Green synthesis and biological screening of some fluorinated pyrazole chalcones in search of potent anti-inflammatory and analgesic agents

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Background and objective
Fluorinated pyrazoles are widely studied for their anti-inflammatory activities. A new series of fluorinated pyrazole chalcones (**4a-g** and **5a-g**) were synthesized and screened for anti-inflammatory and analgesic activities.
Materials and methods
Fluorinated pyrazole chalcones were synthesized using polyethylene glycol 400 (PEG-400) as an alternative reaction medium. The anti-inflammatory activity of compounds **4a-g** and **5a-g** were assessed by the carrageenan paw edema model in rats. Analgesic activity was studied by the tail-flick method in rats.
Result and conclusion
Among the series, compound **5f** was found to be the most potent anti-inflammatory agent, whereas compounds **4c**, **4f**, **4g**, **5a**, **5c**, **5d**, and **5g** showed significant anti-inflammatory activity comparable to the reference standard diclofenac sodium. Three compounds **4d**, **4f**, and **5c** showed significant analgesic activity comparable to the reference standard aspirin. From the result, compounds **4c**, **4f**, **5a**, **5c**, and **5f** have found biologically active members with an interesting dual anti-inflammatory and analgesic profile. Anti-inflammatory activities are supported by the docking study to analyze the possible interactions with the cyclooxygenase-2 enzyme.

Keywords:
ADME, analgesic activity, anti-inflammatory, molecular docking, PEG-400, pyrazole chalcones

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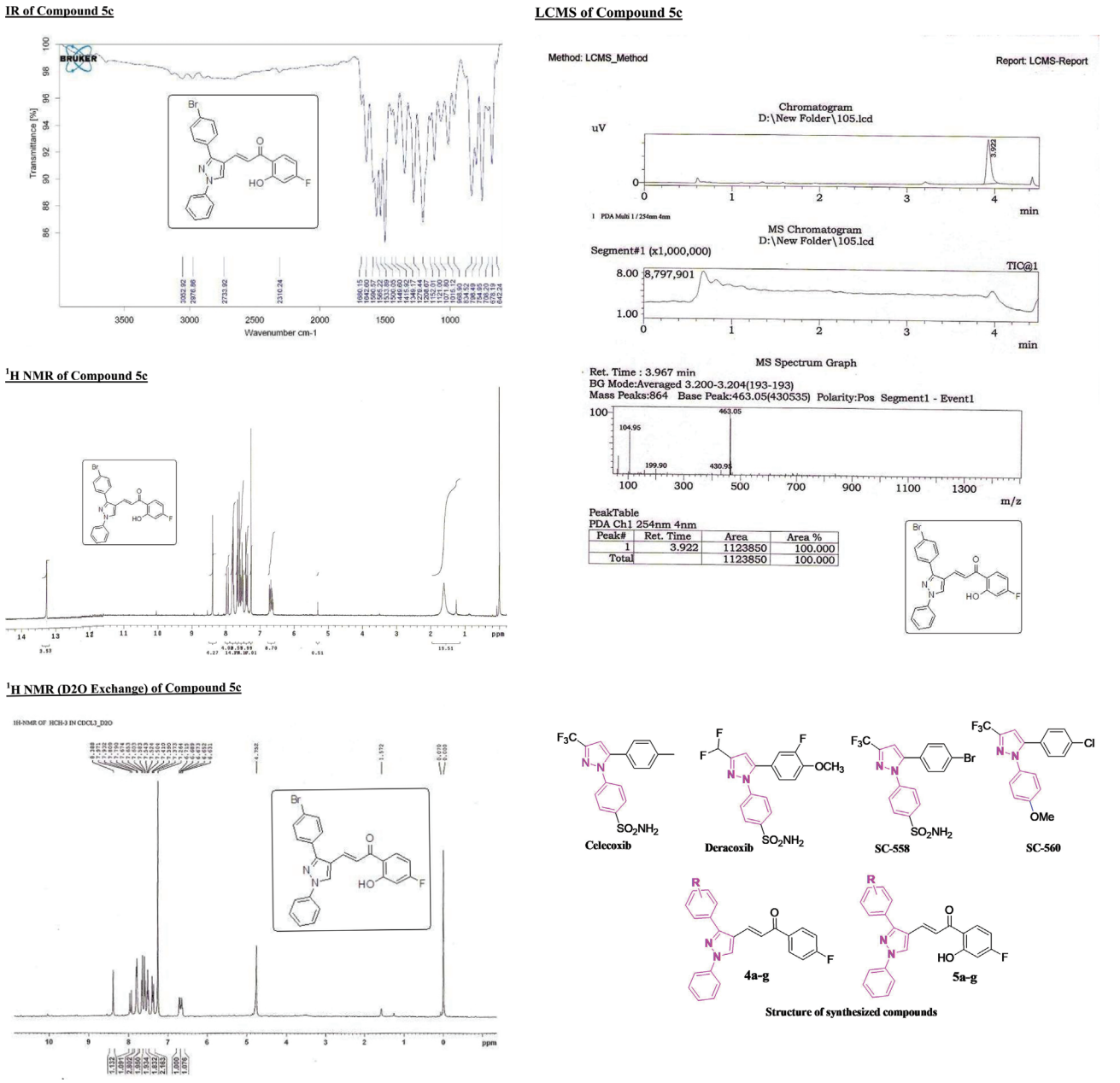
Introduction

NSAIDs, such as naproxen, ibuprofen, diclofenac, flurbiprofen, indomethacin, and aspirin are commonly used to reduce pain and inflammation in different arthritic and postoperative conditions [1]. NSAIDs have four major activities, viz., anti-inflammatory, antipyretic, analgesic, and uricosuric [2]. Their anti-inflammatory effect is mainly due to their ability to inhibit the activities of cyclooxygenases (COX), enzymes that mediate the production of prostaglandins from arachidonic acid [3,4], which is a dietary fatty acid. However, inhibition of COX may lead to undesirable side effects such as gastric ulceration, bleeding, and renal function suppression [5]. Therefore, there is a necessity of designing the new target molecules and for the development of anti-inflammatory as well as analgesic agents as an alternative to NSAIDs. The development of alternatives to NSAIDs is being attempted all over the world.

