MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2025.20.3.13612

Synthesis and Cytotoxic Activity of Methoxylated Chalcones in Breast Cancer MCF-7 and Prostate Cancer DU-145 Cell Lines

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Received January 06, 2024; **Accepted** November 17, 2025; **Available online** November 20, 2025

ABSTRACT. Chalcones, a class of naturally occurring compounds, exhibit a broad spectrum of biological activities, including anticancer properties. In this study, a series of methoxylated chalcones were synthesized and evaluated for cytotoxic activity against breast cancer MCF-7 and prostate cancer DU-145 cell lines. The synthetic route involved Claisen-Schmidt condensation, leading to various methoxy-substituted chalcone derivatives. The structures of the synthesized chalcones were confirmed through UV-Vis, ^{1}H-NMR, ^{13}C-NMR and HRMS. Cytotoxicity was assessed using the PrestoBlue assay, with 4-bromochalcone (compound **2**) displaying the highest cytotoxic activity against MCF-7 cancer cell lines (IC_{50} = 26.99 μM). These results indicate that methoxylated chalcones hold promise as potential lead compounds for the development of new anticancer agents targeting breast cancer.

Keywords: Cytotoxic activity, DU-145 cell lines, MCF-7 cell lines, methoxylated chalcones

INTRODUCTION

Breast and prostate cancers are among the most prevalent cancers in the world, affecting women and men, respectively. Because of their high incidence, recurrence rates, and resistance to conventional therapies, these cancers continue to present major public health challenges, despite notable advancements in early detection, diagnosis, and treatment (De Silva & Alcorn, 2022; Chong et al., 2022). Breast cancer, particularly estrogen receptor-positive (ER+) subtypes such as MCF-7, and prostate cancer, including androgen receptor-negative (AR-) lines such as DU-145, often require chemotherapies. However, treatment effectiveness can diminish over time as resistance develops (Metsiou et al., 2019; Alimirah et.al, 2006). The need for new and more potent anticancer agents is therefore critical in addressing these challenges. Among promising candidates in anticancer research are chalcones.

Chalcones, natural compounds within the flavonoid family, are characterized by their open-chain structure, which consists of two aromatic rings linked by an α,β-unsaturated carbonyl system. These compounds have attracted considerable attention in medicinal chemistry due to their extensive range of biological activities, including anti-inflammatory, antimicrobial, antioxidant, and anticancer properties (Salehi et al., 2021; Mezgebe et al., 2023). Methoxylated chalcone, which have methoxy groups at various positions on the aromatic ring, have demonstrated promising anticancer activity across several cancer lines, including ACHN (renal carcinoma), Panc 1 (pancreatic carcinoma), Calu 1 and H460 (non-small cell lung carcinoma), and HCT 116 (colon carcinoma) (Bandgar et al., 2010). Furthermore, methoxylated chalcone derived from phenothiazine have demonstrated potential against human oral squamous cell carcinoma (OSCC) cell lines (Ca9-22, HSC-2, HSC-3, and HSC-4) (Gul et al., 2018). The presence of methoxy substituents can significantly influence the physicochemical properties and biological activities of chalcones, potentially enhancing their cytotoxic effects on cancer cells.

Despite these findings, research on the effects of methoxylated chalcone compounds against breast cancer MCF-7 and prostate cancer DU-145 cancer cell lines remains limited. Recent studies have shown that methoxylated chalcones based on benzothizaole-imidazopyridine and chalcone-linked thiazole-imidazopyridine derivatives exhibit anticancer activity against these cell lines (Kumar et al., 2021; Suma et al., 2020). This study aims to further explore the potential of methoxylated chalcones as anticancer agents against MCF-7 and DU-145 cells, contributing

to ongoing efforts to develop effective cancer therapies.

EXPERIMENTAL SECTION

Material and Methods

The UV (Ultraviolet) spectrum was determined using a double-beam spectrophotometer (DU-88OOR-UV/Vis, Drawell, China). The ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) spectra were obtained on an Agilent DD2 spectrometer (Agilent Technologies, Santa Clara, CA, USA) at 500 MHz (^{1}H) and 125 MHz (^{13}C). High-resolution mass spectra (HRMS) were obtained with ESI-TOF Waters LCT Premier XE mass spectrometer (Waters Micromass, Milford, MA, USA). Thin-layer chromatography (TLC) analysis employed silica gel plates (Merck Kieselgel 60 GF254, 0.25 thickness), with spots visualized under UV light. Ethyl acetate and *n*-hexane (both technical grade) were used for purification.

