ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis, Pharmacological Evaluation and Docking Study of Novel 3-Phenyl-5-Aryl-4, 5-Dihydro-1h-Pyrazole-1-Carbaldehyde as Anti-Inflammatory Agents.

Anna Pratima G Nikalje[1]*, Gaurav Gaikwad[1], Rajesh Nawale[2], Jaiprakash Sangshetti[1], and Firoz A Khan[1].

[1]Department of Pharmaceutical Chemistry, Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Bagh, P.B. No. 33, Aurangabad-431001, Maharashtra, India.
[2]Department of Pharmacology, Government College of Pharmacy, Aurangabad-431001, Maharashtra, India.

ABSTRACT

A series of novel 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde derivatives **4(a-j)** were obtained by refluxing chalcones **3(a-j)** and hydrazine hydrate in presence of formic acid in milder reaction conditions. The synthesized compounds **4(a-j)** were investigated for in-vivo anti-inflammatory activity in Carrageenan induced rat paw edema model. Some of the synthesized derivatives exhibited good anti-inflammatory activity compaired with diclofenac, while some derivatives have shown comparable anti-inflammatory activity to that of diclofenac. All the synthesized derivatives were found to be potent anti-inflammatory agents. Some of the derivatives were evaluated for ulcerogenic potential and their ulcer index was found to be less than the standard drug diclofenac. The molecular docking analysis was performed to understand the binding interactions of these compounds to COX-2 enzyme. The results from the present investigation suggests that 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde as a promising template for the design of new anti-inflammatory agents.

Keyword: Pyrazloe; carbaldehyde; Anti-inflammatory; NSAIDs; Rat paw edema; Molecular docking.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent [1]. Non sterodial anti-inflammatory drugs (NSAIDs) are one kind of therapeutics, widely used in the world because of their high efficacy in reducing pain and inhibiting inflammation. NSAIDs such as Celecoxib and Diclofenac can inhibit the enzyme cyclooxygenase (COX-1 and COX-2), which catalyze the biotransformation of arachidonic acid to prostaglandins (PGs) and to Thromboxane A_2. These are the mediators of pain, inflammation and fever. Hence, the development and discovery of new agents that can inhibit the COX-1 and COX-2 activity will be of importance for the controlling inflammation. Clinical studies have suggested that selective COX-2 inhibitors could cause typical COX-mediated side effects such as gastrointestinal injury, high blood pressure, and hypersensitivity this may be due to the presence of carboxyl group. Furthermore, concerns have been raised about the cardiovascular safety of selective COX-2 inhibitors and some of them, like Vioxx or Bextra, have been withdrawn from the market. Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals [2]. Pyrazolines have been reported to show a broad spectrum of biological activities including antibacterial [3], antifungal [4], anti-inflammatory [5, 6, 7] analgesic [8], antipyretic [9], diuretic [10a,b] and antidepressant activities [11]. They may prove to be clinically useful compounds and extensive studies have been devoted to aryl pyrazole derivatives such as Celecoxib, a well-known COX-2 inhibitor.

In our search for synthesis of novel NSAID with similar or greater efficacy than other NSAIDs and with little or no gastric side effects, we synthesized a series of 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde **4(a-j)** and evaluated their ability to inhibit carrageenan induced paw edema in rats. Among the numerous 1,5-diarylpyrazoles studied as possible selective COX-2 inhibitors, only few are substituted at the position 4 of the heterocyclic ring, whereas most have a substituent at the position 3.Therefore, in the present work, novel 1,3, 5-trisubstituted pyrazoline derivatives were designed and synthesized having1-carbaldehyde group, 3-phenyl and 5-aryl group **(Fig. 1)** with the aim of improving their anti-inflammatory activity and reducing their ulcerogenic properties. The aldehyde group is metabolized in vivo by the NAD (P) dependent aldehyde dehydrogenase (ALDH1) to corresponding carboxylic acids, which are excreted from the body as such or as conjugates [12]. The compounds were designed in such a way to get the butterfly structure, an essential feature, required for anti inflammatory activity. The synthesized compounds were investigated for in-vivo anti-inflammatory activity in Carrageenan induced rat paw edema model. Some of the derivatives were evaluated for ulcerogenic potential and their ulcer index was found to be less than the standard drug diclofenac. Molecular docking study was also performed using VLife MDS 4.3 to understand the binding interactions of these compounds.

