UDC 547.853.3:547.732:547.496.2

SYNTHESIS AND THE ANTIMICROBIAL ACTIVITY OF ETHYL 5-METHYL-2-(ALKYLTHIO)-4-OXO-3,4-DIHYDROTHIENO [2,3-d]PYRIMIDINE-6-CARBOXYLATES

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Key words: thiophene; pyrimidine; thiourea

By alkylation of the products of diethyl 3-methyl-5-{[(methylthio)carbonothioyl]amino}-2,4-thiophenedicarboxylate interaction with benzylamines the novel derivatives of ethyl 5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylates have been obtained. It has been found that the signal of the CH_2-group adjacent to the nitrogen atom in position 3 of thieno[2,3-d]pyrimidine system is always observed in the range of 5.35-5.40 ppm, while the position of the signal of methythylene-group connected with the sulfur atom much depends upon the structure of the radical attached to this group. IR-spectra of all the compounds contain the intensive C=O stretching band at 1721-1678 cm^{-1}; the spectra of the compounds with amide function contain bands of stretching N–H of 3280-3263 cm^{-1}, while nitriles have the band of stretching C≡N vibrations near 2250 cm^{-1}. It has been determined that all of the compounds are mostly active against the strain of Candida aibicans fungi. The most resistant microorganism was found to be the strains of Staphylococcus aureus. The only exception is the derivative modified with the thioacetic acid residue in position 2 and unsubstituted benzyl in position 3, which appeared to be highly active against Staphylococcus aureus strain. Amides of thioactetic acid modified in position 3 with 3,4-dichlorobenzyl substituent and thioacetamide substituents in position 2 are active against Pseudomonas aeruginosa, as well as the compound, which contains 3-chlorobenzyl substituent in position 3 and p-chlorobenzotiol substituents in position 2 of thieno[2,3-d]pyrimidine.

СИНТЕЗ ТА АНТИМІКРОБНА АКТИВНІСТЬ ПОХІДНИХ ЕТИЛ 5-МЕТИЛ-2-(АЛКІЛТІО)-4-ОКСО-3,4-ДИГІДРОТІЄНО[2,3-d]ПІРИМІДИН-6-КАРБОКСИЛАТІВ

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Ключові слова: *тіофен; піримідин; тіосечовина*

Шляхом алкілування продуктів взаємодії діетил 3-метил-5-{[(метилтіо)карбонотіоїл]аміно}-2,4-тіофендикарбоксилату з бензиламінами отримані нові похідні етил 5-метил-2-(алкілтіо)-4-оксо-3,4-дигідротієно[2,3-d] піримідин-6-карбоксилатів. Встановлено, що в спектрах ^{1}H ЯМР сигнал групи CH_2, з'єднаної з атомом Нітрогену у положенні 3 тієно[2,3-d]піримідинової системи, для усіх кінцевих сполук знаходиться в діапазоні 5,35-5,4 м.ч., в той же час положення сигналу метиленової групи біля атома Сульфуру значно залежить від будови радикалу, безпосередньо зв'язаного з нею. В ІЧ-спектрах для усіх сполук характерними є інтенсивні смуги валентних коливань C=O 1721-1678 $см^{-1}$, для сполук з амідним фрагментом наявні смуги валентних коливань N–H 3280-3263 $см^{-1}$, для нітрилів спостерігається смуга валентних коливань C≡N при 2250 $см^{-1}$. Встановлено, що всі сполуки найбільше пригнічують ріст грибів Candida aibicans. Найменш активною більшість сполук виявилась до штамів Staphylococcus aureus. Виключенням є лише сполука із залишком тіооцтової кислоти у положенні 2 та незаміщеним бензильним замісником у положенні 3, яка проявила значну активність по відношенню до штаму Staphylococcus aureus. Аміди тіооцтової кислоти з 3,4-дихлоробензильним замісником у положенні 3 та тіоацетамідними замісниками у положенні 2 є активними по відношенню до Pseudomonas aeruginosa, так як і сполука, що містить 3-хлоробензильний замісник у положенні 3 тієно[2,3-d]піримідину та п-хлоробензотіольний замісник у положенні 2.

