ACYLATED THIOAMIDES AND RELATED COMPOUNDS AS POTENTIAL ANTITUBERCULOTICS

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Some N-acylthioamides, thiobenzoylthioureas, and thiobenzoylureas were synthesized. The substances prepared were tested for their *in vitro* antimycobacterial activity. The structure of thiobenzoylureas was studied by synthetic and spectral methods.

The antimycobacterial activity of aromatic thioamides can be changed by substitution of the aromatic ring[1,2] and the functional group[3]. Substitution on the nitrogen atom of the functional group affects the antimycobacterial activity quite substantially, but, unfortunately, substitution with an aryl group which most distinctly increased the antimycobacterial activity[3] was also frequently accomapnied by an increase in toxicity[4]. This motivated us to attempt the preparation of such N-substituted thioamides the substituent of which withdrew the electrons of the functional groups similarly as an aryl group, but did not increase toxicity at the same time. For this purpose acetyl and its homologues were selected. In order to compare the biological activities of the preparations obtained in this manner, thioamides were also condensed with isothiocyanates and isocyanates, because in comparison with the literature[5], at least in some instances, it was possible to expect an increase in antimycobacterial activity.

The acylation of thioamides with anhydrides or aliphatic acyl chlorides is known[6-8]. The condensation of thioamides with isothiocyanates is also described[9]. Relatively least atention has been devoted to condensation reactions of thioamides with aryl-isocyanates[10,11]. The present paper is devoted to the extension of the experimental material from this field of substances, both from the synthetic point of view, as well as that of antimycobacterial activity determined *in vitro*.

EXPERIMENTAL

Using a general method[12] nitriles were prepared from benzylmethylamine (Koch-Light) or piperidine (Loba), formaldehyde, and potassium cyanide (both Lachema) which were converted in crude state to thioamides with hydrogen sulfide[13], after dissolution in a mixture of pyridine

and triethylamine. The thioamides were acylated with acid anhydrides in the described manner[6]. The reaction product was crystallized twice from a water–ethanol mixture (1 : 4). The condensation of thioamides with isothiocyanates was carried out in alkaline 50% ethanol, according to literature[9]. A double crystallization from water–ethanol gave analytically pure preparations.

The method used earlier for the condensation of thioamides with isothiocyanates[9] was adapted for the reaction of thioamides with isocyanates. Isocyanate (0·01 mol) was added in small portions to a stirred solution of 0·01 mol of thioamide and 0·01 mol KOH in 50 ml of 50% ethanol. After 15 min the mixture was diluted with 50 ml of water and the corresponding thiobenzoylurea was set free by careful neutralization of the solution with hydrochloric acid, under cooling. The crude product was recrystallized from dimethyl sulfoxide–water. The results of the analyses and melting points of all newly prepared substances are given in Table I.

The thioacetamide employed for the syntheses was of commercial origin (Lachema), the same as acetic, propionic, and butyric anhydrides which were distilled before synthesis. Methyl isothiocyanate (Fluka), phenyl isothiocyanate (Lachema), *p*-chlorophenyl isothiocyanate (Fluka), allyl isothiocyanate (Merck), phenyl isocyanate (Riedel–De Haen), *p*-chlorophenyl isocyanate and *m*-chlorophenyl isocyanate (both Merck) were of commercial origin and they were used without further purification.

In order to confirm the structures of N-thiobenzoylureas a solution of 0·01 mol of N-thiobenzoyl-N′-phenylurea in alcohol was mixed with a solution of 0·01 mol of $Hg(ClO_4)_2$ in 80% alcohol. The resulting solution rapidly produced a black precipitate of HgS. In order to complete the reaction the mixture was neutralized and mildly heated. The solution was filtered while still warm, then diluted with water and allowed to stand for crystallization. The sedimented white crystals melted at 198°C. The melting point of their mixture with N-benzoyl-N′-phenylurea prepared in a different manner[14] was undepressed and the analytical results of both preparations agreed well (within the limits of experimental error).

