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Synthesis and Biological Evaluation of Pyrano[2,3-*f*]chromene-4,8-dione Derivatives as Potential Anticancer Agents

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Abstract Based on the hit 5-hydroxy-2-methyl-10-propyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**1**), a series of pyrano[2,3-*f*]chromene-4,8-dione derivatives was designed and synthesized using phloroglucinol as starting material. Meanwhile, a regioselective synthetic route was developed for 5-methoxy-2,3-dihydro-4*H*,8*H*-pyrano-[2,3-*f*]chromene-4,8-dione products(**11**a—**11**f), and their structures were further confirmed by nuclear Overhauser effect(NOE). The evaluation of anticancer activities of these compounds against four human cancer cell lines, including human glioma cell line (SHG-44), human lung cancer cell line(H1299), breast cancer cell line(MCF7) and human colon carcinoma cell line(HCT-116) *in vitro* shows that 5-methoxy-2,2-dimethyl-9-chloro-10-trifluormethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**e) possesses the best anticancer activities with IC_{50} values of 6.68, 7.90, 5.16 and 4.82 μmol/L, respectively. Finally, the preliminary structure-activity relationships(SARs) were summarized, which could pave the way for generating more potent anticancer agents with drug-like properties.

Keywords Anticancer activity; Phloroglucinol; Pyrano[2,3-*f*]chromene-4,8-dione

1 Introduction

With more than 14 million new cases per year, cancer has emerged as one of the most devastating diseases worldwide, threatening the public health seriously[1]. Consequently, it is extremely urgent to introduce new small molecular drugs for cancer to shorten the prolonged course of treatment and make the patient's life more tolerable. In this regard, naturally occurring products(such as camptothecin, vincristine and paclitaxel) and their related functionalized structural alterations have a profound impact on the successful treatment of various cancers. This also promotes a great number of medicinal chemists to devote their extensive efforts to the development of anticancer agents from natural products[2—4].

Coumarin derivatives possess a variety of biological properties, such as anti-HIV[5], anti-inflammatory[6], anti-hepatitis C virus[7,8], immunosuppressive[9], antipsychotics[10], antiplatelet[11], monoamine oxidase B inhibitors[12], coronary vasodilators[13], and bone anabolic agents[14]. Moreover, the minimum side effects in company with multi-drug reversal activities of coumarin analogues have rendered it an attractive anticancer subject. Many research groups independently reported a series of functionalized coumarin derivatives encompassing potent anticancer activities[15—17], which typically performed diverse pharmacological mechanisms on different human cancer cell lines.

In our previous study for screening anti-HIV agents derived from calanolide A[18], we serendipitously found that 5-hydroxy-2-methyl-10-propyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**1**) was moderately active against four human cancer cell lines, including human glioma cell line (SHG-44), human lung cancer cell line(H1299), breast cancer cell line(MCF7) and human colon carcinoma cell line (HCT-116), with the half maximal inhibitory concentrations (IC_{50}'s) being 10.48, 14.45, 9.73 and 12.17 μmol/L, respectively. The authors suggested that the pyrano[2,3-*f*]-chromene-4,8-dione core may play a crucial role in the anticancer activities of the hit, and thus the subsequent structural modification was mainly focused on the substitution patterns around the core and the influence of intramolecular hydrogen bond on the anticancer activities. Several design strategies(Fig.1) were carried out to improve the pharmacological activities of the hit(**1**), including the following: (i) the design of homologues at 2-position of the chroman-4-one moiety;

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(ii) blocking out the intramolecular hydrogen bond at 4- and 5-position of the core; (iii) introduction of electron-withdrawing(such as halogen, aryl, or trifluoromethyl) or electron-donating substituents(such as alkyl, benzyl, or chloromethyl) at 9- or/and 10-position of the coumarin ring, and (iv) reduction of the carbon-carbon double bond of the coumarin core. All the twenty-nine newly prepared compounds were subjected for the biological evaluation of anticancer activities against the four human cancer cell lines mentioned above. Furthermore, the preliminary structure-activity relationships (SARs) of these pyrano[2,3-*f*]chromene-4,8-dione derivatives were also summarized, which prompted us to explore more potent compounds with *in vivo* bioactivities.

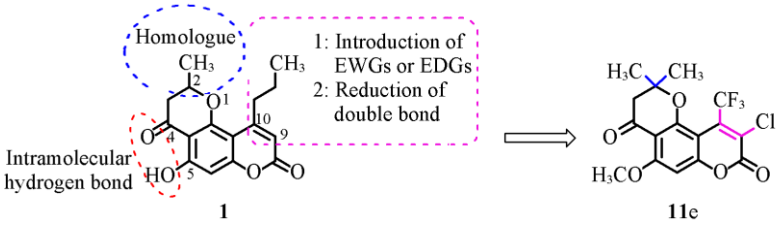


Fig.1 Design strategies of pyrano[2,3-*f*]chromene-4,8-dione derivatives

EWG: electron-withdrawing group; EDG: electron-donating group.

