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Synthesis and *In-vitro* Antifungal Evaluation of 5- Pyrazolones

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Abstract: A series of 4-chloro-3-methyl-*N*-(substituted phenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1- carbothioamide were synthesized using earmark reaction of thiosemicarbazides and ethyl-2-chloroacetoacetate in DMF. All structures of the synthesized compounds were distinguished on the basis of IR, ^{1}H-NMR, ^{13}C-NMR and also elemental analysis. Synthesized Compounds were examined their potent antifungal activity using disc diffusion technique against three fungal pathogens viz *Aspergillus niger, Candida albicans* and *Curvularia*. Fluconazole were used as standard drug respectively. All compounds exhibited good to moderate activity.

Keywords: Antifungal activity, disc diffusion technique, pyrazolones, thiosemicarbazides.

INTRODUCTION

In recent years, fungal infections spreaded worldwide infection to life threatening systemic ailments involving the internal organs ranging from minor superficial skin and mucous membrane. The search for new, effective and safe nuclei leads to important modifications in the existing drugs by increasing their efficacy as well as formulating new bioactive agents by molecular modifications. The role of medicinal chemistry is essential and sustainable for previous and current generation. In the practice of medicinal chemistry developed from an empirical organic synthesis of new compound based on the modification of structure and identifies their biological activity [1, 2]. Moreover, various heterocyclic and biologically active compounds have five-member nitrogen, sulphar, oxygen containing heterocyclic ring [3]. Pyrazolone is a five membered lactum ring, containing two nitrogen and one ketonic group in its structure.

Pyrazolones have acquired versatile importance as drug substances in pharmaceutical industry of their biological importance. For instance, various pyrazolones drugs, viz. phenazone, propyphenazone, ampyrone and metamizole are useful antipyretic and analgesic drugs [4]. Therefore, pyrazolones possess antimicrobial, antifungal [5], anti-mycobacterial [6, 7], antibacterial [8], anti-inflammatory [9], antitumor [10], gastric secretion stimulatory [11], anti-depressant [12] and antifilarial activities [13]. Many attempts have been made to synthesize, characterize and to study biological activity of pyrazolones [14]. Interest in the chemistry of a new organic photochromic compound containing pyrazolone-ring was synthesized and characterized by Liu *et al.* They evaluated their photocromic properties were related to the photoisomerization and time-dependent UV-vis spectra, and these compounds exhibited good antibacterial activities [15]. Atudosie *et al.* [16] have been reported the synthesis of new 5- substituted-2-[2-(2-substituted-10*H*-phenothiazin-10-yl)-2-oxoethyl]-2,4-dihydro-3*H*-pyrazol-3 one containing phenothiazine unit by reaction of *N*-chloroacetyl compound, ethyl acetoacetate with hydrazine hydrate and their were evaluated antiproliferative activity. In the recent years, the chemistry and antibacterial activity of pyrazolone have been investigated and synthesized to be novel pyrazolones from easily available starting materials and their broad range of antimicrobial and anti-inflammatory activity were evaluated [17 - 23]. The study was aimed at exploring our synthesis of some new biologically active pyrazolone derivatives by the reaction of thiosemicarbazide and ethyl-2-chloro acetoacetate.

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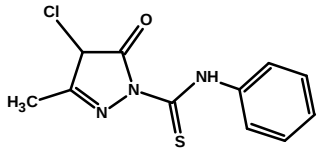
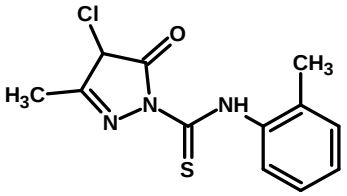
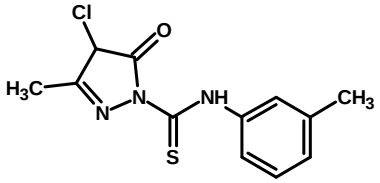
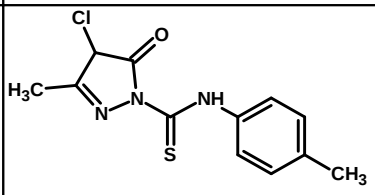
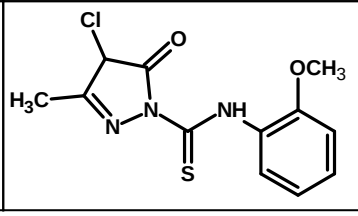
MATERIALS AND METHODS

