Summary of the Project Report

Name of the Minor Project:	Synthesis of new Antimalarials: Pyrazoles and Pyrazolines using Ultrasonic Reactor
Name of Investigators:	Dr. M. B. Bagade Department of Chemistry S.K. Porwal College, Kamptee.-441001
Name of Co-investigators:	Dr. S. J. Sharma Department of Electronics R.T. M. Nagpur University, Nagpur.
Sanction Letter No. & Date:	F.47-1763/10 dated 2.5.2011

INTRODUCTION:

Pyrazoles are the five membered heterocyclic compounds. Pyrazole nucleus is a pharmaceutically important and immerging heterocyclic with broad spectrum of activity. Some pyrazole derivatives were observed to exhibit herbicidal activity (1, 2), antidiabatic and antibacterial activity (3). Some of the pyrazoles also show pesticidal activity (4) and anti-inflammatory activity (5).

Like pyrazoles, pyrazolines are also five member heterocyclic ring compound. Pyrazoline have many applications in varied fields. Some pyrazoline derivatives show antibacterial activity (6), fungicidal activity (7, 8). Some of the pyrazoline derivatives are well known for their anti-

inflammatory activity (9), antiviral (10), herbicidal (11) and antimicrobial activity (12). Some pyrazoline derivatives show analgesic activity and antitubercular activity (13).

Due to vital biological role of Pyrazole and Pyrazoline derivatives own thought of interest to synthesize pyrazole and pyrazoline derivatives.

The sound waves having frequencies higher than those to which human ear can to respond i.e.>16 KHz. It is consider to lie between 5 MHz for gases and 500 MHz for liquids and solids. Ultrasound waves have verities of applications in the field of engineering, medicine and science. However in the recent years it has received attention in chemistry particularly for the synthesis of various compounds. Ultrasound irradiation is a green method for the synthesis giving better yield in shorter time. The term sonochemistry is used to describe the effect of ultrasonic sound waves to chemical reactivity. The types of ultrasound waves which are used in chemistry are basically divided into Power Ultrasound between 20 and 100 KHz, which are used for cleaning, plastic welding and to affect chemical reactivity, and 'High Frequency Ultrasound' in the range of 2-10 MHz, which is used in metal scanning, chemical analysis, in the study of relaxation phenomenon.

Power ultrasound provides a form of energy for the modification of chemical reactivity which is different from the normally used heat, light and pressure.

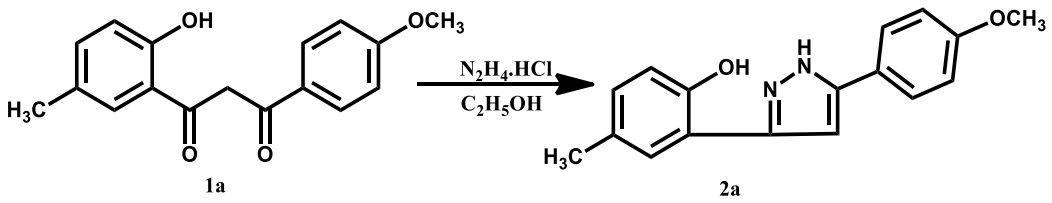
EXPERIMENTAL:

Purity of the compounds was checked on silica gel G plates using iodine as detecting agent. Melting points were determined in open capillaries.

1) **Synthesis of 3-(2-hydroxy-5-methylphenyl)-5-(4′-methoxyphenyl) pyrazole (2a) from 2-hydroxy-5-methyl-4′-methoxyphenyl dibenzoyl methane(1a)**:

   a) Conventional method: Dibenzoyl methane (1a) (0.01 mol; 2.84g) and hydrazine hydrate (0.01 mol; 0.5 g; 0.45ml) were dissolved in ethanol (10ml). The mixture was refluxed for 3 hrs, cooled and poured in cold water. The product separated was crystallized from ethanol to get pyrazole (2a).

b) Sonication Method: Dibenzoyl methane (1a) (0.01 mol; 2.84g) and hydrazine hydrate (0.01 mol; 0.5 g; 0.45ml) were dissolved in ethanol (10ml). The mixture was irradiated by an ultrasonic generator in a water bath at 30-35^{O}C for 5-6 min cooled and poured in cold water. The product separated was crystallized from ethanol to get pyrazole (2a).

