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In vitro and *in silico* studies on novel N-substituted-3,5-diaryl-pyrazoline derivatives as COX-2 inhibitors and anti-inflammatory agents

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The presence of a reactive α,β-unsaturated keto group along with substituted aryl ring improves biological profile of pyrazoline nucleus. Considering this fact a study was planned to synthesize novel pyrazoline derivatives incorporated with chalcone backbone and their evaluation as COX-2 inhibitors and anti-inflammatory agents. Bovine serum albumin denaturation assay was used to measure *in vitro* anti-inflammatory activity. Molecular docking study was performed using Schrödinger-Maestro 9.0 molecular docking software and cyclooxygenase-2 (COX-II) receptor PDB ID: 4-COX. Some of the synthesized compounds showed remarkable anti-inflammatory potential. The compound (E)-3-(4-hydroxyphenyl)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)prop-2-en-1-one **6d** was found to be the most potent anti-inflammatory agents with 69.88% inhibition of protein denaturation. The outcome of docking study also supported results of *in vitro* anti-inflammatory activity and docking score for compound **6d** was found to be –6.70379 which was comparable to the co-crystallized ligand. The results reveal that the synthesized compound can serve as potential lead for the development of novel anti-inflammatory agents.

Keywords: Heterocycles, Pyrazoline, Chalcone, Anti-Inflammatory, Cyclooxygenase-II

Cyclooxygenases which catalyzes the synthesis of inflammatory mediators through arachidonic acid occurs in two isoforms COX-1 and COX-2 (Fig. 1). COX-1 present in gastrointestinal tract is responsible for gastric safety and platelet aggregation[1,2]. The COX-2 is responsible for inflammation and recently many researchers synthesized and investigated anti-inflammatory potential of various COX inhibitors[3,4]. The COX inhibitor seeks great attention of researchers not only for anti-inflammatory effect but also for cancer and Alzheimer's diseases[5,6]. Most of the anti-inflammatory compounds inhibit either form of COX or both, however selective COX-2 inhibitors offer advantage of gastric safety[7,8].

Heterocyclic ring systems offer wide range of biological activities and five-membered heterocyclic compounds containing nitrogen acquired great place in synthetic medicinal chemistry[9-11]. Pyrazolines belong to nitrogen containing five-membered heterocyclic category. Pyrazoline derivatives offers diversified biological activities such as antimicrobial[12], antinociceptive[13], antiamoebic[14], antidepressant[15], anticancer[16] and anti-inflammatory activity[17-19].

Previously, we have reported the synthesis and biological evaluations of different pyrazoline derivatives[20-23], and in the present work, we synthesized pyrazoline derivatives containing reactive chalcones backbone and investigated their potency as anti-inflammatory agent. The presence of a reactive α-β unsaturated keto group along with substituted aryl ring improves biological profile of pyrazoline nucleus[24,25].

The proposed chalcones derivatives were synthesized *via* base catalyzed condensation of substituted aryl aldehydes with substituted acetophenone according to reported procedures[26,27], further cyclization of propenone derivatives was achieved using acetylated hydrazine-hydrate as shown in Scheme 1.

Experimental Section

Melting points were determined on a capillary melting point apparatus (Lab Hosp). IR spectra were determined with a Thermo-Electron FT-IR spectrophotometer within range 400-4000 cm^{-1}. ^1H NMR spectra were recorded on a Bruker's AVANCE-III 500 MHz and 400 MHz FT NMR spectrometers. The mass spectra were measured on a Bruker micro TOF QII mass spectrometer coupled to waters acquity LC system. Elemental analyses were

performed on a Leco 932 CHNSO instrument. Thin layer chromatography (TLC) was performed on Merck aluminum-packed silica gel plates.

Initial raw materials were purchased from local supplier of Loba Chemie & S D Fine Chem Limited. All other solvents & reagents were of the highest purity and anhydrous.

