Benzothiazole compounds. XIII. Synthesis and antitubercular activity of some *N*-[1-(6-R-2-benzothiazolylamino)-2,2,2-trichloroethyl]formamide, -acetamide, -chloroacetamide, and -benzamide derivatives

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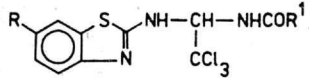
The preparation of *N*-[1-(6-R-2-benzothiazolylamino)-2,2,2-trichloroethyl]formamides, -acetamides, -chloroacetamides, and -benzamides from the original *N*-(1,2,2,2-tetrachloroethyl)amides and 2-amino-6-R-benzothiazoles (R = Cl, Br, NO_2, SCN, CH_3) is described. Their structures were proved by ^{1}H-n.m.r. spectra. When tested for antitubercular activity, the best results were obtained against the representatives of both the typical and atypical mycobacteria. With some compounds also the acute toxicity was investigated.

Описывается синтез *N*-[1-(6-R-2-бензотиазолиламино)-2,2,2-трихлорэтил]формамидов, -ацетамидов, -хлорацетамидов и -бензамидов, исходя из *N*-(1,2,2,2-тетрахлорэтил)амидов и 2-амино-6-R-бензотиазолов (R = = Cl, Br, NO_2, SCN, CH_3). Их структура была подтверждена интерпретацией спектров ^{1}H-ЯМР. При испытаниях на противотуберкулезное действие самые хорошие результаты были получены в случае представителя типичных и нетипичных микробактерий. Некоторые из соединений были испытаны также на острую токсичность.

On the basis of the results obtained in our previous works [1, 2] we studied the synthesis of new benzothiazole compounds. 2-Amino-6-R-benzothiazoles (R = Cl,

Table 1

Characterization of the synthesized *N*-[1-(6-R-2-benzothiazolylamino)-2,2,2-trichloroethyl]formamides, -acetamides, -chloroacetamides, and -benzamides



Compound	R	R^1	Formula	*M*	Calculated/found						Yield %	M.p. °C
					% C	% H	% N	% S	% Cl	% Br		
I	Cl	H	$C_{10}H_7ON_3SCl_4$	359.08	33.44	1.96	11.70	8.93	39.49	—	57	229—230
					33.43	2.01	11.66	8.87	39.78			
II	Cl	CH_3	$C_{11}H_9ON_3SCl_4$	373.11	35.41	2.43	11.26	8.59	38.01	—	59	246—248
					35.54	2.52	11.22	8.38	37.96			
III	Cl	C_6H_5	$C_{16}H_{11}ON_3SCl_4$	435.16	44.16	2.54	9.65	7.36	32.58	—	68	231—233
					44.32	2.55	9.51	7.26	32.45			
IV	Br	H	$C_{10}H_7ON_3SCl_4Br$	403.54	29.76	1.74	10.41	7.94	26.35	19.80	46	224—226
					29.90	1.90	10.59	8.11	26.11	19.61		
V	Br	CH_3	$C_{11}H_9ON_3SCl_3Br$	417.55	31.64	2.17	10.06	7.67	25.47	19.13	52	250—252
					31.67	2.20	10.19	7.50	25.21	19.34		
VI	Br	C_6H_5	$C_{16}H_{11}ON_3SCl_3Br$	479.62	40.06	2.31	8.76	6.68	22.17	16.66	55	233—234
					40.11	2.40	8.96	6.80	22.04	16.52		
VII	NO_2	H	$C_{10}H_7O_3N_4SCl_3$	369.63	32.49	1.90	15.15	8.67	28.77	—	47	250—251
					32.53	1.85	15.26	8.70	28.89			
VIII	NO_2	CH_3	$C_{11}H_9O_3N_4SCl_3$	383.65	34.44	2.36	14.60	8.35	27.72	—	45	250—252
					34.31	2.37	14.46	8.23	27.74			
IX	NO_2	C_6H_5	$C_{16}H_{11}O_3N_4SCl_3$	445.71	43.11	2.48	12.57	7.19	23.86	—	52	230—231
					43.02	2.45	12.59	7.09	23.88			
X	SCN	H	$C_{11}H_7ON_4S_2Cl_3$	381.70	34.61	1.84	14.67	16.79	27.86	—	53	195—197
					34.50	1.93	14.56	16.52	27.99			

