International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
February - March, 2013, Vol. 2, No.2, pp 349-354
ISSN: 2278-0238

Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW 4-BROMO-3, 5-DIARYL-1-PHENYL-2-PYRAZOLINE DERIVATIVES AS ANTIOXIDANT AND ANTI-INFLAMMATORY AGENTS

Anjan Kumar*, Sradhasini Rout, Dillip Kumar Sahoo & B.V.V. Ravi Kumar

Roland Institute of Pharmaceutical sciences, Khodasinghi, Berhampur, Ganjam, Odisha-760010

***Corresponding Author: Email**: akd009@rediffmail.com

(Received: August 17, 2012; **Accepted:** January 06, 2013**)**

ABSTRACT

A number of substituted 1, 3-diphenylprop-2-en-1-one were prepared by Claisen-Schmidt condensation of p-substituted acetophenone with o,m,p-substituted aryl aldehydes which undergoes bromination and subsequent cyclization with phenyl hydrazine to yield 4-bromo-3(substituted phenyl)-5(substituted phenyl)-1-phenyl-2-pyrazoline **(3a-l)**. The structures of compounds were confirmed by elemental analysis, IR, ^{1}HNMR and mass spectral data. The synthesized compounds **(3a-l)** were screened for anti-oxidant and anti-inflammatory activity. The free radical scavenging properties were screened by using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. The antioxidant activity of compound **3b** was found to be the strongest. The IC_{50} values of the synthesized compounds ranged between 8.87 and 81.07 (g mL-1). The anti-inflammatory activities were evaluated by using diclofenac sodium as a standard dug. All the compounds (20mg/kg po) possess significant anti-inflammatory activity, as reflected by their ability to provide protection (66-99%) against carragenan induced edema in rat paw. The anti-inflammatory activity of compound **3g** was found to be the highest. The safety of substituted bromo-pyrazolines is reflected by toxicity studies.

Keywords: 1, 3-diphenylprop-2-en-1-one, antioxidant activity, anti-inflammatory activity, IC_{50} values, elemental analysis, IR, ^{1}HNMR and mass spectral data.

INTRODUCTION

Pyrazolines are well known important nitrogen containing five member heterocyclic compounds. Pyrazoline systems are known to be biologically active and are important constituents of many pharmaceutical and agrochemical products[1]. 1,3,5-trisubstituted pyrazolines represent a very important class of biologically active agents and the focus of a significant amount of research interest. They possess a broad spectrum of biological activities *such as* antibacterial[2], antifungal[3], antitubular[4], antitumor[5], antidepressant[6], anticonvulsant[7], insecticidal[8], herbicidal[9], antiparasitic[10], antipyretic[11],antidiabetic[12], antiacetylcholinesterase[13], molluscicidal[14], antinociceptive[15], anti-inflammatory[16], analgesic[17], antiangiogenic[18] and antioxidant[19].Pyrazolines are used extensively as useful synthons in organic synthesis. Among various pyrazoline derivatives, 2-pyrazolines seem to be the most frequently studied pyrazoline type compounds. In view of immense activity of these compounds substituted derivatives of 3, 5- diaryl-1-phenyl-2-pyrazolines have been synthesized.

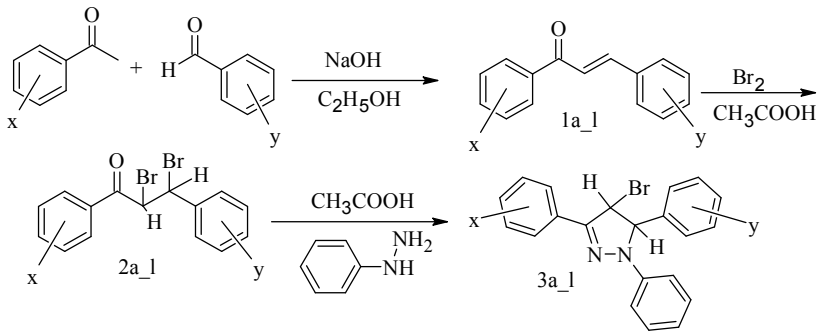
A chemical synthesis of these compounds involves the base catalyzed Claisen-Schmidit condensation of p-substituted acetophenones with o,p-substituted aldehydes to give chalcones,which undergo a subsequent cyclization reaction with phenylhydrazines affording 2-pyrazolines[20]. The newly

synthesize 2-pyrazolines were characterized on the basis of melting points, IR, Mass and ^{1}HNMR spectral data.

