FUNCTIONAL *IN VIVO* PROFILE OF THE CONFORMATIONALLY RESTRICTED ARYLPIPERAZINE ANALOGUES MP349 AND MP401 AT SEROTONIN 5-HT$_{1A}$ RECEPTORS

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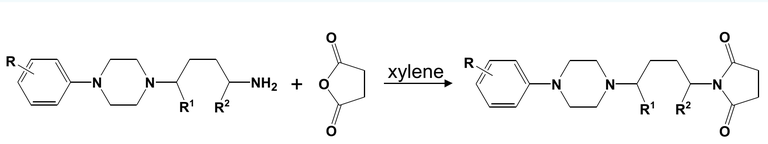
INTRODUCTION

Arylpiperazines are of great importance to many different biological targets, particulary central nervous system receptors. The most thoroughly studied group, called long chain arylpiperazines (LCAPs), can be found as serotonin receptor ligands, especially 5-HT$_{1A}$ and 5-HT$_{2A}$ ones. The majority of them (to list only the most common: buspirone used in the treatment of anxiety; gepirone, ipsapirone, tandospirone, flesinoxan in various phases of clinical studies; NAN-190, WAY100635 or MP3022 frequently used as pharmacological tools) contain a flexible aliphatic chain of different length, which connects an arylpiperazine fragment with a second terminal pharmacophoric group. Due to a high conformational freedom of these agents, it is difficult to find their reliable structure-activity relationships and to predict their conformation within the receptor pocket.

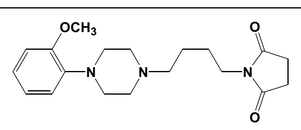
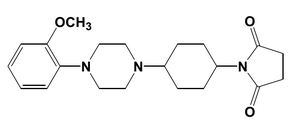
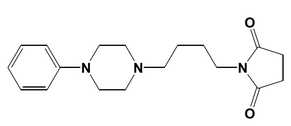
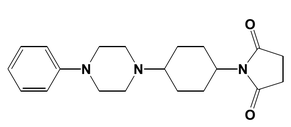
In our earlier studies we showed that 5-HT$_{1A}$ receptor affinities of rigid compounds (obtained by replacing an aliphatic linker with a 1,4-disubstituted cyclohexane ring) were only slightly lower than their chain analogues e.g. NAN-190, MP3022 and MM77. Interestingly, all the constrained derivatives in functional *in vivo* tests acted as antagonists of postsynaptic 5-HT$_{1A}$ receptors (1, 2). Moreover, compound **1b** (MP349) a rigid analogue of MM77, behaved like an antagonist also at presynaptic 5-HT$_{1A}$ receptors (3), becoming the first full antagonist from the arylpiperazine group with a precisely defined 3-D structure. Since all the previously tested constrained derivatives contained a 4-(2-methoxyphenyl)-1-piperazinyl fragment, we wondered whether the methoxy substituent or frozen extended linear conformation determined the full antagonistic profile of **1b**. To answer this question, new analogues **2a** and **2b** (MP401) devoid of 2-methoxy substituent were designed and synthesized.

SYNTHESIS OF INVESTIGATED COMPOUNDS AND THEIR 5-HT$_{1A}$ RECEPTOR AFFINITY



R = H, 2-OCH_3,
$R^1 = R^2$ = H, $-(CH_2)_2-$

The structure of the newly synthesized compounds was confirmed by ^{1}H NMR spectra and elemental analysis. The observed coupling constants in the cyclohexane ring allowed us to assign a diequatorial conformation for compound **2b**. For biological assays, the investigated compounds were converted into hydrochloride salts.

Comp.	Structure	K_i (nM)±SEM 5-HT$_{1A}$
1a MM77		6.4±0.3
1b MP349		15±3
2a		7.4±0.3
2b MP401		43±6

The affinity of the investigated compounds at 5-HT$_{1A}$ receptors was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT.

The flexible compound **2a**, like its 2-methoxy analogue **1a** (MM77), demonstrated very high 5-HT$_{1A}$ receptor affinity. Compound **2b** with restricted conformational freedom, an analogue of **1b** (MP349), possessed still high 5-HT$_{1A}$ receptor affinity, yet slightly lower than that of the parent compound.

IN VIVO RESULTS

Compounds **2a** and **2b** were tested *in vivo* in models commonly used for evaluation of the 5-HT$_{1A}$ receptor functional activity.

Presynaptic 5-HT$_{1A}$ receptors activity

8-OH-DPAT, an agonist of 5-HT$_{1A}$ receptors, induces hypothermia in mice, the effect mediated by presynaptic 5-HT$_{1A}$ receptors, which is abolished by antagonists of 5-HT$_{1A}$ receptors, such e.g., WAY100635.

Postsynaptic 5-HT$_{1A}$ receptors activity

8-OH-DPAT, agonist of these receptors induces a lower lip retraction (LLR) in normal rats, and a behavioral syndrome (flat body posture - FBP, and forepaw treading - FT) in reserpinized rats - the effects connected with stimulation of the postsynaptic 5-HT$_{1A}$ receptors. Those symptoms are sensitive to 5-HT$_{1A}$ receptor antagonists e.g., WAY100635.

The ability to mimic the 8-OH-DPAT-induced action by the tested compounds was regarded as a 5-HT$_{1A}$ agonistic activity. Their antagonistic 5-HT$_{1A}$ activity was assessed on the basis of the blockade of the above mentioned 8-OH-DPAT-induced effects.

