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Synthesis and the antimicrobial activity study of the novel derivatives of 4-oxo- and 4-thio-5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines

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ABSTRACT

The methods for preparation of novel derivatives of ethyl 5-(5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-6-yl)-1,2,4-oxadiazole-3-carboxylate, 3-substituted 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-d]pyrimidin-4(3H)-ones and 4-(alkylthio)-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-d]pyrimidines starting from 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylic acid have been developed. The results of the antimicrobial activity screening, performed by agar well diffusion method, for the compounds synthesized showed that ethyl 5-(5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidin-6-yl)-1,2,4-oxadiazole-3-carboxylate and its 3 p-methylbenzyl derivative together with 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-d]pyrimidine-4(3H)-thione and its 4-S-alkyl derivatives are active against the Candida albicans fungi, while 2-[5-methyl-4-oxo-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-d]pyrimidin-3(4H)-yl]-N-phenylacetamide significantly inhibited the growth of Staphylococcus aureus and Bacillus subtilis bacterial strains.

Keywords: thiophene, pyrimidine, 1,2,4-oxadiazole, thione, alkylation.

INTRODUCTION

The derivatives of thieno[2,3-*d*]pyrimidine substituted at position 6 with the heterocyclic radicals are known as useful for treatment of obesity, and CNS discords [1]; for the similar compounds significant radical scavenging [2], antiviral properties [3], and fatty acids metabolism regulation activity [4] have been reported. The recent investigations of our research group allowed determining of some 6-hetarylthieno[2,3-*d*]pyrimidines with antimicrobial activity [5-8]. Therefore as the part of our work enlargement the novel derivatives of 4-oxo- and 4-thio-5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines were obtained and their antimicrobial activity has been studied.

EXPERIMENTAL SECTION

Chemical part

Melting points (°C) were measured with a with a Kofler melting point apparatus and were not corrected. Elemental analysis were within ±0.4% of the theoretical value. ^{1}H spectral data was obtained at 200 MHz on Varian Mercury-200 spectrometer using TMS as an internal standard. Mass-spectral analyses were obtained on a PE SCIEX API 150EX device.

5-Methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid (1) and 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one were obtained by the previously reported methods [5,9].

Ethyl 5-(5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)-1,2,4-oxadiazole-3-carboxylate (2).
To the suspension of 1.5 g of 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid **1** in 5 ml of anhydrous DMF 1.2 g of 1,1'-carbonyldiimidazole was added and the mixture was heated at 90°C till the clear

solution formation and then for 15 minutes more. Then 1.2 g of ethyl 2-oximinooxamate was added to the reaction mixture and it was heated for 3-4 hours at 130°C. After the cool reaction mixture was diluted with 20 ml of cold water and the precipitate formed was filtered off and crystallized from ethanol.

This compound was obtained in 54% yield as a brown solid, mp 228-230˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.64 (3H, t, CH_3); 2.46 (3H, s, CH_3); 4.19 (2H, q, CH_2); 8.12 (2H, m, CH + NH). Anal. calcd. for $C_{12}H_{10}N_4O_4S$: H, 3.29; C, 47.06; N, 18.29; S, 10.47. Found: H, 3.44; C, 47.15; N, 18.37; S, 10.64.

General method for synthesis of 3-alkyl-5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones (4-6a-c).
To the 0.0005 mole of the 5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one **2** or **3** in 2.5 ml of DMF 0.0005 mole of K_2CO_3 and the corresponding alkylating agent was added. The reaction mixture was stirred and heated at 50-60°C for 8 hours and then cooled and quenched with water. The precipitate formed was filtered off and crystallized from 2-propanol.

Ethyl 5-[5-methyl-3-(4-methylbenzyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl]-1,2,4-oxadiazole-3-carboxylate (4).
This compound was obtained in 85% yield as a brown solid, mp 193-195˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.29 (3H, t, CH_3); 2.21 (2H, s, CH_3); 2.88 (3H, s, CH_3); 4.39 (2H, q, CH_2); 5.11 (2H, s, CH_2); 7.13 (2H, d, Ar-H); 7.27 (2H, d, Ar-H); 8.79 (1H, s, CH). Anal. calcd. for $C_{20}H_{18}N_4O_4S$: H, 4.42; C, 58.53; N, 13.65; S, 7.81. Found: H, 4.53; C, 58.58; N, 13.78; S, 7.88.

