Primary anthelmintic screening of 1-(2-alkylthio-6-benzothiazolylaminomethyl)-5-(3,4-R-phenyl)-1,2,3,4-tetrazoles

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The new 1-(2-alkylthio-6-benzothiazolylaminomethyl)-5-(3,4-R-phenyl)-1,2,3,4-tetrazoles were prepared by Mannich reaction from 2-alkylthio-6-aminobenzothiazoles with 5-phenylsubstituted 1,2,3,4-tetrazoles and formaldehyde. The compounds prepared were tested against *Trichinella spiralis*, *Aspiculuris tetraptera*, *Nematospiroides dubius* and *Heterakis spumosa*.

Посредством реакции Манниха 2-алкилтио-6-аминобензотиазолов с 5-фенил-1,2,3,4-тетразолами и формальдегидом были получены новые 1-(2-алкилтио-6-бензотиазолиламинометил)-5-(3,4,-R-фенил)-1,2,3,4-тетразолы и была проверена их эффективность против *Trichinella spiralis*, *Aspiculuris tetraptera*, *Nematospiroides dubius* и *Heterakis spumosa*.

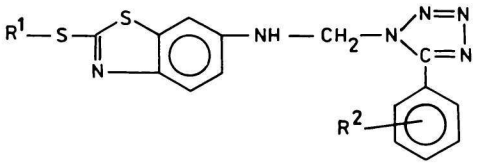
The biological activity of the starting 2-alkylthio-6-aminobenzothiazoles [1—3] and compounds obtained by their reaction with substituted tetrazoles [4, 5] stimulated the preparation of another series of these compounds with a presumed biological activity against helminths (Table 1).

Loss of the effect on *Heterakis spumosa* due to extension of the hydrocarbon chain by one CH_2 group with *II* in relation to *I* is quite rare in the group of compounds with an unsubstituted tetrazole ring (*I*—*X*), similarly as the 100 % increase of activity of this compound against *Nematospiroides dubius*. A negligible effect against *Aspiculuris tetraptera* contrasted with the isobutyl derivative *IV*, having a 100 % activity [6]. Branching of the chain in *IV* was associated with the activity loss on *Aspiculuris tetraptera*. In the group of substituted tetrazoles chlorinated derivatives *XI*, *XII* revealed considerably higher activity than *XIII* bearing a nitro group.

Comparison of biological effects of the starting compounds *XIV* and *XV* with the average effect of derivatives prepared let us to conclude that binding of benzothiazole molecules to tetrazole ones was not reflected by a noticeable biological effect. However, separate results of some tests, *e.g.* with substances *IV*

Table 1

Primary anthelmintic screening of compounds *I*—*XV*



Compound	R^1	R^2	Activity/% on			
			TS	AT	ND	HS
I	CH_3CH_2	H	0	0	0	25
II	$CH_3(CH_2)_2$	H	0	0	50[a] 50[b]	0
III	$CH_3(CH_2)_3$	H	0	40	0	0—70
IV	$(CH_3)_2CHCH_2$	H	0	0	0	100[a] 20[b]
V	$CH_3(CH_2)_4$	H	0	0	0	0
VI	$(CH_3)_2CHCH_2CH_2$	H	0	0	15	0
VII	$CH_3(CH_2)_7$	H	*c*	*c*	0	0
VIII	$CH_3(CH_2)_8$	H	*c*	40	0	0
IX	$CH_2=CH—CH_2$	H	0	0	0	20
X	$C_6H_5—CH_2$	H	0	0	0	20
XI	$(CH_3)_2CHCH_2$	3-Cl	0	0	50	0
XII	$(CH_3)_2CHCH_2$	3,4-Cl_2	10	0	60	20
XIII	$(CH_3)_2CHCH_2$	4-NO_2	0	0	0	10
XIV	6-Amino-2-isobutylthio-benzothiazole	—	0	0	20	60[a] 45[b]
XV	5-Phenyl-1,2,3,4-tetrazole	—	0	0	55	40

a) First screening, *b*) second screening, *c*) not tested for the lack of substances.

TS — *Trichinella spiralis*, AT — *Aspiculuris tetraptera*, ND — *Nematospiroides dubius*, HS — *Heterakis spumosa*.

and *XII* showed that the former is a very effective and the latter a medium effective compound. Scattered results with the respective compounds might be due to their application in suspension. Some greater particles can pass the intestinal tract without being dissolved and consequently, not utilized. Another rationalization might be due to physiological behaviour [7], *i.e.* the individual host organism has both certain defensive ability against helminths and variability. This physiological dependence is then manifested by various invasion intensity in the laboratory animal. As known, the helminth cannot succeed to invade the host organism at any time. The low infectility is, therefore, associated with an easier manifestation of high activity of the compound under investigation.

Experimental

2-R-thio-6-aminobenzothiazoles were synthesized from potassium 6-amino-2-mercaptobenzothiazole and the respective R-halides according to [1—3]. The final products with the exception of *VI, XI—XIII* have already been prepared [8] by the Mannich reaction from 2-R-thio-6-aminobenzothiazoles and 5-phenyl-1,2,3,4-tetrazoles with formaldehyde.

