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STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS: PART V. THIOSEMICARBAZONES AND RELATED COMPOUNDS

BY

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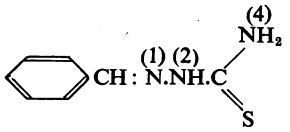
It has been reported (Domagk, Behnisch, Mietzsch, and Schmidt, 1946; Domagk, 1948) that some thiosemicarbazones of cyclic aldehydes and ketones show antituberculous activity *in vitro*. In some instances, activity *in vivo* was also demonstrated in experimental animals, and certain compounds have been used in the treatment of lupus and pulmonary tuberculosis (Moncorps and Kalkoff, 1947; Domagk, 1948; Kuhlmann, 1948) with results which are claimed to be encouraging. We have undertaken a systematic investigation of derivatives of thiosemicarbazide both *in vitro* and *in vivo*, using methods which have been described in earlier papers in this series (Martin, 1946; Hoggarth and Martin, 1948a). The compounds which have shown most interest are all thiosemicarbazones of monosubstituted benzaldehydes, the general formula of which may be written as follows:



RESULTS

The test method consisted of the infection of mice by the intravenous route, and their treatment by drugs administered orally twice daily by syringe and catheter at doses ranging downwards from the maximum tolerated. The results are presented in the form used in preceding papers.

(i) *Substitution in the aryl residue.*—Thiosemicarbazones of variously substituted benzaldehydes are listed in Tables I and II. The parent aldehydes in Table I carry one substituent. In some instances the same substituent has been placed in the three possible positions with respect to the thiosemicarbazone group. The compounds in Table II have two substituents in the aryl ring.

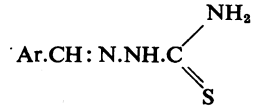
(ii) *Thiosemicarbazones of heterocyclical aldehydes.*—The results are given in Table III. Com-

TABLE I

ANTITUBERCULOUS TESTS ON SOME THIOSEMICARBAZONES OF MONOSUBSTITUTED BENZALDEHYDES

Doses given twice daily by syringe and catheter.

Compounds of the form:



No.	Ar	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
5669	phenyl	0.25	+0.1	1.7
		0.5	+1.1	
		0.5	+2.7	3.0
5670	*p*-chlorophenyl	0.25	−0.3	1.7
		0.5	+2.1	
		0.5	+3.3	3.0
6056	*p*-nitrophenyl	0.5	+4.3	1.7
		1.0	+6.7	
		0.5	+7.2	2.2
		1.0	+8.7	
6267	*m*-nitrophenyl	0.1	+0.1	1.7
		0.25	−0.3	
5704	*o*-nitrophenyl	0.25	−0.3	1.7
		0.5	+0.9	
		1.0	−0.1	
6198	*p*-aminophenyl	1.0	+3.3	1.4
6087	*p*-methylamino-phenyl	1.0	+4.1	1.6
		3.0	+2.4	
6147	*p*-ethylamino-phenyl	0.25	+3.2	1.4
		0.5	+3.5	
5672	*p*-dimethyl-aminophenyl	1.0	+4.7	1.6
		1.5	+5.1	1.7
		2.0	+6.5	

TABLE I—contd.

No.	Ar	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
6148	*p*-methylethylaminophenyl	0.25 0.5	+2.2 +2.9	1.4
5916	*p*-diethylaminophenyl	0.25 0.5	+0.9 +3.4	1.9
5961	N-*p*-pyrrolidinophenyl	0.25 0.5	0 +5.7	2.2
6473	*p*-di-β-chloroethylaminophenyl	0.1 0.25	−0.4 +0.5	1.7
6478	*p*-ethyl-β-chloroethylaminophenyl	1.0	+2.2	1.2
6082	*p*-hydroxyphenyl	10.0 10.0 10.0 20.0	+8.4 +8.1 +10.8 +11.2	2.8 1.3 2.0
5958	*o*-hydroxyphenyl	5.0 10.0	+0.3 −0.8	1.4 1.6
6057	*p*-anisyl	2.5 5.0 5.0	+6.1 +8.4 +10.1	1.4 2.2 1.2
6083	*m*-anisyl	1.0 2.0 5.0	1.3 1.5 4.5	1.4
6253	*o*-anisyl	0.25 0.5	0 +0.7	1.4
6524	*p*-phenetidyl	2.0	+4.5	1.4
6772	*p-iso*propoxyphenyl	1.0 2.0	+4.2 +5.6	1.4
6462	*p*-methylmercaptophenyl	0.25 0.5	+2.8 +4.7	1.4
8574	*p*-methylsulphonylphenyl	5.0*	+10.0	3.3
8388	*p*-ethylsulphonylphenyl	5.0 10.0 10.0	+12.7 +11.6 +9.8	2.7 1.5
8580	*p-n*-propylsulphonylphenyl	10.0	+9.3	2.8
6463	*p*-thiocyanophenyl	0.5 1.0	+4.2 +5.6	1.4

