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Psychotropic activity evaluation of bromantane — *N*-(camphan-2-yl)anilines new structural analogues in *in vivo* tests

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The aim of the work was the synthesis of bromantane — *N*-(camphan-2-yl)anilines new structural analogues and the study of their psychotropic properties.

Materials and methods. The synthesized compounds were identified using the NMR spectroscopy. The purity of the compounds was confirmed by a GC-MS analysis. Psychotropic properties of N-(camphan-2-yl)anilines were studied in the experiments on the Wistar rats aged 18-20 weeks, the mice (12 weeks old) obtained from the "Stolbovaya" nursery using the following tests: the "Open Field", the "Elevated Plus Maze", the "Novel Object Recognition", the "Vogel Conflict", the "Tail Suspension", the "Tightrope suspension", the "Extrapolation" test.

Results. A series of new camphor aromatic amines, structural analogues of bromantane, were obtained and their psychotropic effects were evaluated by *in vivo* biological studies. Based on the results, it was possible to identify a leader compound, i.e. (1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(4-ethylphenyl)bicyclo[2.2.1.1]heptan-2-amine (4e), which had a pronounced anxiolytic effect. Substance 4f showed cognitive properties in the "Novel Object Recognition" and the "Extrapolation" tests. The fact was established by the indices of the time of learning a novel object and the time of diving.

Conclusion. The obtained data testify to the prospect of searching for substances with a psychotropic action in the range of aromatic camphor amines. Substance 4f, which deserves an in-depth study of the spectrum and mechanism of its psychotropic action, should be singled out separately.

Keywords: bromantane; arylamines of monoterpenoid ketones; camphor; anxiolytic action; antidepressant action; cognitive action

Abbreviations: NMR — nuclear magnetic resonance; GC-MS — gas chromatography-mass spectrometry; TLC — thin layer chromatography; OF — the "Open field" test; EPM — the "Elevated plus maze" test; NOR — the" Novel Object Recognition" test; EP — the "Extrapolation" test; TS — the "Tail suspension" test; TRS — "Tightrope suspension" test.

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Оценка психотропной активности новых структурных аналогов бромантана — *N*-(камфан-2-ил)анилинов — в тестах *in vivo*

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Цель. Синтез новых структурных аналогов бромантана — *N*-(камфан-2-ил)анилинов и изучение их психотропных свойств.

Материалы и методы. Синтезированные соединения идентифицировали с применением ЯМР-спектроскопии. Чистота соединений подтверждалась ГЖХ-МС анализом. Психотропные свойства *N*-(камфан-2-ил)анилинов изучены в экспериментах на крысах линии Wistar в возрасте 18–20 нед., мышах (12 нед.), полученных из питомника «Столбовая» с использованием тестов: «Открытое поле», «Приподнятый крестообразный лабиринт» (ПКЛ), «Тест Распознавание нового объекта», «Питьевой конфликтный тест Фогеля», «Подвешивание мышей за хвост», «Удержание на канатике», «Тест экстраполяционного избавления».

Результаты. Был получен ряд новых ароматических аминов камфоры — структурных аналогов бромантана, и в ходе биологических исследований *in vivo* оценено их психотропное действие. По итогам исследований удалось выделить соединение-лидер, а именно (1*R*,2*R*,4*R*)-1,7,7-триметил-*N*-(4-этилфенил)бицикло[2.2.1]гептан-2-амин (4е), которое оказывало выраженное анксиолитическое действие. Вещество 4е в тестах «Распознавание нового объекта» и в «Тесте экстраполяционного избавления» проявило когнитивные свойства, что было установлено по показателям времени изучения нового объекта и по времени подныривания.

Заключение. Полученные данные свидетельствуют о перспективности поиска веществ с психотропным действием в ряду ароматических аминов камфоры. Отдельно стоит выделить вещество 4е, которое заслуживает углублённого изучения спектра и механизма его психотропного действия.

Ключевые слова: бромантан; ариламины монотерпеноидных кетонов; камфора; анксиолитическое действие; антидепрессивное действие; когнитивное действие

Список сокращений: ЯМР — ядерный магнитный резонанс; ГЖХ-МС — газовая хромато-масс-спектрометрия; ТСХ — тонкослойная хроматография; ОП — тест «Открытое поле»; ПКЛ — тест «Приподнятый крестообразный лабиринт»; РНО — тест «Распознавание нового объекта»; ТЭИ — тест экстраполяционного избавления; ПМХ — тест «Подвешивание мышей за хвост».

INTRODUCTION

A high pace of modern life, psycho-emotional stresses, consequences of viral and somatic diseases are the reasons for the growth of anxiety and depression, and the decrease in the quality of life. According to the world health organization (WHO)[1], about half of the population experience feelings of anxiety and worry at certain intervals in their lives. About 970 million people worldwide suffer from mental disorders [2]. Anxiety and depressive pathologies are the most common types of mental disorders. More than 300 million people in the world are estimated as suffering from anxiety. Cardiovascular, metabolic, respiratory and many other diseases are often associated with anxiety and depression [1].

[1] When an anxious state requires special attention. Available from: https://rg.ru/2024/04/10/kogda-trevozhnoe-sostoianie-trebuet-osobogo-vnimaniia.html

[2] About 4 million Russians suffer from mental illnesses. Available from: https://www.interfax.ru/russia/945840

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Therefore, despite the availability of more than 160 neuropsychotropic drugs in circulation according to the DrugBank[3] database [2–4], the search for the substances aimed at the correction of psychoemotional (anxiety-depressive) states is an urgent task for synthetic chemists and pharmacologists [2–4]. But the problem of the effective and safe treatment of patients with such pathologies is still unsolved [5–7], so the search for the substances with anxiolytic, antiphobic and antidepressant effects continues [8–10].

A drug derivative of adamantane, bromantane, has been used in domestic medical practice for quite a long period of time [11–13]. It has a wide spectrum of action: it increases a physical and mental performance, has an anti-asthenic effect, and has anxiolytic and antidepressant effects [14–16]. The literature also describes bromantan related experimental preparations — ADK-910 (chlodantane), ADK-918 [17]. Unlike bromantan, these compounds belong to amides of carboxylic acids by their chemical structure. In this regard, the spectrum of their pharmacological action is somewhat different. It should be noted that the presence of adamantane fragment in their structure remains unchanged.

Guided by the general idea of creating bioisosteric structural analogues of bromantane, different variants of replacing the adamantane fragment with other hydrocarbon radicals were taken into account. In particular, by a bioisosteric replacement of the adamantane framework fragment with a fragment of norcamphan, for some antiviral agents, the problem of hepatotoxicity of functional adamantane derivatives and their ability to cause Reye's syndrome was solved [18]. A specific example of this kind is the transition from the rimantadine molecule to the deutiforin molecule [18] (Fig. 1).

In the authors' opinion, this approach is also justified for the directed modification of the bromantane molecule. From the total number of the considered variants of hydrocarbon radicals, a special attention was drawn by camphane. Camphane, like adamantane, is a ten-carbon carbocyclic radical of the framework structure and contains 3 methyl groups in its structure, which undergo hydroxylation in the course of a microsomal oxidation, which leads to a decrease in the level of a potential hepatotoxicity of the obtained substances. At the same time, a rapid hydroxylation and a subsequent conjugation may provide a higher level of the obtained substances clearance compared to bromantane. Finally, the availability of the starting compound plays an important role: camphor is of a natural origin and is a component of essential oils [19]. Thus, new structural analogues of bromantane — *N*-(camphan-2-yl)anilines (see Fig. 1) became the objects of the research in this work.

