



## Antidepressant-like activity of the phenylpiperazine pyrrolidin-2-one derivatives in mice

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### Abstract:

The present study was designed to investigate the central nervous system activity of 23 novel phenylpiperazine pyrrolidin-2-one derivatives. These compounds had marked antiarrhythmic and hypotensive activities and revealed affinity for  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. These effects may be related to their  $\alpha$ -adrenolytic properties. We assessed their antidepressant-like effect in the forced swimming test, influence of spontaneous locomotor activities and binding to 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors. Our study demonstrated the strong antidepressant-like activity of compound **EP-65** in the forced swimming test. The effect of **EP-65** was stronger than results obtained with the classical antidepressants imipramine and mianserin. Other compounds, **EP-41**, **EP-42**, **EP-44**, **EP-47**, **EP-48**, **EP-49**, **EP-50**, **EP-62**, **EP-66**, **EP-70**, **EP-75** and **EP-76**, showed significantly weaker activities in this test. Compound **EP-42** showed the strongest affinity for 5-HT<sub>1A</sub> ( $K_i = 24.5$  nM), and compound **EP-50** showed the strongest affinity for the 5-HT<sub>2</sub> receptor ( $K_i = 109.1$  nM). All tested compounds significantly suppressed the spontaneous locomotor activity of mice. Currently, it is not possible to determine which mechanisms are involved in the witnessed antidepressant-like activity of novel phenylpiperazine pyrrolidin-2-one derivatives.

### Key words:

1-[3-(4-arylpiperazin-1-yl)-2-hydroxypropyl]-pyrrolidin-2-one derivatives,  $\alpha$ -adrenoceptor blocking activity, 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors binding, antidepressant-like activity

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## Introduction

The monoamine theory states that depression may be due to reduced levels of monoamine or neuronal activity in the brain based on primary pharmacological effects of antidepressants. Most antidepressants exert important actions on the metabolism of monoamine neurotransmitters and their receptors, particularly norepinephrine and serotonin. Apart from antidepressants such as tricyclic antidepressants, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, drugs with the receptor

activity, for example nefazodone, trazodone, mirtazapine and mianserin play an important role in the therapy of the depression [12, 22].

It is widely accepted that cerebral  $\alpha$ -adrenergic (especially  $\alpha_2$ ) and serotonin receptors (especially 5-HT<sub>1</sub> and 5-HT<sub>2</sub>) are involved in the depression and action of atypical antidepressants [6, 34]. Drugs that have antagonistic effects at 5-HT<sub>2A</sub> receptors may contribute to antidepressant and anxiolytic activity [6, 8]. On the other hand, drugs that have antagonistic effects at presynaptic 5-HT<sub>1</sub> subtype autoreceptors and  $\alpha_2$ -adrenolytics may contribute to enhanced neuronal release

of serotonin or norepinephrine, respectively, which initiates the antidepressant effect [12, 27].

Our earlier research has shown that pyrrolidin-2-one derivatives with arylpiperazine have marked antiarrhythmic and hypotensive activities and revealed affinity for  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors. Antiarrhythmic and hypotensive effects may be related to their  $\alpha$ -adrenolytic properties [17–19, 24]. Many studies showed that arylpiperazine can condition affinity to  $\alpha$ -adrenoceptors; 5-HT<sub>1A</sub>/5-HT<sub>2A</sub> receptors and compounds with arylpiperazine had antidepressant-like effects [1, 14, 15, 21, 26, 31, 35] or antipsychotic and anxiolytic activity [10, 16, 32].

It is possible that new pyrrolidin-2-one derivatives with arylpiperazine will have antidepressant activities. In this study, we assessed the antidepressant effect (in the forced swimming test) and sedation effect, as well as the 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptor binding of 23 pyrrolidin-2-one derivatives.

