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Research Article

Synthesis and Evaluation of Chalcone Derivatives as Inhibitors of Neutrophils' Chemotaxis, Phagocytosis and Production of Reactive Oxygen Species

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Inhibitory effects on neutrophils' chemotaxis, phagocytosis and production of reactive oxygen species (ROS) are among the important targets in developing anti-inflammatory agents and immunosuppressants. Eight series of chalcone derivatives including five newly synthesized series were assessed for their inhibitory effects on chemotaxis, phagocytosis and ROS production in human polymorphonuclear neutrophils (PMNs). Inhibition of PMNs' chemotaxis and phagocytosis abilities were investigated using the Boyden chamber technique and the Phagotest kit, respectively, while ROS production was evaluated using luminol- and lucigenin-based chemiluminescence assay. The new derivatives (4d and 8d), which contain 4-methylaminoethanol functional group were active in all the assays performed. It was also observed that some of the compounds were active in inhibiting chemotaxis while others suppressed phagocytosis and ROS production. The information obtained gave new insight into chalcone derivatives with the potential to be developed as immunomodulators.

Key words: chalcones, chemotaxis, inhibitors, phagocytosis, reactive oxygen species, structure-activity relationship

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Immune system is a complex network of cells involves in an organized reactions to preserve host tissues from external aggressions. Among the cascade of events that happen in respond to these external stimuli are chemotaxis, phagocytosis and oxidative burst. The endogenous and exogenous mediators for instance interleukin-8 (IL-8) and formyl-methionyl-leucyl-phenylalanine (fMLP) released during tissue injury act as chemo-attractants in the recruitment of phagocytes to the site of injury (1). Phagocytes, primarily neutrophils, will then engulf the intruders through phagocytosis. This process is made possible by the presence of Fc receptors and β2 integrins, which will bind to immunoglobulins (IgG) or complement-coated particles, respectively (2). Activation of neutrophils' killing mechanisms, which involve the enzymatic and oxidative processes follows (3). In the oxidative process, a rise in oxygen consumption by neutrophils led to respiratory burst where a variety of microbiostatic and microbiocidal reactive oxygen species (ROS) are generated, namely superoxide (O_2^-) (4), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and hydroxyl radical (OH•) (5). Nonetheless, the exact mechanism on how these ROS evoke their antimicrobial function is still not fully understood.

Although the above processes are desired to preserve host tissues from infections and injuries, overexpression on these reactions is undesirable and can lead to many pathological conditions such as rheumatoid arthritis (6), tumour (7) and atherosclerosis (8). Anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants such as tacrolimus and cyclosporin are currently used to treat these conditions. Nevertheless, problems such as gastric ulceration caused by NSAIDs (9) and poor bioavailability of tacrolimus (10) are still remain unresolved, and hence, drugs with better safety profiles and bioavailability are much needed.

Synthesis of chalcone (1,3-diaryl-2-propen-1-one) has been a considerable interest due to various pharmacological activities exhibited by natural chalcones, namely anticarcinogenic properties of xanthohumol (11), chemopreventive effect of isoliquiritigenin (12) and anti-adipogenic of butein (13). On the other hand, for synthetic chalcone derivatives among the activities reported are antitumour (14), antimicrobial (15) and anti-inflammatory (16). These activities mainly due to their α,β-unsaturated ketone moiety and various substituents introduced to the two aryl rings (17). The role of chalcone derivatives in inhibiting different steps in the inflammatory cascades has been explored extensively (18). Methoxy and hydroxyl groups in the diaryl rings are among the functional groups of interest in promoting the anti-inflammatory effect (19–21). Nevertheless, the study on inhibitory effects of chalcone derivatives on chemotaxis, phagocytosis and oxidative burst of neutrophils is still lacking.

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This study aimed to synthesize five series of new chalcone derivatives. The inhibitory activities of these new derivatives and another three series, which were synthesized previously on polymorphonuclear neutrophils (PMNs) chemotaxis, suppression of PMNs phagocytosis, and inhibition of intracellular and extracellular ROS production in PMNs and human whole blood were also examined. Results from these assays help in designing better derivatives, which can be potentially used to modulate immune response during inflammation.

Methods and Materials

General methods

^{1}H 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-d6 as solvent. High resolution mass spectra (HRMS) were determined by the electrospray ionization mass spectrometry (ESI-MS) on MicroTOF-Q mass spectrometer (Bruker, Coventry, UK). Microanalyses data were obtained from the Fison EA 1108 elemental analyser. Infrared spectra were recorded using KBr disc on a Perkin Elmer 400 (FTIR) spectrometer. Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck, Kuala Lumpur, Malaysia), and thin layer chromatography (TLC) was carried out on precoated silica plates (kiesel gel 60 F254, 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.

Syntheses of compounds **1a**, **1b**, **1c**, **2a**, **2b**, **2c**, **3a**, **3b**, **3c**, **4a**, **4b**, **4c**, **4e**, **5b**, **5c** and **6e** were reported previously (22,23), whereas compounds **4d**, **5d**, **6d**, **6f**, **6g**, **7d**, **7e**, **7h**, **7i**, **8d** and **8e** were prepared in this study using the sodium hydroxide-catalysed Claisen–Schmidt condensation reaction.

