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Synthesis and preliminary biological assessment of novel chalcone derivatives derived from Duff's formylated mephenesin

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There is an urgent need in the medical community today for the discovery of anti-carcinogenic and anti-oxidative medicines that are more effective and have fewer adverse effects. To accomplish this goal, novel chalcone compounds (**A1–A6**) were produced by subjecting the Duff formylated mephenesin (**I**) and substituted aromatic ketones to a Claisen-Schmidt condensation reaction. Through the use of well-established spectroscopical techniques, the basic chemical structures of the produced derivatives were deduced. The MTT assay was used to determine how effective the produced chalcone derivatives were *in vitro* as neoplastic inhibitory agents against five different malignant cell lines, including HepG2, A549, MCF-7, HeLa, and Ovcar-3. In a similar manner, the anti-oxidative impact of chalcone derivatives was examined *in vitro* by making use of the DPPH moiety. According to the data, the derivative with the potent electronegative substituent, **A2**, exhibited the most potent anti-neoplastic impact compared to the other derivatives, with 50% inhibitory concentration (IC50) values that were quite near to those of the gold standard (doxorubicin). This effect was shown especially in relation to the HepG2 and A549 cells. In addition, the derivative **A2** showed that it had a significant antioxidative impact on the examined free radical moieties, with percentage inhibition (Pi) values that were close to those of the standard (ascorbic acid).

KEYWORDS

Chalcone derivatives; claisen-schmidt condensation; Duff formylation; anticancer; antioxidant.

Introduction

Cancer is a significant problem on a worldwide scale. It is a term that refers to a category of disorders in which cells proliferate uncontrollably and have the potential to invade other organs or spread across the body [1-6]. It is a lethal condition that ranks only behind disorders of the heart and blood vessels in terms of the mortality and morbidity that it causes [7]. One in five men and one in six women may receive a cancer diagnosis at some point in their lives, according to the World Health Organization (WHO) [8]. It is important to point out that established registries of mortality and incidence have not dropped lately, and unfortunately, it is expected that cancer will continue to be the main cause of death in the years to come [9]. This is something that should be highlighted, as it is crucial. Even while cancer research has produced several innovative and effective solutions, the treatments that are now in use have apparent practical concerns, such as limited specificity, lethal side effects, and cancer that is resistant

to many drugs. Current scientific and commercial priorities place a high premium on the discovery of new neoplastic inhibitory medicines that exhibit the characteristics of potency, safety, and selectivity [10, 11]. The complex state known as oxidative stress affects biological systems and is characterized by unchecked production of free radical moieties and insufficient endogenous and external antioxidative activities in the body to capture and eliminate these reactive moieties [12]. When the body's natural and external antioxidative processes fail to successfully get rid of reactive molecules, it experiences oxidative stress. The oxidative stress has been proven to have a strong connection with the beginning or the onset of a broad range of diseases, including but not limited to cancer, diabetes mellitus, cardiovascular disease, and others according to many studies conducted on individuals. These findings highlight the significance of achieving a suitable equilibrium between antioxidants and free moieties that are reactive to the environment to lessen the impact on the body's physiological and pathological processes that may otherwise occur [13]. Antioxidants are seen as a potential treatment for the damage brought on by the free radicals and harmful moieties. Too far, however, only a select few antioxidants have been approved for use in clinical settings because of the significant adverse effects and poor effectiveness associated with their usage. As a result, there is an urgent need for innovative antioxidants that are more effective while having less negative side effects [14].

Given that their chemistry is straightforward and that chemical synthesis is a reasonably simple process, substances with an enone system inside their molecules are still of a significant amount of interest. They are of significant value in biological research, and have the potential to serve as precursors in organic chemistry [15,16]. Chalcones, also known as chalconoids and 1,3-diaryl-2-propen-1-ones, are compounds that may be found in nature as well as those that have been artificially created. In chalcones, aromatic residues are connected by *α,β*-unsaturated chain of an electrophile that is made out of three carbon atoms [17,18]. Molecules that include a 1,3-diaryl-2-propen-1-one residue are found to have several biological actions, and as a result, they are of special significance in the field of pharmacology [19-22]. This is shown by the findings that have been published in the relevant scientific journals. Among the most notable biological actions of chalcones are their capabilities as anticancer agents, antibacterial agents, antiulcer agents, analgesics, antipyretics, anti-inflammatory agents, anti-fungal drugs, cytotoxic agents, anti-viral drugs, anti-malarial agents, anti-tuberculosis drugs, anti-parasitic agents, and anti-malarial agents and anti-hepatotoxic agents [23,24]. Chalcones feature an extended conjugate system, which makes it easier for them to attach to biomolecules that are active, such as enzymes and DNA [25]. Chalcones are a class of organic compounds. The presence of a carbonyl group with *a,β*-unsaturated double bond, which functions as a Michael acceptor and makes it easier for chalcones to engage with sulfhydryl groups found in the presence of cysteine or thiol groups, is a necessary condition for this interaction to take place. It is believed that this interaction is responsible for the diverse biological features that these chemicals possess [26]. In addition, the chemical structure of chalcones, particularly the substituents placed on their two aromatic residues, has a significant influence on the pharmacological action of these substances. Its capacity to modulate molecular pathways associated with cancer, which in turn positively influences apoptosis, metastasis, and the response to cellular stress, is one of the reasons why this substance is beneficial. There are a lot of different methoxy chalcones with anticancer characteristics, and because

of the existence of a methoxy group is beneficial for this activity [27, 28]. These chemicals provide an important skeleton for the structure for the development of novel anticancer drugs since chalcones have biological efficacy in laboratory and animal models, in malignancies that are amenable to treatment as well as in the case of cancers that are resistant to therapy. In addition, 1,3-diaryl-2-propen-1-ones are a crucial family of naturally occurring tiny compounds that have a chemotherapeutic activity on cancer [29].

