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Research Article

SYNTHESIS OF SUBSTITUTED 2-PYRAZOLINES AS POTENTIAL ANTIMALARIAL AGENTS

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ABSTRACT

A series of substituted 2-pyrazolines derivatives were synthesized by refluxing from various substituted chalcones and nicotinic acid hydrazide in two steps. Compounds were confirmed by physical and spectral data such as M.P., R_f, elemental analyses, IR, ^{1}H NMR and Mass spectral data and were evaluated for antimalarial activity against chloroquine-sensitive (MRC-2) and chloroquine-resistant (RKL-9) *P. falciparum* strains. Compounds 4d (IC50=0.050 µM for MRC-2 and IC =0.413 µM for RKL-9) displayed better antiplasmodial activity than the chloroquine. The *in vitro* cytotoxicity study conducted on the human HepG2 cell line (>30µM) and showed good selectivity index.

Keywords: Pyrazolines, Chalcones, Antimalarial, Synthesis, Nicotinic acid hydrazide

1. INTRODUCTION

Plasmodium falciparum malaria continues to be a global health problem, especially in developing countries such as sub-Saharan Africa. It is responsible for over 1 million deaths per year [1]. The rapid spread of resistance to available anti-malarial drugs, especially chloroquine (CQ) and related quinoline-based agents, has highlighted the need to identify alternative anti-malarial compounds.

Pyrazolines and their derivatives have been found to possess a broad spectrum of biological activities such as antibacterial [2–3], antidepressant [4] anticonvulsant [5–6] antihypertensive [7] antioxidant [8] antitumor [9] and anticancer activities [10-11]. Recently these classes of compounds are reported to possess potential antiviral activity against flavivirus [12] and HIV [13]. In the last five years there has been an extensive focus of research towards the investigation of antiplasmodial potential of the pyrazoline ring with promising results [14-18]. Our literature survey revealed that these classes of compounds are yet to be explored for their possible antimalarial activities.

In view of these observations it was thought of interest to synthesize some novel trisubstituted pyrazolines carrying noicotinic moiety

2. EXPERIMENTAL

All Melting points were determined by open capillary apparatus and were uncorrected. IR spectra were recorded on a FT-IR Shimadzu DZU 8400S spectrophotometer in KBr disks. Elemental analyses were done on a Perkin-Elmer 2400C, H, N analyzer and values were found to be within the acceptable limits of the calculated values.

The ^{1}H-NMR spectra of the synthesized compounds in $CDCl_3$/DMSO were recorded at 400 MHz by Bruker Advance II 400 NMR spectrometer. Chemical shift values are given in (ppm) scale using tetramethylsilane (TMS) as an internal standard. The FAB mass spectra (at room temperature) were recorded on TOF MS ES$^+$ mass spectrometer. The melting point of the synthesized compounds was determined using an open capillary and are uncorrected. Progress of reaction and purity of synthesized compounds was ascertained by thin layer chromatography (TLC) using Silica gel G and Iodine vapors as detecting agent.

2.1.Synthetic Scheme

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

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

To a solution of substituted acetophenone (16 mmole) in 10 mL of methanol on an ice bath, freshly prepared 2N 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.

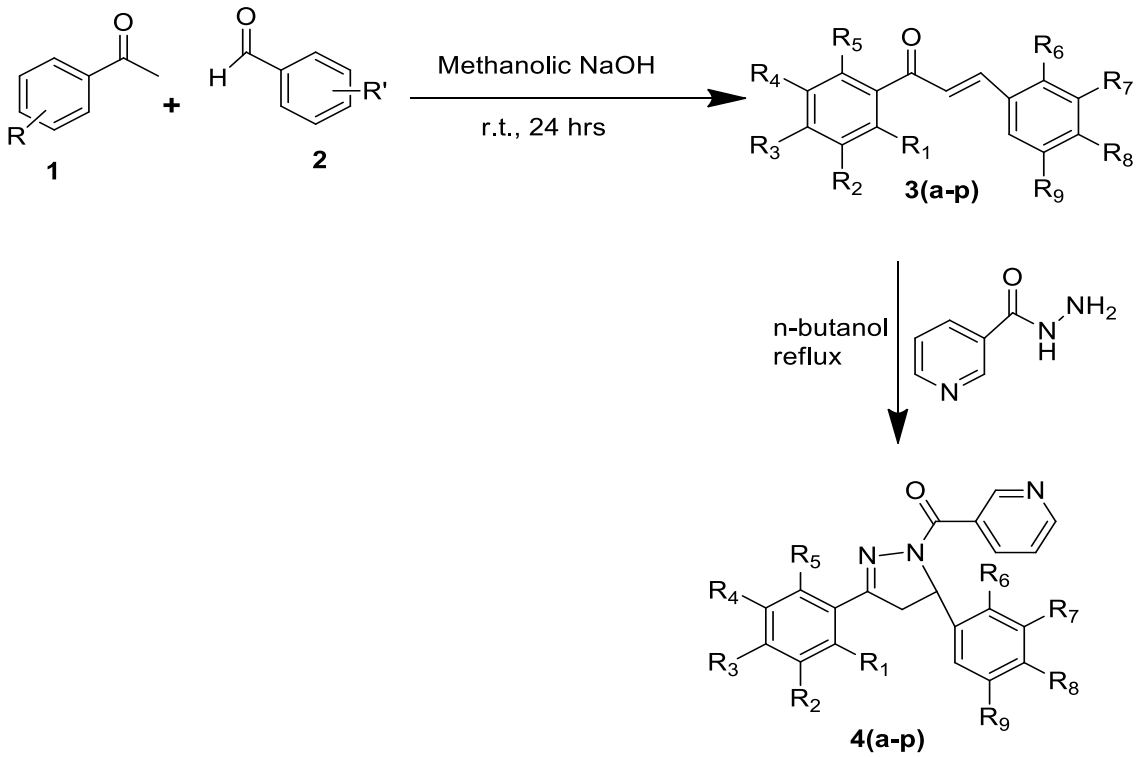

Fig. 1: Scheme for the synthesis of 1, 3, 5-trisubstituted pyrazolines 4 (a-p).

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

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

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 [18]. The product was recrystallized from methanol or ethanol/ water. The purity of the product was checked by TLC using ethyl acetate and hexane (4:6) as mobile phase and iodine vapors as detecting agent.

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

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.

2.2. Biological Activity

2.2.1. In vitro anti-malarial assay

The *in vitro* antimalarial activities of the compounds were assessed against CQ sensitive and resistant isolates of *P. falciparum* and compared with clinically used antimalarial drug chloroquine. The half maximal inhibitory concentrations (IC_{50}) were obtained [18]. In brief, the cultures of asynchronous parasites of *P. falciparum* (MRC-2 and RKL-9) were synchronized using 5% aqueous solution of sorbitol. All other stages except rings were degenerated. Degenerated stages had been removed by centrifuge for 5 minutes at 1500 rpm. Parasitemia was adjusted to about 1% for assay by diluting with fresh washed RBCs. The synthesized compounds were dissolved in 100µL of dimethylsulfoxide (DMSO) and required dilutions were made with a RPMI-1640 medium.

