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The effect of 3,7-diazabicyclo[3.3.1]nonanes containing monoterpenoid moieties on the physical activity of mice

Anastasiya A. KOTLYAROVA [3 *], Konstantin Yu. PONOMAREV [1], Ekaterina A. MOROZOVA [1], Dina V. KORCHAGINA [2], Evgeniy V. SUSLOV [1], Alla V. PAVLOVA [1], Tatyana G. TOLSTIKOVA [1], Konstantin P. VOLCHO [1], Nariman F. SALAKHUTDINOV [1]

1 Department of Medicinal Chemistry, Novosibirsk Institute of Organic Chemistry of Siberian Branch of Russian Academy of Sciences, Lavrentjev av. 9, 630090, Novosibirsk, Russia
2 Department of Physical Organic Chemistry, Novosibirsk Institute of Organic Chemistry of Siberian Branch of Russian Academy of Sciences, Lavrentjev av. 9, 630090, Novosibirsk, Russia
3 Department of Experimental Pharmacology, Research Institute of Clinical and Experimental Lymphology - a branch of the Institute of Cytology and Genetics of Siberian Branch of Russian Academy of Sciences, Acad. Timakova str., 2, 630060, Novosibirsk, Russia
* Corresponding Author. E-mail: kotlyarova.anastasiya@yandex.ru (A.K.); Tel. +7-913-489 20 02.

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ABSTRACT: The aim of this work is to study the effect of agents combining fragments of monoterpenoids and 3,7-diazabicyclo[3.3.1]nonane (bispidine) on the performance of mice. Physical endurance was studied using two tests: exhaustive swimming with a load of 7% of body weight (after single dosing) and exhaustive treadmill running (after single dosing and a 7-day administration). It has been shown for the first time that derivatives of monoterpenoids with 3,7-diazabicyclo[3.3.1]nonane moiety containing methyl substituents at positions 1 and 5 have a stimulatory effect on the performance of mice, which exceeds the effect of the reference drug bromantane after single dosing. Compound K1-458, which contains (–)-myrtenal residues linked to a bispidine fragment via amino groups, is the most effective in increasing the duration of running and swimming at a dose of 100 mg/kg after single intragastric administration. It has been also established that the LD_{50} for these compounds exceeds 1,000 mg/kg.

KEYWORDS: Actoprotectors; treadmill; bromantane; monoterpenoids; bispidine.

1. INTRODUCTION

Actoprotectors are drugs that enhance body resistance to physical loads without an increase in oxygen consumption or heatp roduction [1]. In other words, actoprotectors present nature and synthetic adaptogens with a significant capacity to improve physical performance [2]. Nowadays, the use of actoprotectors for improvement of physical performance is relevant not only in relation to extreme conditions, sports and military medicine, but also when considering pharmacotherapy of an average healthy individual. There are five fundamentally different ways of improving physical activity [3]: improved performance as a result of additional body stimulation (a group of psychomotor stimulants, doping drugs); increase in performance due to elimination of the drawbacks of the body functional systems, enhancement of its nonspecific resistance (correction of the tolerability of extreme loads on the body); improved performance as a result of elimination of the causes of its decrement (correction of fatigue processes and the mechanisms of performance impairment); improved physical activity due to acceleration of post-exercise recovery processes; enhanced performance as a result of body adaptation to increasing physical loads (pharmacology of adaptive processes). The use of a sole pharmacological agent for implementation of each of the ways seems to be impossible. In this regard, the arsenal of drugs is quite large. Based on their mechanism of action, actoprotectors can be divided into psychostimulants, energy-producing compounds, and metabolic regulators [1].

