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Synthesis And Assessment of Antimicrobial Potential of Some Novel 1, 3, 5-Trisubstituted Pyrazolines Derivatives

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KEYWORDS:
Pyrazoline, antibacterial, nuclear magnetic resonance (NMR), infrared (IR), chalcones, derivatives

ABSTRACT
The chalcones and Pyrazoline demonstrate diverse pharmacological profiles, including antimalarial activity with distinct mechanisms of action, making them highly intriguing options. The synthesis of the intended compounds 3a-3p and 4a-4p was accomplished using the widely recognized Claisen-Schmidt reaction. The resulting products were purified by recrystallization from methanol, resulting in a yield of 60-70%. The required product was synthesized by refluxing chalcone and nicotinic acid hydrazide in n-butanol or methanol, respectively, during the second stage. The synthesized compounds were subjected to physico-chemical characterisation, including investigation of their melting point, FT-IR, 1H-NMR mass spectrum, and elemental properties. The results demonstrated complete concordance between the observed values and the expected values, therefore validating the anticipated structures of the produced molecules. The IR spectra of the synthesized compounds exhibited absorption bands that are indicative of the expected structure of the produced molecules. The NMR spectra of the synthesized compounds exhibited signals corresponding to both aliphatic and aromatic protons, which are indicative of the expected structure of the produced compounds. The synthesized compounds were assessed for their antibacterial activity. The compounds were further assessed for their in vitro antifungal efficacy. The outcomes of the in vitro antibacterial and antifungal activity of the produced compounds (4a – 4p) are exhibited potential antimicrobial and antifungal activity.

INTRODUCTION

Medicinal or Pharmaceutical Chemistry is a scientific discipline that combines chemistry and biology to focus on the creation, production, and advancement of substances used for medical treatment. Annually, a substantial number of novel compounds are produced and subjected to screening to evaluate their pharmacological activity. These compounds are classified into many categories based on their chemical structure or pharmacological function.

Illnesses produced by organisms from the Kingdom Monera, Fungi, and Protista pose significant threats to the well-being of humans, plants, and cattle. These inflict significant destruction. The majority of bacteria and protozoa are single-celled creatures, with a high tendency for mutation. This complicates the therapy by rendering the utilization of current medications ineffective (Thomas et al., 1998).

An obstacle in the advancement of future malarial chemotherapy is the need to create molecules that are both inventive in terms of their chemical structure and specific in their molecular target. Various strategies for antimalarial drug discovery are currently being utilized. These include enhancing the effectiveness of existing drugs through combination therapy, creating analogs of existing drugs, investigating potent compounds derived from natural sources such as plants, repurposing drugs originally developed for other diseases, and assessing the potential of drug-resistance reversers (chemosensitizers) and new chemotherapeutic targets. These techniques are expected to offer promising potential in the quest for secure and effective novel medications for malaria therapy (Pandeya et al., 1999).

Microorganisms are present everywhere. The relationship between humans and these bacteria is often symbiotic, since they form part of the microbial flora in the human stomach and skin. However, the tissues of healthy animals and plants are predominantly devoid of

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microorganisms. This is
Attained by employing a variety of general and targeted defensive measures. Microorganisms can cause infections when they violate the body's defenses by expressing virulence factors and adapting to a harmful lifestyle. This can occur as a result of sickness, unintentional trauma, or the implantation of medical equipment.
An infection refers to the infiltration of microorganisms into a host organism, followed by the rapid multiplication of these invading organisms and the subsequent response of the host to these bacteria. On the other hand, an infectious illness is characterized by an infection that leads to the emergence of clinical symptoms. The several pathways by which bacteria can enter the body, known as portals of entry, include the skin, respiratory system, digestive tract, urinogenital tract, and conjunctiva.

A significant proportion of bacteria exhibit susceptibility to antibiotics, and the majority of infectious infections are treatable. Despite the medical advancements made in the previous century, infectious illnesses remain the second most common cause of mortality. Only a maximum of six highly contagious illnesses, namely pneumonia, TB, diarrheal diseases, malaria, measles, and more recently HIV/AIDS, are responsible for 50% of all untimely deaths, primarily affecting children and young people. Approximately 14 to 17 million individuals perish annually as a result of infectious illnesses, with the overwhelming majority residing in underdeveloped nations. Antimicrobial drugs, once discovered, have become extensively utilized and have demonstrated remarkable efficacy in treating bacterial illnesses. The antibacterial agents might possess either bacteriostatic or bactericidal properties.

