



# Synthesis and 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor activity of N-(4-arylpiperazin-1-yl)alkyl derivatives of 2-azaspiro[4.4]nonane and [4.5]decane-1,3-dione

Jolanta Obniska<sup>1</sup>, Ewa Tatarczyńska<sup>2</sup>, Agnieszka Nikiforuk<sup>2</sup>,  
Sijka Charakchieva-Minol<sup>3</sup>, Beata Duszyńska<sup>3</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, Medical College of Jagiellonian University, Medyczna 9, PL 30-688 Kraków, Poland

<sup>2</sup>Department of New Drugs Research, <sup>3</sup>Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

**Correspondence:** Jolanta Obniska e-mail: mfobnisk@cyf-kr.edu.pl

---

## Abstract:

Two series of N-(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane (**5–10**) and [4.5]decane-1,3-dione (**11–16**) derivatives were synthesized and their serotonin 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor affinities were determined. Compounds with the methylene spacer (**5–7** and **11–13**) exhibited low 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor affinity, in contrast to their ethylene analogues regarded as potent 5-HT<sub>1A</sub> ligands, especially those containing a cyclohexane moiety (**14–16**; *K<sub>i</sub>* = 5.1, 2.7 and 4.3 nM, respectively) in the 3-position of the pyrrolidine-2,5-dione ring. Moreover, derivatives with 3-chloro substituent (**10** and **14**) showed distinct affinity for 5-HT<sub>2A</sub> receptors. The functional activity of compounds **10**, **14**, **15** and **16** was tested *in vivo* in the commonly used animal models. In those experiments, the tested compounds showed features of agonists of pre- and postsynaptic (**14**), agonists of presynaptic and antagonists of postsynaptic (**10**, **15**), or agonists of postsynaptic (**16**) 5-HT<sub>1A</sub> receptors. Additionally, **10** and **16** exhibited properties of potential 5-HT<sub>2A</sub> receptor antagonists. The above results suggested a crucial role of the spacer between the amide fragment and 4-arylpiperazine moiety, as well as of the size of the cycloalkyl ring at the 3-position of pyrrolidine-2,5-dione ring in functional 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> properties.

## Key words:

5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor ligands, 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione, arylpiperazine, structure-activity relationship

---

## Introduction

Serotonin (5-HT) plays an important role in many physiological and pathophysiological processes in the brain. These processes are mediated by a specific interaction of 5-HT with seven major receptor classes. The 5-HT<sub>1A</sub> receptor subtype is one of the best characterized; it is well established that these receptors are involved in such psychiatric disorders as depression and anxiety [22]. Several classes of compounds are

known to bind to 5-HT<sub>1A</sub> receptor sites. Among them, 4-arylpiperazines that are linked to a terminal cyclic amide *via* a long chain are effective as antianxiety and antidepressant drugs [3, 12]. Many studies into the structure-activity relationship of such long-chain arylpiperazine derivatives as 5-HT<sub>1A</sub> receptors ligands have been carried out [5, 19–21]. The authors indicated that the nature of the aryl ring at N4 nitrogen atom of the piperazine moiety and the length of the alkyl chain, as well as the terminal cyclic amide frag-

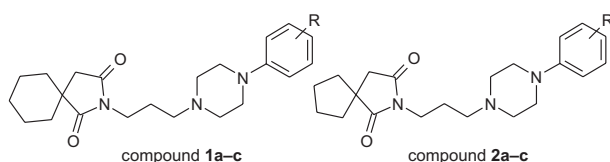
ment play an important role in the 5-HT<sub>1A</sub> receptor-ligand binding.

In our previous papers [14, 16], we described the synthesis and 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor affinity of a new 3-spirocycloalkyl pyrrolidine-2,5-dione with a 4-arylpiperazine moiety connected to the amide fragment with a propylene spacer. As shown in Figure 1, some of them displayed high 5-HT<sub>2A</sub> and low-to-moderate 5-HT<sub>1A</sub> receptors affinity. In this series of compounds, the affinity for both 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors depended on the size of the cycloalkyl ring; in fact, cyclohexane derivatives (**1a–c**) were slightly more active than their cyclopentane analogues (**2a–c**). In an *in vivo* study, compounds **1a–c** were demonstrated to act as 5-HT<sub>2A</sub> receptor antagonists. The radioligand binding and functional data prompted us to synthesize and test some new compounds in which we changed the length of the spacer between the 4-arylpiperazine moiety and the amide fragment from propylene (**1a–c** and **2a–c**) to methylene (**5–7** and **11–13**) or to ethylene (**8–10** and **14–16**). Furthermore we examined the influence of those modifications on 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor activities *in vitro*, and described the pharmacological properties of the most active derivatives *in vivo*.

