Heterocyclic Letters
Vol. 8| No.2|297-302|Feb-April |2018
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY OF NOVEL PYRAZOLINE DERIVATIVES

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ABSTRACT:
A new series of Chalcones **(2a-i)** were prepared by reacting 9-anthraldehyde and substituted ketones in alcohol medium in presence of NaOH. The Chalcones undergoes selective cyclization with Benzhydrazide (**1**) in glacial acetic acid medium to yield the title compounds 1,3,5-trisubstituted Pyrazolines **(3a-i).** The new compounds were assigned on the basis of ^{1}H-NMR, IR and Mass spectral data. The newly synthesized compounds were evaluated for *In-Vitro* anti-inflammatory activity by bovine serum and egg albumin method. Some of the tested compounds 3a, 3h, 3f showed promising activity against bovine serum albumin denaturation when compared to the standard diclofenac sodium.

KEYWORDS: Chalcones, Pyrazolines, Benzhydrazide, 9-anthraldehyde, anti-inflammatory activity.

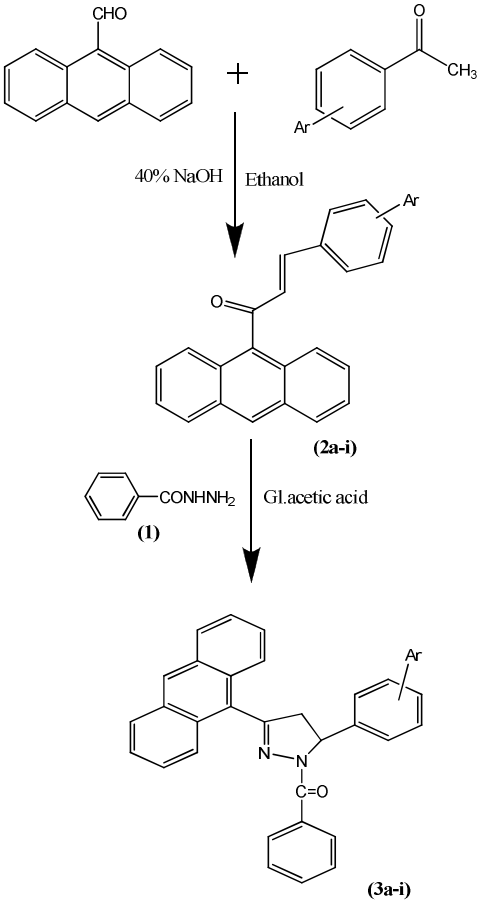
INTRODUCTION:

Pyrazoline is five-membered heterocyclic compound having two adjacent nitrogen atoms within the ring, play an important role in medicinal chemistry. The dihydro derivative of Pyrazole is known as Pyrazoline. Depending on the position of the double bond, can exist in three separate forms: 1-pyrazoline, 2-pyrazoline and 3-pyrazoline[I]. Changes in their structure have offered a high degree of diversity that has proven useful for the development of new therapeutic agents having improved potency and lesser toxicity.
Pyrazolines have been found to possess antifungal[II], antibacterial[II], antidepressant[III], anticonvulsant[III], anti-inflammatory[IV], anti-viral[V] properties. Pyrazolines are also acting as hole transporting material in OELD (organic electroluminescent device) because of formation of π conjugated system due to one of the nitrogen atom.
A considerable variety of methods are available in literature for the synthesis of Chalcones. The most convenient method is the one, that involves the Claisen-Schmidt condensation[VI] of equimolar quantities of an aryl methyl ketones with aryl aldehydes in presence of alcoholic alkali. Chalcones are 1,3-diphenyl-2-propene- 1-one, in which two aromatic rings are linked

by a three carbon α, β- unsaturated carbonyl system. These are abundant in edible plants and are considered to be precursors of flavonoids and isoflavonoids.

Chalcones are α, β-unsaturated ketones containing the reactive ketoethylenic group – CO – CH= CH –. Presence of α, β-unsaturated carbonyl system in chalcone makes it biologically active. Chalcones are useful synthons in the synthesis of a large number of bioactive molecules such as Pyrazolines, isoxazoles etc

A new series of Chalcones **(2a-i)** were synthesized by reacting 9-anthraldehyde and substituted ketones in alcohol medium in presence of NaOH as base 1,3,5-trisubstituted Pyrazolines **(3a-i)** were prepared by reacting Benzhydrazide **(1)** and Chalcones **(2a-i)** in glacial acetic acid medium.**(Scheme-01).** All the new compounds were evaluated for *In-Vitro* anti-inflammatory activity.



Scheme-01

EXPERIMENTAL

MATERIALS AND METHODS

IR spectra were recorded by using Bruker Alpha IR Spectrometer using KBr pellets and frequencies were expressed in $cm^{-1.}$ The ^{1}H-NMR spectra were recorded on Bruker Avance II 400 NMR Spectrometer. All spectra were obtained in $CDCl_3$ and DMSO. Chemical shift values are reported as values in ppm relative to TMS (δ=0) as internal standard. Mass spectra were recorded on ESI. Melting points were determined by open capillary method and are uncorrected. The reactions were monitored and checked by TLC.

