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Biological activity of 4-ethynyl-, 4-oxy-, 4-butoxypropylpyperidine and azaheterocyclic compounds

Abstract. The immune system of humans and higher animals performs an important function of maintaining the constancy of the internal environment of the body by detecting and eliminating foreign substances of an antigenic nature, both endogenously created and exogenously penetrating. This function of the immune system is performed by factors of innate and acquired immunity. As the name suggests, immunostimulants are the agents boosting the immune response. The new chemical series of compounds is promising for the search new effective immunostimulating drugs. Acute toxicity and the effect on peripheral blood hemogram parameters and on the subpopulation composition of lymphocytes were determined in the compounds (piperidine-containing derivatives): 1-(3-n-butoxypropyl)-piperidine-3-methyl-4-spiro-5'-imidazolidine-2',4'-dione,1-(2-ethoxyethyl)-4-carboxy-4-hydroxypiperidine, bis[1-(2-ethoxyethyl)-4-ethynyl-4-hydroxypiperidine) dihydrochloride and, 1:1 complexes of β-cyclodextrin with 1-(2-ethoxyethyl)-4-(2-ethoxyethoxyl)-4-ethynylpiperidine, 1-(2-ethoxyethyl)-4-(2-methoxyethoxyl)-4-ethynylpiperidine, 7-(2-methoxyethyl)-2,2-dimethyl-3-thia-7-azabicyclo[3.3.1]nonane or 3-(2-morpholinoethyl)-7-(3-isopropoxypropyl) diazabicyclo[3.3.1] nonane with β-cyclodextrin. Compound - 1-(3-n-butoxypropyl) piperidine-3-methyl-4-spiro-5'-imidazolidine-2',4'-dione, exceeds the immunostimulating activity of the comparison drug of levamizole by 3.1 times and shows low toxicity.

Key words: piperidine-containing derivatives, immunostimulating activity, acute toxicity, rat, hemogram, leukogram, phenotyping of leukocytes.

Introduction

The immune system of humans and higher animals performs an important function of maintaining the constancy of the internal environment of the body by detecting and eliminating foreign substances of an antigenic nature, both endogenously created (cells modified by viruses, xenobiotics, malignant cells, etc.) and exogenously penetrating (primarily microbes, parasites, viruses). This function of the immune system is performed by factors of innate and acquired immunity. The immune cells are involved in the process of destroying antigens: neutrophils, eosinophils, basophils, monocytes/macrophages, dendritic cells, NK cells, T and B lymphocytes, etc. Immunodeficiency diseases develop if the number and functional activity of the immune system cells are disturbed. These diseases are treated using a complex of immunotherapy methods, one of which is the use of immunotropic medicines [1; 2]. There are main groups of immunotropic medicines: immunomodulators and immunostimulants. As the name suggests, immunostimulants are the agents boosting the immune response. Today immunostimulants of microbial, thymic, bone marrow, cytokine, nucleic, animal, plant and synthetic origin are known. On the pharmaceutical market of the Republic of Kazakhstan, immunostimulants of domestic production are represented only by biologically active supplements that are not pharmaceuticals [3-6].

Thus, screening of new synthetic immunostimulants in the Republic of Kazakhstan is

relevant and necessary. The laboratory of Synthetic and Natural Medicinal Compounds chemistry at the A.B. Bekturov Institute of Chemical Sciences has accumulated vast experience in the field of synthesis and chemical transformations of complex heterocycles; new data have been obtained allowing important conclusions about the relationship of the fine chemical structure of synthesized compounds with their reactivity, spectral characteristics and biological activity [7]. The stimulus for searching new immunostimulating medicines among complex heterocycles was the manifestation of weak immunostimulating activity by the Prosidol. In the field of biological research, the Department of Biophysics and Biomedicine of the Faculty of Biology and Biotechnology of Al-Farabi KazNU has plenty of experience.

