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SYNTHESIS AND THE ANTIMICROBIAL ACTIVITY 1-*N*-ALKYLATED DERIVATIVES OF 3-*N*-SUBSTITUTED 1*H*-THIENO[3,2-*d*]PIRIMIDINE-2,4-DIONES

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Key words: thiophene; pyrimidine; aromatic compounds; amides; antimicrobial substances

Two approaches for synthesis of a great variety of 3-N-substituted 1H-thieno[3,2-d]pyrimidine-2,4-diones have been investigated. The first one is based on the interaction of methyl 3-aminothiophene-2-carboxylate with isocyanates, which is a good way for preparation of 3-N-aryl-1H-thieno[3,2-d]pyrimidine-2,4-diones. The key step of the other one, which allows introduction of different alkyl substituents in position 3, is oxidation of 4-oxo-2-thioxo-2,3-dihydrothieno[3,2-d]pyrimidines prepared by interaction of 3-isothiocyanatothiophene-2-carboxylate and the primary aliphatic amines with hydrogen peroxide. Alkylation of the intermediates obtained in both ways resulted in 1-N-alkyl-3-N-substituted 1H-thieno[3,2-d]pyrimidine-2,4-diones. ^{1}H NMR spectra of the target molecules contain the signals of thiophene cycle protons H-6 (δ 8.02-8.18 ppm) and H-7 (δ 7.06-7.15 ppm) together with the signal of CH_2 groups in position 1 of the heterocyclic system in the range of δ 4.70-5.20 ppm. The antimicrobial activity of the compounds synthesized has been investigated by the agar well diffusion method. It has been determined that the compound with phenyl substituents in position 3 and o-methylbenzyl substituent in position 1 is the most active antimicrobial agent. The 1-N-alkyl derivatives of 2,4-dioxo-1,4-dihydro-2H-thieno[3,2-d]pyrimidine-3-yl)propanoic acid benzyl amide appeared to be active against the strains of Staphylococcus aureus and Bacillus subtilis.

СИНТЕЗ ТА АНТИМІКРОБНА АКТИВНІСТЬ 1-N-АЛКІЛОВАНИХ ПОХІДНИХ 3-N-ЗАМІЩЕНИХ 1H-ТІЄНО [3,2-d]ПІРИМІДИН-2,4-ДІОНІВ

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Ключові слова: *тіофен; піримідин; ароматичні сполуки; аміди; антимікробні засоби*

Досліджені два підходи до синтезу похідних 3-N-заміщених 1H-тієно[3,2-d]піримідин-2,4-діонів, які здатні забезпечити велике хімічне розмаїття. Згідно з першим з них, заснованим на взаємодії метилового естеру 3-амінотіофен-2-карбонової кислоти з ізоціанатами, були отримані 3-N-арил заміщені 1H-тієно[3,2-d] піримідин-2,4-діони. Другий підхід, який надає можливість одержати складні алкільні замісники у положенні 3, заснований на окисненні гідрогену пероксидом 4-оксо-2-тіоксо-2,3-дигідротієно[3,2-d]піримідинів, отриманих шляхом взаємодії метилового естеру 3-ізотіоціанатотіофен-2-карбонової кислоти з похідними первинних аліфатичних амінів. Шляхом алкілування отриманих напівпродуктів були синтезовані 1-N-алкіл-3-N-заміщені 1H-тієно[3,2-d]піримідин-2,4-діони. Спектри ^{1}H ЯМР отриманих кінцевих сполук містять сигнали протонів тіофенового циклу у вигляді двох дублетних сигналів протонів H-6 (δ 8.02-8.18 м.ч.) і H-7 (δ 7.01-7.36 м.ч.) та сигнали протонів CH_2 групи замісника в положенні 1 гетероциклічної системи в області δ 4.50-5.25 м.ч. Антимікробну активність отриманих сполук вивчали методом дифузії в агар. Встановлено, що найбільшу антимікробну дію чинить сполука, яка містить фенільний замісник у положенні 3 та о-метилбензильний – у положенні 1 молекули. Досліджені 1-N-алкіловані похідні 3-N-заміщених 1H-тієно[3,2-d]піримідин-2,4-діонів виявились активними по відношенню до штамів Staphylococcus aureus та Basillus subtilis.

