

# Bioscreening of the spacerized complex BIS (2-Pyridyl)-3-(1,2,4-Triazolyl) propane and 11-Hydroxy-1,1-Ethylidenediphosphonic acid

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**Abstract**—scientific work is devoted to the study of the biological activity spacerized complex bis(2-Pyridyl) -3- (1,2,4-Triazolyl) Propane And 11-Hydroxy-1,1-Ethylidenediphosphonic Acid in the dose range from 5 to 200 mg / kg. The results of the study of acute toxicity showed that spacing of the HDA+BTP complex reduces its toxicity by 1.4 times in comparison with characteristics of its precursor components. It was found that the test compound exhibits a dose-dependent hypotensive and vasorelaxating effect, sedative, anxiogenic and analgesic effects.

**Keywords**—spacerized complex bis (2-Pyridyl) -3- (1,2,4-Triazolyl) Propane and 11-Hydroxy-1,1-Ethylidenediphosphonic acid, bioscreening, rats

## I. INTRODUCTION

Diphosphonate ligands are capable of providing various methods of bonding with metal cations, what creates the prerequisites for obtaining coordination compounds with original molecular and supramolecular structures, and 1,2,4-triazoles are good matrix for creation of coordination compounds with different nuclearity and topology. 1-Hydroxy-1,1-ethylidene diphosphonic acid (HDA) and bis (2-pyridyl-1,2,4-triazolyl-3) propane (BTP) were used as base for obtaining their adduct (HDA + BTP) – a new generation hybrid compound with new biological properties.

## II. PROBLEM STATEMENT

The components of bisphosphonates and 1,2,4-triazole have a big variety of different useful biological properties. That gives their derivatives a great potential to search for different types of biological activity in them. It has been shown that isolated single administration of HDA and BTP in the dose range from 5 to 200 mg/kg has analgesic activity, sedative and anxiogenic effects on the central nervous system and ability to change the indicators of the

cardiorespiratory system [1-4]. In this regard, the screening of biological activity of HDA, BTP and HDA+BTP in the dose range from 5 to 200 mg / kg is appropriate and useful.

## III. RESEARCH QUESTIONS

The introduction of newly synthesized chemicals into clinical practice is feasible only after detailed study of their specific pharmacological activity and safety. This circumstance is the reason for the optimization of methodological approaches of identification the biological effects of tested substances and for using a big variety of methods, the choice of which depends on goals, objectives of the study, generally accepted international requirements and the ability to assess physiological effects.

In general, the study raises such questions as:

- 1) determination of the acute toxicity of GDK + BTP and its influence on male and female rats.
- 2) assessment of the influence of 5 and 50 mg/kg doses of GDK + BTP on the work of cardiorespiratory system of rats.
- 3) assessment of the influence of 5 and 50 mg/kg doses of GDK + BTP on the behavioral changes of rats.
- 4) assessment of the influence of 5 and 50 mg/kg doses of GDK + BTP on the pain sensitivity of rats.
- 5) determination of the influence of 5 and 50 mg/kg doses of GDK + BTP on the metabolic rate and biochemical parameters of blood.

## IV. PURPOSE OF THE STUDY

The aim of the study was to conduct bioscreening of spacer complex bis(2-pyridyl)-3-(1,2,4-triazolyl)propane and 11-hydroxy-1,1-ethylidenediphosphonic acid and to establish the main direction of influence of the newly synthesized compound on biological activity.

## V. EXPERIMENTAL

The experimental group consisted of 100 healthy sexually matured albino Wistar rats of both sexes (FSUE "Rappolovo" Nursery for laboratory animals) weighing 180-200 g. Their quarantine was conducted under standard vivarium conditions, which included maintaining the temperature at 18-22<sup>0</sup> C, using the "Rehofix MK 2000" bedding based on corn cobs, providing a natural 12-hour light-dark cycle, free access to water (GOST 33215-2014 "Guide for the maintenance and care of laboratory animals. Rules for the equipment of premises and organization of procedures") and complete granular feed (GOST R-50258-92).

The first group was used for biological control (C); second to fifth groups were experimental (E) and received intraperitoneal injections of HDA+BTP at a concentration of 5, 50, 100 and 200 mg / kg, respectively.

