p-PYRIDOYLAMINOBENZOIC ACIDS AND THEIR DERIVATIVES AS POSSIBLE TUBERCULOSTATS*†

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ABSTRACT

p-Pyridoylaminobenzoic acids, their glycine and DL-methionine conjugates and their esters, hydrazides and aldehyde derivatives of the hydrazides totalling 105 *compounds have been synthesised and screened for* in vitro *activity against* Mycrobacterium tuberculosis $H_{37}R_v$ *strain. Out of these,* 19 *compounds have shown inhibition of growth of the organism at dilutions varying from* 1 *in* 10000 (100 γ/*ml.*) *to* 1 *in* 10000000 (0.1 γ/*ml.*). *Three compounds which were highly active* **in vitro** (0.1 γ *to* 1 γ/*ml.*) *were found to be ineffective,* **in vivo** *in experimental tuberculosis of mice tested by the drug-diet method.*

Although the existing potent antituberculosis agents could be used successfully, there are many problems still to be faced to render the treatment more effective. While the development of bacterial resistance, which is a major problem, can be prevented or atleast delayed by the simultaneous or alternating combination of drugs, there is need for newer drugs which, used singly, can eliminate the tubercle bacilli rapidly and radically.

In a search for new antituberculosis agents, N-acetyldipeptides and their derivatives[1], pyridoylaminoacids and their derivatives[2], pyridylacrylic acids, pyridylpropionic acids, furylacrylic acids and their derivatives[3], chlorophenoxyacetic acids and their derivatives[4] and pyridoyldipeptides and their derivatives[5] have been synthesised in our laboratory and screened for *in vitro* antituberculosis activity with encouraging results. The present work consists of the synthesis and testing of the following types of compounds.

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(A) *p*-Isonicotinoylaminobenzoic acid, its glycine and DL-methionine conjugates, their esters, hydrazides and hydrazone derivatives (39 compounds).

(B) *p*-Nicotinoylaminobenzoic acid, its glycine and DL-methionine conjugates, their esters, hydrazides and hydrazone derivatives (39 compounds).

(C) *p*-Picolinoylaminobenzoic acid, its glycine and DL-methionine conjugates, their esters, hydrazides and hydrazone derivatives (27 compounds).

The scheme of synthesis given in Chart I is self-explanatory.

While isonicotinic acid[6] and picolinic acid[7] were prepared by the permanganate oxidation of γ- and α-picolines respectively, nicotinic acid was available from stock. The acid chlorides of the pyridine carboxylic acids (II) have been prepared by treating their potassium salts with thionyl chloride in dry benzene[8]. The acid chloride on condensation with ethyl *p*-aminobenzoate gave the corresponding ethyl *p*-pyridoylaminobenzoates (III) in good yields. Ethyl esters of glycine and DL-methionine conjugates (VIII) of *p*-pyridoylaminobenzoic acids have been prepared *via* the acid azide method[9], *i.e.*, by the action of ethyl ester of the aminoacid on the acid azides of *p*-pyridoylaminobenzoic acids.

Screening for *in vitro* tuberculostatic activity was carried out by the serial dilution method using surface culture technique by Professor M. Sirsi. The substances were first dissolved in ethylene glycol and further required dilutions were prepared in the Youman's media. The test organism was *Mycobacterium tuberculosis* $H_{37}R_v$ strain. The degree of inhibition or growth was observed at weekly intervals. The degree of inhibition or growth seen at the end of three weeks was recorded. The results of *in vitro* screening are mentioned in the experimental part and discussed at the end.

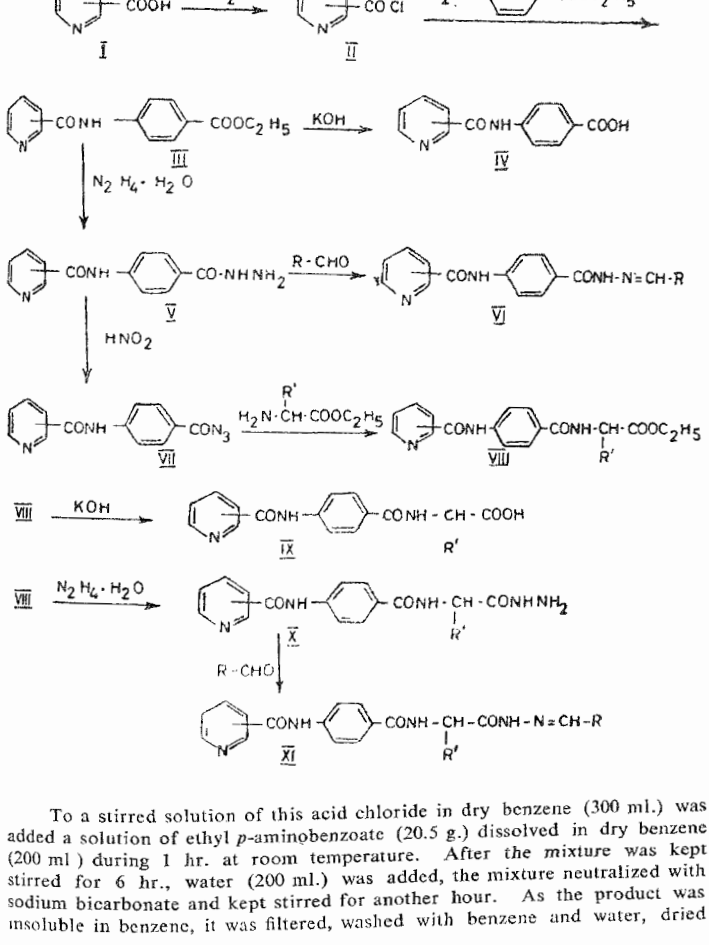
EXPERIMENTAL*

(A) *p*-ISONICOTINOYLAMINOBENZOIC ACID, ITS GLYCINE AND DL-METHIONINE CONJUGATES AND THEIR DERIVATIVES

Ethyl p-*isonicotinoylaminobenzoate*[10] (*Componnd No.* 1)

To a suspension of potassium isonicotinate (20 g.) in dry benzene (100 ml.), cooled to 0°C, was slowly added redistilled thionyl chloride (40 ml) over a period of 10 min. After addition, the reaction mixture was refluxed for 1 hr. The crude isonicotinoyl chloride obtained on removal of benzene and excess of thionyl chloride was used as such for further condensation.