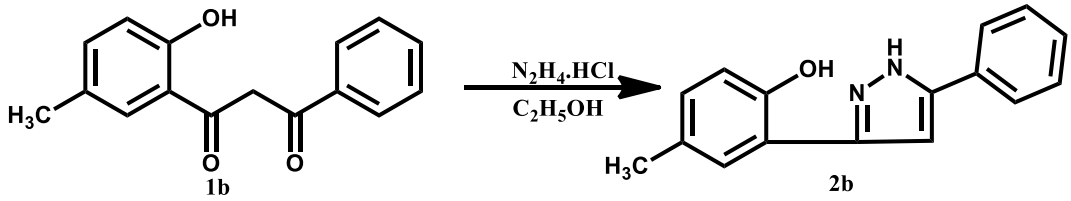


1a 2a

Synthesis of 3-(2-hydroxy-5-methylphenyl)-5-phenyl pyrazole (2b) from 2-hydroxy-5-methyl-phenyl dibenzoyl methane (1b):

a) Conventional method: Dibenzoyl methane (1b) (0.01 mol; 2.84g) and hydrazine hydrate (0.01 mol; 0.5 g; 0.45ml) were dissolved in ethanol (10ml). The mixture was refluxed for 3 hrs cooled and poured in cold water. The product separated was crystallized from ethanol to get pyrazole (2b).

b) Sonication Method: Dibenzoyl methane (1b) (0.01 mol; 2.84g) and hydrazine hydrate (0.01 mol; 0.5 g; 0.45ml) were dissolved in ethanol (10ml). The mixture was irradiated by an ultrasonic generator in a water bath at 30-35^{O}C for 5-6 min cooled and poured in cold water. The product separated was crystallized from ethanol to get pyrazole (2b).



1b 2b

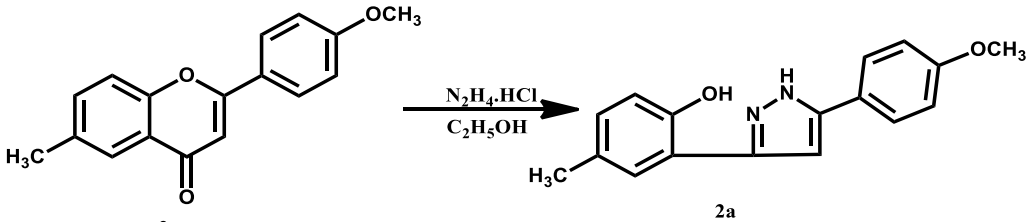
Synthesis of 3-(2-hydroxy-5-methylphenyl)-5-(4′-methoxyphenyl) pyrazole (2a) from 4′-methoxy-6-methyl Flavone (3a):

a) Conventional method: 4′methoxy-6-methyl Flavone (3a) (0.01mol; 2.66g) and hydrazine hydrate (0.02mol; 1.0g; 0.9ml) were dissolved in ethanol (10ml) the mixture was refluxed for 1 hr, cool and poured in cold water. The product separated was crystallized from ethanol to get

pyrazole (2a). The purity of the compound was checked on the basis of mp, mmp, and TLC.

b) Sonication Method: 4′methoxy-6-methyl Flavone (3a) (0.01mol; 2.66g) and hydrazine hydrate (0.02mol; 1.0g; 0.9ml) were dissolved in ethanol (10ml). The mixture was irradiated by an ultrasonic generator in a water bath at 30-35^{O}C for 10 min, cool and poured in cold water.

The product separated was crystallized from ethanol to get pyrazole (2a). The purity of the compound was checked on the basis of mp, mmp, and TLC.



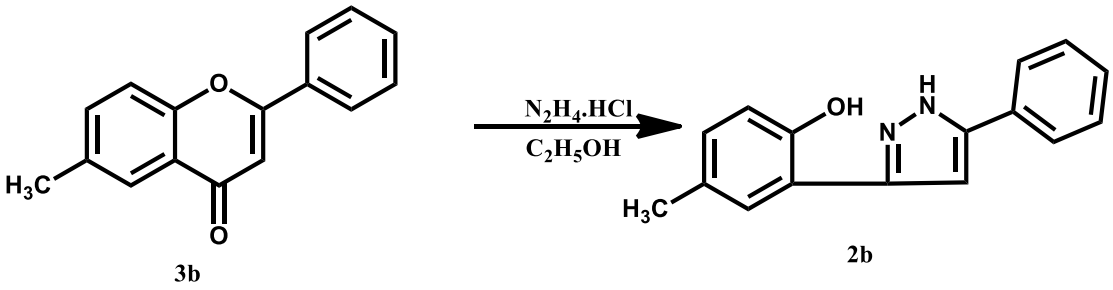
3a 2a

Synthesis of 3-(2-hydroxy-5-methylphenyl)-5-phenyl pyrazole (2b) from 6-methyl Flavone (3b):

