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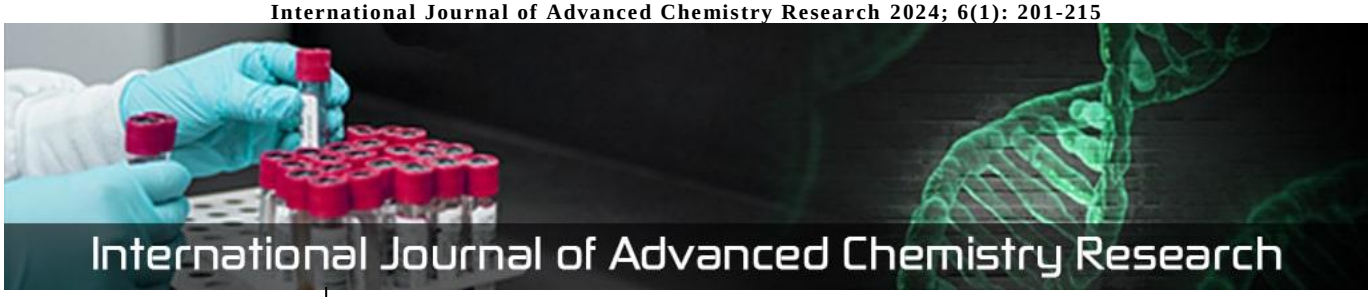

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Design and synthesis of some novel 1, 3, 5-trisubstituted pyrazolines derivatives for antimicrobial activities

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Abstract
In this study, chalcones and pyrazoline derivatives were synthesized and evaluated for their multifarious pharmacological profiles, particularly their antimicrobial and antimalarial activities. The synthesis of compounds 3a-3p and 4a-4p was achieved through the Claisen-Schmidt reaction and subsequent reactions with nicotinic acid hydrazide, yielding products with high purity and anticipated structures confirmed by physico-chemical characterization methods including FT-IR, 1H-NMR, mass spectral, and elemental analyses. The compounds displayed solubility in various organic solvents but were insoluble in water. Antimicrobial activity assays against Gram-positive and Gram-negative bacteria, as well as antifungal activity tests, demonstrated that several compounds exhibited mild to good activity. Notably, compounds 4d, 4h, 4l, 4n, and 4p showed significant antibacterial activity, while 4i, 4k, 4l, and 4p were most effective against fungi. Furthermore, the *in vitro* antimalarial activity identified compound 4p as the most promising candidate. Compounds 4d and 4p are highlighted as potential leads for further analog synthesis and structure-activity relationship studies to enhance antimicrobial efficacy. These findings suggest promising directions for the development of new therapeutic agents based on chalcone and pyrazoline scaffolds.

Keywords: Chalcones, pyrazoline, antimicrobial activity, antimalarial activity, structure-activity relationships

Introduction
Medicinal or Pharmaceutical Chemistry is a branch of science at the interface of chemistry and biology, which deals with the design, synthesis and development of agents for therapeutic use. Each year, hundreds of thousands of new molecules are synthesized and screened for their pharmacological activity. These molecules are categorized into different classes depending upon their chemical structure or pharmacological activity. One of the challenges of future malarial chemotherapy is to develop compounds that are innovative with respect to the chemical scaffold and molecular target. Many approaches to antimalarial drug discovery currently being deployed include optimization of therapy with available drugs including combination therapy, developing analogs of the existing drugs, evaluation of potent agents from natural products especially plants, use of compounds originally developed against other diseases, and evaluation of drug-resistance reversers (Chemosensitizers) as well as new chemotherapeutic targets. It is hope that these approaches might present good prospective in the search for safe and efficient new drugs for the treatment of malaria (Pandeya *et al.* 1999) [11].

Microorganisms are ubiquitous. The association of humans with such microorganisms is generally harmonious as microbial flora in human gut and skin. In spite of this, the tissues of healthy animals and plants are essentially microbe-free. This is achieved through provision of a number of non-specific and specific defense mechanisms. Violation of these defenses by microorganisms, through the expression of virulence factors and adaptation to a pathogenic mode of life, or following disease, accidental trauma or implantation of medical devices may lead to the establishment of microbial infections (Acharya *et al*., 2007) [1].

An infection is invasion of a host organism by microorganisms, proliferation of the invading organisms and host reaction to those microorganisms where as an infectious disease is infection with outbreak of clinical symptoms. Common routes of invasion for microorganisms, also called portal of entry are skin, respiratory tract, intestinal tract, urinogenital tract and conjunctiva (Acharya *et al*., 2010) [2].

Many of bacteria are sensitive to antibiotics and most infectious diseases are curable. Infectious diseases are still, despite the medical advances made during the last century, second leading cause of death. No more than six deadly infectious diseases - pneumonia, tuberculosis, diarrhoeal diseases, malaria, measles and more recently HIV/AIDS - account for half of all premature deaths, killing mostly children and young adults. Between 14 and 17 million people die each year due to infectious diseases - nearly all live in developing countries. Since their discovery antimicrobial agents are widely used and proven extremely effective in the treatment of bacterial infections. The antibacterial agents may be bacteriostatic or bactericidal in nature.

The rapid rise in microbial resistance to the traditional antibiotics has necessitated a continuing search for new classes of compounds with novel modes of antibacterial activity. Considering all the scaffolds of heterocyclic ring, pyrazoline among the various 5- membered heterocyclic compound derivatives have received significant attention in the recent years due to their diverse pharmacological and biological activities such as antifungal, antidepressant, anticonvulsant, anti-inflammatory, antibacterial, antitubercular, anticancer, human acyl-CoA: cholesterol acyltransferase inhibitors and analgesic properties (Bansal 2001, Acharya *et al.* 2010, Joshi *et al.* 2010) [13, 2, 14]. The stability and broad range of promising pharmacological properties inspired chemists to synthesize and study more pyrazoline derivatives since structural modifications can lead to different bioactivity. A number of pyrazolines prepared from hydrazines, arylhydrazines and a few acid hydrazides were reported in literature. Literature reports indicated that additional ester group, amide group or in general carbonyl group can enhance many of the bioactivities of pyrazolines. Among the various synthetic strategies reported, one pot cyclic condensation between chalcones and appropriate acid hydrazide seems to be more promising and challenging. This led to the planning of the synthesis of the above mentioned new 2- pyrazolines using similar strategy.

Thus the present work aims at the synthesis of some new pyrazolin derivatives through the reaction between appropriate acid hydrazide and chalcones.

Experimental

All the other chemicals used were obtained from Sigma-Aldrich, Spectrochem and High Media.

Physico-Chemical Data of the Synthesized Compounds

The structures of synthesized compounds were determined using melting points, infrared spectroscopy (IR), ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR) and elementary analysis. The melting point of the synthesized compounds were determined using an open capillary and are uncorrected.

IR spectra were recorded on a FT-IR Shimadzu DZU 8400S spectrophotometer in KBr disks and Elemental analysis was 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 CDCl3/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. All these above analysis were done at SAIF, Punjab University, Chandigarh. Progress of reaction and purity of synthesized compounds was ascertained by thin layer chromatography (A) using Silica gel G and Iodine vapors as detecting agent.

Chemistry

The synthesis of the designed compounds (4a-4p) was performed in a manner as outlined in Figure 1.

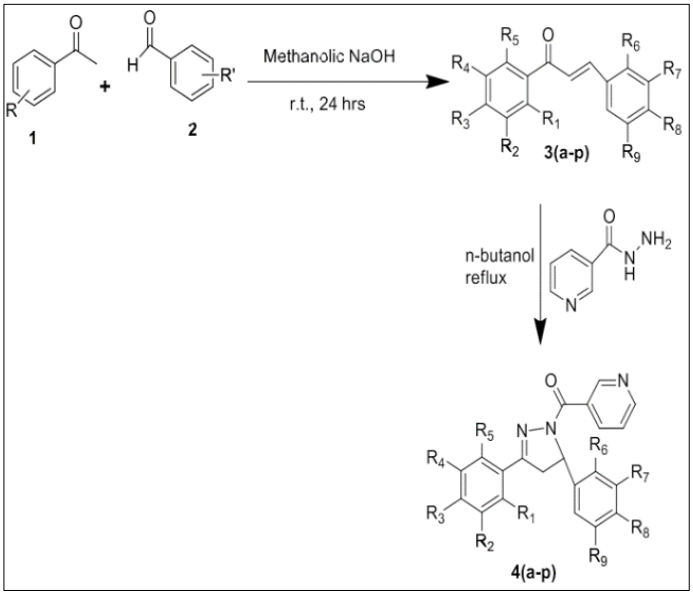


Fig 1: Scheme for the synthesis of substituted 2-pyrazolines 4 (a-p).

