INVESTIGATIONS IN THE PYRIDAZINE SERIES FOR COMPOUNDS POSSESSING TUBERCULOSTATIC ACTIVITY

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Numerous derivatives of pyridazine are known which possess various biological activities, although there are little data concerning the tuberculostatic activity of this class of compounds. Thus by analogy with isoniazide and pyridazamide, a series of derivatives of pyridazine carbonic acid and also a series of hydrazones of 3-hydrazine pyridazine were synthesized [1]. The halide [2] and mercapto-derivatives of pyridazone [3] which possess tuberculostatic activity are well-known. 4,5-Dimercaptopyridazone-6, substituted in relation to the mercapto groups with methylfuran and methyl thiophene, and also 4-mercapto-5-haloidpyridazone-6 have been described in patents as antituberculous compounds [3].

It was of interest to follow the change in tuberculostatic activity of the 4,5-dihaloid-substituted pyridazone-6 when a molecule of various substitutes was introduced into position number 1, and also when the halides in positions number 4 and 5 were replaced by other groups. In addition it would be desirable to obtain derivatives of pyridazine, which are analogous, of the tuberculostatic thiocarbanilides. Similar analogs containing the pyrimidine ring, obtained by us previously [4], showed a high degree of antituberculous activity.

In the present study derivatives of dibromopyridazone-6 containing various substitutes in positions 1, 4 and 5 (Table 1) and also substituted thioureas of the pyridazine series (Table 2) were obtained.

4,5-Dichloro-(I) [2], 4,5-dibromo-(II) [2], 1-phenyl-4,5-dibromo-(IV) [5], 1-cyclohexyl-4,5-dibromo-(V) [5], 1-(n-nitrophenyl)-4,5-dibromopyridazone-6 (VI) [6], and 1-(M-oxy-n-carbethoxyphenyl)-4,5-dibromopyridazone-6 (IX) were synthesized by the usual method, i.e. by condensation of dichloro- or dibromomuconic acid with hydrazine hydrate and substituted hydrazines.

The methyl group was introduced into position number 1 of 4,5-dibromopyridazone-6 by the action of methyl iodide (compound III) [5].

1-(n-Aminophenyl)-4,5-dibromopyridazone-6 (VII) [6] was formed by the reduction of compound VI, and 1-(n-acetaminophenyl)-4,5-dibromopyridazone-6 (VIII) was produced by acetylation of compound VII.

1-Phenyl-4,5-dibromopyridazone was converted into 1-phenyl-4-nitro-5-oxypyridazone-6 (XI) by the action of sodium nitrite in aqueous dimethylformamide [5]. Compound XI was converted into 1-phenyl-4-nitro-5-bromopyridazone-6 (X) [8] by the action of phosphorus tribromide, and it was converted into 1-phenyl-4-nitro-5-aminopyridazone-6 (XII) [7] by the action of ammonium.

1-Phenyl-4-nitro-5-(o-chlorophenylamino)-pyridazone-6 (XIV) was obtained by the interaction between compound X and o-chloraniline, and it was then reduced to 1-phenyl-4-amino-5-(o-chlorophenylamino)-pyridazone-6 (XV).

1-Phenyl-4,5-diamino-pyridazone-6 (XIII) [8] was formed by the reduction of compound XII.

An attempt was made to obtain the substituted thiourea arising from the diaminopyridazone of compound XIII. However, in non-polar solvents and under normal conditions no interaction between compound XIII and n-methoxyphenyl isothiocyanate occurred. The original substances were isolated unchanged. On boiling the components in alcohol, 4,4'-dimethoxythiocarbanilide and the unchanged amine of compound XIII were isolated. It is possible to explain the formation of thiocarbanilide by the partial cleavage of the ori-

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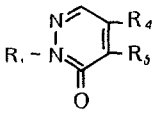
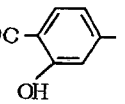
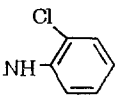
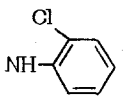
TABLE 1



