Synthesis and antimicrobial activity of 2-alkylthio-6-aminobenzothiazoles

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S-Alkyl (C_{1-16}) derivatives of 6-amino-2-mercaptobenzothiazole were synthesized and tested for antimicrobial activity against dermatophytes and bacteria. The antimycobacterial activity against typical and atypical tuberculosis mycobacteria was found to be most significant.

Были синтезированы *S*-алкил (C_{1-16}) производные 6-амино-2-меркаптобензтиазола. Соединения были испытаны на их антимикробиальное действие по отношению к дерматофитам и бактериям. Наиболее значительным оказалось антимикобактериальное действие на типичные и нетипичные туберкулезные микобактерии.

2-Alkylthio-6-aminobenzothiazoles are interesting from the view-point of their biological activity and possible utilization in further syntheses as intermediates. The so far known 2-alkylthio-6-aminobenzothiazoles with lower alkyls showed high antimicrobial activity [1] and this fact urged us to continue the investigation of this group of compounds.

2-Alkylthio-6-aminobenzothiazoles *I—XIX*, presented in Table 1, were synthesized by treatment of potassium salt of 6-amino-2-mercaptobenzothiazole with alkyl halides. Majority of the final products were low melting (derivatives with higher alkyls were wax-like) and were purified by pouring onto crushed ice under vigorous stirring. After removal of last residues of organic solvents the products solidified forming small granules. Twofold purification, described in Experimental, was sufficient for obtaining a sample of anal. grade and relatively stable in colour. Though the products became light-brown when exposed to light, their melting points, percentual composition, and biological activity were unchanged even after one year. Further crystallization of the synthesized compounds from the mixture of acetone and water led to greater stability in colour.

Table 1

2-Alkylthio-6-aminobenzothiazoles

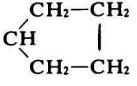
Compound	Alkyl	Formula	*M*	Calculated/found				Halogen of the reagent	Yield %	M.p. °C
				% C	% H	% N	% S			
I	CH_3	$C_8H_8N_2S_2$	196.29					I	96.8	107—108 111 [1]
II	C_2H_5	$C_9H_{10}N_2S_2$	210.31					I	94.1	78—80 74 [1]
III	$(CH_2)_2CH_3$	$C_{10}H_{12}N_2S_2$	224.34	53.56 53.86	5.39 5.41	12.49 12.70	28.59 28.48	I	89.3	64—65.5
IV	$CH(CH_3)_2$	$C_{10}H_{12}N_2S_2$	224.34	53.56 53.75	5.39 5.42	12.49 12.45	28.59 28.65	Br	84.0	69—71
V	$CH_2CH=CH_2$	$C_{10}H_{10}N_2S_2$	222.32					Br	73.8	58—60 48 [1]
VI	$(CH_2)_3CH_3$	$C_{11}H_{14}N_2S_2$	238.37	55.42 55.45	5.92 5.94	11.76 11.78	26.91 26.80	I	83.1	37.5—39
VII	$CH_2CH(CH_3)_2$	$C_{11}H_{14}N_2S_2$	238.37	55.42 55.08	5.92 5.93	11.76 11.78	26.91 26.75	I	90.1	59—60
VIII	$CH(CH)_3CH_2CH_3$	$C_{11}H_{14}N_2S_2$	238.37	55.42 55.16	5.92 5.68	11.76 11.57	26.91 26.61	I	91.5	45—47
IX		$C_{12}H_{14}N_2S_2$	250.38	57.55 57.87	5.64 5.66	11.11 10.87	25.61 25.52	I	87.1	64—66
X	$(CH_2)_5CH_3$	$C_{13}H_{18}N_2S_2$	266.43	58.61 58.46	6.81 6.91	10.51 10.22	24.07 24.11	I	73.6	66—68

Table 1 (Continued)

