Original Research Article | J Adv Med Biomed Res. 2025; 33(Special Issue-162): 110-122

Special Issue: Second International Conference for Pharmaceutical Sciences (SICPS 2025), University of Misan, Maysan, 62001, Iraq

Journal of Advances in Medical and Biomedical Research | ISSN:2676-6264

Design, Synthesis, In Silico Studies, and Pharmacological Evaluation of New Chalcone Derivatives as Anticancer and Antioxidant Agents

Zainab Y. Kadhim[1*], Sarah A. Al-khafaji[2], Rusul N. Mankhi[3], Manar A. Abddulameer[4]

1. *Department of Physiology, College of Veterinary Medicine, Al-Muthanna University, Samawah, Iraq*
2. *Department of Microbiology, College of Veterinary Medicine, Al-Muthanna University, Samawah, Iraq*
3. *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Misan, Maysan, Iraq*
4. *Department of Sciences, College of Basic Education, Al-Muthanna University, Samawah, Iraq*

Article Info

10.30699/jambr.33.162.110

Received: 2025/10/08;
Accepted: 2025/11/19;
Published Online: 29 Dec 2025;

Use your device to scan and read the article online

***Corresponding author:**
Rusul N. Mankhi,
Department of Pharmaceutical Chemistry, College of Pharmacy, University of Misan, Maysan, Iraq

Email: rusul.naaem@uomisan.edu.iq

ABSTRACT

Background & Objective: Chalcones are promising compounds in the pharmaceutical field due to their antioxidant and anticancer properties. The aim of this study was to synthesize two novel chalcone derivatives (A_1 and A_2) and evaluate their biological activities, including antioxidant potential and cytotoxicity against cancer cells.

Materials & Methods: The chemical structures of compounds A_1 and A_2 were confirmed using spectroscopic techniques including Proton Nuclear Magnetic Resonance (^{1}H-NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Ultraviolet-Visible (UV-Vis) spectroscopy. A hemolysis assay was conducted to assess biocompatibility. Antioxidant activity was measured using the DPPH radical scavenging assay at various concentrations (12.4-1000µg/ml). Cytotoxicity was evaluated against human breast cancer cells (MCF-7).

Results: Both A_1 and A_2 exhibited low hemolytic activity (4.09% and 3.99% respectively, at 100µg/ml), indicating good biocompatibility. Compound A_1 exhibited more potent antioxidant activity than A_2. Cytotoxicity assays demonstrated that both compounds were more toxic to MCF-7 cancer cells, with IC_{50} values for the produced compounds A1, A2, and Tamoxifen were 34.67µg/ml, 28.34µg/ml, and 15.48µg/ml, respectively, indicating potential selective anticancer activity.

Conclusion: Compounds A_1 and A_2 exhibited promising antioxidant and anticancer properties, with minimal hemolytic effects and selective toxicity toward cancer cells, making them potential candidates for further pharmaceutical development.

Keywords: Sulfonamide Chalcone Derivatives, Antioxidant, Hemolysis, In silico Docking, GC-MS, 1H-NMR, UV-Vis, MCF-7 Breast Cancer

1. Introduction

Chalcones are naturally occurring α, β-unsaturated aromatic compounds widely distributed in plants, from ferns to higher plants (1). They are known for diverse biological activities, including cytotoxicity (2), analgesic, antipyretic, and anti-inflammatory effects (3), as well as antibacterial (4), antifungal, insecticidal (5), and antimutagenic properties (6). Structurally, chalcones consist of two aromatic rings linked by an, β-unsaturated carbonyl group, existing primarily as the E isomer (7, 8), and can be synthesized both naturally and artificially (9, 10). Modern green chemistry approaches, such as microwave-assisted synthesis, enable thw rapid and efficient preparation of chalcone derivatives with high yield and purity (11-16).

Despite the well-documented biological activities of chalcones, a need remains for the systematic design and evaluation of new derivatives with enhanced anticancer and antioxidant properties, particularly those targeting breast cancer cell lines. In this context, the current study focuses on the synthesis of two novel chalcone derivatives, A_1 and A_2, and the assessment of their anticancer, antioxidant, and hemolytic activities, addressing the gap in correlating structural modifications with biological efficacy.

2. Materials and Methods

2.1 General Experiment Information

In this study, chalcone derivatives were synthesized from *N*-(4-acetylphenyl) methanesulfonamide and various aldehydes, whereas terephthalaldehyde and 1*H*-pyrrole-2-carbaldehyde. This study was conducted at the Research Laboratory of Chemistry, Department of Physiology, College of Veterinary Medicine, and University of Al-Muthanna. ^{1}H-NMR spectra were recorded using a BRUKER spectrophotometer (300MHz) in the Laboratories of the College of Engineering in the Islamic Republic of Iran. Tetramethylsilane (TMS) is the standard liquid for expressing chemical alteration values in δ (ppm), and used with Dimethylsulfoxide-d_6 as a solvent. At Sharif University of Technology in Tehran, Iran, researchers utilized an Agilent Technologies mass selective detector model 5973 to get the GC-MS spectra at 70 eV.

2.2 Synthesis of (mono-bis) Chalcones (A1, A2) (17)

(A) Traditional method: Equimolar amount of the reaction between 0.01 moles of *N*-(4-acetylphenyl) methanesulfonamide, 0.01 moles of various aldehyde in 30 ml of ethanol, and a catalytic amount of sodium hydroxide (10%) produced the chalcones (A_1, A_2). With a magnetic stirrer, the mixture was mixed at 25 ºC for (4 hours A_1, 6 hours A_2). After observing the reaction, vacuum evaporation is used to remove the solvent. The material was mixed with ice-cold water in a glass. The precipitate was also collected via filtration. Lastly, the products were recrystallized by a suitable solvent.

(B) The use of microwaves to irradiate method: Equal quantity (0.01 mol) of 0.01 moles of various aldehyde, ethanol in small amounts (3 ml) was used for mixing and dissolving 0.01 moles. Aqueous sodium hydroxide (10%) was then added and blended with this solution. The entire reaction mixture was exposed to 180 watts of microwave radiation for roughly 2 minutes A_1, and 6 minutes A_2. This is a list of all chalcone derivatives' spectrum data:

***N,N'*-(3,3'-(1,4-phenylene)bis(acryloyl))bis(4,1-phenylene))dimethansulfonami de(A_1)**

Conventional method yielded 79%, microwave irradiation yielded 89%, it was made by reacting terephthalaldehyde (0.01mole, 1.34gm) compound with *N*-(4-acetylphenyl) methanesulfonamide (0.02mole, 2.13gm), m.p=148-149^{0}C using methanol, recrystallize.

***N*-(4-(3-(1H-pyrrol-2-yl) acryloyl)phenyl)methanesulfonamide(A_2)**

Conventional method yield 88%, microwave irradiation yield 91%, *N*-(4-acetylphenyl)methanesulfonamide, (0.01mole, 2.13gm) is synthesized by treating it with 1*H*-pyrrole-2-carbaldehyde(0.01mole, 0.95gm), m.p=184-186^{0}C using chloroform, recrystallize.

