# СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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## SYNTHESIS AND THE ANTIMICROBIAL ACTIVITY OF 5-METHYL-6-(2-METHYL-1,3-THIAZOL-4-YL)-3-PHENYLTHIENO[2,3-*d*]PYRIMIDINE-2,4(1*H*,3*H*)-DIONES

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*Key words: thiophene; pyrimidine; alkylation; thiazole*

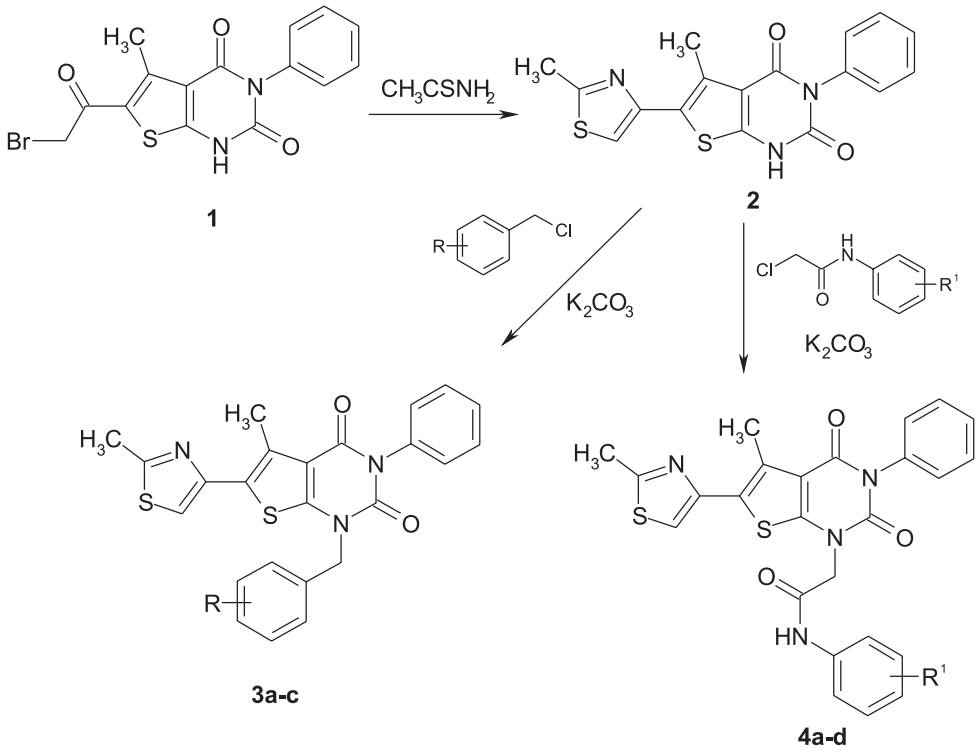
*By the reaction of 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione with thioacetamide 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione was obtained; further the compound was modified by alkylation of its position 1 with benzyl chlorides and chloroacetamides. The structures of the compounds obtained have been confirmed by $^1$H NMR and mass-spectral data. All the $^1$H NMR spectra of the compounds obtained have the signals of thiazole cycle at 7.62-7.57 ppm, for 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d] pyrimidine-2,4(1H,3H)-diones the signals of the methylene group protons are observed in the range of 5.13-5.21 ppm for benzyl substituted derivatives and 4.78-4.82 ppm for the compounds with the acetamide fragment; the last ones also contain the sharp signal of NH proton in the range of 9.68-10.41 ppm. 5-Methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-dione showed the antimicrobial activity against Staphylococcus aureus higher than the reference drugs Metronidazole and Streptomycin, it also appeared to be moderately active against Pseudomonas aeruginosa and Candida albicans fungi. The antimicrobial activity of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-d]pyrimidine-2,4(1H,3H)-diones is inferior to the activity of the compound with the hydrogen atom in position 1; the highest activity has been determined for the derivative with 4-methylbenzyl substituent in position 1, which inhibits the growth of Staphylococcus aureus and Candida albicans.*

In the last years the interest to derivatives of 6-hetarylthieno[2,3-*d*]pyrimidines has grown. They were reported as adenosine $A_{2A}$ receptors antagonists [8], as the compounds with antioxidant [10] activity and acetyl-CoA carboxylase (ACC) inhibitors [7]. Some compounds of the similar structure are potential anti-viral agents [9]. Our last investigations also confirm the expediency of searching novel antimicrobial agents in the range of thieno [2,3-*d*]pyrimidine derivatives modified in position 6 with the aromatic heterocyclic substituents [2, 3, 4, 12]. We have also found that in some cases the presence of the electron-withdrawing substituent in position 6 of thieno [2,3-*d*]pyrimidine together with alkylation of the nitrogen atom in position 1 promotes the antimicrobial activity of the compounds [11].

