Scientific paper

(*E*)-1-(4-Hydroxyphenyl)-3-(substituted-phenyl) prop-2-en-1-ones: Synthesis, *In Vitro* Cytotoxic Activity and Molecular Docking Studies

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Abstract

A series of chalcone compounds (**2**–**11**) were designed and synthesized to determine their cytotoxic effects. The structures of **2**–**11** were fully characterized by their physical and spectral data. The *in vitro* cytotoxic effects of **2**–**11** were evaluated against human ovarian cancer (A2780), breast cancer (MCF-7) and prostate cancer (PC-3 and LNCaP) cell lines. The activity potentials of compounds were further evaluated through molecular docking studies with AutoDock4 and Vina softwares. All the compounds (except compound **5**) showed significant cytotoxic effects at high doses in all cancer cell lines. Among all the compounds studied, one compound i.e. compound **2** demonstrated dose-dependent activity, particularly against A2780/LNCaP cancer cell lines. The most effective compounds **8**, **9**, **10** and **11** reduced the cell viability of A2780, MCF-7, PC-3 and LNCaP cells by 50–98%, while other compounds **2**, **4** and **7** reduced the cell viability of A2780 cells by 70–90% at concentrations of 50 and 100 μM.

Keywords: Chalcone; cytotoxic; A2780; MTT assay; molecular docking

1. Introduction

The chalcones or phenyl styryl ketones are unsaturated ketones, containing the reactive keto-ethylenic group. Chalcone is an important chemo type that has attracted great research interest for decades due to the abundant natural chalcone-based compounds, the easy synthesis and derivatization, and most importantly, the diverse biological activities of various chalcone-based compounds.[1–6] The well documented biological activities of chalcones include anti-HIV,[7] antibacterial,[8] anti-cancer and antioxidant,[9–11] antituberculosis agents,[12] anti-proliferative,[13] antiplatelet,[14,15] and antimalarial.[16,17] Numerous studies have attempted to elucidate the mechanisms of action and target interactions responsible for these biological activities.[18–20] Additionally, these compounds have applications in a variety of areas including nonlinear optical materials,[21,22] dye sensitized solar cell,[23,24] and optoelectronic and fluorescence materials.[25–27]

There are several methods available for the synthesis of chalcones. The Claisen–Schmidt condensation reaction, one of the most widely used chalcone synthesis methods, was used in the synthesis of compounds in this article by using sodium/potassium hydroxide.[28–36] The base-cata-

lysed other method of chalcone synthesis include Claisen–Schmidt reaction.[37] The acid catalyzed methods that have been used to synthesize chalcones includes Friedel–Crafts acylation,[38] silica-sulfuric acid,[39] dry HCl,[40, 41] boron trifluoride-diethyletherate (BF_3-Et_2O),[42] Aldol condensation,[43] Suzuki coupling reaction,[44] Julia–Kocienski olefination[45] and Wittig reaction.[46] Among these the direct aldol condensation and Claisen–Schimdt condensation still occupy prominent position.

In addition to the wide potential applications of chalcone derivatives, their ability to be easily isolated from natural plants as well as their synthetic accessibility with many synthesis procedures, as mentioned above, increased the studies on these compounds.

Herein, we have synthesized a series of chalcone derivatives in order to evaluate their cytotoxic effects against four human cancer cell lines namely ovarian (A2780), prostate (PC-3 and LNCaP), and breast (MCF-7). For this reason, the synthesis of target compounds was achieved by the condensation of *para*-hydroxyacetophenone with benzaldehyde using sodium hydroxide as a condensing agent. These compounds **2**–**11** were confirmed by using mass (MALDI-TOF-MS), FT-IR, elemental analysis, ^{1}H, ^{13}C-APT NMR spectroscopy.

Disruption of the microtubules can lead to apoptosis induction as it has vital importance to the continuation of the cell cycle. Therefore, the literature emphasizes that tubulin inhibitors can be used as effective anticancer drugs.[47,48] Binding site for colchicine is well determined in the tubule.[49] Potential tubulin inhibition effects with synthesized molecules were determined by tubulin-colchicine complex. The interactions of compounds with the tubulin-colchicine complex were put forthed via molecular docking studies. And their possible anti-cancer properties were investigated against A2780, MCF-7, PC-3 and LNCaP cell lines by using MTT assay method.[50–53] Our results indicate that these compounds displayed strong cytotoxic activity on these cell lines.

2. Experimental

2. 1. Synthesis

All aldehydes and solvents used in the present study were provided by Sigma-Aldrich and Merck. ^{1}H and ^{13}C-APT NMR spectra, infrared analysis and microanalysis were acquired using a Bruker DPX-400 MHz spectrometer, a Perkin Elmer FT-IR spectrometer and a LECO 932 CHNS-O apparatus, respectively. A Bruker microflex LT MALDI-TOF MS spectrometer was used to obtain mass spectra. In cell culture studies, the human prostate (PC-3 and LNCaP), breast (MCF-7), and ovarian cancer cell lines were provided by ATCC (the American Type Culture Collection), new-born calf serum and Dulbecco's modified Eagle's medium (DMEM) were provided by Hyclone (Waltham, MA, USA); and penicillin, trypsin, streptomycin. Nuve MN-120 as biological safety cabinet, Panasonic as C_{O2} Incubator, BioTEK spectrophotometer as microplate reader, Inverted Microscope SOIF-XDS for maintenance and control of cells and Nuve for Sterilization were used.

2. 1. 1. General Synthesis Method of (*E*)-1-(4-Hydroxyphenyl)-3-(substituted-phenyl) prop-2-en-1-one Compounds

Appropriate *para*-hydroxyacetophenone (1.00 equiv.) was placed in a round bottom flask with ethanol (50 mL) and 60% sodium hydroxide (25 mL). After 30 min, aldehyde (1.015 equiv.) was added to the reaction mixture. The solution was let to stir overnight. The mixture was then poured into an ice/water mixture. Concentrated HCl was added until the solution reached an acidic pH (until about pH 6). Upon addition of the acid a precipitate formed. The precipitate was filtered and washed with plenty of water to pH neutral. The product was recrystallized in ethanol.

(*E*)-1-(4-Hydroxyphenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (2)

Yield: 92%; yellow solid; MALDI-MS: *m/z* calcd: 254.29; found: 255.58 $[M+H]^+$. FT-IR (KBr) ν_{max} (cm^{-1}): 3294 ν_{OH}, 3000 and 3018 $\nu_{Ar\text{-}CH}$, 2829, 2903 and 2965 $\nu_{Aliphatic\text{-}CH}$, 1652 $\nu_{C=O}$, 1512, 1573, 1594 and 1606 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 3.84 (3H, s, H^{17} (-OCH_3)), 6.91–6.93 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.01–7.04 (1H, d, Ar-H^{14}), 7.35–7.39 (1H, t, Ar-H^{15}), 7.42–7.43 (1H, d, Ar-H^{12}), 7.47–7.48 (1H, d, Ar-H^{16}), 7.65–7.69 (1H, d, *J* = 15.6 Hz, H^9 (-CH=)), 7.91–7.95 (1H, d, *J* = 15.6 Hz, H^{10} =CH-)), 8.09–8.11 (2H, d, *J* = 8.8 Hz, Ar-$H^{2,6}$), 10.43 (1H, s, H^7 (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 55.75 (C^{17} (-OCH_3)), 113.64 (Ar-C^{14}), 115.85 (Ar-$C^{3,5}$), 116.90 (Ar-C^{12}), 122.01 (Ar-C^{16}), 122.80 (C^9 (-CH=)), 129.55 (Ar-C^1), 130.37 (Ar-C^{15}), 131.73 (Ar-$C^{2,6}$), 136.77 (Ar-C^{11}), 143.21 (C^{10} (=CH-)), 160.11 (Ar-C^{13}), 162.71 (Ar-C^4), 187.59 (C^8 (-C=O)). Anal. Calcd for $C_{16}H_{14}O_3$: C, 75.58; H, 5.55. Found: C, 75.69; H, 5.49%.

