Benzothiazole compounds. I. Synthesis and antimicrobial investigation of 6-substituted allyl (2-benzothiazolylthio)acetates

[a]V. SUTORIS, [b]P. FOLTÍNOVÁ, and [a]G. BLÖCKINGER

[a]*Department of Organic Chemistry and Biochemistry, Faculty of Natural Sciences, Komenský University, 801 00 Bratislava*

[b]*Department of Microbiology, Faculty of Natural Sciences, Komenský University, 886 04 Bratislava*

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Allyl (6-X-2-benzothiazolylthio)acetates (X = H, NO_2, NH_2, Cl, Br, NHR, $N(R)_2$; R = CH_3, C_2H_5, $CH_2CH{=}CH_2$, $CH_2C{\equiv}CH$) were synthesized and their antimicrobial efficiencies studied. Some derivatives (X = H, NO_2, NH_2, Br) showed significant positive microbiological effect. Alkylation of the amino group resulted in a decrease of the antimicrobial efficiency.

Some derivatives of 2-mercaptobenzothiazole showed pesticidal properties, mainly as herbicides, fungicides, insecticides, and defoliants [1—8]. 2-Mercaptobenzothiazole [9, 10] showed good antimicrobial efficiency [11—14]. For these reasons we have started to study systematically the benzothiazole derivatives from the point of view of their pesticidal effects as well as of their effect on various types of microbacteria and tuberculous bacteria. The starting compound for the synthesis of the described derivatives, 2-mercaptobenzothiazole, was prepared by purification [15] of the technical grade 2-mercaptobenzothiazole which contained benzothiazole, aniline, and high-molecular polycondensates. 2-Mercapto-6-nitrobenzothiazole was prepared by nitration [16] and hydroiodic acid was used for its reduction to 6-amino-2-mercaptobenzothiazole. 6-Halo derivatives were prepared from 6-amino-2-mercaptobenzothiazole by diazotization and subsequent Sandmeyer reaction.

Allyl (6-X-2-benzothiazolylthio)acetates (Table 1, compounds *I—III*, *XI*, *XII*) were prepared from 6-substituted 2-mercaptobenzothiazoles (X = H, NO_2, NH_2, Cl, Br) or from their potassium salts, and allyl chloroacetate in ethanol. The use of potassium salts excludes the formation of the tautomeric (N and S) isomers in the course of syntheses.

The alkylation of amino group (Table 1, compounds *IV—X*) was accomplished by treatment of allyl (6-amino-2-benzothiazolylthio)acetates with the appropriate alkyl halogenides in benzene in the presence of triethylamine. Allyl (6-alkylamino-2-benzothiazolylthio)-acetates and allyl (6-dialkylamino-2-benzothiazolylthio)acetates could be isolated only by purification on the column of Al_2O_3 in benzene because distillation under reduced pressure (180°C/1 torr) caused their decomposition.

Antimicrobial efficiency of the derivatives of allyl (2-benzothiazolylthio)acetate was tested on the following microorganisms: *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium bovis* BCG, *Candida pseudotropicalis*, intracellular parasite *Trypanosoma cruzi*, and *Euglena gracilis*.

Table 1

Analytical data of the synthesized allyl (6-X-2-benzothiazolylthio)acetates

No.	X	Formula	*M*	Calculated/found % C	% H	% N	% S	% Hal.	Yield [%]	M.p. [°C] Solvent (n_D^{20})
I	$-H$	$C_{12}H_{11}O_2NS_2$	265.3	54.38 54.19	4.18 4.28	5.28 5.41	24.20 24.32		85	49–50 Ether–Petroleum ether (1 1)
II	$-NO_2$	$C_{12}H_{10}O_4N_2S_2$	310.3	46.49 46.70	3.25 3.31	9.03 9.16	20.68 20.48		85	87–88 Ethanol (80%)
III	$-NH_2$	$C_{12}H_{12}O_2N_2S_2$	280.5	51.47 51.25	4.32 4.21	10.03 9.91	22.85 23.00		90	89 Ethanol (70%)
IV	$-NHCH_3$	$C_{13}H_{14}O_2N_2S_2$	294.4	53.10 53.26	4.80 4.70	9.53 9.40	21.80 22.04		74	79 Ether–Petroleum ether (1 1)
V	$-N(CH_3)_2$	$C_{14}H_{16}O_2N_2S_2$	308.4	54.59 54.46	5.23 5.31	9.09 8.94	20.81 20.87		65	Highly viscous liquid
VI	$-NHC_2H_5$	$C_{14}H_{16}O_2N_2S_2$	308.4	54.59 54.73	5.23 5.15	9.09 9.23	20.81 21.10		71	74–75 Ether–Petroleum ether (1 1)
VII	$-N(C_2H_5)_2$	$C_{16}H_{20}O_2N_2S_2$	336.4	57.19 57.28	6.00 5.87	8.33 8.51	19.08 19.30		68	Highly viscous liquid
VIII	$-NHCH_2CH=CH_2$	$C_{15}H_{15}O_2N_2S_2$	319.3	56.47 56.59	4.73 4.61	8.78 8.68	20.10 20.21		64	Highly viscous liquid
IX	$-N(CH_2CH=CH_2)_2$	$C_{16}H_{20}O_2N_2S_2$	360.5	60.05 60.18	5.60 5.66	7.78 7.87	17.81 17.95		62	(1.6459)
X	$-NHCH_2C\equiv CH$	$C_{15}H_{14}O_2N_2S_2$	318.4	56.65 56.75	4.40 4.31	8.81 8.97	20.16 20.35		68	50–52 Ether–Petroleum ether (1 1)
XI	$-Cl$	$C_{12}H_{10}O_2S_2NCl$	299.8	48.20 48.41	3.37 3.22	4.34 4.45	21.44 21.30	Cl 11.85 11.80	56	Highly ous liquid
XII	$-Br$	$C_{12}H_{10}O_2S_2NBr$	344.2	41.80 41.75	2.93 2.87	4.06 4.13	18.63 18.52	Br 23.23 23.03	49	44–46 Ethanol (60–70%)