Chalcone comprises a class of compounds with important therapeutic potential. Chalcone and its derivatives exhibit various pharmacological properties including anti-inflammatory [6], antimicrobial, antioxidant [7], analgesic [8], antiproliferative [9], antitumor [10], and anticancer activities [11]. Furthermore, pyrazole derivatives are reported as potent bioactive molecules [12]. The well-known pyrazole derivatives like Celecoxib, Deracoxib, SC-558, and SC-560 (Fig. 1) are COX inhibitors with less gastrointestinal side effects [13]. The survey of literature also shows that the compounds containing pyrazole moiety exhibited excellent anti-inflammatory [14], analgesic, antimicrobial [15], anti-infective [16], and antitumor [17] activities. In addition to this, the presence of an enone function in chalcone with pyrazole moiety also enhanced the biological activity [18]. The fluorinated pyrazole derivatives are recently reported as anti-inflammatory [19,20], analgesic, antioxidant [21,22], anti-infective [23], and antitubercular agents [24].

Figure 1



Structures of known NSAIDs and title compounds with its common pharmacophore features.

On the other hand, PEG solvents are known to be inexpensive, easily available, thermally stable, recyclable, biologically compatible, nontoxic, and a water-soluble compound that does not hydrolyze on long storage [25,26]. Due to these advantages, PEGs of different molecular weights are extensively used as solvents or vehicles in various pharmaceutical industries. The use of PEG as a green and alternative reaction medium in organic reactions is relatively recent [27–29]. In view of these observations, we report herein the synthesis of some new series of fluoro-substituted pyrazole chalcones using PEG-400 and evaluate them as a potential template for dual anti-inflammatory and analgesic agents.

Materials and methods

Materials and reagents

Melting points were determined with a digital thermometer and were uncorrected. Infrared (IR) spectra were recorded on an Fourier-Transform Infrared (FT-IR) spectrometer (PerkinElmer, Waltham, Massachusetts, United States) using the KBr disk method. Proton nuclear magnetic resonance (^{1}HNMR) spectra were recorded on ^{1}HNMR (Varian-NMR-mercury 300 MHz) spectrometer in $CDCl_3$ as a solvent. All chemical shifts (δ) are quoted in parts per million downfield from tetramethylsilane (TMS) and coupling constants

(J) are given in hertz. Abbreviations used in the splitting pattern were as follows: s=singlet, d=doublet, t=triplet, q=quintet, m=multiplet. The mass spectra were obtained with a (Shimadzu, Kyoto, Japan) LCMS-2010 EV. All the reagents and solvents used were of analytical grade and were used as supplied, unless otherwise stated. Thin-layer chromatography was performed on precoated silica plates (Merkskiesegel 60F254, sheet thickness 0.2 mm). The spots could be visualized easily under ultraviolet light.

Synthesis of fluoro-pyrazole chalcones (4a-g)

A mixture of substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **3** (1 mmol/l) and 4-fluoro-acetophenone (1 mmol/l) was dissolved in 15 ml PEG-400. To this mixture, sodium hydroxide (20%, 1 ml) was added and the reaction mixture was stirred at 40–50°C temperature for 1 h. The completion of the reaction was monitored by Thin-Layer Chromatography (TLC). The reaction mixture was then poured into 100 ml ice-cold water. The product was separated, filtered, and processed out. The products obtained were purified by recrystallization from ethanol to afford pure compounds **4a-g**.

1-(4-Fluoro-phenyl)-3-[3-(4-fluoro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***4a***

Yield 85%; M.P. 180°C, IR (KBr): 1659, 1523, 1495, 1209, 832, 3058, 3126 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ8.36 (s, 1 H, H-pyrazole), 8.0 (d, 2 H, ArH), 7.85 (d, 1 H, *J=15* Hz, -CH=CH-), 7.8 (d, 2 H, ArH, *J*=7.5 Hz), 7.7 (dd, 2 H, ArH), 7.5 (t, 2 H, ArH, *J*=7.8 Hz and 8.1 Hz), 7.35 (d, 1 H, *J=15* Hz, -CH=CH-), 7.2 (m, 5 H, N-ArH); ^{13}CNMR (100 MHz, DMSO-d_6, δ in ppm): 187.2, 166.6, 164.0, 163.2, 160.7, 138.8, 134.1, 131.5, 131.0, 128.8, 128.3, 127.2, 121, 118, 115.8, 115.6; LCMS: m/e 387 (M^{+1}).

1-(4-Fluoro-phenyl)-3-[3-(4-chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***4b***

Yield 90%; M.P. 170°C, IR (KBr): 1653, 1581, 1489, 1200, 816, 3117, 3181 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ8.36 (s, 1 H, H-pyrazol), 8.0 (d, 2 H, ArH), 7.84 (d, 1 H, *J=15* Hz, -CH=CH-), 7.8 (d, 2 H, ArH, *J*=8.1 Hz), 7.65 (d, 2 H, ArH, *J*=8.1 Hz), 7.16 (t, 2 H, ArH), 7.30 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.36–7.54 (m, 5 H, N-ArH); LCMS: m/e 403 (M^{+1}).