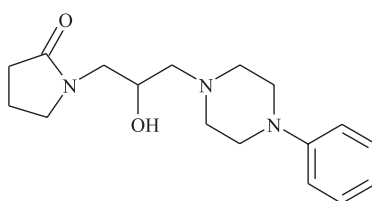
## Materials and Methods

### Animals

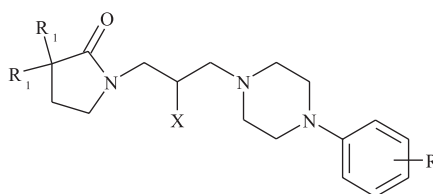
Experiments were carried out on male Albino-Swiss mice (body weight 18–26 g). Animals were housed in constant temperature facilities, exposed to 12:12 h light-dark cycle and maintained on a standard pellet diet and tap water given *ad libitum*. All procedures were conducted according to the Animal Care and Use Committee Guidelines and approved by the Ethical Committee of Jagiellonian University. Control and experimental groups consisted of 6–8 animals each.

### Drugs

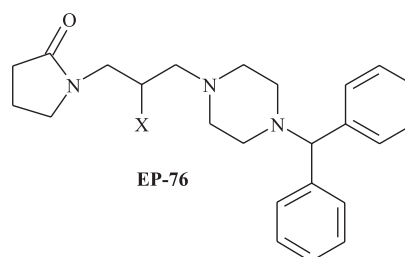
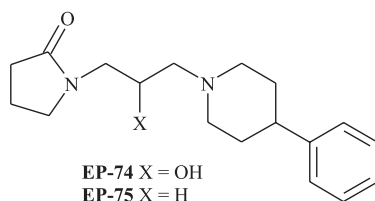
The tested compounds (Fig. 1) were synthesized by Katarzyna Kulig and Barbara Malawska in the De-



**MG-1** 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-pyrrolidin-2-one



Comp.	<b>EP-40</b>	<b>EP-41</b>	<b>EP-42</b>	<b>EP-43</b>	<b>EP-44</b>	<b>EP-45</b>	<b>EP-46</b>	<b>EP-47</b>	<b>EP-48</b>	<b>EP-49</b>
X	-OH	H	H	-OH	H	H	H	H	H	H
R	2-OH	H	2-OMe	2-O- <i>i</i> -Pr	2-Cl	2-F	2-OEt	2-Me	3-OMe	3-Cl
R <sub>1</sub>	H	H	H	H	H	H	H	H	H	H
Comp.	<b>EP-50</b>	<b>EP-61</b>	<b>EP-62</b>	<b>EP-63</b>	<b>EP-64</b>	<b>EP-65</b>	<b>EP-66</b>	<b>EP-70</b>	<b>EP-73</b>	
X	H	H	H	H	H	H	H	OH	OH	
R	4-Cl	2-CF <sub>3</sub>	4-OMe	4-OH	2,4-diF	2-Cl, 5-OMe	3-OH	2-OEt	4-OMe	
R <sub>1</sub>	H	H	H	H	H	H	H	Ph	Ph	



**Fig. 1.** Schematic structure of MG-1 and new pyrrolidin-2-one derivatives

partment of Physicochemical Drug Analysis, Pharmaceutical Faculty, Jagiellonian University. The syntheses of tested compounds were described in previous papers [17–19, 24]. Imipramine (Imipraminum hydrochloricum, Polpharma, Poland), ritanserin and WAY-100635 (Sigma) were dissolved in 0.9% sodium chloride. Mianserin (Organon) was suspended in 0.5% methylcellulose (Loba-Chemie, Germany). The investigated compounds and reference drugs were administered intraperitoneally.

