Synthesis and cytotoxic evaluation of novel chromenes and chromene(2,3-d)pyrimidines

Mahmoud N. M. Yousif,1* Abdel-Rahman B. A. El-Gazzar1, Ahmed A. Fayed1, May A. El-Manawaty2, Nabil M. Yousif1

1Photochemistry Department, National Research Centre, Cairo, Egypt.
2Department of Pharmacognosy, National Research Centre, Cairo, Egypt.

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ABSTRACT
The synthesis of novel compounds starting from 2-amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile 2 has been studied. Diarylidene cyclohexanone reacts with malononitrile to afford compound 2. Compound 2 reacts with benzoyl chloride to afford compound 3. N-(8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,6,7,8-tetrahydro-4H-chromen-2-yl)benzamide 3 reacts with acetic anhydride to afford compound 4. Compound 2 reacts with acetic anhydride to afford 9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d]pyrimidin-4-one 5. Chromene derivative 2 reacts with formic acid to give compound 6. Compounds 4–6 react with phosphorus oxychloride to give compounds 7a–c. Chromeno[2,3-d]pyrimidine derivatives 7a–c react with hydrazine hydrate to afford compounds 8a–c. Chromeno[2,3-d]pyrimidine derivatives 8a,b react with xylose and glucose to give compounds 9a–d. Chromeno[2,3-d]pyrimidine derivatives 9a–d react with acetic anhydride to give compounds 10a–d. Screening of most of the synthesized compounds against A-549, CaCo-2, and HT-29 cell lines were done. 2-Amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile 2 gives high cytotoxic activity against A-549 and HT-29 cancer cell lines as compared to doxorubicin as the reference drug.

INTRODUCTION
Chromenes have recently gained the attention of many researchers due to their various applications. Chromene derivatives have shown different remarkable biological activities against various targets. 4-Substituted-4H-chromenes have shown significant anticancer activity (Aridoss et al., 2012). Also, 4-substituted-4H-chromenes have anticoagulant activity (Bonsignore et al., 1993) and are used as regulators of the potassium cation channel (Jin et al., 2004). 2-Amino-6-bromo-4-(nitromethyl)-4H-chromene-3-carbonitrile (Ia) and 2-amino-6-bromo-4-(1-nitroethyl)-4H-chromene-3-carbonitrile (Ib) have afforded good cytotoxic activity with IC50<4 µg/ml and they have activity four times more than the standard drug Etoposide (Zonouzi et al., 2013).

In addition, 4H-chromene derivatives have shown spasmolytic, diuretic, anticoagulant, and antianaphylactic activities (Ghorbani-Vaghei et al., 2011). 4H-Chromene derivatives bind to the Bcl-2 protein and initiate apoptosis in cancer cells. The Bcl-2 protein improves neoplastic cell proliferation by preventing normal cell turnover. Increasing Bcl-2 gene expressions are present in many types of human cancers and can result in cancer cell resistance to chemotherapy and radiotherapy. Therefore, Bcl-2 protein-binding compounds are promising compounds as anticancer agents (Ghorbani-Vaghei et al., 2011).

*Corresponding Author
Mahmoud N. M. Yousif, Photochemistry Department, National Research Centre, Cairo, Egypt. E-mail: mahmoud_nabil18@yahoo.com

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Aminochromene derivatives have also shown antihypertensive and anti-ischemic behavior (Ghorbani-Vaghei et al., 2011).

Chromenes are also used as food additives, cosmetic agents, and potent biodegradable agrochemical (Subbareddy et al., 2017). They are used as antifungal, anti-HIV, antimalarial, antibacterial, antioxidant, and anti-influenza virus agents (Subbareddy et al., 2017). The chromene derivative MX58151 has been used in the treatment of drug-resistant cancers (Fig. 1) (Subbareddy et al., 2017). In addition, chromene derivative EPC2407 is used in phase I/II clinical trials as a vascular disrupting anti-tumoral drug for the treatment of advanced solid tumors (Fig. 1) (Subbareddy et al., 2017). Chromone derivative HA14-1 is used as an inhibitor of acute myeloid leukemia. Ethyl 2-amino-4-(1H-indol-3-yl)-4H-chromene-3-carboxylate II is used as an anti-human immunodeficiency virus reverse transcriptase (anti-HIV-1 RT) (Fig. 1) (Subbareddy et al., 2017). N-(4-Chlorophenyl)-8-methoxy-2-methyl-4-(2-methyl-1H-indol-3-yl)-4H-chromene-3-carboxamide III has high antibacterial activity against Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa. Compound III has a minimum inhibitory concentration in the range of 9.3–18.7 mg/ml (Subbareddy et al., 2017).

