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Scientific paper

Synthesis and *In Vitro* Cytotoxicity of Novel Halogenated Dihydropyrano[3,2-*b*]Chromene Derivatives

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Abstract

Lung and breast cancers are among the most common cancers. In the present work, initially, 6-bromo-; and 6-chloro-3-hydroxychromone compounds were prepared. In the next step, a series of 8-bromo-; and 8-chloro-dihyropyrano[3,2-*b*] chromene derivatives were synthesized by one-pot three component reaction of these two compounds, aromatic aldehydes, and ethyl cyanoacetate in the presence of triethylamine in EtOH at reflux conditions. The synthesized compounds were tested for their *in vitro* cytotoxic activity against A549 (lung cancer) and MCF-7 (breast cancer) cell lines. It was found that some compounds have high to moderate cytotoxicity, which makes them potential candidates for further studies. This study can be the basis for further studies to design and synthesis potent anticancer compounds and investigating their mechanism of action.

Keywords: Chromone derivatives; 6-bromo-3-hydroxychromone; 6-chloro-3-hydroxychromone; Three-component reactions; Cytotoxicity; Cancer cell line

1. Introduction

Compounds with a fused benzene and 4-pyrone ring are called 4*H*-1-benzopyran-4-one, 4*H*-chromen-4-one or chromone. Chromones are a group of naturally occurring compounds that are ubiquitous in nature, especially in plants.[1,2] They are present in various flavonoids as a core structure. For instance, 3-hydroxyflavone (a type of hydroxyflavone) is a compound with a phenyl group in the 2-position and a hydroxyl group in the 3-position in the pyrone ring of chromone scaffold. Moreover, the chromone derivatives with a hydroxyl group in the 3-position in the pyrone ring are called 3-hydroxychromone. Chromone derivatives display a wide range of biological activities including antifungal,[3] antimicrobial,[4] antiobesity,[5] antiviral,[6,7] anti inflammatory,[8] anticancer,[9,10] antioxidant,[11] and protein kinase inhibitory.[12] In addition, chromones are good bidentate ligands able to coordinate metal ions. These compounds are widely investigated as fluorescent membrane probes and fluorescent chelators.[13,14]

Cancer, a worldwide health problem, is the second cause of death. Lung cancer is the leading cause of cancer death in males and females. Breast cancer is also one of the most common cancers that ranks as the second cause of deaths from cancer among females.[15] In spite of the existence of several strategies for cancer therapy, searching for new chemotherapeutic agents continues. It is because of the complex nature of cancer and occurring drug resistance in cancerous cells.[16]

Herein, we have synthesized 6-bromo-; and 6-chloro-3-hydroxychromone **(3a,b)** at first step. Then, we prepared 8-halopyrano[3,2-*b*]chromen-10(4*H*)-one derivatives **(6a-j)** by one-pot three component reactions of **(3a,b)**, aromatic aldehydes, and ethyl cyanoacetate in the presence of triethylamine in ethanol as solvent and at reflux conditions. Biological evaluation was also carried out for screening the potential cytotoxic activity of the compounds by MTT assay.

2. Experimental

2. 1. General Methods

All chemicals and reagents used in current study were obtained from commercial sources and used without further purification. All melting points were determined on Electrothermal-9100 apparatus and are uncorrected. IR spectra were recorded on a Bruker FTIR (Alpha model) spectrophotometer using KBr pallets. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on a Bruker AVANCE III 300 MHz spectrometer in DMSO-d_6, with TMS as an internal standard. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). Reactions were monitored by thin layer chromatography (TLC) on the Aluminium-backed silica gel sheets (GF254) and visualized in UV light (254 nm). Elemental analyses were performed using a Heraeus CHN-O-Rapid analyzer.

2. 2. Preparation of 6-bromo-; and 6-chloro-3-hydroxychromone (3a,b)

These compounds were prepared according to literature procedures which presented by Spadafora and et al. and represented in Scheme 1.[13] The isolated products were crystallised from ethanol.

6-Bromo-3-hydroxy-4H-chromen-4-one (3a)

Pale yellow powders; yield: 88%; mp 205–206 °C; IR (KBr) ν 3101 (OH), 1623 (C=O), 1601 cm^{-1} (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 9.35 (br, 1H, OH), 8.27 (s, 1H, CH), 8.17 (d, 1H, *J*=3 Hz, ArH), 7.89 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.62 (d, 1H, *J*=9 Hz, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 171.96 (C=O), 154.60 (C3), 142.50 (C2), 141.70, 136.38, 127.40, 124.72, 121.59, 117.35; Anal. calcd. for $C_9H_5BrO_3$: C, 44.85; H, 2.09%. Found: C, 44.64; H, 1.96%.

6-Chloro-3-hydroxy-4H-chromen-4-one (3b).

