Synthesis and Antimicrobial Activity of Some Trisubstituted 2-Pyrazolines Derivatives

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ABSTRACT

A series of 1,3,5-trisubstituted 2-pyrazolines derivatives were synthesized by refluxing from various substituted chalcones and 4-hydrazinobenzenesulfonamidehydrochloridein two steps. Compounds were confirmed by physical and spectral data such as M.P., R_f, 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.Compound 4o and 4p showed better activity than standard drugs.

Keywords: Pyrazolines, Chalcones, Antimicrobial, Synthesis, Antibacterial, Antifungal.

INTRODUCTION

Despite the rapid progress of science, the treatment of infectiousdiseases still remains a serious problem of concern to the scientificcommunity, mainly because of the wide range of factorsleading to the emergence of these diseases and also the increasednumber of pathogenic microorganisms with resistance to multipledrugs, including the Gram positive bacteria [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].

Pyrazolines and their derivatives have been found to possess a broad spectrum of biological activities such as antibacterial [5-6], antidepressant [7], anticonvulsant [8-9], antihypertensive [10], antioxidant [11], antitumor [12] and anticancer activities [13-14]. Recently these classes of compounds are reported topossess potential antiviral activity against flavivirus [15] and HIV [16].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.

MATERIAL AND METHOD0053

Material

Melting points were determined by open capillaryapparatus and were uncorrected. IR spectra were recorded on a FT-IR Shimadzu DZU 8400S spectrophotometerin 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. 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.

Methods

The synthesis of all the compounds**4a-p**was performed in a manner as outlined in Fig.1 and Table 1.

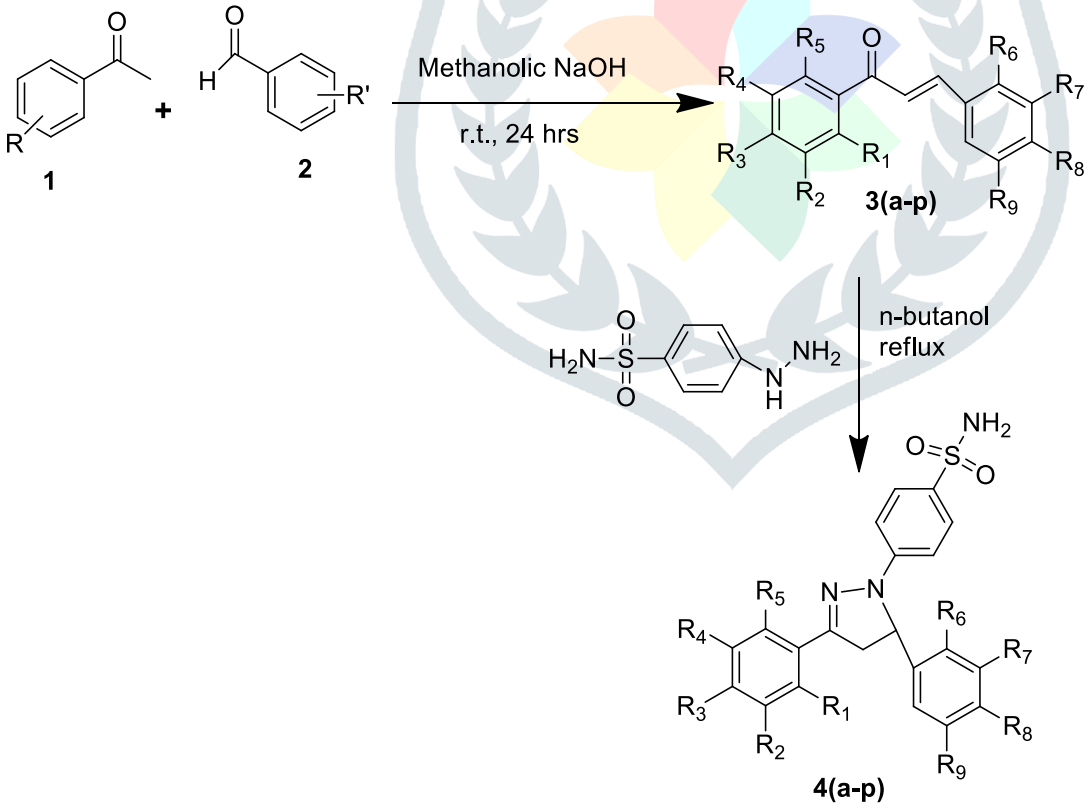

Fig 1.Scheme for the synthesis of 1, 3, 5-trisubstituted pyrazolines 4 (a-p).

