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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME SUBSTITUTED 2-PYRAZOLINES SULPHONAMIDE HYBRID COMPOUNDS

Krishna Mehta*, Alok Pal Jain
RKDF Collage of Pharmacy, NH-12, Hoshangabad Road, Bhopal (M.P.)

*Corresponding Author's E mail: *kpdmehta3@gmail.com*

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ABSTRACT
A series of substituted 2-pyrazolines derivatives were synthesized by refluxing from various substituted chalcones and nicotinic acid hydrazide in two steps. The synthesized compounds were characterized by elemental analyses, IR, ^{1}H NMR and Mass spectral data and were evaluated for antibacterial activity against four different bacterial species and antifungal activity against two different fungal species. Some of them have shown significant activity when compared with the standard.

Keywords: Pyrazoline, Antimicrobial, Synthesis, Sulphonamide, Chalcone.

INTRODUCTION

Bacterial infections have increased dramatically in recent years. Bacteria have been the cause of some of the most deadly diseases and widespread epidemics in human civilization. Bacterial diseases such as tuberculosis, typhus, plague, diphtheria, typhoid fever, cholera, dysentery, and pneumonia have taken a high toll on humanity. [1]

The rapid emergence of multidrug resistant pathogenic bacteria has become a serious health threat worldwide. It has been postulated that the development of resistance to known antibiotics could be overcome by identifying new drug targets *via* genomics, improving existing antibiotics and by identifying new antibacterial agents with novel structure and mode of action.[2] Since in the last two decades the incidence of invasive fungal infections has risen sharply, it has become imperative to enlarge the number of antifungal drugs with more potent activity and less toxicity.[3-4] With the increase in

resistance of bacteria to antibiotic treatment, attention has focused on developing novel approaches to antimicrobial therapy.[5-6]

Pyrazolines and their derivatives have been found to possess a broad spectrum of biological activities such as antibacterial[7], antidepressant [7-8] anticonvulsant[9-10] antihypertensive [11] antioxidant [12] antitumor[13] and anticancer activities.[14-15] Recently these classes of compounds are reported to possess potential antiviral activity against flavivirus [16] and HIV [17]

Therefore, a study was initiated to explore the activity of this class of compound. The present work reports the synthesis of new 1,3,5-trisubstituted 2-pyrazolines derivatives and their *in vitro* antibacterial and antifungal screening as a part of our program aimed at the development of new heterocyclic compounds with potential biological activities.

MATERIALS AND METHODS

Material

Experimental

IR spectra were recorded on a FT-IR Shimadzu DZU 8400S spectrophotometer in KBr disks. Elemental analysis were done on a Perkin-Elmer 2400C, H, N analyzer and values were found to be within the acceptable limits of the calculated values. The ^{1}H-NMR spectra of the synthesized compounds in $CDCl_3$/DMSO were recorded at 400 MHz by Bruker Advance II 400 NMR spectrometer. Chemical shift values are given in (ppm) scale using tetramethylsilane (TMS) as an internal standard. Significant ^{1}H-NMR data are written in order: number of protons, multiplicity (b, broad; s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet), coupling constants in Hertz, assignment. The FAB mass spectra (at room temperature) were recorded on TOF MS ES$^+$ mass spectrometer. The melting point of the synthesized compounds were determined using an open capillary and are uncorrected. Progress of reaction and purity of synthesized compounds was ascertained by thin layer chromatography (TLC) using Silica gel G and Iodine vapors as detecting agent.

Chemistry

The synthesis of 1,3,5-trisubstituted pyrazoline derivatives **4 (a-p)** were carried out as outlined in Fig.1 and Table 1. In the first step, syntheses of chalcones **3 (a-p)** were carried out by the well-known Claisen-Schmidt reaction and products were purified by recrystallization from methanol (60–70% yield). In the second step, chalcone and nicotinic acid hydrazide were refluxed in n-butanol in order to synthesize the desired product.

Purity of the compounds was checked on TLC plates (silica gel G) which were visualized by exposing to iodine vapours.

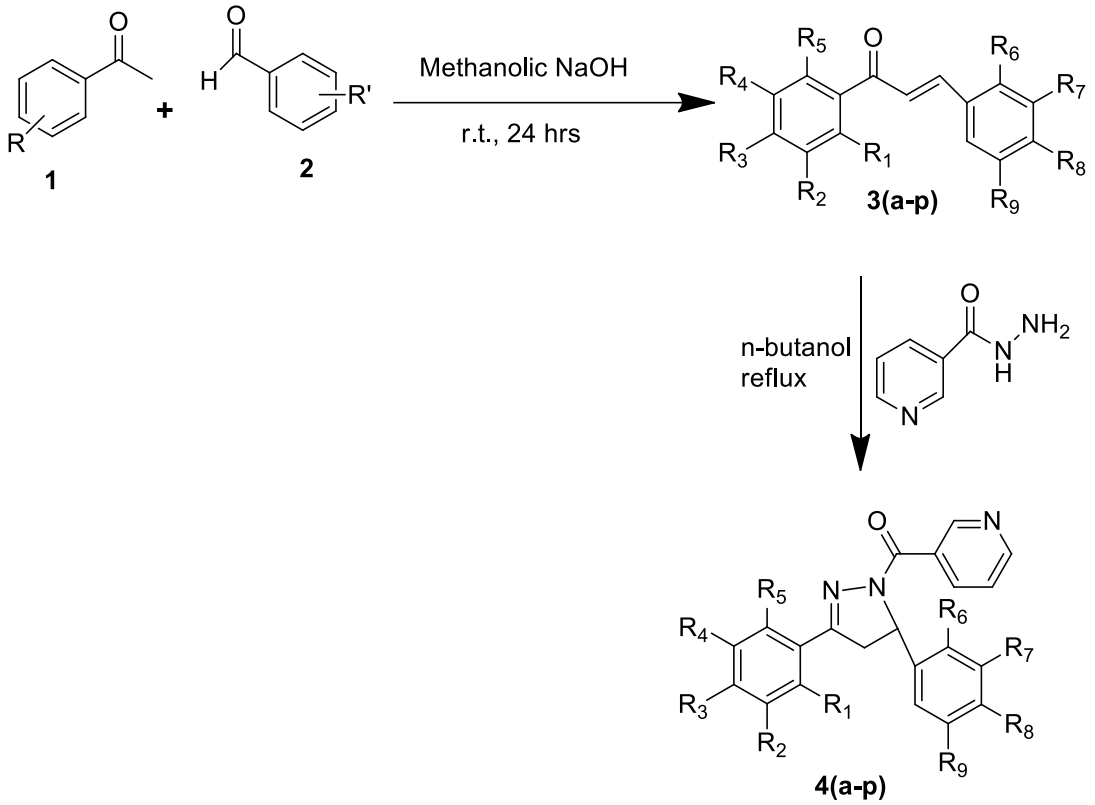

Fig 1. Scheme for the synthesis of 1, 3, 5-trisubstituted pyrazolines 4 (a-p).