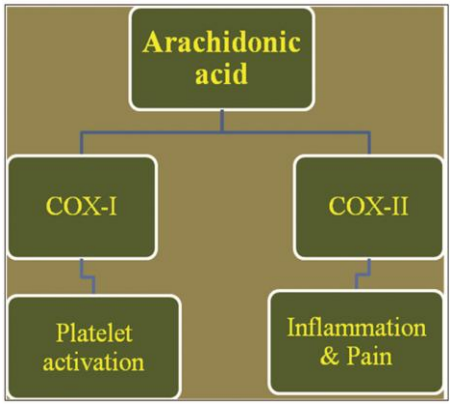


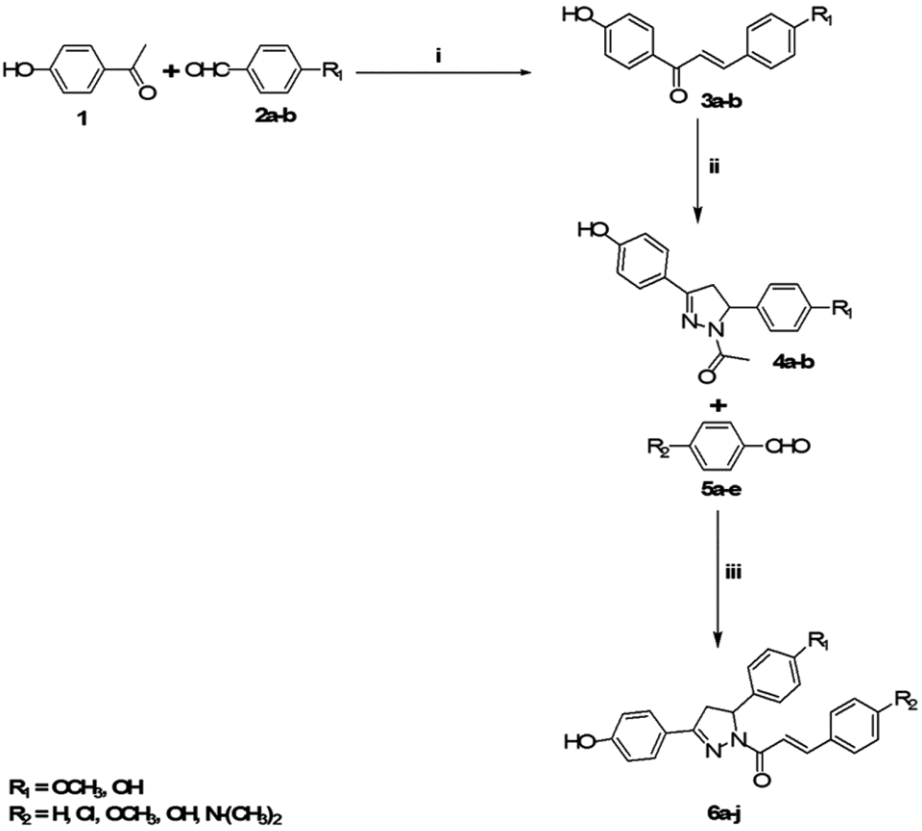
Fig. 1 — Inflammatory cascade of COX *via* arachidonic acid

Synthesis of chalcones derivatives (3a-b)

An equimolar mixture of 4-hydroxy acetophenone (**1**) and appropriate benzaldehyde derivatives (**2a-b**) in ethanol is stirred magnetically. Then NaOH solution (10%) was added drop wise to the reaction mixture with vigorous stirring. The assembly was set over the cold water bath to maintain reaction temperature from 20 to 25°C. The reaction mixtures allowed to stir 3-4 h (depending upon aryl substitution) and then cooled at room temperature and refrigerated overnight. The precipitate of crude chalcones (**3a-b**) were filtered, dried and recrystallized by rectified spirit.

Synthesis of pyrazoline derivatives (4a-b)

A 0.01 mol solution of chalcones **(3a-b)** in acetic acid (25 mL), hydrazine hydrate (0.02 mol) and 50 mL of ethanol was refluxed for 8-16 h. After completion of reaction the product was poured into crushed ice. The solid which separated out was filtered, washed with cold water, dried and recrystallized using methanol.



Reaction conditions: (i) NaOH solution (10%), ethanol, stir 3 h; (ii) Hydrazine hydrate (0.02 mol), ethanol, acetic acid, reflux 8-16 h; (iii) NaOH solution (10%), ethanol, stir 3 h; keep overnight.

