СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

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SYNTHESIS, THE ANTI-INFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF 6-(1*H*-BENZIMIDAZOL-2-YL)-5-METHYL-4-(ALKYLTHIO)THIENO[2,3-*d*]PYRIMIDINES

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An effective method for the synthesis of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidine-4(3H)-thione starting from the corresponding 4-oxo analogue has been developed. For enlarging the chemical diversity the alkylation of 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidine-4(3H)-thione with benzyl chlorides, chloroacetamides and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles has been carried out; as a result, the series of 6-(1H-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-d]pyrimidines have been obtained. For the compounds obtained the screening of the antimicrobial activity by the "agar well" diffusion method and the screening of the anti-inflammatory activity using the carrageenan-induced paw edema model in rats have been performed. The results of the tests have shown that alkylation of the sulphur atom in position 4 of thieno[2,3-d]pyrimidine improves the antimicrobial properties. Most of 6-(1H-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-d]pyrimidines appeared to be active against the strains of Candida albicans fungi. The compounds containing the chlorine atom in the phenyl radical of the thioacetamide fragment in position 4 of thieno[2,3-d]pyrimidine have been found to be active against Staphylococcus aureus, Escherichia coli and Bacillus subtilis. After the treatment of rats with 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidine-4(3H)-one, 6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidine-4(3H)-thione and 2-{[6-(1H-benzimidazol-2-yl)-5-methylthieno[2,3-d]pyrimidin-4-yl]thio}-N-phenylacetamide in the doses of 1, 10 and 50 mg/kg a statistically significant and dose-dependent reduction of edema was not observed compared to the control pathology. The anti-inflammatory activity of the test compounds was about 20%, and it was 2-2.5 times less than the activity of the reference drug (diclofenac sodium).

The results of studying 6-hetarylthieno[2,3-*d*]pyrimidines have shown their potential as the group of promising biologically active compounds. The main types of the activities studied for the compounds with heterocycles in position 6 of thieno[2,3-*d*]pyrimidine were the binding activity of A_{2A} adenosine receptors [18] and the inhibitory activity of acetyl-KoA carboxylase [20]; their antioxidant [13], antiviral and antimicrobial activities were also studied [1-3, 19, 22].

Derivatives of benzimidazole are well known biologically active compounds; the antimicrobial [7, 14, 17, 24] and anti-inflammatory agents [6, 11, 15, 16, 21] were reported for derivatives of this class. Modification of position 6 of thieno[2,3-*d*]pyrimidine with the benzimidazole fragment can be a good way for constructing the novel biologically active compounds with the antimicrobial and anti-inflammatory activity. Therefore, we decided to use the compound previously synthesized [23] as a precursor for the synthesis of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione, which could be a perfect building-block for modification opening an access for a great variety of biologically active substances.

Materials and Methods

Chemical Part

All solvents and reagents were obtained from the commercial sources. The melting points (°C) were measured with a Kofler hot-bench apparatus. The ^{1}H NMR-spectra were measured with a Varian Mercury device (200 MHz) and the ^{13}C NMR-spectra were recorded on a Varian Gemini (300 MHz) spectrometer in DMSO-d_6 using TMS as an internal standard (chemical shifts were in ppm). LC/MS was recorded with a PE SCIEX API 150EX chromatograph equipped with a mass-spectrometer. The mass spectra were recorded on a Varian 1200L mass spectrometer (direct input, EI, 70 eV). The nitrogen microanalysis was performed by Kjeldahl method.

5-Methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid (1) was obtained according to the reported methods [12].

6-(1*H*-Benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4-one (2). To the suspension of 0.025 Mole of acid 1 in 30 ml of DMF add 0.026 Mole of 1,1'-carbonyldiimidazole, heat the reaction mixture at 50-70°C till complete evolution of carbon dioxide, and then heat additionally for 15 min. After that add 0.025 Mole of

ortho-phenylenediamine to the clear solution, and heat the reaction mixture at 130°C for 3-5 h. After cooling dilute the reaction mixture with water, filter the precipitate formed and wash with 50% 2-propanol-water solution. Yield – 62%. M. p. >300°C. ^{1}H NMR: 2.89 (3H, s., CH_3); 7.21 (2H, d., J = 4,3, Ar-H); 7.61 (2H, m., Ar-H); 8.13 (1H, s., CH), 12.59 (2H, br.s., NH). ^{13}C NMR: 15.49 (CH_3); 123.78; 124.51; 134.27; 146.35; 147.18; 158.73; 164.82. LC/MS, m/z: 283 [M+H]+. Found, %: N 19.93. $C_{14}H_{10}N_4OS$. Calculated, %: N 19.84. M.w. 282.33.