2.3 Hemolysis Assay

The samples of healthy human blood were centrifuged at 1350 rpm for 10 minutes. To achieve the concentration of 2% w/v RBCs, the normal saline concentration (0.9%) was used for washing the (RBCs), equal amounts of distilled water and 0.9% normal saline (positive control) and the suspension was spread out using a 2% RBC suspension (1.25m) (negative control) A_1 and A_2 Compounds at a concentration (25, 50, 75, 100µg/ml) were incubated at 37^{0}C for three hours with (1.25ml) of normal saline and (1.25ml) of 2% RBCs **(18)**. At (900 rpm), the sample was centrifuged for 12min, and the hemolytic ratio (HR) was evaluated by using the supernatant. Liberated hemoglobin concentration was valued by scaling the absorbance of the supernatant solutions at 538nm. The following equation was used for calculating HR **(19)**:

$$\text{Hemolysis (\%)} = (D_s - D_n)/(D_w - D_n) \times 100$$

D_s: the absorbance of the sample

D_n: the absorbance of the saline

D_w: the absorbance of the distilled water

2.4 Antioxidant Action

Amount of 2ml of the compound solution prepared was mixed at a concentration of (12.4, 50, 200, 400, 750, 800, 1000µg/ml) with 2ml of a solution of (2, 2-Dipheny-l-Picrylhydrazyl) (DPPH) prepared at a concentration of (0.004g) 40ppm in 100ml of a mixture of methanol and dimethyl sulfoxide in a ratio of 1:1) and the mix was left in the dark for a while. After two hours, the absorbance was recorded using a spectrometer in the visible and ultraviolet regions at a wavelength of 517nm **(20)**. Positive control was used as a standard solution, which is Ascorbic acid **(21)**. The action of the compounds was expressed as a percentage of inhibition of free radicals using the following equation was calculated.

DPPH radical scavenging activity (%) = (Abs control - Abs sample/ Abs control) × 100

Were

Abs $_{\text{Control}}$ =absorbance of DPPH radical + methanol: DMSO (1:1)

Abs $_{\text{Sample}}$ = absorbance of DPPH radical + sample

2.5 Docking Studies

Computational docking studies were performed using MOE (Molecular Operating Environment, version 2015). The crystal structure of EGFR (PDB ID: 1XKK) was retrieved from the Protein Data Bank https://www.rcsb.org/. Water molecules and the co-crystallized ligand were removed, and hydrogen atoms were added. The protein structure was energy-minimized using the MMFF94x force field to relieve steric clashes.

Ligands (chalcone derivatives A_1 and A_2) were prepared via energy minimization and generation of multiple conformations. Docking was performed using

the Triangle Matcher placement method, and the top 10 poses were evaluated using the London dG scoring function. The best-scoring pose for each ligand was selected for further interaction analysis, including hydrogen bonds, hydrophobic interactions, and π–π stacking with key active site residues.

2.6 *In Vitro* Anti-breast Cancer Activity

MCF-7 cells (human breast cancer cell line) were obtained from the National Cell Bank of Iran (Pasteur Institute, Iran) and cultured in RPMI-1640 medium (Gibco) supplemented with 10% FBS and antibiotics (100 U/mL penicillin, 100 µg/mL streptomycin) at 37 °C in 5% CO_2. Cells were passaged using trypsin/EDTA and PBS. For cytotoxicity assessment, the MTT assay was employed. Briefly, cells were seeded in 96-well plates at 1.4×10^4 cells/well and allowed to attach for 24 h. Cells were then treated with compounds at concentrations of 6.25–400 µg/mL for 24 h. Following treatment, 200 µL of MTT solution (0.5 mg/mL in PBS) was added and incubated for 4 h at 37 °C. The supernatant was removed, and chalcone compounds were solubilized in 100 µL DMSO. Absorbance was measured at 570 nm using an ELISA reader (BioTek, USA). IC_{50} values were calculated from dose-response curves (22).

3. Result

3.1 Chemistry

Chalcone compound characterization

Chalcones are a family of polyphenolic chemicals obtained from plants called flavonoids. According to studies, several chalcones exhibit a range of cytoprotective and modulatory properties that may have therapeutic value in treating various disorders. Their physicochemical characteristics appear to determine how active they are biologically. (Mono-bis) Chalcones (A_1, A_2) are synthesized from *N*-(4-acetylphenyl) methanesulfonamide, various aldehydes, and the presence of sodium hydroxide (10%) (23); as shown in Figure 1. Chalcone is produced using the Claisen-Schmidt condensation process, which involves an acid or base catalyst-assisted cross-aldol condensation of aldehydes and ketones, followed by a dehydration reaction (24). The general mechanism of these reactions is described in Figure 2.

3.2 Spectroscopic Studies

3.2.1 ^{1}H-NMR spectral for (mono-bis) chalcones (A_1, A_2)

Some representative ^{1}H-NMR spectra of the (mono-bis) chalcones (A_1, A_2), as shown in Figures 3 and 4. The ^{1}H-NMR spectra of A_1 showed a signals singlet at chemical shift δ (*s*, 3.35ppm, 6H) for protons of the methyl group (CH_3). Also showed a singlet signal at chemical shift δ (*s*, 12.63ppm, 1H) for amine proton (NH). It exhibited a doublet signal at δ 7.63(2H, *d*, *J*=9Hz, CO=CH), 8.20(1H, *d*, *J*=9Hz, Ar-C=H). Finally, the spectrum showed multiple signals at chemical shift δ (*m*, 7.51-7.87ppm, 12H) for aromatic proton (25). The ^{1}H-NMR spectra of A_2 showed signals singlet at chemical shift δ (*s*, 3.47ppm, 3H) for protons of the methyl group (CH_3), and showed a singlet signal at chemical shift δ (*s*,10.28ppm, 1H) for protons amine (NH). Also showed a singlet signal at chemical shift δ (*s*, 10.32ppm, 1H) for amine proton (NH). It exhibited a doublet signal at δ 7.54(1H, *d*, *J*=9Hz, CO=CH), 7.72(1H, *d*, *J*=9Hz, Ar–C=H), finally the spectrum showed signals multiple at chemical shift δ(*m*, 6.86-7.67ppm, 12H) for aromatic proton (26).

3.2.2 Mass spectra for (mono-bis) chalcones (A_1, A_2)

The mass spectrum of *N,N'*-(3,3'-(1,4-phenylene)bis(acryloyl))bis(4,1-phenylene))dimethanesulfonamide(A_1). Showed the molecular ion peak at m/z=524, and shown the important fragmentation peaks in 65.1m/z, 92.1m/z, 119.1m/z, 165.1m/z, 214.1m/z, 257.1m/z, 331.1m/z, 364.1m/z, 399.1m/z (27). The mass spectrum of *N*-(4-(3-(1*H*-pyrrol-2-yl)acryloyl)phenyl)methanesulfonamide (A_2). showed the molecular ion peak at m/z=290 shown the important fragmentation peaks in 51.1m/z, 77.1m/z, 104.1m/z, 121.1m/z, 148.0m/z, 167.0m/z, 195.0m/z, 207.1m/z , 240.0m/z (28). As shown in Figures 5 and 6.

3.2.3 UV–Vis spectra for (mono-bis) chalcones (A_1, A_2)

The functional group (-C=O-CH=CH-) usually absorbs at the wavelength range from (208-328) nm (n-π*, π-π*), as shown in Figures 7 and 8. The electronic spectrum of compound A_1 exhibits transitions at 342.50, 304.50, and 293.50nm, respectively; these bands may be due to n-π* of (-C=O-CH=CH-) and π-π* transition. The electronic spectrum of A_2 also exhibits three transitions at 319.00, 302.00, and 266.50nm; all these transitions may be due to n-π* and π-π*, respectively (29).

3.3. Thin-layer Chromatography (TLC) for (mono-bis) chalcones (A_1, A_2)

TLC was used to determine the end of the synthesis of chalcones was identified using TLC, and the value of R_f was calculated every 30 minutes. Each response comes from a single, obvious channel.

3.4. Hemolysis Study for (mono-bis) chalcones (A1, A2)

The amount of hemoglobin released from the RBCs was used to assess the hemolysis ratio of synthetic chalcone derivatives (30). Compounds A_1 and A_2 had hemolysis ratio percentages of 4.09% and 3.99%, respectively, at a concentration of 100µg/ml. All chalcones had hemolysis ratio percentages of less than 10%; this finding suggests that using them in the human body in this form is safe. As shown in Table 1 and Figure 9.

