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SYNTHESIS AND BIOLOGICAL ACTIVITY OF 6-(1,3-BENZOXAZOL-2-YL)-5-METHYLTHIENO-[2,3-*d*]PYRIMIDINES

S. V. Vlasov,[1,*] S. N. Kovalenko,[1] T. P. Osolodchenko,[2] E. B. Lenitskaya,[1] and V. P. Chernykh[1]

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An approach to the synthesis of new 6-(1,3-benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones and their 4-thioanalogs was developed. Some of the synthesized compounds exhibited antimicrobial activity against strains of *Bacillus subtilis* bacteria and *Candida albicans* fungi.

Keywords: thiophene, pyrimidine, benzoxazole, acetamides, sulfur.

Approaches to the synthesis of biologically active thieno[2,3-*d*]pyrimidines with an azole substituent in the 6-position have recently been attracting increasing attention. Similar compounds were proposed as adenosine A_{2A} receptor antagonists [1]. Related structures also turned out to be acetyl-CoA carboxylase inhibitors [2]. Studies of the antioxidant [3] and antiviral [4] properties of several 6-hetarylthieno[2,3-*d*]pyrimidines discovered similar compounds with antimicrobial activity [5 – 8]. Currently, two principal approaches to introducing a heterocycle in the 6-position of thieno[2,3-*d*]pyrimidines are known. The first uses 6-bromothieno[2,3-*d*]pyrimidines in reactions with boronic acids [1, 4] or organotin compounds [2]. The second involves modification of functional groups in the 6-position of the heterocyclic core [1, 3, 5 – 8]. In turn, benzoxazole derivatives possessed highly promising biological activity such as antimicrobial [9 – 16] and anti-inflammatory [17 – 19].

The present work continues our research on the use of 5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid (**I**) to synthesis 6-heterylthieno[2,3-*d*]pyrimidines. Use of *o*-aminophenol in reactions with acid imidazolides is known to be an effective method for preparing benzoxazoles [20-22] although it often requires additional dehydration after the acylation step.

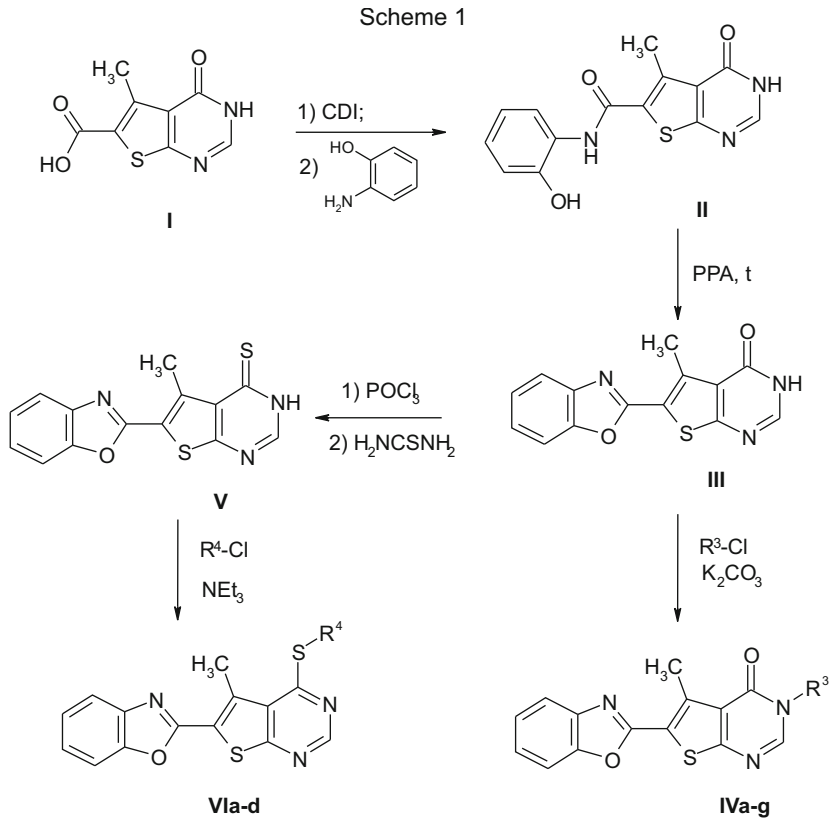
The 6-(1,3-benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-ones were built from *o*-aminophenol and acid **I** using 1,1′-carbonyldiimidazole (CDI) as a condensing reagent to produce *N*-(2-hydroxyphenyl)-5-methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxamide **II**, which was cyclized with heating in polyphosphoric acid (PPA) at 180°C for 3 h to give 6-(1,3-benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-one (**III**) (Scheme 1).