2-[5-Methyl-4-oxo-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-*N*-phenylacetamide (5)
This compound was obtained in 79% yield as a white solid, mp > 300˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.94 (2H, s, CH_3); 4.87 (3H, s, CH_2); 7.01 (1H, t, Ar-H); 7.31 (2H, t, Ar-H); 7.57 (2H, d, Ar-H); 8.55 (1H, s, CH); 10.46 (1H, s, NH). Anal. calcd. for $C_{23}H_{17}N_5O_3S$: H, 3.86; C, 62.29; N, 15.79; S, 7.23. Found: H, 3.97; C, 62.55; N, 15.85; S, 7.35.

5-Methyl-3-{[3-(4-methylphenyl)-1,2,4-oxadiazol-5-yl]methyl}-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*] pyrimidin-4(3*H*)-one (6a)
This compound was obtained in 73% yield as a white solid, mp 235-237˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.34 (1H, s, CH_3); 2.91 (2H, s, CH_3); 5.63 (3H, s, CH_2); 7.32 (2H, d, Ar-H); 7.57 (3H, m, Ar-H); 7.83 (2H, d, Ar-H); 8.04 (2H, d, Ar-H); 8.80 (1H, s, CH). Anal. calcd. for $C_{25}H_{18}N_6O_3S$: H, 3.76; C, 62.23; N, 17.42; S, 6.65. Found: H, 3.81; C, 62.29; N, 17.27; S, 6.65.

3-{[3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl]methyl}-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*] pyrimidin-4(3*H*)-one (6b)
This compound was obtained in 76% yield as a white solid, mp 225-227˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.95 (2H, s, CH_3); 5.66 (3H, s, CH_2); 7.52-7.67 (5H, m, Ar-H); 7.96 (2H, d, Ar-H); 8.06 (2H, m, Ar-H); 8.79 (1H, s, CH). Anal. calcd. for $C_{24}H_{15}ClN_6O_3S$: H, 3.01; C, 57.32; N, 16.71; S, 6.38. Found: H, 3.08; C, 57.49; N, 16.78; S, 6.49.

3-{[3-(2,3-Dimethoxyphenyl)-1,2,4-oxadiazol-5-yl]methyl}-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno [2,3-*d*]pyrimidin-4(3*H*)-one (6c)
This compound was obtained in 63% yield as a white solid, mp 222-224˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.93 (2H, s, CH_3); 3.72 (3H, s, OCH_3); 3.83 (3H, s, OCH_3); 5.65 (3H, s, CH_2); 7.12-7.35 (3H, m, Ar-H); 7.50-7.62 (2H, m, Ar-H); 8.04 (2H, m, Ar-H); 8.79 (1H, s, CH). Anal. calcd. for $C_{26}H_{20}N_6O_5S$: H, 3.81; C, 59.08; N, 15.90; S, 6.07. Found: H, 3.86; C, 59.11; N, 15.92; S, 6.09.

5-Methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidine-4(3*H*)-thione (7)
To the compound **3** (2 g) 15 ml of $POCl_3$ was added and the mixture was boiled for 48 hours till the formation of the clear solution. Then the $POCl_3$ excess was distilled off at reduced pressure. The residual solid was treated with cold water and then filtered off and dried at room temperature. To the suspension of 1.5 g of the chloro derivative prepared in the previous step in 5 ml of DMF 0.4 g of thiourea was added and the mixture was heated at 130°C for 3 hours. After the cool reaction mixture wad diluted with water and the precipitate formed was filtered off.

This compound was obtained in 56% yield as a yellow solid, mp > 300˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.22 (2H, s, CH_3); 7.51 (3H, m, Ar-H); 8.04 (2H, m, Ar-H); 8.07 (2H, m, Ar-H); 8.29 (1H, s, CH); 13.95 (1H, br s, NH). LC-MS: m/z (MH^+) 327. Anal. calcd. for $C_{15}H_{10}N_4OS_2$: H, 3.09; C, 55.20; N, 17.17; S, 19.65. Found: H, 3.15; C, 55.24; N, 17.18; S, 19.71.