THE AIM of the work was the synthesis of bromantane — *N*-(camphan-2-yl)anilines new structural analogues and the study of their psychophysiological effect on animals

MATERIALS AND METHODS

Methods of preparation and analysis

Reagents from "Alfa Aesar" (UK) and solvents from "Komponent-Reactiv" (Russia) were used for the synthesis. The starting camphor anils had been synthesized as described before [20].

The target compounds were synthesized from the corresponding camphor anils (6b, d-z) by their reduction with a combination of sodium borohydride ($NaBH_4$) and nickel (II) chloride hexahydrate ($NiCl_2{\times}6H_2O$) in 95% ethanol according to the known procedure [21, 22] (Fig. 2).

4.75 g (20 mmol) of $NiCl_2{\times}6H_2O$ dissolved in 70 mL of 95% EtOH was added to the solution of the starting anil 6a-i (10 mmol) in 30 mL of 95% ethanol (EtOH) in one step. The resulting mixture was stirred until a clear solution was formed, and then cooled in a bath of dry ice and 95% EtOH to -40°C. 3.78 g (100 mmol) of $NaBH_4$ was added to the resulting solution in several steps while stirring and maintaining the temperature to -30°C under the argon atmosphere. Upon the completion of the addition, the reaction mixture was stirred for another 60 min under the same conditions and then left overnight at room temperature. The next day, the resulting mixture was treated with 15 mL of a 3N sodium hydroxide aqueous solution, filtered and distilled off with EtOH under the reduced pressure. The cube residue was extracted with diethyl ether (Et_2O); the combined organic extracts were dried in anhydrous sodium sulfate (Na_2SO_4), filtered and evaporated. The technical product remaining in the cube was purified by column chromatography with a gravity isocratic elution with a mixture of cyclohexane: methyl tertiary butyl ether (MTBE) (13:1 by volume) on the silica gel. To obtain analytical samples, cyclohexane was used as an eluent.

The NMR spectroscopy, gas chromatography-mass spectrometry, and thin-layer chromatography (TLC) were used to identify the obtained compounds.

The ^1H and ^{13}C NMR spectra of the compounds were recorded on an AVANCE 600 NMR spectrometer (BRUKER, Germany) (operating frequencies of 600 and 150 MHz, respectively) in deuterobenzene (C_6D_6). The chemical shifts of the ^1H and ^{13}C nuclei are given relative to Me_4Si or solvent signals (C_6D_6: δ_H 7.16 ppm, δC 128.0

[3] DrugBank. Available from: https://go.drugbank.com/drugs

ppm). Two-dimensional NMR spectra (^{1}H-^{1}H COSY, ^{1}H-^{1}H NOESY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC) were recorded using the Z-gradient pulse technique (mixing time — 700 ms). Signals in the ^{1}H and ^{13}C NMR spectra of the products were attributed using 2D experiments ^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC.

Macherey-Nagel Alugram Xtra Sil G/UV$_{254}$ plates (Germany) were used for TLC. The spots were visualized in the UV light. R_f values of the substances are given for the eluent (cyclohexane : MTBE=13 : 1 vol.).

A GC-MS analysis was performed on a Chromatech-Crystal 5000 instrument (Russia) under conditions of the electron impact ionisation with an intensity of 70 eV.

It should be noted that the reduction of 4-bromo-*N*-[(1*R*,4*R*)-camphan-2-ylidene]aniline under these conditions leads to the formation of product 4a due to the hydrogenolysis of the C-Br bond [21]. That is why in this work, there is no study of the psychotropic activity of the product, which is the closest to bromantane in the structure.

In vivo studies

All biological activity studies were performed in accordance with the requirements of the current guidelines and in accordance with Article 11 of Federal Law 61 dated 12.04.2010 and according to the requirements of the EAEU.

The study of a psychotropic activity was performed on 69 male Wistar rats aged 20–22 weeks, weighing 300–350 g, and on 18 12-week-old mice weighing 24–28 g. The laboratory animals were obtained from the specialized nursery “Stolbovaya” of Russia (Moscow region, Chekhov district, Stolbovaya settlement). The conditions of the laboratory animals were in accordance with all the requirements of laboratory practice for preclinical studies in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 199n “On approval of the rules of good laboratory practice” dated 01 Apr 2016, GOST 33044-2014; Interstate Standard Principles of Good Laboratory Practice, and in accordance with the Decree of the Chief State Sanitary Doctor of the Russian Federation No. 51 “On approval of SR 2.2.1.3218-14 “Sanitary and Epidemiological Requirements for the Establishment of the Laboratory Animal Facility” dated 29 Aug 2014.

After the animals had been admitted to the vivarium of the Scientific Centre for Innovative Medicines of Volgograd State Medical University, they were quarantined for 14 days. The animals had a free access to food (standard rodent pellet diet GOST R 51849-2001, LLC “Laboratoryfodder”, Moscow, Russia) and water (GOST “Drinking water” 2874-82 and SanPiN 2.1.4.1074-01 “Drinking water. Hygienic requirements for water quality of centralized drinking water supply systems”). The rats were kept in plastic cages (545×395×200 mm, Type: T/4B, LLC “MEST”, Moscow), at the temperature regime of 20–22°C and the relative humidity of 40–60%. Soft wood chips were used as bedding. The light regime was 12 h of light and 12 h of darkness. The compounds were tested on the animals in equivalent doses taken in proportion to the molecular weight of the substance: 1/100, 1/30 and 1/10 of the molecular weight (Table 1).

Ethics approval

The study was approved by the Local Ethical Committee of the Volgograd State Medical University (IRB registration number 00005839 IORG 0004900 [OHRP]). Minutes of the committee meeting No. 88 dated 06 Oct 2023.

Assessment of psychophysiological state of animals

To assess the psychophysiological state of the animals, the following tests were used: the Open Field (OF) test, the Elevated Plus Maze (EPM) test, the Novel Object Recognition (NOR) test, the Extrapolation (EP) test, the Vogel conflict (VC) test, the Tail suspension (TS) test, the Tightrope suspension (TRS) test.

The animals (rats) were divided into 22 groups of 3 individuals each, except for the control group, which had 6. In the study of the anxiolytic activity, diazepam widely used in clinical practice, was used as a reference drug. Fluoxetine was used as a reference drug in the study of antidepressant properties. Phenotropil was chosen as a reference drug in the study of physical endurance and a cognitive function. The study substances were administered in two equimolar doses of 1/10 and 1/30 of their molecular weight (MW), and the substances that showed their psychotropic activity were studied at a dose of 1/100 of MW. The animals in the control group received a 0.9% sodium chloride (NaCl) solution. The mice were divided into 3 groups of 6 individuals each for the TS test.

Motor and exploratory activities of the animals were evaluated in the OF test. The setup for the “Open Field” test for rats is a circular area 97 cm in diameter, bounded by 40 cm high boards, divided by markings into 25 equal sectors, at the intersection of which, there are 16 holes (2 cm in diameter). The installation is illuminated with steady light (400 lux). To clean the setup and reduce the influence of foreign odours on behaviour (due to the presence of the previous animal), before each test, the setup is wiped with a cloth moistened with water, followed by a cloth moistened with a 5% alcohol solution and a dry rag. The next animal is tested after a complete drying of the unit surface.

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After placing a test animal in the centre of the setup, the following parameters were recorded for 3 minutes: the number of squares crossed (a spontaneous motor activity), the total number of posts and surveyed foot-holes (an approximate exploratory activity), and the number of crossings of the setup central squares [23].