TABLE 1. Yields and Melting Points of **IV** and **VI**

Compound	mp, °C	Yield, %	Molecular formula
IVa	239 – 241	83	$C_{21}H_{15}N_3O_2S$
IVb	238 – 240	77	$C_{22}H_{17}N_3O_2S$
IVc	> 300	79	$C_{22}H_{16}N_4O_3S$
IVd	> 300	67	$C_{22}H_{15}BrN_4O_3S$
IVe	> 300	78	$C_{24}H_{20}N_4O_3S$
IVf	> 300	71	$C_{23}H_{17}ClN_4O_3S$
IVg	298 – 300	82	$C_{24}H_{20}N_4O_5S$
VIa	271 – 273	74	$C_{22}H_{16}N_4O_2S_2$
VIb	248 – 250	69	$C_{23}H_{18}N_4O_2S_2$
VIc	253 – 255	73	$C_{25}H_{22}N_4O_2S_2$
VId	239 – 241	86	$C_{24}H_{20}N_4O_4S_2$

[1] National University of Pharmacy, 53 Pushkinskaya St., 61002 Kharkiv, Ukraine.
[2] I. I. Mechnikov Institute of Microbiology and Immunology, National Academy of Medical Sciences of Ukraine, 14 – 16 Pushkinskaya St., 61057 Kharkiv, Ukraine.
* e-mail: sergiy.vlasov@gmail.com

Scheme 1



IV: R^3 = Bn (a), R^3 = 4-CH_3-Bn (b); R^3 = $CH_2CONHPh$ (c), R^3 = CH_2CONH(4-Br-Ph) (d), R^3 = CH_2CONH(2,4-diCH_3-Ph) (e), R^3 = CH_2CONH(2-Cl-4-CH_3-Ph) (f), R^3 = CH_2CONH(3,5-diOCH_3-Ph) (g).
VI: R^3 = $CH_2CONHPh$ (a), R^3 = CH_2CONH(4-CH_3-Ph) (b), R^3 = CH_2CONH(4-i-Pr-Ph) (c), R^3 = CH_2CONH(3,5-diOCH_3-Ph) (d).

TABLE 2. PMR Spectra of **IV** and **VI**, DMSO-d_6, δ, ppm

Compound	CH_2 (s, 2H)	NH (br.s, 1H)	Thiophene CH_3 (c, 3H)	Aliphatic protons	Aromatic protons
IVa	5.15	-	2.92	-	7.16 – 7.46 (m, 7H), 7.63 – 7.78 (m, 2H), 8.71 (s, 1H)
IVb	5.13	-	2.97	2.25 (c, 3H)	7.15 (d, 2H), 7.27 (d, 2H), 7.37 – 7.47 (m, 2H), 7.72 – 7.85 (m, 2H), 8.73 (s, 1H)
IVc	4.86	10.48	2.97	-	7.06 (t, 2H), 7.25 – 7.47 (m, 4H), 7.58 (d, 2H), 7.71 – 7.83 (m, 2H), 8.51 (s, 1H)
IVd	4.86	10.54	2.97	-	7.38 – 7.55 (m, 6H), 7.71 – 7.83 (m, 2H), 8.51 (s, 1H)
IVe	4.87	9.73	2.98	2.18 (c, 3H), 2.21 (c, 3H)	6.89 – 7.04 (m, 2H), 7.24 (d, 1H), 7.35 – 7.47 (m, 2H), 7.72 – 7.82 (m, 2H), 8.51 (s, 1H)
IVf	4.95	10.01	2.97	2.26 (c, 3H)	7.12 (d, 1H), 7.33 (d, 1H), 7.35 – 7.47 (m, 2H), 7.56 (d, 1H), 7.74 – 7.83 (m, 2H), 8.52 (s, 1H)
IVg	4.84	10.44	2.97	3.65 (c, 6H)	6.23 (m, 1H), 6.82 (m, 2H), 7.36 – 7.48 (m, 2H), 7.75 – 7.85 (m, 2H), 8.51 (s, 1H)
VIa	4.34	10.39	3.15	-	7.04 (t, 1H), 7.22 – 7.47 (m, 4H), 7.59 (d, 2H), 7.72 – 7.84 (m, 2H), 8.80 (s, 1H)
VIb	4.35	10.30	3.20	2.23 (c, 3H)	7.10 (d, 2H), 7.40 – 7.53 (m, 4H), 7.78 – 7.89 (m, 2H), 8.85 (s, 1H)
VIc	4.35	10.26	3.20	1.15 (d, 6H), 2.82 (m, 1H)	7.16 (d, 2H), 7.38 – 7.56 (m, 4H), 7.77 – 7.90 (m, 2H), 8.84 (s, 1H)
VId	4.33	10.34	3.15	3.68 (c, 6H)	6.20 (m, 1H), 6.83 (m, 2H), 7.33 – 7.47 (m, 2H), 7.71 – 7.85 (m, 2H), 8.80 (s, 1H)

Compound **III** was further modified via alkylation of the 3-N atom using DMF and K_2CO_3 to produce compounds **IV** (Tables 1 and 2). The regioselectivity of this reaction was evaluated from NOESY spectra of **IVa** and **IVc**, in which a distinct cross peak was observed for through-space coupling of alkyl methylene protons with the 2-H proton of the pyrimidine core.

