Benzothiazole compounds. V. Synthesis and antimicrobial effects of some esters of (2-benzothiazolylthio)acetic acid and 6-substituted (2-benzothiazolylthio)formic acid

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Some esters of (2-benzothiazolylthio)acetic and (6-X-2-benzothiazolylthio)formic acids (X = H, NO_2, NH_2, $NH_3^+Cl^-$, Cl, Br, I) have been synthesized and their antimicrobial effects investigated. Depending upon the concentration applied the compounds show bactericidal or bacteriostatic activity.

Continuing our previous studies of the antimicrobial activity of some esters of 6-substituted (2-benzothiazolylthio)acetic acid [1—3] we have synthesized esters of hitherto unknown (2-benzothiazolylthio)formic and (2-benzothiazolylthio)acetic acid. Some esters of (2-benzothiazolylthio)acetic acid (*e.g.* methyl, ethyl, propyl, and isopropyl) were studied in connection with the oxidation of the sulfur linked to the carbon at the position 2 [4—6]. It has been shown that ethyl (2-benzothiazolylthio)acetate is a more active fungicide [7] against *Pythium aphanidermatum* than the hitherto used zinc ethylene—bis-dithiocarbamate.

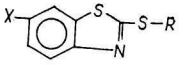
We have previously shown that the efficiency of the esters of 6-substituted (2-benzothiazolylthio)acetic [1, 2], propionic, and butyric [3] acids depends primarily upon the nature of the alkyl in the ester group and only secondarily upon the substituents in the position 6. For better understanding of the effect of the alkoxy carbonyl group upon the antibacterial activity, the esters of (6-X-2-benzothiazolylthio)formic acid, not containing the methylene bridge, were compared with those of (2-benzothiazolylthio)-acetic acid.

Since methyl, ethyl, allyl, and propargyl (6-X-2-benzothiazolylthio)acetates [1, 3] were found to be most active antibacterial agents, analogs of (6-X-2-benzothiazolylthio)-formic acid (Table 1) have been purposefully synthesized.

The synthesis of *IX*, *XI*, *XV*, *XVI*, *XXI*, *XXII*, *XXIV*, and *XXV* was accomplished

Table 1

Synthesized alkyl (2-benzothiazolylthio)acetates and alkyl (6-X-2-benzothiazolylthio)formates



No.	R	X	Formula	*M*	Calculated/found					Yield [%]	M.p. [°C] Solvent B.p. [°C/torr] $[n_D^{20}]$
					% C	% H	% N	% S	% Hal.		
I	CH_2COOCH_3	H	$C_{10}H_9O_2NS_2$	239.3	50.25	3.79	5.85	26.82		90	74—75
					50.41	3.65	5.76	26.68			Ether—Petroleum ether 1 : 1
II	$CH_2COOC_2H_5$	H	$C_{11}H_{11}O_2NS_2$	253.3	52.21	4.38	5.53	25.34		93	43
					52.36	4.20	5.33	25.45			Ether—Petroleum ether 1 : 1
III	$CH_2COOCH_2CH=CH_2$	H	$C_{12}H_{11}O_2NS_2$	265.3	54.38	4.18	5.28	24.20		85	49—50
					54.19	4.29	5.41	24.32			Ether—Petroleum ether 1 : 1
IV	$CH_2COOCH_2C\equiv CH$	H	$C_{12}H_9O_2NS_2$	263.3	54.79	3.44	5.32	24.38		84	62
					54.52	3.58	5.27	24.39			Ethanol
V	$CH_2COOC_4H_9$	H	$C_{13}H_{15}O_2NS_2$	281.4	55.56	5.38	4.98	22.82		85	(188/5)
					55.80	5.50	4.81	22.84			
VI	*i*-$CH_2COOC_4H_9$	H	$C_{13}H_{15}O_2NS_2$	281.4	55.56	5.38	4.98	22.82		78	(197/5)
					55.59	5.27	5.17	23.00			
VII	*sec*-$CH_2COOC_4H_9$	H	$C_{13}H_{15}O_2NS_2$	281.4	55.56	5.38	4.98	22.82		76	(193/3)
					55.36	5.47	4.78	22.59			
VIII	*sec*-$CH_2COOC_8H_{17}$	H	$C_{17}H_{23}O_2NS_2$	337.5	60.58	6.87	4.15	19.02		78	(200/5)
					60.39	6.72	4.32	19.24			
IX	CH_2COOCH_2-Fu*	H	$C_{14}H_{11}O_3NS_2$	305.3	55.12	3.63	4.59	21.02		80	[1.6108]
					55.26	3.49	4.71	20.84			
X	$COOCH_3$	H	$C_9H_7O_2NS_2$	225.3	48.03	3.13	6.22	20.49		59	60—61
					48.01	3.35	6.19	20.50			Ethanol
XI	$COOCH_3$	NO_2	$C_9H_6O_4N_2S_2$	270.3	40.00	2.24	10.37	23.74		86	132—133
					40.20	2.38	10.56	24.02			Acetone—Water 5 : 1
XII	$COOCH_3$	NH_2	$C_9H_8O_2N_2S_2$	240.3	40.98	3.35	11.56	26.70		80	240—242
					45.15	3.52	11.56	26.60			Ethanol
XIII	$COOCH_3$	Cl	$C_9H_6O_2NS_2Cl$	259.7	41.57	2.32	5.38	24.66	Cl	78	119—120
					41.80	2.49	5.56	24.34	13.66		Acetone—Water 5 : 1
									13.47		

