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Chalcone Derivatives: Synthesis and Cytotoxicity Assays

Asraa H. Ahmed[1], Ahmed Ahmed[1], Haikel Jelassi[2]

[1]Department of Chemistry, College of Science, Al-Nahrain University, Jadriah, Baghdad, Iraq.
[2]Laboratory on Energy and Matter for Nuclear Sciences Development, Tunisia. National Center for Nuclear Sciences and Technologies, Sidi Thabet Technopark 2020, Ariana, Tunisia.

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Abstract

The research included the formation of chalcone derivatives, which are organic materials with biological importance, on which this research is based. The Chalcone derivatives were synthesised from the Claisen-Schmidt condensation reaction. Compounds I-III were synthesised by reacting p-aminoacetophenone with (4-methylbenzaldehyde, 2,3-dimethylbenzaldehyde, and cinnamaldehyde), respectively, in an ethanolic solution of KOH (30%). Chalcones were characterised by Fourier transform infrared spectroscopy (FT-IR) and proton nuclear magnetic resonance (1H NMR). The cytotoxicity effect was demonstrated in human melanoma cells A-375 and HdFn normal cells, and the MTT results showed that the cytotoxicity of Chalcone derivatives was greater than HdFn cells. The ability to use Chalcone derivatives as sunscreen showed a promising result.

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*Corresponding author: ahmed.ahmed@nahrainuniv.edu.iq

1. Introduction

Chalcones are a large class of natural compounds found widely in fruits, vegetables, spices, tea, and soy-based foods. They are thought to be progenitors of flavonoids and isoflavonoids [1]. Most chalcone moieties' biological characteristics and distinctive conjugated molecular architecture have generated significant interest [2-4]. Many of the chalcone derivatives have important pharmacological properties, such as analgesic [5], arthritis [6], anti-inflammatory [7], anti-malarial [8], anti-fungal [9], and anti-cancer [10]. Chalcone compounds are characterized by their ability to absorb ultraviolet rays, which allows them to be used as sunscreen agents [11]. Chalcones is an aromatic ketone consisting of two aromatic rings united through a three-carbon α, ß-unsaturated carbonyl system [12]. The IUPAC name is 1,3-diphenyl-2-propen-1-one derivative [13-15]. It is readily produced by reacting acetophenone derivatives with benzaldehyde in an alkaline Claisen-Schmidt condensation process [16-18]. The characteristics of chalcones depend on the presence of α, ß-unsaturated groups and appropriately substituted groups on the ring [19].
In this work, we synthesized three chalcone derivatives and the cytotoxic effect of Chalcone derivatives C (I, II, and III) was evaluated against different cell lines: A-375 (a human melanoma cell line (epithelial melanoma) initiated through explant culture of a solid tumour from a 54-year-old female) [20] and the normal HdFn cells (the Human Dermal Fibroblast of the Neonate (HDFn) is a human normal cell line isolated from the Neonate (HDFn) is a human normal cell line isolated from neonatal foreskin that has research applications) [21] was carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay method. [22,23].

2. Materials and Methods

All chemicals used in the research were supplied by international companies and used without purification. MTT Kit (Intron Biotech (Korea)), FT-IR register with an 8300 spectrophotometer Shimadzu at a frequency ranging from 400 to 4000 cm1 in the laboratories of the Chemistry Department of the College of Science at AL-Nahrain University, 1H-NMR spectroscopy in DMSO-d6 was measured using a Bruker 400 MHz NMR spectrometer at Sharif University of Technology, Department of Chemistry/Iran. The melting points of all compounds were determined by using the

