Vitamin B₁₂ Hydrophobic Derivative Exhibits Bioactivity: Biomedical and Photophysical Study

O. A. Gromova¹ · L. A. Maiorova^{2,1} · D. S. Salnikov² · V. I. Demidov³ · A. G. Kalacheva³ · I. Yu. Torshin¹ · T. E. Bogacheva³ · A. N. Gromov¹ · O. A. Limanova³ · T. R. Grishina³ · S. M. Jafari⁴ · O.I. Koifman^{2,5}

Accepted: 28 October 2021 / Published online: 24 November 2021

© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Vitamin B_{12} (cyanocobalamin) and some of its hydrophilic derivatives are used as antidotes and also to treat megaloblastic anemia, nerve myelination disorders, and liver pathology. This work presents results of a comparative experimental study of cyanocobalamin and its derivatives aquacobalamin and heptamethyl ester of cyanoaquacobyrinic acid. In a model of thiosemicarbazide seizures in rats, aquacobalamin contributed to a lengthening of the seizure latency period while cyanocobalamin contributed to a decrease in the seizure latency period and to a decrease in seizures. Histological study of the brain samples showed that all investigated compounds exhibited an antispasmodic effect as well as neuroprotective and myelinating effects. For the first time it was shown that a derivative of vitamin B_{12} , which has hydrophobic substituents—heptamethyl ester of cyanoaquacobyrinic acid, also exhibits biological activity and, therefore, is of interest for further research. Analysis of changes in the electronic absorption spectra recorded during the interaction of aquacobalamin with thiosemicarbazide indicated possibility of direct interaction of thiosemicarbazide with aquacobalamin.

 $\textbf{Keywords} \ \ Vitamin \ B_{12} \cdot A quacobalamin \cdot Heptamethyl \ ester \ of \ cyanoaquacobyrinic \ acid \ \cdot \ Thiosemicarbazide \ \cdot \ Antidotes$

1 Introduction

Vitamins represent important class of micronutrients vital for humans. Each vitamin affects the expression levels or function of a specific set of genes and proteins/enzymes [5] and thus has a certain range of specific biologic functions and certain compartmentalization in the organism. Vitamin

☑ O. A. Gromova unesco.gromova@gmail.com; maiorova.larissa@gmail.com

L. A. Maiorova unesco.gromova@gmail.com; maiorova.larissa@gmail.com

- ¹ Institute of Pharmacoinformatics, Federal Research Center "Computer Science and Control" of Russian Academy of Sciences, Moscow, Russia
- ² Institute of Macroheterocyclic Compounds, Ivanovo State University of Chemistry and Technology, Ivanovo, Russia
- ³ Ivanovo State Medical Academy, Ministry of Health of Russia, Ivanovo, Russia
- ⁴ Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
- ⁵ G.A. Krestov Institute of Solution Chemistry, Russian Academy of Sciences, Ivanovo, Russia

 B_{12} , being compartmentalized predominantly in the liver, intestinal epithelium, bone marrow and nerve cells, is fundamental for folate metabolism (including neutralization of homocysteine), DNA methylation and nucleotide biosynthesis. Derangement of folate metabolism and DNA methylation that occurs against the background of vitamin B_{12} deficiency contributes to carcinogenesis and megaloblastic anemia. In neurology, vitamin B_{12} is used to treat pain and restore myelin sheath of neurons [15].

Currently, much attention is given to the study of the properties of vitamin B_{12} derivatives which might expand the area of their practical applications. From the chemical point of view, vitamin B ₁₂ (cyanocobalamin) is a unique natural cobalt complex which contains a substituted corrin ring and 5.6-dimethylbenzimidazole, a nucleotide base. Cobalt in vitamin B_{12} can take different oxidation states: Co(III), Co(II), Co(I). Thanks to this, vitamin B_{12} is able to act as a catalyst in redox processes. The biological functions of vitamin B_{12} as well as the possibility of its chemical modification make it a promising tool in the design and development of new drugs. Aquacobalamin, by binding the cyanide base to form harmless cyanocobalamin, can also be used as an antidote for cyanide poisoning and overdose of sodium nitroprusside (in addition



to the above-mentioned effects of vitamin B_{12}). [1]. Diaquacobinamide, in which the ribonucleotide base of aquacobalamin is replaced by a water molecule, can also act as an antidote for poisoning with cyanide [1], hydrogen sulfide [16], and methyl mercaptan [6]. Heptamethyl ester of cyanoaquacobyrinic acid (HECAA) forms strong complexes with cyanide and hydrogen sulfide in aqueous solutions [2].