The pyranopyrimidines have also shown various pharmacological activities, e.g., antibacterial activity, antifungal activity, antigenotoxic activity, antiplatelet activity, antithrombotic activity, and analgesic and anti-inflammatory activity (Chaker et al., 2017).

All the aforementioned biological activities and our previous work (El-Gazzar et al., 2008; Fayed and Yousif, 2019; Fayed et al., 2019a; Nemr et al., 2019; Soliman et al., 2014; Yousif et al., 2017; 2018, Yousif et al., 2019a; 2019b; 2019c; 2020; 2021) directed us to prepare novel chromene derivatives and measure the cytotoxic activity of the prepared compounds.

4-H-Chromene derivative (I) has been synthesized from aromatic aldehyde, malononitrile, and phenol derivatives in a one-pot three-component reaction (El-Maghrawy et al., 2014).

![Diagram of chromone derivative synthesis](image)

**Experimental section**

The apparatus used was as in a previously reported study (Yousif et al., 2019b). Compound 1 (diarylidine cyclohexanone) was prepared according to previously known literature (Kumar et al., 2011).

2-Amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile 2

A mixture of diarylidine cyclohexanone (0.01 mmol), malononitrile (0.01), and 5-ml triethylamine in 50 ml absolute ethanol was refluxed for 8 hours. Then, the reaction mixture was cooled and filtered. The precipitate was crystalized from ethanol.

Yield: 95%; m.p. 244–246°C; IR (KBr) cm⁻¹: 2,215 (CN), 3,210 (NH); 1H NMR (DMSO) δ/ppm: 1.74 (t, 2H, J = 7.1 Hz, CH₃), 2.04 (t, 2H, J = 7.1 Hz, CH₂), 2.10 (m, 2H, CH₂), 2.46 (brs, 2H, NH), 3.91 (s, 1H, CHAr), 5.23 (s, 1H, CH=), 7.27–7.51 (m, 8H, Ar). 13C NMR (DMSO) δ/ppm: 22.19, 26.97, 27.06 (3CH₃), 39.38 (CH), 115.8, 119.8, 126.0, 127.3, 128.5, 129.24, 129.27, 129.7, 129.9, 130.9, 131.4, 131.5 (12 aromatic C=), 132.8 (CN), 133.3, 135.2, 135.4 (3 C=), 141.27 (=C-O), 160.6 (=C=NH). MS (m/z): 409.3 (M+, 23%). Anal. calcd. for C₉H₈ClN₂O₂: C, 70.18; H, 4.32; N, 5.46; Found: C, 70.43; H, 4.50; N, 5.67.

N-(8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,6,7,8-tetrahydro-4H-chromen-2-yl)benzalamide 3

A mixture of compound 2 (0.01 mol) and benzoyl chloride (0.01 mol) in 50-ml pyridine was refluxed for 4 hours. The reaction mixture was cooled and filtered. The precipitate was crystalized from ethanol. Yield: 50%; m.p. 184°C–186°C; IR (KBr) cm⁻¹: v, 1,660 (C=O), 2,215 (CN), 3,210 (NH); 1H NMR (DMSO) δ/ppm: 1.51 (t, 2H, J = 7.1 Hz, CH₃), 1.84 (t, 2H, J = 7.1 Hz, CH₂), 1.93 (m, 2H, CH₂), 2.34 (brs, 1H, NH), 4.10 (s, 1H, CHAr), 4.41 (s, 1H, CH=), 7.12–7.40 (m, 13H, Ar). 13C NMR (DMSO) δ/ppm: 21.0, 24.92, 26.02 (3CH₃), 40.20 (CH), 110.10, 116.20, 118.1,118.4, 114.40, 124.80, 125.3, 126.1, 126.48, 127.8, 128.15, 129.57, 129.60, 129.91, 130.10, 130.9, 131.32, 131.40 (18 aromatic C=), 131.6 (CN), 132.1, 133.2, 134.1 (3 aromatic C=), 141.27 (=C-O), 160.6 (=C=NH), 165.23 (C=O), MS (m/z): 513.4 (M+, 17%). Anal. calcd. for C₂₆H₂₁Cl₂N₂O₂: C, 70.18; H, 4.32; N, 5.46; Found: C, 70.43; H, 4.50; N, 5.67.