Naturally occurring azaheterocyclic structures have found widespread clinical use, albeit that now a day the majority of the pharmacologically active compounds are synthetic in nature. The remarkable ability of azaheterocyclic nuclei to serve both as biomimetics and reactive pharmacophores has largely contributed to their unique value as traditional key elements of numerous drugs [8]. Azaheterocycles are common structural units in marketed drugs and in medicinal chemistry targets in the drug discovery process. Almost two third of top small molecule drugs contain at least one azaheterocyclic fragment in their structures. Basically antibiotics, antifungal, anticonvulsants, antipyretics, non-steroidal anti-inflammatory drug, cytostatic drug, antihistamine, psychoactive, antihypertensive drug [9; 10].

The uncontrolled growth of cells in the body, started due to certain stimuli, lays the foundation of cancer, anticancer drugs either kill cancer cell or modify their growth. Cancer or neoplastic disease may be regarded as a family of related disorders. A common feature in different forms of cancer is an abnormal and uncontrolled cell division, frequently at a rate greater than that of most normal body cells. Among the heterocyclic compounds, five-member heterocyclic moieties fused with Aromatic ring system with nitrogen atom possess wide spectrum of pharmacological activity [11]. Heterocycles like indole, pyrimidine, pyridine, quinoline etc. are an integral part of huge number of natural and synthetic compounds and play important roles in the biological system. For developing the suitable leads for anticancer drugs introduction of appropriate substituents at C-3 of indole, C-5 of pyrimidine, C-2 of pyridine and quinoline is required. The substituents carry nitrogen and oxygen as two ligating sites along with hydrophobic moieties, the essential requirement for multiple target ligands. The structure-activity relationship studies point that the contribution of phenylglycinol moiety as a part of side chain at C-3 of indole and C-5 of pyrimidines seems to be crucial for exhibiting anticancer activities [12; 13].

The heterocyclic compounds are enjoying their importance as being the center of activity. The nitrogen-containing heterocyclic compounds were found in abundance in most of the medicinal compounds and the presence of three nitrogen heteroatoms in five-membered ring systems defines an interesting class of compounds [14]. The first Schiff base compounds were reported by Hugo Schiff in 1864. In recent years, the chemistry of Schiff bases contains N-donor atom which has been extensively studied and has acquired a great interest because of the azomethine C=N linkage essential for biological activity [15]. 1,2,4-triazoles constitute broad realization due to their useful application in different areas of biological activity, and as industrial intermediates, it is effectively used in polymers, dyestuff, photographic chemicals, and agricultural chemicals [16]. There is some biological activity of 1,2,4-triazole such as antimicrobial [17], anti-inflammatory [18], and antioxidant [19].

Cyclophosphamide, a widely employed alkylating agent and a major constituent of combination chemotherapy regimens; it causes myelosuppression, which limits chemotherapy's dose intensification and precludes the administration of optimal treatments for cancer patients [20].

The practical significance of the study is that azaheterocyclic compounds contain nitrogen atom in the ring. They are of vital importance in the race to improve our understanding of basic chemistry which underlies nearly all of the important life-processes and a large proportion of transformations leading to and creating the increasingly sophisticated products, which enhance our society today. A large number of azaheterocyclic compounds are well known and this number is increasing rapidly because they hold a special place among pharmaceutically significant natural products and synthetic compounds. Their study is of great interest both from the theoretical as well as practical standpoint. Azaheterocyclic compounds are very widely distributed in nature and are essential to life in various ways. Compounds such as alkaloids, antibiotics, essential amino acids, vitamins, haemoglobin, hormones and a large number of synthetic drugs and dyes contain azaheterocyclic ring systems. Knowledge of azaheterocyclic chemistry is useful in biosynthesis as well as in drug metabolism. There are also a large number of azaheterocyclic compounds with other important

practical applications as antioxidants, vulcanization accelerators, copolymers, solvents photographic sensitizer and developers, dyestuffs and many are valuable intermediates in synthesis. Azaheterocycles are omnipresent extremely in all branches of chemistry and biochemistry as well as in our lives. Another important property of azaheterocyclic compounds is their extraordinarily participation in a wide range of reactions. Depending upon pH of the medium, they may behave as acids or bases, forming anions or cations. Some interact readily with electrophilic reagents, other with nucleophiles, yet others with both. Some are readily oxidized, but resist reduction, while others can be readily hydrogenated but are stable towards the action of oxidizing agents. The ability of many azaheterocyclic compounds to produce stable complexes with metal ions has great biochemical significance. All these results prove that azaheterocyclic compounds are excellent scaffolds for obtaining a wide variety of compounds and speeding up research activity. The majority of pharmaceuticals and biologically active agrochemicals are azaheterocyclic [21].