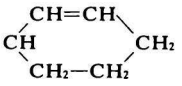
Compound	Alkyl	Formula	*M*	Calculated/found % C	% H	% N	% S	Halogen of the reagent	Yield %	M.p. °C
XI		$C_{13}H_{14}N_2S_2$	262.40	59.50 59.74	5.38 5.50	10.68 10.55	24.44 24.16	Br	87.0	95—96.5
XII	$(CH_2)_6CH_3$	$C_{14}H_{20}N_2S_2$	280.46	59.96 59.89	7.19 7.36	9.99 9.89	22.87 22.94	I	69.3	42—44.5
XIII	$(CH_2)_7CH_3$	$C_{15}H_{22}N_2S_2$	294.48	61.18 61.42	7.53 7.50	9.51 9.46	21.78 21.58	I	58.1	47—49
XIV	$(CH_2)_8CH_3$	$C_{16}H_{24}N_2S_2$	308.51	62.29 62.06	7.84 7.77	9.08 8.80	20.78 20.49	I	75.8	38—40
XV	$(CH_2)_{15}CH_3$	$C_{23}H_{38}N_2S_2$	406.70	67.93 68.24	9.42 9.72	6.89 6.62	15.77 15.40	I	84.0	61.5—63.5
XVI	$CH_2C_6H_5$	$C_{14}H_{12}N_2S_2$	272.38	61.73 61.76	4.44 4.32	10.29 10.12	23.54 23.34	Cl	91.9	83—85
XVII	$CH_2C_6H_4CH_3$	$C_{15}H_{14}N_2S_2$	286.42	62.90 63.23	4.93 5.03	9.78 9.51	22.38 22.10	Br	87.7	118.5—120.5
XVIII	CH_2CH_2OH	$C_9H_{10}N_2OS_2$	226.31					I	95.0	107—108 103—104 [1]
XIX	CH_2COOH	$C_9H_8N_2O_2S_2$	240.30	45.26 44.98	3.36 3.53	11.66 11.48	26.68 26.82	Cl	93.6	210—212

Table 2

Antimicrobial activity (MIC in μg/ml) of some synthesized compounds

Compound	Dermatophytes									Bacteria			
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
I	500	—	500	500	—	250	500	—	—	>500	>500	>500	>500
II	250	—	250	500	—	250	250	—	—	>500	>500	>500	>500
III	250	>100	500	500	>100	250	250	100	>100	100	250	250	250
IV	100	>100	250	250	100	100	100	50	>100	250	>500	>500	500
V	250	>100	500	500	>100	250	250	100	>100	500	>500	250	500
VII	500	>100	250	500	>100	250	250	100	>100	100	>500	>500	500
IX	500	—	>500	>500	—	500	250	—	—	>500	>500	>500	500
XVI	>500	—	>500	>500	—	500	500	—	—	>500	>500	>500	>500
XVIII	>500	—	>500	>500	—	>500	>500	—	—	>500	>500	>500	>500
XIX	>500	—	>500	>500	—	>500	>500	—	—	>500	>500	>500	>500

1. Trichophyton rubrum; 2. Trichophyton rosaceum; 3. Trichophyton gypseum; 4. Microsporum gypseum; 5. Microsporum cookei; 6. Epidermophyton floccosum; 7. Trichophyton Kaufmann—Wolf; 8. Trichophyton fávi-formae; 9. Trichophyton violaceum; 10. Candida albicans; 11. Escherichia coli; 12. Salmonella typhi-murium; 13. Staphylococcus aureus.

— Not tested.

Some compounds were tested for antifungal activity against dermatophytes. They were active only in high concentrations (Table 2). The isopropyl derivative *IV* showed the highest activity.

Moderate antimicrobial activity against *Candida albicans, Escherichia coli, Salmonella typhi-murium,* and *Staphylococcus aureus* exhibited only the compounds with the alkyls C_3 and C_4 in higher concentrations (Table 2). The *n*-propyl and isobutyl derivatives *III* and *VII* were observed to be most active.

The synthesized compounds were active against both the typical and atypical tuberculosis mycobacteria (Table 3). The compounds *I—XIV*, *XVI*, and *XVII* inhibited the growth of *M. tuberculosis* $H_{37}R_v$ already in 10 μg/ml concentration. The most active were the compounds with medium alkyls, mainly the *n*-hexyl derivative *X*. The MIC (5 μg/ml) of this compound against *M. tuberculosis* $H_{37}R_v$ was close to the values of the preparations used in practice and its activity (MIC 5 μg/ml) against *M. kansasii* was even higher [2].

With three representatives of the synthesized compounds the acute toxicity on mice was examined orientatively [3]. The obtained values were lower than those of Isoniazid, except the toxicity of the benzyl derivative *XVI* which was approximately two times higher after 48 h than that of Isoniazid (Table 4).

