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Synthesis, characterization and anti-oxidant, anti-inflammatory activities of some novel chalcones and 1,3,5-trisubstituted pyrazolines

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ABSTRACT

A systematic investigation of pyrazoline class of heterocyclic lead revealed that pyrazoline containing pharmacoactive agents play important role in medicinal chemistry. Some new1-[3-(2,4-dichloro-5-fluorophenyl)-5-(aryl)-4,5-dihydro-1H-pyrazol-1-yl] ethanone derivatives were synthesized by reacting chalcones derivatives with hydrazine hydrate in glacial acetic acid. The structural elucidation of compounds was confirmed by ^1H NMR and IR analysis. The synthesized compounds were screened for the antioxidant activity by DPPH method and Hydrogen peroxide method. Also the synthesized compounds were screened for the anti-inflammatory activity by egg albumin denaturation method and Heat induced hemolytic method.

Keywords: 1, 3, 5-Triaryl-2-pyrazolines, Antioxidant activity, Anti- inflammatory, Chalcones, 2, 4-dichloro 5 – fluoro acetophenone.

INTRODUCTION

The prevalence of pyrazolines cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic a lead compound. Pyrazolines possess a broad spectrum of biological activities such as analgesic, anti-inflammatory, antimicrobial, tranquilizing, muscle relaxant, psycho-analeptic, anticonvulsant, antihypertensive and antidepressant activities[1].In view of such biological reports, the present work is aimed to design and develop the synthesis of some new potential -[3-(2,4-dichloro-5-fluorophenyl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone derivatives were synthesized by reacting chalcones with hydrazine hydrate in glacial acetic acid. Due to prolonged refluxing with glacial acetic acid, acetylation of one of the nitrogen atom of the pyrazoline nucleus has occurred and explores the evaluation of their in-vitro antioxidant activity & anti-inflammatory activity.

The biological properties of fluorine or multi fluorine containing compounds have been recently investigated. Owing to their unique properties, such as high thermal stability and lipophilicity, fluoro-organic compounds have been frequently used as biorelated materials, medicines and agrochemicals [2].

MATERIALS AND METHODS

The chemicals and solvents used for the experimental work were procured from E. Merck, India, and S.D Fine Chemicals, India. Silica gel GF_{254} used for analytical chromatography (TLC) was obtained from E. Merck. Melting points were determined in an open glass capillary using a Kjeldahl flask containing paraffin and are uncorrected. The proton magnetic resonance spectra (^1H NMR) were recorded on a BRUKER-AMX 400 MHz (Bruker, Germany) in dimethylsulfoxide-d6/$CDCl_3$ using tetramethylsilane as an external standard. Chemical shifts (δ) are expressed n ppm. The infrared spectra of compounds were recorded in KBr on Bruker infrared spectrophotometer. Purity of the compounds was checked by TLC using silica gel G.ELICO-SL 164 double beam UV-Visible

Spectrophotometer was used for measuring absorbance of the samples in screening for antioxidant activity and *invitro* anti-inflammatory activity of the synthesized compounds.

STEP: 1- Procedure for Synthesis of Chalcone derivatives from 2, 4-dichloro 5-fluoro acetophenone:
0.005mol (1g) 2, 4-dichloro5-fluoro acetophenone of and equimolar quantity of various substituted aromatic aldehydes are taken in round bottom flask containing 20ml of ethanol; and then mixed with 1 ml of 40% KOH by maintaining the temperature below 20^0C with continuous stirring on magnetic stirrer and then the reaction was monitored by TLC; after completion of reaction (48hrs),the reaction mixture was filtered and then washed with ice cold water, dried and then recrystallized with ethanol.

STEP: 2-Preparation of1, 3, 5-trisubstituted Pyrazolines
0.002 mol (658 mg) of chalcone derivatives (compound1a to 1d) and 0.008 mol (3.12ml) hydrazine hydrate were taken in a Round Bottom Flask containing glacial acetic acid as solvent and Refluxed for 70-80 hrs at 140^0C by monitoring the reaction with TLC, and then poured into crushed ice with continuous stirring; filtered the precipitate and dried. All the compounds (2a-2d) were purified by washing with petroleum ether and ethyl acetate (8:2).