The natural affinity of vitamin B_{12} to the bone marrow and nerve cells can, in principle, be used for targeted delivery of drugs. Submicron capsules can reduce the loss of vitamin B_{12} in the presence of oxidants [17] and during targeted delivery [3, 4]. Previously, vitamin B_{12} was successfully encapsulated in micron-sized nano-engineered polymer capsules [13]. The process was accompanied by formation of two-dimensional and three-dimensional micro- and nanoaggregates [10, 20, 21, 29] due to the ability of such compounds to self-assemble [22–24, 26, 31]. These ensembles are formed in a confined space at nanolevel and their properties often differ from those of the initial compounds [11, 12, 14, 25, 27, 28, 30].

Chemoreactomic analysis is an effective method for a preliminary assessment of the potential pharmacological properties of the compounds [18, 19]. Chemoneurocytological analysis of vitamin B_{12} derivatives shown, in particular, that cyanocobalamin and aquacobalamin can have significant neuroprotective effects [7]. One of the aim of the present paper is to corroborate these findings in an experimental study on rats. Derivatives of vitamin B_{12} with hydrophobic substituents have not yet found wide application in medicine. So the other aim of the article is whether a) one of such derivatives exhibits biological activity and b) both derivatives under study in this work exhibit antidote properties towards thiosemicarbazide.

2 Materials and methods

The dicyanocobyrinic acid heptamethyl ester $((CN)_2Cby(OCH_3)_7)$ was prepared by refluxing a vitamin B_{12} methanol/sulfuric acid (1.0 M) solution for four days (Salnikov D.S. synthesis). Cyanoaquacobyrinic acid heptamethyl ester $(CN)(H_2O)Cby(OCH_3)_7$ (Fig. 5a) was prepared by vacuum drying of $(CN)_2Cby(OCH_3)_7$ aqueous solution (pH 4,0 and 25 0 C). The structure and purity of the ester were confirmed by ¹H NMR, elemental analysis, MALDI-TOF mass spectroscopy.

The values of the equilibrium constants, K, were obtained by fitting titration curve to Eq. 1:

$$A_t = \left(A_0 + A_\infty \times K \times [L]\right) / (1 + K \times [L]) \tag{1}$$

where A_0 and A_{∞} correspond to absorbance values at 0% and 100% formation of complexes between thiosemicarbazide and vitamin B_{12} derivative, respectively, and A_t is the absorbance at any thiosemicarbazide concentration [L]. The medical study was carried out using a model of thiosemicarbazide seizures in rats on 30 white male rats (weight 200–300 g). All the substances studied were injected intramuscularly at dose of 60 µg/kg for 18 days. The animals were divided into 5 groups: the first group (n=6, intact control); the second group (n=6, control with the model of primary generalized seizures); the third group (n=6, cyanocobalamin); the fourth group (n=6, aquacobalamin); the fifth group (n=6, HECAA). Cyanocobalamin was purchased (registration number P No. 015993/01, JSC "Borisovskiy Plant of Medical Preparations," Republic of Belarus), the rest of the compounds were synthesized in our lab.

To study the anticonvulsant properties of the substances, an experimental model of primary generalized seizures caused by chemical exposure was reproduced [8, 9]. In the experimental groups (second, third, fourth, fifth), the seizure model was reproduced by intraperitoneal administration of thiosemicarbazide at a dose of 28 mg/kg of body weight. In all groups, the latent time to the first seizure, the number and the nature of the peculiarities of the seizures (flinching, arena running, clonic seizures, tonic–clonic seizures with lateral position and tonic extension ending in death) and mortality within 90 min were registered. Statistical data processing was carried out using the program "Statistica 10.0."

Histological analysis of sectional material (rat brain) was carried out on a model of primary generalized seizures in rats caused by thiosemicarbazide. By means of craniotomy, the entire brain was removed and fixed in a 10% solution of neutral formalin, after 1 day, using frontal incisions, the area of the precentral gyrus of the forebrain, cerebellum, and brain stem were isolated. After the secondary fixation and washing of the material, the nerve tissue was wired (dehydrated) using 99% isopropyl alcohol. Subsequently, the pieces of the brain were embedded in paraffin, and histological Sects. $5-6 \mu m$ thick made on a Microm sled microtome were stained with hematoxylin and eosin. Duplicate sections were stained according to the Nissl method and impregnated with silver using a kit of reagents from BioVitrum (Russia).

