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Biocidal Activity of Benzothiazole and Arylaminomalonate Derivatives

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Abstract—The biocidal activity of some benzothiazoles, arylaminomalonates, and their phosphorylated derivatives against iron-oxidizing bacteria of the genus *Siderococcus*.

Keywords: phosphonates, aminomalonates, benzothiazoles, biocidal activity, iron-oxidizing bacteria, *Siderococcus*

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INTRODUCTION

One of the reasons for the low quality of drinking water is increased concentration of iron, which may be caused by biocorrosion of equipment. Biocorrosion is the result of oxidation of metals by various microorganisms. Compounds formed in this process impair performance of materials and constructions and are also harmful to the environment and human health.

One of the main species inducing biocorrosion are iron-oxidizing bacteria. Their vital activity is believed to be responsible for corrosion of water pipes. Iron bacteria proliferate on the inner pipe surface to form slimy agglomerates impregnated with iron(III) hydroxide and lumps that are difficult to separate from the pipe surface. As a result of growth of iron bacteria, the inner diameter of water pipes decreases by 20% [1], whereas iron hydroxide deposits could clog pipes completely.

Although iron-oxidizing bacteria themselves are not harmful to human health, metabolites released as a result of their vital activity may be hazardous, and increased metal content of water could give rise to the risk of chronic diseases [2]. In addition, growth of iron-oxidizing bacteria creates favorable conditions for the growth of other microorganisms, e.g., thionic bacteria, which also belong to the group of corrosive micro-organisms.

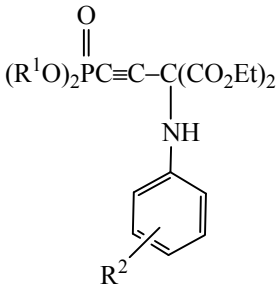
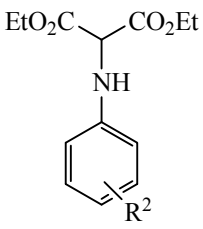
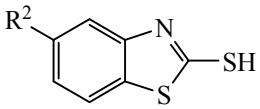
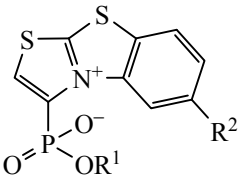
Thus, iron bacteria cause severe damage to human economic activity. Therefore, an important problem is search for efficient and inexpensive methods for the protection of metal constructions from biocorrosion, in particular by the use of biocides.

At present, biocides are extensively studied as biocorrosion inhibitors [3]. Biocides belong to different classes of organic and inorganic compounds, and mechanisms of their action are also different. Organic inhibitors are adsorbed only on the metal surface, whereas corrosion products do not adsorb than. Therefore, these inhibitors are used in acid etching of metals to remove rust, dross, and scales. The most widely used organic corrosion inhibitors are aliphatic and aromatic compounds containing nitrogen, sulfur, and oxygen atoms.

Positive results [4] in preventing biocorrosion were obtained with the aid of bactericidal additives, such as benzothiazole-2-thiol and its zinc salt, benzothiazolyl disulfide, tetramethylthiuram disulfide, dithiocarbamic acid esters, salicylanilide zinc and nickel salts, methylnaphthol, and some thiophene derivatives. Numerous studies also deal with biocidal properties of benzothiazoles [5, 6].

There are chemical compounds that interfere with the synthesis of cell components; they are structural analogs of the corresponding compounds or antimeta-

Table 1. Structures of the compounds tested

Compound no.	Formula	R^1	R^2
1		Et	*p*-Cl
2		Et	*m*-OCH_3
3		Me	H
4		Me	*m*-CH_3
5		Me	*p*-F
6		Et	*p*-OCH_3
7			*p*-Cl
8			H
9			*p*-F
10			*p*-Br
11			*p*-OCH_3
12			CH_3
13			NH_2
14			Cl
15		Et	*p*-CH_3
16		Et	*p*-NH_2
17		Et	*p*-Cl
18		Et	*m*-OCH_3

bolites. For example, malonates are ctructural analogs of succinate. The presence of malonic acid even in a low concentration suppresses the transformation of succinate to fumarate. In this case, the normal metabolite (succinate) competes with its structural analog (malonate) for the active site of succinate dehydrogenase, i.e., structural similarity of inhibitors to cellular metabolites underlies competitive inhibition [7].

The goal of the present study was to estimate the biocidal activity of benzothiazole-2-thione and 2-(arylamino)malonate derivatives.

RESULTS AND DISCUSSION

In this work we studied the biocidal activity of benzothiazole and 2-(arylamino)malonate derivatives [8] which were synthesized at the Department of Organic Chemistry of the St. Petersburg State Institute of Technology.

The test culture was iron-oxidizing bacteria of the genus *Siderococcus* from the collection of the Department of Technology of Microbiological Synthesis (St. Petersburg State Institute of Technology). This bacterial strain was harvested from the waterworks of Shlisselburg town.