The following reagents were used in the synthesis of chalcones: acetophenone p.a (Merck, Germany), 4'-methoxyacetophenone p.a. (Sigma Aldrich, USA), benzaldehyde p.a. (Merck, Germany), 4-methoxybenzaldehyde p.a. (Sigma Aldrich, USA), 3,4-dimethoxybenzaldehyde p.a. (Sigma Aldrich, USA), 4-bromobenzaldehyde p.a. (Sigma Aldrich, USA), 4-metylbenzaldehyde p.a. (Sigma Aldrich, USA), ethanol p.a. (Merck, Germany), NaOH (Merck, Germany).

For the cytotoxic assay, cisplatin (Sigma Aldrich, USA), penicillin-streptomycin (Gibco Thermo, Uppsala, Sweden), phosphate-buffered saline (Gibco Thermo, Uppsala, Sweden), PrestoBlue™ cell viability reagent (Thermo Fischer Scientific, Uppsala, Sweden), RPMI medium (Gibco Thermo, Uppsala, Sweden), fetal bovine serum (Gibco Thermo, Uppsala, Sweden), trypsin-EDTA (Gibco Thermo, Uppsala, Sweden), and trypan blue (Gibco Thermo Fischer Scientific, Uppsala, Sweden) were used.

Synthesis of Chalcones (1-9)

A mixture of acetophenone **(10-11)** (1 mmol) and benzaldehyde **(12-16)** (1 mmol) was dissolved in 5 mL of ethanol, followed by the addition of NaOH (15 mmol, 0.6 mg). The reaction mixture was stirred for 12 hours at room temperature and then refrigerated overnight. The resulting yellow solid was filtered, washed with water, and dried. Purification was performed by column chromatography (silica gel, ethyl acetate in hexane) to isolate chalcone compounds **(1-9)**. Chalcones set **(1-4)** were synthesized by reacting acetophenone **(10)** (1 mmol, 0.12 mL) with benzaldehyde **(12)** (1 mmol, 0.1 mL), 4-bromobenzaldehyde **(13)** (1 mmol, 0.185 g), 4-methylbenzaldehyde **(14)** (1 mmol, 0.12 mL), and 4-methoxybenzaldehyde **(15)** (1 mmol, 0.12 mL). Chalcones set **(5-9)** were obtained from reactions of 4'-methoxyacetophenone **(11)** (1 mmol, 0.15 g) with benzaldehyde **(12)** (1 mmol, 0.1 mL), 4-bromobenzaldehyde **(13)** (1 mmol, 0.185 g), 4-methylbenzaldehyde **(14)** (1 mmol, 0.12 mL), 4-methoxybenzaldehyde **(15)** (1 mmol, 0.12 mL), and 3,4-dimethoxybenzaldehyde **(16)** (1 mmol, 0.166 g). The synthesized chalcones were characterized by UV, ^{1}H-NMR, ^{13}C-NMR and HRMS spectroscopy. The spectral data for chalcones **(1-9)** are presented as follows:

Chalcone **(1)**, yellow solid (yield 86%). UV (methanol, λ_{max} 234 and 323 nm). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.03 (*d*, *J* = 8.1 Hz, H-2'/6'), 7.82 (*d*, *J* = 15.7 Hz, H-β), 7,65 (*dd*, *J* = 7.6 and 3.8 Hz, H-2/6), 7.6 (*t*, *J* = 8.2 Hz, H-4'), 7.54 (*d*, *J* = 15.7 Hz, H-α), 7.51 (*t*, *J* = 7.3 Hz, H-3'/5'), 7.42 (*m*, H-3/4/5). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 190.7 (C=O), 145.0 (C-β), 138.4 (C-1'), 135.0 (C-1), 132.9 (C-4'), 130.7 (C-4), 128.8 (C-3'/5'), 129.1 (C-2'/6'), 128.6 (C-2/6), 128.6 (C-3/5) 122.2 (C-α). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{15}H_{12}O$ $[M+H]^+$ 209.0966 found 209.0963 m/z. Spectroscopic data compared with literature (Marcovicz et al., 2022).