General procedure for the synthesis of chalcone derivatives

Synthesis of the chalcone derivatives was achieved by the steps outlined in Scheme 1. An amount of 10 mmol of the respective ketones was added to a solution of the respective aldehydes (10 mmol) in ethanol (15 mL). A solution of 50% NaOH was added dropwise, and the reaction mixture was stirred at room temperature (27 °C) for 2–24 h accordingly. The appearance of precipitate and colour changes of the reaction mixture are an indicative of product formation, and reaction completion was monitored by TLC. Upon completion, the reaction mixture was poured into ice (50 mL), which has been acidified with concentrated HCl (1 mL), extracted with ethyl acetate (50 mL), washed with water (2 × 150 mL), dried and concentrated *in vacuo* to give oils and solids. The crude products were further purified either by column chromatography or recrystallization.

(E)-3-(4-methyaminoethanolphenyl)-1-(5-methyl-2-furyl)-2-propen-1-one (4d)

This compound was obtained by reacting 2-acetyl-5-methylfuran (1.24 g, 10 mmol) with *N*-methyl-*N*-(2-hydroxyacetyl)-4-aminobezaldehyde (2.15 g, 12 mmol) to give clear crystals (0.91 g, 32%). R_F 0.22 (EtOAc-PE 2:3 v/v); mp: 131–132 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.79 (d, J = 16 Hz, 1H), 7.53 (d, J = 8 Hz, 2H), 7.21 (d, J = 1.8 Hz, 1H), 7.19 (d, J = 10.2 Hz, 1H), 6.75 (d, J = 9 Hz, 2H), 6.19 (d, J = 3 Hz, 1H), 3.86 (t, J = 6 Hz, 2H), 3.58 (t, J = 6 Hz, 2H), 3.07 (s, 3H), 2.44 (s, 3H), 1.81 (s, 1H), ^{13}C NMR (500 MHz, $CDCl_3$): δ = 177.71, 157.43, 152.93, 151.37, 143.97, 130.47, 123.16, 118.54, 116.41, 112.05, 109.07, 60.23, 54.60, 38.97, 14.18; $IR_{Vmax/cm-1}$ (ATR): 3374.1, 1624.80, 1581.48, 1261.58; HRMS (ESI) m/z: 286.14 $[M + H]^+$, 308.12 $[M + Na]^+$.

(E)-3-(4-methyaminoethanolphenyl)-1-(2-furyl)-2-propen-1-one (5d)

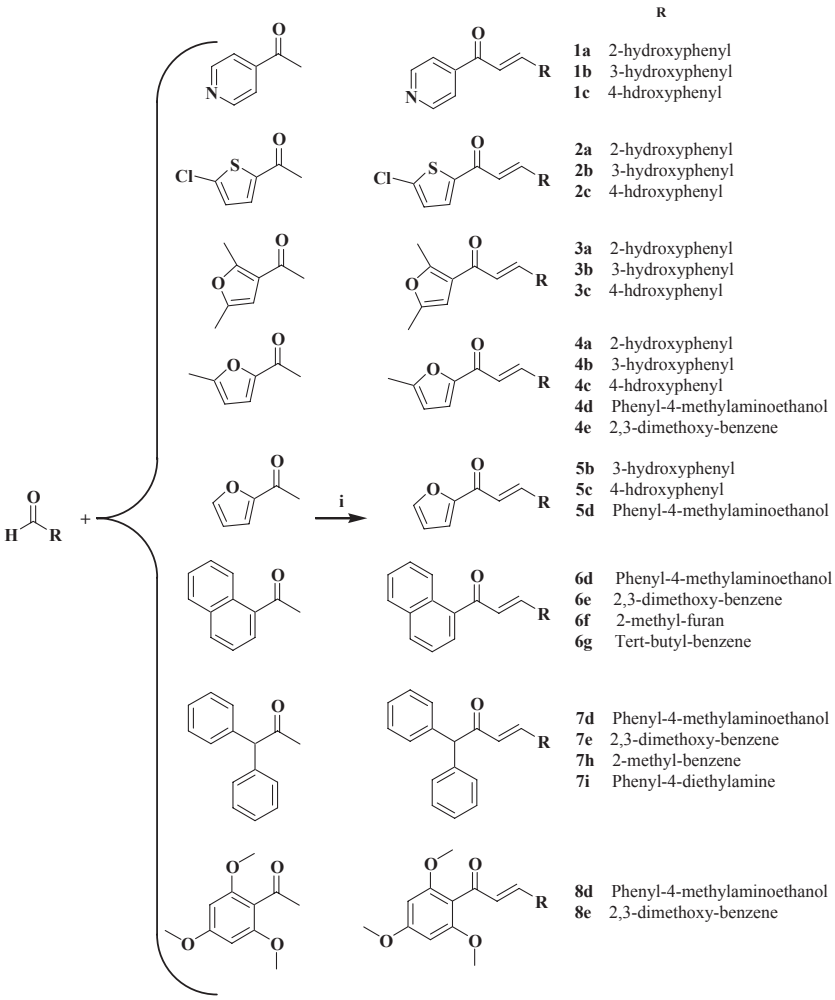
This compound was obtained by reacting 2-furyl methyl ketone (1 mL, 10 mmol) with *N*-methyl-*N*-(2-hydroxyacetyl)-4-aminobezaldehyde (1.79 g, 12 mmol) to give dark brown crystals (2.2 g, 81%). R_F 0.61 (EtOAc-PE 1:3 v/v); mp: 150–151 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.84 (d, J = 13.0 Hz, 1H), 7.63 (dd, J = 0.5 Hz, 1H), 7.55 (d, J = 7.5 Hz, 2H), 7.28 (d, J = 0.5 Hz, 1H), 7.27 (d, J = 4.0 Hz, 1H), 6.75 (d, J = 7.5 Hz, 2H), 6.57 (d, J = 1.5 Hz, 1H), 3.87 (t, J = 4.5 Hz, 2H), 3.59 (t, J = 5.0 Hz, 2H), 3.09 (s, 3H), 1.59 (s, 1H), ^{13}C NMR (500 MHz, $CDCl_3$) δ = 178.33, 154.23, 151.52, 145.89, 144.65, 130.60, 123.10, 116.46, 116.13, 112.29, 112.08, 109.07, 60.26, 54.58, 38.96; HRMS (ESI) m/z: 294.09 $[M + Na]^+$.

