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HYDRAZIDE OF *o*-HYDROXYBENZOIC ACID AND ITS ERIVATIVES. SYNTHESIS AND PROPERTIES

Abstract. The article presents the results of a study by the authors of the article on the development of new ways of synthesis and study of the biological activity of hydrazide derivatives of o-hydroxybenzoic acid. Methods for the preparation of hydrazone, oxadiazole, thiosemicarbazide, 1,2,4-triazole-3-thionic derivatives and methods for their further modification are described. The condensation reaction of hydroxybenzoic acid hydrazides with 1-deoxy-2,3,4,6-tetra-O-acetyl-D-glucopyranosyl isothiocyanate synthesized their corresponding acetylated glycosyl-containing thiosemicarbazide derivatives. The structures of the synthesized compounds were studied by ^{1}H and ^{13}C NMR spectroscopy, as well as by the data of two-dimensional spectra of COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C). The values of chemical shifts, multiplicity, and integrated intensity of 1H and 13C signals in one-dimensional NMR spectra were determined. Using spectra in the formats COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C), homo- and heteronuclear interactions were established, confirming the structure of the compounds under study. The results of evaluating their antimicrobial, anti-inflammatory and cytotoxic activity (*in vitro*) on cultures of human monocytic cell lines *MonoMac-6* and *THP-1Blue* are described.

Key words: o-hydroxybenzoic acid hydrazide, thiosemicarbazide, hydrazone, oxodiazole

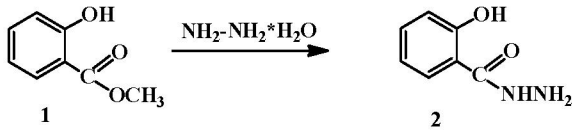
Introduction. Modification of the structure of known bioactive substances widely used in medical practice is still one of the main approaches in the search for new drugs. One of the promising substrates for the modification of substrates is the molecule of o-hydroxybenzoic acid (salicylic acid (SA). Many derivatives of o-hydroxybenzoic acid (salicylates) have been used in medical practice as non-steroidal anti-inflammatory drugs since the middle of the last century (since 1876) [1-5]. They combine this action with an analgesic and antipyretic effect. Derivatives such as acetylsalicylic acid (ASA, aspirin), sodium salicylate, salicylamide (SAM), methyl salicylate are used in medicine as analgesics (analgesic), antipyretics (antipyretic) and antiplatelet agents (antithrombotic) [1-4]. P-hydroxybenzoic acid methyl ester (methyl-paraben, nipagin, methyl-4-hydroxybenzoate) has been used as a preservative and antiseptic [3-5].

Most drugs of the salicylate group also have similar side effects: a damaging effect on the mucous membrane of the gastrointestinal tract, impaired renal function, and some others [2-6]. In the scientific literature there is evidence of antimicrobial, antifungal [6–9], psychotropic, antitumor, antipyretic, antiflammatory and cytotoxic properties of various hydroxybenzoic acid derivatives [10-14].

According to recent data, derivatives of SA can be considered as bioregulators, which are synthesized by the body itself and perform protective functions. And this allows us to rethink the role of SA in the pathophysiology of humans and animals. In recent years, interest in derivatives of o- and p-hydroxybenzoic acids has increased again [6-12], due to the relevance of the search for new antibacterial drugs. In this regard, an important task is the development of new and improvement of known methods for the synthesis of new derivatives of SC. In this regard, we found it interesting to study hydrazone, oxadiazole, thiosemicarbazide,

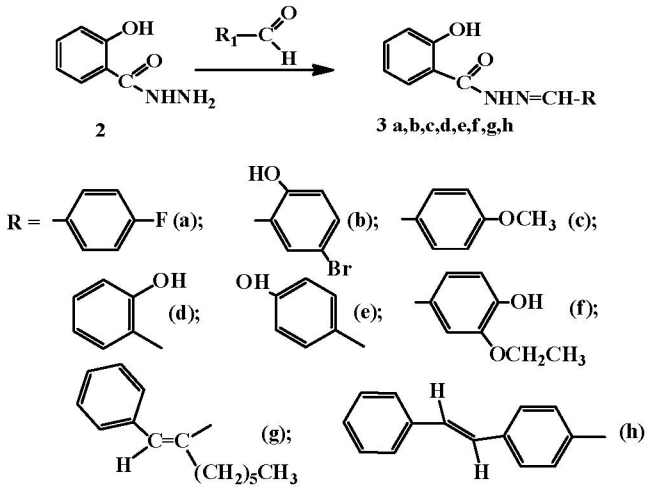
1,2,4-triazole-3-thionic, and other derivatives of these synthons. New derivatives of SC should be characterized by increased biological activity in combination with low toxicity and less pronounced side effects.

In this work, we generalized some of our own results of the synthesis of o-hydroxybenzoic acid hydrazide derivatives (salicylic acid (SA). These studies are aimed at searching for new antimicrobial derivatives and ways for their further modification. The initial o-hydroxybenzoic acid hydrazide (**2**) was obtained by the interaction of methyl salicylate (**1**) with hydrazine hydrate [15-17].



The obtained **2** was subsequently reacted with various functionally substituted aromatic aldehydes to produce new N-arylidenehydrazones (**3**).

The condensation reaction was carried out by heating equimolar amounts of aldehydes and (**2**) in ethanol at 60–70°C for 3-5 hours [15-17].



The products of reaction **3** are white crystallizable substances that are soluble in many organic solvents; the yield of compounds is 70-90%.

The structure of all the obtained N-arylidenehydrazones of *o*-hydroxybenzoic acid was proved using IR and ^{1}H, ^{13}C NMR spectroscopy, and the composition was determined by elemental analysis. Spin-spin interactions in these substances, carried out between H-H and H-C atoms through one or more bonds, were established using the two-dimensional spectra of COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) (figures 1 and 2) [15,16].

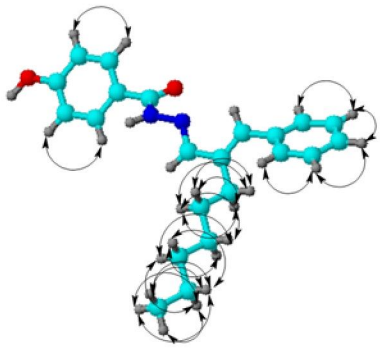


Figure 1 – Correlation of COZY (^{1}H-^{1}H) of compound **3f**

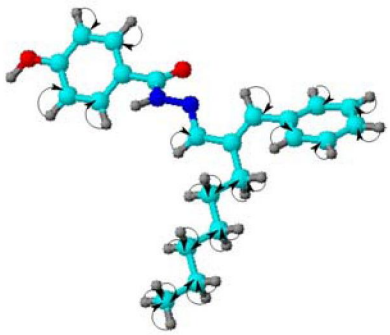


Figure 2 – Correlation of HMQC (^{1}H-^{13}C) of compound **3f**

To prove the spatial structure of the derivatives of N-arylidenehydrazones of p-hydroxybenzoic acid, an X-ray diffraction study of N-(5-bromo-2-hydroxybenzylidene)-4-hydroxybenzohydrazide (**3b**) was performed (figure 3).

