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### SYNTHESIS AND ANTIBACTERIAL STUDIES OF 4-CHLORO-3-METHYL-N- (SUBSTITUTED PHENYL)-5-OXO-4,5-DIHYDRO-1H-PYRAZOLE-1- CARBOTHIOAMIDE

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**Abstract:** Pyrazolone derivatives are an important class of heterocyclic compounds that occur in many drugs and synthetic products. It has a particular value due to their broad spectrum of biological activities and their utility as synthetic tools in the design of various bioactive molecules. It is also exhibited analgesic, antipyretic and anti-inflammatory activity. A series of 4-chloro-3-methyl-N-(substituted phenyl)-5-oxo-4,5-dihydro-1H-pyrazole-1-carbothioamide (3a-g) were synthesized by the reaction of thiosemicarbazides (1) and ethyl-2-chloroacetoacetate (2) in DMF. The synthesized compounds were characterized on the basis of IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and elemental analysis. All synthesized Compounds were screened for antibacterial activity using disc diffusion technique against four bacterial pathogens viz *Staphylococcus aureus*, *Escherichia coli*, *Bacillus anthracis* and *Pseudomonas aeruginosa*. Chloramphenicol was used as standard drug respectively. All compounds exhibit moderate activity.

**Keywords:** Pyrazolones, Thiosemicarbazides, Antimicrobial Activity, Disc Diffusion Technique.



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## INTRODUCTION

Medicinal chemistry is the discipline concerned with determining the influence of chemical structure on biological activity and in the practice of medicinal chemistry developed from an empirical one involving organic synthesis of new compound based largely on the modification of structure and then identifies their biological activity [1, 2]. Antibacterial agents are the greatest contribution of present century to chemotherapy. They have great importance in the developing countries where infectious diseases are predominates. Various biologically active synthetic compounds have five-member nitrogen-containing heterocyclic ring in their structures [3]. Pyrazolone is a five membered lactum ring, containing two nitrogen and one ketonic group in its structure. Pyrazolones have gained importance as drug substances in pharmaceutical industry in view of their biological importance. For instance, the pyrazolones, viz. phenazone, propyphenazone, ampyrone and metamizole are useful antipyretic and analgesic drugs [4]. In addition, pyrazolones possess antimicrobial, antifungal [5], antimycobacterial [6, 7], antibacterial [8], anti-inflammatory [9], antitumor [10], gastric secretion stimulatory [11], antidepressant [12] and antifilarial activities [13].

Biological activities of 2-amino-4-substituted pyrano [2,3-c]pyrazole-3-carbonitriles have been considerable research directed for synthesis of derivatives of this ring system [14]. Interest in the chemistry of a new organic photochromic compound containing pyrazolone-ring, 1-phenyl-3-methyl-4-(4-bromobenzal)-pyrazolone-5 thiosemicarbazone (PM<sub>4</sub>BrBP-TSC) was synthesized and characterized by *Guangfei Liu et. al.* They showed that the photochromic properties were related to the photoisomerization and Time-dependent UV-Vis spectra, and these compound exhibited good antibacterial activities [15]. *Lavinia Baciu-Atudosie et al.* [16] have been reported the synthesis of new 5-substituted-2-[2-(2-substituted-10H-phenothiazin-10-yl)-2-oxoethyl]-2,4-dihydro-3H-pyrazol-3-one containing a phenothiazine unit by reaction of N-chloroacetyl compound, ethyl acetoacetate with hydrazine hydrate and they were evaluated antiproliferative activity. The chemistry and antibacterial activity of some substituted pyrazolone have been investigated in recent years and it was thought worldwide to synthesized novel pyrazolones from easily available starting materials and evaluations of their possible antimicrobial and anti-inflammatory activity [17-22]. Keeping in view of this and in continuation of our search on biologically potent molecules [23], we hereby reported the synthesis of some new biologically active pyrazolone derivatives by the reaction of thiosemicarbazide and ethyl-2-chloro acetoacetate.

