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Synthesis and antimicrobial activity of 3-(thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)propanoic and butanoic acids

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ABSTRACT

The interaction of 4-hydrazinylthieno[2,3-d]pyrimidines with dicarboxylic acids anhydrides has been studied. It was found that for ethyl 4-hydrazinyl-5-methylthieno[2,3-d]pyrimidine-6-carboxylate this reaction proceeds with the Dimroth rearrangement even in the mild conditions. The most suitable conditions for this reaction was boiling in the glacial acetic acid media. Using 1,1'-carbonyldiimidazole as the coupling reagent the series of 3-[8-(ethoxycarbonyl)-9-methy lthieno[3,2-e][1,2,4]triazolo[1,5-c]pyrimidin-2-yl]propanoic acid amides were synthesized. The compounds obtained showed the moderate antimicrobial activity.

Key words: thiophene, pyrimidine, cyclization, rearrangement, antimicrobial.

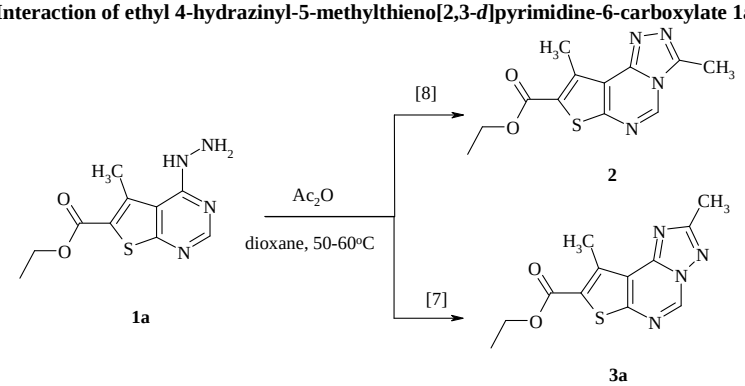
INTRODUCTION

The derivatives of thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine were reported as the biologically active compounds. For the compounds with the similar structure the anti-inflammatory [1,2], antimicrobial [3-6] and anticancer activities [7] were reported. In some cases these compounds are the products of Dimroth rearrangement [1-3].

The reported information displayed that the study of ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate interaction with dicarboxylic acids anhydrides may be a promising way to the novel biologically active compounds.

Earlier it was proposed that interaction of ethyl 4-hydrazino-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **1a** [9] with Ac_2O at heating in 1,4-dioxane media resulted in the product of the structure **2** (M.p. = 177-179°C) [8] (Scheme 1).

Scheme 1. Interaction of ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate 1a with Ac_2O



1a **2** **3a**

On the other hand the authors [7] have reported the preparation of the isomeric compound with the structure **3a** (M.p. = 178–180°C) using aminonitrile cyclization. An interesting fact was the resembling of the melting points for the both products isolated by the authors [8] and [7]. However, the structure of the product obtained was not fully confirmed yet.

MATERIALS AND METHODS

The starting ethyl 4-hydrazino-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **1a** and 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine **1b** were prepared by the known methods [9,10].

Method for preparation of 3-(8,9-dimethylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)propanoic acid 3b.
To 0.4 g (0.002 mole) of 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine **1b** 0.206 g (0.0021 моль) of succinic anhydride and 7 ml of DMF were added. The reaction mixture was stirred and heated at 120°C for 3.5 hours. Then the reaction mixture was diluted with water and the precipitate formed was filtered off and washed with water.
Yield 59 %. M.p. 193-195 °C. ^{1}H NMR (200 MHz, DMSO-d_6): 2.48 (3H, s, CH_3); 2.57 (3H, s, CH_3); 2.80 (2H, t, CH_2); 3.12 (2H, t, CH_2); 9.49 (1H, s, CH); 12.22 (1H, s, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 12.57; 13.23; 23.75; 31.27; 120.40; 126.27; 135.22; 136.54; 148.49; 151.62; 166.53; 173.42. Found, %: C 52.19, H 4.54, N 20.36. $C_{12}H_{12}N_4O_2S$. Calculated, %: C 52.16, H 4.38, N 20.28. M.w. 276.32.

