), 2.86–2.72 (m, 4H, piperazine $2CH_2$), 2.44–2.28 (m, 3H, cyclohexane axial H-4 and pyrrolidin-2-one H-5 and H-5′), 2.10–1.95 (m, 4H, cyclohexane equatorial H's and pyrrolidin-2-one H-4 and H-4′), 1.87–1.52 (m, 2H, cyclohexane equatorial H's), 1.58–1.38 (m, 4H, cyclohexane axial H's). **16**·$1.5C_4H_4O_4$: colorless crystals, mp 217–219 °C. Anal. ($C_{21}H_{31}N_3O$·$1.5C_4H_4O_4$) C, H, N.

5.1.14. 1-[4-(4-Phenylpiperazin-1-yl)butyl]pyrrolidin-2-one (19). Compound **19** was prepared by the general procedure in 79% yield as a yellow oil, $R_f = 0.25$ (SiO_2, $CHCl_3$/MeOH = 19/1); ^1H NMR (60 MHz) δ 7.2 (t, $J = 8$ Hz, 2H, Ar-H), 7.0–6.6 (m, 3H, Ar-H), 3.6–3.0 (cluster, 8H), 2.8–1.8 (cluster, 10H), 1.8–1.1 (m, 4H, $–CH_2–(CH_2)_2–CH_2–$). **19**·$1.45C_4H_4O_4·H_2O$: colorless crystals, mp 117–119 °C. Anal. ($C_{18}H_{27}N_3O$·$1.45C_4H_4O_4·H_2O$) C, H, N.

5.1.15. *trans*-1-[4-(4-Phenylpiperazin-1-yl)cyclohexyl]-pyrrolidin-2-one (20). Compound **20** was prepared by the general procedure in 61% yield as colorless crystals, mp 186–188 °C, $R_f = 0.21$ (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^1H NMR (300 MHz) δ 7.25 (td, $J = 7.0$, 1.8 Hz, 2H, phenyl H-3 and H-5), 6.92 (dd, $J = 8.7$, 1.0 Hz, 2H, phenyl H-2 and H-6), 6.84 (tt,

J = 7.3, 1.0 Hz, 1H, phenyl H-4), 4.00–3.86 (m, 1H, cyclohexane axial H-1), 3.33 (t, J = 7.0 Hz, 2H, pyrrolidin-2-one H-3 and H-3′), 3.26–3.14 (m, 4H, piperazine $2CH_2$), 2.80–2.68 (m, 4H, piperazine $2CH_2$), 2.43–2.27 (m, 3H, cyclohexane axial H-4 and pyrrolidin-2-one H-5 and H-5′), 2.08–1.88 (m, 4H, cyclohexane equatorial H's and pyrrolidin-2-one H-4 and H-4′), 1.88–1.71 (m, 2H, cyclohexane equatorial H's), 1.57–1.37 (m, 4H, cyclohexane axial H's). **20**·$0.75C_4H_4O_4$: colorless crystals, mp 217–219 °C. Anal. ($C_{20}H_{29}N_3O·0.75C_4H_4O_4$) C, H, N.

5.1.16. 1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}pyrrolidin-2-one (21). Compound **21** was prepared by the general procedure in 74% yield as colorless crystals, mp 80–82 °C, R_f = 0.18 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^1H NMR (60 MHz) δ 7.1 (t, J = 8 Hz, 1H, Ar-H.), 6.9–6.5 (m, 3H, Ar-H.), 3.7–2.8 (cluster, 8H), 2.8–1.8 (cluster, 10H), 1.7–1.1 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–). **21**·$C_4H_4O_4$: colorless crystals, mp 126–128 °C. Anal. ($C_{18}H_{26}N_3OCl·C_4H_4O_4$) C, H, N.

