Synthesis, *in vitro* antibacterial and antifungal activity of some n-acetylated and non-acetylated pyrazolines

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Abstract: Fourteen new N-acetylated and non-acetylated pyrazoline derivatives were synthesized by reacting chalcones with hydrazine in the presence of absolute ethanol however reaction was carried out in the presence of glacial acetic acid to afford N-acetylated pyrazolines. The chemical structures of the synthesized pyrazolines were confirmed by FTIR, ^{1}HNMR, ^{13}CNMR and mass spectroscopic data. The pyrazolines **(1-14)** were screened for antibacterial activity against ten bacterial strains using seven Gram-positive and three Gram-negative bacteria and antifungal activity against *Aspergillus Flavus, Aspergillus Niger* and *Aspergillus pterus.* Pyrazolines **(1-14)** found to exhibit good to excellent antimicrobial activities compared to the Levofloxacin and fluconazole used as standard drugs.

Keywords: Pyrazolines; antibacterial activity; antifungal activity.

INTRODUCTION

Chalcones are naturally bioactive (Walton and Jean 1945) and are eminent intermediates for synthesizing a variety of heterocyclic compounds. Nitrogen containing heterocycles are commonly establish in beneficial structures (pharmacophores) (Wess and Sickenberger 2001, Muegge 2002, Anies *et al.,* 1994) nevertheless their inclusion occasionally acquire special problems (multistep sequences, lack of simplification, preparation commencing acyclic precursors, etc.); hence, only a restricted number of strategies have been effectively useful in the preparation of heterocyclic scaffolds (Collins 2002, Gordeev *et al.,* 1996, Lorsbach *et al.,* 1998, Munoz *et al.,* 2000, Pelish *et al.,* 2001, Nagashima and Kanematsu 1990). The progress of novel, quick, and sparkling synthetic routes to focused such compounds is consequently of enormous meaning to both medicinal and synthetic chemists (Teague *et al.,* 1999, Armstrong *et al.,* 1996, Stuart 2000).

The combretastatin-A4 an antitumor drug isolated from the African willow tree Combretum caffrum come with drawbacks as a possible antitumor drug; the natural product has limited bioavailability and is poorly soluble in biological media. N-acetylated and non-acetylated pyrazoline analogs of the combretastatins are suitable in terms of biological activity and aqueous solubility and they have an advantage of being readily accessible (Johnson *et al.,* 2007). A number of 3,5-diaryl-4,5-dihydropyrazoles, their 1-acetylated derivatives having a 3,4,5-trimethoxyphenyl moiety combined with a variety of substituted phenyl rings was synthesized and evaluated for antitumor activity. 5-(3,4,5-Trimethoxyphenyl) pyrazoline was found to possess potent antiproliferative activity against SR and MDA-MB-435 with GI_{50} inhibitory values in nanomolar range. Structure activity relationships revealed that introduction of a (hydroxy)acetyl group at N-1 of inactive 5-(3,4,5-trimethoxy-phenyl)pyrazolines results in a clear in vitro activating effect (Congiu *et al.,* 2010). Several pyrazoline derivatives are reported to have anti-inflammatory (Bansal *et al.,* 2001), anticancer (Manna *et al.,* 2005), antidiabetic (Jin *et al.,* 2004) and antidepressant properties (Prasad *et al.,* 2005). N-acetylated pyrazolines are inhibitors of kinesin spindle protein (KSP); potentially helpful for the healing of cancer (Christopher *et al.,* 2005).

Indoloquinoxaline pyrazolines have been synthesized by refluxing chalcones with substituted acid hydrazides as potential antioxidant, anti-inflammatory and antihistamic agents (Sridevi *et al.,* 2011). Pyrazoles are also an important class of five-membered heterocyclic compounds and were found to have potential pharmacological activities. Therefore, a number of synthetic routes for their synthesis have been described in chemistry literature (Sharshira *et al.,* 2011). The most popular method for pyrazolines synthesis is based on the reaction between α, β-unsaturated aldehydes and ketones with hydrazines (Shaharyar *et al.,* 2006,). But, a series of specially substituted derivatives have been synthesized rarely.