3.5 Antioxidant activity for (mono-bis) chalcones (A_1, A_2)

It is common practice to test the capacity of synthetic compounds to function as free radical scavengers and to gauge antioxidant activity using the standard and stable

free radical known as DPPH (31). Accepting an electron or hydrogen lowers the DPPH intensity. DPPH radical scavenging assays are used to measure the in vitro radical scavenging capacities of A_1 and A_2. By turning the initial deep purple DPPH solution into a yellow tint, we could determine how active the samples were when they were less active. As shown in Table 2, the antioxidant activity of the samples is assessed and contrasted with that of the reference antioxidant ascorbic acid. A_1 exhibits 89.78-30.78%, A_2 exhibits 77.45-25.99%, and conventional ascorbic acid exhibits 98.67-89.78% at concentrations of 12.4-1000µg/ml. When compared to the conventional vitamin C, the aforementioned reported activity is lower than that of the A_1 and A_2 (32). Figure 10 displays the results for the DPPH free radical scavenging activity of the A_1, A_2, and standard (sorbic).

3.6 Docking Studies of (mono-bis) chalcones (A_1, A_2)

To determine whether chemicals may bind to a specific protein (PDB: 1XKK) and thereby prevent breast cancer, the software MOE 2015 was utilized. Wethen tested these compounds in vitro, as they showed vigorous activity against the examined protein. Protein binding to these produced compounds is seen in Figure 11 and Table 3.

3.7 In Vitro Anti-breast Cancer Activity for (mono-bis) chalcones (A_1, A_2)

The present study demonstrates that the growth inhibition percentage gradually increases with rising concentrations of the three compounds (Tamoxifen, A_1, A_2) as shown in Table 4, indicating a directly proportional relationship between concentration and anticancer activity (33). Tamoxifen exhibited high efficacy across all tested concentrations, achieving over85% inhibition at 100 µg/ml and exceeding 97% even at the lowest concentration (6.25 µg/ml). This is expected, as Tamoxifen is a well-known and effective drug against MCF-7 cells and was used here as a positive control. Compound A_1 demonstrated potent inhibitory activity that increased with concentration, ranging from 47.45% at 400 µg/ml to 96.17% at 6.25 µg/ml. Notably, its efficacy at lower concentrations became very close to that of Tamoxifen, suggesting the potency and high cytotoxicity of this compound against cancer cells (34).

Compound A_2 showed lower efficacy than A_1 at most concentrations; however, it still possessed a notable effect, achieving an inhibition percentage of 95.87% at 6.25 µg/ml. This indicates that both compounds possess anticancer properties, with a slight superiority of A_1 at medium and low concentrations (35), as shown in Figure 12.

Table 1. The results of the hemolysis ratio (%) for (mono-bis) chalcones (A_1, A_2) at different concentrations.

No	HR (%)			
	25µg/ml	**50µg/ml**	**75µg/ml**	**100µg/ml**
A_1	0.45%	0.99%	2.01%	3.99%
A_2	0.63%	1.85%	2.67%	4.09%

Table 2. DPPH Radical Scavenging Activity of the Synthesized Compound.

No	% RSA (radical scavenging activity) at seven different concentrations(µg/ml)						
	12.4µg/ml	**50µg/ml**	**200µg/ml**	**400µg/ml**	**750µg/ml**	**800µg/ml**	**1000µg/ml**
A_1	30.78	44.90	63.12	66.45	76.78	85.65	**89.78**
A_2	25.99	38.06	43.98	50.43	57.89	67.31	**77.45**
Sorbic	88.56	89.56	91.45	93.34	95.23	96.91	**98.67**

Table 3. Docking parameters of the binding of (mono-bis) chalcone compounds with the Epidermal Growth Factor Receptor [EGFR] protein.

No	ID	Binding Energy ($kcalmol^{-1}$)	rmsd-refine	E(kal/mol)	Interaction	Distance	Bonding
A_1	1XKK	-9.4854	0.6929	-4.0	H-acceptor	2.79	Ala722: N Lig: O
				-0.7	Pi-H	4.53	Val726: CG1 Lig: 6-ring
A_2	1XKK	-9.0573	1.7203	-2.0	H-acceptor	2.99	Cys797: N Lig: O

Table 4. Growth inhibition percentages of Anticancer and cytotoxicity (MCF-7 cell line) against different concentrations of (mono-bis) chalcones (A_1, A_2) compounds.

Conce.	MCF-7 cell line		
	Tamoxifen	A_1	A_2
	Mean± SD	Mean± SD	Mean± SD
400µg/ml	97.72 ±0.99b	96.17 ±0.41d	95.87 ±0.35d
200µg/ml	96.60 ±1.04b	95.33±0.68d	89.78 ±4.85d
100µg/ml	95.33 ±2.88b	94.60±3.05d	75.12 ±5.01c
50µg/ml	94.14±1.11b	84.68±2.47c	63.27 ±2.89b
25µg/ml	85.38 ±1.18b	64.12±3.57b	55.48±1.47b
12.50µg/ml	73.15±4.56a	53.55±0.47a	50.50±2.67a
6.25µg/ml	67.94±2.03a	47.45±4.63a	42.71±2.77a

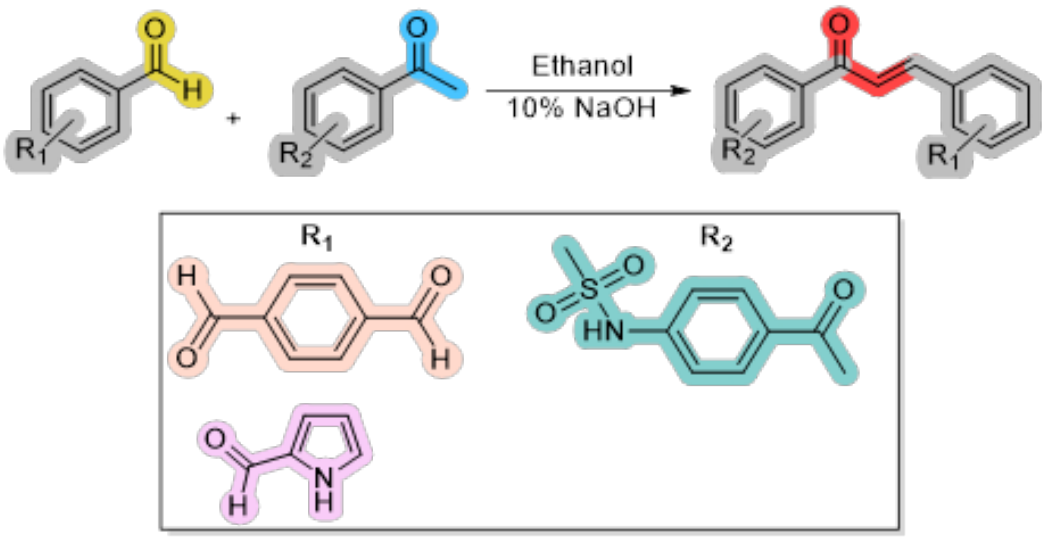

Figure 1. Synthesis of (mono-bis) chalcones (A_1, A_2) (**Prepared by Authors, 2025**).