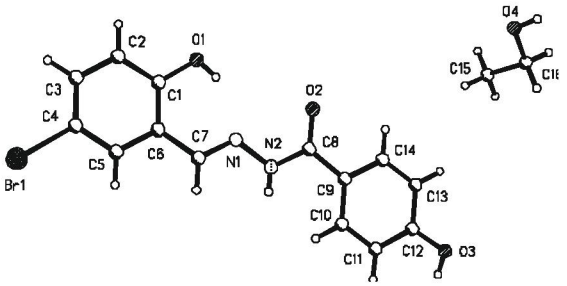
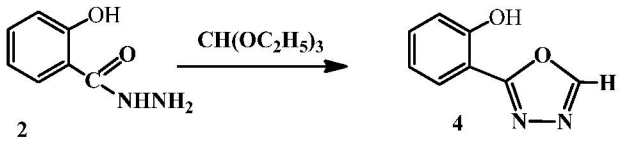


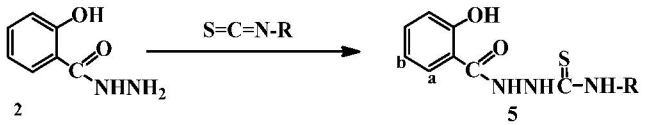
Figure 3 – The spatial structure of the molecule. N-(5-bromo-2-hydroxybenzylidene)-4-hydroxybenzohydrazide (**3b**)

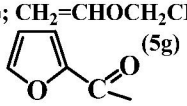
The carbonyl group and the hydrazide fragment are convenient synthons for heterocyclization. The study of the scintillation efficiency of a large number of various organic substances has led to the discovery of new, very promising classes of compounds, one of which is the class of oxadiazoles [2-5]. In this regard, it was of interest to us to synthesize new oxadiazole derivatives based on **2** and orthoformate. Orthoform ether is widely used in organic chemistry for the synthesis of various heterocyclic systems [9-13]. The interaction of **2** with a triple amount of orthoformate ester while boiling the reaction mixture led to the formation of 2-substituted 1,3,4-oxadiazole (**4**) [17]:



The synthesized compound **4** is a crystalline substance soluble in many organic solvents. In the ^{1}H NMR spectrum of compound **4**, the signals of α and β protons of the aromatic ring are recorded in the field of weak fields: Hα doublet at 7.98 ppm. and Hβ 6.98 ppm. The proton of aromatic hydroxyl appears singlet at 10.53 ppm. The methylene proton signal appears in the region of 9.22 ppm. narrow singlet.

The addition of hydrazides to various isothiocyanates is one of the convenient methods for the synthesis of substituted thiosemicarbazides [4,10-12], which are interesting substrates for the formation of various heterocycles, including heterocyclic ensembles. We carried out a series of nucleophilic addition reactions of *o*-hydroxybenzoic acid hydrazide to various isothiocyanates. The reactions were carried out in an alcohol medium at equimolar ratios of the reactants. The synthesized compounds **5** are white crystalline substances that are readily soluble in polar organic solvents [18,19].

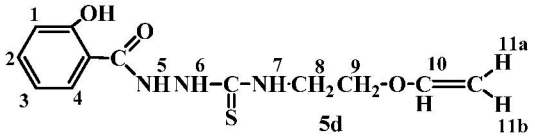


R = CH_2=CH=CH_2-(5a); CH_3CH_2- (5b); C_6H_5- (5c); CH_2=CHOCH_2CH_2- (5d);
CH_2=CHC(O)- (5e); CH_2=C(CH_3)C(O) - (5f);  **(5g)**

In the IR spectra of synthesized compounds **5**, an absorption band appears in the region of 1330–1310 cm^{-1}, which is characteristic of the -NH-CS group of the thiosemicarbazide fragment, and absorption bands of the amide group C(O)NH appear in the region of 1690–1675 cm^{-1} and –NH- groups in the region of 3390-3360 cm^{-1}.

In the ^{1}H NMR spectrum of allylthiosemicarbazide o-hydroxybenzoic acid (**5a**), signals and protons of the aromatic ring are recorded in the field of weak fields: doublet H at 7.78 ppm. (J_{HH}=8.7 Hz) and doublet H at 6.81 ppm. The proton of aromatic hydroxyl manifests itself as a singlet at 10.09 ppm. Amide and thioamide N-H protons are also written out in the field of weak fields in the form of three singlets in the region of 10.06 ppm. (H_1), 9.25 ppm. (H_2) and 8.2 ppm. (H_3). The methine proton H_5 of the vinyl fragment appears as a complex multiplet in the region of 5.82 ppm. The methylene protons H_{6a} and H_{6b} of the same vinyl fragment are manifested by two doublets in the region of 5.04 ppm. and 5.14 ppm with the spin-spin interaction constant J_{H6aH6b}=17.27 Hz. Methylene protons of the NCH_2-fragment manifest themselves in the

region of 4.09 ppm. in the form of an expanded triplet. The ratio of integrated intensities corresponds to structure 10a.



5d

When analyzing the ^{1}H NMR spectrum of compound **5d**, characteristic signals of protons of the aromatic ring are observed. So, signals of aromatic protons H_1 - H_4 are registered in the field of weak fields: doublet H_1 at 6.97 ppm, triplet H_2 at 7.45 ppm, triplet H_3 at 6.92 ppm, doublet H_4 at 7.87 ppm The signals of the four methylene protons H_8, H_9 of the oxyethyl moiety appear as two triplets in the region of 3.70 ppm. and 3.80 ppm. The methine proton H_{10} of the vinyl residue is written out as a doublet of doublets in the region of 6.50 ppm. The methylene protons H_{11a} and H_{11b} of the same vinyl fragment are manifested by two doublets in the region of 4.00 ppm. and 4.21 ppm The proton of aromatic hydroxyl appears singlet at 8.30 ppm. Amide and thioamide N-H protons are written out as three singlets in the region of 11.92 ppm. (H_5), 10.58 ppm. (H_6) and 9.55 ppm. (H_7) [15-19].

In order to study the spatial structure and stereochemistry of salicylic acid hydrazide derivatives, an X-ray diffraction study of salicylic acid N-furanoylthiosemicarbazide (**5g**) was carried out. A general view of the 9g molecule is shown in figure 4.

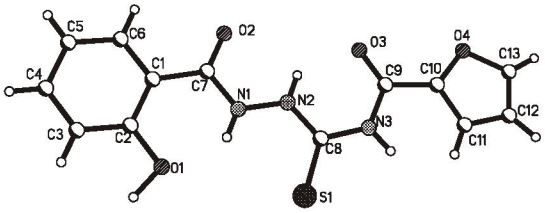
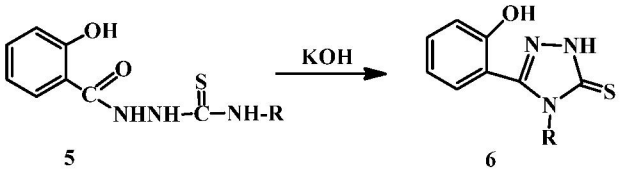


Figure 4 – The spatial structure of the molecule. Salicylic Acid N-Furanoylthiosemicarbazide (**5g**)

The phenolic cycle is flat with an accuracy of ± 0.003 Å. The furan ring is flat with an accuracy of ± 0.002 Å. The phenolic cycle, relative to the main skeleton of the molecule, is slightly developed, almost lying in the plane of the remaining atoms. This position of the phenolic cycle is explained by the presence of a strong intramolecular hydrogen bond N1H ... O1, which holds the hydroxyl group and does not allow the cycle to take an energetically favorable position, i.e. turn around 900. Also, the molecules in the package are connected by another intermolecular H-bond, which forms endless ribbons along the crystallographic axis b.