In order to construct the novel 6-hetarylthieno[2,3-*d*] pyrimidine-2,4(1*H*,3*H*)-diones we performed the reaction of previously reported 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **1** [4] with thioacetamide in the acetic acid medium and as the result 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **2** was obtained. Further alkylation of compound **2** was performed with benzyl chlorides and chloroacetamides in the DMF medium promoted with potassium carbonate (Scheme). The series of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **3** and **4** were isolated as white crystalline solids (Tab. 1).

In the $^1$H NMR spectrum of compound **2** the signals of thiazole and thiophene methyl groups are very close to each other 2.57 (3H, s, $CH_3$) and 2.67 (3H, s, $CH_3$); the signal of aromatic proton of thiazole is observed at 7.57 ppm. In all of the $^1$H NMR spectra of compounds **3** and **4** the signals of the methylene group protons are observed in the range of 5.13-5.21 ppm for compounds **3**, and 4.78-4.82 ppm for derivatives **4**. The sharp signals of acetamide NH are present in the spectra of compounds **4** in the region of 9.68-10.41 ppm.

For all of the compounds obtained the screening of the antimicrobial activity by the agar diffusion method has been performed; the results are given in Tab. 3. The screening showed that 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione **2** displayed the highest antimicrobial activity; its acti-




Scheme

vity against *Staphylococcus aureus* was higher than that of the reference drugs Metronidazole and Streptomycin; it also appeared to be moderately active against *Pseudomonas aeruginosa* and *Candida albicans* fungi. Alkylation of position 1 of the thieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione fragment decreases the antimicrobial activity.

Among 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **3** and **4** the highest activity was revealed by derivative **3b** with the 4-methylbenzyl substituent in position 1, which moderately inhibited the growth of *Staphylococcus aureus* and *Candida albicans*. It is noteworthy that none of the compounds tested inhibited the growth of *Bacillus subtilis* strain.

**Materials and Methods**

**Chemical Part**

The melting points (°C) were measured with a Kofler melting point apparatus and were not corrected. The IR spectrum was recorded on a Bruker Tensor 27 spectrometer in KBr. $^1$H NMR spectra were recorded on a Varian Mercury (200 MHz) spectrometer in DMSO-$d_6$ using TMS as an internal standard (chemical shifts are in ppm). LC/MS was recorded with a PE SCIEX API

Table 1

Physicochemical properties of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **3** and **4**

| Compd. No. | R | R$^1$ | Mol. formula M.w. | Yield %, in the alkylation step | M.p., °C | N% calc. / found |
|---|---|---|---|---|---|---|
| 3a | H | – | $C_{24}H_{19}N_3O_2S_2$ 445.57 | 76 | 279-280 | 9.43 / 9.67 |
| 3b | 4-Me | – | $C_{25}H_{21}N_3O_2S_2$ 459.59 | 83 | 272-274 | 9.14 / 9.18 |
| 3c | 4-F | – | $C_{24}H_{18}FN_3O_2S_2$ 463.56 | 71 | 253-255 | 9.06 / 9.32 |
| 4a | – | 4-Me | $C_{26}H_{22}N_4O_3S_2$ 502.62 | 71 | > 300 | 11.15 / 11.29 |
| 4b | – | 2,4-diMe | $C_{27}H_{24}N_4O_3S_2$ 516.65 | 79 | >300 | 10.84 / 10.97 |
| 4c | – | 4-OEt | $C_{27}H_{24}N_4O_4S_2$ 532.64 | 86 | >300 | 10.52 / 10.79 |
| 4d | – | 3,5-diOMe | $C_{27}H_{24}N_4O_5S_2$ 548.64 | 77 | 293-295 | 10.21 / 10.32 |

