

# Neuropharmacological characteristics of antidepressant action of a new 3-substituted thietane-1,1-dioxide derivative

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## Abstract

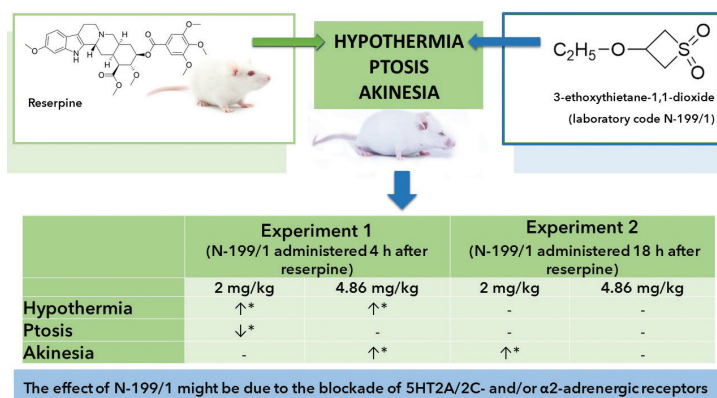
**Introduction:** Due to severe burden of depressive disorders and a low rate of remission in patients receiving antidepressant therapy, there is an urgent need for developing novel agents with antidepressant action and a fundamentally new mechanism of action. 3-ethoxythietane-1,1-dioxide (N-199/1) is a new molecule that showed significant antidepressant properties when administered intraperitoneally once or repeatedly. The aim of the present study was to investigate the mechanism of action of N-199/1, using reserpine test.

**Materials and methods:** N-199/1 (2 mg/kg and 4.86 mg/kg) and the reference drugs (imipramine and fluoxetine) were administered once intraperitoneally to outbred male mice 4 h (Experiment 1) and 18 h (Experiment 2) after a single intraperitoneal injection of reserpine (2.5 mg/kg). The severity of reserpine-induced symptoms (hypothermia, ptosis and akinesia) was assessed.

**Results and discussion:** N-199/1 potentiated reserpine-induced hypothermia at both doses and reduced ptosis at a dose of 2 mg/kg when administered 4 h after reserpine. N-199/1 increased the duration of reserpine akinesia at a dose of 2 mg/kg when administered 18 h after reserpine and at a dose of 4.86 mg/kg when administered 4 h after reserpine. The effect of N-199/1 resembled the effect of fluoxetine and was dose-dependent.

**Conclusion:** Based on the results obtained, it can be assumed that the antidepressant action of N-199/1 is due to its serotonin-positive properties, and probably the blockade of serotonin 5HT<sub>2A/2C</sub> receptors and/or  $\alpha$ <sub>2</sub>-adrenergic receptors. The effect of N-199/1 is dose-dependent and resembles the effect of fluoxetine.

### Graphical abstract:



## Keywords

animal outbred strain, antidepressants, [reserpine](#), [thietane](#).

## Introduction

According to the World Health Organization (2017), depressive disorders affect more than 300 million people worldwide and are among the leading causes of disability and suicide. Although there are effective treatments for depression, about 30% of patients receiving antidepressant therapy do not achieve full remission (Sanches et al. 2021); therefore, the development of new antidepressants with a fundamentally new mechanism of action is needed.

3-substituted thietane-1,1-dioxides are a new promising chemical class synthesized at the Department of Pharmaceutical Chemistry with courses of Analytical and Toxicological Chemistry of Bashkir State Medical University (BSMU, Head of the Department – Elena E. Klen, Professor, Doctor of Pharmaceutical Sciences). Amongst thietane dioxide derivatives, compounds with antidepressant action have been already found (Ivanova et al. 2011a, 2011b; Klen et al. 2017), such as 3-ethoxythietane-1,1-dioxide (laboratory code N-199/1). N-199/1 showed significant antidepressant properties after single (Khaliullin et al. 2020) and repeated intraperitoneal (i.p.) administration to outbred male mice in a wide range of doses (Mavlyutov and Gaisina 2020) in the forced swimming test and in the tail suspension test, and is of interest for studying its mechanism of action.

There is still neither single unifying theory that could explain the heterogeneity of depression symptoms, nor universal animal models that make it possible to replicate them (Czéh et al. 2016). In recent years, the involvement of the hypothalamic-pituitary-adrenal axis, glutamatergic system, neuroplasticity and inflammatory reactions in the pathogenesis of depression has been shown. Nevertheless, the first developed theory of depression – monoaminergic – remains the most widely accepted and valid in the development of novel antidepressants. All current antidepressants either way affect monoamine release; therefore the development of novel antidepressants should include studying their effect on the central monoaminergic system. A commonly used method for screening antidepressant agents is the reserpine test, which evaluates the effect of the test compounds on noradrenergic, dopaminergic, and serotonergic neurotransmission by their ability to reverse reserpine-induced symptoms (hypothermia, ptosis, akinesia) (Czéh et al. 2016; Raupp-Barcaro et al. 2018). In the present study, we investigated the mechanism of the antidepressant action of N-199/1 in the test of neuropharmacological interaction with [reserpine](#).

