2-Alkylthio-6-benzoylaminobenzothiazoles

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2-Alkylthio-6-benzoylaminobenzothiazoles have been synthesized by alkylation of 6-benzoylamino-2-benzothiazolinethione and benzoylation of 2-alkylthio-6-aminobenzothiazole, respectively. The compounds exhibited antimycobacterial, anthelmintic, and antifungal activities. Especially noticeable was the high antimycobacterial activity against atypical tuberculous mycobacteria in connection with low acute toxicity.

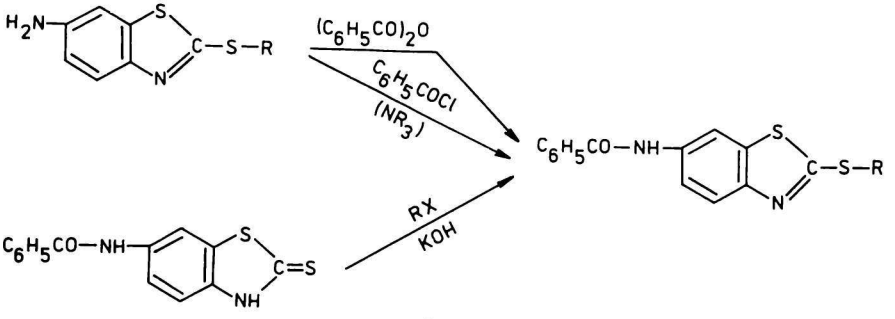
2-Алкилтио-6-бензоиламинобензотиазолы были синтезированы посредством алкилирования 6-бензоиламино-2-бензотиазолинтиона и бензоилирования 2-алкилтио-6-аминобензотиазола соответственно. Полученные соединения проявляли антимикобактериальную, антигельминтную и антифунгальную активности. Особенно стоит отметить высокую антимикобактериальную активность по отношению к нетипичным туберкулезным микобактериям при собственной низкой ядовитости.

The knowledge about the biological activity of 2-alkylthio-6-aminobenzothiazoles [1] and substituted benzamides prompted us to synthesize 2-alkylthio-6-benzoylaminobenzothiazoles from these two structural units.

So far, only 2-(2-chloroethyl)thio-6-benzoylaminobenzothiazole and its starting compound 6-benzoylamino-2-benzothiazolinethione [2] have been prepared. According to the original method of *Kuznetsova et al.*, 6-benzoylamino-2-benzothiazolinethione was prepared by the reaction of benzoyl chloride with 6-amino-2-benzothiazolinethione in dioxan in the presence of pyridine. The new method developed by us employs pyridine as the medium and thus shortens the reaction time.

2-Alkylthio-6-benzoylaminobenzothiazoles can be prepared in three ways: benzoylation of 2-alkylthio-6-aminobenzothiazole with benzoic anhydride, ben-

zoylation of 2-alkylthio-6-aminobenzothiazole with benzoyl chloride in the presence of tertiary amine, or alkylation of 6-benzoylamino-2-benzothiazolinethione with alkyl halide in the presence of potassium hydroxide (Scheme 1).



Scheme 1

The third way is the most advantageous one as it starts from one intermediate and thus the laborious purification of 2-alkylthio-6-aminobenzothiazoles is omitted. This method was applied also by *Kuznetsova* and coworkers [2] in the preparation of 6-benzoylamino-2-(2-chloroethylthio)benzothiazole. They used sodium hydroxide and the mixture of alcohol—water (volume ratio = 1 : 3) as the reaction medium to create the alkali salt of 6-benzoylamino-2-mercaptobenzothiazole. Application of potassium hydroxide in the synthesis made possible to increase the portion of ethanol in the reaction mixture (ethanol—water (volume ratio = 3 : 1)) and thus decrease the volume of the reaction mixture as well as obtain crude products of high purity. The compound *VIII* (Table 3) was prepared in all the above ways, the highest yield resulted from the procedure *A* (see Experimental).

The synthesized compounds were tested for antimycobacterial and anthelmintic activities.

The antimycobacterial activity of 2-alkylthio-6-benzoylaminobenzothiazoles (Table 1) is more differentiated according to the alkyl on sulfur bound in the position 2 than that of the starting 2-alkylthio-6-aminobenzothiazoles and analogous 6-acetylamino-2-alkylthiobenzothiazoles. Contrary to these groups of compounds, only the derivatives with alkyls of 2 to 5 C atoms have been active. The activity of the derivatives with branched alkyls was higher. 6-Benzoylamino-2-(2-hydroxyethylthio)benzothiazole (*XVIII*) differs markedly in its activity from the basic 2-alkylthio-6-aminobenzothiazole: while the amine is completely inactive, the *N*-benzoyl derivative belongs to two most active compounds [1, 3]. *Sec*-butyl derivative (*VIII*) [4] is considered to be the best as it was not only more active against some atypical tuberculous mycobacteria (*M. avium*, *M. tbc.* INH-resistant) than isonicotinohydrazide (INH) but had also eight-times lower acute toxicity than INH.