### 5-HT<sub>1A</sub> receptor binding experiments

[<sup>3</sup>H]8-Hydroxy-2-(di-n-propylamino)-tetralin ([<sup>3</sup>H]8-OH-DPAT, spec. act. 106 Ci/mmol, NEN Chemicals) was used for labeling 5-HT<sub>1A</sub> receptors. The membrane preparation and assay were carried out accordingly as previously published [5, 25] with slight modifications. Briefly, the cerebral cortex tissue was homogenized in 20 vol. of 50 mM Tris-HCl buffer (pH 7.7 at 25 °C) using Ultra-Turrax<sup>®</sup> T 25 and was then centrifuged at 32,000 × g for 10 min. The supernatant fraction was discarded, and the pellet was resuspended in the same volume of Tris-HCl buffer followed by centrifugation. Before the third centrifugation, samples were incubated at 37°C for 10 min. The final pellet was resuspended in Tris-HCl buffer containing 10 μM pargyline, 4 mM CaCl<sub>2</sub> and 0.1% ascorbic acid. One milliliter of the tissue suspension (9 mg of wet weight), 100 μl of 10 μM serotonin (for unspecific binding), 100 μl of [<sup>3</sup>H]8-OH-DPAT and 100 μl of analyzed compound were incubated at 37°C for 15 min. Incubation was followed by rapid vacuum filtration through Whatman GF/B glass filters. The suspension was then washed 3 times with 5 ml of a cold buffer (50 mM Tris-HCl, pH 7.7) using a Brandel cell harvester. The final [<sup>3</sup>H]8-OH-DPAT concentration was 1 nM, and the concentrations of the analyzed compounds ranged from 10<sup>-10</sup> to 10<sup>-4</sup> M.

### 5-HT<sub>2</sub> receptor binding experiments

[<sup>3</sup>H]Ketanserin (spec. act. 60 Ci/mmol, NEN Chemicals) was used for labeling 5-HT<sub>2</sub> receptors. The assay was performed according to the method of Laysen et al. [5, 20] with slight modifications. The cerebral cortex tissue was homogenized in 20 vol. of 50 mM Tris-HCl buffer (pH 7.7 at 25°C) and centrifuged at 32,000 × g for 20 min. The resulting pellet was resuspended in the same quantity of buffer, preincubated at

37°C for 10 min and centrifuged for 20 min. The final pellet was resuspended in 50 vol. of the same buffer. One milliliter of the tissue suspension, 100 μl of 1 μM mianserin (displacer), 100 μl of [<sup>3</sup>H]ketanserin and 100 μl of the analyzed compound were incubated at 37°C for 20 min, followed by rapid vacuum filtration through Whatman GF/B glass filters. The filtrate was then washed three times with 5 ml of a cold Tris-HCl buffer. The final [<sup>3</sup>H]ketanserin concentration was 0.6 nM, and the concentrations of analyzed compounds ranged from 10<sup>-10</sup> to 10<sup>-4</sup> M.

### Forced swimming test in mice

The forced swimming test experiments were carried out according to the slightly modified method of Porsolt et al. [2, 28]. Mice were dropped individually into glass cylinders (height 25 cm, diameter 10 cm) filled with water to a height of 10 cm (maintained at 23–25°C) and left there for 6 min. After an initial 2 min period of vigorous activity, each animal assumed an immobile posture. The total duration of immobility was recorded during the final 4 min of the 6 min testing period. Mice were judged to be immobile when they remained floating passively in the water, making only small movements to keep their heads above the water. Imipramine and mianserin were used as reference compounds. The tested compounds and reference drugs were given *ip* 45 min before the experiments. Ritanserin (a 5HT<sub>2</sub> antagonist) and WAY 100635 (a 5HT<sub>1A</sub> antagonist) were administered *ip* 60 min before the test (dose: ritanserin – 4 mg/kg, WAY 100635 – 0.1 mg/kg)

### Spontaneous locomotor activity

Spontaneous locomotor activity in mice was measured with circular photoresistor actometers (32 cm in diameter). The investigated compounds were injected *ip* at a dose range of 2.5–30 mg/kg. Thirty minutes after the injection of the investigated compounds, mice were placed in actometers for 30 min. Each crossing of the light beam was recorded automatically. The number of impulses was noted after 20 min.