Scheme 1 — Synthesis of novel pyrazoline derivatives **(6a-j)**. (i) NaOH solution (10%), ethanol, stir 3 h; (ii) Hydrazine hydrate (0.02 mol), ethanol, acetic acid, reflux 8-16 h; (iii) NaOH solution (10%), ethanol, stir 3 h; kept overnight.

General method for the synthesis of final compounds (6a-j)

Same procedure was adopted which was used to synthesize chalcones derivatives (**3a-b**); however this time pyrazoline derivatives (**4a-b**) and appropriate aromatic benzaldehydes (**5a-e**) were used instead of 4-hydroxy acetophenone (**1**) and benzaldehydes (**2a-b**), respectively.

(E)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)-3-phenylprop-2-en-1-one (6a)

Yield 55%, mp 68-70°C, Elemental Calculated for $C_{24}H_{20}N_2O_2$ (MW = 368.43): Found: C, 78.21; H, 5.45; N, 7.56. IR (ATR): 3026, 2924, 1758, 1702, 1652, 966, 746, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 4.07 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.42 (1H, d, J = 6.8 Hz), 6.87-6.95 (3H, 6.90 (ddd, J = 8.3, 1.1, 0.5 Hz), 6.91 (d, J = 15.6 Hz)), 7.15-7.31 (5H, 7.18 (tt, J = 7.7, 1.5 Hz), 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz)), 7.34-7.49 (5H, 7.44 (dddd, J = 8.0, 7.3, 2.0, 0.4 Hz), 7.37 (dddd, J = 8.0, 1.5, 1.5, 0.4 Hz), 7.46 (tt, J = 7.3, 1.5 Hz)), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz), 7.84 (1H, d, J = 15.6 Hz). LC-MS: m/z % 369 $[M+H]^+$, 100%.

(E)-3-(4-chlorophenyl)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)prop-2-en-1-one (6b)

Yield 68%, mp 63-64°C, Elemental Calculated for $C_{24}H_{19}ClN_2O_2$ (MW = 402.87): Found: C, 71.53; H, 4.76; N, 6.96. IR (ATR): 3025, 2927, 1758, 1702, 1652, 966, 746, 682, 692. ^{1}H NMR (500MHz, $CDCl_3$): δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 4.07 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.33 (1H, d, J = 6.8 Hz), 6.81-6.93 (3H, 6.90 (ddd, J = 8.3, 1.1, 0.5 Hz), 6.85 (d, J = 15.6 Hz)), 7.15-7.31 (5H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.18 (tt, J = 7.7, 1.5 Hz)), 7.53-7.61 (4H, 7.56 (ddd, J = 8.1, 1.4, 0.5 Hz), 7.58 (ddd, J = 8.3, 1.8, 0.5 Hz)), 7.65 (2H, ddd, J = 8.1, 1.4, 0.5 Hz), 7.80 (1H, d, J = 15.6 Hz). LC-MS: m/z % 403 $[M+H]^+$, 100%.

(E)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)-3(4methoxyphenyl)prop-2-en-1-one (6C)

Yield 62%, mp 64-66°C, Elemental Calculated for $C_{25}H_{22}N_2O_3$ (MW = 398): Found: C, 75.34; H, 5.58; N, 7.04. IR (ATR): 3025, 2927, 2815, 1758, 1702, 1652, 966, 746, 682. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 3.78 (3H, s), 4.06 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.32 (1H, d, J = 6.8 Hz), 6.81 (1H, d, J = 15.6 Hz), 6.90 (2H, ddd, J = 8.3, 1.1, 0.5 Hz), 7.15-7.31 (7H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.21 (ddd, J = 8.8, 1.2, 0.5 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.18 (tt, J = 7.7, 1.5 Hz)), 7.50 (2H, ddd, J = 8.8, 1.7, 0.5 Hz), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz), 7.72 (1H, d, J = 15.6 Hz). LC-MS: m/z % 399 $[M+H]^+$, 100%.

