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Antituberculosis activity of some nitrofuran derivatives

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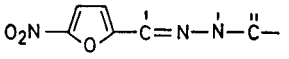
A series of 4,6-disubstituted 2-(5-nitrofurfurylidenehydrazino)-1,3,5-triazines has been synthesized. Some of these compounds possessed good antibacterial activity against *Mycobacterium tuberculosis in vitro* and moderate activity *in vivo* (the activity *in vivo* was observed only with high dosages compared with compounds in clinical use and the results were also irregular). The most active compounds are triazinyl-hydrazones of 5-nitro-2-furaldehyde in which the triazine ring is substituted with two amino-groups, at least one of which is an (α-branched chain alkyl)- or cycloalkylamine. Structure- anti-tuberculosis activity relations for these compounds are discussed.

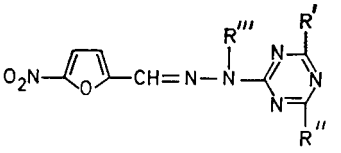
Many hundreds of nitrofurans have been reported in the literature (Paul & Paul, 1964; Ellis & West, 1967) but so far no nitrofurans have been claimed to have anti-tuberculosis activity *in vivo* (Schnitzer, 1964; Ellis & West, 1967). We now wish to report such anti-tuberculosis activity in a series of diamino-1,3,5-trizainylhydrazones of 5-nitro-2-furaldehyde.

Dodd, Cramer, & Ward (1950) noted that the basic structure I usually conferred *in vivo* antibacterial activity. It occurred to us that triazinylhydrazones of 5-nitro-2-furaldehyde would conform to such structure requirements. Accordingly, a number



(I)

of compounds of Type II and some simple analogues were synthesized and we now report the unusual pharmacological and microbiological properties of a wide range of triazinylhydrazones of 5-nitro-2-furaldehyde and other aldehydes.‡



(II)

In the first instance the readily accessible 2-chloro-4,6-di(substituted amino)-1,3,5-triazines (Pearlman & Banks, 1948; Thurston, Dudley & others, 1951) were reacted with three molecular proportions of hydrazine hydrate in refluxing ethanol to give the corresponding new di(substituted amino)triazinylhydrazines which, after isolation by

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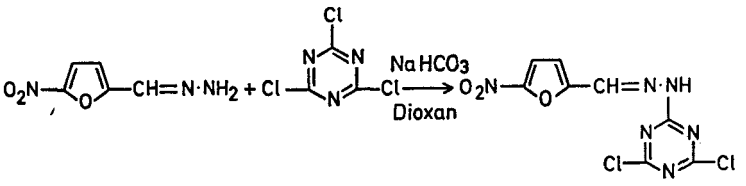
‡ During the course of this work Hirao & Kato (1964) described the preparation of several nitrofurans of this type (cpds 22, 26, 28, 45, 51 and 55).

one of two techniques described, readily condensed with 5-nitro-2-furaldehyde to give compounds of Type III (see Table 1). The analogous aldehydes: 2-furaldehyde, β-(5-nitrofur-2-yl)acrylaldehyde, 5-nitrothiophen-2-carbaldehyde, and 5-nitropyrrole-2-carbaldehyde, gave the corresponding hydrazones of Type V (see Table 3).

In a similar manner methylhydrazine and hydroxyethylhydrazine gave the corresponding substituted diaminotriazinylhydrazines which likewise condensed with 5-nitro-2-furaldehyde to give compounds of Type IV (see Table 2).

Investigation of triazinylhydrazones with substituents other than amino-groups on the triazine ring was made difficult by the inaccessibility of many of the required intermediates. However it was found that 5-nitro-2-furaldehyde hydrazone (ICI, 1959) reacts with cyanuric chloride to give the stable dichloro-derivative VI. Using



(VI)

the method of Senier (1886) this dichloro-derivative was treated with hot glacial acetic acid to give the corresponding dihydroxy-compound. Reaction of the dichloro-derivative VI with sodium methoxide gave the dimethoxy-derivative. The chlorine atoms could also be replaced by amino-groups and a combination of such reactions led to successive replacement of the chlorine atoms in VI to give mixed chloro-amino- or hydroxy-amino-triazinylhydrazones. The di-xylyl derivative was prepared in a similar manner to the diaminotriazinylhydrazines from the 2-chloro-4,6-di(2,4-xylyl)-1,3,5-triazine described by CIBA (1961). The trichloromethyl group in 2-amino-4-methyl-6-trichloromethyl-1,3,5-triazine (Kreutzberger, 1957) was replaced by the hydrazino-group by reaction with hydrazine; subsequent condensation with 5-nitro-2-furaldehyde gave the desired product. Typical preparative details are given in the experimental section and the melting point and analysis of the compounds are listed in Tables 1, 2, 3, and 4.