White powders; yield: 91%; mp 218–219 °C (lit.[17] 216 °C); IR (KBr) ν 3296 (OH), 1629 (C=O), 1605 cm^{-1} (C=C); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 9.36 (br, 1H, OH), 8.26 (s, 1H, CH), 8.05 (d, 1H, *J*=3 Hz, ArH), 7.80 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.71 (d, 1H, *J*=9 Hz, ArH); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 172.10 (C=O), 154.26 (C3), 142.45 (C2), 141.73, 133,74, 129.49, 124.29, 124.25, 121.44; Anal. calcd. for $C_9H_5ClO_3$: C, 54.99; H, 2.56%. Found: C, 54.83; H, 2.41%.

2. 3. General Procedure for the Preparation of Dihydropyrano[3,2-*b*]chromene derivatives (6a-j)

A mixture of 6-bromo-; or 6-chloro-3-hydroxychromone **(3a,b)** (2 mmol), aromatic aldehydes **(4a-j)** (2 mmol) and ethyl cyanoacetate **(5)** (2.1 mmol), and three drops of triethylamine in ethanol (10 mL) were added to a 50 mL round bottomed flask equipped with a magnetic stirring bar and a reflux condenser. It was stirred and refluxed for 1 h. The progress of the reaction was monitored by TLC using hexane/ethyl acetate as an eluent. After completion of the reaction, the mixture was cooled and the obtained crude product was filtered, washed with ethanol and crystallized from ethanol to give the pure solid sample for analysis.

Ethyl 2-amino-8-bromo-10-oxo-4-phenyl-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6a)

white powders; yield: 90%; mp 200–201 °C; IR (KBr) ν 3431, 3315 (NH_2), 3027 (CH, aromatic), 2982 (CH, aliphatic), 1686, 1650 (2C=O), 1611 (C=C), 1193 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.13 (d, 1H, *J*=3 Hz, ArH), 7.89 (br, 2H, NH_2), 7.85 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.50 (d, 1H, *J*=9 Hz, ArH), 7.36–7.21 (m, 5H, ArH), 4.93 (s, 1H, CH), 3.97 (q, 2H, *J*=6 Hz, O$\underline{CH_2}CH_3$), 1.04 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 167.99 (C-10, CO), 167.75 (ester CO), 160.04 (C-2), 153.95, 153.51, 143.36, 137.28, 133.35, 129.01, 128.22, 127.71, 125.23, 121.31 (C-8), 118.21, 75.38 (C-3), 59.42 (O$\underline{CH_2}CH_3$), 41.03 (C-4), 14.57 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{21}H_{16}BrNO_5$: C, 57.03; H, 3.65; N, 3.17%. Found: C, 56.79; H, 3.49; N, 3.21%.

Ethyl 2-amino-8-bromo-4-(4-chlorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6b)

white powders; yield: 92%; mp 239–241 °C; IR (KBr) ν 3468, 3333 (NH_2), 3062 (CH, aromatic), 2984 (CH, aliphatic), 1708, 1671 (2C=O), 1622 (C=C), 1192 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.13 (d, 1H, *J*=3 Hz, ArH), 7.93 (br, 2H, NH_2), 7.87 (dd, 1H, *J*=6 Hz, 3 Hz, ArH), 7.52 (d, 1H, *J*=9 Hz, ArH), 7.40–7.33 (m, 4H, ArH), 4.96 (s, 1H, CH), 3.97 (q, 2H, *J*=6 Hz, O$\underline{CH_2}CH_3$), 1.04 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 167.87 (C-10, CO), 167.80 (ester CO), 159.99 (C-2), 153.97, 152.81, 142.34, 137.34, 133.40, 132.29, 130.14, 128.96, 127.73, 125.24, 121.32 (C-8), 118.24, 74.99 (C-3), 59.48 (O$\underline{CH_2}CH_3$), 40.48 (C-4), 14.60 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{21}H_{15}BrClNO_5$: C, 52.19; H, 3.17; N, 2.94%. Found: C, 52.22; H, 2.98; N, 2.69%.

Ethyl 2-amino-8-bromo-10-oxo-4-(p-tolyl)-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6c)

white powders; yield: 87%; mp 209–210 °C; IR (KBr) ν 3396, 3291 (NH_2), 3023 (CH, aromatic), 2984 (CH, aliphatic), 1680, 1661 (2C=O), 1615 (C=C), 1196 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.14 (d, 1H, *J*=3 Hz, ArH), 7.90–7.86 (m, 3H, NH_2, ArH), 7.53 (d, 1H, *J*=9 Hz, ArH), 7.16 (q, 4H, *J*=9 Hz, ArH), 4.89 (s, 1H, CH), 3.97 (q, 2H, *J*=6 Hz, O$\underline{CH_2}CH_3$), 2.24 (s, 3H, CH_3), 1.07 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 168.02 (C-10, CO), 167.76 (ester CO), 160.01 (C-2), 153.96, 153.78, 140.42, 137.29, 136.88, 133.29, 129.59,

128.06, 127.73, 125.24, 121.34 (C-8), 118.21, 75.49 (C-3), 59.44 ($O\underline{CH_2}CH_3$), 40.61 (C-4), 21.07 (CH_3), 14.62 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{22}H_{18}BrNO_5$: C, 57.91; H, 3.98; N, 3.07%. Found: C, 58.02; H, 3.71; N, 2.89%.