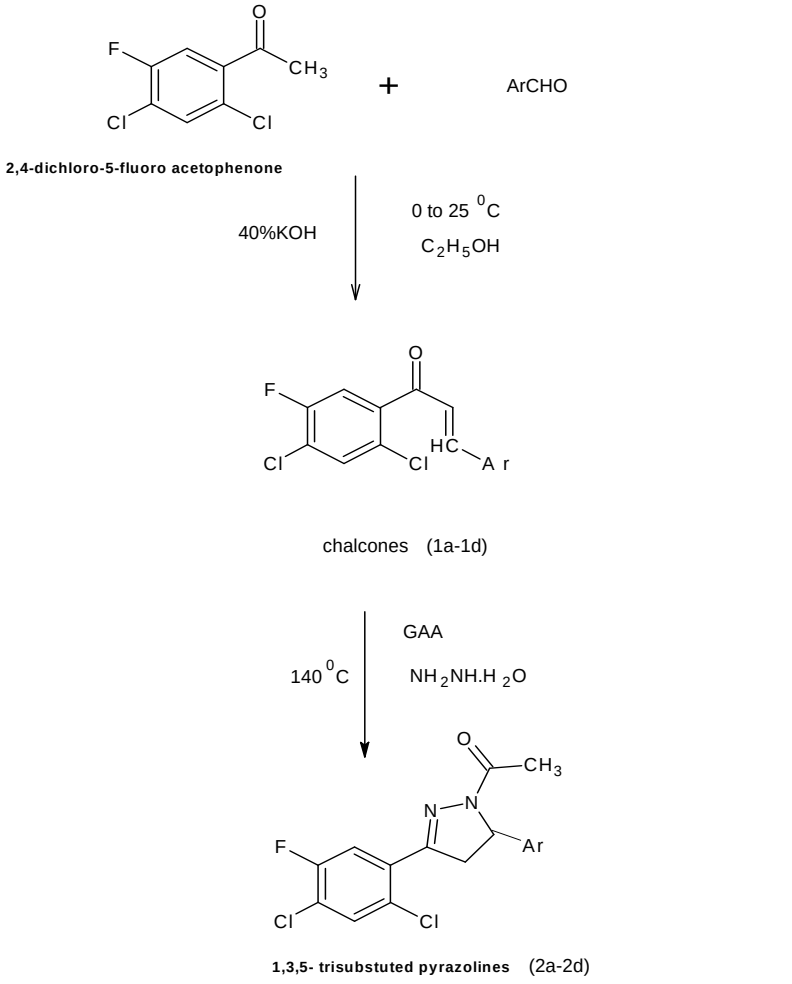
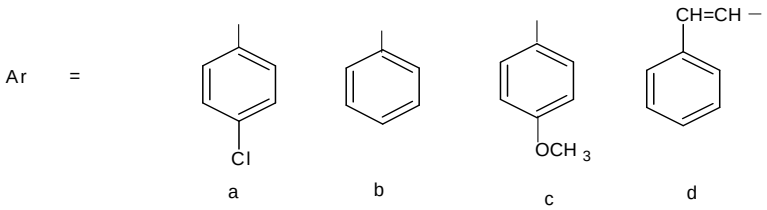


Figure 1: Synthetic Scheme



ANTI-OXIDANT ACTIVITY

1. DPPH Method [3]**:**

To 3 ml of various concentrations of test/ standard solution, 1 ml solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added. Simultaneously blank samples were prepared for each concentration without addition of 0.1mM of DPPH solution and equal amount of methanol was added to each blank sample. 3 ml of methanol and 1 ml of 0.1 mM DPPH was added and used as control. Ascorbic acid was used as standard for comparison. After incubation for 20 minutes in dark, absorbance was recorded at 517 nm. % scavenging was calculated using the formula,

$$\% \textbf{ Scavenging } = (\mathbf{A°} - \mathbf{A})/\mathbf{A°} \times \mathbf{100}$$

Where
A^O=Absorbance of the control
A=Absorbance of the test or standard

Graph was plotted by taking concentration (µg/ml) on x-axis and percentage scavenged/ inhibition on y-axis.

2. Hydrogen Peroxide method [4]**:**

The ability of the synthesized compounds to scavenge H_2O_2 was determined by the following procedure. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). The concentration of $H_2O_2$was determined by absorption at 230 nm using a UV Visible spectrophotometer. Test solutions were added to a H_2O_2solution (0.6 ml 40mM). The absorbance of H_2O_2 at 230 nm was determined after 10 minutes against blank solution containing phosphate buffer and test compound without H_2O_2. Control solution was prepared by taking a solution of H_2O_2 in phosphate buffer (pH 7.4) and its absorbance was measured. The percentage of H_2O_2 scavenging by the test and the standard was calculated using the following formula.

$$\% \boldsymbol{Scavenged} = [(\boldsymbol{A} - \boldsymbol{A1})/\boldsymbol{A}]\mathbf{100}$$

Where
A=absorbance of the control,
A1= absorbance of the test /standard

Procedure

To 1.4 ml of the test or standard solution, 0.6ml of the 40mM H_2O_2 is added and allowed to stand for 10 minutes. The absorbance of the above solution is measured at 230nm. Ascorbic acid was taken as standard. Graph was plotted by taking concentration (µg/ml) on x-axis and percentage scavenged/ inhibition on y-axis.