## MATERIALS AND METHODS

### General

All chemicals employed in the present work were purchased from Aldrich Chemicals (USA), and were used as received. All solvents used were of reagent grade and were purified by standard

procedures prior to their use. All melting points were determined in open capillaries and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck) with acetone/n-hexane (1:3). The IR spectra (in KBr pellets) were recorded on a spectrophotometer.  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra were recorded ( $\text{CDCl}_3$ ) on a Bruker DRX 300 (300MHz, FT NMR) and varian (300MHz) spectrometer. The Mass spectra were recorded on a Jeol -SX 102 spectrometer. Elemental analyses were performed on a Flash EA 1112 series CHNS-O Analyzer. Commercial grade solvents and reagents were used without further purification.

### **General procedure for the synthesis of thiosemicarbazide (1a-g)**

N-(substituted phenyl) thiosemicarbazide has been synthesized in two steps.

#### **1. Synthesis of N-(substituted phenyl) isothiocyanate:**

A three-necked 200 mL round bottom flask equipped with a mechanical stirrer and a dropping funnel, leaving the third neck open or loosely stoppered, was cooled in a freezing mixture of ice and salt. A concentrated aqueous ammonia solution (20 mL) was introduced into the flask through the dropping funnel, followed by carbondisulphide (10 mL) and resulting mixture was continuously stirred. Substituted aniline (0.01M, 0.92 mL), dissolve in ethanol (20 mL) was run through the dropping funnel in a duration of about 20 minutes, when a heavy precipitate of ammonium tolyldithiocarbamate salt separated. This salt was transferred into a 2 litre round bottom flask with 100 mL water. A solution of lead nitrate was added in the resulting solution and the reaction mixture was continuously stirred till the lead sulphide precipitated out. The reaction mixture was finally distilled by steam distillation process.

The white shining crystals of m-methyl phenyl isothiocyanate has been separated out from the distillate and dried over anhydrous calcium chloride. It was purified by recrystallization with diethyl ether.

#### **2. Synthesis of N-(substituted phenyl) thiosemicarbazide:**

Solution of isothiocyanatobenzene (0.01M) in ethanol was taken in a round bottom flask. A solution of hydrazine hydrate (0.01M) in ethanol was added to it at once, followed by continuous shaking. An exothermic reaction accompanied by vigorous effervescences was observed. The Contents of the Flask were refluxed on a water bath for 4-5 hours, resulting in the formation of a white precipitate of phenyl thiosemicarbazide, which was filtered and washed repeatedly with ethanol. The product was found to be soluble in dioxane, dimethylsulphoxide and dimethylformamide.

### 3. General procedure for the synthesis of 3-methyl-*N*-(substituted phenyl)-5-oxo-4, 5-dihydro-1*H*-pyrazole-1-carbothioamide (3a-g)

Take intimate mixture of phenyl thiosemicarbazide (0.01 mole, 1.67 gm), ethyl-2-chloro acetoacetate (0.01 mole, 1.378 mL) and dimethyl formamide (DMF) 25 mL were taken in a round bottom flask fitted with air condenser and refluxed for 10 hrs at 80-90 °C. Now mixture was melted and obtained transparent colour. The progress of the reaction was checked by TLC. Refluxing was continued till the reaction was completed. After completion of reaction mixture was allowed to stand overnight, next day excess of solvent was distilled off and the resultant residue was poured on crushed ice with few drops of H<sub>2</sub>SO<sub>4</sub>. The solid precipitated were filtered and recrystallized with ethanol. Yield= 70%, M.P. = 145°C

#### 4-chloro-3-methyl-5-oxo-*N*-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (3a)

Yield-70%, M.P.-145°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3250 (N-H-Str), 1693 (C=O), 1425 (C-N-Str), 1159 (C=S), 1610(C=N); <sup>1</sup>HNMR=(300MHz,DMSO) $\delta$ =1.1(3H,s), 2.6(2H,s), 4.5(1H,s), 6.49(2H,t), 6.69(1H,t) 7.05(2H,m)ppm; <sup>13</sup>C NMR:  $\delta$  183.1, 173.2, 156.9, 140.4, 128.8, 125.9, 124.7, 36.2, 20.6; MS(EI) m/z=267.02[M]<sup>+</sup> · Anal. calcd. for C<sub>11</sub>H<sub>11</sub>N<sub>3</sub>OS: C,56.63; H,4.75; N,18.01; Found: C,55.43; H, 3.64; N, 17.07.