2 Experimental

2.1 Materials and Instruments

All reagents and solvents were obtained from commercially available source(Aladdin) and were used without further purification. Melting points were determined on an X-4 binocular microscope(Gongyi Tech. Instrument Co., Gongyi City, China) and were uncorrected. ^{1}H NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer(Varian, USA) at the ambient temperature in DMSO-d_6 or Acetone-d_6 with tetramethylsilane(TMS) as the internal standard. Splitting patterns were displayed in the following order: s, singlet; d, doublet; t, triplet; m, multiplet. High-resolution mass spectrometer(HRMS) measurements were carried out by LC/MSD TOF using a column of C_{18}(rapid resolution, 3.5 μm, 2.1 mm× 30 mm) at a flow rate of 0.40 mL/min(Agilent, USA). Column chromatography was carried out on silica gel(200—300 mesh) from Qingdao Ocean Chemicals(Qingdao, China).

2.2 General Procedure for the Preparation of Target Compounds 4a—4l and 6a—6j

Intermediates **3**[19] and **5**[20] were prepared according to the corresponding literature, respectively.

To a stirring solution of the intermediate **3** or **5**(5 mmol) in 10 mL of trifluoroacetic acid(TFA) were successively added *β*-keto ester(5.5 mmol) and *p*-toluenesulfonic acid(0.5 mmol) at room temperature. The corresponding mixture was refluxed for 2 h. After completion of the reaction, the mixture was cooled to room temperature, with 30 mL of H_2O added, and then extracted with ethyl acetate(15 mL×3). The organic phases were combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography(petroleum ether/ethyl acetate) to afford the angular compound.

5-Hydroxy-2,10-dimethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4a**): a light yellow solid; yield 38%; m. p. 219—221 °C; ^{1}H NMR(300 MHz, DMSO-d_6), *δ*: 12.46(s, 1H), 6.45(s, 1H), 6.10(s, 1H), 4.78—4.88(m, 1H), 2.75—3.02(m, 2H), 2.52(s, 3H), 1.51(d, *J*=5.7 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{14}H_{13}O_5$: 261.0763, found 261.0754.

5-Hydroxy-2-methyl-10-ethyl-2,3-dihydro-4*H*,8*H*-pyrano-[2,3-*f*]chromene-4,8-dione(**4b**): a light yellow solid; yield 36%; m. p. 136—138 °C; ^{1}H NMR(300 MHz, acetone-d_6), *δ*: 12.54 (s, 1H), 6.35(s, 1H), 6.00(s, 1H), 4.87—4.99(m, 1H), 2.85—3.06(m, 4H), 1.64(d, *J*=6.0 Hz, 3H), 1.27(t, *J*=7.2 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{15}H_{15}O_5$: 275.0919, found 275.0913.

5-Hydroxy-2,9,10-trimethyl-2,3-dihydro-4*H*,8*H*-pyrano-[2,3-*f*]chromene-4,8-dione(**4c**): a light yellow solid; yield 24%; m. p. 187—189 °C; ^{1}H NMR(300 MHz, DMSO-d_6), *δ*: 12.44(s, 1H), 6.43(s, 1H), 4.79—4.90(m, 1H), 2.74—3.02(m, 2H), 2.52(s, 3H), 2.05(s, 3H), 1.52(d, *J*=6.0 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{15}H_{15}O_5$: 275.0919, found 275.0911.

5-Hydroxy-2-methyl-2,3,9,10,11-pentahydro-2*H*-cyclopenta-[*c*]pyrano[2,3-*f*]chromene-4,8-dione(**4d**): a light yellow solid; yield 45%; m. p. 220—222 °C; ^{1}H NMR(300 MHz, DMSO-d_6), *δ*: 12.26(s, 1H), 6.48(s, 1H), 4.78—4.87(m, 1H), 3.16—3.27(m, 2H), 2.74—3.01(m, 2H), 2.66(t, *J*=7.2 Hz, 2H), 2.05(m, 2H), 1.50(d, *J*=6.0 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{16}H_{15}O_5$: 287.0919, found 287.0915.

5-Hydroxy-2-methyl-2,3,9,10,11,12-hexahydro-4*H*,8*H*-cyclohexa[*c*]pyrano[2,3-*f*]chromene-4,8-dione(**4e**): a light yellow solid; yield 19%; m. p. 188—190 °C; ^{1}H NMR(300 MHz, DMSO-d_6), *δ*: 12.42(s, 1H), 6.43(s, 1H), 4.77—4.86(m, 1H), 3.00—3.04(m, 2H), 2.74—2.96(m, 2H), 2.39(t, *J*=7.2 Hz, 2H), 1.66—1.71(m, 4H), 1.50(d, *J*=6.0 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{17}H_{17}O_5$: 301.1076, found 301.1071.

