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Synthesis and In-vitro Anti-inflammatory Activity of some 1- (4-methylsulphonyl amino methyl) phenyl -3, 5-diaryl-pyrazolines

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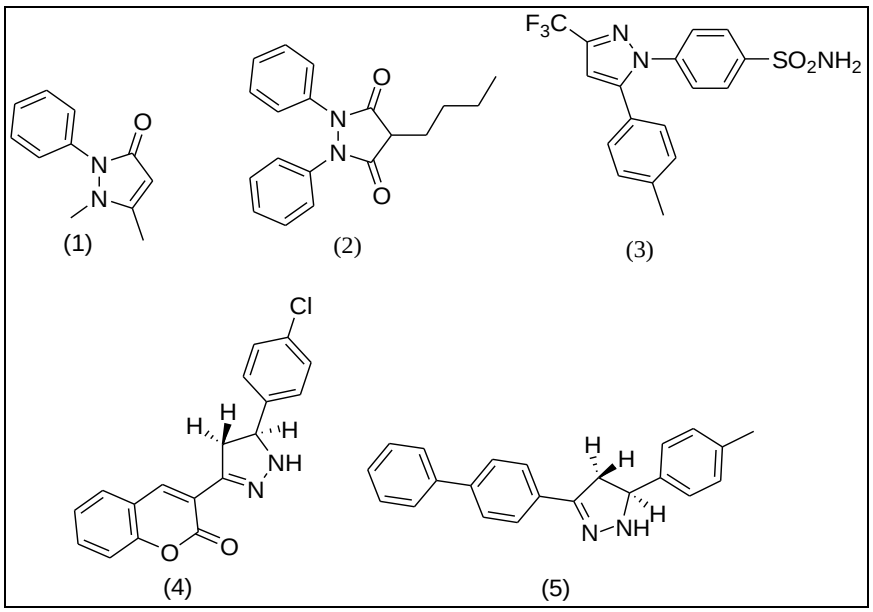
Abstract : New 1- (4-methylsulphonyl amino methyl) phenyl -3, 5-diaryl-pyrazolines (**3a-j**) bearing aryl and sulphanoamido pharmacophores were synthesized following convenient synthetic protocol as cyclocondensation of 1,3-diaryl 2-propene-1-ones (chalcones) (**2a-j**) and 4-(sulphonylamino methyl)-phenyl hydrazine hydrochloride (1) in ethanol and TEA . The 2-propene-1-ones (**1a-j**), required were freshly prepared by following Claisen-Schimidt condensation of substituted acetophenones and aryl aldehydes in alcoholic KOH. Synthesized intermediates and final compounds were characterized by FT I.R, ^{1}H NMR, MASS spectroscopic techniques and C, H, N & S analysis. Synthesized titled compounds were evaluated for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Some of the synthesized compound showed good anti-inflammatory activity as compared to standard Diclofenac sodium.
Keywords: Chalcones, Trisubstitued pyrazolines, in vitro anti-inflammatory activity, HRBC membrane stabilization.

Introduction

Pyrazoline derivatives constitute an interesting class of organic heterocyclic compounds with diverse pharmacological applications as antibacterial[1, 2, 3] antifungal[4], antitumor [5] and anti-tubercular agents[6]. Literature survey revealed that many pyrazoline derivatives have been found to possess clinical applications as NSAIDS. Antipyrine **(1)**, 2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one, was the first pyrazoline derivative used in the treatment of pain and inflammation. Phenyl butazone **(2)** and its potent metabolite celocoxib **(3)**, a prototype of pyrazoline-dione NSAIDs, are potent anti-inflammatory agents. However their use became restricted due to their GI side effects [7] besides these some pyrazoline derivatives, **(4)** and **(5)** are also reported in the literature as potent anti-inflammatory agents.[8, 9]