(E)-3-(4-methyaminoethanolphenyl)-1-(naphthalen-1-yl)-2-propen-1-one (6d)

This compound was obtained by reacting 1-acetonaphthone (1.52 mL, 10 mmol) with *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (2.15 g, 12 mmol) to give reddish-orange oil (1.76 g, 53%). R_F 0.44 (EtOAc-PE 1:1 v/v); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.26 (dd, J = 6.6 & 4.2 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.91 (dd, J = 4.8 & 5.4 Hz, 1H), 7.71 (dd, J = 7.2 & 6.6 Hz, 1H), 7.52 (m, 4H), 7.46 (d, J = 11.4 Hz, 2H), 7.10 (d, J = 15.6 Hz,1H), 6.74 (dd, J = 12.0 & 11.4 Hz, 2H), 3.85 (t, J = 5.4 Hz, 2H), 3.58 (t, J = 6.0 Hz, 2H), 3.07 (s, 3H), 2.06 (s, 1H); ^{13}C NMR (500 MHz, $CDCl_3$): δ = 196.41, 151.56, 147.15, 138.06, 133.81, 130.80, 130.58, 128.34, 127.11, 126.48, 126.31, 125.83, 124.61, 122.67, 112.05, 60.25, 54.52, 39.00; HRMS (ESI) m/z: 354.14 $[M + Na]^+$, 685.30 $[2M + Na]^+$.

(Z)-3-(5-methyl-2-furyl)-1-(naphthalen-1-yl)-2-propen-1-one (6f)

This compound was obtained by reacting 1-acetonaphthone (1.52 mL, 10 mmol) with 5-methyl furfural (1 mL, 10 mmol) to give brown crystals (2.2 g, 84%). R_F 0.38 (EtOAc-PE 1:3 v/



Scheme 1: Reagents and conditions: (i) NaOH, EtOH, r.t.

v); mp: 138–139 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.39 (d, J = 7.5 Hz, 1H), 8.00 (d, J = 7.5 Hz, 1H), 7.91 (d, J = 17.5 Hz, 1H), 7.77 (d, J = 18.5 Hz, 1H), 7.55 (m, 3H), 7.31(d, J = 7.5 Hz, 1H), 7.16 (d, J = 7.5 Hz, 1H), 6.65 (s, 1H), 6.14 (s, 1H), 2.34 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ = 195.20, 156.29, 149.93, 137.45, 133.85, 131.85, 131.36, 130.53, 128.40, 127.32, 126.89, 126.39, 125.81, 124.58, 122.73, 118.40, 109.47, 14.01; HRMS (ESI) m/z: 285.06 $[M + Na]^+$; Anal. calcd for $C_{18}H_{14}O_2$: C 82.42, H 5.38, found C 82.76, H 5.45.

(Z)-3-(4-tert-butyl-phenyl)-1-(naphthalen-1-yl)-2-propen-1-one (6g)

This compound was obtained by reacting 1-acetonaphthone (1.52 mL, 10 mmol) with 4-tert-butylbenzaldehyde (1.7 mL, 10 mmol) to give light yellow crystals (2.4 g, 76%). R_F 0.47 (EtOAc-PE 2:3 v/v); mp: 112–113 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=8.33 (d, J = 7.0 Hz, 1H), 8.02 (d, J = 8.50 Hz, 1H), 7.94 (d, J = 7.0 Hz, 1H), 7.78 (d, J = 6.5 Hz, 1H), 7.57 (m, 6H), 7.45 (d, J = 8.5 Hz, 2H), 7.30 (d, J = 16.5 Hz, 1H), 1.36 (s, 9H); ^{13}C NMR (500 MHz, $CDCl_3$): δ=196.03, 154.47, 146.06, 137.32, 133.86, 131.87, 131.44, 130.55, 127.38, 127.01, 126.46, 126.00, 125.72, 124.56, 34.98, 31.16; HRMS (ESI) m/z: 337.12 $[M + Na]^+$; Anal. calcd for $C_{23}H_{22}O$: C 87.86, H 7.05, found C 88.22, H 7.27.

