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PEG mediated synthesis and biological evaluation of asymmetrical pyrazole curcumin analogues as potential analgesic, anti-inflammatory and antioxidant agents

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The new series of asymmetrical pyrazole curcumin analogues 4a-g were synthesized by using polyethylene glycol (PEG-400) as a green reaction medium and evaluated for their in *vivo* analgesic and *in vitro* antioxidant (H_2O_2, DPPH, Ferrous reducing power and Nitric oxide scavenging activity) and anti-inflammatory activities. All the compounds synthesized 4a-g showed the potential to demonstrate analgesic activity as compared to the standard ibuprofen. Among the tested series, compounds 4e and 4b exhibited good hydrogen peroxide scavenging activity as compared to the standard butylated hydroxyl toluene (BHT). Compounds 4b, 4d, 4f and 4g showed good DPPH free radical scavenging activity. Compounds 4b, 4c, 4d, 4e and 4g showed excellent ferrous reducing power activity whereas

all the compounds showed better nitric oxide scavenging activity than standard ascorbic acid. Additionally, all the synthesized compounds were also screened for their *in vitro* anti-inflammatory activity. Compounds 4b, 4d, 4f and 4g showed good anti-inflammatory activity as compared to standard diclofenac sodium.

Keywords: Asymmetrical Pyrazole Curcumin, PEG-400, Analgesic activity, Antioxidant activity, Anti-inflammatory activity.

Curcumin is a β-diketone constituent of the turmeric that is obtained from the powdered rhizome of *Curcuma longa*. Curcumin has a wide range of interesting biological activities such as anti-inflammatory, antioxidant, antiviral, cutaneous wound healing, hypocholesterolemic effects in diabetic patients, anti-angiogenic and stimulatory response to stress-induced biological activity (1,2). Curcumin has been demonstrated to possess preventative activity against Aβ-aggregation in Alzheimer's model (3). Evidence from both *in vitro* and *in vivo* studies show that β-diketone moiety is responsible for instability and weak pharmacokinetic profiles of curcumin. Recently, synthetic modifications of curcumin, which were aimed at enhancing its bioactivities, suggested that the stability and metabolic profile of curcumin could be enhanced by deleting β-diketone moiety. Recent studies from several independent groups demonstrated that the curcumin analogues without β-diketone either retained or increased various biological activities such as anticancer (4, 5), antibacterial (6) and anti-inflammatory (7).

The survey of literature reveals that many curcumin analogues have been used as lead molecules for the design and development of array of therapeutic agents including anti-inflammatory, anticancer, antioxidant, antiviral, and antimicrobials (8). Several analogues of curcumin have been synthesized and evaluated for multifunctional pharmacological application in a variety of diseases such as liver fibrosis, inflammation, cardiovascular diseases, and cancer (9-11). Several curcuminoid pyrazoles have been synthesized and described as new therapeutic agents in inflammatory bowl diseases targeting the matrix metalloproteinases(12). Novel series of curcumin analogues were synthesized and tested as anticancer and antiangiogenic agents (13).

Variety of traditional non-steroidal anti-inflammatory drugs (NSAIDs), such as naproxen, ibuprofen, diclofenac, flurbiprofen, indomethacin and aspirin are available in the market which have some limitations in clinical use like gastrointestinal (GI) haemorrhage and ulceration, addiction, tolerance especially for opiates(14). The present day NSAIDs are having side effects. The development of alternatives to NSAIDs is being attempted all over the world.

Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function, or excessive quantities, the state called oxidative stress. These species react indiscriminately with almost every type of biomolecules found in living cell such as, sugars, amino acids, phospholipids, DNA bases, organic

acids and may deviate the cells from its normal physiological functions(15). Oxidative stress is also proposed to be involved in the process of aging both by including damage to mitochondrial DNA and by other mechanisms (16, 17), and leads to the initiation of various diseases such as cancer (18), ischemia-reperfusion injury (19), atherosclerosis (20), cardiovascular (21), inflammation (22), and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (23,24). Antioxidants are the compounds capable of scavenging the free radicals; for this antioxidant therapy is one of the recent options.

