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RESEARCH ARTICLE

Synthesis and structure-activity relationship study: Anticancer chalcones derived from 4′-morpholinoacetophenone

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ABSTRACT

The aim of this research was to synthesize chalcone derivatives containing morpholine ring and to investigate their antiproliferative activity. The 3-aryl-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one derivatives were synthesized and evaluated for their *in vitro* antiproliferative activities on C6 cells (Rat glioma cell line) and HeLa cells (Human cervix adenocarcinoma), using the BrdU ELISA assay. The calculation of ADME properties of new chalcone derivatives was used by topological polar surface area (TPSA), absorption (ABS%) and Lipinski's rules. Most of the compounds exhibited significantly antiproliferative activity on two cell lines. Among the screened compounds, compound **7, 10, 11** and **12** revealed higher antiproliferative activity than cisplatin which was used as reference drug. Compound **11** on HeLa cell and compound **10** on C6 cell line are the most effective compared with cisplatin.

Keywords: Chalcone; antiproliferative activity; cytotoxic; morpholine

1. INTRODUCTION

Cancer is commonly known as the second major cause of death at a ratio of 20-25% in developing countries after the cardiovascular disease [1]. The basic principle of the chemotherapy is to inhibit the proliferation and growth of tumor cells without damaging the patient's normal cells and tissues, later to kill tumor cells. But, it is not possible to stop or destroy cancer cells selectively without affecting healthy cells though the physiological and anatomical structure of cancer cells are different from normal cells. Therefore, anticancer drugs which are also cytotoxic agents, cause severe side effects such as hair loss, disorder of gastrointestinal system (vomitting, diarrhea etc.), liver toxication and damage to bone marrow and also drug-resistance to tumor cells occur at long term therapy [2]. Thus, new anticancer agents are urgently needed for cancer therapy.

Flavonoids which are classified as flavonol, flavonone, flavanonol, flavanol, flavone, chalcone etc. are known phytochemical natural products have different pharmacological activities including anticancer, anti-HIV, antimicrobial, antiinflammatory [3, 4]. Chalcones are a significant part of plants belonging to the flavonoids family [5]. Two aryl groups are attached by three-carbons with α,β-unsaturated carbonyl

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system. These compounds have been known to display interesting pharmacological activities such as anticancer, anti-inflammatory, antibacterial, antifungal, antimicrobial, antimalarial and anti-HIV activities [6-13]. Therefore in this research, we synthesized some novel 1,3-diaryl-2-propen-1-ones with the hope of discovering more active and selective anticancer agents. The calculation of topological polar surface area (TPSA), absorption (ABS%) and Lipinski's rule of five the was proved that these compounds **1-12** were drug-likeness compared to cisplatin.

2. MATERIALS AND METHODS

2.1. Chemistry

All chemicals and solvents were in analytical grade and purchased from Sigma-Aldrich, Merck or Roche. All chemical reactions were monitored with thin layer chromatography (TLC) using Merck silica gel 60 F_{254} plates. Melting points were determined by EZ-Melt melting point apparatus and were uncorrected. Electronic spectra were recorded in DMF on a PG Instruments T80+ UV-visible Spectrophometer. IR spectra were determined with a Bruker Alpha 4000 FTIR spectrophotometer. Elemental analyses (CHNS) were performed on a VarioMICRO elemental analyzer. ^{1}H, ^{13}C, DEPT-90, DEPT-135, DEPT, APT, HETCOR, HMBC, NMR spectra were recorded on a Bruker Unity-400 spectrometer. The chemical shift values are given in ppm and following abbreviations were used: s = singlet, d = doublet, dd = doublet to doublet, t = triplet, m = multiplet. The coupling constants (J) are given in Hertz. The mass spectra of all compounds were recorded on a Agilent 1260 infinity LC. 6210 HPLC-TOF/MS spectrometer in electrospray mode. The mass spectra of compounds **1, 2** and **12** were recorded on a Agilent 1100 MSD spectrometer in order to detect the fragments.

2.2. General Synthesis Method

The quantities of substituted benzaldehyde and 4'-morpholino acetophenone were dissolved in methanol, three equivalents of NaOH was added to the mixture and stirred at the room temperature. The resulting solution was stirred for 2 days and kept in refrigerator overnight. The reaction mixture was extracted with dichloromethane. Organic phase was dried with anhydrous $MgSO_4$. The solvent was evaporated and the crude product recrystallized from *n*-hexane/DCM [14].

2.2.1. *(2E)-3-(furan-3-yl)-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (1).* Green crystal, 69%, 180-182°C. UV/Vis λ_{max}(nm): 264, 358. FTIR ν_{max}(cm^{-1}): 1644 (C=O); 1580, 1542, 1515, 1472 (C=C); 1188 (C-O-C morpholine); 1126 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6, δ ppm): 3.32 (t, 4H, Hb morpholine); 3.75 (t, 4H, Ha morpholine); 6.68 (dd, 1H, J_1=2.0 Hz, $J_{2=}$1.6 Hz, Hd B ring); 7.03 (d, 2H, *J*=8.8 Hz, Ha A ring); 7.06 (d, 1H, *J*=3.2 Hz, Hb B ring); 7.50 (d, 1H, *J*=15.6 Hz, Hα proton); 7.56 (d, 2H, *J*=15.2 Hz, Hβ proton); 7.89 (d, 1H, *J*=0.8 Hz, Hc B ring); 7.99 (d, 2H, *J*=9.2 Hz, Hb A ring). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.2, 66.3 (-NCH_2CH_2O-); 119.4, 129.4 (-CH=CH-CO-); 113.4, 113.6, 116.4, 127.9, 130.8, 146.2, 151.9, 154.5 (Ar-C); 186.4 (C=O). TOF/MS(m/z) 284.1296 [M+H]$^+$. MS: m/z= 283 (M$^+$), 255, 225, 197, 190, 162, 132, 121, 86, 77, 65, 51, 39. Anal. calc. for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94. Found: C, 71.84; H, 6.01; N, 5.11 %.