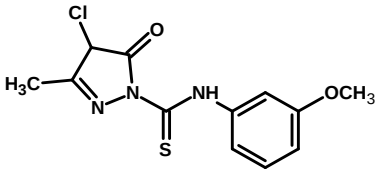
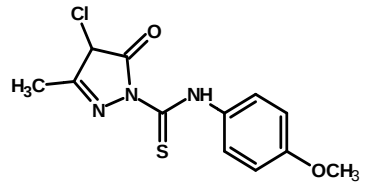
All materials were of commercial reagent grade and purchased from Sigma-Aldrich. All reaction were monitored by column chromatography with E-Merck silica precoated plates and visualization was executed by ultraviolet and iodine chamber. Melting points were measured by open glass capillary and are uncorrected. ^{1}H and ^{13}C NMR spectra in $CDCl_3$ were taped on a BRUKER AVANCE II 129 400 MHz FT-NMR spectrometer (Bruker Bio Spin, Switzerland) at 400 and 100 MHz, respectively. IR spectra were taped using Perkin Elmer FTIR spectrophotometer. Elemental analysis was performed on Elementarvario MICRO cube CHN analyser. All yields refer to isolated products.

Literature revealed that more possibilities to finding a suitable derivative, which would express their activity more than already known drugs containing N-N and semicarbazone moiety. With the alarming trends in bacterial resistance to many pyrazolone derivatives it has become necessary to synthesize some novel pyrazolone for bioassay of antifungal activity and the need for drugs with more specific antifungal activity. Therefore it was considered of interest to combine all the above-mentioned bioactive heterocyclic rings attach together in a molecular framework of pyrazolone in a proper manner to enhance their biological activity. Put on all ideas in mind and continuation of earlier studies, we synthesized 4- chloro-3-methyl-5-oxo-*N*-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, 4-chloro-3- methyl-*N*-(2-methylphenyl)-5-oxo-4,5-dihydro-1*H* pyrazole-1-carbothioamide and *N*-(2- methoxyphenyl)-4-chloro -3-methyl-5-oxo-4,5-dihydro-1*H* pyrazole-1-carbothioamide with engrossing structural features. Moreover, the antifungal potentiality of pyrazolone and to probe the structure activity- relationship, all the fabricated molecules were evaluated for their antifungal activities in Table **1**.

Table 1. Physico-chemical data of compounds (3a-g).

S. No	Comp	Structure	Yield %	M.P. °C	Molecular formula	Colour	Ref. (M.P.) °C
1	3a		70	146-149	$C_{11}H_{10}ClN_3OS$	White	144-148 [23]
2	3b		67	225-227	$C_{12}H_{12}ClN_3OS$	Creamish white	224-227 [23]
3	3c		68	175-179	$C_{12}H_{12}ClN_3OS$	Pinkish white	170-172[23]
4	3d		60	188-190	$C_{12}H_{12}ClN_3OS$	Light yellow	170-175 [23]
5	3e		64	235-238	$C_{12}H_{12}ClN_3O_2S$	Creamish white	240-242 [23]

(Table 1) contd.....