EXPERIMENTAL

Chemical

All melting points are uncorrected.

2-Hydrazino-4,6-dipiperidino-1,3,5-triazine. 2-Chloro-4,6-dipiperidino-1,3,5-triazine (Pearlman & Banks, 1948) (17·6 g) was dissolved in ethanol (50 ml) under reflux and then treated with hydrazine hydrate (100%) (9·4 ml). The mixture was refluxed for 1 h during which time the product deposited. On completion of the reaction the liquor was cooled and the product was collected by filtration and washed well with water. The solid was recrystallized from a mixture of light petroleum (b.p. 100–120°) and ethanol (10:1) to give the hydrazine (12·4 g), m.p. 130°. (Found: C, 56·1; H, 8·3; N, 35·3. $C_{13}H_{25}N_7$ Requires: C, 56·3; H, 8·4; N, 35·35%).

2-Ethylamino-4-hydrazino-6-isopropylamino-1,3,5-triazine. 2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (21·6 g) was suspended in ethanol (150 ml) under reflux and then treated with hydrazine hydrate (100%) (15 ml). The mixture was refluxed for 1 h. Ethanol (50 ml) was removed by distillation and water (100 ml) was

added to the residue. The aqueous ethanolic solution of the residue was extracted with chloroform (2 × 50 ml, 2 × 25 ml) and the chloroform extract was washed with water (25 ml) and dried over anhydrous sodium sulphate. Removal of the chloroform gave the hydrazine (21·1 g) as a syrup which could not be crystallized and which was reacted directly to give the hydrazone.

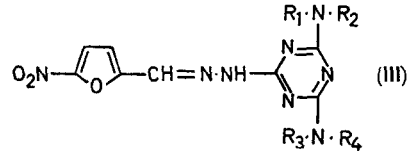
2-*Ethylamino*-4-*isopropylamino*-6-(5-*nitrofurfurylidenehydrazino*)-1,3,5-*triazine.* Crude 2-ethylamino-4-hydrazino-6-isopropylamino-1,3,5-triazine (21·1 g) was dissolved in ethanol (100 ml) and treated with 5-nitro-2-furaldehyde (14·1 g). The mixture was refluxed for ½ h and then cooled to 5°. The yellow product which precipitated was collected by filtration and washed with ethanol (20 ml). The solid was recrystallized from nitromethane (125 ml) and then ethanol (250 ml) to give the hydrazone (12 g), m.p. 199–201° (Found: C, 46·7; H, 5·4; N, 33·6. $C_{13}H_{18}N_8O_3$ Requires: C, 46·7; H, 5·4; N, 33·5%).

2,4-*Dichloro*-6-(5-*nitrofurfurylidenehydrazino*)-1,3,5-*triazine.* A solution of cyanuric chloride (6·2 g) in dioxan (50 ml) was prepared and to this vigorously stirred solution was added simultaneously a solution of 5-nitro-2-furaldehyde hydrazone (ICI, 1959) in dioxan (150 ml) at 30° and a solution of sodium bicarbonate (2·8) in water (50 ml). The addition was completed in 15 min and the mixture was then stirred a further 2 h. The yellow product was collected by filtration and washed with aqueous dioxan. The solid was dried to give the crude hydrazone (7·3 g), which was then extracted with nitroethane in a Soxhlet apparatus. The nitroethane extract deposited crystals of the pure hydrazone, m.p. > 300° (Found: C, 31·6; H, 1·3; Cl, 23·5, $C_8H_4Cl_2N_6O_3$ Requires: C, 31·7; H, 1·3; Cl, 23·4%).

2,4-*Dihydroxy*-6-(5-*nitrofurfurylidenehydrazino*)-1,3,5-*triazine.* 2,4-Dichloro-6-(5-nitrofurfurylidenehydrazino)-1,3,5-triazine (5 g) was extracted with glacial acetic acid (500 ml) in a sintered glass Soxhlet apparatus for 24 h. The extract was cooled slightly and the decomposition product removed by filtration. On further cooling the filtrate deposited the product which was collected by filtration and dried. The dry material (2·3 g) was extracted with nitromethane for 8 h in a Soxhlet apparatus and on completion of the extraction the nitromethane extract deposited yellow crystals of the hydrazone (0·4 g), m.p. > 300° (Found: C, 36·1; H, 2·45; N, 31·7. $C_8H_6N_6O_5$ Requires: C, 36·1; H, 2·3; N, 31·6).