Prompted by the above mentioned biological properties of pyrazolines, it was contemplated to prepared new N-acetylated and nonacetylated pyrazoline derivatives. All of the synthesized compounds were evaluated against bacterial and fungal strains.

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MATERIALS AND METHODS

General

Melting points were determined by a digital Gallenkamp (SANYO) model MPD.BM 3.5 equipment and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded in acetone-d_6 at 300MHz and 75MHz respectively with a Bruker spectrophotometer. FTIR spectra were recorded on Perkin Elmer Spectrum BX spectrophotometer as KBr pellets. Bioactivities were carried out at the Riphah Institute of Pharmaceutical Sciences Islamabad, Pakistan. All chemicals used were of analytical grades.

Synthesis of pyrazolines

To a reaction flask containing NaOH (2.5mmol) as a solid was added absolute ethanol (25 mL). A solution of phenyl hydrazine (250 mmol, 10 mL) in absolute ethanol was added into the reaction flask and refluxed the resulting mixture for 3h with continuous stirring. The reaction mixture was allowed to cool to room temperature and quenched with dilute HCl (5mL). The reaction mixture concentrated to dryness in vacuum to afford the product as a solid. The crude solid was recrystallized from a mixture of methanol and n-hexane (Kabli *et al.,* 1992).

Antibacterial activity

In vitro evaluation of antibacterial activity of the pyrazolines **(1-14)** was carried out by agar well diffusion assay against ten different Gram positive and Gram negative bacteria. The seven were Gram negative *viz. Proteus mirabilis, Escherichia coli, Pseudomonas putida, Pseudomonas aeruginosa, Salmonalla typhi, Shigella flexineri* and *Klebsiella pneumoniae* and three were Gram positive *viz Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* (Okeke *et al.,* 2001). Antibacterial activity was determined by using the Mueller Hinton Agar (MHA). The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5Mc-Farland.

A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by 6 mm sized borer to make the wells. The sample dilutions were prepared by dissolving each sample (1.0mg) in 1.0 mL of DMSO used as negative control in this bioassay. The equimolar concentration of Levofloxacin (1.0mg/ml), a broad spectrum antibiotic (positive control) was prepared. These plates were incubated at 37 °C for 24 hours. Antibacterial activity of the pyrazolines **(1-14)** was determined by measuring the diameter of zone of inhibition (mm, ± standard deviation) and presented by subtracting the activity of the negative control in table 2.

Antifungal activity

Antifungal activity of the pyrazolines **(1-14)** was determined by using three fungal strain; *Aspergillus flavus, Aspergillus nigar* and *Aspergillus pterus* using poison plate method (Shastri and Varudkar, 2009). Potato dextrose agar (PDA) plates were equipped by using pour plate technique for each compound. A 2% concentration of the synthesized compounds in DMSO as a solvent was used. A 2% solution of fluconazole was used as standard. A drug free control was included and plates were observed for growth after 48 h of static incubation at 30°C and results are presented in table 3.

RESULTS

In this study, 14 new pyrazolines five of them non acetylated and remaining nine N-acetylated pyrazolines were synthesized. The substituted chalcones were treated with hydrazine (80%) in the presence of absolute ethanol to afford non acetylated pyrazolines **(1-5).** Acetylated pyrazolines **(6-14)** were prepared when chalcones were condensed with hydrazines in the presence of glacial acetic acid Scheme I. Table 1 presented various substituents of acetylated and non acetylated pyrazolines.

This reaction was possibly involved in the transitional structure of hydrazones and successive addition of N-H on the double bond of the propenone moiety. The chemical structures of the synthesized pyrazolines were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The FTIR data were very informative and provided evidence for the formation of the expected structures (table 3). The ^{1}H NMR and ^{13}C NMR spectral data were also consistent with the assigned structures. Mass spectral results showed molecular ion peaks, which were in concurrence with their molecular formula.

