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Synthesis and Antimicrobial Activity of *o*- and *p*-Hydroxybenzoic Acid Thiosemicarbazides

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Abstract—The *ortho*- and *para*-hydroxybenzoic acid thiosemicarbazide derivatives which are potentially biologically active substances were obtained by the reaction of the corresponding hydrazides with various isothiocyanates. Their structures were determined using IR, ^{1}H NMR spectroscopy, and mass spectrometry. Pronounced antimicrobial activity of synthesized derivatives was revealed.

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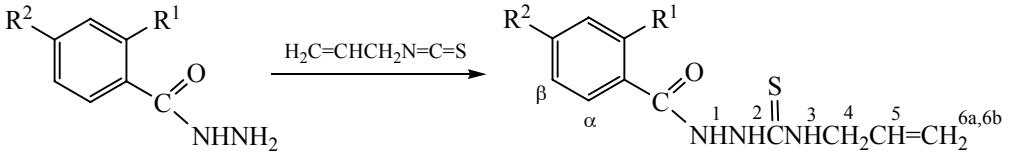
The retrieval of chemical substances possessing antibacterial activity is carried out now on the ground of certain scientific principles and quantitative approaches to predicting the structure of compounds, and in essence their purposeful synthesis is performed. In the development of research in this area several trends can be traced, one of them is the introduction of pharmacophore fragments ino the structure of the desired molecule. Such fragments include the hydrazide and thiosemicarbazide groups and many of their derivatives [1–3]. It is known also that thiosemicarbazide derivatives possess a wide range of biological effects: anticonvulsant, hypoglycemic, anti-inflammatory and antibacterial [4, 5]. One of the first antiviral drugs with the thiosemicarbazide fragment was *tibon* (the *para*-acetaminobenzaldehyde thioacetazone or thiosemicarbazone) [5]. Thiosemicarbazide derivatives are also common among the tuberculostatic preparations [6–8].

In this regard, we were interested in the synthesis of new thiosemicarbazide derivatives based on the salycilic and *p*-hydroxybenzoic acid hydrazides, and in the study of their antimicrobial activity. Special attention deserve in this respect the salicylic acid hydrazide derivatives which are widely used as anti-pyretic, antirheumatic, anti-inflammatory, analgesic and tubercocidal drugs [4, 5]. Initial hydrazides **I** and **II** were obtained by hydrazinolysis of methyl salicylate and nipagin (methyl *o*- and *p*-hydroxybenzoates).

The hydrazides addition to various isothiocyanates is one of the convenient methods of the synthesis of substituted thiosemicarbazides, which are of great interest not only in terms of a possible study of biological activity, but also as the starting compounds for the synthesis on this ground of the pharmacologically important nitrogen-containing heterocycles. For example, we have described [9] the synthesis of salicylic *N*-2-vinyloxyethylthiosemicarbazide and a possibility of its cyclization to 1,2,4-triazole-3(4*H*)-thione.

In continuation of our research and to expand the range of new biologically active substances, we performed a reaction of nucleophilic addition of *o*- and *p*-hydroxybenzhydrazides **I** and **II** to allyl isothiocyanate. The reaction was carried out in alcohol at the equimolar ratio of reagents.

Synthesized compounds **III** and **IV**, respectively, are white crystalline substances, readily soluble in polar organic solvents. The IR spectra of the synthesized compounds contain an absorption band at 1330–1310 cm^{-1} characteristic of –NH–CS group of the thiosemicarbazide fragment, the absorption bands of the amide group C(O)NH appear at 1690–1675 cm^{-1}, and of NH group, at 3390–3360 cm^{-1}.

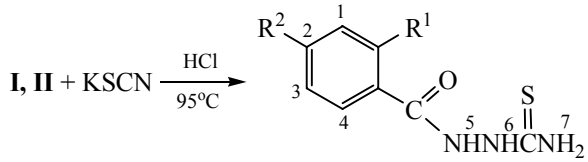


I, II **III, IV**

R^1 = OH, R^2 = H (**I, III**); R^1 = H, R^2 = OH (**II, IV**).

In the ^{1}H NMR spectrum of the *p*-hydroxybenzoic acid allylthiosemicarbazide **IV** the signals of α and β protons of the aromatic ring are in a weak field: H_α doublet at 7.78 ppm [$J(H_\alpha H_\beta)$ = 8.7 Hz] and H_β doublet at 6.81 ppm. Aromatic hydroxyl proton gives rise to a singlet at 9.10 ppm. Amide and thioamide N–H protons also give downfield signals: three singlets, at 6.10 (H^1), 9.25 (H^2) and 8.2 ppm (H^3). The methine proton H^5 of vinyl fragment appears as a complex multiplet at 5.82 ppm. The methylene protons H^{6a} and H^{6b} of the same vinyl fragment are manifested by two doublets at 4.5 and 5.14 ppm with the spin-spin coupling constant $J(H_{6\alpha}H_{6\beta})$ = 17.27 Hz. Methylene protons of the NCH_2 resonate at 4.9 ppm as a broad triplet.