The use of erythropoietin (EPO) is likely to be restricted in oncology because it increases the risk of death and serious cardiovascular events. Therefore, it is necessary to search for compounds that promote hematopoiesis, enhance immunity, potentiate anticancer effects, and detoxify anticancer drugs [22]. Azaheterocyclic componds isolated and may be a source of beneficial compounds, as they have been reported to induce hematopoiesis.

The majority of heterocycle compounds and typically common heterocycle fragments present in most pharmaceuticals currently marketed, alongside with their intrinsic versatility and unique physicochemical properties, have poised them as true cornerstones of medicinal chemistry. Apart from the already marketed drugs, there are many other being investigated for their promising activity against several malignancies. In particular, anticancer research has been capitalizing on the intrinsic versatility and dynamic core scaffold of these compounds. Nevertheless, as for any other promising anticancer drugs, heterocyclic compounds do not come without shortcomings. In this research provide for a concise overview of heterocyclic active compounds and families and their main applications in medicine. We shall focus on those suitable for therapy while simultaneously addressing main biochemical modes of action, biological targets, structure-activity relationships as well as intrinsic limitation issues in the use of these compounds.

Materials and methods

Test compounds. Piperidine-containing derivatives: 1:1 complex of β-cyclodextrin with 1-(2-ethoxyethyl)-4-(2-ethoxyethoxyl)-4-ethynylpiperidine (1); 1:1 complex of β-cyclodextrin with 1-(2-ethoxyethyl)-4-(2-methoxyethoxyl)-4-ethynylpiperidine (2); 1-(3-n-butoxypropyl) piperidine-3-methyl-4-spiro-5'-imidazolidine-2',4'-dione (3); 1-(2-ethoxyethyl)-4-carboxy-4-hydroxypiperidine (4); bis[1-(2-ethoxyethyl)-4-ethynyl-4-hydroxypiperidine) dihydrochloride (5); 1:1 complex of β-cyclodextrin with 7-(2-methoxyethyl)-2,2-dimethyl-3-thia-7-azabicyclo[3.3.1]nonane (6) and 1:1 complex of β-cyclodextrin with 3-(2-morpholinoethyl)-7-(3-isopropoxypropyl)diazabicyclo[3.3.1]nonane (7), had been firstly synthesized in the Laboratory of chemistry of synthetic and natural medicinal compounds of the A.B. Bekturov Institute of Chemical Sciences.

Method of peripheral blood hemogram analysis. To evaluate the peripheral blood hemogram, 80 adult female laboratory albino rats 10-15 weeks of age weighing 210-280 g were used. Animals arrived from one breeding station in one time (the biological clinic of the Faculty of Biology and Biotechnology of Al-Farabi KazNU). The studies were performed in accordance with the "Rules for conducting preclinical (nonclinical) studies of biologically active substances" and "Ethical principles and recommendations for scientific experiments on animals" [23]. All animals were managed under uniform conditions (sawdust litter, room temperature 22-24°C, natural light mode) and received standard feed rations. The animals were divided into 10 groups of 8 specimens. The 10th group of animals was intact. With a 24-h interval, cyclophosphamide hydrochloride was administered to all experimental groups of animals at a dose of 10 mg/kg in 1% normal saline in a volume of 0.2 – 0.21 ml. After 72 h, the animals were injected with: 1, 2, 3, 4, 5, 6, 7th experimental group – 0.1-0.12 ml of 1% solution of compound 1, 2, 3, 4, 5, 6, 7 (the dose is 5 mg/kg in normal saline); the 8th placebo group – 0.1 – 0.12 ml of normal saline; 9th control group – 0.1 – 0.12 ml of 1% methyluracil solution (administration dose 5 mg/kg in normal saline). Blood sampling was at 09.00 in the morning from the rat orbital vein into VF-052SDK hematological tubes with EDTA (K2) 7 days after the injection of the test compounds under mild ether anesthesia. Blood was tested on an Abacus Junior VET hematological analyzer (Diatron, Denmark). Double cytological control was used on blood smears. Blood smears were colored by

the Giemsa method and counted under a SA3300S microscope under immersion (magnification 7 x 100), 100 cells for each smear sample, then the relative number of cells of each type was converted to an absolute value [24].

