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ORIGINAL PAPER

Synthesis of some novel pyrano[2,3-*f*]chromenone derivatives

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Abstract A new series of pyrano[2,3-*f*]chromenone derivatives were synthesized starting from resorcinol. Resorcinol reacted with 3-chloropropanoic acid to give 7-hydroxychroman-4-one and reaction of the later compound with 2-methylbut-3-yn-2-ol led to the formation of 7-((2-methylbut-3-yn-2-yl)oxy)chroman-4-one. The obtained compound tolerated the cyclization reaction through Claisen rearrangement by heating in DMF to afford 8,8-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one. Reaction of the later compound with various aromatic aldehydes gave the title compounds in good yields. All products were evaluated for their cytotoxic activity on the blood tumor cell line K562 and compared to reference drug, etoposide. Most of them exhibited low inhibitory activity against tumor cell line and among them, the compound possessing three methoxy groups on aromatic ring showed better cytotoxic effect.

Keywords Pyranochromenone · Resorcinol · Claisen rearrangement

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Introduction

Pyranochromenes or pyranobenzopyrans are one of the most important class of heterocycles attracting considerable interest in organic and natural product synthesis [1]. At this juncture, promising biological properties such as antitubercular [2], BACE1 inhibitory [3], anticancer (in human leukemia) [4], and antimicrobial activities [5] of pyranochromenes have attracted medicinal chemists attention. Also, they are widely present in naturally occurring compounds such as deguelin (**a**), glyinflanin K (**b**), and glyasperin M (**c**) (Fig. 1) possessing a wide range of biological and pharmacological properties [6–8].

For example, deguelin (**a**, Fig. 1) was originally isolated from African plant *Mundulea sericea* and has depicted strong anticancer activity in various in vitro and in vivo studies for a number of cancers [9–11] including prostate [12], lung [13, 14], and breast [15, 16]. It is believed that it induces apoptosis in premalignant and malignant human bronchial epithelial (HBE) cells with no effect on the growth of normal HBE cells. Thus, it is an attractive candidate agent for chemoprevention of cancer [13].

Brilliant results connected to exclusive antitumor activity of deguelin have inspired researchers to develop its application and investigate more details [11, 17]. Herein, in continuation of our research on the synthesis of chromene derivatives [18–20], we report an efficient method for the preparation of novel pyrano[2,3-*f*]chromenone **3** via the reaction of 8,8-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one **1** and various aromatic aldehydes **2** in the presence of LDA and HMPA in THF at −78 °C (Scheme 1).

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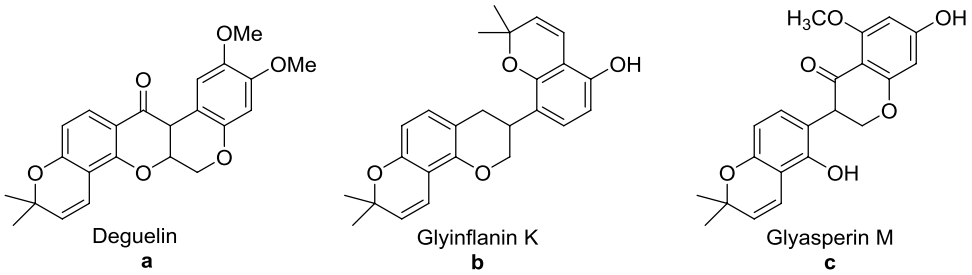
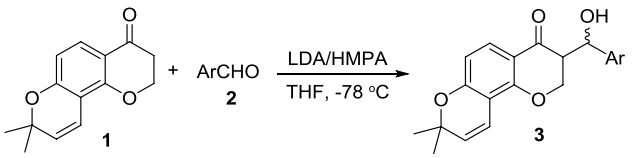
Fig. 1 Structure of deguelin (**a**) and glyinflanin K (**b**)





Scheme 1 Synthesis of pyrano[2,3-*f*]chromenone derivatives **3**

Experimental section

Chemistry

^{1}H-NMR spectra were measured using a Bruker 500 spectrometer (Germany) and chemical shifts were expressed as δ (ppm) with tetramethylsilane as internal standard. The IR spectra were obtained on a Shimadzu IR prestige-21. Mass spectra were recorded on Agilent 6410 Triple Quad. LC/MS and Finnigan MAT TSQ-70. The purity of all compounds was confirmed by TLC using different mobile phases. The elemental analysis was performed with an Elementar Analysensystem GmbH *VarioEL* CHNS mode which were within ±0.4 % of theoretical values for C, H, and N. All chemicals were obtained from Merck and Aldrich and 7-hydroxychroman-4-one **6** was prepared according to our previous report through the reaction of resorcinol **3** and 3-chloroacetic acid **4** [21].

