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2-Hydroxyethylammonium Salts of Organylsulphanyl(sylphonyl)acetic Acids as Novel Pharmacologically Active Compounds

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Abstract

Preparative methods of the synthesis of organylsulphanyl(sulphonyl)acetic acids and their 2-hydroxyethylammonium salts developed by the authors are presented in this review. The latter salts represent a new class of pharmacologically active substances possessing antiaggregant, membrane-stabilizing, antioxidant, cytostatic, cardiotropic and hypocholesterolemic activity. Compounds with combined immunotropic and antitumour activity promising for the development of up-to-date remedies for treating immunodeficient, autoimmune, oncological and other diseases were found.

Key words: organylsulphanyl(sulphonyl)acetic acids, 2-hydroxyethylammonium salts, synthesis, pharmacological activity

Contents

INTRODUCTION

Aroxyalkancarboxylic acids and their derivatives since the second half of the last century were widely used as efficient growth-promoting factor for agricultural plants and as herbicides for weed control in the crops of different cultures [1]. The studies performed within the 1980–1990 at the Irkutsk Institute of Organic Chemistry of the USSR Academy of Sciences together with a number of research institutes and institutions of medical and agricultural profile demonstrated that the 2-hydroxyethylammonium salt aroxyacetic acids exhibit a highly specific biological activity to be promising for applications in medicine and agriculture. So, tris-(2-hydroxyethyl) ammonium-2-methylphenoxyacetate with the general formula such as

$RR_1C_6H_3OCH_2COO^- \cdot N^+H(CH_2CH_2OH)_3$

where R = 2-CH_3, R_1 = H (**I**) correspond to medicinal preparation trecresan and R = 2-CH_3, R_1 = 4-Cl (**II**) correspond to chlorocresacin. Being non-toxic (LD_{50} = 3000 and 1500 mg/kg,

respectively), compounds **I** and **II** exhibit adaptogenic, haematopoiesis modulating and immunomodulatory properties [2–18]. They cause the resistance of an animal organism to increase in the course of cytotoxic hypoxia, hyper- and hypothermia, intoxication with alcohol, heavy metal salts, organophosphorus compounds, when exposed to electromagnetic radiation. Chlorocresacin **II** efficiently suppresses tumour cell proliferation, mastocytoma, melanoma, lymphoma, hepatoma, and inhibits metastasis of hepatoma cells in the lungs, surpassing well-known drug 5-FU in these properties [16].

Compound **I** under name of "cresacin" is used in agriculture in order to increase crop productivity and resistivity, in order to increase the productivity of livestock, poultry, fish farming, bee-keeping, and as a stimulator of the growth of useful micro-organisms [19–28].

We synthesized sulphur-containing analogs of these compounds such as alkanolammonium salts of organylsulphanyl(sulphonyl) acetic acid (ASA) *via* the reaction between corresponding acids with mono-, di- and triethanolamine, dimethyl- and diethylethanolamine, 1-(4-nitrophenyl)-2-amino-1,3 dioxypropanol with the yield amounting to 70–90 % at 60–70 °C in an alcoholic medium according to the scheme [29–38]

$RS(O)_nCH_2COOH + NR_1R_2(CH_2CH_2OH)$

$\rightarrow RS(O)_nCH_2\overset{\ominus}{C}OO \cdot \overset{\oplus}{H}NR_1R_2(CH_2CH_2OH)$

R = Ar, Het; R_1, R_2 = H, Me, CH_2CH_2OH, $CH(CH_2OH)CH(OH)C_6H_4$–NO_2-4; $n = 0$–2

Being non-toxic compounds (LD_{50} = 1300–6000 mg/kg), ASA exhibit a high and diverse biological activity, which is comparable with, and often greater than the activity alkanolammonium salts aroxyacetic acids related to them. In [28], we presented the results of testing and using the ASA as novel high-efficient growth promoting agents in the biotechnological processes of cultivation useful bacteria, fungi, in the production of feed and baker's yeast, citric acid, food, therapeutic-and-prophylactic drug "Bifidumbakterin", in the technology of brewing malt, growing oak and mulberry silkworms. This paper is concerned discussing the efficient methods we developed for the synthesis of organylsulphanyl(sulphonyl) acetic acid as a raw materials for ASA. A high and manifold pharmacological activity of the ASA is demonstrated therein, which opens up the prospects for the development of advanced drugs based on them.

SYNTHESIS OF INITIAL ORGANYLSULPHANYL(SULPHONYL)ACETIC ACIDS

Unlike 2-hydroxyethylamines (commercial products) we performed the synthesis of the second component of ASA, aryl(hetaryl)-sulphanyl(sulphonyl)acetic acids, with 90 % yield using the most common method for obtaining alkyl and a number of arylsul-phanyl acetic acids [29] based the reaction between organylthiols and monochloroacetic acid in an aqueous alkaline medium at 90–100 °C during 1.5–2 h:

$RSH + HalCH_2COOH \rightarrow RSCH_2COOH$

R= Ph, 2-MeC_6H_4, 4-MeC_6H_4, 2-ClC_6H_4, 4-ClC_6H_4, 4-$MeOC_6H_4$, 4-FC_6H_4, 4-$CF_3C_6H_4$, 4-BrC_6H_4, 4-$NO_2C_6H_4$, 2,4-$Cl_2C_6H_3$, 2,5-$Cl_2C_6H_3$, 2-Me, 4-ClC_6H_3, 3,4-$Me_2C_6H_3$, 2,4-$Me_2C_6H_3$, 3-(2-$HOOCC_6H_4NHCO)C_6H_4$, 3-(3-$HOOCC_6H_4NHCO)C_6H_4$, indole-3-yl, pyridine-2-yl, pyrimidine-2-yl, 5-butyl-pyrimidine-2-yl, quinoline-2-yl, purine-8-yl, 1,3-benzimidazol-2-yl, 1,3-benzothiazolyl.