Nissl staining was carried out as follows. Straightened sections of nerve tissue were placed in a 0.1% solution of toluidine blue or thionine, which was then heated twice until vapors appeared. After cooling, the tissue samples were rinsed in water and 70% alcohol then in 96% alcohol, xylene, balsam. The samples were stained with ready-made dyes in the kit "Toluidine Blue Nissl Modification" from the Biovitrum company (Russia).

Evaluation of pathological changes in the rat brain during modeling of primary generalized seizures took into account the degree of circulatory disorders, damage to the pathways, structural changes in pyramidal neurons of the cerebral cortex (cerebral hemispheres) and piriform neurons (Purkinje cells) of the cerebellum. The morphometric study of histological sections was carried out on a BioVision image analyzer (Vision Microscopy, Austria) and consisted in counting damaged piriform neurons in the cerebellar cortex in 10 different fields of vision. Micrographs were obtained using an MC200 research microscope (Micros, Austria) and a DCM900 digital eyepiece camera (UK).

Photophysical studies of derivatives of vitamin B_{12} were performed on a Cary 60 spectrophotometer in a sealed quartz cell. Preliminary comparative studies have shown that oxygen does not affect the interaction of the vitamin B_{12} derivatives studied with thiosemicarbazide. Therefore, all further experiments were carried out in aerobic conditions.

3 Results

3.1 The medical study using a model of thiosemicarbazide seizures in rats

In animals of the control (second) group introduction of thiosemicarbazide at 28 mg/kg dose resulted in primary generalized convulsions in 100% of cases. The convulsions were manifested in the form of flinching, dressage running, clonic convulsions, tonic–clonic convulsions with lateral position, and tonic extension. Mortality in this group of animals was 100%.

The results of the study showed that the administration of cyanocobalamin (third group) at the indicated dose on the thiosemicarbazide seizure model significantly reduced the severity of convulsive seizures according to frequencies of the features "flinching" (p = 0.02) and "tonic extension ending in death" (p = 0.027) as compared to the control group (Table 1). The lethality of animals in the third group was 50%.

In animals injected with aquacobalamin (fourth group), the latency period to seizures significantly increased (p=0.008), the number of seizures (p=0.01) and the severity of seizures according to occurrence of tonic extension ending in death (p=0.027) were decreased in comparison with the control group (Table 1, Fig. 1a). The lethality of animals in the fourth group was 50% (Table 1, Fig. 1b). As a result of a comparative assessment of the duration, nature and severity of seizures in the third (who received cyanocobalamin) and the fourth (receiving aquacobalamin) groups of animals, no significant differences were observed.

In the fifth group of rats (injected with HECAA), the latency period to seizures was significantly lengthened (p=0.006) while the severity of flinching was decreased (p=0.02) compared to the control group of animals and to the third group (cyanocobalamin, see Table 1 and Fig. 1a). The lethality of animals in the fifth observation group was 67% (Table 1, Fig. 1b). As a result of a comparative assessment of the duration, nature and severity of seizures in the

Table 1 Assessm	tent of the experiment	ental groups								
Group	Latency period	Number of	Survival time	Severity of sei	izures					Duration of
	prior to seizures (min)	seizures	(min)	1—flinching	2—"wild" arena run- ning	3—clonic con- vulsions	4—tonic–clonic convulsions with lateral position	5—tonic exten- sion	6—tonic exten- sion resulting in death	convulsions (sec)
Control (2 nd group)	52.5±3.21	3.67±0.76	72.67 ± 5.98	1 ± 0.0	1 ± 0.0	1 ± 0.0	1 ± 0.0	0.67 ± 0.21	0.83 ± 0.17	161.0±56.51
Cyano-cobal- amin (3 rd group)	65.33±5.67	2.83 ± 0.54	76.67 ± 8.11	$0.33 \pm 0.21^{*.}$	² 0.83 ± 0.17	0.67 ± 0.21	0.83 ± 0.17	0.83 ± 0.17	$0.17 \pm 0.17^{*2}$	118.67 ± 33.75
Aqua-cobalamin (4 th group)	$73.67 \pm 4.52^{*1}$	$1.5 \pm 0.22^{*1}$	74.67 ± 8.41	0.67 ± 0.21	1 ± 0.0	0.83 ± 0.17	1 ± 0.0	0.67 ± 0.21	$0.17 \pm 0.17 *^{1}$	79.5 ± 16.81
HECAA# ¹ (5 th group)	$71.83 \pm 2.6^{*3}$	2.0 ± 0.37	$81.25 \pm 0.55 \#^{1}$	1 ± 0.0	1 ± 0.0	1 ± 0.0	0.83 ± 0.17	1 ± 0.0	0.33 ± 0.21	103.83 ± 19.68
* 1-groups com	pared: control and	aquacobalamin; *	² —groups: control	and cyanocoba	lamin; * ³ —gro	ups: control and I	HECAA. # ¹ —grouj	ps: cyanocobalam	in and HECAA	



77



Fig. 1 Latent period to seizures (a) and survival (b) in comparison groups

fifth and the fourth groups, no significant differences were observed.

