JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Effects of diarylpentanoid analogues of curcumin on chemiluminescence and chemotactic activities of phagocytes

Ibrahim Jantan[a], Syed Nasir Abbas Bukhari[a], Nordin Haji Lajis[b], Faridah Abas[b], Lam Kok Wai[a] and Malina Jasamai[a]

[a]Drug and Herbal Research Center, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Kuala Lumpur and [b]Institute of Bioscience, Universiti Putra Malaysia, Selangor, Malaysia

Keywords
chemotactic activity; chemiluminescence; curcumin diarylpentanoid analogues; immunomodulatory; polymorphonuclear leukocytes

Correspondence
Ibrahim Jantan, Drug and Herbal Research Center, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.
E-mail: ibj@pharmacy.ukm.my

Received September 8, 2011
Accepted November 9, 2011

doi: 10.1111/j.2042-7158.2011.01423.x

Abstract

Objectives A series of 43 curcumin diarylpentanoid analogues were synthesized and evaluated for their inhibitory effects on the chemiluminescence and chemotactic activity of phagocytes *in vitro*.

Methods The effects of the compounds on the respiratory burst of human whole blood and isolated human polymorphonuclear leukocytes (PMNs) were evaluated using a luminol-based chemiluminescence assay and their effect on chemotactic migration of PMNs was investigated using the Boyden chamber technique.

Key findings Compounds **6**, **17**, **25** and **30** exhibited significant inhibitory activity on the oxidative burst of PMNs. The presence of methoxy groups at positions 2 and 5, and methoxylation and fluorination at positions 4 and 2 of both phenyl rings, respectively, may contribute significantly to their reactive oxygen species inhibition activity. Compounds **7**, **17**, **18**, **24** and **32** showed strong inhibition of the chemotaxis migration of PMNs. Chlorination at various positions of both phenyl rings of cyclohexanone diarylpentanoid resulted in compounds with potent inhibitory effects on PMN migration.

Conclusions The results suggest that some of these diarylpentanoid analogues are able to modulate the innate immune response of phagocytes at different steps, emphasizing their potential as a source of new immunomodulatory agents.

Introduction

Professional phagocytes such as polymorphonuclear neutrophils (PMNs), peripheral blood mononuclear and macrophage cells play important roles in our innate immune defence against infectious microbes. Several steps are involved in phagocytosis: migration of phagocyte cells to the site of infection and adherence towards vascular endothelial cells, followed by degradation of the pathogen.[1] Chemotaxis is the movement of phagocytes to the site of infection and is the first step of the immune response. The ability of these cells to be chemotactically attracted to the site of the initial microbial invasion or to an inflammatory focus is fundamental for the full activation of the immune response that follows.[2] Accumulation of phagocytes at the infection site can be induced by endogenous chemoattractants such as interleukin 8, leukotriene B4, platelet activating factor, or exogenous chemoattractants such as formyl methionyl-leucyl-phenylalanine (fMLP) derived from bacterial cell products.[3]

The PMNs adhere stably to the endothelium cells because these express all three CD11/CD18 leucocyte integrins on their cell surfaces.[4] Binding of a receptor-specific ligand may lead to, besides signal-transduction events, internalization of the receptor–ligand complex, which leads to a subsequent down-regulation of surface receptor expression. Phagocytosis will occur at the site of infection by means of phagocyte–ligand interaction, leading to a sequence of events known as oxidative burst. The oxidative burst involves increased oxygen consumption and generation of highly reactive superoxide anion radicals (O_2^-) by the NADPH–oxidase complex. O_2^- is further dismutated to hydrogen peroxide (H_2O_2), either spontaneously or enzymatically by superoxide dismutase. Highly damaging hydroxyl radicals (OH^-) are formed from O_2^- and H_2O_2 via a Fenton reaction. In the phagosome, H_2O_2 and myeloperoxidase enzyme (MPO) activate a halogenating system, giving rise to hypochlorous acid (HClO), which is a potent bactericidal

agent (myeloperoxidase–H_2O_2–Cl^- system). MPO is also involved in the production of highly toxic nitric oxides (NO).[5] Besides the defensive roles during the infections, when excessively or inappropriately deployed the phagocyte–microbe interactions can damage host tissues and contribute to the pathogeny of various immune and non-immune chronic inflammatory diseases, including some rheumatoid disorders. Therefore, inhibitors of phagocyte reactive oxygen species (ROS) production can be used in the treatment of a variety of disorders, including inflammation.[6]

Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3,5-dione) is the most studied curcuminoid of the species *Curcuma*. Curcumin is well known for its anti-inflammatory effects and over the past few decades its numerous biological and pharmacological activities have been reported.[7,8] Curcumin has been shown to inhibit the metabolism of arachidonic acid and the activities of cyclooxygenase-2 (COX-2), lipoxygenase, proinflammatory cytokines, inducible nitric oxide (iNOS), protein kinases, transcription factors such as nuclear factor-kB and release of steroids.[9–11] Other activities of curcumin include inhibition of low-density lipoprotein oxidation, reduction of blood cholesterol level, inhibition of platelet aggregation, suppression of thrombosis and myocardial infarction, treatment of rheumatoid arthritis, inhibition of HIV replication, protection from liver injury, as well as anticancer and immunomodulatory activities.[12–14]

Curcumin is known to have a poor bioavailability, as orally administered curcumin undergoes hepatic conjugation, leading to the formation of glucoronides and sulphates, and systematic administration causes it to undergo reduction.[15] Many studies have been carried out to improve the bioavailability of curcumin by modifying its molecular structure, i.e. eliminating the unstable β-diketone moiety and modifying the heptadiene linker while retaining the phenolic OH groups.[16,17] The presence of the β-diketone moiety will result in rapid metabolism by aldo-keto reductase in the liver, thus limiting the beneficial effects of curcumin on many types of disease. Various curcumin analogues have been synthesized and evaluated for activity against known biological targets in order to investigate their structure–activity relationships (SAR). Recent SAR studies on curcumin analogues have demonstrated anti-inflammatory, anticancer, antioxidant, anti-tyrosinase and antiosteoporosis properties.[17–20] The presence of phenolic OH on both phenyl ring structures has been shown to be important for antioxidant activity.[21] Our previous studies on synthesized diarylpentanoids and pyrazoline derivatives have revealed that these compounds inhibit NO production in IFN-γLPS-activated RAW 264.7 and U937 cells. More importantly, one of the compounds suppressed both the iNOS gene and enzyme expression while displaying a strong inhibitory effect on MCP-1 and IL-10 secretion and gene expression.[22,23]

