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Synthesis of 1,3,5-trisubstituted pyrazolines as potential antimalarial and antimicrobial agents

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Abstract

A series of novel 1,3,5-trisubstituted pyrazolines derivatives have been synthesized from chalcones and nicotinic acid hydrazide in two steps. In first step, chalcones were prepared by treatment of 4-hydroxy acetophenone with different substituted benzaldehyde by Claisen-Schimidt Condensation. In second step, various pyrazoline derivatives were prepared by reflux reaction of chalcones with nicotinic acid hydrazide in ethanolic solution. Compounds were confirmed by elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectral data and were evaluated for antimalarial and antibacterial activity. Compounds 5n (IC_{50}=0.022 μM for MRC-2 and IC_{50}=0.192 μM for RKL-9) displayed better antiplasmodial activity than the chloroquine (CQ) against chloroquine-sensitive (MRC-2) and chloroquine-resistant (RKL-9) *P. falciparum* strains. The *in vitro* cytotoxicity study conducted on the human HepG2 cell line (>30μM) and selectivity index (100-220) indicate that this series presents an interesting selective antiplasmodial profile. Further, *in vitro* heme crystallization inhibition assay showed compound 5e inhibited formation of β-hematin more efficiently than CQ. In addition, antibacterial and antifungal evaluations were conducted, compounds 5c, 5i and 5j displayed better antibacterial activity against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* than ciproafloxacin. Antifungal activity of compound 5l against *A. niger* (MIC-3.25μg/ml) and *C. albicans* (MIC-6.5μg/ml) was found to be better than the standard drug fluconazole.

Keywords: Pyrazolines, Claisen-Schmidt condensation, antimicrobial, antimalarial, antifungal, cytotoxicity assay, heme crystallization inhibition assay

1. Introduction

Malaria continues to present a major health challenge in many resource-limited countries, with an estimated 212 million cases of malaria worldwide and 429 000 deaths estimated in 2015[1,2]. Most deaths result from infection with *Plasmodium falciparum*, although *Plasmodium vivax* also contributes substantially to the overall morbidity[3]. In absence of effective vaccines, treatment of malaria only relies on chemotherapy[4]. The current antimalarial chemotherapy has various drawbacks such as drug resistance, toxicity, and higher cost which heightened alarms about malaria in the international health community[5]. Frontline drugs (Chloroquine, mefloquine etc.) are becoming futile due to the emergence and spread of drug resistance[6]. The newer chemotherapeutic agents such as artesunate and arteether suffer with its lower abundance also get trapped by the resistance problem[7,8]. The dearth of new affordable drugs has not only intricate the clinical management of malaria in endemic areas, but has also resulted increased mortality rate. This state emphasizes the need for urgent discovery of new chemical entities.

Considering all the scaffolds of heterocyclic ring, pyrazoline among the various 5-membered heterocyclic compound derivatives have received significant attention in the recent years due to their diverse pharmacological and biological activities such as antifungal[9,10], antidepressant[11,12], anticonvulsant[12,13], anti-inflammatory[14,15], antibacterial[16-18], antitubercular[18], anticancer[19-21], human acyl-CoA: cholesterol acyltransferase inhibitors[22] and analgesic properties[23]. 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[24-28]. Quite recently, based on pharmacophore mapping studies Acharya *et al*.[28,29] explored antimalarial potential of 1,3,5-trisubstituted pyrazoline by targeting haem detoxification pathway of malaria parasite.

In order to carry on with investigation of the antiplasmodial potential of new pyrazoline derivatives, we focused on evaluation of influence of substitution of position 5 of the pyrazoline ring toward this activity. All the synthesized compounds were evaluated for *in vitro* antiplasmodial activity against chloroquine-sensitive (MRC-2) and chloroquine-resistant (RKL-9) *P. falciparum* strains. In order to assess compounds selectivity, cytotoxicity study was performed on human HepG2 cell line. The compounds were also evaluated for β-hematin formation inhibition activity to known possible mechanism of action. In parallel, to investigate potential of pyrazoline scaffold in other pharmacological profile, synthesized compounds have been screened for antimicrobial and antifungal activity.

2. Results and discussion

2.1. Chemistry

The synthesis of 1,3,5-trisubstituted pyrazoline scaffolds were carried out as outlined in Fig.1. In the first step, chalcones (3a-o) were synthesized by reacting 1-(4-Hydroxy-phenyl) -ethanone with different substituted aldehydes by the well-known Claisen- Schmidt reaction and products were purified by crystallization from methanol (60–80% yield). In the second step, desirable 1,3,5-trisubstituted pyrazolines (5a–o) were prepared by refluxing chalcone and nicotinic acid hydrazide in *n*-butanol.

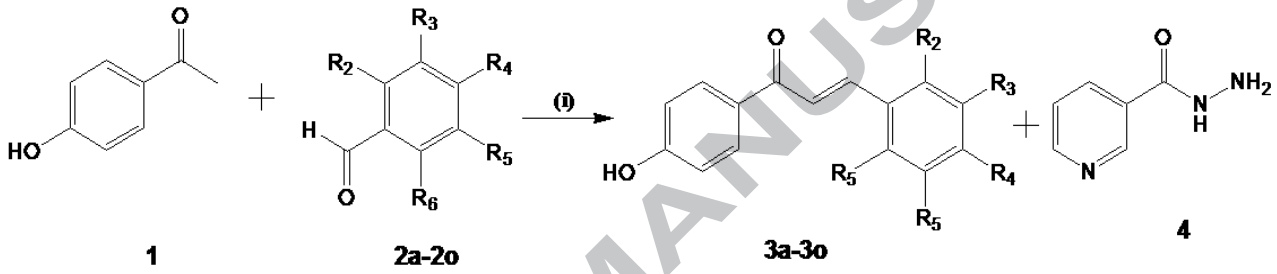
During the synthesis of 1,3,5-trisubstituted pyrazolines, it has been observed that refluxing time or moreover the rate of the reaction is greatly influenced by the structure and position of the substituents. It is supposed that the formation of 2-pyrazolines by chalcone takes place *via* an initial formation of an aryl hydrazone with a subsequent nucleophilic attack of nitrogen upon the carbon-carbon double bond at β position. Hence, whatever the factors will improve the electropositive character of β carbon will ultimately accelerate the rate of the reaction and consequently will reduce the refluxing time for the 2-pyrazoline formation. Compounds 5h, 5i and 5l took least refluxing time (8-9 h) in their preparation since they have been formed from halogenated chalcones (3h, 3i and 3l). Compounds 5a, 5b, 5c, 5m and 5n took 16-18 h for complete conversion from alkylated chalcones 3a, 3b, 3c, 3m and 3n followed by alkoxy substituted (5d, 5e, 5k and 5o) chalcones which took 19-22 h for complete conversion into corresponding 2-pyrazolines. Apart from the structure and position of the substituents, solvent used in the reaction also profoundly affect the course of the reaction. Lower acidity of the *n*-butanol compared to other solvent like acetic acid used in condensation between chalcone and an acid hydrazide, improve the yield quantitatively. It is due to the alkaline nature of final products which get protonated by the solvent like acetic acid and washed away by water.

Structures of compounds 3a–o were confirmed by IR, NMR data as well as their distinct R_f values in TLC analysis. As the chalcones are formed, C=C is introduced adjacent to a carbonyl group result in delocalization of the π electrons in the C=O and C=C bonds. This conjugation increases the single bond character of the C=O and C=C bonds in the resonance hybrid and hence lowers their force constants, resulting in a lowering of the frequencies of carbonyl and double-bond absorption. Characteristics IR signals in the ranges 1685-1695 cm^{-1} for C=O and 1645-1655 cm^{-1} for C=C were observed for chalcones.

Structures of compounds 5a–o were confirmed by IR and NMR techniques. All of the 1,3,5-trisubstituted pyrazolines possesses a similar basic skeletal structure. As soon as 2-pyrazolines are

formed C=O disappeared from the chalcones and pyrazoline ring is formed. Therefore, IR signals were assigned by comparing the spectra of the products (5a–o) with their corresponding chalcones.

Proton NMR signals were assigned by comparing the spectra of the products (5a–o) with their corresponding chalcones. Signals around 1.8-1.9 for 2H (CH_2) and 4.7-4.9 for 1H, (CH) of pyrazoline ring were assigned. These signals clearly showing the formation of pyrazoline ring. Similarly, signal around 155, 40 and 39 were obtained for C_3, C_4, and C_5 respectively, for pyrazoline ring which further support the ring formation.