S. No	Comp	Structure	Yield %	M.P. °C	Molecular formula	Colour	Ref. (M.P.) °C
6	3f		58	245-249	$C_{12}H_{12}ClN_3O_2S$	Cream	256-258 [23]
7	3g		60	232-235	$C_{12}H_{12}ClN_3O_2S$	Creamish white	240-244 [23]

The synthetic pathway of the reaction, ethylacetoacetate with thiosemicarbazide to gave direct regain to the desired 4 -chloro-3-methyl-5-oxo-*N*-phenyl-4,5- dihydro-1*H*-pyrazole-1-carbothioamide, 4-chloro-3 -methyl-*N*-(2-methylphenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide and *N*-(2- methoxyphenyl)-4-chloro -3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide. The initial preparation of precursors *N*-(substituted phenyl) thiosemicarbazide. The reactive ethylacetoacetate were approchable via the reaction of an equimolar quantity of thiosemicarbazide containing H, CH_3 and OCH_3 group in dimethylformamide at 80-90°C, which resulted in the formation of 4-chloro-3-methyl-5-oxo-*N*-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide, 4-chloro-3-methyl-*N*-(2-methylphenyl)-5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide and *N*-(2- methoxyphenyl)-4-chloro -3-methyl- 5-oxo-4,5-dihydro-1*H*-pyrazole-1-carbothioamide in good yield.

General Procedure for the Synthesis of Pyrazolones: [3a-g]

Equimolar mixture of phenyl thiosemicarbazide (0.01 mole, 1.67 gm) (1) and ethyl- 2-chloro acetoacetate (0.01 mole, 1. 28 mL) (2) were refluxed for 10 h in DMF (20 mL). Now mixture was refrigerated with water and solidified filtrate were recrystallized with ethanol.

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, ν_{max} cm^{-1}): 3330-3000(N-H), 1215(C=S), 3020-1731(C-H), 1483-1402(C-N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.49(1H, s, C-H), 7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 44.40, 38.12, 24.56; MS(EI) m/z=267.02$[M]^+$, Anal. calcd. for $C_{11}H_{10}ClN_3OS$: C,49.35; H,3.76; N,15.69; Found: C,49.75; H, 3.94; N, 15.97.

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, ν_{max} cm^{-1}): 3178(N-H-Str), 1699 (C=O), 1452 (C-N-Str), 1157 (C=S), 1602 (C=N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.59(3H, s, Ar-CH_3), 7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 62.8, 44.40, 38.12, 24.56; MS(EI) m/z=281.04$[M]^+$, Anal. calcd. for $C_{12}H_{12}ClN_3OS$: C,51.15; H,4.29; N,14.91; Found: C,51.33; H, 4.94; N, 14.99.

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, ν_{max} cm^{-1}): 3265 (N-H-Str), 1672 (C=O), 1476 (C-N-Str), 1166 (C=S), 1620 (C=N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.59(3H, s, Ar-CH_3), 7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 62.8, 44.40, 38.12, 24.56; MS(EI) m/z=281.04$[M]^+$, Anal. calcd. for $C_{12}H_{12}ClN_3OS$: C,51.15; H,4.29; N,14.91; Found: C,51.34; H, 4.64; N, 14.97.

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, ν_{max} cm^{-1}): 3256 (N-H-Str), 1668 (C=O), 1482 (C-N-Str), 1141 (C=S), 1605 (C=N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.59(3H, s, Ar-CH_3),

7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 62.8, 44.40, 38.12, 24.56; MS(EI) m/z=281.04$[M]^{+\cdot}$ Anal. calcd. for $C_{12}H_{12}ClN_3OS$: C,51.15; H,4.29; N,14.91; Found: C,51.19; H, 4.49; N, 14.99.

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

Yield-64%, M.P.-235°C, IR(KBr, ν_{max} cm^{-1}): 3300 (N-H-Str), 1686 (C=O), 1463 (C-N-Str), 1163 (C=S), 1615 (C=N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.49(3H, s, Ar-OCH_3), 7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 62.8, 44.40, 38.12, 24.56; MS(EI) m/z=297.03$[M]^{+\cdot}$ Anal. calcd. for $C_{12}H_{12}ClN_3O_2S$: C,48.40; H,4.06; N,14.11; Found: C, 48.60; H, 4.24; N, 14.67.