In 1907, Paul Ehrlich coined the word chemotherapy, originally to describe treatment for parasites, but now it encompasses the use of specific chemical compounds that target bacteria or cancer cells.
In order for an antimicrobial agent to effectively combat a specific microorganism, it must satisfy two requirements: an essential objective that is vulnerable to a little dosage of the antibiotic
The presence of the micro-organism is essential, and the antibiotic must effectively enter the bacterial envelope and reach the intended target in a enough amount (Sithambaram et al., 2016).

METHODOLOGY

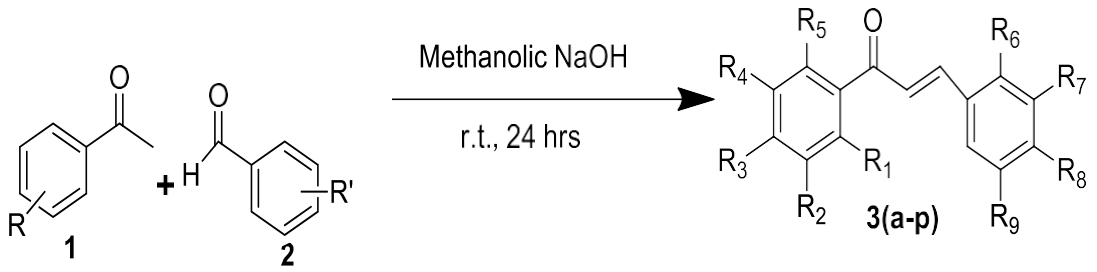
All the other chemicals used were obtained from Sigma-Aldrich, Spectrochem and High Media.

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 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 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.



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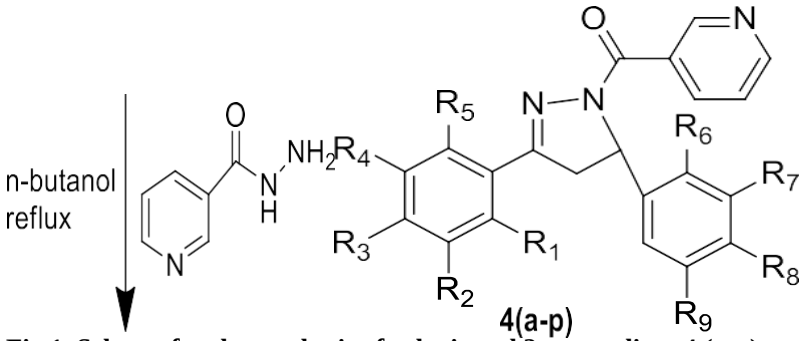
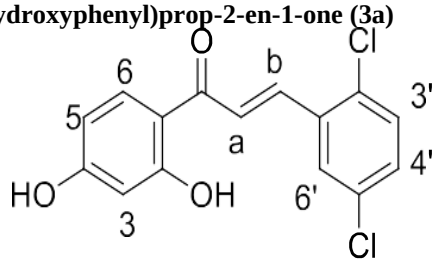


Fig 1. Scheme for the synthesis of substituted 2-pyrazolines 4 (a-p).

General method of synthesis of Chalcone (3a – 3p)
Chalcones are synthesized by Claisen-Schmidt condensation (Furniss *et al.*, 1989; Kumar *et al.*, 2010) of aldehyde and ketone by base catalyzed or acid catalyzed followed by dehydration to yield chalcones.

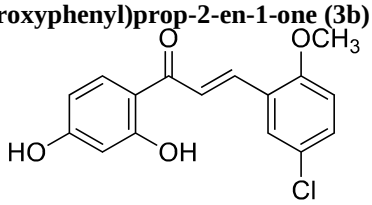
General procedure for the synthesis of chalcones (3a-3p)
To a solution of substituted acetophenone (16 mmole) in 10 mL of methanol on an ice bath, freshly prepared 2 N methanolic NaOH solution (60 mL) was added and stirred for 10 min. To this, appropriate aldehyde (16 mmole) was added and stirred at 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)