## Materials and methods

### Experimental animals

The present study is a part of the BSMU research project "Study of the biological activity of thietanes" and was approved by the Local Ethics Committee of BSMU (Minutes No. 9, 2013). All the animal procedures were carried out in accordance with the Rules of Good Laboratory Practice of the Eurasian Economic Union in the field of drugs (Decision No. 81 of the Council of the Eurasian Economic Commission dated November 3, 2016 "On Approval of Rules of Good Laboratory Practice of the Eurasian Economic Union in the Sphere of Medicine Circulation") and the International Recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No.123, 1986). The experiments were performed on white outbred male mice (18–25 g). The animals were maintained in a 12-h light regime (08:00–20:00) with free access to water and food.

### Drugs and treatment

N-199/1 was synthesized at the Department of Pharmaceutical Chemistry with courses of Analytical and Toxicological Chemistry of BSMU. [Reserpine](#) was purchased from Sigma Aldrich, USA. [Fluoxetine](#) (Apo-Fluoxetine, capsules 20 mg, Apotex INC, Canada) and [imipramine](#) (Melipramine, tablets 25 mg, Egis Pharmaceuticals PLC, Hungary) were used as the reference drugs. [Reserpine](#), N-199/1 and the reference drugs were suspended with 1–2 drops of [Tween-80](#) (Panreac Quimica S.A.U., Spain) and diluted in [saline](#) (0.2 ml per 20 g of body weight). The control group received an equivalent volume of [saline](#).

### Experimental design

The animals were divided into 6 experimental groups (6 in each group): Group 1 (control) received two injections of [saline](#), i.p., Group 2 – [reserpine](#) (2.5 mg/kg) and [saline](#), i.p., Groups 3 – [reserpine](#) (2.5 mg/kg) and [imipramine](#) (10 mg/kg), i.p., Group 4 – [reserpine](#) (2.5 mg/kg) and [fluoxetine](#) (10 mg/kg), i.p., Group 5 – [reserpine](#) (2.5 mg/kg) and N-199/1 (2 mg/kg), i.p., and Group 6 – [reserpine](#) (2.5 mg/kg) and N-199/1 (4.86 mg/kg), i.p.

### Experiment 1 (according to Wang et al. (2014))

[Reserpine](#) was injected i.p. to Groups 2–6 at a dose of 2.5 mg/kg while Group 1 received [saline](#). Four hours later,

mice were treated with saline (Groups 1–2), imipramine at a dose of 10 mg/kg (Group 3), fluoxetine at a dose of 10 mg/kg (Group 4) and N-199/1 at doses of 2 mg/kg (Group 5) and 4.86 mg/kg (Group 6). Reserpine-induced symptoms were assessed 4, 4.5, 5, 5.5, 6, 12, 22, and 24 h after reserpine administration (Fig. 1).

**Experiment 2 (according to Bourin (1990))**

Saline (Groups 1–2), imipramine at a dose of 10 mg/kg (Group 3), fluoxetine at a dose of 10 mg/kg (Group 4), and N-199/1 at doses of 2 mg/kg (Group 5) and 4.86 mg/kg (Group 6) were administered i.p. to mice 18 h after the reserpine injection (2.5 mg/kg). Reserpine-induced hypothermia, ptosis and akinesia were measured 18, 18.5, 19, 19.5, and 20 h after the administration of reserpine (Fig. 2).

**Assessment of reserpine-induced symptoms (hypothermia, ptosis and akinesia)**

Rectal temperature was measured using a TW2-193 electrothermometer (Braintree Scientific, USA) and then deltas ( $\Delta t$ ) were calculated using the formula  $\Delta t = t(t) - t(0)$ ,

where  $t(t)$  is the body temperature at a given point in time, and  $t(0)$  is the rectal temperature at the time point of 0 h.

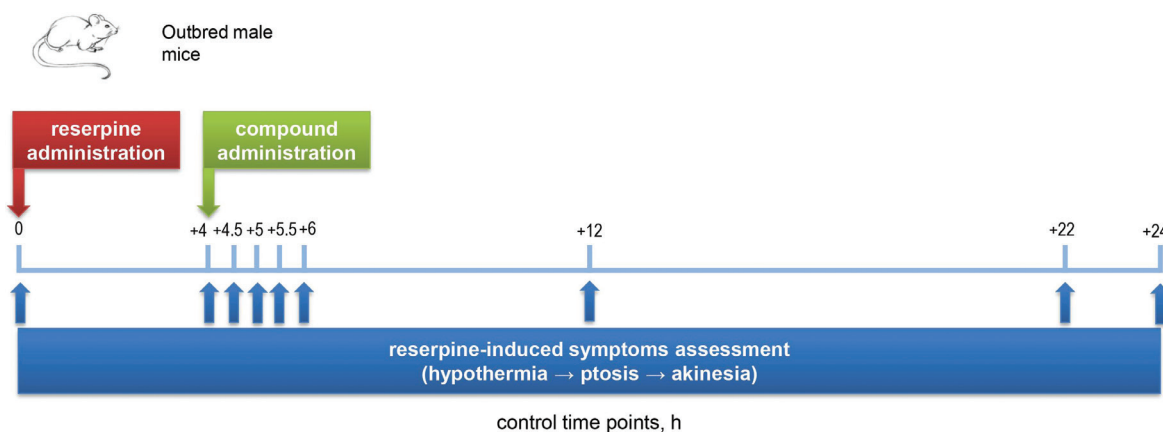
Ptosis was assessed in points (0–4) according to the scale of B. Rubin:

- 4 – eyes open
- 3 – eyes 3/4 open
- 2 – eyes 1/2 open
- 1 – eyes 1/4 open
- 0 – eyes closed

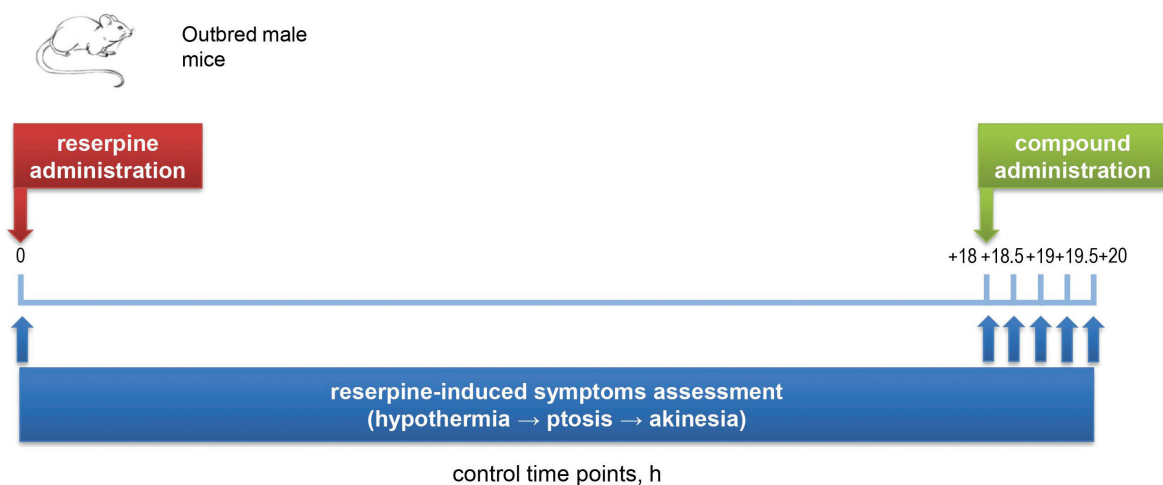
The duration of akinesia was measured in s for 1 min. The animals were placed in the center of a black circle (7.5 cm in diameter), and the total time during which the mice remained within the circle was measured (Wang et al. 2014).

**Statistical analysis**

The statistical analysis was performed using the STATISTICA 10.0 software (StatSoft, USA). The normality of distribution was assessed, and descriptive statistics, such as median [Me], interquartile range [IQR], and standard



**Figure 1.** Design of Experiment 1.



**Figure 2.** Design of Experiment 2.

deviation were calculated. Since the data distribution was abnormal, nonparametric tests were used to compare the samples, such as Kruskal-Wallis, Mann-Whitney (p1), Friedman, Wilcoxon (p2). The results were considered statically significant at a p-level < 0.05.

## Results

### Experiment 1

**Reserpine** significantly reduced the rectal temperature of animals by 1.3–5.0 °C (p1 = 0.004) compared with the control group and by 0.9–6.2 °C (p2 = 0.028) compared with the base level  $\Delta t_{0-4}$ . Hypothermia was observed throughout the experiment and reached its maximum value 22 h after **reserpine** administration (Fig. 3).

N-199/1 at doses of 2 mg/kg and 4.86 mg/kg potentiated the reserpine-induced hypothermia throughout the experiment by 1.7–3.05 °C and 1.2–4.35 °C, respectively, compared with the reserpine group (p1 < 0.05). The rectal temperature decreased in dynamics as well by

