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Synthesis and antimicrobial activity study of thieno[3,2-*e*][1,2,4]triazolo [4,3-*c*]pyrimidin-3(2*H*)-one derivatives

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ABSTRACT

Cyclization 4-hydrazinothieno[2,3-d]pyrimidines with 1,1'-carbonyldiimidazole was proposed as the novel and highly effective approach for synthesis of the series of thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-3(2H)-ones. Heterocyclic systems obtained were modified by alkylation with chloroacetamides (DMF-K_2CO_3), and produced 2-(3-oxothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-2(3H)-yl)-N-acetamides. Antimicrobial activity screening for all of the compounds obtained has been performed by the agar well diffusion method. The highest antimicrobial activity was determined for ethyl 9-methyl-3-oxo-2,3-dihydrothieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidine-8-carboxylate ***2d,*** *which showed the activity against Proteus vulgaris similar to Synthomycine. Some of the compounds also showed better activity than Metronidazole against the strain of Candida albicans fungi.*

Key words: thiophene, pyrimidine, carbonyldiimidazole, triazole, antimicrobial activity

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INTRODUCTION

In the last years, many works devoted to antimicrobial activity study for thieno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidines have been published. Some of these compounds displayed good antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*, but the better antifungal activity against *Candida albicans* and *Candida parapsilosis* [1]. Derivatives of this series with stronger activity than Ampicillin towards *Bacillus Subtilis, Shigella dysenteriae* and *Bacillus subtilis* [2,3], and also stronger activity than Nystatin against *Macrophomina phaseolina, Alternaria alternata* and *Fusarium equiseti* [2,4] have been reported. Thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-3(2*H*)-thiones also appeared to be the promising objects for antimicrobial investigation; they had the effect comparable with the activity of Ampicillin against *Escherichia coli* [5]; their analogues modified at position 3 with thioglycolic acid amide fragment are active against *Candida albicans*, with no significant antibacterial activity [6].

The preparation methods and pharmacological activity of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one derivatives are intensively studied nowadays [7,8]; some of them were reported as the compounds with anti-inflammatory activity [8]. The proposed ways for their preparation are either interaction of 4-chlorothieno[2,3-*d*]pyrimidines with semicarbazide hydrochloride [7], or cyclization of 4-hydrazinothieno[2,3-*d*]pyrimidines with ethyl chloroformate in pyridine [8]. Both methods require rigorous reaction conditions, but give the low yields of the target products. Therefore, the aim of our research was to develop an effective, rapid and safe method for preparation of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one derivatives as the potent antimicrobial substances.

EXPERIMENTAL SECTION

Melting points (°C) were measured with a with a Koeffler melting point apparatus and were not corrected. Elemental analysis were within ±0.4% of the theoretical value. ^{1}H and ^{13}C NMR spectral data were reordered at 200 and 75 MHz respectively on Varian Mercycry-200 and Varian Geminy-300 spectrometers using TMS as an internal standard. LC/MS-spectral analyses were obtained on a PE SCIEX API 150EX device equipped with mass spectrometer. Mass spectrum was recorded on GS/MS spectrometer Varian 1200L (70 eV) using direct input of sample.

Starting 4-hydrazinothieno[2,3-*d*]pyrimidines **1a-d** were obtained by the previously reported methods [2,9-13].

General method for preparation of compounds 2a-d.
To the solution of 0.042 mole of 1,1'-carbonyldiimidazole in 50 ml of anhydrous DMF 0.028 mole of the corresponding 4-hydrazinothieno[2,3-d]pyrimidine **1** was added. The mixture was stirred and heated at 120°C for 2.5 hours. After, the cold reaction mixture was quenched with water, and the precipitate formed was filtered off and crystallized from 2-propanol-DMF mixture.

8,9-Dimethylthieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one 2a. This compound was obtained in 72% yield as a white solid, mp 295-296˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 8.51 (1 H, s, CH), 12.25 (1 H, br.s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.54, 12.94, 118.16, 127.01, 133.95, 135.15, 138.54, 148.75, 149.36; ms (EI): m/z (M^+) 220. Anal. calcd. for : H, 3.66; C, 49.08; N, 25.44; S, 14.56. Found: H, 3.74 C, 49.19; N, 25.79; S, 14.34.