Table 1 (Continued)

No.	R	X	Formula	*M*	Calculated/found					Yield [%]	M.p. [°C] Solvent B.p. [°C/torr] [n_D^{20}]
					% C	% H	% N	% S	% Hal.		
XIV	$COOCH_3$	I	$C_9H_6O_2NS_2I$	351.2	30.79 30.65	1.72 1.98	3.98 3.82	18.26 18.45	I 36.14 36.30	75	112–114 Acetone–Water 5 : 1
XV	$COOC_2H_5$	H	$C_{10}H_9O_2NS_2$	239.3	50.25 50.51	3.79 3.90	5.85 5.92	26.82 26.78		60	64–66 Ethanol
XVI	$COOC_2H_5$	NO_2	$C_{10}H_8O_4N_2S_2$	284.3	42.28 42.51	2.83 2.73	9.85 9.69	22.55 22.38		80	137–138 Acetone–Water 5 : 1
XVII	$COOC_2H_5$	NH_2	$C_{10}H_{10}O_2N_2S_2$	254.3	47.22 47.28	3.96 3.99	11.02 11.04	25.21 25.15		72	214–216 Ethanol
XVIII	$COOC_2H_5$	$NH_3^+Cl^-$	$C_{10}H_{11}O_2N_2S_2Cl$	290.8	41.27 41.45	3.81 4.10	9.62 9.78	22.03 21.87		73	276–278 Ethanol–Chloroform 1 : 1
XIX	$COOC_2H_5$	Cl	$C_{10}H_8O_2NS_2Cl$	273.7	43.83 43.78	2.94 3.06	5.10 5.11	23.42 23.27	Cl 12.95 13.00	64	120–122 Acetone–Water 5 : 1
XX	$COOC_2H_5$	Br	$C_{10}H_8O_2NS_2Br$	318.2	37.74 37.42	2.53 2.81	4.40 4.90	20.15 20.03	Br 25.11 25.65	71	118–120 Acetone–Wator 5 : 1
XXI	$COOCH_2CH=CH_2$	H	$C_{11}H_9O_2NS_2$	251.3	52.63 52.74	3.61 3.45	3.57 3.61	25.54 25.41		85	[1.6798]
XXII	$COOCH_2CH=CH_2$	NO_2	$C_{11}H_8O_4N_2S_2$	269.3	44.63 44.72	2.72 2.60	9.45 9.60	21.65 21.68		75	118–119 Ethanol
XXIII	$COOCH_2CH=CH_2$	NH_2	$C_{11}H_{10}O_2N_2S_2$	266.3	49.66 49.70	3.78 3.48	10.52 10.61	24.10 24.31		76	184 Ethanol–Water 2 : 1
XXIV	$COOCH_2C\equiv CH$	H	$C_{11}H_7O_2NS_2$	249.3	53.05 52.89	2.83 2.92	5.62 5.60	25.75 25.61		74	98 Ethanol
XXV	$COOCH_2C\equiv CH$	NO_2	$C_{11}H_6O_4N_2S_2$	294.3	44.93 44.80	2.05 1.92	9.52 9.47	21.80 21.62		80	146–147 Ethanol–Tetrahydrofuran 3 : 1
XXVI	$COOCH_2C=CH$	NH_2	$C_{11}H_8O_2N_2S_2$	264.3	50.04 50.16	3.04 3.10	10.60 10.48	24.28 24.09		81	194–196 Ethanol–Tetrahydrofuran 3 : 1

*Fu = 2-furyl.