To assess the anxiolytic activity of the compounds under study, the Elevated plus Maze (EPM) test is used to evaluate the level of anxiety in the animals.

The EPM installation consists of 4 (50×14 cm) arms, diverging cross-shaped from the central platform (10×10 cm) at right angles: two opposite, open, without walls (the height of the edge is 1 cm) and two closed, dark, fenced on the sides with walls 30 cm high. The cross-shaped arena of the maze is mounted on a trolley with stoppers, which provides lifting the arena to a height of 55 cm. The installation is illuminated with steady light (400 lux). Before landing the next animal, the installation was treated in the same way as the "Open Field" had been treated.

When the animals land on the central, brightly lit, elevated platform, an aversive environment for them is created; and they tend to move to a more comfortable closed arm from it. After the familiarization with the closed arm, which is not dangerous for them, the reflex of curiosity and assessment of a possible danger in other zones of the EPM is triggered.

The tested animal is placed on the central EPM platform with its head towards the open arm. And during 3 minutes, the parameters of the rodent behaviour in the conditions of a variable stressogenicity (under a free choice of comfortable conditions) are recorded: a latent period of the exit from the central platform to any arm, a number of visits and time of stay in the open arms (a potentially stressful zone of the installation), a frequency of visits and time of stay of the animals in closed arms, a sum of transitions between the arms of the installation. The time of the exit from the central site (a latent period) can be interpreted as an indicator of the speed of decision making, in addition, the central EPM zone is also a lighted space, and the time spent in it is summed up with the time in the open arms [24].

A cognitive function was assessed using the Novel Object Recognition (NOR) test. The test is based on the novelty and curiosity of animals and consists of two sessions: training and reproduction. In the training session, two identical objects are placed in a home cage without a top netting, 10 cm apart and 10 cm from each of the crate walls, and the animal explores the crate and the objects in it for 3 min. The time associated with the exploration of each object is recorded. After training, the animals are placed in home cages. In the playback session, 1 h after training, the animals were re-housed in a home cage without the top net, but one of the objects was replaced with a new one. The objects are different in shape, colour and texture but are approximately the same size. The caged animal freely explores the objects and the environment for 3 min. The animal finds the new object more interesting than the old one, the memory trace of which allows it to pay more attention to the new object (a novelty preference). The time spent exploring each object is recorded. After each test, the box is alcoholized with a 5% alcohol solution. The time spent examining the objects is presented in seconds. The NOR test reflects the state of episodic memory [25].

To assess cognitive functions under stress conditions, the Extrapolation (EP) test was used. The EP test setup is a cylindrical container (height 40 cm, diameter 35 cm) into which water with a temperature of 18–19°C is poured up to a certain level. In the centre of the container, a cylinder is vertically fixed, the lower part of which is lowered into the water by 2.5 cm. The test is conducted in 3 stages: training, the first reproduction and the second reproduction. At the stage of 'training', the animal is placed with its tail down into the inner cylinder, and within 3 min, it has to solve the following task: to dive under the edge of the cylinder, after which it is removed from the installation. After 24 h, the animals that failed to solve the "extrapolation task" within 3 min, are excluded during the first reproduction. The second reproduction is carried out 24 h after the first reproduction, i.e. 48 h after training.

The following indices are recorded in the EP test: a latent period (LP) of diving, immobilization time, a number of jumps (rapid upward body movements). Shorter time spent on the EP task of the animals of the same group receiving the investigated substance, compared to the animals of the control group, is considered as better memorization and execution of the strategy of getting rid of the aversive environment. The increase in the latent period of diving is interpreted as a loss (forgetting) of the animal's skill of active escape from the aversive environment.

The presence of the myorelaxant effect was assessed by the results of the Tightrope suspension test. To perform the test, a 0.5 cm diameter nylon rope is placed horizontally at a height of 95 cm from the cage tray filled with a 5 cm layer of wood chips. The forelegs of the animal are placed on the rope and the hind limbs are released. The time of keeping the rat on the rope is recorded. For each rat, 3 repeated landings were performed 10 sec apart. The duration of the retention was summed. A longer holding time on the rope compared to that of the control animals is considered as an increase in strength and absence of the myorelaxant

action, and *vice versa***,** a shorter holding time on the rope indicates a decrease in strength and/or a myorelaxant action of the tested substance.

The Vogel Conflict test is based on the conflict between the instinct to the quench thirst and the avoidance of punishment. It is used to determine the anxiolytic effect of the tested substances. The animal is deprived of water intake for 18 h. After 18 h, a drinker is placed in the chamber and the animals' approach to take water, but receive a weak electric shock. The number of approaches of the animals in the control group that received a physiological solution or test substances 60 min prior to testing, is recorded. Under the influence of anxiolytic substances, the animals make more approaches to the water drinker.

The Tail Suspension test is a classic test for determining the antidepressant effect of substances. The test setup was a rectangular chamber 46 cm high and 30 cm wide. During the test, the animal was suspended by 1/3 of the tail at a height of 10-15 cm from the ground with an adhesive tape. The duration of the observation is 6 min. The duration of the immobilization (desperation behaviour, the animal does not make any movement, just hangs down) is evaluated in seconds. The total duration of the immobilization during the 6 minutes of testing is taken into account. If the animal climbs on its tail, it is excluded from the experiment. During the test, a video recording of the test was used, which makes it possible to observe the animal while it is out of sight, as well as the possibility to perform an additional analysis of the animal's behaviour based on the video recording.

Statistical processing

The results were processed and analyzed using Microsoft Excel 2021 (Microsoft Corporation, USA).

Statistical processing of the obtained results was carried out by methods of descriptive and analytical statistics. The distribution of quantitative indicators was evaluated using the Shapiro-Wilk and Dunn criteria. Numerical values were presented as an arithmetic mean and a standard error of the mean. The differences between the indicators in the group were considered statistically significant at p **<0.05.**

RESULTS

Characterization of target products

The structures of the studied compounds were confirmed by NMR spectroscopy, thin layer chromatography and gas chromatography-mass spectrometry.

(1*R,*2*R,*4*R*)-1,7,7-Trimethyl-N-phenylbicyclo[2.2.1] heptane-2-amine (4a). The yield is 80%; according to the GC-MS data, the content of the target product is 99.6%. Physicochemical and NMR spectra data of the obtained sample correspond to those published before [21, 26].