8,9,10,11-Tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one 2b. This compound was obtained in 76% yield as a white solid, mp 265-267˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.82 (4 H, m, $2CH_2$), 2.82 (4 H, m, $2CH_2$), 8.53 (1 H, s, CH), 12.46 (1 H, br.s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 21.79; 22.59; 24.69; 24.77, 117.11; 129.27; 134.96; 136.84; 138.60; 148.88; 150.56; lcms: m/z (MH^+) 247. Anal. calcd. for $C_{11}H_{10}N_4OS$: H, 4.09; C, 53.64; N, 22.75; S, 13.02. Found: H, 4.24; C, 53.83; N, 22.75; S, 13.14.

9,10,11,12-Tetrahydro-8*H*-cyclohepta[4,5]thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one 2c. This compound was obtained in 73% yield as a white solid, mp 245-246˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.31-1.95 (6H, m, $3CH_2$), 2.88 (2H, m, CH_2), 3.15 (2H, m, CH_2), 8.49 (1 H, s, CH), 12.16 (1 H, br.s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 26.85, 27.44, 27.85, 29.40, 31.68, 118.17, 134.47, 135.11, 138.72, 140.87, 148.72; lcms: m/z (MH^+) 261. Anal. calcd. for $C_{12}H_{12}N_4OS$: H, 4.65; C, 55.37; N, 21.52; S, 12.32. Found: H,4.82; C, 55.59; N, 21.56; S, 12.36.

Ethyl 9-methyl-3-oxo-2,3-dihydrothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-8-carboxylate 2d. This compound was obtained in 59% yield as a white solid, mp 289-291˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.3 (3H, t, J=7.2, CH_3), 2.81 (3H, s, CH_3), 4.31 (2H, q, J=7.2, CH_2), 8.73 (1 H, s, CH), 12.65 (1 H, br.s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.06, 14.60, 61.36, 119.15, 125.51, 138.40, 138.79, 139.67, 148.63, 154.57, 161.61; lcms: m/z (MH^+) 279. Anal. calcd. for $C_{11}H_{10}N_4O_3S$: H, 3.62; C, 47.48; N, 20.13; S, 11.52. Found: H, 3.90; C, 47.50; N, 20.28; S, 11.39.

General method for synthesis of compound 3.
To the suspension of 0.001 mole of compound **2** in 3 ml of DMF 0.0011 mole of corresponding chloroacetamide and 0.0015 mole of K_2CO_3 was added. The mixture was stirred and heated at 130°C during the period of 6 hours. Then to the cold reaction mixture 10 ml of water was added. The precipitate formed was filtered off and crystallized from ethanol-DMF mixture.

2-(8,9-Dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-phenylacetamide 3a. This compound was obtained in 58% yield as a white solid, mp 274-276˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.78 (2 H, s, CH_2), 7.05 (1 H, t, H-4 $_{Ar}$), 7.31 (2 H, t, H-3+H-5 $_{Ar}$), 7.56 (2 H, d, H-2+H-6 $_{Ar}$), 8.63 (1 H, s, CH), 10.21 (1 H, br.s, NH); lcms: m/z (MH^+) 354. Anal. calcd. for $C_{17}H_{15}N_5O_2S$: H, 4.28; C, 57.78; N, 19.82; S, 9.07. Found: H, 4.37 C, 57.52; N, 25.79; S, 9.21.

2-(8,9-Dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-(4-methylphenyl)acetamide 3b. This compound was obtained in 81% yield as a white solid, mp 233-235˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.22 (3 H, s, CH_3), 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.76 (2 H, s, CH_2), 7.20 (2 H, d, H-2+H-6 $_{Ar}$), 7.43 (2 H, d, H-3+H-5 $_{Ar}$), 8.64 (1 H, s, CH), 10.12 (1 H, br.s, NH). Anal. calcd. for $C_{18}H_{17}N_5O_2S$: H, 4.66; C, 58.84; N, 19.06; S, 8.73. Found: H, 4.72; C, 58.99 ; N, 19,22; S, 8.67.