Synthesis of non acetylated pyrazolines

1-(5-furan-2-yl)-1-phenyl-4, 5-dihydro-1H-pyrazol-3-yl) phenol (1)

Compound **1** was synthesized by following the general procedure by, furan-2-yl, 2'-hydroxychalcone (0.856g, 4 mmol) and phenyl hydrazine (0.432mL, 4 mmol). Yield 63%, M.p. 138ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 3.64 (dd, J = 7.4 Hz, 4.3 Hz, 2H, 4-CH_2), 6.42-7.63 (m, 11H, Aromatic-H), 5.59 (dd, J=3.9Hz, 1H, 5-CH), 9.10 (s, 1H, OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ (ppm) 161.0 (C-OH), 109.0–145.1 (Aromatic-C), 59.8 (C-5), 37.0 (C-4); HRMS: For calcd $[M^+]$ = $C_{19}H_{16}O_2N_2$ 304.0; found, 304.10 m/z (100%).

1-(1, 5-diphenyl-4, 5-dihydro-1H-pyrazol-3-yl) phenol (2)

Compound **2** was prepared using, 3'-hydroxychalcone (0.896g, 4 mmol) and phenyl hydrazine (0.432mL, 4 mmol). Yield 56%, M.p. 126ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) = 3.69 (dd, J=7.4Hz, 4.3Hz, 2H, 4-CH_2), 6.77-7.50 (m, 14H, Aromatic-H), 5.59(dd, J=3.9Hz, 1H, 5-CH), 9.11 (s, 1H, OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 161.0 (C-OH), 115.0-145.1

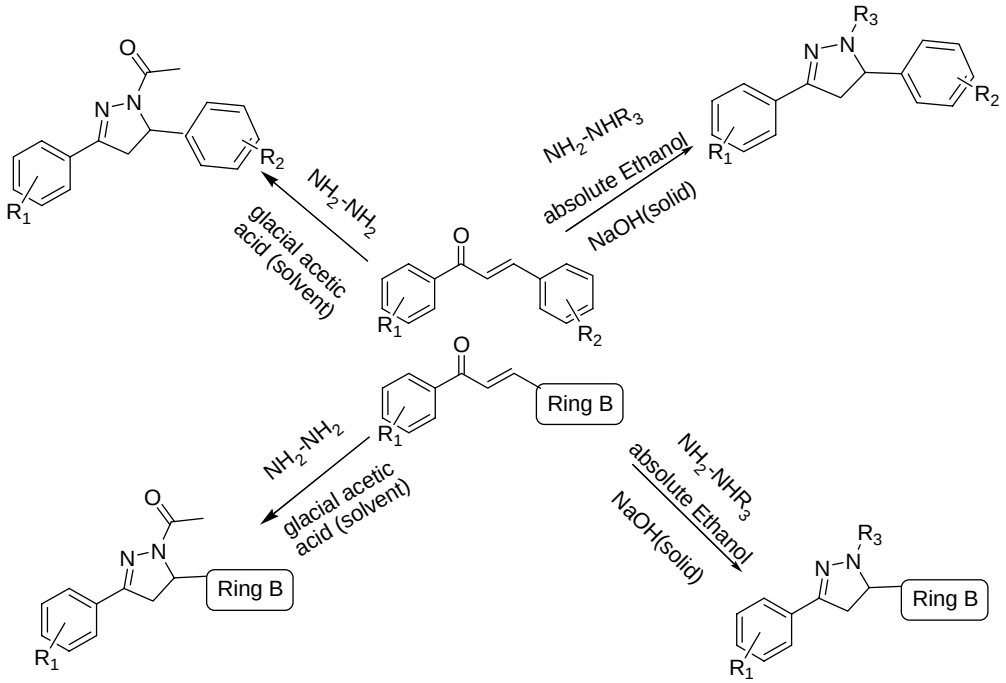
(Aromatic-C), 61.8 (C-5), 40.0 (C-4); HRMS:For calcd $[M^+]$ =$C_{21}H_{18}ON_2$ 314.0; found, 314.10 m/z (100%).

