Heterocyclic Letters
Vol. 9| No.3|333-337|May-July|2019
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS AND IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF PYRAZOLINE DERIVATIVES

Chaithra R Shetty, M.Vijay Kumar*, K.Chaithra, Ashwini, B.C.Revanasiddappa

**Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences of Nitte (Deemed to be University), Paneer, Deralakatte, Mangalore-575 018,Karnataka, India*
Email: vijaym24520@gmail.com

ABSTRACT

A new series of Pyrazoline derivatives **(3a-i)** were synthesized by reacting Chalcones **(2a-i)** and benzhydrazide **(1)** in glacial acetic acid medium. The synthesized compounds were constituted on the basis of spectral data. The title compounds **(3a-i)** were evaluated for *In-vitro* anti-inflammatory activity by human red blood cell (HRBC) stabilization method. Some of the tested compounds **3e,3g** have shown high anti-inflammatory activity.

KEYWORDS: Chalcones, Pyrazolines, Anti-inflammatory activity, Human red blood cell, Diclofenac sodium.

INTRODUCTION

A number of nitrogen containing heterocyclic compounds are presently available in the market because of their wide utility in the treatment of so many diseases. Pyrazolines are emerged as one of the important nitrogen containing heterocyclic compound with five membered ring structure. Since these compounds possess wide range of biological activity, chemical reactivity and industrial application, the research for synthesis of novel pyrazolines derivatives remain indispensible.

The pyrazoline nucleus is pervasive feature of the medicinal compound present in the market which possessing many pharmacological and physiological activities, hence they are useful compound in dug research. At present so many Pyrazolines molecules are available in the market viz., Anturane (uricosuric), Indoxacarb (insecticide), Phenazone (analgesic), Tandearil (anti-inflammatory) etc. Pyrazoline were reported to possess antibacterial[I], antioxidant[II], antifungal[III], anti-inflammatory[IV], analgesic[V], anti-depressant[VI] activities.

Generally Pyrazolines were synthesised by the very common intermediate chalcones. These later compounds were used in the synthesis of so many five, six and seven membered heterocyclic compounds. A well known Claisen-Schmidth condensation reaction is used for the synthesis of chalcones by varying the proportions of base in the alcohol medium. The presence of the keto group in these compounds was found to be responsible for so many activities.

Based on the above pharmacological profile of both chalcones and pyrazolines, in the present work it was planned to synthesize a novel series of pyrazolines from the key intermediate chalcones and their subsequent evaluation for anti-inflammatory activity.

EXPERIMENTAL

The melting points of the compounds were recorded by open capillary method. IR spectra were recorded by using Alpha Bruker FT-IR Spectrometer and frequencies are expressed in cm^{-1}.^{1}H-NMR spectra were recorded by using 400 MHz Bruker Avance-II NMR Spectrometer obtained in $CDCl_3$ and DMSO solution. Mass Spectrum was recorded on GC-MS Perkin Elmer Clarus 680 Spectrometer obtained by electron impact ionisation method.

Synthesis of Pyrazoline derivatives (3a-i)

Chalcones (0.01 mol) **(2a-i)** were dissolved in 30 ml of glacial acetic acid and benzhydrazide **(1)** was added. The reaction contents were refluxed for 12-21 hrs, cooled and poured into crushed ice. The solid separated was filtered off, dried and recrystallized from alcohol. The physical data of Pyrazolines **(3a-i)** is given in table-1.

(5-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl) methanone 3a: IR(KBr): 752(C-Cl), 1544 (C=C), 1603 (C=N), 1648 (C=O), 3041 (C-H). ^{1}H-NMR (400 MHz) δ (ppm)**:** 5.14-5.16 (dd,H_A,1H), 6.82 (dd,H_B,1H), 6.85-6.87 (dd, H_X, 1H), 6.96-8.83 (m, Ar-H,13H). **MS(m/z):** 405 (M^+).

(3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) (phenyl)methanone 3c: IR(KBr): 733 (C-Cl), 1523 (C=C), 1629 (C=N), 1643 (C=O), 3067 (C-H). ^{1}H-NMR (400 MHz) δ (ppm)**:** 5.52-5.53(dd,H_A,1H), 6.86-6.88 (dd,H_B,1H), 6.91-6.93 (dd, H_X, 1H), 7.03-8.00 (m, Ar-H,13H).**MS(m/z):**377 (M^+).