* This compound proved toxic at this dose, and ten of the twenty mice present originally in the treated group died in the first few days of the experiment.

pound No. 6060 is the thiosemicarbazone corresponding to 5-nitrofurfural semicarbazone ("Furacin"). The latter was also tested under the same conditions (doses 0.5 and 1.0 mg. per 20 g. mouse) and was inactive.*

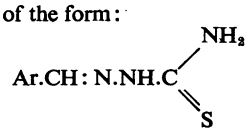
(iii) *Aliphatic thiosemicarbazones.*—Thiosemicarbazones derived from a few aliphatic aldehydes (heptaldehyde, cinnamaldehyde, *p*-dimethylamino-

TABLE II

ANTITUBERCULOUS TESTS ON SOME THIOSEMICARBAZONES OF DISUBSTITUTED BENZALDEHYDES

Doses given twice daily by syringe and catheter.

Compounds of the form:



No.	Ar	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
5887	2:4-dinitrophenyl	0.5 1.0 2.0	+0.5 +0.8 +1.9	1.4 1.6
6465	3-nitro-4-dimethylaminophenyl	0.25 0.5	+0.1 0	1.0
6464	3-nitro-4-diethylaminophenyl	0.25 0.5	+0.4 +1.4	1.2
6475	2-chloro-4-di-β-chloroethylaminophenyl	2.0	+0.8	1.7
6468	4-ethylamino-*m*-tolyl	0.1 0.25	+1.1 +1.0	1.3
6476	4-di-β-chloroethylamino-*o*-tolyl	1.0 3.0	−1.9 +1.3	1.7
6222	4-hydroxy-*m*-anisyl	8.0	+0.6	1.4
6255	3:4-dimethoxyphenyl	1.0 2.0 2.0	+1.4 +2.9 +3.1	1.4
6266	4-ethoxy-*m*-anisyl	5.0	+5.3	1.4
6197	3:4-methylenedioxyphenyl	0.5 1.0	+1.9 +3.2	1.4

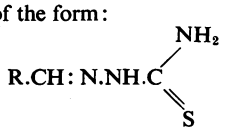
* It has been shown recently that "Furacin" has no therapeutic effect in tuberculous guinea-pigs (Wolinsky, E., Wetzel, V., and Steenken, W., 1949, *Proc. Soc. exp. Biol. Med.*, **70**, 483).

TABLE III

ANTITUBERCULOUS TESTS ON SOME THIOSEMICARBAZONES OF HETEROCYCLICAL ALDEHYDES

Doses given twice daily by syringe and catheter.

Compounds of the form:



No.	R	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
6060	2-(5-nitrofur-furyl)	1.0 2.0	−1.6 −3.2	1.7
6116	2-quinolyl	2.5 5.0	+0.9 +1.1	1.2
6099	4-quinolyl	1.0 5.0	+1.6 +6.2	1.2

cinnamaldehyde, and dextrose) were all without any activity and results are not given in detail.

(iv) *Substitution in the thiosemicarbazone group.*—The hydrogen atoms of the thiosemicarbazide residue—i.e., those on nitrogen atoms N^2 and N^4, and also that on the sulphur atom in *iso*thiosemicarbazide (: N.N: C(SH)NH_2)—were replaced by alkyl groups, singly or in pairs. The parent aldehyde for most of these derivatives was *p*-dimethylaminobenzaldehyde or *p*-anisaldehyde. Results with these compounds are reported in Table IV *a* and *b*.