СИНТЕЗ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ 1-N-АЛКИЛИРОВАННЫХ ПРОИЗВОДНЫХ 3-N-ЗАМЕЩЕННЫХ 1H-ТИЕНО[3,2-d]ПИРИМИДИН-2,4-ДИОНОВ

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Ключевые слова: *тиофен; пиримидин; ароматические соединения; амиды; противомикробные средства*

Было исследовано два подхода к синтезу производных 3-N-замещенных 1H-тиено[3,2-d]пиримидин-2,4-дионов, которые способны обеспечить большое химическое разнообразие. Согласно первому из них, основанному на взаимодействии метилового эфира 3-аминотиофен-2-карбоновой кислоты с изоцианатами, были получены 3-N-арил замещенные 1H-тиено[3,2-d]пиримидин-2,4-дионы. Второй подход, который дает возможность получить разнообразные алкильные заместители в положении 3, основан на окислении пероксидом водорода 4-оксо-2-тиоксо-2,3-дигидротиено[3,2-d]пиримидинов, полученных путем взаимодействия метилового эфира 3-изотиоцианатотиофен-2-карбоновой кислоты с производными первичных алифатических аминов. Путем алкилирования полученных полупродуктов были синтезированы 1-N-алкил-3-N-замещенные 1H-тиено[3,2-d]пиримидин-2,4-дионы. Спектры ^{1}H ЯМР полученных конечных соединений содержат сигналы протонов тиофенового цикла в виде двух дублетных сигналов H-6 (δ 8.02-8.18 м.д.) и H-7 (δ 7.01-7.36 м.д.) и сигналы протонов CH_2 группы заместителя в положении 1 гетероциклической системы в области δ 4.50-5.25 м.д. Противомикробную активность полученных соединений изучали методом диффузии в агар. Установлено, что наиболее высоким противомикробным действием обладает соединение, которое содержит фенильный заместитель в положении 3 и о-метилбензильный – в положении 1 молекулы. Исследованные 1-N-алкилированные производные 3-N-замещенных 1H-тиено[3,2-d]пиримидин-2,4-дионов оказались активными по отношению к штаммам Staphylococcus aureus и Bacillus subtilis.

Nowadays much information is published about the chemical and pharmacological properties of thiophene and pyrimidine. But the only few of them describe the synthesis and properties of thieno[3,2-*d*] pyrimidine-2,4-diones. Because of the possible high biological activity of the similar compounds development of their preparation methods, together with investigations of their biological activity are of great importance.

Among the derivatives of thieno[3,2-*d*]pyrimidine-2,4-dione the substances with analgesic and antiinflammatory [1], antiulcer [2], antitumor [3, 4] activities have been found. Some of them were reported as the drugs against diabetes mellitus complications [5]. Thieno[3,2-*d*]pyrimidine-2,4-diones are coronary and peripheral blood vessels dilatants [6]; they are also selective serotonin receptors antagonists and adrenergic α-blockers [7], all the abovementioned make thieno[3,2-*d*]pyrimidine-2,4-diones useful for treatment of cardiac and blood circulatory system diseases.

The derivatives of thieno[3,2-*d*]pyrimidine-2,4-dione are mainly obtained by the reaction of methyl 3-aminothiophene-2-carboxylate with aryisocyanates as the second carbonyl component for the pyrimidine ring closure [1, 3]. The interaction of ethyl (2-carbamoylthiophene-3-yl-amino)acetic acid with 1,1'-carbonyldiimidazole is a good way for synthesis of ethyl (2,4-dioxo-3,4-dihydro-2*H*-thieno[3,2-*d*] pyrimidine-1-yl)-acetic acid [5].

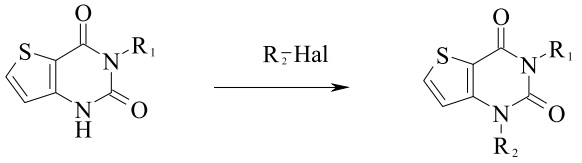
In this work we propose two approaches for synthesis of 3-*N*-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones. According to the first one (Method A) 3-*N*-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones were prepared by interaction of methyl 3-aminothiophene-2-carboxylate with **1** with isocyanates in pyridine (Scheme 1).

The second approach (Method B) was based on oxidation of 3-*N*-substituted 2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidine-4(3*H*)-ones [8] prepared by the method reported by us previously. The advantages of this method are the high yields (up to 76-80%), the short reaction time and the great chemical diversity of the target thieno[3,2-*d*]pyrimidine-2,4-diones.