The HDA+BTP bioscreening was performed an hour after 0.2 ml injection of specified substance. At the same time the animals of control group received the same volume injections of physiological saline. After that the indicators of the inflammation, metabolism, work of cardiorespiratory system, biochemical parameters of blood, behavioral and pain reactions have been reported in animals.

The acute toxicity of HDA+BTP was studied on rats with a single intraperitoneal injection of the test compounds in the dose range from 5 to 500 mg / kg. LD20 and LD50 was determined experimentally, LD100 was determined with probit analysis and graphically. The investigation of the influence of HDA+BTP on work of cardiorespiratory system included registration of heart rate (HR), respiratory rate (BH), systolic (SDA) and diastolic blood pressure (DBP), microcirculation indicators (MC). Blood pressure, HR and BH of rats were recorded using the NIBP200A system (Biopac Systems, Inc., USA). Data was recorded and processed with computer by using the "AcqKnowledge 4.2 for MP150" program. The MC indicators were recorded with the LDF 2.20.0.507WL program using the LAKK-02 laser blood flow analyzer in the second version (manufactured by NPP Lazma, Russia). Non-oscillatory basal blood flow parameters (such as perfusion index (PM, perf. Units), standard deviation (flux, standard deviation, perf. Units), coefficient of variation (CV,%)) were chosen as the indicators, analyzed by the LDF method. The amplitudes of blood flow oscillations of different frequency ranges were determined by using the wavelet analysis of the LDF signal. Endothelial vibrations, which is caused by periodic contractions of the endothelial cell cytoskeleton, characterized by the lowest frequency (0.0095-0.02 Hz). Endothelial vibrations show the effect of humoral-metabolic factors on the microvascular bed and characterize the state of nutritional blood flow. Myogenic fluctuations (0.07-0.15 Hz) are caused by the periodic activity of smooth muscle fibers of arterioles, which lead to changes of their lumen diameter (vasomotion). Neurogenic fluctuations (0.02-0.046 Hz), which show the effect of sympathetic regulatory activity, are superimposed on such a frequency of constriction and dilatation of microvessels. High-frequency oscillations

include respiratory (0.15-0.4 Hz) and pulse (0.8-0.16 Hz) fluctuations. Respiratory waves are represented by periodic changes in pressure in the venous section of the vascular bed caused by respiratory excursions of the chest. Pulse fluctuations in blood flow are caused by changes in intravascular pressure, which are more or less synchronized with cardiac rhythm.

Animal blood from experimental group was collected from the tail vein in the morning on an empty stomach; hematological and biochemical parameters of that blood were examined on a Mythic 18 hematology analyzer (Orphee, Switzerland). Laboratory parameters, which characterize the state of protein, lipid and carbohydrate metabolism, were analyzed in blood serum with an ERBA-XL-180 automatic biochemical analyzer (ErbaLachema, Czech Republic) using proprietary reagent kits designed to work with this device.

Investigation of HDA+BTP influence on the central nervous system of male and female rats was conducted in models of studying the tranquilizing (anxiolytic) activity of substances - in the open field test (IR Actimeter installation and Actitrack 2.0 software, PanLab Harvard Apparatus, Spain) and "elevated cruciform labyrinth" (installation of "Open Science", Russia). Antidepressant activity was examined with the test of learned helplessness of Porsolt (installation of "Open Science", Russia). The influence of HDA+BTP on pain and inflammation development was evaluated with 3 algological tests tail-flick pain sensitivity models (LE7106 Tail-flick Meter installation, Panlab, Spain), "forceps" (Randall-Selitto test, BIO- installation RP-R, BioSeb, France) and a "hot plate" ("hotplate", BIOCHIP installation, Bioseb, France).

The acute inflammatory reaction was reproduced by subplanetary injection of 0.1 ml of a 2% aqueous solution of formalin into the rat left hind paw ("formalin test"). Limb edema was measured using the method of water plethysmometry.

For a given period of time the metabolic rate of rats was studied with Actitrak software module that evaluates motor activity in metabolic cells (manufactured by Panlab, Barcelona, Spain), which record vertical and horizontal motor activity (raising animals on their lower legs), traveled distance and speed characteristics of movement. Fluid and food intake evaluation was performed with the Compulse software module using the same cells.