In order to obtain new thiosemicarbazide derivatives we synthesized monosubstituted thiosemicarbazide derivatives by the reaction of *o*- and *p*-hydroxybenzhydrazides **I** and **II** with potassium thiocyanate.



VI, VI

R^1 = OH, R^2 = H (**I, V**); R^1 = H, R^2 = OH (**II, VI**).

The reaction was performed in dilute hydrochloric acid at 95°C for 4 h. Basic physical and chemical characteristics and the elemental analysis data of synthesized compounds **III–VIII** are listed in Table 1.

The IR spectra of *o*- and *p*-hydroxybenzoic acid thiosemicarbazides **V** and **VI** contain absorption bands of stretching vibrations of NH_2 groups in the region of 3305–3240 cm^{-1}. In the regions of 1660 cm^{-1} and 1270 cm^{-1} there are absorption bands of carbonyl (C=O) and thiocarbonyl (C=S) groups, respectively.

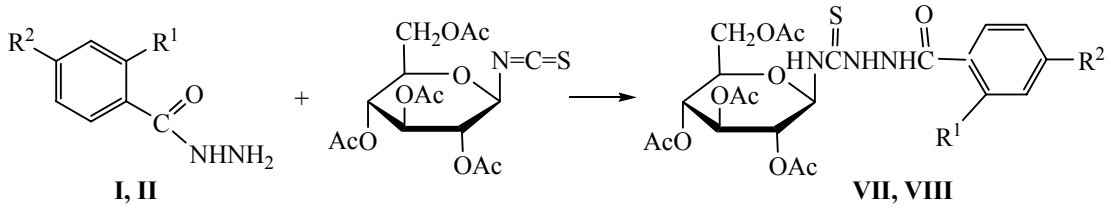
The analysis of ^{1}H NMR spectrum of compound **V** reveals characteristic signals of the aromatic ring. Thus, in a weak field the signals are present of aromatic protons H^1–H^4: H^1 doublet at 6.89 ppm, H^2 triplet at 7.42 ppm, H^3 doublet at 6.93 ppm, and H^4 doublet at 7.81 ppm. Aromatic hydroxyl proton resonates as a singlet at 9.42 ppm. Amide and thioamide N–H protons give three singlets at 11.89 ppm (H^5), 10.52 ppm (H^6), and 7.9 ppm (H^7).

As known, glucosyl isothiocyanates play an important role in carbohydrate chemistry as synthons for producing various biologically active compounds [10]. The introduction of carbohydrate residues in the structure of biologically active substances increases the solubility of the latter in water and leads to a sharp decrease in the toxicity [11, 12]. Therefore by the

Table 1. Physicochemical constants and elemental analysis data of the synthesized compounds **III–VIII**

Comp. no.	Yield, %	mp, °C	Found, %			Formula	Calculated, %		
			C	H	N		C	H	N
III	85	213–215	52.84	5.72	16.96	$C_{11}H_{13}N_3O_2S$	52.57	5.21	16.72
IV	53	215–216	52.90	5.80	17.12	$C_{11}H_{13}N_3O_2S$	52.57	5.21	16.72
V	84	217–218	45.67	4.55	20.12	$C_8H_9N_3O_2S$	45.49	4.29	19.89
VI	52	219–220	45.86	4.49	19.98	$C_8H_9N_3O_2S$	45.49	4.29	19.89
VII	96	136–137	48.95	5.43	7.95	$C_{22}H_{27}N_3O_{11}S$	48.79	5.03	7.76
VIII	57	145–146	49.11	5.45	8.10	$C_{22}H_{27}N_3O_{11}S$	48.79	5.03	7.76

condensation of hydrazides **I** and **II** with 1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate, which was obtained *in situ* from tetra-*O*-acetyl-α-D-glucopyranosyl bromide and lead thiocyanate, we synthesized the corresponding acetylated glucosyl-containing thiosemicarbazide derivatives of *o*- and *p*-hydroxybenzoic acids (**VII, VIII**), potentially possessing a biological activity.



I, II **VII, VIII**

R^1 = OH, R^2 = H (**I, VII**); R^1 = H, R^2 = OH (**II, VIII**).

It is shown that glucosyl isothiocyanate readily reacts with nucleophilic reagents and forms in a high yield the N-substituted acetylated glucosyl-containing thiosemicarbazide derivatives of *o*- and *p*-hydroxybenzoic acid **VII** and **VIII**, respectively. The synthesized compounds are white crystalline substances, soluble in polar organic solvents.