General method of synthesis of Chalcone (3a - 3p)

Chalcones are synthesized by Claisen-Schmidt condensation (Furniss *et al.*, 1989; Kumar *et al.*, 2010) [4, 8] of aldehyde and ketone by base catalyzed or acid catalyzed followed by dehydration to yield chalcones (Figure 2).

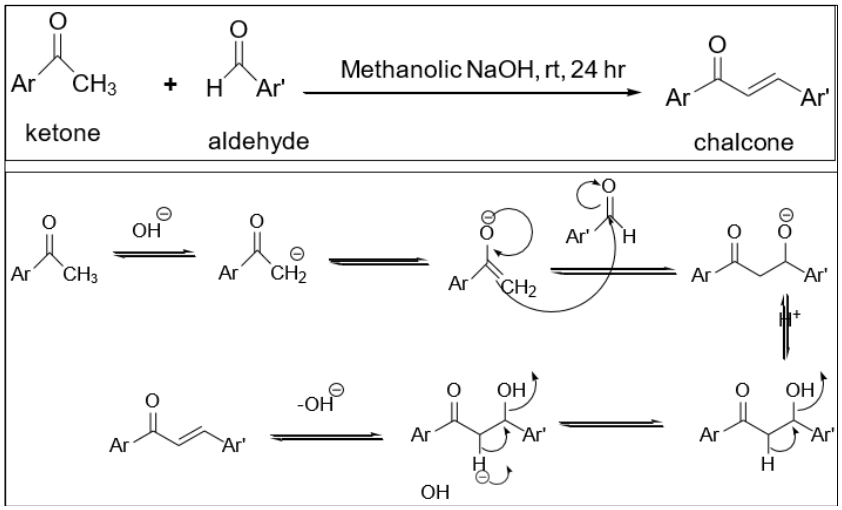


Fig 2: Mechanism of reaction for synthesis of chalcone derivatives (3a-3p)

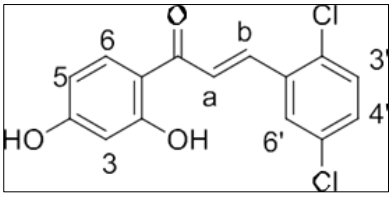
Table 1: Different substitutions on synthesized substituted 2-pyrazolines 4 (a-p)

S. No	Comp. No.		R1	R2	R3	R4	R5	R6	R7	R8	R9
1	3a	4a	OH	-	OH	-	-	Cl	-	-	Cl
2	3b	4b	OH	-	OH	-	-	OCH3	-	-	Cl
3	3c	4c	OH	-	OH	-	-	-	OH	-	-
4	3d	4d	OH	-	OH	-	-	-	OCH3	OCH3	-
5	3e	4e	Cl	-	Cl	-	-	Cl	-	-	Cl
6	3f	4f	Cl	-	Cl	-	-	OCH3	-	-	Cl
7	3g	4g	Cl	-	Cl	-	-	-	OH	-	-
8	3h	4h	Cl	-	Cl	-	-	-	OCH3	OCH3	-
9	3i	4i	OCH3	-	-	Cl	-	Cl	-	-	Cl
10	3j	4j	OCH3	-	-	Cl	-	OCH3	-	-	Cl
11	3k	4k	OCH3	-	-	Cl	-	-	OH	-	-
12	3l	4l	OCH3	-	-	Cl	-	-	OCH3	OCH3	-
13	3m	4m	-	Cl	OCH3		OH	Cl	-	-	Cl
14	3n	4n	-	Cl	OCH3	-	OH	OCH3	-	-	Cl
15	3o	4o	-	Cl	OCH3	-	OH	-	OH	-	-
16	3p	4p	-	Cl	OCH3	-	OH	-	OCH3	OCH3	-

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

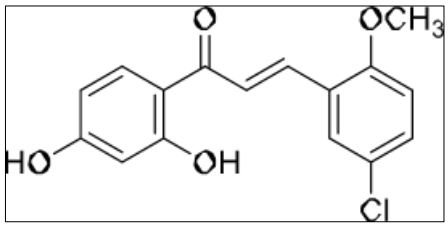
To a solution of substituted acetophenone (16 mmole) in 10 mL of methanol on an ice bath, freshly prepared 2 N methanolic NaOH solution (60 mL) was added and stirred for 10 min. To this, appropriate aldehyde (16 mmole) was added and stirred at room temperature for 12-24 hr. The reaction mixture was cooled 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)



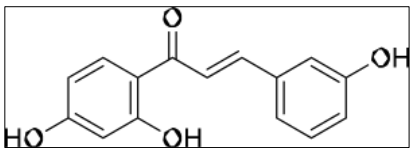
Synthesized by above method from 2,4-dihydroxyacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 85%, White solid; mp 165-167 °C; *Rf* (EtOAc/Hex 4:6) 0.45; IR (KBr) νmax/cm^{-1} 3440 (O-H), 1669 (C=O), 1597 (Ar C=C), 750 (C-Cl), 3063, 2931, 1625, 1415, 1325, 1296, 1134, 1153, 1046, 982, 759, 737 (Ar); ^{1}H-NMR (CDCl3, 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 C15H10Cl2O3: 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)



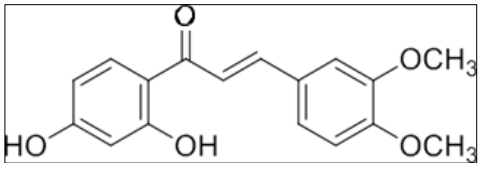
Synthesized by above method from 4-Hydoxyacetophenone (16 mmol) and 2- methoxy,5-chlorobenzaldehyde (16 mmol); Yield 70%, yellow crystalline solid; mp 112- 114°C; *Rf* (EtOAc/Hex 4:6) 0.47; IR (KBr) max/cm^{-1} 3441 (O-H), 1645 (C=O), 1595 (Ar C=C), 760 (C-Cl), 3060, 2965, 1645, 1434, 1323, 1296, 982, 759 (Ar); ^{1}H-NMR (CDCl3, 400 MHz), (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, OCH3-2'); FAB-MS *m/z* 304.06 [M +H]$^{+}$; Anal. Calcd for C16H13ClO4: 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)



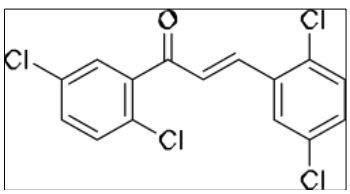
Synthesized by above method from 4-Hydroxyacetophenone (16 mmol) and 4- hydroxybenzaldehyde (16 mmol); Yield 65%, Yellow solid; mp 124-126°C; *Rf* (EtOAc/Hex 4:6) 0.36; IR (KBr) νmax/cm^{-1} 3440 (O-H), 1661 (C=O), 1592 (Ar C=C), 750 (C-Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ^{1}H-NMR (CDCl3, 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 C15H12O4: 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)



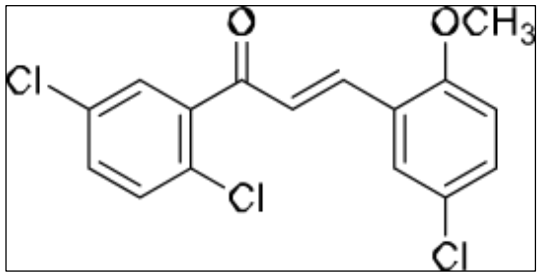
Synthesized by above method from 4-Hydroxyacetophenone (16 mmol) and 4- hydroxybenzaldehyde (16 mmol); Yield 76%, White solid; mp 108-110 °C; *Rf* (EtOAc/Hex 4:6) 0.34; IR (KBr) νmax/cm^{-1} 3435 (O-H), 1661 (C=O), 1592 (Ar C=C), 760 (C-Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ^{1}H-NMR (CDCl3, 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, OCH3-3', 4'); FAB-MS *m/z* 300.08 [M +H]$^{+}$; Anal. Calcd for C17H16O5: 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)