(1*R*,2*R*,4*R*)-1,7,7-Trimethyl-*N*-(4-methylphenyl) bicyclo[2.2.1]heptan-2-amine (4b). The yield is 88%, the target product content (as determined by GC-MS) is 100%, the diastereomeric composition (as determined by GC-MS) is 99:1% (*ekzo-/endo-*). The mass spectrum is: (*ekzo*-form) (Eu, 70 eV), m/z (Iotn (%)): 243 [M] (100), 172 (71), 133 (62), 120 (54), 107 (40), 95 (35), 91 (18). The mass spectrum is: (*endo*-form) (Eu, 70 eV), *m/z* (Iotn (%)): 243 [M] (100), 172 (82), 133 (65), 120 (65), 107 (58), 95 (57), 91 (26). The ^{1}H NMR spectrum is: (C_6D_6, δ, m.d.): 0.73 (s, 3 H, C(7)Me camphan), 0.82 (s, 3 H, C(1) Me camphan), 0.91 (s, 3 H, C(7)Me camphan), 0.99–1. 04 (m, 1 H, C(5)H_{endo} camphan), 1.06–1.10 (m, 1 H, C(6) H_{endo} camphan), 1.42–1.47 (m, 1 H, C(6)H_{ekzo} camphan), 1.52–1.65 (m, 3 H, C(3)H_{ekzo}, C(4)H, C(5)H_{ekzo} camphan), 1.68–1.71 (m, 1 H, C(3)H_{endo} camphan), 2.23 (s, 3 H, C(4) $_{Ar}CH_3$), 3.16–3. 19 (m, 1 H, C(2)H_{endo} camphan), 3.38 (s, 1 H, NH), 6.47–6.49 (m, 2 H, C(2)$_{Ar}$H, C(6)$_{Ar}$H), 6.99–7.01 (m, 2 H, C(3)$_{Ar}$H, C(5)ArH). The ^{13}C NMR spectrum is: (C_6D_6, δ, m.d.): 12.67 (C(1)Me camphane), 20.89 (C(7) Me camphane), 20.97 (C(7)Me camphane), 20.98 (C(4)$_{Ar}$CH3), 28.01 (C(5) camphan), 37.18 (C(6) camphan), 41.30 (C(3) camphan), 45. 82 (C(4) camphan), 47.57 (C(1) camphan), 49.29 (C(7) camphan), 62.32 (C(2) camphan), 113.82 (C(2)$_{Ar}$, C(6)$_{Ar}$), 126.12 (C(4)$_{Ar}$), 130.33 (C(3)$_{Ar}$, C(5)$_{Ar}$), 146.84 (C(1)$_{Ar}$). R_f=0.86.

(1*R*,2*R*,4*R*)-1,7,7-Trimethyl-N-(2-ethylphenyl) bicyclo[2.2.1]heptan-2-amine (4c). The yield is 70%; according to the GC-MS data, the content of the target product is 99.7%. Physicochemical and NMR data of the obtained sample correspond to those published before [21].

(1*R*,2*R*,4*R*)-1,7,7-Trimethyl-N-(4-methoxyphenyl) bicyclo[2.2.1.1]heptan-2-amine (4d). The yield is 85%; and according to GC-MS, the target product content is 99.5%. Physicochemical and NMR data of the obtained sample correspond to those published before [21].

(1*R*,2*R*,4*R*)-1,7,7-Trimethyl-N-(4-ethoxyphenyl) bicyclo[2.2.1]heptan-2-amine (4e). The yield is 83%, the target product content (as determined by GC-MS) is 100%, the diastereomeric composition (as determined by GC-MS) is 100:0% (*ekzo-/endo-*). The mass spectrum (*ekzo*-form) (Eu, 70 eV), *m/z* (Iotn (%)) is: 273 [M] (100), 202 (31), 163 (50), 150 (30), 137 (23), 95 (29). The ^{1}H NMR spectrum (C_6D_6, δ, m.d.) is: 0.74 (s, 3 H, C(7)Me camphan), 0.84 (s, 3 H, C(1)Me camphan), 0.95 (s, 3 H, C(7)Me camphan), 1.01-1.05 (m, 1 H, C(5)N_{endo} camphan), 1. 08-1.12 (m, 1 H, C(6)H_{endo} camphan), 1.20 (t, 3 H, C(4)$_{Ar}$OSH2CH3, J=7.0 Hz), 1.44–1.49 (m, 1 H, C(6) H_{ekzo} camphan), 1.56–1.66 (m, 3 H, C(3)H_{ekzo}, C(4)H, C(5)

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H_{ekzo} camphan), 1.68–1.73 (m, 1 H, C(3)H_{endo} camphan), 3.13–3.15 (m, 1 H, C(2)H_{endo} caffman), 3. 26 (s, 1 H, NH), 3.37 (k, 2 H, C(4)$_{Ar}$OSH2CH3, J=7.0 Hz), 6.47–6.49 (m, 2 H, C(2)$_{Ar}$H, C(6)$_{Ar}$H), 6.86–6.89 (m, 2 H, C(3)$_{Ar}$H, C(5)$_{Ar}$H). The ^{13}C NMR spectrum (C6D6, δ, m.d.): 12.70 (C(1)Me camphan), 15.55 (C(4)$_{Ar}$OSH$_2$CH$_3$), 20.93 (C(7) Me camphan), 20.99 (C(7)Me camphan), 28.05 (C(5) camphan), 37.25 (C(6) camphan), 41.38 (C(3) camphan), 45. 87 (C(4) camphan), 47.60 (C(1) camphan), 49.27 (C(7) camphan), 62.99 (C(2) camphan), 64.28 (C(4)$_{Ar}$OSH$_2$CH$_3$), 114.81 (C(2)$_{Ar}$, C(6)$_{Ar}$), 116.39 (C(3)$_{Ar}$, C(5)$_{Ar}$), 143.34 (C(1)$_{Ar}$), 152.17 (C(4)$_{Ar}$). R_f=0.58.

((1*R*,2*R*,4*R*)-1,7,7-Trimethyl-N-(4-ethylphenyl) bicycle[2.2.1]heptane-2-amine (4e). The yield is 78%, the target product content (as determined by GC-MS) is 99.1%, the diastereomeric composition (as determined by GC-MS) is 100:0% (*ekzo-/endo-*). The mass spectrum (*ekzo*-form) (Eu, 70 eV), *m/z* (Iotn (%)) is: 243 [M] (100), 158 (87), 93 (76), 41 (30). The ^{1}H NMR spectrum (C_6D_6, δ, m.d.) is: 0.72 (s, 3 H, C(7)Me camphan), 0.83 (s, 3 H, C(1) Me camphan), 0.91 (s, 3 H, C(7)Me camphan), 0.99-1.03 (m, 1 H, C(5)H_{endo} camphan), 1.06–1.11 (m, 1 H, C(6)H_{endo} camphan), 1.20 (t, 3 H, C(4)$_{Ar}$CH$_2$CH$_3$, J=7.6 Hz), 1.43–1.47 (m, 1 H, C(6)H_{ekzo} camphan), 1.54–1.65 (m, 3 H, C(3)H_{ekzo}, C(4)H, C(5)H_{ekzo} camphan), 1.69–1.72 (m, 1 H, C(3)H_{endo} camphan), 2. 55 (k, 2 H, C(4)$_{Ar}$CH2CH3, J=7.6 Hz), 3.18–3.20 (m, 1 H, C(2)H_{endo} camphan), 3.43 (widespread s, 1 H, NH), 6.50–6.53 (m, 2 H, C(2)$_{Ar}$H, C(6)$_{Ar}$H), 7.04–7.06 (m, 2 H, C(3)$_{Ar}$H, C(5)$_{Ar}$H). The ^{13}C NMR spectrum (C_6D_6, δ, m.d.): 12.67 (C(1)Me camphane), 16.88 (C(4)$_{Ar}$CH$_2$CH$_3$), 20.88 (C(7)Me camphane), 20.96 (C(7)Me camphane), 28.01 (C(5) camphan), 28.86 (C(4)$_{Ar}$CH2CH3), 37.19 (C(6) camphan), 41. 35 (C(3) camphan), 45.83 (C(4) camphan), 47.59 (C(1) camphan), 49.28 (C(7) camphan), 62.37 (C(2) camphan), 113.86 (C(2)$_{Ar}$, C(6)Ar), 129.16 (C(3)$_{Ar}$, C(5)$_{Ar}$), 133.04 (C(4)$_{Ar}$), 147.07 (C(1)$_{Ar}$). R_f =0.87.

