SYNTHESIS OF SOME NEW BIOACTIVE 1-N-ACID HYDRAZIDE SUBSTITUTED PYRAZOLINES

Sadaf Sadiq Khan and Aurangzeb Hasan*
Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
flavonoids@hotmail.com

Abstract : A series of 14 new bioactive 1-N-acid hydrazide substituted pyrazolines were synthesized by cyclization of variably substituted chalcones and different acid hydrazides, using acetic acid as a solvent. The chemical structure of the compounds was characterized by FTIR, EIMS and ^{1}H NMR spectroscopy. The antibacterial activities of these compounds were evaluated by agar well diffusion method. 1-N-picolinic acid hydrazide pyrazoline was found to be more active as compared to the standard antibiotic Roxithromycin.

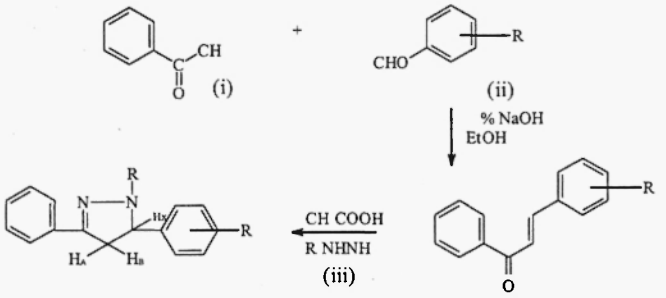
Introduction

Pyrazolines and their derivatives display various biological properties such as pesticidal, fungicidal, insecticidal, anti-inflammatory, antiarthritic, antidepressant and antiviral activities[1-3]. Considerable interest has been focused on the pyrazolines structure, which has known to possess a broad spectrum of biological activities such as antinociceptive, antibacterial, antioxidant and antiamoebic[4-8]. Moreover, these heterocyclic compounds, in addition to biological activities have also shown some industrial applications like bleaching agents, dyes, optical brighteners and various fluorescent whitening agents [1,3, 9-12]. Earlier studies by Gabriele Murineddu, *et al.* [13] also demonstrated the analgesic and anti-inflammatory activities of acid hydrazides.

The present study is therefore devoted to the synthesis of pyrazolines from variably substituted chalcones which are also associated with diverse biological activities [14-18] and different acid hydrazides. This study was carried out in the quest to prepare pyrazolines not synthesized earlier, that may possess new and/or enhanced biological and other industrial properties. We report the synthesis of a series of 14 new 1-N-acid hydrazide substituted pyrazolines which were characterized by physical and FTIR, EIMS and 1H NMR spectroscopic data and have also been evaluated for their antibacterial activities

Results and Discussions

1,3-Diphenyl-2-propen-1-ones (chalcones) **1a-f** were synthesized by condensing acetophenone (**i**) with appropriate benzaldehyde derivatives (**ii**) in dilute ethanolic sodium hydroxide solution at room temperature.1-N-acid hydrazide substituted -2-pyrazolines (**2g-t**) were synthesized by the reaction of appropriate 1,3-Diphenyl-2-propen-1-one derivatives (**1a-f**) and acid hydrazides (**iii**) according to the following **Scheme-1**. Physical data of synthesized pyrazolines (**2g-t**) are listed in **Table-1**.



2-pyrazolines (**2g-t**) Chalcones (**1a-f**)

Scheme-1

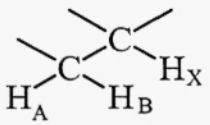
Table-1 : Structure and physical data of pyrazolines (2g-t)