In order to study the properties of new potentially bioactive substances, we [19-21] carried out a number of preparatively convenient chemical transformations with the obtained thiosemicarbazides, which led to the production of 1,2,4-triazole-3-thions (**6**).



5 6

R = CH_2=CH-CH_2- (6a); CH_3CH_2- (6b); C_6H_5- (6c); CH_2=CHOCH_2CH_2- (6d)

The thiosemicarbazides (**5**) of o-hydroxybenzoic acid were cyclized in **6** by boiling for 2–3 hours in an aqueous solution of caustic potassium followed by acidification with acetic acid.

In the IR spectra of the obtained new triazoles **6b** there is no absorption band characteristic of the amide carbonyl (C = O) and in the region of 1272 cm-1 there is an absorption band for the thiocarbonyl group (C = S).

When analyzing the ^{1}H NMR spectrum of compound **6b**, characteristic signals of the protons of the aromatic ring are observed. So, signals and protons of the aromatic ring are registered at 7.48 ppm. ($J_{H□H□}$ = 8.6 Hz) and 6.93 ppm. in the form of two doublets, respectively. In a weak field, signals of proton aromatic

hydroxyl are recorded at 10.09 ppm. and thioamide N-H proton at 13.79 ppm. in the form of two small broadened singlets. Signals of methyl group protons resonate at 1.15 ppm. (J_{HH} = 7.1 Hz) as a triplet and a methylene group at 4.01 ppm. (J_{HH} = 7.2 Hz) as a quartet.

In order to establish the spatial structure of the synthesized triazoles **6**, an X-ray diffraction study of the 4-ethyl-5-(2-hydroxyphenyl)-1,2,4-triazole-3-thione (**6b**), 4-(2-hydroxyethyl)-5- molecule was carried out (2-hydroxyphenyl)-2H-1,2,4-triazolo-3(4H)-thione (**6d**) and crystalline hydrate 4-allyl-3-(4-hydroxy-phenyl)-1H-1,2,4-triazole-5(4H)-thione (**6a**), a general view of them is shown in figures 5-7 [20-22].

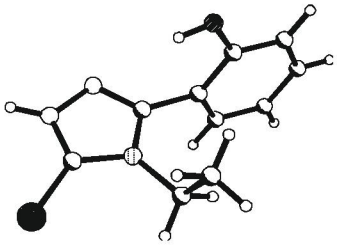


Figure 5 – Molecule structure. 4-ethyl-5-(2-hydroxyphenyl)-1,2,4-triazole-3-thione (**6b**)

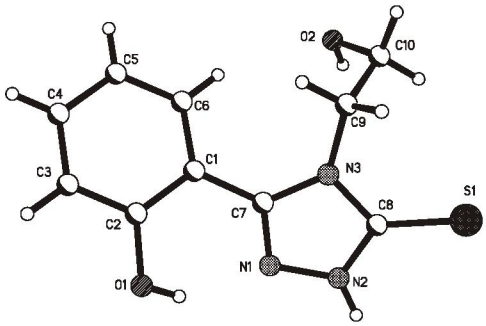


Figure 6 – The spatial structure of the molecule. 4-(2-hydroxyethyl)-5-(2-hydroxyphenyl)-2H-1,2,4-triazolo-3(4H)-thione (**6d**)

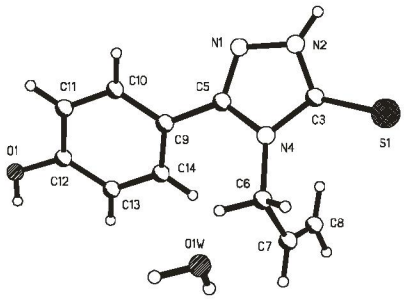
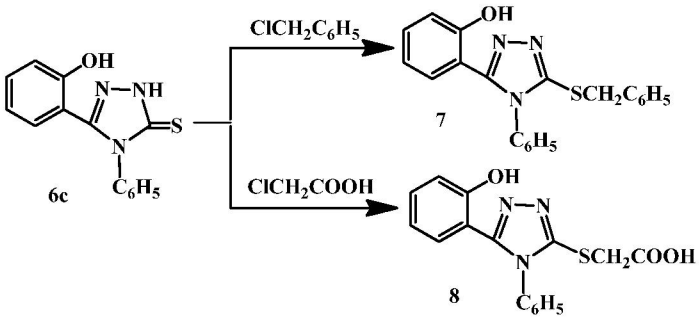


Figure 7 – Molecule structure. 4-allyl-3-(4-hydroxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (**6a**)

The five-membered triazole cycle $N_1N_2N_3C_7C_8$ of compound **6d** is flat with an accuracy of ±0.0028 Å. The sulfur atom leaves the plane of these atoms at +0.079 Å. The flattening of this cycle occurs in our opinion due to the conjugation of the electron densities of double bonds N_1=N_2 and C_8=S_1. The atoms of the phenolic cycle are coplanar with an accuracy of ±0.0012 Å, the output of the O1 atom is -0.074 Å from the plane of the remaining atoms. The phenolic cycle is in equatorial orientation relative to the triazole cycle (torsion angle $N_1C_7C_1C_2$ = 32,84°). An intramolecular hydrogen bond O_1HO_1... N_1 is observed in the molecule, which significantly affects the rotation of the phenolic ring relative to the five-membered ring. The bulky substituent at the N_3 atom is oriented equatorially.

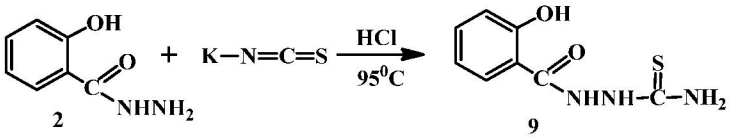
The triazole ring is flat of compounds **6a** with an accuracy of ± 0.005 Å, the allyl group is rotated perpendicular to it, the p-hydroxyphenyl substituent at – 42.7°. A similar reversal of the phenyl cycle is observed in the molecule of 4-amino-3-(4-ethoxyphenyl)-1,2,4-triazole-3-thione (torsion angle $N_1C_5C_9C_{10}$-41,5°) [23]. However, it should be noted that the turn of the phenyl substituent from the packing of molecules in the crystal can vary significantly. For example, in the 3-phenyl-4,5-di-hydro-1,2,4-triazole-3-thione molecule, the torsion angle is $N_1C_5C_9C_{10}$-7,9° [24], and in the 4-allyl-5- (2- hydroxyphenyl) -2,4-dihydro-1,2,4-triazole-3-thione torsion angle $N_1C_5C_9C_{10}$-78,8° [25]. In the crystal, the molecules are connected by intermolecular hydrogen bonds, forming flat ribbons along the c axis, which, in turn, are crosslinked by hydrogen bonds.