2-(8,9-Dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-(4-fluorophenyl)acetamide 3c. This compound was obtained in 72% yield as a white solid, mp 274-276˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.76 (2 H, s, CH_2), 7.12 (2 H, t, H-2+H-6 $_{Ar}$), 7.55 (2 H, t, H-3+H-5 $_{Ar}$), 8.64 (1 H, s, CH), 10.22 (1 H, br.s, NH); lcms: m/z (MH^+) 372. Anal. calcd. for $C_{17}H_{14}FN_5O_2S$: H, 3.80; C, 54.98; N, 18.86; S, 8.63. Found: H, 3.83; C, 55.19; N, 18.88; S, 8.51.

2-(8,9-Dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-(4-methylbenzyl)acetamide 3d. This compound was obtained in 85% yield as a white solid, mp 255-257˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.21 (3 H, s, CH_3), 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.22 (2 H, d, CH_2), 4.57 (2 H, s, CH_2), 7.19 (4 H, m, Ar-H), 8.48 (1 H, m, NH), 8.64 (1 H, s, CH); δ ; lcms: m/z (MH^+) 382. Anal. calcd. for $C_{19}H_{19}N_5O_2S$: H, 5.02; C, 59.83; N, 18.36; S, 8.41. Found: H, 5.24; C, 59.72; N, 18.52; S, 8.49.

2-(8,9-Dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-(2,4-dimethylphenyl)acetamide 3e. This compound was obtained in 61% yield as a white solid, mp 239-241˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.21 (3 H, s, CH_3), 2.22 (3 H, s, CH_3), 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.72 (2 H, s, CH_2), 7.02 (1 H, d, H-6 $_{Ar}$), 7.29 (2 H, m, H-3+H-5 $_{Ar}$), 8.62 (1 H, s, CH), 10.02 (1 H, br.s, NH). Anal. calcd. for $C_{19}H_{19}N_5O_2S$: H, 5.02; C, 59.83; N, 18.36; S, 8.41. Found: H, 4.87; C, 59.98; N, 18.39; S, 8.59.

2-(8,9-Dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-(4-ethylphenyl)acetamide 3f. This compound was obtained in 58% yield as a white solid, mp 231-232˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.12 (3 H, t, CH_3), 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 2.52 (2H, m, CH_2), 4.72 (2 H, s, CH_2), 7.12 (2 H, d, H-2+H-6 $_{Ar}$), 7.45 (2 H, d, H-3+H-5 $_{Ar}$), 8.64 (1 H, s, CH), 10.02 (1 H, br.s, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ 12.52, 12.90, 15.28, 27.59, 48.90, 117.74, 120.05, 127.18, 127.87, 134.60, 134.80, 136.12, 137.73, 139.44, 147.82, 150.00. Anal. calcd. for $C_{19}H_{19}N_5O_2S$: H, 5.02; C, 59.83; N, 18.36; S, 8.41. Found: H, 5.25; C, 59.82; N, 18.32; S, 8.22.

***N*-(4-Bromophenyl)-2-(8,9-dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetamide 3g.** This compound was obtained in 89% yield as a white solid, mp 261-263˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.76 (2 H, s, CH_2), 7.49 (4 H, m, Ar-H), 8.63 (1 H, s, CH), 10.31 (1 H, br.s, NH). Anal. calcd. for $C_{17}H_{14}BrN_5O_2S$: H, 3.26; C, 47.23; N, 16.20; S, 7.42. Found: H, 3.12; C, 47.55; N, 16.25 S, 7.27.

***N*-(2,4-Difluorophenyl)-2-(8,9-dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetamide 3h.** This compound was obtained in 73% yield as a white solid, mp 234-236˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.84 (2 H, s, CH_2), 7.04 (1 H, m, Ar-H), 7.32 (1 H, m, Ar-H), 7.76 (1 H, m, Ar-H), 8.64 (1 H, s, CH), 10.04 (1 H, br.s, NH); lcms: m/z (MH^+) 390. Anal. calcd. for $C_{17}H_{13}F_2N_5O_2S$: H, 3.37; C, 52.44; N, 17.99; S, 8.23. Found: H, 3.52; C, 52.61; N, 18.20; S, 8.27.

***N*-(3-Chloro-4-methoxyphenyl)-2-(8,9-dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetamide 3i.** This compound was obtained in 92% yield as a white solid, mp 293-295˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 3.81 (3 H, s, OCH_3), 4.76 (2 H, s, CH_2), 7.08 (1 H, d, H-5), 7.43 (1 H, dd, H-6), 7.81 (1 H, dd, H-2), 8.64 (1 H, s, CH), 10.07 (1 H, br.s, NH); lcms: m/z (MH^+) 418. Anal. calcd. for $C_{18}H_{16}ClN_5O_3S$: H, 3.86; C, 51.74; N, 16.76; S, 7.67. Found: H, 4.07; C, 51.92; N, 16.93; S, 7.55.

