Antimycobacterially active 2-alkylthio-6-formamidobenzothiazoles and 6-formamido-2-benzothiazolinethione

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By the formylation of 2-alkylthio-6-aminobenzothiazoles and 6-amino-2-benzothiazolinethione with 85 % formic acid, corresponding 6-formamido derivatives were synthesized. Compounds are antimycobacterially active, mainly against atypical strains of tuberculosis mycobacteria.

С помощью формилирования 2-алкилтио-6-аминобензотиазолов и 6-амино-2-бензотиазолинтиона 85 % муравьиной кислотой были синтезированы соответствующие 6-формамидо производные. Эти соединения проявляли антимикобактериальную активность, главным образом, по отношению к нетипичным штаммам туберкулезных микобактерий.

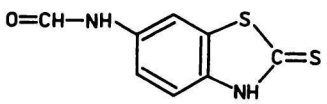
2-Alkylthio-6-aminobenzothiazoles are antimicrobially active compounds. Their activity against mycobacteria [1], anaerobic sporulating bacteria [2] and yeast-like microorganisms [3] has been determined. The above-mentioned compounds (as aromatic amines) show colour changes during longer storage.

2-Alkylthio-6-formamidobenzothiazoles and 6-formamido-2-benzothiazolinethione (Table 1), unknown yet, were synthesized with the aim to study their antimycobacterial activity.

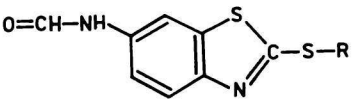
Formylation of 2-alkylthio-6-aminobenzothiazoles (in case of isopentyl derivative *IX* of the corresponding ammonium chloride) and of 6-amino-2-benzothiazolinethione was carried out with 85 % formic acid under reflux. The excess of formic acid was used as a solvent. Reaction time 10 min showed to be optimal for majority of the derivatives. In case of carboxymethyl derivative *XV* the reaction mixture solidified in 7 min, and in case of 6-formamido-2-benzothiazolinethione (*I*) in 2 min. Prolongation of the reaction time to 30 min was unfavourable, as crude product with wider interval of melting point was isolated from the reaction mixture. Repeated crystallization of such a product from different solvents gave only 6.6 % of pure product (in case of n-propyl derivative *III*).

Table 1

Characterization of the prepared compounds *I*—*XV*



I



II–*XV*

Compound	R	Formula	M_r	w_i(calc.)/% w_i(found)/%				Yield	M.p.
				C	H	N	S	%	°C
I	—	$C_8H_6N_2OS_2$	210.28	45.26	3.80	13.20	30.21	89.5	257—259.5
				45.56	3.67	13.08	30.32		(decomposition)
II	C_2H_5	$C_{10}H_{10}N_2OS_2$	238.33	50.40	4.23	11.75	26.91	69.2	89—91
				50.70	4.21	11.55	26.67		
III	$(CH_2)_2CH_3$	$C_{11}H_{12}N_2OS_2$	252.36	52.35	4.79	11.10	25.41	96.4	64—67
				52.31	4.70	11.16	25.70		
IV	$CH(CH_3)_2$	$C_{11}H_{12}N_2OS_2$	252.36	52.35	4.79	11.10	25.41	97.1	93—95
				52.38	4.76	10.96	25.16		
V	$CH_2CH{=}CH_2$	$C_{11}H_{10}N_2OS_2$	250.34	52.78	4.04	11.19	25.61	79.2	78.5—81
				52.58	3.89	11.01	25.35		
VI	$(CH_2)_3CH_3$	$C_{12}H_{14}N_2OS_2$	266.39	54.11	5.30	10.52	24.07	91.3	58—59
				54.11	5.31	10.36	23.89		
VII	$CH_2CH(CH_3)_2$	$C_{12}H_{14}N_2OS_2$	266.39	54.11	5.30	10.52	24.07	97.5	82—84
				54.01	5.25	10.53	24.24		
VIII	$(CH_2)_4CH_3$	$C_{13}H_{16}N_2OS_2$	280.41	55.68	5.75	9.99	22.87	95.2	66.5—68
				55.38	5.71	9.76	22.96		
IX	$(CH_2)_2CH(CH_3)_2$	$C_{13}H_{16}N_2OS_2$	280.41	55.68	5.75	9.99	22.87	96.4	69—70.5
				55.85	5.87	10.12	23.14		
X	$CH(CH_2)_4$ cyclo	$C_{13}H_{14}N_2OS_2$	278.40	56.09	5.07	10.06	23.03	94.9	119.5—121.5
				56.39	5.07	9.91	22.98		