The tests were done in 96 well plate using CQ sensitive and resistant isolates. Different concentrations of synthesized compounds were dispensed in 96 well plate in triplicate. The first well in all the rows was without any drug and considered as control. The synchronized parasites were inoculated to all the wells, including control wells. The plates were incubated in a CO_2 incubator at 37°C for 24-30 h depending on the maturation of the schizont, thereafter; smears were prepared from all the wells, fixed with methanol, stained with Giemsa's stain and examined under light microscope, 100 x oil immersion. Growth of parasites in the test wells was compared to that of negative controls and the inhibition of parasite growth was expressed as a percentage. The halfmaximal inhibitory Concentration (IC_{50}) responses were estimated by the probit method.

2.2.2. Cell cytotoxicity assay

Toxicity is an important consideration in any drug development program [18], therefore we studied cytotoxicity of these compounds against (Table 2) HepG2 cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at ACTREC, Tata Memorial Centre, Mumbai. Briefly, cell cultures were routinely maintained in RPMI 1640 medium supplemented with 10% bovine foetal calf serum (FCS). For cytotoxicity evaluations, 5 x 10 cells in 180 ml medium were seeded in each well of a 96-well plate and incubated at 37°C under a 5% CO_2 atmosphere for 1 h. Aliquots of 20 µL of serial dilutions of the test compounds (stocks in DMSO) were added to the wells. Untreated wells received 20 µL of culture medium while additional solvent controls were prepared with medium containing DMSO to account for any possible effect of DMSO on cell viability. The plates were then incubated at 37°C under an atmosphere of 5% CO_2 for seven days and 20 µL MTT (5 mg/ml) was added to each well. The plates were further incubated for an additional 4 h at 37°C under an atmosphere of 5% CO_2 and then centrifuged for 10 minutes at 800 G. The supernatant was carefully aspirated from each well without disturbing the pellet and the cells were washed wit h 150 µl of phosphate buffered saline (PBS) followed by centrifugation for 10 minutes at 800 G. The supernatant was again carefully aspirated and the plates were dried at 37°C for an hour. The 100 µL ethanol was added to each well to solubilize the resultant formazan crystals, aided by a gentle mechanical shacking for 1-2 h.

Absorbance were measured on a Universal Microplate Reader (ELx800 UV, Bio-tek Instrument) at a wavelength of 570 nm and the percentage cell growth in drug treated well were calculated and plotted against log drug concentration to determine the corresponding IC_{50} values by non-linear regression analysis.

3. RESULTS AND DISCUSSION

3.1. Synthesis of compounds

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 R_f 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 -OCH_3 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-H_x and 4-H_y protons of pyrazoline derivatives. The fragmentation pattern obtained in the mass spectra was also according to the anticipated structures. All the above results confirmed the formation of the synthesized compounds. These signals clearly showing the formation of pyrazoline ring.

3.1.1. Spectral data of 3-(2',5'-dichlorophenyl)-1-(2,4-dihydroxyphenyl)prop-2-en-1-one (3a)

Synthesized by method described in 2.1.1., from 2,4-dihydroxyacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); *Yield* 85%, White solid; ***MP*** 165-167°C; R_f (EtOAc/Hex 4:6) 0.45; ***IR (KBr)*** ν_{max}/cm^{-1}: 3440 (O-H), 1669 (C=O), 1597 (Ar C=C), 750 (C–Cl), 3063, 2931, 1625, 1415, 1325, 1296, 1134, 1153, 1046, 982, 759, 737 (Ar) ; ***^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)***: 11.62 (2H, s, OH-2,4), 7.76 (1H, d, *J* 16, H-b), 7.69 (2H, dd, *J* 6.8 and 4.5, H-6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 7.21 (4H, m, *J* 4.8, H-3, 5, 3', 4'); ***FAB-MS m/z***: 308.14 $[M+H]^+$; *Anal. Calcd for* $C_{15}H_{10}Cl_2O_3$: C, 58.28; H, 3.26. Found: C, 58.98; H, 3.12

3.1.2. Spectral data of 3-(5'-chloro-2'-methoxyphenyl)-1-(2,4-hydroxyphenyl)prop-2-en-1-one (3b)

Synthesized by method described in 2.1.1., from 4-Hydoxyacetophenone (16 mmol) and 2-methoxy,5-chlorobenzaldehyde (16 mmol); *Yield* 70%, yellow crystalline solid; ***MP*** 112-114°C; R_f (EtOAc/Hex 4:6) 0.47; ***IR (KBr)*** ν_{max}/cm^{-1}: 3441 (O-H), 1645 (C=O), 1595 (Ar C=C), 760 (C–Cl), 3060, 2965, 1645, 1434, 1323, 1296, 982, 759 (Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm):*** 10.60 (2H, s, OH-2,4), 7.76 (1H, d, *J* 15.6, H-b), 7.69-7.60 (4H, m, H-3, 5, 6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 6.81 (2H, dd, *J* 5.2, H-3', 4'), 3.81 (3H, s, OCH_3-2'); ***FAB-MS m/z***: 304.06 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{13}ClO_4$: C, 63.06; H, 4.30; Found: C, 63.41; H, 4.58.

3.1.3. Spectral data of 3-(3'-hydroxyphenyl)-1-(2,4-hydroxyphenyl)prop-2-en-1-one (3c)

Synthesized by method described in 2.1.1., from 4-Hydroxyacetophenone (16 mmol) and 4-hydroxybenzaldehyde (16 mmol); *Yield* 65%, Yellow solid; ***MP*** 124-126°C; R_f (EtOAc/Hex 4:6) 0.36; ***IR (KBr)*** ν_{max}/cm^{-1}: 3440 (O-H), 1661 (C=O), 1592 (Ar C=C), 750 (C–Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm):*** 9.95 (3H, s, OH-2,4, 3'), 7.71 (1H, d, *J* 15.3, H-b), 7.61-7.54 (4H, m, H-3, 5, 6, 6'), 7.31 (1H, d, *J* 16.0, H-a), 7.21-7.15 (4H, m, *J* 4.8, H-2, 2', 4', 5') ***FAB-MS m/z***: 256.08 $[M+H]^+$; *Anal. Calcd for* $C_{15}H_{12}O_4$: C, 70.31; H, 4.72; Found: C, 70.37; H, 4.12;

3.1.4. Spectral data of 3-(3', 4'-dimethoxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (3d)

Synthesized by method described in 2.1.1., from 4-Hydroxyacetophenone (16 mmol) and 4-hydroxybenzaldehyde (16 mmol); *Yield* 76%, White solid; ***MP*** 108-110°C; R_f (EtOAc/Hex 4:6) 0.34; ***IR (KBr)*** ν_{max}/cm^{-1}: 3435 (O-H), 1661 (C=O), 1592 (Ar C=C),