(E)-3-(4-hydroxyphenyl)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)prop-2-en-1-one (6d)

Yield 64%, mp 67-68°C, Elemental Calculated for $C_{24}H_{20}N_2O_3$ (MW = 384): Found: C, 74.96; H, 5.23; N, 7.31. IR (ATR): 3026, 2924, 1758, 1702, 1652, 966, 746, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 4.07 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.42 (1H, d, J = 6.8 Hz), 6.87-6.95 (3H, 6.90 (ddd, J = 8.3, 1.1, 0.5 Hz), 6.91 (d, J = 15.6 Hz)), 7.15-7.31 (5H, 7.18 (tt, J = 7.7, 1.5 Hz), 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz)), 7.34-7.49 (5H, 7.44 (dddd, J = 8.0, 7.3, 2.0, 0.4 Hz), 7.37 (dddd, J = 8.0, 1.5, 1.5, 0.4 Hz), 7.46 (tt, J = 7.3, 1.5 Hz)), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz), 7.84 (1H, d, J = 15.6 Hz). LC-MS: m/z % 385 $[M+H]^+$, 100%.

(E)-3-(4-(dimethylamino)phenyl)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1 yl)prop-2-en-1-one (6e)

Yield 59%, mp 60-62°C, Elemental Calculated for $C_{26}H_{25}N_3O_2$ (MW = 411): Found: C, 75.91; H, 6.11; N, 10.20. IR (ATR): 3026, 2924, 2850, 1758, 1702, 1652, 966, 746, 692. ^{1}H NMR (500MHz, $CDCl_3$): δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 2.85 (6H, s), 4.06 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.32 (1H, d, J = 6.8 Hz), 6.81 (1H, d, J = 15.6 Hz), 6.90 (2H, ddd, J = 8.3, 1.1, 0.5 Hz), 7.15-7.31 (7H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.21 (ddd, J = 8.8, 1.2, 0.5 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.18 (tt, J = 7.7, 1.5 Hz)), 7.50 (2H, ddd, J = 8.8, 1.7, 0.5 Hz), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz), 7.72 (1H, d, J = 15.6 Hz). LC-MS: m/z % 412 $[M+H]^+$, 100%.

(E)-1-(5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-phenylprop-2-en-1-one (6f)

Yield 67%, mp 59-61°C, Elemental Calculated for $C_{24}H_{19}ClN_2O_2$ (MW = 402): Found: C, 71.57; H, 4.74 N, 6.94. IR (ATR): 3025, 2927, 1758, 1702, 1652, 966, 746, 682, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ

2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 4.07 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.33 (1H, d, J = 6.8 Hz), 6.81-6.93 (3H, 6.90 (ddd, J = 8.3, 1.1, 0.5 Hz), 6.85 (d, J = 15.6 Hz)), 7.15-7.31 (5H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.18 (tt, J = 7.7, 1.5 Hz)), 7.53-7.61 (4H, 7.56 (ddd, J = 8.1, 1.4, 0.5 Hz), 7.58 (ddd, J = 8.3, 1.8, 0.5 Hz)), 7.65 (2H, ddd, J = 8.1, 1.4, 0.5 Hz), 7.80 (1H, d, J = 15.6 Hz). LC-MS: m/z % 403 $[M+H]^+$, 100%.

(E)-3-(4-chlorophenyl)-1-(5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)prop-2-en-1-one (6g)

Yield 73%, mp 62-63°C, Elemental Calculated for $C_{24}H_{18}Cl_2N_2O_2$ (MW = 436): Found: C, 65.89; H, 4.16 N, 6.42. IR (ATR): 3025, 2927, 1758, 1702, 1652, 966, 746, 682, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 4.07 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.33 (1H, d, J = 6.8 Hz), 6.81-6.93 (3H, 6.90 (ddd, J = 8.3, 1.1, 0.5 Hz), 6.85 (d, J = 15.6 Hz)), 7.15-7.31 (5H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.53-7.61 (4H, 7.56 (ddd, J = 8.1, 1.4, 0.5 Hz), 7.58 (ddd, J = 8.3, 1.8, 0.5 Hz)), 7.65 (2H, ddd, J = 8.1, 1.4, 0.5 Hz). LC-MS: m/z % 437 $[M+H]^+$, 100%.

