Supplementary Material (ESI) for New Journal of Chemistry

Immunomodulatory effects of functionalised chalcones on pro-inflammatory cytokine release from lung epithelial cells

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SUPPORTING INFORMATION

Contents

1.0 General Experimental

4-Bromobenzaldehyde (99% purity), 4'-methoxyacetophenone (99% purity) and 2-acetylthiophene (≥98% purity) were purchased from Aldrich and used without further purification. Anisaldehyde (≥99.5% purity), piperonal (≥99% purity), 4-aminoacetophenone (99% purity) and PEG300 were purchased from Fluka and used without further purification. 4-(methylthio)benzaldehyde (97% purity) and 2-acetylpyrrole (98% purity) were purchased from Alfa Aesar and used without further purification. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker AV500 instrument in 5 mm NMR tubes. Samples were recorded in DMSO-d_6 solution in ppm (δ) and referenced to the internal residual partially-deuterated DMSO septet at 2.50 ppm (^{1}H NMR) and 39.52 ppm (^{13}C NMR). Mass spectrometry measurements were performed on a Waters Micromass Autospec Mass Spectrometer.

2.0 Synthesis

2.1 General route for the synthesis of chalcones

The respective aryl-methyl ketone (4.12 mmol) was added to a stirred solution of PEG300 (5-6 ml) and crushed sodium hydroxide (NaOH) (4.12 mmol) at ambient temperature. The respective aryl-aldehyde (4.12 mmol) was then added and the reaction mixture was left stirring at room temperature for 2 h. Water (100 ml) was added, the precipitate was collected by suction filtration and dried on a high vacuum line.

2.2 Characterisation of chalcones

1: δ_H (500 MHz, DMSO) 11.95 (s, 1H, NH), 7.79 (d, 2H, 8.82 Hz), 7.62 (d, 1H, 15.81 Hz), 7.54 (d, 1H, 15.58 Hz), 7.32 (dd, 1H, 3.78 Hz, 1.37 Hz), 7.13 (dd, 1H, 2.18 Hz, 1.49 Hz), 7.00 (d, 2H, 8.82 Hz), 6.25 (dd, 1H, 3.67 Hz, 2.18 Hz), 3.81 (s, OCH_3). δ_C (125 MHz, DMSO) 177.93, 160.92, 140.57, 133.15, 130.27, 127.53, 126.00, 120.63, 116.91, 114.34, 110.04, 55.31. MS (EI^+, 70 eV, 200 °C) for $C_{14}H_{13}NO_2$ ([M^+]): calcd: 227.0946; found: 227.0944 (100%).

2: δ_H (500 MHz, DMSO) 8.30 (dd, 1H, 3.78 Hz, 1.15 Hz), 8.03 (dd, 1H, 4.93 Hz, 1.15 Hz), 7.85 (d, 2H, 8.94 Hz), 7.75 (d, 1H, 15.46 Hz), 7.70 (d, 1H, 15.69 Hz), 7.30 (dd, 1H, 4.81 Hz, 3.67 Hz), 7.02 (d, 2H, 8.82 Hz), 3.82 (s, OCH_3). δ_C (125 MHz, DMSO) 181.53, 161.40, 145.77, 143.10, 135.13, 133.21, 130.81, 128.81, 127.13, 119.37, 114.41, 54.86. MS (EI^+, 70 eV, 200 °C) for $C_{14}H_{12}O_2S$ ([M^+]): calcd: 244.0558; found: 244.0554 (100%), 243.0485 (58%).

3: δ_H (500 MHz, DMSO) 7.91 (d, 2H, 8.71 Hz), 7.78 (d, 2H, 9.05 Hz), 7.72 (d, 1H, 15.46 Hz), 7.58 (d, 1H, 15.81 Hz), 6.98 (d, 2H, 8.71 Hz), 6.62 (d, 2H, 8.71 Hz), 6.10 (s, NH_2), 3.80 (s, OCH_3). δ_C (125 MHz, DMSO) 185.90, 160.83, 153.69, 141.34, 130.96, 130.24, 127.79, 125.58, 119.92, 114.31, 112.72, 55.29. MS (EI^+, 70 eV, 200 °C) for $C_{16}H_{15}NO_2$ ([M^+]): calcd: 253.1103; found: 253.1094 (100%).

4: δ_H (500 MHz, DMSO) 8.15 (d, 2H, 9.28 Hz), 7.83 (d, 2H, 9.05 Hz), 7.80 (d, 1H, 15.46 Hz), 7.68 (d, 1H, 15.46 Hz), 7.07 (d, 2H, 9.05 Hz), 7.01 (d, 2H, 8.71 Hz), 3.86 (s, OCH_3), 3.81 (s, OCH_3). δ_C (125 MHz, DMSO) 187.26, 163.03, 161.21, 143.12, 130.77, 130.68, 130.64, 127.46, 119.49, 114.37, 113.95, 55.51, 55.33. MS (EI^+, 70 eV, 200 °C) for $C_{17}H_{16}O_3$ ($[M^+]$): calcd: 268.1099; found: 268.1105 (100%).