A series of 43 diarylpentanoids has been synthesized based on the chemical structure of curcumin by eliminating the β-diketone and modifying it into conjugated double bonds, i.e. two identical aromatic ring regions separated by five carbon linkers. To the best of our knowledge, curcumin-related diarylpentanoid analogues have not been investigated for their immunomodulatory effects. We therefore evaluated these compounds for their effects on the ROS production of human whole blood and isolated PMNs, and chemotaxis of PMNs induced by the bacterial peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMLP).

Materials and Methods

Chemicals and equipment

Serum opsonized zymosan A (*Sacromyces cerevisae* suspensions and serum), luminol (3-aminophthalhydrazide), phosphate buffer saline tablet (PBS), Hanks balance salt solutions ($HBSS^{++}$), fetal calf serum (PAA Laboratories, USA), Ficoll, Hanks balance salt solution ($HBSS^-$), *N*-formyl-methionylleucyl-phenylalanine (fMLP), acetyl salicylic acid (purity > 99%), ibuprofen (purity > 99%), dimethylsulfoxide (DMSO), methanol and ammonium chloride of analytical grades were purchased from Sigma (St Louis, MO, USA). Chemiluminescense measurements were carried out on a Luminoskan Ascent luminometer (Thermo Scientific, UK). fMLP was stored as a stock solution of 10^8 M in DMSO at –80°C and diluted in Hanks solution prior to assay. Haematoxylin and xylene for staining were obtained from BDH (UK). A Boyden 48-well chamber with a 2 μm polycarbonate membrane filter separating the upper and lower compartments was purchased from Neuro Probe (Cabin John, MD, USA). A CO_2 incubator (Shell Lab, USA) and light microscope (Leitz Watzler, Germany) were used in this assay.

Synthesis of diarylpentanoid analogues

The diarylpentanoid analogues of curcumin were synthesized by direct coupling of the appropriate aromatic aldehyde with the three ketones, acetone (**1–17**), cyclohexanone (**18–33**) or cyclopentanone (**34–43**) at a molar ratio of 1 : 2, using the base-catalysed Claisen–Schmidt condensation reaction. Figure 1 illustrates the general synthesis of diarylpentanoids. Briefly, the appropriate aromatic aldehyde (20 mmol, 2 equiv.) and the appropriate ketone (10 mmol, 1 equiv.) were mixed and dissolved in 15 ml of ethanol and stirred at 5°C for a few minutes. Four drops of 40% NaOH solution in ethanol was added dropwise over several minutes. The mixture was stirred at room temperature for 10 h. The reaction was neutralized with dilute HCl to form a precipitate, which was subjected to further purification by recrystallization from appropriate solvents or column chromatography. The structures of the synthesized compounds

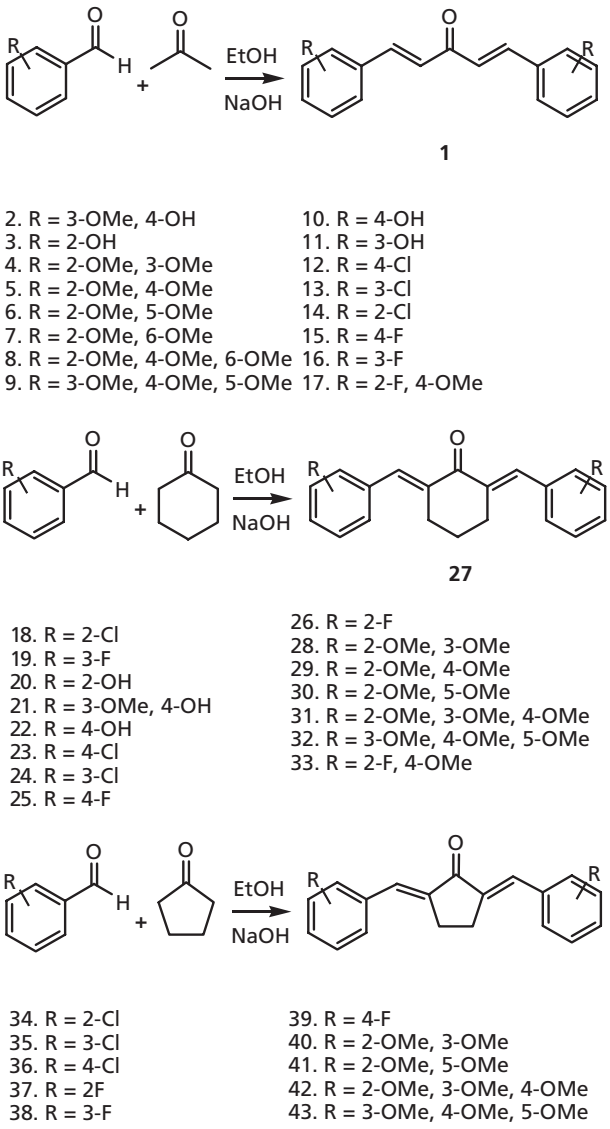

Figure 1 General synthesis of diarylpentanoid analogues.

were elucidated by spectroscopic techniques and comparison of their spectroscopic data made with those of related compounds. All the chemicals used for synthesis of 2,6-bisbenzylidene derivatives were of research grade, purchased from Sigma-Aldrich, Merck and Acros Organics. Analytical TLC plates (silica gel F254 precoated, 0.2 mm thickness) were obtained from Merck. Melting points were determined using a hot-stage melting point apparatus equipped with a microscope, XSP-12 model 500X, and were uncorrected. IR spectra were recorded on a Perkin Elmer RX1 FT-IR spectrometer as KBr disk or thin film. Electron impact mass spectra (EIMS) were performed on a Thermo Finnigan POLARISQ spectrometer at 70 ev. The ^{1}H and ^{13}C spectra were carried out on a Varian 500 MHz NMR spectrometer.

