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Effects of Synthetic Chalcone Derivatives on Oxidised Palmitoyl Arachydonoyl Phosphorylcholine-Induced Proinflammatory Chemokines Production

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Oxidised 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) induces the production of proinflammatory chemokines has been widely studied for its role in vascular inflammation. There is increasing evidence on the role of chalcones as potential anti-inflammatory agents but less is known about its effects on OxPAPC-induced chemokines production and the involvement of unfolded protein response (UPR) signalling, particularly through XBP1 pathway. The present study sought to investigate the inhibitory potential of synthetic chalcone derivatives on the release of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1), induced by OxPAPC through XBP1 signalling pathway on differentiated U-937 macrophages. The effects of synthetic chalcone derivatives on the chemokines productions were investigated using enzyme-linked immunosorbent assays, while the inhibitions of XBP1 signalling were detected using western immunoblot. Results show that all the three tested synthetic chalcone derivatives inhibited OxPAPC-induced chemokines production in a concentration-dependent manner. Compound **1.5** exhibited the strongest inhibition of IL-8 and MCP-1 at 61.4 ± 4.23% and 63.8 ± 2.16%, respectively. Compound 1.5 also achieved the lowest IC_{50} values for both IL-8 (18.33 ± 1.59 μM) and MCP-1 (8.42 ± 1.05 μM) inhibitions. For XBP1 protein expression, both compound **1.4** and **1.5** exhibited significant concentration-dependent suppression of the protein expressions. The results suggest that synthetic chalcone derivatives may serve as potential alternatives for future development of anti-inflammatory agents, particularly in vascular inflammation.

Introduction

Chemokines play various important roles in inflammatory processes. Diverse factors such as oxidised phospholipids (OxPLs) [1], ultraviolet radiation exposure [2] and endotoxin [3] are known to be involved in the generation of chemokines. Overproduction of proinflammatory chemokines such as interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) has been associated with various inflammatory diseases including atherosclerosis [4]. MCP-1 is widely known to play important role in the accumulation and transmigration of mononuclear cells such as monocytes to the sites of inflammation [5] while IL-8 is responsible for monocytes arrestment to vascular endothelium [6,7]. IL-8 and MCP-1 are chemokines that are widely known to play a major role in vascular inflammations [8]. These chemokines can be induced by conventional pro-inflammatory inducers such as lipopolysaccharide (LPS) through activation of NF-kB pathway. Similarly, oxidised phospholipid such as oxidised 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) can induce the release of these chemokines. However, induction of IL-8 and MCP-1 by OxPAPC is independent of the classic nuclear factor kappa b (NFκβ) pathway and therefore unlike LPS, OxPAPC did not induce certain pro-inflammatory cytokines such as TNF-alpha and IL-1β. Moreover, stimulation with OxPAPC will generally result in more prolonged upregulation of these chemokines [9].

Oxidised phospholipids (OxPLs), the bioactive components of oxidised low density lipoprotein (OxLDL) particles, have received considerable attentions in their effects on vascular inflammatory events [10,11,12]. Oxidised 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine (OxPAPC) in particular is present at high concentration in atherosclerotic plaques [13] and is thought to contribute to the inflammatory processes induced by OxLDL. OxPAPC has been shown to induce the expressions of inflammatory genes such as IL-8 and MCP-1 in human endothelial cells through UPR pathway[14], which is one of the pathways that are activated during endoplasmic reticulum stress. The levels of OxPAPC-induced

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IL-8 and MCP-1 release were also reported in human THP-1 cell [15]. Furthermore, an *in vivo* study carried out by Furnkranz and co-workers showed that topical administration of OxPAPC on C57BL/6J mice carotid arteries resulted in enhanced mRNA expression of MCP-1 and keratinocyte-derived chemokine (KC) [16], of which the latter is a murine chemokine with closest resemblance to IL-8 in human system.

