SOME FURTHER STUDIES ON TUBERCULOSTATIC COMPOUNDS

By E. M. BAVIN, D. J. DRAIN, M. SEILER and D. E. SEYMOUR

From the Research Department, Herts Pharmaceuticals, Ltd., Welwyn Garden City

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INTRODUCTION

Continuing our examination of the tuberculostatic activity of substances related to *p*-aminosalicylic acid, we report on some further derivatives of this substance and on the activity of some heterocyclic compounds.

Table I lists the compounds which have been examined together with their inhibitory concentrations against a standard inoculum of 0·001 mg./ml. of *M. tuberculosis* H37Rv strain. The culture medium and technique used for the determination of *in vitro* activity was similar to that described previously.[1] Table II lists the acute toxicity and the *in vivo* activity of some of the compounds, the latter being determined by the mouse corneal test of Rees and Robson.[2] The corneal test was carried out on groups of 10 animals and a positive sign in the column marked "activity" indicates that more than 50 per cent. of the animals under test were protected from the development of corneal lesions after a period of 30 days treatment. A more detailed estimate of the degree of protection was obtained in some cases by microscopical examination of the eyes and the figure in parenthesis indicates the percentage of eyes protected. In some instances, a number of animals were eliminated from the test owing to the presence of non-tuberculous lesions.

RESULTS

(*a*) *p-Aminosalicylic acid derivatives.* Earlier work with esters of *p*-aminosalicylic acid had indicated to us that they were not sufficiently active to justify extended trial. However, reports by Freire *et al.*[3,4,5] that the phenyl ester (phenyl-4-aminosalicylate) has an *in vitro* and *in vivo* tuberculostatic activity many times greater than *p*-aminosalicylic acid and at least equal to that of streptomycin, prompted us to re-examine this compound and a series of related aryl esters. The results as given in the tables indicate that the aryl esters have an *in vitro* activity of the same order as *p*-aminosalicylic acid; this activity being maintained *in vivo* with 3 typical members of the group (Compounds No. 77, 82 and 83). No marked difference in *in vivo* activity was observed according to whether the substance was administered by oral or subcutaneous route. The poor protective power of the butyl ester confirms reports by others[6] that the alkyl esters have little *in vivo* activity, and although the aromatic esters are probably the most useful members of the group, they do not appear to offer the advantages over *p*-aminosalicylic acid reported by the French workers. Compound 108 is of interest in so far as it can be regarded as a conjugate of 2 molecules of *p*-aminosalicylic acid, this

TABLE I

TUBERCULOSTATIC ACTIVITIES *in vitro*

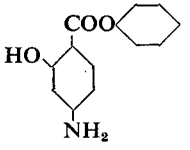
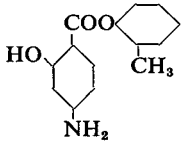
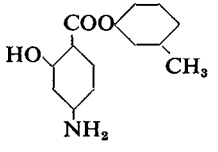
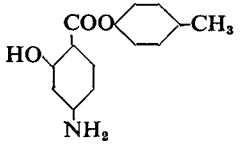
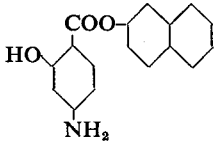
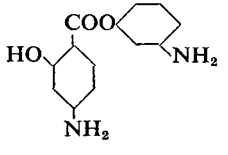
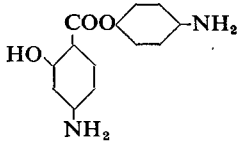
No.	Name	Formula	*In vitro* activity mg./100 ml.
	(a) *Derivatives of* p-*Aminosalicylic acid*		
77	Phenyl-4-aminosalicylate		0·0487–0·0243
84	*o*-Cresyl-4-aminosalicylate		0·0243–0·0121
85	*m*-Cresyl-4-aminosalicylate		0·0243–0·0121
82	*p*-Cresyl-4-aminosalicylate		0·0243–0·0121
83	β-Naphthyl-4-aminosalicylate		0·0121–0·006
88	*m*-Aminophenyl-4-aminosalicylate		0·0243–0·0121
98	*p*-Aminophenyl-4-aminosalicylate		0·0487–0·0243