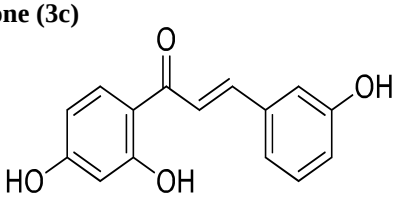
Synthesized by above method from 2,4-dihydroxyacetophenone (16 mmol) and 2,5-dichlorobenz aldehyde (16 mmol); Yield 85%, White solid; mp 165–167°C; *Rf* (EtOAc/Hex 4:6) 0.45; IR (KBr) νmax/cm^{-1} 3440 (O-H), 1669 (C=O), 1597 (Ar C=C), 750 (C–Cl), 3063, 2931, 1625, 1415, 1325, 1296, 1134, 1153, 1046,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

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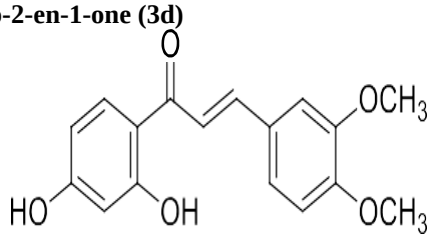
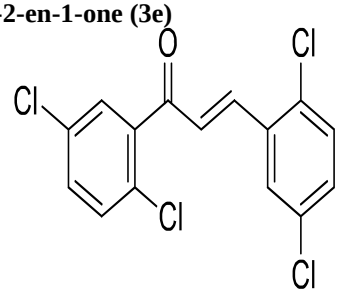
MHz), δ (ppm) 9.95 (3H, s, OH-2,4, 3'), 7.71 (1H, d, *J* 15.3, H-b), 7.61-7.54 (4H, m, H-3, 5, 6, 6'), 7.31 (1H, d, *J* 16.0, H-a), 7.21-7.15 (4H, m, *J* 4.8, H-2, 2', 4', 5') FAB-MS *m/z* 256.08 $[M+H]^+$; Anal. Calcd for C15H12O4: C, 70.31; H, 4.72; Found: C, 70.37; H, 4.12;

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



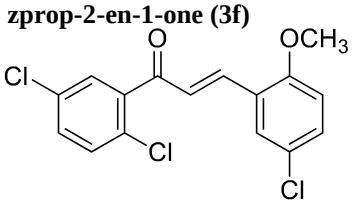
Synthesized by above method from 4-Hydroxyacetophenone (16 mmol) and 4-hydroxybenzaldehyde (16 mmol); Yield 76%, White solid; mp 108–110°C; *Rf* (EtOAc/Hex 4:6) 0.34; IR (KBr) νmax/cm^{-1} 3435 (O-H), 1661 (C=O), 1592 (Ar C=C), 760 (C–Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ^{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)



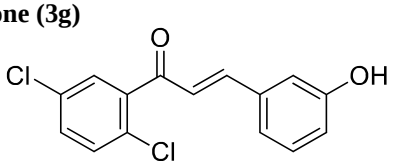
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 69%, White crystalline solid; mp 138–140°C; *Rf* (EtOAc/Hex 4:6) 0.38; IR (KBr) νmax/cm^{-1} 1662 (C=O), 1598 (Ar C=C), 743 (C–Cl), 3064, 2930, 1627, 1415, 1325, 1296, 1134, 1117, 1047, 982, 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-dichloroph henyl) zprop-2-en-1-one (3f)



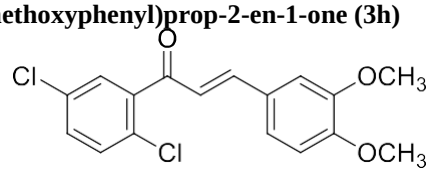
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 5- chloro, 2-methoxybenzaldehyde (16 mmol); Yield 67%, Creamy-coloured fine needles; mp 148–150°C; *Rf* (EtOAc/Hex 4:6) 0.79; IR (KBr) νmax/cm^{-1} 1662, 1216 (C=O), 1594 (C=C), 1261, 1026 (C–O), 742 (C–Cl), 3061, 1591, 1457, 1384, 1296, 1194, 980, 854, 756 (Ar); ^{1}H-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)