6-(1*H*-Benzimidazol-2-yl)-5-methylthieno[2,3-*d*] pyrimidine-4(3*H*)-thione 4. To 4.5 g (0.016 Mole) of compound 2 add 15 ml of phosphorous oxychloride, and heat the reaction mixture while stirring for 3 h. After cooling pour the reaction mixture on the crashed ice, filter the precipitate of compound 3, and wash with plenty of cold water. Dry compound 3 at room temperature for 2-3 days. To the suspension of 4 g (0.013 Mole) of the chloro-derivative 3 in anhydrous DMF add 1.21 g (0.016 Mole) of thiourea, and reflux the reaction mixture for 2 h. After cooling dilute the reaction mixture with water, filter the precipitate formed, and then dissolve in 10 ml of 10% water solution of sodium hydroxide. Stir the clear solution at 50°C for 1 h. Filter the precipitate of compound 4 formed after neutralization, and wash with plenty of water. Yield – 72%. M. p. >300°C. ^{1}H NMR: 3.12 (3H, s., CH_3); 7.24 (2H, m., Ar-H); 7.61 (2H, m., Ar-H); 8.20 (1H, s., CH), 12.73 (2H, br.s., $NH_{benzimidazole}$), 13.89 (2H, br.s., NH). LC/MS, m/z: 299 [M+H]+. Found, %: N 18.90. $C_{14}H_{10}N_4S_2$. Calculated, %: N 18.78. M.w. 298.39.

The general method for preparation of 6-(1*H*-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-*d*]pyrimidines (8-10). To 0.15 g (0.0005 Mole) of the mixture of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione **4** in 4 ml of DMF add 0.08 ml (0.0006 Mole) of triethylamine and 0.0005 Mole of the corresponding alkylating agent. Heat the reaction mixture while stirring at 100°C for 4 h. After cooling dilute the reaction mixture with water. Filter the precipitate formed, and recrystallize from ethanol.

The study of the anti-inflammatory activity

The anti-inflammatory activity was studied at the premises of the Central Research Laboratory of the National University of Pharmacy. The acute carrageenan-induced edema was studied in albino rats weighing 150-180 g [4, 10]. "Ortofen" tablets (diclofenac sodium) were used for the experiment as the reference drug in the dose of 8 mg/kg according to the pharmacologically active component. The dose of the reference drug was calculated by Yu.R.Rybolovlev method [4] based on the daily human dose.

At the first stage of screening the compounds studied were introduced to rats intragastrically in the dose of 10 mg/kg; this dose corresponded to the dose of the reference drug. Then at the second stage of the screening experiment the compounds were introduced as finely dispersed suspensions stabilized with Tween-80 in the doses of 1 mg/kg and 50 mg/kg. The compounds were introduced 1 hour before the injection of carrageenan solution. The animals of the positive control group were treated with distilled water. The paw volume was measured in 1, 2, 3, 4 and 5 hours using the mechanical oncometer. The anti-inflammatory activity (AIA) activity as % was determined by the ability of the test compound to suppress the inflammatory reaction in experimental animals compared to the control ones. The calculations were performed according to the following formula:

$$AIA = \frac{P_c - P_e}{P_c} \times 100\%,$$

where: P_c – is the mean difference between the volumes of the swollen and normal paw in the control group; P_e – is the mean difference between the volumes of the swollen and normal paw in the experimental group.