TABLE I (*continued*)

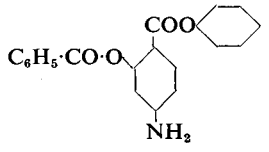
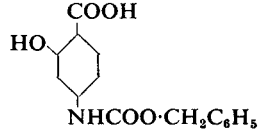
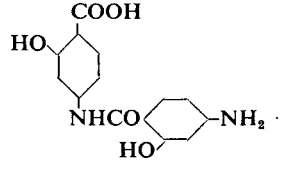
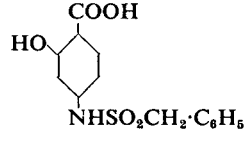
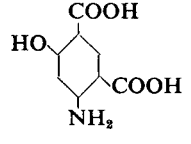
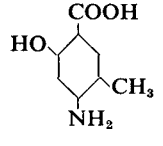
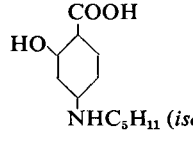
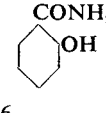
No.	Name	Formula	*In vitro* activity mg./100 ml.
80	Phenyl-4-amino-2-benzoyloxybenzoate		0·195–0·0975
106	4-Carbobenzyloxy-aminosalicylic acid		0·39–0·195
108	4-(4′-Amino-2′-hydroxybenzamido)-salicylic acid		0·0975–0·0487
123	4-Benzylsulphonamido-salicylic acid		0·39–0·195
102	4-Amino-6-hydroxy-*iso*phthalic acid		0·0121–0·006
72	4-Amino-5-methyl-salicylic acid		0·0975–0·0487
120	4-*iso*Amylaminosalicylic acid		0·0487–0·0243
	(b) *Amides and Thio-amides:*		
34	2-Hydroxybenzamide (salicylamide)		>25

TABLE I (*continued*)

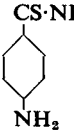
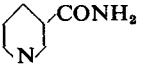
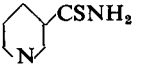
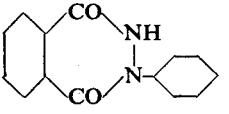
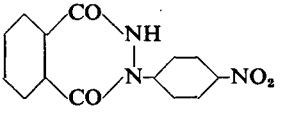
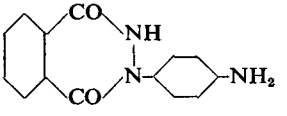
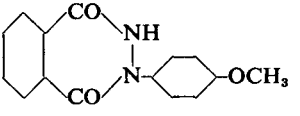
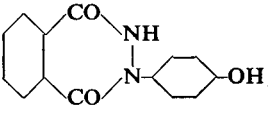
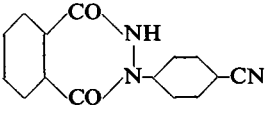
No.	Name	Formula	*In vitro* activity mg./100 ml.
74	4-Aminobenzamide		0·78–0·39
50	4-Aminobenzthioamide		0·0975–0·0487
79	Nicotinamide		>12·5
78	Nicotinthioamide		3·125–1·56
	(c) *Phthalazine Derivatives:*		
127	1:4-Diketo-3-phenyl-tetrahydrophthalazine		0·0243–0·0121
233	1:4-Diketo-3-(*p*-nitrophenyl)-tetrahydrophthalazine		0·0243–0·0121
256	1:4-Diketo-3-(*p*-aminophenyl)-tetrahydrophthalazine		0·78–0·39
257	1:4-Diketo-3-(*p*-methoxyphenyl)-tetrahydrophthalazine		1·56–0·78
261	1:4-Diketo-3-(*p*-hydroxyphenyl)-tetrahydrophthalazine		0·78–0·39
263	1:4-Diketo-3-(*p*-cyanophenyl)-tetrahydrophthalazine		1·56–0·78

TABLE I (*continued*)