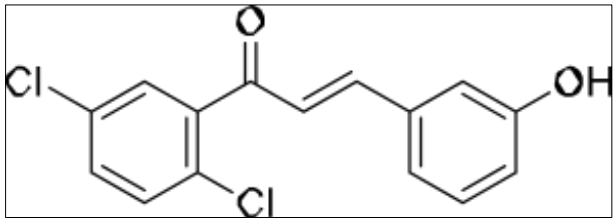
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 69%, White crystalline solid; mp 138-140 °C; *Rf* (EtOAc/Hex 4:6) 0.38; IR (KBr) νmax/cm^{-1} 1662 (C=O), 1598 (Ar C=C), 743 (C-Cl), 3064, 2930, 1627, 1415, 1325, 1296, 1134, 1117, 1047, 982, 819, 759, 737 (Ar); ^{1}H-NMR (CDCl3, 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 C15H8Cl4O: 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)



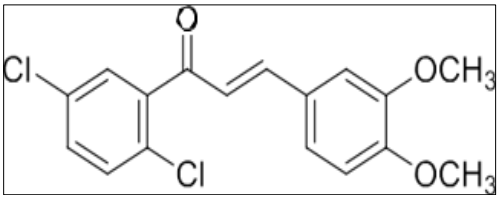
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 5- chloro, 2-methoxybenzaldehyde (16 mmol); Yield 67%, Creamy-coloured fine needles; mp 148-150°C; Rf (EtOAc/Hex 4:6) 0.79; IR (KBr) νmax/cm-1 1662, 1216 (C=O), 1594 (C=C), 1261, 1026 (C-O), 742 (C-Cl), 3061, 1591, 1457, 1384, 1296, 1194, 980, 854, 756 (Ar); 1H-NMR (CDCl3, 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, OCH3-2'); FAB-MS m/z: 341.27 [M +H]+; Anal. Calcd for C16H11Cl3O2: 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)



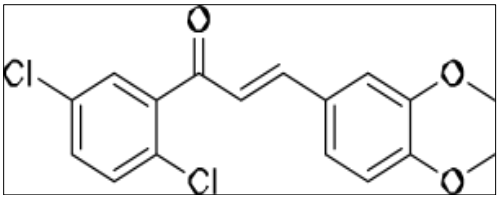
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); Yield 60%, White amorphous solid; mp 141-144 °C; *Rf* (EtOAc/Hex 4:6) 0.42; IR (KBr) νmax/cm^{-1} 3441 (O-H), 1663 (C=O), 1589 (Ar C=C), 745 (C-Cl), 3060, 2945, 1620, 1320, 1298, 1139, 1153, 1046, 982, 759, 737 (Ar); ^{1}H-NMR (CDCl3, 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 C15H10Cl2O2: 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)



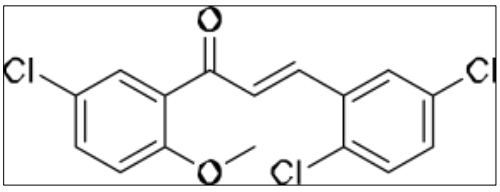
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 3, 4-dimethoxybenzaldehyde (16 mmol); Yield 69%, white amorphous solid; mp 115-118°C; Rf (EtOAc/Hex 4:6) 0.67; IR (KBr) νmax/cm-1 1653 (C=O), 1605 (Ar C=C), 1547 (COC=C), 1261, 1025 (C-O), 1150 (C-Cl), 3051 (Ar C-H), 2932, 2841 (C-H), 1524, 1472, 1147, 969 (Ar); 1H-NMR (CDCl3, 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, OCH3-3', 4'). FAB-MS m/z 322.02 [M +H]+; Anal. Calcd for C16H12Cl2O3: C, 59.46; H, 3.74;. Found: C, 59.23; H, 3.42;

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



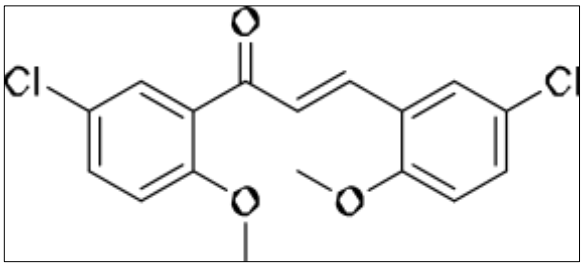
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 3, 4-dimethoxybenzaldehyde (16 mmol); Yield 69%, white amorphous solid; mp 115-118°C; *Rf* (EtOAc/Hex 4:6) 0.67; IR (KBr) νmax/cm^{-1} 1653 (C=O), 1605 (Ar C=C), 1547 (COC=C), 1261, 1025 (C-O), 1150 (C-Cl), 3051 (Ar C-H), 2932, 2841 (C-H), 1524, 1472, 1147, 969 (Ar); ^{1}H-NMR (CDCl3, 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, OCH3-3', 4'). FAB-MS *m/z* 322.02 [M +H]$^{+}$; Anal. Calcd for C16H12Cl2O3: 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)



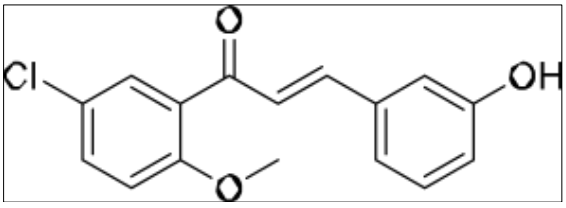
Synthesized by above method from 2- methoxy, 5-chloro-acetophenone (16 mmol) and 2, 5-dichlorobenzaldehyde (16 mmol); Yield 66%, Yellow solid; mp 105-107 °C; *Rf* (EtOAc/Hex 4:6) 0.32; IR (KBr) νmax /cm^{-1} 1650 (C=O), 1585 (Ar C=C), 1517 (COC=C), 1268, 1029 (C-O), 1150 (C-Cl), 3058 (Ar C-H), 2933 (C-H), 1619, 1512, 969, 810, (Ar); ^{1}H-NMR (CDCl3, 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, OCH3-2). FAB-MS *m/z* 339.38 [M +H]$^{+}$; Anal. Calcd for C16H11Cl3O2: 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)



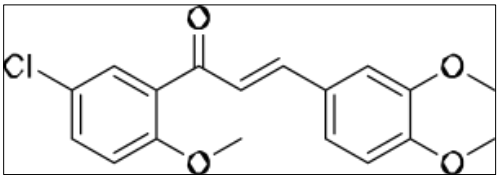
Synthesized by above mentioned method A from 2-methoxy, 5-chloroacetophenone (16 mmol) and 2-methoxy, 5-chlorobenzaldehyde (16 mmol); Yield 3.7 g, 69%, Yellow solid; mp 107-109°C; *Rf* (EtOAc/Hex 4:6) 0.35; IR (KBr) νmax /cm^{-1} 1655 (C=O), 1580 (Ar C=C), 1519 (COC=C), 1264, 1025 (C-O), 1157 (C-Cl), 3052 (Ar C-H), 2930 (C-H), 1611, 1517, 960, 815, (Ar); ^{1}H-NMR (CDCl3, 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, OCH3-2), 3.85 (3H, s, OCH3-2'), FAB-MS *m/z* 339.38 [M +H]$^{+}$; Anal. Calcd for C17H14Cl2O3: 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)



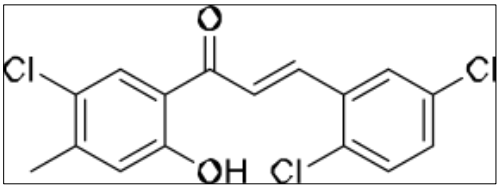
Synthesized by above method from 2-methoxy,5-chloroacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); Yield 69%, yellow cryastalline solid; mp 135-137 °C; *Rf* (EtOAc/Hex 4:6) 0.34; IR (KBr) νmax/cm^{-1} 3445 (O-H), 1665 (C=O), 1568 (Ar C=C), 748 (C-Cl), 3063, 2941, 1625, 1291, 1134, 1150, 980, 754 (Ar); ^{1}H-NMR (CDCl3, 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, OCH3-2) FAB-MS *m/z* 288.06 [M +H]$^{+}$; Anal. Calcd for C16H13ClO3: 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)