Compound	R_1	R_4	R_5	Tuberculostatic activity (μg/ml) Strain $H_{37}R_V$ without serum	with serum
I	H	Cl	Cl	30	250
II	H	Br	Br	10[1]	
III	CH_3	Br	Br	30	125
IV	C_6H_5	Br	Br	<0,06	8
V	C_6H_{11}	Br	Br	4	30—60
VI	$NO_2C_6H_4$ (n)	Br	Br	1	30
VII	$NH_2C_6H_3$ (n)	Br	Br	0,5—1	4
VIII	$CH_3CONHC_6H_4$ (n)	Br	Br	0,5	1
IX		Br	Br	8	>500
X	C_6H_5	NO_2	Br	0,25—1	30
XI	C_6H_5	NO_2	OH	500	1 000
XII	C_6H_5	NO_2	NH_2	500	1 000
XIII	C_6H_5	NH_2	NH_2	1 000	1 000
XIV	C_6H_5	NO_2		500	1 000
XV	C_6H_5	NH_2		>1,5[2]	>30[2]

*Data in the literature [2].

† In high concentrations precipitation of the compound is hindered.

ginal isothiocyanate under these conditions and the subsequent interaction of the n-anisole formed with isothiocyanate. The formation of n-anisole was confirmed by thin-layer chromatography of the reaction mixture on aluminium oxide in the presence of an indicator. When diaminopyridazone (XIII) was heated with n-methoxyphenyl isothiocyanate in dimethylformamide at 80-90° for 1 hour, 5-phenyl-2-mercaptoimidazo-(4,5-d)-pyridazone-4 (XVI) and 4,4'-dimethoxythiocarbanilide were isolated. The formation of these compounds can be explained by the following method. n-Methoxyphenyl isothiocyanate reacts with diaminopyridazine XIII to form 5-phenyl-2-mercaptoimidazo-(4,5-d)-pyridazone-4 (XVI) and n-anisole. The latter reacts with isothiocyanate which has not participated in the reaction to form the symmetrical thiocarbanilide.

The structure of the isolated imidazopyridazone of XVI has been determined by elementary analysis and identification with the compound obtained on fusion of the diaminopyridazone of XIII with thiourea (i.e. by a method similar to the well-known method for obtaining 2-mercapto benzimidazoles):

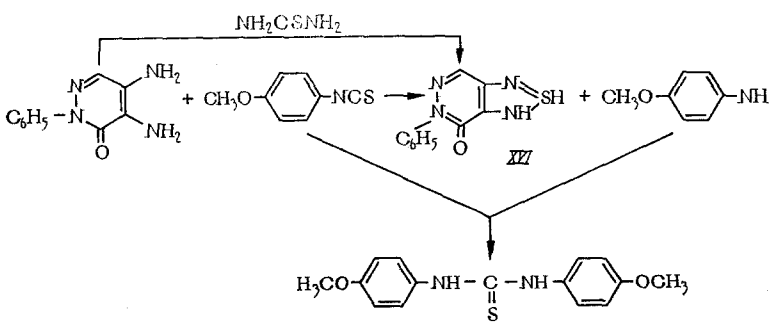
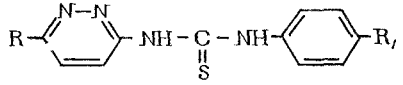