*7-((2-Methylbut-3-yn-2-yl)oxy)chroman-4-one (**8**)*

DBU (0.31 mL, 2.08 mmol) was added drop wise to a solution of 2-methylbut-3-yn-2-ol 7 (0.53 mL, 5.44 mmol) in anhydrous CH3CN (13 mL) under argon at −5 °C. Then, trifluoroacetic anhydride (0.76 mL, 5.44 mmol) was added to the above-mentioned mixture over 30 min while keeping the temperature just below 0 °C. Then, it was allowed to stir at 0 °C for 1 h to form 2-methyl-3-butyn-2-triflate. Separately, DBU (0.31 mL, 2.08 mmol) and $CuCl_2{\cdot}2H_2O$ (0.02 g, 0.10 mmol) was added to a solution of 7-hydroxychroman-4-one 6 (0.22 g, 1.34 mmol) in CH_3CN (2 mL) under argon at −5 °C and allowed to be stirred for 30 min. Then, it was added to the freshly prepared 2-methyl-3-butyn-2-triflate solution over 50 min while keeping the temperature below 0 °C and allowed to be stirred for 30 min at 0 °C. Next, small amount of water was added to the reaction mixture and the solution was concentrated under reduced pressure and the residue was extracted with EtOAc (3 × 5 mL), washed with 1 N HCl, saturated $NaHCO_3$, and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure and the residue was purified by column chromatography using silicagel and hexane/ethyl acetate (4:1) as eluent to obtain 7-((2-methylbut-3-yn-2-yl)oxy)chroman-4-one **8**.

Pale yellow oil; yield: 97 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.72 (s, 6H, 2 × CH_3), 2.67 (s, 1H, C≡CH), 2.78 (t, J = 6.3 Hz, 2H, H_3), 4.53 (t, J = 6.3 Hz, 2H, H_2), 6.80 (dd, J = 8.8, 2.3 Hz, 1H, H_6), 6.88 (d, J = 2.3 Hz, 1H, H_8), 7.84 (d, J = 8.8 Hz, 1H, H_5). IR (film) ν 1,650 (C=O) cm^{-1}. Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13. Found: C, 73.19; H, 6.29.

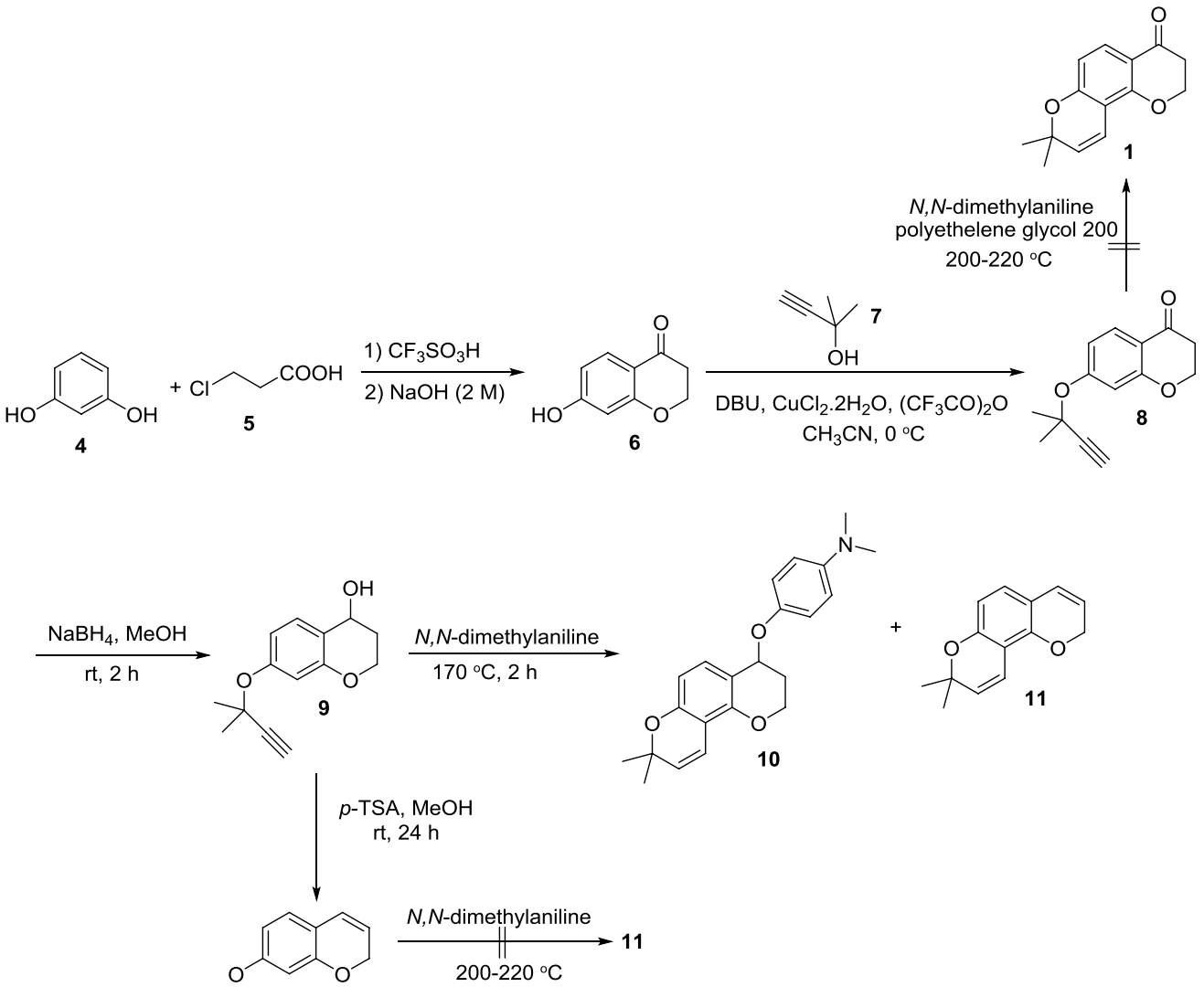
*7-((2-Methylbut-3-yn-2-yl)oxy)chroman-4-ol (**9**)*