(3-(4-fluorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl) (phenyl)methanone 3h: IR(KBr): 748(C-Cl), 1543(C=C), 1616 (C=N), 1652 (C=O), 3064 (C-H). ^{1}H-NMR (400 MHz) δ (ppm)**:** 3.72 (s, OCH_3, 3H), 5.11(dd,H_A,1H), 5.56 (dd,H_B,1H), 6.84 (dd, H_X,1H), 6.93-7.88 (m, Ar-H,13H).

(5-(2,4-dichlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)(phenyl)methanone 3i: IR(KBr): 744 (C-Cl), 1522 (C=C), 1599 (C=N), 1646 (C=O), 3057 (C-H). ^{1}H-NMR (400 MHz) δ (ppm)**:** 6.70 (dd,H_A,1H), 6.72-6.73 (dd,H_B,1H), 6.78-6.80 (dd, H_X, 1H), 6.87-8.78 (m, Ar-H,13H).**MS(m/z):**413(M^+).

***In-vitro* Anti-inflammatory activity HRBC membrane stabilization method[VII]**

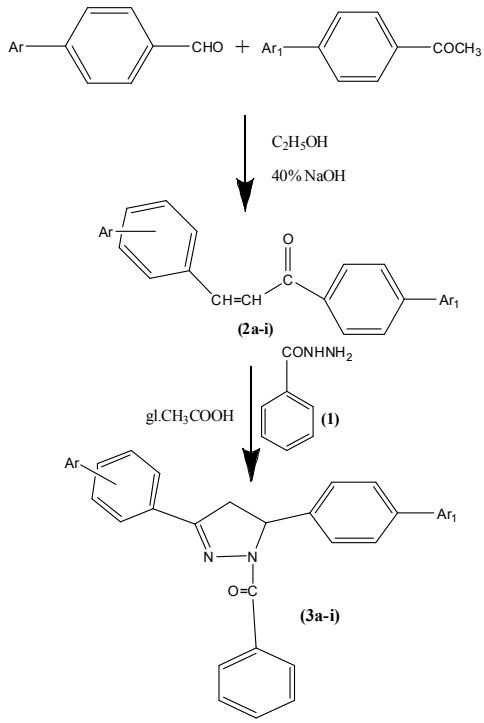
- The test sample consists of stock erythrocyte (RBC) suspension (0.50 ml) is mixed with 5 ml of hypotonic solution (50 mM NaCl) in 1ml of phosphate buffer saline (pH 7.4) containing the synthesized pyrazoline compounds with different concentrations (10-50 μg/ml)
- Standard solution (4.5ml) consist of 1ml of diclofenac sodium in normal saline, 1ml of phosphate buffer (7.4pH), 0.5ml of 10%v/v HRBC suspension and 2ml of hypotonic saline (0.25%w/v)
- Control sample (4.5ml) consists of RBC mixed with hypotonic buffered saline solution alone.
- All the above contents were incubated at room temperature for 20 min and centrifuged at 3000 rpm for 20 min and the absorbance of supernatant was measured at 560 nm. The results are showed in table-2. The percentage membrane stabilization was calculated according to the formula:

(Abs of test solution – Abs of drug control) x 100

RESULTS AND DISCUSSION

The reaction sequence for the preparation of the title compounds Pyrazolines **(3a-i)** is outlined in **Scheme-1.** Initially the key intermediates chalcones were prepared by the very

Scheme-01



common Claisen-Schmidt condensation reaction. In the next step, these chalcones will undergo selective cyclization with benzhydrazide to yield the title compounds. Designed series of the molecules were characterized by spectral data before evaluating for *In-Vitro* membrane stabilizing anti-inflammatory activity. The inhibition of hypotonicity induced HRBC membrane lysis i.e, stabilisation of HRBC membrane was taken as a measure of the anti-inflammatory activity and it was found to be concentration dependent. All the compounds were tested at a concentration of 10-50 µgm/ml. Some of the tested compounds **3e, 3g** showed very potent activity when compared to standard diclofenac sodium. The other compounds **3c, 3d, 3h, 3i** also exhibited good activity.

