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Original article

Active conformation of some arylpiperazine postsynaptic 5-HT_{1A} receptor antagonists

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Abstract

The synthesis and pharmacological properties of novel conformationally restricted arylpiperazine (**2b**–**4b**) or 1,2,3,4-tetrahydroisoquinoline (**5b** and **6b**) derivatives of the known, flexible 5-HT_{1A} receptor ligands **2a**–**6a** ($K_i = 0.95$–7 nM) with different intrinsic activities are reported. Replacement of a tetramethylene chain with a 1e,4e-disubstituted cyclohexane ring in the structure of flexible ligands resulted in insignificant diminution of the 5-HT_{1A} receptor affinity in the case of **2b**–**4b** ($K_i = 15$–52 nM), whereas derivatives **5b** and **6b** were practically inactive ($K_i > 1354$ nM). The results of in vivo behavioural tests showed that **2a** and **3a** acted as postsynaptic 5-HT_{1A} receptor partial agonists. Like the flexible **4a**, the new rigid compounds **2b**–**4b** showed features of postsynaptic 5-HT_{1A} receptor antagonists. Since all possible conformations of the constrained compounds belong to an extended family—as indicated by molecular modelling studies—our hypothesis that such conformations are responsible for the blockade of postsynaptic 5-HT_{1A} receptors has been confirmed. Determination of regions explored by terminal amide, or imide and hydrocarbon groups of the restricted compounds as well as the results of in vitro and in vivo studies allowed us to discuss the bioactive conformations of flexible ligands.

Keywords: postsynaptic 5-HT_{1A} antagonists; rigid analogs of arylpiperazine 5-HT_{1A} receptor ligands; conformational analysis

1. Introduction

Arylpiperazines are of great importance to many different biological targets, especially central nervous system receptors. In the case of serotonin (5-HT) receptors, compounds containing this moiety represent the biggest and thoroughly examined class of 5-HT_{1A} receptor ligands. However, simple arylpiperazines are classified as non-selective 5-HT receptors (and other ones) ligands, 4-substituted derivatives show good selectivity and high affinity for 5-HT_{1A} receptors. The majority of them contain a flexible aliphatic chain of different length, which connects the arylpiperazine fragment with the second terminal pharmacophoric group. Structural modifications within long-chain arylpiperazines (LCAPs) occur mainly at the two opposite ends of a molecule and have been described in many original papers [1–6] and reviews [7]. Aliphatic chain modifications have also been extensively studied. At first, the influence of the aliphatic chain length of some simple 4-alkyl-1-arylpiperazines on their 5-HT_{1A} receptor activity has been discussed [8,9]. Additionally, in a number of series of arylpiperazine ligands, the aliphatic chain length has been optimized with respect to the relative position of the above mentioned basic pharmacophoric groups [6]. Moreover, groups other than methylene, i.e. heteroatoms (O, NH, S) [10–13], carbonyl or the amide fragment [14–20], and multiple bonds [21] have also been introduced to the spacer. Although some of these modifications increase the rigidity of a link chain, the whole molecule still remains flexible, so a prediction of its conformation within the receptor pocket is highly speculative. In other classes of 5-HT_{1A} receptor ligands (ergolines, tryptamines, aminotetralins with the exception of aryloxyalkylamines) such problem does not exist, and the conformationally constrained model ligands have already been described [22–25].

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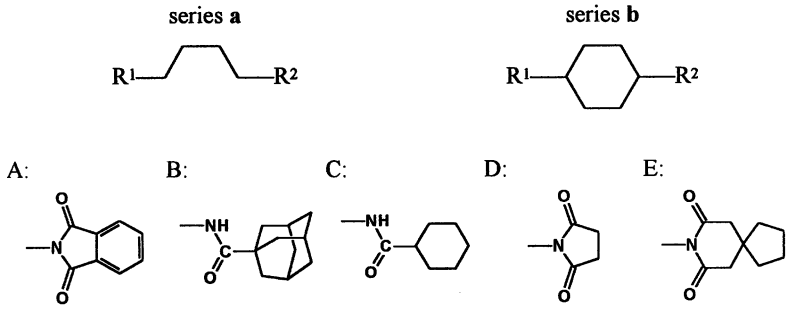
Among LCAPs there are compounds which show different 5-HT_{1A} receptor functional activities, i.e. agonistic, partial agonistic or antagonistic one. The most frequently investigated member of the LCAPs is buspirone (Buspar®, Bristol–Myers Squibb), used in the treatment of anxiety (a second generation anxiolytic). Arylpiperazine 5-HT_{1A} receptor agents such as gepirone, ipsapirone, tandospirone, flesinoxan and many others, in various phases of clinical studies are regarded as potential drugs in the therapy of anxiety, depression, Alzheimer's disease, learning and memory disfunctions etc. [26,27]. Moreover, several other agents, such as, e.g. NAN-190 (**1a**), WAY 100635 or MP 3022, are frequently used as pharmacological tools [28–30]. It is noteworthy that the majority of the above mentioned compounds contain a flexible, 4-membered aliphatic spacer. Hence our general concept was to generate rigid analogues of well-known 5-HT_{1A} receptor agents with limited conformational freedom by replacing the aliphatic chain with a 1,4-substituted cyclohexane ring. Our earlier studies [31] allowed us to determine a bioactive conformation of **1a**, a well-known antagonist of postsynaptic 5-HT_{1A} receptors, using this constrained analogues approach. These findings encouraged us to extend our studies to other 5-HT_{1A} receptor ligands.

At present we wish to report a synthesis of conformationally restricted derivatives (**2b**–**6b**, Table 1) of the five, previously described, active in vivo 5-HT_{1A} receptor ligands (**2a**–**6a**). We chose two postsynaptic antagonists (**2a** and **4a**) [32,33] and three partial agonists (**3a**, **5a** and **6a**) [15,34,35] which contain a 4-(2-methoxyphenyl)-1-piperazinyl (oMPP) fragment (**2a**, **3a** and **4a**) or a 1,2,3,4-tetrahydroisoquinolin-2-yl (THIQ) moiety (**5a** and **6a**)—a tool system recently used for studying the ligand–5-HT_{1A} receptor interactions [35]. The aim of the present study was to determine the influence of conformational constraints in biologically

Table 1
Structure and binding data on the 5-HT_{1A} receptors of the investigated compounds



Compound	R^1	R^2	$K_i \pm$ SEM (nM)
1a	oMPP	A	0.55 ± 0.14[a]
1b	oMPP	A	8 ± 2[a]
2a	oMPP	B	7 ± 1[b]
2b	oMPP	B	52 ± 2
3a	oMPP	C	4.0 ± 0.2[c]
3b	oMPP	C	44 ± 5
4a	oMPP	D	6.4 ± 0.3[d]
4b	oMPP	D	15 ± 3
5a	THIQ	B	0.95 ± 0.04[e]
5b	THIQ	B	1354 ± 4
6a	THIQ	E	5.0 ± 0.2[f]
6b	THIQ	E	2356 ± 156

[a] Data from ref [31]. [b] The result reported in ref [32] is 0.4±0.03. [c] The result reported in ref [15] is 0.6±0.1. [d] Data from ref [33]. [e] Data from ref [34]. [f] Data from ref [35].