An integral assessment of body composition was performed using the bioimpedansometry three-component model, which include analysis of fat mass, extracellular mass (connective tissue, extracellular fluid) and active cell mass (muscle and organ cells, nerve cells) and total body fluid (ImpediVet, Australia). The data of three repetitions of the experiment were used for statistical processing. Since the distribution of variable values differed from normal, there were used a nonparametric methods of statistics. The data, obtained in the experiment, were calculated, statistically processed and graphically depicted using the MicrosoftExcel program and the StatSoft \ STATISTICA 8 software package. The reliability of statistical differences between the

control (intraperitoneal injection of physiological saline) and experimental groups (with different doses of BTP + HDA administration of 5, 50, 100, 200 mg / kg) was determined using the Mann-Whitney test and one-way analysis of variance (ANOVA) with the Tukey posterior test and Dunn's multiple comparisons test.

## VI. RESULTS AND DISCUSSION

Studies of acute toxicity after a single administration of the HDA+BTP complex showed that LD20 (at which the first animal mortality was observed) was 200 mg / kg, LD50 was 350 mg / kg, and the absolute lethal dose, which was calculated by the probit analysis method, was 570 mg / kg. It allowed attributing this complex to hazard class 3 - moderately toxic substances.

### A. Changes of parameters of the cardiorespiratory system work under the influence of HDA+BTP complex

It was found that after administration of 5mg / kg dose of HDA+BTP reducing of the systolic and diastolic blood pressure parameters was not significant and reliable, but at a dose of 50 mg / kg it decreases by 6.8% ( $p \leq 0.05$ ) and 7.4% ( $p \leq 0.05$ ) respectively (results was compared with those of the control group of rats). After administration of 5mg / kg dose of HDA+BTP HR increased by 8.4% ( $p \leq 0.05$ ), and with a dose of 50 mg / kg it tended to decrease (by 4.5%). While using the HDA+BTP complex at a dose of 5 mg / kg the respiratory rate significantly increased by 11.0% ( $p \leq 0.05$ ), and at a dose of 50 mg / kg it was almost unchanged. Using of 100mg/kg and 200mg/kg doses led to increased blood pressure and heart rate.

Studies of HDA+BTP influence on the microcirculation showed that with 5 mg/kg dose administration there were increasing amplitudes of endothelial (Ae, by 13%,  $p \leq 0.05$ ) and pulse fluctuations (Ap, by 12%,  $p \leq 0.05$ ), as well as PM - by 8% ( $p \leq 0.05$ ) in comparison with the parameters of control group animals, which indicates an increasing perfusion in tissues, caused by growth of the metabolic activity of the endothelium and increasing inflow of arterial blood into the microbore. Administration of 50 mg/kg dose of HDA+BTP complex led to a significant increase in tissue perfusion (by 27%,  $p \leq 0.05$ ) due to an increase in the activity of regulatory mechanisms for microcirculation control: in comparison with data of the control group of animals, Ae increased by 47% ( $p \leq 0.01$ ), An - 20% ( $p \leq 0.01$ ), Am - 24% ( $p \leq 0.01$ ), ( $p \leq 0.05$ ), Ap - 14%, ( $p \leq 0.05$ ), PM 26% ( $p \leq 0.05$ ), and Ar decreased by 15% ( $p \leq 0.05$ ).

The results showed an increase in tissue perfusion caused by increase in endothelium-dependent vasodilation, a decrease in peripheral resistance, an increase in blood flow to the nutritive microvascular bed, and an improvement in venous outflow.

Increasing of administrated doses to 100 and 200 mg/kg led to decreasing of all MC indicators. However, these changes were not significant.

The cardiorespiratory system of male rats was more sensitive to the HDA+BTP complex than female.

