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Research Article

Effects of Novel Diarylpentanoid Analogues of Curcumin on Secretory Phospholipase A_2, Cyclooxygenases, Lipo-oxygenase, and Microsomal Prostaglandin E Synthase-1

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Arachidonic acid and its metabolites have generated a heightened interest due to their significant role in inflammation. Inhibiting the enzymes involved in arachidonic acid metabolism has been considered as the synergistic anti-inflammatory effect. A series of novel curcumin diarylpentanoid analogues were synthesized and evaluated for their inhibitory effects on activity of secretory phospholipase A_2, cyclooxygenases, soybean lipo-oxygenase as well as microsomal prostaglandin E synthase-1. Among the curcumin analogues, compounds 3, 6, 9, 12, and 17 exhibited strong inhibition of secretory phospholipase A_2 activity, with IC_{50} values ranging from 5.89 to 11.02 μM. Seven curcumin analogues 1, 3, 6, 7, 9, 11, and 12 showed inhibition of cyclooxygenases-2 with IC_{50} values in the range of 46.11 to 94.86 μM, which were lower than that of curcumin. Compounds 3, 6, 7, 12, and 17 showed strong inhibition of lipo-oxygenase enzyme activity. Preliminary screening of diarylpentanoid curcumin analogues for microsomal prostaglandin E synthase-1 activity revealed that four diarylpentanoid curcumin analogues 5, 6, 7, and 13 demonstrated higher inhibition of microsomal prostaglandin E synthase-1 activity with IC_{50} ranging from 2.41 to 4.48 μM, which was less than that of curcumin. The present results suggest that some of these diarylpentanoid analogues were able to inhibit the activity of these enzymes. This raises the possibility that diarylpentanoid analogues of curcumin might serve as useful starting point for the design of improved anti-inflammatory agents.

Key words: chemical biology, drug discovery, enzymatic mechanism

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A number of studies have shown the involvement of aberrant arachidonic acid metabolism in inflammation. Membrane phospholipids, the main source of arachidonic acid, are hydrolyzed by secretory phospholipase A_2 ($sPLA_2$); the liberated arachidonic acid is additionally metabolized by cyclooxygenase (COX) and lipo-oxygenase (LOX) (1). Metabolism of arachidonic acid gives rise to key lipid mediators. COX enzymes catalyze the first step in the biosynthesis of prostaglandin and thromboxane. Lipo-oxygenase enzymes catalyze arachidonic acid leading to the production of leukotrienes and lipoxins. These mediators play a fundamental role during inflammation. Leukotrienes and prostaglandins are recognized to uphold the inflammatory reaction, while the lipoxins are mainly anti-inflammatory in nature. Arachidonic acid and its metabolites have newly generated a heightened interest owing to increase in evidence regarding their important role in cancer biology and various types of cardiovascular diseases. New advances in the field of inflammation have given insight into the modulation of arachidonic acid metabolism. Inhibiting the enzymes involved in arachidonic acid metabolism has been considered as the synergistic anti-inflammatory effect with enhanced spectrum of activity (2). Therefore, inhibitors of these enzymes have initially been of interest for the treatment of inflammation.

Curcumin 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione,diferuloylmethane) is a natural phenolic compound, isolated as a yellow pigment from turmeric (dry rhizomes of *Curcuma longa*), which is frequently used as a food colorant, spice, and conventional medicine in India, China, and some Asian countries. Curcumin is renowned for its anti-inflammatory effects, and over the past few decades, its several biological and pharmacological activities have been reported. As per recent reviews, curcumin is a multitarget pleiotropic agent, showing a wide range of biological activities (3). Curcumin and its derivatives exhibit a wide variety of pharmacological activities, viz. antibacterial, antifungal, antiviral, anti-HIV anti-inflammatory, anti-Parkinson's, anti-Alzheimer's, anti-angiogenesis, free radical scavenging activity, antirheumatic, antimalarial, anticancer, antiprotozoan, antimutagenic, wound treatment, hepatoprotective activity, anti-leishmanial activity,

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antibacterial, antiviral, antioxidant, and amyloid heart disease (4–6).

Curcumin has been revealed to inhibit the metabolism of arachidonic acid and the activities of cyclooxygenase-2 (COX-2), lipo-oxygenase, proinflammatory cytokines, inducible nitric oxide (iNOS), protein kinases, transcription factors such as nuclear factor-kB, and release of steroids (7,8). Curcumin has been shown to inhibit COX-2 expression in gastrointestinal cancer cells and mouse skin (9). A number of previous studies have also indicated that curcumin affects the formation of COX- and LOX-dependent metabolites and decreases activities of PLA_2 (10). Curcumin is recognized to have a poor bioavailability, as orally administered curcumin goes through hepatic conjugation, resulting in the formation of glucuronides and sulfates, whereas systemic administration causes it to undergo reduction (11). Due to these limitations, its potential use as a therapeutic agent is seriously affected. As a result, plentiful approaches have been undertaken to synthesize new analogues and derivatives to enhance bioavailability of curcumin by altering its molecular structure, that is, eliminating the unstable β-diketone moiety and modifying the heptadiene linker while retaining the phenolic OH groups (12,13). Latest reports have demonstrated that the β-diketone moiety of curcumin is a specific substrate for liver aldoketo reductases. The presence of β-diketone moiety may add to the rapid metabolism of curcumin, thus limiting its beneficial effects in many types of disease. Recent studies have illustrated that the curcumin analogues are of great chemical and pharmacological interest owing to their anti-inflammatory properties (14).

Thus, a series of 17 curcumin-like diarylpentanoids has been synthesized, derived from the chemical structure of curcumin by eliminating the β-diketone and modifying it into conjugated double bonds, that is, two identical aromatic ring regions separated by five carbon linkers (Figure 1). To the best of our knowledge, curcumin-related diarylpentanoid analogues have not been investigated for their effects on enzymes of arachidonic acid metabolism. Therefore, we evaluated these compounds for their effects on the $sPLA_2$, COX, LOX, and microsomal prostaglandin E synthase-1 (mPGES-1).

Methods and Materials

Materials

Chemicals used in $sPLA_2$ assay include 1,2-bis(heptanoylthio)-phosphatidylcholine, 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), and recombinant human PLA_2-V from Cayman Chemicals, Ann Arbor, MI, USA. $CaCl_2$, KCl, and HCl were purchased from Merck, Darmstadt, Germany. Curcumin, dimethyl sulphoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), and $SnCl_2$ were obtained from Sigma-Aldrich (Steinheim, Germany). Cyclooxygenase activity was determined using a COX Inhibitor Screening Kit from Cayman Chemicals. Soybean lipo-oxygenase, arachidonic acid, and potassium hydroxide were also obtained from Cayman Chemicals. Human recombinant microsomal PGE synthase-1 enzyme and prostaglandin H_2 (PGH_2) were purchased from Cayman Chemicals. Prostaglandin E_2 (PGE_2) EIA kit used to quantify the PGE_2 concentration was also purchased from Cayman Chemicals.