The UV spectrum of N-acetylthiobenzamide was measured in a $4 \cdot 10^{-3}$ mol dm^{-3} ethanolic solution on a Pye–Unicam SP-8-100 instrument. The IR spectra were recorded on a Specord IR-75 instrument in dichloromethane, in KBr cells of 0·6 mm strength, and also using the KBr technique. The antimycobacterial activity was tested *in vitro* by a method described earlier[2,15].

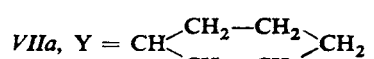
RESULTS AND DISCUSSION

All the substances in this paper have the general formula Y—C(S)—NH—X. By combining substituents X and Y the substances mentioned below can be derived, which were used for antimycobacterial tests. The following substances were prepared and tested (the preparations prepared according to other authors have the reference in brackets): *Ia*(16), *Ib*(6), *Ic*(6), *Id*(6), *Ie*(9), *If*, *Ig*(9), *Ih*, *Ii*(17), *Ij*, *Ik*, *IIa*(18), *IIb*(6), *IIc*(6), *IId*(6), *IIg*, *IIh*, *IIi*(11), *IIj*, *IIk*, *IIIa*(19), *IIIb*(8), *IIIc*, *IIId*,*IIIg*(9), *IIIh*, *IIIi*(20), *IIIj*, *IIIk*, *IVa*(21), *IVb*(8), *IVc–f*, *IVg*(9), *IVi*(20), *IVj*, *Va*(22), *Vb*, *Vc*, *Ve–i*, *VIa*, *VIb*(7), *VIc*(23), *VId*(23), *VIi–k*, *VIIa*, *VIIIa*. The results of the analyses of newly prepared compounds and their melting points are given in Table I.

Y—C(S)—NH—X

Ia–k, Y = C_6H_5
IIa–k, Y = C_6H_4-CH_3(*p*)
Va–h, Y = C_6H_4-Cl(*m*)
VIa–k, Y = CH_3

IIIa–k, Y = C_6H_4-OCH_3(*p*)
IVa–j, Y = C_6H_4-Cl(*p*)

VIIa, Y = 
VIIIa, Y = CH_2—$N(CH_3)$—C_7H_7

In formulae *I*—*VIII*:

a, X = H
b, X = CO—CH_3
c, X = CO—C_2H_5
d, X = CO—C_3H_7
e, X = O(S)—NH—CH_3
f, X = C(S)—NH—CH_2—CH=CH_2
g, X = C(S)—NH—C_6H_5
h, X = C(S)—NH—C_6H_4-Cl(*p*)
i, X = C(O)—NH—C_6H_5
j, X = C(O)—NH—C_6H_4-Cl(*p*)
k, X = C(O)—NH—C_6H_4-Cl(*m*)

From the structural point of view, all the substances used in this paper can be classified into three groups: Acylated thioamides, N-thioacylated thioureas, and N-thioacylated N′-arylureas. The structure of N-acylthioamides has been solved already[24–26]. The red colour of these substances is due to the sharp maximum which is in the case of *Ib* at 476 nm. Its molar decadic absorption coefficient is $1{\cdot}7 \cdot 10^2$ $dm^3 \cdot mol^{-1} cm^{-1}$. The substances prepared by us had similar properties to those described earlier[6] and therefore we consider that their structure is analogous. The structure of N-thiobenzoylthioureas also seems to be solved[9] and it is probable that the remaining substances of this group listed in Table I have the same structure as the basic N-thiobenzoyl-N′-phenylthiourea.

In the case of substances formed on reaction of isocyanates and thioamides the situation seems less clear. Their structure has not been studied in detail so far. The molecule of thioamide has two nucleophilic centres onto which isocyanate can be bound, *i.e.* the atoms S and N. Therefore, we may expect the formation of two series of derivatives, represented by formulae *IX* and *X*.