1-(4-Fluoro-phenyl)-3-[3-(4-bromo-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***4c***

Yield 87%; M.P. 166°C, IR (KBr): 1657, 1588, 1493, 1207, 3053, 3120 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ8.36 (s, 1 H, H-pyrazol), 8.02 (d, 2 H, ArH), 7.85 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.8 (d, 2 H, ArH, *J*=8.1 Hz), 7.64 (d, 2 H, ArH, *J*=8.4 Hz), 7.16 (t, 2 H, ArH, *J*=8.4 Hz), 7.30 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.4–7.56 (m, 5 H, N-ArH); LCMS: m/e 447 (M^+).

1-(4-Fluoro-phenyl)-3-[3-(4-methyl-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***4d***

Yield 82%; M.P. 140°C, IR (KBr): 1622, 1587, 1493, 1212, 821, 3055, 3114 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ8.35 (s, 1 H, H-pyrazol), 8.0 (d, 2 H, ArH), 7.90 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.8 (d, 2 H, ArH, *J*=8.1 Hz), 7.6 (d, 2 H, ArH, *J*=8.1 Hz), 7.51 (t, 2 H, ArH, *J*=7.8 Hz), 7.27 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.14–7.4 (m, 5 H, N-ArH), 2.4 (s, 3 H, $-CH_3$); LCMS: m/e 383 (M^{+1}).

1-(4-Fluoro-phenyl)-3-[3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***4e***

Yield 84%; M.P. 138°C, IR (KBr): 1652, 1577, 1403, 1208, 823, 2983, 3115 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ8.34 (s, 1 H, H-pyrazol), 8.0 (d, 2 H, ArH), 7.91 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.8 (d, 2 H, ArH, *J*=8.1 Hz), 7.6 (d, 2 H,ArH, *J*=8.1 Hz), 7.5 (t, 2 H, ArH, *J*=7.8 Hz), 7.30 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.2 (m, 5 H, N-ArH), 3.9 (s, 3 H, $-OCH_3$); LCMS: m/e 399 (M^{+1}).

1-(4-Fluoro-phenyl)-3-[3-(3-nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***4f***

Yield 78%; M.P. 190°C, IR (KBr): 1661, 1597, 1499, 1342, 1299, 1216, 819, 3063, 3129 cm^{--1}; ^{1}H NMR ($CDCl_3$): δ8.64 (s, 1 H, ArH,), 8.42 (s, 1 H, H-pyrazol), 8.32 (dd, 1 H, ArH, *J*=8.7 Hz), 8.04 (d, 2 H, ArH, 7.2 Hz), 7.98 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.84 (d, 1 H, ArH, *J*=7.5 Hz), 7.80 (dd, 2 H, ArH, *J*=7.5 Hz), 7.7 (t, 1 H, ArH, *J*=8.1 Hz), 7.39 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.2-7.6 (m, 5 H, N-ArH); LCMS: m/e 414 (M^{+1}).

1-(4-Fluoro-phenyl)-3-[3-phenyl-1-phenyl-1H-pyrazol-4-yl]-propenone ***4g***

Yield 90%; M.P. 165°C, IR (KBr): 1656, 1579, 1488, 1207, 750, 3120, 3165 cm^{-1}; ^{1}HNMR ($CDCl_3$): δ8.38 (s, 1 H, H-pyrazol), 8.0 (d, 2 H, ArH), 7.91 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.8 (d, 2 H, ArH, *J*=7.8 Hz), 7.7 (dd, 2 H, ArH, *J*=7.5 Hz), 7.5 (m, 5 H, N-ArH), 7.4 (t, 1 H, ArH, *J*=8.1 Hz), 7.30 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.14 (t, 2 H, ArH, *J*=8.7 Hz); LCMS: m/e 369 (M^{+1}).

Synthesis of hydroxyl-fluoro-pyrazole chalcones (5a-g)

A mixture of substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **3** (1 mmol/l) and 4-fluoro-2-hydroxy-

acetophenone (1 mmol/l) was dissolved in 15 ml PEG-400. To this mixture, sodium hydroxide (20%, 1 ml) was added and the reaction mixture was stirred at 40–50°C temperature for 1 h. The completion of the reaction was monitored by TLC. The reaction mixture was then poured into 100 ml ice-cold water. The product was separated, filtered, and processed out. The products obtained were purified by recrystallization from ethanol to afford pure compounds **5a-g**.

1-(4-Fluoro-2-hydroxy-phenyl)-3-[3-(4-fluoro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***5a***

MP: 142°C, IR (cm^{-1}): 1658, 1599, 1524, 1492, 1230, 832, 2918, 3126; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 8.34 (s, 1 H, H-pyrazole), 7.33–7.37 (d, 1 H, *J*=16 Hz, -CH=CH-), 7.85–7.89 (d, 1 H, *J*=16 Hz, -CH=CH-), 6.89–7.90 (m, 12 H, ArH), 10.37 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=403 (M^{+1}).

3-[3-(4-Chloro-phenyl)-1-phenyl-1H-pyrazol-4-yl]- 1-(4-fluoro-2-hydroxy-phenyl)-propenone ***5b***

MP: 148°C, IR (cm^{-1}): 1658, 1599, 1524, 1492, 1230, 832, 2918, 3126; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 8.34 (s, 1 H, H-pyrazole), 7.33–7.37 (d, 1 H, *J*=16 Hz, -CH=CH-), 7.85–7.89 (d, 1 H, *J*=16 Hz, -CH=CH-), 6.89–7.90 (m, 12 H, ArH), 10.35 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=419 (M^{+1}).

3-[3-(4-Bromo-phenyl)-1-phenyl-1H-pyrazol-4-yl]- 1-(4-fluoro-2-hydroxy-phenyl)-propenone ***5c***

MP: 156°C, IR (cm^{-1}): 3639, 2983, 2835, 1676, 1635, 1571, 1533, 1200, 1118, 832; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 8.40 (s, 1 H, H-pyrazole), 7.36–7.41 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.93–7.98 (d, 1 H, *J*=15 Hz, -CH=CH-), 6.5–8.0 (m, 12 H, ArH); 10.4 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=463 (M^+).

