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SYNTHESIS AND *INVITRO* ANTIMICROBIAL ACTIVITY OF SOME NOVEL 3, 5-DISUBSTITUTED-PHENYL-4, 5-DIHYDRO-1-PHENYL-2-PYRAZOLINE DERIVATIVES

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Abstract: A series of some novel 3-(substituted-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline derivatives(4a-j) have been synthesized by the treatment of 1-(substituted-2-hydroxy-phenyl)-3-(4'-dimethylamino-phenyl)-prop-2-en-1-one (2-hydroxychalcones) (3a-j) with phenyl hydrazine and few drops of glacial acetic acid using ethanol as a solvent by conventional method. In 65-75% yield with high purity, characterizationof newly synthesized compounds was confirmed by the IR, ^{1}HNMR and Mass spectral analysis. All these newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against four different pathogens such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis* and in vitro antifungal activity against *Aspergillus niger*, *Penicillium chrysogenum*, *Fusarium moneliforme* and *Aspergillus flavus*, using Penicillin and Greseofulvin using Peniciline and Greseofulvin as standard drugs by agar cup method and Poison plate method, respectively.

Keywords:2-Hydroxychalcone, phenyl hydrazine, 1-phenyl-2-pyrazoline, antimicrobial activity.

1. INTRODUCTION

In recent time millions of heterocyclic compounds were synthesized due to their specific activity are employed in the treatment of many infectious diseases. Their use in the treatment is attributed to their inherent toxicity of various pathogens. Among a wide range of heterocyclic compounds that have been explored for the development of pharmaceutically important molecules. Most of the heterocyclic compounds are well known due to their biological importance.Out of these Pyrazolines are important five member heterocyclic compounds containing two different nitrogen atoms, out of these two nitrogenfirst nitrogen atom is basic in nature and second nitrogen atom neutral in nature, shows variety of biological applications and various methods have been worked out for the synthesis of pyrazoline derivatives. Several pyrazoline derivatives possess important pharmacological activities [1-2]and therefore they are useful materials in drug research. Moreover pyrazolines have played an important and crucial role in the development of theory in heterocyclic chemistry and also are extensively useful in organic chemistry.Most of thePyrazoline derivativesdisplay a broad spectrum of pharmacological activities such as antibacterial[3], antifungal[4],antimalerial[5],antitubercular[6], cytotoxic[7],anti-inflammatory[8-10], antioxidant[11-12], anticancer[13],anti-candidal[14],anticonvulsant[15]agents. Numerous pyrazoline derivatives have been found to possess considerable biological activities [16-17]. The literature survey shows interesting biological activities of pyrazoline derivatives therefore; our interest to synthesize the new pyrazoline derivatives may have good biological importance. In view of these observations, in the present investigation we report herein, the synthesis of a number of novel 3,5-disubstituted-4, 5-dihydro-1-phenyl-2-Pyrazoline derivatives **(4a-j),** having halogen, hydroxy and

dimethylamino groups with an aim to find new most active antibacterial and antifungal agents.

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Materialsand Methods

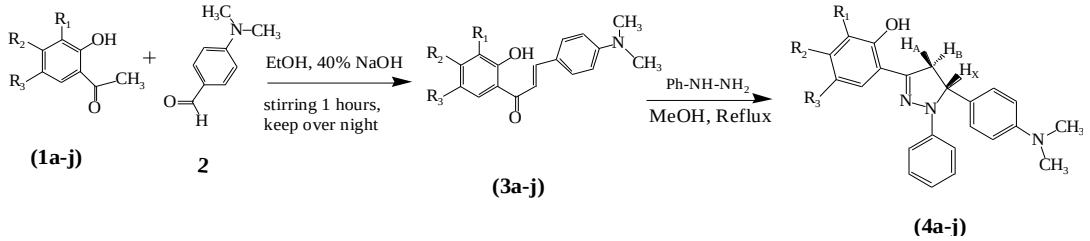
All the solvents and reagents were obtained from commercial sources and were used without further purification. The melting points were determined by Open Capillary method and are uncorrected. The mass spectra were obtained with a Shimadzu GC-MS spectrophotometer. The IR spectra in KBr were recorded on Shimadzu Spectrophotometer and ^{1}HNMR spectra were recorded in DMSO on Avance 300 MHz Spectrometer using TMS as internal standard. The chemical shift values are expressed in part per million (ppm) downfield from the internal standard and signals are quoted as s (singlet), d (doublet), t (triplet) and m (multiplet). Thin-layer chromatography (TLC) was used to monitor the progress of all reactions and to check the purity of compounds by using ethyl acetate and petroleum ether as an eluent in the ratio of (3:7 v/v).