The above described triazole reaction allowed us to synthesize a number of interesting compounds for screening for biological activity [26]. The alkylation of triazole **11c** with benzyl chloride in an alcohol solution and monochloracetic acid in an aqueous medium in the presence of potassium hydroxide gave 5-S-substituted triazoles **7, 8, 13, 14**.



In the ^{1}H NMR spectrum of compound **8**, along with the proton signals of two aromatic rings, 6.79-7.49 ppm. and two hydroxyl groups of 10.16 and 12.95 ppm. there is an intense singlet at 4.07 ppm related to the methylene group CH_2.

In order to obtain new thiosemicarbazide derivatives, we [17, 26] also synthesized monosubstituted thiosemicarbazide derivatives by the reaction of o-hydroxybenzoic acid hydrazide (**2**) with potassium thiocyanate. The reaction was carried out in a dilute solution of hydrochloric acid at 95^{0}C for 4 hours.

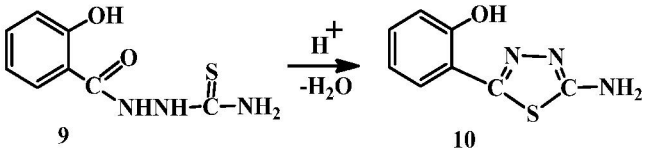


The IR spectrum of thiosemicarbazide o-hydroxybenzoic acid (**9**) contains absorption bands of stretching vibrations of the NH2 group in the region of 3305-3240 cm^{-1}. In the region of 1660 and 1270 cm^{-1}, absorption bands of carbonyl C=O and thiocarbonyl C=S groups are present, respectively.

When analyzing the NMR spectrum of compound **9**, characteristic signals of the protons of the aromatic ring are observed. So, signals of aromatic protons H_1-H_4 are recorded in the field of weak fields: doublet H1 at 6.89 ppm, triplet H2 at 7.42 ppm, doublet H_3 at 6.93 ppm, doublet H_4 at 7.81 ppm The proton of aromatic hydroxyl appears singlet at 9.42 ppm. Amide and thioamide N-H protons are written out as three singlets in the region of 11.89 ppm. (H_5), 10.52 ppm. (H_6) and 7.9 ppm. (H_7).

In organic chemistry, the synthesis of cyclic sulfur-containing compounds is given great importance in view of the presence of valuable biological properties. Derivatives of the cyclic system of 1,3,4-thiadiazole are widely used as medicines, oxidation inhibitors, cyanine dyes and complexing agents with metals. The main method for the synthesis of the thiadiazole system is the cyclization of thiosemicarbazide in a strongly acidic medium [27].

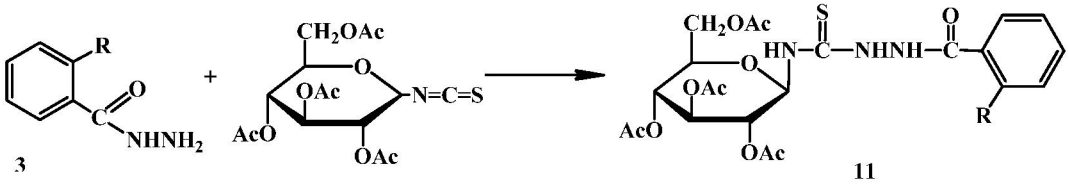
To obtain 5-(o-hydroxyphenyl)-2-(amino)-1,3,4-thiadiazole (**10**), we [26] cyclized the thiosemicarbazide of o-hydroxybenzoic acid (**9**) in an acidic medium (H_2SO_4) for 20 hours As a result of the corresponding treatment, 5-(2-hydroxyphenyl)-2-(amino)-1,3,4-thiadiazole (**10**) was isolated in 75.6% yield, and so on. 265-266^{0}C.



In the IR spectrum of 5-(2-hydroxyphenyl)-2-(amino)-1,3,4-thiadiazole (**10**) there are no absorption bands of the C=S group. Absorption bands: at 3305-3255 cm^{-1} are caused by stretching vibrations of the NH_2 group, 1605cm^{-1} are characteristic of the C=N bond. Absorption bands are also observed in the regions of 1515, 1480, 1390, 1235, 1040, and 860 cm^{-1}, characteristic of vibrations of the 1,3,4-thia-diazole ring [28], and absorption bands at 1320–1300 cm^{-1} are valence C-N bond vibrations in aromatic amines

As is known, glycosyl isothiocyanates play a large role in the chemistry of carbohydrates, being synthons in the synthesis of various biologically active compounds [30]. In addition, it is known that the introduction of carbohydrate residues into the structure of biologically active substances leads to an increase in their solubility in water, a sharp decrease in toxicity [31] and prolonged action of drugs [32].

In connection with this condensation of hydrazides **3, 4** with 1-deoxy-2,3,4,6-tetra-O-acetyl-D-glucopyranosyl isothiocyanate, the corresponding acetylated glycosyl-containing thiosemicarbazide derivatives of o-hydroxybenzoic acid were synthesized (**11**) [16,33,34].



It was found that 1-isothiocyano-1-deoxy-2,3,4,6-tetra-O-acetyl-D-glucopyranose quite readily reacts with o-hydroxybenzoic acid hydrazide in an o-xylene solution at room temperature. The synthesized compound **11** was obtained with a yield of 57.8% and after recrystallization from a mixture of isopropanol-hexane is a white crystalline substance, readily soluble in many organic solvents, except saturated hydrocarbons.

All compounds **3** were tested to evaluate their anti-inflammatory activity (in vitro) on cultures of human MonoMac-6 monocytic cell lines. The anti-inflammatory effect was evaluated as the ability of a compound to inhibit lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF) in MonoMac-6 cells. The cells were treated with the compound for 30 min, then LPS (0.5 μg / ml) was added to the cell culture. Cytokine levels were evaluated after 24-hour incubation. Cytokines were measured in cell supernatants using an enzyme immunoassay (ELISA). The effective concentration, which suppresses the response by 50% (IC50), was found using regression analysis using dose-dependent curves. As a result, it was found that all the studied hydrazone do not suppress LPS-induced production of IL-6 and TNF in MonoMac-6 cells.

Compounds 3 were studied to evaluate their effect on the activity of the neutrophil elastase enzyme (EC 3.4.21.37) (table 1). Elastase activity was evaluated as the ability of a compound to hydrolyze a synthetic substrate N-methylsuccinyl-Ala-Ala-Pro-Val-7-amino-4-methylcoumarin (*Calbiochem*). The formation of a florescent product was measured with excitation of 355 nm and emission of 460 nm on a *Fluoroskan Ascent FL* instrument. An effective concentration causing a 50% inhibition of enzyme activity (IC50) was found by regression analysis.