* All melting points are uncorrected and *d* denotes decomposition. Infrared spectra of all the compounds taken in the Perkin-Elmer Infracord Model 137B showed the characteristic absorptions.



To a stirred solution of this acid chloride in dry benzene (300 ml.) was added a solution of ethyl *p*-aminobenzoate (20.5 g.) dissolved in dry benzene (200 ml) during 1 hr. at room temperature. After the mixture was kept stirred for 6 hr., water (200 ml.) was added, the mixture neutralized with sodium bicarbonate and kept stirred for another hour. As the product was insoluble in benzene, it was filtered, washed with benzene and water, dried

and crystallised from aqueous ethanol m.p. 154-5°C. (Reported[10] m.p. 140°C). Yield: 30 g.; 91%. (Found: 66 18; H, 5.09; N, 10.79. $C_{15}H_{14}O_3N_2$ requires: C, 66.66; H, 5.22; N, 10.36%)

p-*Isonicotinoylaminobenzoic acid*[10] (*Compound No.* 2)

Ethyl *p*-isonicotinoylaminobenzoate (1 g.) was stirred with potassium hydroxide solution (1N, 20 ml.) for 3 to 4 hr. at room temperature. The precipitated acid was filtered, washed, dried and crystallised from ethanol. m p. 350°C.(d). (Reported[10] m p 350°C.). Yield · 0.4 g.: 45%.

p-Isonicotinoylaminobenzoic acid hydrazide[10] (*Compound No.* 3)

A mixture of ethyl *p*-isonicotinoylaminobenzoate (20 g.) dissolved in ethanol (400 ml, 90%) and hydrazine hydrate (22 g., 99%) was refluxed for 6 to 8 hr. Alcohol and excess of hydrazine hydrate were distilled off under suction and the solid residue obtained was crystalised from ethyl alcohol. m.p. 246-8°C. (Reported[10] m.p. 235-7°C.). Yield: 12 g.: 63%. (Found: C, 60.60; H, 4 90; N, 21 69. $C_{13}H_{12}O_2N_4$ requires: C, 60 93; H, 4.72; N, 21.86%).

Aldehyde derivatives of p-*isonicotinoylaminobenzoic acid hydrazide*

These were prepared by refluxing in each case, *p*-isonicotinoylaminobenzoic acid hydrazide (1 g.) and an equimolar amount of the aldehyde in absolute ethanol (100 ml.) for 8 hr. The hydrazones which precipitated on cooling were filtered, washed with a little alcohol, dried and crystallised from ethanol. Table 1 contains the compounds prepared (Nos. 4 to 13) in this series.

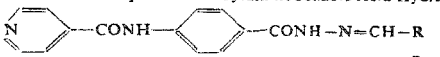
Ethyl p-*isonicotinoylaminobenzoylglycinate* (*Compound No.* 14)

To a stirred solution of *p*-isonicotinoylaminobenzoic acid hydrazide (12.8 g.) in dilute hydrochloric acid (90 ml., 1 : 1), maintained at 0°C., was added a previously cooled solution of sodium nitrite (25 g.) in water (60 ml.) over a period of 30 min. After 2 hr. of stirring, the mixture was neutralized with sodium carbonate solution and the solid azide filtered, washed and dried over phosphorus pentoxide in a vacuum desiccator. Yield: 12.6 g.; 94%.

To a stirred solution of *p*-isonicotinoylaminobenzoic acid azide (12.3 g.) in dimethylformamide (DMF) (100 ml.) was added ethyl glycinate (7.11 g.) dissolved in DMF (10 ml.) over a period of 30 min. at room temperature. After stirring for 10 hr., the solution was filtered and the solvent partly removed under suction. Addition of either benzene-petrol mixture (1 : 1) or water gave the required product which was filtered and crystallised from ethanol. m p. 207-8°C. Yield: 12.6 g.; 83.6%. (Found: C, 62.46; H, 5.54; N, 12.92 $C_{17}H_{17}O_4N_3$ requires: C, 62.38; H, 5 23; N, 12.84%).