EXPERIMENTAL

Melting points were determined in open capillary tubes are uncorrected. IR spectra were recorded on SHIMADZU FTIR affinity series-I using KBr. ^{1}HNMR spectra were recorded on BRUKNER AVANCE II 400 NMR SPECTROMETER using $CDCl_3$ as solvent and TMS as an internal standard. Peak values are shown in δ ppm. The purity of the synthesized compounds was checked by TLC on silica gel plates.

Compound	x	y
a	H	H
b	*p*-Br	*p*-NO2
c	H	*p*-NO2
d	*p*-Cl	*m*-Cl
e	H	*p*-Br
f	*p*-Br	*m*-Cl
g	H	*m,p*- OCH3
h	H	*m,p*-Cl
i	H	*p*-F
J	*p*-Cl	*p*-CH3
k	H	*p*-Cl
l	*p*-Cl	*p*-Br



Scheme of Synthesis

General procedure for the preparation of substituted 4-bromo-3, 5-diaryl-1-phenyl-2-pyrazolines (3a-l).

To about 20-30 ml of acetic acid taken in a conical flask, equimolar amount of substituted chalcones were added followed by addition of equimoles of bromine drop wise under stirring for 4 to 5 hrs carefully. The mixture was kept in an ice bath for one hour and then the solid mass was filtered with suction through buchner funnel. To this equimoles of phenyl hydrazine was added and reflux for 5-6 hrs. The semisolid mixture was kept in deep freezer for an overnight. The solid mixture so formed was filtered out and neutralized by 1% NaOH solution. Recrystallization of the product had been done using a suitable solvent. The completion of the reaction was monitored by TLC.

Method:

Antioxidant activity

The antioxidants react with the stable free radical. 1,1-diphenyl-2-picrylhydrazyl (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration using the DPPH method illustrated by Blois et al[21]. The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in methanol was at 517 nm. 3.0 ml of sample solution (Compound + DMSO) samples of various concentrations of the synthesized compounds in methanol were separately added to a 1 ml solution of DPPH radical in methanol (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and allowed to stand for 30 min after which the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer (Shimadzu UV-1800). Inhibition of free radical DPPH as percentage (I %) was calculated as follows:

$I\% = 100 \times (A_{blank} - A_{sample})/A_{blank}$

where A_{blank} is the absorbance of the control (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. IC50 value (g ml^{-1}) is the effective concentration at which DPPH radicals

are scavenged by 50%. This was obtained by interpolation and using linear regression analysis.

Anti-inflammatory activity

Animals

Albino wistar rates (150-200g) of either sex were used. The animals housed under standard laboratory conditions maintained at 25±1°C and under 12/12 hour light/dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India limited.) and water *ad libitum*. The experimental protocols were approved by Institutional Animal Ethics Committee (Regn No: 926/ab/06/CPESEA).

Acute toxicity study

Acute toxicity of 4-bromo-3,5-diaryl-1-phenyl-2-pyrazolines (3a-l) derivatives were determined in albino wistar rats with the staircase method[22]. Each group of 6 animals was fasted for 24 hour prior to the administration of the test compounds. The test compounds, 3a-l were administered orally in doses up to 2000 mg/kg by suspending in 1 % C.M.C solution and were kept under observation for a period of 24 hour.

Carrageenan induced paw edema

The anti-infammatory activities of 3a-l were assessed *in vitro* for their percent inhibition of paw edema in carageenan model of inflammation in albino wistar rats using the method illustrated by Winter et al[23]. After 16 hours of fast the rats were divided into different groups of six each. Carrageenan (0.1 mL, 1%) was administered into the plantar surface of the right hind paw of the animals. The experimental groups, negative control group (1% CMC), and positive control group (20 mg/kg/po Diclofenac) were given either the control drug and test compounds orally, 1 hour prior to the administration of the carrageenan. Before injection of carrageenan , the average volume (V_o) of the right hind paw of each rat was calculated from 3 readings that did not deviate more than 3%. After injection of the phlogistic agent, the paw volume (V_t) was measured after 1st, 2nd,3rd,4th ,5th and 6th hours with the aid of a digital plethysmometer. The edema was expressed as an increase in the volume of paw and percentage inhibition of acute edema was obtained as follows:

% inhibition = [1-(ΔV experimental/ ΔV control)] x 100

Where,

$\Delta V = V_t - V_o$ = Mean paw volume

RESULT AND DISCUSSIONS

4-bromo-1,3,5-triphenyl-2-pyrazoline (3a) (Yield 90%, M.P. 183°C), **IR (KBr), CM^{-1} :** 3201 (CHBr-*str*), 3022, 3003, (aromatic C-H *str*), 2949 (aliphatic C-H *str*) 1608, 1489, 1448 (aromatic C⋯C), 1577 (C=N), 1398 (aliphatic C-H *ben*), 1319 (C-N), 680 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.72 (d, 1H, CH_a (Pyraz)), 7.03(d, 1H, CH_b (Pyraz)), 7.24-8.02 (m, Ar-H,15H)**Elemental analysis: obs % .(cal %.):** C 66.77(66.85)% H 4.41(4.54)% Br 21.78(21.18) % N 7.23(7.42).% Mass (m/z):374 (M^+-3).

4-bromo-5-(4-nitrophenyl)-3-(4-bromophenyl)-1-phenyl-2-pyrazoline (3b) (Yield 94%, M.P. 188°C), **IR (KBr), CM^{-1} :** 3124 (CHBr-*str*), 3032, 3012, (aromatic C-H *str*), 2935 (aliphatic C-H *str*) 1607, 1489, 1448 (aromatic C⋯C), 1598 (C=N), 1387 (aliphatic C-H *ben*), 1312 (C-N), 1521(*asym*) & 1335(*sym*)(C-NO_2), 576, 578 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.2917 (d, 1H, CH_a (Pyraz)), 6.8303(d, 1H, CH_b (Pyraz)), 7.5997-7.8027 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %):** C 50.24(50.33)% H 3.13(3.02)% Br 31.81(31.89)% N 8.29(8.38)% O 6.31(6.38)% . Mass (m/z): 504 (M^++3)

4-bromo-5-(4-nitrophenyl)-1,3-diphenyl-2-pyrazoline (3c) (Yield 87%, M.P. 188°C), **IR (KBr), CM^{-1} :** 3190 (CHBr-*str*), 3088, 3026, (aromatic C-H *str*), 2943 (aliphatic C-H *str*) 1606, , 1578 (aromatic C⋯C), 1340 (C=N), (aliphatic C-H *ben*), 1541(asym),1326 (sym,C-NO_2) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.8171 (d, 1H, CH_a (Pyraz)), 6.9928(d, 1H, CH_b (Pyraz)), 7.0712-7.9426 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 66.77(66.85) H 4.41(4.54) Br 21.78(21.18) N 7.23(7.42). Mass (m/z):- 423 (M^++1)

4-bromo-5-(3-chlorophenyl)-3-(4-chlorophenyl)-1-phenyl-2-pyrazoline (3d) (Yield 90 %, M.P. 188°C), **IR (KBr), CM^{-1} :** 3199 (CHBr-*str*), 3047, 3022, (aromatic C-H *str*), 3001 (aliphatic C-H *str*) 1608, 1490, 1446 (aromatic C⋯C), 1579 (C=N), 1400 (aliphatic C-H *ben*), 1323 (C-N), 815, 854 (C-Cl), 567 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.8643 (d, 1H, CH_a (Pyraz)), 6.9791(d, 1H, CH_b (Pyraz)), 7.0221-7.7163 (m, Ar-H,13H) **Elemental analysis: obs % .(cal %.):-** C 56.44(56.53)% H 3.29(3.39)% Br 17.83(17.91)% Cl 15.79(15.89)% N 6.19(6.28)% Mass (m/z):- 446(M^+)

4-bromo-5-(4-bromophenyl)-1,3-diphenyl-2-pyrazoline (3e) (Yield 85%, M.P. 190°C), IR (KBr), **CM^{-1} :** 3199 (CHBr-*str*), 3047, 3020, (aromatic C-H *str*), 2953 (aliphatic C-H *str*)

