

## Synthesis, Antibacterial and Antifungal Activities of 3-Aryl-5-(pyridin-3-yl)-4,5-dihydropyrazole-1-carbothioamide Derivatives

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A new series of 3-aryl-5-(pyridin-3-yl)-1-thiocarbamoyl-2-pyrazoline derivatives (**4a-j**) were prepared by the reaction of azachalcones **3a-j** with thiosemicarbazide in ethanolic sodium hydroxide. The structure of synthesized compounds were confirmed by <sup>1</sup>H NMR and Mass spectral data. Their antibacterial activities against *Escherichia coli* (CTP 7624), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12229), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 1156) and *Micrococcus luteus* (ATCC 9341) were investigated. Antifungal activity of compounds against *Candida albicans* and *Candida glabrata* were found to be inactive. Compounds **4a-j** were also evaluated for antituberculosis activity against *Mycobacterium tuberculosis H<sub>37</sub>Rv* (ATCC 27294) in BACTEC 12B using a broth microdilution assay and Microplate Alamar Blue Assay (MABA). The preliminary results showed that compounds **4e**, **4d** and **4g** had 87%, 93% and 92% inhibitory effect respectively.

**Keywords:** Azachalcones, 2-Pyrazoline, Antimycobacterial agents, Antifungal

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### INTRODUCTION

Antibiotics are among the most prescribed drugs in the world today, and since their development and commercialization, have saved countless millions of lives. The ideal antimicrobial agents are selective in only targeting the microorganism but not host cells. Resistance to antimicrobial agents is now recognized as a major global public health problem. In addition, because of the increased number of immunocompromised patients (AIDS, cancer and transplants), primary and opportunistic fungal infections continue to increase rapidly, and as a consequence, invasive fungal infections constitute a major cause of mortality for these

patients. Although there are new classes of compounds that are now frequently used to treat fungal infections, the frequency of deeply invasive microbial agents has increased 10 fold during the past decade. Moreover, many infections are actually refractory to antimicrobial therapy. With the emergence of new bacterial strain resistant to many currently available antibiotic treatments, there is increasing interest in the discovery of novel antibacterial agents [1,2]. Certain small heterocyclic molecules are known as pharmacophores of a number of biologically active and medicinally useful molecules [3,4]. Electron-rich nitrogen heterocycles play an important role in diverse biological activities. Introducing a pyrazolidine ring in place of  $\beta$ -lactam ring in penicillins and cephalosporins results in enhanced activity [5]. A second nitrogen in the five-membered ring also influences the

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antibacterial or pharmacokinetic properties [6,7].

2-Pyrazoline derivatives [8-14] have been reported in the literature to exhibit various pharmacological activities such as antibacterial, antifungal, herbicidal and anticholinergic. Considering the above discussion and in continuation of our previous work in pyrazoline derivatives [15], herein we report the synthesis of novel pyrazoline derivatives (**4a-j**) with possible antimicrobial and antifungal activities.

## EXPERIMENTAL

### Chemistry

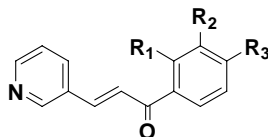
Melting points were determined on a Kofler hot stage apparatus, and are uncorrected. The Infrared spectra were acquired on a Nicolet 550-FT spectrograph (KBr disks). The mass spectra were run on a Finigan-MAT TSQ-70

spectrophotometer at 70 eV. <sup>1</sup>H NMR spectra were recorded on a Varian unity plus 400 MHz instrument and tetramethylsilane was used as an internal standard.

**General procedure for the synthesis of 1-Aryl-3-(pyridin-3-yl)prop-2-ene-1-ones (3a-j).** To a mixture of 3-pyridinecarboxaldehyde (21.4 g, 0.2 mol) and 40 ml NaOH 10% in ethanol (20 ml), different acetophenone derivatives was added slowly under cooling (5 °C) during 30 min. After addition was completed, reaction mixture was stirred for 4 h keeping the temperature below 10 °C. The resulting solid was collected by filtration, washed thoroughly with ice-cold water, dried in a vacuum dessicator and recrystallized from ethanol-water to give **3a-j**. Physical, analytical and spectral data for these compounds are given in Table 1.