Table 1

Antimycobacterial activity and acute toxicity of 2-alkylthio-6-benzoylaminobenzothiazoles compared to that of isonicotinohydrazide and 2-mercaptobenzothiazole

Compound	MIC for *Mycobacterium*								DTM
	tbc. $H_{37}R_v$		*tbc.* INH-R		*avium*		*kansasii*		mg kg^{-1}
	{*a*}	{*b*}	{*a*}	{*b*}	{*a*}	{*b*}	{*a*}	{*b*}	
I	—	>200	—	>200	—	>200	—	>200	—
II	33	103.8	11	34.6	>33	>103.8	>33	>103.8	—
III	33	108.4	11	36.1	>33	>108.4	>33	>108.4	—
IV	3.7	12.2	3.7	12.2	1.2	3.9	3.7	12.2	250
V	33	107.7	11	35.9	>33	>107.7	3.7	12.1	—
VI	>33	>113.0	33	113.0	>33	>113.0	>33	>113.0	—
VII	3.7	12.7	3.7	12.7	3.7	12.7	3.7	12.7	250
VIII	0.4	1.4	1.2	4.1	0.4	1.4	1.2	4.1	1000
IX	33	117.6	33	117.6	>33	>117.6	>33	>117.6	—
X	>33	>117.6	3.7	13.2	>33	>117.6	>33	>117.6	—
XI	>33	>117.0	3.7	13.1	3.7	13.1	3.7	13.1	—
XII	>33	>122.2	>33	>122.2	>33	>122.2	>33	>122.2	—
XIII	>33	>126.9	>33	>126.9	>33	>126.9	>33	>126.9	—
XIV	>33	>131.5	>33	>131.5	>33	>131.5	>33	>131.5	—
XV	>33	>136.2	>33	>136.2	>33	>136.2	>33	>136.2	—
XVI	>33	>124.2	>33	>124.2	>33	>124.2	>33	>124.2	—
XVII	>33	>128.9	>33	>128.9	>33	>128.9	>33	>128.9	—
XVIII	1.2	4.0	1.2	4.0	1.2	4.0	1.2	4.0	250
INH	0.4	0.5	33	45.3	11	15.1	1.2	1.6	125
2-MBT	11	18.4	11	18.4	3.7	6.2	33	55.2	250

MIC = minimum inhibitory concentration ({*a*} = *a* 10^5/(mol dm^{-3}); {*b*} = *b*/(μg cm^{-3})), DTM = *dosis tolerata maxima*, — = not tested, INH = = isonicotinohydrazide, 2-MBT = 2-mercaptobenzothiazole.

The anthelmintic activity was tested on four model helminths: *Nematospiroides dubius, Aspiculuris tetraptera,* and *Heterakis spumosa,* which are parasites in different parts of host intestine; *Trichinella spiralis,* which has more complex developing cycle, was used as a model of tissue helminths. In the first screening, the compounds *VI*, *XVII*, and *XVIII* were active against *Aspiculuris tetraptera* and the compounds *II*, *III*, *XVI*, *XVII*, and *XVIII* exhibited activity against *Heterakis spumosa* (Table 2). The results of the repeated tests were variable, practically not reproducible, therefore, the compounds were not subjected to further screening.

Experimental

Antimycobacterial activity was determined in a liquid semisynthetic Šula medium [5] on strains of *Mycobacterium (M) tuberculosis* $H_{37}R_v$ (Collection of Research Institute of Preventive Medicine, Bratislava), *M. tuberculosis* INH-R and *M. avium* (Collection of KHS, Brno) and *M. kansasii PKG 8* (Collection of Dr. Runyon, Salt Lake City, USA). The compounds in concentrations: $c/(\text{mol dm}^{-3})$: 0.4×10^{-5}, 1.2×10^{-5}, 3.7×10^{-5}, 11×10^{-5}, and 33×10^{-5} were added into the medium in the form of DMSO solution (1 % of the volume of the medium). Isonicotinohydrazide (INH, Izoniazid, Jenapharm, GDR) and 2-mercaptobenzothiazole (2-MBT) were tested under the identical experimental conditions [6]. The methyl derivative was tested in concentration $\varrho = 200\ \mu\text{g cm}^{-3}$.

Acute toxicity of compounds *(Dosis tolerata maxima,* DTM) was determined according to the method described by *Wagner* [7]. After administration of compounds to white mice by oesophageal sound, 95 % decay was followed, compared to both the group of mice to which only the solvent and that to which INH and 2-MBT, respectively, were administered.