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 procedure 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[17]. 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); Yield65%, 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); Yield69%, 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); Yield60%, 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); Yield71%, 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 method for the synthesis of 3,5-diaryl-1-(p-sulfamylphenyl)-2-pyrazolines(4a-4p):

Appropriate chalcones**3a – 3p** (0.001 mol) and 4-hydrazinobenzenesulfonamidehydrochloride (0.001 mol) were dissolved in ethanol (25 ml) andrefluxed for 24 h. After completion of the reaction, refluxing condenser wasremoved and the reaction solution was concentrated to 10–15 ml. It was left atroom temperature to give crystalline compound. It was filtered andrecrystallised to give pure compound [18].For preparing pyrazoline a smallamount of NaOAc was added to refluxing alcohol.

3-(2",4" -hydroxyphenyl)-5-(2',5'-dichlorophenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4a)

Synthesized by above method from chalcone**3a**; Recrystallized from methanol; Yield 46%, off white amorphous solid; mp 210-213°C; R_f = 0.74, (toluene/ethyl acetate/formic acid, 5:4:1); IR (KBr) ν_{max}/cm^{-1} 3254 (O-H), 3315 & 3252 (NH_2), 1605 (C=N), 1159 (SO_2N<), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.05 (2H, s, 2",4"-OH), 7.87 (1H, d, *J 12.3* H-6"), 7.65 (2H, m, H-8, 10), 7.40-7.48 (3H, m, H-3', 4', 6'), 7.10 (2H, s, SO_2NH_2), 6.82-6.86 (4H, m, H-7, 11, 3", 5"), 5.68 (1H, m, H-5), 3.96 (1H, m, 4-H_y), 3.10 (1H, m, 4-H_x); FAB-MS *m/z*: 477.72 $[M+H]^+$; Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_4S$: C, 52.73; H, 3.58; N, 8.78; Found: C, 52.49; H, 3.26; N, 8.36.

3-(2",4" -hydroxyphenyl)-5-(2'-methoxy,5'-chlorophenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4b)

Synthesized by above method from chalcone**3b**; Recrystallized from methanol; Yield 35%, off white amorphous solid; mp 219-222°C; R_f = 0.75, (toluene/ethyl acetate/formic acid, 5:4:1); IR (KBr) ν_{max}/cm^{-1} 3254 (O-H), 3315 & 3252 (NH_2), 1605 (C=N), 1159 (SO_2N<), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.05 (2H, s, 2",4"-OH), 7.87 (1H, d, *J 12.3* H-6"), 7.65 (2H, m, H-8, 10), 7.40-7.48 (3H, m, H-3', 4', 6'), 7.10 (2H, s, SO_2NH_2), 6.82-6.86 (4H, m, H-7, 11, 3", 5"), 5.68 (1H, m, H-5), 3.96 (1H, m, 4-H_y), 3.73 (3H, s, OCH_3-2'), 3.10 (1H, m, 4-H_x); FAB-MS *m/z*: 461.72 $[M+H]^+$; Anal. Calcd for $C_{22}H_{20}ClN_3O_4S$: C, 57.70; H, 4.40; N, 9.18 Found: C, 57.29; H, 4.73; N, 9.83.

3-(2",4" -hydroxyphenyl)-5-(3'-hydroxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4c)