1-(5-(3, 4-dimethoxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl) phenol (3)

Compound **3** was synthesized by the general procedure using, 3, 4 dimethoxychalcone (1.136g, 4 mmol) and hydrazine (0.128mL, 4 mmol). Yield 72%, M.p. 122ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 3.64 (dd, J=7.4 Hz, 4.3 Hz, 2H, 4-CH_2), 3.91 (dd, J =3.8Hz, 1H, 5-CH), 6.74-7.52 (m, 7H, Aromatic-H), 7.1 (d, J=4.7Hz, 1H, N-H), 3.84 (s, 6H, 2-OCH_3), 9.1 (s, 1H,Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 169.0 (C-OH), 125.0–149.1 (Aromatic-C), 65.0 (C-5), 40.0 (C-4); HRMS: For calcd $[M^+]$ =$C_{17}H_{18}O_3N_2$ 270.0; found, 270.10 m/z (100%).

1-(2-(furan-2-yl)-4-(4-hydroxyphenyl) ethanone (4)

Compound **4** was synthesized by the general procedure using, 3-(furan-2-yl), 2'-hydroxychalcone (0.856g, 4mmol) and hydrazine (0.128mL, 4mmol). Yield 84%, M.p. 159ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)=



Scheme I Synthesis of Acetylated and non acetylated pyrazolines **(1-14)**

Table 1: Various substituents of non acetylated and acetylated pyrazolines

Entry (pz)	R_1	R_2	R_3	Ring B
Non-Acetylated Pyrazolies				
1	2-OH	-	C_6H_5	Furan-2-yl
2	3-OH	H	C_6H_5	-
3	2-OH	3,4-diOCH_3	H	-
4	4-OH	-	H	Furan-2-yl
5	4-OH	4-Cl	H	-
Acetylated Pyrazolies				
6	2-OH	H	$OCCH_3$	-
7	2-OH	-	$OCCH_3$	Furan-2-yl
8	4-OH	3-Br	$OCCH_3$	-
9	2-OH	3-Cl	$OCCH_3$	-
10	2-OH	2-OH	$OCCH_3$	-
11	2-OH	4-CH_3	$OCCH_3$	-
12	4-OH	2-F	$OCCH_3$	-
13	4-OH	4-F	$OCCH_3$	-
14	4-OH	3-OCH_3,4-OH	$OCCH_3$	-

Table 2: Antibacterial bioassay screening of Pyrazolines **(1-14)**

Entry	*P. mirabilis*	*B. subtilis*	*E. coli*	*S. aureus*	*P. putida*	*P. aeruginosa*	*S. typhi*	*M. luteus*	*S. flexineri*	*K. pneumoniae*
1	14mm	04mm	10mm	10mm	08mm	10mm	07mm	04mm	05mm	09mm
2	10mm	-	12mm	09mm	10mm	13mm	11mm	08mm	11mm	12mm
3	11mm	07mm	15mm	11mm	15mm	-	10mm	-	-	07mm
4	17mm	08mm	11mm	-	13mm	11mm	13mm	10mm	09mm	14mm
5	16mm	05mm	10mm	15mm	15mm	-	10mm	-	11mm	10mm
6	14mm	-	14mm	07mm	11mm	14mm	14mm	09mm	14mm	15mm
7	10mm	-	11mm	08mm	10mm	15mm	10mm	10mm	10mm	11mm
8	24mm	06mm	13mm	10mm	24mm	22mm	16mm	16mm	23mm	15mm
9	21mm	03mm	12mm	09mm	21mm	24mm	19mm	-	21mm	17mm
10	16mm	10mm	14mm	12mm	16mm	15mm	16mm	14mm	16mm	17mm
11	14mm	08mm	12mm	-	14mm	12mm	14mm	08mm	14mm	16mm
12	26mm	11mm	17mm	15mm	26mm	25mm	17mm	11mm	24mm	18mm
13	22mm	05mm	15mm	10mm	22mm	23mm	15mm	13mm	21mm	15mm
14	17mm	07mm	13mm	13mm	17mm	14mm	17mm	17mm	17mm	19mm
Standard	30mm	25mm	32mm	34mm	36mm	28mm	35mm	40mm	26mm	36mm