(*E*)-1-(4-Hydroxyphenyl)-3-(2,4-dimethoxyphenyl) prop-2-en-1-one (3)

Yield: 83%; yellow solid; MALDI-MS: *m/z* calcd: 284.31; found: 284.48. FT-IR (KBr) ν_{max} (cm^{-1}): 3144 ν_{OH}, 3005 and 3013 $\nu_{Ar\text{-}CH}$, 2835 and 2934 $\nu_{Aliphatic\text{-}CH}$, 1645 $\nu_{C=O}$, 1506, 1541, 1569, 1590 and 1600 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 3.85 (3H, s, H^{18} (-OCH_3)), 3.91 (3H, s, H^{17} (-OCH_3)), 6.64–6.65 (2H, m, Ar-H^{13}, H^{15}), 6.89–6.91 (2H, d, *J* = 8.4 Hz, Ar-$H^{3,5}$), 7.72–7.76 (1H, d, *J* = 15.6 Hz, H^9 (-CH=)), 7.89–7.91 (1H, d, *J* = 8.8 Hz, Ar-H^{16}), 7.93–7.97 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.02–8.05 (2H, d, *J* = 8.4 Hz, Ar-$H^{2,6}$), 10.36 (1H, s, H^7 (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 55.98 (C^{18} (-OCH_3)), 56.26 (C^{17} (-OCH_3)), 98.74 (Ar-C^{13}), 106.70 (Ar-C^{15}), 115.77 (Ar-$C^{3,5}$), 116.55

(Ar-C^{11}), 119.55 (C^{9} (-CH=)), 129.96 (Ar-C^{1}), 130.35 (Ar-C^{16}), 131.37 (Ar-$C^{2,6}$), 137.93 (C^{10} (=CH-)), 160.22 (Ar-C^{14}), 162.35 (Ar-C^{12}), 163.28 (Ar-C^{4}), 187.65 (C^{8} (-C=O)). Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.89; H, 5.72%.

(*E*)-1-(4-Hydroxyphenyl)-3-(3,4-dimethoxyphenyl) prop-2-en-1-one (4)

Yield: 77%; yellow solid; MALDI-MS: *m/z* calcd: 284.31; found: 285.38 $[M+H]^{+1}$. FT-IR (KBr) ν_{max} (cm^{-1}): 3112 ν_{OH}, 3026 and 3071 $\nu_{Ar\text{-}CH}$, 2835 and 2956 $\nu_{Aliphatic\text{-}CH}$, 1641 $\nu_{C=O}$, 1506, 1541, 1581, 1591 and 1608 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s, H^{18} (-OCH_3)), 3.88 (3H, s, H^{17} (-OCH_3)), 6.91–6.93 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.01–7.03 (1H, d, *J* = 8.4 Hz, Ar-H^{16}), 7.35–7.38 (1H, d, *J* = 9.6 Hz, Ar-H^{15}), 7.53 (1H, s, Ar-H^{12}), 7.64–7.68 (1H, d, *J* = 15.6 Hz, H^{9} (-CH=)), 7.79–7.83 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.08–8.10 (2H, d, *J* = 8.4 Hz, Ar-$H^{2,6}$), 10.39 (1H, s, H^{7} (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 56.05 (C^{17} (-OCH_3)), 56.20 (C^{18} (-OCH_3)), 111.09 (Ar-C^{12}), 112.02 (Ar-C^{15}), 120.13 (C^{9} (-CH=)), 124.10 (Ar-C^{16}), 128.20 (Ar-C^{11}), 129.83 (Ar-C^{1}), 131.54 (Ar-$C^{2,6}$), 143.68 (C^{10} (=CH-)), 149.49 (Ar-C^{13}), 151.50 (Ar-C^{14}), 162.49 (Ar-C^{4}), 187.52 (C^{8} (-C=O)). Anal. Calcd for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.89; H, 5.72%.

(*E*)-1-(4-Hydroxyphenyl)-3-(2,3,4-trimethoxyphenyl) prop-2-en-1-one (5)

Yield: 79%; yellow solid; MALDI-MS: *m/z* calcd: 314.34; found: 315.42 $[M+H]^{+1}$. FT-IR (KBr) ν_{max} (cm^{-1}): 3392 ν_{OH}, 3005 and 3050 $\nu_{Ar\text{-}CH}$, 2830 and 2938 $\nu_{Aliphatic\text{-}CH}$, 1657 $\nu_{C=O}$, 1530, 1580, and 1606 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 3.79 (3H, s, H^{18} (-OCH_3)), 3.87 (3H, s, H^{19} (-OCH_3)), 3.89 (3H, s, H^{17} (-OCH_3)), 6.90–6.94 (3H, m, Ar-$H^{3,}$ H^{15}), 7.75–7.80 (2H, m, H^{9} (-CH=), Ar-H^{16}), 7.75–7.88 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.03–8.05 (2H, d, *J* = 8 Hz, Ar-$H^{2,6}$), 10.37 (1H, s, H^{7} (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 56.51 (C^{19} (-OCH_3)), 60.94 (C^{18} (-OCH_3)), 61.98 (C^{17} (-OCH_3)), 108.90 (Ar-C^{15}), 115.82 (Ar-$C^{3,5}$), 120.91 (C^{9} (-CH=)), 121.65 (Ar-C^{11}), 123.73 (Ar-C^{16}), 129.76 (Ar-C^{1}), 131.50 (Ar-$C^{2,6}$), 137.58 (C^{10} (=CH-)), 142.21 (Ar-C^{13}), 153.42 (Ar-C^{12}), 155.96 (Ar-C^{14}), 162.52 (Ar-C^{4}), 187.57 (C^{8} (-C=O)). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.71; H, 5.71%.

(*E*)-1-(4-Hydroxyphenyl)-3-(2,4,5-trimethoxyphenyl) prop-2-en-1-one (6)

Yield: 72%; yellow solid; MALDI-MS: *m/z* calcd: 314.34; found: 314.48. FT-IR (KBr) ν_{max} (cm^{-1}): 3307 ν_{OH}, 3028 and 3067 $\nu_{Ar\text{-}CH}$, 2827 and 2929 $\nu_{Aliphatic\text{-}CH}$, 1639 $\nu_{C=O}$, 1505, 1544, 1583, and 1613 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s, H^{18} (-OCH_3)), 3.88 (3H, s, H^{19} (-OCH_3)), 3.91 (3H, s, H^{17} (-OCH_3)), 6.76 (1H, s, H^{13}), 6.90–6.92 (2H, d, *J* = 8.4 Hz, Ar-$H^{3,5}$), 7.50 (1H, s, H^{16}), 7.71–7.75 (1H, d, *J* = 15.6 Hz, H^{9} (-CH=)), 7.98–8.02 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.04–8.06 (2H, d, *J* = 8.4 Hz, Ar-$H^{2,6}$), 10.29 (1H, s, H^{7} (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 56.33 (C^{17} (-OCH_3)), 56.94 (C^{18} (-OCH_3)), 56.96 (C^{19} (-OCH_3)), 98.28 (Ar-C^{13}), 111.73 (Ar-C^{16}), 115.16 (Ar-C^{11}), 115.74 (Ar-$C^{3,5}$), 119.49 (C^{9} (-CH=)), 130.09 (Ar-C^{1}), 131.38 (Ar-$C^{2,6}$), 137.73 (C^{10} (=CH-)), 143.65 (Ar-C^{12}), 153.07 (Ar-C^{15}), 154.53 (Ar-C^{14}), 162.32 (Ar-C^{4}), 187.69 (C^{8} (-C=O)). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.83; H, 5.82%.

