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Synthesis and Antimicrobial Activity of Some Pyrazoline Derivatives

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The objective of present study is to synthesize and screening of antimicrobial activity of some derivatives of pyrazoline. 1*H*-[4-nitro phenyl-5-(substituted phenyl)]pyrazoline has been used as a precursor to synthesize some biologically active heterocycles. Reaction of 1-(4-nitrophenyl)-3-(substituted phenyl) prop-2-en-1-one with hydrazine hydrate gave 1*H*-(3-nitrophenyl-5-(substituted phenyl) pyrazoline which on reaction with benzoyl chloride in pyridine gave 1-benzoyl-(4-nitrophenyl)-5-(substituted phenyl) prazoline and on reaction with acetic acid yields 1-acetyl-(4-nitrophenyl)-5-(substituted phenyl) pyrazoline derivatives. Several derivatives have been synthesized and screened for their antimicrobial efficacy against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Antifungal activity against, *Aspergillus flavus*, *Fusarium oxisporum*, *Aspergillus niger* and *Trichoderma viridae*.

Key Words: Pyrazoline, Antifungal activity, Antibacterial activity.

INTRODUCTION

Heterocyclic compounds play important role in diverse bilogical activities[1]. A second nitrogen in five membered ring also influences the antibacterial or pharmaceutical properties[2,3]. 2-Pyrazoline derivatives have been reported in the literature to exhibit various pharmacological activities such as antibacterial, antifungal, herbicidal and anticholinergic[4-10].

In this communication, we report the synthesis of some pyrazoline derivatives **III(a-l)** and **IV(a-l)** with possible antimicrobial activities.

EXPERIMENTAL

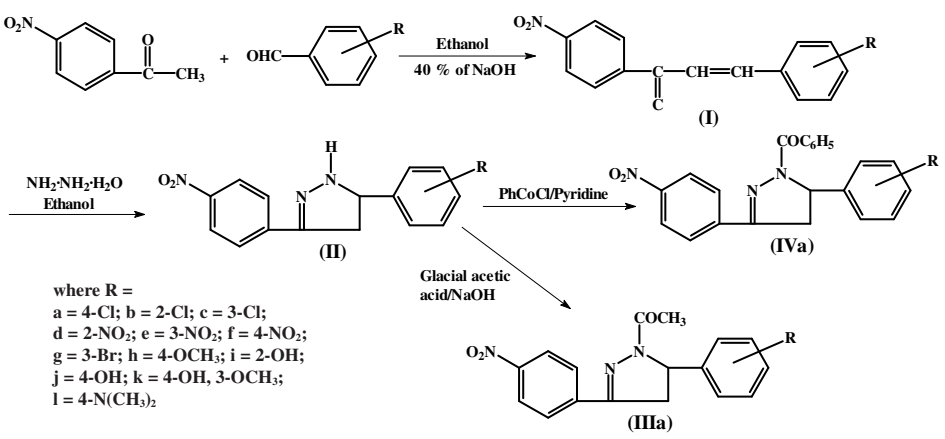
All the melting points have been determined in a open capillary and are uncorrected. The completion of reactions were monitored on TLC. The IR spectra have been recorded in KBr pellets on a Shimadzu 8201PC spectrophotometer (ν_{max} in cm^{-1}) ^1H NMR spectra on a AVANCE II 400 NMR spectrometer (chemical shift in δ ppm), elemental analysis has been carried out on a Carlo Elba 1108 model analyzer.

Synthesis of 1-(4-nitrophenyl)-3-substituted phenyl prop-2-en-1-one: *p*-Nitro acetophenone (0.01 mol) was dissolved in ethanol. Aromatic substituted aldehyde (0.01 mol) was added and the solution was heated to boiling. To this hot solution

40 % NaOH was added with constant stirring. A yellow orange coloured mass was obtained which was kept overnight and acidified by 10 % HCl. Washed with 10 % $NaHCO_3$ followed by water and crystalized from ethanol to give **Ia**, m.p. 205 ºC, yield 69 %. m.f. $C_{15}H_{10}NO_3Cl$ and m.w. 287.5. IR spectrum (nujol, ν_{max}, cm^{-1}): 3050 (C-H), 1672 (C=O) stretching), 1600 (Ar-NO_2 syn. *str.*), 1548 (asym in -CH=CH-), 1180 (C-N *str.*). ^1H NMR ($CDCl_3$) in δ ppm 6.42 (unsym m, 4H nitro substituted benzene ring), 6.02 (sym. m, 4H chloro substituted benezene ring), 2.56 (S, 2H, CH=CH).