(v) *Ketone thiosemicarbazones.*—Some thiosemicarbazones derived from aliphatic and aromatic ketones (e.g., acetophenone, *p*-methoxyacetophenone, 5-diethylaminopentane-2-one) were found to be inactive.

(vi) *Benzaldehyde semicarbazones and benzalaminoguanidines.*—No activity was found in the semicarbazones of benzaldehyde, *p*-hydroxy, *p*-methoxy-, or *p*-dimethylaminobenzaldehyde, nor in the benzalaminoguanidines derived from the two last-named aldehydes.

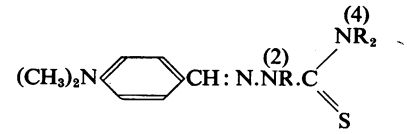
(vii) *Related compounds.*—The inactivity of the compounds mentioned in paragraph (vi) above indicated that the thioureido portion of the thiosemicarbazide group was essential. We have therefore examined other compounds in which the spatial relationship between an aryl nucleus and a thioureido group was similar to that in the benzaldehyde thiosemicarbazones. These compounds (21 in

TABLE IV

ANTITUBERCULOUS TESTS ON SOME SUBSTITUTED THIOSEMICARBAZIDE DERIVATIVES OF *p*-DIMETHYLAMINOBENZALDEHYDE

Doses given orally twice daily by syringe and catheter.

(*a*) Compounds of the form:

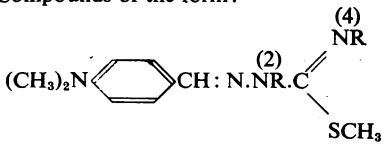


No.	Substituents on N^2	Substituents on N^4	Substituents on N^4	Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
5873	H	CH_3	H	1.0 2.0	−0.2 +0.3	1.9
5874	H	C_2H_5	H	5.0	+1.6	1.9
5875	H	(*n*) C_3H_7	H	1.0 2.0	+0.7 +0.7	1.2
5876	H	(*iso*) C_3H_7	H	7.0	−0.8	1.9
5877	H	(*n*) C_4H_9	H	5.0 10.0 10.0	−1.5 −0.9 +0.1	2.2 1.9
5878*	H	(*iso*) C_4H_9	H	1.0 5.0 5.0 2.5 5.0	+2.5 +5.9 +5.7 +3.3 +8.5	2.2 1.9 2.2
6258*	H	(*sec*) C_4H_9	H	1.0 2.0	−0.2 −1.0	1.4
6067	CH_3	CH_3	H	1.0	+1.0	1.6
6068	CH_3	C_2H_5	H	0.5 1.0	+1.6 +0.5	1.6
6069	CH_3	(*iso*) C_3H_7	H	0.5	+0 4	1.6
6070	CH_3	(*n*) C_4H_9	H	10.0	+1.9	1.6
6290*	H	CH_3	CH_3	0.5 1.0	−0.8 −0.5	1.4

* Compounds analogous to Nos. 5878, 6258, and 6290, but prepared from *p*-anisaldehyde in place of *p*-dimethylaminobenzaldehyde, were inactive.

TABLE IV—contd.

(*b*) Compounds of the form:



No.	Substituents on		Dose (mg. per 20 g. mouse)	Increased mean survival time (days)	Increase required for significance (days)
	N^2	N^4			
6085	H	H	0.5 1.0	−0.3 +0.2	1.6
6080	H	CH_3	0.5 1.0	0 −0.7	1.4
6081	H	(*iso*) C_3H_7	1.0 2.0	−0.4 −0.4	1.5
6086	CH_3	CH_3	0.5 1.0	−0.7 +0.4	1.4

all) fall into the first six classes listed below. The last category (phenyl thioureas) represents the simplest possible combination of an aryl nucleus and a thioureido group. All these compounds were completely inactive.

