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Synthesis and antimicrobial activity of some novel pyrazoles

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ABSTRACT

New substituted pyrazoles were prepared from o-hydroxyacetophenone and cinnamic acids as starting materials through1,3-diketones as intermediates. These intermediates on reaction with hydrazines in alkaline media, finally converted into corresponding pyrazoles. The synthesized compounds were characterized by their physical properties, IR, NMR and LC Mass spectroscopic studies. The antimicrobial activity of synthesized pyrazoles was assessed by agar cup method and filter paper disc method. In the most cases pyrazole having Chloro substitution on the styryl ring was found to be more efficient than the remaining against Xanthomonas Campestris and Aspergillus Niger species.

***Keywords*:** Pyrazoles, Phenyl hydrazine, 1,3-diketone , antibacterial activity, anti- fungal activity, *Xanthomonas Campestris and Aspergillus Niger*

INTRODUCTION

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and are also called as Azoles [1]. Recently Pyrazole derivatives have been found in nature [1], β-[1-pyrazolyl]alanine was isolated from the seeds of water melons [Citurllus lanatus]. The best described property of almost every group of pyrazoles is in the treatment of inflammation and inflammation associated disorders, such as arthritis [2]. Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial[3], antiviral[4], antitumor[5,6], antihistaminic[7], antidepressant[8], insecticides[9] and fungicides[9].

Several pyrazole derivatives have been found to possess significant activities such as 5-α-red-uctase inhibitor[10], antiproliferative[11], antiparasitic[12], herbicides[13]. A good number of pyrazoles have also been reported to have interesting biological activities like anti-inflammatory[14] and antiprotozoal[15-16] which render them valuable active ingredients of medicine and plant protecting agents. Further, current literature indicates 1,2-pyrazole derivatives to possess various biological activities [17].

Until now, the binding of heterocyclic compounds with acyclic sugar moiety forming thus the acyclonucleosides have commanded the world-wide attention of many research groups because of their high potential to exhibit chemotherapeutic activity [18,19]. Substituted pyrazole and its analogs have been used as precursors for synthesis of various biologically active molecules. In the recent years, the efficiency of microwave chemistry in dramatically reducing reaction times has recently been proven in several different fields of organic chemistry [20], microwave-

assisted organic synthesis has shown significant improvement in the generation of combinatorial libraries of small molecules [21].

Taking into consideration the important biological activities of pyrazoles, we have decided to devote some attention for the synthesis and antimicrobial activity of new substituted pyrazoles by following literature [22] methods.

MATERIALS AND METHODS

3.1. Materials and physical measurements:

Melting points were measured by a Stuart Scientific melting point apparatus in open capillaries and are uncorrected. Infrared spectra (KBr discs) were recorded on a Bruker Alpha (FTIR) Spectrometer. ^{1}H-NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz using DMSO-d_6 and $CDCl_3$ as a solvent with TMS as an internal standard. Mass spectra was recorded on an agilent-1100 periods LC-MSD. Elemental analysis was performed using a (EURO EA 3000 instrument). Acme silica gel-G and Merck silica gel (100 to 200, 60 to 120 meshes) were used for analytical TLC and Column chromatography respectively. All other analytical grade chemicals and solvents were obtained from commercial sources and used as received standard procedure.

3.2. Chemistry:

We have prepared the novel pyrazoles in three steps, using cinnamic acids and *o*-hydroxyacetophenone as the starting materials. The desired cinnamic acids were prepared from commercially available benzaldehydes and melonic acid using Knoevengal-Doebner reaction conditions [23].

Later the above synthesized cinnamic acids were treated with *o*-hydroxy- acetophenone in pyridine solution using $POCl_3$ as condensing agent, to obtain 2'-cinnamoyloxyacetophenones. These 2'-cinnamoyloxyacetophenones were treated with powdered KOH in pyridine to get 1,3-diketones, which was then finally converted into the desired pyrazoles by treating with alkaline hydrazine. The clear procedure for the preparation of desired pyrazoles was given below.