5: δ_H (500 MHz, DMSO) 8.16 (d, 2H, 9.05 Hz), 7.81 (d, 1H, 15.46 Hz), 7.65 (s, 1H), 7.63 (d, 1H, 14.89 Hz), 7.31 (dd, 1H, 8.25 Hz, 1.49 Hz), 7.07 (d, 2H, 8.82 Hz), 6.98 (d, 1H, 7.90 Hz), 6.10 (s, 2H), 3.86 (s, OCH_3). δ_C (125 MHz, DMSO) 187.18, 163.10, 149.38, 148.07, 143.15, 130.81, 130.63, 129.36, 125.71, 119.95, 113.93, 108.48, 106.91, 101.60, 55.53. MS (EI^+, 70 eV, 200 °C) for $C_{17}H_{14}O_4$ ($[M^+]$): calcd: 282.0892; found: 282.0893 (100%).

6: δ_H (500 MHz, DMSO) 7.91 (d, 2H, 8.71 Hz), 7.81 (d, 1H, 15.58 Hz), 7.77 (d, 2H, 8.36 Hz), 7.57 (d, 1H, 15.69 Hz), 7.29 (d, 2H, 8.48 Hz), 6.63 (d, 2H, 8.71 Hz), 2.5 (s, SCH_3). δ_C (125 MHz, DMSO) 185.85, 158.43, 158.13, 153.45, 141.03, 131.60, 131.04, 129.00, 125.62, 121.32, 112.94, 14.25. MS (EI^+, 70 eV, 200 °C) for $C_{16}H_{15}NOS$ ($[M^+]$): calcd: 269.0874; found: 269.0872 (100%).

7: δ_H (500 MHz, DMSO) 11.92 (s, NH), 7.58 (d, 1H, 1.65 Hz), 7.56 (d, 2H, 1.78 Hz), 7.35 (dd, 1H, 3.57 Hz, 1.37 Hz), 7.27 (dd, 1H, 7.96 Hz, 1.78 Hz), 7.13 (m, 1H), 6.97 (d, 1H, 7.96 Hz), 6.25 (dd, 1H, 3.70 Hz, 2.33 Hz), 6.09 (s, 2H). δ_C (125 MHz, DMSO) 177.87, 149.08, 148.05, 140.62, 133.17, 129.43, 126.08, 125.13, 121.17, 117.11, 110.05, 108.49, 106.77, 101.54. MS (EI^+, 70 eV, 200 °C) for $C_{14}H_{11}NO_3$ ($[M^+]$): calcd: 241.0739; found: 241.0738 (100%).

8: δ_H (500 MHz, DMSO) 11.92 (s, NH), 7.79 (d, 2H, 8.92 Hz), 7.61 (d, 1H, 15.51 Hz), 7.55 (d, 1H, 15.64 Hz), 7.47 (m, 2H), 7.40 (m, 2H), 7.34 (m, 1H), 7.32 (m, 1H), 7.13 (m, 1H), 7.08 (d, 2H, 8.92 Hz), 6.25 (m, 1H), 5.18 (s, 2H). δ_C (125 MHz, DMSO) 177.88, 159.96, 140.46, 136.76, 133.12, 130.26, 128.45, 127.92, 127.76, 127.72, 125.99, 120.75, 116.91, 115.16, 110.02, 69.35. MS (EI^+, 70 eV, 200 °C) for $C_{20}H_{17}NO_2$ ($[M^+]$): calcd: 303.1259; found: 303.1259 (100%).

9: δ_H (500 MHz, DMSO) 11.95 (s, NH), 7.77 (d, 2H, 8.48 Hz), 7.65 (d, 1H, 15.58 Hz), 7.60 (d, 1H, 15.69 Hz), 7.34 (dd, 1H, 3.78 Hz, 1.37 Hz), 7.30 (d, 2H, 8.48 Hz), 7.15 (dd, 1H, 2.52 Hz, 1.26 Hz), 6.26 (dd, 1H, 3.78 Hz, 2.41 Hz), 2.51 (s, SCH_3). δ_C (125 MHz, DMSO) 177.76, 141.16, 140.23, 133.11, 131.31, 128.98, 126.24, 125.60, 122.00, 117.20, 110.12, 14.24. MS (EI^+, 70 eV, 200 °C) for $C_{14}H_{13}NOS$ ($[M^+]$): calcd: 243.0718; found: 243.0720 (100%).