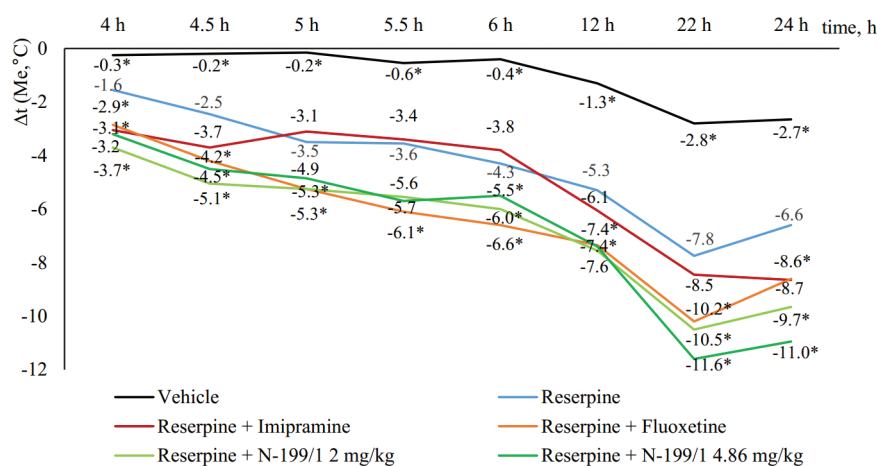
1.35–6.8 °C in Group 5 and by 1.3–8.4 °C in Group 6 (p2 < 0.05) compared with the base level (Fig. 3).

**Imipramine** did not change the value of reserpine-induced hypothermia compared with the reserpine group. In dynamics, the rectal temperature of the animals decreased, starting from the timepoint of 12 h, by 3.0–5.6 °C (p2 = 0.028) in relation to the base level (Fig. 3).

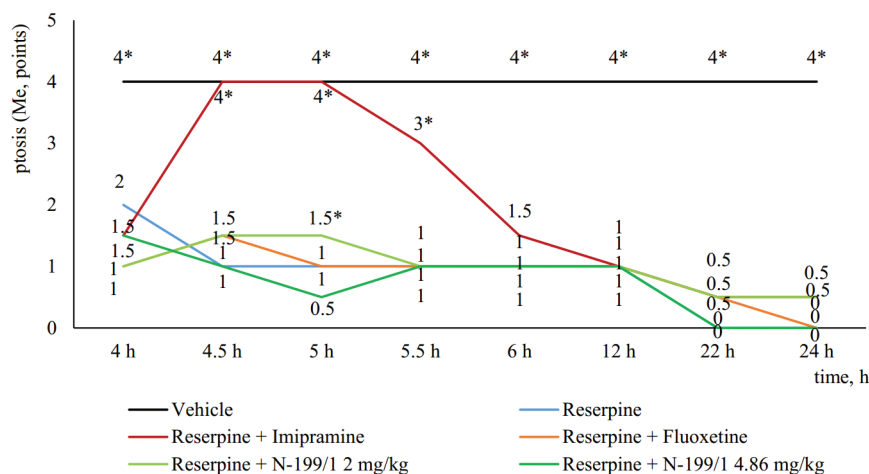
**Fluoxetine** potentiated the effect of **reserpine** throughout the experiment, reducing the body temperature of the animals by 1.75–2.55 °C compared with the reserpine group (p1 < 0.05). Compared with the base level, the rectal temperature decreased by 1.35–7.35 °C (p2 = 0.028, Fig. 3).

Reserpine-induced ptosis was 0–2 points during the experiment, reaching the maximum value (0 points) 22 h after **reserpine** administration (Fig. 4).

N-199/1 reduced a degree of **reserpine** ptosis by 0.5 points (5 h, p1 = 0.045) at a dose of 2 mg/kg and did not change it at a dose of 4.86 mg/kg compared with the reserpine group. The effects of two doses were statistically different 5 h after **reserpine** injection (p1 = 0.020). In dynamics, the value of ptosis did not change in Group 5, whereas increased by 1–1.5 points in Group 6 (p2 < 0.05, Fig. 4).



**Figure 3.** Effect of N-199/1 on reserpine-induced hypothermia when administered 4 h after **reserpine**. Note: \* – p < 0.05 for the Mann-Whitney test compared with the reserpine group (Group 2).



**Figure 4.** Effect of N-199/1 on reserpine-induced ptosis when administered 4 h after **reserpine**. Note: \* – p < 0.05 for the Mann-Whitney test compared with the reserpine group (Group 2).

Imipramine reduced a degree of ptosis by 2–3 points compared with Group 2 ( $p1 < 0.05$ ) and by 1.5–2.5 points compared with the initial timepoint of 4 h ( $p2 = 0.028$ ) 4.5–5.5 h after reserpine administration (Fig. 4).

Fluoxetine did not affect the value of ptosis throughout the experiment compared with Group 2. In dynamics, the ptosis score increased by 1 point 24 h after reserpine administration in comparison with the timepoint of 4 h ( $p2 = 0.028$ , Fig. 4).

Four hours after reserpine administration, the animals developed akinesia, the duration of which sequentially increased from 27 s to the maximum of 60 s (22 h,  $p2 = 0.028$ , Fig. 5).

N-199/1 did not affect reserpine-induced akinesia throughout the experiment at a dose of 2 mg/kg.