R—C(S)—NH—C(O)—NH—R′ *IX*

R—C(=NH)—S—C(O)—NH—R′ *X*

The first step in the elucidation of the structure of the given group of substances is the localization of the acyl group. A proof consists in the reaction of *Ii* with Hg^{2+} ions, which proceeds as in equation (*A*).

$$C_6H_5—C(S)—NH—C(O)—NH—C_6H_5 + Hg^{2+} + H_2O \rightarrow$$
$$\rightarrow HgS + 2\,H^+ + C_6H_5—C(O)—NH—C(O)—NH—C_6H_5 \qquad (A)$$

The isolation of the reaction product from the filtrate, its analysis and the undepressed mixture melting point (with authentic N-benzoyl-N′-phenylurea) represent chemical proof of the localization of the acyl and thus proof of the validity of struc-

TABLE I

Melting points and analyses of the newly prepared compounds

Compound	Formula (M.w.)	Melting point, °C (solvent)	Calculated/found			
			% C	% H	% N	% Cl
If	$C_{11}H_{12}N_2S_2$ (236·3)	67—68 (ethanol–water)	55·93 55·59	5·08 4·85	11·86 12·19	
Ih	$C_{14}H_{11}ClN_2S_2$ (306·7)	144—146 (ethanol–water)	54·90 55·35	3·52 3·49	9·15 8·93	11·43 11·04
Ik	$C_{14}H_{11}ClN_2OS$ (290·6)	210—211 (ethanol–water)	57·80 58·14	3·81 3·77	9·62 9·49	— —
IIg	$C_{15}H_{14}N_2S_2$ (286·4)	131—134 (ethanol–water)	62·90 63·06	4·89 4·81	9·79 9·50	
IIh	$C_{15}H_{13}ClN_2S_2$ (320·7)	152—154 (ethanol–water)	56·25 56·39	4·06 3·90	8·75 8·53	10·94 10·52
IIj	$C_{15}H_{13}ClN_2OS$ (304·7)	249 (dimethyl sulfoxide–water)	59·12 59·65	4·29 4·17	9·19 9·45	11·62 11·80
IIk	$C_{15}H_{13}ClN_2OS$ (304·7)	205—207 (dimethyl sulfoxide–water)	59·12 59·56	4·29 4·23	9·19 9·41	11·62 11·36
IIIc	$C_{11}H_{13}NO_2S$ (223·3)	143—144 (ethanol–water)	58·90 58·32	5·82 5·85	6·25 6·55	
IIId	$C_{12}H_{15}NO_2S$ (237·3)	115—117 (ethanol–water)	60·75 61·27	6·36 6·18	5·90 5·84	
IIIh	$C_{15}H_{13}ClN_2OS_2$ (336·7)	158—160 (ethanol–water)	53·57 53·38	3·87 3·75	8·33 8·28	10·42 10·53
IIIj	$C_{15}H_{13}ClN_2O_2S$ (320·7)	218—220 (dimethyl sulfoxide–water)	56·16 56·40	4·08 3·95	8·73 8·65	11·05 10·90
IIIk	$C_{15}H_{13}ClN_2O_2S$ (320·7)	197—198 (acetone–water)	56·16 56·28	4·08 4·25	8·73 9·02	11·05 10·85
IVc	$C_{10}H_{10}ClNOS$ (227·5)	98—101 (ethanol–water)	52·76 52·78	4·96 4·51	6·15 6·11	15·55 15·45
IVd	$C_{11}H_{12}ClNOS$ (241·5)	108·5—110 (ethanol–water)	54·63 54·84	5·41 5·42	5·79 5·49	14·65 14·55
IVe	$C_9H_9ClN_2S_2$ (244·6)	132—135 (ethanol–water)	44·20 43·81	3·69 3·55	11·48 11·47	14·34 14·22
IVf	$C_{11}H_{11}ClN_2OS$ (270·6)	92·5—94 (ethanol–water)	48·89 46·68	4·04 3·97	10·37 10·41	12·90 12·53
IVj	$C_{14}H_{10}Cl_2N_2OS$ (325·1)	245 (dimethyl sulfoxide–water)	51·71 51·95	3·10 2·80	8·61 8·23	21·77 21·53