1,5-bis(2,5-dimethoxyphenyl)-1,4-pentadiene-3-one (compound 6)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.98 (d, 2H, J = 15.5 Hz), 6.88 (d, 2H, J = 15.5 Hz), 6.84 (d, 4H, J = 8.0 Hz), 6.82 (s, 2H), 3.96 (s, 6H, OCH_3), 3.85 (s, 6H, OCH_3); mp 105–106°C; MS (EI): $[M]^+$ at *m/z* 354. UV (MeOH): λ_{max} 396 (ε 20 000), 316 (21 000), 207 (38 300) nm; IR (KBr, cm^{-1}): 1493, 1578, 1611.

1,5-bis(2,6-dimethoxyphenyl)-1,4-pentadiene-3-one (compound 7)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 8.17 (d, 2H, J = 16.3 Hz), 7.59 (d, 2H, J = 16.3 Hz), 7.36 (t, 2H, J = 8.5 Hz), 6.75 (d, 4H, J = 8.5 Hz), 3.90 (s, 12H); mp 153–155°C; MS (EI): $[M]^+$ at *m/z* 354. UV (MeOH): λ_{max} 366 (ε 28 100), 213 (42 900) nm; IR (KBr, cm^{-1}): 1568, 1604, 1637.

1,5-bis(2-fluoro, 4-methoxyphenyl)-1,4-pentadiene-3-one (compound 17)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.84 (d, 2H, J = 15.5 Hz), 7.81 (m, 2H), 7.24 (d, 2H, J = 15.5 Hz), 6.90 (m, 4H), 3.91 (s, 6H); mp 115–117°C; EIMS: $[M]^+$ at *m/z* 330. UV (MeOH): λ_{max} 357 (ε 33 900), 325 (17 600), 200 (37 800) nm; IR (KBr, cm^{-1}): 1105, 1508, 1612.

2,6-bis(2-chlorobenzylidene)-cyclohexanone (compound 18)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.85 (s, 2H), 7.56 (m, 4H), 7.45 (m, 4H), 2.87 (t, 4H, J = 6.5 Hz), 1.78 (q, 2H, J = 6.5 Hz); mp 90–92°C; (EI): $[M]^+$ at *m/z* 343. UV (MeOH): λ_{max} 313 (ε 21 700), 203 (46 000) nm; IR (KBr, cm^{-1}): 1051, 1576, 1603.

1,5-bis(3-chlorobenzylidene)-cyclohexanone (compound 24)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.65 (s, 2H), 7.56 (s, 2H), 7.51 (t, 4H, J = 5.5 Hz), 7.44 (m, 2H), 2.99 (m, 4H), 1.86 (quintet, 2H, J = 6.0 Hz); mp 136–138°C; EIMS: $[M]^+$ at *m/z* 343. UV (MeOH): λ_{max} 323 (ε 38 100), 234 (21 400), 207 (58 400) nm; IR (KBr, cm^{-1}): 1092, 1577, 1606.

1,5-bis(4-fluorobenzylidene)-cyclohexanone (compound 25)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.68 (s, 2H), 7.56 (s, 2H), 7.49 (d, 4H, J = 8.3 Hz), 7.42 (d, 4H, J = 8.3 Hz), 2.99 (t, 2H, J = 5.5 Hz), 1.83 (quintet, 2H, J = 6.0 Hz); mp 86–88°C; EIMS: $[M]^+$ at *m/z* 310. UV (MeOH): λ_{max} 328 (ε 35 400), 230 (21 000), 201 (36 600) nm; IR (KBr, cm^{-1}): 1158, 1507, 1608.

1,5-bis(2,5-dimethoxybenzylidene)-cyclohexanone (compound 30)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.89 (s, 2H), 7.02 (d, 2H, J = 8.5 Hz), 6.97 (d, 4H, J = 8.5 Hz), 3.84 (s, 6H), 3.80 (s, 6H), 2.92 (t, 4H, J = 6.0 Hz), 1.78 (quintet, 2H, J = 6.0 Hz); mp 161–163°C; EIMS: $[M]^+$ at *m/z* 394. UV (MeOH): λ_{max} 376 (ε 8700), 313 (11 000), 279 (9400), 202 (30 100) nm; IR (KBr, cm^{-1}): 1494, 1582, 1603.

1,5-bis(3,4,5-trimethoxybenzylidene)-cyclohexanone (compound 32)

^{1}H NMR (500 MHz, acetone-d_6) δ in ppm: 7.64 (s, 2H), 6.86 (s, 4H), 3.90 (s, 12H), 3.79 (s, 6H), 2.82 (t, 4H, J = 6.5 Hz), 1.77 (quintet, 2H, J = 6.5 Hz); mp 147–149°C; EIMS: $[M]^+$ at *m/z* 454. UV (MeOH): λ_{max} 357 (ε 18 300), 547 (10 400), 200 (42 900) nm; IR (KBr, cm^{-1}): 1505, 1604, 1579.