## Materials and Methods

### CHEMICAL PART

The synthesis of compounds **8–10** and **14–16** is shown in Figure 2. Melting points (m.p.) were deter-



- 1a** R = H Ki [nM]\*: 5-HT<sub>1A</sub> = 535 ± 30, 5-HT<sub>2A</sub> = 27 ± 3  
**1b** R = 2-F Ki [nM]\*: 5-HT<sub>1A</sub> = 368 ± 75, 5-HT<sub>2A</sub> = 46 ± 12  
**1c** R = 3-Cl Ki [nM]\*: 5-HT<sub>1A</sub> = 106 ± 12, 5-HT<sub>2A</sub> = 15 ± 3  
**2a** R = H Ki [nM]\*\*: 5-HT<sub>1A</sub> = 692 ± 26, 5-HT<sub>2A</sub> = 29 ± 4  
**2b** R = 2-F Ki [nM]\*\*: 5-HT<sub>1A</sub> = 354 ± 16, 5-HT<sub>2A</sub> = 137 ± 10  
**2c** R = 3-Cl Ki [nM]\*\*: 5-HT<sub>1A</sub> = 107 ± 8, 5-HT<sub>2A</sub> = 26 ± 4

Fig. 1. Chemical structure of compounds **1a–c** and **2a–c** [16], \*\*[14]

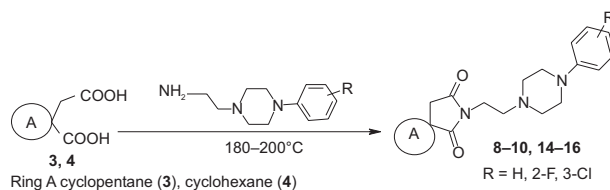


Fig. 2. Synthesis of compounds **8–10** and **14–16**

mined in an electrothermal digital m.p. apparatus and are uncorrected. The chemical structures of the obtained compounds were confirmed by elemental and spectral analyses. <sup>1</sup>H NMR spectra were obtained with a Varian Mercury spectrometer operating at 300 MHz. Chemical shifts were reported as parts per million (δ ppm) from (CH<sub>3</sub>)<sub>4</sub>Si (TMS) as an internal standard. Signal multiplicities are represented as: s (singlet), d (doublet), t (triplet), m (multiplet). The purity of the compounds was checked by a thin-layer chromatography (TLC) on Merck silica gel GF<sub>254</sub> aluminium sheets, using the following mobile phases: S<sub>1</sub> butanol : acetic acid : H<sub>2</sub>O (5:4:1), and S<sub>2</sub> chloroform : isopropanol : 25% ammonia (9:11:2). Spots were detected by absorption of UV light. The results of elemental analyses for C, H, N were within ± 0.4% of the theoretical values.

The synthesis and physicochemical data of compounds **5–7** and **11–13** were described earlier [15, 17] but it should be noted that none of them were tested for their 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptors affinity.

The starting 1-carboxy-1-cyclopentane-acetic acid (**3**) and 1-carboxy-1-cyclohexane-acetic acid (**4**) were obtained by the method described by Scott et al. [25]. The appropriately substituted 1-(2-aminoethyl)-4-arylpiperazines were synthesized on the basis of the method described earlier [10]. The N-[2-(4-arylpiperazin-1-yl)-ethyl]-2-aza-spiro[4.4]nonane (**8–10**) and [4.5]decane-1,3-dione derivatives (**14–16**) were obtained in a one-pot cyclization of the acids **3**, **4** with the appropriately substituted 1-(2-aminoethyl)-4-arylpiperazines by heating them at ca. 180°C for 1.5–2 h.

**General procedure for the preparation of the N-[2-(4-arylpiperazin-1-yl)-ethyl]- derivatives of 2-azaspiro[4.4]nonane- (8–10) and [4.5]decane-1,3-dione (14–16):** Appropriately substituted 1-(2-aminoethyl)-4-arylpiperazine (0.001 mol) was dissolved in 20 ml of water, and 0.01 mol of the 1-carboxy-1-cyclopentane- or 1-carboxy-1-cyclohexane-acetic acid was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. After

complete removal of water, the temperature of the reaction mixture was raised up to 180°C and maintained for 1.5 h. The crude products were crystallized from ethanol. Free bases were converted into hydrochloride salts in anhydrous ethanol saturated with HCl gas. The obtained precipitates of the salts were crystallized from anhydrous ethanol.

**N-[2-(4-Phenylpiperazin-1-yl)-ethyl]-2-azaspiro[4.4]nonane-1,3-dione hydrochloride (8):** White crystals, mp 220–222°C (EtOH); Yield: 63% (2.38 g);  $R_f = 0.75$  (S<sub>1</sub>),  $R_f = 0.96$  (S<sub>2</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.62–1.68 (m, 4H, C<sub>4</sub>H<sub>8</sub>), 1.69–2.13 (m, 2H, C<sub>4</sub>H<sub>8</sub>), 2.15–2.18 (t, 2H, C<sub>4</sub>H<sub>8</sub>,  $J = 4.12$  Hz), 2.60 (s, 2H, imide), 2.63–2.68 (m, 6H, 4H, N(CH<sub>2</sub>)<sub>2</sub>, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 3.13–3.17 (t, 4H, N(CH<sub>2</sub>)<sub>2</sub>,  $J = 5.08$  Hz), 3.69–3.73 (t, 2H, CH<sub>2</sub>–CH<sub>2</sub>,  $J = 6.46$  Hz), 6.84–6.95 (m, 3H, C<sub>6</sub>H<sub>5</sub>), 7.28–7.32 (m, 2H, C<sub>6</sub>H<sub>5</sub>), 12.80 (br. s, 1H, NH<sup>+</sup>); Anal. Calcd. for C<sub>20</sub>H<sub>28</sub>ClN<sub>3</sub>O<sub>2</sub> C: 63.55, H: 7.47, N: 11.12. Found: C: 63.83, H: 7.40, N: 11.04.