TABLE I

Aldehyde Derivatives of *p*-Isonicotinoylaminobenzoic Acid Hydrazide



R=Aldehye residue

Compound No.	R— Aldehyde residue	Mol. formula	m.p.°C	Found/Required (%) C	H	N
4	C_6H_5-	$C_{20}H_{16}O_2N_4$	292 – 3(d)	69 31 69.76	4.77 4 68	16.41 16 27
5	*o*-OH C_6H_4-	$C_{20}H_{16}O_3N_4$	289 – 90(d)	66.36 66.66	4.28 4.48	15.69 15.55
6	*m*-OH C_6H_4-	$C_{20}H_{16}O_3N_4$	310 (d)	66.90 66.66	4,88 4 48	15.49 15.55
7	*p*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	334 – 5(d)	66.22 66 66	4 83 4 48	15.22 15.55
8	C_5H_4N-3-	$C_{19}H_{15}O_2N_5$	285 (d)	65.80 66.08	3 99 4 38	19.89 20.28
9	C_5H_4N-4-	$C_{19}H_{15}O_2N_5$	280 – 1(d)	66.33 66.08	4.33 4 38	19.80 20.28
10	*p*-MeO C_6H_4-	$C_{21}H_{18}O_3N_4$	290 (d)	67 30 67.37	4.83 4.85	14 62 14.96
11	*p*-$Me_2N.C_6H_4$-	$C_{22}H_{21}O_2N_5$	294 (d)	67.72 68 20	5.50 5.46	18.28 18 08
12	C_6H_5 CH = CH-	$C_{22}H_{18}O_2N_4$	291 (d)	71.25 71.34	4.96 4.90	15.21 15.13
13	C_4H_3O-2-	$C_{18}H_{14}O_3N_4$	318 (d)	64.28 64.67	4 38 4.22	16.37 16.76

Note: d means decomposition

p-*Isonicotinoylaminobenzoylglycine* (*Compound No.* 15)

This was prepared by the hydrolysis of ethyl *p*-isonicotinoylaminobenzoylglycinate (0.5 g.) with potassium hydroxide solution (1N, 10 ml.) and isolated in the usual manner. m.p. 298°C.(d). Yield : 0.2 g. ; 43.8%. (Found : C, 59.74 ; H, 4.07 ; N, 13.68. $C_{15}H_{13}O_4N_3$ requires : C, 60.20 ; H, 4.38 ; N, 14.04%).

p-Isonicotinoylaminobenzoylglycine hydrazide (*Compound No.* 16)

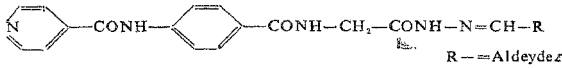
This was prepared by refluxing ethyl *p*-isonicotinoylaminobenzoylglycinate (8 g.) and hydrazine hydrate (7.3 g.; 99%) in ethanol (400 ml., 90%) in the usual way. m.p. above 300°C. (d). Yield: 7.1 g.; 92.7%. (Found: C, 57.07; H, 5.12; N, 22.08. $C_{15}H_{15}O_3N_5$ requires: C, 57.50; H, 4.83; N, 22.35%).

Aldehyde derivatives of p-*isonicotinoylaminobenzoylglycine hydrazide*

The hydrazones were prepared in good yields in the usual way. Table 2 contains the compounds prepared (Nos. 17 to 26) in this series.

TABLE 2

Aldehyde Derivatives of *p*-Isonicotinoylaminobenzoylglycine Hydrazide



R = = Aldeyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m.p.°C	Found/Required (%) C	H	N
17	C_6H_5-	$C_{22}H_{19}O_3N_5$	262–3	65.40 65.83	4.45 4.77	17.14 17.45
18	*o*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	268	63.54 63.30	4.31 4.59	16.78 16.78
19	*m*-OH C_6H_4-	$C_{22}H_{19}O_4N_5$	280 (d)	63.17 63.30	4.14 4.59	17.00 16.78
20	*p*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	310–11 (d)	62.94 63.30	4.27 4.59	17.12 16.78
21	C_5H_4N-3-	$C_{21}H_{18}O_3N_6$	282 (d)	62.38 62.68	4.52 4.51	20.73 20.89
22	C_5H_4N-4-	$C_{21}H_{18}O_3N_6$	288 (d)	62.30 62.68	4.40 4.51	20.85 20.89
23	*p*-MeO.C_6H_4-	$C_{23}H_{21}O_4N_5$	268–9 (d)	64.16 64.03	4.50 4.91	16.63 16.23
24	*p*-Me_2N.C_6H_4-	$C_{24}H_{24}O_3N_6$	281 (d)	64.91 64.85	5.09 5.44	19.26 18.91
25	C_6H_5-CH=CH-	$C_{24}H_{21}O_3N_5$	260–1 (d)	67.51 67.44	4.50 4.95	16.50 16.38
26	C_4H_3O-2-	$C_{20}H_{17}O_4N_5$	285 (d)	61.60 61.38	4.37 4.38	18.37 17.89

Note: d means decomposition

Ethyl p-*isonicotinoylaminobenzoyl-DL-methionate* (*Compound No.* 27)

To a stirred solution of *p*-isonicotinoylaminobenzoic acid azide (6.5 g.) in DMF (80 ml.) was added ethyl DL-methionate (6 5 g.) in DMF (10 ml.) over a period of 30 min. at room temperature. The filrered solution after 10 hr. of stirring, was concentrated and diluted with benzene-petrol mixture (1 : 1) to give the required product which was filtered, washed, dried and crystalised from ethanol. m.p. 182°C. Yield : 7.8 g. ; 79.8%. (Found : C, 60.32 ; H, 6.23 ; N, 10.47. $C_{20}H_{23}O_4N_3S$ requires : C, 59 85 ; H, 5.74 ; N, 10.47%).

p-*Isonicotinoylaminobenzoyl-DL-methionine* (*Compound No.* 28)

Ethyl *p*-isonicotinoylaminobenzoyl-DL-methionate (0.5 g) was hydrolysed with potassium hydroxide solution (1N, 10 ml.) and the acid isolated in the usual way. m.p. 144-5°C. Yield : 0 24 g. ; 50%. (Found : N, 10.96. $C_{18}H_{19}O_4N_3S$ requires : N, 11.26%).

p-*Isonicotinoylaminobenzoyl*-DL-*methionine hydrazide* (*Compound No.* 29)

This was prepared by treating ethyl *p*-isonicotinoylamino-DL-methionate (8 g.) in ethanol (300 ml., 90%) with hydrazine hydrate (6 g., 99%) in the usual way. m.p. 222-23°C. Yield : 6 8 g. ; 87.7%. (Found : C, 55.43 ; H, 5.65 ; N, 18.27. $C_{18}H_{21}O_3N_5S$ requires : C, 55.82 ; H, 5.43 ; N, 18.09%).