All the newly synthesized 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazolinecompounds were tested for their antimicrobial activities by agar cup method and Poison plate method, respectively.

General method for the Synthesis of 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazolinederivatives

An equimolar reaction mixture of (0.001mol) substituted 2′-hydroxychalcone and phenyl hydrazine (0.001mol) in methanol (20 ml), few drops of glacial acetic acid was refluxed for 8 hours. The progress of the reaction was monitored by using TLC [eluent: ethyl acetate; petroleum ether (3:7)]. After completion of reaction, the reaction mixture was distilled to remove the excess of solvent and the reaction mixture was poured on crushed ice. The solid crude product obtained was filtered, washed with cold water, dried and recrystallized by using ethanol to get corresponding 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazoline compoundsin 65-75 % yield.

Scheme-1:Synthesis of3-(substituted-phenyl)-5-(4'-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazolinederivatives



(1a-j) **2** **(3a-j)** **(4a-j)**

Table-1: Physical data of synthesized3-(substituted-phenyl)-5-(4'-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazolinederivatives (4a-j)

Sr.No.	Entry	R_1	R_2	R_3	Molecular Formula	Molecular weight	Yield in (%)	Melting Point ^{0}C
1	**4a**	Cl	H	Cl	$C_{23}H_{22}OCl_2N_3$	428	75	135-137
2	**4b**	I	H	Cl	$C_{23}H_{22}OClIN_3$	518	65	142-144
3	**4c**	Br	H	Cl	$C_{23}H_{22}OBrClN_3$	472	65	152-154
4	**4d**	Br	H	Br	$C_{23}H_{22}OBr_2N_3$	516	68	137-139
5	**4e**	H	H	Br	$C_{23}H_{23}OBrN_3$	437	75	151-153
6	**4f**	H	H	Cl	$C_{23}H_{23}OClN_3$	392	72	127-129
7	**4g**	Br	CH_3	Cl	$C_{24}H_{24}OBrClN_3$	485	70	136-138
8	**4h**	I	CH_3	Cl	$C_{24}H_{24}O\ IClN_3$	532	66	189-191
9	**4i**	Cl	H	CH_3	$C_{24}H_{24}OClN_3$	405	67	194-196
10	**4j**	Cl	H	Br	$C_{23}H_{22}OBrClN_3$	472	65	111-113

Antimicrobial Activity

In vitro antibacterial activity

All the newly synthesized 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazoline compounds (**4a-j**) were assessed for their antibacterial and antifungal activities against four different strains of bacteria such as *E. coli*, *S typhi*, *S. aureus* and *B. subtilis* and four fungi like *Aspergillus niger*, *Penicillium chrysogenum, Fusarium moneliforme and Aspergillus flavus*. The test for antibacterial activity was carried by agar cup method[18](cup size 8 mm) with nutrient agar as medium where as antifungal activity was carried out by using potato-dextrose agar (PDA) medium by same agar cup plate method. All newly synthesized compounds were dissolved in DMSO and used as control concentration of each test compound was 100µg/ml. The experiments were performed in triplicate in order to minimize the errors. Zone of inhibition were recorded after incubation at 37 ^{0}C for 24 hrs, zone of inhibition produced by each compound was measured in mm.After incubation plates were observed for the zone of inhibition of bacterial growth around the agar cup. Results were recorded by measuring the zone of inhibition in millimeter (mm) using zone reader.