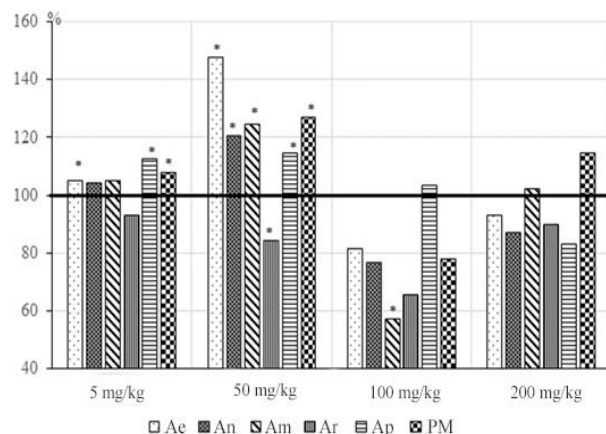


Fig. 1. Microcirculation under the influence of bis (2-pyridyl) -3- (1,2,4-triazolyl) propane and 1-hydroxy-1,1-ethylidene diphosphonic acid in different concentrations (compared to the data of the control group taken as 100%).

Notes: \* - the significance of differences in indicators in animals of the experimental group (compared to the data of the control group) was assessed by the Mann-Whitney criterion at  $p \leq 0.05$ ; Ae are the amplitudes of endothelial genesis, An is the amplitude of neurogenic oscillations, Am is the amplitude of myogenic oscillations, Ar is the amplitude of respiratory oscillations, Ap is the amplitude of pulse oscillations, PM is the perfusion index.

There were no significant changes in MC (except for Am, which decreased by 38%,  $p \leq 0.05$ ) of female rats, but male rats had their MC - parameters decreased after the administration of 100 mg/kg dose. On the contrary after using the 200 mg/kg dose the significant increase of all main parameters of MC was recorded.

### B. Changes in hematological and biochemical parameters of blood under the influence of the HDA+BTP complex

Hematological blood tests, which was conducted an 1 hour after single administration of HDA+BTP in doses of 5, 50, 100 and 200 mg/kg, showed no significant changes in the number of red blood cells, white blood cells, white blood cell count, hemoglobin level and platelet count.

After a single injection, no significant changes of the indicators of protein, carbohydrate and lipid metabolism were found in serum.

### C. Changes in behavioral reactions under the influence of the HDA+BTP complex

It was shown that in the "open field" test HDA+BTP in doses of 50 and 100 mg/kg had a sedative effect, which significantly reduced the total distance traveled by males and females. In this test the substance showed a "U" - shaped dose-response relationship, which was most prominent at a dose of 50 mg/kg. In the "elevated cruciform labyrinth" test 100 mg dose of HDA+BTP showed a strong anxiogenic effect, in contrast to the 200 mg dose, which showed decreasing of the effect. In the Porsolt's learned helplessness test, the 50 mg dose of HDA+BTP provided a depressing effect on rats (observations showed, that immobility time periods became longer than time of active swimming), what could be the result of the sedative effect of the substance, so the curve of "dose-effect" had a "U" - shaped form.

*D. Changes in pain sensitivity and inflammation under the influence of HDA+BTP complex*

The “hot plate” test showed that 50 mg dose administration caused an increase in the latent period of pain response (LPPR). The increase of LPPR by 22.58%,  $p \leq 0.05$  compared to the data of control group is shown in table I. The rest of doses of the compound were not active at all.

The tail-flick test showed an increase of LPPR by 84,47% ( $p \leq 0.05$ ) after 100 mg dose injection. Doses of 50 mg/kg and 200 mg/kg made LPPR increase by 54,25% and 27,25% respectively. The increasing of maximum applied force, which causes a pain reaction in rats, by 99.50% ( $p \leq 0.05$ ) was registered after 50 mg/kg administration. The injection of 100 mg/kg dose led to the increasing of maximum applied force by 83,24 % ( $p \leq 0,05$ ). Doses of 5 mk/kg and 200 mk/kg caused an increase of LPPR indicators by 11,07 % ( $p \leq 0,05$ ) and 24,18 % ( $p \leq 0,05$ ) respectively.

The results of water plethysmometry test showed that after administration of doses of 50 and 100 mg/kg there was the maximum decrease in the index of growth of limb edema of rats (it was increased by 41,65% and 40,62% in comparison with control data), but this changes were not reliable, so the results are presented in table 1. After 5 mg/kg dose injection received results were significantly lower than control data by 28,49%, and 200 mg/kg dose administration caused increase of index of edema growth by 65,65% in comparison with data of control group.