5-Hydroxy-2,10-dimethyl-9-chloro-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4f**): a light yellow solid; yield 42%; m. p. 201—203 °C; ^{1}H NMR(300 MHz, DMSO-d_6), *δ*: 12.51(s, 1H), 6.52(s, 1H), 4.81—4.91(m, 1H), 2.77—3.04(m, 2H), 2.71(s, 3H), 1.53(d, *J*=6.3 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{14}H_{12}ClO_5$: 295.0373, found 295.0365.

5-Hydroxy-2,10-dimethyl-9-benzyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4g**): a light yellow solid; yield 28%; m. p. 180—182 °C; ^{1}H NMR(300 MHz, DMSO-d_6), *δ*: 12.49(s, 1H), 7.16—7.30(m, 5H), 6.47(s, 1H), 4.77—4.86(m, 1H), 3.94(s, 2H), 2.73—3.01(m, 2H), 2.54(s, 3H), 1.49(d, *J*=6.3 Hz, 3H); HRMS(ESI): *m/z* $[M+H]^+$ calcd. for $C_{21}H_{19}O_5$: 351.1232, found 351.1228.

5-Hydroxy-2-methyl-10-chloromethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4**h): a white solid; yield 36%; m. p. 160—162 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.47(s, 1H), 6.53(s, 1H), 6.46(s, 1H), 4.92—5.09(m, 2H), 4.82—4.90 (m, 1H), 2.77—3.03(m, 2H), 1.54(d, J=6.3 Hz, 3H); HRMS (ESI): m/z[M+H]$^+$ calcd. for $C_{14}H_{12}ClO_5$: 295.0373, found 295.0368.

5-Hydroxy-2,10-dimethyl-9-phenyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4**i): a light yellow solid; yield 39%; m. p. 205—207 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.53(s, 1H), 7.36—7.47(m, 3H), 7.25—7.28(m, 2H), 6.51(s, 1H), 4.79—4.91(m, 1H), 2.76—3.03(m, 2H), 2.35(s, 3H), 1.49(d, J=6.3 Hz, 3H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{20}H_{17}O_5$: 337.1076, found 337.1070.

5-Hydroxy-2-methyl-10-phenyl-2,3-dihydro-4*H*,8*H*-pyrano-[2,3-*f*]chromene-4,8-dione(**4**j): a light yellow solid; yield 37%; m. p. 147—149 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.46(s, 1H), 7.35—7.45(m, 5H), 6.56(s, 1H), 6.01(s, 1H), 4.40—4.48(m, 1H), 2.54—2.83(m, 2H), 0.75(d, J=6.3 Hz, 3H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{19}H_{15}O_5$: 323.0919, found 323.0912.

5-Hydroxy-2-methyl-10-(4-nitrophenyl)-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4**k): a light yellow solid; yield 29%; m. p. 240—242 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.44(s, 1H), 8.30(d, J=9.0 Hz, 2H), 7.67(d, J=8.7 Hz, 2H), 6.60(s, 1H), 6.14(s, 1H), 4.43—4.50(m, 1H), 2.57—2.83(m, 2H), 0.73(d, J=6.3 Hz, 3H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{19}H_{14}NO_7$: 368.0770, found 368.0764.

5-Hydroxy-2-methyl-10-trifluormethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**4**l): a light yellow solid; yield 65%; m. p. 130—132 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.54(s, 1H), 6.82(s, 1H), 6.61(s, 1H), 4.76—4.91(m, 1H), 2.78—3.07(m, 2H), 1.47(d, J=6.3 Hz, 3H); HRMS(ESI): m/z [M+H]$^+$ calcd. for $C_{14}H_{10}F_3O_5$: 315.0480, found 315.0476.

5-Hydroxy-2,2-dimethyl-10-propyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**a): a light yellow solid; yield 23%; m. p. 108—110 °C; ^{1}H NMR(300 MHz, acetone-d_6), δ: 12.49(s, 1H), 6.32(s, 1H), 5.98(s, 1H), 2.99(s, 2H), 2.93(t, J=7.5 Hz, 2H), 1.66—1.76(m, 2H), 1.63(s, 6H), 1.06(t, J=7.2 Hz, 3H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{17}H_{19}O_5$: 303.1232, found 303.1227.

5-Hydroxy-2,2,10-trimethyl-2,3-dihydro-4*H*,8*H*-pyrano-[2,3-*f*]chromene-4,8-dione(**6**b): a light yellow solid; yield 25%; m. p. 183—185 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.37(s, 1H), 6.43(s, 1H), 6.09(s, 1H), 2.98(s, 2H), 2.52(s, 3H), 1.50(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{15}H_{15}O_5$: 275.0919, found 275.0913.