Lymphocyte phenotyping by indirect immunofluorescence. Blood fractions were separated with ficoll-urografin. Lymphocytes phenotyping was made by the conventional indirect fluorescence method using monoclonal antibodies: ICO 111 (CD3), ICO 101 (CD4), ICO 31 (CD8), ICO 180 (CD20) (SPC MedBioSpektr, Moscow) [8]. Cell complexes with antibodies were suspended in 20 µl of a working FITC-conjugates of secondary antibodies solution (FITC-anti-mouse manufactured by: SPC MedBioSpektr, Moscow) and incubated for 30 min at 4°C in a humid thermostat. The cells were fixed with a cell fixation solution (8% formalin solution and 4% paraformaldehyde solution). The cell suspension was placed in the wells on a glass slide. Since the reaction results were registered on luminescent microscopes, the slides were not covered with cover slips, but the objective was carefully placed into the well using a 50% glycerol solution as an immersion medium. A 100x microscope objective and a 10x eyepiece were used. Glowing cells were counted within 24 h after setting the reaction, keeping preparations in a dark room. Microscopy was performed in a dark room. The results were statistically processed with the average value, mean error and Student's confidence interval.

When statistically processing the research results, the samples were compared by Student's t test.

In addition, the acute toxicity of the new compounds was measured by intraperitoneal administration them to white outbred mice of both sexes and weighing 18-23 g.

Results and discussion

1. Acute toxicity indices of new synthetic compounds. In general, all compounds of the investigated series showed a very low acute toxicity index. The level of acute toxicity of compounds 4 and 6 was very low – more than 8.71 times lower than that of the reference substance. The acute toxicity indices of compounds 1, 2, 7 ranged from 787.045 ± 8.38 to 851.138 ± 1.38 mg/kg of animal body weight, which characterized the compounds as low-toxic. Only the compound 5 showed acute toxicity below 500 mg/kg – 396.278 ± 3.28 mg/kg, however, the toxicity level was 3.45 times lower than that of the reference substance (Table 1).

Table 1 – Acute toxicity of compounds

No.	Number of compounds	Acute toxicity index (LD_{50}), mg/kg	Acute toxicity index (LD_{50}) relative to the standard compound methyluracil
1	1	851.138 ± 1.38	7.41
2	2	787.045 ± 8.38	6.85
3	3	446.68 ± 3.24	3.89
4	4	> 1000	>8.71
5	5	396.278 ± 3.28	3.45
6	6	> 1000	>8.71
7	7	790.678 ± 8.74	6.89
8	Levamisole	114.74 ± 0.71	1

All new synthesized compounds showed low acute toxicity.

2. Screening results for immunostimulating activity of new synthetic compounds

Control values for intact animals were within the physiological range. The erythrocyte index was (9.5 ± 1.4) $\cdot 10^{12}$/L of blood with a hemoglobin content (145.7 ± 1.2) g/l of blood and, accordingly, hematocrit (39.8 ± 1.9) %. The platelet count was also within the normal range (659.6 ± 36.0) $\cdot 10^{9}$/L of blood, the thrombocyte level was (12.6 ± 0.3) %, the leukocyte indicator was (11.18 ± 1.22) $\cdot 10^{9}$/L of blood with a lymphocytes count (6.93 ± 0.89) $\cdot 10^{9}$/L of blood or 62.9 ± 0.1% according to the blood leukogram. Granulocytic leukocytes were (3.97 ± 0.9) $\cdot 10^{9}$/L of blood with a percentage of (31.9 ± 0.4) %. The indicator of monocytes was (0.68 ± 0.03) $\cdot 10^{9}$/L of blood, which according to the blood leukogram was 6.1 ± 0.5%. In general, all peripheral blood counts are within normal limits (Table 2).