(Z)-3-(4-methyaminoethanolphenyl)-1-diphenyl-2-propen-1-one (7d)

This compound was obtained by reacting 1,1-diphenylacetone (2.10 g, 10 mmol) with *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde (1.8 g, 10 mmol) to give light brown solids (1.25 g, 34%). R_F 0.48 (EtOAc-PE 1:3 v/v); mp: 128–129 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.83 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 7.5 Hz, 1H), 7.52 (d, J = 7.50 Hz 2H), 7.35 (m, 10H), 6.70 (d, J = 9.5 Hz, 2H), 5.40 (s, 1H), 3.58 (m, 4H), 2.30 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ = 197.93, 154.08, 152.45, 145.21, 130.48, 130.08, 129.28, 128.60, 128.49, 128.30, 111.97, 62.97, 60.17, 54.51, 38.93; HRMS (ESI) m/z: 372.19 $[M + H]^+$; Anal. calcd for $C_{25}H_{25}NO_2$: C 80.83, H 6.78, N 3.77, found C 80.85, H 6.60, N 3.73.

(E)-3-(2,4-dimethoxyphenyl)-1-diphenyl-2-propen-1-one (7e)

This compound was obtained by reacting 1,1-diphenylacetone (2.10 g, 10 mmol) with 2,3-dimethoxybenzaldehyde (1.7 g, 10 mmol) to give clear crystals (2.8 g, 78%). R_F 0.45 (EtOAc-PE 2:3 v/v); mp: 121–122 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.02 (d, J = 16.0 Hz, 1H), 7.35 (m, 10H), 7.12 (d, J = 8.0, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.94 (d, J = 8 Hz, 1H), 6.88 (d, J = 16.0 Hz, 1H), 5.48 (s, 1H), 3.88 (s, 3H), 3.75 (s, 3H). ^{13}C NMR (500 MHz, $CDCl_3$): δ = 197.83, 153.16, 148.86, 138.75, 138.27, 129.29, 128.70, 127.15, 126.81, 124.17, 119.41, 114.23, 62.89, 61.26, 55.90; HRMS (ESI) m/z: 381.09 $[M + Na]^+$; Anal. calcd for $C_{24}H_{22}O_3$: C 80.42, H 6.19, found C 80.22, H 6.08.

(Z)-1,1,5-triphenyl-3-hexen-2-one (7h)

This compound was obtained by reacting 1,1-diphenylacetone (2.10 g, 10 mmol) with 2-phenylpropionaldehyde (1.34 mL, 10 mmol) to give white powder (2.8 g, 86%). R_F 0.72 (EtOAc-PE 2:3 v/v); mp: 156–157 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.66 (d, J = 8.0 Hz, 1H), 7.30 (m, 10H), 7.11 (m, 5H), 6.88 (d, J = 8.0 Hz, 1H), 4.95 (s, 1H), 3.65 (m, 1H), 1.61 (d, J = 6.0 Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ=207.71, 153.41, 143.06, 140.14, 128.65, 128.60, 127.26, 126.94, 126.21, 125.92, 122.98, 63.00, 41.82, 16.97; Anal. calcd for $C_{24}H_{22}O$: C 88.31, H 6.79, found C 88.15, H 6.97.

(Z)-3-(4-diethylaminophenyl)-1-diphenyl-2-propen-1-one (7i)

This compound was obtained by reacting 1,1-diphenylacetone (2.10 g, 10 mmol) with 4-diethylamino benzaldehyde (1.76 g, 10 mmol) to give light red solids (1.25 g, 34%). R_F 0.55 (EtOAc-PE 1:3 v/v); mp: 106–107 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.84 (d, J = 7.5 Hz, 2H), 7.74 (d, J = 8.50, 1H), 7.61 (d, J = 7.0 Hz, 1H), 7.35 (m, 10H), 6.70 (d J = 7.0 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1H), 5.41 (s, 1H), 3.45 (q, J = 7.0 Hz 4H), 1.23 (t, J = 10.0 Hz, 6H). ^{13}C NMR (500 MHz, $CDCl_3$): δ=190.10, 149.68, 144.40, 141.32, 130.74, 129.30, 128.57, 128.49, 128.09, 126.93, 110.60, 62.91, 44.50, 12.57; HRMS (ESI) m/z: 370.21 $[M + H]^+$; Anal. calcd for $C_{26}H_{27}NO$: C 84.51, H 7.37, N 3.79, found C 84.53, H 7.67, N 3.81.