The use of adamantane derivatives presents a promising direction for the synthesis and study of actoprotective activity. A whole range of pharmacological agents with psychostimulant, antiviral, immunotropic and antitumor activity, as well as adaptogenic properties and antiparkinsonian activity have

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been developed based on adamantane [4–8]. The mechanism of action of adamantane derivatives that belong to the class of actoprotectors (bromantane, chlodantan) is related to the increased synthesis of dopamine and inhibition of the process of lipid peroxidation [9–11]. Bromantane (Figure 1) enhances GABAergic transmission and supresses expression of the genes that control the synthesis of GABA transporters [2]. The neuroprotective effect of bromantane is also known to be a result of increased synthesis of effector kinases of the mitogen-activated cascade (ERK1/ERK2), as well as increased expression of the genes of BDNF and NGF neurotrophic factors in different parts of the brain (striatum, hypothalamus, and hippocampus in rats) [11]. Bromantane is a doping agent with a psychostimulant effect; it is included in the WADA's list of prohibited substances [12].

One of the approaches of medical chemistry to the synthesis of effective drugs with low toxicity is the wide use of natural compounds [13]. Monoterpenoids present secondary metabolites of plants and possess native biological activity. Among them, compounds with anti-inflammatory, antiviral, antibacterial and other types of activities have been found [14]. Some monoterpenoids have a pronounced effect on the nervous system. For example, (-)-menthone [15], (-)-myrtenol [16], (-)-isopulegol showed an anticonvulsant activity [17], geraniol demonstrated the capacity to reduce the negative symptoms of Parkinson syndrome in animal models of Parkinson's disease [18, 19]. However, despite high availability of monoterpenoids, their rapid metabolism and low selectivity often limit the use of these compounds as medications without prior chemical modification.

Agonists and antagonists of various receptors of the central nervous system, blockers of Na^+, K^+ and Ca^{2+} channels that are active at nanomolar concentrations were found among different bispidine derivatives (3,7-diazabicyclo[3.3.1]nonane) [20]. For example, H 345/52 was the first compound to show high capacity to block potassium channels while demonstrating low selectivity for calcium channels involved in heartbeat regulation [21]. Compound 3-(6-chloropyridin-3-yl)-bispidine showed analgesic activity at a dose of 0.62 μmol/kg in the hot plate test and turned out to be an agonist of nicotinic acetylcholine receptors with high affinity [22] (Figure 1). Some bispidine derivatives have been shown to act as highly effective antagonists of orexin receptors: for example, compound **1** (Figure 1) [23]. Orexin receptor antagonists can be used for the treatment of drug abuse, mood or eating disorders, and various cognitive dysfunctions [24, 25]. Thus, many bispidine derivatives have an effect on the CNS at low doses, which makes this fragment a promising scaffold to use in further research regarding the study of actoprotective properties, including improvement of cognitive functions.

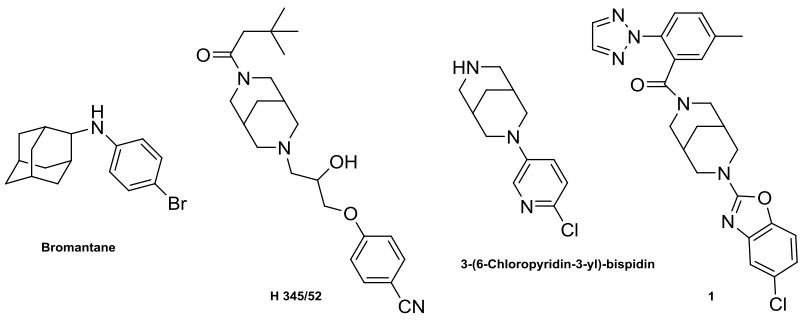

Figure 1. Some biologically active derivatives of bispidine and adamantane.

Joining the fragments of monoterpenoids and bispidines in a single molecule seems to be a promising approach, which might allow enhancement of the useful biological properties inherent to them or result in the emergence of new ones, in particular, actoprotective properties. It should be noted that, as far as we know, there are no data regarding modification of bispidines with monoterpenoids and the study of their biological properties to date. In this work, we studied for the first time the actoprotective activity of monoterpenoid derivatives in two modes of administration: acute and subchronic (after a 7-day administration).