(E)-1-(5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-(4 methoxyphenyl)prop-2-en-1-one (6h)

Yield 66%, mp 66-68°C, Elemental Calculated for $C_{25}H_{21}ClN_2O_3$ (MW = 432): Found: C, 69.38; H, 4.88 N, 6.46. IR (ATR): 3025, 2815, 2927, 1758, 1702, 1652, 966, 746, 682, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 3.78 (3H, s), 4.06 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.32 (1H, d, J = 6.8 Hz), 6.81 (1H, d, J = 15.6 Hz), 7.15-7.31 (7H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.21 (ddd, J = 8.8, 1.2, 0.5 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.50 (2H, ddd, J = 8.8, 1.7, 0.5 Hz), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz). LC-MS: m/z % 433 $[M+H]^+$, 100%.

(E)-1-(5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-(4 hydroxyphenyl)prop-2-en-1-one (6i)

Yield 70%, mp 61-62°C, Elemental Calculated for $C_{24}H_{19}ClN_2O_3$ (MW = 418): Found: C, 68.84; H, 4.56; N, 6.68. IR (ATR): 3026, 2924, 1758, 1702, 1652, 966, 746, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 4.07 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.42 (1H, d, J = 6.8 Hz), 6.87-6.95 (3H, 6.90 (ddd, J = 8.3, 1.1, 0.5 Hz), 7.15-7.31 (5H, 7.18 (tt, J = 7.7, 1.5 Hz), 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz)), 7.34-7.49 (5H, 7.44 (dddd, J = 8.0, 7.3, 2.0, 0.4 Hz), 7.37 (dddd, J = 8.0, 1.5, 1.5, 0.4 Hz), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz), 7.84 (1H, d, J = 15.6 Hz). LC-MS: m/z % 419 $[M+H]^+$, 100%.

(E)-1-(5-(4-chlorophenyl)-3-(4-hydroxyphenyl)-4,5-dihydropyrazol-1-yl)-3-(4 (dimethylamino)phenyl)prop-2-en-1-one (6j)

Yield 59%, mp 65-67°C, Elemental Calculated for $C_{26}H_{24}ClN_3O_2$ (MW = 445): Found: C, 70.01; H, 5.43; N, 9.43. IR (ATR): 3026, 2924, 2850, 1758, 1702, 1652, 966, 746, 692. ^{1}H NMR (500MHz, $CDCl_3$) : δ 2.58 (1H, dd, J = 17.2, 6.8 Hz), 3.11 (1H, dd, J = 17.2, 7.8 Hz), 2.85 (6H, s), 4.06 (1H, ddd, J = 7.8, 6.8, 6.8 Hz), 4.32 (1H, d, J = 6.8 Hz), 6.90 (2H, ddd, J = 8.3, 1.1, 0.5 Hz), 7.15-7.31 (7H, 7.26 (dddd, J = 7.7, 7.6, 1.9, 0.6 Hz), 7.21 (ddd, J = 8.8, 1.2, 0.5 Hz), 7.26 (dddd, J = 7.6, 1.5, 1.2, 0.6 Hz), 7.50 (2H, ddd, J = 8.8, 1.7, 0.5 Hz), 7.58 (2H, ddd, J = 8.3, 1.8, 0.5 Hz), 7.72 (1H, d, J = 15.6 Hz). LC-MS: m/z % 446 $[M+H]^+$, 100%.

***In vitro* anti-inflammatory activity (Anti-denaturation assay)**

Bovine serum albumin denaturation method was used to estimate *in vitro* anti-inflammatory activity of the synthesized compounds [28]. Different aliquots of the synthesized derivatives were incubated with 0.5% w/v of bovine serum albumin for 20 min at 37°C and then temperature was raised up to 57°C for 30 min. After cooling, the turbidity was measured at 660 nm using UV-Visible spectrophotometer following addition of phosphate buffered saline. Diclofenac sodium was used as reference drug. The % inhibition of protein denaturation was calculated as follows:

% Inhibition =

100 - [(optical density of test solution - optical density of product control)
÷ (optical density of test control)] × 100