2,4-*Dimethoxy*-6-(5-*nitrofurfurylidenehydrazino*)-1,3,5-*triazine.* 2,4-Dichloro-6-(5-nitrofurfurylidenehydrazino)-1,3,5-triazine (20 g) was suspended in methanol (350 ml). To this suspension a solution of sodium methoxide, prepared from sodium (30·6 g) and dry methanol (650 ml), was added dropwise over 1½ h. On completion of the addition the solution was allowed to stand at room temperature for 4 h. The solid which precipitated was discarded and the resulting clear liquor was cooled to give a yellow product. Evaporation of the mother liquor gave further crops of the product. The solid was recrystallized from nitroethane to give the hydrazone (5 g), m.p. 260° (dec.) (Found: C, 40·65; H, 3·4; N, 28·7. $C_{10}H_{10}N_6O_5$ Requires: C, 40·8; H, 3·4; N, 28·6%).

2-*Chloro*-4-*diethylamino*-6-(5-*nitrofurfurylidenehydrazino*)-1,3,5-*triazine.* 2,4-Dichloro-6-(5-nitrofurfurylidenehydrazino)-1,3,5-triazine (6·1 g) was dissolved in dimethyl sulphoxide (100 ml) at 50°. To this solution at 50° ± 2°, a solution of diethylamine (3·0 g) in chloroform (10 ml) was added dropwise during ½ h. On completion of the addition the solution was allowed to stand at room temperature for 1 h and was then poured into water (200 ml) with stirring. The yellow product which deposited was



(III)

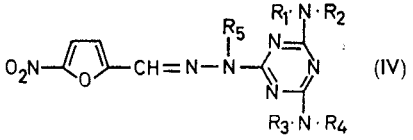
Table 1. *Diaminotriazinylhydrazones of 5-nitro-2-furaldehyde*

Cpd no.	R_1	R_2	R_3	R_4	m.p. °C	Formula	Found C	Found H	Found N	Found S	Required C	Required H	Required N	Required S
1	H	H	H	H	*a* >200 (dec.)	$C_8H_8N_8O_3 \cdot C_4H_4O_4$	37·8	3·45	29·5		37·9	3·2	29·5	
2	Me	H	H	H	*b* 221	$C_9H_{10}N_8O_3 \cdot \frac{1}{2}H_2SO_4 \cdot \frac{3}{4}H_2O$	31·7	4·0	32·7	4·65	31·7	3·8	32·9	4·70
3	Et	H	H	H	244–6 (dec.)	$C_{10}H_{12}N_8O_3$	41·2	4·4			41·1	4·1		
4	Pr^i	H	H	H	237–9 (dec.)	$C_{11}H_{14}N_8O_3 \cdot \frac{1}{2}H_2O$	42·1	4·7			41·9	4·8		
5	C_3H_5	H	H	H	225 (dec.)	$C_{11}H_{12}N_8O_3$	43·3	4·2	36·7		43·4	4·0	36·8	
6	Bu^s	H	H	H	164	$C_{12}H_{16}N_8O_3$	44·95	5·15	34·7		45·0	5·0	35·0	
7	Bu^t	H	H	H	199–200	$C_{12}H_{16}N_8O_3$	45·3	5·05	35·0		45·0	5·0	35·0	