2. RESULTS AND DISCUSSION

2.1. Synthesis of bispidine derivatives

For the synthesis of target substances, nesessary starting compounds were synthesized (Figure 2). The synthesis of 1,3-diazaadamantane (**2**) and 1,5-dimethyl-3,7-diazabicyclo [3.3.1]nonan-9-one (**3**) was carried

out according to the procedure [26]. 2-(Bromomethyl)-6,6-dimethylbicyclo [3.1.1]hept-2-ene (**4**) (Figure 2) was obtained according to the procedure [27]. The acid chloride **5** was synthesized from myrtenal according to [28] through acid **6**.

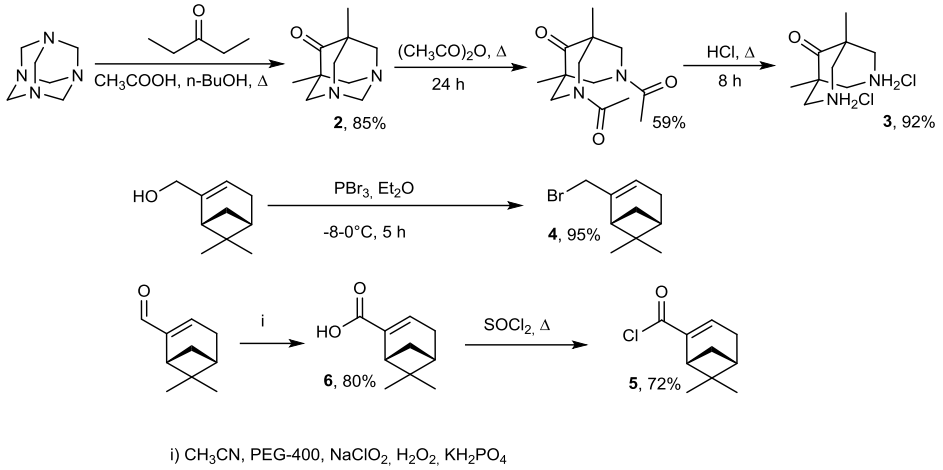

Figure 2. Synthesis of diazaadamantane **2**, bispidinone **3** and bromo derivative **4**.

Amide K1-456 was obtained by the interaction of acid chloride **5** with diazaadamantane **2** in a mixture of water-benzene in the presence of $NaHCO_3$. The yield of product K1-456 was 86%.

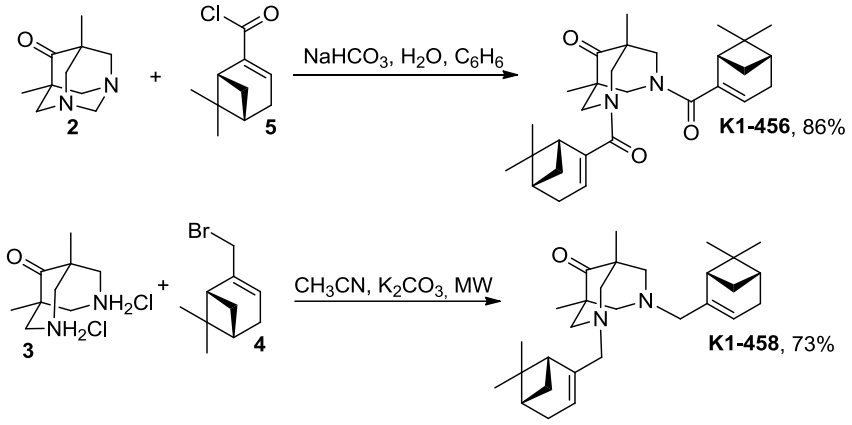

Figure 3. Synthesis of compounds K1-456 and K1-458.

The reaction of compound **3** with bromide **4** in acetonitrile in the presence of K_2CO_3 at microwave heating at 70°C led to K1-458 with a yield of 73% (Figure 3). The structure of K1-456 and K1-458 was established using ^1H and ^{13}C NMR spectra and HRMS and elemental analysis data.