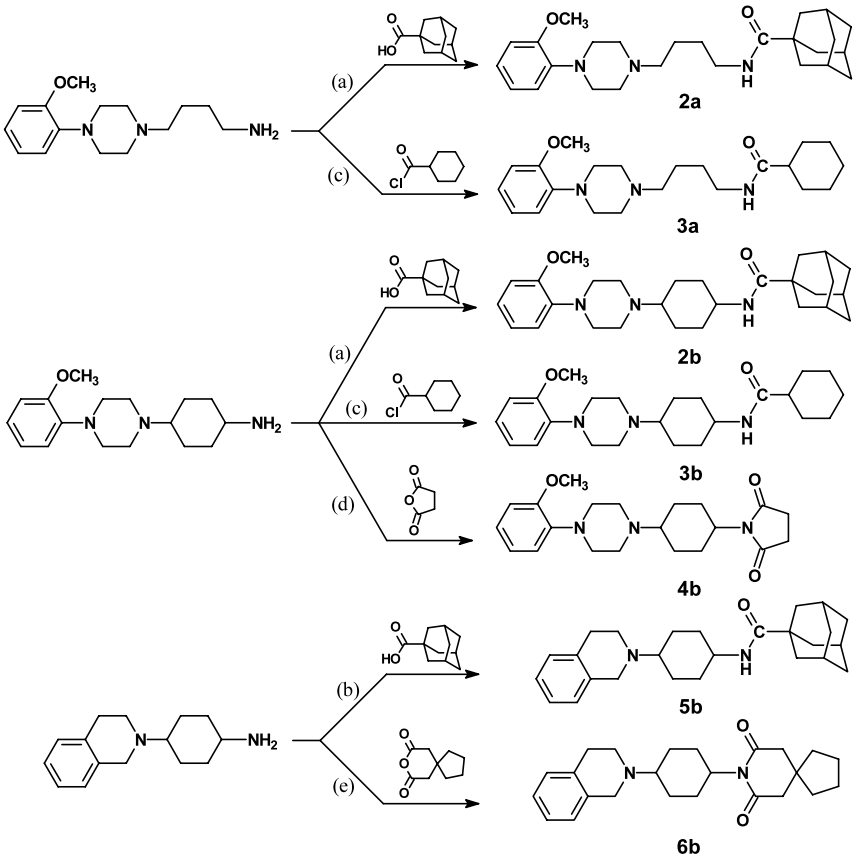


Fig. 1. Reagents and conditions: (a) Ph_3P, NBS, Et_3N, CH_2Cl_2, 0–25 °C; (b) BOP, Et_3N, CH_3CN, room temperature; (c) 20% K_2CO_3–$CHCl_3$, room temperature; (d) xylene, reflux; (e) pyridine, reflux.

active structures on their 5-HT_{1A} receptor binding and intrinsic activity. On the basis of pharmacological and conformational analysis results we were able to discuss bioactive conformations of the investigated compounds.

2. Chemistry

Synthetic routes to the target compounds, i.e. flexible **2a** and **3a**, as well as the new constrained arylpiperazines **2b**–**6b**, prepared in this work, are showed in Fig. 1. The synthesis of starting amines had been previously described [1,31] (see Section 6). Amides **2a** and **2b** were generated in high yields under mild conditions from 1-adamantanecarboxylic acid and the corresponding amine in the presence of the equivalent amounts of triphenylphosphine, *N*-bromosuccinimide and triethylamine. Amide **5b** was obtained by a direct reaction between 4-[2-(1,2,3,4-tetrahydroisoquinolinyl)]-cyclohexylamine and 1-adamantanecarboxylic acid in the presence of benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP). Compounds **3a** and **3b** were prepared by simple acylation of the respective amine in an alkaline medium. Imides **4b** and **6b** were yielded upon a prolonged reflux of the appropriate substituted cyclohexylamine with succinic or 3,3-tetramethyleneglutaric anhydride. The structures of all those new compounds were elucidated from their analytical and spectroscopic data. 2D ^{1}H-NMR spectra and decoupling experiments allowed us to calculate the appropriate coupling constants in the cyclohexane ring, and to assign a diequatorial 1e,4e-chair conformation for compounds **2b**–**6b**. For pharmacological assays, the investigated compounds were converted into hydrochloride or fumarate salts.

3. Pharmacological results

3.1. In vitro experiments

The affinity at 5-HT_{1A} receptors for all the newly synthesized compounds **2b**–**6b**, as well as for the previously described reference arylpiperazines **2a** [32] and **3a** [15] was determined by standard competitive displacement assays using rat brain hippocampus with [^{3}H]-8-OH-DPAT, a 5-HT_{1A} receptor agonist, as a competitive ligand. The results of those assays and the previously published data from our laboratory on compounds **1a**, **1b** and **4a**–**6a** are displayed in Table 1.

Flexible arylpiperazines **2a** and **3a** showed a high nanomolar affinity ($K_i = 7$ and 4 nM, respectively); however, the K_i values for those compounds reported by Glennon and coworkers [15,32] were slightly lower (0.4 and 0.6 nM, respectively). Rigid arylpiperazines **2b**–**4b** demonstrated a good 5-HT_{1A} receptor affinity ($K_i = 15–52$ nM), whereas tetrahydroisoquinoline derivatives **5b** and **6b** were practically inactive ($K_i >$ 1354 nM).