***N*-(2,3-Dihydro-1,4-benzodioxin-6-yl)-2-(8,9-dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetamide 3j.** This compound was obtained in 55% yield as a white solid, mp 278-280˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 4.18 (4 H, s, $(CH_2)_2$), 4.74 (2 H, s, CH_2), 6.77 (1 H, d, H-5), 6.94 (1 H, dd, H-6), 7.18 (1 H, dd, H-2), 8.63 (1 H, s, CH), 10.05 (1 H, br.s, NH); lcms: m/z (MH^+) 412. Anal. calcd. for $C_{19}H_{17}N_5O_4S$: H, 4.16; C, 55.47; N, 17.02; S, 7.79. Found: H, 4.37; C, 55.15; N, 17.28; S, 7.86.

8,9-Dimethyl-2-[2-(6-methyl-2,3-dihydro-4*H*-1,4-benzoxazin-4-yl)-2-oxoethyl]thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-one 3k. This compound was obtained in 64% yield as a white solid, mp 219-221˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.18 (3 H, s, CH_3), 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 3.92 (2 H, m, CH_2), 4.27 (2 H, m, CH_2), 5.19 (2 H, s, CH_2), 6.79 (2 H, m, Ar-H), 7.83 (1 H, br s, Ar-H), 8.65 (1 H, s, CH). Anal. calcd. for $C_{20}H_{19}N_5O_3S$: H, 4.68; C, 58.67; N, 17.10; S, 7.83. Found: H, 4.49; C, 58.65; N, 17.38; S, 7.88.

***N*-(5-Chloro-2-methoxyphenyl)-2-(8,9-dimethyl-3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetamide 3l.** This compound was obtained in 81% yield as a white solid, mp 185-187˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.40 (3 H, s, CH_3), 2.42 (3 H, s, CH_3), 3.86 (3 H, d, OCH_3), 4.93 (2 H, s, CH_2), 7.09 (2 H, m, Ar-H), 8.05 (1 H, m, Ar-H), 8.65 (1 H, s, CH), 9.79 (1 H, br.s, NH). Anal. calcd. for $C_{18}H_{16}ClN_5O_3S$: H, 3.86; C, 51.74; N, 16.76; S, 7.67. Found: H, 3.89; C, 51.91; N, 16.81; S, 7.70.

***N*-(2-Ethyl-6-methylphenyl)-2-(3-oxo-8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetamide 3m.** This compound was obtained in 61% yield as a white solid, mp >300˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.07 (3 H, t, CH_3), 1.84 (4 H, m, $2CH_2$), 2.13 (3 H, s, CH_3), 2.53 (2 H, m, CH_2), 2.86 (4 H, m, $2CH_2$), 4.75 (2 H, s, CH_2), 7.08 (3 H, m, Ar-H), 8.68 (1 H, s, CH), 9.30 (1 H, br.s, NH). Anal. calcd. for $C_{22}H_{23}N_5O_2S$: H, 5.50; C, 62.69; N, 16.61; S, 7.61. Found: H, 5.83; C, 62.94; N, 16.70; S, 7.90.

Methyl 4-{[(3-oxo-8,9,10,11-tetrahydro[1]benzothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)acetyl] amino} benzoate 3n. This compound was obtained in 85% yield as a white solid, mp 276-277˚C; ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.80 (4 H, m, $2CH_2$), 2.83 (4 H, m, $2CH_2$), 3.81 (3 H, s, $COOCH_3$), 4.84 (2 H, s, CH_2), 7.70 (2 H, d, H-2+H-6 $_{Ar}$), 7.92 (2 H, d, H-3+H-5 $_{Ar}$), 8.68 (1 H, s, CH), 10.59 (1 H, br.s, NH). Anal. calcd. for $C_{21}H_{19}N_5O_4S$: H, 4.38; C, 57.66; N, 16.01; S, 7.33. Found: H, 4.15; C, 57.81; N, 16.34; S, 7.52.

