Benzothiazole compounds. VIII. Synthesis and microbiological activity of *N*-substituted 6-amino-2-methylthiobenzothiazoles

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6-Alkylamino-, 6-dialkylamino-, 6-arylamino-, 6-diarylamino-, 6-(*N*-alkyl-*N*-nitrosoamino)-, and 6-(*N*-aryl-*N*-nitrosoamino)-2-methylthiobenzothiazoles, not described up to now, were synthesized and their antibacterial activities were studied. Their structures were proved by evaluation of the i.r. and p.m.r. spectra. The highest antibacterial activity was found with 6-methylamino-2-methylthiobenzothiazole (*I*), 6-(*p*-nitrophenylamino)-2-methylthiobenzothiazole (*XIV*), 6-(di-*p*-nitrophenylamino)-2-methylthiobenzothiazole (*XV*), and 6-(2,4-dinitrophenylamino)-2-methylthiobenzothiazole (*XVII*). The minimum inhibition concentration for *Bacillus subtilis* and *Escherichia coli* was 50 μg/ml.

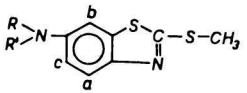
Были синтезированы до сих пор не описанные *N*-алкил-, *N*-диалкил-, *N*-арил-, *N,N*-диарил-, *N*-алкил-*N*-нитрозо-, *N*-арил-*N*-нитрозопроизводные 6-амино-2-метилтиобензтиазола и исследованы их антибактериальные свойства. Структура была подтверждена на основании ИК и ПМР спектров. Самое сильное антибактериальное действие проявляют 6-(метиламино)-2-метилтиобензтиазол (*I*), 6-(*п*-нитрофениламино)-2-метилтиобензтиазол (*XIV*), 6-ди(*п*-нитрофениламино)-2-метилтиобензтиазол (*XV*) и 6-(*о*, *п*-динитрофениламино)-2-метилтиобензтиазол (*XVII*) при минимальной ингибирующей концентрации для *Bacillus subtilis* и *Escherichia coli* 50 μг/мл.

It is not possible to form an unambiguous opinion on the contribution of an amino group in the position 6 of the benzothiazole derivatives to antibacterial activity on the basis of our studies [1—4]. Allyl (6-amino-2-benzothiazolylthio)acetates, allyl (6-bromo-2-benzothiazolylthio)acetates, and allyl (6-nitro-2-benzothiazolylthio)acetates [1] had approximately the same activity though the individual substituents influenced the aromatic system differently. 6-Amino-2-methylthiobenzothiazole was chosen in this work to study the effect of amino group whose electronic properties were altered by substitutions.

The existence of thiol and thione forms of 2-mercaptobenzothiazole [7, 8] makes the formation of *S* and *N* derivatives possible. In alkali medium the equilibrium is shifted to the side of the thiol form. Therefore, potassium salt of 6-amino-2-mercaptobenzothiazole was prepared in the first step of our syntheses.

The p.m.r. spectra of some 6-alkylamino-2-methylthiobenzothiazoles were taken to ascertain *S*—*N* tautomerism and the effect of the alkyl on this tautomerism. It was proved unambiguously that *S* derivatives were involved since the p.m.r. spectra of 6-amino-2-methylthiobenzothiazole and 6-alkyl-amino-2-methylthiobenzothiazole (alkyl = C_2H_5, $CH_2CH=CH_2$, $CH_2C_6H_5$) showed only one signal belonging to the *S*-methyl group [9]. The chemical shift of the CH_3 group was with all derivatives very similar (from 2.5 to 2.46 δ). For 6-amino-2-methylthiobenzothiazole the signal of the NH_2 group was at 3.75 δ.

The aromatic part of the p.m.r. spectra was similar with all derivatives.



The resonance signal of the proton *a* ($\delta = 7.63$, d, $J_{ac} \sim 8$ Hz) was observed at the lowest field, the signal of the proton *b* ($\delta = 6.98$, d, $J_{bc} \sim 2$ Hz) was in the middle, and the signal of the proton *c* ($\delta = 6.74$, dd) was observed at the highest field. With the individual derivatives also the signals of the appropriate *N*-alkyl group were observed.