Table 1 (Continued)

Compound	R	Formula	M_r	w_i(calc.)/% w_i(found)/%				Yield	M.p.
				C	H	N	S	%	°C
XI	$(CH_2)_5CH_3$	$C_{14}H_{18}N_2OS_2$	294.44	57.11	6.16	9.51	21.78	76.1	70—71
				57.11	6.16	9.44	21.86		
XII	$(CH_2)_6CH_3$	$C_{15}H_{20}N_2OS_2$	308.47	58.41	6.54	9.08	20.79	97.3	68—70
				58.10	6.56	8.81	20.74		
XIII	$(CH_2)_7CH_3$	$C_{16}H_{22}N_2OS_2$	322.49	59.59	6.88	8.69	19.88	99.2	68.5—70.5
				59.35	7.01	8.46	19.72		
XIV	$(CH_2)_8CH_3$	$C_{17}H_{24}N_2OS_2$	336.52	60.67	7.19	8.32	19.06	93.6	72.5—74.5
				60.53	7.43	8.08	18.88		
XV	CH_2COOH	$C_{10}H_8N_2OS_2$	268.32	44.76	3.01	10.44	23.90	91.9	214—216
				44.78	2.78	10.25	23.87		

By this method new antimycobacterially active compounds were prepared, which in contrast to the starting 2-alkylthio-6-aminobenzothiazoles can be stored easier, their appearance, elemental analysis and melting point do not change after 6-month storage. Similarly as in the case of its native group of 2-alkylthio-6-aminobenzothiazoles the whole series is antimycobacterially effective (except for 2-carboxymethyl derivative *XV*), however, the activity is less pronounced, and less differentiated among corresponding derivatives in comparison with the series of starting compounds [1].

From 2-alkylthio-6-formamidobenzothiazoles the best effect was shown by n-heptyl (*XII*) followed by n-pentyl (*VIII*) derivative, while in the parent series 6-amino-2-n-hexylthiobenzothiazole was the most effective compound. Newly synthesized compounds are the most effective against the atypical strain of tuberculosis mycobacteria *Mycobacterium avium*. Activity of compounds *I, VI, VIII, IX, XI,* and *XII* was twice as high as activity of commercially used Isoniazide (isonicotinohydrazide, INH) and equal to that of Ethionamide (2-ethylisonicotinothioamide, ETA).

On the contrary, in the series of 6-acetamido-2-alkylthiobenzothiazoles [2], only cyclopentyl derivative was highly effective against *M. avium*. The activity of acetamido derivatives against the atypical strains *M. kansasii* and *M. fortuitum* was again much lower than the activity of the newly synthesized compounds. On the contrary, the activity of 2-alkylthio-6-formamidobenzothiazoles against typical tuberculosis mycobacteria *M. tuberculosis* $H_{37}R_v$ was in some cases as far as 10 times lower than the activity of the corresponding acetamides [2].

6-Formamido-2-benzothiazolinethione (*I*) showed good efficiency against atypical tuberculosis mycobacteria, its activity equals the activity of the commercially used Ethionamide, while against typical tuberculosis mycobacteria *M. tuberculosis* $H_{37}R_v$ the same compound is five times less active than Ethionamide (Table 2).