5-Hydroxy-2,2-dimethyl-10-ethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**c): a white solid; yield 33%; m. p. 179—181 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.40(s, 1H), 6.45(s, 1H), 6.07(s, 1H), 3.00(s, 2H), 2.88—2.95(m, 2H), 1.51(s, 6H), 1.21(t, J=7.2 Hz, 3H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{16}H_{17}O_5$: 289.1076, found 289.1071.

5-Hydroxy-2,2-dimethyl-2,3,9,10,11-pentahydro-2*H*-cyclo-penta[*c*]pyrano[2,3-*f*]chromene-4,8-dione(**6**d): a white solid; yield 37%; m. p. 236—238 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.18(s, 1H), 6.46(s, 1H), 3.25(t, J=7.2 Hz, 2H), 2.97(s, 2H), 2.66(t, J=7.2 Hz, 2H), 2.00—2.10(m, 2H), 1.48(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{17}H_{17}O_5$: 301.1076, found 301.1069.

5-Hydroxy-2,2-dimethyl-2,3,9,10,11,12-hexahydro-4*H*,8*H*-cyclohexa[*c*]pyrano[2,3-*f*]chromene-4,8-dione(**6**e): a light yellow solid; yield 21%; m. p. 225—227 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.34(s, 1H), 6.41(s, 1H), 3.00(t, J=5.1 Hz, 2H), 2.96(s, 2H), 2.38(t, J=5.7 Hz, 2H), 1.67—1.69(m, 4H), 1.49(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{18}H_{19}O_5$: 315.1232, found 315.1227.

5-Hydroxy-2,2,10-trimethyl-9-chloro-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**f): a light yellow solid; yield 42%; m. p. 227—229 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.42(s, 1H), 6.50(s, 1H), 3.00(s, 2H), 2.71(s, 3H), 1.52(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{15}H_{14}ClO_5$: 309.0530, found 309.0525.

5-Hydroxy-2,2,10-trimethyl-9-benzyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**g): a white solid; yield 31%; m. p. 191—193 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.40(s, 1H), 7.16—7.30(m, 5H), 6.44(s, 1H), 3.93(s, 2H), 2.97(s, 2H), 2.54(s, 3H), 1.49(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{22}H_{21}O_5$: 365.1389, found 365.1383.

5-Hydroxy-2,2-dimethyl-10-chloromethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**h): a white solid; yield 38%; m. p. 185—187 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.38(s, 1H), 6.51(s, 1H), 6.46(s, 1H), 4.99(s, 2H), 3.00(s, 2H), 1.54(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{15}H_{14}ClO_5$: 309.0530, found 309.0522.

5-Hydroxy-2,2-dimethyl-10-phenyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**i): a light yellow solid; yield 29%; m. p. 231—233 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.35(s, 1H), 7.44—7.46(m, 3H), 7.30—7.33(m, 2H), 6.54(s, 1H), 5.98(s, 1H), 2.78(s, 2H), 0.96(s, 6H); HRMS(ESI): m/z [M+H]$^+$ calcd. for $C_{20}H_{17}O_5$: 337.1076, found 337.1069.

5-Hydroxy-2,2-dimethyl-10-trifluormethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**6**j): a white solid; yield 22%; m. p. 178—180 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.45(s, 1H), 6.80(s, 1H), 6.59(s, 1H), 3.02(s, 2H), 1.46(s, 6H); HRMS(ESI): m/z[M+H]$^+$ calcd. for $C_{15}H_{12}F_3O_5$: 329.0637, found 329.0631.

2.3 Procedure for the Preparation of Target Compound 7

The intermediate **5**(2 mmol)and cinnamic acid(2.2 mmol) were dissolved in 5 mL of CF_3SO_3H. The corresponding mixture was then stirred at room temperature for 2 h. After completion of the reaction, 20 mL of H_2O was added to the mixture, and then the mixture was extracted with ethyl acetate (10 mL×3). The organic phases were combined, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/ethyl acetate) to give 5-hydroxy-2,2-dimethyl-10-phenyl-2,3,9,10-tetrahydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**7**) as a white solid; yield 41%; m. p. 128—130 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 12.64(s, 1H), 7.39—7.52(m, 5H), 6.09(s, 1H), 5.65—5.70(m, 1H), 3.10—3.19(m, 1H),

2.85—2.99(m, 2H), 2.68—2.74(m, 1H), 1.46(s, 3H), 1.43(s, 3H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{20}H_{19}O_5$: 339.1232, found 339.1226.