Table 1: Different substitutions on synthesized 1, 3, 5-trisubstituted pyrazolines 4 (a-p)

S.No	Comp. No.		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
1	**3a**	**4a**	OH	-	OH	-	-	Cl	-	-	Cl
2	**3b**	**4b**	OH	-	OH	-	-	OCH_3	-	-	Cl
3	**3c**	**4c**	OH	-	OH	-	-	-	OH	-	-
4	**3d**	**4d**	OH	-	OH	-	-	-	OCH_3	OCH_3	-
5	**3e**	**4e**	Cl	-	Cl	-	-	Cl	-	-	Cl
6	**3f**	**4f**	Cl	-	Cl	-	-	OCH_3	-	-	Cl
7	**3g**	**4g**	Cl	-	Cl	-	-	-	OH	-	-
8	**3h**	**4h**	Cl	-	Cl	-	-	-	OCH_3	OCH_3	-
9	**3i**	**4i**	OCH_3	-	-	Cl	-	Cl	-	-	Cl
10	**3j**	**4j**	OCH_3	-	-	Cl	-	OCH_3	-	-	Cl
11	**3k**	**4k**	OCH_3	-	-	Cl	-	-	OH	-	-
12	**3l**	**4l**	OCH_3	-	-	Cl	-	-	OCH_3	OCH_3	-
13	**3m**	**4m**	-	Cl	OCH_3		OH	Cl	-	-	Cl
14	**3n**	**4n**	-	Cl	OCH_3	-	OH	OCH_3	-	-	Cl
15	**3o**	**4o**	-	Cl	OCH_3	-	OH	-	OH	-	-
16	**3p**	**4p**	-	Cl	OCH_3	-	OH	-	OCH_3	OCH_3	-

General method for the synthesis of chalcones (3a-p)

To a solution of substituted acetophenone (16 mmole) in 10 mL of methanol on an ice bath, freshly prepared 2 N methanolic NaOH solution (60 mL) was added and stirred for 10 min. To this, appropriate aldehyde (16 mmole) was added and stirred at room temperature for 12-24 hr. The reaction mixture was cooled on an ice bath, neutralized with diluted HCl and the precipitate was washed three times with 50 mL distilled water to give the crude product. The product was recrystallized from methanol or ethanol/ water. The purity of the product was checked by TLC using ethylacetate and hexane (4:6) as mobile phase and iodine vapors as detecting agent.

3-(2',5'-dichlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (3a)

Synthesized by above method from 2,4-dihydroxyacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 85%, White solid; mp 165–167°C; R_f (EtOAc/Hex 4:6) 0.45; IR (KBr) ν_{max}/cm^{-1} 3440 (O-H), 1669 (C=O), 1597 (Ar C=C), 750 (C–Cl), 3063, 2931, 1625, 1415, 1325, 1296, 1134, 1153, 1046, 982, 759, 737 (Ar) ; ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.62 (2H, s, OH-2,4), 7.76 (1H, d, *J* 16, H-b), 7.69 (2H, dd, *J* 6.8 and 4.5, H-6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 7.21 (4H, m, *J* 4.8, H-3, 5, 3', 4'); FAB-MS *m/z* 308.14 $[M+H]^+$; Anal. Calcd for $C_{15}H_{10}Cl_2O_3$: C, 58.28; H, 3.26. Found: C, 58.98; H, 3.12

3-(5'-chloro-2'-methoxyphenyl)-1-(2,4-hydroxyphenyl)prop-2-en-1-one (3b) Synthesized by above method from 4-Hydoxyacetophenone (16 mmol) and 2-methoxy,5-chlorobenzaldehyde (16 mmol); Yield 70%, yellow crystalline solid; mp 112–114°C; R_f (EtOAc/Hex 4:6) 0.47; IR (KBr) ν_{max}/cm^{-1} 3441 (O-H), 1645 (C=O), 1595 (Ar C=C), 760 (C–Cl), 3060, 2965, 1645, 1434, 1323, 1296, 982, 759 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.60 (2H, s, OH-2,4), 7.76 (1H, d, *J* 15.6, H-b), 7.69-7.60 (4H, m, H-3, 5, 6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 6.81 (2H, dd, *J* 5.2, H-3', 4'), 3.81 (3H, s, OCH_3-2'); FAB-MS *m/z* 304.06 $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}ClO_4$: C, 63.06; H, 4.30; Found: C, 63.41; H, 4.58.

3-(3'-hydroxyphenyl)-1-(2,4-hydroxyphenyl)prop-2-en-1-one (3c)

Synthesized by above method from 4-Hydroxyacetophenone (16 mmol) and 4-hydroxybenzaldehyde (16 mmol); Yield 65%, Yellow solid; mp 124–126°C; R_f (EtOAc/Hex 4:6) 0.36; IR (KBr) ν_{max}/cm^{-1} 3440 (O-H), 1661 (C=O), 1592 (Ar C=C), 750 (C–Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.95 (3H, s, OH-2,4, 3'), 7.71 (1H, d, *J* 15.3, H-b), 7.61-7.54 (4H, m, H-3, 5, 6, 6'), 7.31 (1H, d, *J* 16.0, H-a), 7.21-7.15 (4H, m, *J* 4.8, H-2, 2', 4', 5') FAB-MS *m/z* 256.08 $[M+H]^+$; Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; Found: C, 70.37; H, 4.12;

3-(3', 4'-dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3d)

Synthesized by above method from 4-Hydroxyacetophenone (16 mmol) and 4-hydroxybenzaldehyde (16 mmol); Yield 76%, White solid; mp 108–110°C; R_f (EtOAc/Hex 4:6) 0.34; IR (KBr) ν_{max}/cm^{-1} 3435 (O-H), 1661 (C=O), 1592 (Ar C=C), 760 (C–Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.55 (1H, s, OH-4, 2), 7.73 (1H, d, *J* 15.3, H-b), 7.66-7.55 (4H, m, H-3, 5, 6, 6'), 7.35 (1H, d, *J* 16.0, H-a), 7.25-7.21 (3H, m, *J* 4.4, H-2, 2', 5'), 3.74 (6H, s, OCH_3-3', 4'); FAB-MS *m/z* 300.08 $[M+H]^+$; Anal. Calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37; Found: C, 67.28; H, 5.70;

3-(2', 5'-dichlorophenyl)-1-(2, 5-dichlorophenyl)prop-2-en-1-one (3e)

Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 69%, White crystalline solid; mp 138–140°C; R_f (EtOAc/Hex 4:6) 0.38; IR (KBr) ν_{max}/cm^{-1} 1662 (C=O), 1598 (Ar C=C), 743 (C–Cl), 3064, 2930, 1627, 1415, 1325, 1296, 1134, 1117, 1047, 982, 819, 759, 737 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.76 (1H, d, *J* 15.7, H-b), 7.69 (2H, dd, *J* 6.8 and 4.5, H-6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 7.21-7.15 (4H, m, *J* 4.8, H-3, 4, 3', 4') FAB-MS *m/z*: 345.93 $[M+H]^+$; Anal. Calcd for $C_{15}H_8Cl_4O$: C, 52.06; H, 2.33. Found: C, 52.59; H, 2.29.