### Statistical analysis

Data are expressed as the mean ± SEM and evaluated by one-way analysis of variance (ANOVA) followed by the Duncan test; *p* < 0.05 was considered significant.

## Results

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

Table 1 shows the binding profiles ( $\alpha_1$ ,  $\alpha_2$ , 5-HT<sub>1A</sub>, 5-HT<sub>2</sub> receptors) of all compounds and mianserin. Compound **EP-42** showed the strongest affinity for the 5-HT<sub>1A</sub> receptor and had weak affinity for the 5-HT<sub>2</sub> receptor. On the other hand, compound **EP-50** showed the strongest affinity for the 5-HT<sub>2</sub> receptor. Results indicate that compounds **EP-42**, **EP-43**, **EP-44**, **EP-46** and **EP-49** showed stronger affinity for 5-HT<sub>1A</sub> receptors than mianserin. Only compound

**EP-50** had stronger affinity for the 5-HT<sub>2</sub> receptor than mianserin (Tab. 1).

### Forced swimming test in mice

In this test, thirteen compounds showed significant activity: **EP-41**, **EP-42**, **EP-44**, **EP-47**, **EP-48**, **EP-49**, **EP-50**, **EP-62**, **EP-65**, **EP-66**, **EP-70**, **EP-75** and **EP-76**, (Tab. 2a, 2b).

The most potent effect was produced by compound **EP-65**, which significantly reduced the immobility time in this test at doses 2.5 mg/kg (by 32.2%), 5 mg/kg (by 46.6%), 10 mg/kg (by 51.7%) and 20 mg/kg (by 31.2%) (Tab. 2b). The effect was stronger than the results obtained with classical antidepressants imipramine, mianserin and reference compound **MG-1**, (Tab. 3).

Compounds **EP-41** and **EP-47** significantly reduced immobility time in the forced swimming test (by 14 and 23%, respectively) at 5 mg/kg doses; **EP-76** was affective at both doses (5 and 10 mg/kg) by 29.5–25.3%. Compounds **EP-49**, **EP-62**, **EP-70** and **EP-75** were significantly active only at 10 mg/kg doses (by 16.7, 26.1, 27.4 and 24.9%, respectively) (Tab. 2a, 2b).

Compounds **EP-42**, **EP-44**, **EP-48** and **EP-66** significantly reduced the immobility time at 20 mg/kg doses (by 21.1, 21.1, 40.4 and 25.7%, respectively), and compound **EP-50** was effective at doses of 20 and 30 mg/kg by 30 and 24%, respectively (Tab. 2a, 2b). Classical antidepressant imipramine and mianserin were significantly active in this test at doses of 10 and 20 mg/kg (Tab. 3).

### Effect of combined administration of EP-65 and ritanserin or WAY100635 in the forced swimming test

The effect of compound **EP-65** on the total duration of the immobility time and the effect of pre-treatment with ritanserin or WAY100635 on the effect produced in the forced swimming test in mice is shown in Figure 2. **EP-65** at a 10 mg/kg dose significantly reduced (by 51.7%) the immobility time in the forced swimming test in mice. Administration of ritanserin or WAY100635 had no effect on the immobility time (data not shown), although it antagonized the effect elicited by **EP-65** in this test (by 39.5% and 29.8, respectively) (Fig. 2).