4. Preparation of new substituted pyrazoles: (11-15)

General procedure:

Hydrazine was dissolved in sodium hydroxide solution in a round bottom flask with a mechanical stirrer. The flask was cooled to 15 ^oC (ice bath) and diketone was added in portion wise with stirring, continued for one hour at same temperature. Water was added with stirring to the reaction mixture to dissolve the in-organic salts, transfer the contents of the flask to a separating funnel and shaked it by adding ether. Separate the layers and extract the aqueous layer with four portions of ether. Wash the combined ethereal extracts with saturated sodium chloride solution, dry over anhydrous potassium carbonate and remove the ether on a rotary evaporator. The pale yellow solid was obtained in good yield. The product was dried and purified by column chromatography over silica gel using Hex-EtOAc as eluent. The resultant substituted pyrazole, was recrystallized from ethyl acetate and hexane.

4.1). 3-(2-Hydroxy phenyl)-5-(4-chlorostyryl)-1-phenylpyrazole (11):

Following the general procedure, **11** was obtained from 1-(2-hydroxyphenyl)-5-(4-chlorophenyl)-3-hydroxy-2,4-pentadiene-1-one (1.8 gm,), phenylhydrazine (1 gm) NaOH (6.15 ml) as yellowish solid (1.4 gm,) MP: 108– 110 ^oC; **IR(KBr)** υ_{max}: 3321, 1638, 1618, 1558, 1449, 1020, 1219, 771, 635 cm^{-1}; **^{1}H-NMR (DMSO-d_6)**: δ 12.21(1H, br, s, Ar-OH), 7.70 – 6.79 (Ar-H, m,), 6.62 (1H, d, J=16.0 Hz, H-α), 7.72 (1H, d, J=16.0 Hz, H-β), 6.37 (1H, s, H-4); **LC-MS (ESI, negative ion mode)**: m/z- 371.1 $(M-H)^-$; **Elemental analysis**: Analysis: Calcd. for $C_{23}H_{17}N_2OCl$: C-74.09; H-4.56; N-7.51; Cl-9.53 %. Found: C-74.11; H-4.70; N-7.48; Cl-9.56 %.

4.2) 3-(2-Hydroxy phenyl)-5-(4-methoxystyryl)-1-phenylpyrazole (12):

Following the general procedure, **12** was obtained from 1-(2-hydroxyphenyl)-5-(4-methoxyphenyl)-3-hydroxy-2,4-pentadiene1one (1.6gm,), phenylhydrazine (1 gm) NaOH (6.15 ml) as brownish yellow solid (1.5 gm,), MP: 115 – 116 ^oC;**IR(KBr)** υ_{max}: 3330, 2945, 2833, 1631, 1618, 1565, 1488, 1410, 1388, 1297, 1219, 1175, 1017, 771 cm^{-1}. **^{1}H-NMR ($CDCl_3$)**: δ 12.38 (1H, br, s, Ar-OH), 7.84 – 6.80 (Ar-H,m), 6.46 (1H, d, J=16.0 Hz, H-α), 7.66 (1H, d, J=16.0 Hz, H-β), 6.35 (1H,s, - H-4), 3.82 (3H, s, Ar-OCH_3).; **LC-MS (ESI, negative ion mode)**: m/z- 367 $(M-H)^-$; **Elemental analysis**: Analysis: Calcd. for $C_{24}H_{20}N_2O_2$: C-78.26; H-5.43; N-7.60;%; Found: C-78.30; H-5.57; N-7.48; %.

4.3) 3-(2-Hydroxy phenyl)-5-(4-chlorostyryl)-1H-pyrazole (13):
Following the general procedure, **13** c was obtained from 1-(2-hydroxyphenyl)-5-(4-chlorophenyl)-3-hydroxy-2,4-pentadiene-1-one (4 gm, 1.5 mmol), hydrazine HCl (1 gm 1.05 mmol), NaOH (6.15 ml) as yellow solid (2.8 g,), MP: 129- 130 °C; **IR(KBr)** υ_{max}: 3448, 3315, 1642, 1570, 1450, 1228, 1114, 1020, 772, 661 cm^{-1}; **^{1}H-NMR (DMSO-d_6)**: δ 14.3 (N-H) 12.32(1H, br, s, Ar-OH), 7.82 – 6.82 (Ar-H, m), 6.58 (1H, d, J=16.0 Hz, H-α), 7.78 (1H, d, J=16.0 Hz, H-β), 6.38 (1H, s, H-4); **LC-MS (ESI, negative ion mode)**: m/z- 295.0 $(M-H)^-$; **Elemental analysis**: Analysis: Calcd. for $C_{17}H_{13}N_2OCl$: C-68.80; H-4.38; N-9.44; Cl-11.97 %. Found: C-68.84; H-4.45; N-9.48; Cl-11.89 %.