4-bromochalcone **(2)**, yellow solid (yield 55%). UV (methanol, λ_{max} 219 and 316 nm). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.01 (*d*, *J* = 8.5 Hz, H-2'/6'), 7.74 (*d*, *J* = 15.7 Hz, H-β), 7.60 (*t*, *J* = 5.5 Hz, H-4'), 7.45-7.55 (*m*, 7H H-2/3/5/6/3'/5'/α). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 190.4 (C=O), 143.5 (C-β), 138.1 (C-1'), 133.9 (C-1), 133.1 (C-4'), 132.4 (C-3/C-5), 129.9 (C-3'/C-5'), 128.8 (C-2/C-6), 128.7 (C-2'/C-6'), 124.9 (C-4), 122.7 (C-α). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{15}H_{11}BrO$ $[M+H]^+$ 287.0072 found 287.0078 m/z. Spectroscopic data compared with literature (Marcovicz et al., 2022).

4-methylchalcone **(3)**, yellow solid (yield 70%). UV (methanol, λ_{max} 230 nm and 311 nm). ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.02 (*dd*, *J* = 8.2 and 1.3 Hz, H-2'/6'), 7.80 (*d*, *J* = 15.7 Hz, H-β), 7.58 (*tt*, *J* = 8.7 Hz, 2.1 Hz, H-4'), 7.55 (*d*, *J* = 8.2 Hz, H-3'/5'), 7.51 (*d*, *J* = 7.8 Hz, H-2/6), 7.50 (*d*, *J* = 15.7 Hz, H-α), 7.23 (*d*, *J* = 7.8 Hz, H-3/5), 2.40 (*s*, 4-CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 190.8 (C=O), 145.1 (C-β), 141.3 (C-1'), 138.5 (C-1), 132.8 (C-4'), 132.3 (C-4), 129.8 (C-2'/6'), 128.7 (C-3'/5'), 128.6 (C-2/6), 128.6 (C-3/C-5), 121.2 (C-α), 21.7 (4-CH_3). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{16}H_{14}O$ $[M+H]^+$ 223.1122 found 223.1122. Spectroscopic data compared with literature (Talniya & Sood, 2016).

4-methoxychalcone **(4)**, yellow solid (yield 33%). UV (methanol, λ_{max} 241 and 343 nm).^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 8.05 (*d*, *J* = 8.9 Hz, H-2'/6'), 7.80 (*d*, *J* = 15.6 Hz, H-β), 7.64 (*dd*, *J* = 2.4 and 4.5 Hz, H-2/6), 7.54 (*d*, *J* = 15.6 Hz, H-α), 7.40 (*m*, H-3'/4'/5'), 6.98 (*d*, *J* = 8.9 Hz, H-3/5), 3.86 (*s*, 4-

OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 188.9 (C=O), 163.6 (C-4′), 144.1 (C-β), 135.2 (C-1′), 131.2 (C-1), 131.0 (C-2′/C-6′), 130.5 (C-α), 129.1 (C-2/C-6), 128.5 (C-3/C-5) 122.0 (C-4), 114.0 (C-3′/C-5′), 55.6 (4-OCH_3). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{16}H_{14}O_2$ $[M+H]^+$ 239.1072 found 239.1072. Spectroscopic data compared with literature (Acho et al., 2024)

4′-methoxychalcone **(5)**, yellow solid (yield 73%). UV (methanol, λ_{max} 227 and 319 nm).^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 8.04 (*d*, *J* = 8.5 Hz, H-2′/6′), 7.80 (*d*, *J* = 15.6 Hz, H-β), 7.63 (*dd*, *J* = 3.1 and 5.9 Hz, H-2/6), 7.54 (*d*, *J* = 15.6 Hz, H-α), 7.39 (*m*, H-3/4/5), 6.97 (*d*, *J* = 8.5 Hz, H-3′/5′), 3.86 (*s*, 4′-OCH_3). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 188.7 (C=O), 163.4 (C-4′), 143.9 (C-β), 135.1 (C-1′), 131.1 (C-1), 130.9 (C-2′/C-6′), 130.8 (C-α), 129.0 (C-2/C-6), 128.4 (C-3/C-5) 121.9 (C-4), 113.9 (C-3′/C-5′), 55.5 (4′-OCH_3). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{16}H_{14}O_2$ $[M+H]^+$ 239.1072 found 239.1064. Spectroscopic data compared with literature (Harmastuti et al., 2012).