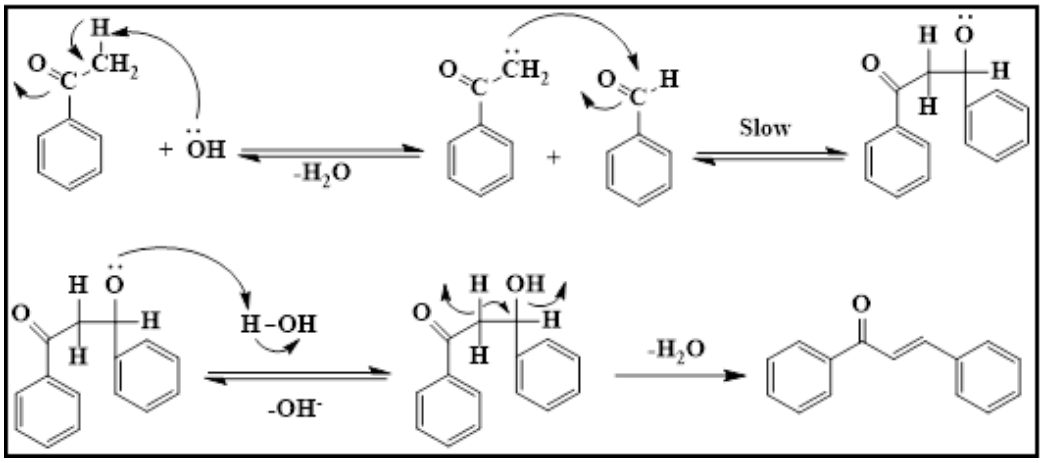

Figure 2. Mechanism of formation of chalcones (**Prepared by Authors, 2025**).

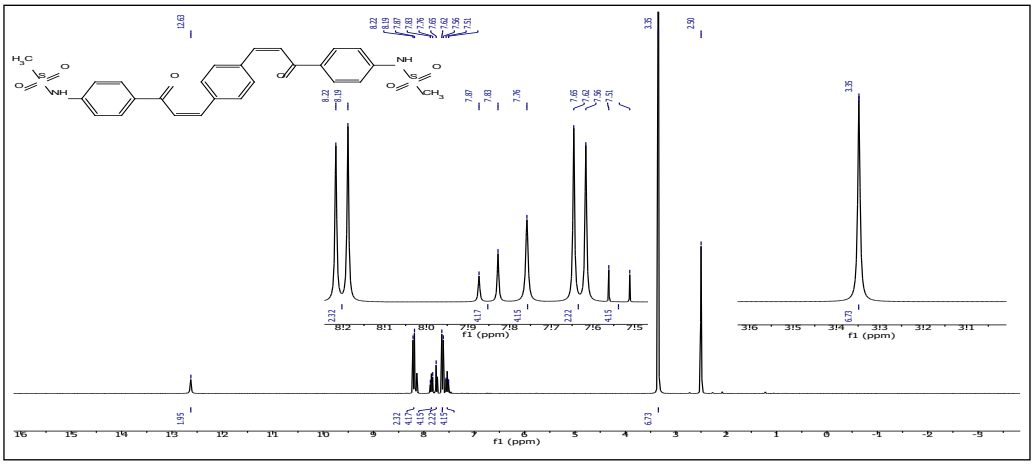

Figure 3. [1]H-NMR spectrum (300MHz) of *N,N'*-(3,3'-(1,4-phenylene)bis(acryloyl)) bis(4,1-phenylene))dimethanesulfonamide(A_1) (**Prepared by Authors, 2025**).

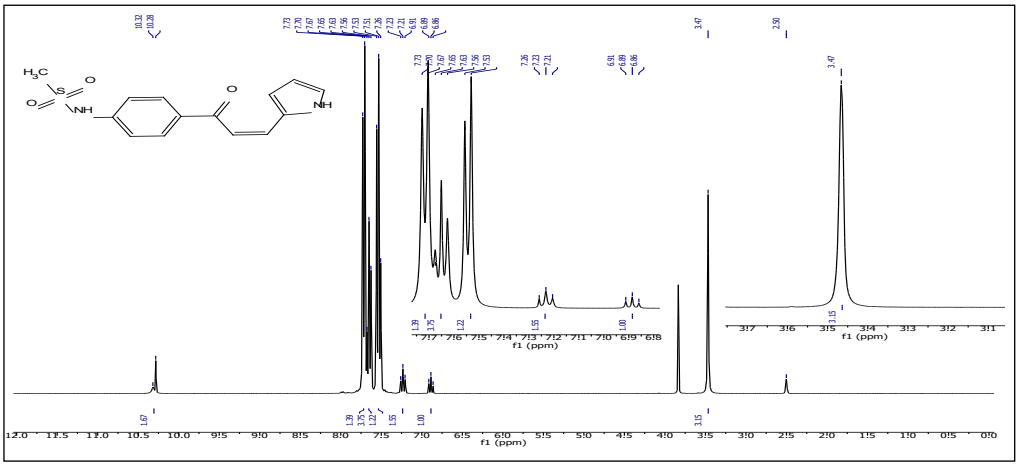

Figure 4. [1]H-NMR spectrum (300MHz) of *N*-(4-(3-(1*H*-pyrrol-2-yl)acryloyl)phenyl) methanesulfonamide(A_2) (**Prepared by Authors, 2025**).

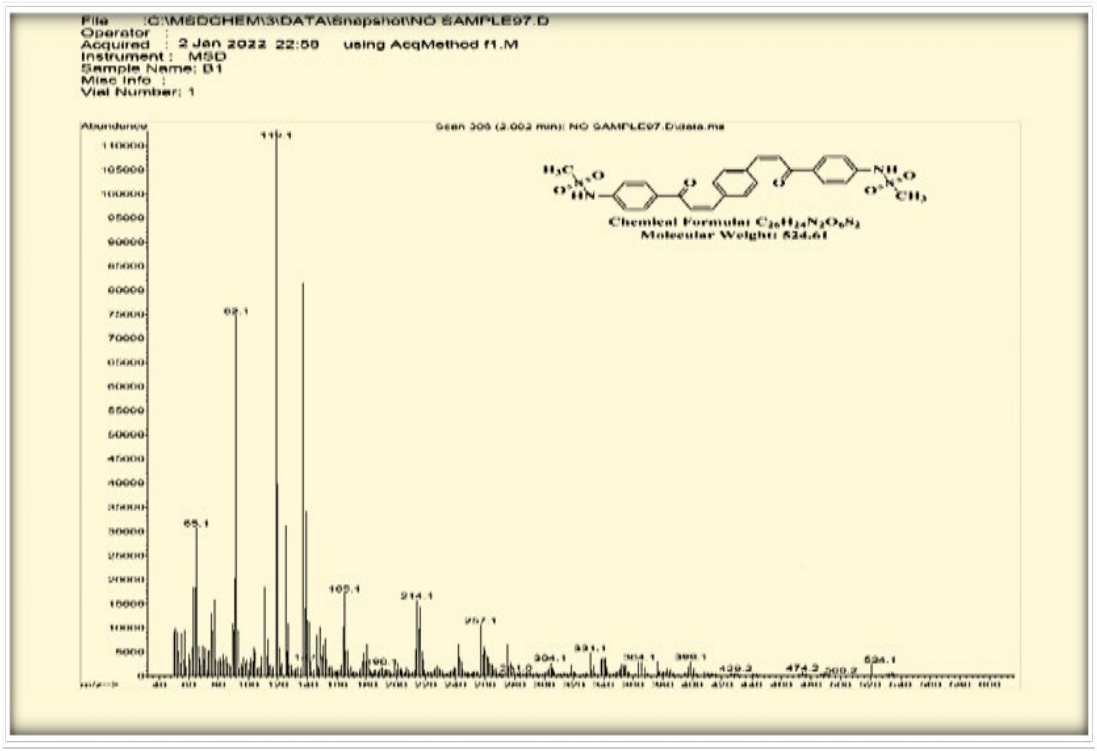

Figure 5. Mass spectrum of *N,N′*-(3,3'-(1,4-phenylene)bis(acryloyl))bis(4,1-phenyl ene))dimethan esulfonamide(A_1). (**Prepared by Authors, 2025**).

