Flexible and corresponding conformationally constrained arylpiperazines: synthesis, binding to serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, $_1$-adrenergic and dopaminergic D$_2$ receptors, and in vivo 5-HT$_{1A}$ functional characteristics

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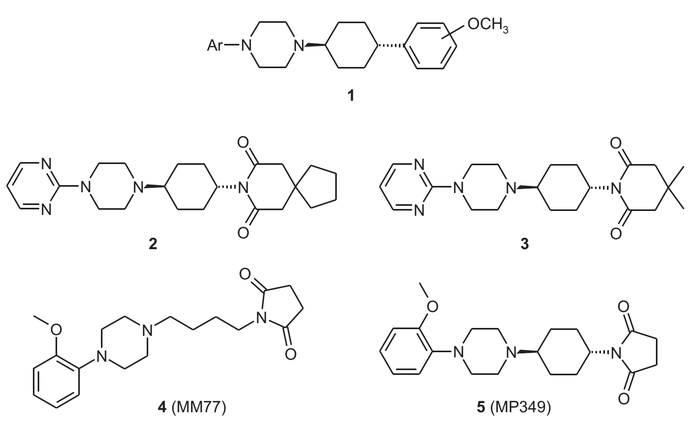
INTRODUCTION

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 respect of long-chain arylpiperazines (LCAPs), this approach has been rarely used 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 and multiple bonds, whereas in more rigid derivatives, a polymethylene chain was incorporated in a cyclic ring [1-6].

Recently, Perrone et al. described trans-4-[4-(methoxyphenyl)-cyclohexyl]-1-arylpiperazines (**1**) as a new class of 5-HT$_{1A}$ receptor ligands showing high affinity (*K*i ~0.02 nM) and selectivity for the dopaminergic D$_2$ and $_1$-adrenergic receptors [3]. A few selected compounds examined in the [^{35}S]GTP S binding assay at the human cloned 5-HT$_{1A}$ receptors demonstrated full or partial agonistic properties. On the other hand, similar structural rigidification 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) [5].

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 [2,4]. 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 [7,8]), turned out to be a highly potent, full (pre- and postsynaptic) 5-HT$_{1A}$ receptor antagonist [4,8]. Moreover, it revealed pronounced selectivity (at least 150-fold to 5-HT$_{2A}$, D$_1$, D$_2$ and benzodiazepine and 15-fold to $_1$ receptors) and - like a parent compound - also demonstrated anxiolytic-like activity in some animal models [8].

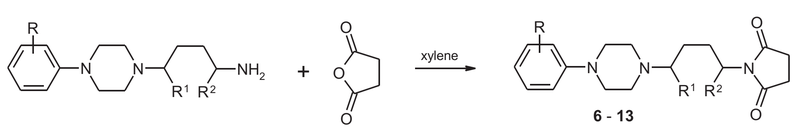


All the above-mentioned rigid derivatives of the highly active flexible 5-HT$_{1A}$ receptor agents, shared a common linear polycyclic structure. Despite the fact that the extended conformation of N1-substituted N4-arylpiperazines is postulated to be bioactive, these compounds presented diverse affinity 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$_{2A}$, D$_2$ and $_1$ receptors was investigated.

CHEMISTRY

The structures of the investigated compounds are shown in Table 1, and the way of their synthesis is illustrated below. 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 **7**, **9** and **11**-**13**, 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]piperazine2 and 1-(2-methoxyphenyl)-4-[4-(2-succinimido)cyclohexyl]piperazine [4].



R = H, *m*-Cl, *m*-CF$_3$, *m*-OCH$_3$, *p*-OCH$_3$

R^1 = R^2 = H or R^1, R^2 = —(CH$_2$)$_2$—

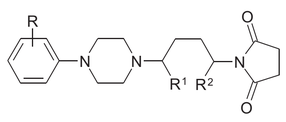
PHARMACOLOGY

The compounds were tested in competition binding experiments for native serotonin 5-HT$_{1A}$, 5-HT$_2$, $_1$-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. The hypothermia produced by the compounds tested in mice (and reduced by WAY100635) 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, i.e. flat body posture (FBP) and forepaw treading (FT), in reserpinized rats was tested. 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.

Table 1. Structure and binding affinity data on serotonin (5-HT$_{1A}$, 5-HT$_{2A}$), $_1$-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$_{2A}$		$_1$		D$_2$	
4 (MM77)	*o*-OMe	H	H	6.4	0.3*	1510	95*	11.9	1*	490	50*
5 (MP349)	*o*-OMe	–(CH$_2$)$_2$–		15.2	3.2**	11575	20**	234	15**	2606	160**
6	H	H	H	7.4	0.3	1100	50	117	10	15000	1900
7	H	–(CH$_2$)$_2$–		43	6	2560	60	162	26	14000	2400
8	*m*-Cl	H	H	32	2	121	14	60	5	7800	600
9	*m*-Cl	–(CH$_2$)$_2$–		5.4	0.9	440	20	23	8	5300	120
10	*m*-CF$_3$	H	H	21	1	245	28	483	36	31000	2100
11	*m*-CF$_3$	–(CH$_2$)$_2$–		4	0.5	462	18	505	62	27000	1900
12	*m*-OMe	–(CH$_2$)$_2$–		27	2	3450	110	230	18	NT	
13	*p*-OMe	–(CH$_2$)$_2$–		145	15	18600	200	1930	32	NT	

*Ref. 10, **Ref. 4. NT - not tested.

