CCA-22 547.497.1

Syntheses in the 4-Substituted Thiosemicarbazide Series

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Received July 28, 1956

In connection with the investigations of tuberculostatic activity of arylthiosemicarbazides and arylthiosemicarbazones new 4-substituted thiosemicarbazides (I—XVIII) were synthesized. Condensation of these with aldehydes or ketones afforded the corresponding 4-substituted thiosemicarbazones (XIX—LVI). The attempted acetylation of *p*-nitrobenzaldehyde-4-phenylthiosemicarbazone and *o*-chlorobenzaldehyde-4-phenylthiosemicarbazone gave besides acetanilide N,N.-diacetyl-*p*-nitrobenzylidenhydrazine (LVII) and N,N-diacetyl-*o*-chlorobenzylidenehydrazine (LIX). The 4-substituted thiosemicarbazides and thiosemicarbazones were tested for their tuberculostatic activity.

Preliminary investigations in the series of 4-substituted thiosemicarbazides and thiosemicarbazones have shown that some of these substances possess a remarkable tuberculostatic activity[24]. Therefore it was decided to prepare other hitherto unknown 4-substituted thiosemicarbazides and their derivatives and investigate their activity.

Thiosemicarbazides could be synthesized by one of the following general methods[20]: by action of hydrazine hydrate on isothiocyanates or by action of hydrazine hydrate on substituted thioureas. As the latter reaction in many cases does not proceed smoothly and is accompanied by different by-products, we have chosen the first route which, if conducted under proper conditions, leads to thiosemicarbazides in excellent yields.

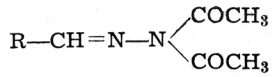
$$R—NCS + NH_2NH_2 = R—NH—CS—NH—NH_2$$

For the preparation of isothiocyanates we have chosen the method of decomposing the ammonium salts of dithiocarbamic acids with lead nitrate, as this method is simple and requires no special precautions (as in the case of thiophosgenation). The formation of ammonium salts of dithiocarbamic acids from *m*-nitroaniline and *o*-chloroaniline failed because of the low basicity of both amines. It is interesting that *p*-chloroaniline (pK_b 12,0) with almost the same basicity as *o*-chloroaniline (pK_b 12,05) could be readily converted into the salt of dithiocarbamic acid. Both before mentioned isothiocyanates were therefore prepared from the corresponding disubstituted thioureas[15, 23].

The 4-substituted thiosemicarbazides were easily prepared from the corresponding isothiocyanates, by dissolving the latter compounds in ethanol and

adding aqueous hydrazine hydrate in small excess. The yields were almost quantitative. 4-(4'-methoxyphenyl)- and 4-(4'-chlorophenyl)-thiosemicarbazide have been already described in the literature but they were prepared in a different way, e. g. from disubstituted thioureas. Also 4-(2'-naphtyl)-thiosemicarbazide has been mentioned in literature[7] but neither method of preparation nor melting-point have been mentioned. The 4-substituted thiosemicarbazides are stable and crystalline substances which readily undergo condensations with aldehydes and ketones to form stable 4-substituted thiosemicarbazones. Some of the 4-substituted thiosemicarbazides have been already proposed for the identification and characterisation of aldehydes and ketones[8, 25, 26, 27] as the obtained derivatives are well-crystallised substances with high melting-points and are therefore very suitable for the identification of carbonyl compounds.

We have tried to acetylate some of the earlier synthesized 4-substituted thiosemicarbazones under mild conditions with acetic anhydride, but the reaction failed and instead of the expected acetylated compounds the molecule has split in two parts. Thus we have obtained from *p*-nitrobenzaldehyde-4-phenylthiosemicarbazone acetanilide and *N,N-diacetyl-p-nitrobenzylidenehydrazine* (LVII), identified by an authentic specimen which has been synthesized by acetylation of known *p*-nitrobenzylidenehydrazine. Similarly we obtained from *o*-chlorobenzaldehyde-4-phenylthiosemicarbazone acetanilide and N,N-*diacetyl-o-chlorobenzylidenehydrazine* (LIX).



LVII R: p-$NO_2C_6H_4$-
LIX R: o-Cl-C_6H_4-

It has been found that substituted hydrazines possess strong physiological action and isonicotinic acid hydrazide has been found very effective in treatment of tuberculosis. After the discovery of tuberculostatic activity of thiosemicarbazones[3, 4, 5, 12], *p*-acetylaminobenzaldehyde thiosemicarbazone (Conteben, Tibione) has been introduced as clinically effective tuberculostatic agent and many related thiosemicarbazones were made and tested (*inter alia*:[2, 6, 10, 13, 14, 16, 18, 19, 22]). Among them highest tuberculostatic activity was found to reside in a series of *p*-substituted benzaldehyde derivatives[21].