A solution of compound **8** (0.15 g, 0.65 mmol) in MeOH (5 mL) was added to a stirred suspension of $NaBH_4$ (45.4 mg, 1.2 mmol) in MeOH (1 mL) and the mixture was stirred at room temperature for 2 h. Then, small amount of water was added to the reaction mixture, MeOH was removed under reduce pressure and the pH of solution was adjusted to pH = 3 using 1 N HCl. Next, the crude product was extracted with EtOAc (3 × 5 mL), washed with water, saturated $NaHCO_3$, and brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure to give the product which was purified by column chromatography using silicagel and hexane/ethyl acetate (3:1) as eluent to give pure 7-((2-methylbut-3-yn-2-yl)oxy)chroman-4-ol **9**.

Pale yellow oil; yield: 85 %. IR (film) ν 3,300 (OH) cm^{-1}.

*4-((8,8-Dimethyl-3,4-dihydro-2H,8H-pyrano[2,3-f]chromen-4-yl)oxy)-N,N-dimethylaniline (**10**) and 8,8-dimethyl-2H,8H-pyrano[2,3-f]chromene (**11**)*

A solution of compound **9** (0.46 g, 0.2 mmol) in anhydrous *N*,*N*-dimethylaniline (0.5 mL) was heated at 170 °C under argon for 2 h. After cooling to room temperature, water was added to the reaction mixture and extracted with ethyl acetate (3 × 15 mL). The combined organic phase was dried



Scheme 2 Investigation of various routes for the preparation of 8,8-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one **1**

over Na_2SO_4 and solvent was evaporated under reduced pressure to obtain crude oil which was purified by column chromatography using silicagel and hexane/ethyl acetate (7:1) as eluent to separate two compounds **10** and **11**.

*4-((8,8-Dimethyl-3,4-dihydro-2H,8H-pyrano[2,3-f]chrome n-4-yl)oxy)-N,N-dimethylaniline (**10**)*

Pale yellow oil; yield: 65 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.43 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.04 (m, 1H, H_3), 2.23 (m, 1H, H_3), 2.93 (s, 6H, $N(CH_3)_2$), 4.01 (t, $J = 6.3$ Hz, 1H, H_2), 4.16 (m, 1H, H_4), 4.21 (t, $J = 5.1$ Hz, 1H, H_2), 5.59 (d, $J = 9.9$ Hz, 1H, H_9), 6.30 (d, $J = 8.3$, Hz, 1H, H_6), 6.63 (d, $J = 8.3$ Hz, 1H, H_5), 6.69 (d, $J = 9.9$ Hz, 1H, H_{10}), 6.70 (d, $J = 8.6$ Hz, 2H, N–Ar), 7.03 (d, $J = 8.6$ Hz, 2H, N–Ar). ESI-Mass *m/z* 374 (M^++23). Anal. Calcd for $C_{22}H_{25}NO_3$: C, 75.19; H, 7.17; N, 3.99. Found: C, 75.38; H, 7.09; N, 4.18.

*8,8-Dimethyl-2H,8H-pyrano[2,3-f]chromene (**11**)*

Pale yellow oil; yield: 15 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.43 (s, 6H, 2 × CH_3), 4.82 (dd, $J = 3.6$, 1.7 Hz, 2H, H_2), 5.60 (d, $J = 10.0$ Hz, 1H, H_9), 5.63 (dt, $J = 10.0$, 3.6 Hz, 1H, H_3), 6.34 (d, 1H, $J = 8.3$ Hz, H_6), 6.36 (dt, $J = 10.0$, 1.7 Hz, 1H, H_4), 6.60 (d, 1H, $J = 10.0$ Hz, H_{10}), 6.73 (d, 1H, $J = 8.3$ Hz, H_5). ESI-Mass *m/z* 237 (M^++23). Anal. Calcd for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78.29; H, 6.69.

*7-((2-Methylbut-3-yn-2-yl)oxy)-2H-chromene (**15**)*

A mixture of compound **9** (0.46 g, 0.2 mmol) and catalytic amount of *p*-TsOH in THF (5 mL) was heated at refluxed for 2 h. Then, small amount of saturated $NaHCO_3$ was added to the reaction mixture and solvent was removed

under reduce pressure at room temperature. The crude product was extracted with ethyl acetate (3 × 15 mL) and the combined organic phase was washed with brine. After drying over Na_2SO_4, the solvent was removed under reduced pressure to give the residue which was purified by column chromatography using silicagel and hexane/ethyl acetate (8:1) as eluent to obtain compound **15**.