Table No. 2: Antibacterial activity data of 3-(substituted-phenyl)-5-(4'-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazolinederivatives (4a-j)

Sr. No.	Entry	molecular formula	Antibacterial activity (Zone of Inhibition in mm)			
			Escherichia coli	*Salmonella typhi*	*Staphylococcus aureus*	*Bacillus subtilis*
1	**4a**	$C_{23}H_{22}OCl_2N_3$	11	16	22	13
2	**4b**	$C_{23}H_{22}OClIN_3$	12	15	21	13
3	**4c**	$C_{23}H_{22}OBrClN_3$	11	16	--	17
4	**4d**	$C_{23}H_{22}OBr_2N_3$	--	--	20	16
5	**4e**	$C_{23}H_{23}OBrN_3$	--	14	18	--
6	**4f**	$C_{23}H_{23}OClN_3$	12	--	--	14
7	**4g**	$C_{24}H_{24}OBrClN_3$	--	17	21	--
8	**4h**	$C_{24}H_{24}O\ IClN_3$	11	--	--	14
9	**4i**	$C_{24}H_{24}OClN_3$	12	--	18	--
10	**4j**	$C_{23}H_{22}OBrClN_3$	10	--	21	--
+ve Control DMSO			**-ve**	**-ve**	**-ve**	**-ve**
Penicilline			**12**	**20**	**34**	**22**

(-- = No Antibacterial activity

In vitro antifungal activity

The antifungal activity of substituted 3,5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazoline compounds (**4a-j)** were screened against four plant pathogenic and mold fungi, such as *Aspergillus niger, penicillium chrysogenum, Fusarium moneliforme and Aspergillus flavus.* The antifungal activities of the synthesized 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazoline compounds were assessed by poisoned plate method[9]. Griseofulvin (100µg/disc) was used as standard drug for the antifungal test. Potato Dextrose Agar (PDA) was[19] used as basal medium for test fungi. The compound 100 µg were mixed with sterilized potato dextrose agar (PDA) medium at 40 ^{0}C of the rate 100 mg/mL PDA. The medium was poured in sterilized Petri-plates and allowed solidified PDA media and then incubated at 30 ^{0}C for 72 hours. The growth of fungal area was measured in mm after 4 days of incubation at 30 ^{0}C. A control set was maintained using only PDA with DMSO as growth medium. Results were measured as the growth of fungi (does not show antifungal activity), reduced growth of fungi (to observed moderate antifungal activity), and no growth of fungi (antifungal activity observed in the area).

Table No. 3: Antifungal activity data of 3-(substituted-phenyl)-5-(4'-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazolinederivatives (4a-j)

Sr. No.	Entry	molecular formula	Antifungal activity (Zone of Inhibition in mm)			
			Aspergillus niger	*penicillium chrysogenum*	*Fusarium moneliforme*	*Aspergillus flavus*
1	**4a**	$C_{23}H_{22}OCl_2N_3$	-ve	-ve	-ve	-ve
2	**4b**	$C_{23}H_{22}OClIN_3$	-ve	-ve	-ve	-ve
3	**4c**	$C_{23}H_{22}OBrClN_3$	-ve	RG	-ve	RG
4	**4d**	$C_{23}H_{22}OBr_2N_3$	RG	-ve	RG	-ve
5	**4e**	$C_{23}H_{23}OBrN_3$	RG	RG	-ve	-ve
6	**4f**	$C_{23}H_{23}OClN_3$	-ve	-ve	-ve	RG
7	**4g**	$C_{24}H_{24}OBrClN_3$	-ve	-ve	-ve	-ve
8	**4h**	$C_{24}H_{24}O\ IClN_3$	-ve	-ve	-ve	RG
9	**4i**	$C_{24}H_{24}OClN_3$	RG	RG	RG	-ve
10	**4j**	$C_{23}H_{22}OBrClN_3$	-ve	-ve	RG	-ve
+ve Control DMSO			**+ve**	**+ve**	**+ve**	**+ve**
-ve Control (Griseofulvin)			**-ve**	**-ve**	**-ve**	**-ve**

[+ve = No growth (Antifungal activity absent), RG = Reduced Growth (more than 50 % but less than 90 % i. e.Moderate Activity), -ve = No Growth (Antifungal Activity Observed 90 %)]