Compound #	R_1	R_2	Formula	M.P ^{0}C	Yield (%)	R_f	Purification Solvent
2g		-H	$C_{21}H_{17}N_3O$	158-160	71	89	Ethanol
2h		-H	$C_{21}H_{17}N_3O$	146-148	75	97	"
2i		-H	$C_{21}H_{17}N_3O$	148-150	76	93	"
2j	"	$-OCH_3$	$C_{22}H_{19}N_3O_2$	130-132	78	87	"
2k	"	-NO2	$C_{21}H_{16}N_4O_3$	136-138	72	94	"
2l	"	$-N(CH_3)_2$	$C_{23}H_{22}N_4O$	127-129	72	94	"
2m	"	-F	$C_{21}H_{16}N_3OF$	168-169	88	91	"
2n	"	-Cl	$C_{21}H_{16}N_3OCl$	138-140	87	92	"
2o		-H	$C_{29}H_{24}N_2O$	170-172	80	85	"
2p	"	$-NO_2$	$C_{29}H_{23}N_3O_3$	120-122	65	93	"
2q	"	-Cl	$C_{29}H_{23}N_2OCl$	98-100	71	92	"
2r		-H	$C_{22}H_{18}N_2O$	158-160	84	91	"
2s	"	$-NO_2$	$C_{22}H_{17}N_3O_3$	136-138	75	86	"
2t		-H	$C_{22}H_{17}N_2OF$	128-130	73	92	"

1-N-Picolinoyl-3, 5-diphenyl-2-pyrazoline (**2g**) was obtained as yellow granules. The molecular formula and molecular weight of the compound (**2g**) are $C_{21}H_{17}N_3O$ and 327 respectively. The FTIR spectrum showed stretching frequencies at 1583 cm^{-1} 1281 cm^{-1} 1506 cm^{-1} 3034 cm^{-1} 1690 cm^{-1} which are characteristics of C=N, C-C, C=C Ar., CH_2, C=O of the aromatic rings respectively.

The mass spectrum of 1-N-Picolinoyl-3, 5-diphenyl-2-pyrazoline (**2g**) showed characteristics peaks of molecular ion peak M at m/z 327 which is found in good agreement with molecular weight. The characteristic peaks at m/z 106 and 78 appeared due to the formation of the picolinoyl and pyridine segments. The base peak in the mass spectrum appeared at m/z 327 which is also the molecular ion peak.

The ^{1}H NMR spectrum of 1-N-Picolinoyl-3, 5-diphenyl-2-pyrazoline (**2g**) showed three doublet of doublets at 3.17 ppm, 3.77 ppm, 5.60 ppm, with large coupling constant 4.70,17.6 Hz, 11.69,17.6 Hz and 4.71 ,11.6 Hz which are characteristic and showed following type of arrangement of protons.



The aromatic protons appeared as a multiplet in the range of 7.23-7.47 ppm. The characteristic peak for the picolinoyl protons appear as doublet of doublet of 4-H between 7.81-10.37. Interpretation of FTIR, mass, NMR, spectral data and physical constants confirmed the structure of (**2g**) as 1-N-Picolinoyl-3, 5-diphenyl-2-pyrazoline. Similarly all other pyrazolines (**2h-t**) were characterized on the basis of their physical and spectral data.

Experimental Protocols

All melting points were determined in open capillaries using Gallenkemp melting point apparatus and are un-corrected. All chemicals were supplied by Fluka and Sigma Aldarich (Germany). R_f values were calculated by using pre-coated silica gel aluminum backed plates Kieselgel 60 F_{254} Merck (Germany), in ethyl acetate: pet-ether (1:9). FTIR spectra were recorded on Bio-Rad Merlin using KBr discs. ^{1}H NMR spectra were recorded on GEQE 3400 Spectrometer (Oxford Magnet) using TMS as internal standard. EIMS were recorded on VG: 70 SE Mass Spectrometer. Purity of each compound was monitored by TLC.

General Procedure for the Preparation of 1, 3-Diphenyl-2-propen-1-ones (1a)

1, 3-Diphenyl-2-propen-1-one derivatives were synthesized by condensing acetophenone (0.01mol) with appropriate benzaldehyde derivatives (0.01mol) according to Claisen Schmidt condensation reaction [19-20]

General Procedure for the Preparation of 1-N-picolinoyl-1, 3-Diphenyl-2-pyrazoline (2g)