General procedure for the synthesis of 1,3,5-trisubstituted Pyrazolines (3a-i)

A solution of 9-anthraldehyde Chalcones **(2a-i)** (0.01 mol) and Benzhydrazide **(1)** (0.01 mol) in glacial acetic acid (25 ml) was refluxed for about 20-30 hrs. Excess of solvent was removed under reduced pressure and the reaction mixture was poured into ice cold water. The product which was obtained is filtered, washed with water, dried and recrystallized from ethanol. The physical data of compounds **(3a-i)** is given in **table-1.**

(3-(anthracen-9-yl)-5-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanone 3c: IR (KBr), (ν_{max} cm^{-1}): 1443(C=C), 1590(C=N), 1657(C=O), 3046(C-H). ^{1}H-NMR (400 MH_Z, DMSO-d_6) δ (ppm)**:** 1.67 (s, CH_3, 3H), 2.36-2.38 (dd, 1H, H_A), 2.42-2.44(dd, 2H, H_B,), 7.28-7.30(dd, 1H, H_X), 7.33-8.43 (m, Ar-H, 18H). **MS(m/z):** 440.54(M+).

(3-(anthracen-9-yl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl) methanone 3f: IR (KBr), (ν_{max} cm^{-1}): 1521(C=C), 1654(C=O), 3036(CH). ^{1}H-NMR (400 MH_Z, DMSO-d_6) δ (ppm)**:** 2.35-2.45 (dd, 1H, H_A), 6.87-6.90 (dd, 2H, H_B, H_X), 7.44-9.01(m, Ar-H, 18H), 11.55 (s, 1H, OH).**MS(m/z):** 442.51(M+).

(3-(anthracen-9-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanone 3h: IR (KBr), (ν_{max} cm^{-1}): 1487(C=C), 1591(C=N), 1665(C=O), 3035(C-H). ^{1}H-NMR (400 MH_Z, DMSO-d_6) δ (ppm)**:** 2.12-2.21(dd, 1H, H_A), 2.62-2.68 (dd, 1H, , H_B), 3.96-4.04 (dd, 1H, H_X), 7.40-8.91 (m, Ar-H, 18H). **MS(m/z):** 471.51 (M+).

(3-(anthracen-9-yl)-5-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanone 3i: IR (KBr), (ν_{max} cm^{-1}): 1569(C=C), 1603(C=N), 1650(C=O), 3011(C-H). ^{1}H-NMR (400 MH_Z, DMSO-d_6) δ (ppm)**:** 2.12-2.15(dd, 1H, H_A), 3.58-3.65 (dd, 1H, H_B), 3.91-3.99 (dd, 1H, H_X), 7.09-8.33 (m, Ar-H, 18H). **MS(m/z):** 505.40(M+).

RESULTS AND DISCUSSION:

A new series of substituted Pyrazoline derivatives **(3a-i)** were prepared by reacting 9-anthraldehyde Chalcones **(2a-i)** and benzhydrazide (**1)** in glacial acetic acid medium. The excess glacial acid was removed by distillation under reduced pressure. The key intermediate 9-anthraldehyde Chalcones **(2a-i)** were prepared by the well known Claisen-Schmidt condensation reaction. The completion of the reaction is monitored by TLC. The reaction contents were cooled and poured into the crushed ice at room temperature. The precipitated solid compound is filtered and washed repeatedly with water and recrystallized by using alcohol. The reaction sequence is depicted in **Scheme-01.**

The IR spectrum of compound **3c** showed absorption band at 3046 cm^{-1} due to aliphatic –CH stretch. The absorption band at 1657 cm^{-1} (C=O stretch) and 1590 cm^{-1} (C=N stretch). The other prominent absorption bands in IR spectrum were observed at 1443cm^{-1} (C=C stretch).The ^{1}H-NMR spectrum of compound **3c** showed a doublet of doublet at δ2.36-2.38 , δ 2.42-2.44 and δ 7.28-7.30 corresponding to three proton of Pyrazolines in H_A,H_B, H_X pattern. The methyl protons is observed at δ 1.67 and appeared as singlet, integrating for three protons. Aromatic protons resonated as multiplets at δ 7.33-8.43. Further evidence for the formation of Pyrazolines **(3c)** was obtained by recording its mass spectrum. The mass spectrum of the compound **(3c)** showed molecular ion peak at m/z 444.56 (M+) in conformity with the molecular formula $C_{31}H_{28}N_2O$.