Thus, cobalamin derivatives studied on the model of primary generalized seizures in rats significantly tended to increase the survival rate and to decrease the severity and duration of seizures. At the same time, aquacobalamin contributed to a greater extent to the lengthening of the latent period of seizures and the number of seizures (which corresponds to a "faster" antidote-like action), and cyanocobalamin—to reduce the severity of seizures (which corresponds to a more "long-term" tissue-protective effect).

3.2 Histological analysis

A pathohistological study of the sectional material of rat brain took into account the degree of circulatory disorders, damage to the pathways, structural changes in pyramidal neurons of the cerebral cortex (cerebral hemispheres), and piriform neurons (Purkinje cells) of the cerebellum.

In all observations of the control group (group No. 2), after the reproduction of primary generalized seizures, all parts of the brain had significant circulatory disorders characterized by stasis of erythrocytes in the capillaries, the formation of fibrin-erythrocyte thrombi in the lumens of small veins of the gray and white matter of the brain with the development of pronounced perivascular edema of the nervous tissue (Fig. 2a). In the pial and intracerebral arteries, there was a change in the contours of the elastic membrane of the intima against the background of narrowing of the vascular lumen, which characterizes persistent spasm of the arterial link (Fig. 2b).

Pathohistological examination of the gray matter of the cerebral hemispheres and cerebellum revealed the predominance of the ischemic type of neuronal damage. In many



Fig. 2 Hemostasis (a) and spasm of intracerebral arteries (b) in microvasculature (A) and perivascular edema of the nervous tissue of the cerebral cortex (B). Staining with hematoxylin and eosin. Magnification \times 480

fields of vision there were whole groups of pyknomorphic and hyperchromic neurons with an increase in the activity of microglial elements in the damaged areas. When brain slices were impregnated with silver, myelin fibers were unevenly colored, indicating disintegration processes of the myelin sheath of the nerve fibers.

Pathohistological study of the effects of cyanocobalamin (group 3) showed a less pronounced diffused focal hemostasis of capillaries, a plethora of intracerebral and pial veins (Fig. 3a) and an increase in the lumen of arteries. Analysis of structural changes in neurocytes showed a decrease in the number of cells irreversibly damaged by ischemia and structural changes in nerve cells were more reversible as represented by focal fusion of the Nissl lumps (Fig. 3b). Myelinated nerve fibers in many fields of view retained clear contours, with only single foci of dechromination (Fig. 3c). A similar histological picture was observed in the other two groups. With the introduction of aquacobalamin (group 4), a moderately expressed spasm of small.

Reversible changes in nerve cells in groups 3 and 4 prevailed over deep lesions that were characterized by swelling and deformation of the cell nuclei, diffuse small focal chromatolysis of the cytoplasm. The impregnation of the conducting pathways of the brain with silver in most cases showed the presence of only local damage to the myelin nerve fibers.

Injections of HECAA (group 5) corresponded to moderate circulatory disorders in the form of diffuse focal hemostasis in capillaries and venules, perivascular edema of the nervous tissue. Damage to the neurons of the cerebral hemispheres and cerebellum was of a diffuse focal nature with a predominance of ischemic changes in the form of hyperchromia and a decrease in the volume of cytoplasm while preserving the nuclei, a macroglial



Fig. 3 Pathohistological study of the effects of cyanocobalamin (group 3). (a) Dilation, acute plethora of the venule of the molecular layer of the cerebellum. Staining with hematoxylin and eosin, Magnification \times 480. (b) Vacuolization of the cytoplasm, swelling of the axons of the neurons of the brain stem. Nissl staining with toluidine

blue, magnification \times 1200. (c) Myelin fibers of the white matter of the cerebral hemisphere without damage over a considerable extent. Silver impregnation, magnification \times 1200. (d) Hemostasis, pericapillary edema (A), plethora and venule dilation (B). Staining with hematoxylin and eosin, magnification \times 480

🖄 Springer

reaction with moderate astrocyte hypertrophy (Fig. 4a). Damage to myelin fibers was of a focal nature with loss of stained areas of insignificant extent (Fig. 4b).