Method for preparation of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid 3c.
A) To 1.0 g (0.004 mole) of the starting ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **1a** 0.4 g (0.004 mole) of succinic anhydride was added. The reaction mixture was stirred in the absolute 1,4-dioxane (20 ml) at 70-80°C for 3-4 hours. Then the cool reaction mixture was diluted with water and the precipitate formed was filtered of and washed with plenty of water.

B) To 1.0 g (0.004 mole) of ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **1a** 0.4 g (0.004 mole) of succinic anhydride and 5 ml of DMF were added. The reaction mixture was stirred and heated at 120°C for 3.5 hours. Then the cool reaction mixture was diluted with water and the precipitate formed was filtered of and washed with plenty of water.

C) To 1.0 g (0.004 mole) of ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **1a** 0.4 g (0.004 mole) of succinic anhydride and 10 ml of glacial acetic were added. The reaction mixture was boiled for 3-5 hours. Then the cool reaction mixture was diluted with water and the precipitate formed was filtered off and washed with water.

Yield: **A:** 69%, **B:** 57%, **C:** 83%. M.p. 205-207 °C. ^{1}H NMR (200 MHz, DMSO-d_6): 1.34 (3H, t, $COOCH_2\underline{CH_3}$); 2.81 (2H, t, CH_2); 2.96 (3H, s, CH_3); 3.14 (2H, t, CH_2); 4.33 (2H, q, $COO\underline{CH_2}CH_3$); 9.69 (1H, s, CH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.13; 14.84; 23.72; 31.15; 61.59, 120.79, 126.19; 139.40; 140.21; 149.16; 155.05; 161.63; 166.95; 173.30. Found, %: C 50.45, H 4.36, N 16.82. $C_{14}H_{14}N_4O_4S$. Calculated, %: C 50.29, H 4.22, N 16.76. M.w. 334.36.

4-[8-(Ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]butanoic acid 3d was obtained similar to the **A** route for synthesis of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid **3c**.

Yield 64 %. M.p. 180-182 °C. ^{1}H NMR (200 MHz, DMSO-d_6): 1.33 (3H, t, $COOCH_2\underline{CH_3}$); 2.01 (2H, quint, CH_2); 2.37 (2H, t, CH_2); 2.93 (2H, t, CH_2); 2.96 (3H, s, CH_3); 4.34 (2H, q, $COO\underline{CH_2}CH_3$); 9.69 (1H, s, CH); 12.10 (1H, s, COOH). ^{13}C NMR (75 MHz, DMSO-d_6): 14.13; 14.85; 22.78; 27.42; 32.92; 61.58; 120.80; 126.03; 139.42; 140.22; 149.14; 154.97; 161.61; 167.40; 174.15. Found, %: C 52.02, H 4.78, N 16.18. $C_{15}H_{16}N_4O_4S$. Calculated, %: C 51.72, H 4.63, N 16.08. M.w. 348.38.

Method for preparation of 4-[2-(5,6-dimethylthieno[2,3-*d*]pyrimidin-4-yl)hydrazinyl]-4-oxobutanoic acid 4.
To 0.2 g (0.001 mole) 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine **1b** 0.103 g (0.001 mole) of succinic anhydride and 5 ml of absolute 1,4-dioxane were added. The reaction mixture was heated at 70°C and stirred for 2 hours after the precipitation of the product. Then the cool reaction mixture was diluted with water; the precipitate formed was filtered off and washed with water.