a) Conventional method: 6-methyl Flavone (3b) (0.01mol; 2.66g) and hydrazine hydrate (0.02mol; 1.0g; 0.9ml) were dissolved in ethanol (10ml) the mixture was refluxed for 1 hr, cool and poured in cold water. The product separated was crystallized from ethanol to get pyrazole (2b). The purity of the compound was checked on the basis of mp, mmp, and TLC.

b) Sonication Method: 6-methyl Flavone (3b) (0.01mol; 2.66g) and hydrazine hydrate (0.02mol; 1.0g; 0.9ml) were dissolved in ethanol (10ml). The mixture was irradiated by an ultrasonic generator in a water bath at 30-35^{O}C for 10 min, cool and poured in cold water.

The product separated was crystallized from ethanol to get pyrazole (2b). The purity of the compound was checked on the basis of mp, mmp, and TLC.



3b 2b

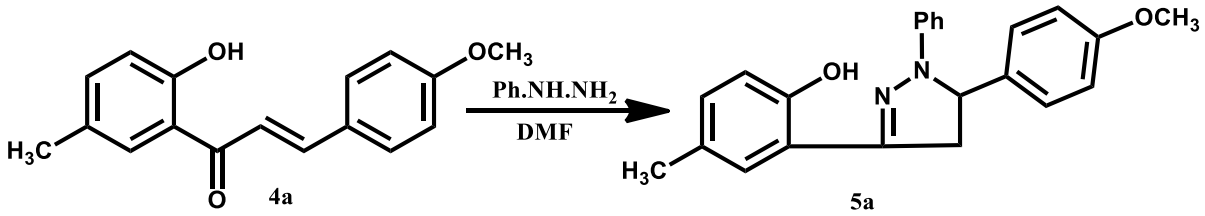
Pyrazole (2a) is light pink fibrous shining compound mp 170^{O}C. Its alcoholic solution gives green coloration with neutral $FeCl_3$ solution. Found C, 72.12; cal 72.85, H found 5.65 cal 5.7, N 9.9 cal 10% having mol formula C_{17} H_{16} O_2 $N_{2.}$

(2a) is light pink fibrous shining compound mp 156^{O}C. Its alcoholic solution gives green coloration with neutral $FeCl_3$ solution.

Synthesis of 1-phenyl-3(2-hydroxy-5-methylphenyl)-5- methoxyphenyl pyrazolines (5a) from 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a):

a) Conventional method: 2′-hydroxy-4-methoxy-5'-methyl Chalcone (4a) (0.01 mol; 2.68 g) and phenylhydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water; the product separated was crystallized form ethanol- acetic acid to get pyrazolines (5a)

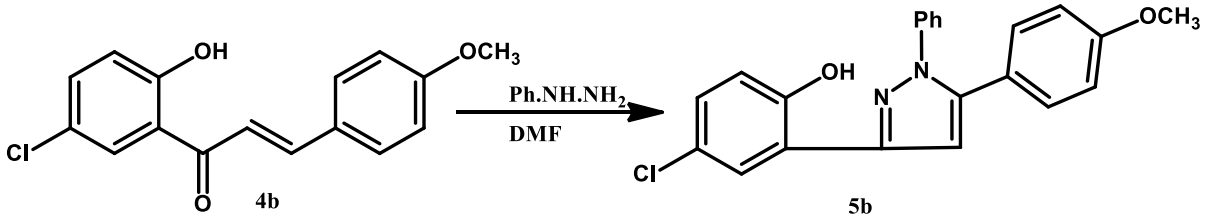
b) Sonication Method: 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a) (0.01 mol;2.68 g) and phenylhydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) . The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 10 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (5a).