1608, 1490, 1446 (aromatic C⋯C), 1577 (C=N), 1400 (aliphatic C-H *ben*), 1323 (C-N), 581 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.0222 (d, 1H, CH_a (Pyraz)), 7.0171(d, 1H, CH_b (Pyraz)), 7.3089-8.1268 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 5.20(55.29)% H 3.45 (3.54)% Br 35.12(35.03)% N 6.06 (6.14)% Mass (m/z):- 458 (M^++1)

4-bromo-5-(3-chlorophenyl)-3-(4-bromophenyl)-1-phenyl-2-pyrazoline (3f) (Yield 89%, M.P. 186°C), **IR (KBr), CM^{-1} :** 3086, 3068, (aromatic C-H *str*), 2922 (aliphatic C-H *str*) 1605, 1473, 1456 (aromatic C⋯C), 1597 (C=N), 1393 (aliphatic C-H *ben*), 1317 (C-N), 682 (C-Cl),581 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.7427 (d, 1H, CH_a (Pyraz)), 6.9771(d, 1H, CH_b (Pyraz)), 7.0035-7.8243 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 51.33(51.41)% H 3.11(3.08)% Br 32.48(32.57)% C l7.15(7.23)% N 5.63(5.71)%. Mass (m/z):- 492 (M^++1)

4-bromo-5-(3,4-dimethoxyphenyl)-1,3-diphenyl-2-pyrazoline (3g) (Yield 89 %, M.P. 186°C), **IR (KBr), CM^{-1} :** 3201 (CHBr-*str*), 3022, 3003, (aromatic C-H *str*), 2949 (aliphatic C-H *str*) 1608, 1489, 1448 (aromatic C⋯C), 1577 (C=N), 1398 (aliphatic C-H *ben*), 1319 (C-N), 578 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 3.8760 (m,6H,2x(OCH_3)), 6.6746 (d, 1H, CH_a (Pyraz)), 6.8184(d, 1H, CH_b (Pyraz)), 6.9984-7.6077 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 66.77(66.85) H 4.41(4.54) Br 21.78(21.18) N 7.23(7.42) Mass (m/z):- 437 (M^+-1)

4-bromo 5-(3, 4-Dichloro phenyl)-1, 3-diphenyl-2-Pyrazoline (3h) (Yield 86%, M.P. 186°C), **IR (KBr), CM^{-1} :** 3201 (CHBr-*str*), 3022, 3001, (aromatic C-H *str*), 2951 (aliphatic C-H *str*) 1607, 1485, 1579 (C=N), 1397 (aliphatic C-H *ben*), 1331 (C-N), 787, 783 (C-Cl), 587 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.6791 (d, 1H, CH_a (Pyraz)), 6.8233 (d, 1H, CH_b (Pyraz)), 7.2987-7.9035 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 56.44(56.53)% H 3.29(3.39)% Br17.83(17.91)% Cl 15.79(15.89)% N 6.19(6.28)%. Mass (m/z):- 445 (M^+-1)

4-bromo-5-(4-fluorophenyl)-1,3-diphenyl-2-pyrazoline (3i) (Yield 92 %, M.P. 157° C), **IR (KBr), CM^{-1} :** 3188 (CHBr-*str*), 3064, 3041, (aromatic C-H *str*), 2987 (aliphatic C-H *str*) 1604, 1514, 1448 (aromatic C⋯C), 1371 (C=N), 1305 (aliphatic C-H *ben*), 1165 (C-F), 587 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.7912 (d, 1H, CH_a (Pyraz)), 6.8791(d, 1H, CH_b (Pyraz)), 7.2303-7.9028 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 55.27(55.29)% H 3.45(3.54)% Br 35.12(35.03)% N 6.21(6.14)%. Mass (m/z):- 397 (M^++2)

4-bromo-3-(4-chlorophenyl)-5-(4-methylphenyl)-2-pyrazoline (3j) (Yield 91%, M.P. 185°C), **IR (KBr), CM^{-1} :** 3187 (CHBr-*str*), 3062, 3026, (aromatic C-H *str*), 2927 (aliphatic C-H *str*) 1608, 1492, 1446 (aromatic C⋯C), 1597 (C=N), 1392 (aliphatic C-H *ben*), 1223 (C-N), 756(C-Cl), 577 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 2.1725 (3H, CH_a) 6.6746 (d, 1H, CH_a (Pyraz)), 6.8320(d, 1H, CH_b (Pyraz)), 7.2009-7.8921 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 66.77(66.85) H 4.41(4.54) Br 21.78(21.18) N 7.23(7.42).