Immunosuppressive syndrome was induced by intoxication of animals with three times administration of sodium cyclophosphamide with an interval of 24 h. After the intoxication, the condition of the animals externally manifested a violation of psychomotor reactions, appetite and increased tear secretion. Stool disturbance was observed, the hair was dirty, sometimes hyperreactivity and aggressive reactions were observed in rats.

The blood hemogram has changed in the following parameters: 1) a critical decrease in the common leukocyte count; 2) decrease in the erythrocyte and platelet counts from (9.5 ± 1.46) 10^{12} /l of blood in intact animals to (4.93 ± 1.3) $\cdot 10^{12}$/L of blood, i.e., 1.93 times; 4) the hemoglobin decreased by 1.93 times (from 140.7 ± 16.7 g/l to 90.75 ± 12.0 g/l). The hematocrit volume decreased by 1.88 ($p \leq 0.05$), from (39.8 ± 6.3) % to (21.21 ± 2.58) %. Critical values recorded in changes in platelet cells – (70.5 ± 4.3) $\cdot 10^9$/L with the values for intact animals of (650.6 ± 36.0) $\cdot 10^9$/L, i.e., 9.23 times ($p \leq 0.05$). In the blood leukogram, changes occurred in all cell populations counts.

The total leukocyte count decreased from those of intact animals (11.18 ± 1.22) $\cdot 10^9$/L to the values of the experimental groups – from (1.82 ± 0.33) $\cdot 10^9$/L to (4.26 ± 0.15) $\cdot 10^9$/L; there was a release of immature granulocytes: metamyelocytes from (2.36 ± 0.71) % to (4.00 ± 1.24) %, myelocytes from (0.9 ± 0.08) % to (2.88 ± 0.25) %. In addition, the relative count of stab neutrophils increased from (5.2 ± 0.3) % to (18.0 ± 0.9) %. The segmented leukocytes increased from (20.8 ± 0.94) % to (42.2 ± 0.54) %. Particularly significant changes in counts of other granulocytic leukocytes were not found. Decrease was registered among agranulocytes.

It was in the lymphocytic population of cells that a critical decrease was in the range from (62.0 ± 0.11)% to (20.4 ± 6.72)%. The monocyte count increased by 3-4%. Thus, cyclophosphamide intoxication leads to an immunosuppressive syndrome with a shift to the left in the blood leukogram.

Further conducted a study on new azaheterocyclic compounds: the 1:1 complex of β-cyclodextrin with 1-(2-ethoxyethyl)-4-(2-ethoxyethoxy)-4-ethynylpyridine (1), the 1:1 complex of β-cyclodextrin with 1-(2-ethoxyethyl)-4-(2-methoxyethoxy)-4-ethynylpyridine (2), 1-(3-n-butoxypropyl)-piperidine-3-methyl-4-Spiro-5'-imidazolidine-2',4'-dione (3), 1-(2-ethoxyethyl)-4-carboxy-4-oxypiperidine (4), bis(1-(2-ethoxyethyl)-4-ethyl-4-oxypiperidine) dihydrochloride (5), 1:1 β-cyclodextrin complex with 7-(2-methoxyethyl)-2,2-dimethyl-3-TIA-7-azabicyclo[3.3.1]nananom (6), complex 3-(2-morpholinoethyl)-7-(3-isopropoxypropyl) diazabicyclo[3.3.1]nonane with β-cyclodextrin (1:1) (7) for immunostimulating activity.