The 2(4)-nitro- and [(2,4-dinitrophenyl)sulphonyl]acetic acids were obtained with a high yield *via* the reaction between fluoro(chloro)-2(4)nitrobenzenes or 2,4-dinitrochlorobenzene and thioglycolic acid at 80 °C in an alcoholic alkali solution. At the same time the authors of [30] described a method for the synthesis of [(4-nitrophenyl)sulphanyl]-acetic acid which is carried out in an anhydrous medium in the presence of an alkali metal [30]:

$4\text{-}NO_2C_6H_4Cl(F) + HSCH_2COOH \xrightarrow[-HHlg]{NaOH} 4\text{-}NO_2C_6H_4SCH_2COOH$

$2,4\text{-}(NO_2)_2C_6H_3Cl + HSCH_2COOH \xrightarrow[-HHlg]{NaOH} 2,4\text{-}(NO_2)_2C_6H_3SCH_2COOH$

A method for the synthesis of the methyl ester of chloromethylsulphanylacetic acid which reacts with 4-chlorothiophenol to produce [(4-chlorophenylmethyl)sulphanyl]acetic acid with a high yield. In order to obtain α-chlorosulphide we first used a chlormethylating system such as paraformaldehyde–trimethylchlorosilane [31]:

$(CH_2O)_n + (CH_3)_3SiCl + CH_3OOCCH_2SH \rightarrow CH_3OOCCH_2SCH_2Cl \xrightarrow{4\text{-}ClC_6H_4SH,\ H_2O} 4\text{-}ClC_6H_4SCH_2SCH_2COOH$

Taking into account a high biological activity of indole derivatives we synthesized a series of 1H-, 1R-indol-3-yl-sulphanylacetic acids according to an original technoque [32, 33], without using any unstable, allergenic and difficult of access indolthiols. Indole-3-yl-sulphanylacetic acids synthesized with a yield of 80 % *via* the reaction between indole-3-yl-isothiuronium iodides (obtained *in situ* from indoles, thiourea and iodine at a ratio equal to 1 : 2 : 1, respectively, in the presence of potassium iodide) and monochloroacetic acid with alkali in an alcoholic medium. The purity level of the compounds with no additional purification amounted up to 94–99 % (Scheme 1).

Using this advanced approach to the synthesis of indolylsulphanylacetic acid *via* the reaction between 1-substituted pyrrole derivatives and iodine, thiourea, as well as halocarboxylic acids, we first obtained 1-benzylpyrrole-2-sulphanylacetic acid and 1-methylpyrrole-2-sulphanylacetic acid with a 59–68 % yield [34] (Scheme 2).

In order to synthesize arylsulphonylacetic acids containing hexavalent sulphur atom researchers commonly use a method known from the literature consisting in the oxidation of arylsulphanylacetic acids by the excess of an oxidant, but there is often a mixture of products obtained. We have developed a method for the selective oxidation of arylsulphanylacetic acids by 30 % H_2O_2 in glacial acetic acid at a ratio between the reactants amounting to 1 : 2 : 2, *via* two stages: holding the mixture at a room temperature within the range of 3–24 h and boiling for 15 min at the final stage. This procedure allowed increasing the yield (65–93 %) and the purity of the target products [35, 36]:

$$RSCH_2COOH \xrightarrow[CH_3COOH]{H_2O_2} RS(O)_2CH_2COOH$$

R = C_6H_5, 4-$CH_3C_6H_4$, 4-$ClCC_6H_4$, 4-FC_6H_4, 4-$NO_2C_6H_4$, 2,4-$Cl_2C_6H_3$, 2,5-$Cl_2C_6H_3$, 2-CH_3-4-ClC_6H_3

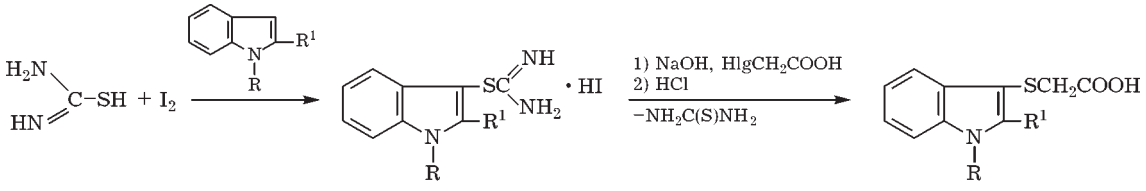
4-Methyl- and 4-chlorophenylsulphonylacetic acid were also obtained with the yield of 70 % *via* the oxidation of corresponding arylsulphanylacetic acids by 30 % H_2O_2 in acetic anhydride.

The most convenient preparative method for the synthesis of arylsulphonylacetic acids and their esters, with no using free thiols consists in the condensation of sodium arylsulphinates with monochloroacetic acid. *Via* varying the ratio between the reactants, temperature, time, nature of the solvent, the condensation products were obtained with the yield of 85–93 %, and high purity [35, 36]:

$RC_6H_5SO_2Na + ClCH_2COOH \rightarrow RC_6H_5SO_2CH_2COOH + NaCl$

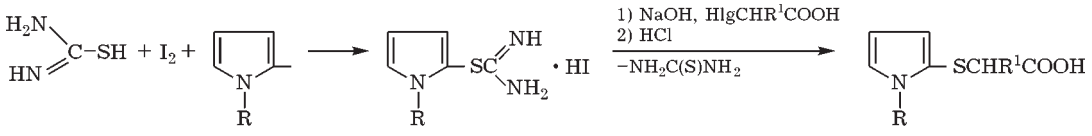
R = H, 4-CH_3, 4-Cl

In order to synthesize arylsulphonylalkanecarboxylic acids with branched carbon chain we used the method of alkylation by alkyl halides and benzyl chloride in the presence of sodium hydroxide. Depending on the ratio between the reactants one can observe the formation of mono- or dialkylation products with the yield amounting to 73–85 % [36] (Scheme 3).



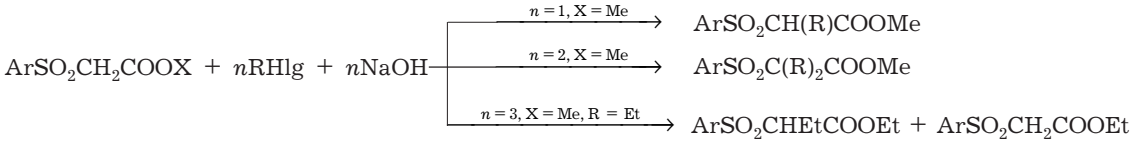
Hlg = Cl, Br; R = H, CH_3, CH_2Ph; R^1 = H, CH_3, C_2H_5

Scheme 1.



Hlg = Cl, Br; R = Me, Bz; R^1 = Alk

Scheme 2.



Scheme 3.