(1*R*,2*R*,4*R*)-1,7,7-Trimethyl-N-(2-methoxyphenyl) bicyclo[2.2.1.1]heptan-2-amine (4f). The yield is 72%, the target product content (as determined by GC-MS) is 100%, the diastereomeric composition (as determined by GC-MS) is 100:0% (*ekzo-/endo-*). The mass spectrum (*ekzo*-form) (Eu, 70 eV), *m/z* (Iotn (%)) is: 259 [M] (100), 188 (89), 136 (83), 95 (70), 41 (20). The ^{1}H NMR spectrum (C_6D_6, δ, m.d.): 0.73 (c, 3 H, C(7)Me camphan), 0.93 (c, 3 H, C(1)Me camphan), 1.03 (ddd, 1 H, C(5)H_{endo} camphan, J=12.5, 9.4, 4.4 Hz), 1.05 (c, 3 H, C(7)Me camphan), 1.11 (ddd, 1 H, C(6)H_{endo} camphan, J=12.5, 9.4, 3.8 Hz), 1. 47 (dd, 1 H, C(6)H_{ekzo} camphan, J=12.5, 11.6, 4.4 Hz), 1.58 (dd, 1 H, C(4)H camphan, *J*=4.3 Hz), 1.59-1. 70 (m, 2 H, C(3)H_{ekzo}, C(5)H_{ekzo} camphan), 1.75 (dd, 1 H, C(3)H_{endo}, *J*=12.8, 8.3 Hz), 3.25 (dd, 1 H, C(2)N_{endo} cafman, J=8.3, 6.1, 4.8 Hz), 3.34 (s, 3 H, C(2)$_{Ar}$OCH3), 4.54 (e, 1 H, NH J=6.1), 6.59 (dd, 1 H, C(3)$_{Ar}$H, J=7.9, 1.4 Hz), 6. 70 (dd, 1 H, C(6)$_{Ar}$H, J=7.9, 1.4 Hz), 6.73 (ddd, 1 H, C(4)$_{Ar}$H, J=7.9, 7.5, 1.5 Hz), 7.01 (ddd, 1 H, C(5)$_{Ar}$H, J=7.9, 7.5, 1.5 Hz). 13C NMR spectrum (C_6D_6, δ, m.d.): 12.74 (C(1)Me camphan), 20.95 (C(7)Me camphan), 20.99 (C(7)Me camphan), 28.05 (C(5)caffman), 37.21 (C(6)camphan), 41.52 (C(3) camphan), 45.92 (C(4)camphan), 47.64 (C(1)camphan), 49.40 (C(7)camphan), 55.46 (C(2)$_{Ar}$OCH3), 61. 91 (C(2) camphan), 110.16 (C(3)$_{Ar}$), 110.68 (C(6)$_{Ar}$), 116.35 (C(4)$_{Ar}$), 122.16 (C(5)$_{Ar}$), 122.16 (C(5)$_{Ar}$), 139.12 (C(1)$_{Ar}$), 147.41 (C(2)$_{Ar}$). R_f =0.82.

(1*R*,2*R*,4*R*)-1,7,7-Trimethyl-N-(2,5-dimethoxyphenyl) bicyclo[2.2.1.1]heptan-2-amine (4z). The yield is 64%, the target product content (as measured by GC-MS) is 99.8%, and the diastereomeric composition (as measured by GC-MS) is 100:0% (*ekzo-/endo-*). The mass spectrum of (*ekzo*-form) (Eu, 70 eV), *m/z* (Iotn (%)) is: 289 [M] (100), 274 (23), 218 (36), 179 (31), 166 (33), 153 (29), 138 (23), 95 (50), 41 (21). The ^{1}H NMR spectrum of (C_6D_6, δ, m.d.) is: 0.70 (s, 3 H, C(7)Me camphan), 0.92 (s, 3 H, C(1)Me camphan), 0.94-0.98 (m, 1 H, C(5)H_{endo} camphan), 1.02-1.07 (m, 1 H, C(6)H_{endo} camphan), 1.03 (s, 3 H, C(7)Me camphan), 1. 40-1.45 (m, 1 H, C(6)H_{ekzo} camphan), 1.54-1.61 (m, 2 H, C(4)H caffman, C(5)H_{ekzo} camphan), 1.64-1.68 (m, 1 H, C(3)H_{ekzo}), 1.73 (dd, 1 H, C(3)H_{endo}, *J*=12.9, 8.3 Hz), 3.23 (ddd, 1 H, C(2)H_{endo} camphan, J=8.3, 6.2, 4.7 Hz), 3.39 (c, 3 H, C(2)$_{Ar}$OCH3), 3. 53 (c, 3 H, C(5)$_{Ar}$OCH3), 4.59 (e, 1 H, NH J=6.2), 6.18 (dd, 1 H, C(4)$_{Ar}$H, J=8.6, 2.9 Hz), 6.55 (e, 1 H, C(6)$_{Ar}$H, J=2.9). The 13C NMR spectrum (C_6D_6, δ, m.d.) is: 12.73 (C(1)Me camphan), 20.90 (C(7) Me camphan), 20.95 (C(7)Me camphan), 27.96 (C(5) camphan), 37.12 (C(6) camphan), 41.35 (C(3) camphan), 45.89 (C(4)camphan), 47.61 (C(1)camphan), 49.38 (C(7) camphan), 55.49 (C(5)$_{Ar}$OCH3), 56.09 (C(2)$_{Ar}$OCH3), 61. 82 (C(2)camphan), 98.50 (C(4)$_{Ar}$), 99.39 (C(6)$_{Ar}$), 110.55 (C(3)$_{Ar}$), 140.13 (C(1)$_{Ar}$), 142.27 (C(2)$_{Ar}$), 156.20 (C(5)$_{Ar}$). R_f=0.65.

Results of *in vivo* studies

In the OF test, the animals administered with substances 4c and 4h had a higher (53.7±1.6 and 52.3±2.0, respectively) motor activity (a number of crossed squares) compared to the control group (38.5±0.8), they were more in the central squares of the setup than others. A higher exploratory activity was registered in the animals injected with substance 4h in all doses, while substances 4c, 4d and 4e increased an exploratory activity only in low (1\100 of MW) doses (Fig. 3).

In the EPM test, i.e. under the conditions of stressogenicity, the behaviour of animals receiving compounds 4c and 4f was particularly different from

the control group (Fig. 4). It is possible to note a decrease in the LP of leaving the central site in the animals receiving compounds 4c (1.3±0.3) and 4g (1.7±0.7), which may indicate a rapid decision making and an increased exploratory activity. There was an increase in the frequency of going into the open arm and the time spent in the open arm, especially for compound 4f (26 mg/kg; 130±10). The animals receiving compounds 4c (9 mg/kg; 5±1), 4f (2.5±1.5) at all doses, 4g (9 and 26 mg/kg; 4.3±1.6 and 3.5±0.9), and 4h (10 and 29 mg/kg; 4.5±2 and 2.8±1.6) were less likely to move between the arms, also indicating a reduced anxiety.

The recording of transitions between the arms, especially the exits to and stays in the open arms, makes it possible to judge the depressant (diazepam) or anxiolytic, antiphobic action (compound 4e).

All the animals, except those receiving diazepam and compound 4e, quickly left the central site. A pre-injection of the investigated substances did not interfere with the decision to leave the central site quickly under the conditions of variable aversiveness. The animals under the influence of compound 4e stayed in the open arms longer than others (130±10), and the animals receiving compound 4c repeatedly moved from one arm to another without lingering on the open surfaces of the EPM. Under the influence of diazepam, the animals moved little both in the OF and in the EPM tests, but after entering the central area or after crossing it, they stayed longer on the open surface.