8	C_5H_9*c*	H	H	H	218	$C_{13}H_{16}N_8O_3 \cdot \frac{1}{2}H_2O$	45·6	5·1	33·1		45·7	5·0	32·8	
9	s-C_5H_{11}	H	H	H	138–140 (dec.)	$C_{13}H_{18}N_8O_3$	46·8	5·6	33·8		46·7	5·4	33·5	
10	Me·CH_2·CH(Et)	H	H	H	167 (dec.)	$C_{13}H_{18}N_8O_3 \cdot \frac{1}{2}H_2O$	45·3	5·6	32·6		45·5	5·6	32·6	
11	C_6H_{11}*d*	H	H	H	218–222 (dec.)	$C_{14}H_{18}N_8O_3$	48·4	5·45	32·4		48·5	5·2	32·3	
12	Ph	H	H	H	282	$C_{14}H_{12}N_8O_3$	49·4	3·7	33·1		49·4	3·55	32·9	
13	*o*-MeO·C_6H_4	H	H	H	268–9 (dec.)	$C_{15}H_{14}N_8O_4 \cdot H_2O$	48·1	4·2	30·1		48·4	4·3	30·1	
14	*m*-CF_3·C_6H_4	H	H	H	246 (dec.)	$C_{15}H_{11}F_3N_8O_3$	44·4	2·6	27·3		44·1	2·7	27·5	
15	Ph·CH_2	H	H	H	215	$C_{15}H_{14}N_8O_3$	50·7	4·3	31·5		50·8	4·0	31·6	
16	Pr^i	H	Me	H	203–5 (dec.)	$C_{12}H_{16}N_8O_3$	45·0	5·2	34·9		45·0	5·0	35·0	
17	Pr^i	H	Et	H										
18	Pr^i	H	Pr^i	H	220–2	$C_{14}H_{20}N_8O_3$	48·5	5·9	32·45		48·25	5·8	32·2	
19	Pr^i	H	Bu^s	H	207–9 (dec.)	$C_{15}H_{22}N_8O_3$	49·5	6·4	30·85		49·7	6·1	30·9	
20	Pr^i	H	Ph	H	186–8 (dec.)	$C_{17}H_{18}N_8O_3$	53·55	5·0	29·1		53·4	4·7	29·3	
21	Et	Et	H	H	254 (dec.)	$C_{12}H_{16}N_8O_3$	45·0	4·9			45·0	5·0		
22	Et	H	Et	H	220–2	$C_{12}H_{16}N_8O_3$	44·85	5·2			45·0	5·0		
23	Et	Et	Et	H	185–7	$C_{14}H_{20}N_8O_3$	48·3	5·8			48·25	5·8		
24	Et	Et	Et	Et	111–2	$C_{16}H_{24}N_8O_3$	51·3	6·55			51·05	6·4		
25	Me	Me	H	H	267–9 (dec.)	$C_{10}H_{12}N_8O_3$	41·1	4·15	38·1		41·1	4·1	38·35	
26	Me	H	Me	H	249–250	$C_{10}H_{12}N_8O_3$	41·0	4·3	38·1		41·1	4·1	38·25	
27	Me	Me	Me	H	250–2 (dec.)	$C_{11}H_{14}N_8O_3$	43·1	4·7			43·1	4·6		
28	Me	Me	Me	Me	201–2	$C_{12}H_{16}N_8O_3$	44·9	4·9	35·2		45·0	5·0	35·0	
29	Me	H	Et	H	234–7 (dec.)	$C_{11}H_{14}N_8O_3$	43·2	4·55			43·1	4·6		
30	Me	H	Pr^n	H	*b* 178–185	$C_{12}H_{16}N_8O_3 \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{2}H_2O$	37·9	5·1	29·7		38·1	4·8	29·6	
31	Me	H	Bu^n	H	168–170	$C_{13}H_{18}N_8O_3$	46·5	5·3	33·7		46·7	5·4	33·5	
32	Me	H	Bu^s	H	171–2	$C_{13}H_{18}N_8O_3$	46·6	5·6	33·6		46·7	5·4	33·5	
33	Me	H	Bu^t	H	219–222 (dec.)	$C_{13}H_{18}N_8O_3$	46·4	5·4	33·6		46·7	5·4	33·5	