Ethyl 2-amino-8-bromo-4-(4-fluorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6d)
cream powders; yield: 90%; mp 214–215 °C; IR (KBr) ν 3466, 3381 (NH_2), 3044 (CH, aromatic), 2991 (CH, aliphatic), 1707, 1684 (2C=O), 1659 (C=C), 1194 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.13 (d, 1H, *J*=3 Hz, ArH), 7.91–7.86 (m, 3H, NH_2, ArH), 7.52 (d, 1H, *J*=9 Hz, ArH), 7.38–7.34 (m, 2H, ArH), 7.15 (t, 2H, *J*=9 Hz, ArH), 4.96 (s, 1H, CH), 3.97 (q, 2H, *J*=6 Hz, $O\underline{CH_2}CH_3$), 1.04 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 167.92 (C-10, CO), 167.80 (ester CO), 163.33 (C-2), 160.12, 159.97, 153.97, 153.11, 139.52, 137.32, 133.33, 130.22, 130.11, 127.73, 125.24, 121.32 (C-8), 118.22, 115.89, 115.61, 75.26 (C3), 59.44 ($O\underline{CH_2}CH_3$), 40.55 (C-4), 14.58 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{21}H_{15}BrFNO_5$: C, 54.80; H, 3.29; N, 3.04%. Found: C, 54.59; H, 2.98; N, 3.11%.

Ethyl 2-amino-8-bromo-4-(4-methoxyphenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6e)
yellow powders; yield: 86%; mp 211–213 °C; IR (KBr) ν 3423, 3297 (NH_2), 3069 (CH, aromatic), 2983 (CH, aliphatic), 1686, 1656 (2C=O), 1632 (C=C), 1189 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.13 (d, 1H, *J*=3 Hz, ArH), 7.89–7.85 (m, 3H, NH_2, ArH), 7.52 (d, 1H, *J*=9 Hz, ArH), 7.21 (d, 2H, *J*=9 Hz, ArH), 6.87 (d, 2H, *J*=9 Hz, ArH), 4.87 (s, 1H, CH), 3.98 (q, 2H, *J*=6 Hz, $O\underline{CH_2}CH_3$), 3.71 (s, 3H, OCH_3), 1.07 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 168.05 (C-10, CO), 167.75 (ester CO), 159.98 (C-2), 158.84, 153.96, 153.85, 137.26, 135.39, 133.22, 129.22, 127.72, 125.24, 121.31 (C-8), 118.19, 114.38, 75.64 (C-3), 59.42 ($O\underline{CH_2}CH_3$), 55.48 (OCH_3), 40.17 (C-4), 14.64 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{22}H_{18}BrNO_6$: C, 55.95; H, 3.84; N, 2.97%. Found: C, 55.98; H, 3.56; N, 2.73%.

Ethyl 2-amino-8-chloro-10-oxo-4-phenyl-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6f)
white powders; yield: 91%; mp 190–192 °C; IR (KBr) ν 3466, 3411 (NH_2), 3028 (CH, aromatic), 2982 (CH, aliphatic), 1687, 1662 (2C=O), 1612 (C=C), 1193 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.03 (t, 1H, *J*=3 Hz, ArH), 7.90 (br, 2H, NH_2), 7.82–7.77 (m, 1H, ArH), 7.63 (q, 1H, *J*=3 Hz, ArH), 7.37–7.22 (m, 5H, ArH), 4.95 (s, 1H, CH), 3.97 (q, 2H, *J*=6 Hz, CH_2), 1.05 (t, 3H, *J*=6 Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 168.00 (C-10, CO), 167.92 (ester CO), 160.06 (C-2), 153.61, 143.38, 134.63, 133.35, 130.34, 129.04 (C-8), 128.22, 127.71, 124.89, 124.64, 121.22, 75.41 (C-3), 59.43 ($O\underline{CH_2}CH_3$), 41.03 (C-4), 14.58 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{21}H_{16}ClNO_5$: C, 63.40; H, 4.05; N, 3.52%. Found: C, 63.27; H, 3.85; N, 3.49%.

