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SYNTHESIS AND ANTIOXIDANT ACTIVITY OF NOVEL PYRAZOLINE DERIVATIVES

B.C. REVANASIDDAPPA*, M.VIJAY KUMAR, HEMANTH KUMAR

Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences of Nitte -Deemed to be University, Paneer, Deralakatte, Mangalore-575 018, Karnataka, India

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Correspondence:

B.C. Revanasiddappa M.Pharm, Ph.D
Department of Pharmaceutical Chemistry,
NGSM Institute of Pharmaceutical Sciences of Nitte -Deemed to be University,
Paneer, Deralakatte, Mangalore-575 018,
Karnataka, India

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ABSTRACT

Plan: *A novel series of pyrazolines were synthesized through chalcones. The synthesized compounds were evaluated for various antioxidant activities.*
Preface: *Pyrazolines belongs to five membered nitrogen classes of compounds. Pyrazolines were reported with wide spread of chemotherapeutic activities.*
Methodology*: A new series of Chalcones **(2a-j)** were prepared by reacting substituted aldehydes and ketones in alcohol medium in presence of NaOH. The chalcones **(2a-j)** undergoes selective cyclization with benzhydrazide **(1)** in glacial acetic acid medium to yield the title compounds 1, 3, 5-trisubstituted Pyrazolines **(3a-j)**. The new compounds were assigned on the basis of ^{1}H-NMR, IR and Mass spectral data. The newly synthesized compounds were screened for their In-Vitro antioxidant activity by DPPH, super oxide and nitric oxide methods.*
Outcome: *Some of the tested compounds **3e, 3g, and 3f** showed moderate activity when compared to the standard drug ascorbic acid.*

1. INTRODUCTION

Nitrogen containing heterocyclic compounds has been continuing to attract the researchers in the field of chemistry. The fascination towards the nitrogen containing heterocyclic compounds attributes due to their diversified biological activities. The dihydro derivative of Pyrazole is known as Pyrazoline. These are 5-membered heterocyclic compounds and various methods have been worked out for their synthesis. The ring is quite stable and has inspired chemists to carryout various structural variations in the ring.. Pyrazolines are reported to possess analgesic[1], anti-inflammatory[2], antimicrobial[3], antiamoebic[4], antipyretic[5] and antidepressant[6] activities. The pyrazoline nucleus is the ubiquitous feature of various compounds possessing many pharmacological and physiological activities and therefore they are useful material in drug research.

Corresponding author email: revan@nitte.edu.in
Mobile- 09448953198
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Chalcones are basically 1, 3-diaryl-2-propen-1-ones and are natural or synthetic compounds prepared by well known Claisen-Schmidt condensation reaction. The presence of reactive α, β- unsaturated keto group in chalcones is found to be responsible for their biological activity. Chalcones represent an essential group of natural as well as synthetic products and they are reported to possess wide range of activities such as antimalarial[7], antileshmanaial[8], cytotoxic[9], antitubercular[10], anti-inflammatory[11], antibacterial[12] etc. Moreover, chalcones are useful synthons in the synthesis of a large number of bioactive molecules such as pyrazolines. These compounds found application in the synthesis of various heterocyclic compounds.

Based on the above pharmacological profile of both chalcones and pyrazolines, and as part of our continuous efforts in this area[13-16], in the present work we have planned to synthesize a new series of pyrazolines from the key intermediate chalcones and their subsequent evaluation for antioxidant activities.

2. MATERIALS AND METHODS

Melting points were determined in open capillary tube and are uncorrected. All the chemicals were obtained of analytical grade purity. IR spectra were recorded by using Alpha Bruker IR spectrometer using a thin film on KBr pellet technique and frequencies are expressed in $cm^{-1.}$ ^{1}H-NMR spectra were recorded on Bruker Avance II 300 NMR spectrometer. All spectra were obtained in DMSO. All the chemical shift are expressed in δ ppm relative to TMS (δ=0) as internal standard. Mass spectra were recorded on Perkin Elmer Clarus 680 GC-MS spectrometer. TLC was used to monitor the progress of the reaction. TLC was done on precoated 0.2 mm Merck silica gel 60 plates.