PHARMACOLOGICAL ACTIVITY OF ASA

It is known that the initial organylsulphanyl(sulphonyl) acetic acids intended for the synthesis of ASA, some their derivatives and 2-hydroxyethylamins exhibit separate types of pharmacological activity. So, 2-methyl-4-chloro- and [(2,4-dichlorophenyl)sulphanyl]acetic acids possess anaesthetic properties [37]. Hypotensive activity is demonstrated by fluorine-containing phenylsulphanylacetic acid [38]. Acids those contain the 4-chlorphenylsulphanyl group exhibit anaesthetic and hypocholesterolemic activity [39], inhibit releasing histamine from blood leukocytes; these compounds could be used as antiallergic agents [40], they were recommended for the treatment of cardiovascular diseases [41]. Cyclohexylphenylethylidenesulphinyl(sulphonyl)acetic acids, as well as their alkyl esters and amides exhibit antithrombotic activity; they positively affect the cholesterol and triglyceride metabolism [42]. Phenyl substituted sulphanylacetic acids exhibit antiarrhythmic activity [43]. The hydrazides of chloro, methoxy, ethoxy, nitro substituted acridine-9-sulphanylacetic acids being moderately toxic compounds exhibit neurotropic, anti-inflammatory, analgesic, antimicrobial and antihypoxic activity [44].

Cytostatic activity is exhibited by the hydrochlorides of 5,6-dimethylbenzimidazole-2-sulphanyl-2-aminobenztiazolyl-3-sulphanyl-, 1,3-dimethylxanthineyl-8-sulphanylacetic acid hydrazides [45]. An influence of benzimidazolyl 2-sulphanylacetic acid derivatives containing thiethane cycle upon humoral and cellular immunity was studied. It was found that depending on the structure, these compounds exert either immunostimulatory, or immunosuppressive effects [46].

The 2-hydroxyethylamines as a second component for the synthesis of ASA also exhibit the properties of biologically active compounds [47, 48]. They represent part of a number of physiologically important substances and drugs. So, the 2-hydroxyethylammonium fragment present in the molecule of choline, acetylcholine and colamine. Monoethanolamine is a part cephalins and dimethylethanolamine is a part of lecithin belonging to the class of phospholipids. The latter play an important role in living organisms, participating in the formation of nerve cells and blood plasma. The monoethanolamine represents an intermediate in the biosynthesis and metabolism of such amino acid as glycine necessary for the formation of various organs and tissues. The NCH_2CH_2O fragment is included in phospholipids, in coenzyme Q, in vitamin B_{12}. An antioxidant action of ethanolamines was reported. The inhibitory effect of ethanolamines increases with increasing the number of hydroxyl groups in the molecule of an amino alcohol.

Amino alcohols normalize carbohydrate metabolism, stimulate the accumulation of glycogen in the liver, the biosynthesis of nucleic acids and proteins [47, 48]. An influence of mono-, di- and triethanolamine upon the zeta potential of blood platelets was investigated. At the concentration values equal to 10^{-3}–10^{-4} M these substances inhibit the functional activity of thrombocyte platelets, in this case the highest activity is observed for triethanolamine [49]. Basing on dimethyl, diethylethanolamine, pyridyl- and morpholyletanolamine, drugs those exhibit antiallergic and antihistaminic properties were synthesized [48]. The activity of these compounds structurally related to histamine, but non-toxic as opposed to the latter, could be explained by an increased ability to form complexes with proteins and metal ions.

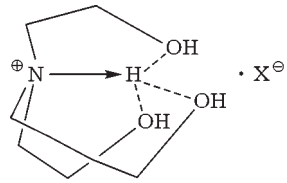
As the chemotherapy of cancer is concerned, compounds have found application therein with so-called yperite nitrogen ($NHCH_2CH_2Cl$) or aziridine cores those are hydrolyzed in an organism to give ethanolamines forming metal complexes to exert a specific alky-

lating effect on cancer cells [50]. Alkanolamines are actively involved in the intracellular metabolism of various systems both in the form of complexes with metal ions (if this is necessary for introducing deficient metals into the intracellular medium), and as a part of organic compounds with a built-in nitrogen yperite (when used for binding unwanted metal ions) [48].

Screening was performed for the physiological activity of about 100 ASA those we synthesized. It was found that having a low toxicity level they exhibit adaptogenic, hematopoietic and immunomodulatory activity, antitumour, antimetastatic, antiaggregation properties; these compounds cause increasing the resistance of animals against hypoxia, hyper- and hypothermia, sonication, and γ-irradiation.

A high biological activity of ASA, as well as of related alkanolammonium salts aroxyacetic acids exceeding the activity of parent acids and alkanolamines, is, to all appearance, connected with their unusual structure. Using an X-ray diffraction method, by the example of trecresan tris-(2-hydroxyethyl)ammonium-4-chlorophenylsulphanyl acetate and we established an original compact tricyclic (2,8,9-trihydroprotatrane) structure of these compounds containing N → H bond and intramolecular trifurcation bonds H···OH [51, 52] (Scheme 4).

Such a compact structure of triethanolammonium salts of the aroxy and organylsulphanyl (sulphonyl)acetic acids causes, to all appearance, increasing their ability of efficiently penetrating the cellular barriers. A similar mono or bicyclic endostructure could be theoretically exhibited also by the salts of other alkanolamines (monoethanolamine and methylethanolamine, diethanolamine and dimethylethanolamine) with organylsulphanyl(sulphonyl)acetic acids those we also investigated as novel pharmacologically active agents.



X = 2−CH_3−$C_6H_4OCH_2COO$, 4−Cl−$C_6H_4SCH_2COO$

Scheme 4.

Antithrombotic, hypocholesterolemic and protect adaptative properties

The formation of thrombocyte platelet-based and other cell aggregates clogging small vessels and violating the system of blood microcirculation is connected with changes in the functional activity of the platelets. Under the action of 2-hydroxyethylammonium salts of arylsulphanylacetic acids at the concentration ranging within 10^{-5}–10^{-7} M, researchers in all the cases observed an increase in electrokinetic zeta potential of platelets and the inhibition of platelet aggregation induced by adenosine diphosphate (ADP) [49, 53–59]. The effect of the compounds depends on the nature, number and position of substituents in the aromatic ring and increases in the case of electronegative substituents (4-Cl, 4-NO_2, 2,3-Cl_2, 3,5-Cl_2). The influence of the alkanolammonium fragment of ASA was investigated by the example of the 2-hydroxyethylammonium salts of 4-chlorophenylsulphanylacetic acid. The inhibition of platelet aggregation at the concentration values equal to 10^{-6} M amounted to 36, 40 and 45 % for mono-, di- and triethanolammonium salts, respectively. With increasing the number of hydroxyethyl substituents, to all appearance, an increase in affinity occurs for the cell membranes of platelets. The alkanolammonium salts of organylsulphanyl-(sulphonyl)acetic acids, those include an hexavalent sulphur atom (the sulphonyl group), are active at much lower concentration values comparing to their analogues containing a divalent (sulphanyl) sulphur atom [49, 53–55]. Like the derivatives of sulphur-containing acids, the tris-(2-hydroxyethyl)ammonium salt of phenylselenoacetic acid inhibits the platelet aggregation when ADP, thrombin and phospholipase C are used as inducers [58].