General methods

^1H and ^{13}C NMR spectra were recorded with a JEOL ECP spectrometer operating at 500 MHz, with Me_4Si as internal standard and $CDCl_3$ or DMSO-d^6 as the solvent. High-resolution mass spectra (HRMS) were determined by the electrospray ionization mass spectrometry (ESI-MS) on MicroTOF-Q mass spectrometer (Bruker, Bremen, Germany). Microanalyses data were obtained from the Fison EA 1108 elemental analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Infrared spectra were recorded using KBr disc on a Perkin Elmer 400 (FTIR) spectrometer (Waltham, MA, USA). Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck), and thin-layer chromatography (TLC) was carried out on precoated silica plates (kiesel gel 60 F_{254}, BDH). Melting points were determined on an electrothermal instrument and are uncorrected. Compounds were visualized by illumination under ultraviolet (UV) light (254 nm) or by the use of vanillin stain followed by charring on a hotplate.

General procedure for synthesis of diarylpentanoid analogues

The diarylpentanoid analogues of curcumin were synthesized by direct coupling of the appropriate aromatic aldehyde with the three ketones, namely cyclohexanone, acetone, and cyclopentanone, at a molar ratio of 1:2, under base-catalyzed Claisen–Schmidt condensation reaction. Figure 1 illustrates the general synthesis of diarylpentanoids. Briefly, the appropriate aromatic aldehyde (20 mmol, two equivalent) and the appropriate ketone (10 mmol, one equivalent) were mixed and dissolved in 15 mL of ethanol in a single-necked round-bottomed flask and stirred at 5 °C for a few minutes. Into this solution, a 40% NaOH solution in ethanol was then added drop wise over several minutes. The mixture was then allowed to stir at room temperature (27 °C) for 1–24 h. The appearance of precipitate and color changes of the reaction mixture served as an indicative marker of product formation. Reaction was monitored by TLC, and on completion, the reaction was quenched by adding acidified ice to the mixture. The diarylpentanoid curcumin analogues were isolated by column chromatography or recrystallization.

2,6-Bis[4-(methylaminoethanol)benzylidine] cyclohexanone (1)

Cyclohexanone (1.00 mL, 10 mmol) with *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (3.58 g, 20 mmol)

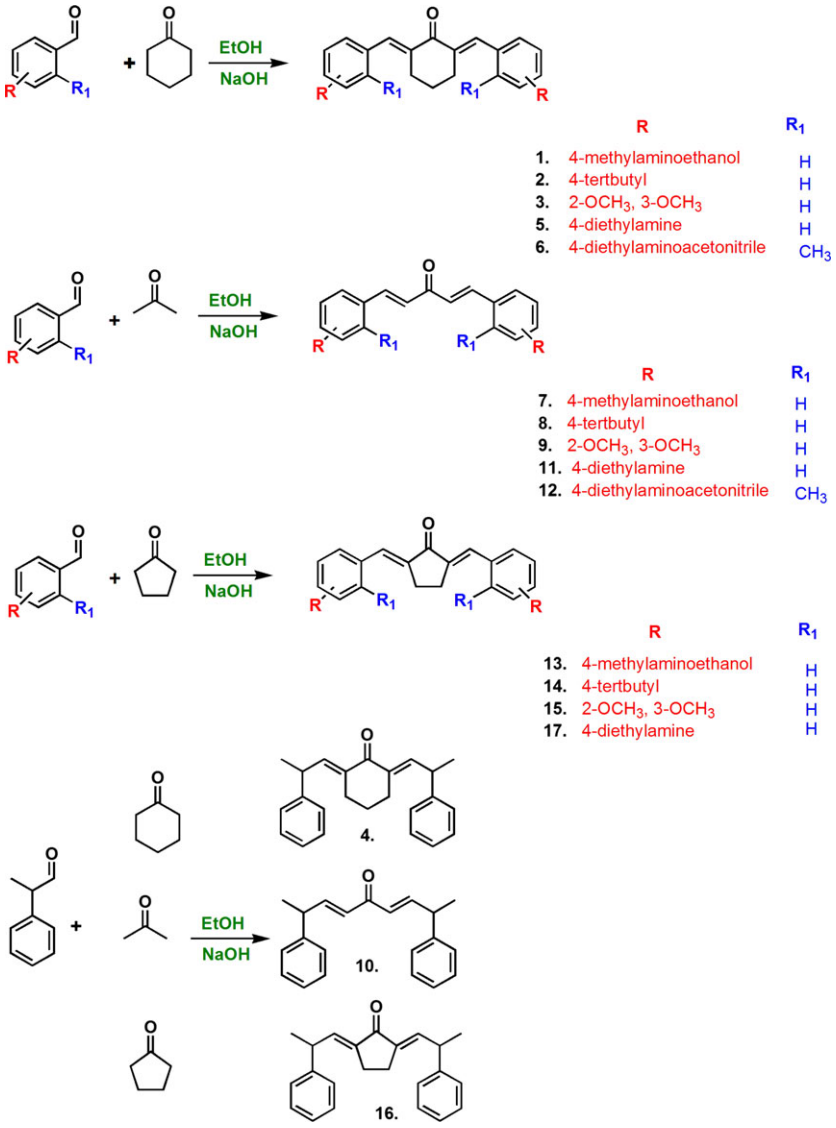

Figure 1: Synthesis scheme of diarylpentanoid analogues of curcumin.

were reacted to obtain compound **1** as dark red solids (2.89 g, 63%). mp: 80–81 °C; ^{1}H NMR (500 MHz, CDCl3) δ: 7.99 (s, 2H), 7.50 (d, J = 8 Hz, 4H), 6.76 (d, J = 8 Hz, 4H), 3.89 (t, J = 13.0, 4H), 3.62 (t, J = 7.5, 4H), 3.13 (s, 6H), 2.61 (t, J = 24.0 Hz, 4H), 1.70 (m, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.42, 153.94, 147.42, 137.77, 132.60), 125.30, 111.77, 60.01, 54.36, 39.18, 28.77, 23.60; $IR_{Vmax/cm^{-1}}$ (ATR) 3347.5, 1583.92, 1520.85, 1241.43; HRMS (ESI) m/z: 421.24 $[M+H]^+$; microanalysis calculated for $C_{26}H_{32}N_2O_3$ (420.54), C:74.26%, H:7.67%, N:6.66%. Found C: 74.42%, H: 7.62%, N: 6.99%.

2,6-Bis(2,3-dimethoxybenzylidene)cyclohexanone (3)

Cyclohexanone (1 mL, 10 mmol) and 2,3-dimethoxybenzaldehyde (3.32 g, 20 mmol) were reacted to give yellow crystals of 2,6-bis(2,3-dimethoxybenzylidene)cyclohexanone (3.23 g, 82%). ^{1}H NMR (500 MHz, CDCl3) δ: 7.95 (s, 2H), 7.07 (d, J = 8 Hz, 2H), 6.94 (m, J = 4 Hz, 4H), 3.90 (s, 6H), 3.84 (s, 6H), 2.95 (t, J = 24 Hz, 4H), 1.37 (m, J = 21 Hz, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.32, 152.88, 148.37, 137.55, 132.48, 130.49, 123.44, 122.17, 112.80, 61.13, 55.88, 28.73, 23.31; $IR_{Vmax/cm^{-1}}$ (ATR) 1576.5, 1471.78, 1257.27; HRMS (ESI) m/z 417.16 $[M+Na]^+$; microanalysis calculated for $C_{24}H_{26}O_5$ (394.46), C:73.08%, H:6.64%. Found C: 73.58%, H: 6.77%.