TABLE 2



Compound	R	R_1	Melting point (in degrees; alcohol)	Found (%)				Empirical formula	Calculated (%)				Tuberculostatic activity (μg/ml)	
				C	H	N	S		C	H	N	S	without serum	with serum
XVII	CH_3O	H	194—5	55,41	4,63	21,72	12,50	$C_{12}H_{12}N_4OS$	55,36	4,64	21,52	12,31	>30[2]	>250[2]
XVIII		OCH_3	201—201,5	53,81	5,23	19,32	11,28	$C_{13}H_{14}N_4O_2S$	53,77	4,86	19,29	11,04	125	1 000
XIX		OC_4H_9	189—90	58,05	6,28	16,54	9,90	$C_{16}H_{20}N_4O_2S$	57,80	6,06	16,85	9,64	15÷30	500
XX		OC_4H_9-i	173—173,5	57,89	6,13	17,04	9,86	$C_{16}H_{20}N_4O_2S$	57,80	6,06	16,85	9,64	2÷4	125
XXI		OC_2H_5	201—2	55,41	5,62	19,01	10,62	$C_{14}H_{16}N_4O_2S$	55,24	5,29	18,40	10,53	60	500
XXII		OC_5H_{11}-i	168—168,5	59,37	6,28	15,85	9,20	$C_{17}H_{22}N_4O_2S$	58,93	6,40	16,17	9,25	<0,06	125
XXIII		OC_3H_7	194,5—195	56,85	5,84	—	10,33	$C_{15}H_{16}N_4O_2S$	56,58	5,69	17,59	10,07	4	>60[2]
XXIV		Br	202—4	42,74	3,45	—	9,27	$C_{18}H_{11}BrN_4OS$	42,48	3,26	16,51	9,45	60	>1 000
XXV		CH_3	193,5—194	57,30	5,23	20,63	11,88	$C_{13}H_{14}N_4OS$	56,91	5,14	20,42	11,68	>60[2]	>125[2]
XXVI	C_4H_9O	H	147,5—148,5	59,77	6,05	18,23	10,47	$C_{15}H_{16}N_4OS$	59,57	6,00	18,52	10,60	2	60
XXVII		OCH_3	161—2	57,95	6,10	16,87	9,77	$C_{16}H_{20}N_4O_2S$	57,80	6,06	16,85	9,64	—	30
XXVIII		OC_3H_7	152—3	59,80	6,48	15,56	8,72	$C_{18}H_{24}N_4O_2S$	59,97	6,71	15,54	8,89	2—8	60
XXIX		CH_3	144,5—145	60,44	6,20	17,38	10,15	$C_{16}H_{20}N_4OS$	60,73	6,37	17,70	10,13	0,5—1	60—120
XXX		OC_4H_9	148—9	61,30	7,0	15,46	8,47	$C_{19}H_{26}N_4O_2S$	60,93	6,99	14,96	8,56	8	250
XXXI		Br	170—1	47,22	4,65	14,37	8,67	$C_{15}H_{17}BrN_4OS$[1]	47,24	4,75	14,69	8,40	15—30	1 000
XXXII		OC_2H_5	164—5	58,53	6,52	16,29	9,0	$C_{17}H_{22}N_4O_2S$	58,93	6,40	16,17	9,25	30	120
XXXIII		OC_4H_9-i	162—3	60,92	6,66	14,86	8,37	$C_{19}H_{26}N_4O_2S$	60,93	6,99	14,96	8,56	60—120	250—500
XXXIV		OC_5H_{11}-i	154—154,5	61,44	6,82	14,86	8,13	$C_{20}H_{28}N_4O_2S$	62,08	7,26	14,42	8,25	60	250

*Found, %: Br 20.84. Calculated, %: Br 20.95.

†At high concentrations precipitation of the compound is hindered.

It was possible to obtain substituted thioureas containing the pyridazine ring, 1-(6'-alkoxy-3'-pyridazinyl)-3-arylthiourea (see Table 2; XVII-XXXIV), from 3-amino-6-methoxy- and 3-amino-6-butoxypyridazine on heating with arylisothiocynates in anhydrous benzene of ethyl acetate.

3-Amino-6-methoxypyridazine was obtained by heating 3-amino-6-chloropyridazine with sodium methoxide for 20 hours in an autoclave at 120° and 8 atmospheres of pressure [9, 10]. The product was isolated by extraction with methylene chloride and a yield of 70% was obtained.

3-Amino-6-butoxypyridazine was synthesized from 3-amino-6-chloropyridazine by heating with sodium butylate at 150-160° [11].

The arylisothiocyanates used in the reaction were obtained by heating the symmetrically di-substituted thiocarbanilides with acetic anhydride [12].

A study was conducted of the tuberculostatic activity of the compounds obtained. It was shown that the 4,5-dibromo-derivatives of pyridazone-6 (see Table 1) possess marked bacteriostatic activity in relation to Mycobacterium tuberculosus. This was apparently on account of the presence of bromine atoms in the molecule. Substitution of two halide atoms into other groups led to a sharp decrease in the tuberculostatic activity of the compounds. Introduction of an aromatic radical into position number 1 of the dibromo-derivatives of pyridazone-6 increases the activity of the compounds.