**Tab. 1.** Affinity towards 5HT<sub>1A</sub> and 5HT<sub>2</sub> serotonin receptors in the rat cerebral cortex

Compound	[ <sup>3</sup> H]8-hydroxy-DPAT 5 HT <sub>1A</sub>	[ <sup>3</sup> H]ketanserin 5 HT <sub>2</sub>
	K <sub>i</sub> (nM)	
<b>EP-40</b>	163.5 ± 24.1	3880 ± 1250
<b>EP-41</b>	169.2 ± 34.7	580 ± 100
<b>EP-42</b>	24.5 ± 6.1	1850 ± 800
<b>EP-43</b>	91.1 ± 9.4	3090 ± 1300
<b>EP-44</b>	48.5 ± 12.8	440 ± 50
<b>EP-45</b>	419.9 ± 13.7	744.5 ± 59.6
<b>EP-46</b>	55.3 ± 10.1	680 ± 30
<b>EP-47</b>	133.4 ± 9.3	290 ± 10
<b>EP-48</b>	597.1 ± 40.2	491.1 ± 47.7
<b>EP-49</b>	90.2 ± 7.8	180 ± 10
<b>EP-50</b>	1100 ± 456	109.1 ± 13.3
<b>EP-61</b>	18800 ± 3700	37400 ± 1300
<b>EP-62</b>	11600 ± 1800	7800 ± 1900
<b>EP-63</b>	56900 ± 13000	1100 ± 500
<b>EP-64</b>	14900 ± 2700	3600 ± 1000
<b>EP-65</b>	1100 ± 400	350 ± 20
<b>EP-66</b>	439.4 ± 1.2	3100 ± 900
<b>EP-70</b>	435.7 ± 51.2	5800 ± 1600
<b>EP-73</b>	48300	51300
<b>EP-74</b>	22400	14600
<b>EP-75</b>	53200	15300
<b>EP-76</b>	12500	5800
<b>MG-1</b>	2500 ± 1500	13530 ± 2750
Mianserin [22]	97	121

**Tab. 2a.** Effect of tested compounds on the total duration of immobility in the forced swimming test (Porsolt test) and influence on spontaneous locomotor activity of mice

Compound	Dose (mg/kg)	Reduced immobility time in Porsolt test $\pm$ SEM (%)	Inhibition of locomotor activity $\pm$ SEM (%)
<b>EP-40</b>	10	not active	28 $\pm$ 15.2*
	20		45 $\pm$ 26***
	30		65 $\pm$ 16.1****
<b>EP-41</b>	2.5	0	2 $\pm$ 9.5
	5	14 $\pm$ 5.9*	11 $\pm$ 8.2
	10	10 $\pm$ 17.3	30 $\pm$ 16.4**
	30	0	63 $\pm$ 18.3***
<b>EP-42</b>	10	0	0
	20	21 $\pm$ 11.4*	20 $\pm$ 18.8
	30	3 $\pm$ 4	26 $\pm$ 12*
<b>EP-43</b>	5	not active	31 $\pm$ 17.5***
	10		57 $\pm$ 16****
	20		67 $\pm$ 15.5****
<b>EP-44</b>	10	0	20 $\pm$ 11.1
	20	21 $\pm$ 10.7*	20 $\pm$ 25.9
	30	0	38 $\pm$ 12.5*
<b>EP-45</b>	5	not active	20 $\pm$ 10.2
	10		39 $\pm$ 13.5***
	20		48 $\pm$ 11.5****
<b>EP-46</b>	5	not active	20 $\pm$ 8.6
	10		50 $\pm$ 19.8****
	20		59 $\pm$ 12.5****
<b>EP-47</b>	2.5	0	–
	5	23 $\pm$ 5.3***	13 $\pm$ 11.9
	10	8 $\pm$ 6.7	52 $\pm$ 10.1****
	20	7 $\pm$ 7.5	49 $\pm$ 14.8****
<b>EP-48</b>	10	11 $\pm$ 3.9	0
	20	40 $\pm$ 14.2***	47 $\pm$ 12.1***
	30	10 $\pm$ 4.7	67 $\pm$ 8.1****
<b>EP-49</b>	5	0	9 $\pm$ 11
	10	17 $\pm$ 8.5*	36 $\pm$ 19.1***
	20	0	64 $\pm$ 3.1****
<b>EP-50</b>	5	1 $\pm$ 10.4	13 $\pm$ 12.1
	10	11 $\pm$ 6.9	12 $\pm$ 8
	20	30 $\pm$ 4.5**	24 $\pm$ 5.9
	30	25 $\pm$ 11.9*	40 $\pm$ 18***
<b>EP-61</b>	5	not active	7 $\pm$ 5.7
	10		28 $\pm$ 6.9*
	20		48 $\pm$ 10.7**