Aldehyde derivatives of p-*isonicotinoylaminobenzoyl-DL-methionine hydrazide*

The hydrazones were prepared in the usual manner. Table 3 contains the compounds prepared (Nos. 30 to 39) in this series.

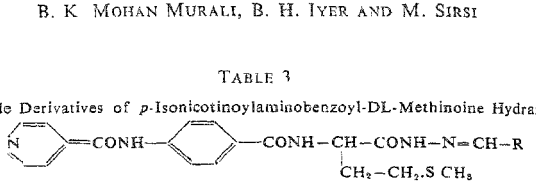
(B) *p*-NICOTINOYLAMINOBENZOIC ACID, ITS GLYCINE AND DL-METHIONINE CONJUGATES AND THEIR DERIVATIVES

Ethyl p-*nicotinoylaminobenzoate*[11] (*Compound No.* 40)

To a stirred solution of nicotinoyl chloride in dry benzene (300 ml.), prepared from potassium nicotinate (15 g.) and thionyl cloride (30 ml.) in the usual way, was added a solution of ethyl *p*-aminobenzoate (15.4 g.) dissolved in benzene (200 ml.) during a period of 1 hr. at room temperature and the product was isolated in the usual manner. m.p. 130-1°C. (Reported[11] m.p. 128°C.). Yield 22 g. ; 89.4%. (Found : C, 66.27 ; H, 5.65 ; N, 10.07. $C_{15}H_{14}O_3N_2$ requires : C, 66 66 ; H, 5.22 ; N, 10.37%).

TABLE 3

Aldehyde Derivatives of *p*-Isonicotinoylaminobenzoyl-DL-Methinoine Hydrazide



—R = Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m. p. °C	Found/Required (%) C	H	N
30	C_6H_5-	$C_{25}H_{25}O_3N_5S$	245 (d)	63.48 63.16	4 86 5.26	14 52 14 73
31	*o*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	240 (d)	61.31 61.09	5.26 5.09	13 82 14 26
32	*m*-OH C_6H_4-	$C_{25}H_{25}O_4N_5S$	263 (d)	60.73 61.09	5.35 5.09	13 96 14.26
33	*p*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	269 – 70 (d)	60 61 61.09	5.00 5 09	13 96 14 26
34	C_5H_4N-3-	$C_{24}H_{24}O_3N_6S$	237 (d)	60 36 60 50	4.70 5 04	17 40 17 65
35	C_5H_4N-4-	$C_{24}H_{24}O_3N_6S$	232 (d)	60 12 60.50	4 70 5.04	17.39 17.65
36	*p*-MeO.C_6H_4-	$C_{26}H_{27}O_4N_5S$	257 – 8 (d)	61 42 61.79	5.64 5.35	14.26 13.87
37	*p*-$Me_2N.C_6H_4$-37	$C_{27}H_{30}O_3N_8S$	256 (d)	62.73 62.55	5 59 5.79	16 62 16 22
38	C_6H_5-CH=CH	$C_{27}H_{27}O_3N_5S$	235 – 6 (d)	64.65 64.67	5.06 5.39	14 00 13.97
39	C_4H_3O-2-	$C_{23}H_{23}O_4N_3S$	206	59.27 59.34	5.11 4.95	14.70 15.05

Note: ↓ means decomposition

p-*Nicotinoylaminobenzoic acid*[12, 13, 14] (*Compound No.* 41)

This was obtained by the hydrolysis of compound No. 40 (1 g.) with potassium hydroxide solution (1N, 20 ml.). m.p. 315°C.(d). (Reported m.p. 299°C.[12]; 293-4°C.[13]; and 300-2°C.[14]). Yield: 0.35 g.; 39%. (Found; C, 64.00; H, 4.18; N, 11.61. $C_{13}H_{10}O_3N_2$ requires: C, 64.46; H, 4.16; N, 11.56%).

p-*Nicotinoylaminobenzoic acid hydrazide* (*Compound No.* 42)

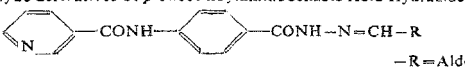
This was obtained by treating ethyl *p*-nicotinoylaminobenzoate (20 g.) with hydrazine hydrate (22 g. ; 99%) in the usual way. m.p. 242-4°C. Yield : 13 g. ; 69%. (Found : C, 60.53 ; H, 4.45 ; N, 21.72. $C_{13}H_{12}O_2N_4$ requires : C, 60.93 ; H, 4.72 ; N, 21.87%).

Aldehyde derivatives of p-*nicotinoylaminobenzoic acid hydrazide*

These were prepared in the usual manner. Table 4 contains the compounds prepared (Nos. 43 to 52) in this series.