5.1.17. *trans*-1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]cyclohexyl}pyrrolidin-2-one (22). Compound **22** was prepared by the general procedure in 50% yield as colorless crystals, mp 170–172 °C, R_f = 0.19 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^1H NMR (300 MHz) δ 7.15 (t, J = 8.1 Hz, 1H, phenyl H-5), 6.86 (t, J = 2.2 Hz, 1H, phenyl H-2), 6.82–6-74 (m, 2H, phenyl H-4 and H-6), 4.00–3.88 (m, 1H, cyclohexane axial H-1), 3.33 (t, J = 6.9 Hz, 2H, pyrrolidin-2-one H-3 and H-3′), 3.28–3.14 (m, 4H, piperazine $2CH_2$), 2.82–2.64 (m, 4H, piperazine $2CH_2$), 2.43–2.28 (m, 3H, cyclohexane axial H-4 and pyrrolidin-2-one H-5 and H-5′), 2.07–1.91 (m, 4H, cyclohexane equatorial H's and pyrrolidin-2-one H-4 and H-4′), 1.87–1.72 (m, 2H, cyclohexane equatorial H's), 1.57–1.37 (m, 4H, cyclohexane axial H's). **22**·$1.1C_4H_4O_4$: colorless crystals, mp 206–208 °C. Anal. ($C_{20}H_{28}N_3OCl·1.1C_4H_4O_4$) C, H, N.

5.1.18. 1-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]butyl}pyrrolidin-2-one (23). Compound **23** was prepared by the general procedure in 33% yield as a yellow oil, R_f = 0.16 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^1H NMR (60 MHz) δ 7.55–6.8 (m, 4H, Ar-H.), 3.5–3.0 (m, 8H), 2.7–1.7 (cluster, 10H), 1.7–1.1 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–). **23**·$1.6C_4H_4O_4$:colorless crystals, mp 165–167 °C. Anal. ($C_{19}H_{26}N_3OF_3·1.6C_4H_4O_4$) C, H, N.

5.1.19. *trans*-1-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}pyrrolidin-2-one (24). Compound **24** was prepared by the general procedure in 53% yield as colorless crystals, mp 162–164 °C, R_f = 0.27 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^1H NMR (300 MHz) δ 7.33 (t, J = 7.9 Hz, 1H, phenyl H-5), 7.10 (s, 1H, phenyl H-2), 7.12–7.01 (m, 2H, phenyl H-4 and H-6), 4.02–3.87 (m, 1H, cyclohexane axial H-1), 3.33 (t, J = 7.0 Hz, 2H, pyrrolidin-2-one H-3 and H-3′), 3.30–3.18 (m, 4H, piperazine $2CH_2$), 2.82–2.66 (m, 4H, piperazine $2CH_2$), 2.44–2.28 (m, 3H, cyclohexane axial H-4 and pyrrolidin-2-one H-5 and H-5′), 2.08–1.92 (m, 4H, cyclohexane equatorial H's and pyrrolidin-2-one H-4 and H-4′), 1.88–1.73 (m, 2H, cyclohexane equatorial H's), 1.58–1.38 (m, 4H, cyclohexane axial H's). **24**·$C_4H_4O_4$: colorless crystals, mp 215–217 °C. Anal. ($C_{21}H_{28}N_3OF_3·C_4H_4O_4$) C, H, N.

5.1.20. General procedure for the preparation of compounds 17, 18, and 25–30. A solution of the appropriate 4-aryl-1-[4-(2-phthalimido)butyl]piperazine[11] or 4-aryl-1-[4-(2-phthalimido)cyclohexyl]piperazine[2] (1 mmol) in glacial acetic acid (2.26 g) was heated to 60 °C and zinc dust (5.5 mmol) was added all at once with stirring. The reaction mixture was refluxed with stirring for 1 h, then filtered hot and washed with glacial acetic acid. Next the mixture was concentrated under reduced pressure, alkalized by saturated $NaHCO_3$ solution, and extracted with $CHCl_3$ (3 × 30 ml). The combined extracts were washed with saturated $NaHCO_3$ solution then with water and dried ($MgSO_4$). After evaporation of the solvent, the residue was purified by column chromatography. For pharmacological assays free bases were converted into the hydrochloride salts in acetone solution by the treatment with excess Et_2O saturated with gaseous HCl.

5.1.21. 2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-2,3-dihydro-1*H*-isoindol-1-one (17). Compound **17** was prepared by the general procedure in 38% yield as a yellow oil, R_f = 0.37 (Al_2O_3, ethyl acetate); ^1H NMR (60 MHz) δ 7.9–7.6 (m, 1H, Ar-H), 7.5–7.1 (m. 3H, Ar-H), 6.8 (s, 4H, Ar-H), 4.3 (s, 2H, dihydroisoindol-1-one CH_2), 3.7 (s, 3H, OCH_3), 3.6 (t, J = 6 Hz, 2H, –CH_2–dihydroisoindol-1-one), 3.2–2.7 (m, 4H, piperazine $2CH_2$), 2.7–2.1 (m, 6H, piperazine $2CH_2$ and –CH_2–piperazine), 1.8–1.3 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–). **17**·2HCl·$0.25H_2O$: colorless crystals, mp 227–229 °C. Anal. ($C_{23}H_{29}N_3O_2·2HCl·0.25H_2O$) C, H, N.

