4,5-DIHYDRO-1H-PYRAZOLE-1-CARBALDEHYDE: SYNTHESIS, ANTI-INFLAMMATORY ACTIVITY AND DOCKING STUDY

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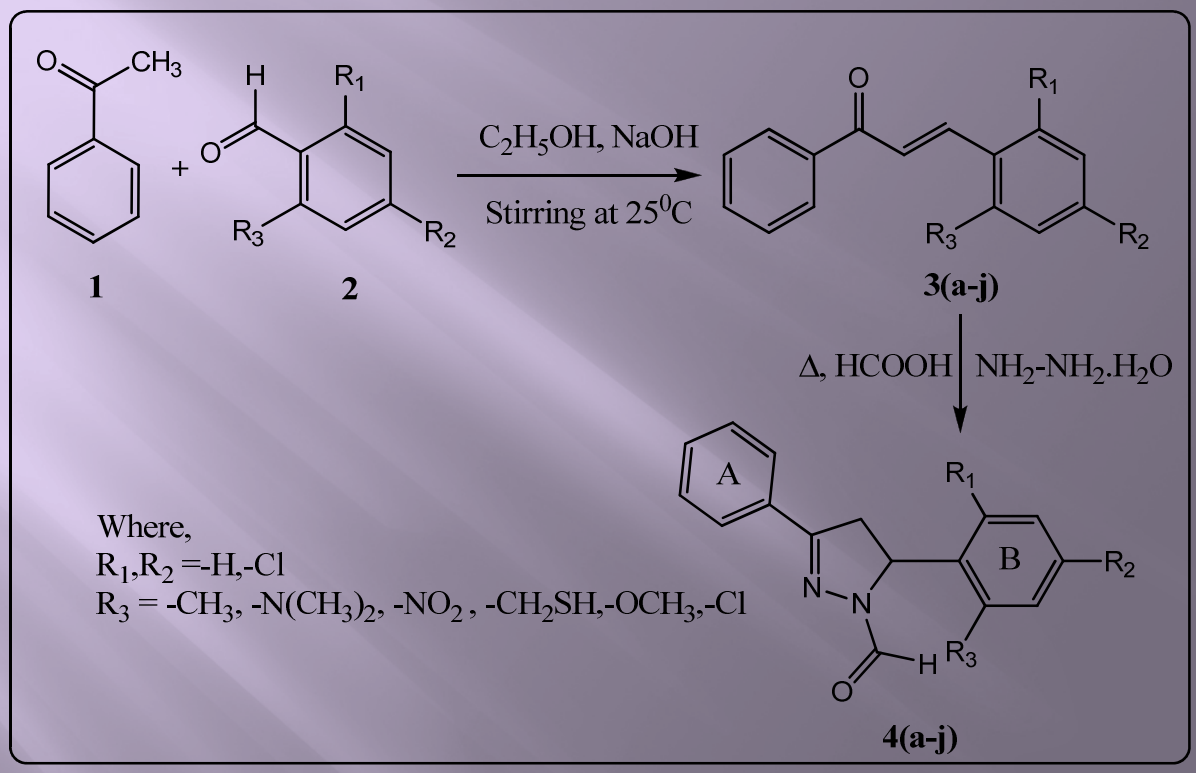
INTRODUCTION

- 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.
- Development and discovery of new agents that can inhibit the COX-1 and COX-2 activity will be of importance for the controlling inflammation.
- 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.
- The synthesized compounds were evaluated for in-vitro and in-vivo anti-inflammatory activity with ulcerogenic evaluation, molecular ocking study was also performed.

MATERIALS AND METHODS

1. Chemistry

The chalcones **3(a-j)** were obtained via condensation 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 the 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.



Scheme 1 Scheme of synthesis

2. Pharmacological evaluation

The synthesized compounds were evaluated for

- *in-vivo* anti-inflammatory activity by carrageenan induced rat paw edema model
- The ulcerogenic toxicity study

3. Molecular docking study

To identify potential anti-inflammatory lead compounds among compounds **4(a-j)**, docking calculations were performed using VLifeMDS 4.3 into the 3D structure of the catalytic site of COX-2 enzyme (PDB code: 6COX).

RESULT AND DISCUSSION

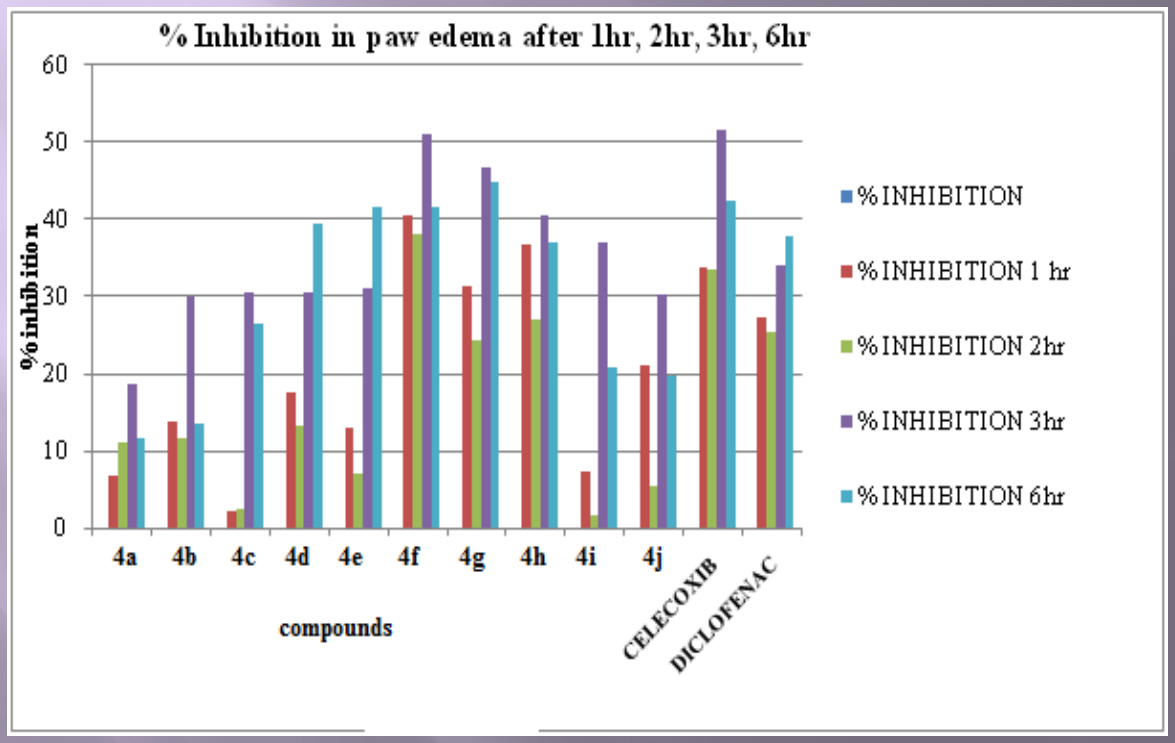
1. *in-vivo* anti-inflammatory activity by Carrageenan induced rat paw edema model