S.No.	Comp. No.			R_2	R_3	R_4	R_5	R_6
1	2a	3a	5a	C_2H_5	H	H	H	H
2	2b	3b	5b	i-C_3H_7	H	H	H	H
3	2c	3c	5c	CH_3	H	CH_3	H	CH_3
4	2d	3d	5d	OCH_3	H	H	OCH_3	H
5	2e	3e	5e	H	OCH_3	OCH_3	H	H
6	2f	3f	5f	H	H	$OCH_2C_6H_5$	H	H
7	2g	3g	5g	H	CH_3	OCH_3	H	H
8	2h	3h	5h	H	H	Br	H	H
9	2i	3i	5i	H	H	F	H	H
10	2j	3j	5j	H	H	NO_2	H	H
11	2k	3k	5k	H	H	OCH_3	H	H
12	2l	3l	5l	H	Cl	H	H	H
13	2m	3m	5m	H	H	i-C_3H_7	H	H
14	2n	3n	5n	H	H	C_3H_7	H	H
15	2o	3o	5o	H	H	Oi-C_3H_7	H	H

Reagents and conditions: (i) Methanolic NaOH, stirred at RT 20-24 hr (ii) n-butanol, reflux 8-22 hr

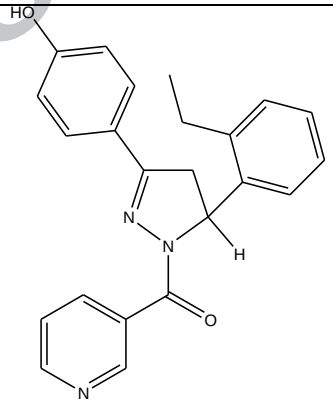
Fig 1. Scheme for the synthesis of 1, 3, 5-trisubstituted pyrazolines

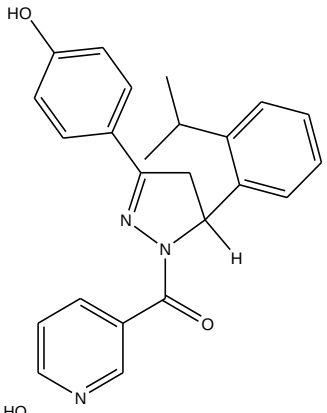
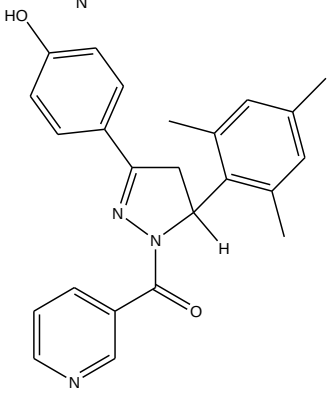
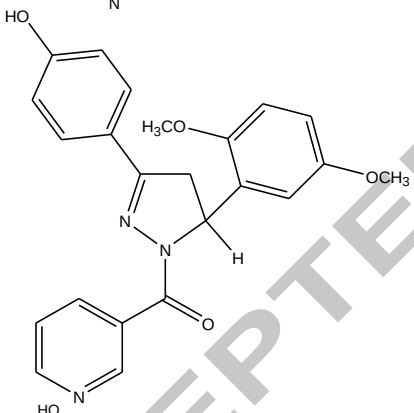
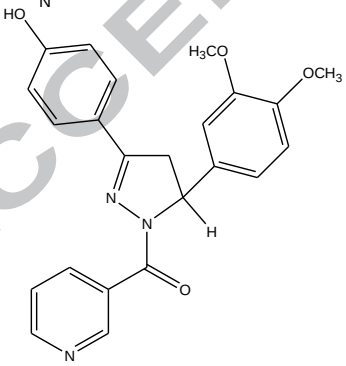
2.2 Biological evaluation

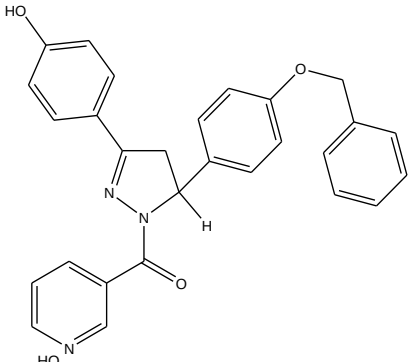
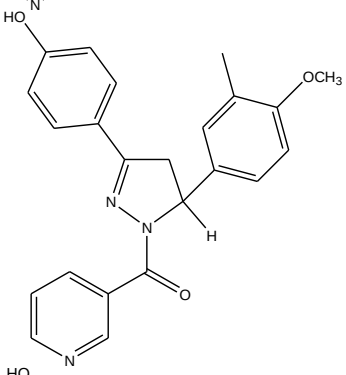
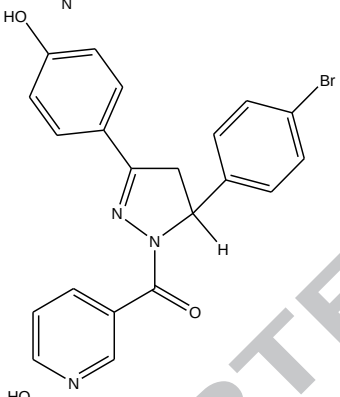
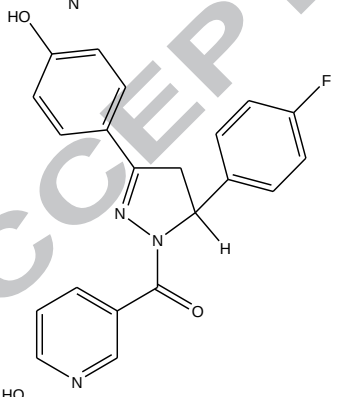
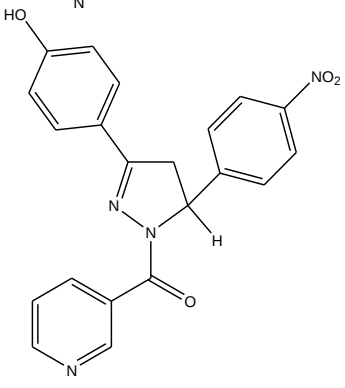
2.2.1 In vitro antiplasmodial activity

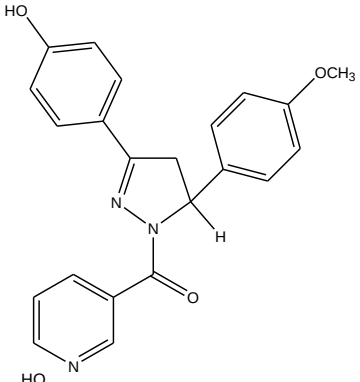
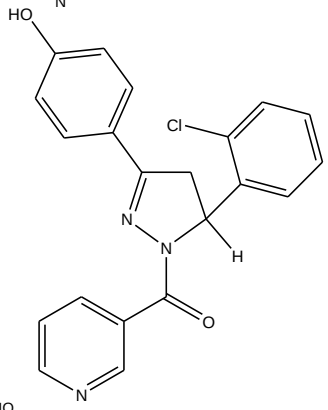
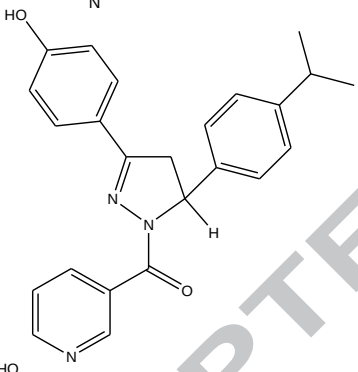
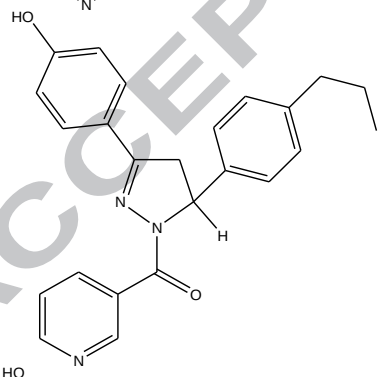
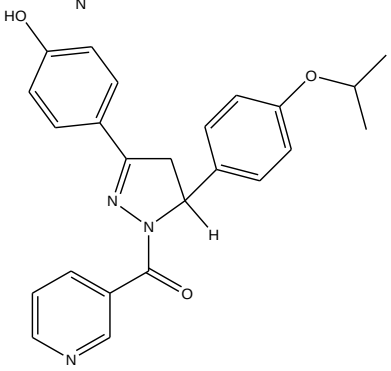
All the synthesized compounds (5a to 5o) were tested *in vitro* on Chloroquine (CQ) - sensitive isolates (MRC-2) and CQ-resistant isolates (RKL-9) of *P. falciparum* malaria parasite. For each compound, the inhibitory concentration 50% (IC_{50}) was determined, as presented in Table 1. The inhibitory concentration (IC_{50}) was ranged from 0.02 to 2.5 μM for Chloroquine (CQ) - sensitive (MRC-2) and 0.19 to 2.9 μM for CQ-resistant (RKL-9) *P. falciparum* strains. Compounds 5d, 5e, 5m, 5n and 5o of this series have shown better activity than chloroquine (IC_{50} = 0.05 μM) against chloroquine sensitive strain (MRC-2) of *P. falciparum* and except 5m (almost similar to chloquine) rest four compounds have shown better activity than chloroquine (IC_{50} = 0.4 μM) against chloroquine resistant strain (RKL-9) of parasite. Compound 5n was found to be most potent against sensitive (IC_{50} = 0.022 μM) as well as resistant (IC_{50} = 0.192 μM) strains of *P. falciparum*. For compound 5n, R4 is propyl, 5m, R4 is isopropyl, 5o R4 is alkoxy, 5e and 5d has methoxy group at meta, para and ortho, meta position respectively. Interestingly, compounds with para substitution showed better antiplasmodial activity except those with electron withdrawing groups (compound 5f, 5h, 5i, 5j, and5l) as well as three carbons aliphatic chain (compound 5n) enhances the potency while branching of alkyl (compound 5m, 5o) and step down of one carbon (compound 5a) decreases the activity against both strains.