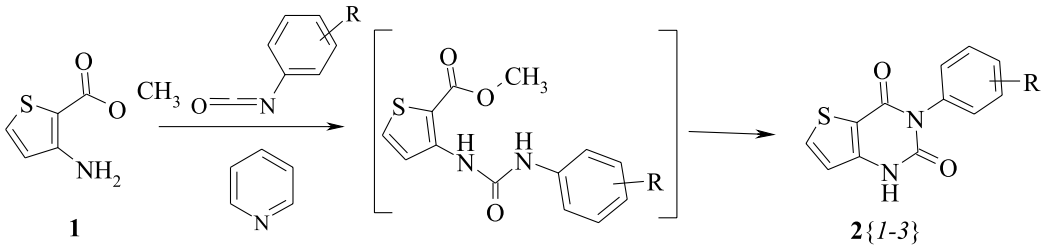
As an example (2,4-dioxo-1,4-dihydro-2*H*-thieno [3,2-*d*]pyrimidin-3-yl)propanoic acid **4** has been obtained according to Method B. The introduction of 1,1'-carbonyldiimidazole promotes amidation of **4** and allows obtaining of the corresponding benzyl amide **5** (Scheme 2).

Alkylation of 3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones **2** and **5** with alkyl halides, α-bromoketones, and chloroacetamides is a versatile reaction to achieve a great chemical diversity in this range of compounds (Scheme 3).

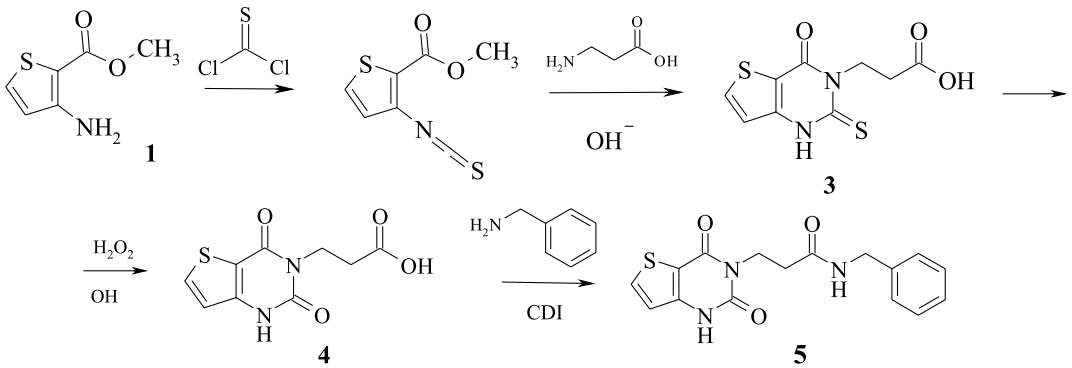
The characteristics of 1-*N*-alkyl-3-*N*-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones **6**{*1–23*} are presented in Table 1.



Scheme 3



Scheme 1



Scheme 2

Table 1

Physical and chemical properties of 1-N-alkyl-3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones **6**

