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MICROWAVE ASSISTED SYNTHESIS OF SOME NOVEL 2-PYRAZOLINE DERIVATIVES AS POSSIBLE ANTIMICROBIAL AGENTS

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Abstract: Some new [3-(4-phenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanones and 3-substituted phenyl-5-substituted phenyl-4,5-dihydro-pyrazole-1-carbothioamides have been synthesized employing microwave techniques and evaluated for antimicrobial activity. Substituted acetophenones **(1)** were reacted with appropriately substituted benzaldehydes **(2)** in the presence of ethanol to furnish substituted chalcones **(3a-f)**. These chalcones were further treated with isonicotinic acid hydrazide (INH) to afford substituted [3-(4-phenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanones **(4a-f)**. Reaction of these chalcones with thiosemicarbazide yielded substituted 3,5-diphenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamides **(5a-f)**. The structures of newly synthesized compounds **(4a-f)** and **(5a-f)** have been confirmed by suitable spectroscopic techniques such as IR and ^{1}H NMR. All the compounds were screened for their antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* and for antifungal activity against *Candida albicans* and *Aspergillus niger.* The compounds exhibited moderate antibacterial and good antifungal activities. Compound **4b** and **4d** showed significant antifungal activity against *A. niger* and *C. albicans*, respectively.

Keywords: 2-pyrazoline, thiosemicarbazide, antifungal, antibacterial

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. The therapeutic problem has achieved increasing importance in hospitalized patients, in immuno suppressed patients with AIDS or undergoing anticancer therapy and organ transplants. In spite of a large number of antibiotics and chemotherapeutics available for medical use, the emergence of old and new antibiotic resistance developed in the last decades, has created a substantial medical need for new classes of antibacterial agents. A potential approach to overcome the resistance problem is to design innovative agents with a different mode of action, so that no cross resistance with the present therapeuticals can occur (1, 2).

Infectious diseases are one of the leading causes of death worldwide. During the past few decades, new infectious diseases have appeared and old ones previously thought to be controlled have reemerged (3) and thus, despite of many significant developments in the antimicrobial therapy, many problems remain to be solved for most of the antimicrobial drugs available (4). Hence, discovery of novel antimicrobial agents with better pharmacological profile is still highly desirable.

Historically, the use of anti-infective agents can be credited with saving more human lives than any other area of medicinal therapy discovered to date. It is a highly valued medical science, which has shaped modern humanity in a phenomenal fashion (5).

In recent decades, the problems of multi-drug resistant microorganism have reached an alarming stage in many countries around the world. A number of recent clinical reports describe the increasing occurrence of meticillin-resistant *S. aureus* and other antibiotic-resistant human pathogenic microorganisms in the United States and European countries. Infections caused by these microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to an escalating search for novel antimicrobial agents (6). Drug

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resistant bacteria are an increasing threat to public health, as highlighted by a recent estimate that in the US, methicillin-resistant *Staphylococcus aureus* (MRSA) may contribute to more deaths than HIV (7). Methicillin-resistant strains of *S. aureus* were initially documented in the 1960s (8) and have been associated with higher mortality rates than their drug-sensitive counterparts. Similar challenges are posed by the emergence of multidrug and extensively-drug resistant tuberculosis (MDR-TB and XDR-TB, respectively). Antibiotic resistance can result from large genomic changes, such as the acquisition of entire plasmids or mobile elements encoding resistance factors. Recent studies are, however, revealing the important role which small mutations play in the evolution of resistance. For example, only 35 point-mutations distinguish a vancomycin resistant strain of *S. aureus* from its sensitive counterpart, and these mutations evolved in just 3 months within an infected patient (9).

