Benzothiazole compounds
XXII. Synthesis of 3- and 2,3-substituted benzothiazolium salts, investigation of their antimicrobial and growth-regulating activity

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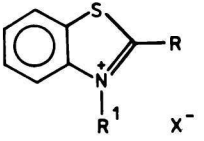
3-Substituted and 2,3-disubstituted benzothiazolium salts were prepared by treatment of benzothiazole or 2-alkylbenzothiazoles with reactive halides or dialkyl sulfates. The antibacterial, antifungal, and antiprotozoal activity of the salts is reported. 3-Ethyl-, 3-propyl-, and 3-butylbenzothiazolium salts were found to be active against such strains of *Staphylococcus aureus* which are resistant to some antibiotics. Several compounds showed good growth--stimulating and/or inhibiting activity.

3-Замещенные и 2,3-дизамещенные соли бензотиазола были получены действием на бензотиазол или 2-алкилбензотиазолы реакционными галогенидами или диалкил сульфатами. Сообщается о антибактериальной, антигрибковой и антипротозойной активности этих солей. 3-Этил-, 3-пропил- и 3-бутилбензотиазолиевые соли оказались активными по отношению к таким штаммам *Staphylococcus aureus*, которые резистентны к некоторым антибиотикам. Некоторые соединения проявили хорошую рост-стимулирующую или ингибирующую активность.

Our previous results [1—3] of investigations into growth-regulating and antimicrobial properties of benzothiazolium salts encouraged us to synthesizing more derivatives of this type. The effects of substituents in positions 2 and 3 on biological activity were studied. 3-Substituted and 2,3-disubstituted benzothiazolium salts given in Table 1 were prepared, like previous series of compounds of this type [1—3], by treatment of benzothiazole, 2-methyl- or 2-propylbenzothiazole with

Table 1

Characterization of the prepared benzothiazolium salts



Compound	R	R^1	X	Formula	M_r	w_i(calc.)/w_i(found)				Yield	M.p.
						% C	% H	% N	% S	%	°C
I	H	C_2H_5	Br	$C_9H_{10}BrNS$	244.15	44.27	4.12	5.73	13.13	52	210—211
						44.12	4.15	5.64	13.21		
II	H	C_2H_5	$C_2H_5SO_4$	$C_{11}H_{15}NO_4S_2$	289.37	45.65	5.22	4.83	22.15	76	123—125
						45.75	5.25	4.78	22.20		
III	H	C_3H_7	I	$C_{10}H_{12}INS$	305.18	39.35	3.96	4.59	10.51	72	158—160
						39.32	3.77	4.63	10.41		
IV	H	C_4H_9	I	$C_{11}H_{14}INS$	319.20	41.39	4.42	4.38	10.04	70	112—114
						41.34	4.41	4.28	9.98		
V	H	$CH_2COOC_3H_7$	Cl	$C_{12}H_{14}ClNO_2S$	271.76	53.03	5.19	5.15	11.79	40	163—165
						53.19	5.14	5.25	11.61		
VI	H	$CH_2COOC_5H_{11}$	Br	$C_{14}H_{18}BrNO_2S$	344.27	48.84	5.26	4.06	9.31	62	154—155
						48.68	5.12	4.18	9.32		
VII	H	$CH_2COOC_7H_{15}$	Br	$C_{16}H_{22}BrNO_2S$	372.33	51.61	5.95	3.76	8.61	67	162—163
						51.65	5.88	3.69	8.56		
VIII	H	CH_2CH_2COOH	Br	$C_{10}H_{10}BrNO_2S$	288.16	41.68	3.49	4.86	11.12	52	214—217
						41.60	3.48	4.93	11.08		
IX	H	$CH_2CH_2COOCH_3$	Br	$C_{11}H_{12}BrNO_2S$	302.19	43.72	4.00	4.63	10.61	57	182—184
						43.63	3.91	4.51	10.66		

Table 1 (Continued)