Isolation of human polymorphonuclear leukocytes

For isolation of PMNs, venous blood was obtained in heparin-containing tubes by aseptic vein puncture from healthy human volunteers aged ≥18 years old who fulfilled the following inclusion criteria: non-smoker, fasted overnight and did not take any medicine or supplements. The blood was centrifuged initially at 170*g* to remove the platelet-rich plasma and then at 1000*g* to eliminate platelet-poor plasma. The buffy coat of white cells was diluted with PBS (pH 7.2). Dextran was added and the mixture was left for 45 min at room temperature (26°C) for sedimentation. The supernatant was centrifuged by Ficoll-gradient separation and then washed twice with distilled water to remove red blood cells. A pellet of PMNs was collected from the tube base. The cells were suspended in HBSS (pH 7.4) with Ca^{2+} and Mg^{2+} ($HBSS^{++}$). Cell suspensions were counted using a haemocytometer and light microscope and diluted with HBSS to obtain a final cell suspension of 1×10^6/ml.[24] The use of human blood was approved by the Human Ethical Committee of UKM (approval no. FF-220–2008).

Cell viability

Cell viability was determined by the standard trypan blue exclusion method. The PMNs and macrophage cells (1×10^6/ml) were incubated with 6.25 or 100 μg/ml of synthetic compounds, each in triplicate at room temperature for 2 h. The blue dye uptake was an indication of cell death. The percentage viability was calculated from the total cell counts.[24]

Chemiluminescence assay

Luminol-based chemiluminescence assay was carried out as described by Jantan *et al*.[25] The luminol was used as a probe to detect the level of ROS affected by the synthetic compound. The molecular weight of luminol is relatively small and it can enter the cells and then react with intracellular ROS.[26] For the assay, 25 μl diluted whole blood (1 : 50 dilution in sterile PBS, pH 7.4) or 25 μl PMN (1×10^6/ml) suspended in $HBSS^{++}$ were incubated with 25-μl serial dilutions of synthetic compounds in DMSO and H_2O (5 : 95 ratio) (6.25–100.00 μg/ml). The cells were stimulated with 25 μl of opsonized zymosan followed by 25 μl of luminol (7×10^5 M) and then $HBSS^{++}$ was added to adjust the final volume to 200 μl. The final concentrations of the samples in the mixture were 12.5, 6.25, 3.13, 1.56 and 0.78 μg/ml. Tests were performed in white 96-well microplates, which were incubated at 37°C for 50 min in the thermostatically controlled chamber of the luminometer. The control wells contained 0.6% DMSO, $HBSS^{++}$, luminol and cells but no compound. Curcumin and acetylsalicylic acid, a non-steroidal anti-inflammatory drug (NSAID), were used as positive controls. The final concentration of DMSO in the mixture was 0.6%, to eliminate the effect of the solvent on the chemiluminescence. The luminometer results were monitored as the chemiluminescence reading per luminometer unit (RLU) with peak and total integral values set with repeated scans at 30 s intervals and 1 s points measuring time. The inhibition percentage (%) for each compound was calculated using the following formula:

$$\text{inhibition}\,(\%) = \frac{(\text{RLU}_{\text{control}} - \text{RLU}_{\text{sample}}) \times 100\%}{\text{RLU}_{\text{control}}}$$

Chemotaxis assay

The assay was performed using a modified 48-well Boyden chamber with formyl-methionyl-leucyl-phenylalanine (fMLP) as a chemotaxin, as previously described by Sacerdote *et al*.[27] Briefly, aliquots of 25 μl of fMLP (10^{-8} M) were added to the lower chamber. Serial dilutions (5 μl) of each compound in DMSO and H_2O (5 : 95 ratio) (6.25–100 μg/ml) were added six times each ($n = 6$) to the upper chamber containing 45 μl PMNs (1×10^6 cells per ml) suspended in $HBSS^-$ and incubated for 1 h at 37°C in a CO_2 incubator. The final concentrations of the samples in the mixture were 10, 5, 2.5, 1.25 and 0.625 μg/ml. The final concentration of DMSO in the reaction mixture was fixed at 0.5% to avoid interference with the chemotactic study. Migrated cells that had adhered to the distal part of the filters were fixed and stained by haematoxylin and xylene. The cell migration distance was measured by using a light microscope. The chemoattractant buffer (DMSO and HBSS, 1 : 1 ratio) was added as a control and curcumin and ibuprofen were used as positive controls. The chemotactic effect of each compound was measured at different concentrations. The percentage inhibition (%) was calculated using the following formula:

$$\frac{(\text{distance travelled by control} - \text{distance travelled by sample}) \times 100\%}{\text{distance travelled by control}}$$

Statistical analysis

All the data were analysed using Statistical Package for Social Sciences (SPSS) version 15.0. Each sample was measured in triplicate and the data presented as mean ± standard error of mean (SEM). The IC_{50} values were calculated using GraphPad Prism 5 software. The values were obtained from at least three determinations. Data were analysed 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 reactive oxygen species generation

The cell viability test carried out to evaluate the cytotoxicity of the 43 curcumin-like diarylpentanoid analogues on PMNs at 6.25 and 100 μg/ml indicated that the cells were viable (>90%) after 2 h of incubation. Preliminary screening of the compounds on the whole blood showed that compounds **3**, **6**, **17**, **19**, **25**, **28** and **30** exhibited high inhibitory activity for luminol-enhanced chemiluminescence with IC_{50} values ranging from 5.7 to 10.5 μM (Table 1). Phagocyte cells, upon activation by serum opsonized zymosan (SOZ), release ROS. These were then quantified by the luminol-enhanced chemiluminescence assay. Fγc receptors on the surface of the phagocyte cells recognize SOZ and the interaction that occurs initiates the process of respiratory burst. The luminol was used as a probe to detect the specific ROS effected by the compounds. The molecular weight of luminol is relatively small and it can enter the cells and then react with intracellular and extracellular ROS such as H_2O_2, OH^- and HClO, which are primarily produced at a later phase of oxidative burst by phagocytic MPO.[5] The inhibitory activity of the compounds may be due to their ability to block the complement receptors and to inhibit NADPH oxidase.