Antimirobical resistance threatens the health of many throughout the world, since both old and new infectious diseases remain a formidable public health threat. To what extent drug resistance and globalization are similarly related remains unclear. The breakout of Severe Acute Respiratory Syndrome (SARS) in the spring of 2003 illustrates how an infectious disease with limited therapeutic options can spread rapidly across national borders. With globalization booming, it is important to understand international patterns of resistance. Resistance mechanisms may develop over months or years. Once established, a single resistance mechanism can often allow a bacterium to resist multiple drugs. Drug resistance raises the cost of treatment for infectious diseases, sometimes manifold, as well as increasing morbidity and mortality from such diseases (10).

Small ring heterocycles containing nitrogen, sulfur and oxygen have been under investigation for a long time because of their important medicinal properties (11). Also, 2-pyrazolines have been reported to possess a variety of significant and diverse pharmacological activities such as antibacterial (12-14), antifungal (15, 16), antiviral (17), antitubercular (18, 19), antidepressant (20, 21), antiamoebic (22, 23), anti-inflammatory (24), anticonvulsant (25), analgesic (26) and anticancer (27) activity. In light of these findings, it was felt worthwhile to synthesize some new 2-pyrazoline derivatives and evaluate them for their antimicrobial potential.

On the other hand, microwave assisted organic reactions have emerged as a new 'lead' in organic synthesis with important advantages like highly acclerated rate of reaction along with improvement in yield and quality of products (28). Thus, keeping in view the advantages of these techniques, and immense biological importance of pyrazolines, it was felt worthwhile to study the reaction under microwave irradiation and to screen the target compounds for antimicrobial activity.

EXPERIMENTAL

Melting points were determined in open capillary tubes and are uncorrected. All the chemicals and solvents (ethanol and acetone) used were of laboratory grade and solvents were purified by suitable methods (29). IR spectra (KBr, cm^{-1}) were recorded on a JASCO FT/IR-410 spectrometer. ^1H NMR spectra was recorded on Brucker 300 MHz NMR spectrometer (chemical shifts in δ ppm) using TMS as an internal standard. The purity of the compounds was ascertained by thin layer chromatography on aluminium plates precoated with silica gel G (Merck) in various solvent systems using iodine vapors as detecting agent. Reactions were carried out in a Daewoo KOG-370A domestic microwave oven at 2450 MHz. Elemental analysis was done using Carlo Erba 1106 CHN analyzer.

General method

The title compounds were prepared in the following steps:

General procedure for synthesis of chalcones **(3a-f)**

A solution of acetophenone/substituted acetophenone (0.01 mol) and appropriately substituted benzaldehyde (0.02 mol) in dry ethanol (20 mL) were taken in a beaker. Sodium hydroxide was added in catalytic quantities (1-2 pellets) and the reaction mixture was zapped in microwave oven for 30 s to 2 min at 210 watts (i.e. 30% microwave power) and then cooled in ice bath. The product obtained was filtered and washed with ethanol (5 mL) followed by washings with water and recrystallization from acetone. The purity of the compounds was checked by TLC using methanol : water (8 : 2, v/v) as solvent system.

Synthesis of [3-(4-phenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanones **(4a-f)**

A mixture of chalcone **(3a-f)** (0.001 mol) and isoniazide (0.001 mol) was zapped inside a microwave oven for 8 to 10 min at 640 watts (i.e. 80% microwave power) in the presence of piperidine (1-2 mL) as a catalyst. After cooling, the solution was poured onto crushed ice and the product