2.1. General procedure for synthesis of chalcones (2a-j)[13]

A mixture of substituted aromatic aldehydes [0.01 mol] and substituted ketones [0.01 mol] in ethanol [20 ml] were stirred together for 24 hr, in presence of 40% NaOH [5 ml]. The reaction mixture was poured into crushed ice and acidified with dilute HCl. The precipitated compound obtained was filtered, washed with water and recrystallized from suitable solvents.

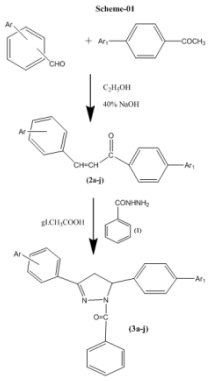
2.2. Synthesis of substituted 1, 3, 5-trisubstituted Pyrazolines (3a-j)[16]

Chalcones **(2a-j)** [0.01 mol] and benzhydrazide (**1)** [0.01mol] were dissolved in glacial acetic acid [20ml], and the reaction mixture is refluxed for about 10-18 hrs. The reaction mixture is cooled and poured into 100 ml of ice cold water with constant stirring. The solid which is precipitated is filtered, washed with cold water, dried and recrystallized by using alcohol. The physical data of pyrazolines **(3a-j)** is given in Table 1.

Table 1: Physical data of Pyrazoline Derivatives (3a-j)

Comp.	*Ar-CHO*	*Ar^1-$COCH_3$*	*Molecular weight*	*M.P (^{0}C)*	*Yield (%)*
3a	4-Cl	4-Cl	395.28	105-07	66
3b	4-Cl	4-OH	376.84	95-97	54
3c	4-Cl	4-NO_2	405.83	116-18	58
3d	4-Cl	4-CH_3	374.86	124-26	62
3e	4-Br	4-Cl	439.73	137-39	68
3f	4-Br	4-OH	421.29	112-14	69
3g	4-Br	4-NO_2	449.04	154-56	66
3h	4-Br	4-CH_3	419.31	177-79	68
3i	4-OH	4-CH_3	356.42	130-32	63
3j	4-OH	4-Br	421.29	143-45	64

3c: IR (KBr) ν (cm^{-1}): 1501(C=C), 1582(C=N), 1658(C=O), 3095 (C-H), **^{1}H-NMR (300 MHz, DMSO-d$_6$):** δ 7.28-7.32 (dd, 1H, H_A), 7.37-7.39 (dd, 2H, H_B, H_X), 7.71-8.08 (m, Ar-H, 13H). **MS (m/z):** 405 (M^+). **3i: IR (KBr) *ν* (cm^{-1})**: 1512(C=C), 1588(C=N), 1699(C=O), 3097(C-H), 3353 (OH). **^{1}H-NMR (300 MHz, DMSO-d$_6$):** δ 2.08 (s, CH_3, 3H), 7.47-7.49 (dd, 3H, H_A, H_B, H_X), 7.63-8.10 (m, Ar-H, 13H), 10.34 (s, 1H, OH). **MS (m/z):** 356 (M^+) **3j: IR (KBr) *ν* (cm^{-1})**: 1512(C=C), 1589C=N), 1642(C=O), 3089(C-H), 3382(OH). **^{1}H-NMR (300 MHz, DMSO-d$_6$)** 6.89-6.91 (dd, 3H, H_A, H_B,H_X), 7.50-8.08 (m, Ar-H, 13H), 10.41 (s, 1H, OH). **MS (m/z):** 421 (M^+).



2.3. Antioxidant Activity

2.3.1. Superoxide anion scavenging activity[17]

About 1 ml of nitro blue tetrazolium (NBT) solution (156µM NBT in 100mM phosphate buffer, pH 7.4), 1 ml NADH solution (468µM in 100m M phosphate buffer, pH 7.4) and 0.1 ml of sample solution (10-50µg/ml) in water is mixed. The reaction is started by adding 100µl of phenazinemethosulphate (PMS) solution (60µM PMS in 100m M phosphate buffer, pH 7.4) to the mixture. The reaction mixture is incubated at 25°C for 5 minutes, and the absorbance was recorded at 560 nm using ELISA plate reader and the results were calculated using the formula given in Eqn.1. Ascorbic acid was used as standard drug material. The results obtained by super oxide activity are given in Table 2 and Figure 1. The percentage scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = \frac{\text{Control - Test}}{\text{Control}} \times 100 \quad \ldots\ldots\ldots \text{ Eqn } \ldots\ldots (1)$$