(-) No activity,

Table 3: Antifungal bioassay screening of Pyrazolines **(1-14)**

Entry	*Aspergillus flavus*	*Aspergillus niger*	*Aspergillus pterus*
1	12mm	-	15mm
2	11mm	05mm	13mm
3	13mm	04mm	16mm
4	09mm	-	10mm
5	15mm	06mm	15mm
6	19mm	09mm	11mm
7	29mm	18mm	30mm
8	18mm	-	15mm
9	16mm	11mm	17mm
10	17mm	-	14mm
11	31mm	19mm	33mm
12	19mm	-	17mm
13	23mm	13mm	28mm
14	17mm	-	14mm
Standard	37mm	23mm	36mm

(-) No activity

3.64 (dd, J=7.4Hz, 4.3Hz, 2H, 4-CH_2), 3.9 (dd, J=3.8Hz, 1H, 5-CH), 6.42-7.85 (m, 7H, Aromatic-H); 2.84 (s, 3H, CH_3), 9.1 (s, 1H, Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 160.0 (C-OH), 110.0–142.1 (Aromatic-C), 52.0 (C-5), 42.0 (C-4); HRMS: For calcd $[M^+]$ = $C_{17}H_{14}O_2N_2$ 278.0; found, 278.10 m/z (100%).

1-(5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-1-yl)ethanone (5)

Compound **5** was synthesized by the general procedure using, 4-chloro, 4'-hydroxychalcone (1.032g, 4mmol) and hydrazine (0.128mL, 4mmol). Yield 75%, M.p. 192ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) = 3.68 (dd, J=7.7Hz, 4.5Hz, 2H, 4-CH_2), 4.81 (dd, J=3.9Hz, 1H, 5-CH), 6.83-7.86 (m, 8H, Aromatic-H), 9.10 (s, 1H, Ar-OH), 5.1 (d, J=4.8Hz, 1H, N-H), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 160.0 (C-OH), 110.0–142.1 (Aromatic-C), 52.0 (C-5), 42.0 (C-4); HRMS: For calcd $[M^+]$=$C_{15}H_{13}ON_2Cl$, 272.0; found, 272.10 m/z (100%) and [M+2] 274.10 m/z (32%).

Synthesis of acetylated pyrazolines

1-(3-(2-hydroxyphenyl)-5-phenyl-4, 5-dihydro-1H-pyrazol-1-yl) ethanone (6)

Compound **6** was synthesized by the general procedure using, 2'-hydroxychalcone (0.896g, 4 mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL,4mmol); Yield 83%, M.p. 151ºC; ^{1}HNMR (300 MHz, DMSO-d_6): δ(ppm)=3.64 (dd, J=7.4Hz, 4.3Hz, 2H, 4-CH_2), 3.90 (dd, J=3.8Hz, 1H, 5-CH), 6.42-7.85 (m, 9H, Aromatic-H); 2.84 (s, 3H, CH_3), 9.10 (s, 1H, Ar-OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 160.0 (C-OH), 110.0–142.1 (Aromatic-C), 52.0 (C-5), 42.0 (C-4); HRMS: For calcd $[M^+]$ = $C_{17}H_{16}O_2N_2$, 280.0; found, 280.10 m/z (100%).

1-(5-(furan-2-yl)-3-(2-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-1-yl)ethanone (7)

Compound **7** was synthesized by the general procedure using, 2'-hydroxychalcone (0.856g, 4 mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4mmol); Yield 73%, M.p. 147ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)= 3.64 (dd, J=7.5Hz, 4.4Hz, 2H, 4-CH_2), 4.9 (dd, J=4.8Hz, 1H, 5-CH), 6.42-7.75 (m, 7H, Aromatic-H); 2.1 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 163.0 (C-OH), 110.0–141.1 (Aromatic-C), 52.0 (C-5), 42.0 (C-4); HRMS: For calcd [M^+] = $C_{15}H_{14}O_3N_2$, 270.0; found, 270.10 m/z (100%).