Compound	R_1	R_2	Molecular formula M.w.	N, % calc./ found	M.p., °C	Yield,%
6{*1*}	Ph	$-CH_2CONH(CH_2)_2Ph$	$C_{22}H_{19}N_3O_3S$ 405.48	10.36 / 10.48	255-257	90
6{*2*}	Ph	$-CH_2COPh$	$C_{20}H_{14}N_2O_3S$ 362.41	7.73 / 7.88	198-200	75
6{*3*}	Ph	$-CH_2$(2-Me-C_6H_4)	$C_{20}H_{16}N_2O_2S$ 348.43	8.04 / 8.24	252-254	70
6{*4*}	$-C_6H_4$-4-Me	$-CH_2CO$-1-*N*-(2-Me-C_5H_9N)	$C_{22}H_{25}N_3O_3S$ 411.53	10.21 / 10.32	265-267	85
6{*5*}	$-C_6H_4$-4-Me	$-CH_2COPh$	$C_{21}H_{16}N_2O_3S$ 376.44	7.44 / 7.52	199-201	80
6{*6*}	$-C_6H_4$-4-Me	$-CH_2$(2-Me-C_6H_4)	$C_{21}H_{18}N_2O_2S$ 362.45	7.73 / 7.80	280-282	75
6{*7*}	$-C_6H_4$-4-Me	$-CH_2CONHCH_2$(4-Me-C_6H_4)	$C_{23}H_{21}N_3O_3S$ 419.51	10.02 / 10.10	267-269	90
6{*8*}	$-C_6H_4$-4-Me	$-CH_2CONH$(4-MeO-C_6H_4)	$C_{22}H_{19}N_3O_4S$ 421.48	9.97 / 10.06	265-268	95
6{*9*}	$-C_6H_4$-4-OEt	$-CH_2CO$-1-*N*-(2-Me-C_5H_9N)	$C_{22}H_{25}N_3O_4S$ 427.53	9.83 / 9.91	281-283	85
6{*10*}	$-C_6H_4$-4-OEt	$-CH_2CONH$(4-EtO-C_6H_4)	$C_{24}H_{23}N_3O_5S$ 465.53	9.03 / 9.14	291-293	90
6{*11*}	$-C_6H_4$-4-OEt	$-CH_2$(2,4,6-*tri*Me-C_6H_2)	$C_{24}H_{24}N_2O_3S$ 420.53	6.66 / 6.75	248-250	80
6{*12*}	$-(CH_2)_2CONHBn$	Bn	$C_{23}H_{21}N_3O_3S$ 419.51	10.02 / 10.12	261-263	70
6{*13*}	$-(CH_2)_2CONHBn$	$-CH_2$(3-NO_2-C_6H_4)	$C_{23}H_{20}N_4O_5S$ 464.50	12.06 / 12.14	240-242	75
6{*14*}	$-(CH_2)_2CONHBn$	$-CH_2CONH(CH_2)_2$(3,4-diMeO-C_6H_3)	$C_{28}H_{30}N_4O_6S$ 550.64	10.17 / 10.23	212-214	90
6{*15*}	$-(CH_2)_2CONHBn$	$-CH_2CONH$(4-Et-C_6H_4)	$C_{26}H_{26}N_4O_4S$ 490.59	11.42 / 11.53	264-266	95
6{*16*}	$-(CH_2)_2CONHBn$	$-CH_2COOBn$	$C_{25}H_{23}N_3O_5S$ 477.54	8.80 / 8.92	210-212	75
6{*17*}	$-(CH_2)_2CONHBn$	$-CH_2$(2-CN-C_6H_4)	$C_{24}H_{20}N_4O_3S$ 444.52	12.60 / 12.70	243-245	70
6{*18*}	$-(CH_2)_2CONHBn$	$-CH_2$(4-NO_2-C_6H_4)	$C_{23}H_{20}N_4O_5S$ 464.50	12.06 / 12.12	211-213	75
6{*19*}	$-(CH_2)_2CONHBn$	$-CH_2CONH$(4-MeO-3-Cl-C_6H_3)	$C_{25}H_{23}ClN_4O_5S$ 527.00	10.63 / 10.73	268-270	85
6{*20*}	$-(CH_2)_2CONHBn$	$-CH_2CONH$(2-EtO-C_6H_4)	$C_{26}H_{26}N_4O_5S$ 506.58	11.06 / 11.14	239-241	90
6{*21*}	$-(CH_2)_2CONHBn$	$-CH_2CONH$(2,4,6-*tri*Me-C_6H_2)	$C_{27}H_{28}N_4O_4S$ 504,61	11.10 / 11.18	251-253	95
6{*22*}	$-(CH_2)_2CONHBn$	$-CH_2CONH$(2,6-*di*Me-C_6H_3)	$C_{26}H_{26}N_4O_4S$ 490.59	11.42 / 11.49	273-275	85
6{*23*}	$-(CH_2)_2CONHBn$	$-CH_2CON$(Me)Ph	$C_{25}H_{24}N_4O_4S$ 476.56	11.76 / 11.84	270-272	90

The structures of all the compounds obtained have been confirmed by ^{1}H NMR spectra (Table 2). The thiophene protons H6 and H7 in thieno[3,2-d]pyrimidine structures **6***{1-23}* are sometimes concealed by other signals, but usually clearly observed as doublets in the range of δ 8.02-8.18 ppm (H6) and 7.01-7.36 ppm (H7). The signals of pyrimidine NH disappear, but the new singlet signals of NCH_2 are observed in the range of

Table 2

Data of ^{1}H NMR-spectra of 1-*N*-alkyl-3-*N*-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones 6

