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Synthesis, Characterization, Anti-Oxidant and Anti Inflammatory Activity Evaluation of Chalcones and Pyrazoline Derivatives

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ABSTRACT

Pyrazolines display a broad spectrum of potential pharmacological activities. Hence pyrazolines are used extensively as useful synthons in organic synthesis. 4-Amino acetophenone was diazotized then followed by coupling with morpholine as a means of protection of amine group in 4-Amino acetophenone. The obtained product is then made to react with different aldehydes in the presence of 40% KOH solution as catalyst to yield chalcone derivatives. These are then subjected for cyclization by treating with hydrazine hydrate. The structures were proposed based on ^{1}H NMR and IR spectral data. All the compounds are screened for Anti-oxidant and anti Inflammatory activity.

Key words: Chalcones, 4-Amino acetophenone, Pyrazolines, Anti-oxidant, Anti- Inflammatory.

INTRODUCTION

In Medicinal chemistry, derivatives of 4-Amino Acetophenone found to have diverse therapeutic applications. Many 4-Amino Acetophenone derivatives have been developed as chemo therapeutic agents and are widely used. 4-Amino Acetophenone moiety carrying compounds exhibit various activities like anti-bacterial, anti-fungal, anticancer, anti-convulsion, anti-inflammatory, anti-oxidant etc[1-4].

Chalcone is an aromatic ketone and an enone that forms the central core for a variety of important biological compounds, which are known collectively as chalcones or chalconoids. These are coloured compounds because of the presence of the chromophore -CO-CH=CH-. Chalcones bears a very good synthon so that variety of novel heterocycles with good pharmaceutical profile can be designed. Chalcones can be prepared by Claisen-Schmidt condensation between an aromatic aldehyde and an aromatic ketone in the presence of sodium hydroxide as a catalyst.

Chalcones are popular intermediates for synthesizing various heterocyclic compounds. The compounds with the backbone of chalcones have been reported to possess various biological activities such as anti-microbial, anti-inflammatory of chemical mediators release, inhibition of leukotriene B4, inhibition of tyrosinases an inhibition of aldose reductase activities. The presence of a reactive α,β-unsaturated keto function in chalcones is found to be responsible for their biological activities[5].

Heterocyclic compounds are well known for their wide range of biological applications out of which pyrazolines occupy unique position due to dominant applications. Pyrazolines are well known and important nitrogen-containing five-membered heterocyclic compounds. Several pyrazoline derivatives have been found to possess considerable biological activities, which stimulated research activity in this field. Considerable attention has been focused on Pyrazolines and substituted Pyrazolines due to their interesting biological activities. They have found to possess anti-fungal, anti-depressant, anti-convulsant, anti-inflammatory, anti-bacterial, anti-cancer, antioxidant, anti-pyretic, anti-neoplastic activities, anti-viral, anti-amoebic, anti-cholinergic, antidiabetic, anti-HIV, antimalarial, anaxiolytic, antiparasitic, anti-allergic, anti-microbial, anti-tuberculosis, tyrosinase inhibitor, hypoglycemic, hypotensive, immunosuppressive, anti-tumor properties[6,7]. Pyrazoline is dihydropyrazole which is a five membered heterocyclic compound containing two nitrogen atoms in adjacent position having only one endocyclic double bond. Among all the pyrazoline derivatives 2-pyrazoline has gained the most importance because of its diverse biological activities. 2-Pyrazolines display a broad spectrum of potential pharmacological activities and are present in a number of pharmacologically active molecules such as phenazone/ amidopyrene/ methampyrone (analgesic and antipyretic), azolid/ tandearil (anti-inflammatory), indoxacarb (insecticidal), anturane (uricosuric), etc. In addition, pyrazolines have played a crucial part in the development of theory in hetero cyclic chemistry and also used extensively in organic synthesis.

MATERIALS AND METHODS

The Identification and characterization of synthesized compounds were carried out by the following procedure to ascertain that all the prepared compounds were of different chemical nature than the respective parent compounds. This involved the determination of the melting point, solubility characters, and their behaviour in Thin Layer Chromatography (TLC) studies as compared to that of their parent compounds and Nuclear Magnetic Resonance (^{1}H NMR) data, Infra-red spectroscopy. The melting point was determined for the synthesized compounds were taken in open capillary tubes by using Arson digital melting point apparatus which were uncorrected.

The TLC was done on precoated aluminium plates of silica gel 60 F_{254} obtained from MERCK and visualization of spots was done by using UV TLC visualization chamber.IR Spectra recorded on BRUKER,^{1}H NMR spectra of the compounds recorded on BRUKER-AMX 400 MHz, UV Visible spectrophotometer of Lab India was used for screening the Synthesized compounds for antioxidant activity.

Methodology

Step 1: Diazotization and coupling of amine Group in 4-Amino Acetophenone with morpholine

0.01 mol of 4-Amino acetophenone is dissolved in a mixture of 3ml of Conc.HCl and 6ml water. Then dissolve 1.35gm of sodium nitrite in 6ml of water . Both the above solutions are cooled to 0-5^{o}C by keeping in an ice bath and also by the addition of few pieces of ice to the 4-Amino acetophenone solution. Now sodium nitrite solution is added dropwise to the mixture of 4-Amino acetophenone in Conc. HCl and water with continuous stirring in order to avoid rise in temperature during the reaction. After the completion of sodium nitrite addition, morpholine is added to the reaction mixture with continuous stirring at the same temperature i.e., 0-5^{o}C until a yellow coloured precipitate is obtained. This is then filtered and washed with ice cold water and dried.