Table-2: Data of super oxide radical scavenging activity of Pyrazoline derivatives (3a-j)

	Percentage inhibition										
Conc.	*Std*	*3a*	*3b*	*3c*	*3d*	*3e*	*3f*	*3g*	*3h*	*3i*	*3j*
10µg/ml	32.06	20.32	17.24	11.21	8.95	18.98	16.69	21.33	14.21	22.22	18.41
20µg/ml	40.24	28.36	24.55	19.45	18.66	30.21	31.21	33.11	25.08	31.01	31.45
30µg/ml	55.65	40.25	33.54	30.11	33.1	45.74	44.21	45.1	38.33	42.55	43.21
40µg/ml	67.31	54.25	45.78	45.08	45.21	58.81	58.21	58.21	52.11	51.30	55.21
50µg/ml	75.22	62.32	52.11	53.1	59.21	70.21	68.33	69.24	65.1	65.21	68.11
IC_{50}	*16.39*	*28.10*	*36.88*	*36.64*	*33.36*	*23.98*	*24.81*	*23.81*	*28.57*	*27.10*	*25.46*

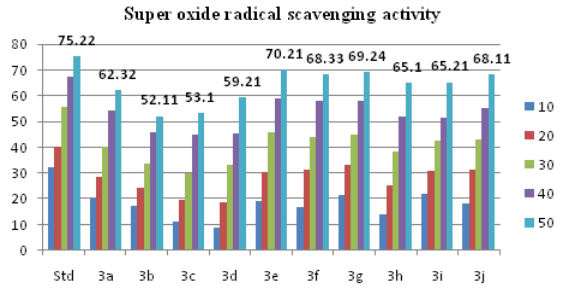


Fig-1: Data of super oxide radical scavenging activity

2.3.2. DPPH free radical scavenging activity[18]

The free radical scavenging activity of the title compounds was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. 0.2mM solution of DPPH in methanol was prepared and 100µl of this solution was added to title compounds (10-50µg/ml). After 30 minutes, absorbance was measured at 517nm using ELISA plate reader. Ascorbic acid was used as standard drug material. The results by DPPH radical scavenging assay is given in Table 3 and Figure 2.

Table-3: Data of DPPH radical scavenging activity of Pyrazoline derivatives (3a-j)

	Percentage inhibition										
Conc.	*Std*	*3a*	*3b*	*3c*	*3d*	*3e*	*3f*	*3g*	*3h*	*3i*	*3j*
10µg/ml	33.66	25.31	8.22	11.23	18.32	17.44	14.76	18.22	10.58	14.33	21.33
20µg/ml	42.01	32.88	19.77	24.55	29.11	30.11	29.31	28.24	21.11	25.11	32.11
30µg/ml	53.22	45.03	33.10	39.55	37.06	39.11	31.14	43.12	32.44	37.22	45.11
40µg/ml	62.88	54.18	45.69	47.11	46.01	50.12	44.03	55.02	45.03	48.06	55.02
50µg/ml	72.14	67.84	59.85	56.12	62.31	65.49	58.33	68.02	55.08	60.12	68.02
IC_{50}	*17.16*	*24.66*	*32.91*	*32.72*	*30.91*	*28.22*	*34.22*	*25.92*	*35.19*	*31.38*	*24.89*

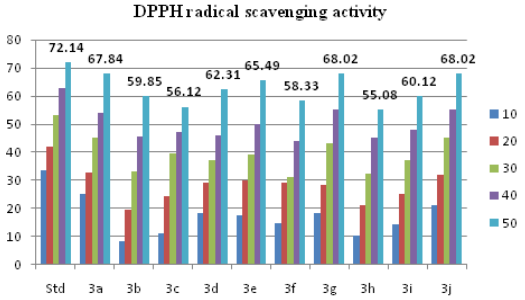


Fig-2: Data of DPPH radical scavenging activity

2.3.3. Nitric oxide radical scavenging activity[19]

Sodium nitroprusside (1ml of 10mM) is mixed with 1ml of test compounds at different concentration (10-50 µg/ml) in phosphate buffer (pH 7.4). The mixture is incubated at 25°C for 150 min. 1ml of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) is added to all the incubated solution. Absorbance is read at 546 nm using ELISA plate reader. Ascorbic acid was used as standard drug material. The results by nitric oxide radical scavenging assay are given in Table 4 and Figure 3.