**N-[2-[4-(2-Fluorophenyl)-piperazin-1-yl]-ethyl]-2-azaspiro[4.4]nonane-1,3-dione hydrochloride (9):** White crystals, mp 190–192°C (EtOH); Yield: 55% (2.18 g);  $R_f = 0.77$  (S<sub>1</sub>),  $R_f = 0.95$  (S<sub>2</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.65–1.97 (m, 6H, C<sub>4</sub>H<sub>8</sub>), 2.13–2.20 (m, 2H, C<sub>4</sub>H<sub>8</sub>), 2.86 (s, 2H, imide), 3.01–3.05 (t, 2H, N(CH<sub>2</sub>)<sub>2</sub>,  $J = 9.87$  Hz), 3.34–3.36 (d, 2H, CH<sub>2</sub>–CH<sub>2</sub>,  $J = 4.68$  Hz), 3.45–3.49 (d, 2H, N(CH<sub>2</sub>)<sub>2</sub>,  $J = 12.93$  Hz), 3.66–3.3.74 (t, 2H, CH<sub>2</sub>–CH<sub>2</sub>,  $J = 11.55$  Hz), 3.95–3.98 (t, 4H, N(CH<sub>2</sub>)<sub>2</sub>,  $J = 5.36$  Hz), 6.98–7.14 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 12.90 (br. s, 1H, NH<sup>+</sup>); Anal. Calcd. for C<sub>20</sub>H<sub>27</sub>ClN<sub>3</sub>O<sub>2</sub> C: 60.66, H: 6.87, N: 10.61. Found: C: 60.95, H: 6.87, N: 10.53.

**N-[2-[4-(3-Chlorophenyl)-piperazin-1-yl]-ethyl]-2-azaspiro[4.4]nonane-1,3-dione hydrochloride (10):** White crystals, mp 233–235°C (EtOH); Yield: 70% (2.88 g);  $R_f = 0.80$  (S<sub>1</sub>),  $R_f = 0.94$  (S<sub>2</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.69–1.98 (m, 4H, C<sub>4</sub>H<sub>8</sub>), 2.10–2.98 (m, 2H, C<sub>4</sub>H<sub>8</sub>), 2.25–2.35 (m, 2H, C<sub>4</sub>H<sub>8</sub>), 2.64 (s, 2H, imide), 3.02–3.13 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 3.39–3.43 (d, 2H, CH<sub>2</sub>–CH<sub>2</sub>,  $J = 12.93$  Hz), 3.61–3.69 (m, 6H, 4H, N(CH<sub>2</sub>)<sub>2</sub>, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 7.04–7.13 (m, 2H, C<sub>6</sub>H<sub>4</sub>), 7.24–7.41 (m, 2H, C<sub>6</sub>H<sub>4</sub>), 12.98 (br. s, 1H, NH<sup>+</sup>); Anal. Calcd. for C<sub>20</sub>H<sub>27</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub> C: 58.31, H: 6.61, N: 10.20. Found: C: 58.04, H: 6.47, N: 10.16.

**N-[2-(4-Phenylpiperazin-1-yl)-ethyl]-2-azaspiro[4.5]decane-1,3-dione hydrochloride (14):** White crystals, mp 228–230°C (EtOH); Yield: 59% (2.31 g);  $R_f = 0.79$  (S<sub>1</sub>),  $R_f = 0.96$  (S<sub>2</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.24–1.83 (m, 10H, C<sub>5</sub>H<sub>10</sub>), 2.83 (s, 2H, imide), 2.98 (br. s, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 3.18–3.33 (d, 2H, CH<sub>2</sub>–CH<sub>2</sub>,  $J = 4.68$  Hz), 3.62–3.77 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 3.92–3.95 (t,

4H, N(CH<sub>2</sub>)<sub>2</sub>,  $J = 5.36$  Hz), 6.94–7.01 (m, 3H, C<sub>6</sub>H<sub>5</sub>), 7.32–7.35 (m, 2H, C<sub>6</sub>H<sub>5</sub>), 12.97 (br. s, 1H, NH<sup>+</sup>); Anal. Calcd. for C<sub>21</sub>H<sub>30</sub>ClN<sub>3</sub>O<sub>2</sub> C: 64.35, H: 7.71, N: 10.75. Found: C: 64.30, H: 7.94, N: 10.75.

**N-[2-[4-(2-Fluorophenyl)-piperazin-1-yl]-ethyl]-2-azaspiro[4.5]decane-1,3-dione hydrochloride (15):** White crystals, mp 224–226°C (EtOH); Yield: 67% (2.75 g);  $R_f = 0.79$  (S<sub>1</sub>),  $R_f = 0.90$  (S<sub>2</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.29–1.79 (m, 10H, C<sub>5</sub>H<sub>10</sub>), 2.51 (s, 2H, imide), 2.90 (br. s, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 3.18–3.22 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 3.35–3.62 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 3.79–3.9 (m, 4H, N(CH<sub>2</sub>)<sub>2</sub>), 7.04–7.20 (m, 4H, C<sub>6</sub>H<sub>4</sub>), 10.95 (br. s, 1H, NH<sup>+</sup>); Anal. Calcd. for C<sub>21</sub>H<sub>29</sub>FCIN<sub>3</sub>O<sub>2</sub> C: 61.52, H: 7.13, N: 10.25. Found: C: 61.71, H: 7.27, N: 10.49.