Pale yellow oil; yield: 75 %. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.65 (s, 6H, 2 × CH_3), 2.59 (s, 1H, C≡CH), 4.81 (dd, J = 3.6, 1.5 Hz, 2H, H_2), 5.68 (dt, J = 9.8, 3.6 Hz, 1H, H_3), 6.41 (dt, J = 9.8, 1.5 Hz, 1H, H_4), 6.73 (d, J = 2.4, 1H, H_8), 6.71 (dd, J = 8.8, 2.4 Hz, 1H, H_6), 6.86 (d, J = 8.8 Hz, 1H, H_5). ESI-Mass *m/z* 237 (M^++23). Anal. Calcd for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78.27; H, 6.72.

*8,8-Dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**1**)*

A solution of compound **8** (0.05 g, 0.22 mmol) in DMF (3 mL) was heated at reflux for 6 h. After completion of reaction, water was added to the reaction mixture and the crude was extracted with dichloromethane (3 × 10 mL). After drying over Na_2SO_4, the organic layers were evaporated under reduced pressure and the pure compound **1** was obtained by flash chromatography using silicagel and hexane/ethyl acetate (5:1) as eluent.

Pale yellow solid; yield: 80 %, mp 120–122 °C. ^{1}H-NMR ($CDCl_3$) δ 1.46 (s, 6H, 2 × CH_3), 2.78 (t, J = 6.4 Hz, 2H, H_3), 4.57 (t, J = 6.4 Hz, 2H, H_2), 5.62 (d, J = 10.0 Hz, 1H, H_9), 6.47 (d, J = 8.7 Hz, 1H, H_6), 6.63 (d, J = 10.0 Hz, 1H, H_{10}), 7.74 (d, J = 8.7 Hz, 1H, H_5). IR (film) ν 1,760 (C=O) cm^{-1}. MS *m/z* (%) 230 (M^+, 19), 192 (33), 177 (100), 137 (54), 107 (32), 67 (53). Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.03; H, 6.13. Found: C, 73.22; H, 6.28.

Procedure for the synthesis of pyrano[2,3-*f*]chromenone derivatives (**3**)

A solution of compound **1** (0.17 mmol) in dry THF (0.1 mL) was added to a solution of LDA (0.7 mL, 0.5 mmol) in the mixture of THF:Hex (2:1) at −78 °C and stirred for 30 min. Then, aromatic aldehyde **2** (0.24 mmol) and HMPA (0.1 mL) was added to the above-mentioned solution and the reaction mixture was stirred for 2 h. Next, it was quenched with saturated aq. NH_4Cl (1 mL) and extracted with EtOAc (3 × 20 mL). The extract was washed successively with water (1 mL), 10 % HCl (1 mL), water, saturated aq. $NaHCO_3$ and brine. Organic phase was dried over Na_2SO_4 and solvent was evaporated in vacuo to give an oily product which was purified by TLC chromatography (silica gel, hexane/EtOAc: 5/1) to give pure compound **3** as mixture of diastereomers.

*3-(Hydroxy(phenyl)methyl)-8,8-dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**3a**)*

Yield: 50 % as a mixture of diastereomers (*anti*/*syn*: 40/60). ^{1}H-NMR ($CDCl_3$) δ 1.44 (s, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 2.98 (m, 1H, H_3), 3.14 (m, 1H, H_3), 3.95 (m, 1H, H_2), 4.01 (d, J = 7.9 Hz, 1H, H_2), 4.29 (dd, J = 11.5, 5.2 Hz, 1H, H_2), 4.52 (t, J = 11.5 Hz, 1H, H_2), 5.12_{anti} (d, J = 9.1 Hz, 1H, CH), 5.59 (d, J = 10.0 Hz, 1H, H_9), 5.77_{syn} (s, 1H, CH), 6.48 (d, J = 8.5 Hz, 1H, H_6), 6.50 (d, J = 8.6 Hz, 1H, H_6), 6.53 (d, J = 10.0 Hz, 1H, H_9), 6.83 (d, J = 10.0 Hz, 1H, H_{10}),7.57 (m, 3H, H_{10}, Ar), 7.62 (d, J = 8.5 Hz, 2H, Ar), 7.72_{syn} (d, J = 8.6 Hz, 1H, H_5), 7.72 (m, 1H, Ar), 7.76_{anti} (d, J = 8.7 Hz, 1H, H_5), 8.26 (m, 4H, Ar), 8.32 (d, J = 8.5 Hz, 1H, Ar). IR (film) ν 3,500 (OH), 1,675 (C=O) cm^{-1}; ESI-Mass *m/z* 359 (M^++23); Anal. Calcd for $C_{21}H_{20}O_4$: C, 74.98; H, 5.99. Found: C, 74.76; H, 6.13.