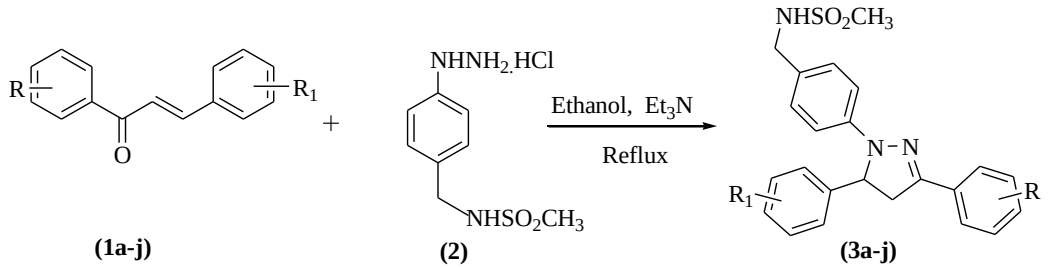


Various trisubstituted pyrazolines have been synthesized and have displayed anti-inflamatory activity.[10-15]. Most of the reported anti-inflammatory pyrazolines are effective in small doses and have less ulceration side effect. However there was scanty information on synthesis of 1, 3, 5-triaryl pyrazolines with methylsulphonyl amino methyl COX-2 selective pharmacophore. Considering therapeutic significance of the pyrazolines and the above finding here it was thought worthwhile to synthesize a new series of 1-[(4-methylsulphonylamino methyl) phenyl]-3, 5-diaryl-pyrazolines with the hope to obtain better COX-2 selective anti-inflammatory agents.

Materials and methods

Experimental

Reactions were monitored by thin layer chromatography. TLC was performed with Merk precoated TLC plates, silica gel 60F254 with thickness of 0.25mm and spots were visualized by irradiation with ultraviolet light (254 nm). Melting points were determined by open capillary method and are uncorrected. IR spectra were recorded on Bruker alpha ATR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were carried out on Bruker apparatus at DRX-300 MHz, using TMS as internal reference and DMSO-d_6 as medium. Chemical shifts (δ values) are expressed in parts per million (ppm). Mass spectra have been scanned on DART-MS (ESI^+) and on JMS 100LC, AccuTOF spectrometers. Elemental analysis was performed on Perkin–Elmer 2400 CHNS Elemental analyzer at SAIF CDRI Luckhnow, India. Starting materials **1a-g** was synthesized according to known procedures.[16, 17]



Scheme-1

Synthesis 1- (4-methylsulphonylamino methyl) phenyl -3, 5-diaryl-pyrazolines (3a-g).

A mixture of 4-(methylsulphonyl methyl) pheny hydrazine hydrochoride **(4)** (20 mmol), chalcone (20 mmol) and triethyl amine (20 mmol) was dissolved in ethanol (25 mL) and the solution was heated at reflux with stirring for 12 h. The reaction mixture was concentrated in vacuum. The crude solid appeared was filtered

and crystalized from ethanol. Similarly other compounds of the series were prepared and their characteristic physical data is recorded.

1- (4-methylsulphonylamino methyl) phenyl -3, 5- diphenyl pyrazolines (3a)

Yield 86%, mp 152-153°C**. IR (cm $^{-1}$):** 3206 (N-H stretch) 3022 (Ar-H, strech, aromatic), 2882 (C-H strech aliphatic assym.), 2819 (C-H, aliphatic symm.), 1709 (C=N), 1677 (C=C), 1503 (N-H bend), 1326 (S=O assym.) and 1104 (S=O symm.).**^{1}H NMR** (DMSO-d_6, 400MHz) δ (ppm) **:** 2.79(s, 3H, -SO_2CH_3), 3.80 (s, 2H, -CH2), 3.05 (1H, dd, J= 4.9, 17.8 Hz, 4-H $_{trans}$) 3.66 (1H,dd, J = 11.1, 18.0Hz, 4-H $_{cis}$) 5.66 (1 HH,dd, J = 4.8, 10.9 Hz, 5-H), 6.96-7.95 (m, 14H, Ar-H), 12.73 (s,1H,-NH, exchangeable with D2O). **MS** (ESI^+ mode): m/z (% intensity) **:** 406.15. **Elemental Analysis : M.F-:$C_{23}H_{23}N_3O_2S$** Found% (Calculated %) **:** C, 68.12 (68.09); H, 5.72 (5.73); N, 10.36 (10.32); O, 7.89 (7.87) ; S, 7.91(7.89).