Synthesis of 1-phenyl-3(2-hydroxy-5-chlorophenyl)-5-methoxyphenyl pyrazolines (5b) from 2′-hydroxy-4-methoxy-5′-chloro Chalcone (4b):

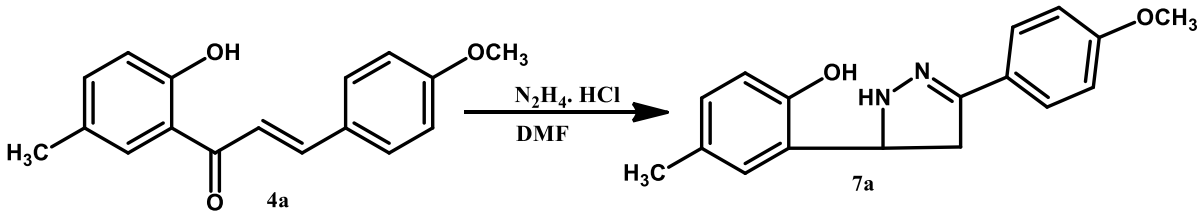
a) Conventional method: 2′-hydroxy-4-methoxy-5'-chlor Chalcone (4b) (0.01 mol; 2.68 g) and phenyl hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water; the product separated was crystallized form ethanol- acetic acid to get pyrazolines (5b)

b) Sonication Method: 2′-hydroxy-4-methoxy-5′-chloro Chalcone (4b) (0.01 mol; 2.68 g) and phenyl hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml). The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 10 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (5b).



Synthesis of 3-(4-methoxy phenyl)-5-(2-hydroxy-5-methyl phenyl) Δ^2-pyrazoline (7a) 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a): **a)Conventional method:** 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a) (0.01 mol;2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7a)

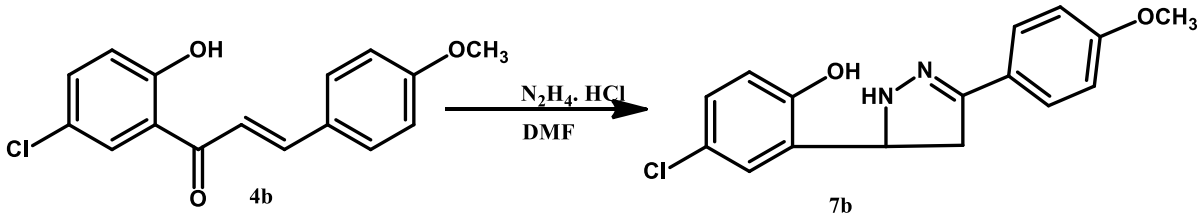
b) Sonication Method: 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml). The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 10 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7a)



Synthesis of 3-(4-methoxy phenyl)-5-(2-hydroxy-5-chlor phenyl) Δ^2-pyrazoline (7b) 2′-hydroxy-4-methoxy-5′-chloro Chalcone (4b):

a) Conventional method: 2′-hydroxy-4-methoxy-5'-chloro Chalcone (4b) (0.01 mol;2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7b)

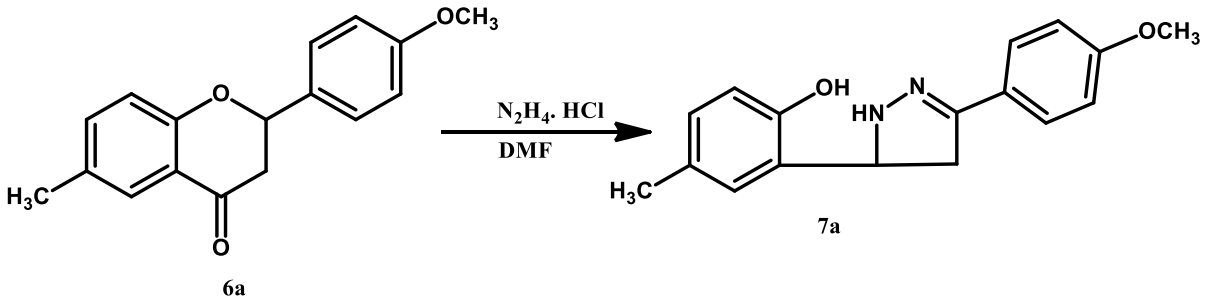
b) Sonication Method: 2′-hydroxy-4-methoxy-5′chloro Chalcone (4b) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) . The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 10 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7b).



