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The Synthetic of Halogens (F) & Compere With (Cl) at Meta-positions of Aromatic Rings In Chalcones on Their *In Vitro* Anti-inflammatory Activity

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ABSTRACT

Scientists have been increasingly interested in recent years in finding new anti-inflammatory drugs. Chalcone term is given to the flavonoid compounds bearing the 1,3-diphenyl-2-propen-1-one framework. Generally, chalcones are precursors of flavonoids with two aromatic rings joined together through three carbons, α, β-unsaturated carbonyl system. In plants, chalcones are converted to the respective (2S)-flavanones by enzymatic reaction of chalcone isomerase. Based on the close chemical and biogenetic relationship between flavanones and chalcones, they are considered as natural products. For anti-inflammatory activity of chalcones, activated macrophages play an important role and compounds with that inhibit nitro oxide production by macrophages have been found potential for the prevention and treatment of inflammatory disorders. Some functional groups such as dimethylamine, methoxy and butoxy groups increase the electron density of the B-ring resulting in significant loss of anti-inflammatory activity. Therefore, in this project we synthesised five compounds for chalcones containing halogens (-Cl, -F) at meta-positions on aromatic rings in chalcones and tested for their anti-inflammatory activity. The synthesized compounds were purified by column chromatography and characterised by 1H-NMR, 13C-NMR, FTIR, Mass and UV spectra. Further evaluation of their in vitro anti-inflammatory activity were carried out using RAW 264.7 mouse macrophages. The test dose of chalcones were determined was cytotoxicity (MTT) assay on RAW264.7 mouse macrophages. The results showed that the halogen substitution at meta-positions on aromatic rings improved the anti-inflammatory activity for the compound (E)-1,3-bis(3-**chlorophenyl) prop-2-en-1-one (III*)* shows the best activity. The table below showed the compounds activity with IC_{50} values.**

Chalcone	IC_{50} value
C	> 100 μM
C-I *(E)-1-(3-chlorophenyl)-3-phenylprop-2-en-1-one*	> 100 μM
C-II *E)-3-(3-chlorophenyl)-3-phenylprop-2-en-1-one*	> 100 μM
C-III*(E)-1, 3-bis (3-chlorophenyl) prop-2-en-1-one*	29.7 μM
C-IV *(E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one*	75 μM
C-V *(E) -3-(3-fluorophenyl)-1-phenylprop-2-en-1-on*	73 μM

1Background

The chemistry of chalcones has generated intensive scientific studies throughout the world. The name “Chalcones” was given by Kostanecki and Tambor [1]. Chalcones are also known as benzyl acetophenone or benzylideneacetophenone. In chalcones, two aromatic rings are linked by an aliphatic three carbon chain. Chalcones (trans-1, 3-diaryl-2-propen-1-ones) are α, β-unsaturated ketones consisting of two aromatic rings (ring A and B) having diverse array of substituents. Rings are

interconnected by a highly electrophonic three carbon α, β-unsaturated carbonyl system that assumes linear or nearly planar structure [2-4].

They contain the ketoethylenic group (–CO–CH=CH-). Chalcones possess conjugated double bonds and a completely delocalized π-electron system on both benzene rings. the other hand, the chalcones with meta- (i.e. 2′,4′, 3′,5′) substitutions show significant decrease in activities (around 25% of the control) even at the concentration of IC_{50}>200 μM. It demonstrates that the substitution of two hydroxyl groups on chalcone rings is very important structural feature for their antioxidant and radical scavenging activities. For anti-inflammatory activity of chalcones, activated macrophages play an important role and compounds with excess inhibition for production of NO by macrophages have been found more potential for the treatment and prevention of inflammatory diseases. Some functional groups such as dimethylamine, methoxy and butoxy groups will increase the electronic density on the B-ring, resulting in decreased inhibition of the nitrite production

2 Materials and Methods

2.1 Chemicals

All the chemicals including ketones (acetophenone, 3-chloro acetophenone, 3-floro acetophenone), aldehydes (benzaldehyde, 3-chloro benzaldehyde and 3-floro benzaldehyde), and sodium hydroxide were of analytical grade and bought from Sigma Aldrich and used without further purification. Deionized double-distilled water was used throughout the experiments. Absolute ethanol from Sigma Aldrich was also used without further distillation. The resulting (E)-chalcone, (E)-1-(3-chlorophenyl)-3-phenylprop-2-en-1-one, (*E*)-1,3-bis(3-chlorophenyl)prop-2-en-1-one, (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one, (*E*)-1,3-bis(3-fluorophenyl)prop-2-en-1-one products were synthesized and purified based on previously reported literatures (86-88).

The materials and chemicals used for the chemical synthesis and cell culture assays in this study are recorded below in Table 3.1

Table 2.1Materials and chemicals that used for chemical synthesis and biological assay**.**

Materials and Chemicals	Brand / Supplier
3-chlorobenzaldehyde	Darmstadt , Germany
3-chloroacetophenone	Darmstadt , Germany
3-fluoroacetophenone	Darmstadt, Germany
3-fluoro benzaldehyde	Darmstadt, Germany
Acetophenone	Sigma chemicals Co. (St. Louis, MO, USA)
Benzaldehyde	Sigma chemicals Co. (St. Louis, MO, USA)
Methanol	Darmstadt, Germany
Ethyl acetate	Sigma chemicals Co. (St. Louis, MO, USA)
n-Hexane	Sigma chemicals Co, (St. Louis, MO, USA)
Dulbecco's Modified Eagle Medium (DMEM), supplemented with10% heat-inactivated FBS, and 100U/ml of penicillin/streptomycin Interferon-gamma (IFN-γ) 12.5 UI/ml Lipopolysacharide (LPS) 5µg/ml	Sigma chemicals Co, (St. Louis, MO, USA)
Dimethyl sulfoxide (DMSO) 0.1% 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) 0.25% trypsin-EDTA	Sigma chemicals Co, (St.Louis, MO, USA)