*3-(Hydroxy(4-methoxyphenyl)methyl)-8,8-dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**3b**)*

Yield: 63 % as a mixture of diastereomers (*anti*/*syn*: 76/24). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 2.98 (m, 1H, H_3), 3.07 (m, 1H, H_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.04 (t, J = 11.5 Hz, 1H, H_2), 4.09 (dd, J = 11.5, 5.4 Hz, 1H, H_2), 4.40 (dd, J = 11.5, 5.4 Hz, 1H, H_2), 4.52 (m, 1H, H_2), 4.93_{syn} (d, J = 9.0 Hz, 1H, CH), 5.57_{anti} (d, J = 10.0 Hz, 1H, CH), 5.59 (d, J = 10.0 Hz, 1H, H_9), 6.51 (m, 3H, H_9, H_{10}, H_{10}), 6.48 (d, 1H, J = 8.7 Hz, H_6), 6.51 (m, 1H, H_6), 6.91 (d, J = 9.0 Hz, 2H, Ar), 6.94 (d, J = 8.5 Hz, 2H, Ar), 7.28 (d, J = 9.0 Hz, 2H, Ar), 7.34 (d, J = 8.5 Hz, 2H, Ar), 7.73_{syn} (d, J = 8.6 Hz, 1H, H_5), 7.77_{anti} (d, J = 8.7 Hz, 1H, H_5). IR (film) ν 500 (OH), 1,675 (C=O) cm^{-1}; ESI-Mass m/z 389 (M^++23). Anal. Calcd for $C_{22}H_{22}O_5$: C, 72.12; H, 6.05. Found: C, 72.23; H, 6.26.

*3-(Hydroxy(3-methoxyphenyl)methyl)-8,8-dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**3c**)*

Yield: 60 % as a mixture of diastereomers (*anti*/*syn*: 66/35). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.45 (s, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 3.02_{anti} (ddd, J = 10.0, 9.1, 5.7 Hz, 1H, H_3), 3.12_{syn} (ddd, J = 10.0, 8.5, 3.0 Hz, 1H, H_3), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.02 (m, 1H, H_2), 4.07 (dd, J = 11.0, 5.8 Hz, 1H, H_2), 4.36 (dd, J = 11.0, 5.2 Hz, 1H, H_2), 4.50 (dd, J = 11.0, 7.4 Hz, 1H, H_2), 4.95_{anti} (d, J = 9.1 Hz, 1H, CH), 5.56_{syn} (d, J = 3.0 Hz, 1H, CH), 5.57 (d, J = 8.1 Hz, 1H, H_9), 5.59 (d, J = 10.0 Hz, 1H, H_9), 6.46 (d, J = 8.7 Hz, 1H, H_6), 6.51 (d, J = 8.7 Hz, 1H, H_6), 6.52 (d, J = 10.0 Hz, 1H, H_{10}), 6.54 (d, J = 10.0 Hz, 1H, H_{10}), 6.51 (s, 1H, Ar), 6.52 (s, 1H, Ar), 6.82–6.99 (m, 4H, Ar), 7.29 7.31 (m, 2H, Ar), 7.73 (d, J = 8.7 Hz, 1H, H_5),

7.77 (d, $J = 8.7$ Hz, 1H, H_5). IR (film) ν 3,450 (OH), 1,760 (C=O) cm^{-1}. ESI-Mass *m/z* 389 (M^++23), Anal. Calcd for $C_{22}H_{22}O_5$: C, 72.12; H, 6.05. Found: C, 72.25; H, 5.88.

*3-((3,4-Dimethoxyphenyl)(hydroxy)methyl)-8,8-dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**3**)*

Yield: 65 % as a mixture of diastereomers (*anti*/*syn*: 55/45). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 3.01 (m, 1H, H_3), 3.09 (m, 1H, H_3), 3.89 (s, 6H, 2 × OCH_3), 3.91 (s, 6H, 2 × OCH_3), 3.99 (dd, $J = 10.7$, 5.6 Hz, 1H, H_2), 4.07 (dd, $J = 10.7$, 8.2 Hz, 1H, H_2), 4.41 (dd, 1H, $J = 11.3$, 4.9 Hz, H_2), 4.63 (t, $J = 7.2$ Hz, 1H, H_2), 4.92 (d, $J = 9.4$ Hz, 1H, CH), 5.57 (d, $J = 9.4$ Hz, 1H, CH), 5.60 (d, $J = 10.0$ Hz, 1H, H_6), 6.48 (d, $J = 8.7$ Hz, 1H, H_6), 6.48 (d, $J = 10.1$ Hz, 1H, H_9), 6.51 (d, $J = 8.6$ Hz, 1H, H_9), 6.53 (d, $J = 8.6$ Hz, 1H, H_{10}), 6.55 (d, $J = 10.1$ Hz, 1H, H_{10}), 6.85 (m, 2H, Ar), 6.91 (m, 4H, Ar), 7.74 (d, $J = 8.6$ Hz, 1H, H_5), 7.78 (d, $J = 8.7$ Hz, 1H, H_5). IR (film) ν 3,500 (OH), 1,760 (C=O) cm^{-1}. ESI-Mass *m/z* 419 (M^++23); Anal. Calcd for $C_{23}H_{24}O_6$: C, 69.68; H, 6.10. Found: C, 69.52; H, 6.31.