Table 1 (Continued)

Compound	R	R^1	Formula	*M*	Calculated/found						Yield %	M.p. °C
					% C	% H	% N	% S	% Cl	% Br		
XI	SCN	CH_3	$C_{12}H_9ON_4S_2Cl_3$	395.71	36.42	2.29	14.15	16.20	26.87	—	57	240—242
					36.30	2.40	13.99	16.01	26.71			
XII	SCN	C_6H_5	$C_{17}H_{11}ON_4S_2Cl_3$	457.79	44.60	2.42	12.23	14.00	23.23	—	60	232—234
					44.53	2.50	12.11	14.01	23.41			
XIII	SCN	CH_2Cl	$C_{12}H_8ON_4S_2Cl_4$	430.17	33.50	1.87	13.02	14.90	32.97	—	58	207—209
					33.61	2.03	13.11	14.77	33.24			
XIV	CH_3	CH_2Cl	$C_{12}H_{11}ON_3SCl_4$	387.13	37.23	2.86	10.85	8.28	36.89	—	59	208—210
					37.37	3.01	10.97	8.07	36.87			
XV	CH_3	H	$C_{11}H_{10}ON_3SCl_3$	338.66	39.01	2.97	12.40	9.46	31.40	—	52	224—226
					39.18	2.96	12.47	9.63	31.42			
XVI	CH_3	CH_3	$C_{12}H_{12}ON_3SCl_3$	352.67	40.86	3.42	11.91	9.09	30.15	—	48	233—234
					40.61	3.52	11.82	9.11	30.26			
XVII	CH_3	C_6H_5	$C_{17}H_{14}ON_3SCl_3$	414.74	49.23	3.40	10.13	7.73	25.64	—	61	214—216
					49.30	3.44	9.98	7.55	25.80			

Br, NO_2, SCN, CH_3) [3, 4] and *N*-(1,2,2,2-tetrachloroethyl)formamide, -acetamide, -chloroacetamide, and -benzamide [5] prepared from *N*-(1-hydroxy-2,2,2-trichloroethyl)formamide, -acetamide, -chloroacetamide, and -benzamide [5—8] by chlorination with thionyl chloride were the starting components. The prepared chloro derivatives were used for further reaction immediately because of their instability.

The hitherto obtained knowledge on fungicidal activity of the *N*-(1,2,2,2-tetrachloroethyl)formamide compounds and their bis-derivatives indicated that the activity was dependent on the appropriate substituents and bridges in bis-derivatives, respectively [9—11]. The synthesized *N*-[1-(6-R-2-benzothiazolylamino)-2,2,2-trichloroethyl]formamides, -acetamides, -chloroacetamides, and -benzamides *I*—*XVII* (Table 1) were tested for antitubercular activity. The compounds *IX*, *X*, *XIII*, and *XV* showed good activity on a representative of atypical

Table 2

Antimycobacterial activity of compounds (MIC, μg/ml)

Compound	*M. tuberculosis* $H_{37}R_v$	*M. kansasii* *PKG 18*
I	25	50 (25)
II	100	100
III	50	>100
IV	25	50
V	50	50
VI	—	—
VII	>100	>100
VIII	25	50
IX	10	25 (10)
X	25	25
XI	50 (25)	25
XII	100 (50)	>100
XIII	10	10
XIV	100	>100
XV	10	50
XVI	>100	>100
XVII	>100	>100
2-MBT	25	50
Isoniazide	1	10
Ethionamide	1	10

The minimal inhibition concentration was read in Šule medium after 14 days incubation at 37°C.

The values in brackets mean partial inhibition concentration.

The standards used: 2-MBT — 2-mercaptobenzothiazole; Isoniazide — isonicotinohydrazide; Ethionamide — 2-ethylisonicotinothioamide.

mycobacteria which, at present, represent a serious therapeutical problem of mycobacteriosis. These compounds will be the subject of our further investigations. It is evident from the results in Table 2 that the activity against *Mycobacterium (M) tuberculosis* $H_{37}R_v$, a typical strain sensitive to antituberculotics (AT) (from the collection of the Research Institute of Epidemiology and Microbiology, Bratislava) and against *M. kansasii PKG 18*, an atypical strain resistant to AT (from the collection of E. H. Runyon, Salt Lake City, Utah, USA) was almost the same. The active compounds showed at the same experimental conditions values comparable with those of the known AT (Table 2).