3-(5'-chloro-2'-methoxyphenyl)-1-(2,5-dichlorophenyl)prop-2-en-1-one (3f)

Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 5-chloro, 2-methoxybenzaldehyde (16 mmol); Yield 67%, Creamy-coloured fine needles; mp 148–150°C; R_f (EtOAc/Hex 4:6) 0.79; IR (KBr) ν_{max}/cm^{-1} 1662, 1216 (C=O), 1594 (C=C), 1261, 1026 (C–O), 742 (C–Cl), 3061, 1591, 1457, 1384, 1296, 1194, 980, 854, 756 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.74 (1H, d, *J* 15.7, H-b), 7.65 (1H, d, *J* 6.8, H-6), 7.34 (1H, d, *J* 15.9, H-a), 7.26-7.30 (4H, m, H-3, 4, 4', 6'), 6.81 (1H, d, *J* 5.2, H-3'), 3.89 (3H, s, OCH_3-2'); FAB-MS *m/z*: 341.27 $[M+H]^+$; Anal. Calcd for $C_{16}H_{11}Cl_3O_2$: C 56.25, H 3.25 Found C 56.23, H 3.92.

1-(2,5-dichlorophenyl)-3-(3'-hydroxyphenyl)prop-2-en-1-one (3g)

Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); Yield 60%, White amorphous solid; mp 141–144°C; R_f (EtOAc/Hex 4:6) 0.42; IR (KBr) ν_{max}/cm^{-1} 3441 (O-H), 1663 (C=O), 1589 (Ar C=C), 745 (C–Cl), 3060, 2945, 1620, 1320, 1298, 1139, 1153, 1046, 982, 759, 737 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.62 (1H, s, OH-3'), 7.70 (1H, d, *J* 15.7, H-b), 7.61 (2H, dd, *J* 6.5 and 4.4, H-6, 6'), 7.32 (1H, d, *J* 16.0, H-a), 7.21-7.11 (4H, m, H-3, 4, 2', 4', 5'); FAB-MS *m/z* 292.01 $[M+H]^+$; Anal. Calcd for $C_{15}H_{10}Cl_2O_2$: C, 61.46; H, 3.44. Found: C C, 61.98; H, 3.12

1-(2,5-dichlorophenyl)-3-(3',4'-dimethoxyphenyl)prop-2-en-1-one (3h)

Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 3, 4-dimethoxybenzaldehyde (16 mmol); Yield 69%, white amorphous solid; mp 115-118°C; R_f (EtOAc/Hex 4:6) 0.67; IR (KBr) ν_{max}/cm^{-1} 1653 (C=O), 1605 (Ar C=C), 1547 (COC=C), 1261, 1025 (C-O), 1150 (C-Cl), 3051 (Ar C-H), 2932, 2841 (C-H), 1524, 1472, 1147, 969 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.81 (1H, d, *J* 15.5, H-b), 7.75 (1H, d, *J* 8.5, H-6), 7.61 (1H, d, *J* 15.1, H-a), 7.40 (1H, d, *J* 6.8, H-4), 7.15 (1H, dd, *J* 2.3 and 8.5, H-6, 6'), 7.01 (1H, d, *J* 2.3, H-2'), 6.98 (1H, d, *J* 5.1 H-3), 6.84 (1H, d, *J* 8.1, H-5'), 3.82 (6H, s, OCH_3-3', 4'). FAB-MS *m/z* 322.02 [M +H]$^+$; Anal. Calcd for $C_{16}H_{12}Cl_2O_3$: C, 59.46; H, 3.74;. Found: C, 59.23; H, 3.42;

1-(5-chloro-2-methoxyphenyl)-3-(2',5'-dichlorophenyl)prop-2-en-1-one (3i)

Synthesized by above method from 2- methoxy, 5-chloro-acetophenone (16 mmol) and 2, 5-dichlorobenzaldehyde (16 mmol); Yield 66%, Yellow solid; mp 105-107°C; R_f (EtOAc/Hex 4:6) 0.32; IR (KBr) ν_{max} /cm^{-1} 1650 (C=O), 1585 (Ar C=C), 1517 (COC=C), 1268, 1029 (C-O), 1150 (C-Cl), 3058 (Ar C-H), 2933 (C-H), 1619, 1512, 969, 810, (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 7.81 (1H, d, *J* 15.7, H-b), 7.71 (1H, d, *J* 8.3, H-6), 7.60 (1H, d, *J* 15.4, H-a), 7.56 (1H, d, *J* 6.4, H-4), 7.40 (1H, d, *J* 5.9, H-3), 7.10 (1H, dd, *J* 2.6 and 8.4, H-6'), 7.06 (1H, d, *J* 1.9, H-3'), 6.90 (1H, d, *J* 8.8, H-4'), 3.76 (3H, s, OCH_3-2). FAB-MS *m/z* 339.38 [M +H]$^+$; Anal. Calcd for $C_{16}H_{11}Cl_3O_2$: C, 56.25; H, 3.25; Found: C, 56.68; H, 3.39

1-(5-chloro-2-methoxyphenyl)-3-(5'-chloro-2'-methoxyphenyl)prop-2-en-1-one (3j)

Synthesized by above mentioned method A from 2- methoxy, 5-chloroacetophenone (16 mmol) and 2-methoxy, 5-chlorobenzaldehyde (16 mmol); Yield 3.7 g, 69%, Yellow solid; mp 107-109°C; R_f (EtOAc/Hex 4:6) 0.35; IR (KBr) ν_{max} /cm^{-1} 1655 (C=O), 1580 (Ar C=C), 1519 (COC=C), 1264, 1025 (C-O), 1157 (C-Cl), 3052 (Ar C-H), 2930 (C-H), 1611, 1517, 960, 815, (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 7.86 (1H, d, *J* 15.7, H-b), 7.74 (1H, d, *J* 8.3, H-6), 7.61 (1H, d, *J* 15.4, H-a), 7.54 (1H, d, *J* 6.4, H-4), 7.46 (1H, d, *J* 5.9, H-3), 7.10 (1H, dd, *J* 2.6 and 8.4, H-6'), 7.04 (1H, d, *J* 1.9, H-3'), 6.92 (1H, d, *J* 8.8, H-4'), 3.80 (3H, s, OCH_3-2), 3.85 (3H, s, OCH_3-2'), FAB-MS *m/z* 339.38 [M +H]$^+$; Anal. Calcd for $C_{17}H_{14}Cl_2O_3$: C, 60.55; H, 4.18; Found: C, 60.29; H, 4.57