On the other hand, PEG solvents are known to be inexpensive, easily available, thermally stable, recyclable, biological compatible, non-toxic and 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. However, PEG polymer of low molecular weight differs significantly from polymers of high molecular weight in their physico-chemical properties, higher toxicity and possibly genotoxicity (27). The toxicity data of different molecular weight PEG's are also available and some of them are already approved for internal consumption by the USA FDA (28). The use of PEG as a green and alternative reaction medium in organic reactions is relatively recent (29).

The researcher's recent success on the development of new selective eco-friendly methodologies using polyethylene glycol (PEG-400) (30, 31) as a green solvent for the preparation of biologically active compounds, they report the synthesis of some new series of asymmetrical pyrazole curcumin analogues (APCAs) using aq.NaOH in PEG-400 as green alternative reaction medium and evaluate them as analgesic agent along with assessing their antioxidant and anti-inflammatory potential. To the best of our knowledge this is the first report on the *in vivo* analgesic study of newly synthesized of asymmetrical pyrazole curcumin analogues (APCAs).

Methods and Materials

Instrumentation

Melting points were determined with digital thermometer and were uncorrected. IR spectra were recorded on FT-IR spectrometer (Perkin Elmer) using KBr disc method. ^{1}HNMR spectra were recorded on ^{1}HNMR (Varian-NMR-mercury 300 MHz) spectrometer in $CDCl_3$ as solvent. All chemical shifts (δ) are quoted in parts per million downfield from 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 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 pre-coated silica plates (Merks kiesegel 60F254, 0.2 mm thickness) sheet. The spots could be visualized easily under UV light.

Chemistry

Although, curcumin is non-toxic and has promising biological activities, preclinical and clinical studies have indicated that poor bioavailability and pharmacokinetic profile are due to its instability under physiological condition which has limited its application in anti-cancer therapies (32-34).To overcome these barriers, several research groups have synthesized curcumin analogues. Robinson et al. (4) have synthesized enone and dienone analogues, Ahn et al. (35) have synthesized the bis-alkynyl/alkyl, pyridine and thiophene derivatives as curcumin analogues. Woo et al. (36) have synthesized curcumin mimics containing enone and amide ring which were shown to possess increased anti-angiogenic activity. Bandgar et al. (37) have synthesized novel curcumin mimics incorporating olefin as well as aromatic, alicyclic or heteroaromatic amide moities and evaluated for antioxidant, cytotoxic and antimicrobial activity. Bandgar et al. (38) have also synthesized novel curcumin analogues and evaluated as anti-inflammatory, anti-cancer and anti-oxidant agents.

Herein, we synthesized the new curcumin analogues containing enone and amide including pyrazole moiety. The title compounds asymmetrical pyrazole curcumin analogues (APCAs) were prepared by the acylation of 3-aminoacetophenone (**1**) with fluoro acyl chloride to afford corresponding amide (**2**), which on Claisen-Schmidt condensation with various substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**3a-g)** and NaOH in PEG-400 furnished APCAs (**4a-g)** in good to excellent yields (**Scheme-1**). The completion of the reaction was monitored by TLC. The substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes (**3a-g**) were prepared by the Vilsmeir-Haack reaction (39) of various substituted aryl hydrazones from various substituted acetophenones. All the synthesized compounds were characterized by IR, ^{1}HNMR and Mass spectroscopy.

General procedure for the preparation N-(3-Acetyl-phenyl)-4-fluoro-benzamide (2)

1-(3-Amino-phenyl)ethanone **1** (1 g, 7.40 mM) was suspended in 20 mL of 5 % of sodium hydroxide solution in round bottom flask and added 2 mL 4-fluorobenzoyl chloride, 0.5 mL at a time, with constant shaking and stirred vigorously for 10 min, reaction mixture was heated under reflux on water bath at 70-80^{o}C for 30 min until the odor of the 4-fluorobenzoyl chloride was disappeared. Make sure that the mixture has an alkaline pH. Filter off the solid benzoyl derivative and recrystallized it from petroleum ether and ethyl acetate to obtain compound **2**.