2.2. Evaluation of acute toxicity

Administration of the compounds (K1-456, K1-458) under study at a dose of 500, 700, and 1,000 mg/kg did not result in animal death and had no effect on the physical and psycho-emotional state of animals, body weight and the amount of food and water consumed. Thus, the LD_{50} value for these compounds exceeds 1,000 mg/kg.

2.3. Determination of physical performance

Porsolt forced swimming test was originally used for assessment of depressive behavior [34]. However, this test is also widely used to evaluate the effectiveness of performance-enhancing drugs in unavoidable stress situations. The forced swimming test presents a severe type of stress, which has physical and emotional components [31].

The forced swimming test demonstrated that all of the studied compounds improve the performance in mice. Compound K1-458 at a dose 100 mg/kg body weight significantly increases the duration of

swimming. Increase in swimming duraion upon administration of K1-458 and reference drug bromantane was observed only at the trend level (by 20%). K1-456 at a dose of 100 and 50 mg/kg resulted in a 7% and 6% increase in swimming duration in mice, respectively. However, the changes were observed only at the trend level (Figure 4).

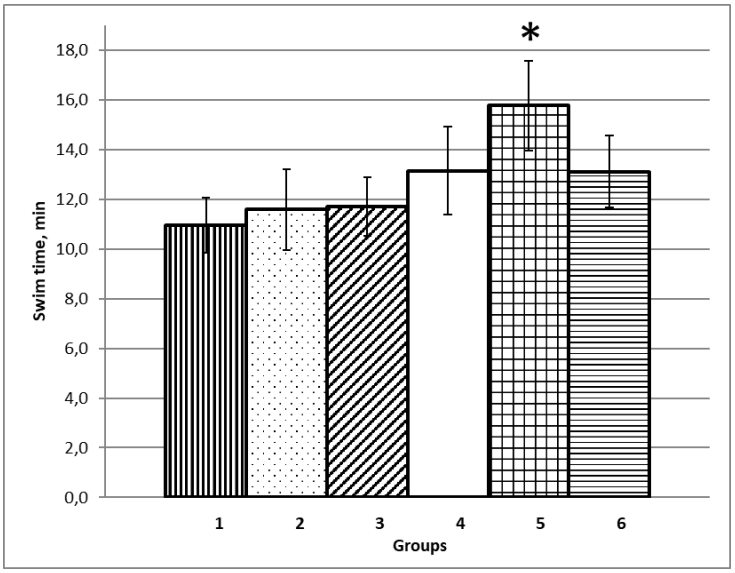

Figure 4. Effects of derivatives of monoterpenoids on the swimming time of forced swimming test in mice. Average data are presented as mean ± standard deviation, statistical analysis was conducted by Mann-Whitney U-test; a shows significant the difference against the control group (1) ($p<0.05$).

Designation of groups: 1- Control, 2 - K1-456, 50 mg/kg, 3 - K1-456, 100 mg/kg, 4 - K1-458, 50 mg/kg, 5 - K1-458, 100 mg/kg, 6 - Bromantane, 50 mg/kg.

Next, the performance of mice was studied using exhaustive treadmill running test 1, 6 and 24 hours after a single administration of the compounds [35]. Then, the compounds were administered for 7 days, and the animals were tested on a treadmill 1 hour after administration (Figure 5).