TABLE 4

Aldehyde derivatives of *p*-Nicotinoylaminobenzoic Acid Hydrazide



—R = Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m. p. °C	Found/Required (%) C	H	N
43	C_6H_4-	$C_{20}H_{16}O_2N_4$	269	69 52 69 76	4 57 4 68	15.86 16 27
44	*o*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	251	66 34 66 66	4 26 4 48	15.49 15.55
45	*m*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	304 (d)	67 18 66 66	3.99 4 48	15.46 15.55
46	*p*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	315 – 7 (d)	66.29 66.66	4.12 4 48	15.10 15 55
47	C_5H_4N-3-	$C_{19}H_{15}O_2N_5$	270	66.30 66 08	4.48 4.38	20.18 20.28
48	C_5H_4N-4-	$C_{19}H_{15}O_2N_5$	288	66 04 66.08	4 08 4.38	20.10 20.28
49	*p*-MeO.C_6H_4-	$C_{21}H_{18}O_3N_4$	285	67.57 67.37	4.88 4.85	15.27 14.96
50	*p*-Me_2N.C_6H_4-	$C_{22}H_{21}O_2N_5$	279	67.75 68 20	5.26 5.46	18.32 18.08
51	C_6H_5-CH=CH-	$C_{22}H_{18}O_2N_4$	281	70.98 71.34	4.58 4.90	15.28 15.13
52	C_4H_3O-2-	$C_{18}H_{14}O_3N_4$	276 – 7	64.51 64.67	4.12 4.22	16.92 16.76

Note : d means decomposition

Ethyl p-*nicotinoylaminobenzoylglycinate* (*Compound No.* 53)

This was obtained by condensing ethyl glycinate (5.67 g.) with *p*-nicotinoylaminobenzoic acid azide (9.8 g.) in DMF at room temperature in the usual way as described earlier (cf., Compound No. 14). m.p. 218°C. Yield: 9.8 g.; 82.5%. (Found: C, 62.41; H, 4.90; N, 13.29. $C_{17}H_{17}O_4N_3$ requires: C, 62.38; H, 5.23; N, 12.84%).

p-*Nicotinoylaminobenzoylglycine* (*Compound No.* 54)

This was prepered by the hydrolysis of the compound No. 53 (1 g.) with potassium hydroxide solution (1N, 20 ml) in the usual way. m.p. 295°C. (d). Yield: 0.3 g.; 32%. (Found: C, 59.99; H, 4.29; N, 13.68. $C_{15}H_{13}O_4N_3$ requires: C, 60.20; H, 4.38; N, 14.04%).

p-*Nicotinoylaminobenzoylglycine hydrazide* (*Comound No.* 55)

This was prepared by refluxing ethyl *p*-nicotinoylaminobenzoylglycinate (10 g.) in ethanol (500 ml., 90%) with hydrazine hydrate (9.2 g., 99%) m.p. above 300°C. (d). Yield: 7 g.; 73.1%. (Found. C, 57.18; H, 4.52; N, 22.44. $C_{15}H_{15}O_3N_5$ requires: C, 57.50; H, 4.83; N, 22.35%).

Aldehyde derivatives of p-*nicotinoylaminobenzoylglycine hydrazide*

These were prepared in the usual way. Table 5 contains the compounds prepared (Nos. 56 to 65) in this series.

Ethyl p-*nicotinoylaminobenzoyl-DL-methionate* (*Compound No.* 66)

This was prepared in the usual way by treating ethyl DL-methionate (6.98 g.) with *p*-nicotinoylaminobenzoic acid azide (7 g.) in DMF at room temperature. m.p. 187-8°C Yield: 8 g.; 76.1%. (Found: C, 60.10; H, 5.55; N, 10.72. $C_{20}H_{23}O_4N_3S$ requires: C, 59.85; H, 5.74; N, 10.47%).

p-*Nicotinoylaminobenzoyl-DL-methionine* (*Compound No.* 67)

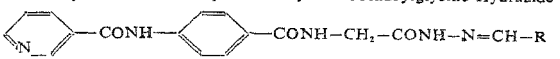
This was prepared by the hydrolysis of compound No. 66 (0.5 g.) with potassium hydroxide solution (1N, 10 ml.) in the usual way. m.p. 232-4°C. Yield: 0.25 g.; 51%. (Found: C, 58.41; H, 5.54; N, 10.99. $C_{18}H_{19}O_4N_3S$ requires: C, 57.92; H, 5.09; N, 11.26%).

p-*Nicotinoylaminobenzoyl-DL-methionine hydrazide* (*Compound No.* 68)

This was prepared in the usual way by refluxing ethyl *p*-nicotinoylaminobenzoyl-DL-methionate (6 g.) and hydrazine hydrate (4.5 g., 99%) in ethanol. m.p. softens at 175°C. and melts at 200-1°C. Yield: 4.8 g.; 90.5%. (Found: C, 55.52; H, 5.66; N, 18.05. $C_{18}H_{21}O_3N_5S$ requires: C, 55.82; H, 5.43; N, 18.09%).

TABLE 5

Aldehyde derivatives of *p*-Nicotinoylaminobenzoylglycine Hydrazide



—R=Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m. p. °C	Found/Required (%) C	H	N
56	C_6H_5-	$C_{22}H_{19}O_3N_5$	260	65.54 66.83	4.74 4.77	16.94 17.45
57	*o*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	261	62.90 63.30	4.63 4 59	16.44 16.78
58	*m*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	310 (d)	62.90 63.30	4.49 4.59	16.44 16.78
59	*p*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	310 (d)	63.09 63.30	4.96 4.59	16.44 16.78
60	C_5H_4N-3-	$C_{21}H_{18}O_3N_6$	275 (d)	62.22 62.68	4.80 4.51	21.24 20.89
61	C_5H_4N-4-	$C_{21}H_{18}O_3N_6$	254 (d)	62.32 62.68	4.74 4.51	21.08 20.89
62	*p*-MeO.C_6H_4-	$C_{23}H_{21}O_4N_5$	266–7(d)	64.39 64.03	4.49 4.91	16.07 16.23
63	*p*-$Me_2N.C_6H_4$-	$C_{24}H_{24}O_3N_6$	266–(d)	64.55 64 85	5.50 5.44	19 21 18.91
64	C_6H_5-CH=CH-	$C_{24}H_{21}O_3N_5$	263–4	67.38 67.44	5.16 4.95	16.08 16.38
65	C_4H_3O-2-	$C_{20}H_{17}O_4N_5$	282 (d)	61·10 61.38	4.58 4.38	17.45 17.89

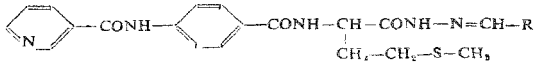
Note: d means decomposition

ldehyde derivatives of p-*nicotinoylaminobenzoyl-DL-methionine hydrazide*

The hydrazones were prepared in the usual manner. Table 6 contains e compounds prepared (Nos. 69 to 78) in this series.