General procedure for the preparation of asymmetrical pyrazolecurcumin analogues (4a-g)

The substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes **3a-g** were prepared by Vilsmeier-Haack reaction on acetophenone hydrazones obtained from various substituted acetophenones according to literature method (39). A mixture of substituted 1,3-diphenyl-1*H*-pyrazole-4-carbaldehydes **3a-g** (1 mM) and N-(3-Acetyl-phenyl)-4-fluoro-benzamide **2** (1 mM) was dissolved in 15 mL PEG-400. To this mixture, NaOH (20%, 1mL) was added and the reaction mixture was stirred at 40-50^{o}C temperature for 1h. The reaction mixture was then poured into 100 mL ice cold water. The product was separated out; it was filtered and processed out. The products obtained were purified by recrystallization from ethanol to afford pure compounds **4a-g**.

Analgesic activity

The analgesic activity was evaluated using the acetic acid induced writhing method (40) using groups of six animals each. A solution of acetic acid (1% v/v) in distilled water was prepared and injected intraperitoneally in a volume of 0.1 mL. One group was kept as control, and the animals of the other group were pretreated with the test compounds, 1 h before the acetic acid (1% v/v) treatment. The writhing episodes were recorded for 15 min; stretching movements consisting of arching of the back, elongation of body, and extension of hind limbs were counted.

Preparation of test compounds

After suspending the test compounds in 1.0% aq. solution of gum acacia, the test samples were administered to the test animals orally (5 mg/kg). The positive and negative control group animals received the same experimental handling as those of the test groups except that the drug-treated control group animals received only appropriate volumes of vehicle and of the reference drug, Ibuprofen (15 mg/kg, p.o).

Pharmacology

Swiss albino mice of either sex weighing 20–25 g were obtained from Animal house, Luqman College of Pharmacy, Gulberga, Karnataka, India. All the animals were housed under standard ambient conditions of temperature (25 ± 2^{o}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 Ethical Committee (IAEC) of Luqman College of Pharmacy, Gulberga, 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.

Antioxidant activity:

Hydrogen Peroxide (H_2O_2) Scavenging activity

The hydrogen peroxide scavenging assay was performed by the reported method (41). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). The 1 mM concentrations of various compounds (4a-g) were added to a hydrogen peroxide solution (0.6 mL, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min. against a blank solution containing phosphate buffer without drug. The percentage scavenging of hydrogen peroxide of synthetic compounds and standard compounds was calculated using the following formula:

$$\% \text{ scavenged } [H_2O_2] = [(A_0 - A_1)/A_0] \times 100$$

Where,

A_0 = the absorbance of the control

A_1 = the absorbance in the presence of the sample of MO and standards.

DPPH radical scavenging activity

The ability of compounds to scavenge DPPH radical was assessed using literature method (42) with slight modification. Briefly, 1 ml of synthesized compounds (4a-g) as 100mM was mixed with 3.0 mL DPPH (0.5 mmol/L in methanol), the resultant absorbance was recorded at 517 nm after 30 min. incubation at 37^{O}C. The percentage of scavenging activity was derived using the following formula,

Percentage of inhibition (%) = [(Acontrol - Asample) / Acontrol] x 100

Where,

A control - absorbance of DPPH

A sample - absorbance reaction mixture (DPPH with Sample).

Ferrous reducing power

The reducing ability of compounds was measured according to the reported method [43]. 100mM of the synthesized compounds (1.0 mL) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The mixture was incubated at 50 ^{O}C for 20 min. with TCA (10%: 2.5 mL). Then mixture was centrifuged at 3 000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (1%) and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power. The reducing power of compounds was compared with that of standard antioxidant.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging was carried out as per the reported method [42]. Nitric oxide radicals were generated from sodium nitroprusside solution. 1 mL of 10 mM sodium nitroprusside was mixed with 1 mL of 100 mM synthesized compounds in phosphate buffer (0.2 M pH 7.4). The mixture was incubated at 25 ^{O}C for 150 min. After incubation the reaction mixture mixed with 1.0 mL of pre-prepared Griess reagent (1% sulphanilamide, 0.1% napthylethylenediamine dichloride and 2% phosphoric acid). The absorbance was measured at 546 nm and percentage of inhibition was calculated using the same formula as above. The decreasing absorbance indicates a high nitric oxide scavenging activity.