9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d]pyrimidin-4-one 4

A mixture of compound 3 (0.01) and 30-ml acetic anhydride was refluxed for 12 hours. The reaction mixture was cooled and filtered. The precipitated filtered crystalized from ethanol. Yield: 56%; m.p. 270–272°C; IR (KBr) cm⁻¹: v, 1,675 (C=O), 1,620 (C=N); 1H NMR (DMSO) δ/ppm: 1.21 (t, 2H, J = 7.1 Hz, CH₂), 1.64 (t, 2H, J = 7.1 Hz, CH₂), 1.73 (m, 2H, CH₂), 2.51 (brs, 1H, NH), 4.10 (s, 1H, CHAr), 4.73 (s, 1H, CH=), 7.21–7.35 (m, 13H, Ar). MS (m/z): 513.4 (M+, 29%). Anal. calcd. for C₂₆H₂₁Cl₂N₂O₂: C, 70.18; H, 4.32; N, 5.46; Found: C, 70.20; H, 4.42; N, 5.49.

9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d]pyrimidin-4-one 5

A mixture of compound 2 (0.01 mol) and 30-ml acetic anhydride was refluxed for 10 hours. The reaction mixture was cooled and filtered. The precipitate filtered crystalized from ethanol. Yield: 55%; m.p. 260°C–262°C; IR (KBr) cm⁻¹: v, 1,655 (C=O), 1,630 (C=N); 1H NMR (CDCl₃) δ/ppm: 1.32 (t, 2H, J = 7.1 Hz, CH₃), 1.51 (t, 2H, J = 7.1 Hz, CH₂), 1.62 (m, 2H, CH₂), 1.70 (s, 3H, CH₃), 2.91 (brs, 1H, NH), 3.80 (s, 1H, CHAr), 4.91 (s, 1H, CH=), 7.32–7.45 (m, 8H, Ar). MS (m/z): 451.3 (M⁺, 31%). Anal. calcd. for C₁₅H₁₃ClN₂O₂: C, 66.53; H, 4.47; N, 6.21; Found: C, 66.63; H, 4.50; N, 6.29.

9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d]pyrimidin-4-one 6

A mixture of compound 2 (0.01 mol) and 30-ml formic acid was refluxed for 10 hours. The reaction mixture was cooled...
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and filtered. The precipitate crystalized from ethanol. Yield: 60%; m.p. 224°C–226°C; IR (KBr) cm⁻¹, ν: 1,675 (C=O), 1,615 (C=N); ¹H NMR (CDCl₃) δ/ppm: 1.41 (t, 2H, J = 7.1 Hz, CH₂), 1.62 (t, 2H, J = 7.1 Hz, CH₂), 3.81 (s, 1H, CHAr), 4.42 (brs, 1H, NH), 5.23 (s, 1H, CH=), 7.13–7.25 (m, 8H, Ar), 8.43 (s, 1H, NCH); ¹³C NMR (DMSO) δ/ppm: 23.6, 23.9, 25.9 (3 CH₂), 32.4 (CHAr), 124.0, 124.9 (2 C=), 126.1, 126.8, 126.9, 127.4, 127.9, 128.3, 129.4, 130.1, 135.1, 137.2, 138.3, 139.8 (12 Ar C), 140.1, 146.9, 147.9, 148.2 (4 C=), 150.4 (C=N), 162.3 (C=O). MS (m/z): 437.3 (M⁺, 41%). Anal. calcd. for C₂₄H₁₈Cl₂N₂O₂: C, 65.92; H, 4.15; N, 6.41; Found: C, 66.03; H, 4.20; N, 6.49.