10: δ_H (500 MHz, DMSO) 12.00 (s, NH), 7.80 (d, 2H, 8.59 Hz), 7.73 (d, 1H, 15.69 Hz), 7.64 (d, 2H, 8.48 Hz), 7.61 (d, 1H, 15.69 Hz), 7.38 (dd, 1H, 3.89 Hz, 1.49 Hz), 7.17 (dd, 1H, 2.41 Hz, 1.37 Hz), 6.27 (dd, 1H, 3.78 Hz, 2.41 Hz). δ_C (125 MHz, DMSO) 177.56, 139.31, 134.21, 133.02, 131.80, 130.43, 126.59, 123.90, 123.33, 117.63, 110.23. MS (EI^+, 70 eV, 200 °C) for $C_{13}H_{10}NOBr$ ($[M^+]$): calcd: 274.9946; found: 274.9941 (100%), 276.9920 (97%).

11: δ_H (500 MHz, DMSO) 8.14 (d, 2H, 8.94 Hz), 7.84 (d, 2H, 8.94 Hz), 7.80 (d, 1H, 15.35 Hz), 7.67 (d, 1H, 15.58 Hz), 7.46 (d, 2H, 7.45 Hz), 7.40 (dd, 2H, 7.79 Hz, 7.45

Hz), 7.34 (dd, 1H, 8.25 Hz, 7.45 Hz), 7.08 (t, 4H, 8.36 Hz, 8.71 Hz), 5.18 (s, 2H), 3.86 (s, OCH_3). δ_C (125 MHz, DMSO) 187.27, 163.09, 160.28, 143.06, 136.73, 130.79, 130.67, 130.66, 128.48, 127.95, 127.79, 127.66, 119.63, 115.22, 113.98, 69.39, 55.56. MS (EI^+, 70 eV, 200 °C) for $C_{23}H_{20}O_3$ ($[M^+]$): calcd: 344.1412; found: 344.1425 (100%).

12: δ_H (500 MHz, DMSO) 8.16 (d, 2H, 9.05 Hz), 7.89 (d, 1H, 15.46 Hz), 7.81 (d, 2H, 8.48 Hz), 7.67 (d, 1H, 15.58 Hz), 7.31 (d, 2H, 8.48 Hz), 7.08 (d, 2H, 8.94 Hz), 3.86 (s, OCH_3), 2.52 (s, SCH_3). δ_C (125 MHz, DMSO) 187.25, 163.15, 142.73, 141.69, 131.19, 130.84, 130.54, 129.25, 125.54, 120.85, 113.98, 55.54, 14.17. MS (EI^+, 70 eV, 200 °C) for $C_{17}H_{16}SO_2$ ($[M^+]$): calcd: 284.0871; found: 284.0874 (100%).

3.0 Testing

Compound names ns135A2, ns135A3, ns135A4, ns135A5 and ns135C5 correspond to compounds **1, 2, 3, 4** and **5** respectively. Compound names ns136A3, ns136B1, ns136B2, ns136B3, ns136B4, ns136D2 and ns136D3 correspond to compounds **6, 7, 8, 9, 10, 11** and **12** respectively.

3.1 Cytotoxicity studies

The cytotoxicity of the chalcone derivatives on A549 lung epithelial cells was determined using acid phosphatase and lactate dehydrogenase assays. The A549 cells were seeded at 1.2 X 10^5 per well in 24 well cell culture plates and allowed to grow confluent in humidified atmosphere in air at 37°C, 5% CO2. The culture medium were replaced with serum-free medium and incubated for 24 h. The A549 cells were then exposed to the chalcone derivatives at 300 μM, 200 μM, 100 μM and 50 μM (DMSO < 1% [v/v]). After 24 hours incubation at 37°C, 5% CO2, acid phosphatase (Fig. S1) and lactate dehydrogenase assays (Fig. S2) were performed. The concentration range that was used for further investigation was 100 μM, 50 μM, 10 μM and 1 μM.

Fig. S1 The level of acid phosphatase of A549 lung epithelial cells after 24 h treatment with chalcone derivatives. Data presented are means ± SD ,n=1. * significantly different from unstimulated control (*P<0.01).