Alkylation of the NH_2 group by alkyl halogenides proceeded to either the first or the second degree according to the molar ratio of the reactants. Treatment of one mole of 6-amino-2-methylthiobenzothiazole with one mole of alkyl halogenide resulted in 6-alkylamino-2-methylthiobenzothiazole. When two moles of the alkyl halogenide were employed, a mixture of mono- and dialkylamino derivatives was obtained. For a quantitative alkylation to the second degree, three moles of the reagent had to be used for one mole of the amine. To prevent salt formation of the starting amine [10], an equivalent amount of triethylamine was added to the reaction mixture of 6-amino-2-methylthiobenzothiazole and alkyl halogenide.

6-(*N*-Alkyl-*N*-nitrosoamino)-2-methylthiobenzothiazole and 6-(*N*-aryl-*N*-nitrosoamino)-2-methylthiobenzothiazole were prepared by nitrosation of 6-alkylamino- and 6-arylamino-2-methylthiobenzothiazoles. Their structures were proved by evaluation of their i.r. spectra, mainly by the missing bands belonging to stretching vibrations ν(N—H) which were observed in the spectra of initial 6-alkylamino-2-methylthiobenzothiazoles. Strong bands belonging to the stretching vibrations of the NO group appeared in the region 1440—1450 cm^{-1} The effect of a steric factor on the course of nitrosation could be observed in the reaction of 6-(2,4-dinitrophenylamino)-2-methylthiobenzothiazole. The nitro group in *o*-position to nitrogen of the secondary amino group hinders the substitution of proton by a nitroso group and the reaction does not proceed at all.

6-Alkylamino-2-methylthiobenzothiazoles and 6-dialkylamino-2-methylthiobenzothiazoles were viscous liquids, 6-arylamino-2-methylthiobenzothiazoles and 6-(*N*-aryl-*N*-nitrosoamino)-2-methylthiobenzothiazoles were yellow to red solids insoluble in water, soluble in alcohol, ether, chloroform, acetone, and other organic solvents (Table 1).

The effect of substituents on electronic spectra of the benzothiazole system was followed. Study of the u.v. spectra showed that the electron shell of the system was strongly influenced by introduction of the amino group into the position 6. Substitution of hydrogens on the amino group by alkyls caused only a small shift of bands (the shift between C_2H_5 and C_3H_7 was approximately 5 nm and that between CH_3 and C_2H_5 even smaller). The greatest effect had the CH_3 group.

With the compounds *I—IV, VI—IX, XI, XII, XIV, XV,* and *XVII* (Table 2) antibacterial

Table I

Characterization of the synthesized substances

No.	R	R'	Formula	*M*	Calculated/found				Yield %	M.p., °C
					% C	% H	% N	% S		
I	CH_3	H	$C_9H_{10}N_2S_2$	210.3	51.47	4.80	13.31	30.49	70	Highly
					51.59	4.52	13.54	30.20		viscous liquid
II	CH_3	CH_3	$C_{10}H_{12}N_2S_2$	224.3	53.62	5.40	12.48	28.53	73	Highly
					53.49	4.47	12.49	28.36		viscous liquid
III	C_2H_5	H	$C_{10}H_{12}N_2S_2$	224.3	53.62	5.40	12.48	28.53	65	Highly
					53.70	5.32	12.67	28.64		viscous liquid
IV	C_2H_5	C_2H_5	$C_{12}H_{16}N_2S_2$	252.5	57.19	6.39	11.09	25.39	74	1.6873
					57.02	6.48	11.22	25.46		
V	C_2H_5	NO	$C_{10}H_{11}ON_3S_2$	253.3	47.47	4.38	16.98	25.31	54	94—95
					47.30	4.26	17.14	25.40		
VI	C_3H_7	H	$C_{11}H_{14}N_2S_2$	238.4	55.50	5.92	11.75	26.90	73	1.6943
					55.42	5.75	11.92	26.70		
VII	C_3H_7	C_3H_7	$C_{14}H_{20}N_2S_2$	280.4	60.04	8.57	10.05	22.88	66	1.6856
					60.21	8.42	10.19	22.91		
VIII	$CH_2CH=CH_2$	H	$C_{11}H_{12}N_2S_2$	236.4	55.97	5.12	11.84	27.13	65	1.7
					55.83	5.24	12.01	27.31		
IX	$CH_2CH=CH_2$	$CH_2CH=CH_2$	$C_{14}H_{16}N_2S_2$	276.4	60.92	5.84	10.14	23.20	75	1.6818
					60.80	5.70	10.27	23.26		
X	$CH_2CH=CH_2$	NO	$C_{11}H_{11}ON_3S_2$	265.3	49.85	4.14	15.83	24.17	57	50—52
					49.72	4.23	15.70	24.30		