Ar.CH: N.NH.CS.NH_2
(Benzaldehyde thiosemicarbazones)
Ar.CO.NH.NH.CS.NH_2
1-Benzoylthiosemicarbazides
Ar.SO_2NH.NH.CS.NH_2
1-Benzenesulphonylthiosemicarbazides
Ar.CH: N.NH.CS.NH.N: CH.Ar
Dibenzalthiocarbhydrazides
Ar.NH.CS.NH.CS.NH_2
Phenyldithiobiurets
Ar.NH.C(NH).NH.CS.NH_2
Phenylguanylthioureas
Ar.CO.NH.NH.CS.NH.CO.Ar
1 : 4-Dibenzoylthiosemicarbazides
Ar.NH.CS.NH_2
Phenylthioureas

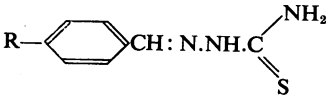
(Ar = an aryl residue usually with a *para* substituent which was either Cl, NO_2, HO, CH_3O, $(CH_3)_2N$, CH_3, or $NH_2.SO_2$.)

TABLE V

FURTHER ANTITUBERCULOUS TESTS ON THE MORE ACTIVE THIOSEMICARBAZONES OF MONOSUBSTITUTED BENZALDEHYDES

Drugs (mixed with powdered food) administered to groups of 20 mice for the first fourteen days of the test.

Compounds of the form :



No.	R	Estimated daily intake (mg.) of drug per 20 g. mouse	Observed mean survival time (days)	Increased mean survival time (days)	Increase required for significance (days)	First death (days)	Last death* (days)
5672	$N(CH_3)_2$	1.5 2.0	20.7 24.2	3.8 6.5	1.5 2.1	17 18	26 29(5)
6057	OCH_3	5.0 10.0 5.0 5.0	23.8 25.9 20.7 25.3	6.1 8.2 3.8 6.6	2.1 1.5 2.0	21 14 16 17	29(1) 29(3) 25(2) 33
6082	OH	10.0 20.0 2.0 5.0 10.0	29.6 30.0 22.4 27.2 30.5	10.8 11.2 2.8 7.6 10.9	2.0 3.0	19 20 18 20 22	35(2) 35(5) 30 31 33
8388	$SO_2C_2H_5$	2.0 5.0 10.0	32.7 34.8 35.4	13.1 15.2 15.8	3.0	25 26 28	40 50 50

* Figures in parentheses give number of survivors at the termination of the experiment. In all the above experiments all control mice were dead by the 22nd day except for a single mouse which survived to the 27th day in one test.

(viii) *Further comparisons of active compounds.*—Examination of Table I reveals that highest activity is shown by the thiosemicarbazones of *p*-hydroxybenzaldehyde (6082), *p*-anisaldehyde (6057), and the group of *p*-alkylsulphonylbenzaldehydes (8388, 8574, 8580). With all these compounds increases up to ten or twelve days in the mean survival time have been recorded, and it was felt desirable to compare these compounds directly one with another.

For this purpose we have preferred to administer the drugs mixed with the powdered food over a period of fourteen days, as previously described (Hoggarth and Martin, 1948b). We find that, on the whole, the effects observed with the two methods of administration (when the total daily intake of drug is the same) do not differ greatly from one another. The results are given in Table V.

The toxicities of the compounds in Table V are of interest in connexion with their therapeutic effects. In general the highest doses quoted in this table were the highest found to be tolerated without serious toxic effects by uninfected mice. We have not attempted to determine median lethal doses, but the observations recorded in Table VI were made.

TABLE VI

TOXIC EFFECTS OF THIOSEMICARBAZONES NOS. 5672, 6057, 6082, 8388

Drugs given by syringe and cathether twice daily to groups of twelve mice for three weeks.

No.	Dose (mg. per 20 g.)	Effect
5672	1.0	None
	2.0	Growth slightly retarded but no deaths
	4.0	Failure to gain weight (4 deaths)
6057	4.0	None
	6.0	Growth retarded but no deaths
	8.0	
	10.0	
6082	10.0	None
8388	4.0	None
	6.0	Growth slightly retarded but no deaths
	8.0	
	10.0	

From these limited observations, 6082 appears to be the least toxic, and in another experiment mice were given food containing 0.25% of this substance (estimated daily intake of drug 10 mg. per 20 g.) for 7 weeks without obvious toxic effects. When this concentration of 8388 was added to the food, the animals at first refused to eat it but later did so, without apparent harm, for 120 days.