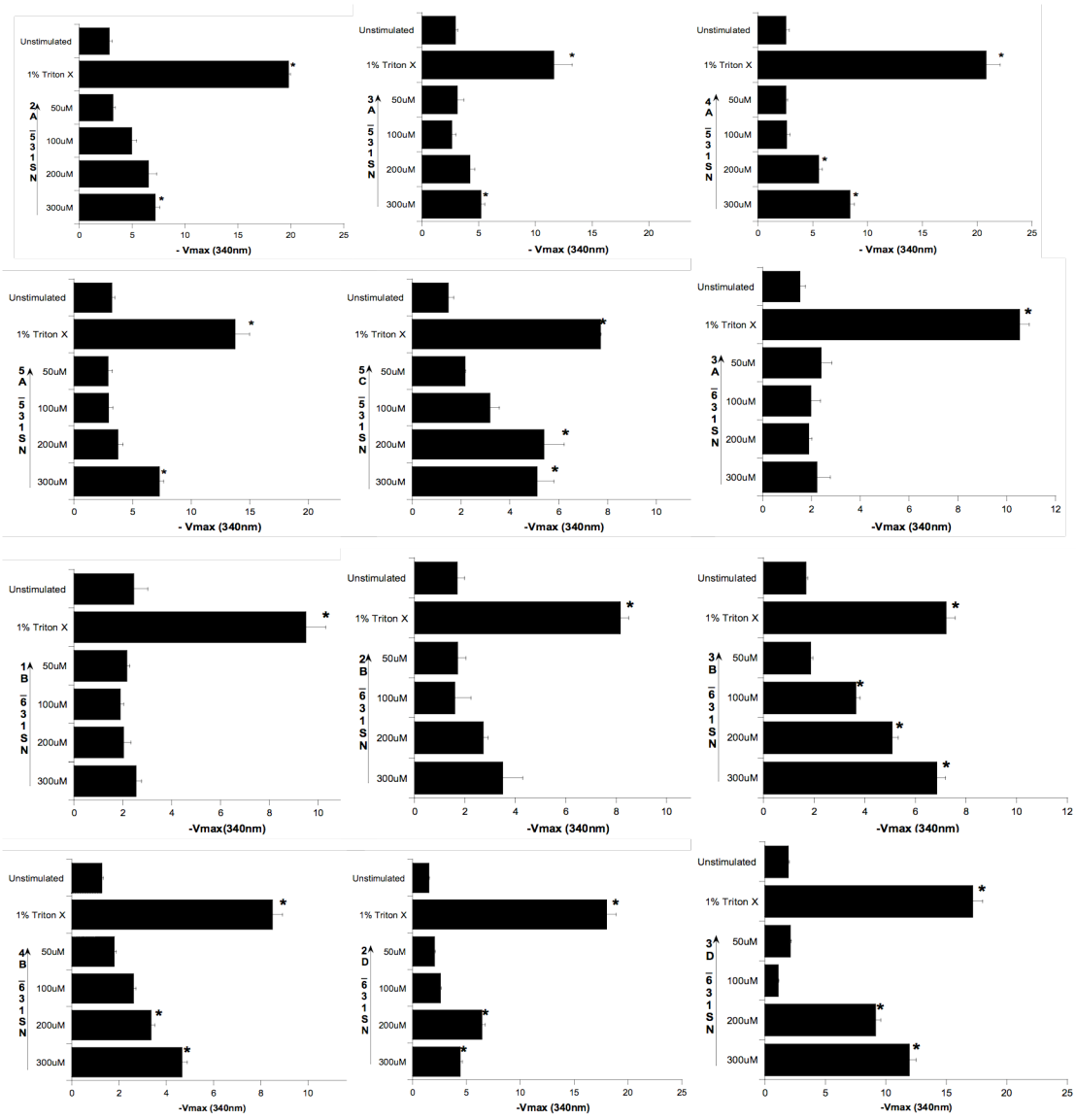

Fig. S2 The activity of lactate dehydrogenase (LDH) released from A549 lung epithelial cells after 24 h treatment with chalcone derivatives. Data presented are means ± SD ,n=1. * significantly different from unstimulated control (*P<0.01).

3.2 Effect of chalcones on A549 cells

The chalcone derivatives were studied to determine if they had any pro-inflammatory effect on A549 lung epithelial cells. Briefly, the A549 cells were seeded at 1.2 X 10^5 per well in 24 well cell culture plates and allowed to grow confluent in humidified atmosphere in air at 37°C, 5% CO_2. The culture medium were replaced with serum-free medium and incubated for 24 h. The A549 cells were incubated in the presence of 100 µM, 50 µM, 10 µM and 1 µM of the chalcone derivatives for 24 h. The cell supernatants were collected and assayed for IL-6 and IL-8 using ELISA (Fig. S3 and Fig. S4).