**General procedure for the synthesis of 3-aryl-5-(pyridine-3-yl)-4,5-dihydropyrazole-1-carbothioamide derivatives (4a-j).** To a solution of NaOH (1 g, 0.025 mol)

**Table 1.** Physical and Spectral Data of 1-Aryl-3-(pyridin-3-yl)prop-2-en-1-ones (**3a-j**)

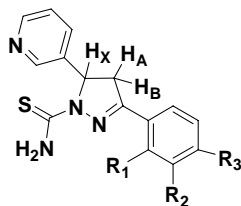


Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Yield (%)	M.P. (°C)	Formula (MW)	<sup>1</sup> H NMR (CDCl <sub>3</sub> ), MS
<b>3a</b>	H	H	H	41	100-102	C <sub>14</sub> H <sub>11</sub> NO, 209	7.4 (dd, 1H, J = 8 and 4 Hz, pyridine-H <sub>5</sub> ), 7.59 (m, 4H, 3H-phenyl and 1H-vinyl-H), 7.79 (d, 1H, J = 15.6 Hz, vinyl-H), 7.95 (d, 1H, J = 8 Hz, pyridine-H <sub>4</sub> ), 8.02 (m, 2H, aromatic-H), 8.64 (d, 1H, J = 4 Hz, pyridine-H <sub>6</sub> ), 8.86 (d, 1H, J = 1.2 Hz, pyridine-H <sub>2</sub> ). MS m/z (%): 209 (M <sup>+</sup> , 25), 180 (75), 151 (10), 132 (25), 105 (20), 76 (32).
<b>3b</b>	H	CH <sub>3</sub>	H	39	69-71	C <sub>15</sub> H <sub>13</sub> NO, 223	2.4 (s, 3H, CH <sub>3</sub> ), 7.64 (m, 8H, 4H-phenyl-H, 2H-vinyl-H and 2H-pyridine-H <sub>5,4</sub> ), 8.62 (d, 1H, J = 4 Hz, pyridine-H <sub>6</sub> ), 8.81 (d, 1H, J = 1.2 Hz, pyridine-H <sub>2</sub> ). MS m/z (%): 223 (M <sup>+</sup> , 60), 195 (62), 191 (30), 132 (30), 118 (65), 78 (10).
<b>3c</b>	H	H	OMe	36	106-108	C <sub>15</sub> H <sub>13</sub> NO <sub>2</sub> , 239	4.9 (s, 3H, OMe), 7.00 (d, 2H, J = 8 Hz, aromatic-H <sub>3,5</sub> ), 7.37 (dd, 1H, J = 8 and 4 Hz, pyridine-H <sub>5</sub> ), 7.62 (d, 1H, J = 15.6 Hz, H <sub>3</sub> ), 7.78 (d, 1H, J = 15.6 Hz, H <sub>2</sub> ), 7.95 (d, 1H, J = 8 Hz, pyridine-H <sub>4</sub> ), 8.05 (d, 2H, J = 8 Hz, aromatic-H <sub>2,6</sub> ), 8.63 (dd, 1H, J = 4 and 1.2 Hz, pyridine-H <sub>6</sub> ), 8.86 (d, 1H, J = 1.2 Hz, pyridine-H <sub>2</sub> ). MS m/z (%): 239 (M <sup>+</sup> , 60), 209 (90), 196 (40), 167 (50), 134 (70), 92 (20), 76 (30).