General procedure for the preparation of compounds 7a–c
A mixture of compounds 4–6 (0.01 mol), 30-ml phosphorus oxychloride, and 2 g phosphorous pentachloride was refluxed for 6 hours. Then, the reaction mixture was cooled and filtered. The precipitate was filtered and crystalized from ethanol to give compound 7a–c.

4-chloro-9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 7a
Yield: 50%; m.p. 100°C–102°C; IR (KBr) cm⁻¹, ν: 1,635 (C=N); ¹H NMR (CDCl₃) δ/ppm: 1.34 (t, 2H, J = 7.1 Hz, CH₂), 1.71 (t, 2H, J = 7.1 Hz, CH₂), 1.95 (m, 2H, CH₂), 3.82 (brs, 1H, NH), 4.21 (s, 1H, CHAr), 5.43 (s, 1H, CH=), 7.21–7.45 (m, 8H, Ar); MS (m/z): 437.3 (M⁺, 41%). Anal. calcd. for C₂₃H₂₁Cl₂N₂O: C, 63.92; H, 4.08; N, 5.96; Found: C, 64.02; H, 4.15; N, 6.02.

General procedure for the preparation of compounds 8a–c
A mixture of compounds 7a–c (0.01 mol), 1-ml hydrazine hydrate in 30-ml dioxane was refluxed for 4 hours.
Then, the reaction mixture evaporated under reduced pressure. The residue was crystallized from ethanol to give compounds 8a–c.

9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-4-hydrazinyl-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 8a

Yield: 60%; m.p. 224°C–226°C; IR (KBr) cm⁻¹: 3,038, 2,932, 1,701, 1,515, 1,223. 1H NMR (CDCl₃) δ/ppm: 7.29 (t, 2H, J=7.1 Hz, CH₂), 1.78 (m, 2H, CH₂), 5.10 (brs, 3H, NH, NH₂), 3.71 (s, 1H, CHAr), 5.53 (s, 1H, CH=), 7.17–7.35 (m, 13H, Ar). MS (m/z): 527.4 (M⁺, 20%). Anal. calcd. for C₃₁H₂₂Cl₂N₂O: C, 63.74; H, 4.89; N, 8.49; Found: C, 63.90; H, 4.97; N, 8.70.

6-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)hexane-1,2,3,4,5-pentaol 9b

Yield: 65%; m.p. 130°C–132°C; IR (KBr) cm⁻¹: 3,134 (NH), 3,130 (OH); 1H NMR (DMSO) δ/ppm: 1.02 (t, 2H, J=7.1 Hz, CH₂), 1.13 (brs, 5H, 5OH), 2.82 (t, 2H, J=7.1 Hz, CH₂), 2.84 (m, 2H, CH₂), 3.02 (d, 1H, J=7.0 Hz, CHO), 3.33 (q, 1H, J=7.0 Hz, CHO), 3.49 (t, 1H, J=7.0 Hz, CHO), 3.49 (t, 1H, J=7.0 Hz, CHO), 3.82 (s, 1H, CHAr), 4.20 (d, 2H, J=7.0 Hz, CHO), 4.50 (brs, 1H, NH), 6.10 (s, 1H, CH=), 7.20 (d, 1H, J=6.2 Hz, NCH=), 7.30–7.36 (m, 13H, Ar). Anal. calcd. for C₃₁H₂₂Cl₂N₂O: C, 62.89; H, 5.10; N, 8.21.

5-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)pentane-1,2,3,4-tetraol 9c