Table 1. Physical and analytical data of the synthesized compounds

Comp no.	R_1	R_2	Reaction time (min)	Mol. formula	Yield (%)	Mol. wt.	M.p. (^oC)	R_f value
4a	4-OCH_3	H	10	$C_{22}H_{19}N_3O_2$	83	357	82-83	0.73
4b	4-Cl	H	10	$C_{21}H_{16}ClN_3O$	85	361	103-104	0.72
4c	3-NO_2	H	10	$C_{21}H_{16}N_4O_3$	92	372	135-136	0.76
4d	4-OCH_3	4-Cl	10	$C_{22}H_{18}ClN_3O_2$	84	391	143-145	0.68
4e	4-Cl	4-Cl	10	$C_{21}H_{15}Cl_2N_3O$	82	396	153-155	0.77
4f	3-NO_2	4-Cl	10	$C_{21}H_{15}ClN_4O_3$	90	406	145-146	0.72
5a	4-OCH_3	H	6	$C_{17}H_{17}N_3OS$	2	311	160-162	0.71
5b	4-Cl	H	5.5	$C_{16}H_{14}ClN_3S$	85	315	122-123	0.72
5c	3-NO_2	H	6	$C_{16}H_{14}N_4O_2S$	89	326	128-130	0.69
5d	4-OCH_3	4-Cl	6	$C_{17}H_{16}ClN_3OS$	71	345	125-127	0.72
5e	4-Cl	4-Cl	5.5	$C_{16}H_{13}Cl_2N_3S$	73	350	146-148	0.70
5f	3-NO_2	4-Cl	6	$C_{16}H_{13}ClN_4O_2S$	90	360	148-150	0.68

thus obtained was filtered and recrystallized using dichloromethane-methanol (1 : 1, v/v).

Synthesis of 3-substituted phenyl-5-substituted phenyl-4,5-dihydro-pyrazole-1-carbothioamides **(5a-f)**

A mixture of the chalcone **(3a-f)** (0.022 mol) and thiosemicarbazide (0.02 mol) was dissolved in acetone (5 mL) and ethanol (5 mL), respectively. Basic alumina (4 g) was added and stirred vigorously. After 5 min, the solvent was removed under vacuum and the dry powder was irradiated in a microwave oven for the appropriate time, at 650 W. After completion of the reaction the product was eluted with acetone. Removal of the solvent under reduced pressure yielded the product which was recrystallized from acetone-ethanol mixture (1 : 1, v/v).

Physical data of synthesized compounds are presented in Table 1.

[3-(4-Methoxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanone **(4a)**

IR (KBr, cm^{-1}): 3410, 3041, 1919, 1649, 1594, 1500, 818. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.62 (3H, s, Ar-OCH_3), 3.45 (2H, d, CH_2 of 2-pyrazoline), 5.85 (1H, t, CH of 2-pyrazoline), 6.84-7.04 (4H, m, Ar-H), 7.20-7.30 (5H, m, Ar-H), 8.46-8.95 (4H, m, Ar-H of pyridine). Analysis: for $C_{22}H_{19}N_3O_2$, found % (calculated %): C, 73.88 (73.93); H, 5.32 (5.36); N, 11.74 (11.76).

[3-(4-Chlorophenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanone **(4b)**

IR (KBr, cm^{-1}): 3412, 3048, 1917, 1645, 1592, 1498, 812. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.48 (2H, d, CH_2 of 2-pyrazoline), 5.81 (1H, t, CH of 2-pyrazoline), 7.20-7.30 (5H, m, Ar-H), 7.32-7.49 (4H, m, Ar-H), 8.48- 8.94 (4H, m, Ar-H of pyridine). Analysis: for $C_{21}H_{16}ClN_3O$, found % (calculated %): C, 69.68 (69.71); H, 4.42 (4.46); N, 11.59 (11.61).

[3-(3-Nitrophenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanone **(4c)**

IR (KBr, cm^{-1}): 3411, 3052, 1921, 1649, 1591, 1496, 814. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.46 (2H, d, CH_2 of 2-pyrazoline), 5.88 (1H, t, CH of 2-pyrazoline), 7.20-7.30 (5H, m, Ar-H), 7.91-7.95 (4H, m, Ar-H), 8.46- 8.94 (4H, m, Ar-H of pyridine). Analysis: for $C_{21}H_{16}N_4O_3$, found % (calculated %): C, 67.71 (67.73); H, 4.31 (4.33); N, 15.01 (15.05).