Picolinic acid hydrazide (0.013 mol) 1.78g was added to 1,3-Diphenyl-2-propen- 1-one (chalcone) **1a-f** (0.01 mol) 2.08g in acetic acid (15 ml) and the mixture was refluxed under constant stirring for 3 hours at 100-110°C until the cyclization is completed and a deep orange colour developed. The reaction mixture was diluted with ice-cold water, extracted with ethyl acetate and the solvent was removed under reduced pressure. This procedure afforded the product **2g** which was purified by crystallization from ethanol. IR (KBr) (υ_{max}/cm^1):1585, 1281, 1506, 3034, 1690. ^{1}H NMR ($CDCl_3$) δH: 3.17(dd, 1H, HA), 3.77(dd, 1H, H_B), 5.60(dd, 1H, H_X), 7.237.47(m10H, $H_{arom.}$), 7.81-10.37(dds, 4H, $H_{picolinoyl}$). EIMS (m/z, %): 327(M^+, 11.9), 221(28.2), 106(12.2), 78(100), 51(15.3).

1-N-Isonicotinoyl-1, 3-Diphenyl-2-pyrazoline (2h) IR (KBr) (υ_{max}/cm^1): 1593, 1302, 1491, 3000, 1636. ^{1}HNMR ($CDCl_3$) δH: 3.27(dd, 1H, H_A), 3.84(dd, 1H, H_B), 5.80(dd, 1H, H_X), 7.23-7.43(m10H, $H_{arom.}$), 7.6-8.76 (dds, 4H, $H_{isonicotinoyl}$). EIMS (m/z, %): 327(M^+, 100), 221(21.2), 106(68), 78(32.4), 51(16).

1-N-Nicotinoyl-1, 3-Diphenyl-2-pyrazoline (2i) IR (KBr) (υ_{max}/cm^1): 1587, 1200, 1476, 3197, 1346. ^{1}HNMR ($CDCl_3$) δH: 3.27(dd, 1H, HA), 3.84(dd, 1H, H_B), 5.82(dd, 1H, H_X), 7.23-7.42(dd, 1H, H_X), 7.23-7.42(10H, m, $H_{arom.}$), 7.71-8.68, 9.29(2H, 2ds, 1H, s, $H_{nicotinoyl}$). EIMS (m/z, %): 327(M^+, 11.9), 106(100), 78(43.2), 51(10.8).

1-N-Nicotinoyl-3-phenyl-5-(p-methoxyphenyl)-2-pyrazoline (2j) IR (KBr) (υ_{max}/cm^1): 1595, 1230, 1495, 3090, 1650. ^{1}HNMR ($CDCl_3$) δH: 3.26(dd, 1H, H_A), 3.80(dd, 1H, H_B), 5.80(dd, 1H, H_X), 7.23-7.40(9H, m, $H_{arom.}$), 7.70-8.68, 9.20(2H, 2ds, 1H, s, $H_{nicotinoyl}$), 3.70(3H, s, OCH_3). EIMS (m/z, %): 357(M^+, 40), 106(100), 78(47), 51(12).

1-N-Nicotinyl-3-phenyl-5-(m-nitrophenyl)-2-pyrazoline (2k) IR (KBr) (υ_{max}/cm^1): 1580, 1190, 1480, 3102, and 1630. ^{1}HNMR ($CDCl_3$) δH: 3.26(dd, 1H, H_A), 3.81(dd, 1H, H_B), 5.80(dd, 1H, H_X), 7.23-7.41(9H, m, $H_{arom.}$), 7.72-8.80, 9.22(2H, 2ds, 1H, s, $H_{nicotinoyl}$). EIMS (m/z, %): 372(M^+, 39), 106(100), 78(50), 51(14).

1-N-Nicotinyl-3-phenyl-5-(p-N, N-dimethylphenyl)-2-pyrazoline (2l) IR (KBr) (υ_{max}/cm^1): 1597, 1225, 1496, 3159, 1644, and 1300. ^{1}HNMR ($CDCl_3$) δH: 3.36(dd, 1H, H_A), 3.91(dd, 1H, H_B), 5.86(dd, 1H, H_X), 7.23-7.50(9H, m, $H_{arom.}$), 7.73-8.78, 9.30(2H, 2ds, 1H, s, $H_{nicotinoyl}$), 2.91(6H, s, N $(CH_3)_2$). EIMS (m/z, %): 370(M^+, 50), 106(100), 78(47), 51(20).