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

Yield-58%, M.P.-245°C, IR(KBr, ν_{max} cm^{-1}): 3245 (N-H-Str), 1660 (C=O), 1455 (C-N-Str), 1145 (C=S), 1612 (C=N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.49(3H, s, Ar-OCH_3), 7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 62.8, 44.40, 38.12, 24.56; MS(EI) m/z=297.03$[M]^{+\cdot}$ Anal. calcd. for $C_{12}H_{12}ClN_3O_2S$: C, 48.40; H, 4.06; N, 14.11; Found: C, 48.93; H, 4.44; N, 14.27.

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

Yield-60% , M.P.-232°C, IR(KBr, ν_{max} cm^{-1}): 3223 (N-H-Str), 1630 (C=O), 1435 (C-N-Str), 1159 (C=S), 1606 (C=N), 755 (C-Cl); ^{1}HNMR=(400MHz, $CDCl_3$)δ=1.32(3H, s, CH_3), 2.26(1H, s, NH), 3.49(3H, s, Ar-OCH_3), 7.22-7.67(Ar-5H, m)ppm; ^{13}C NMR: 169.13, 145.33, 137.81, 129.71, 126.09, 124.19, 77.31, 61.44, 62.8, 44.40, 38.12, 24.56; MS(EI) m/z=297.03$[M]^{+\cdot}$ Anal. calcd. for $C_{12}H_{12}ClN_3O_2S$: C, 48.40; H, 4.06; N, 14.11; Found: C, 48.55; H, 4.34; N, 14.55.

Disc Diffusion Assay

It is done by using Kirby-Bauer method to determine the antibacterial susceptibility at a fixed concentration [24]. For this, few colonies of organism were inoculated in 2-5 ml broth and grown for 2.5 hours. Before inoculation, dried agar plates to prevent flow of inoculated material during incubation. A sterile cotton swab is dipped into the bacterial suspension and used to evenly spread the diluted culture on the agar surface. After the inoculation dried, impregnated discs were placed on the agar surface with flamed forceps and gently pressed down to ensure contact [25]. The sterile (6 mm diameter) discs impregnated with fixed doses 600µg/ml were assassible on the pre-inoculated accede. The seeded petri-dishes were incubated within 30 minutes at 37°C for 48 hours. Similar plates were prepared for the standard drug, the reference antifungal drug fluconazole was used. Dimethylformamide was used as control solvent in the assay. The zone of inhibition is directly proportional to the degree of sensitivity of fungal strain and the concentration of compound. The antifungal activity of the compounds was calculated by using the formula as given below:

$$\%\ \text{Activity index} = \frac{\text{Zone of inhibition by test compound (diameter)}}{\text{Zone of inhibition by standard (diameter)}} \times 100$$

Determination of Minimum Inhibitory Concentration (MIC) Value

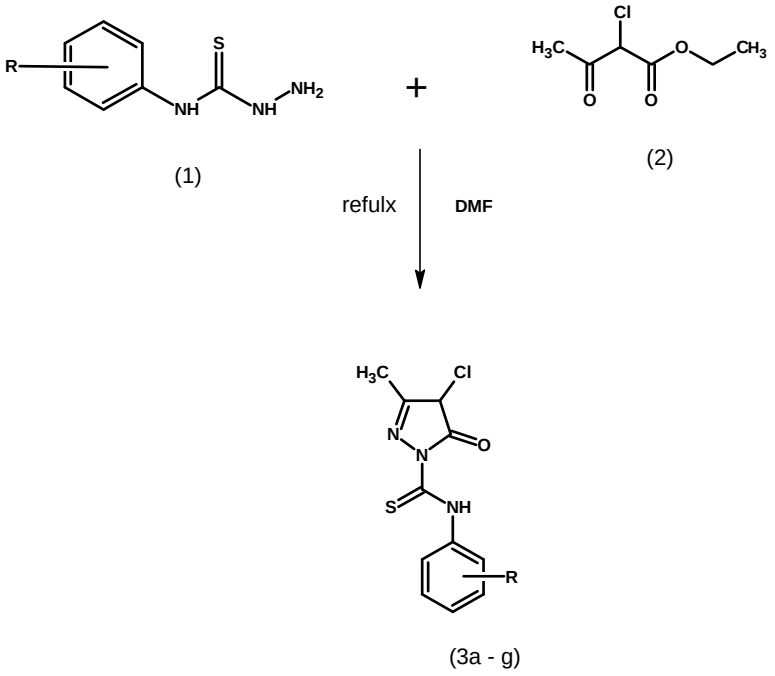
The antibacterial screening was determined using disc diffusion method by measuring zone of inhibition in mm.