Compound	Chemical shift, δ, ppm.		
	NH	Aliphatic protons	Aromatic protons
6{*1*}	8.20 t (1H)	4.70 s (2H, CH_2), 2.70-2.80 m (2H, CH_2), 1.10-1.20 m (2H, CH_2)	8.10 d (1H, H6), 7.06-7.50 m (11H, H7 Ar-H)
6{*2*}	-	5.20 s (2H, CH_2)	8.12 d (2H, Ar-H), 8.08 d (1H, H6), 7.40-7.76 m (6H, Ar-H), 7.32 d (1H, H7), 7.12-7.30 m (2H, Ar-H)
6{*3*}	-	5.20 s (2H, CH_2), 2.30 s (3H, CH_3)	8.08 d (1H, H6), 7.32-7.52 m (5H, Ar-H), 7.08-7.22 m (3H, Ar-H), 7.06 d (1H, H7), 6.96 d (1H, Ar-H)
6{*4*}	-	4.90 m (2H, CH_2), 3.90-4.30 m (2H, CH_2), 2.10 s (3H, CH_3), 1.42-1.70 m (9H, $4CH_2$+CH), 0.80 d (3H, CH_3)	8.10 d (1H, H6), 7.06-7.20 m (4H, Ar-H), 7.04 d (1H, H7)
6{*5*}	-	5.20 s (2H, CH_2), 2.30 s (3H, CH_3)	8.02-8.10 q (3H, H6 Ar-H), 7.75 t (1H, Ar-H), 7.58 t (2H, Ar-H), 7.20-7.36 m (3H, Ar-H), 7.10 d (2H, H7 Ar-H)
6{*6*}	-	5.20 s (2H, CH_2), 2.20 s (3H, CH_3), 2.12 s (3H, CH_3)	8.06 d (1H, H6), 7.04-7.22 m (7H, Ar-H), 7.02 d (1H, H7), 6.90 d (1H, Ar-H)
6{*7*}	8.60 t (1H)	5.20 s (2H,CH_2), 4.20-4.30 m (2H, CH_2), 2.40 s (3H, CH_3), 2.20 s (3H, CH_3)	8.10 d (1H, H6), 7.20-7.30 m (3H, Ar-H), 7.04-7.15 m (6H, H7 Ar-H)
6{*8*}	10.10 s (1H)	4.80 s (2H, CH_2), 3.60 s (3H, OCH_3), 2.60 s (3H, CH_3)	8.18 d (1H, H6), 7.46 d (2H, Ar-H), 7.20-7.30 q (3H, Ar-H), 7.12 m (2H, H7Ar-H), 6.90 d (2H, Ar-H)
6{*9*}	-	4.90 s (2H, CH_2), 4.10-4.21 m (2H, CH_2), 2.40 s (3H, CH_3), 1.40-1.70 m (6H, $3CH_2$), 1.22 (3H, CH_3), 1.10-1.20 s (3H, CH_2+CH)	8.10 d (1H, H6), 6.90-7.20 m (5H, H7 Ar-H)
6{*10*}	10.02 s (1H)	4.90 s (2H, CH_2), 3.96-4.02 m (4H, $2CH_2$), 1.10-1.22 m (6H, $2CH_3$)	8.10 d (1H, H6), 7.45 d (2H, Ar-H), 7.32 d (2H, Ar-H), 7.10 d (2H, Ar-H), 7.02 d (1H, H7), 6.80 d (2H, Ar-H)
6{*11*}	-	5.20 s (2H, CH_2), 4.02-4.10 m (2H,CH_2), 2.10 t (9H, $3CH_3$), 1.22 t (3H, CH_3)	8.02 d (1H, H6), 6.80-7.10 m (7H, H7 Ar-H)
6{*12*}	8.32 t (1H)	5.25 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.40-2.50 m (2H, CH_2)	8.06 d (1H, H6), 7.15-7.30 m (11H, H7 Ar-H)
6{*13*}	8.32 t (1H)	5.30 s (2H, CH_2), 4.20-4.30 m (4H, $2CH_2$), 2.40-2.50 m (2H, CH_2)	8.20 s (1H, Ar-H), 8.10 m (2H, H6 Ar-H), 7.52-7.78 m (2H, Ar-H), 7.15-7.30 m (6H, H7 Ar-H)
6{*14*}	8.32 t (1H) 8.20 t (1H)	4.60 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 3.70 d (6H, $2OCH_3$), 3.15-3.25 m (2H, CH_2), 2.60 t (2H, CH_2), 2.48-2.52 m (2H, CH_2)	8.05 d (1H, H6), 7.18-7.32 m (5H, Ar-H), 7.06 d (1H, H7), 6.64-6.84 m (3H, Ar-H)
6{*15*}	10.00 s (1H) 8.32 t (1H)	4.82 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.30-2.40 m (4H, $2CH_2$), 1.20 t (3H, CH_3)	8.10 d (1H, H6), 7.42 d (2H, Ar-H), 7.20-7.30 m (7H, Ar-H), 7.10 d (1H, H7)
6{*16*}	8.32 t (1H)	5.20 s (2H, CH_2), 4.80 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.30-2.40 m (2H, CH_2)	8.02 d (1H, H6), 7.15-7.30 m (11H, H7 Ar-H)
6{*17*}	8.32 t (1H)	5.35 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.30-2.40 m (2H, CH_2)	8.08 d (1H, H6), 7.88 d (1H, Ar-H), 7.42-7.60 m (2H, Ar-H), 7.04-7.22 m (7H, H7 Ar-H)
6{*18*}	8.34 t (1H)	5.40 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.30-2.40 m (2H, CH_2)	8.06-8.18 q (3H, H6 Ar-H), 7.58 d (2H, Ar-H), 7.18-7.30 m (6H, H7 Ar-H)
6{*19*}	9.36 s (1H) 8.34 t (1H)	4.80 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 3.80 s (3H, OCH_3), 2.30-2.40 m (2H, CH_2)	8.08 d (1H, H6), 7.70 d (1H, Ar-H), 7.12-7.42 m (7H, Ar-H), 7.10 d (1H, H7)
6{*20*}	9.32 s (1H) 8.34 t (1H)	4.95 s (2H, CH_2), 4.10-4.20 m (6H, $3CH_2$), 2.40-2.50 m (2H, CH_2), 1.20 t (3H, CH_3)	8.08 d (1H, H6), 7.80 d (1H, Ar-H), 7.16-7.30 m (6H, Ar-H), 7.00-7.10 m (2H, H7 Ar-H), 6.80-6.90 m (1H, Ar-H)
6{*21*}	9.40 s (1H) 8.34 t (1H)	4.80 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.40-2.50 m (2H, CH_2), 2.25 s (3H, CH_3), 2.10 s (6H, $2CH_3$)	8.08 d (1H, H6), 7.12-7.40 m (6H, H7 Ar-H), 6.80 s (2H, Ar-H)
6{*22*}	9.50 s (1H) 8.34 t (1H)	4.80 s (2H, CH_2), 4.10-4.20 m (4H, $2CH_2$), 2.40-2.50 m (2H, CH_2), 2.20 s (6H, $2CH_3$)	8.08 d (1H, H6), 7.01-7.36 m (6H, H7 Ar-H), 6.80 s (3H, Ar-H)
6{*23*}	8.32 t (1H)	4.50 s (2H, CH_2), 4.25 d (2H, CH_2), 4.20 t (2H, CH_2), 2.40-2.50 m (5H, CH_2), 2.40-2.50 m (3H, CH_3)	8.08 d (1H, H6), 7.31-7.56 m (5H, Ar-H), 7.22-7.30 m (6H, H7 Ar-H)

