WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 8, 1915-1920. Research Article ISSN 2277– 7105

SYNTHESIS, ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF NOVEL PYRAZOLINES DERIVATIVES

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Article Received on
02 June 2015,

Revised on 25 June 2015,
Accepted on 19 July 2015

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ABSTRACT

A simple, clean and efficient method has been developed for synthesis of novel 3-(-2'-hydroxy substituted phenyl)-5-(2'-hydroxy substituted phenyl)-2-pyrazoline and N-phenyl pyrazolines are synthesized by refluxing polyhydroxy chalcones with hydrazine hydrate or phenyl hydrazine in ethanol for 3-4 hours. An excellent yield (70-78%) was obtained. This method gives remarkable advantages such as mild reaction conditions, less reaction times, excellent yields and involves the nonchromatographic isolation procedure. The compounds were found to have antibacterial and antifungal activitity.

KEYWORDS*:* Polyhydroxy chalcones, Hydrazine hydrate, Phenyl hydrazine and biological activity.

INTRODUCTION

The scientific and traditional interests of pyrazolines[1] have been reported for their biological and pharmacological efficiency. Different types of pyrazoline derivatives have been reported as antimicrobial[2], antiinflammatery[3], antipyretic and analgesics[4], fungicidal[5], antiamoebic[6], anticancer, antitubercular[7] and antidiabetic[8] activity. There are several substituted pyrazolines having bleaching property or act as luminescent and fluorescent agents.[9] They are also useful as biodegradable agrochemicals.[10]

Literature survey reveals several synthetic protocols for the synthesis of these compounds and the presence of this core in any molecule plays a key role in enhancing the activity. Phenyl ring containing halogen and methyl groups have shown significant biological activities or enhance the biological activities of heterocyclic derivatives drastically.[11,12,13] An especially

popular procedure is based on the reaction of *α, β*-unsaturated aldehydes and ketones with hydrazines[14,15] Such a glamour history prompted us to review the synthesis of pyrazolines as an urgent need which can possess biological and medicinal importance.

These compounds are still of interest from medical point of view due to their physiological, biological and pharmaceutical properties. We have prepared and tested bioactivity of some new pyrazoline and N-phenyl pyrazolines.

EXPERIMENTAL

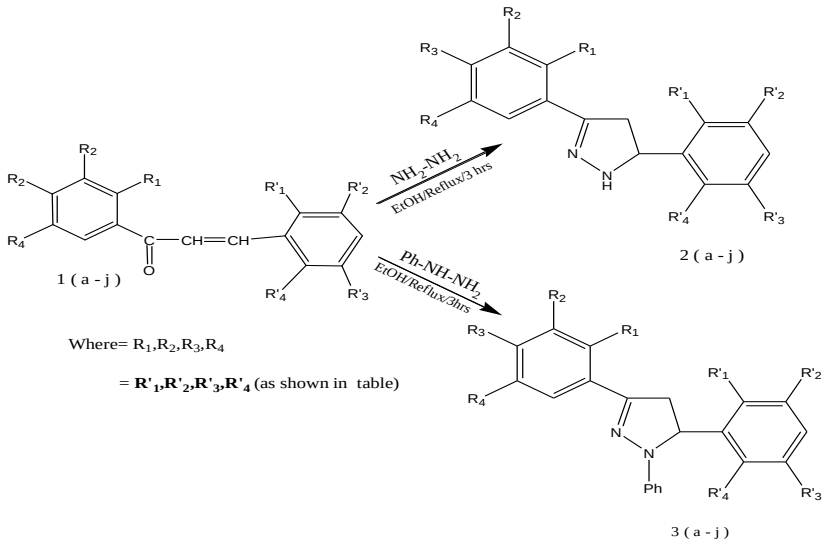
All aldehydes and ketones (actophenone) were obtained from a freshly opened container and used without further purification to use. Melting point was determined in open capillary tubes and is uncorrected. IR spectra were recorded on Perkin Elmer FTIR spectrophotometer in KBr disc, ^{1}H NMR spectra were recorded on variant 300 MHz spectrophotometer in $CDCl_3$ using TMS as the internal standard. The chemical shifts have been expressed in $^{\delta}$- ppm scale, the melting points and other data were recorded in Table 1.

Experimental Procedure

A mixture of chalcone (0.01 mmol) and phenyl hydrazine (0.01mol) or hydrazine hydrate (0.01mol) in ethanol (15 ml) was refluxed for 3 hours. After completion of the reaction as indicated by TLC, the solvent was evaporated on rota-vapour, the residue was poured on crushed ice and the solid obtained which was filtered.