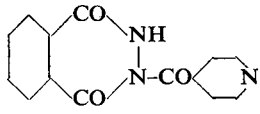
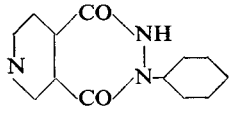
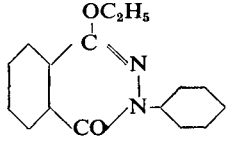
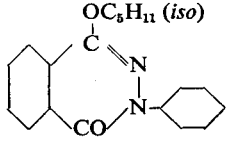
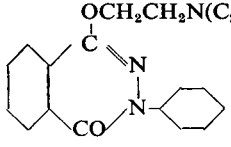
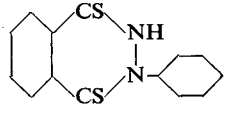
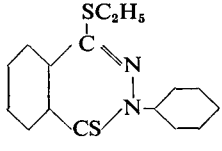
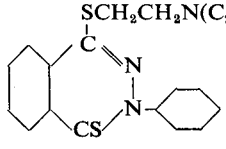
No.	Name	Formula	*In vitro* activity mg./100 ml.
238	1:4-Diketo-3-*iso*nicotinyltetrahydrophthalazine		0·0243–0·0121
232	6-Aza-1:4-diketo-3-phenyltetrahydrophthalazine		3·125–1·56
152	1-Ethoxy-4-keto-3-phenyl-3:4-dihydrophthalazine		3·125–1·56
153	1-*iso*Amyloxy-4-keto-3-phenyl-3:4-dihydrophthalazine		3·125–1·56
207	1-β-Diethylaminoethoxy-4-keto-3-phenyl-3:4-dihydrophthalazine hydrochloride		1·56–0·78
129	1:4-Dithio-3-phenyltetrahydrophthalazine		0·39–0·195
223	1-Ethylthio-3-phenyl-4-thio-3:4-dihydrophthalazine		0·0975–0·0487
224	1-Diethylaminoethylthio-3-phenyl-4-thio-3:4-dihydrophthalazine hydrochloride		1·56–0·78

TABLE I (*continued*)

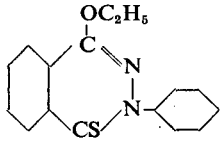
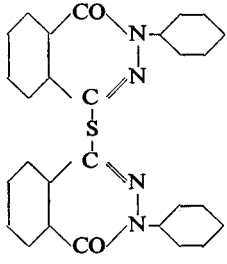
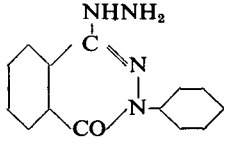
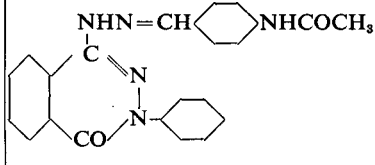
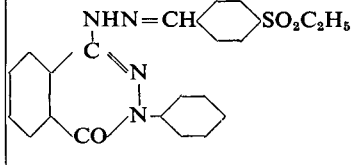
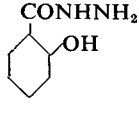
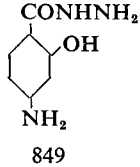
No.	Name	Formula	*In vitro* activity mg./100 ml.
227	1-Ethoxy-4-thio-3-phenyl-3:4-dihydrophthalazine		0·0243–0·0121
225	1:1′-Bis-(4-keto-3-phenyl-3:4-dihydrophthalazinyl)-sulphide		0·0975–0·0487
228	1-Hydrazino-4-keto-3-phenyl-3:4-dihydrophthalazine		3·125–1·56
230	1-(*p*-Acetamidobenzalhydrazino)-4-keto-3-phenyl-3:4-hydrophthalazine		0·0975–0·0487
231	1-(*p*-Ethylsulphonylbenzalhydrazino)-4-keto-3-phenyl-3:4-dihydrophthalazine		0·0975–0·0487
	(d) *Acid Hydrazides:*		
185	2-Hydroxybenzhydrazide		0·78–0·39
116	4-Amino-2-hydroxybenzhydrazide		0·0975–0·0487