2.2.2. (2E)-1-[4-(morpholin-4-yl)phenyl]-3-(thiophen-3-yl) prop-2-en-1-one (2). Yellow crystal, 45%, 156-158°C. UV/Vis λ_{max}(nm): 244, 227, 358. FTIR ν_{max}(cm^{-1}): 1641 (C=O); 1598, 1574, 1444, 1422 (C=C); 1188 (C-O-C morpholine); 1120 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6, δ ppm): 3.33 (t, 4H, Hb morpholine), 3.75 (t, 4H, Ha morpholine); 7.04 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.19 (dd, 1H, J_1=3.6 Hz, $J_{2=}$3.6 Hz, Hc B ring); 7.57 (d, 1H, J_1=15.2 Hz, Hα proton); 7.66 (d, 1H, J_1=3.2 Hz, Hb B ring); 7.76 (d, 1H, *J*=5.2 Hz, Hd B ring); 7.84 (d, 2H, *J*=15.2 Hz, Hβ proton); 8.01 (d, 2H, *J*=9.2 Hz, Hb A ring). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.2, 66.3 (-NCH_2CH_2O-); 127.8 140.5 (-CH=CH-CO-); 113.6, 121, 129.1, 130.2, 130.8, 132.5, 135.5, 154.5 (Ar-C); 186.5 (C=O). TOF/MS(m/z) 299.0991 [M+H]$^+$. MS: m/z= 299 (M$^+$), 271, 241, 213, 190, 157, 137, 131, 109, 86, 77, 65, 51, 39. Anal. calc. for $C_{17}H_{17}NO_2S$: C, 68.20; H, 5.72; N, 4.68; S, 10.09. Found: C, 68.02; H, 5.53; N, 4.46; S, 10.39 %.

2.2.3. *(2E)-1-[4-(morpholin-4-yl)phenyl]-3-phenylprop-2-en-1-one (3).* Green crystal, 67%, 173-175°C. UV/Vis λ_{max}(nm): 227, 264, 304, 354, 357. FTIR ν_{max}(cm^{-1}): 1643 (C=O); 1583, 1515, 1495, 1445 (C=C); 1220 (C-O-C morpholine); 1118 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.33 (t, 4H, Hb morpholine); 3.75 (t, 4H, Ha morpholine); 7.04 (d, 2H, *J*= 9.2 Hz, Ha A ring); 7.44-7.48 (m, 3H, Hb,c,d B ring); 7.68 (d, 1H, *J*=15.6 Hz, Hα proton); 7.88 (dd, 2H, J_1= J_2=2.4 Hz, Ha,e B ring); 7.94 (d, 1H, *J*= 15.6 Hz, Hβ proton); 8.09 (d, 2H, *J*=8.8 Hz, Hb A ring). ^{13}C-NMR: (100 MHz) (DMSO-d_6/TMS) δ ppm: 47.2, 66.3 (-NCH_2CH_2O-); 122.6, 142.7 (-CH=CH-CO-); 113.54, 127.9, 129.14, 129.34, 130.7, 131.04, 135.5, 154.6 (Ar-C); 187 (C=O). TOF/MS (m/z) 293.4150 [M+H]$^+$. Anal. calc. for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.50; H, 6.55; N, 4.96 %.

2.2.4. *(2E)-3-(4-chlorophenyl)-1-[4-(morpholin-4-yl)phenyl] prop-2-en-1-one (4).* Yellow crystal, 69%, 203-205°C. UV/Vis λ_{max}(nm): 230, 311, 355. FTIR ν_{max}(cm^{-1}): 1644 (C=O); 1583, 1487, 1445 (C=C); 1188 (C-O-C morpholine); 1079 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.36 (t, 4H, Hb morpholine); 3.89 (t, 4H, Ha morpholine); 6.94 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.40 (d, 2H, *J*=8.4 Hz, Hb,d B ring); 7.55 (d, 1H, *J*=15.6 Hz, Hα proton); 7.59 (d, 2H, *J*=8.4 Hz, Ha,e B ring); 7.76 (d, 1H, *J*=15,6 Hz, Hβ proton); 8.03 (d, 2H, *J*=9.2 Hz, Hb A ring). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.4, 66.5 (-NCH_2CH_2O-); 122.4, 141.8 (-CH=CH-CO-); 113.3, 128.6, 129.2, 129.4, 131.2, 133.8, 135.9, 154.3 (Ar-C); 187.8 (C=O). TOF/MS(m/z) 327.1158 $[M+H]^+$. Anal. calc. for $C_{19}H_{18}ClNO_2$: C, 69.62; H, 5.53; N, 4.27. Found: C, 69.47; H, 5.52; N, 4.57 %.

2.2.5. *(2E)-3-(2,4-dichlorophenyl)-1-[4-(morpholin-4-yl) phenyl]prop-2-en-1-one (5).* Yellow crystal, 42%. 135-137°C. UV/Vis λ_{max}(nm): 235, 300, 355. FTIR ν_{max}(cm^{-1}): 1649 (C=O); 1583, 1547, 1464 (C=C); 1186 (C-O-C morpholine); 1120 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.35 (t, 4H, Hb morpholine), 3.75 (t, 4H, Ha morpholine); 7.04 (d, 2H *J*=9.2 Hz, Ha A ring); 7.53-7.65 (dd, 1H, J_1=8.8 Hz, J_2=2.0 Hz, Hb B ring), 7.74 (d, H, *J*=2.0 Hz, Hd B ring), 7.90 (d, H, *J*=15.6 Hz, Hα proton); 8.03 (d, 1H, *J*=8.8 Hz, Ha B ring); 8.10 (d, 2H, *J*=9.2 Hz, Hb A ring). 8.30 (d, 1H, *J*=15.2 Hz, Hβ proton). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.1, 66.3 (-NCH_2CH_2O-); 126, 136.1 (-CH=CH-CO-); 113.5, 127.5, 128.3, 129.9, 130.2, 131.3, 132.2, 135.4, 135.6, 154.7 (Ar-C); 186.5 (C=O). TOF/MS(m/z) 361.0685 $[M+H]^+$. Anal. calc. for $C_{19}H_{17}Cl_2NO_2$: C, 63.00; H, 4.73; N, 3.87. Found: C, 63.17; H, 4.78; N, 4.17 %.

2.2.6. *(2E)-3-(3,4-dichlorophenyl)-1-[4-(morpholin-4-yl) phenyl]prop-2-en-1-one (6).* Yellow crystal, 67%, 177-179°C. UV/Vis λ_{max}(nm): 230, 235, 300, 355. FTIR ν_{max}(cm^{-1}): 1648 (C=O); 1592, 1547, 1516, 1470 (C=C); 1189 (C-O-C morpholine); 1116 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.36 (t, 4H, Hb morpholine); 3.89 (t, 4H, Ha morpholine); 6.93 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.45-7.52 (m, 2H, Hb,e B ring); 7.55 (d, 1H, *J*=15.6 Hz, Hα proton); 7.69 (d, 1H, *J*=15.6 Hz, Hβ proton); 7.73 (s, 1H, Ha B ring); 8.02 (d, 2H, *J*=8.8 Hz, Hb A ring). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.1, 66.3 (-NCH_2CH_2O-); 126, 136.1 (-CH=CH-CO-); 113.5, 127.5, 128.3, 129.9, 130.2, 131.3, 132.2, 135.4, 135.6, 154.7 (Ar-C); 186.5(C=O). TOF/MS(m/z) 361.0645 $[M+H]^+$. Anal. calc. for $C_{19}H_{17}Cl_2NO_2$: C, 63.00; H, 4.73; N, 3.97. Found: C, 62.78; H, 4.70; N, 4.12 %.