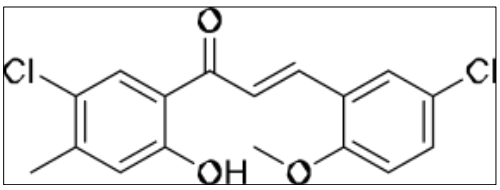
Synthesized by above method from 2-methoxy, 5-chloroacetophenone (16 mmol) and 3,4-dimethoxybenzaldehyde (16 mmol); Yield 71%, Pale yellow solid; mp 117- 119 °C; *Rf* (EtOAc/Hex 4:6) 0.49; IR (KBr) νmax /cm^{-1} 1645 (C=O), 1589 (Ar C=C), 1512 (COC=C), 1265, 1025 (C-O), 1151 (C-Cl), 3064 (Ar C-H), 2941 (C-H), 1611, 1518, 967, 811 (Ar); ^{1}H-NMR (CDCl3, 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, OCH3-2), 3.71 (6H, s, OCH3-3',4'). FAB-MS *m/z* 332.08 [M +H]$^{+}$; Anal. Calcd for C18H17ClO4: 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)



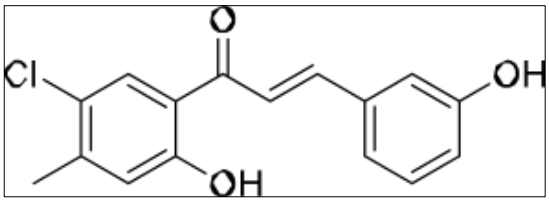
Synthesized by above method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 71%, White amorphous solid; mp94-97 °C; *Rf* (EtOAc/Hex 4:6) 0.67; IR (KBr) νmax/cm^{-1} 3447 (O-H), 1660 (C=O), 1596 (Ar C=C), 740 (C-Cl), 3064, 2965, 1647, 1434, 1320, 980, 759 (Ar, CH3); ^{1}H-NMR (CDCl3, 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, CH3-4); FAB-MS *m/z* 339.98 [M +H]$^{+}$; Anal. Calcd for C16H11Cl3O2: 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)



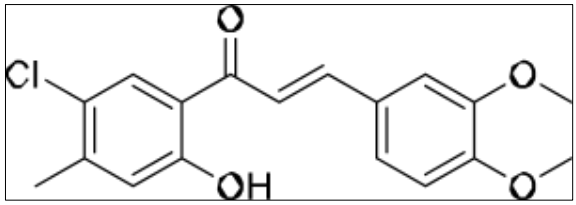
Synthesized by above method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2-methoxy, 5-dichlorobenzaldehyde (16 mmol); Yield 68%, White solid; mp 137-139°C; *Rf* (EtOAc/Hex 4:6) 0.48; IR (KBr) νmax/cm^{-1} 3431 (O-H), 1657 (C=O), 1586 (Ar C=C), 760 (C-Cl), 3064, 2964, 1643, 1434, 985, 753 (Ar); ^{1}H-NMR (CDCl3, 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, OCH3-2'), 2.34 (3H, s, CH3-4); FAB-MS *m/z* 336.03 [M +H]$^{+}$; Anal. Calcd for C17H14Cl2O3: 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; *Rf* (EtOAc/Hex 4:6) 0.31; IR (KBr) νmax/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 (CDCl3, 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, CH3-4); FAB-MS *m/z* 288.38 [M +H]$^{+}$; Anal. Calcd for C16H13ClO3: 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; *Rf* (EtOAc/Hex 4:6) 0.76; IR (KBr) νmax /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 (CDCl3, 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, OCH3-3', 4'), 2.32 (3H, s, CH3-4). FAB-MS *m/z* 332.07 [M +H]$^{+}$; Anal. Calcd for C18H17ClO4: C, 64.97; H, 5.15; Found: C, 64.23; H, 5.67

General method for synthesis of substituted 2-pyrazolines (4a-4p)

The substituted 2-pyrazolines (4a-4p) were synthesized according to the scheme depicted in Figure 3 (Ozdemir *et al.*, 2008) [10].

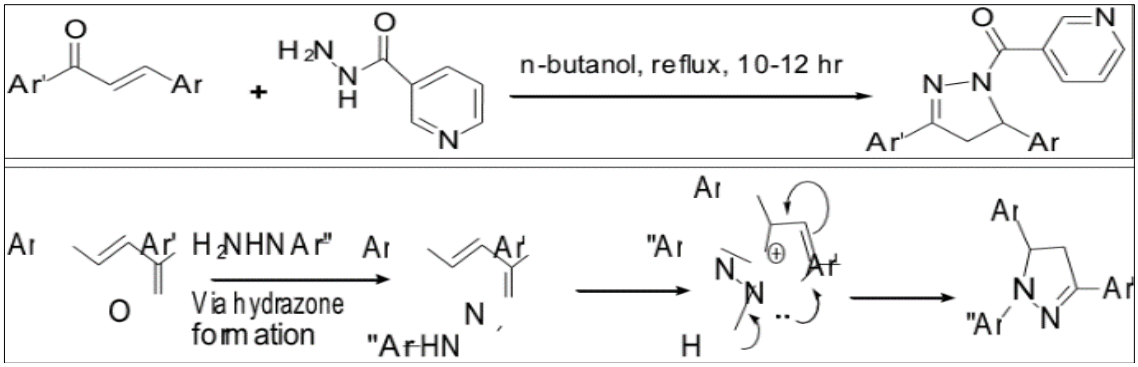
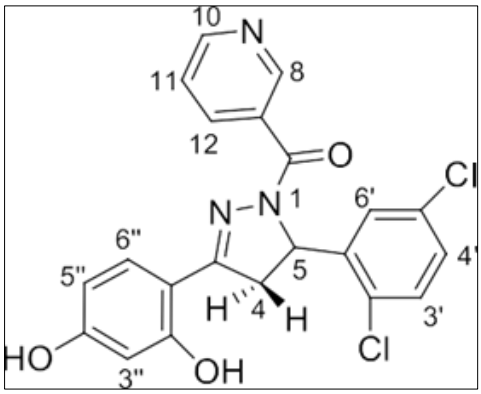


Fig 3: Scheme and mechanism of reaction for synthesis of designed compounds (4a-4p)

In this method, chalcone and nicotinic acid hydrazide were refluxed in *n*-butanol in order to synthesize the desired product (Kini and Gandhi, 2008) [7]. Factors such as the structure and position of the substituents have profoundly influenced the rate of the reaction. The generally accepted interpretation of this reaction, involves the initial formation of an aryl hydrazone with subsequent nucleophilic attack of nitrogen upon the carbon-carbon double bond at β position. Hence the electropositive nature of β carbon may control the overall rate of the reaction. The electropositive nature of β carbon is controlled by the aromatic ring directly connected to it. Halogens being electron withdrawing in nature significantly increase the positive character of β carbon lead to faster reaction while electron donating alkyl and alkoxy groups contributed for slower reaction.