TABLE I (*continued*)

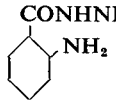
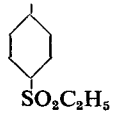
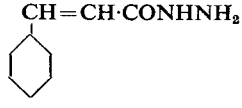
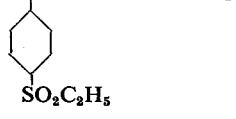
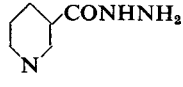
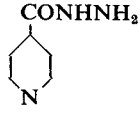
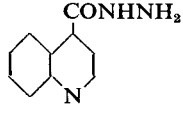
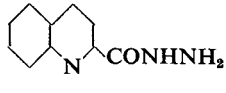
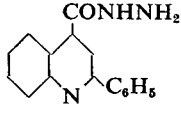
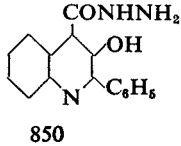
No.	Name	Formula	*In vitro* activity mg./100 ml.
186	2-Aminobenzhydrazide		>12·5
191	4-Ethylsulphonylbenzhydrazine		3·125–1·56
184	Cinnamic acid hydrazide		>12·5
188	4-Ethylsulphonylcinnamic acid hydrazide		6·25–3·125
187	Nicotinyl hydrazide		1·56–0·78
181	*iso*Nicotinyl hydrazide		0·0008–0·0004
195	Cinchoninyl hydrazide*		6·25–3·125
252	Quinoline-2-carboxyhydrazide		0·39–0·195
194	2-Phenylcinchoninyl hydrazide		0·39–0·195
189	3-Hydroxy-2-phenylcinchoninyl hydrazide		1·56–0·78

TABLE I (*continued*)

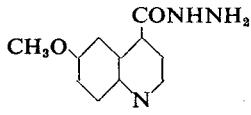
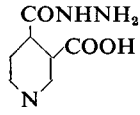
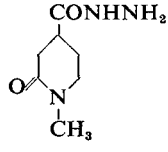
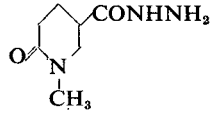
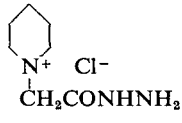
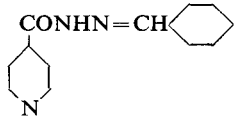
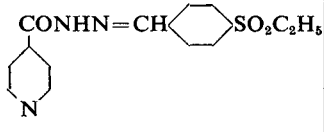
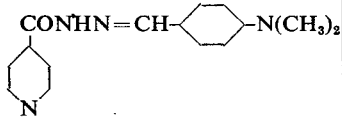
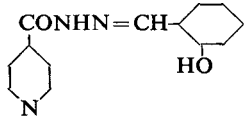
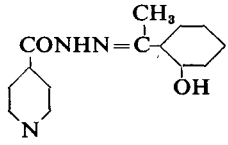
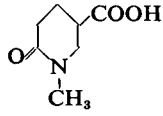
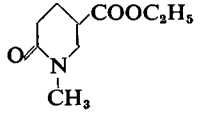
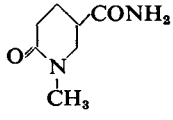
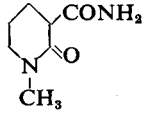
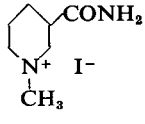
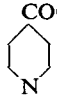
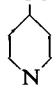
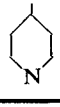
No.	Name	Formula	*In vitro* activity mg./100 ml.
196	Quininic acid hydrazide*		1·56–0·78
197	3-Carboxy-*iso*nicotinyl hydrazide		>12·5
190	1-Methyl-2-pyridone-4-carboxyhydrazide		0·0487–0·0243
204	1-Methyl-2-pyridone-5-carboxyhydrazide		0·78–0·39
222	Pyridinium acethydrazide chloride		>12·5
250	Benzal-*iso*nicotinyl-hydrazone		0·0002–0·0001
192	4-Ethylsulphonylbenzal-*iso*nicotinyl hydrazone		0·006–0·003
212	4-Dimethylaminobenzal-*iso*nicotinyl hydrazone		0·003–0·0017
213	2-Hydroxybenzal-*iso*-nicotinyl hydrazone		0·003–0·0017