2.2.7. *(2E)-3-(4-fluorophenyl)-1-[4-(morpholin-4-yl)phenyl] prop-2-en-1-one (7).* Yellow crystal, 70%, 197-199°C. UV/Vis λ_{max}(nm): 230, 306, 354, 358. FTIR ν_{max}(cm^{-1}): 1645 (C=O); 1580, 1503, 1446, 1417 (C=C); 1224 (C-F); 1187 (C-O-C morpholine); 1116 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.33 (t, 4H, Hb morpholine), 3.75 (t, 4H, Ha morpholine); 7.04 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.30 (d, 2H, Ha,e B ring); 7.70 (d, 1H, *J*=15.6 Hz, Hα proton); 7.91 (d, 1H, *J*=15.2 Hz, Hβ proton); 7.94-7.98 (m, 2H, Hb,d B ring); 8,09 (d, 2H, *J*=9.2 Hz, Hb A ring). ^{13}C-NMR: (100 MHz) (DMSO-d_6/TMS) δ ppm: 47.2, 66.3 (-NCH_2CH_2O-); 122.6, 141.5 (-CH=CH-CO-); 116.2, 116.4, 127.9, 131, 131.4, 132.2, 154.6, 162.4 (Ar-C); 186.9 (C=O). TOF/MS(m/z) 311.1459 $[M+H]^+$. Anal. calc. for ($C_{19}H_{18}FNO_2$): C, 73.29; H, 5.83; N, 4.50; Found: C, 73.37; H, 5.85; N, 4.81 %.

2.2.8. *(2E)-3-[4-(methylsulfanyl)phenyl]-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-one (8).* Yellow crystal, 71%, 145-147°C. UV/Vis λ_{max}(nm): 227, 250, 358. FTIR ν_{max}(cm^{-1}): 1646 (C=O); 1580, 1548, 1489, 1443 (C=C); 1219 (C-O-C morpholine); 1115 (C-N-C morpholine). ^{1}H-NMR ($CDCl_3$, δ ppm): 2.54 (s, 3H, -SCH_3); 3.36 (t, 4H, Hb morpholine); 3.89 (t, 4H, Ha morpholine); 6.94 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.27 (d, 2H, *J*=8.0 Hz, Hb,d B ring); 7.55-7.59 (m, 3H, Ha,e B ring, Hα proton); 7.78 (d, 1H, *J*=15.6 Hz, Hβ); 8.03 (d, 2H, *J*=8.8 Hz, Hb A ring). ^{13}C-NMR: (100 MHz)($CDCl_3$ /TMS) δ ppm: 14.69 (-SCH_3); 47.21, 66.33 (-NCH_2CH_2O-); 186.9 (C=O); 141.8, 122.4 (-CO-CH=CH-); 154.3, 135.9, 133.8, 131.2, 129.4, 129.2, 128.6, 113.3 (Ar-C). TOF/MS(m/z) 340.1344 $[M+H]^+$. Anal. calc. for $C_{20}H_{21}NO_2S$: C, 70,77; H, 6.24; N, 4.13; S, 9.45. Found: C, 70,24; H, 6.03; N, 5.09; S, 9.88%.

2.2.9. *(2E)-3-(4-methoxyphenyl)-1-[4-(morpholin-4-yl) phenyl]prop-2-en-1-one (9).* Yellow crystal, 79 %, 138-140°C. UV/Vis λ_{max}(nm): 227, 354, 368, 372. FTIR ν_{max}(cm^{-1}): 1643 (C=O); 1598, 1564, 1509, 1445 (C=C); 1224 (C-F); 1193 (C-O-C morpholine); 1117 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.34 (t, 4H, Hb morpholine); 3,87 (s, 3H, -OCH_3); 3.88 (t, 4H, Ha morpholine ring); 6,93 (d, 2H, *J*=8.8 Hz, Ha A ring); 6,95 (d, 2H, *J*=8.0 Hz Hb,d B ring); 7.47 (d, 1H, *J*=15.6 Hz, Hα proton); 7,62 (d, 2H, *J*=8.4 Hz, Ha,e B ring); 7.80 (d, 1H, *J*=15,6 Hz, Hβ proton); 8.03 (d, 2H, *J*=8.8 Hz, Hb A ring). ^{13}C-NMR: (100 MHz) (DMSO-d_6/TMS) δ ppm: 42.8, 50.6, 61.9 (Aliphatic C); 114.8, 138.4 (-CH=CH-CO-); 108.7, 109.6, 124.4, 123.2, 125.2, 125.8, 149.3, 156.6 (Ar-C); 188.2 (C=O). TOF/MS(m/z) 325.1666 $[M+H]^+$. Anal. calc. for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.00; H, 6.54; N, 4.47 %.

2.2.10. *(2E)-1-[4-(morpholin-4-yl)phenyl]-3-(4-nitrophenyl) prop-2-en-1-one (10).* Orange crystal, 53 %, 143-145°C. UV/Vis λ_{max}(nm): 227, 312, 388. FTIR ν_{max}(cm^{-1}): 1654 (C=O); 1594, 1448, 1420 (C=C); 1190 (C-O-C morpholine); 1108 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.34 (t, 4H, Hb morpholine); 3.75 (t, 4H, Ha morpholine); 7.05 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.75 (d, 1H, *J*=15.2 Hz, Hα proton); 8.10 (d, 2H, *J*=8.8 Hz, Ha,e B ring); 8.12 (d, 2H, *J*=8.8 Hz, Hb A ring); 8.15 (d, 1H, *J*=14.4 Hz, Hβ proton); 8.28 (d, 2H, *J*=8.8 Hz, Hb,d B ring). ^{13}C-NMR: (100 MHz) (DMSO-d_6/TMS) δ ppm: 47.1, 66.3 (-NCH_2CH_2O-); 126.8, 142.0 (-CH=CH-CO-); 113.5, 124.3, 127.5, 130, 131.3, 139.8, 148.3, 154.7 (Ar-C); 186.6 (C=O).The HMBC spectrum displayed correlations of H-4/C-3; H-4/C-5; H-5/C-6; H-8/C-7; H-8/C-9; H-8/C-10; H-9/C-7; H-9/C-8; H-9/C-10; H-12/C-10; H-12/C-13. In DEPT 90 spectrum, only CH's signal was dedected and it was positive. Therefore CH positive signals of **10** were detected at 113.46, 124.34, 126.80, 130.07, 131.27, 139.88 ppm. The negative signals in DEPT 135 were indicated that CH_2 group of morpholine were at 47.15 and 66.3 ppm. TOF/MS (m/z) 338.1327 $[M+H]^+$. Anal. calc. for $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28. Found: C, 67.40; H, 5.31; N, 8.24 %.