Step 2: Preparation of chalcones

0.005 mol of diazotized 4-Amino Acetophenone coupled with morpholine and

equimolar amount of aldehydes in a round bottomed flask containing 50ml of ethanol, and then 1ml of 40% KOH is added at the room temperature with continuous stirring on magnetic stirrer for 2hrs. The reaction mixture is monitored by TLC during the reaction. After the completion of the reaction the reaction mixture is poured into crushed ice with a little amount of dilute HCl in order to neutralize potassium hydroxide. Then the solid is filtered and washed with cold water, dried and recrystallized from ethanol.

Step 3: Preparation of pyrazolines

0.003mol of chalcones and 0.024mol (1.2ml) of hydrazine hydrate in a round bottomed flask containing 30ml ethanol as a solvent is refluxed for 2hrs at 60°C by monitoring with TLC. After the completion, the reaction mixture is poured into crushed ice and the solid is filtered and dried.

Antioxidant activity

DPPH Method :(1, 1-diphenyl-2-picrylhydrazyl[8]

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 0.1mM 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 %Scavenged = [(A-A1)/A] x 100

Where A=Absorbance of the control ;

A1=Absorbance of the test or standard

Hydrogen Peroxide method[9]

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.

%Scavenged = [(A-A1)/A] x 100

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

Invitro Anti-inflammatory activity

Inhibition of Bovine Albumin Denaturation Method[10]

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

% Inhibition = [(A-A1)/A] x 100

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

Heat Induced Haemolytic Method[11]

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 suspension and equal amount of normal saline was added to each blank sample. Equal amount of 1% RBC's suspension and normal saline was added and used as control. All these samples were taken into centrifuge tubes and incubated in water bath at 56°C 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

% Inhibition = [(A-A1)/A] x 100

Where A=absorbance of the control.

A1= absorbance of the test /standard.

Calculation of IC_{50} values[12]

$IC_{50}$was calculated using Graphpad prism software. In order to calculate IC_{50} initially XY data table was created. Then the logarithm of the concentration of the inhibitor was entered into X and response was entered into Y. From the data table click Analyze, choose nonlinear regression, then choose the panel of equations "Dose response curves-Inhibition" and then choose the equation "log (Inhibitor) v/s normalized response-variable slope". Then we will get IC_{50} values for the given data.

RESULTS AND DISCUSSIONS

Compound 2A: (2E)-1-{4-[(E)-morpholin-4-yldiazenyl] phenyl}-3-(4-nitrophenyl) prop-2-en-1-one:

Molecular formula: $C_{19}H_{18}N_4O_4$, Molecular Weight: 366.37, Physical state: crystalline powder, Colour: Green, Melting Point: 216-218°C, Rf Value: 0.47 (n-hexane: Ethyl acetate; 6:4), Solubility: Chloroform, DMSO, Ethyl acetate, Percentage yield : 87%.

Table 1: Physico-chemical characterization data of synthesized compounds

S. No.	Compound Code	Molecular formula	Mol.wt	%Yield	M.P	Rf Value	Colour
1	2A	$C_{19}H_{18}N_4O_4$	366.37	87%	216-218 °C	0.47*	Green
2	2B	$C_{19}H_{18}N_4O_4$	366.37	85%	136-139°C	0.7*	Green
3	2C	$C_{20}H_{21}N3O_2$	335.39	70%	137-140°C	0.57*	Orange
4	2D	$C_{22}H_{25}N_3O_5$	411.45	86%	126-129°C	0.52*	Green
5	2E	$C_{19}H_{18}F\ N_3O_2$	339.36	88.20%	102-104°C	0.6*	Cream
6	3A	$C_{19}H_{20}N_6O_3$	380.4	87.60%	140-142°C	0.62**	Light yellow
7	3B	$C_{19}H_{20}N_6O_3$	380.4	70%	76-78°C	0.58**	Light yellow
8	3C	$C_{21}H_{23}N_5O$	349	86.50%	56-58°C	0.45**	Dark yellow
9	3E	$C_{19}H_{20}F\ N_5O$	353.39	88.30%	68-70°C	0.35**	Dark yellow

Solvent used: * = n-Hexane: Ethyl acetate (6:4) ** = n-Hexane: Ethyl acetate (4:6)

Table 2: The percentage scavenging activity by DPPH Method

Conc	% scavenging activity(Mean ± SEM)									
µg/ml	Std	2A	2B	2C	2D	2E	3A	3B	3C	3E
20	84.26 ±0.176	49.29 ±0.159	45.43 ±0.088	58.43 ±0.296	63.50 ±0.288	55.50 ±0.288	50.30 ±0.650	44.46 ±0.290	58.73 ±0.218	46.40 ±0.305
40	85.40 ±0.208	50.26 ±0.091	49.35 ±0.224	64.60 ±0.305	67.90 ±0.450	58.73 ±0.371	51.56 0.808	49.33 ±0.333	61.33 ±0.333	49.96 ±0.983
60	86.36 ±0.185	53.63 ±0.120	50.46 ±0.218	67.50 ±0.288	70.40 ±0.305	62.06 ±0.560	55.80 0.416	52.60 ±0.264	65.86 ±0.466	54.53 ±0.290
80	89.30 ±0.152	56.53 ±0.088	55.43 ±0.120	71.66 ±0.240	73.63 ±0.272	64.60 ±0.305	58.73 0.371	55.80 ±0.416	71.93 ±0.520	60.70 ±0.351
100	90.40± 0.115	61.33 ±0.145	60.63 ±0.272	72.06 ±0.581	76.60 ±0.305	65.86 ±0.466	64.66 0.240	61.67 ±0.338	74.96 ±0.548	65.93 ±0.405
120	91.33± 0.176	66.40 ±0.115	62.83 ±0.120	74.60 ±0.305	78.86 ±0.466	69.13 ±0.592	66.83 0.440	65.30 ±0.208	81.63 ±0.317	69.16 ±0.088
IC_{50}µg/ml	2.80	58.61	64.20	42.81	36.30	50.62	55.42	60.02	32.34	54.90

