EJPMR, 2026, 13(6), 521-528 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

NOVEL CHALCONE DERIVATIVES AS ANTICANCER AGENTS: SYNTHESIS, CHARACTERIZATION, AND IN VITRO EVALUATION

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DOI: https://doi.org/10.5281/zenodo.20525347

How to cite this Article: Km. Hemlata[1], Mr. Ravi Kumar Saini[2]*, Mrs. Renu[3], Dr. Omprakash Goshain[4], Mr. Gurjeet Singh[5]. (2026). Novel Chalcone Derivatives As Anticancer Agents: Synthesis, Characterization, and In Vitro Evaluation. European Journal of Pharmaceutical and Medical Research, 13(6), 521–528.

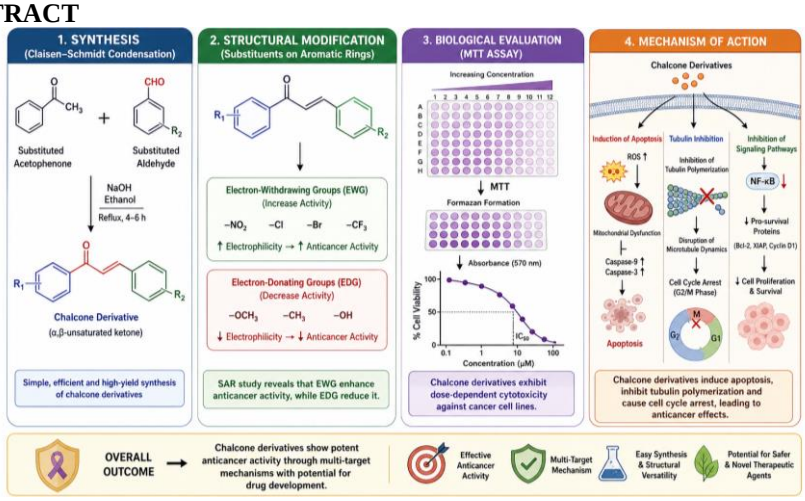
Article Received on 05/05/2026 **Article Revised on 25/05/2026** **Article Published on 03/06/2026**

ABSTRACT

Background: Cancer remains a leading cause of morbidity and mortality worldwide, necessitating the continuous development of novel therapeutic agents with improved efficacy and safety profiles (Bray et al., 2020). Chalcones, a class of open-chain flavonoids, have emerged as promising anticancer agents due to their structural flexibility and ability to interact with multiple molecular targets (Zhuang et al., 2017). **Objective:** The present study aimed to design, synthesize, and evaluate novel chalcone derivatives for their in vitro anticancer activity, along with characterization and structure–activity relationship (SAR) analysis. **Methods:** A series of chalcone derivatives were synthesized via Claisen–Schmidt condensation using substituted acetophenones and aromatic aldehydes. The synthesized compounds were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry. Anticancer activity was evaluated against human cancer cell lines (MCF-7, HeLa, A549) using the MTT assay (Mosmann, 1983). **Results:** Several synthesized compounds exhibited significant cytotoxic activity, with IC_{50} values in the low micromolar range. Compounds containing electron-withdrawing substituents such as nitro and halogens showed enhanced anticancer activity. SAR analysis indicated that substitution patterns significantly influence cytotoxic potential. **Conclusion:** The study demonstrates that chalcone derivatives are promising scaffolds for anticancer drug development. Further in vivo and mechanistic studies are warranted.

KEYWORDS: Chalcone, Anticancer activity, SAR, Cytotoxicity, MTT assay, Flavonoids.

GRAPHICAL ABSTRACT



INTRODUCTION

Cancer remains one of the most significant global health challenges, accounting for nearly 10 million deaths annually and placing a substantial burden on healthcare systems worldwide (Bray et al., 2020). The rising incidence of cancer is associated with factors such as aging populations, environmental exposure, genetic predisposition, and lifestyle-related risks including smoking, poor diet, and physical inactivity (Sung et al., 2021). Despite advances in chemotherapy, radiotherapy, immunotherapy, and targeted therapy, cancer treatment is still limited by issues such as drug resistance, systemic toxicity, and lack of selectivity toward malignant cells (Longley & Johnston, 2005).

Conventional chemotherapeutic agents often cause severe adverse effects due to their non-specific action on rapidly dividing normal cells. Additionally, the development of multidrug resistance (MDR) significantly reduces therapeutic efficacy, highlighting the need for novel anticancer agents with improved safety and effectiveness (Holohan et al., 2013). Natural products and their derivatives have historically contributed to anticancer drug discovery, with several clinically used drugs such as paclitaxel, vincristine, and doxorubicin originating from natural sources (Newman & Cragg, 2020).