2.4 Synthetic Procedure of the Target Compounds 11a—11f

*2.4.1 Preparation of 5-Methoxy-2,2-dimethyl-4-oxo-3,4-dihydro-2H-chromen-7-yl 4-Methylbenzenesulfonate(**9**)*

The intermediate **5**(5 mmol), potassium carbonate(35 mmol) and *p*-TsCl(5 mmol) were dissolved in 50 mL of acetone. The corresponding yellow mixture was refluxed for 5 h under ambient atmosphere to give the crude product **8**. When the mixture was cooled to room temperature, potassium carbonate (15 mmol) and dimethyl sulfate(5.5 mmol) were added, and then the mixture was refluxed for 20 h. After completion of the reaction, the mixture was cooled to room temperature, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography(petroleum ether/ethyl acetate) to give compound **9** as a yellow oil; yield 64%(in two steps); ^{1}H NMR(300 MHz, DMSO-d_6), δ: 7.83(d, J=8.4 Hz, 2H), 7.50(d, J=8.1 Hz, 2H), 6.23(s, 2H), 3.64(s, 3H), 2.66(s, 2H), 2.43(s, 3H), 1.33(s, 6H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{19}H_{21}SO_6$: 377.1059, found 377.1051.

*2.4.2 Preparation of 7-Hydroxy-5-methoxy-2,2-dimethyl-2,3-dihydrochromen-4-one(**10**)*

The intermediate **9**(3 mmol) prepared above was dissolved in 25 mL of THF. Then, tetrabutylammonium fluoride trihydrate(TBAF $3H_2O$) was added to the solution. The corresponding mixture was stirred at room temperature for 3 h. After the completion of the reaction, the mixture was concentrated under reduced pressure. To the residue was added 50 mL of ethyl acetate. The solution was washed with water(25 mL×3) and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography(petroleum ether/ethyl acetate) to afford compound **10** as a light-yellow solid; yield 77%; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 10.42(s, 1H), 5.99(d, J=2.1 Hz, 1H), 7.50(d, J=2.1 Hz, 1H), 3.71(s, 3H), 2.53(s, 2H), 1.32(s, 6H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{12}H_{15}O_4$: 223.0970, found 223.0965.

*2.4.3 Preparation of Target Compounds **11a—11f***

Compounds **11**a—**11**f were prepared by the same method as those of compounds **4**a—**4**l.

5-Methoxy-2,2-dimethyl-10-propyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**a): a light yellow solid; yield 53%; m. p. 146—148 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 6.64(s, 1H), 5.97(s, 1H), 3.87(s, 3H), 2.70(s, 2H), 2.54(t, J=7.5 Hz, 2H), 1.63—1.70(m, 2H), 1.40(s, 6H), 0.95(t, J=6.9 Hz, 3H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{18}H_{21}O_5$: 317.1389, found 317.1382.

5-Methoxy-2,2,10-trimethyl-2,3-dihydro-4*H*,8*H*-pyrano-[2,3-*f*]chromene-4,8-dione(**11**b): a white solid; yield 61%; m. p. 182—184 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 6.63(s, 1H), 5.99(s, 1H), 3.87(s, 3H), 2.70(s, 2H), 2.27(s, 3H), 1.40(s, 6H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{16}H_{17}O_5$: 289.1076, found 289.1071.

5-Methoxy-2,2-dimethyl-10-phenyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**c): a white solid; yield 72%; m. p. 193—195 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 8.06—8.09(m, 2H), 7.55—7.59(m, 3H), 6.93(s, 1H), 6.80(s, 1H), 3.93(s, 3H), 2.74(s, 2H), 1.43(s, 6H); HRMS(ESI): *m/z* [M+H]$^+$ calcd. for $C_{21}H_{19}O_5$: 351.1232, found 351.1225.

5-Methoxy-2,2,9-trimethyl-10-trifluormethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**d): a white solid; yield 64%; m. p. 170—172 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 6.72(s, 1H), 3.91(s, 3H), 2.74(s, 2H), 2.03(s, 3H), 1.43(s, 6H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{17}H_{15}F_3O_5$: 356.0872, found 356.0868.

5-Methoxy-2,2-dimethyl-9-chloro-10-trifluormethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**e): a light yellow solid; yield 43%; m. p. 165—167 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 6.84(s, 1H), 3.93(s, 3H), 2.77(s, 2H), 1.43(s, 6H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{16}H_{13}ClF_3O_5$: 377.0404, found 377.0397.

5-Methoxy-2,2-dimethyl-10-pentafluorethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**f): a white solid; yield 63%; m. p. 108—110 °C; ^{1}H NMR(300 MHz, DMSO-d_6), δ: 6.82(s, 1H), 6.77(s, 1H), 3.92(s, 3H), 2.75(s, 2H), 1.42(s, 6H); HRMS(ESI): *m/z*[M+H]$^+$ calcd. for $C_{17}H_{14}F_5O_5$: 393.0761, found 393.0754.