Another modification pathway used an intermediate 4-chloro derivative to prepare 6-(1,3-benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione (**V**) as reported earlier for similar structures [8]. Alkylation of the S atom of thione **V** produced a series of 4-S-alkyl derivatives (**VI**) (Tables 1 and 2).

Screening of **III**-**VI** for antimicrobial activity used an agar diffusion method (well diffusion method) [23 – 25].

The results found that the tested compounds were active mainly against *Bacillus subtilis* and *Candida albicans* (Table 3) and, in isolated instances, even more active than the reference drugs (metronidazole and synthomycin). The most active compound with respect to breadth and strength of antimicrobial activity was **IVg**.

TABLE 3. Screening Data for Antimicrobial Activity of **III**-**VI** by the Well Diffusion Method

Compound	Average diameter (mm) of growth inhibition zone, number of test repetitions $n = 3$*					
	Staphylococcus aureus ATCC 25923	*Escherichia coli* ATCC 25922	*Proteus vulgaris* ATCC 4636	*Pseudomonas aeruginosa* ATCC 27853	*Bañillus subtilis* ATCC 6633	*Candida albicans* ATCC 653/885
III	14	14	–	13	16	19
V	16	16	–	–	19	21
IVa	–	–	14	17	14	20
IVb	15	14	14	14	14	14
IVc	16	18	15	17	21	14
IVd	14	14	13	13	16	16
IVe	14	–	–	–	16	15
IVf	14	–	–	–	16	17
IVg	20	16	15	16	19	20
VIa	15	15	–	–	15	15
VIb	14	15	–	–	15	13
VIc	14	–	–	–	16	16
VId	13	–	–	–	–	14
Metr.**	14	14	–	–	16	14
Synt.***	14	17	17	17	17	–

* Averages of three tests are given; ** Metr. is metronidazole (DMSO solution, 30 μg/mL); *** Synt. is synthomycin (H_2O solution, 30 μg/mL).

Screening of **III**, **V**, and **VIa** for anti-inflammatory activity used a rat-paw carrageenan-induced edema model [26]. These tests showed that the compounds at the studied doses did not exhibit significant anti-inflammatory activity (Table 4). Administration of **III**, **V**, and **VIa** at doses of 1 and 50 mg/kg did not cause a statistically significant dose-dependent reduction of edema vs. the controls.

EXPERIMENTAL CHEMICAL PART

All solvents and reagents were obtained commercially. Melting points (°C) were measured using a Kofler apparatus. PMR spectra were recorded in DMSO-d_6 with TMS internal standard on a Varian Mercury instrument (200 MHz). ^{13}C NMR and NOESY spectra were recorded in DMSO-d_6 with TMS internal standard on a Varian Gemini instrument (300 MHz). GC-MS analysis used a PE SCIEX API 150EX chromatograph with a mass-selective detector.

5-Methyl-4-oxo-3,4-dihydrothieno[2,3-*d*]pyrimidine-6-carboxylic acid (I) was prepared by the literature method [27].

6-(1,3-Benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-one (III). A suspension of **I** (10 g, 0.048 mol) in anhydrous DMF (30 mL) was treated with CDI (8.4 g, 0.052 mol), heated until the reagents were fully dissolved and for another 15 – 20 thereafter. The resulting imidazolide was treated with *o*-aminophenol (5.2 g, 0.048 mol), heated at 120 – 130°C for 3 – 4 h, cooled, and diluted with H_2O. The precipitate of **II** was filtered off. Compound **II** (10 g) was treated with PPA (35 mL), heated at 180°C until a homogeneous solution formed and for another 3 h thereafter, cooled, poured onto ice, and treated with conc. NaOH solution until slightly basic. The precipitate was filtered off and rinsed with copious amounts of H_2O to produce **III**, which did not require further purification. Yield 8.3 g (61.1%); mp > 300°C; PMR spectrum (DMSO-d_6), δ, ppm: 2.96 (c, 3H, CH_3), 7.29 – 7.47 (m, 2H, Ar-H), 7.67 – 7.84 (m, 2H, Ar-H), 8.17 (s, 1H, CH), 12.57 (br.s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 15.78; 111.31; 120.11; 124.58; 125.65; 126.14; 139.63; 141.53; 148.33; 150.06; 158.61; 165.99. HPLC-MS, *m*/*z*: 283.3 (MH^+). $C_{14}H_9N_3O_2S$.