1-(5-chloro-2-methoxyphenyl)-3-(3'-hydroxyphenyl)prop-2-en-1-one (3k)

Synthesized by above method from 2-methoxy,5-chloroacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); Yield 69%, yellow cryastalline solid; mp 135–137°C; R_f (EtOAc/Hex 4:6) 0.34; IR (KBr) ν_{max}/cm^{-1} 3445 (O-H), 1665 (C=O), 1568 (Ar C=C), 748 (C–Cl), 3063, 2941, 1625,

1291, 1134, 1150, 980, 754 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 11.54 (1H, s, OH-2'), 7.71 (1H, d, *J* 15.7, H-b), 7.65 (2H, dd, *J* 6.5 and 4.4, H-6, 6'), 7.30 (1H, d, *J* 16.0, H-a), 7.24-7.15 (4H, m, H-3, 4, 2', 4'), 6.94 (1H, d, *J* 8.0, H-5'); 3.70 (3H, s, OCH_3-2) FAB-MS *m/z* 288.06 $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}ClO_3$: C, 66.56; H, 4.54. Found: C, 66.39; H, 4.40

1-(5-chloro-2-methoxyphenyl)-3-(3',4'-dimethoxyphenyl)prop-2-en-1-one (3l)

Synthesized by above method from 2-methoxy, 5-chloroacetophenone (16 mmol) and 3,4-dimethoxybenzaldehyde (16 mmol); Yield 71%, Pale yellow solid; mp 117-119°C; R_f (EtOAc/Hex 4:6) 0.49; IR (KBr) ν_{max} /cm^{-1} 1645 (C=O), 1589 (Ar C=C), 1512 (COC=C), 1265, 1025 (C-O), 1151 (C-Cl), 3064 (Ar C-H), 2941 (C-H), 1611, 1518, 967, 811 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 7.84 (1H, d, *J* 15.9, H-b), 7.58 (1H, d, *J* 15.6, H-a), 7.54 (1H, d, *J* 6.4, H-4), 7.48 (1H, d, *J* 5.4, H-3), 7.10 (2H, dd, *J* 2.6 and 8.4, H-6,6'), 7.22 (1H, d, *J* 4.3, H-2'), 6.91 (1H, d, *J* 8.1, H-5') 3.79 (3H, s, OCH_3-2), 3.71 (6H, s, OCH_3-3',4').. FAB-MS *m/z* 332.08 $[M+H]^+$; Anal. Calcd for $C_{18}H_{17}ClO_4$: C, 64.97; H, 5.15. Found: C, 64.36; H, 5.45

1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2',5'-dichlorophenyl)prop-2-en-1-one (3m)

Synthesized by above method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 71%, White amorphous solid; mp 94–97°C; R_f (EtOAc/Hex 4:6) 0.67; IR (KBr) ν_{max}/cm^{-1} 3447 (O-H), 1660 (C=O), 1596 (Ar C=C), 740 (C–Cl), 3064, 2965, 1647, 1434, 1320, 980, 759 (Ar, CH_3); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 11.57 (1H, s, OH-2), 7.75 (1H, d, *J* 15.5, H-b), 7.68 (2H, dd, *J* 6.8, 7.8 , H-6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 7.62-7.53 (2H, m, H-4', 2), 7.41-7.23 (2H, m, H-3',6'), 6.71 (1H, d, *J* 8.1, H-5), 2.31 (3H, s, CH_3-4); FAB-MS *m/z* 339.98 $[M+H]^+$; Anal. Calcd for $C_{16}H_{11}Cl_3O_2$: C, 56.25; H, 3.25; Found: C, 56.54; H, 3.65.

1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(5'-chloro-2'-methoxyphenyl)prop-2-en-1-one (3n)

Synthesized by above method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2-methoxy, 5-dichlorobenzaldehyde (16 mmol); Yield 68%, White solid; mp 137–139°C; R_f (EtOAc/Hex 4:6) 0.48; IR (KBr) ν_{max}/cm^{-1} 3431 (O-H), 1657 (C=O), 1586 (Ar C=C), 760 (C–Cl), 3064, 2964, 1643, 1434, 985, 753 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 10.35 (1H, s, OH), 7.70 (1H, d, *J* 15.2, H-b), 7.69 (2H, dd, *J* 6.8, 7.8 , H-6', 2), 7.32 (1H, d, *J* 16.0, H-a), 7.24 (1H, d, *J* 4.2, H-5), 6.82 (2H, dd, *J* 5.3,7.1 H-3', 4'), 2.84 (3H, s, OCH_3-2'), 2.34 (3H, s, CH_3-4); FAB-MS *m/z* 336.03 $[M+H]^+$; Anal. Calcd for $C_{17}H_{14}Cl_2O_3$: 60.55; H, 4.18;; Found: C, 60.67; H, 4.38.

1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(3'-hydroxyphenyl)prop-2-en-1-one (3o)

Synthesized by above mentioned method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); Yield 69%, yellow solid; mp 183–185°C; R_f (EtOAc/Hex 4:6) 0.31; IR (KBr) ν_{max}/cm^{-1} 3445 (O-H), 1665 (C=O), 1568 (Ar C=C), 760 (C–Cl), 3063, 2941, 1625, 1291, 1134, 1150, 980, 754 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.54 (1H, s, OH), 7.71 (1H, d, *J* 15.7, H-b), 7.65 (2H, dd, *J* 6.5 and 4.4, H-6, 6'), 7.30 (1H, d, *J* 16.0, H-a), 7.24 (4H, m, *J* 4.5, H-3, 4, 2', 4'), 6.94 (1H, d, *J* 8.0, H-5'), 2.37 (3H, s, CH_3-4); FAB-MS *m/z* 288.38 $[M+H]^+$; Anal. Calcd for $C_{16}H_{13}ClO_3$: C, 66.56; H, 4.54; Found: C, 63.29; H, 4.73

1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(3',4'-dimethoxyphenyl)prop-2-en-1-one (3p)

