IRSTI 34.45.21; 34.43.35

[1*]L.K. Baktybayeva, [1]M.K. Tauassarova, [2]B.K. Darrell, [3]V.K. Yu, [3]A.G. Zazybin

[1]al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Oklahoma State University, Center for Health Sciences, Stillwater, Oklahoma City, USA
[3]A.B. Bekturov Institute of Chemical Sciences, Almaty, Kazakhstan;
Kazakh-British Technical University, School of Chemical Engineering, Almaty, Kazakhstan
[*]e-mail: Layilia.Baktybaeva@kaznu.kz

Immunostimulating properties of the azaheterocyclic compounds BIV-3, BIV-4, BIV-7

Abstract: In animals and humans, immune system performs an important function to maintain the constancy of the body internal environment, carried out by recognizing and eliminating alien substances of antigenic nature from the body. This immune system function is carried out with the congenital and acquired immunity factors. Different types of radiation, heavy metal salts, vitamin and micronutrient deficiency, stressful situations, age-related changes in the lympho-myeloid complex, therapy with anti-tuberculosis, antibacterial, hormonal, cytostatic drugs and a number of other factors lead to the development of immune diseases. These diseases can be treated with a set of immunotherapy methods; use of immunostimulants is one of them. Today, immunostimulants are distinguished as of microbial, thymic, bone marrow, cytokine, nucleic, plant and synthetic origin. Azaheterocyclic compounds comprise a class of compounds that have demonstrated significant biological activities against various human diseases. We suggest that azaheterocyclic compounds with a piperidine nucleus are perspective for the search for new effective immunostimulating drugs. To study their immunostimulating activity, the following compounds were taken: **BIV-3** – 1-(3-butoxypropyl)-3-methylpiperidine 4-spiro 5'-imidazolidine-2',4'-dione, **BIV-4** – 1-(2-ethoxyethyl)-4-hydroxy-4-dimethoxyphosphorylpiperidine, **BIV-7** – complex of 3-(2-morpholinoethyl)-7-(3-isopropoxypropyl)-3,7-diazabicyclo [3.3.1] nonane with β-cyclodextrin. The comparison drug was methyluracil. Results of the studies are presented in this paper.
Key words: azaheterocyclic compounds, immunostimulating properties.

Introduction

In animals and humans, immune system performs an important function to maintain the constancy of the body internal environment, carried out by recognizing and eliminating alien substances of antigenic nature from the body. This immune system function is carried out with the congenital and acquired immunity factors. Different types of radiation, heavy metal salts, vitamin and micronutrient deficiency, stressful situations, age-related changes in the lympho-myeloid complex, therapy with anti-tuberculosis, antibacterial, hormonal, cytostatic drugs and a number of other factors lead to the development of immune diseases. These diseases can be treated with a set of immunotherapy methods; use of immunostimulants is one of them.

Today, immunostimulants are distinguished as of microbial, thymic, bone marrow, cytokine, nucleic, plant and synthetic origin. Many immunostimulants have a wide range of side effects. For example, a naturally occurring drug sodium nucleinate is widely used to stimulate the bone marrow cells proliferation [1]. Sodium nucleinate is able to stimulate both congenital and acquired immunity factors. All drugs from the group of nucleic acids are very effective inducers of interferon. Based on sodium nucleinate a number of synthetic drugs have been developed: poludan – polyadenylic-uridine acid complex; inosine pranobex (isoprinosine) – a complex of inosine with acetylamido-benzoic acid; methyluracil and riboxine – a complex compound consisting of hypoxanthine – riboside. Methyluracil is the most accessible and widely used synthetic immunostimulator [2]. We should remember that both synthetic and natural nucleic acid drugs containing precursors for the synthesis of DNA and RNA chains induce growth and multiplication of both eukaryotic and prokaryotic cells. Therefore, despite their strong immunostimulating properties, due to stimulation of growth and