Biological Assay

The antifungal activity of all synthesized compounds (3a-g) were tested against three fungal strains, viz; *A. niger, C. albicans* and *Curvularia,* using disc diffusion technique. Compounds (**3a-g**) were liquefy in dimethylformamide at different concentrations and the discs were dipped into the respective compounds and put on the petri dishes of specified organisms against a control of dimethylformamide. After 48 hours, the zone of inhibition was mascurated in mm. and the details of antifungal activity were furnished in Table **2**.

RESULTS AND DISCUSSION

Synthetic route of the target compounds (3a-g) is depicted in Scheme **1**. Here, we reported newly synthesized biologically active pyrazolone derivatives by the reaction of thiosemicarbazide and ethyl-2-chloro acetoacetate with DMF as a solvent. Dimethylformamide is a polar (hydrophilic) aprotic solvent with a high boiling point as compared to ethanol. It can easily react with $NHNH_2SCN$ reactant compound and makes it possible to react with ethyl-2- choloro acetoacetate and leads to the formation of target compounds (3a-g).



Where R = (I) H (with respect to thiosemicarbazide)

(II) CH_3, (*o, m, p* position with respect to thiosemicarbazide)

(III) OCH_3 (*o, m, p* position with respect to thiosemicarbazide)

Scheme. 1. Synthesis of substituted pyrazolone derivatives.

Table 2. Results of *in-vitro* antifungal activity observed for the synthesized pyrazolone compounds through disc diffusion assay.

S.No.	Comp	Inhibition zone in diameter (mm)		
		Fungal strains		
		A. niger	*C. albicans*	*Curvularia*
1	3a	12	13	13
2	3b	11	12	12
3	3c	-	13	14
4	3d	15	12	11
5	3e	16	-	14
6	3f	16	13	16
7	3g	18	17	15
Fluconazole		24	22	23

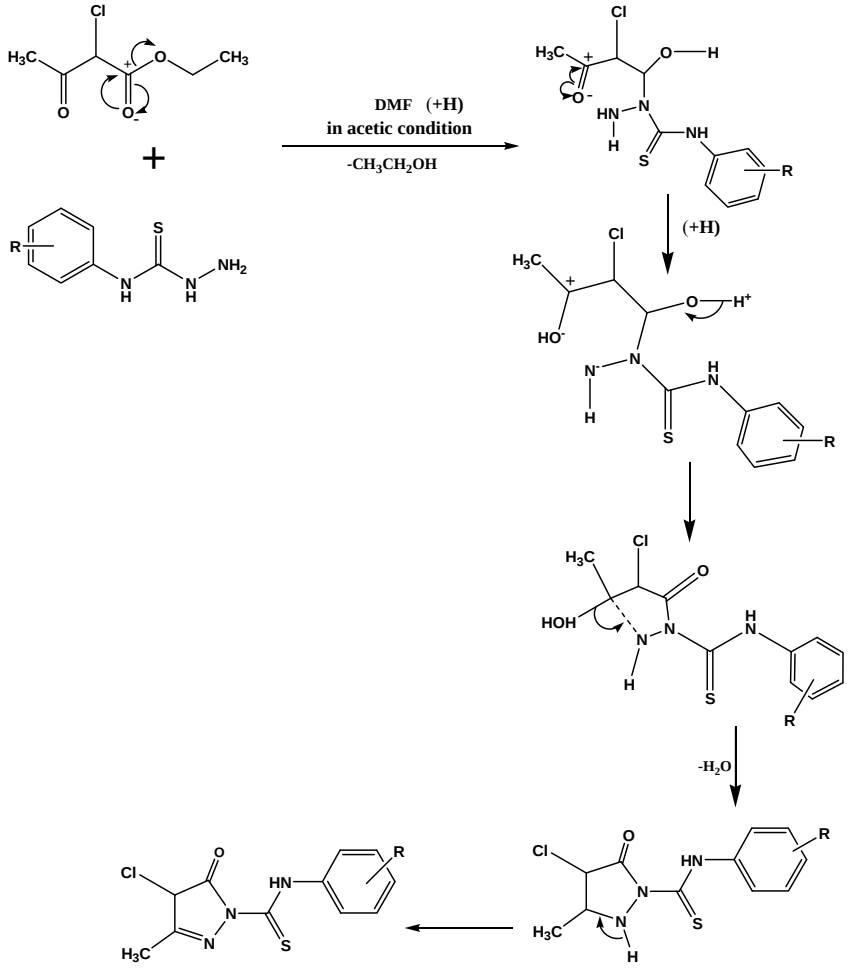
Antifungal susceptibility of compounds was determined in terms of zone of growth inhibitions. (-) means not shown activity. Inhibition zone diameters in millimeters at 600 µg /mL concentration of compounds.