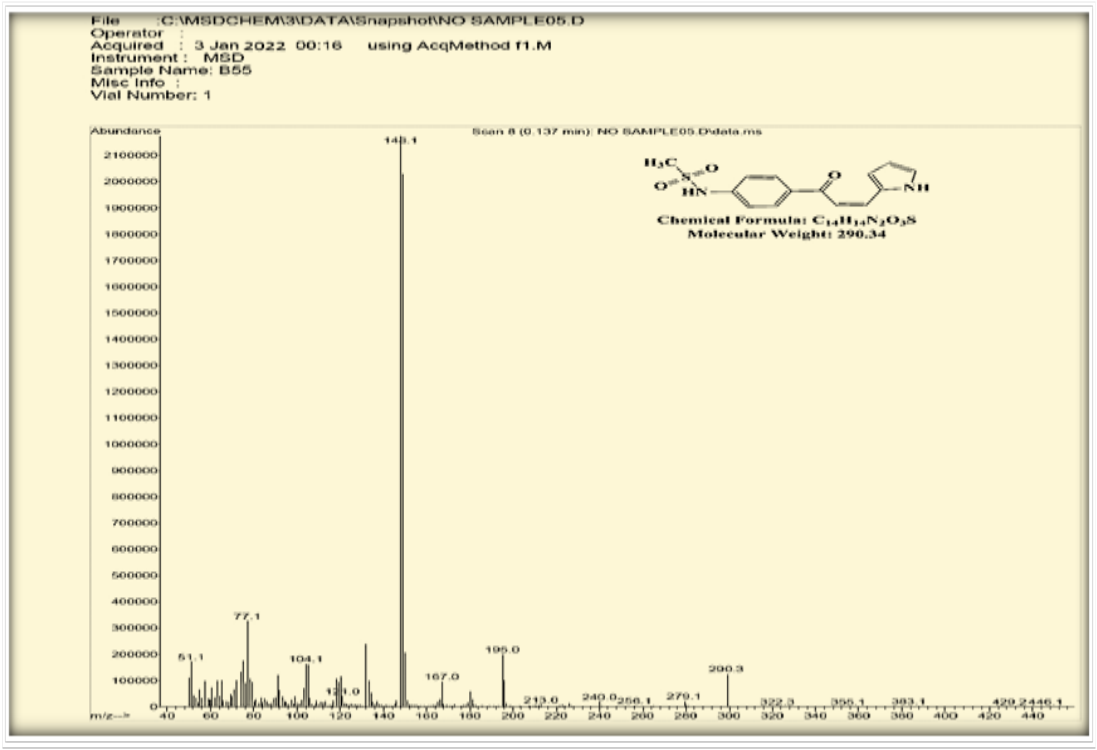

Figure 6. Mass spectrum of *N*-(4-(3-(1*H*-pyrrol-2-yl)acryloyl)phenyl)methanesulfon amide(A_2) (**Prepared by Authors, 2025**).

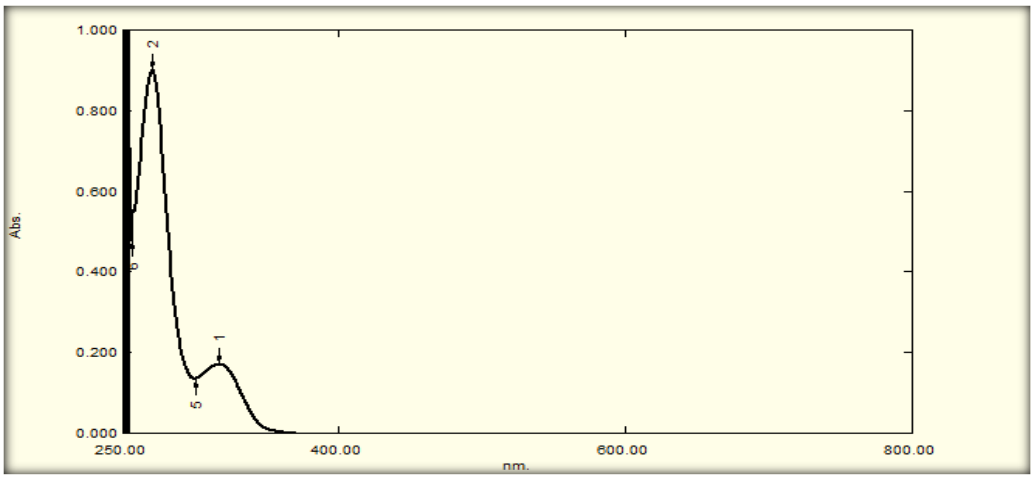

Figure 7. UV spectra of *N,N'*-(3,3'-(1,4-phenylene)bis(acryloyl))bis (4,1-phenylene)) dimethanesulfonamide(A_1) (**Prepared by Authors, 2025**).

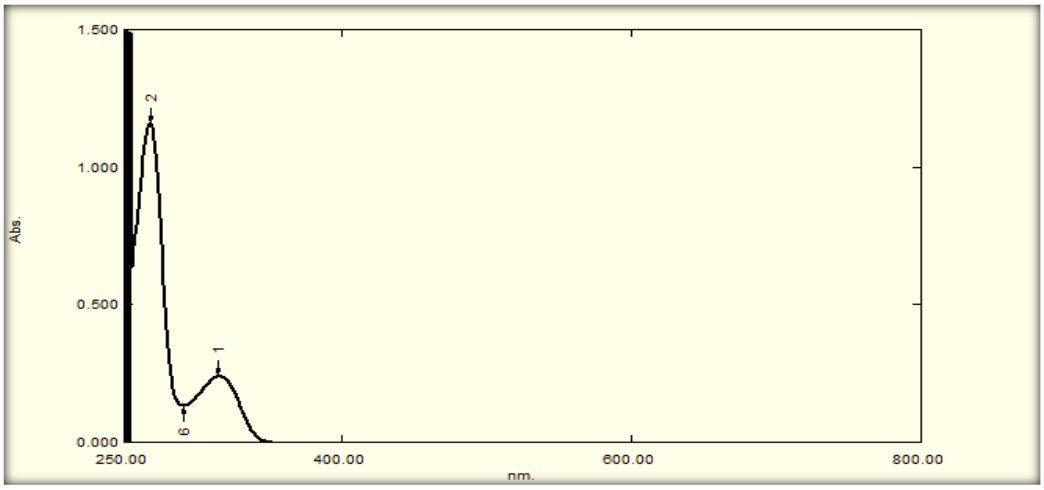

Figure 8. UV spectra of *N*-(4-(3-(1*H*-pyrrol-2-yl)acryloyl)phenyl)methanesulfon amide(A_2) (**Prepared by Authors, 2025**).

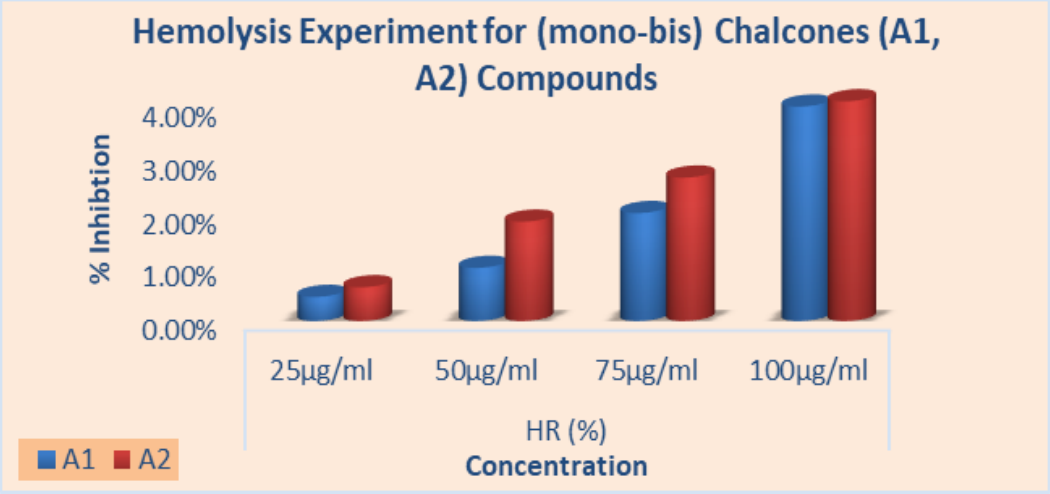

Figure 9. Measurement of hemolysis for (mono-bis) chalcone compounds towards blood cells at different concentrations (**Prepared by Authors, 2025**).