Table 1 – The inhibitory activity of hydrazones against human neutrophil elastase

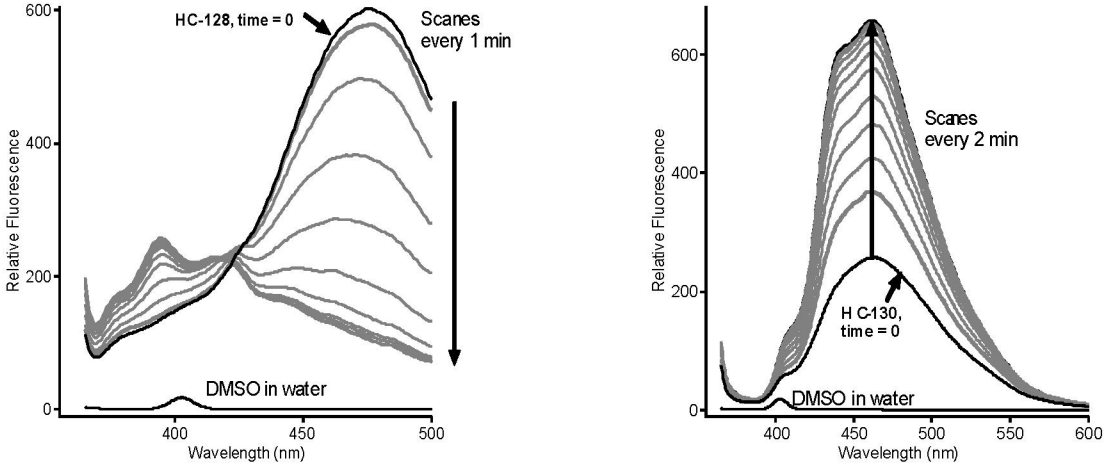
No	Compound	IC_{50}, μM
3a	N-(4-fluorobenzylidene)-2-hydroxybenzohydrazide	N.D.[a]
3b	N-(5-bromo-2-hydroxybenzylidene)-2-hydroxybenzohydrazide	33,3
3c	2-hydroxy-N-(4-methoxybenzylidene)benzohydrazide	N.A.
3d	2-hydroxy-N-(2-hydroxybenzylidene)benzohydrazide	N.A.
3e	2-hydroxy-N-(4-hydroxybenzylidene)benzohydrazide	97,8
3g	N-(3-ethoxy-4-hydroxybenzylidene)-2-hydroxybenzohydrazide	78,8
3f	N-(2-benzylidene-ethyl styrene)-2-hydroxybenzohydrazide	N.D.[a]
3h	2-hydroxy-N-(4-((E)styryl)benzylidene)benzohydrazide	N.D.[б]

[a], the compounds are rapidly hydrolyzed, which is accompanied by either a decrease or an increase in intrinsic fluorescence at 460 nm; [б] the compound has a high level of intrinsic fluorescence.

As a result, it was shown that some hydrazones, in particular N-(5-bromo-2-hydroxybenzylidene)-2-hydroxybenzohydrazide (**3b**), N-(3-ethoxy-4-hydroxyben-zylidene)-2-hydroxybenzohydrazide (**3g**), 2-hydroxy-N-(4-hydroxybenzylidene) benzohydrazide) (**3e**) inhibits neutrophil elastase activity.

Study of the fluorescence spectra of the compounds N-(2-benzylidene-octylene)-2-hydroxybenzohydroside (**3f**) and 2-hydroxy-N-(4-((E)styryl) benzylidene)benzohydrazide (**3h**) during their spontaneous hydrolysis, were recorded on a Perkin Elmer LS50B instrument at λ_{ex}=355 nm. N-(2-Benzylidene-ethylstyrene)-2-hydroxybenzohydroside (**3f**) has a specific fluorescence peak (λ_{ex}=355 nm; λ_{em}=475 nm). In an aqueous medium, **3f** is almost completely hydrolyzed in 10 min, which is accompanied by the disappearance of fluorescence in the region of 460–475 nm and the appearance of a peak of weaker fluorescence at λ=395 nm (fig. 8A). 2-Hydroxy-N-(4-((E)styryl)benzylidene)benzohydrazide (**3h**) has a specific fluorescence peak (λ_{ex}=355 nm; λ_{em}=460 nm). In an aqueous medium, **3h** is almost completely hydrolyzed in 20 min, which is accompanied by an increase in fluorescence at λ=460 nm (figure 8B).

As a result, it was found that spontaneous hydrolysis of hydrazone **3f** and **3h**, proceeding presumably in the imino group >C=N-, is accompanied by a change in the fluorescence spectra of the solution. The synthesized compounds underwent biological tests for the presence of antimicrobial activity under the supervision of PhD. Akhmetova S.B. on the basis of the Department of Microbiology of the Karaganda State Medical University.



A

A. Spectra **3f** (50 μM solution in water with 0.5% DMSO) were taken every 1 min

B

B. Spectra **3h** (5 μM solution in water with 0.5% DMSO) were taken every 2 min

Figure 8 – Kinetics of the fluorescence spectra of hydrazides **3f** and **3h** at λ_{ex}=355 nm (gap width 5 nm)

The obtained experimental data allow us to conclude that substances **3a** and **3b** exhibit weak antibacterial activity against strains of gram-positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, gram-negative strains of *Escherichia coli*, *Pseudomonas aeruginosa* and to yeast *Candida albicans* (for comparison, bacteria and nystatin for the yeast *C.albicans*).

The synthesized compounds **5a, 5g, 6d** passed biological tests for antimicrobial activity [16]. As a result of the bioscreening, it was found that the compound N-allyl-2-(2-hydroxybenzoyl)-hydrazinocarbothioamide **(5a)** exhibits pronounced activity against gram-positive strains (*Staphylococcus aureus, Bacillus subtilis*). Substances **5g** and **6d** exhibit moderate antibacterial activity against gram-positive (*Staphylococcus aureus, Bacillus subtilis*). Compounds **5a, 5g, 6d** show a moderately pronounced activity against the gram-negative strain of *Escherichia coli* and *Pseudomonas aeruginosa* with a pronounced antifungal effect against yeast *Candida albicans*.

Compound **6d** was tested for growth-promoting properties in the biotechnology laboratory of Kazakh Research Institute of fruit growing and viticulture (Almaty). The test was carried out on the processes of common beans, a verified concentration of rhizogenesis regulators -10, 50 and 100 mg/l. An analysis of the data showed that the test growth regulator at different concentrations contributes to better root formation of beans compared to water. The greatest effect on the root formation of the test compound compared to the standard preparation (akpinol) was obtained at a concentration of 50 mg/l. The tested preparation showed rhizogenic activity on bean processes, but it depended on the concentration of the growth regulator. Compounds **5a-d** and **6a-d** were tested to evaluate their anti-inflammatory and cytotoxic activity (*in vitro*) on cultures of human monocytic *MonoMac-6* and *THP-1Blue* cell lines.