4-bromo-5-(4-chlorophenyl)-1,3-diphenyl-2-pyrazoline (3k) (Yield 93%, M.P. 190°C), **IR (KBr), CM^{-1} :** 3128 (CHBr-*str*), 3053, 3047, (aromatic C-H *str*), 2958 (aliphatic C-H *str*) 1605, 1490, 1446 (aromatic C⋯C), 1595 (C=N), 1409 (aliphatic C-H *ben*), 1307 (C-N), 729, 579 (C-Br),**^{1}H NMR ($CDCl_3$), δ,ppm:** 6.3185 (d, 1H, CH_a (Pyraz)), 6.6906(d, 1H, CH_b (Pyraz)), 7.0027-8.0470 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 61.15(61.11)% H 4.07(4.15)% Br 19.27(19.36)% Cl 8.50(8.59)% N 6.71(6.79)%. Mass (m/z):- 415 (M^++4)

4-bromo-5-(4-bromophenyl)-3-(4-chlorophenyl)- 2-pyrazoline (3l) (Yield 87%, M.P. 189°C), **IR (KBr), CM^{-1} :** 3170 (CHBr-*str*), 3085, 3049, (aromatic C-H *str*), 2922 (aliphatic C-H *str*) 1608, 1489, 1597 (C=N), 1401 (aliphatic C-H *ben*), 1321 (C-N), 773, 572 (C-Br) **^{1}H NMR ($CDCl_3$), δ,ppm:** 6.7274(d, 1H, CH_a (Pyraz)), 6.7659(d, 1H, CH_b (Pyraz)), 7.0305-8.0282 (m, Ar-H,13H), **Elemental analysis: obs % .(cal %.):** C 51.33(51.41)% H 3.11(3.08)% Br 32.48(32.57)% C l7.15(7.23)% N 5.63(5.71)% Mass (m/z):- 491 (M^++1)

Antioxidant Activity:

The percentage inhibition of free radicals (degree of discoloration) indicates the free radical scavenging activities of the test drug was calculated against the control on the basis of experimental data obtained and represented in fig-1A&1B. It has been found that the standard antioxidant ascorbic acid reduce and decolorize 1,1-diphenyl-2-picrylhydrazyl by their hydrogen donating ability with an IC50 value of 15.43 µg ml. In the present study, the new brom-pyrazolines derivatives were able to

show significant scavenging activity against the stable free radical DPPH. The IC_{50} value ranges from 8.87 g ml−1 to 81.07 g ml−1. The anti-oxidant activity data showed that compound **(3b)** having 4-bromo and 4-nitro group in the phenyl ring at C-3 and C-5 respectively of pyrazoline nucleus posses highest activity followed by compound **(3l)** having 4-Cl group in the phenyl ring at C-3 and 4-Br group at C-5. It was interesting to note that halogen atom as well as elctron with drawing group attached in the phenyl ring at C-3 showed maximum antioxidant activity.

Anti-inflammatory activity:

The percent inhibition of edema was calculated against the control on the basis of experimental data obtained. The percent inhibition was calculated from 1st hour to 6th hour, and it was found to be showing significant anti-inflammatory activity after 4th hour. The compounds showed anti-inflammatory activity ranging from 55.77% to 94.27%, where as standard drug diclofenac showed 93.63% inhibition after 6th hour. The anti-inflammatory activity data showed that compound **(3g)** having 3,4-dimethoxy group in the phenyl ring at C-5 of pyrazoline nucleus posses highest