2,6-Bis(4-diethyl-aminobenzylidene)cyclohexanone (5)

Cyclohexanone (1 mL, 10 mmol) and 4-diethylaminobenzaldehyde (3.54 g, 20 mmol) were reacted to yield light red solid powder of 2,6-bis(4-diethyl-aminobenzylidene)

cyclohexanone (2.12 g, 51%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (s, 2H), 7.69 (d, J = 8 Hz, 4H), 6.59 (d, J = 8.5 Hz, 4H), 3.94 (q, J = 6.5 Hz, 8H), 2.33 (t, J = 7 Hz, 4H), 1.61 (m, J = 11 Hz, 2H), 1.32 (t, J = 6.5 Hz, 4H).; ^{13}C NMR (500 MHz, $CDCl_3$) δ: 189.73, 152.21, 148.09, 124.48, 130.77, 110.95, 44.56, 29.06, 23.79, 12.40; $IR_{Vmax/cm^{-1}}$ (ATR) 2974.2, 1588.15, 1522.45, 1272.49; HRMS (ESI) m/z: 417.28 $[M+H]^+$.

2,6-Bis[2-methyl-4(N-ethyl-N-ethylacetonitrile) aniline]cyclohexanone (6)

Cyclohexanone (1 mL, 10 mmol) and 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-aminobenzaldehyde (4.32 g, 20 mmol) were reacted to give a dark red solid compound **6** (2.55 g, 52%). mp: 180–181 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.61 (s, 2H), 6.71 (d, J = 2.4 Hz, 2H), 6.70 (d, J = 3.0 Hz, 2H), 6.61 (s, 2H), 3.71 (t, J = 6.6 Hz, 4H), 3.49 (q, J = 7.2 Hz, 4H), 2.77 (t, J = 6.6 Hz, 4H), 2.50 (m, 6H), 2.36 (s, 6H), 1.11 (t, J = 7.2 Hz, 6H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.41, 151.21, 142., 139.54, 139.21, 134.77, 123.58, 119.51, 113.76, 109.45, 55.55, 44.71, 29.24, 28.81, 20.24, 16.15, 12.47; $IR_{Vmax/cm^{-1}}$ (ATR) 1583.92, 1520.85, 1241.43; HRMS (ESI) m/z: 495.27 $[M+H]^+$; microanalysis calculated for $C_{32}H_{38}N_4O$ (494.67), C: 77.70%, H: 7.74%, N: 11.33%. Found C: 77.91%, H: 7.78%, N: 11.42%.

(1Z,4Z)-1,5-Bis [4-(methylaminoethanol)phenyl]-1,4-pentadiene-3-one (7)

Acetone (0.73 mL, 10 mmol) and *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (3.58 g, 20 mmol) were reacted, and a dark brown powder of (1Z,4Z)-1,5-Bis [4-(methylaminoethanol)phenyl]-1,4-pentadiene-3-one (2.12 g, 55%) was obtained. RF 0.56 (EtOAc-PE 1:3 v/v); mp: 80–81 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.74 (d, J = 8.5 Hz, 4H), 7.51 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 6.79 (d, J = 9.0 Hz, 2H), 6.76 (d, J = 6.0 Hz, 2H), 3.88 (t, J = 4.0 Hz, 4H), 3.62 (m, 4H), 3.14 (s, 3H), 3.09 (s, 3H), 2.36 (s, 2H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.32, 153.90, 151.40, 130.17, 122.44, 121.43, 112.10, 60.20, 60.13, 38.93; $IR_{Vmax/cm^{-1}}$ (ATR) 3391.9, 1746.0, 1516.42, 1048.69-1351.89; HRMS (ESI) m/z: 381.21 $[M+H]^+$.

(1Z,4Z)–1-5-Bis[2,3(dimethoxy)phenyl]-1,4-pentadien-3-one (9)

Acetone (0.73 mL, 10 mmol) and 2, 3-dimethoxybenzaldehyde (3.32 g, 20 mmol) were reacted to yield pale yellow solid (1Z,4Z)–1-5-bis[2,3(dimethoxy)phenyl]-1,4-pentadien-3-one (1.32 g, 37%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.06 (d, J = 16 Hz, 2H), 7.27 (d, J = 7.5 Hz, 2H), 7.18 (d, J = 16 Hz, 2H), 7.11 (t, J = 5 Hz, 2H), 6.99 (d, J = 7.5 Hz, 2H), 3.91 (s, 6H); ^{13}C NMR (500 MHz, CDCl3) δ: 189.72, 153.20, 148.83, 137.92, 129.10, 126.94, 124.23, 119.14, 114.14, 61.36, 55.92; $IR_{Vmax/cm^{-1}}$ (ATR) 2974.2, 1715.59, 1469.64, 1369.16; HRMS (ESI) m/z: 291.17 $[M+H]^+$.

(1E,4E)-1,5-Bis[2-methyl-4(N-ethyl-N-ethylacetonitrile)aniline]-1,4-pentadiene-3-one (12)

The reaction of acetone (0.73 mL, 10 mmol) with 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-aminobenzaldehyde (4.32 g, 20 mmol), followed by purification (by column chromatography) yielded a dark red solid compound **12** (2.95 g, 64%). mp: 178–179 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.82 (d, J = 15.5 Hz, 2H), 7.70 (d, J = 4.8 Hz 2H), 6.65 (d, J = 15.5 Hz, 2H), 6.63 (d, J = 2.4 Hz, 2H), 6.52 (s, 2H), 3.66 (q, J = 9 Hz, 8H), 2.75 (t, J = 7.2 Hz, 4H), 2.38 (s, 6H), 1.12 (t, J = 2.6 Hz, 6H); ^{13}C NMR (500 MHz, CDCl3) δ: 187.85, 148.94, 140.88, 139.86, 128.74, 128.65, 123.07, 119.94, 113.17, 110.50, 56.26, 44.61, 23.07, 16.24, 12.46; $IR_{Vmax/cm^{-1}}$ (ATR) 1746.0, 1516.42, 1048.69-1351.89; HRMS (ESI) m/z: 455.27 $[M+H]^+$; microanalysis calculated for $C_{29}H_{34}N_{4O}$ (454.61), C: 76.62%, H: 7.54%, N: 12.32%. Found C: 76.69%, H: 7.79%, N: 12.41%.

2,5-Bis[4-(methylaminoethanol)benzylidene] cyclopentanone (13)

This compound was obtained by reacting cyclopentanone (0.88 mL, 10 mmol) with *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (3.58 g, 20 mmol). Compound **13** was isolated as red solid (2.92 g, 72%). mp: 221–223 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.97 (s, 2H), 7.56 (d, J = 8.0 Hz, 4H), 7.35 (d, J = 9 Hz, 4H), 3.93 (t, J = 8.0 Hz, 4H), 3.62 (t, J = 11.5 Hz, 4H), 3.52 (s, 4H), 2.99 (s, 6H); ^{13}C NMR (500 MHz, CDCl3) δ: 195.92, 154.84, 137.83, 136.97, 136.51, 134.00, 123.72, 61.37, 55.92, 38.38, 26.74; $IR_{Vmax/cm^{-1}}$ (ATR) 3369.5, 1741.10, 1169.92; HRMS (ESI) m/z: 407.17 $[M+H]^+$; microanalysis Calculated for $C_{25}H_{30}N_2O_3$ (406.52), C: 73.86%, H: 7.44%, N: 6.89%. Found C: 73.69%, H: 7.65%, N: 6.53%.