TABLE I
(*Continued*)

Compound	Formula (M.w.)	Melting point, °C (solvent)	Calculated/found			
			% C	% H	% N	% Cl
Vb	C_9H_8ClNOS	110–113	50·47	4·20	6·53	—
	(213·5)	(ethanol–water)	50·28	3·86	6·63	—
Vc	$C_{10}H_{10}ClNOS$	106–107	52·76	4·86	6·15	15·55
	(227·5)	(ethanol–water)	52·96	4·48	6·17	15·75
Ve	$C_9H_9ClN_2S_2$	107–109	44·26	3·69	11·48	14·34
	(244·7)	(ethanol–water)	44·63	3·57	11·48	14·31
Vf	$C_{11}H_{11}ClN_2S_2$	77–78	48·89	4·04	10·37	12·90
	(270·6)	(ethanol–water)	48·63	3·82	10·08	12·40
Vg	$C_{14}H_{11}ClN_2S_2$	139–141	54·90	3·59	9·15	11·44
	(306·7)	(ethanol–water)	55·09	3·44	9·07	11·14
Vi	$C_{14}H_{11}ClN_2OS$	110–112	58·03	3·82	9·66	11·89
	(290·7)	(dimethyl sulfoxide–water)	57·71	3·77	9·51	11·48
Vk	$C_{14}H_{10}Cl_2N_2OS$	202–204	51·70	3·10	8·61	21·77
	(325·1)	(dimethyl sulfoxide–water)	52·20	2·93	8·40	21·54
VIi	$C_9H_{10}N_2OS$	175–177	55·65	5·19	14·42	
	(194·8)	(dimethyl sulfoxide–water)	55·70	5·14	14·55	
VIj	$C_9H_9ClN_2OS$	215–217	47·26	3·79	12·24	15·52
	(229·2)	(dimethyl sulfoxide–water)	47·56	3·92	12·70	14·91
VIk	$C_9H_9ClN_2OS$	186–188	47·26	3·79	12·24	—
	(229·2)	(dimethyl sulfoxide–water)	47·35	3·98	12·23	—
VIIa	$C_7H_{14}N_2S$	96–97·5	53·13	8·91	17·69	
	(158·2)	(ethanol–water)	53·12	8·77	17·95	
VIIIa	$C_{10}H_{14}N_2S$	79–81	61·82	7·26	14·42	
	(194·3)	(ethanol–water)	62·41	7·19	14·99	

ture *IX*. This chemical proof was completed by the determination of the prevalent tautomer by infrared spectral measurements of the given group of substances (see Table II), in which broad absorption bands in the 3 360 cm^{-1} region are clearly visible in consequence of the stretching vibration of the N—H bonds, as well as an intensive band in the 1 730–1 710 cm^{-1} (CH_2Cl_2) and about the 1 695 cm^{-1} (KBr) region, representing the stretching vibration of the carbonyl group.

Both groups of vibrations again agree with formula *IX*. The stretching vibrations of the C=C bonds of the aromatic ring appear as an intensive band within a narrow

range of about 1 600 cm^{-1}. The position of the carbonyl band is slightly dependent on the nature of the substituents of both nuclei; the frequency of this vibration increases with their electron-accepting properties. Finally, polarographic oxidation of N-thiobenzoyl-N'-phenylureas[27] also agrees with the structure *IX*, because the substance of structure *X* could not produce anodic oxidation waves.