[3-(4-Methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanone **(4d)**

IR (KBr, cm^{-1}): 3410, 3042, 1917, 1646, 1592, 1498, 814. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.42 (2H, d, CH_2 of 2-pyrazoline), 3.62 (3H, s, Ar-OCH_3), 5.81 (1H, t, CH of 2-pyrazoline), 6.84-7.04 (4H, m, Ar-H), 7.22-7.33 (4H, m, Ar-H), 8.42- 8.86 (4H, m, Ar-H of pyridine). Analysis: for $C_{22}H_{18}ClN_3O_2$,

found % (calculated %): C, 67.39 (67.43); H, 4.61 (4.63); N, 10.69 (10.72).

[3-(4-Chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanone **(4e)**

IR (KBr, cm^{-1}): 3412, 3040, 1916, 1649, 1592, 1496, 812. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.42 (2H, d, CH_2 of 2-pyrazoline), 5.92 (1H, t, CH of 2-pyrazoline), 7.22-7.33 (4H, m, Ar-H), 7.35-7.68 (4H, m, Ar-H), 8.42- 8.86 (4H, m, Ar-H of pyridine). Analysis: for $C_{21}H_{15}Cl_2N_3O$, found % (calculated %): C, 63.62 (63.65); H, 3.81 (3.82); N, 10.58 (10.60).

[3-(3-Nitrophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanone **(4f)**

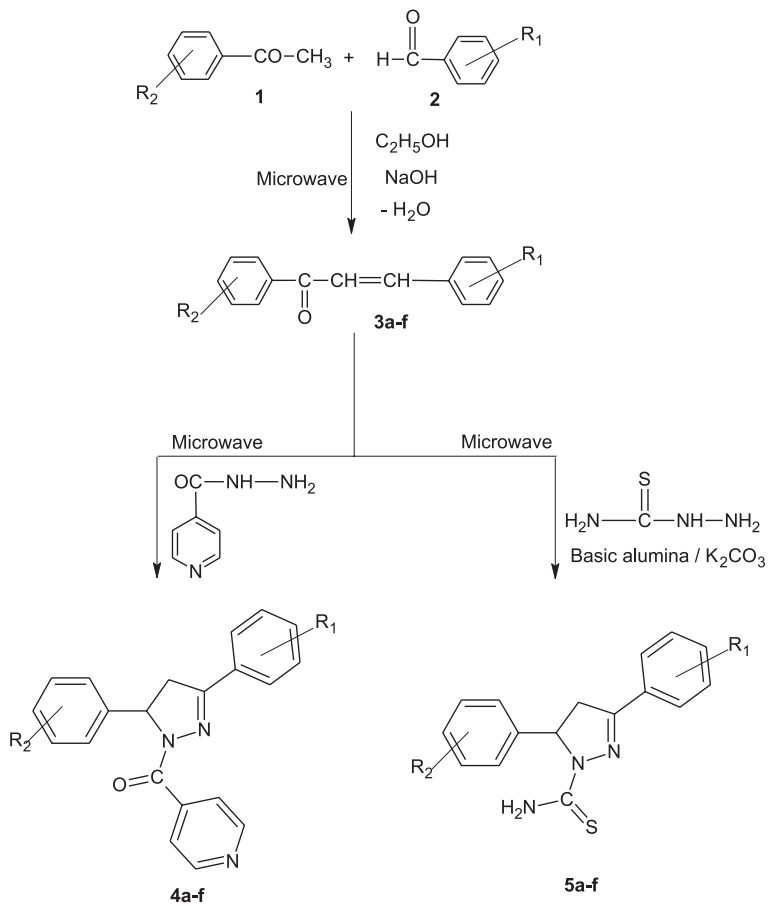
IR (KBr, cm^{-1}): 3408, 3040, 1918, 1652, 1591, 1498, 818. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.42 (2H, d, CH_2 of 2-pyrazoline), 5.92 (1H, t, CH of 2-pyrazoline), 7.22-7.33 (4H, m, Ar-H), 7.91-7.95 (4H, m, Ar-H), 8.42- 8.86 (4H, m, Ar-H of pyridine). Analysis: for $C_{21}H_{15}ClN_4O_3$, found % (calculated %): C, 61.98 (62.00); H, 3.69 (3.72); N, 13.72 (13.77).