СИНТЕЗ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ ПРОИЗВОДНЫХ ЭТИЛ 5-МЕТИЛ-2-(АЛКИЛТИО)-4-ОКСО-3,4-ДИГИДРОТИЕНО[2,3-d]ПИРИМИДИН-6-КАРБОКСИЛАТОВ

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Ключевые слова: *тиофен; пиримидин; тиомочевина*

Путем алкилирования продуктов взаимодействия диэтил 3-метил-5-{[(метилтио)карбонотиоил]амино}-2,4-тиофендикарбоксилата с бензиламинами получены новые производные этил 5-метил-2-(алкилтио)-4-оксо-3,4-дигидротиено[2,3-d]пиримидин-6-карбоксилатов. Установлено, что в спектрах ^{1}H ЯМР сигнал группы CH_2, связанной с атомом азота в положении 3 тиено[2,3-d]пиримидиновой системы, для всех конечных соединений находится в диапазоне 5,35-5,4 м.д., в то же время положение сигнала метиленовой группы возле атома серы значительно зависит от строения радикала, непосредственно связанного с ней. В ИК-спектрах для всех соединений характерными являются интенсивные полосы валентных колебаний C=O 1721-1678 $см^{-1}$, для соединений с амидным фрагментом присутствуют полосы валентных колебаний N–H 3280-3263 $см^{-1}$, для нитрилов наблюдается полоса валентных колебаний C≡N при 2250 $см^{-1}$. Установлено, что все соединения наиболее активно подавляют рост грибов Candida aibicans. Большинство соединений оказалось наименее активным по отношению к штаммам Staphylococcus aureus. Исключением из этого является соединение с остатком тиоуксусной кислоты в положении 2 и незамещенным бензильным заместителем в положении 3, которое проявило значительную активность по отношению к штамму Staphylococcus aureus. Амиды тиоуксусной кислоты с 3,4-дихлорбензильным заместителем в положении 3 и тиоацетамидными заместителями в положении 2 являются активными по отношению к Pseudomonas aeruginosa так же как и соединение, содержащее 3-хлорбензильный заместитель в положении 3 тиено[2,3-d]пиримидина и п-хлорбензотиольный заместитель в положении 2.

It has been recently published that esters of 5-methyl-2-thioxothieno[2,3-d]pyrimidine-6-carboxylic acid display affinity to histamine receptors of 5-HT_3 subtype and could be used as medicines for treatment of Bowel disease. They may also bind 5-HT_{1A} subtype receptors, which make them useful for treatment of stress induced ulcer [1]. As the result of *in vivo* investigation of S-glycosides of ethyl ester of 3-amino-5-methyl-2-thioxothieno[2,3-d]pyrimidine-6-carboxylic acid some compounds with a high range of anti-inflammatory activity have been found [2]. Esters of 2-S-alkylthiothieno[2,3-d]pyrimidine-6-carboxylic acid may also be applied as the intermediates for preparation of fused systems of heterocycles with the antimicrobial activity [3].

Since the number of works dedicated to investigations of the biological activity of 5-methyl-2-thioxothieno[2,3-d]pyrimidine-6-carboxylic acid is not enough, we devoted our work to the synthesis and research of the antimicrobial activity of some derivatives of ethyl 5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno [2,3-d]pyrimidine-6-carboxylate.

Synthesis of the target compounds has been carried out by cyclization of the starting dithiocarbamate **1** [4,5], with primary, amines [4]. Mostly amines of benzyl type were used. The above mentioned procedure resulted in the corresponding 3-R-5-methyl-4-oxo-2-thioxo-1,2,3,4-tertrahydrothieno[2,3-d]pyrimidine-6-carboxylates **2** as the crude products, which were suitable for further use without additional purification.