Data are presented as the means  $\pm$  SEM of 6–8 mice per group (in percent of control). Results were analyzed by one-way ANOVA followed by Duncan test. \*  $p < 0.05$ ; \*\*  $p < 0.02$ ; \*\*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.001$  vs. respective control

**Tab. 2b.** Effect of tested compounds on the total duration of immobility in the forced swimming test (Porsolt test) and influence on spontaneous locomotor activity of mice

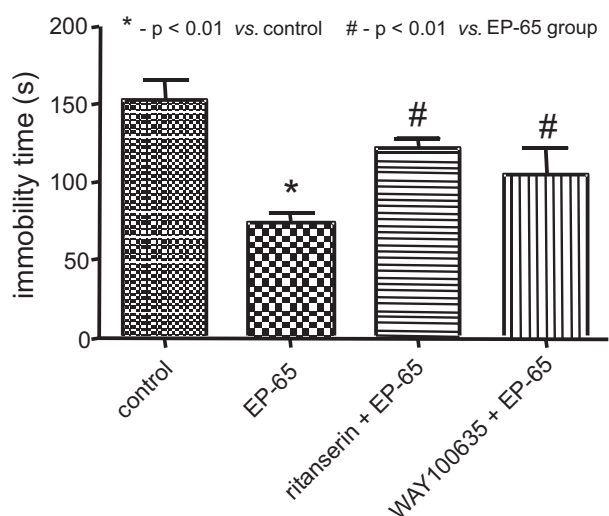
Compound	Dose (mg/kg)	Reduced immobility time in Porsolt test $\pm$ SEM (%)	Inhibition of locomotor activity $\pm$ SEM (%)
<b>EP-62</b>	2.5	14 $\pm$ 13.3	0
	5	21 $\pm$ 10.6	5 $\pm$ 8.1
	10	26 $\pm$ 10.4*	26 $\pm$ 5.5*
	20	0	47 $\pm$ 11.4***
<b>EP-63</b>	5	not active	6 $\pm$ 7.7
	10		28 $\pm$ 9.6
	20		32 $\pm$ 8.5*
	30		50 $\pm$ 10.5***
<b>EP-64</b>	5	not active	14 $\pm$ 8.1
	10		31 $\pm$ 13.5*
	20		44 $\pm$ 11.9**
<b>EP-65</b>	2.5	32 $\pm$ 19.8*	5 $\pm$ 3.1
	5	47 $\pm$ 22.8***	7 $\pm$ 5.9
	10	52 $\pm$ 8***	23 $\pm$ 4.5*
	20	31 $\pm$ 16.2*	29 $\pm$ 5.9**
	30	0	51 $\pm$ 15.7***
<b>EP-66</b>	5	13 $\pm$ 8.7	5 $\pm$ 5.6
	10	21 $\pm$ 11.2	21 $\pm$ 4.6*
	20	26 $\pm$ 13.6*	24 $\pm$ 6.3*
	30	3 $\pm$ 8.7	41 $\pm$ 7.8***
<b>EP-70</b>	5	21 $\pm$ 9	4 $\pm$ 2.8
	10	27 $\pm$ 11.5*	7 $\pm$ 3.1
	20	16 $\pm$ 12.5	25 $\pm$ 7.7*
	30	6 $\pm$ 11.9	32 $\pm$ 4.6**
<b>EP-73</b>	5	not active	5 $\pm$ 7.2
	10		14 $\pm$ 8.2
	20		30 $\pm$ 8.6*
	30		42 $\pm$ 8.6**
<b>EP-74</b>	5	not active	5 $\pm$ 5.6
	10		24 $\pm$ 4.7*
	20		37 $\pm$ 11.1**
	30		49 $\pm$ 11.4***
<b>EP-75</b>	5	14 $\pm$ 8.5	5 $\pm$ 9.9
	10	25 $\pm$ 8.3*	10 $\pm$ 8.7
	20	17 $\pm$ 17.6	34 $\pm$ 7.5*
<b>EP-76</b>	2.5	18 $\pm$ 19.2	–
	5	29 $\pm$ 10.6**	8 $\pm$ 7.4
	10	25 $\pm$ 10*	8 $\pm$ 4.3
	20	7 $\pm$ 12.7	36 $\pm$ 9**