2.2.11. *(2E)-1-[4-(morpholin-4-yl)phenyl]-3-[4-(trifluoromethyl)phenyl]prop-2-en-1-one (11).* Yellow crystal, 71 %, 130-132°C. UV/Vis λ_{max}(nm): 227, 292, 358. FTIR ν_{max}(cm^{-1}): 1651 (C=O); 1585, 1517, 1449, 1416 (C=C); 1159 (C-O-C morpholine); 1104 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.72 (t, 4H, Hb morpholine); 3.90 (t, 4H, Ha morpholine); 6.95 (d, 2H, *J*=8.8 Hz, Ha A ring); 7.63-7.69 (m, 3H, Ha,e B ring, Hα proton); 7.76 (d, 2H, *J*=8.0 Hz, Hb,d B ring); 7.81 (d, 1H, *J*=15.6 Hz, Hβ proton); 8.04 (d, 2H, *J*=9.2 Hz, Hb A ring). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.4, 66.5 (-NCH_2CH_2O-); 123.5, 140.4 (-CH=CH-CO-); 113.4, 127.5, 128.3, 129.6, 130.7, 130.8, 133.5, 135.4, 154.4 (Ar-C); 187.4 (C=O). TOF/MS (m/z) 362.1358 $[M+H]^+$. Anal. calc. for $C_{20}H_{18}F_3NO_2$: C, 66.48; H, 5.02; N, 3.88. Found: C, 66.71; H, 5.05; N, 4.18 %.

2.2.12. *(2E)-1-[4-(morpholin-4-yl)phenyl]-3-[4-(trifluoromethoxy)phenyl]prop-2-en-1-one (12).* Yellow crystal, 75 %, 119-121°C. UV/Vis λ_{max}(nm): 227, 297, 358. FTIR ν_{max}(cm^{-1}): 1647 (C=O); 1602, 1581, 1504, 1448 (C=C); 1157 (C-O-C morpholine); 1119 (C-N-C morpholine). ^{1}H-NMR (DMSO-d_6/TMS, δ ppm): 3.33 (t, 4H, Hb morpholine); 3.74 (t, 4H, Ha morpholine); 7.04 (d, 2H, *J*=9.2 Hz, Ha A ring); 7.44 (d, 2H, Hd,c B ring); 7.69 (d, 1H, *J*=15.6 Hz, Hα proton); 7.97 (d, 1H, *J*=15.6 Hz, Hβ proton); 8.02 (d, 2H, *J*=8.8 Hz, Hb A ring); 8.09 (d, 2H, *J*=8.8 Hz, Ha,e B ring). ^{13}C-NMR: (100 MHz)(DMSO-d_6/TMS) δ ppm: 47.2, 66.3 (-NCH_2CH_2O-); 123.8, 140.9 (-CH=CH-CO-); 113.5, 119.2, 121.7, 127.8, 131.02, 131.1, 134.8, 149.7, 154.6 (Ar-C); 186.9 (C=O).TOF/MS (m/z) 377.1464 $[M+H]^+$. MS: m/z= 377(M^+), 349, 292, 291, 215, 190, 187, 132, 105, 103, 102, 86, 77, 65, 51. Anal. calc. for $C_{20}H_{18}F_3NO_3$: C, 63.66; H, 4.81; N, 3.71. Found: C, 64.16; H, 4.84; N, 3.96 %.

2.3. Cell Culture Studies

Cisplatin was used as positive control. Stock solution of compounds were prepared in DMSO and diluted with Dulbecco's modified eagle medium (DMEM). DMSO final concentration is below 1 % in all tests. C6 cells (Rat glioma cell l ine) and HeLa cells (Human cervix adenocarcinoma) were kindly provided by Prof.Dr. Nazlı Arda and Associate Prof. Ali Karagoz (Department of Molecular Biology, Istanbul University, Turkey). The cancer cell lines were grown in Dulbecco's modified eagle medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 2 % penicilin streptomycin. The medium was changed twice a week.

2.3.1. Antiproliferative Activity Assay

Antiproliferative effects of the chalcones were investigated on HeLa and C6 cell lines using BrdU ELISA assay kit (15,16). Cultured cells were grown in 96-well plates (COSTAR, Corning, USA) at a density of 3 x 10^4 cells $well^{-1}$. In each experimental set, cells were plated in triplicate and replicated twice. The cell lines were determined in each compounds with eight concentrations (at 5, 10, 20, 30, 40, 50, 75 and 100 µM) as well as positive controls DMSO and cisplatin, for 24 h at 37 °C in a humidified atmosphere of 5% CO_2. Cells were then incubated for overnight before applying the BrdU Cell Proliferation ELISA assay reagent (Roche, Germany) according to manufacturer's procedure. The amount of cell proliferation was assessed by determining the A450 nm by using a microplate reader (Ryto, China). Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. All assays were repeated at least twice using HeLa and C6 cell lines. Percentage of inhibition of cell proliferation was calculated as follows: $[1-(A_{samples}/A_{control})]\times 100$.

2.4. Cell cytotoxicity

LDH assay was carried out using LDH cytotoxicity detection kit (Roche Diagnostics GmbH, Mannheim, Germany) according to protocol in the user's manual. The highest dose was used

as the concentration of 100 µland determined cytotoxicity (%) on HeLa cell line. Samples and 5-FU (5-fluorouracil) were incubated with 100 µl of HeLa cell suspension having 5×10^3 cell*ml^{-1} in 96 well plate at 37 °C for overnight in 5% CO_2 atmosphere. All the control and tested substances were tested in triplicates and twice and mean ± SEM of the absorbance value were taken to calculate cytotoxicity.

Cytotoxicity % =(Triplicate absorbance – low control) /(High control – low control) x 100

2.5. Statistical analysis

The results of investigation *in vitro* are means ± SD of nine measurement. Differences between groups were tested with ANOVA. *p* values of <0.01 were considered as significant.