Ethyl 2-amino-8-chloro-4-(4-chlorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6g)
cream powders; yield: 93%; mp 238–240 °C; IR (KBr) ν 3470, 3335 (NH_2), 3065 (CH, aromatic), 2984 (CH, aliphatic), 1706, 1672 (2C=O), 1654 (C=C), 1194 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.01 (d, 1H, *J*=3 Hz, ArH), 7.93 (br, 2H, NH_2), 7.79 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.62 (d, 1H, *J*=9 Hz, ArH), 7.41–7.33 (m, 4H, ArH), 4.97 (s, 1H, CH), 3.97 (q, 2H, *J*=6 Hz, $O\underline{CH_2}CH_3$), 1.05 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 167.93 (C-10, CO), 167.88 (ester CO), 159.99 (C-2), 153.60, 152.83, 142.35, 134.66, 133.38, 132.29, 130.36, 130.16, 128.97 (C-8), 124.86, 124.62, 121.18, 75.00 (C-3), 59.48 ($O\underline{CH_2}CH_3$), 40.47 (C-4), 14.60 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{21}H_{15}Cl_2NO_5$: C, 58.35; H, 3.50; N, 3.24%. Found: C, 58.11; H, 3.37; N, 3.19%.

Ethyl 2-amino-8-chloro-10-oxo-4-(p-tolyl)-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6h)
yellow powders; yield: 88%; mp 196–197 °C; IR (KBr) ν 3460, 3313 (NH_2), 3049 (CH, aromatic), 2995 (CH, aliphatic), 1687, 1660 (2C=O), 1607 (C=C), 1191 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.00 (d, 1H, *J*=3 Hz, ArH), 7.87 (br, 2H, NH_2), 7.74 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.57 (d, 1H, *J*=9 Hz, ArH), 7.15 (q, 4H, *J*=9 Hz, ArH), 4.87 (s, 1H, CH), 3.97 (q, *J*=6 Hz, 2H, $O\underline{CH_2}CH_3$), 2.23 (s, 3H, CH_3), 1.06 (t, *J*=6 Hz, 3H, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 168.02 (C-10, CO), 167.85 (ester CO), 160.01 (C-2), 153.73, 153.55, 140.42, 136.87, 134.55, 133.26, 130.31, 129.57 (C-8), 128.05, 124.82, 124.59, 121.12, 75.47 (C-3), 59.43 ($O\underline{CH_2}CH_3$), 40.61 (C-4), 21.05 (CH_3), 14.60 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{22}H_{18}ClNO_5$: C, 64.16; H, 4.41; N, 3.40%. Found: C, 64.23; H, 4.17; N, 3.39%.

Ethyl 2-amino-8-chloro-4-(4-fluorophenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6i)
white powders; yield: 89%; mp 208–210 °C; IR (KBr) ν 3469, 3334 (NH_2), 3068 (CH, aromatic), 2985 (CH, aliphatic), 1707, 1672 (2C=O), 1653 (C=C), 1194 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 7.98 (d, 1H, *J*=3 Hz, ArH), 7.91 (br, 2H, NH_2), 7.75 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.58 (d, 1H, *J*=9 Hz, ArH), 7.38–7.33 (m, 2H, ArH), 7.19–7.12 (m, 2H, ArH), 4.95 (s, 1H, CH), 3.96 (q, 2H, *J*=6 Hz, $O\underline{CH_2}CH_3$), 1.04 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 167.92 (C-10, CO), 167.89 (ester CO), 163.34 (C-2), 160.12, 159.98, 153.57, 153.08, 139.56, 139.52, 134.59, 133.30, 130.21 (C-8), 124.83, 124.60, 121.11, 115.88, 115.60, 75.25 (C-3), 59.43 ($O\underline{CH_2}CH_3$), 40.55 (C-4), 14.57 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{21}H_{15}ClFNO_5$: C, 60.66; H, 3.64; N, 3.37%. Found: C, 60.39; H, 3.67; N, 3.13%.

Ethyl 2-amino-8-chloro-4-(4-methoxyphenyl)-10-oxo-4,10-dihydropyrano[3,2-b]chromene-3-carboxylate (6j)
yellow powders; yield: 87%; mp 207–208 °C; IR (KBr) ν 3419, 3295 (NH_2), 3072 (CH, aromatic), 2984 (CH, ali-