The anti-inflammatory effect was evaluated as the ability of the compound to suppress lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor (TNF) in *MonoMac-6* cells, as well as NF-κB-dependent production of alkaline phosphatase (ALP)) in transfected *THP-1Blue* cells. The cells were treated with the compound for 30 minutes, then LPS (0.5 μg/ml) was added to the cell culture. Cytokine or alkaline phosphatase levels were evaluated after 24-hour incubation. Cytokines were measured in cell supernatants using an enzyme immunoassay (ELISA). AP production was measured using a specific Quanti-BlueTM substrate. The level of cytotoxicity was evaluated using the chemiluminescent *CellTiter-Glo* kit. The effective concentration, which suppresses the response by 50% (IC50), was found using regression analysis using dose-dependent curves (at least 5 concentrations). As a result, the investigated thiosemicarbazides **5a-d** and 1,2,4-triazolthione 6a-d were non-cytotoxic and inactive in the anti-inflammatory test *in vitro* at concentrations <100 μM.

Compounds **5a-c** were tested for antioxidant (antiradical) activity. The antiradical effect of the samples was carried out against the DPPH radical. Under the conditions of this test system, sample **5c** showed

pronounced antiradical activity, for which a concentration was determined that could reduce the optical density of a 100 μM solution of the DPPH radical by 50%. For N-phenyl-2-(2-hydroxybenzoyl)hydrazine-carbothioamide (**5c**), the IC50 (DPPH) was 15.5 μM.

The compound N-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-2-(2-hydroxybenzoyl)hydrazinocarbothioamide (**11**) passed a screening test for antimicrobial activity. It was found that this compound exhibits moderate antibacterial activity against gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and gram-negative *Escherichia coli* strain, as well as yeast *Candida albicans.*

Thus, the above material demonstrates the feasibility and prospects of the search for highly effective biologically active substances among the new multifunctional derivatives of o-hydroxybenzoic acid hydrazides. The functionality of o-hydroxybenzoic acid hydrazide and its derivatives emphasize the need to continue work in the field of synthesis of biologically active substances, which may ultimately lead to the identification of new biologically active drugs.

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о-ГИДРОКСИБЕНЗОЙ ҚЫШҚЫЛ ГИДРАЗИДІ МЕН ОНЫҢ ТУЫНДЫЛАРЫ. СИНТЕЗ ЖӘНЕ ҚАСИЕТТЕРІ

Аннотация. Мақалада авторлардың *о*-гидроксилбензой қышқыл гидразид туындыларының биологиялық белсенділігін синтездеудің және зерттеудің жаңа тәсілдерін жасау нәтижелері ұсынылған. *о*-Гидроксибензой қышқылының гидразидтерінің құрылысының өте үлкен синтетикалық және биологиялық потенциалы бар екені, олар оның жаңа биологиялық белсенді қосыларын синтездеу әдістемелерін жасауға мүмкіндік беретіні көрсетілді. Оларға келесідей ұқсас қосалқы зиянды әсерлердің тән екені көрсетілді: адамның іш-құрылысының жұқа қабатына, бүйректің дұрыс жұмысты істеу қызметіне және тағы басқаларға. Ғылыми әдебиетте оксибензой қышқылының әр түрлі туындыларының микробқа қарсы, ісікке қарсы, антипиретті, антифломаторлы және цитотоксикалық әсерлері туралы деректер бар екені анықталды. Әдебиеттердегі салицил қышқылының әртүрлі туындыларының қасиеттері туралы мәліметтері бойынша қышқылдық топтар негізінде орын алмасулар олардың жоғары температураны төмендететін, аналгетикалық, қабынуға қарсылық қасиеттерінің сақталатынын және олардың құрылысында жаңа биологиялық белсенділіктердің пайда болатынын анықталды. Ал салицил қышқылының фенилді тобы бойынша орыналмасулар оларда туберкулезге қарсылық, фунгицидтік, микробтарға қарсылық, антидепрессанттық әкелетіні көрсетілді. Осы тұрғыда олардың көптеген туындыларында, бастапқы субстратқа тән, ауруды қойдыратын, дене ыстығын төмендеткізетін қасиеттердің сақталатыны анықталды (R" = NH_2, Cl, Br, NO_2 және басқалар). Осы қатардағы көптеген биологиялық белсенді заттар негізінен молекуланың бастапқы құрылысымен сутектік байланыстарды түзетін аминді және амидті топтардан, және молекулалардың әр түрлі рецепторлармен қажетті конформациялық пен сәйкестілікті қамтамасыз ететін ароматты топтардан тұратыны көрсетіледі. Гидразонды, оксадиазолды, тиосемикарбазидті, 1,2,4-триазол-3-тионды туындыларды дайындау әдістері және оларды әрі қарай өзгерту әдістері сипатталған. Гидразон туындыларына альдегид ретінде әртүрлі ароматтық альдегидтер қолданылған: 4-фторбензальдегид, 5-бром-2-гидроксибензальдегид, 4-метоксибензальдегид, 2- и 4-гидроксибензальдегидтер. *о*-Гидроксибензой қышқыл гидразидінің тиосемикарбазидті туындылары аллил-, этил-, фенил-, акрилоил-, фураноилизотиоцианаттарды қолдана отырып синтездеген. 1-дезокси-2,3,4,6-тетра-О-ацетил-β-D-глюкопиранозил изотиоцианаты бар гидроксибензой қышқылы гидразидтерінің конденсация реакциясы олардың сәйкес ацетилденген гликозилі бар тиосемикарбазид туындылары синтезделді. 1-Изотиоциано-1-дезокси-2,3,4,6-тетра-О-ацетил-β-D-глюкопираноздың *о*-гидроксибензой қышқыл гидразидімен бөлме температурасында және о-ксилол ерітісінде реакцияға жеңіл түсетіні анықталды. Синтезделген қосылыстардың құрылымы ^{1}H және ^{13}C ЯМР спектроскопиясымен, сонымен қатар COZY (^{1}H-^{1}H) және HMQC (^{1}H-^{13}C) екі өлшемді спектрлерінің мәліметтерімен зерттелінді. Бірөлшемді ЯМР спектрлеріндегі ^{1}H және ^{13}C сигналдарының химиялық ығысуының, көбейтуінің және интегралды қарқындылығының мәні анықталды. Спектрлерді COZY (^{1}H-^{1}H) және HMQC (^{1}H-^{13}C) форматында қолдану, гомо- және гетеронуклеарлық әрекеттесулер құрылды, бұл зерттелетін қосы-

лыстардың құрылымын растайды. Синтезделген қосылыстардың ішіндегі бесеуінің: N-(5-бром-2-гидроксибензилиден)-4-гидроксибензогидразидтің, N-фураноилтиосемикарбазидтің, 4-этил-5-(2-гидроксифенил)-1,2,4-триазол-3-тионның, 4-(2-гидроксиэтил)-5-(2-гидроксифенил)-2*H*-1,2,4-триазоло-3(4*H*)-тионның (6d), 4-аллил-3-(4-гидроксифенил)-1*H*-1,2,4-триазол-5(4*H*)-тионның кеңістіктік құрылымы рентгенқұрылымды анализ арқылы дәлелденген. Моно микробқа қарсы, қабынуға қарсы және цитотоксикалық белсенділігін (*in vitro*) адамның моноцитарлық Mono Mac-6 және THP-1Blue жасушалық желілері культураларында бағалау нәтижелері сипатталған. Қабынуға қарсы белсенділік нәтижесінде барлық зерттелген гидразондар *MonoMac-6* жасушаларында IL-6 және TNF өнімдерін жоймайтыны анықталды. Гидразон туындыларының нейтрофил эластаза ферментінің белсенділігіне әсерін бағалау нәтижесінде (EC 3.4.21.37) кейбір туындылар, атап айтқанда N-(5-бромо-2-гидроксибензилиден)-2-гидроксибензогидразид (3b), N-(3-этокси-4-гидроксибензилиден)-2-гидрокси-бензогидразид, 2-гидрокси-N-(4-гидроксибензилиден)бензогидразид нейтрофил эластазасының белсенділігін тежейтіндігі анықталған. Микробқа қарсы жүргізілген биоскрининг нәтижесі бірнеше қосылыстардың айқын және орташа белсенділіктерінің бар екендігін көрсеткен. N-Фенил-2-(2-гидроксибензоил)-гидразинкарботиоамид ДФПГ-радикалына қарсы айқын антирадикалды белсенділікті көрсетті.

