Benzothiazole compounds. XVI. Preparation and antimycobacterial activity of *N'*,*N'*-bis[(2-thioxo--3-benzothiazolinyl)methyl]hydrazides

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The reaction of 2-mercaptobenzothiazole with formaldehyde and hydrazides of acids resulted in bis derivatives of Mannich bases. The relationship between the formation of bis derivatives and the α effect in hydrazides is explained. The prepared compounds were tested for antimycobacterial activity.

При реакции 2-меркаптобензтиазола с формальдегидом и гидразидами кислот образуются бис производные оснований Манниха. Объясняется взаимосвязь между их образованием и α-эффектом в гидразидах. Приготовленные соединения были испытаны на антимикобактериальную активность.

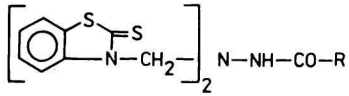
In the previous work [1] we presented the results of studies of the reactions of 2-mercaptobenzothiazole (2-MBT) with formaldehyde and primary amines and found a relationship between the basicity of amines and formation of mono and bis derivatives, respectively. The aim of the present work was to find out if the same relationship is valid when hydrazides of acids are used as amino components of the Mannich reaction. Regarding the basicity of hydrazides of acids it can be assumed that, by the reaction of the above-mentioned components, mono derivatives of Mannich bases will be formed.

With regard to the earlier proved activity [2] of the compound *XII* (Table 3), the prepared bases were tested for antimycobacterial activity in order to find out the influence of the acid part of the molecule on the activity of compounds.

By the Mannich reaction of 2-MBT with aliphatic and aromatic hydrazides of acids and formaldehyde, bis derivatives are formed (Table 1).

According to the conclusions of the work [1] regarding the pK_B values of hydrazides (acetohydrazide 10.76, benzohydrazide 9.90, isonicotinohydrazide 12.30) we assumed that mono derivatives would be formed similarly as in the

Table 1. Characterization of the prepared compounds



Compound	R	Formula	*M*	Calculated/found				Yield %	M.p. °C
				% C	% H	% N	% S		
I	CH_3	$C_{18}H_{16}N_4OS_4$	432.61	49.97	3.72	12.95	29.64	21	205—207
				49.50	3.79	12.64	29.55		
II	CH_3CH_2	$C_{19}H_{18}N_4OS_4$	446.64	51.09	4.06	12.54	28.71	20	225—227
				50.75	4.18	12.31	28.32		
III	$CH_3(CH_2)_2$	$C_{20}H_{20}N_4OS_4$	460.67	52.14	4.37	12.16	27.84	32	207—209
				51.94	4.30	12.14	28.11		
IV	$(CH_3)_2CH$	$C_{20}H_{20}N_4OS_4$	460.67	52.14	3.37	12.16	27.84	30	236—238
				51.84	4.33	12.00	28.03		
V	$CH_3(CH_2)_3$	$C_{21}H_{22}N_4OS_4$	474.69	53.13	4.67	11.80	27.01	30	209—211
				53.29	4.64	11.81	27.31		
VI	$CH_3(CH_2)_6$	$C_{24}H_{28}N_4OS_4$	516.77	55.78	5.46	10.84	24.81	20	185—187
				55.80	5.55	10.69	24.50		
VII	Phenyl	$C_{23}H_{18}N_4OS_4$	494.68	55.84	3.66	11.32	25.92	55	241—243
				55.92	3.68	11.31	25.73		
VIII	4-Tolyl	$C_{24}H_{20}N_4OS_4$	508.71	56.66	3.96	11.01	25.21	35	333—335
				56.72	4.20	10.82	24.75		
IX	Salicyl	$C_{23}H_{18}N_4O_2S_4$	510.68	54.09	3.55	10.97	25.11	76	200—203
				53.85	3.35	10.79	25.09		
X	3,4,5-$C_6H_2(OH)_3$	$C_{23}H_{18}N_4O_4S_4$	542.68	50.90	3.34	10.32	23.63	50	Decomposition
				50.63	3.20	10.00	23.41		
XI	3-NO_2 phenyl	$C_{23}H_{17}N_5O_3S_4$	539.68	51.18	3.17	12.97	23.76	75	236—238
				50.70	3.28	13.01	23.36		
XII	4-Pyridyl	$C_{22}H_{17}N_5OS_4$	495.67	53.30	3.45	14.13	25.87	80	229—231
				53.34	3.58	14.31	26.10		

reactions with primary amines of comparable basicity (pK_B of aniline 9.42, *p*-toluidine 8.93, *o*-nitraniline 14.28 [3]). The discrepancy between the assumed and the real course of reactions can be explained by the supposition that the formation of mono and bis derivatives, respectively, is dependent not only on basicity but also on nucleophility of the used hydrazides of acids. It is known that the basicity of amines is equal to nucleophility, however, the nucleophility of hydrazides of acids increases due to the α effect [4]. The connection between the formation of bis derivatives in the Mannich reactions and the α effects in the hydrazides of acids is proved also by the differences in the nucleophility of these compounds in comparison with primary amines [4].