reproduction of malignant, bacterial and other cells, many sodium nucleinate derivatives are prohibited in many countries of the world. Immunostimulants of bone marrow origin are known by high cost due to the unique ingredients and cause severe allergic diseases. Cytokine origin drugs can cause a non-controlled inflammatory reaction up to a fatal outcome. There is a continuous search for new effective immunostimulants. Among them, we should mention polyoxidonium, immunophane, immunomax and other. Laboratory of Pharmaceutical Chemistry of A.B. Bekturov Institute of Chemical Sciences monitors new synthetic immunostimulants in Kazakhstan. The laboratory staff has accumulated vast experience in the field of synthesis and chemical transformations of 4-oxopiperidines, have obtained new data, which allows making important conclusions about the relationship between the fine chemical structures of synthesized compounds and their reactivity, spectral characteristics and biological activities. Drugs with high anesthetic, antiarrhythmic, anti-allergic and other types of activity have been found, which have significant advantages over common medical products. In addition, the laboratory is engaged in the search for new immunostimulants. Stimulus for search among the piperidine derivatives was the manifestation of immunostimulating activity by prosedol drug, which is a derivative of piperidine and was obtained in this laboratory for the first time [3].

Thus, **the purpose** of our research was to study effect of BIV-3, BIV-4, BIV-7 on leukopoiesis and oxygen-dependent links of neutrophil defense reactions and adhesive properties of peritoneal macrophages.

Materials and methods

To study immunostimulating activity, the following compounds were taken: **BIV-3** – 1-(3-butoxypropyl)-3-methylpiperidine 4-spiro 5'-imidazolidine-2',4'-dione, **BIV-4** – 1-(2- ethoxyethyl)-4-hydroxy-4-dimethoxyphosphorylpiperidine, **BIV-7**-complex of 3-(2-morpholinoethyl)-7-(3-isopropoxypropyl)-3,7-diazabicyclo [3.3.1] nonane with β-cyclodextrin. The comparison drug was methyluracil [2]. Immunostimulating activity of the compounds was determined by ability of the test compounds to stimulate leukopoiesis to normal values of the blood leukogram and the phagocytic ability of the granulocytes were evaluated. Evaluation of the monocytes and granulocytes absorption function does not give an idea of the phagocytosis stages process peculiarities, which ensure the killing the of phagosome content. To assess the intracellular microbicidal nature of phagocytic cells, indirect methods are widely used to evaluate the production of superoxide radicals, in particular, the reduction test for nitro blue tetrazolium (NBT). It is also important to assess the adhesive ability of the phagocytic cells. The adhesion of a phagocyte to a foreign object serves as a trigger mechanism for the development of subsequent stages of the phagocytic process – antigen uptake, when the particle is captured and immersed in the cytoplasm of the phagocyte for intracellular disintegration of the absorbed particle.

Healthy adult laboratory mice of both sexes, 8-10 weeks of age, weighing 18-20 g were used to determine acute toxicity. The deviation in groups by the initial body weight did not exceed ± 10%. All experiments held at the same time of day (from 9:00 am). Immunostimulating activity was studied on laboratory mature adult albino rats of both sexes, 10-15 weeks of age with a body weight of 210-280 g, 48 individuals. All animals were obtained at the same time from the vivarium of laboratory animals at the Faculty of Biology and Biotechnology of the al-Farabi Kazakh National University. Studies were conducted in accordance with the current "Rules for preclinical (nonclinical) studies of biologically active substances" [4]. All animals were in the same habitat conditions (wood litter from sawdust, room temperature 22-24°C, 12-hour light) and feeding (standard briquetted food). Laboratory rats were divided into 6 groups of 8 individuals. The 6th group of animals was intact. No compounds were administered to animals of the intact group. All other experimental groups of animals were injected with sodium cyclophosphamide (OJSC "Kievmedpreparat", Ukraine, powder for solution for injection): intramuscularly at a dose of 30 mg/kg in saline solution of 0.5 ml, three times with a daily interval. On the 6^{th}, 8^{th} and 10^{th} day of the experiment the 1-5th group of animals was intramuscularly injected: 1st, 2nd, 3rd – at a dose of 1/10 LD_{50} of BIV-3, BIV-4, BIV-7 in saline in the volume of 0.5 ml intramuscularly, the fourth – in 0.5 ml (0.4 mg/kg) solution of methyluracil (control group), the 5th – saline 0.5 ml (placebo group). The test chemical compounds, the reference drug and saline were administered 3 times at a daily interval. Blood sampling was carried out from the orbital vein of rats on the 12^{th} day of the experiment, anesthetized with mild ether anesthesia at 09:00 am. The blood was tested on a hematological analyzer for laboratory animals "Abacus junior VET" (Diatron, Denmark). To ensure a double cytological control the blood smears were made to evaluate leukogram. Blood smears were

stained by Romanowsky-Giemsa, and the SA3300C microscope under the immersion (magnification 7x100) was counted on 100 cells per each smear, then the relative amount of each cell type was recalculated into an absolute value [5; 6]. The statistical processing of the data was carried out using Student's *t*-test.