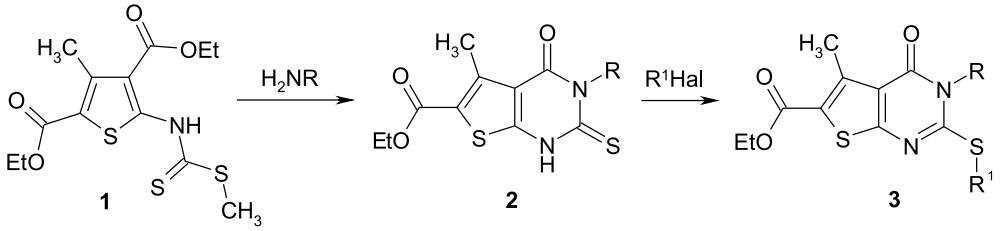
Target molecules **3** were prepared by alkylation of intermediates **2** with different alkyl halides (like benzyl chlorides and derivatives of chloroacetic acid). The radicals R and R^1 and physicochemical properties of compounds **3** are listed in Table 1.

The structures of all compounds **3** were determined by ^{1}H NMR and IR-spectral data (Tables 2 and 3). In all ^{1}H NMR-spectra of compounds **3** the signal of the methyl group is observed in the range of 2.65-2.85 ppm together with the signals of the carbethoxy-group protons as a triplet of CH_3 at 1.15-1.38 ppm and a quartet of CH_2-group at 4.2- 4.6 ppm. The signal of the CH_2-group adjacent to the nitrogen-atom in position 3 of thieno[2,3-d]pyrimidine system is always observed in the range of 5.35-5.40 ppm. The position of the methythylene-group attached to the atom of sulfur much depends upon the structure of R^1; for example, for benzylated derivatives its position is located in the range of 4.43-4.47 ppm, for compounds with the benzoyl-group attached to CH_2 it is shifted downfield to 4.78-4.85 ppm. For compounds **3h**, **3k**, **3p** and **3q** with the carbethoxy-group this signal is found at 3.8-4.05 ppm, nitriles **3g** and **3m** – 4.20-4.35 ppm, carboxamides **3d**, **3l**, **3r-3u** – 3.85-4.1 ppm; for acid **3e** the signal $C\underline{H}_2COOH$ is located at 4.03 ppm. For compounds **3t** and **3u** with the phenylethyl fragment two signals of methylene-groups are present – 4.2 (2H, t., $NC\underline{H}_2CH_2$ Ph) and 3.03 (2H t., $NCH_2C\underline{H}_2$Ph). The integral intensity of all the signals present in the region the aromatic protons resonance well correlates with the number of protons in the structure of compounds **3**. For compound **3e** the signal of the carboxyl-group OH-proton is observed at 12.8 ppm; amides **3d, 3l, 3r-3u** show the signals of NH-proton in the range of 7.9-8.7 ppm.

IR-spectra of all compound **3** show the stretching bands of C–H in the region of 3091-2853 cm^{-1}; for all of these compounds the intensive C=O stretching band is present at 1721-1678 cm^{-1}. The spectra of compounds **3** with the amide function contain bands of stretching N–H 3280-3263 cm^{-1}, while nitriles have the band of stretching C≡N vibrations near 2250 cm^{-1}.

The antimicrobial activity of compound **3** were investigated by the agar well diffusion method [6, 7]. The antimicrobial effect was measured by the diameter of the growth inhibition zone based on the known data about active antibiotics applied for the well diffusion method against susceptible microorganism strains [7, 8]. To evaluate the antimicrobial activity the following criteria were used: in the case of the inhibition zone absence or its diameter was less than 10 mm either the bacteria strains were considered to be resistant or the concentration of the compound tested was rather low for the inhibition effect; the diameter of the inhibition zone was 10-15 mm – the low sensitivity of the bacteria strain to the compound in the given concentration; the diameter of the inhibition zone of 15-25 mm was considered as the sign of the substance activity against the microorganism strain; the diameter of the inhibition zone of 25 mm or more was considered as the evidence of the high antimicrobial activity of the compound tested. The results of the antimicrobial activity assay are presented in Table 4.