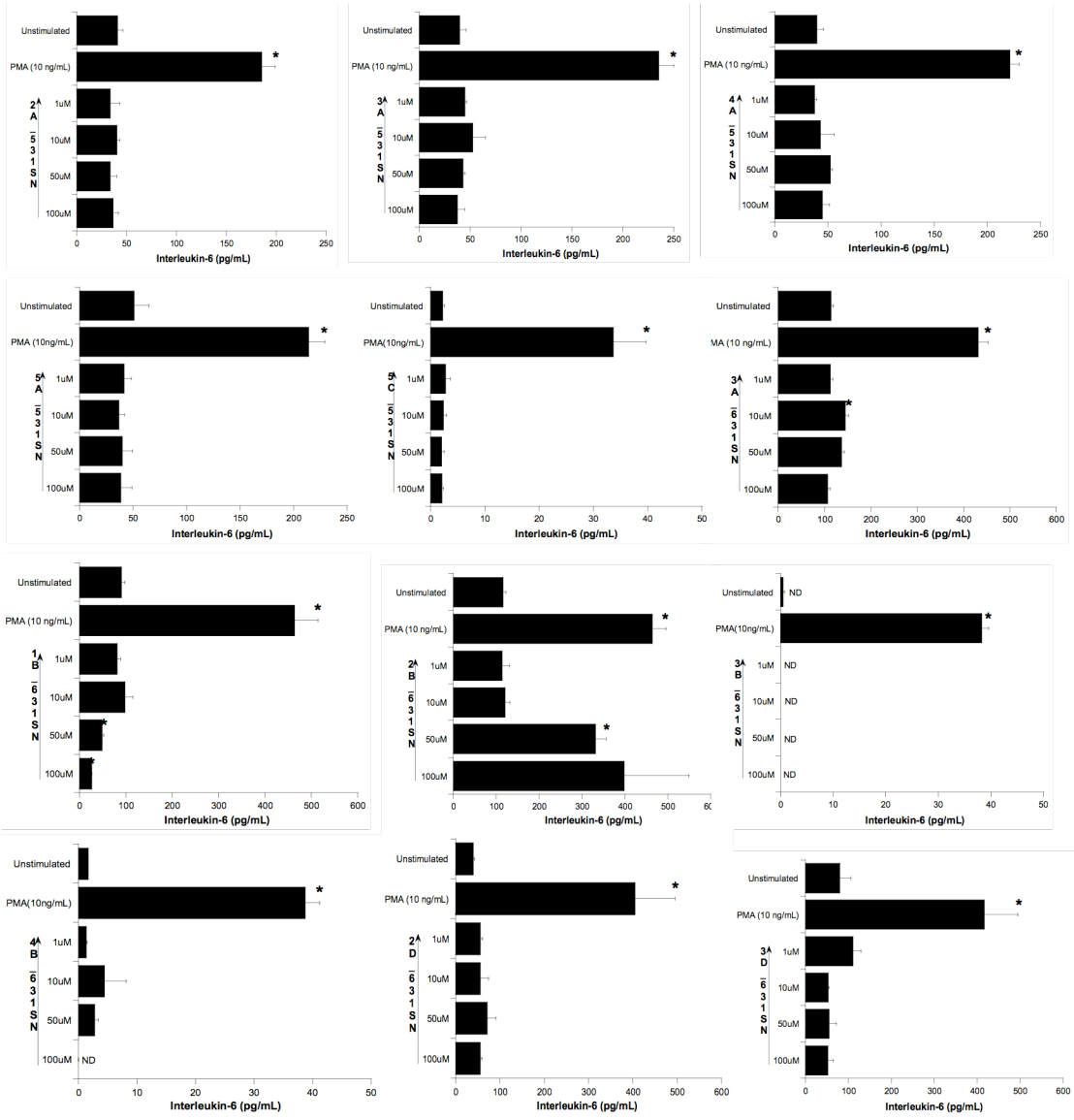

Fig. S3. The level of interleukin-6 of A549 lung epithelial cells after 24 h treatment with chalcones. Data presented are means ± SD ,n=1. * significantly different from unstimulated control (*P<0.01).