760 (C–Cl), 3068, 2931, 1621, 1435, 1312, 1213, 1115, 1153, 1046, 982, 751(Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm)***: 11.55 (1H, s, OH-4, 2), 7.73 (1H, d, *J* 15.3, H-b), 7.66-7.55 (4H, m, H-3, 5, 6, 6'), 7.35 (1H, d, *J* 16.0, H-a), 7.25-7.21 (3H, m, *J* 4.4, H-2, 2', 5'), 3.74 (6H, s, OCH_3-3', 4'); ***FAB-MS m/z:*** 300.08 $[M+H]^+$; *Anal. Calcd for* $C_{17}H_{16}O_5$: C, 67.99; H, 5.37; Found: C, 67.28; H, 5.70;

3.1.5. Spectral data of 3-(2', 5'-dichlorophenyl)-1-(2, 5-dichlorophenyl)prop-2-en-1-one (3e)

Synthesized by method described in 2.1.1., from 2,5-dichloroacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); *Yield* 69%, White crystalline solid; ***MP*** 138-140°C; R_f (EtOAc/Hex 4:6) 0.38; ***IR (KBr) ν_{max}/cm^{-1}***: 1662 (C=O), 1598 (Ar C=C), 743 (C–Cl), 3064, 2930, 1627, 1415, 1325, 1296, 1134, 1117, 1047, 982, 819, 759, 737 (Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm):*** 7.76 (1H, d, *J* 15.7, H-b), 7.69 (2H, dd, *J* 6.8 and 4.5, H-6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 7.21-7.15 (4H, m, *J* 4.8, H-3, 4, 3', 4') ***FAB-MS m/z:*** 345.93 $[M+H]^+$; *Anal. Calcd for* $C_{15}H_8Cl_4O$: C, 52.06; H, 2.33. Found: C, 52.59; H, 2.29.

3.1.6. 3-(5'-chloro-2'-methoxyphenyl)-1-(2,5-dichlorophenyl)prop-2-en-1-one (3f)

Synthesized by method described in 2.1.1., from 2,5-dichloroacetophenone (16 mmol) and 5-chloro, 2-methoxybenzaldehyde (16 mmol); *Yield* 67%, Creamy-coloured fine needles; ***MP*** 148-150°C; R_f (EtOAc/Hex 4:6) 0.79; ***IR (KBr) ν_{max}/cm^{-1}***: 1662, 1216 (C=O), 1594 (C=C), 1261, 1026 (C–O), 742 (C–Cl), 3061, 1591, 1457, 1384, 1296, 1194, 980, 854, 756 (Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm):*** 7.74 (1H, d, *J* 15.7, H-b), 7.65 (1H, d, *J* 6.8, H-6), 7.34 (1H, d, *J* 15.9, H-a), 7.26-7.30 (4H, m, H-3, 4, 4', 6'), 6.81 (1H, d, *J* 5.2, H-3'), 3.89 (3H, s, OCH_3-2'); ***FAB-MS m/z:*** 341.27 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{11}Cl_3O_2$: C 56.25, H 3.25 Found C 56.23, H 3.92.

3.1.7. Spectral data of 1-(2,5-dichlorophenyl)-3-(3'-hydroxyphenyl)prop-2-en-1-one (3g)

Synthesized by method described in 2.1.1., from 2,5-dichloroacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); *Yield* 60%, White amorphous solid; ***MP*** 141-144°C; R_f (EtOAc/Hex 4:6) 0.42***; IR (KBr) ν_{max}/cm^{-1}***: 3441 (O-H), 1663 (C=O), 1589 (Ar C=C), 745 (C–Cl), 3060, 2945, 1620, 1320, 1298, 1139, 1153, 1046, 982, 759, 737 (Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm)***: 11.62 (1H, s, OH-3'), 7.70 (1H, d, *J* 15.7, H-b), 7.61 (2H, dd, *J* 6.5 and 4.4, H-6, 6'), 7.32 (1H, d, *J* 16.0, H-a), 7.21-7.11 (4H, m, H-3, 4, 2', 4', 5'); ***FAB-MS m/z***: 292.01 $[M+H]^+$; *Anal. Calcd for* $C_{15}H_{10}Cl_2O_2$: C, 61.46; H, 3.44. Found: C C, 61.98; H, 3.12

3.1.8. Spectral data of 1-(2,5-dichlorophenyl)-3-(3',4'-dimethoxyphenyl)prop-2-en-1-one (3h)

Synthesized by method described in 2.1.1., from 2,5-dichloroacetophenone (16 mmol) and 3, 4-dimethoxybenzaldehyde (16 mmol); *Yield* 69%, white amorphous solid; ***MP*** 115-118°C; R_f (EtOAc/Hex 4:6) 0.67; ***IR (KBr) ν_{max}/cm^{-1}***: 1653 (C=O), 1605 (Ar C=C), 1547 (COC=C), 1261, 1025 (C-O), 1150 (C-Cl), 3051 (Ar C-H), 2932, 2841 (C-H), 1524, 1472, 1147, 969 (Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz), δ (ppm):*** 7.81 (1H, d, *J* 15.5, H-b), 7.75 (1H, d, *J* 8.5, H-6), 7.61 (1H, d, *J* 15.1, H-a), 7.40 (1H, d, *J* 6.8, H-4), 7.15 (1H, dd, *J* 2.3 and 8.5, H-6, 6'), 7.01 (1H, d, *J* 2.3, H-2'), 6.98 (1H, d, *J* 5.1 H-3), 6.84 (1H, d, *J* 8.1, H-5'), 3.82 (6H, s, OCH_3-3', 4'). ***FAB-MS m/z:*** 322.02 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{12}Cl_2O_3$: C, 59.46; H, 3.74;. Found: C, 59.23; H, 3.42;

3.1.9. Spectral data of 1-(5-chloro-2-methoxyphenyl)-3-(2',5'-dichlorophenyl)prop-2-en-1-one (3i)

Synthesized by method described in 2.1.1., from 2-methoxy, 5-chloro-acetophenone (16 mmol) and 2, 5-dichlorobenzaldehyde (16 mmol); *Yield* 66%, Yellow solid; ***MP*** 105-107°C; R_f (EtOAc/Hex 4:6) 0.32; ***IR (KBr) ν_{max} /cm^{-1}***: 1650 (C=O), 1585 (Ar C=C), 1517 (COC=C), 1268, 1029 (C-O), 1150 (C-Cl), 3058 (Ar C-H), 2933 (C-H), 1619, 1512, 969, 810, (Ar); ***^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm)***: 7.81 (1H, d, *J* 15.7, H-b), 7.71 (1H, d, *J* 8.3, H-6), 7.60 (1H, d, *J* 15.4, H-a), 7.56 (1H, d, *J* 6.4, H-4), 7.40 (1H, d, *J* 5.9, H-3), 7.10 (1H, dd, *J* 2.6 and 8.4, H-6'), 7.06 (1H, d, *J* 1.9, H-3'), 6.90 (1H, d, *J* 8.8, H-4'), 3.76 (3H, s, OCH_3-2). ***FAB-MS m/z***: 339.38 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{11}Cl_3O_2$: C, 56.25; H, 3.25; Found: C, 56.68; H, 3.39