Phagocytic, microbicidal and adhesive activity was done according to the generally accepted method of studying the spontaneous NBT test with the reduction of nitro blue tetrazolium in diformazan [7]. The reaction results were evaluated by an immersion objective (magnification 7x100) on a SA3300C microscope. The percentage of cells having diformazan in the form of granules or solid deposits was detected. The mean cytochemical coefficient (MCC) was calculated by G.Astaldi and L.Verg formula. Evaluation of adhesive properties was performed by a standard method on rat peritoneal mast cells. The index of adhesion of peritoneal macrophages (IA-PM) was equal to the percentage of cells attached to the glass Goryaev's chamber filled with Tyrode's solution in a Petri dish. The number of cells on the Goryaev's chamber was counted on a SA3300C microscope under an amplification of 7x10x40 [7].

The biological activity and acute toxicity of new compounds were compared with the data of the reference drug methyluracil.

Results and discussion

Results of chemical studies

Saturated azaheterocycles polyfunctional derivatives, especially piperidines, as one of the most promising families of chemical compounds because of their high pharmacological potential and simple synthesis, are subject of Research of the Pharmaceutical companies and Universities. Introduction into the piperidine molecule another pharmacophoric fragments leads to new biological activities. A functionalization of N-alkoxyalkyl piperidin-4-ones is carried out by us. For synthesis of spiro bicyclic having two-pharmacophore cycle – hydantoin and N-alkoxyalkylpiperidines Bucherer-Bergs reaction had been choosed. Interaction of N-alkoxyalkyl piperidin-4-ones with sodium cyanide and ammonium carbonate in an aqueous alcoholic solution is carried out in a single stage in sealed vials at 75°C for 4 hours. Target 1-(3-Butoxypropyl)-3-methylpiperidine hydantoin (I [8], BIV-3) was obtained in a yield of 40% yield, m.p.102-106°C.

Introduction of phosphate moiety leads to the appearance of new biological properties. α-Oxiphosphonates easily were obtained by interaction of N-alkoxyalkyl piperidin-4-ones with dimethylphosphite in the presence of sodium methylate in hexane. 1-(2-Ethoxyethyl)-4-(dimethylphosphoryl)-4-hydroxypiperidine (II [9], BIV-4) had been prepared, yield 93%, m.p. 112-113°S.

Simultaneous condensation of N-alkoxyalkyl piperidin-4-ones with paraformaldehyde and (2-morpholinoethyl) amine in methanol + acetic acid led to corresponding 3,7-diazabicyclo [3.3.1] nonane-9-ones with a yield of 40-60%. To search of potential BAS reduction of 3,7-diazabicyclo [3.3.1] nonane-9-one in a Wolff-Kishner reaction by hydrazine hydrate in triethylene glycol in the presence of KOH gave the corresponding 3,7-diazabicyclononane in a yield of 57%. Complex of 3-(3-i-propoxypropyl)-7-(2-morpholinoethyl)-3,7-diazabicyclo [3.3.1] nonan with β-cyclodextrin [10] is BIV-7.

Results of biological studies

1. New synthetic drugs acute toxicity indicators

In general, all compounds of the study series showed a very low acute toxicity index. In accordance with the received acute toxicity indicators, only one investigated BIV-3 compound can be attributed to the compounds of the first toxicity group. Its acute toxicity was 446.68 ± 3.24 mg/kg, but the BIV-3 compound was less toxic than the comparative drug methyluracil 1.12 times. The acute toxicity level of the BIV-7 compound was more than 500 mg/kg and this compound can be attributed to compounds with moderate acute toxicity. Acute toxicity of the BIV-7 compound was 790.68 ± 8.74 mg/kg and was less than that of the reference preparation in 1.99 times. The least acute toxicity was shown by BIV-4 compound. It can be attributed to low-toxic compounds. The acute toxicity of the BIV-4 compound was more than 1000 mg/kg and was 2.51 times less than the acute toxicity of the methyluracil comparative drug (Table 1). All test compounds were less toxic than the methyluracil [8].