#### 4-chloro-3-methyl-*N*-(2-methylphenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (3b)

Yield-67%, M.P.-225°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3178(N-H-Str), 1699 (C=O), 1452 (C-N-Str), 1157 (C=S), 1602 (C=N); <sup>1</sup>HNMR=(300MHz,DMSO) $\delta$ =1.02(3H,s), 2.3(2H,s), 2.39(3H,s), 4.3(1H,s), 6.44(1H,d), 6.60(1H,t), 6.89(2H,dd)ppm; <sup>13</sup>C NMR:  $\delta$  182.0, 173.2, 155.8, 141.1, 134.6, 129.8, 126.8, 125.5, 124.6, 35.8, 20.9, 12.9; MS(EI) m/z=281.04[M]<sup>+</sup> · Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>OS: C,52.28; H,5.30; N,15.99; Found: C,51.03; H, 4.94; N, 14.27.

#### 4-chloro-3-methyl-*N*-(3-methylphenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (3c)

Yield-68%, M.P.-179°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3265 (N-H-Str), 1672 (C=O), 1476 (C-N-Str), 1166 (C=S), 1620 (C=N); <sup>1</sup>HNMR=(300MHz,DMSO) $\delta$ =1.09(3H,s), 2.22(2H,s), 2.41(3H,s), 4.20(1H,s), 6.37(2H,d), 6.52(1H,d), 6.91(1H,dd)ppm, <sup>13</sup>C NMR:  $\delta$  182.9, 172.2, 156.1, 139.7, 138.1, 128.9, 126.5, 125.8, 122.8, 36.4, 21.1, 20.4; MS(EI) m/z=281.04[M]<sup>+</sup> · Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>OS: C,51.28; H,4.29; N,14.91; Found: C,51.03; H, 4.04; N, 14.27.

#### 4-chloro-3-methyl-*N*-(4-methylphenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (3d)

Yield-60%, M.P.-190°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3256 (N-H-Str), 1668 (C=O), 1482 (C-N-Str), 1141 (C=S), 1605 (C=N); <sup>1</sup>HNMR=(300MHz,DMSO) $\delta$ =1.20(3H,s), 2.32(2H,s), 2.61(3H,s), 4.10(1H,s), 6.54(2H,t), 6.85(2H,t)ppm; <sup>13</sup>C NMR:  $\delta$  183.3, 172.5, 155.7, 136.9, 134.7, 130.5, 126.1, 36.4, 21.3, 20.3; MS(EI) m/z=281.04[M]<sup>+</sup> · Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>OS: C,51.20; H,4.50; N,15.89; Found: C,51.13; H, 3.99; N, 15.77.