In order to perform extended studies of pharmacological activity *in vitro* and *in vivo* the authors have chosen the most active compounds as it follows: tris-(2-hydroxyethyl)ammonium-4-chlorophenylsulphonyl acetate (sulfacetamine, SFA) [55] with the formula
4-$ClC_6H_4SO_2CH_2COO^- \cdot HN^+(CH_2CH_2OH)_3$
and tris-[(2-hydroxyethyl)ammonium-indol-3-yl-sulphanyl acetate (indacetamine, INA) with the formula [59] 3-Ind-S-$CH_2COO^- \cdot N^+H(CH_2CH_2OH)_3$.

In the presence of SFA at the concentration ranging within 10^{-5}–10^{-7} M, the ADP induced platelet aggregation *in vitro* demonstrated a 80–85 % inhibition level; the surface charge of the platelets in this case exhibited a 30–35 % increase in 15 min. The platelet aggregation induced by serotonin, epinephrine, histamine, and thrombin was also almost completely inhibited by sulfacetamine.

As far as the *in vivo* experiments are concerned, when the SFA was administered intravenously to rabbits at a dose of 1 mg/kg, after 15 min passed, the ADP induced platelet aggregation demonstrated a 57 % decrease as compared to the control group. The platelet charge increased in this case by 35 % as compared to intact platelets. Compound SFA delayed the onset of blood clotting time from 50 to 234 s already in 15 min. The thromboplastin-time test demonstrated that the effect of the compound exhibited also after 15 min with the duration up to 24 h. In the case of preliminary SFA administering to rats at a dose of 2 mg/kg intravenously, and the subsequent rapid thrombin administration (at a dose causing death in 70 % of animals), the animal survival level exhibited a 50–65 % increase [49, 53–55].

One of the most promising ways to affect the thrombotic process consists in the use of drugs combining antiaggregant and antioxidant action. The antioxidant effect of SFA was studied in comparison with ionol. As the experimental test, we used a method based on the inhibition of weak chemiluminescence (CL) induced by iron ions (II) in plasma enriched by thrombocites. Being at concentration equal to 10^{-6} M, the SFA inhibits the induced plasma chemiluminescence by 57 % (ionol at the 10^{-3} M concentration exhibits only 15–20 % inhibition level). The advantage of SFA consists also in high and membrane protective and antithrombotic activity.

Under unfavourable conditions connected with external and internal factors (hypoxia, temperature effects, radiation, intoxication, cardiovascular disease), there are changes occurring in the integrity and functional activity of erythrocyte membranes, which results in the activation of coagulation system, disordering the processes of blood microcirculation, hemodynamic changes. An influence of SFA upon the resistance of rabbit erythrocyte membranes with respect to *in vitro* chemical, saponin and ultrasonic hemolysis was studied. At the concentration values ranging within 10^{-5}–10^{-7} M the SFA caused an increase in the resistance of erythrocyte membranes by 12–39 % with respect to HCl, by 5–12 % with respect to saponins, by 17–40 % with respect to ultrasonic hemolysis. Increasing the erythrocyte resistance with respect to damaging factors under the action of sulfacetamine could be caused, to all appearance, by an influence of the substance upon the strength of the lipid-protein complexes of erythrocyte membranes [49, 53–55].

A high protective effect was demonstrated by indacetamine (INA) [59]. With the incubation of erythrocytes with INA at the concentration ranging within 10^{-4}–10^{-2} M the cataphoretic mobility increased by 9–20 %. INA at the concentration ranging within 10^{-2}–10^{-10} M increased the resistance of red blood cell membranes with respect to extreme chemical and mechanical damage *in vitro* (HCl, saponins, bacterial endotoxin *Salmonella thyphimurium* (ET), ultrasound, γ-radiation). At the concentration ranging within 10^{-4}–10^{-8} M the protective effect of INA in ultrasound exposure was equal to 19–49 % that against the action of saponins amounted to 18–39 %. In the case of the pre-incubation of 5 % rabbit erythrocyte suspension for 30 min with INA at 10^{-2}–10^{-4} M concentration prior to the irradiation at a dose of 500 kRad the hemolysis level was equal to 0.718 % with respect to a control sample (100 % for the control). In the case of the radiation damage of erythrocyte membranes there is haemoglobin liberation from the cells observed to occur, so the level of destruction can be judged by the amount of free haemoglobin released. At the concentration of 10^{-6}–10^{-5} M the INA reduced the release level of free haemoglobin from the cells by 36–49 % in 60 min, whereas at the concentration of 10^{-4} M a 39–60 % reduction was observed in 180 min.

At a dose of 0.2 mg/kg, INA exerted an effect on the intensity of the reactions of lipid peroxidation within isolated erythrocyte membranes initiated by Fe^{2+} ions. Under the influence of the INA, the erythrocyte membrane ability of peroxidation was inhibited. In 60 min after INA administration, the content of the reactive products of thiobarbituric acid oxida-

tion decreased by 29 %, in 120 min this value decreased by 37.7 %. Increasing the antioxidant properties of the membranes of red blood cells, to all appearance, is one of the basic mechanisms of the protective action of INA.

Taking into account an important role of platelets in haemostasis and in disseminated intravascular coagulation (DIC), we investigated the effect of INA on the platelet membranes. In the in vitro experiments we revealed a high protective effect of INA which consisted in the inhibition of platelet aggregation release reaction, in blocking the reaction of endogenous arachidonic acid release and metabolism, in increasing the concentration of free SH groups on the membranes. At the concentration values equal to 10^{-3}–10^{-2} M the INA caused platelet aggregation induced by ADP to reduce by 16–80 %, for that induced by thrombin this value was equal to 10–44 %, for that induced by phospholipase C – by 29–37 %. The rate of aggregation in this case decreased by 27–73, 29–81 and 36–53 %, respectively. The maximal inhibitory effect of the compound exerted on the aggregation induced by arachidonic acid and ET amounted to 38–73 and 15–74 %, respectively.