Table 1. The effect of the tested compounds on body temperature in mice

Treatment	Dose (mg/kg)	t S.E.M. C 30 min	60 min	90 min	120 min
Vehicle	-	-0.1 0.1	-0.0 0.1	-0.0 0.1	-0.0 0.1
2a	2.5	-1.2 0.1^b	-1.3 0.1^b	-1.3 0.1^b	-1.2 0.1^b
	5	-2.1 0.3^b	-2.0 0.3^b	-1.4 0.4^b	-1.2 0.2^b
Vehicle	-	-0.1 0.2	0.0 0.2	0.1 0.1	-0.1 0.1
2b	2.5	-0.3 0.2	-0.3 0.1	-0.2 0.1	-0.2 0.1
	5	-2.1 0.3^b	-1.6 0.2^b	-1.3 0.2^b	-1.1 0.2^a
Vehicle	-	-0.1 0.1	0.1 0.1	0.1 0.1	-0.1 0.1
WAY100635	0.1	-0.2 0.1	0.2 0.1	0.1 0.1	0.2 0.1

The tested compounds were administered 30 min before the test. Absolute initial mean body temperatures were within the range 36 ± 0.4°C ; n = 8 - 9 mice per group; ^{a}p < 0.05, ^{b}p < 0.01 vs respective vehicle group.

Table 2. The effect of WAY 100635 on the hypothermia induced by the tested compounds in mice

Treatment and dose (mg/kg)	t S.E.M. C 30 min	60 min
Vehicle + vehicle	-0.0 0.1	-0.0 0.1
Vehicle + **2a** (2.5)	-2.0 0.2^b	-1.9 0.2^b
WAY 100635 (0.1) + **2a** (2.5)	-0.7 0.2^{aA}	-0.5 0.2^A
Vehicle +vehicle	-0.1 0.1	-0.1 0.1
Vehicle + **2b** (5)	-1.9 0.2^b	-1.4 0.1^b
WAY 100635 (0.1) + **2b** (5)	-1.5 0.3^b	-0.7 0.2^{bA}
Vehicle +vehicle	0.1 0.1	0.1 0.1
8-OH-DPAT (5)	-1.3 0.1^b	-0.7 0.1^b
WAY 100635 (0.1) + 8-OH-DPAT (5)	-0.1 0.1^A	0.1 0.1^A

WAY 100635 was administered 15 min before the compounds studied. Body temperature was recorded 30 and 60 min after injection of the tested compounds. Absolute initial mean body temperatures were within the range 36.5 ± 0.3°C; n = 8 - 9 mice per group; ^{a}p < 0.05, ^{b}p < 0.01 vs respective vehicle + vehicle group, ^{A}p < 0.01 vs respective vehicle + tested compound group.

Table 3. Induction of lower lip retraction (LLR) by the investigated compounds (A) and their effect on the 8-OH-DPAT - induced LLR (B) in rats

Treatment	Dose (mg/kg)	Mean S.E.M. LLR score A	B
Vehicle	-	0.1 0.1	2.8 0.1
2a	5	1.4 0.1^a	1.4 0.2^a
	10	2.5 0.3^a	NT
Vehicle	-	0.1 0.1	2.7 0.1
2b	5	2.1 0.3^a	NT
	10	2.2 0.3^a	1.9 0.2
WAY 100635	0.1	0.1 0.1	0.3 0.2^a

The investigated compounds were administered 15 min before test (A) or 45 min before 8-OH-DPAT (1 mg/kg); n = 6 rats per group. ^{A}p < 0.01 vs vehicle (A) or vs vehicle + 8-OH-DPAT (B).

Table 4. Induction of behavioral syndrome by the investigated compounds (A) and their effect on the 8-OH-DPAT-induced behavioral syndrome (B) in reserpine-pretreated rats.

Treatment	Dose (mg/kg)	Mean S.E.M. behavioral score A FBP	FT	B FBP	FT
Vehicle	-	0.1 0.1	0.1 0.1	14.5 0.3	13.2 0.2
2a	5	0.0 0.0	0.5 0.2	4.7 1.0^b	4.8 0.9^b
	10	1.2 0.5^a	0.5 0.5	1.7 0.9^b	3.0 0.9^b
Vehicle	-	0.1 0.1	0.1 0.1	14.5 0.3	13.2 0.2
2b	5	0.0 0.0	1.0 0.5	2.7 0.8^b	2.3 0.7^b
	10	1.5 0.4	2.7 0.7^a	2.0 1.1^b	0.7 0.5^b
WAY 100635	0.1	0.0 0.0	0.0 0.0	0.8 0.4^b	1.2 0.7^b

Reserpine (1 mg/kg) was administered 18 h before the test. The investigated compounds were administered 3 min before the test (A) or 60 min before 8-OH-DPAT (5 mg/kg) (B); n = 6 rats per group; ^{a}p < 0.05, ^{b}p < 0.01 vs vehicle (A) or vs vehicle + 8-OH-DPAT (B).

CONCLUSIONS

Functional *in vivo* activity of the investigated 5-HT$_{1A}$ receptor ligands

Comp.	presynaptic	postsynaptic
1a	–	antagonist
1b	antagonist	antagonist
2a	agonist	partial agonist
2b	agonist	partial agonist

The removal of the methoxy substituent from the arylpiperazine part of the ligand structure resulted in the alteration of its *in vivo* profile. Both new compounds with the phenylpiperazine fragment, flexible **2a** and rigid **2b** behaved as agonists of presynaptic 5-HT$_{1A}$ receptors, while described earlier compound **1b** exhibited an antagonistic activity at these receptors. At the postsynaptic 5-HT$_{1A}$ receptors the investigated ligands **2a** and **2b** revealed also some agonistic features, in contrast to their 2-methoxy counterparts, described as the postsynaptic 5-HT$_{1A}$ receptor antagonists.

From the above results we can conclude that the presence of the 2-methoxy group in arylpiperazine pharmacophore of the ligand **1b** seems to be responsible for its full antagonism at 5-HT$_{1A}$ receptors.

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