1-N-Nicotinoyl-3-phenyl-5-(m-fluorophenyl)-2-pyrazoline (2m) IR (KBr) (υ_{max}/cm^{1}): 1575, 1187, 1476, 3170, 1632, and 1120. ^{1}HNMR ($CDCl_3$) δH: 3.35(dd, 1H, H_A), 3.89(dd, 1H, H_B), 5.80(dd, 1H, H_X), 7.30-7.40(9H, m, $H_{arom.}$), 7.70-8.70, 9.29(2H, 2ds, 1H, s, $H_{nicotinoyl}$). EIMS (m/z, %): 345(M^+, 48), 106(100), 78(51), 51(20).

1-N-Nicotinyl-3-phenyl-5-(p-chlorophenyl)-2-pyrazoline (2n) IR (KBr) (υ_{max}/cm^{1}): 1582, 1220, 1482, 3140, 1660, 695. ^{1}HNMR ($CDCl_3$) δH: 3.37(dd, 1H, H_A), 3.86(dd, 1H, H_B), 5.82(dd, 1H, H_X), 7.29-7.45(9H, m, $H_{arom.}$), 7.69-8.82, 9.30(2H, 2ds, 1H, s, $H_{nicotinoyl}$). EIMS (m/z, %): 361($M^{+\cdot}$ 60), 106(100), 78(60), 51(40).

1-N-Benzeliloyl-3, 5-diphenyl-2-pyrazoline (2o) IR (KBr) (υ_{max}/cm^{1}): 1536, 1298, 1490, 3034, 1697. ^{1}HNMR ($CDCl_3$) δH: 3.25(dd, 1H, H_A), 3.78(dd, 1H, H_B), 5.80(dd, 1H, H_X), 4.30(1H, s, $H_{Aliphatic}$), 7.23-7.62(20H, m, $H_{arom.}$). EIMS (m/z, %): 416($M^{+\cdot}$ 100), 249(70), 221(40), 167(80), 77(60), 51(40)

1-N-Benzeliloyl-3-phenyl-5-(m-nitrophenyl)-2-pyrazoline (2p) IR (KBr) (υ_{max}/cm^{1}): 1550, 1282, 1485, 3020, 1695. ^{1}HNMR ($CDCl_3$) δH: 3.28(dd, 1H, H_A), 3.79(dd, 1H, H_B), 5.92(dd, 1H, H_X), 4.32(1H, s, $H_{Aliphatic}$), 7.21-7.61(20H, m, $H_{arom.}$). EIMS (m/z, %): 461($M^{+\cdot}$ 100), 294(68), 266(42), 167(82), 77(68), 51(42).

1-N-Benzeliloyl -3-phenyl-5-(4-chlorophenyl)-2-pyrazoline (2q) IR (KBr) (υ_{max}/cm^{1}): 1570, 1295, 1499, 3035, 1680, 695. ^{1}HNMR ($CDCl_3$) δH: 3.26(dd, 1H, H_A), 3.82(dd, 1H, H_B), 5.84(dd, 1H, H_X), 4.29(1H, s, $H_{Aliphatic}$), 7.21-7.65(20H, m, $H_{arom.}$). EIMS (m/z, %): 450($M^{+\cdot}$ 100), 283(60), 255(38), 167(90), 77(70), 51(32).

1-N-Benzoyl-3,5-diphenyl-2-pyrazoline (2r) IR (KBr) (υ_{max}/cm^{1}): 1594, 1244, 1493, 3060, 1690. ^{1}HNMR ($CDCl_3$) δH: 3.23(dd, 1H, H_A), 3.81(dd, 1H, H_B), 5.83(dd, 1H, H_X), 7.27-7.30(10H, m, $H_{arom.}$),7.37-7.88(5H, m, $H_{Benzoyl}$). EIMS (m/z, %): 326($M^{+\cdot}$ 100), 221(27.2), 105(100), 222(26), 77(59.1).

