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Coumarin-Based Bioactive Compounds: Facile Synthesis and Biological Evaluation of Coumarin-Fused 1,4-Thiazepines

Mehdi Khoobi[1,2], Alireza Foroumadi[2,3], Saeed Emami[4], Maliheh Safavi[3,5], Gholamreza Dehghan[6], Babak H. Alizadeh[7], Ali Ramazani[1], Sussan K. Ardestani[5] and Abbas Shafiee[3,*]

[1]*Chemistry Department, Zanjan University, P.O. Box 45195-313, Zanjan, Iran*
[2]*Drug Design & Development Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran*
[3]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran*
[4]*Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran*
[5]*Department of Biochemistry, Institute of Biochemistry and Biophysics, University of Tehran, P.O. Box 13145-1365, Tehran, Iran*
[6]*Department of Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran*
[7]*Department of Chemistry, Iranian Research Institute of Plant Protection (IRIPP), Tehran, Iran*
**Corresponding author: Abbas Shafiee, ashafiee@ams.ac.ir*

As part of our ongoing studies on the synthesis of bioactive coumarin compounds, we synthesized a series of new coumarin-fused 1,4-thiazepines using a simple method. The biological activity of target compounds along with 3-(2-hydroxybenzylidene)chroman-2,4-dione intermediates was screened by evaluation of their antioxidant and cytotoxic activities. The antioxidant activity was assessed using two methods, namely, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and ferric reducing antioxidant power (FRAP) assay. 4-Methoxyphenolic compound 5d in thiazepine series showed the most potent scavenging activity, while the 4-bromophenolic derivative 5b was the most efficient compound in FRAP assay. Also, the result of cytotoxic evaluation using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay demonstrated that compound 5b is at least twofold more potent than etoposide against MCF-7, SK-N-MC, and MDA-MB 231 cell lines.

Key words: antioxidant activity, coumarin, cytotoxic activity, phenolic compounds, thiazepine

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Currently, cancer is a serious clinical problem that poses significant socioeconomical effects on the human healthcare and is the second leading cause of death in most countries (1). Apart from the use of surgery and radiotherapy, chemotherapy has still been an important treatment modality for cancers. Enormous progress has been made in the chemotherapy of cancer with the development of novel anticancer agents including paclitaxel, docetaxel, imatinib, and sorafenib (2). However, owing to toxicity and drug-resistance problems with current chemotherapeutic agents, it remains a great challenge to discover and develop more effective anticancer drugs (1).

Among potential chemotherapeutic agents, heterocyclic compounds represent an outstanding type of anticancer drug candidate (3). Over the last two decades, synthesis of N and S containing heterocyclic compounds especially thiazepines retained the interest of researchers because of the unique structural properties and broad spectrum of biological activities of these compounds. The aryl- and heteroaryl-fused 1,4-thiazepines are a privileged structure with fascinating pharmacological properties, such as antiarrhythmic, antispasmodic, angiogenic, and CNS activities, and therefore represent promising synthetic targets (4). On the other hand, coumarin rings has been found as a key structural unit in many bioactive natural products and pharmaceuticals (5). However, to the best of our knowledge, the coumarin-fused 1,4-thiazepine that combines coumarin and thiazepine fragments in one molecule has not been reported previously.

Synthetic approaches to 1,4-thiazepine are varied and involve addition, condensation, coupling, rearrangement, and thermolysis methodologies in multistep synthesis (6). As a part of our ongoing studies on the synthesis of bioactive chromene and coumarin compounds (7–12), herein, we wish to report the synthesis of novel 5-(2-hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[*4,3-e*][*1,4*]thiazepin-6(5*H*)-one derivatives (**5a**–**e**) using a simple method in two steps and their screening for antioxidant and cytotoxic activities.

Experimental Section

Chemistry

All starting materials, reagents, and solvents were purchased from Merck AG (Darmstadt, Germany). 4-Hydroxycoumarin derivatives **2a**–**c** were prepared according to the literature method (13). The purity of

the synthesized compounds was confirmed by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F254 plates were applied for analytical TLC. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded using a Bruker 500 spectrometer (Bruker, Rheinstetten, Germany), and chemical shifts are expressed as δ (ppm) with tetramethylsilane as internal standard. The IR spectra were obtained on a Nicolet 550-FTIR spectrometer (potassium bromide disks). Elemental analyses were carried out on a CHN-O-rapid elemental analyzer (GmbH, Hanau, Germany) for C, H, and N, and the results are within ±0.4% of the theoretical values.