1-(5-(3-bromophenyl-3-(4-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-1-yl) ethanone (8)

Compound **8** was synthesized by the general procedure using, 3-bromo, 4'-hydroxy chalcone (0.302g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4mmol); Yield 78%, M.p. 171ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)= 3.67 (dd, J=7.8Hz, 4.5Hz, 2H, 4-CH_2), 4.8 (dd, J=3.8Hz, 1H, 5-CH), 6.42-7.75 (m, 7H, Aromatic-H); 2.2 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 162.0 (C-OH), 110.0–141.1 (Aromatic-C), 52.0 (C-5), 42.0 (C-4); HRMS: For calcd [(M^+] =$C_{17}H_{15}O_2N_2Br$, 358.0; found, 358.10 m/z (100%) and [M+2] 360.10 m/z (97%).

1-(5-(3-chlorophenyl)-3-phenyl-4, 5-dihydro-1H-pyrazol-1-yl) ethanone (9)

Pyrazoline **9** was synthesized by the general procedure using, 3-chloro, 2'-hydroxy chalcone (1.032g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24 mL, 4mmol); Yield 74%, M.p. 160ºC; ^{1}HNMR (300 MHz, DMSO-d_6): δ (ppm)= 3.68 (dd, J=7.8Hz, 4.5Hz, 2H, 4-CH_2), 4.7 (dd, J=3.8Hz, 1H, 5-CH), 6.42-7.75 (m, 8H, Aromatic-H); 2.2 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 164.0 (C-OH), 169(C-N), 112.1–140.3 (Aromatic-C), 51.3(C-5), 43.5 (C-4); HRMS: For calcd [M^+]=$C_{17}H_{15}O_2N_2Cl$, 316.0; found, 316.10 m/z (100%) and [M+2] 318.10 m/z (32%).

1-(3, 5-bis (2-hydroxyphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (10)

Compound **10** was synthesized by the general procedure using, 2, 2'-dihydroxychalcone (0.96g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4mmol); Yield 84%, M.p 164ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)= 3.68 (dd, J=7.8Hz, 4.5Hz, 2H, 4-CH_2), 4.7 (dd, J=3.8Hz, 1H, 5-CH), 6.42-7.75 (m, 8H, Aromatic-H); 2.2 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 163.0 (C-OH), 168 (C-N), 109.3–139.2 (Aromatic-C), 52.0 (C-5), 42.0 (C-4); HRMS: For calcd [M^+] = $C_{17}H_{16}O_3N_2$, 296.0; found, 296.10 m/z (100%).

1-(3-(2-hydroxyphenyl)-5-p-tolyl-4, 5-dihydro-1H-pyrazol-1-yl) ethanone (11)

It was synthesized by the general procedure using, 4-methyl,2'-hydroxy chalcone (0.238g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4mmol); Yield 79%, M.p 155ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)= 3.68 (dd, J=7.7Hz, 4.2Hz, 2H, 4-CH_2), 4.7 (dd, J=3.9Hz, 1H, 5-CH), 6.93-7.53 (m, 8H, Aromatic-H); 2.1 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH), ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 160.0 (C-OH), 168 (C-N), 110.0–139.1 (Aromatic-C), 66.0 (C-5), 41.0 (C-4); HRMS: For calcd [M^+] = $C_{18}H_{18}O_2N_2$, 294.0; found, 294.10m/z (100%).

1-(5-(2-fluorophenyl)-3-(4-hydroxyphenyl)-4,-5-dihydro-1H-pyrazol-1-yl) ethanone (12)

Pyrazoline **12** was synthesized by the general procedure using, 2-fluoro,4'-hydroxy chalcone (0.968g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4 mmol); Yield 84%, M.p 157ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)= 3.68 (dd, J=7.6Hz, 4.5Hz, 2H, 4-CH_2), 4.6 (dd, J=3.8Hz, 1H, 5-CH), 6.93-7.53 (m, 8H, Aromatic-H); 2.1 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 160.0 (C-OH), 168 (C-N), 110.0–139.1 (Aromatic-C), 66.0 (C-5), 41.0 (C-4); HRMS: For calcd [M^+] = $C_{17}H_{15}O_2N_2F$, 298.0; found, 298.10m/z (100%).