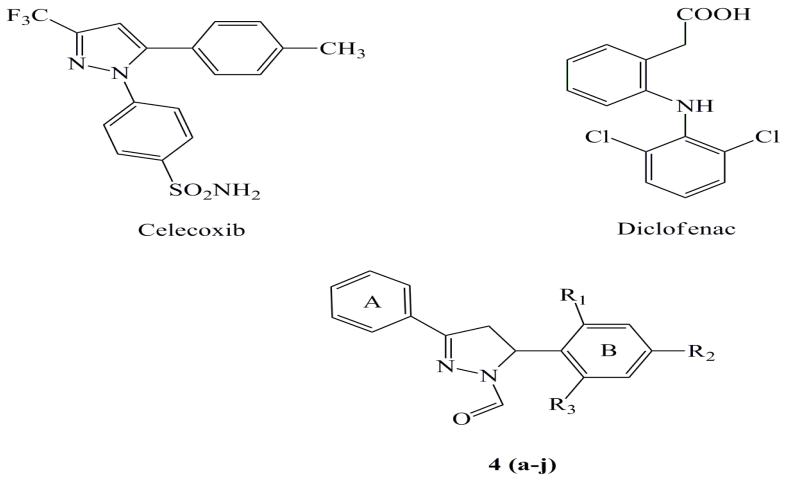


Figure 1: Structure of Celecoxib, Diclofenac and target compound

MATERIALS AND METHODS

All the chemicals used for synthesis were of Merck, Sigma, Research lab, Qualigens and Hi media. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on

JASCO FTIR (PS 4000) using KBr pallet and Brucker Avance II (400 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm), using TMS as an internal standard. The mass spectra were recorded on 410 Prostar Binary LC with 500 MS IT PDA Detectors. Elemental analyses (C, H, and N) were undertaken with a Shimadzu's FLASHEA112 analyzer and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. Digital plethysmometer (Ugo Basil 7140, Italy) was used for evaluation of anti-inflammatory activity.

The synthetic protocols employed for the synthesis of 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde are presented in **Scheme 1**. The chalcones **3(a-j)** were obtained via reaction of acetophenone and substituted benzaldehyde, in presence of aqueous alkali. The synthesized chalcones were refluxed with hydrazine hydrate in presence of formic acid to give target compounds **4(a-j)**. The purity of the synthesized compounds was checked by TLC and melting points were determined in open capillary tubes and are uncorrected. The physical characterization data of the synthesized compounds are presented in **Table 1**. The data obtained from IR, ^{1}H NMR, Elemental analysis and Mass spectroscopy confirmed the proposed structures.

Table 1: Physical Characterization data of 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde 4 (a-j)

Code	R_1	R_2	R_3	Molecular formula	Molecular weight	% Yield	M.P. ^{0}C	R_f value
4a	H	H	H	$C_{16}H_{14}N_2O$	250	90	170	0.33
4b	H	OCH_3	H	$C_{17}H_{16}N_2O_2$	280	75	120	0.48
4c	H	CH_3	H	$C_{17}H_{16}N_2O$	264	80	80	0.63
4d	Cl	Cl	H	$C_{16}H_{12}Cl_2N_2O$	319	78	169	0.61
4e	Cl	H	Cl	$C_{16}H_{12}Cl_2N_2O$	319	81	160	0.83
4f	H	CH_2SH	H	$C_{17}H_{16}N_2OS$	296	88	130	0.54
4g	Cl	H	H	$C_{16}H_{13}ClN_2O$	284	72	110	0.56
4h	H	Cl	H	$C_{16}H_{13}ClN_2O$	284	80	120	0.63
4i	H	$N(CH_3)_2$	H	$C_{18}H_{19}N_3O$	293	60	140	0.53
4j	H	NO_2	H	$C_{16}H_{13}N_3O_3$	295	65	165	0.66

Solvent of recrystallization was ethanol; Eluants used in TLC were benzene: methanol (4.5:0.5) for all compounds

Experimental

General procedure for synthesis of chalcones **3(a-j)**

Equimolar quantities of acetophenone**1** (0.01mole) and substituted aromatic aldehydes **2** (0.01 mole) were dissolved in ethanol (30 ml) and ice-cold solution of NaOH (10 ml, 10%) was added in portion keeping the temperature 25ºC with continuous stirring on magnetic stirrer. The reaction mixture was corked and kept in ice chest overnight. The completion of reaction was monitored by TLC. The product obtained was filtered, washed with cold water until neutral to litmus paper and finally washed with 5 ml absolute ethanol. The dried compounds were recrystallized from absolute ethanol.

General procedure [13] for the synthesis of 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde **4(a-j)**

A mixture of chalcones **3(a-j)** (5.0 mmoles), hydrazine hydrate (15.0 mmoles) in presence of formic acid (25 ml) was refluxed for 3 hr in heating mantle. The completion of reaction was monitored by TLC. The reaction mixture was then poured into crushed ice and water. The precipitate obtained was separated by filtration, washed with water and crystallized from absolute ethanol to obtain 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde **4 (a-j)**. The structures of the final compounds were confirmed by the spectral data and elemental analysis.