4.4) 3-(2-Hydroxy phenyl)-5-styryl-1H-pyrazole (14):
Following the general procedure, **14** was obtained from 1-(2-hydroxyphenyl)-5-phenyl-3-hydroxy-2,4-pentadiene-1-one (1g, 1 mmol), hydrazine HCl (300 mg 1.05 mmol), NaOH (1.8 ml) as pale yellow solid (1.3 g,), MP: 124-125 °C.**; IR(KBr)** υ_{max}: 3412, 3314, 1638, 1615, `580, 1449, 1219, 1114, 1020, 772 cm^{-1}; **^{1}H-NMR ($CDCl_3$)**: δ 14.36 (N-H) 12.08 (1H, br, s, Ar-OH), 7.88 – 6.9 (Ar-H, m), 6.62 (1H, d, J=16.0 Hz, H-α), 7.76 (1H, d, J=16.0 Hz, H-β), 6.48 (1H, s, - H-4); **LC-MS (ESI, negative ion mode)**: m/z- 261 $(M-H)^-$; **Elemental analysis**: Analysis: Calcd. for $C_{17}H_{14}N_2O$: C-77.86; H-5.34; N-10.68 %. Found: C-77.78; H-5.70; N-10.48 %.

4.5) 3-(2-Hydroxy phenyl)-5-styryl-1-phenyl pyrazole (15):
Following the general procedure, **15** was obtained from 1-(2-hydroxyphenyl)-5-phenyl-3-hydroxy-2,4-pentadiene-1-one (870 mg 1 mmol), phenylhydrazine HCl (520 mg 1.05 mmol), NaOH (3.12 ml) as pale yellow solid (1.3 g,), MP: 110-111°C; **IR(KBr)** υ_{max}: 3312, 1633, 1610, 1565, 1487, 1449, 1219, 1016, 772 cm^{-1}; **^{1}H-NMR ($CDCl_3$)**: δ 12.12 (1H, br, s, Ar-OH), 7.76 – 6.6 (Ar-H, m,), 6.72 (1H, d, J=16.0 Hz, H-α), 7.81 (1H, d, J=16.0 Hz, H-β), 6.42(1H, s, H-4); **LC-MS (ESI, negative ion mode)**: m/z- 337 $(M-H)^-$; **Elemental analysis**: Analysis: Calcd. for $C_{23}H_{18}N_2O$: C-81.65; H-5.32; N-8.28 % . Found: C-81.69; H-5.39; N-8.38 %.

5. Antimicrobial activity:
Preparation of Inoculum:
The gram positive (Bacillus subtilis and Staphylococcus aureus) and gram negative bacteria (Escherichia coli, Xanthomonas campestris) were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A nm). The fungal inoculums (Aspergillus niger and A. flavus) was prepared from 5 to 10 day old culture grown on Potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A nm) to obtain a final concentration of approximately 10^5 spores/ml.

5.1. Anti-bacterial activity:
Determination by Agar cup method:
The antibacterial activity of pyrazole derivatives was studied by agar cup method [24, 25]. Glass Petri dishes used were sterilized and Nutrient broth was used as basal medium for testing bacteria. The Nutrient broth medium was prepared by taking Beet extract (1 gm/lit), Yeast extract (2 gm/lit), peptone (5.0 gm/it), NaCl (5 gm/lit) Agar (15 gm/lit) and with pH (7.0), and plated into Petri dishes, allowed to solidification. The selected Bacterial culture, single colony was inoculated in to broth medium and kept for incubation for overnight at 25 ^{0}C. The overnight Bacterial culture was spread evenly over the entire surface and left undisturbed for few minutes to percolate the culture. Wells (4 mm) were created using a sterile borer into the solidified agar medium. The selected compounds were added to each well (100 & 50 µL) at peripheral and the reference compound (streptomycine) was added at the centre. Thus the prepared plates were incubated at room temperature (at about 25 ^{0}C) for about 3-5 days. After incubation period the plates were collected and record the inhibition zone in mm (from the margin of the well to surface of inhibition).Dimethyl sulphoxide (DMSO) was used as solvent to prepare the stock solutions (5 mg in 0.5 mL) of the compounds initially and also to maintain proper control. A control well was also pl) respectively.