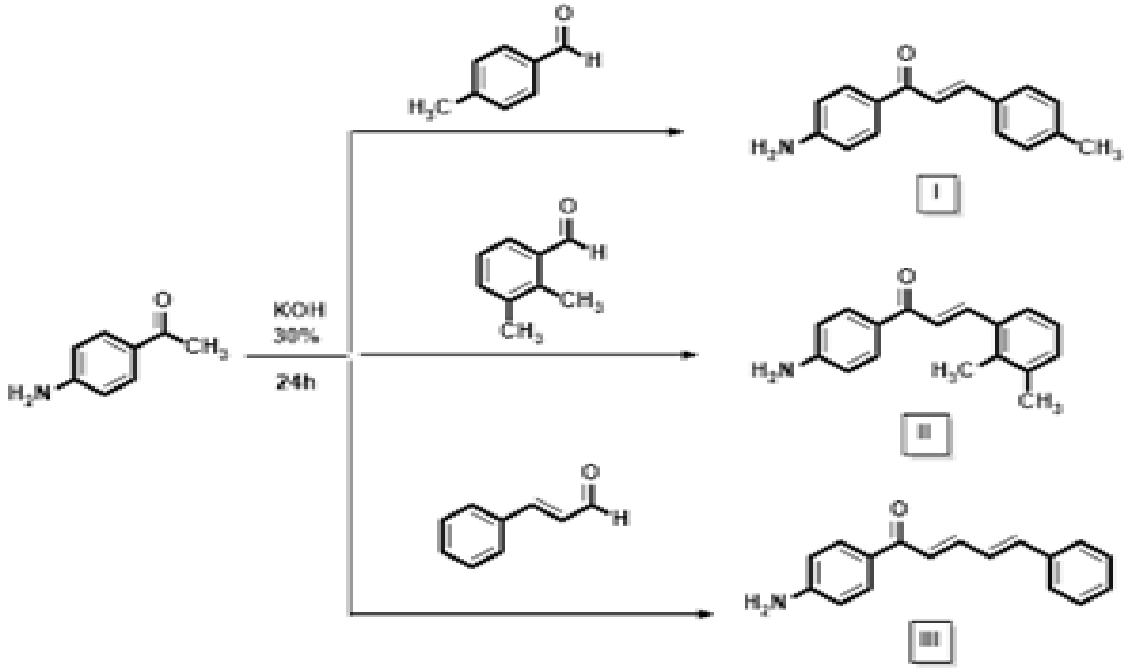
digital melting point (stone, Staffordshire, ST15 0SA, UK) in the laboratories of the Department of Chemistry, College of Sciences, AL-Nahrain University, an inverted microscope (Olympus (Japan), a CO2 incubator (Gallenkamp (England), laminar Laminar flow (K and K (Korea), a microtiter plate reader (Bio-Rad (Germany), a sensitive balance (Sartorius (Germany), a hemocytometer (Sigma (USA), and a thermofisher (Japan).

3. Theory and Formula

3.1. Experimental Work

3.1.1. Synthesis of Chalcone derivatives (I, II, III):

A mixture of p-aminoacetophenone (0.25g, 0.00184 mol) with aldehyde (0.3 ml, 0.00184 mol) in an ethanolic solution of KOH (30%) was stirred overnight at room temperature. The solution was kept in the refrigerator for 2 to 3 hours. The mixture was then poured into ice-cold water and acidified with diluted hydrochloric acid (20%). The product formed was filtered and washed with hot ethanol.



Scheme 1: Chemical structure of chalcone derivatives I, II, III.

3.2. Cytotoxicity Assays

The cytotoxicity of Chalcone derivatives was assessed using the MTT test at various concentrations: 25, 50, 100, 200, and 400 µg/mL. The investigation employed normal (non-cancerous) HdFn cells and the human A-375 melanoma cancer cell line. 1640 Medium—Roswell Park Memorial Institute (RPMI)—a 100-ml ready-to-use package. This trial used RPMI the entire time. As shown by the manufacturer, L-glutamine and 4-(2-hydroxyethyl)-1piperazine-ethane sulfonic acid (HEPES) were previously added to the medium. The following components were added to the medium to finish it: streptomycin (0.001 g), sodium bicarbonate (1%), and penicillin G (103 IU). 1.5 × 103 cells per well in 96-well plates were cultured in a humidified incubator at 37 °C with 5% CO2. The colourimetric Microculture Tetrazolium Assay (MTT) method was employed to evaluate the cytotoxic effects of the substances produced. The cultured cells' regular media was removed, and 200 ml of fresh medium containing different sample concentrations (25, 50, 100, 200, and 400 g/ml) was added. The cells were then incubated at 37 °C with 5% CO2. After 24 hours, the MTT reagent was added to each well, and the mixture was incubated for 4 hours. The next step was to add 100 mL of DMSO to each well. Using a smaller plate to measure absorbance at 575 nm, MTT degeneration to formazan was detected. ELISA spectrophotometers (Thermofisher, Japan) were utilized to determine the optical density (OD) in every well. Every experiment was conducted in triplicate, and the mean value, expressed as IC50 ± SD, was found [24].

4. Results and Discussion

Chalcone derivatives were prepared using the Claessen-Schmidt condensation method, as shown

in Scheme 1. Table 1 shows some of the physical properties of the Chalcone derivatives I, II, III.