(*E*)-1-(4-Hydroxyphenyl)-3-(1,1′-biphenyl)prop-2-en-1-one (7)

Yield: 65%; yellow solid; MALDI-MS: *m/z* calcd: 300.36; found: 300.54. FT-IR (KBr) ν_{max} (cm^{-1}): 3162 ν_{OH}, 3026 and 3059 $\nu_{Ar\text{-}CH}$, 2824 and 2960 $\nu_{Aliphatic\text{-}CH}$, 1652 $\nu_{C=O}$, 1515, 1553, 1595, and 1609 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 6.91–6.94 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.41–7.43 (1H, t, H^{20}), 7.49–7.51 (2H, t, $H^{19,21}$), 7.72–7.79 (5H, m, H^{9} (-CH=), Ar-$H^{12,16}$, Ar-$H^{18,22}$), 7.94–7.98 (3H, m, H^{10} (=CH-), Ar-$H^{13,15}$), 8.09–8.11 (2H, d, *J* = 8.4 Hz, Ar-$H^{2,6}$), 10.41 (1H, s, H^{7} (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 115.88 (Ar-$C^{3,5}$), 122.54 (C^{9} (-CH=)), 127.20 (C^{20}), 127.53 (Ar-$C^{12,16}$), 128.43 (Ar-$C^{13,15}$), 129.43 (Ar-$C^{18,22}$), 129.61 (Ar-C^{1}), 129.87 (Ar-$C^{19,21}$), 131.69 (Ar-$C^{2,6}$), 134.53 (Ar-C^{11}), 139.75 (Ar-C^{14}), 142.27 (Ar-C^{17}), 142.70 (C^{10} (=CH-)), 162.75 (Ar-C^{4}), 187.55 (C^{8} (-C=O)). Anal. Calcd for $C_{21}H_{16}O_2$: C, 83.98; H, 5.37. Found: C, 84.05; H, 5.42%.

(*E*)-1-(4-Hydroxyphenyl)-3-(3,5-difluorophenyl)prop-2-en-1-one (8)

Yield: 66%; yellow solid; MALDI-MS: *m/z* calcd: 260.24; found: 261.31 $[M+H]^{+}$. FT-IR (KBr) ν_{max} (cm^{-1}): 3223 ν_{OH}, 3026 and 3100 $\nu_{Ar\text{-}CH}$, 2829 and 2972 $\nu_{Aliphatic\text{-}CH}$, 1657 $\nu_{C=O}$, 1516, 1593, and 1613 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 6.91–6.93 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.29–7.34 (1H, Ar-H^{14}), 7.63–7.67 (1H, d, *J* = 15.6 Hz, H^{9} (-CH=)), 7.70–7.72 (2H, m, Ar-H^{12}, Ar-H^{16}), 8.03–8.06 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.11–8.13 (2H, d, *J* = 8.4 Hz, Ar-$H^{2,6}$), 10.49 (1H, s, H^{7} (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 105.80 (Ar-C^{14}), 112.02 (Ar-C^{12}), 112.21 (Ar-C^{16}), 115.86 (Ar-$C^{3,5}$), 125.25 (C^{9} (-CH=)), 129.25 (Ar-C^{1}), 131.93 (Ar-$C^{2,6}$), 139.22 (Ar-C^{11}), 140.58 (C^{10} (=CH-)), 161.96 (Ar-C^{15}), 162.96 (Ar-C^{4}), 164.40 (Ar-C^{13}), 187.30 (C^{8} (-C=O)). Anal. Calcd for $C_{15}H_{10}F_2O_2$: C, 69.23; H, 3.87. Found: C, 69.29; H, 3.92%.

(*E*)-1-(4-Hydroxyphenyl)-3-(2-fluoro-4-chlorophenyl) prop-2-en-1-one (9)

Yield: 58%; yellow solid; MALDI-MS: *m/z* calcd: 276.69; found: 276.98. FT-IR (KBr) ν_{max} (cm^{-1}): 3270 ν_{OH}, 3026 and 3067 $\nu_{Ar\text{-}CH}$, 2865 and 2943 $\nu_{Aliphatic\text{-}CH}$, 1647 $\nu_{C=O}$, 1514, 1572, 1595 and 1605 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 6.91–6.93 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.40–7.42 (1H, d, *J* = 9.6 Hz, Ar-H^{15}), 7.55–7.57 (1H, d, *J* = 10.4 Hz, Ar-H^{16}) 7.70–7.74 (1H, d, *J* = 15.6 Hz, H^{9} (-CH=)), 7.95–7.99 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.06–8.08 (2H, d, *J* = 8 Hz, Ar-$H^{2,6}$), 8.15 (1H, s, Ar-H^{13}), 10.46 (1H, s, H^{7} (Ph-

OH)). ^{13}C-APT NMR (DMSO-d_6) δ 115.95 (Ar-$C^{3,5}$), 117.32 (Ar-C^{13}), 122.11 (Ar-C^{11}), 125.02 (Ar-C^{15}), 125.80 (C^9 (-CH=)), 129.19 (Ar-C^1), 130.65 (Ar-C^{16}), 131.83 (Ar-$C^{2,6}$), 133.10 (C^{10} (=CH-)), 136.03 (Ar-C^{14}), 159.78 (Ar-C^{12}), 162.98 (Ar-C^4), 187.16 (C^8 (-C=O)). Anal. Calcd for $C_{15}H_{10}ClFO_2$: C, 65.11; H, 3.64. Found: C, 65.17; H, 3.69%.

(*E*)-1-(4-Hydroxyphenyl)-3-(3-(trifluoromethyl)phenyl)prop-2-en-1-one (10)

Yield: 69%; yellow solid; MALDI-MS: *m/z* calcd: 292.26; found: 293.19 $[M+H]^+$. FT-IR (KBr) ν_{max} (cm^{-1}): 3330 ν_{OH}, 3013 and 3067 $\nu_{Ar\text{-}CH}$, 2882 and 2981 $\nu_{Aliphatic\text{-}CH}$, 1646 $\nu_{C=O}$, 1514, 1557, 1597 and 1606 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 6.92–6.94 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.67–7.71 (1H, t, Ar-H^{15}), 7.75–7.79 (1H, d, *J* = 15.6 Hz, H^9 (-CH=)), 7.77 (1H, s, Ar-H^{16}), 8.09–8.18 (4H, m, Ar-$H^{2,6}$, H^{10} (=CH-)), Ar-H^{14}), 8.33 (1H, s, Ar-H^{12}), 10.46 (1H, s, H^7 (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ = 115.88 (Ar-$C^{3,5}$), 124.51 (Ar-C^{12}), 125.40 (C^9 (-CH=)), 126.90 (Ar-C^{14}), 129.35 (Ar-C^1), 130.10 (Ar-C^{17}), 130.38 (Ar-C^{15}), 130.42 (Ar-C^{13}), 131.91 (Ar-$C^{2,6}$), 133.28 (Ar-C^{16}), 136.55 (Ar-C^{11}), 141.39 (C^{10} (=CH-)), 162.90 (Ar-C^4), 187.43 (C^8 (-C=O)). Anal. Calcd for $C_{16}H_{11}F_3O_2$: C, 65.76; H, 3.79. Found: C, 65.83; H, 3.75%.