***N*-(2-methoxyphenyl)-4-chloro-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide  
(3e)**

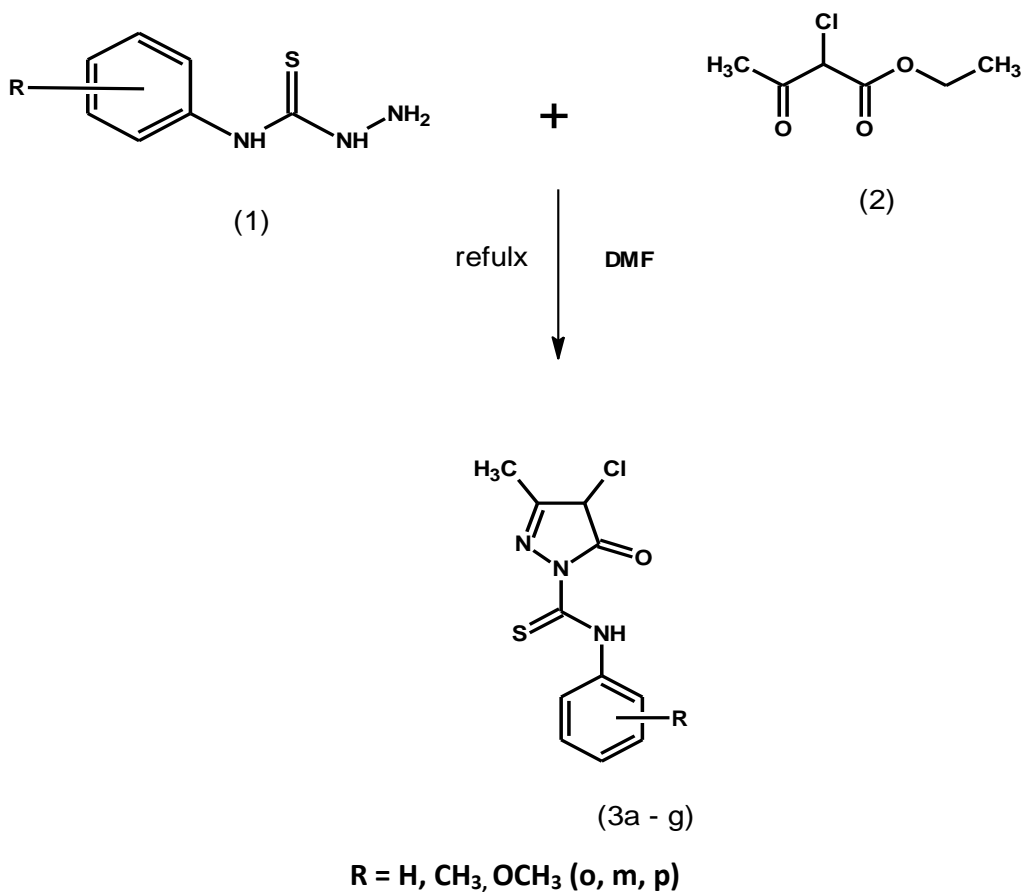
Yield-64%, M.P.-175°C, IR(KBr,  $\nu_{\max}$   $\text{cm}^{-1}$ ): 3300 (N-H-Str), 1686 (C=O), 1463 (C-N-Str), 1163 (C=S), 1615 (C=N);  $^1\text{H NMR}$ =(300MHz,DMSO) $\delta$ =1.10(3H,s), 2.23(2H,s), 3.75(3H,s), 4.15(1H,s), 6.42(1H,d), 6.55(1H,dd), 6.60(1H,dd), 6.66(1H,d)ppm,  $^{13}\text{C NMR}$ :  $\delta$  185.0, 173.7, 158.8, 156.7, 126.3, 125.8, 121.6, 115.5, 56.6, 36.5, 20.6; MS(EI)  $m/z$ =297.03[M] $^+$  · Anal. calcd. for  $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ : C,48.74; H,4.98; N,14.96; Found: C, 48.43; H, 4.64; N, 13.77.