As a result of statistical processing of data, the following data were obtained. The highest activity was shown by compound 3. Chemical compound 3 stimulated leukopoiesis bringing the total leukocyte count from (1.90 ± 0.51) $\cdot 10^9$/L of blood to (4.26 ± 0.15) $\cdot 10^9$/L of blood and was higher than in other experimental groups, this value was also at the control group level with the administration of the reference substance, but 2.62 times lower than the value for intact animals (11.18 ± 1.22) $\cdot 10^9$/L of blood. In the group taking the compound 3, the relative count of segmented neutrophils was (36.4 ± 0.7) % with an absolute index (1.56 ± 0.23) × 10^9/l of blood, which correlated with the control group (1.84 ± 0.13) $\cdot 10^9$/L and higher counts for the intoxication group – (0.04 ± 0.04) $\cdot 10^9$/L blood by 46 times ($p \leq 0.01$). The relative lymphocyte count after administration of compound 3 recovered to (45.4 ± 0.6) % from the value during the period of intoxication (8.9 ± 0.2) %, i.e., 5.1 times ($p \leq 0.05$). The absolute lymphocyte count increased to (1.93 ± 0.15) $\cdot 10^9$/L of blood, higher than for the control group (0.23 ± 0.11) $\cdot 10^9$/L of blood, but below the counts for the intact group (6.93 ± 0.89) $\cdot 10^9$/L of blood (3.59 times). The next compound to stimulate the total leukocyte count restoration rate was compound 7. It restored the total leukocyte count to (3.58 ± 0.11) $\cdot 10^9$/L of blood. But it was 1.2 times lower than that of the control group (4.37 ± 0.52) $\cdot 10^9$/L of blood. But recovery of the relative lymphocyte count was not observed (Table 3).

The relative lymphocyte count was (29.8 ± 0.9) % – below the count of intact animals (62.0 ± 0.1)% by 2.08 times and below the count of animals with the administration of compound 3 (45.4 ± 0.6) %. The segmented neutrophils increased more intensively. It was (38.7 ± 0.8) % in the group taking the compound 7 with a relative count of (1.39 ± 0.04) $\cdot 10^9$/L of blood and was comparable to that of the group taking the compound 3 (36.4 ± 0.7)% with the relative count (1.56 ± 0.11) $\cdot 10^9$/L of blood.

Compounds 1, 4, 5 did not have leukopoiesis-stimulating activity. Animals administered with compound 2 died on the 14-32 day of observation after the start of the experiment.

Table 2 – Leukogram peripheral blood of rats

Number of compounds	Leukocytes $\cdot 10^9$/L	Myelocytes $\cdot 10^9$/L %	Metamyelocytes $\cdot 10^9$/L %	Neutrophils $\cdot 10^9$/L %		Eosinophils $\cdot 10^9$/L %	Basophils $\cdot 10^9$/L %	Lymphocytes $\cdot 10^9$/L %	Monocytes $\cdot 10^9$/L %
				banded	segmented				
1	2.63±0.92	0.07±0.00 2.8±0.2	0.11±0.01 4.0±0.1	0.37±0.11 14.2±0.3	1.00±0.18 38.2±1.5	0.04±0.00 1.5±0.1	0.03±0.00 1.3±0.5	0.68±0.09 26.0±0.7	0.32±0.03 12.0±0.1
2	1.82±0.33	0.02±0.00 1.2±0.2	0.05±0.00 3.2±0.5	0.33±0.29 18.0±0.9	0.74±0.16 40.6±1.7	0.04±0.00 2.2±0.2	0.04±0.00 2.2±0.08	0.37±0.08 20.4±0.5	0.22±0.00 12.2±0.2
3	4.26±0.15	0.04±0.00 0.9±0.01	0.12±0.00 2.9±0.1	0.36±0.43 8.4±0.7	1.56±0.23 36.4±0.7	0.09±0.00 2.2±0.3	0.06±0.00 1.5±0.6	1.93±0.05 45.4±0.6	1.00±0.03 2.3±0.2
4	2.68±0.28	0.05±0.00 2,0±0,01	0.11±0.01 4.2±0.1	0.34±0.24 12.8±0.7	1.13±0.7 42.2±0.5	0.08±0.00 2.8±0.3	0.05±0.00 1.7±0.6	0.60±0.01 22.4±1.8	0.32±0.02 11.9±0.4
5	2.70±0.32	0.03±0.00 1.0±0.1	0.07±0.01 2.5±0.2	0.35±0.69 12.8±0.2	1.08±0.68 40.1±0.5	0.05±0.00 1.7±0.1	0.03±0.00 1.0±0.09	0.77±0.08 28.4±2.4	0.34±0.02 12.5±0.3
7	3.58±0.33	0.05±0.00 1.4±0.1	0.08±0.00 2.3±0.1	0.44±0.03 12.4±0.3	1.39±0.14 38.7±0.8	0.05±0.00 1.5±0.3	0.07±0.00 2.0±0.7	1.07±0.01 29.8±0.9	0.43±0.04 11.9±0.2
control	4.37±0.52	0.12±0.00 2.8±0.2	0.22±0.00 5.0±0.3	0.73±0.03 16.8±0.9	1.84±0.13 42.0±1.3	0.10±0.00 2.4±0.1	0.04±0.00 1.0±0.01	0.23±0.00 28.2±0.5	0.08±0.00 1.8±0.1
intact	11.18±1.22	0 0	0 0	0.58±0.04 5.2±0.3	2.33±0.25 20.8±0.9	0.46±0.03 4.1±0.9	0.20±0.02 1.8±0.1	6.93±0.09 62.0±0.1	0.68±0.00 6.1±0.5
placebo	1.90±0.51	0.10±0.02 5.3±0.2	0.13±0.01 7.0±0.1	0.27±0.03 14.1±0.1	0.78±0.69 40.9±0.6	0.04±0.00 2.0±0.1	0.03±0.00 1.8±0.2	0.17±0.03 8.9±0.2	0.38±0.00 20.0±0.5