(*E*)-1-(4-Hydroxyphenyl)-3-(3-(trifluoromethyl)-4-chlorophenyl)prop-2-en-1-one (11)

Yield: 73%; yellow solid; MALDI-MS: *m/z* calcd: 326.70; found: 327.73 $[M+H]^+$. FT-IR (KBr) ν_{max} (cm^{-1}): 3315 ν_{OH}, 3029, 3060 and 3076 $\nu_{Ar\text{-}CH}$, 2837 and 2961 $\nu_{Aliphatic\text{-}CH}$, 1647 $\nu_{C=O}$, 1515, 1553, 1595 and 1603 $\nu_{C=C}$. ^{1}H NMR (DMSO-d_6) δ 6.91–6.93 (2H, d, *J* = 8.8 Hz, Ar-$H^{3,5}$), 7.74–7.78 (1H, d, *J* = 15.6 Hz, H^9 (-CH=)), 7.81–7.83 (1H, d, *J* = 8.4 Hz, Ar-H^{15}), 8.10–8.14 (1H, d, *J* = 15.6 Hz, H^{10} (=CH-)), 8.12–8.14 (2H, d, *J* = 8.4 Hz, Ar-$H^{2,6}$), 8.21–8.23 (2H, d, *J* = 8.4 Hz, Ar-H^{16}), 8.39 (1H, s, Ar-H^{12}), 10.52 (1H, s, H^7 (Ph-OH)). ^{13}C-APT NMR (DMSO-d_6) δ 115.88 (Ar-$C^{3,5}$), 124.17 (Ar-C^{17}), 125.10 (C^9 (-CH=)), 127.53 (Ar-C^{13}), 128.33 (Ar-C^{15}), 129.29 (Ar-C^1), 131.93 (Ar-$C^{2,6}$), 132.17 (Ar-C^{14}), 132.58 (Ar-C^{16}), 134.30 (Ar-C^{12}), 135.24 (Ar-C^{11}), 140.30 (C^{10} (=CH-)), 162.93 (Ar-C^4), 187.35 (C^8 (-C=O)). Anal. Calcd for $C_{16}H_{11}F_3O_2$: C, 58.82; H, 3.09. Found: C, 58.93; H, 3.14%.

2. 2. *In Vitro* Cytotoxic Activity

Changes in cell viability of compounds at concentrations such as 1, 5, 25, 50 and 100 μM were determined by MTT analysis. This method is based on the principle of MTT dye breaking down the tetrazolium ring, and in the first stage, MTT is actively absorbed into living cells and the reaction is catalyzed by mitochondrial succinate dehydrogenase. Finally, it is reduced to the blue-violet water-insoluble form. Formazan formation, a marker of cell viability, occurs only in living cells with active mitochondria. The value determined spectrophotometrically is related to the number of live cells. 0.5 mg/mL MTT working solution in sterile PBS was prepared from the stock MTT solution and added to 96 well plates. The optical densities of the cells in the plates were kept in the incubator for 3 hours and then scanned at the 550 nm wavelength with ELISA device (Synergy HT USA). The average of the absorbance values obtained from the control wells was accepted as 100% live cells. The absorbance values obtained from the solvent and agent and wells were proportional to the control absorbance value and were considered as percent viability.[50–53]

In this study, human prostate cancer (PC-3, LNCaP), human breast cancer (MCF-7) and human ovarian cancer cell lines (A2780) were selected as cell types. Feeding of all cells was done using RPMI-1640 medium (prepared by adding 10% FCS, 100 U/mL penicillin and 0.1 mg/mL streptomycin) in 25 cm^2 culture flasks. The cells of the medium were kept twice at 37 °C in the carbon dioxide (5% CO_2) incubator and the medium was changed twice a week. When cells were combined, they were removed from the flasks using trypsin-EDTA solution and transferred to 96-well plates and used for 3-(4,5-dimethylthiazole-2-yl) diphenyltetrazolium bromide (MTT) analysis. Relative cell viability (%) was expressed as a percentage relative to untreated control cells. Each value represents an average of 10 measurements. All cellular results were obtained against negative control cells.[51–53]

In cell culture experiments, solutions of the compounds in dimethyl sulfoxide (DMSO) were used. In the comparison of the obtained results for this reason, the effects of the substances against DMSO were determined by statistical analysis. The same amounts of solvent (DMSO) were added to the wells containing the concentrations of the tested compounds at 1, 5, 25, 50 and 100 μM and were incubated in a CO_2 incubator (Panasonic, Japan) for 24 hours at 37 °C. After incubations, viability of the cells was determined using 0.4% tryphan blue in a hemocytometer.

IBM SPSS Statistics 22.0 (Windows) package program was used for statistical analysis. Conformity to normal distribution was evaluated with the Shapiro Wilk test. Comparison of quantitative variables between groups was measured by Kruskal Wallis H test. When significant statistical differences between the groups were determined, multiple comparisons between the groups were made with the Bonferroni-corrected Mann Whitney U test. Data were presented as mean ± standard deviation. $p < 0.05$ value was considered statistically significant. $LogIC_{50}$ values were calculated by using Graphpad prism 6 program in computer environment according to the obtained MTT results.

2. 3. Molecular Docking Studies

Ligands were energy-minimized using ChemOffice on Windows 10 operating system. Grid box points as size

of 60·60·60 Å^3 and a regular space of 0.375 Å were determined by centering on colchicine. "Tubulin-colchicine complex" pdb file (PDB ID: 4O2B) was get (https://www.rcsb.org/) and was modified using the Maestro.[54] Lamarckian Genetic Algorithm was preferred and standard settings used for all compounds. Docking scores were obtained using both AutoDock 4.2 software,[55] and AutoDock Vina software.[56] To validate the Autodock program, the co-crystallized ligand (PDB ID: LOC) was redocked on the target and RMSD value of 0.42 was found for tubulin-colchicine complex.

3. Results and Discussion

3. 1. Synthesis

In this work, 1-(4-hydroxyphenyl)-3-(3-substituted-phenyl)prop-2-en-1-one compounds **2**–**11** were prepared by the interaction of *para*-hydroxyacetophenone (**1**) with substitute aldehydes (3-methoxy, 2,4-dimethoxy, 3,4-dimethoxy, 2,3,4-trimethoxy, 2,4,5-trimethoxy, 4-phenyl, 3,5-difluoro, 4-chloro-2-fluoro, 3-(trifluoromethyl) phenyl, 4-chloro-3-(trifluoromethyl) benzaldeyhe) in the presence of ethanol and aqueous NaOH at room temperature.[28] The structures of compounds **2**–**11** were determined by MS, FT-IR, microanalysis, and 1D (^{1}H and ^{13}C-APT) NMR spectroscopic methods. The synthetic pathway of **2**–**11** and their numbering for ^{1}H and ^{13}C-APT NMR characterizations is shown in Scheme 1.

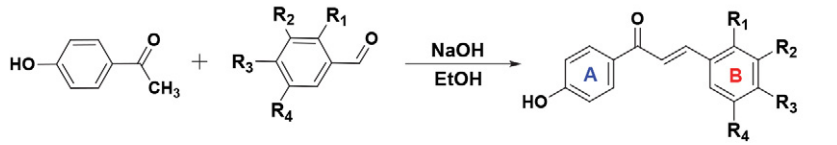
The compounds have only primary, tertiary and quaternary (–CH, –CH_3 and –C) carbon atoms in their structure. Therefore, ^{13}C-APT NMR analyzes were performed. These carbons provided easier assignment of NMR spectra.

When the proton and carbon NMR spectra of **2**–**11** are investigated, the methoxy proton and carbon peaks (H/C) of compounds containing methoxy as side groups have been observed at 3.84 / 55.75 ppm for **2** (number 17 proton and carbon peaks); 3.91 / 56.26 and 3.85 / 55.98 for **3**; 3.88 / 56.05 and 3.83 / 56.20 for **4** (number 17 and 18 proton and carbon peaks, respectively); 3.89 / 61.98, 3.79 / 60.94 and 3.87 / 56.51 for **5**; and 3.91 / 56.33, 3.83 / 56.94 and 3.88 / 56.94 for **6** (number 17, 18 and 19 proton and carbon peaks, respectively).