Table 2

Data of $^1$H NMR-spectra of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones 3 and 4

| Compd. No. | Chemical shift, δ, ppm. | | | |
|---|---|---|---|---|
| | NH | Thiophene $CH_3$ (3H, s.) Thiazole $CH_3$ (3H, s.) | Aliphatic protons | Aromatic protons |
| 3a | – | 2.52 + 2.64 | 5.21 (2H, s, $CH_2$); | 7.25-7.53 (10H, m, Ar-H); 7.62 (1H, s, CH thiazole) |
| 3b | – | 2.58 + 2.64 | 2.26 (3H, s, $CH_3$); 5.13 (2H, s, $CH_2$); | 7.1-7.5 (9H, m, Ar-H); 7.62 (1H, s, CH thiazole) |
| 3c | – | 2.52 + 2.64 | 5.13 (2H, s, $CH_2$); | 7.05-7.5 (9H, m, Ar-H); 7.62 (1H, s, CH thiazole) |
| 4a | 10.21 (1H, s) | 2.64+2.66 | 2.23 (3H, s, $CH_3$); 4.80 (2H, s, $CH_2$); | 7.0-7.5 (9H, m, Ar-H); 7.67 (1H, s, CH thiazole) |
| 4b | 9.68 (1H, s) | 2.63+2.66 | 2.15 (3H, s, $CH_3$); 2.22 (3H, s, $CH_3$); 4.82 (2H, s, $CH_2$); | 6.8-7.5 (9H, m, Ar-H); 7.67 (1H, s, CH thiazole) |
| 4c | 10.18 (1H, s) | 2.64+2.66 | 1.28 (3H, t, $CH_3$); 3.97 (2H, q, $CH_2$); 4.78 (2H, s, $CH_2$); | 6.86 (2H, d, Ar-H); 7.27 (2H, d, Ar-H); 7.46 (5H, m, Ar-H); 7.64 (1H, s, CH thiazole) |
| 4d | 10.41 (1H, s) | 2.63+2.66 | 3.67 (6H, s, $2OCH_3$); 4.80 (2H, s, $CH_2$); | 6.23 (1H, m, Ar-H); 6.79 (2H, d, Ar-H); 7.27 (2H, d, Ar-H); 7.44 (3H, m, Ar-H); 7.65 (1H, s, CH thiazole) |

150EX chromatograph equipped with a mass-spectrometer using the column C18 (100×4 мм), the cycle of analysis was 25 min.

**The method for preparation of 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione 2**

To 2 g of thioacetamide in glacial acetic acid add 9 g of 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-*d*]pyrimidin-2,4(1*H*,3*H*)-dione **1** and reflux the mixture till formation of a blue-violet precipitate and then additionally for 2-3 hours. After cooling dilute the mixture with water, filter the precipitate formed. Next suspend the precipitate in water, alkalify with the concentrated ammonia solution, and boil the suspension for 1 hour. Filter the precipitate formed, wash with plenty of water and dry.

Table 3

The antimicrobial activity of 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **2**, **3** and **4**

| Compnd. No. | Diameter of the growth inhibition zone*, mm | | | | | |
|---|---|---|---|---|---|---|
| | Staphylococcus aureus ATCC 25923 | Escherichia coli ATCC 25922 | Proteus vulgaris ATCC 4636 | Pseudomonas aeruginosa ATCC 27853 | Bacillus subtilis ATCC 6633 | Candida albicans ATCC 653/885 |
| 2 | +++ | + | + | + | – | ++ |
| 3a | – | – | – | – | – | – |
| 3b | ++ | + | + | + | – | ++ |
| 3c | – | – | – | – | – | – |
| 4a | + | – | – | – | – | – |
| 4b | – | – | – | – | – | + |
| 4c | – | – | – | – | – | – |
| 4d | + | – | – | – | – | + |
| Metr. ** | + | + | – | – | ++ | + |
| Strept.*** | ++ | ++ | – | – | ++ | – |

* "–" – diameter of the growth inhibition zone is less than 10 mm; "+" – diameter of the growth inhibition zone is 10-15 mm; "++" – diameter of the growth inhibition zone is 15-20 mm; "+++" – diameter of the growth inhibition zone is more than 20 mm;
** Metr. – Metronidazole DMSO solution (the concentration is 30 μg/ml);
*** Strept. – Streptomycin $H_2O$ solution (the concentration is 30 μg/ml).

M.p. > 300°C.

Yield – 75%.