2. Screening results of immunostimulating activity of new synthetic compounds

In order to investigate the immunostimulating activity of compounds in animals, it was necessary to induce an artificial immunosuppressive syndrome. The control values obtained from intact animals admitted to the experiment were within the physiological norm. The leukocyte count (9.83 ± 1.51) · 10^9/L blood, with lymphocyte (5.40 ± 1.1) · 10^9/L blood, which was (56.9 ± 0.9)%. Granulocytic leukocytes had a value (3.64 ± 0.9) · 10^9/L of blood with a percentage ratio in the blood leukogram of 37.0 ± 1.4%. The monocyte index (0.7 ± 0.0) · 10^9/L of blood was minimal, which according to the leukogram was (7.0

± 0.4)%. Also, the numbers of erythrocytes and platelets were normal. The erythrocyte index was (7.8 ± 1.4) · 10^{12}/L blood with a hemoglobin content (158.7 ± 1.2) g/L blood and accordingly a hematocrit (39.8 ± 1.9)%. The platelet score was also normal (350.6 ± 3.6) · 10^{9}/L of blood and thrombocrit was (12.6 ± 0.3)%. In general, the main blood indicators obtained from the animals during experiment were normal.

Table 1 – Acute toxicity of the compounds

No.	Compound name	Acute toxicity index (LD_{50}), mg/kg	The acute toxicity index (LD_{50}) relative to the methyluracil
1	Methyluracil	398.24 ± 0.52	1
2	BIV-3	446.68 ± 3.24	1.12
3	BIV-4	> 1000 mg / kg	>2.51
4	BIV-7	790.68 ± 8.74	1.99

Directed immunosuppressive effect of sodium cyclophosphamide led to a mielodepression effect with impairment of blood counts on the first day after injection. The total leukocyte count was (4.15 ± 1.2) · 10^{9}/L of blood, i.e. with a decrease of 2.19 times ($p \leq 0.05$) and on the 3rd day after injection, the level of leukocytes was (2.69 ± 0.54) · 10^{9}/L of blood, which was a 3.40-fold decrease in comparison with control ($p \leq 0.01$). According to the blood leukogram, significant negative changes can be noted in the cell pools of lymphocytes, granulocytes, and monocytes. The parameters of immunocompetent cells-lymphocytes from the control value (5.50 ± 1.1) · $10^{9/L}$ of blood decreased on the 1st day to (2.46 ± 0.75) · 10^{9}/L of blood and reached the 3rd day (1.99 ± 0.18) · 10^{9}/L of blood, i.e. in 2.74 times ($p \leq 0.05$) (Table 2).

Changes that are even more significant occurred in the cellular populations of granulocytes. The level of granulocytic leukocytes of blood decreased from 3.64 ± 0.9 ·10^{9}/L to (1.33 ± 0.18) · 10^{9}/L in 1st day, i.e. in 5.28 times ($p \leq 0.01$). Decrease in granulocytes in 3 days after injection of cyclophosphamide was (23.75 ± 8.55)%, i.e. in 1.68 times. A considerable decrease in the absolute granulocyte count (5.28 times) compared to a moderate decrease in the relative value of granulocytic leukocytes (1.68 times) can be explained by a substantial impairment in the overall leukocyte index, which affected the absolute values of the cells in the blood (Table 2).

A certain increase in monocytes was observed on the first day after the injection of cyclophosphamide, which can be explained by the mass death of cells and an increase in the functional load. The index of intact animals (7.0 ± 0.4)% on the first day after the injection of cyclophosphamide became (7.05 ± 4.6)%, but on the third day after injection it fell to (0.6 ± 0.0%), i.e. 10 times compared to control value ($p \leq 0.01$) (Table 2).