Synthesized by above method from chalcone**3c**; Yield 38%, Pale yellow solid; mp 265-267°C; IR (KBr) ν_{max}/cm^{-1} 3212 (O-H), 3334& 3278 (NH_2), 1612 (C=N), 1151 (SO_2N<), 1110 (C–Cl), 3041, 2954 (C-H), 1501, 1462, 923, 811, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.05 (3H, s, 2",4", 3'-OH), 7.82 (1H, d, *J 10.3* H-6"), 7.20-7.15 (1H, m, H-2', 5'), 7.10 (2H, s, SO_2NH_2), 7.61 (2H, dd, *J 12.3* and 6.2, H-8, 10), 6.94 (2H, d, *J* 7.1, H-2'), 6.85-6.76 (6H, m, H-7, 11, 4',6', 3", 5"), 5.92 (1H, dd, *J 12.5* and 6.2, H-5), 3.85 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.08 (1H, dd, *J 17.5* and 4.6, 4-H_x); FAB-MS *m/z*: 425.39 [M +H]$^+$; Anal. Calcd for $C_{21}H_{19}N_3O_5S$: C, 59.28; H, 4.50; N, 9.88; Found: C, 59.10; H, 4.29; N, 9.98;

3-(2",4"-hydroxyphenyl)-5-(3',4'-dimethoxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^{2}Pyrazoline(4d)

Synthesized by above method from chalcone**4d**; Yield 42%, white amorphous solid; mp 256-258°C; IR (KBr) ν_{max}/cm^{-1} 3245 (O-H), 3334& 3278 (NH_2), 1612 (C=N), 1151 (SO_2N<), 1110 (C–Cl), 3041, 2954 (C-H), 1501, 1462, 923, 811, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.65 (2H, s, 2",4"-OH), 7.87 (1H, d, *J 10.4* H-6"), 7.67 (2H, dd, *J 10.3* and 4.2, H-8, 10), 7.10 (2H, s, SO_2NH_2), 6.92-6.85 (7H, m, H-7, 11, 2', 4',6', 3", 5"), 5.92 (1H, t, H-5), 3.89 (1H, dd, *J 17.5* and 11.3, 4-H_y), 3.75 (6H, s, OCH_3-3', 4'), 3.01 (1H, dd, *J 17.4* and 4.9, 4-H_x); FAB-MS *m/z*: 469.79 [M +H]$^+$; Anal. Calcd for $C_{23}H_{23}N_3O_6S$: C, 58.84; H, 4.94; N, 8.95Found: C, 58.27; H, 4.93; N, 8.45

3-(2",5"-dichorophenyl)-5-(2',5'-dichlorophenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(5e)

Synthesized by above method from chalcone **5e**; Yield 42%, white solid; mp 226-228°C; IR (KBr) ν_{max}/cm^{-1} 3312& 3261 (NH_2), 1630 (C=N), 1159 (SO_2N<), 1101 (C–Cl), 3031, 2934 (C-H), 1462, 923, 818, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.87 (2H, dd, *J 10.4* and 4.1, H-2", 6"), 7.67 (2H, dd, *J 10.3* and 4.2, H-8, 10), 7.10 (2H, s, SO_2NH_2), 6.92-6.85 (7H, m, H-7, 11, 2', 4',6', 3", 5"), 5.92 (1H, t, H-5), 3.89 (1H, dd, *J 17.5* and 11.3, 4-H_y), 3.01 (1H, dd, *J 17.4* and 4.9, 4-H_x); FAB-MS *m/z*: 453.79 [M +H]$^+$; Anal. Calcd for $C_{21}H_{15}Cl_4N_3O_2S$: C, 60.91; H, 5.11; N, 9.27; Found: C, 60.27; H, 5.93; N, 9.45

3-(2",5"-dichorophenyl)-5-(5'-chloro-2'-methoxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(5f)

Synthesized by above method from chalcone**3f**; Yield 44%, Pale yellow solid; mp 218-220°C; IR (KBr) ν_{max}/cm^{-1} 3301& 3258 (NH_2), 1637 (C=N), 1151 (SO_2N<), 1105 (C–Cl), 3036, 2926 (C-H), 1461, 926, 794 (Ar); ^{1}H-NMR

($CDCl_3$, 400 MHz), δ (ppm) 7.87 (2H, dd, *J 10.4* and 4.1, H-2", 6"), 7.67 (2H, dd, *J 10.3* and 4.2, H-8, 10), 7.10 (2H, s, SO_2NH_2), 6.90-6.83 (7H, m, H-7, 11, 3', 4', 6', 3", 5"), 5.87 (1H, t, H-5), 3.80 (1H, m, 4-H_y), 3.70 (3H, s, OCH_3-2'), 3.04 (1H, m, 4-H_x); FAB-MS *m/z*: 511.35 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}Cl_3N_3O_3S$: C, 51.73; H, 3.55; N, 8.23; Found: C, 51.10; H, 3.39; N, 8.37