Molecular docking study

Molecular docking study was performed using Schrödinger-Maestro 9.0 software and PDB ID: 4COX as 3D structure of COX-2 receptor. The docking software was run to check and delete (if present) water molecules, ions and cofactors from crystal structure of receptor. Hydrogen atoms were added and charges were assigned to the receptor

structures along with bond orders. The ligands were sketched in ACD/Chem Sketch (Freeware) and saved as MDL mol files. Further ligands were prepared using "LigPrep" (Schrödinger) and optimized by generating lowest energy for the ligand under the OPLS force field model. Receptor grid was calculated for prepared proteins so that various ligand poses may bind within the active site of receptor. The grids of receptor were generated in Glide by using default parameters of van der Waals scaling factor, charge cutoff and OPLS force field model. Cubic box of specific dimensions around the active site residues was generated for further process of molecular docking. Docking of designed ligand with optimized receptor (4COX) was performed using standard precision (SP) of Glide Docking in Schrödinger-Maestro 9.0. Final scoring for energy-minimized poses was displayed as Glide score.

Results

Chemistry

The synthesis of proposed compounds was performed as shown in Scheme 1. The intermediate chalcones **3a-b**, were obtained by Claisene Schmidt condensation reaction between 4-hydroxy acetophenone (**1**) and different aromatic aldehydes (**2a-b**) in a basic medium with 96% ethanol. The solution of appropriate chalcones (**3a-b**) in acetic acid and hydrazine hydrate along with 50 mL of ethanol was refluxed for 8-16 h to obtain pyrazoline derivatives (**4a-b)**. Final compounds (**6a-j**) were synthesized by condensing pyrazoline derivatives (**4a-b)** with different aromatic aldehydes (**5a-e**). The electron withdrawing substituent offered high yield as shown in Table 1.

Biological evaluation (*in vitro* anti-inflammatory activity)

Inhibition of bovine serum albumin denaturation method was employed to evaluate the anti-inflammatory activity of the novel pyrazoline derivatives and compared with diclofenac sodium as reference standard. All compounds showed potent anti-inflammatory activity as % inhibition of protein denaturation was measured ≥ 50 and summarized in Table 2. Compound **6d** offered maximum anti-inflammatory potential as inhibition of protein denaturation was found to be 69.88% for compound **6d**.

Molecular docking studies

The pharmacological results further established by molecular docking study using Schrödinger-Maestro 9.0 molecular docking software. The proposed compounds were docked to COX-2 receptor (PDB ID: 4COX) crystal structure. The outcomes of molecular docking study supported the findings of *in vitro* anti-inflammatory activity. The compound **6d** which possessed most potent anti-inflammatory activity also showed prompt binding interaction with receptor sites in molecular docking study as shown in Fig. 2. The glide score of compound **6d** was found to be -6.70379 comparable to the co-crystallized ligand. Particularly, compounds with electronegative substituent showed higher binding affinity which may be due to the hydrogen binding and secondary interactions (Fig. 3).

Table 1 — % Yield of synthesized pyrazoline derivatives.

S. No.	Compd	R1	R2	Yield (%)
1	**6a**	H	H	55
2	**6b**	H	Cl	68
3	**6c**	H	OCH_3	62
4	**6d**	H	OH	64
5	**6e**	H	$N\text{-}(CH_3)_2$	59
6	**6f**	Cl	H	67
7	**6g**	Cl	Cl	73
8	**6h**	Cl	OCH_3	66
9	**6i**	Cl	OH	70
10	**6j**	Cl	$N\text{-}(CH_3)_2$	59

Table 2 — Results of anti-inflammatory activity and docking score of the synthesized compounds

S. No.	Compd	Activity (% inhibition of protein denaturation)				Docking Score
		50 μg/mL	100 μg/mL	400 μg/mL	800 μg/mL	
1	**6a**	11.31	22.62	45.62	64.62	-6.11211
2	**6b**	12.12	23.73	45.55	63.11	-6.22318
3	**6c**	13.06	23.79	45.78	64.34	-6.19265
4	**6d**	14.12	24.57	48.38	69.88	-6.70379
5	**6e**	11.51	23.18	44.84	64.84	-6.25265
6	**6f**	13.12	23.97	46.16	64.25	-6.13252
7	**6g**	13.75	22.73	47.25	65.14	-6.35516
8	**6h**	14.83	25.29	51.15	65.55	-6.96201
9	**6i**	12.71	23.61	46.04	64.16	-6.21421
10	**6j**	13.65	22.71	45.82	63.89	-6.24341