General method for synthesis of the compounds 4-(alkylthio)-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines (8a,b).
To the suspension of the compound **7** in 3 ml of DMF 0.0008 mole of triethylamine and 0.0008 mole of the corresponding alkylating agent ware added. The reaction mixture was stirred at 90°C for 5 hours. The cool reaction mixture was diluted with water and the precipitate formed was filtered off and crystallized from 2-propanol.

4-(Benzylthio)-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidine (8a)
This compound was obtained in 88% yield as a white solid, mp 203-204˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.13 (2H, s, CH_3); 4.67 (3H, s, CH_2); 7.25-7.40 (2H, m, Ar-H); 7.45-7.62 (6H, m, Ar-H); 8.04 (2H, m, Ar-H); 8.96 (1H, s, CH). Anal. calcd. for $C_{22}H_{16}N_4OS_2$: H, 3.87; C, 63.44; N, 13.45; S, 15.40. Found: H, 3.93; C, 63.50; N, 13.58; S, 15.51.

2-{[5-Methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4-yl]thio}-*N*-phenylacetamide (8b)
This compound was obtained in 74% yield as a white solid, mp 243-244˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.17 (2H, s, CH_3); 4.37 (3H, s, CH_2); 7.04 (1H, t, Ar-H); 7.31 (2H, t, Ar-H); 7.5-7.64 (5H, m, Ar-H); 8.04 (2H, m, Ar-H); 8.87 (1H, s, CH); 10.39 (1H, br s, NH). LC-MS: m/z (MH^+) 460. Anal. calcd. for $C_{23}H_{17}N_5O_2S_2$: H, 3.73; C, 60.11; N, 15.24; S, 13.95. Found: H, 3.77; C, 60.18; N, 15.25; S, 13.99.

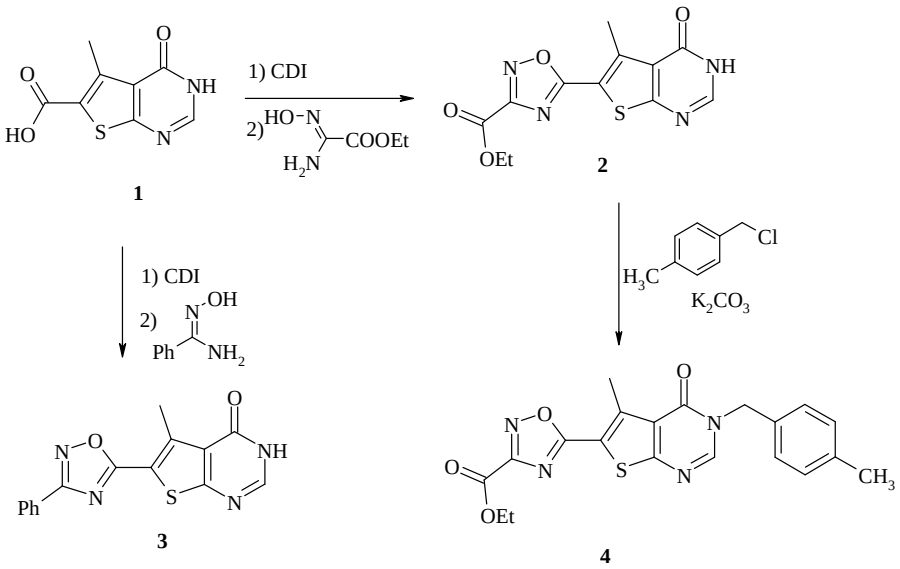
Antimicrobial activity study
According to the WHO recommendations [10-15] the following microorganisms test-strains have been used *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC653/885. Bacterial concentration was 10^7 CFU/ml (determined by McFarland standard). Overnight cultures kept for 18-24 h at 36ºC ± 1ºC were used. The bacterial suspension was inoculated onto the entire surface of a Mueller-Hinton agar (Dagestan Scientific research institute of nutrient media). The compounds were introduced to the wells in the form of DMSO solution in concentrations 100 μg/ml; the open wells were filled with 0.3 ml of the solution.

For evaluation of antimicrobial activity the following criteria were used: in the case of inhibition zone absence or its diameter less than 10 mm either the bacteria strains were considered to be resistant or the concentration of the tested compound rather low for inhibition effect; the diameter of inhibition zone 10-15 mm — low sensitivity of the bacteria strain to the compound in the given concentration; the diameter of inhibition zone 15-25 mm was considered as the sign of the substance activity against the microorganism strain; the diameter of inhibition zone 25 mm or more was considered as the evidence of the high antimicrobial activity of the compound tested.