3-(2",5"-dichorophenyl)-5-(3'-hydroxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4g)

Synthesized by above method from chalcone**3g**; Yield 41%, white solid; mp 224-227°C; IR (KBr) ν_{max}/cm^{-1} 3223 (O-H), 3330& 3276 (NH_2), 1617 (C=N), 1157 ($SO_2N<$), 1130 (C–Cl), 3046, 2923 (C-H), 1501, 1462, 923, 810, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.65 (1H, s, 4"-OH), 7.87 (2H, dd, *J 10.4* and 4.1, H-2", 6"), 7.67 (2H, dd, *J 10.3* and 4.2, H-8, 10), 7.10 (2H, s, SO_2NH_2), 6.90-6.83 (7H, m, H-7, 11, 3', 4', 6', 3", 5"), 5.87 (1H, t, H-5), 3.80 (1H, m, 4-H_y), 3.04 (1H, m, 4-H_x); FAB-MS *m/z*: 511.35 $[M+H]^+$; Anal. Calcd for $C_{21}H_{17}Cl_2N_3O_3S$: C, 54.55; H, 3.71; N, 9.09; Found: C, 54.62; H, 3.45; N, 9.63

3-(2",5"-dichorophenyl)-5-(3',4'-dimethoxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4h)

Synthesized by above method from chalcone**3h** Yield 38%, white solid; mp 287-289°C; IR (KBr) ν_{max}/cm^{-1} 3323& 3267 (NH_2), 1610 (C=N), 1150 ($SO_2N<$), 1139 (C–Cl), 3041, 2925 (C-H), 1520, 1461, 923, 790 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 6.98-6.85 (7H, m, H-7, 11, 2', 5', 6'), 7.45 (2H, d, J 3.2 H-3", 4"), 7.69 (2H, dd, *J 10.3* and 4.1, H-8, 10), 7.16 (2H, s, SO_2NH_2), 5.81 (1H, t, H-5), 3.82 (1H, m, 4-H_y), 3.72 (3H, s, OCH_3-3', 4'), 3.04 (1H, m, 4-H_x); FAB-MS *m/z*: 505.76 $[M+H]^+$; Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_4S$: C, 54.55; H, 4.18; N, 8.30; Found: C, 54.83; H, 4.57; N, 8.07.

3-(2"methoxy, 5"-chorophenyl)-5-(2',5'-dichlorophenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4i)

Synthesized by above method from chalcone**3i,** Yield 44%, Pale yellow solid; mp 240-242°C; IR (KBr) ν_{max}/cm^{-1} 3326& 3260 (NH_2), 1613 (C=N), 1145 ($SO_2N<$), 1130 (C–Cl), 3046, 2920 (C-H), 1522, 1456, 920, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.75-7.40 (7H, m, H-8, 10, 3', 4', 6', 4", 6"), 7.12 (2H, s, SO_2NH_2), 6.95-6.89 (3H, m, H-7, 11, 3") 5.87 (1H, t, H-5), 3.80 (1H, m, 4-H_y), 3.04 (1H, m, 4-H_x); FAB-MS *m/z*: 511.23 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}Cl_3N_3O_3S$: C, 51.72; H, 3.50; N, 8.27; Found: C, 51.46; H, 3.83; N, 8.53

3-(2"methoxy,5"-chorophenyl)-5-(2'-methoxy,5'-chlorophenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4j)

Synthesized by above method from chalcone**3j**; Yield: 39%,brownish yellow amorphous solid; mp 261-263°C; IR (KBr) ν_{max}/cm^{-1} 3321& 3245 (NH_2), 1610 (C=N), 1149 ($SO_2N<$), 1156 (C–Cl), 3048, 2928 (C-H), 1520, 1452, 928, 799 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.72-7.42 (7H, m, H-8, 10, 3', 4', 6', 4", 6"), 7.14 (2H, s, SO_2NH_2), 6.96-6.88(3H, m, H-7, 11, 3") 5.82 (1H, t, H-5), 3.85 (1H, m, 4-H_y), 3.71 (3H, s, OCH_3-2'), 3.08 (1H, m, 4-H_x); FAB-MS *m/z*: 505.84 $[M+H]^+$; Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_4S$: C, 54.55; H, 4.18; N, 8.30; Found: C, 54.30; H, 4.29; N, 8.84