At a dose of 4.86 mg/kg, N-199/1 significantly increased the duration of akinesia by 23.5 s half an hour after its administration (4.5 h;  $p2 = 0.028$ ). At the timepoint of 5 h, the duration of akinesia reached its maximum value and persisted until the end of the experiment (Fig. 5). Compared with

Group 2, the duration of akinesia in Group 6 was significantly increased by 12.5 s at the timepoint of 4.5 h ( $p1 = 0.020$ ).

Imipramine did not affect the duration of akinesia compared with Group 2, but reduced it in dynamics by 12.5 s half an hour after its administration (4.5 h,  $p2 = 0.043$ ) compared with the base level.

The effect of fluoxetine was comparable to the effect of reserpine at all timepoints ( $p1 > 0.05$ , Fig. 5).

### Experiment 2

Reserpine caused the development of hypothermia of  $-9.5\text{ }^{\circ}\text{C}$  ( $p1 = 0.002$  compared with Group 1), which gradually decreased to  $-4.6\text{ }^{\circ}\text{C}$  by 20 h after reserpine administration ( $p1 = 0.004$ ,  $p2 = 0.028$ , Fig. 6).

Hypothermia decreased by  $1.8\text{--}5.1\text{ }^{\circ}\text{C}$  18.5–20 h after reserpine injection compared with the base level ( $\Delta t0\text{-}18$ ) in Group 6 ( $p2 = 0.028$ ) and did not change in Group 5. There was no significant difference between the groups which had received N-199/1 (2 mg/kg or 4.86 mg/kg) and

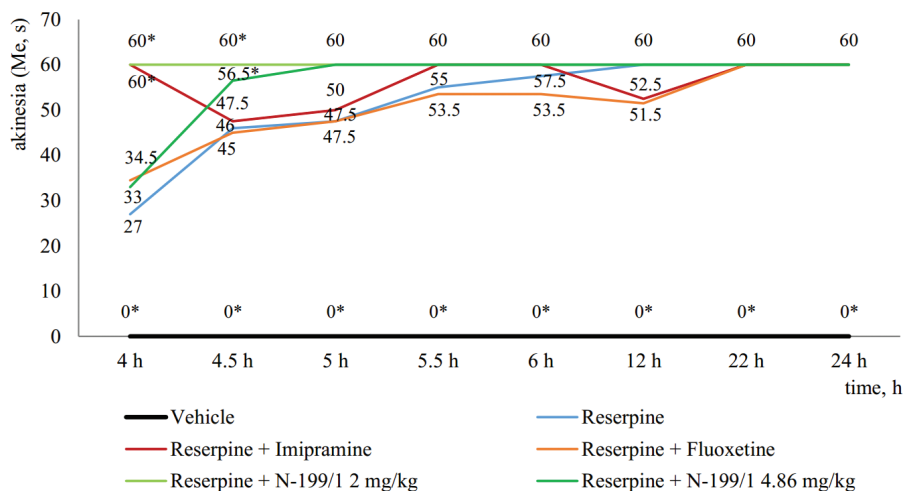


Figure 5. Effect of N-199/1 on reserpine-induced akinesia when administered 4 h after reserpine. Note: \* –  $p < 0.05$  for the Mann-Whitney test compared with the reserpine group (Group 2).

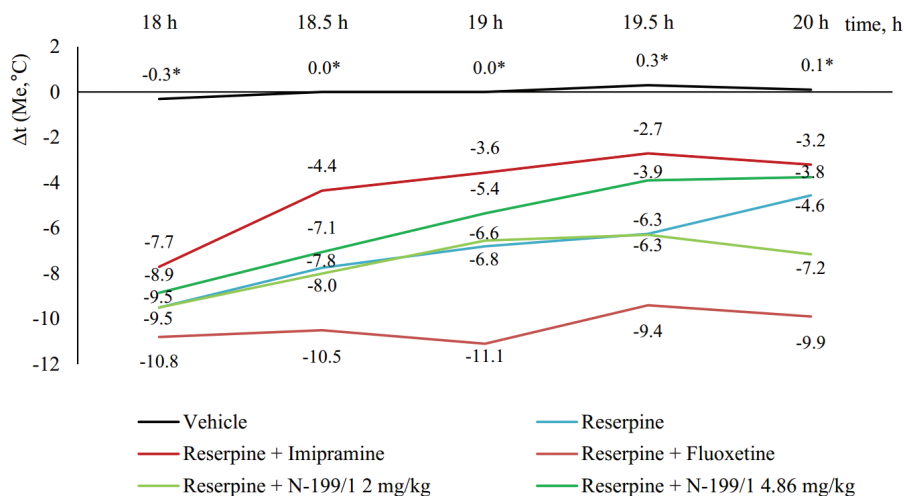


Figure 6. Effect of N-199/1 on reserpine-induced hypothermia when administered 18 h after reserpine. Note: \* –  $p < 0.05$  for the Mann-Whitney test compared with the reserpine group (Group 2).

reserpine group at all time points ( $p1 > 0.05$ ), while in Group 5 (N-199/1, 2 mg/kg), hypothermia was more pronounced than in Group 3 (imipramine,  $p1 < 0.05$  19–20 h after reserpine injection, Fig. 6).