Synthesized by above mentioned method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 3,4-methoxy benzaldehyde (16 mmol); Yield 78%, white solid; mp 123-125°C; R_f (EtOAc/Hex 4:6) 0.76; IR (KBr) ν_{max} /cm^{-1} 1650 (C=O), 1589 (Ar C=C), 1517 (COC=C), 1265, 1024 (C-O), 1155 (C-Cl), 3055 (Ar C-H), 2939 (C-H), 1612, 1519, 975, 818, (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.82 (1H, d, *J* 16, H-b), 7.54 (1H, d, *J* 16, H-a), 7.54 (1H, d, *J* 6.4, H-4), 7.48 (1H, d, *J* 5.4, H-3), 7.10 (2H, dd, *J* 2.6 and 8.4, H-6, 6'), 7.22 (1H, d, *J* 4.3, H-2'), 6.91 (1H, d, *J* 8.1, H-5'), 3.70 (6H, s, OCH_3-3', 4'), 2.32 (3H, s, CH_3-4). FAB-MS *m/z* 332.07 $[M+H]^+$; Anal. Calcd for $C_{18}H_{17}ClO_4$: C, 64.97; H, 5.15; Found: C, 64.23; H, 5.67

General procedure for the synthesis of 1, 3, 5- trisubstituted pyrazolines (4a-4p)

To the solution of the appropriate chalcone **4a – 4p** (4 mmole) in 10 mL of *n*-butanol, (0.55 g, 4 mmole) of nicotinic acid hydrazide was added and the reaction mixture was refluxed for 8–10 hr. The excess of solvent was removed under reduced pressure and the reaction mixture was cooled on an ice bath. The products precipitated out at low temperature were washed five times with 50 mL distilled water, reconstituted in minimum amount of methanol and dried under reduced pressure (Kini and Gandhi, 2008). This product was further purified by crystallization from the ethanol-DMF mixture (1:1). Purity of the products was checked by TLC using mixture of acetone and petroleum ether (40:60 V/V) as mobile phase.

(5-(2',5'-dichlorophenyl)-3-(2",4"-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4a)

Synthesized by above method from chalcone **3a** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 19h reflux; Yield 58%, Pale yellow solid; mp 137-139°C; IR (KBr) ν_{max}/cm^{-1} 3221 (O-H), 1665 (N-

C=O), 1596 (Ar C=C), 1560 (C=N stretching), 1260, 1091 (C–O), 1320, 1215 (C-N), 1107, 777 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.05 (1H, s, 2", 4"-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 7.90 (1H, d, *J 12.3* H-6"), 7.59-7.55 (2H, m, H-11, 4'), 7.43-7.39 (2H, m, H-3', 6'), 6.80 (2H, d, *J* 7.6, H-3", 5"), 5.92 (1H, dd, *J 12.3* and 6.2, H-5), 3.89 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.10 (1H, dd, *J 17.8* and 4.8, 4-H_x); FAB-MS *m/z*: 427.45 $[M+H]^+$; Anal. Calcd for $C_{21}H_{15}Cl_2N_3O_3$: C, 58.89; H, 3.53; N, 9.81; Found: C, 58.54; H, 3.57; N, 9.32;

(5-(5'-chloro-2'-methoxyphenyl)-3-(2",4"-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4b)

Synthesized by above method from chalcone **3b** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 65%, White solid; mp 145-147°C; IR (KBr) ν_{max}/cm^{-1} 3440 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C–O), 1215 (C-N), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.05 (1H, s, 2",4"-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 7.86 (1H, dd, *J 12.3* H-6"), 7.23 (1H, dd, *J* 7.4 and 3.2, H-4', 6'), 6.85-6.89 (3H, m, H-3', 3", 5"), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.70 (3H, s, OCH_3-2"), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H_x); FAB-MS *m/z*: 407.34 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}ClN_3O_4$: C, 62.34; H, 4.28; N, 9.91 Found: C, 62.50; H, 4.41; N, 9.21

(5-(3'-hydroxyphenyl)-3-(2",4"-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4c)

Synthesized by method C from chalcone **3c** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 8h reflux; Yield 68%, Pale yellow solid; mp 165-167°C; IR (KBr) ν_{max}/cm^{-1} 3421 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C–O), 1215 (C-N), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.05 (3H, s, 2",4", 3'-OH), 9.02 (1H, s, 8-H), 8.72 (1H, d, *J* 3.5, 10-H), 8.22 (1H, d, *J* 7.4, 12-H), 7.87 (1H, dd, *J 12.* H-6"), 7.59 (1H, d, *J* 7.6 H-11), 7.24 (1H, d, *J* 4.4, H-5'), 6.99 (1H, d, *J* 7.6, H-2'), 6.85-6.87 (4H, m, H-4',6', 3", 5"), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H_x); FAB-MS *m/z*: 375.76 $[M+H]^+$; Anal. Calcd for $C_{21}H_{17}N_3O_4$: C, 67.19; H, 4.56; N, 11.19 Found: C, 67.78; H, 4.53; N, 11.64

5-(3',4'-Dimethoxyphenyl)-3-(2",4"-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4d)

Synthesized by method above from chalcone **3d** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 69%, Light yellow solid; mp 156-159°C; IR (KBr) ν_{max}/cm^{-1} 3415 (O-H), 1668 (N-C=O), 1591 (Ar C=C), 1560 (C=N), 1262, 1096 (C–O), 1210 (C-N), 1102 (C–Cl), 3041, 2954 (C-H), 1501, 1467, 922, 815, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.05 (1H, s, 2",4"-OH), 9.02 (1H, s, 8-H), 8.70 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 7.82 (1H, dd, *J 12.3* H-6"), 6.89 (1H, d, 3.2, H-2'), 6.83-6.86 (3H, m, H-5', 3", 5"), 6.89 (1H, dd, J 6.7 and 3.2, H-6'), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.70 (3H, s, OCH_3-3', 4'), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H_x); FAB-MS *m/z*: 419.31 $[M+H]^+$; Anal. Calcd for $C_{23}H_{21}N_3O_5$: C, 65.86; H, 5.05; N, 10.02; Found: C, 65.39; H, 5.18; N, 10.37

(5-(2', 5'-dichlorophenyl)-3-(2", 5"-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4e)

Synthesized by above mentioned method from chalcone **3e** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 10h reflux; Yield 59%, Brown solid; mp: 189-191°C; IR (KBr) ν_{max}/cm^{-1} 1658 (N-C=O), 1587 (Ar C=C), 3083 (Ar C-H), 2934 (C-H), 1637, 1496 (C=N), 817, 738 (Ar CH bend); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.10 (1H, s, H-8), 8.75 (1H, d, *J* 4.5, H-10), 8.11 (1H, d, *J* 7.4, H-12), 7.72 (3H, m, H-6", 6', 11), 7.41-7.52 (4H, m, H-3',4',3",4"), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 12.5, 4-H_y), 3.08 (1H, dd, *J* 17.5 and 5.1, 4-H_x); FAB-MS *m/z*: 464.96 $[M+H]^+$; Anal. Calcd for $C_{21}H_{13}Cl_4N_3O$: C 54.22, H 2.82, N 9.03. Found: C 54.40, H 2.67, N 9.54.

