

## Synthesis of Some $\gamma$ -Picolinyl-1,2,4,5-Dithiadiazine and Their Antimicrobial Activity

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3-Aryl/alkylimino - 5- $\gamma$ -picolinyl-5-phenylimino - 1,2,4,5-dithiadiazines (V) have been synthesized following the interaction of phenylimino-chloromethane sulphenyl chloride (III) and 1-aryl/alkyl-4- $\gamma$ -picolinyl thiosemicarbazides (II). The latter, in turn, have been prepared by the condensation of aryl/alkyl isothiocyanates (I) and isoniazid. The structures of all these compounds were established on the basis of elemental analysis, equivalent weight determination and IR and PMR spectral data. The synthesized compounds were assayed for their antimicrobial activity against gram-positive as well as gram-negative microorganisms such as *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis*, *A. aerogenes* and *B. megatherium*.

**Key words:** Synthesis,  $\gamma$ -picolinyl-1,2,4,5-dithiadiazine, antimicrobial activity.

### INTRODUCTION

Synthesis of various dithiadiazines are reported in the literature<sup>1-4</sup>. Some of them are shown to possess antifungal activity.<sup>1</sup> Isoniazid is itself a potent drug; therefore, we felt it important to incorporate some part of it as a substituent in new heterocyclic compounds and study its antimicrobial activity. With this aim, we have prepared 1,2,4,5-dithiadiazines (Va–Vg) and tested them for their antimicrobial activity against some gram-positive and gram-negative organisms, and the results are described in the present communication.

### RESULTS AND DISCUSSION

Isoniazid and *p*-tolyl isothiocyanate (Ia) was reacted in chloroform medium for 1.5 h. On cooling the reaction mixture and on removal of chloroform by vacuum distillation a colourless solid (IIa) was separated. It was washed with petroleum ether (60–80°C) and recrystallized from ethanol, m.p. 164°C. The elemental analysis indicated the m.f. of the product as C<sub>14</sub>H<sub>14</sub>N<sub>4</sub>OS. The product is insoluble in water but soluble in organic solvents. The compound was found desulphurizable with soluble alkaline plumbite indicating the presence of >C=S group. The IR spectral analysis of the product indicated the presence of  $\nu(\text{NH})$  (3320 cm<sup>-1</sup>),  $\nu(\text{C=O})$  (1680, 1540 cm<sup>-1</sup>),  $\nu(\text{C=S})$  (1350 cm<sup>-1</sup>),  $\nu(\text{C—N})$  (1280 cm<sup>-1</sup>)<sup>5-7</sup>. The PMR spectrum of the product showed peak due to methyl proton ( $\delta$  2.3), NH proton ( $\delta$  7.95 and 10.7 ppm), aromatic proton ( $\delta$  7.1–7.5 ppm) and pyridyl proton ( $\delta$  8.8–9.5 ppm).

On the basis of all the above facts the compound (IIa) has been assigned the structure 1-*p*-tolyl-4- $\gamma$ -picolinyl thiosemicarbazide. The other thiosemicarbazides (IIb–IIg) were prepared by extending this reaction to other aryl/alkyl isothiocyanates in good yield (Table-1).

TABLE-I  
FORMATION OF 1-ARYL/ALKYL-4- $\gamma$ -PICOLINYL THIOSEMICARBAZIDE

Aryl isothiocyanates I	1-Aryl/alkyl-4- $\gamma$ -picolinyl thiosemicarbazide II	Yield (%)	m.p. (°C)	Analysis %, found (calcd.) S
<i>p</i> -Tolyl isothiocyanate ( <b>Ia</b> )	1- <i>p</i> -Tolyl-4- $\gamma$ -picolinyl thiosemicarbazide ( <b>IIa</b> )	87	164	11.01 (11.19)
<i>o</i> -Tolyl isothiocyanate ( <b>Ib</b> )	1- <i>o</i> -Tolyl-4- $\gamma$ -picolinyl thiosemicarbazide ( <b>IIb</b> )	91	170	11.23 (11.18)
<i>m</i> -Tolyl isothiocyanate ( <b>Ic</b> )	1- <i>m</i> -Tolyl-4- $\gamma$ -picolinyl thiosemicarbazide ( <b>IIc</b> )	91	184	10.94 (11.18)
Phenyl isothiocyanate ( <b>Id</b> )	1-Phenyl-4- $\gamma$ -picolinyl thiosemicarbazide ( <b>IIId</b> )	85	194	11.85 (11.76)
<i>o</i> -Chlorophenyl-isothiocyanate ( <b>Ie</b> )	1- <i>o</i> -Chlorophenyl-4- $\gamma$ -picol -inyl thiosemicarbazide ( <b>IIe</b> )	75	162	9.90 (10.06)
<i>p</i> -Chlorophenyl-isothiocyanate ( <b>If</b> )	1- <i>p</i> -Chlorophenyl-4- $\gamma$ -picol inyl thiosemicarbazide ( <b>IIIf</b> )	83	186	10.17 (10.06)
<i>t</i> -Butyl-isothiocyanate ( <b>Ig</b> )	1- <i>t</i> -Butyl-4- $\gamma$ -picolinyl thiosemicarbazide ( <b>IIg</b> )	63	180	12.50 (12.69)

Note: All the compounds give satisfactory C, H and N analysis.