Table1: Physical properties of Chalcone derivatives I, II, III.

No.	IUPAC names	M.p °C	Color
I	(E)-1-(4-aminophenyl)-3-(p-tolyl)prop-2-en-1-one	167-169	Yellowish white
II	(E)-1-(4-aminophenyl)-3-(2,3-dimethylphenyl)prop-2-en-1-one	100-102	Dark red
III	(2E,4E)-1-(4-aminophenyl)-5-phenylpenta-2,4-dien-1-one	83-85	red

4.1. Infrared Spectra

The infrared spectra (FT-IR) of compounds I, II, and III were recorded with an FT-IR register with an 8300 spectrophotometer at Shimazu. The essential investigative bands were identified in Table 2 and Figures 1, 2, 3, 2, and 3. The FT-IR spectra of chalcones I, II, and III accord a strong band at 1640–1655 cm-1, which belongs to the conjugated carbonyl group of the aromatic ring. Another strong band belongs to the aliphatic carbon-carbon double bond and appeared at 1590–1600 cm-1. The appearance of this peak is the result of the reaction of acetophenone with aldehyde to form the CO-CH=CH- ketoethylenic group of Chalcone compounds. The symmetrical and asymmetrical vibration bands of NH2 appear at (3350–3220) cm-1 and disappear from the peak of the aldehyde.

Table 2: Chalcone derivatives I, II, and III FT-IR spectrum data.

No.	NH_2 (Sym & asym)	C-H (aromatic)	C-H (Aliphatic)	C=C (Aromatic)	C=C (Aliphatic)	C=O
I	3293-3353	3020	2855-2908	1507	1593	1654
II	3225-3354	3001	2835-2926	1499	1590	1642
III	3237-3355	3022	2909-2971	1490	1590	1652

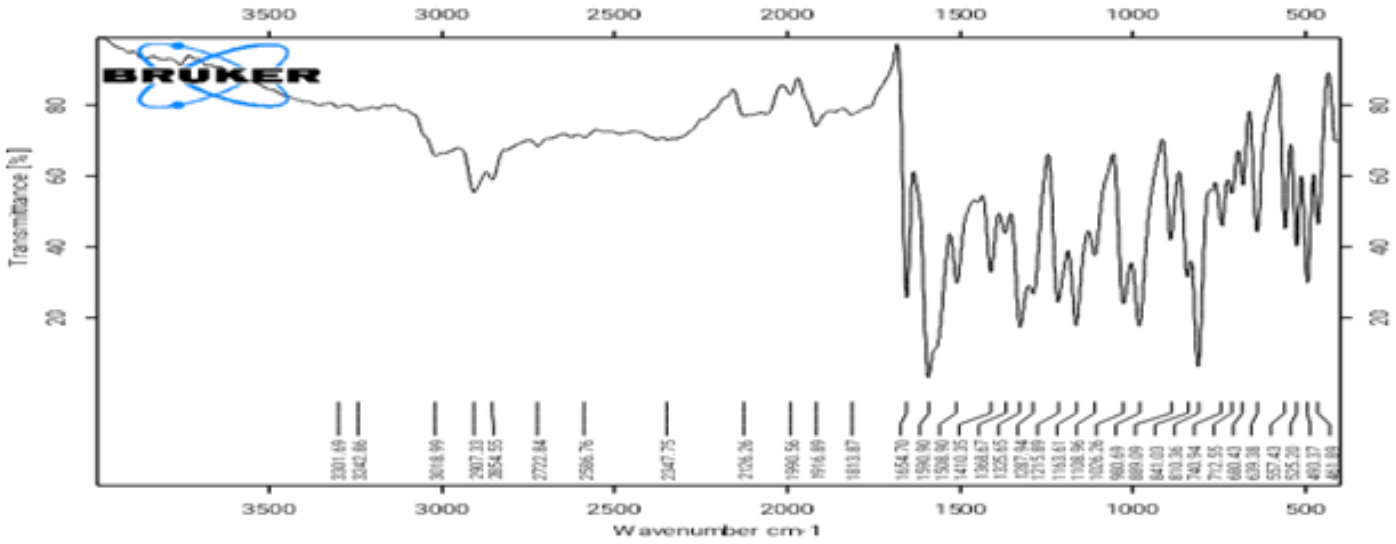


Figure 1: FT-IR of compound I.