The derivatives with R = Cl, Br (*I—V*) and varying R' (H, CH_3, C_6H_5) showed average antitubercular activity. With the compounds where R = NO_2 (*VII—IX*) the activity decreased from *IX* to *VII* in dependence on the R' (C_6H_5, CH_3, H). The compounds with R = SCN (*X—XIII*) and varying R' showed the highest activity in average. The most active compound was *XIII* where R' = CH_2Cl; the activity decreased with the compound *XII* where R' = C_6H_5. The group of compounds with R = CH_3 (*XIV—XVII*) and varying R' was the least active group except the compound *XV* which was fairly well active. Replace of the formamide group caused loss of activity. Among the compounds where R' = CH_3 (*II*, *V*, *VIII*, *XI*, *XVI*) and R varied (Cl, Br, NO_2, SCN, CH_3), *II* and *XVI* were found to be inactive. This finding indicated the influence of R. The group of compounds with R' = H (*I*, *IV*, *VII*, *X*, *XV*) showed the highest activity. It can be stated that the antitubercular activity of the synthesized compounds was dependent on the effects of the substituents R and R'. The toxicity of some of the investigated compounds, *dosis tolerata maxima* (DTM), was compared with the toxicity of isoniazide. As seen from Table 3, the toxicity was in most cases lower or the same as that of the standard.

Table 3

Acute toxicity of compounds (DTM, mg/kg) after administration *per os* in dimethyl sulfoxide to white mice

Compound	24 h	48 h
I	500	250
IV	>1000	1000
VIII	125	<60
X	250	60
XI	250	125
XIII	1000	250
XV	500	500
Isoniazide	125	125

Table 4

^{1}H-n.m.r. chemical shifts (δ) and interaction constants J (Hz)

Compound	R	R^1	N—H	H-4	H-5	H-7	C—H	R/R^1	J_{NH-CH}	J_{45}	J_{57}
II	Cl	CH_3	8.935	7.277	7.081	7.650	6.725	1.934	8.5	8.7	2.0
			8.848						9.0		
III	Cl	C_6H_5	9.266	7.440	7.230	7.816	7.088	7.3—7.9	8.7	8.7	2.2
			8.816						8.8		
IV[a]	Br	H	9.206	7.375	7.375	7.926	6.863	8.150	9.0	—	—
			9.154						9.5		
VI[a]	Br	C_6H_5	8.665	7.403	7.403	7.955	7.106	7.2—8.0	8.5	—	—
			8.233						9.0		
VIII	NO_2	CH_3	9.471	7.558	8.084	8.723	6.938	1.926	8.8	8.9	2.5
			8.985						9.1		
IX	NO_2	C_6H_5	9.411	7.612	8.106	8.753	7.200	7.3—7.9	8.2	8.8	2.5
X[a]	SCN	H	9.346	7.548	7.548	8.119	6.900	8.175	8.8	—	—
			9.260						8.8		
XII[a]	SCN	C_6H_5	8.703	7.536	7.536	8.103	7.125	7.3—7.9	8.7	—	—
			8.431						8.7		
XIII	SCN	CH_2Cl	9.275	7.542	7.502	8.099	6.885	4.203	8.9	8.6	1.5
XIV	CH_3	CH_2Cl	9.155	7.329	7.025	7.460	6.850	2.278	9.0	8.3	1.7
			8.835					4.200	8.8		
XV	CH_3	H	9.125	7.318	7.012	7.450	6.850	2.273	10.0	8.0	1.5
			8.889					8.125	8.2		
XVI	CH_3	CH_3	8.805	7.307	7.013	7.450	6.838	2.274	8.9	8.1	1.4
			8.700					1.915	8.0		
XVII	CH_3	C_6H_5	9.198	7.343	7.015	7.475	~7.000	2.288	8.4	8.4	1.5
			8.548					7.3—7.9	8.6		

a) Apparently simple spectra ($\Delta\delta_{4-5} \cong 0$).