The anxiolytic properties of the camphor derivative — compound 4e ((1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(4-ethylphenyl)bicyclo[2.2.1]heptan-2-amine), which had the most pronounced anxiolytic effect in the EPM test, were evaluated in the Vogel conflict test. The substance increased the number of attempted punished water draws compared to the animals that received a NaCl solution 60 min before testing (control group), but statistically significantly less than the reference drug diazepam (Fig. 5A).

These data, together with the results obtained in the OF and EPM tests, indicate the anxiolytic properties of substance 4f.

Depressive disorders are not rare in persons with anxiety disorders. Therefore, the presence (or absence) of antidepressant properties of compound 4f was assessed by the duration of the immobilization in the EPM test. Compared to the control animals, the immobilization time was statistically significantly shorter in the mice treated with compound 4f (Fig. 5B).

Amnesic and myorelaxant actions are characteristic for the substances with anxiolytic effects similar to benzodiazepines [27, 28]. For this purpose, cognitive effects were studied in the Novel Object Recognition and Extrapolation tests, as well as in the Tightrope suspension test, and the effect of the substance on the physical strength was tested.

The examination of the compound 4f effect on the attention and short-term memory in the Novel Object Recognition test showed that the animals in the control group learnt the objects more (A1: 9.3±0.3; A2: 7.7±1.8) than the animals receiving compound 4f in the first phase of testing (Fig. 5C). However, after 1 hour in the second stage of testing, most animals that had been administered the compound in all three dosages (1/100: 9±0.3, 1/30: 7.7±2.9 and 1/10: 7.5±2.5 of molecular weight) studied the novel object more, suggesting that the compound under study enhances the animals' attention and short-term memory (Fig. 5D).

In the Extrapolation test, the animals receiving compound 4f at a dose of 9 mg/kg were faster in the extrapolation escape task on day 2 (3.3±2.3) than on day 1 (13.3±10.8), and also faster compared to the control group (9.7±4.1) (Fig. 5E). Taken together, the compound in the Novel Object Recognition and Extrapolation tests indicated an improvement in the cognitive function.

In the course of the experiment on the Tightrope suspension, it was found out that the animals receiving compound 4f at a dose of 26 mg/kg were keping on the rope longer (89.7±14.3) than the animals of the control group (47.7±12.7), which indicates the absence of myorelaxing properties in this substance.

DISCUSSION

The structural features of camphor, a typical representative of natural monoterpenoids, in combination with its low toxicity, were the basis for the choice of this compound as a starting material for a directed structural modification to obtain pharmacologically active monoterpenoid aromatic amines with low toxicity.

The modification was carried out by obtaining intermediates — substituted camphor anils, by the reduction of which the studied compounds had been obtained. Herewith, the known methods of the anil synthesis, involving the use of catalytic systems and condensing agents, lead to obtaining difficult to isolate and, often, contaminated products, which reduces the yield of pure substances. To solve this problem, an original method of synthesis in the presence of an *in situ* obtained catalytic complex was developed. That provides a technological approach to the preparation of intermediates [29], where the catalytic system performs an additional function of an acceptor of the water released during the condensation.

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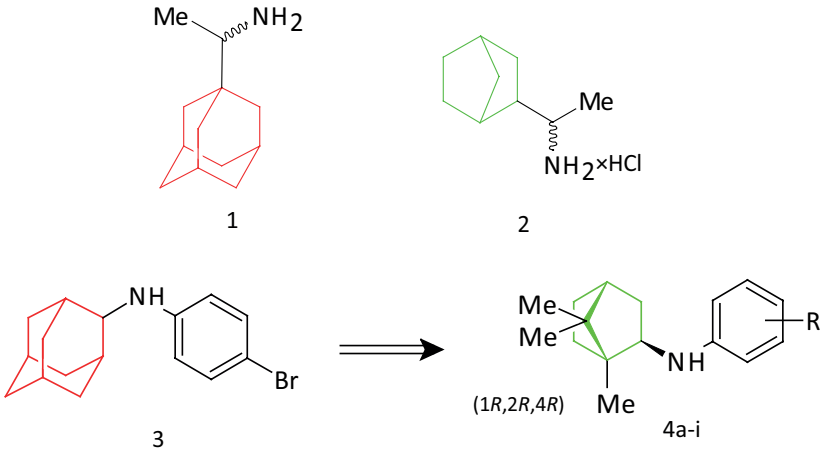

Figure 1 – Chemical structure of rimantadine (1), deutiforin (2), bromantane (3) and the investigated compounds (4 a–z — N-(camphan-2-yl)anilines)

Note: R=H (4a), 4-Me (4b), 2-Et (4c), 4-OMe (4d), 4-OEt (4d), 4-Et (4e), 2-OMe (4j), 2,5-(OMe)2 (4h).

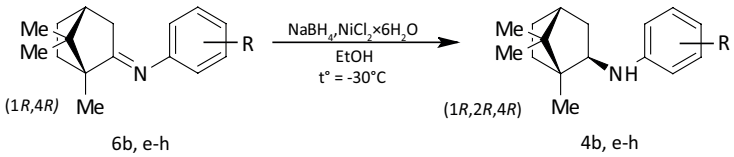

Figure 2 – Reaction of target compounds formation

Note: 4b — 88%, 4e — 83%, 4f — 78%, 4g — 72%, 4i — 64%.

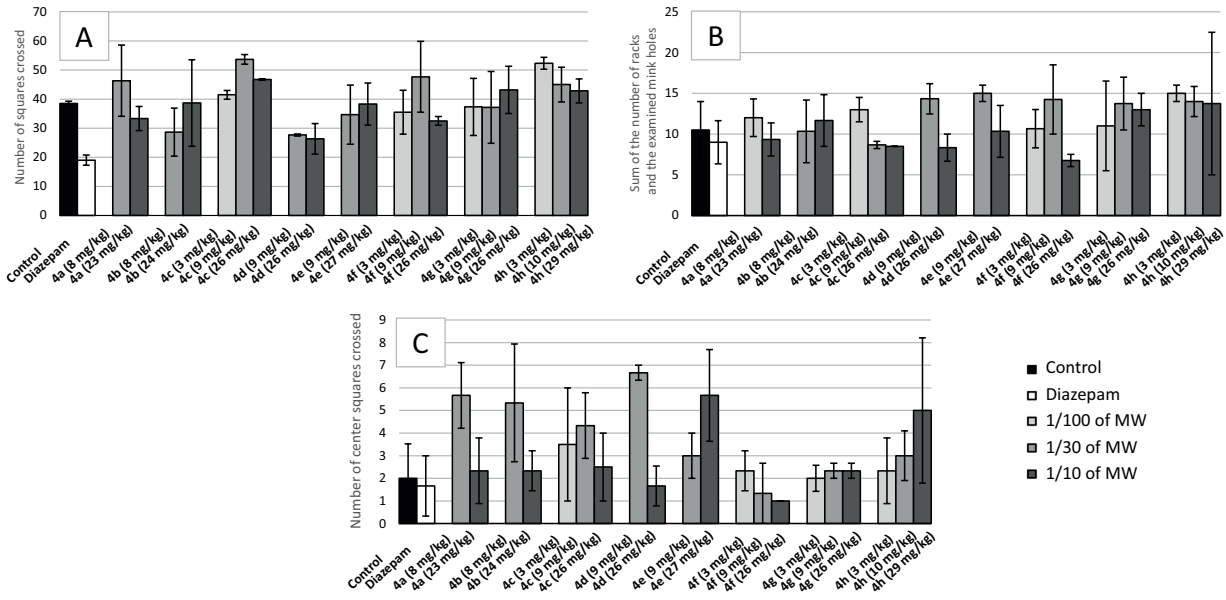

Figure 3 – Effect of the tested substances on animal behaviour in the "Open Field" test

Notes: A) a number of crossed squares; B) total exploratory activities (a total number of peeks into holes, unsupported stands and supported stands); C) a number of crossings of central sectors. * — differences are statistically significant compared to control group animals.