Table-4: Data of nitric oxide radical scavenging activity of Pyrazoline derivatives (3a-j)

	Percentage inhibition										
Conc.	*Std*	*3a*	*3b*	*3c*	*3d*	*3e*	*3f*	*3g*	*3h*	*3i*	*3j*
10µg/ml	24.29	20.11	18.66	17.01	17.22	22.02	14.11	17.22	20.88	24.33	22.01
20µg/ml	38.12	31.02	28.33	28.09	33.18	35.11	26.11	28.46	31.02	32.11	32.02
30µg/ml	55.18	43.55	40.11	40.12	46.11	46.11	34.88	35.06	43.44	43.08	44.08
40µg/ml	67.97	55.26	52.08	52.18	58.22	58.07	44.05	41.02	55.08	55.66	57.69
50µg/ml	80.21	62.88	63.09	66.28	74.11	70.22	57.01	55.08	63.09	63.08	68.02
IC_{50}	*17.77*	*26.77*	*28.48*	*27.55*	*23.05*	*23.09*	*34.24*	*36.57*	*26.73*	*26.28*	*24.45*

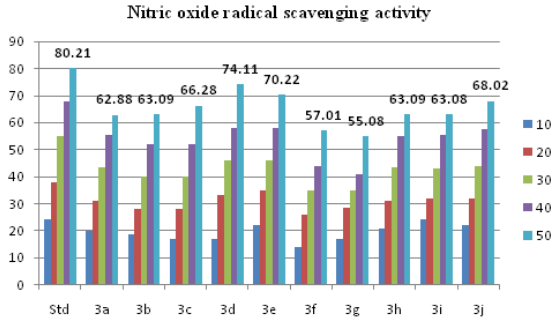


Fig-3: Data of nitric oxide radical scavenging activity

3. RESULTS AND DISCUSSION

3.1. Chemistry

The aim of the present study was to design and synthesize a new series of pyrazolines and to evaluate for various antioxidant activities. The key intermediate chalcones have been synthesized by Claisen–Schmidt condensation between appropriate aromatic aldehydes and ketones in presence of alcoholic basic medium. These chalcones undergoes selective cyclization with benzhydrazide in glacial acetic acid medium. Synthetic route leading to the formation of target compounds are summarized in **Scheme-01.** The physicochemical properties of pyrazoline derivatives are presented in table-1.

The final structures of these compounds were proven on the basis of melting points and spectroscopic data. The ^{1}H-NMR spectrum of compound **3c** showed δ 7.28-7.32, δ 7.37-7.39 corresponding to three protons of pyrazoline in H_A,H_B, H_X pattern. The signals for aromatic protons were observed in the region δ 7.71-8.08. The IR spectra of compound **3c** showed the appearance of C=O band at 1658 cm^{-1} and the other bands at 3095 and 1582cm^{-1} corresponds to the C-H and C=N respectively. The mass spectrum of compound **3c** showed molecular ion peak at M/z = 405.83(M+), which is in agreement with the molecular formula $C_{22}H_{16}N_3O_3Cl$. Mass spectrum of pyrazolines was in good agreement with their suggested structure.

3.2. Antioxidant activity

The evaluation of antioxidant activity results (figure-1, 2, 3) revealed that most of the tested compounds exhibited moderate to weak antioxidant activity (in all the three methods) when compared with the positive control ascorbic acid. Among them, compounds possessing electron withdrawing groups like nitro, chloro, bromo on the phenyl ring influences the antioxidant activity. The compounds **3e, 3f, 3g** showed moderate activity, when compared to the other tested compounds. The comparative result of the antioxidant activity indicated that compounds with bromo substituents are showed moderate activity than other derivatives.

4. CONCLUSION

A series of pyrazolines encompassing benzhydrazide derivatives were synthesized and evaluated for *In-Vitro* antioxidant activity. Results of the present study showed that, the tested pyrazolines derivatives exhibited moderate to weak activity in all the three methods, when compared to standard drug ascorbic acid. None of the tested compounds was found to be potent in comparison to standard. The study further requires structural modifications of these compounds, to make them valid lead compounds, which would possess better activity and may be quite useful for the pharmaceutical industries.

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