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Antiradical and Antimicrobial Activity of Thiosemicarbaside and 1,2,4-Triazole Derivatives of Hydroxybenzoic Acid

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Abstract—The antimicrobial and antioxidant activity of synthesized biologically active compounds have been evaluated in comparison with the standard antioxidant, ascorbic acid. The antioxidant activity of the compounds has been studied by their ability to interact with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$). The proposed test system has revealed the pronounced antiradical activity of 2-(2-hydroxybenzoyl)-*N*-phenylhydrazinecarbothioamide (**1**) (IC_{50}(DPPH) = 15.5 μM) and 2-(4-hydroxybenzoyl)*N*-phenylhydrazinecarbothiamide (**2**) (IC_{50}(DPPH) = 31.7 μM). A weak antibacterial activity of these compounds has been shown against gram-positive and gram-negative test strains.

Keywords: antioxidant, antiradical, antimicrobial activity, strain, free radical, thiosemicarbazide, 1,2,4-triazole, DPPH

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INTRODUCTION

Salicylic acid derivatives (salicylates) entered clinical practice at the end of the 19th century and are widely used to date. Salicylates, such as acetylsalicylic acid (aspirin), sodium salicylate, salicylanilide, salicylamide (SAM), methyl salicylate are used in medicine as analgesic, antipyretic, antiplatelet, antioxidant, antiproliferative, and cytotoxic agents [1–3]. These compounds also showed antitumor activity [4–6]. According to recent data, the salicylic acid derivatives can be considered as bioregulators, which are synthesized in the body and perform protective functions. These properties of salicylates allow for rethinking their role in the pathophysiology of humans and animals.

The presence of the OH and COOH groups in the neighboring positions of the benzene ring provides great opportunities for chemical transformations of salicylic acid. Salicylic acid is a natural phenolic hormone, which plays an important role in protecting plants from various types of fungi and pathogens. According to the literature data on various derivatives of salicylic acid [7, 8], the following relationships in their structure–biological activity can be noted.

(a) All substitutions in the acidic groups (sites I and II) provided the preservation of antipyretic, analgesic, and anti-inflammatory properties and the appearance of new types of activity (R' = OCH_3, OC_3H_7-i, NH_2, $NHCH_2CH_2$, COOR, etc. [9–11].

(b) Substitutions in the phenyl ring (site III) led to the appearance of antituberculosis, fungicidal, antifungal, antidepressant, and other properties. Many preparations also retain analgesic and antipyretic properties of the original substrate [12–14].

A set of substituted salicylic acid amides that contained the screening *tert*-butyl substituents at the *ortho*-position was obtained in the Vorozhtsov Novosibirsk Institute of Organic Chemistry of the Siberian Branch of the Russian Academy of Sciences (NIOH SB RAS) by directed synthesis based on the osalmid and paracetamol structures [15]. The authors studied the antioxidant properties of the synthesized compounds and proposed two types of interaction, i.e., the interaction with peroxyl radicals and the destruction of hyperoxides with the formation of molecular products.

A targeted search for new effective salicylic acid-based therapeutic agents with increased biological activity, low toxicity, and less pronounced side effects is still an urgent task. A promising direction for creating new bioactive salicylic acid derivatives is the synthesis of hybrid molecules that combine several functional groups in their structure, which independently

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Table 1. Chemical formulas of studied bioactive compounds

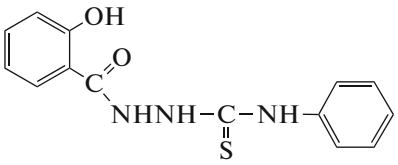
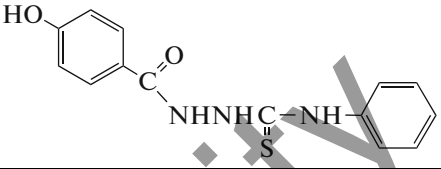
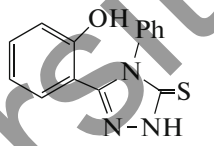
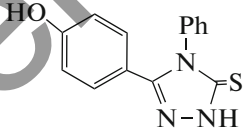
No.	Name of compound	Structural formula
1	2-(2-Hydroxybenzoyl)-*N*-phenylhydrazincarbothioamide (**1**)	
2	2-(4-Hydroxybenzoyl)-*N*-phenylhydrazincarbothioamide (**2**)	
3	3-(2-Hydroxyphenyl)-4-phenyl-1*H*-1,2,4-triazole-5(4*H*)-thion (**3**)	
4	3-(4-Hydroxyphenyl)-4-phenyl-1*H*-1,2,4-triazole-5(4*H*)-thion (**4**)	