Note: the numerator is the total number of cells in 1 µl. of blood the denominator is the relative cell content in%.

Table 3 – The content of leukocytes and lymphocytes in the peripheral blood of rats is normal, after lead salt intoxication and treatment

Number of compounds	Leukocytes, $\cdot 10^9$/L	Lymphocytes, $\frac{\cdot 10^9/L}{\%}$	CD3 $^+$-typed, $\frac{\cdot 10^9/L}{\%}$	CD8 $^+$-typed, $\frac{\cdot 10^9/L}{\%}$	CD20 $^+$-typed, $\frac{\cdot 10^9/L}{\%}$	CD4 $^+$-typed, $\frac{\cdot 10^9/L}{\%}$
1	2.63±0.92	$\frac{0.68\pm0.49}{26.0\pm0.7}$	$\frac{0.11\pm0.00}{16.2\pm0.2}$	$\frac{0.21\pm0.00}{31.1\pm1.23}$	$\frac{0.11\pm0.00}{16.9\pm0.71}$	$\frac{0.14\pm0.00}{21.0\pm0.35}$
2	1.82±0.33	$\frac{0.37\pm0.18}{20.4\pm0.5}$	$\frac{1.85\pm0.00}{9.1\pm0.2}$	$\frac{5.12\pm0.00}{25.1\pm1.1}$	$\frac{8.26\pm0.00}{11.1\pm0.2}$	$\frac{8.37\pm0.00}{16.5\pm0.32}$
3	4.26±0.15	$\frac{1.93\pm0.15}{45.4\pm0.6}$	$\frac{0.31\pm0.00}{16.2\pm0.4}$	$\frac{0.98\pm0.00}{50.9\pm0.7}$	$\frac{0.45\pm0.00}{23.5\pm0.4}$	$\frac{0.47\pm0.00}{24.5\pm0.9}$
4	2.68±0.28	$\frac{0.60\pm0.11}{22.4\pm1.8}$	$\frac{0.06\pm0.00}{10.0\pm0.04}$	$\frac{0.29\pm0.00}{48.8\pm0.65}$	$\frac{0.06\pm0.00}{10.4\pm0.3}$	$\frac{0.18\pm0.00}{30.5\pm0.7}$
5	2.70±0.32	$\frac{0.77\pm0.18}{28.4\pm2.4}$	$\frac{0.08\pm0.00}{10.8\pm0.6}$	$\frac{0.21\pm0.00}{27.4\pm0.2}$	$\frac{0.07\pm0.00}{9.4\pm0.6}$	$\frac{0.16\pm0.00}{20.4\pm0.3}$
7	3.58±0.33	$\frac{1.07\pm0.98}{29.8\pm0.9}$	$\frac{0.12\pm0.00}{11.3\pm0.91}$	$\frac{0.53\pm0.00}{49.6\pm1.64}$	$\frac{0.07\pm0.00}{6.3\pm0.27}$	$\frac{0.39\pm0.00}{36.4\pm0.33}$
levamisole	4.37±0.52	$\frac{0.67\pm0.11}{28.2\pm0.5}$	$\frac{0.06\pm0.00}{26.2\pm2.2}$	$\frac{0.07\pm0.00}{30.7\pm1.93}$	$\frac{0.05\pm0.00}{21.8\pm0.8}$	$\frac{0.05\pm0.00}{23.3\pm0.4}$
intact	11.18±1.22	$\frac{6.93\pm0.89}{62.0\pm0.1}$	$\frac{0.64\pm0.00}{9.19\pm0.13}$	$\frac{1.82\pm0.00}{26.3\pm0.85}$	$\frac{0.81\pm0.00}{11.72\pm0.48}$	$\frac{1.05\pm0.00}{15.21\pm0.21}$
placebo	1.90±0.51	$\frac{0.17\pm0.03}{8.9\pm0.2}$	$\frac{0.03\pm0.00}{17.3\pm0.4}$	$\frac{0.11\pm0.00}{63.0\pm0.9}$	$\frac{0.03\pm0.00}{15.8\pm0.08}$	$\frac{0.07\pm0.00}{38.5\pm0.4}$