Spectral data of compounds 4(a-j)

5-Diphenyl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde (4a)

IR (KBr, *v*-max in cm^{-1}): 3084 (C-H of aromatic), 2806 (C-H of aldehyde), 1656 (C=O), 1604 (C=N), 1219 (C-N); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.21-3.88(dd, 2H, CH_2), 5.59-5.54(dd, 1H, CH), 7.27-7.78(m,10H,Ar-H), 9(s, 1H, CHO); MS m/z: 251(M+1); Anal Calcd. For $C_{16}H_{14}N_2O$ C, 76.78; H, 5.64; N, 11.19; Found C, 76.75; H,5.60; N,11.13

5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde (4b)

IR (KBr, *v*-max in cm^{-1}): 3048(C-H of aromatic), 2938(C-H of aliphatic), 2844(C-H of aldehyde), 1664(C=O), 1595(C=N), (1251) C-N, 1212 (C-O-C); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.18-3.74(dd, 2H, CH_2); 3.78(s, 1H, OCH_3), 5.47-5.52(dd, 1H, CH), 6.58-7.75 (m, 9H, Ar-H), 8.94(s, 1H, CHO)MS m/z: 281(M+1); Anal Calcd. For $C_{17}H_{16}N_2O_2$ C, 72.84; H, 5.75; N, 9.99; Found C, 72.81; H,5.74; N,9.98.

3-Phenyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (4c)

IR (KBr, *v*-max in cm^{-1}): 3061(C-H Aromatic), 2905(C-H aliphatic), 2734(C-H aldehyde), 1654(C-H aldehyde), 1654 (C=O), 1595 (C=N), 1240(C-N); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 2.35(s, 3H, CH_3), 3.17-3.72(dd, 2H, CH_2), 5.47-5.52(dd, 1H, CH), 6.59-7.72(m, 9H, Ar-H), 8.48(s, 1H, CHO); MS m/z: 265(M+1); Anal Calcd. For $C_{17}H_{16}N_2O$,C 77.25; H, 6.10; N, 10.60; Found C,77.30; H,6.13; N,10.02.

5-(2, 4-Dichlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde (4d)

IR (KBr, *v*-max in cm^{-1}): 3055(C-H aromatic), 2925(C-H of alphatic), 2839(C-H of aldehyde), 1665(C=O), 1595(C=N),1235(C-N) 754(C-Cl); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.40-3.61(dd, 2H, CH_2), 5.44-5.53(dd, 1H, CH), 6.21-7.68(m, 8H, Ar-H), 8.93(s, 1H, CHO); MS m/z: 320(M+1); Anal Calcd. For $C_{16}H_{12}Cl_2N_2O$ C, 60.21; H, 3.79; N, 8.78; Found C,60.31; H,3.80; N,8.75

5-(2,6-Dichlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde (4e)

IR (KBr, *v*-max in cm^{-1}): 3065(C-H aromatic), 2940(C-H of alphatic), 2849(C-H of aldehyde), 1665(C=O), 1595(C=N), 1240(C-N), 768(C-Cl); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.48-3.67(dd, 2H, CH_2), 5.44-5.61(dd, 1H, CH), 7.21-7.68(m, 8H, Ar-H), 8.56(s, 1H, CHO); MS m/z: 320(M+1); Anal Calcd. For $C_{16}H_{12}Cl_2N_2O$ C, 60.21; H, 3.79; N, 8.78; Found C,60.21; H,3.80; N,8.75.

5-(4-(Mercapto methyl) phenyl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbaldehyde (4f)

IR (KBr, *v*-max in cm^{-1}): 3033(C-H aromatic), 2920(C-H aliphatic), 2875(C-H of aldehyde), 1670(C=O), 1597(C=N),1245(C-N), 753(C-S); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.4(s, 1H, SH), 3.42-3.61(dd, 2H,CH_2), 5.44(s, 1H, CH), 7.30-7.7.61(m,9H,Ar-H), 8.92(s, 1H, CHO); MS m/z: 297(M+1); Anal Calcd. For $C_{17}H_{16}N_2OS$ C, 68.89; H, 5.44; N, 9.45; Found C,68.91; H,5.41; N,9.42.