General procedure for synthesis of 3-(2-hydroxybenzylidene)chroman-2,4-dione derivatives (3)

A mixture of 4-hydroxycoumarin derivative **2** (10 mmol) and appropriate 2-hydroxybenzaldehyde (12 mmol) in ethanol (15 mL) was refluxed for 0.5–1 h. The progress of reaction was followed by TLC. After completion of the reaction, the mixture was allowed to cool and crystals of corresponding product were formed. Immediately, the precipitated solid was filtered off and washed with ethanol. If after the cooling of mixture, appropriate crystals or solid did not form, the solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography to give the pure product **3**.

3-(2-Hydroxybenzylidene)chroman-2,4-dione (3a)

Yellow crystal (0.79 g, 30%); mp 173–175 °C; IR (KBr, 1/cm) ν_{max} 3431 (OH), 1721 (C = O); ^{1}H NMR ($CDCl_3$) δ 6.89 (td, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H), 7.38 (td, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.54 (m, 2H), 7.60 (dd, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H), 7.67 (td, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H), 7.97 (s, 1H), 11.73 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 117.0, 117.9, 118.6, 119.1, 125.1, 126.4, 129.0, 132.5, 133.6, 137.5, 144.2, 154.5, 158.0, 163.2, 195.8. Anal. calcd for $C_{16}H_{10}O_4$: C, 72.18; H, 3.79; Found: C, 72.36; H, 3.97.

3-(5-Bromo-2-hydroxybenzylidene)chroman-2,4-dione (3b)

Yellow crystal (1.20 g, 35%); mp 194–196 °C; IR (KBr, 1/cm) ν_{max} 3440 (OH), 1721 (C = O); ^{1}H NMR ($CDCl_3$) δ 6.89 (td, ^3J = 7.5 Hz, ^4J = 1.0 Hz, 1H), 7.06 (dd, ^3J = 8.5 Hz, ^4J = 1.0 Hz, 1H), 7.30 (d, J = 8.5 Hz, 1H), 7.50 (dd, ^3J = 8.0 Hz, ^4J = 2.0 Hz, 1H), 7.55 (td, ^3J = 7.5 Hz, ^4J = 2.0 Hz, 1H), 7.52 (m, 2H), 7.86 (s, 1H), 11.65 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 117.7, 118.7, 119.2, 119.3, 127.4, 131.1, 132.3, 136.3, 137.7, 142.6, 153.3, 157.3, 163.2, 195.6. Anal. calcd for $C_{16}H_9BrO_4$: C, 55.68; H, 2.63; Found: C, 55.89; H, 2.34.

3-(2-Hydroxy-3-methoxybenzylidene)chroman-2,4-dione (3c)

Colorless solid (0.88 g, 35%); mp 217–219 °C; IR (KBr, 1/cm) ν_{max} 3428 (OH), 1723 (C = O); ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H), 6.88 (t, J = 7.5 Hz, 1H), 7.05 (d, J = 8.2 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.53 (m, 2H), 7.94 (s, 1H), 11.72 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.3, 115.3, 118.5, 118.9, 119.1, 120.2, 124.9, 126.7, 132.5, 137.5, 144.2, 144.4, 147.3, 153.1, 157.5, 163.2, 196.2; Anal. calcd for $C_{17}H_{12}O_5$: C, 68.92; H, 4.08; Found: C, 68.70; H, 4.37.

3-(2-Hydroxy-5-methoxybenzylidene)chroman-2,4-dione (3d)

Colorless solid (0.88 g, 35%); mp 158–160 °C; IR (KBr,1/cm) ν_{max} 3443 (OH), 1703 (C=O); ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H), 6.88 (t, J = 7.5 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 7.29 (t, J = 8.0 Hz, 1H), 7.53 (m, 2H), 7.94 (s, 1H), 11.72 (bs, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.3, 115.3, 118.5, 118.9, 119.1, 120.2, 124.9, 126.7, 132.5, 137.5, 144.2, 144.4, 147.3, 153.1, 157.5, 163.2, 196.2; Anal. calcd for $C_{17}H_{12}O_5$: C, 68.92; H, 4.08; Found: C, 68.73; H, 4.31.