Table 2 – Animals blood leukogram after intoxication

Parameter	General leukocyte rate (WBC, · 10^{9}/L)	Abs. number of granulocytes (GRA, · 10^{9}/L) relative indicators granulocytes (GR, %)	Abs. number of lymphocytes (LYM, · 10^{9}/L) relative indicators lymphocytes (LY, %)	Abs. number of monocytes (MID, · 10^{9}/L) relative indicators monocytes (MI, %)
Intact animals	9.83±2.51	3.64±0.9 37.0±1.4	5.50±1.1 56.9±0.9	0.7±0.0 7.0±0.4
Immunodepression, 1st day	4.15±1.2	1.33±0.18 33.55±4.45	2.46±0.75 58.95±0.85	0.36±0.28 7.05±4.6
Immunodepression, 3rd day	2.69±0.54	0.685±0.355 23.75±8.55	1.99±0.18 75.65±8.55	0.015±0.005 0.6±0.0

No significant changes were observed in red blood cells as it was in the leukocyte cell populations. Some fluctuations of erythrocyte cells from (6.5 ± 1.4) · 10^{12}/L, decrease in the first day to (4.71 ± 1.37) · 10^{12}/L by 1.38 times and a slight increase to (5.80 ± 0.27) · 10^{12}/L on the third day after the injection of cyclophosphamide was noticed. Also, the same pattern of fluctuations on the first day and the third day after the injection of sodium cyclophosphamide was observed in the values of hemoglobin, hematocrit, the average volume of erythrocytes, the average hemoglobin content in erythrocyte cells, and

the erythrocytes distribution amplitude. Decrease in indices 1.2-1.6 times was observed on the 1st day. Significant changes were recorded in platelet counts, which naturally affects the values of platelet crit count, the average platelet count and the amplitude of platelet distribution. On the first day after the injection of sodium cyclophosphamide, platelet levels fell to (245.0 ± 126.0) · 10^9/L of blood, when the value for intact animals (350.0 ± 32.2) · 10^9/L of blood, i.e. 1.43 times (Table 3).

By the 3rd day after injection, the platelet count decreased to (74.5 ± 39.5) · 10^9/L of blood from the baseline value (350.0 ± 32.2) ·10^9/L of blood, i.e. 4.7 times ($p \leq 0.05$). This indicator is critical and characterized by spontaneous abdominal hemorrhage and other hemophilic disorders.

Thus, it shows that cyclophosphamide caused myelo suppression and the most sensitive cells were leukocyte cells and platelets. Lymphocytes, granulocytes and further monocytes were killed first among the leukocytic cells. Then animals having myelo depressive syndrome, were injected with azaheterocyclic compounds under the code "BIV". Based on the acute toxicity index, the therapeutic dose of the new compounds was calculated: TD_{50} (BIV-3) = 4.5 mg/kg; TD_{50} (BIV-4) = 10.0 mg/kg; TD_{50} (BIV-7) = 8.0 mg/kg, which was used. The therapeutic dose of the standard compound was TD_{50} (methyluracil) = 4.0 mg/kg.

After the treatment with the immunostimulating compound with methyluracil and new synthetic compounds of the BIV series, the following results were obtained. The BIV-3 compound showed the highest activity, it increased the total leukocyte count to (7.31 ± 1.54) ·10^9/L and exceeded the comparison drug 2.6 times. The overall leukocyte count returned to normal, the leukocyte formula was almost back to normal. The number of myelocytes decreased to 0.9%, metamyelocytes – 2.9%, stab neutrophils – 8.4%. There was intensive recovery of granulocytes. The next in immunostimulating activity scale was the BIV-7 compound. It was inferior in activity to the BIV-3 compound, but it was more active than BIV-4 and the reference drug 1.3 times. After the treatment, the BIV-7 compound increased the overall leukocyte count 1.9 times to 3.69 ± 0.31 × 10^9/L compared to the control group (Table 3).