3-(2"methoxy,5"-chorophenyl)-5-(3'-hydroxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4k)

Synthesized by above method from chalcone**3k**; Yield 45%, reddish brown amorphous solid; mp 258-260°C; IR (KBr) ν_{max}/cm^{-1} 3282 (O-H), 3329& 3240 (NH_2), 1617 (C=N), 1141 ($SO_2N<$), 1150 (C–Cl), 3040, 2921 (C-H), 1524, 921, 770 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.25 (1H, s, 2'-OH), 7.72-7.42 (7H, m, H-8, 10, 2', 4', 6', 4", 6"), 6.95-6.85 (3H, m, H-7, 11, 3", 5') 7.10 (2H, s, SO_2NH_2), 5.84 (1H, t, H-5), 3.86 (1H, m, 4-H_y), 3.09 (1H, m, 4-H_x); FAB-MS *m/z*: 457.64 $[M+H]^+$; Anal. Calcd for $C_{22}H_{20}ClN_3O_4S$: C, 57.70; H, 4.40; N, 9.18; Found: C, 57.07; H, 4.94; N, 9.75

3-(2"methoxy,5"-chorophenyl)-5-(3',4'-dimethoxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4l)

Synthesized by above method from chalcone**3l**; Yield 30%, white solid; mp 222-224°C; IR (KBr) ν_{max}/cm^{-1} 3322& 3246 (NH_2), 1610 (C=N), 1146 ($SO_2N<$), 1155 (C–Cl), 3041, 2934 (C-H), 1525, 932, 789 (Ar);; ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 7.70-7.46 (7H, m, H-8, 10, 2', 5', 6', 4", 6"), 6.93-6.86 (3H, m, H-7, 11, 3") 7.15 (2H, s, SO_2NH_2), 5.88 (1H, t, H-5), 3.82 (1H, m, 4-H_y), 3.70 (3H, s, OCH_3-3', 4'), 3.04 (1H, m, 4-H_x); FAB-MS *m/z*: 501.49 $[M+H]^+$; Anal. Calcd for $C_{24}H_{24}ClN_3O_5S$: C, 57.42; H, 4.82; N, 8.37; Found: C, 57.28; H, 4.58; N, 8.43

3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(2',5'-dichlorophenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4m)

Synthesized by above method from chalcone**3m**; Yield 43%, off white amorphous solid; mp 282-284°C; IR (KBr) ν_{max}/cm^{-1} 3223 (O-H), 3330& 3276 (NH_2), 1617 (C=N), 1157 ($SO_2N<$), 1130 (C–Cl), 3046, 2923 (C-H), 1501, 1462, 923, 810, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 11.09 (1H, s, 2"-OH), 7.77-7.45 (6H, m, H-8, 10, 3', 4', 6', 6"), 7.15 (2H, s, SO_2NH_2), 6.94-6.89 (3H, dd, J 5.4 and 2.4, H-7, 11) 5.83(1H, t, H-5), 3.81

(1H, m, 4-H_y), 3.06 (1H, m, 4-H_x), 2.89 (3H, s, CH_3-4"); FAB-MS *m/z*: 511.47 $[M+H]^+$; Anal. Calcd for $C_{22}H_{18}Cl_3N_3O_3S$: C, 51.73; H, 3.55; N, 8.23; Found: C, 51.08; H, 3.78; N, 8.34

3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(5'-chloro-2'-methoxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4n)