Table 1—*continued*

Cpd no.	R_1	R_2	R_3	R_4		m.p. °C	Formula	Found C	Found H	Found N	Found S	Required C	Required H	Required N	Required S
34	Me	H	C_5H_9*c*	H		136 (dec.)	$C_{14}H_{18}N_8O_3$	48·5	5·3	32·55		48·5	5·2	32·3	
35	Me	H	s-C_8H_{17}	H		153–5	$C_{17}H_{26}N_8O_3$	52·2	6·65			52·3	6·7		
36	Me	H	Ph	H		236 (dec.)	$C_{15}H_{14}N_8O_3$	50·8	4·1	31·5		50·8	4·0	31·6	
37	Et	H	C_3H_6OMe	H		179–182	$C_{14}H_{20}N_8H_4 \cdot \frac{1}{2}H_2SO_4 \cdot 2H_2O$	37·7	5·7		3·75	37·5	5·6		3·6
38	Pr^i	H	C_3H_5	H		201–2	$C_{14}H_{18}N_8O_3$	48·7	5·5	32·2		48·5	5·2	32·4	
39	Pr^n	H	C_2H_4OH	H		152–4	$C_{13}H_{18}N_8O_4$	44·4	5·2			44·55	5·2		
40	C_3H_5	H	C_3H_5	H		189–190 (dec.)	$C_{14}H_{16}N_8O_3$	48·9	4·65	32·5		48·8	4·7	32·6	
41	Pr^i	H	C_3H_6OMe	H		106–7 (dec.)	$C_{15}H_{22}N_8O_4 \cdot \frac{1}{2}H_2O$	46·7	6·0	28·9		46·5	6·0	29·0	
42	Bu^s	H	Bu^s	H	*b*	185–7	$C_{16}H_{24}N_8O_3 \cdot \frac{1}{2}H_2SO_4 \cdot 2H_2O$	41·5	6·4	24·2	3·6	41·6	6·6	24·3	3·4
43	Bu^t	H	Bu^t	H		269–271 (dec.)	$C_{16}H_{24}N_8O_3$	51·3	6·4	29·4		51·05	6·4	29·8	
44	$C_{12}H_{25}$	H	$C_{12}H_{25}$	H		145–6	$C_{32}H_{56}N_8O_3 \cdot \frac{1}{4}H_2O$	63·8	9·2	18·3		63·5	9·4	18·5	
45	C_5H_9*c*	H	C_5H_9*c*	H	*b*	259 (dec.)	$C_{18}H_{24}N_8O_3 \cdot \frac{1}{2}H_2SO_4 \cdot H_2O$	46·2	5·85	24·0	3·4	46·25	5·8	24·0	3·4
46	C_6H_{11}*d*	H	C_6H_{11}*d*	H		218–220 (dec.)	$C_{20}H_{28}N_8O_3$	56·2	6·7	25·9		56·1	6·6	26·15	
47	Ph	H	Ph	H		255–8 (dec.)	$C_{20}H_{16}N_8O_3$	57·4	3·9			57·6	3·9		
48	Ph·CH_2	H	$PhCH_2$	H		203–4	$C_{22}H_{20}N_8O_3$	59·2	4·5			59·4	4·5		
49	H	H	$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2$			282 (dec.)	$C_{12}H_{14}N_8O_3$	45·5	4·6	35·1		45·3	4·4	35·2	
50	H	H	$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2$			270 (dec.)	$C_{13}H_{16}N_8O_3$	46·8	5·1	33·8		47·0	4·85	33·7	
51	Me	H	Et	Et		220–1 (dec.)	$C_{13}H_{18}N_8O_3$	46·8	5·5			46·7	5·4		
52	Pr^i	H	Me	Me		194–5	$C_{13}H_{18}N_8O_3$	46·7	5·5	33·45		46·7	5·4	33·5	
53	Pr^i	H	Et	Et		172–4	$C_{15}H_{22}N_8O_3$	49·9	6·0	31·2		49·7	6·1	30·9	
54	Et	Et	$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2$			188–200 (dec.)	$C_{16}H_{22}N_8O_3$	51·3	6·2	30·0		51·3	5·9	29·9	
55	$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot$		$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2$			218 (dec.)	$C_{18}H_{24}N_8O_3$	54·2	6·2	27·9		54·0	6·0	28·0	
56	$CH_2 \cdot CH_2 \cdot O \cdot CH_2 \cdot CH_2$		$CH_2 \cdot CH_2 \cdot O \cdot CH_2 \cdot CH_2$			262 (dec.)	$C_{16}H_{20}N_8O_5 \cdot \frac{1}{2}C_2H_5OH$	47·9	5·5	26·3		47·8	5·4	26·3	
57	$HO \cdot [CH_2]_2$	$HO \cdot [CH_2]_2$	$HO \cdot [CH_2]_2$	$HO \cdot [CH_2]_2$		190–2	$C_{16}H_{24}N_8O_7 \cdot H_2O$	42·2	5·7	24·3		41·9	5·7	24·45	

a Maleate. *b* Sulphate. *c* Cyclopentyl. *d* Cyclohexyl.

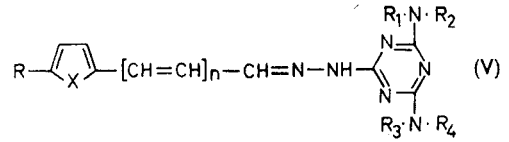


(IV)