1-**(4-methylsulphonylamino methyl) phenyl -3 (4-cholro) phenyl, 5-(4-methoxy) phenyl pyrazolines (3b) Yield 75 %, mp 89-91 °C. IR (cm $^{-1}$):** 3226 (N-H stretch) 3044 (Ar-H, strech, aromatic), 2900 (C-H strech aliphatic assym.), 2836 (C-H, aliphatic symm.), 1721 (C=N), 1688 (C=C), 1522 (N-H bend), 1342 (S=O assym.) and 1114 (S=O symm.). **^{1}H NMR** (DMSO-d6, 400MHz) δ (ppm) : 2.93 (s, 3H, -SO2CH3), 3.95 (s, 2H, -CH2), 3.17 (1H, dd, J=5.2, 18.2Hz, 4-H trans) 3.79 (1H,dd, J = 11.2, 18.1Hz, 4-H cis) 5.71 (1 HH,dd, J = 5.0, 10.8 Hz, 5-H), 7.35-8.21 (m, 12H, Ar-H), 12.80 (s,1H,-NH, exchangeable with D2O). **MS** (ESI^+ mode): m/z (% intensity) **:** 472.12 (M+2, 36).470.12 (M^+, 26). **Elemental Analysis : M.F-$C_{24}H_{24}ClN_3O_3S$:** Found% (Calculated %) **:** C, 62.82 (61.33); H,5.01 (5.15); N, 9.55 (8.94); O,10.19 (10.21);S, 7.29 (6.82).

1- (4-methylsulphonylamino methyl) phenyl -3-(4-chloro) phenyl, 5-(4-fluro) phenyl pyrazolines (3c) Yield 82%, mp 102-104 °C. IR (cm $^{-1}$): 3223 (N-H stretch) 3039 (Ar-H, strech, aromatic), 2898(C-H strech aliphatic assym.), 2832 (C-H, aliphatic symme.), 1719 (C=N), 1686 (C=C), 1520 (N-H bend), 1339 (S=O assym.) and 1112 (S=O symm.). **^{1}H NMR** (DMSO-d6, 400MHz) δ (ppm) : 2.89(s, 3H, -SO2CH3), 3.91 (s, 2H, -CH2), 3.13 (1H, dd, J=5.0, 18.1Hz, 4-H trans) 3.72 (1H,dd, J = 10.8, 18.1Hz, 4-H cis) 5.66 (1 H, dd, J = 5.0, 10.8 Hz, 5-H), 7.12-8.10 (m, 12H, Ar-H), 12.70 (s,1H,-NH, exchangeable with D2O) **MS** (ESI^+ mode): m/z (% intensity) **:** 441.13 (M+2, 13).439.12 (M^+, 40), 336 (55),281 (50) and 251 (100).**Elemental Analysis : M.F-**$C_{23}H_{22}ClN_3O_2S$**:** Found% (Calculated %) **:** C, 62.82 (62.79); H,5.01 (5.04); N, 9.50 (9.51); S, 7.24 (7.23).

1- (4-methylsulphonylamino methyl) phenyl -3 (4-chloro) phenyl, 5-phenyl pyrazolines (3d)

Yield 0.12 g (83%), Yellow solid, mp 148–150 °C.IR (cm $^{-1}$): 3220 (N-H stretch) 3036 (Ar-H, strech, aromatic), 2896(C-H strech aliphatic assyme.), 2829 (C-H, aliphatic symme.), 1713 (C=N), 1683 (C=C), 1515 (N-H bend), 1336 (S=O assym.) and 1110 (S=O symm.) **^{1}H NMR** (DMSO-d6, 400MHz) δ (ppm) : 2.89(s, 3H, -SO2CH3), 3.91 (s, 2H, -CH2), 3.13 (1H, dd, J=5.0, 18.1Hz, 4-H trans) 3.72 (1H,dd, J = 10.8, 18.1Hz, 4-H cis) 5.66 (1 H, dd, J = 5.0, 10.8 Hz, 5-H), 6.91-7.95 (m, 13H, Ar-H), 12.70 (s,1H,-NH, exchangeable with D2O). **MS** (ESI^+ mode): m/z (% intensity) **:** 441.13 (M+2, 13).439.12 (M^+, 40), 336 (55),281 (50) and 251 (100).**Elemental Analysis : M.F-**$C_{23}H_{22}ClN_3O_2S$**:** Found% (Calculated %) **:** C, 62.82 (62.79); H,5.01 (5.04); N, 9.55 (9.51); S, 7.20 (7.23).