*3-(Hydroxy(3,4,5-trimethoxyphenyl)methyl)-8,8-dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**3e**)*

Yield: 70 % as a mixture of diastereomers (*anti*/*syn*: 31/69). ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.44 (s, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 3.01_{anti} (m, 1H, H_3), 3.10_{syn} (ddd, $J = 11.0$, 5.5, 3.6 Hz, 1H, H_3), 3.86 (s, 6H, 2 × OCH_3), 3.87 (s, 6H, 2 × OCH_3), 3.89 (s, 6H, 2 × OCH_3), 4.05 (m, 1H, H_2), 4.12 (dd, $J = 11.0$, 5.5 Hz, 1H, H_2), 4.41 (dd, $J = 11.0$, 5.2 Hz, 1H, H_2), 4.53 (t, 1H, $J = 11.5$ Hz, H_2), 4.90_{anti} (d, $J = 9.1$ Hz, 1H, CH), 5.59 (d, $J = 10.1$ Hz, 1H, H_9), 5.60 (d, 1H, $J = 10.1$ Hz, H_9), 5.61_{syn} (d, $J = 3.2$ Hz, 1H, CH), 6.49 (d, $J = 7.5$ Hz, 1H, H_6), 6.50 (d, $J = 8.7$ Hz, 1H, H_6), 6.56 (d, $J = 10.1$ Hz, 1H, H_{10}), 6.59(d, $J = 10.1$ Hz, 1H, H_{10}), 6.64 (s, 1H, Ar), 6.81 (s, 1H, Ar), 6.85 (s, 1H, Ar), 7.15 (s, 1H, Ar), 7.74 (d, 1H, $J = 7.5$ Hz, H_5), 7.76 (d, 1H, $J = 8.7$ Hz, H_5). IR (film) ν 3,350 (OH), 1,760 (C=O) cm^{-1}. ESI-Mass *m/z* 449 (M^++ 23). Anal. Calcd for $C_{24}H_{26}O_7$: C, 67.59; H, 6.15. Found: C, 67.40; H, 6.29.

*3-(Hydroxy(4-nitrophenyl)methyl)-8,8-dimethyl-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (**3f**)*

Yield: 31 % as a mixture of diastereomers (*anti*/*syn*: 55/45). ^{1}H-NMR (500 MHZ, $CDCl_3$) δ 1.44 (s, 6H, 2 × CH_3), 1.47 (s, 6H, 2 × CH_3), 2.36 (m, 1H, H_3), 3.02 (m, 1H, H_3), 3.95 (m, 1H, H_2), 4.10 (d, $J = 7.7$ Hz, 1H, H_2), 4.29 (dd, $J = 11.5$, 5.0 Hz, 1H, H_2), 4.54 (t, $J = 11.5$ Hz, 1H, H_2), 5.60_{anti} (d, $J = 10.1$ Hz, 1H, CH), 5.78_{syn} (d, $J = 3.7$ Hz, 1H, CH), 6.51 (d, $J = 8.7$ Hz, 1H, H_9), 5.65 (d, $J = 9.9$ Hz, 1H, H_9), 6.51 (d, $J = 8.7$ Hz, 1H, H_{10}), 6.54 (d, $J = 9.9$ Hz, 1H, H_{10}), 6.82 (d, $J = 7.5$ Hz, 1H, H_6), 6.85 (d, $J = 7.5$ Hz, 1H, H_6), 7.55 (d, $J = 7.5$ Hz, 1H, H_5), 7.57 (d, $J = 8.5$ Hz, 2H, Ar), 7.72 (d, $J = 7.5$ Hz, 1H, H_5), 7.74 (d, $J = 8.7$ Hz, 2H, Ar), 8.27 (d, $J = 8.5$ Hz, 2H, Ar), 8.32 (d, $J = 8.7$ Hz, 2H, Ar). IR (film) ν 3,500 (OH), 1,760 (C=O) cm^{-1}. ESI-Mass *m/z* 404 (M^++ 23); Anal. Calcd for $C_{21}H_{19}NO_6$: C, 66.14; H, 5.02; N, 3.67. Found: C, 66.27; H, 4.87; N, 3.43.