Percentage of inhibition (%) =[(Acontrol - Asample) / Acontrol] x 100

Where,

A control - absorbance of Nitric oxide

A sample - absorbance reaction mixture (Nitric oxide with Sample).

In vitro anti-inflammatory activity by Protein denaturation method [44]

The reaction mixture (10 mL) consisted of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 4 mL of synthesized compounds (100mM). Similar

volume of double-distilled water served as control. Then the mixtures were incubated at (37^{O}C ± 2) in a incubator for 15 min and then heated at 700^{O}C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the 100 mM was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% inhibition = 100 x (Vt / Vc - 1)

Where, Vt = absorbance of test sample,

Vc = absorbance of control.

Results and discussion

Spectral analysis

All the synthesized compounds were characterized by IR, ^{1}H NMR, and MS. The IR spectrum of the titled compounds showed absorption due to –NH stretching at ~3350 cm-1, amide carbonyl group at ~1645 cm-1.^{1}H NMR spectrum (300 and 400 MHz) recorded in DMSO-d6 showed a typical singlet at δ ~ 9-10 (for -NH) and a typical 1H-1H coupling constant in between 12-16 Hz showing *trans* stereochemistry of the double bond.

N-(3-Acetyl-phenyl)-4-fluoro-benzamide (2)

Yield: 78%; MP: 115-116^{o}C; **IR** (KBr): 3476, 3296, 3077, 2968, 1709, 1609, 1499, 1281, 1178, 1061, 862 cm $^{-1}$. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 8.36 (broad s, 1H, -NH), 8.16 (s, 1H, ArH), 8.10 (d, 1H, ArH,*J* = 8.1 Hz), 7.72 (d, 1H, ArH,*J* = 7.8 Hz), 7.47 (t, 1H, ArH,*J* = 7.8 Hz), 7.96-7.7.13 (m, 4H, ArH), 2.60 (s, 3H, CH_3). **MS:** m/e 258 (M+1).

4-Fluoro-N-(3-{3-[3-(4-fluoro-phenyl)-1-phenyl-1H-pyrazol-4-yl]-acryloyl}-phenyl)-benzamide (4a)

Yield: 82%; mp: 210-212^{0}C; **IR** (KBr): 3744, 3292, 3117, 3058, 1649, 1595, 1529, 1409, 1241, 842, 751cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 7.17-7.98 (m, 17H, Ar-H); 7.39 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.88 (d, 1H, *J*=15.0 Hz, -CH=CH-); 8.18 (s, 1H, pyrazole-H); 8.39 (s, 1H, N-H, D_2O exchangeable); **MS:** m/e 506 (M+1).

N-(3-{3-[3-(4-Chloro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-acryloyl}-phenyl)-4-fluoro-benzamide (4b)

Yield: 80%; mp: 217-218^{0}C; **IR** (KBr): 3821, 3743, 3314, 3114, 1651, 1593, 1527, 1418, 1239, 841, 757 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 7.18 (t, 2H, ArH, *J* = 8.4 Hz); 7.65 (d, 2H, ArH, *J* = 8.4 Hz); 8.02 (s, 1H, ArH); 7.16-8.0 (m, 12H, Ar-H); 7.37 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.81 (d, 1H, *J*=15.0 Hz, -CH=CH-); 8.19 (s, 1H, pyrazole-H); 8.37 (s, 1H, N-H, D_2O exchangeable). **^{13}C NMR** (100 MHz, DMSO-d_6, δ in ppm): 188.8, 164.5, 151.6, 151.6, 139.5, 138.8, 138.1, 134.0, 133.5, 130.9, 130.7, 130.0, 129.0, 127.2, 124.8, 123.7, 121.9, 119.9, 118.7, 117.7, 115.4, 115.2. **MS:** m/e 522 (M+1).