TABLE 6

Aldehyde derivatives of *p*-Nicotinoylaminobenzoyl-DL-Methionine Hydrazide



—R = Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m. p. °C	Found/Required (%) C	H	N
69	C_6H_5-	$C_{25}H_{25}O_3N_5S$	265 (d)	62.88 63.16	5.24 5.26	14.33 14.73
70	*o*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	238 (d)	60.68 61.09	4.79 5.09	14.27 14.26
71	*m*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	269–70 (d)	61.08 61.09	4.75 5.09	14.07 14.26
72	*p*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	262–3 (d)	60.91 61.09	5.26 5.09	13.86 14.26
73	C_5H_4N-3-	$C_{24}H_{24}O_3N_6S$	228–9	60.16 60.50	5.46 5.04	17.78 17.65
74	C_5H_4N-4-	$C_{24}H_{24}O_3N_6S$	233	60.37 60.50	5.32 5.04	17.78 17.65
75	*p*-MeO.C_6H_4-	$C_{26}H_{27}O_4N_5S$	228–9 (d)	61.80 61.79	5.37 5.35	13.68 13.87
76	*p*-Me_2N.C_6H_4-	$C_{27}H_{30}O_3N_6S$	243–5 (d)	62.92 62.55	5.97 5.79	16.34 16.22
77	C_6H_5-CH=CH-	$C_{27}H_{27}O_3N_5S$	238–9 (d)	64.52 64.67	5.27 5.39	14.26 13.97
78	C_4H_3O-2-	$C_{23}H_{23}O_4N_5S$	188	59.58 59.34	5.01 4.95	14.80 15.05

Note: d means decomposition

(C) *p*-PICOLINOYLAMINOBENZOIC ACID, ITS GLYCINE AND DL-METHIONINE CONJUGATES AND THEIR DERIVATIVES

Ethyl p-picolynoylaminobenzoate (*Compound No.* 79)

To a suspension of potassium picolinate (10 g.) in dry benzene (60 ml.), cooled to 0°C., was added slowly redistilled thionyl chloride (20 ml.) over a period of 10 min. After the addition, the reaction mixture was kept as such for half an hour and then the benzene and excess of thionyl chloride were removed by distillation under suction.

To a stirred solution of this crude picolinoyl chloride in dry benzene (150 ml.), was added a solution of ethyl *p*-aminobenzoate (10.25 g.) in dry benzene (150 ml.), and the product isolated in the usual way. m.p. 135°C. Yield: 13.5 g.; 82.5%. (Found: C, 67.01; H, 4.79; N, 10.32. $C_{15}H_{14}O_3N_2$ requires: C, 66.66; H, 5.22; N, 10.36%).

p-*Picolinoylaminobenzoic acid* (*Compound No.* 80)

This was prepared by the hydrolysis of the compound No. 79 (1 g.) with potassium hydroxide solution (1N, 20 ml.) in the usual way. m.p. 265-7°C. Yield: 0.12 g.; 14%. (Found: N, 11.61. $C_{13}H_{10}O_3N_2$ requires: N, 11.56%)

p-*Picolinoylaminobenzoic acid hydraziae* (*Compound No.* 81)

This was obtained by refluxing ethyl *p*-picolinoylaminobenzoate (20 g.) and hydrazine hydrate (22 g., 99%) in ethanol in the usual way. m.p. 247-8°C. Yield: 15 g.; 79.1%. (Found: C, 60.61; H, 4.72; N, 22.18. $C_{13}H_{12}O_2N_4$ requires C, 60.93; H, 4.72; N, 21.87%).

Aldehyde derivatives of p-picolinoylaminobenzoic acid hydrazide

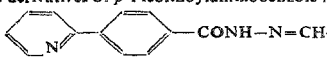
The hydrazones were prepared in the usual way. Table 7 contains the compounds prepared (Nos. 82 to 87) in this series.

Ethyl p-*picolinoylaminobenzoylglycinate* (*Compound No.* 88)

This was prepared by condensing ethyl glycinate (8.1 g.) with *p*-picolinoylaminobenzoic acid azide (14 g.) in DMF at room temperature in the usual way. m.p. 172-3°C. Yield: 15 g.; 87.5%. (Found: C, 62.05; H, 5.17; N, 12.53. $C_{17}H_{17}O_4N_3$ requires: C, 62.38; H, 5.23; N, 12.84%).