***N*-(3-methoxyphenyl)-4-chloro-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide  
(3f)**

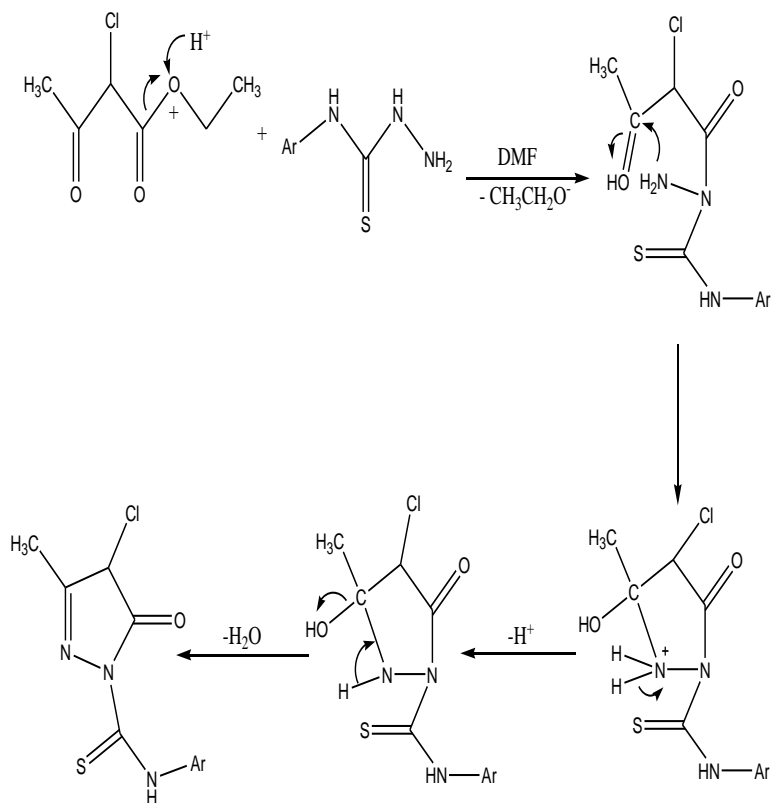
Yield-58%, M.P.-165°C, IR(KBr,  $\nu_{\max}$   $\text{cm}^{-1}$ ): 3245 (N-H-Str), 1660 (C=O), 1455 (C-N-Str), 1145 (C=S), 1612 (C=N);  $^1\text{H NMR}$ =(300MHz,DMSO) $\delta$ =1.12(3H,s), 2.33(2H,s), 3.85(3H,s), 4.35(1H,s), 6.02(1H,s), 6.10(1H,d), 6.22(1H,d), 6.97(1H,dd)ppm,  $^{13}\text{C NMR}$ :  $\delta$  183.7, 172.7, 156.3, 162.9, 141.6, 130.8, 118.6, 112.1, 110.9, 56.8, 36.8, 21.4; MS(EI)  $m/z$ =297.03[M] $^+$  · Anal. calcd. for  $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ : C, 49.84; H, 5.18; N, 16.36; Found: C, 48.93; H, 5.04; N, 15.17.

***N*-(4-methoxyphenyl)-4-chloro-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide  
(3g)**

Yield-60%, M.P.-182°C, IR(KBr,  $\nu_{\max}$   $\text{cm}^{-1}$ ): 3223 (N-H-Str), 1630 (C=O), 1435 (C-N-Str), 1159 (C=S), 1606 (C=N);  $^1\text{H NMR}$ =(300MHz,DMSO) $\delta$ =1.05(3H,s), 2.30(2H,s), 3.81(3H,s), 4.32(1H,s), 6.44(2H,t), 6.59(2H,t)ppm,  $^{13}\text{C NMR}$ :  $\delta$  183.2, 172.7, 156.1, 159.1, 132.2, 127.3, 115.4, 57.0, 36.2, 20.2; MS(EI)  $m/z$ =297.03[M] $^+$  · Anal. calcd. for  $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ : C, 48.64; H, 4.98; N, 15.99; Found: C, 48.43; H, 4.64; N, 14.87.



Scheme – 1 Synthesis of substituted pyrazolone derivatives

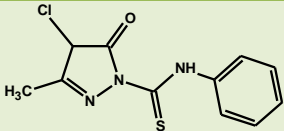
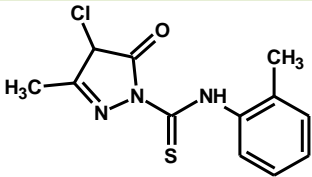
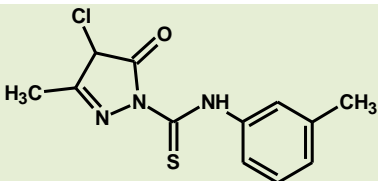
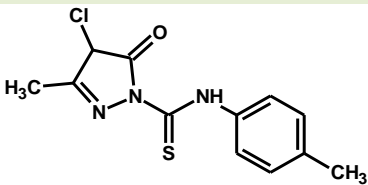
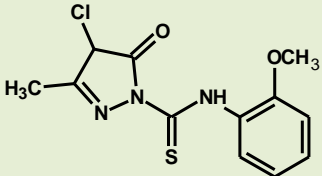
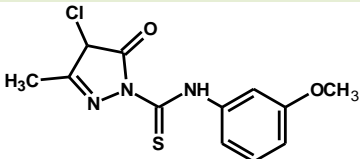
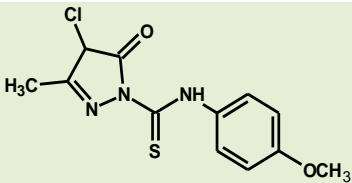