We have found that also some of the synthesized compounds exhibit remarkable tuberculostatic effect. The tuberculostatic activity was measured *in vitro* on the liquid culture medium Sula with the strain of *Mycobacterium tuberculosis* H 37 Rv. As reference substance Solvoteben (diethylammonium salt of the thiosemicarbazone of benzaldehyde-4-carboxylic acid) was used. The samples were prepared as described before[24]. The incubation temperature was 37° and the incubation time was 14 days. We found that some of the tested substances showed complete inhibition in the concentration of 5 γ/ml. No definite pattern of structure-activity relationship could be established.

TABLE I

4-Substituted thiosemicarbazides *R—NH—CS—NH—NH₂*

Compound No.	R	Yield, %	M. p., °C	Formula	Analyses, % N		Inhibition effect (γ/ml)
					calc'd	found	
I	2′-methoxyphenyl-	96.5	159	$C_8H_{11}ON_3S$	21.30	21.34	—
II	4′-methoxyphenyl-	90.3	154(a)	$C_8H_{11}ON_3S$	21.30	21.34	—
III	2′-ethoxyphenyl-	90.6	137,5	$C_9H_{13}ON_3S$	19.89	19.80	—
IV	4′-ethoxyphenyl-	91.2	149—50	$C_9H_{13}ON_3S$	19.89	19.92	—
V	4′-dimethylaminophenyl-	88.5	171—2	$C_9H_{14}N_4S$	26.64	26.48	—
VI	1′-naphtyl-	85.5	139	$C_{11}H_{11}N_3S$	19.34	19.29	5
VII	2′-naphtyl-	89.5	172—3	$C_{11}H_{11}N_3S$	19.34	19.58	—
VIII	Benzoyl-	83.5	151—2	$C_8H_9ON_3S$	21.52	21.57	—
IX	2′,3′-dimethylphenyl-	87	160	$C_9H_{13}N_3S$	21.52	21.70	> 5
X	2′,4′-dimethylphenyl-	92	152	$C_9H_{13}N_3S$	21.52	21.29	> 5
XI	2′,5′-dimethylphenyl-	86	162	$C_9H_{13}N_3S$	21.52	21.78	> 5
XII	3′,4′-dimethylphenyl-	77.2	119	$C_9H_{13}N_3S$	21.52	21.59	> 5
XIII	3′-nitrophenyl-	87	175	$C_7H_8O_2N_4S$	26.40	26.21	5
XIV	2′-chlorophenyl-	87	130—1	$C_7H_8N_3SCl$	20.84	20.89	—
XV	3′-chlorophenyl-	89	120(b)	$C_7H_8N_3SCl$	20.84	21.00	—
XVI	4′-chlorophenyl-	90	187—8(c)	$C_7H_8N_3SCl$	20.84	20.84	5
XVII	Benzyl-	85.5	129	$C_8H_{11}N_3S$	23.18	23.24	—
XVIII	Cyclohexyl-	90.8	147	$C_7H_{15}N_3S$	24.25	24.16	—

(a) Busch and Ulmer[9] give m. p. 144°
(b) Guha and Ray[17] give m. p. 130°
(c) Busch and Ulmer[9] give m. p. 180°

TABLE II

4-Substituted thiosemicarbazones R—NH—CS—NH—N=CH—R'

