Benzothiazole compounds. II. Preparation and biological activity of the esters of (6-nitro-2-benzothiazolylthio)acetic acid

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Esters of (6-nitro-2-benzothiazolylthio)acetic acid were synthesized and their biological activity on microorganisms was studied. Esters as well as free acid exhibited antibacterial activity against both gram-positive and gram--negative bacteria. The inhibition of protozoa and yeast-like microorganisms required higher concentrations of above compounds than the inhibition of bacteria.

After previous finding [1] that the allyl (6-X-benzothiazolylthio)acetates (X = H, NO_2, NH_2, NHR, $N(R)_2$, Cl, Br; R = CH_3, C_2H_5, $CH_2CH{=}CH_2$, $CH_2C{\equiv}CH$) possess antibacterial properties our attention was devoted to the preparation of alkyl (6-nitro--2-benzothiazolylthio)acetates. So far, these compounds have not been studied. We studied the effect of changing the nature of the alkyl group on the antibacterial activity of the synthesized esters.

Experimental

Characterization of the prepared compounds is in Table 1.

Melting points were determined on a Kofler block. Infrared spectra were measured with a Zeiss UR-20 spectrophotometer in the range 3700—700 cm^{-1} in paraffin oil. The instrument was calibrated using the standard spectrum of polystyrene. Reading accuracy of absorption bands (Table 2) was ± 1 cm^{-1}. For the testing of biological activity the compounds were dissolved in ethyl alcohol or dimethyl sulfoxide. The results are summarized in Table 3.

(6-Nitro-2-benzothiazolylthio)acetic acid (I)

A solution of 2-mercapto-6-nitro-benzothiazole (6.4 g; 0.03 mole) in 5% KOH (80 ml) was mixed with chloroacetic acid (2.9 g; 0.03 mole) and the mixture was heated on a water bath at 100°C for 2 hours. After cooling the mixture was acidified with hydrochloric acid (congo paper). The formed precipitate was filtered, washed with water, and crystallized from 80% methyl alcohol.

Table 1

Characterization of the synthesized esters of (6-nitro-2-benzothiazolylthio)acetic acid

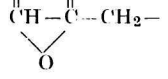
No.	R	Formula	*M*	Calculated/found				Yield [%]	M.p. [°C] (n_D^{20})
				% C	H	N	% S		
I	H–	$C_9H_6O_4N_2S_2$	270.3	40.00 39.87	2.24 2.32	10.31 10.21	23.74 23.86	76	225–227
II	CH_3–	$C_{10}H_8O_4N_2S_2$	284.3	42.23 42.46	2.83 2.70	9.85 9.98	22.57 22.41	82	166
III	C_2H_5–	$C_{11}H_{10}O_4N_2S_2$	298.3	44.33 44.20	3.38 3.46	9.40 9.56	21.51 21.39	86	97
IV	C_3H_7–	$C_{12}H_{12}O_4N_2S_2$	312.3	46.16 46.21	3.87 3.84	8.97 8.90	20.55 20.53	88	93
V	$CH\equiv C-CH_2$–	$C_{12}H_8O_4N_2S_2$	308.3	46.79 46.86	2.94 3.06	9.09 9.26	20.81 20.76	79	144
VI	C_4H_9–	$C_{13}H_{14}O_4N_2S_2$	326.4	47.89 47.82	4.32 4.36	8.59 8.75	19.66 19.49	83	83
VII	*n*-C_4H_9–	$C_{13}H_{14}O_4N_2S_2$	326.4	47.89 47.77	4.32 4.21	8.59 8.59	19.66 19.60	80	70–72
VIII	*i*-C_4H_9–	$C_{13}H_{14}O_4N_2S_2$	326.4	47.89 47.94	4.32 4.27	8.59 8.86	19.66 19.61	82	63–65
IX	*sec*-C_8H_{17}–	$C_{17}H_{22}O_4N_2S_2$	382.5	3.42 3.39	5.80 5.67	7.33 7.18	16.74 16.45	77	(1.5840)
X		$C_{14}H_{14}O_5N_2S_2$	354.5	47.49 47.63	3.98 4.11	7.90 7.87	18.11 18.10	85	95–96