The 1-*p*-tolyl-4- $\gamma$ -picolinyl-thiosemicarbazide (**IIa**) and phenylimino chloromethane sulphenyl chloride<sup>8</sup> (**III**) in equimolar proportion were refluxed in chloroform medium for 1 h. The evolution of hydrochloric acid gas was observed and tested with moist blue litmus paper. Cooling the reaction mixture and distilling off chloroform afforded a sticky mass, which on washing with petroleum ether followed by addition of a little ethanol gave a pale yellow solid (**IVa**). It was crystallized from ethanol, m.p. 164°C. It was acidic to litmus. On determination of equivalent weight it was found to be monohydrochloride (**IVa**). Basification with aqueous ammonia afforded a free base (**Va**), crystallized from aqueous ethanol, m.p. 156°C. On the basis of elemental analysis of the products with m.p. 156°C, the m.f. was found to be C<sub>21</sub>H<sub>17</sub>N<sub>5</sub>OS<sub>2</sub>.

The IR spectrum of (**Va**) showed the presence of  $\nu(\text{NH})$  (3255 cm<sup>-1</sup>),  $\nu(\text{C=O})$  (1680 cm<sup>-1</sup>),  $\nu(\text{C=N})$  (1600 cm<sup>-1</sup>),  $\nu(\text{N-N})$  (1216 cm<sup>-1</sup>),  $\nu(\text{C-S})$  (687 cm<sup>-1</sup>), and  $\nu(\text{S-S})$  (439 cm<sup>-1</sup>). The PMR spectra of the product showed peaks due to Ar-CH<sub>3</sub> ( $\delta$  2.3 ppm), NH proton ( $\delta$  10.6 ppm), aromatic protons ( $\delta$  7.2-7.8) and pyridyl protons at ( $\delta$  8.5-9.6 ppm), respectively.

On the basis of all the above facts, the compound (**Va**) has been assigned the structure as 3-*p*-tolylimino-5- $\gamma$ -picolinyl-6-phenylimino-1,2,4,5-dithiadiazine. The other compounds from (**Vb-Vg**) were prepared by extending the above reaction to other 1-aryl/alkyl-4- $\gamma$ -picolinyl thiosemicarbazides and the related compounds (**Vb-Vg**) were isolated in good yield (Table-2).

Majority of the title compounds (**IIa-IIg**) were highly active (15 mm zone of inhibition with 50  $\mu\text{g/mL}$ ), moderately active (15 mm zone with 100  $\mu\text{g/mL}$ ) against test organism except **IIa**, **IIg** which were inactive. Compound **IIb** showed

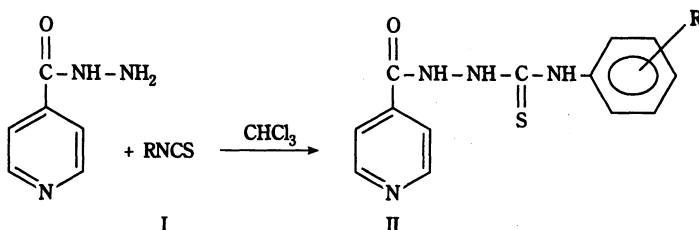
TABLE-2  
 FORMATION OF 3-ARYL/ALKYL IMINO-5- $\gamma$ -PICOLINYL-6-PHENYL-IMINO-1,2,4,5-DITHIAZINES (V)  
 Reactant: 1-Aryl/Alkyl-4- $\gamma$ -Picolinyl-Thiosemicarbazide (II) and Phenyl Imino Chloromethane Sulphonyl Chloride (III) (0.01 Mole Each)