On the whole it can be concluded that the synthesis of the title compounds has fulfilled its aim. In comparison with 2-alkylthio-6-aminobenzothiazoles compounds with higher melting point and better consistence (loose compounds) were prepared with only slightly lower activity. Newly synthesized compounds did not change their appearance (consistence, elemental composition and melting point) after 6-month storage. In comparison with 6-acetamido-2-alkylthiobenzothiazoles the newly synthesized group of compounds is less effective against typical strains and more effective against the atypical strains of tuberculosis mycobacteria [4].

As 2-alkylthio-6-formamidobenzothiazoles and 2-alkylthio-6-aminobenzothiazoles are similar in their antimycobacterial activity, additional research of biological activity of the newly synthesized compounds is due, because 2-alkyl-

Table 2

Antimycobacterial activity of the synthesized compounds in comparison with the activity of known antituberculotics

Compound	MIC/(μg cm^{-3}) Against *Mycobacterium*			
	tuberculosis $H_{37}R_v$	*kansasii* PKG 8	*avium* 16/18	*fortuitum* 1021
I	25	25	25	50
II	100	100	100	>100
III	50	50(25)	50	50
IV	50	50	100	100
V	100(50)	50	100	100
VI	25	25	25	50
VII	25	50(25)	50	100
VIII	10	25(10)	25	50
IX	25(10)	25	25	50
X	25	50	50	100
XI	10(5)	25(10)	25	50
XII	10(5)	25	25	>100
XIII	25(10)	25	50	>100
XIV	100(50)	50	50	>100
XV	>100	>100	>100	>100
INH	1	5	50	25
ETA	5	25	25	50

MIC = minimal inhibitory concentration. Partial inhibitory concentration is given in the brackets.

thio-6-aminobenzothiazoles have already proved further antimicrobial activity [3, 4].

Experimental

Starting compounds were prepared according to the known procedure [1]. Physical constants, analytical data, and yields of the synthesized compounds are given in Table 1. Melting points were determined on a Kofler block.

Antimycobacterial activity of compounds was determined in liquid Šula medium by a dilution test [5], dimethyl sulfoxide was used as a solvent. Their resulting concentrations ϱ/(μg cm^{-3}): 0.5, 1, 5, 10, 25, 50 and 100. All strains (*Mycobacterium tuberculosis* $H_{37}R_v$, *M. kansasii PKG 8, M. avium* 16/18, and *M. fortuitum* 1021) were obtained from the collection of the Research Institute of Preventive Medicine, Centre for Epidemiology and Microbiology. The activity of tested compounds was compared with the activity of

Isoniazide (Jenapharm, GDR) and Ethionamide (Trécator, Teraplix, Paris) tested under the same conditions. In case of sedimentation the results were controlled by microscopical preparation.

6-Formamido-2-benzothiazolinethione (I)

6-Amino-2-benzothiazolinethione [6] (5.45 g; 0.03 mol) and 85 % formic acid (7.5 cm^3, 9.0 g; 0.166 mol) were heated under reflux. After 2 min the reaction mixture was left aside for 30 min and then dispersed in 300 cm^3 of cold water. The sucked product was washed with water to neutral reaction.

2-Alkylthio-6-formamidobenzothiazoles II—XV

2-Alkylthio-6-aminobenzothiazole (0.03 mol) and 85 % formic acid (7.5 cm^3, 9.0 g; 0.166 mol) were heated under reflux for 10 min. After cooling to room temperature the reaction mixture was poured on 100 g of ice, and filled up with water to 200 cm^3. From the reaction mixture solid product precipitated.

In the case of preparation of carboxymethyl derivative (*XV*) the reaction time was shortened to 7 min, when the reaction mixture solidified. Isopentyl derivative was prepared from the corresponding ammonium chloride, the crude product was greasy and solidified after 48 h.

The prepared compounds were crystallized from the mixture ethanol—water ($\varphi_r = 1:1$ to 5 : 1). n-Propyl derivative (*III*) was crystallized from the mixture benzene—cyclohexane ($\varphi_r = 1:3$) and isopentyl derivative (*IX*) from the mixture ethyl acetate—cyclohexane—petroleum ether. In all cases active carbon was used.

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