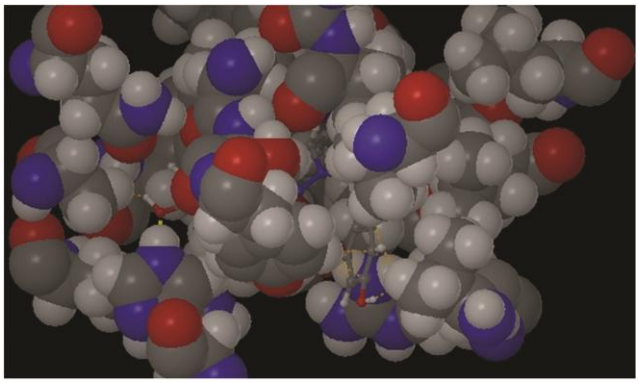


Fig. 2 — Docking pose of compound 6d (CPK model)

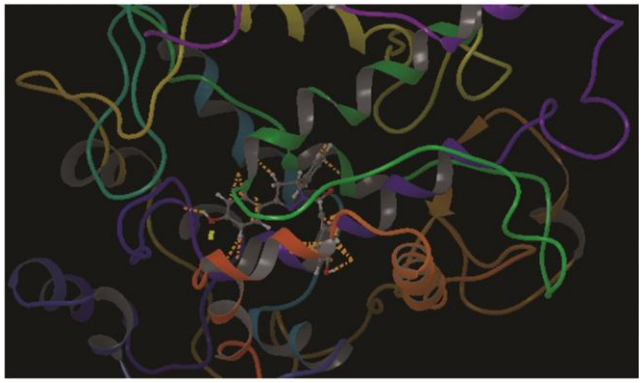


Fig. 3 — Interaction of compound 6d with binding sites of receptor (hydrogen bond depicted as yellow line)

Discussion

The kinetic of reactions revealed that haloginated derivatives took less time as compared to non-halogenated derivatives. The refluxing reaction involves formation of an aryl hydrazone with successive nucleophilic attack of nitrogen on the carbon-carbon double bond at β position. Therefore the electropositive β carbon may be considered as prime rate controlling factor since this carbon is greatly affected by the aromatic ring directly connected to it. Electron withdrawing group such as, halogen increases the positive character of β carbon resulting faster completion of reaction.

The structures of compounds were confirmed by MS, NMR & IR techniques. Proton NMR signals around δ value 3.17 and 3.71 ppm recorded as doublet of doublets (dd) were assigned to $–CH_2$ protons of pyrazoline. The single proton of pyrazoline –CH interacting with neighbored CH_2 protons and signal appeared as triplet (δ value 4.07 ppm).

The synthesized compounds possess chalcones backbone along with central pyrazoline ring and therefore offer desired pharmacological response. Substitution on heterocyclic nitrogen, electron withdrawing capacity and steric effect of substituted groups modulate pharmacological response of synthesized derivatives.

Compounds **6d** exhibited good interaction with crystal structure of COX-II receptor (PDB ID: 4COX) and this affinity attributed to the formation of hydrogen bond with amino acid residue of receptor. The binding affinity of compound may also be due the hydrophobic interaction of phenyl rings with LEU-352, GLY-526, ALA-527 and LEU-531 residue of receptor. The π–π interaction between phenyl ring of compound **6d** and receptor also contributed towards the drug-receptor interaction.