(5-(5'-chloro-2'-methoxyphenyl)-3-(2",5"-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4f)

Synthesized by above mentioned method from chalcone **3f** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 70%, Brown solid; mp: 195-197°C; IR (KBr) ν_{max}/cm^{-1} 1658 (N-C=O), 1587 (Ar C=C), 3083 (Ar C-H), 2934 (C-H), 1637, 1496 (C=N), 817, 738 (Ar CH bend); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.02 (1H, s, 8-H), 8.71 (1H, d, *J* 3.5, 10-H), 8.25 (1H, d, *J* 7.4, 12-H), 7.84 (1H, d, *J* 6.5, H-6"), 7.53-7.48 (3H, m, H-11, 3', 4'), 7.36 (1H, d, *J* 7.1 H-6'), 7.22 (1H, dd, *J 8.3* and 6.4, H-4'), 6.85 (1H, dd, *J 6.3* and 6.2, H-3'), 5.92 (1H, dd, *J 12.3* and 6.2, H-5), 3.90 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.81 (3H, s, OCH_3-2'), 3.15 (1H, dd, *J 17.8* and 4.8, 4-H_x); FAB-MS *m/z*: 459.96 $[M+H]^+$; Anal. Calcd for $C_{22}H_{16}Cl_3N_3O_2$: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.42; H, 3.29; N, 9.48

(3-(2",5"-dichlorophenyl)-5-(3'-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4g)

Synthesized by method from chalcone **3g** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 14 hrs reflux; Yield 67%, Pale yellow solid; mp 165-167°C; IR (KBr) ν_{max}/cm^{-1} 3414 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 10.02 (1H, s, 3'-OH), 9.02 (1H, s, 8-H), 8.73 (1H, d, *J* 3.7, 10-H), 8.16 (1H, d, *J* 7.1, 12-H), 7.48 (2H, d, *J* 4.4, H-3", 4''), 7.68 (2H, d, *J* 7.6, H-6", 11), 7.22 (1H, dd, *J 8.1* and 6.2, H-4'), 7.01 (1H, d, *J* 5.1, H-2'), 6.83-6.78 (2H, m, H-4', 6'), 5.95 (1H, dd, *J 12.1* and 6.8, H-5), 3.83 (1H, dd, *J 17.7* and 11.6, 4-H_y), 3.18 (1H, dd, *J 17.1* and 4.3, 4-H_x); FAB-MS *m/z*: 412.54 $[M+H]^+$; Anal. Calcd for $C_{21}H_{15}Cl_2N_3O_2$: C, 61.18; H, 3.67; N, 10.19. Found: C, 61.01; H, 3.97; N, 10.74

(3-(2",5"-dichlorophenyl)-5-(3',4'-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4h)

Synthesized by method above from chalcone **3h** (4 mmol) and nicotinic acid hydrazide (4 mmol); 68%, white solid; mp 178-180°C; IR (KBr) ν_{max}/cm^{-1} 1660 (N-C=O), 1596 (Ar C=C), 1560 (C=N), 1260, 1092 (C–O), 1215 (C-N), 1108, 776 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 11.05 (1H, s, 4'-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.16 (1H, d, *J* 7.2, 12-H), 7.58 (2H, dd, *J* 7.6 & 6.2, H-11), 7.48 (2H, d, *J* 4.8, H-3", 4''), 6.87-6.70 (3H, m, H-2', 5', 6'), 5.93 (1H, dd, *J 12.3* and 6.2, H-5), 3.82 (1H, dd, *J 17.1* and 11.2, 4-H_y), 3.82 (6H, s, OCH_3-3', 4'), 3.10 (1H, dd, *J 17.8* and 4.8, 4-H_x); FAB-MS *m/z*: 455.48 $[M+H]^+$; Anal. Calcd for $C_{23}H_{19}Cl_2N_3O_3$: C, 60.54; H, 4.20; N, 9.21 Found: C, 60.94; H, 4.76; N, 9.63

(3-(5"-chloro-2"-methoxyphenyl)-5-(2',5'-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4i)

Synthesized by above mentioned method from chalcone **3i** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 13 hrs reflux; Yield 63%, Light-yellow solid; mp 142-145°C; IR (KBr) ν_{max}/cm^{-1} 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.80 (H, s, H-6"), 7.56-7.60 (2H, m, H-4', 11), 7.37-7.43 (3H, m, H-3', 6', 4"), 6.99 (1H, d, *J* 5.1, H-3'), 5.95(1H, dd, *J* 10.5 and 6.1, H-5), 3.90 (1H, dd, *J* 17.3 and 6.1, 4-H_y), 3.82 (3H, s, OCH_3-2"), 3.10 (1H, dd, *J* 17.5 and 8.5, 4-H_x); FAB-

MS *m/z*: 459.37 $[M+H]^+$; Anal. Calcd for $C_{22}H_{16}Cl_3N_3O_2$: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.86; H, 3.55; N, 9.16;

(3-(5"-chloro-2"-methoxyphenyl)-5-(5'-chloro-2'-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4j)

Synthesized by above mentioned method from chalcone **3j** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 62%, Light-yellow solid; mp 152-155°C; IR (KBr) ν_{max}/cm^{-1} 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.72 (2H, t, *J* 8.3, H-6", 11), 7.32-7.38 (4H, m, H-3", 4", 4', 6'), 6.83 (1H, d, *J* 5.5, H-3'), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 6.5, 4-H_y), 3.87 (6H, s, OCH_3-2',2"), 3.08 (1H, dd, *J* 17.5 and 8.1, 4-H_x); FAB-MS *m/z*: 456.52 $[M+H]^+$; Anal. Calcd for $C_{23}H_{19}Cl_2N_3O_3$: C, 60.54; H, 4.20; N, 9.21; Found: C, 60.13; H, 4.19; N, 9.56.