TABLE 7

Aldehyde derivatives of *p*-Picolinoylaminobenzoic Acid Hydrazide



—R = Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m. p. °C	Found/Required (%) C	H	N
82	C_6H_5-	$C_{20}H_{16}O_2N_4$	247–8	70.11 69.76	4.60 4.68	15 86 16.27
83	*o*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	245–6	66.53 66.66	4.25 4.48	15.23 15.55
84	*m*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	270–1 (d)	66.22 66.66	4.45 4.48	15.17 15 55
85	*p*-OH.C_6H_4-	$C_{20}H_{16}O_3N_4$	311–2 (d)	66.65 66.66	4.41 4.48	15.19 15.55
86	C_5H_4N-3-	$C_{19}H_{15}O_2N_5$	262–4	66.03 66.08	4.13 4.38	19 97 20.28
87	C_5H_4N-4-	$C_{19}H_{15}O_2N_5$	269–70	66.51 66.08	3 95 4.38	19.92 20.28

Note: d means decomposition

p-*Picolinoylaminobenzoylglycine* (*Compound No.* 89)

This was prepared by the hydrolysis of the compound No. 88 (0.5 g.) with potassium hydroxide solution (1N, 10 ml.) in the usual way. m.p. 235°C. (d). Yield : 0.24 g. ; 50%. (Found : C, 60.13 ; H, 4.63 ; N, 14.21. $C_{15}H_{13}O_4N_3$ requires : C, 60.20 ; H, 4.38 ; N, 14.04%).

p-*Picolinoylaminobenzoylglycine hydrazide* (*Compound No.* 90)

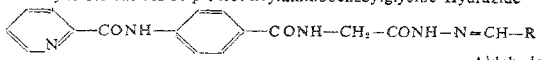
This was prepared from ethyl *p*-picolinoylaminobenzoylglycinate (12 g.) and hydrazine hydrate (11 g., 99%) in the usual manner. m.p. 257°C. Yield : 10.3 g.; 91%. (Found: C, 57.97; H, 4.79; N, 22.15. $C_{15}H_{15}O_3N_5$ requires : C, 57.50 ; H, 4.83 ; N, 22.35%).

Aldehyde derivatives of p-*picolinoylaminobenzoylglycine hydrazide*

The hydrazones were prepared in the usual way. Table 8 contains the compounds prepared (Nos. 91 to 96) in this series.

TABLE 8

Aldehyde derivatives of *p*-Picolinoylaminobenzoylglycine Hydrazide



=Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m.p. °C	Found/Required (%) C	H	N
91	C_6H_5-	$C_{22}H_{19}O_3N_5$	265	65.70 65.83	4 42 4 77	17 92 17.45
92	*o*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	263–4(d)	63.10 63 30	4 69 4.59	17.25 16.78
93	*m*-OH.C_6H_4-	$C_{22}H_{19}O_4N_5$	255	63 11 63.30	4.19 4 59	16.92 16.78
94	*p*-OH.C_6H_4	$C_{22}H_{19}O_4N_5$	270–1(d)	62.81 63.30	4 58 4.59	16.83 16.78
95	C_5H_4N-3-	$C_{21}H_{18}O_3N_6$	278 (d)	63.19 62.68	4 78 4 51	20 74 20.89
96	C_5H_4N-4-	$C_{21}H_{18}O_3N_6$	255 (d)	62.40 62.68	4 37 4 51	20.71 20 89

Note: d means decomposition

Ethyl p-*picolinoylaminobenzoyl-DL-methionate* (*Compound No.* 97)

This was prepared by treating *p*-picolinoylaminobenzoic acid azide (6 g.) with ethyl DL-methionate (6 g.) in DMF at room temperature in the usual way. m.p. 148–49°C. Yield: 6.5 g.: 72.2%, (Found: C, 59.53; H, 583; N, 10.25. $C_{20}H_{23}O_4N_3S$ requires: C, 59.85; H, 5.74; N, 10.47%).

p-*Picolinoylaminobenzoyl-DL-methionine* (*Compound No.* 98)

Hydrolysis of the compound No. 97 (0.5 g.) with potassium hydroxide solution (1N, 10 ml.) in the usual way gave the required acid. m.p. 192°C. Yield: 0.16 g.; 33%. (Found: N, 11.32. $C_{18}H_{19}O_4N_3S$ requires: N, 11.26%).

p-Picolinoylaminobenzoyl-DL-methionine hydrazide (*Compound No.* 99)

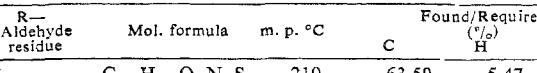
This was obtained in the usual way by refluxing ethyl *p*-picolinoylaminobenzoyl-DL-methionate (12 g.) with hydrazine hydrate (9 g., 99%) in ethanol. m.p. 206-7°C. Yield: 10.1 g.; 87%. (Found: C, 55.69; H, 5.27; N, 17.72. $C_{18}H_{21}O_3N_5S$ requires: C, 55.82; H, 5.43; N, 18.09%).

Aldehyde derivatives of p-*picolinoylaminobenzoyl-DL-methinonine hydrazide*

The hydrazones were prepared in the usual way. Table 9 contains the compounds prepared (Nos. 100 to 105) in this series.

TABLE 9

Aldehyde derivatives of *p*-Picolinoylaminobenzoyl-DL-Methionine Hydrazide



—R = Aldehyde residue

Compound No.	R— Aldehyde residue	Mol. formula	m. p. °C	Found/Required (%) C	H	N
100	C_5H_6-	$C_{25}H_{25}O_3N_5S$	210	63.59	5.47	14 67
				63 16	5.26	14.73
101	*o*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	215–6	60.59	4.95	14.70
				61.09	5.09	14 26
102	*m*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	210	60.67	5 22	14.56
				61.09	5 09	14.26
103	*p*-OH.C_6H_4-	$C_{25}H_{25}O_4N_5S$	248	61 31	5.14	14.32
				61.09	5.09	14.26
104	C_5H_4N-3-	$C_{24}H_{24}O_3N_6S$	228–9	60.46	4.62	17.68
				60.50	5.04	17.65
105	C_5H_4N-4-	$C_{24}H_{24}O_3N_6S$	241–2 (d)	60.19	5.42	17.26
				60.50	5.04	17.65

Note: d means decomposition

Results of in vitro *Screening*

A total of 105 compounds have been synthesised and tested for *in vitro* antituberculosis activity by the surface culture technique, using the virulent strain of *Mycrobacterium tuberculosis* $H_{37}R_v$. Out of this, 19 compounds have shown complete inhibition of growth of the organism at a dilution of 1 in 10,000 (100 γ/ml.). Twenty compounds were partially inhibitory at this dilution. Among the 19 active compounds, some have inhibited the growth even at higher dilutions. The screening data of all the compounds are summarised in Table 10.