Table 3

LC/MS-spectra of 1-N-alkyl-3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones **6**

Compound	MS (m/z)
6{*1*}	405, 301, 285, 257, 138, 110
6{*4*}	411, 382, 299, 271, 258, 165, 138
6{*9*}	427, 138, 126, 98, 44
6{*19*}	526, 421, 370, 343, 328, 183, 157, 138, 106
6{*23*}	370, 341, 328, 236, 223, 195, 181, 138, 106

δ 4.50-5.25 ppm for compounds **6**. The multiplet signals of aromatic protons are clearly seen in the range of δ 6.90-8.18 ppm. The position and the splitting of signals well correlate with the structures of compounds.

For 3-*N*-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones **2** and **5** because of the tautomerism with the possible transfer of proton between the nitrogen in position 1 and the oxygen in position 2 the regioselectivity of their alkylation is an important problem. It has been reported that for 1-N-alkylated derivatives of 3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones the signal of NCH_2 group protons is observed in the range of δ 5.20–5.38 ppm [4], while for 2-propoxy-3-propyl-3*H*-thieno[3,2-*d*]pyrimidine-4-one prepared by the reaction of 2-methylthio-3-propyl-3*H*-thieno[3,2-*d*]pyrimidine-4-one with sodium propylate the signal of OCH_2 protons is located at δ 4.38-4.48 ppm [9]. The comparison of the reported data with our results show that compounds **6** are the products with the substituents at the nitrogen atom in position 1.

In the LC/MS-spectra of the compounds synthesized the signals of molecular ions are cleanly observed. The further fragmentation mostly occurs for the fragment in position 1 (Table 3).

As the part of investigations on the biological activity of 1-*N*-alkyl-3-*N*-substituted 1*H*-thieno[3,2-*d*] pyrimidine-2,4-diones the antimicrobial activity of some compounds from this range has been studied.