5.2. Antifungal Activity:
Determination by filter paper disc method:
The antifungal activity was tested by disc diffusion method [26, 27]. The potato dex-
trose agar was used as basal medium for testing fungi. The potato dextrose agar medium was prepared by taking yeast extract (3 gm/lit), peptone (10 gm/it), Dextrose (20 gm/lit) Agar (15 gm/lit) distilled water (1 lit) and with pH (6.0), and plated into Petri dishes, allowed to solidification. The potato dextrose agar plates were inoculated with

each fungal culture (10 days in old) by point inoculation. The filter paper discs (5 mm in diameter) impregnated with 100 µl and 50 µl concentrations of the extracts were placed on test organism-seeded plates. DMSO was used to dissolve the tested compounds and was completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent DMSO followed by drying off was used as negative control and Nystatin (10 µg) used as positive control. The activity was determined after 72 hrs of incubation at 28 °C. The diameters of the inhibition zones were measured in mm.

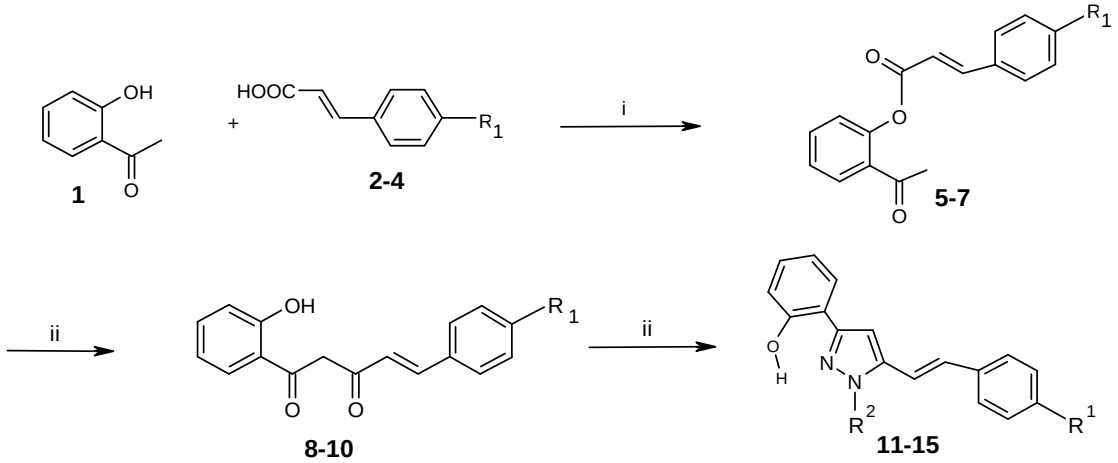
RESULTS AND DISCUSSION

3.1. Chemistry

Following our experience in the synthesis of organic compounds, we have decided to devote some attention for the synthesis new substituted pyrazoles in three steps followed by the study of their anti-bacterial and anti-fungal activities.

The first step of synthetic method involve preparation of the desired 2'-cinnamoyl- oxyacetophenones from commercially available 2-hydroxyacetophenone with various cinnnamic acids in pyridine solution using $POCl_3$ as condensing agent in best yields. These resulting esters were then converted into corresponding 1,3-diketones by stirring their pyridine solution in the presence of powdered KOH in good yield. Finally the desired pyrazoles were prepared in better yields from 1,3-diketones by using alkaline solution of hydrazine.

By adopting the above general methodology, the following di- and tri-substituted pyrazoles were synthesized according scheme-1 and the results have been represented in table-1.



(11) R_2=Ph ; R_1= Cl, (13) R_2 =H ;R_1=Cl, (15) R_2=Ph; R_1=H
(12) R_2=Ph ; R_1=OCH_3, (14) R_2=H; R_1=H,

[Reagents & Conditions: (i) Pyridine; $POCl_3$, rt. 4 hrs. (ii) Dry pyridine, KOH, rt. 1-2 hrs. (iii) NaOH, Ether, 1h Stirring At 15 ^{0}C.

Sceme-1

Table-1

Compound	R_1	R_2	M.P.(^{0}C)	Yield (%)
11	Cl	Ph	108-110	98.3
12	OCH_3	Ph	115-116	97.5
13	Cl	H	129-130	98.7
14	H	H	124-125	100
15	H	Ph	110-111	99.6

The synthetic pyrazoles were characterized by their spectral studies. In the IR spectra, the ring system showed adsorption bands in the region of 1631-1642 cm^{-1} for -C =N group. The aromatic skeleton of pyrazoles appears in the region 1410-1615 cm^{-1}. The –OH stretching at 3312-3330 cm^{-1} and –NH stretching at 3412-3448 cm^{-1} were noticed.