Synthesized by above method from chalcone**3n**; Yield 40%, off white solid; mp 287-289°C; IR (KBr) ν_{max}/cm^{-1} 3245 (O-H), 3334& 3278 (NH_2), 1612 (C=N), 1151 (SO_2N<), 1110 (C–Cl), 3041, 2954 (C-H), 1501, 1462, 923, 811, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.56 (1H, s, 2"-OH), 7.71-7.47 (6H, m, H-8, 10, 3', 4', 6', 6"), 7.10 (2H, s, SO_2NH_2), 6.91-6.85 (3H, dd, J 5.4 and 2.4, H-7, 11) 5.85(1H, t, H-5), 3.81 (1H, m, 4-H_y), 3.78 (3H, s, OCH_3-2'), 3.06 (1H, m, 4-H_x), 2.91 (3H, s, CH_3-4"); FAB-MS *m/z*: 505.39 $[M+H]^+$; Anal. Calcd for $C_{23}H_{21}Cl_2N_3O_4S$: C, 54.55; H, 4.18; N, 8.30Found: C, 54.28; H, 4.82; N, 8.47

3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(3'-hydroxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4o)

Synthesized by above method from chalcone**3o**; Yield 31%, off white solid; mp 296-298°C; IR (KBr) ν_{max}/cm^{-1} 3267 (O-H), 3330& 3278 (NH_2), 1612 (C=N), 1151 (SO_2N<), 1110 (C–Cl), 3041, 2954 (C-H), 1501, 1462, 923, 794 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.25 (2H, s, 2", 3'-OH), 7.60-7.63 (3H, m, H-8, 10, 6"), 6.85-6.40 (4H, m, H-2', 4', 6', 5"), 7.12 (2H, s, SO_2NH_2), 6.88-6.82 (3H, dd, J 5.4 and 2.4, H-7, 11) 5.85 (1H, t, H-5), 3.83 (1H, m, 4-H_y), 3.12 (1H, m, 4-H_x), 2.84 (3H, s, CH_3-4"); FAB-MS *m/z*: 457.39 $[M+H]^+$; Anal. Calcd for $C_{22}H_{20}ClN_3O_4S$: C, 57.70; H, 4.40; N, 9.18; Found: C, 57.75; H, 4.25; N, 9.87

3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(3',4'-dimethoxyphenyl)-1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline(4p)

Synthesized by above method from chalcone**3p**; Yield 53%, white solid; mp 231-234°C; IR (KBr) ν_{max}/cm^{-1} 3438 (O-H),3330& 3276 (NH_2), 1151 (SO_2N<), 1581(Ar C=C), 1610 (C=N), 1238, 999 (C–O), 1212 (C-N), 1121 (C–Cl), 3049, 2945(C-H), 1565, 812, 773 (Ar); ^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm) 10.12 (1H, s, 2"-OH), 7.72-7.50 (6H, m, H-8, 10, 2', 5', 6', 6"), 7.14 (2H, s, SO_2NH_2), 6.92-6.87 (3H, dd, J 5.4 and 2.4, H-7, 11) 5.81(1H, t, H-5), 3.84 (1H, m, 4-H_y), 3.79 (6H, s, OCH_3-3', 4'), 3.10 (1H, m, 4-H_x), 2.79 (3H, s, CH_3-4"); FAB-MS *m/z*: 501.96 $[M+H]^+$; Anal. Calcd for $C_{24}H_{24}ClN_3O_5S$: C, 57.42; H, 4.82; Cl, 7.06; N, 8.37 Found: C, 57.54; H, 4.87; N, 8.56

***In vitro* antimicrobial activity**

Minimal Inhibitory Concentrations (MICs, µg/ml) were determined on different microbesusing Broth Micro Dilution procedure according to the recommendations of National Committees for Clinical Laboratory Standards (NCCLS). MIC was defined as the lowest concentration ofcompound that inhibited visible growth of

microbes after incubation at 35ºC for 24 h for bacteria and 48 h for fungi (Clinical and Laboratory Standards Institute, 2002, 2006). Strains of gram negative bacteria *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 51) and gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121) and fungal strains *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 8189) were used for testing antibacterial and antifungal activities. Bacterial strains were grown in Mueller–Hinton Broth and fungal strains were grown in Sabouraud Liquid medium. The inoculum densities of 5 x 10^5 CFU/ml for bacteria and 0.5–2.5 x 10^3 CFU/ml for fungi wereprepared. Ciproafloxacin and fluconazole were used as standard antibiotic powder. The synthesizedcompounds were dissolved in DMSO and further dilutions were prepared in sterile distilled water ofvarious concentrations by twofold serial dilution method to obtain the required concentration.Ciproafloxacin and fluconazolewere diluted in sterile distilled water. After dilution was completed,microbe suspensions were inoculated into each well of row. MIC values were given as µg/ml. Theturbidity was monitored by the unaided eye and the lowest concentration, at which no growth wasseen, recorded and considered as MIC of that particular compound.