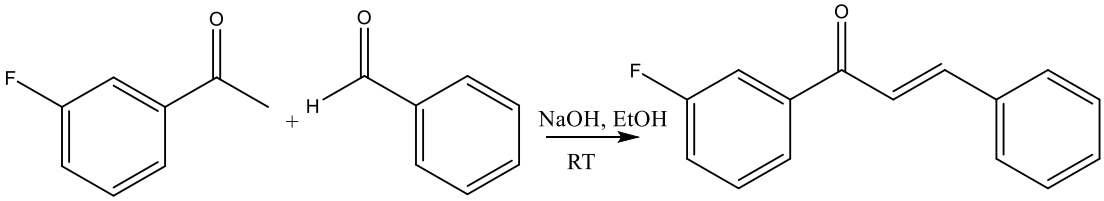
2.2 Instrumentation

The synthesized chalcones were kept in a desiccator and related melting points were determined by Electro Thermal Digital Melting point apparatus model IA 9100 (0-400) $^{\circ}C$. The IR of the products were recorded by using Perkin Elmer GX spectrophotometer in the range of 400-4000 cm^{-1} and the spectrophotometer is attached with Attenuated Total Reflectance (ATR) sample holder. Nuclear Magnetic Resonance (NMR) for ^{1}H and ^{13}C experiments were performed with Joel-ECP 400 MHz and bench top NMR (50 MHz) spectrometer using $CDCl_3$ and DMSO-d_6 as solvents. UV-visible absorption spectrum of the compounds were recorded using UV-VIS spectrophotometer using quartz cuvette. Multi-Analyte ELIS Array Kits from Qiagen were used for anti-inflammatory tests.

3. Synthetic routes

3.1 **Synthesis of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one (IV)**

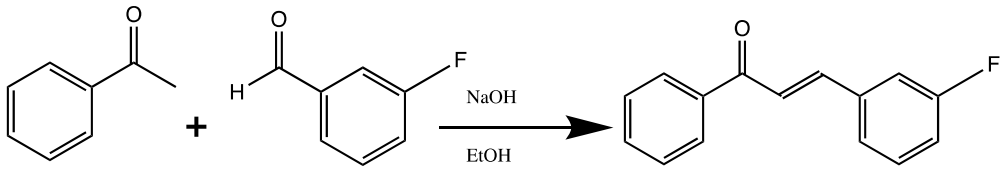
The (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one was prepared by the stirring mixture of 3-flouro acetophenone (8.57 mMol, 1.2 g), and benzaldehyde (8.57 mMol, 0.9 g) in minimum amount of ethanol at room temperature for 8 hours. After completion of reaction using then thin layer chromatography (TLC), 40% sodium hydroxide was added slowly and some residue was formed. The solid was filtered and washed with cold ethanol. The product, (E)-1-(3-chlorophenyl)-3-phenylprop-2-en-1-one, was then recrystallized from ethanol and dried using rotavap White powder was obtained in ca 25% yield Melting point; (62-63˚C) (Scheme 3.1).



Scheme 3.1. Synthesis of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one (i)

3.2 Synthesis of (E) -3-(3-fluorophenyl)-1-phenylprop-2-en-1-on

The (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-one was prepared from the stirring mixture of acetophenone (8.57 mMol, 1.2 g) and 3-flourobenzaldehyde (8.57 mMol, 1 g) in minimum amount of ethanol at room temperature for 8 hours. After completion of reaction using then thin layer chromatography (TLC), 40% sodium hydroxide was added slowly until the residue was formed. The solid was filtered and washed with cold ethanol to remove the unreacted starting materials. The product, (E)-3-(3-fluorophenyl)-1-phenylprop -2-en-1-one, was then recrystallized from ethanol and dried Yalow powder was obtained in ca 79% yield (m.p.78-89˚C). (Scheme 3.5).



Scheme 3.2. Synthesis of (E) -3-(3-fluorophenyl) -1-phenylprop-2-en-1-one (ii)

3.3 *In-vitro* anti-inflammatory studies

The *in-vitro* anti-inflammatory studies were carried out using *in-vitro* model of lipopolysaccharide and/or IFNγ- induced inflammation in RAW 264.7 cells with the objective of obtaining an insight on structure activity relationships (89, 90). All of the synthesized (E)-1-(3-chlorophenyl)-3-phenylprop-2-en-1-one, (E)-1,3-bis(3-chlorophenyl) prop-2-en-1-one, (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one, and (E)-1,3-bis(3-fluorophenyl) prop-2-en-

1-one were subjected to RAW 264.7 cells for their anti-inflammatory activities. These tests were carried out by Microbial Culture Collection Unit (UNiCC), International Medical University (IMU).

3.4 Cell culture and treatment of RAW 264.7mouse macrophages

Mouse leukaemic macrophage cells (RAW 264.7),were purchased from cell line bank of China and cultured in RPMI 1640 medium supplemented with10% fetal bovine serum, 1% of 2 mM L-glutamine, 50 IU/mL penicillin and 50 µg/mL streptomycin, under atmosphere of CO_2 (5%), at 37 °C. The cells were harvested with trypsin-EDTA and diluted to a suspension in fresh medium. In all experiments, macrophages were incubated in the presence of various concentrations of linalool, which added 1 h before LPS (1 mg/mL) stimulation(85).

Preparation of compounds:

In preparation step, concentration of compounds was calculated based on molecular weight, density and volume. DMSO was used to prepare the stock solutions, and serial dilutions in different concentrations of 100, 50, 25, 12,5, 6.25, and 3.125 µl were prepared for dose response and IC_{50}. 20 µl of DMSO 1% was prepared and transferred to the vehicle wells. Then 20 µl of compounds was transferred to each respective well. The plate was incubated at 37 °C and CO_2 (5%) for 4 hours (85,86).

Treatment with LPS:

For LPS preparation, the 10 µl of stock solution was thawed at room temperature before diluting. Then, 990 µl of complete media was added to the tube to achieve 10 µl/ml in 1 ml concentration. 20 µl of supernatant of each well except for blank and vehicle without LPS was remove 20 µl of 10 µl/ml was then added to each well to achieve 1 µl/ml of LPS inside the well. The plates were then incubated 37 °C and CO_2 (5%) for 20 hours(85,86).