All of compounds **3** possessed the antimicrobial activity, but they were mostly active against the strain of *Candida albicans* fungi. The most antifungal activity was displayed by compounds **3e**, **3o** and **3s,** which



Scheme

Table 1

Physicochemical properties of ethyl
5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate derivatives **3**

Compound	R	R^1	Mol. formula M.w.	Yield %, in the alkylation step	M.p., °C	N% calc./found
3a	Bn	CH_3	$C_{18}H_{18}N_2O_3S_2$ 374.48	76	180	7.48/7.57
3b	Bn	CH_2COPh	$C_{25}H_{22}N_2O_4S_2$ 478.59	87	159-161	5.85/5.99
3c	Bn	p-ClBn	$C_{24}H_{21}ClN_2O_3S_2$ 485.03	85	137-139	5.78/5.92
3d	Bn	CH_2CONH(Cyclohexyl)	$C_{25}H_{29}N_3O_4S_2$ 499.66	94	261-262	8.41/8.57
3e	Bn	CH_2COOH	$C_{19}H_{18}N_2O_5S_2$ 418.49	61	192-193	6.69/6.85
3f	4-FBn	CH_2COPh	$C_{25}H_{21}FN_2O_4S_2$ 496.58	82	176-178	5.64/5.72
3g	4-FBn	p-ClBn	$C_{24}H_{20}ClFN_2O_3S_2$ 503.02	89	162-164	5.57/5.87
3h	4-FBn	CH_2COOEt	$C_{21}H_{21}FN_2O_5S_2$ 464.54	70	177-179	6.03/6.17
3i	2-ClBn	CH_2COPh	$C_{25}H_{21}ClN_2O_4S_2$ 513.04	85	163-165	5.46/5.67
3g	2-ClBn	CH_2CN	$C_{19}H_{16}ClN_3O_3S_2$ 433.94	68	166-168	9.68/9.78
3k	2-ClBn	CH_2COOEt	$C_{21}H_{21}ClN_2O_5S_2$ 480.99	73	128-130	5.82/5.95
3l	2-ClBn	$CH_2CONHBn$	$C_{26}H_{24}ClN_3O_4S_2$ 542.08	93	175-176	7.75/7.92
3m	3-ClBn	CH_2CN	$C_{19}H_{16}ClN_3O_3S_2$ 433.94	62	183-185	9.68/9.98
3n	3-ClBn	CH_2COPh	$C_{25}H_{21}ClN_2O_4S_2$ 513.04	85	164-165	5.46/5.55
3o	3-ClBn	p-ClBn	$C_{24}H_{20}Cl_2N_2O_3S_2$ 519.47	78	140-145	5.39/5.67
3p	3-ClBn	CH_2COOEt	$C_{21}H_{21}ClN_2O_5S_2$ 480.99	82	152-155	5.82/5.93
3q	3,4-diClBn	CH_2COOEt	$C_{21}H_{20}Cl_2N_2O_5S_2$ 515.44	88	191-193	5.43/5.49
3r	3,4-diClBn	$CH_2CONHBn$	$C_{26}H_{23}Cl_2N_3O_4S_2$ 576.52	93	215-217	7.29/7.44
3s	3,4-diClBn	CH_2CONH(Cyclohexyl)	$C_{25}H_{27}Cl_2N_3O_4S_2$ 568.55	90	218-220	7.39/7.49
3t	CH_2CH_2Ph	$CH_2CONHBn$	$C_{27}H_{27}N_3O_4S_2$ 521.66	78	212-213	8.06/8.22
3u	CH_2CH_2Ph	CH_2CONH(Cyclohexyl)	$C_{26}H_{31}N_3O_4S_2$ 513.68	83	197-198	8.18/8.35

showed the most diameters of the growth inhibition zones. The most resistant microorganisms were determined to be the strains of *Staphylococcus aureus* and *Proteus vulgaris*. The only exception was the derivative **3e** modified with the thioacetic acid residue in position 2, which appeared to be highly active against *Staphylococcus aureus* strain. Amides of thioactetic acid modified in position 3 with 3,4-dichlorobenzyl substituent **3r** and **3s** were active against *Pseudomonas aeruginosa*, as well as compound **3o** with 3-chlorobenzyl substituents in position 3 and *p*-chlorobenzotiol substituents in position 2.