2,5-Bis(4-diethylaminobenzylidene)cyclopentanone (17)

Cyclopentanone (0.88 mL, 10 mmol) and 4-diethylaminobenzaldehyde (3.54 g, 20 mmol) were reacted to give compound **17** as dark brown powder (2.76 g. 69%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.03 (s, 2H), 7.53 (d, J = 4 Hz, 4H), 6.71 (d, J = 8.5 Hz, 4H), 3.43 (q, J = 7 Hz, 4H), 3.08 (s, 4H), 1.22 (t, J = 7 Hz, 12H); ^{13}C NMR (500 MHz, CDCl3) δ: 196.02, 148.30, 133.49, 123.49, 133.08, 132.88; 111.33, 44.46, 26.69, 12.66; $IR_{Vmax/cm^{-1}}$ (ATR) 2977.8, 1575.07, 1513.89, 1264.85; MS (ESI) m/z: 403.22 $[M+H]^+$; microanalysis calculated for $C_{27}H_{34}N_2O$ (402.57), C: 80.55%, H: 8.51%, N: 6.96%. Found C: 80.48%, H: 8.27%, N: 6.77%.

Secretory phospholipase A_2 –V (sPLA$_2$-V) activity assay

Human recombinant sPLA$_2$-V was employed as an enzyme source. The activity of sPLA2 enzyme was

determined by a photometric assay based on the Ellman's method (15). In brief, the hydrolysis of sn-2 ester bond of the substrate 1,2-bis(heptanoylthio)-glycerophosphocholine by PLA_2-V was followed by the exposure of free thiols. These thiols activated the alteration of DTNB (5,5–dithio-bis-(2-nitrobenzoic acid) to 2-nitro-5-thiobenzoic acid, which was detected photometrically at 405 nm. Later, the assay was progressed in an aqueous buffer solution (pH 7.5) containing KCl (94 mM), $CaCl_2$ (9 mM), Tris (24 mM), and Triton-X 100 (280 μM). Earlier to the assay, substrate and PLA2-V were resuspended in assay buffer, and DTNB was dissolved in an aqueous solution of Tris–HCl (pH 8). Enzyme and DTNB yielded final concentrations of 100 ng/mL and 87 μM, correspondingly. Assays were carried out in 96-well microliter plates, at room temperature containing DTNB, substrate solution, and the respective test substance. 100% activity of the enzyme was calculated by adding substrate and enzyme only. Dimethyl sulphoxide served as negative control and was inactive at the concentration used in the assay (1.7% v/v).

Lipo-oxygenase activity assay

The inhibitory activity of the compounds against purified LOX enzyme was determined by a standard colorimetric assay (16). Soybean lipo-oxygenase was employed as an enzyme source. The assay was progressed in a 0.1 M Tris–HCl buffer solution at pH 7. Immediately preceding the assay, LOX enzyme was resuspended in assay buffer. Substrate was dissolved in equal volume of potassium hydroxide, vortexed, and diluted with the HPLC grade water to obtain the final concentration of 1 mM. Assays were performed in 96-well microliter plates at room temperature containing substrate, enzyme, and respective test substance. 100% activity of enzyme was calculated by adding the substrate and enzyme only. 90 μL of LOX and 10 μL of respective test sample were added to determine the activity. The reaction was started by adding substrate solution to all wells and incubated for 5 min. Then, 100 μL of chromogen solution was added to all wells to stop enzyme catalysis and incubated for 5 min. After that, the plate cover was removed and the absorbance was measured at 490 nm using Tecan® Infinite Pro 200 microplate reader (Mannedorf, Switzerland). Dimethyl sulphoxide served as a negative control. This solvent was inactive at the concentration used in the assay (1.7% v/v).

Cyclooxygenase activity assay

The inhibitory effects of the curcumin analogues on COX-1 and COX-2 activities were determined using a COX inhibitor screening kit (17). Reaction mixtures were prepared in 100 mM Tris–HCl buffer, pH 8.0 containing 1 mM heme and COX-1 (ovine) or COX-2 (human recombinant) and preincubated for 10 min in a water bath at 37 °C. The reaction was initiated by the addition of 10 μL arachidonic acid. Final concentration of arachidonic acid in reaction mixture was 100 μM. After 2 min, the reaction was terminated by adding 1 M HCl, and finally, PGE_2 was quantified by an ELISA method. The test compounds were dissolved in DMSO and diluted to the desired concentration with potassium phosphate buffer of pH 7.4. Following transfer to a 96-well plate coated with a mouse anti-rabbit IgG, the tracer prostaglandin acetylcholine esterase and primary antibody (mouse anti-PGE_2) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and the wells were washed with 10 mM potassium phosphate buffer containing 0.05% Tween-20. Ellman's reagent (200 μL) was added to each well, and the plate was incubated at room temperature for 60 min and measured the absorbance at 412 nm using Tecan® Infinite Pro 200 microplate reader. A standard curve with PGE_2 was generated from the same plate, which was used to quantify the PGE_2 levels produced in the presence of test samples. Percent inhibition was calculated by the comparison of compound treated to control incubations. All determinations were performed in duplicate.

Microsomal prostaglandin E synthase-1 activity assay

Microsomal prostaglandin E synthase-1 activity was assessed, as described previously (18). Human recombinant mPGES-1 was used as enzyme source. Prostaglandin H_2 (PGH_2) served as the substrate for the enzyme mPGES-1. Reduced glutathione was also employed, which served as catalyst for the enzyme. Hundred millimolar $KHPO_4$ buffer at pH 7.0 supplemented with 2 mM EDTA and 2.5 mM reduced GSH served as assay buffer. Microsomal prostaglandin E synthase-1 activity was determined using 96-well non-binding plate. 100 μL of reaction buffer containing mPGES-1 was added to each well of a 96-well non-binding plate. Afterward, the test samples were added to their respective wells. The 96-well plate was covered with the plate sealer and incubated for 30 min at 20 °C. After incubation, the enzyme reaction was initiated by adding 20 μL of cold 2.8 μM PGH_2 to all wells and incubated again for 30 seconds at room temperature. At set time, the reaction was quenched by adding 20 μL of $SnCl_2$ solution in 1 N HCl. The activity of mPGES-1 enzyme was calculated by measuring the concentration of product (PGE_2). Prostaglandin E_2 concentration was measured by enzyme-linked immunosorbent assay. The procedure was performed according to protocol supplied by the manufacturer. The sample was transferred to a 96-well plate coated with a goat antimouse IgG. Afterward, 50 μL of tracer prostaglandin acetylcholine esterase and 50 μL of monoclonal antibody were added and incubated for 60 min at room temperature. Reaction mixtures were removed and washed with the washing buffer. Ellman's reagent (200 μL) was added to each well and incubated at room temperature in the dark on an orbital shaker for 60–90 min, and the absorbance of the solution at 420 nm was measured using a plate reader. All determinations were performed in duplicate.