3.5 Cell viability assay by MTT

The cell viability was evaluated by MTT assay (87). 100 µl of supernatant from each well was transferred to a new plate for nitrate assay. 50 µl of MTT was then added to the current plate for cell viability assay. The plate was then incubated at 37 °C and CO_2 (5%) for 3-4 hours. After about 4 hours, the wells were decanted and 100 µl of DMSO 100% was

added to each well. Purple color was observed for viable cells, and the plate was then measured at 550 and 570 nm with 630 nm as reference. The results were interpreted in percentage of cell viability based on control.

3.6 Inhibitory effect on LPSEc induced nitric oxide production

The cells were seeded in a 96- well plate with 5 × 10^3 cells/well and allowed to settle down for 24 h at 37 °C in a humidified atmosphere containing 5% carbon dioxide.Then the medium was replaced with fresh medium containing 100 ng lipopolysaccharide (LPSEc) together with various concentrations of compounds and then incubated for 48 hours. Nitric oxide (NO) production was determined by measuring the accumulation of nitrite in the culture supernatant using the Griess reagent. The absorbance of the solution at 570 nm was measured using microplate reader. The compounds that showed anti-inflammatory effect were proceed to phase 3. The standard drug used in this study was flurbiprofen.

Preparation of Griess reagent:

For preparation of Griess reagent, a 1:1 mixture of 2% naphtylethylenediaminedihydrochloride (NED) and 2% sulphanilamide were dissolved in 5% phosphoric acid at room temperature for 15-30 minutes and under dark conditions. 50 µl of sulphanilamide solution was added to the wells containing the supernatant and incubate for 5-10 minutes under dark conditions. 50 µl of NED solution was then added to the same wells and incubated for 5-10 minutes under dark conditions. Purple color (Magenta) was observed and the plate was measured at 540 nm. The results was then interpreted in percentage of inhibition of IC_{50}value(86.87).

3.6 Profiling of differential expression of cytokines/chemokines

The effect of active compounds (identified in previous level) on differential expression of cytokines and/or chemokines (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-17α, IFN-γ, TNF-α, G-CSF and GM-CSF) in cell supernatant solution was determined as per the protocol present in mouse inflammatory cytokines multi-analytic ELISA array kit from Qiagen.

4.The characterization of the compound (E)-1-(3-fluorophenyl) prop-2-en-1-one

The IR spectrum of compound (IV), (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one, also showed the presence of (C-H) stretching, C=O, C=C, (=C-H) bending and C-F

functional groups at 3030, 1683,1585, 1249 and 779 cm^{-1}, respectively as has shown in Fig 4.1.

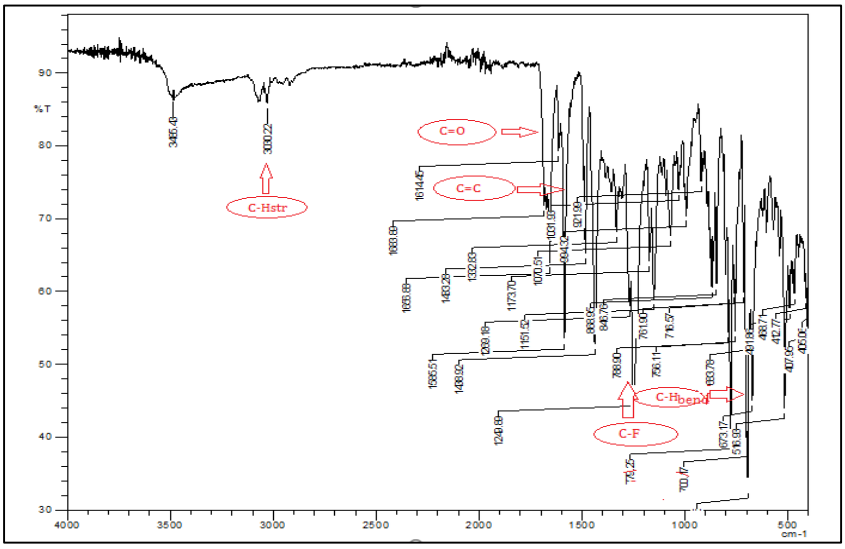


Figure 4.1. Infra-red (IR) spectrum of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one

The ^{1}H NMR of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one showed the vinyl protons at chemical shifts of 7.49 and 7.23 δ ppm, respectively. The aromatic ring protons were also observed in overlap to each other at chemical shifts of about .08, 6.97, 6.89, and 6.84 δ ppm as shown in δppm . The UV-visible spectrum of compound showed the electron transaction of π π* at maximum UV 256 nm (Figure 4.2).

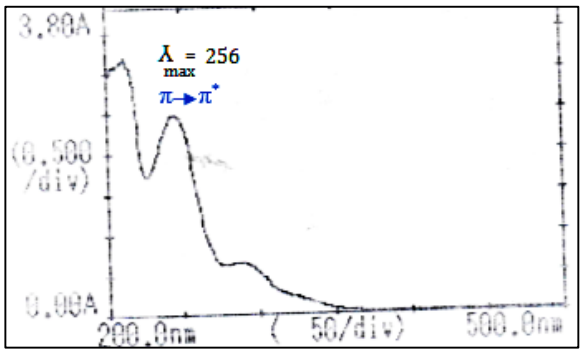


Figure 4.2UV-visible spectrum of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one

The LC-MS fraction pattern of product IV, also showed the $(M+H)^{+}$, and $(M+K)^{+}$ clustersat m/z of 228.29, 265.34, respectively (Figure 4.3).