(Z)-2',4',6'-trimethoxy-4-methylaminoethanol-chalcone (8d)

This compound was obtained by reacting 2,4,6-trimethoxyacetophenone (2.10 g, 10 mmol) with *N*-methyl-*N*-(2-hydroxyacetyl)-4-aminobezaldehyde (2.15 g, 12 mmol) to give orange solids (1.15 g, 31%). R_F 0.21 (EtOAc-PE 1:1 v/v); mp: 140–141 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.34 (d, J = 9.0 Hz, 1H), 7.25 (d, J = 7.7 Hz, 1H), 7.00 (m, 4H), 6.16 (s, 2H), 3.86 (m, 7H), 3.77 (s, 9H), 0.71 (t, J = 14.3 Hz, 1H), ^{13}C NMR (500 MHz, $CDCl_3$): δ = 194.85, 162.58, 159.02, 153.25, 148.63, 139.17, 130.43, 129.51, 124.39, 113.96, 111.96, 90.86, 61.49, 56.06, 55.65; $IR_{Vmax/cm-1}$ (ATR) 3381.86, 1641.06, 1565.82, 1088.80, 1176.93; HRMS (ESI) m/z: 394.11 $[M + Na]^+$, 765.23 $[2M + Na]^+$.

(E)-2',4',6'-trimethoxy-4-(2,3-dimethoxy)-chalcone (8e)

This compound was obtained by reacting 2,4,6-trimethoxyacetophenone (2.10 g, 10 mmol) with 2,3-dimethoxybenzaldehyde (1.99 g, 12 mmol) to give yellow crystals (1.52 g, 42%). R_F 0.37 (EtOAc-PE 1:2 v/v); mp: 119–124 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ=7.68 (d, J = 16.0 Hz, 1H), 7.19 (d, J = 19.0 Hz, 1H), 6.96 (m, 3H), 6.16 (s, 2H), 3.86 (s, 6H), 3.83 (s, 6H), 3.05 (s, 3H); ^{13}C NMR (500 MHz, $CDCl_3$): δ = 195.07, 162.20, 158.77, 151.41, 146.01, 130.53, 124.78, 123.19, 112.16, 90.88, 56.10, 55.63, 54.73; $IR_{Vmax/cm-1}$ (ATR) 1666.52, 1583.83, 1120.92; HRMS (ESI) m/z: 381.08 $[M + Na]^+$, 739.18 $[2M + Na]^+$.

Isolation of human polymorphonuclear neutrophils

Polymorphonuclear neutrophils (PMNs) used in this study were isolated as described in our previous work (24). Briefly, blood obtained from healthy volunteers (aged > 18 years old) was centrifuged to get white cells, which were then diluted with phosphate buffer saline (PBS). Dextran was added, and the mixture was left to sediment. The supernatant was centrifuged again and washed with distilled water, PMNs pellets were then collected. The use of human blood was approved by the Ethics Committee of the Universiti Kebangsaan Malaysia (approval no: FF-220-2008).

Cell viability

Cell viability was determined by the standard trypan blue exclusion method. The PMNs and macrophages cells (1 × 106/mL) were incubated with 6.25 or 100 μg/mL of test compounds, each in triplicate at room temperature (27 °C) for 1–2 h. The blue dye uptake was an indication of cell death. The percentage viability was calculated from the total cell counts. The concentration of compounds at which viability was > 90% and was used for the assays (24).

Chemotaxis assay

This assay was performed as described in our previous work (24). Briefly, formyl-methionyl-leucyl-phenylalanine (fMLP), a chemotaxin was added to the modified 48-well Boyden lower chamber whereas various concentrations of test compounds (10.00, 5.00, 2.50, 1.25 and 0.63 μg/mL) in DMSO were added to the upper chamber. The inhibitory activity of test compounds on the movement of PMN cells towards chemotaxin was calculated from the distance of the cell migration.

Phagocytosis assay

Phagotest kit was used to determine the suppressive effect of test compounds on PMNs phagocytic activity. This assay was carried out on compounds **4d**, **4e**, **5d**, **6d**, **6e**, **6f**, **6g**, **7d**, **7e**, **7h**, **7i**, **8d** and **8e** only, according to the protocol given by the manufacturer (25). Briefly, heparinized whole blood was mixed using a vortex mixture, and 100-μL aliquots were incubated in an ice bath (0 °C) for 10 min prior to the addition of *E. coli*. The mixture was mixed well incubated further at 37 °C for 10 min after the addition of *E. coli* (20 μL) and test compounds (20 μL). Phagocytosis was quenched by adding 100 μL of ice-cold quenching solution to the mixture at the end of the incubation period. The mixture was washed twice with washing solution (3 mL), and the supernatant was discarded. The whole blood was lysed by adding the lysing solution (2 mL), spun (250 g at 4 °C) for 5 min, the supernatant was discarded and the sample was washed. Finally, DNA staining solution (200 μL) was added, the mixture was mixed and incubated for 10 min on ice. The cell suspension was analysed using the flow cytometry blue–green excitation light (488 nm argon-ion laser, FACS Canto II/V96101153) within 60 min of the last procedure undertaken. A control was used to set marker for fluorescence, and percentage of phagocytosis above the marker was determined.