IR (KBr): 3435, 3191, 3062, 2911, 1719, 1654, 1567, 1529, 1503, 1454, 1437, 1375, 1352, 1297, 1273, 1173, 1155, 1121, 1058, 1028, 986, 961, 911, 851, 766, 734, 711, 700, 686, 664, 617, 579, 535, 495 $cm^{-1}$.

$^1H$ NMR (200 MHz, DMSO-$d_6$): 2.57 (3H, s, $CH_3$), 2.67 (3H, s, $CH_3$), 7.27 (2H, m, Ar-H), 7.42 (3H, m, Ar-H), 7.57 (1H, s, CH thiazole), 12.36 (1H, br. s, NH).

LC/MS: m/z ($MH^+$) 356.

Found, %: N 11.99. $C_{17}H_{13}N_3O_2S_2$. Calculated, %: N 11.82. M. 355.44.

**The general method for synthesis of 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno [2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones 3 and 4.**

To the suspension of 0.15 g of 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione and 0.06 g of potassium carbonate add 0.00045 mole of the corresponding alkylating agents and stir the mixture when heating (60-80°C) for 5-7 hours. After cooling dilute the reaction mixture with water and filter the precipitate formed.

**The study of the antimicrobial activity**

According to the WHO recommendations [1, 5, 6] the following test-strains have been used: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC653/885. The 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 Mueller-Hinton agar (Dagestan Scientific 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

By the reaction of 3-phenyl-6-(α-bromoacetyl)-5-methylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione with thioacetamide 5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione was obtained; further the compound was modified by alkylation of its position 1 with benzyl chlorides and chloroacetamides. 5-Methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-dione showed the antimicrobial activity against *Staphylococcus aureus* higher than the reference drugs Metronidazole and Streptomycin, it also appeared to be moderately active against *Pseudomonas aeruginosa* and *Candida albicans* fungi. For 1-alkyl-5-methyl-6-(2-methyl-1,3-thiazol-4-yl)-3-phenylthieno[2,3-*d*]pyrimidine-2,4(1*H*,3*H*)-diones the lower antimicrobial activity was found than that for the compound with unsubstituted position 1; the highest activity in this range was exhibited by compound **3b** with 4-methylbenzyl substituent at position 1. It moderately inhibits the growth of *Staphylococcus aureus* and *Candida albicans*.

REFERENCES


1. *Бактеріологічний контроль поживних середовищ. Інформаційний лист МОЗ України №05.4.1/1670. – К., 2001.*
2. *Власов С.В., Заремба О.В., Коваленко С.М. та ін. // ЖОрФХ. – 2011. – Т. 9, №4. – С. 24-30.*
3. *Власов С.В., Коваленко С.М., Федосов А.І., Черних В.П. // ЖОрФХ. – 2011. – Т. 9, №3. – С. 51-55.*
4. *Власов С.В., Коваленко С.М., Черних В.П. // ЖОрФХ. – 2013. – Т. 11, №2. – С. 41-46.*
5. *Волянський Ю.Л., Гриценко І.С., Широбоков В.П. та ін. Вивчення специфічної активності антимікробних лікарських засобів: Метод. рекоменд. – К., 2004. – 38 с.*
6. *Некрасова Л.С., Свита В.М., Глушкевич Т.Г. та ін. Визначення чутливості мікроорганізмів до антибактеріальних препаратів: Метод. рекоменд. – К., 2007. – 79 с.*
7. *Пат. US2013/123231 (2013). Заявл.: 09.11.2012. Опубл.: 16.05.2013. [Електронний ресурс] Режим доступу http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2013123231A1&KC=A1&FT=D&ND=3&date=20130516&DB=EPODOC&locale=en_EP.*
8. *Пат. US 2007208040 (2007). Заявл.: 02.03.2007. Опубл.: 06.09.2007. [Електронний ресурс] Режим доступу http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2007208040A1&KC=A1&FT=D&ND=3&date=20070906&DB=EPODOC&locale=en_EP.*
9. *Пат. US 2013102601 (2013). Заявл.: 18.10.2013. Опубл.: 25.04.2013. [Електронний ресурс] Режим доступу http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2013102601A1&KC=A1&FT=D&ND=3&date=20130425&DB=EPODOC&locale=en_EP.*
10. *Kotaiah Y., Harikrishna N., Nagaraju K., Venkata Rao C. // Eur. J. Med. Chem. – 2012. – Vol. 58. – P. 340-345.*
11. *Tkachenko O.V., Vlasov S.V., Kovalenko S.M. et al. // News of Pharmacy. – 2013. – №4. – С. 5-10.*
12. *Vlasov S.V., Kovalenko S.M., Chernykh V.P., Krolenko K.Yu. // J. Chem. Pharm. Res. – 2014. – Vol. 6, №6. – P. 22-27.*