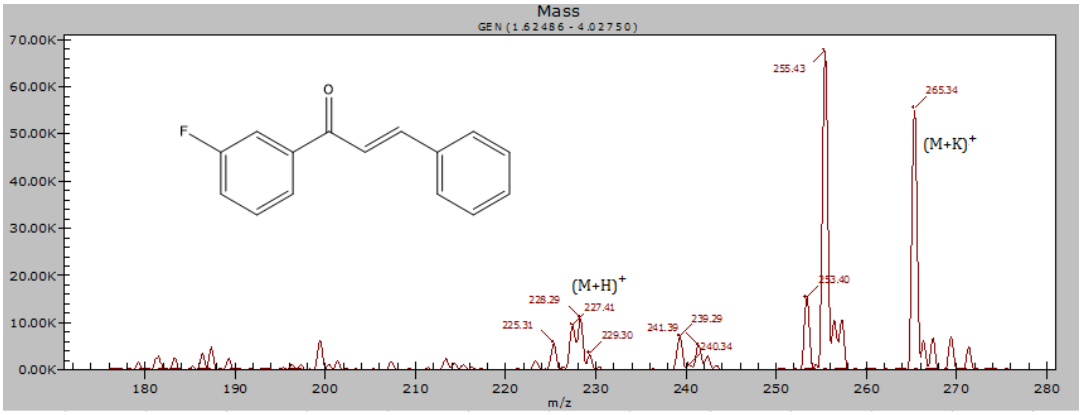


Figure 4.3LC-MS spectrum of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one (IV

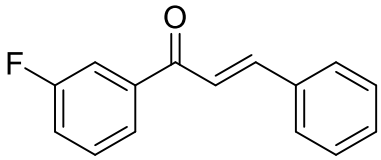


Figure 4.4Based on the above spectral data, the structure of (E)-1-(3-fluorophenyl)-3-phenylprop-2-en-1-one

4.1The characterization of the compound (E)3-(3-fluorophenyl)-1phenylprop-2-en-1-one

The IR spectrum of compound (V), (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-one, also showed the presence of $(C\text{-}H)_{stretching}$, C=O, C=C, $(=C\text{-}H)_{bending}$ and C-F functional groups at 3065, 1657,1586, 1246 and 779 cm^{-1}, respectively as has shown in Fig 4.4.

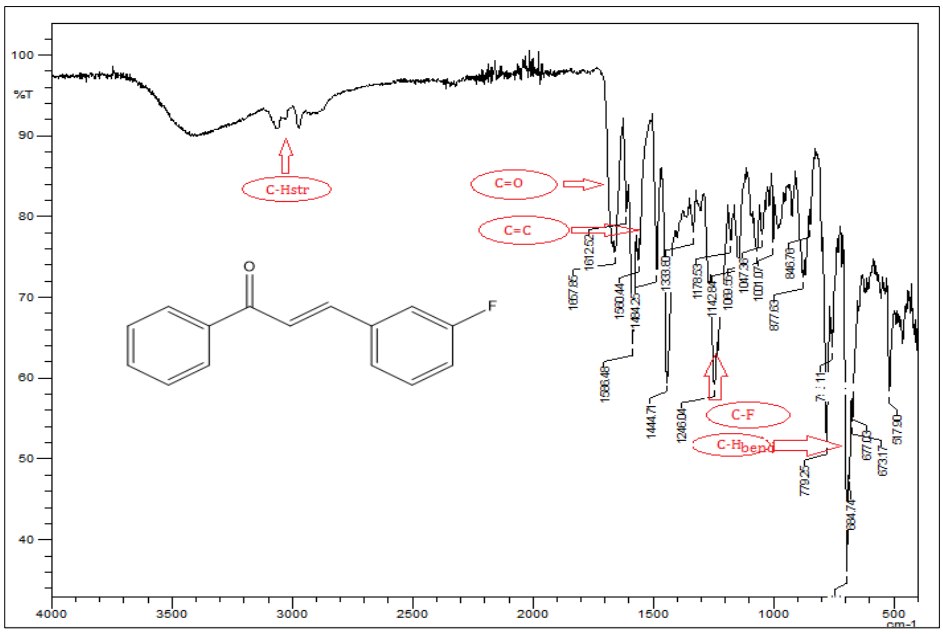


Figure 4.4Infra-red (IR) spectrum of (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-one

The ^{1}H NMR of (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-one (V) showed the vinyl protons at chemical shifts of 7.79 and 7.38 δ ppm, respectively. The aromatic rings protons were also observed in overlap to each other at chemical shifts of about7.68, 7.24, 7.11,6.81, and 6.71 δ δppm .

The UV-visible spectrum of compound (V) showed the electron transaction of $\pi \rightarrow \pi^*$ corresponds to benzoyl moiety at maximum UV band of 250 nm as shown in Figure 4.5

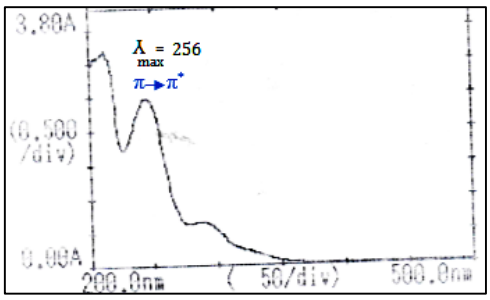


Figure 4.5UV-visible spectrum of (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-one

The LC-MS fraction pattern of product V, was in agreement with the expected structure showing $(M+H)^+$, $(M+C_2H_5)^+$, and $(M+K)^+$ clusters at m/z of 228.34 ,255.45 and 265.36, respectively (Figure 4.6).