Table 2

Data of ^{1}H NMR-spectra of ethyl
5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate derivatives **3**

Compound	Chemical shift, δ, ppm.				
	NH OH	CH_3 (3H, s.)	OCH_2CH_3 (3H, t. + 2H, q.)	aliphatic protons	aromatic protons
1	2	3	4	5	6
3a	-	2.3	1.37 + 4.33	2.58 (3H, t., SCH_3) 5.31 (2H, s., CH_2Ph)	7.30 (5H, m., Ar-H)
3b	-	2.78	1.35 + 4.3	5.3 (2H, s., NCH_2Ph) 4.85(2H,s., SCH_2COPh)	7.30 (5H, m., Ar-H) 7.55 (2H, t., H-5′+ H-3′) 7.68 (1H, t., H-4′) 8.02 (2H, d., H-2′+ H-6′)
3c	-	2.83	1.37 + 4.3	5.25(2H, s., CH_2Ph) 4.47 (2H, s., SCH_2(4-ClPh))	7.30 (9H, m., Ar-H)
3d	7.97 (NH)	2.83	1.3 + 4.3	1.1-1.8 (10 H, m., cyclohexyl) 3.89 (2H, s., SCH_2CO) 5.3 (2H, s., NCH_2Ph) 3.52 (1H, m., NHCHcyclohexyl)	7.30 (5H, m., Ar-H)
3e	12.8 (OH)	2.85	1.22 + 4.32	4.03 (2H, s., SCH_2COOH) 5.25 (2H, s.,NCH_2Ph)	7.30 (5H, m., Ar-H)
3f	-	2.75	1.15 + 4.25	4.8 (2H, s., SCH_2COPh) 5.32 (2H, s., NCH_2(4-FPh))	7.06 (2H, t., H-2 + H-6) 7.46 (2H, q., H-3 + H-5) 7.55 (2H, t., H-5′ + H-3′) 7.64 (1H, t. H-4′) 8.07 (2H, d., H-2′ + H-6′)
3g	-	2.82	1.15 + 4.32	4.43 (2H, s., SCH_2(4-ClPh)) 5.2 (2H, s., NCH_2(4-FPh))	7.38 (2H, d., H-3′ + H-5′) 7.31 (2H, q., H-3 + H-5) 7.27 (2H, d., H-2′ + H-6′) 7.0 (2H, t., H-2 + H-6)
3h	-	2.83	1.25 + 4.2 1.35 + 4.4	3.8 (2H, s., SCH_2COOEt) 5.3 (2H, s., NCH_2(4-FPh))	7.46 (2H, q., 3-H + 5-H) 7.06 (2H, t., 2-H + 6-H)
3i	-	2.8	1.35+4.75	4.78 (2H, s., SCH_2COPh) 5.4 (2H, s., NCH_2(2-ClPh))	6.88-7.48 (4H, m., 2-ClPh-H) 7.54 (2H, t., H-5′ + H-3′) 7.64 (1H, t. H-4′) 8.07 (2H, d., H-2′ + H-6′)
3g	-	2.83	1.4+4.32	4.20 (2H, s., SCH_2CN) 5.35 (2H, s., NCH_2(2-ClPh))	6.88-7.48 (4H, m., Ar-H)
3k		2.85	1.25 + 4.2 1.35 + 4.4	3.92 (2H, s.,SCH_2COOEt) 5.37 (2H, s., NCH_2(2-ClPh))	7.9-7.5 (4H, m., Ar-H)
3l	8.6 (NH)	2.85	1.42 + 4.35	5.3 (2H, s., NCH_2(2-ClPh)) 4.1 (2H, s., SCH_2CO) 4.32 (2H, s., $NHCH_2Ph$)	6.9 -7.5 (9H, m., Ar-H)
3m	-	2.83	1.34 + 4.31	4.35 (2H,s., SCH_2CN) 5.27 (2H, s., NCH_2(3-ClPh))	7.2-7.4 (4H, m., Ar-H)
3n	-	2.81	1.38 + 4.6	4.27 (2H, s., SCH_2COPh) 5.42 (2H, s., NCH_2(3-ClPh))	6.85-8.1 (9H, m., Ar-H)
3o	-	2.84	1.38+4.22	4.45 (2H, s., $NHCH_2$(4-ClPh)) 5.25 (2H, s., NCH_2(3-ClPh))	7.10-7.45 (8H, m., Ar-H)
3p	-	2.79	1.25 + 4.2 1.37 + 4.3	3.98(2H, s., SCH_2COOEt) 5.3 (2H, s., NCH_2(3-ClPh))	7.2-7.4 (4H, m., Ar-H)
3q	-	2.65	1.25 + 4.3 1.35 + 4.4	4.05(2H, s., SCH_2COOEt) 5.32 (2H, s., NCH_2(3,4-diCl-Ph))	7.3-7.6 (3H, m., Ar-H)
3r	8.7 (NH)	2.82	1.42+4.3	4.05 (2H,s., SCH_2CO) 5.32 (2H, s., $NHCH_2Ph$) 4.3 (2H, s., NCH_2(3,4-diCl-Ph))	7.2-7.65 (5H, m., Ar-H)