Compound No.	R	R'	M. p., °C	Formula	Analyses, % N		Inhibition effect (γ/ml)
					calc'd	found	
XIX	2'-methoxyphenyl-	phenyl-	183	$C_{15}H_{15}ON_3S$	14.73	14.78	—
XX	2'-methoxyphenyl-	*p*-methoxyphenyl-	164	$C_{16}H_{17}O_2N_3S$	13.32	13.15	—
XXI	4'-methoxyphenyl-	phenyl-	189—90	$C_{15}H_{15}ON_3S$	14.73	14.88	—
XXII	4'-methoxyphenyl-	*p*-methoxyphenyl-	168	$C_{16}H_{17}O_2N_3S$	13.32	13.40	—
XXIII	2'-ethoxyphenyl-	phenyl-	157	$C_{16}H_{17}ON_3S$	14.04	14.00	—
XXIV	2'-ethoxyphenyl-	*p*-methoxyphenyl-	192	$C_{17}H_{19}O_2N_3S$	12.76	12.88	—
XXV	4'-ethoxyphenyl-	phenyl-	190	$C_{16}H_{17}ON_3S$	14.04	13.89	—
XXVI	4'-ethoxyphenyl-	*p*-methoxyphenyl-	164	$C_{17}H_{19}O_2N_3S$	12.76	12.73	—
XXVII	4'-dimethylaminophenyl	phenyl-	219—20	$C_{16}H_{18}N_4S$	18.78	18.64	—
XXVIII	4'-dimethylaminophenyl	*p*-methoxyphenyl-	189—90	$C_{17}H_{20}ON_4S$	17.06	17.17	—
XXIX	1'-naphtyl-	*n*-pentyl-	113.5	$C_{17}H_{21}N_3S$	13.94	14.14	> 5
XXX	1'-naphtyl-	*m*-methoxy-*p*-hydroxyphenyl	209	$C_{19}H_{17}O_2N_3S$	11.96	11,87	5
XXXI	1'-naphtyl-	*p*-methoxyphenyl-	209	$C_{19}H_{17}ON_3S$	12.53	12.47	—
XXXII	1'-naphtyl-	phenyl-	210	$C_{18}H_{15}N_3S$	13.76	13.95	—
XXXIII	2'-naphtyl-	phenyl-	203	$C_{18}H_{15}N_3S$	13.76	13.78	—
XXXIV	2'-naphtyl-	*p*-methoxyphenyl-	182	$C_{19}H_{17}ON_3S$	12.53	12.38	—
XXXV	2',3'-dimethylphenyl-	phenyl-	206	$C_{16}H_{17}N_3S$	14.83	14.88	—
XXXVI	2',3'-dimethylphenyl-	*p*-methoxyphenyl-	218	$C_{17}H_{19}ON_3S$	13.41	13.18	—
XXXVII	2',4'-dimethylphenyl-	phenyl-	208	$C_{16}H_{17}N_3S$	14.83	14.73	—
XXXVIII	2',4'-dimethylphenyl-	*p*-methoxyphenyl-	193	$C_{17}H_{19}ON_3S$	13.41	13.41	—
XXXIX	2',5'-dimethylphenyl-	phenyl-	177	$C_{16}H_{17}N_3S$	14.83	14.89	—
XL	2',5'-dimethylphenyl-	*p*-methoxyphenyl-	176	$C_{17}H_{19}ON_3S$	13.41	13.30	—
XLI	3',4'-dimethylphenyl-	phenyl-	191	$C_{16}H_{17}N_3S$	14.83	14.75	—

XLII	3′,4′-dimethylphenyl-	*p*-methoxyphenyl-	170	$C_{17}H_{19}ON_3S$	13.41	13.43	—
XLIII	3′-nitrophenyl-	*p*-methoxyphenyl-	199	$C_{15}H_{14}O_3N_4S$	16.96	16.79	—
XLIV	2′-chlorophenyl-	phenyl-	188	$C_{14}H_{12}N_3SCl$	14.50	14.66	—
XLV	2′-chlorophenyl-	*p*-methoxyphenyl-	202	$C_{15}H_{14}ON_3SCl$	13.14	13.21	—
XLVI	3′-chlorophenyl-	phenyl-	148	$C_{14}H_{12}N_3SCl$	14.50	14.46	—
XLVII	3′-chlorophenyl-	*p*-methoxyphenyl-	168	$C_{15}H_{14}ON_3SCl$	13.14	13.26	—
XLVIII	4′-chlorophenyl-	phenyl-	200—1	$C_{14}H_{12}N_3SCl$	14.50	14.42	—
XLIX	4′-chlorophenyl-	*p*-methoxyphenyl-	192—3	$C_{15}H_{14}ON_3SCl$	13.14	13.14	—
L	Benzyl-	phenyl-	136	$C_{15}H_{15}N_3S$	15.60	15.47	—
LI	Benzyl-	*p*-methoxyphenyl-	155	$C_{16}H_{17}ON_3S$	14.04	14.06	—
LII	Cyclohexyl-	phenyl-	176	$C_{14}H_{19}N_3S$	16.03	16.32	—
LIII	Cyclohexyl-	*p*-methoxyphenyl-	179	$C_{15}H_{21}ON_3S$	14.42	14.30	—

4-Substituted thiosemicarbazones R—NH—CS—NH—N=R′

LIV	1′-naphtyl-	Cyclopentyl-	191	$C_{16}H_{17}N_3S$	14.83	15.01	> 5

4-Substituted thiosemicarbazones R—NH—CS—NH—N$\langle^{R_1}_{R_2}$

Compound No.	R	R_1	R_2	M. p., °C	Formula	Analyses, % N		Inhibition effect (γ/ml)
						calc'd	found	
LV	1′-naphtyl-	methyl-	methyl-	179	$C_{14}H_{15}N_3S$	16.33	16.07	5
LVI	1′-naphtyl-	methyl-	ethyl-	126	$C_{15}H_{17}N_3S$	15.49	15.53	> 5

EXPERIMENTAL

All melting-points were determined with Kofler's heating microscope. All compounds decompose at the melting-point.

Preparation of isothiocyanates

Isothiocyanates were prepared from the corresponding amines using a generally applicable procedure[28]. The only exception was made in the case of preparation of 2-naphtylisothiocyanate where the necessary quantity of ethanol was doubled because of the low solubility of 2-naphtylamine. *m*-Nitrophenyl-[23] and *o*-chlorophenylisothiocyanate[15] were prepared from the corresponding disubstituted thioureas. For the preparation of benzoylisothiocyanate the procedure of Ambelang and Johnson[1] was used.