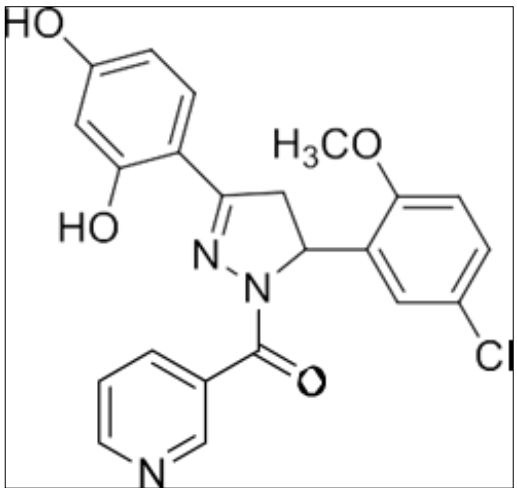
To the solution of the appropriate chalcone 3a - 3p (4 mmole) in 10 mL of *n*- butanol, (0.55 g, 4 mmole) of nicotinic acid hydrazide was added and the reaction mixture was refluxed for 8-10 hr. The excess of solvent was removed under reduced pressure and the reaction mixture was cooled on an ice bath. 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. 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)



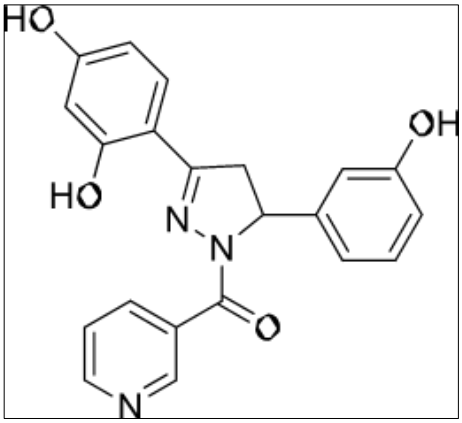
Synthesized by above method from chalcone 3a (4 mmol) and nicotinic acid hydrazide (4 mmol) after 19h reflux; Yield 58%, Pale yellow solid; mp 137-139 °C; IR (KBr) νmax/cm^{-1} 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1560 (C=N stretching), 1260, 1091 (C-O), 1320, 1215 (C-N), 1107, 777 (C-Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR (CDCl3, 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-Hy), 3.10 (1H, dd, *J 17.8* and 4.8, 4-Hx); FAB-MS *m/z*: 427.45 [M +H]$^+$; Anal. Calcd for C21H15Cl2N3O3: 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)



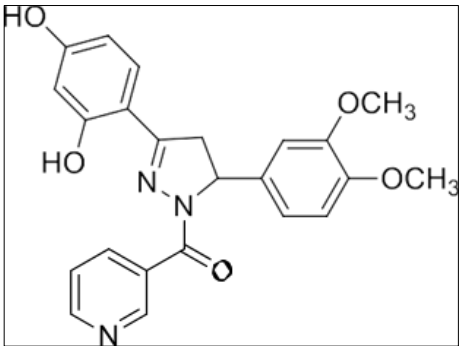
Synthesized by above method from chalcone 3b (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 65%, White solid; mp 145-147 °C; IR (KBr) □max/cm^{-1} 3440 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C-O), 1215 (C-N), 1107 (C-Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR (CDCl3, 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-Hy), 3.70 (3H, s, OCH3-2"), 3.11 (1H, dd, *J 17.5* and 4.6, 4-Hx); FAB- MS *m/z*: 407.34 [M +H]$^+$; Anal. Calcd for C22H18ClN3O4: 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)



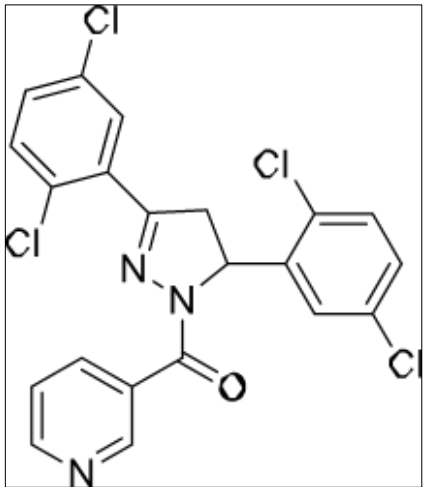
Synthesized by method C from chalcone 3c (4 mmol) and nicotinic acid hydrazide (4 mmol) after 8h reflux; Yield 68%, Pale yellow solid; mp 165-167 °C; IR (KBr) νmax/cm^{-1} 3421 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C-O), 1215 (C-N), 1107 (C-Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{1}H-NMR (CDCl3, 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-Hy), 3.11 (1H, dd, *J 17.5* and 4.6, 4-Hx); FAB-MS *m/z*: 375.76 [M +H]$^{+}$; Anal. Calcd for C21H17N3O4: 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)



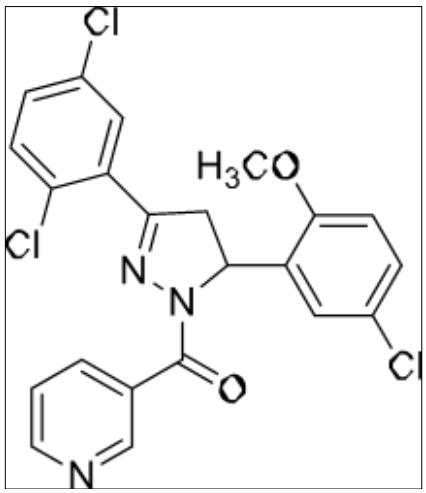
Synthesized by method above from chalcone 3d (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 69%, Light yellow solid; mp 156-159°C; IR (KBr) νmax/cm^{-1} 3415 (O-H), 1668 (N-C=O), 1591 (Ar C=C), 1560 (C=N), 1262, 1096 (C-O), 1210 (C-N), 1102 (C-Cl), 3041, 2954 (C-H), 1501, 1467, 922, 815, 798 (Ar); ^{1}H-NMR (CDCl3, 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-Hy), 3.70 (3H, s, OCH3-3', 4'), 3.11 (1H, dd, *J 17.5* and 4.6, 4-Hx); FAB-MS *m/z*: 419.31 [M +H]$^{+}$; Anal. Calcd for C23H21N3O5: 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) νmax/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 (CDCl3, 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-Hy), 3.08 (1H, dd, *J* 17.5 and 5.1, 4-Hx); FAB-MS *m/z*: 464.96 [M +H]$^{+}$; Anal. Calcd for C21H13Cl4N3O: 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)

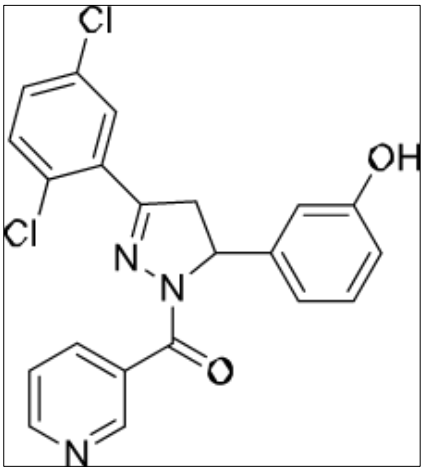


Synthesized by above mentioned method from chalcone **3f** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 70%, Brown solid; mp: 195-197 °C; IR (KBr) νmax/cm^{-1} 1658 (N-C=O), 1587 (Ar C=C), 3083 (Ar C-H), 2934 (C-H), 1637, 1496 (C=N), 817, 738 (Ar CH bend); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 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-Hy), 3.81 (3H, s, OCH3-2'), 3.15 (1H, dd, *J 17.8* and 4.8, 4-Hx); FAB-MS *m/z*: 459.96 [M +H]$^+$; Anal. Calcd for C22H16Cl3N3O2: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.42; H, 3.29; N, 9.48

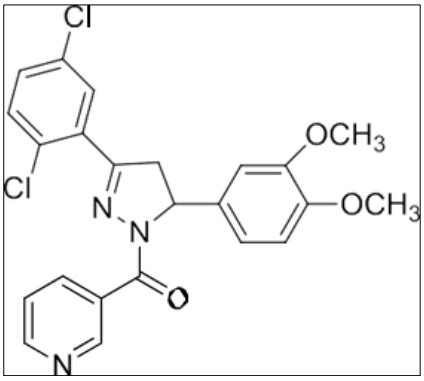
(3-(2",5"-dichlorophenyl)-5-(3'-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1- yl)(pyridin-3-yl)methanone (4g)

Synthesized by method from chalcone **3g** (4 mmol) and nicotinic acid hydrazide (4 mmol)



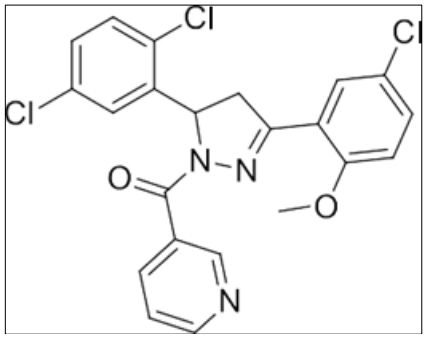
after 14 hrs reflux; Yield 67%, Pale yellow solid; mp 165-167 °C; IR (KBr) νmax/cm^{-1} 3414 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C-O), 1215 (C-N), 1108 (C-Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 10.02 (1H, s, 3'-OH), 9.02 (1H, s, 8-H), 8.73 (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-Hy), 3.18 (1H, dd, *J 17.1* and 4.3, 4-Hx); FAB-MS *m/z*: 412.54 [M +H]$^+$; Anal. Calcd for C21H15Cl2N3O2: 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)

