

## SYNTHESIS AND *IN-VITRO* ANTIMICROBIAL STUDIES OF SOME NEW PYRAZOLONES

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### ABSTRACT

In the present scenario antibacterial agents are the greatest contribution of chemotherapy. They have great importance in the developing countries where infectious diseases predominate. 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. In the present study, a series of 3-methyl-*N*-(substituted phenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (**3a-g**) were synthesized by the reaction of thiosemicarbazides (**1**) and ethylacetoacetate (**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 antimicrobial activities using disc diffusion technique against four bacterial pathogens viz *Staphylococcus aureus*, *Escherichia coli*, *Bacillus anthracis* and *Pseudomonas aeruginosa* & two fungal pathogens namely *Candida albicans*, *Aspergillus niger*. Chloramphenicol and Fluconazole were used as standard drug respectively. The compounds exhibit moderate activity.

**Keywords:** Pyrazolones, Thiosemicarbazides, Antimicrobial activity, Disc diffusion technique.

### INTRODUCTION

In recent decades, the problem of microbial infections has reached alarming levels in the developing countries around the world. Studies on the influence of structure on activity showed that sometimes, minor changes in the nuclei enhance the pharmacological profile multifold than the parent molecule. The search for new, effective and safe nuclei has led to improvements in the existing drugs by increasing their potency, duration of action as well as minimizing their toxic effect. This is achieved by creating new biologically active agents by molecular modifications.

Heterocyclic compounds are acquiring more importance in recent years because of their pharmacological activities. Pyrazolones have a particular value due to their broad spectrum of biological activity and their wide ranging utility as synthetic tools in the design of various bioactive molecules. Pyrazolone is a five membered lactum ring, containing two nitrogen and one ketonic group in its structure. The chemistry of pyrazolone was started by Knorr in 1883 and reported the first pyrazolones derivative [1]. Antipyrine was the first pyrazolones derivative for clinical use and was synthesized in 1883 [2, 3]. 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]. They also serve as precursors for dyes, pigments, pesticides and chelating agents [14], besides finding applications in the extraction and separation of various metal ions [15-18]. They are also employed in chromatography, petrochemical industry, as laser materials and <sup>1</sup>H NMR shift reagents [19].

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 [20]. These compounds were first obtained by *H. Otto* in 1974 [21] by adding malononitrile to 4-arylidene-3-methyl-2-pyrazolin-5-one. In 1981 [22] *S. Abdou* have been reported alternate synthesis of this product. *Gokcen Eren et. al.* reported 1-phenyl-3-trifluoromethyl-5-(3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1*H*-pyrazoles and their *in-vitro* inhibitory activities on COX-1 and COX-2 isoforms were evaluated using a purified enzyme assay [23]. *Kuppusamy Sujatha* have been developed some pyrazolone derivatives to possess promising

antiviral activity against ruminant virus [24]. The chemistry and antimicrobial activity of some substituted pyrazolone has been investigated in recent years and it was thought world wide to synthesize novel pyrazolones from easily available starting materials and evaluations of their possible antimicrobial and anti-inflammatory activity [25-30]. Keeping in view of this and in continuation of our search on biologically potent molecules [31], we hereby reported the synthesis of some new biologically active pyrazolone derivatives by the reaction of thiosemicarbazide and ethylacetoacetate.

### MATERIALS AND METHODS

#### General

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) was recorded on a spectrophotometer. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were recorded (CDCl<sub>3</sub>) on a Bruker DRX 300 (300MHz, FT NMR) and varian (300MHz) spectrometer. The Mass spectra was 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.

The synthetic route has been highlighted in **Scheme 1**. For the synthesis of the titled compounds, *N*-phenyl hydrazine carbothioamide (**1**) was prepared by reacting phenyl isothiocyanate with hydrazine hydrate in the presence of ethanol. The reaction of equimolar quantities of thiosemicarbazide with ethylacetoacetate (**2**) were refluxed in the DMF for 10 hrs resulted in the target compound (**3**).

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

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

#### 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 tolyldithio carbamate 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.

#### 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.

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

Take an intimate mixture of phenyl thiosemicarbazide (0.01 mole, 1.67 gm), ethylacetoacetate (0.01 mole, 1.28 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 yellow 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= 80%, M. P. = 130°C

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

Yield-80%, M. P.-120°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=233.06[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,56.43; H, 4.64; N, 18.07.

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

Yield-77%, M. P.-215°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=247.08[M]<sup>+</sup>.Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>OS: C,58.28; H,5.30; N,16.99; Found: C,58.03; H, 4.94; N, 16.27.

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

Yield-78%, M. P.-159°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=247.08[M]<sup>+</sup>.Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>OS: C,57.28; H,4.90; N,17.99; Found: C,57.03; H, 4.94; N, 17.27.