The resistance of platelet membrane with respect to disintegrant factors depends on the level of free sulphhydryl groups. After incubating the platelets with INA at the doses of $1.5 \cdot 10^{-3}$–$1.2 \cdot 10^{-2}$ M for 15 min at 37 °C, the concentration of free SH groups increased ranging from $0.68 \cdot 10^{-5}$ to $3.07 \cdot 10^{-5}$ M ($0.65 \cdot 10^{-5}$ M for control).

A positive impact of the INA on central hemodynamics and myocardial contractile function was revealed in the case of DIC development in rabbits. In the case of endotoxin DIC syndrome one observes changing the arterial pressure, cardiac output (CO) and total peripheral vascular resistance (TPVR). When INA administered intravenously at a dose of 25 mg/kg, starting from the 31 min after administration, a steady increase was observed in blood pressure during 5 h, with increasing the TPVR within 2 h, whereas the CO did not changed during this time. In this case one could observe also improving the main parameters of the basic contractile myocardial function.

The pathogenesis of atherosclerosis is closely associated with thrombosis, and hypercholesterolemia those contribute to the development of cardiovascular lesions. For the treatment of these diseases, it is promising to use the drugs those combine antithrombotic, anti-oxidant and hypocholesterolemic activities with a low toxicity level. The hypocholesterolemic activity of SFA [60] and INA [61] was studied in outbred white rats. Experimental hyperlipidaemia was created by a single intraperitoneal administering non-ionic surfactant Tween-80 to the animals at a dose of 200 mg per 100 g of body mass (Tween model) in 1 mL of distilled water. Preparations SFA and INA were administered intraperitoneally at the concentration of 25 and 50 mg/kg in 5 min after the administration of the detergent. The efficiency of the compounds was evaluated after 1 day to compare with clofibrate administered at the same doses. It was found that SFA caused a 53 % decrease of total cholesterol at a dose of 25 mg/kg and a 63 % decrease of that at a dose of 50 mg/kg, whereas INA at a dose of 25 mg/kg caused a 36 and 42 % decrease of total blood cholesterol and triglyceride levels, respectively, as compared with the control group. The reference drug at a dose of 25 and 50 mg/kg caused decreasing the cholesterol level by 25 and 27 %, respectively, whereas for triglycerides a 20 % decrease was observed. The hypocholesterolemic action of SFA and INA did not accompanied by undesired side effects, being exhibited not only by reducing the total cholesterol level, but also by lowering the blood levels of low density atherogenic lipoproteins to a greater extent than the reduction of the cholesterol level, which is especially important. The lipoprotein composition of blood returned to normal, and the cholesterol content verged towards the physiological norm.

The problems of increasing the adaptive capacity of an organism under various extreme conditions are now becoming increasingly important. We investigated the protective properties of the SFA with respect to hypoxia and load in the case of intraperitoneal administering the drug to outbred albino mice. The drug was used during 1 month at a dose of 4 and 60 mg/kg. The resistance with respect to hypoxia was detected by means of a pressure chamber Ku-7, with determining maximum lifting height and maximum staying the animals

at an altitude of 12 000 m before the onset of the lateral position of the animals. The resistance of the animals with respect to the physical activity was judged by the time they swim in water at the temperature of 18 °C till first diving into the water. It was revealed that the optimal dose amounted to 4 mg/kg, in this case the maximum duration of staying the animals at the altitude equal to 12 000 m was 2.5–3 times greater than the duration value for the control group. The maximum ceiling of the "lifting" the tested animals was 3000–4000 m higher than that for the control group. The medicinal preparation SFA also caused increasing the resistance with respect to physical stress. So, at a dose of 4 mg/kg increased the duration of mice swimming exhibited a more than two-fold increase as compared to the intact animals.

Immunoactive properties

Selective immunomodulators represent a novel and urgent part of immunopharmacology. All the existing drugs, both immunostimulants, and immunosuppressants exert a nonspecific action. In this case, immunosuppressants are characterized by severe toxicity with a high risk of adverse reactions and the presence of a whole series of contraindications.

The urgent problem consists in developing novel immunoactive drugs for the treatment autoimmune, immune deposit, immunodeficiency, lymphoproliferative, cancer, allergic diseases, complications in the case of organ and bone marrow transplantation, and other diseases connected with the activation of the immune system, and with the production of various antibodies.

There are currently no drugs approved for medical use those are able to selectively alter the balance of Th1/Th2 cytokines in the right direction [62].

Basing on screening the properties of immunoactive ASA *in vitro* and *in vivo* in a series of tris-(2-hydroxyethyl)ammonium salts of aryl- and indole-3-yl-sulphanyl(sulphonyl)acetic acids, compounds were revealed with the potential ability of altering the balance between the production of pro-/anti-inflammatory cytokines Th1 or Th2 cells to form a cellular or humoral immunity, respectively, with minimal potential of undesired effects on various body systems, including the processes of haematopoiesis and immunopoiesis [63–72].

The most detailed studies were performed concerning the most promising compounds for the development of novel immunomodulators such as indacetamine (INA) [63-66] and its structural analog tris-(2-hydroxyethyl)ammonium-1-benzyl-indol-3-yl-sulphanyl acetate (BM) [67–72] with the following formula:

1-Benz-3-Ind-S-$CH_2COO^- \cdot N^+H(CH_2CH_2OH)_3$

The distinctive feature of INA and BM consists in the fact that they demonstrate pronounced antiproliferative activity in vitro and immunosuppressive properties *in vivo* at a relatively low toxicity. Indacetamine exhibits pronounced erythropoiesis and immunopoiesis modulating properties. For example, in mice with different clinical variants of immunopathology (immunodeficiency, anaemia, immune complex glomerulonephritis), this compound causes the proliferative differentiation parameters of the hematopoietic erythropoiesis precursors to restore. In particular, the INA inhibits the production of pro-inflammatory cytokines IL-1β, TNFα, causes the number of early erythroid precursors to decrease, and stimulates the amount of macrophage precursors of the granuloid series. Owing to these, anaemia could be eliminated and the immune function could be restored. In the experimental model of autoimmune disease (immune deposit glomerulonephritis) the INA exhibits a pronounced therapeutic effect (eliminates anaemia, causes increasing the body mass, reducing the erythrocyte sedimentation rate and proteinuria), which is connected with the suppression of pro-inflammatory cytokines and the reduction of erythropoiesis hyperplasia from the early stages of erythron differentiation in sick mice. According to the morphological studies, the compound inhibits the development of mesangial proliferative glomerulonephritis.