Biology

Reagents and chemicals

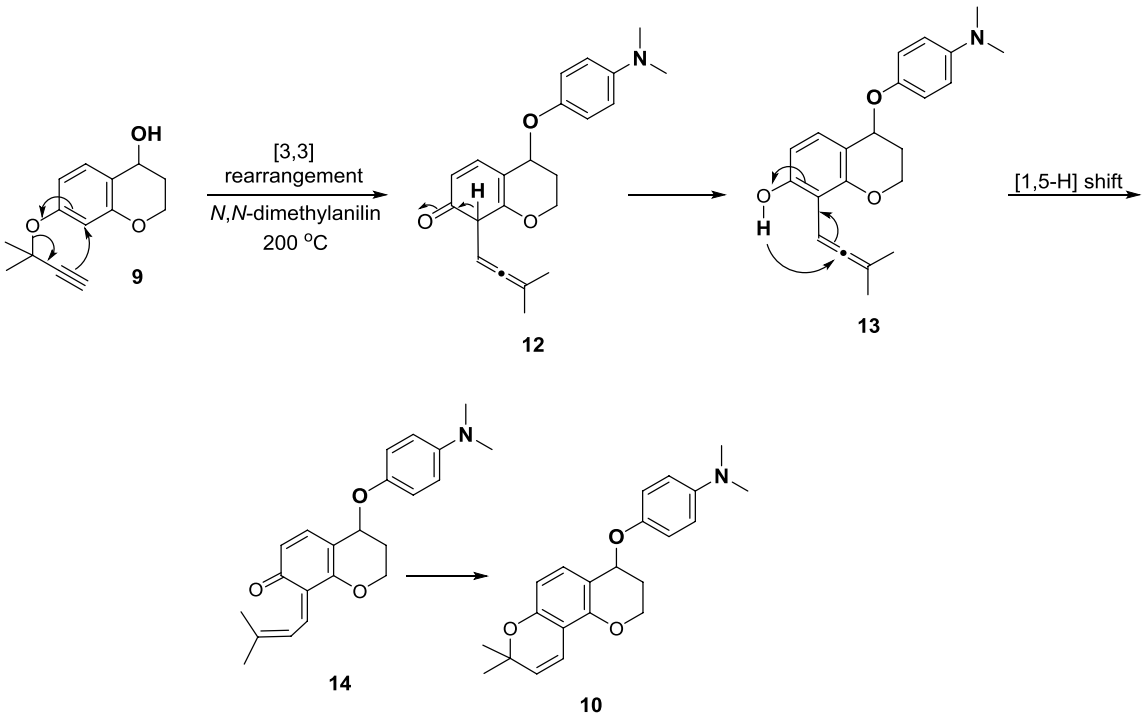
RPMI 1640 and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY). 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), trypsin–EDTA solution and dimethyl sulphoxide (DMSO) were obtained from Sigma (Saint Louis, MO, USA). Penicillin/streptomycin was purchased from Invitrogen (San Diego, CA, USA).

Cell lines and cell culture

Human blood cancer cell line K562 was obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran and cultured at a density of 3–5 × 10 4/mL RPMI 1640 medium supplemented with FBS (10 %, v/v), streptomycin (100 µg/mL), and penicillin (100 U/mL) and kept at 37 °C in a 5 % CO_2 humidified atmosphere. Drug treatments were usually done 24 h after ceding the cells.

Cytotoxicity assay

The in vitro cytotoxic activity of all synthesized compounds was determined against human blood cancer cell line K562 using MTT colorimetric assay [22]. 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 for 24 and 48 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) and incubated for 4 h. The viable cell number is directly proportional to the formation of formazan. After that, they were dissolved in isopropyl alcohol and the absorbance of each well was measured using a microplate reader at 492 nm. 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.



Scheme 3 The formation of compound **10**

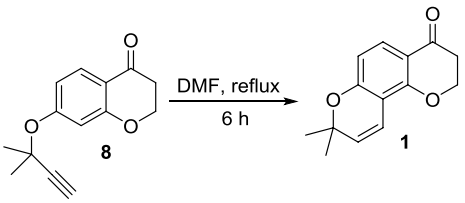
Results and discussion

Chemistry

To achieve our goal, we tried to establish the best condition for the preparation of 8,8-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one **1** and based on our previous experience [21], resorcinol **4** was found as a versatile starting material (Scheme 2).

Accordingly, 7-hydroxychroman-4-one **6** was prepared by the condensation reaction of resorcinol **4** and 3-chloropropanoic acid **5** [21]. Then, focusing on the Godfrey et al. report on alkylation of phenolic hydroxyl [23], 7-((2-methylbut-3-yn-2-yl)oxy)chroman-4-one **8** was prepared via the reaction of **6** and 2-methyl-3-butyn-2-ol **7** in the presence of DBU, $(CF_3CO)_2O$, and $CuCl_2$ in acetonitrile at 0 °C in good yield (85 %). Compound **8** was heated in *N*,*N*-dimethylaniline and polyethelene glycol 200 at 200–220 °C to give the cyclized product **1**, but no rearrangement was occurred.

Alternatively, we changed our route and reacted compound **8** with $NaBH_4$ in MeOH to obtain 7-((2-methylbut-3-yn-2-yl)oxy)chroman-4-ol **9** and heated the later compound in boiling *N*,*N*-dimethylaniline which afforded cyclized products 4-((8,8-dimethyl-3,4-dihydro-2*H*,8*H*-pyrano[2,3-*f*]chromen-4-yl)oxy)-*N*,*N*-dimethylaniline **10** and 8,8-dimethyl-2*H*,8*H*-pyrano[2,3-*f*]chromene **11** as major and minor products, respectively. Obviously, this route could not direct us to compound **1**. The formation of compound **10** has been depicted in Scheme 3. It is presumed that heating **9** led to [3]—sigmatropic rearrangement and intermediates **12** and **13** were formed. Finally, [1,5-H] shift gave **14** following with cyclization to obtain the related compound **10**.