Table 1. *In vitro* antiplasmodial activity, cell cytotoxicity and selectivity Index of the 1,3,5-trisubstituted pyrazoline derivatives

S.	Compound	IC_{50} ((μM))[a] ± SD		Cytotoxicity IC_{50} (μM)[d]	SI[e]
		MRC-2[b]	RKL-9[c]		
5a		0.081 ± 0.051	0.471 ± 0.27	35.39	75.14

5b		0.110 ± 0.014	1.650 ± 0.114	49.68	30.12
5c		0.094 ± 0.022	0.544 ± 0.334	54.54	100.26
5d		0.040 ± 0.034	0.330 ± 0.063	56.76	172
5e		0.030 ± 0.055	0.251 ± 0.054	53.57	213.43

5f		0.214 ± 0.121	2.550 ± 0.051	11.42	4.48
5g		0.063 ± 0.019	0.779 ± 0.153	44.78	57.48
5h		0.141 ± 0.213	0.970 ± 0.166	58.71	60.5
5i		2.500 ± 0.220	2.902 ± 0.091	52.43	18.07
5j		1.566 ± 0.051	2.441 ± 0.027	18.62	7.63

5k		0.059 ± 0.010	0.556 ± 0.035	42.44	76.33
5l		0.240 ± 0.043	2.332 ± 0.048	47.74	20.47
5m		0.034 ± 0.033	0.465 ± 0.021	38.74	83.31
5n		0.022 ± 0.015	0.192 ± 0.024	41.56	216.46
5o		0.038 ± 0.045	0.337 ± 0.021	45.43	138.93

Ref.	Chloroquine	0.050 ± 0.031	0.401 ± 0.102	27.00	67.33

[a] Concentration corresponding to 50% growth inhibition of the parasite.
[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 line. Values are the mean of one experiment in duplicate
[e]SI: Selectivity Index (IC_{50} value of Cytotoxicity activity / IC_{50} values of antiplasmodial activity against RKL-9)
Ref. – Reference compound i.e. chloroquine

2.2.2 Cytotoxicity and selectivity index

Cytotoxicity experiments performed on HepG2 cell line and determine their selectivity indexes so as to validate their real potential as selective antiplasmodial (Table 1). Among the series, only two compounds 5f (IC_{50} = 11.42 μM) and 5j (IC_{50} = 18.62 μM) were found to be more toxic than chloroquine (IC_{50} = 27.0 μM) rest thirteen compounds did not displayed cytotoxicity properties in comparison with the standard chloroquine. Selectivity indexes for CQ-resistant (RKL-9) *P. falciparum* strains ranging between 4 and 217. Selectivity ratios of two most potent hit compounds 5e and 5n were 213.43 and 216.46 indicates their selectivity towards antiplasmodial activity.

2.2.3 In vitro Heme Crystallization Inhibition Assay

Compounds were studied for their ability to inhibit the crystallization of hematin to β-hematin (the synthetic equivalent of hemozoin) *in vitro* using a colorimetric β-hematin inhibition assay. The results are given in Table 2. Compound 5a, 5b, 5c, 5m and 5n having alkyl substitutions, inhibited the formation of β-hematin in the range of 48-81%. Among these compounds 5n inhibited 81% formation of β-hematin which is quite near to CQ (87%) Interestingly, compounds 5f, 5i and 5j which displayed least active *in vitro* antiplasmodial results also showed very less (<5%) inhibition of β-hematin formation, this might be due to their inability to accumulate significantly within the parasite's digestive vacuole, which is the definitive site of hemoglobin catabolism and therefore the site of action of hemozoin formation inhibitors. Compounds having alkoxy substitution, e.g. 5d, 5e, 5g and 5k and 5o significantly inhibited β-hematin formation in the range of 54-89%. Among these compounds 5e inhibited 89% formation of β-hematin which is more than CQ (87%). Except a few, most of the synthesized compounds in this study, especially having alkyl and alkoxy substitutions accumulated significantly within the parasite's digestive vacuole which

indicates, chloroquine and 1,3,5- trisubstituted pyrazolines (5a–o) may have a similar antiplasmodial mode of action. This finding further strengthens the hypothesis that heme polymerization inhibition may be a possible mode of action for this series of compounds (5a–o).

Table 2. *In vitro* heme crystallization inhibition assay of synthesized compounds 5a-5o

Comp.	% Inhibition of β-hematin Formation*
5a	53 ± 0.24
5b	57 ± 0.39
5c	67 ± 0.29
5d	71 ± 0.67
5e	89 ± 0.71
5f	< 5
5g	66 ± 0.24
5h	76 ± 1.00
5i	< 5
5j	< 5
5k	54 ± 0.03
5l	31 ± 0.06
5m	48 ± 0.54
5n	81 ± 0.62
5o	78 ± 0.71
CQ	87 ± 2.65

*Average of duplicate determinations and equivalents of compounds (relative to heamatin)

2.5. *In vitro* antimicrobial activity

All the target compounds were evaluated for their *in vitro* antimicrobial activity against *Staphylococcus aureus* (MTCC 3160) and *Bacillus subtilis* (MTCC 121) representing Gram-positive bacteria, and *Pseudomonas aeruginosa* (MTCC 741) and *Escherichia coli* (MTCC 51) representing Gram-negative bacteria. Compounds were also evaluated for their *in vitro* antifungal activity against *Candida albicans* (MTCC 227) and *Aspergillus niger* (MTCC 8189). The results of *in vitro* antibacterial as well as antifungal activities of compounds (5a–5o) are summarized in Table 3.

Table 3. ***In vitro*** **antimicrobial activity of synthesized compounds 5a–5o (MIC in μg/ml)**

Compound	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*A. niger*	*C. albicans*
5a	50	50	12.5	25	50	100
5b	25	12.5	3.25	12.5	25	50
5c	12.5	12.5	6.5	6.5	25	50
5d	25	25	25	12.5	12.5	75
5e	50	50	50	25	6.5	75
5f	50	50	25	12.5	12.5	3.25
5g	25	25	12.5	6.5	25	50
5h	6.5	12.5	25	12.5	25	50
5i	12.5	3.25	6.5	12.5	25	12.5
5j	6.5	12.5	6.5	25	25	12.5
5k	50	75	25	25	12.5	50
5l	75	12.5	50	3.25	3.25	6.5
5m	12.5	50	3.25	12.5	25	12.5
5n	12.5	50	12.5	12.5	25	25
5o	50	75	6.5	50	12.5	6.5
Ciprofloxacin[a]	25	25	12.5	12.5	-	-
Fluconazole[b]	-	-	-	-	25	12.5

* Values are mean ± SD of three replicates
[a]ciprofloxacin was used as a positive control against bacteria species
[b]fluconazole was used as a positive control against fungi species

Compounds 5c, 5h, 5i, 5j, 5m and 5n showed better antimicrobial effects with a MIC value of 12.5, 6.5, 12.5, 6.5, 12.5 and 12.5 μg/ml respectively, than ciproafloxacin (MIC = 25 μg/ml) against *S. aureus*. Similarly, 5b, 5d and 5g showed activity comparable to the standard against *S. aureus*. In case of *B. subtilis*, compounds 5b, 5c, 5h, 5i, 5j and 5l with MIC values in the range 3.25–12.5 μg/ml exhibited better antibacterial activity than all other compounds and standard drug ciproafloxacin (MIC = 25 μg/ml).

In general, it has been observed that synthesized compounds displayed better antibacterial performance against the Gram-negative bacteria than the Gram-positive bacteria. Compounds 5b, 5c, 5i, 5j, 5m and 5o showed superior activity with MIC value of 3.25, 6.5, 6.5, 6.5, 3.25 and 6.5 μg/ml than ciproafloxacin (MIC = 12.5 μg/ml) against *E. coli*. Likewise, 5a, 5g, 5n showed antibacterial activity equipotent with the standard drug against *E. coli*. In case of *P. aeruginosa*, the compounds 5c, 5g and 5l with MIC values in the range 3.25–12.5 μg/ml exhibited better activity than all other compounds and the standard drug. Compounds 5b, 5c, 5h, 5i and 5j showed excellent

broad spectrum activity in the range 3.25-25 μg/ml concentration, against all the tested Gram-positive and Gram-negative bacterial strains.

The results of the antifungal activities of synthesized compounds (5a–o) were summarized in Table 3. In comparison with standard antifungal drug, fluconazole, compounds 5b, 5c, 5g, 5h, 5i, 5j, 5m and 5n showed equivalent inhibitory activity against *A. niger* and the compounds 5d, 5e, 5f, 5k, 5l and 5o exhibited greater antifungal activity than standard drug against *A. niger*. Compounds 5e and 5l with MICs 6.5 and 3.25 μg/ml, respectively were most active against *A. niger*. Similarly, compounds 5f, 5l and 5o with MICs 3.25, 6.5 and 6.5 μg/ml respectively, showed greater activity than standard drug against *C. albicans*. Compounds 5i, 5j and 5m displayed a similar level of activity than standard drug against *C. albicans*.

3. Conclusion

A new series of 1,3,5-trisubstituted pyrazolines was prepared in two steps through a simple and efficient synthesis pathway. Among these fifteen compounds four antiplasmodial hit compounds were identified from an *in vitro* screening. Compound 5n was identified as most potent antiplasmodial hit, displaying an IC_{50}=0.022 μM against CQ-sensitive (MRC-2) and IC_{50}=0.192 μM against CQ-resistant (RKL-9) *P. falciparum* strains. The structure activity relationships clearly show that the substitution at para position with electron releasing groups has great influence on antiplasmodial activity as compared to electron withdrawing groups. Most of the compounds have shown safe toxicological profile toward HepG2 cell line and high selectivity indexes compared to chloroquine indicating as selective antiplasmodial agents. In order to assess the mechanism of antiplasmodial effect, compounds were also evaluated for *in vitro* heme crystallization inhibition assay. Compound 5e (89%) and 5n (81%) significantly inhibit the β-hematin formation.