Statistical analysis

All the experiments are conducted three times, and all data are presented as the mean ± standard error of mean (SEM). The IC_{50} values were calculated using GraphPad Prism 5 software (Graphpad Software, Inc., La Jolla, CA, USA). The values were obtained from at least three determinations. Data were analyzed using a one-way analysis of variance (ANOVA) for multiple comparisons. $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Inhibition of $sPLA_2$-V

Eicosanoids are the oxygenated metabolites of arachidonic acid (AA) with broad implications in a number of diseases. Upon stimulation of neutrophils, PLA_2 cleaves arachidonic acid from membrane phospholipids at sn-2 position and generates lysophospholipid and free fatty acid. Subsequently, the free fatty acid is converted into biologically active prostaglandins, prostacyclins, thromboxanes, and leukotrienes, collectively known as eicosanoids (19). The release of arachidonic acid by PLA_2 from membrane phospholipids is typically the rate-limiting step for further arachidonic acid metabolism and eicosanoid generation. In this context, there is an increased support for strategies that prevent inflammatory reaction. Mixed results have been achieved by inhibiting selective pathways of eicosanoid production, that is, the LOX and COX pathways (20). Numerous types of PLA_2s are found in human system. Among these PLA_2 enzymes, $sPLA_2$s play a key role in the pathogenesis of inflammatory diseases, and elevated levels of $sPLA_2$s are detected in many inflammatory conditions (21). So, the inhibition of PLA_2 is an important strategy for the prevention of inflammation. Thus, the effects of diarylpentanoid curcumin analogues at concentrations of 1.25–20 μg/mL on the activity of $sPLA_2$ were determined by photometric assay based on Ellman's method. Their percentage inhibitions (%) are shown in Table 1. Ten curcumin analogues **1**, **3**, **5**, **6**, **7**, **9**, **11**, **12**, **15,** and **17** strongly inhibited the activity of $sPLA_2$ with percentage inhibition ranging from 74.09% to 86.93%. Dimethyl sulphoxide was used as a control and was inactive at the concentration used in the assay (1.70% v/v). Curcumin and dexamethasone were used as positive controls. The IC_{50} values of the compounds on the inhibition of PLA_2 are shown in Table 1. Curcumin was used as a reference compound, which inhibited the activity of sPLA2 by 81.57% (IC_{50} 11.10 μM). Several previous studies had been conducted for the other isoforms of PLA2, which indicated that curcumin decreased activities of PLA_2, and various isoforms of PLA_2 enzymes exhibited more than 70% homology (17,22). Compounds **3**, **5**, **6**, **9**, **12,** and **17** strongly inhibited $sPLA_2$-V in a dose-dependent manner (Figure 2), with IC_{50} values in the range of 5.89–12.09 μM, which are lower or comparable to that of curcumin (11.10 μM).

Structure–activity analysis of the diarylpentanoid analogues indicated that compounds bearing 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-amino, diethylamino, and *N*-methyl-*N*-(2-hydroxyethyl)-4-amino substitutions at position 4 of benzene ring showed good inhibition of $sPLA_2$ activity in all three types of curcumin analogues bearing acetone, cyclopentanone, and cyclohexanone. However, 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-amino bearing analogues demonstrated stronger inhibition of $sPLA_2$, in fact almost a twofold improvement in inhibition of $sPLA_2$ relative to curcumin. Compounds of cyclohexanone ring system were more active than cyclopentanone or acetone bearing analogues. Among these three, the most active was compound **6,** which belongs to cyclohexanone ring system. Methoxylation at positions 2 and 3 of both phenyl rings in acetone and cyclohexanone demonstrated stronger inhibitory activity; however, methoxylation at same position in cyclopentanone system did not show such strong inhibition. The introduction of 4-tert-butyl substitution reduced the inhibitory effect in all three types of curcumin analogues bearing acetone, cyclopentanone, and cyclohexanone.

Table 1: Inhibition of secretory phospholipase A_2-V ($sPLA_2$-V) activity by curcumin analogues

Compounds	$sPLA_2$-V% inhibition	IC_{50} (μM)
1	79.01 ± 1.59	15.90 ± 0.71
2	49.44 ± 0.90	53.28 ± 2.50
3	83.64 ± 2.10	11.02 ± 0.50
4	48.96 ± 1.20	94.71 ± 4.23
5	79.89 ± 2.30	12.09 ± 1.68
6	82.92 ± 1.80	5.89 ± 0.20
7	80.13 ± 2.03	16.36 ± 0.52
8	51.68 ± 1.10	51.88 ± 2.30
9	86.05 ± 2.40	10.38 ± 1.69
10	44.73 ± 0.89	125.38 ± 7.23
11	75.74 ± 3.15	16.06 ± 1.59
12	86.93 ± 4.10	5.98 ± 0.43
13	70.78 ± 0.97	19.13 ± 0.98
14	42.80 ± 2.20	83.21 ± 5.10
15	74.09 ± 1.78	16.45 ± 0.52
16	22.11 ± 5.20	ND
17	78.69 ± 1.19	10.18 ± 0.74
Curcumin	81.57 ± 1.56	11.10 ± 0.30
Dexamethasone	77.66 ± 1.03	0.61 ± 0.01

Values are the mean ± SD; $n = 3$. ND, not determined.

Inhibition of cyclooxygenases

The enzyme cyclooxygenase (COX) catalyzes the first two steps in the biosynthesis of prostaglandins (PGs) from the substrate arachidonic acid. Prostaglandins play important roles in health as well as in disease state, in the gastrointestinal tract, renal, skeletal, and ocular systems. Two isoforms of this enzyme exist, which are COX-1 and COX-2 (23). The constitutive COX-1 is well recognized to be housekeeping enzyme and is responsible for maintaining normal physiological function; in contrast, COX-2 is an inducible enzyme and its expression is activated by a diversity of stimuli such as mitogens, oncogenes, tumor promoters, growth factors, tissue damage, and inflammatory

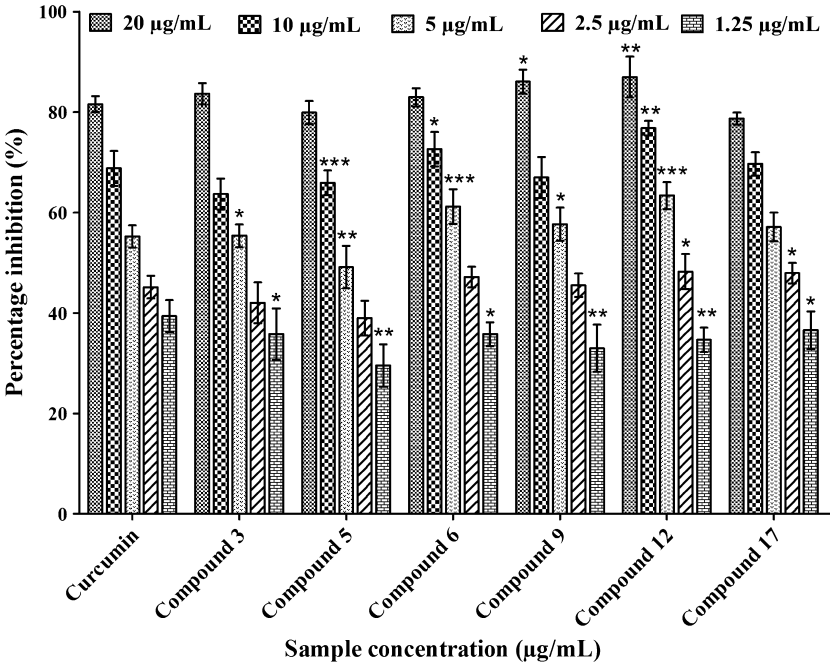

Figure 2: Concentration-dependent inhibitory effects of diarylpentanoid analogues on activity of secretory phospholipase A_2-V. Graph represents the % inhibition by most active compounds along with curcumin. *p < 0.05, **p < 0.01, ***p < 0.001 are significant difference with respect to curcumin. The data shown are an average of three independent experiment, and values are mean ± SD.