1- (4-methylsulphonylamino methyl) phenyl -3 phenyl, 5-(4-methoxy) phenyl pyrazolines (3e) Yield 80%, mp 84-86 °C. IR (cm $^{-1}$): 3225 (N-H stretch) 3032 (Ar-H, strech, aromatic), 2893(C-H strech aliphatic assyme.), 2826 (C-H, aliphatic symme.), 1714 (C=N), 1682 (C=C), 1512 (N-H bend), 1332 (S=O assym.) and 1109 (S=O symm.). **^{1}H NMR** (DMSO-d6, 400MHz) δ (ppm) : 2.86(s, 3H, -SO2CH3), 3.88 (s, 2H, -CH2), 3.11 (1H, dd, J=5.2, 18.2Hz, 4-H trans) 3.70 (1H,dd, J = 10.9, 17.9Hz, 4-H cis) 5.65 (1 H, dd, J = 5.0, 10.8 Hz, 5-H), 7.10-8.12 (m, 13H, Ar-H), 12.70 (s,1H,-NH, exchangeable with D2O). **MS** (ESI^+ mode): m/z (% intensity) **:** 436.16.**Elemental Analysis : M.F-**$C_{24}H_{25}N_3O_3S$**:** Found% (Calculated %) **:** C, 66.15 (66.18); H,5.81 (5.79); N, 9.66 (9.65); O,11.08 (11.02); S, 7.34 (7.36).

1- (4-methylsulphonylamino methyl) phenyl -3 (4-fluro) phenyl, 5-phenyl pyrazolines (3f)

Yield 84 %, mp 120-122°C. IR (cm $^{-1}$): 3215 (N-H stretch) 3030 (Ar-H, strech, aromatic), 2890(C-H strech aliphatic assym.), 2822 (C-H, aliphatic symm.), 1711 (C=N), 1680 (C=C), 1508 (N-H bend), 1330 (S=O assym.) and 1108 (S=O symm.). **^{1}H NMR** (DMSO-d6, 400MHz) δ (ppm) : 2.81(s, 3H, -SO2CH3), 3.82 (s, 2H, -CH2), 3.07 (1H, dd, J=4.80, 17.8Hz, 4-H trans) 3.67 (1H,dd, J = 11.0, 17.9Hz, 4-H cis) 5.61 (1 H, dd, J = 4.9.0, 10.8 Hz, 5-H), 6.98-8.00 (m, 13H, Ar-H), 12.58 (s,1H,-NH, exchangeable with D2O). **MS** (ESI^+ mode): m/z (% intensity) **:** 424.15 (M+1).**Elemental Analysis : M.F-**$C_{23}H_{22}FN_3O_2S$**:** Found% (Calculated %) **:** C, 65.25(65.23); H,5.27 (5.24); N, 9.94 (9.92); S, 7.55 (7.57).

1- (4-methylsulphonylamino methyl) phenyl -3 (4-methyl) phenyl, 5-phenyl pyrazolines (3g)

Yield 68%, mp 135-137°C. IR (cm^{-1}): 3210 (N-H stretch) 3027 (Ar-H, strech, aromatic), 2887 (C-H strech aliphatic assym.), 2820 (C-H, aliphatic symm.), 1710 (C=N), 1678 (C=C), 1505 (N-H bend), 1327 (S=O assym.) and 1107 (S=O symm.). **^{1}H NMR** (DMSO-d6, 400MHz) δ (ppm) : 2.15 (s, 3H, -CH_3) 2.79(s, 3H, -SO2CH3), 3.80 (s, 2H, -CH2), 3.05 (1H, dd, J=4.8, 17.8 Hz, 4-H trans) 3.66(1H,dd, J = 10.8, 18.0Hz, 4-H cis) 5.60 (1 H, dd, J = 4.9, 10.8 Hz, 5-H), 6.82-7.85 (m, 13H, Ar-H), 12.50 (s,1H,-NH, exchangeable with D2O). **MS** (ESI^+ mode): m/z (% intensity) **:** 420.17 (M+1). **Elemental Analysis : M.F-**$C_{23}H_{22}ClN_3O_2S$**:** Found% (Calculated %) **:** C, 62.82 (62.79); H,5.01 (5.04); N, 9.55 (9.51); S, 7.29 (7.23).