***In Vitro* Anti-Inflammatory Activity**

1.Inhibition of Egg Albumin Denaturation Method [5,6]**:**

To 2ml of various concentrations of test or standard solutions 2.8ml of normal saline (pH=7.4) and 0.2ml of 1% egg albumin solution was added. Simultaneously blank samples were prepared for each concentration without addition of 1% egg albumin solution and equal volume of normal saline (pH7.4) was added to each blank sample. To 4.8ml of normal saline (pH 7.4), 0.2ml of 1% egg albumin solution was added and used as control. The test/standard samples were incubated for 15 min at 70^0C.Then the tubes were cooled under running tap water and then absorbance was recorded at 660nm. % inhibition of denaturation of egg albumin was calculated using the formula

$$\% \boldsymbol{Inhibition} = [(\boldsymbol{A} - \boldsymbol{A1})/\boldsymbol{A}]\mathbf{100}$$

Where
A=absorbance of the control,
A1= absorbance of the test /standard

2.Heat induced hemolytic method [7]:

To 1ml of various concentrations of test or standard solutions, 1ml of 1% RBC's suspension was added. Simultaneously blank samples were prepared for each concentration without addition of 1% RBC's solution and equal amount of normal saline was added to each blank sample. Equal amount of 1%RBC's solution and normal saline was added and was used as control.

All these samples were taken into centrifuge tubes and incubated in water bath at 56^0C for 30 min. The tubes were cooled under running tap water and then centrifuged at 2500 rpm for 15 min and absorbance of supernatant was taken at 560 nm.% inhibition was calculated using formula

$$\% \boldsymbol{Inhibition} = [(\boldsymbol{A} - \boldsymbol{A1})/\boldsymbol{A}]\mathbf{100}$$

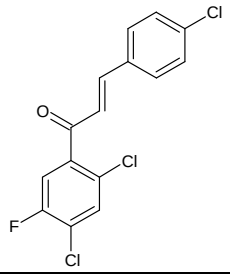
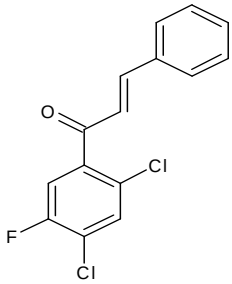
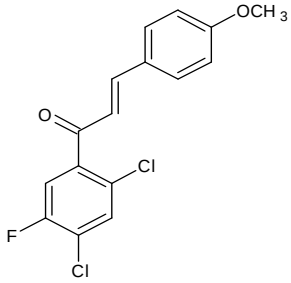
Where
A=absorbance of the control,
A1= absorbance of the test /standard.

IC_{50} Values:
IC_{50} was calculated using Graphpad prism software

Statistical analysis:
All the data was expressed as mean ± SEM. Statistical significance was tested by using one way ANOVA followed by the Tukey's test using computer based fitting program (Graph pad prism 5)

RESULTS AND DISCUSSION

Table 1: List of synthesized compounds

S. No.	Compound Code	Structure	IUPAC name
1.	1a		3-(4-chlorophenyl)-1-(2,4-dichloro-5-fluorophenyl)prop-2-en-1-one
2.	1b		1-(2,4-dichloro-5-fluorophenyl)-3-phenylprop-2-en-1-one
3.	1c		1-(2,4-dichloro-5-fluorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one

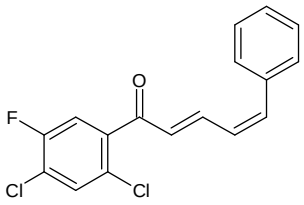
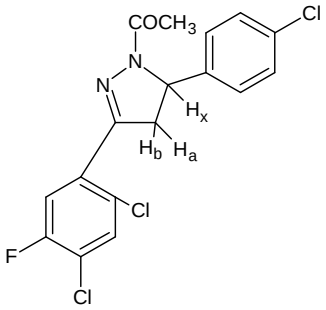
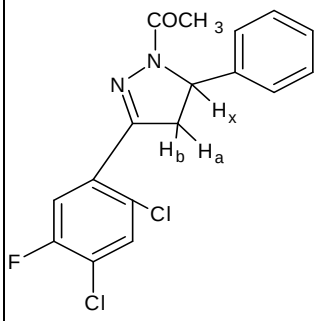
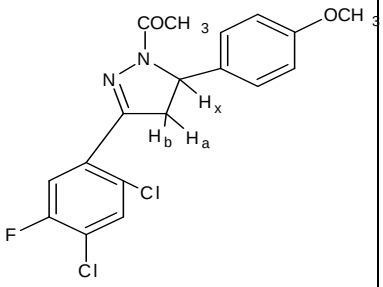
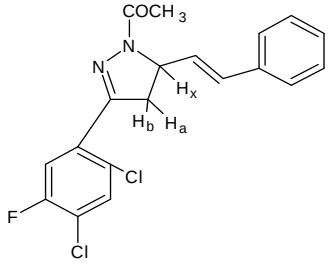
4.	1d		1-(2,4-dichloro-5-fluorophenyl)-5-phenylpenta-2,4-dien-1-one
5.	2a		1-[5-(4-chlorophenyl)-3-(2,4-dichloro-5-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone
6.	2b		1-[3-(2,4-dichloro-5-fluorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone
7.	2c		1-[3-(2,4-dichloro-5-fluorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone
8.	2d		1-{3-(2,4-dichloro-5-fluorophenyl)-5-[(*E*)-2-phenylethenyl]-4,5-dihydro-1*H*-pyrazol-1-yl}ethanone