7-Hydroxy-3-(2-hydroxybenzylidene)chroman-2,4-dione (3e)

Yellow solid (0.84 g, 30%); mp 240–242 °C; IR (KBr, 1/cm) ν_{max} 3260 (OH), 1685 (C = O); ^{1}H NMR (DMSO-d_6) δ 6.32 (s, 1H), 6.37 (d, ^3J = 8.8 Hz, 1H), 7.42 (t, ^3J = 7.5 Hz, 1H), 7.48 (d, ^3J = 7.5 Hz, 1H), 7.63 (d, ^3J = 8.8 Hz, 1H), 7.70 (t, ^3J = 7.5 Hz, 1H), 7.82 (d, ^3J = 7.5 Hz, 1H), 8.26 (s, 1H), 11.48 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.9, 109.0, 114.3, 116.7, 118.8, 125.3, 128.4, 129.9, 133.5, 135.0, 142.8, 154.1, 158.5, 163.8, 165.7, 192.4; Anal. calcd for $C_{16}H_{10}O_5$: C, 68.09; H, 3.57; Found: C, 68.23; H, 3.31.

6-Fluoro-3-(2-hydroxybenzylidene)chroman-2,4-dione (3f)

Yellow crystal (0.99 g, 35%); mp 156–158 °C; IR (KBr, 1/cm) ν_{max} 3429 (OH), 1722 (C = O); ^{1}H NMR ($CDCl_3$) δ 7.04 (dd, ^3J = 9.0 Hz, ^4J = 4.5 Hz, 1H), 7.23 (dd, ^3J = 8.5 Hz, ^4J = 3.0 Hz, 1H), 7.28 (dt, ^3J = 8.5 Hz, ^4J = 3.0 Hz, 1H), 7.39 (t, J = 7.5 Hz, 1H), 7.44 (d, J = 7.2 Hz, 1H), 7.62 (d, J = 7.2 Hz, 1H), 7.68 (t, J = 7.2 Hz, 1H), 7.99 (s, 1H), 11.53 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 116.9, 117.1, 117.7, 120.0, 120.1, 125.2, 125.4, 129.2, 134.0, 144.7, 147.3, 153.8, 155.7, 157.3, 159.4, 195.5; Anal. calcd for $C_{16}H_9FO_4$: C, 67.61; H, 3.19; Found: C, 67.28; H, 3.41.

6-Fluoro-3-(2-hydroxy-3-methoxybenzylidene)chroman-2,4-dione (3g)

Yellow crystal (0.94 g, 30%); mp 193–195 °C; IR (KBr, 1/cm) ν_{max} 3417 (OH), 1718 (C = O); ^{1}H NMR ($CDCl_3$) δ 4.02 (s, 3H), 7.05 (m, 1H), 7.19 (d, ^3J = 8.0 Hz, 1H), 7.23 (m, 2H), 7.32 (m, 2H), 7.99 (s, 1H), 11.49 (s, 1H, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.4, 115.5, 116.9, 117.1, 118.4, 119.9, 120.0, 120.3, 125.1, 125.2, 126.2, 144.9, 147.3, 153.8, 155.7, 157.3, 159.4, 195.5; Anal. calcd for $C_{17}H_{11}FO_5$: C, 64.97; H, 3.53; Found: C, 65.10; H, 3.39.

General procedure for synthesis of 5-(2-hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[4,3-*e*][1,4]thiazepin-6(5*H*)-one derivatives (5)

A mixture of 3-(2-hydroxybenzylidene)chroman-2,4-dione derivatives **3** (0.5 mmol) and 2-aminoethanethiol hydrochloride (1.5 mmol) was

dissolved in ethanol (2 mL). The mixture was stirred at room temperature overnight. The precipitated solid was filtered off and washed with water to give pure thiazepine derivative **5**.