In vitro anti-inflammatory activity by HRBC membrane stabilization method

Fresh whole human blood was collected and it was mixed with equal volumes of sterilized Alsever's solution (Dextrose 2%, Sodium citrate 0.8%, Citric acid 0.05%, Sodium chloride 0.42%, and Distilled water 100mL). This blood solution was centrifuged at 3000 rpm for 10min and was washed three times with equal volume of normal saline. The volume of the blood is measured and reconstituted as 10% v/v suspension with normal saline. The reaction mixture consists of 1.0mL of test sample of different concentrations in normal saline and 0.5mL of 10% HRBC suspension, 1mL of 0.2M phosphate buffer, 1mL hyposaline were incubated at 37°C for 30 min and centrifuged at 3,000 rpm for 30 mins. The hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560nm. Each experiment was performed in triplicate. Dichlorofenac sodium was used as standard and distilled water as control in this study.[18] Where the blood control represents 100% lysis or zero percent stability, the percentage of HRBC hemolysis calculated by formula,

% Hemolysis = (O.D of Test Sample / O.D of Control) × 100.

The concentration of a compound, where 50% of its maximal effect is observed (EC_{50}) using graph pad prism was measured.

Results and discussion

Chemistry

The 2-propene-1-ones (Chalcones) (**1a-j**), required were freshly prepared by following Claisen-Schimidt condensation of substituted acetophenones and aryl aldehydes in alcoholic KOH. The 2-propene-1-ones (**1a-j**), synthesized were allowed to react with 4-methyl sulphonyl amino methyl phenyl hydrazine hydrochloride in alcohol in the presence of organic base triethyl amine in good yields (68-85%) **(Scheme 1**).The ring B or C possesses substituent at 4th postion and genrally an electron withdrawing group. The structures of all new trisubstituted pyrazolines **3a-g** have been elucidated by elemental analyses, I.R., ^{1}HNMR and Mass spectroscopic measurements. In the ^{1}H-NMR spectra of compounds **3a-g** along with other diagnostic peak pyrazoline C=N stretching band (1721-1709 cm^{-1}) is recorded. In the ^{1}H-NMR spectra of compounds 3a-g the three protons attached to the C-4 and C-5 carbon atoms of the 2-pyrazoline ring system gave an ABX spin system. Chemical shifts and and the coupling constants values certainly prove a 2-pyrazoline structure. Mass spectra of synthesized compounds are in good agreement with structures proposed. The formation of the products can be satisfactorily explained by initial formation of an arylhydrazone with subsequent attack of nitrogen upon the carbon-carbon double bond and cyclization to give 2-pyrazoline.

Anti-inflammatory activity.

Anti-inflammatory agents inhibit the cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. Because human red blood cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure in estimating anti-inflammatory activity.[18] Anti-inflammatory activity done by Human Red Blood Cell (HRBC), DFS stabilizes the membrane, thereby reducing the hemolysis. Thus with the increase in the component are prevented from leaking, thus as the concentration of DFS increases, the O.D decreases thereby decreasing the effect of the tonicity caused by hypo saline. Thus, HRBC membrane stabilization method[19] was used to estimate anti-inflammatory activity. The result of the in vitro membrane stabilization activity of synthesized pyrazolines **(3a-g)** is presented in **Table 1**, **Fig.1** and **Fig.2.** According to these results all the compounds showed dose dependent inhibition of hemolysis. Compound **5b** (EC_{50} = 4.47± 0.06), **5c** (EC_{50} = 4.57± 0.07) displayed very good activity among the series as

compared to standard Diclofenac sodium (EC_{50} = 13.24). Other compound **5d** (EC_{50} = 6.95± 0.46), **5e** (EC_{50} = 10.11± 0.08) & **5f** (EC_{50}= 9.45± 0.12) also showed good anti-inflammatory activity as compared to standard DCS.

Table1. In vitro anti-inflammatory activity of synthesized compounds.