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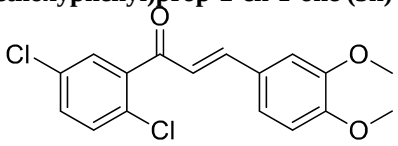
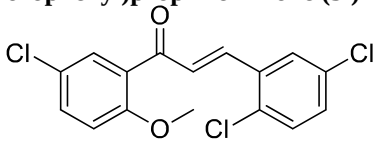
0.67; IR (KBr) νmax/cm^{-1} 1653 (C=O), 1605 (Ar C=C), 1547 (COC=C), 1261, 1025 (C-O), 1150 (C-Cl), 3051 (Ar C-H), 2932, 2841 (C-H), 1524, 1472, 1147, 969 (Ar); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 7.81 (1H, d, *J* 15.5, H-b), 7.75 (1H, d, *J* 8.5, H-6), 7.61 (1H, d, *J* 15.1, H-a), 7.40 (1H, d, *J* 6.8, 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-(2,5-dichlorophenyl)-3-(3',4'-dimethoxyphenyl)prop-2-en-1-one (3h)



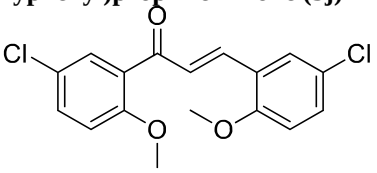
Synthesized by above method from 2,5-dichloroacetophenone (16 mmol) and 3, 4-dimethoxybenzaldehyde (16 mmol); Yield 69%, white amorphous solid; mp 115-118°C; *Rf* (EtOAc/Hex 4:6) 0.67; IR (KBr) νmax/cm^{-1} 1653 (C=O), 1605 (Ar C=C), 1547 (COC=C), 1261, 1025 (C-O), 1150 (C-Cl), 3051 (Ar C-H), 2932, 2841 (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)



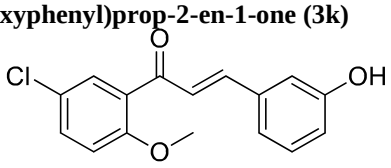
Synthesized by above method from 2- methoxy, 5-chloro-acetophenone (16 mmol) and 2, 5-dichlorobenzaldehyde (16 mmol); Yield 66%, Yellow solid; mp 105-107°C; *Rf* (EtOAc/Hex 4:6) 0.32; IR (KBr) νmax /cm^{-1} 1650 (C=O), 1585 (Ar C=C), 1517 (COC=C), 1268, 1029 (C-O), 1150 (C-Cl), 3058 (Ar C-H), 2933 (C-H), 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)



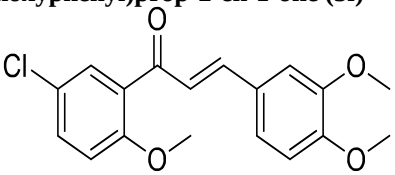
Synthesized by above mentioned method A from 2-methoxy, 5- chloroacetophenone (16 mmol) and 2-methoxy, 5-chlorobenzaldehyde (16 mmol); Yield 3.7 g, 69%, Yellow solid; mp 107-109°C; *Rf* (EtOAc/Hex 4:6) 0.35; IR (KBr) νmax /cm^{-1} 1655 (C=O), 1580 (Ar C=C), 1519 (COC=C), 1264, 1025 (C-O), 1157 (C-Cl), 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)



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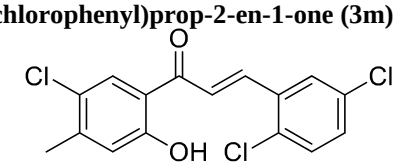
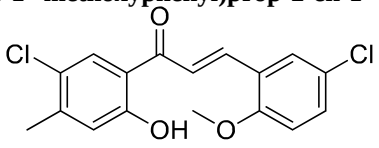
Synthesized by above method from 2-methoxy, 5-chloroacetophenone (16 mmol) and 3,4-dimethoxybenzaldehyde (16 mmol); Yield 71%, Pale yellow solid; mp 117- 119°C; *Rf* (EtOAc/Hex 4:6) 0.49; IR (KBr) νmax /cm^{-1} 1645 (C=O), 1589 (Ar C=C), 1512 (COC=C), 1265, 1025 (C-O), 1151 (C-Cl), 3064 (Ar C-H), 2941 (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)



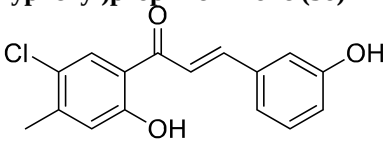
Synthesized by above method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); Yield 71%, White amorphous solid; mp 94–97°C; *Rf* (EtOAc/Hex 4:6) 0.67; IR (KBr) νmax/cm^{-1} 3447 (O-H), 1660 (C=O), 1596 (Ar C=C), 740 (C–Cl), 3064, 2965, 1647, 1434, 1320, 980, 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)