Table 2 continued

1	2	3	4	5	6
3s	7.9(NH)	2.8	1.32+4.3	1.1-1.8 (10 H, m., cyclohexyl) 3.85 (2H,s., SCH_2CO) 5.3 (2H, s., NCH_2 Ph) 3.55 (1H, NH–CH cyclohexyl)	7.37-7.65 (3H, m., Ar-H)
3t	8.62(NH)	2.81	1.38+4.3	4.07 (2H, s., SCH_2CO) 4.22 (2 H, t., NCH_2CH_2 Ph) 4.34 (2H, s., $NHCH_2$ Ph) 3.03 (2H t., NCH_2CH_2Ph)	7.30 (10H, m., Ar-H)
3u	7.95(NH)	2.83	1.3+4.2	1.1-1.9 (10 H, m., cyclohexyl) 3.97 (2H,s., SCH_2CO) 4.22 (2 H, t., NCH_2CH_2 Ph) 3.03 (2H, t., NCH_2CH_2 Ph) 3.55 (1H, m., NHCH cyclohexyl)	7.3-7.4 (5H, m., Ar-H)

Table 3

Data of IR-spectra of ethyl
5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate derivartives **3**

Compound	Wavenumber, ν, cm^{-1}				
	ν N-H	ν C-H	ν C≡N	ν C=O	ν C=N ν C=C
1	2	3	4	5	6
3a	-	3085 3066 3032 2976 2973 2886	-	1711 1683	1537 1540 1470 1390 1377 1363 1336 1324
3b	-	3063 3034 3007 2983 2914	-	1686	1595 1579 1509 1467 1381 1365 1338
3c	-	3063 3033 2992 2969 2930 2919 2868 2709	-	1718 1686	1594 1540 1509 1471 1406 1388 1372 1358 1335
3d	3277	3091 3033 2989 2931 2853	-	1715 1681 1637	1561 1516 1468 1393 1376 1363 1339 1309
3e		2976 2938 2738 2677	-	1744 1697	1586 1538 1508 1469 1374 1335 1302
3f	-	2981 2909	-	1719 1688	1510 1469 1449 1364 1338 1311
3g	-	2991 2972 2933	-	1715 1686	1603 1540 1509 1490 1471 1444 1433 1406 1387
3h	-	3067 2978 2935	-	1722 1692	1604 1540 1510 1470 1434 1388 1377 1365 1338 1319
3i	-	3059 2973 2924		1715 1686	1579 1537 1509 1467 1448 1365 1326 1293 1251
3g	-	3062 2977 2941 2902	2250	1718	1594 1591 1469 1443 1376 1334
3k	-	3064 2980 2936	-	1719 1691	1537 1511 1468 1443 1402 1385 1368 1335 1315
3l	3288	3064 3029 2989 2929 2872	-	1717 1678	1517 1468 1443 1391 1376 1332
3m	-	2991 2942	2250	1710 1687	1517 1473 1434 1375