Table 2. *Substituted diaminotriazinylhydrazones of 5-nitro-2-furaldehyde*

Cpd no.	R_1	R_2	R_3	R_4	R_5	m.p. °C.	Formula	Found C	Found H	Found N	Found S	Required C	Required H	Required N	Required S
58	Me	H	Me	H	Me	*b* 248–9	$C_{11}H_{14}N_8O_3 \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{4}H_2O$	36·6	4·5	31·2	4·5	36·7	4·3	31·1	4·45
59	Et	H	Pr^i	H	Me	*b* 271 (dec.)	$C_{14}H_{20}N_8O_3 \cdot H_2SO_4$	37·8	5·1	25·2	7·2	37·7	5·0	25·2	7·2
60	Pr^i	H	Pr^i	H	Me	146–7	$C_{15}H_{22}N_8O_3$	49·7	6·1	30·9		49·6	6·3	30·8	
61	$C_{12}H_{25}$	H	$C_{12}H_{25}$	H	Me	*b* 237	$C_{33}H_{56}N_8O_3 \cdot H_2SO_4$	55·7	8·2	15·7	4·2	55·8	8·2	15·8	4·5
62	Ph	H	Ph	H	Me	235	$C_{21}H_{18}N_8O_3$	58·4	4·1	25·8		58·6	4·2	26·0	
63	MeO·$[CH_2]_3$	H	C_6H_{11}*d*	H	Me	*b* 252 (dec.)	$C_{19}H_{28}N_8O_3 \cdot H_2SO_4 \cdot H_2O$	42·9	5·7	20·9	5·8	42·85	5·95	21·0	6·0
64	$C_{12}H_{25}$	H	HO·$[CH_2]_2$	HO·$[CH_2]_2$	Me	115–7	$C_{25}H_{42}N_8O_5$	56·2	8·1	20·9		56·3	7·75	21·0	
65	Ph·CH_2	H	$CH_2 \cdot CH_2 \cdot O \cdot CH_2 \cdot CH_2$		Me	178–180	$C_{20}H_{22}N_8O_4$	54·7	5·2	25·6		54·8	5·1	25·6	
66	Me	Me	Me	Me	Me	211–2	$C_{13}H_{18}N_8O_3$	46·9	5·65			46·7	5·4		
67	Et	Et	Et	Et	Me	138–140	$C_{17}H_{26}N_8O_3$	52·1	6·6	29·0		52·3	6·7	28·7	
68	$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2$		$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2$		Me	232	$C_{19}H_{26}N_8O_3$	54·9	6·45	26·8		55·05	6·3	27·0	
69	Et	H	Et	H	HO·$[CH_2]_2$	211–2 (dec.)	$C_{14}H_{20}N_8O_4$	46·1	5·5	30·7		46·1	5·5	30·75	
70	Et	H	Pr^i	H	HO·$[CH_2]_2$	187–8	$C_{15}H_{22}N_8O_4$	47·8	6·1	29·35		47·6	5·9	29·6	
71	Et	MeCO	Et	Me·CO	Me·CO·O·$[CH_2]_2$	191–2	$C_{20}H_{26}N_8O_7$	49·2	5·1	22·9		49·0	5·3	22·85	

b sulphate. *d* cyclohexyl.

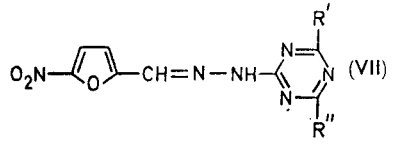


(V)

Table 3. *Diaminotriazinylhydrazones of various other aldehydes*

Cpd no.	R	X	n	R_1	R_2	R_3	R_4	m.p.	°C.	Formula	Found C	Found H	Found N	Found S	Required C	Required H	Required N	Required S
72	NO_2	O	1	Me	H	Pr^i	H	*b* 258	(dec.)	$C_{14}H_{18}N_8O_3 \cdot H_2SO_4 \cdot H_2O$	36·5	5·0	24·4		36·4	4·8	24·2	
73	NO_2	O	1	Me	Me	Me	Me	244–6	(dec.)	$C_{14}H_8N_8O_3$	48·7	5·1			48·5	5·2		
74	H	O	0	Me	H	Pr^i	H	*e* 170–4	(dec.)	$C_{12}H_{17}N_7O \cdot HCl \cdot H_2O$	43·9	6·2	29·7		43·7	6·1	29·7	
75	H	O	0	Et	H	Et	H	100–112		$C_{12}H_{17}N_7O$	52·2	6·3			52·35	6·2		
76	H	O	0	Me	Me	Me	Me	210–1		$C_{12}H_{17}N_7O$	52·3	6·4			52·35	6·2		
77	NO_2	S	0	Pr^i	H	Pr^i	H	183·5		$C_{14}H_{20}N_8O_2S$	46·1	5·7	30·9	8·9	46·1	5·5	30·8	8·8
78	NO_2	NH	0	Et	H	Pr^i	H	*b* 182	(dec.)	$C_{13}H_{19}N_9O_2 \cdot \frac{1}{2}H_2SO_4 \cdot \frac{1}{2}H_2O$	40·3	5·5	32·4	4·0	39·9	5·4	32·2	4·1
79	NO_2	NH	0	Pr^i	H	Pr^i	H	148–9		$C_{14}H_{21}N_9O_2 \cdot \frac{1}{4}H_2O$	48·1	6·2	36·0		47·9	6·2	35·8	

b sulphate. *e* hydrochloride.



(VII)

Table 4. *Di-substituted triazinylhydrazones of 5-nitro-2-furaldehyde*

Cpd no.	R′	R″	m.p.	°C.	Formula	Found C	Found H	Found Cl	Found N	Required C	Required H	Required Cl	Required N
80	Cl	Cl											
81	OH	OH											
82	MeO	MeO											
83	Cl	NH·Pr^i	131–5		$C_{11}H_{12}ClN_7O_3$	40·6	3·9	10·7	30·1	40·6	3·7	10·9	30·1
84	Cl	NEt_2											
85	OH	$NHPr^i$	*e* 253–5	(dec.)	$C_{11}H_{13}N_7O_4 \cdot HCl \cdot 1{\cdot}6H_2O$	35·3	4·55		26·4	35·6	4·7		26·3
86	OH	NEt_2											
87	2,4-Xylyl	2,4-Xylyl	189–190		$C_{24}H_{22}N_6O_3$	65·0	4·95		19·1	65·1	5·1		19·0
88	Me	NH_2	240	(dec.)	$C_9H_9N_7O_3 \cdot \frac{1}{4}H_2O$	40·3	3·7		36·5	40·4	3·6		36·6

e hydrochloride.