5-(2-Chlorophenyl)-3-phenyl-4, 5-dihydro-1h-pyrazole-1-carbaldehyde (4g)

IR (KBr, *v*-max in cm^{-1}): 3056(C-H of aromatic), 2930(C-H of aliphatic), 2880(C-H of aldehyde), 1670(C=O), 1595(C=N), 1240(C-N), 740(C-Cl); ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.45-3.65(dd, 2H, CH_2), 6.2(s, 1H, CH),7.29-7.70(m, 9H, Ar-H), 8.72(s, 1H, CHO); MS m/z: 285(M+1); Anal Calcd. For $C_{16}H_{13}ClN_2O$ C, 67.49; H, 4.60; N, 9.84; Found C,67.51; H,4.61; N,9.82

5-(4-Chlorophenyl)-3-phenyl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde (4h)

IR (KBr, *v*-max in cm^{-1}): 3036(C-H of aromatic), 2935(C-H of aliphatic), 2856(C-H of aldehyde), 1665(C=O), 1597(C=N), 1235(C-N), 740(C-Cl) ; ^1H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.42-3.59(dd, 2H, CH_2), 5.56(dd, 1H, CH),7.39-7.70(m, 9H, Ar-H), 8.72(s, 1H, CHO); MS m/z: 285(M+1); Anal Calcd. For $C_{16}H_{13}ClN_2O$ C, 67.49; H, 4.60; N, 9.84; Found C,67.41; H,4.59; N,9.82

5-(4-(Dimethylamino) phenyl)-3-phenyl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde (4i)

IR (KBr, *v*-max in cm^{-1}): 3065(C-H of aromatic), 2930(C-H of aliphatic), 1670(C=O), 1595(C=N), 1235(C-N); ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.10(s, 3H, CH_3), 3.40-3.56(dd, 2H, CH_2), 5.42(dd, 1H,CH), 6.68-7.69(m, 9H,Ar-H), 8.90(s, 1H, CHO); MS m/z: 294(M+1); Anal Calcd. For $C_{18}H_{19}N_3O$ C, 73.69; H, 6.53; N, 14.32; Found C,73.68; H,6.52; N,14.32.

5-(4-Nitrophenyl)-3-phenyl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde (4j)

IR (KBr, *v*-max in cm^{-1}): 3061(C-H Aromatic), 2905(C-H aliphatic), 2734(C-H aldehyde), 1654(C-H aldehyde), 1654 (C=O), 1595 (C=N), 1240(C-N); ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 3.42-3.65(dd, 2H, CH_2), 5.59(dd, 1H,CH), 7.42-7.89(m, 9H,Ar-H), 8.78(s, 1H, CHO) ; MS m/z: 296(M+1); Anal Calcd. For $C_{16}H_{13}N_3O_3$ C, 65.08; H, 4.44; N, 14.23; Found C, 65.10; H,4.45; N,14.22.

Molecular Docking study

To identify potential anti-inflammatory lead compounds among compounds **4(a-j)**, docking calculations were performed using V Life MDS 4.3 into the 3D structure of the catalytic site of COX-2 enzyme (PDB code: 6COX). Docking procedure was followed using the standard protocol implemented in V Life MDS 4.3 [14]. The conformers thus obtained, were optimized (MMFF) till they reached a rms gradient energy of 0.001 kcal/mol Å. Docking of the conformers of each molecule, into the COX-2 modeled protein was done by positioning with the active site of cavity 1. The complexes were then minimized using the MMFF method, till they reached an rms gradient of 0.1 kcal/mol/ Å. The binding energy in kcal/mol and the ligand–receptor interaction energy obtained after docking the ligands into the enzyme active site can be defined as:

$$E = InterEq + InterEvdW + IntraEq + IntravdW + IntraEtor$$

Where,

InterEq: Intermolecular electrostatic energy of complex,
InterEvdW: Intermolecular vdW energy of complex,
IntraEq: Intramolecular electrostatic energy of ligand,
IntraEvdW: Intramolecular vdW energy of ligand and
IntraEtor: Intramolecular torsion energy of ligand.

The new derivatives obtained by the above mentioned procedure were undertaken for the anti-inflammatory studies by the carragenan induced rat paw edema model. Celecoxib and diclofenac were used as standard drugs. The standard and test compounds were given intraperitoneally at dose level of 10 mg/kg.Carrageenan induced rat paw edema is a non-specific inflammation resulting from a complex of diverse mediators. Since edema of this type is highly sensitive to NSAIDs, carrageenan induced rat paw edema has been accepted as a useful model for studying new anti-inflammatory agents. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs [15].

Anti-inflammatory activity

The animals were procured under the CPCSEA number CPCSEA/IAEC/Pharm. Chem/14/2011-12/56 approved by Institutional Animal Ethics Committee (IAEC). Swiss Albino rats (150-200 g) were supplied by Wockhardt Ltd Aurangabad. The animals were housed in stainless steel cages, divided into groups of five animals each and deprived of food but not water 24 h before the experiment. The anti-inflammatory activity of the compounds under investigation was studied using carrageenan induced rat paw oedema. A suspension of the test compounds **4 (a-j)** and standard drugs (celecoxib and diclofenac) in carboxy methyl cellulose (CMC) solution (0.5% w/v in water) was administered intraperitoneally in a dose level of 10 mg/kg. Control animals were treated similarly with CMC solution (0.5% w/v in water). After 1 h, 0.1 mL of freshly prepared 1% carrageenan solution was injected into the sub plantar region of the left hind paw of rats according to the method of Winter et al [16]. The volume was measured before and after carrageenan treatment at 1,2,3,6 h

with the help of digital plethysmometer (Ugo Basil 7140, Italy). Paw edema volume was compared with vehicle control group and percent reduction was calculated by formula (Vc-Vt/Vc) × 100,
Where Vc = paw volume of control group, Vt = paw volume of test group

Ulcerogenic activity

Adult albino rats weighing 150–200 g were divided into different groups consisting of five animals in each group. Animals were deprived of food but not water 24 h before the experiment.