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Table 1. Continued

<b>3d</b>	H	H	Br	45	120-123	C <sub>14</sub> H <sub>10</sub> BrNO, 289	7.74 (m, 8H, 4H-phenyl-H, 2H-vinyl-H and 2H-pyridine-H <sub>4,5</sub> ), 8.62 (d, 1H, J = 4 Hz, pyridine-H <sub>6</sub> ), 8.84 (d, 1H, J = 1.2 Hz, pyridine-H <sub>2</sub> ) MS m/z (%): 289 (M <sup>+</sup> , 40), 260 (45), 207 (70), 181 (90), 153 (91), 130 (80), 103 (73), 76 (90).
<b>3e</b>	H	Br	H	35	115-117	C <sub>14</sub> H <sub>10</sub> BrNO, 289	7.4 (m, 3H, phenyl-H), 7.54 (d, 1H, J = 16 Hz, vinyl-H), 7.74 (d, 1H, J = 8 Hz, phenyl-H), 7.81 (d, 1H, J = 16 Hz, vinyl-H), 7.96 (t, 2H, J = 8.4 Hz, pyridine-H <sub>4,5</sub> ), 8.65 (d, 1H, J = 4 Hz, pyridine-H <sub>6</sub> ), 8.87 (s, 1H, pyridine-H <sub>2</sub> ). MS m/z (%): 289 (M <sup>+</sup> , 70), 260 (45), 207 (80), 190 (90), 153 (30), 130 (93), 76 (52).
<b>3f</b>	Br	H	H	39	112-114	C <sub>14</sub> H <sub>10</sub> BrNO, 289	7.36 (m, 3H, 2H-phenyl-H and H-vinyl-H), 7.45 (m, 3H, 2H-phenyl-H and vinyl-H), 7.66 (d, 1H, J = 8 Hz, pyridine-H <sub>5</sub> ), 7.89 (dd, 1H, J = 6.8 and 2 Hz, pyridine-H <sub>4</sub> ), 8.62 (dd, 1H, J = 4 and 1.7 Hz, pyridine-H <sub>6</sub> ), 8.87 (d, J = 1.7 Hz, 1H, pyridine-H <sub>2</sub> ). MS m/z (%): 289 (M <sup>+</sup> , 90), 262 (45), 210 (70), 181 (90), 153 (50), 130 (80), 107 (83), 76 (85).
<b>3g</b>	Cl	H	H	42	99-100	C <sub>14</sub> H <sub>10</sub> ClNO, 243	7.38 (dd, 1H, J = 4 and 8 Hz, pyridine-H <sub>5</sub> ), 7.5 (m, 2H, J = 8 Hz, aromatic-H), 7.6 (d, 1H, J = 16 Hz, vinyl-H), 7.81 (d, 1H, J = 16 Hz, vinyl-H), 7.97 (m, 3H, 1H-pyridine-H <sub>4</sub> , 2H-aromatic-H), 8.46 (dd, 1H, J = 4 and 8 Hz, pyridine-H <sub>6</sub> ), 8.86 (d, 1H, J = 1.6 Hz, pyridine-H <sub>2</sub> ). MS m/z (%): 243 (M <sup>+</sup> , 40), 214 (45), 161 (70), 135 (91), 107 (80), 76 (90).
<b>3h</b>	Cl	H	Cl	52	120-122	C <sub>14</sub> H <sub>9</sub> Cl <sub>2</sub> NO, 277	7.38 (m, 2H, aromatic-H), 7.49 (m, 4H, 1H-phenyl and 2H-vinyl-H& 1H-pyridine-H <sub>5</sub> ), 7.9 (d, 1H, J = 8 Hz pyridine-H <sub>4</sub> ), 8.62 (d, 1H, pyridine-H <sub>6</sub> ), 8.76 (d, 1H, J = 1.6 Hz, pyridine-H <sub>2</sub> ). MS m/z (%): 277 (M <sup>+</sup> , 25), 248 (75), 163 (80), 146 (25), 114 (20), 76 (32).
<b>3i</b>	F	H	H	45	92-95	C <sub>14</sub> H <sub>10</sub> FN, 227	7.2 (m, 2H, aromatic-H), 7.36 (dd, 1H, J = 4 and 8 Hz, pyridine-H <sub>5</sub> ), 7.58 (d, 1H, J = 16 Hz, vinyl-H), 7.8 (d, 1H, J = 16 Hz, vinyl-H), 7.95 (d, 1H, J = 8 Hz, pyridine-H <sub>4</sub> ), 8.75 (m, 2H, aromatic-H), 8.63 (dd, 1H, J = 8 and 1.2 Hz pyridine-H <sub>6</sub> ), 8.86 (d, 1H, J = 1.2 Hz, pyridine-H <sub>2</sub> ). MS m/z (%): 227 (M <sup>+</sup> , 60), 198 (90), 167 (50), 125 (70), 113 (20), 85 (30).
<b>3j</b>	H	H	F	50	105-107	C <sub>14</sub> H <sub>10</sub> FN, 227	7.4 (dd, 1H, J = 4 and 8 Hz, pyridine-H <sub>5</sub> ), 7.59 (m, 4H, 3H-phenyl and 1H-vinyl-H), 7.79 (d, 1H, vinyl-H), 7.95 (d, 1H, J = 8 Hz, pyridine-H <sub>4</sub> ), 8.02 (m, 2H, aromatic-H), 8.64 (d, 1H, pyridine-H <sub>6</sub> ), 8.86 (d, 1H, pyridine-H <sub>2</sub> ) MS m/z (%): 227 (M <sup>+</sup> , 60), 198 (90), 167 (50), 125 (70), 113 (20), 85 (30).