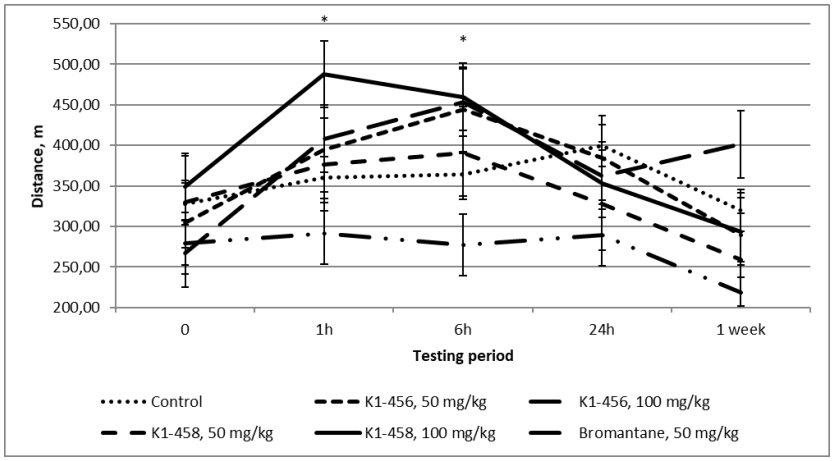

Figure 5. Effects of monoterpenoid derivatives on the endurance of mice in a treadmill running test. Average data are presented as mean ± standard deviation, statistical analysis was conducted by Mann-Whitney U-test; a shows significant the difference against the control group ($p<0.05$).

h – hour

According to the data obtained, it was found for the first time that derivatives of monoterpenoids, compounds with 3,7-diazabicyclo[3.3.1]nonane moiety, namely compound K1-458 at a dose of 100 mg/kg, significantly increase the running time compared to the control group 1 hour and 6 hours after oral administration (by 35% and 26%, respectively). Compound K1-458 at a dose of 50 mg/kg increases performance at the trend level 1 hour and 6 hours after administration by 4% and 7%, respectively. The

reference drug bromantane did not increase the running distance in comparison with the control group but significantly increased the running distance 6 hours and 1 week after administration within the group when compared to the initial test.

Compound K1-456 at a dose of 100 mg/kg showed a tendency to a decrease in the running time by an average of 23.5% compared to the control group. Compound K1-456 at a dose of 50 mg/kg enahcnes performance by 22% 6 hours after administration. However, due to a large error of the mean (SE±73.25), the latter value is not considered significant.

After seven days of administration, none of the synthesized compounds had an effect on the duration of running at the studied doses. Bromantane increases the distance of running by 25% compared to the control group but without significant differences.

3. CONCLUSION

It was shown for the first time that derivatives of monoterpenoids with 3,7-diazabicyclo[3.3.1]nonane moiety containing methyl substituents at positions 1 and 5 can improve physical endurance of animals in exhaustive swimming and running tests while exceeding the effect of the reference drug bromantane. The most pronounced actoprotective activity has been shown for the compound K1-458, which contains residues of (-)-myrtenol linked to a bispidine fragment via amino groups, at a dose of 100 mg/kg after single administration.

Thus, bispidinone derivatives containing monoterpenoid residues present a novel promising class of organic compounds for the search for new actoprotectors.

4. MATERIALS AND METHODS

4.1. Chemistry

4.1.1. Synthesis of test compounds

All reagents used in the work had a purity of at least 95%. Solvents were dried and distilled before use according to standard methods.

All synthesized compounds were purified using column chromatography: silica gel (SiO_2; 60–200 μ; Macherey–Nagel); hexane/EtOAc 100/0 → 0/100 (increments of 2%).

GC: 7820A gas chromatograph (Agilent Tech., USA); flame ionization detector; HP-5 capillary column (Ø 0.25 mm × 30 m×0.25 μm); He as a carrier gas (flow rate 2 mL min^{-1}, flow division 99:1).

HRMS: DFS Thermo Scientific spectrometer in a full-scan mode (15-500 m/z, 70 eV electron ionization, direct sample injection).

Elemental analysis: Carlo-Erba 1106-Elemental analysis instrument.

^1H- and ^{13}C-NMR spectra: Bruker Avance – III 600 spectrometer [600.30 MHz (^1H) and 150.95 MHz (^{13}C) in $CDCl_3$]; chemical shifts in ppm rel. to residual chloroform [δ_H 7.24ppm, δ_C 76.90 ppm], J in Hz. The structures of the products were determined by analyzing their ^1H NMR spectra, J-modulated ^{13}C NMR spectra (JMOD) and ^{13}C -^1H-type 2D heteronuclear correlation with one bond (HSQC, ^1J 145 Hz) and long-range spin–spin coupling constants (HMBC, $^{2,3}J$ 7 Hz) and ^1H -^1H double-resonance spectra(COSY, NOESY). Numeration of atoms in the compounds is given for assigning the signals in the NMR spectra and does not coincide with that for the names according to the nomenclature of the compounds.