In the ^{1}H-NMR spectra the characteristic signals for the H-4 was observed around δ 6.35-6.48. The Resonance signal to β-H (δ 7.66-7.81) appeared at higher frequency values than that of α-H (δ 6.72-6.46) due to the mesomeric deshielding effect of highly electronegative nitrogen atoms of pyrazole ring. The Trans configuration of C_α-C_β double bond was assigned from the coupling constant values, $J_{H\alpha\text{-}H\beta} \approx 16.00$ Hz of all pyrazoles. The hydroxyl protons resonances at δ ≈ 12.18-12.38 assigned to Ar–OH groups and the higher frequency value of this from the normal value is due to the intramolecular hydrogen bond of –OH with nitrogen atom of pyrazole ring. In some cases N-H peaks have been observed at the range of δ 14.30-14.36 ppm.

Most of the pyrazole derivatives prepared for the present work showed intense molecular ion at respective molecular weights. We examined their molecular mass using LC mass spectrometry and got corresponding molecular ion peak for each compound in the negative ion mode.

The synthetic pyrazoles were tested for their Anti-bacterial and anti-fungal activities in the laboratory by two common methods i.e. agar cup method and filter paper disc methods respectively.

The anti-bacterial activities of pyrazoles (**11-15**) were studied *in vitro* at the concentration of 100 & 50 µg against two bacterial stains. The screening results indicated that all the compounds exhibited antibacterial activities to the tested bacteria. It was noted that the pyrazole (**11**) shows greater inhibitory activity against bacteria Xanthomonas campestris compared to the remaining pyrazole derivatives (**12--15**). It has also been observed that the pyrazoles with Cl substituent exhibited better bacterial effect than the other pyrazoles against the bacteria Agro bacterium tumafeciens.

The results of diameter of zone inhibition (in mm) of synthetic pyrazole have been incorporated in Table-2.

Table-2: Antibacterial activity of pyrazoles

Compound	R_1	R_2	Conc. (µL)	Zone of inhibition (mm) *Xanthomonas campestris*	Zone of inhibition (mm) *Agrobacterium tumafeciens*
11	Cl	Ar	50	5.5	5.4
			100	14.2	11.3
12	OMe	Ar	50	5.6	4.7
			100	11.2	9.1
13	Cl	H	50	6.6	4.2
			100	13.8	10.9
14	H	H	50	6.0	4.3
			100	10.8	7.8
15	H	Ar	50	7.5	4.6
			100	11.6	8.6
Streptomycine			10	15	12

Table-3: Antifungal activity of pyrazoles

Compound	R_1	R_2	Conc. (µL)	Zone of inhibition (mm) *Aspergillus niger*	Zone of inhibition (mm) *Penicillum chrysogenium*
11	Cl	Ar	50	7.9	6.6
			100	15.6	12.4
12	OMe	Ar	50	7.3	6.1
			100	13.2	11.4
13	Cl	H	50	6.6	6.4
			100	14.4	11.7
14	H	H	50	6.4	5.3
			100	10.3	10.1
15	H	Ar	50	7.7	5.6
			100	10.6	10.8
Nystatin			10	19	13

The antifungal activities of pyrazoles (**11-15**) were studied *in vitro* at the concentration of 100 & 50 µg against two fungal stains. The screening results indicated that all the compounds exhibited antifungal activities to the tested fungi. It was noted that the pyrazole (**11-13**) with chloro and methoxyl groups showed a greater inhibitory activity against both fungi compared to the remaining pyrazole derivatives (**14,15**).

From the results it was concluded that the pyrazole derivative with chloro or methoxyl group was responsible for the greater antifungal effects. The results of diameter of zone of inhibition (in mm) of synthetic pyrazoles have been incorporated in Table-3.

CONCLUSION

We have synthesized some novel substituted pyrazoles present work in very good yields. These were synthesized and they were characterized by spectroscopic methods. The substituted pyrazoles have tested for their anti-microbial activity, where they showed modest activity against Xanthomonas and Aspergillus species.

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