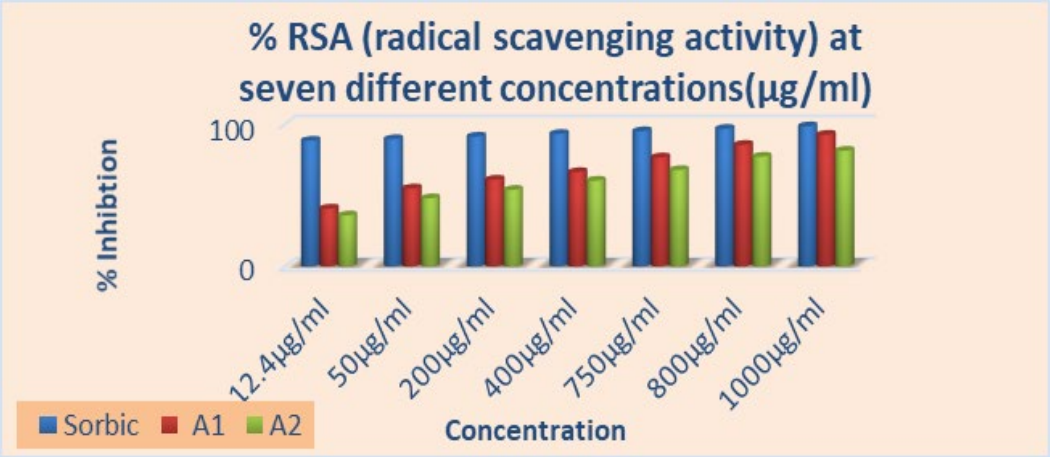

Figure 10. Radical scavenging activity (RSA) for the synthesized compounds (**Prepared by Authors, 2025**).

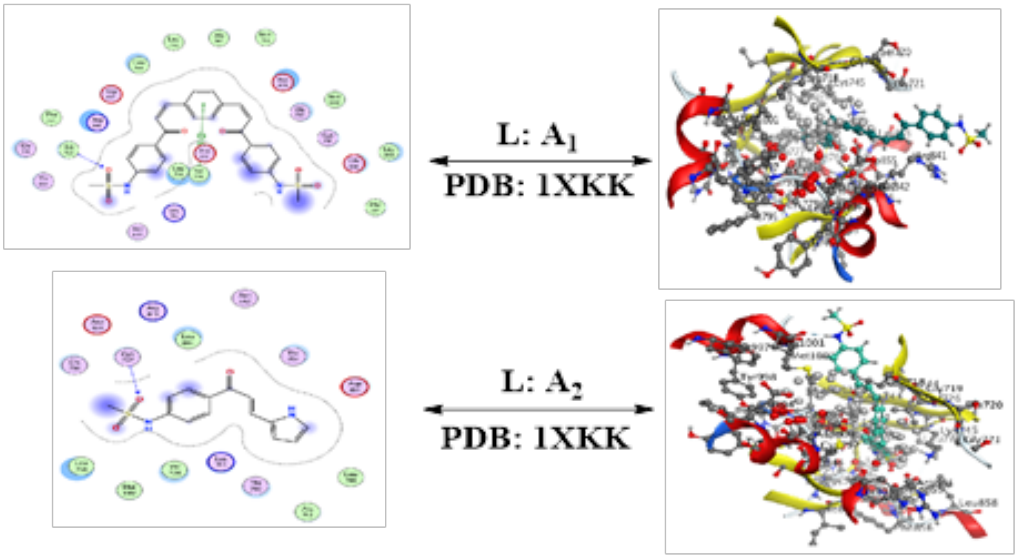

Figure 11. The interaction mode of compounds (A_1, A_2) with active site amino acids of the protein (PDB 1XKK) (**Prepared by Authors, 2025**).

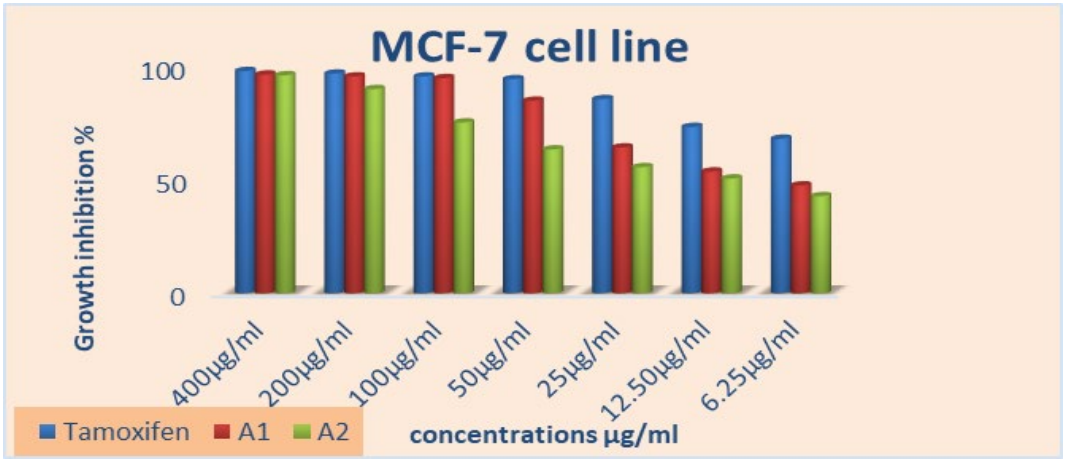

Figure 12. Anti-proliferative activity in cancer cells (MCF-7 cell line). Cultured with various concentrations of (mono-bis) chalcones (A_1, A_2) compounds after 24 hrs. The results are presented as the means ± SD. $p<0.05$ (**Prepared by Authors, 2025**).

4. Discussion

The synthesis of mono- and bis-chalcone derivatives (A_1 and A_2) molecules is reported in this work. According to (^{1}H-NMR), (GC-MS), and (UV-Vis), the products showed excellent biological potential, including antioxidant, low hemolytic toxicity, and anticancer effects against MCF-7 breast cancer cells. The hemolysis assay, with both A_1 (4.09%) and A_2 (3.99%) at 100µg/ml, demonstrated values far below the 10% cytotoxicity threshold in the hemolysis experiment, suggesting good biocompatibility and suitabilityfor systemic use, confirming high safety (36). Both chalcones demonstrated concentration-dependent antioxidant activity in the DPPH experiment, at 1000 µg/ml A_1 (89.78%) beating A_2 (77.45%) **(37)**. Free radicals are neutralized, and cytoprotective potential is provided by their enone and phenolic structures, which promote hydrogen donation (38). According to anticancer studies, both compounds suppressed MCF-7 cell growth proportionately at all concentrations, which was comparable to Tamoxifen. Where IC_{50} values for the produced compounds A_1, A_2, and Tamoxifen were 34.67µg/ml, 28.34µg/ml, and 15.48µg/ml, respectively. The highest toxicity compound to MCF-7 cells was compound A_1 (39).