RESULTS AND DISCUSSION

Recently we have reported the synthesis of 5-methyl-6-(3-aryl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one derivatives, which was performed by cyclization of generated *in situ* imidazolide of 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid **1** with aromatic amidoximes [5]. It is also known that cyclization of ethyl 2-oximinooxamate with carboxylic acids promoted with coupling-reagents or their chlorides is the way for preparation of 1,2,4-oxadiazole cycle with carbethoxy group at its position 3 [16-18].

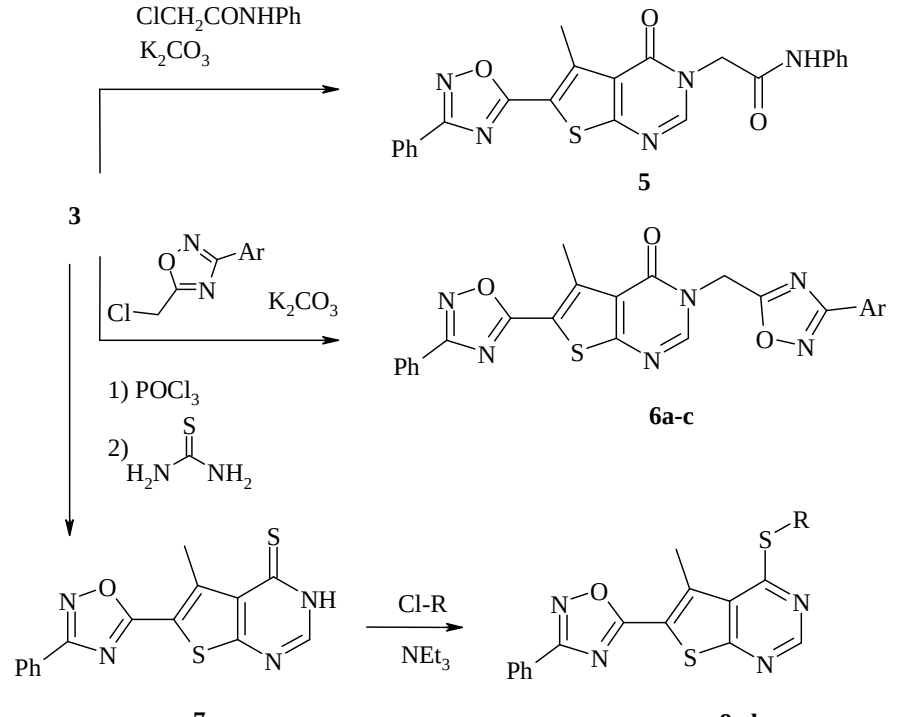
As the part of our research program of 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid **1** modification we performed the 1,1'-carbonyldiimidazole promoted interaction of this compound with ethyl 2-oximinooxamate (scheme 1). As the result of this reaction performed at heating 140°C for 3-4 hours we isolated the product **2**. The ^{1}H NMR spectrum of the compound **2** contains the signals of carbethoxy group protons as 1.64 ppm (3H, t, CH_3) and 4.19 ppm (2H, q, CH_2), together with the intense singlet of the pyrimidine ring proton at 8.12 ppm.



Scheme 1. Synthesis of ethyl 5-(5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)-1,2,4-oxadiazole-3-carboxylate derivatives 2,4 and 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one 3

Further the product **2** was alkylated with *p*-methylbenzyl chloride in DMF-K_2CO_3 conditions, which resulted in the compound **4**. In the ^{1}H NMR spectrum of the compound **4** the signal of CH_2 benzylic fragment is observed at 5.11 ppm, which well correlates with the data obtained for the similar heterocyclic systems. It was earlier confirmed by the NOESY experiments that benzylation of 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones in the same conditions occurs at position 3 of thieno[2,3-*d*]pyrimidine system [5].

In order to enlarge the variety of the 5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines we have modified the compound **3**, obtained using the previously reported method [5] with 2-chloro-N-phenylacetamide and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles [19] (scheme 2). The resulted derivatives **5** and **6 a-c** were isolated in high yields (scheme 2).



