СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

Recommended by Doctor of Pharmacy, professor S.V.Kolisnyk

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SYNTHESIS AND THE STUDY OF THE ANTIMICROBIAL ACTIVITY OF 3-AMINO-5-METHYL-2-(ALKYLTHIO)-4-OXO-*N*-ARYL-3,4-DIHYDROTHIENO[2,3-*d*]PYRIMIDINE-6-CARBOXAMIDES

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Key words: thiophene; pyrimidine; amides; alkylation

By alkylation of 3-amino-5-methyl-4-oxo-N-aryl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-6-carboxamides with substituted benzylchlorides and chloroacetic acid the series of novel derivatives of 3-amino-5-methyl-2-(alkylthio)-4-oxo-N-aryl-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamides have been obtained. The structures of these compounds have been confirmed by ^{1}H NMR spectral data and the elemental analysis. For all of the products obtained the ^{1}H NMR spectra contain the signals of the amino group as the sharp singlet in the region of 5.75-5.84 ppm, the signal of the methyl group at position 5 of the thieno[2,3-d]pyrimidine system (2.68-2.75 ppm); the signal of the carboxamide NH group, which position varies from 9.67 ppm to 10.61 ppm depending on the structure of the substituent of the amide aromatic cycle, is also observed. In the spectra the signals of the benzyl CH_2 group protons are located in the region of 4.23-4.29 ppm, while the same signal for the derivative of thioacetic acid is observed at 3.82 ppm. The antimicrobial activity screening for the compounds obtained has been performed by the agar well diffusion method. In general, it has been found that all of the compounds tested appeared to be more active than the reference drugs against the strains of both Proteus vulgaris and Pseudomonas aeruginosa; 3-amino-2-(benzylthio)-5-methyl-N-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-d]pyrimidine-6-carboxamide was the most active compound, which moderately inhibited the growth of all test-strains of microorganisms, and showed even higher activity than the reference drugs streptomycin and metronidazole against Bacillus subtilis and Candida albicans fungi.

It was reported that some derivatives of thieno[2,3-*d*] pyrimidin-4(1*H*)-one showed the antimicrobial activity [1-4, 6, 8-10]. The compounds with modified amino and thioxo groups at positions 2 and 3 of the core heterocyclic system [3], as well as the derivatives containing these groups included into the other fused heterocyclic system [1, 6, 9] are known among them. Our special attention was paid to the derivatives containing the carboxamide group at α-position of the thiophene ring. Earlier we reported about the triheterocyclic systems containing similar heterocyclic fragments as the effective antimicrobials [1, 9]. However, the simpler compounds with the free amino group at position 3 were not prepared previously and were not used for antimicrobial trials. Therefore, the synthesis and study of the antimicrobial activity of derivatives of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide were the aim of our research work.

Materials and Methods

Chemical Part

All of the solvents and reagents were received from the commercial sources. Melting points (°C) were determined with a Kofler (Hotbench) melting point apparatus. ^{1}H NMR spectra were recorded using a Varian Mercury (200 MHz) spectrometer in DMSO-d_6 with TMS as an internal standard. Chemical shifts (δ) are reported in ppm. Elemental analysis was performed by Kjeldahl method.

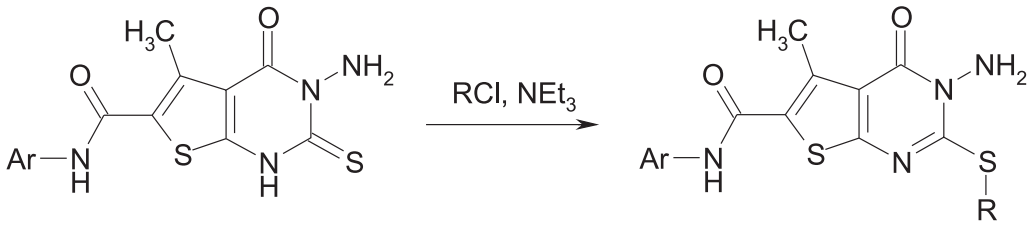
3-Amino-5-methyl-4-oxo-N-phenyl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-d]pyrimidine-6-carboxamides (1) were obtained by the methods previously reported [1, 9].

The general method for the synthesis of 3-amino-5-methyl-2-(alkylthio)-4-oxo-N-aryl-3,4-dihydrothieno [2,3-d]pyrimidine-6-carboxamides (2)

To the suspension of 0.00045 mol of the starting compound **1** in dimethylformamide add 0.0005 mol of triethylamine and 0.0005 mol of the corresponding chloroderivative. Heat the reaction mixture (70°C) with stirring for 5-8 hours. After cooling the reaction mixture dilute it with water. Filter the precipitate of compound **2** formed and wash with water. Purify the products by boiling in 2-propanol.