Table 3

Antimycobacterial activity (MIC in μg/ml) of the synthesized compounds

Compound	*Mycobacterium*				
	tuberculosis $H_{37}R_v$	*kansasii*	*avium*	*fortuitum*	*bovis*
I	100 (50)	>100	100	>100	—
II	50 (25)	>100	100	100	—
III	50 (25)	50	50	50	—
IV	50 (25)	50	50	50	—
V	25 (10)	50	50	50	—
VI	25	50 (25)	25	25	—
VII	50 (25)	50	50	50	—
VIII	50 (25)	50	25	50	—
IX	25 (10)	25	25	25	—
X	5	5	50	25	10 (5)
XI	10	25	25	25	—
XII	25	25	>100	100	—
XIII	50 (25)	50 (25)	25	50 (25)	—
XIV	10	10	25	50	5
XV	100	100	>100	>100	—
XVI	25 (10)	25	25	25	—
XVII	10	10	50	100	—
XVIII	>100	>100	>100	>100	—
XIX	>100	>100	>100	>100	—
2-MBT	25	50	50	100	10
Isoniazid	1	10	10	50	1
Ethionamide	1	10	25	50	10

Partial inhibition concentration is given in parentheses.
— Not tested.

Table 4

Acute toxicity of compounds (DTM in mg/kg) administered *per os* in dimethyl sulfoxide to white mice

Compound	After 24 h	After 48 h
V	500	500
IX	1000	500
XVI	250	60
Isoniazid	125	125

Experimental

Physical constants, analytical data, and yields of the synthesized compounds are presented in Table 1. Melting points were determined on a Kofler block.

The activities against dermatophytes [4], yeast-like microorganism *Candida albicans* [5], and bacteria [5] were determined after the methods described in the cited literature. The results are given in Table 2.

Antimycobacterial activity against tuberculosis mycobacteria was followed in a liquid Šula medium by the dilution test [6] using dimethyl sulfoxide as solvent; incubation at 37°C for 14 days. The resulting concentrations in the medium were 1, 5, 10, 25, 50, and 100 μg/ml. *Mycobacterium (M) tuberculosis* $H_{37}R_v$ (sensitive to antituberculotics), *M. avium*, and *M. bovis* from the collection of the Research Institute of Preventive Medicine, Centre of Epidemiology and Microbiology, Bratislava, *M. kansasii PKG* (photochromogenic atypical mycobacterium) from the collection of Dr. E. H. Runyon (Salt Lake City, Utah, USA), and *M. fortuitum* from the collection of Professor Hauduroy (Lausanne) were used for tests. The activities of compounds were compared to those of 2-mercaptobenzothiazole [7], Isoniazid (Jenapharm, GDR), and Ethionamide (Trécator, Teraplix, Paris) (Table 3).

Dosis tolerata maxima (DTM) was investigated after *Wagner* [3] by administration of compounds to white mice with oesophageal sound. The 100% survival by the appropriate group of test animals was followed after 24 and 48 h.

2-Alkylthio-6-aminobenzothiazoles (I—XVIII)

In a three-necked flask provided with a stirrer and a reflux, 6-amino-2-mercaptobenzothiazole (9.1 g; 0.050 mol) was dissolved in the mixture of dimethylformamide [8] (25 ml) and dimethyl sulfoxide (15 ml), respectively, and the solution of potassium hydroxide (6.3 g; 0.112 mol) in water (10 ml). The temperature of the reaction mixture rised to 30—35°C and then the appropriate alkyl halide (0.055 mol) was added. The temperature increased to 40—45°C and after 30 min stirring without heating, the reaction mixture was poured onto ice (800 g). The formed 2-alkylthio-6-aminobenzothiazole was sucked and dried between filtration paper at laboratory temperature.

The compounds were purified by crystallization using charcoal first from the mixture of ethanol—water and then from acetone—water. The less soluble derivatives were crystallized twice from the mixture of acetone—ethanol.

The hexadecyl derivative *XV* was prepared by heating for 150 min on a water bath. The low melting compounds (mainly the crude products) were dried between filtration paper at low temperatures (5—10°C).

6-Amino-2-(carboxymethylthio)benzothiazole (XIX)

To the solution of 6-amino-2-mercaptobenzothiazole (9.1 g; 0.050 mol), prepared by the above described procedure, aqueous solution (30 ml) of chloroacetic acid (5.3 g; 0.057 mol) and of potassium hydroxide (3.1 g; 0.055 mol) was added. After 1 h stirring diluted (1:1) hydrochloric acid (20 ml) was added to the reaction mixture to precipitate the free acid in the form of yellowish powder.

The compound was purified by dissolving in 10% sodium hydroxide solution, decolouration with charcoal, and precipitation with diluted (1 : 1) hydrochloric acid.

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