**Table 2.** Physical and Spectral Data of 3-Aryl-5-(pyridin-3-yl)-1-thiocarbamoyl-2-pyrazolin Derivatives (**4a-j**)

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Yield (%)	M.P. (°C)	Formula (MW)	<sup>1</sup> H NMR (CDCl <sub>3</sub> ), MS
<b>4a</b>	H	H	H	45	163-165	C <sub>15</sub> H <sub>14</sub> N <sub>4</sub> S, 282	3.22 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.89 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.07 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 7.01 (brs, 1H, NH), 7.1 (brs, 1H, NH), 7.27 (m, 1H, pyridine-H), 7.46 (m, 3H, aromatic-H), 7.54 (m, 1H, pyridine-H), 7.73 (m, 2H, aromatic-H), 8.54 (m, 2H, pyridine-H). MS m/z (%): 282 (M <sup>+</sup> , 100), 268 (8), 249 (53), 222 (25), 193 (15), 104 (19).
<b>4b</b>	H	CH <sub>3</sub>	H	38	167-169	C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> S, 296	2.39 (s, 3H, CH <sub>3</sub> ), 3.22 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.89 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.07 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 6.2 (brs, 1H, NH), 7.19 (brs, 1H, NH), 7.29 (m, 4H, pyridine-H and phenyl), 7.53 (m, 2H, pyridine-H and phenyl), 8.54 (m, 2H, pyridine-H). MS m/z (%): 296 (M <sup>+</sup> , 40), 263 (28), 236 (30), 208 (18), 179 (42), 145 (100), 103 (28).
<b>4c</b>	H	H	OMe	38	164-166	C <sub>16</sub> H <sub>16</sub> N <sub>4</sub> O S, 312	3.18 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.81 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 3.92 (s, 3H, OCH <sub>3</sub> ), 6.05 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 6.94 (d, 2H, J = 8 Hz, aromatic-H), 7.22 (m, 1H, pyridine-H), 7.54 (m, 1H, pyridine-H), 7.62 (d, 2H, J = 8 Hz, aromatic-H), 8.54 (m, 2H, pyridine-H). MS m/z (%): 312 (M <sup>+</sup> , 58), 279 (38), 252 (40), 238 (15), 177 (60), 145 (100), 133 (26), 103 (42).

and azachalcon derivatives **3a-j** (0.01 mol) in ethanol (25 ml), thiosemicarbazide (0.92 g, 0.01 mol) was added slowly under stirring. After addition was completed, the reaction mixture was refluxed for 1 h and the solution was added to crushed ice. The resulting solid was washed with ether and water then filtered. The crystals were washed thoroughly with ice-cold water, dried and recrystallized from appropriate solvent to give **4a-j**. Physical, analytical and spectral data for these

compounds are given in Table 2.

### Microbiology

Compounds for antimicrobial studies were dissolved in dimethylsulphoxide at 12 mg ml<sup>-1</sup> and stored at -20 °C. To avoid interference with the solvents, the highest DMSO concentration was 1%.