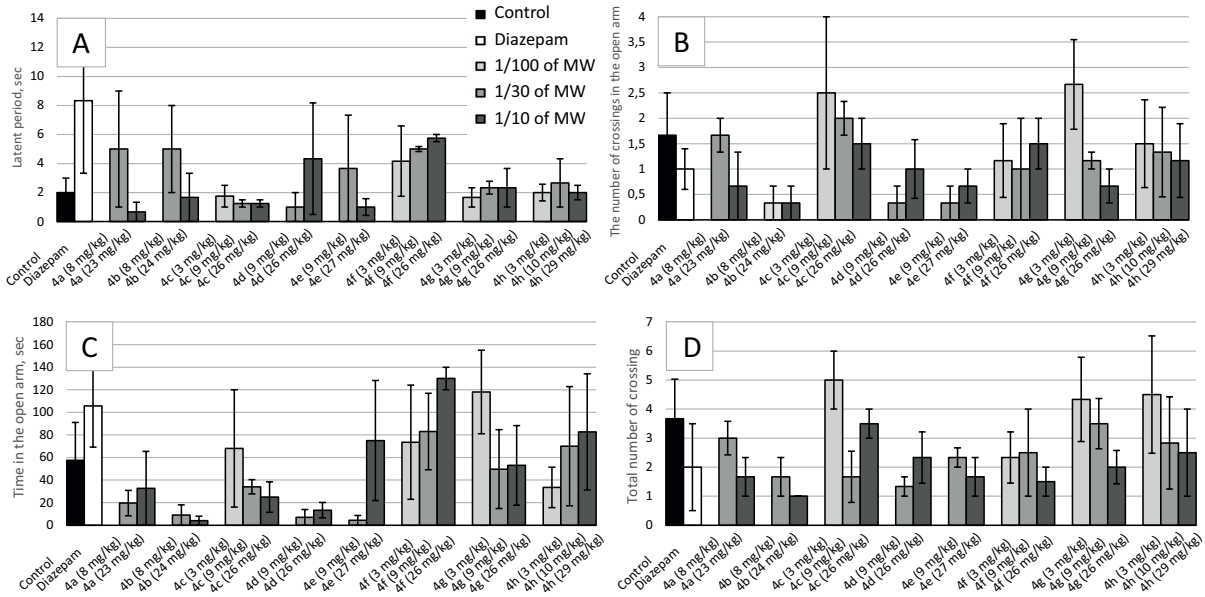

Figure 4 – Effect of the investigated substances on animal behaviour in the Elevated Plus Maze test

Note: A) a latent period of leaving the central site; B) frequency of animal exits to the open arm of the facility; C) time of animal stay in the open arm of the facility; D) a total number of transitions between the arms of the facility (the sum of transitions, entrances to the open arm and entrances to the closed arm). * — the differences are statistically significant compared to the animals of the control group.

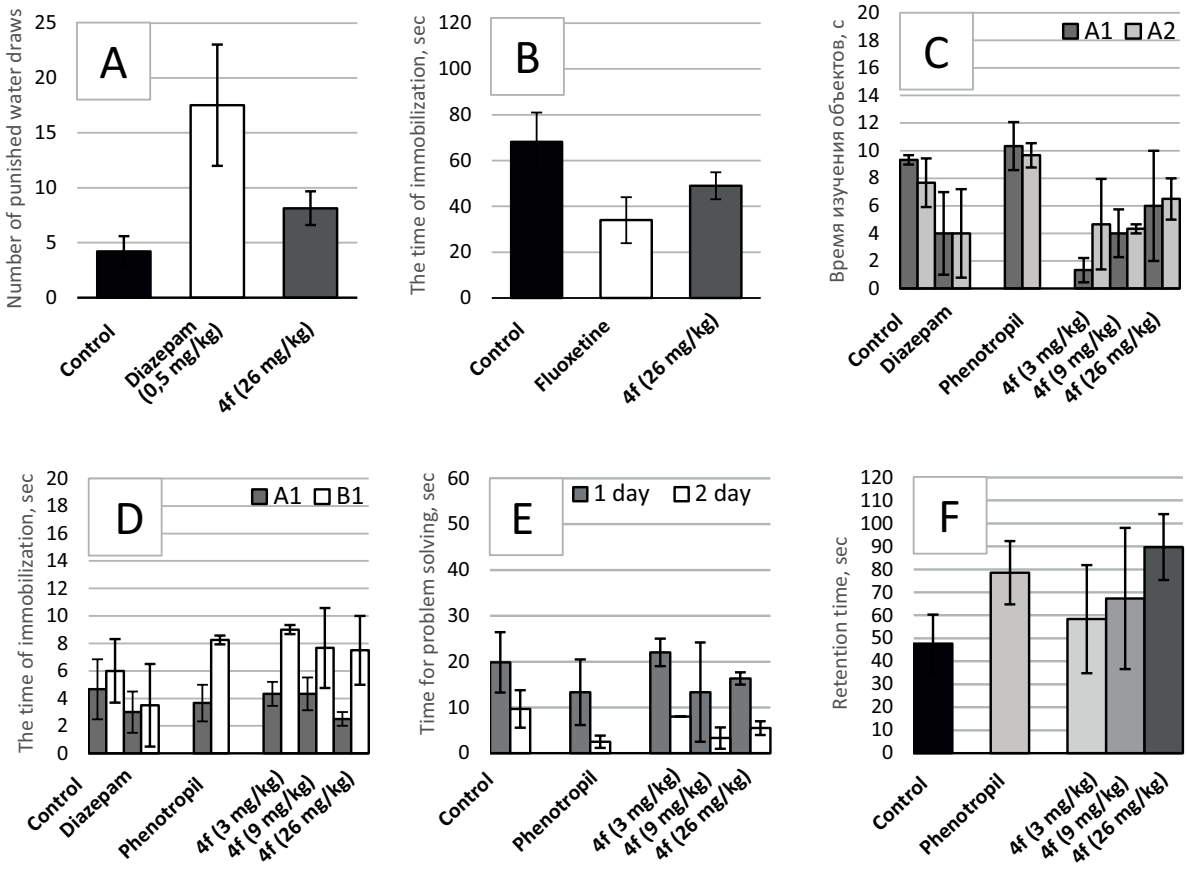

Figure 5 — Behaviour of animals in the tests studied

Note: A) The Vogel conflict test; B) The Tail suspension test; C) The Novel Object Recognition test; the training session; D) The RNO test; the reproduction session; E) The Extrapolation test; F) The tightrope suspension test. * — the differences are statistically significant compared to the group of animals. # — the differences are statistically significant in comparison with the time of learning the object A1 in the NOR test and in comparison with the time of solving the extrapolation task on day 1 in the Extrapolation test.