2.5 Pharmacological Evaluation

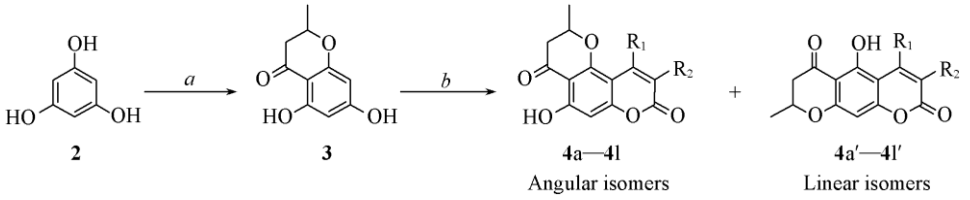
All the twenty-nine synthesized compounds were evaluated for their anticancer activities against SHG-44, H1299, MCF7 and HCT-116 *in vitro*. Teniposide[21], a semi-synthetic analogue of podophyllotoxin, was employed as the positive control. For a typical pharmacological screening, all the cells were cultured in a DMEM medium, which was supplemented with 10% fetal bovine serum(FBS), and then plated in 96-well plates in a 180 μL of culture medium at 1×10^4 cells per well. After incubated at 37 °C, 5% CO_2 and 100% relative humidity for 24 h, the cells were treated with different concentrations of the tested compounds. The plates were incubated for an additional 48 h. Subsequently, the antiproliferative activities of individual compound against the four human cancer cell lines mentioned above were evaluated *via* the methyl thiazolyl tetrazolium(MTT) assay. The absorbance of each well was measured at 492 nm on an RT6100 Microplate Reader. The data expressed as IC_{50} values were the mean of three independent experiments.

3 Results and Discussion

3.1 Synthesis

The synthesis of pyrano[2,3-*f*]chromene-4,8-dione derivatives(**4**a—**4**l) was illustrated in Scheme 1. Initially, the reaction of commercially available phloroglucinol(**2**) with dry crotonic acid in the presence of CH_3SO_3H and P_2O_5 afforded 5,7-dihydroxy-2-methylchroman-4-one(**3**)[19], which was then cyclized with various *β*-keto esters in trifluoroacetic acid(TFA)

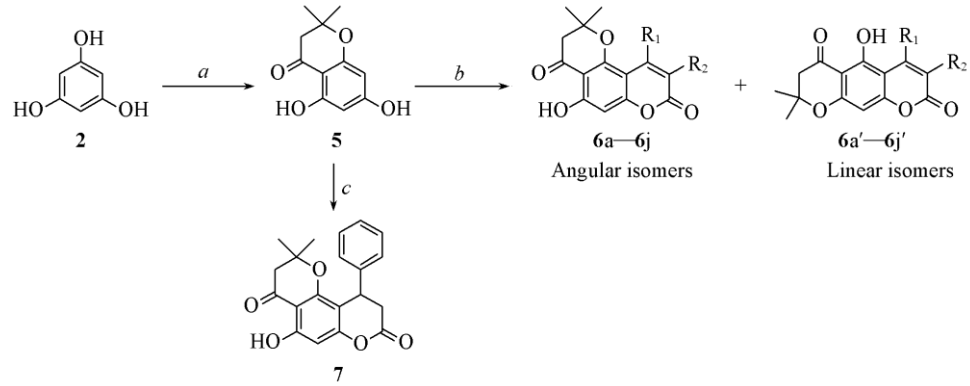
using *p*-toluenesulfonic acid(*p*-TsOH) as the catalyst to deliver the desired angular isomers(**4a—4l**) with the unavoidable linear byproducts(**4a′—4l′**). It should be mentioned that a pair of regioisomers(such as **4**a and **4**a′) can be structurally identified by the chemical shifts of the hydroxyl groups[18], with those of **4**a and **4**a′ being 12.46 and 13.74, respectively. Similarly, Friedel-Crafts acylation of phloroglucinol(**2**) with 3,3-dimethylacrylic acid in the presence of boron trifluoride diethyl etherate (BF_3 Et_2O) gave 5,7-dihydroxy-2,2-dimethyl-4-chromanone (**5**)[20], which can be readily converted to the target compounds **6a—6j** *via* Pechmann reactions, as exemplified by Scheme 2. Additionally, dihydrocoumarin **7** was successfully synthesized by treatment of compound **5** with cinnamic acid in CF_3SO_3H at room temperature for 2 h.



4a(a′): R_1=CH_3, R_2=H; **4**b(b′): R_1=C_2H_5, R_2=H; **4**c(c′): R_1=CH_3, R_2=CH_3; **4**d(d′): R_1=R_2=propylidene; **4**e(e′): R_1=R_2=butylidene; **4**f(f′): R_1=CH_3, R_2=Cl; **4**g(g′): R_1=CH_3, R_2=benzyl; **4**h(h′): R_1=$ClCH_2$, R_2=H; **4**i(i′): R_1=CH_3, R_2=C_6H_5; **4**j(j′): R_1=C_6H_5, R_2=H; **4**k(k′): R_1=4-NO_2-C_6H_4, R_2=H; **4**l(l′): R_1=CF_3, R_2=H

Scheme 1 Syntheses of target compounds 4a—4l

Reagents and conditions: *a*. dry crotonic acid, CH_3SO_3H, P_2O_5, 70 ºC, 45 min; *b*. *β*-keto ester, *p*-TsOH, TFA, reflux, 2 h.