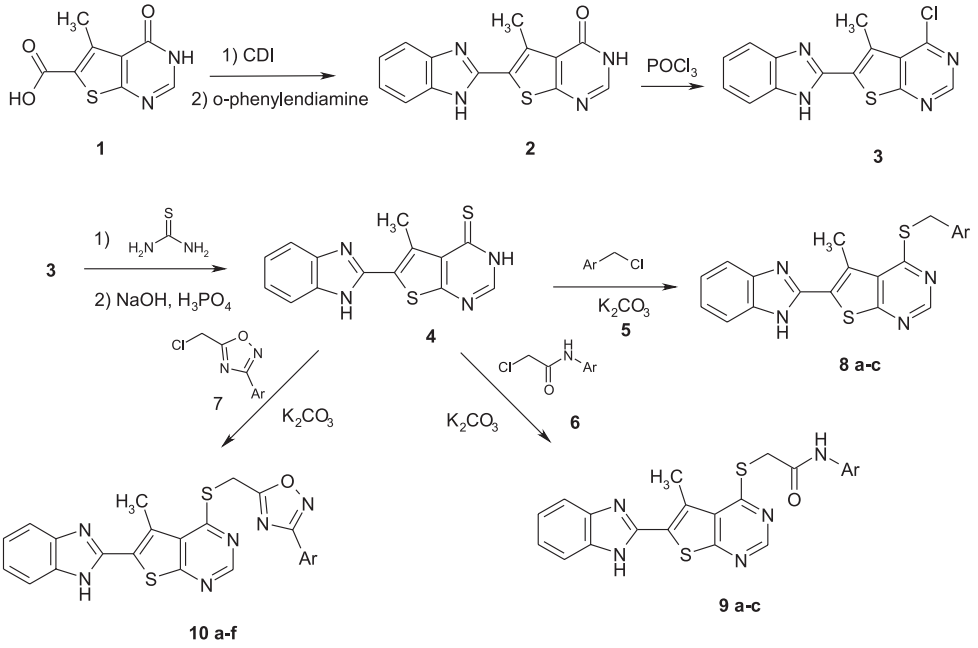
The study of the antimicrobial activity

The microbiological experiment was performed by the Laboratory of Biochemistry of Microorganisms and Nutrient Media of the State Institution "Institute of Microbiology and Immunology named after I.I.Mechnikov of the National Academy of Medical Sciences of Ukraine". According to the WHO recommendations [5, 7, 9] to estimate the activity of the test compounds such strains of microorganisms 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. The inoculum suspension was prepared using a Densi-La-Meter apparatus (made by PLIVA-Lachema, Czech Republic; at the wavelength of 540 nm). The cultures were synchronized using low temperature conditions (4°C). The microbial load was 10^7 cells per 1 ml of the medium and was determined according to McFarland standard. The 18 to 24-hour culture of microorganisms was used for the test. Mueller-Hinton agar was used ("HIMedia Laboratories Pvt. Ltd India") for bacteria. The strains of *Candida albicans* were cultivated using Sabouraud agar ("HIMedia Laboratories Pvt. Ltd India"). The compounds studied were introduced as aliquots of DMSO solution in the volume of 0.3 ml and the concentration of 100 μg/ml).

Results and Discussion

Earlier we reported that application of 1,1'-carbonyldiimidazole (CDI) for the interaction of 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid with aromatic amidoximes could be a good one-pot method for preparing 5-methyl-6-(3-aryl-1,2,4-oxadiazol-5-yl) thieno[2,3-*d*]pyrimidine-4(3*H*)-ones [1]. Using the same methodology we focused our efforts on construction of the benzimidazole ring in position 6 of thieno[2,3-*d*] pyrimidine [23]. In this paper the preparation of 4-thio analogues of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno [2,3-*d*]pyrimidine-4(3*H*)-one as the promising biologically active compounds was described (Scheme).

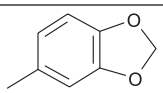
Compound **2** was isolated as the result of CDI promoted *one-pot* interaction of acid **1** with *o*-phenylenediamine in anhydrous DMF. The further modification of **2** was based on the earlier proposed strategy of substitution of the oxo-group in position 4 of thieno[2,3-*d*] pyrimidine with a chlorine atom easily producing the



Scheme

Table 1

The physiochemical characteristics of 6-(1*H*-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-*d*]pyrimidines **8-10**

Compound	Ar	Mol. Formula, M.м.	Yield %, at the alkylation stage	M. p., °C	N% calc. / found	LC/MS* MS**
8a	C_6H_5-	$C_{21}H_{16}N_4S_2$ 388.52	73	>300	14.42 / 14.45	389.2*
8b	4-F-C_6H_4-	$C_{21}H_{15}FN_4S_2$ 406.51	72	283-285	13.78 / 13.89	407.2*
8c	4-OCF_3-C_6H_4-	$C_{22}H_{15}F_3N_4OS_2$ 472.51	79	267-268	11.86 / 11.95	473.2*
9a	C_6H_5-	$C_{22}H_{17}N_5OS_2$ 431.54	67	>300	16.23 / 16.28	432.2*
9b	4-CH_3-C_6H_4-	$C_{23}H_{19}N_5OS_2$ 445.57	66	>300	15.72 / 15.87	446.3*
9c	2,4-diF-C_6H_3-	$C_{22}H_{15}F_2N_5OS_2$ 467.52	79	>300	14.98 / 15.10	468.1*
10a	4-CH_3-C_6H_4-	$C_{24}H_{18}N_6OS_2$ 470.58	58	258-260	17.86 / 17.92	470**
10b	4-Cl-C_6H_4-	$C_{23}H_{15}ClN_6OS_2$ 491.00	63	282-284	17.12 / 17.26	490**
10c	2-Cl-C_6H_4-	$C_{23}H_{15}ClN_6OS_2$ 491.00	66	185-187	17.12 / 17.19	490**
10d	2,3-di(OCH_3)-C_6H_3-	$C_{25}H_{20}N_6O_3S_2$ 516.60	68	249-251	16.27 / 16.30	516**
10e		$C_{24}H_{16}N_6O_3S_2$ 500.56	64	252-253	16.79 / 16.86	500**
10f	3-Cl-4-F-C_6H_4-	$C_{23}H_{14}ClFN_6OS_2$ 508.99	90	279-280	16.51 / 16.59	508**

Note: * – For LC/MS data the peaks of quasi-molecular ions are given $[MH]^+$; ** – For MS data the peaks of molecular ions are given $[M]^+$.