Compound	Percentage hemolysis at different concentrations				(EC_{50} ± SD)
	1ug/ml	5ug/ml	25ug/ml	50ug/ml	
5a	60.12	63.19	76.68	85.88	**18.78 ± 0.02**
5b	73.61	80.36	85.27	91.41	**4.47 ± 0.06**
5c	71.77	78.57	85.88	95.70	**4.57 ± 0.07**
5d	69.32	76.68	84.66	90.18	**6.95 ± 0.46**
5e	64.41	73.61	84.04	87.73	**10.11 ± 0.08**
5f	65.03	75.46	80.98	86.50	**9.45 ± 0.12**
5g	67.48	71.77	73.61	84.04	**16.3 ± 0.04**
DCS	61.34	63.19	65.64	70.55	**13.24 ± 0.12**

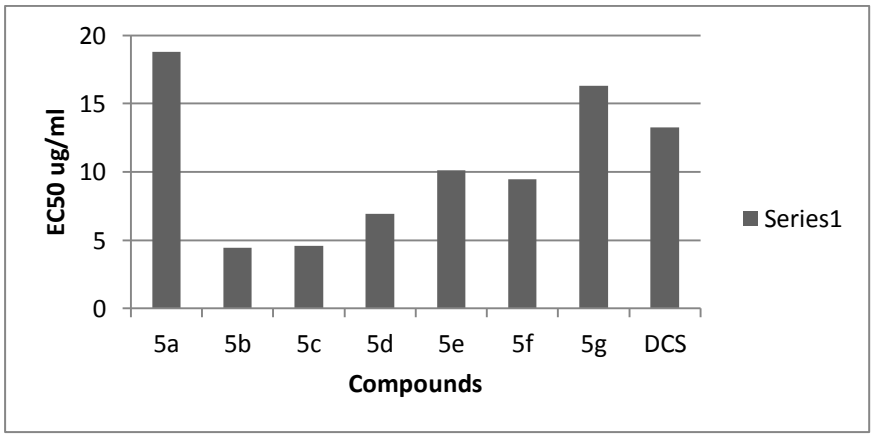
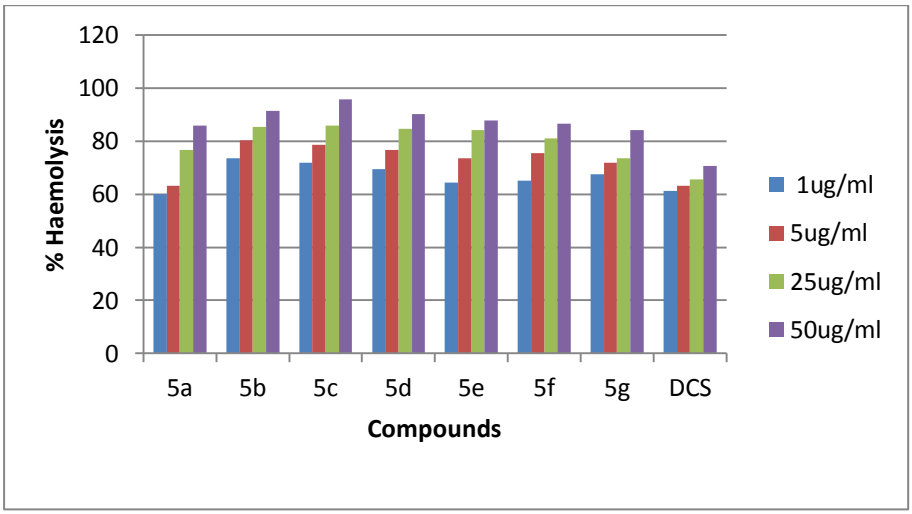


Fig.1. EC50 values of compounds (5a-g) and standard.



.Fig.2. Percent hemolysis by compounds (5a-g) and standard at various concentrations.

3. Conclusion

A new series of methylsulphonylamino methyl pharmacophore possesing trisubstituted pyrazoline derivatives has been synthesized by convenient synthetic protocols and characterized by different spectral and elemental analyses. The newly synthesized pyrazoles **(3a-g)** showed potential anti-inflammatory

activity compared with Standard Diclofenac sodium. The compounds **3b** and **3c** displayed most potential anti-inflammatory activity.