Yield 71 %. M.p. 187-188 °C. ^{1}H NMR (200 MHz, DMSO-d_6): 2.39 (4H, s, $2CH_2$); 2.44 (3H, s, CH_3); 2.48 (3H, s, CH_3); 8.30 (1H, s, CH); 8.56 (1H, s, NH); 9.98 (1H, s, NH). Found, %: C 47.81, H 4.70, N 19.22. $C_{12}H_{14}N_4O_3S$. Calculated, %: C 47.72, H 4.58, N 19.04. M.w. 294.33.

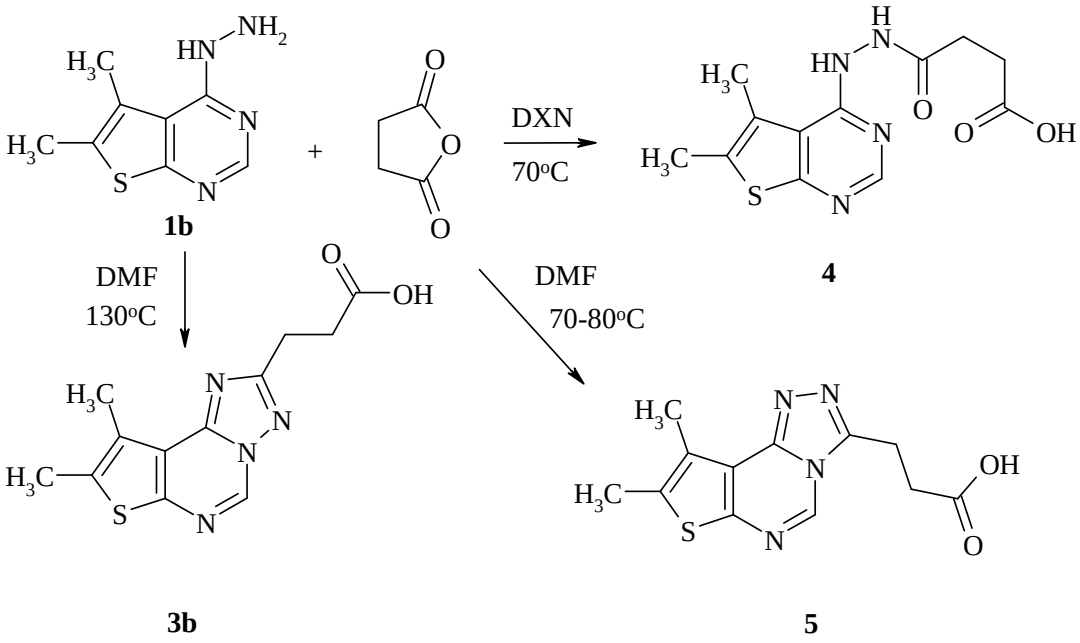
Method for preparation of 3-(8,9-dimethylthieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3-yl)propanoic acid 5.
To 0.2 g (0.001 mole) of 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine **1b** 0.103 g (0.001 mole) of succinic anhydride and 5 ml of DMF were added. The reaction mixture was heated at 70-80°C and stirred for 1-1.5 hours. Then the cool reaction mixture was diluted with water and the product precipitated was filtered off and washed with water.

Yield 73 %. M.p. 290-291 °C. ^{1}H NMR (200 MHz, DMSO-d_6), δ: 2.49 (3H, s, CH_3); 2.59 (3H, s, CH_3); 2.88 (2H, t, CH_2); 3.37 (2H, t, CH_2); 9.18 (1H, s, CH); 12.33 (COOH). ^{13}C NMR (75 MHz, DMSO-d_6), δ: 12.90; 13.24; 19.58; 30.63; 118.90; 127.12; 134.48; 134.79; 145.63; 145.98; 147.69; 173.27. Found, %: C 52.12, H 4.57, N 20.46. $C_{12}H_{12}N_4O_2S$. Calculated, %: C 52.16, H 4.38, N 20.28. M.w. 276.32.