Synthesis of 1*H*-3-(4-nitrophenyl)-5-(substituted phenyl) pyrazoline (II): 4-Nitro substituted chalcone (**I**) dissolved in ethanol (25 mL) and hydrazine (0.02 mol) was added to it. The reactin mixture was refluxed for 2 h, cooled, concentrated and allowed to stand overnight. The resulting solid which separated out was recrystallized from ethanol to yield 1*H*-3-(4-Nitrophenyl)-5-(4-chlorophenyl) pyrazoline **II(a)** with m.p. 109 ºC, yield 72 % m.f. $C_{15}H_{11}N_3O_2Cl$ m.w. 300.5. IR (nujol, ν_{max}, cm^{-1}): 3401 (N-H *str.*), 3152 (Ar-H *str.*), 2095 (Ali-H *str.*), 1675 (C=N *str.*), 1320 (C-N *str.*), 1170 (C-O *str.*), 928 (N-O *str.*), 835 (N-N *str.*), 759 (Ar-Cl *str.*). ^1H NMR ($CDCl_3$): δ 6.03 (sym, m, 4H, chloro substituted benzene ring) δ 6.51 (unsym. m, 4H, nitro substituted benzene ring), δ 7.39 (s, 1H, N-H), δ 2.54 (s, 2H, CH_2), δ 2-41 (s, 1H, CH).

Synthesis of 1-acetyl-3-(4-nitrophenyl)-5-(substituted phenyl)pyrazoline IIIa: Compound **IIa** and acetic acid (10 mL) were refluxed for 2 h. The reaction mixture was then concentrated, allowed to cool. The solid filtered, washed with water and recrystallized from ethanol to get **IIIa** (**Scheme-I**); IR (nujol, ν_{max}, cm^{-1}): 3049 (Ar-H *str.*), 2915 (Ali C-H *str.*), 1549 (C=N *str.*), 1270 (C-N *str.*), 1165 (C-O *str.*), 921 (N-O *str.*), 829 (N-N *str.*), 401 (C-H bending out-of-plane). ^1H NMR ($CDCl_3$) δ 4H, 6.54 (unsym. *meta*, nitro substituted benzene ring) 6.08 (sym. m, 4H, chloro substituted benzene ring) δ 2.48 (s, 2H, CH_2), δ 2.21 (1H, CH), 4.02δ (s, 3H, $COCH_3$).



(**Scheme-I**)**:** Reaction sequence of synthesized pyrazoline derivatives

Synthesis of 1-benzoyl-3-(4-nitrophenyl)-5-(substituted phenyl) pyrazoline IVa: 1*H*-3-(4-Nitrophenyl)-5-(4-chlorophenyl)pyrazoline **IIa** was dissolved in pyridine and benzoyl chloride stirred at room temperature for 1 h, after which the reaction mixture was treated with cold dilute HCl. The resulting solid was filtered, washed successively with water, cold NaOH (2 %) and water and finally crystallized from glacial acetic acid to obtain **IVa**, (yield 79 % m.p. 215 ºC) (**Scheme-I**). IR (nujol, ν_{max}, cm^{-1}): 3010 (Ar C-H *str.*), 2845 (Ali C-H *str.*), 1672 (C=N *str.*), 1402 (C-H bending in plane), 1319 (C-N *str.*), 1165 (C-O *str.*), 915 (N-O *str.*), 830 (N-N *str.*), 659 (C-H bending out-of-plane). ^{1}H NMR ($CDCl_3$) δ 6.23 (unsym. m, 4H) nitro substituted benzene ring) 6.41 (sym. m, 4H, chloro substituted benzene ring), 3.02 δ (Co s, CH_2) 2.46, δ (s, 1H, C-H), δ 7.38 (m, 6H, COC_6H_5).

RESULTS AND DISCUSSION

The reaction of *p*-nitro acetophenone and aromatic substituted aldehyde in ethanol gave 1-(4-nitro phenyl-3-substituted phenyl)prop-2-en-1-one **I**(**a-l**). In this step, chalcones are formed. Further, the compound **I**(**a-l**) on treatment with hydrazine hydrate in ethanol gave **II**(**a-l**) that refluxed in acetic acid has given 1-acetyl-3-(4-nitrophenyl)-5-substituted phenyl)pyrazoline **III**(**a-l**).