Imipramine reduced reserpine hypothermia in dynamics by 3.4–5.0 °C compared with the base level  $\Delta t_{0-18}$  ( $p2 = 0.028$ ), and, statistically insignificantly (by 1.4–3.6 °C) compared with Group 2 ( $p1 > 0.05$ ) at all time points after reserpine administration (Fig. 6).

In Group 4, the body temperature of the animals remained at the same level ( $p2 > 0.05$ ) throughout the experiment and was lower than in the reserpine group by 2.8–5.4 °C, but statistically insignificantly (Fig. 6). At the same time, the effect of fluoxetine significantly differed from the effect of imipramine 18.5 h ( $p1 = 0.045$ ) and 20 h ( $p1 = 0.045$ ) after reserpine administration.

Reserpine induced ptosis, which was 0–1 points at all time points (18–20 h after its administration) ( $p1 < 0.05$ , Fig. 7).

Neither N-199/1, nor imipramine affected ptosis.

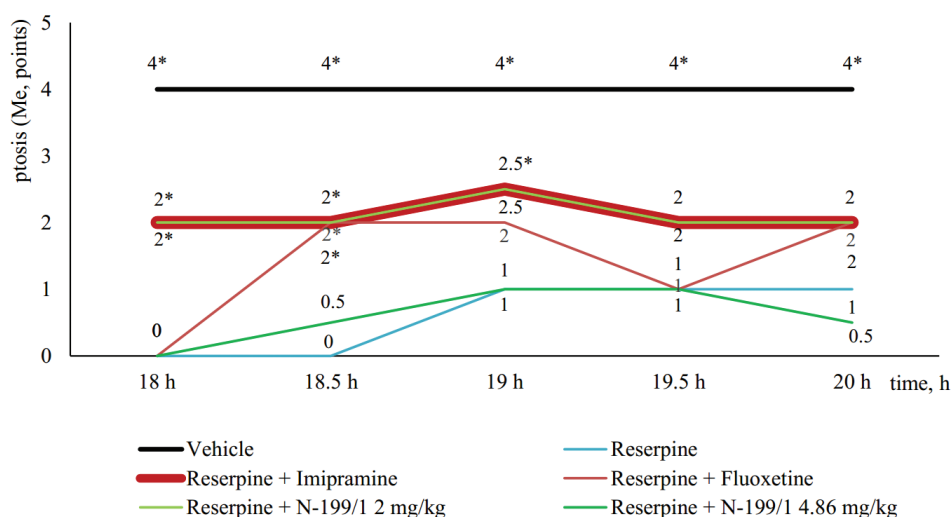
Fluoxetine reduced reserpine ptosis by 2 points compared with Group 2 at the timepoint of 18.5 h ( $p1 = 0.036$ ) and by 1–2 points compared with the timepoint of 18 h ( $p2 = 0.043$ , Fig. 7).

18 h after the administration of reserpine, the animals developed akinesia lasting 60 s ( $p1 = 0.001$ ), the duration of which gradually decreased and by the end of the experiment (20 h) was 41.5 s ( $p2 = 0.046$ , Fig. 8).

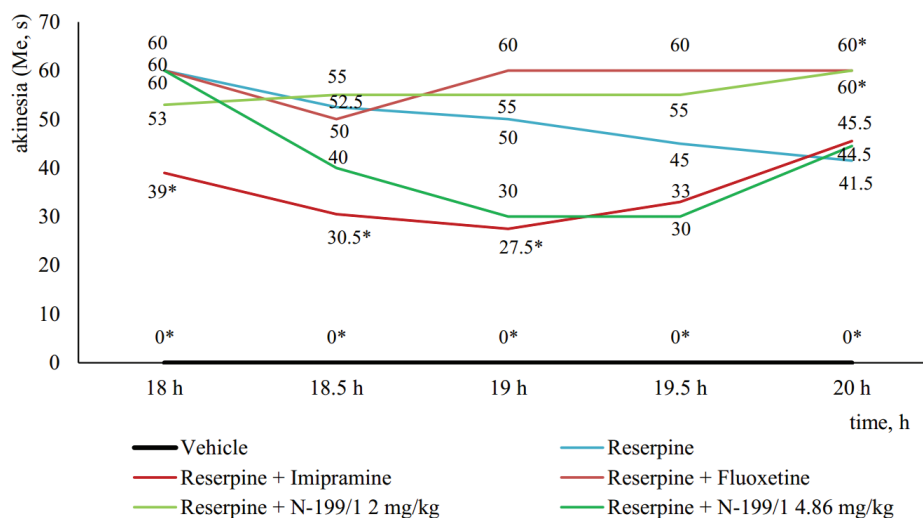
N-199/1 (2 mg/kg) counteracted a decrease in akinesia compared with Group 2 (20 h,  $p1 = 0.010$ ), maintaining it constant throughout the experiment ( $p2 > 0.05$ ). At a dose of 4.86 mg/kg, N-199/1 did not affect the duration of reserpine-induced akinesia (Fig. 8).