As tests for antimycobacterial activity (Table 2) only slight differences were found between the effectiveness of aliphatic and aromatic acid parts of the

Table 2

Antimycobacterial activity (MIC in μg/ml) of the prepared compounds

Compounds	*M. tuberculosis* $H_{37}R_v$	*M. kansasii*
I	50	100
II	50	50
III	50	100
IV	50	100
V	50	100
VI	25	50
VII	50	50
VIII	50	50
IX	50	100
X	25	100
XI	50	100
XII	1	10
2-MBT	25	10

molecules. The activity of the compound *XII* containing a heterocyclic acid residue differed significantly. When comparing its structure with that of the compound *VII* (Table 1) it is evident that the difference in the activities of these two most similar compounds is due to the nitrogen atom in the aromatic ring, bound in *para* position to the hydrazide group. The activity of *XII* differed from that of isonicotinohydrazide (INH) (Table 3). This difference is especially significant with resistant strains of mycobacteria, which points to the activity of benzothiazolinethione parts of the molecules. In the series of these tests also the activity of 2-MBT (Table 2) was examined. Regarding the similar activity of *XII* and the used antituberculotics (Table 3), the acute toxicity of *XII* was informatively determined. Dosis tolerata

Table 3

Comparison of the activity of the compound *XII* (MIC in μg/ml) with the used medicaments

Compounds	*M. tuberculosis* $H_{37}R_v$	INH-R[a] 3161[b]	INH-R 19811	*M. bovis*	*M. avium*	*M. kansasii*
XII	1	1	0.5	1	25	10
INH	0.1	25	10	1	25	10
Ethionamide	1	10	1	5	25	5
Etambutol	1	1	1	1	10	25

a) INH-resistant strain of mycobacteria.
b) The number of the strain from the collection.

maxima was after 24 h 1000 mg/kg and after 48 h 500 mg/kg. With INH this value was 125 mg/kg after 24 and 48 h, respectively.

The activity of all compounds prepared was examined also on *Mycobacterium avium* and *M. fortuitum.* The MIC values were 100 μg/ml, excepted the compound *XII* where this value was 25 μg/ml for both types.

Experimental

2-Mercaptobenzothiazole was obtained from the rubber accelerator Kaptax according to the purification method after *Sutoris* and coworkers [5]. Hydrazides of acids were prepared by the reaction of hydrazine hydrate with the appropriate chlorides and esters, respectively anhydrides of acids. The aliphatic hydrazides were obtained in 16—40% yields, the aromatic ones in 40—90%. For the activity tests the following strains were used: a virulent strain *M. tuberculosis* $H_{37}R_v$ (sensitive to antituberculotics), *M. avium, M. bovis 42 Z/75* from the Research Institute of Preventive Medicine, Centre of Epidemiology and Microbiology, Bratislava, *M. tuberculosis INH-R* (INH-resistant) No. 3161 and No. 19811 from the collection of the Institute of Transpiration Illness, Podunajské Biskupice (Czechoslovakia) as well as strains of *M. kansasii PKG-8* (an atypical mycobacterium) from the collection of Dr. Runyon (Salt Lake City, Utah, USA), and *M. fortuitum* from the collection of Professor Hauduroy (Faculty of Medicine, Lausanne, Switzerland). For the *in vitro* tests the classical dilution method modified for mycobacteria was used [6]. Dosis tolerata maxima was determined after *Wagner* [7] by simple dosing of compounds to mice by oesophageal probe. At different concentrations of compounds, 100% survival of animals was followed.

N',N'-bis[(2-Thioxo-3-benzothiazolinyl)methyl]hydrazides of acids

Hydrazide of acid (0.01 mol), 2-MBT (0.02 mol), and absolute ethanol (20 ml) were heated until a solution was formed. Then 34% formaldehyde (0.025 mol) was added and the mixture was heated under reflux for 30 min. Then it was allowed to stay at room

temperature for 20 h. The crystalline compound was sucked on a Büchner funnel and washed with dry ethanol (15 ml) and ether (15 ml).

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