General method for synthesis of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid amides 6.
To 0.3 g (0.9 mmole) of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid **3c** 0.160 g (0.99 mmole) of CDI and 3 ml of anhydrous DMF were added. The reaction mixture was stirred and heated at 50-70°C for 20 minutes. Then to the solution 0.9 mmole of the corresponding amine was added and the reaction mixture was additionally stirred for 3-5 hours. Then the cool reaction mixture was diluted with water and the precipitate formed was filtered off and crystallized from ethanol.

Antimicrobial activity study. Microbiological experiment was performed by "The microorganism biochemistry and nutrient media laboratory of Institute microbiology and immunology n.I.I.Mechnikov NAMS of Ukraine". According to the WHO recommendations [11,12] to estimate the activity of the compounds tested the following strains of microorganisms were 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. The inoculum suspension was prepared using Densi-La-Meter apparatus (made by PLIVA-Lachema, Czech Republic; 540-nm wavelength). The cultures were synchronized using the low temperature conditions (4 ^{o}C). Inoculum's density was 10^7 cells in 1 ml of media and was determined by comparing with McFarland standard. 18 to 24-hour old culture of the microorganism was used for the test. Mueller-Hinton agar was employed («HIMedia Laboratorles Pvt. Ltd India») for bacteria. The strain of *Candida albicans* was cultivated using Sabouraud agar («HIMedia Laboratorles Pvt. Ltd India»). The studied compounds were introduced as 0.3 ml DMSO (100 µg/ml) solution aliquots.

Scheme 2. Interaction of 4-hydrazinyl-5,6-dimethylthieno[2,3-*d*]pyrimidine 1b with succinic anhydride

