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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY STUDIES OF SOME SUBSTITUTED PYRRAZOLINES

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ABSTRACT

A number of substituted Chalcones were synthesized from 2-acetyl pyrrole by condensing with various substituted benzaldehydes in presence of NaOH as a base. Pyrazolines derivatives were prepared by the condensation of chalcones with Hydrazine. Hydrate /Phenyl hydrazine in the presence of Glacial Acetic acid as the catalyst. The structures of the final synthesized compounds were confirmed by IR, ^{1}H NMR & MASS spectra. Compounds have been evaluated for antifungal, and antimicrobial activity.

KEYWORDS: Chalcones, Pyrazolines, Antimicrobial activity.

INTRODUCTION

The chemistry of heterocyclic compounds is one of the most complex and intriguing branches of modern-day chemistry. As we know that cyclic organic compound containing all carbon atoms in a ring formation is referred to as a carbocyclic compound. If at least one atom other than carbon, forms a part of ring system then it is designated as a heterocyclic compound. Usually these compounds contain nitrogen, oxygen, Sulphur in the heterocyclic ring system but compounds containing other than these heteroatoms are also widely known.

A vast majority of the compounds produced in nature have heterocyclic ring system as a part of their structure. Many of these compounds are very essential to life. Different compounds such as alkaloids, antibiotics, essential amino acids, vitamins, hemoglobin, hormones, dyes contain heterocyclic ring systems. Also, a significant proportion of the synthesized compounds in pharmaceutical industries have these ring systems.[2]

Combining these natural as well as synthetic heterocyclic compounds molecules which have a profound physiological effect on the living system is been recognized in developing new synthetic drugs. Furthermore, all biological processes are chemical in nature. Such fundamental manifestations of life as the provision of energy, transmission of nerve impulses, sight, metabolism and the transfer of hereditary information are all based on chemical reactions involving the participation of many heterocyclic compounds, such as vitamins, enzymes, coenzymes.

These heterocyclic compounds have also shown wide spectrum of activity against many infectious diseases due to their inherent toxicity against various pathogens. Although the heterocyclic compounds were recognized lately, their biological activities attracted the researchers. This has led to intensive research in heterocyclic field to yield new derivatives. Pertaining to one such area is Pyrazolines.

The present work deals synthesis of Chalcones from 2-acetyl pyrrole by condensing with various substituted benzaldehydes in presence of NaOH as a base followed by synthesis of substituted Pyrazolines, upon reaction with Chalcones in presence of Glacial acetic acid as a catalyst.

Chalcones are readily synthesized by the base catalysed Claisen-Schmidt condensation reaction. An aldehydes and appropriate ketones react readily in presence of solvent like ethanol and NaOH as a base, which undergoes a subsequent cyclization reaction with Glacial acetic acid afford pyrazolines.[4]

MATERIALS AND METHODS

All the chemicals used to synthesize the title compounds were of laboratory grade and purchased from Fusion Scientific Company Bangalore. All the reactions were carried out under prescribed laboratory conditions. Melting points of the synthesized compounds were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (60F) using chloroform: methanol (9:1) solvent system. The developed chromatographic plates were visualized under UV at 260nm. IR spectra were recorded using KBr on Josco FTIR model 8400 spectrophotometer, 1H NMR spectra in DMSO on a BRUKER FT-NMR instrument using TMS as internal standard.

A. Synthesis of chalcones

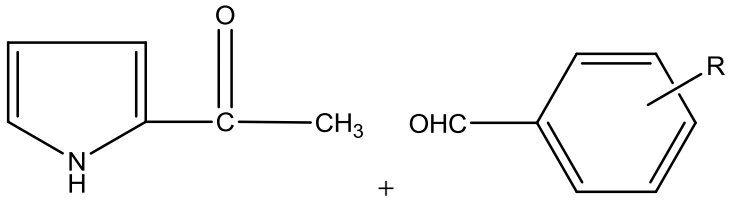
A mixture of 2-acetyl pyrrole [0.01 mol] and substituted benzaldehydes [0.01 mol] in ethanol [20 ml] were stirred together for 24 h, in presence of 20% NaOH [4 ml]. The mixture was poured into crushed ice and acidified with 5%HCl. The product [substituted chalcones] obtained was filtered, washed with water and re-crystallized from suitable solvents.

B. Synthesis of substituted pyrazolines

A mixture of substituted chalcones [0.01 mol] in 20 ml of Glacial acetic acid and phenyl hydrazine, hydrazine hydrate [0.01 mol], were added and refluxed for 5-8 hrs, 16-20 hrs respectively.