The IC_{50} values of these compounds on the oxidative burst of human whole blood were comparable to that of curcumin (7.6 μM) and lower than that of acetylsalicylic acid (18.6 μM), both used as positive controls (Table 1). Acetylsalicylic acid was used as a positive control in a previous study by Parij *et al*.,[28] who showed that acetylsalicylic acid inhibits luminol-amplified chemiluminescence of human neutrophils. The compounds were further evaluated for their effects on the oxidative burst of PMNs. Of the seven compounds, compounds **6**, **17**, **25** and **30** were the most potent against PMNs, with IC_{50} values ranging from 5.2 to 8.3 μM, respectively. Their IC_{50} values were higher than that of curcumin (3.8 μM) but lower than that of acetylsalicylic acid (9.5 μM) (Table 1). The results showed that the compounds inhibit ROS generation during the metabolic phase of phagocytosis in a dose-dependent manner, i.e. as the concentration of the samples increased the percentage inhibition increased. Figure 2 shows the dose-dependent ROS-inhibitory effect of compounds **6**, **17**, **25** and **30**.

Table 1 Percentage inhibition at 12.5 μg/ml and IC_{50} values (μmol/l) of ROS inhibitory activity of synthetic diarylpentanoid analogues on human whole blood and PMNs assayed by luminol-amplified chemiluminescence

	Mean ± SEM		IC_{50} values	
Compound	Whole blood	PMNs	Whole blood	PMNs
1	36.1 ± 1.4	46.6 ± 0.4	–	–
2	42.9 ± 0.5	54.3 ± 0.1	–	30.8 ± 0.5
3	71.6 ± 0.8	89.6 ± 0.3	7.5 ± 0.6	12.4 ± 0.2
4	56.8 ± 5.0	56.5 ± 1.4	19.0 ± 6.4	31.2 ± 0.7
5	14.3 ± 1.4	16.5 ± 0.1	–	–
6	76.5 ± 1.0	91.0 ± 0.3	10.5 ± 0.5	5.2 ± 0.7
7	53.0 ± 0.5	62.9 ± 0.1	34.3 ± 0.3	24.5 ± 0.3
8	42.7 ± 2.0	54.2 ± 0.6	–	27.2 ± 0.3
9	42.2 ± 1.7	54.0 ± 0.5	–	27.2 ± 0.4
10	57.6 ± 1.2	67.1 ± 0.4	43.2 ± 2.4	32.2 ± 0.4
11	54.0 ± 1.6	60.3 ± 0.5	43.9 ± 3.1	41.9 ± 0.7
12	37.8 ± 1.3	49.9 ± 0.4	–	–
13	37.7 ± 1.4	49.9 ± 0.4	–	–
14	34.7 ± 5.3	49.5 ± 1.6	–	–
15	61.5 ± 1.4	43.2 ± 0.3	26.2 ± 1.0	–
16	58.4 ± 2.8	50.7 ± 0.8	27.8 ± 3.6	37.4 ± 1.1
17	88.8 ± 0.3	82.3 ± 0.1	5.7 ± 0.4	8.3 ± 0.2
18	41.7 ± 1.1	32.3 ± 0.3	–	–
19	84.2 ± 0.9	80.9 ± 0.3	9.0 ± 1.0	12.4 ± 0.3
20	50.8 ± 3.0	34.3 ± 0.9	36.7 ± 3.9	–
21	58.9 ± 0.5	47.3 ± 0.1	23.6 ± 1.1	–
22	54.3 ± 0.1	43.8 ± 0.1	38.2 ± 0.8	53.5 ± 1.0
23	35.2 ± 5.6	28.3 ± 1.6	–	51.0 ± 6.7
24	31.7 ± 3.8	28.1 ± 1.5	–	–
25	87.4 ± 2.8	84.7 ± 0.8	6.3 ± 0.3	5.7 ± 0.1
26	52.7 ± 0.9	43.6 ± 0.3	35.1 ± 1.2	44.7 ± 0.2
27	46.2 ± 1.7	41.4 ± 0.5	–	–
28	73.3 ± 0.5	71.2 ± 0.2	10.4 ± 0.3	10.5 ± 0.1
29	57.2 ± 0.4	45.2 ± 0.1	23.5 ± 0.5	–
30	76.8 ± 2.1	86.8 ± 0.6	8.9 ± 0.7	6.7 ± 0.4
31	50.7 ± 2.0	43.3 ± 0.6	24.7 ± 1.3	–
32	54.3 ± 1.8	44.4 ± 0.5	23.1 ± 1.2	–
33	59.2 ± 1.1	45.8 ± 0.3	25.8 ± 0.5	–
34	38.2 ± 2.5	39.7 ± 0.7	–	–
35	40.4 ± 1.1	40.3 ± 0.3	–	–
36	38.3 ± 0.6	39.7 ± 0.2	–	–
37	50.6 ± 2.7	57.8 ± 0.8	38.9 ± 3.1	29.9 ± 0.3
38	52.7 ± 1.3	43.9 ± 0.4	36.0 ± 1.3	–
39	45.6 ± 1.8	44.7 ± 0.5	–	–
40	51.5 ± 3.0	43.6 ± 0.9	29.3 ± 1.7	–
41	50.0 ± 1.4	43.1 ± 0.4	30.0 ± 1.2	–
42	45.7 ± 1.6	47.5 ± 0.5	–	–
43	42.1 ± 2.8	46.5 ± 0.8	33.0 ± 2.8	–
Curcumin	80.5 ± 2.4	93.5 ± 0.3	7.8 ± 0.3	3.8 ± 0.2
Aspirin	71.9 ± 0.4	92.5 ± 0.3	18.6 ± 0.2	9.5 ± 0.3