There are three techniques for modulating natural chalcones used to create compounds with superior anticancer properties: modifying the presence of substituents on the two aromatic residues (acetophenone and aldehyde); replacing the residues of aromatic compounds with heterocycles; creating molecular hybrids by linking chalcones in conjunction with other compounds that have anticancer activities; these are three methods [16].

This study's goal is to create various new chalcone-4'-propoxy-1,2-diol derivatives (**A1-A6**). The neoplastic inhibiting impact of the produced derivatives (**A1-A6**) was investigated *in vitro* by the use of an MTT-based assay on five different malignant cell lines, including HepG2, A549, Ovcar-3, HeLa, and MCF-7.

Experimental

Chemicals and instruments

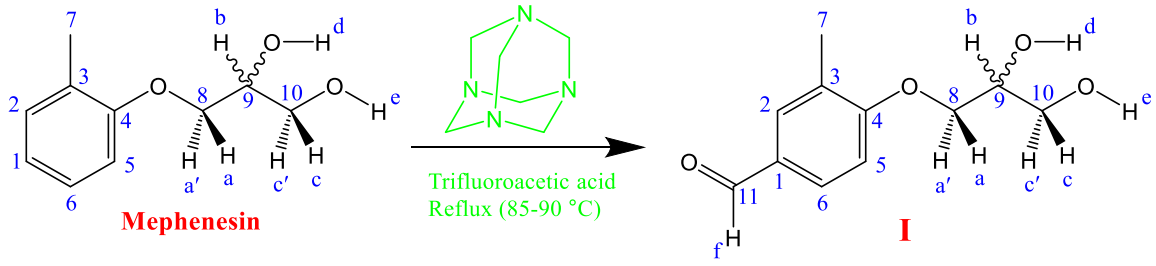
To create chalcone derivatives, chemical substances, solvents, and reagents in addition to the biological evaluation systems that were acquired from a variety of worldwide resources, such as Hyper Chemical Limited, Thomas Baker, CDH, Sigma-Aldrich, Scharlau and others. The purchased biological and chemical agents were used right away without being further purified. To determine melting points, a Stuart's SMP3 melting temperature instrument was used in open capillary tubes without pre-correction. TLC, or thin-layer chromatography, was used to assess the lack of impurity in the synthesized derivatives and monitor the synthesis progress. An aluminum-backed TLC with a silica gel 60 F_{254} coating sheet and a mixture of methanol: ethylacetate: n-hexane (1:3:3) are the stationary and mobile phases of this technique. The IR spectra were obtained with the use of an ATR-FTIR spectrophotometer (Schimadzu, Japan), ^{1}H-NMR and ^{13}C-NMR spectral patterns of the newly manufactured substances were recorded on an Inova reveals the Ultra Shield operating at respective frequencies of 500 and 125 MHz.

Chemical pathways

Synthesis of compound (I) 4-(2,3-dihydroxypropoxy)-3-methylbenzaldehyde; Duff formylation of mephenesin (Scheme 1)

To a solution of mephenesin 3.64 g (20 mmol) in 15 mL of trifluoroacetic acid, the amount of hexamethylenetetramine that was added was 2.8 g (20 mmol). The solution was heated at a reflux temperature of (85–90 °C) for 12 h. After it had been brought down to room temperature, the solution was swirled for 15 minutes while it was concentrated to roughly 2/3 of its initial volume and blended with 60 mL of cold water. The resulting liquid was made basic with Na_2CO_3 to a pH of 8 after the stirring was completed [30]. After that, the mixture was extracted with 100 mL of *n*-butanol three times [31,32]. After being dried on $MgSO_4$, the solution was filtered before being concentrated under decreasing pressure until it reached dryness, and then recrystallized from chloroform-heptane to afford a yellow powder 2.73 g (13 mmol) (65 % yield) of 4-(2,3-dihydroxypropoxy)-3-methylbenzaldehyde (mp 94–96 °C). R_f = 0.35; IR ν_{max} (cm^{-1}): 3360 (br, alkyl diol O-H), 2927, 2883 (m, alkyl C-H), 1674 (s, aryl aldehyde C=O), 1600, 1581, 1500 (s, aromatic

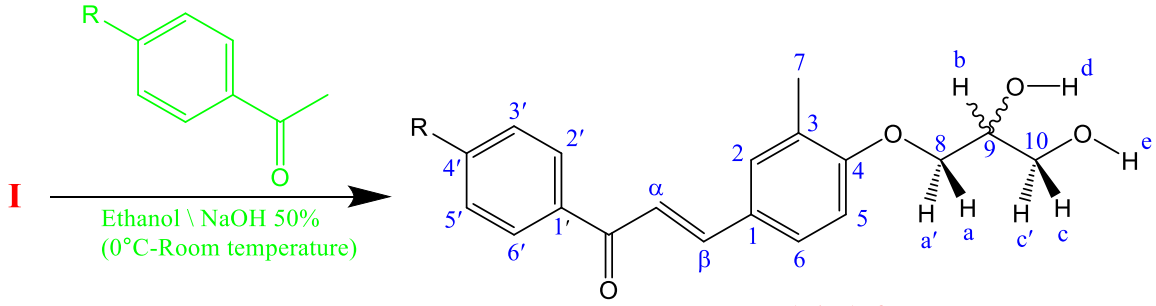
C=C), 1257, 1033 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 9.91 (1H, s, f-H), 7.69 (1H, d, H-6), 7.52 (1H, s, H-2), 6.92 (1H, d, H-5), 4.88 (1H, s, H-d), 4.63 (1H, s, H-e), 3.88 (3H, m, H-a, H-a′, H-b), 3.47 (2H, m, H-c, H-c′), 2.09 (3H, s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 190.1 (C-11), 163.7 (C-4), 130.7 (C-1, C-2, C-6), 72.6 (C-8, C-9), 64.1 (C-10), and 16.3 (C-7).