3.2. In vivo experiments

Compounds which showed a 5-HT_{1A} receptor affinity up to $K_i = 52$ nM were tested in vivo. To determine postsynaptic 5-HT_{1A} agonistic effects of the tested compounds, their ability to induce lower lip retraction (LLR) in rats and behavioural syndrome, i.e. flat body posture (FBP) and forepaw treading (FT), in reserpinized rats was tested [36,37]. The ability of the investigated compounds to inhibit those symptoms produced by the 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT) was regarded as a postsynaptic antagonistic activity.

Table 2
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^{-1})	Mean ± S.E.M. LLR score	
		A	B
Vehicle		0.1 ± 0.1	2.8 ± 0.1
2a	8	0.6 ± 0.4	1.1 ± 0.2 [a]
	16	0.9 ± 0.2 [b]	0.6 ± 0.2 [a]
Vehicle		0.1 ± 0.1	2.8 ± 0.1
2b	8	0.0 ± 0.0	1.5 ± 0.1 [a]
	16	0.0 ± 0.0	0.9 ± 0.3 [a]
Vehicle		0.1 ± 0.1	2.7 ± 0.1
3a	8	1.3 ± 0.1 [c]	1.5 ± 0.2 [a]
	16	2.0 ± 0.3 [c]	1.1 ± 0.2 [a]
Vehicle		0.1 ± 0.1	2.9 ± 0.1
3b	4	0.2 ± 0.1	1.1 ± 0.2 [a]
	8	0.1 ± 0.1	0.6 ± 0.3 [a]
Vehicle		0.1 ± 0.1	2.7 ± 0.1
4a [d]	4	0.1 ± 0.1	1.6 ± 0.1 [a]
	8	0.1 ± 0.1	1.1 ± 0.2 [a]
	16	0.0 ± 0.0	0.3 ± 0.1 [b]
Vehicle		0.1 ± 0.1	2.8 ± 0.1
4b	0.125	0.1 ± 0.1	1.8 ± 0.8 [e]
	0.25	0.0 ± 0.0	1.2 ± 0.2 [a]
	0.5	0.1 ± 0.1	0.7 ± 0.1 [b]

[a] $P < 0.01$ versus vehicle + 8-OH-DPAT.
[b] $P < 0.05$ versus vehicle.
[c] $P < 0.01$ versus vehicle.
[d] Data from Ref. [33].
[e] $P < 0.05$ versus vehicle + 8-OH-DPAT.

3.2.1. Lower lip retraction in rats

Compounds **2a** and **3a**, given alone in doses of 8–16 mg kg^{-1}, induced LLR in rats, the maximum score being 30 and 67%, respectively, at the highest doses used. The remaining compounds **2b** (8–16 mg kg^{-1}), **3b** (4–8 mg kg^{-1}), the previously described **4a** (4–16 mg kg^{-1}) [33] and **4b** (0.125–0.5 mg kg^{-1}), given alone, showed no activity in that test (Table 2A). At the same time, **2a**, **2b**, **3a** (8–16 mg kg^{-1}), **3b** (4–8 mg kg^{-1}), the previously investigated **4a** (4–16 mg kg^{-1}) [33] and **4b** (0.125–0.5 mg kg^{-1}) inhibited the LLR induced by 8-OH-DPAT in rats in a dose-dependent manner. At the highest doses used, those compounds attenuated the effect of 8-OH-DPAT by 59 (**3a**)–89% (**4a**) (Table 2B).

3.2.2. Behavioural syndrome in reserpinized rats

Of the tested compounds, **2a** (8–16 mg kg^{-1}) and **3a** (4–8 mg kg^{-1}) administered alone induced FBP (but not FT) in reserpine-pretreated rats, the maximum score being 62 and 36%, respectively, after the highest doses used. Compounds **2b** (8–16 mg kg^{-1}), **3b** (4–8 mg kg^{-1}), the previously investigated **4a** (4–16 mg kg^{-1}) [33] and **4b** (0.125–0.5 mg kg^{-1}) did not evoke changes in the behaviour in reserpinized rats (Table 3A). The 8-OH-DPAT-induced FBP and FT were dose-dependently reduced by **2a** (8–16 mg kg^{-1}), **3a**, **3b** (4–8 mg kg^{-1}), the previously described **4a** (4–16 mg kg^{-1}) [33] and **4b** (0.125–0.5 mg kg^{-1}). An almost complete blockade of the behavioural symptoms induced by 8-OH-DPAT was observed after administration of **4a** and **4b** at the highest doses (Table 3B).

4. Molecular modelling and discussion

In order to better understand structural requirements for a compound to interact with the 5-HT_{1A} receptor, in the present paper we have described new, constrained analogues of five known 5-HT_{1A} agents. The synthesis of these compounds extends the group of 5-HT_{1A} receptor ligands which may be used for studying ligand–receptor interactions. Introduction of a 1,4-disubstituted cyclohexane ring in place of the flexible and stereochemically difficult to define tetramethylene chain significantly limits the spatial arrangement of pharmacophoric fragments in ligands; however, some conformational freedom still exists (Fig. 2). In Fig. 3, possible rotations in the molecule were marked by the respective dihedral angles τ_1, τ_2, τ_3 or τ_4. It is generally accepted that bioactive conformation of the oMPP fragment is perpendicular; therefore, like in our previous paper, we fixed angle $\tau_1 = 180°$. The remaining rotations were studied by a semi-empirical AM1 method, and the resultant energy profiles are presented in Fig. 3. All possible minimum energy

Table 3
Induction of behavioural syndrome by the investigated compounds (A) and their effect on the 8-OH-DPAT-induced behavioural syndrome (B) in reserpine-pretreated rats