3.1.10. Spectral data of 1-(5-chloro-2-methoxy phenyl)-3-(5'-chloro-2'-methoxyphenyl)prop-2-en-1-one (3j)

Synthesized by method described in 2.1.1., from 2-methoxy, 5-chloroacetophenone (16 mmol) and 2-

methoxy, 5-chlorobenzaldehyde (16 mmol); *Yield* 3.7 g, 69%, Yellow solid; ***MP*** 107-109°C; R_f (EtOAc/Hex 4:6) 0.35; ***IR (KBr)*** ν_{max} ***/cm^{-1}***: 1655 (C=O), 1580 (Ar C=C), 1519 (COC=C), 1264, 1025 (C-O), 1157 (C-Cl), 3052 (Ar C-H), 2930 (C-H), 1611, 1517, 960, 815, (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 7.86 (1H, d, *J* 15.7, H-b), 7.74 (1H, d, *J* 8.3, H-6), 7.61 (1H, d, *J* 15.4, H-a), 7.54 (1H, d, *J* 6.4, H-4), 7.46 (1H, d, *J* 5.9, H-3), 7.10 (1H, dd, *J* 2.6 and 8.4, H-6'), 7.04 (1H, d, *J* 1.9, H-3'), 6.92 (1H, d, *J* 8.8, H-4'), 3.80 (3H, s, OCH_3-2), 3.85 (3H, s, OCH_3-2'), ***FAB-MS m/z***: 339.38 $[M+H]^+$; *Anal. Calcd for* $C_{17}H_{14}Cl_2O_3$: C, 60.55; H, 4.18; Found: C, 60.29; H, 4.57

3.1.11.Spectral data of 1-(5-chloro-2-methoxy phenyl)-3-(3'-hydroxyphenyl)prop-2-en-1-one (3k)

Synthesized by method described in 2.1.1., from 2-methoxy,5-chloroacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); *Yield* 69%, yellow cryastalline solid; ***MP*** 135-137°C; R_f (EtOAc/Hex 4:6) 0.34; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3445 (O-H), 1665 (C=O), 1568 (Ar C=C), 748 (C–Cl), 3063, 2941, 1625, 1291, 1134, 1150, 980, 754 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz), δ (ppm)***: 11.54 (1H, s, OH-2'), 7.71 (1H, d, *J* 15.7, H-b), 7.65 (2H, dd, *J* 6.5 and 4.4, H-6, 6'), 7.30 (1H, d, *J* 16.0, H-a), 7.24-7.15 (4H, m, H-3, 4, 2', 4'), 6.94 (1H, d, *J* 8.0, H-5'); 3.70 (3H, s, OCH_3-2) ***FAB-MS m/z***: 288.06 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{13}ClO_3$: C, 66.56; H, 4.54. Found: C, 66.39; H, 4.40

3.1.12. Spectral data of 1-(5-chloro-2-methoxyphenyl)-3-(3',4'-dimethoxyphenyl) prop-2-en-1-one (3l)

Synthesized by method described in 2.1.1., from 2-methoxy, 5-chloroacetophenone (16 mmol) and 3,4-dimethoxybenzaldehyde (16 mmol); *Yield* 71%, Pale yellow solid; ***MP*** 117-119°C; R_f (EtOAc/Hex 4:6) 0.49; ***IR (KBr)*** ν_{max} ***/cm^{-1}***: 1645 (C=O), 1589 (Ar C=C), 1512 (COC=C), 1265, 1025 (C-O), 1151 (C-Cl), 3064 (Ar C-H), 2941 (C-H), 1611, 1518, 967, 811 (Ar); **^{1}H-NMR (CDCl$_3$, 400 MHz), δ (ppm)** 7.84 (1H, d, *J* 15.9, H-b), 7.58 (1H, d, *J* 15.6, H-a), 7.54 (1H, d, *J* 6.4, H-4), 7.48 (1H, d, *J* 5.4, H-3), 7.10 (2H, dd, *J* 2.6 and 8.4, H-6,6'), 7.22 (1H, d, *J* 4.3, H-2'), 6.91 (1H, d, *J* 8.1, H-5') 3.79 (3H, s, OCH_3-2), 3.71 (6H, s, OCH_3-3',4'). ***FAB-MS m/z***: 332.08 $[M+H]^+$; *Anal. Calcd for* $C_{18}H_{17}ClO_4$: C, 64.97; H, 5.15. Found: C, 64.36; H, 5.45

3.1.13.Spectral data of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(2',5'-dichlorophenyl)prop-2-en-1-one (3m)

Synthesized by method described in 2.1.1., from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2,5-dichlorobenzaldehyde (16 mmol); *Yield* 71%, White amorphous solid; ***MP*** 94-97°C; R_f (EtOAc/Hex 4:6) 0.67; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3447 (O-H), 1660 (C=O), 1596 (Ar C=C), 740 (C–Cl), 3064, 2965, 1647, 1434, 1320, 980, 759 (Ar, CH_3); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 11.57 (1H, s, OH-2), 7.75 (1H, d, *J* 15.5, H-b), 7.68 (2H, dd, *J* 6.8, 7.8 , H-6, 6'), 7.34 (1H, d, *J* 16.0, H-a), 7.62-7.53 (2H, m, H-4', 2), 7.41-7.23 (2H, m, H-3',6'), 6.71 (1H, d, *J* 8.1, H-5), 2.31 (3H, s, CH_3-4); ***FAB-MS m/z***: 339.98 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{11}Cl_3O_2$: C, 56.25; H, 3.25; Found: C, 56.54; H, 3.65.

3.1.14.Spectral data of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(5'-chloro-2'-methoxy-phenyl)prop-2-en-1-one (3n)

Synthesized by method described in 2.1.1., from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 2-methoxy, 5-dichlorobenzaldehyde (16 mmol); *Yield* 68%, White solid; ***MP*** 137-139°C; R_f (EtOAc/Hex 4:6) 0.48; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3431 (O-H), 1657 (C=O), 1586 (Ar C=C), 760 (C–Cl), 3064, 2964, 1643, 1434, 985, 753 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.35 (1H, s, OH), 7.70 (1H, d, *J* 15.2, H-b), 7.69 (2H, dd, *J* 6.8, 7.8 , H-6', 2), 7.32 (1H, d, *J* 16.0, H-a), 7.24 (1H, d, *J* 4.2, H-5), 6.82 (2H, dd, *J* 5.3,7.1 H-3', 4'), 2.84 (3H, s, OCH_3-2'), 2.34 (3H, s, CH_3-4); ***FAB-MS m/z***: 336.03 $[M+H]^+$; *Anal. Calcd for* $C_{17}H_{14}Cl_2O_3$: 60.55; H, 4.18;; Found: C, 60.67; H, 4.38.