5-(2-Hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[4,3-*e*][1,4]thiazepin-6(5*H*)-one (5a)

White solid (0.14 g, 85%); mp 223–225 °C; IR (KBr, 1/cm) ν_{max} 3441 (OH), 1705 (C=O); ^{1}H NMR (DMSO-d_6) δ 2.60–2.73 (m, 2H), 2.79–2.83 (m, 2H), 5.36 (s, 1H), 7.34 (td, ^3J = 8.0 Hz, ^4J = 2.0 Hz, 1H), 7.43–7.44 (m, 2H), 7.48–7.52 (m, 2H), 7.67 (d, ^3J = 7.5 Hz, 1H), 7.74 (td, ^3J = 8.0 Hz, ^4J = 1.5 Hz, 1H), 7.98 (bs, 2H, OH, NH), 8.06 (dd, ^3J = 8.0 Hz, ^4J = 1.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 28.8, 36.3, 38.3, 95.3, 101.1, 113.5, 116.7, 116.7, 121.3, 122.8, 124.8, 126.2, 129.3, 129.9, 133.1, 149.4, 152.0, 156.3, 159.9; Anal. calcd for $C_{18}H_{15}NO_3S$: C, 66.44; H, 4.65; N, 4.30; Found: C, 66.11; H, 4.97; N, 4.16.

5-(5-Bromo-2-hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[4,3-*e*][1,4]thiazepin-6(5*H*)-one (5b)

White solid (0.18 g, 88%); mp 265–267 °C; IR (KBr, 1/cm) ν_{max} 3441 (OH), 1705 (C = O); ^{1}H NMR (DMSO-d_6) δ 2.63–2.72 (m, 2H), 2.85–2.88 (m, 2H), 5.39 (s, 1H), 7.41 (d, ^3J = 8.5 Hz, 1H), 7.48–7.53 (m, 2H), 7.62 (dd, ^3J = 8.5 Hz, ^4J = 2.5 Hz, 1H), 7.73 (t, ^3J = 8.0 Hz, 1H), 7.90 (d, ^4J = 2.5 Hz, 1H), 7.91 (bs, 2H, OH, NH), 8.05 (d, ^3J = 8.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 26.9, 35.8, 38.2, 100.9, 113.3, 116.7, 117.6, 119.0, 122.8, 123.9, 124.8, 132.0, 132.1, 133.2, 148.7, 152.0, 156.1, 159.7; MS, *m/z* (%) 405 (M^+ + 2, 6), 403 (M^+, 6), 329 (100), 327 (93), 248 (20), 220 (17), 163 (23); Anal. calcd for $C_{18}H_{14}BrNO_3S$: C, 53.48; H, 3.49; N, 3.46; Found: C, 53.69; H, 3.78; N, 3.21.

5-(2-Hydroxy-3-methoxyphenyl)-2,3-dihydro-1*H*-chromeno[4,3-*e*][1,4]thiazepin-6(5*H*)-one (5c)

White solid (0.15 g, 85%); mp 203–205 °C; IR (KBr, 1/cm) ν_{max} 3444 (OH), 1727 (C = O); ^{1}H NMR (DMSO-d_6) δ 2.56–2.69 (m, 2H), 2.82–2.85 (m, 2H), 3.95 (s, 3H), 5.35 (s, 1H), 7.15 (dd, ^3J = 8.0 Hz, ^4J = 1.3 Hz, 1H), 7.19 (d, ^3J = 7.0 Hz, 1H), 7.28 (t, ^3J = 8.0 Hz, 1H), 7.52–7.55 (m, 2H), 7.74 (bs, 2H, OH, NH), 7.76 (dt, ^3J = 7.0 Hz, ^4J = 1.6 Hz, 1H), 7.96 (dd, ^3J = 7.0 Hz, ^4J = 1.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 26.7, 36.4, 38.2, 56.2, 95.3, 111.7, 113.6, 116.7, 120.6, 121.9, 122.5, 124.8, 126.0, 133.0, 139.0, 147.4, 151.9, 156.1, 159.8; Anal. calcd for $C_{19}H_{17}NO_4S$: C, 64.21; H, 4.82; N, 3.94; Found: C, 64.58; H, 4.49; N, 3.71.