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

Yield-70%, M. P.-180°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=247.08[M]<sup>+</sup>. Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>OS: C,58.20; H,5.50; N,16.89; Found: C,58.13; H, 4.99; N, 16.77.

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

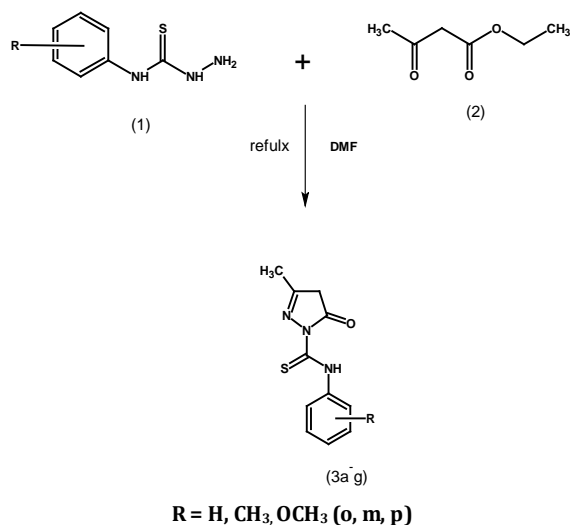
Yield-65%, M. P.-155°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3300 (N-H-Str), 1686 (C=O), 1463 (C-N-Str), 1163 (C=S), 1615 (C=N); <sup>1</sup>HNMR=(300MHz,DMSO) $\delta$ =1.10(3H,s), 2.23(2H,s), 3.75(3H,s), 4.15(1H,s), 6.42(1H,d), 6.55(1H,dd), 6.60(1H,dd), 6.66(1H,d)ppm, <sup>13</sup>C NMR:  $\delta$  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=263.07[M]<sup>+</sup>.Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S: C,54.74; H,4.98; N,15.96; Found: C, 54.43; H, 4.64; N, 15.77.

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

Yield-68%, M. P.-145°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3245 (N-H-Str), 1660 (C=O), 1455 (C-N-Str), 1145 (C=S), 1612 (C=N); <sup>1</sup>HNMR=(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, <sup>13</sup>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=263.07[M]<sup>+</sup>.Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S: C, 55.84; H, 5.18; N, 16.36; Found: C, 54.93; H, 5.04; N, 16.17.

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

Yield-70%, M. P.-162°C, IR(KBr,  $\nu_{\max}$  cm<sup>-1</sup>): 3223 (N-H-Str), 1630 (C=O), 1435 (C-N-Str), 1159 (C=S), 1606 (C=N); <sup>1</sup>HNMR=(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, <sup>13</sup>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=263.07[M]<sup>+</sup>.Anal. calcd. for C<sub>12</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>S: C, 54.64; H, 5.98; N, 15.99; Found: C, 54.43; H, 5.64; N, 15.87.



Scheme 1: Synthesis of pyrazolone derivatives

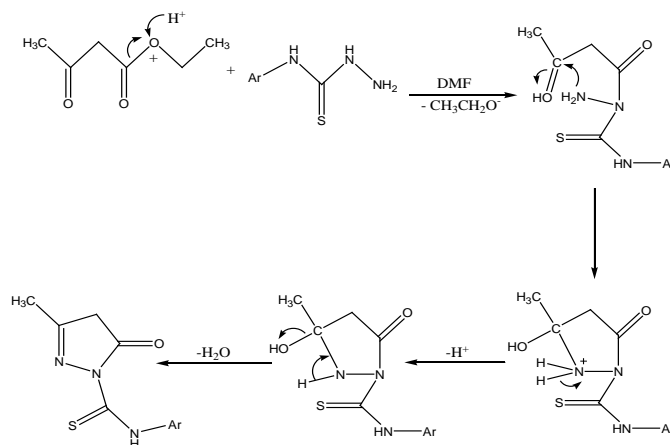
#### Microbiology

All the compounds were screened for their *in vitro* antimicrobial activity at the Birla institute of Medical Research and College of Life Sciences, Gwalior, against 24h old cultures of bacterial and fungal pathogens. Antimicrobial activity was determined against, *Escherichia coli*, *Bacillus anthracis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* bacterial strains and *Candida albicans*, *Aspergillus niger* fungal strains using the disc diffusion assay. For this, a sterile filter paper disc (6 mm) impregnated with fixed doses 600 $\mu$ g/mL of the synthesized compounds under investigation were placed upon the seeded petridishes. Similar discs were prepared for

the standard drugs, chloramphenicol, fluconazole and the solvent control, dimethyl formamide. The plates were incubated for 24h at 37.8C for the bacterial strains and 48 h at 37.8°C for the fungal strains. The zone of inhibition, observed around the disc after incubation was measured. The results are presented in Table-II.