Anthelmintic activity was tested *in vivo* on white mice (VELAZ, Prague) as hosts. The invasion stages of the individual species of helminths were obtained according to the procedures [8—10] as modified in [11]. The mice were invaded *per os.* The tested compounds were applied as follows: in the case of enteronematodes immediately after the prepatent period, in the case of *T. spiralis* on the fourth day after invasion. The compounds dispersed in Dorfmann solution were applied ($w = 150$ mg/kg) by stomach sound thrice during 3 days. The activity of compounds was determined by the method of critical controlled test [10]: in the case of enteronematodes on the eighth day after the first application of the compound, in the case of *T. spiralis* on the fortieth day after invasion.

6-Benzoylamino-2-benzothiazolinethione

To the mixture of 6-amino-2-benzothiazolinethione (18.2 g; 0.1 mol) and benzoyl chloride (16.8 g; 0.12 mol), dry pyridine (50 cm³, 49 g; 0.62 mol) was added, bringing about an exothermic reaction. The mixture was left to stand for 10 min and then it was heated on a water bath for 15 min, while it became completely solid. Cold water (500 cm³) was added and the mixture was acidified with concentrated hydrochloric acid to pH = 5. The solid portion was sucked and crystallized from the mixture of acetone—ethanol—water (volume ratio = 3:2:1) using charcoal. 6-Benzoylamino-2-benzothiazolinethione, m.p. =

Table 2

Anthelmintic activity/% (*in vivo*)

Helminths	Test No.	Compounds													
		II	*III*	*IV*	*V*	*VI*	*VII*	*XI*	*XII*	*XIII*	*XIV*	*XV*	*XVI*	*XVII*	*XVIII*
Aspiculuris tetraptera	*1*	0	0	0	0	70	0	0	0	0	0	0	0	50	60
	2	—	—	—	—	0	—	—	—	—	—	—	—	—	—
Heterakis spumosa	*1*	30	50	40	15	10	20	10	10	15	25	30	85	90	65
	2	70	0	—	—	—	0	—	—	—	—	0	15	20	—

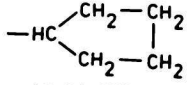
— = not tested.

Table 3

2-Alkylthio-6-benzoylaminobenzothiazoles

Compound	Alkyl	Formula	M_r	w_i(calc.)/% / w_i(found)/%				Yield	M.p.
				C	H	N	S	%	°C
I	$-CH_3$	$C_{15}H_{12}N_2OS_2$	300.40	59.97	4.03	9.33	21.35	95.0	191—191.5
				60.14	3.87	9.25	21.44		
II	$-C_2H_5$	$C_{16}H_{14}N_2OS_2$	314.43	61.12	4.49	8.91	20.39	94.5	145.5—146.5
				60.92	4.51	8.86	20.35		
III	$-(CH_2)_2CH_3$	$C_{17}H_{16}N_2OS_2$	328.46	62.17	4.91	8.53	19.52	70.1	130—132
				62.17	4.84	8.53	19.55		
IV	$-CH(CH_3)_2$	$C_{17}H_{16}N_2OS_2$	328.46	62.17	4.91	8.53	19.52	50.0	154—156
				61.97	4.98	8.54	19.29		
V	$-CH_2CH=CH_2$	$C_{17}H_{14}N_2OS_2$	326.44	62.55	4.32	8.58	19.64	65.1	134—136
				62.30	4.25	8.56	19.60		
VI	$-(CH_2)_3CH_3$	$C_{18}H_{18}N_2OS_2$	342.48	63.13	5.30	8.18	18.72	90.8	139—140
				62.81	5.27	8.02	18.65		
VII	$-CH_2CH(CH_3)_2$	$C_{18}H_{18}N_2OS_2$	342.48	63.13	5.30	8.18	18.72	71.6	151.5—153
				63.22	5.34	8.16	18.62		
VIII	$-CH(CH_3)C_2H_5$	$C_{18}H_{18}N_2OS_2$	342.48	63.13	5.30	8.18	18.72	72.2 (*A*)	148—149.5
				63.13	5.30	8.20	18.90	69.8 (*B*)	
								68.6 (*C*)	
IX	$-(CH_2)_4CH_3$	$C_{19}H_{20}N_2OS_2$	356.51	64.01	5.65	7.86	17.99	89.3	151—153
				64.23	5.69	7.90	17.80		

Table 3 (Continued)