Additionally, to investigate potential of pyrazoline scaffold in other pharmacological profile, synthesized compounds have been screened for antimicrobial against a panel of *S. aureus, B. subtilis. E.coli, P. aeruginosa* and antifungal activity against a panel of *A. niger, C. albicans.* These observations indicated that the 1,3,5-trisubstituted pyrazolines constitute attractive chemical scaffold for the establishment of new chemical entities with antimalarial potential.

4. Experimental

4.1. General

Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Agilent Cary 630 FTIR Spectrometer in KBr. ^{13}C and ^{1}H NMR spectra were recorded on Bruker

DRX - 300 instruments in DMSO-d_6 at 27°C. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. The coupling constants (*J*) are given in Hertz. Spin multiples are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet) and m (multiplet). Mass spectra were recorded on a JEOL Sx 102/DA-6000 mass spectrometer using fast atomic bombardment (FAB). Elemental analysis was undertaken with Elemental vario EL III Carlo Erba 1108 analyzer. The results of elemental analysis (C, H, and N) were within ± 0.4% of the calculated values. The progress of the reaction and purity of the obtained compounds was monitored by thin layer chromatography (TLC) on pre-coated silica gel $60F_{254}$ (mesh) and a solvent system by using a mixture of ethyl acetate and hexane (3:7 v/v) for chalcones and using a mixture of acetone and petroleum ether (40:60 v/v) as mobile phase for 1, 3, 5- trisubstituted pyrazolines. The spots were detected by exposure to the UV lamp at 254 nm. Overall synthesis of titled 1, 3, 5-trisubstituted pyrazolines (5a-5o) were performed as outlined in fig.1.

4.2. General procedure for the synthesis of chalcones (3a to 3o)

To the solution of (0.05 mole, 6.8 g) of 1-(4-Hydroxy-phenyl) -ethanone (1) in 30 ml of methanol on an ice bath, freshly prepared 2 N methanolic NaOH solution (50 ml) was added and stirred for 20 min. To this 0.05 mole of appropriate aldehyde (2a–l) was added and the reaction mixture was stirred at room temperature for 20-24 h. The reaction mixture was cooled in an ice bath and neutralized with dilute hydrochloric acid. The obtained precipitate was separated by filtration and washed with distilled water to give the crude product. The product (3a-o) so obtained was recrystallized from 75% methanol. The purity of the products was checked on TLC by using a mixture of ethyl acetate and hexane as mobile phase.

4.2.1. *3-(2-Ethyl-phenyl)-1-(4-hydroxy-phenyl)-propenone (3a)*

Prepared by the above method from 2a (0.05 mole, 6.6 ml) and 1 (0.05 mole, 6.8 g); yield: 72% as pale yellow crystalline solid; R_f = 0.64 in EtOAc/hexane, 3:7; mp: 202–204 ^oC. FTIR (neat) ν_{max}: 3085 (CH, aromatic), 767 (CH, substituted phenyl ring), 1686 (C=O), 1640 (C=C, conjugated to C=O), 1590 (C---C str, aromatic), 2961–2835 (CH), 1443 (CH); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 7.3 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 6.4 Hz), 7.1 (m, 3H, ArH), 7.25 (d, 1H, ArH, *J* = 6.9 Hz), 2.55 (m, 2H, methylene), 1.25 (t, 3H, methyl, *J* = 7.4 Hz).

4.2.2. *1-(4-Hydroxy-phenyl) -3-(2-isopropyl-phenyl) -propenone (3b)*

Prepared by the above method from 2b (0.05 mole, 7.6 ml) and 1 (0.05 mole, 6.8 g); yield: 63% as off white crystalline solid; R_f = 0.66 in EtOAc/hexane, 3:7; mp: 199–201 ^oC. FTIR (neat) ν_{max}: 3094 (CH, aromatic), 765(CH, substituted phenyl ring), 1689 (C=O), 1642 (C=C, conjugated to C=O), 1584 (C---C str, aromatic ring), 2956–2835 (C-H), 1444(C-H def); ^{1}H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 7 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 6.5 Hz), 7.08 (dd, 2H, ArH, *J* = 5.5 Hz, *J* = 6.2 Hz), 7.03 (s, 1H, ArH), 7.22(d, 1H, ArH, *J* = 6.7 Hz), 3.10 (m, 1H, methine), 1.3 (dd, 6H, methyl, *J* = 7.1 Hz, *J* = 8 Hz).

4.2.3. ***1-(4-Hydroxy-phenyl) -3-(2,4,6-trimethyl-phenyl) -propenone (3c)***

Prepared by the above method from 2c (0.05 mole, 7.4 ml) and 1 (0.05 mole, 6.8 g); yield: 65% as yellow crystalline solid; R_f = 0.62 in EtOAc/hexane, 3:7; mp: 246–248 ^oC. FTIR (neat) ν_{max}: 3083 (C-H, aromatic ring), 776 (C-H, substituted phenyl ring), 1688 (C=O), 1641 (C=C, conjugated to C=O), 1578 (C---C straromatic ring), 2960–2848 (C-H) 1437 (C-H def); ^{1}H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 6.6 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.1 Hz), 2.35 (s, 9H, methyl), 6.65 (d, 2H, ArH, *J* = 6.4 Hz).

4.2.4. ***3-(2,5-Dimethoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone (3d)***

Prepared by the above method from 2d (0.05 mole, 8.3 g) and 1 (0.05 mole, 6.8 g); yield: 69% as light brown crystalline solid; R_f = 0.55 in EtOAc/hexane, 3:7; mp: 211-213 ^oC. FTIR (neat) ν_{max}: 3085 (C-H, aromatic ring), 767 (C-H, substituted phenyl ring), 1689 (C=O), 1638 (C=C, conjugated to C=O), 1579 (C---C straromatic ring), 2962–2843 (C-H), 1447 (C-H def); ^{1}H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 6.4 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.2 Hz), 3.70 (s, 6H, methoxy), 6.65 (m, 3H, ArH).

4.2.5. ***3-(3,4-Dimethoxy-phenyl)-1-(4-hydroxy-phenyl)-propenone (3e)***

Prepared by the above method from 2e (0.05 mole, 8.3 g) and 1 (0.05 mole, 6.8 g); yield: 70% as pale yellow crystalline solid; R_f = 0.52 in EtOAc/hexane, 3:7; mp: 219-221 ^oC. FTIR (neat) ν_{max}: 3095 (C-H, aromatic ring), 764 (C-H, substituted phenyl ring), 1690 (C=O), 1642 (C=C, conjugated to C=O), 1580 (C---C str of aromatic ring), 2970–2834 (C-H), 1433 (C-H def); ^{1}H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 6.5 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.2 Hz), 3.75 (s, 6H, methoxy), 6.65 (m, 3H, ArH).

4.2.6. ***3-(4-Benzyloxy-phenyl)-1-(4-hydroxy-phenyl)-propenone (3f)***

Prepared by the above method from 2f (0.05 mole, 10.6 g) and 1 (0.05 mole, 6.8 g); yield: 66% as yellow crystalline solid; R_f = 0.47 in EtOAc/hexane, 3:7; mp: Decomposed at 232 ^oC. FTIR (neat) ν_{max}: 3094 (C-H, aromatic ring), 771 (C-H, substituted phenyl ring), 1692 (C=O), 1639 (C=C, conjugated to C=O), 1589 (C---C str aromatic ring), 2964–2825 (C-H), 1446 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6.3 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.2 Hz), 5.20 (s, 2H, methylene), 7.25 (m, 5H, ArH).

4.2.7. ***1-(4-Hydroxy-phenyl)-3-(4-methoxy-3-methyl-phenyl)-propenone (3g)***

Prepared by the above method from 2g (0.05 mole, 7.3 g) and 1 (0.05 mole, 6.8 g); yield: 76% as yellow amorphous solid; R_f = 0.52 in EtOAc/hexane, 3:7; mp: 219-221 ^oC. FTIR (neat) ν_{max}: 3095 (C-H, aromatic ring), 764 (C-H, substituted phenyl ring), 1690 (C=O), 1642 (C=C, conjugated to C=O), 1580 (C---C str, aromatic ring), 2970–2834 (C-H), 1433 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6.5 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.3 Hz), 3.7 (s, 3H, methoxy), 2.4 (s, 3H, methyl), 6.6 (s, 1H, ArH), 7.0 (m, 2H, ArH).

4.2.8. ***3-(4-Bromo-phenyl)-1-(4-hydroxy-phenyl)-propenone (3h)***

Prepared by the above method from 2h (0.05 mole, 9.3 g) and 1 (0.05 mole, 6.8 g); yield: 62% as off white crystalline solid; R_f = 0.48 in EtOAc/hexane, 3:7; mp: 174–175 ^oC. FTIR (neat) ν_{max}: 3095 (C-H, aromatic ring), 765 (C-H, substituted phenyl ring), 1693 (C=O), 1640 (C=C, conjugated to C=O), 1584 (C---C straromatic ring), 2957–2828 (C-H), 1447 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6.4 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.3 Hz), 7.20 (m, 2H, ArH), 7.4 (m, 2H, ArH).