Yield: 70%; m.p. 220°C–222°C; IR (KBr) cm⁻¹: 3,142 (C=O), 3,240 (NH), 3,325 (OH); 1H NMR (DMSO) δ/ppm: 1.12 (t, 2H, J=7.1 Hz, CH₂), 1.54 (brs, 4H, 4OH), 2.21 (s, 3H, CH₃), 2.31 (2H, J=7.1 Hz, CH₂), 2.51 (m, 2H, CH₂), 3.12 (t, 1H, J=7.0 Hz, CHO), 3.40 (t, 1H, J=7.0 Hz, CHO), 3.52 (q, 1H, J=7.0 Hz, CHO), 3.82 (s, 1H, CHAr), 4.18 (d, 2H, J=7.0 Hz, CHO), 4.78 (brs, 1H, NH), 6.14 (s, 1H, CH=), 7.17 (d, 1H, J=6.2 Hz, NCH=), 7.25–7.34 (m, 8H, Ar), 7.81 (s, 1H, NCH); 13C NMR (DMSO) δ/ppm: 21.2, 22.1, 24.2 (3 CH₃), 70.1, 71.3, 73.2 (3 CHOH), 75.1 (CHOH), 123.1, 124.2 (2 C=), 126.5, 127.1, 127.5, 128.1, 128.5, 128.9, 131.9, 131.1, 134.2, 136.2, 140.5, 145.4 (13 Ar C), 146.3, 146.7, 147.1, 148.2 (4 C=), 152.4 (C=N). Anal. calcd. for C₃₂H₂₃Cl₂N₂O: C, 63.87; H, 4.47; N, 12.41; Found: C, 63.95; H, 4.56; N, 12.59.

General procedure for the preparation of compounds 9a–d

A mixture of compounds 8a,b (0.01 mol), 40-ml ethanol, 5-ml distilled water, 1-ml acetic acid, and glucose or xylose (0.01 mol) was refluxed for 6 hours. The reaction mixture evaporated under reduced pressure. The residue crystallized from ethanol to give compounds 9a–d.

5-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)pentane-1,2,3,4-tetraol 9d

Yield: 60%; m.p. 170°C–172°C; IR (KBr) cm⁻¹: 3,038, 2,932, 1,701, 1,515, 1,223. 1H NMR (CDCl₃) δ/ppm: 1.02 (t, 2H, J=7.1 Hz, CH₂), 1.23 (brs, 4H, 4OH), 2.62 (t, 2H, J=7.1 Hz, CH₂), 2.91 (m, 2H, CH₂), 3.06 (d, 1H, J=7.0 Hz, CHO), 3.40 (q, 1H, J=7.0 Hz, CH₂), 3.52 (t, 1H, J=7.0 Hz, CHO), 3.90 (s, 1H, CHAr), 4.18 (d, 2H, J=7.0 Hz, CH₂OH), 4.48 (brs, 1H, NH), 6.14 (s, 1H, CH=), 7.17 (d, 1H, J=6.2 Hz, NCH=), 7.25–7.34 (m, 13H, Ar). Anal. calcd. for C₃₃H₂₄Cl₂N₂O: C, 63.74; H, 4.89; N, 8.49; Found: C, 63.90; H, 4.97; N, 8.70.

General procedure for the preparation of compounds 10a–d

A mixture of compounds 9a–d (0.01 mol) and 10-ml acetic anhydride was refluxed for 6 hours. Then, the reaction mixture was poured into water and the solid formed filtered, dried, and crystallized from ethanol to give compounds 10a–d.
1-(2-Acetyl-8-(2-chlorobenzylidene)-12-(2-chlorophenyl)-5-phenyl-2,3,8,10,11,12-hexahydro-9H-chromeno[3,2-e][1,2,4]triazolo[4,3-c]pyrimidin-3-yl)butane-1,2,3,4-tetrayl pentaacetate 10a

Yield: 60%; m.p. 130°C–132°C; IR (KBr) cm⁻¹: ν: 1,644 (C=N), 1,744 (C=O); ¹H NMR (CDCl₃) δ/ppm: 1.12, 1.68 (2s, 12H, 4CH₂), 2.01 (s, 9H, 3CH₃CO), 2.23 (t, 2H, J=7.1 Hz, CH₂), 2.35 (t, 2H, J=7.1 Hz, CH₂), 2.58 (m, 2H, CH₂), 4.28 (s, 1H, CHAr), 4.97, 5.08, 5.14 (3d, 3H, J=7 Hz, 3CHO), 5.27 (s, 1H, CH=), 5.95 (d, 1H, J=7 Hz, CHN), 6.10 (m, 1H, CHO), 7.19–7.47 (m, 13H, Ar). Anal. calcd. for C₃₁H₂₆Cl₂N₄O₁₂: C, 58.71; H, 5.04; N, 6.37; Found: C, 58.90; H, 5.19; N, 6.50.

Cytotoxic activity

The cytotoxic activity was carried out based on a previously reported procedure (Yousif et al., 2019c).