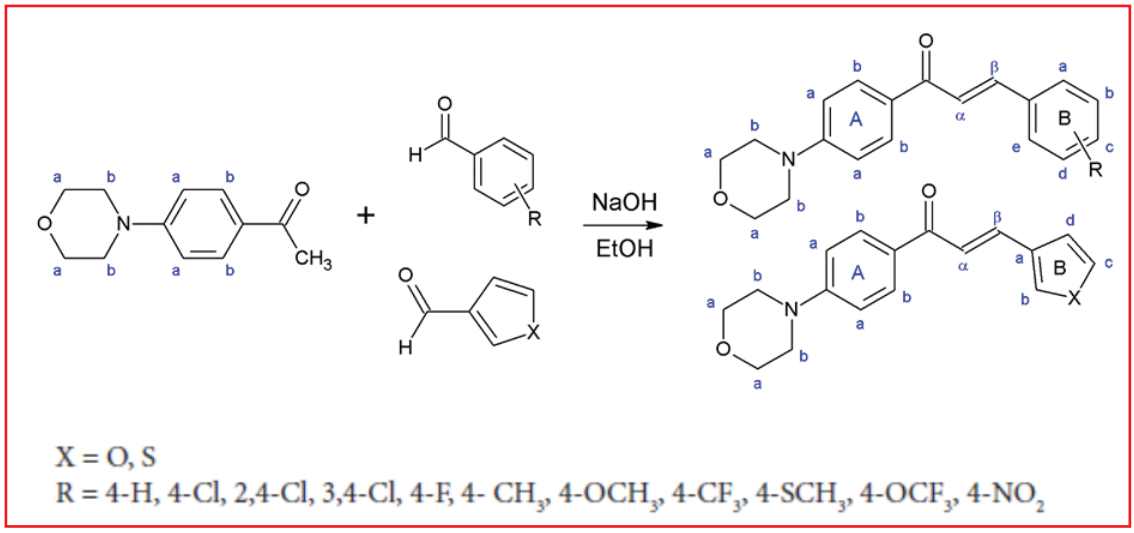
The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological function. In this paper, IC_{50} and IC_{75} values were determined using ED50 plus v1.0.

3. RESULTS AND DISCUSSION

3.1.Synthesis of compounds

3-aryl-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-ones were synthesized by Claisen-Schmidt condensation between 4'-morpholineacetophenone and various benzaldehydes in the presence of NaOH in methanol (Scheme 1). **1-12** were purified by hexane-DCM mixture. The yields were in the range of 45-79%. **3, 4, 7** and **9** were synthesized by Gopalakrishnan *et al* [17]. Chalcones which were colored compound observed three absorption bands at 227-235, 250-311 and 355-372 nm. The band at 355-372 nm indicated the presence of cinnamyl group. The absorption band at 227-235 nm due to benzoyl groups, were evaluated according to the exchange of chromophore groups. The IR spectrum showed the characteristic bands in the region of 1661-1641 cm^{-1} indication a carbonyl band as previously reported [7]. Aromatic and aliphatic =C-H streching bands were observed at 3180-2982 cm^{-1} and 2970-2835 cm^{-1} respectively. The aromatic C-Cl and C-F streching band were at 1105-1090 cm^{-1} and 1235-1120 cm^{-1} respectively [18].

In the ^1H NMR spectrum, protons at α,β unsaturated carbonyl system of **1-12** resonated doublet at 7.47-7.90 ppm and the other doublet at 7.56-8.09 ppm which confirmed the presence of chalcone moiety. The coupling constants of the vincylic system *(J=15.2-15.6 Hz)* confirmed the trans configuration of the chalcones. The ^1H NMR spectra of **8** and **9** carrying -SCH_3 and -OCH_3 groups showed a CH_3 signals at 2.54 and 3.87 ppm, respectively. ^{13}C NMR spectra of **1-12** displayed signals in the range 42.8 and 66.3 ppm which represent the methylene (CH_2) group of morpholine of the chalcone moiety. The ^{13}C NMR spectra indicated that the carbonyl signal was at 186.4-188.2 ppm. It is known that the signals of CH and CH_3 are positive while the CH_2 signal is negative in the DEPT135 spectrum. In DEPT 90 spectrum, only CH's signal is detected and positive. Therefore, CH's positive signals of **12** were detected at 113.3, 121.69, 123.89, 140.9, 131.1, 131.03 ppm. The negative signals in DEPT 135 were indicated that the CH_2 group of morpholine were at 47.15 and 66.3 ppm (Figure 1a). The HMBC spectrum of **12** displayed correlations of H-5/C-7; H-5/C-6; H-4/C-3; H-4/C-8; H-9/C-7; H-9/C-10; H-8/C-7; H-8/C-10; H-8/C-5; H-11/C-10; H-12/C-14 (Figure 1b and 1c).