washed with water and dried. The dried solid was extracted with chloroform in a Soxhlet apparatus and the chloroform extract evaporated to low bulk. The crystals (2·9 g) which separated were collected by filtration and recrystallized from methanol (100 ml) to give yellow needles of the hydrazone (1·4 g), m.p. 215–6° (dec.) (Found: C, 42·6; H, 4·2; Cl, 10·6; N, 28·6. $C_{12}H_{14}ClN_7O_3$ Requires: C, 42·4; H, 4·15; Cl, 10·4; N, 28·9%).

2-*Diethylamino*-4-*hydroxy*-6-(5-*nitrofurfurylidenehydrazino*)-1,3,5-*triazine*. 2-Chloro -4-diethylamino-6-(5-nitrofurfurylidenehydrazino)-1,3,5-triazine (10 g) was dissolved in glacial acetic acid (100 ml) and the solution refluxed for 1½ h. The cooled solution deposited the hydrochloride of the product which was collected by filtration and washed with chloroform. The hydrochloride was dissolved in 50/50 aqueous methanol (150 ml) and neutralized with 2N sodium bicarbonate solution (70 ml). The precipitated base was collected by filtration and washed with water. The dried product was recrystallized from methanol (1200 ml) to give the hydrazone (3·5 g) as yellow plates, m.p. 257–263° (Found: C, 44·7; H, 4·8; N, 30·5. $C_{12}H_{15}N_7O_4$ Requires: C, 44·85; H, 4·7; N, 30·5%).

BIOLOGICAL METHODS

In vitro *assay for antituberculosis activity*

The compounds were dissolved in a suitable organic solvent at different concentrations. An exact amount of each solution was added to a hundredfold quantity of Youmans medium (Youmans & Karlson, 1947) containing 1·5% Noble—Agar (Difco) and 10% bovine serum. After thorough mixing, the compound-containing medium was distributed in culture-tubes (5 ml per tube) and slanted. After solidification the tubes were inoculated with standardized suspensions of tubercle bacilli using a wild strain (A_5) as well as an isoniazid-resistant (INH_r) and a streptomycin-resistant (Str_r) strain. The culture tubes were then incubated at 37° and read after 3 weeks. The minimal inhibitory concentration was determined which is the lowest concentration at which no growth occurred. Controls containing the same amount of solvent were included in each experiment.

In vivo *assay of antituberculosis activity*

Albino mice, 20 g, of a strain susceptible to tuberculosis were infected intravenously with 0·2 ml of a suspension containing 0·8 mg virulent tubercle bacilli (wild strain A_5). Treatment was started 3 days after the infection and continued till the 33rd day after the infection. The compounds were suspended in 0·5% carboxymethylcellulose and given by stomach tube in dosages of 400, 200, 100 and 50 mg/kg during 5 days of the week. Groups of 10 mice were used for each dose-level. The life span after infection was noted and the median-survival time of each group compared with that of the untreated controls. A therapeutic effect was demonstrated by a significant increase of the survival time of the treated animals.

RESULTS AND DISCUSSION

Of the compounds described in this paper all those which exhibited *in vitro* activity at a concentration of less than 10 μg/ml against at least one of the strains mentioned in the previous section are listed in Table 5.

Table 5. *Antituberculosis activity of nitrofuran derivatives*

Cpd no.	*Mycobacterium tuberculosis* Minimum inhibitory concentration after 21 days (μg/ml) A_5	INH_r	Str_r	Activity *in vivo* A_5
3	0·5	10	2·5	—
4	0·3	3	0·3	++
5	1	10	1	—
6	0·25	2·5	0·25	++
7	0·25	2·5	0·5	+
8	0·1	1	0·25	+
9	0·25	2·5	0·5	—
10	1	10	1	—
11	1	2·5	0·5	—
12	0·25	1	0·25	—
13	0·1	1	0·1	—
14	0·25	0·5	0·25	—
15	1	2·5	2·5	—
16	0·25	1	0·25	++
17	0·3	1	0·3	++
18	0·25	1	0·25	+++
19	0·25	2·5	0·25	+
20	1	5	1	—
21	0·3	3	1	—
22	0·25	1	0·25	—
23	0·25	2·5	0·5	—
29	1	10	1	—
30	1	10	10	—
31	1	10	10	—
32	0·1	1	0·1	++
33	0·5	2·5	2·5	—
34	1	10	2·5	—
36	0·25	0·5	1	—
37	2·5	5	5	—
38	0·25	2·5	0·25	++
39	5	10	5	N.T.
40	1	10	1	—
42	0·5	>5	1	—
43	1	10	10	—
45	1	10	1	—
46	1	10	5	—
47	1	10	10	—
49	0·25	2·5	0·5	—
50	0·25	2·5	1	+
52	0·1	1	0·1	—
53	0·5	2·5	1	—
54	1	10	2·5	—
55	1	10	1	—
56	1	10	10	N.T.
59	0·03	0·3	0·03	+
60	0·03	0·1	0·03	+
62	0·3	0·3	0·3	—
63	0·3	<1	0·3	—
64	3	10	3	—
65	1	3	3	—
70	1	3	0·3	—
72	1	1	1	—
73	3	3	3	—
77	0·5	1	0·5	—
83	0·1	2·5	0·25	—
Isoniazid	0·05–0·1	>100	0·05–0·1	++++