1-(4-Fluoro-2-hydroxy-phenyl)-3-(1-phenyl-3-p-tolyl-1H-pyrazol-4-yl)-propenone ***5d***

MP: 192°C, IR (cm^{-1}): 1658, 1599, 1524, 1492, 1230, 832, 2918, 3126; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 2.46 (s, 3 H, CH_3), 8.34 (s, 1 H, H-pyrazole), 7.33–7.37 (d, 1 H, *J*=16 Hz, -CH=CH-), 7.85–7.89 (d, 1 H, *J*=16 Hz, -CH=CH-), 6.89–7.90 (m, 12 H, N-ArH), 10.04 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=399 (M^{+1}).

1-(4-Fluoro-2-hydroxy-phenyl)-3-[3-(4-methoxy-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***5e***

M.P.: 208°C, IR (cm^{-1}): 3775, 2979, 2734, 1689, 1635, 1567, 1535, 1206,1110, 753; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 3.87 (s, 3 H, $-OCH_3$); 8.25 (s, 1 H, H-pyrazole), 6.83–6.88 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.74–7.79 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.0–8.0 (m, 12 H, ArH); 10.06 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=415 (M^{+1}).

1-(4-Fluoro-2-hydroxy-phenyl)-3-[3-(3-nitro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-propenone ***5f***

M.P.: 195°C, IR (cm^{-1}): 1658, 1599, 1524, 1492, 1230, 832, 2918, 3126; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 8.34 (s, 1 H, H-pyrazole), 7.33–7.37 (d, 1 H, *J*=16 Hz, -CH=CH-), 7.85–7.89 (d, 1 H, *J*=16 Hz, -CH=CH-), 6.89–7.90 (m, 12 H, N-ArH), 10.39 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=430 (M^{+1}).

3-(1,3-Diphenyl-1H-pyrazol-4-yl)-1-(4-fluoro-2-hydroxy-phenyl)-propenone ***5g***

M.P.: 126°C, IR (cm^{-1}): 3183, 2833, 1665, 1593, 1517, 1216, 1117,750; ^{1}HNMR (300 MHz, $CDCl_3$, δ in ppm): 8.52 (s, 1 H, 5*H*-pyrazole), 7.37–7.42 (d, 1 H, *J*=15 Hz, -CH=CH-), 7.93–7.98 (d, 1 H, *J*=15 Hz, -CH=CH-), 6.5–8.0 (m, 13 H, ArH); 10.06 (s, 1 H, D_2O exchangeable, -OH); LCMS: *m/z*=385 (M^{+1}).

Pharmacology

Male Wister albino rats weighing 200–250 g were obtained from Animal House, Luqman College of Pharmacy, Gulbarga (Karnataka, India) and used throughout the study. All the animals were housed under standard ambient conditions of temperature (25 ±2°C) and relative humidity of 50±5%. A 12/12-h light/dark cycle was maintained. All the animals were allowed to have free access to water and standard palletized laboratory animal diet 24 h before pharmacological studies. The experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethics Committee of Luqman College of Pharmacy, Gulbarga, constituted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA/ 346), Government of India.

Anti-inflammatory activity by carrageenan-induced rat paw edema method

The anti-inflammatory activity of compounds **4a-g** and **5a-g** was assessed by the carrageenan paw edema model in rats [30]. Compounds **4a-g** and **5a-g** (100 mg/kg), diclofenac sodium (50 mg/kg), and vehicle (Tween 80) were administered orally 1 h before the injection of carrageenan (0.1 ml; 2% w/v in saline) into the

subplantar area of the right hind paw of all animals. The volume of injected paws was measured at 0, 1, 2, and 3 h after induction of edema using a plethysmometer. The volume of edema was expressed for each animal as the difference between 0 and 1, 2, and 3 h volume. The percent inhibition of edema was calculated for each group with respect to its control group. The anti-inflammatory activity was calculated using the formula:

Percentage inhibition of edema: V_c–V_t/V_c×100.

where V_c and V_t denote a mean increase in paw volume of control and drug-treated animals at 1, 2, and 3 h, respectively.

Analgesic activity by the tail-flick method in rats

Before the study, Wister albino rats were screened for sensitivity test by placing the tip of the tail on the radiant heat source [31]. The animals were divided into nine groups of six rats each. Each animal of the groups received one of the following compounds **4a-g** and **5a-g** (25 mg/kg), aspirin (25 mg/kg) and 1% w/v of tween 80 (2 ml/kg) in orally. Analgesia was assessed with a tail-flick apparatus (Analgesiometer). The basal reaction time was measured initially and another set of four measures was taken at 30, 60, and 90 min interval and the reaction of the animals considered as the 1-h postdrug reaction time. A cutoff period of 10 s was observed to prevent tissue damage of the tail of the animals.

Statistical analysis

All data generated from the animal experiments were calculated as mean±SEM. The one-way analysis of variance followed by Dunnett's multiple comparison tests was used to find out the statistical difference between the treatment and the standard.

Molecular docking

Molecular docking study is carried out on the PyRx program based on AutoDock software (The Scripps Research Institute, La Jolla, California, USA) [32] and visualization is carried out on the Discovery studio visualizer version 19.1.0.18287. For docking study PDB : 3LN1 was taken from the RCSB protein data bank (https://www.rcsb.org) developed by radiograph diffraction with resolution: 2.4 Å is used which is a structure of celecoxib bound at the COX-2 active site. The software-generated binding affinity scores were obtained and analyzed.