5-(2-Hydroxy-5-methoxyphenyl)-2,3-dihydro-1*H*-chromeno[4,3-*e*][1,4]thiazepin-6(5*H*)-one (5d)

White solid (0.14 g, 80%); IR (KBr, 1/cm) ν_{max} 3442 (OH), 1720 (C = O); ^{1}H NMR (DMSO-d_6) δ 2.40–2.49 (m, 2H), 2.56–2.59 (m, 2H), 3.80 (s, 3H), 5.25 (s, 1H), 7.00 (dd, ^3J = 9.0 Hz, ^4J = 3.0 Hz, 1H), 7.15 (d, ^4J = 3.0 Hz, 1H), 7.36 (d, ^3J = 9.0 Hz, 1H), 7.47–7.51 (m, 2H), 7.73 (dt, ^3J = 7.5 Hz, ^4J = 1.5 Hz, 1H), 8.03 (dd, ^3J = 7.5 Hz, ^4J = 1.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.60, 36.5, 40.5, 55.6, 100.6, 113.3, 113.5, 115.1, 116.6, 117.50, 122.4, 122.6, 124.6, 132.8, 143.6, 151.8, 156.1, 156.8, 159.8; Anal. calcd for $C_{19}H_{17}NO_4S$: C, 64.21; H, 4.82; N, 3.94; Found: C, 64.56; H, 4.51; N, 3.69.

9-Hydroxy-5-(2-hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[4,3-*e*][1,4]thiazepin-6(5*H*)-one (5e)

White solid (0.13 g, 78%); mp 190–193 °C; IR (KBr, 1/cm) ν_{max} 3431 (OH), 1698 (C = O); ^{1}H NMR (DMSO-d_6) δ 2.56–2.69 (m, 2H), 2.83–2.86 (m, 2H), 5.34 (s, 1H), 6.83 (d, ^4J = 2.2 Hz, 1H), 6.93 (dd, ^3J = 8.7 Hz, ^4J = 2.2 Hz, 1H), 7.33 (t, ^3J = 7.5 Hz, 1H), 7.39–7.46 (m, 2H), 7.63 (d, ^3J = 7.5 Hz, 1H), 7.71 (bs, 1H, NH), 7.89 (d, ^3J = 8.7 Hz, 1H), 10.36 (bs, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.2, 36.9, 38.8, 97.8, 102.8, 105.7, 114.0, 117.0, 121.9, 124.7, 126.4, 129.6, 130.3, 150.0, 154.4, 157.6, 160.8, 162.6; Anal. calcd for $C_{18}H_{15}NO_4S$: C, 63.33; H, 4.43; N, 4.10; Found: C, 63.10; H, 4.71; N, 3.82.

Biological activity

Cytotoxic assay

Human cancer cell lines including MCF-7, SK-N-MC, and MDA-MB 231 were obtained from Pasture institute, Tehran (Iran). Cells were maintained in RPMI 1640 with added 10% FBS, 1% L-glutamine, and penicillin/streptomycin, and then cells were incubated at 37 °C in a 5% concentration of CO_2. Cells were harvested by trypsin/EDTA and resuspended in fresh medium. Cell survival was determined by MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] colorimetric assay (14). Exponentially growing cells (4×10^4 cells/well) were seeded in 96-well plates in RPMI with 10% FBS and incubated for 24 h. After treatment of cells with different concentrations of test compounds **3a–g** and **5a–e** for 24 h at 37 °C, the medium was removed and phenol red-free medium with FBS was added to cells. Then, MTT solution was added to each well (2 mg/mL), followed by 4-h incubation. The viable cell number is directly proportional to the production of formazan, which, following solubilization with isopropyl alcohol, can be measured spectrophotometrically at 570 nm by an ELISA plate reader. In each plate, there were control wells (cells without test compounds) and blank wells (the medium only or 0.1% DMSO). The percentage of cell viability versus controls was assessed by the formula: [1–(absorbance of treated cells/absorbance of control cells)] ×100.