3-(4-Methoxyphenyl)-5-phenyl-4,5-dihydropyrazole-1-carbothioamide **(5a)**

IR (KBr, cm^{-1}): 3431, 3059, 1869, 1655, 1215, 816. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.49 (2H, d, CH_2 of 2-pyrazoline), 3.62 (3H, s, Ar-OCH_3), 6.18 (1H, t, CH of 2-pyrazoline), 7.18 (2H, s, C=S-NH_2), 6.88-7.10 (4H, m, Ar-H), 7.30-7.51 (5H, m, Ar-H). Analysis: for $C_{17}H_{17}N_3OS$, found % (calculated %): C, 66.52 (66.57); H, 5.46 (5.50); N, 13.46 (13.49).

3-(4-Chlorophenyl)-5-phenyl-4,5-dihydropyrazole-1-carbothioamide **(5b)**

IR (KBr, cm^{-1}): 3432, 3058, 1872, 1658, 1214, 818. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.46 (2H, d, CH_2 of 2-pyrazoline), 6.19 (1H, t, CH of



Scheme 1. Synthesis of title compounds **(4a-f)** and **(5a-f)**

Table 2. Antimicrobial activity-sensitivity testing of compounds **(4a-f)** and **(5a-f)**

Compound No.	Zone of inhition in mm					
	Antibacterial activity				Antifungal activity	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
4a	10	11	8	8	22	24
4b	13	15	9	9	23	26
4c	10	12	8	9	19	22
4d	12	13	9	9	26	22
4e	11	10	9	9	17	20
4f	10	09	8	8	17	20
5a	09	11	9	9	19	22
5b	10	12	8	8	17	20
5c	10	12	9	9	16	19
5d	11	10	8	8	15	18
5e	10	10	9	9	14	17
5f	09	10	9	9	16	19
Ciprofloxacin	26	26	28	25	-	-
Fluconazole	-	-	-	-	26	25

2-pyrazoline), 7.18 (2H, s, C=S-NH_2), 7.30-7.51 (5H, m, Ar-H), 7.29-7.60 (4H, m, Ar-H). Analysis: for $C_{16}H_{14}ClN_3S$, found % (calculated %): C, 60.81 (60.85); H, 4.45 (4.47); N, 13.29 (13.31).

3-(3-Nitrophenyl-5-phenyl-4,5-dihydropyrazole-1-carbothioamide **(5c)**

IR (KBr, cm^{-1}): 3428, 3058, 1870, 1658, 1218, 814. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.46 (2H, d, CH_2 of 2-pyrazoline), 6.19 (1H, t, CH of 2-pyrazoline), 7.18 (2H, s, C=S-NH_2), 7.30-7.51 (5H, m, Ar-H), 7.95-8.06 (4H, m, Ar-H). Analysis: for $C_{16}H_{14}N_4O_2S$, found % (calculated %): C, 58.85 (58.88); H, 4.29 (4.32); N, 17.14 (17.17).

3-(4-Methoxyphenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide **(5d)**

IR (KBr, cm^{-1}): 3431, 3058, 1868, 1654, 1212, 814. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.42 (2H, d, CH_2 of 2-pyrazoline), 3.62 (3H, s, Ar-OCH_3), 6.19 (1H, t, CH of 2-pyrazoline), 7.18 (2H, s, C=S-NH_2), 7.33-7.41 (4H, m, Ar-H), 7.68-7.14 (4H, m, Ar-H). Analysis: for $C_{17}H_{16}ClN_3OS$, found % (calculated %): C, 59.01 (59.04); H, 4.62 (4.66); N, 12.11 (12.15).