**N-[2-[4-(3-Chlorophenyl)-piperazin-1-yl]-ethyl]-2-azaspiro[4.5]decane-1,3-dione hydrochloride (16):** White crystals, mp 252–254°C (EtOH); Yield: 75% (3.19 g);  $R_f = 0.78$  (S<sub>1</sub>),  $R_f = 0.93$  (S<sub>2</sub>); <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 1.30–1.89 (m, 10H, C<sub>5</sub>H<sub>10</sub>), 2.57 (s, 2H, imide), 2.60–2.65 (m, 6H, 4H, N(CH<sub>2</sub>)<sub>2</sub>, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 3.11–3.15 (t, 4H, N(CH<sub>2</sub>)<sub>2</sub>,  $J = 4.68$  Hz), 3.66–3.70 (t, 2H, CH<sub>2</sub>–CH<sub>2</sub>,  $J = 6.32$  Hz), 6.77–6.89 (m, 3H, C<sub>6</sub>H<sub>4</sub>), 7.15–7.20 (t, 1H, C<sub>6</sub>H<sub>4</sub>,  $J = 8.11$  Hz), 12.30 (br. s, 1H, NH<sup>+</sup>); Anal. Calcd. for C<sub>21</sub>H<sub>29</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub> C: 59.21, H: 6.86, N: 9.86. Found: C: 59.01, H: 6.69, N: 9.79.

## PHARMACOLOGICAL PART

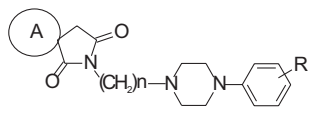
All the experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

### *In vitro* studies

#### 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> binding assays

The affinity of the investigated compounds for 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors *in vitro* was assessed on the basis of their ability to displace [<sup>3</sup>H]-8-OH-DPAT (170 Ci/mmol, NEN Chemicals, USA) and [<sup>3</sup>H]-ketanserin (88 Ci/mmol, NEN Chemicals, USA), respectively. Radioligand binding experiments were carried out on the rat brain using tissues from the hippocampus and from the cortex, and affinity for 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors, respectively, according to the previously published procedures [4].  $K_i$  values were determined from at least three competition binding experiments in which 10–14 concentrations of the tested compounds, run in triplicate, were used. The

**Tab. 1.** Chemical structure and the 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor affinities of the investigated compounds **5–16**



Compound	Ring A	n	R	K <sub>i</sub> SEM (nM)	
				5-HT <sub>1A</sub>	5-HT <sub>2A</sub>
<b>5</b>	cyclopentane	1	H	500 ± 34	2580 ± 68
<b>6</b>	cyclopentane	1	2-F	220 ± 15	1410 ± 20
<b>7</b>	cyclopentane	1	3-Cl	750 ± 6	1620 ± 120
<b>8</b>	cyclopentane	2	H	70 ± 2	390 ± 16
<b>9</b>	cyclopentane	2	2-F	120 ± 10	930 ± 72
<b>10</b>	cyclopentane	2	3-Cl	10 ± 1	30 ± 1
<b>11</b>	cyclohexane	1	H	650 ± 26	1380 ± 82
<b>12</b>	cyclohexane	1	2-F	270 ± 12	1600 ± 36
<b>13</b>	cyclohexane	1	3-Cl	275 ± 5	72 ± 8
<b>14</b>	cyclohexane	2	H	5.1 ± 0.2	86 ± 5
<b>15</b>	cyclohexane	2	2-F	2.7 ± 0.3	192 ± 23
<b>16</b>	cyclohexane	2	3-Cl	4.3 ± 0.7	15 ± 0.5
	Ketanserin			1933 ± 219	1.5 ± 0.2

Cheng and Prusoff equation [6] was used for K<sub>i</sub> calculations.

### In vivo experiments

The experiments were performed on male Wistar rats (280–310g) or male Albino Swiss mice (24–28 g). The animals were kept at a room temperature of 20 ± 1°C, and had free access to food (standard laboratory pellets, LSM) and tap water. All the investigations were conducted in the light phase, of a natural day-night cycle (from January to March), between 9 a.m. and 2 p.m. Each experimental group consisted of 6–8 animals/dose, and all the animals were used only once. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemical Inc.), *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) and (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (±)-(DOI) were used as aqueous solutions. The investigated compounds (**10**, **14**, **15**, **16**) were suspended in 1% aqueous solution of Tween 80. The suspensions were neutralized using a few drops of 0.1 M NaOH.

8-OH-DPAT and WAY 100635 were injected subcutaneously (*sc*), **10**, **14**, **15**, **16** and (±)-DOI were given intraperitoneally (*ip*), in a volume of 2 ml/kg (rats) and 10 ml/kg (mice). The obtained data were analyzed by one-way analysis of variance followed by Dunnett's test (when only one drug was given) or by Newman-Keuls test (when two drugs were administered).

### Body temperature in mice

The effects of the tested compounds given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In separate experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **10**, **14** or **16** was tested. WAY 100635 was administered 15 min before the tested compounds and the rectal body temperature was recorded 30 and 60 min after their injection. In another experiment, the effect of **15** (which did not change mouse body temperature) on the 8-OH-DPAT (5 mg/kg)-induced hypothermia was assessed. Compound **15** was administered 45 min prior to 8-OH-DPAT, and rectal body temperature was measured 15, 30, 45, and 60 min after 8-OH-DPAT injection. The results were expressed as a change in body temperature ( $\delta t$ ) with respect to the basal body temperature, as measured at the beginning of the experiment.