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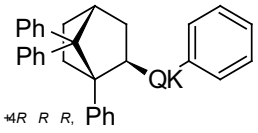
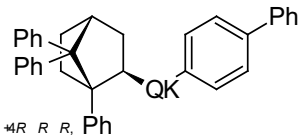
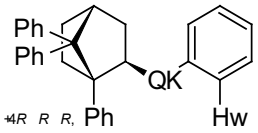
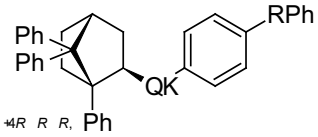
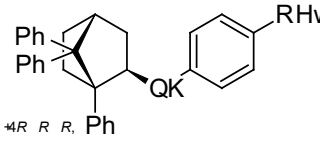
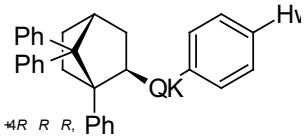
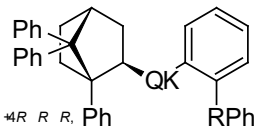
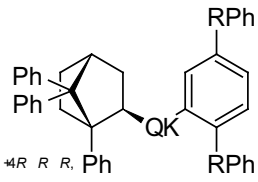
Table 1 – Obtained compounds, their names, structural formulas, molecular weight and doses for *in vivo* study

Sequence number	Name Molecular weight, g/mol; equimolar doses (mg/kg)	Structural formula	Molecular weight, g/mol; equimolar doses (mg/kg)
4a	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(phenyl) bicycle[2.2.1]heptane-2-amine		229.36 1/30 (8) 1/10 (23)
4b	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(4-methylphenyl) bicycle[2.2.1]heptane-2-amine		243.39 1/30 (8) 1/10 (24)
4c	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(2-ethylphenyl) bicycle[2.2.1]heptane-2-amine		257.41 1/100 (3) 1/30 (8) 1/10 (24)
4f	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(4-methoxyphenyl) bicyclo[2.2.1.1]heptan-2-amine		259.39 1/30 (8) 1/10 (26)
4g	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(4-ethoxyphenyl) bicyclo[2.2.1.1]heptan-2-amine		273.42 1/30 (9) 1/10 (27)
4h	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(4-ethylphenyl) bicycle[2.2.1]heptane-2-amine		257.41 1/100 (3) 1/30 (9) 1/10 (26)
4i	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(2-methoxyphenyl) bicyclo[2.2.1.1]heptan-2-amine		259.39 1/100 (3) 1/30 (9) 1/10 (26)
4j	(1*R*,2*R*,4*R*)-1,7,7-trimethyl-*N*-(2,5-dimethoxyphenyl)bicyclo[2.2.1.1]heptan-2-amine		289.41 1/100 (3) 1/30 (10) 1/10 (29)

In the reduction process of the obtained intermediates, a special attention was paid to the proper selection of the hydrogenating agent and to the identification of limitations to the application of the technique in terms of steric factors hindering hydrogenation. The competition manifests itself in the form of an activating action of donor substituents in the aromatic ring depending on their structure, amount and location relative to the exocyclic nitrogen atom. A high yield of the target compounds was accompanied by obtaining a by-product of the aromatic ring reduction, so the most effective method of the target compounds isolation was the method of column chromatography. The nature and location of the substituent in the aromatic ring also influenced the activity of the compounds. All the studied camphor aromatic amines, except for substance 4a, contained donor substituents represented by lower alkyl and alkoxyl groups. A high activity expected from

alkoxy-substituted aromatic amines was not justified, and the substituted derivatives 4c and 4f were the most active. In both cases, the ethyl substituent located in positions providing a coordinated orientation with the exocyclic nitrogen, led to an increase in the activity of the substances. However, the shielding effect of the ethyl substituent in the ortho-position slightly reduced the activity of the amine, whereas the para-ethyl substituted product showed the highest activity.

According to the results of the biological activity study, compound 4f should be singled out in the Elevated plus maze (EPM) test (significantly longer dwell time in the open arms compared to the animals of other groups, including the control group). In the Vogel conflict test, the animals receiving the same compound made more approaches to punished water draws than the animals of the control group. In the Tail suspension test, the animals administered with compound 4f recorded a shorter period of immobilization, and in the Extrapolation test, active behaviour and a rapid task solution of getting rid of the aversive environment indicated an antidepressant effect. The animals receiving compound 4f performed better in the Novel Object Recognition and the Extrapolation tests and, respectively, in the first and second tests, learnt more about the novel object and were quicker to dive under the lower edge of the cylinder and get rid of the aversive environment, reflecting good attention, short-term memory and quick decision making. In the Tightrope suspension test, the animals held on longer in comparison with the animals of the control group, and at a dose of 26 mg/kg, and longer than the animals receiving the reference drug phenotropil. Thus, the performed study made it possible to obtain substance 4f ((1*R*,2*R*,4*R*)-1,7,7-trimethyl-N-(4-ethylphenyl)bicyclo[2.2.1.1]heptan-2-amine) with pronounced anxiolytic, antidepressant and nootropic actions in a series of camphan derivatives and possibly increasing physical performance. Compound 4f is promising for a further in-depth study of its psychotropic properties.

Study limitations

The study was conducted on a relatively small group of animals, which can reduce a statistical power and make it difficult to identify rare or weak effects. The experiment covered a limited period of time, which makes it impossible to assess long-term effects of the substances.

CONCLUSION

In the OF test, compounds 4c with an ethyl substituent in the second position of the phenyl ring and 4i, having two methoxy groups in the phenyl ring, increased a motor activity, and in the Elevated plus maze (EPM) test, the animals made more transitions between the arms, indicating their psychostimulant effect.

Compound 4f in the Elevated plus maze (EPM) test increased the time spent in the open arms, and in the Vogel conflict test, there was a greater number of attempts of punished water draws than in the animals of the control group, but fewer than in the animals receiving the comparison drug diazepam.

The 4f substance insignificantly decreased the immobilization time in the Tail suspension and Extrapolation tests compared to the control. When assessing the effects of the substance on the cognitive function, an increase in the time to explore an unknown object and a decrease in the time to solve an extrapolative escape were noted, which can be regarded as an improvement in the cognitive function. In the Tightrope suspension test, a significantly longer retention of the animals was observed.

The presented data indicate that compound 4f had a moderate anxiolytic effect without myorelaxant, amnesic and, at the same time, antidepressant effects. The presented data indicate the prospect of a further search for substances with psychotropic properties in the range of camphan derivatives and the expediency of in-depth study of specific activities and an action mechanism of the compound (1R,2R,4R)-1,7,7trimethyl-N-(4-ethylphenyl)bicyclo(2,2,1,1)heptan-2-amine (4e).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ОРИГИНАЛЬНАЯ СТАТЬЯ
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AUTHORS' CONTRIBUTION

Andrey A. Vernigora — implementation of the research process, synthesis of chemicals; Leila L. Brunilina — creation and preparation of the manuscript; Andrey V. Kazhberov — implementation of the research process, including isolation and purification of chemical structures; Nikita S. Bolokhov — implementation of the research process, including experiments with animals, creation and preparation of the manuscript, application of statistical methods for the analysis of research data; Aleksander A. Pokhlebin — implementation of the scientific research process, including experiments with animals; Alina A. Sokolova — implementation of the research process, including performing experiments with animals; Vladislav E. Pustynnikov — implementation of the research process, including performing experiments with animals; Ivan N. Tyurenkov — control, leadership and mentoring in the process of planning and conducting research; Ivan A. Novakov — control, leadership and mentoring in the planning process; Vyacheslav I. Krasnov, Dmitry N. Polovyanenko — implementation of the scientific research process, including identification of chemical structures.

All the authors confirm their authorship compliance with the ICMJE international criteria (all the authors made significant contributions to the conceptualization, research and preparation of the article, read and approved the final version before the publication).

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