Chemiluminescence assay

This assay was performed as described in our previous work (24). Briefly, PMNs suspended in Hanks' balance salt solution ($HBSS^{++}$) or blood diluted with PBS was incubated with the test compounds, luminol and opsonized zymosan in dimethyl sulphoxide (DMSO) and distilled water. The inhibitory effect of test compounds on intracellular ROS production by PMNs and blood cells was calculated from the luminometer readings. The inhibitory effect of test compounds on extracelluar ROS production was studied using similar procedure as described above, but luminol was replaced with lusigenin, and the assay was performed on human whole blood.

Statistical analysis

All data were analysed using the Statistically Package for Social Sciences (SPSS, IBM, Armonk, NY, USA). Each sample was measured in triplicate, and the data are presented as means ± standard error of mean (SEM). GraphPad Prism 5 Software (GraphPad Software Inc., San Diego, CA, USA) was used to determine the IC_{50} values for the active test compounds. The values were obtained from at least three determinations. Data were analysed using a one-way analysis of variance (ANOVA) for multiple comparisons, and $p < 0.05$ was considered to be statistically significant.

Result and Discussion

Chemistry

Claisen–Schmidt base-catalysed condensation reaction was employed to synthesize five series of chalcone derivatives (Scheme 1). The respective aldehydes and ketones used in the reaction were obtained commercially. Compounds **4d**, **5d**, **6d**, **7d** and **8d** were synthesized without prior protection of the hydroxyl group on the *N*-methyl-*N*-(2-hydroxyethyl)-4-aminobenzaldehyde as about similar yield was obtained when the hydroxyl was protected with *p*-toulenesulphonic acid prior to the synthesis of compound **4d**. The compounds synthesized were characterized by ^{1}H and ^{13}C NMR, IR and MS. Purity of these compounds were verified by microanalysis, and melting point data obtained. *E* and *Z* configurations assigned at position 2 and 3 of the 2-propen-1-one were based on the *J* values of 7.19–16.5 Hz obtained from the ^{1}H NMR (26).

Pharmacology

The chalcone derivatives synthesized were evaluated for their immunomodulatory properties by chemotaxis, luminol- and lucigenin-amplified chemiluminescence and phagotest assays. These assays were carried out on PMNs and human whole blood using ibuprofen (27) and aspirin (28) that were used as reference compounds based on reports on their roles in inflammatory process. The effect of test compounds on PMNs and human whole blood cells' viability were assessed at concentrations of 6.25 and 100.0 μg/mL. The compounds were rendered non-toxic if more than 90% of the cells were viable after 2 hours incubation. Structure-activity relationships (SAR) of the derivatives were deduced based on their activities in the assays performed.

Inhibition of PMN chemotaxis

The inhibitory effects of the test compounds on PMNs towards exogenous chemo-attractant, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) were investigated at 10 μg/mL, and test compounds which gave more than 50% inhibition were investigated further at serial concentrations of 5.00, 2.50, 1.25 and 0.63 μg/mL to determine their IC_{50} values (Table 1).

Compounds **2a**, **2b**, **2c**, **3b**, **3c**, **4a**, **4d**, **4e**, **5c**, **5d**, **6d**, **7d**, **7e**, **7i**, **8d** and **8e** displayed strong inhibition of PMNs migration towards fMLP with percentage inhibition ranging from 64.2 to 80.8%. Compound **4d** was the most active with 80.0% inhibition but with a moderate IC_{50} value of 9.5 μM. Compounds **3b** and **7i** on the other hand displayed strong inhibition, 79.1 and 80.0%, respectively, and IC_{50} values (6.7 and 7.5 μM) comparable to that of ibuprofen. Moreover, compounds **2c**, **4a**, **4d**, **4e**, **5c**, **8d** and **8e** exhibited strong inhibition than control (>68%) but have moderate IC_{50} values (8.7-26.4 μM).

The presence of aromatic ring A did not govern inhibitory activity of the test compounds as lack of activity was observed in series **1** despite having pyridine ring in their structures. The position of hydroxyl group on ring B also

Table 1: Percentage inhibition at 10 μg/mL and IC_{50} values (μM) of chalcone derivatives on PMNs chemotaxis