The following criteria have been used in indicating the relative *in vivo* activities.

— = no activity observed up to a dose of 400 mg/kg per day. + = significant increase in survival-time of mice over controls at dose levels of 200–400 mg/kg. ++ = significant increase in survival-time of mice over controls at dose levels of 100 mg/kg per day and higher. +++ = significant increase in survival-time of mice over controls at dose levels of 50 mg/kg per day and higher. ++++ = significant increase in survival-time for mice over controls at a dose of 5 mg/kg per day. N.T. = not tested.

Chemical structure—biological activity relations

(a) *Diaminotriazinylhydrazones III, IV, and V.* The *in vitro* tests show a distinct relation between the antituberculosis activity and the structure of the diamino-triazinylhydrazones studied. In the series of amino(monosubstituted amino)triazinyl-hydrazones of Type III (cpds 1–15, Table 1) activity is quickly reached with the ethyl derivative (cpd 3) and only begins to fall with the 1-ethylpropyl derivative (cpd 10). The aryl derivatives (cpds 12–15) exhibit activity of a similar order to that of the C_5 alkyl derivative (cpd 8). A similar rise and fall of activity with increasing chain-length is noticed with the series of di(mono substituted amino)triazinylhydrazones of Type III (cpds 16–20, 22, 26, and 29–48, but particularly in the series formed by cpds 26, 22, 18, 42, 43, 45 and 44). The beneficial effect on activity of a secondary or tertiary alkyl group in this series is clearly illustrated by a comparison of cpds 16 with 30 and of 31 with 32 and 33; and many of the most active compounds synthesized contain an α-branched-chain component. The variation of one (substituted amino)-group when the other is the isopropylamino-group leads to no significant alteration in activity (cpds 16–19).

Amino (di-substituted amino)triazinylhydrazones of Type III are comparable with the isomeric di(monosubstituted amino)-compounds (cf. cpds 21 and 25 with 22 and 26).

The (monosubstituted amino) (disubstituted amino)triazinylhydrazones of Type III (except the trimethyl derivative, cpd 27) retain high activity (cpds 23, 52, and 53), but substitution of all four hydrogen atoms in the series of di(disubstituted amino) triazinylhydrazones leads to a weakening of activity (cpds 24, and 54–7). Relatively few compounds of Type III with high *in vitro* activity have *in vivo* activity. *In vivo* activity is essentially restricted to those compounds which possess at least one (α-branched-chain alkyl)amino-group or cycloalkylamino-group, and either two or three unsubstituted hydrogen atoms on the two amino-groups (cpds 4, 6–8, 16–19, 32 and 38).

The introduction of a methyl substitutent on the hydrazine nitrogen atom in compounds of Type IV (Table 2) led to an almost ten-fold increase in *in vitro* activity, closely approaching the activity of isoniazid. *In vivo* however the activity was reduced (cpds 59, 60 and 62). On the other hand introduction of a hydroxyethyl substituent in this position produced little effect (cpds 69 and 70). Two vinylogues (cpds 72 and 73) and a thiophene analogue (cpd 77) showed little difference in *in vitro* activity but *in vivo* activity was negligible. Furan and nitropyrrole analogues were without significant activity (cpds 74–6, and 78–9).

(b) *Other triazinylhydrazones type VII.* Replacement of the amino-group of a diaminotriazinylhydrazone by a halogen atom (as in cpd 83, Table 4; cf. cpd 4) maintained *in vitro* activity but not *in vivo* activity; but replacement of one amino-group by a hydroxyl group (as in cpd 85) resulted in loss of activity. Replacement of both amino-groups also caused loss of activity.

The structural requirements for antituberculosis activity are clearly quite critical. The nitro-group is essential, as is one amino-group or (substituted amino)-group on the triazine ring; for *in vivo* activity two amino-groups or (substituted amino)-groups are necessary.

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