TABLE I (*continued*)

No.	Name	Formula	*In vitro* activity mg./100 ml.
214	2-Hydroxyacetophenone-*iso*nicotinyl hydrazone		0·0121–0·006
	(e) *Miscellaneous Pyridine Compounds:*		
136	1-Methyl-2-pyridone-5-carboxylic acid		>12·5
137	Ethyl-1-methyl-2-pyridone 5-carboxylate		3·125–1·56
135	1-Methyl-2-pyridone-5-carboxyamide		0·78–0·39
144	1-Methyl-2-pyridone-3-carboxyamide		0·39–0·195
138	Nicotinamide methiodide		6·25–3·125
198	*iso*Nicotinic acid		3·125–1·56
203	*iso*Nicotinamide		>12·5
210	*iso*Nicotin-hydroxamic acid hydrochloride		>12·5

* We wish to thank Professor F. S. Spring, of the Royal Technical College, Glasgow, for kindly supplying compounds 195 and 196.

TABLE II

TUBERCULOSTATIC ACTIVITIES *in vivo*
(Mouse Corneal Test)

No.	Name	LD50 mg./g.	*In vivo* activity. Mouse corneal test. Dosage mg./g.	Route	Activity
	(a) *Derivatives of* p-*Amino-salicylic acid:*				
77	Phenyl-4-aminosalicylate	>5 (oral)	1·5	Gastric tube	+
		>3 (subcutaneous)	1·5	Subcutaneous	+
82	*p*-Cresyl-4-aminosalicylate	5 (subcutaneous)	2·0	In diet	+
			2·0	Subcutaneous	+
			1·0	In diet	+
83	β-Naphthyl-4-aminosalicylate	>5 (oral)	2·0	In diet	+
		>5 (subcutaneous)	1·5	Subcutaneous	+
			1·0	In diet	+
115	*n*-Butyl-4-aminosalicylate	5 (oral)	2·0	In diet	−
		5 (subcutaneous)	1·5	Subcutaneous	−
108	4(4′-Amino-2′-hydroxybenzamido) salicylic acid	—	2·0	In diet	+
			1·0	In diet	+
123	4-Benzylsulphonamidosalicylic acid	4 (oral)	—		
		4·5 (subcutaneous)	2·0	In diet	+
125	3:-Di-iodo-4-aminosalicylic acid*	0·5 (subcutaneous)	0·4	In diet	+
	(b) *Amides:*				
79	Nicotinamide	2·5 (oral)	—	Oral	−†
		2·75 (subcutaneous)			
	(c) *Phthalazine Derivatives:*				
1:4-	Diketo-3-phenyltetrahydro-phthalazine	—	0·5	In diet	+
	(d) *Acid Hydrazides:*				
116	4-Amino-2-hydroxybenzhydrazide	0·5 (oral)			
		0·35 (subcutaneous)	0·2	In diet	−
181	*iso*Nicotinyl hydrazide	0·1 (oral)	0·008	In diet	+ (100)
		0·1 (subcutaneous)	0·004	In diet	+ (100)
			0·002	In diet	+ (55)
190	1-Methyl-2-pyridone-4-carboxy-hydrazide	—	0·008	In diet	+ (75)
			0·004	In diet	− (40)
192	4-Ethylsulphonylbenzal *iso*nicotinyl hydrazone	—	0·008	In diet	+ (57)
			0·004	In diet	− (30)
213	2-Hydroxybenzal-*iso*nicotinyl hydrazone	>10 (oral)	1·0	In diet	+ (100)
	(e) *Miscellaneous Pyridine Compounds:*				
137	Ethyl-1-methyl-2-pyridone-5-carboxylate	—	0·5	In diet	−
135	1-Methyl-2-pyridone-5-carboxamide	—	0·5	In diet	−

* We have previously reported the *in vitro* activity of this compound.[8]
† Rees and Robson—personal communication.