The solid was recrystallized from ethanol to expecting the pure product the results were summarized in Table 2. The structures of the products were confirmed by IR and ^{1}H NMR spectral data and were compared with authentic samples prepared according to the literature method (Scheme 1)

Sr.	R_1	R_2	R_3	R_4	R_1'	R_2'	R_3'	R_4'
1	OH	H	OH	H	OH	H	Cl	H
2	OH	Br	OH	Br	OH	Cl	H	H
3	H	I	OH	I	OH	H	Cl	H
4	OH	I	H	CH_3	OH	H	Cl	H
5	OH	I	CH_3	Cl	OH	H	Cl	CH_3
6	H	CH_3	OH	I	OH	H	Cl	H
7	OH	I	H	Cl	OH	Cl	H	H
8	H	CH_3	OH	I	OH	H	Cl	CH_3
9	OH	I	CH_3	Cl	OH	H	Cl	H
10	OH	I	H	Cl	OH	H	Cl	H



SCHEME; Synthesis of pyrazolines

Table 1: Antimicrobial activity data of pyrazoline derivatives 2(a-e) and 3(a-e)

Entry	Zone of inhibition in mm		% of germination after 12hrs.	
	Bacteria		Fungi	
	S.aureus	*E.coli*	*H.torulosum*	*A.niger*
2a	14	-	-	10
2b	--	08	10	10
2c	08	12	12	15
2d	07	12	13	-
2e	14	11	10	10
3a	08	13	12	17
3b	08	19	15	14
3c	07	16	-	08
3d	15	15	17	12
3e	06	12	14	14
Control	00	00	00	00
Tetracycline	20	20	--	--

-- = Not show zone inhibition

Table 2: Physical data of the synthesized compounds[a]

Entry	Mol.Formula	Yield [b](%)	M.P([0]C)	Halogen analysis % Found (Calcu.)	
				X=Cl,Br,I	N
2a	$C_{15}H_{13}ClN_2O_3$	78	162-164	11.35 (11.63)	9.00(9.29)
2b	$C_{15}H_{11}Br_2ClN_2O_3$	72	180-182	42.71(42.28)	6.86(6.06)
2c	$C_{15}H_{11}ClI_2N_2O_2$	74	241-243	53.11(53.52)	5.09(5.18)
2d	$C_{16}H_{14}ClIN_2O_2$	70	118-120	38.09(37.88)	6.93(6.54)
2e	$C_{17}H_{15}Cl_2IN_2O_2$	74	196-198	41.51(41.96)	5.31(5.87)
2f	$C_{16}H_{14}ClIN_2O_2$	73	185-187	37.33(37.88)	6.11(6.54)
2g	$C_{15}H_{11}Cl_2IN_2O_2$	77	117-119	44.89(44.05)	6.36(6.24)
2h	$C_{17}H_{16}ClIN_2O_3$	76	188-190	36.93(36.68)	6.10(6.33)

2i	$C_{16}H_{13}Cl_2IN_2O_2$	75	187-189	42.31(42.71)	6.86(6.05)
2j	$C_{15}H_{11}Cl_2IN_2O_2$	71	126-129	44.81(44.05)	6.77(6.24)
3a	$C_{21}H_{17}ClN_2O_3$	78	140-142	09.11(09.39)	7.00(7.36)
3b	$C_{21}H_{15}Br_2ClN_2O_3$	73	193-195	36.79(36.25)	5.89(5.20)
3c	$C_{21}H_{15}ClI_2N_2O_2$	76	170-172	46.31(46.91)	5.00(4.54)
3d	$C_{22}H_{18}ClIN_2O_2$	76	143-145	32.83(32.16)	5.99(5.56)
3e	$C_{22}H_{17}Cl_2N_2O_2$	74	204-206	36.89(36.39)	5.78(5.20)
3f	$C_{22}H_{18}Cl_2I_2N_2O_2$	75	195-197	32.00(32.16)	5.87(5.55)
3g	$C_{21}H_{15}Cl_2IN_2O_2$	77	126-128	37.93(37.66)	5.92(5.33)
3h	$C_{23}H_{20}ClIN_2O_2$	73	210-212	24.79(24.46)	5.31(5.40)
3i	$C_{22}H_{17}Cl_2IN_2O_2$	71	179-181	36.30(36.69)	5.93 (5.20)
3j	$C_{21}H_{15}Cl_2IN_2O_2$	76	103-051	37.96(37.66)	5.00(5.33)

[a]*Reaction conditions*: **1(a-j)** (0.01 mmol) and hydrazine hydrate or phenylhydrazine(0.01mmol) in ethanol at reflux temperature. [b]Isolated yields.