Compounds	Mean ± SEM	IC_{50} values (μM)
1a	35.2 ± 1.4	–
1b	36.8 ± 2.2	–
1c	44.3 ± 0.8	–
2a	76.6 ± 1.4	10.7 ± 0.9
2b	64.2 ± 1.7	14.9 ± 1.0
2c	69.2 ± 0.8	26.4 ± 0.6
3a	39.2 ± 0.8	–
3b	79.1 ± 2.5	6.7 ± 0.3
3c	64.9 ± 1.7	17.8 ± 2.4
4a	80.0 ± 1.4	12.2 ± 0.6
4b	53.4 ± 1.4	35.1 ± 1.2
4c	30.9 ± 2.9	–
4d	80.8 ± 0.8	9.5 ± 0.3
4e	70.8 ± 0.8	12.7 ± 0.8
5b	54.2 ± 0.8	32.0 ± 1.7
5c	77.4 ± 0.8	9.6 ± 1.3
5d	67.5 ± 2.5	11.0 ± 0.5
6d	65.0 ± 1.4	15.3 ± 1.2
6e	54.2 ± 0.8	21.5 ± 1.2
6f	53.3 ± 1.7	30.0 ± 3.2
6 g	48.3 ± 3.6	–
7d	65.8 ± 0.8	9.9 ± 1.1
7e	65.0 ± 1.4	12.3 ± 0.4
7 h	42.5 ± 2.9	–
7i	80.0 ± 1.4	7.5 ± 0.4
8d	74.2 ± 0.8	10.5 ± 0.3
8e	79.2 ± 0.8	8.7 ± 0.3
Ibuprofen	65.8 ± 2.20	6.6 ± 0.8

Values are presented as mean ± SD (n = 3). (–): IC_{50} values were not determined.

was not important as inhibitory activity was observed at *ortho*, *meta* and *para* substitutions (compounds **2a**, **2b**, **2c**, **3b**, **3c**, **4a** and **5c**). On the other hand, 4-methylaminoethanol and 2,3-dimethoxy substituents on ring B are viewed important as most compounds with these functional groups displayed strong inhibitory activity towards PMNs chemotaxis.

Phagocytic activity of PMNs

The ability of the test compounds to inhibit the ingestion of FITC-labelled *E. coli* suspension by PMNs at the concentrations of 100.0 and 6.25 μg/mL was investigated. The suppressive effect of the test compounds was dose dependent as the effect was only observed at higher concentration (100 μg/mL) of the compounds. Strong suppressive effect on FITC-labelled *E. coli* suspension ingestion by PMNs was displayed by compounds **4d** and **8d** with phagocytic activity of 21.43% and 19.12%, respectively (Table 2).

Compounds which bore dimethoxy group on ring B showed strong suppressive effect on PMNs phagocytic activity. On the hand, naphthalene group on ring A was not favourable as only moderate inhibitory activity was observed on test compounds containing this functional group.

Table 2: Percentage of PMNs phagocytic activity at 100 and 6.25 μg/mL of chalcone derivatives

	Mean ± SEM	
Compounds	100 μg/mL	6.25 μg/mL
4d	21.43 ± 1.58	86.53 ± 0.93
4e	31.73 ± 0.39	89.03 ± 0.93
5d	36.45 ± 0.39	88.93 ± 0.93
6d	51.91 ± 1.73	86.93 ± 0.93
6e	47.63 ± 0.39	90.73 ± 0.93
6f	63.90 ± 1.25	87.13 ± 0.93
6 g	42.59 ± 0.48	90.43 ± 0.93
7d	46.18 ± 0.77	89.83 ± 0.93
7e	44.15 ± 2.19	91.23 ± 0.93
7 h	50.96 ± 0.57	89.63 ± 0.93
7i	24.62 ± 0.48	85.23 ± 0.93
8d	19.12 ± 3.85	93.43 ± 0.93
8e	39.34 ± 3.91	85.13 ± 0.93
Negative control	97.43 ± 1.43	

Values are presented as mean ± SD (n = 3).

Inhibition of intracellular and extracellular ROS productions

ROS productions in PMNs and human whole blood were activated by serum opsonized zymosan (SOZ), and the ability of the test compounds to inhibit this process was investigated using a luminol and a lucigenin probes. Luminol and lucigenin were used to detect the presence of intracellular and extracellular ROS, respectively. Test compounds which showed percentage inhibition of more than 50% were investigated further at serial concentrations of 12.5, 6.25, 3.13, 1.56 and 0.78 μg/mL to determine their IC_{50} values (Table 3).

In general, these chalcone derivatives were more active inhibiting both intracellular and extracellular ROS productions in human whole blood rather than in PMNs. Compounds **4a**, **4b**, **4d**, **7d**, **7e**, **7i**, **8d** and **8e** showed lower or comparable IC_{50} values with controls on intracellular ROS production in human whole blood inhibition while only compound **4a**, **7i** and **8d** were active in PMNs. Only five series of test compounds were assayed for inhibitory effect on extracellular ROS production, and most of the compounds tested displayed lower IC_{50} values than that of controls both in human whole blood and in PMNs. Among all the compounds tested, compound **7i** was the most active in inhibiting intracellular and extracellular ROS productions both in human whole blood and PMNs.