Scheme 1. General synthetic pathway

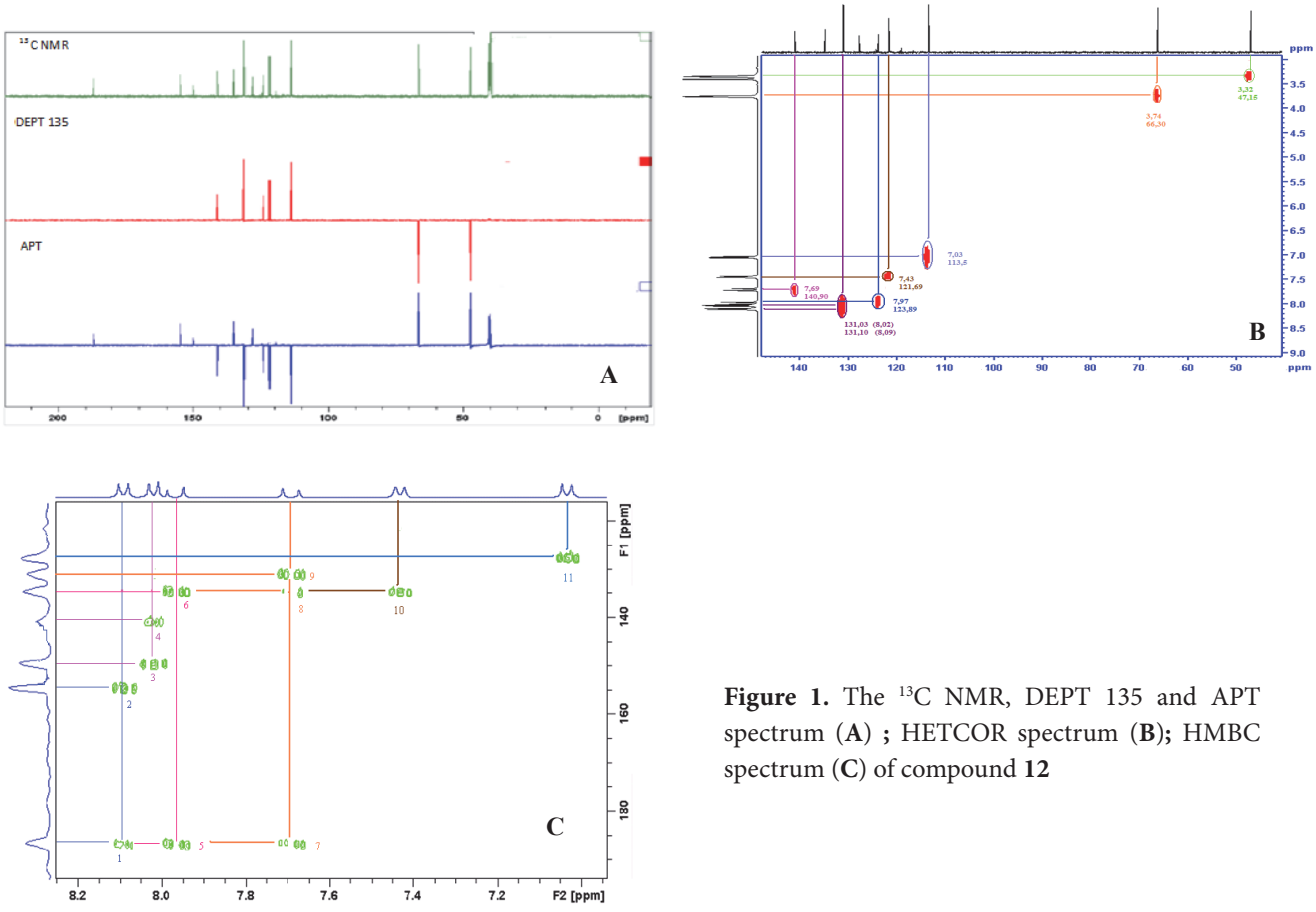


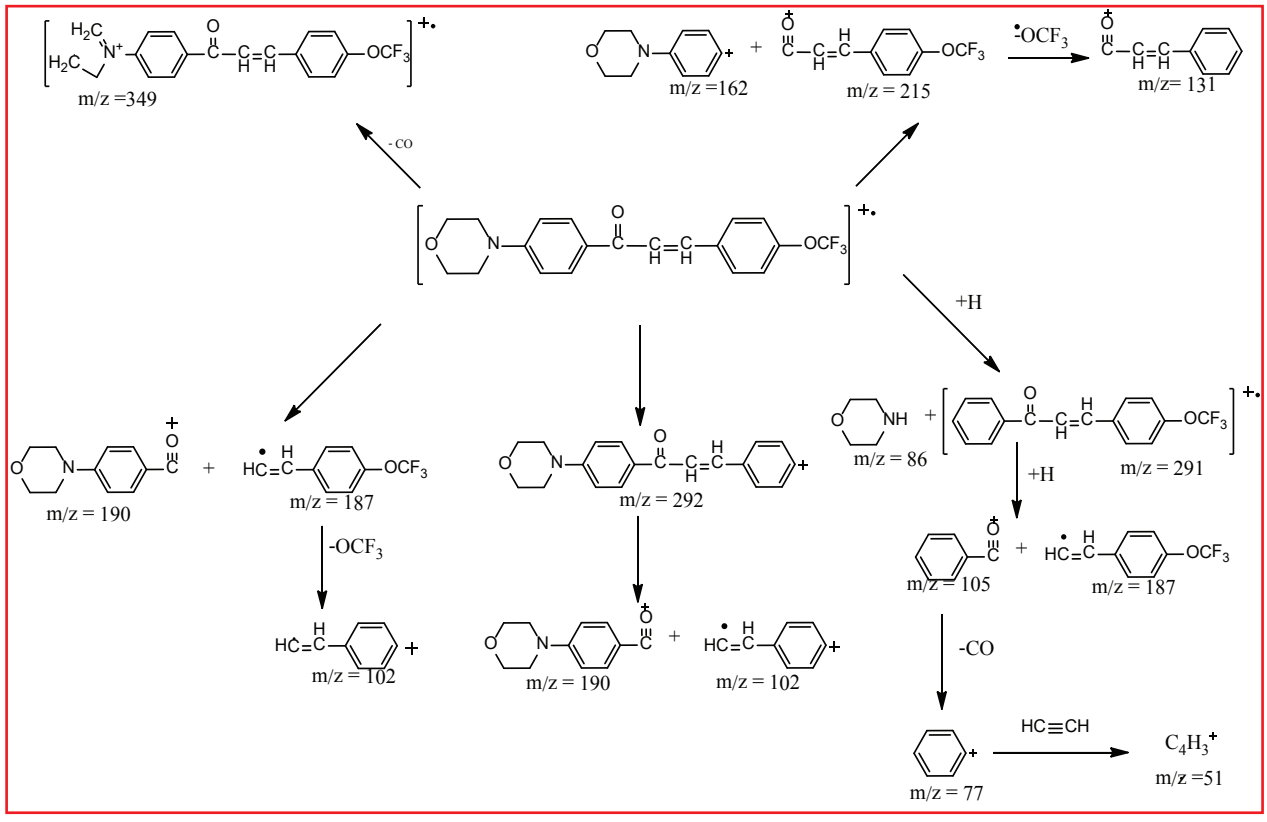
Figure 1. The ^{13}C NMR, DEPT 135 and APT spectrum (**A**) **;** HETCOR spectrum (**B**)**;** HMBC spectrum (**C**) of compound **12**

The mass fragmentation of compound containing thiophene and furan rings were according to literature [18,19]. The cleavage between carbonyl group and 4-morpholinophenyl ring gave the fragment at m/z 163 and also the fragmentation of the single bond between carbonyl and ethylene group produced an ion m/z 190 (Scheme 2). The loss of -OCF_3 (m/z 85) became the base peak of spectrum of compound **12**. The mass spectrum of morpholine showed that the ring was cleaving as a result of the loss of carbonyl group as given in literature [20,21]. And another important fragment was the cleavage of the morpholine ring (m/z 86) from the structure (compound **1, 2** and **12**) .

3.2. Antiproliferative activity results

The 3-aryl-1-[4-(morpholin-4-yl)phenyl]prop-2-en-1-ones **1-12** were evaluated *in vitro* for their antiproliferative activity on two cancer cell lines, HeLa and C6 cells, using the BrdU ELISA assay (Table 1). The activities of compounds **3**, **4**, **7**, **8**, **9**, **10**, **11**, **12** that have substituent at the four position of the phenyl ring were compared with cisplatin and 5-FU (Figure 2a). According to Figure 2a, the antiproliferative activities of the substituents on HeLa cell line showed the following order (at 5μM):11> 12> 7> 9> 5-FU> 10> 8> cisplatin> 4> 3, i.e., CF_3> OCF_3> F> OCH_3> 5-FU> NO_2> SCH_3> cisplatin> Cl> H to compared with cisplatin and 5-FU. This results showed that F atom was important for activity. However, the activities of compounds **1** and **2** (at 5μM) which were the bioisomer of phenyl ring the antiproliferative effects of thiophene ring were the higher than furan ring on HeLa cell line (Figure 2b). According to Figure 2c, compound **5** which has 2,4-dichlorophenyl ring have higher activity than compound **4** which has 4-chloro phenyl ring and compound **3** which has phenyl ring.