The antimicrobial activity of compounds **6**{*1-3, 5, 6, 12-16*} has been investigated by the agar well diffusion method [10, 11]. The antimicrobial effect has been 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. For evaluation of the antimicrobial activity the following criteria have been used: in the case of the inhibition zone absence or its diameter less than 10 mm either the bacteria strains are considered to be resistant or the concentration of the tested compound rather low for the inhibition effect; the diameter of the inhibition zone of 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 is considered as the sign of the substance activity against the microorganism strain; the diameter of the inhibition zone of 25 mm or more is considered as the evidence of the high antimicrobial activity of the compound studied. The results of the antimicrobial activity assay are given in Table 4.

It has been found that all compounds **6**{*1-3, 5, 6, 12-16*} are active against *Staphylococcus aureus* and *Bacillus subtilis*. While compounds **6**{*2, 3, 5, 6*} showed the high activity against the strain of *Escherichia coli.*

Table 4

Antimicrobial properties of 1-N-alkyl-3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones concentration 100 μg per ml*

Compound	Staphylococcus aureus ATCC 25923	Escherichia coli ATCC 25922	Pseudomonas aeruginosa ATCC 27853	Proteus vulgaris ATCC 4636	Bacillus subtilis ATCC 6633	Candida albicans ATCC 653/885
6{1}	++	–	–	–	++	+
6{2}	++	++	++	–	++	+
6{3}	++	+++	++	–	++	+
6{5}	++	++	++	–	++	+
6{6}	++	++	++	–	++	+
6{12}	++	+	+	–	++	+
6{13}	++	+	+	–	++	+
6{14}	++	+	+	–	++	+
6{15}	++	+	+	–	++	+
6{16}	++	+	+	–	++	+
DMSO	+	–	–	–	+	–

* "–" – diameter of the growth inhibition zone less than 10 mm; "+" – diameter of the growth inhibition zone 10-15 mm; "++" – diameter of the growth inhibition zone 15-25 mm; "+++" – diameter of the growth inhibition zone more than 25 mm.

It has been found that the most active compound is **6**{*3*}, which contains the phenyl substituent in position 3 and o-methoxybenzyl in position 1 of the molecule. Compounds **6**{*12-16*} showed a weak antimicrobial action in relation to the strains of *Escherichia coli* and *Pseudomonas aeruginosa.* Compounds **6**{*1-3, 5, 6, 12-16*} revealed the moderate antifungal activity against the strain of *Candida albicans.* All compounds **6**{*1-3, 5, 6, 12-16*} appeared to be inactive against *Proteus vulgaris.*

Experimental Part

The melting points (°C) were measured with a Buchi B-520 melting point apparatus and were not corrected. Elemetal analyses (N) were measured with a EuroVector Euro EA-3000 apparatus.

^{1}H-NMR spectra were recorded on a Varian Mercury 200 (200 MHz) spectrometer in DMSO-d_6 using TMS as an internal standard (chemical shifts are reported in ppm).

LC/MS-spectral analyses were obtained on a PE SCIEX API 150EX chromatograph equipped with a mass spectrometer. Compounds **6**{*1, 4, 9, 19, 23*} were prepared to ≥95% purity by LC/MS.

All the solvents and reagents were obtained from commercial sources and were used without additional purification. Methyl 3-aminothiophene-2-carboxylate **1** was obtained from "*Sigma-Aldrich*" (USA).

General method for synthesis of 3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones 2{*1-3*}.

Dissolve methyl 3-aminothiophene-2-carboxylate **1** (0.0015 mole) in 2 ml of pyridine and add 0.0016 mole of the corresponding isocyanate. Heat the mixture at reflux till the reaction is completed (TLC control). Then dilute the reaction mixture with water and acidify with concentrated hydrochloric acid. Filter the precipitate formed, wash with water and crystallize from 2-propanol : DMF mixture.

*3-Phenyl-1H-thieno[3,2-d]pyrimidine-2,4-dione **2**{1}:* Yield – 80%. M.p. – 186-188°C. ^{1}H NMR, d, ppm: 11.82 s (1H, NH); 8.08 d (1H, H6); 7.42-7.50 m (3H, Ar-H); 7.24-7.30 m (2H, Ar-H); 6.96 d (1H, H7). Anal. for $C_{12}H_8N_2O_2S$: calc. N, 11.47; found N, 11.54.

*3-(4-Methylphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione **2**{2}:* Yield – 75%. M.p. – 188-200°C. ^{1}H NMR, δ, ppm: 11.82 s (1H, NH); 8.08 d (1H, H6); 7.24 d (2H, Ar-H); 7.12 d (2H, Ar-H); 6.96 d (1H, H7); 2.30 s (3H, CH_3). Anal. for $C_{13}H_{10}N_2O_2S$: calc. N, 10.85; found. N, 10.96.