Mean ± SEM = Mean ± Standard Error Mean, IC_{50}=Half maximal inhibitory concentration

IR (KBr disk)

γ (cm-1), C-H Stretching (Morpholine) - 3104.94 Cm^{-1}**,** C-H Stretching (Aromatic) - 2980.08 Cm^{-1} **,** C-H Stretching (olefins) - 3076.89 Cm^{-1}, C=O - 1658.49 Cm^{-1} ,C=C Stretching (Olefin) - 1598.09 Cm^{-1} , C=C (Aromatic) - 1517.45 Cm-1, N=O (Nitro) - 1431.77 Cm^{-1}, C-H Bending (Aromatic) - 832.65 Cm^{-1} C-H Stretching (Morpholine) - 3104.94 Cm^{-1}**,** C-H Stretching (Aromatic) - 2980.08 Cm^{-1} **,** C-H Stretching (olefins) - 3076.89 Cm^{-1} , C=O - 1658.49 Cm^{-1} ,C=C Stretching (Olefin) - 1598.09 Cm^{-1} , C=C (Aromatic) - 1517.45 Cm^{-1} , N=O (Nitro) - 1431.77 Cm^{-1} , C-H Bending (Aromatic) - 832.65 Cm^{-1} .

^{1}H NMR ($CDCl_3$)

δ(ppm)3.893 to 3.884 (8H,m,morpholine protons),7.575 (2H,d,CC&CE Ar-H, *J*=8.8Hz), 7.693 (1H,d,Ci Olefinic proton=15.6 Hz), 7.801

Table 3: The percentage scavenging activity by Hydrogen Peroxide Method

Conc	% scavenging activity(Mean ± SEM)									
µg/ml	Std	2A	2B	2C	2D	2E	3A	3B	3C	3E
20	89.7 ±0.351	44.53 ±0.290	33.46 ±0.290	48.7 ±0.351	50.2 ±0.099	52.6 ±0.305	47.9 ±0.493	37.83 ±0.440	55.6 ±0.305	46.4 ±0.305
40	92.6 ±0.305	46.86 ±0.466	35.06 ±0.066	51.36 ±0.317	55.2 ±0.416	57.767 ±0.39	49.6 ±0.305	40.76 ±0.145	61.86 ±0.466	58.733 ±0.371
60	94.46 ±0.240	50.23 ±0.392	37.73 ±0.371	54.86 ±0.466	59.4 ±0.305	59.267 ±0.266	52.6 ±0.305	42.36 ±0.317	63.63 ±0.317	60.53 ±0.290
80	95.06 ±0.066	53.76 ±0.392	42.7 ±0.351	58.7 ±0.351	61.36 ±0.317	61.567 ±0.296	58.23 ±0.120	45.63 ±0.3179	67.46 ±0.290	62.23 ±0.185
100	95.36 ±0.185	55.83 ±0.440	47.6 ±0.305	60.4 ±0.305	64.93 ±0.520	63.267 ±0.133	59.03 ±0.088	47.26 ±0.176	70.63 ±0.202	62.8 ±0.115
120	96.2 ±0.100	57.83 ±0.440	49.53 ±0.290	63.43 ±0.296	69.4 ±0.305	64.4 ±0.305	60.5 ±0.288	48.56 ±0.296	72.6 ±0.305	65.56 ±0.296
IC_{50} µg/ml	2.2	62.41	116.02	24.23	20.18	32.41	50.11	120.06	16.28	40.92

Mean ± SEM = Mean ± Standard Error Mean, IC_{50}=Half maximal inhibitory concentration

Table 4: The percentage scavenging activity by Egg AlbuminDenaturation Method

Conc	% Inhibition(Mean ± SEM)									
µg/ml	Std	2A	2B	2C	2D	2E	3A	3B	3C	3E
20	76.83 ±0.441	25.86 ±0.592	32.56 ±0.260	36.40 ±0.305	33.4 ±0.305	30.6 ±0.305	28.4 ±0.305	23.8 ±0.416	40.7 ±0.351	29.93 ±0.520
40	78.2 ±0.115	30.46 ±0.290	34.26 ±0.176	37.30 ±0.251	35.7 ±0.152	41.63 ±0.318	33.46 ±0.290	35.46 ±0.290	42.2 ±0.200	31.66 ±0.176
60	79.30 ±0.173	36.63 ±0.318	37.36 ±0.318	43.26 ±0.176	40.2 ±0.416	43.56 ±0.296	41.63 ±0.318	42.5 ±0.288	45.5 ±0.288	33.26 ±0.176
80	80.06 ±0.066	43.46 ±0.290	43.33 ±0.166	48.43 ±0.233	47.4 ±0.305	45.16 ±0.088	43.7 ±0.351	44.36 ±0.272	47.5 ±0.288	35.33 ±0.176
100	81.40 ±0.115	46.40 ±0.305	44.53 ±0.290	51.46 ±0.290	55.56 ±0.296	46.56 ±0.296	46.4 ±0.305	45.26 ±0.176	52.36 ±0.318	48.46 ±0.290
120	82.63 ±0.202	51.30 ±0.351	47.40 ±0.305	56.73 ±0.371	61.8 ±0.416	54.3 ±0.351	51.63 ±0.318	46.6 ±0.305	58.53 ±0.290	53.73 ±0.371
IC_{50} µg/ml	1.02	116.32	134.04	86.43	84.22	104.08	114.02	128.4	82.06	106.28

Mean ± SEM = Mean ± Standard Error Mean, IC_{50}=Half maximal inhibitory concentration

(2H,d,C2&C6Ar-H,J=9Hz), 7.841 (1H,d,CiiOlefinic proton=16), 8.071 (2H,d,CB&CF Ar-H,J=8.4 Hz), 8.29 (2H,d,C3&C5Ar-H,J=8.4 Hz).