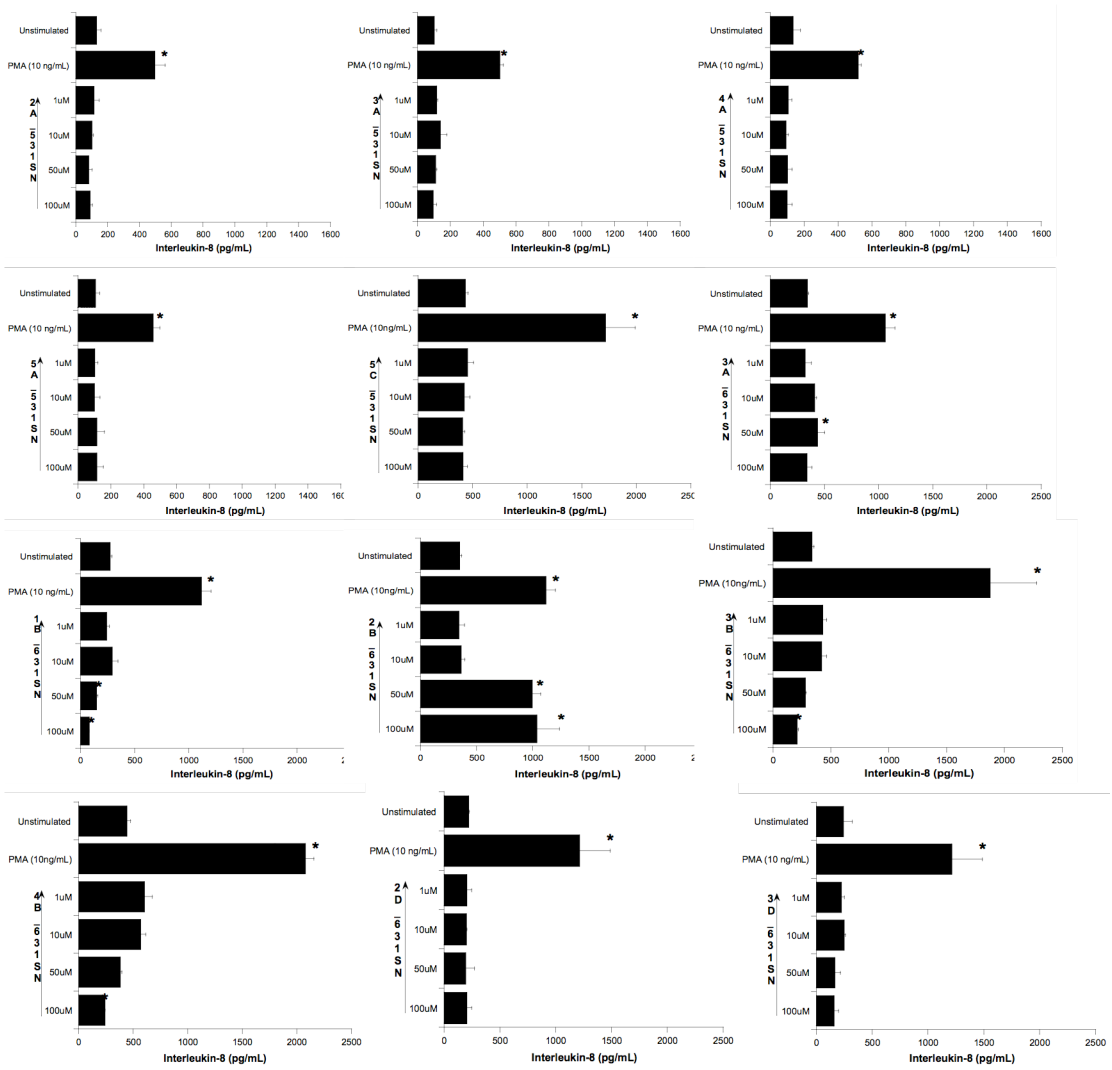

Fig. S4. The level of interleukin-8 of A549 lung epithelial cells after 24 h treatment with chalcones. Data presented are means ± SD ,n=1. * significantly different from unstimulated control (*P<0.01).

3.3 Effect of chalcones on thrombin mediated IL-6 and IL-8 production of A549 lung epithelial cells

The chalcone derivatives were studied to determine if they had any immunomodulatory effect on the protease mediated pro-inflammatory cytokines release from A549 cells. A549 cells were seeded at 1.2 X 10^5 per well in 24 well cell culture plates and allowed to grow confluent in humidified atmosphere in air at 37°C, 5% CO_2. The culture medium were replaced with serum-free medium and incubated for 24 h. The A549 cells were then pre-incubated with chalcone derivatives at 100 µM, 50 µM, 10 µM and 1 µM for 2 hours and followed by thrombin stimulation (2 unit/mL) for 24 hours. The cell supernatants were collected and assayed for IL-6 and IL-8 using ELISA (Fig. S5).