4-bromo-4-′methoxychalcone **(6)**, yellow solid (yield 57%). UV (methanol, λ_{max} 232 nm and 323 nm). ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 8.03 ppm (*d*, *J* = 8.8 Hz, H-2′/6′), 7.73 ppm(*d*, *J* = 15.7 Hz, H-β), 7.57-7.48 ppm (*m,* H-α/2/3/5/6), 6.99 (*d*, *J* = 8.8 Hz, H-3′/5′), 3,89 (*s*, 4′-OH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 188.5 (C=O), 163.6 (C-4′), 142.6 (C-β), 134.1 (C-1′), 132.3 (C-2′/C-6′), 131.0 (C-2/C-6), 130.9 (C-3/C-5), 129.8 (C-1), 124.7 (C-4), 122.5 (C-α), 114.0 (C-3′/C-5′), 55.6 (4′-OCH_3). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{16}H_{13}O_2Br$ $[M+H]^+$ 317.0177 found 317.0183. Spectroscopic data compared with literature (Elfi & Mulyani, 2022)

4-methyl-4′-methoxychalcone **(7)**, yellow solid, (yield 71%). UV (methanol, λ_{max} 231 nm and 323 nm). ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 8.03 (*d*, *J* = 8.9 Hz, H-2′/6′), 7.78 (*d*, *J* = 15.6 Hz, H-β), 7.54 (*d*, *J* = 7.8 Hz, H-2/6), 7.50 (*d*, *J* = 15.6 Hz, H-α), 7.22 (*d*, *J* = 7.8 Hz, H-3/5), 6.98 (*d*, *J* = 8.9 Hz, H-3′/5′), 3.88 (*s*, 4′-OCH_3), 2.39 (*s*, 4-CH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 188.9 (C=O), 163.4 (C-4′), 144.2 (C-β), 140.9 (C-1′), 132.4 (C-1), 131.3 (C-4), 130.9 (C-2′/C-6′), 129.8 (C-2/C-6), 128.5 (C-3/C-5), 121.0 (C-α), 113.9 (C-3′/C-5′), 55.6 (4′-OCH_3), 21.6 (4-CH_3). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{17}H_{16}O_2$ $[M+H]^+$ 253.1238 found 253.1229. Spectroscopic data compared with literature (Perdana et al., 2015)

4,4′-dimethoxychalcone **(8)**, yellow solid, (yield 59%). UV (methanol, λ_{max} 233 nm and 345 nm). ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 8.01 (*d*, *J* = 8.9 Hz, H-2′/6′), 7.76 (*d*, *J* = 15.6 Hz, H-β), 7.57 (*d*, *J* = 8.7 Hz, H-2/6), 7.41 (*d*, *J* = 15.6 Hz, H-α), 6.95 (*d*, *J* = 8.9 Hz, H-3′/5′), 6.90 (*d*, *J* = 8.7 Hz, H-3/5), 3.84 (*s*, 4′-OH_3), 3.82 (*s*, 4-OH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 188.7 (C=O), 163.3 (C-4′), 161.5 (C-4), 143.8 (C-β), 131.4 (C-1′), 130.7 (C-2′/6′), 130.2 (C-2/C-6), 127.8 (C-1), 119.5 (C-α), 114.4 (C-3′/C-5′), 113.8 (C-3/C-5), 55.4 (4′-OCH_3), 55.0 (4-OCH_3),. High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{17}H_{17}O_3$ $[M+H]^+$ 269.1178 found 269.1174. Spectroscopic data compared with literature (Perdana et al., 2015).