Table 2

Effective concentrations (μg/ml) of *I*—*XXVI*

No.	MIC		BCG		MIC	*Euglena grac.*		*Tryp. cruzi*	
	B. subtilis	*E. coli*	-static	-cidal	*Candida*	lethal	-static	lethal	-static
I	>100	>100	50	100	100	500	250	>100	100
II	50	50	50	100	100	500	250	>100	100
III	10	50	10	50	100	100	>50	100	50
IV	50	50	50	100	>100	500	250	>100	100
V	>100	>100	50	100	>100	500	250	>100	100
VI	100	100	50	100	>100	250	125	>100	100
VII	>100	>100	100	>100	>100	500	250	>100	>100
VIII	100	>100	100	>100	>100	>500	500	>100	100
IX	100	100	100	>100	>100	500	250	>100	100
X	10	50	10	100	100	250	125	100	50
XI	100	100	50	100	100	500	250	100	100
XII	10	50	50	100	100	500	250	100	100
XIII	100	100	50	100	100	500	250	100	100
XIV	100	100	100	100	100	500	250	100	100
XV	50	100	50	100	100	500	250	100	100
XVI	100	100	100	100	100	500	250	100	100
XVII	100	100	50	100	100	500	250	100	100
XVIII	100	100	100	100	100	500	125	100	100
XIX	100	100	100	100	100	500	250	100	100
XX	100	100	100	100	100	250	125	100	50
XXI	50	100	50	100	100	250	125	100	50
XXII	50	50	50	100	100	500	250	100	100
XXIII	50	50	50	100	100	500	250	100	100
XXIV	100	100	50	100	100	500	250	100	50
XXV	100	100	100	100	100	500	250	100	100
XXVI	100	100	100	100	100	500	250	100	100

using triethylamine in acetone. Other compounds were obtained using, as the base, potassium hydroxide in alcohol [1—3]. 2-Mercapto-6-bromo- and 2-mercapto-6-iodobenzothiazoles were prepared from 2-mercapto-6-aminobenzothiazole. The used alkyl chloroformates were prepared from the corresponding alcohols and phosgene [8]. The produced esters were isolated from diluted acetone either as solids (Table 1; compounds *I*, *IV*, *X—XX*, *XXII—XXVI*), or liquids by extraction with ether. Compounds not bearing substituents in the position 6 (*V—VIII*) could be distilled under reduced pressure without decomposition. Compounds *IX* and *XXI* decomposed during attempted distillation and therefore they were purified by chromatography on alumina using benzene as the mobile phase.

Antibacterial, fungicidal, and antiprotozoal effects of the synthesized compounds were tested *in vitro*. Gram-positive (*Bacillus subtilis*), gram-negative (*Escherichia coli*) and *Mycobacterium bovis* BCG bacteria were used as substrates. As for the last mentioned bacteria, our attention was focused on the concentration at which the agents showed bactericidal or bacteriostatic activity. Of the group of protozoa the intracellular parasite of the type *Trypanosoma cruzi* was used. The effects upon yeasts of the group of *Candida pseudotropicalis* and *Euglena gracilis* were also investigated. The tested compounds were used as solutions in ethanol and dimethyl sulfoxide. The results of the evaluation of the efficiency made by comparing the minimum inhibitory concentration (MIC) are summarized in Table 2.

As far as their microbial activity is concerned compounds *III* and *X* were found to be most efficient of the esters tested. While *Bacillus subtilis* and *Escherichia coli* were affected by them at the concentration of 50—10 μg/ml a noticeable effect of these substances upon *Candida pseudotropicalis* was observed at 100 μg/ml.

On the other hand compounds *III*, *X*, *XX*, *XXI*, and *XXIV* were most effective against crithidium forms of *Trypanosoma cruzi*. Antimicrobial effects were observed also in the case of the esters *II*, *IV*, *XII*, *XV*, *XXI*, *XXII*, and *XXIII*.

At lower concentrations the compounds under investigation show bacteriostatic effectiveness, which at higher concentrations becomes bactericidal. The most toxic to *Euglena gracilis* was found to be allyl (2-benzothiazolylthio)acetate *III*.