Table 2. Optical absorption of 100 μM DPPH$^{\bullet}$ solution after 10-min incubation with the studied compound at their final concentration of 50 μM

No.	Name of compound	Optical absorption, arb. units
1	2-(2-Hydroxybenzoyl)-*N*-phenylhydrazincarbothioamide (1)	0.049
2	2-(4-Hydroxybenzoyl)-*N*-phenylhydrazincarbothioamide (2)	0.297
3	3-(2-Hydroxyphenyl)-4-phenyl-1*H*-1,2,4-triazole-5(4*H*)-thion (3)	0.82
4	3-(4-Hydroxyphenyl)-4-phenyl-1*H*-1,2,4-triazole-5(4*H*)-thion (4)	0.882
	Control (DPPH solution without studied compound)	1.038

or synergistically act on the oxidation processes of substrates in the lipid or aqueous phase.

The goal of this work was to study the antimicrobial and antiradical activity of the thiosemicarbazide and triazole derivatives of *ortho*- and *para*-hydroxybenzoic acids (**1**–**4**) on the model of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and to expand the arsenal of antioxidant agents of synthetic origin based on its analog, salicylic acid.

RESULTS AND DISCUSSION

The research objects are shown in Table 1.

Earlier, we synthesized derivatives of salicylic acid (**1**–**4**) that contained the hydroxyl group in the *para*-position [16, 17], the thioamide and 1,2,4-triazole groups, and the phenyl ring in their structure. The antiradical properties of compounds (**1**–**4**) were evaluated for the first time to identify active antioxidants.

Among compounds (**1**–**4**), only 2-(2-hydroxybenzoyl)-*N*-phenylhydrazinium (**1**) and 2-(4-hydroxybenzoyl)-*N*-phenylhydrazinium (**2**) reduce the optical density of the initial solution of the DPPH radical by more than 50% (Table 2), thus being promising for further research.

In the second series of experiments, we studied the ability of compounds **1** and **2** to interact with the radical at different concentrations (from 2.5 to 50 μM) (Table 3).

The dependence of the optical density of the DPPH$^{\bullet}$ solution on the concentration of compounds **1** and **2** is shown in Fig. 1.

Using the calibration curves (Fig. 1), we evaluated the concentrations of 2-(2-hydroxybenzoyl)-*N*-phenylhydrazinium (**1**) and 2-(4-hydroxybenzoyl)-*N*-phenylhydrazinium (**2**), capable of 50% reduction in optical density of 100 μM DPPH$^{\bullet}$ solution. The

Table 3. Optical absorption of 100 μM DPPH• solution after 10-min incubation with compounds **1** and **2** at their final concentrations of 50, 25, 20, 15, 10, 5, and 2.5 μM in the reaction mixture

No.	Final concentration of compounds **1** and **2**, μM	Optical absorption, arb. units, for compound **1**	Optical absorption, arb. units, for compound **2**
1	50	0.048	0.315
2	25	0.294	0.575
3	20	0.413	0.644
4	15	0.562	0.708
5	10	0.698	0.758
6	5	0.848	0.817
7	2.5	0.929	0.858
	Control (DPPH solution without studied compound)	1.038	1.038

IC_{50}(DPPH) values were 15.5 μM, and 31.7 μM for compounds (**1**) and (**2**), respectively.

Ascorbic acid was used as the reference compound with an antioxidant effect, for which we studied the ability to interact with the DPPH radical at various concentrations (from 2.5 to 50 μM) (Table 4). Ascorbic acid is assigned to water-soluble antiradical compounds, which can interact with free radicals of fatty acid components of lipids [18, 19].

Using the calibration curve (Fig. 2), we evaluated the concentration of ascorbic acid, which can reduce the optical density of 100 μM DPPH• solution by 50%. The IC_{50}(DPPH) value for ascorbic acid was 19.9 μM.