**СИНТЕЗ ТА АНТИМІКРОБНА АКТИВНІСТЬ 5-МЕТИЛ-6-(2-МЕТИЛ-1,3-ТІАЗОЛ-4-ІЛ)-3-ФЕНІЛТІЄНО[2,3-d]ПІРИМІДИН-2,4(1H,3H)-ДІОНІВ**

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***Ключові слова:*** *тіофен; піримідин; алкілування; тіазол*

*Шляхом реакції 3-феніл-6-(α-бромацетил)-5-метилтієно[2,3-d]піримідин-2,4(1H,3H)-діону з тіоацетамідом отримано 5-метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діон, який далі модифікували шляхом алкілювання положення 1 бензилхлоридами та хлороацетамідами. Структура одержаних сполук була підтверджена даними $^1$H ЯМР та масспектроскопії. Для одержаних сполук у спектрах $^1$H ЯМР спостерігається сигнал протону тіазолу біля 7.62-7.67 м.ч., для 1-алкіл-5-метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діонів присутні сигнали протонів метиленових груп у діапазоні 5.13-5.21 м.ч. для 1-бензилпохідних та при 4.78-4.82 м.ч. для сполук із ацетамідним фрагментом, для останніх також характерні чіткі сигнали протонів NH в діапазоні 9.68-10.41 м.ч. 5-Метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діон виявив значну антимікробну активність, яка перевищила препарати порівняння метронідазол та стрептоміцин по відношенню до штаму Staphylococcus aureus, також він показав помірну пригнічуючу активність до росту Pseudomonas aeruginosa та грибів Candida albicans. Антимікробна активність 1-алкіл-5-метил-6-(2-метил-1,3-тіазол-4-іл)-3-фенілтієно[2,3-d]піримідин-2,4(1H,3H)-діонів поступається активності сполуки із незаміщеним положенням 1; найбільшу активність у цьому ряду проявила сполука, яка містить 4-метилбензильний замісник у положенні 1; вона помірно пригнічує ріст Staphylococcus aureus та Candida albicans.*

**СИНТЕЗ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ 5-МЕТИЛ-6-(2-МЕТИЛ-1,3-ТИАЗОЛ-4-ИЛ)-3-ФЕНИЛТИЕНО[2,3-d]ПИРИМИДИН-2,4(1H,3H)-ДИОНОВ**

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***Ключевые слова:*** *тиофен; пиримидин; алкилирование; тиазол*

*Путем реакции 3-фенил-6-(α-бромацетил)-5-метилтиено[2,3-d]пиримидин-2,4(1H,3H)-диона с тиоацетамидом получен 5-метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дион, который в дальнейшем модифицировали с помощью алкилирования положения 1 бензилхлоридами и хлорацетамидами. Структура полученных соединений была подтверждена данными $^1$H ЯМР и масс-спектроскопии. Для полученных соединений в спектрах $^1$H ЯМР наблюдается сигнал протона тиазола при 7.62-7.67 м.д., для 1-алкил-5-метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дионов присутствуют сигналы протонов метиленовых групп в диапазоне 5.13-5.21 м.д. для 1-бензил производных и при 4.78-4.82 м.д. для соединений с ацетамидным фрагментом, для них также характерны четкие сигналы протонов NH в диапазоне 9.68-10.41 м.д. 5-Метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дион проявил значительную противомикробную активность, которая превысила препараты сравнения метронидазол и стрептомицин по отношению к штамму Staphylococcus aureus, также он показал умеренную подавляющую активность роста Pseudomonas aeruginosa и грибов Candida albicans. Противомикробная активность 1-алкил-5-метил-6-(2-метил-1,3-тиазол-4-ил)-3-фенилтиено[2,3-d]пиримидин-2,4(1H,3H)-дионов уступает активности соединения с незамещенным положением 1; наибольшую активность в этом ряду проявило соединение, которое содержит 4-метилбензильный заместитель в положении 1; оно умеренно угнетает рост Staphylococcus aureus и Candida albicans.*