In-Vitro anti-inflammatory activity[VII]

Protein denaturation by bovine serum albumin method

Test solution (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of test solution. Control solution (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml of distilled water. Product control (0.5ml)

consists of 0.45ml of distilled water and 0.05ml of test solution. Standard solution (0.5ml) consists of 0.45ml of bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium . All of the above solutions were adjusted to pH 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20minutes and heated at 57°C for 3 minutes. After cooling, add 2.5ml of phosphate buffer to the above solution. The absorbance of the solutions was measured using UV-Visible spectrophotometer at 416nm. The results of the compounds **(3a-i)** are showed in table-2.The percentage inhibition of protein denaturation was calculated using the formula.

% inhibition = $100 \times [V\,t / V_C - 1]$

Where, Vt = absorbance of the test sample, Vc = absorbance of control

Protein denaturation by egg albumin method

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations (10-50 µg/mL) of Pyrazoline derivatives. A similar volume of double-distilled water served as the control. Next, the mixtures were incubated at 37 ± 2°C in a BOD incubator for 15minutes and then heated at 70°C for five minutes. After cooling, their absorbance was measured at 660 nm by using the vehicle as a blank. Diclofenac sodium was used as the reference drug and treated similarly for the determination of absorbance. The results of the compounds **(3a-i)** are showed in table-3. The percentage inhibition of egg albumin denaturation was calculated by using the following formula.

% inhibition = $100 \times [V\,t / V_C - 1]$

Where, Vt = absorbance of the test sample, Vc = absorbance of control.

CONCLUSION:

The present work was mainly focused on efficient synthesis of Pyrazolines incorporated with 9-anthraldehyde moiety. The new compounds were evaluated for *In-Vitro* anti-inflammatory activity. Some of the tested compounds **3h** showed very good anti-inflammatory activity in the bovine serum albumin denaturation method, when compared to the standard. The other compounds showed significant activity. In the egg albumin method all the tested compounds showed weak to moderate activity. Compounds **3d, 3h** showed good activity when compared to standard.

ACKNOWLEDGEMENTS

The authors of thankful to authorities of NGSM Institute of Pharmaceutical Sciences, Nitte- Deemed to be University, Mangalore for providing all the necessary facilities.

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Received on March 23, 2018.

Table-1: Physical data of Compounds (3a-i)

Comp	**Ar-$COCH_3$**	**Molecular formula**	**Molecular Weight**	**MP (^{0}C)**	**Yield (%)**
3a	2,4-$(Cl)_2$	$C_{30}H_{24}Cl_2N_2O$	495.40	172-74	67
3b	3,4-$(OCH_3)_2$	$C_{32}H_{30}N_2O_3$	486.56	158-60	75
3c	4-CH_3	$C_{31}H_{28}N_2O$	440.54	165-67	61
3d	4-NH_2	$C_{30}H_{27}N_3O$	441.52	185-87	68
3e	4-OCH_3	$C_{31}H_{28}N_2O_2$	456.53	179-81	67
3f	4-OH	$C_{30}H_{26}N_2O_2$	442.51	146-48	62
3g	C_6H_5	$C_{30}H_{26}N_2O_2$	426.51	135-37	67
3h	4-NO_2	$C_{30}H_{25}N_3O_3$	471.51	122-24	69
3i	4-Br	$C_{30}H_{25}BrN_2O$	505.40	150-52	71

Table-2: Data of Anti-inflammatory activity by bovine serum albumin denaturation method

Conc. (µgm/ml)	**Standard**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
10	52.7	22.53	4.63	27.1	33.43	25.12	18.81	20.53	30.71	17.34
20	53.97	23.22	12.12	28.84	38.45	28.22	30.96	28.34	39.13	28.22
30	57.32	29.7	28.4	30.15	44.32	36.82	34.72	34.76	44.74	33.82
40	67.7	34.03	31.33	34.73	48.54	39.64	38.43	38.32	48.33	38.21
50	70.7	41.6	35.15	37.24	56.86	46.93	44.43	47.33	54.32	44.32
IC_{50}	**24.65**	**59.89**	**65.38**	**64.35**	**38.63**	**50.07**	**52.17**	**50.72**	**39.63**	**52.79**

Table-3: Data of Anti-inflammatory activity by egg albumin denaturation method

Conc. (µgm/ml)	**Standard**	**3a**	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**
10	41.22	41.33	38.23	48.4	42.13	28.43	55.7	35.35	55.7	40.33
20	64.24	53.65	42.13	51.53	51.53	49.44	57.89	38.2	58.92	42.13
30	68.45	67.33	49.44	54.73	54.72	53.63	61.33	40.32	60.33	44.24
40	70.57	73.67	58.93	58.63	57.84	54.73	62.14	72.66	70.54	63.16
50	72.66	78.93	67.89	60.32	61.33	62.12	68.47	78.96	82.12	76.8
IC_{50}	**22.81**	**23.28**	**31.19**	**29.59**	**30.30**	**32.65**	**24.12**	**28.96**	**21.47**	**29.28**