3.1.15.Spectral data of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(3'-hydroxyphenyl)prop-2-en-1-one (3o)

Synthesized by method described in 2.1.1., from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 3-hydroxybenzaldehyde (16 mmol); *Yield* 69%, yellow solid; ***MP*** 183-185°C; R_f (EtOAc/Hex 4:6) 0.31; ***IR (KBr)*** ν_{max}***/cm^{-1}:*** 3445 (O-H), 1665 (C=O), 1568 (Ar C=C), 760 (C–Cl), 3063, 2941, 1625, 1291, 1134, 1150, 980, 754 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ***

(ppm): 11.54 (1H, s, OH), 7.71 (1H, d, *J* 15.7, H-b), 7.65 (2H, dd, *J* 6.5 and 4.4, H-6, 6'), 7.30 (1H, d, *J* 16.0, H-a), 7.24 (4H, m, *J* 4.5, H-3, 4, 2', 4'), 6.94 (1H, d, *J* 8.0, H-5'), 2.37 (3H, s, CH_3-4); ***FAB-MS m/z***: 288.38 $[M+H]^+$; *Anal. Calcd for* $C_{16}H_{13}ClO_3$: C, 66.56; H, 4.54; Found: C, 63.29; H, 4.73

3.1.16. Spectral data of 1-(5-chloro-2-hydroxy-4-methylphenyl)-3-(3',4'-dimethoxyphenyl)prop-2-en-1-one (3p)

Synthesized by method described in 2.1.1., from 3-chloro, 6-hydoxy, 4-methylacetophenone (16 mmol) and 3,4-methoxy benzaldehyde (16 mmol); *Yield* 78%, white solid; ***MP*** 123-125°C; R_f (EtOAc/Hex 4:6) 0.76; ***IR (KBr)*** ν_{max} ***/cm^{-1}***: 1650 (C=O), 1589 (Ar C=C), 1517 (COC=C), 1265, 1024 (C-O), 1155 (C-Cl), 3055 (Ar C-H), 2939 (C-H), 1612, 1519, 975, 818, (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 7.82 (1H, d, *J* 16, H-b), 7.54 (1H, d, *J* 16, H-a), 7.54 (1H, d, *J* 6.4, H-4), 7.48 (1H, d, *J* 5.4, H-3), 7.10 (2H, dd, *J* 2.6 and 8.4, H-6, 6'), 7.22 (1H, d, *J* 4.3, H-2'), 6.91 (1H, d, *J* 8.1, H-5'), 3.70 (6H, s, OCH_3-3', 4'), 2.32 (3H, s, CH_3-4). ***FAB-MS m/z***: 332.07 $[M+H]^+$; *Anal. Calcd for* $C_{18}H_{17}ClO_4$: C, 64.97; H, 5.15; Found: C, 64.23; H, 5.67

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

Synthesized by method described in 2.1.2., from chalcone **3a** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 19h reflux; *Yield* 58%, Pale yellow solid; ***MP*** 137-139°C; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1560 (C=N stretching), 1260, 1091 (C–O), 1320, 1215 (C-N), 1107, 777 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.05 (1H, s, 2", 4"-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 7.90 (1H, d, *J 12.3* H-6"), 7.59-7.55 (2H, m, H-11, 4'), 7.43-7.39 (2H, m, H-3', 6'), 6.80 (2H, d, *J* 7.6, H-3", 5"), 5.92 (1H, dd, *J 12.3* and 6.2, H-5), 3.89 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.10 (1H, dd, *J 17.8* and 4.8, 4-H_x); ***FAB-MS m/z:*** 427.45 $[M+H]^+$; *Anal. Calcd for* $C_{21}H_{15}Cl_2N_3O_3$: C, 58.89; H, 3.53; N, 9.81; Found: C, 58.54; H, 3.57; N, 9.32;

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

Synthesized by method described in 2.1.2., from chalcone **3b** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 65%, White solid; ***MP*** 145-147°C; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3440 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C–O), 1215 (C-N), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.05 (1H, s, 2",4"-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 7.86 (1H, dd, *J 12.3* H-6"), 7.23 (1H, dd, *J* 7.4 and 3.2, H-4', 6'), 6.85-6.89 (3H, m, H-3', 3", 5"), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.70 (3H, s, OCH_3-2"), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H_x); ***FAB-MS m/z:*** 407.34 $[M+H]^+$; *Anal. Calcd for* $C_{22}H_{18}ClN_3O_4$: C, 62.34; H, 4.28; N, 9.91 Found: C, 62.50; H, 4.41; N, 9.21

3.1.19.Spectral data of (5-(3'-hydroxyphenyl)-3-(2",4"-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4c)

Synthesized by method described in 2.1.2., from chalcone **3c** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 8h reflux; *Yield* 68%, Pale yellow solid; ***MP*** 165-167°C; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3421 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1565 (C=N), 1260, 1091 (C–O), 1215 (C-N), 1107 (C–Cl), 3045, 2950 (C-H), 1505, 1467, 922, 815, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 11.05 (3H, s, 2",4", 3'-OH), 9.02 (1H, s, 8-H), 8.72 (1H, d, *J* 3.5, 10-H), 8.22 (1H, d, *J* 7.4, 12-H), 7.87 (1H, dd, *J 12.* H-6"), 7.59 (1H, d, *J* 7.6 H-11), 7.24 (1H, d, *J* 4.4, H-5'), 6.99 (1H, d, *J* 7.6, H-2'), 6.85-6.87 (4H, m, H-4',6', 3", 5"), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H_x); ***FAB-MS m/z:*** 375.76 $[M+H]^+$; *Anal. Calcd for* $C_{21}H_{17}N_3O_4$: C, 67.19; H, 4.56; N, 11.19 Found: C, 67.78; H, 4.53; N, 11.64.

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

Synthesized by method described in 2.1.2., from chalcone **3d** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 69%, Light yellow solid; ***MP*** 156-159°C; ***IR (KBr)*** ν_{max}***/cm^{-1}***: 3415 (O-H), 1668 (N-C=O), 1591 (Ar C=C), 1560 (C=N), 1262, 1096 (C–O), 1210 (C-N),

1102 (C–Cl), 3041, 2954 (C-H), 1501, 1467, 922, 815, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.05 (1H, s, 2",4"-OH), 9.02 (1H, s, 8-H), 8.70 (1H, d, *J* 3.9, 10-H), 8.26 (1H, d, *J* 7.2, 12-H), 7.82 (1H, dd, *J 12.3* H-6"), 6.89 (1H, d, 3.2, H-2'), 6.83-6.86 (3H, m, H-5', 3", 5"), 6.89 (1H, dd, J 6.7 and 3.2, H-6'), 5.95 (1H, dd, *J 12.3* and 6.2, H-5), 3.88 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.70 (3H, s, OCH$_3$-3', 4'), 3.11 (1H, dd, *J 17.5* and 4.6, 4-H$_x$); ***FAB-MS m/z:*** 419.31 [M +H]$^+$; *Anal. Calcd for* $C_{23}H_{21}N_3O_5$: C, 65.86; H, 5.05; N, 10.02; Found: C, 65.39; H, 5.18; N, 10.37