The study of the antimicrobial activity

The microbiological experiment was performed at the premises of the laboratory of Biochemistry of Mic-



1a-e **2a-j**

1a: Ar = 2-Pyridyl; **1b**: Ar = 2-MeC_6H_4; **1c**: Ar = 4-MeC_6H_4; **1d**: Ar = 4-FC_6H_4; **1e**: 4-$CH_3OC_6H_4$; **2a**: Ar = 2-Pyridyl, R = $C_6H_5CH_2$;
2b: Ar = 2-Pyridyl, R = 4-$CH_3C_6H_4CH_2$; **2c**: Ar = 2-MeC_6H_4, R = $C_6H_5CH_2$; **2d**: Ar = 4-MeC_6H_4, R = $C_6H_5CH_2$;
2e: Ar = 4-MeC_6H_4, R = 4-$CH_3C_6H_4CH_2$; **2f**: Ar = 4-MeC_6H_4, R = $HOOCCH_2$; **2g**: Ar = 4-FC_6H_4, R = $C_6H_5CH_2$;
2h: Ar = 4-FC_6H_4, R = 4-$CH_3C_6H_4CH_2$; **2i**: Ar = 4-$CH_3OC_6H_4$, R = $C_6H_5CH_2$; **2j**: Ar = 4-$CH_3OC_6H_4$, R = 4-$CH_3C_6H_4CH_2$.

Scheme

roorganisms and Nutrient Media at the Institute of Microbiology and Immunology named after I.I.Mechnikov at NAMS of Ukraine. According to the WHO recommendations [5, 7] such test-strains as *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC653/885 were used. 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 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 0.3 ml of the solution.

Results and Discussion

The target compounds **2** (scheme) were obtained by alkylation of 3-amino-5-methyl-4-oxo-*N*-aryl-2-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxamide derivatives **1** with the substituted benzyl chlorides and chloroacetic acid. Starting compounds **1 a-e** were obtained by the methods previously reported [1, 9], and their purity appeared to be sufficient for further transformations. All of the compounds **2** were isolated as crystalline solids (Table 1).

The structure of the compounds obtained was confirmed by ^{1}H NMR spectroscopic method (Table 2). For all of the compounds **2** their ^{1}H NMR spectra contain the signals of the amino group as the sharp singlet in the region of 5.75-5.84 ppm, the signal of the methyl group at position 5 of the thieno[2,3-*d*]pyrimidine sys-

Table 1

Physico-chemical properties of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides **(2a-j)**

Compd. No.	Ar	R	Mol. formula M.w.	Yield %, in the alkylation step	M.p., °C	N% calc. / found
2a	2-Pyridyl	$C_6H_5CH_2$	$C_{20}H_{17}N_5O_2S_2$ 423.52	79	264-266	16.54 / 16.67
2b	2-Pyridyl	4-$CH_3C_6H_4CH_2$	$C_{21}H_{19}N_5O_2S_2$ 437.55	83	216-217	16.01 / 16.23
2c	2-MeC_6H_4	$C_6H_5CH_2$	$C_{22}H_{20}N_4O_2S_2$ 436.56	77	25-256	12.83 / 12.90
2d	4-MeC_6H_4	$C_6H_5CH_2$	$C_{22}H_{20}N_4O_2S_2$ 436.56	69	240-241	12.83 / 13.02
2e	4-MeC_6H_4	4-$CH_3C_6H_4CH_2$	$C_{23}H_{22}N_4O_2S_2$ 450.59	83	256-258	12.43 / 12.38
2f	4-MeC_6H_4	CH_2COOH	$C_{17}H_{16}N_4O_4S_2$ 404.47	53	189-190	13.85 / 13.97
2g	4-FC_6H_4	$C_6H_5CH_2$	$C_{21}H_{17}FN_4O_2S_2$ 440.52	69	278-279	12.72 / 12.75
2h	4-FC_6H_4	4-$CH_3C_6H_4CH_2$	$C_{22}H_{19}FN_4O_2S_2$ 454.55	75	297-299	12.33 / 12.56
2i	4-$CH_3OC_6H_4$	$C_6H_5CH_2$	$C_{22}H_{20}N_4O_3S_2$ 452.56	83	252-253	12.38 / 12.49
2j	4-$CH_3OC_6H_4$	4-$CH_3C_6H_4CH_2$	$C_{23}H_{22}N_4O_3S_2$ 466.58	88	289-291	12.01 / 12.12

Table 2

Data of ^{1}H NMR-spectra of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides **(2a-j)**