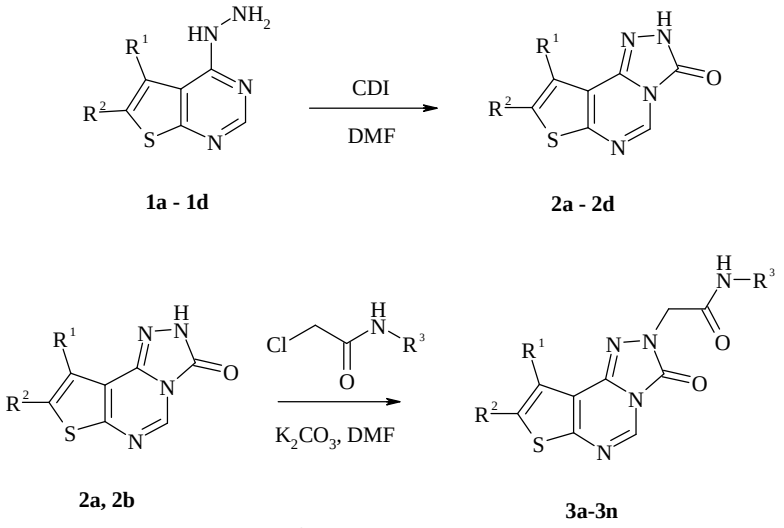
Antimicrobial activity study

According to the WHO recommendations [14,15] the following microorganisms test-strains have been used for the test *Staphylococcus aureus* ATCC 25923, *Esherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillis 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 0.3ml 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

In order to apply less toxic cyclizing agent we tried 1,1'-carbonyldiimidazole as the more safe analog of ethyl chloroformate. It was found that heating of this reagent with hydrazines **1** [2,9-13] in anhydrous DMF for 2.5 hours resulted in formation of the products **2a-d** (scheme).



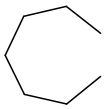
1a - 1d **2a - 2d**

2a, 2b **3a-3n**

Scheme. Synthesis and alkylation of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-ones

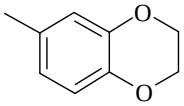
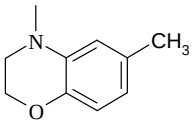
All of the thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-ones **2** were isolated in good yields and sufficient purity (table 1). Analytical samples of **2** were crystallized from 2-propanol-DMF mixture. In their ^{1}H NMR spectra the only one signal of NH proton is observed in the region 12.16-12.65 ppm; the signal of the pyrimidine cycle proton is located at 8.49-8.73 ppm. The mass-spectral data obtained also well corresponded with the structure of the compounds **2**.

Table 1. Thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-ones 2a-d

Compound	R^1/R^2	Mol. formula, M.w.	Yield, %	MS (M^+) and LC/MS (MH^+)
2a	CH_3/CH_3	$C_9H_8N_4OS$ 220.25	72	220 (M^+)
2b		$C_{11}H_{10}N_4OS$ 246.29	76	247 (MH^+)
2c		$C_{12}H_{12}N_4OS$ 260.32	73	261 (MH^+)
2d	CH_3/COOEt	$C_{11}H_{10}N_4O_3S$ 278.29	59	279 (MH^+)

Analysis of the reported data suggested that introduction of acetamide fragment promotes the antimicrobial properties of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidines [6]. Therefore, the further modification of compounds **2a** and **2b** was performed by their alkylation with chloroacetamides, as the result the range of compounds **3** was isolated (table 2). The ^{1}H NMR spectra of these compounds contain the signals methylene group of acetamide fragment at 4.57-5.19 ppm and also the signals of aromatic protons at 6.77-8.05 ppm.

Table 2. 2-(3-Oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-acetamides 3a-n