### Lower lip retraction (LLR) in rats

The LLR was assessed according to the method described by Berendsen et al. [2]. The rats were individually placed in cages (30 × 25 × 25 cm), and they were scored three times (at 15, 30 and 45 min) after the administration of the tested compounds or 8-OH-DPAT as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = clearly visible. The total maximum score amounted to 3/rat. In separate experiment, the effect of the investigated compounds or WAY 100635 on LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The investigated compounds or WAY 100635 were administered 45 min and 15 min, respectively, prior to 8-OH-DPAT, and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

**Tab. 2.** The effect of the investigated compounds and WAY 100635 on the body temperature in mice

Treatment	Dose mg/kg	$\Delta t$ SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	–	–0.1 ± 0.1	0.0 ± 0.1	–0.2 ± 0.1	0.0 ± 0.1
<b>10</b>	5	–0.5 ± 0.2	–0.4 ± 0.1	–0.5 ± 0.1	–0.3 ± 0.1
	10	–0.9 ± 0.1 <sup>b</sup>	–0.8 ± 0.1 <sup>b</sup>	–1.1 ± 0.2 <sup>b</sup>	–0.8 ± 0.2 <sup>b</sup>
<b>14</b>	10	–0.6 ± 0.1 <sup>a</sup>	–0.5 ± 0.2	–0.3 ± 0.1	–0.2 ± 0.1
	20	–1.0 ± 0.1 <sup>b</sup>	–1.1 ± 0.1 <sup>b</sup>	–1.6 ± 0.1 <sup>b</sup>	–0.8 ± 0.1 <sup>b</sup>
Vehicle	–	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1
<b>15</b>	10	–0.3 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1	0.1 ± 0.1
	20	–0.4 ± 0.2	–0.3 ± 0.1	–0.4 ± 0.2	–0.2 ± 0.2
<b>16</b>	10	–0.2 ± 0.1	–0.3 ± 0.1	–0.5 ± 0.2	–0.3 ± 0.1
	20	–1.2 ± 0.1 <sup>b</sup>	–1.3 ± 0.2 <sup>b</sup>	–1.1 ± 0.2 <sup>b</sup>	–1.2 ± 0.2 <sup>b</sup>
Vehicle	–	0.1 ± 0.1	0.0 ± 0.1	–0.1 ± 0.1	–0.2 ± 0.1
WAY 100635	0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1

The investigated compounds (*ip*) and WAY 100635 (*sc*) were administered 30 min before the test. The absolute mean initial body temperatures were within a range of 36.3 ± 0.5 °C, <sup>a</sup> p < 0.05, <sup>b</sup> p < 0.01 vs. vehicle

**Tab. 3.** The effect of WAY 100635 on the hypothermia induced by compounds **10**, **14**, **16** and 8-OH-DPAT in mice

Treatment and dose (mg/kg)	$\Delta t$ SEM (°C)	
	30 min	60 min
Vehicle + vehicle	0.0 ± 0.1	–0.1 ± 0.1
Vehicle + <b>10</b> (10)	–0.9 ± 0.2 <sup>b</sup>	–0.7 ± 0.1 <sup>b</sup>
WAY 100635 (0.1) + <b>10</b> (10)	–0.3 ± 0.1 <sup>B</sup>	–0.3 ± 0.1 <sup>A</sup>
Vehicle + vehicle	–0.1 ± 0.1	0.0 ± 0.1
Vehicle + <b>14</b> (20)	–1.0 ± 0.1 <sup>b</sup>	–1.1 ± 0.1 <sup>b</sup>
WAY 100635 (0.1) + <b>14</b> (20)	–0.5 ± 0.1 <sup>A</sup>	–0.4 ± 0.1 <sup>B</sup>
Vehicle + vehicle	–0.1 ± 0.1	–0.1 ± 0.1
Vehicle + <b>16</b> (20)	–0.9 ± 0.1 <sup>b</sup>	–1.0 ± 0.1 <sup>b</sup>
WAY 100635 (0.1) + <b>16</b> (20)	–0.4 ± 0.2 <sup>A</sup>	–0.4 ± 0.2 <sup>B</sup>
Vehicle + vehicle	0.1 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT (5)	–1.0 ± 0.1 <sup>b</sup>	–0.7 ± 0.2 <sup>a</sup>
WAY 100635 (0.1) + 8-OH-DPAT (5)	–0.1 ± 0.1 <sup>B</sup>	0.1 ± 0.1 <sup>B</sup>

WAY 100635 was administered (*sc*) 15 min before the investigated compounds (*ip*) or 8-OH-DPAT (*sc*). The absolute mean initial body temperatures were within a range of 36.3 ± 0.5 °C, <sup>a</sup> p < 0.05, <sup>b</sup> p < 0.01 vs. vehicle + vehicle; <sup>A</sup> p < 0.05, <sup>B</sup> p < 0.01 vs. vehicle + investigated compound

**Tab. 4.** Induction of lower lip retraction (LLR) by the investigated compounds and WAY 100635 (**A**) and their effect on the 8-OH-DPAT-induced LLR (**B**) in rats

Treatment	Dose mg/kg	Means SEM LLR score	
		A	B
Vehicle	–	0.1 ± 0.1	2.8 ± 0.1
<b>10</b>	10	0 ± 0	1.2 ± 0.2 <sup>b</sup>
	20	0.3 ± 0.1	1.2 ± 0.2 <sup>b</sup>
<b>14</b>	10	1.0 ± 0.2 <sup>b</sup>	2.3 ± 0.2
	20	2.6 ± 0.2 <sup>b</sup>	NT
Vehicle	–	0.1 ± 0.1	2.8 ± 0.2
<b>15</b>	10	1.3 ± 0.2 <sup>b</sup>	2.8 ± 0.2
	20	2.0 ± 0.2 <sup>b</sup>	NT
<b>16</b>	5	0.1 ± 0.1	1.8 ± 0.3 <sup>b</sup>
	10	0.4 ± 0.2	0.6 ± 0.3 <sup>b</sup>
WAY 100635	0.1	0.1 ± 0.1	0.3 ± 0.2 <sup>b</sup>