3,4,4′-Trimethoxychalcone **(9),** yellow solid, (yield 78%). UV (methanol, λ_{max} 234 nm and 355 nm). ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 8.00 (*d*, *J* = 8.4 Hz, H-2′/6′), 7.72 (*d*, *J* = 15.5 Hz, H-β), 7.39 (*d*, *J* = 15.5 Hz, H-α), 7.19 (*dd*, *J* = 2.0 and 8.0 Hz, H-6), 7.10 (*d*, *J* = 2.0 Hz, H-2), 6.93 (*d*, *J* = 8.5 Hz, H-3′/5′), 6.85 (*d*, *J* = 8.0 Hz, H-5), 3.91 (*s*, 3-OCH_3), 3.88 (*s*, 4-OCH_3), 3.83 (*s*, 4′-OCH_3). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm): 188.9 (C=O), 163.3 (C-4′), 151.2 (C-3), 149.2 (C-4), 144.1 (C- β), 131.3 (C-1′), 130.7 (C-2′/C-6′), 128.1 (C-1), 123.0 (C-6), 119.8 (C-α), 113.8 (C-3′/C-5′), 111.1 (C-5), 110.1 (C-2), 56.0 (3-OCH_3), 55.9 (4-OCH_3), 55.5 (4′-OCH_3). High-resolution mass spectrometry (HRMS) [electrospray ionization-time-of-flight (ESI-TOF)] *m/z* calculated for $C_{18}H_{19}O_4$ $[M+H]^+$ 299.1283 found 299.1274. Spectroscopic data compared with literature (Rehana et al., 2019).

Cytotoxic Test

A resazurin-based colorimetric assay was conducted using PrestoBlue™ reagent to assess the cytotoxic properties of synthesized chalcones (Fareza et al., 2021; Boncler et al., 2014). MCF-7 and DU-145 cancer cell lines were obtained from the Central Laboratory of Padjadjaran University, Indonesia. Cells with at least 70% confluence were prepared. After removing the culture media, cells were rinsed twice with 1 mL of PBS, treated with 1 mL of trypsin-EDTA, and incubated for 5 minutes to disperse the cell layer. The cell suspension was then centrifuged, and the pellet was resuspended in fresh media. A trypan blue solution was added to the cell suspension for viability analysis. The hemocytometer was cleaned with 70% ethanol, and 10 μL of the trypan blue-cell mixture was loaded into the hemocytometer. Viable cells per mL were calculated to determine cell density. Subsequently, 17,000 cells per well were seeded into 96-well plates and incubated for 24 hours at 37°C in a 5% CO_2 gas content. Cisplatin was used as a positive control drug.

RESULTS AND DISCUSSION

Synthesis and Characterization of Chalcones

Chalcone compounds **1-9** were synthesized via the Claisen-Schmidt condensation reaction under strong alkaline conditions using acetophenone **(10–11)** and

benzaldehyde (12–16) (Figure 1). The synthesis of the chalcone reaction mechanism involves several reaction stages, namely the attack of an alpha hydrogen by a base on acetophenone to form an enolate ion. The enolate ion, acting as a nucleophile, then attacks the electrophilic carbonyl group of benzaldehyde, resulting in a β-hydroxy ketone intermediate. Furthermore, the β-hydroxy ketone compound will undergo dehydration to form an unsaturated α,β-carbonyl conjugate (Harmastuti et al., 2012). In synthesizing chalcone (1) without substituents, a yield of 86% was obtained, indicating that the selected reaction conditions were quite optimal. In general, the influence of substituents, both electron-withdrawing and electron-donating, on acetophenone and benzaldehyde can affect the yield in chalcone synthesis (Donaire-Arias et al., 2023). The presence of electron-donating substituents such as bromo (-Br), methyl (-CH_3), and methoxy (-OCH_3) groups on benzaldehyde can reduce the electrophilic properties of benzaldehyde, resulting in a lower yield of chalcone which can be seen from the yield of chalcone derivatives, namely 4-bromochalcone (55%), 4-methylchalcone (70%), and 4-methoxychalcone (33%) compared to unsubstituted chalcone (86%). On the other hand, the addition of electron-pushing groups to acetophenone can actually increase the yield obtained, such as in the compounds 4-bromo-4′-methoxychalcone (57%) and 4,4′-dimethoxychalcone (7) (59%) when compared to 4-bromochalcone (55%) and 4-methoxychalcone (33%). Adding electron-pushing groups to acetophenone can increase its nucleophilicity.