For docking protein file is prepared by adding missing atoms and residues in the protein. Grid for docking was selected where the co-crystallized ligand was attached. Interactions generated with the co-crystallized ligand and the designed molecule were studied.

Results and discussion

General chemistry

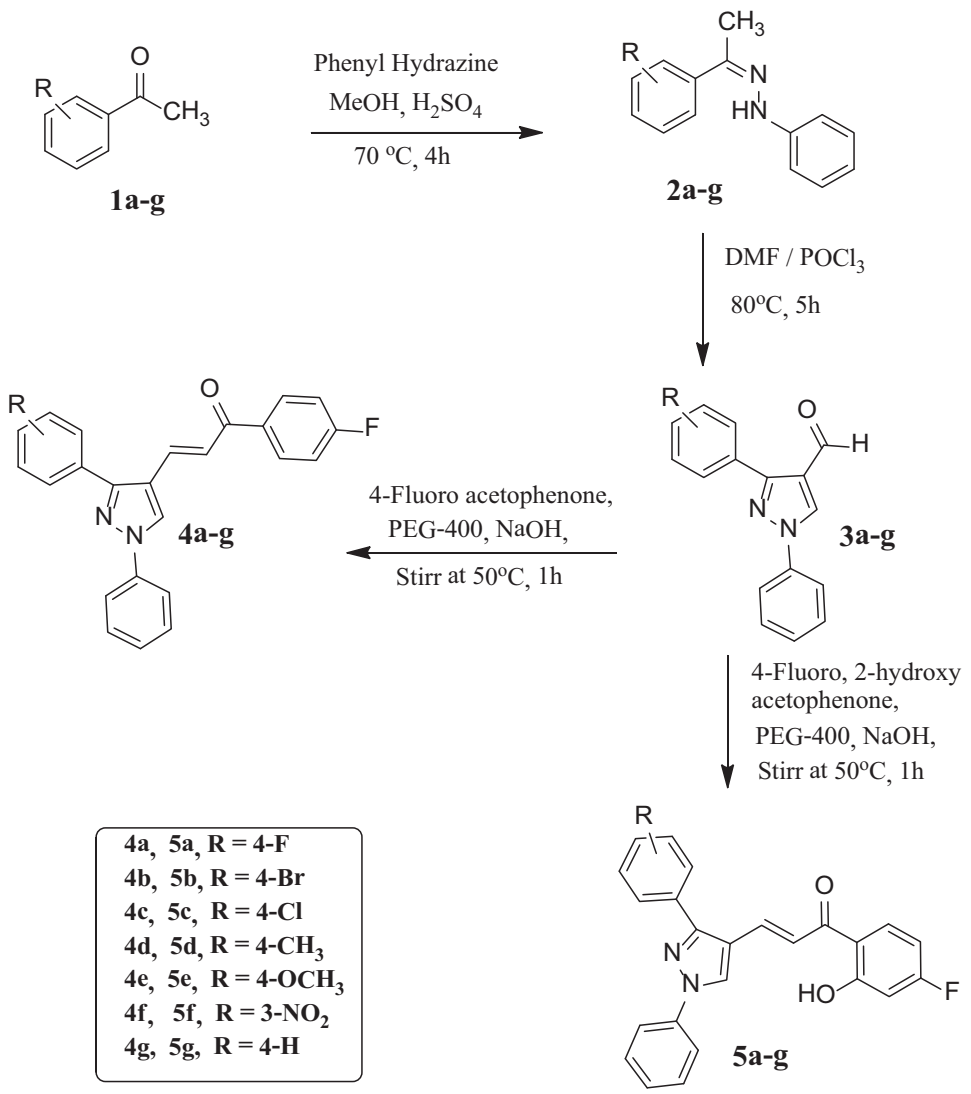
In the present investigation, pyrazole chalcones were prepared as outlined in Scheme 1. The substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes **3a-g** were prepared by the Vilsmeier-Haack reaction on acetophenone hydrazone **2** obtained from various substituted acetophenone **1** according to the literature method [33]. The pyrazole chalcones **4a-g** and **5a-g** were prepared by the reaction of various substituted pyrazole aldehydes **3a-g** with 4-fluoro-acetophenone and 4-fluoro-2-hydroxy-acetophenone, respectively, in PEG-400 and aqueous NaOH. The completion of the reaction was monitored by TLC.

All the synthesized compounds were characterized by IR, ^{1}HNMR, and mass spectroscopy. The IR spectra of pyrazole chalcones showed characteristic bands at ~1650–1670 cm^{-1} due to >C=O stretching vibration. Lowering of normal >C=O frequency was observed due to the presence of –C=C stretching in chalcones. ^{1}HNMR spectra of the compounds showed characteristic doublet signals at ~7.3 and ~7.9 δ ppm due to alkene α, β-protons, respectively. The coupling constant for alkene α, β-protons were found to be ~15–16 Hz. As the typical values of J_{H-H} for E-alkene protons are ~15–18 Hz and for Z-alkene protons are ~10 Hz. It can be concluded that the synthesized chalcones are E-isomers which show trans stereochemistry at the double bond. However, these doublets coalesced with aromatic protons. The phenolic proton was observed as a singlet at ~10–13 δ ppm due to hydrogen bonding with the adjacent carbonyl group, while other aromatic and aliphatic protons were found at the expected regions. These newly synthesized compounds are also confirmed by Liquid Chromatography–Mass Spectrometry (LC-MS) analysis and mass peaks were obtained at expected *m/e* values (M^+ or M^{+1}).

In-vivo anti-inflammatory activity

All these newly synthesized pyrazole chalcones were evaluated for their anti-inflammatory activity at 100 mg/kg postoperative against the carrageenan-induced paw edema method in Wistar rats and were compared with the standard drug diclofenac sodium. The protocol of animal experiments has been approved by the Institutional Animal Ethics Committee. Each test compound was dosed orally (100 mg/kg body

Scheme 1



PEG-mediated synthesis of fluorinated pyrazole chalcones.