The carbonyl stretching vibrations, carbonyl protons and carbonyl carbon peaks of the starting compound aldehydes were not observed in the FT-IR, ^{1}H and ^{13}C-APT NMR spectra of the synthesized compounds **2**–**11**, respectively. The carbon numbers and the integration of proton signals in the NMR spectra of **2**–**11** support the structures of synthesized compounds. The ^{1}H and ^{13}C-APT NMR spectra of compound **2** are given as examples in Figure 1. The spectra of other compounds are given in Figures S1–30 in the Supplementary Information file.

3. 2. *In Vitro* Cytotoxic Activity

The chalcone compounds contain two phenyl rings shown as A and B in the scaffold (see Scheme 1). By chang-



	2	3	4	5	6	7	8	9	10	11
R_1	-H	-OCH_3	-H	-OCH_3	-OCH_3	-H	-H	-F	-H	-H
R_2	-OCH_3	-H	-OCH_3	-OCH_3	-H	-H	-F	-H	-CF_3	-CF_3
R_3	-H	-OCH_3	-OCH_3	-OCH_3	-OCH_3	-Ph	-H	-Cl	-H	-Cl
R_4	-H	-H	-H	-H	-OCH_3	-H	-F	-H	-H	-H

R_1 : 17 for 2, 5 and 6
R_2 : 17 for 2, 4, 8 and 11, 18 for 5
R_3 : 18 for 3, 4 and 6 19 for 5,
R_4 : 19 for 6

Scheme 1. Synthetic pathway of **2**–**11** and their numbering for ^{1}H and ^{13}C-APT NMR characterizations

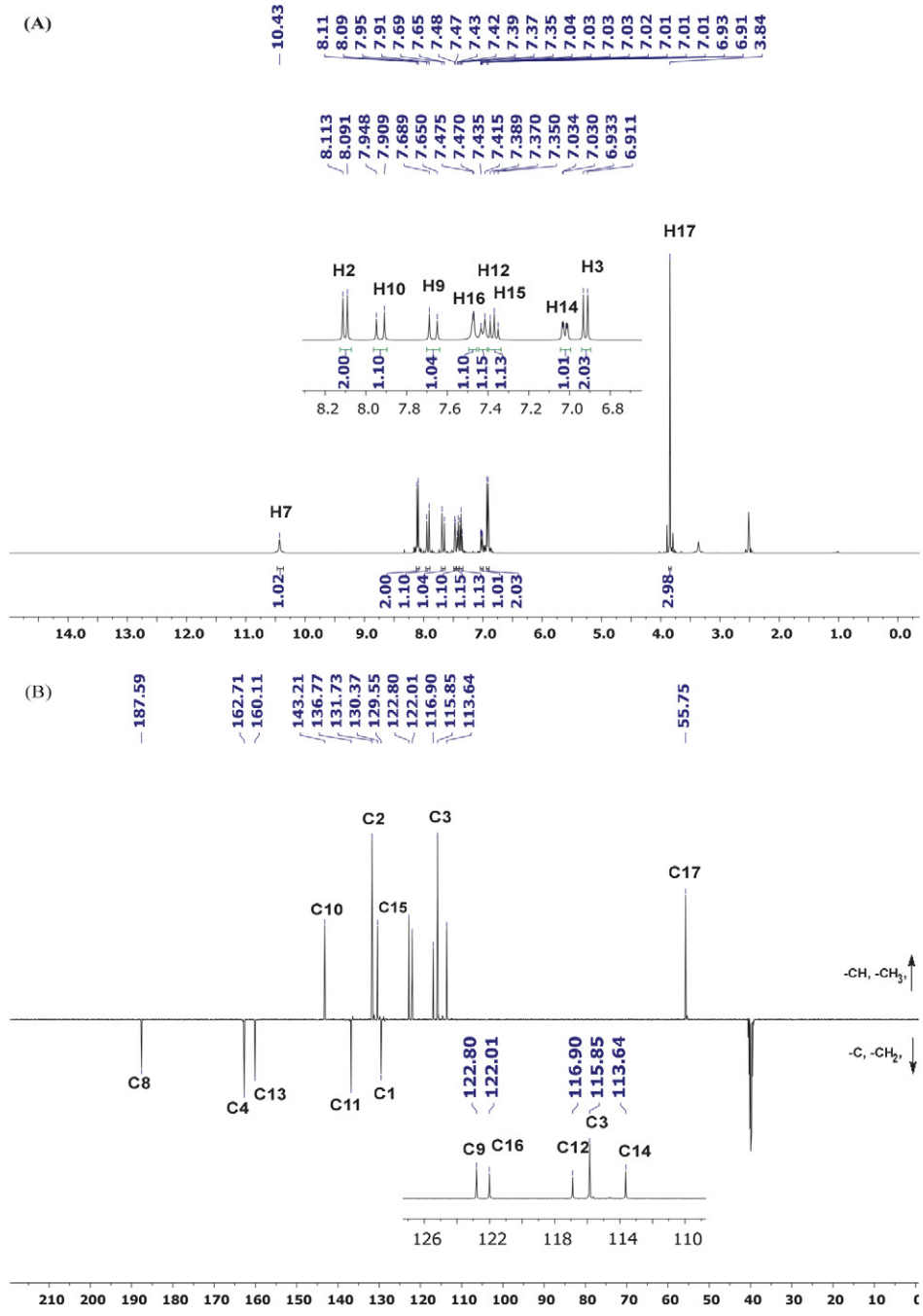


Figure 1. (A) ^{1}H NMR spectrum and (B) ^{13}C-APT NMR spectrum of **2** (DMSO-d_6)

ing the side groups on the phenyl rings of these compounds, biologically active molecules with different effects are formed. Numerous pure chalcones isolated from different plants have been approved for clinical studies in the treatment of cancer, viral and cardiovascular disorders. For example, the methochalcone compound containing methoxy group in the 2 and 4 positions of ring A and in the 4 positions of ring B has been approved for clinical use as a choleretic and diuretic agent. Similarly, some chalcone derivatives have been clinically tested and approved to show various biological activities, including anti-ulcer/mucoprotective activity (sofalkonin), vascular protection (hesperidin methyl-chalcone), anti-microbial activity (isobavachalcone), anti-cancer effect (flavokawain A), inflammatory action (cardamonin) and anti-diabetes-acting compound (4-hydroxyderricin) can be given as examples.[18–20] Each of these compounds has different side groups in rings A and B. This causes different biological activity. Therefore, in this study, a series of chalcone compounds with various groups in the side chains were syn-

thesized and their cytotoxic effects on different cancer cells were investigated. For this purpose, compounds having the -OH functional group in ring A and different side groups in ring B were synthesized.

Changes in cell viability rates of compounds **2**–**11** against A2780, LNCaP, PC-3 and MCF-7 cell lines are presented in Tables 1 and 2.

When the cytotoxic effects of the compounds **2**–**6** with the methoxy group(s) at different positions of the ring B against A2780 cells were examined, it was found that the compound **2** caused a significant decrease in cell viability in a dose-dependent manner ($p < 0.05$). In other compounds, cytotoxic effects were observed only for 25, 50 and 100 μM concentrations ($p < 0.05$). When the structure-activity relationship between the compound **2** with one methoxy group and the compounds **3**, **4**, **5** and **6** with two or three methoxy groups was examined, different cytotoxic effects were observed at different concentrations. Compound **2** exhibited cytotoxic effect at all concentrations. However, other compounds with more than one methoxy group did not show this effect. Despite these results, compounds carrying more than one methoxy group caused significant reductions at high concentrations. In particular, the effect of the compound **4** on cell viability is noteworthy.

In terms of IC_{50} values, it is seen that the compound **2** has an IC_{50} value of 10 μM, and the compound **4** has an IC_{50} value of 12.61 μM.