Table 1 (Continued)

No.	R	R′	Formula	*M*	Calculated/found % C	% H	% N	% S	Yield %	M.p., °C n_D^{20}
XI	$CH_2C_6H_5$	H	$C_{15}H_{14}N_2S_2$	286.4	62.98 62.76	7.39 7.47	9.77 9.82	22.35 22.19	73	82—85
XII	$CH_2C_6H_5$	$CH_2C_6H_5$	$C_{22}H_{20}N_2S_2$	376.5	70.27 70.38	5.36 5.19	7.45 7.59	17.08 17.14	41	1.6948
XIII	$CH_2C_6H_5$	NO	$C_{15}H_{13}ON_3S_2$	315.4	57.19 57.08	4.95 4.79	13.31 13.47	20.65 20.55	45	98
XIV	$C_6H_4NO_2$-*p*	H	$C_{14}H_{11}O_2N_3S_2$	317.4	53.04 53.16	3.49 3.60	13.24 13.21	20.20 20.36	71	79—81
XV	$C_6H_4NO_2$-*p*	$C_6H_4NO_2$-*p*	$C_{20}H_{14}O_4N_4S_2$	438.5	54.83 54.70	3.22 3.15	12.77 12.52	14.63 14.68	76	69—71
XVI	$C_6H_4NO_2$-*p*	NO	$C_{14}H_{10}O_3N_4S_2$	346.4	48.59 48.47	2.91 3.06	17.88 17.78	16.38 16.22	49	79—80
XVII	$C_6H_3(NO_2)_2$-*o,p*	H	$C_{14}H_{10}O_4N_4S_2$	362.4	46.44 46.52	2.78 2.90	15.45 15.62	17.69 17.49	25	205

Table 2

Biological activity of *N*-substituted 6-amino-2-methylthiobenzothiazoles (μg/ml)

No.	MIC		BCG		Lethal concentration		MIC
	Bacillus subtilis	*Escherichia coli*	Bactericidal conc.	Bacteriostatical conc.	*Trypanosoma cruzi*	*Euglena gracilis*	*Candida pseudo-tropicalis*
I	50	50	100	50	>100	>125	>100
II	50	100	100	50	>100	125	>100
III	50	100	100	50	>100	250	>100
IV	50	100	100	50	>100	125	>100
V	100	100	—	—	—	—	—
VI	50	100	100	100	>100	125	>100
VII	100	100	100	>50	>100	250	>100
VIII	100	100	>100	100	>100	500	>100
IX	100	100	>100	100	>100	>500	>100
X	100	100	—	—	—	—	—
XI	50	100	100	50	>100	125	>100
XII	100	100	100	>50	>100	>500	>100
XIII	100	100	—	—	—	—	—
XIV	50	50	100	50	>100	125	>100
XV	50	50	100	50	100	125	>100
XVI	50	100	—	—	—	—	—
XVII	50	50	100	50	>100	125	>100