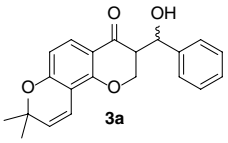
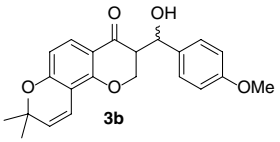
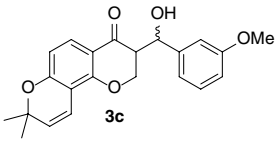
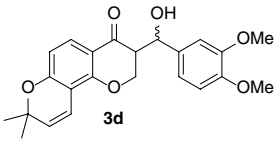
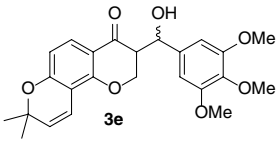
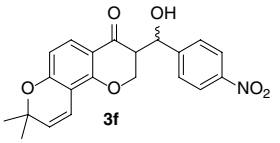
Scheme 4 Synthesis of 8,8-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one **1**

Another effort directed us to dehydration reaction of **9** in the presence of *p*-TSA to obtain compound **15** which was heated in *N*,*N*-dimethylaniline leading to no cyclized product **11**.

Finally, after examination of several procedures, we perceived that 8,8-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromen-4-one **1** could be prepared in high yield by heating 7-((2-methylbut-3-yn-2-yl)oxy)chroman-4-one **8** in DMF at reflux (Scheme 4). It seems that Claisen rearrangement easily took place in DMF to produce **1**.

Next, different pyrano[2,3-*f*]chromenone derivatives **3a–f** (Table 1), bearing hydroxyl group at benzylic position, were prepared by treatment of **1** and aromatic aldehydes **2**

Table 1 Synthesis and percentage growth inhibition values of products **3a–f** against K562 tumor cell line at the concentrations of 50 and 100 µg/mL for 48 and 72 h

Entry	Product **3**	48 h		72 h	
		50 µg/mL	100 µg/mL	50 µg/mL	100 µg/mL
1.	 **3a**	4.7 ± 0.3	30 ± 5.6	11.6 ± 1.6	31.8 ± 2.6
2.	 **3b**	7 ± 1.5	6.7 ± 0.5	20 ± 3.5	32 ± 3.4
3.	 **3c**	7.8 ± 2.1	12.1 ± 1.7	16.4 ± 2.4	9.3 ± 1.3
4.	 **3d**	5.9 ± 1.1	32.3 ± 5.3	7.5 ± 1.2	22.2 ± 3.6
5.	 **3e**	6 ± 0.8	42.9 ± 7.2	7.3 ± 0.8	54 ± 5.8
6.	 **3f**	7.1 ± 0.6	17.6 ± 3.1	30 ± 4.6	37.6 ± 4.1
7.	Etoposide (10 µg/mL)		45.3 ± 6.7		68 ± 8.3

Percent growth inhibition values of cell proliferation at different concentrations; mean ± SD values of three independent experiments are reported

in the presence of LDA and HMPA at −78 °C in THF. It is worth mentioning that all reactions proceeded either with electron-withdrawing or electron-donating para- and meta-substituted benzaldehydes and desired products were obtained in good yields.

All products **3a-f** were obtained as a mixture of two *syn*/*anti* diastereomers based on the S-configuration or R-configuration of C_{11} benzylic hydroxyl group (Fig. 2) and the corresponding configuration at C_{11} was distinguished by the chemical shift of proton H_{11} which was shifted downfield in *syn*-isomer. Also, H_{11}–H_3 coupling constant was smaller in comparison to similar coupling in anti-isomer. Because of the formation of hydrogen bond between hydroxyl group at C_{11} and oxygen of carbonyl group (Fig. 2), the H_{11}–H_3 relationship of the *syn*-isomer is axial-equatorial and that of the anti-isomer is di-axial. Thus, the observed coupling constant between H_{11} and H_3 of *syn*-isomer was lower than that of anti-isomer in accordance with the data reported in the literature [24].

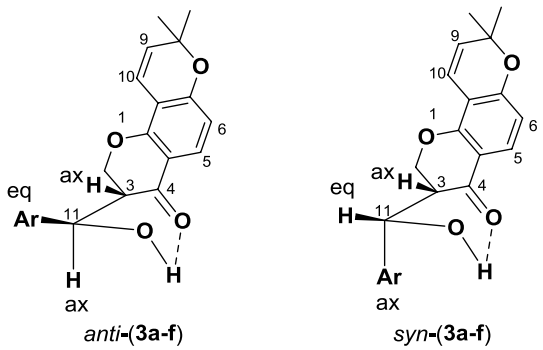


Fig. 2 *Syn*/*anti* diastereomers of compound **3**

Biological investigation

The in vitro cytotoxic activity of all pyrano[2,3-*f*]chromenones **3a–f** were evaluated against blood cancer human cell line K562 according to the literature [22] and

compared with the reference drug etoposide (Table 1). According to percentage growth inhibition values of compounds **3a–f**, most of the compounds have no effect on the cell line. Our results revealed that the cytotoxicity is improved by increasing the number of methoxy groups in aromatic ring. Hence, compound **3e** showed better activity against blood cancer human cell line K562 (Entry 5, Table 1).

Conclusion

In summary, we have developed an efficient synthetic approach for the synthesis of novel pyrano[2,3-*f*]chromenone derivatives as analogues of deguelin, starting from resorcinol. All products were obtained as a mixture of *syn*/*anti* diastereomers which were demonstrated from their ^{1}H NMR spectra.

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