Table 2

Frequencies of the characteristic i.r. absorption bands of the esters of (6-nitro-2-benzothiazolylthio)acetic acid

No.	$\tilde{\nu}$(C=O)	$\tilde{\nu}$(NO_2)	$\tilde{\nu}$(NO_2)	$\tilde{\nu}$(2-benzothiazolylthio-ring)		
I	1730 s	1336 s	1519 s	1600 w	1578 w	1405 m
II	1739 s	1334 s	1523 s	1601 w	1577 w	1406 m
III	1741 s	1335 s	1522 s	1601 w	1579 w	1404 m
IV	1732 s	1332 s	1523 s	1602 w	1575 w	1405 m
V	1747 s	1335 s	1523 s	1602 w	1578 w	1405 m
VI	1732 s	1331 s	1520 s	1601 w	1576 w	1405 m
VII	1754 s	1335 s	1527 s	1604 w	1580 w	1406 m
VIII	1730 s	1334 s	1527 s	1602 w	1578 w	1407 m
IX	1759 s	1337 s	1580 s	1603 w	1580 w	1403 m
X	1737 s	1335 s	1601 s	1601 w	1579 w	1405 m

s — strong; m — medium; w — weak.

Table 3

Effective concentrations (μg/ml) of the esters of (6-nitro-2-benzothiazolylthio)acetic acid

No.	MIC			BCG		*Euglena grac.*		*Tryp. cruzi*	
	B. subtilis	*E. coli*	*C. pseudotrop.*	-cidal	-static	lethal	-static	lethal	-static
I	10	50	>100	50	10	500	125	100	> 50
II	50	50	>100	50	10	>500	500	>100	100
III	50	50	>100	50	10	>500	500	>100	100
IV	50	50	>100	100	50	>500	500	>100	100
V	50	50	>100	100	50	>500	500	>100	>100
VI	50	50	>100	100	50	>500	500	>100	100
VII	50	50	>100	100	50	500	>250	>100	100
VIII	100	100	>100	100	50	>500	500	>100	100
IX	100	>100	>100	>100	100	>500	500	>100	>100
X	100	100	>100	100	>50	>500	>250	>100	>100

Esters of (6-nitro-2-benzothiazolylthio)acetic acid (II—X)

To 2-mercapto-6-nitrobenzothiazole (21.2 g; 0.1 mole) in ethyl alcohol (400 ml) KOH (5.6 g; 0.1 mole) was added. The mixture was kept at 30—40°C under stirring for 30 minutes. The ester of chloroacetic acid (0.1 mole) was then added dropwise to the reaction mixture during another 30 minutes at the same temperature. The reaction mixture was then stirred at 60—70°C for 3 hours. Ethanol (50—70%) was then evaporated under reduced pressure and the residue was poured into cold water (500 ml). Insoluble portion (*II—VIII* and *X*) was then filtered and dried. The pure product in the form of yellow crystals was obtained by crystallization from ethanol.

2-Octyl (6-nitro-2-benzothiazolylthio)acetate (*IX*) was extracted from water solution with the mixture of ether and 1,2-dichloroethane (1 1, v/v). After drying the extract

with Na_2SO_4 and evaporation of the solvents the residue was dissolved in 50 ml of the mixture of benzene and ethanol (4 : 1, v/v) and purified by chromatography on the column of Al_2O_3.

Discussion

The present study deals with the antibacterial activity of the individual alkyl (6-nitro-2-benzothiazolylthio)acetates. Although the other derivatives of this acid were reported to be biologically active [2—6], the above compounds have not been investigated so far.

Some alkyl (2-benzothiazolylthio)acetates were prepared by *Kucherov* [7] by treatment of 2-mercaptobenzothiazole in sodium hydrogen carbonate solution with the esters of chloroacetic acid. *Svetlaeva* and co-workers [8] prepared the esters by the reaction of (2-benzothiazolylthio)acetic acid with the corresponding alcohol in the presence of sulfuric or hydrochloric acid. The yields varied between 52 and 75%. The prepared esters were used in the studies of their oxidation to sulfones.