5.1.22. *trans*-2-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]cyclohexyl}-2,3-dihydro-1*H*-isoindol-1-one (18). Compound **18** was prepared by the general procedure in 87% yield as colorless crystals, mp 162–164 °C, R_f = 0.31 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^1H NMR (300 MHz) δ 7.85 (d, J = 6.9 Hz, 1H, dihydroisoindol-1-one H-7), 7.57–7.41 (m, 3H, dihydroisoindol-1-one H-4, H-5 and H-6), 7.05–6.89 (m, 3H, phenyl H-3, H-4 and H-5), 6.87 (d, J = 7.9 Hz, 1H, phenyl H-6), 4.35 (s, 2H, dihydroisoindol-1-one CH_2), 4.33–4.18 (m, 1H, cyclohexane axial H-1), 3.87 (s, 3H, OCH_3), 3.30–3.05 (m, 4H, piperazine $2CH_2$), 2.98–2.80 (m, 4H, piperazine $2CH_2$), 2.60–2.44 (m, 1H, cyclohexane axial H-4), 2.22–2.07 (m, 2H, cyclohexane equatorial H's), 2.07–1.92 (m, 2H, cyclohexane equatorial H's), 1.71–1.50 (m, 4H, cyclohexane axial H's). **18**·2HCl·H_2O: colorless crystals, mp 258–260 °C. Anal. ($C_{25}H_{31}N_3O·2HCl·H_2O$) C, H, N.

5.1.23. 2-[4-(4-Phenylpiperazin-1-yl)butyl]-2,3-dihydro-1*H*-isoindol-1-one (25). Compound **25** was prepared by the general procedure in 44% yield as a yellow oil, R_f = 0.34 (SiO_2, $CHCl_3/CH_3OH$ = 19/1); ^1H NMR (60 MHz) δ 7.9–7.6 (m, 1H, Ar-H), 7.6–7.0 (m. 5H, Ar-H), 7.0–6.6 (m, 3H, Ar-H), 4.3 (s, 2H, dihydroisoindol-1-one CH_2), 3.6 (t, J = 6 Hz, 2H, –CH_2– dihydroisoindol-1-one), 3.3–2.9 (m, 4H, piperazine $2CH_2$), 2.8–2.0 (m, 6H, piperazine $2CH_2$ and –CH_2–piperazine), 2.0–1.3 (m, 4H, –CH_2–(CH_2)$_2$–CH_2–). **25**·2HCl: color-

less crystals, mp 224–226 °C. Anal. ($C_{22}H_{27}N_3O\cdot 2HCl$) C, H, N.

5.1.24. *trans*-2-[4-(4-Phenylpiperazin-1-yl)cyclohexyl]-2,3-dihydro-1*H*-isoindol-1-one (26). Compound **26** was prepared by the general procedure in 51% yield as colorless crystals, mp 218–220 °C, $R_f = 0.14$ (SiO_2, $CHCl_3$/CH_3OH = 97/3); ^1H NMR (300 MHz) δ 7.86 (dd, $J = 6.5$, 1.7 Hz, 1H, dihydroisoindol-1-one H-7), 7.56–7.42 (m, 3H, dihydroisoindol-1-one H-4, H-5 and H-6), 7.26 (t, $J = 7.2$ Hz, 2H, phenyl H-3 and H-5), 6.94 (d, $J = 8.7$ Hz, 2H, phenyl H-2 and H-6), 6.86 (t, $J = 7.3$ Hz, 1H, phenyl H-4), 4.35 (s, 2H, dihydroisoindol-1-one CH_2), 4.32–4.18 (m, 1H, cyclohexane axial H-1), 3.30–3.16 (m, 4H, piperazine $2CH_2$), 2.85–2.71 (m, 4H, piperazine $2CH_2$), 2.50–2.36 (m, 1H, cyclohexane axial H-4), 2.16–1.90 (m, 4H, cyclohexane equatorial H's), 1.70–1.48 (m, 4H, cyclohexane axial H's). **26**·2HCl: colorless crystals, mp 255–257 °C. Anal. ($C_{24}H_{29}N_3O\cdot 2HCl$) C, H, N.