Optical rotation ($[\alpha]_D$): polAAr 3005 spectrometer; MeOH soln. concentration g/100 mL; specific rotation is expressed as deg ·mL ·g^{-1} ·dm^{-1}.

Microwave reactor: Monowave 300 from Anton Paar.

3,7-Bis-(((1R,5S)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)methyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one (K1-458) (Figure 3)

A mixture of the hydrochloric acid salt of diamine **3** (0.82 g (3.4 mmol)), 2.5-times excess of bromo-derivative **4** and 6-time excess of potassium carbonate in 6 ml of acetonitrile was heated in a microwave reactor to 70°C and held for 60 minutes. After cooling the reaction mixture, the precipitate was separated, washed with ethyl acetate. The organic phases are combined, the solvent is distilled. The yield of the product after chromatography was 1.10 g (73%). Optical rotation, elemental analysis, HRMS and ^1H and ^{13}C data are given in Supplementary information.

3,7-Bis-((1R,5S)-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-carbonyl)-1,5-dimethyl-3,7-diazabicyclo[3.3.1]nonan-9-one (K1-456) (Figure 3)

To a mixture of 0.36 g (1.98 mmol) of diazaadamantane **2** and 0.42 g (4.95 mmol) of $NaHCO_3$ in 14 ml of benzene and 4 ml of water was added 0.91 g (4.95 mmol) of acid chloride (5) over 10 minutes. The mixture was stirred for 6 hours at room temperature. The aqueous phase was separated, washed with benzene; the organic phases are combined, the solvent is distilled off. After chromatography, 0.79 g of product was obtained (86%). Optical rotation, elemental analysis, HRMS and ^{1}H and ^{13}C data are given in Supplementary information.

4.2. The studied compounds

The subject of the study was derivatives of monoterpenoids with easily accessible 3,7-diazabicyclo[3.3.1]nonane (bispidine) moiety containing methyl substituents at positions 1 and 5 of the carbon moiety with pinane fragments linked to nitrogen atoms at positions 3 and 7 of the bispidine molecule: compounds K1-456 and K1-458 (Figure 3).

Bromantane, which is widely used in clinical and experimental medical practice for improvement of physical endurance and performance [29], was used as a reference drug; it was administered intragastrically at a dose of 50 mg/kg similar to the compounds under study.

4.3. Experimental animals

Outbred CD-1 mice of both sexes weighing 25-35 g were used in the experiments. The animals were obtained from the vivarium at the Federal Research Center Institute of Cytology and Genetics SB RAS, where they were kept under standard vivarium conditions with free access to water and standard pelleted food. After quarantine, the animals were randomized by weight and divided into groups of 8-10 mice of the same sex each. Work with animals was carried out in strict accordance with the Order of the Ministry of Health of the Russian Federation No. 199n of April 1, 2016 "Principles of good laboratory practice" and the provisions of Directive 2010/63/EU on the protection of animals used for scientific purposes and the Council of the European Union dated September 22, 2010 on the protection of animals used for scientific purposes.

4.4. Evaluation of acute toxicity

Acute toxicity is considered as the toxic effect of the compoundat single-dose or multiple-dose administration for a period not exceeding 24 hours, which can be manifestedas impairment of physiological functions or disruption of organ morphology in experimental animals, as well as animal death [30].

The acute toxicity of the compounds under study was determined in mice of both sexes, with a weight of 25-35 g, randomized in 3 groups of 8 animals each.

Bispidine derivatives (K1-456, K1-458) were administered intragastrically once at a single dose of 500 mg/kg, 700 mg/kg or 1,000 mg/kg body weight. After administration of the compounds, mice were examined for the presence of impairments every hour for the first six hours and for the next 14 days on a daily basis (changes in breathing, diarrhea, convulsions, salivation, fatigue, changes in motor activity, coma, aggressiveness, discharge from the eyes and ears, injuries, food or water refusal, death, and etc.).