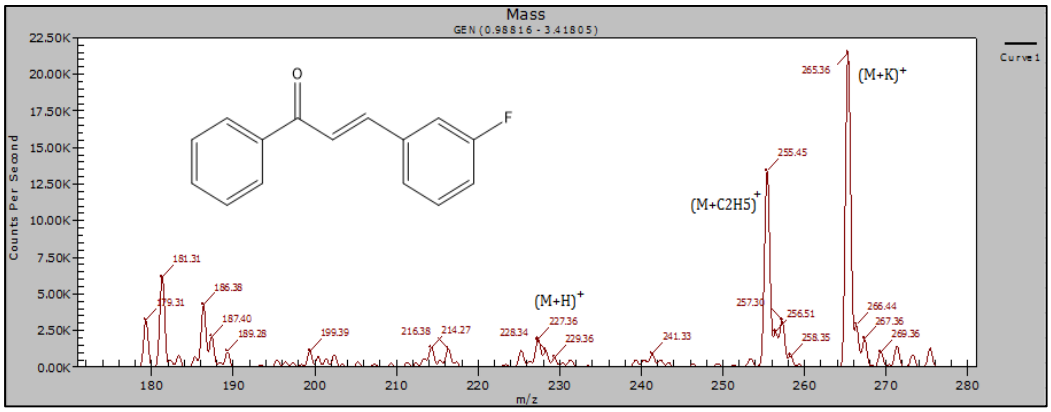


Figure 4.6LC-MS spectrum of (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-one

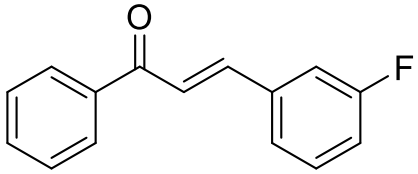


Figure 4.7Based on the above spectral data, the structure of (E)-3-(3-fluorophenyl)-1-phenylprop-2-en-1-onewas assigned.

4.2 *In vitro* anti-inflammatory activity of chalcones

4.2.1The chalcones were tested for their effect on viability of RAW 264.7 cells using MTT assay.

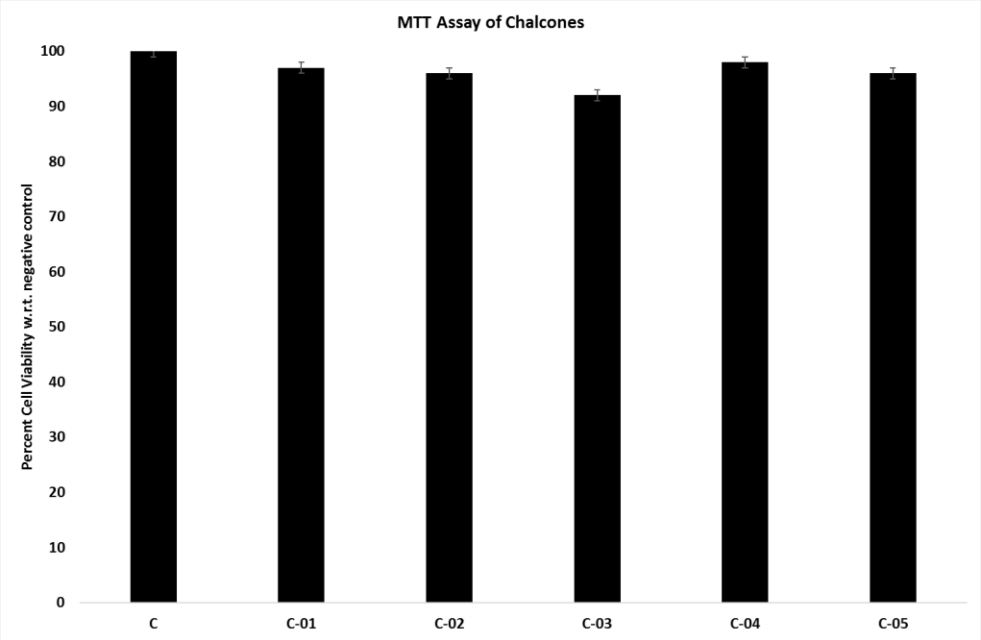


Figure 4.7Cytotoxic effect of chalcones onRAW 264.7 cell line. RAW 264.7 cells were treated with chalcones at a concentration of 100 µM.

Cell viability was determined using MTT assay. All the compounds were found to be non-toxic on RAW 264.7 cells. For all in vitro experiments, the activities of halogen substituted chalcones (C-I to C-V) were compared with unsubstituted simple chalcone (C). The MTT assay results revealed that all the chalcones at 100 µM (the highest concentration used in subsequent studies) do not decrease the cell viability of RAW 264.7 cells indicating that all the chalcones were non-toxic.

4.2.2 Nitro oxide inhibiting test

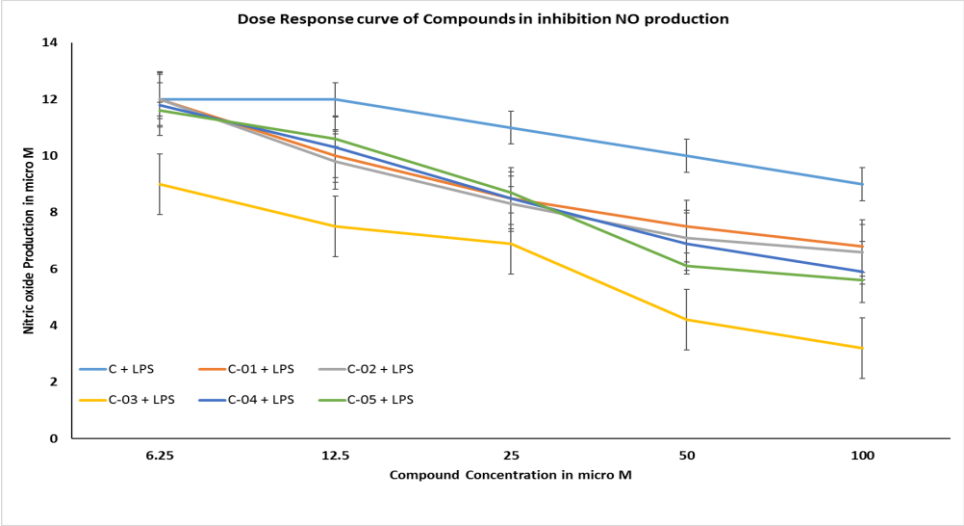


Figure 4.8*In vitro* anti-inflammatory activity chalcones in inhibiting NO production in RAW 264.7 cell line.