SCHEME 1 The synthesis of (**I**) 4-(2,3-dihydroxypropoxy)-3-methylbenzaldehyde from Mephenesin

Standard operating method of operation for the preparation of α, β-unsaturated ketones (chalcones A1-A6) via Claisen-Schmidt reaction [33] (Scheme 2)

One mmol (0.21 g) of 4-(2,3-dihydroxypropoxy)-3-methylbenzaldehyde (**I**) was dissolved in 5 mL of ethanol and kept stirring at 0 ˚C, and then 0.5 mL of 40% NaOH solution was gradually added while swirling constantly for 10 minutes. The substituted acetophenone derivative (1 mmol) was introduced into the mixture undergoing the reaction in a drop by drop manner. The reaction mixture had been diluted with 5 mL of water and stored in the refrigerator overnight after being swirled continuously for a full day at room temperature. To extract chalcone in its purest form, the produced precipitate was cleaned with ice water after being filtered many times, and then recrystallized by the use of ethanol [34].



A1:R=H; **A2**:R=CF3; **A3**:R=Cl
A4:R=Br; **A5**:R=F; **A6**:R=NO_2

SCHEME 2 The synthesis of substituted chalcones (**A1-A6**) from (**I**) 4-(2,3-dihydroxypropoxy)-3-methylbenzaldehyde

3-(4-(2,3-Dihydroxypropoxy)-3-methylphenyl)-1-phenylprop-2-en-1-one (A1): White powder 0.187 g (60 % yield) (mp 144-146˚C). R_f = 0.5; IR ν_{max} (cm^{-1}): 3417 (br, alkyl diol O-H), 2935, 2885 (m, alkyl C-H), 1651 (s, C=O), 1596, 1562, 1496 (s, aromatic C=C), 1261, 1033 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 7.97 (3H, d, H-2′, H-6′, H-β), 7.46 (5H, m, H-6, H-3′, H-4′, H-5′, H-α), 7.11 (1H, s, 2-H), 6.74 (1H, d, 5-H),

4.85 (1H, s, d-H), 4.66 (1H, s, e-H), 3.85 (1H, m, b-H), 3.76 (2H, m, H-a, H-a′), 3.45 (2H, m, H-c, H-c′), 2.10 (3H, s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 189.7 (C-11), 158.7 (C-4), 145.4 (C-β), 138.2 (C-1′), 134.2 (C-4′), 129.7 (C-1, C-2, C-6, C-2′, C-3′, C-5′, C-6′), 123.1 (C-3, C-α), 112.1 (C-5), 71.6 (C-8, C-9), 63.3 (C-10), and 16.2 (C-7).

3-(4-(2,3-Dihydroxypropoxy)-3-methylphenyl)-1-(4-(trifluoromethyl)phenyl)prop-2-en-1-one (A2): Light yellow powder 0.274 g (72 % yield) (mp 158-160°C). R_f = 0.55; IR ν_{max} (cm^{-1}): 3282 (br, alkyl diol O-H), 2924, 2870 (m, alkyl C-H), 1668 (s, C=O), 1600, 1543, 1508 (s, aromatic C=C), 1253, 1107 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 8.01 (3H, d, H-2′, H-6′, H-β), 7.60 (4H, d, H-6, H-3′, H-5′, H-α), 7.19 (1H, s, H-2), 6.80 (1H, d, H-5), 4.79 (1H, s, H-d), 4.59 (1H, s, H-e), 3.81 (1H, m, H-b), 3.72 (2H, m, H-a, H-a′), 3.48 (2H, m, H-c, H-c′), 2.09 (3H, s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 190.5 (C-11), 156.7 (C-4), 144.4 (C-β), 140.2 (C-1′), 135.2 (C-4′), 129.4 (C-1, C-2, C-6, C-2′, C-3′,C- 5′, C- 6′), 124.2 (C-3, C-α, C-CF_3), 112.1 (C-5), 70.8 (C-8, C-9), 64.2 (C-10), and 17.1 (C-7).

1-(4-Chlorophenyl)-3-(4-(2,3-dihydroxypropoxy)-3-methylphenyl)prop-2-en-1-one (A3): Off white powder 0.256 g (74 % yield) (mp 178-180°C). R_f = 0.53; IR ν_{max} (cm^{-1}): 3275 (br, alkyl diol O-H), 2916, 2850 (m, alkyl C-H), 1653 (s, C=O), 1600, 1543, 1492 (s, aromatic C=C), 1257, 1097 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 7.91 (3H, d, H-2′, H-6′, H-β), 7.55 (4H, d, H-6, H-3′, H-5′, H-α), 7.19 (1H, s, H-2), 6.75 (1H, d, H-5), 4.83 (1H, s, H-d), 4.62 (1H, s, H-e), 3.78 (1H, m, H-b), 3.69 (2H, m, H-a, H-a′), 3.51 (2H, m, H-c, H-c′), 2.15 (3H, s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 191.0 (C-11), 157.2 (C-4), 145.4 (C-β), 141.2 (C-1′), 136.2 (C-4′), 130.1 (C-1, C-2, C-6, C-2′, C-3′,C-5′, C- 6′), 123.9 (C-3, C-α), 111.4 (C-5), 71.1 (C-8, C-9), 63.9 (C-10), and 16.5 (C-7).