In the ^{1}H-n.m.r. spectra of all compounds studied, two doublets and one doublet, respectively, with the interaction constants of 8—10 Hz were observed in the region of 9.5—8.2 δ (Table 4). These signals belonged to two NH groups and proved unambiguously that by alkylation of 2-amino-6-R-benzothiazoles with the appropriate chloro derivatives, *N*-[1-(6-R-2-benzothiazolylamino)-2,2,2-trichloroethyl]amides *I*—*XVII* were obtained. The observed relatively sharp NH signals as well as their split with the vicinal proton of C—H group indicated the presence of a strong electric field about the N atom. This strong electric field eliminated quadrupole broadening of the NH signals even at the absence of an immediate exchange of protons [12]. Also the values of interaction constants ~9 Hz were in accordance with those given in the literature [12].

Chemical shifts of aromatic protons of the benzothiazole skeleton were calculated after the ABX approximation of the first order for all derivatives except *IV*, *VI*, *X*, and *XII*. The interaction of *para* hydrogens was neglected because in benzothiazole derivatives J_{47} was ~0.4 Hz [13], which was in the range of resolution capability of the apparatus. The spectra of the mentioned derivatives were apparently simple ABX systems with $\Delta\delta_{AB} \cong 0$. We failed to obtain more precise spectral data because of their low solubility in other solvents.

The resonance signals of the C—H group were found in the region of 7.2—6.7 δ in the form of multiplet. This was caused by different conformers formed by

Table 5

Wavenumbers of the characteristic vibrations (cm^{-1})

Compound	ν(NH)	ν(C=O)	$\nu_{as}(NO_2)$, $\nu_s(NO_2)$	ν(C≡N)
I	3300 sh, 3210 m	1694 sh, 1666 s		
II	3235 m	1689 sh, 1680 s		
III	3325 m, 3245 m	1655 s		
IV	3211 m	1694 sh, 1667 s		
V	3235 m	1699 sh, 1677 s		
VI	3335 m, 3251 m	1654 s		
VII	—	—		
VIII	3332 m, 3230 m	1687 sh, 1676 s	~1505 s, 1333 s	
IX	3425 m, 3171 m	1664 s, 1655 sh	~1525 s, 1335 s	
X	3225 m	1696 sh, 1670 s		2161 m
XI	3385 m, 3260 m	1700 sh, 1676 s		2162 m
XII	3300 m	1653 sh, 1650 s		2159 m
XIII	3304 m, 3195 m	1689 s, ~1655 sh		2160 m
XIV	3325 m, 3205 m	1688 sh, 1683 s		
XV	3175 m	1686 sh, 1680 s		
XVI	3240 m	1688 sh, 1679 s		
XVII	3310 m, 3250 m	1653 sh, 1650 s		

s — strong, m — medium, sh — shoulder.

rotation about the C—N bonds as well as by the possibility of formation of inter- and intramolecular hydrogen bonds. Beside the above-mentioned signals also signals belonging to the appropriate functional groups R and R^1 were present in the spectra.

The wavenumbers of the characteristic absorption bands of the compounds *I—XVII* are listed in Table 5. In the region of the stretching vibrations of N—H and C=O bonds split absorption bands were observed. It could be explained by the presence of different conformers formed by rotation about the C—N bonds in the side chain on the benzothiazole ring as well as by intramolecular hydrogen bonds of the >C=O...H—N< or =N...H—N< types. The geometry of the above conformers and the nature of hydrogen bonds could not be investigated in detail because of the negligible solubility of the compounds in organic solvents (carbon tetrachloride, chloroform).