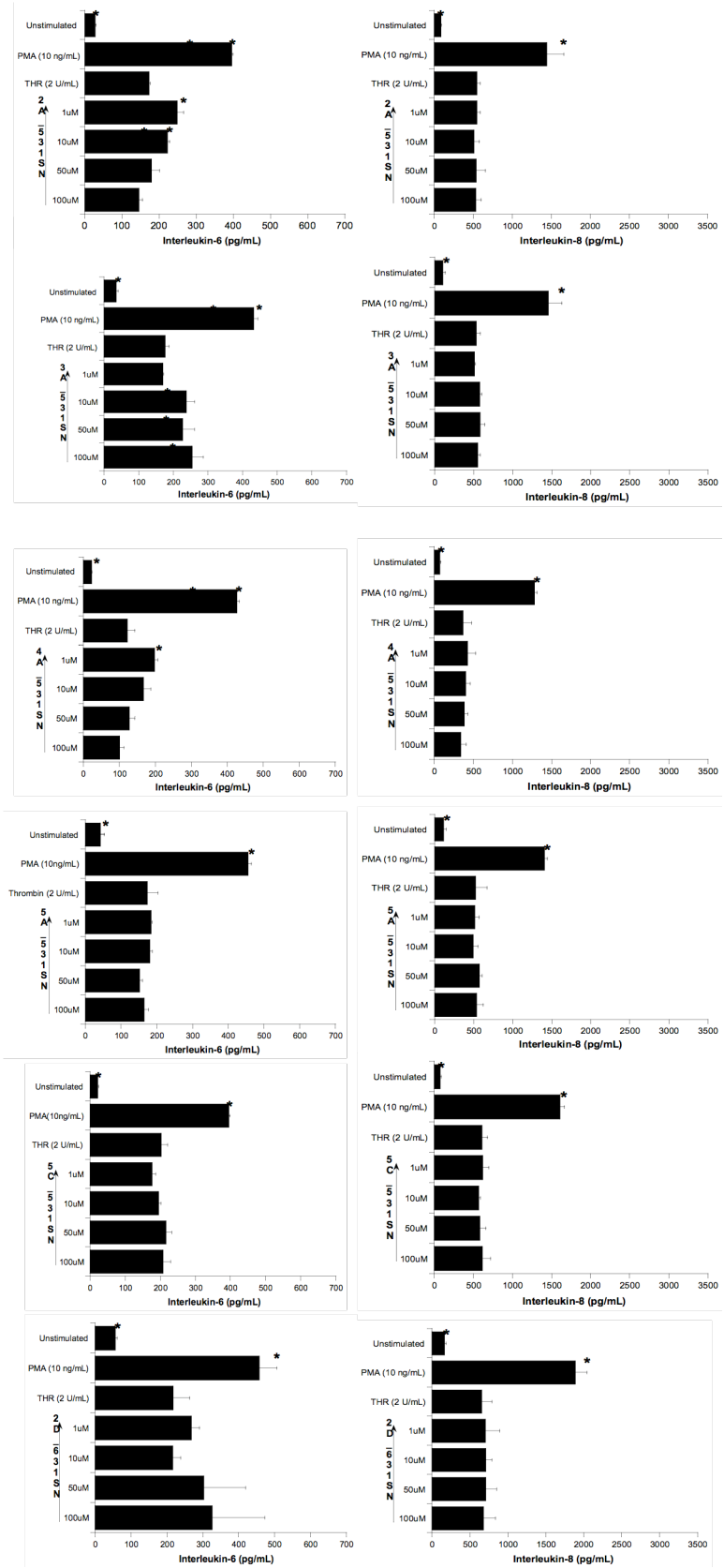

Fig. S5 The level of interleukin-6 and interleukin-8 of A549 lung epithelial cells after 2 h pre-incubation with chalcone derivatives and followed by 24 h co-incubation with thrombin. Data presented are means ± SD ,n=1. * significantly different from thrombin control (*P<0.008).

3.4 Effect of chalcones on protease activity

Azocoll assay was performed to determine if the chalcone derivatives inhibited the proteolytic activity of the proteases. The azocoll collagen substrates (4 mg/mL) were co- incubated with 2 unit/mL of Thrombin and 100 µg/mL of Trypsin respectively in the presence of 100 µM of chalcone derivatives at 37°C for 45 mins. The supernatants were then collected and the absorbance was monitored at 520nm (Fig. S6).

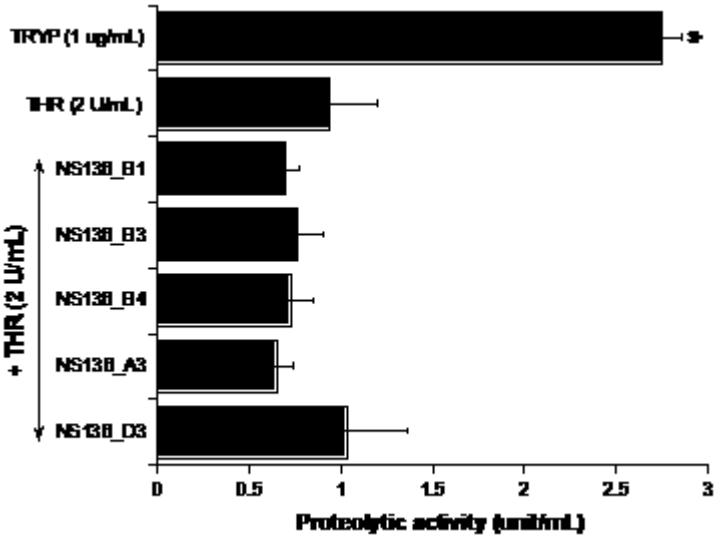

Fig. S6 Proteolytic activity of thrombin co-incubated with chalcone derivatives. Data presented are means ± SD ,n=1. * significantly different from thrombin control (*P<0.008).

4.0 Computational analysis

A computer-assisted conformational study was made on some selected chalcones (compounds **1**, **3**, and **6-10**) using the program GAUSSIAN03. In a first step the three-dimensional models of the investigated compounds were assembled using the atoms and structural fragments from GAUSSVIEW 3.0. Geometry optimisation was then performed at RHF/3-21G level of theory. After the structures were determined at RHF/3-21G level of theory, partially relaxed scan calculations were run for every 15° rotation of the torsional angles ϕ and θ at RHF/3-21G level of theory. Energy profiles of compounds **1**,**3**, and **6-10** at RHF/3-21G level of theory are given in Fig. S7. In order to obtain a better degree of accuracy, the minima obtained for each compound from the relaxed scan calculations were further optimised at RHF/6-31+G** level of theory.