Table 2: Physico-chemical characterization data of synthesized compounds

S. No.	Code	Molecular formula	Molecular weight	Melting point (^{0}c)	% yield (%)	R_fValue	λ_{max} (nm)	Colour
1.	1a	$C_{15}H_8Cl_3FO$	329.5	97-102	63.2	0.66*	450	White
2.	1b	$C_{15}H_9Cl_2FO$	295	200-206	86	0.54*	570	White
3.	1c	$C_{16}H_{11}Cl_2FO$	325	140-147	62	0.63**	580	White
4.	1d	$C_{17}H_{11}Cl_2FO$	320	75-80	96	0.56*	510	Brown
5.	2a	$C_{17}H_{12}Cl_2N_2FO$	385	103-105	68	0.62*	590	White
6.	2b	$C_{17}H_{13}Cl_2NFO$	350	260-264	60	0.73*	630	White
7.	2c	$C_{18}H_{15}Cl_2N_2O_2F$	381	246-252	65	0.61**	650	Greenish yellow
8.	2d	$C_{17}H_{12}Cl_2N_3O_2F$	376	110-116	83.3	0.65*	560	Brown

**n-Hexane: Ethyl acetate (8:2)* ***n-Hexane: Ethyl acetate (6:4)*

Compound 1a:3-(4-chlorophenyl)-1-(2,4-dichloro-5-fluorophenyl) prop-2-en-1-one
^{1}H NMR Values: ^{1}H NMR ($CDCl_3$): δ 7.543(1H, d, C-a, J=6.4HZ), 7.313(1H, d, C-b, J=6.4HZ), 7.292(2H, d, C-2, C-6, Ar-H J=8.4HZ), 7.100(2H, d, C-3, C-5Ar, H J=8.4HZ), 7.527(1H, d, C-3', ArH, J=7.4HZ), 7.498(1H, d, C-6', Ar-H, J=7.4HZ). **IR (KBR):** 1604.57Cm^{-1}(C=C, Str), 1668.57Cm^{-1}(C=O, Str), 778.89Cm^{-1}(Ar-Cl), 1250.52Cm^{-1}(Ar-F), 2919.70Cm^{-1}(C-H, Ar-Str), 1460.70Cm^{-1}(C=C, Ar-Str), 881.58Cm^{-1}(C-H, Ar-Bending).

Compound 1b:1-(2,4-dichloro-5-fluorophenyl)-3-phenylprop-2-en-1-one
^{1}HNMRValues: ^{1}HNMR($CDCl_3$):δ 6.402(1H, d, C-b, J=6.4HZ), 6.859(1H, d, C-a, J=6.4HZ), 7.756 & 7.276 (2H, d, C-2, C-6, Ar-H, J=8.8HZ), 7.219-7.182 (2H, d, C-6',C-4, Ar-H, J=7.6HZ), 7.143-7.053(3H, d, C-3, C-5, C-3', J= 7.6HZ). **IR (KBR):**1598.01Cm^{-1}(C=C, Str), 1663.79Cm^{-1}(C=O, Str), 792.27Cm^{-1}(Ar-Cl), 1253.03Cm^{-1}(Ar-F), 3457.20Cm^{-1}(C-H, Ar- Str), 1463.48Cm^{-1}(C=C, Ar- Str), 883.30Cm^{-1}(C-H, Ar-Bending).

Compound 1c:1-(2,4-dichloro-5-fluorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one
^{1}H NMR Values: ^{1}HNMR($CDCl_3$): δ 7.958(1H, d, C-b, J=6.4), 7.678(1H, d, C-a, J=6.4), 7.176(2H, d, C-3', C-6', Ar-H, J=8.4), 7.065-6.849(2H, d, C-2, C-6,Ar-H, J=8.4), 6.738-6.572(2H, d, C-3, C-5, Ar-H, J=8.4), 3.491(3H, S, -Co-CH_3). **IR (KBR):**1609.11Cm^{-1}(C=C, Str), 1685.80Cm^{-1}(C=O, Str), 730.91Cm^{-1}(Ar- Cl), 1247.12Cm^{-1}(Ar-F), 3528.99Cm^{-1}(C-H, Ar- Str), 1465.33Cm^{-1}(C=C, Ar-Str), 882.56Cm^{-1}(C-H, Ar-Bending), 1179.11Cm^{-1}(C-O-C).