N-(3-{3-[3-(4-Bromo-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-acryloyl}-phenyl)-4-fluoro-benzamide (4c)

Yield: 79%; mp: 248-250^{0}C; **IR** (KBr): 3821, 3743, 3314, 3114, 1651, 1593, 1527, 1501, 1418, 239, 841,757 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 7.38-8.40 (m, 17H, Ar-H); 7.39 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.79 (d, 1H, *J*=15.0 Hz, -CH=CH-); 9.4 (s, 1H, pyrazole-H); 10.5 (s, 1H, N-H, D_2O exchangeable); **MS:** m/e 568 (M+2).

4-Fluoro-N-{3-[3-(1-phenyl-3-*p*-tolyl-1*H*-pyrazol-4-yl)-acryloyl]-phenyl}-benzamide (4d)

Yield: 86%; mp: 140-141^{0}C; **IR** (KBr): 3853, 3743, 3392, 3245, 3123, 2924, 1645, 1577, 1533, 1422, 1239, 845, 749 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 2.41 (s, 3H, Methyl-H); 7.19 (t, 2H, ArH, *J* = 8.4 Hz), 7.28-8.03 (m, 15H, Ar-H); 7.30 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.90 (d, 1H, *J*=15.0 Hz, -CH=CH-); 8.14 (s, 1H, pyrazole-H); 8.37 (s, 1H, N-H, D_2O exchangeable); **MS:** m/e 502 (M+1).

4-Fluoro-N-(3-{3-[3-(4-methoxy-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-acryloyl}-phenyl)-benzamide (4e)

Yield: 88%; mp: 209-211^{0}C; **IR** (KBr): 3853, 3743, 3392, 3245, 3123, 2924, 1645, 1577, 1533, 1422, 1239, 845, 749 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 2.91 (s, 3H, Methoxy-H); 7.16-8.03 (m, 17H, Ar-H); 7.29 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.89 (d, 1H, *J*=15.0 Hz, -CH=CH-); 8.14 (s, 1H, pyrazole-H); 8.37 (s, 1H, N-H, D_2O exchangeable); **MS:** m/e 518 (M+1).

4-Fluoro-N-(3-{3-[3-(3-nitro-phenyl)-1-phenyl-1*H*-pyrazol-4-yl]-acryloyl}-phenyl)-benzamide (4f)

Yield: 78%; mp: 211-213^{0}C; **IR** (KBr): , 3739, 3290, 3108, 1644, 1590, 1526, 1500, 1409, 1352, 844, 757 cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 7.69 (t, 1H, ArH, *J* = 8.1 Hz); 8.06 (d, 1H, ArH, *J* = 7.2 Hz); 8.18 (s, 1H, ArH); 8.29 (d, 1H, ArH, *J* = 8.1 Hz); 7.18-8.31 (m, 13H, Ar-H); 7.42 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.87 (d, 1H, *J*=15.0 Hz, -CH=CH-); 8.42 (s, 1H, pyrazole-H); 8.66 (s, 1H, N-H, D_2O exchangeable). **MS:** m/e 533 (M+1).

N-{3-[3-(1,3-Diphenyl-1*H*-pyrazol-4-yl)-acryloyl]-phenyl}-4-fluoro-benzamide(4g)

Yield: 86%; mp: 224-225^{0}C; **IR** (KBr): 3819, 3739, 3313, 3116, 3062, 1652, 1593, 1526, 1500, 1426, 1226, 843, 756cm^{-1}. **^{1}H NMR** (300 MHz, $CDCl_3$, δ in ppm): 7.17-8.02 (m, 18H, Ar-H); 7.37 (d, 1H, *J*=15.0 Hz, -CH=CH-); 7.77 (d, 1H, *J*=15.0 Hz, -CH=CH-); 8.13 (s, 1H, pyrazole-H); 8.39 (s, 1H, N-H, D_2O exchangeable). **MS:** m/e 488 (M+1).