The chalcones (C-I to -V) were found to more active than simple chalcone (C) in reducing nitric oxide production in RAW 264.7 cells. These results indicate that halogens at meta-position on aromatic rings of chalcones have positive influence on their anti-inflammatory activity. Among mono-substituted chalcones, fluorine showed positive influence than chlorine in inhibiting NO production in RAW 264.7 cells. Disubstitutedchalcone (C-III) was found to be more potent than mono-substituted chalcones. The IC_{50} values of chalcones were shown in the following Table 4.2

Chalcone	IC_{50} value
C	> 100 μM
C-01	> 100 μM
C-02	> 100 μM
C-03	29.7 μM
C-04	75 μM
C-05	73 μM

Table 4.1 IC_{50} values of chalcones in inhibiting NO production by RAW 264.7 cells

From the Table 4.1, it is clearly evident that chalcones substituted with mono- substituted fluorine and disubstituted chlorine were found to be more potent than simple chalcone (C). Since di-chlorsubstitutedchalcone (C-III) was found to be more potent, it's influence on the expression cytokines were determined using multi-analytie ELISA kit from Qiagen whose results were shown in Fig 4.29.

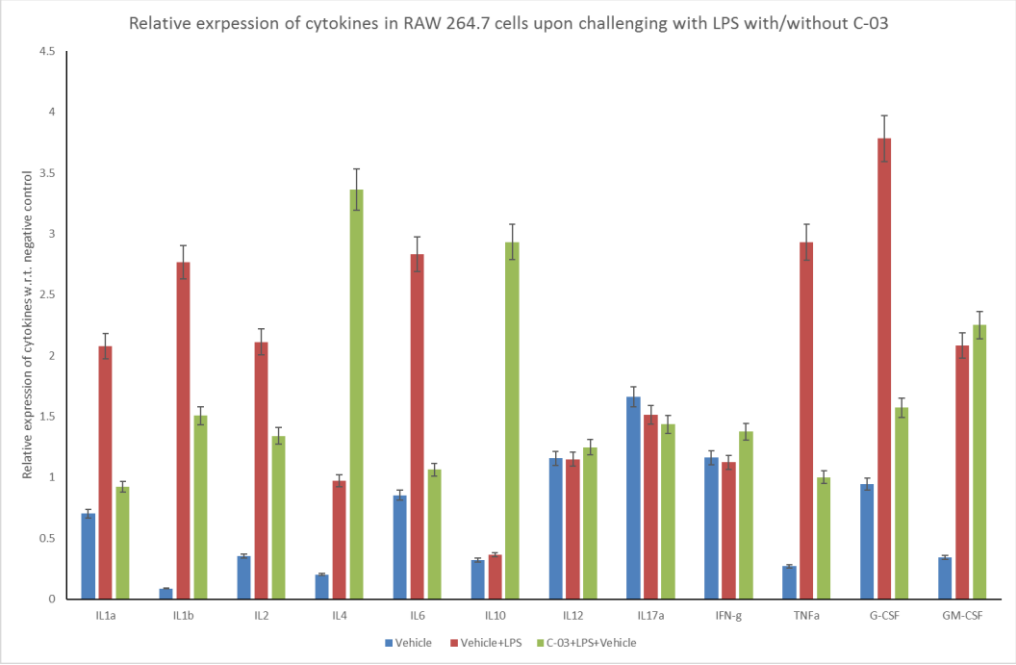


Figure4.9Relative expression of cytokines in RAW 264.7 cells treated with LPS with and without disubstitutedchalcone (C-III)

Interleukin (IL)-1α, IL-1β, IL-2, IL-6, TNF-α, G-CSF and GM-CSF are pro-inflammatory cytokines. IL-4, IL-10, IFN-γ and anti-inflammatory cytokines. The remaining cytokines IL-12 and IL-17α could be either pro- or anti-inflammatory cytokines. From the figure 4.24; it is clearly evident that C-03 at a concentration of 25μM reduced the expression of pro-inflammatory cytokines (IL-1α, IL-1β, IL-2, IL-6, TNF-α and G-CSF). The chalcone C-III at a concentration also increased expression of anti-inflmmatory cytokines such as IL-4 and IL-10. The chalcone C-III does not possess any effect on expression of cytikines, IL-12, IL-17α and IFN-γ.

CONCLUSION

In the present study, five chalcones were synthesized in which the halogens (Cl and F) were substituted at meta positions on aromatic rings. All the compounds were purified well by using column chromatography technique, and then characterised using physical and spectral data such as ^{1}H and ^{13}C NMR, Mass and FTIR spectra. Furthermore, *in vitro* anti-inflammatory activity of chalcones were determined in RAW 264.7 mouse macrophage cells using Greiss reagent. The in vitro anti-inflammatory activity of chalcones were compared with simple chalcone to determine the influence of halogens on anti-inflammatory activity. The results showed that the halogen substitution at meta-positions on aromatic rings improved the anti-inflammatory activity of chalcones, the order of activity being di-chloro substituted >monofluoro substituted >monochloro substituted > simple chalcone. The toxicity of chalcones were determined using MTT assay and the chalcones were found to be non-toxic against RAW264.7 cells at the concentration of 100 μM, the highest concentration used in *in vitro* assays. The dichloro-substituted chalcone (C-III) was found to be the most potent and tested for its influence on expression of cytokines using multianalyte ELISA array kit. The chalcone, C-III (E)-1,3-bis(3-chlorophenyl)prop2-en-1one, reduced the expression of a few pro-inflammatory cytokines, increased the expression of a few anti-inflammatory cytokines and few more cytokines were unaffected.

FUTURE WORK RECOMMENDATIONS

The aim of this study was to synthesise meta-halogen substituted chalcones and test their influence on *in vitro* anti-inflammatory activity and differential expression of cytokines. The limited time and funding available only five chalcones could be synthesised. Further work should be carried out to synthesise more chalcones to obtain meaningful structure activity relationship of chalcones in eliciting anti-inflammatory activity. The metabolic stability of chalcones and their efficacy in *in vivo* inflammatory model should also be carried out.