The effect of compounds on the bacterial growth was estimated by standard agar well diffusion and serial dilution methods. The inoculum was prepared on a selective solid nutrient medium using a McFarland standard no. 5. The concentration of bacterial suspension was 1.5×10^8 CFU/mL.

The formulas of the synthesized benzothiazole and arylaminomalonate derivatives are given in Table 1. Table 2 shows the results of studying the biocidal activity of 18 compounds against iron-oxidizing bacteria of the genus *Siderococcus* by the serial dilution assay. The highest biocidal activity was found for compound **6** [minimum inhibitory concentration (MIC) 3.13 mg/mL].

Table 2. Biocidal activity of benzothiazole and arylaminomalonate derivatives

Compound no.	Concentration, mg/mL								
	50.00	25.00	12.50	6.25	3.13	1.56	0.78	0.39	0.20
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+
6	–	–	–	–	–	+	+	+	+
7	–	–	–	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+
9	–	–	–	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+
13	+	+	+	+	+	+	+	+	+
14	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+
16	+	+	+	+	+	+	+	+	+
17	+	+	+	+	+	+	+	+	+
18	+	+	+	+	+	+	+	+	+

Table 3. Biocidal activity of benzothiazole and arylaminomalonate derivatives

Compound no.	Inhibition zone diameter, mm	Compound no.	Inhibition zone diameter, mm
1	No inhibition	**10**	No inhibition
2	No inhibition	**11**	No inhibition
3	21.5±0.5	**12**	22.5±1.5
4	17.0±1.0	**13**	16.0±2.0
5	16.0±2.0	**14**	23.0±2.0
6	23.0±2.0	**15**	16.0±0.5
7	14.5±0.5	**16**	No inhibition
8	20.0±0.1	**17**	12.5±0.5
9	No inhibition	**18**	14.5±0.5

The MIC value for compounds **7** and **9** was 12.50 mg/mL. Compounds **1**–**5**, **8**, **9**, and **12**–**18** in the concentration range from 50.00 to 0.20 mg/mL did not inhibit the growth of iron bacteria.

Table 3 contains the results of the agar well diffusion assay for the compounds at a concentration of 50 mg/mL. Petri dishes inoculated with the test culture were incubated for 48 h at 28°C. The biocidal

Table 4. Inhibition zone diameters, mm

Compound no.	Concentration,[a] mg/mL						
	50.000	20.000	10.000	1.000	0.100	0.010	0.001
3	21.5±0.5	23.0±0.5	17.0±0.5	16.0±0.1	15.5±0.5	–	–
6	23.0±2.0	17.0±0.5	19.5±0.5	17.5±0.5	18.0±0.5	–	–
8	20.0±0.1	20.0±0.1	20.0±0.1	18.5±0.5	17.0±1.0	–	–
12	22.5±1.5	21.5±0.5	20.5±0.5	18.5±0.5	18.5±0.5	–	–
14	23.0±2.0	20.0±0.1	17.5±0.5	17.5±0.5	16.5±0.5	–	–

[a] "–" stands for the absence of inhibitory effect.

activity was evaluated by the inhibition zone diameter. Compounds **3**, **6**, **8**, **12**, and **14** showed the highest activity, and these compounds were assayed for minimum inhibitory concentration, which was estimated at 0.1 mg/mL.

Taking into account published data and structural similarity of malonates to succinates, it may be presumed that the compounds under study are involved in the Krebs cycle, thus interfering with the synthesis of cellular components. However, not all aminomalonates showed biocidal activity. According to the data in Table 3, compounds **6**–**8** inhibited the growth of iron bacteria only at the maximum concentration (50 mg/mL), whereas compounds **9**–**11** turned out to be inactive.

Phosphorylated aminomalonates **1**–**6** lack CH group, so that they cannot be entangled in the Krebs cycle. Nevertheless, most phosphorylated aminomalonates did exhibit biocidal activity (compounds **3**–**5**). These findings allowed us to presume that the biocidal properties originate not only from the structural similarity between malonate and succinate fragments; probably, in this case, the active site is the amine fragment. To verify this assumption, it is necessary to estimate biocidal activity of the initial amines.

Benzothiazole-2-thiones showed high biocidal activity. For example, compound **12** turned out to be eactive even at a concentration of 0.1 mg/mL. However, the activity of phosphorylated benzothiazole-2-thiones (compounds **15**–**18**) was lower, presumably due to substitution of highly biologically active thione fragment.

CONCLUSIONS

The highest biocidal activity against iron-oxidizing bacteria was found for compounds of the arylaminomalonate and benzothiazole series. The minimum inhibitory concentration of diethyl 2-anilino-2-(dimethoxyphosphorylethynyl)malonate (**3**), diethyl 2-(diethoxyphosphorylethynyl)-2-(4-methoxyanilino)-malonate (**6**), diethyl 2-anilinomalonate (**8**), 5-methyl-1,3-benzothiazole-2-thiol (**12**), and 5-chloro-1,3-benzothiazole-2-thiol (**14**) was estimated at 0.1 mg/mL.

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