3.1.21.Spectral data of (5-(2', 5'-dichlorophenyl)-3-(2",5"-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)methanone (4e)

Synthesized by method described in 2.1.2., from chalcone **3e** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 10h reflux; *Yield* 59%, Brown solid; ***MP*** 189-191°C; ***IR (KBr) ν_{max}/cm^{-1}***: 1658 (N-C=O), 1587 (Ar C=C), 3083 (Ar C-H), 2934 (C-H), 1637, 1496 (C=N), 817, 738 (Ar CH bend); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 9.10 (1H, s, H-8), 8.75 (1H, d, *J* 4.5, H-10), 8.11 (1H, d, *J* 7.4, H-12), 7.72 (3H, m, H-6", 6', 11), 7.41-7.52 (4H, m, H-3',4',3",4"), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 12.5, 4-H$_y$), 3.08 (1H, dd, *J* 17.5 and 5.1, 4-H$_x$); ***FAB-MS m/z:*** 464.96 [M +H]$^+$; *Anal. Calcd for* $C_{21}H_{13}Cl_4N_3O$: C 54.22, H 2.82, N 9.03. Found: C 54.40, H 2.67, N 9.54.

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

Synthesized by method described in 2.1.2., from chalcone **3f** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 70%, Brown solid; ***MP*** 195-197°C; ***IR (KBr) ν_{max}/cm^{-1}***: 1658 (N-C=O), 1587 (Ar C=C), 3083 (Ar C-H), 2934 (C-H), 1637, 1496 (C=N), 817, 738 (Ar CH bend); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 9.02 (1H, s, 8-H), 8.71 (1H, d, *J* 3.5, 10-H), 8.25 (1H, d, *J* 7.4, 12-H), 7.84 (1H, d, *J* 6.5, H-6"), 7.53-7.48 (3H, m, H-11, 3', 4'), 7.36 (1H, d, *J* 7.1 H-6'), 7.22 (1H, dd, *J 8.3* and 6.4, H-4'), 6.85 (1H, dd, *J 6.3* and 6.2, H-3'), 5.92 (1H, dd, *J 12.3* and 6.2, H-5), 3.90 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.81 (3H, s, OCH$_3$-2'), 3.15 (1H, dd, *J 17.8* and 4.8, 4-H$_x$); ***FAB-MS m/z:*** 459.96 [M +H]$^+$; *Anal. Calcd for* $C_{22}H_{16}Cl_3N_3O_2$: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.42; H, 3.29; N, 9.48.

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

Synthesized by method described in 2.1.2., from chalcone **3g** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 14 hrs reflux; *Yield* 67%, Pale yellow solid; ***MP*** 165-167°C; ***IR (KBr) ν_{max}/cm^{-1}***: 3414 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.02 (1H, s, 3'-OH), 9.02 (1H, s, 8-H), 8.73 (1H, d, *J* 3.7, 10-H), 8.16 (1H, d, *J* 7.1, 12-H), 7.48 (2H, d, *J* 4.4, H-3", 4''), 7.68 (2H, d, *J* 7.6, H-6", 11), 7.22 (1H, dd, *J 8.1* and 6.2, H-4'), 7.01 (1H, d, *J* 5.1, H-2'), 6.83-6.78 (2H, m, H-4', 6'), 5.95 (1H, dd, *J 12.1* and 6.8, H-5), 3.83 (1H, dd, *J 17.7* and 11.6, 4-H$_y$), 3.18 (1H, dd, *J 17.1* and 4.3, 4-H$_x$); ***FAB-MS m/z:*** 412.54 [M +H]$^+$; *Anal. Calcd for* $C_{21}H_{15}Cl_2N_3O_2$: C, 61.18; H, 3.67; N, 10.19. Found: C, 61.01; H, 3.97; N, 10.74

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

Synthesized by method described in 2.1.2., from chalcone **3h** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 68%, white solid; ***MP*** 178-180°C; ***IR (KBr) ν_{max}/cm^{-1}***: 1660 (N-C=O), 1596 (Ar C=C), 1560 (C=N), 1260, 1092 (C–O), 1215 (C-N), 1108, 776 (C–Cl), 3045, 2956 (C-H), 1502, 1465, 922, 816, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 11.05 (1H, s, 4'-OH), 9.07 (1H, s, 8-H), 8.71 (1H, d, *J* 3.9, 10-H), 8.16 (1H, d, *J* 7.2, 12-H), 7.58 (2H, dd, *J* 7.6 & 6.2, H-11), 7.48 (2H, d, *J* 4.8, H-3", 4''), 6.87-6.70 (3H, m, H-2', 5', 6'), 5.93 (1H, dd, *J 12.3* and 6.2, H-5), 3.82 (1H, dd, *J 17.1* and 11.2, 4-H$_y$), 3.82 (6H, s, OCH$_3$-3', 4'), 3.10 (1H, dd, *J 17.8* and 4.8, 4-H$_x$); ***FAB-MS m/z:*** 455.48 [M +H]$^+$; *Anal. Calcd for* $C_{23}H_{19}Cl_2N_3O_3$: C, 60.54; H, 4.20; N, 9.21 Found: C, 60.94; H, 4.76; N, 9.63.

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

Synthesized by method described in 2.1.2., from chalcone **3i** (4 mmol) and nicotinic acid hydrazide (4 mmol) after 13 hrs reflux; *Yield* 63%, Light-yellow solid; ***MP*** 142-145°C; ***IR (KBr) ν_{max}/cm^{-1}***: 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984

(trans ethylenic H), 816, 736 (Ar C-H bend); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.80 (H, s, H-6"), 7.56-7.60 (2H, m, H-4', 11), 7.37-7.43 (3H, m, H-3', 6', 4"), 6.99 (1H, d, *J* 5.1, H-3'), 5.95(1H, dd, *J* 10.5 and 6.1, H-5), 3.90 (1H, dd, *J* 17.3 and 6.1, 4-H$_y$), 3.82 (3H, s, OCH$_3$-2"), 3.10 (1H, dd, *J* 17.5 and 8.5, 4-H$_x$); ***FAB-MS m/z:*** 459.37 [M +H]$^+$; *Anal. Calcd for* $C_{22}H_{16}Cl_3N_3O_2$: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.86; H, 3.55; N, 9.16;

3.1.26.Spectral data of (3-(5"-chloro-2"-methoxyphenyl)-5-(5'-chloro-2'-methoxy phenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridine -3-yl)methanone (4j)

Synthesized by method described in 2.1.2., from chalcone **3j** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 62%, Light-yellow solid; ***MP*** 152-155°C; ***IR (KBr) ν_{max}/cm^{-1}***: 1645 (N-C=O), 1622, 1579 (C=N), 1596 (Ar C=C), 1252, 1027 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)*** 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.72 (2H, t, *J* 8.3, H-6", 11), 7.32-7.38 (4H, m, H-3", 4", 4', 6'), 6.83 (1H, d, *J* 5.5, H-3'), 5.91 (1H, dd, *J* 10.2 and 6.5, H-5), 3.92 (1H, dd, *J* 17.2 and 6.5, 4-H$_y$), 3.87 (6H, s, OCH$_3$-2',2"), 3.08 (1H, dd, *J* 17.5 and 8.1, 4-H$_x$); ***FAB-MS m/z:*** 456.52 [M +H]$^+$; *Anal. Calcd for* $C_{23}H_{19}Cl_2N_3O_3$: C, 60.54; H, 4.20; N, 9.21; Found: C, 60.13; H, 4.19; N, 9.56.