DPPH radical scavenging assay

Several concentrations of test compounds **3b**–**g** and **5a**–**e** in DMSO were prepared. The compound solution (1.0 mL) was added to the methanolic DPPH solution (2.0 mL, 0.1 mM), and the mixture was kept in the dark for 15 min. The absorbance at 517 nm was then measured by an UV/visible spectrophotometer. The percent scavenging activity was calculated using the following formula: inhibition (%) = $100 \times (Abs_{control} - Abs_{compound})/Abs_{control}$. The DPPH radical scavenging activity of compounds was expressed in terms of IC_{50} (mM), which is obtained from linear regression plot between concentrations of test compound and percent inhibitions (15).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay reagent was prepared by adding 10 vol of 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate and 16 mL glacial acetic acid), 1 vol of 10 mM 2,4,6-tripyridyl-*s*-triazine prepared in 40 mM HCl, and 1 vol of 20 mM $FeCl_3$. The mixture was diluted to 1/3 with methanol and prewarmed at 37 °C. This reagent (3 mL) was mixed with 0.1 mL diluted test compounds **3b**–**g** and **5a**–**e**. The mixture was shaken and incubated at 37 °C for 8 min, and the absorbance was read at 593 nm. A blank with only 0.1 mL methanol was used for calibration. The difference in absorbance between the tested sample and the blank reading was calculated, and the data were expressed as mM of ferric reduced to ferrous form (16).

Results and Discussion

Chemistry

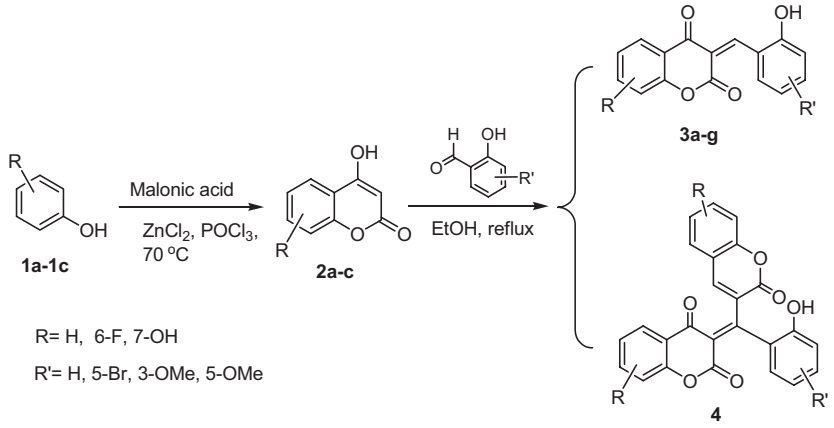
The synthetic routes for synthesis of key intermediates 3-(2-hydroxybenzylidene)chroman-2,4-diones **3a**–**g** and target compounds 5-(2-hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[*4,3-e*][*1,4*]thiazepin-6(5*H*)-one derivatives **5a**–**e** are depicted in Schemes 1 and 2, respectively.

First, 4-hydroxycoumarin derivatives **2a**–**c** were prepared by the literature method using an appropriately substituted phenol and malonic acid, $ZnCl_2$ as Lewis acid, and phosphorus oxychloride ($POCl_3$) as condensing agent (13). Then, compounds **2a**–**c** were converted to the corresponding benzylidene derivatives **3a**–**g** using a simple aldol condensation with appropriate 2-hydroxybenzaldehyds refluxing in absolute ethanol (Scheme 1). The low yields (20–40%) of the desired benzylidene derivatives **3a**–**g** may be relatively because of the formation of bis-coumarins **4**. Reaction of 2-aminoethanethiol hydrochloride with benzylidene derivatives **3a**–**g** in ethanol at room temperature gave the desired coumarin-fused thiazepines **5a**–**e** (Scheme 2). It seems that slightly acidic conditions provided by the excess 2-aminoethanethiol hydrochloride were necessary for the reaction to proceed. The structures of all compounds were ascertained by their spectroscopic data (^{1}H- and ^{13}C-NMR, MS) and elemental analyses. Based on ^{1}H-NMR spectra of the crude product, it was concluded that the compound **5** was the only product of this reaction and formation of **6** was not detected. An indication for this interpretation is the existence of just one singlet signal in 5.25–5.39 ppm.