The results of morphometric analysis showed significant differences only in comparison with the control group (group 2), while between groups 3, 4, and 5, corresponding to various derivatives of vitamin B12, there were no significant differences (Table 2).

In general, the model of primary generalized seizures had morphological confirmation in all cases of observation and was characterized by circulatory disorders, edema of the nervous tissue, damage to neurocytes, and demyelination of the pathways. Administration of vitamin B_{12} preparations to laboratory animals in groups 3, 4, and 5 prior to reproduction of the model did influence the levels of cerebral circulatory disorders and was characterized by a decrease in the severity of hemostasis in vessels and edema of the nervous tissue. All the compounds studied showed an antispasmodic effect, neuroprotective effect, and stimulated lower level of damage to the myelin fibers of the brain. In group 5 (HECAA), a relatively high neuroglia reactivity was revealed.

3.3 Photophysical studies of the interaction of thiosemicarbazide with vitamin B₁₂ derivatives

In accordance with the results of behavioral tests (Table 1), aquacobalamin in a greater extent contributed to the lengthening of the latent period of seizures and of the number of seizures (which corresponds to a "faster" antidote-like action of vitamin B_{12}). It is known that the derivatives of vitamin B_{12} are used as antidotes against a number of compounds. In this regard, we hypothesized that the studied derivatives of vitamin B₁₂ can act as antidotes against thiosemicarbazide poisoning. To test this hypothesis, a study was carried out on the possibility of interaction of thiosemicarbazide with aquacobalamin (Fig. 5a) and heptamethyl ester of cyanoaquacobyrinic acid (Fig. 5b).

Spectral analysis indicated that the addition of thiosemicarbazide to aquacobalamin in aqueous solution at pH 6.86 resulted in disappearance of the peaks of the initial aquacobalamin spectrum (Fig. 5c, spectrum 1) and appearance of two new peaks at 304 nm and at 540 nm (Fig. 5, spectrum 2). The resulting electronic spectrum (spectrum 2) is characteristic of cobalamin complexes formed with the participation of the Co^{3+} ion. The experiments were performed in the



Fig. 4 Pathogistology of group 5 (HECAA). Silver impregnation. (a) Hypertrophied obese astrocytes. Magnification × 1200. (b) Focal demyelination of nerve fibers. Magnification × 480

Table 2 Results of morphometric analysis of structural changes in neurons in different groups structural changes		Intact neurons	Reversible changes in neurons	Irreversible changes in neu- rons
	2nd group (control)	27.8%	32.5%	39.7%
	3rd group (cyanocobalamin)	32.4%	35.4%	32.2%
	4th group (aquacobalamin)	31.7%	35.0%	33.3%
	5th group (HECAA)	29.3%	35.9%	34.8%

Content courtesy of Springer Nature, terms of use apply. Rights reserved.





Fig. 5 Structures of vitamin B12 (aquacobalamin, a) and heptamethyl ester of cyanoaquacobyrinic acid (b) Changes in the electronic absorption spectrum (pH=6.86, t=150C, reaction time—10 min) recorded during the interaction of 50 μ M of aquacobalamin (c) and

heptamethyl ester of cyanoaquacobyrinic acid (d) with 2.7 mM of thiosemicarbazide (the arrows show the direction of the change in optical density during the reaction). The insert in (c) shows dependence of absorption value at 567 nm on thiosemicarbazide concentration (L)

presence and absence of oxygen and we found that the presence of oxygen in the reaction medium does not affect the supposed reaction between thiosemicarbazide and aquacobalamin. Thus, the reaction probably involves complexation and not reduction of the Co^{3+} ion.

Formation of the complex between aquacobalamin and thiosemicarbazide is, most likely, reversible. The shape of the titration curve registered for this reaction strongly supports this supposition (Fig. 5c, insert). Fitting the titration curve to Eq. (1) gave the best fit with $K=1\pm0.1 \text{ M}^{-1}$. Thus, the calculated binding constant was rather low. That is, thiosemicarbazide might bind to aquacobalamin in animal experiments only partially. In similar experiments, it was found that for a hydrophobic derivative of vitamin B_{12} (HECAA) the binding with thiosemicarbazide was even weaker. Very small changes

were observed for this reaction (Fig. 5d). Because of it, the titration curve was not suitable for fitting by Eq. 1. In this case, we suppose that the equilibrium constant was 3 times weaker in comparison to that of aquacobalamin.

Thus, the effects of vitamin B_{12} derivatives observed in the present series of experiments cannot be explained exclusively by direct interactions with the thiosemicarbazide molecule.