5.1.25. 2-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-2,3-dihydro-1*H*-isoindol-1-one (27). Compound **27** was prepared by the general procedure in 52% yield as colorless crystals, mp 111–113 °C, $R_f = 0.10$ (SiO_2, $CHCl_3$/CH_3OH = 49/1); ^1H NMR (60 MHz) δ 8.0–7.7 (m, 1H, Ar-H), 7.6–7.0 (m, 4H, Ar-H), 7.0–6.6 (m, 3H, Ar-H), 4.3 (s, 2H, dihydroisoindol-1-one CH_2), 3.6 (t, $J = 6$ Hz, 2H, $-CH_2-$dihydroisoindol-1-one), 3.3–2.9 (m, 4H, piperazine $2CH_2$), 2.7–2.2 (m, 6H, piperazine $2CH_2$ and $-CH_2-$piperazine), 1.9–1.3 (m, 4H, $-CH_2-(CH_2)_2-CH_2-$). **27**·HCl: colorless crystals, mp 209–211 °C. Anal. ($C_{22}H_{26}N_3OCl\cdot HCl$) C, H, N.

5.1.26. *trans*-2-{4-[4-(3-Chlorophenyl)piperazin-1-yl]cyclohexyl}-2,3-dihydro-1*H*-isoindol-1-one (28). Compound **28** was prepared by the general procedure in 78% yield as colorless crystals, mp 214–216 °C, $R_f = 0.32$ (SiO_2, $CHCl_3$/CH_3OH = 19/1); ^1H NMR (300 MHz) δ 7.89 (dd, $J = 6.7$, 1.8 Hz, 1H, dihydroisoindol-1-one H-7), 7.59–7.46 (m, 3H, dihydroisoindol-1-one H-4, H-5 and H-6), 7.19 (t, $J = 8.1$ Hz, 1H, phenyl H-5), 6.91 (t, $J = 2.1$ Hz, 1H, phenyl H-2), 6.86–6.79 (m, 2H, phenyl H-4 and H-6), 4.38 (s, 2H, dihydroisoindol-1-one CH_2), 4.35–4.22 (m, 1H, cyclohexane axial H-1), 3.30–3.18 (m, 4H, piperazine $2CH_2$), 2.82–2.72 (m, 4H, piperazine $2CH_2$), 2.50–2.40 (m, 1H, cyclohexane axial H-4), 2.16–1.95 (m, 2H, cyclohexane equatorial H's), 1.76–1.50 (m, 4H, cyclohexane axial H's). **28**·HCl·0.8H_2O: colorless crystals, mp 269–271 °C. Anal. ($C_{24}H_{28}N_3OCl\cdot HCl\cdot 0.8H_2O$) C, H, N.

5.1.27. 2-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]butyl}-2,3-dihydro-1*H*-isoindol-1-one (29). Compound **29** was prepared by the general procedure in 34% yield as a yellow oil, $R_f = 0.21$ (SiO_2, ethyl acetate/CH_3OH = 9/1); ^1H NMR (60 MHz) δ 7.9–7-6 (m, 1H, Ar-H), 7.7–7.2 (m, 3H, Ar-H), 7.2–6.8 (m, 4H, Ar-H), 4.3 (s, 2H, dihydroisoindol-1-one CH_2), 3.6 (t, $J = 6$ Hz, 2H, $-CH_2-$dihydroisoindol-1-one), 3.3–2.9 (m, 4H, piperazine $2CH_2$), 2.7–2.0 (m, 6H, piperazine $2CH_2$ and $-CH_2-$piperazine), 2.0–1.3 (m, 4H, $-CH_2-(CH_2)_2-CH_2-$). **29**·HCl·0.25H_2O: colorless crystals, mp 203–205 °C. Anal. ($C_{23}H_{26}N_3OF_3\cdot HCl\cdot 0.25H_2O$) C, H, N.