1-Aryl/alkyl-4- $\gamma$ -picolinyl thiosemicarbazide, (II)	3-Aryl/alkylimino-5- $\gamma$ -picolinyl-6-phenyl-imino-1,2,4,5-dithiadiazine hydrochloride, (IV)	Yield %	m.p. (°C)	Eq. wt. found (calcd.)	3-Aryl/alkylimino-5- $\gamma$ -picolinyl-6-phenyl-imino-1,2,4,5-dithiadiazine (free base) (V)	m.p. (°C)	% Analysis found (calcd.) (S)
1- <i>p</i> -Tolyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IIa)	3- <i>p</i> -Tolylimino . . . , (IVa)	53	164	452 (455.5)	3- <i>p</i> -Tolylimino . . . , (Va)	156	15.21 (15.27)
1- <i>o</i> -Tolyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IIb)	3- <i>o</i> -Tolylimino . . . , (IVb)	60	160	454 (455.5)	3- <i>o</i> -Tolylimino . . . , (Vb)	100	15.08 (15.27)
1- <i>m</i> -Tolyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IIc)	3- <i>m</i> -Tolylimino . . . , (IVc)	48	168	450.8 (455.5)	3- <i>m</i> -Tolylimino . . . , (Vc)	152	15.25 (15.27)
1-Phenyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IId)	3-Phenylimino . . . , (IVd)	50	174	436 (441.5)	3-Phenylimino . . . , (Vd)	168	14.28 (14.56)
1- <i>o</i> -Chlorophenyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IIe)	3- <i>o</i> -Chloro-phenylimino . . . , (IVe)	52	156	468 (476)	3- <i>o</i> -Chloro-phenylimino . . . , (Ve)	120	14.28 (14.56)
1- <i>p</i> -Chlorophenyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IIf)	3- <i>p</i> -Chloro-phenylimino . . . , (IVf)	58	160	472 (476)	3- <i>p</i> -Chloro-phenylimino . . . , (Vf)	150	14.33 (14.56)
1- <i>t</i> -butyl-4- $\gamma$ -picolinyl-thiosemicarbazide (IIg)	3- <i>t</i> -Butylimino . . . , (IVg)	52	156	413 (421.5)	3- <i>t</i> -Butylimino . . . , (Vg)	140	16.23 (16.62)

Note: All the compounds gave satisfactory C, H and N analyses.

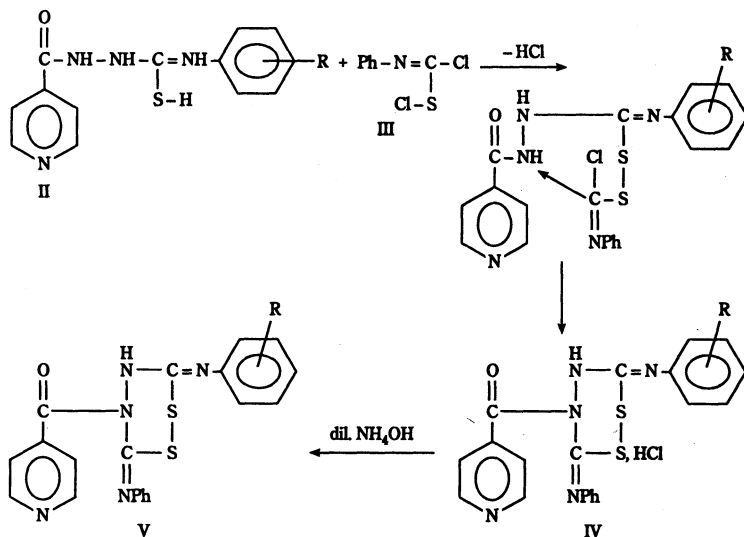
high activity against *B. subtilis* and *B. megatherium* while **IIf** showed very high activity against *A. aerogenes* and *B. megatherium*. In dithiadiazine series again **Vf** is highly active against *S. aureus*, *B. subtilis*, *B. megatherium* and *A. aerogenes* and **Vd** is active against *S. aureus* and *S. typhi*.

The formation of **II**, **IV** and **V** can be shown as follows:



where **Ia, IIa** = R = *p*-tolyl, **Ib, IIb** = R = *o*-tolyl,  
**Ic, IIc** = R = *m*-tolyl, **Id, IId** = R = phenyl,  
**Ie, IIe** = R = *o*-chloro-phenyl, **If, IIIf** = R = *p*-chlorophenyl  
**Ig, IIg** = R = tert-butyl

Mechanism of formation of **IV** and **V** is shown below:



where **IIa, IVa, Va** R = *p*-tolyl,  
**IIb, IVb, Vb** R = *o*-tolyl  
**IIc, IVc, Vc** R = *m*-tolyl  
**IId, IVd, Vd** R = phenyl  
**IIe, IVe, Ve** R = *o*-chloro-phenyl  
**IIIf, IVf, Vf** R = *p*-chloro-phenyl  
**IIg, IVg, Vg** R = tert-butyl

## EXPERIMENTAL

The melting points are recorded using hot paraffin bath and are uncorrected. IR spectra are recorded on Perkin-Elmer instrument in Nujol mull and as KBr pellets. PMR are recorded using TMS as internal standard. Phenyl iminochloromethane sulphenyl chloride was prepared following Ottmann and Hook's method.<sup>8</sup>

### Formation of 1-*p*-tolyl-4-( $\gamma$ -picolinyl)thiosemicarbazide (IIa)

Isoniazid (0.01 mole) *p*-tolyl-isothiocyanate (0.01 mole) and chloroform (20 mL) were refluxed for 1.5 h. On distilling off chloroform a solid residue was obtained and washed with petroleum ether. It was crystallized from ethanol; a colourless crystalline solid (IIa) was obtained, m.p. 164°C. (Found C, 58.75; H, 4.52; N, 19.46; S, 11.01%, C<sub>14</sub>H<sub>14</sub>N<sub>4</sub>OS requires C, 58.74; H, 4.89; N, 19.57; S, 11.19%).