Compound 1d:1-(2,4-dichloro-5-fluorophenyl)-5-phenylpenta-2, 4-dien-1-one
^{1}H NMR Values: ^{1}HNMR($CDCl_3$):δ 7.259-7.218 (5H, m, Ar-H), 6.994 (2H, d, C-b, C-d, J=18HZ), 7.120 & 7.492 (2H, d, C-a, C-c, J=14.8HZ), 7.357 (2H, d, C-3', C-6', Ar-H, J=7.4HZ). **IR (KBr):** 1597.13Cm^{-1}(C=C, Str), 1660.18Cm^{-1}(C=O, Str), 728.96Cm^{-1} (Ar-Cl), 1256.21Cm^{-1} (Ar-F), 3425.46Cm^{-1} (C-H, Ar- Str), 1597.13Cm^{-1} (C=C, Ar-Str), 884.54Cm^{-1}(C-H, Ar-Bending).

Compound 2a:1-[5-(4-chlorophenyl)-3-(2,4-dichloro-5-fluorophenyl)-4, 5-dihydro-1*H*-pyrazol-1-yl] ethanone
^{1}H NMR Values: ^{1}H NMR ($CDCl_3$): δ 2.394(3H, s, CO -CH_3,), 3.4 (1H, dd, Ha), 3.970 (1H, dd, Hb), 5.5 (1H, dd, Hx), 7.2(2H, d, C-2, C-6, Ar-H, J=6.4HZ), 7.16(2H, d, C-3, C-5, Ar-H, J=8.4HZ), 7.6(1H, d, C-6', Ar-H, J=8.4HZ), 7.4(1H, d, C-3', Ar-H, J=8.4HZ), (J_{ab}=17.2, J_{ax}=8.4, J_{bx}=12.4HZ). **IR (KBr):** 1440.23Cm^{-1}(C=O, Str)**,** 730.46Cm^{-1}(Ar-Cl), 1251.92Cm^{-1}(Ar-F), 2924.65Cm^{-1}(C-H, Ar-Str)**,** 1382.19Cm^{-1}(C=C, Ar-Str)**,** 825.14Cm^{-1}(C-H, Ar-Bending)**,** 1667.11Cm^{-1}(C=N)**,** 1093.93Cm^{-1}(C_5-N_1, Str-pyrazoline).

Compound 2b:1-[3-(2,4-dichloro-5-fluorophenyl)-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone
^{1}H NMR Values: ^{1}H NMR ($CDCl_3$): δ 3.943(3H, s, CO -CH_3), 1.999 (1H, dd, Ha), 3.219 (1H, dd, Hb), 5.134 (1H, dd, Hx), 7.2 (2H, d, C-2,C-6, Ar-H, J=8.4 HZ), 7.141 (3H, d, C-3, C-5, C-6', Ar-H, J=8.4HZ), 6.858(1H, d, C-4, Ar-H J=8.8HZ), 7.4(1H, d, C-3', Ar-H J=8.4), (J_{ab}=14.8, J_{ax}=6, J_{bx}=12HZ). **IR (KBr):** 1467.09Cm^{-1}(C=O, Str), 728.78Cm^{-1}(Ar-Cl), 1256.72Cm^{-1}(Ar-F), 3419.40Cm^{-1}(C-H, Ar-Str), 1546.42Cm^{-1}(C=C, Ar-Str), 886.11Cm^{-1}(C-H, Ar-Bending), 1695.91Cm^{-1}(C=N),1097.08Cm^{-1}(C_5-N_1,Str-pyrazoline).

Compound 2c:1-[3-(2,4-dichloro-5-fluorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethanone
^{1}H NMR Values:^{1}H NMR ($CDCl_3$): δ 1.693(3H, s, CO -CH_3), 2.053(3H, s, OCH_3), 2.236 (1H, dd, Ha), 2.212 (1H, dd, Hb), 5.074 (1H, dd, Hx), 7.158-7.054 (2H, d, C-2, C-6, Ar-H, J=8.4HZ), 6.874-6.729(2H, d, C-3, C-5, Ar-H, J=8.4HZ), 7.262(1H, d, C-6', Ar-H, J=6.4HZ), 7.74(1H, d, C-3', Ar-H, J=6.4HZ), (J_{ab}=15, J_{ax}=6.4, J_{bx}=12HZ). **IR (KBr):**1440.23Cm^{-1}(C=O, Str), 730.46Cm^{-1}(Ar-Cl), 1251.92Cm^{-1}(Ar-F), 2924.65Cm^{-1}(C-H, Ar-Str), 1382.19Cm^{-1}(C=C, Ar-Str), 825.14Cm^{-1}(C-H, Ar-Bending), 1667.11Cm^{-1}(C=N), 1093.93Cm^{-1}(C_5-N_1, Str-pyrazoline), 1197.39Cm^{-1}(C-O-C).