2. RESULTS AND DISCUSSION:

In the present work a series of some novel 3-(substituted-2-hydroxy-phenyl)-5-(4'-(dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline derivatives(**4a-j**) were synthesized by cyclization of corresponding 3-(4'-dimethylamino-phenyl)-1-(substituted-2-hydroxy-phenyl)-propenone(2-hydroxy-chalcone) derivatives(**3a-j**) and phenyl hydrazine. The uses of different chalcone for the synthesis of pyrazoline derivatives have been investigated. The presence of halogen, hydroxy and dimethylamino groups in different position of benzene ring of the chalcone and the use of phenyl hydrazine resulted in synthesis of new pyrazoline derivatives with significantly high yield.All these product of 1-phenyl-2-pyrazoline derivatives didn't give pink coloration with concentrated H_2SO_4 solution. The structures of newly synthesized compounds have been confirmed by IR, ^{1}H NMR and Mass spectral data. The IR spectrum of compound **4a** exhibited peaks due to group >C=N,Ar-OH and >C=C and C-N 1603 cm^{-1}, 3343 cm^{-1}, 1495 cm^{-1} and 1153 cm^{-1} respectively. Compounds containing the halogen group, such as chloro, bromo and iodo showed an absorption band in the region of 1392–1383 cm^{-1}, 787–756 cm^{-1} and 695–650cm^{-1}, respectively.Its ^{1}H NMR spectrum showed pairs of doublets of doublet in the region δ3.1-3.3 (dd, 1H, CH_A pyrazoline) and δ 3.6-3.8 (dd, 1H, CH_B pyrazoline) respectively, due to germinal and vicinal coupling of CH_2 protons of the pyrazoline ring. Further, the -CH proton of the ring resonated as a doublet of doublets at δ 5.2-5.3 (dd, 1H, CH_X pyrazoline) due to vicinal coupling with the two non-equivalent proton of the methylene group at position C_4 of the pyrazoline ring, absence of singlet at δ 7.0-7.5 due to (N-H) and singlet at δ 11.10-12.30 due to proton of *o*-hydroxyl group. These observations are in agreement with the spectral dataas reported.[20-21]

In vitro antibacterial activity

All these newly synthesized compounds were screened for their antibacterial activity against the selected four different pathogens, such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis*. The antibacterial activity data presented in **Table No. 2** The study revealed that the compounds with two nitrogen atoms in pyrazoline ring and halogen atoms, hydroxyl group on phenyl ring attached at 3-position of pyrazoline ring and 4-dimethylamino-phenyl group on 5-position of pyrazoline exhibited greater antibacterial activity.Among these, the compound 3-(3, 5-dichloro-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4a)**and3-(3-chloro-5-iodo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4b)** showed potent antibacterial activity against all the four strains of bacteria. The compound 3-(3, 5-dichloro-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4a)**, exhibited greater antibacterial activity against *Staphylococcus aureus* as compared to standard with a zone of inhibition of 22

mm.The compounds 3-(3-chloro-5-iodo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline(**4b),** 3-(3-chloro-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4f)**and 3-(5-chloro-3-methyl-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline**(4i)**showed maximum activity against *E. coli*as compared to standard with a zone of inhibition of 12 mm, 12 mm, 12 mm, and compounds of 3-(3, 5-dibromo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline(**4d)**, 3-(5-bromo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline(**4e)** and 3-(5-bromo-3-chloro-4-methyl-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4g)**do not biologically active against *E.coli*. The compounds 3-(3, 5-dichloro-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline(**4a)**, 3-(3-chloro-5-iodo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4b),**3-(5-bromo-3-chloro-4-methyl-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4g)**and3-(3-bromo-5-chloro-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline**(4j)**showed significant activity against *Staphylococcus aureus*,as compared to standard with a zone of inhibition of 22 mm, 21 mm, 21 and 21 mm.while 3-(3-chloro-4-methyl-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline(**4f)**and 3-(3-chloro-5-Iodo-4-methyl-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4h)**do not showed activity in comparison with standard drugs (Penicilin). The compounds containg N-phenyl ring and halogen, hydroxyl group on phenyl ring attached at 3-position of pyrazoline ring and 4′-dimethylamino-phenyl group on 5-position of pyrazoline **4b,4f** and **4i** are significant antibacterial activity against E. coli, as comparison with (peniciline) standard drug. Thus the main structural feature responsible for antibacterial activity is thetwo nitrogen atoms of pyrazoline ring,halogen,hydroxyl and dimethylamino groups substituted in pyrazoline ring. The presence of halogen, hydroxyl and dimethylamino groups resulted in an increase of antibacterial activity of the synthesized pyrazoline compounds.