Ar = C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>5</sub>-R

R = H, CH<sub>3</sub>(o,m,p), OCH<sub>3</sub>(o,m,p)

Mechanism of substituted pyrazolone derivatives

Table-I Physical characterization data of compounds 3a-g

| S.No | Comp. | Structure   | Yield % | M.P. °C | Molecular formula  | Colour         |
|------|-------|---|---------|---------|--|----------------|
| 1    | 3a    |    | 70      | 145     | C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> OCl               | White          |
| 2    | 3b    |    | 67      | 225     | C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> OCl               | creamish white |
| 3    | 3c    |    | 68      | 179     | C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> OCl               | Pinkish white  |
| 4    | 3d    |   | 60      | 190     | C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> OCl               | Light yellow   |
| 5    | 3e    |  | 64      | 175     | C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> Cl | Creamish white |
| 6    | 3f    |  | 58      | 165     | C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> Cl | Cream          |
| 7    | 3g    |  | 60      | 182     | C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> Cl | Creamish white |



## Microbiology

All the compounds were screened for their *in vitro* antibacterial activity at the Birla institute of Medical Research and College of Life Sciences, Gwalior, against 24h old cultures of bacterial and fungal pathogens. Antibacterial activity was determined against, *Escherichia coli*, *Bacillus anthracis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* bacterial strains using the disc diffusion assay. For this, a sterile filter paper disc (6mm) impregnated with fixed doses 600µg/mL of the synthesized compounds under investigation were placed upon the seeded petridishes. Similar discs were prepared for the standard drugs, chloramphenicol and the solvent control dimethyl formamide. The plates were incubated for 24h at 37.8 °C. The zone of inhibition, observed around the disc after incubation was measured. The results are presented in **Table-II**.

### Disc diffusion test

The compounds were dissolved in dimethylformamide (DMF) and 600µg/ml of each compound was tested using a filter paper disc diffusion method with Mueller-Hinton agar media. For each experiment, DMF alone was used as a control and found inactive in the culture medium. Ampicillin was used as reference standard drug. Growth inhibition was examined after 24 h of incubation at 37 °C; antibacterial activity was determined by measuring the zone of inhibition (ZOI) in millimeters around the each disk.

### Preparation of anti-microbial Suspension (1mg/mL)

The antibacterial activities of the synthesized compounds were screened *in vitro* using the disc diffusion technique against different human pathogens at 600µg/mL. All of the compounds tested, showed moderate activity.

**Table-II: Antibacterial activity data in MIC (µg/mL) of the compounds 3a-g**

| S.No.           | Comp | Inhibition zone in diameter (mm) |                  |                    |                     |
|-----------------|------|----------------------------------|------------------|--------------------|---------------------|
|                 |      | Bacterial strains                |                  |                    |                     |
|                 |      | <i>E. coli</i>                   | <i>S. aureus</i> | <i>B.anthrecis</i> | <i>P.aeruginosa</i> |
| 1               | 3a   | 16                               | 18               | 14                 | 17                  |
| 2               | 3b   | 17                               | 15               | 12                 | 18                  |
| 3               | 3c   | 16                               | 15               | 14                 | 15                  |
| 4               | 3d   | 12                               | 16               | 11                 | 16                  |
| 5               | 3e   | 14                               | 16               | 14                 | 15                  |
| 6               | 3f   | 17                               | 17               | 14                 | 17                  |
| 7               | 3g   | 19                               | 19               | 15                 | 19                  |
| Chloramphenicol |      | 23                               | 24               | 26                 | 24                  |

## Interpretation

After incubation, the disc showing no visible growth were considered to be representing the MIC. The details of results are furnished in **Table-II**.