Compound 2d:1-{3-(2, 4-dichloro-5-fluorophenyl)-5-[2-phenylethenyl]-4,5-dihydro-1*H*-pyrazol-1-yl} ethanone

^{1}H NMR Values: ^{1}H NMR ($CDCl_3$): δ 3.484(3H, s, CO -CH_3,), 2.392 (1H, dd, Ha), 2.049 (1H, dd, Hb), 5.289 (1H, dd, Hx), 7.508 (2H, d, C-3', C-6', Ar-H, J=8.4HZ), 7.350(5H, m, Ar-H), 6.616(2H, d, CH=CH, J=14HZ). **IR (KBr):**1464.88Cm^{-1}(C=O, Str), 728.74Cm^{-1}(Ar-Cl), 1255.12Cm^{-1}(Ar-F), 2928.78Cm^{-1}(C-H, Ar-Str), 1379.04Cm^{-1}(C=C, Ar-Str), 836Cm^{-1}(C-H, Ar-Bending), 1666.92Cm^{-1}(C=N), 1095.38Cm^{-1}(C_5-N_1, Str-pyrazoline).

Table 3: DPPH scavenging activity

Conc. (µg/ml)	% Scavenged ± SEM								
	Ascorbic Acid	1a	1b	1c	1d	2a	2b	2c	2d
20	88.2± 0.219	36.21± 0.418	15.7± 0.336	50.6± 0.329	39.1 ± 0.315	28.7± 0.273	27.6 ± 0.326	50.9± 0.186	37.2 ± 0.254
40	89.1± 0.193	41.3± 0.212	26.6± 0.127	52.9± 0.136	43.5± 0.325	40.4± 0.459	34.5± 0.323	58.3± 0.278	42.7± 0.289
60	89.8± 0.148	44.3± 0.247	22.2± 0.136	58.4± 0.645	47.5± 0.453	45.6± 0.156	37.5± 0.452	60.2± 0.789	46.5± 0.410
80	90.2± 0.298	46.4± 0.145	24.5± 0.345	63.8± 0.194	49.6± 0.456	46.7± 0.245	39.6± 0.365	64.3± 0.452	48.5± 0.542
100	91.2± 0.145	48.5± 0.178	27.7± 0.178	69.8± 0.452	50.6± 0.147	49.6± 0.423	40.4± 0.752	69.1± 0.124	50.7± 0.275
120	92.5± 0.127	50.2± 0.425	31.2± 0.782	74.2± 0.214	51.7± 0.324	55.4± 0.782	43.6± 0.124	70.5± 0.145	52.5± 0.235
IC_{50}(µg/ml)	10.14	128.56	220.47	36.45	80.48	120.78	150.15	34.76	140.12

**All the values are average of three readings, Mean±SEM*
SEM = Standard Error Mean
IC_{50}= Half maximal inhibitory concentration

Table 4: H_2O_2 Scavenging activity

Conc. (µg/ml)	% Scavenged ± SEM*								
	Ascorbic Acid	1a	1b	1c	1d	2a	2b	2c	2d
20	88.2± 0.219	36.2± 0.654	34.4± 0.698	60.6± 0.241	32.1± 0.354	37.9± 0.542	19.3± 0.752	54.3± 0.354	31.2± 0.564
40	89.1± 0.193	31.6± 0.543	38.2± 0.542	63.9± 0.123	34.5± 0.345	40.4± 0.654	23.5± 0.456	58.3± 0.524	35.7± 0.345
60	92.8± 0.148	44.2± 0.124	44.2± 0.243	65.4± 0.657	37.5± 0.785	45.6± 0.546	26.7± 0.345	60.2± 0.345	37.5± 0.123
80	94.2± 0.298	46.5± 0.543	46.8± 0.654	66.8± 0.765	40.6± 0.675	46.7± 0.645	28.6± 0.546	64.3± 0.567	40.5± 0.134
100	96.4± 0.145	48.3± 0.456	48.7± 0.654	67.8± 0.678	45.7± 0.345	49.6± 0.789	30.4± 0.543	69.4± 0.578	46.7± 0.786
120	98.5± 0.127	50.4± 0.123	49.4± 0.234	69.5± 0.345	48.5± 0.543	55.4± 0.564	32.6± 0.678	70.4± 0.567	49.5± 0.134
IC_{50}(µg/ml)	10.14	80.43	124.67	20.34	143.56	106.45	164.56	50.87	140.34