phatic), 1686, 1656 (2C=O), 1632 (C=C), 1192 cm^{-1} (C-O); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.00 (d, 1H, *J*=3 Hz, ArH), 7.86 (brs, 2H, NH_2), 7.76 (dd, 1H, *J*=9 Hz, 3 Hz, ArH), 7.59 (d, 1H, *J*=9 Hz, ArH), 7.22 (d, 2H, *J*=9 Hz, ArH), 6.88 (d, 2H, *J*=9 Hz, ArH), 4.87 (s, 1H, CH), 3.98 (q, 2H, *J*=6 Hz, O$\underline{CH_2}CH_3$), 3.70 (s, 3H, OCH_3), 1.07 (t, 3H, *J*=6 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 168.05 (C-10, CO), 167.86 (ester CO), 159.98 (C-2), 158.84, 153.84, 153.57, 135.40, 134.55, 133.19, 130.30, 129.22 (C-8), 124.84, 124.60, 121.13, 114.37, 75.63 (C-3), 59.42 (O$\underline{CH_2}CH_3$), 55.47 (OCH_3), 40.17 (C-4), 14.63 ($OCH_2\underline{CH_3}$); Anal. calcd. for $C_{22}H_{18}ClNO_6$: C, 61.76; H, 4.24; N, 3.27%. Found: C, 61.51; H, 4.29; N, 3.01%.

2. 4. Cell Culture

All the cell lines were purchased from the Iranian Biological Resource Center (IBRC, Tehran, Iran) and cultured in Dulbecco's modified Eagle's medium (DMEM, Biosera, France) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco, USA) and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin, Biosera, France). The cells were incubated at 37 °C, 5% CO_2, and 95% relative humidity.

2. 4. 1. The Cytotoxic Assay

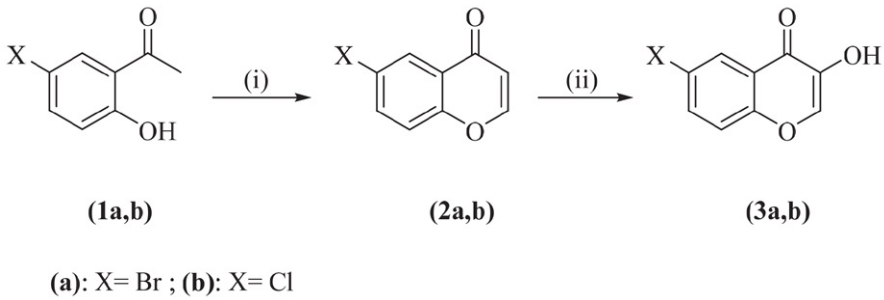
The synthesized compounds were dissolved in DMSO to prepare stock solutions. Afterwards, different concentrations (from 0.2 to 1000 µM) were prepared by diluting the appropriate amounts of the stock solutions in DMEM (without FBS). The final amounts of DMSO were kept below 1%.

The cells with a confluency of about 80% were harvested with trypsin-EDTA (Biosera, France); and 1×10^4 cells were cultured in each well of a 96-well culture microplate. The microplates were then incubated in the same conditions mentioned above for 24 h. The next day, the media was removed from the wells and replaced with 100 µL of the prepared concentrations of the synthesized compounds or doxorubicin (as the standard). At least 3 wells of the microplate were used for each concentration. The cell controls were treated with DMEM containing the same percent of DMSO without the compounds. The microplates were further incubated for 24 h. Finally, 10 µL of the MTT solution (5 mg/ml, Melford, England) was added to the wells, the microplates were incubated for 3 h protected from light, formed formazan crystals were solubilized in 100 µL DMSO, and the absorbance was measured at 570 nm in a multiplate reader. The experiment was repeated 3 times. GraphPad® Prism version 5 was used to calculate the IC_{50} values from the mean percent of viable cells.[16]