4 Conclusion

A comparative study of vitamin B_{12} , aquacobalamin and heptamethyl ester of cyanoaquacobyrinic acid on a model of thiosemicarbazide seizures in rats showed that aquacobalamin, to a greater extent than the other compounds, contributed to the lengthening of the latent period of seizures, and cyanocobalamin—to reduction of the severity of the seizures.

The study of the rat brain showed that all compounds investigated exhibited an antispasmodic effect, neuroprotective and myelinating effects, which can partially be explained by antidote-like action of the compounds.

It is important that the derivative of vitamin B_{12} , which has hydrophobic substituents in its composition, heptamethyl ester of cyanoaquacobyrinic acid, also exhibits vitamin activity and, therefore, is of interest for further research, including for targeted delivery of the compound in capsules.

The results presented here are the second stage of a comprehensive project aimed at studying the possibility of targeted delivery of drugs based on vitamin B_{12} . At the first stage, before experimental studies on animals, a study was carried out by the method of chemoneurocytological analysis [7]. In addition, studies on the aggregate behavior and functional properties of compounds at the air–water interface, in thin films on solid supports and in capsules are started. At the third stage, a study of the biological effect of drugs in capsules on animals will be presented.

Acknowledgements This work was financially supported by the grant of the Russian Science Foundation (N20-12-00175), Ivanovo State University of Chemistry and Technology; synthesis of compounds was supported by a grant of the Russian Ministry of Education and Science (FZZW-2020-0008).

Funding This work was financially supported by the grant of the Russian Science Foundation (N20-12–00175), Ivanovo State University of Chemistry and Technology; synthesis of compounds was supported by a grant of the Russian Ministry of Education and Science (FZZW-2020–0008).

Declarations

Informed Consent All authors consent for publication of article.

Research Involving Humans and Animals Statement The protocol of study was approved by the Biomedical Ethic Expert Committee of Ivanovo State Medical Academy (protocol #2 of 12 February 2020) under the institutional and the international ethical guidelines. Injections and care were given in accordance with standard recommendations of veterinary practice by qualified personnel with additional checks for health and welfare of the animals.

Conflict of Interest None.

References

 Chan, A., Balasubramanian, M., Blackledge, W., Mohammad, O. M., Alvarez, L., Boss, G. R., & Bigby, T. D. (2010). Cobinamide is superior to other treatments in a mouse model of cyanide poisoning. *Clinical toxicology (Philadelphia)*, 48(7), 709–717. https://doi.org/10.3109/15563650.2010.505197