Compound	Alkyl	Formula	M_r	w_i(calc.)/% / w_i(found)/%				Yield	M.p.
				C	H	N	S	%	°C
X	$-CH_2CH_2CH(CH_3)_2$	$C_{19}H_{20}N_2OS_2$	356.51	64.01	5.65	7.86	17.99	85.0	134.5—136
				63.73	5.42	7.58	17.88		
XI		$C_{19}H_{18}N_2OS_2$	354.49	64.38	5.12	7.90	18.09	53.3	165—166.5
				64.25	5.13	7.90	18.12		
XII	$-(CH_2)_5CH_3$	$C_{20}H_{22}N_2OS_2$	370.54	64.83	5.98	7.56	17.31	91.9	133.5—134
				64.67	6.08	7.56	17.33		
XIII	$-(CH_2)_6CH_3$	$C_{21}H_{24}N_2OS_2$	384.56	65.59	6.29	7.28	16.68	95.3	132—133.5
				65.30	6.35	7.23	16.63		
XIV	$-(CH_2)_7CH_3$	$C_{22}H_{26}N_2OS_2$	398.59	66.29	6.58	7.03	16.09	94.6	124—125
				66.11	6.47	7.02	15.91		
XV	$-(CH_2)_8CH_3$	$C_{23}H_{28}N_2OS_2$	412.62	66.95	6.84	6.79	15.54	99.1	135—136
				67.10	7.03	6.85	15.34		
XVI	$-CH_2C_6H_5$	$C_{21}H_{16}N_2OS_2$	376.50	66.99	4.28	7.44	17.03	91.2	178.5—179.5
				66.78	4.22	7.35	16.85		
XVII	$-CH_2-C_6H_4-p\text{-}CH_3$	$C_{22}H_{18}N_2OS_2$	390.53	67.66	4.65	7.17	16.42	89.7	182.5—184
				67.54	4.59	7.09	16.42		
XVIII	$-CH_2CH_2OH$	$C_{16}H_{14}N_2O_2S_2$	330.43	58.16	4.27	8.48	19.41	82.8	163—165
				58.44	4.29	8.41	19.40		

247—249 °C, was obtained in 20.8 g (72.7 %) yield; Ref. [2] gives m.p. 247—248 °C and 72 % yield.

2-Alkylthio-6-benzoylaminobenzothiazoles (I—XVIII)

Procedure A (I—XVIII)

To 6-benzoylamino-2-benzothiazolinethione (8.8 g; 0.03 mol) ethanol (75 cm^3), solution of potassium hydroxide (2.3 g; 0.036 mol) in water (25 cm^3), and alkyl iodide (0.036 mol) (in the case of *IV*, *V*, *XVII* alkyl bromide and in the case of *XVI* alkyl chloride) were added. The reaction mixture was refluxed for 5 to 45 min according to the nature of the alkyl halide. After cooling the mixture to 5 °C, 2-alkylthio-6-benzoylaminobenzothiazole precipitated from the solution.

Further purification was not necessary in the case of *IV*, *V*, *VI*, *X*, and *XVI*. The other compounds were crystallized from ethanol (*I*, *XIII*, *XV*), benzene (*IX*), ethyl acetate (*XVII*), mixture of ethanol—water in the volume ratio of 2:1 to 18:1 (*II*, *III*, *VII*, *VIII*, *XI*, *XII*, *XIV*, *XVIII*) by using charcoal.

Characterization of the synthesized 2-alkylthio-6-benzoylaminobenzothiazoles is presented in Table 3.

Procedure B (VIII)

The mixture of 6-amino-2-*sec*-butylthiobenzothiazole (7.15 g; 0.03 mol) and benzoic anhydride (22.6 g; 0.10 mol) was left to react at 80 °C for 10 min. After cooling 5 % solution of potassium hydroxide (200 cm^3) was added to the crushed melt. After dissolution of benzoic acid and discharge of the solution of its potassium salt, the dark sticky residue was crystallized from the mixture of ethanol—water (volume ratio = 5:1) using charcoal. 6-Benzoylamino-2-*sec*-butylthiobenzothiazole of m.p. = 148.5—150.5 °C, identical with the compound *VIII* prepared after the procedure *A*, was obtained in 69.8 % yield.

Procedure C (VIII)

The mixture of 6-amino-2-*sec*-butylthiobenzothiazole (4.75 g; 0.02 mol), benzoyl chloride (2.8 g; 0.02 mol), *N*,*N*-diethylaniline (3.00 g; 0.02 mol), and benzene (25 cm^3) was refluxed for 30 min. After removal of benzene by distillation under reduced pressure ethanol and water in the volume ratio of 5:1 (150 cm^3) was added to the distillation residue, the solution was decolorized with charcoal at boiling and after filtration ice water (500 g) was added. The precipitate was washed first with 0.5 % hydrochloric acid (500 cm^3), then with water (500 cm^3) and crystallized from the mixture of ethanol—water (volume ratio = = 5:1) using charcoal.

6-Benzoylamino-2-*sec*-butylthiobenzothiazole, m.p. = 148.5—150.5 °C, the mixed melting point of which with the samples prepared according to the procedures *A* and *B* gave no depression, was obtained in 68.6 % yield.

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