Antimicrobial activities of compounds were tested using

## Synthesis, Antibacterial and Antifungal Activities of

Table 2. Continued

<b>4d</b>	H	H	Br	40	200-202	C <sub>15</sub> H <sub>13</sub> BrN <sub>4</sub> S, 362	3.2 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.8 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.07 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 7.21 (m, 2H, pyridine-H), 7.49 (d, 2H, J = 8 Hz, aromatic-H), 7.61 (d, 2H, J = 8 Hz, aromatic-H), 8.54 (m, 2H, pyridine-H). MS m/z (%): 362 (M <sup>+</sup> , 60), 327 (38), 301 (72), 288 (25), 223 (30), 192 (18), 178 (94), 143 (100), 137 (15), 103 (31).
<b>4e</b>	H	Br	H	39	195-197	C <sub>15</sub> H <sub>13</sub> BrN <sub>4</sub> S, 362	3.18 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.87 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.09 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 6.2 (brs, 1H, NH), 7.15 (brs, 1H, NH), 7.28 (m, 1H, pyridine-H), 7.32 (m, 1H, aromatic-H), 7.59 (m, 3H, aromatic-H), 7.91(d, 1H, J = 8 Hz, pyridine-H), 8.54 (m, 2H, pyridine-H). MS m/z (%): 362 (M <sup>+</sup> , 60), 327 (38), 301 (72), 288 (25), 223 (30), 192 (18), 178 (94), 143 (100), 137 (15), 103 (31).
<b>4f</b>	Br	H	H	35	180-180	C <sub>15</sub> H <sub>13</sub> BrN <sub>4</sub> S, 362	3.4 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 4.06 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.05 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 7.37 (m, 3H, aromatic-H), 7.56 (dd, 1H, J = 1.2 and 7.6 Hz, pyridine-H), 7.59 (m, 1H, aromatic-H), 7.68 (d, 1H, J = 7.6 Hz, pyridine-H), 8.54 (d, 1H, J = 4.8 Hz, pyridine-H), 8.54 (d, 1H, J = 2 Hz, pyridine-H). MS m/z (%): 362 (M <sup>+</sup> , 60), 327 (38), 301 (72), 288 (25), 223 (30), 192 (18), 178 (94), 143 (100), 137 (15), 103 (31).
<b>4g</b>	H	H	Cl	48	176-178	C <sub>15</sub> H <sub>13</sub> ClN <sub>4</sub> S, 317	3.19 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.81 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.08 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 6.19 (brs, 1H, NH), 7.109 (brs, 1H, NH), 7.26 (m, 1H, pyridine-H), 7.42 (d, 2H, aromatic-H), 7.53 (m, 1H, pyridine-H), 7.66 (m, 2H, aromatic-H), 8.54 (m, 2H, pyridine-H). MS m/z (%): 317 (M <sup>+</sup> , 100), 327 (38), 256 (70), 243 (18), 228 (50), 178 (94), 150 (100), 127 (15), 103 (31).
<b>4h</b>	Cl	H	Cl	52	190-192	C <sub>15</sub> H <sub>12</sub> Cl <sub>2</sub> N <sub>4</sub> S, 351	3.36 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 4.06 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.06 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 6.15 (brs, 1H, NH), 7.07 (brs, 1H, NH), 7.26 (m, 1H, Aromatic-H), 7.32 (dd, 1H, J = 2 and 8.4 Hz, pyridine-H), 7.48 (d, 1H, Aromatic-H), 7.54 (dd, 1H, J = 8 Hz, pyridine-H), 7.67 (d, 1H, J = 8.4 Hz, Aromatic-H), 8.54 (m, 2H, pyridine-H). MS m/z (%): 351 (M <sup>+</sup> , 100), 335 (38), 291 (80), 261 (40), 243 (18), 228 (50), 181 (94), 143 (100), 127 (15), 103 (31).