conditions (23). The inhibition of COX-1 results in undesirable side-effects, whereas COX-2 inhibition provides therapeutic effects in pain, inflammation, cancer, and neuropathologic conditions like Alzheimer's and Parkinson diseases (24,25). The inhibition of the enzymes COX-1 and COX-2 is considered as one of the mechanisms of anti-inflammatory actions. The inhibitory effect of curcumin analogues on the cyclooxygenase activity was determined at concentration of 40 µg/mL for both isoforms of cyclooxygenase, that is, COX-1 and COX-2. COX-1 and COX-2 inhibitory activity was evaluated using the COX inhibitor screening assay kit *in vitro* (17). Percentage inhibition and IC_{50} were calculated, and the results are exhibited in the Table 2. The COX-1 and COX-2 inhibitory effect of these diarylpentanoid analogues was compared with that of pure curcumin.

Table 2: Inhibition of COX-1 and COX-2 activity by curcumin analogues

	COX% inhibition		IC_{50} (µM)	
Compounds	COX-1	COX-2	COX-1	COX-2
1	89.41 ± 0.67	61.73 ± 1.02	22.08 ± 0.42	46.55 ± 1.35
2	71.51 ± 1.07	39.02 ± 1.23	43.22 ± 0.28	ND
3	84.34 ± 0.87	63.51 ± 0.33	33.33 ± 0.17	53.23 ± 1.27
4	52.68 ± 1.33	32.55 ± 2.31	194.0 ± 3.14	ND
5	74.97 ± 0.95	41.98 ± 0.67	38.52 ± 0.48	ND
6	82.55 ± 0.38	55.78 ± 0.87	16.65 ± 0.35	57.75 ± 0.58
7	88.20 ± 0.59	67.91 ± 1.33	23.62 ± 0.38	56.57 ± 1.37
8	68.50 ± 1.23	34.24 ± 1.43	53.24 ± 0.76	ND
9	79.82 ± 1.02	58.20 ± 0.34	41.51 ± 0.49	70.71 ± 1.36
10	49.29 ± 2.01	22.52 ± 2.01	194.6 ± 2.40	ND
11	78.88 ± 1.21	51.93 ± 0.77	37.70 ± 0.31	94.86 ± 2.16
12	86.62 ± 0.57	65.13 ± 0.65	17.40 ± 0.60	46.11 ± 1.4 8
13	72.46 ± 1.04	44.27 ± 1.03	35.86 ± 0.24	ND
14	66.51 ± 1.45	31.55 ± 1.45	60.01 ± 0.97	ND
15	77.90 ± 0.96	36.04 ± 0.46	40.24 ± 0.36	ND
16	46.65 ± 2.48	33.83 ± 0.25	319.64 ± 4.12	ND
17	75.35 ± 1.73	44.88 ± 1.12	31.96 ± 0.54	ND
Curcumin	83.06 ± 1.43	51.53 ± 2.6	31.31 ± 0.45	96.36 ± 1.43
Indomethacin*	94.06 ± 0.45	75.11 ± 0.13	0.21 ± 0.03	3.24 ± 0.01

*30 µM concentration.
Values are the mean ± SD; n = 3. ND, not determined.

The result showed that the curcumin analogues had a affinity toward COX-1. Five compounds (**1**, **3**, **6**, **7,** and **12**) exhibited strong inhibitory effect on activity of COX-1 with extent of inhibition ranging from 82.55% to 89.41%. Effect of pure curcumin was also observed on the activity of COX-1 and was used as a reference compound. Indomethacin served as positive control. Four compounds (**1**, **6**, **7,** and **12**) illustrated better COX-1 inhibitory activity than that shown by curcumin with IC_{50} values in the range of 16–22 μM, which was on the lower side than that of curcumin (31.31 μM), whereas the inhibitory activity of compound **17** was comparable with the curcumin with an IC_{50} value of 31.96 μM. Compound **6** exhibited the highest COX-1 inhibition (IC_{50} 16.65 μM) followed by **12** (IC_{50} 17.40 μM). The COX-1 inhibitory effect of **6** was twofold when compared to curcumin (IC_{50} 31.31 μM).

In case of COX-2, in contrast to COX-1, curcumin analogues were less efficient in suppressing the activity of COX-2. Seven compounds (**1**, **3**, **6**, **7**, **9**, **11,** and **12**) exhibited COX-2 inhibitory activity with IC_{50} values in the range of 46.11–94.86 μM. COX-2 inhibitory activity of these compounds was better than that of curcumin (IC_{50} 96.36 μM). The highest COX-2 inhibition was exhibited by compound **12** with an IC_{50} value of 46.11, which was twofold greater when compared to curcumin (IC_{50} 96.36 μM). Thus, analogue **12** showed significant enhancement in the selectivity toward COX-2 enzyme (COX-2/COX-1 = 2.6) when compared to curcumin (COX-2/COX-1 = 3.07). Compounds **1**, **3**, **6,** and **7** also showed better selectivity toward COX-2. It was observed that the compounds inhibited COX activity in a dose-dependent manner; there was an increase in the inhibitory activity of compounds with increase in the concentration. The results of the present study suggest that curcumin analogues act as non-selective inhibitors of COX enzymes and showed anti-inflammatory effects. The structure–activity relationship studies (SAR) revealed that diarylpentanoid analogues (**5**, **11,** and **17**) with diethylamine group at position 4 of both phenyl rings showed better COX-1 inhibition without having effect on COX-2. The conversion of diethylamine groups at position 4 of phenyl ring into methoxy groups (**3** and **9**) improved both COX-1 and COX-2 inhibitory activity; however, compound **15** with similar substitution pattern but contains cyclopentanone linker did not show improvement toward COX-2. The presence of *N*-methyl-*N*-(2-hydroxyethyl)-4-amino at position 4 of the both phenyl rings of cyclohexanone and acetone diarylpentanoid analogues resulted in compounds **1** and **7**, which exhibited strong COX-2 inhibitory activity than curcumin. Compound **12** acetone diarylpentanoid analogue with 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-amino substitution at position 4 of both phenyl rings showed strongest COX-2 inhibition.

Inhibition of lipo-oxygenase

Eicosanoids are oxygenated metabolites of arachidonic acid (AA) with broad implications in a variety of diseases. Arachidonic acid is a substrate for the lipo-oxygenases. Lipo-oxygenase is a family of non-heme iron-containing dioxygenases. The upregulation of LOX has been associated with various adverse effects (26). Lipo-oxygenase family exists in three isoforms: LOX-5, LOX-12, and LOX-15. Together, they catalyze the deoxygenation of arachidonic acid into hydroperoxyeicosatetraenoic acids (HpETEs). In the end, this is followed by their conversion to their corresponding hydroxyeicosatetraenoic acids (HETEs), resulting in the formation of the leukotrienes (LKs), lipoxins (LOs), and hepoxilins (HOs) (27). Inhibitors of LOX have attracted attention initially as potential agents for the treatment for inflammatory and allergic diseases and certain types of cancer. For this reason, we evaluated the ability of curcumin analogues to inhibit soybean lipo-oxygenase, which converts linoleic to 13-hydroperoxylinoleic acid. Soybean lipo-oxygenase is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) in a qualitatively similar manner to that of the rat mast cell lipo-oxygenase and can be used as a reliable screen for such activity (28).