RESULTS AND DISCUSSION

The affinity of phenylpiperazine analogues, in which an *o*-methoxy group of MM77 and MP349 has been removed (**6**, **7**) or replaced with *m*-Cl (**8**, **9**) or *m*-CF$_3$ (**10**, **11**) 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, i.e. 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 **12** and **13** 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 [9].

Regarding 5-HT$_{2A}$ receptors, new arylpiperazines were at least 60 times less active, except for the two flexible *m*-Cl (**8**) and *m*-CF$_3$ (**10**) 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 results of the $_1$ 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 $_1$ receptors, and were thus unselective 5-HT$_{1A}$/ $_1$ ligands. Improved selectivity was obtained in the case of *m*-CF$_3$-substituted analogues (S $_1$/5-HT$_{1A}$ = 23 and 126 for **10** and **11**, respectively), since a significant reduction in their $_1$ 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 it comes out of the above results, rigidification of the investigated group of LCAPs maintaining high 5-HT$_{1A}$ receptor affinity, had weaker influence on 5-HT$_{2A}$ receptors, and practically did not change the affinity for $_1$ 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 [4]. However that conclusion cannot be generalized, since the similarly constrained compounds (**1**) reported by Perrone et at., evaluated in in vitro assays, were classified as agonists or partial agonists [3]. Nevertheless, those ligands were devoid of an imide portion in the terminal fragment and contained various arylpiperazine moieties. Interestingly, of all the investigated compounds, 1-(2-methoxyphenyl)piperazine derivative showed the weakest agonistic properties, since it only partly stimulated [^{35}S]GTPS binding (Emax = 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 to note 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 (**6**-**11**) were tested in vivo in mice and rats to establish their functional activity at pre- and postsynaptic 5-HT$_{1A}$ receptor. All the compounds produced a decrease in mouse body temperature. This effect was reduced or abolished by WAY 100635 in the case of **6**-**9** only, hence those compounds were classified as agonists of presynaptic 5-HT$_{1A}$ receptors.

In behavioral models used to assess the function at postsynaptic 5-HT$_{1A}$ receptors, compounds **6**-**11** given alone induced LLR in rats; moreover, **8**-**11** produced flat body posture (FBP) and forepaw treading (FT) in reserpinized rats.

On the other hand - like partial agonists of postsynaptic 5-HT$_{1A}$ receptors - compounds **6**-**9** attenuated both symptoms of behavioral syndrome, and **6**, **8** and **9** inhibited LLR induced by 8-OH-DPAT. The *m*-CF$_3$ derivatives **10** and **11** 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 **6**-**11** can be classified as partial agonists of postsynaptic 5-HT$_{1A}$ receptors, and that the intrinsic activity of **10** and **11** is higher than that of **6**-**9**.

As can be inferred from our functional in vivo study, the effects induced by **6**-**11** were not identical, however, similar within pairs of the tested ligands, i.e. flexible and constrained analogues showed the same functional activity at pre- and postsynaptic sites (Table 2). 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 **6**-**11** 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 [4,8,10,11]. These 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 is bound to occur about the role of a position of a methoxy group in the aromatic ring in functional activity of **5**. For that reason, the *meta* isomer (**12**) was additionally evaluated. In vivo experiments demonstrated that **12** (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 13 (*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 2. 5-HT$_{1A}$ receptor functional profile of the tested compounds

Compound	Presynaptic	Postsynaptic
4 (MM77)	–	antagonist
5 (MP349)	antagonist	antagonist
6	agonist	partial agonist
7	agonist	partial agonist
8	agonist	partial agonist
9	agonist	partial agonist
10	–	partial agonist
11	–	partial agonist

CONCLUSIONS

A series of 1-aryl-4-[4-(2-succinimido)butyl]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$_{2A}$) $_1$-adrenergic and dopaminergic D$_2$ receptors.

The new compounds **6**-**12** exhibited nanomolar 5-HT$_{1A}$ receptor affinity (Ki = 4-44 nM) , which indicates that the extended linear arrangement, frozen in a cyclohexane ring, reflects the most probable bioactive conformation of flexible molecules. Their affinity for other receptors was: controlled by substituents in the aromatic ring ($_1$), or influenced also by rigidification (5-HT$_{2A}$), or very low (D$_2$, *K*i = 5.3-31 M).

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 (**10** and **11**) 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 connected with the engagement of o-methoxy group in ligand-receptor interactions.

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