REFERENCES:

1. Khatib S, Nerya O, Musa R, Shmuel M, Tamir S, Vaya J. Chalcones as potent tyrosinase inhibitors: the importance of a 2, 4-substituted resorcinol moiety. Bioorganic & Medicinal Chemistry. 2005;13(2): 433-41.
2. Nowakowska Z. A review of anti-infective and anti-inflammatory chalcones. European Journal of Medicinal Chemistry. 2007; 42(2):125-37.
3. Go M, Wu X, Liu X. Chalcones: an update on cytotoxic and chemoprotective properties. Current medicinal chemistry. 2005;12(4): 483-99.
4. Syam S, Abdelwahab SI, Al-Mamary MA, Mohan S. Synthesis of chalcones with anticancer activities. Molecules. 2012;17(6):6179-95.
5. Ducki S. The development of chalcones as promising anticancer agents. Drugs. 2007;10(1):
6. Tatsuzaki J, Bastow KF, Nakagawa-Goto K, Nakamura S, Itokawa H, Lee K-H. Dehydrozingerone, Chalcone, and Isoeugenol Analogues as *in Vitro* Anticancer Agents#. Journal of Natural Products. 2006; 69(10): 1445-9.
7. Boumendjel A, Ronot X, Boutonnat J. Chalcones derivatives acting as cell cycle blockers: potential anti cancer drugs? Current Drug Targets. 2009;10(4): 363-71.
8. Sashidhara KV, Kumar A, Kumar M, Sarkar J, Sinha S. Synthesis and *in vitro* evaluation of novel coumarin–chalcone hybrids as potential anticancer agents. Bioorganic &Medicinal Chemistry Letters. 2010; 20(24): 7205-11.
9. Bandgar BP, Gawande SS, Bodade RG, Gawande NM, Khobragade CN. Synthesis and biological evaluation of a novel series of pyrazole chalcones as anti-inflammatory, antioxidant and antimicrobial agents. Bioorganic & Medicinal Chemistry. 2009; 17(24): 8168-73.
10. Bandgar BP, Gawande SS, Bodade RG, Totre JV, Khobragade CN. Synthesis and biological evaluation of simple methoxylated chalcones as anticancer, anti-inflammatory and antioxidant agents. Bioorganic & Medicinal Chemistry. 2010; 18(3): 1364-70.
11. Hsieh H-K, Lee T-H, Wang J-P, Wang J-J, Lin C-N. Synthesis and anti-inflammatory effect of chalcones and related compounds. Pharmaceutical research. 1998;15(1):39-46.
12. Hsieh H-K, Tsolt, Wang JP, Lin Cn. Synthesis and Anti-inflammatory Effect of Chalcones. Journal of Pharmacy and Pharmacology. 2000; 52(2): 163-71.
13. Liaras K, Geronikaki A, Glamočlija J, Ćirić A, Soković M. Thiazole-based chalcones as potent antimicrobial agents. Synthesis and biological evaluation. Bioorganic & Medicinal Chemistry. 2011;19(10):3135-40.
14. Solankee A, Kapadia K, Ćirić A, Soković M, Doytchinova I, Geronikaki A. Synthesis of some new S-triazine based chalcones and their derivatives as potent antimicrobial agents. European Journal of Medicinal Chemistry. 2010; 45(2): 510-8.

15. Siddiqui ZN, Musthafa TM, Ahmad A, Khan AU. Thermal solvent-free synthesis of novel pyrazolyl chalcones and pyrazolines as potential antimicrobial agents. Bioorganic & Medicinal Chemistry Letters. 2011; 21(10): 2860-5.
16. Choudhary AN, Juyal V. Synthesis of chalcone and their derivatives as antimicrobial agents. Int Pharmaceut Sci. 2011; 3:125-8.
17. Selway JT. Antiviral activity of flavones and flavans. Progress in Clinical and Biological Research. 1986; 213: 521.
18. El-Subbagh HI, Abu-Zaid SM, Mahran MA, Badria FA, Al-Obaid AM. Synthesis and biological evaluation of certain α, β-unsaturated ketones and their corresponding fused pyridines as antiviral and cytotoxic agents. Journal of Medicinal Chemistry. 2000; 43(15): 2915-21.
19. Binder D, Noe C, Holzer W, Rosenwirth B. Thiophene as a structural element of physiologically active substances. 12. Thiophene analogs of antiviral chalcones. Archiv der Pharmazie. 1985;318(1):49.
20. Tadigoppula N, Korthikunta V, Gupta S, Kancharla P, Khaliq T, Soni A, et al. Synthesis and insight into the structure–activity relationships of chalcones as antimalarial agents. Journal of Medicinal Chemistry. 2012; 56(1): 31-45.
21. Ferrer R, Lobo G, Gamboa N, Rodrigues J, Abramjuk C, Jung K, et al. Synthesis of [(7-chloroquinolin-4-yl) amino] chalcones: potential antimalarial and anticancer agents. Scientia Pharmaceutica. 2009; 77(4): 725-42.
22. Ohnogi H, Sugiyama K, Enoki T, Kobayashi E, Sagawa H, Kato I. Chalcone Compounds. Google Patents; 2009.
23. Visavadiya NP, Narasimhacharya A. Ameliorative effects of herbal combinations in hyperlipidemia. Oxidative Medicine and Cellular Longevity. 2011; 2011.
24. Raj CGD, Sarojini BK, Bhanuprakash V, Yogisharadhya R, Swamy BEK, Raghavendra R. Studies on radioprotective and antiviral activities of some bischalcone derivatives. Medicinal Chemistry Research. 2012;21(9):2671-9.
25. Wilson C. Synthesis and Characterisation of Chalcones. J Asian Med chem. 1938;61:2303.
26. Claisen L, Claparede A. Ber., 1881, 14, 2463; b) Claisen L. Ber; 1887.
27. Datta S, Murtiv, Seshadri T. Syenthesis of 2'-Methoxyflavanones. Natl Inst Science Commumnation Dr Ks Krishnan Marg,New Delhi 110 012, INDIA; 1971. p. 614-&.
28. Makrandi J, Kumar S. An Efficient Synthesis of 2'-Hydroxychalcones. Asian Journal of Chemistry. 2004;16(2):1189.
29. Lin W-W, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. The Journal of Clinical Investigation. 2007; 117(5): 1175-83.
30. Rojas J, Payá M, Dominguez JN, Ferrandiz ML. The synthesis and effect of fluorinated chalcone derivatives on nitric oxide production. Bioorganic & MedicinalChemistry Letters. 2002; 12(15): 1951-4.
31. Ferrero-Miliani L, Nielsen O, Andersen P, Girardin S. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. Clinical & Experimental Immunology. 2007; 147(2): 227-35.
32. Ortolan XR, Fenner BP, Mezadri TJ, Tames DR, Corrêa R, de Campos Buzzi F. Osteogenic potential of a chalcone in a critical-size defect in rat calvaria bone. Journal of Cranio-Maxillofacial Surgery. 2014; 42(5): 520-4.
33. Sharma J, Al-Omran A, Parvathy S. Role of nitric oxide in inflammatory diseases. Inflammopharmacology. 2007;15(6):252-9.
34. Laveti D, Kumar M, Hemalatha R, Sistla R, GM Naidu V, Talla V, et al. Anti-inflammatory treatments for chronic diseases: a review. Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy). 2013;12(5):349-61.