Curcumin was used as a reference compound. Curcumin has also been reported to inhibit 5-LOX activity both in *in vitro* and *in vivo* models (6,29). Inhibitory effect of curcumin on soybean LOX has also been reported in a study conducted by Katsori *et al.* (28). The effects of the diarylpentanoid analogues on the activity of LOX were determined at concentration ranging 2.5–40 μg/mL, and their percentage inhibitions are shown in Table 3. Preliminary screening of the compounds on the activity of LOX showed that compounds **3**, **6**, **7**, **9**, **12**, **15,** and **17** strongly inhibited the activity of LOX with percentage inhibitions ranging from

Table 3: Inhibition of lipo-oxygenase activity by curcumin analogues

Compounds	Lipo-oxygenase% inhibition	IC_{50} (μM)
1	54.45 ± 1.95	82.44 ± 0.71
2	36.03 ± 2.97	ND
3	77.42 ± 1.41	47.83 ± 0.51
4	36.27 ± 3.72	ND
5	42.82 ± 2.72	ND
6	80.53 ± 1.43	30.02 ± 0.21
7	62.57 ± 1.51	56.42 ± 0.52
8	38.21 ± 2.03	ND
9	70.29 ± 1.49	62.86 ± 1.69
10	30.12 ± 2.44	ND
11	52.04 ± 1.88	95.07 ± 4.24
12	75.10 ± 1.14	41.13 ± 0.43
13	52.27 ± 2.58	89.51 ± 0.98
14	23.16 ± 3.68	ND
15	62.17 ± 1.77	63.76 ± 1.84
16	27.86 ± 4.86	ND
17	62.29 ± 1.86	56.76 ± 0.74
Curcumin	66.29 ± 1.59	57.77 ± 0.21
NDGA*	94.65 ± 1.48	9.23 ± 0.33

*16 μM concentration.
Values are the mean ± SD; n = 3. ND, not determined.

64.2% to 80.53%. Dimethyl sulphoxide was used as negative control and did not show any effect on activity of enzyme. Compounds **7** and **15** (62.57% and 62.17%) were equipotent to curcumin (66.29%) at 40 μg/mL. Among the analogues tested, **3**, **6**, **9,** and **12** were the strong inhibitors with IC_{50} values ranging from 30.02 to 62.86 μM, which are lower or comparable to the value for curcumin (57.7 μM). Among all curcumin analogues, **6** exhibited highest inhibitory activity. The extent of % inhibition was 80.53 with an IC_{50} value of 30.02 μM. From the results, it can be concluded that the compounds inhibited LOX activity in a dose-dependent manner, that is, as the concentration of the compound increased, there was a decrease in the activity of enzyme and vice versa, as shown in Figure 3.

Structure–activity analysis of the diarylpentanoid analogue (compounds **3**, **5,** and **15**) indicates that the presence of methoxy groups at positions 2 and 3 on the phenyl ring demonstrated stronger inhibitory activity against LOX. Among the diarylpentanoid analogue, compound bearing 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-amino groups at position 4 of both phenyl rings (compounds **6** and **12**) exhibited the strongest inhibition of LOX activity than the curcumin. The presence of diethylamino group at position 4 (compound **17**) of both phenyl rings also shows inhibition of LOX in cyclopentanone ring system. However, substitution of phenyl rings with 4-tert-butyl produced compounds with lower inhibitory activity.

Inhibition of microsomal prostaglandin E synthase-1

Prostaglandin E_2 is a potent lipid mediator, that is, closely related with inflammation and cancer. Arachidonic acid is transformed to PGH_2 by COX, and it is this PGH_2 which then acts as substrate for microsomal PGE synthase-1 (mPGES-1) and gets converted to PGE_2. mPGES-1 is the major PGE_2 synthase under pathological conditions related to inflammation and cancer (30). mPGES-1 expressed in response to pro-inflammatory stimuli results in increased formation of PGE_2 during inflammation (31). Latest advances in genetic and pharmacologic inhibition of mPGES-1 indicate a crucial role of mPGES-1 in the development and maintenance of inflammatory disorders, pain, fever, and cardiovascular diseases, and suggest mPGES-1 inhibitors as alternatives to non-steroidal anti-inflammatory drugs showing comparable anti-inflammatory effectiveness while being essentially free of severe side-effects (30,32).

The effect of curcumin analogue on mPGES-1 activity was assessed, as described previously (18). Purified human recombinant mPGES-1 was used as enzyme source to investigate the direct effects of curcumin analogue on the activity of mPGES-1. PGE_2 concentration was assessed by ELISA. Pure curcumin effect was also determined and used as a reference. Curcumin has been shown to lower production of PGE_2 both *in vitro* and *in vivo* in earlier demonstrations (33,34). Well-known mPGES-1 inhibitor MK-886 was used as control. All curcumin analogues were screened to assess the effect on the activity of mPGES-1 at concentrations ranging from 0.625 to 10 μg/mL. Dimethyl sulphoxide was used as a solvent to dissolve the curcumin analogue and also as negative control. Percentage inhibitions and IC_{50} values were calculated. Results are demonstrated in the Table 4 in the form of % inhibition and IC_{50} values. The curcumin analogues inhibited the activity of the mPGES-1. The% inhibition of mPGES-1

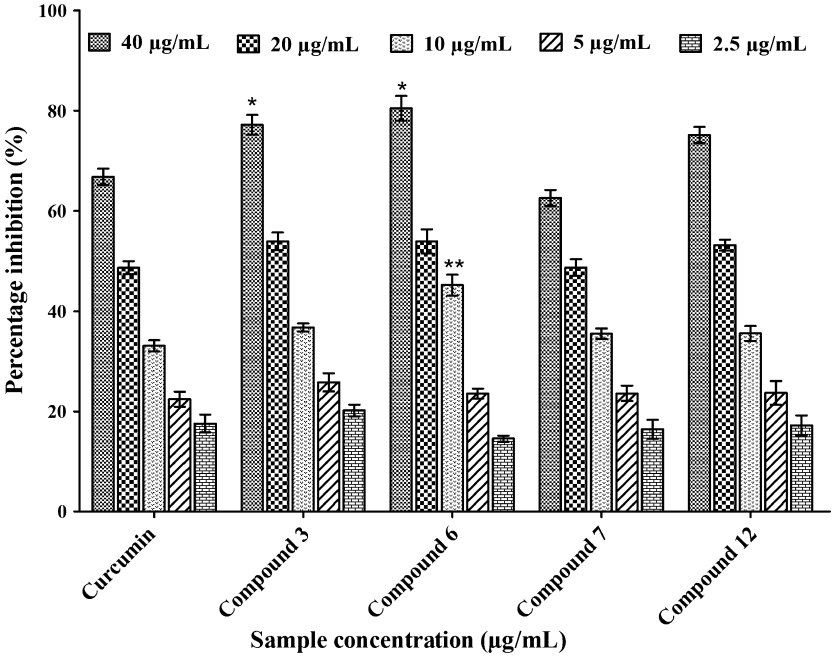

Figure 3: Concentration-dependent inhibitory effects of diarylpentanoid analogues on activity of lipo-oxygenase. Graph represents the % inhibition by most active compounds along with curcumin. *$p < 0.05$, **$p < 0.01$ are significant difference with respect to curcumin. The data shown are an average of three independent experiment, and values are mean ± SD.