The synthesized chalcones were characterized by UV-Vis, ^{1}H-NMR, ^{13}C-NMR, and HRMS spectroscopy. The UV spectra of the chalcones displayed two wavelength absorption peaks, indicating the presence of benzoyl and cinnamoyl groups (Aksöz & Ertan, 2012). The chalcone molecule in the benzoyl group exhibits a characteristic signal for symmetrical 1,4-disubstituted benzene in its ^{1}H-NMR spectra (Table 1). The signals for the aromatic regions H-2'/6' and H-3'/5' on the chemical shift will show up as two signals each for ^{2}H. In the cinnamoyl group, the ABX coupling system of the 1,3,4-trisubstituted aromatic ring was observed as three distinct signals. A singlet signal for ^{1}H in the ^{1}H-NMR spectrum indicated the presence of a methoxy group. The *trans* configuration of the chalcone molecule was confirmed by the coupling constants (15–16 Hz) of the H-α and H-β protons in the unsaturated carbonyl system. The ^{13}C-NMR spectrum (Table 2) shows the presence of 15 carbon signals in the chalcone basic structure. In general, the appearance of two signals with high intensity is characteristic for carbon signals in the C-2'/6' and C-3'/5' benzoyl groups, representing the presence of two symmetrical carbon atoms. The methoxy and carbonyl group in the chalcone compound will appear at a chemical shift of around 55 Hz and 188 Hz, respectively.

Evaluation of Cytotoxic Activity

The cytotoxic effects of chalcones 1-9 were assessed on breast cancer MCF-7 and prostate cancer DU-145 cell lines using the PrestoBlue assay (Fareza et al., 2021). Cisplatin, a standard chemotherapeutic agent for various cancers, including breast and prostate cancer, served as a positive control (Brown et al., 2019; Huang et al., 2021). Table 3 displays the cytotoxic results of the chalcone compounds 1–9.

Substituents in the chalcone framework, particularly electron-donating and electron-withdrawing groups, had a significant impact on anticancer activity. For example, the presence of methoxy or methyl groups at C-4 in chalcone 3, 4, and 5 decreased cytotoxicity against both DU-145 and MCF-7 cells compared to chalcone (1). Moving the methoxy group from C-4 (chalcone 4) to C-4′ (chalcone 5) further diminished cytotoxicity, especially in DU-145 cells. Karthikeyan et al. (2015) reported that electron-donating groups such as methoxy in ring A and di/tri-methoxy groups in ring B can increase anticancer activity in chalcones. However, additional methoxy substitutions in chalcones 5, 7, and 9 showed reduced activity, particularly in DU-145 cells, contrary to Bandgar et al. (2010), who reported increased activity with similar substitutions. It has also been reported that chalcones α,β-unsaturated carbonyl moiety play a significant role in its anticancer activity (Silva et al, 2018).

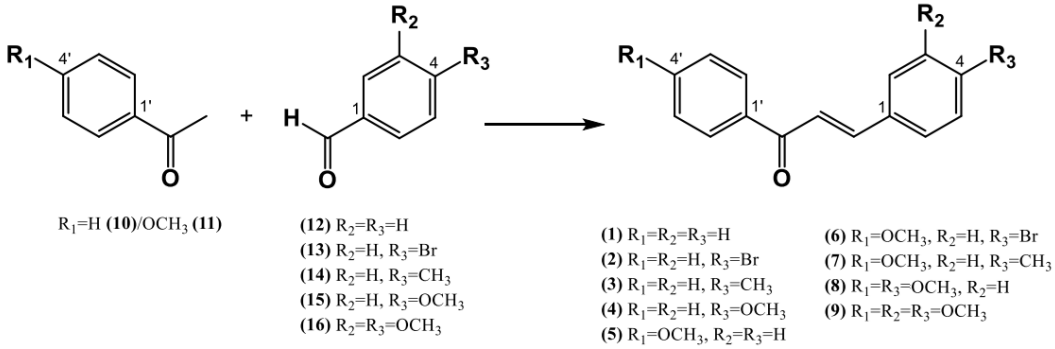

Figure 1. Synthesis of chalcone with various acetophenones and benzaldehydes

Table 1. ^{1}H-NMR (500 MHz, $CDCl_3$) data of Chalcones (**1-9**)