Compound	R	R¹	X	Formula	M_r	w_i(calc.)/w_i(found)				Yield	M.p.
						% C	% H	% N	% S	%	°C
X	CH_3	CH_3	CH_3SO_4	$C_{10}H_{13}NO_4S_2$	275.34	43.62	4.75	5.08	23.29	74	138—141
						43.67	4.65	5.08	23.31		
XI	CH_3	C_2H_5	$C_2H_5SO_4$	$C_{12}H_{17}NO_4S_2$	303.40	47.50	5.64	4.61	21.13	72	197—201
						47.39	5.69	4.73	21.24		
XII	CH_3	C_3H_7	I	$C_{11}H_{14}INS$	319.19	41.39	4.42	4.38	10.04	57	173—174
						41.27	4.36	4.34	9.91		
XIII	CH_3	C_4H_9	I	$C_{12}H_{16}INS$	333.23	43.25	4.83	4.20	9.62	53	186—188
						43.27	4.83	4.19	9.64		
XIV	CH_3	$CH(CH_3)C_2H_5$	I	$C_{12}H_{16}INS$	333.23	43.25	4.83	4.20	9.62	51	176—179
						43.15	4.92	4.11	9.69		
XV	CH_3	$CH_2C_6H_5$	I	$C_{15}H_{14}INS$	367.25	49.05	3.84	3.81	8.73	68	209—211
						49.07	3.81	3.70	8.75		Decomposition
XVI	C_3H_7	CH_3	I	$C_{11}H_{14}INS$	319.19	41.39	4.42	4.38	10.04	61	247—249
						41.22	4.41	4.28	9.96		
XVII	C_3H_7	CH_3	CH_3SO_4	$C_{12}H_{17}NO_4S_2$	303.40	47.50	5.64	4.61	21.13	52	135—138
						47.38	5.59	4.61	21.01		
XVIII	C_3H_7	C_2H_5	I	$C_{12}H_{16}INS$	333.23	43.25	4.83	4.20	9.62	50	176—178
						43.14	4.92	4.13	9.53		
XIX	C_3H_7	$CH_2CH=CH_2$	Br	$C_{13}H_{16}BrNS$	298.25	52.35	5.40	4.69	10.75	51	167—168
						52.21	5.50	4.70	10.62		

reactive halides or dialkyl sulfates in dimethylformamide—acetone (mass ratio = 2:1) mixture with yields 40—76 %. Methyl or propyl group in position 2 have no substantial effect on quaternization reaction of nitrogen in position 3. The reaction and its yield depend more on the reactivity of the alkylating agent (Table 1).

The results of antimicrobial tests of benzothiazolium salts are summarized in Table 2. It is evident that the compounds are active mainly against gram-positive bacterial strains (*Staphylococcus aureus, Bacillus subtilis*). There is little or no activity against gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa*). Antifungal activity (*Microsporum gypseum, Trichophyton rubrum*) is significant mainly with the derivatives *I*—*IV* The remaining derivatives are less active against fungi tested. The compounds *I*—*IV* are the most active against protozoal strain *Tritrichomonas foetus* as well.

Table 2

Antimicrobial activity of the prepared benzothiazolium salts

Compound	Minimum inhibitory amount a/(μg/disc)				Fungicidal/fungistatical mass concentration ϱ/(μg cm^{-3})		Protozocidal/ protozostatical mass concentration ϱ/(μg cm^{-3})
	1	*2*	*3*	*4*	*5*	*6*	*7*
I	3.1	3.1	200	>200	50/12.5	12.5/>3.1	50/12.5
II	3.1	3.1	200	>200	50/12.5	12.5/>3.1	50/12.5
III	3.1	12.5	200	>200	50/12.5	12.5/>3.1	50/12.5
IV	3.1	12.5	200	>200	50/12.5	12.5/>3.1	50/12.5
V	12.5	12.5	200	200	200/50	200/12.5	50/12.5
VI	50	50	>200	>200	200/>50	200/>50	50/>12.5
VII	50	200	>200	>200	>200/200	200/>50	50/>12.5
VIII	>200	>200	>200	>200	>200/>200	>200/>200	800/200
IX	200	200	>200	>200	>200/>200	>200/200	200/50
X	200	200	>200	>200	>200/200	200/50	200/50
XI	12.5	12.5	>200	>200	>200/200	200/>50	200/50
XII	12.5	50	>200	>200	>200/200	200/>50	200/50
XIII	12.5	50	>200	>200	>200/200	200/>50	200/50
XIV	50	50	200	200	200/50	200/50	
XV	50	50	200	>200	200/50	200/50	50/>12.5
XVI	12.5	50	>200	>200	>200/200	200/>50	200/50
XVII	12.5	12.5	>200	>200	>200/200	200/>50	200/50
XVIII	12.5	50	>200	>200	>200/200	200/>50	200/50
XIX	12.5	50	>200	>200	>200/200	200/>50	200/50

1. Staphylococcus aureus ATCC 6538; 2. Bacillus subtilis ATCC 6051; 3. Escherichia coli ATCC 9637; 4. Pseudomonas aeruginosa ATCC 10145; 5. Microsporum gypseum; 6. Trichophyton rubrum; 7. Tritrichomonas foetus.

The derivatives *I*—*IV* were tested for additional five strains of *Staphylococcus aureus*, differently sensitive to antibiotics (Table 3). Standard antibiotical tests were carried out to check the sensitivity of the strains to antibiotics and the results are included in Table 3 for comparison. The compounds I—IV were found to be active also against such strains of *Staphylococcus aureus* which are rather resistant to antibiotics. Hence, after additional investigation, there is possibility of using these derivatives for making antistaphylococcal preparations with great advantage of their solubility in water.