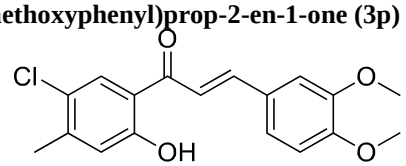
Synthesized by above method from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2-methoxy, 5-dichlorobenzaldehyde (16 mmol); Yield 68%, White solid; mp 137–139°C; *Rf* (EtOAc/Hex 4:6) 0.48; IR (KBr) νmax/cm^{-1} 3431 (O-H), 1657 (C=O), 1586 (Ar C=C), 760 (C–Cl), 3064, 2964, 1643, 1434, 985, 753 (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 2 (Ozdemir *et al.*, 2008).

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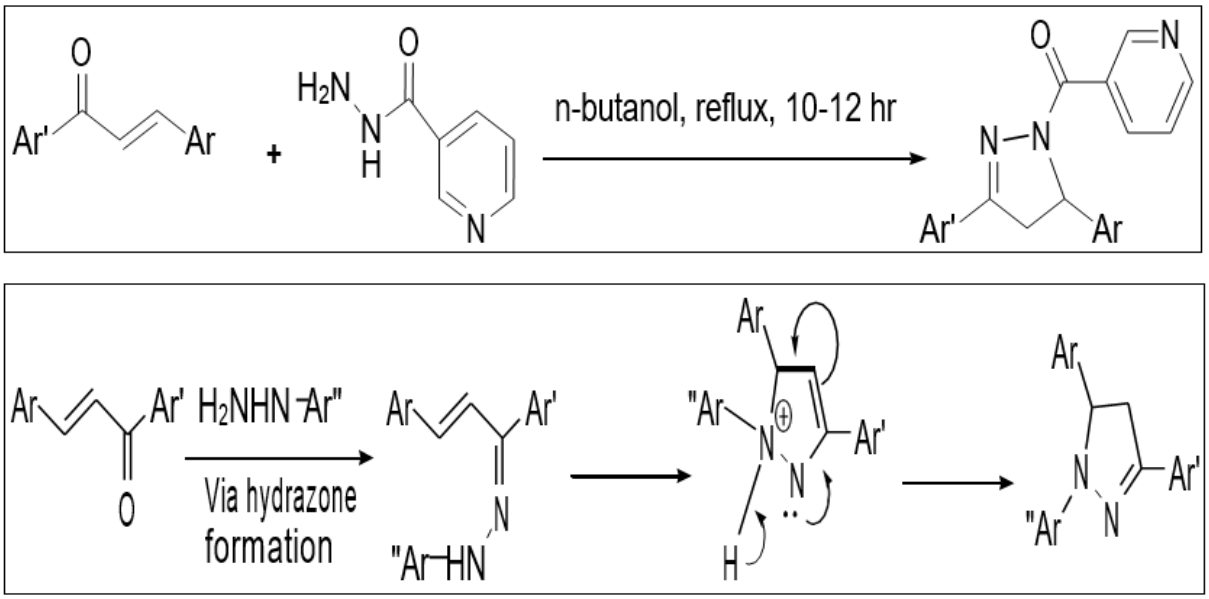


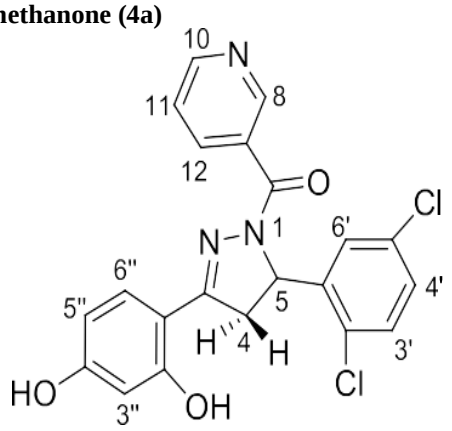
Figure 2: Scheme and mechanism of reaction for synthesis of designed compounds

(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). 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;