The preparation BM inhibits *in vitro* a mitogene-stimulated proliferation of mice spleen cells and human blood lymphocytes, of the tumour cells of different lines, as well as a testosterone-stimulated proliferation of hematopoietic stem cells in the bone marrow of mice [67]. The BM is an immunosuppressant drug with anticancer effect. It was demonstrated that the prepa-

ration can suppress the Th2 dependent activation of B cells (inhibition of IgE antibody production), *i. e.* the drug exhibits anti-allergic properties.

Immunosuppressant BM possesses a low potentiality for accumulation, it does not inhibit the formation of blood, and it does not exhibit incidental nephrotoxicity and hepatotoxic properties of the known immunosuppressive drugs cyclosporine A and azathioprine [67].

It should be noted that the INA [63–65] and BM [67–69] are able of selective influence upon the proportion between the function of T or B cell immunity system, causing the deviation of the immune response in a particular direction (Th1 or Th2).

Antitumour activity

A cytostatic activity was investigated *in vitro* for aryl-substituted ASA against the ascites tumour cells of limfadenoma NK/Ly, Ehrlich's adenocarcinoma, Fischer's limfadenoma and sarcoma 37, according to the inhibition level of nucleic acid synthesis in tumour cells [73].

Compounds were revealed those possess a high cytotoxic activity at the doses of 1–10 μg/mL comparable to the activity of known anticancer drug "Novembihin". In the *in vivo* experiments (in outbred albino mice) with four strains of transplantable tumours demonstrated the highest activity of tris-(2-hydroxyethyl)ammonium salt of 2-methyl-4-chlorophenylsulphanylacetic and 3,4-dimethylsulphonylacetic acids: the substances inhibited the growth of Ehrlich, Fisher's sarcoma 37 strains within the range of 68–88 %. In this case the cancerostatic activity arylsulphonylacetic acid salts was higher as compared with that for the corresponding arylsulphanyl derivatives [73].

The investigation was performed concerning the antitumor activity of tris-(2-hydroxyethyl)ammonium salts of 1-methylindol-3-ylsulphanyl and BM at doses ranging within 3–300 μg/mL in linear and hybrid mice [70]. The study was conducted using a ^{3}H-thymidine technique for the cells of mastocytoma P815, melanoma B16, lymphoma L1210, and hepatoma G27. As a reference drug we used cisplatin widely used in oncology, at a dose of 6 mg/ml. In tumour cells of the four types, the highest dose-dependent cytotoxic effect was demonstrated by BM. The maximum effect of this compound (the inhibition level of tumour growth amounting to 89–99 %) was achieved at a dose of 150 μg/mL for all the types of tumour cells used, which indicated the absence of any differential sensitivity of tumour cells with respect to this compound.

When *in vivo* studying the antitumour activity concerning lymphoid L1210, melanoma B16 and hepatoma G2 tumours the BM and tris-(2-hydroxyethyl)ammonium-1-methylindol-yl-3-sulphanyl acetate reduced the amount of lung metastases within the range of 17–43 %. We revealed that the BM exhibits antimetastatic effect comparable to that of cisplatin. We have determined the efficiency of combined using BM together with cisplatin (half a dose) with respect to melanoma B16 metastasis: the combination effect was significantly higher (about 70 %) as compared to using each of the drugs separately (26.2 and 49.4 %, respectively) due to the manifestation of the potentiating effect. As opposed to cisplatin, the BM is non-toxic.

Toxicological studies

The investigation of the acute toxicity of ASA was performed in white outbred mice by means of intraperitoneal administration. Most of the synthesized compounds are non-toxic with respect to warm-blooded animals (LD_{50} = 1300–6000 mg/kg) to be promising for advanced biomedical tests.

In the series of (2-hydroxyethyl)ammonium arylsulphonylacetic acid salts there is a tendency observed of increasing the toxicity in the case of introducing the second substituent to the benzene ring of the anion and with decreasing the number of 2-hydroxyethyl groups in the ammonium fragment. More detailed studies were performed concerning the toxicity of the compounds those are promising for practical use as potential drugs.

So, the toxicity level of SFA after intraperitoneal administration is equal to 6000 mg/kg. ASA compounds containing heterocyclic radical are generally less toxic than their analogues with an aromatic radical. Using intraperitoneal and *per os* administration in outbred white mice, studies were performed concerning the acute

and chronic toxicity of INA. In the case of intraperitoneal INA administration LD_{50} = 3000 mg/kg, whereas when the substance administered *per os* the value was found to amount to 4466 mg/kg. The substance does not exert either blistering or resorptive effect. In the chronic experiment, in the case of intraperitoneal INA injection at a dose of 10 mg/kg, no abnormalities were revealed in the tissues of animal organs. Indacetamine has no allergenic, mutagenic, teratogenic and cytotoxic properties.

A detailed study was performed concerning acute and chronic toxicity of BM [71, 72]. In determining the acute toxicity of this substance the mean lethal dose for mice depending on gender were equal to 1300–1500 mg/kg, those for rats amounting to 470–700 mg/kg, which allows one to attributed to this compound to the III class of hazard. Owing to its low capacity of accumulation, this substance does not accumulate in animals at the concentration of hazard. The introduction of the drug to the animals during six months resulted in the only minor changes on the ECG. When studying the allergenic and mutagenic properties of the BM compound using different experimental models, no undesirable effects such as mutagenicity and allergy manifestation were revealed. In animal experiments, BM exerts mild analgesic and anti-inflammatory effects, having no significant influence upon the central nervous and cardiovascular systems, without affecting the rate and rhythm of breathing.

The investigation of the pharmacokinetics of the compound BM demonstrated that it is rapidly delivered to the systemic rabbit bloodstream when administered intragastrically to be registered within 24 h [72]. Its absolute bioavailability is equal to 0.71. The maximum content of the compound BM was detected in kidney and liver, whereas the minimum content was revealed in cardiac and skeletal muscle as well as in the brain. Biochemical studies of the liver and kidney condition demonstrate its safety as a medicinal preparation.

CONCLUSION

The (2-hydroxyethyl)ammonium salts of organylsulphanyl(sulphonyl)acetic acids, preparative methods for obtaining those from available raw materials were developed by the authors of this paper represent a novel class of pharmacologically active substances having a high antiaggregation, membrane stabilizing, antioxidant, anti-metastatic, cardiotropic, hypocholesterolemic activities. We have revealed non-toxic drugs (INA, BM) with immunotropic and antitumour activity those are promising for developing advanced medicinal preparations in order to treat immune deficiency, autoimmunity, cancer and other diseases.