4.2.9. ***3-(4-Fluoro-phenyl)-1-(4-hydroxy-phenyl)-propenone (3i)***

Prepared by the above method from 2i (0.05 mole, 6.6 mL) and 1 (0.05 mole, 6.8 g); yield: 68% as pale yellow crystalline solid; R_f = 0.45 in EtOAc/hexane, 3:7; mp: 181-183 ^oC. FTIR (neat) ν_{max}: 3094 (C-H, aromatic ring), 759 (C-H, substituted phenyl ring), 1690 (C=O), 1641 (C=C, conjugated to C=O), 1592 (C---C str, aromatic ring), 2960–2810 (C-H), 1444 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6.5 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.5 Hz), 7.30 (m, 2H, ArH), 7.0 (m, 2H, ArH).

4.2.10. ***1-(4-Hydroxy-phenyl)-3-(4-nitro-phenyl)-propenone (3j)***

Prepared by the above method from 2j (0.05 mole, 7.6 g) and 1 (0.05 mole, 6.8 g); yield: 74% as yellow crystalline solid; R_f = 0.49 in EtOAc/hexane, 3:7; mp: 222–223 ^oC. FTIR (neat) ν_{max}: 3094 (C-H, aromatic ring), 765 (C-H, substituted phenyl ring), 1688 (C=O), 1643 (C=C, conjugated to C=O), 1585 (C---C straromatic ring), 2945–2823 (C-H), 1456 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6.6 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.6 Hz), 7.55 (m, 2H, ArH), 8.2 (m, 2H, ArH).

4.2.11. ***1-(4-Hydroxy-phenyl)-3-(4-methoxy-phenyl)-propenone (3k)***

Prepared by the above method from 2k (0.05 mole, 6.1 ml) and 1 (0.05 mole, 6.8 g); yield: 70% as straw yellow amorphous solid; R_f = 0.53 in EtOAc/hexane, 3:7; mp: 217–218^oC. FTIR (neat) ν_{max}: 3103 (C-H, aromatic ring), 766 (C-H, substituted phenyl ring), 1695 (C=O), 1640 (C=C, conjugated to C=O), 1589 (C---C str, aromatic ring), 2972–2848 (C-H), 1432 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6.5 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.8 Hz), 7.2 (m, 2H, ArH), 6.7 (m, 2H, ArH), 3.7 (s, 3H, methoxy).

4.2.12. ***3-(2-Chloro-phenyl)-1-(4-hydroxy-phenyl)-propenone (3l)***

Prepared by the above method from 2l (0.05 mole, 5.6 ml) and 1 (0.05 mole, 6.8 g); yield: 79% as pale yellow amorphous solid; R_f = 0.56 in EtOAc/hexane, 3:7; mp: 229–231 ^oC. FTIR (neat) ν_{max}: 3097 (C-H, aromatic ring), 775 (C-H, substituted phenyl ring), 1691 (C=O), 1642 (C=C, conjugated to C=O), 1586 (C---C str, aromatic ring), 2961–2834 (C-H), 1448 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.40 (d, 1H, 1-ethylene, *J* = 6.9 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.5 Hz), 7.20 (m, 2H, ArH), 7.0 (m, 2H, ArH).

4.2.13. ***1-(4-Hydroxy-phenyl)-3-(4-isopropyl-phenyl)-propenone (3m)***

Prepared by the above method from 2m (0.05 mole, 7.5 g) and 1 (0.05 mole, 6.8 g); yield: 62% as yellow amorphous solid; R_f = 0.66 in EtOAc/hexane, 3:7; mp: decomposed at 248 ^oC. FTIR (neat) ν_{max}: 3095 (C-H, aromatic ring), 764 (C-H, substituted phenyl ring), 1690 (C=O), 1642 (C=C, conjugated to C=O), 1580 (C---C str, aromatic ring), 2970–2834 (C-H), 1433 (C-H def); ^1H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.45 (d, 1H, 1-ethylene, *J* = 6 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.2 Hz), 3.1 (dd, 6H, methyl, *J* = 5.4 Hz, *J* = 7.4 Hz), 1.3 (m, 1H, methine), 7.2 (m, 2H, ArH), 7.0 (m, 2H, ArH).

4.2.14. ***1-(4-Hydroxy-phenyl)-3-(4-propyl-phenyl)-propenone (3n)***

Prepared by the above method from 2n (0.05 mole, 7.3 g) and 1 (0.05 mole, 6.8 g); yield: 73% as yellow amorphous crystalline solid; R_f = 0.59 in EtOAc/hexane, 3:7; mp: 206-208 ^oC. FTIR (neat)

ν_{max}: 3095 (C-H, aromatic ring), 764 (C-H, substituted phenyl ring), 1690 (C=O), 1642 (C=C, conjugated to C=O), 1580 (C---C str, aromatic ring), 2970–2834 (C-H), 1433 (C-H def); ^{1}H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 6.5 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.5 Hz), 2.6 (t, 2H, methylene, *J* = 6.1 Hz), 1.7 (m, 2H, methylene), 1.1 (t, 3H, methyl, *J* = 8.2 Hz), 7.2 (m, 2H, ArH), 7.0 (m, 2H, ArH).

4.2.15. ***1-(4-Hydroxy-phenyl)-3-(4-isopropoxy-phenyl)-propenone (3o)***

Prepared by the above method from 2o (0.05 mole, 7.9 g) and 1 (0.05 mole, 6.8 g); yield: 70% as pale yellow amorphous solid; R_f = 0.49 in EtOAc/hexane, 3:7; mp: 233-234 ^{o}C. FTIR (neat) ν_{max}: 3095 (C-H, aromatic ring), 764 (C-H, substituted phenyl ring), 1690 (C=O), 1642 (C=C, conjugated to C=O), 1580 (C---C str, aromatic ring), 2970–2834 (C-H), 1433 (C-H def); ^{1}H NMR (300 MHz, d): 5.1(s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.55 (m, 2H, ArH), 7.44 (d, 1H, 1-ethylene, *J* = 6.6 Hz), 8.25 (d, 1H, 1-ethylene, *J* = 7.4 Hz), 4.0 (m, 1H, methine), 1.4 (dd, 6H, methyl, *J* = 5.6 Hz, *J* = 6.1 Hz), 7.2 (m, 2H, ArH), 6.7 (m, 2H, ArH).

4.3. ***General procedure for the synthesis of 1, 3, 5- trisubstituted pyrazolines (5a-5o)***

To the solution of (4 mmole) of appropriate chalcone (3a–o) in 10 ml of n-butanol, nicotinic acid hydrazide (4) (4 mmole, 0.55 g) was added and the reaction mixture was refluxed for 8–22 h. Progress of the reaction was monitored in every 60 min interval on silica coated TLC plates using a mixture of acetone and petroleum ether (40:60 V/V) as mobile phase. The excess of solvent was removed under reduced pressure and the reaction mixture was cooled in an ice bath. The product precipitated out at low temperature was washed with distilled water, reconstituted in a minimum amount of methanol and dried under reduced pressure. All the 1, 3, 5 – trisubstituted pyrazolines (compounds 5a-5o) were synthesized by this method. Corresponding reflux time is given to individual products.

4.3.1.***[5-(2-Ethyl-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5a)***

Prepared by the above method from 3a (4 mmole, 1.01 g) and 4 (4 mmole, 0.55 g) after 17h reflux; yield: 87% as shiny white amorphous solid; mp: 188-190 ^{o}C. FTIR (neat) ν_{max}: 3087 (C-H, aromatic ring), 765 (C-H, substituted phenyl ring), 1667 (C=O), 1591 (C---C str, aromatic ring), 2960–2836 (C-H), 1440 (C-H def), 1609 (C=N), 3334 (C-N); ^{1}H NMR (300 MHz, d): 7.0-7.1(m, 4H, ArH), 2.7 (m, 2H, methylene), 1.2 (m, 3H, methyl CH), 5.1(s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H,

ArH), 1.8 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 124, 130.4, 115.8, 159.6, 116 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 40 and 39.2 (C_3, C_4, and C_5), 168 (1C, C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 142, 138.7, 127.7, 126.4, 125.5 and 127.1 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 22.4 (1C, methylene), 16.6 (1C, methyl); ESI-MS (m/z): 371.16 (M^+); Anal. Calcd for $C_{23}H_{21}N_3O_2$: C, 74.37; H, 5.70; N, 11.31. Found: C, 74.31; H, 5.65; N, 11.27

4.3.2.*[3-(4-Hydroxy-phenyl)-5-(2-isopropyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5b)*

Prepared by the above method from 3b (4 mmole, 1.06 g) and 4 (4 mmole, 0.55 g) after 18h reflux; yield: 81% as white crystalline solid; mp: 154-155 ^{o}C. FTIR (neat) ν_{max}: 3092 (C-H, aromatic ring), 761(C-H, substituted phenyl ring), 1670 (C=O), 1580 (C---C str, aromatic ring), 2960–2830 (C-H), 1442 (C-H def), 1605 (C=N), 3335 (C-N); ^{1}H NMR (300 MHz, d): 7.05 (m, 4H, ArH), 3.1 (m, 1H, methine), 1.3 (dd, 6H, methyl CH, J = 6.5 Hz, J = 8 Hz), 5.0 (s, 1H, Ar-OH), 7.4 (m, 2H, ArH), 6.8 (m, 2H, ArH), 2.0 (m, 2H, CH_2 pyrazoline), 4.8 (m, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 125, 130.4, 115.8, 159.6, 115.8 and 129.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 40.2 and 40 (C_3, C_4, and C_5), 168.2 (1C, C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 140.1, 147.3, 126.1, 126.2, 126 and 126.8 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 25.5 (1C, methine), 24.7 (2C, methyl); ESI-MS (m/z): 385.18 (M^+); Anal. Calcd for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.72; H, 5.99; N, 10.87

4.3.3. *[3-(4-Hydroxy-phenyl)-5-(2,4,6-trimethyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5c)*

Prepared by the above method from 3c (4 mmole, 1.06 g) and 4 (4 mmole, 0.55 g) after 16h reflux; yield: 90% as off white amorphous solid; mp: 166-168 ^{o}C. FTIR (neat) ν_{max}: 3081 (C-H, aromatic ring), 778 (C-H, substituted phenyl ring), 1667 (C=O), 1580 (C---C str, aromatic ring), 2965–2846 (C-H) 1437 (C-H def) 1620 (C=N) 3343 (C-N); ^{1}H NMR (300 MHz, d): 2.45 (m, 9H, C-H methyl), 6.6 (m, 2H, ArH), 5.2 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.6 (m, 2H, ArH), 1.9 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 130.4, 115.8, 158.6, 115.8 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 40.5 and 32.4 (C_3, C_4, and C_5), 168 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 17.9 (2C, methyl on $C_{2''}$ and $C_{6''}$), 21.0 (1C, methyl on $C_{4''}$),

141, 136, 127.4, 135.5, 127 and 136 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$); ESI-MS (m/z): 385.18 (M^+); Anal. Calcd for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.7; H, 6.0; N, 10.87.