It is gathered from this study that 4-methylaminoethanol and 2,3-dimethoxy substituents on ring B were favourable in inhibiting human whole blood extracellular ROS produc-

Table 3: Inhibitory activity of the synthetic chalcone derivatives on intracellular and extracellular ROS productions

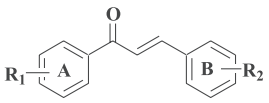
Compounds	Intracellular IC_{50} values (μM)		Extracellular IC_{50} values (μM)	
	Whole blood	PMNs	Whole blood	PMNs
1a	74.7 ± 3.5	–	–	–
1b	–	–	–	–
1c	–	–	–	–
2a	42.3 ± 2.0	32.3 ± 0.4	–	–
2b	–	–	–	–
2c	–	–	–	–
3a	25.7 ± 1.3	13.3 ± 0.8	–	–
3b	32.8 ± 1.7	25.6 ± 1.3	–	–
3c	33.8 ± 1.5	24.9 ± 1.1	–	–
4a	10.9 ± 1.0	6.2 ± 0.6	–	–
4b	18.2 ± 2.0	11.7 ± 1.2	–	–
4c	27.5 ± 2.1	20.2 ± 1.5	–	–
4d	14.6 ± 0.8	13.6 ± 0.0	16.4 ± 2.9	17.5 ± 3.0
4e	26.7 ± 1.4	19.4 ± 0.9	18.5 ± 0.8	16.3 ± 0.7
5b	63.9 ± 4.0	49.1 ± 3.0	–	–
5c	41.4 ± 0.7	31.5 ± 0.5	–	–
5d	30.5 ± 1.6	19.7 ± 0.9	70.1 ± 6.4	61.8 ± 5.6
6d	30.0 ± 1.3	28.1 ± 2.1	19.9 ± 2.0	16.4 ± 1.6
6e	30.3 ± 1.8	25.6 ± 1.1	–	34.2 ± 9.2
6f	–	–	–	–
6g	23.7 ± 5.9	16.5 ± 3.2	22.2 ± 2.9	25.7 ± 3.3
7d	14.5 ± 0.3	13.5 ± 1.0	8.6 ± 0.3	7.0 ± 0.2
7e	12.2 ± 1.3	14.8 ± 0.8	14.9 ± 6.7	12.1 ± 5.5
7h	–	–	–	–
7i	6.9 ± 0.7	4.8 ± 0.5	7.3 ± 1.1	17.6 ± 2.5
8d	17.3 ± 0.9	9.6 ± 0.1	10.9 ± 1.1	12.5 ± 1.3
8e	14.3 ± 4.1	13.4 ± 4.2	12.6 ± 1.4	10.2 ± 1.1
Aspirin	18.7 ± 0.2	9.6 ± 0.1	50.2 ± 0.8	–
Negative control	0.0			

Values are presented as mean ± SD (n = 3). (–): IC_{50} values were not determined.

tion as the activity was retained except for compound **5d**, regardless of functional groups on ring A. Moreover, inhibitory activities of the test compounds diminished with naphthalene or furan functional groups on ring A.

Summary of SAR

Overall, size of the diaryl rings has little influence on the activities of the test compounds as weak or strong inhibitions were observed in both bulky substituents (naphthalene and diphenyl) and small groups (thiophene and furan). Nevertheless, inhibitory activities at different stages of the immune response were observed with variation of substituents on the diaryl rings (Figure 1). Migration of PMNs towards chemo-attractant was mainly inhibited by the presence of hydroxyl, 4-methylaminoethanol, dimethoxy and 4-diethylamine groups in ring B. On the other hand, phagocytic activity of PMNs were suppressed primarily only by compounds, which bore trimethoxy and methyl-furan groups in ring A and 4-methylaminoethanol group in ring B. Moreover,



- Pyridine as ring A was not desirable for most activity assayed
- Naphthalene group in ring A gave moderate or low activity in most assays

- Positions of OH group in ring B were not crucial for chemotaxis of PMNs
- 4-methylaminoethanol and dimethoxy groups in ring B gave desired effects in most assays

Figure 1: Summary of chalcone derivatives SAR on the assays carried out.

Table 4: Physicochemical properties of compound **8d**

Properties	Optimal range (29,30)	Values for compound **8d**[a]
MW	<500	371.43
Clog*P*	<5	3.31
H-bond donors	<5	1
H-bond acceptors	<10	6
Polar surface area (PSA) [Å^2]	<140	68.24

[a]Calculated with Molinspiration property engine v.2011.04 (http://www.molinspiration.com).

both intracellular and extracellular ROS productions were inhibited exclusively only by compound containing a 4-diethylamine substituent. Furthermore, an absence of methyl group on furan in ring A diminished activities of the compound altogether as observed in **5d** (absence of methyl group) in contrast to **4d** (with methyl group).

Compound **8d** was found to have high potency in most of the assays carried out. It can be depicted from its physicochemical properties (Table 4) that this compound has potential to be a new lead compound in developing inhibitors of neutrophils' chemotaxis, phagocytosis and ROS production.

Conclusion

Five series of chalcone derivatives were successfully synthesized using the Claisen–Schmidt base-catalysed condensation reaction. It was observed that phenyl-4-methylaminoethanol and dimethoxy substituents contribute to the inhibition of PMNs chemotaxis towards fMLP, suppressive effect of phagocytic activity of phagocytes and preventing intracellular and extracellular ROS productions. Compounds **4d** and **8d** that posses the 4-methylaminoethanol functional group were active in all the four assays performed. This active compound adds to the reservoir of potential immunomodulatory and anti-inflammatory agents worth investigating.

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