The anthelmintic activity was investigated *in vivo* in the primary screening on four model helminths: *Nematospiroides dubius, Aspiculuris tetraptera, Heterakis spumosa,* and *Trichinella spiralis*. The first three helminths parasitize in various parts of the host intestine, the fourth one was a tissue model. All compounds were tested on white mice (Velaz, Prague) as host organisms. The invasion stadia of individual species of helminths were obtained according to [9—11], or by modification of the procedure [12]. Mice were invaded *per os*, the tested compounds were applied immediately after the prepatent period when testing enteronematodes, and on the fourth day after invasion when testing *T. spiralis*. The Dorfmann solution suspended compounds were applied by means of a stomach probe three times 150 mg/kg each for three days. Activity of compounds was estimated by the critical controlled test method [11] at the eighth day after the first application with enteronematodes and at the 40th day after invasion with *T. spiralis*.

1-(2-Isoamylthio-6-benzothiazolylaminomethyl)-5-phenyl--1,2,3,4-tetrazole (VI)

2-Isoamylthio-6-ammoniobenzothiazole chloride (2.8 g; 10 mmol), or sulfate (3.5 g; 10 mmol) was mixed with 5-phenyl-1,2,3,4-tetrazole (1.4 g; 10 mmol) and ethanol (20 cm^3) and heated to 35 °C. A 30 % aqueous formaldehyde (2 cm^3; 20 mmol) was added to the stirred mixture, followed by NaOH (0.4 g; 10 mmol) in water (3 cm^3). Crystallization of the final product occurred after 5 min of stirring at 35—37 °C. The product was filtered off after additional 5 min and dropwise washed with ethanol (10 cm^3). Yield = 1.7 g (41 %), m.p. = 99—102 °C.

For $C_{20}H_{22}N_6S_2$ (M_r = 410.5) w_i(calc.): 58.51 % C, 5.39 % H, 20.46 % N, 15.62 % S; w_i(found): 58.63 % C, 5.34 % H, 19.89 % N, 16.15 % S.

1-(2-Isobutylthio-6-benzothiazolylaminomethyl)-5-(3-chlorophenyl)--1,2,3,4-tetrazole (XI)

2-Isobutylthio-6-aminobenzothiazole (2.3 g; 10 mmol) was heated with sodium salt of 5-(3-chlorophenyl)-1,2,3,4-tetrazole (2.0 g; 10 mmol) and ethanol to 37 °C. At this temperature a 30 % aqueous formaldehyde (2 cm^3; 20 mmol) and after a 1-min interval conc. hydrochloric acid (1 cm^3; 10 mmol) were added with stirring. The mixture was stirred another 5 min, then water (5 cm^3), acetone (10 cm^3), and light petroleum (10 cm^3) were added, the mixture was cooled to − 10 °C and allowed to stand for 24 h at this temperature. The separated crystals were filtered off and dropwise washed with ethanol (15 cm^3). Yield = 2.4 g (56 %), m.p. = 118—120 °C.

For $C_{19}H_{19}N_6S_2Cl$ (M_r = 431) w_i(calc.): 52.95 % C, 4.44 % H, 19.49 % N, 14.88 % S, 8.22 % Cl; w_i(found): 53.04 % C, 4.37 % H, 19.42 % N, 14.14 % S, 8.37 % Cl.

1-(2-Isobutylthio-6-benzothiazolylaminomethyl)-5-(3,4-dichlorophenyl)--1,2,3,4-tetrazole (XII)

2-Isobutylthio-6-aminobenzothiazole (2.3 g; 10 mmol), 5-(3,4-dichlorophenyl)--1,2,3,4-tetrazole (2.1 g; 10 mmol), and ethanol (25 cm^3) were heated with stirring to 37—40 °C. Then 30 % aqueous formaldehyde (2 cm^3; 20 mmol) was added and the white crystalline product, which begins to separate within 15 min was cooled to 20 °C, filtered off and washed with ethanol (15 cm^3). Yield = 3.0 g (65 %), m.p. = 136—138 °C.

For $C_{19}H_{18}N_6S_2Cl_2$ (M_r = 465.4) w_i(calc.): 49.03 % C, 3.89 % H, 18.05 % N, 13.77 % S, 15.23 % Cl; w_i(found): 49.08 % C, 3.92 % H, 17.84 % N, 14.24 % S, 15.78 % Cl.

1-(2-Isobutylthio-6-benzothiazolylaminomethyl)-5-(4-nitrophenyl)--1,2,3,4-tetrazole (XIII)

A 30 % aqueous formaldehyde (2 cm^3; 20 mmol) was added to a stirred and to 40 °C heated mixture consisting of 2-isobutylthio-6-aminobenzothiazole (2.3 g; 10 mmol), 5-(4-nitrophenyl)-1,2,3,4-tetrazole (1.9 g; 10 mmol), and ethanol (30 cm^3). The mixture was cooled to 20 °C and the separated crystals were filtered off after 24 h and washed with ethanol (15 cm^3).

For $C_{19}H_{19}N_7S_2O_2$ (M_r = 441.5) w_i(calc.): 51.68 % C, 4.33 % H, 22.20 % N, 14.52 % S; w_i(found): 52.41 % C, 4.40 % H, 21.97 % N, 14.62 % S.

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