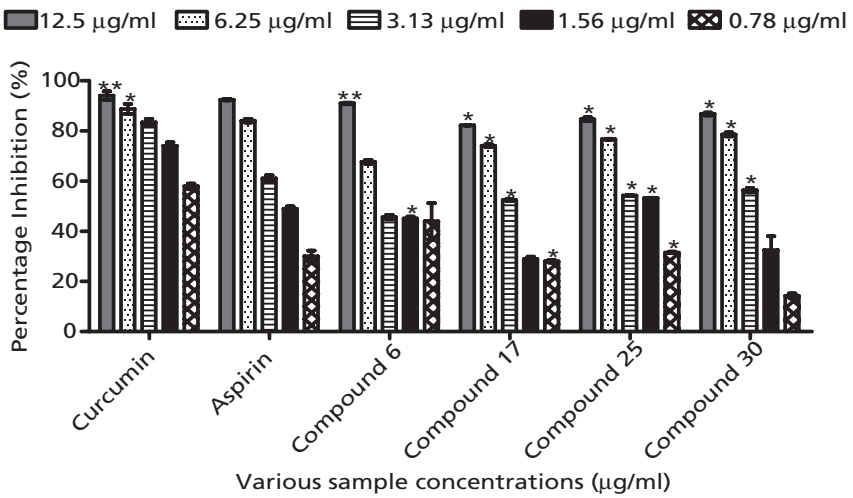

Figure 2 Effects of curcumin-related diarylpentanoid analogues on phagocytic ROS production by luminol-amplified chemiluminescence. Percentage inhibition in oxidative burst in response to various concentrations of compounds, as compared to curcumin and aspirin with isolated PMNs. Significance of differences with respect to control: $*P < 0.05$, $**P < 0.01$.

Structure–activity analysis of diarylpentanoid analogues as inhibitors of ROS production

Structure–activity analysis of the acetone diarylpentanoid analogues indicated that compounds bearing hydroxyl groups at position 2 on both the phenyl ring structures (e.g. compound **3**) possess high inhibitory activity against ROS production in human whole blood. However, those with hydroxyl groups present at other positions of the ring do not show such strong inhibition. The analogue with methoxy groups at positions 2 and 5 of the phenyl ring structures (compound **6**) demonstrated stronger inhibitory activity than the positive controls, in fact almost a twofold improvement in ROS inhibition of PMNs relative to acetylsalicylic acid. Methoxylation at position 4 and fluorination at position 2 of both phenyl rings (compound **17**) produce compounds with strong inhibition on the oxidative burst of whole blood and PMNs. The introduction of the cyclopentanone ring as a rigid linker chain in the structures reduces the inhibitory effect of the compound with hydroxyl groups at position 2 on both their phenyl ring structures (compound **20**). However, the presence of a cyclohexanone ring has little effect on the compound with methoxy groups at positions 2 and 5 of their phenyl ring structure (compound **30**). These result suggest that the presence of two methoxyl groups at these positions (compounds **6** and **30**) are important structural features for ROS-inhibitory activity. Compounds with methoxy groups at positions 2 and 3 on both their phenyl ring structures (compound **28**) also show strong ROS-inhibitory activity in PMNs. The presence of electron-withdrawing fluoride at position 4 (compound **25**) on both phenyl ring structures strongly increases the inhibitory effect of the diarylpentanoid analogues with a linker containing a six-member ring. The presence of a fluoride group at position 3 (compound **19**) also showed strong inhibitory activity but to a lesser extent than compound **25**. The results suggest that cyclohexanone containing linker may play an important role in the ROS inhibitory activity of the analogues. However, cyclopentanone containing analogues exhibited lower ROS inhibitory activity than the cyclohexanone and the acetone analogues.

The present study shows that the bioactive compounds were able to inhibit the luminol chemiluminescence produced by a myeloperoxidase–H_2O_2–Cl^- system. Interestingly, the diarylpentanoid analogues, which showed strong ROS-inhibitory activity, have been shown in a recent study to be able to strongly suppress NO production, i.e. compounds **3**, **6**, **17**, **19**, **25**, **28** and **30** strongly inhibit NO production in IFN-γ/LPS-activated macrophage cells.[20] Other studies – by Ko *et al*.[29] and Rojas *et al*.[30] – showed that chalcone derivatives with methoxy groups at positions 2 and 5 of their phenyl ring structures potently inhibit NO production in activated RAW 264.7 cells. Liang *et al*.[17] showed that in the presence of a 3-methoxy group, cyclohexanone-containing analogues of diarylpentanoids exhibit strong anti-inflammatory properties through inhibiting the LPS-induced TNF-α and IL-6 release in macrophages. It was therefore suggested that the substitution patterns of the phenyl rings of diarylpentanoid analogues for increased inhibitory effects on ROS, NO, TNF-α and IL-6 release in macrophages have high degree of similarity.

Inhibition of PMN chemotaxis

The effects of the diarylpentanoid analogues at 10 μg/ml on the migration of PMNs towards the chemoattractant *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) were determined and their percentage inhibitions (%) are shown in Table 2. Compounds **2**, **7**, **16**, **17**, **18**, **23**, **24**, **32**, **34** and **42** strongly inhibited migration of PMNs with percentage inhibitions ranging from 64.2 to 81.7%. Chemoattractant buffer (DMSO and HBSS, 1 : 1 ratio) was used as a control and curcumin and ibuprofen were used as positive controls. In a study to determine the effect of selected NSAIDs in blocking the migration of PMNs, ibuprofen was found to be the most effective drug.[31] The IC_{50} values of the compounds on PMN chemotaxis are shown in Table 2. Compounds **7**, **17**, **18**, **24** and **32** strongly inhibit migration of PMNs, with IC_{50} values of 2.0–6.4 μM, which is lower or comparable to the values for curcumin (5.1 μM) and ibuprofen (6.7 μM) (Table 2). All the active compounds showed a dose-dependent effect as exemplified by compounds **7**, **17**, **18**, **24** and **32** (Figure 3).