and antiprotozoal activities were tested *in vitro*. As test-organisms both gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria were used. The activity was followed also on a mycobacterial strain (*Mycobacterium bovis* BCG). From the group of protozoa intracellular parasite *Trypanosoma cruzi* was used. Moreover, the compounds were tested for lethal effect on *Euglena gracilis* and in low concentrations also for a bleaching effect and for the effect on a yeast organism *Candida pseudotropicalis*. The antibacterial effect was higher than the antiprotozoal effect with all compounds tested. An important finding is the fact that both on G^+ and G^- bacteria the inhibition effects were observed. Compounds *I, IX, XV,* and *XVII* were found to be most effective; the minimum inhibition concentration (MIC) against *Bacillus subtilis* and *Escherichia coli* was 50 μg/ml. The antimycobacterial activity of individual substances was fairly similar. All compounds, except *VIII* and *IX*, acted as bactericides already at the concentration 100 μg/ml. The compounds *VIII* and *IX* acted bacteriostatically at this concentration. Otherwise, 50 μg/ml caused bacteriostasis of a different degree. The intracellular parasite *Trypanosoma cruzi* was proved to be insensitive. Lethal effect was achieved at concentrations higher than 100 μg/ml. The values of lethal concentrations on *Euglena gracilis* varied in the range 500—125 μg/ml. The compounds *XI, IX,* and *XII* were proved to be least toxic; the lethal effect was achieved at higher concentrations than 500 μg/ml. With the compounds *V, X, XIII,* and *XVI,* only antibacterial activity was followed. The values of minimum inhibition concentrations were approximately the same as with the group of compounds mentioned above.

The antibacterial tests showed that there was no evident relationship between the effect of the substituent in the position 6 and the activity of the compound.

Experimental

Melting points (Kofler), indices of refraction, and analytical data of the synthesized compounds are presented in Table 1. Infrared spectra were measured on a UR-20 (Zeiss, Jena) spectrophotometer in the region 3800—700 cm^{-1} Polystyrene foil was used for calibration. The liquid samples were measured in a chloroform solution and the solids in Nujol. The p.m.r. spectra were taken on a Tesla 487 apparatus at 80 MHz; 6% solutions ($CDCl_3$) with an isotopic enrichment 99.5% were measured. Tetramethylene was used as an internal standard. The chemical shift was read with an accuracy of ±0.02 p.p.m. and the interaction constants J with an accuracy of ±1.6 Hz. The u.v. spectra were measured on a Perkin—Elmer spectrophotometer in the region 400—200 nm using methanol as standard. The concentration of samples in methanol was 5×10^{-5} M. Microbiological activities were determined according to [5, 6] and are given in Table 2.

6-Methylamino-2-methylthiobenzothiazole (I)

6-Amino-2-mercaptobenzothiazole (18.2 g; 0.1 mole) and potassium hydroxide (5.6 g; 0.1 mole) were dissolved in ethanol (300 ml) under stirring at 50—60°C. After cooling to 20—25°C methyl iodide (14.2 g; 0.1 mole) was added dropwise and the temperature was risen to 50—60°C for 1 hr. Then triethylamine (20.2 g; 0.2 mole) and methyl iodide (14.2 g; 0.1 mole) were added dropwise. After stirring for 2 hrs at room temperature and 1 hr at 40°C, the reaction mixture was poured into glacial water (600—700 ml) and extracted with ether. After drying with sodium sulfate and distilling of ether under reduced pressure, highly viscous 6-methylamino-2-methylthiobenzothiazole was isolated. It was purified through a column of silica gel using benzene as eluent.

The compounds *III, VI, VIII, XI, XIV,* and *XVII* were prepared in the same way. The compounds *XI, XIV,* and *XVII* were crystallized from chloroform.

6-Dimethylamino-2-methylthiobenzothiazole (II)

The preparation was the same as in the case of the compound *I* except that the amount of triethylamine was 30.3 g (0.3 mole) and that of methyl iodide 42.6 g (0.3 mole).

The compounds *IV*, *VII*, *IX*, *XII*, and *XV* were prepared by the same method. The derivative *XV* was crystallized from ethanol.

6-(N-Ethyl-N-nitrosoamino)-2-methylthiobenzothiazole (V)

6-Ethylamino-2-methylthiobenzothiazole (2.2 g; 0.01 mole) was dissolved in ethanol (50 ml) and the solution was cooled to −10°C. Concentrated hydrochloric acid (10 ml) was added slowly under stirring and then the solution of sodium nitrite (2.1 g; 0.03 mole) in water (12 ml) was added dropwise. The mixture was allowed to stand for 1 hr and diluted with water. An orange to red colloidal solution was formed from which 6-(*N*-ethyl-*N*-nitrosoamino)-2-methylthiobenzothiazole precipitated. This was crystallized from ethanol.

The compounds *X*, *XIII*, and *XVI* were prepared by the same method.

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