4.3.4. *[5-(2,5-Dimethoxy-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5d)*

Prepared by the above method from 3d (4 mmole, 1.14 g) and 4 (4 mmole, 0.55 g) after 21h reflux; yield: 78% as dark brown solid; mp: 212-214 ^oC. FTIR (neat) ν_{max}: 3090 (C-H, aromatic ring), 763 (C-H, substituted phenyl ring), 1664 (C=O), 1577 (C---C str, aromatic ring), 2960–2840 (C-H), 1446 (C-H def), 1615 (C=N), 3330 (C-N); ^1H NMR (300 MHz, d): 3.7 (s, 6H, C-H methoxy), 6.6 (s, 1H, ArH), 6.3 (s, 1H, ArH), 6.5 (s, 1H, ArH), 5.0 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H, ArH), 1.7 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 124, 130.4, 115.8, 159.6, 116 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 40 and 35.8 (C_3, C_4, and C_5), 168 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 129, 152.8, 114.6, 113.1, 154 and 113.6 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 56.3 (1C, methoxy on $C_{2''}$), 56.0 (1C, methoxy on $C_{5''}$); ESI-MS (m/z): 403.15 (M^+); Anal. Calcd for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42. Found: C, 68.44; H, 5.21; N, 10.4

4.3.5. *[5-(3,4-Dimethoxy-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5e)*

Prepared by the above method from 3e (4 mmole, 1.14 g) and 4 (4 mmole, 0.55 g) after 22h reflux; yield: 91% as mud brown solid; mp: 202-204 ^oC. FTIR (neat) ν_{max}: 3098 (C-H, aromatic ring), 761 (C-H, substituted phenyl ring), 1672 (C=O), 1581 (C---C str, aromatic ring), 2970–2833 (C-H), 1432 (C-H def), 1622 (C=N), 3341 (C-N); ^1H NMR (300 MHz, d): 3.73 (s, 6H, C-H methoxy), 6.5 (s, 1H, ArH), 6.66 (s, 1H, ArH), 6.57 (s, 1H, ArH), 5.0 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.9 (m, 2H, ArH), 1.9 (m, 2H, CH_2 pyrazoline), 4.8 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 130.4, 116, 161.6, 115.8 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 39.9 and 45.7 (C_3, C_4, and C_5), 168.3 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 135.8, 113.6, 147.1, 145.6, 114.6 and 120.3 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 56.9 (2C, methoxy on $C_{3''}$ and $C_{4''}$); ESI-MS (m/z): 403.15 (M^+); Anal. Calcd for $C_{23}H_{21}N_3O_4$: C, 68.47; H, 5.25; N, 10.42. Found: C, 68.43; H, 5.22; N, 10.4

4.3.6. *[5-(4-Benzyloxy-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5f)*

Prepared by the above method from 3f (4 mmole, 1.32 g) and 4 (4 mmole, 0.55 g) after 17h reflux; yield: 93% as brownish yellow amorphous solid; mp: 178-180 ^{o}C. FTIR (neat) ν_{max}: 3090 (C-H, aromatic ring), 767 (C-H, substituted phenyl ring), 1671 (C=O), 1587 (C---C str, aromatic ring), 2960–2820 (C-H), 1440 (C-H def), 1612 (C=N), 3344 (C-N); ^{1}H NMR (300 MHz, d): 7.0 (m, 2H, ArH), 6.8 (m, 2H, ArH), 5.2 (s, 2H, methylene), 7.6 (m, 5H, $C_{b''}$-H, $C_{c''}$-H, $C_{d''}$-H, $C_{e''}$-H and $C_{f''}$-H), 5.0 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H, ArH), 1.7 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 122.8, 130.4, 115.8, 159.6, 116.6 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 38.5 and 45.4 (C_3, C_4, and C_5), 167.6 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 134.8, 128.0, 113.6, 160, 113.6 and 128 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 78 (1C of –OCH_2–), 141, 127.3, 128.7, 127.4, 129 and 127.3 ($C_{a'}$, $C_{b'}$, $C_{c'}$, $C_{d'}$, $C_{e'}$ and $C_{f'}$); ESI-MS (m/z): 449.17 (M^+); Anal. Calcd for $C_{28}H_{23}N_3O_3$: C, 74.82; H, 5.16; N, 9.35. Found: C, 74.8; H, 5.13; N, 9.34.

4.3.7. *[3-(4-Hydroxy-phenyl)-5-(4-methoxy-3-methyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5g)*

Prepared by the above method from 3g (4 mmole, 1.07 g) and 4 (4 mmole, 0.55 g) after 18h reflux; yield: 81% as pale yellow solid; mp: 203-205 ^{o}C. FTIR (neat) ν_{max}: 3099 (C-H, aromatic ring), 768 (C-H, substituted phenyl ring), 1666 (C=O), 1574 (C---C str, aromatic ring), 2950–2810 (C-H), 1445 (C-H def), 1605 (C=N), 3335 (C-N); ^{1}H NMR (300 MHz, d): 2.35 (s, 3H, methyl), 3.7 (s, 3H, methoxy), 6.6-6.8 (m, 3H, ArH), 5.0 (s, 1H, aromatic C-OH), 6.79 (m, 2H, ArH), 7.36 (m, 2H, ArH), 1.85 (m, 2H, CH_2 pyrazoline), 4.8 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 130.4, 115, 159.6, 115.8 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.7, 37.8 and 39.6 (C_3, C_4, and C_5), 168.9 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 12.3 (1C, methyl), 56.5 (1C, methoxy), 134.9, 126, 128.4, 127.7, 128.7 and 126 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$); ESI-MS (m/z): 387.16 (M^+); Anal. Calcd for $C_{23}H_{21}N_3O_3$: C, 71.30; H, 5.46; N, 10.85. Found: C, 71.24; H, 5.40; N, 10.81.

4.3.8. *[5-(4-Bromo-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5h)*

Prepared by the above method from 3h (4 mmole, 1.21 g) and 4 (4 mmole, 0.55 g) after 9h reflux; yield: 84% as brownish yellow solid; mp: 146-148 ^{o}C. FTIR (neat) ν_{max}: 3092 (C-H, aromatic ring), 761 (C-H, substituted phenyl ring), 1670 (C=O), 1582 (C---C str, aromatic ring), 2955–2825 (C-H), 1440 (C-H def), 1612 (C=N), 3344 (C-N); ^{1}H NMR (300 MHz, d): 7.07 (m, 2H, ArH), 7.5 (m, 2H, ArH), 5.0 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H, ArH), 1.8 (m, 2H, CH_2 pyrazoline), 4.8 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 130.4, 115.8, 159.6, 115.8 and 131 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 41 and 45.4 (C_3, C_4, and C_5), 168 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 141.5, 129.2, 131.6, 121, 131.6 and 129.2 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$); ESI-MS (m/z): 421.04 (M^+); Anal. Calcd for $C_{21}H_{16}BrN_3O_2$: C, 59.73; H, 3.82; N, 9.95. Found: C, 59.7; H, 3.81; N, 9.92.

4.3.9. *[5-(4-Fluoro-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5i)*

Prepared by the above method from 3i (4 mmole, 0.97 g) and 4 (4 mmole, 0.55 g) after 8h reflux; yield: 82% as off white amorphous solid; mp: 230-232 ^{o}C. FTIR (neat) ν_{max}: 3090 (C-H, aromatic ring), 765 (C-H, substituted phenyl ring), 1675 (C=O), 1588 (C---C str, aromatic ring), 2955–2810 (C-H), 1441 (C-H def), 1615 (C=N), 3336 (C-N); ^{1}H NMR (300 MHz, d): 7.4 (m, 2H, ArH), 6.7 (m, 2H, ArH), 5.5 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H, ArH), 2.0 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 133.4, 115.8, 159.6, 120.1 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 39.9 and 45.4 (C_3, C_4, and C_5), 167.9 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 138, 128.6, 115, 160, 115.3 and 128.6 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$); ESI-MS (m/z): 361.12 (M^+); Anal. Calcd for $C_{21}H_{16}FN_3O_2$: C, 69.80; H, 4.46; N, 11.63. Found: C, 69.76; H, 4.42; N, 11.6.