Conclusions

Novel N-substituted-3,5-diaryl-pyrazoline derivatives were synthesized in the present study. These compounds were characterized and investigated for their *in vitro* anti-inflammatory activity. The compound *(E)-3-(4-hydroxyphenyl)-1-(3-(4-hydroxyphenyl)-5-phenyl-4,5-dihydropyrazol-1-yl)prop-2-en-1-one* **(6d)** was identified as most potent anti-inflammatory agent. The outcome of docking study also supported the results of *in vitro* anti-inflammatory activity. The *in-vitro* cyclooxygenase (COX) inhibition assays (enzyme chemiluminescent kit) and *in-vivo* anti-inflammatory activity (carrageenan-induced paw edema method) are in progress.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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Conflict of Interest

No conflict of interest associated with this work

References

1 Dubois R N, Abramson S B, Crofford L, Gupta R A, Simon L S, Van De Putte L B & Lipsky P E, *FASEB J*, 12 (1998) 1063.
2 Kaufmann W E, Worley P F, Pegg J, Bremer M & Isakson P, *Proc Natl Acad Sci*, 93 (1996) 2317.
3 Kawada N, Moriyama T, Kitamura H, Yamamoto R, Furumatsu Y, Matsui I, Takabatake Y, Nagasawa Y, Imai E & Wilcox C S, *Clin Exp Nephrol*, 16 (2012) 25.
4 Fitzgerald G A & Patrono C, *Engl J Med*, 345 (2001) 433.
5 Bakhle Y S, *Br J Pharmacol*, 134 (2001) 1137.
6 Aisen P S, *J Pain Symp Manag*, 23 (2002) S35.

7 Clive M D, Jeffrey S & Stoff M D, *N Engl J Med*, 310 (1984) 563.
8 Patrono C, Patrignani P & Garcia Rodriguez L A, *J Clin Invest*, 108 (2001) 7.
9 Jain D K, Goyal N & Bhadoriya U, *Asian J Chem*, 25 (2013) 789.
10 Agrawal O P, Alam P, Alam N, Sharma P, Solanki S & Bhadoriya U, *I J P F A*, 2 (2011) 9.
11 Saini L, Jain A & Bhadoriya U, *A J P L S*, 1 (2011) 232.
12 Abdel-Wahab B F, Abdel-Aziz H A & Ahmed E M, *Eur J Med Chem*, 44 (2009) 2632.
13 Kaplancikli Z A, Zitouni G T, Zdemir A O & Chevallet P, *Eur J Med Chem*, 44 (2009) 2606.
14 Budakoti A, Bhat A R & Azam A, *Eur J Med Chem*, 44 (2009) 1317.
15 Gokhan-Kelekc N, Koyunoglu S & Yabanoglu S, *Bioorg Med Chem*, 17 (2009) 675.
16 Aouatef T, Zafer A, Dahmane T, Leyla Y, Zerrin C, Ozlem A, Merve B & Gulhan T Z, *Turk J Chem*, 40 (2016) 641.
17 Barsoum F F, Hosni H M & Girgis A S, *Bioorg Med Chem*, 14 (2006) 3929.
18 Rathish I G, Javed K, Ahmad S & Bano S, *Bioorg Med Chem Lett*, 19 (2009) 255.
19 Khode S, Maddi V, Aragade P & Palkar M, *Eur J Med Chem*, 44 (2009) 1682.
20 Bhadoriya U & Jain D K, *Int J Pharm Sci Res*, 7 (2016) 1874.
21 Bhadoriya U & Sarkar B, *J D D T*, 3 (2013) 42.
22 Sarkar B, Patel R & Bhadoriya U, *J A P E R*, 1 (2011) 43.
23 Jain D K, Bhadoriya U & Lodhi J L, *Asian J Chem*, 25 (2013) 1387.
24 Abdel-Aziz H A, Al-Rashood K A, Tahir K H & Suddek G M, *Eur J Med Chem*, 80 (2014) 416.
25 Kumar S, Bawa S, Drabu S & Kumar R, *Bentham Science*, 4 (2009) 154.
26 Fioravanti R, Bolsaco A, Manna F, Rossi F, Orallo F, Ortuso F, Alcaro S & Cirilli R, *Eur J Med Chem*, 45 (2010) 6135.
27 Ali M A, Shaharyar M, Siddiqui D, Sriram P & Yogeeswari E D, *Acta Pol Pharma Drug Res*, 63 (2007) 423.
28 Mizushima Y & Kobayashi M, *J Pharm Pharmacol*, 20 (1968) 169.