The structures of the synthesized compounds (3a-g) were determined on the basis of their FT-IR, ^{1}H-NMR, ^{13}C-NMR and elemental analysis. The IR spectra of target compounds (3a-g) showed the presence of these groups N-H, C=O, C- N, C-Cl and C=S absorptions band at 3,200-3,430 cm^{-1}, 1,600 cm^{-1}, 750 cm^{-1}, 1400-1490 cm^{-1} and 1,150 $cm,^{-1}$ respectively. In ^{1}H- NMR spectra, all the compounds were characterized due to the presence of aromatic protons (Ar-CH) expected multiplet near δ 7.22-7.67 ppm. Another N-H protons also exhibited singlets at δ 2.20- 2.30 ppm,

respectively whereas, the pyrazolone methyl protons seemed high as a singlet near δ 1.32 ppm. ^{13}C NMR spectra recorded signals correspond to thiosemicarbazide moiety and other aromatic corbons. The mass spectrum of pyrazolone **(3a)** sustained molecular ion peak at m/z= 267.02 $(M)^+$, with the molecular formula $C_{11}H_{10}ClN_3OS$. And all compounds gave satisfactory elemental analysis.

Antifungal Activity

The antifungal activities were found of compounds (**3a-g**) against *Candida albicans, Aspergillus niger* and *Curvularia* fungal strain. Table **2** shows result of *in- vitro* antifungal bioassay. The reference antifungal drug was Fluconazole. Compounds **3d** and **3g** were assigned to inhibit the growth of *Aspergillus niger* and *Candida albicans,* respectively, compounds **3c** and **3f** exhibited remarkable inhibition on *Curvularia,* respectively. Here we can conclude that different methyl and methoxy group on aromatic ring increases the antifungal activity of different derivatives.



Proposed Mechanism of Pyrazolones Formation

CONCLUSION

In the present work, substituted pyrazolones have been successfully synthesized by using thiosemicarbazide and ethyl-2-chloro acetoacetate as a starting materials. The synthesized compounds were deduced by spectral analysis (^1H -NMR, ^{13}C -NMR, IR, Mass) and elemental analysis. The result of screening clearly indicated the nature of substitution in newly synthesized compounds affected *in-vitro* antifungal activity. The presence of electron-withdrawing group on the aromatic ring of thiosemicarbazide increases the antifungal activity of tested compounds. Here, electron-donating group were also shows moderate activity against tested pathogens such as *Aspergillus niger, Candida albicans and Curvularia.* All the synthesized compounds exposed better antifungal activities against a wide range of microorganisms.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers web site along with the published article.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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