Synthesis, Characterization and Biological activities of 1,3,5-trisubstituted pyrazolines

S. Jayaprakash and A. Faritha*
P.G.and Research Dept of Chemistry, Periyar E.V.R. College, Tiruchirappalli-23

ABSTRACT

α, β -unsaturated ketone derivatives generally known with the common name Chalcones have been prepared by Claisen-Schmidt condensation reaction using acetanilide and various substituted benzaldehydes. They are further condensed with nicotinic acid hydrazide to form corresponding pyrazolines. All these compounds are characterized by CHN analysis and spectral techniques such as FT-IR and ^{1}H NMR. The synthesized compounds are subjected to antibacterial studies.

Key words: Pyrazole, Chalcones, Nicotinic Acid Hydrazide, Anti bacterial activity

1. INTRODUCTION

Substituted pyrazoles are important synthetic targets in the pharmaceutical industry because the pyrazole motif makes up the core structure of numerous biologically active compounds.[1]

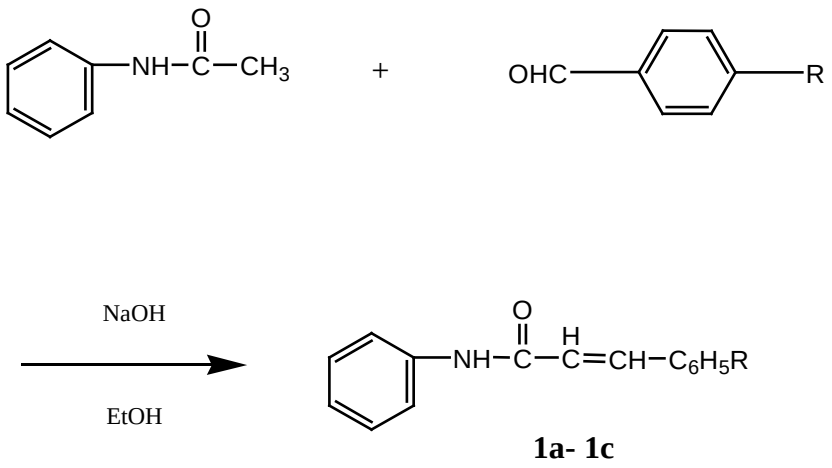
A systematic investigation of this class of compounds reveals that pyrazole containing pharmaco active agents play important role in medicinal chemistry and display anti-inflammatory, antibacterial, antifungal, antiviral, antitubercular, antioxidant, antiandrogenic activities.[2-8] 1,3,5-trisubstituted pyrazoline derivatives of nicotinic acid hydrazides are found to possess good antibacterial activity.[9] In the present study certain new pyrazolines have been synthesized by the reactions of chalcones with nicotinic acid hydrazides. The chalcones are obtained from acetanilide and substuted benzaldehydes by Claisen-Schmidt condensation reaction. The structures of the synthesized compounds are assigned on the basis of elemental analysis, IR, ^{1}H NMR spectral data.

2. EXPERIMENTAL METHODS

All the chemicals and reagents are purchased from MERCK. Melting points of the synthesised compounds are determined in open capillaries and are uncorrected. TLC is used to monitor the reaction and to check the purity. IR spectra are recorded on a Schimatzu 8201 pc (4000-400cm^{-1}). The ^{1}H NMR spectra are recorded on BRUKER AVANCE III 400 MHz multi nuclei solution NMR spectrometer at ambient temperature with TMS as internal standard. Elemental analyses are performed for C, H and N and are found to be within ± 0.5% of the theoretical values.

2.1. General procedure for the synthesis of derivatives of N,3-diphenylacrylamide (1a – 1c)

A mixture of acetanilide (0.01 mol) and various substituted benzaldehyes (0.01mol) in ethanol was stirred in the presence of NaOH for 8 to 10 hrs. The reaction mixture was poured into crushed ice and neutralized with dil. HCl. The solid obtained was filtered and recrystallised from ethanol to obtain pure chalcones. The purity of the product was checked on TLC by using the mixture of toluene and ethyl acetate as mobile phase. (Scheme-1)