No	δ_H (multiplicity, J Hz)								
	1	2	3	4	5	6	7	8	9
H-1	-	-	-	-	-	-	-	-	-
H-2	7.65 (*dd*, 7.6&3.8)	7.45-7.55 (*m*, overlap)	7.51 (*d*, 7.8)	7.64 (*dd*,2.4&4.5)	7.63 (*dd*, 3.1&5.9)	7.57-7.48 (*m*)	7.54 (*d*, 7.8)	7.57 (*d*, 8.7)	7.10 (*d*, 2.0)
H-3	7.42 (*m*)	7.45-7.55 (*m*, overlap)	7.23 (*d*, 7.8)	7.40 (*m*)	7.39 (*m*)	7.57-7.48 (*m*)	7.22 (*d*, 7.8)	6.90 (*d*, 8.7)	-
H-4	7.42 (*m*)	-	-	-	7.39 (*m*)	-	-	-	-
H-5	7.42 (*m*)	7.45-7.55 (*m*, overlap)	7.23 (*d*, 7.8)	7.40 (*m*)	7.39 (*m*,	7.57-7.48 (*m*)	7.22 (*d*, 7.8)	6.90 (*d*, 8.7)	6.85 (*d*, 8.0)
H-6	7,65 (*dd*, 7.6&3.8)	7.45-7.55 (*m*, overlap)	7.51 (*d*, 7.8)	7.64 (*dd*, 2.4&4.5)	7.63 (*dd*, 3.1&5.9)	7.57-7.48 (*m*)	7.54 (*d*, 7.8)	7.57 (*d*, 8.7)	7.19 (*dd*, 2.0&8.0)
H-1′	-	-	-	-	-	-	-	-	-
H-2′	8.03 (*d*,8.1)	8.01 (*d,* 8.5)	8.02 (*dd*, 8.2&1.3)	8.05 (*d*, 8.9)	8.04 (*d*, 8.5)	8.03 (*d*, 8.8)	8.03 (*d*, 8.9)	8.01 (*d*, 8.9)	8.00 (*d*, 8.4)
H-3′	7.51 (*t*, 7.3)	7.45-7.55 (*m*, overlap)	7.55 (*d,* 8.2)	6.98 (*d*, 8.9)	6.97 (*d*, 8.5)	6.99 (*d*, 8.8)	6.98 (*d*, 8.9)	6.95 (*d*, 8.9)	6.93 (*d*, 8.5)
H-4′	7.6 (*t*,8.2)	7.60 (*t*, 5.5)	7.58 (*tt*, 8.7 & 2.1)	7.40 (*m*)	-	-	-	-	-
H-5′	7.51 (*t,* 7.3)	7.45-7.55 (*m*. overlap)	7.55 (*d,* 8.2)	6.98 (*d*, 8.9)	6.97 (*d*, 8.5)	6.99 (*d*, 8.8)	6.98 (*d*, 8.9)	6.95 (*d*, 8.9)	6.93 (*d*, 8.5)
H-6′	8.03 (*d*, 8.1)	8.01 (*d,* 8.5)	8.02 (*dd*, 8.2&1.3)	8.05 (*d*, 8.9)	8.04 (*d*, 8.5)	8.03 (*d*, 8.8)	8.03 (*d*, 8.9)	8.01 (*d*, 8.9)	8.00 (*d*, 8.4)
H-α	7.54 (*d*, 15.7)	7.45-7.55 (*m*, overlap)	7.50 (*d*, 15.7)	7.54 (*d*, 15.6)	7.54 (*d*, 15.6)	7.57-7.48 (*m*)	7.50 (*d*, 15.6)	7.41 (*d*, 15.6)	7.39 (*d*, 15.5)
H-β	7.82 (*d*,15.7)	7.74 (*d,* 15.7)	7.80 (*d*, 15.7)	7.80 (*d*, 15.6)	7.80 (*d*, 15.6)	7.73 (*d*, 15.7)	7.78 (*d*, 15.6)	7.60 (*d*, 15.6)	7.72 (*d*, 15.5)
3-OCH_3	-	-	-	-	-	-	-	-	3.91 (*s*)
4-OCH_3	-	-	-	63.86 (*s*)	-			3.82 (*s*)	3.88 (*s*)
4′-OCH_3	-	-	-	-	3.86 (*s)*	3.89 (*s*)	3.88 (*s*)	3.84 (*s*)	3.83 (*s)*
4-CH_3	-	-	2.40 (*s*)	-	-	-	2.39 (*s*)	-	-

Table 2. ^{13}C-NMR (125 MHz, $CDCl_3$) data of Chalcones (**1-9**)