Endoplasmic reticulum (ER) stress is characterised as imbalanced of cellular protein modifications. Various stimuli which initiate ER stress include accumulation of unfolded proteins, deprivation of glucose, disruption in calcium homeostasis as well as accumulation of free cholesterol and oxidised phospholipids [17,18]. Continual ER stress causes activation of unfolded protein response (UPR) signalling, which involves inositol requiring enzyme (IRE) 1, RNA-depending protein kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor (ATF) 6 [19]. Among the three signalling arms, IRE1 is the most evolutionarily conserved pathway, whereby activation of IRE1 is responsible for X-box protein (XBP) 1 activation. It is linked to various inflammatory disorders such as atherosclerosis [20], inflammatory bowel disease [21] and colitis [22]. Besides, it was reported that UPR is responsible in effector cells of the immune response through the enhanced release of proinflammatory mediators [23,24].

Chalcones (1,3-diphenyl-2-propen-1-one) are natural polyphenolic compounds biosynthesized abundantly in fruits, soy beans, spices and vegetables. They are composed of open-chain flavanoids which are joined by α,β-unsaturated ketone moiety [25]. Chalcones have been a considerable interest among scientists due to their broad range of pharmacological activities and simple synthesis. The anti-inflammatory effects of chalcones have been reported extensively. For example, hydroxychalcones were shown to be effective in inhibition of lipopolysaccharide (LPS)-induced tumour necrosis factor-α (TNF-α) [26] and LPS-induced inducible nitric oxide synthases (iNOS) [27] on RAW 264.7 macrophages.

Nevertheless, synthetic chalcone derivatives have not been extensively studied in OxPAPC-induced proinflammatory chemokines release. Therefore, this study was aimed to investigate the inhibitory effects of synthetic chalcone derivatives on the release of IL-8 and MCP-1 induced by OxPAPC on differentiated U-937 macrophages and the mechanism of action. Results obtained demonstrated that the synthetic chalcones derivatives tested were able to reduce the release of IL-8 and MCP-1 through inhibition of XBP1 signalling.

Materials and methods

Chemicals and reagents

All cell culture reagents were purchased from Invitrogen (Burlington, ON, Canada). 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphocoline (PAPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA) and ELISA kits were purchased from eBioscience (San Diego, CA, USA). Other chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise stated.

Synthesis of chalcone derivatives

Synthetic chalcone derivatives used in this study were synthesised by members of research group using Claisen-Schmidt condensation reaction and reported previously (Table 1). Briefly, an amount of 10 mmol of the respective ketones was added to a solution of the respective aldehydes (10 mmol) in ethanol (15 mL). NaOH (50%) solution was added dropwise as catalyst, and the reaction mixture was stirred at room temperature for 2-24 h accordingly. Reaction completion was monitored by TLC and appearance of precipitate and colour changes of the reaction mixture were an indicative of product formation. Upon completion, the reaction mixture was poured into acidified ice, extracted with ethyl acetate (50 mL), washed with water (2 x 150 mL), dried and concentrated *in vacuo* to give oils and solids. The crude products were further purified either by column chromatography or recrystallization[28].

All compounds were characterised as reported previously[28]. ^{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 ionisation 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.

4-(4-Diethylamino-phenyl)-1,1-diphenyl-but-3-en-2-one **(1.2)** [28]
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%). RF 0.55 (EtOAc-PE 1:3 v/v); mp: 106–107 °C; ^{1}H NMR (500 MHz, $CDCl_3$): d = 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, $CDCl_3$): d=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.

1,1,5-Triphenyl-hex-3-en-2-one **(1.4)** [28]
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%). RF 0.72 (EtOAc-PE 2:3 v/v); mp: 156 – 157 °C; ^{1}H NMR (500 MHz, $CDCl_3$): d = 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, $CDCl_3$): d=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.

3-(5-methyl-furan-2-yl)-1-naphthalen-1-yl-propenone **(1.5)** [28]
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%). RF 0.38 (EtOAc-PE 1:3 v/v); mp: 138 – 139 °C; ^{1}H NMR (500 MHz, $CDCl_3$): d = 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, $CDCl_3$): d = 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.