In vitro antifungal activity

The newly synthesized 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazoline compounds (**4a-j**) were evaluated for their *in vitro* antifungal activity and comparison with standard drugs by the Poison plate method against four different pathogens such as *Aspergillus niger*, *penicillium chrysogenun*, *Fusarium moneliforme* and *Aspergillus flavus*. The antifungal activity data presented in **Table No.3**The study revealed that the newly synthesized 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazoline compounds exhibited good antifungal activity as comparison with (Greseofulvin) standard drugs against four different pathogens.Among these, the compound 3-(3, 5-dichloro-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4a),**3-(3-chloro-5-iodo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (**4b)**and3-(5-bromo-3-chloro-4-methyl-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4g)** showed more than 90% reduction in growth in all the four strains.The compounds 3-(3, 5-dibromo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4d)**, 3-(5-bromo-2-hydroxy-phenyl)-5-(4′-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (**4e)** shows reduced the growth of fungal and other compounds shows moderate activityas compare to zone of inhibition.From these result it is clear that the presence of two nitrogen atoms of pyrazoline ring,N-phenyl, halogen, hydroxyl and dimethylamino groups resulted in an increase of antifungal activity of the synthesized pyrazoline compounds.

Spectroscopic data of synthesized compounds

3-(3, 5-Dichloro-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4, 5-dihydro-1-phenyl-2-pyrazoline (4a);Yield 75 % and M. P**.** 135-137^{0}C ,IR (KBr): 1603 cm-^{1} (C=N), 3343 cm^{-1} (Ar-OH), 1153cm^{-1}(C-N), 772 cm^{-1} (C-Cl);^{1}HNMR (CDCl3): δ 3.0 (s ,6H, 2CH_3), δ 3.15 (dd, 1H, CH_A, pyrazoline), δ 3.63 (dd, 1H, CH_B, pyrazoline), δ 5.23 (dd 1H, CH_X, pyrazoline), δ 6.73-7.12 (m, 11H ,Ar-H), δ11.21 (s, 1H, OH), M.S. (m/z): m+z =428 (M^+).

3-(3-Bromo-5-Chloro-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (4c);Yield 65 % and M.P. 152-154^{0}C, IR (KBr): 1601 cm^{-1} (C=N), 3329 cm^{-1} (Ar-OH), 1134 cm^{-1} (C-N), 765 cm^{-1} (C-Cl); ^{1}H NMR ($CDCL_3$): δ 2.9 (s, 6H, 2CH_3), δ 3.16 (dd, 1H, CH_A, pyrazoline), δ 3.59 (dd, 1H, CH_B, pyrazoline),δ5.19 (dd 1H, CH_X, pyrazoline), δ 6.7-7.5 (m, 11H, Ar-H), δ11.21 (s, 1H, OH); M.S. (m/z): m+1=472(M^+)

3-(3,5-dibromo-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-

pyrazoline (4d);Yield 68 %, and M. P. 137-139^{0}C,IR(KBr): 1598 cm^{-1} (C=N), 3328 cm^{-1} (Ar-OH), 1156 cm^{-1} (C-N) 768 cm^{-1} (C-Br); ^{1}H NMR (CDCl3): δ 3.0 (s, 6H, 2CH$_3$), δ 3.0 (dd, 1H, CH$_A$, pyrazoline), δ 3.60 (dd, 1H, CH$_B$, pyrazoline), δ 5.20 (dd 1H, CH$_X$, pyrazoline), δ 6.7-7.6 (m, 11H, Ar-H), δ 12.1 (s, 1H, OH); M.S. (m/z): m+2 =516 (M$^+$)

3-(5-bromo-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (4e);Yield 75 % and M. P. 151-153^{0}C, IR (KBr): 1597 cm^{-1}(C=N), 3333 cm^{-1}(Ar-OH), 1158 cm^{-1} (C-N) 772 cm^{-1} (C-Br); ^{1}H NMR (CDCL$_3$): δ3.0 (s, 6H, 2CH$_3$), δ3.11 (dd, 1H, CH$_A$, pyrazoline), δ 3.58 (dd, 1H, CH$_B$, pyrazoline), δ5.21 (dd 1H, CH$_X$, pyrazoline),δ 6.69-7.25 (m, 12H, Ar-H),δ11.31 (s, 1H, OH); M.S. (m/z): m+1=437 (M$^+$)