Data are presented as the means  $\pm$  SEM of 6–8 mice per group (in percent of control). Results were analyzed by one-way ANOVA followed by Duncan test. \*  $p < 0.05$ ; \*\*  $p < 0.02$ ; \*\*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.001$  vs. respective control

**Tab. 3.** Effect of reference compounds MG-1, imipramine and mianserin on the total duration of immobility in the forced swimming test and influence on spontaneous locomotor activity of mice

Compound	Dose (mg/kg)	Reduced immobility time (%) in Porsolt test $\pm$ SEM (%)	% Inhibition of locomotor activity $\pm$ SEM (%)
MG-1	5	0	5 $\pm$ 5
	10	11 $\pm$ 8.3	21 $\pm$ 6.3
	20	8 $\pm$ 9.6	29 $\pm$ 3.9**
Imipramine	5	15 $\pm$ 9.2	–
	10	34 $\pm$ 10.2**	–
	20	49 $\pm$ 18.1****	–
Mianserin	5	13 $\pm$ 5.9	1 $\pm$ 8
	10	22 $\pm$ 10.7*	6 $\pm$ 5.8
	20	36 $\pm$ 9.5***	18 $\pm$ 10.9

Data are presented as the means  $\pm$  SEM of 6–8 mice per group. Results were analyzed by one-way ANOVA followed by Duncan test. \*  $p < 0.05$ ; \*\*  $p < 0.02$ ; \*\*\*  $p < 0.01$ ; \*\*\*\*  $p < 0.001$  vs. respective control



**Fig. 2.** Effect of EP-65 and pretreatment with ritanserin (4 mg/kg) or WAY 100635 (0.1 mg/kg) on immobility time in the forced swim test

### Spontaneous locomotor activity

All compounds tested significantly suppressed the spontaneous locomotor activity of mice.

The most potent effect was produced by compound **EP-43**, which significantly decreased spontaneous locomotor activity at a dose of 5 mg/kg (by 30.8%) and at doses of 10–20 mg/kg (by 57.4–66.9%) (Tab. 2a).

Compounds **EP-40**, **EP-41**, **EP-45**, **EP-46**, **EP-47**, **EP-49**, **EP-61**, **EP-62**, **EP-64**, **EP-65**, **EP-66** and **EP-**

**74** significantly suppressed the spontaneous locomotor activity at doses of 10 mg/kg, and compounds **EP-48**, **EP-63**, **EP-70**, **EP-73**, **EP-75**, **EP-76** and **MG-1** suppressed activity of mice at doses of 20 mg/kg (Tab 2a, 2b and 3). Compounds **EP-42**, **EP-44** and **EP-50** were significantly active in this test after administration *ip* at doses of 30 mg/kg (Tab. 2a).

### Discussion

The present study demonstrated the potent antidepressant-like activities of several phenylpiperazine pyrrolidin-2-one derivatives in the forced swimming test in mice. The forced swimming test is widely used as a reliable animal model of depression to screen new antidepressants [9, 29]. Most conventional antidepressant drugs act *via* distinct mechanisms, including serotonergic, noradrenergic and/or dopaminergic systems to increase serotonin, norepinephrine and dopamine synaptic availability, as evidenced by the forced swimming test [27, 30].