Compound **II**(**a-l**) was stirred with benzoyl chloride in pyridine at room temperature and gave 1-benzoyl-3-(4-nitrophenyl)-5-(substituted phenyl) pyrazoline **IV**(**a-l**). The physical characteristic data of synthesized pyrazoline derivatives are given in Table-1.

TABLE-1
PHYSICAL CHARACTERISTIC DATA OF
SYNTHESIZED PYRAZOLINE DERIVATIVES

Comp.	m.f.	m.w.	Yield	m.p.	Elemental analysis (%)					
					C		H		N	
					Calcd.	Found.	Calcd.	Found.	Calcd.	Found.
IIIa	$C_{17}H_{14}N_3O_3Cl$	343.75	75	186	59.39	59.39	4.10	4.10	12.22	12.21
IIIb	$C_{17}H_{14}N_3O_3Cl$	343.75	76	136	59.39	59.38	4.10	4.09	12.22	12.20
IIIc	$C_{17}H_{14}N_3O_3Cl$	343.75	71	145	59.39	59.38	4.10	4.09	12.22	12.20
IIId	$C_{17}H_{14}N_3O_5$	354.29	69	158	57.63	57.61	3.98	3.97	15.81	15.80
IIIe	$C_{17}H_{14}N_3O_5$	354.29	74	160	57.63	57.62	3.98	3.96	15.81	15.80
IIIf	$C_{17}H_{14}N_3O_5$	354.29	78	165	57.63	57.62	3.98	3.98	15.81	15.79
IIIg	$C_{17}H_{14}N_3O_3Br$	287.29	73	190	52.72	52.72	3.64	3.63	10.84	10.83
IIIh	$C_{18}H_{17}N_3O_4$	331.38	72	180	65.23	65.22	5.17	5.17	12.69	12.69
IIIi	$C_{18}H_{17}N_3O_4$	326.31	81	168	66.25	66.24	4.63	4.62	12.87	12.86
IIIj	$C_{18}H_{17}N_3O_4$	326.31	75	170	66.25	66.25	4.63	4.63	12.87	12.86
IIIk	$C_{18}H_{17}N_3O_5$	355.32	76	182	60.89	60.84	4.82	4.81	11.83	11.81
IIIl	$C_{19}H_{20}N_4O_3$	352.37	81	198	64.76	64.75	5.72	5.72	15.90	15.89

IVa	$C_{22}H_{16}N_3O_3Cl$	405.82	80	184	65.11	65.10	3.97	3.96	10.35	10.34
IVb	$C_{22}H_{16}N_3O_3Cl$	405.82	79	189	65.11	65.11	3.97	3.97	10.35	10.34
IVc	$C_{22}H_{16}N_3O_3Cl$	405.82	81	182	65.11	65.10	3.97	3.97	10.35	10.35
IVd	$C_{22}H_{16}N_4O_5$	416.36	78	170	63.46	63.46	3.87	3.86	10.09	10.08
IVe	$C_{22}H_{16}N_4O_5$	416.36	76	167	63.46	63.45	3.87	3.86	10.08	10.08
IVf	$C_{22}H_{16}N_4O_5$	416.36	75	176	63.46	63.46	13.8	3.87	10.09	10.08
IVg	$C_{22}H_{16}N_3O_3Br$	449.37	74	140	58.80	58.79	3.80	3.57	9.35	9.34
IVh	$C_{23}H_{19}N_3O_4$	449.37	69	190	61.47	61.46	4.26	4.25	3.35	9.35
IVi	$C_{22}H_{17}N_3O_4$	387.37	68	167	68.21	68.20	4.42	4.42	10.84	10.84
IVj	$C_{22}H_{17}N_3O_4$	387.37	70	168	68.21	68.20	4.42	4.41	10.84	10.84
IVk	$C_{23}H_{20}N_3O_4$	418.39	72	185	66.02	66.01	4.81	4.79	10.04	10.03
IVl	$C_{24}H_{22}N_3O_3$	400.40	75	189	71.98	71.97	5.53	5.53	10.49	10.48

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED PYRAZOLINE DERIVATIVES III(**a-1**) AND IV (**a-1**) AGAINST VARIOUS BACTERIA AT TWO DIFFERENT CONCENTRATIONS, (in ppm)