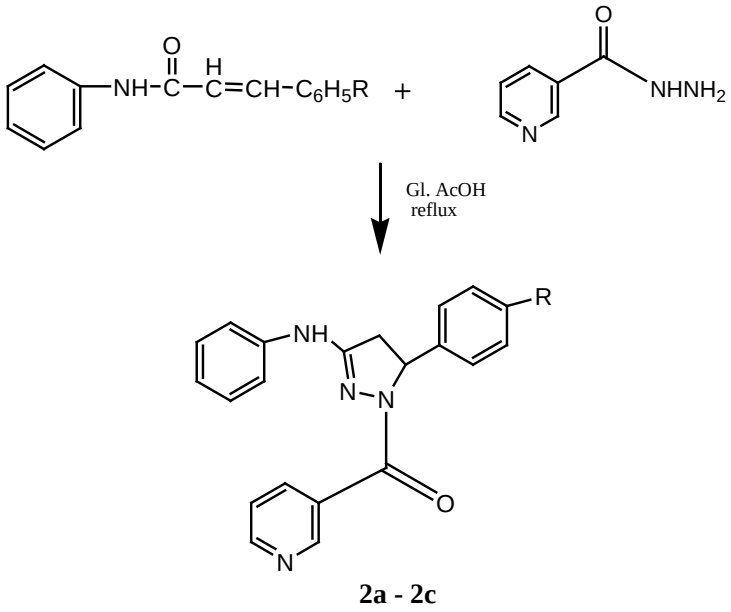


1a- 1c

Scheme-1 **R**: a= H, b = Cl, c = NO_2

2.2. General procedure for the synthesis of substituted (5-phenyl-3-(phenylamino)- 4,5-dihydropyrazol-1-yl)(pyridin-3-yl)methanones (2a – 2c)

To the solution of the appropriate chalcone (0.01mol) **(1a-1c)** in 25 ml ethanol, nicotinic acid hydrazide (0.01mol) and catalytic amount of glacial acetic acid were added and the reaction mixture was refluxed for 20-28 hrs. The excess of solvent was removed under reduced pressure and the reaction mixture was poured into ice cold water. The product obtained was filtered, washed with water and recrystallised with suitable solvents. The purity of the product was checked on TLC by using mixture of acetone and petroleum ether as mobile phase.(Scheme-2)



2a - 2c

Scheme-2 **R:** a= H , b= Cl , c = NO_2

2.2.1. (5-phenyl-3-(phenylamino)-4,5-dihydropyrazol-1-yl)(pyridin-3-yl)methanone *(2a)*

IR (KBr, cm^{-1}): 3124(NH), 3055(Ar-C-H), 1641(C=O), 1591(C=N),

^{1}H NMR (400 MHz, DMSO-d6, δ (ppm)): 3.48(dd,1H, Ha), 3.77 (dd,1H, Hb), 6.14 (dd,1H, Hx), 7.03-7.64(m, 14H, Ar), 9.25 (s,1H, NH);

2.2.2. (5-(4-chlorophenyl)-3-(phenylamino)-4,5-dihydropyrazol-1-yl)(pyridin-3-yl) methanone *(2b)*

IR (KBr, cm^{-1}): 3120(NH), 3059(Ar-C-H), 1645(C=O), 1593(C=N),

^{1}H NMR (400 MHz, DMSO-d6, δ (ppm)): 3.39(dd,1H, Ha), 3.70 (dd,1H, Hb), 6.17 (dd,1H, Hx), 7.07-8.13(m, 13H, Ar), 9.18 (s,1H, NH);

2.2.3. (5-(4-nitrophenyl)-3-(phenylamino)-4,5-dihydropyrazol-1-yl)(pyridin-3-yl) methanone *(2c)*

IR (KBr, cm^{-1}): 3446(NH), 3032(Ar-C-H), 1622(C=O), 1560(C=N),

^{1}H NMR (400 MHz, DMSO-d6, δ (ppm)): 3.81(dd,1H, Ha), 4.02 (dd,1H, Hb), 6.13 (dd,1H, Hx), 7.08-8.31(m, 13H, Ar), 9.21 (s,1H, NH);

Table-1: Physico-chemical characterization of the synthesized compounds

Compo-und	R	M.P. ^oC ±2	M.W.	Yield %	M.F.	Elemental Analysis Calculated(found) %
2a	H	208	342	62	$C_{21}N_4H_{18}O$	C73.67(73.35);H5.30(4.84); N16.36(15.92)
2b	Cl	218	376	70	$C_{21}N_4H_{17}ClO$	C66.93(66.90);H4.85(4.49); N14.87(14.40)
2c	NO_2	202	387	55	$C_{21}N_5H_{17}O_3$	C65.11(64.86);H4.42(3.92); N18.08(17.60)

3. IN-VITRO ANTIBACTERIAL ASSAY

In-vitro antibacterial activity was examined for the series of synthesized compounds against clinical strains of multidrug resistant. Amongst three microorganisms investigated, two Gram-negative bacteria were *Klebsiella pneumonia & Psuedomonas aeruginosa* and one gram-positive bacteria was Methicillin Resistant *Staphylococcus aureus*. All the microorganisms were maintained at 4˚C on nutrient agar slants.

3.1. Media Preparation and Antibacterial Activity:

The antimicrobial assay was performed by agar well diffusion method for synthesized compounds. The test microorganisms were seeded into Mueller Hinton agar by spread plate 10µl (10^6). For agar well diffusion method, the well (0.7 cm) was loaded with 50µl of the test compound on the seeded agar plate. The plates were incubated overnight at 37˚C. Microbial growth was determined by measuring the diameter of zone of inhibition. The result was obtained by measuring the zone diameter (Table 2).