1-Phenyl-4,5-dibromopyridazone-6 was studied during experimental tuberculosis in white mice. The compound was toxic in large doses and not active in tolerable doses (5 mg or lower per animal).

It follows from Table 2 that the 1-(6'-alkoxy-3'-pyridazinyl)-3-arylthioureas containing the butoxy group in the pyridazine ring are more active than similar compounds possessing the methoxy group (XXVI and XVII, XXIX and XXV, XXX and XIX). However, the methoxypyridazinyl thioureas containing alkoxy groups with a branched radical in the benzene ring, on the contrary, are more active than the corresponding butoxy-derivatives. The tuberculostatic activity of the methoxypyridazinyl arylthioureas increases with increase in length of the radical of the alkoxy group in the benzene ring.

Butoxypyridazinyl thioureas which do not contain a substitute in the benzene ring, and also those with a methyl group in the n-position of the benzene ring (XXVI and XXIX) also showed high tuberculostatic activity. One should note that in the presence of blood serum there is a sharp decrease in the tuberculostatic activity of the substituted thioureas of the pyridazine series.

EXPERIMENTAL

1-n-Acetylaminophenyl-4,5-dibromopyridazone-6 (VIII). A 4 g quantity of compound VII was heated in the presence of excess acetic acid until it had dissolved, and the mixture was cooled. A 3 g quantity of compound VIII (67%) was isolated, mp 268-270° (from alcohol). Found, %: C 37.70, 2.51; N 10.62; Br 41.89. $C_{12}H_9N_3O_2Br_2$. Calculated, %: C 37.32; H 2.34; N 10.85; Br 41.33.

1-(M-Oxy-n-carbethoxy)-phenyl-4,5-dibromopyridazone-6 (IX). A mixture of 7.67 g of dibromomuconic acid and 5 g of the ethyl ester of n-hydrazine salicylic acid in 50 ml of acetic acid was boiled for 5 hours. The precipitate obtained on cooling was washed with acetic acid and ether. A 7 g quantity of compound IX was isolated (56.7%), mp 157-158° (from alcohol). Found, %: C 37.98; H 2.77; N 7.06; Br 38.51. $C_{13}H_{10}N_2Br_2O_4$. Calculated, %: C 37.33; H 2.41; N 6.70; Br 38.30.

1-Phenyl-4-nitro-5-(o-chlorophenylamino)-pyridazone-6 (XIV). A 5.5 g quantity of 1-phenyl-4-nitro-5-bromo-pyridazone-6 (X) was dissolved in 50 ml of dimethylformamide, and 2.34 g of o-chloraniline and 1 ml of triethylamine were added, and the mixture was heated for 5 hours in a boiling water bath. The solution was diluted with an equal volume of water and the precipitate was extracted. After recrystallization from alcohol 3.6 g of compound XIV (56%) was obtained, mp 175-177° (from alcohol). Found, %: C 55.98; H 3.22; N 16.60; Cl 10.58. $C_{16}H_{11}N_4O_3Cl$. Calculated, %: C 56.06; H 3.24; N 16.35; Cl 10.35.

1-Phenyl-4-amino-5-(o-chlorophenylamino)-pyridazone-6 (XV). A 3.6 g quantity of NH_4Cl, 2 ml of an 18% solution of HCl, and 9.6 g of iron filings were added in portions to 3.6 g of compound XIV in 120 ml of alcohol. The reaction mixture was boiled for 5 hours, filtered, and the precipitate formed on cooling was filtered by suction and washed with water. A 1.8 g quantity of compound XV (54.8%) was obtained, mp 233-235° (from alcohol). Found, %: C 61.15; H 4.34; N 17.22; Cl 11.21. $C_{16}H_{13}N_4OCl$. Calculated, %: C 61.44; H 4.19; N 17.91; Cl 11.35.