The EGFR enzyme is a molecular target found in the tested MCF-7 cells. So, Chalcones were docked in an EGFR model to research their affinity and binding mode with the enzyme. The docked inhibitor (A_1) was found to bind to the active site of the enzyme, suggesting that the studied compound may exhibit a competitive inhibition-binding mode. In terms of structure, mono-chalcone A_2 had a single reactive site and reduced affinity (40), whereas bis-chalcone A1 showed greater potency due to its dual α, β-unsaturated ketone and methane sulfonamide groups, which improved multi-target interactions.

Both compounds' α, β-unsaturated ketone moiety functions as a Michael acceptor, creating covalent adducts with cysteine residues and causing cancer cells to undergo apoptosis **(41)**.

5. Conclusion

The new chalcone compounds A_1 and A_2 were successfully synthesized by two different methods (conventional method and microwave irradiation) in good yields (79, 89-88, 91%). They demonstrated anti-proliferative, anti-oxidant, and anti-breast cancer (MCF-7) cell activities. Molecular docking studies further supported the experimental findings. The chemical structure of the synthesized compounds was demonstrated. Using 1HNMR, UV-Vis, and GC-MS, according to antioxidant experiments in vitro. The A1 exhibits a potent DPPH radical antioxidant activity. The chalcones were more toxic towards the MCF-7 cells.

6. Declarations

6.1 Acknowledgments

The authors would like to thank to the Department of Physiology and Department of Microbiology, College of Veterinary Medicine, Al-Muthanna University, Iraq, for their partial assistance. Also, the authors are grateful to the Veterinary Medicine College, Al-Muthanna University, for allowing the authors to use the equipment in the Biochemistry laboratory.

6.2 Ethical Considerations

Not applicable.

6.3 Authors' Contributions

Methodology, software, R.N; writing—original draft preparation, Z.Y, R.N, S.A, M.A; writing—review and editing, S.A, M.A; supervision, project administration, Z.Y. All authors reviewed, edited, and approved the final version of the manuscript.

6.4 Conflict of Interest

The authors declare no conflict of interest.

6.5 Fund or Financial Support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

7. Publisher's Note

This article is part of the Special Issue arising from the Second International Conference for Pharmaceutical Sciences (SICPS 2025), College of Pharmacy, University of Misan, Iraq (29 Nov – 1 Dec 2025, see https://uomisan.edu.iq/pharmacy/conference/). All manuscripts in this issue were peer-reviewed and accepted for publication in *Journal of Advances in Medical and Biomedical Research (J Adv Med Biomed Res)*.

References

1. Wijayanti LW, Swasono RT, Lee W, Jumina J. Synthesis and Evaluation of Chalcone Derivatives as Novel Sunscreen Agent. Molecules. 2021;26(9):2698. [PMCID] [DOI:10.3390/molecules26092698] [PMID]
2. Elavarasan M, Thendral MT, Shafi SS. Synthesis, Characterisation and Antimicrobial Activity of Some New Chalcones. Int J Pharm Sci Res. 2018;9(5):1969-73.
3. Jumina J, Styaningrum RW, Siswanta D, Triono S, Priastomo Y, Harizal H, et al. Synthesis and preliminary evaluation of several chalcone derivatives as sunscreen compounds. Chem J Mold. 2019;14(2):90-6. [DOI:10.19261/cjm.2019.624]
4. Aguiar ASN, Dias PGM, Queiroz JE, Firmino PP, Custódio JMF, Dias LD, et al. Insights on potential photoprotective activity of two butylchalcone derivatives: synthesis, spectroscopic characterization and molecular modeling. Photonics 2023;10(3):228. [DOI:10.3390/photonics10030228]
5. Rocha S, Ribeiro D, Fernandes E, Freitas M. A systematic review on anti-diabetic properties of chalcones. Curr Med Chem. 2020;27(14):2257-321. [PMID] [DOI:10.2174/0929867325666181001112226]
6. Sooknual P, Pingaew R, Phopin K, Ruankham W, Prachayasittikul S, Ruchirawat S, et al. Synthesis and neuroprotective effects of novel chalcone-triazole hybrids. Bioorg Chem. 2020; 105:104384. [DOI:10.1016/j.bioorg.2020.104384] [PMID]
7. Custodio JMF, Guimaraes-Neto JJA, Awad R, Queiroz JE, Verde GMV, Mottin M, et al. Molecular modelling and optical properties of a novel fluorinated chalcone. Arab J Chem. 2020;13(1):3362-71. [DOI:10.1016/j.arabjc.2018.11.010]
8. Borges ID, Danielli JAV, Silva VEG, Sallum LO, Queiroz JE, Dias LD, et al. Synthesis and structural studies on (E)-3-(2,6-difluorophenyl)-1-(4-fluorophenyl)prop-2-en-1-one: a promising nonlinear optical material. RSC Adv. 2020;10(38):22542-55. [DOI:10.1039/D0RA03634J][PMID][PMCID]
9. Custodio JMF, Perez CN, Valverde C, Osorio FAP, Napolitano HB. Enhanced nonlinear optics properties of a bromine chalcone from a novel polymorph. Chem Phys Lett. 2020;738: 136852. [DOI:10.1016/j.cplett.2019.136852]
10. Elkanzi NAA, Hrichi H, Alolayan RA, Derafa W, Zahou FM, Bakr RB. Synthesis of Chalcones Derivatives and Their Biological Activities: A Review. ACS Omega. 2022; 7(32):27769-86. [PMID] [PMCID] [DOI:10.1021/acsomega.2c01779]
11. Ahn S, Truong VN-P, Kim B, Yoo M, Lim Y, Cho SK, et al. Design, synthesis, and biological evaluation of chalcones for anticancer properties targeting glycogen synthase kinase 3 beta. Appl Biol Chem. 2022;65(1):17. [DOI:10.1186/s13765-022-00686-x]
12. Li T, Li W, Yang X, Chen G, Jin X, Chen W, et al. Design, Synthesis, anticancer evaluation and in silico studies of 2,4,6-trimethoxychalcone derivatives. Saudi Pharm J. 2023;31(1):65-84. [PMID] [PMCID] [DOI:10.1016/j.jsps.2022.11.006]
13. Henry EJ, Bird SJ, Gowland P, Collins M, Cassella JP. Ferrocenyl chalcone derivatives as possible antimicrobial agents. J Antibiot (Tokyo). 2020;73(5):299-308. [DOI:10.1038/s41429-020-0280-y] [PMID]
14. Farghaly TA, Masaret GS, Muhammad ZA, Harras MF. Discovery of thiazole-based-chalcones and 4-hetarylthiazoles as potent anticancer agents: Synthesis, docking study and anticancer activity. Bioorg Chem. 2020;98: 103761. [DOI:10.1016/j.bioorg.2020.103761] [PMID]
15. Gao F, Huang G, Xiao J. Chalcone hybrids as potential anticancer agents: Current development, mechanism of action, and structure-activity relationship. Med Res Rev. 2020;40(5):2049-84. [DOI:10.1002/med.21698] [PMID]
16. El-Wakil MH, Khattab SN, El-Yazbi AF, El-Nikhely N, Soffar A, Khalil HH. New chalcone-tethered 1,3,5-triazines potentiate the anticancer effect of cisplatin against human lung adenocarcinoma A549 cells by enhancing DNA damage and cell apoptosis. Bioorg Chem. 2020;105:104393. [DOI:10.1016/j.bioorg.2020.104393] [PMID]
17. Ahmad MR, Sastry VG, Bano N, Anwar S. Synthesis of novel chalcone derivatives by conventional and microwave irradiation methods and their pharmacological activities. Arab J Chem. 2016;9:S931-S5. [DOI:10.1016/j.arabjc.2011.09.002]
18. Ridha AA, Kashanian S, Azandaryani AH, Rafipour R, Mahdavian E. New Folate-Modified Human Serum Albumin Conjugated to Cationic Lipid Carriers for Dual Targeting of Mitoxantrone against Breast Cancer. Curr