RESULTS AND DISCUSSION

Diarylidene cyclohexanone 1 reacts with malononitrile in triethylamine to produce 2-Amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile 2. Compound 2 has been previously reported (Wang et al., 2004a; Jin et al., 2005; Wang et al., 2004b; Kumar et al., 2011). The method of preparation of compound 2 was a modified method, by using triethylamine as a weak base instead of sodium methoxide in a solvent-free reaction. The proposed structure is in agreement with spectral data. The IR of compound 2 shows the absorption band for CN group and NH group and shows the disappearance of carbonyl group absorption band. Mass spectroscopy for compound 2 shows a molecular ion peak at m/z 409.

2-amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile 2 reacts with benzoyl chloride to afford N-(8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-3-cyano-5,6,7,8-tetrahydro-4H-chromen-2-yl) benzamide 3. Compound 3 is heated under reflux in acetic anhydride to give 9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d] pyrimidin-4-one 4. Also, 2-amino-8-(2-chlorobenzylidene)-4-(2-chlorophenyl)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile 2 is heated under reflux in acetic anhydride to give 9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d] pyrimidin-4-one 5. Compound 2 reacts with formic acid to afford 9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-3,5,6,7,8,9-hexahydro-4H-chromeno[2,3-d] pyrimidin-4-one 6. The spectral data of compounds 3–6 are compatible with the proposed structure. The IR spectrum of compound 3 shows the absorption band for carbonyl group. The ¹³C NMR of compound 3 shows a characteristic signal for carbonyl group at δ 165.23 ppm. The IR of compound 4 shows the disappearance of the absorption band for cyano group (CN). The mass spectrum for compound 4 shows a molecular ion peak at m/z 513. The IR spectrum of compounds 5,6 shows the disappearance of the absorption band of cyano functional group. The mass spectrum of compound 5 shows a molecular ion peak at m/z 451. The ¹³C NMR of compound 6 shows a signal at δ 162.3 ppm characteristic for carbonyl group.

Chlorination of compounds 4–6 using phosphorous pentachloride and phosphorus oxychloride affords 4-chloro-9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 7a, 4-chloro-9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 7b, and 4-chloro-9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 7c respectively. Also, compounds 7a–c react with hydrazine hydrate to give...
Scheme 1
9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-4-hydrazinyl-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 8a, 9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-4-hydrazinyl-2-methyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 8b, and 9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-4-hydrazinyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidine 8c, respectively. The structures of compounds 7a–c and 8a–c were elucidated from 1H NMR, IR, and mass spectral data. The IR of compounds 7a–c shows the disappearance of the absorption band of carbonyl function group. The 13C NMR of compound 7c shows the disappearance of signal for carbonyl group. Also, the IR of compounds 8a–c shows the appearance of the absorption band of NH, NH2 groups. The mass spectrum of compound 8a shows a molecular ion peak at m/z 527.

Compounds 8a–b react with xylose and glucose to afford 5-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)pentane-1,2,3,4-tetraol 9a, 6-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-phenyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)hexane-1,2,3,4,5-pentao 9b, 5-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)pentane-1,2,3,4-tetraol 9c, and 6-(2-(9-(2-chlorobenzylidene)-5-(2-chlorophenyl)-2-methyl-6,7,8,9-tetrahydro-5H-chromeno[2,3-d]pyrimidin-4-yl)hydrazono)hexane-1,2,3,4,5-pentao 9d, respectively. In addition, compounds 9a–d were acetylated using acetic anhydride to afford acetylated sugar derivatives 10a–d. The spectral data of compounds 9a–d and 10a–d are compatible with the proposed structure. The IR spectrum of compounds 9a–d shows the absorption band for hydroxyl group. Also, the IR of compounds 10a–d shows the absorption band for carbonyl group and disappearance of absorption band for hydroxyl group, indicating acetylation of hydroxyl groups of compounds 9a–d. The 13C NMR of compound 10c shows a signal at δ 161.2 ppm indicating carbonyl function group.