Synthesis of 3-(4-methoxy phenyl)-5-(2-hydroxy-5-methyl phenyl) Δ^2-pyrazoline (7a) from 4′-methoxy-6--methyl flavanone (6a):

a) Conventional method: 4′-methoxy-6--methyl flavanone (6a) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water; the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7a)

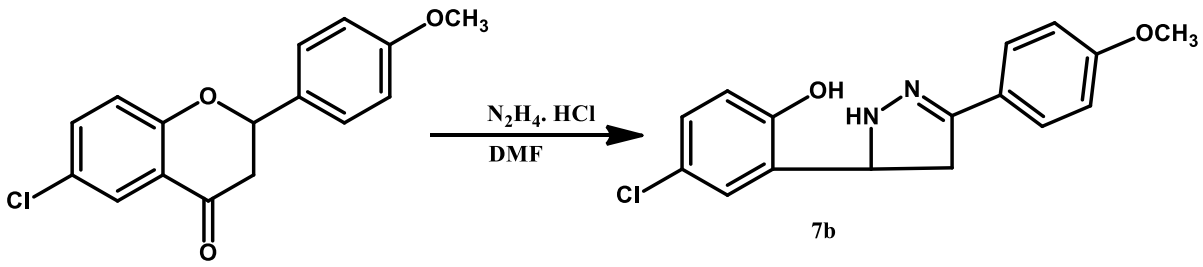
b) Sonication Method: 4′-methoxy-6-methyl Chalcone (4a) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml). The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 10 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7a).



6a 7a

Synthesis of 3-(4-methoxy phenyl)-5-(2-hydroxy-5-chloro phenyl) Δ^2-pyrazoline (7b) from 4′-methoxy-6-chlor flavanone (6b): **a) Conventional method:** 4′-methoxy-6-chlor flavanone (6b) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water; the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7b)

b) Sonication Method: 4′-methoxy-6-chloro Chalcone (4b) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.37 g) were dissolved in dimethylformamide (DMF) (15ml) . The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 10 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (7b).

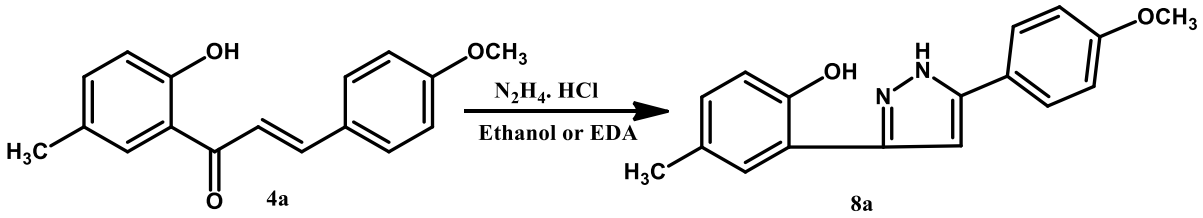


6b 7b

Synthesis of 3-(2-hydroxy-5-methyl phenyl) -5-(4′-methoxy phenyl)-Δ^2-pyrazoline (8a) from 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a):

a) Conventional method: 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a) (0.01 mol;2.68 g) and hydrazine hydrate (0.02mol; 1.0 g,0.9 ml) were dissolved in ethylenediamine (EDA) (10ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water; the product separated was crystallized form ethanol- acetic acid to get pyrazolines (8a)

b) Sonication Method: 2′-hydroxy-4-methoxy-5′-methyl Chalcone (4a) (0.01 mol; 2.68 g) and hydrazine hydrate (0.02mol; 1.0g, 0.9 ml) were dissolved in ethylenediamine (EDA) (10ml). The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 5 min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (8a).

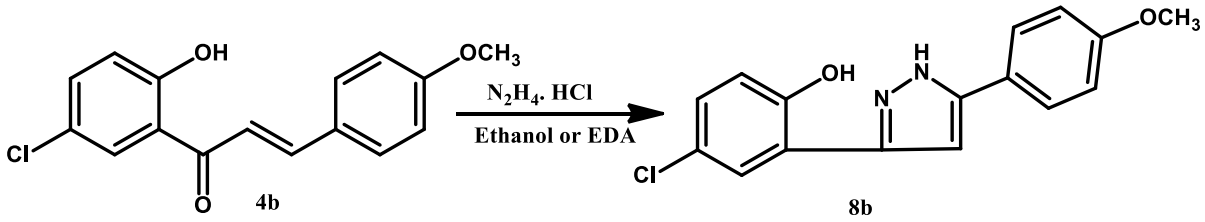


4a 8a

Synthesis of 3-(4-methoxy phenyl)-5-(2-hydroxy-5-chlor phenyl) Δ^2-pyrazoline (8b) from 2′-hydroxy-4-methoxy-5′-chloro Chalcone (4b):