"dimer" being an attempt to produce a condensed molecule with the aim of maintaining *in vivo* activity with a reduced dose. Further studies on these lines are continuing and will be reported later. Nuclear substitution in the 5-position of the *p*-aminosalicylic acid molecule does not markedly reduce the tuberculostatic activity as is shown by compounds 72 and 102, the latter being a known by-product in some of the commercial methods of synthesis of *p*-aminosalicylic acid.[7]

We have previously reported[8] the *in vitro* activity of the hydrazide of *p*-aminosalicylic acid (Compound No. 116). The low *in vivo* activity of this substance and that of the other aromatic hydrazides examined (Compounds 185, 186 and 191) confirms the reports by others[9,10] on the low activity of the hydrazides of aromatic acids.

(*b*) *Nicotinamide.* Earlier work by one of us[11] confirmed that nicotinamide is active by the mouse survival test at a dose level of 0·9 mg./g. The inactivity of the substance *in vitro* and by the corneal test is the only instance we have encountered to date where the 2 *in vivo* tests do not correlate. Preliminary results of clinical trial with nicotinamide indicate that the substance, although displaying activity, is not so effective as *d*-aminosalicylic acid or streptomycin.[12]

(*c*) *Phthalazine derivatives.* Following a report by Buu-Hoi *et al.*[13] that the *iso*amyl ether of 1:4-diketo-3-phenyltetrahydrophthalazine is more active than streptomycin in a mouse survival test, we synthesised this compound (No. 153) and other related ethers (152 and 207), and found them all to exhibit low *in vitro* activity. The high *in vitro* activity of the 1:4-diketo-3-phenyltetrahydrophthalazine (No. 127) itself, coupled with its promising behaviour in the mouse corneal test, led us to synthesise further compounds of this series, the *in vitro* results of which are given in Table I.

(*d*) *Acid hydrazides and derivatives.* Following the recent reports[14,15] from the United States that *iso*nicotinyl hydrazide possesses an outstandingly high antitubercular activity *in vitro*, in animals and in man, we directed our attention to a further series of hydrazides and other derivatives of this compound. We confirm the high *in vitro* activity of *iso*nicotinyl hydrazide (Compound 181) and also show that the substance exhibits remarkable protective power in the mouse corneal test. From the series of derivatives reported in this paper, and from other published work,[10] it is apparent that *iso*nicotinyl hydrazide is another example of a substance displaying specificity for tuberculostatic activity. The substituted aldehyde and ketone *iso*nicotinyl hydrazones (No. 250, 192, 212, 213 and 214) however, show comparable *in vitro* tuberculostatic activity, but it is conceivable that these derivatives may owe their activity to breakdown to *iso*nicotinyl hydrazide.

The authors wish to thank Professor Knox and Professor J. M. Robson of Guy's Hospital, London, for their interest and advice, and the Directors of Herts Pharmaceuticals Ltd., for permission to publish these results.

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DISCUSSION

The paper was presented by MR. D. E. SEYMOUR.

DR. F. HARTLEY (London) commented on the statement that the substituted aldehyde and ketone *iso*nicotinyl hydrazones might owe their activity to breakdown to *iso*nicotinyl hydrazide. Hydrazones, generally speaking, would be expected to be very stable compounds. He suggested that if Mr. Seymour would measure the stability of those hydrazones to oxidising agents and to acid hydrolysis he would probably find them to be stable. If that were so the evaluation of one or other of the four compounds should be further pursued.

MR. D. E. SEYMOUR, in reply, said he thought that the compounds might break down. Their stability was being studied in detail. Since the paper was written, further work had been done with a few of the compounds, in particular the benzaldehyde derivative, which was more active than isoniazid *in vitro* but not so active *in vivo*. American workers had reported similar results with some of the derivatives.