General method for preparing 3-alkyl-6-(1,3-benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidin-4(3*H*)-ones (IV). A suspension of **III** (0.15 g, 0.00052 mol) in DMF was treated with alkylating agent (0.00052 mol) and K_2CO_3 (0.075 g, 0.00052 mol), heated and stirred at 60°C for 5 – 8 h, cooled, and diluted with H_2O. The resulting precipitate was filtered off. Compounds **IV** were purified by refluxing in lower alcohols.

6-(1,3-Benzoxazol-2-yl)-5-methylthieno[2,3-*d*]pyrimidine-4(3*H*)-thione (V). Compound **III** (8 g, 0.028 mol) was treated with phosphoryl chloride (30 mL), stirred and refluxed until a homogeneous solution formed and for another 4 h thereafter, cooled, and poured onto ice. The resulting precipitate of the chloro-derivative was filtered off, dried at room

TABLE 4. Screening Data for Anti-inflammatory Activity (AIA) of **III**, **V**, and **VIa** in the Carrageenan Edema Model in Rats (n = 5)

Group	Dose, mg/kg	Paw edema dynamics, mm							
		1 h	AIA, %	2 h	AIA, %	3 h	AIA, %	4 h	AIA, %
CP	–	11.4 ± 1.0	–	26.4 ± 2.1	–	36.6 ± 1.5	–	37.0 ± 3.1	–
Orthophen	8	10.2 ± 2.4	11	11.2 ± 1.6*	58	17.4 ± 3.3*	53	22.4 ± 3.3*	40
III	1	10.2 ± 1.5	11	25.4 ± 2.8	4	33.4 ± 1.9	9	34.6 ± 2.0	7
	50	17.8 ± 3.7	–56	33.6 ± 1.5*	–27	38.8 ± 2.2	–6	35.4 ± 1.4	4
V	1	16.8 ± 2.9	–47	28.6 ± 3.4	–8	30.6 ± 2.5	16	31.4 ± 1.8	15
	50	17.2 ± 4.0	–51	28.4 ± 5.3	–8	35.6 ± 3.4	3	38.2 ± 2.0	–3
VIa	1	8.6 ± 1.3	25	25.0 ± 2.1	5	34.6 ± 2.2	6	35.0 ± 1.9	5
	50	14.4 ± 2.6	–26	27.2 ± 2.7	–3	35.0 ± 2.0	4	32.2 ± 1.4	13

* Difference statistically significant vs. CP (control pathology), $p < 0.05$; n is the number of animals in the group.

temperature, and used for subsequent reactions. The intermediate chloro-derivative (8 g, 0.026 mol) was treated with thiourea (2.5 g, 0.032 mol) and DMF (20 mL), stirred and refluxed for 2 – 3 h, cooled, and diluted with H_2O (20 – 30 mL). The resulting precipitate was filtered off. Crude product **V** was dissolved in a two-fold molar excess of aqueous base and heated for 10 – 15 min. The product precipitated after acidification to neutrality and was filtered off and rinsed on the filter with a large volume of H_2O. Yield 6.3 g (74.6%); mp > 300°C; PMR spectrum (DMSO-d_6), δ, ppm: 3.22 (c, 3H, CH_3), 7.31 – 7.48 (m, 2H, Ar-H), 7.70 – 7.85 (m, 2H, Ar-H), 8.22 (s, 1H, CH), 14.00 (br.s, 1H, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 18.02; 111.34; 120.20; 120.66; 125.69; 126.34; 132.60; 140.72; 141.39; 146.48; 149.99; 158.42; 164.19; 180.19. HPLC-MS, *m*/*z*: 300.2 (MH^+). $C_{14}H_9N_3OS_2$.

General method for preparing 4-alkylthio-6-(1,3-benzoxazol-2-yl)-5-methylthieno[2,3-***d*]**pyrimidines (VI).** A suspension of **V** (0.15 g, 0.0005 mol) was treated with Et_3N (0.00055 mol) and alkylating reagent (0.0005 mol), heated at 120°C for 5 – 7 h, cooled, and diluted with H_2O. The precipitate was filtered off. The compounds were further purified in refluxing EtOH.

EXPERIMENTAL BIOLOGICAL PART

Antimicrobial activity of the tested compounds was assessed according to WHO recommendations [23 – 25]. Compounds were added as DMSO solutions (0.3 mL) at a concentration of 100 ìg/mL with agar diffusion from wells.

Anti-inflammatory activity was studied in rats from the Central Research Laboratory of the National University of Pharmacy. Animals were kept according to current rules for instrumentation, equipment, and vivariums [28, 29]. Animals were handled according to the *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes* [30]. The standard suite of Statistica 6.0 programs was used for the mathematical calculations [31].

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