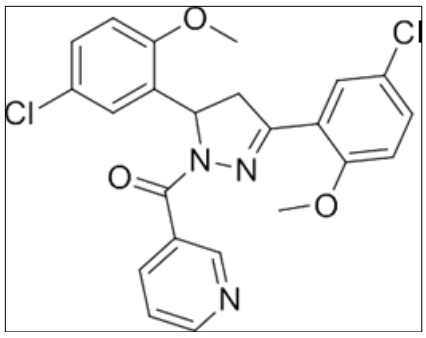


Synthesized by method above from chalcone 3h (4 mmol) and nicotinic acid hydrazide (4 mmol); 68%, white solid; mp 178-180 °C; IR (KBr) nmax/cm^{-1} 1660 (N- C=O), 1596 (Ar C=C), 1560 (C=N), 1260, 1092 (C-O), 1215 (C-N), 1108, 776 (C-Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR (CDCl3, 400 MHz), d (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-Hy), 3.82 (6H, s, OCH3-3', 4'), 3.10 (1H, dd, *J 17.8* and 4.8, 4-Hx); FAB-MS *m/z*: 455.48 [M +H]$^+$; Anal. Calcd for C23H19Cl2N3O3: 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)



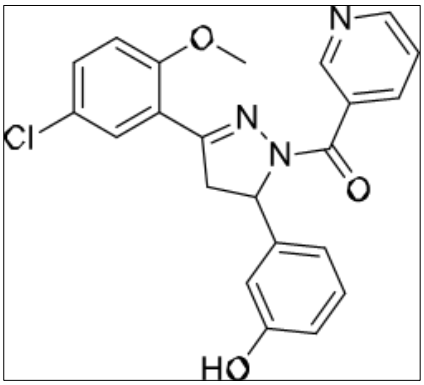
Synthesized by above mentioned method from chalcone 3i (4 mmol) and nicotinic acid hydrazide (4 mmol) after 13 hrs reflux; Yield 63%, Light-yellow solid; mp 142-145 °C; IR (KBr) nmax/cm^{-1} 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C-O), 1121 (C-Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR (CDCl3, 400 MHz), d (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-Hy), 3.82 (3H, s, OCH3-2"), 3.10 (1H, dd, *J* 17.5 and 8.5, 4-Hx); FAB-MS *m/z*: 459.37 [M +H]$^+$; Anal. Calcd for C22H16Cl3N3O2: 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) nmax/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 (CDCl3, 400 MHz), d (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-Hy), 3.87 (6H, s, OCH3-2',2"), 3.08 (1H, dd, *J* 17.5 and 8.1, 4-Hx); FAB-MS

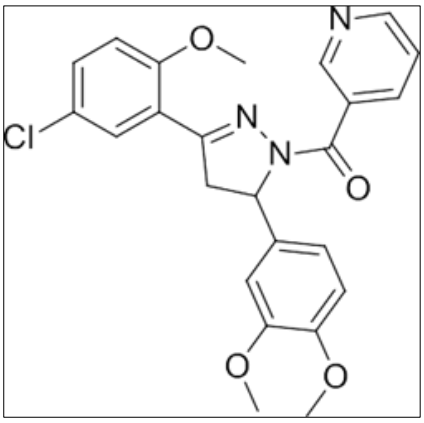
m/z: 456.52 [M +H]$^+$; Anal. Calcd for C23H19Cl2N3O3: C, 60.54; H, 4.20; N, 9.21; Found: C, 60.13; H, 4.19; N, 9.56.

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



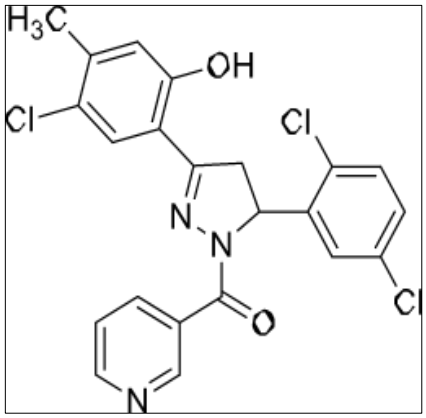
Synthesized by method from chalcone 3k (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 58%, Pale yellow solid; mp 173-175 °C; IR (KBr) nmax/cm^{-1} 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C-O), 1215 (C-N), 1108 (C-Cl), 3045, 2950 (C-H), 1502, 1465, 922, 816, 798 (Ar); ^{1}H-NMR (CDCl3, 400 MHz), d (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-Hy), 3.81 (3H, s, OCH3-2”), 3.16 (1H, dd, *J 17.8* and 4.8, 4-Hx); FAB-MS *m/z*: 407.29 [M +H]$^+$; Anal. Calcd for C22H18ClN3O3: 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)



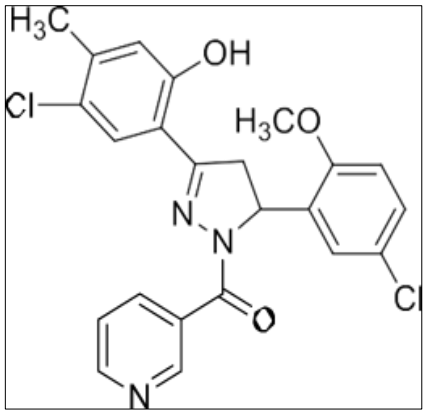
Synthesized by method from chalcone 3l (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 67%, Pale yellow solid; mp 193-195 °C; IR (KBr) nmax/ cm^{-1} 1668 (N-C=O), 1594 (Ar C=C), 1265, 1090 (C-O), 1560 (C=N), 1219 (C-N), 1101 (C-Cl), 3049, 2953 (C-H), 1501, 1468, 920, 816, 798 (Ar); ^{1}H-NMR (CDCl3, 400 MHz), d (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-Hy), 3.80 (3H, s, OCH3-2’), 3.85 (6H, s, OCH3-3”, 4”), 3.16 (1H, dd, *J 17.8* and 4.8, 4-Hx); FAB-MS *m/z*: 451.13 [M +H]$^+$; Anal. Calcd for C23H20ClN3O4: 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 (4 m)



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) nmax/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); 1H-NMR (CDCl3, 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-Hy), 2.85 (3H, s, CH3-4), 3.11 (1H, dd, J 16.5 and 8.5, 4-Hx); FAB-MS m/z: 459.03 [M +H]+; Anal. Calcd for C22H16Cl3N3O2: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.74; H, 3.27; N, 9.56

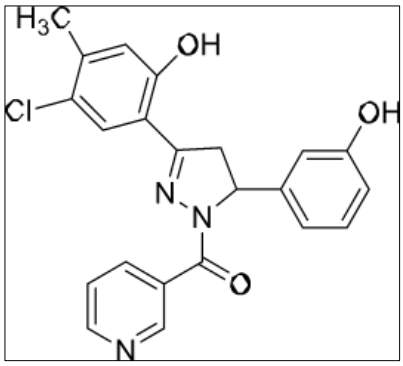
(3-(5”-chloro-2”-hydroxy-4”-methylphenyl)-5-(5’-chloro-2’-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4n)



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) νmax/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-