The evaluation of the antiradical actions of samples (**1**–**4**) against the DPPH radical under the used conditions showed that samples **1** and **2** had a more pronounced antiradical activity. We evaluated the concentration of these compounds, which led to a 50% reduction in the optical density of a 100 μM DPPH• solution. The IC_{50}(DPPH) values for 2-(2-hydroxybenzoyl)-*N*-phenylhydrazinium (**1**) and 2-(4-hydroxybenzoyl)-*N*-phenylhydrazinium (**2**) were 15.5 and 31.7 μM, respectively.

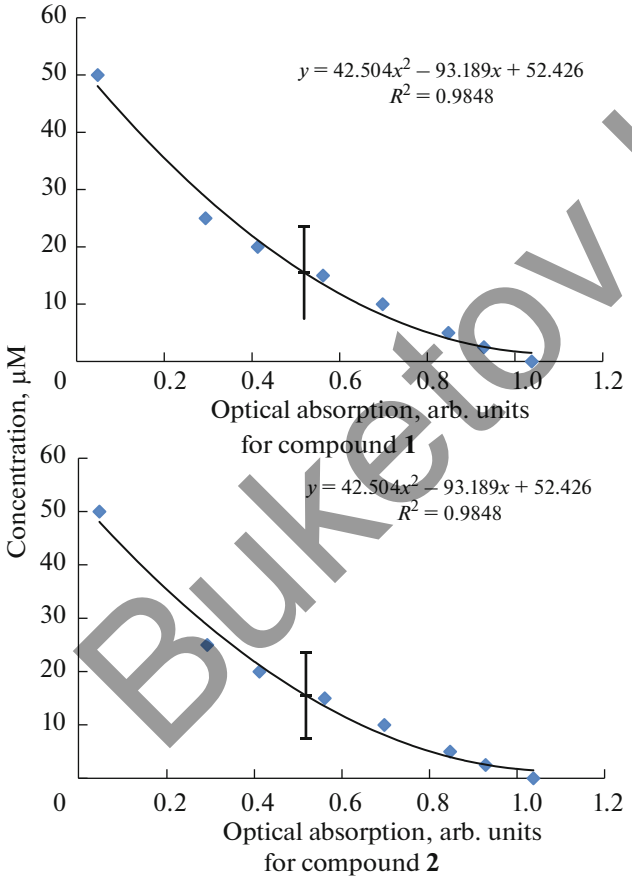


Fig. 1. Dependence of optical absorption of the DPPH• solution on the concentration of 2-(2-hydroxybenzoyl)-*N*-phenyl-hydrazinecarbothioamide (**1**) and 2-(4-hydroxybenzoyl)-*N*-phenyl-hydrazinecarbothioamide (**2**).

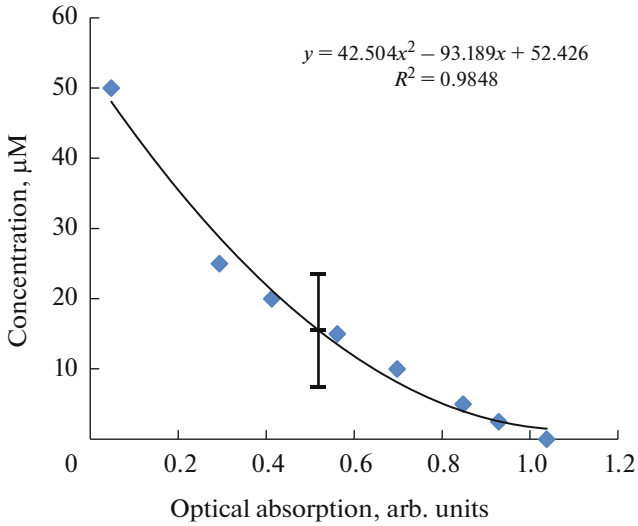


Fig. 2. Dependence of optical absorption of the DPPH• solution on the concentration of ascorbic acid.