a) Conventional method: 2′-hydroxy-4-methoxy-5′-chloro Chalcone (4a) (0.01 mol; 2.68 g) and hydrazine hydrate (0.02mol; 1.0 g, 0.9 ml) were dissolved in ethylenediamine (EDA) (10ml) and the mixture was refluxed for 3hrs. The mixture was cooled & poured in cold water; the product separated was crystallized form ethanol- acetic acid to get pyrazolines (8b)

b) Sonication Method: 2′-hydroxy-4-methoxy-5′-chloro Chalcone (4b) (0.01 mol; 2.68 g) and hydrazine hydrochloride (0.02mol; 1.0g, 0.9ml) were dissolved in ethylenediamine (EDA) (10ml). The mixture was irradiated by an ultrasonic generator in a water bath at 35-40^{O}C for 5min the mixture was cooled & poured in cold water , the product separated was crystallized form ethanol- acetic acid to get pyrazolines (8b).



RESULT AND DISCUSION:

Properties of 3-(2-hydroxy-5-methyl phenyl)-5-(4′-methoxy phenyl) Pyrazole (2a): It is light pink compound, mp 170^{o}C; its alcoholic solution gives green coloration with neutral $FeCl_3$ solution. Form analytical data molecular formula is found to be $C_{17}H_{16}O_2N_2$ and molecular wt.280.

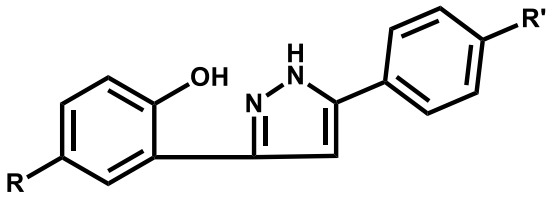
Elemental analysis, found: C, 72.12; H,5.65; N,9.90 calculated: C,72.83; H,5.71; N,10.0. NMR spectra: δ2.40(s, 3H, Ar-CH_3); δ3.83(s, 3H, Ar-OCH_3); δ 6.65 (s, 1H, C-H); 6.88-7.9 (m, 7H, Ar-H) PMR above 10 corresponding to NH & OH shifts were not recognized.

IR: 3200-2950 (broad H bonded OH, NH & C=N overlap), 2850 (O-CH3), 1680 (-C=N), 1600 (benzene ring), 1490-1510 (NH), 1430 (O-H bending), 1390 (-CH3 sym. deformation) and other picks at 840,820,720 for substituted (o,m,p) benzene rings.

From analytical and spectral data the compound 2a was assigned the structure as 3-(2-hydroxy-5-methyl phenyl)-5-(4′-methoxy phenyl) Pyrazole.

2-Hydroxy-5-methyl dibenzoyl methane was also irradiated with hydrazine hydrate in sonicator for 5 min in ethanol, the product 2b was characterized.

Similar results were obtained on irradiating flavones 3a and 3b with hydrazine hydrate in sonicator in ethanol for 10 min to get corresponding 2a and 2b.



(2)

2a. R = CH_3 and R' =OCH_3

2b. R = CH_3 and R' =H

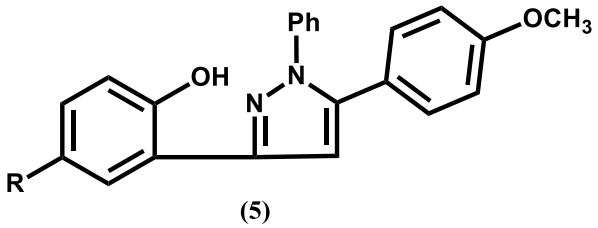
Reaction of 2′-hydroxy-4-methoxy-5′-methyl Chalcone(4a) with phenyl hydrazine hydrochloride was carried out in dimethlformamide and the mixture was irradiated in sonicator for10 min. the product 1-phenyl-3-(2-hydroxy-5-methyl phenyl)-5-(4'-methoxy phenyl)pyrazolines(5a-b) was characterized on the basis of elemental analysis and spectral data.