1-(4-Bromophenyl)-3-(4-(2,3-dihydroxypropoxy)-3-methylphenyl)prop-2-en-1-one (A4): yellow powder 0.278 g (71 % yield) (mp 175-177°C). R_f = 0.56; IR ν_{max} (cm^{-1}): 3282 (br, alkyl diol O-H), 2954, 2924 (m, alkyl C-H), 1653 (s, C=O), 1600, 1543, 1508 (s, aromatic C=C), 1257, 1103 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 8.05 (3H, d, H-2′, H-6′, H-β), 7.65 (4H, d, H-6, H-3′, H-5′, H-α), 7.17 (1H, s, H-2), 6.85 (1H, d, H-5), 4.77 (1H, s, H-d), 4.55 (1H, s, H-e), 3.84 (1H, m, H-b), 3.75 (2H, m, H-a, H-a′), 3.44 (2H, m, H-c, H-c′), 2.05 (3H, s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 188.5 (C-11), 154.6 (C-4), 145.4 (C-β), 136.1 (C-1′), 131.2 (C-6, C-2′, C-3′,C- 5′, C- 6′) 127.2 (C-1, C-2, C-4′), 122.2 (C-3, C-α), 114.1 (C-5), 71.0 (C-8, C-9), 63.8 (C-10), and 16.6 (C-7).

3-(4-(2,3-Dihydroxypropoxy)-3-methylphenyl)-1-(4-fluorophenyl)prop-2-en-1-one (A5): Off white powder 0.234 g (71 % yield) (mp 179-181°C). R_f = 0.52; IR ν_{max} (cm^{-1}): 3282 (br, alkyl diol O-H), 2920, 2870 (m, alkyl C-H), 1643 (s, C=O), 1604, 1543, 1508 (s, aromatic C=C), 1253, 1041 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 7.91 (3H, d, H-2′, H-6′, H-β), 7.55 (4H, d, H-6, H-3′, H-5′, H-α), 7.14 (1H, s, H-2), 6.83 (1H, d, H-5), 4.83 (1H, s, H-d), 4.55 (1H, s, H-e), 3.78 (1H, m, H-b), 3.68 (2H, m, H-a, H-a′), 3.51 (2H, m, H-c, H-c′), 2.13 (3H, s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 189.8 (C-11), 166.7 (C-4′), 155.7 (C-4), 144.9 (C-β), 134.2 (C-1′), 131.2 (C-6, C-2′, C- 6′), 127.1 (C-1, C-2), 123.8 (C-3, C-α), 114.1 (C-5, C-3′,C- 5′), 71.1 (C-8, C-9), 63.8 (C-10), and 15.9 (C-7).

3-(4-(2,3-Dihydroxypropoxy)-3-methylphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (A6): Light yellow powder 0.225 g (63 % yield) (mp 181-183°C). R_f = 0.46; IR ν_{max} (cm^{-1}): 3263 (br, alkyl diol O-H), 2924, 2850 (m, alkyl C-H), 1654 (s, C=O), 1600, 1543, 1515 (s, aromatic C=C), 1257, 1087 (s, ether C-O-C); ^{1}H-NMR (ppm, 500 MHz, DMSO-d_6): δ= 8.30 (2H, d, H-3′, H-5′), 8.04 (3H, d, H-2′, H-6′, H-β), 7.56 (2H, d, H-6, H-α), 7.14 (1H, s, H-2), 6.82 (1H, d, H-5), 4.81 (1H, s, H-d), 4.56 (1H, s, H-e), 3.80 (1H, m, H-b), 3.70 (2H, m, H-a, H-a′), 3.50 (2H, m, H-c, H-c′), 2.07 (3H,

s, H-7); ^{13}C-NMR (ppm, 125 MHz, DMSO-d_6): δ= 189.5 (C-11), 154.2 (C-4, C-4′), 144.6 (C-β, C-1′), 131.4 (C-6, C-2′, C- 6′), 126.9 (C-1, C-2) 122.9 (C-3, C-α, C-3′, C- 5′), 114.1 (C-5), 71.1 (C-8, C-9), 63.7 (C-10), and 16.1 (C-7).