REFERENCES

1 Melnikov N. N., Pestitsidy, Khimiya, Moscow, 1987.
2 Vyshkovskiy G. L. (Ed.), Registr Lekarstvennykh Sredstv Rossii (Handbook), Informkhim, Moscow, 1995.
3 Platonova R. N., Voronkov M. G., Olkhovenko V. P., Polyachenko V. M., Karpova N. I., Parfenov G. G., Dyakov V. M., Semenova N. V., Platonova A. T., Ratner E. T., *Dokl. RAN*, 226, 6 (1976) 1433.
4 Voronkov M. G., Batoev Ts. Zh., Tsibekmitova G. Ts., Semenova N. V., *Dokl. RAN*, 284, 1 (1985) 241.
5 Voronkov M. G., Semenova N. V., Kazaimirovskaya V. B., Kholdeeva L. N., Moskvitina L. T., Nepsha V. D., Novikova N. V., *Khim-Farm. Zh.*, 20, 8 (1986) 952.
6 RU Pat. No. 2099051, 1994.
7 Kazaimirovskaya V. B., Semenova N. V., Moskvitina L. T., Nepsha V. D., Novikova N. V., Voronkov M. G., *Eksperim. Klin. Farmakol.*, 58, 3 (1995) 37.
8 Shirinskiy V. S., Kolesnikova O. P., Kudaeva O. T., Semenova N. V., Zhuk E. A., Mirskova A. N., Tuzova M. N., Sukhenko T. G., Voronkov M. G., Kozlov V. A., *Eksperim. Klin. Farmakol.*, 56, 3 (1993) 42.
9 Kolesnikova O. P., Kudaeva O. T., Sukhenko T. G., Limonov V. L., Kozlov V. A., Mirskova A. N., Voronkov M. G., *Dokl. RAN*, 391, 3 (2003) 410.
10 Voronkov M. G., Mirskova A. N., Levkovskaya G. G., *Dokl. RAN*, 385, 3 (2002) 411.
11 Kassin V. Yu., Nikolaev M. P., Mironova L. D., Rolik I. S., Konyushko O. I., Mirskova A. N., Voronkov M. G., *Ros. Otorinolaringol.*, 5, 12 (2004) 85.
12 Shibanov P. D., Ganopolskiy V. P., Zarubina I. V., Zhumashev A. B., Elistratov A. A., *Neyronauki*, 5, 3 (2006) 43.
13 Voronkov M. G., Kolesnikova O. P., Mirskova A. N., *Khim-Farm. Zh.*, 41, 5 (2007) 13.
14 Voronkov M. G., Rasulov M. M., *Khim-Farm. Zh.*, 41, 1 (2007) 3.
15 Voronkov M. G., Sofronov G. A., Starchenko D. A., Adamovich S. N., Mirskova A. N., *Dokl. RAN*, 385, 3 (2002) 125.
16 Kolesnikova O. P., Mirskova A. N., Adamovich S. N., Mirskov R. G., Kudaeva O. T., Voronkov M. G., *Dokl. RAN*, 425, 4 (2009) 556.
17 Mirskova A. N., Adamovich S. N., Mirskov R. G., Voronkov M. G., *Dokl. RAN*, 433, 5 (2009) 710.
18 Voronkov M. G., Nurbekov M. K., Bobkova S. N., Karaulova L. K., Susova M. I., Rasulov M. M., *Dokl. RAN.*, 431, 2 (2010) 261.