Table.2. Antibacterial activity of synthesized compound:

Zone of inhibition in diameter

Bacteria	2a	2b	2c
MRSA	-	-	-
K. pneumoniae	-	14mm	-
P. aeruginosa	11mm	16mm	12mm

4. RESULTS AND DISCUSSION:

The present study describes the synthesis, characterization and antibacterial evaluation of certain pyrazolines **2a – 2c.** Assignment of selected characteristic IR bands[10,11] provides significant indications for the formation of cyclized pyrazoline analogues of nicotinic acid hydrazide **2a - 2c** . . The IR spectra of the compounds showed $\upsilon_{C=N}$ stretching at 1560-1591 cm^{-1} . In addition, the absorption bands at 1425-1456 cm^{-1} were attributed to υ_{N-N} stretching vibration, which also confirms the formation of pyrazoline derivatives.

In the ^{1}H NMR spectra of these compounds , the three hydrogen atoms attached to the C-4 and C-5 carbon atoms of the pyrazoline ring gave an ABX spin system.[12] The CH_2 protons of the pyrazoline ring resonated as a pair of doublet of doublets around δ 3.56ppm (Ha) and δ 3.83 ppm (Hb). The CH (Hx) proton appeared as a doublet of doublets around δ 6.15 ppm due to vicinal coupling with the two magnetically non-equivalent protons of the C-4 carbon of pyrazoline ring. The J values were calculated for these signals and found to be around 18 Hz and 5 Hz for signal around δ 3.56 ppm and 18 Hz and 12 Hz for signals around δ 3.83 ppm respectively. The 'dd' pattern of Hx proton (5-H, δ around 6.15 ppm) of pyrazoline ring showed J value around 12 Hz and 5 Hz. The –NH proton appeared as a singlet near δ 9.25 ppm and other aromatic protons were observed at the expected regions.

The results of antibacterial screening showed that compound-**2b** was active against both the two gram negative bacteria *K. pneumonia & Pseudomonas aeruginosa* (Table.1) All the three

compounds showed activity against *P.aeruginosa*. The compounds **2a** & **2c** were found to be less active against *P. aeruginosa* while the compound **2b** showed higher activity when compared with **2a & 2c**. None of the compounds were active against the gram positive bacteria *Methicillin Resistant Staphylococcus aureus*.

5. CONCLUSION

Presence of electron with drawing (p-Cl) substituent on phenyl ring increased the antibacterial activity of the compound **2b** against both the two gram negative bacteria. The role of electron with drawing groups in improving antibacterial activities is supported by the studies of Mustafa et al[13] and Konda et al.[14] The behaviour of the pyrazole compound **2a** with the unsubstituted phenyl ring when compared to other compounds with the substituents in the phenyl ring towards the microorganisms led to the conclusion that the antimicrobial activity of such compounds may increase with the introduction of a specific group.

6. REFERNCES:

[1] J Elguero; P Goya;N Jegerovic; AMS Silva,Targets Heterocycl.Syst., **2002**, 6, 52.

[2] AP Guzman; RT Webster; MC Allen et al. *J. Bioorg.Med.Chem Lett*., **2001**, 11, 803.

[3] SG Roelfvan; C Arnold; K Welinga, *J. Agric. Food Chem*. **1979**, 84.

[4] HZ Katri; SA Vunii, *J. Indian Chem*. Soc., **1981**, 58, 168

[5] Rajendra; Krushanji Wanare, *J. Chem.Pharm. Res.*, **2011**, 3, 136.

[6] Meghasham; Narayanrao Narule, *J.Chem. Pharm. Res.*, **2011**, 3, 38.

[7] Singaravel; Mohan; Sarkkarai ; Ananthan, *J.Chem. Pharm.Res.*, **2011**, 3,402.

[8] G Amr ael; NA Lalif; M Abdalla, *Bioorg.Med.Chem*., **2006**, 14, 373.

[9] A. Faritha; AJA Nasser; AP Ahamed; N Thajuddin, *J. Chem. Pharm. Res.*, **2014**, 6, 189.

[10] S Parekh; D Bhavsar; M Savant et al. *Eur.J.Med.Chem*., **2011**, 46, 1942.

[11] GJ Nee Pant; P Singh; BS Rawat et al. *Spectrochim. Acta.*, **2011**, A78, 1075.

[12] R Gupta; N Gupta; A Jain. *Indian J. of Chem*., **2010**, 49B, 351

[13] YAH Mostafa; AA Radwan; AEHN Kfafy. *Arch.Pharmacol Res*., **2008**, 31, 279.

[14] SG Konda; BM Shaik; SA Chavan; BS Dawane. *Chinese Chem Lett.*, **2011**, 1, 65