Assessment of the bioactivities

Neoplastic suppressive results

MCF-7, HepG2, A549, HeLa and Ovcar lines of cells were coming from the Pasteur Institute (Tehran, Iran). Dulbecco's Modified Eagle Medium (GMEM; Gibco, Life Technologies, Waltham, MA, USA) was used for the propagation and maintenance of the cells. FBS (BioWest SAS, Nuaille, France) and PSF (antibiotic antimycotic solution; Sigma-Aldrich®, St. Louis, MO, USA) were mixed into the medium at a certain strength of 10% and 1%, respectively. The incubator was humidified with 5% carbon dioxide, and the temperature was set to 37°C. When the cells had achieved about 75% confluency, they were removed from the culture dish by treating it with 0.1% ethylene diamine tetraacetic acid (Merck, Darmstadt, Germany) and 0.25% trypsin (Gibco, Invitrogen, Waltham, MA, USA) in phosphate-buffered saline (PBS) at 37°C. Following that, the cells were re-suspended in DMEM that had 10% FBS and 1% PSF added to it. Cells were seeded onto the 96-well plates at a density of 5000 cells per well prior to the investigations, and the plates were allowed to rest for 24 hours before the research began. Phosphate buffered saline, sometimes known as PBS, was used to wash the cells, which has a pH of 7.4, and fresh media with varying amounts were utilized of chalcone derivatives (**A1–A6**) along with the standard doxorubicin (500, 250, 125, 62.5, 31.25, and 0 µg/mL) for 72 h. The 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction test was used to ascertain the cell viability assay [35,36]. To assess the cytotoxic impact at different concentrations, MTT was used. MTT (0.5 mg/ml in PBS) was put in each well after it had been incubated for the first 72 hours (37°C, 5% CO_2 in a humid environment). After that, the plate was kept in an incubator for an additional 4 hours at a temperature of 37°C. At 37°C, the formed dissolving formazan in 100 µL of DMSO with light trembling, and the absorbance was assessed using an ELISA reader at 570 nm. The average of three separate trials served as the basis for the results. Then, the concentrations of doxorubicin and chalcone derivatives (**A1-A6**) that which led to a 50% reduction in cell viability (i.e. IC_{50} values) were determined. Table 1 presents the findings.

DPPH Radical-Scavenging Assay of the chalcone derivatives (A1-A6)

DPPH Radical Scavenging Assay for Determining Antioxidant Activity

The 1,1-diphenyl-2-picryl hydrazide (DPPH) radical scavenging technique was used to assess the *in vitro* antioxidant capacity of produced chalcone derivatives (**A1-A6**) using ascorbic acid as a reference . The vivid blue-purple hue and 517 nm absorbance are both due to the DPPH radical, which has an unusual electron. A stable free radical may be quantified spectrophotometrically from the absorbance decline after accepting an electron from an antioxidant molecule and decolorizing [37]. In a solution of 0.1 mM DPPH containing methanol, seven different chemicals were evaluated to see how well they neutralized free radicals. The test chalcone compound samples and ascorbic acid were mixed together with a same amount of a methanolic solution of DPPH, and the mixture was left to incubate without light or visibility at room temperature for forty minutes. The concentrations used were (31, 25, 6, 125, 250, and 500 µg/mL). Using a UV spectrophotometer set to 517 nm, test samples that included the reference

medication ascorbic acid were used to measure the absorbance after incubation to determine the products' antiradical potency. The outcomes of the experiment were represented as the mean of these three determinations, which were carried out three times over. The subsequent equation was used to compute the percentage inhibitions (Pi) and the drugs' IC_{50} [38]:

(Absorbance of sample/Absorbance of control) / 100 equals % Inhibition (Pi).

The conventional medication ascorbic acid's IC_{50} was discovered to be 23.27 µg/mL. Compound **A2** demonstrated excellent radical scavenging or high antioxidant activity among the six novel compounds tested for antioxidant activity using the DPPH technique, with an IC_{50} value less than 50 µg/ml at 45.89 µg/ml. Others, however, exhibit IC_{50} values that range from 57.39 µg/ml for **A6** to 88.50 µg/mL for **A1**, indicating intermediate antioxidant activity [39-41]. None of the compounds, however, demonstrated more than average radical scavenging activity. Table 2 lists the data of antioxidant activity.

Results and discussion

Consideration of the importance of the bioactivities effects that are inhibitive to neoplastic growth

The well-established MTT-dye test procedure was used to evaluate the preliminary neoplastic inhibiting impact of the pure produced chalcone derivatives on five different malignant cell lines *in vitro*. These cells were made up of HepG2, which is a human Caucasian hepatocyte carcinoma cell line; A549 which is a human Caucasian lung carcinoma cell line, HeLa, which is a human cervix epitheloid carcinoma cell line, Ovcar-3 which is a high grade ovarian serous adenocarcinoma cell line and MCF-7 which is a human breast cancer cell line. The test used doxorubicin on the positive side and DMSO on the negative side as the positive and negative dominions, respectively. According to the results of the investigation, which are shown in Table 2, the created derivatives exhibited an anti-neoplastic impact on several investigated malignant cell lines. Despite this, the prepared derivatives were not as powerful as doxorubicin, with IC_{50} values ranging between 0.23 ± 0.01 µM and 1.5 ± 0.06 µM. In contrast to the other produced derivatives, **A2** had a more potent anti-neoplastic impact towards all of the examined malignant cell lines, with a potency that was comparable to that of doxorubicin. This was particularly the case with regard to the A549 and HepG2 cell lines. The inclusion of trifluoromethyl group, which had been added in the formula for the skeleton, to position 4' of **A2**, may be responsible for this amazing effect. Numerous research publications stress the positive impact of trifluoromethyl substitution in the aromatic system [42-44], which has been shown to have an inhibiting influence on the development of malignant tumors.