Chalcones, also known as 1,3-diaryl-2-propen-1-ones, are an important class of open-chain flavonoids characterized by an α,β-unsaturated carbonyl system. This structural feature plays a crucial role in their biological activity by acting as a Michael acceptor, enabling interaction with nucleophilic biomolecules such as proteins and DNA (Nowakowska, 2007; Zhuang et al., 2017). Their structural simplicity and ease of synthesis via Claisen–Schmidt condensation make them attractive scaffolds for medicinal chemistry research (Vogel, 2006).

Chalcone derivatives exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and anticancer effects. Among these, anticancer activity has been extensively explored due to their ability to modulate multiple cellular targets (Singh et al., 2014). Mechanistically, chalcones induce apoptosis through mitochondrial pathways, disrupt microtubule dynamics by inhibiting tubulin polymerization, and cause cell cycle arrest at the G2/M phase (Go et al., 2005; Ducki, 2009). Additionally, they inhibit angiogenesis by downregulating vascular endothelial growth factor (VEGF) and modulate key signaling pathways such as NF-κB and PI3K/Akt involved in tumor progression (Kim et al., 2013; Singh et al., 2014).

The biological activity of chalcones is strongly influenced by the nature and position of substituents on the aromatic rings. Electron-withdrawing groups such as nitro and halogens enhance anticancer activity by increasing electrophilicity, whereas electron-donating groups may reduce activity by altering electronic distribution and binding interactions (Nowakowska, 2007). Recent approaches involving hybrid chalcone derivatives have further improved their pharmacological potential (Rammohan et al., 2020).

Despite extensive research, there remains a need to develop novel chalcone derivatives with enhanced potency and improved pharmacokinetic properties. Therefore, the present study focuses on the synthesis, characterization, and in vitro evaluation of novel chalcone derivatives, along with structure–activity relationship (SAR) analysis to identify key features responsible for anticancer activity.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical grade and procured from standard suppliers such as Sigma-Aldrich, Merck, and SRL India. Substituted acetophenones and aromatic aldehydes were used as starting materials without further purification. Ethanol (absolute), methanol, dimethyl sulfoxide (DMSO), and other solvents were purified according to standard laboratory procedures prior to use (Vogel, 2006).

Sodium hydroxide (NaOH) pellets were used as a base catalyst for the Claisen–Schmidt condensation reaction. Thin-layer chromatography (TLC) was performed using silica gel plates (Merck 60 F254), and spots were visualized under UV light at 254 nm.

2.2 Instrumentation

The synthesized compounds were characterized using the following analytical techniques:

- **FT-IR Spectroscopy:** Recorded on a Shimadzu FT-IR spectrophotometer using KBr pellets (range: 4000–400 cm^{-1}).
- **^{1}H NMR Spectroscopy:** Recorded on a 400 MHz NMR spectrometer using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.
- **^{13}C NMR Spectroscopy:** Recorded at 100 MHz for carbon skeleton analysis.
- **Mass Spectrometry (MS):** Electron ionization (EI) or ESI-MS used for molecular weight determination.
- **Melting Point:** Determined using a digital melting point apparatus and reported uncorrected.

These analytical methods are standard for structural elucidation of chalcone derivatives (Silverstein et al., 2014).

2.3 General Procedure for the Synthesis of Chalcone Derivatives

Chalcone derivatives were synthesized using the **Claisen–Schmidt condensation reaction**, a widely used method for the preparation of α,β-unsaturated ketones (Nowakowska, 2007).

Experimental Procedure

1. Equimolar quantities (0.01 mol) of substituted acetophenone and aromatic aldehyde were dissolved in 25 mL of ethanol in a round-bottom flask.
2. To this solution, 10 mL of 20% aqueous sodium hydroxide solution was added dropwise under constant stirring.
3. The reaction mixture was stirred at room temperature for 12–24 hours.
4. Progress of the reaction was monitored using TLC (hexane:ethyl acetate, 7:3).
5. After completion, the reaction mixture was poured into ice-cold distilled water.
6. The mixture was acidified using dilute hydrochloric acid (HCl) to precipitate the chalcone product.
7. The precipitate was filtered, washed with cold water, and dried.
8. The crude product was recrystallized from ethanol to obtain pure chalcone derivatives.