**Cytotoxic activity**

The cytotoxic activity of the new synthesized compounds was carried out against three different cancer cell lines, namely adenocarcinomic human alveolar basal epithelial cells A-549, human epithelial colorectal adenocarcinoma cells CaCo-2, and human colorectal adenocarcinoma cell line HT-29, using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Yousif et al., 2019c). The results are presented in Table 1 as cytotoxic activity of the synthesized compounds at 100 μM on the three cell lines. The results show that compounds 9b,d and 10b,d have moderate cytotoxic activity toward A-549 cell lines when compared to doxorubicin as the reference drug. Compounds 3–6, 7b, and 8a–b have a weak cytotoxic activity toward A-549 cell lines. Compound 2 has high cytotoxic activity toward CaCo-2 cell lines when compared to doxorubicin as the reference drug. Compounds 5, 6, 9b, and 10b have a weak cytotoxic activity toward CaCo-2 cell lines. Compound 2 shows high cytotoxic activity toward HT-29 cell lines. Compounds 3, 5, 6, 7a–b, 8a–b, 9b,d, and 10b,d show a weak cytotoxic activity toward HT-29 cell lines.

**Table 1.** Percentage cytotoxicity of compounds on human tumor cancer cell lines at 100 μM.

| Compound | A-549     | CaCo-2   | HT-29   |
|----------|-----------|----------|---------|
| 2        | –         | 88.3 ± 1.3 | 76.4 ± 1.6 |
| 3        | 16.6 ± 4.6 | –        | 33.1 ± 4.1 |
| 4        | 17.3 ± 10.6 | 0        | 0       |
| 5        | 27.4 ± 6.9 | 0.7 ± 0.9 | 2.0 ± 1.4 |
| 6        | 37.4 ± 8.8 | 24.6 ± 4.1 | 20.4 ± 2.9 |
| 7a       | –         | –        | 5.1 ± 3.8 |
| 7b       | 21.0 ± 2.5 | 0        | 3.3 ± 1.7 |
| 8a       | 10.2 ± 6.5 | –        | 10.3 ± 8.1 |
| 8b       | 25.3 ± 1.5 | 0        | 9.9 ± 3.5 |
| 9b       | 45.9 ± 5.7 | 7.5 ± 2.2 | 0.5 ± 0.9 |
| 9d       | 47.8 ± 0.3 | –        | 32.9 ± 6.6 |
| 10b      | 44.8 ± 10.1 | 7.0 ± 4.2 | 8.7 ± 11.8 |
| 10d      | 52.5 ± 21.0 | 0        | 3.0 ± 2.2 |

Doxorubicin 100 100 100

\( p \leq 0.01, n = 3. \)

Results are shown as average percentage cytotoxicity ± standard deviation.

From the aforementioned biological activity, we can deduce the structural activity relationship. The presence of the amino group at position 2 and the cyano group at position 3 in compound 2 increases the cytotoxic activity toward CaCo-2 and HT-29 cell lines. The presence of the hydrazine group linked to glucose in compounds 9b,d makes the cytotoxic activity moderate toward A-549 cell lines. The presence of the triazolo ring linked to acetylated glucose in compound 10b,d makes the cytotoxic activity moderate toward A-549 cell lines. The disappearance of the amino group in compound 3 and the presence of the pyrimidine ring linked to chromene afford a weak cytotoxic activity toward A-549 cell lines. The presence of the pyrimidine ring linked to chromene and chlorame at position 4 in compound 7b makes cytotoxic activity weak toward A-549 cell lines. Also, the presence of the triazolo ring linked to chromene and hydrazine function group at position 4 in compound 8a,b makes the cytotoxic activity weak toward A-549 cell lines. The presence of the pyrimidine ring linked to chromene in compounds 5,6 makes the cytotoxic activity toward CaCo-2 cell lines weak. Also, the presence of the pyrimidine ring linked to the chromene and hyrazino function group and linked to glucose in compound 9b makes cytotoxic activity weak toward CaCo-2 cell lines. In addition, the presence of the pyrimidine ring and triazolo ring linked to chromene and acetylated glucose in compound 10b makes the cytotoxic activity weak toward CaCo-2 cell lines.

**CONCLUSION**

Novel compounds derived from chromene have been synthesized and structurally elucidated using mass spectroscopy, infrared, and nuclear magnetic resonance spectroscopy. Screening of most of the synthesized compounds against A-549, CaCo-2, and HT-29 cell lines has been made.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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