**Table 2.** Continued

<b>4i</b>	F	H	H	37	150-152	C <sub>15</sub> H <sub>13</sub> FN <sub>4</sub> S, 300	3.19 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.89 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.08 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 7.14 (t, 4H, J = 8.8 Hz, aromatic-H), 7.53 (dd, 1H, J = 8 Hz, pyridine-H), 7.74 (dd, 1H, J = 8 and 5.2 Hz, Pyridine-H), 8.54 (d, 1H, J = 2 Hz, pyridine-H). MS m/z (%): 300 (M <sup>+</sup> , 60), 281 (70), 240 (52), 207 (25), 190 (18), 183 (38), 143 (100), 137 (15), 105 (31).
<b>4j</b>	H	H	F	39	163-165	C <sub>15</sub> H <sub>13</sub> FN <sub>4</sub> S, 300	3.15 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>AB</sub> = 16 Hz, H <sub>A</sub> ), 3.87 (dd, 1H, J <sub>AB</sub> = 16 Hz, J <sub>BX</sub> = 12 Hz, H <sub>B</sub> ), 6.09 (dd, 1H, J <sub>AX</sub> = 3.6 Hz, J <sub>BX</sub> = 12 Hz, H <sub>X</sub> ), 7.14 (m, 4H, aromatic-H), 7.53 (dd, 1H, J = 8 Hz, pyridine-H), 7.74 (dd, 1H, J = 8 Hz, J = 5.2 Hz, Pyridine-H), 8.54 (d, 1H, J = 2 Hz, pyridine-H). MS m/z (%): 300 (M <sup>+</sup> , 60), 281 (70), 240 (52), 207 (25), 190 (18), 143 (100), 137 (15), 105 (31).

agar dilution method on solid cultivation method [16]. Tested microorganism strains were *Escherichia coli* (CTP 7624), *Staphylococcus aureus* (ATCC 6538), *Staphylococcus epidermidis* (ATCC 12229), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 1156), *Micrococcus luteus* (ATCC 9341), *Candida albicans* and *Candida glabrata*. Test with gram positive and gram negative bacteria were carried out in Muller Hilton agar. Antifungal activity was evaluated in Sabouraud Dextrose Agar. The compounds were diluted in the test medium to obtain final concentration ranging between 0.004-0.256 mg ml<sup>-1</sup>. Drug free control were included. The MICs were determined after 24 h and 48 h of the static incubation at 35 °C. Gentamicin was used as standard antibacterial agent whereas ketoconazole was used as antifungal. The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 3.

Antimycobacterial evaluation was carried out in Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL USA, which is a part of National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.25 µg ml<sup>-1</sup> against *M. tuberculosis H<sub>37</sub>Rv* in BACTEC 12B medium using the BACTEC 460 radiometric system.

Compounds showing at least 90% inhibition at 6.25 µg ml<sup>-1</sup> in this primary screening were retested at lower concentrations against *M. tuberculosis H<sub>37</sub>Rv* to determine the minimum inhibitory concentration (MIC) in a broth microdilution Alamar Blue assay (MABA). The MIC was defined as the lowest concentration causing a decrease in fluorescence of 90% relative to controls. The observed data on the antimycobacterial activity of the compound **4a-j** and control drugs are given in Table 4.

## RESULTS AND DISCUSSION

1-Aryl-3-(pyridin-3-yl)-prop-2-ene-1-ones (**3a-j**) and 3-Aryl-5-(pyridin-3-yl)-4,5-dihydropyrazole-1-carbothioamide derivatives (**4a-j**) were prepared according to Scheme 1. The azachalcones were prepared by reacting acetophenone derivatives with 3-pyridinecarboxaldehyde in the presence of a base by conventional Claisen-Schmidt condensation [17]. Reaction of chalcones and thiosemicarbazide in a solution of sodium hydroxide and ethanol led to pyrazolines (**4a-j**).