Discussion

Our investigation of thiosemicarbazones and related compounds has shown that marked activity against an acute infection with *Mycobacterium tuberculosis* in mice is limited to the thiosemicarbazones of substituted benzaldehydes or heterocyclical aldehydes. Substitution of the hydrogen atoms of the thiosemicarbazide residue by alkyl groups usually abolishes activity, and the few thiosemicarbazones derived from alkyl-substituted thiosemicarbazides which are active are usually less so than the parent compound. Activity has not been found amongst the many closely related compounds examined. The relationship between activity and the type and position of substituents in the aromatic nucleus of benzaldehyde thiosemicarbazones may be inferred from Tables I and II. For highest activity a *para* substituent is necessary, though a few substituents in the *meta* position confer activity. High activity is found with alkylsulphonyl, nitro, amino, hydroxy, mercapto, and thiocyano groups in the *para* position, but only low activity with a *para* chloro group. Alkylation of the *para* hydroxy group does not result in significantly higher activity. Amongst the *p*-aminobenzaldehyde thiosemicarbazones the peak of activity is shown by the dimethylamino compound. We have examined three thiosemicarbazones of *p*-alkylsulphonyl benzaldehydes, all of which are highly active; the ethylsulphonyl compound is possibly the most active. The methylsulphonyl compound is more toxic than the other two. Introduction of a second substituent in the aryl residue usually reduces activity greatly. The activity of quinoline-4-aldehyde thiosemicarbazone is within the range of activity of the benzaldehyde thiosemicarbazones.

The response observed with the most active compounds of this type (Nos. 6057, 6082, 8388, 8574, and 8580) under the particular conditions of our routine test was better than that obtained with any other synthetic substance (including *p*-aminosalicylic acid) which we have yet examined. When the drugs were given mixed with the food for the first fourteen days only of the test, it has proved possible with most of these compounds to keep all treated animals alive until after the death of the last control animal. The treated animals did eventually die with marked tuberculous lesions of the lungs.

The activity *in vitro* of all the compounds mentioned above was determined by our usual method (Hoggarth and Martin, 1948a), but the results are

not given here in order to save space. We agree with the general statement of Domagk and his co-workers that the compounds with most activity *in vitro* in this series will inhibit growth of the tubercle bacillus at concentrations of 1: 50,000–1: 100,000. In our *in vitro* test we commonly find that the concentration at which growth is equal to controls is not less than 1/9 or 1/27 of the concentration for complete inhibition—e.g., No. 6082 will completely inhibit growth at 1: 81,000 but has no inhibitory effect at 1: 2,000,000. With certain compounds of this series—e.g., Nos. 6056, 6099, 6255, 6266, 8388—partial growth took place even at 1: 1,000, the highest concentration tested, but inhibition of growth could be detected at a concentration of 1: 729,000 or 1: 2,000,000. We believe this unusually wide range of partial activity to be a real phenomenon, as it has been observed repeatedly with the substances mentioned and also occasionally with substances having no therapeutic action. Many compounds of high activity *in vitro* were devoid of activity *in vivo*.

The substances tested clinically by the German workers may be identified by the key given by Domagk (1947). These correspond to our numbers 8388, 6057, and the acetyl derivative of our number 6198. We find that the thiosemicarbazone of *p*-ethylsulphonylbenzaldehyde (8388) is superior in its therapeutic effect to any of the other compounds of its class which we have examined. This is a reflection of its marked persistence in the blood stream (Spinks, 1949).

Summary

Antituberculous activity in mice has been investigated in certain derivatives of thiosemicarbazide and related compounds. After a study of about one hundred such compounds, therapeutic activity has been found to be confined to thiosemicarbazones of substituted benzaldehydes and heterocyclical aldehydes.

Our thanks are due to Dr. N. Barton of these laboratories for samples of phenyldithiobiuret compounds originally prepared by him for antimalarial test.

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