Ulcerogenic activity was evaluated after oral administration of a suspension of test compounds **4b**, **4c**, **4f** and standard drug diclofenac in carboxy methyl cellulose solution (0.5% w/v in water) in a dose level of 100 mg/kg. Control animals were treated similarly with CMC solution (0.5% w/v in water). After 5 h the rats were sacrificed by decapitation, the stomachs were removed, collected, opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage of each stomach was examined. The mucosal damage was assessed according to following formula and scoring system [17].

Formula: $UI= UN+US+UP\times10^{-1}$

Where,

UI= Ulcer index; UN= Average number of ulcers per animal
US= Average of severity score ; UP= Percentage of animals with ulcers

Severity Score

0 = Normal colored stomach; 0.5 = Red coloration.
1 = Spot ulcer ; 1.5 = Hemorrhagic streak.
2 = Ulcers ≥ 3 but ≤5 ; 3 = ulcers > 5.

RESULTS AND DISCUSSION

Anti-inflammatory activity

Table 2: Results of anti-inflammatory activity of title compounds 4 (a-j) against Carrageenan induced rat paw edema model in rats.

Compound Code	Mean paw volume in ml ± SEM (% Inhibition)				
	0 hr	1 hr	2hr	3hr	6hr
Control	1.63±0.03	2.61±0.15	2.59±0.07	3.41±0.08	2.83±0.17
4a	1.50±0.08	2.43±0.27 (6.89)	2.3±0.15 (11.19)	2.77±0.06** (18.76)	2.50±0.08 (11.66)
4b	1.56±0.05	1.79±0.14** (31.41)	1.96±0.08* (24.32)	1.75±0.1** (46.68)	1.56±0.20** (44.87)
4c	1.48±0.24	1.65±0.05** (36.78)	1.89±0.05** (27.02)	2.14±0.16** (40.44)	1.78±0.13** (37.10)
4d	1.53±0.05	2.15±0.16 (17.62)	2.25±0.15 (13.12)	2.37±0.12** (30.49)	1.71±0.11** (39.57)
4e	1.49±0.03	2.27±0.02 (13.02)	2.41±0.1 (6.94)	2.35±0.03** (31.02)	1.65±0.28** (41.69)
4f	1.53±0.06	1.55±0.02** (40.61)	1.6±0.05** (38.22)	1.67±0.04** (51.02)	1.65±0.07** (41.69)
4g	1.57±0.03	2.25±0.21 (13.79)	2.29±0.15 (11.58)	2.39±0.12** (29.91)	2.45±0.24 (13.42)
4h	1.54±0.04	2.55±0.03 (2.29)	2.53±0.13 (2.31)	2.37±0.11** (30.49)	2.08±0.11* (26.50)
4i	1.46±0.08	2.42±0.06 (7.27)	2.55±0.2 (1.54)	2.15±0.17** (36.95)	2.24±0.11 (20.84)
4j	1.54±0.02	2.06±0.12 (21.07)	2.45±0.09 (5.40)	2.38±0.05** (30.20)	2.27±0.09 (19.78)
Celecoxib	1.53±0.06	1.73±0.16** (33.71)	1.72±0.13** (33.59)	1.65±0.12** (51.62)	1.63±0.17** (42.40)
Diclofenac	1.53±0.03	1.90±0.05** (27.20)	1.93±0.13** (25.48)	2.25±0.22** (34.01)	1.76±0.12** (37.80)

ISSN: 0975-8585

All the synthesized compounds are screened for anti-inflammatory activity at dose of 10 mg/kg intraperitoneally in carrageenan induced rat paw oedema model. Standard drug (celecoxib and diclofenac) and test compounds were injected intra peritoneally at dose 10 mg/kg. The activity assessed after 1, 2, 3, 6 h of drug administration. The synthesized derivatives **4b**, **4c**, **4f** and **4i** showed excellent anti-inflammatory activity, more than diclofenac but less than celecoxib while the derivatives **4d**, **4e**, **4h**, and **4j** showed comparable anti-inflammatory with diclofenac. All synthesized compounds exhibited moderate to good anti-inflammatory activity. The results of anti-inflammatory activity are presented in **Table 2**. Those compounds which exhibited good anti-inflammatory activity were further tested for ulcerogenic activity at dose level of 100 mg/kg. Graphical presentation of results of anti-inflammatory activity is shown in **Fig. 2**.