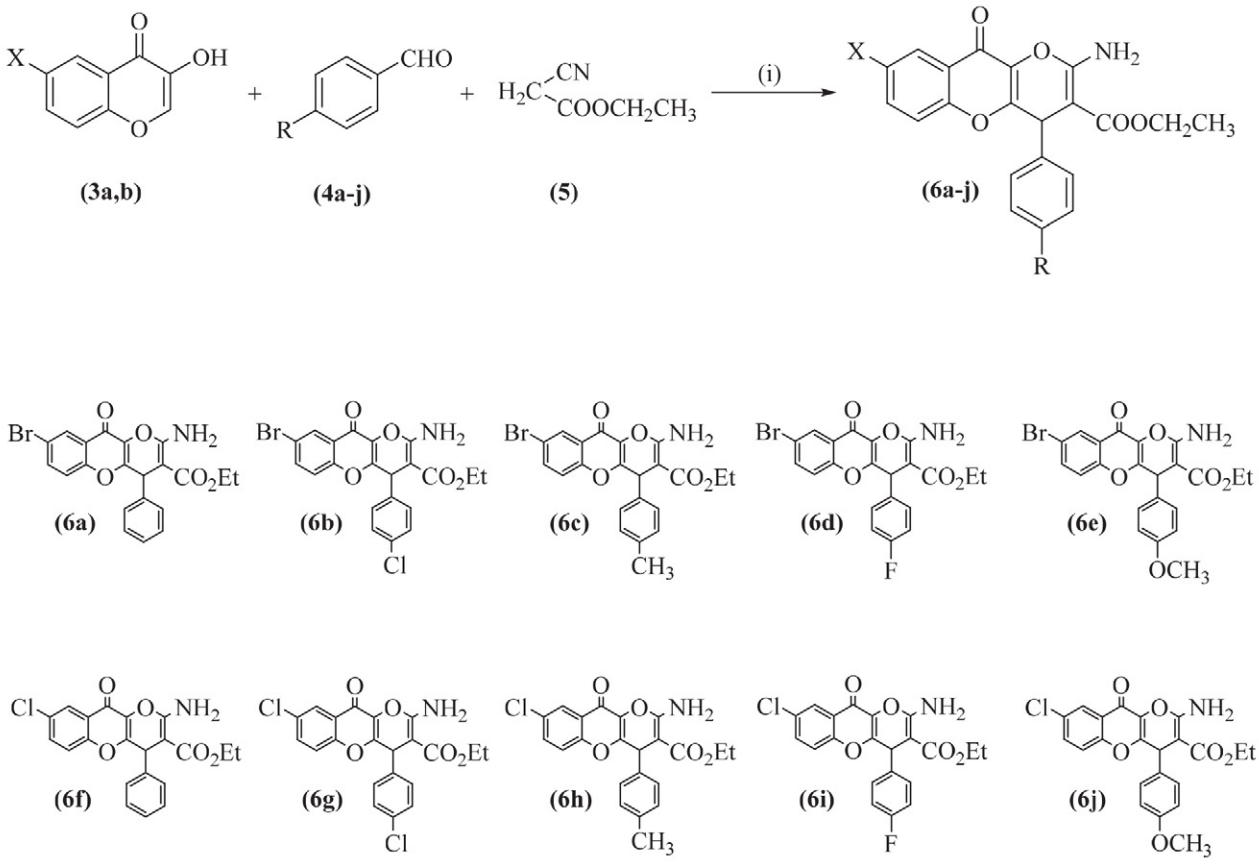
3. Results and Discussions

To prepare (6a-j) derivatives, 3-hydroxychromone derivatives were used as substrates (Scheme **1**). The condensation of 5'-bromo-; or 5'-chloro-2'-hydroxyacetophenone **(1a,b)** with N,N-dimethylformamide-dimethylacetal (DMF-DMA) was irradiated under microwave conditions, then refluxed with concentrated HCl. This reaction led to the production of 6-bromo-; and 6-chloro-chromone derivatives **(2a,b)**, which formed epoxy chromones upon treatment with H_2O_2/NaOH in methylene chloride. This undergoes ring opening with concentrated HCl afforded 6-bromo-; and 6-chloro-3-hydroxychromones **(3a,b)** in good yields.[13] These compounds were fully characterized by standard spectroscopic techniques (IR, ^{1}H and ^{13}C NMR) and elemental analyses.

Following our previous works on multi-component reactions to reach potentially bioactive scaffolds,[18,19] we have synthesized a novel one-pot three component reaction for the synthesis of 8-halopyrano[3,2-*b*]chromen-10(4*H*)-one **(6a-j)** including 6-bromo-; and 6-chloro-3-hydroxychromones **(3a,b)**, aromatic aldehydes **(4a-j)**, and ethyl cyanoacetate **(5)** in the presence of three drops of Et_3N in ethanol as the solvent and at reflux conditions. After completion of the reaction, the crude product was purified by recrystallization and a series of ethyl 8-halo-4,10-dihydropyrano[3,2-*b*]chromene-3-carboxylate derivatives **(6a-j)** were prepared in 86–93% yields (Scheme **2**). The structures of compounds **(6a-j)** were determined on the basis of their elemental analyses, ^{1}H and ^{13}C NMR and IR spectroscopic data. In similar studies heteroannelation of cyclic ketones were conducted by use of catalysts like sodium carbonate, sodium saccharine and poly(4-vinylpyridine); [20–22] however in the present study we man-



Scheme 1. Synthesis of 6-bromo-; and 6-chloro-3-hydroxychromone. Reagents and conditions: (i): 1) DMF-DMA/ MW; 2) HCl (con.), CH_2Cl_2, reflux; (ii): 1) H_2O_2, NaOH, CH_2Cl_2, ice-bath; 2) HCl (con.), reflux.



Scheme 2. Synthesis of dihydropyrano[3,2-*b*]chromene derivatives. Reagents and conditions: (i): triethylamine, EtOH, reflux.

aged to synthesized halogenated dihydropyranochromene by one pot three component reaction without any catalyst in good yields.