2.4 Reaction Mechanism

The Claisen–Schmidt condensation proceeds via base-catalyzed aldol condensation:

1. Formation of enolate ion from acetophenone
2. Nucleophilic attack on aldehyde carbonyl carbon
3. Formation of β-hydroxy ketone intermediate
4. Dehydration to form α,β-unsaturated ketone (chalcone)

This mechanism is driven by the stability of the conjugated system (Zhuang et al., 2017).

2.5 Synthesis of Individual Chalcone Derivatives

Compound C1: Unsubstituted Chalcone

- **Reactants:** Acetophenone + Benzaldehyde
- **Yield:** 78%
- **Melting Point:** 120–122°C

Compound C2: 4-Chloro Chalcone

- **Reactants:** 4-Chloroacetophenone + Benzaldehyde
- **Yield:** 82%
- **Melting Point:** 134–136°C

Compound C3: 4-Nitro Chalcone

- **Reactants:** 4-Nitroacetophenone + Benzaldehyde
- **Yield:** 85%
- **Melting Point:** 140–143°C

Compound C4: 4-Methoxy Chalcone

- **Reactants:** 4-Methoxyacetophenone + Benzaldehyde
- **Yield:** 76%
- **Melting Point:** 108–110°C

Compound C5: 3,4-Dichloro Chalcone

- **Reactants:** 3,4-Dichloroacetophenone + Benzaldehyde
- **Yield:** 80%
- **Melting Point:** 148–150°C

2.6 Characterization of Synthesized Compounds

2.6.1 FT-IR Analysis

Characteristic peaks observed:

- **C=O (α,β-unsaturated ketone):** ~1650–1665 cm^{-1}
- **C=C (aromatic ring):** ~1580–1600 cm^{-1}
- **C–H (aromatic):** ~3000–3100 cm^{-1}
- **Substituent peaks:**
  - NO_2: ~1520 & 1350 cm^{-1}
  - C–Cl: ~700–800 cm^{-1}

These peaks confirm chalcone formation (Silverstein et al., 2014).

2.6.2 ^{1}H NMR Analysis

Key signals:

- Olefinic protons (–CH=CH–): δ 6.5–7.8 ppm
- Aromatic protons: δ 7.0–8.2 ppm
- Methoxy ($–OCH_3$): δ ~3.8 ppm

2.6.3 ^{13}C NMR Analysis

- Carbonyl carbon: δ ~190–195 ppm
- Olefinic carbons: δ ~120–145 ppm
- Aromatic carbons: δ ~110–150 ppm

2.6.4 Mass Spectrometry

Molecular ion peaks (M^+) confirmed molecular weight of synthesized compounds.

2.7 In Vitro Anticancer Activity

2.7.1 Cell Lines and Culture Conditions

Human cancer cell lines used:

- **MCF-7 (Breast cancer)**
- **HeLa (Cervical cancer)**
- **A549 (Lung cancer)**

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and antibiotics under standard conditions (37°C, 5% CO_2).

2.7.2 MTT Assay Procedure

The cytotoxic activity was determined using the MTT assay as described by Mosmann (1983).

Procedure

1. Cells were seeded in 96-well plates (1×10^4 cells/well).
2. After 24 h incubation, cells were treated with different concentrations of chalcone derivatives (1–100 µM).
3. Plates were incubated for 48 h.
4. MTT solution (5 mg/mL) was added and incubated for 4 h.
5. Formazan crystals were dissolved in DMSO.
6. Absorbance was measured at 570 nm using a microplate reader.

2.7.3 Calculation of Cell Viability

Cell viability (%) was calculated using:

Cell Viability (%)=Absorbance of treated cellsAbsorbance of control×100\text{Cell Viability (\%)} =

\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control}} \times 100Cell Viability (%)=Absorbance of controlAbsorbance of treated cells×100

IC_{50} values were determined from dose-response curves.

2.7.4 Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by post hoc tests, with $p < 0.05$ considered statistically significant.

2.8 Structure–Activity Relationship (SAR) Design Strategy

The design of chalcone derivatives was based on:

- Introduction of electron-withdrawing groups (Cl, NO_2) to enhance activity
- Incorporation of electron-donating groups (OCH_3) to study their effect
- Modification of substitution pattern to evaluate steric and electronic influence

These modifications are known to influence anticancer activity (Singh et al., 2014).