Compd. No.	Chemical shift, δ, ppm.				
	NH_2 (2H, s)	Thiophene CH_3 (3H, s)	NH (1H, br.s)	Aliphatic protons	Aromatic protons
2a	5.76	2.72	10.59	4.29 (2H, s, CH_2);	7.16 (1H, t); 7.29 (3H, m); 7.43 (2H, m); 7.82 (1H, t); 8.05 (1H, d); 8.36 (1H, d)
2b	5.76	2.72	10.61	2.25 (3H, s, CH_3); 4.24 (2H, s, CH_2);	7.13 (3H, m); 7.31 (2H,d); 7.82 (1H, t); 8.05 (1H, d); 8.36 (1H, d)
2c	5.76	2.68	10.08	2.26 (3H, s, CH_3); 4.28 (2H, s, CH_2);	7.14 (2H, d); 7.29 (3H, m); 7.44 (2H, m); 7.53 (2H, d)
2d	5.76	2.75	9.67	2.23 (3H, s, CH_3); 4.29 (2H, s, CH_2);	7.09-7.48 (9H, m)
2e	5.76	2.68	10.10	2.25 (6H, s, $2CH_3$); 4.23 (2H, s, CH_2);	7.13 (4H, m); 7.31 (2H, d); 7.54 (2H, d)
2f	5.84	2.68	10.07	2.25 (3H, s, CH_3); 3.82 (2H, s, CH_2);	7.12 (2H, d); 7.52 (2H, d)
2g	5.76	2.70	10.22	4.29 (2H, s, CH_2);	7.09-7.50 (6H, m); 7.68 (2H, m)
2h	5.75	2.70	10.21	2.25 (3H, s, CH_3); 4.24 (2H, s, CH_2);	7.15 (4H, m); 7.31 (2H, m); 7.67 (2H, m)
2i	5.76	2.69	10.03	3.72 (3H, s, OCH_3); 4.28 (2H, s, CH_2);	6.90 (2H, d); 7.29 (3H, m); 7.44 (2H, m); 7.56 (2H, d)
2j	5.76	2.69	10.04	2.25 (3H, s, CH_3); 3.72 (3H, s, OCH_3) 4.23 (2H, s, CH_2);	6.90 (2H, d); 7.10 (2H, d); 6.32 (2H, d); 7.56 (2H, d);

tem (2.68-2.75 ppm); the signal of the carboxamide NH group, which position varies from 9.67 ppm to 10.61 ppm depending on the structure of the substituent of the amide aromatic cycle, is also observed. In the spectra the signals of benzyl CH_2 group protons are located in the region of 4.23-4.29 ppm, while the same signal for the derivative of thioacetic acid is observed at 3.82 ppm.

As to the spectrum and efficacy of antimicrobial properties, the highest activity in the range of compounds **2** (Table 3) was revealed by compound **2d**, which moderately inhibited the growth of all test-strains of microorganisms and showed even higher activity than the reference drugs against *Bacillus subtilis* and *Candida albicans* fungi. All of the 4-methylphenyl amides **2e** and **2f** tested also exhibited a moderate antimicrobial activity. Its noteworthy that most of the compounds studied, namely **2a**, **2d-2g** and **2i** appeared to be more active than the reference drugs against *Proteus vulgaris* and *Pseudomonas aeruginosa*; it agreed with the previously reported typical manner of thieno[2,3-*d*]pyrimidines [10].

Table 3

The antimicrobial activity of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamides **(2a-j)***

Compnd. No.	*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*
2a	++	++	+	+	++	+
2b	++	+	–	+	++	+
2c	–	–	–	–	–	–
2d	++	++	++	++	++	++
2e	++	++	++	++	++	+
2f	++	++	++	++	++	+
2g	+	+	+	++	++	+
2h	–	–	–	–	–	–
2i	++	++	++	++	++	+
2j	+	+	–	–	+	+
Metr.**	+	+	–	–	++	+
Strept.***	++	++	–	–	++	–

* "–" – diameter of the growth inhibition zone is less than 10 mm; "+" – diameter of the growth inhibition zone is 10-14 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).

CONCLUSIONS

1. The synthesis of novel derivatives of 3-amino-5-methyl-2-(alkylthio)-4-oxo-*N*-aryl-3,4-dihydrothieno [2,3-*d*]pyrimidine-6-carboxamides has been performed by alkylation of 3-amino-5-methyl-4-oxo-*N*-aryl-2-thioxo-1,2, 3,4-tetrahydrothieno[2,3-*d*]pyrimidine-6-carboxamides.

2. Their antimicrobial activity screening has shown that almost all of the compounds obtained appeared to be more active than the reference drugs against both *Proteus vulgaris* and *Pseudomonas aeruginosa* strains. Compound **2d** – 3-amino-2-(benzylthio)-5-methyl-*N*-(4-methylphenyl)-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide has been identified as the most active one, which moderately inhibited the growth of all of the test strains and showed the high activity against *Bacillus subtilis* and *Candida albicans* fungi.