Condensation of chalcones with thiosemicarbazide can lead to two different pyrazolines (**4** or **9**) as shown in Scheme 2. Through the route A, the reaction probably goes through the

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**Table 3.** Minimum Inhibitory Concentration (MIC)  $\mu\text{g ml}^{-1}$  of Compounds Against Selected Bacteria<sup>a</sup>

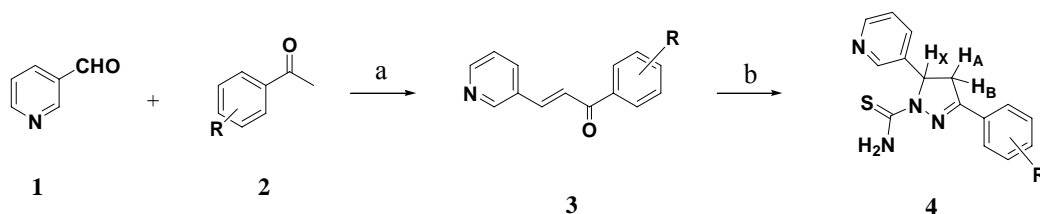
Compounds	<i>Pseudomonas aeruginosa</i> (ATCC 9027)	<i>Staphylococcus aureus</i> (ATCC 6538)	<i>Escherichia coli</i> (CTP 7624)	<i>Staphylococcus epidermidis</i> (ATCC12229)	<i>Micrococcus luteus</i> (ATCC 9341)	<i>Bacillus subtilis</i> (ATCC 1156)
<b>3a</b>	-	>128	-	-	-	-
<b>3b</b>	-	-	-	-	-	-
<b>3c</b>	-	-	-	-	-	-
<b>3d</b>	-	>128	-	-	-	-
<b>3e</b>	-	-	-	-	-	-
<b>3f</b>	-	-	-	-	-	-
<b>3g</b>	-	-	-	-	-	-
<b>3h</b>	-	>64	>64	>64	>64	-
<b>3i</b>	-	>64	-	>32	>32	>32
<b>3j</b>	-	>128	>128	>128	>128	-
<b>4a</b>	-	>128	>128	>128	>128	-
<b>4b</b>	-	>128	-	-	-	-
<b>4c</b>	-	-	-	-	-	-
<b>4d</b>	-	-	-	-	-	-
<b>4e</b>	-	-	-	-	-	-
<b>4f</b>	-	-	-	-	-	-
<b>4g</b>	-	>128	-	-	-	-
<b>4h</b>	-	-	-	-	-	-
<b>4i</b>	-	-	-	-	-	-
<b>4j</b>	-	-	>128	-	-	-
<b>Gentamicin</b>	8	0.5	8	16	-	0.5

<sup>a</sup>Agar dilution method was used to determine the MICs.

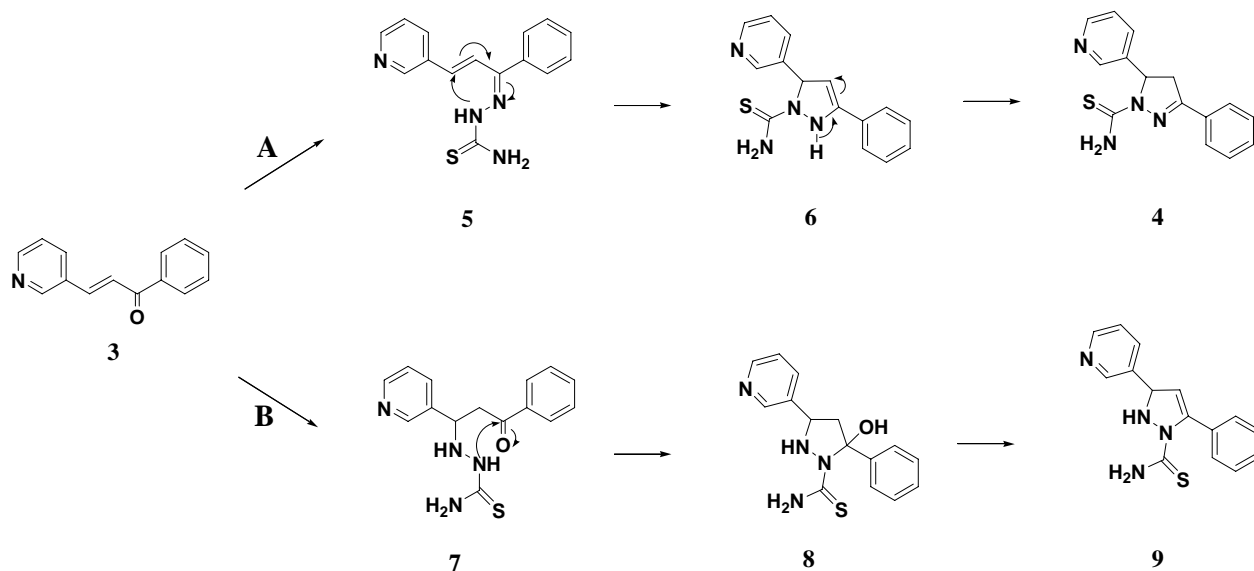
**Table 4.** Antimycobacterial Results of **4a-j** Against *M. tuberculosis H<sub>37</sub>Rv*