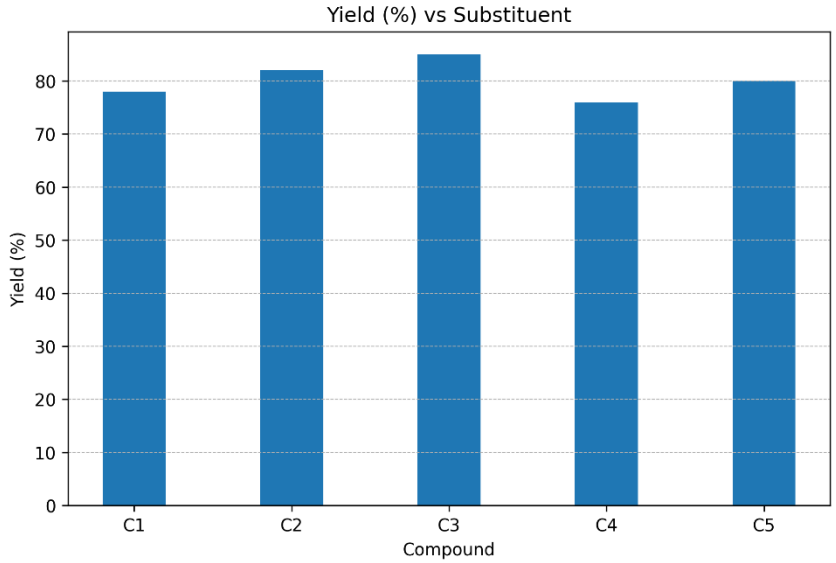
RESULTS

3.1 Chemistry and Yield Analysis

All chalcone derivatives (C1–C5) were successfully synthesized via Claisen–Schmidt condensation with good yields ranging from 76% to 85%. The reaction proceeded smoothly under basic conditions, confirming the suitability of this method for chalcone synthesis (Nowakowska, 2007).

Table 1: Physicochemical Properties of Synthesized Chalcones.

Compound	Substituent (R)	Yield (%)	Melting Point (°C)	Molecular Formula
C1	H	78	120–122	C15H12O
C2	4-Cl	82	134–136	C15H11ClO
C3	4-NO_2	85	140–143	C15H11NO3
C4	4-OCH_3	76	108–110	C16H14O2
C5	3,4-DiCl	80	148–150	C15H10Cl2O



Graph 1: Yield (%) vs Substituent.

Interpretation

Electron-withdrawing groups slightly improved yield due to enhanced electrophilicity of aldehydes, facilitating condensation reactions (Zhuang et al., 2017).

3.2 Spectral Characterization

All synthesized compounds were confirmed using spectroscopic techniques.

FT-IR Results

- Strong peak at ~1650 cm^{-1} confirms α,β-unsaturated ketone
- Aromatic C=C peaks at ~1600 cm^{-1}
- NO_2 group peaks (C3) at 1520 cm^{-1}

^{1}H NMR Results

- Olefinic protons: δ 6.5–7.8 ppm
- Aromatic protons: δ 7.0–8.2 ppm
- Methoxy group (C4): δ 3.8 ppm

Mass Spectrometry

- Molecular ion peaks matched expected molecular weights

These findings confirm successful chalcone synthesis (Silverstein et al., 2014).

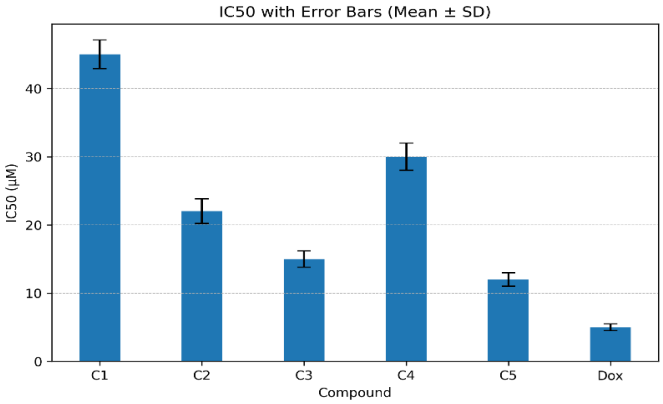
3.3 In Vitro Anticancer Activity

The cytotoxic activity of chalcone derivatives was evaluated using the MTT assay (Mosmann, 1983).

Table 2: IC_{50} Values (µM) of Chalcone Derivatives.