When the cytotoxic effects of these compounds in LNCaP cancer cell lines were examined, it was found that compound **2** decreased cell viability in the cell line depending on the dose ($p < 0.05$). Other compounds containing the methoxy group also had an effect at only 25, 50 and 100 μM concentrations ($p < 0.05$). Compound **4** caused decreases in cell viability at only 100 μM concentrations ($p < 0.05$). Although it causes a significant decrease at this concentration, its cytotoxic effect does not seem to be as strong as in A2780 cells. Especially for compound **2**, which acts on dose-dependent reduction of cell viability against A2780 and LNCaP cell lines, the IC_{50} value is 10.9 μM against A2780 cell lines, while this value is 1.823 μM against LNCaP cell lines. Considering the results, compound **2** seems to have a better effect against A2780 cell lines than LNCaP cell lines.

When the cytotoxic effects of these compounds on PC-3 and MCF-7 cancer cell lines were examined, it was observed that especially the compound **2** did not produce a cytotoxic effect in both cell lines. A similar situation was observed for the compound **5**. Compound **6** showed an effect at only 100 μM concentrations in both cells ($p < 0.05$). These compounds, which contain the methoxy group, appear to have very different effects in different cells. Overall, the results show that methoxy-containing compounds **2**–**6** are more effective against A2780 and LNCaP cells ($p < 0.05$).

Table 1. Cell viability (in %) of compounds **2**–**11** against human cancer cell lines ($p^* < 0.05$).

	A2780 Human Cancer Cell Lines						
	Control	**Solvent**	**1 μM**	**5 μM**	**25 μM**	**50 μM**	**100 μM**
2	100 ± 9.67	91.25 ± 8.16	**61.82 ± 8.12***	**51.25 ± 5.58***	**44.80 ± 9.21***	**30.18 ± 6.94***	**23.74 ± 4.11***
3	100 ± 9.67	91.25 ± 8.16	109.5 ± 12.31	100.9 ± 13.09	**66.66 ± 6.29***	**64.64 ± 7.02***	**47.49 ± 5.16***
4	100 ± 9.67	91.25 ± 8.16	92.18 ± 8.06	77.25 ± 10.05	**37.64 ± 6.68***	**10.77 ± 2.06***	**6.54 ± 2.19***
5	100 ± 9.67	91.25 ± 8.16	97.22 ± 8.65	85.48 ± 7.77	**68.95 ± 6.09***	**68.88 ± 8.01***	**14.42 ± 2.26***
6	100 ± 9.67	91.25 ± 8.16	199.5 ± 9.31	90.58 ± 8.1	**65.33 ± 5.27***	**66.34 ± 6.3***	**30.26 ± 5.36***
7	100 ± 9.67	91.25 ± 8.16	81.56 ± 10.17	**66.07 ± 8.72***	**11.35 ± 2.36***	**13.98 ± 3.24***	**11.64 ± 4.41***
8	100 ± 9.67	91.25 ± 8.16	85.62 ± 7.16	96.10 ± 11.29	**6.78 ± 2.03***	**4.74 ± 1.41***	**4.60 ± 1.12***
9	100 ± 9.67	91.25 ± 8.16	102.6 ± 13.28	**60.41 ± 10.25***	**4.75 ± 2.02***	**3.23 ± 1.26***	**2.45 ± 0.96***
10	100 ± 9.67	91.25 ± 8.16	103.8 ± 14.06	101.0 ± 11.64	**26.38 ± 4.11***	**6.91 ± 3.09***	**4.67 ± 1.01***
11	100 ± 9.67	91.25 ± 8.16	104.1 ± 10.21	97.87 ± 12.17	**5.70 ± 3.06***	**4.51 ± 2.41***	**4.14 ± 2.13***
	MCF-7 Human Breast Cancer Cell Lines						
	Control	**Solvent**	**1 μM**	**5 μM**	**25 μM**	**50 μM**	**100 μM**
2	100 ± 8.76	94.22 ± 7.87	106.6 ± 9.75	105.1 ± 14.21	97.2 ± 11.12	105.2 ± 10.11	93.97 ± 13.91
3	100 ± 8.76	94.22 ± 7.87	103.5 ± 9.51	84.89 ± 8.74	84.76 ± 11.41	81.64 ± 6.43*	**67.24 ± 7.79***
4	100 ± 8.76	94.22 ± 7.87	103.3 ± 11.5	98.59 ± 13.54	86.02 ± 13.41	**28.45 ± 6.97***	**29.17 ± 9.98***
5	100 ± 8.76	94.22 ± 7.87	118.3 ± 17.7	109.0 ± 15.62	105.8 ± 14.5	106.7 ± 17.9	107.3 ± 12.4
6	100 ± 8.76	94.22 ± 7.87	10.75 ± 11.37	99.77 ± 10.23	78.81 ± 9.40	83.88 ± 12.84	**29.04 ± 5.42***
7	100 ± 8.76	94.22 ± 7.87	99.66 ± 13.68	102.9 ± 14.81	102.0 ± 17.9	93.00 ± 9.72	**37.22 ± 6.38***
8	100 ± 8.76	94.22 ± 7.87	93.04 ± 12.31	**66.94 ± 10.23***	**16.26 ± 4.81***	**11.15 ± 2.37***	**11.34 ± 3.46***
9	100 ± 8.76	94.22 ± 7.87	96.83 ± 12.56	103.4 ± 16.66	**21.15 ± 5.67***	**11.37 ± 3.06***	**12.11 ± 4.21***
10	100 ± 8.76	94.22 ± 7.87	92.46 ± 9.99	**70.47 ± 5.25***	**21.93 ± 3.30***	**23.42 ± 4.81***	**23.69 ± 2.46***
11	100 ± 8.76	94.22 ± 7.87	105.6 ± 14.5	106.2 ± 15.88	**31.97 ± 5.64***	**21.74 ± 3.77***	**21.25 ± 4.27***

μM: Micromolar.

Table 2. Cell Viability (in %) of compounds **2**–**11** against cancer cell lines ($p^* < 0.05$).