6a: Ar = 4-CH_3-C_6H_4-; 6b: Ar = 4-Cl-C_6H_4-; 6c: Ar = 2,3-di(OCH_3)-C_6H_4-; 8a: R = C_6H_5-CH_2-; 8a: R = $C_6H_5NHCOCH_2$-

Scheme 2. Synthesis of 3-alkyl-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones 5,6a-c and 4-(alkylthio)-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines 8a,b

The other way for modification of the compound **3** was the exchange of the oxo-group in position 4 of thieno[2,3-*d*]pyrimidine with the sulphur. The transformation was performed similarly to the method proposed earlier [6]. The main problem for this procedure was extremely low solubility of the compound **3** in $POCl_3$, which critically complicated the chlorination step. To accomplish the chlorination 48 hours of the compound **3** boiling in phosphorus oxychloride was required. The unstable 4-chloroproduct was immediately reacted with thiourea in DMF. Then ^1H NMR spectrum of the isolated product **7** contains the signal of NH proton at 13.95 ppm, which is typical for such thiones [6]; mass-spectrum of the derivative **7** has the peak of quasimolecular ion (m/z [MH^+] = 327) well corresponding with its molecular weight.

The product **7** obtained was then alkylated with benzyl chloride and N-phenylchloroacetamide using DMF-triethylamine conditions. As the result the 4-S-alkyl derivatives **8a** and **8b** were isolated (scheme 2). The antimicrobial activity study for the novel derivatives of 5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines was performed by agar well diffusion method; the results of antimicrobial activity study are listed in the table.

Table. Antimicrobial activity of 5-methyl-6-(1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidines 2, 4, 5, 6a-c, 7 and 8a,b (concentration 100 µg/ml) *

Comp. №	*Staphylococcus aureus ATCC 25923*	*Escherichia coli ATCC 25922*	*Proteus vulgaris ATCC 4636*	*Pseudomonas aeruginosa ATCC 27853*	*Bacillus subtilis ATCC 6633*	*Candida albicans ATCC 653/885*
2	16	13	growth	16	15	21
4	18	15	growth	15	18	21
5	20	16	16	16	22	growth
6a	15	14	growth	growth	17	growth
6b	15	14	growth	growth	17	growth
6c	15	15	growth	growth	17	growth
7	growth	growth	18	18	19	22
8a	19	16	17	17	16	22
8b	19	16	17	16	15	20
Metr. **	14	14	growth	growth	16	14
Strept.***	15	16	growth	growth	17	growth

** – the average values for 3 experiments are listed in the table;*
*** Metr. — Metronidazole, DMSO solution (concentration 30 µg/ml);*
**** Strept. — Streptomycin, H_2O solution (concentration 30 µg/ml).*

The results of antimicrobial activity study showed that most of the compounds like the derivatives of ethyl 5-(5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)-1,2,4-oxadiazole-3-carboxylate **2** and **4** together with 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)-4-thiothieno[2,3-*d*]pyrimidines **7, 8a, 8b** are active against the *Candida albicans* fungi. At the same time 3-alkyl-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones **5** and **6a-c** showed no activity against the fungi strain. But 2-[5-methyl-4-oxo-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-*N*-phenylacetamide **5** significantly inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis* bacterial strains. Their activity was higher than the activity of the reference drugs metronidazole and streptomycin.

CONCLUSION

Using the transformations of 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid the novel derivatives of ethyl 5-(5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidin-6-yl)-1,2,4-oxadiazole-3-carboxylate, 3-substituted 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-4(3*H*)-ones and the derivatives of 4-(alkylthio)-5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidine were obtained. The results of their antimicrobial activity study showed that the compounds with ethyl 1,2,4-oxadiazole-3-carboxylate substituent in position 6 of thieno[2,3-*d*]pyrimidine system and 5-methyl-6-(3-phenyl-1,2,4-oxadiazol-5-yl)-4-thiothieno[2,3-*d*]pyrimidines are active against the *Candida albicans* fungi, while 2-[5-methyl-4-oxo-6-(3-phenyl-1,2,4-oxadiazol-5-yl)thieno[2,3-*d*]pyrimidin-3(4*H*)-yl]-*N*-phenylacetamide was determined to be active against the strains of *Staphylococcus aureus* and *Bacillus subtilis* bacteria.

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