Table 4. Optical absorption of 100 μM DPPH$^{\bullet}$ solution after 10-min incubation with ascorbic acid at its final concentrations of 50, 25, 20, 15, 10, 5, and 2.5 μM in the reaction mixture

No.	Final concentration of ascorbic acid, μM	Optical absorption, arb. units
1	50	0.029
2	25	0.418
3	20	0.51
4	15	0.635
5	10	0.694
6	5	0.791
7	2.5	0.865
	Control (DPPH solution without studied compound)	1.028

Table 5. Antimicrobial activity of compounds (**1**–**4**)

Compound	*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
1	18 ± 1	16 ± 1	14 ± 1	12 ± 1	13 ± 1
2	12 ± 1	11 ± 1	10 ± 1	–	12 ± 1
3	13 ± 1	13 ± 1	12 ± 1	–	11 ± 1
4	12 ± 1	14 ± 1	12 ± 1	–	11 ± 1
Gentamicin	26 ± 1	24 ± 1	23 ± 1	24 ± 1	–
Nystatin	–	–	–	–	22 ± 1

According to our data, the IC_{50}(DPPH) value for the reference sample (ascorbic acid) was 19.9 μM. Thus, 2-(2-hydroxybenzoyl)-*N*-phenylhydrazine carbothioamide (**1**) (IC_{50}(DPPH) = 15.5 μmM), is not inferior in its activity to the reference sample.

According to the literature data [20], the IC_{50}(DPPH) values (μM) for known antioxidants were 49 (glutathione), 27 (hydroquinone), 28 (trolox), 28 (α-tocopherol), and 8 (quercetin). Thus, the antiradical activity of samples **1** and **2** is comparable to that of known antioxidants.

To continue research on the detection of a pronounced biological activity of the synthesized compounds, we performed primary screening tests of compounds (**1**–**4**) for their antimicrobial activity against the gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria and the *Candida albicans* yeast strain by the diffusion in agar method.

The antimicrobial activity of compounds (**1**–**4**) was evaluated by the diameter of zones of growth retardation of the test strains (mm). The diameter of these zones in the Petri dish less than 10 mm (and continuous growth), 10–15, 15–20, and over 20 mm were considered as the absence of antibacterial activity, weak activity, moderate activity, and pronounced activity, respectively. Each sample was tested in three parallel experiments [21].

The results of studying the antimicrobial activity of the samples are shown in Table 5.

EXPERIMENTAL

We used DPPH (Sigma-Aldrich); 100 μM methanol solution of the DPPH radical was used to study the antiradical activity of samples (**1**–**4**). To choose compounds with the pronounced antiradical activity, 100 μM methanol solution of DPPH$^{\bullet}$ (2 mL) was mixed with 5 μM methanol solution of the studied compound (20 μL). The final concentration of the studied compound in the reaction mixture was 50 μM. After 10 min, the optical absorption in the reaction mixture was measured at 515 nm. The compounds, which were able to reduce the optical absorption by more than 50%, were tested in the reaction with the DPPH radical at the different final concentrations (50, 25, 20, 15, 10, 5, and 2.5 μM), and the concentration, which led to the decrease in the optical absorption by 50% (IC_{50}(DPPH) was determined.

The antimicrobial activity of the above samples was studied against strains of gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative strains of *Escherichia coli* and *Pseudomonaus aeruginosa*, and yeast fungus *Candida albicans* by diffusion in agar (wells). Gentamicin for bacteria and nystatin for the yeast fungus *C. albicans* were used as the reference preparations by the diffusion in the agar method.

The cultures were grown on a liquid medium, pH 7.3 ± 0.2, at a temperature from 30 to 35°C for 18–20 hours. The cultures were diluted with a sterile isotonic 0.9% sodium chloride solution (1 : 1000), added

by 1 mL in each dish that contained an appropriate elective nutrient medium for the test strains, and sown using the continuous lawn method. After drying, wells with a size of 6.0 mm were formed on the agar surface, and the solution of the reference sample, (gentamicin or nystatin) was added. The equivolume amount of ethyl alcohol was used as the control. The cultures were incubated at 37°C and counted after 24 h.

CONCLUSIONS

(1) It was found that the antiradical activity of compounds 1 and 2 have a pronounced antiradical effect, which is comparable to that of the known antioxidants.

(2) All studied compounds were found to have weak antimicrobial activity against gram-positive and gram-negative test strains.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain studies that use humans and animals as objects of research.

Conflict of Interests

The authors state that there is no conflict of interests.

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