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References:

1. Satish Babulal Jadhav, Achyut S. Munde, Mohd. Shafique,Shantilal D. Rathod Synthesis, Characterization and Antimicrobial Screening of Some Novel N-Substituted-2-Pyrazolines, Derived from Chalcones, International Journal of ChemTech Research, 2017,10 (1), 139-147.
2. R. Kalirajan, S.U.Sivakumar, S. Jubie, B. Gowramma, B. Suresh, Synthesis and Biological evaluationof some heterocyclic derivatives of Chalcones, Int. J. Chem Tech Res.2009,1 (1), 27-34.
3. Sarveswari S., Vijayakumar V., Synthesis and Antibacterial Screening of 3-(4, 5-Dihydro-5-Aryl-1HPyrazol-3-YL)-4-Hydroxyquinolin-2(1H)-Ones, Int. J. Chem Tech Res. 2015, 8(6), 782-788.
4. Raj Narayan Sharma, K.P. Sharma, S.N. Dixit, Synthesis, Charecterization, and Biological Activities of some New Pyrazoline Derivatives, derived from Ethyl-2-(2, 5-dichloroanilido) Acetohydrazide,, International Journal of ChemTech Research,2010,2(3),1415-1425.
5. Muhammed Karabacak, Mehlika Dilek Altıntop , Halil İbrahim Çiftçi , Ryoko Koga,Masami Otsuka , Mikako Fujita and Ahmet Özdemir , Synthesis and Evaluation of New Pyrazoline Derivatives as Potential Anticancer Agents, Molecules, 2015, 20, 19066-19084.
6. Aftab Ahmada, Asif Husainb, Shah Alam Khanc, Mohd. Mujeebd, Anil Bhandarie, Synthesis, antimicrobial and antitubercular activities of some novel pyrazoline derivatives, Journal of Saudi Chemical Society, 2016, ss20(5), 577–584.
7. William, D. A.; Lemke, T. L. Foye's Principle of Medicinal Chemistry, 5th ed.; Williams and Wilkins: Lippincott, Philadelphia, 2002, 751.
8. Khode, S.; Maddi, V.; Aragade, P.; Palkar, M.; Ronad, P. K.; Mamledesai, S.; Thippeswamy, A. H. M.; Satyanarayana, D. Eur. J. Med. Chem. 2009, 44, 1682-8.
9. Mohammad, A.; Amir, A. Bioorg. Med. Chem. Lett. 2006, 16, 2812-2816.
10. Barsoum, F.F.; Girgis, A.S., Eur J Med Chem. 2009, 44 (5), 2172-2177.
11. Amir, M.; Kumar, H.; Khan, S.A. Bioorg Med Chem Lett. 2008, 18 (3), 918-922.
12. Rathish, I.G.; Javed, K.; Ahmad, S.; Bano, S.; Alam, M.S.; Pillai ,K.K.; Singh, S.; Bagchi ,V.Bioorg. Med. Chem. Lett. 2008, 21, 212-213.
13. Rani P, Srivastava VK, Kumar A. Eur. J. Med. Chem. 2004, 39(5),449-452.
14. Kelekc, N. G.; Yabanoglu, S.; Kupeli, E.; Salgın, U.; Ozgen, O.; Ucar, G.; Erdem, Y.; Kendi, E.; Yesiladaf, A.;Bilgin ,A. A. Bioorg. Med. Chem. 2007, 15, 5775-5786.
15. Khode S, Maddi V, Aragade P, Palkar M, Ronad P. K, Mamledesai S, Thippeswamy A. H. M, Satyanarayana, D. Eur. J. Med. Chem. 2009, 449(4),1682-8.
16. Suvitha Syam, Siddig Ibrahim Abdelwahab, Mohammed Ali Al-Mamary, Syam Mohan, Synthesis of Chalcones with Anticancer Activities, Molecules, 2012, 17, 6179-6195.
17. R. Kalirajan, S.U.Sivakumar, S. Jubie, B. Gowramma and B. Suresh, Synthesis and Biological evaluation of some heterocyclic derivatives of Chalcones, International Journal of ChemTech Research, 2009,1,1, 27-34.
18. Yerramsetty Nagaharika, Valluri kalyani, Shaik Rasheed, Ramadosskarthikeyan, Anti-inflammatory activity of leaves of Jatropha gossypifolia L. by hrbc membrane stabilization method, Journal of Acute Disease ,2013,156-158.
19. Joseph Mahimaidoss a, Charles Antony , Alex Ramani Vincent , Phytochemical screening and bioactivity studies of Phyllanthus wightianus, Journal of Pharmacy Research, 2013, 6,188-192.

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