(3-(5"-chloro-2"-methoxyphenyl)-5-(3'-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4k)

Synthesized by method from chalcone **3k** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 58%, Pale yellow solid; mp 173-175°C; IR (KBr) ν_{max}/cm^{-1} 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2950 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.04 (1H, s, 3'-OH), 9.04 (1H, s, 8-H), 8.69 (1H, d, *J* 3.9, 10-H), 8.18 (1H, d, *J* 7.2, 12-H), 7.82 (2H, d, *J* 7.6, H-6"), 7.61 (1H, dd, *J* 12.6 and 6.4, H-11), 7.36 (1H, d, *J* 7.1, H-3"), 7.25 (1H, t, *J* 7.6, H-5'), 6.99-7.04 (2H, m, H-2', 3"), 6.75-6.87 (2H, m, H-4', 6'), 5.93 (1H, dd, *J* 12.3 and 6.2, H-5), 3.89 (1H, dd, *J* 17.5 and 11.6, 4-H_y), 3.81 (3H, s, OCH_3-2"), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H_x); FAB-MS *m/z*: 407.29 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}ClN_3O_3$: C, 64.79; H, 4.45; N, 10.30 Found: C, 64.34; H, 4.65; N, 10.15

(3-(5"-chloro-2"-methoxyphenyl)-5-(3',4'-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4l)

Synthesized by method from chalcone **3l** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 67%, Pale yellow solid; mp 193-195°C; IR (KBr) ν_{max}/ cm^{-1} 1668 (N-C=O), 1594 (Ar C=C), 1265, 1090 (C–O), 1560 (C=N), 1219 (C-N), 1101 (C–Cl), 3049, 2953 (C-H), 1501, 1468, 920, 816, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.04 (1H, s, 8-H), 8.69 (1H, d, *J* 3.9, 10-H), 8.18 (1H, d, *J* 7.2, 12-H), 7.66 (2H, d, *J* 7.6, H-6", 11-H), 6.85-6.90 (4H, m, H-2", 3', 4', 6'), 5.93 (2H, dd, *J 12.3* and 6.2, H-5, 5"),

3.89 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.80 (3H, s, OCH_3-2'), 3.85 (6H, s, OCH_3-3", 4"), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H_x); FAB-MS *m/z*: 451.13 $[M+H]^+$; Anal. Calcd for $C_{23}H_{20}ClN_3O_4$: C, 63.79; H, 4.91; N, 9.30; Found: C, 63.12; H, 4.47; N, 9.67

(3-(5"-chloro-2"-hydroxy-4-methylphenyl)-5-(2',5'-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4m)

Synthesized by above method from chalcone **3m** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 62%, Light-yellow powder; mp 179-181°C; IR (KBr) ν_{max}/cm^{-1} 3218 (O-H), 1641 (N-C=O), 1623, 1574 (C=N), 1591 (Ar C=C), 1255, 1024 (C–O), 1125 (C–Cl), 2913 (C-H), 1471, 1320, 1239 (C-N), 945 (trans ethylenic H), 822, 764 (Ar C-H bend); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 10.04 (1H, s, 6"-OH), 9.10 (1H, s, H-8), 8.72 (1H, d, *J* 4.3, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.62-7.56 (3H, m, H-11, 4', 2"), 7.39-7.42 (2H, m, H-3', 6'), 6.40 (1H, s, H-5"), 5.99 (1H, dd, *J* 10.3 and 6.3, H-5), 3.91 (1H, dd, *J* 17.1 and 6.4, 4-H_y), 2.85 (3H, s, CH_3-4), 3.11 (1H, dd, *J* 16.5 and 8.5, 4-H_x); FAB-MS *m/z*: 459.03 $[M+H]^+$; Anal. Calcd for $C_{22}H_{16}Cl_3N_3O_2$: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.74; H, 3.27; N, 9.56

(3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(5'-chloro-2'-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4n)

Synthesized by above mentioned method from chalcone **3n** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 42%, Light-yellow solid; mp 169-172°C; IR (KBr) ν_{max}/cm^{-1} 3215 (O-H), 1649 (N-C=O), 1622, 1585 (C=N), 1590 (Ar C=C), 1252, 1012 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 11.10 (1H, s, 6"-OH), 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.76 (2H, t, *J* 8.3, H-6', 11), 7.38-7.42 (4H, m, H-3', 4', 2", 5"), 5.95 (1H, d, *J* 10.2 H-5), 3.98 (1H, dd, *J* 17.2 and 6.5, 4-H_y), 3.85 (6H, s, OCH_3-4",2'), 3.03 (1H, dd, *J* 17.5 and 8.1, 4-H_x), 2.85 (3H, s, CH_3-4); FAB-MS *m/z*: 455.08 $[M+H]^+$; Anal. Calcd for $C_{23}H_{19}Cl_2N_3O_3$: C, 60.54; H, 4.20; N, 9.21; Found: C, 60.63; H, 4.84; N, 9.53

(3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(3'-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4o)

Synthesized by above method from chalcone **3o** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 59%, Pale yellow solid; mp 127-129°C; IR (KBr) ν_{max}/cm^{-1} 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2950 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ(ppm) 10.05 (2H, s, 6', 3"-OH), 9.09 (1H, s, 8-H), 8.63 (1H, d, *J* 3.9, 10-H), 8.10 (1H, d, *J* 7.2, 12-H), 7.65-7.60 (2H, m, H-2", 11), 6.78-6.84 (2H, m, H-4', 6'), 7.20-7.05 (2H,

m, H-2', 5'), 6.43 (1H, s, H-5"), 5.95 (1H, dd, *J* 12.5 and 6.5, H-5), 3.87 (1H, dd, *J* 17.6 and 11.6, 4-H_y), 3.80 (3H, s, OCH_3-4'), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H_x), 2.32 (3H, s, CH_3-4); FAB-MS *m/z*: 407.58 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}ClN_3O_3$: C, 64.79; H, 4.45; N, 10.30 Found: C, 64.19; H, 4.95; N, 10.73

(3-(5"-chloro-2"-hydroxy-4"-methoxyphenyl)-5-(3',4'-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4p)

Synthesized by above method from chalcone **3p** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 55%, Pale yellow powder; mp 187-190°C; IR (KBr) ν_{max}/cm^{-1} 3227 (O-H), 1668 (N-C=O), 1594 (Ar C=C), 1265, 1090 (C–O), 1219 (C-N), 1101 (C–Cl), 3049, 2953 (C-H), 1501, 1468, 920, 816, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 9.04 (1H, s, 8-H), 8.64 (1H, d, *J* 3.9, 10-H), 8.19 (1H, d, *J* 7.2, 12-H), 7.64-7.60 (2H, m, H-2', 11), 6.84-6.90 (3H, m, H-2', 5', 6'), 5.93 (1H, dd, *J 12.3* and 6.2, H-5) 3.89 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.85 (6H, s, OCH_3-3', 4'), 3.80 (3H, s, OCH_3-4"), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H_x); FAB-MS *m/z*: 451.13 $[M+H]^+$; Anal. Calcd for $C_{24}H_{22}ClN_3O_5$: C, 61.61; H, 4.74; N, 8.98; Found: C, 61.12; H, 4.50; N, 8.45

***In vitro* antimicrobial activity**

All the synthesized compounds **4(a-p)** were screened *in vitro* against representatives of two gram positive bacteria *Staphylococcus aureus*, and *Bacillus subtilis* and two gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* by disk diffusion method. The test organisms were first cultured in Nutrient broth and incubated for 24 hrs at 37^0C and then freshly prepared bacterial cells were spread onto the Muller Hinton agar plates in a laminar flow cabinet. The test compounds which were previously dissolved in DMF at concentration of 50µg /mL, 100µg/mL and 150µg/mL were then soaked onto sterile disks of Whatman filter paper (5 mm diameter). The disks were then placed onto the surface of the previously prepared inoculated plates and incubated. After 24 hrs of incubation at 37^0C, the diameter of zone of inhibition was measured for each concentration in mm as shown in table. The activity was compared with standard antibiotic norfloxacin (positive control) and a disk impregnated with DMF was used as a negative control.