3. 1. The Cytotoxic Assay

To evaluate the cytotoxic activity of the synthesized compounds, MTT assay was used after treating MCF-7 (breast cancer) and A549 (lung cancer) cell lines. The compounds had three or four aromatic rings, and based on Nagai et. al, having more than two aromatic rings leads to a higher tumor specificity.[9] The IC_{50} values range from 36 μM (compound **6f**) to 631 μM (compound **6j**) for MCF-7 and 56 μM (compound **6f**) to 558 μM (compound **6j**) for A549 cells (Table 1). The most potent compound is compound **6f** (Fig.**1**). Compared to compound **6a**, which has a Br atom in C8, **6f** (the compound with a Cl atom in this position) is about 11 and 7 times stronger on MCF-7 and A549, respectively. Placing any moiety on para position of aromatic aldehydes (R) leads to a decrease in the cytotoxicity when X is a chlorine atom, however, when X is a bromine atom, adding an R moiety results in a more potent compound especially when the R group is a chlorine atom (compound **6b**). In our previous study, a halogen group substitution on the carbon 4 of the phenyl ring decreased the cytotoxic activity of the compound except when it was Cl, interestingly it is also understood that chromenes bearing chlorine and bromine have quite much cytotoxic effect in comparison with ones that lack Cl and Br.[19] Since compound **6f** can be a promising candidate as a cytotoxic agent, it was tested on SW480 (a colorectal cancer cell line) and HUVEC (Human Umbilical Vein Endothelial Cells). The IC_{50} values were 10.8 ± 1.5 and 57.2 ± 3.2 μM, respectively. By comparison of our compounds with benzo[*h*] chromene derivatives, it is revealed that fused ring at 2,3-position may boost antitumor activity of compound.[23] So a rationale in our future work is to fuse ring at 2,3 position and making new compounds which may have stronger cytotoxic activity. One study showed that inclusion of thienyl group next to chromene ring make these compounds more potent and also selective against prostate cancer.[22] In another study it is found that a chlorophenyl moiety on central dihyrobenzo[*h*]pyrano[3,2-*c*]chromene ring has a positive impact on cytotoxicity, which is also effective in our compounds.[24] It is also revealed that electron withdrawing group at the four position of the phenyl ring at the 1-position of 1*H*-benzo[*f*]chromene enhance the antitumor activity of the compounds.[25] This trend is also in accordance with our finding. Generally, it seems that modifications at the C-4 and C-6 positions of

Table 1. The IC_{50} (μM) values of the compounds on two cancer cell lines measured by MTT assay method (mean ± standard error of mean).

Compd. NO.	A549	MCF-7
6a	414.8 ± 2.1	389.0 ± 18
6b	120.5 ± 0.3	115.5 ± 1.2
6c	155 ± 1.2	169.1 ± 0.5
6d	142.3 ± 0.5	168.5 ± 1.5
6e	150.5 ± 8.9	175.8 ± 1.9
6f	56 ± 0.3	35.8 ± 3.8
6g	107.8 ± 6.4	179.8 ± 5.8
6h	272.3 ± 8.9	232.1 ± 3
6i	230.6 ± 4.8	328.8 ± 16.6
6j	558.2 ± 8.7	631.2 ± 8.5
Doxorubicin	7. 9 ± 0.2	6.4 ± 0.1

chromenes have impacts on anticancer activity of these compounds.[26]

4. Conclusion

The present study describes the synthesis and investigation of cytotoxic activities of a series of novel halopyranochromene derivatives. We have synthesized some derivatives by one-pot three component reactions of 6-bromo-; or 6-chloro-3-hydroxychromone, aromatic aldehydes, and ethyl cyanoacetate in the presence of triethylamine in ethanol as solvent and at reflux conditions. Some of the compounds showed moderate cytotoxicity on the two cancer cell lines (A549 and MCF-7). The best com-