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

The antimicrobial 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.



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)  
Machenism of Pyrazolone derivatives

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

S. No.	Comp.	Struture	Yield %	M. P. °C	Molecular formula	Colour
1	3a		80	120	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> OS	cream
2	3b		77	215	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> OS	Pinkish white
3	3c		78	159	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> OS	Brown
4	3d		70	180	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> OS	Redish brown
5	3e		65	155	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	Creamish white
6	3f		68	145	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	Cream
7	3g		70	162	C <sub>12</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	Creamish white

Table II: Antimicrobial activity data in MIC ( $\mu\text{g/mL}$ ) of the compounds 3a-g

S. No.	Comp	Inhibition zone in diameter (mm)					
		Bacterial strains			Fungal strains		
		<i>E. coli</i>	<i>S. aureus</i>	<i>B. anthracis</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
1	3a	17	19	15	16	17	16
2	3b	18	16	13	18	15	18
3	3c	18	16	14	16	17	18
4	3d	14	16	12	17	13	15
5	3e	15	17	15	15	16	17
6	3f	17	16	13	18	18	19
7	3g	19	19	13	20	15	14
	Chloramphenicol	23	24	26	24	-	-
	Fluconazole	-	-	-	-	24	26

### Preparation of media

All media employed in bacteriological studies were prepared by dissolving the required amount of individual components of the subjective media in distilled water and then autoclaved at 121°C for 20 minutes. For solid medium, agar was added to the broth @1.6% prior to autoclaving. The fresh media plates were prepared by pouring lukewarm (40°C) autoclaved agar medium in sterile petri-plates inside a laminar flow cabinet.

### 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.

## RESULTS AND DISCUSSION

### Chemistry

The synthetic route to title compounds 3a-g is depicted in Scheme 1. In present study, we have conducted our reaction using dimethylformamide. In the previous literature most of the pyrazolone derivatives prepared by using ethanol as a solvent. R. Khan et al. [32] have been used ethanol for the synthesis of pyrazolone. Now we hereby reported the synthesis of some new biologically active pyrazolone derivatives by the reaction of thiosemicarbazide and ethylacetoacetate with DMF 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 ethylacetoacetate and lead to the formation of target compounds 3a-g. We have also tried the reaction with ethanol, acetic acid and n-butenol but they take more time and low yield due to less polarity and there was not found significant product, so we have used DMF because of its polarity is more than ethanol and others and reaction was completed less timing with good yield.

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 3,200-3,430  $\text{cm}^{-1}$ , 1,600-1,750  $\text{cm}^{-1}$ , 1400-1490  $\text{cm}^{-1}$  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  $m/z = 233.06(\text{M})^+$ , which is in agreement with the molecular formula  $\text{C}_{11}\text{H}_{11}\text{N}_3\text{OS}$ . 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. It was observed that these synthesized compounds showed weak to good antibacterial activities against the tested bacteria at 600 $\mu\text{g/mL}$ . Compounds 3a, 3b and 3g were shown to inhibit the growth of *Escherichia coli*,

*Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively; compounds 3b and 3f exhibited good activities on *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, while compounds 3c and 3g inhibited the growth of *Escherichia coli* and *Pseudomonas aeruginosa*, and less activity shows on *Bacillus anthracis*, respectively. These fig. were slightly lower than those of chloramphenicol. Amongst the new products compound 3f exhibited similar activities as chloramphenicol on the corresponding bacteria. 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 3e, 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, 3e), it showed much higher activity than that of 3c, 3f at the 3-position of benzene, this may be caused by electronic hindrance.

### Antifungal activity bioassay

The antifungal activities of compounds 3a-g against *Candida albicans* and *Aspergillus niger* fungal strain. The results of the *in-vivo* bioassay against are given in Table-II. Fluconazole was used as a reference antifungal drug. Compounds 3a, 3b and 3f were shown to inhibit the growth of *Aspergillus niger*, respectively; compounds 3c and 3f exhibited good activities on *Candida albicans*, respectively. Here 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 3-position of benzene (3c, 3f), it showed much higher activity than other compounds.

## CONCLUSION

In the present study, a mild and effective method for the preparation of seven novel pyrazolone derivatives employing phenyl thiosemicarbazide and ethyl acetoacetate as the starting materials is described. 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 assays against *Escherichia coli*, *Bacillus anthracis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and antifungal activity assays against *Candida albicans*, *Aspergillus niger*. The results showed that the synthesized compounds possessed weak to good antibacterial and antifungal activities against the tested bacteria and fungi, with compounds 3a, 3b 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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