## RESULT AND DISCUSSION

### Chemistry

The synthetic route of target compounds **3a-g** is depicted in **Scheme 1**. Now we hereby reported the synthesis of some new biologically active pyrazolone derivatives by the reaction of thiosemicarbazide and ethyl-2-chloro acetoacetate with ethanol as a solvent. Dimethylformamide is a polar (hydrophilic) aprotic solvent with a high boiling point as compare to ethanol. It can easily react with  $\text{NHNH}_2\text{SCN}$ , which make it possible for  $\text{SCN}^-$  to readily react with ethyl-2-chloro acetoacetate and lead to the formation of target compounds **3a-g**. The structures of the compounds **3a-g** were established on the basis of their spectroscopic data. The IR (KBr) spectra of the target compounds **3a-g** showed characteristic N-H, C=O, C-N and C=S absorptions at  $3,200\text{--}3,430\text{ cm}^{-1}$ ,  $1,600\text{--}1,750\text{ cm}^{-1}$ ,  $1400\text{--}1490\text{ cm}^{-1}$  and  $1,150\text{ cm}^{-1}$  respectively. The  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ) spectra of these compounds exhibited the expected multiplet near  $\delta$  7.01-7.05 ppm due to the presence of aromatic protons. The N-H protons appeared singlets at  $\delta$  4.07-4.58 ppm respectively, whereas the pyrazolone methyl protons showed up near  $\delta$  2.50 ppm as a singlet.  $^{13}\text{C}$  NMR spectra recorded signals corresponding to thiosemicarbazide moiety and other aromatic carbons. The mass spectrum of **3a** showed molecular ion peak at  $m/z = 267.02(\text{M})^+$ , which is in agreement with the molecular formula  $\text{C}_{11}\text{H}_{11}\text{N}_3\text{OSCl}$ . Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part and characterization is provided in **Table-I**.

### Antibacterial Activity Bioassay

The *in vitro* antibacterial screening data of the pyrazolone derivatives are provided in **Table-II**. All the synthesized compounds exhibited different antibacterial activities for all four bacterial strains at  $600\mu\text{g/mL}$ . Compounds **3a**, **3f** and **3g** were shown to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively; compounds **3a** and **3f** exhibited good activities on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, and rest of the compounds shown moderate antibacterial activity against the bacterial strain and less activity shows on *Bacillus anthracis*, respectively. These figures were slightly lower than those of chloramphenicol. Although, a definite structure activity relationship could not be established with the limited experimental data and available compounds, it appears that with the incorporation of  $-\text{NHNH}_2\text{SCN}$  in the resulting products **3** might have a positive influence, enhancing the antibacterial activity of the designed compounds. From the activity differences between **3b** and **3g**, we can conclude that different methyl and methoxy group positions on benzene can result in different activity: when  $-\text{CH}_3$  and  $-\text{OCH}_3$  was substituted at the 2 and 4-

position of benzene (**3b**, **3g**), it showed much higher activity than that of **3d**, **3f**, it may be occurred by electronic hindrance.

## CONCLUSION

In summary, a series of substituted pyrazolones have been successfully synthesized using thiosemicarbazide and ethyl-2-chloro acetoacetate as the starting materials. The synthesized compounds were characterized by spectral data ( $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , IR, Mass) and elemental analysis. The compounds were subjected to *in-vitro* antibacterial activity against *Escherichia coli*, *Bacillus anthracis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results showed that the synthesized compounds possessed good antibacterial activities against the tested bacteria, with compounds **3a**, **3f** and **3g** displaying good activity. Preliminary bioassays indicated that some of these compounds are also associated with good inhibitory activities against MIC at a concentration of  $600\mu\text{g/mL}$ . Further studies are currently underway to establish a definite structure activity relationship.

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