Table 2

Effective concentration (μg/ml) of allyl (6-X-2-benzothiazolylthio)acetates

No.	MIC			BCG	Lethal concentration	
	Bacillus subtilis	*Escherichia coli*	*Candida pseudotropicalis*		*Trypanosoma cruzi*	*Euglena gracilis*
I	10	50	100	10	50	100
II	10	50	100	10	100	500
III	10	50	100	10	50	100
IV	50	50	>100	50	>100	500
V	50	50	>100	50	>100	500
VI	50	100	>100	50	>100	500
VII	50	50	>100	50	>100	250
VIII	50	50	>100	50	>100	>500
IX	50	50	>100	50	>100	250
X	50	100	>100	50	>100	500
XI	50	50	100	50	100	>500
XII	10	10	100	10	100	250

The compounds used for testing were dissolved in ethanol or dimethyl sulfoxide.

The results of testing, *i.e.* the minimal inhibition concentrations (MIC) of compounds, are in Table 2.

As evident from the values in Table 2, some of the tested compounds are effective in a wide range. Inhibition effect on gram-positive (*Bacillus subtilis*) as well as on gram-negative bacteria (*Escherichia coli*) was observed at favourable concentrations. The best results were obtained with compounds *I*—*III*, *XI*, and *XII*. The MIC values of these compounds for the tested strains of non-specific bacterial flora varied between 10 and 50 μg/ml. The growth of the acid-resistant BCG was inhibited also at 10 μg/ml concentration of these compounds. The growth of the yeast test-organism, *Candida pseudotropicalis*, was inhibited at 100 μg/ml concentration. For the *in vitro* forms of the intracellular parasite *Trypanosoma cruzi* (originator of the incurable disease Chagas) the lethal concentration of these compounds was 50—100 μg/ml.

Neither the compound *I* nor the compound *III* tested *in vivo* was effective on the experimental Chagas disease at mice.

The effect of the compounds *IV*—*X* on the used test-organisms was somewhat lower than that of the allyl (2-benzothiazolylthio)acetate or allyl (6-amino-2-benzothiazolylthio)-acetate. The MIC values for both non-specific strains of bacterial flora were in the range of 50—100 μg/ml and for *Mycobacterium bovis* BCG 50 μg/ml. *Candida pseudotropicalis* was affected only at concentrations above 100 μg/ml. Lethal effect on the *in vitro* forms of the intracellular parasite *Trypanosoma cruzi* was observed at concentrations higher than 100 μg/ml, *i.e.* higher than with compounds *I*—*III*, *XI*, and *XII*.

Compounds *III* and *XI* were least toxical for *Euglena gracilis*. For lethal effect more than 500 μg/ml was required; none of the tested compounds caused depigmentation.

Experimental

Characterization of the synthesized compounds is in Table 1.

Very pure 2-mercaptobenzothiazole

Technical grade 2-mercaptobenzothiazole (100 g, melt) was dissolved in diluted ammonia (60 ml 25% ammonia in 2 l water) at boiling and diluted hydrogen peroxide (2 ml 30% H_2O_2 in 100 ml hot water) was added. This solution was poured into hot water (6 l; 80—90°C) and sodium sulfite (1 g) was added. The hot solution was decanted from the solid material and the 2-mercaptobenzothiazole was precipitated with concentrated hydrochloric acid (60 ml). The precipitate was filtered and washed 3 times with cold water. Then it was worked up again in the above-described manner and precipitated with acetic acid (50 ml). The precipitate, washed 3 times with hot water, was again dissolved in the solution of sodium hydroxide and sodium sulfite (6 l H_2O, 20 g NaOH, and 2 g Na_2SO_3) and the colourless clear solution was carefully decanted from the impurity. The crystalline product was obtained by precipitation with diluted acetic acid (50 ml CH_3COOH and 300 ml H_2O) at boiling. Yield 65%, m.p. 181—183°C.