TABLE 1 The results of the study that evaluated the neoplastic inhibiting effects of the produced chalcone derivatives

Compound Symbol	IC50 (µM) ± SD (n=3)				
	HepG2	A549	Ovcar-3	HeLa	MCF-7
Doxorubicin	0.200 ± 0.01	0.30 ± 0.01	0.06 ± 0.002	0.076 ± 0.003	0.06 ± 0.002
A1	0.80 ± 0.02	0.80 ± 0.03	1.02 ± 0.06	1.5 ± 0.06	0.98 ± 0.04
A2	0.63 ± 0.02	0.23 ± 0.01	0.61 ± 0.03	0.93 ± 0.05	0.55 ± 0.02
A3	0.58 ± 0.02	0.53 ± 0.03	0.74 ± 0.04	1.4 ± 0.08	0.88 ± 0.03
A4	0.94 ± 0.05	0.5 ± 0.01	1.3 ± 0.04	1.3 ± 0.04	0.68 ± 0.02
A5	0.5 ± 0.02	0.63 ± 0.02	0.7 ± 0.04	1.4 ± 0.05	0.78 ± 0.02
A6	0.4 ± 0.01	0.73 ± 0.03	0.73 ± 0.04	1.5 ± 0.06	0.98 ± 0.03

Free radicals trapping effect

To test the antioxidative impact of the pure produced chalcone derivatives, their ability to capture DPPH radical moieties *in vitro* was examined. As presented in Table 2, the produced chalcone derivatives exhibited a trapping effect toward the free harmful moieties, although one that was less potent than that of vitamin C, with IC_{50} values ranging from 45.89 µg/mL and 88.50 µg/Ml.

TABLE 2 Antioxidant quality possessed by the molecules that were synthesized (IC_{50} in µg / mL)

Compound	IC_{50} ± SD (n=3)
A1	88.50 ± 3.5
A2	45.89 ± 1.9
A3	65.64 ± 3.1
A4	73.93 ± 1.7
A5	78.04 ± 2.8
A6	57.39 ± 2.1
Ascorbic acid	23.27 ± 1.2

Conclusion

The preparation of novel chalcone derivatives and investigation of the neoplastic-inhibiting and antioxidative activities of these compounds *in vitro* were the primary goals of this particular body of study. According to the results of the study, the produced chalcone derivatives did have an inhibiting effect on the progression of neoplastic growth in each of five examined distinct malignant cell lines, but this effect was much less than the norm. In particular targeting A549 and HepG2 cells, the derivative with the potent electronegative substituent known as **A2** displayed a substantial neoplastic inhibitory effect with efficacy comparable to the standard. In addition, as compared to other derivatives, **A2** had a much stronger antioxidative action on the examined free radical moieties, with a potency that was almost identical to the standard.

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Conflict of Interest

The authors declare that they have no competing interests in this article.

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References

[1] N. Zarei, M.R. Rezaie, A. Jomehzadeh, Radiation hazards investigation of photon scattering by Elekta 6 MeV Linac during liver cancer treatment, *Chem. Methodol.*, **2022**, *6*, 582-590. [crossref], [Google Scholar], [Publisher]

[2] A. Ahmadi, H. Ghanbari, M.M. Salem, A.M. Milani Fard, K. Barkhordari, The outcome of the correction of anterior vocal cord web by flap technique using real anterior vocal cord base in frontolateral laryngectomy in patients with glottis cancer, *Eurasian J. Sci. Technol.*, **2022**, *2*, 262-267. [crossref], [Google Scholar], [Publisher]

[3] M. Rezaie, A. Nouraddini Shahabadi, S. Mohammadi, Monte Carlo investigation of

organs activation in proton and heavy ions cancer therapy by spallation process, *Int. J. Adv. Biol. Biomed. Res.*, **2022**, *10*, 117-125. [crossref], [Google Scholar], [Publisher]

[4] F. Mohajer, G. Mohammadi Ziarani, A. Badiei, New advances on modulating nanomagnetic cores as the MRI-monitored drug release in cancer, *J. Appl. Organomet. Chem.*, **2021**, *1*, 143-147. [crossref], [Google Scholar], [Publisher]

[5] M. Karimi, Considering the main center for treatment and counseling of cancer patients, *Int. J. Adv. Stu. Hum. Soc. Sci.*, **2021**, *10*, 146-150. [crossref], [Google Scholar], [Publisher]

[6] T. Emamian, R. Emamia, Child birth right with child health and social benefit in family support law of 2013, *J. Law Political Stud.*, **2022**, *2*, 190-202. [crossref], [Google Scholar], [Publisher]

[7] K.V. Sashidhara, A. Kumar, M. Kumar, J. Sarkar, S. Sinha, Synthesis and in vitro evaluation of novel coumarin–chalcone hybrids as potential anticancer agents, *Bioorganic Med. Chem. Lett.*, **2010**, *20*, 7205–7211. [crossref], [Google Scholar], [Publisher]

[8] L. Zhang, Z. Xu, Coumarin-containing hybrids and their anticancer activities, *Eur. J. Med. Chem.*, **2019**, *181*, 111587. [crossref], [Google Scholar], [Publisher]

[9] M.K. Bashir, Y.F. Mustafa, M.K. Oglah, Synthesis and antitumor activity of new multifunctional coumarins, *Period. Tche Quim.*, **2020**, *17*, 871–883. [Pdf], [Google Scholar], [Publisher]

[10] Y.F. Mustafa, M. K. Bashir, M. K. Oglah, R. R. Khalil, E. T. Mohammed, Bioactivity of some natural and semisynthetic coumarin derived compounds, *NeuroQuantology*, **2021**, *19*, 99–112. [crossref], [Google Scholar], [Publisher]

[11] M.J. Ansari, S.A. Jasim, T.Z. Taban, D.O. Bokov, M.N. Shalaby, M.E. Al-Gazally, H.H. Kzar, M.T. Qasim, Y.F. Mustafa, M. Khatami, Anticancer drug-loading capacity of green synthesized porous magnetic iron nanocarrier and cytotoxic effects against human cancer cell line, *J. Clust. Sci.*, **2023**, *34*, 467–477. [crossref], [Google Scholar], [Publisher]