Table 2

The NMR-spectral data of 6-(1*H*-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-*d*]pyrimidines **8-10**

Compound	Chemical shift, δ, ppm			
	NH	CH_3 thiophene (3H, s.)	Aliphatic protons	Aromatic protons
8a	12.89 (1H, br.s)	2.97	4.64 (2H, s, CH_2)	7.08-7.74 (9H, m, Ar-H); 8.87 (1H, s, $CH_{pyrimidine}$)
8b*	12.87 (1H, br.s)	2.98	4.65 (2H, s, CH_2)	7.06-7.31 (4H, m, Ar-H); 7.46-7.76 (4H, m, Ar-H); 8.88 (1H, s, $CH_{pyrimidine}$)
8c*	12.90 (1H, br.s)	2.98	4.68 (2H, s, CH_2)	7.12-7.40 (4H, m, Ar-H); 7.48-7.76 (4H, m, Ar-H); 8.88 (1H, s, $CH_{pyrimidine}$)
9a*	10.36 (1H, s); 12.90 (1H, br.s)	3.07	4.36 (2H, s, CH_2)	7.04 (1H, t, J = 7.3 Hz, Ar-H); 7.20-7.36 (4H, m, Ar-H); 7.53-7.70 (4H, m, Ar-H); 8.81 (1H, s, $CH_{pyrimidine}$)
9b	10.29 (1H, s); 12.95 (1H, br.s)	3.05	2.23 (3H, s, CH_3); 4.33 (2H, s, CH_2)	7.10 (2H, d, J = 8.1 Hz, Ar-H); 7.18-7.33 (2H, m, Ar-H); 7.46 (2H, d, J = 8.1 Hz, Ar-H); 7.53-7.76 (2H, m, Ar-H); 8.80 (1H, s, $CH_{pyrimidine}$)
9c*	10.14 (1H, s); 12.95 (1H, br.s)	3.05	4.35 (2H, s, CH_2)	7.02 (1H, m, Ar-H); 7.15-7.40 (3H, m, Ar-H); 7.51-7.85 (3H, m, Ar-H); 8.81 (1H, s, $CH_{pyrimidine}$)
10a	12.96 (1H, br.s)	3.05	2.34 (3H, s, CH_3); 5.03 (2H, s, CH_2)	7.21-7.38 (4H, m, Ar-H); 7.66 (2H, m, Ar-H); 7.85 (2H, d, J = 8.2 Hz, Ar-H); 8.80 (1H, s, $CH_{pyrimidine}$)
10b	–	3.05	5.04 (2H, s, CH_2)	7.21-7.30 (2H, m, Ar-H); 7.55-7.69 (4H, m, Ar-H); 7.96 (2H, d, J = 8.5 Hz, Ar-H); 8.79 (1H, s, $CH_{pyrimidine}$)
10c	12.95 (1H, br.s)	3.05	5.07 (2H, s, CH_2)	7.17-7.35 (2H, m, Ar-H); 7.42-7.77 (5H, m, Ar-H); 7.84 (2H, dd, J = 7.3, 1.8 Hz, Ar-H); 8.80 (1H, c, $CH_{pyrimidine}$)
10d	12.95 (1H, br.s)	3.05	3.70 (3H, s, OCH_3); 3.83 (3H, s, OCH_3); 5.04 (2H, s, CH_2)	7.11-7.39 (5H, m, Ar-H); 7.51-7.78 (2H, m, Ar-H); 8.81 (1H, s, $CH_{pyrimidine}$)
10e	12.93 (1H, br.s)	3.05	5.02 (2H, s, CH_2); 6.11 (2H, s, OCH_2O)	7.05 (1H, d, J = 8.5 Hz, Ar-H); 7.21-7.34 (2H, m, Ar-H); 7.40 (1H, s, Ar-H); 7.48-7.75 (3H, m, Ar-H); 8.80 (1H, s, $CH_{pyrimidine}$)
10f	12.98 (1H, br.s)	3.05	5.05 (2H, s, CH_2)	7.19-7.35 (2H, m, Ar-H); 7.52-7.76 (3H, m, Ar-H); 7.93-8.02 (1H, m, Ar-H); 8.09 (1H, dd, J = 7.0, 2.1 Hz, Ar-H); 8.79 (1H, s, $CH_{pyrimidine}$)