Autoxidation of PAPC

Autoxidation of PAPC was carried out using the method previously described [13]. PAPC (1 mg) was dissolved in high performance liquid chromatography (HPLC)-grade methanol and evaporated under a nitrogen stream. The dried PAPC was left to autoxidise at room temperature for 7 days. Both the purity of PAPC and the autoxidation status of OxPAPC were analysed and monitored using the positive ion-electrospray ionization-mass spectrometry (ESI-MS).

Cell culture and cell differentiation

U-937 (ATCC® CRL-1593.2 ™) cell line was purchased from American Type Culture Collection (ATCC) (Manassas, VA, United States). U-937 mononuclear cell line was grown in the Rosewell Park Memorial Institute-1640 (RPMI-1640) culture media, enriched with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) penicillin G/streptomycin in a 75 cm^2 cell culture flask. The cultured cells were maintained at 37°C humidified atmosphere with 5% CO_2. Prior to treatment with the tested compounds, U-937 cells (5 x 10^5 cells/mL) were incubated with phorbol myristate acetate (PMA) at concentration 200 nM/mL for 24 hours to allow differentiation from monocytes to macrophage-like phenotypes. Subsequently, cells were incubated with serum free media overnight for recovery phase [29].

Measurement of cell viability

The cytotoxic effect of the synthetic chalcone derivatives was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [30]. Differentiated U-937 macrophages were treated with different concentrations of synthetic chalcone derivatives and simvastatin (6.25, 12.5, 25, 50 and 100 μM) in the presence and absence of OxPAPC for 24 hours. Subsequently, MTT salt reagent was added and cells were further incubated for 4 hours. The formazan crystals were dissolved in DMSO and analysed at 565 nm using Tecan's Infinite® 200PRO NanoQuant microplate reader (Tecan Trading AG, Mannedorf, Switzerland). The concentrations of test compounds at viability more than 90% [31] were chosen and used for subsequent assays.

Determination of chemokines release

The chalcone derivatives were dissolved in dimethylsulphoxide (DMSO) and further diluted in serum-free RPMI media prior to use at the desirable concentrations. The highest concentration of DMSO used in cell culture was at 0.1%. Differentiated U-937 macrophages were pre-treated with synthetic chalcone derivatives at concentration 6.25, 12.5, 25, and 50 μM (2 hours), followed by stimulation with 30 μg/mL of OxPAPC for 6 hours and 9 hours for IL-8 and MCP-1 quantifications, respectively. Preliminary work in our laboratory showed that significant level of IL-8 and MCP-1 were started to be observed from the respective time points forward (supplementary fig. I & II). A negative control group received OxPAPC (30 μg/mL) whilst a positive control group received simvastatin and vehicle control group received 0.1% DMSO. Supernatants were collected and enzyme-linked immunosorbent assay (ELISA) was carried out according to the manufacturer's instructions. The percentage of inhibition (%) at highest treatment concentrations (50 μM) and IC_{50} values for respective compounds were determined. Percentages of inhibition (%) were calculated using formula as follows:

$$\% \text{ inhibition} = \frac{Cn - Cx}{Cn} \times 100\%$$

Where C*n* is the concentration of chemokines (pg/mL) at 0 μM treatment (negative control), while C*x* is the concentration of chemokines (pg/mL) at corresponding concentrations (μM).

Western immunoblot

XBP1 protein expressions was determined using western immunoblot. Cells were pre-treated with test compounds with concentrations ranging from 6.25 to 50 μM, followed by stimulation of OxPAPC for 2 hours. Cells treated with complete media and OxPAPC only were used as negative controls while cells treated with 4-phenylnutyric acid (4-PBA) [32] were used as positive controls. Total protein lysates of 1 mg/mL were loaded and separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and transfer onto polyvinylidene difluoride (PVDF) membranes. The western immunoblot techniques used were as previously described [33]. Rabbit polyclonal anti-XBP1 antibody (1: 5000, abcam, Cambridge, MA) was used to detect XBP1 protein expressions (42 kDa) while mouse monoclonal anti-beta actin antibody (1: 10,000, abcam, Cambridge, MA) was used as loading control. The established bands were visualised using enhanced chemiluminescence substrate (ECL) (Perkin Elmer, USA) and the relative intensity of each band was quantified using Fusion-Capt Advance Software (Vilber Loumat, Germany).