Compound 2B

(2E)-1 -{4-[(E)-morpholin-4-yldiazenyl] phenyl}-3- (3-nitrophenyl)prop-2-en-1 -one

Molecular Formula

$C_{19}H_{18}N_4O_4$, Molecular Weight : 366.37, Physical state : crystalline powder ,Colour: Green ,Melting Point: 136-1 39°C R_fValue : 0.7 (n-hexane:Ethylacetate;4:6) ,Solubility : Chloroform, DMSO, Ethyl acetate, Percentage yield : 85%.

IR (KBr disk)

γ (cm^{-1}),C-H Stretching (olefins) - 3073.45 Cm^{-1},C-H Stretching (morpholine) - 2963.93 Cm^{-1},C-H Stretching (Aromatic) - 2990.21 Cm^{-1},C=C (Aromatic) - 1522.56 Cm^{-1},C-H Bending (Aromatic) - 805.79 Cm^{-1}, C=O - 1660.04 Cm^{-1},N=O (Nitro) - 1437.85 Cm^{-1} , C=C Stretching (Olefin)- 1607.01 Cm^{-1}

^{1}H NMR ($CDCl_3$)

δ (ppm)3.911 to 3.885 (8H,m,morpholine protons), 7.593 to7.558 (2H,d,CC&CE Ar-H,J=8.4Hz), 7.633 (1H,t,C5Ar-H,J=8 Hz), 7.705 (1H,d,C6Ar-H,J=8.9 Hz), 7.854 (1H,d, CiOle finicproton ,J=15.6 Hz), 7.929 (1H,d, CiiOlefinic proton,J=14 Hz), 8.084 (2H,d,CB&CF Ar-H,J=8.8Hz), 8.263 (1H,d,C4 Ar-H,J=8.4Hz), 8.518(1H,s,C6 Ar-H).

Compound 2C

(2E)-3-(4-methylphenyl)-1-{4-[(E)-morpholin-4-yldiazenyl]phenyl}prop-2-en-1-one Molecular Formula : $C_{20}H_{21}N_3O_2$, Molecular Weight : 335.39, Physical state : crystalline powder, Colour:Orange,Melting Point :137-140°C,R_f Value:0.57(n-hexane:Ethylacetate;6:4) , Solubility: Chloroform, DMSO, Ethyl acetate, Percentage yield : 70%.

IR (KBr disk)

γ (cm^{-1}),C-H Stretching (olefins) - 3052.25 Cm^{-1}, C-H Stretching (morpholine) - 2971.19 Cm^{-1}, C-H Stretching (Aromatic) - 2903.18 Cm^{-1},C-H Stretching (CH3) - 2854.95 Cm^{-1},C=O - 1649.71 Cm^{-1},C=C Stretching (Olefin) - 1588.87 Cm^{-1},C=C (Aromatic) - 1562.54 Cm^{-1},C-H Bending (Aromatic) - 810.55 Cm^{-1}

^{1}H NMR ($CDCl_3$)

δ(ppm) 2.396 (3H,s,-CH3 protons on C4 of aldehyde), 3.876 (8H,m,morpholine protons), 7.258(2H,d,CC&CEAr-H,J=8.8Hz), 7.505(1H,d,Ci Olefinicproton, J=12Hz),7.560 to 7.531

Table 5: The percentage scavenging activity by Egg Albumin Denaturation Method

Conc	% Inhibition(Mean ± SEM)									
µg/ml	Std	2A	2B	2C	2D	2E	3A	3B	3C	3E
20	53.36 ±0.318	29.6 ±0.305	27.56 ±0.296	35.83 ±0.441	33.7 ±0.351	31.2 ±0.115	31.9 ±0.435	30.43 ±0.296	35.5 ±0.288	34.23 ±0.145
40	62.4 ±0.305	31.53 ±0.290	35.76 ±0.393	40.53 ±0.290	35.86 ±0.466	32.63 ±0.318	34.7 ±0.251	32.26 ±0.218	38.63 ±0.318	35.36 ±0.318
60	67.86 ±0.466	35.43 ±0.296	37.7 ±0.351	48.93 ±0.520	37.56 ±0.296	34.36 ±0.318	36.86 ±0.466	34.6 ±0.305	40.46 ±0.290	37.7 ±0.351
80	71.9 ±0.493	42.46 ±0.290	40.46 ±0.290	50.2 ±0.200	42.2 ±0.611	37.86 ±0.466	39.36 ±0.318	37.5 ±0.288	41.53 ±0.290	40.4 ±0.305
100	74.6 ±0.305	44.2 ±0.200	42.26 ±0.176	51.16 ±0.088	49.46 ±0.290	42.6 ±0.305	43.46 ±0.290	43.73 ±0.371	45.6 ±0.305	42.46 ±0.240
120	75.56 ±0.296	45.86 ±0.466	44.4 ±0.305	52.46 ±0.290	54.8 ±0.416	48.33 ±0.333	49.86 ±0.240	48.53 ±0.290	55.16 ±0.166	50.66 ±0.176
IC_{50} µg/ml	13.02	128.01	174.51	90.2	102.01	126.04	130.45	142.05	100	164.23