- El-Shalakany, H. H., Hamza, M. S., & Silaev, A. H. (2018). Novel selective spectrophotometric method for hydrosulfide (HS⁻) ions assessment using vitamin B₁₂ precursor, aquacyanocobyrinic acid heptamethyl ester. *Europe Chemical Bulletin*, 7(8), 203–209. https://doi.org/10.1016/j.aca.2006.04.030
- Erokhina, S. L., Pastorino, L., Di Lisa, D., Kiiamov, A. G., Tayurskii, D. A., Iannotta, S., Erokhin, V., & Faizullina, A. R. (2021).
 3D structure reconstruction of nanoengineered polymeric capsules using Coherent X-Ray diffraction imaging. *MethodsX*, *8*, 101230. https://doi.org/10.1016/j.mex.2021.101230
- Erokhina, S. I., Ricci, V., Iannotta, S., & Erokhin, V. V. (2020). Modification of the porous glass filter with LbL technique for variable filtration applications. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 606, 125459. https://doi. org/10.1016/j.colsurfa.2020.125459
- Gromova, O.A., Torshin, I.Yu. Micronutrients and Reproductive Health. Management. GEOTAR-Media, 2019, 672 pp., ISBN 978–5–9704–5149–6 (In Russian)
- Hendry-Hofer, T. B., Ng, P. C., McGrath, A. M., Soules, K., Mukai, D. S., Chan, A., Maddry, J. K., White, C. W., Lee, J., Mahon, S. B., Brenner, M., Boss, G. R., & Bebarta, V. S. (2021). Intramuscular cobinamide as an antidote to methyl mercaptan poisoning. *Inhalation Toxicology*, *33*(1), 25–32. https://doi.org/ 10.1080/08958378.2020.18661236
- Gromova, O. A., Torshin, I. Y., Maiorova, L. A., Koifman, O. L., Salnikov, D. S.(2021) Bioinformatic and chemoneurocytological analysis of the pharmacological properties of vitamin B12 and some of its derivatives. *Journal of Porphyrins and Phthalocyanines*, 25(09), 835–842. https://doi.org/10.1142/S10884246215006 44.
- Gromova, O. A., Demidov, V. I., Kalacheva, A. G., Torshin, I. Yu., Grishina, T. R., & Bogacheva, T. E. (2020). Study of the anticonvulsant and remyelinating potential of dexketoprofen in a model of primary generalized seizures in rats. *Neurology, neuropsychiatry, psychosomatics.*, *12*(4), 84–90. https://doi.org/10. 14412/2074-2711-2020-4-84-90
- 9. Guidelines for conducting preclinical studies of drugs. Part 1, Moscow, Grif i K, 2012, 944 pp. (In Russian)
- Karlyuk, M. V., Krygin, Y. Y., Maiorova, L. A., Ageeva, T. A., & Koifman, O. I. (2013). Formation of two-dimensional (M) and three-dimensional (V) nanoaggregates of substituted cobalt porphyrin in the Langmuir layers and Langmuir-Schaefer films. *Russian Chemical Bulletin, 62*, 471–479. https://doi.org/10.1007/ s11172-013-0066-5
- Kharitonova, N. V., Maiorova, L. A., & Koifman, O. I. (2018). Aggregation behavior of unsubstituted magnesium porphyrazine in monolayers at air-water interface and in Langmuir-Schaefer films. *Journal of Porphyrins and Phthalocyanines*, 22(6), 509– 520. https://doi.org/10.1142/S1088424618500505
- Maiorova, L. A., Kobayashi, N., Zyablov, S. V., Bykov, V. A., Nesterov, S. I., Kozlov, A. V., Devillers, ChH., Zavyalov, A. V., Alexandriysky, V. V., Orena, M., & Koifman, O. I. (2018). Magnesium Porphine Supermolecules and Two-Dimensional Nanoaggregates Formed Using the Langmuir-Schaefer Technique. ACS Langmuir, 34(31), 9322–9329. https://doi.org/10.1021/acs.langm uir.8b00905
- Maiorova, L. A., Erokhina, S. I., Pisani, M., Barucca, G., Marcaccio, M., Koifman, O. I., Salnikov, D. S., Gromova, O. A., Astolfi, P., Ricci, V., & Erokhin, V. (2019). Encapsulation of vitamin B₁₂ into nanoengineered capsules and soft matter nanosystems for targeted delivery *Colloids Surf.*, B. 2019; 182C:110366. https:// doi.org/10.1016/j.colsurfb.2019.110366
- Maiorova, L. A., Vu, T. T., Gromova, O. A., Nikitin, K. S., & Koifman, O. I. (2018). Nanostructured Stable Floating M-Mono- and

Bilayers and Langmuir-Schaefer Films of 5,10,10-Triphenylcorrole. *BioNanoScience*, 8(1), 81–89. https://doi.org/10.1007/ s12668-017-0424-0

- Rebrov V.G., Gromova O.A. Vitamins, macro- and microelements, Geotar Med, 2008, 968 pp. ISBN. 978–5–9704–0814–8 (In Russian).
- Salnikov, D. S., Makarov, S. V., Eldikvan, R., Kucherenko, P. N., & Boss, G. R. (2014). Kinetics and Mechanism of the Reaction of Hydrogen Sulfide with Diaquacobinamide in Aqueous Solution. *European Journal of Inorganic Chemistry*, 25, 4123–4133. https:// doi.org/10.1002/ejic.201402082
- Salnikov, D. S., Makarov, S. V., & Koifman, O. I. (2021). The radical versus ionic mechanisms of reduced cobalamin inactivation by tert-butyl hydroperoxide and hydrogen peroxide in aqueous solution. *New Journal of Chemistry*, 45, 535–543. https://doi. org/10.1039/D0NJ04231E
- Torshin, I. Yu., Maiorova, L. A., Uvarova, E. V., Tapilskaya, N. I., & Gromova, O. A. (2020). Chemoreactom analysis of inositol stereoisomers: different profiles of the pharmacological action of myo-inositol and D-chiro-inositol in disorders of the female reproductive system. *Questions of gynecology, obstetrics and perinatology, 19*(5), 57–69. https://doi.org/10.20953/ 1726-1678-2020-5-57-69
- Torshin, I. Yu., Gromova, O. A., Maiorova, L. A., Grishina, T. R., Fedotova, L. E., Gromov, A. N., & Sardaryan, I. S. (2021). Chemoreactom analysis of cytidyldiphosphocholine indicates synergistic combinations of neuroprotectors. *Neurology, neuropsychiatry, psychosomatics, 13*(2), 144–156. https://doi.org/10.14412/2074-2711-2021-2-144-156
- Valkova, L. A., Glibin, A. S., & Valli, L. (2008). Quantitative analysis of compression isotherms of fullerene C 60 Langmuir layers. *Colloid Journal*, 70(1), 6–11. https://doi.org/10.1134/ S1061933X0801002X
- Valkova, L. A., Glibin, A. S., Koifman, O. I., & Erokhin, V. V. (2011). The influence of molecular structure and π-system extent on nano- and microstructure of Langmuir layers of copper azaporphyrins. *Journal of Porphyrins and Phthalocyanines*, *15*(10), 1044–1051. https://doi.org/10.1142/S1088424611004026
- Valkova, L. A., Valli, L., Casilli, S., Giancane, G., Borovkov, N. Yu., Sibrina, G. V., Glibin, A. S., Koifman, O. I., Pisani, M., & Rustichelli, F. (2008). Nanoaggregates of copper porphyrazine in floating layers and Langmuir-Schaefer films. *ACS Langmuir, 24*, 4857–4864. https://doi.org/10.1021/la703585p
- Valkova, L. A., Shabyshev, L. S., Feigin, L. A., Akopova, O. B. (19997). Preparation and x-ray study of Langmuir-Blodgett films of liquid crystal 4.5'bis(4-decyloxybenzoyloxybenzylidenamino)