Treatment	Dose (mg kg^{-1})	Mean ± S.E.M. behavioural score			
		A		B	
		Flat body posture	Forepaw treading	Flat body posture	Forepaw treading
Vehicle		0.2 ± 0.1	0.2 ± 0.2	15.0 ± 0.0	13.0 ± 0.7
2a	8	5.0 ± 1.5 [a]	0.3 ± 0.2	6.8 ± 1.1 [b]	8.5 ± 1.4 [b]
	16	9.3 ± 1.7 [a]	0.8 ± 0.3	4.0 ± 1.4 [b]	3.2 ± 0.5 [b]
Vehicle		0.2 ± 0.1	0.2 ± 0.2	15.0 ± 0.0	13.0 ± 0.7
2b	8	0.0 ± 0.0	0.0 ± 0.0	12.2 ± 1.4	11.8 ± 0.7
	16	0.0 ± 0.0	0.0 ± 0.0	11.4 ± 1.5	13.6 ± 0.7
Vehicle		0.2 ± 0.1	0.2 ± 0.2	13.4 ± 0.8	12.8 ± 0.7
3a	4	5.2 ± 0.9 [a]	0.1 ± 0.1	11.5 ± 1.1	7.5 ± 0.8 [b]
	8	5.4 ± 0.7 [a]	0.0 ± 0.0	8.0 ± 1.4 [c]	3.8 ± 1.3 [b]
Vehicle		0.2 ± 0.1	0.2 ± 0.2	13.4 ± 0.8	12.8 ± 0.7
3b	4	0.1 ± 0.1	0.1 ± 0.1	12.4 ± 0.4	6.6 ± 1.0 [b]
	8	0.1 ± 0.1	0.0 ± 0.0	6.0 ± 1.1 [b]	2.8 ± 0.7 [b]
Vehicle		0.2 ± 0.1	0.2 ± 0.1	15.0 ± 0.0	12.3 ± 1.0
4a [d]	4	0.2 ± 0.2	0.1 ± 0.1	11.8 ± 1.3 [c]	8.3 ± 1.5
	8	0.1 ± 0.1	0.1 ± 0.1	2.3 ± 1.0 [b]	6.8 ± 1.6 [c]
	16	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0 [b]	1.3 ± 0.7 [b]
Vehicle		0.2 ± 0.1	0.2 ± 0.2	14.8 ± 0.2	13.0 ± 0.9
4b	0.125	0.0 ± 0.0	0.1 ± 0.1	13.0 ± 0.5	7.5 ± 1.4 [b]
	0.25	0.1 ± 0.2	0.1 ± 0.1	11.7 ± 0.7 [c]	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]

[a] $P < 0.01$ versus vehicle.
[b] $P < 0.01$ versus vehicle + 8-OH-DPAT.
[c] $P < 0.05$ versus vehicle + 8-OH-DPAT.
[d] Data from Ref. [33].

conformations were constructed and optimized on the basis of those calculations (see Table 4).

Although the number of predominant conformations of compounds **2b** and **3b** are 16 and 24, respectively, all of them, as well as these of compounds **4b**–**6b**, belong to a family of extended conformations. Since neither differences in the energy of individual conformers (1.96–4.27 kcal mol^{-1}) nor energy barriers of the analysed rotatable bonds (up to 4 kcal mol^{-1}) are very high, we assumed that each of the presented conformations of the investigated compounds may interact with the 5-HT$_{1A}$ receptor. Hence the entire group of low-energy conformers for each investigated compound was overlapped by oMPP or THIQ fragments. Finally, we summed up the van der Waals' volumes of terminal hydrocarbons and amide/imide moieties (Table 4); in Fig. 3 these regions are marked in cyan and magenta, respectively. Thus we have determined the maximum possible space explored by the pharmacophores mentioned above.

The 5-HT$_{1A}$ receptor binding data presented in Table 1 indicate that introduction of structural rigidity to the aliphatic spacer of ligands containing the oMPP moiety (compounds **1a**–**4a**) results in a few-fold diminution of affinity (compounds **1b**–**4b**). Nevertheless, the constrained analogues are still good 5-HT$_{1A}$ receptor ligands. The same modification of ligands with the THIQ fragment (compounds **5a** and **6a**) leads to an almost complete loss of the 5-HT$_{1A}$ receptor affinity of compounds **5b** and **6b**. Their non-constrained analogues **5a** and **6a** showed a high 5-HT$_{1A}$ receptor affinity, which can be explained by a different mode of complex ligand–receptor formation. A comparison between the 5-HT$_{1A}$ receptor affinities of arylpiperazine **2b** ($K_i = 52$ nM) and tetrahydroisoquinoline **5b** ($K_i = 1354$ nM) suggests that such a big difference in their binding properties may stem from the spatial arrangement of the aromatic ring of heterocyclic amine moieties. Superimposition of both these amine fragments using a common basic nitrogen atom reveals that planes of their benzene rings are perpendicular (Fig. 3). Compound **2b** with a 1-adamantyl group shows binding affinity comparable to that of compound **3b**, which contains a cyclohexyl fragment. As indicated by molecular modelling, regions accessible to both adamantyl and cyclohexyl substituents are almost the same (341.1 and 320.1 Å^3, respectively), though their own volumes differ

significantly (135 and 83 Å^3, respectively). Thus we may state, that inspite of a large hydrophobic receptor pocket in which an adamantyl group may be placed, the cyclohexyl substituent is big enough to stabilize the ligand–5-HT$_{1A}$ receptor complex. On the other hand, rigid imide **4b** with the smallest hydrophobic part of the molecule represented by an ethylene fragment (78 Å^3) demonstrates the highest 5-HT$_{1A}$ receptor affinity. It may be concluded that in the presently described series of constrained arylpiperazines the terminal hydrophobic substituent slightly disturbs formation of the ligand–5-HT$_{1A}$ receptor complex. Although the calculated volumes accessible to an amide or an imide part of the molecule are practically the same (Table 4), the results of binding studies suggest that the imide group is preferred to the amide one at the 5-HT$_{1A}$ receptor site probably due to additional electrostatic interactions involving the second carbonyl group (compare **1b** and **4b** vs. **2b** and **3b**, Table 1).

The described results of in vivo experiments clearly show that the investigated arylpiperazine derivatives have a different intrinsic activity at postsynaptic 5-HT$_{1A}$ receptors. Flexible compounds **2a** and **3a** may be classified as partial agonists of these receptors. These data are somewhat at variance with the findings of

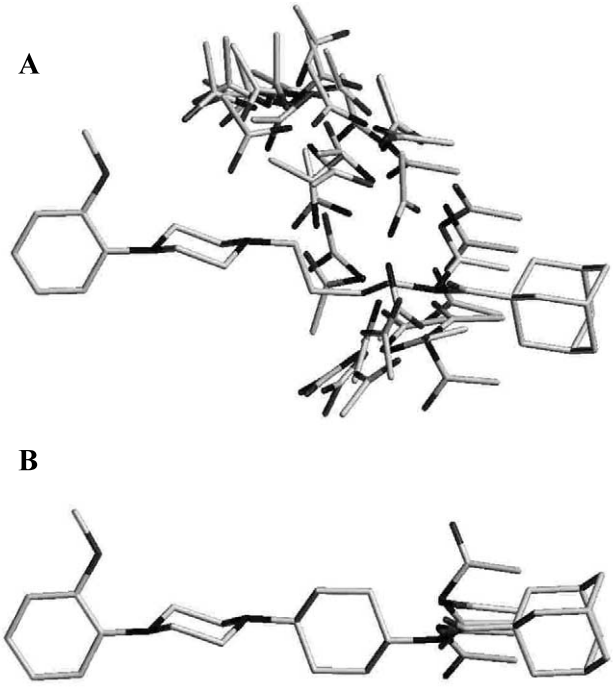


Fig. 2. Results of a conformational analysis (a random search procedure) applied to the flexible compound **2a** (A) and its constrained analogue **2b** (B). In both cases conformers were superimposed using oMPP fragments. For simplification, hydrogen atoms have been omitted and full structures are shown only for a global energy minima. Local energy minimum conformers are represented solely by N–CO–C fragments.