Biological studies

In vivo analgesic activity

The seven asymmetrical pyrazole curcumin analogues shown in **Scheme-1** were tested for *in vivo* analgesic activity by using the acetic acid induced writhing method. The assays were carried out as per the reported methods and the results are summarized in **Table 1**. The analgesic activity result revealed that all the tested compounds (**4a-g)** showed potent analgesic activity in the range of 80-96% as compared to the standard Ibuprofen (62%). The entire series of APCAs (**4a-g)** exhibited

excellent analgesic activity at 5 mg/kg p.o. When their activities were compared with Ibuprofen, it was determined that all the compounds showed analgesic activity more than standard Ibuprofen. The compound carrying methoxy- and chloro- substituent at *p*-position of phenyl ring (**4e** and **4b)** appeared as the most active compound in this series. However, all other compounds were also the most notable ones because they showed a remarkable analgesic activity as compared to the reference drug Ibuprofen. All the data were analyzed by one-way ANOVA test followed by Dunnet's test in the acetic acid induced writhing model.

***In vitro* anti-inflammatory activity**

All the synthesized compounds were evaluated for anti-inflammatory activity by protein denaturation method and results are presented in **Table 1**. The results revealed that the synthesized compounds **4b, 4g, 4d** and **4f** showed good inhibition (85.19 – 79.31%) as compared with standard diclofenac sodium (90.21%) whereas rest of the compounds showed moderate inhibition.

In vitro antioxidant activity

Reactive oxygen species and nitrogen species contribute to the pathophysiology of anti-inflammatory (45) conditions. Taking into the account of multifactorial character of oxidative stress which is involved in many pathological states, we have evaluated antioxidant activity of synthesized asymmetrical pyrazole curcumin analogues (APCAs) against reactive oxygen species such as hydrogen peroxide, DPPH, ferrous reducing power and nitric oxide radical scavenging activity and results are presented in **Table 1**. All the synthesized compounds showed good to moderate scavenging activity against hydrogen peroxide. The antioxidant activity result revealed that the compound **4e** (88.45%) was found to possess excellent inhibition of H_2O_2 scavenging activity followed by compound **4b** (71.12%) as compared to the BHT (88.42%). The remaining compounds **4a**, **4d**, **4f** and **4g** showed (46-61%) moderate inhibition of H_2O_2 scavenging activity except compound **4c** (22%).

In case of DPPH free radical scavenging activity, compounds **4f, 4b, 4d** and **4g** exhibited good activity (42.32 – 36.80%) as compared to standard ascorbic acid (42.98) whereas rest of the compounds showed moderate activity. Compounds **4d, 4e, 4g, 4b** and **4c** showed excellent ferrous reducing power activity (55.55 – 44.44%) as compared to standard ascorbic acid (42.44%), whereas the remaining compounds **4a** and **4f** showed moderate activity (33.33%).

The nitric oxide radical scavenging activity result revealed that all the synthesized curcumin analogues (77.94 – 32.35%) were found showning excellent activity than standard ascorbic acid (32.32%). Among the series, the compounds **4b, 4a, 4c** and **4f** showed more potent nitric oxide radical scavenging activity. The wide variation in the free radical scavenging potential for the tested compounds may be due to the variation in the proton–electron transfer by the derivatives due to difference in their structures

As the reactive oxygen species are involved in pain and inflammation, the analgesic agents should have both antioxidant and analgesic activity. The NSAIDs like Ibuprofen and Aspirin have both

analgesic and antioxidant activities (46). In case of our synthesized compounds, compounds **4b, 4d, 4e, 4f** and **4g** showed potent analgesic as well as good antioxidant and anti-inflammatory activity, suggesting that the analgesic activity of the compound might be partly connected to its antioxidant and anti-inflammatory activity.