3.1.27.Spectral data of (3-(5"-chloro-2"-methoxyphenyl)-5-(3'-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(pyridin-3-yl)metha -none (4k)

Synthesized by Synthesized by method described in 2.1.2., nicotinic acid hydrazide (4 mmol); *Yield* 58%, Pale yellow solid; ***MP*** 173-175°C; ***IR (KBr) ν_{max}/cm^{-1}***: 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2950 (C-H), 1502, 1465, 922, 816, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.04 (1H, s, 3'-OH), 9.04 (1H, s, 8-H), 8.69 (1H, d, *J* 3.9, 10-H), 8.18 (1H, d, *J* 7.2, 12-H), 7.82 (2H, d, *J* 7.6, H-6"), 7.61 (1H, dd, *J* 12.6 and 6.4, H-11), 7.36 (1H, d, *J* 7.1, H-3"), 7.25 (1H, t, *J* 7.6, H-5'), 6.99-7.04 (2H, m, H-2', 3"), 6.75-6.87 (2H, m, H-4', 6'), 5.93 (1H, dd, *J* 12.3 and 6.2, H-5), 3.89 (1H, dd, *J* 17.5 and 11.6, 4-H$_y$), 3.81 (3H, s, OCH$_3$-2"), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H$_x$); ***FAB-MS m/z:*** 407.29 [M +H]$^+$; *Anal. Calcd for* $C_{22}H_{18}ClN_3O_3$: C, 64.79; H, 4.45; N, 10.30 Found: C, 64.34; H, 4.65; N, 10.15

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

Synthesized by method described in 2.1.2., from chalcone **3l** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 67%, Pale yellow solid; ***MP*** 193-195°C; ***IR (KBr) ν_{max}/ cm^{-1}***: 1668 (N-C=O), 1594 (Ar C=C), 1265, 1090 (C–O), 1560 (C=N), 1219 (C-N), 1101 (C–Cl), 3049, 2953 (C-H), 1501, 1468, 920, 816, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 9.04 (1H, s, 8-H), 8.69 (1H, d, *J* 3.9, 10-H), 8.18 (1H, d, *J* 7.2, 12-H), 7.66 (2H, d, *J* 7.6, H-6", 11-H), 6.85-6.90 (4H, m, H-2", 3', 4', 6'), 5.93 (2H, dd, *J 12.3* and 6.2, H-5, 5"), 3.89 (1H, dd, *J 17.5* and 11.6, 4-H$_y$), 3.80 (3H, s, OCH$_3$-2'), 3.85 (6H, s, OCH$_3$-3", 4"), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H$_x$); ***FAB-MS m/z:*** 451.13 [M +H]$^+$; *Anal. Calcd for* $C_{23}H_{20}ClN_3O_4$: C, 63.79; H, 4.91; N, 9.30; Found: C, 63.12; H, 4.47; N, 9.67

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

Synthesized by method described in 2.1.2., from chalcone **3m** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 62%, Light-yellow powder; ***MP*** 179-181°C; ***IR (KBr) ν_{max}/cm^{-1}***: 3218 (O-H), 1641 (N-C=O), 1623, 1574 (C=N), 1591 (Ar C=C), 1255, 1024 (C–O), 1125 (C–Cl), 2913 (C-H), 1471, 1320, 1239 (C-N), 945 (trans ethylenic H), 822, 764 (Ar C-H bend); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm):*** 10.04 (1H, s, 6"-OH), 9.10 (1H, s, H-8), 8.72 (1H, d, *J* 4.3, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.62-7.56 (3H, m, H-11, 4', 2"), 7.39-7.42 (2H, m, H-3', 6'), 6.40 (1H, s, H-5"), 5.99 (1H, dd, *J* 10.3 and 6.3, H-5), 3.91 (1H, dd, *J* 17.1 and 6.4, 4-H$_y$), 2.85 (3H, s, CH$_3$-4), 3.11 (1H, dd, *J* 16.5 and 8.5, 4-H$_x$); ***FAB-MS m/z:*** 459.03 [M +H]$^+$; *Anal. Calcd for* $C_{22}H_{16}Cl_3N_3O_2$: C, 57.35; H, 3.50; N, 9.12; Found: C, 57.74; H, 3.27; N, 9.56

3.1.30.Spectral data of (3-(5"-chloro-2"-hydroxy-4"-methylphenyl)-5-(5'-chloro-2'-methoxy phenyl)-4,5-dihydro-1H-pyrazol-1-yl) (pyridine-3-yl)methanone (4n)

Synthesized by method described in 2.1.2., from chalcone **3n** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield*

42%, Light-yellow solid; ***MP*** 169-172°C; ***IR (KBr)*** ν_{max}/cm^{-1}: 3215 (O-H), 1649 (N-C=O), 1622, 1585 (C=N), 1590 (Ar C=C), 1252, 1012 (C–O), 1121 (C–Cl), 2917 (C-H), 1473, 1384, 1225 (C-N), 984 (trans ethylenic H), 816, 736 (Ar C-H bend); ***^{1}H-NMR (CDCl$_3$, 400 MHz), δ (ppm)***: 11.10 (1H, s, 6"-OH), 9.12 (1H, s, H-8), 8.77 (1H, d, *J* 4.9, H-10), 8.12 (1H, d, *J* 7.2, H-12), 7.76 (2H, t, *J* 8.3, H-6', 11), 7.38-7.42 (4H, m, H-3', 4', 2", 5"), 5.95 (1H, d, *J* 10.2 H-5), 3.98 (1H, dd, *J* 17.2 and 6.5, 4-H_y), 3.85 (6H, s, OCH_3-4",2'), 3.03 (1H, dd, *J* 17.5 and 8.1, 4-H_x), 2.85 (3H, s, CH_3-4); ***FAB-MS m/z:*** 455.08 $[M+H]^+$; *Anal. Calcd for* $C_{23}H_{19}Cl_2N_3O_3$: C, 60.54; H, 4.20; N, 9.21; Found: C, 60.63; H, 4.84; N, 9.53