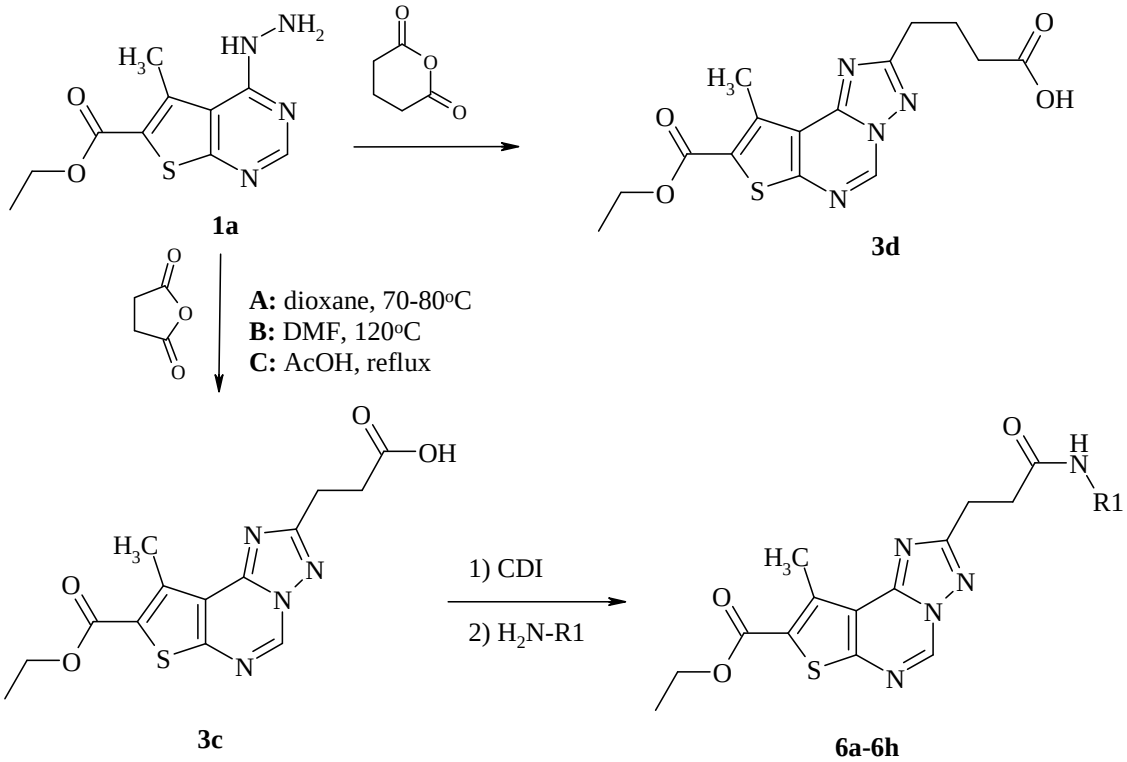


RESULTS AND DISCUSSION

To identify the structure of the **1a** cyclization product with Ac_2O we have obtained the NOESY spectrum of the sample. For to the structure **2** the protons of triazole ring CH_3 should be in the close proximity with CH proton of pyrimidine cycle, therefore the corresponding cross-peak should be observed for these groups of protons. But the spectrum contained no cross peaks for these groups of protons. This may be the evidence of thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine **3a** structure for the compound obtained and the easiness of the rearrangement ethyl 4-hydrazino-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate in the reaction with carboxylic acids anhydrides, even in mild conditions. Therefore the next step of our research was the study of the reaction between 4-hydrazinyllthieno[2,3-*d*]pyrimidines and dicarboxylic acid anhydrides.

As the results of these experiments it was found that ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate **1a** interaction with succinic and glutaric anhydrides required the higher temperature to form the single products. The NOESY spectra of the isolated compounds contained no cross-peaks of pyrimidine ring proton with any of the other groups of protons. But as the source of misinterpretation the not enough distance between the protons for NOE observation was possible. Therefore we have decided to study this interaction in details.

Scheme 3. Interaction of ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate 1a with succinic and gutaric anhydride; synthesis of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid amides 6



The first step was base on the application of the simple hydrazide **1b** [10] for the reaction. But the low solubility of the compound **1b** complicated the cyclization reaction and the product **4** was isolated. For better solubility of the starting compound **1b** its interaction with succinic anhydride was performed in DMF media. As the result of starting reagents heating at 70-80°C for 1-1.5 hours the single product, which showed the signal of CH proton of pyrimidine ring at 9.18 ppm and the cross-peak of this signal with the downfield peak of succinic CH_2 group. The longer term heating resulted in the mixture of the isomeric products, which was established by LC-MS. The signal of pyrimidine CH proton for the other isomeric compound was observed at 9.49 ppm, and no cross-peak with succinic acid residue was found for this signal in the NOESY-spectrum.

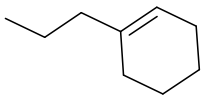
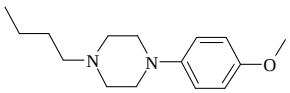
The heating of the hydrazide **1b** with succinic anhydride in DMF media at 120°C for 3.5 hours resulted in the single product **3b**. Probably the presence of only electron-donating substituents in the thiophene ring is not favorable for pyrimidine ring opening and complicates Dimroth rearrangement step.

The obtained information about the reaction was used for **3c** derivative synthesis optimization. The following reaction may be performed in 1,4-dioxane (70-80°C), but this solvent appeared to be hardly suitable for the preparation of the greater amounts (from 2 g of the starting hydrazide **1a**). It is also possible to obtain the target

compound **3c** in DMF at 120°C; the disadvantage of this method is the possibility of the product resinification. The most suitable way for the synthesis was boiling of the starting compounds in the glacial acetic acid, which gave the highest yield of the target acid **3c**.

The presence of the carboxyl group in the molecule of the compound **3c** was favorable for chemical diversity enlargement by amidation reaction using CDI as the coupling reagent. As the result of this combinatorial stage starting from the compound **3c** the series of amides **6** was prepared (table 1).