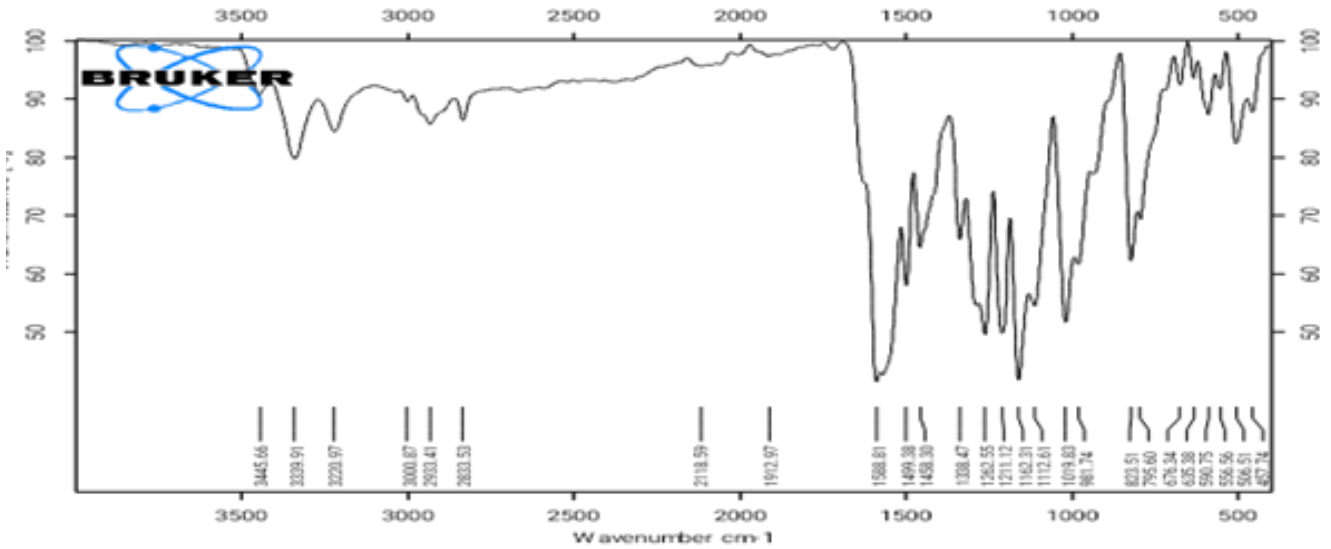


Figure 2: FT-IR of compound II.

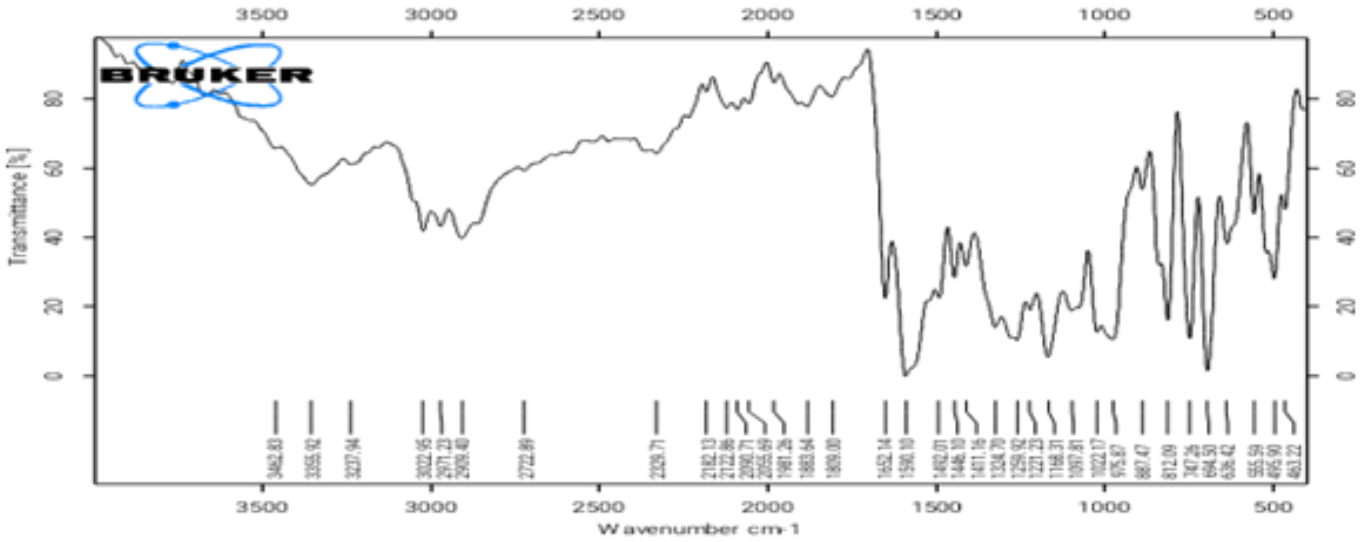


Figure 3: FT-IR of compound III.