All compounds showed higher activities than cisplatin. The antiproliferative effects of substituents were secondly investigated on C6 cell line. The activities of **3**, **4**, **7**, **8**, **9**, **10**, **11**, **12** which had para subtituent at phenyl ring were compared with between themselves. According to Figure 3a, the antiproliferative activities of the substituents aganist C6 cell line showed the following order (at 5 μM): 5-FU> 10> 12> 11> cisplatin> 3> 8> 7> 4> 9, i.e., 5-FU> *p*-NO_2> *p*-OCF_3>



Scheme 2. The mass fragmentation of compound **12** which was selected as a model compound

Table 1. IC_{50} and IC_{75} values of samples and cisplatin[a])

Compounds	HeLa		C6	
	IC_{50} [μM]	IC_{75} [μM]	IC_{50} [μM]	IC_{75} [μM]
1	-[b])	30.39	-	36.55
2	-	19.09	2.77	45.80
3	68.27	79.16	7.36	45.32
4	24.31	49.42	10.31	47.89
5	-	7.77	-	13.86
7	-	12.66	-	39.50
8	-	32.65	14.50	54.26
9	-	14.95	18.93	50.43
10	-	29.26	-	42.37
11	-	-	-	49.48
12	-	-	-	34.42
Cisplatin	43.89	68.28	19.54	54.54
5-FU	-	5.77	-	26.08

[a]) The IC_{50} and IC_{75} values were determined by using BrdU method. –[b]) $IC_{50<}$ 0 μM.

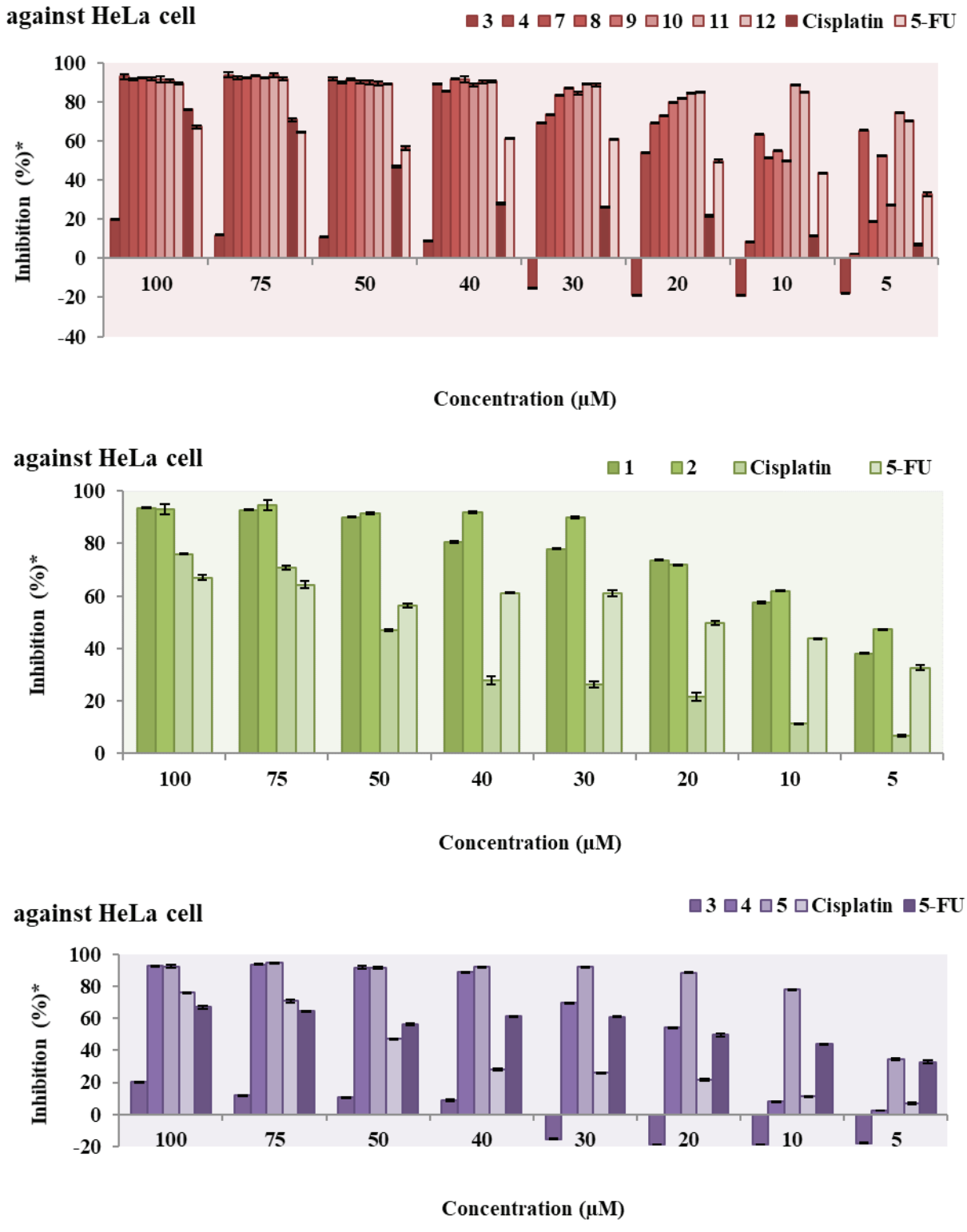


Figure 2. Antiproliferative activity of samples, cisplatin and 5-FU on HeLa cell line.