5.1.28. *trans*-2-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}-2,3-dihydro-1*H*-isoindol-1-one (30). Compound **30** was prepared by the general procedure in 81% yield as colorless crystals, mp 187–188 °C, $R_f = 0.18$ (SiO_2, $CHCl_3$/CH_3OH = 97/3); ^1H NMR (300 MHz) δ 7.85 (dd, $J = 6.9$, 1.8 Hz, 1H, dihydroisoindol-1-one H-7), 7.56–7.42 (m, 3H, dihydroisoindol-1-one H-4, H-5 and H-6), 7.34 (t, $J = 7.9$ Hz, 1H, phenyl H-5), 7.11 (s, 1H, phenyl H-2), 7.10–7.02 (m, 2H, phenyl H-4 and H-6), 4.35 (s, 2H, dihydroisoindol-1-one CH_2), 4.33–4.18 (m, 1H, cyclohexane axial H-1), 3.33–3.20 (m, 4H, piperazine $2CH_2$), 2.87–2.70 (m, 4H, piperazine $2CH_2$), 2.51–2.35 (m, 1H, cyclohexane axial H-4), 2.15–1.91 (m, 4H, cyclohexane equatorial H's), 1.70–1.47 (m, 4H, cyclohexane axial H's). **30**·HCl·H_2O: colorless crystals, mp 259–261 °C. Anal. ($C_{25}H_{28}N_3OF_3\cdot HCl\cdot H_2O$) C, H, N.

5.2. 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 non-linear regression (Prism, GrafPad Software Inc., San Diego, USA), using the Cheng–Prusoff equation[29] to calculate K_i values.

5.2.1. Serotonin 5-HT$_{1A}$, 5-HT$_7$, and α_1-adrenergic binding assays. Radioligand studies with native 5-HT$_{1A}$, 5-HT$_7$, and α_1-adrenergic receptors were conducted according to the methods previously described by us[5,30,31] Briefly: 5-HT$_{1A}$ assays used rat hippocampal membranes, [^{3}H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals) and 5-HT, for non-specific binding; 5-HT$_7$ assays used rat hypothalamic membranes, [^{3}H]-5-CT (102.0 Ci/mmol, Amersham) and serotonin, for non-specific binding; α_1 assays used rat cortical membranes, [^{3}H]-prazosine (25.0 Ci/mmol, Amersham), and phentolamine for non-specific binding.

5.3. 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 room temperature (20 ± 1 °C) on a natural day–night cycle (April–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.) and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexane-carboxamide trihydrochloride (WAY

100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were used as aqueous solutions. Compounds **5**–**10** and **13**–**30** were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc), compounds **5**–**10** and **13**–**30** 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).

5.3.1. Body temperature in mice. The effect of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) was 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 investigated compounds was tested. WAY 100635 was administered 15 min before compounds **5**–**10** and **13**–**30**, 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.

5.3.2. Lower lip retraction in rats. The lower lip retraction (LLR) was assessed according to the method described by Berendsen et al.[19] The rats were individually placed in cages ($30 \times 25 \times 25$ 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, and 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.

5.3.3. Conflict drinking test (Vogel test) in rats. A modification of the method of Vogel et al.[21] was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a plexiglass box ($27 \times 27 \times 50$ cm), equipped with a grid floor of stainless steel bars and with a drinking bottle containing tap water. After the initial adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for a further 10 min adaptation period during which they had free access to the drinking bottle. Afterwards, they were allowed a 30 min free-drinking session in their home cage. After another 24 h period of water deprivation, the rats (those that drank water the day before) were placed again in the test chamber and allowed to drink for 30 s. Immediately afterwards, their drinking attempts were punished with an electric shock (0.5 mA). Impulses were released every 2 s (timed from the moment when a preceding shock was delivered) in 1 s periods, between the grid floor and the spout of the drinking bottle. The number of shocks accepted throughout a 5 min experimental session was counted by an experimenter who observed a behavioral reaction (e.g., body jerks) of rats to an electric shock. The tested compounds were administered 60 min before the test.

5.3.4. Shock threshold and free-drinking tests. To control the possibility of drug-induced changes in the perception of a stimulus or in the thirst drive, which might have contributed to the activity in the conflict drinking test, stimulus threshold measurements and a free-drinking experiment were also carried out. In both these cases, the rats were treated before the experiment in the same manner as described in the conflict drinking test, including two 24 h water deprivation periods separated by 30 min of water availability. In the shock threshold test, the rats were placed individually in the box and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise at 15 s shock-free intervals by increasing manually the current (0.1, 0.2, 0.3, 0.4, and 0.5 mA); the shock lasted for 1 s and was delivered through the grid floor until a rat showed an avoidance reaction (jump, or jerk) to the electric stimulus.

In the free-drinking test, each animal was allowed to drink from the water spout. Licking was not punished. The total amount of the water (ml) consumed during 5 min was recorded for each rat. The tested compound was administered 60 min before tests.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.09.060.

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