Table 1. The properties 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid amides 6

Compnd. №	R1	Molecular formula M.w., M.p., ºC	Yield, %
6a	-$CH_2C_6H_5$	$C_{21}H_{21}N_5O_3S$ 423.50 211-212	64
6b	-CH_2(4-$CH_3C_6H_5$)	$C_{22}H_{23}N_5O_3S$ 437.52 224-226	57
6c	-CH_2(4-$C_2H_5C_6H_5$)	$C_{23}H_{25}N_5O_3S$ 451.55 220-221	83
6d	-CH_2(4-FC_6H_5)	$C_{21}H_{20}FN_5O_3S$ 441.49 226-228	77
6e	-CH_2(2-ClC_6H_5)	$C_{21}H_{20}ClN_5O_3S$ 457.94 233-234	64
6f	-CH_2(4-$OCH_3C_6H_5$)	$C_{22}H_{23}N_5O_4S$ 453.52 201-202	80
6g		$C_{22}H_{27}N_5O_3S$ 441.56 225-227	92
6h		$C_{28}H_{35}N_7O_4S$ 565.70 184-186	87

^{1}H NMR spectra of amides **6** contain the signals of methyl protons of thiophene nuclei in the range 2.94-2.99 ppm and the signals of aliphatic protons of propanoic acid residue, which are observed as two triplets at 2.62-2.78 and 3.16-3.24 ppm. The signal of pyrimidine CH is present in the region 9.67-9.70 ppm, and the NH protons give their peak in the region from 7.88 to 8.51 ppm, which position much depends upon the amide hydrocarbon radical (table 2).

Table 2. ^{1}H NMR spectra of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid amides 6

Compound. №	Chemical shift, δ, ppm			
	CH, NH	CH_3 (3H, c.)	Aliphatic protons	Aromatic protons
6a *	9.69 (s, CH); 8.47 (t, NH);	2.94	1.32 (3H, t, CH_3); 2.74 (2H, t, CH_2); 3.17 (2H, t, CH_2); 4.17-4.45 (4H, m, 2CH_2);	7.19 (5H, m)
6b	9.70 (s, CH); 8.41 (t, NH);	2.99	1.34 (3H, t, CH_3); 2.21 (3H, s, CH_3); 2.73 (2H, t, CH_2); 3.24 (2H, t, CH_2); 4.20 (2H, d, NHCH_2); 4.36 (2H, q, OCH_2);	6.92-7.13 (4H, m, Ar-H);
6c	9.67 (s, CH); 8.42 (t, NH);	2.94	1.09 (3H, t, CH_3); 1.32 (2H, t, CH_3); 2.48 (2H, m, CH_2); 2.72 (2H, t, CH_2); 3.16 (3H, t, CH_2); 4.20 (2H, d, NHCH_2); 4.36 (2H, q, OCH_2);	6.91-7.17 (4H, m, Ar-H);
6d	9.69 (s, CH); 8.46 (t, NH);	2.99	1.34 (3H, t, CH_3); 2.74 (2H, t, CH_2); 3.18 (2H, t, CH_2); 4.24 (2H, d, NHCH_2); 4.36 (2H, q, OCH_2);	7.05 (2H, t, H-2' + H-6'); 7.23 (2H, m, H-3' + H-5');
6e	9.69 (s, CH); 8.51 (t, NH);	2,96	1.32 (3H, t, CH_3); 2.78 (2H, t, CH_2); 3.18 (2H, t, CH_2); 4.22-4.42 (4H, m, 2CH_2);	7.08-7.42 (4H, m, Ar-H);
6f	9.70 (s, CH); 8.38 (t, NH);	2.98	1.34 (3H, t, CH_3); 2.72 (2H, t, CH_2); 3.17 (2H, t, CH_2); 3.68 (3H, s, OCH_3); 4.18 (2H, d, NHCH_2); 4.36 (2H, q, OCH_2);	6.76 (2H, t, H-3' + H-5'); 7.10 (2H, m, H-2' + H-6');
6g	9.69 (s, CH); 7.88 (t, NH);	2.98	1.34 (3H, t, CH_3); 1.45 (4H, m); 1.71-2.05 (6H, m); 2.62 (2H, t, CH_2); 3.03-3.18 (4H, m); 4.34 (2H, q, OCH_2); 5.31 (1H, c, =CH);	-
6h **	9.68 (s, CH); 7.96 (t, NH);	2.98	1.31 (3H, t, CH_3); 1.53 (2H, t, CH_2); 2.18 (2H, m); 2.64 (2H, t, CH_2); 2.79-3.21 (12H, m); 3.64 (3H, s, OCH_3); 4.32 (2H, q, OCH_2);	6.68-6.88 (4H, m, Ar-H);