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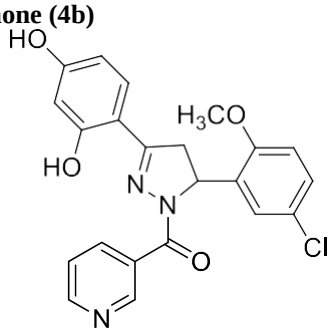
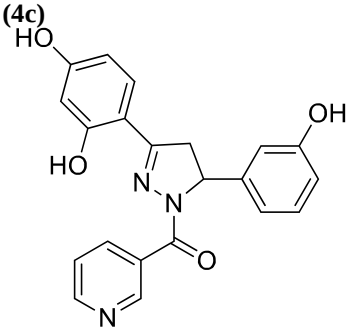
(5-(5'-chloro-2'-methoxyphenyl)-3-(2",4"-hydroxy phenyl)-4,5-dihydro-1H-pyrazol- 1-yl)(pyridin-3-yl) methanone (4b)



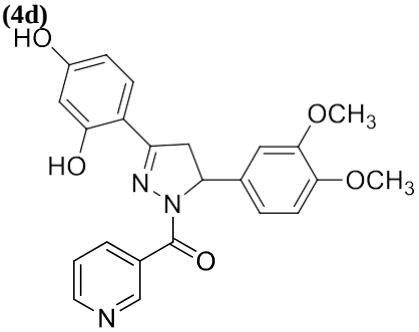
Synthesized by above method from chalcone **3b** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 65%, White solid; mp 145-147°C; IR (KBr) νmax/cm^{-1} 3440 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C–O), 1215 (C-N), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ^{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)metha none (4c)



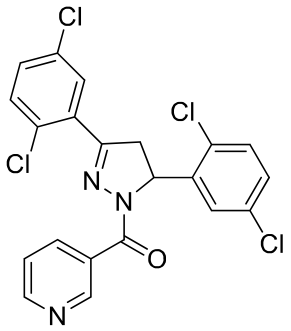
Synthesized by method C from chalcone **3c** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 8h reflux; Yield 68%, Pale yellow solid; mp 165-167°C; IR (KBr) νmax/cm^{-1} 3421 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C–O), 1215 (C-N), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 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)metha none (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)metha none (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,

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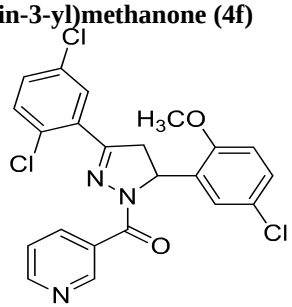
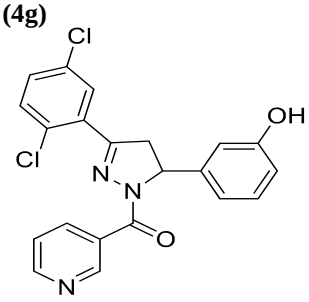
738 (Ar CH bend); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 9.10 (1H, s, H-8), 8.75 (1H, d, *J* 4.5, H-10), 8.11 (1H, d, *J* 7.4, H-12), 7.72 (3H, m, H-6", 6', 11), 7.41-7.52 (4H, m, H-3',4',3",4"), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 12.5, 4-Hy), 3.08 (1H, dd, *J* 17.5 and 5.1, 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)



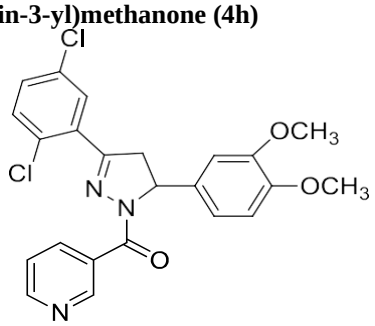
Synthesized by above mentioned method from chalcone **3f** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 70%, Brown solid; mp: 195-197°C; IR (KBr) νmax/cm^{-1} 1658 (N-C=O), 1587 (Ar C=C), 3083 (Ar C-H), 2934 (C-H), 1637, 1496 (C=N), 817, 738 (Ar CH bend); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 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)



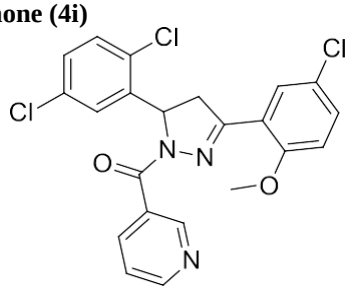
Synthesized by method from chalcone **3g** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 14 hrs reflux; Yield 67%, Pale yellow solid; mp 165-167°C; IR (KBr) νmax/cm^{-1} 3414 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 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) νmax/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), δ (ppm) 11.05 (1H, s, 4'-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.16 (1H, d, *J* 7.2, 12-H), 7.58 (2H, dd, *J* 7.6 & 6.2, H-11), 7.48 (2H, d, *J* 4.8, H-3", 4''), 6.87-6.70 (3H, m, H-2', 5', 6'), 5.93 (1H, dd, *J 12.3* and 6.2, H-5), 3.82 (1H, dd, *J 17.1* and 11.2, 4-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