In order to reveal the substances with pronounced biological activity among the synthesized derivatives, we carried out primary screening test of compounds **II–V, VII** for antimicrobial activity against gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) strains by the agar diffusion method. The reference preparations were gentamicin for bacteria and nystatin for fungi. Antimicrobial activity of compounds **II–V, VII** was estimated by measuring the diameter (mm) of the zone of growth inhibition of the test strains. As a result of bioscreening it was found that the studied compound **III** showed a pronounced activity against gram-positive strains (*Staphylococcus aureus*, *Bacillus subtilis*) (Table 2). Compounds **IV**, **V,** and **VII** exhibit moderately strong antibacterial activity only against gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) strains. Compounds **II** and **VII** show moderate activity against gram-negative strain of *Escherichia coli* and yeast fungus *Candida albicans*. Table 2 lists the numerical values of diameters of the zones of growth inhibition of the test strains (mm).

Thus, proceeding from *o*- and *p*-hydroxylbenzhydrazides we obtained thiosemicarbazide derivatives very promising in terms of biological activity, among which are found substances with strong and moderate antimicrobial activity.

EXPERIMENTAL

IR spectra were recorded on a Fourier-transform AVATAR-320 NICOLET spectrometer from the tablets with KBr. ^{1}H NMR spectra were recorded on a

Table 2. Antimicrobial activity of samples[a]

Compound	*S. aureus* 505	*Bacillus Subtilis*	*Pseudomonas aeruginosa*	*E. coli* M–17	*Candida Albicans*
III	24±0.1	26±0.1	14±0.2	20±0.1	18±0.2
IV	18±0.1	20±0.1	–	14±0.1	11±0.3
V	20±0.1	20±0.2	–	11±0.2	–
VII	23±0.2	22±0.1	–	17±0.1	16±0.1
Gentamicin	26±0.1	24±0.1	24±0.1	23±0.2	–
Nystatin	–	–	–	–	22±0.1

[a] "–" means absence of the zone of growth inhibition. The diameters of zones of growth inhibition is less than 10 mm and the continuous growth in the cup was assessed as the absence of antibacterial activity, 10–15 mm is weak activity, 15–20 mm is moderate activity, more than 20 mm is expressed activity.

Bruker DRX500 spectrometer at a frequency of 500 MHz in a solution of DMSO-d_6 relative to internal TMS. Melting points were determined on a Boetius unit. The reaction progress was monitored and the purity of the compounds obtained was checked by TLC on Silufol UV-254 plates in the system isopropyl alcohol–benzene–25% ammonia solution 10:5:2. The plates were developed with the iodine vapor.

4-Allyl-1-(2-hydroxybenzoyl]thiosemicarbazide (III). To a stirred solution of 1.52 g (0.01 mol) of *o*-hydroxybenzhydrazide in 20 ml of ethanol was added dropwise 1.1 ml (0.011 mol) of allyl isothiocyonate. The mixture was stirred for 60 min at 50–60°C. The completion of the reaction was monitored by TLC. At cooling a powdery white crystalline substance precipitated. The recrystallization from 2-propanol gave 2.14 g (85%) of compound **III**, mp 213–215°C.

4-Allyl-1-(4-hydroxybenzoyl)thiosemicarbazide (IV) was obtained similarly in 53% yield, mp 215–216°C (from 2-propanol).

1-(2-Hydroxybenzoyl)thiosemicarbazide (V). A mixture of 1.66 g (0.01 mol) of *o*-hydroxybenzhydrazide **I**, 1.4 g (0.015 m) of potassium thiocyanate, 1.5 ml of hydrochloric acid, and 20 ml of water was heated with stirring for 4 h at 95°C. The reaction mixture was left for a day at room temperature. The solution was alkalinized to pH = 6–7, the precipitate formed was filtered off. The recrystallization from ethanol gave 1.78 g (84%) of of *o*-hydroxybenzoic acid thiosemicarbazide, mp 217–218°C.

1-(4-Hydroxybenzoyl)thiosemicarbazide VI was obtained similarly to **V** in 52% yield, mp 219–220°C (from 2-propanol).

4-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1-(2-hydroxybenzoyl)thiosemicarbazide (VII). To a solution of 1-isothiocyano-1-deoxy-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in *o*-xylene, obtained *in situ* by 8-h boiling of 1.32 g (3.2 mmol) acetobromoglucose with 1.61 g (5 mmol) of lead thiocyanate, was added 0.5 g (0.003 mol) of *o*-hydroxybenzhydrazide and the mixture was stirred at room temperature for about 2 h till the absence of glucosyl isothiocyanate (TLC monitoring). The solution was evaporated to give a white powdery substance. The yield of crude substance 1.72 g (96%). After two recrystallizations from benzene a crystalline product was obtained, mp 136–137°C.

4-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-1-(4-hydroxybenzoyl)thiosemicarbazide (VIII) was obtained similarly to compound **VII** in 57% yield, mp 145–146°C (from 2-propanol).

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