**All the values are average of three readings, Mean±SEM*
SEM = Standard Error Mean
IC_{50}= Half maximal inhibitory concentration

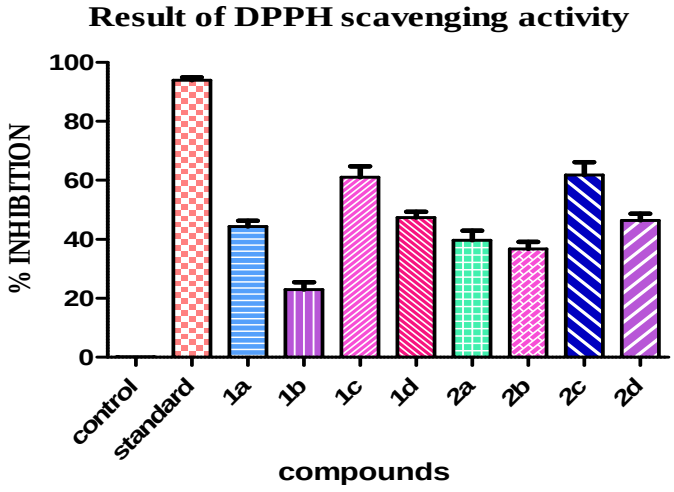


Figure 2: DPPH Scavenging activity

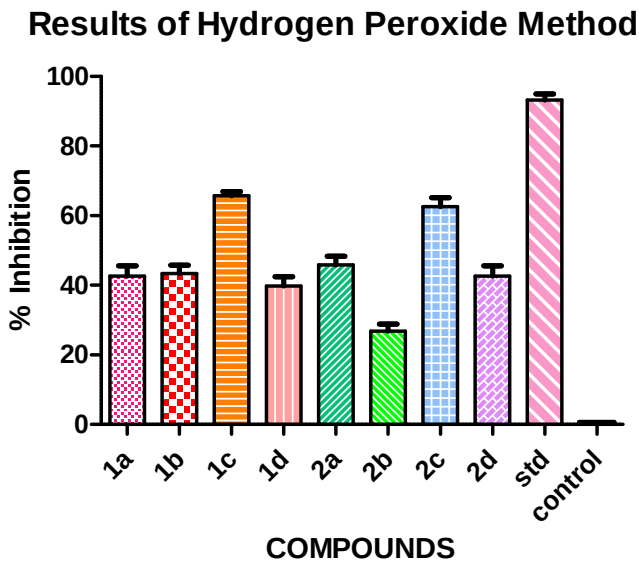


Figure 3: H_2O_2 Scavenging activity

Table 5: Egg Albumin Denaturation Method

Conc. (µg/ml)	% Inhibition ± SEM*								
	Diclofenac sodium	1a	1b	1c	1d	2a	2b	2c	2d
20	75.3± 0.364	31.8± 0.561	34.2± 0.543	33.1± 0.245	33.9± 0.572	35.7± 0.642	19.3± 0.023	54.9± 0.324	31.7± 0.531
40	81.4± 0.234	40.0± 0.195	38.0± 0.542	36.1± 0.231	35.0± 0.632	38.6± 0.753	23.2± 0.521	58.3± 0.423	36.8± 0.324
60	86.0± 0.321	42.9± 0.215	44.9± 0.213	43.4± 0.231	37.8± 0.345	46.9± 0.245	26.5± 0.632	60.1± 0.634	38.7± 0.125
80	94.0± 0.423	46.7± 0.632	48.0± 0.102	50.9± 0.094	40.8± 0.324	49.7± 0.235	28.7± 0.421	64.30± 0.354	43.9± 0.521
100	96.4± 0.624	51.6± 0.532	50.5± 0.425	57.8± 0.129	45.4± 0.634	60.3± 0.187	30.7± 0.452	69.5± 0.634	45.5± 0.524
120	98.0± 0.245	59.4± 0.942	58.5± 0.542	65.3± 0.674	49.5± 0.567	46.5± 0.954	32.5± 0.364	70.6± 0.854	52.9± 0.482
IC_{50} (µg/ml)	7.873	86.0	85.20	69.2	165.0	74.25	280.0	15.0	130.0

**All the values are average of three readings, Mean±SEM*
SEM = Standard Error Mean
IC_{50}= Half maximal inhibitory concentration