Table 6

Comparison of biological activity with ν(C=O)

Compound	R	R^1	ν(C=O)		MIC, μg/ml	
			cm^{-1} [a]	cm^{-1} [b]	*M. tuberculosis* $H_{37}R_v$	*M. kansasii* PKG 18
III	Cl	C_6H_5	1655	1655	50	>100
I	Cl	H	1666	1680	25	50 (25)
II	Cl	CH_3	1680	1684.5	100	100
VI	Br	C_6H_5	1654	1654	—	—
IV	Br	H	1667	1680.5	25	50
V	Br	CH_3	1677	1688	50	50
IX	NO_2	C_6H_5	1655	1659.5	10	25 (10)
VIII	NO_2	CH_3	1676	1681.5	25	50
XII	SCN	C_6H_5	1650	1651.5	100 (50)	>100
X	SCN	H	1670	1683	25	25
XI	SCN	CH_3	1676	1688	50 (25)	25
XIII	SCN	CH_2Cl	1689	1689	10	10
XVII	CH_3	C_6H_5	1650	1651.1	>100	>100
XVI	CH_3	CH_3	1679	1683.5	>100	>100
XV	CH_3	H	1680	1683	10	50
XIV	CH_3	CH_2Cl	1683	1685.5	100	>100

a) Band with the highest intensity.
b) Mean values of wavenumbers (from Table 5).

Comparison of biological activities of the studied compounds with the stretching vibrations of C=O, which could be taken for the measure of electron density on the C=O bond (Table 6), led to the following findings. The biological activity (when tested on the above-mentioned microorganisms) of the compounds with R = Cl, Br, NO_2 and varying R^1 decreased with the increasing electron density on the C=O bond. With the compounds where R = SCN, the biological activity increased strikingly with the increasing electron density on the C=O bond due to the influence of the R^1 substituents. This indicated a different mechanism of chemical processes at biochemical interaction of the 6-SCN derivatives from that of the above-mentioned 6-Cl, 6-Br, and 6-NO_2 derivatives. The 6-CH_3 derivatives had in general low biological activity which did not change with the changed electron density on the C=O bond due to the influence of the R^1 substituents. The only exception was the compound *XV* (R^1 = H) which showed increased biological activity when compared with that of other derivatives of this series. It is probable that the carbonyl group in the compounds where R = CH_3 does not participate in the biochemical process which is deciding for the activity of these compounds.

Experimental

The results of elemental analysis and the physicochemical constants of the synthesized compounds are presented in Table 1.

Antimycobacterial activity was followed by the diluting method in the Šule liquid medium according to the procedure used at the screening of antitubercular activity [14]. The results are in Table 2.

The acute toxicity was investigated by administration of some of the synthesized compounds (DTM) to white mice with oesophageal sound after *Wagner* [15] in 1000, 500, 250, 125, and 60 mg/kg (Table 3). The compounds were dissolved in dimethyl sulfoxide and 0.5 ml/mouse was administered.

The ^{1}H-n.m.r. spectra of the studied compounds were measured on a Tesla BS 487 apparatus with the working frequency of 80 MHz. The compounds were measured in deuterated dimethyl sulfoxide (DMSO) (concentration 10%) at 25°C using hexamethyldisiloxan (HMDSO) as internal standard. The chemical shifts and the interaction constants were read with the accuracy of ±0.002 p.p.m. and ±0.3 Hz, respectively. The results are presented in Table 4.

The i.r. spectra were taken on a Perkin—Elmer 567 spectrophotometer in paraffin oil suspensions. The results are in Table 5.

N-[1-(6-Chloro-2-benzothiazolylamino)-2,2,2-trichloroethyl]benzamide (III)

2-Amino-6-chlorobenzothiazole (10 g; 0.054 mole) was dissolved in dry acetone (200 ml) and triethylamine (5.4 g; 0.053 mole) was added. At the temperature of the

mixture (40—50°C), the solution of *N*-(1,2,2,2-tetrachloroethyl)benzamide (15.5 g; 0.054 mole) in dry acetone (90 ml) was added dropwise at stirring. After 24 h staying at room temperature, the formed crystalline triethylammonium hydrogen chloride was filtered and the filtrate was evaporated *in vacuo*. The isolated product was crystallized from the mixture of ethanol—acetone (2 : 1).

The compounds *I*, *II*, *IV*—*XVII* (Table 1) were prepared similarly. In the case of *IX*, *XI*, *XII*, and *XVII*, the filtrate (after removing triethylammonium hydrogen chloride) was poured onto crushed ice and the product was isolated after 24 h staying in refrigerator.

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