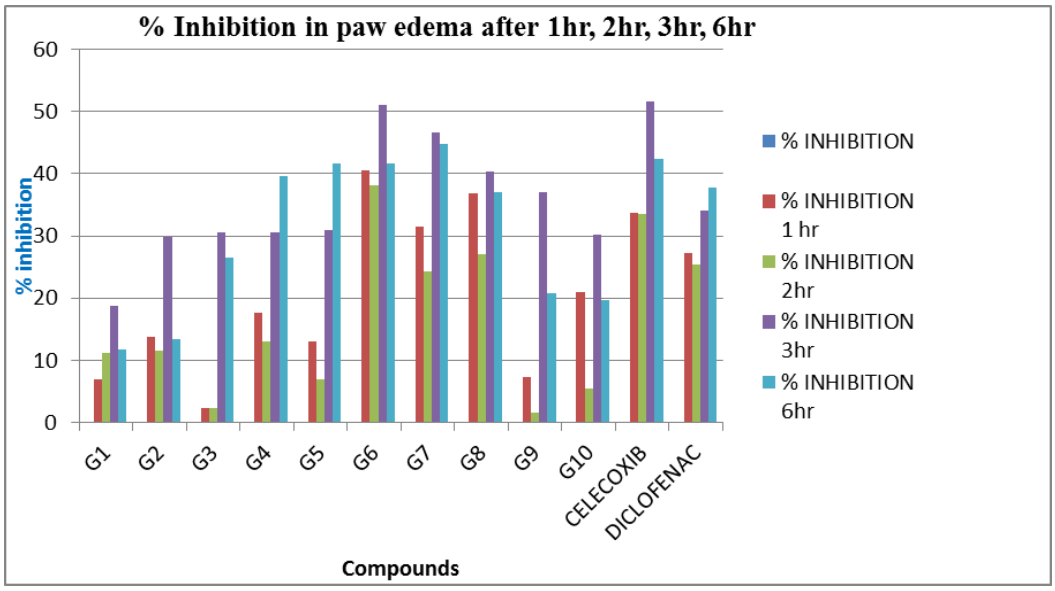


Figure 2: Graph of anti-inflammatory activity

Ulcerogenic activity

The major side effect of NSAIDs is gastric ulceration. The ulcerogenic liability was evaluated for **4b, 4c, 4f** at dose level of 100mg/kg. The gastric ulcerogenic potential was evaluated by calculating the ulcer index in treated and control animals. Diclofenac was used as standard drug for ulcerogenic potential studies. Results are given in **Table 3** that indicates these three compounds cause less gastric ulceration at the above mentioned oral dose as compared to diclofenac. Hence gastric tolerance to these compounds was better than that of standard drug diclofenac.

Table 3: Ulcerogenic effects of synthesized compounds in comparison to diclofenac

Group	Dose mg/kg	Ulcer index (mean±SEM)
Control	0.5% sodium CMC	0
Diclofenac	100	18.95±1.214*
4f	100	13.18±1.206**
4b	100	8.286±1.171**
4c	100	10.63±0.314**

The Results are expressed as mean±SEM (n=5). Data analyzed by one way ANOVA followed by Dunnett's test. $^{**}P < 0.01$, $^{*}P < 0.05$ significant from control

Molecular Docking study

The docking score along with number of hydrophobic, hydrogen bonding and the binding energy of compounds with COX-2 enzyme is presented in **Table 4**. All synthesized compounds fitted well into the binding pocket displayed good binding energies compared to the active celecoxib. The compound **4h** (-75.34 kcal/mol) and **4j** (-75.73 kcal/mol) had shown better binding when compared with celecoxib (-73.20 kcal/mol). The compounds **4g** (TYR130), **4j** (ASP125 and ALA151) and celecoxib (ASP125 and ARG469) were showing two

ISSN: 0975-8585

hydrogen bonding interaction each. All the synthesized compounds **4(a-j)** have shown good hydrophobic interactions with active site residues like ARG44, GLU46, ASP125, THR129, TYR130, ALA151, LEU152, PRO153 and ARG469.The superimposition of COX-2 enzyme with compounds **4g, 4h, 4j** and celecoxib are in **Figure 3**.