Түйін сөздер: *о*-гидроксибензой қышқылы гидразиді, тиосемикарбазид, гидразон, оксодиазол.

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ГИДРАЗИД *о*-ГИДРОКСИБЕНЗОЙНОЙ КИСЛОТЫ И ЕГО ПРОИЗВОДНЫЕ. СИНТЕЗ И СВОЙСТВА

Аннотация. В статье представлены результаты исследования по разработке новых путей синтеза и исследования биологической активности гидразидных производных *о*-гидроксибензойой кислоты. Показано, что структура гидразидов *п*-гидроксибензойной кислоты обладает большим синтетическим и биологическим потенциалом, что дает возможность разработки методов синтеза новых биологически активных веществ. Им присущи и сходные побочные эффекты: повреждающее действие на слизистую оболочку желудочно-кишечного тракта, нарушение функции почек и некоторые другие. В научной литературе имеются данные об антимикробных, антифунгальных, противоопухолевых, антипиретических, антифламоторных и цитотоксических свойствах различных производных оксибензойной кислоты. Из анализа литературных данных по различным производным салициловой кислоты многие замещения по кислотным группам обеспечивали сохранение жаропонижающих, анальгезирующих, противовоспалительных свойств и появлению новых видов активности. Замещения по фенильному кольцу молекулы салициловой кислоты приводили к появлению противотуберкулезных, фунгицидных, противогрибковых, антидепрессантных и др. свойств. При этом во многих препаратах также сохраняется обезболивающее, жаропонижающее свойства исходного субстрата (R” = NH_2, Cl, Br, NO_2 и др). Установлено, что большинство биологически активных структур этого ряда включают в себя аминную или амидную группы, обеспечивающие образование водородных связей с целевой природной молекулой, а также ароматический фрагмент, отвечающий за необходимую конформацию и комплементарность молекулы тому или иному рецептору. Описаны методы получения гидразоновых, оксадиазоловых, тиосемикарбазидных, 1,2,4-триазол-3-тионовых производных и пути их дальнейшей модификации. Для гидразоновых производных в качестве альдегидов были использованы различные ароматические альдегиды как 4-фторбензальдегид, 5-бром-2-гидроксибен-зальдегид, 4-метоксибензальдегид, 2- и 4-гидроксибензальдегиды. Тиосемикарбазидные производные гидразида *о*-гидроксибензойной кислоты синтезированы с использованием аллил-, этил-, фенил-, акрилоил-, фураноилизотиоцианатов. Реакцией конденсации гидразидов гидроксибензойных кислот с 1-дезокси-2,3,4,6-тетра-О-ацетил-β-D-глюкопиранозилизотиоцианатом были синтезированы их соответствующие ацетилированные гликозилсодержащие тиосемикарбазидные производные. Установлено, что 1-изотиоциано-1-дезокси-2,3,4,6-тетра-О-ацетил-β-D-глюкопираноза довольно легко реагирует с гидразидом *о*-гидроксибензойной кислоты при комнатной температуре в растворе *о*-ксилола. Исследованы строения синтезированных соединений методами ЯМР ^{1}H и ^{13}C спектроскопии, а также данными двумерных спектров COSY (^{1}H-^{1}H) и HMQC (^{1}H-^{13}C). Определены значения химических сдвигов, мультиплетность и интегральная интенсивность сигналов ^{1}H и ^{13}C в одномерных спектрах ЯМР. С помощью спектров

в форматах COSY (^{1}H-^{1}H) и HMQC (^{1}H-^{13}C) установлены гомо- и гетероядерные взаимодействия, подтверждающие структуру исследуемых соединений. Пространственное строение 5-ти соединений: N-(5-бром-2-гидроксибензилиден)-4-гидроксибензогидразида, N-фураноилтиосемикарбазида, 4-этил-5-(2-гидроксифенил)-1,2,4-триазол-3-тиона, 4-(2-гидроксиэтил)-5-(2-гидроксифенил)-2*H*-1,2,4-триазоло-3(4*H*)-тиона, 4-аллил-3-(4-гидроксифенил)-1*H*-1,2,4-триазол-5(4*H*)-тиона доказаны с помощью рентгеноструктурного анализа. Описаны результаты оценки их антимикробной, противовоспалительной и цитотоксической активности (*in vitro*) на культурах человеческих моноцитарных линий клеток MonoMac-6 и THP-1Blue. В результате противовоспалительной активности выявлено, что все изученные гидразоны не подавляют ЛПС-индуцированную продукцию IL-6 и TNF в клетках *MonoMac-6*. В результате оценки влияния гидразоновых производных на активность фермента эластазы нейтрофилов (EC 3.4.21.37) показано, что некоторые производные, в частности N-(5-бром-2-гидроксибензилиден)-2-гидроксибензогидразид, N-(3-этокси-4-гидроксибен-зилиден)-2-гидроксибензогидразид, 2-гидрокси-N-(4-гидроксибензилиден)-бензогидразид подавляют активность эластазы нейтрофилов. Проведенный биоскрининг на антимикробную активность выявил несколько соединений с выраженной и умеренно-выраженной активностью. Соединение N-фенил-2-(2-гидроксибензоил)гидразин-карботиоамид показал выраженную антирадикальную активность в отношении ДФПГ-радикала.

Ключевые слова: гидразид *о*-гидроксибензойной кислоты, тиосемикарбазид, гидразон, оксодиазол.

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REFERENCES

[1] Lisina SV, Brel AK, Mazanova LS, Spasov AA (2008) Synthesis and antipyretic activity of new derivatives of salicylic acid [Sintez i zharoponizhayushchaya aktivnost' novykh proizvodnykh salitsilovoy kisloty] 10:24-26 (in Russ.).

[2] Belikov VG (1993) Synthetic and natural medicines [Sinteticheskiye i prirodnyye lekarstvennyye sredstva] Higher School, Moscow. ISBN: 5060029859 (in Russ.).

[3] Mashkovsky MD (2012) Medicines [Lekarstvennyye sredstva] RIA New Wave LLC, Moscow. ISBN: 5786402878 (in Russ.).

[4] Mashkovsky MD (1998) Medications of the 20th century [Lekarstva XX veka] New Wave LLC, Moscow. ISBN: 5-7864-0072-7 (in Russ.).

[5] Belikov VG (1985) Pharmaceutical Chemistry [Farmatsevticheskaya khimiya] Higher School, Moscow. ISBN: 5-06-003253-1 (in Russ.).