	LNCaP Human Prostate Cancer Cell Lines						
	Control	**Solvent**	**1 μM**	**5 μM**	**25 μM**	**50 μM**	**100 μM**
2	100 ± 8.99	93.17 ± 10.12	**42.86 ± 5.21***	**38.13 ± 3.26***	**34.88 ± 6.78***	**24.44 ± 4.12***	**20.21 ± 3.96***
3	100 ± 8.99	93.17 ± 10.12	103.8 ± 14.4	92.66 ± 13.29	**59.60 ± 9.87***	**47.28 ± 8.86***	**38.22 ± 9.16***
4	100 ± 8.99	93.17 ± 10.12	96.94 ± 9.26	99.02 ± 11.23	78.82 ± 10.87	70.03 ± 14.69	**58.95 ± 8.26***
5	100 ± 8.99	93.17 ± 10.12	77.79 ± 10.86	74.92 ± 12.22	**51.56 ± 8.26***	**45.94 ± 9.13***	**39.18 ± 7.75***
6	100 ± 8.99	93.17 ± 10.12	81.98 ± 10.34	86.62 ± 11.2	**54.5 ± 8.7***	**46.21 ± 8.6***	**38.12 ± 9.6***
7	100 ± 8.99	93.17 ± 10.12	95.72 ± 14.26	90.69 ± 12.25	90.82 ± 13.23	99.67 ± 10.51	**61.55 ± 8.29***
8	100 ± 8.99	93.17 ± 10.12	86.25 ± 9.12	85.55 ± 8.26	**55.74 ± 9.41***	**48.52 ± 7.26***	**39.05 ± 7.29***
9	100 ± 8.99	93.17 ± 10.12	96.00 ± 9.26	97.42 ± 13.41	**52.40 ± 8.24***	**51.00 ± 9.12***	**41.92 ± 7.66***
10	100 ± 8.99	93.17 ± 10.12	91.28 ± 8.21	83.96 ± 9.22	**51.78 ± 7.42***	**10.14 ± 2.45***	**11.52 ± 3.11***
11	100 ± 8.99	93.17 ± 10.12	88.21 ± 9.77	80.90 ± 9.29	**62.67 ± 7.39***	**14.78 ± 2.88***	**10.78 ± 3.21***
	PC-3 Human Prostate Cancer Cell Lines						
	Control	**Solvent**	**1 μM**	**5 μM**	**25 μM**	**50 μM**	**100 μM**
2	100 ± 9.13	92.36 ± 8.13	110.2 ± 13.97	103.3 ± 13.2	96.57 ± 11.2	108.8 ± 15.2	90.97 ± 9.96
3	100 ± 9.13	92.36 ± 8.13	101.26 ± 7.34	110.7 ± 12.7	76.93 ± 8.96	**70.95 ± 9.34***	**5.67 ± 1.22***
4	100 ± 9.13	92.36 ± 8.13	99.53 ± 10.41	98.96 ± 8.41	**67.72 ± 7.34***	**62.73 ± 6.13***	**23.98 ± 3.81***
5	100 ± 9.13	92.36 ± 8.13	103.5 ± 12.32	95.55 ± 13.21	99.24 ± 8.99	93.53 ± 14.22	92.23 ± 7.49
6	100 ± 9.13	92.36 ± 8.13	102.6 ± 7.14	109.6 ± 11.5	79.94 ± 8.86	85.96 ± 8.33	**5.67 ± 1.22***
7	100 ± 9.13	92.36 ± 8.13	112.4 ± 10.85	85.97 ± 9.34	89.10 ± 8.45	80.97 ± 7.78	**11.14 ± 2.39***
8	100 ± 9.13	92.36 ± 8.13	88.59 ± 9.29	91.12 ± 11.29	**55.90 ± 8.21***	**7.98 ± 1.14***	**3.61 ± 1.22***
9	100 ± 9.13	92.36 ± 8.13	**70.11 ± 8.28***	**65.34 ± 7.92***	**64.46 ± 9.12***	**2.68 ± 0.78***	**1.81 ± 0.84***
10	100 ± 9.13	92.36 ± 8.13	82.71 ± 9.21	80.33 ± 8.93	**38.16 ± 6.23***	**1.90 ± 0.37***	**1.73 ± 0.22***
11	100 ± 9.13	92.36 ± 8.13	92.08 ± 10.12	87.54 ± 11.98	72.65 ± 9.93	**1.85 ± 0.42***	**1.65 ± 0.34***

μM: Micromolar.

Although compound **7** significantly reduced cell viability at 5, 25, 50 and 100 μM concentrations in A2780 cell lines, it caused significant reductions in cell viability at only 100 μM concentration in other cell lines ($p < 0.05$). The IC_{50} value of the compound **7** against A2780 cell lines is 6.898 μM. In terms of structure-activity relationship, the fact that the compound contains a phenyl ring at the *para* position caused a significant decrease in cell viability. The $logIC_{50}/IC_{50}$ values of the compounds are given in Table 3. When the cytotoxic activities of the compounds **8**, **9**, **10** and **11**, which contain -F, -Cl, $-CF_3$ groups separately or together, were observed against four cell lines, there was a significant decrease in cell viability, especially at concentrations of 50 and 100 μM ($p < 0.05$). In particular, these

Table 3. The $logIC_{50}/IC_{50}$ (50% inhibition-causing concentration) values (μM) of compounds 2–11 against A2780, LNCaP, PC-3 and MCF-7 cancer cell lines

Comp.	**A2780** $\mathbf{LogIC_{50}}$ **/** $\mathbf{IC_{50}}$ **(μM)**	**LNCaP** $\mathbf{LogIC_{50}}$ **/** $\mathbf{IC_{50}}$ **(μM)**	**PC-3** $\mathbf{LogIC_{50}}$ **/** $\mathbf{IC_{50}}$ **(μM)**	**MCF-7** $\mathbf{LogIC_{50}}$ **/** $\mathbf{IC_{50}}$ **(μM)**
2	1.037 / 10.9	0.2607 / 1.823	–	3.537 / 3445
3	1.911 / 81.4	1.674 / 47.24	1.763 / 57.88	2.285 / 192.9
4	1.101 / 12.61	2.097 / 125.1	1.747 / 55.91	1.67 / 46.78
5	1.712 / 51.53	1.548 +/ 35.35	2.1066 / 27.616	–
6	1.818 / 65.71	1.605 / 40.29	1.853 / 71.36	1.935 / 86.12
7	0.8387 / 6.898	2.403 / 253	1.866 / 73.52	2.18 / 151.2
8	0.9888 / 9.746	1.635 / 43.19	1.259 / 18.16	0.9071 / 8.075
9	0.7853 / 6.1	1.684 / 48.34	1.112 / 12.93	1.164 / 14.57
10	1.153 / 14.24	1.242 / 17.47	1.049 / 11.18	1.062 / 11.54
11	1.027 / 10.64	1.3198 / 20.84	1.312 / 20.49	1.32 / 20.9
Paclitaxel	0.7516 / 5.645	–	–	–
Tamoxifen	–	–	–	1.308 / 20.33
Docetaxel	–	0.7792 / 6.014	1.229 / 16.95	–

μM: Micromolar.

Table 4. Molecular docking binding scores of some compounds, within the tubulin-colchicine complex (PDB ID: 4O2B) active site. Residues participating in H-bonds with the compounds are shown.

Comp.	Auto dock Result				Vina Result
	Residues participating H-bonds Receptor			Docking Score	Docking Score
	A Chain	B Chain	Estimated Inhibition Constant, *Ki*	Estimated Free Energy of Binding (kcal/mol)	
2	Gln11, Asn101	Gln247, Lys254	1.91 μM	−7.80	−8.4
3	Gln11	Gln247, Lys254	1.96 μM	−7.79	−8.2
4	Gln11, Asn101	Gln247, Lys254	1.77 μM	−7.85	−8.2
5	Gln11, Asn101	Gln247, Lys254	1.25 μM	−8.05	−8.4
6	Gln11, Asn101	Gln247, Lys254	1.07 uM	−8.15	−8.2
7	Gln11, Asn101	Gln247, Lys254	22.01 μM	−10.45	−8.9
8	Gln11, Asn101	Gln247, Lys254	3.48 μM	−7.45	−8.5
9	Gln11, Asn101	Gln247, Lys254	1.16 μM	−8.10	−8.3
10	Gln11, Asn101	Gln247, Lys254	2.25 μM	−7.70	−9.3
11	Gln11	Gln247, Lys254	1.25 μM	−8.05	−9.7