The confirmation of the analogous structure of all three groups of substances enables comparison of the effect of the substituent on the antimycobacterial activity of the preparations synthesized. The quantitative parameter of this activity is the minimal inhibition concentration (M.I.C.) which stops the growth of the mycobacteria. In this field our attention was focused to the most common strains, $H_{37}Rv$ and PKG 8. The results of the experiments are presented in Table III. From the literature[28] it is known that an increasing polarization of the thiocarbonyl bond brings about an increase in the antimycobacterial activity. However, it may be expected at the same time – owing to the non-polar components of the cell membrane of the mycobacterium[29] – that the polar substances will penetrate only with difficulty from the aqueous medium into the cell of the mycobacterium. Therefore it cannot be expected that more polarization of the C=S bond, due to the withdrawal of the electrons from the functional group, will affect the biological activity. In fact, all the substituents used in this paper affect the functional group in this manner, but according to Table III it is evident that the effect does not have a dominant character. We believe that the low solubility of thiobenzoylureas in water determines the decrease of the antimycobacterial activity in comparison with the basic thioamide, but that it increases the antimycobacterial activity of N-thioacetylureas in which an increase in lipophilicity may be expected in comparison with thioacetamide. The high antimycobacterial activity of N-thiobenzoylthioureas is probably due to the superposition of the activity of thioamide and isothiocyanate[5].The most striking example is the comparison of the activities of compounds *IIIa* and *IIIh*, where the last mentioned compound has an antimycobacterial activity equalling that of Ethionamide. Only in the group of N-acylthioamides the striking increase in the antimyco-

TABLE II

Stretching vibrations (in cm^{-1}) of newly prepared N-thiobenzoylureas in CH_2Cl_2[a]

Compound	*Ik*	*IIj*	*IIIj*	*IIIk*	*Vi*	*VIi*	*VIj*	*VIk*
ν(N—H)	3 357	3 370	3 360	3 360	3 356	3 364	3 357	3 368
ν(C=O)	1 724	1 714	1 728	1 716	1 723	1 607	1 598	1 731

[a] KBr technique gave for *IVj* ν(C=O) = 1 690 cm^{-1} and for *Vk* ν(C=O) = 1 694 cm^{-1}.

bacterial activity, referred to the basic thioamide, can be attributed predominantly to the increase in polarization of the bonds of the functional group. Even though at present the effect of the substituent on the antimycobacterial activity of a substance[28] can be predicted only very roughly, a tenfold increase in antimycobacterial activity of thioamides after acetylation, or by condensation of thioamides with isothiocyanates opens up further possible directions in the development of the chemistry of the group of biologically active substances discussed in this paper.

TABLE III

Minimal inhibitory concentration of the prepared substances, against selected mycobacterial strains

Compound	M.I.C., 10^4 mol dm^{-3}		Compound	M.I.C., 10^4 mol dm^{-3}	
	$H_{37}Rv$	PKG 8		$H_{37}Rv$	PKG 8
Ia	3·3	10	*IIIk*	>10	>10
Ib	0·37	1·1	*IVa*	3·3	10
Ic	0·37	1·1	*IVb*	0·37	3·3
Id	0·37	1·1	*IVc*	0·37	1·1
Ie	1·1	1·1	*IVd*	0·37	3·3
If	1·1	1·1	*IVe*	0·12	1·1
Ig	0·37	0·37	*IVf*	0·12	1·1
Ih	0·37	0·37	*IVg*	1·1	3·3
Ii	10	10	*IVi*	10	>10
Ik	10	10	*IVj*	3·3	>10
IIa	3·3	10	*Va*	2·4	10
IIb	0·37	3·3	*Vb*	1·1	3·3
IIc	1·1	3·3	*Vc*	0·37	3·3
IId	0·37	1·1	*Ve*	0·37	1·1
IIg	1·1	1·1	*Vf*	0·37	0·6
IIi	10	>10	*Vg*	1·1	3·3
IIj	10	3·3	*Vi*	3·3	10
IIk	10	10	*Vk*	10	>10
IIIa	3·3	10	*VIa*	10	>10
IIIb	0·37	1·1	*VIi*	1·1	3·3
IIIc	0·37	1·1	*VIj*	1·1	3·3
IIId	0·37	1·1	*VIk*	1·1	3·3
IIIg	1·1	1·1	*VIIa*	3·3	10
IIIh	0·12	0·37	*VIIIa*	3·3	3·3
IIIi	0·37	1·1	I.N.H.[a]	0·041	0·37
IIIj	10	>10	Ethionamid	0·12	0·37

[a] Isonicotinic acid hydrazide.

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