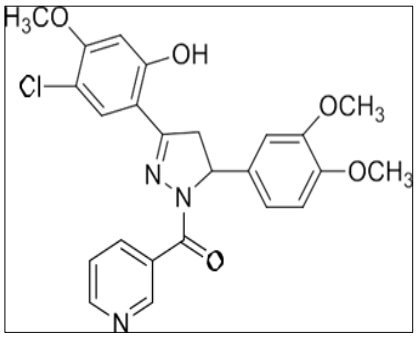
H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); 1H-NMR (CDCl3, 400 MHz), δ (ppm) 11.10 (1H, s, 6”-OH), 9.12 (1H, s, H-8), 8.77 (1H, d, J 4.9, H-10), 8.12 (1H, d, J 7.2, H-12), 7.76 (2H, t, J 8.3, H-6’, 11), 7.38-7.42 (4H, m, H-3’, 4’, 2”, 5”), 5.95 (1H, d, J 10.2 H-5), 3.98 (1H, dd, J 17.2 and 6.5, 4-Hy), 3.85 (6H, s, OCH3-4”,2’), 3.03 (1H, dd, J 17.5 and 8.1, 4-Hx), 2.85 (3H, s, CH3-4); FAB-MS m/z: 455.08 [M +H]+; Anal. Calcd for C23H19Cl2N3O3: 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) nmax/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 (CDCl3, 400 MHz), d (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-Hy), 3.80 (3H, s, OCH3-4’), 3.16 (1H, dd, *J 17.8* and 4.8, 4-Hx), 2.32 (3H, s, CH3-4; FAB-MS *m/z*: 407.58 [M+H]$^{+}$; Anal. Calcd for C22H18ClN3O3: 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) nmax/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 (CDCl3, 400 MHz), d (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-Hy), 3.85 (6H, s, OCH3-3’, 4’), 3.80 (3H, s, OCH3-4”), 3.16 (1H, dd, *J 17.8* and 4.8, 4-Hx); FAB-MS *m/z*: 451.13 [M +H]$^{+}$; Anal. Calcd for C24H22ClN3O5: C, 61.61; H, 4.74; N, 8.98; Found: C, 61.12; H, 4.50; N, 8.45

Antimicrobial Evaluation

Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae, protozoa, and the infectious agents at the borderline of life that are called viruses. It is concerned with their form, structure, reproduction, physiology, metabolism and classification. It includes the study of their distribution in nature, their relationship to each other and to other living organisms, their effects on human beings, animals and plants, and their reactions to physical and chemical agents.

Microorganisms are closely associated with the health and welfare of human beings. Some microorganisms are beneficial and others are detrimental (Tripathi, 2003) [12]. For example, microorganisms are involved in making of yoghurt, cheese, and wine, in production of penicillin, interferon and alcohol, microorganisms can cause disease, spoil food and deteriorate materials like iron pipes, glass lenses and wood pilings. A number of microbial species are responsible for mild to severe infectious diseases in man. Drugs, which are helpful in combating the infections diseases caused by microbes, are known as anti-microbial agents. Anti-microbial agents are chemical substance of either natural or synthetic origin, which suppress the growth of different microorganisms and may eventually destroy them. As literature reveals that pyrazoline compounds showed anti-microbial activities, anti-microbial screening of the synthesized compounds was carried out to explore their potential as anti-microbial agents.

Methods of Antimicrobial Evaluation

After the development of desired new drug molecules, with different structure, an *in vitro* screening is done necessary to uncover the desired activity of the compounds. The inhibition of the microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficacy of the synthesized compounds.

The following two are the methods available for screening of the antimicrobial agent:

1. Turbidimetric/photometric/tube dilution method.
2. Agar diffusion/cup-plate/cylinder plate method.

Turbidimetric method

In this method a graded concentration of the antimicrobial substance in sterile fluid nutrient media is prepared. All of them are inoculated with a loop of specific microorganism. A positive control, a negative control and a blank is also maintained. They are incubated at 37 °C for 24 hours or necessary conditions depending on the organism chosen. Among the different concentrations of the substance, the least one, which inhibits the growth of the microorganism, is noted visually or by measuring the percentage transmittance or absorbance at 530 nm against a blank. By this method minimum inhibitory concentration (MIC) for the newly synthesized compound is determined.

Agar diffusion method

This method gives the extent of growth of the microorganism, inoculated into a solid nutrient agar bed by the antimicrobial substance. The test substance is kept in a cup made-up of agar bed and diffuses to inhibit the growth of microorganism. The diameter of zone of inhibition measured in comparison with suitable drug substance is considered as potency of that substance. The diameter of zone of inhibition is directly proportional to the concentration of the drug substances added into the cup, thickness of the agar bed, and diffusion coefficient of the antimicrobial substance into the agar cup, sensitivity of the microorganism to the test substance and temperature.

The appropriate media is sterilized and cooled to 42 °C, incubated with the test organism, mixed uniformly and poured into Petri dishes and cooled to room temperature. Bores are made into it specified test solution is added and left at room temperature for 30 minutes. Then incubated at 37 °C for 24 hours. The zone of inhibition is measured in mm after 24 hrs (J. H. Jorgensen *et al.* 1999, NCCLS, 1993) [6, 9].

Materials and Methods

Method followed: Cup plate method.

Requirements: Petri plates, glass syringes, cork borers, inoculation loop, cotton.

Working procedure

Stock solution of the synthesized compounds and standard drug ciprofloxacin used were prepared in dimethyl sulfoxide taken in the concentration of 100 µg/ml.

Microorganism used

Standard cultures of *Staphylococcus aureus (Gram +ve), Bacillus subtilus (Gram +ve), Escherichia coli (Gram -ve), Pseudomonas aeruginosa (Gram -ve). Aspergillus niger* species and *candida albicans* were obtained from Department of Microbiology, Barkatullah University, Bhopal. Staining technique and bio-chemical reaction identified the microorganism.

The microorganisms, which were maintained by sub culturing, was used at regular intervals in nutrient agar medium.

Preparation of Inoculum

The suspension of all the organisms were prepared as Mac-Farland Nephelometer standard (Baily and Scott 1990) [15]. A 24 hrs old culture was used for the preparation of bacterial suspension. Suspension of organisms were made in sterile isotonic solution of sodium chloride and turbidity was adjusted.

Ingredients of Media

SI. No.	Ingredients	Weight in gm
1	Beef extract	10.0
2	Peptone	10.0
3	Agar	20.0
4	Distilled water	1000 ml
	pH	5.4

Preparation of assay media

The above mentioned quantities of different ingredients were accurately weighed and dissolved in appropriate amount of distilled water. Media so prepared was sterilized by autoclaving at 121 °C for 15 min.

Procedure

The petri plate were washed thoroughly and sterilized in hot air oven at 160 °C for one hr 30 ml of sterile nutrient agar medium was poured into sterile Petri dishes and allow to solidify. The petri plates were incubated at 37 °C for 24 hrs to check for sterility. The medium was seeded with the organism by spread plate method using sterile cotton swabs. Bores were made on the medium using sterile borer and 0.1 ml of the Ciprofloxacin at a concentration of 100µg/ml was taken as standard reference. A control having only DMSO in the cup was maintained in each plate. The petri plates were kept in refrigerator at 4 °C for 15 min, allowing diffusion to take place. Agar diffusion, the petri plate were incubated at 37 °C for 24 hrs and zone of inhibition were observed.

Antimicrobial activity of all the compounds were carried out against microorganisms. The Mean Zone of inhibition of the derivatives is reported for all compounds against different micro-organism. All the synthesized compounds were purified, characterized and screened for their antimicrobial ctivity. They were tested against two gram positive (*Staphylococcus aureus*, and *Bacillus subtilis*) and two gram negative (*Escherichia coli* and *Pseudomonas aeruginosa)* organisms.

The antifungal activity tested against *Candida albicans* and *Aspergillus niger*. The activity of the derivatives were performed by cup plate method at different concentration level. Ketoconazole was used as standard drug at concentration remains same.

Results and Discussion

Synthesis of Pyrazoline Derivatives

The strategy to synthesise compounds 3(a-p) and 4(a-p) has been shown 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.

Structures of compounds 3(a-p) and 4(a-p) were confirmed by IR, NMR data as well as their distinct Rf values in TLC analysis. Distinct stretching band of -C=C- aromatic appears in 1491-1603 cm-1 region. Out-of-plane bending vibrations occurring in 637-986 cm-1 region could be ascribed to trans-olifinic structure. 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 ^{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. Chemical shifts between 6.54-7.80 ppm (Multiplets), 5.99-5.75 ppm (Singlet) and 3.76-3.96 ppm (Singlet) regions, ascribed to benzene, Ar O-H and -OCH3 protons respectively, indicating presence of mentioned protonic groups in chalcones are in conformity of

infrared inferences regarding success of the condensation reactions leading to formation of chalcones under study. Signals around d value 3.1 and 3.9 ppm recorded as doublet of doublets (dd) were assigned to 4-Hx and 4-Hy 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. These signals clearly showing the formation of pyrazoline ring.