Statistical analysis

Data were obtained from at least three independent experiments and analysed using the GraphPad Prism5 software (GraphPad Software Inc., San Diego, CA, USA). Values were presented as mean ± standard error of mean (SEM). Statistical test analysis of variance (ANOVA) and Bonferroni post hoc test were used for multiple comparisons and data analysis with p-values < 0.05 was regarded as statistically significant.

Results

Cytotoxic effect of synthetic chalcone derivatives on PMA-differentiated U-937 cells

All the 6 synthetic chalcone derivatives were tested towards differentiated U-937 macrophages at concentrations ranging from 0 to 100 μM. Compounds **1.2**, **1.4** and **1.5** showed 90 % or higher

percentage of cell viability and were used in the subsequent assays (Fig. 1).

Inhibitory effect of synthetic chalcone derivatives on OxPAPC-induced IL-8 secretion

All tested compounds inhibited IL-8 release induced by OxPAPC in a concentration-dependent manner. Compound **1.5** at the maximum concentration of 50 μM showed the strongest inhibition (61.39 ± 4.23%) of OxPAPC-induced IL-8 release and achieved the lowest IC_{50} value of 18.33 ± 1.59 μM. Compound **1.2** and **1.4** similarly inhibited OxPAPC-induced IL-8 with percentage of inhibition of 51.66 ± 0.40% and 45.46 ± 1.78%, respectively. Moreover, the percentage of inhibition for compound **1.5** at 50 μM showed comparable effects to the inhibition of simvastatin at concentration 25 μM. The percentage of inhibition and IC_{50} values for each tested compound are presented in Table 2.

Inhibitory effect of synthetic chalcone derivatives on OxPAPC-induced MCP-1 secretion

All the tested compounds inhibited MCP-1 release induced by OxPAPC in a concentration-dependent manner. Among the three synthetic chalcone derivatives tested, compound **1.5** showed the highest percentage of inhibition (63.79 ± 2.16%) and also achieved the lowest IC_{50} value, recorded at 13.05 ± 1.37 μM. On the other hand, compound **1.2** and **1.4** demonstrated relatively moderate inhibition at 48.23 ± 4.16% and 54.79 ± 1.81%, respectively.The percentage of inhibition and IC_{50} values for each tested compound are presented in Table 3.

Suppression of XBP1 protein expression

Differentiated U-937 macrophages incubated with media only without stimulation of OxPAPC showed no induction of XBP1 protein expression, while 4-PBA (0.4 μM) used as positive controls showed significant reduction in XBP1 protein expression. Data obtained from western immunoblot analysis were as shown in fig. 2. The intensity of each bands were normalised with respect to beta actin protein expressions while statistical analysis was carried out with respect to negative control. From the results obtained, both compounds **1.4** and **1.5** showed significant reduction of XBP1 protein expressions in a concentration dependent manner. Cells treated with these 2 compounds showed significant reduction of XBP1 protein expressions at concentration 25 and 50 μM. Interestingly, compound **1.4** at 50 μM while compound **1.5** at concentrations 25 and 50 μM showed no significant difference in XBP1 protein expression compared with positive control, suggesting a comparable effect of these compounds with 4-PBA in the suppression of XBP1 protein expressions.

Discussion

Previous findings reported that concentrations of individual component of OxPAPC ranging from 1 to 50 μM are available in human and rabbit atherosclerotic plaques [34]. A preliminary study carried out in our lab (data not shown) showed that OxPAPC at concentration 30 μg/ml of OxPAPC, which is within the pathophysiological concentration, was able to induce significant increase in IL-8 and MCP-1 production. This concentration was selected to investigate the effect of synthetic chalcones on oxidised phospholipids-induced chemokine production. In the present study, compounds **1.2**, **1.4** and **1.5** were shown to inhibit the production of IL-8 and MCP-1 as well as the expression of XBP1 induced by OxPAPC.