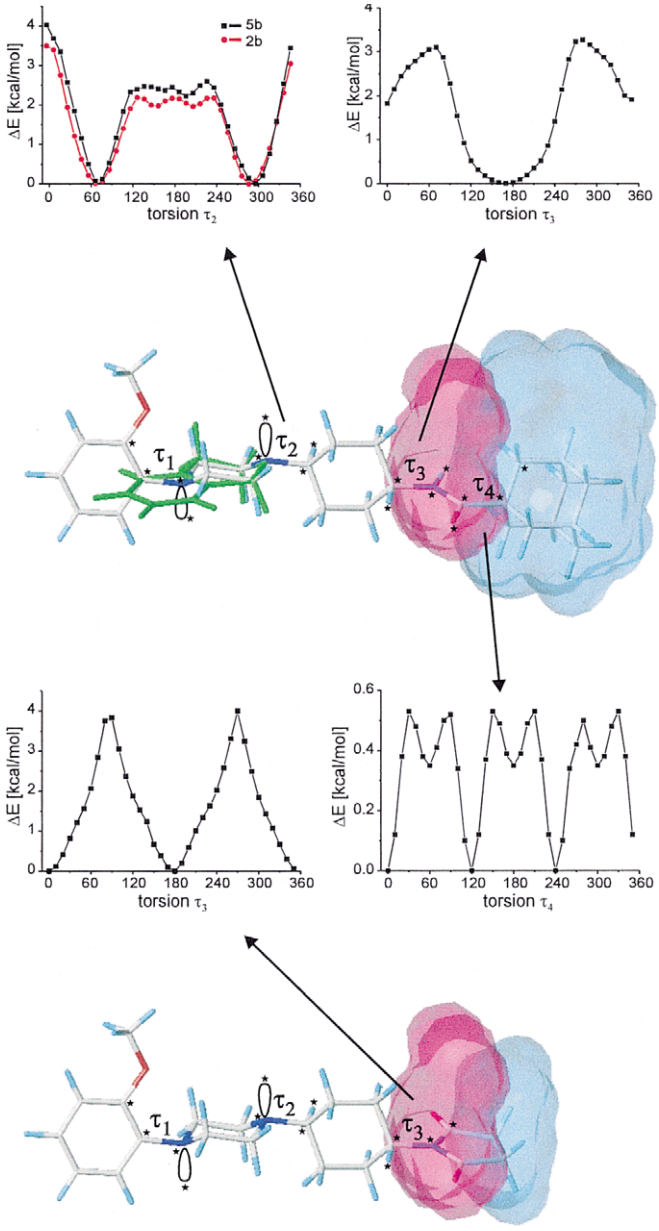


Fig. 3. Global energy minima of compounds **2b** and **4b** and a superimposition mode of the THIQ group (in green) with the oMPP fragment. The dihedral angles τ_1–τ_4 are defined, and the respective rotation energy profiles are shown. Regions penetrated by an amide (or imide) group and a terminal hydrophobic part of a molecule during rotations of τ_2, τ_3 and τ_4 are marked in magenta and cyan, respectively.

Raghupathi et al. [32] who pointed to an antagonistic activity of **2a** in a 5-HT$_{1A}$ postsynaptic coupled adenylyl cyclase assay (rat hippocampal membranes). The reason for such a discrepancy is difficult to explain, though certain differences between our experimental conditions and those provided by Raghupathi et al. [32] consisting in the use of substantially different functional models (in vivo tests vs. in vitro assays), should be taken into account. On the other hand, our results on **3a** are coherent with the previous findings of

El-Bermawy et al. [15] which showed that **3a** acted as a postsynaptic 5-HT_{1A} receptor partial agonist in an in vitro functional assay (i.e. the forskolin-stimulated adenylate cyclase, using rat hippocampal membrane homogenates). The previously described flexible compound **4a** exhibited features of a postsynaptic 5-HT_{1A} antagonist [33] in in vivo behavioural tests.

Restriction of conformational freedom in the structure of partial agonists **2a** and **3a** affected their functional profiles. Rigid analogues **2b** and **3b** demonstrated a postsynaptic 5-HT_{1A} antagonistic activity. Contrariwise, in the case of the postsynaptic 5-HT_{1A} receptor antagonist **4a**, the introduced structural modification did not change the intrinsic activity of the constrained compound **4b**. In our previous study, a similar structural modification of **1a**, a postsynaptic 5-HT_{1A} receptor antagonist, afforded its rigid analogue **1b** which represented very well a bioactive conformation of the parent compound [31]. To compare directly in vivo activities of both pairs of compounds **1a** versus **1b** and **4a** versus **4b**, we also present their ED_{50} values for inhibition of the 8-OH-DPAT-induced effects (Table 5). The ED_{50} values for **1a** were somewhat lower than those determined for **1b**, but were of the same order of magnitude [31,33]. In contrast, the ED_{50} values for **4a** [33] were distinctly higher than those for **4b**. Moreover, the ED_{50} values for the latter were extremely low; thus rigid compound **4b** turned out to be a very interesting new 5-HT_{1A} antagonist with high in vitro and in vivo potency.

5. Conclusions

Summing up, we have found that all the three new rigid oMPP derivatives (**2b**–**4b**) are postsynaptic 5-HT_{1A} receptor antagonists, whereas their flexible analogues have features of partial agonists **2a** and **3a** or antagonist **4a** of these receptors. On the basis of present studies it may be anticipated that the imide fragment of antagonist **4a** can be found within the volumes defined in Fig. 3, whereas the terminal amide and hydrocarbon groups of partial agonists **2a** and **3a** are located outside these regions. These findings may create a basis for further receptor modelling and docking studies with other flexible 5-HT_{1A} ligands of the arylpiperazine type. Moreover, on the grounds of conformational searches we have managed to confirm our hypothesis that extended conformations are responsible for the blockade of postsynaptic 5-HT_{1A} receptors.