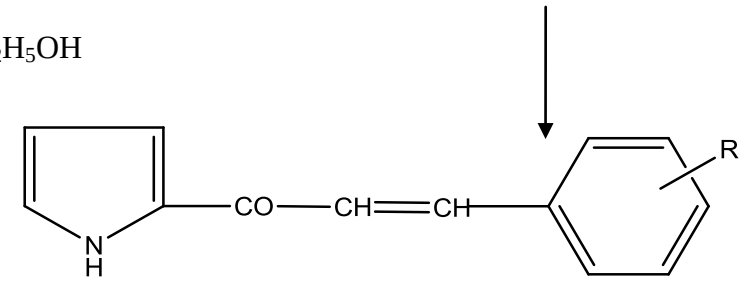
After the completion of the reaction, the reaction mixture was poured into 250 ml of ice cold water. The solid separated is filtered and washed with cold water. The separated compound is recrystallized by using ethanol / chloroform. Chloroform: Ethyl acetate (9:1) is the solvent system for TLC.

Figure 1: Scheme of synthesis



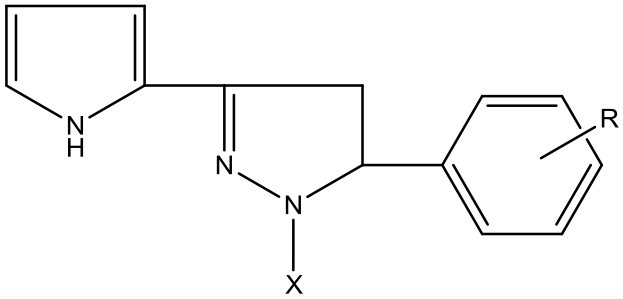
2-Acetyl Pyrrole Substituted Benzaldehydes

20% NaOH in C_2H_5OH



A) $C_6H_5NHNH_2$

B) NH_2NH_2



Substituted pyrazolines

X A= H / B = C_6H_5 →

R = Cl, F, CH_3, OH, NO_2

The list of new pyrazolines derivatives synthesized

1. 5-(2-chlorophenyl)-3-(1H-pyrrol-2yl)-4,5-dihydro-1H-pyrazole (**PZ1)**
2. 5-(4-flurophenyl)-3-(1H-pyrrol-2yl)-4,5-dihydro-1H-pyrazole (**PZ2)**
3. 5-(4-methoxyphenyl)-3-(1H-pyrrol-2yl)-4,5-dihydro-1H-pyrazole (**PZ3)**
4. 3-(1H-pyrrol-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole (**PZ4)**

Details of new substituted pyrazoline

Pyrazolines	Compound [Ar-CHO]	Physical State	Molecular Formula	Mol Wt	MP (°C)	% Yield
PZ1	2 - Cl	Light yellow crystals	$C_{19}H_{16}ClN_3$	321	140-142	70
PZ2	4 – F	Yellow crystals	$C_{19}H_{16}FN$	305.	158-160	74
PZ3	p-OCH_3	Light yellow crystals	$C_{14}H_{15}N_3O$	241	138-140	78
PZ4	pyrrol-2-yl	Light green crystals	$C_{14}H_{12}N_4$	236	120-122	60

Spectral data of synthesized compounds

5-(2-chlorophenyl)-3-(1H-pyrrol-2yl)-4,5-dihydro-1H-pyrazole (PZ1)

IR (KBr in cm^{-1}): 782 (C-Cl), 1643 (C=C), 1329 (C-N), 3040(C-H); ^{1}H NMR (DMSO, δ ppm): 7.40 to 7.32.(S, 2H of NH), 7.26 to 7.211 (m, 7H, Ar-H), 3.0-3.7(dd,1H of Ha), 3.8-4.2(dd,1H of Hb), 5.7-5.8(dd,1H of Hc).

5-(4-flurophenyl)-3-(1H-pyrrol-2yl)-4,5-dihydro-1H-pyrazole(PZ2)

IR(KBr in cm^{-1}): 3040 (C-H), 1639 (Ar C=C), 1324.3 (C-N), 1407 (C-F), 1683 (C=N), ^1H NMR (DMSO, δ ppm): 3.064 to 3.124 (dd, 1H, Ha), 3.786 to 3.859 (dd, 1H, Hb), 5.213 to 5.261 (dd, 1H, Hc), 6.76 to 7.31 (m, 7H, Ar-H), 7.5-7.6(s,2Hof NH)

5-(4-methoxyphenyl)-3-(1H-pyrrol-2yl)-4,5-dihydro-1H-pyrazole(PZ3)

IR(KBr in cm^{-1}): 1642 (C=N), 1506 (Ar C=C), 2972 (C-H), 3286 (N-H), 3075 (Ar C-H); ^1H NMR (DMSO, δ ppm): 7.28 to 7.12(m,7h of Ar-H)3.0 to 3.124 (dd, 1H of Ha),3.7-3.8(dd,1H of Hb), 3.9 (dd,1H of of Hc), 7.2-7.5(s,2H of NH), 2.3(S,IH of OCH_3)