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СИНТЕЗ ТА ДОСЛІДЖЕННЯ АНТИМІКРОБНОЇ АКТИВНОСТІ 3-АМІНО-5-МЕТИЛ-2-(АЛКІЛТІО)-4-ОКСО-*N*-АРИЛ-3,4-ДИГІДРОТІЄНО[2,3-*d*]ПІРИМІДИН-6-КАРБОКСАМІДІВ

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

Шляхом алкілування 3-аміно-5-метил-4-оксо-N-арил-2-тіоксо-1,2,3,4-тетрагідротієно[2,3-d] піримідин-6-карбоксамідів заміщеними бензилхлоридами та хлороцтовою кислотою були одержані нові похідні з ряду 3-аміно-5-метил-2-(алкілтіо)-4-оксо-N-арил-3,4-дигідротієно[2,3-d] піримідин-6-карбоксамідів. Структура одержаних сполук була підтверджена даними ^{1}H ЯМР спектроскопії та елементного аналізу. Для всіх одержаних сполук у спектрах ^{1}H ЯМР спостерігаються сигнали протонів аміногрупи у вигляді чіткого синглету в діапазоні 5.75-5.84 м.ч., сигнал метильної групи у положенні 5 тієно[2,3-d]піримідинової системи при 2.68-2.75 м.ч. та сигнал протону NH карбоксамідної групи, положення якого варіює від 9.67 м.ч. до 10.61 м.ч. в залежності від природи замісників у ароматичному ядрі аміду. Також у спектрах проявляються сигнали протонів груп CH_2 бензильних замісників у діапазоні 4.23-4.29 м.ч., для похідного тіооцтової кислоти цей сигнал знаходиться при 3.82 м.ч. Скринінг антимікробної активності одержаних сполук проводили шляхом дифузії в агар, використовуючи «метод колодязів». Встановлено, що більшість зі сполук виявилась активнішою за препарати порівняння одразу по відношенню до Proteus vulgaris та Pseudomonas aeruginosa, найбільш активною сполукою виявися 3-аміно-2-(бензилтіо)-5-метил-N-(4-метилфеніл)-4-оксо-3,4-дигідротієно[2,3-d] піримідин-6-карбоксамід, який помірно пригнічував ріст усіх тест-штамів мікроорганізмів та перевищив активність препаратів порівняння Стрептоміцину та Метронідазолу по відношенню до Bacillus subtilis та грибів Candida albicans.

СИНТЕЗ И ИССЛЕДОВАНИЕ ПРОТИВОМИКРОБНОЙ АКТИВНОСТИ 3-АМИНО-5-МЕТИЛ-2-(АЛКИЛТИО)-4-ОКСО-*N*-АРИЛ-3,4-ДИГИДРОТИЕНО[2,3-*d*]ПИРИМИДИН-6-КАРБОКСАМИДОВ

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

Путем алкилирования 3-амино-5-метил-4-оксо-N-арил-2-тиоксо-1,2,3,4-тетрагидротиено [2,3-d]пиримидин-6-карбоксамидов замещенными бензилхлоридами и хлоруксусной кислотой были получены новые производные ряда 3-амино-5-метил-2-(алкитио)-4-оксо-N-арил-3,4-дигидротиено[2,3-d]пиримидин-6-карбоксамидов. Структура полученных соединений была подтверждена данными ^{1}H ЯМР спектроскопии и элементного анализа. Для всех полученных

соединений в спектрах ^{1}H ЯМР наблюдаются сигналы протонов аминогруппы в виде четкого синглета в диапазоне 5.75-5.84 м.д., сигнал метильной группы в положении 5 тиено[2,3-d] пиримидиновой системы при 2.68-2.75 м.д. и сигнал протона NH карбоксамидной группы, положение которого варьирует от 9.67 м.д. до 10.61 м.д. в зависимости от природы заместителей в ароматическом ядре амида. Также в спектрах проявляются сигналы протонов групп CH_2 бензильных заместителей в диапазоне 4.23-4.29 м.д., для производного тиоуксусной кислоты этот сигнал находится при 3.82 м.д. Скрининг противомикробной активности полученных соединений проводили путем диффузии в агар, используя «метод колодцев». Установлено, что большинство соединений оказалось активнее препаратов сравнения сразу по отношению к Proteus vulgaris и Pseudomonas aeruginosa, наиболее активным соединением является 3-амино-2-(бензилтио)-5-метил-N-(4-метилфенил)-4-оксо-3,4-дигидротиено[2,3-d]пиримидин-6-карбоксамид, который умеренно угнетал рост всех тест-штаммов микроорганизмов и превысил активность препаратов сравнения Стрептомицина и Метронидазола по отношению к Bacillus subtilis и грибам Candida albicans.