3-(3-Chloro-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (4f); Yield 72 % and M. P. 151-153 ^{0}C, IR (KBr): 1592 cm^{-1}(C=N), 3340 cm^{-1}(Ar-OH), 1162 cm^{-1} (C-N) 772 cm^{-1} (C-Cl); ^{1}H NMR (CDCL$_3$): δ3.01 (s, 6H, 2CH$_3$), δ3.10 (dd, 1H, CH$_A$, pyrazoline), δ 3.52 (dd, 1H, CH$_B$, pyrazoline), δ5.23 (dd 1H, CH$_X$, pyrazoline), δ 6.70-7.50 (m, 12H, Ar-H), δ 11.30 (s, 1H, OH); M.S. (m/z): m+1=392 (M$^+$)

3-(5-bromo-3-Chloro-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (4g); Yield 70 % and M. P. 151-153 ^{0}C, IR (KBr): 1598 cm^{-1}(C=N), 3335 cm^{-1}(Ar-OH), 1169 cm^{-1} (C-N) 687 cm^{-1} (C-Br); ^{1}H NMR (CDCL$_3$): δ 3.02 (s, 6H, 2CH$_3$), δ3.09 (dd, 1H, CH$_A$, pyrazoline), δ 3.62 (dd, 1H, CH$_B$, pyrazoline), δ5.28 (dd 1H, CH$_X$, pyrazoline), δ 6.65-7.50 (m, 12H, Ar-H), δ 11.30 (s, 1H, OH); M.S. (m/z): m+1=485 (M$^+$)

3-(3-Chloro-5-methyl-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (4i); Yield 67 % and M. P. 194-196^{0}C, IR (KBr): 1598 cm^{-1}(C=N), 3342 cm^{-1}(Ar-OH), 1152 cm^{-1} (C-N) 774 cm^{-1} (C-Cl); ^{1}H NMR (CDCL$_3$): δ2.9-3.01 (s, 6H, 2CH$_3$), δ3.10 (dd, 1H, CH$_A$, pyrazoline), δ 3.56(dd, 1H, CH$_B$, pyrazoline),δ5.26(dd 1H, CH$_X$, pyrazoline), δ 6.65-7.35 (m, 12H, Ar-H), δ 11.35 (s, 1H, OH); M.S. (m/z): m+1=405 (M$^+$)

3-(3-Chloro-5-bromo-2-hydroxy-phenyl)-5-(4'-dimethylamino-phenyl)-4,5-dihydro-1-phenyl-2-pyrazoline (4j); Yield 65 % and M. P. 111-113^{0}C IR (KBr): 1595 cm^{-1}(C=N), 3348 cm^{-1}(Ar-OH), 1154 cm^{-1} (C-N) 771 cm^{-1} (C-Cl); ^{1}H NMR (CDCL$_3$): δ3.0 (s, 6H, 2CH$_3$), δ3.08 (dd, 1H, CH$_A$, pyrazoline), δ 3.51(dd, 1H, CH$_B$, pyrazoline), δ5.20(dd 1H, CH$_X$, pyrazoline), δ6.59-7.45 (m, 12H, Ar-H), δ 11.21 (s, 1H, OH); M.S. (m/z): m+1=472 (M$^+$)

3. CONCLUSION

In this work, we have demonstrated the synthesis of 3, 5-disubstituted-4, 5-dihydro-1-phenyl-2-pyrazolinecompounds using simple experimental procedure with high yields, relatively short reaction time, easily work up and low cost.All the synthesized compounds were screened for their *in vitro* antibacterial and antifungal activities. From the result of antibacterial and antifungal activities, it can be concluded that the title compounds and the ring system, presence ofhalogen, hydroxyl, dimethylamino groups and two nitrogen atoms of pyrazoline ring are responsible for the antibacterial and antifungal effects. The obtained results in all these assays during the study will be certainly useful to go for further research for drug designing might provide interesting and additional synthesizing of new effective derivatives.

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Conflict of interest

The authors declareno conflict of interest.

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