Table 3 continued

1	2	3	4	5	6
3m	-	2991 2942	2250	1710 1687	1517 1473 1434 1375
3n	-	3083 3064 2980 2920 2871	-	1683	1597 1579 1509 1464 1435 1396 1378 1370 1355
3o	-	2979 2937	-	1719 1692	1595 1573 1537 1510 1493 1468 1436 1412 1376 1358 1334 1314 1298
3p	-	2988 2933 2906 2872	-	1721 1693	1542 1510 1473 1437 1388 1377 1366 1336 1309
3q	-	2981 2931 2873	-	1719 1689	1560 1537 1509 1469 1432 1400
3r	3263	3089 3031 2981 2935 2871	-	1717 1681	1562 1538 1511 1469 1431 1399 1366 1350 1331
3s	3273	3093 2986 2933 2854	-	1717 1680	1562 1517 1470 1394 1375 1352
3t	3271	3066 3027 2990 2932 2869 2285	-	1715 1681	1604 1586 1552 1533 1511 1468
3u	3980	3090 3024 2986 2932 2854	-	1718 1687	1561 1516 1468 1453 1396 1377 1366 1337 1303

Table 4

Antimicrobial properties of ethyl 5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate derivartives **3**

Compound	The growth inhibition zone diameter, mm experiments were performed in triplicate, n=3					
	Staphylococcus aureus ATCC 25923	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 4636	*Basillus subtilis* ATCC 6633	*Candida albicans* ATCC 653/885
3a	15, 16, 15	15, 14, 15	17, 17, 16	16, 15, 14	17, 16, 16	20, 19, 18
3b	14, 15, 14	16, 17, 16	17, 17, 16	15, 16, 16	18, 19, 18	17, 16, 17
3c	14, 15, 14	14, 15, 14	13, 12, 13	12, 12, 13	17, 17, 17	20, 21, 22
3d	14, 15, 14	14, 15, 14	13, 12, 13	12, 12, 13	17, 17, 17	20, 21, 22
3e	25, 22, 23	15, 15, 16	17, 18, 18	16, 15, 16	18, 19, 20	24, 22, 22
3f	15, 15, 14	14, 13, 14	16, 16, 17	15, 14, 14	16, 16, 16	14, 14, 14
3g	15, 14, 15	18, 17, 17	16, 17, 16	15, 15, 15	18, 19, 19	20, 21, 20
3h	17, 18, 18	13, 13, 14	15, 15, 15	13, 14, 13	15, 16, 16	17, 18, 17
3i	14, 15, 15	12, 13, 13	13, 12, 13	12, 12, 13	15, 15, 15	19, 18, 18
3g	18, 18, 19	16, 15, 15	16, 18, 17	15, 15, 16	17, 18, 18	16, 16, 17
3k	14, 15, 15	17, 18, 17	15, 17, 17	13, 14, 14	16, 15, 16	16, 17, 17
3l	15, 15, 15	16, 15, 16	14, 13, 14	12, 13, 13	14, 15, 14	20, 19, 19
3m	18, 17, 18	16, 16, 17	16, 16, 16	15, 16, 14	19, 18, 18	17, 16, 16
3n	14, 15, 15	13, 13, 13	14, 13, 14	14, 13, 13	16, 15, 16	19, 20, 19
3o	14, 14, 15	16, 17, 17	22, 21, 20	17, 17, 18	18, 19, 19	23, 24, 23
3p	15, 15, 15	13, 14, 14	15, 16, 16	14, 13, 14	15, 16, 17	18, 19, 19
3q	14, 15, 15	14, 13, 14	14, 14, 14	12, 13, 12	15, 17, 16	14, 15, 14
3r	15, 16, 15	14, 13, 13	20, 18, 19	16, 17, 16	16, 15, 16	18, 19, 19
3s	15, 16, 15	14, 14, 13	20, 20, 21	15, 16, 16	15, 16, 17	22, 21, 22
3t	15, 14, 14	14, 13, 14	16, 17, 16	15, 14, 14	15, 16, 16	20, 19, 19
3u	15, 14, 15	14, 14, 14	15, 14, 14	13, 13, 13	16, 17, 16	17, 18, 17