Table 3 – Animals blood leukogram after injection of the test compounds, where: numerator is the total number of cells in 1μl of blood, while denominator is the relative content of cells in %

Parameter	General leukocyte index (WBC, ·10^9/L)	Abs. number of granulocytes (GRA, · 10^9/L) relative indicators granulocytes (GR, %)	Abs. number of lymphocytes (LYM, · 10^9/L) relative indicators lymphocytes (LY, %)	Abs. number of monocytes (MID, · 10^9/L) relative indicators monocytes (MI, %)
Intact group	9.83±2.51	3.64±0.93 37.01±1.43	5.50±1.10 56.9±0.98	0.70±0.00 7.0±0.41
Immunodepression	2.69±0.54	0.69±0.41 23.76±8.55	1.99±0.18 75.65±6.23	0.02±0.01 0.61±0.00
BIV-3	7.31±1.54	3.39±0.36 46.44±0.35	2.58±0.11 35.46±2.35	1.33±0.01 18.24±1.25
BIV-4	2.77±0.34	1.41±0.12 52.24±4.22	0.76±0.01 28.22±2.52	0.53±0.00 19.66±1.81
BIV-7	3.69±0.31	1.75±0.42 48.73±6.23	1.07±0.01 29.84±3.54	0.77±0.00 21.52±5.35
Control group	2.81±0.54	1.19±0.23 42.44±3.29	1.26±0.01 44.99±3.65	0.36±0.00 12.71±1.23
Placebo group	1.91±0.54	1.16±0.87 60.92±6.28	0.17±0.00 8.95±1.29	0.57±0.00 30.23±4.57

Similarly, as after treatment with the previous compound in the peripheral blood, a small amount of myelocytes and metamyelocytes was observed. BIV-4 compound showed activity comparable to the activity of the methyluracil. The overall leukocyte count was (2.77 ± 0.34) ·10^9/L. Thus, the newly synthesized BIV-3 compound exceeded the leucopoiesis stimulating activity of methyluracil. Cyclophosphamide intoxication led to the acquired immunosuppressive syndrome with a drop in the NBT-indicator to 1.09

± 0.01 assorted units; with an average cytochemical coefficient of 0.49 ± 0.01 conventional units, and the adhesion index of peritoneal macrophages 5.21 ± 0.04% [2].

NBT-test evaluates the functional state of neutrophils. It reflects the degree of activation of oxygen-dependent metabolism, primarily the hexomonophosphate shunt and the associated production of free oxygen radicals. When assessing the NST-test, the maximum index was for the BIV-7 compound, 7.02 ± 0.01 standard units, against the control 1.09 ± 0.01 conventional units ($p \leq 0.05$), exceeding methyluracil by 3.36 times and compound BIV-3 by 3.49 times. The compound showed a comparable high activity, but was inferior to the BIV-4 compound. The highly active BIV-4 compound exceeded the BIV-7, BIV-3 and methyluracil compounds in activity 1.73, 3.49 and 3.36 times, respectively (Figure 1, a).

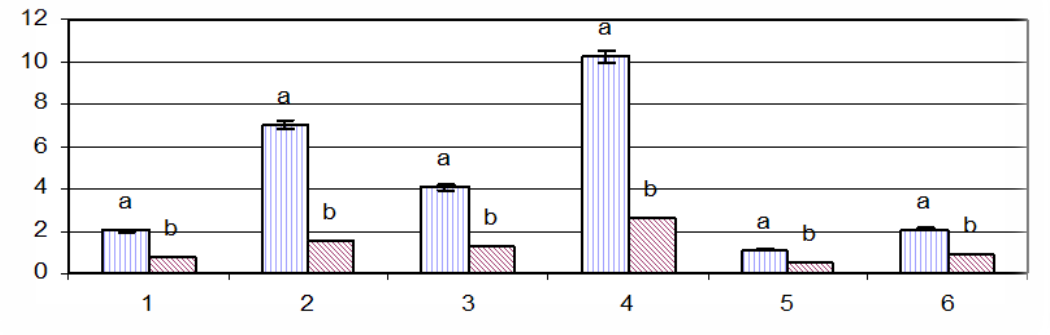

Figure 1 – Effect of compounds on the NST-indicator (a) and MCC-coefficient (b) neutrophils in peripheral blood of rats. On the abscissa axes – BIV-3 (1), BIV-4 (2), BIV-7 (3), intact (4), control (5), methyluracil (6); on the axes of ordinates – NBT-indicator and MCC-coefficient, standard units

To reflect the degree of activity of the enzyme systems of phagocytic neutrophils, the mean cytochemical coefficient (MCC) was calculated. MCC reflects the energy processes that ensure the production of bio-oxidants with bactericidal action. In this connection, the MCC in the spontaneous NBT test serves as an additional criterion for evaluating the bactericidal activity of neutrophils. According to the MCC, the BIV-4 compound had a maximum value of 1.53 ± 0.002 units, exceeding the metryluracil 1.66 times, BIV-3 compounds 1.91 times. A BIV-7 compound, comparable in value to the BIV-4 compound, was similar (Figure 1, b).