Antifungal assay: The synthesized compounds **4(a-p)** were screened *in vitro* against strains of *candida albicans* and *Aspergillus niger* by disk diffusion technique. The Potato Dextrose Agar medium was for culturing of microbes and the freshly prepared fungal spores were spread onto the plates of same media in a laminar flow cabinet. The test compounds which were previously dissolved in DMF at concentration of 50µg /mL, 100µg/mL and 150µg/mL were then soaked onto sterile disks of Whatman filter paper (5

mm diameter). The disks were then placed onto the surface of the previously prepared inoculated plates and incubated for 48 hrs at 37^0C. After incubation the diameter of zone of inhibition was measured for each concentration in mm as shown in table 2. The activity was compared with standard antibiotic nystatin (positive control) and a disk impregnated with DMF was used as a negative control.

RESULTS AND DISCUSSIONS

Synthesis of compounds 4a-4p

The procedure used to synthesize a series of 16 pyrazoline derivatives is outlined in Figure 1. Structures of compounds **3(a-p)** and **4(a–p)** were confirmed by IR, NMR data as well as their distinct R_f values in TLC analysis. The IR spectra of synthesized compounds showed absorption bands which are characteristic of the anticipated structure of the synthesized compounds. Carbonyl stretching band of aldehydes and methyl ketones which generally occurs in 1680-1700 cm^{-1} range, is absent in the infrared spectra of products and a new band appears in 1630-1655 cm^{-1} region could be assigned to α, β-unsaturated ketonic group in the synthesized compounds. The signal at 1350-1300 (asymmetric) and 1120-1100 (symmetric) arise due to SO_2 group in sulfonamide chalcones. Two strong bands between 3321-3382 cm^{-1} and 3413-3485 cm^{-1} regions ascribe to amide –NH- stretching in sulfonamide chalcones. Other characteristic bands of substituted groups, viz, $\nu_{C\text{-}Cl}$, $\nu_{O\text{-}H}$, $\nu_{C\text{-}O\text{-}C}$, and $\nu_{C=N}$ in product chalcones are displayed in 725-750 cm^{-1}, 3550-3200 cm^{-1}, 1260-1000 cm^{-1} and 1260-1000 cm^{-1} regions of infrared spectra, respectively. The ^{1}H-NMR spectra of the synthesized compounds show signals for both aliphatic and aromatic protons, characteristic of the anticipated structure of the synthesized compounds. A singlet arising in 8.34-9.68 ppm region could be attributed to amide (-NH-) proton. Two doublets appearing in 7.25-7.77 ppm (J ~16 Hz, Ha) and 7.22-7.49 ppm (J ~16 Hz, Hb) regions may be due to trans-olifinic protons. The large J value (17 Hz) clearly reveals the trans geometry for the chalcones. Signals around δ value 3.1 and 3.9 ppm recorded as doublet of doublets (dd) were assigned to 4-H_x and 4-H_y protons of pyrazoline derivatives. The fragmentation pattern obtained in the mass spectra was also according to the anticipated structures. All the above results confirmed the formation of the synthesized compounds.

In vitro antimicrobial activity

All the target compounds were evaluated for their *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* representing Gram positive bacteria, and *Pseudomonas aeruginosa*, *Escherichia coli* representing Gram-negative bacteria using ciprofloxacin as standard. Compounds were also evaluated for their *in vitro* antifungal activity against *Candida albicans, Aspergillus niger* using

Ketoconazole as standard. The results of *in vitro* antibacterial as well as antifungal activities of compounds (**4a–4p**) are summarized in Table 2.

Table 2: Antibacterial and Antifungal activity of synthesized compounds 4a-4p

Compound Code	Zone of inhibition in mm					
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E.coli***	***A. niger***	***C. albicans***
4a	11	09	09	10	15	**16**
4b	10	10	08	11	13	14
4c	13	14	10	12	**16**	15
4d	14	**16**	**19**	**16**	**17**	**19**
4e	06	10	08	09	15	**16**
4f	09	09	08	10	10	13
4g	14	13	12	12	08	09
4h	13	**16**	**16**	15	09	12
4i	**16**	11	13	14	**16**	15
4j	13	14	13	12	09	12
4k	15	14	**17**	15	**16**	14
4l	12	**16**	**19**	15	**19**	**16**
4m	11	10	07	15	13	11
4n	15	14	**16**	12	12	**16**
4o	14	12	**16**	13	12	14
4p	**18**	**16**	**19**	**16**	**17**	**19**
Control	00	00	00	00	00	00
Ciprofloxacin	19	17	20	20	-	-
Ketoconazole	-	-	-	-	22	22

Note: The zone of inhibition was measured in mm from the one end to another end of inhibition zone at three different diagonals and the average value is recorded.
Note: **'-'** denotes no activity, 6-11 mm poor activity, 12-15 mm moderate activity, 16-19 mm and above good activity

All the dilutions of standard drug as well as synthesized compounds were prepared in the same manner as for the antibacterial activity. The Mean Zone of inhibition of the derivatives is reported for all compounds against different micro-organism. All test tube were incubated in an electrically heated incubator at 26±1°C for 72 hrs, and then examined for growth.

Ciprofloxacin has shown maximum activity against S. aureus, B. subtilis, P. aeruginosa and E. coli with the zone of inhibition of 19mm, 17mm, 20mm and 20mm while Ketoconazole has shown maximum activity against Aspegallus niger and Candida albicans with zone of inhibition of 22mm and 22mm

In accordance with the data obtained from antibacterial activity, all the synthesized 1,3,5-trisubstituted pyrazoline derivatives have showed mild to good activity against tested organisms. Among these 1,3,5-trisubstituted pyrazoline derivatives, compound **4a, 4b, 4e, 4f, 4m** showed mild activity and compound **4c, 4g. 4i, 4j, 4k, 4o** showed moderate activity and **4d, 4h, 4l, 4n, 4p** showed good activity against bacteria.

In accordance with the data obtained from antifungal activity, compound **4g, 4h, 4j** showed mild activity and compound **4a-4f, 4m-4o** showed moderate activity and **4i, 4k, 4l, 4p** showed good activity against fungi.

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