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

Synthesized by method described in 2.1.2., from chalcone **3o** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 59%, Pale yellow solid; ***MP*** 127-129°C; ***IR (KBr)*** ν_{max}/cm^{-1}: 3221 (O-H), 1665 (N-C=O), 1596 (Ar C=C), 1260, 1092 (C–O), 1215 (C-N), 1108 (C–Cl), 3045, 2950 (C-H), 1502, 1465, 922, 816, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz) δ (ppm)***: 10.05 (2H, s, 6', 3"-OH), 9.09 (1H, s, 8-H), 8.63 (1H, d, *J* 3.9, 10-H), 8.10 (1H, d, *J* 7.2, 12-H), 7.65-7.60 (2H, m, H-2", 11), 6.78-6.84 (2H, m, H-4', 6'), 7.20-7.05 (2H, m, H-2', 5'), 6.43 (1H, s, H-5"), 5.95 (1H, dd, *J* 12.5 and 6.5, H-5), 3.87 (1H, dd, *J* 17.6 and 11.6, 4-H_y), 3.80 (3H, s, OCH_3-4'), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H_x), 2.32 (3H, s, CH_3-4); ***FAB-MS m/z:*** 407.58 $[M+H]^+$; *Anal. Calcd for* $C_{22}H_{18}ClN_3O_3$: C, 64.79; H, 4.45; N, 10.30 Found: C, 64.19; H, 4.95; N, 10.73.

3.1.32.Spectral data of (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 method described in 2.1.2., from chalcone **3p** (4 mmol) and nicotinic acid hydrazide (4 mmol); *Yield* 55%, Pale yellow powder; ***MP*** 187-190°C; ***IR (KBr)*** ν_{max}/cm^{-1}: 3227 (O-H), 1668 (N-C=O), 1594 (Ar C=C), 1265, 1090 (C–O), 1219 (C-N), 1101 (C–Cl), 3049, 2953 (C-H), 1501, 1468, 920, 816, 798 (Ar); ***^{1}H-NMR (CDCl$_3$, 400 MHz), δ (ppm)***: 9.04 (1H, s, 8-H), 8.64 (1H, d, *J* 3.9, 10-H), 8.19 (1H, d, *J* 7.2, 12-H), 7.64-7.60 (2H, m, H-2', 11), 6.84-6.90 (3H, m, H-2', 5', 6'), 5.93 (1H, dd, *J 12.3* and 6.2, H-5) 3.89 (1H, dd, *J 17.5* and 11.6, 4-H_y), 3.85 (6H, s, OCH_3-3', 4'), 3.80 (3H, s, OCH_3-4"), 3.16 (1H, dd, *J 17.8* and 4.8, 4-H_x); ***FAB-MS m/z:*** 451.13 $[M+H]^+$; *Anal. Calcd for* $C_{24}H_{22}ClN_3O_5$: C, 61.61; H, 4.74; N, 8.98; Found: C, 61.12; H, 4.50; N, 8.45.

3.2. *In vitro* Anti-malarial screening

All the 16 synthesized compounds **4(a-p)** were tested *in vitro* on Chloroquine (CQ) - sensitive isolates (MRC-2) and CQ-resistant isolates (RKL-9) of *P. falciparum* malaria parasite. For each compound, the inhibitory concentration 50% (IC_{50}) was determined, as presented in Table 2.

The inhibitory concentration (IC_{50}) was ranged from 0.050 to 4.539 μM for Chloroquine (CQ) -sensitive (MRC-2) and 0.413 to 5.59 μM for CQ-resistant (RKL-9) *P. falciparum* strains. Compounds **4c** and **4d** have shown better activity than CQ against CQ sensitive strain (MRC-2) of *P. falciparum* (IC_{50} = 0.058 μM) and against CQresistant strain (RKL-9) of parasite (IC_{50} = 0.468 μM). Compound **4d** was found to be most potent against sensitive (IC_{50} = 0.050 μM) as well as resistant (IC_{50} = 0.413 μM) strains of *P. falciparum*. Compound **4o** was found to be least active in this series of compounds.

Compound **4d** has methoxy group at meta, para position of one ring and hydroxyl group on ortho and para position of second ring. It indicates that electron withdrawing groups enhances the activity against both strains in this series of compounds.

3.3 Cell Cytotoxicity Assay

Cytotoxicity experiments performed on HepG2 cell line and determine their selectivity indexes so as to validate their real potential as selective antiplasmodial (Table 1). The maximum concentration of DMSO in any well was 0.1% and did not affect cell growth. The two compounds (**4c** and **4d**) were selected for this study which were found to be less toxic than CQ (Table 2). Yet for all the compounds, the cytotoxic/antiplasmodial ratios were >1, indicating better selectivity against *P. falciparum* even though a long cell-drug exposure time (72 hr) was given for cytotoxicity test. Selectivity ratios of the two compounds **4c** and **4d** were found to be 88.17 and 83.07 for CQ-resistant strain of *P. falciparum* (RKL-9) respectively.

Table 2: *In vitro* antimalarial activity of synthesized compounds against MRC-2 and RKL-9 strains of *P. falciparum*

S. No.	Compd	IC_{50} (μM)[a] ±SD		Cytotoxicity IC_{50} (μM)[d]	SI[e]
		MRC-2[b]	RKL-9[c]		
1	4a	0.092±0.052	0.579±0.248	-	-
2	4b	0.061±0.040	0.535±0.010	-	-
3	4c	**0.058±0.065**	**0.448±0.046**	39.50	88.17
4	4d	**0.050±0.17**	**0.413±0.27**	35.97	83.07
5	4e	0.412±0.036	0.538±0.058	-	-
6	4f	0.73±0.025	0.938±0.038	-	-
7	4g	1.036±0.148	1.142±0.042	-	-
8	4h	1.639±0.017	2.167±0.013	-	-
9	4i	1.765±0.076	2.039±0.154	-	-
10	4j	1.017±0.27	1.432±0.27	-	-
11	4k	1.047±0.125	1.134±0.164	-	-
12	4l	1.058±0.136	1.067±0.054	-	-
13	4m	1.626±0.036	2.832±0.056	-	-
14	4n	2.91±0.027	2.543±0.45	-	-
15	4o	3.226±0.078	3.865±0.246	-	-
16	4p	2.57±0.019	2.267±0.37	-	-
33	CQ	0.058±0.065	0.468±0.043	29	61.96

[a] *Concentration corresponding to 50% growth inhibition of the parasite, where efficacy in at least one strain is submicromolar results are in bold.*
[b] *Chloroquine sensitive strain of P. falciparum, IC_{50}, μM± SD, n=2*
[c] *Chloroquine resistant strain of P. falciparum. IC_{50}, μM± SD, n=2*
[d] *Cytotoxicity against HepG2 cell, Values are the mean of one experiment in duplicate*
[e] *SI: Selectivity index (IC_{50} values of cytotoxic activity / IC_{50} values of antimalarial activity against RKL-9).*
-, not determined

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