In Group 3, reserpine akinesia was less pronounced than in Group 2 by 12–22 s (18–19 h,  $p1 < 0.05$ ) and did not change when compared to the 18 h timepoint ( $p2 > 0.05$ , Fig. 8).

Fluoxetine increased the duration of reserpine-induced akinesia 20 h after reserpine administration compared



**Figure 7.** Effect of N-199/1 on reserpine-induced ptosis when administered 18 h after reserpine. **Note:** \* –  $p < 0.05$  for the Mann-Whitney test compared with the reserpine group (Group 2).



**Figure 8.** Effect of N-199/1 on reserpine-induced akinesia when administered 18 h after reserpine. **Note:** \* –  $p < 0.05$  for the Mann-Whitney test compared with the reserpine group (Group 2).

with Group 2 ( $p_1 = 0.006$ ) and did not change it in dynamics (Fig. 8).

## Discussion

All known antidepressants cause their therapeutic effect through the involvement of the central monoaminergic system. We studied the effect of N-199/1 on central noradrenergic, serotonergic and dopaminergic neurotransmissions in the test of neuropharmacological interaction with **reserpine**. Considering that the activity of antidepressants in the reserpine test may depend not only on their mechanism of action, but also on the dosing schedule in relation to **reserpine**, we conducted 2 experiments: in the first experiment the compound and the reference drugs were administered 4 h after **reserpine** (according to Wang et al. (2014)), in the second –18 h afterwards (according to Bourin (1990)).

The non-selective monoamine reuptake inhibitor **imipramine** (a tricyclic antidepressant) and the selective serotonin reuptake inhibitor **fluoxetine** were used as reference drugs (Mashkovsky et al. 1983).

4 h after administration, **reserpine** caused the development of hypothermia, which gradually increased throughout the experiment and was maximum 22 h after administration ( $30.1^\circ\text{C}$ ).

It was shown that the drugs increasing the monoamine concentration in the synaptic cleft and/or stimulate  $\beta$ -adrenergic receptors, including tricyclic antidepressants, have the ability to reverse reserpine-induced hypothermia (Mashkovsky et al. 1983; Bourin 1990). **Mianserin**, which acts on  $\alpha_2$ -adrenergic receptors, does not antagonize reserpine effects, or enhances them due to the strong central antagonistic action against **serotonin** (Mashkovsky et al. 1983). The ability to potentiate reserpine hypothermia was also shown for **clomipramine**, **prazosin**, **propranolol**, and **yohimbine** (Bourin 1990).

N-199/1 at both doses exacerbated reserpine hypothermia when administered 4 h after **reserpine**, causing an effect similar to that of **fluoxetine**. It is known that **norepinephrine** plays an important role in the effect of **fluoxetine** (Cryan et al. 2004), which is realized not only by inhibiting **serotonin** reuptake, but also by blocking 5HT<sub>2A/2C</sub> receptors, leading to an increase in the extracellular levels of other catecholamines (**dopamine**, **norepinephrine**) (Koch et al. 2002). In our experiment, **fluoxetine** enhanced the effect of **reserpine** when administered 4 h later, probably increasing **norepinephrine** and **dopamine** release and thus worsening the depletion of central catecholamine stores, which may be associated with the blockade of 5HT<sub>2A/2C</sub> receptors, rather than inhibition of the **serotonin** transporter protein.

Thus, N-199/1, as well as **fluoxetine**, can block neuronal reuptake of **serotonin** and/or 5HT<sub>2A/2C</sub> receptors, or, like other multimodal antidepressants, inhibit other central receptors ( $\alpha_2$ -adrenergic receptors) involved into monoaminergic neurotransmission regulation.

**Imipramine** counteracted reserpine hypothermia during the first 2 h after its administration (at the timepoints of 4.5–6 h), maintaining the body temperature of the animals at  $35.1\text{--}34.5^\circ\text{C}$  ( $p_2 > 0.05$ ), which is consistent with the results obtained by Bourin (1990).

When administered 18 h after **reserpine**, neither N-199/1 nor the reference drugs significantly changed the severity of reserpine hypothermia. However, it was found that the effects of **imipramine** and **fluoxetine** were oppositely directed, which indicates that antidepressants with different mechanisms of action have different effect on reserpine hypothermia when administered both 4 and 18 hours after **reserpine**. N-199/1 acted in a dose-dependent manner, counteracting the reversal of reserpine hypothermia over time at a dose of 2 mg/kg like **fluoxetine**, and insignificantly decreasing reserpine hypothermia at a dose of 4.86 mg/kg similar to **imipramine**, which suggests that the mechanism of action of N-199/1 depends on a dose.