5-Phenyl-2-mercaptomidazo-(4,5-d)-pyridazone-4 (XVI). A. A mixture of 2 g of compound XIII, 1.54 g of n-methoxyphenyl isothiocyanate and 15 ml of dimethylformamide was heated for 1 hour at 80-90° and poured into water. The resulting precipitate was separated. A 2.2 g quantity of a mixture of compounds was obtained, mp 182-240°. By means of thin-layer chromatography on aluminium oxide it was shown that this mixture consists of two compounds. In a chloroform-methanol (95:5) system on development in iodine vapor one of the substances was determined with an indicator as 4,4'-dimethoxythiocarbanilide. The second substance remained as a spot at the starting point. The mixture was dissolved in a 5% solution of NaOH. The undissolved precipitate was removed by filtration. A 1 g quantity of 4,4'-dimethoxythiocarbanilide was obtained, mp 187-188° (from alcohol). According to data in the literature [13] the melting point of this compound is 186-188°. The alkaline mother liquor was acidified with dilute hydrochloric acid. A 1.2 g quantity of compound XVI was isolated, mp 305-307° (from the experiment), R_f value of 0.216 in a system of methanol-10% aqueous ammonia (90:10) on development with iodine vapor. Found, %: C 54.28; H 3.72; N 22.62; S 12.96. $C_{11}H_8N_4OS$. Calculated, %: C 54.10; H 3.30; N 22.94; S 13.11.

B. A mixture of 0.8 g of compound III and 0.3 g of thiourea was fused until ammonia ceased to evolve. The warm product of fusion was dissolved in a 5% solution of NaOH, and the filtered solution was acidified with dilute hydrochloric acid. A 0.5 g quantity of compound XVI was obtained, mp 306-308° (from alcohol), R_f 0.216 in a system of methanol-10% ammonia (90:10) on development with iodine vapor. Found, %: C 54.23; H 3.50; N 22.65; S 13.23. $C_{11}H_8N_4OS$. Calculated, %: C 54.10; H 3.30; N 22.94; S 13.11.

3-Amino-6-methoxypyridazine. A solution of 13 g of 3-amino-6-chloropyridazine and sodium methoxide (prepared from 6.1 g of sodium and 300 ml methanol) was heated for 20 hours in an autoclave at 120° at a pressure of 8 atmospheres. The sodium chloride precipitate was removed by filtration. The filtrate was extracted under vacuum to dryness and extracted with methylene chloride. After evaporation of the extract the precipitate was removed by filtration and washed with a small quantity of methylene chloride. A 8.13 g quantity (70%) of 3-amino-6-methoxypyridazine was obtained, mp 103-105°. According to data in the literature this compound has a melting point of 103-105°.

1-(6'-Methoxy-3'-pyridazinyl)-3-(n-ethoxyphenyl)-thiourea (XXI). A solution of 1.41 g of n-ethoxyphenyl isothiocyanate in 10 ml of anhydrous benzene was added stepwise to a solution of 1 g of 3-amino-6-methoxypyridazine heated to boiling in 15 ml of benzene, and the mixture was heated for 2 hours with mixing. After cooling to room temperature the precipitate of compound XXI was removed by filtration and washed with dry ether. The mother liquor was evaporated under vacuum and the precipitate was washed with dilute hydrochloric acid and ether. A 2 g quantity of compound XXI (82.5% theoretical) was obtained, mp 201-202° (alcohol). Compounds XVII-XX and XXII-XXV were obtained by an analogous method.

1-(6'-Butoxy-3'-pyridazinyl)-3-(n-methoxyphenyl)-thiourea (XXVII). A 1.48 g quantity of n-methoxyphenyl isothiocyanate in 5 ml of ethyl acetate was added gradually with mixing to a solution of 1.5 g of 3-amino-6-butoxypyridazine in 10 ml of ethyl acetate heated to boiling, and the mixture was heated for 4 h. After cooling to room temperature the precipitate was removed by filtration, washed with ether, and recrystallized from alcohol. A 1.83 g quantity (65.5% theoretical) of compound XXVII was obtained, mp 161-162°.

Compounds XXVI, XXVIII–XXXIV were obtained in an analogous manner.

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