Note: * – ^{13}C NMR **8b**: 16.99; 32.75; 115.64; 115.92; 127.08; 129.06; 130.36; 131.69; 131.81; 133.59; 145.86; 153.01; 160.28; 163.51; 165.29; 165.77; **8c**: 16.96; 32.59; 121.50; 123.32; 127.07; 129.07; 130.36; 131.62; 137.13; 145.83; 147.97; 152.96; 165.05; 165.80; **9a**: 16.85; 35.07; 120.18; 123.27; 124.01; 127.48; 129.07; 129.51; 130.40; 139.37; 145.96; 152.74; 165.09; 166.07; **9c**: 16.82; 34.39; 104.09; 104.41; 104.45; 104.77; 111.34; 111.59; 111.63; 123.18; 126.40; 127.55; 129.43; 130.37; 145.93; 152.71; 164.86; 166.02; 166.81.

4-thio analogue in the reaction with thiourea [22]. An interesting fact is a relative stability of isothiuromiun salt, which requires the additional alkaline hydrolysis for cleavage of cyanamide in the case of the benzimidazole substituent. The procedure proposed allowed isolating 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione **4** in the satisfactory yield.

Further modification of compound **4** obtained was performed by its alkylation with benzyl chlorides **5**, chloroacetamides **6** and 3-aryl-5-(chloromethyl)-1,2,4-oxadiazoles **7**. As the result the series of 4-S-alkyl derivatives **8-10** were obtained. The structures and the data for compounds **8-10** are given in Tab. 1.

The ^{1}H NMR-spectra of compounds **8-10** contained the signals of the methylene groups of alkyl residues in the range of 4.33-5.07 ppm, their positions much depended upon the structure of the adjacent radical. For the spectra of derivatives **8-10** the signals of NH proton of benzimidazole at 12.87-12.98 ppm were typical; the signal of the methyl group of the thiophene ring was observed in the range of 2.97-3.07 ppm. The signals of aromatic protons were in accordance with the substitution of the aromatic fragments of the molecules (Tab. 2).

The results of studying the anti-inflammatory activity

The screening of the anti-inflammatory activity for some 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine derivatives obtained was performed using the model of carrageenan-induced paw edema in rats [4]. The main representatives of the compounds obtained, namely 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-one **2**, 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione **4** and 2-{[6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4-yl]thio}-*N*-phenylacetamide **9a**, which showed the best solubility, were chosen for the experiment. According to the data reported by Di Rosa and co-workers [9]

Table 3

The results of screening (the first stage) of the anti-inflammatory activity for derivatives of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidines 2, 4 and 9a (carrageenan-induced paw edema model in rats, the dose of 10 mg/kg), (n = 6)

Experimental groups	Dose, mg/kg	Edema rate, mm							
		1 hour	AIA,%	2 hours	AIA,%	3 hours	AIA,%	4 hours	AIA,%
Control	–	8.8±1.4	–	19.0±2.0	–	25.4±2.0	–	23.4±1.5	–
"Ortofen"	8	**3.7±1.0***	**58**	**3.7±1.9***	**81**	**4.8±1.6***	**81**	**4.3±2.2***	**82**
2	10	9.0±2.0	-2	21.8±3.5	-15	26.3±2.7	-4	24.3±1.9	-4
4	10	11.3±2.8	-29	16.5±3.5	13	22.5±2.2	11	22.5±3.1	4
9a	10	9.3±1.5	-6	18.8±3.7	1	25.8±3.3	-2	24.0±2.3	-3

Note: * – statistically significant differences compared to the control, $p<0.05$; n – number of animals in the group.

the inflammation is caused by the influence of biogenic amines, kinins and prostaglandins. The dependence of the exudation process on different mediators in the course of this experiment allows predicting the possible mechanism of action for the test compounds.

The screening studies showed that all test compounds in the doses studied did not reveal a significant anti-inflammatory activity. After the treatment of rats with the compounds selected for screening in the doses of 1, 10 and 50 mg/kg a statistically significant and dose-dependent reduction of edema was not observed compared to the control pathology. Only at the second stage of the screening experiment a statistically significant reduction of the paw edema was observed for compound **2** (1 mg/kg) during the second hour of the experiment and for compounds **4** and **9a** (50 mg/kg), which both showed the anti-inflammatory effect, during the third hour of the experiment. The anti-inflammatory activity of the test compounds was about 20%, and it was 2-2.5 times less than the activity of the reference drug (diclofenac sodium) (Tab. 3, 4).