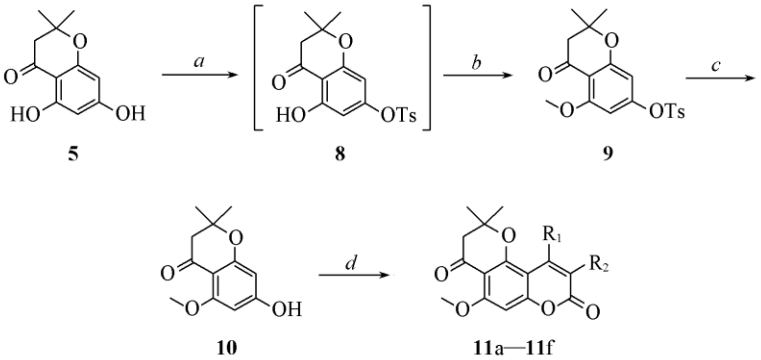


6a(a′): R_1=*n*-C_3H_7, R_2=H; **6**b(b′): R_1=CH_3, R_2=H; **6**c(c′): R_1=C_2H_5, R_2=H; **6**d(d′): R_1=R_2=propylidene; **6**e(e′): R_1=R_2=butylidene; **6**f(f′): R_1=CH_3, R_2=Cl; **6**g(g′): R_1=CH_3, R_2=benzyl; **6**h(h′): R_1=$ClCH_2$, R_2=H; **6**i(i′): R_1=C_6H_5, R_2=H; **6**j(j′): R_1=CF_3, R_2=H

Scheme 2 Syntheses of target compounds 6a—6j and 7

Reagents and conditions: *a*. 3,3-dimethylacrylic acid, BF_3 Et_2O, 70 ºC, 2.5 h; *b*. *β*-keto ester, *p*-TsOH, TFA, reflux, 2 h; *c*. cinnamic acid, CF_3SO_3H, r. t., 2 h.

A regioselective synthetic route was explored for the synthesis of the target compounds **11**a—**11**f, as depicted in Scheme 3. Selective protection of the hydroxyl group of compound **5** with subsequent methylation in the presence of anhydrous potassium carbonate in refluxing acetone produced the intermediate **9** in one pot, which underwent deprotection by tetrabutylammonium fluoride trihydrate(TBAF $3H_2O$) to produce the mono-methylated product **10**. Finally, regioselective cyclization of compound **10** with a series of *β*-keto esters offered the target compounds **11**a—**11**f. Furthermore, the structures of these compounds were confirmed by nuclear Overhauser effect(NOE). As presented in Fig.2, ^{1}H NMR conformational analysis of the compound **11**a in DMSO-d_6 had revealed a strong enhancement between the protons of methoxy group and the proton of the benzene ring.



11a: R_1=*n*-C_3H_7, R_2=H; **11**b: R_1=CH_3, R_2=H; **11**c: R_1=C_6H_5, R_2=H; **11**d: R_1=CF_3, R_2= CH_3; **11**e: R_1=CF_3, R_2= Cl; **11**f: R_1= CF_3CF_2, R_2=H

Scheme 3 Syntheses of target compounds 11a—11f

Reagents and conditions: *a*. *p*-TsCl, K_2CO_3, acetone, reflux, 5 h; *b*. $(CH_3)_2SO_4$, K_2CO_3, acetone, reflux, 20 h; *c*. TBAF $3H_2O$, THF, r. t., 3 h; *d*. *β*-keto ester, *p*-TsOH, TFA, reflux, 2 h.

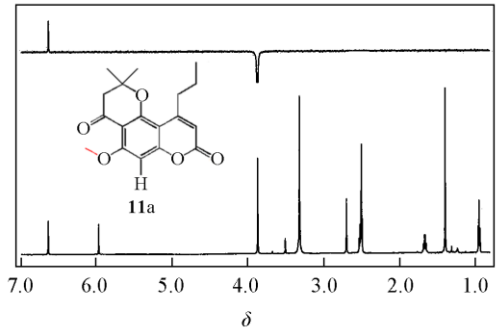


Fig.2 NOE spectrum of compound 11a

3.2 Anticancer Activity

Twenty-nine newly synthesized pyrano[2,3-*f*]chromene-4,8-dione derivatives were screened for their anticancer activities against SHG-44, H1299, MCF7 and HCT-116 cell lines with teniposide as a reference, and the results were summarized in Table 1. It reveals that our synthesized compounds exerted good sensitivity profiles against SHG-44 and H1299 cell lines. At the same time, compounds **4**e, **6**e and compound **7** were totally inactive against MCF7 and HCT-116 cell lines. Several target compounds(**4**h, **6**h, **11**d, **11**e and **11**f), which had more potency than that of the hit **1**, exhibited remarkable anticancer properties at micromolar level against the four cancer cell lines. Other compounds(**4**f, **4**l, **6**a, **6**f, **6**j, and **11**a) also gave their anticancer activities to some extent. In particular, 5-methoxy-2,2-dimethyl-9-chloro-10-trifluormethyl-2,3-dihydro-4*H*,8*H*-pyrano[2,3-*f*]chromene-4,8-dione(**11**e) presented the most potent antiproliferative activities against the four human cancer cell lines with IC_{50} values of 6.68, 7.90, 5.16 and 4.82 μmol/L, respectively. The result encourages us that compound **11**e may have the potential as a lead compound.