3-(4-Chlorophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide **(5e)**

IR (KBr, cm^{-1}): 3431, 3056, 1872, 1652, 1218, 818. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.42 (2H, d, CH_2 of 2-pyrazoline), 6.19 (1H, t, CH of 2-pyrazoline), 7.18 (2H, s, C=S-NH_2), 7.33-7.41 (4H, m, Ar-H), 7.45-7.60 (4H, m, Ar-H). Analysis: for $C_{16}H_{13}Cl_2N_3S$, found % (calculated %): C, 54.85 (54.86); H, 3.71 (3.74); N, 11.98 (12.00).

3-(3-Nitrophenyl)-5-(4-chlorophenyl)-4,5-dihydropyrazole-1-carbothioamide **(5f)**

IR (KBr, cm^{-1}): 3432, 3056, 1869, 1651, 1218, 818. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.42 (2H, d, CH_2 of 2-pyrazoline), 3.62 (3H, s, Ar-OCH_3), 6.19 (1H, t, CH of 2-pyrazoline), 7.18 (2H, s, C=S-NH_2), 7.33-7.41 (4H, m, Ar-H), 7.95-8.06 (4H, m, Ar-H). Analysis: for $C_{16}H_{13}ClN_4O_2S$, found % (calculated %): C, 53.20 (53.26); H, 3.61 (3.63); N, 15.48 (15.53).

Biological activity

The antimicrobial activity was determined using disc diffusion method (30) by measuring the inhibition zone in mm. All the newly synthesized compounds, i.e. **(4a-f)** and **(5a-f)** were screened *in vitro* for their antibacterial activity against two Gram-positive strains (*Staphylococcus aureus* and *Bacillus subtilis)* and two Gram-negative strains (*Escherichia coli* and *Pseudomonas aeruginosa)* at a concentration of 500 µg/mL. Antifungal activity was tested against *Candida albicans* and *Aspergillus niger* at a concentration of 500 µg/mL. Ciprofloxacin (10 µg/disc) was used as a standard drug for antibacterial screening and fluconazole (10 µg/disc) was used as a standard for antifungal screening. All the

synthesized compounds exhibited moderate antibacterial activities and significant antifungal activities. Each experiment was done in triplicate and the average reading was taken.

RESULTS

Chalcones **(3a-f)** were prepared by following the standard protocol (31) and were reacted with isoniazide to yield [3-(4-phenyl)-5-phenyl-4,5-dihydropyrazol-1-yl](pyridine-4-yl)methanones **(4a-f).** Another series of 3-substituted phenyl-5-substituted phenyl-4,5-dihydro-pyrazole-1-carbothioamides **(5a-f)** was synthesized by reacting the chalcones with thiosemicarbazide, respectively. The synthetic procedure for preparation of title compounds is given in Scheme 1. The assigned structure and molecular formula of the newly synthesized compounds **(4a-f)** and **(5a-f)** were confirmed and supported by ^{1}H NMR and IR data as well as elemental analysis, which was in full agreement with proposed structures. The compounds were screened *in vitro* for their antibacterial and antifungal potential by disc diffusion assay against selected pathogenic bacteria and human pathogenic fungi. The results of antibacterial and antifungal activities expressed in terms of zone of inhibition are reported in Table 2.

DISCUSSION AND CONCLUSION

Some novel 2-pyrazoline derivatives **(4a-f** and **5a-f)** have been synthesized and evaluated for antimicrobial activities. The results of antimicrobial studies of newly synthesized compounds revealed that they possess antibacterial activities to certain extent and significant antifungal activities. Compound **4b** with chloro substitution, was found to be the most potent compound of the series with antifungal activity better than that of the standard drug, i.e. fluconazole, against *A. niger.* It was followed by compound **4d** which depicted equipotent action as that of the same standard drug against *C. albicans*. The rest of compounds have shown moderate activities against tested fungal strains. In general, compounds **4a-f** have depicted more potent activities than compounds **5a-f**. Even though, the synthesized compounds did not exhibit appreciable antibacterial activity, the data reported in this paper may be helpful guide for the medicinal chemists who are working in this area.

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