Pharm Biotechnol. 2020;21(4):305-15. [PMID] [DOI:10.2174/1389201020666191114113022]

19. Kadhim ZY, Alqaraghuli HGJ, Abd MT. Synthesis, Characterization, Molecular Docking, In Vitro Biological Evaluation and In Vitro Cytotoxicity Study of Novel Thiazolidine-4-One Derivatives as Anti-Breast Cancer Agents. Anticancer Agents Med Chem. 2021;21(17):2397-406. [PMID] [DOI:10.2174/1871520621666210401100801]
20. Kadhim ZY, Magtoof MS. Synthesis, characterization, novel oxazolone derivatives of study cytotoxicity, antioxidant and antibacterial activity in vitro. HIV Nursing. 2022;22(2):528-33.
21. Mankhi RN, Abdul-Rida NA. New Fenofibrate Derivatives As Anticancer And Antioxidant Agents: Synthesis, In Silico Study And Biological Evaluation. Kimya Problemleri. 2025;23(2):286-93. [DOI:10.32737/2221-8688-2025-2-286-293]
22. Singh D, Kaundal V, Aggarwal N, Jindal S, Ankalgi AD, Goyal K. A concise review on synthesis, anti-inflammatory and antioxidant activities of chalcone. Asian J Pharm Res. 2022;12(1):37-44. [DOI:10.52711/2231-5691.2022.00007]
23. Septianingtyas D, Zafira N, Zulhipri, Kurniadewi F, Dianhar H. Green synthesis of chalcones derivatives. InAIP Conference Proceedings. 2021. (Vol. 2331, No. 1, p. 040020). Melville, NY, USA: AIP Publishing LLC. [DOI:10.1063/5.0042002]
24. Jawad AM, Salih MNM, Helal TA, Obaid NH, Aljamali NM. Review on chalcone (preparation, reactions, medical and bio applications). Int J Chem Synth Chem Reac. 2019;5(1):16-27.
25. Khairul WM, Daud AI, Augustine E, Arshad S, Razak IA. FT-IR, NMR and X-ray crystallography dataset for newly synthesized alkoxy-chalcone featuring (E)-1-(4-ethylphenyl)-3-(4-(heptyloxy) phenyl) prop-2-en-1-one. Chem Data Collect. 2020;28:100473. [DOI:10.1016/j.cdc.2020.100473]
26. Lee Y, Koh D, Lim Y. 1 H and 13C NMR spectral assignments of 25 ethyl 2-oxocyclohex-3-enecarboxylates. Magn Reson Chem. 2018;56(12):1188-200. [DOI:10.1002/mrc.4778] [PMID]
27. Lee MS, Yang YL, Wu CY, Chen YL, Lee CK, Tzean SS, et al. Efficient identification of fungal antimicrobial principles by tandem MS and NMR database. J Food Drug Anal. 2019; 27(4):860-8. [DOI:10.1016/j.jfda.2019.06.003] [PMID] [PMCID]
28. Müller WH, Verdin A, De Pauw E, Malherbe C, Eppe G. Surface-assisted laser desorption/ionization mass spectrometry imaging: A review. Mass Spectrom Rev. 2022; 41(3):373-420. [DOI:10.1002/mas.21670] [PMID] [PMCID]
29. Lavakumar S, Vivekanand PA, Prince AAM. Simultaneous analysis of octylmethoxycinnamate and butylmethoxydibenzoylmethane in sunscreen products by a validated UV-spectrophotometric method. Mater Today Proc. 2021;36:893-7. [DOI:10.1016/j.matpr.2020.07.025]
30. Kadhim ZY, Alqaraghuli HGJ. Synthesis, Docking Study, and Structural Characterization of New Bioactive Thiazolidine-4-one Derivatives as Antibacterial and Antioxidant Agents. Russ J Bioorg Chem. 2025;51(2):729-42. [DOI:10.1134/S1068162024605007]
31. Bhattacharya S, Sherje AP. Development of resveratrol and green tea sunscreen formulation for combined photoprotective and antioxidant properties. J Drug Deliv Technol. 2020;60: 102000. [DOI:10.1016/j.jddst.2020.102000]
32. Almeida-Neto FWQ, da Silva LP, Ferreira MKA, Mendes FRS, de Castro KKA, Bandeira PN, et al. Characterization of the structural, spectroscopic, nonlinear optical, electronic properties and antioxidant activity of the N-{4'-[(E)-3-(Fluorophenyl)-1-(phenyl)-prop-2-en-1-one]}-acetamide. J Mol Struct. 2020;1220: 128765. [DOI:10.1016/j.molstruc.2020.128765]
33. Siddiqui L, Hawsawi MB, Chotana GA, Saleem RSZ. Bis-Chalcones: Recent Reports of Their Diverse Applications in Biological and Material Sciences. ACS Omega. 2024;9(41): 42061-90. [DOI:10.1021/acsomega.4c04635] [PMID] [PMCID]
34. Sager A, Abbood AF, Abaies JK, Khazal FA. A Design, Molecular Docking, ADMET Studies, Synthesis, Characterization, and Invitro Pharmacological Evaluation of Tetrazole Derivatives. Iraqi J Pharm Sci. 2025; 34(1):10-25.
35. Constantinescu T, Lungu CN. Anticancer Activity of Natural and Synthetic Chalcones. Int J Mol Sci. 2021;22(21):11306. [PMCID] [DOI:10.3390/ijms222111306] [PMID]
36. Dos Santos MB, Bertholin Anselmo D, de Oliveira JG, Jardim-Perassi BV, Alves Monteiro D, Silva G, et al. Antiproliferative activity and p53 upregulation effects of chalcones on human breast cancer cells. J Enzyme Inhib Med Chem. 2019;34(1):1093-9. [DOI:10.1080/14756366.2019.1615485] [PMID] [PMCID]

37. Coskun D, Aydin İ, Cinar-Asa S, Coskun MF, Ari F. A dual-targeted attack: 2-benzofuran chalcone-tamoxifen combination induces apoptosis and suppresses metastatic behavior in ER+ breast cancer. Results Chem. 2025;18: 102683. [DOI:10.1016/j.rechem.2025.102683]

38. Salum KA, Alidmat MM, Khairulddean M, Kamal NNSNM, Muhammad M. Design, synthesis, characterization, and cytotoxicity activity evaluation of mono-chalcones and new pyrazolines derivatives. J Appl Pharm Sci. 2020;10(8):020-36.

39. Kuttithodi AM, Nikhitha D, Jacob J, Narayanankutty A, Mathews M, Olatunji OJ, et al. Antioxidant, Antimicrobial, Cytotoxicity, and Larvicidal Activities of Selected Synthetic Bis-Chalcones. Molecules. 2022;27(23):8209. [DOI:10.3390/molecules27238209] [PMID] [PMCID]

40. Ouyang Y, Li J, Chen X, Fu X, Sun S, Wu Q. Chalcone Derivatives: Role in Anticancer Therapy. Biomolecules. 2021;11(6):894. [DOI:10.3390/biom11060894] [PMID] [PMCID]

41. Lai SL, Mustafa MR, Wong PF. Panduratin A induces protective autophagy in melanoma via the AMPK and mTOR pathway. Phytomedicine. 2018;42:144-51. [DOI:10.1016/j.phymed.2018.03.027] [PMID]

How to Cite This Article:

Kadhim Z Y, Al-khafaji S A, Mankhi R N, Abddulameer M A. Design, Synthesis, In Silico Studies, and Pharmacological Evaluation of New Chalcone Derivatives as Anticancer and Antioxidant Agents. J Adv Med Biomed Res. 2025;33(162):110-20.

Download citation:
BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks

Send citation to:
Mendeley Zotero RefWorks