Note: the numerator is the total number of cells in 1 μl. of blood the denominator is the relative cell content in%.

Analysis of lymphocytic subpopulations showed the following: In case of intoxication, the lymphocytes have decreased from the count for intact animals (6.99 ± 0.89) ·10^9/L of blood to (0.17 ± 0.03) ·10^9/L of blood by 40.76 times. But the subpopulation composition was changed towards an increase in the subpopulation of $CD8^+$ defined to (63.0 ± 0.9)% and $CD4^+$ defined to (38.5 ± 0.4) %.

The rest of the subpopulation counts were not high. After administering the test compounds, the following results were obtained: The most effective compound was the compound 3. The lymphocytes have increased from (0.17 ± 0.03) ·10^9/L of blood to (1.93 ± 0.15) ·10^9/L of blood, i.e., 11.35 times. The number of $CD8^+$ -defined cells was (50.9 ± 0.7) %, with a uniform distribution of $CD20^{+-}$ defined cells up to (23.5 ± 0.4) % and $CD4^+$ – defined cells (24.5 ± 0.9) %. Smaller count of lymphocytes was recorded in animals treated with compound 4. The absolute lymphocyte count was (0.60 ± 0.11) ·10^9/L of blood. The relative count of $CD8^+$ defined cells was high (48.8 ± 0.65) %. Also at a high level was the count of $CD4^+$ -defined cells (30.5 ± 0.7) %. Although the absolute count of $CD20^+$ defined cells was low and amounted to (10.4 ± 0.3) %. The count of $CD3^+$ defined cells was also not high and amounted to (10.0 ± 0.04) %. In the group with administering the compound 7, the level of $CD20^+$ defined cells was very low (6.3 ± 0.27) %, and the $CD3^+$ defined cells were (11.3 ± 0.91) % – not very high. Count of $CD8^+$ defined cells was high (49.6 ± 1.64) % and the $CD4^+$ defined cells too (36.4 ± 0.33) %. An almost uniform distribution of lymphocytic subpopulations was in the groups with methyluracil administration.

The absolute count was (0.23 ± 0.11) ·10^9/l of blood. The relative counts of CD3+ – and CD20+ defined cells was (26.2 ± 2.2)% and (21.8 ± 0.8)%, respectively. The level of CD8+ defined cells was higher, amounting to (30.7 ± 1.93)%, and slightly lower was the level of CD4+ defined cells – (23.3 ± 0.4)%.

Conclusion

1. The new piperidine-containing derivatives, related to mono- and bicyclic azaheterocycles are promising for the search new effective immunostimulating drugs.

2. Spirojunction of piperidine and imidazoline rings in one molecule turned out to be the most successful for immunostimulating activity, 1-(3-n-butoxypropyl) piperidine-3-methyl-4-spiro-5'-imidazolidine-2',4'-dione exceeds the activity in 3,1 time and has a lower toxicity of the reference drug levamisole.

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