In vitro antimicrobial activity

The derivatives were screened for antibacterial and antifungal activity using ciprofloxacin and Ketoconazole as standards. Ciprofloxacin has shown maximum activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* with the zone of inhibition of 19mm, 17mm, 20 mm and 20 mm while Ketoconazole has shown maximum activity against *Aspegallus niger* and *Candida albicans* with zone of inhibition of 22 mm and 22 mm (Table 2 and Fig 4-6).

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

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.

However, further studies on activity and long term toxicity are to be carried out before any conclusion are drawn, as these categories of drug are known to have potential antimicrobial activity. Testing on different models can further substantiate the antimicrobial activity of the synthesized analogues.

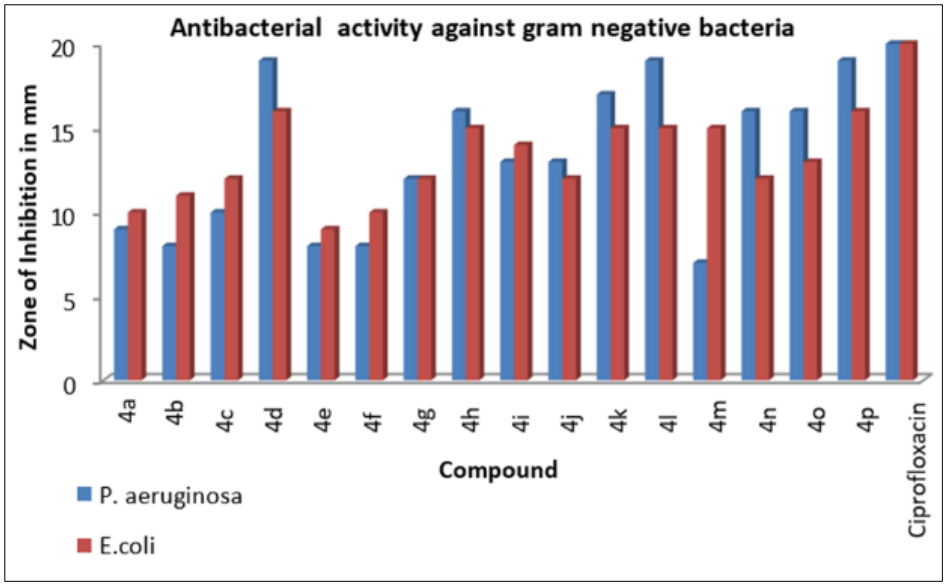


Fig 4: Representation of Zone of Inhibition of the derivatives by Bar graph (Gram negative organisms)

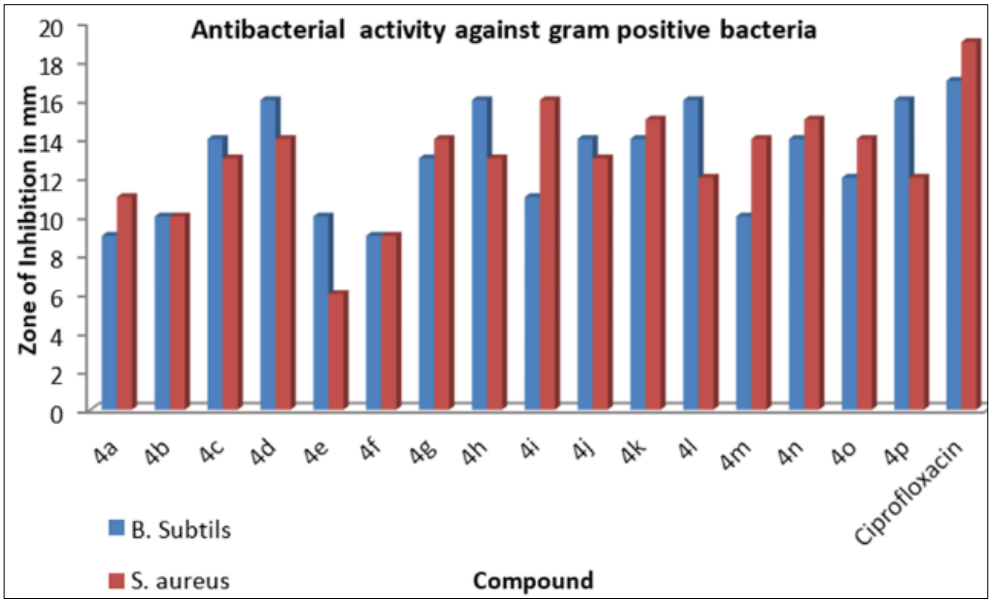


Fig 5: Representation of Zone of Inhibition of the derivatives by Bar graph (Gram positive organisms)

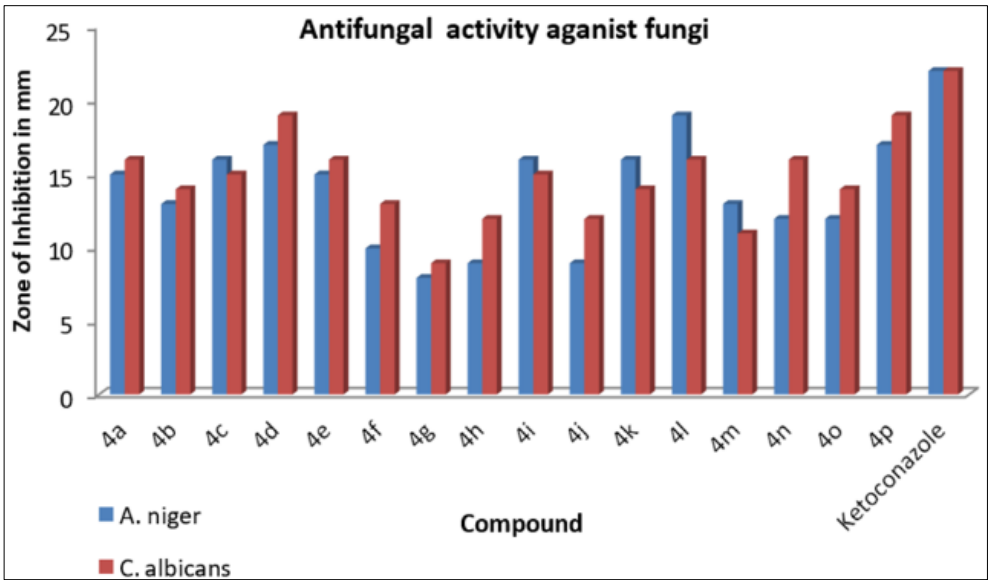


Fig 6: Representation of Zone of Inhibition of the derivatives by Bar graph (fungal organisms)

Conclusion

In this context, chalcones and Pyrazoline are promising candidates, as these individually possess multifarious pharmacological profiles including antimalarial activities with different mode of action. The substitution on these two pharmacophores into novel scaffolds and evaluation of their biological activities have not yet been reported.

The strategy to synthesis of designed compounds 3a-3p and 4a-4p has been shown in Fig.

1-3 and Table 4.1. In the first step, syntheses of chalcones 3a-3p 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 or methanol respectively, 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. Physico-chemical characterization, melting point, FT-IR, ^{1}H-NMR mass spectral and elemental analysis of the synthesized compounds were done. The results showed that the observed values are in full agreement with the expected values and confirm the anticipated structures of synthesized compounds. The IR spectra of synthesized compounds showed absorption bands which are characteristic of the anticipated structure of the synthesized compounds. The NMR spectra of synthesized compounds showed signals for both aliphatic and aromatic protons, characteristic of the anticipated structure of the synthesized compounds. The fragmentation patterns obtained in the mass spectra also confirm the anticipated structures of the synthesized compounds. All the synthesized compounds were found to be soluble in most of the organic solvents (Chloroform, DMSO, ethyl acetate, acetone and dichloromethane) and insoluble in water. All the synthesized compounds were evaluated for their *in vitro* antimicrobial activity against *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121) representing Gram positive 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 synthesized compounds (4a-4p) are summarized in Table 2. 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.

In conclusion, 2-pyrazoline compounds were successfully synthesized and tested for their *in vitro* antimalarial activity, and the compound 4p was the most promising compound identified from the study. Compounds 4d and 4p could serve as basic formats to synthesize new analogues for antimicrobial evaluation and study of structure-activity relationships. These results offer new possibilities for further improvements in the antimicrobial performance of these derivatives.

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