Table 2 Percentage inhibition at 10 μg/ml and IC_{50} values (μmol/l) of synthetic diarylpentanoid analogues on PMN chemotaxis

Compound.	Mean ± SEM	IC_{50} values
1	60.8 ± 7.3	16.4 ± 4.4
2	70.0 ± 2.5	7.5 ± 1.6
3	73.3 ± 0.8	18.2 ± 0.4
4	53.3 ± 1.7	21.6 ± 1.3
5	47.5 ± 2.9	–
6	78.3 ± 0.8	10.9 ± 0.3
7	80.8 ± 0.8	6.4 ± 0.7
8	75.0 ± 2.5	11.0 ± 0.3
9	71.7 ± 2.2	10.8 ± 1.6
10	80.8 ± 2.2	12.7 ± 2.1
11	55.8 ± 3.0	37.1 ± 3.1
12	65.0 ± 1.4	17.7 ± 0.3
13	30.8 ± 1.7	–
14	13.3 ± 5.1	–
15	64.2 ± 0.8	9.3 ± 1.5
16	76.7 ± 2.2	7.0 ± 0.5
17	73.3 ± 1.7	5.7 ± 0.6
18	79.2 ± 0.8	3.8 ± 0.3
19	77.5 ± 1.4	14.7 ± 0.1
20	59.2 ± 0.8	13.9 ± 0.1
21	40.8 ± 0.8	–
22	58.3 ± 0.8	10.1 ± 2.3
23	70.0 ± 3.8	7.4 ± 3.8
24	81.7 ± 2.2	2.0 ± 0.1
25	56.7 ± 4.6	22.8 ± 6.9
26	60.0 ± 2.9	12.1 ± 1.6
27	60.8 ± 1.7	15.3 ± 0.6
28	69.2 ± 0.8	10.9 ± 0.5
29	25.8 ± 0.8	–
30	77.5 ± 1.4	10.6 ± 0.5
31	62.5 ± 1.4	12.0 ± 0.4
32	77.5 ± 2.9	5.5 ± 0.2
33	48.3 ± 0.8	–
34	57.5 ± 2.5	7.4 ± 0.9
35	63.3 ± 1.7	15.2 ± 1.7
36	53.3 ± 3.6	21.6 ± 3.1
37	67.5 ± 1.4	12.5 ± 1.3
38	78.3 ± 1.7	11.1 ± 2.9
39	60.8 ± 0.8	11.5 ± 0.7
40	48.3 ± 0.8	–
41	65.0 ± 1.4	12.9 ± 0.7
42	64.2 ± 2.2	8.1 ± 1.0
43	54.2 ± 0.8	17.4 ± 0.4
Curcumin	80.5 ± 0.6	5.1 ± 0.4
Ibuprofen	65.8 ± 2.2	6.70 ± 2.2

All values were significant at $P \leq 0.05$ when compared with the respective control.

Structure–activity analysis of diarylpentanoid analogues as inhibitors of PMN chemotaxis

Compound **17**, which showed strong inhibition on the oxidative burst of whole blood and PMNs, was the most effective acetone diarylpentanoid analogue in inhibiting PMN migration. The presence of methoxy groups at positions 2 and 6 of the phenyl rings of the acetone diarylpentanoid analogue (compound **7**) may contribute to the high inhibitory effect on PMN migration. Fluorination at position 3 of both phenyl rings (compound **16**) produced a compound with strong inhibition of PMN chemotaxis. Compound **2**, which has a similar substitution pattern to curcumin on its phenyl ring structures but contains a five-carbon linker, showed weaker activity than the latter. Chlorination at various positions of both phenyl rings of cyclohexanone diarylpentanoid analogues resulted in compounds with potent inhibitory effects on PMN migration (compounds **18**, **23** and **24**). In fact, the 3-chlorinated analogue (compound **24**) was the most potent compound amongst the 43 diarylpentanoid analogues tested, exhibiting more than fourfold and threefold increases in PMN chemotaxis relative to curcumin and ibuprofen, respectively. Trimethoxylation of the phenyl rings of the cyclohexanone diarylpentanoid analogues strongly contributed to increased inhibitory activity on PMN chemotaxis, as shown by compound **32**, which contains methoxy groups at positions 3, 4 and 5 of both phenyl ring structures. Trimethoxylation of both phenyl rings of the cyclopentanone-containing linker (compound **42**) could also significantly increase the inhibitory effect of the diarylpentanoid analogues. The presence of an electron-withdrawing chloride at position 2 of both phenyl ring structures of the cyclopentanone diarylpentanoid analogue resulted in a compound (compound **34**) with strong inhibitory activity on chemotactic migration of PMNs.

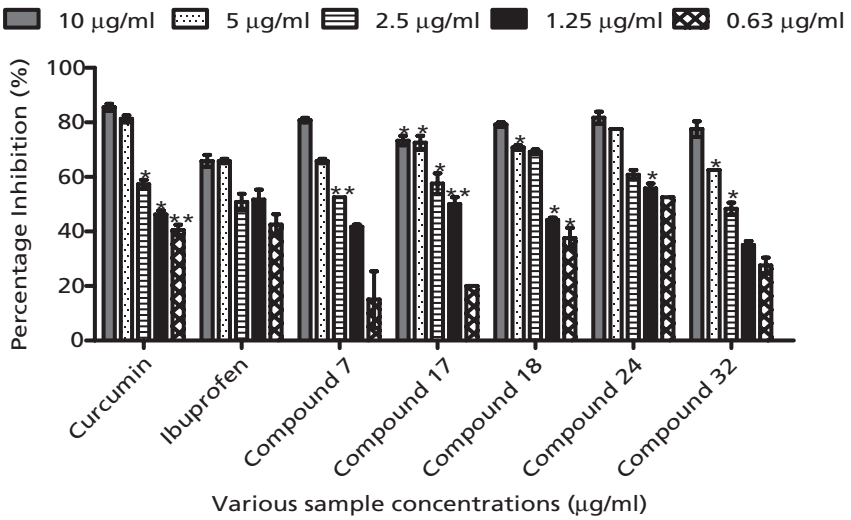

Figure 3 Effects of curcumin-related diarylpentanoid analogues on migration of PMNs towards the chemoattractant fMLP. Percentage inhibition by most active compounds along with curcumin and ibuprofen on migration of PMNs. Significance of differences with respect to control: $*P < 0.05$, $**P < 0.01$.