Table 1 Anticancer activities of the target compounds against four human cancer cell lines[a]

Compd.	IC_{50}/(μmol L^{-1})[b]			
	SHG-44	H1299	MCF7	HCT-116
Teniposide	0.68	0.95	0.27	0.49
1	10.48	14.45	9.73	12.17
4a	16.51	26.70	11.49	18. 35
4b	11.49	13.72	10.16	20.06
4c	20.74	18.56	27.93	45.28
4d	35.75	26.03	54.46	>100
4e	30.91	34.13	>100	>100
4f	9.47	11.63	10.46	9.02
4g	28.02	22.16	36.20	58.37
4h	7.58	9.93	9.76	8.76
4i	23.04	46.48	33.65	67.46
4j	30.94	18.57	36.40	45.83
4k	27.09	16.58	33.26	39.57
4l	9.19	8.49	10.78	7.49
6a	12.67	12.04	9.16	13.64
6b	14.90	23.59	14.32	15.82
6c	12.06	11.73	10.03	17.29
6d	30.05	23.57	46.82	>100
6e	27.58	25.30	>100	>100
6f	7.02	10.39	10.96	9.15
6g	23.84	19.38	29.05	48.52
6h	7.43	9.58	9.54	8.32
6i	27.49	16.30	37.47	51.03
6j	9.01	8.32	10.94	7.80
7	58.36	27.81	>100	>100
11a	10.39	9.82	8.35	11.37
11b	12.85	17.40	11.39	14.08
11c	20.29	13.38	28.63	45.82
11d	9.20	8.24	9.91	7.36
11e	**6.68**	**7.90**	**5.16**	**4.82**
11f	8.26	9.47	7.62	10.85

a. The antiproliferative activities of individual compound against four human cancer cell lines were evaluated through the MTT assay; *b*. the data were the mean of three independent experiments.

Next, the preliminary structure–activity relationships (SARs) around the pyrano[2,3-*f*]chromene-4,8-dione core were explored. It can be concluded that the 2,2-dimethyl-substituted homologues(**6**a—**6**c) do not significantly improve the anticancer activities compared with the hit **1**. By contrast, the products formed by blocking out the intramolecular hydrogen bond at 4- and 5-position of the core have a positive effect on the anticancer activities, as demonstrated by compounds **11**a and **11**d—**11**f. Compounds **4**h and **6**h bearing a chloromethyl group at 9-position of the core may act as bioalkylating agents, possessing apparently distinguishing anticancer activities against the four cancer cell lines. Most importantly, the introduction of strong electron-withdrawing groups at 9- or/and 10-position of the pyrano[2,3-*f*]chromene-4,8-dione core(**4**l, **6**j and **11**d—**11**f) is more favorable to enhancing the antiproliferative activities compared with the compounds bearing electron-donating groups(**4**a—**4**e, **6**b—**6**e and **11**b). It should be mentioned that the introduction of aromatic substituent at 9-position of the pyrano[2,3-*f*]chromene-4,8-dione core or reduction of the carbon-carbon double bond of the coumarin ring results in several compounds with poor anticancer activities(**4**j, **4**k, **6**i, **7** and **11**c).

4 Conclusions

In our work, a series of compounds was designed, synthesized and evaluated for their anticancer activities against SHG-44, H1299, MCF7 and HCT-116 cell lines *in vitro*. Some compounds(**4**h, **6**h, **11**d, **11**e and **11**f) displayed outstanding antiproliferative activities at micromolar level. It should be noted that compound **11**e presented the strongest cytocoxicity on the four sensitive cell lines with the IC_{50} values of 6.68, 7.90, 5.16 and 4.82 μmol/L, respectively. The preliminary SARs showed that blocking out the intramolecular hydrogen bond as well as introduction of strong electron-withdrawing groups at 9- or/and 10-position of the pyrano[2,3-*f*]chromene-4,8-dione core was crucial to preserve the best anticancer activities. In summary, compound **11**e encompassing the pyrano[2,3-*f*]-chromene-4,8-dione core is a promising lead compound, which can be further structurally modified in the next study to afford more effective and drug-like anticancer agents with *in vivo* bioactivities.

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