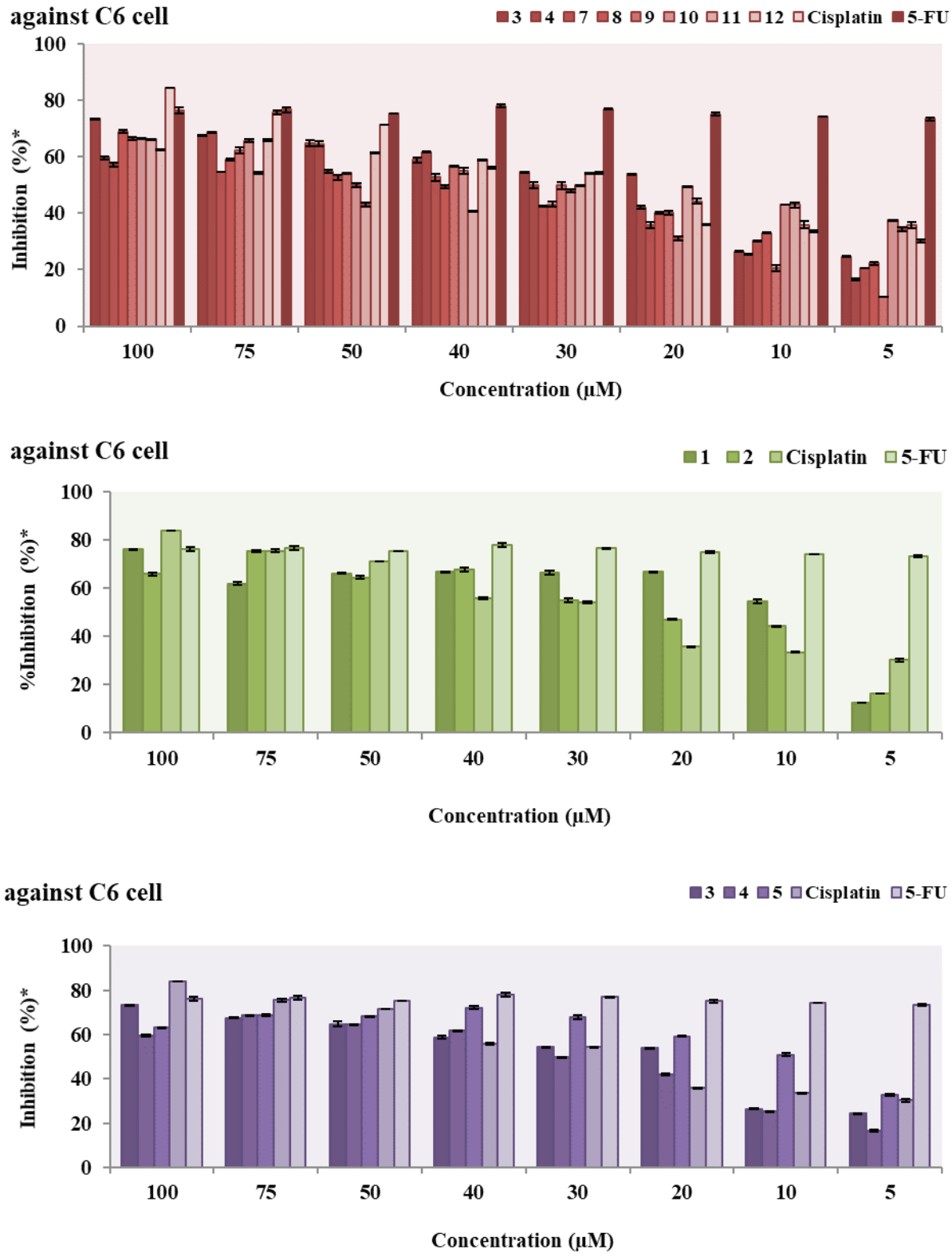


Figure 3. Antiproliferative activity of samples, cisplatin and 5-FU on C6 cell line.

p-CF_3> cisplatin> H> *p*-SCH_3> *p*-F> p-Cl > *p*-OCH_3. The substitution of NO_2 in phenyl ring **10** increased the inhibitor activity as compared to the standard cisplatin. Sato et al. reported that some compounds carrying nitro group could not show carcinogenicity and mutagenicity [22]. But in our research, our nitro compound **10** had potent antiproliferative activity. Organofluorine compounds are gained much more importance in the cancer therapy [23]. In our study, **7, 11** and **12** having flour atom, showed the highest activity on HeLa cell line.

However, according to Figure 3b, the antiproliferative effects of thiophene ring were the higher than furan ring on C6 cell line (at 5μM). Compound **5** which had 2,4-dichlorophenyl ring showed higher activities than **4** which has 4-chlorophenyl ring and compound **3** which has phenyl ring (Figure 3c).

3.3. Cytotoxic effects results

At 100 μg/mL concentration that was the highest dose at antiprolifertaive activity test were determined cytotoxicity (%) on HeLa cell line. 5-FU were used as positive control. Test results were given (Table 2). According to results, cytotoxicity (%) values of **2** were shown as quite low than 5-FU. In addition to, cytotoxicity (%) values of **10** was the same with cytotoxicity (%) values 5-FU. Low to high compared with 5-FU toxicity values were as follows respectively: compound **2>4~5-FU>6>7> 5~9>8>12>11.**

Table 2. Cytotoxicity (%) values of samples on HeLa cell.

Compound	Cytotoxicity (%)*
1	18
2	1
5	18
6	6
7	17
8	27
9	18
10	4
11	38
12	28
5-FU	4

*each substance was tested twice in triplicates aganist cell lines. Data show avarage of 2 individual experiments ($p<0.01$ for cytotoxicity (%) values of all samples)

3.4. Druglikeness properties

The number of hydrogen bond acceptors (n-ON) and donors (n-OHNH) are within the Lipinski's rules, n-ON<10 and n-OHNH<5. The calculated logP must be smaller than 5. In our study the logP values of all synthesized compounds were smaller than 5 except compound **6** (logP: 5.043). The molecular weight of chalcone derivatives are at the range of 283.327 Da and 377.362 Da. These derivatives could be a new potential anticancer agents according to calculated data. The calculation of absorption (% ABS) according to Zhao et al. (24), TPSA of compound **1-12** and Lipinski's rule of five were given (Table 3).

Table 3 . Drug-likeness properties of the synthesized chalcone derivatives *)

Compound	logP	TPSA	% ABS	MW	nON	nOHNH	Solubility	Drug likeness	Drug-score
1	3.168	29.543	98.807	299.395	3	0	-3.84	2.73	0.47
2	2.526	42.683	94.274	283.327	4	0	-3.6	2.72	0.5
3	3.76	29.543	98.807	293.366	3	0	-3.94	-0.33	0.34
4	4.437	29.543	98.807	327.811	3	0	-4.68	3.43	0.4
5	4.864	29.543	98.807	362.256	3	0	-5.42	3.79	0.33
6	5.043	29.543	98.807	362.256	3	0	-5.42	3.09	0.32
7	3.923	29.543	98.807	311.356	3	0	-4.26	1.75	0.34
8	4.193	29.543	98.807	339.46	3	0	-4.79	2.92	0.39
9	3.816	38.777	95.622	323.392	4	0	-3.96	2.89	0.46
10	3.718	75.367	82.998	338.363	6	0	-4.45	0.46	0.17
11	4.655	29.543	98.807	361.363	3	0	-4.72	-4.69	0.2
12	4.729	38.777	95.622	377.362	4	0	-4.97	-7.97	0.18

*) These parameters were determined with Molinspiration Calculation software and Preadmet software

4. CONCLUSIONS

We reported the synthesis and evaluation of a series of antiproliferative activity carrying 2-propen-1-one group. The results of the activity studies showed that the most of the synthesized chalcone derivatives had antiproliferative activities on two cell lines, especially HeLa compared with cisplatin. (*2E*)-1-[4-(morpholin-4-yl)phenyl]-3-(4-nitrophenyl)prop-2-en-1-one **10** for C6 cell line and (*2E*)-1-[4-(morpholin-4-yl)phenyl]-3-[4-(trifluoromethyl) phenyl]prop-2-en-1-one **11** for HeLa cell line were the most effective compounds than cisplatin with comparison of IC_{50}.

All these examples showed antiproliferative activity results were quite remarkable, and % cytotoxicity in combination with low values of the results increased the importance of processing.

5. ACKNOWLEDGEMENTS

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Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at http://dx.doi.org/10.12991/mpj.2017.18.

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