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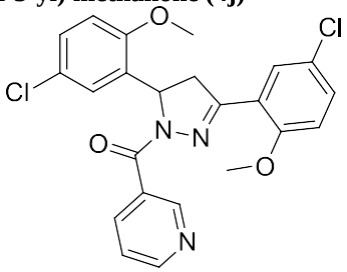
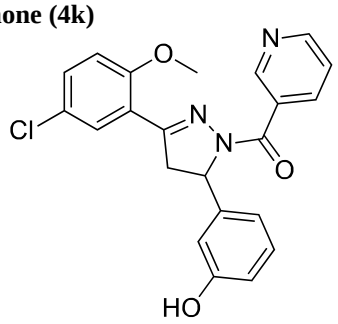
13 hrs reflux; Yield 63%, Light-yellow solid; mp 142-145°C; IR (KBr) νmax/cm^{-1} 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.80 (H, s, H-6"), 7.56-7.60 (2H, m, H-4', 11), 7.37-7.43 (3H, m, H-3', 6', 4"), 6.99 (1H, d, *J* 5.1, H-3'), 5.95(1H, dd, *J* 10.5 and 6.1, H-5), 3.90 (1H, dd, *J* 17.3 and 6.1, 4-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)



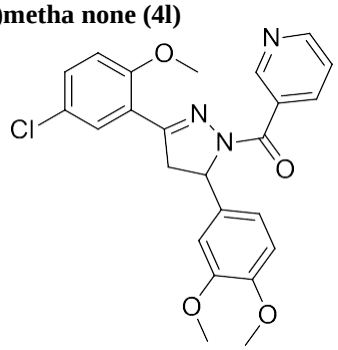
Synthesized by above mentioned method from chalcone **3j** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 62%, Light-yellow solid; mp 152-155°C; IR (KBr) νmax/cm^{-1} 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-NMR (CDCl3, 400 MHz), δ (ppm) 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.72 (2H, t, *J* 8.3, H-6", 11), 7.32-7.38 (4H, m, H-3", 4", 4', 6'), 6.83 (1H, d, *J* 5.5, H-3'), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 6.5, 4-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) νmax/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), δ (ppm) 10.04 (1H, s, 3'-OH), 9.04 (1H, s, 8-H), 8.69 (1H, d, *J* 3.9, 10-H), 8.18 (1H, d, *J* 7.2, 12-H), 7.82 (2H, d, *J* 7.6, H-6"), 7.61 (1H, dd, *J* 12.6 and 6.4, H-11), 7.36 (1H, d, *J* 7.1, H-3"), 7.25 (1H, t, *J* 7.6, H-5'), 6.99-7.04 (2H, m, H-2', 3"), 6.75-6.87 (2H, m, H-4', 6'), 5.93 (1H, dd, *J* 12.3 and 6.2, H-5), 3.89 (1H, dd, *J* 17.5 and 11.6, 4-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)metha none (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) νmax/ 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), δ (ppm) 9.04 (1H, s, 8-H), 8.69 (1H, d, *J* 3.9, 10-H), 8.18 (1H, d, *J* 7.2, 12-H), 7.66 (2H, d, *J* 7.6, H-6", 11-H), 6.85-6.90 (4H, m, H-2", 3', 4', 6'), 5.93 (2H, dd, *J 12.3* and 6.2, H-5, 5"), 3.89 (1H, dd, *J 17.5* and 11.6, 4-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 (4m)

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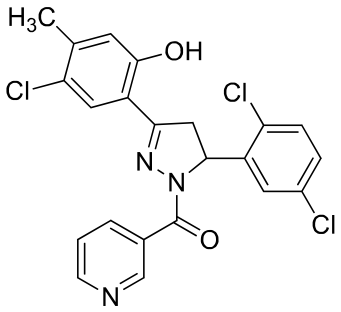
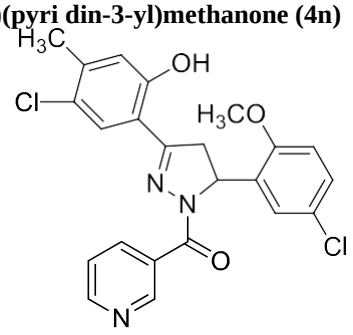