Mean ± SEM = Mean ± Standard Error Mean, IC_{50}=Half maximal inhibitory concentration

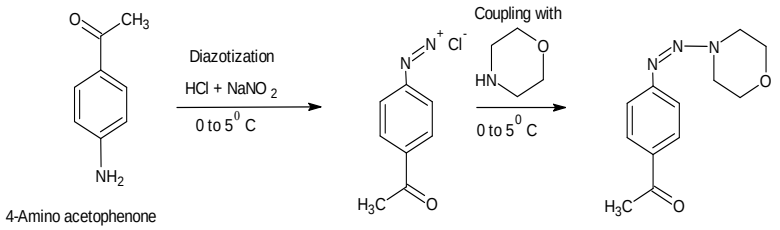

Fig. 1: Protection of Amine group in 4-Amino Acetophenone

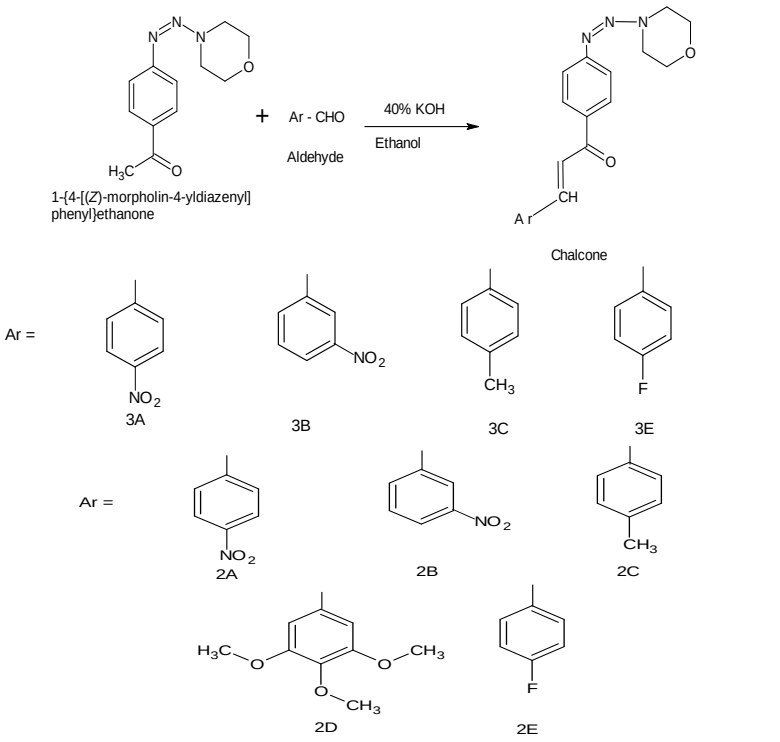

Fig. 2: Synthesis of Chalcones

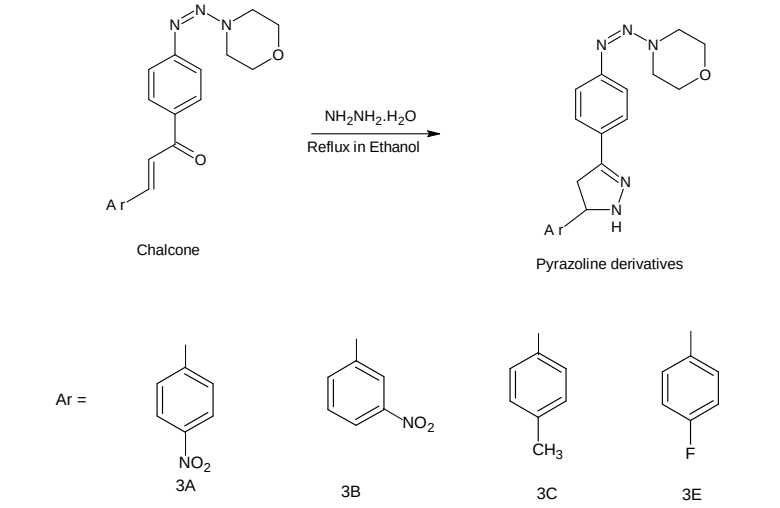

Fig. 3: Cyclisation of chalcones to pyrazolines

(4H,m,C2,C3,C5,C6Ar-H,J=8.4Hz), 7.818(1H,d, CiiOlefinic proton,J=14.6Hz), 8.054(2H,d, CB&CF Ar-H,J=8.4 Hz).

Compound 2D :(2E)-3-(3, 4, 5-tri methoxy phenyl)-1-{4-[(E)-morpholin-4-yldiazenyl] phenyl}prop-2-en-1-one

Molecular Formula : $C_{22}H_{25}N_3O_5$,Molecular Weight : 411.45, Physical state: crystalline powder , Colour: Green ,Melting Point : 126-129°C,Rf Value: 0.52 (n-hexane:Ethylacetate; 4:6) Solubility : Chloroform, DMSO, Ethyl acetate. Percentage yield : 86 %.

IR (KBr disk)

γ (cm_{-1}),C-H Stretching (olefins)-2995.03 Cm^{-1},C-H Stretching (morpholine)- 2973.24 Cm^{-1}, C-H Stretching (Aromatic) - 2937.50 Cm^{-1},C=O - 1656.15 Cm^{-1}, C=C (Aromatic) - 1602.99 Cm^{-1}, C=C Stretching (Olefin) - 1502.80 Cm^{-1}, C-O Stretching (Ether) - 1326.88 Cm^{-1} , C-O-C Stretching - 1248.83 Cm^{-1}, C-H Bending (Aromatic) - 836.89 Cm^{-1}.