dibenzo-18-crown-6. Bull. Russ. Acad. Sci.: Phys. (IZVESTIYA AKADEMII NAUK SERIYA FIZICHESKAYA), 61(3): 631–636. WOS:A1997WZ50000048

- Valkova, L. A., Shabyshev, L. S., Feigin, L. A., & Akopova, O. B. (1996). Formation and X-ray diffraction investigation of Langmuir-Blodgett films of liquid crystalline substituted crown esters. *Molecular Crystals and Liquid Crystals Science and Technology Section C, Molecular Materials Print, 6*(4), 291–298.
- Valkova, L. A., Menelle, A., Borovkov, NYu., Erokhin, V. V., & Pisani, M. (2003). Small-angle X-ray scattering and neutron reflectivity studies of Langmuir–Blodgett films of copper tetratert-butyl-azaporphyrines. *Journal of Applied Crystallography*, 36, 758–762. https://doi.org/10.1107/S0021889803004965
- Valkova, L. A., Betrencourt, C., Hochapfel, A., Myagkov, I. V., & Feigin, L. A. (1996). Monolayer Study of Monensin and Lasalocid in the Gas State. *Molecular Crystals and Liquid Crystals*, 287, 269–273. https://doi.org/10.1080/10587259608038763
- Valkova, L., Borovkov, N., Kopranenkov, V., Pisani, M., Bossi, M., & Rustichelli, F. (2002). Some features of the molecular assembly of copper porphyrazines. *Materials Science and Engineering.*, 22, 167–170. https://doi.org/10.1016/S0928-4931(02) 00166-2
- Valkova LA, Borovkov N. Yu., Pisani M, & Rustichelli F. (2001) Three-dimensional structure of the copper porphyrazine layers at the air-water interface. *Thin Solid Films*. 401, 267–272. https:// doi.org/10.1016/S0040-6090(01)01475-4
- Valkova, L. A., Glibin, A. S., & Koifman, O. I. (2011). Influence of the Solvent Nature on the Structure of Two-Dimensional Nanoaggregates in Langmuir Layers of Copper Tetra-tert-butyltetra benzotriazaporphyrin. *Macroheterocycles*, 4(3), 222–226. https:// doi.org/10.6060/mhc2011.3.13
- Valkova, L. A., Zyablov, S. V., Erokhin, V. V., & Koifman, O. I. (2010). Nanoaggregates in floating layers of azaporphyrins. *Journal of Porphyrins and Phthalocyanines*, 14, 513–522. https://doi. org/10.1142/S1088424610002380
- Vu, T. T., Maiorova, L. A., Berezin, D. B., & Koifman, O. I. (2016). Formation and study of nanostructured M-monolayers and LS-films of triphenylcorrole. *Macroheterocycles*, 9(1), 73–79. https://doi.org/10.6060/mhc151205m

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH ("Springer Nature").

Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users ("Users"), for smallscale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use ("Terms"). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.

These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.

We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.

While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

- 1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
- 2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
- 3. falsely or misleadingly imply or suggest endorsement, approval, sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
- 4. use bots or other automated methods to access the content or redirect messages
- 5. override any security feature or exclusionary protocol; or
- 6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.

These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.

To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.

Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.

If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com