Conclusion

Some of these derivatives were able to modulate the innate immune response of phagocytes at different levels of ROS production, emphasizing their potential for use as chemical leads for the development of new immunomodulatory agents. Further studies are required to elucidate the types of receptor involved in the inhibition of ROS production by the diarylpentanoid analogues, by using other activators such as phorbal 12-myristate 13-acetate.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by the Ministry of Higher Education, Malaysia, under the Fundamental Research Grant Scheme (FRGS) (grant number 03-FRGS0029-2010).

References

1. Beutler B. Innate immunity: an overview. *Mol Immunol* 2004; 40: 845–859.
2. Stephens L *et al*. Moving towards a better understanding of chemotaxis. *Curr Biol* 2008; 18: 485–494.
3. Eeden SF *et al*. The use of flow cytometry to measure neutrophil function. *J Immunol Methods* 1999; 232: 23–43.
4. Nagahata H *et al*. Analysis of the functional characteristics of L-selectin and its expression on normal and CD18-deficient bovine neutrophils. *Immunol Cell Biol* 2000; 78: 264–271.
5. Arnhold J. Free radicals-friends or foes? Properties, functions and secretion of human myeloperoxidase. *Biochem* 2004; 69: 4–9.
6. Hougee S *et al*. Oral administration of the NADPH-oxidase inhibitor apocynin partially restores diminished cartilage proteoglycan synthesis and reduces inflammation in mice. *Eur J Pharmacol* 2006; 531: 264–269.
7. Itokawa H *et al*. Recent advances in the investigation of curcuminoids. *Chin Med* 2008; 3: 1–11.
8. Gupta SC *et al*. Multitargeting by curcumin as revealed by molecular interaction studies. *Nat Prod Rep* 2011; DOI: 10.1039/c1np00051a.
9. Kohli K *et al*. Curcumin; a natural anti-inflammatory agent. *Indian J Pharmacol* 2005; 37: 141–147.
10. Shakibaei M *et al*. Suppression of NF-kB activation by curcumin leads to inhibition of expression of cyclooxygenase-2 and matrix metalloproteinase-9 in human articular chondrocytes; Implications for the treatment of osteoarthritis. *Biochem Pharmacol* 2007; 73: 1434–1445.
11. Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in

chronic diseases: an age-old spice with modern targets. *Trends Pharmacol Sci* 2009; 30: 85–94.
12. Aggarwal BB *et al*. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res* 2003; 23: 363–398.
13. Allam G. Immunomodulatory effects of curcumin treatment on murine chistosomiasis mansoni. *Immunobiology* 2009; 214: 712–727.
14. Gupta SC *et al*. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. *Cancer Metastasis Rev* 2010; 29: 405–434.
15. Anand P *et al*. Bioavailability of curcumin: problems and promises. *Mol Pharmacol* 2007; 4: 807–818.
16. Straganz GD, Nidetzky J. Reaction coordinate analysis for β-diketone cleavage by the non-heme Fe^{2+}-dependent dioxygenase dke1. *J Am Chem Soc* 2005; 127: 12306–12314.
17. Liang G *et al*. Synthesis, crystal structure and anti-inflammatory properties of curcumin analogues. *Eur J Med Chem* 2009; 44: 915–919.
18. Fuchs JR *et al*. Structure-activity relationship studies of curcumin analogues. *Bioorg Med Chem Lett* 2009; 19: 2065–2069.
19. Park SK *et al*. Synthesis of substituted triazolyl curcumin mimics that inhibit RANKL-induced osteoclastogenesis. *Bioorg Med Chem Lett* 2011; 21: 3573–3577.
20. Lee KH *et al*. Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities. *Eur J Med Chem* 2009; 44: 3195–3200.
21. Parvathy KS *et al*. Antioxidant, antimutagenic and antibacterial activities of curcumin-β-diglucoside. *Food Chem* 2009; 115: 265–271.
22. Lam KW *et al*. Synthesis and evaluation of DPPH and anti-inflammatory activities of 2,6-bisbenzylidenecyclohexanone and pyrazoline derivatives. *Med Chem Res* DOI 10.1007/s00044-010-9521-0.
23. Tham CL *et al*. A synthetic curcuminoid derivative inhibits nitric oxide and proinflammatory cytokine synthesis. *Eur J Pharmacol* 2010; 628: 247–254.
24. Koko WS *et al*. *In vitro* immunomodulating properties of selected Sudanese medicinal plants. *J Ethnopharmacol* 2008; 118: 26–34.
25. Jantan I *et al*. Inhibition of chemiluminescence and chemotactic activity of phagocytes *in vitro* by the extracts of selected medicinal plants. *J Nat Med* 2011; 65: 400–405.
26. Magrisso MJ *et al*. Model components of luminol chemiluminescence generated by PMNL. *J Biochem Biophys Methods* 1995; 30: 257–269.
27. Sacerdote P *et al*. *In vivo* and *in vitro* treatment with the synthetic cannabinoid CP55,940 decreases the *in vitro* migration of macrophages in the rat: involvement of both CB1 and CB2 receptors. *J Neuroimmunol* 2000; 109: 155–163.
28. Parij N *et al*. Effects on non-steroidal anti-inflammatory drugs on the luminol and lucigenin chemiluminescence of human neutrophiles. *Eur J Pharmacol* 1998; 352: 299–305.
29. Kao HH *et al*. Structure–activity relationship studies on chalcone derivatives: the potent inhibition of chemical mediators release. *Bioorg Med Chem* 2003; 11: 105–111.
30. Rojas J *et al*. Synthesis and inhibitory activity of dimethylamino-chalcone derivatives on the induction of nitric oxide synthase. *Eur J Med Chem* 2002; 37: 699–705.
31. Spisani S *et al*. Inhibition of human leukocytes locomotion by anti-inflammatory drugs. *Specialia* 1978; 1: 803–804.