Chalcone derivatives have been shown to inhibit proinflammatory chemokines production induced by LPS and TNF-α. Naringenin, a chalcone derivative, was shown to suppress the LPS-induced MCP-1 in RAW 264 macrophages [35]. Moreover, 4'-hydroxychalcone was reported to inhibit IL-8 production induced by TNF-α in K562 cell line [36]. To the best of the authors' knowledge, the three tested synthetic chalcone derivatives used in this study were shown for the first time to inhibit the release of IL-8 and MCP-1 induced by pathophysiological concentration of OxPAPC. It was observed that compound **1.5** with naphthalene functional group has the highest inhibitory activity in OxPAPC-induced IL-8 and MCP-1 as compared to compound **1.2** and compound **1.4** with diphenyl functional groups. It is possible that naphthalene functional group plays a more important role in the inhibitory activity of synthetic chalcone derivatives on proinflammatory chemokines production. Moreover, in our previous studies[28] it was observed that methyl-furan group is very important and all the compounds bearing methyl-furan group were found strong inhibitor of phagocytic activity of polymorphonuclear neutrophils (PMNs) so strong activity of compound **1.5** in present study can also be result of the presence of methyl-furan group. On other hand, comparison of two compounds (**1.2** and **1.4**) bearing same diphenyl moiety shows that substitution pattern on aldehyde ring is also very important and effective.

Simvastatin was shown to be able to reduce chemokines level and therefore were used as a reference drug for inhibition of IL-8 and MCP-1. Based on a research carried out by Rezaie-Majd *et al.* [37], treatment with simvastatin was able to reduce the serum levels of IL-8 and MCP-1 tested on patients with hypercholesterolemia. Moreover, simvastatin was demonstrated to inhibit the production of MCP-1 induced by LPS on human peripheral blood mononuclear cells and human endothelial cells in a dose-dependent manner [38]. It also effectively inhibits IL-1-induced IL-8 release on epithelial cells [39]. A preliminary work carried out in our laboratory showed that simvastatin was able to inhibit chemokines production induced by OxPAPC (supplementary fig. III), which led to its use as a positive control in the present study. Simvastatin at concentration of 25 μM was used as it is the highest non-toxic concentration towards the treated differentiated U-937 macrophagess based on MTT assay.

Activations of IRE1 signalling cause activation of XBP1, forming isoform of spliced XBP1, the functionally active transcription factor in the UPR pathway. XBP1 signalling was chosen in the present study due to the fact that the expression of XBP1 is ubiquitous, specifically within endothelial cells [14] and macrophages [40]. The activated XBP1 plays dual roles, either promoting or inhibiting inflammation, depending on the cell type and context. Previous research has shown that expression of XBP1 is correlated with

development of vascular inflammations. Based on a study carried out by Zeng et al. [41], it was reported that the enhanced expression of activated XBP1 leads to increased of inflammatory atherosclerotic lesions. To the best of the authors' knowledge, the synthetic chalcones derivatives tested have shown for the first time to cause inhibition on XBP1 protein expression tested on differentiated U-937 macrophages.

Conclusions

The present study demonstrated that the synthetic chalcone derivatives possessed potential anti-inflammatory effects by inhibiting OxPAPC-induced proinflammatory chemokines release through inhibition of XBP-1 pathway.