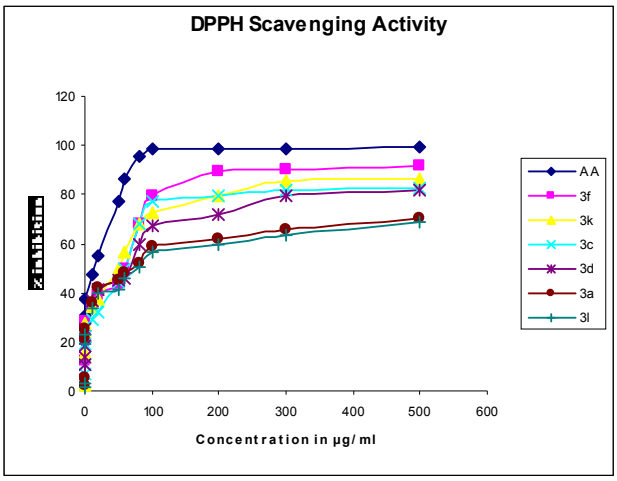


Fig -1A

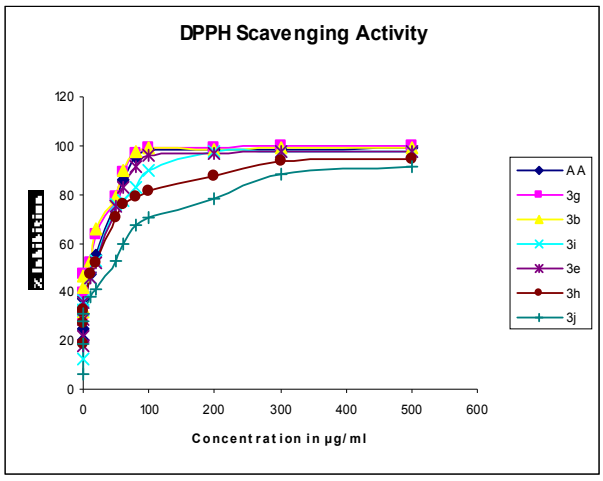


Fig-1B

Table-1: Anti-inflammatory activity

Compound	% inhibition ± SEM 1hr	2hr	3hr	4hr	5hr	6hr
Standard	23.32±2.3***	47.13±3.6***	64.35±3.55***	75.21±2.21***	81.17±1.77***	93.63±1.01***
3_a	11.07±4.35	18.03±1.89**	20.18±1.156*	31.47±2.223*	36.59±2.476**	55.77±1.768**
3_b	18.18±2.526*	33.46±3.426	59.57±1.644**	69.93±2.804**	77.01±0.660**	85.13±1.856***
3_c	15.01±3.585	26.32±1.345**	37.59±1.149*	61.92±1.282**	65.83±1.037*	76.25±1.483**
3_d	16.07±3.585	26.87±1.765*	30.95±1.159*	50.33±2.254**	57.8±2.007*	70.25±1.484**
3_e	14.98±2.483	26.18±2.673*	43.95±1.567*	63.39±1.286**	67.8±1.098*	77.15±2.786**
3_f	14.48±1.472	27.18±2.427*	29.95±1.157*	52.39±2.283**	61.81±2.237*	72.25±0.48**
3_g	20.09±1.622	41.98±2.526*	61.75±1.376*	76.93±2.804**	80.66±1.574**	94.27±0.360***
3_h	16.07±3.585	21.03±2.724**	28.18±1.159*	37.47±2.283*	36.59±3.476**	65.84±1.463**
3_i	16.09±2.584	31.28±2.724*	51.45±1.159*	65.89±2.283**	68.83±2.007*	79.27±1.484**
3_j	16.71±1.789	25.03±2.724**	28.18±1.159*	47.47±2.283*	56.59±3.476**	68.84±1.347**
3_k	15.07±3.585	27.48±1.897*	47.43±1.67*	64.91±1.631**	69.81±0.097*	78.15±1.457**
3_l	19.09±2.327	40.98±1.522*	57.75±1.325*	73.95±0.287**	78.66±1.43**	89.24±0.630***

activity (94.27%) followed by compound(**3b**) showing (87.24%) having 4-Cl group in the phenyl ring at C-3 and 4-Br group at C-5. All the bromo pyrazoline derivatives exhibited encouraging anti-inflammatory activity as shown in Table-1. The low toxicity of synthesized compounds was evident from the observation that there was no mortality in rat at doses up to 2000mg/kg.