Conclusion

In conclusion, a series of new asymmetrical pyrazole curcumin analogues (APCAs) (**4a-g**) have been synthesized with excellent yields without formation of any detectable side products by using PEG-400 as an alternative reaction medium and were characterized by IR, ^{1}H NMR and mass spectrometry. All the newly synthesized compounds (**4a-g**) were evaluated for their analgesic, antioxidant and anti-inflammatory potential. The result of analgesic activity revealed that all the compounds were found to be shown better activity than standard Ibuprofen, suggesting that all the synthesized APCAs were found to be superior over the standard Ibuprofen. In case of antioxidant activity, compounds **4e** was found to be potent whereas compound **4b** was found to be comparable H_2O_2 scavenging activity to standard BHT. The compounds **4f, 4b, 4d** and **4g** were found to be good DPPH free radical scavenging activity as compared to standard ascorbic acid. In ferrous reducing power activity, compounds **4d, 4e, 4g, 4b** and **4c** were found to be superior than standard ascorbic acid, whereas all the synthesized compounds were found potent nitric oxide radical scavenging activity than standard ascorbic acid. The result of anti-inflammatory activity revealed that the compounds **4b, 4g, 4d** and **4f** showed pronounced anti-inflammatory activity which was comparable to diclofenac sodium. In general, compounds **4b, 4d, 4e, 4f** and **4g** were found to be the most active analgesic agents in addition to having potential antioxidant as well as anti-inflammatory activity. Thus, these compounds constitute an interesting template for the evaluation of new analgesic agents and may be helpful for the design of new therapeutic tools.

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Conflict of Interest

Authors have no conflict of interest.

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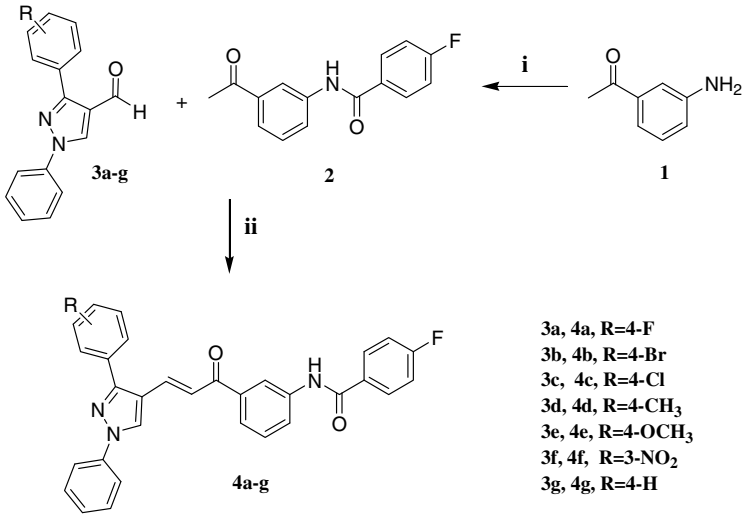

Scheme1. Reagents and conditions: (**i**) 4-Fluorobenzoyl chloride, NaOH, 70-80^{o}C,30 min. (**ii**) NaOH, PEG-400, stirr, 40-50^{o}C, 1 h.

Entry	Analgesic activity (*in vivo*)		Anti-inflammatory activity (*in vitro*)	Antioxidant activity (*in vitro*)			
	No.of Writhings ± SEM	% inhibition	% inhibition	H_2O_2 (%)	DPPH (%)	Ferrous reducing power (%)	Nitric oxide radical scavenging activity (%)
Control	26.00±0.68	--	--	--	--	--	--
Ibuprofen	10.00±0.57**	62	--	--	--	--	--
4a	5.16±0.70**	80	65.51	51.03	34.84	33.33	69.11
4b	1.05±0.22**	96	85.19	71.12	40.85	44.44	77.94
4c	2.16±0.30**	92	60.89	22.75	34.84	44.44	67.74
4d	2.16±0.30**	92	79.31	46.75	39.29	55.55	44.08
4e	1.00±0.25**	96	55.17	88.45	30.30	55.55	32.35
4f	2.00±0.44**	92	79.31	61.79	42.32	33.33	62.5
4g	3.83±0.40**	85	82.75	53.10	36.80	55.55	38.29
Diclofenac sodium	--	--	90.21	--	--	--	--
BHT	--	--	--	88.42	--	--	--
Ascorbic acid	--	--	--	--	42.98	42.44	32.32

Table 1. Analgesic, anti-inflammatory and antioxidant activity of compounds **4a-g**.

SEM – standard error mean; N = 6; *P< 0.05, **P<0.01, ***P<0.001;
BHT –butylatedhydroxytoluene; --, Not determined;