^{1}H NMR ($CDCl_3$)

δ(ppm) 3.875 (8H,m,morpholine protons), 3.904 (3H,s,-OCH3 on C4), 3.925 (6H,s,-OCH_3 on C3&C5), 6.872 (2H,s, C2&C6Ar-H), 7.453 (1H,d,Ci

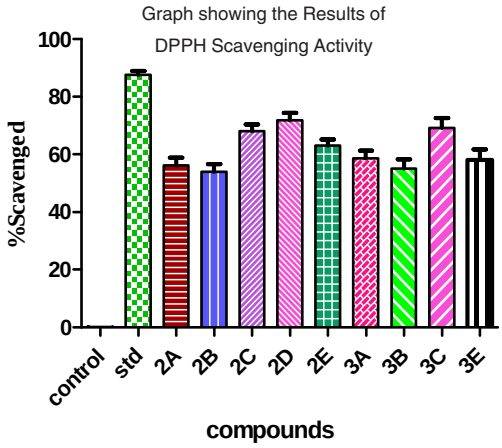

Fig. 4: The percentage scavenging activity by DPPH Method

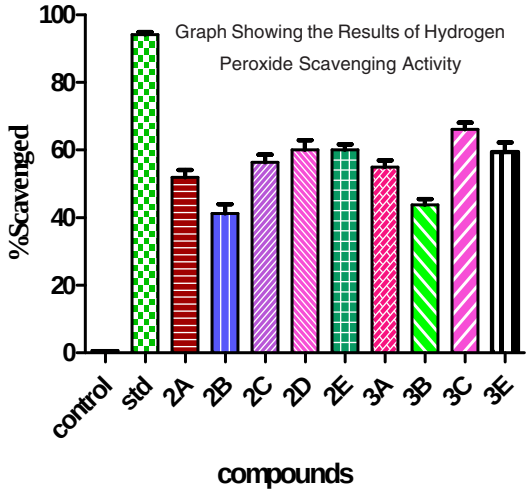

Fig. 5: The percentage scavenging activity by Hydrogen Peroxide Method

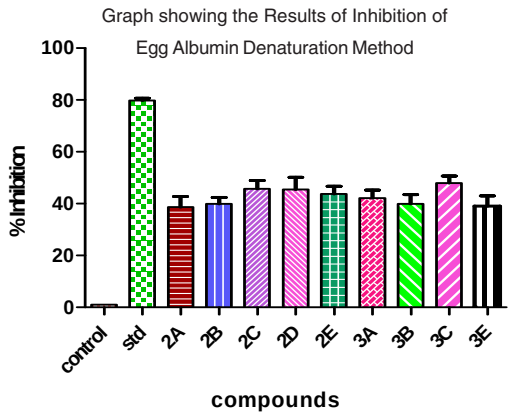

Figure 6: The percentage scavenging activity by Egg Albumin Denaturation Method

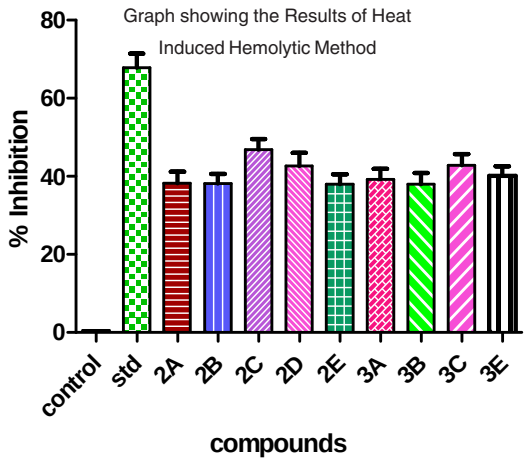

Fig. 7: The percentage scavenging activity by Heat Induced hemolytic Method

Olefinicproton,J=15.6Hz), 7.559 (2H,d,CC&CE Ar-H,J=8.4Hz), 7.742(1H,d, CiiOlefinicproton, J=15.6Hz), 8.052 (2H,d,CB&CF Ar-H,J=8.4Hz).

Compound 2E: (2E)-3-(4-fluorophenyl)-1-{4-[(E)-morpholin-4-yldiazenyl]phenyl}prop-2-en-1-one

Molecular Formula: $C_{19}H_{18}FN_3O_2$, Molecular Weight: 339.36, Physical state: Crystalline powder, Colour: Cream, Melting Point: 102-104°C,R_f Value: 0.6 (n-hexane: Ethylacetate; 6:4), Solubility: Chloroform, DMSO, Ethyl acetate, Percentage yield: 88.2%

IR (KBr disk)

γ (cm^{-1}),C-H Stretching (olefins) - 3066.52 Cm^{-1},C-H Stretching (morpholine) - 2974.73 Cm^{-1}, C-H Stretching (Aromatic) - 2866.95 Cm^{-1},C=O - 1656.66 Cm^{-1}, C=C Stretching (Olefin) - 1596.46 Cm^{-1},C=C (Aromatic) - 1510.09 Cm^{-1}, C-F Stretching - 1332.37 Cm^{-1} C-H Bending (Aromatic) - 827.50 Cm^{-1}

^{1}H NMR ($CDCl_3$)

δ(ppm) 3.874 (8H,m,morpholine protons), 7.218 (2H,d,C3&C5Ar-H,J=8.4 Hz), 7.470 (1H,d,Ci Olefinicproton=15.6 Hz), 7.555 (2H,d,C2&C6Ar-H,J=8.4 Hz),7.654 to 7.619(2H,d,C3&C5Ar-H,J=8.4 Hz),7.796 (1H,d,Cii Olefinicproton=15.6Hz), 8.051 (2H,d,CB&CF Ar-H,J=8.4Hz).