The accepted phenylpiperazine antidepressants nefazodone and trazodone have weak inhibitory actions on serotonin transports, and nefazodone may have a minor effect on norepinephrine transport [12]. Additionally, both drugs may inhibit presynaptic 5-HT<sub>1</sub> subtype autoreceptors to enhance neuronal release of serotonin and exert at least a partial agonist effect on postsynaptic 5-HT<sub>1</sub> receptors. Nefazodone also has a significant direct antagonistic effect on 5-HT<sub>2A</sub> receptors that may contribute to antidepressant and anxiolytic activity [12, 13]. Trazodone also blocks cerebral  $\alpha_1$ -adrenergic and H<sub>1</sub>-histamine receptors, possibly contributing to its tendency to induce priapism and sedation, respectively [12, 13].

Mirtazapine and mianserin are structural analogs of 5-HT with potent antagonistic effects at several postsynaptic 5-HT receptor types (including 5-HT<sub>2A</sub>, 5-HT<sub>2C</sub>, and 5-HT<sub>3</sub> receptors); they can lead to gradual down-regulation of 5-HT<sub>2A</sub> receptors. These drugs limit the effectiveness of inhibitory  $\alpha_2$ -adrenergic heteroreceptors on serotonergic neurons as well as inhibitory  $\alpha_2$  autoreceptors and 5-HT<sub>2A</sub> heteroreceptors on noradrenergic neurons [11, 12]. These effects may enhance the release of amines and contribute to the antidepressant effects of these drugs [12]. Trazodone and mirtazapine are not active in the forced swim test [2,



11]. In contrast, mianserin is active in the forced swim test [4, 23, 33, 34].

In our study, the most potent effect observed in the forced swimming test was produced by compound **EP-65**. The effect was stronger than the results obtained with classic antidepressants, such as imipramine and mianserin. This compound had affinities for  $\alpha$ -receptors ( $K_i$ :  $\alpha_1 = 114.6$  nM,  $\alpha_2 = 142.8$  nM) and 5-HT<sub>2</sub> receptors ( $K_i = 350$  nM) and a weak affinity for 5-HT<sub>1A</sub> receptors. Recent preclinical and clinical studies have reported a key role for 5-HT<sub>2</sub> receptors in the pathology of depression as well as the action of many antidepressants [3, 7, 8]. Ritanserin and WAY 100635 partially antagonized the antidepressant-like activity of **EP-65**. These results indicate the participation of the serotonergic system in the antidepressant-like mechanism of action of **EP-65**. In our earlier studies, this compound antagonized the response (increase blood pressure) elicited by epinephrine, norepinephrine and methoxamine [18, 19]. This is evidence that **EP-65** has  $\alpha$ -adrenolytic properties. It is possible that **EP-65** blocks  $\alpha_2$ -adrenergic heteroreceptors on serotonergic neurons and  $\alpha_2$  autoreceptors on noradrenergic neurons, thereby leading to its antidepressant-like activity. At this stage, however, further experiments are needed to elucidate the exact mechanism of action of compound **EP-65**.

Compounds **EP-41**, **EP-42**, **EP-44**, **EP-47**, **EP-48**, **EP-49**, **EP-50**, **EP-62**, **EP-66**, **EP-70**, **EP-75** and **EP-76** showed weak activities in the forced swimming test, but they exhibited  $\alpha$ -adrenolytic properties [17–19] and stronger affinities for 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors. All tested compounds significantly suppressed spontaneous locomotor activity in mice. The sedative effects of these compounds likely result from the  $\alpha$ -adrenolytic properties of 5-HT<sub>1A</sub> and 5-HT<sub>2</sub> receptors, but this needs further research.

In conclusion, preliminary experiments demonstrated that these novel arylpiperazine pyrrolidin-2-one derivatives showed antidepressant-like activities. Many studies showed that compounds with affinity for 5-HT<sub>1A</sub> or 5-HT<sub>2A</sub> receptors have been shown to decrease immobility in the forced swimming test [1, 14, 15, 21, 26, 31, 35]. Our study demonstrated the strong antidepressant-like activity of compound **EP-65**, for which the effect was stronger than the results obtained with the classic antidepressants imipramine and mianserin.

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