Effect of compounds on the adhesion index of peritoneal macrophages is presented on Figure 2.

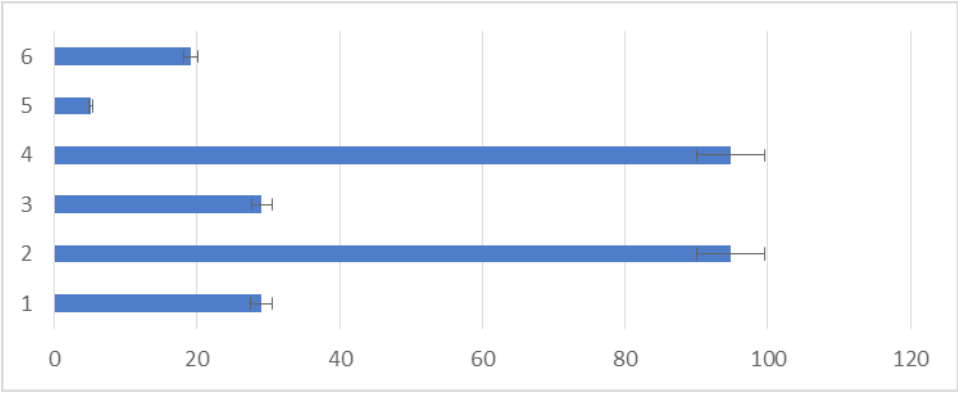

Figure 2 – Effect of compounds on the adhesion index of peritoneal macrophages. The ordinate is the BIV-3 (1), BIV-4 (2), BIV-7 (3), intact (4), placebo (5), control (6); on the axis of abscissa – the index of adhesion index, % of active cells

According to the adhesion index, the best result was shown by BIV-4 compound, 94.81 ± 0.07%, against the control 5.21 ± 0.04% ($p \leq 0.05$), which exceeded the control value 18.2 times and methyluracil 4.46 times. The IA-PM of the BIV-4 compound was almost identical to that of the intact animals, i.e. BIV-4 in a 10-day time interval restored the adhesive activity of cells of animals subjected to immunosuppressive stress.

Conclusion

1. Azaheterocyclic compounds with a piperidine nucleus are perspective for the search for new effective immunostimulating drugs. They have high immunostimulating activity and low acute toxicity.

2. The **BIV-3** – 1-(3-butoxypropyl)-3-methylpiperidine-4-spiro-5'-imidisolidine-2',4'-dione compound stimulates leukopoiesis and has low toxicity.

3. **BIV-4** – 1-(2-ethoxyethyl)-4-hydroxy-4-dimethoxyphosphorylpiperidine and **BIV-7** complexes of 3-(2-morpholinoethyl)-7-(3-isopropoxypropyl)-3,7-diazabicyclo [3.3.1] nonane with β-cyclodextrin stimulate intracellular microbicidal activity, the effectiveness of enzyme systems and the adhesive properties of phagocytic cells and are characterized by low toxicity.

References

1. Khaitov R.M., Pinegin B.V. (2003) Immunomodulators: the mechanism of action and clinical application. *Immunology*, vol. 24, no. 4, pp.196-203.

2. Mashkovsky M.D. (1993) Preparations, correcting the processes of immunity (immunomodulators, immunocorrectors). In the book: Medicines (Manual for doctors), Part II. pp. 192-209.

3. Praliev K.D., Yu V.K., Fomicheva E.E., Baktybayeva L.K., Svambaev E.A., Tuleukhanov S.T. (2007) Immunostimulants of the N-alkoxyalkyl piperidine series. *Chemical Journal of Kazakhstan*, no. 2, pp. 180-187.

4. Order of the Minister of Health of the Republic of Kazakhstan No. 745 from November 19, 2009 "On approval of the Rules for preclinical (non-clinical) studies of biologically active substances"

5. Giemsa G. (1904) Eine Vereinfachung und Vervollkommnung meiner Methylenazur-Methylenblau-Eosin-Färbemethode zur Erzielung der Romanowsky-Nochtschen Chromatinfärbung. *Centralbl f Bakt etc*., no. 37, pp. 308-311.