The investigated compounds (*ip*) and WAY 100635 (*sc*) were administered 15 min before the test (**A**), or 45 min before 8-OH-DPAT (1mg/kg, *sc*) (**B**). <sup>b</sup> p < 0.01 vs. vehicle (A) or vs. vehicle + 8-OH-DPAT (B); NT – not tested

**Tab. 5.** The effect of compounds **10**, **16** and ketanserin on the (±)-DOI-induced head twitch response in mice

Treatment	ID <sub>50</sub> (mg/kg, <i>ip</i> ) <sup>a</sup>
<b>10</b>	8.3 (6.1–11.2)
<b>16</b>	4.3 (3.2–5.8)
Ketanserin	0.12 (0.07–0.2)

<sup>a</sup> ID<sub>50</sub> – the dose inhibiting the head twitches in mice by 50%; confidence limits (90%) given in parenthesis. The investigated compounds were administered *ip* 60 min before (±)-DOI (2.5 mg/kg, *ip*)

**Tab. 6.** Functional *in vivo* 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor activity of the investigated compounds

Compound	5-HT <sub>1A</sub> activity		5-HT <sub>2A</sub> activity
	presynaptic	postsynaptic	
<b>10</b>	agonist	antagonist	antagonist
<b>14</b>	agonist	agonist	NT
<b>15</b>	non active	agonist	NT
<b>16</b>	agonist	antagonist	antagonist

NT – not tested

### Head twitch response in mice

In order to habituate mice to the experimental environment, each animal was randomly transferred to a 12 cm (diameter) × 20 cm (height) glass cage, lined with sawdust, 20 min before treatment. Head twitches were induced in mice by (±)-DOI (2.5 mg/kg). Immediately after the treatment, the head twitches were counted for 20 min [7]. The investigated compounds were administered 60 min before (±)-DOI.

## Results and Discussion

The results of the binding studies with compounds **5–16** are summarized in Table 1. They show that the novel arylpiperazines exhibit significant but diversified affinities for 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptors, probably due to the length of the spacer and the size of the cycloalkyl ring at the amide fragment. In contrast to the previously obtained propylene derivatives (**1a–c** and **2a–c**), which were found to be potent 5-HT<sub>2A</sub> receptor ligands [14, 16], the new compounds with the ethylene chain and cyclohexane ring (**14**, **15**, **16**) are the most potent 5-HT<sub>1A</sub> receptor ligands ( $K_i = 5.1$ , 2.7 and 4.3 nM, respectively), whereas cyclopentane analogues (**8**, **9**, **10**) are slightly less active ( $K_i = 70$ , 120 and 10 nM, respectively). In the case of the series with a methylene bridge (**5–7** and **11–13**), the shortening of the spacer resulted in low affinity for both 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors ( $K_i = 220–750$  nM and 390–2580 nM, respectively). Among those compounds, only 3-chloro derivative (**13**) produced an increase in 5-HT<sub>2A</sub> receptor affinity ( $K_i = 72$  nM), but did not significantly influence  $K_i$  values at the 5-HT<sub>1A</sub> receptor.

Regarding 5-HT<sub>2A</sub> receptors, the results of binding studies demonstrated that the affinity for this receptor was, as a rule, lower than that for 5-HT<sub>1A</sub> receptor. The introduction of an electron withdrawing 3-chloro substituent produced the most positive effect and increased the affinity for 5-HT<sub>2A</sub> receptor. Except for compound **7**, the other 3-Cl derivatives **10**, **13**, **16** demonstrated high affinity ( $K_i = 15–72$  nM) for 5-HT<sub>2A</sub> receptors.

The compounds **10**, **14**, **15**, **16** active *in vitro* ( $K_i \leq 10$  nM) were further tested in several *in vivo* models to determine their functional profile at 5-HT<sub>1A</sub> and

5-HT<sub>2A</sub> receptors. As was shown previously, the hypothermia induced by the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT in mice depended primarily on the stimulation of presynaptic 5-HT<sub>1A</sub> receptors [11, 13] and was abolished by such 5-HT<sub>1A</sub> receptor antagonists as WAY 100635 [9]. Thus, the hypothermia produced by the tested compounds and reduced by WAY 100635 was regarded as a measure of presynaptic 5-HT<sub>1A</sub> receptor agonistic activity. Moreover, the effect of compounds which, like WAY 100635, did not modify mouse body temperature in the 8-OH-DPAT-induced hypothermia was assessed to measure presynaptic 5-HT<sub>1A</sub> receptor antagonistic activity. To determine the postsynaptic 5-HT<sub>1A</sub> receptor agonistic effect of the tested 5-HT<sub>1A</sub> 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<sub>1A</sub> receptors [1, 2]; moreover, it was shown that the latter symptom was sensitive only to 5-HT<sub>1A</sub> receptor antagonists [9, 18, 23]. Additionally, the 5-HT<sub>2A</sub> antagonistic activity of **10** and **16** was assessed by testing their ability to antagonize the (±)-DOI-induced head twitches in mice, the effect being connected with selective stimulation of central 5-HT<sub>2A</sub> receptors [7, 26].