The following generalisations are made from the results of antituberculosis tests.

(i) *p*-Pyridoylaminobenzoic acids and their amino acid conjugates are inactive in general.

(ii) The ethyl esters of all the three *p*-pyridoylaminobenzoic acids are highly active. Conjugation with either glycine or DL-methionine destroys the activity.

(iii) All the three hydrazides of *p*-pyridoylaminobenzoic acids are active. While the hydrazides of the glycine conjugates are active, those of DL-methionine are inactive.

(iv) Formation of hydrazones destroys the activity in most of the cases.

(v) Esters, acids, hydrazides and hydrazones of picolinoyl series are more active than the corresponding derivatives from nicotinoyl and isonicotinoyl series.

In Vivo *Activity*

Among the compounds tested, ethyl *p*-isonicotinoylaminobenzoate (Compound No. 1), ethyl *p*-nicotinoylaminobenzoate (Componnd No. 40) and ethyl *p*-picolinoylaminobenzoate (Compound No. 79) were found to exhibit *in vitro* antituberculosis activity equivelent to those of *p*-aminosalicylic acid, streptomycin and isoniazid. They were therefore tested for *in vivo* potency in experimental tuberculosis of mice by the drug-diet method and compared with isoniazid treated animals. None of the compounds was found to be active *in vivo*. They neither showed any survivors nor prolonged the life span of the treated mice compared to the controls.

ACKNOWLEDGEMENT

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TABLE 10

Results of Screening for Inhibition of Growth *in Vitro* of Mycobacterium Tuberculosis $H_{37}R_0$

Serial No.	Compound	*p*-Isonicotinoylaminobenzoic acid derivatives	*p*-Isonicotinoylaminobenzoyl-glycine derivatives	*p*-Isonicotineylaminobenzoyl-DL-methionine derivatives	*p*-Nicotinoylaminobenzoic acid derivatives	*p*-Nicotinoylaminobenzoyl-glycine derivatives	*p*-Nicotinoylaminobenzoyl-DL-methionine derivatives	*p*-Picolinoylaminobenzoic acid derivatives	*p*-Picolinoylaminobenzoyl-glycine derivatives	*p*-Picolinoylaminobenzoyl-DL-methionine derivatives	Total number of active compounds
1	Ethyl ester	=	+	+	=	+	+	≡	−	±	4
2	Acid	+	+	+	+	+	+	±	+	+	1
3	Hydrazide	−	±	+	−	−	+	±	−	+	5
	HYDRAZONES WITH										
4	Benzaldeyde	+	±	+	+	+	+	±	±	+	0
5	*o*-Hydroxybenzaldehyde	+	±	+	+	−	+	±	±	+	1
6	*m*-Hydroxybenzaldehyde	+	±	+	+	+	+	±	±	+	0
7	*p*-Hydroxybenzaldehyde	+	±	+	+	+	+	±	−	+	1
8	Nicotinaldehyde	+	±	+	+	+	+	±	−	+	1
9	Isonicotinaldehyde	+	±	+	−	+	+	−	−	+	3
10	*p*-Methoxybenzaldehyde	+	±	+	+	+	+	×	×	×	0

11 *p*-Dimethylamino benzaldehyde	+	±	+	+	−	+	×	×	×	1
12 Cinnamaldehyde	+	±	+	+	+	±	×	×	×	1
13 2-Furanaldehyde	+	±	+	+	−	+	×	×	×	1
Compounds in each series :										
Total No.	13	13	13	13	13	13	9	9	9	
Serial Nos.	1-13	14-26	27-39	40-52	53-65	67-78	79-87	88-96	97-105	
Active compounds in each serie										
Total No.	2	0	0	3	4	1	4	5	0	19
Serial Nos.	1, 3	0	0	40, 42, 48	55, 57 63, 65	77	79, 80, 81, 87	88, 90, 94, 95, 96	0	

LEGEND

− indicates complete inhibition at 1 : 10,000 dilution

= indicates complete inhibition at 1 : 1 million dilution.

≡ indicates complete inhibition at 1 : 10 million dilution.

± indicates partial inhibition at 1 : 100,000 dilution.

± indicates partial inhibition at 1 : 10,000 dilution.

\+ indicates no inhibition at 1 : 10,000 dilution.

× compounds not prepared.

Ethyl-*p*-aminobenzoate inhibits the growth of *M.t. in vitro* at 1 : 10 million dilution.

p-Aminobenzoic acid and all the aldehydes, excepting *p*-dimethylaminobenzaldehyde are inactive at 1 : 10,000 dilution. *p*-Dimethylaminobenzaldehyde shows partial inhibition at this dilution.

Note : Out of 105 compounds (*i.e* , 9 esters, 9 acids, 9 hydrazides and 78 hydrazones) synthesised and tested, 19 compounds have completely inhibited the growth of *M.t.* at 1 : 10,000 dilution. Twenty compounds were partially inhibitory at this dilution. Among the active compounds some have shown inhibition even at higher dilutions.

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