Table 3: ^{1}H NMR spectrum data of Chalcone derivatives I, II and III.

No.	Data (δ/ppm)
I	6.62(d,1H, CO=CH), Doublet 1H of 8.24(d,1H, CH=CH-Ar) 3.40(s,2H, NH2), 6.62-7.77(m, 8H, Ar-H), 2.42(s,3H, CH3-Ph
II	7.68(d,1H, CO=CH), 7.92(d,1H, CH=CH-Ar), 3.87(s,2H, NH2), 6.59-7.71(m,5H, Ar-H), 2.30-2.40(s,2H, CH3-Ar)
III	7.23(d,1H, CO=CH), 7.80(d,1H, CH=CH-Ar, 4.14(s,2H, NH2), 6.62-7.64(m,9H, Ar-H)

4.2. ^{1}H NMR spectra of Chalcone derivatives

The 1H NMR spectrum of Chalcone derivatives, as shown in Table 3 and Figures 4, 5, and 6, showed the presence of a doublet proton bound to C=C-Ar, which belongs to the ketoethylenic group of chalcones at 7.80, 7.92, and 8.24 δ/ppm, and at 6.62, 7.23, and 7.68 δ/ppm, the appearance of a doublet proton linked to the CO=CH carbonyl group of Chalcone derivatives.

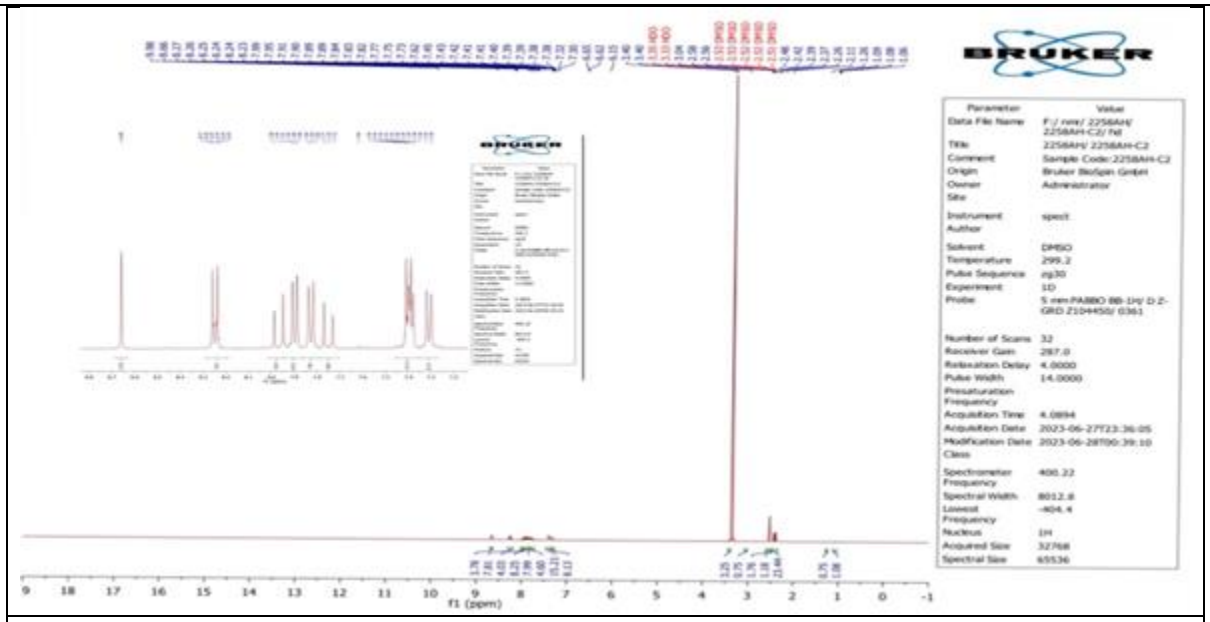


Figure 4: ^{1}H NMR of compound I.