Reserpine-induced ptosis can be antagonised by agents with serotonergic/ $\alpha$ -adrenomimetic activity, including tricyclic antidepressants (Bourin 1990). The most pronounced antagonism of **reserpine** effects is produced by monoamine oxidase (MAO) inhibitors, whereas multimodal antidepressants that act on receptors (such as **mianserin**) either do not reverse reserpine symptoms or reduce them insignificantly, or enhance the effect of **reserpine** (Mashkovsky et al. 1983; Bourin 1990).

N-199/1 slightly reduced reserpine ptosis at a dose of 2 mg/kg, had no effect at a dose of 4.86 mg/kg when administered 4 h after **reserpine**, and at neither doses when administered 18 h after **reserpine**. The effect of N-199/1 was less pronounced compared with the both reference drugs ( $p_1 < 0.05$ ). This might suggest that N-199/1 increases monoamines levels not by inhibiting reuptake or MAO, but by blocking  $\alpha$ -adrenergic receptors and/or other receptors involved in the regulation of monoaminergic neurotransmission.

Moreover, in both experiments, 1 h after the administration of N-199/1 (at the point +5 h/+19 h), there was a statistically significant difference between the groups having received N-199/1 at a dose of 2 mg/kg and 4.86 mg/kg, which indicates that N-199/1 acts in a dose-dependent manner.

The development of akinesia under the influence of **reserpine** is associated with reduced activity of the dopaminergic neurons (de Freitas et al. 2016); accordingly, drugs with dopaminergic activity can decrease the duration of reserpine-induced akinesia (Bourin 1990).

N-199/1 worsened reserpine-induced akinesia at a dose of 2 mg/kg when administered 18 h after **reserpine** and at a dose of 4.86 mg/kg when administered 4 h after **reserpine**. There was a significant difference between the two doses 19–20 h after **reserpine** injection (Experiment 2), which indicates that the effect of the molecule is dose-dependent.

The action of N-199/1 on reserpine akinesia was similar to the action of **fluoxetine**: **fluoxetine** did not change the duration of akinesia when administered 4 h after **reserpine** and increased it when administered 18 h after **reserpine** (at

the timepoints of 19–20 h, statistically significant at 20 h). An increase in the duration of akinesia indicates reserpine-induced depletion of neuronal storage in dopaminergic terminals due to the combined impairment of vesicular dopamine uptake (de Freitas et al. 2016) and an increase in dopamine release caused by the blockade of 5HT<sub>2A/2C</sub> receptors (Stahl 2013) under the influence of fluoxetine.

The duration of akinesia in the imipramine group was twice longer than in the reserpine group at the timepoint of 4 h (before imipramine administration) and did not differ from it at the timepoints of 4.5–24 h. At the same time, 0.5 h after administration (at the timepoint of 4.5 h) imipramine insignificantly reduced akinesia. Thus, imipramine counteracted reserpine akinesia 4.5 h after reserpine administration. 18 h after administration of reserpine (Experiment 2), there was also an initial significant difference between the reserpine and imipramine groups. Nonetheless, imipramine did not affect the duration of reserpine-induced akinesia.

## Conclusion

The results obtained in the test of neuropharmacological interaction with reserpine showed that antidepressants with different mechanisms of action (imipramine and fluoxetine) have different effects on reserpine-induced hypothermia, ptosis and akinesia when administered both 4 and 18 h after reserpine.

N-199/1, like fluoxetine, potentiated the severity of reserpine-induced hypothermia (at both doses when administered 4 h after reserpine) and akinesia (at a dose of 2 mg/kg when administered 18 h after reserpine and at a dose of 4.86 mg/kg when administered 4 h after reserpine). N-199/1 also reduced ptosis at a dose of 2 mg/kg when administered 4 h after reserpine, like imipramine. The results

obtained suggest that the mechanism of antidepressant action of N-199/1 may be associated with direct blockade of receptors (probably 5HT<sub>2A/2C</sub> or  $\alpha$ <sub>2</sub>-adrenergic receptors) rather than monoamine reuptake inhibition.

There was a statistically significant difference between the effects of N-199/1 at a dose of 2 mg/kg and 4.86 mg/kg in tests of reserpine-induced hypothermia, ptosis and akinesia, which indicates that N-199/1 acts in a dose-dependent manner. Thus, the resemblance to fluoxetine was observed in the group of animals that had received N-199/1 at a dose of 2 mg/kg. This suggests that different doses of N-199/1 cause the effect through different mechanisms.

The results obtained in the study of N-199/1 mechanism of action when administered once intraperitoneally at doses of 2 mg/kg and equimolar 10 mg/kg of amitriptyline (4.86 mg/kg) allow us to conclude that the antidepressant-like action of N-199/1 is due to its serotonin-positive properties, and probably the blockade of serotonin 5HT<sub>2A/2C</sub> receptors and/or  $\alpha$ <sub>2</sub>-adrenergic receptors. The effect of N-199/1 is dose-dependent and resembles the effect of fluoxetine (at a dose of 2 mg/kg).

## Conflict of interests

The authors declare no conflict of interests.

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