The results of studying the antimicrobial activity

All compounds synthesized were tested for the antimicrobial activity using the agar well diffusion method [5, 7, 9]. The results of the experiment are presented in Tab. 5.

The results of the antimicrobial activity screening showed that most of the compounds obtained exhibited the moderate activity against most of the strains of microorganisms. It is noteworthy that the alkylation of the sulphur atom in position 4 of thieno[2,3-*d*]pyrimidine increases the antimicrobial activity, and introduction of halogens to the substituent at the sulphur atom improves the activity for all compounds **8-10**. Some of compounds **8b, 9b, 9c** and **10f** appeared to be highly active against the strain of *Candida albicans*. Compound **10b** was active against both *Staphylococcus aureus* and *Escherichia coli* strains; compound **10c** inhibited the growth of *Bacillus*

Table 4

The results of screening (the second stage) of the anti-inflammatory activity for derivatives of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidines 2, 4 and 9a (carrageenan-induced paw edema model in rats, the doses of 1 mg/kg and 50 mg/kg), (n = 5)

Experimental groups	Dose, mg/kg	Edema rate, mm									
		1 hour	AIA,%	2 hours	AIA,%	3 hours	AIA,%	4 hours	AIA,%	5 hours	AIA,%
Control	–	11.4±1.0	–	26.4±2.1	–	36.6±1.5	–	37.0±3.1	–	34.0±3.9	–
"Ortofen"	8	10.2±2.4	11	**11.2±1.6***	**58**	**17.4±3.3***	**53**	**22.4±3.3***	**40**	**21.4±2.9***	**37**
2	1	11.8±1.3	-4	21.6±2.4	18	**28.8±2.6*/****	**21**	27.4±4.0	26	25.8±4.7	24
	50	13.4±1.7	-18	33.2±1.7*	-26	36.4±1.3	1	34.8±2.0	6	34.8±2.0	-2
4	1	16.6±2.4*	-46	33.0±2.2	-25	40.0±1.5	-9	38.8±1.8	-5	36.4±2.6	-7
	50	13.4±2.3	-18	20.2±2.8	24	**29.8±1.3*/****	**19**	33.4±2.5	10	31.6±1.9	7
9a	1	22.0±1.6*	-93	38.4±2.8*	-46	41.0±2.7	-12	42.6±2.2	-15	40.6±2.5	-19
	50	11.2±2.1	2	**20.4±1.9*/****	23	31.2±2.0	15	29.6±2.2	20	29.6±3.9	13

Note: * – statistically significant differences compared to the control, $p<0.05$; ** – statistically significant differences compared to the group treated with "Ortofen", $p<0.05$; n – number of animals in the group.

Table 5

The results of screening of the antimicrobial activity for derivatives of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidines 4, 8-10

Compound	The average diameters of the growth inhibition zone (mm); number of the repeated experiments n = 3					
	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
4	13	14	growth	growth	16	15
8a	15	14	14	16	17	growth
8b	14	17	15	14	16	21
8c	18	17	15	14	19	14
9a	14	growth	growth	growth	14	15
9b	12	16	13	16	growth	20
9c	16	14	growth	growth	14	19
10a	14	16	15	14	19	14
10b	23	20	15	14	18	13
10c	16	17	15	17	21	17
10d	16	17	15	15	16	16
10e	19	17	14	13	18	growth
10f	17	18	17	18	18	19
Metr.*	14	14	growth	growth	16	14
Strept.*	15	16	growth	growth	17	growth

Note: *– Metr. – Metronidazole, DMSO solution with the concentration of 30 μg/ml; * – Strept. –Streptomycin, H_2O solution with the concentration of concentration 30 μg/ml.

subtilis strain, and **8c, 10a** and **10f** were less active against this microorganism strain.

CONCLUSIONS

An effective method for the synthesis of 6-(1*H*-benzimidazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione has been developed, and the alkylation of the compounds obtained has been performed; as a result, the series of 6-(1*H*-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-*d*]pyrimidines have been obtained. In the screening of the anti-inflammatory activity using the carrageenan-induced paw edema model in rats in the doses of 1, 10 and 50 mg/kg for the selected compounds a statistically significant and dose-dependent reduction of edema was not observed compared to the control pathology. For the compounds obtained the screening of the antimicrobial activity by the "agar well" diffusion method has been performed. The results of the antimicrobial activity tests have shown that the alkylation of the sulphur atom in position 4 of thieno[2,3-*d*]pyrimidine improves the antimicrobial properties compared to the starting thione. Most of 6-(1*H*-benzimidazol-2-yl)-5-methyl-4-(alkylthio)thieno[2,3-*d*]pyrimidines appeared to be active against the strain of *Candida albicans* fungi and the compounds containing the chlorine atom in the alkyl radical inhibited the growth of *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* strains.