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Notes and references

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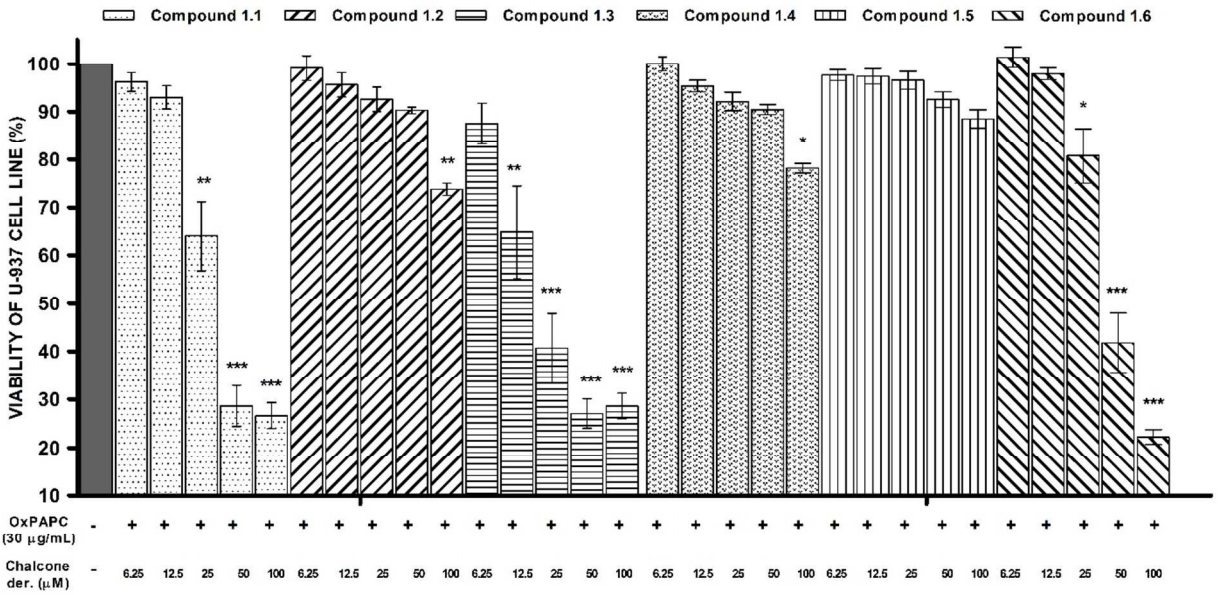
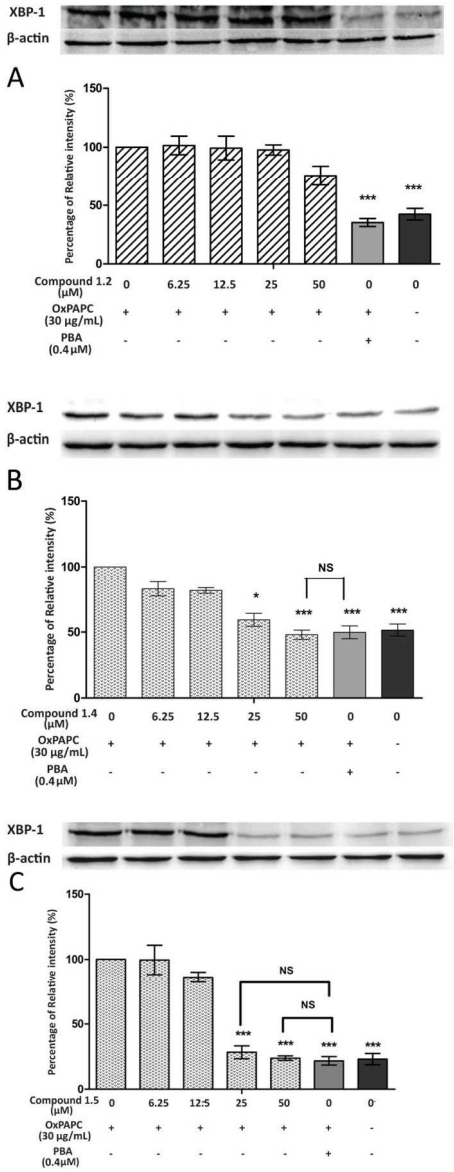


Fig. 1 Effects of synthetic chalcone derivatives on viability of PMA-differentiated U-937 macrophages. Data represent mean ± SEM of at least 3 independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus negative control



Compound 1.2 (A), compound 1.4 (B) and compound 1.5 (C) inhibit OxPAPC-induced expression of XBP-1 in PMA-differentiated U937 macrophages. Data represent mean ± SEM of at least 3 independent experiments. Data *p < 0.05, ***p < 0.001 versus negative control, while NS represent no significant different versus 4-PBA (positive control).