Table 4: Inhibition of microsomal prostaglandin E synthase-1 (mPGES-1) activity by curcumin analogues

Compounds	mPGES-1% inhibition	IC_{50} (μM)
1	81.38 ± 2.83	4.96 ± 0.47
2	69.38 ± 0.83	8.95 ± 1.03
3	74.07 ± 1.54	7.34 ± 0.76
4	56.07 ± 1.49	20.27 ± 2.11
5	85.20 ± 2.89	2.41 ± 0.24
6	79.92 ± 2.83	4.48 ± 0.80
7	80.02 ± 2.50	4.23 ± 0.26
8	48.72 ± 1.67	36.99 ± 4.32
9	59.44 ± 3.63	14.75 ± 2.25
10	42.77 ± 2.44	60.71 ± 5.85
11	71.75 ± 2.67	6.98 ± 0.79
12	67.65 ± 0.83	7.89 ± 0.21
13	82.12 ± 2.83	3.78 ± 0.24
14	51.96 ± 1.44	24.48 ± 3.22
15	67.24 ± 2.20	10.43 ± 1.84
16	70.21 ± 1.83	7.48 ± 1.89
17	79.93 ± 2.20	5.48 ± 0.74
Curcumin	83.17 ± 1.63	4.88 ± 0.54
MK 886*	91.83 ± 2.20	1.91 ± 0.8

*30 μM concentration.
Values are the mean ± SD; n = 3.

activity varied for different analogues at different concentrations. Compounds (**1**, **5**, **6**, **7**, **13**, and **17**) showed strong inhibitory effect on mPGES-1 activity with IC_{50} values ranging from 2.4 to 5.4 μM, respectively. The effects of pure curcumin were also investigated on mPGES-1 activity. It was observed that curcumin inhibited mPGES-1 activity in a concentration-dependent manner, with an IC_{50} value of 4.88 μM. Previous studies showed that curcumin effectively inhibited PGE_2 formation and mPGES-1 directly with IC_{50} values ranging from 1 to 5 μM (35,36). So, our findings are somehow consistent with previous studies. The four diarylpentanoid curcumin analogues (**5**, **6**, **7,** and **13**) demonstrated strong inhibition of mPGES-1 activity with IC_{50} values ranging from 2.41 to 4.48 μM, which was less than that of curcumin (4.88 μM). While the inhibitory activity of compound **1** was comparable to curcumin, compound 1 showed almost similar IC_{50} value (4.96 μM). Among all tested curcumin analogues, compound **5** exhibited the strongest inhibitory effect with 85.20% reduction in mPGES-1 activity and with an IC_{50} of 2.41 μM, followed by **13**, **7,** and **6**, respectively. The results indicate that the potency of compound **5** to inhibit the mPGES-1 was twofold greater when compared to curcumin (4.88 μM). The results showed that the compounds inhibit activity of mPGES-1 in a dose-dependent manner, that is, as the concentration of the samples increased the activity of mPGES-1 decreased and vice versa (Figure 4).

Structure–activity analysis of the diarylpentanoid analogues indicated that compounds (**5** and **17**) bearing diethylamino substitutions at position 4 of both phenyl rings were showing strong inhibition of mPGES-1 activity. In fact, the 4-diethylamino analogue (compound **5**) was the most potent compound among the 17 diarylpentanoid analogues tested. Compound **11,** which has a similar substitution pattern on its phenyl ring structures but contains an acetone linker, showed weaker activity than the latter. The presence of *N*-methyl-*N*-(2-hydroxyethyl)-4-amino substitutions at position 4 of both phenyl rings resulted in a compounds (**1**, **7**, **13**) with strong inhibitory activity. Substitution of 2-methyl-*N*-ethyl-*N*-(2-cyanoethyl)-4-amino at position 4 of both phenyl rings (compound **6**) produced a compound

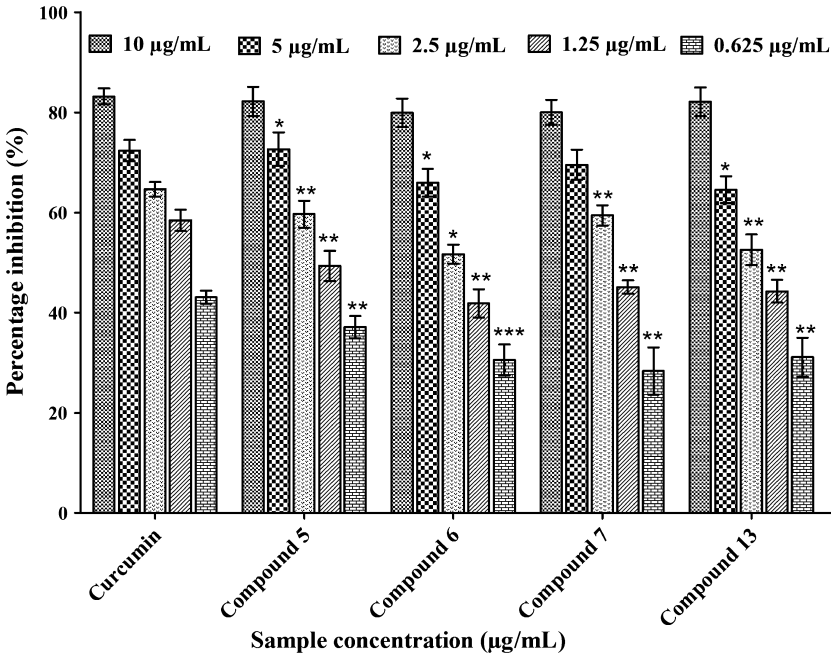

Figure 4: Concentration-dependent inhibitory effects of diarylpentanoid analogues on activity of microsomal prostaglandin E synthase-1. Graph represents the % inhibition by most active compounds along with curcumin. *p < 0.05, **p < 0.01, ***p < 0.001 are significant difference with respect to curcumin. The data shown are an average of three independent experiment, and values are mean ± SD.

with strong inhibition of mPGES-1 activity. Compound **12**, which has a similar substitution pattern on its phenyl ring structures but contains an acetone linker, showed weaker activity than the latter.

Conclusions

In summary, we presented a series of novel diarylpentanoid analogues of curcumin and evaluated their potential inhibitory activities against the enzymes involved in biosynthesis of arachidonic acid and its metabolites. Out of the series employed, several derivatives were able to inhibit the activity of PLA_2, COX, LOX, and mPGES-1. Inhibition of these enzymes by diarylpentanoid analogues enlightens the anti-inflammatory property of these compounds. It raises the possibility that diarylpentanoid analogues of curcumin might serve as a useful starting point for the design of new and improved anti-inflammatory agents.

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