6. Bezrukov A.V. Coloring by Romanowsky: to the question of priority / To the 120th anniversary of the discovery of the Romanowsky effect, p. 12.

7. Khaitov R.M., Pinegin B.V., Latysheva T.V. (2002) Methodical instructions for testing new immunomodulating medications. *Herald of the Scientific center of expertise and state control of medicines*, no. 1, pp. 11-21.

8. Yu V.K., Praliev K.D., Fomicheva E.E., Baktybayeva L.K., Svambayev E.A., Tuleukhanov S.T. (2007) Immunostimulants based on N-alkoxypiperidines. *Chemical Journal of Kazakhstan*, no. 2, pp. 180-187.

9. Yu V.K., Praliyev K.D. (1997) Preliminary patent 5011 RK. 1-(2-Ethoxyethyl)-4-(dimethylphosphoryl)-4-hydroxypiperidine possess plant growth-stimulating activity. *Bull RK*, no. 3.

10. Praliyev K.D., Yu V.K., Zhaxibaeva Zh.M., Togyzbaeva N.A., Kemelbekob U.S., Baktybayeva L.K., Svambayev E.A. (2008) Preliminary patent 19832 RK. Complex of 3-(3-i-propoxypropyl)-7-(2-morpholinoethyl)-3,7-diazabicyclo[3.3.1]nonan with β-cyclodextrin and its precursors synthesis. *Bull RK*, no. 8.

11. Zimin Yu.S., Borisova N.S., Timerbaeva G.R., Gimadieva A.R., Mustafin A.G. (2016) Obtaining, toxicity and anti-inflammatory activity of complex compounds of uracil derivatives with polyfunctional acids. *Chemical-pharmaceutical journal*, vol. 50, no. 10, pp. 16-24.

12. Nozdrachev A.D. (2007) A large workshop on human and animal physiology of man and animals. Physiology of visceral systems. *M.: Publishing center* Academy, vol. 2.

13. Leskov V.P. (1999) Immunostimulants. *Allergy, asthma and clinical immunology*, no. 4, pp. 12-25.

14. Khaitov R.M., Pinegin B.V. (1996) Immunomodulators and some aspects of their clinical use. *Clinical medicine*, vol. 74, no. 8, pp. 7-12.

12. Kharkevich D.A. (2002) Pharmacology. Textbook for high schools. *M.: GEOTAR-MED*.

13. Hadden J.W. (1993) Immunostimulants. *Immunology Today*, vol. 14, pp. 275-280.

14. Werner G.H., Jolles P. (1996) Immunostimulating agents: what next? A review of their present and potential medical applications. *European Journal of Immunology*. vol. 242, pp. 1-19.

15. Mikhailova A.A. (1996) Individual myelopeptides are "new generation" drugs used for immunorehabilitation. *International Journal of Immunoreability*, no. 2. pp. 27-31.

16. Petrov RV, Khaitov RM, Nekrasov A.V. (1999) Polyoxidonium – immunomodulator of the last generation: results of a three-year clinical application. *Allergy, asthma and clinical immunology*, no. 3, pp. 3-6.

17. Ershov F.I. (1998) Antiviral drugs. Directory. *M.: Medicine*.

18. Petrov R.V. (1987) Immunological mobilization. *M.: Molodaja gvardija*.

19. Khaitov R.M., Pinegin B.V. (2000) Modern immunomodulators: the basic principles of their use. *Immunology*, no. 5, pp. 4-7.

20. Petrov R.V. (1994) Immunorehabilitation and strategy of medicine. *International Journal of Immunoreability*, Suppl. 1, pp. 5-6.

21. Khaitov P.M., Gushchin IS, Pinegin B.V., Zebrev A.I. (1999) Experimental study of the immunotropic activity of pharmacological preparations. *Herald of the Scientific center of expertise and state control of medicines*, no. 1, pp. 31-36.

22. Gaetke L.M., Chow C.K. (2003) Copper toxicity, oxidative stress and antioxidant nutrients. *Toxicology*, vol. 189, pp. 147-163.