TABLE I. SHOWING EFFECTS OF THE SPACER-BOUND COMPLEX OF BIS (2-PYRIDYL) -3- (1,2,4-TRIAZOLYL) PROPANE AND 11-HYDROXY-1,1-ETHYLIDENE DIPHOSPHONIC ACID ON RAT PAIN SENSITIVITY AND INFLAMMATION

Doses (mg / kg) / groups	Hot plate, with (M±m) and % of control	Nippers, pain threshold for tail compression, g, (M ± m) and% of control	Tail-flick, latent period of tail reaction, with (M ± m) and% of control	Water plethysmometry, V, ml
Control	4,47±1,03 100 %	403,39± 163,80 100 %	3,24±0,82 100 %	0,28±0,09 100 %
E-5	4,92±0,65 110, 1 %	448,06± 192,43 111,1 %	3,77±0,89 116,4 %	0,20±0,04 71,4 %
E-50	5,48±0,86 122,6 % $p < 0,02$	804,75± 276,50 199,5 % $p < 0,005$	4,99±1,37 154,3 % $p < 0,005$	0,16±0,06 57,1 %
E-100	4,81±0,75 107,6 %	739,19± 189,03 183,3 % $p < 0,005$	5,97±1,47 184,3 % $p < 0,005$	0,17±0,09 60,7 %
E-200	4,17±0,99 93,3 %	500,94± 149,99 124,2 % $p < 0,02$	4,12±1,21 127,2 % $p < 0,04$	0,46±0,18 164,3 %

<sup>a</sup> Note: the significance of differences in indicators (compared with the control group at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively) was determined with one-way analysis of variance (ANOVA) with the Tukey posterior test and the Dunn multiple comparison test.

*H. Changes in the metabolic rate in rats under the influence of the HDA+BTP complex*

Bioimpedanceometry (ImpediVet, ImpediMed, Switzerland) showed that single administration of HDA+BTP in 5, 50, 100 and 200 mk/kg doses to lab rats did not lead to a redistribution of active cell mass, adipose tissue in the body and redistribution of intracellular and extracellular fluid.

**VII. CONCLUSION**

The results of the study of acute toxicity showed that spacing of the HDA+BTP complex reduces its toxicity by 1.4 times in comparison with characteristics of its precursor components (according to LD100), so the newly synthesized complex became less toxic and more safe than the starting material, which provide the basis for creation of new substance. It can also be assumed that the toxicity of the complex was caused by the presence of HDA and BTP.

It was found that using HDA+BTP in dose range from 5 to 100 mg/kg can cause pronounced biological effect. So doses of 5 and 50 mg/kg had a dose-dependent hypotensive, a vasorelaxating and a negative chronotropic effects. Doses of 50 and 100 mg/kg had sedative and analgesic effects, and the 5 mg/kg dose exhibited an anxiolytic activity. So the 50 mg/kg dose showed the maximum biological activity of this complex.

A comparative analysis of the effects of HDA+BTP and its components (HDA and BTP) allowed us to establish that the hypotensive and vasodilating effects, which was caused by the influence of the complex, is lower than the influence of its separated components. It was found that BTP mostly determines the vasoactive properties of the complex. The negative chronotropic effect of the HDA+BTP complex is caused by summation of the effects of both substances.

A comparative analysis of the effects of BTP + HDA and its components (HDA and BTP) showed that HDA+BTP has a sedative effect in completely different doses (50 and 100 mg / kg) than its predecessors (5 mg / kg (HDA) and 150 mg / kg (GDK and BTP)), so it was found that there is a shift of sedation range of the components in the complex.

The complex also does not preserve the anxiogenic properties of BTP and HDA, which was detected at doses of 150 and 200 mg/kg, but this effect remains at a 100 mg/kg dose and coincides in effect of HDA. Therefore, HDA determines anxiogenic properties of the complex to a greater extent.

It was revealed that pronounced analgesic effect of HDA+BTP appears only when they are in compound, but it can be assumed that the salt formation of the precursor components (BTP and HDA) leads to the summation and / or potentiation of the analgesic properties.

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