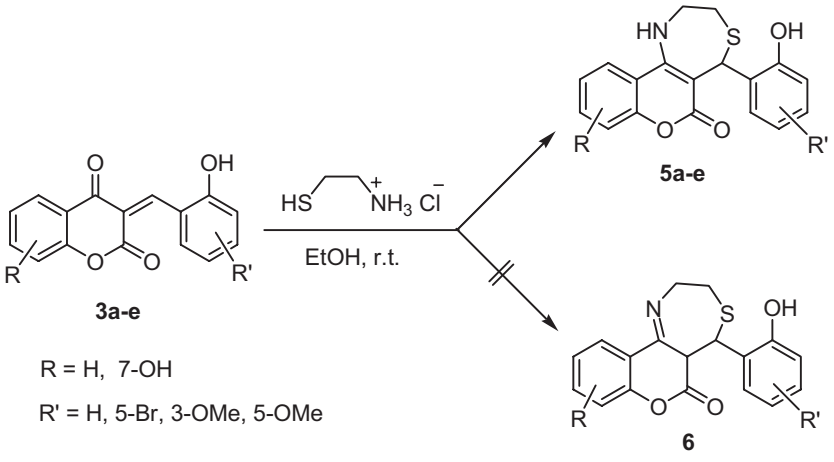
Biological activity

Cytotoxic activity

The cytotoxic activity of compounds **3a**–**g** and **5a**–**e** was evaluated against three human cell lines MCF-7, SK-N-MC, and MDA-MB 231 using MTT assay. The amount of produced purple formazan

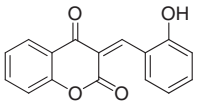
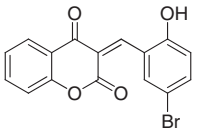
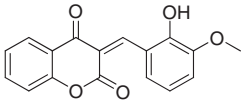
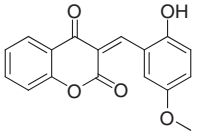
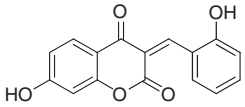
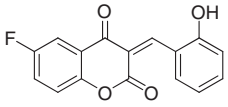
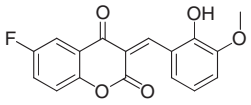
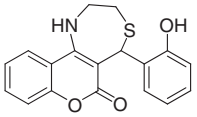
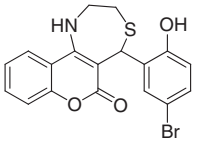
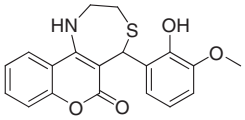
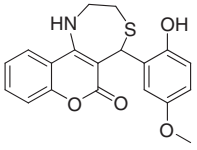
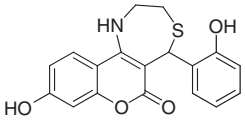


Scheme 1: Synthesis of key intermediates 3-(2-hydroxybenzylidene) chroman-2,4-diones **3a**–**g**.



Scheme 2: Synthesis of 5-(2-hydroxyphenyl)-2,3-dihydro-1*H*-chromeno[*4,3-e*][*1,4*]thiazepin-6(5*H*)-one derivatives **5a**–**e**.

Table 1: Chemical structures and cytotoxic activity (IC_{50}, μg/mL) of compounds **3a**–**g** and **5a**–**e**

Compounds	Structure	MCF-7	SK-N-MC	MDA-MB-231
3a		>100	>100	>100
3b		>100	>100	>100
3c		>100	>100	>100
3d		>100	>100	>100
3e		>100	>100	>100
3f		>100	>100	>100
3g		>100	>100	>100
5a		>100	8.49 ± 0.75	>100
5b		5.35 ± 1.03	3.75 ± 0.75	10.32 ± 2.30
5c		21.78 ± 8.82	15.02 ± 4.45	45.21 ± 12.87
5d		>100	>100	>100
5e		>100	>100	>100
Etoposide		18.43 ± 1.55	14.04 ± 1.05	20.52 ± 2.47

from MTT was assayed by spectrophotometer and is proportional to the number of viable cells. The IC_{50} values (μg/mL) were calculated by linear regression analysis, expressed in mean ± SD. The result of cytotoxic evaluation in comparison with etoposide as a standard drug was summarized in Table 1.

The growth inhibitory concentrations of compounds indicate that 3-(2-hydroxybenzylidene)chroman-2,4-diones **3a**–**g** showed no significant activity against tested cell lines. Among thiazepine derivatives, 4-bromophenolic compound **5b** and 2-methoxyphenolic counterpart **5c** exhibited good cytotoxic activity against all tested cell lines. Although compound **5a** showed no activity (IC_{50} > 100 μg/mL) against MCF-7 and MDA-MB-231 cell lines but had potent inhibitory activity against SK-N-MC cell (IC_{50} < 10 μg/mL). The comparison of IC_{50} values of the most potent compound **5b** and standard drug etoposide demonstrated that compound **5b** was at least twofold more potent than etoposide. Also, the activity of compound **5c** against MCF-7 and SK-N-MC cells was comparable to that of etoposide.