REFERENCES

1. *Власов С.В., Заремба О.В., Коваленко С.М. та ін. // ЖОФХ. – 2011. – Т. 9, №4. – С. 24-30.*
2. *Власов С.В., Коваленко С.М., Федосов А.І. та ін. // ЖОФХ. – 2011. – Т. 9, №3. – С. 51-55.*
3. *Власов С.В., Коваленко С.М., Черних В.П. // ЖОФХ. – 2013. – Т. 11, №2. – С. 41-46.*
4. *Доклінічні дослідження лікарських засобів: Метод. рекоменд. / За ред. О.В.Стефанова. – К., 2001. – 528 с.*
5. *Некрасова Л.С., Свита В.М., Глушкевич Т.Г. та ін. Методичні вказівки «Визначення чутливості мікроорганізмів до антибактеріальних препаратів». – К., 2007. – 79 с.*
6. *Achar K.C.S., Hosamani K.M., Seetharamareddy H.R. // Eur. J. Med. Chem. – 2010. – Vol. 45, №5. – P. 2048-2054.*
7. *American Society for Microbiology. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology. – Washington, 2005. – P. 236.*
8. *Ansari K.F., Lal C. // Eur. J. Med. Chem. – 2009. – Vol. 44, №5. – P. 2294-2299.*
9. *Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Document M100-S22, Vol. 32, No. 3, CLSI, Wayne, PA, January, 2012.*
10. *Di Rosa M., Giround J.P., Williughby D.A. // J. Pathol. – 1971. – Vol. 104, №1. – P. 15-29.*

11. *Evans D., Hicks T.A., Williamson W.R.N. et al. // Eur. J. Med. Chem. – 1996. – Vol. 31, №7-8. – P. 635-642.*
12. *Grinev A.N., Kaplina N.V. // Chem. of Heterocycl. Compnd. – 1985. – Vol. 21, №7. – P. 767-770.*
13. *Kotaiah Y., Harikrishna N., Nagaraju K., Venkata Rao C. // Eur. J. Med. Chem. – 2012. – Vol. 58, №2. – P. 340-345.*
14. *Kumar B.V.S., Vaidya S.D., Kumar R.V. et al. // Eur. J. Med. Chem. – 2006. – Vol. 41, №5. – P. 599-604.*
15. *Leonard J.T., Jeyaseeli L., Kumar M., Sivakumar R. // Asian J. Chem. – 2006. – Vol. 18, №2. – P. 1104-1108.*
16. *Mariappan G., Bhuyan N.R., Kumar P. // Ind. J. of Chem. Section B. – 2011. – Vol. 50B, №9. – P. 1216-1219.*
17. *Mavrova A.T., Anichina K.K., Vuchev D.I. et al. // Eur. J. Med. Chem. – 2006. – Vol. 41, №12. – P. 1412-1420.*
18. *Pat. US 20070208040 (2007); Chem. Abstr., 147, 344106 (2007).*
19. *Pat. US 20130102601 (2013); Chem. Abstr., 158, 620878 (2013).*
20. *Pat. US 20130123231 (2013); Chem. Abstr., 158, 711376 (2013).*
21. *Sravanthi M., Nagaraju N., Manikanta K., Mogalabi Sk. et al. // J. Chem. Pharm. Res. – 2012. – Vol. 4, №8. – P. 3832-3836.*
22. *Vlasov S.V., Kovalenko S.M., Chernykh V.P., Krolenko K.Yu. // J. Chem. Pharm. Res. – 2014. – Vol. 6, №6. – P. 22-27.*
23. *Vlasov S.V., Kovalenko S.M., Chernykh V.P., Krolenko K.Yu. // J. Org. Pharm. Chem.– 2015. – Vol. 13, №2. – P. 30-34.*
24. *Yadav S., Kumar P., Clercq E.D. et al. // Eur. J. Med. Chem. – 2010. – Vol. 45, №12. – P. 5985-5997.*

СИНТЕЗ, ПРОТИЗАПАЛЬНА ТА АНТИМІКРОБНА АКТИВНІСТЬ 6-(1*H*-БЕНЗІМІДАЗОЛ-2-ІЛ)-5-МЕТИЛ-4-(АЛКІЛТІО)ТІЄНО[2,3-*d*]ПІРИМІДИНІВ

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***Ключові слова**: тіофен; піримідин; меркаптани; протизапальні засоби; антибактеріальні засоби*