Table 1 Anti-inflammatory activity of pyrazole chalcones (5a-g and 6a-g) by carrageenan-induced paw edema method in rats

Compounds number	Anti-inflammatory activity paw volume (ml) (mean±SEM)				% Inhibition at different time intervals			Dock scores
	0 h	1 h	2 h	3 h	1 h	2 h	3 h	
Control	1.25±0.01	1.29±0.01	1.32±0.001	1.38±0.001	–	–	–	
Diclofenac	0.85±0.02	0.66±0.02	0.64±0.001	0.58±0.01	48.83	51.51	57.97	–9.2
4a	1.15±0.002	1.19±0.002	1.32±0.003	1.06±0.005	07.75	00.00	23.18	–8.9
4b	0.85±0.003***	0.77±0.004**	0.62±0.002***	0.88±0.001**	40.31	53.00	36.23	–9.0
4c	0.75±0.004**	0.89±0.005**	0.77±0.003**	0.68±0.001**	31.00	41.66	50.72	9.3
4d	1.15±0.002	1.28±0.005	1.22±0.003	1.18±0.002	00.77	07.57	14.49	–8.7
4e	1.25±0.001	1.29±0.001	1.32±0.001	1.38±0.001	00.00	00.00	00.00	–8.5
4f	0.84±0.004***	0.79±0.005**	0.82±0.003**	0.68±0.002**	38.79	37.87	50.72	–9.1
4g	0.55±0.002**	0.59±0.003**	0.45±0.001**	0.68±0.004**	54.26	65.90	50.72	–9.3
5a	0.84±0.004***	0.79±0.005**	0.82±0.003**	0.68±0.002**	38.79	37.87	50.72	–9.0
5b	1.15±0.002	1.12±0.001	1.14±0.003	1.23±0.001	13.17	13.63	10.86	–8.6
5c	0.65±0.004**	0.89±0.005**	0.72±0.002**	0.72±0.002**	31.00	45.45	47.82	–9.2
5d	0.85±0.003***	0.79±0.002**	0.62±0.004***	0.68±0.001**	38.79	53.00	50.72	–9.1
5e	1.22±0.002	1.21±0.003	1.20±0.001	1.20±0.001	06.20	09.09	13.04	–8.5
5f	0.65±0.001**	0.59±0.001**	0.32±0.001***	0.45±0.001***	54.26	75.75	67.39	–9.5
5g	0.84±0.004***	0.79±0.005**	0.82±0.003**	0.68±0.002**	38.79	37.87	50.72	–9.4

n=6. *P value less than 0.05. **P value less than 0.01. ***P value less than 0.001; not determined.

weight) 1 h before the induction of inflammation by carrageenan injection. Diclofenac was utilized as a reference anti-inflammatory drug at a dose of 50 mg/kg. The anti-inflammatory activity was then calculated 1–3 h after induction and is presented in Table 1 as the mean paw volume (ml) in addition to the percentage anti-inflammatory activity (AI%).

A comparative study of the anti-inflammatory activity of test compounds relative to the reference drug at different time intervals indicated the following: after 1 h, compounds **4g** and **5f** were found to be more effective in inhibiting the paw edema with a percentage activity of 54% when compared with that of diclofenac (48%). Five other compounds **4b** (40%), **4f** (38%), **5a** (38%), **5d** (38%), and **5g** (38%) showed significant anti-inflammatory activity, whereas compounds **4c** and **5c** displayed good anti-inflammatory activity (31%) as compared with diclofenac (48%).

After 2 h, four compounds, **4b** (53%), **4g** (65%), **5d** (53%), and **5f** (75%) showed excellent anti-inflammatory activity inhibition and found to be more superior over the reference drug diclofenac (51%). Compound **5c** showed potent anti-inflammatory activity with a percentage activity of 45%, whereas four other compounds **4c** (41%), **4f** (37%), **5a** (37%), and **5g** (37%) showed significant anti-inflammatory activity as compared with diclofenac (51%).

After 3 h, compound **5f** was found to be more effective in inhibiting the paw edema with percentage activity of 67% when compared with that of diclofenac (57%). Seven other compounds, **4c** (50%), **4f** (50%), **4g** (50%), **5a** (50%), **5c** (47%), **5d** (50%), and **5g** (50%) showed significant anti-inflammatory activity, whereas compound **4b** (36%) displayed good anti-inflammatory activity as compared with diclofenac (57%).

Taking the anti-inflammatory activity after 2 h time interval as a criterion for comparison, it can be concluded that four compounds **4b** (53%), **4g** (65%), **5d** (53%), and **5f** (75%) showed higher anti-inflammatory activity than the reference diclofenac (51%), whereas compound **5c** showed potent anti-inflammatory activity with a percentage activity of 45% and four other compounds **4c** (41%), **4f** (37%), **5a** (37%), and **5g** (37%) showed significant anti-inflammatory activity. The SAR study indicated that 2-hydroxy 4-fluoro phenyl substituted pyrazole chalcones (**5a-g**) showed better activity than 4-fluoro phenyl substituted pyrazole chalcones (**4a-g**). Furthermore, the substitution of the electron-withdrawing group ($-NO_2$) at –R significantly enhanced the anti-inflammatory activity.