4.5. Determination of physical performance

The experiment was carried out on mice: males weighing 25-35 g (divided into groups of 8-12 animals each) receiving the test compound in the form of fine suspension dissolved in tween-20 in a volume of 0.2 ml per 10 g of body weight once at doses of 50 and/or 100 mg/kg using the probe. The control received an equal volume of water-tween solution intragastrically.

Physical endurance was studied using two tests: exhaustive swimming with a load of 7% of body weight and exhaustive treadmill running.

Physical endurance in the exhaustive swimming test with a load of 7% of body weight was assessed one hour after administration of the test agents. Prior to the experiment, two training swims have been conducted once daily. The training swim time was 7 minutes with a weight of 7% of the animal's body weight (the training time is 50-60% of the maximum swimming time for mice with this weight) [31, 32]. Control testing was carried out after one day of rest from the last workout. The effect of the compounds under study on physical endurance was assessed based on the time of swimming until reaching fatigue, which is indicated by the immersion of the animal to the bottom, after which the animal was immediately

removed from the water and dried. Water temperature was 24-26°C. Swimming time was recorded in seconds. The technique was carried out after a single administration for the search for the optimal working dose of the test substances.

Physical endurance was assessed using the method of exhaustive running on an FT-200six-track treadmill, Chengdu Technology and Market Co., Ltd. (China) [33]. Prior to that, mice were trained daily for two days to run at a speed of 10 m/min for 10 minutes with a tilt angle of 10°. On the third day, initial testing was performed. The speed was 10 m/min for the first 10 minutes, 15 m/min for the next 5 minutes. The speed was increased by 2 units every 2 minutes starting from the minute 15 (Figure 6).

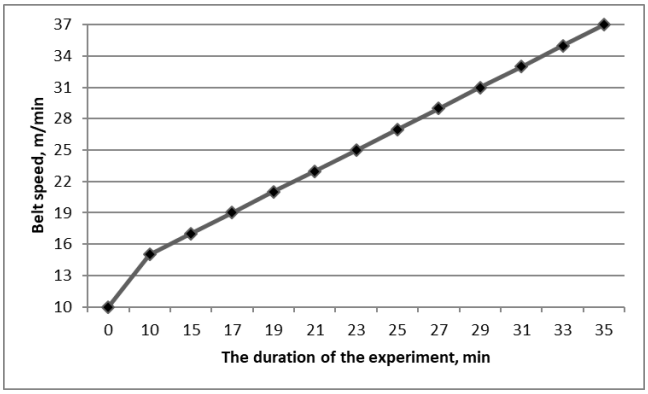

Figure 6. Treadmill testing speed protocol.

In this test, the condition corresponding to exhaustion of animals is defined as being in a "fatigue area" for 5 consecutive seconds. "Fatigue area" is defined as an area that includes the bottom of the treadmill within the size of one grid length with current, as well as the grid itself (it is denoted in the figure by a black rectangle) (Figure 7).

Figure 7. The Treadmill with "fatigue area" (it is denoted in the figure by a black rectangle).

On the fourth and the fifth day, testing was performed using the same method as on the third day 1, 6 and 24 hours after administration of the test compounds, respectively. The running time and distance were recorded. Next, the same mice received the compounds under study for 7 days, after which, they were tested again using the above-mentioned method.

4.6. Statistical analysis

Statistical processing of the data was performed using Statistica 6.0 software. The data are presented as arithmetic mean and standard error of the mean. Comparisons between the groups were made using the non-parametric Mann–Whitney U test. For comparison of changes in a sample within the same group but under different conditions, the Wilcoxon W-test was used. Data were considered significant at $p \leq 0.05$. Differences at the trend level were considered significant at $0.05 < p < 0.1$.

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Conflict of interest statement: The authors declared no conflict of interest.

Ethics committee approval: This study was approved by the Biothical Committee of the N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry of Siberian Branch of Russian Academy of Sciences.

Appendix A. Supplementary Material

Supplementary Material related to this article can be accessed at https://doi.org/10.35333/jrp.2020.136.

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