No C	1	2	3	4	5	6	7	8	9
1	135.0	133.9	138.5	131.2	131.1	129.8	132.4	127.8	128.1
2	128.6	128.8	128.6	129.1	129.0	131.0	129.8	130.2	110.1
3	128.6	132.3	128.6	128.5	128.4	130.9	128.5	113.8	151.2
4	130.7	124.9	132.4	122.0	121.9	124.7	131.3	161.5	149.2
5	128.6	132.4	128.6	128.5	128.4	130.9	128.5	113.8	111.1
6	128.6	128.8	128.6	129.1	129.0	131.0	129.8	130.2	123.0
1′	138.4	138.1	141.3	135.2	135.1	134.1	140.9	131.4	131.3
2′/6′	129.1	128.7	129.8	131.0	130.9	132.3	130.9	130.7	130.7
3′/5′	128.8	129.9	128.7	114.0	113.9	114.0	113.9	114.4	113.8
4′	132.9	133.1	132.8	163.6	163.4	163.6	163.4	163.3	163.3
α	122.2	122.7	121.2	130.5	130.8	122.5	121.0	119.5	119.8
β	145.0	143.5	145.1	144.1	143.9	142.6	144.2	143.8	144.1
3-OCH_3	-	-	-	-	-	-	-	-	56.0
4-OCH_3		-	-	55.6	-	-	-	55.0	55.9
4′-OCH_3		-	-	-	55.5	55.6	55.6	55.4	55.5
4-CH_3		-	21.7	-	-	-	21.6		-
C=O	190.7	190.4	190.8	188.9	188.7	188.5	188.9	188.7	188.9

Table 3. Cytotoxicity of chalcone **1-9**

Chalcones	R	IC_{50} (μM)	
		MCF-7	DU-145
Chalcone (**1**)	R_1=R_2=R_3=H	98.19	190.29
4-Bromochalcone (**2**)	R_1=Br, R_2=R_3=H	26.99	145.98
4-Methylchalcone (**3**)	R_1=CH_3, R_2=R_3=H	110.62	356.07
4-Methoxychalcone (**4**)	R_1= R_2=H, R_3=OCH_3	92.20	299.13
4′-Methoxychalcone (**5**)	R_1=OCH_3, R_2=R_3=H	89.51	951.99
4-Bromo-4′-Methoxychalcone (**6**)	R_1=OCH_3, R_2=H, R_3=Br	993.82	>1500
4-Methyl-4′-Methoxychalcone (**7**)	R_1=OCH_3, R_2=H, R_3=CH_3	>1500	>1500
4,4′-Dimethoxychalcone (**8**)	R_1=R_3=OCH_3 R_2=H	1441.43	>1500
3,4,4′-Trimethoxychalcone (**9**)	R_1=R_2=R_3=OCH_3	80.31	409.42
Cisplatin		39.26	7.59

Among the synthesized chalcones, 4-bromochalcone (**2**) demonstrated the strongest cytotoxic activity against both cell lines. The addition of an electron-withdrawing bromine atom at the 4′ position in ring B (cinnamoyl) increased anticancer activity compared to chalcone **1** and other derivatives (chalcones **3**–**5**) containing methyl or methoxy groups. This result aligns with previous studies showing that halogen substitutions (F, Cl, Br) in chalcone derivatives increase antiproliferative effects (Dias et al., 2013). Notably, 4'-bromochalcone has previously shown anticancer potential with a GI_{50} of 38.1 μM against MCF-7 cells (Prabhakar et al., 2014).

Interestingly, the cytotoxicity of methoxylated chalcones varied between the cell lines. Some compounds exhibited greater activity against MCF-7 cells, while others were more potent against DU-145 cells, suggesting cell line-specific responses. This differential sensitivity may stem from distinct molecular pathways regulating breast and prostate cancer cell survival. Chalcone compounds and their derivatives are known to induce apoptosis by targeting key proteins such as NF-κB, p53, BCRP, VEGF, and EGFR (Das & Manna, 2016; Constantinescu et al., 2021).

CONCLUSIONS

This study demonstrated that some methoxylated chalcones exhibit cytotoxic activity against MCF-7 and DU-145 cancer cell lines, with specific structural modifications enhancing their potency. The findings underscore the potential of these compounds as novel anticancer agents, particularly in cancers with resistance to standard therapies. Notably, brominated chalcone derivates appear to be a good candidates for antibreast cancer agents. Further optimization and mechanistic studies are essential to fully harness their therapeutic potential.

ACKNOWLEDGMENTS

The authors would like to thank Jenderal Soedirman University for the financial support through Sceme Grant for Research Institutions LPPM UNSOED (No. (132/UN23.18/PT.01.02/2022) in the fiscal year of 2022.

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