In-vivo analgesic activity

All these compounds were also evaluated for their analgesic activity at 25 mg/kg postoperative by the radiant heat tail-flick method in rats. The results are summarized in Table 2 and are expressed as percentage elongation at the end of 60 min. All the compounds showed analgesic activity in the range of 27–102.5% and were compared with the standard drug aspirin. The analgesic result showed that the compounds **4d** (102%), **4f** (100%), and **5c** (101%) showed significant analgesic activity whereas compounds **4c**

Table 2 Analgesic activity of pyrazole chalcones (4a-g and 5a-g) on rats by the tail-flick method

Entry	Tail withdrawing time in second (ml) (mean±SEM)				% Inhibition
	0 h	30 min	60 min	90 min	60 min
Control	3.84±0.369	3.78±0.285	4.00±0.362	3.84±0.365	–
Aspirin	4.26±0.418	5.79±0.267	8.71±0.233**	9.20±0.174**	117.75
4a	3.92±0.462	4.59±0.480	5.14±0.564	6.33±0.251	28.50
4b	3.90±0.468	4.57±0.475	5.12±0.561	6.26±0.256	28.00
4c	4.23±0.376	5.28±0.502**	6.17±0.350**	7.84±0.350*	54.25
4d	3.67±0.265	5.01±0.584**	8.10±0.453**	8.90±0.453**	102.50
4e	4.18±0.370	4.84±0.512	6.07±0.352*	7.93±0.355*	51.75
4f	4.15±0.376	5.02±0.526**	8.02±0.450**	8.92±0.453**	100.50
4g	3.92±0.467	4.66±0.470	5.11±0.565	6.31±0.252	27.75
5a	4.13±0.264	4.89±0.512	6.07±0.354*	7.94±0.354*	51.75
5b	3.90±0.463	4.57±0.475	5.12±0.567	6.23±0.256	28.00
5c	3.79±0.260	5.33±0.503**	8.04±0.453**	8.95±0.453**	101.00
5d	3.91±0.468	4.50±0.479	5.15±0.561	6.23±0.257	28.75
5e	3.77±0.244	4.48±0.410	5.22±0.560	6.22±0.256	30.50
5f	3.89±0.369	4.76±0.582	6.13±0.475*	7.24±0.259*	53.25
5g	3.75±0.225	4.08±0.243	4.15±0.207	4.25±0.211	30.75

n=6. **P* value less than 0.05. ***P* value less than 0.01. ****P* value less than 0.001; not determined.

(54%), **4e** (51%), **5a** (51%), and **5f** (53%) showed moderate activity comparable to the reference standard aspirin after 1-h treatment; however, none of them was found to be superior over the reference drug. The SAR study indicated that the substitution of the electron-withdrawing group $-NO_2$ at –R significantly enhanced the analgesic activity. The order of halogen substitution at –R to the activity was 4-Br>4-F>4-Cl.

Figure 2

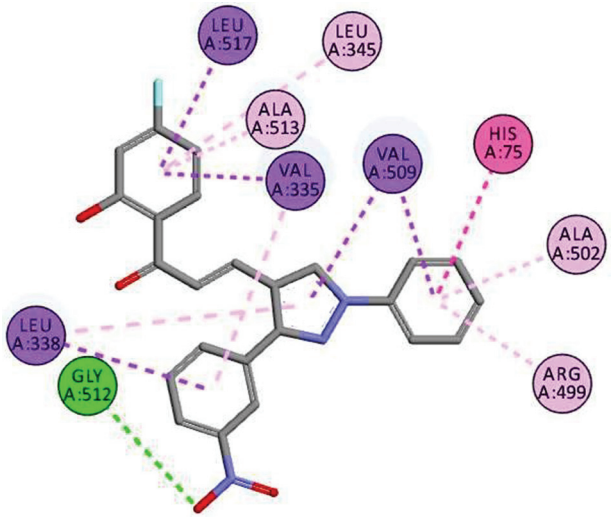


Figure showing binding interactions of molecule **5f** with 3LN1.

Molecular docking analysis

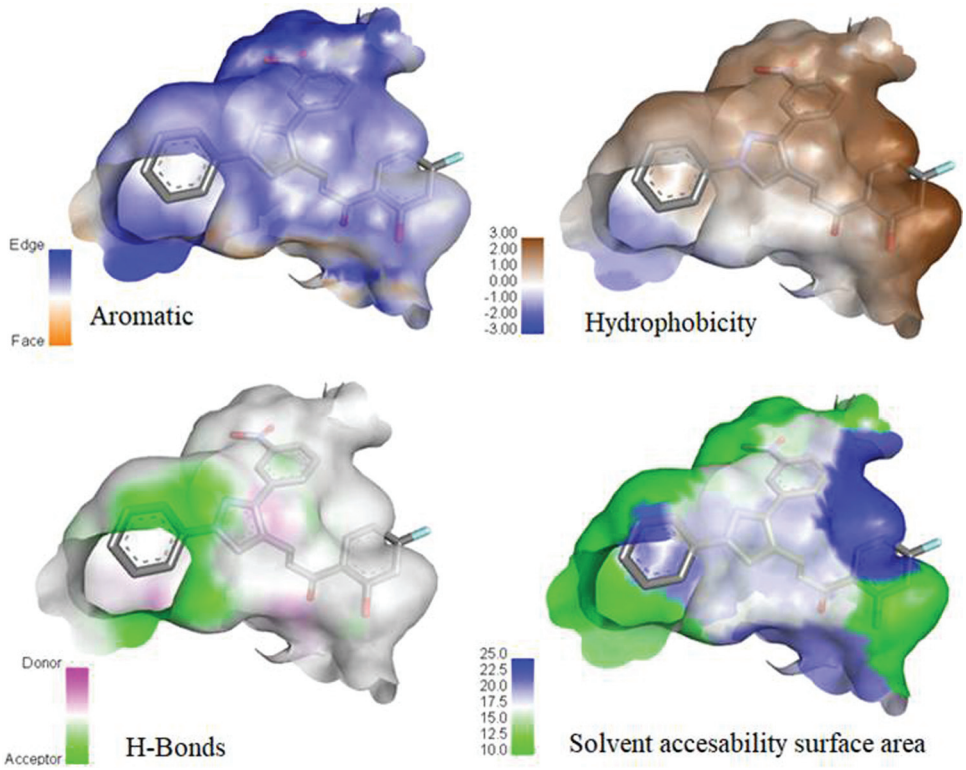
Docking analysis for the anti-inflammatory potential of synthesized derivatives carried out against the COX-2 enzyme **(PDB: 3LN1)**. Compounds (**4a-4g**) have shown significant interactions at the binding site and found interacting with some common amino acids interacting with the co-crystallized ligand (Fig. 1). Binding site contains Leu 370, Tyr 371, Trp 373, Phe 504, Ile 503, Gln 178, Gly 340, Arg 106, Val 102, Met 508, Val 509, Glu 510, Leu 345, Ser 339, Leu 517, Ala 502, Gly 512, Met 99, Ser 516, Leu 345, Tyr 341, His 75, Arg 499, Tyr 334, Val 335, and Ala513 in the proximity of 5 Å. The binding site has a significant capability of aromatic and hydrophobic interaction (Fig. 2). Docking scores of compounds have shown good correlation with actual activities as shown in Table 1. Compound **5f** have shown better docking scores as they have shown high inhibition of the enzyme.