6. Experimental

6.1. Chemistry

Melting points were determined using an Electrothermal Digital Melting Point IA9000 apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Brucker AMX 500 (500 MHz), Varian Mercury 300 (300 MHz) or Varian EM-360L (60 MHz) spectrometers, in $CDCl_3$ solutions. The chemical shifts values (δ)

Table 4
Conformation analysis results for compounds **2b**–**6b** and volumes of amide and hydrophobic regions

Compound	Number of rotatable bonds [a]	Number of conformers	ΔE [b] (kcal mol^{-1})	Volume of amide or imide region (Å^3)	Volume of hydrophobic region (Å^3)
2b	3	16	4.04	121.6	341.1
3b	3	24	4.27	126.9	320.1
4b	2	4	1.96	123.1	78.1
5b	3	12	4.12	106.9	337.6
6b	2	6	2.15	112.2	272.3

[a] Excluding rotation in the oMPP fragment.
[b] Range of differences in the energy of individual conformers.

Table 5
Inhibition of the 8-OH-DPAT-induced effects by **1a**, **1b**, **4a** and **4b**

Effect	ED_{50} (mg kg^{-1})			
	1a [a]	**1b** [a]	**4a** [b]	**4b**
Flat body posture	2.0 (1.2–3.2) [c]	6.3 (3.6–13.9)	5.4 (3.4–8.6)	0.28 (0.18–0.45)
Forepaw treading	2.5 (1.7–4.3)	2.3 (1.4–3.9)	7.0 (4.2–11.6)	0.20 (0.14–0.28)
LLR	1.7 (0.9–3.1)	7.5 (4.8–11.6)	6.0 (3.6–9.9)	0.24 (0.17–0.34)

[a] Data from Ref. [31].
[b] Data from Ref. [33].
[c] Confidence limits (95%) are given in parentheses.

were expressed in ppm relative to Me_4Si as internal standard and the coupling constants J in Hertz (Hz). When necessary, the signals were unambiguously assigned by 2D NMR ($^1H-^1H$) COSY technique or decoupled by irradiation of the NH signal frequency. The spectral data for amines refer to their free bases. All compounds were routinely checked by TLC using Merck Kieselgel 60-F_{254} sheets. Column chromatography separations were carried out on Merck Kieselgel 60 or aluminium oxide 90, neutral (70–230 mesh). Elemental analysis were performed in the Institute of Organic Chemistry, Polish Academy of Sciences (Warsaw, Poland), and were within $\pm 0.4\%$ of the theoretical values. The syntheses of 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine [1], 4-[4-(2-methoxyphenyl)-1-piperazinyl]cyclohexylamine [31] and 4-[2-(1,2,3,4-tetrahydroisoquinolinyl)]cyclohexylamine [31] had been previously reported.

*6.1.1. General procedure for the preparation of **2a** and **2b***

1-Adamantanecarboxylic acid (2 mmol) was dissolved in CH_2Cl_2 (5 mL) and triphenylphosphine (2 mmol) was added under stirring. After 5 min, *N*-bromosuccinimide (2.1 mmol) was added in portions and afterwards the mixture was stirred for 0.5 h at a room temperature (r.t.). A solution of 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine or 4-[4-(2-methoxyphenyl)-1-piperazinyl]cyclohexylamine (2 mmol) and Et_3N (2.2 mmol) in CH_2Cl_2 (3 mL) was then added dropwise. The reaction mixture was stirred for 3 h at a r.t. and left overnight. Then, $CHCl_3$ (20 mL) was added and the solution was washed with a 20% aq. solution of NaOH (10 mL), water (10 mL) and was dried over anhydrous $MgSO_4$. The inorganic precipitate was filtered off, the solvents were evaporated and the amides were separated by column chromatography using SiO_2 and $CHCl_3$ followed by $CHCl_3$–MeOH = 49:1, as eluents.

*6.1.1.1. 4-[4-(1-Adamantanecarboxamido)butyl]-1-(2-methoxyphenyl)piperazine (**2a**).* The title compound was prepared by the general procedure in 70% yield as pale yellow oil: ^{1}H-NMR (60 MHz): δ 7.0 (s, 4H), 5.9 (br s, 1H), 3.8 (s, 3H), 3.6–2.9 (m, 6H), 2.9–2.2 (m, 6H), 2.2–1.4 (cluster, 19H). Complex **2a·$H_4C_4O_4$·H_2O**: m.p. 95–97 °C (C_3H_6O), lit. m.p. 112–117 °C (THF) [32]. Anal. $C_{26}H_{39}N_3O_2\cdot H_4C_4O_4\cdot H_2O$ (C, H, N).

*6.1.1.2. trans-4-[4-(1-Adamantanecarboxamido)cyclohexyl]-1-(2-methoxyphenyl)piperazine (**2b**).* The title compound was prepared by the general procedure in 55% yield as colourless crystals: m.p. 210–212 °C ($CHCl_3$); ^{1}H-NMR (500 MHz): δ 7.02–6.90 (m, 3H, ArH), 6.85 (dd, $J = 8.0$, 1.2, 1H, ArH), 5.36 (d, $J = 8.0$, 1H, NHCO), 3.86 (s, 3H, OCH_3), 3.76–3.68 (m, decoupled leaves td, $J = 11.6$, 4.0, 1H, cyclohexane axial H-4), 3.09 (br s, 4H, piperazine $2CH_2$), 2.76 (app br t, 4H, piperazine $2CH_2$), 2.31 (td, $J = 11.6$, 3.0, 1H, cyclohexane axial H-1), 2.04 (cluster, 5H, cyclohexane equatorial H-3 and H-3′ and adamantane 3CH), 1.98 (app br d, 2H, cyclohexane H-2 and H-2′), 1.83 (d, $J = 2.6$, 6H, adamantane H's), 1.71 (q, $J = 12.2$, 6H, adamantane H's), 1.43 (dddd, $J = 12.9$, 12.8, 3.1, 3.0, 2H, cyclohexane axial H-2 and H-2′), 1.14 (dddd, $J = 12.7$, 12.6, 3.1, 2.9, 2H, cyclohexane axial H-3 and H-3′). Complex **2b·2HCl**: m.p. 289–291 °C (EtOH–Et_2O). Anal. $C_{28}H_{41}N_3O_2\cdot 2HCl$ (C, H, N).