All the results of *in vivo* studies are presented in Tables 2–5. Of the compounds tested, **10**, **14** and **16**, like 8-OH-DPAT, induced hypothermia in mice (Tab. 2), and this effect was reduced by WAY 100635 (Tab. 3). On the other hand, compound **15**, like WAY 100635, did not change body temperature in mice (Tab. 2) but, in contrast to WAY 100635, at the dose up to 10 mg/kg, it did not affect the hypothermia induced by 8-OH-DPAT in mice (data not shown). These results indicate that in the hypothermia model, compounds **10**, **14** and **16** behave like presynaptic 5-HT<sub>1A</sub> receptor agonists, while **15** is inactive in this test. Like 8-OH-DPAT, compounds **14** and **15** evoked LLR in rats, whereas **10** and **16**, like WAY 100635, inhibited the LLR induced by 8-OH-DPAT (Tab. 4). The above results demonstrate that **14** and **15** exhibit features of postsynaptic 5-HT<sub>1A</sub> receptor agonists, while **10** and **16** behave like antagonists of these receptors. Like ketanserin, a reference 5-HT<sub>2A</sub> receptor antagonist, compounds **10** and **16** (which exhibited the highest 5-HT<sub>2A</sub> receptor affinity) inhibited the head twitches induced by (±)-DOI, a 5-HT<sub>2A</sub> receptor agonist, in mice (Tab. 5). Hence, compounds **10** and **16** may be classified as 5-HT<sub>2A</sub> receptor antagonists. However, it has been demonstrated that head twitch

response evoked by (±)-DOI was also inhibited by e.g. the selective antagonists of dopamine D<sub>1</sub> and D<sub>2</sub> receptors or α<sub>1</sub>-adrenoreceptors [8, 24]. In the present paper, for compounds **10** and **16**, only 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor activity was determined. Thus, it cannot be excluded that mechanisms other than 5-HT<sub>2A</sub> receptor blockade are involved in reduction of (±)DOI-induced head twitches by these compounds.

As follows from the *in vivo* results, the tested compounds are characterized by diverse *in vivo* activity at 5-HT<sub>1A</sub> receptors. In fact, in the used models, those compounds showed characteristics of pre- and postsynaptic 5-HT<sub>1A</sub> receptors agonists (**14**), of agonists of presynaptic and antagonists of postsynaptic sites (**10** and **16**), or of postsynaptic 5-HT<sub>1A</sub> receptor antagonists (**15**). Moreover, compounds **10** and **16** exhibited properties of potential 5-HT<sub>2A</sub> receptor antagonists (Tab. 6).

In conclusion, a new series of N-[(4-arylpiperazin-1-yl)-alkyl]-2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione analogues of series **1a–c** and **2a–c** can be regarded as potential ligands of 5-HT<sub>1A</sub> receptors. The obtained results show that both structural features, i.e. the alkyl chain length and the size of the cycloalkyl ring, seem to play a significant role in the binding to 5-HT<sub>1A</sub> receptor sites. Considering the functional profile of the investigated 5-HT<sub>1A</sub> and 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor ligands, it cannot be excluded that they (or at least some of them) are likely to reveal potential psychotropic properties.