93x227mm (300 x 300 DPI)

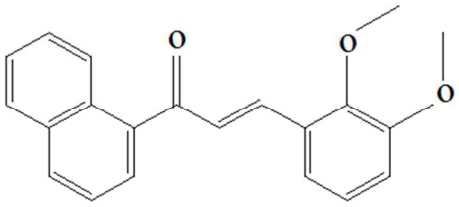
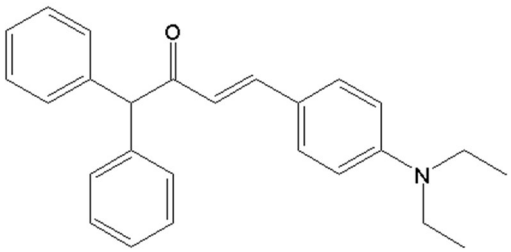
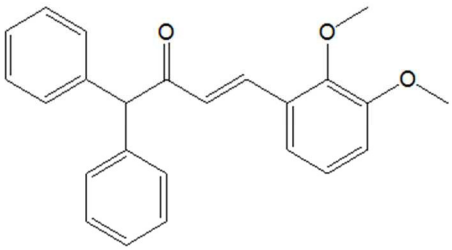
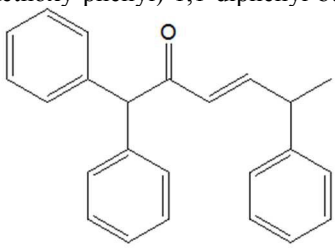
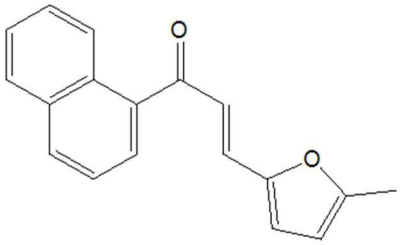
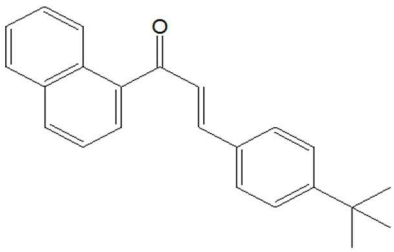
Compound	Molecular structures	Molecular weight
1.1	 3-(2,3-Dimethoxy-phenyl)-1-naphthalen-1-yl-propenone	318.37
1.2	 4-(4-Diethylamino-phenyl)-1,1-diphenyl-but-3-en-2-one	369.21
1.3	 4-(2,3-Dimethoxy-phenyl)-1,1-diphenyl-but-3-en-2-one	358.16
1.4	 1,1,5-Triphenyl-hex-3-en-2-one	326.17
1.5	 3-(5-methyl-furan-2-yl)-1-naphthalen-1-yl-propenone	262.30
1.6	 3-(4-tert-butyl-phenyl)-1-(naphthalen-1-yl)-2-propen-1-one	314.42

Table 1: Structures of synthetic chalcone derivatives

Table 2: Percentage of inhibition (%) and IC_{50} (μM) values of test compounds on IL-8 secretion.

Compound	Percentage of inhibition (%)	IC_{50} (μM)
1.2	51.66 ± 0.40	45.46 ± 1.78
1.4	53.19 ± 4.28	27.32 ± 4.84
1.5	61.39 ± 4.23 ^{NS}	18.33 ± 1.59
Simvastatin (25 μM)	77.64 ± 1.51	5.97 ± 0.93
Negative control (OxPAPC 30μg/ml)	0.00	-

Values are expressed in mean ± SEM; n=3. Percentage inhibition>10% was significant at $p<0.05$ compared with negative control. ^{NS} $p>0.05$ was considered not significant compared with simvastatin.

Table 3: Percentage of inhibition (%) and IC_{50} (μM) values of test compounds on MCP-1 secretion.

Compound	Percentage of inhibition (%)	IC_{50} (μM)
1.2	48.23 ± 4.16	ND
1.4	54.79 ± 1.81	23.45 ± 0.47
1.5	63.79 ± 2.16 ^{NS}	13.05 ± 1.37
Simvastatin (25 μM)	75.46 ± 1.75	8.42 ± 1.05
Negative control	0.00	-

Values are expressed in mean ± SEM; n=3. Percentage inhibition>10% was significant at $p<0.05$ compared with negative control. ^{NS} $p>0.05$ was considered not significant compared with simvastatin. ND represents IC_{50} value not determined.