1-(5-(4-fluorophenyl)-3-(4-hydroxyphenyl)-4,-5-dihydro-1H-pyrazol-1-yl) ethanone (13)

Compound **13** was synthesized by the general procedure using, 4-fluoro,4'-hydroxy chalcone (0.968g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4 mmol); Yield 72%, M.p. 161ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm)= 3.68 (dd, J=7.9Hz, 4.7Hz, 2H, 4-CH_2), 4.10 (dd, J=4.1Hz, 1H, 5-CH), 6.83-7.86 (m, 8H, Aromatic-H); 2.1 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 160.0 (C-OH), 168 (C-N), 113.0–143.2 (Aromatic-C), 63.4 (C-5), 43.2 (C-4); HRMS: For calcd [M^+] = $C_{17}H_{15}O_2N_2F$, 298.0; found, 298.10m/z (100%).

1-(5-(4-hydrox-3-methoxyphenyl)-3(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethanone (14)

It was synthesized by the general procedure using, 3'-hydroxy,4'-methoxy,4-hydroxychalcone (1.24g, 4mmol), hydrazine (0.128mL, 4mmol) and glacial acetic acid (0.24mL, 4mmol); Yield 84%, M.p. 149ºC; ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm)= 3.67 (dd, J=7.8Hz, 4.2Hz, 2H, 4-CH_2), 4.7 (dd, J=3.8Hz, 1H, 5-CH), 6.81-7.81 (m, 7H, Aromatic-H); 2.1 (s, 3H, $OCCH_3$), 9.1 (s, 1H, Ar-OH); ^{13}C NMR (300 MHz, DMSO-d_6): δ(ppm) 158.2 (C-OH), 168 (C-N), 113.2–138.5 (Aromatic-C), 65.3 (C-5), 42.8 (C-4); HRMS: For calcd [M^+]= $C_{18}H_{18}O_3N_2$, 310.0; found, 310.10m/z (100%).

DISCUSSION

Antimicrobial bioassay

Biological activity of a compound is a function of the nature and extent of interactions between the target site and functional groups of the compound. A range of structural features present in the molecule like electronegativity and hydrophobicity are the important determining factors. Molecules with high HOMO (highest occupied molecular orbital) energies are more proficient to donate their electrons and are good hydrogen bond donors than molecules with low-lying HOMOs; thus HOMO is a measure of the ability to develop hydrogen bonding with target site. However, structure activity relationship cannot be established on the basis of single structural feature.

The antibacterial activity results revealed that pyrazolines **(1-14)** exhibited good bacterial growth inhibition against gram negative strain but all of non acetylated and N-acetylated pyrazolines **(1-14)** are inactive against selected gram positive strains. The growth inhibition is also enhanced after acetylation at N1, the N-acetylated pyrazolines **(6-14)** are more hydrophobic than non acetylated pyrazolines **(1-5)** and also good structural analogs in terms of antibacterial activity and solubility in biological media. The *Salmonalla typhi, Klebsiella pneumonia* and *Escherichia coli* are the most resistant bacterial strains among the tested microorganisms. The compounds **(8, 9, 12 and 13)** which possess halogenated benzene ring showed excellent growth inhibition against *Proteus mirabilis, Pseudomonas putida, Pseudomonas aeruginosa* and *Shigella flexineri.* It reflects that electronegative substituents play important role in antibacterial activity.

All of the synthesized compounds showed moderate to good antifungal activity against the selected fungal strains. The non acetylated pyrazolines showed moderate activity compared to standard but again the acetylated pyrazolines showed good growth inhibition. The *Aspergillus niger* is the most resistant against acetylated and non acetylated pyrazolines derivatives. The compound **(7)** possess heterocyclic moiety, **(11)** hydrophobic p-methylphenyl ring and **(13)** p-flourophenyl ring showed excellent antifungal potential against *Aspergillus flavus* and *Aspergillus pterus.*

CONCLUSION

A novel type of N-acetylated and non acetylated pyrazolines were synthesized by coupling of substituted chalcones with hydrazine. The synthesized compounds were characterized by spectroscopic techniques and evaluated for their *in vitro* antibacterial and antifungal activity. In general acetylated compounds show better antibacterial and antifungal activity than non acetylated. The halogen substituted derivatives have higher antibacterial potential than antifungal. The hydrophobic substituents when present enhance the antifungal activity.

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