Table 3

Antibacterial activity of the compounds *I*—*IV* and of some antibiotics against strains *Staphylococcus aureus*

Strain	Minimum inhibitory amount *a*/(μg/disc)				Antibiotic					
	I	*II*	*III*	*IV*	Pn.	Er.	Tc.	Km.	Cph.	Ap.
Man 29/58	3.1	3.1	3.1	3.1	+	+	+	+	+	+
Man 78/71	3.1	3.1	3.1	3.1	±	+	+	+	+	+
CCM 2394	3.1	3.1	3.1	3.1	−	+	+	+	+	−
CCM 2560	3.1	3.1	3.1	3.1	−	+	−	+	+	+
SPA	6.2	6.2	6.2	3.1	−	±	−	−	+	−

Pn. — Penicillin 10 i.u.; Er — Erythromycin 10 μg/disc; Tc. — Tetracycline 30 μg/disc; Km. — Komanycin 30 μg/disc; Cph. — Chloramphenicol 30 μg/disc; Ap. — Amphicillin 20 μg/disc. − = resistance, + = sensitivity, ± = decreased sensitivity.

The results of the antimicrobial tests described in this paper as well as in the previous ones [1—3] allow to conclude that both methyl and propyl group in position 2 cause slightly decreased activity. Longer alkyl chains in ester groups (compounds *VI*, *VII*) are not effective in promoting the activity, either.

Growth-stimulating and inhibiting activity was assessed also in this series of benzothiazole derivatives. The compounds *II*, *X*, and *XIV* were found to show better stimulating activity than indole-3-acetic acid or 2,4-dichlorophenoxyacetic acid, which were used as standards (Table 4). Noticeable inhibiting activity at the concentration 10^{-3} mol dm^{-3} was found for the compounds *I*, *III*, *IV*, and *XIV*, but they cannot be regarded as typical inhibitors.

Some of the prepared compounds could be practically used because of their activity, solubility in water, and simple synthetic availability.

Table 4

Growth-regulating activity of benzothiazolium salts on *Vicia sativa*

Compound	Stimulation		Inhibition	
	$+\Delta l$/mm	c/(mol dm^{-3})	$-\Delta l$/mm	c/(mol dm^{-3})
I	5.4	10^{-7}	13.35	10^{-3}
II	4.5	10^{-11}	11.1	10^{-3}
III	4.0	10^{-7}	14.4	10^{-3}
IV	2.75	10^{-7}	14.35	10^{-3}
V	4.85	10^{-7}		
VI	1.10	10^{-9}		
VII	1.95	10^{-7}		
VIII	4.25	10^{-9}		
IX	1.9	10^{-11}	6.3	10^{-3}
X	3.15	10^{-13}	2.65	10^{-3}
XI	2.85	10^{-9}	2.25	10^{-3}
XII	2.35	10^{-13}	9.15	10^{-3}
XIII	1.80	10^{-9}	3.85	10^{-3}
XIV	3.45	10^{-13}	24.45	10^{-3}
XV	3.9	10^{-9}		
XVI	2.05	10^{-7}	5.3	10^{-3}
XVII	0.45	10^{-5}	6.65	10^{-3}
XVIII	1.80	10^{-9}	5.45	10^{-3}
XIX	3.15	10^{-9}	7.9	10^{-3}
IAA	3.10	10^{-12}	18.55	10^{-6}
2,4-D	4.95	10^{-9}	23.30	10^{-5}
CCC			3.85	10^{-3}

IAA — Indole-3-acetic acid; 2,4-D — 2,4-dichlorophenoxyacetic acid; CCC — 2-chloroethyltrimethylammonium chloride.

Experimental

The general procedure for preparing benzothiazolium salts has been described in the paper [1]. Melting points, yields, and analytical data are contained in Table 1. Antibacterial activity was measured against bacterial strains *Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6051, Escherichia coli ATCC 9637,* and *Pseudomonas aeruginosa ATCC 10145* by the plate diffusional method using Mueller—Hint agar with adjustments necessary for a particular strain [4]. The compounds were tested at various amounts (a/(μg/disc): 200, 50, 3.1, and 0.78). The results are expressed as amounts, which cause a measurable zone of inhibited bacterial growth, *i.e.* minimum inhibitory amounts. The antibacterial test was

extended for *Staphylococcus aureus* strains *Man 29/58, Man 78/71, CCM 2394, CCM 2560,* and *SPA*. Standard commercial antibiotical discs (Lachema, Brno) were used to check the sensitivity of the strains. Antifungal activity against *Microsporum gypseum* and *Trichophyton rubrum,* and antiprotozoal activity against *Tritrichomonas foetus* were determined by the test-tube dilution methods [5]. Growth-regulating activity was assessed using seedlings of vetch (*Vicia sativa*) and the method has been described in the previous papers [1, 6].

References

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