Compound	R^1/R^2	R^3	Mol. formula M.w.	Yield, %*
3a	CH_3/CH_3	Ph	$C_{17}H_{15}N_5O_2S$ 353.41	58
3b	CH_3/CH_3	*p*-MePh	$C_{18}H_{17}N_5O_2S$ 367.43	81
3c	CH_3/CH_3	*p*-FPh	$C_{17}H_{14}FN_5O_2S$ 371.40	72
3d	CH_3/CH_3	p-MeBn	$C_{19}H_{19}N_5O_2S$ 381.46	85
3e	CH_3/CH_3	2,4-diMePh	$C_{19}H_{19}N_5O_2S$ 381.46	61
3f	CH_3/CH_3	*p*-EtPh	$C_{19}H_{19}N_5O_2S$ 381.46	58
3g	CH_3/CH_3	*p*-BrPh	$C_{17}H_{14}BrN_5O_2S$ 432.30	89
3h	CH_3/CH_3	2,4-diFPh	$C_{17}H_{13}F_2N_5O_2S$ 389.39	73
3i	CH_3/CH_3	3-Cl-4-OMePh	$C_{18}H_{16}ClN_5O_3S$ 417.88	92
3j	CH_3/CH_3		$C_{19}H_{17}N_5O_4S$ 411.44	55
3k	CH_3/CH_3		$C_{20}H_{19}N_5O_3S$ 409.47	64
3l	CH_3/CH_3	2-OMe-5-Cl-Ph	$C_{18}H_{16}ClN_5O_3S$ 417.88	81
3m		2-Et-6-MePh	$C_{22}H_{23}N_5O_2S$ 421.52	61
3n		4-COOMePh	$C_{21}H_{19}N_5O_4S$ 437.48	85

** yield is given for alkylation step*

The screening of antimicrobial activity for the compounds **2** and **3** has been performed by agar well diffusion method [16-19]. The results showed that most of the tested compounds were not active against *Pseudomonas aeruginosa* and *Proteus vulgaris*, except of ethyl 9-methyl-3-oxo-2,3-dihydrothieno[3,2-*e*][1,2,4]triazolo[4,3-

c]pyrimidine-8-carboxylate **2d**, which showed the activity against *Proteus vulgaris* similar to the reference drug Synthomycine (table 3).

Table 3. Antimicrobial activity of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-ones 2a-d and 3a-n (concentration 100 µg/ml) *

Compound	*Staphylococcus aureus ATCC 25923*	*Esherichia coli ATCC 25922*	*Pseudomonas aeruginosa ATCC 27853*	*Proteus vulgaris ATCC 4636*	*Bacillis subtilis ATCC 6633*	*Candida albicans ATCC 653/885*
2a	+	=	-	-	++	++
2b	-	-	-	-	-	-
2c	+	+	-	-	++	+
2d	++	+	+	++	++	++
3a	+	+	+	+	++	++
3b	+	+	-	-	++	+
3c	+	+	-	-	+	++
3d	+	+	-	-	++	+
3e	+	+	-	-	++	-
3f	+	+	-	-	++	+
3j	+	+	-	-	++	+
3k	+	+	-	-	++	+
3l	+	+	-	-	++	+
3m	-	-	-	-	-	-
3n	+	+	-	-	++	++
Metr.**	+	+	-	-	++	+
Synt. **	+	++	++	++	++	-

** – - diameter of growth inhibition zone less than 10 mm; + - diameter of growth inhibition zone 10-15 mm; ++ - diameter of growth inhibition zone 15-20 mm; +++- diameter of growth inhibition zone more than 20 mm.*
*** concentration of antibiotics 30 µg/ml; Metr. — Metronidazole (DMSO solution);*
Synt. — Synthomycine (H_2O solution).

Most of the tested compounds inhibited the growth of *Bacillis subtilis* as both of the reference drugs, and only some of them (**2a, 2d, 3a, 3c, 3n**) surpassed the activity of Metronidazole against the strain of *Candida albicans* fungi. The structure of all of the most active compounds contain either a small or an electron-withdrawing substituent, which increases the antimicrobial effect of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-ones.

CONCLUSION

The effective way for cyclization of 4-hydrazinothieno[2,3-d]pyrimidines with 1,1'-carbonyldiimidazole in anhydrous DMF media has been developed. Using this method the series of thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3(2*H*)-ones **2** were obtained. By alkylation of heterocyclic compounds **2** they were transformed to 2-(3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-acetamides **3**. Antimicrobial activity study for all of the compounds obtained allowed to indentify ethyl 9-methyl-3-oxo-2,3-dihydrothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine-8-carboxylate **2d** as the most active derivative, which showed the activity against *Proteus vulgaris* similar to the reference drug Synthomycine. Some of the compounds of the 2-(3-oxothieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-2(3*H*)-yl)-*N*-acetamide series, containing electron-withdrawing groups in the acetamide fragment surpassed the activity of Metronidazole against the strain of *Candida albicans* fungi.

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