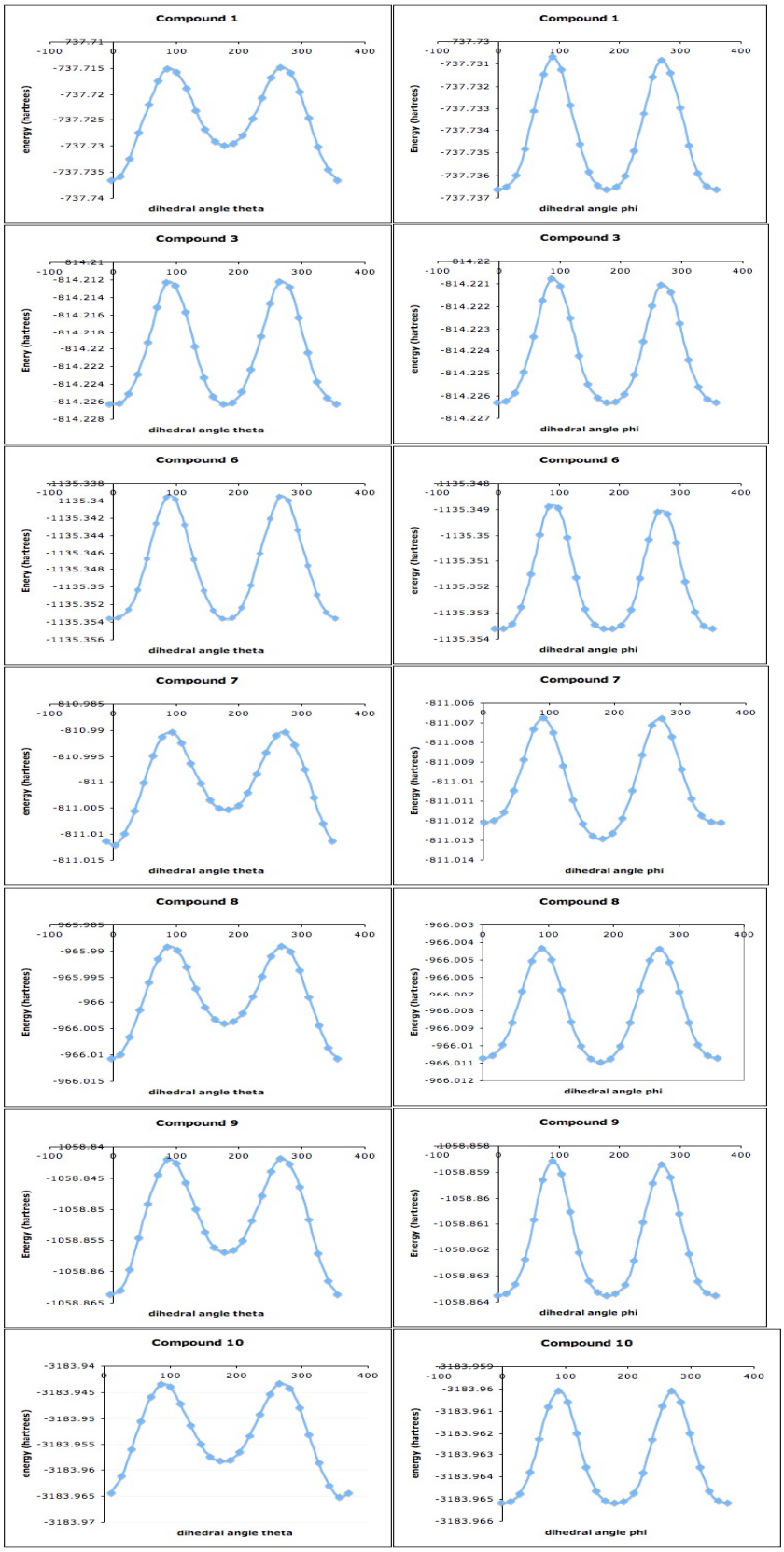

Fig. S7 Energy profiles from partially relaxed scans for compounds **1**, **3** and **6-10** at RHF/3-21G level of theory.

In order to rationalize a possible site of action at the electronic level for compounds **1**, **3**, and **6-10**, we generated LUMO and MEP maps. In a first step, the checkpoint files obtained from optimisation of these compounds at RHF/6-31+G** were first converted into formatted checkpoint files. The total electron density (density=SCF), Electrostatic Potential (potential=SCF) and LUMO (MO=LUMO) cube files were then generated from the formatted checkpoint files using the Cubegen utility program in GAUSSIAN03. Number of points per side in the cube was set to the default value of 80^3 points. MEP surfaces were visualised using GaussView 3.0 by mapping the RHF/6-31+G** electrostatic potentials onto the total electron density (isovalue = 0.001 e/Å). LUMO surfaces were visualised by mapping the RHF/6-31+G** LUMO's onto the total electron density (isovalue = 0.001 e/Å). For the MEP maps, regions with attractive potential appear in red and those of repulsive potential appear in blue. For the LUMO maps regions with the highest absolute value of LUMO are indicated in 'blue', while regions with the lowest values are indicated in 'red'