*6.1.2. General procedure for the preparation of **3a** and **3b***

To a vigorously stirred solution of 4-(4-aminobutyl)-1-(2-methoxyphenyl)piperazine or 4-[4-(2-methoxyphenyl)-1-piperazinyl]cyclohexylamine (2 mmol) in $CHCl_3$ (25 mL) and a 20% aq. solution of K_2CO_3 (25 mL), cyclohexanecarbonyl chloride (3 mmol) was added dropwise. The reaction mixture was stirred at a r.t. for 4 h. The organic layer was separated, washed with water until neutral, and afterwards the product was extracted with a 10% aq. solution of HCl. The water phase was made alkaline, extracted with $CHCl_3$ and dried (K_2CO_3). After evaporation of the solvent, the residue was crystallized to give a pure product.

*6.1.2.1. 4 - [4 - (Cyclohexanecarboxamido)butyl] - 1 - (2-methoxyphenyl)piperazine (**3a**).* The title compound was prepared by the general procedure in 80% yield as colourless crystals: m.p. 128–129 °C ($CHCl_3$–C_6H_{14}); ^{1}H-NMR (60 MHz): δ 7.0 (s, 4H), 6.2 (br s, 1H), 3.9 (s, 3H), 3.5–2.9 (m, 6H), 2.9–2.3 (m, 6H), 2.3–1.0 (cluster, 15H). Complex **3a·0.5$H_4C_4O_4$·1.5H_2O**: m.p. 143–145 °C (C_3H_6O), lit. m.p. (for hydrochloride salt) 190–192 °C (EtOH–Et_2O) [15]. Anal. $C_{22}H_{35}N_3O_2\cdot 0.5H_4C_4O_4\cdot 1.5H_2O$ (C, H, N).

*6.1.2.2. trans - 4 - [4(Cyclohexanecarboxamido)cyclo - hexyl]-1-(2-methoxyphenyl)piperazine (**3b**).* The title compound was prepared by the general procedure in 73% yield as colourless crystals: m.p. 237–239 °C ($CHCl_3$–MeOH); ^{1}H-NMR (200 MHz): δ 7.01–6.82 (m, 4H, ArH), 5.24 (d, $J = 8.2$, 1H, NHCO), 3.85 (s, 3H, OCH_3), 3.70 (m, decoupled leaves td, $J = 11.5$, 4.1, 1H, cyclohexane axial H-4), 3.08 (br s, 4H, piperazine $2CH_2$), 2.75 (app br t, 4H, piperazine $2CH_2$), 2.29 (td, $J = 11.5$, 3.3, 1H, cyclohexane axial H-1), 2.06–1.94 (cluster, 6H, cyclohexane H's), 1.84–1.76 (m, 4H, cyclohexane H's), 1.66 (br s, 1H, cyclohexane H-1′), 1.47–1.35 (m, 4H, cyclohexane H's), 1.31–1.07 (cluster, 4H, cyclohexane H's). Complex **3b·2HCl·2.2H_2O**: m.p. 275–277 °C (C_3H_6O). Anal. $C_{24}H_{37}N_3O_2\cdot 2HCl\cdot 2.2H_2O$ (C, H, N).

*6.1.3. trans-1-(2-Methoxyphenyl)-4-(4-succinimidocyclohexyl)piperazine (**4b**)*

A mixture of 4-[4-(2-methoxyphenyl)-1-piperazinyl]cyclohexylamine (0.5 g, 1.7 mmol) and succinic anhydride (0.19 g, 1.9 mmol) and xylene (20 mL) was refluxed for 3 h. Then the solvent was evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2, $CHCl_3$–MeOH = 49:1) to afford **4b** (0.33 g, 52%) as colourless crystals: m.p. 190–192 °C ($CHCl_3$–MeOH); ^{1}H-NMR (300 MHz): δ 7.25–6.83 (m, 4H, ArH), 3.98 (tt, $J = 12.4$, 3.8, 1H, cyclohexane axial H-4), 3.85 (s, 3H, OCH_3), 3.09 (app br s, 4H, piperazine $2CH_2$), 2.76 (app br t, 4H, piperazine $2CH_2$), 2.65 (s, 4H, $-CH_2CH_2-$ in succinimide), 2.46 (tt, $J = 11.5$, 3.0, 1H, cyclohexane axial H-1), 2.27 (dddd, $J = 12.9$, 12.6, 3.0, 2.8, 2H, cyclohexane axial H's), 2.03 (app br d, 2H, cyclohexane equatorial H's), 1.68 (app br d, 2H, cyclohexane equatorial H's), 1.39 (dddd, $J = 12.9$, 12.6, 3.3, 3.0, 2H, cyclohexane axial H's). Complex **4b·2HCl**: m.p. 294–296 °C (EtOH–C_3H_6O). Anal. $C_{21}H_{29}N_3O_3 \cdot 2HCl$ (C, H, N).

*6.1.4. trans-2-[4-(Adamantanecarboxamido)cyclohexyl]-1,2,3,4-tetrahydroisoquinoline (**5b**)*

1-Adamantanecarboxylic acid (0.18 g, 1 mmol) was dissolved in MeCN (15 mL) and BOP (1 mmol) was added under stirring. To the homogenous solution Et_3N (2 mmol) was added, followed by solution of 4-[2-(1,2,3,4-tetrahydroisoquinolinyl)]cyclohexylamine (0.23 g, 1 mmol) in MeCN (15 mL). The stirring was continued for 6 h at a r.t. and the mixture was left overnight. The solvent was evaporated and the residue was purified by column chromatography (SiO_2, $CHCl_3$–MeOH = 19:1). The imide **5b** (0.37 g, 95%) was obtained as colourless crystals: m.p. 195–197 °C ($CHCl_3$–MeOH); ^{1}H-NMR (500 MHz): δ 7.12–7.07 (m, 3H, THIQ aromatic H's), 7.02–7.00 (m, 1H, THIQ aromatic H), 5.38 (d, $J = 8.0$, 1H, NHCO), 3.77 (s, 2H, THIQ H's-1), 3.77–3.71 (m, decoupled leaves td, $J =$ 11.7, 4.0, 1H, cyclohexane axial H-4), 2.89–2.81 (m, 4H, THIQ H's-3 and H's-4), 2.48 (tt, $J = 11.6$, 3.4, 1H, cyclohexane axial H-1), 2.08–1.98 (cluster, 7H, 4 cyclohexane equatorial H's and 3 adamantane H's), 1.83 (d, $J = 2.6$, 6H, adamantane H's), 1.72 (q, $J = 12.1$, 6H, adamantane H's), 1.34 (dddd, $J = 12.9$, 12.8, 3.1, 3.0, 2H, cyclohexane axial H's), 1.16 (dddd, $J = 12.7$, 12.6, 3.2, 3.1, 2H, cyclohexane axial H's). Complex **5b·HCl·0.4H$_2$O**: m.p. 294–296 °C (C_3H_6O). Anal. $C_{26}H_{36}N_2O \cdot HCl \cdot 0.4\ H_2O$ (C, H, N).