For $C_7H_5S_2N$ (167.22) calculated: 38.39% S, 8.37% N; found: 38.28% S, 8.28% N.

6-Amino-2-mercaptobenzothiazole

2-Mercapto-6-nitrobenzothiazole (2.12 g; 0.01 mole), red phosphorus (0.6 g), and 57% hydroiodic acid (10 ml) were heated at 90—95°C till the exothermic reaction started. After cooling the reaction mixture was dissolved in water (50 ml), purified with charcoal and neutralized with a solution of sodium hydroxide to pH 7. Filtration gave 1.6 g (86%) of 6-amino-2-mercaptobenzothiazole, m.p. 265—268°C.

For $C_7H_6S_2N_2$ (182.24) calculated: 35.23% S, 15.38% N; found: 35.16% S, 15.26% N.

6-Halo-2-mercaptobenzothiazole

6-Amino-2-mercaptobenzothiazole (5.4 g; 0.03 mole) was dissolved in diluted sodium hydroxide (1.2 g; 0.03 mole NaOH and 20 ml H_2O) and a solution of sodium nitrite (4.1 g; 0.06 mole in 20 ml H_2O) was added. This mixture was added dropwise to concentrated hydrochloric acid (100 ml) at 0—5°C. Then the whole content was poured into diluted (1 : 1) hydrochloric acid (100 ml) containing copper(I) halogenide (12 g chloride or 16 g bromide) previously dissolved in it. The solution was slowly heated to 60—70°C and then poured into cool water (1 l). The precipitated 6-halo-2-mercaptobenzothiazole disulfide was filtered off and immediately mixed with the solution of sodium sulfide (20 g Na_2S $9H_2O$ in 250 ml water). Into this solution hydrogen sulfide was introduced at 80°C for 4 hours. The solution was filtered and acidified with diluted acetic acid. The formed precipitate was filtered, dissolved in 10—15% ammonia, filtered again and precipitated with 30—50% acetic acid. The precipitates of 6-halo-2-mercaptobenzothiazole were crystallized from the mixture of ethanol and carbon tetrachloride (1 1). The yield of 6-chloro-2-mercaptobenzothiazole was 56%, m.p. 265—267°C.

For $C_7H_4S_2NCl$ (201.67) calculated: 31.69% S, 6.93% N, 17.54% Cl; found: 31.72% S, 6.86% N, 17.41% Cl.

The yield of 6-bromo-2-mercaptobenzothiazole was 49%, m.p. 245—248°C.

For $C_7H_4S_2NBr$ (246.14) calculated: 26.06% S, 5.69% N, 32.48% Br; found: 25.92% S, 5.74% N, 32.37% Br.

Allyl (6-X-2-benzothiazolylthio)acetates (I—III, XI, XII) (X = H, NO_2, NH_2, Cl, Br)

To 6-X-2-mercaptobenzothiazole (0.1 mole) in ethanol (300—400 ml) potassium hydroxide (5.6 g; 0.1 mole) was added. After 30 minutes' heating at 30—40°C under stirring,

allyl chloroacetate (13.5 g; 0.1 mole) was added dropwise during 10 minutes. The reaction mixture was then heated at 60—70°C for 2 1/2 hours. Two thirds of ethanol were distilled off under reduced pressure and the residue was poured into cool water (800 ml). The precipitated allyl esters were filtered off (allyl (2-benzothiazolylthio)acetate *I* became solid after one-day cooling only).

N-Substituted derivatives of allyl (6-amino-2-benzothiazolylthio)acetate (IV—X) (R = CH_3, C_2H_5, $CH_2CH{=}CH_2$, $CH_2C{\equiv}CH$)

Allyl (6-amino-2-benzothiazolylthio)acetate (28 g; 0.1 mole) was dissolved in anhydrous benzene (300 ml) and triethylamine (10.1 g; 0.1 mole for monosubstituted derivatives and twofold amounts, for disubstituted ones) at 50—60°C under stirring. The appropriate alkyl halogenide (0.1 mole or 0.3 mole) was added dropwise and the reaction mixture was washed with water (100 ml) 4 times. Benzene was distilled off and the brown viscous residue was purified on the column of Al_2O_3 in benzene.

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