Запропонований ефективний підхід до синтезу 6-(1Н-бензімідазол-2-іл)-5-метилтієно[2,3-d] піримідин-4(3Н)-тіону на основі перетворень відповідного 4-оксоаналога. З метою збільшення хімічного розмаїття проведено алкілування 6-(1Н-бензімідазол-2-іл)-5-метилтієно[2,3-d]піримідин-4(3Н)-тіону бензилхлоридами, хлороацетамідами та 3-арил-5-(хлорометил)-1,2,4-оксадіазолами, внаслідок чого були отримані ряди 6-(1Н-бензімідазол-2-іл)-5-метил-4-(алкілтіо) тієно[2,3-d]піримідинів. Для отриманих сполук проведені експерименти із скринінгу антимікробної активності методом дифузії в агар та протизапальної активності на моделі каррагенінового набряку стопи у щурів. Встановлено, що алкілування атома Сульфуру у положенні 4 тієно[2,3-d]піримідину підвищує антимікробну активність, наявність атомів галогенів у замісниках при атомі Сульфуру покращує антимікробну активність. Більшість 6-(1Н-бензімідазол-2-іл)-5-метил-4-(алкілтіо)тієно[2,3-d]піримідинів виявилась активною по відношенню до грибів роду Candida albicans. Сполуки, які містили атом хлору у фенільному радикалі тіоацетамідного фрагменту в положенні 4 тієно[2,3-d]піримідину, виявились активними проти Staphylococcus aureus, Escherichia coli та Bacillus subtilis. При введенні 6-(1Н-бензімідазол-2-іл)-5-метилтієно[2,3-d]піримідин-4(3Н)-ону, 6-(1Н-бензімідазол-2-іл)-5-метилтієно[2,3-d]піримідин-4(3Н)-тіону та 2-{[6-(1Н-бензімідазол-2-іл)-5-метилтієно[2,3-d]піримідин-4-іл]тіо}-N-фенілацетаміду щурам у дозах 1, 10 та 50 мг/кг не спостерігали дозозалежного достовірного зниження набряку відносно контрольної патології. Протизапальна активність зазначених сполук була на рівні 20%, що в 2-2,5 рази менше, ніж активність референтного препарату (диклофенаку натрію).

СИНТЕЗ, ПРОТИВОВОСПАЛИТЕЛЬНАЯ И ПРОТИВОМИКРОБНАЯ АКТИВНОСТЬ 6-(1*H*-БЕНЗИМИДАЗОЛ-2-ИЛ)-5-МЕТИЛ-4-(АЛКИЛТИО)ТИЕНО[2,3-*d*]ПИРИМИДИНОВ

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***Ключевые слова**: тиофен; пиримидин; меркаптаны; противовоспалительные средства; антибактериальные средства*

Предложен эффективный подход для синтеза 6-(1Н-бензимидазол-2-ил)-5-метилтиено[2,3-d] пиримидин-4(3Н)-тиона на основе превращений соответствующего 4-оксоаналога. С целью увеличения химического разнообразия проведено алкилирование 6-(1Н-бензимидазол-2-ил)-5-метилтиено[2,3-d]пиримидин-4(3Н)-тиона бензилхлоридами, хлорацетамидами и 3-арил-5-(хлорометил)-1,2,4-оксадиазолами, в результате чего были получены ряды 6-(1Н-бензимидазол-2-ил)-5-метил-4-(алкилтио)тиено[2,3-d]пиримидинов. Для полученных соединений были проведены эксперименты по скринингу противомикробной активности методом диффузии в агар и противовоспалительной активности на модели каррагенинового отека стопы у

крыс. Установлено, что алкилирование атома серы в положении 4 тиено[2,3-d]пиримидина повышает противомикробную активность, а наличие атомов галогенов в заместителях при атоме серы улучшает противомикробную активность. Большинство 6-(1Н-бензимидазол-2-ил)-5-метил-4-(алкилтио)тиено[2,3-d]пиримидинов оказалось активными по отношению к грибам рода Candida albicans. Соединения, которые содержали атом хлора в фенильном радикале тиоацетамидного фрагмента в положении 4 тиено[2,3-d]пиримидина, оказались активными против Staphylococcus aureus, Escherichia coli и Bacillus subtilis. При введении 6-(1Н-бензимидазол-2-ил)-5-метилтиено[2,3-d]пиримидин-4(3Н)-она, 6-(1Н-бензимидазол-2-ил)-5-метилтиено[2,3-d]пиримидин-4(3Н)-тиона и 2-{[6-(1Н-бензимидазол-2-ил)-5-метилтиено[2,3-d] пиримидин-4-ил]тио}-N-фенилацетамида крысам в дозах 1, 10 и 50 мг/кг не наблюдали дозозависимого достоверного снижения отека относительно контрольной патологии. Противовоспалительная активность данных соединений была на уровне 20%, что в 2-2,5 раза меньше, чем активность препарата сравнения.

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