4.3.10. *[3-(4-Hydroxy-phenyl)-5-(4-nitro-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5j)*

Prepared by the above method from 3j (4 mmole, 1.08 g) and 4 (4 mmole, 0.55 g) after 14h reflux; yield: 80% as brownish yellow amorphous solid; mp: 224-226 ^{o}C. FTIR (neat) ν_{max}: 3094 (C-H, aromatic ring), 767 (C-H, substituted phenyl ring), 1679 (C=O), 1587 (C---C str, aromatic ring), 2945–2823 (C-H), 1450 (C-H def), 1615 (C=N), 3335 (C-N); ^{1}H NMR (300 MHz, d): 7.38 (m, 2H, ArH), 8.14 (m, 2H, ArH), 5.0 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H, ArH), 1.7 (m, 2H, CH_2 pyrazoline), 4.7 (s, 1H, CH of pyrazoline ring), 9.10 (s, 1H, C_b-H of 4-pyridine), 8.85 (s, 1H, C_d-H),

7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 130.4, 115.8, 159.6, 115.8 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 39.9 and 45.4 (C_3, C_4, and C_5), 168 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 148.6, 127.9, 123.1, 146.4, 123.1 and 127.9 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$); ESI-MS (m/z): 388.12 (M^+); Anal. Calcd for $C_{21}H_{16}N_4O_4$: C, 64.94; H, 4.15; N, 14.43. Found: C, 64.90; H, 4.11; N, 14.39.

4.3.11. *[3-(4-Hydroxy-phenyl)-5-(4-methoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5k)*

Prepared by the above method from 3k (4 mmole, 1.02 g) and 4 (4 mmole, 0.55 g) after 19h reflux; yield: 84% as white amorphous solid; mp: 206-207 ^{o}C. FTIR (neat) ν_{max}: 3099 (C-H, aromatic ring), 764 (C-H, substituted phenyl ring), 1675 (C=O), 1586 (C---C str, aromatic ring), 2974–2838 (C-H), 1437 (C-H def), 1627 (C=N), 3338 (C-N); ^{1}H NMR (300 MHz, d): 3.73 (s, 3H, methoxy), 6.95 (m, 2H, ArH), 6.7 (m, 2H, ArH), 5.0 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.9 (m, 2H, ArH), 1.9 (m, 2H, CH_2 pyrazoline), 4.8 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.6, 130.4, 115.8, 159.7, 115.8 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 39.9 and 45.7 (C_3, C_4, and C_5), 168.3 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 134.7, 128.8, 113.4, 161, 113.4 and 128.8 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 56.9 (2C, methoxy on $C_{3''}$ and $C_{4''}$); ESI-MS (m/z): 373.14 (M^+); Anal. Calcd for $C_{22}H_{19}N_3O_3$: C, 70.76; H, 5.13; N, 11.25. Found: C, 70.72; H, 5.10; N, 11.24.

4.3.12. *[5-(2-Chloro-phenyl)-3-(4-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5l)*

Prepared by the above method from 3l (4 mmole, 1.03 g) and 4 (4 mmole, 0.55 g) after 9.5 h reflux; yield: 76% as brown amorphous solid; mp: 162-164 ^{o}C. FTIR (neat) ν_{max}: 3097 (C-H, aromatic ring), 775 (C-H, substituted phenyl ring), 1677 (C=O), 1587 (C---C str, aromatic ring), 2959–2830 (C-H), 1448 (C-H def), 1611 (C=N), 3338 (C-N); ^{1}H NMR (300 MHz, d): 7.02 (s, 1H, ArH), 7.07 (s, 1H, ArH), 7.0 (s, 1H, ArH), 7.2 (s, 1H, ArH), 5.5 (s, 1H, Ar-OH), 6.8 (m, 2H, ArH), 7.4 (m, 2H, ArH), 2.0 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, m, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.8, 133.4, 115.8, 159.6, 120.1 and 130.4 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.6, 39.9 and 45.4 (C_3, C_4, and C_5), 167.9 (1C of C=O), 129.6, 152.5, 148.3, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 142.8, 128.5, 126.4, 127.9, 128.7 and 132.4 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$); ESI-MS (m/z): 377.09 (M^+); Anal. Calcd for $C_{21}H_{16}ClN_3O_2$: C, 66.76; H, 4.27; N, 11.12. Found: C, 66.72; H, 4.23; N, 11.10.

4.3.13. *[3-(4-Hydroxy-phenyl)-5-(4-isopropyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5m)*

Prepared by the above method from 3m (4 mmole, 1.06 g) and 4 (4 mmole, 0.55 g) after 17h reflux; yield: 81% as off white amorphous solid; mp: 184-185 ^{o}C. FTIR (neat) ν_{max}: 3096 (C-H, aromatic ring), 760 (C-H, substituted phenyl ring), 1670 (C=O), 1585 (C---C str, aromatic ring), 2975–2840 (C-H), 1435 (C-H def), 1620 (C=N), 3340 (C-N); ^{1}H NMR (300 MHz, d): 1.25 (dd, 6H, methyl, J = 9 Hz, J = 7.6 Hz), 3.1 (s, 1H, methine), 7.05 (m, 2H, ArH), 7.1 (m, 2H, ArH), 5.0 (s, 1H, Ar-OH), 6.7 (m, 2H, ArH), 7.9 (m, 2H, ArH), 1.9 (m, 2H, CH_2 pyrazoline), 4.9 (s, 1H, CH pyrazoline), 9.15 (s, 1H, C_b-H), 8.85 (s, 1H, C_d-H), 7.65 (s, 1H, C_e-H), 8.35 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.4, 130.5, 115.8, 159.5, 115.8 and 130.5 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.5, 39.6 and 45.0 (C_3, C_4, and C_5), 168.6 (1C of C=O), 129.6, 152.6, 148.5, 124.9 and 137 (C_a, C_b, C_c, C_e and C_f), 139.7, 127.0, 126.4, 146.5, 126.4 and 127.0 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 31.9 (1C, methine), 24.5 (2C, methyl); ESI-MS (m/z): 385.18 (M^+); Anal. Calcd for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.72; H, 6.0; N, 10.71.

4.3.14. *[3-(4-Hydroxy-phenyl)-5-(4-propyl-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5n)*

Prepared by the above method from 3n (4 mmole, 1.06 g) and 4 (4 mmole, 0.55 g) after 17.5h reflux; yield: 78% as off white amorphous solid; mp: 196-197 ^{o}C. FTIR (neat) ν_{max}: 3090 (C-H, aromatic ring), 765 (C-H, substituted phenyl ring), 1678 (C=O), 1584 (C---C str, aromatic ring), 2970–2830 (C-H), 1435 (C-H def), 1625 (C=N), 3330 (C-N); ^{1}H NMR (300 MHz, d): 1.1 (t, 3H, methyl), 1.7 (m, 2H, methylene), 2.5 (m, 2H, C-H methylene), 6.95 (m, 2H, ArH), 7.0 (m, 2H, ArH), 5.1 (s, 1H, Ar-OH), 6.75 (m, 2H, ArH), 7.85 (m, 2H, ArH), 1.95 (m, 2H, CH_2 pyrazoline), 4.85 (s, 1H, CH pyrazoline), 9.15 (s, 1H, C_b-H), 8.8 (s, 1H, C_d-H), 7.65 (s, 1H, C_e-H), 8.3 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.5, 130.5, 116.0, 159.6, 116.0 and 130.5 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.7, 39.8 and 45.2 (C_3, C_4, and C_5), 168.4 (1C of C=O), 129.7, 152.5, 148.4, 124.9 and 137.2 (C_a, C_b, C_c, C_e and C_f), 138.9, 127.6, 128.4, 137, 128.4 and 127.6 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 37.9 (1C, methylene), 25.4 (1C, methylene), 13.9 (1C, methyl); ESI-MS (m/z): 385.18 (M^+); Anal. Calcd for $C_{24}H_{23}N_3O_2$: C, 74.78; H, 6.01; N, 10.90. Found: C, 74.69; H, 6.03; N, 10.84.

4.3.15. *[3-(4-Hydroxy-phenyl)-5-(4-isopropoxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-pyridin-3-yl-methanone (5o)*

Prepared by the above method from 3o (4 mmole, 1.13 g) and 4 (4 mmole, 0.55 g) after 22h reflux; yield: 76% as reddish brown amorphous solid; mp: 215-217 ^{o}C. FTIR (neat) ν_{max}: 3095 (C-H,

aromatic ring), 770 (C-H, substituted phenyl ring), 1660 (C=O), 1580 (C---C str, aromatic ring), 2965–2830 (C-H), 1440 (C-H def), 1620 (C=N), 3345 (C-N); ^{1}H NMR (300 MHz, d): 1.4 (dd, 6H, C-H methyl), 4.0 (m, 1H, C-H methine), 6.95 (m, 2H, ArH), 6.65 (m, 2H, ArH), 5.0 (s, 1H, Ar-OH), 6.9 (m, 2H, ArH), 7.6 (m, 2H, ArH), 1.95 (m, 2H, CH_2 pyrazoline), 4.85 (s, 1H, CH pyrazoline), 9.10 (s, 1H, C_b-H), 8.80 (s, 1H, C_d-H), 7.60 (s, 1H, C_e-H), 8.30 (s, 1H, C_f-H); ^{13}C NMR (300 MHz, d): 123.6, 130.5, 116.2, 159.4, 116.2 and 130.5 ($C_{1'}$, $C_{2'}$, $C_{3'}$, $C_{4'}$, $C_{5'}$ and $C_{6'}$), 155.5, 39.6 and 45.5 (C_3, C_4, and C_5), 168.4 (1C of C=O), 129.3, 152.5, 148.3, 125.1 and 137.2 (C_a, C_b, C_c, C_e and C_f), 134.2, 127.8, 113.9, 157.2, 113.9 and 127.8 ($C_{1''}$, $C_{2''}$, $C_{3''}$, $C_{4''}$, $C_{5''}$ and $C_{6''}$), 70.9 (1C, methine), 22.9 (2C, methyl); ESI-MS (m/z): 401.17 (M^+); Anal. Calcd for $C_{24}H_{23}N_3O_3$: C, 71.80; H, 5.77; N, 10.47. Found: C, 71.76; H, 5.67; N, 10.39.