## References:

- Berendsen HHG, Broekkamp CLE, van Delft AM: Depletion of brain serotonin differently affects behaviours induced by 5-HT<sub>1A</sub>, 5-HT<sub>1C</sub>, and 5-HT<sub>2</sub> receptor activation in rats. *Behav Neural Biol*, 1991, 55, 214–226.
- Berendsen HHG, Jenck F, Broekkamp CLE: Selective activation of 5-HT<sub>1A</sub> receptors induces lower lip retraction in the rat. *Pharmacol Biochem Behav*, 1989, 33, 821–827.
- Blier P, Montigny C: Current advances and trends in the treatment of depression. *Trends Pharmacol Sci*, 1994, 15, 220–226.
- Bojarski AJ, Cegła MT, Charakchieva-Minol S, Mokrosz MJ, Maćkowiak M, Mokrosz JL: Structure-activity relationship studies of CNS agents. Part 9. 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor affinity of some 2- and 3-substituted 1,2,3,4-tetrahydro-β-carbolines. *Pharmazie*, 1993, 48, 289–294.
- Bojarski AJ, Kowalski P, Kowalska T, Duszyńska B, Charakchieva-Minol S, Tatarczyńska E, Kłodzińska A, Chojnacka-Wójcik E: Synthesis and pharmacological evolution of new arylpiperazines. 3-{4-[4-(3-chlorophenyl)-piperazinyl]butyl}-quinazolidin-4-one – a dual serotonin 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor ligand with an anxiolytic – like activity. *Bioorg Med Chem*, 2002, 10, 3817–3827.
- Cheng Y, Prusoff WH: Relationship between the inhibition constant (K<sub>1</sub>) and the concentration of inhibitor which causes 50 per cent inhibition (I<sub>50</sub>) of an enzymatic reaction. *Biochem Pharmacol*, 1973, 22, 3099–3108.
- Darmani NA, Martin BR, Pandey U, Glennon RA: Do functional relationships exist between 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors? *Pharmacol Biochem Behav*, 1990, 36, 901–906.
- Dursun SM, Handley SL: Similarities in the pharmacology of spontaneous and DOI-induced head-shakes suggest 5HT<sub>2A</sub> receptors are active under physiological conditions. *Psychopharmacology*, 1996, 128, 198–205.
- Forster EA, Cliffe IA, Bill DJ, Dover GM, Jones D, Reilly Y, Fletcher A: A pharmacological profile of the selective silent 5-HT<sub>1A</sub> receptor antagonist, WAY-100635. *Eur J Pharmacol*, 1995, 281, 81–88.
- Glennon RA, Naiman NA, Lyon RA, Titeler M: Arylpiperazine derivatives as high affinity 5-HT<sub>1A</sub> serotonin ligands. *J Med Chem*, 1988, 31, 1968–1971.
- Goodwin GM, De Souza RJ, Green AR: The pharmacology of the hypothermic response in mice to 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). A model of presynaptic 5-HT<sub>1</sub> function. *Neuropharmacology*, 1985, 24, 1187–1194.
- Hamon M: Neuropharmacology of anxiety: Perspective and prospects. *Trends Pharmacol Sci*, 1994, 15, 36–39.
- Martin KF, Heal DJ: 8-OH-DPAT-induced hypothermia in rodents: a specific model of 5-HT<sub>1A</sub> autoreceptor function. In: *Serotonin: Molecular Biology, Receptors and Functional Effects*. Ed. Fozard JR, Saxena PR, Birkhauser Verlag, Basel, 1991, 483–490.
- Obniska J, Kołaczkowski M, Charakchieva-Minol S, Nędza K, Dybała M, Bojarski AJ: Synthesis, anticonvulsant properties and 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor affinity of new N-[(4-arylpiperazin-1-yl)-propyl]-2-aza-spiro[4.4]nonane and [4.5]decane-1,3-dione derivatives. *Pharmacol Rep*, 2005, 57, 336–344.
- Obniska J, Kulig K, Zejc A: Synthesis and anticonvulsant properties of new N-piperazinylalkyl imides of succinic acid. *Acta Pol Pharm – Drug Res*, 1998, 55, 223–231.
- Obniska J, Pawłowski M, Kołaczkowski M, Czopek A, Duszyńska B, Kłodzińska A, Tatarczyńska E, Chojnacka-Wójcik E: Synthesis and 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor affinity of new N-[(4-arylpiperazin-1-yl)-propyl] derivatives of 3-spirocyclohexanepyrrolidine-2,5-dione and 3-spiro-β-tetralonepyrrolidine-2,5-dione. *Pol J Pharmacol*, 2003, 55, 553–557.
- Obniska J, Zagórska A: Synthesis and anticonvulsant properties of new N-[(4-phenyl phenylpiperazin-1-yl)-methyl] derivatives of 3-phenylpyrrolidine-2,5-dione and 2-aza-spiro[4.4]nonane-1,3-dione. *Il Farmaco*, 2003, 58, 1227–1234.
- Paluchowska MH, Bojarski AJ, Charakchieva-Minol S, Wesółowska A: Active conformation of some arylpiperazine postsynaptic 5-HT<sub>1A</sub> receptor antagonists. *Eur J Med Chem*, 2002, 37, 273–283.

- 
19. Paluchowska M, Charakchieva-Minol S, Tatarczyńska E, Kłodzińska A, Stachowicz K, Chojnacka-Wójcik E: New 4-[ $\omega$ -(diarylmethylamino)alkyl]-1-arylpiperazines as selective 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptor ligands with differentiated *in vivo* activity. *Pol J Pharmacol*, 2004, 56, 743–754.
  20. Pawłowski M, Chłoń G, Obniska J, Charakchieva-Minol S, Mokrosz MJ: Synthesis, 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor affinity of new 1-phenylpiperazinpropyl derivatives of purine-2,6- and pyrrolidine-2,5-diones. *Il Farmaco*, 2000, 55, 461–468.
  21. Pawłowski M, Katlabi J, Drabczyńska H, Duszyńska B, Charakchieva-Minol S, Dereń-Wesołek A, Tatarczyńska E, Chojnacka-Wójcik E, Mokrosz MJ, Bojarski AJ: New 9- or 10-arylpiperazinoalkyl substituted pyrimido- or diazepino[2,1-*f*]purines with partial or full 5-HT<sub>1A</sub> agonistic activity. *Eur J Med Chem*, 1999, 34, 167–175.
  22. Peroutka SJ, Sleight AJ, McCarthy BG, Pierce PA, Schmidt AW, Hekmatpanah CR: The clinical utility of pharmacological agents that act at serotonin receptors. *J Neuropsychiatry Clin Neurosci*, 1989, 1, 253–262.
  23. Przegaliński E, Filip M, Budziszewska B, Chojnacka-Wójcik E: Antagonism of (+)WAY 100135 to behavioral, hypothermic and corticosterone effects induced by 8-OH-DPAT. *Pol J Pharmacol*, 1994, 46, 21–27.
  24. Schreiber R, Brocco M, Audinot V, Gobert A, Veiga S, Millan MJ: (1-(2,5-Dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT)<sub>2A</sub> receptors: modulation by novel 5-HT<sub>2A/2C</sub> antagonists, D<sub>1</sub> antagonists and 5-HT<sub>1A</sub> agonists. *J Pharmacol Exp Ther*, 1995, 273, 101–112.
  25. Scott KR, Moore JA, Zalusky TB, Nicholson JM: Spiro[4.5] and spiro[4.6]carboxylic acids: cyclic analogues of valproic acid. *J Med Chem*, 1985, 28, 413–417.
  26. Willins DL, Meltzer HY: Direct injection of 5-HT<sub>2A</sub> receptor agonists into the medial prefrontal cortex produces a head-twitch response in rats. *J Pharmacol Exp Ther*, 1997, 282, 699–706.

**Received:**

May 17, 2005; in revised form: January 3, 2006.