Comp.	*B. subtilis*		*E. coli*		*K. pneumoniae*		*S. aureus*	
	50	100	50	100	50	100	50	100
IIIa	++++	++++	+++	+++	++	++	++	++++
IIIb	++	+	++	++	+	++	++	+++
IIIc	+++	+++	+	++	+++	++++	++	+++
IIId	+++	++++	+	++	++	++	+	++
IIIe	++	+	+	++	++	+	+	++
IIIf	+	++	–	+	+	++	++	+++
IIIg	–	++	–	+	–	+	+	++
IIIh	++	++	+	++	+	+	++	+++
IIIi	–	+	+	++	++	++	+	++
IIIj	+	+	+	++	++	+++	+	++
IIIk	++	+	+++	+++	++	+++	++	+++
IIIl	+++	++++	++	+++	+++	+++	+	++
IVa	+++	++++	+++	+++	++++	++++	++	+++
IVb	++	++	+	++	++	++	++	+++
IVc	++	++	+	++	+	+	–	–
IVd	+	–	++	+++	+	+	++	++
IVe	–	–	+	++	+	+	++	++
IVf	+	+	+	++	++	++	++	++
IVg	++	+	+	++	++	++	+	+
IVh	++	++	–	+	+	++	++	++
IVi	++++	+	+	++	++	+++	+++	++++
IVj	++	++	+++	+++	+	+++	++	+++
IVk	++	+	+	++	+	++	+	+
IVl	+++	+++	+	++	++	+++	++	+++
Std.	+++	++++	+++	++++	+++	++++	++++	++++

Std.: Streptomycin; ++++: Strongly active range > 19; +++: moderately active range < 12-18; ++: weakly active range 8-12; +, –: inactive range > 8.

Both series of pyrazoline derivatives **III**(**a-l**) and **IV**(**a-l**) have been screened for their antibacterial activity against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Staphyllococcus aureus* at two different concentrations 50 and 100 ppm, respectivley by filter paper disc plate method and antifungal activity against *Aspergillus niger, Aspergillus flavus, Fusarium oxisporum* and *Trichoderma viridae* by filter paper disc method at two different concentrations (100 and 500 ppm). Standard antifunal drug griseofulvin and antibacterial drug streptomycin have also been screened under the similar conditions for comparison.

The results of antimicrobial activity of **III**(**a-l**) and **IV**(**a-l**) showed that **IIIa** (4-Cl), **IIIc** (2-Cl), **IIId** (2-NO_2, **IVa** (4-Cl), **IVj** (4-OCH_3) and **IVl** [4N-$(CH_3)_2$] are highly active against selected bacteria and fungi and rest of the pyrazoline derivatives have shown good to moderate activity (Tables 2 and 3).

TABLE-3
ANTIFUNGAL ACTIVITY OF THE SYNTHESIZED PYRAZOLINE DERIVATIVES AGAINST VARIOUS FUNGI AT TWO DIFFERENT CONCENTRATION (IN ppm)

Comp.	*A. flavus*		*A. niger*		*F. oxisporum*		*T. viridae*	
	50	100	50	100	50	100	50	100
IIIa	+++	++++	+++	++++	++	+++	++	+++
IIIb	+++	++++	+	++	++	++	++	+++
IIIc	+++	+++	+++	+++	+++	+++	++	+++
IIId	++	++	+	++	++	++	+	++
IIIe	++	++	+	++	++	++	+	++
IIIf	+	+	+	+++	+	++	–	++
IIIg	+	++	++	+++	+	++	++	+++
IIIh	+	+	++	++	+	++	+	++
IIIi	–	+	++	++	+	++	+	++
IIIj	+	++	+	+	+	++	+	++
IIIk	–	+	+	+	+	+	+	++
IIIl	–	–	–	+	+	+	+	++
IVa	+++	++++	++	+++	+++	+++	+	++
IVb	+	++	–	+	+	++	++	+++
IVc	+	++	+	++	++	++	+	+++
IVd	–	+	+	++	++	++	+	++
IVe	+	++	+	++	+	++	++	+++
IVf	+	+	+	++	+	++	+	++
IVg	+	++	+	+	+	+	+	++
IVh	+	++	+	++	++	++	+	+
IVi	++	++	++	+++	++	+	–	+++
IVj	+++	++++	+++	+++	+++	+++	+	++
IVk	–	+	+	+	++	++	+++	++
IVl	+	++	–	++	++	+	+	++++
Std.	+++	++++	+++	++++	++++	++++	+++	++++

Std.: Griseofulvin; ++++: Strongly active range > 19; +++: moderately active range < 12-18; ++: weakly active range 8-12; +, –: inactive range > 8.

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