4.4. *In vitro* antimalarial assay[30,31]

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. 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 100 µL 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 half-maximal inhibitory concentration (IC_{50}) responses were estimated by the probit method.

4.5. Cell cytotoxicity assay

Toxicity is an important consideration in any drug development program; therefore we studied cytotoxicity of these compounds against (Table 1) HepG2 cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[32] 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 10^2 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 with 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 solubilise 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.

$$\% \text{ Toxicity} = \frac{\text{Abs. control} - \text{Abs. comp}}{\text{Abs control}} \text{ x } 100$$

4.6. *In vitro* heme crystallization inhibition assay

The compounds after Schizont Maturation Inhibition Assay, were further screened for the inhibition of the hemozoin formation by the method reported by Ncokazi and Egan (2004)[33]. Heme solution is prepared by dissolving 5.2 mg of haemin chloride (Sigma Chemical Co., USA) in 1 ml of DMSO. Chloroquine and synthesized compounds with different molar equivalents to haemin were prepared by dissolving in DMSO.

The test compounds dissolved in DMSO in doses ranging from 0.12 to 5 M equivalents to haemin chloride in 50 µL of 8 mM and of haemin chloride solution in DMSO, 50 µL DMSO as control were taken for the assay. By adding 100 µL of 8M acetate buffer (pH 5.0) the hemozoin formation was initiated. Culture plates were incubated at 37 °C for 18 h, centrifuged and the soluble fraction of unprecipitated material was collected. Thereafter, 200 µL of DMSO was added to re-suspend the remaining pellet in order to remove unreacted haematin and the plates were centrifuged

again, the DMSO soluble fraction was collected and the residual pellet (which consists of pure precipitate of haematin) was dissolved in 200 µL of 0.1M NaOH. Thereafter 75 µL of it was transferred to new tubes and diluted four times by adding 0.1M NaOH. The amount of haematin was determined spectrophotometrically at 405 nm wavelength. The % inhibition of hemozoin formation was calculated by the below given formula:

$$\% \text{ Inhibition} = 100 - \frac{\text{A (test)}}{\text{A (sample)}} \text{ x } 100$$

The percentage inhibition of hemozoin of the standard drug was compared with the control.

4.7. *In vitro* antimicrobial activity

Minimal Inhibitory Concentrations (MICs, µg/ml) were determined on different microbes using Broth Micro Dilution procedure according to the recommendations of National Committees for Clinical Laboratory Standards (NCCLS) [34-36]. MIC was defined as the lowest concentration of compound that inhibited visible growth of microbes after incubation at 35°C for 24 h for bacteria and 48 h for fungi. Strains of gram negative bacteria *Pseudomonas aeruginosa* (MTCC 741), *Escherichia coli* (MTCC 51) and gram positive bacteria *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 121) and fungal strains *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 8189) were used for testing antibacterial and antifungal activities. Bacterial strains were grown in Mueller–Hinton Broth and fungal strains were grown in Sabouraud Liquid medium. The inoculum densities of 5 x 10^5 CFU/ml for bacteria and 0.5–2.5 x 10^3 CFU/ml for fungi were prepared. Ciproafloxacin and fluconazole were used as standard antibiotic powder. The synthesized compounds were dissolved in DMSO and further dilutions were prepared in sterile distilled water of various concentrations by twofold serial dilution method to obtain the required concentration. Ciproafloxacin and fluconazole were diluted in sterile distilled water. After dilution was completed, microbe suspensions were inoculated into each well of row. MIC values were given as µg/ml. The turbidity was monitored by the unaided eye and the lowest concentration, at which no growth was seen, recorded and considered as MIC of that particular compound. The MICs of the synthesized compounds and standard drug has been summarized in Table 3.

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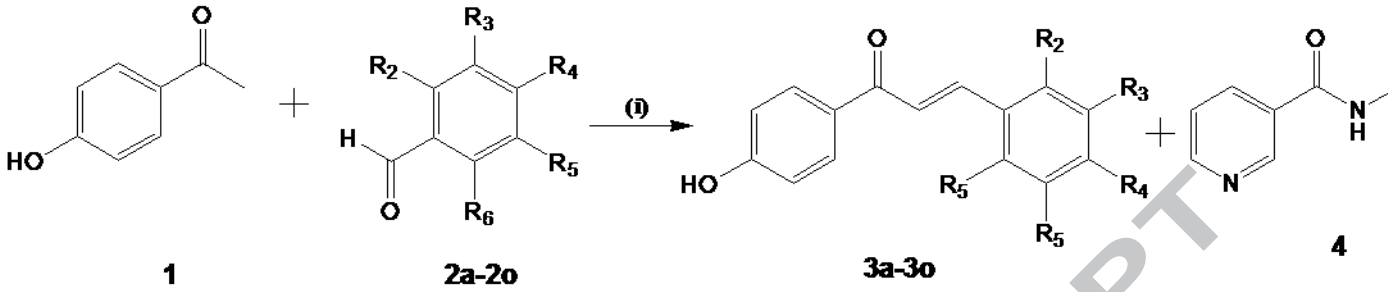
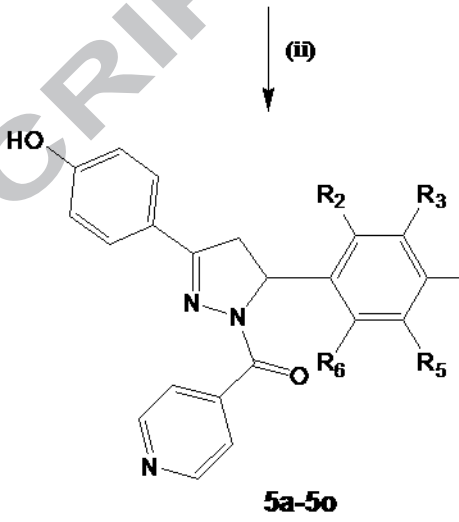
Highlights

- Potential antimalarial 1,3,5-trisubstituted pyrazoline were synthesized
- Compounds were evaluated for *in vitro* antiplasmodial activity against *P. falciparum*.
- *In-vitro* heme crystallization inhibition and cell cytotoxicity assay was assessed.
- Compounds Screened for antibacteria and antifungi activity.



S.No.	Comp. No.			R_2	R_3	R_4	R_5	R_6
1	2a	3a	5a	C_2H_5	H	H	H	H
2	2b	3b	5b	i-C_3H_7	H	H	H	H
3	2c	3c	5c	CH_3	H	CH_3	H	CH_3
4	2d	3d	5d	OCH_3	H	H	OCH_3	H
5	2e	3e	5e	H	OCH_3	OCH_3	H	H
6	2f	3f	5f	H	H	$OCH_2C_6H_5$	H	H
7	2g	3g	5g	H	CH_3	OCH_3	H	H
8	2h	3h	5h	H	H	Br	H	H
9	2i	3i	5i	H	H	F	H	H
10	2j	3j	5j	H	H	NO_2	H	H
11	2k	3k	5k	H	H	OCH_3	H	H
12	2l	3l	5l	H	Cl	H	H	H
13	2m	3m	5m	H	H	i-C_3H_7	H	H
14	2n	3n	5n	H	H	C_3H_7	H	H
15	2o	3o	5o	H	H	Oi-C_3H_7	H	H

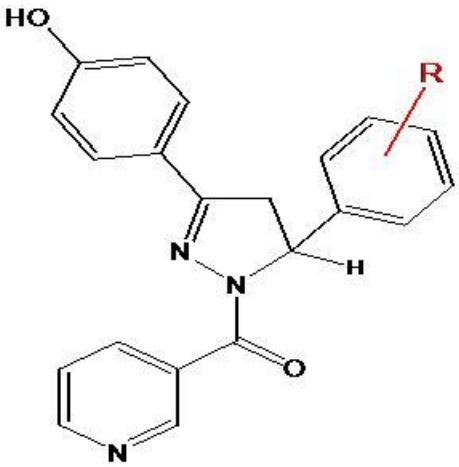


Reagents and conditions: (i) Methanolic NaOH, stirred at RT 20-24 hr (ii) n-butanol, reflux 8-

Fig 1. Scheme for the synthesis of 1, 3, 5-trisubstituted pyrazolines

Graphical abstract

Comp. No.	R	IC_{50} ((μM))± SD	
		MRC-2	RKL-9
5d	2,5-Dimethoxy-	0.040 ± 0.034	0.330 ± 0.063
5e	3,4-Dimethoxy-	0.030 ± 0.055	0.251 ± 0.054
5m	4-isopropyl-	0.034 ± 0.033	0.465 ± 0.021
5n	4-propyl-	0.022 ± 0.015	0.192 ± 0.024
5o	4-isopropoxy-	0.038 ± 0.045	0.337 ± 0.021
Ref.	Chloroquine	0.050 + 0.031	0.401 + 0.102



Potent antimalarial hits