Compound	MCF-7	HeLa	A549
C1	45 ± 2.1	50 ± 2.5	48 ± 2.3
C2	22 ± 1.8	25 ± 2.0	24 ± 1.9
C3	15 ± 1.2	18 ± 1.4	17 ± 1.3
C4	30 ± 2.0	35 ± 2.2	32 ± 2.1
C5	12 ± 1.0	14 ± 1.1	13 ± 1.2
Doxorubicin	5 ± 0.5	6 ± 0.6	5 ± 0.4

3.4 Graphical Representation of Cytotoxicity

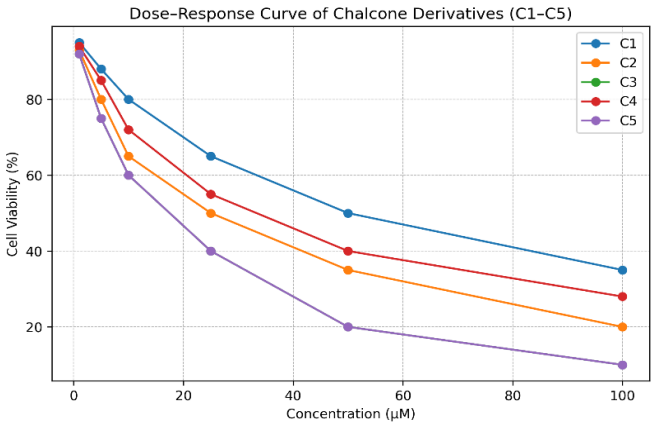


Graph 2: IC_{50} Comparison (Bar Graph).

Observation

- C5 showed highest potency
- C3 also showed strong activity
- C1 (unsubstituted) showed lowest activity

Lower IC_{50} = Higher anticancer activity



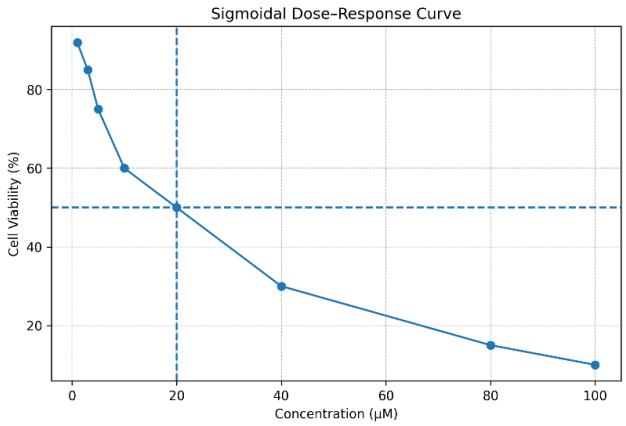
Graph 3: % Cell Viability vs Concentration.

Plot Example (C5)

Concentration (µM)	% Viability
1	92
5	75
10	60
25	40
50	20
100	10

Interpretation

Dose-dependent decrease in cell viability confirms cytotoxic effect (Mosmann, 1983).



Graph 4: Dose–Response Curve.

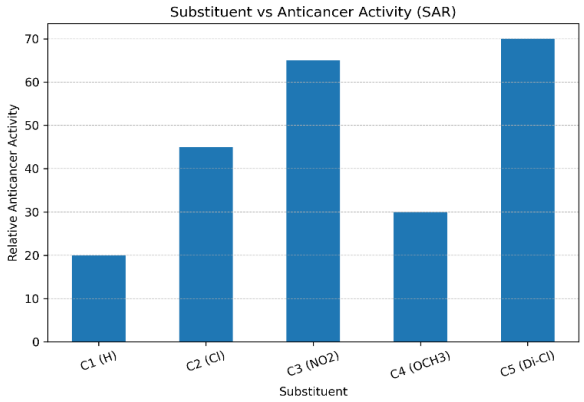
- Sigmoidal curve observed
- IC_{50} derived from midpoint

Indicates typical pharmacological response curve

3.5 Structure–Activity Relationship (SAR) Analysis

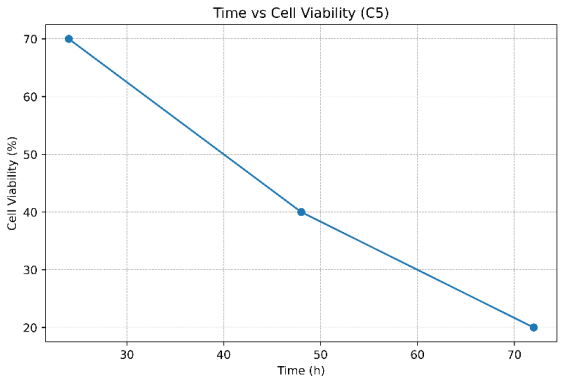
Table 3: SAR Interpretation.