DPPH radical scavenging activity

Many synthetic and natural coumarin derivatives have special ability to scavenge free radicals. Thus, primarily, the free radical scavenging activity of coumarin-based compounds **3b**–**g** and **5a**–**e** was evaluated by DPPH colorimetric method. Several dilutions of compounds were made and assayed to obtain concentration of the sample required to scavenge 50% (IC_{50}) of DPPH-free radical applying suitable regression analysis of the mean values. The results are given in Table 2. Among the tested compounds, thiazepine derivatives **5d** and **5e** showed a significant DPPH radical scavenging activity with IC_{50} values of 0.12 mM. However, their activity was less than quercetin as reference drug. From the results of 3-(2-hydroxybenzylidene)chroman-2,4-diones **3b**–**g** and coumarin-fused thiazepines **5a**–**e**, it is evident that thiazepine formation improved radical scavenging ability of the compounds. Also, the substitution effect on activity was different in these two series of compounds. For example, while the 7-hydroxycoumarin-fused thiazepine analog **5e** showed good scavenging activity, surprisingly benzylidene-7-hydroxycoumarin analog **3e** exhibited very weak activity. In both series, 4-bromo and 4-methoxyphenolic derivatives were more active than 2-methoxyphenolic compounds.

Table 2: Antioxidant activities of compounds **3b**–**g** and **5a**–**e**

Compound	DPPH radical scavenging activity (IC_{50}, mM)	FRAP value (mM Fe^{2+}) (100 μg)
3b	1.56 ± 0.01	39.4 ± 0.6
3c	3.19 ± 0.02	41.8 ± 0.8
3d	1.47 ± 0.01	44.0 ± 1.1
3e	17.03 ± 0.06	30.2 ± 0.5
3f	0.68 ± 0.01	145.7 ± 3.4
3g	0.58 ± 0.01	158.4 ± 5.1
5a	4.52 ± 0.03	41.9 ± 1.9
5b	0.29 ± 0.01	186.4 ± 4.5
5c	0.97 ± 0.01	29.2 ± 0.8
5d	0.12 ± 0.01	89.3 ± 2.3
5e	0.12 ± 0.01	68.1 ± 1.7
Quercetin	7.92 ± 0.3[a]	53.9 ± 1.5

[a]IC_{50} in μM.
DPPH, 1,1-diphenyl-2-picrylhydrazyl; FRAP, ferric reducing antioxidant power.

FRAP ability

The FRAP assay measures the ability of a compound to reduce the ferric 2,4,6-tripyridyl-*s*-triazine complex to the colored ferrous complex. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The FRAP values for test compounds (Table 2) revealed that the fluoro analog of 3-(2-hydroxybenzylidene)chroman-2,4-diones (**3f** and **3g**) and thizepine derivatives **5b**, **5d**, and **5e** exhibited more potent reducing power in comparison with reference drug quercetin as a poly-phenolic flavonoid. Thiazepine compound **5b** having 4-bromophenolic moiety showed the most antioxidant power in FRAP assay. It is noticeable that this compound also showed respectable DPPH radical scavenging activity. Compounds **5d** and **5e** that showed the most potent antioxidant activity in DPPH model had good reducing power but their efficacy was half in respect to that of **5b**.

Conclusion

A series of new coumarin-fused 1,4-thiazepines were synthesized with a simple method in two steps starting from 4-hydroxycoumarins. The biological activity of target compounds along with 3-(2-hydroxybenzylidene)chroman-2,4-dione intermediates was screened by evaluation of their antioxidant and cytotoxic activities. 4-Methoxyphenolic compound **5d** in thiazepine series showed the most potent scavenging activity, while the 4-bromophenolic derivative **5b** was the most efficient compound in FRAP assay. Also, the result of cytotoxic evaluation using MTT assay demonstrated that compound **5b** is at least twofold more potent than etoposide against MCF-7, SK-N-MC, and MDA-MB 231 cell lines. The results revealed that the coumarin-fused 1,4-thiazepine structure could be served as a scaffold for finding new bioactive compounds.

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