3-(1H-pyrrol-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole(PZ4)

IR(KBr in cm^{-1}): 1643.9 (C=N), 1501 (C=C), 3258 (Ar N-H), 2920 (C-H), 3024 (Ar C-H); ^1H NMR (DMSO, δ ppm): 2.3-(s,3H of CH_3), 7.1 to 7.4 (m,7H of Ar- H,), 7.6 (d,2H of NH-H), 3.1-3.2(dd,1H,Ha), 3.4-3.6(dd,1H,Hb), 5.1-5.3(dd,1H.Hc)

Antimicrobial evaluation

1. In vitro evaluation of antibacterial activity of compounds (PZ1 – PZ4)

Broth microdilution method using Mueller-Hinton agar medium was employed to study the preliminary antibacterial activity of synthesized compounds against Gram-positive (*S. aureus and B. Subtilis*) and Gram-negative (*S. typhi and E. coli*) bacteria. The antibacterial activity of the test compounds was compared with Ofloxacin. Solutions of the test compounds and reference drugs were prepared in Muller-Hinton agar. Test compounds, standard drug Ofloxacin were dissolved in dimethyl sulfoxide (DMSO, 1 ml) and the solution was diluted with distilled water (9 ml) to get the concentration level of 200µg/ml. The petri dishes were inoculated with 1-5 x 10^4 colonies forming units (cfu/ml) and incubated at 37 ˚C for 18 h. And finally, the zone of inhibition is measured. The results of the study are described in Table 2.

Table 2: Antibacterial activity of synthesized compounds.

Sl. no.	Compound	Substitution in R	Zone of inhibition (mm)			
			S. aureus	*B. Subtilis*	*S. typhi*	*E. coli*
1	PZ1	2 - Cl	7.7	7.2	6.9	6.5
2	PZ2	4 – F	11.6	11.7	10.5	10.8
3	PZ3	p-OCH_3	8.7	8.4	8.3	8.1
4	PZ4	pyrrol-2-yl	8.8	8.6	8.3	8.9
11	**Ofloxacin**		20.5	19	19.5	20

In Vitro Evaluation of Antifungal Studies of Compounds (PZ1 – PZ4)

Antifungal activities of all the synthesized compounds were preliminarily screened for the in vitro growth inhibitory activity against *A. Niger* and *C. Albicans* by using the disc diffusion method. The fungi were cultured in potato dextrose agar medium. Potato dextrose agar medium (prepared from potato 150 g; dextrose 5 g and agar 2 g in 200 ml of distilled water) was poured in the sterilized Petri plates and allowed to solidify. The plates were inoculated with a spore suspension of *A. Niger* and *C. Albicans* (106 spores/ml of medium). The compounds to be tested were dissolved in acetone to a final concentration of 200µg/ml and soaked in filter paper discs (Whatmann no. 4, 5 mm diameter). These discs were placed on the already seeded plates and incubated at 28 ± 2 ºC for four days. To avoid the activity of the solvent that is used in the test solutions, a solvent only treated plate was maintained, which showed a 1 mm diameter zone of inhibition. Finally, after four days, the zone of inhibition was measured the results are tabulated in table 3. Fluconazole was used as standard.

Table 3: Antifungal Studies of synthesized compounds.

Sl. no.	Compound	Substitution in R	Zone of inhibition (mm)	
			A. Niger	*C. Albicans*
1	PZ1	2 – Cl	8.6	8.8
2	PZ2	4 – F	9.5	9.8
3	PZ3	p-OCH_3	10	10.3
4	PZ4	pyrrol-2-yl	15.1	15.4
11	**Fluconazole**		24.5	25

RESULTS AND DISCUSSION

All the synthesized compounds were purified by successive recrystallization using ethanol. The purity of the synthesized compounds was checked by performing TLC. The structures of the synthesized compounds were determined on the basis of their FTIR and 1HNMR spectral data.

In accordance with the data obtained from antimicrobial activity, all the synthesized compounds have shown good activity against the tested microbes. Among these, compounds bearing heterocyclic ring substitution has shown good activity against all the tested bacteria and fungi.

CONCLUSION

Antibacterial and antifungal activity of the synthesized derivatives was done in comparison with Ofloxacin and Fluconazole as standard to reveal the potency of synthesized derivatives.

All the selected strains of bacteria and fungi namely S. Aureus, B. Subtilis, S. Typhi, E. Coli, C. Albicans and A. Niger showed sensitivity to all derivatives at concentration of 200µg/ml. Among these, compound bearing 4 – F, pyrrol-2-yl, and p-OCH_3 substitution has shown good activity against all the tested bacteria and fungi.

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