General procedure for the preparation of 4-substituted thiosemicarbazides

Isothiocyanate (0.1 mole) was dissolved in 96% ethanol (40—80 ml. are usually necessary). In the case of *p*-ethoxyphenyl-, *p*-dimethylaminophenyl-, 1-naphtyl-2-naphtyl-, *m*-nitrophenyl- and *p*-chlorophenyl-isothiocyanate gentle warming was applied to bring the isothiocyanate in solution but the temperature should not exceed 50°. To the alcoholic solution of isothiocyanate hydrazine hydrate (8 g. of 50% aqueous solution) was added in one portion. The solution was thoroughly mixed and cooled with water. For the preparation of 4-(1'-naphtyl)-, 4-(3',4'-dimethylphenyl)-, 4-benzyl- and 4-cyclohexyl-thiosemicarbazide efficient cooling with ice should be applied because of the exothermic reaction and good solubility of the formed thiosemicarbazide in ethanol. The obtained precipitate was filtered and recrystallised from ethanol.

General procedure for the preparation of 4-substituted thiosemicarbazones

Equimolar quantities of aldehyde or ketone and 4-substituted thiosemicarbazide (quantities corresponding to 0.0025 mole of reactants were used) were refluxed with 96% ethanol (7—10 ml.) for 5—10 min. on a water bath. After cooling, the precipitate was filtered and recrystallised from ethanol.

N,N-*diacetyl*-p-*nitrobenzylidenehydrazine* (LVII)

p-Nitrobenzaldehyde-4-phenylthiosemicarbazone[25] (350 mg.), fused sodium acetate (400 mg.) and freshly distilled acetic anhydride (4 ml.) were refluxed on water bath for 15 min. After cooling, the solution was poured in water and the resulting oil solidified after standing some time. Any solid material was filtered off and recrystallised from ethanol giving colourless microneedles of LVII (35 mg.) with m. p. 163—4°, undepressed in admixture with the synthesized specimen.

Anal. 6.265 mg. subst.: 0.946 ml. N_2 (25°, 743 mm)
$C_{11}H_{11}O_4N_3$ (249.22) calc'd: N 16.86%
found: N 16.91%

The filtrate was made alkaline and continuously extracted with ether for 8 hr. The solvent was then evaporated to dryness, a few ml. of water added, warmed on water bath until a solution was obtained and filtered. After cooling a crystalline precipitate was obtained which, after sublimation at 90—100°/1 mm. had m. p. 115°, not depressed in admixture with an authentic specimen of acetanilide.

An authentic specimen of LVII was prepared by acetylating *p*-nitrobenzalhydrazine[11] for 15 min. as described above. After recrystallisation from ethanol it had m. p. 164°. If the acetylation was carried in the same way but without heating, N-*acetyl*-p-*nitrobenzylidenehydrazine* (LVIII) was obtained. After recrystallisation from ethanol the colourless microneedles had m. p. 197—8°.

Anal. 4.588 mg. subst.: 0.825 ml. N_2 (25°, 743 mm)
$C_9H_9O_3N_3$ (207.19) calc'd: N 20.28%
found: N 20.20%

N,N-*diacetyl-o-chlorobenzylidenehydrazine* (LIX)

o-Chlorobenzaldehyde-4-phenylthiosemicarbazone[25] (200 mg.), fused sodium acetate (200 mg.) and freshly distilled acetic anhydride (2.0 ml.) were refluxed on water bath for 15 min. After cooling the excess of acetic anhydride was destroyed by addition of water. The supernatant solution was decanted and the residual pasty mass recrystallised from ethanol giving colourless needles of LIX (20 mg.) with m. p. 155°.

Anal. 5.995 mg. subst.: 0.628 ml. N_2 (28°, 740 mm)
$C_{11}H_{11}O_2N_2Cl$ (238.67) calc'd: N 11.74%
found: N 11.57%

The decanted solution was made alkaline and continuously extracted with ether for 8 hrs., the ethereal solution evaporated to dryness and the pasty residue sublimed at 100—110°/0.5 mm. giving acetanilide with m. p. 115°, not depressed with an authentic specimen.

Acknowledgments. The author is very much indebted to Professor M. Perpar for her interest in this work and to Dr. S. Banič of the Microbiological Institute of the Faculty of Medicine in Ljubljana for testing the compounds.

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IZVOD

Sinteze u redu 4-supstituiranih tiosemikarbazida

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U vezi s istraživanjem tuberkulostatične aktivnosti tiosemikarbazida pripravljeni su 4-supstituirani tiosemikarbazidi (I–XVIII), te produkti kondenzacije