Sample structure	MIC ( $\mu\text{g ml}^{-1}$ )	%Inhibition	Activity
<b>4a</b>	>6.25	9	+
<b>4b</b>	>6.25	0	-
<b>4c</b>	>6.25	0	-
<b>4f</b>	>6.25	0	-
<b>4e</b>	>6.25	87	+
<b>4d</b>	<6.25	93	+
<b>4g</b>	<6.25	92	+
<b>4h</b>	>6.25	0	-
<b>4i</b>	>6.25	0	-
<b>4j</b>	>6.25	0	-

intermediate formation of thiosemicarbazone **5** and subsequent addition of N-H on the olefinic bond of the propenone moiety gives compound **6**. Then tautomerization of the latter compounds results to the formation of pyrazoline **4**. According to route B, Michael addition of thiosemicarbazide to compound **3** provides compound **7**. Cyclization of the latter, followed by dehydration of the intermediate **8** yields pyrazoline **9**. The differentiation between compounds **4** and **9** was based on <sup>1</sup>H NMR spectral data. In 400 MHz <sup>1</sup>H NMR the signals of the respective protons of the prepared titled compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The CH<sub>2</sub> protons of pyrazoline ring resonated as a pair of doublets of doublets at 3.18-3.4 ppm and 3.81-4.06 ppm. The CH proton appeared as doublet of doublets at 6.05-6.09 ppm due to vicinal coupling



Scheme 1. Reagents and condition: a) NaOH (10%), EtOH, 5 °C, 4 h; b) thiosemicarbazide, NaOH, EtOH, reflux, 1 h



Scheme 2. Proposed mechanism for the formation of pyrazoline

with the two magnetically non-equivalent protons of the methylene group at position 4 of pyrazoline ring ( $J_{AB} = 16$  Hz,  $J_{AX} = 3.6$  Hz,  $J_{BX} = 12$  Hz). According to the above results, we concluded that pyrazoline **4** rather than **9** is formed. It seems that the formation of pyrazoline **4** is favored to regioisomer pyrazoline **9**, because of the stability of **4** in comparison to **9** isomer. Our finding is in good agreement with the previous reported one [18].

The synthesized compounds were evaluated for their *in vitro* antimicrobial activity against several bacterial and fungal agents. The tested compounds were inactive against candida and they did not have significant antibacterial and antifungal activities in comparison to gentamicin and ketoconazole, respectively. However, their antimycobacterial effects showed that among the ten synthesized derivatives, compound **4d** and **4g** with electron rich group in para position of aromatic ring produced highest efficacy and exhibited >90% inhibition in

the primary screen at  $6.25 \mu\text{g ml}^{-1}$  followed by compound **4e** which showed moderate inhibition 87%. In level II, compounds demonstrating at least 90% inhibition in the primary screen were retested at lower concentrations against *M. tuberculosis H<sub>37</sub>Rv* to determine the actual minimum inhibitory concentration (MIC) using MABA and compounds **4d** and **4g** was found to be active against *M. tuberculosis H<sub>37</sub>Rv* at  $6.25 \mu\text{g ml}^{-1}$ .

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