Table 4: Calculated binding docking score for COX-2

Compounds	No. of Hydrogen Bonding	No. of Hydrophobic Bonding	Binding energy
4a	10	0	-67.101875
4b	15	0	-69.416707
4c	13	0	-62.953476
4d	10	0	-62.689241
4e	10	0	-59.301272
4f	10	0	-72.321133
4g	10	2	-65.656723
4h	11	0	-75.341679
4i	17	0	-55.139032
4j	7	2	-75.737225
Celecoxib	13	2	-73.205385

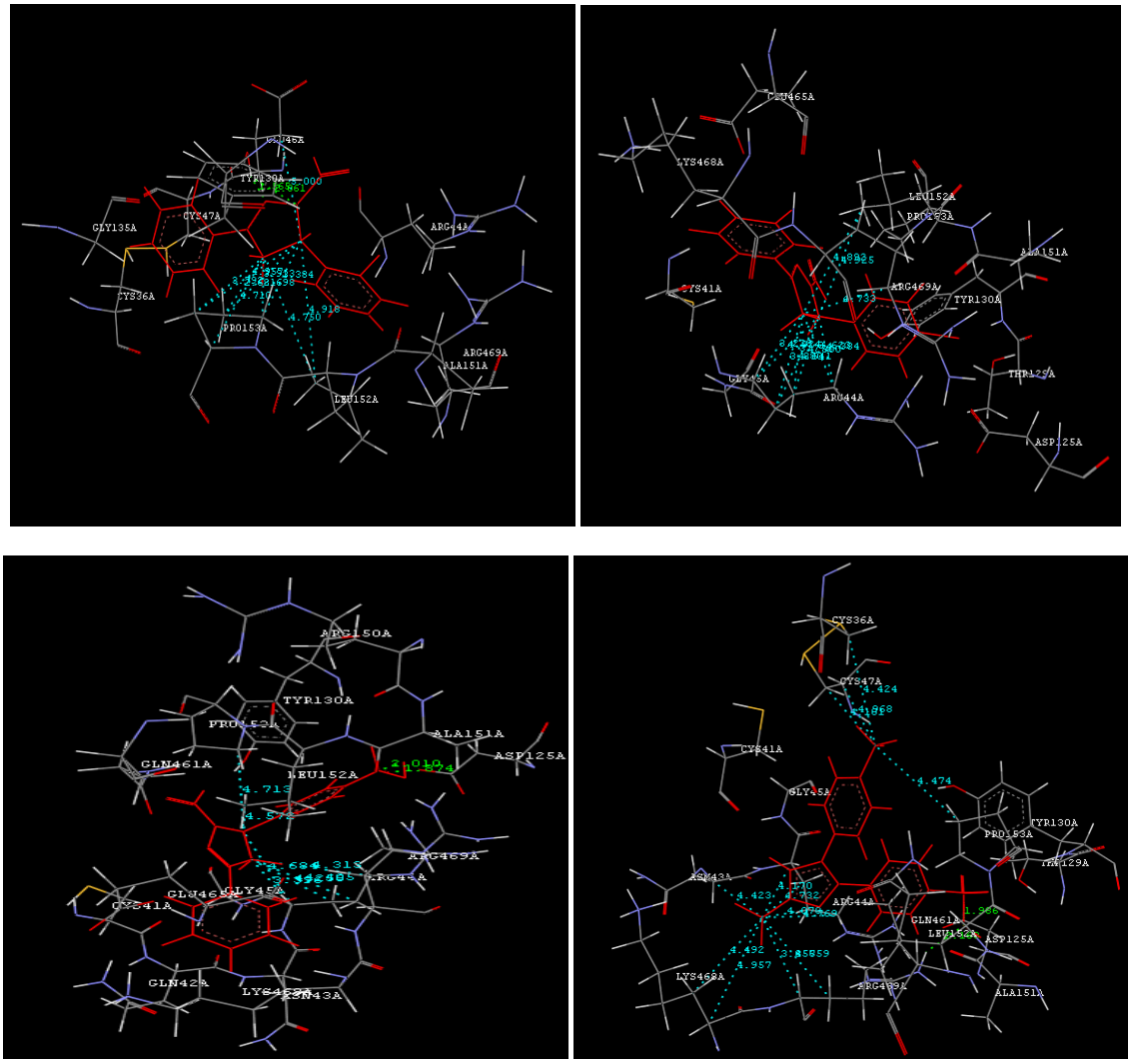
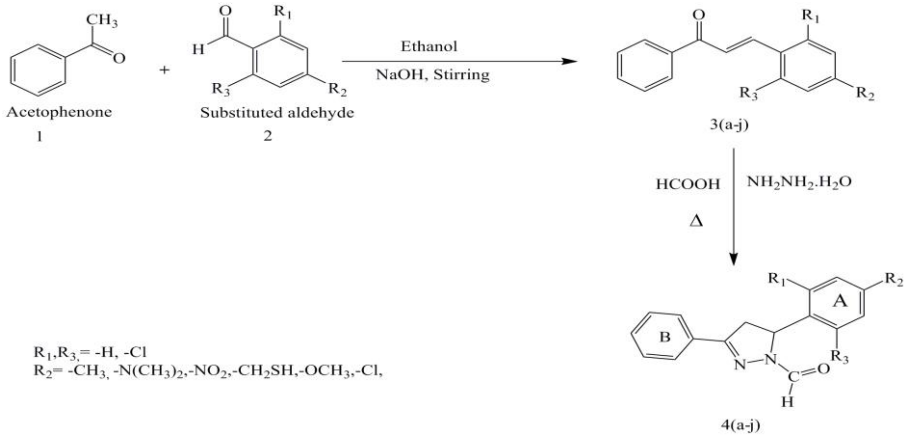