[12] H. Sies, Oxidative stress: Concept and some practical aspects, *Antioxidants*, **2020**, *9*, 852. [crossref], [Google Scholar], [Publisher]

[13] R.R. Khalil, Y.F. Mustafa, Phytochemical, antioxidant and antitumor studies of coumarins extracted from Granny Smith apple seeds by different methods, *Syst. Rev. Pharm.*, **2020**, *11*, 57–63. [crossref], [Google Scholar], [Publisher]

[14] M.K. Oglah, Y.F. Mustafa, M.K. Bashir, M.H. Jasim, Curcumin and its derivatives: A review of their biological activities, *Syst. Rev. Pharm.*, **2020**, *11*, 472–481. [crossref], [Google Scholar], [Publisher]

[15] L. Manolaridis, S.M. Jackson, N.M.I. Taylor, J. Kowal, H. Stahlberg, K.P. Locher, Cryo-EM structures of a human ABCG2 mutant trapped in ATP-bound and substrate-bound states, *Nature*, **2018**, *563*, 426–430. [crossref], [Google Scholar], [Publisher]

[16] S.A. Hasan, A.N. Elias, M.S. Farhan, Synthesis, characterization and antimicrobial evaluation of a series of chalcone derivatives, *Der Pharma. Chem*, **2015**, *7*, 39-42. [Pdf], [Google Scholar], [Publisher]

[17] T. Constantinescu, C.N. Lungu, Anticancer activity of natural and synthetic chalcones, *Int. J. Mol. Sci.*, **2021**, *22*, 11306. [crossref], [Google Scholar], [Publisher]

[18] H. Ur Rashid, Y. Xu, N. Ahmad, Y. Muhammad, L. Wang, Promising anti-inflammatory effects of chalcones via inhibition of cyclooxygenase, prostaglandin E2, inducible NO synthase and nuclear factor κb activities, *Bioorg. Chem.*, **2019**, *87*, 335–365. [crossref], [Google Scholar], [Publisher]

[19] H. Yin, X. Shi, H. Wang, G. Liu, L. Ma, VB1 promoted green synthesis of chalcones and its neuroprotection potency evaluation, *Processes*, **2019**, *7*, 236. [crossref], [Google Scholar], [Publisher]

[20] S.N. Saleh, M.S. Farhan, Synthesis And Evaluation of the Antimicrobial Activity of new Different Chalcones Derived From P-Amino Benzaldehyde Containing

Thiozolidinone Ring, *HIV Nursing*, **2023**, *23*, 760-765. [Pdf], [Google Scholar], [Publisher]
[21] M.M. Al-Tufah, S. Beebaeny, S. Jasim, B.L. Mohammed, Synthesis, Characterization of ethyl dioxoisoindolinyl cyclohexenone carboxylate derivatives from some chalcones and its biological activity assessment, *Chem. Methodol.*, **2023**, *7*, 408-418. [crossref], [Google Scholar], [Publisher]
[22] S. Beebany, S.S. Jasim, M.M. Al-Tufah, S. Samih, H., Arslan, Preparation and identification of new 1, 4-bis (5, 3-substituted-2, 3-dihydro-1H-pyrazol-1-yl) buta-1, 4-dione derivatives with their antibacterial effect evaluation, *Chem. Methodol.*, **2023**, *7*, 123-136. [crossref], [Google Scholar], [Publisher]
[23] N.A.A. Elkanzi, H. Hrichi, R.A. Alolayan, W. Derafa, F.M. Zahou, R.B. Bakr, Synthesis of chalcones derivatives and their biological activities: a review, *ACS omega*, **2022**, *7*, 27769–27786. [crossref], [Google Scholar], [Publisher]
[24] S.N.A. Bukhari, Synthesis and evaluation of new chalcones and oximes as anticancer agents, *RSC advances***m 2022**, *12*, 10307–10320. [crossref], [Google Scholar], [Publisher]
[25] Y. Hu, C. Hu, G. Pan, C. Yu, M.F. Ansari, R. R.Y. Bheemanaboina, Y. Cheng, C. Zhou, J. Zhang, Novel chalcone-conjugated, multi-flexible end-group coumarin thiazole hybrids as potential antibacterial repressors against methicillin-resistant Staphylococcus aureus, *Eur. J. Med. Chem.*, **2021**, *222*, 113628. [crossref], [Google Scholar], [Publisher]
[26] P. Thapa, S.P. Upadhyay, W.Z. Suo, V. Singh, P. Gurung, E.S. Lee, R. Sharma, M. Sharma, Chalcone and its analogs: Therapeutic and diagnostic applications in Alzheimer's disease, *Bioorg. Chem.*, **2021**, *108*, 104681. [crossref], [Google Scholar], [Publisher]
[27] T.T. Komoto, T.M. Bernardes, T.B. Mesquita, L.F.B. Bortolotto, G. Silva, T.A. Bitencourt, S.J. Baek, M. Marins, A.L. Fachin, Chalcones repressed the AURKA and MDR proteins involved in metastasis and multiple drug resistance in breast cancer cell lines, *Molecules*, **2018**, *23*, 2018. [crossref], [Google Scholar], [Publisher]
[28] M.M.A. Hawash, D.C. Kahraman, F. Eren, R.C. Atalay, S.N. Baytas, Synthesis and biological evaluation of novel pyrazolic chalcone derivatives as novel hepatocellular carcinoma therapeutics, *Eur. J. Med. Chem.*, **2017**, *129*, 12–26. [crossref], [Google Scholar], [Publisher]
[29] N. Singh, R. Chandra, Probing the binding interaction of ortho-vanillin derived chalcone with lysozyme: A biophysical studies aided by in silico calculations, *J. Mol. Liq.*, **2020**, *321*, 114490. [crossref], [Google Scholar], [Publisher]
[30] F. Wada, H. Hirayama, H. Namiki, K. Kikukawa, T. Matsuda, New applications of crown ethers. II. Synthesis of 4′-Formylbenzocrown Ethers, *Bull. Chem. Soc. Jpn.*, **1980**, *53*, 1473-1474. [crossref], [Google Scholar], [Publisher]
[31] G. König, M.T. Reetz, W. Thiel, 1-Butanol as a solvent for efficient extraction of polar compounds from aqueous medium: theoretical and practical aspects, *J. Phys. Chem. B*, **2018**, *122*, 6975–6988. [crossref], [Google Scholar], [Publisher]
[32] M.T. Reetz, G. König, n-Butanol: An ecologically and economically viable extraction solvent for isolating polar products from aqueous solutions, *Eur. J. Org. Chem.*, **2021**, *2021*, 6224–6228. [crossref], [Google Scholar], [Publisher]
[33] T.W. Jihad, A.S. Alkazzaz, Synthesis of a series of α, β-unsaturated ketoximes and their corresponding acetate esters, *Iraqi J. Sci.*, **2022**, *63*, 4142–4151. [crossref], [Google Scholar], [Publisher]
[34] T.S. Ghali, J.H. Tomma, Synthesis and characterization of indazol-3-one and thioxo pyrimidines derivatives from mono and twin chalcones, *Iraqi J. Sci.*, **2017**, *58*, 2265-2277. [Pdf], [Google Scholar], [Publisher]
[35] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application