Compound 3A:4-[(E)-{4-[5-(4-nitrophenyl)-4, 5-dihydro-1H-pyrazol- 3yl] phenyl} diazenyl] morpholine

Molecular Formula : $C_{19}H_{20}N_6O_3$, Molecular Weight : 380.4 , Physical state : Crystalline powder , Colour : Light yellow , Melting Point: 140-142°C , R_f Value : 0.35 (n-hexane:Ethylacetate; 6:4) ,Solubility : Chloroform, DMSO, Ethyl acetate, Percentage yield : 87.6%.

IR (KBr disk)

γ (cm^{-1}),C-H Stretching (olefins) - 3355.58 Cm^{-1},N-H Stretching (pyrazoline) - 3105.16 Cm^{-1}, C-H Stretching (morpholine) - 3072.35 Cm^{-1}, C-H Stretching (Aromatic) - 2953.16 Cm^{-1}, N=O (Nitro) - 1598.61 Cm^{-1}, C=C (Aromatic) - 1513.25 Cm^{-1}, C=N Stretching (Pyrazoline) - 1437.94 Cm^{-1}, C5-N1 Stretching (Pyrazoline)- 1156.89 Cm^{-1}, C-H Bending (Aromatic) - 843.41 Cm^{-1}.

^{1}H NMR ($CDCl_3$)

δ(ppm) 3.035 to 2.970 (1H,dd,Hb on C4 of pyrazoline ring), 3.611 to 3.544 (1H,dd,Ha on C4 of pyrazoline ring), 3.863 to 3.819 (8H,m, morpholine protons), 5.069 to 5.018 (1H,m, Hx on C5 of pyrazoline ring), 6.080 (1H,d,N1H proton in pyrazoline ring), 7.264 (2H,d,C2&C6Ar-H,J=8.4Hz), 7.657 to 7.579 (4H,m, CB,CF, CC&CE Ar-H,J=8.4 Hz), 8.225 (2H,d,C3&C5Ar-H,J=8.Hz), Jab=16.4, Jax=5.2, Jbx =10.8.

Compound 3B:4-[(E)-{4-[5-(3-nitrophenyl)-4, 5-dihydro-1H- pyrazol3yl] phenyl}diazenyl] morpholine

Molecular Formula : $C_{19}H_{20}N_6O_3$,Molecular Weight : 380.4 ,Physical state : Crystalline powder ,Colour : Light yellow ,Melting Point: 76-78°C ,R_f Value : 0.58 (n-hexane:Ethylacetate; 4:6) ,Solubility: Chloroform, DMSO, Ethyl acetate, Percentage yield : 70%

IR (KBr disk)

ν (cm^{-1}),C-H Stretching (olefins)-3336.76 Cm^{-1}, C-H Stretching (morpholine) - 3308.19 Cm^{-1}, N-H Stretching (pyrazoline) - 3085.54 Cm^{-1}, C-H Stretching (Aromatic) - 2857.31 Cm^{-1}, C=C (Aromatic) - 1438.17 Cm^{-1}, N=O (Nitro) - 1524.22 Cm^{-1}, C=N Stretching (Pyrazoline) - 1348.84 Cm^{-1}, C5-N1 Stretching (Pyrazoline)- 1154.79 Cm^{-1}, C-H Bending (Aromatic) - 835.42 Cm^{-1}.

^{1}H NMR ($CDCl_3$)

δ(ppm) 3.050 to 2.985 (1H,dd,Ha on C4 of pyrazoline ring), 3.617 to 3.577 (1H,dd,Hb on C4 of pyrazoline ring), 3.857 to 3.550 (8H,m,morpholine protons), 5.083 to 5.032 (1H,m, Hx on C5 of pyrazoline ring), 7.481 to 7.460 (2H,d, CC&CE Ar-H,J=8.4 Hz), 7.557 to 7.518 (2H,d, C4&C6 Ar-H,J=8Hz), 7.662 to 7.642 (2H,d, CB&CF Ar-H,J=8 Hz), 7.789 (1H,d,N1H proton in pyrazoline ring), 8.163 (1H,t, C5 Ar-H,J=8.1Hz), 8.284 (1H,s, C2 Ar-H), Jab=16 , Jax=6 , Jbx =10.8.

Compound 3C:4-[(E)-{4-[5-(4-methylphenyl)-4, 5-dihydro-1H-pyrazol-3-yl] phenyl}diazenyl] morpholine

Molecular Formula:$C_{21}H_{23}N_5O$,Molecular Weight : 349, Physical state : Crystalline powder , Colour : Dark yellow , Melting Point : 56-58°C , R_f Value : 0.45 (n-hexane:Ethylacetate; 6:4) ,Solubility

: Chloroform, DMSO, Ethyl acetate. Percentage yield : 86.5%

IR (KBr disk)

ν (cm^{-1}),C-H Stretching (olefins)-3355.61 Cm^{-1},C-H Stretching (morpholine) - 3333.83 Cm^{-1}, N-H Stretching (pyrazoline) - 3025.31 Cm^{-1}, C-H Stretching (Aromatic) - 2918.99 Cm^{-1}, C-H Stretching (CH3) - 2854.54 Cm^{-1}, C=C (Aromatic) - 1435.06 Cm^{-1}, C=N Stretching (Pyrazoline) - 1348.57 Cm^{-1}. C5-N1 Stretching (Pyrazoline) - 1154.81 Cm^{-1}, C-H Bending (Aromatic) - 814.53 Cm^{-1}

^{1}H NMR ($CDCl_3$)

δ(ppm) 2.391 (3H,s,CH3 on C4), 3.072 to 3.010 (1H,dd, Ha on C4 of pyrazoline ring), 3.487 to 3.420 (1H,dd,Hb on C4 of pyrazoline ring), 3.815 (1H,m, Hx on C5 of pyrazoline ring), 3.855 (8H,m,morpholine protons), 7.192 (1H,d,N1H proton in pyrazoline ring), 7.530 to 7.448 (4H,d, C2,C3, C5&C6 Ar-H,J=8.4 Hz), 7.665 to 7.595 (4H,d, CB,CF, CC&CE Ar-H,J=8.4 Hz), Jab=16.4 , Jax=6.4 , Jbx =10.4.