Figure 3: Docking of compounds 4g (Upper left panel), 4h (Upper right panel), 4j (Lower left panel) and celecoxib (Lower right panel). Ligands are shown in red color. Hydrogen bonds are shown in green color. Hydrophobic bonds are shown in sky blue color.

ISSN: 0975-8585



Scheme 1 Synthetic protocol for target compounds

CONCLUSION

In present research work total 10 derivatives of 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde was synthesized in good yield using moderate reaction conditions, as per the scheme of synthesis. The synthesized compounds were evaluated for anti-inflammatory activity and ulcerogenic activity. It was observed that electron donating groups like $-OCH_3$, $-CH_3$, $-CH_2SH$ and -N (CH_3) as in compound no. **4b**, **4c**, **4f**, and **4i** attached to phenyl ring (B) showed excellent anti-inflammatory activity. Synthesized derivatives that have electron withdrawing groups as in compound no. **4d**, **4e**, **4g**, **4h** having -Cl, and **4j** having nitro group exhibited moderate anti-inflammatory activity. Derivative with unsubstituted phenyl ring (B), as in compound **4a** showed least activity. In summary, a new series of pyrazole carbaldehyde derivatives were synthesized and have been identified as anti-inflammatory agents. Biological evaluation revealed that the all the target compounds displayed potent anti-inflammatory activity. The docking study of synthesized compounds **4(a-j)** have shown good hydrophobic interactions and also revealed good binding energy and shown good interactions with active site of COX-2 enzyme, compound **4h** and **4j** had shown better binding when compared with the standard Celecoxib. Therefore, our finding may aid in the strong future potential of new and safe anti-inflammatory agents for the further investigation.

ACKNOWLEDGMENTS

Authors are grateful to Mrs. Fatma Rafiq Zakaria, Chairman, Maulana Azad Education Trust and Dr. M. H. Dehghan, Principal, Y.B Chavan College of Pharmacy, Aurangabad for encouragement and support. The authors thank Principal of Government College of Pharmacy, for the assistance in pharmacological screening.

REFERENCES

[1] Tortora J, Derrikson B. Principal of anatomy and physiology, USA: John Willey and Sons, Inc 2006;11: 817-819.
[2] Sahu S, Banerjee M, Samantray A, Bahera C, Azam M. Tropical J Pharm Res 2008;961-968.
[3] Nauduri, D.; Reddy, G. Chem Pharm Bull 1998;46:1254–1260.
[4] Korgaokar S, Patil P, Shah M, Parekh H. Indian J Pharm Sci 1996:58:222-225.
[5] Nikalje, A.; Malhotra, P.; Pattan, S. Int J Pharm Pharm Sci 2010;2:21-26.
[6] Delay F (S.A. Fermeinch) Patentschriff (Switz), C.A. 1992; 117:90276 f.
[7] Menozzi G, et al. Farmaco 2003;58:795-808.
[8] Reddy B, Sneshama T, Seenhaiha B. Ind J Chem 1991;30:46-50.
[9] Hiroyuti O, Mocoto L, Hiroshi. N. Eur. Patent appl. Ep. 295695, 1988 (CL.Co7D401/6) J P Appl 1987,87/148919.
[10] a). Rajendra P, Lakshmana R, Mural K. Bioorg Med Chem Lett 2005;15:5030-5034.
[11] Brzozowski Z., Kamiński Z, Angielski S. Acta Pol Pharm 1979;36, 645-650.
[12] R Wang, T Nakajima, T Kawamoto, T Honma. Drug Metabol Dispos 2002;30:69–73.
[13] Bhandari, S.; Dangre, S.; Bothra, K.; Patil, A. Eur J Med Chem 2009;44:4622–4636.
[14] Levai A, Jeko J, Bramhabhatt D. J Heterocyclic Chem 2005;42:1231-1235.
[15] www.Vlifesciences.com.
[16] Khode S, Maddi V, Aragade P, Palkar M. Eur J Med Chem 2009; 44:1682–1688.
[17] Winter C, Risley E, Nuss G. Proc Soc Exp Biol Med 1962;111:544-7.
[18] Vogel H. (Eds.), Drug Discovery and Evaluation. Springer-verlag Publication, Berlin 2. 2000;867-872.