Thus, it is obvious that introduction in position 3 of chlorinated benzyl substituents or the derivative of thioacetic acid in position 2 of ethyl 5-methyl-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylate increases the antimicrobial properties of the compound. It is noteworthy that ethers of thioacetic acid (namely compounds **3h**, **3k**, **3p**, **3q**) show a low activity, which indicates the importance of the OH or NH-acid fragment for compound **3** to be active as an antimicrobial agent.

Experimental Part

Chemical research

The melting points (°C) were measured with a Koeffler melting point apparatus and were not corrected. IR spectra were recorded on Bruker Tensor 27 spectrometer in KBr. ^{1}H NMR spectra were recorded on Bruker DRX-500 (500 MHz) spectrometers in DMSO-d_6 using TMS as an internal standard (chemical shifts are in ppm).

Diethyl methyl-5-{[(methylthio)carbonothioyl] amino}-2,4-thiophenedicarboxylate 1 has been obtained by the known method [4, 5].

General method for synthesis of compounds 3

To 2g (0.0028 mol) of diethyl methyl-5-{[(methylthio)carbonothioyl]amino}-2,4-thiophenedicarboxylate **1** 0.004 mol of the corresponding primary amine was added. The mixture was diluted with DMF and stirred at reflux until the release of methylthiol occurred (about 2-5 hours). Then the reaction mixture was cooled and diluted with water. The mixture obtained was acidified with 1 mL of the concentrated hydrochloric acid and the precipitate formed was filtered and thoroughly washed with a lot of water. Product **2** was additionally purified by reflux in 2-propanol.

To compound **2** 0.9 mmol of DMF, 1 mmol of triethylamine were added together with 1 mmol of an alkylating agent and the mixture was stirred at 70°C for 8-12 hours. After that the reaction mixture was cooled and diluted with water. The precipitate of product **3** formed was filtered and crystallized forming 2-propanol.

Research of the antimicrobial activity

According to the WHO recommendations [6, 7] the following test-strains were used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillis subtilis* ATCC 6633, *Candida albicans* ATCC653/885. The bacterial concentration was 10^7 CFU/mL (determined by McFarland standard). Overnight cultures kept for 18-24 h at 36°C ± 1°C 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 of 100 μg/mL; the open wells were filled with 0.3mL of the solution.

Conclusions

The synthesis of novel derivatives of ethyl 5-methyl-2-(alkylthio)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxylates has been carried out and the compounds obtained have been screened for the antimicrobial activity. As the result, it has been found that introduction of chlorinated benzyl substituents in position 3 of thieno[2,3-d]pyrimidine system or the moiety of thioacetic acid in position 2 promotes the antimicrobial activity, while OH- or NH-acidic fragment of thioacetic acid derivative increases the antimicrobial activity of a molecule.

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Надійшла до редакції 08.01.2013 р.

Acknowledgement

Authors gratefully acknowledge Tatyana P. Osolodchenko, the Head of Microorganism Biochemistry and Nutrient Media laboratory of I.I.Mechnikov Institute of Microbiology and Immunology at NAMS of Ukraine for the microbiological experiment.