RESULTS AND DISCUSSION

Chemistry

The procedure used to synthesize a series of 16 designed compounds (pyrazoline derivatives) is outlined in Figure 1 and Table 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 toα, 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* (MTCC 3160), *Bacillus subtilis* (MTCC 121)representing Grampositive bacteria, and *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 51) representing Gram-negative bacteria. Compounds were also evaluated for their *in vitro* antifungal activity against *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 8189). The results of *in vitro* antibacterial as well as antifungal activities of compounds (**4a–4p**) are summarized in Table 2.

All the dilutions of standard drug as well as synthesized compounds were prepared in the same manner as for the antibacterial activity. The MIC for all synthesized compounds and standard drug was determined by performing all the test in triplicate.

All test tube were incubated in an electrically heated incubator at 26±1°C for 72 hrs, and then examined for growth.

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

Compound Code	MIC (µg/mL)				
	P. aeruginosa	***B. subtilis***	***S. aureus***	***A. niger***	***C. albicans***
4a	12.5	12.5	25	25	12.5
4b	12.5	25	100	25	50
4c	12.5	6.5	6.5	12.5	6.5
4d	3.25	6.5	25	6.5	3.25
4e	25	12.5	50	25	12.5
4f	25	75	25	25	25
4g	12.5	12.5	25	12.5	12.5
4h	3.25	6.5	12.5	12.5	6.5
4i	25	25	75	50	100
4j	50	25	50	50	25
4k	12.5	50	25	25	25
4l	12.5	50	50	12.5	12.5
4m	25	25	25	25	25
4n	12.5	6.5	6.5	25	50
4o	25	6.5	12.5	12.5	6.5

4p	6.5	3.25	3.25	6.5	6.5
Ciprofloxacin[a]	12.5	12.5	25	-	-
Fluconazole[b]	-	-	-	25	25

Reported MIC are the results of triplicate
[a]ciprofloxacin was used as a positive control against bacteria species
[b]fluconazole was used as a positive control against fungi species

Compounds **4c, 4h, 4n, 4oand 4p** showed better antimicrobial effects with a MIC value of 12.5, 6.5, 12.5, 6.5, 12.5 and 3.25 µg/ml respectively than ciproafloxacin (MIC = 25 µg/ml) against *S. aureus*. In case of *B. subtilis*, compounds **4c, 4d, 4g, 4h, 4l, 4o** and **4p**with MIC values in the range 6.5 - 3.25µg/ml exhibited better antibacterial activity than all other compounds and standard drug ciproafloxacin (MIC = 12.5µg/ml). In general, it has been observed that synthesized compounds displayed better antibacterial performance against the Gram-negative bacteria than the Gram-positive bacteria. Compounds **4d, 4h**and**4p** showed superior activity with MIC value of 3.25, 3.25, and 6.5µg/ml than ciproafloxacin (MIC = 12.5 µg/ml) against *P. aeruginosa*.

The results of the antifungal activities of synthesized compounds (4a–p) were summarizedin Table 6.3. In comparison with standard antifungal drug, the compounds **4c, 4d, 4g, 4h, 4l, 4o** and **4p**exhibited greater antifungal activity than standard drug against *A. niger*. Compounds**4p** with MICs 6.5 µg/ml were most active against *A. niger*. Similarly,compounds **4d, 4h, 4o**and**4p** with MICs within range of 3.25 - 6.5 µg/ml, showed greater activitythan standard drug against *C. albicans*.

CONCLUSIONS

The 1-(ρ-sulfamylphenyl)-Δ^2-Pyrazoline derivatives with different substituents were synthesized and characterized by modern analytical techniques (IR, NMR and MS). They were subjected to evaluation for antimicrobial activities. From these results, the compounds **4d, 4h** and**4p** exhibited significant biological activity with reference to standard drugs. It may have potential as novel antimicrobial agents.

ACKNOWLEDGEMENTS

The authors are grateful to management, SRK University, Bhopal for providing all the necessary laboratory facilities.

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