The IR spectrum of compounds **3a-i** showed absorption bands around 1629-1599, 1522–1543cm^{-1} due to stretching vibrations of C=N, C=C groups, respectively. The presence of the carbonyl group showed the absorption band around 1640-1650 cm^{-1}. In mass spectra, molecular ion peak is in agreement with proposed molecular weight. In the ^{1}H-NMR spectra, the aromatic protons were appeared multiplets in the region of δ 6.87-8.78. The pyrazoline protons were appeared as doublet of doublets in the ABX pattern integrating for the presence of three protons.

CONCLUSION

A series of substituted pyrazolines were prepared via the formation of chalcones, which was prepared by the reaction of aromatic aldehydes and aromatic ketones in alcohol medium, in presence of base. Further all the title compounds pyrazolines were prepared by reacting chalcones and benzhydrazide in glacial acetic acid medium and were assigned on the basis of spectral data and all the compounds were tested for anti-inflammatory activity. Some of the synthesized compounds showed good activity and these compounds can be used as very good anti-inflammatory agents.

REFERENCES

I. Bhaskar M, Reddy MK. Synthesis and antibacterial activity of prenyloxy chalcones and prenyloxy aurones. Ind J Het Chem 2011; 21: 49-52.

II. Siddiqui NJ, Wasake C, Idrees M. Synthesis antimicrobial, and antioxidant activities of some novel flavones and pyrazolines derived from chalcones. Ind J Adv Chem Sci. 2017; 5(1): 43-9.

III. Deng H, Yu ZY, Shi GY, Chen MJ, Tao K, Hou T. Synthesis and *in vitro* antifungal evaluation of 1,3,5- trisubstituted-2-pyrazoline derivatives. Chem Bio Drug Design. 2012; 79: 279–89.

IV. Amir M, Kumar H, Khan SA.Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents. Bio-org Med Chem Lett. 2008;18: 918–22.

V. Sridhar S, Rajendraprasad Y. Synthesis and analgesic studies of some new 2-pyrazolines. E-J Chem. 2012; 9: 1810–15.

VI. Ozdemir Z, Kandilci HB, Gumusel B, Calis U, Bilgin AA. Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-thienyl) pyrazoline derivatives. Archiv der Pharmazie, 2008; 341: 701–07.

VII Mounnissamy, VM, Kavimani, S, Balu, V, Drlin, QS. Evaluation of antiinflammatory and membrane stabilizing properties of ethanol extract of *Canjera rehedi*. Iranian. J. Pharmacol Therapeut. 2008; 6, 235–237.

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

Comp.	Ar-CHO	Ar^1-$COCH_3$	Molecular weight	M.P (^{0}C)	Yield (%)
3a	4-NO_2	4-Cl	405	133-35	66
3b	4-Cl	4-NH_2	375	100-02	62
3c	4-Cl	4-Cl	377	110-12	61
3d	4-Br	4-OH	421	124-26	60
3e	4-Br	3-NH_2	420	144-46	62
3f	4-NO_2	3,4-$(OCH_3)_2$	431	118-20	65
3g	4-Cl	4-OH	377	93-95	66
3h	4-F	4-OCH_3	374	158-60	68
3i	4-F	2,4-$(Cl)_2$	413	151-53	59

Table-2: *In-vitro* anti-inflammatory data of Pyrazoline derivatives (3a-i)

Comp	10 µgm/ml	20 µgm/ml	30 µgm/ml	40 µgm/ml	50 µgm/ml	IC_{50}
3a	28.45	33.55	45.88	57.84	64.58	34.61
3b	25.66	35.47	43.21	51.25	60.28	37.72
3c	25.55	36.24	48.24	56.22	68.35	33.56
3d	30.22	41.25	52.22	60.33	68.28	31.29
3e	33.28	45.29	56.33	68.22	80.25	26.88
3f	28.69	35.22	44.29	55.22	68.45	34.23
3g	31.88	40.28	51.59	60.1	71.13	30.82

3h	26.33	37.24	48.25	61.33	70.79	31.96
3i	29.68	38.44	46.32	58.24	67.54	33.09
Std	29.32	38.55	47.36	55.25	66.36	33.81

Received on June 29, 2019.