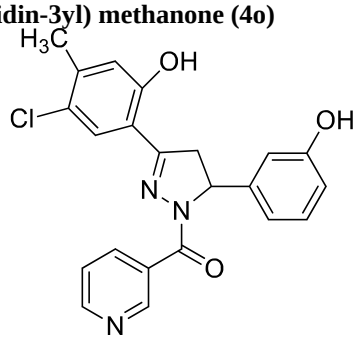
Synthesized by above method from chalcone **3m** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 62%, Light-yellow powder; mp 179-181°C; IR (KBr) νmax/cm^{-1} 3218 (O-H), 1641 (N-C=O), 1623, 1574 (C=N), 1591 (Ar C=C), 1255, 1024 (C–O), 125 (C–Cl), 2913 (C-H), 1471, 1320, 1239 (C-N), 945 (trans ethylenic H), 822, 764 (Ar C-H bend); ^{1}H-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-1yl)(pyri din-3-yl)methanone (4n)



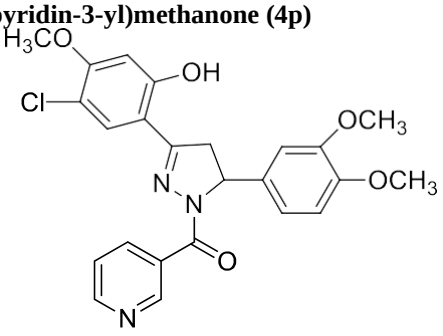
Synthesized by above mentioned method from chalcone **3n** (4 mmol) and nicotinic acid hydrazide (4 mmol); Yield 42%, Light-yellow solid; mp 169-172°C; IR (KBr) νmax/cm^{-1} 3215 (O-H), 1649 (N-C=O), 1622, 1585 (C=N), 1590 (Ar C=C), 1252, 1012 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ^{1}H-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-3yl) 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) νmax/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), δ (ppm) 10.05 (2H, s, 6’, 3”-OH), 9.09 (1H, s, 8-H), 8.63 (1H, d, *J* 3.9, 10-H), 8.10 (1H, d, *J* 7.2, 12-H), 7.65-7.60 (2H, m, H-2”, 11), 6.78-6.84 (2H, m, H-4’, 6’), 7.20-7.05 (2H, m, H-2’, 5’), 6.43 (1H, s, H-5”), 5.95 (1H, dd, *J* 12.5 and 6.5, H-5), 3.87 (1H, dd, *J* 17.6 and 11.6, 4-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) νmax/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), δ(ppm) 9.04 (1H, s, 8-H), 8.64 (1H, d, *J* 3.9, 10-H), 8.19 (1H, d, *J* 7.2, 12-H), 7.64-7.60 (2H,

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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 micro organisms are beneficial and others are detrimental (Tripathi, 2003). For example, micro organisms are involved in making of yogurt, cheese, and wine, in production of penicillin, interferon and alcohol, micro organisms 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, so anti-microbial screening of the synthesized compounds were carried out to explore their potential as anti-microbial agent.

Methods of Antimicrobial Evaluation:

After the development of desired new drug molecules, with different structure, an invitro 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 37^0C 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 ^{0}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 ^{0}C for 24 hours. The zone of inhibition is measured in mm after 24 hrs (J. H. Jorgensen *et al.* 1999, NCCLS, 1993)..

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

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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.
4.1 and Table 4.1. 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 δ 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, 20mm and 20mm while Ketoconazole has shown maximum activity against *Aspegallus niger* and *Candida albicans* with zone of inhibition of 22mm and 22mm..
In accordance with the data obtained from antibacterial activity, all the synthesized 1,3,5- trisubstituted pyrazoline derivatives have showed mild to good activity against tested organisms. Among these 1,3,5-trisubstituted pyrazoline derivatives, compound **4a, 4b, 4e, 4f, 4m** showed mild activity and compound **4c, 4g. 4i, 4j, 4k, 4o** showed moderate activity and **4d, 4h, 4l, 4n, 4p** showed good activity against bacteria.
In accordance with the data obtained from antifungal activity, compound **4g, 4h, 4j** showed mild activity and compound **4a-4f, 4m-4o** showed moderate activity and **4i, 4k, 4l, 4p** showed good activity against fungi.
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.

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 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*

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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**). 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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