1-N-Benzoyl-3-phenyl-5-(m-nitrophenyl)-2-pyrazoline (2s) IR (KBr) (υ_{max}/cm^{1}): 1590, 1250, 1497, 3030, 1695. ^{1}HNMR ($CDCl_3$) δH: 3.25(dd, 1H, H_A), 3.89(dd, 1H, H_B), 5.85(dd, 1H, H_X), 7.27-7.50(9H, m, $H_{arom.}$), 7.37-7.89(5H, m, $H_{Benzoyl}$). EIMS (m/z, %): 371(80), 266(28.2), 105(100), 222(26), 77(54).

1-N-(3-Fluorobenzoyl)-3,5-diphenyl-2-pyrazoline (2t) IR (KBr) (υ_{max}/cm^{1}): 1596, 1540, 1480, 3025, 1685, 1130. ^{1}HNMR ($CDCl_3$) δH: 3.24(dd, 1H, H_A), 3.87(dd, 1H, H_B), 5.81(dd, 1H, H_X), 7.23-7.50(10H, m, $H_{arom.}$), 7.62-7.84(4H, m, $H_{Benzoyl}$). EIMS (m/z, %): 344(70.3), 221(9.7), 123(100), 240(18.3), 95(20.4).

Antibacterial Activity Analysis

Synthesized compound were tested for their antibacterial activity by adopting agar well diffusion method [21]. Bacteria cultures used were *Escherichia coli, Bacillas subtilis, Pseudomonas pickiti* , *Enterobacter aerogenes* and *Micrococcus luteus*, Roxithromycin was used as standard drug. Using 100 µl micropipette, test solutions were poured in respective wells. Different concentrations of test samples, a solution for positive control (Roxithromycin) and one for negative control (DMSO) was applied to each Petri plate. These plates were incubated at 37°C. After 24 hours and 48 hours of incubation the diameter of the clear zones, showing no bacterial growth, around each well was measured. Triplicate plates were prepared for each sample compound. Mean clear zone of these plates was calculated in mm with standard deviation (**Table-2**).

Table-2: Antibacterial activity of pyrazolines (2g-t) against five bacterial strains.

Compound	Zone of Inhibition (mm)					Remarks/ Activity
	E.coli	*B.subtillus*	*P.pickitii*	*M.luteus*	*E.aerogenes*	
ROX	25	22	33	22.5	20	Significant
2g	19	19	26	31.5	29	More active
2h	-	-	-	-	-	-
2i	-	-	-	13.75	-	Significant
2j	-	-	11.5	-	-	"
2k	-	-	12.5	-	-	"
2l	-	-	11.75	-	-	"
2m	-	-	-	-	-	-
2n	-	-	11	-	11.25	Significant
2o	-	-	11	-	-	"
2p	-	-	11.5	-	11	"
2q	-	-	11.5+0.5	-	11.75	"
2r	-	-	-	-	-	-
2s	-	-	11.5	-	11.5	Significant
2t	-	-	10.5	-	-	"

Pyrazoline **2g** was found to be significantly active against *Escherichia coli, Bacillas subtilis* and *Pseudomonas pickiti* bacterial strains. However, the same pyrazoline exhibited even stronger antibacterial activity against *Enterobacter aerogenes* and *Micrococcus luteus* as compared to the standard drug Roxithromycin. Most of the other pyrazolines (**2h-t**) showed some activity only against *Pseudomonas pickiti* and *Enterobacter aerogenes*. Strong antibacterial activity of pyrazoline **2g** may be due to the relative position of the N-atom with respect to the carbonyl group of the acid hydrazide.

Conclusions

It is concluded that among different pyrazolines the presence of different hydrazide moieties, various substituents and their positions are very important from biological activity point of view. Pyrazoline **2g** was found to be significantly active against *Escherichia coli, Bacillas subtilis* and *Pseudomonas pickiti* bacterial strains. However, the same pyrazoline exhibited even stronger antibacterial activity against *Enterobacter aerogenes* and *Micrococcus luteus* as compared to the standard drug Roxithromycin. Most of the other pyrazolines (**2h-t**) showed some activity only against *Pseudomonas pickiti* and *Enterobacter aerogenes*. Strong antibacterial activity of pyrazoline **2g** may be due to the relative position of the N-atom with respect to the carbonyl group of the acid hydrazide

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