Compared to the esters of 6-substituted (2-benzothiazolylthio)acetic acid [1, 3] the analogs *XI*, *XIII*, *XIV*, *XVI*, *XVII*, *XXII*, *XXIII*, *XXV*, *XXVI* derived from formic acid are less efficient antibacterial agents. Inhibitory effects could be achieved with these substances only by using relatively high concentration (100 μg/ml).

Experimental

Analytical figures and physicochemical constants of the synthesized compounds are summarized in Table 1. Alkyl chloroformates were made as described [8]. The results of the antimicrobial tests, carried out according to [9, 10], are in Table 2.

The preparation of alkyl (2-benzothiazolylthio)acetates (*I—IX*) and alkyl (6-X-2-benzothiazolylthio)formates (X = NH_2, Cl, Br, and I) (*XII—XIV*, *XVII*, *XIX*, *XX*, *XXIII*, *XXVI*) was described previously [1—3].

Alkyl (6-X-2-benzothiazolylthio)formates (X, XI, XV, XVI, XXI, XXII, XXIV, XXV) (X = H, NO_2)

To a solution of 2-mercapto-6-X-benzothiazole (0.1 mole) and triethylamine (10.1 g; 0.1 mole) in dry acetone (150 ml) alkyl chloroformate (0.1 mole) was added dropwise

with stirring. After 4 hrs of stirring at ambient temperature the reaction mixture was diluted with an equal volume of water and the solution was cooled for 24 hrs. The separated solid was isolated, dried, and recrystallized. Allyl (2-benzothiazolylthio)formate (*XXI*) was isolated by extraction with ether and viscous residue obtained upon removal of the solvent was purified by chromatography.

(2-Ethoxycarbonylthio-6-benzothiazolyl)ammonium chloride (XVIII)

Ethyl chloroformate (10.8 g; 0.1 mole) was added dropwise to a solution of 2-mercapto-6-aminobenzothiazole (18.2 g; 0.1 mole) in a mixture of acetone and *N*,*N*-dimethylformamide (3 : 1; 200 ml) and the solution was stirred at room temperature for 3 hrs. The separated crystalline (2-ethoxycarbonylthio-6-benzothiazolyl)ammonium chloride was filtered, washed with chloroform, and crystallized from ethanol—chloroform (1 : 1).

2-Mercapto-6-bromobenzothiazole

To a solution of 2-mercapto-6-aminobenzothiazole (5.4 g; 0.03 mole) in 6% sodium hydroxide (20 ml) sodium nitrite (20.5% solution, 20 ml) was added. The resulting mixture was added dropwise with cooling (0—5°C) into concentrated hydrochloric acid (100 ml). The solution of the produced diazonium salt was added dropwise under stirring into a solution of Cu_2Cl_2 (4 g) and potassium bromide (10 g) in diluted (1 : 1) hydrochloric acid (100 ml). The reaction mixture was heated on a water bath (70—80°C) for 1 hr and the separated (6-bromo-2-benzothiazolyl) disulfide was filtered, washed with water until neutral, and mixed with a solution of $Na_2S \cdot 9H_2O$ (20 g) in 250 ml of water. The mixture was heated at 80°C for 4 hrs while a continued stream of hydrogen sulfide was passed through it. The solution was filtered and acidified with 30—50% acetic acid. The separated solid was isolated, dissolved in 10—15% ammonium hydroxide and after filtration acetic acid was again added into the solution. The precipitated 2-mercapto-6-bromobenzothiazole (57%) had m.p. 245—248°C when crystallized from ethanol—carbon tetrachloride (1 : 1).

For $C_7H_4NS_2Br$ (246.14) calculated: 26.06% S, 5.69% N, 32.48% Br; found: 26.27% S, 5.47% N, 32.57% Br.

2-Mercapto-6-iodobenzothiazole

The diazonium salt, made as described above, was added dropwise to a solution of potassium iodide (6 g in 100 ml of water) and the resulting mixture was heated on a boiling water bath for 1 hr. After standing for 24 hrs at room temperature the separated (6-iodo-2-benzothiazolyl) disulfide was filtered, washed with water until neutral, and processed as described above in the preparation of 2-mercapto-6-bromobenzothiazole. The title compound was obtained in 51% yield and melted at 267—269°C (capillary) when crystallized from ethanol—chloroform (1 : 1).

For C_7NS_2I (293.09) calculated: 21.81% S, 4.77% N, 4.33% I; found: 21.75% S, 4.70% N, 4.21% I.

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