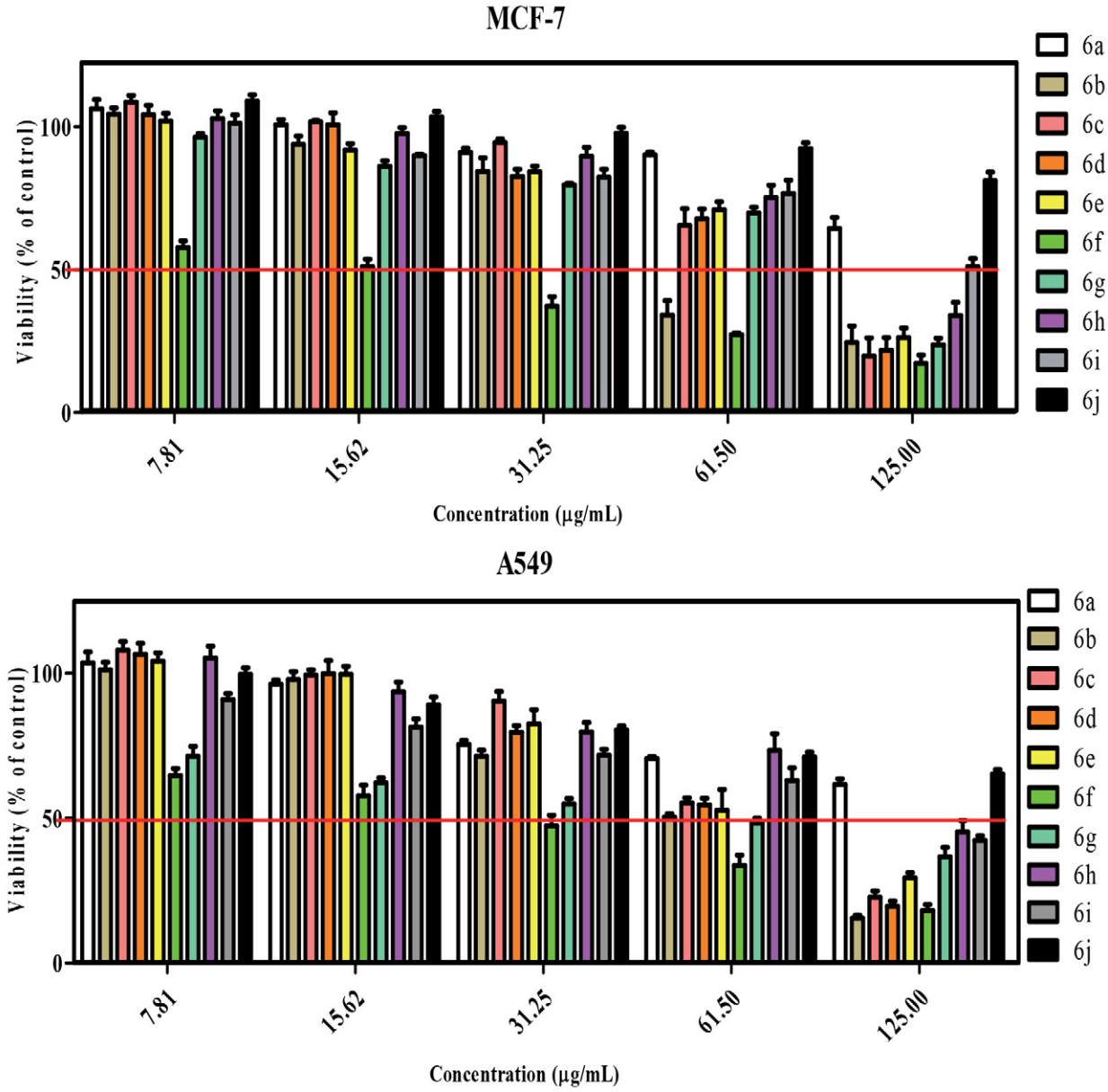


Fig. 1. The viability percent of the cells (MCF-7 and A549) after 24 hours treatment with different concentrations of the synthesized compounds.

pound had a Cl atom (C8) and a phenyl ring (C4) of pyranochromene scaffold (compound **6f**). It is concluded that these compounds can be lead compounds for synthesizing anticancer agents.

Abbreviations

EtOH: ethanol; MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMF-DMA: N,N-dimethylformamide-dimethylacetal; HUVEC: Human Umbilical Vein Endothelial Cells; IC_{50}: 50% Inhibition concentration; KBr: Potassium bromide; ppm: parts per million; TLC: thin layer chromatography.

Supplementary Information

The online version contains supplementary material available at https://doi.….

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Authors' contributions

Ehsan Faghih-Mirzaei and Mehdi Abaszadeh, designed, synthesized and performed experiments, analysed data and wrote the paper. Salehe Sabouri, designed and performed the biologic assay and data analysis and contributed in writing the manuscript. All authors were involved in revising the content, agree to take accountability for the integrity and accuracy of the work, and have read and approved the final manuscript.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Povzetek

Pljučni rak in rak dojk sta med najpogostejšimi raki. V okviru dela smo najprej pripravili 6-bromo- in 6-kloro-3-hidroksikromonske spojine. V naslednjem koraku je bila sintetizirana serija derivatov 8-bromo- in 8-kloro-dihidropirano[3,2-b] kromena s hkratno trikomponentno reakcijo teh dveh spojin, aromatskih aldehidov in etil cianoacetata v prisotnosti trietilamina v EtOH pri pogojih povratnega toka. Sintetizirane spojine so bile testirane za *in vitro* citotoksično delovanje na celičnih linijah A549 (pljučni rak) in MCF-7 (rak dojke). Ugotovljeno je bilo, da imajo nekatere spojine visoko do zmerno citotoksičnost, zato so potencialni kandidati za nadaljnje študije. Ta študija je lahko podlaga za nadaljnje študije za načrtovanje in sintezo močnih proti-rakavih spojin ter raziskovanje njihovega mehanizma delovanja.