The above reaction was extended to synthesize compounds (IIb-IIg).

### Reaction of 1-*p*-tolyl-4-( $\gamma$ -picolinyl thiosemicarbazide with phenylimino chloromethane sulphenyl chloride; Formation of 3-*p*-tolylimino-5- $\gamma$ -picolinyl-6-phenylimino-1,2,4,5-dithiadiazine (Va)

1-*p*-Tolyl-4- $\gamma$ -picolinyl thiosemicarbazide (0.01 mole) was suspended in chloroform (20 mL). To this a solution of phenylimino-chloromethane sulphenyl chloride (0.01 mole) in chloroform was added. The reaction mixture was then refluxed on a water bath for 1 h. The evolution of hydrochloric acid gas was observed. After 1 h, the chloroform was distilled off; a sticky mass was obtained. It was repeatedly washed with petroleum ether (60–80°C), followed by addition of ethanol; a pale yellow solid acidic to litmus was isolated. It was crystallized from ethanol, m.p. 164°C. On basification with dilute ammonia solution a pale yellow free base was obtained and crystallized from aqueous ethanol, m.p. 156°C. (Found C, 59.92; H, 3.98; N, 16.25; S, 15.21%, C<sub>21</sub>H<sub>17</sub>N<sub>5</sub>OS<sub>2</sub> requires C, 60.14; H, 4.05; N, 16.7 and S, 15.27%).

On extending the above reaction to other 1-aryl/alkyl-4- $\gamma$ -picolinyl-thiosemicarbazide, the related 1,2,4,5-dithiadiazines were isolated in good yield.

### Antimicrobial Activity

The title compounds (IIa-IIg and Va-Vg) were screened for their antimicrobial activity against pathogenic bacteria. Organism used include both gram-positive and gram-negative strains like *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis*, *A. aerogenes*, *B. megatherium*. The solvent used was DMF. Sensitivity plates were seeded with a bacterial inoculum of  $1 \times 10^6$  CIU/mL and each well (diameter 10 mm) was loaded with 0.1 mL of test compound solution of variable concentration in DMF. The zones of inhibition were recorded after incubation for 24 h using vernier callipers (Tables 3 and 4).

TABLE-3  
ANTIMICROBIAL ACTIVITY OF 1-ARYL/ALKYL-4- $\gamma$ -PICOLINYL THIOSEMICARBAZIDE (II) AT DIFFERENT CONCENTRATIONS  
(Diameter of inhibition zone in mm)

Organism	1-Aryl/alkyl-4- $\gamma$ -picolinyl thiosemicarbazide (II)						
	IIa	IIb	IIc	IId	IIe	IIf	IIg
<i>E. coli</i>	+	+	+	-	-	++	-
<i>S. aureus</i>	+	+	-	-	-	-	-
<i>S. typhi</i>	+	-	-	-	-	-	-
<i>B. subtilis</i>	+	+++	++	+	+	-	-
<i>A. aerogenes</i>	+	+	+	-	-	+++	++
<i>B. megatherium</i>	+	+++	-	-	-	+++	-

(-) = inactive (less than 12 mm), (+) = weakly active (12-16 mm),  
(++) = moderately active (17-20 mm), (+++) = highly active (21-30 mm).

TABLE-4  
ANTIMICROBIAL ACTIVITY OF 3-ARYL/ALKYL IMINO-5- $\gamma$ -PICOLINYL-6-PHENYL-IMINO-1,2,4,5-DITHIADIAZINE (V) (Diameter of inhibition zone in mm)

Organism	3-aryl/alkyl imino-5- $\gamma$ -picolinyl-6-phenylimino-1,2,4,5-dithiadiazine (V)						
	IIa	IIb	IIc	IId	IIe	IIf	IIg
<i>E. coli</i>	+	-	+	-	+	+	-
<i>S. aureus</i>	+++	-	+	+	-	++	-
<i>S. typhi</i>	++	-	-	-	-	-	-
<i>B. subtilis</i>		-	++	-	-	+++	+
<i>A. aerogenes</i>	++	-	-	++	++	++	++
<i>B. megatherium</i>	-	-	++	-	-	++	-

(-) = inactive (less than 12 mm), (+) = weakly active (12-16 mm),  
(++) = moderately active (17-20 mm), (+++) = highly active (21-30 mm)

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