Our method of synthesis of the esters of (6-nitro-2-benzothiazolylthio)acetic acid is based on the preparation of the potassium salt of 2-mercapto-6-nitrobenzothiazole by its treatment with potassium hydroxide and successive direct addition of the corresponding ester of chloroacetic acid. The reaction was practically completed (judging according to amount of formed potassium chloride) during first 30 minutes at 30—40°C. After subsequent 3-hour heating at 60—70°C and vacuum distillation of ethanol the synthesized compounds were isolated from the water medium.

2-Octyl ester (*IX*), a highly viscous liquid, was extracted from the water medium with the mixture of ether and 1,2-dichloroethane (1 : 1, v/v). This mixture was used because 2-octyl ester dissolved in 1,2-dichloroethane alone separated very slowly from the water phase. 2-Octyl ester decomposed during the vacuum distillation (160°C/1 torr). The prepared esters are new, so far not described compounds.

The i.r. spectra of all prepared esters exhibited strong absorption bands in the region 1760—1730 cm^{-1} associated with the stretching C≡O vibration of the —COOR group. As seen from Table 2, the frequencies of the absorption bands are markedly affected by the presence of alkyl groups. The detailed study of these effects will be the subject of the following study.

Two strong absorption bands associated with asymmetric and symmetric stretching vibrations of $-NO_2$ groups can be observed at 1530—1520 and 1340—1330 cm^{-1}, respectively. The characteristic absorption bands of basic skeleton can be found at ~1603, ~1578, and ~1404 cm^{-1}. Vibration bands at 2139 and 3288 cm^{-1} found in the i.r. spectrum of the compound *V* can be ascribed to C≡C and to C—H stretching vibrations in the $-CH_2C{\equiv}CH$ group. The spectrum of this compound reveals, in contrast to the spectra of the synthesized esters, the absorption band at ~3470 cm^{-1} associated with the stretching —OH vibration in the carboxyl group. The absorption bands of the carbonyl group of this compound were observed at lower frequencies than in the synthesized esters.

(6-Nitro-2-benzothiazolylthio)acetic acid (*I*) exhibited good antibacterial effects when testing the strains of non-specific bacterial flora (*Bacillus subtilis* and *Escherichia coli*) as well as mycobacterial strain *Mycobacterium bovis* BCG. The minimal inhibitory concentrations (MIC) of *I* with the above strains varied from 50 to 10 μg/ml.

When comparing the antibacterial effects of *I* with those of its esters (*II*—*X*; Table 3) it can be seen that they do not differ considerably and that the same effects can be observed on both the gram-positive and the gram-negative bacteria. The MIC value of compounds

II — *VII* was 50 μg/ml both for *Bacillus subtilis* and for *Escherichia coli*. The inhibition of strains of the non-specific bacterial flora required 100 μg/ml concentrations for the compounds *VIII* and *IX*. The tests have also shown that the compounds are bactericidal at the concentrations of 100—50 μg/ml on the mycobacterial strain tested, and that even the lower concentrations are bacteriostatic to different degrees.

In the experiments with the yeast-like strain of *Candida pesudotropicalis* it has been found with all the compounds tested that for the inhibition of its growth concentrations higher than 100 μg/ml were required. The same was true for the inhibition of the intracellular parasite *Trypanosoma cruzi* and its crithidial forms. The lethal effect of the most compounds tested on the *Euglena gracilis* was observed only at the concentrations above 500 μg/ml.

Generally, for the inhibition of protozoa and of yeast-like microorganisms higher concentrations of the compounds tested were needed than for the inhibition of bacteria. The lengthening or the branching of the alkyl chain in esters did not influence substantially the biological activity of the compounds tested.

References

1. Sutoris, V. Foltínová, P., and Blöckinger, G., *Chem. Zvesti* **27**, 698 (1973).
2. Davis, W. H. and Sexton, W. A., *Biochem. J.* **43**, 461 (1948).
3. *US Patent* 2 237 647 (1952).
4. Prilezhaeva, E. N. and Smonina, L. I., *Izv. Akad. Nauk SSSR* **1969**, 693.
5. *US Patent* 2 637 645 (1952).
6. *US Patent* 2 630 381 (1953).
7. Kucherov, V. F., *Zh. Obshch. Khim.* **18**, 752 (1949).
8. Svetlaeva, V. M., Kuznecova, E. A., and Zhuravlev, S. V., *Zh. Obshch. Khim.* **34**, 983 (1964).

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