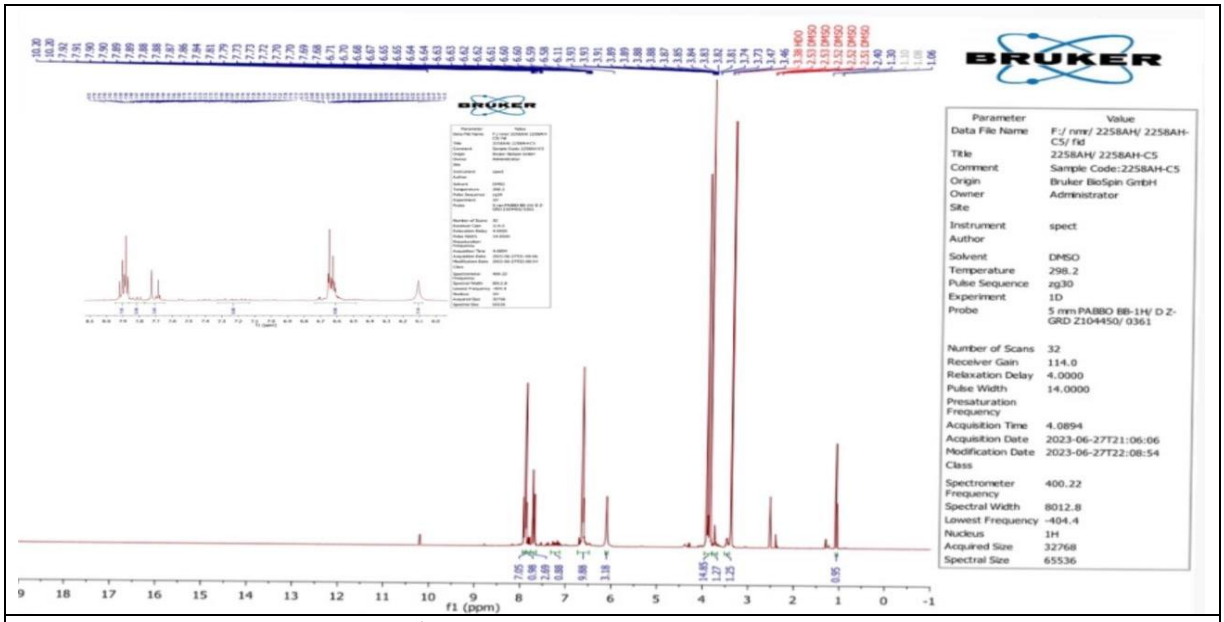


Figure 5: ^{1}H NMR of compound II.