19 Author's Certification No. 638315 USSR, 1977.
20 Khonkhodzhaeva D. A., Voronkov M. G., *Dokl. RAN*, 333, 1 (1993) 124.
21 Voronkov M. G., Semenova N. V., Tseytlina V. R., Yakovleva Z. M., *Dokl. RAN*, 335, 3 (1994) 390.
22 Voronkov M. G., Gorbalinskiy V. A., Dyakov V. M., *Dokl. RAN*, 369, 3 (1999) 831.
23 Voronkov M. G., Dyban A. P., Dyakov V. M., Simbirtsev N. L., *Dokl. RAN*, 364, 4 (1999) 703.
24 Pavel Yu. G., Karus A. L., Kumar Yu. A., Shattshneyder T. K., Voronkov M. G., *Dokl. RAN*, 385, 3 (2002) 419.
25 Mirskova A. N., Levkovskaya G. G., Stupina A. G., Chkhenkeli V. A., Voronkov M. G., *Dokl. RAN*, 390, 2 (2003) 280.
26 Voronkov M. G., Dalmaa G., Tserenpil Sh., Ugtakhbayar O., Chimidzogeol, *Dokl. RAN*, 404, 4 (2005) 562.
27 Makarova L. E., Borovskiy G. B., Bulatova A. M., Voronkov M. G., Mirskova A. N., *Agrokhim.*, 10 (2006)41.
28 Mirskova A. N., Levkovskaya G. G., Mirskov R. G., Voronkov M. G., *Zh. Org. Khim.*, 44, 10 (2008) 1501.
29 Pasto D. J., McMillan D., Murphy T., *J. Org. Chem.*, 30 (1965) 2688.
30 Wittman J., Fromm E., *Ber.*, 4 (1908) 2264.
31 Evstafyeva I. T., Levkovskaya G. G., Mirskova A. N., *Zh. Org. Khim.*, 34, 9 (2008) 1305.
32 Author's Cestification No.1473294, USSR, 1987.
33 Levkovskaya G. G., Rudyakova E. V., Mirskova A. N., *Zh. Org. Khim.*, 38, 11 (2008) 1697.
34 Rudyakova E. V., Mirskova A. N., Levkovskaya G. G., *Zh. Org. Khim.*, 44, 10 (2008) 1539.
35 Mirskova A. N., Levkovskaya G. G., Guseva S. A., Kryukova Yu. I., *Zh. Org. Khim.*, 20, 3 (1984) 602.
36 Levkovskaya G. G., Mirskova A. N., Guseva S. A., Kryukova Yu. I., *Zh. Org. Khim.*, 20, 7 (1984) 1439.
37 Erdman H., Nilsson J., *Acta Chem. Scand.*, 3 (1949) 901.
38 US Pat. No. 89804, 1981.
39 Klimov A. M., Nikulcheva N. G., *Kardiol.*, 6 (1972) 133.
40 US Pat. No. 770062, 1978.
41 UK Pat. No. 13950176, 1980.
42 DE Pat. No. 2546319, 1977.
43 DE Pat. No. 2219923, 1980.
44 Martynovskiy A. A., Samura B. A., Omelyanchik V. N., *Khim.-Farm. Zh.*, 24, 7 (1990) 31.
45 Strokin Yu. V., Krasovskiy I. A., Ovrutskiy V. M., Protsenko L. D., Krezmer A. A., Aleksandrova E. V., Votyakov V. I., Sharykova N. I., Shashikhina M. N., Bukhtiyarova T. A., Zhavrid S. V., Rebyanskaya L. V., *Khim.-Farm. Zh.*, 24, 7 (1990) 45.
46 Khaliullin F. A., Alekhin E. K., Klen E. E., Ryabchinskaya L. A., Kataev V. A., Bogdanova A. Sh., *Khim.-Farm. Zh.*, 35, 1 (2001) 12.
47 Metzler D., Biochemistry, vol. 1, Acad. Press, New York, 1977.
48 Kunaszewska M., *Sci. Bull. of Lodz Techn. Univ.*, 31, 267 (1976)5.
49 Levkovskaya G. G., Kryukova Yu. I., Kazimirovskaya V. B., Kholdeeva L. N., Mirskova A. N., Voronkov M. G., *Khim.-Farm. Zh.*, 18, 4 (1984) 431.
50 Wiliams D. K., *Chem. Rev.*, 72 (1972) 203.
51 Starova G. L., Frank-Kamenetskaya O. V., Fun-damenskiy V. S., Semenova N. V., Voronkov M. G., *Dokl. AN SSSR*, 260, 4 (1981)888.
52 Shklover V. E., Gridunova G. V., Struchkov Yu. T., Voronkov M. G., Kryukova Yu. I., Mirskova A. N., *Dokl. AN SSSR*, 269, 2 (1983) 387.
53 Nefedova T. V., Kazimirovskaya V. B., Levkovskaya G. G., Bryuzgin A. A., Guseva S. A., Mirskova A. N., Voronkov M. G., *Khim.-Farm. Zh.*, 20, 3 (1986) 291.
54 Levkovskaya G. G., Guseva S. A., Kazimirovskaya V. B., Kholdeeva L. N., Voronkov M. G., Mirskova A. N., *Khim.-Farm. Zh.*, 20, 3 (1986) 295.
55 Author's Certification No. 1045569, USSR, 1983.
56 Nefedova T. V., Boyko M. I., Kazimirovskaya V. B., Ivanov A. A., Levkovskaya G. G., Guseva S. A., Bryuzgin A. A., Voronkov M. G., *Dokl. AN SSSR*, 311, 4 (1990) 1000.
57 Nefedova T. V., Kubatiev A. A., Kazimirovskaya V. B., Chernyakhovskaya B. I., Mirskova A. N., Guseva S. A., Levkovskaya G. G., Voronkov M. G., *Khim.-Farm. Zh.*, 22, 10 (1988) 1197.
58 Nefedova T. V., Kubatiev A. A., Martynov A. V., Guseva S. A., Kazimirovskaya V. B., Mirskova A. N., Novikova N. V., Lobanova E. G., Nepsha V. D., Moskvitina L. D., Voronkov M. G., *Khim.-Farm. Zh.*, 21, 9 (1987) 1081.
59 Mirskova A. N., Adamovich S. N., Mirskov R. G., Voronkov M. G., *Dokl. RAN*, 435, 4 (2010) 561.
60 RU Pat. No. 1417426, 1993.
61 RU Pat. No. 2034540, 1995.
62 Khaitov R. M., Il'ina N. I. (Eds.), Allergologiya i Immunologiya: Natsionalnoye Rukovodstvo, GEO-TAR-Media, Moscow, 2009.
63 Kolesnikova O. P., Kudaeva O. T., Sukhenko T. G., Lykov A. P., Gaydul K. V., Limonov V. L., Mirskova A. N., Levkovskaya G. G., Voronkov M. G., Kozlov V. A., *Eksperim. Klin. Farmakol.*, 69, 3 (2006) 47.
64 RU Pat. No. 108100, 1998.
65 Kolesnikova O. P., Mirskova A. N., Adamovich S. N., Kudaeva O. T., Mirskov R. G., Voronkov M. G., *Byull. SO RAMN*, 30, 6 (2010) 12.
66 Kolesnikova O. P., Mirskova A. N., Kudaeva O. T., Goyman E. V., Gavrilova E. D., Perminova O. M., Adamovich S. N., Nauch. Konf. "Meditsinskaya Genomika i Proteomika" (Treatises), Novosibirsk, 2010, p. 21.
67 Limonov V. L., Shurlygina A. V., Robinson M. V., Melnikova E. V., Kolesnikova O. P., Gaydul K. V., Mirskova A. N., Trufakin V. A., *Byull. SO RAMN*, 115, 1 (2005) 70.
68 Kolesnikova O. P., Kudaeva O. T., Nenasheva E. V., Goldina I. A., Goyman E. V., Lykov A. P., Safronova I. V., Limonov V. L., Mirskova A. N., Rudyakova E. V., *Byull. SO RAMN*, 124, 2 (2007) 14.
69 Kolesnikova O. P., Kudaeva O. T., Nenasheva E. V., Goldina I. A., Goyman E. V., Safronova I. V., Limonov V. L., Mirskova A. N., Adamovich S. N., Gaydul K. V., Kozlov V. A., Konf. "Sozdaniye Novykh Lekarstvennykh Preparatov" (Proceedings), Izd-vo Tom. Un-ta, Tomsk, 2007, p. 26.
70 Kolesnikova O. P., Kudaeva O. T., Sukhenko T. G., Lykov A. P., Dolgikh T. V., Gaydul K. V., Mirskova A. N., Levkovskaya G. G., Voronkov M. G., Kozlov V. A., in: VILIM (Doklinicheskiye Issledovaniya), in Gaydul K. V., Kolesnikova O. P. (Eds.), Novosibirsk, 2008, p. 29.
71 Kolesnikova O. P., Kudaeva O. T., Goyman E. V., Lykov A. P., Dolgikh T. V., Gaydul K. V., Mirskova A. N., Levkovskaya G. G., Voronkov M. G., Kozlov V. A., in: VILIM (Doklinicheskiye Issledovaniya), in Gaydul K. V., Kolesnikova O. P. (Eds.), Novosibirsk, 2008, p. 37.
72 Gaydul K. V., Kolesnikova O. P., Kudaeva O. T., Goyman E. V., Gavrilova E. D., Demchuk V. F., Kozlov V. A., in: VILIM (Doklinicheskiye Issledovaniya), in Gaydul K. V., Kolesnikova O. P. (Eds.), Novosibirsk, 2008, p. 123.
73 Voronkov M. G., Mirskova A. N., Levkovskaya G. G., *Dokl. RAN*, 386, 3 (2002) 411.