In binding site analysis (Fig. 3), the high volume of aromatic and hydrophobic sites is found. H-bond donor regions and solvent accessibility surface areas are also in considerable volume.

In-silico Absorption, Distribution, Metabolism and Excretion (ADME)

All the compounds are found acceptable within Lipinski's 'rule of five' or 'drug-likeness.' $CaCO_2$

Figure 3



Binding site analysis and pose of molecule **5f** with 3LN1.

Table 3 ADME parameters of pyrazole chalcones (4a-g and 5a-g)

Entry	BBB	$CaCO_2$	CYP 2C19 inhibition	CYP 2C9 inhibition	CYP 2D6 inhibition	CYP 2D6 substrate	CYP 3A4 inhibition	CYP 3A4 substrate	% HIA	MDCK	% Plasma protein Binding	Skin permeability
4a	0.49	54.54	I	I	NI	NI	I	Weak	99.10	0.08	100	–2.17
4b	0.70	51.92	I	I	NI	NI	I	Weak	99.16	0.10	100	–2.07
4c	0.75	50.7	I	I	NI	NI	I	Weak	99.17	0.05	100	–1.98
4d	0.61	55.08	I	I	NI	NI	I	Weak	99.11	0.12	100	–2
4e	0.29	54.78	I	I	NI	NI	I	Weak	97.94	0.06	100	–2.16
4f	0.06	15.09	NI	I	NI	NI	NI	Weak	98.18	0.04	100	–2.07
4g	0.63	54.84	I	I	NI	NI	I	Weak	99.09	1.46	100	–2.04
5a	0.84	38.41	I	I	NI	NI	I	Weak	96.72	0.04	100	–2.32
5b	1.51	32.41	I	I	NI	NI	I	Weak	97.03	0.04	100	–2.2
5c	1.67	30.97	I	I	NI	NI	I	Weak	97.19	0.04	100	–2.11
5d	1.22	40.8	I	I	NI	NI	I	Weak	96.79	0.04	100	–2.14
5e	0.25	41.45	I	I	NI	NI	I	NI	96.72	0.04	100	–2.28
5f	0.03	12.94	NI	I	NI	NI	NI	Weak	97.46	0.04	100	–2.15
5g	0.62	39.13	I	I	NI	NI	I	Weak	96.71	0.07	100	–2.18

HIA, human intestinal absorption; I, inhibitor; NI, noninhibitor.

(gut–blood barrier) and MDCK cell permeability are considered low if the value is less than 4, average permeability if the value is within 4–70, and high permeability if the value is more than 70 and all the compounds were found to be averagely permeable. Cell permeability is found above 500 for all the molecules which are excellent. BBB is the blood–brain barrier permeability for drugs and acceptable compounds considered central nervous system (CNS) active if the value of BBB is more than 1. All compounds are found CNS inactive as per in-silico predictions. Also predicted Percent Human Oral Absorption found 100% for all the compounds. Celecoxib gets metabolized mainly by CYP 2C9 and all the compounds found inhibitors of CYP 2C9. Percent of human intestinal absorption is also found excellent which is ~96–100%. All the compounds have shown 100% plasma protein binding (Table 3).

Conclusion

The objective of this study was to synthesize and investigate the anti-inflammatory and analgesic activities of a new series of fluorinated pyrazole chalcones using PEG-400 as an alternative reaction medium with the hope of discovering new structure leads serving as a dual anti-inflammatory–analgesic agents. Among the tested series, compounds **4b**, **4g**, **5d**, and **5f** showed excellent anti-inflammatory activity in the range 53–75% and were found to be better than that of standard diclofenac (51%), whereas compounds **4c**, **4f**, **5a**, **5c**, and **5g** displayed significant anti-inflammatory activity (37–45%). These compounds showed anti-inflammatory activity may be due to suppression of COX and reduced prostaglandin formation.

Most of the compounds also exhibited significant analgesic activity. Compounds **4d**, **4f**, and **5c** showed potent analgesic activity in the range of 100–102%, whereas compounds **4c**, **4e**, **5a**, and **5f** showed moderate analgesic activity in the range 51–54% as compared with standard aspirin (117%). Five compounds **4c**, **4f**, **5a**, **5c**, and **5f** showed significant activities in both screens, comparable to those of the standard drugs diclofenac and aspirin. Hence, it can be concluded that the tested fluorinated pyrazole chalcones can be considered as potential anti-inflammatory and analgesic agents.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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