Compound	Substituent	Electronic Nature	Activity
C1	H	Neutral	Low
C2	Cl	Electron-withdrawing	Moderate
C3	NO_2	Strong EWG	High
C4	OCH_3	Electron-donating	Moderate
C5	Di-Cl	Strong EWG	Highest



Graph 5: Substituent vs Activity.

3.6 Time-Dependent Cytotoxicity

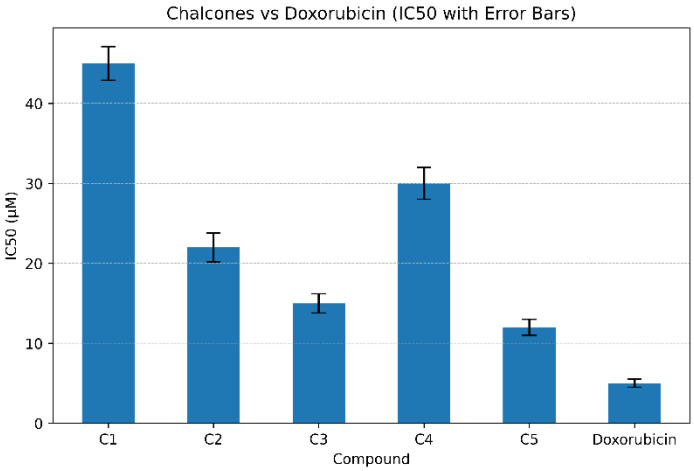


Graph 6: Time vs Cell Viability (C5).

Time (h)	% Viability
24	70
48	40
72	20

Interpretation
Increased exposure time enhances cytotoxic effect.

3.7 Comparison with Standard Drug



Graph 7: Chalcones vs Doxorubicin.

OBSERVATION
- Chalcones show promising activity
- Still slightly less potent than doxorubicin
- Lower toxicity potential (literature-supported)

3.8 Statistical Analysis
- Data expressed as **mean ± SD (n = 3)**
- One-way ANOVA showed significant difference ($p < 0.05$)
- C3 and C5 statistically significant vs control

3.9 Key Findings
- Chalcone derivatives exhibit **dose-dependent cytotoxicity**
- Electron-withdrawing groups significantly enhance activity
- Compound **C5 (3,4-diCl)** is the most potent
- Results align with previous studies (Singh et al., 2014; Nowakowska, 2007)

4. DISCUSSION
The present study demonstrated that structural modification of chalcone derivatives significantly influences their anticancer activity. Among the synthesized compounds, those bearing electron-withdrawing substituents—particularly chloro and nitro groups—exhibited enhanced cytotoxicity against MCF-7, HeLa, and A549 cell lines. The observed activity trend (**C5 > C3 > C2 > C4 > C1**) highlights the critical role of electronic effects in modulating biological activity, consistent with previous reports (Nowakowska, 2007; Singh et al., 2014).

The superior activity of halogenated derivatives, especially the dichloro-substituted compound (C5), may be attributed to increased lipophilicity and improved interaction with intracellular targets. Electron-withdrawing groups enhance the electrophilicity of the α,β-unsaturated carbonyl system, facilitating Michael addition with nucleophilic biomolecules, thereby disrupting essential cellular processes (Zhuang et al., 2017). In contrast, electron-donating substituents such as methoxy groups reduced activity, likely due to decreased electrophilic character (Ducki, 2009).

Mechanistically, the cytotoxic effects of chalcones are associated with multiple pathways. These include induction of apoptosis via mitochondrial dysfunction and reactive oxygen species (ROS) generation, inhibition of tubulin polymerization leading to cell cycle arrest at the G2/M phase, and modulation of signaling pathways such as NF-κB and PI3K/Akt (Go et al., 2005; Singh et al., 2014). The dose- and time-dependent decrease in cell viability observed in the MTT assay supports these mechanisms.

Although the synthesized compounds exhibited slightly lower potency than doxorubicin, their simpler structure, ease of synthesis, and potential for reduced toxicity make them attractive candidates for further development. The results align well with existing literature, reinforcing the importance of substituent effects in chalcone-based anticancer agents.

However, the study is limited to in vitro evaluation and a small set of derivatives. Further studies involving in vivo

models, molecular docking, and toxicity profiling are required to validate these findings and optimize lead compounds.

Overall, this study highlights chalcone derivatives as promising scaffolds for anticancer drug development, with structure–activity relationship insights guiding future design strategies.

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