Compound 3E:4-[(E)-{4-[5-(4-fluorophenyl)-4, 5-dihydro-1H-pyrazol-3-yl] phenyl} diazenyl] morpholine

Molecular Formula:$C_{19}H_{20}FN_5O$, Molecular Weight : 353.39, Physical state : Crystalline powder , Colour : Dark yellow , Melting Point : 68-70°C ,R_f Value: 0.35 (n-hexane:Ethyl acetate; 6:4) , Solubility : Chloroform, DMSO, Ethyl acetate, Percentage yield : 88.3%.

IR (KBr disk)

ν (cm^{-1}),C-H Stretching (olefins) - 3063.27 Cm^{-1}, C-H Stretching (morpholine) - 3036.41 Cm^{-1}, N-H Stretching (pyrazoline) - 2967.44 Cm^{-1},C-H Stretching (Aromatic) - 2855.66 Cm^{-1}, C=C (Aromatic) - 1438.58 Cm^{-1}, C=N Stretching (Pyrazoline) - 1347.59 Cm^{-1}, C5-N1 Stretching (Pyrazoline)- 1156.12 Cm^{-1}, C-F Stretching - 1015.78 Cm^{-1}, C-H Bending (Aromatic) - 839.10 Cm^{-1}.

^{1}H NMR ($CDCl_3$)

δ(ppm) 3.040 to 2.976 (1H,dd,Ha on C4 of pyrazoline ring), 3.510 to 3.442 (1H,dd,Hb on C4 of pyrazoline ring), 3.868 to 3.800 (8H,m,morpholine protons), 4.942 to 4.483 (1H,m, Hx on C5 of pyrazoline ring), 7.050 to 7.007 (2H,d, CC&CE Ar-H,J=8.8 Hz),7.374 (1H,d,N1H proton in pyrazoline ring), 7.476 to 7.454 (4H,d, C2,C3, C5&C6 Ar-H,J=8.8 Hz), 7.664 to 7.643 (2H,d, CB&CF Ar-H,J=8.4 Hz), Jab=16.4 , Jax=6.8 , Jbx =10.8.

The data was analyzed by one way ANOVA using Graph pad prism software. The scavenging activity of the compounds (Table 2&3) and their IC_{50} values were compared with the standard and the significance factor "p" was found to be less than 0.001 for most of the compounds. Among chalcones compound 2D having 3,4,5- trimethoxy substitution and among pyrazolines compound 3C having 4-Methyl has shown highest activity with least IC_{50} values suggesting that electron donating groups aid in scavenging activity. Among chalcones compound 2B and among pyrazolines compound 3B having 3-Nitro substitutions has shown least activity with highest IC_{50}values indicating that electron withdrawing groups at the meta position in the compounds have less scavenging activity. All the other compounds with 4-Nitro, 4- fluoro substituents were found to have intermediate activity.

Similarly the anti inflammatory activity of the compounds and their IC_{50} values were compared with the standard and the significance factor $p < 0.001$ for most of the compounds (Table 4 & 5). Among chalcones compound 2D having 3,4,5-trimethoxy substitution and among pyrazolines compound 3C having 4-Methyl has shown highest activity with least IC_{50}values suggesting that electron donating groups in the compounds aid in good anti inflammatory activity. Among chalcones compound 2B and among pyrazolines compound 3B having 3-Nitro substitutions with highest IC_{50}values has shown least activity indicating that electron withdrawing groups at the meta position in the compounds have less anti inflammatory activity. All the other compounds with 4-Nitro, 4- fluoro substituents were found to have intermediate activity.

CONCLUSION

4- Amino acetophenone was diazotized then followed by coupling with with morpholine as ameans of protection ofamine group in 4-Amino acetophenone. The obtained diazotized product containing the acetyl group is then made to react

with different aldehydes in the presence of 40% KOH solution as catalyst to get chalcone derivatives. These chalcone derivatives are then subjected for cyclization by treating with hydrazine hydrate. All the reactions were monitored by TLC to ascertain the completion of the reaction. All the compounds were found to have good yields. R_f values and melting points of the synthesized compounds were different with each other indicating the difference between the compounds. The structures were proposed based on ^{1}H NMR and IR spectral data.

Anti oxidant activity was performed for the synthesized compounds by DPPH and Hydrogen Peroxide Method, and anti inflammatory activity was performed by Inhibition of Bovine Albumin Denaturation Method and Heat Induced Haemolytic Method. Most ofthe compoundsshowed significant activity with $p < 0.001$ when the data was subjected to one way ANOVA by using Graph pad prism-5 software. Among chalcones compound 2D having 3,4,5- trimethoxy substitution and among pyrazolines compound 3C having 4-Methyl has shown highest activity with least IC_{50}values which is considerable with the standard, suggesting that electron donating groups aid in scavenging activity. Among chalcones compound 2B and among pyrazolines compound 3B having 3-Nitro substitutions has shown least activity with highest IC_{50}values which is considerable with the standard, suggesting that electron withdrawing groups at the meta position in the compounds have less scavenging activity. All the other compounds with 4-Nitro, 4-fluoro substituents were found to have intermediate activity.

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