Table 6: Heat Induced Hemolytic method

Conc. (µg/ml)	% Inhibition ± SEM*								
	Diclofenac sodium	1a	1b	1c	1d	2a	2b	2c	2d
20	55.8± 0.354	37.6± 0.642	44.3± 0.951	38.0± 0.064	32.6± 0.275	35.0± 0.634	43.3± 0.134	49.7± 0.524	31.3± 0.357
40	64.4± 0.362	45.2± 0.263	48.4± 0.367	42.2± 0.625	34.7± 0.268	38.4± 0.106	46.2± 0.361	58.8± 0.265	35.2± 0.026
60	70.5± 0.625	48.6± 0.351	44.2.6± 0.236	48.9± 0.195	37.0± 0.534	46.3± 0.524	49.3± 0.634	60.5± 0.136	37.6± 0.246
80	74.3± 0.624	58.8± 0.365	50.9± 0.135	52.4± 0.654	40.0± 0.354	49.8± 0.064	53.6± 0.306	63.0± 0.629	40.0± 0.135
100	74.5± 0.136	58.9± 0.061	52.2± 0.634	57.7± 0.265	47.3± 0.634	54.1± 0.467	57.1± 0.136	68.1± 0.753	46.8± 0.951
120	76.8± 0.314	59.2± 0.206	54.2± 0.241	59.5± 0.065	49.5± 0.136	57.3± 0.524	58.6± 0.634	70.0± 0.136	49.3± 0.534
IC_{50} (µg/ml)	7.873	58.0	74.4	62.89	151.0	78.18	50.93	22.36	154.7

**All the values are average of three readings, Mean±SEM*
SEM = Standard Error Mean
IC_{50}= Half maximal inhibitory concentration

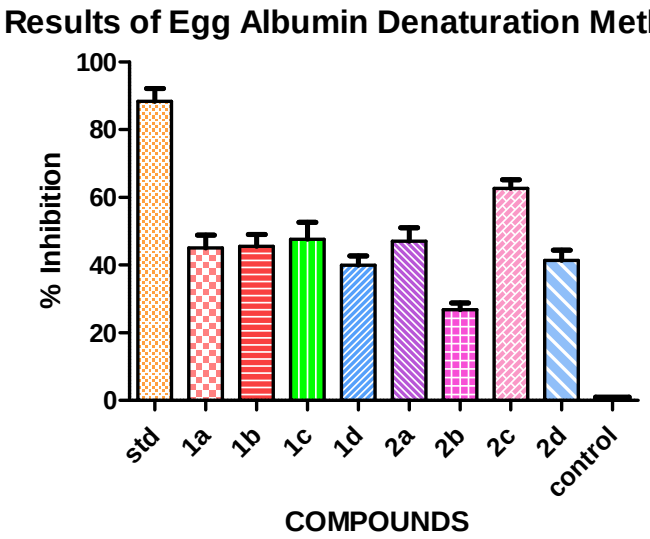


Figure 4: Egg albumin Denaturation activity

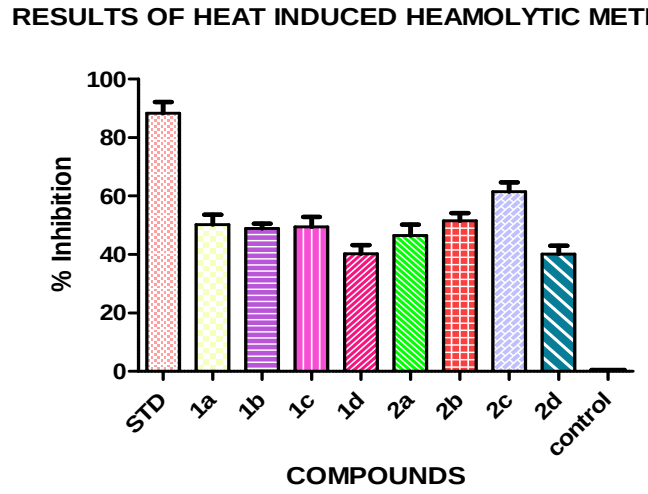


Figure 5: Heat Induced Hemolytic method

CONCLUSION

All the synthesized compounds are characterized by spectral studies. The biological activity data was analyzed by one way ANOVA using Graph pad prism-5 software. The scavenging activity of the compounds was compared with the control and the significance factor “p” was less than 0.001 for all the compounds. The compound having single methoxy group (2C,1C) as a substituent showed the highest scavenging activity suggesting that electron donating groups may aid the scavenging activity (Table 3 &4). The compound with unsubstitution has the least scavenging activity. The other compounds with cinnamaldehye derivative and chloro substituents have intermediate scavenging activity.The scavenging activity of the compounds was compared with the control and the significance factor “p” was less than 0.001 for all of the compounds. The scavenging activity of the compounds was compared with the control and the significance factor “p” was less than 0.001 for all the compounds. The compound with methoxy group (1c, 2c) as a substituent showed the highest inhibition activity suggesting that electron donating groups may aid the activity (Table 5&6).The other compounds with Cinnamaldehyde derivative and chloro derivative have intermediate inhibition activity.

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