to proliferation and cytotoxicity assays, *J. Immunol. Methods,* **1983**, *65*, 55–63. [crossref], [Google Scholar], [Publisher]

[36] F. Denizot, R. Lang, Rapid colorimetric assay for cell growth and survival: modifications to the tetrazolium dye procedure giving improved sensitivity and reliability, *J. Immunol. Methods*, **1986**, *89*, 271–277. [crossref], [Google Scholar], [Publisher]

[37] D. Krasteva, Y. Ivanov, Z. Chengolova, T. Godjevargova, Antimicrobial potential, antioxidant activity, and phenolic content of grape seed extracts from four grape varieties, *Microorganisms*, **2023**, *11*, 395. [crossref], [Google Scholar], [Publisher]

[38] N. Wathoni, C.Y. Shan, W.Y. Shan, T. Rostinawati, R.B. Indradi, R. Pratiwi, M. Muchtaridi. Characterization and antioxidant activity of pectin from Indonesian mangosteen (Garcinia mangostana L.) rind, *Heliyon*, **2019**, *5*, e02299. [crossref], [Google Scholar], [Publisher]

[39] S. Phongpaichit, J. Nikom, N. Rungjindamai, J. Sakayaroj, N. Hutadilok-Towatana, V. Rukachaisirikul, K. Kirtikara, Biological activities of extracts from endophytic fungi isolated from Garcinia plants, *FEMS Microbiol. Immunol.*, **2007**, *51*, 517-525. [crossref], [Google Scholar], [Publisher]

[40] A.E. Harman-Ware, R. Sykes, G.F. Peter, M. Davis, Determination of terpenoid content in pine by organic solvent extraction and fast-GC analysis, *Front. Energy Res.,* **2016**, *4*, 2. [crossref], [Google Scholar], [Publisher]

[41] N. Jadid, D. Hidayati, S.R. Hartanti, B.A. Arraniry, R.Y. Rachman, W. Wikanta, Antioxidant activities of different solvent extracts of Piper retrofractum Vahl. using DPPH assay, In *AIP conference proceedings*, AIP Publishing LLC, **2017**, *1854*, 020019. [crossref], [Google Scholar], [Publisher]

[42] A.S. Sawant, S.S. Kamble, P.M. Pisal, S.S. Sawant, S.V. Hese, K.T. Bagul, R.V. Pinjari, V.T. Kamble, R.J. Meshram, R.N. Gacche, Synthesis and evaluation of N-(4-(substituted)-3-(trifluoromethyl) phenyl) isobutyramides and their N-ethyl analogous as anticancer, anti-angiogenic & antioxidant agents: In vitro and in silico analysis, *Comput. Biol. Chem.*, **2021**, *92*, 107484. [crossref], [Google Scholar], [Publisher]

[43] A. Abula, Z. Xu, Z. Zhu, C. Peng, Z. Chen, W. Zhu, H.A. Aisa, Substitution effect of the trifluoromethyl group on the bioactivity in medicinal chemistry: Statistical analysis and energy calculations, *J. Chem. Inf. Model,* **2020**, *60*, 6242–6250. [crossref], [Google Scholar], [Publisher]

[44] P. Olszewska, D. Cal, P. Zagórski, E.M. Olasik, A novel trifluoromethyl 2-phosphonopyrrole analogue inhibits human cancer cell migration and growth by cell cycle arrest at G1 phase and apoptosis, *Eur. J. Pharmacol.*, **2020**, *871*, 172943. [crossref], [Google Scholar], [Publisher]

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