35. Deshpande AM, Argade NP, Natu AA, Eckman J. Synthesis and Screening of a Combinatorial Library of Naphthalene Substituted Chalcones: Inhibitors of Leukotriene B 4. Bioorganic & Medicinal Chemistry. 1999; 7(6): 1237-40.
36. Perozo-Rondón E, Martín-Aranda RM, Casal B, Durán-Valle CJ, Lau WN, Zhang X, et al. Sonocatalysis in solvent free conditions: An efficient eco-friendly methodology to prepare chalcones using a new type of amino Grafted Zeolites. Catalysis today. 2006; 114(2): 183-7.
37. Bohm BA. Introduction to flavonoids: Harwood Academic Publishers; 1998.
38. Chopra PG. Chalcones: A brief review. International Journal of Research in Engineering and Applied Sciences. 2016; 6(5): 173-85.
39. Narender T, Reddy KP. A simple and highly efficient method for the synthesis of chalcones by using borontrifluoride-etherate. Tetrahedron Letters. 2007; 48(18): 3177-80.
40. Jayapal M, Sreedhar N. Anhydrous K_2CO_3 as Catalyst for the Synthesis of Chalcones under Microwave Irradiation. 2010.
41. Jayapal M, Sreedhar N. Synthesis and characterization of 4-hydroxy chalcones by Aldol condensation using SOCl2/EtOH. Int J Curr Pharm Res. 2010;2(4):60-2.
42. Brun E, Safer A, Carreaux F, Bourahla K, L'Helgoua'Ch J-M, Bazureau J-P, et al. Microwave-Assisted Condensation Reactions of Acetophenone Derivatives and Activated Methylene Compounds with Aldehydes Catalyzed by Boric Acid under Solvent-Free Conditions. Molecules. 2015; 20(6): 11617-31.
43. Rao YK, Fang S-H, Tzeng Y-M. Synthesis and biological evaluation of 3′, 4′, 5′-trimethoxychalcone analogues as inhibitors of nitric oxide production and tumor cell proliferation. Bioorganic & medicinal chemistry. 2009;17(23):7909-14.
44. Rahman MA. Chalcone: A valuable insight into the recent advances and potential pharmacological activities. Chemical Sciences Journal. 2011.
45. Aoki N, Muko M, Ohta E, Ohta S. C-geranylated chalcones from the stems of Angelica keiskei with superoxide-scavenging activity. Journal of Natural Products. 2008; 71(7): 1308-10.
46. Modzelewska A, Pettit C, Achanta G, Davidson NE, Huang P, Khan SR. Anticancer activities of novel chalcone and bis-chalcone derivatives. Bioorganic & Medicinal Chemistry. 2006; 14(10): 3491-5.
47. Boumendjel A, Boccard J, Carrupt P-A, Nicolle E, Blanc M, Geze A, et al. Antimitotic and antiproliferative activities of chalcones: forward structure–activity relationship. Journal of Medicinal Chemistry. 2008; 51(7): 2307-10.
48. Vogel S, Heilmann Jr. Synthesis, cytotoxicity, and antioxidative activity of minor prenylated chalcones from Humulus lupulus. Journal of Natural Products. 2008; 71(7): 1237-41.
49. Enoki T, Ohnogi H, Nagamine K, Kudo Y, Sugiyama K, Tanabe M, et al. Antidiabetic activities of chalcones isolated from a Japanese herb, Angelica keiskei. Journal ofAgricultural and Food Chemistry. 2007; 55(15): 6013-7.
50. Bashir R, Ovais S, Yaseen S, Hamid H, Alam M, Samim M, et al. Synthesis of some new 1, 3, 5-trisubstituted pyrazolines bearing benzene sulfonamide as anticancer and anti-inflammatory agents. Bioorganic & Medicinal Chemistry letters. 2011; 21(14): 4301-5.
6(15): 7167-76.
57. Lorenzo P, Alvarez R, Ortiz MA, Alvarez S, Piedrafita FJ, de Lera AnR. Inhibition of IκB kinase-β and anticancer activities of novel chalcone adamantyl arotinoids. Journal of Medicinal Chemistry. 2008; 51(17): 5431-40.
58. Nowakowska Z, Kędzia B, Schroeder G. Synthesis, physicochemical properties and antimicrobial evaluation of new (E)-chalcones. European journal of Medicinal Chemistry. 2008; 43(4): 707-13.

59. Sivakumar PM, Ganesan S, Veluchamy P, Doble M. Novel chalcones and 1, 3, 5-triphenyl-2-pyrazoline derivatives as antibacterial agents. ChemicalBiology & Drug Design. 2010; 76(5): 407-11.
60. Kumar D, Kumar NM, Akamatsu K, Kusaka E, Harada H, Ito T. Synthesis and biological evaluation of indolyl chalcones as antitumor agents. Bioorganic & Medicinal Chemistry letters. 2010; 20(13): 3916-9.
61. Mahapatra DK, Bharti SK. Therapeutic potential of chalcones as cardiovascular agents. Life Sciences. 2016; 148: 154-72.