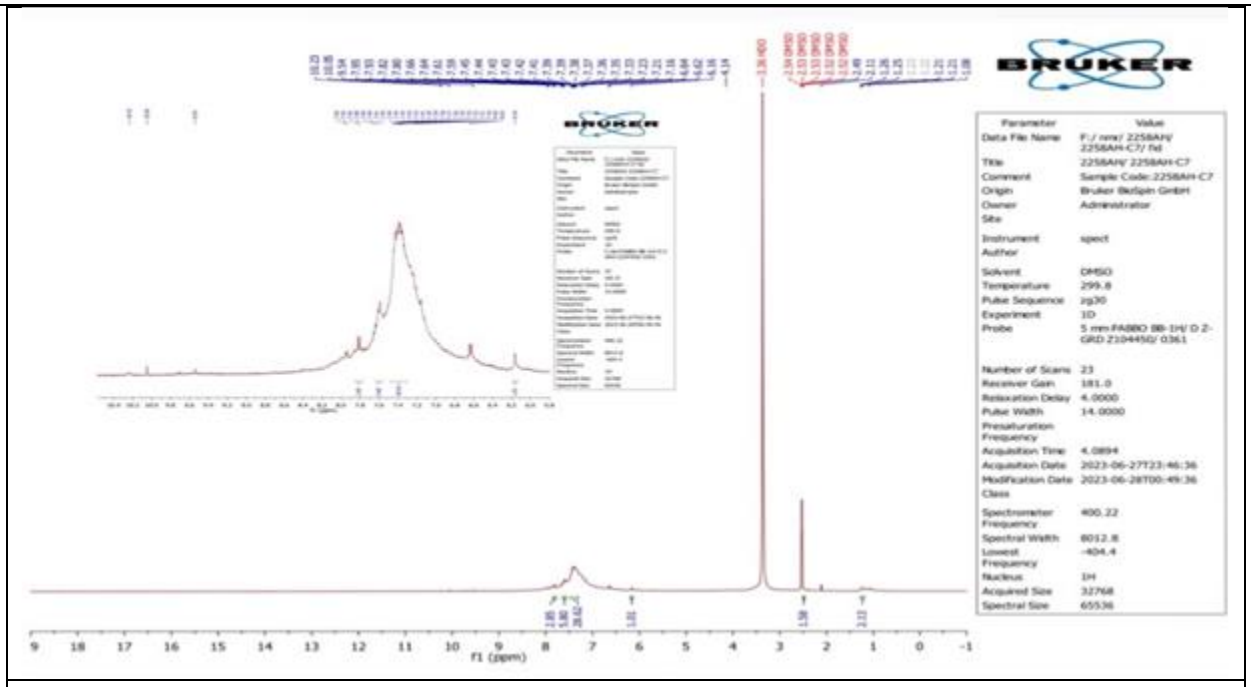


Figure 6: ^{1}H NMR of compound III.

4.3. The Cytotoxicity Assay (MTT) of Chalcone Derivative Compounds

The cytotoxic effect of Chalcone derivatives C (2,5,7) was evaluated against different cell lines (A-375) and normal HdFn cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method. The cell viability was evaluated after 24 hours and treated with different concentrations of each synthesised derivative (25, 50,50,100,200 and 400 µg/ml). The results demonstrate a dose-dependent manner of behaviour on both cell lines. These derivatives were selected based on their inhibition results on Hdfn and A-375 and could be considered the most promising sunscreen agents [25, 26]. The cytotoxicity effect of (I), the inhibition half IC50 values in the instance of normal cells HdFn, as shown in Fig. 7, is 481.1 µg/mL. This suggests a large I concentration is required to kill half of the healthy cells. Still, the cancer cells in A-375 require a significantly lower concentration—33.41 µg/mL—because there are notable variations between HdFn and A-375 cell inhibition, which begins at 25 µg/mL cons., the killing percentage cells (100-96.95=3.05%) for HdFn cell and increase into (100-72.26=23.74%) at 400 µg/mL, while the killing percentage cells for A-375 is (100-86.72=13.28%) at 25 µg/mL rise into (100-42.97=57.03) at 400 µg/mL. Table 4. Fig. 7.

Table 4: Cytotoxicity effects of compound I against A-375 tumor cell line and normal cell line HDFn:

I	HDFn	A-375
Conc. (µg/ml)	Mean ± SD	Mean ± SD
400	72.26 ± 3.52	42.97 ± 4.46
200	85.22 ± 2.27	51.66 ± 2.98
100	92.20 ± 2.77	59.68 ± 1.54
50	96.48 ± 1.29	73.96 ± 1.73
25	96.95 ± 1.14	86.73 ± 1.30

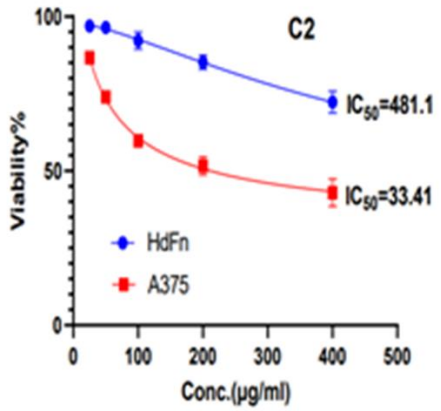


Figure 7: Cytotoxicity effect of compound I on A-375 cells and HDFn cells after incubation for 24 hours at 37°C (Log for the original concentration).

The cytotoxicity effect for II was measured at 400, 200, 100, 50, and 25 µg/mL. The killing percentage for A-375 cells was 38.04, 31.68, 17.98, 7.53, 5.29%, and 28.05, 23.31, 15.67, 13.93, 4.79% for HDFn. The IC50 of A-375 was 121.6, as shown in Table 5. The data suggests that a high concentration of (II) is required to kill half of the melanoma cells, but the IC50 of HDFn is 132.5 for normal cells. As Figure 8 illustrates, the cytotoxicity impact of II was found to be concurrent for both tumour A-375 cells and normal HDFn cells.