It is light brownish crystalline compound, mp 152^{O}C. its alcoholic solution gives green coloration with neutral FeCl3 solution. From analytical data the molecular formula was found to be C_{23} $H_{22}O_2N_2$ and mol wt 358. Elemental analysis found: C, 77.0; H, 6.01; N, 7.68 Calculated: C, 77.09; H, 6.14; N, 7.82.

IR: 3200-2600 cm-1(broad H bonded OH overlap of C=N and Ar-H); 1600-1500 cm-1 (C=N grouping) 1450-1490 cm-1 (-CH=O stretching vib.); 1230-1250 cm-1 (C-O stretching).

From the analytical data and spectra the compound was assigned the structure as 1-phenyl-3-(2-hydroxy-5-methylphenyl-5-(4'-methoxyphenyl) pyrazolines.

2′-Hydroxy-4-methoxy-5′-chloro Chalcone (4b) was irradiated with phenyl hydrazine hydrochloride in DMF for 10 min. The product 1-phenyl-3-(2-hydroxy-5-chloro phenyl)-5-(4′-methoxyphenyl) pyrazolines (5b) was characterized on the basis of similar properties.



(5)

5a. R = CH_3

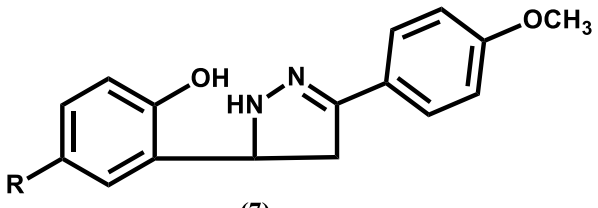
5b. R = Cl

Reaction of 2′-hydroxy-4-methoxy-5′-methyl Chalcone with hydrazine hydrochloride in DMF on irradiation in sonicator for 10 min. gives 3-(4′-methoxyphenyl)-5-(2-hydroxy-5-methylphenyl) pyrazoline was characterized on the basis of elemental analysis.

It is brownish crystalline compound, mp165^{o}C. Its alcoholic solution gives green coloration with ferric chloride solution. It forms acetyl derivative. From the analytical data mol formula was found to be $C_{17}H_{18}O_2N_2$ and mol wt. 282. Elemental analysis found: C, 72.2; H, 6.2; N, 9.7 Calculated: C, 72.3; H,6.4; N,9.9.

Similar results were also obtained on irradiation of 2'-Hydroxy-4-methoxy-5'-chloro Chalcone with hydrazine hydrochloride in DMF for 5 min. The product 3-(4'-methoxyphenyl)-5-(2-hydroxy-5-chlorophenyl) pyrazolines was characterized on the basis similar properties.

Similar results were obtained by irradiating flavanone with hydrazine hydrochloride in DMF.



(7)

7a. R = CH_3

7b. R = Cl

BIOLOGICAL ACTIVITY

The antimicrobial activities were determined by using well diffusion method by measuring zone of inhibition in mm. All newly synthesized compounds were screened for invitro antimicrobial activity against two Gram positive strains (*S.aureus,* and *B.Subtillis*) and two Gram negative strains (*E.coli and K. aerogenes*) at concentration of 100 µl. Amoxicillin was used as a standard drug for antibacterial screening. DMSO was used as control. All the synthesized compounds exhibited moderate to good antibacterial activities. The results are incorporated in following Table.

Compound	R_1	R_2	S. aureus	B. subtilis	E. coli	K. aerogenes
2(a)	CH_3	OCH_3	+++	++	+++	++
2(b)	CH_3	H	+++	+	++++	+
5(a)	CH_3	OCH_3	+++	++	+++	+
5(b)	Cl	OCH_3	+++	++	++++	++
7(a)	CH_3	OCH_3	++	++++	++++	++
7(b)	Cl	OCH_3	++	++	+++	-
8(a)	CH_3	OCH_3	++	++	+++	++
8(b)	Cl	OCH_3	++++	++	+++	+
Amoxicillin			++++	++++	++++	++++

Zone of inhibition: (-) 0-5mm, inactive; (+) 6-10 mm, poor activity; (++) 11-15 mm, moderate activity; (+++) 16-20 mm, good activity; (++++) >20 mm, very good activity.

CONTRIBUTION TO SOCIETY:

The synthesized compounds shows remarkable antimicrobial activity hence these compounds can be further studied as drugs for the various diseases which will benefit the society.

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