*6.1.5. trans-8-{4-[2-(1,2,3,4-Tetrahydroisoquinolinyl)]-cyclohexyl}-8-azaspiro [4,5]decane-7,9-dione (**6b**)*

4-[2-(1,2,3,4-Tetrahydroisoquinolinyl)cyclohexylamine (0.46 g, 2 mmol) and 3,3-tetramethyleneglutaric anhydride (0.34 g, 2 mmol) in anhydrous pyridine (10 mL) were refluxed for 22 h. The solvent was removed under reduced pressure and the residue was treated with water and extracted with $CHCl_3$. The extract was dried over $MgSO_4$, the solvent was evaporated and the residue was purified by column chromatography (Al_2O_3 neutral, $CHCl_3$–C_6H_{14} = 1:1). The product was obtained as a pale yellow oil (0.08 g, 11%); ^{1}H-NMR (300 MHz): δ 7.11–7.07 (m, 3H, THIQ aromatic H's), 7.02–6.99 (m, 1H, THIQ aromatic H), 4.56 (tt, $J = 12.1$, 3.8, 1H, cyclohexane axial H-4), 3.78 (s, 2H, THIQ H's-1), 2.93–2.82 (m, 4H, THIQ H's-3 and H's-4), 2.63 (tt, $J = 11.8$, 3.3, 1H, cyclohexane axial H-1), 2.57 (s, 4H, $2CH_2CO$), 2.39 (dq, $J = 12.6$, 3.3, 2H, cyclohexane axial H's), 2.02 (app br d, 2H, cyclohexane equatorial H's), 1.73–1.60 (cluster, 6H, cyclohexane equatorial 2H's and cyclopentane $2CH_2$), 1.55–1.42 (cluster, 6H, cyclohexane axial 2H's and cyclopentane $2CH_2$). Complex **6b·HCl·2H$_2$O**: m.p. 229–230 °C (C_3H_6O). Anal. $C_{24}H_{32}N_2O_2 \cdot HCl \cdot 2H_2O$ (C, H, N).

6.2. Molecular modelling

All the molecular modelling procedures and computations were performed using SYBYL package version 6.6 (Tripos Associates Inc., St. Louis, USA) run on a Silicon Graphic Indigo II workstation. For the study of the dihedral angles τ_2–τ_4 by the Mopac/AM1 method the structures of compounds **2b**–**6b** were minimized (precise, nomm, gnorm = 0.1) over all the bonds and angles except for the respective torsion which was constrained of values between 0 and 360° with a 10° increment. Exploration of the conformational space of compounds **2a** and **2b** was carried out by a standard random search method (energy cutoff: 3 kcal mol^{-1}) and for maximin2 optimization (Tripos force field) a conjugated gradient method was chosen. Volumes accessible by terminal amide (or imide) and hydrocarbon groups of restricted compounds were generated using a mvolume command.

6.3. Radioligand binding studies

The affinity of the investigated compounds for 5-HT_{1A} receptors was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT (220 Ci mmol^{-1}, Amersham). The experiments were carried out in the hippocampus of rat brain, according to the published procedures [38]. K_i values were determined from at least three competition binding experiments in which 10–14 drug concentrations, run in triplicate, were used. The Cheng and Prusoff [39] equation was used for K_i calculations.

6.4. In vivo studies

The experiments were carried out on male Wistar rats (260–300 g). The animals were kept at an ambient

temperature of 20 ± 1 °C throughout the experiment, and had free access to food (standard laboratory pellets, LSM) and tap water. All experiments were conducted in the light phase on a natural light–dark cycle (from September to October), between 9:00 and 14:00 h 8-OH-DPAT (Research Biochemical Inc.) and reserpine (Ciba, ampoules) were dissolved in saline. The investigated salts of the tested compounds were used in the form of freshly prepared suspensions in a 1% Tween 80. 8-OH-DPAT and reserpine were injected subcutaneously (sc), and the tested compounds intraperitoneally (ip) in a volume of 2 mL kg^{-1}. Each group consisted of six animals. The obtained data were analysed by the Newman–Keuls test.

6.4.1. Lower lip retraction in rats

LLR was assessed according to the method described by Berendsen et al. [37]. The rats were individually placed in cages ($30 \times 25 \times 25$ cm), and were scored three times (at 15, 30 and 45 min after the tested compounds or 8-OH-DPAT administration) as follows: 0 = lower incisors not visible; 0.5 = partly visible; 1 = completely visible. The sum of maximum scores, amounted to three for each rat. The effect of investigated compounds on the LLR induced by 8-OH-DPAT (1 mg kg^{-1}) was tested in a separate experiment. The investigated compounds were administered 45 min before 8-OH-DPAT, and the animals were scored at 15, 30 and 45 min after 8-OH-DPAT administration.

6.4.2. Behavioural syndrome in reserpinized rats

The rats were individually placed in cages ($30 \times 25 \times 25$ cm) 5 min before injection of the tested compounds or 8-OH-DPAT. Observation sessions, lasting 45 s each, began 3 min after drug administration and were repeated every 3 min. Reciprocal forepaw treading and flat body posture were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. The maximum score, summed up over five observation periods, amounted to 15 for each symptom/animal [36]. The effect of the tested compounds on the behavioural syndrome induced by 8-OH-DPAT (5 mg kg^{-1}) was tested in a separate experiment. The investigated compounds were administered 60 min before 8-OH-DPAT, and the animals were scored at 3, 6, 9, 12 and 15 min after 8-OH-DPAT treatment. Reserpine (1 mg kg^{-1}) was administered 18 h before the test.

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