Table 5: Cytotoxicity effects of compound II against A-375 tumor cell line and normal cell line HDFn:

II	HDFn	A-375
Conc. (µg/ml)	Mean ± SD	Mean ± SD
400	71.95± 0.81	61.69 ± 4.22
200	76.69 ± 2.60	68.32 ± 2.49
100	84.33 ± 2.66	82.02 ± 3.90
50	86.07 ± 1.91	92.47 ± 3.81
25	95.21 ± 0.82	94.71 ± 0.43

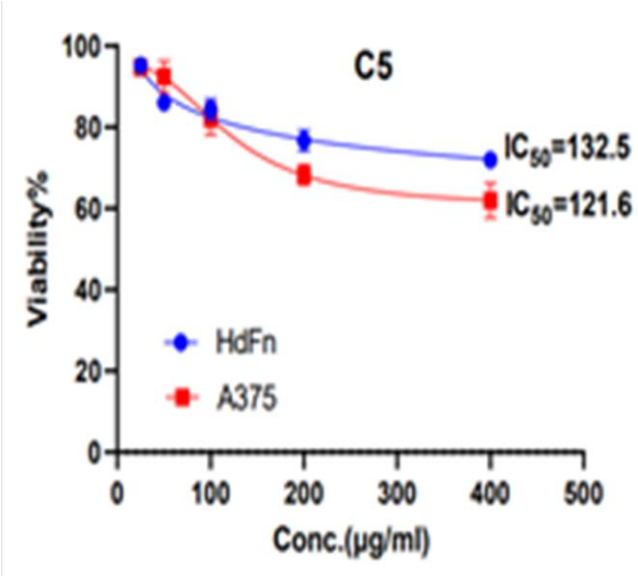


Figure 8: Cytotoxicity effect of compound II on A-375 cells and HDFn cells after incubation for 24 hours at 37 C (Log for the original concentration).

The cytotoxicity effect of III on A-375 cells and HDFn cells was also measured. By MTT assay, the percentage of the killing tumour cells A-375 was (72.34, 60.88, 45.99, 35.31, 4.97%), while the rate of the killing normal cells HDFn was (37.74, 34.03, 28.86, 13.7, .63%). Fig 9. Table 6. shows that the IC50 of A-375 is 64.05, a high concentration of III kills half of melanoma cells, and the IC50 of HDFn is 139.9.

Table 6: Cytotoxicity effects of compound III against A-375 tumor cell line and normal cell line HDFn

III	HDFn	A-375
Conc. (µg/ml)	Mean ± SD	Mean ± SD
400	62.26± 4.62	27.66 ± 2.27
200	65.97 ± 1.10	39.12 ± 3.15
100	71.14 ± 1.83	54.01 ± 3.23
50	86.30 ± 3.74	64.69 ± 4.71
25	95.37 ± 0.90	75.03 ± 4.98

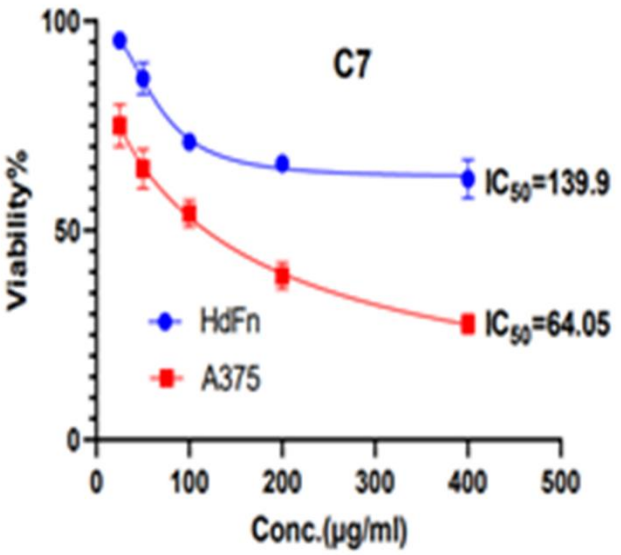


Figure 9: Cytotoxicity effect of compound III on A-375 cells and HDFn cells after incubation for 24 hours at 37ºC (Log for the original concentration).

5. Conclusions

The Chalcone derivatives I, II, and III have been prepared and structurally characterized. All compounds showed significant cytotoxicity against melanoma A-375 cell lines and were compared to normal human cell lines (HdFn). According to the previously reported results, I and III show a good cytotoxicity effect on tumour A-375 cells and normal HDFn cells. Still, II has a weak cytotoxicity effect on cells, which is remarkable. In conclusion, the synthesized compounds have the potential to be developed as novel sunscreen agents.

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