All the synthesized compounds were screened for anti-inflammatory activity at a dose of 10 mg/kg intra peritoneally 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 data of percentage inhibition of anti-inflammatory activity are presented in **Table 1**. Graphical presentation of results of anti-inflammatory activity is shown in **Fig. 1**.

Table 1 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)

Fig.1 Graph of anti-inflammatory activity



2. 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 2,** which indicates that, these three compounds caused 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 2 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**

3. Molecular docking study

All synthesized compounds fitted well into the binding pocket displayed good binding energies compared to the active celecoxib. The docking score along with number of hydrophobic, hydrogen bonding and the binding energy of compounds with COX-2 enzyme is presented in **Table 3**. 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 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 2**.

Table 3 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

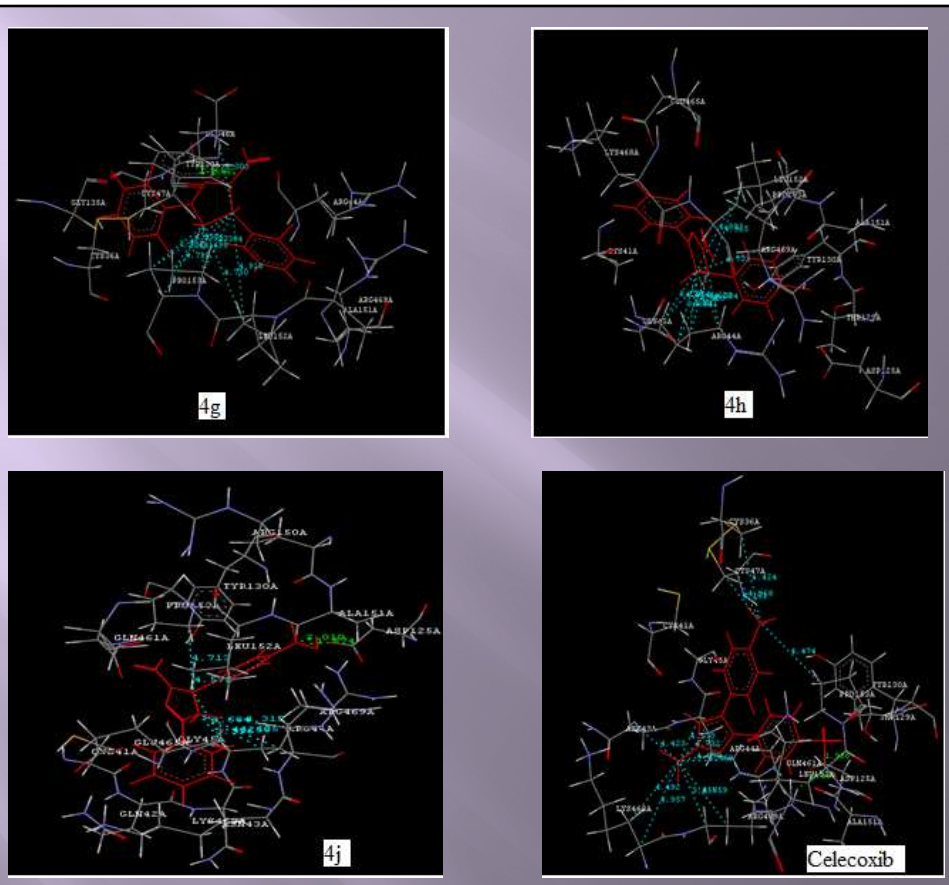


Fig. 3. Docking of compounds **4g**, **4h** , **4j** and **celecoxib** (Lower right panel). Ligands are shown in red color. Hydrogen bonds are shown in green color. Hydrophobic bonds are shown in sky.

CONCLUSION

- In the present research work total 10 derivatives of 3-phenyl-5-aryl-4, 5-dihydro-1H-pyrazole-1-carbaldehyde were synthesized using moderate reaction conditions and 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.
- Derivatives that have electron withdrawing groups as in compound no. **4d**, **4e**, **4g**, **4h** having -Cl, and **4j** having nitro group, attached to phenyl ring (B), exhibited moderate anti-inflammatory activity. Derivative with unsubstituted phenyl ring (B), as in compound **4a** showed least activity.
- The docking study of synthesized compounds also revealed good binding energy and shows good interactions with active site of COX-2 enzyme.

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THANK YOU !