^{13}C NMR (75 MHz, ДМСО-d_6): ***6a**: 14.5; 14.93; 24.25; 32.86; 42.10; 61.62; 120.92; 126.17; 126.66; 127.13; 128.16; 139.54; 140.27; 149.22; 155.07; 161.70; 167.27; 170.73.
****6h**: 14.16; 14.97; 21.97; 22.46; 24.36; 24.70; 27.73; 33.08; 37.25; 37.55; 61.63; 121.79; 126.22; 135.03; 139.53 140.34; 149.31; 155.56; 162.18; 167.34; 170.42.

Most of the tested compounds **3c**, **3d** and amides **6a**-**6h** showed the moderate antimicrobial activity (table 3).

Table 3. Antimicrobial screening data for 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid 3c, 4-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]butanoic acid 3d and 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid amides 6 (concentration 100 µg/ml)

Compnd. №	Diameter of growth inhibition zone in mm number of repeated experiment n=3					
	Staphylococcus aureus ATCC 25923	*Escherichia Coli ATCC 25922*	*Proteus vulgaris ATCC 4636*	*Pseudomonas aeruginosa ATCC 27853*	*Basillus Subtilis ATCC 6633*	*Candida aibicans ATCC 653/885*
3c	14, 14, 15	15, 15, 15	13, 13, 13	16, 15, 17	16, 16, 17	14, 15, 14
3d	growth	16, 16, 16	13, 13, 14	15, 15, 15	14, 14, 14	14, 14, 14
6a	15, 14, 14	17, 16, 17	14, 13, 13	14, 15, 15	17, 17, 18	14, 13, 13
6b	13, 13, 14	16, 16, 16	13, 12, 13	15, 16, 16	14, 14. 15	growth
6c	17, 17, 18	17, 17, 17	growth	14, 14, 15	16, 16, 17	13, 15, 14
6d	14, 14, 14	17, 17, 16	13, 14, 13	17, 15, 16	15, 16, 15	17, 16, 16
6e	16, 16, 17	16, 17, 16	growth	15, 14, 15	17, 15, 17	14, 14, 15
6f	14, 13, 14	17, 16, 17	13, 14, 13	16, 15, 16	15, 15, 15	16, 16, 15
6g	15, 14, 15	15, 15, 16	14, 14, 14	14, 14, 15	14, 14, 15	14, 13, 13
6h	14, 14, 15	17, 17, 16	growth	14, 15, 14	16, 15, 15	14, 14, 14
Metr.**	14, 15, 14	14, 13, 14	growth	growth	16, 15, 16	14, 14, 14
Strept.**	15, 16, 15	15, 16, 17	growth	growth	17, 16, 17	growth

***Metr. – Metronidazole, DMSO solution, concentration 30 µg/ml;*
***Strept. – Streptomycin, H_2O solution, concentration 30 µg/ml;*

CONCLUSION

The interaction of 4-hydrazinylthieno[2,3-*d*]pyrimidines with succinic anhydride showed the easiness of Dimroth rearrangement for ethyl 4-hydrazinyl-5-methylthieno[2,3-*d*]pyrimidine-6-carboxylate. The effective conditions for synthesis of amides of 3-[8-(ethoxycarbonyl)-9-methylthieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl]propanoic acid were developed. The antimicrobial activity study for the compound synthesized revealed their moderate antimicrobial activity.

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