[6] Lisina SV (2006) Successes of modern science [Uspekhi sovremennogo yestestvoznaniya] 11:95 (in Russ.).

[7] Satio H, Tomiioka H, Sato K. (1995) Tuber. Lung. Dis. 76:51 (in Eng.).

[8] Springett VH (1971) Tuber. Lung. Dis. 52:73 (in Eng.).

[9] Dilanyan ER, Hovsepyan TR, Arsenyan FG, Agoronyan AA (1999) Pharmaceutical Chemistry Journal [Khimiko-farmatsevticheskiy zhurnal] 10:15 (in Russ.).

[10] Poplavskaya IA, Khalilova SD (1984) Certificate of authorship [avtorskoye svidetel'stvo] (in Russ.).

[11] Fedorova OV, Mordovsky GG, Rusinov GL, Ovchinnikova IG (1998) Pharmaceutical Chemistry Journal [Khimiko-farmatsevticheskiy zhurnal] 2:11 (in Russ.).

[12] Hogue T, Islam MR (1992) J. Bangladesh Chem. Soc. 2:127 (in Eng.).

[13] Schukina MN, Pershin GN, Sazonova ED (1954) Chemistry and Medicine. Ftivazid [Khimiya i meditsina. Ftivazid] (in Russ.).

[14] Brel AK, Lisina SV, Salomatina YuN, Kovaleva DG (2013) Pharmaceutical Chemistry Journal [Khimiko-farmatsevticheskiy zhurnal] 10:16 (in Russ.).

[15] Alimbaev AS, Zhakina AKh, Gazaliev AM, Nurkenov OA (2007) News of NAS RK. Ser. Chem. [Izvestiya NAN RK. Ser. khim.] 6:50 (in Russ.).

[16] Nurkenov OA, Fazylov SD, Satpaeva ZhB, Seilkhanov TM, Karipova GZh, Isaeva AZh (2014) Synthesis and structure of new derivatives of salicylic acid hydrazide, Russ. J. Gen. Chem. 9:1574-1576. DOI: 10.1134/S1070363214090369 (in Eng.).

[17] Nurkenov OA, Fazylov SD, Akhmetkarimova ZhS, Satpaeva ZhB, Muldakhmetov ZhKh (2012) New 1,3,4-oxazodiazoles based on o- and p-hydroxybenzoic acid hydrazides [Novyye 1,3,4-oksazodiazoly na osnove gidrazidov o- i p-oksibenzoynoy kisloty] Mater. VIII Int. scientific prakt. conf. «Find scientific insights – 2012», Sofia. P. 89-91 (in Russ.).

[18] Alimbaeva AS, Kurapova MYu, Gazaliev AM, Nurkenov OA, Zhakina AKh (2008) Bulletin of the Karaganda State University - Chemistry [Vestnik Karagandinskogo gosudarstvennogo universiteta - Khimiya] 4:48-52 (in Russ.).

[19] Nurkenov OA, Fazylov SD, Zhivotova TS, Satpayeva ZhB, Akhmetova SB, Kurmanbayeva ZhZh, Karipova GZh, Isayeva AZh (2015) Synthesis, chemical transformations and antimicrobial activity of some thiosemicarbazides of o- and p-hydroxybenzoic acid, News of the National academy of sciences of the Republic of Kazakhstan. Ser. chem. and tech. 409:9-14 (in Eng.).

[20] Nurkenov OA, Fazylov SD, Satpaeva ZhB, Turdybekov KM, Karipova GZh, Isaeva AZh, Talipov SA, Ibragimov BT (2015) Synthesis and structure of new 1,2,4 – triazoles derived from *p*-hydroxybenzoic acid hydrazide, Russ. J. Gen. Chem. 1:57-60. DOI: 10.1134/S1070363215010107 (in Eng.).

[21] Nurkenov OA, Satpaeva ZhB, Kulakov IV, Akhmetova SB, Zhaugasheva SK (2012) Synthesis and antimicrobial activity of o- and p-Hydroxybenzoic acid thiosemicarbazides, Russ. J. Gen. Chem. 4:582-585. DOI: 10.1134/S107036321204010X (in Eng.).

[22] Alimbaeva AS, Nurkenov OA, Zhakina AKh, Kulakov IV, Turdybekov DM, Turdybekov KM (2008) News of NAS RK. Series chemistry. [Izvestiya NAN RK. Seriya khimistri] 6:54-59 (in Russ.).

[23] Qi-Chun Ding, Yu-Lin Huang, Miao-Chang Liu, Li-Xue Zhang, Chang-Feng Zhou, Xiao-Qing Cai Z. (2008) Kristallogr. – New Cryst. Struct. 223:289. DOI: 10.1524/ncrs.2008.0124 (in Eng.).

[24] Buzykin BI, Mironova EV, Gubaidullin AT, Litvinov IA, Nabiullin VN (2008) Rus. J. Gen. Chem. 78, 4:634. DOI: 10.1134/S107036320804021X (in Eng).

[25] Karakurt T, Dincer M, Cetin A, Sekerci M (2010) Spectrochim. Acta, Part A. 77:189. DOI:10.1016/j.saa.2010.05.006 (in Eng.).

[26] Nurkenov OA, Kulakov IV, Satpaeva ZhB, Zhakina AKh, Kalugin SA (2011) Bulletin of KazNU [Vestnik KazNU] 322-328 (in Russ.).

[27] Hovsepyan TR, Dilanyan ER, Engoyan AP, Melik-Oganjanyan RG (2004) Chemistry of Heterocyclic Compounds [Khimiya geterotsiklicheskikh soyedineniy] 9:1377-1381 (in Russ.).

[28] Katritsky AR (1966) Physical methods in the chemistry of heterocyclic compounds [Fizicheskiye metody v khimii geterotsiklicheskikh soyedineniy] Mir, Leningrad (in Russ.).

[29] Plattner S (2014) Studying the reducing potencies of antioxidants with the electrochemistry inherently present in electrospray ionization-mass spectrometry, Anal Bioanal Chem. 406(1): 213–224 (in Eng.).

[30] Tashpulatov AA, Rakhmatullaev I, Afanasyev VA, Ismailov N (1988) Journal Organic chemistry [Zhurnal organich khimii] 9:1893 (in Russ.).

[31] Sarymzakova RK, Abdurashitova YuA, Dzhamanbaev ZhA (2006) Vestnik Moscow State University. Ser. 2. Chemistry [Vestnik MGU. Ser. 2. Khimiya] 47:242 (in Russ.).

[32] Stepanenko BN Chemistry and Biochemistry of Carbohydrates: Monosaccharides [Chemistry and Biochemistry of Carbohydrates: Monosaccharides] Higher School, Moscow (in Russ.).

[33] Nurkenov OA, Satpayeva ZhB (2011) Bulletin of KarSU [Vestnik KarGU] 1(61):23-27 (in Russ.).

[34] Nurkenov OA, Ermukhanbetova RA, Satpaeva ZhB (2013) Uzbek Chemical Journal [Uzbekskiy khimicheskiy zhurnal] 1:33-37 (in Russ.).