*3-(4-Ethoxyphenyl)-1H-thieno[3,2-d]pyrimidine-2,4-dione **2**{3}:* Yield – 78%. M.p. – 204-206°C. ^{1}H NMR, δ, ppm: 11.90 s (1H, NH); 8.08 d (1H, H6); 6.90-7.20 m (5H, H7 Ar-H); 4.00-4.10 m (2H, CH_2); 1.20 t (3H, CH_3). Anal. for $C_{14}H_{12}N_2O_3S$: calc. N, 9.72; found. N, 9.82.

4-Oxo-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidine-3-propanoic acid 3 was obtained by the method reported previously [8].

Method for the synthesis of (2,4-dioxo-1,4-dihydro-2*H*-thieno[3,2-*d*]pyrimidine-3-yl)propanoic acid 4.

Dissolve 0.01 mole (2.56 g) of 4-Oxo-2-thioxo-2,3-dihydrothieno[3,2-*d*]pyrimidine-3-propanoic acid **3** in 20 ml of sodium hydroxide solution (0.02 mole) at room temperature. Then add dropwise 0.04 mole of 50% solution of hydrogen peroxide. Stir the reaction mixture additionally overnight. Add 5 ml of concentrated hydrochloric acid. Filter the precipitate formed, wash with water and crystallize from DMF-propanol-2 mixture.

(2,4-Dioxo-1,4-dihydro-2H-thieno[3,2-d]pyrimidine-3-yl)propanoic acid **3**. Yield – 60%. M.p. – 192-194°C. ^{1}H NMR, δ, ppm: 11.80 s (1H, NH); 8.02 d (1H, H6); 6.92 d (1H, H7); 4.02-4.10 m (2H, CH_2); 3.20 s (1H, OH in exchange); 2.32-2.40 m (2H, CH_2). Anal. for $C_9H_8N_2O_4S$: calc. N, 11.66; found N, 11.74.

2,4-Dioxo-1,4-dihydro-2*H*-thieno[3,2-*d*]pyrimidine-3-yl)propanoic acid benzyl amide 5 was obtained by the method reported [8].

2,4-Dioxo-1,4-dihydro-2H-thieno[3,2-d]pyrimidine-3-yl)propanoic acid benzyl amide **5.** Yield – 95%. M.p. – 221-223°C. ^{1}H NMR, δ, ppm: 11.80 s (1H, NH); 8.30 t (1H, NH); 8.02 d (1H, H6); 7.20-7.30 m (5H, Ar-H); 6.92 d (1H, H7); 4.10-4.20 m (4H, $2CH_2$); 2.40-2.50 m (2H, CH_2). Anal. for $C_{17}H_{17}N_3O_3S$: calc. N, 12.24; found N, 12.32.

General method for synthesis of 1-N-alkyl derivatives of 3-N-substituted-1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones 6{*1-23*}.

To the mixture of 0.5 mmole of 3-substituted 3-N-substituted 1*H*-thieno[3,2-*d*]pyrimidine-2,4-dione **2***{1-3}* or **5** and potassium carbonate (1.5 mmole) add 0.6 mmole of the corresponding halocarbon (alkyl halide, α-bromoketone, substituted chloroacetamide, etc.). Stir the reaction mixture at 60°C for 1-2 hours. Then dilute with water (30 ml), filter the precipitate formed, washe with water and crystallize from 2-propanol.

Study of the antimicrobial activity

According to the WHO recommendations [10, 11] the following test-strains have been used *Staphylococcus aureus* ATCC 25923, *Esherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus 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 Mueller-Hinton agar (Dagestan Research Institute of Nutrient Media). The compounds were introduced to the wells in the form of DMSO solution in the concentrations of 100 μg/mL; the open wells were filled with 0.3 mL of the solution.

Conclusions

The novel 1-*N*-alkyl derivatives of 3-*N*-aryl-1*H*-thieno[3,2-*d*]pyrimidine-2,4-diones and (2,4-dioxo-

1,4-dihydro-2*H*-thieno[3,2-d]pyrimidine-3-yl)propanoic acid benzyl amide have been synthesized and screened for the antimicrobial activity. It has been determined that all of the compounds obtained are active against the strains of *Staphylococcus aureus* and *Bacillus subtilis.* The introduction of phenyl substituent in position 3 and *o*-methoxybenzyl in position 1 of 1*H*-thieno[3,2-d]pyrimidine-2,4-dione increases the activity of the compound against *Escherichia coli*.

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