nM: nanomolar, μM: micromolar

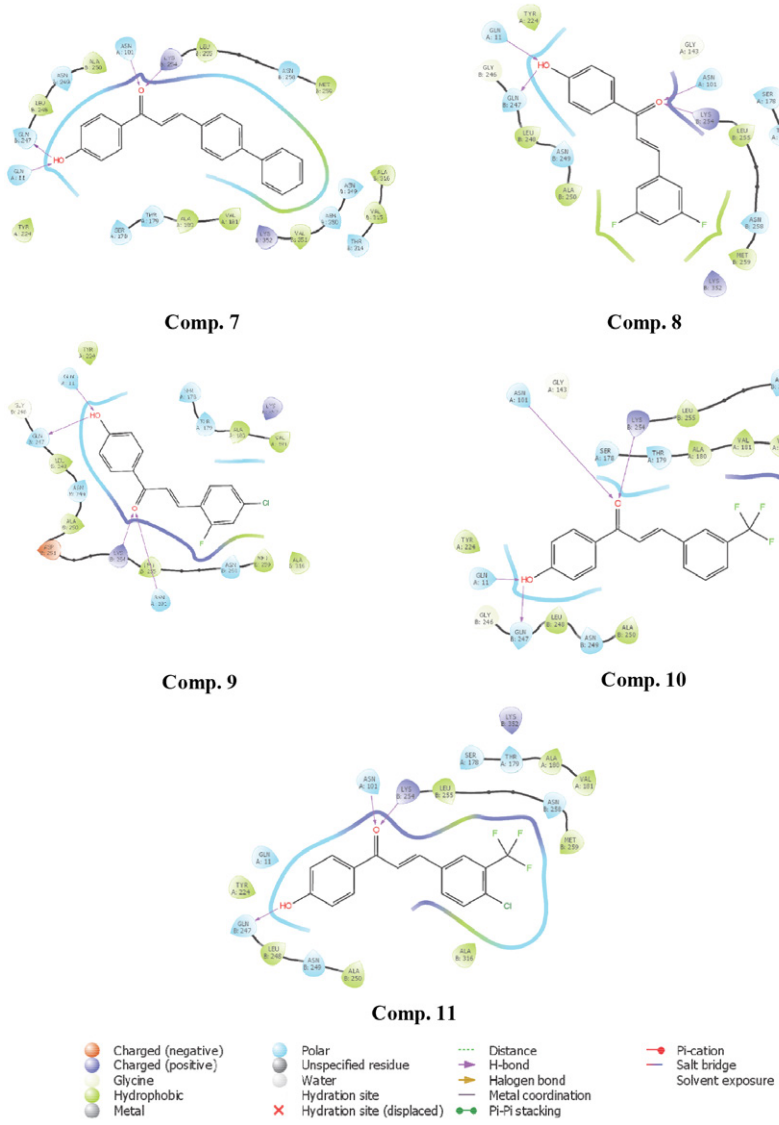


Figure 2. 2D interaction diagram for compounds **7**–**11** at the tubulin binding cavity.

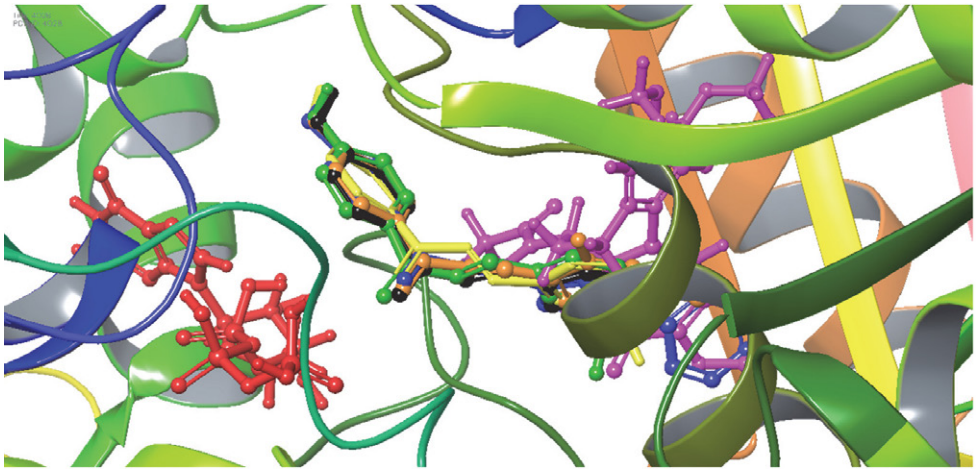


Figure 3. Compounds **7** (blue), **8** (yellow), **9** (orange), **10** (green), and **11** (black), colchicine (pink) and guanosine-5'-triphosphate (red) are presented in the tubulin-colchicine complex binding cavity.

compounds were found to have strong cytotoxic effects ($p < 0.05$) against A2780 and PC-3 cell lines.

According to the results, especially against the A2780 and LNCaP cell lines, the compounds **2**–**7** with electron releasing groups (methoxy / phenyl) in the phenyl ring showed strong cytotoxic effect ($p < 0.05$). Compounds **8**–**11** containing electron withdrawing groups in the phenyl ring were found to have strong cytotoxic effects against four cell lines at different doses ($p < 0.05$).

3. 3. Molecular Docking Studies

According to the X-ray crystallographic structure of tubulin-colchicine complex (PDB ID: 4O2B), main binding site has been determined around small molecules such as colchicine (ligand ID: LOC) and guanosine-5'-triphosphate (ligand ID: GTP) in receptor (https://www.rcsb.org/). It has been declared that colchicine interacts with active site in tubulin as the binding site. It has been previously established that colchicine interacts with Ser178A, Thr179A, Ala180A, Val181A, Cys241B, Leu242B, Leu248B, Ala250B, Asp251B, Lys254B, Leu255B, Asn258B, Met259B, Thr314B, Val315B, Ala316B, Ile318B, Asn350B, Lys352B, Ile378B residues (https://www.ebi.ac.uk/pdbe/). The formation of hydrogen bonds between the hydroxyl group in the phenol ring of all compounds by Gln11A and Gln247B showed binding to the gorge. The Lys254B has a position in the active site to interact with carbonyl of compounds by establishing a hydrogen bond (Table 4).

Docking studies were performed for all compounds and interaction modes for compounds **7**, **8**, **9**, **10** and **11** with enzyme active sites were determined (Figure 2). The binding types and residues were produced showed by Maestro software (Maestro, Schrödinger, LLC, New York, NY, 2020). These compounds binding modes were similar as with colchicine (Figure 3). The results of molecular docking studies were exhibited to be relevant for the results of the *in vitro* activity studies.

4. Conclusion

All the compounds (except compound **5**) showed significant cytotoxic effects at high doses in all cancer cell lines. Among all the compounds studied, one compound i.e. compound **2** demonstrated dose-dependent activity, particularly against A2780/LNCaP cancer cell lines. The most effective compounds **8**, **9**, **10** and **11** reduced the cell viability of A2780, MCF-7, PC-3 and LNCaP cells by 50–98%, while other compounds **2**, **4** and **7** reduced the cell viability of A2780 cells by 70–90% at concentrations of 50 and 100 μM. The results indicate that these compounds have high cytotoxic effect against these human cancer cell lines. Molecular docking studies exhibited the interaction mode of all compounds with tubulin including hydrophobic interactions and hydrogen bonds. Chalcone analogues continue to show hope as an anticancer agent through tubulin inhibition, suggesting that this aspect should be improved. Thus, the next aim of the future study will be to determine activities against various human cancer cell lines and non-tumorigenic epithelial cell lines. *In vivo* experiments will be carried out by selecting those that show significant activity among these compounds.

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Conflict of Interest

Authors declare no conflict of interest

5. References

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Povzetek

Načrtovali in izvedli smo sintezo serije halkonskih spojin (**2**–**11**) z namenom, da določimo njihove citotoksične učinke. Strukture **2**–**11** smo karakterizirali s pomočjo fizikalnih in spektroskopskih podatkov. Citotoksični učinek spojin **2**–**11** smo *in vitro* določali na človeških rakastih celičnih linijah raka jajčnika (A2780), raka dojke (MCF-7) in raka prostate (PC-3 ter LNCaP). Potencialne aktivnosti spojin smo še dodatno raziskali s pomočjo študij molekulskega sidranja s programoma AutoDock4 in Vina. Vse spojine (z izjemo spojine **5**) so pri visokih odmerkih pokazale opazne citotoksične učinke proti vsem rakastim celičnim linijam. Izmed vseh preiskovanih spojin, je spojina **2** izkazala aktivnost, odvisno od koncentracije, še posebej proti A2780/LNCaP rakastim celičnim linijam. Najbolj učinkovite spojine **8**, **9**, **10** in **11** so zmanjšale sposobnost preživetja celic pri A2780, MCF-7, PC-3 ter LNCaP celičnih linijah za 50–98%; spojine **2**, **4** in **7** pa so zmanjšale sposobnost preživetja celic pri A2780 celični liniji za 70–90% pri koncentracijah 50 in 100 μM.