Spectral data

(2d): ^{1}H NMR ($CDCl_3$) δ ppm 2.6 (s,6H, Ar-CH_3),3.5 (dd,1H,-CH_2),4.4 (dd, 1H,-CH_2), 5.2 (t, 1H,CH_x) 7.52- 8.2 (m, 4H, Ar-H) 11.7 (s, 1H, Ar-OH). GC: 3.11(100%) IR max cm^{-1}: 3350 N-H, 1610 -C=N,1500, 1480 CH_2, MS.(m/z): 426(M^+100),33(53),189(25),82(80).

(3d) ^{1}H NMR ($CDCl_3$) δ ppm, 3.5 (dd,1H,-CH_2),4.3 (dd, 1H,-CH_2), 5.0 (t, 1H,CH_x) 7.0-8.5 (m, Ar-H) 11.7-13.0 (s, 1H, -OH) 5.5-8.0 (s,1H, NH), 2.2-2.6 (s,3H,Ar-CH_3).GC: 2.546 (100%),IRmaxcm^{-1}:3400N-H,3290–OH,1610,C=N,1500,1480CH_2MS.(m/z):501(M^+100), 481(50), 332(10,248(25),73(52).

RESULTS AND DISCUSSION

Various methods for the synthesis of pyrazolines have been reported.[16] However, the most convenient method involved is the action of hydrazine hydrate or phenyl hydrazine on α, β unsaturated carbonyl compound like polyhydroxy chalcones. It has well established that all polyhydroxy chalcones react with hydrazine or phenyl hydrazine gives pyrazolines. A series of some novel pyrazoline derivatives were synthesized by refluxing chalcone derivatives and hydrazine hydrate or phenyl hydrazine. To study the generality of this process, variety of examples were illustrated for the synthesis of pyrazolines and results were summarized in Table 1. The reaction is compatible for various substituents such as CH3, OH, Cl and F. This method is also effective for the α, β unsaturated carbonyl compound like polyhydroxy chalcones which form their corresponding pyrazoline derivative in 74~89% of yields (Table 1) The formation of desired product was confirmed by ^{1}H NMR, IR and C,H and N analysis technique. Also melting points were recorded.

Antimicrobial Activity

The information regarding the various species of bacterial used to carry out screening is given below.

(i)*Escherichia coil (E. coil)*

Escherichia coil are gram –negative bacteria they occur in lower portion of the intestine and urinary tract. They causes urinary tract infection, some strains can cause gastro-enteritis.

(ii) *Staphylococcus aureus (S. aureus) S. aureus* are the gram positive non motile cocci arranged in groups. They are parasites in the skin an mucous membranes occurring of human and animals. *S. aureus* produces many toxins that contribute to the bacetrium's pathogenicity by increasing its ability to invade the both or damage tissus. *S. aureus* is the again of toxic shock syndrome a severe infection causing high fever and vomiting and sometimes death. It also produces an entertoxin that cause vomiting and nausea when ingested and is one of the most common of food poising. For establishment of antimicrobial activity of the synthesized compounds, we utilized the reported disk diffusion method. The experiment was performed at a concentration of 150 ppm, we checked the activity of these molecules against different strains of bacteria and fungi as mentioned in Table 2. 10%DMSO was used as solvent control.

Antifungal activity

In the present investigation some of the new synthesized pyrazolines were assessed for their antifungal activity against fungi like *Aspergillus nigar (A.niger), Helminthosporium torulosum (H.toulosum).* The assessment of activity of spore germination method in petridish was fallowed. Spore suspension was prepared from seven days old PDA (Potato dextrose agar) slant cultures. Spore suspensions were placed in small's petridishes. Solutions of different synthesized compounds were prepared in 90:10(V/V) water ethanol and the concentration of compounds (150ppm) was adjusted in spore suspension. Petridishes were placed for incubation period of 12hours under moist chambers. Aqueous ethanol (90:10 v/v) served as control. Percentage germination with effect of these compounds after a period of 12 hours was recorded by observing Petridis directly under microscope. The obtained data of activity of all these tested compounds is shown in Table 2.

CONCLUSION

In conclusion, we have described a general and highly efficient procedure for the preparation of pyrazoline derivatives using without catalyst. The remarkable advantage of this protocol is

mild reaction conditions, excellent yields of product, operational and experimental simplicity. We believe that, this methodology will be a valuable addition to the existing methods for the synthesis of pyrazoline. These pyrazolines were prepared in order to extend the field of pyrazoline chemistry and assess the biological consequences of hydroxyl substituted in aryl rings.

ACKNOWLEDGMENTS

The author are thankful to the Principal of Shri Shivaji College, Parbhani for providing research facilities and Director, IICT Hydrabad for providing spectral analysis. Authors are also thankful Head Department of Botany Science College, Nanded providing necessary facilities for testing biological activity.

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