STATISTICAL ANALYSIS

The test compounds and the standard drugs were administered at a Conc. of 20 mg/Kg po of body weight. Percentage inhibition are expressed in mean ± SEM (n= 6 for each compound). The data were analyzed by One-Way ANOVA, at 99 % confidence interval, followed by Dunnett's Multiple Comparison Test as post hoc analysis * $p < 0.05$ Significant Difference, ** $p < 0.01$ Significant, *** $p < 0.001$ Highly Significant compared to control & student t-test.

CONCLUSION

The plethora of research indicates a wide spectrum of pharmacological activities exhibited by 4-bromo-3,5-diaryl-1-phenyl-2-pyrazoline derivatives. The biological profiles of these new generations of bromo-pyrazolines would represent a fruitful matrix for further development of better medicinal agents.

ACKNOWLEDGEMENT

Author is thankful to Roland Institute of pharmaceutical Sciences, Berhampur for providing necessary facilities for the research work.

REFERENCES

1. Roelfvan S G,Arnold C & Wellnga K, *Journal of Agricultural Food Chem*, 84,(1979),406
2. Shivhare A, Kale A A, Berge D D & Phadkey S , *Indian Journal Pharma Sciences*, *47*, (1985), 155.
3. Das N B & Mitra A S, *Indian Journal Chemical Scociety* 168, (1978), 638.
4. Babu V H, Manna S K, Sineha, Srinivasan K K & Bhat G V, *Indian Journal Heterocyclic Chemistry*,13,(2004),253.
5. Taylor E C, Patel H and Kumar H, *Tetrahedron, 48*, (1992), 8089.
6. Ruhoglu O,Ozdemir Z,Calis U,Gumusel B & Biligin A A, *Arzneim Foresech*, *55*, (2005), 431.
7. Srivastava A V K & Kumar A, *Arzneim Foresech* ,52,(2002),787.
8. Kristopher S S & David M S, *Pestic Biochem and physiol*,81,(2005),136.
9. Basyouni W M, Haygag B and Edin S M, *Egypt Journal Pharmaceutical science*, 339,(1992),585.
10. Nugent R A, Murphy M, Schlachter S T, Dunn J C, Smith R J, Staite N D, Galinet L A, Shields S K, Saspar D G, Richard K A & Rohloff N A, *Journal of Medicinal chemistry*, 36,(1993),134.
11. Aggarwal R, Kumar V & Singh S P, *Indian Journal of chemistry*, 46B, (2007), 1332.
12. Soliman R, Faid-Allah H M & el-Sadany S K, *Journal of Pharma Sciences*, 76, (1987), 626.
13. Holan G, Virgona C T &Watson K G, *Bioorganic Medicinal chemistry, Letter* 6,(1996),77.
14. flora F B,Hanaa M H& Adel S G, *Bioorganic Medicinal chemistry*, Letter 14,(2006),3929.
15. Shafiee A,Bagheri M, Shekarchi M & Abdollahi M, *Journal of Pharma Pharmaceutical sciences*,6(2003),360.
16. R.A Nugent, M.Murphy, S.T.Schlacher, C.J.Dunn, R.J.smith, N.D.Staite, *Journal of Medicinal chemistry*,36 (1993),134.
17. P.H.Chanh, I.Sokan, J.M.Conquelet, J.D.Conquelet, A.Thibault and J.Cros, *Arzneimittel forschung*, 26 (1976), 2050.
18. J. Mojzis, L. Varinska, G. Mojzisova, L. Mirossay, *Pharmacol. Research 57* (2008) 259-265.
19. Tengli A K, Badami S, Kumar B R P, Dongre S K H, Ravi S and Ananda Kumar T, *Indian Journal of heterocyclic chemistry 15*, (2007),333-336.
20. Baker W & Butt V S, *Journal of Indian Chemical Society*, 39, (1949), 2142.
21. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958; 181:1199–200.
22. Ghosh M N, *Fundamentals of experimental Pharmacology,* (scientific Book agency, Calcutta, India), 1981,153.
23. Winter C E, Risley E A & Nuss G W, *Proc Soc Bio Med,* 111, (1962), 544.

