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ORIGINAL RESEARCH

Design, synthesis and biological evaluation of novel furoxan-based coumarin derivatives as antitumor agents

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Abstract

In order to find new anticancer drugs, a series of novel furoxan-based coumarin derivatives (**10a–k**) were synthesized and evaluated for their antiproliferative activities in vitro. All compounds displayed more potent inhibition on human cervical cancer HeLa cell proliferation than coumarin-3-carboxylic acid, and compounds **10b**, **10c**, **10f**, **10h**, and **10i** with IC_{50} values ranging from 0.88 to 5.95 μM were even stronger than doxorubicin ($IC_{50} = 10.21$ μM). The further study showed that compound **10i** exerted the highest antiproliferative activity ($IC_{50} = 0.60$ μM) against human breast cancer MCF-7 cells, and compound **10f** had broader spectrum antiproliferative activity against five cancer cells with IC_{50} values in the low micromolar range of 1.86–9.85 μM. More interestingly, compound **10f** had little effect on normal intestinal epithelial CCD841 cells. Our findings suggest that these novel furoxan-based coumarin derivatives may provide a new framework for the discovery of novel antitumor agents for the intervention of human carcinoma cells.

Keywords Coumarin · Furoxan · Antitumor activity · Synthesis

Introduction

Malignant tumor is one of the leading causes of mortality worldwide. Drug resistance and low selectivity is a common issue to cancer. Hence, it is important to searching for novel anticancer drugs to address these problems.

It is well-known that natural products play a critical role in the drug discovery of virtually all therapeutic areas, especially in the lead identification and drug discovery in oncology, and nearly half of the new drugs introduced into the market are natural products or their derivatives (Newman et al. 2016). Structural modification of a natural molecule is common strategy to improve pharmacological efficacy. Coumarin (Fig. 1) is an important classes of natural products, which isolated from *Umbelliferae*, *Asteraceae*, *Rutaceae*, *Leguminoase sp*, etc (Wang et al. 2017; Cui et al. 2007; Vilar et al. 2006), showed broad spectrum biological activity such as antidepressant, antimicrobial, antioxidant, antitumor, anti-HIV, and anticoagulant (Chen et al. 2007; Adams et al. 2006; Piao et al. 2004; Appendino et al. 2004; Cardellina et al. 1995; Zhou et al. 2009). Several of natural, semisynthetic, or synthetic lead molecules bearing coumarin scaffold have been discovered in various phase of drug development in the recent past. Recently, many coumarin derivatives were widely reported to possess antitumor activity. For instance, psoralen (Fig. 1), a compound extracted from the seeds of psoralea corylifolia, displayed antiproliferative and cytotoxic activity to breast cancer cell EMT6 (Wu et al. 1998) and lung cancer cell A549 (Hsieh et al. 2014). Compound A (Fig. 1) (Kamath et al. 2015), indole-3-carboxylic acid substituent at the C3-position of coumarin, had significant antiproliferative activity on human breast cancer MCF-7 ($IC_{50} = 5.5$ μM) and low inhibition activity on Africa green monkey kidney cell Vero ($IC_{50} > 100$ μM), displayed superior selectivity. Compound B (Fig. 1), 3,4-2H-pyrazole-substituent at the C3-position of coumarin, showed strong inhibition activity on human gastric cancer cell SGC-7901 ($IC_{50} = 2.69$ μM)

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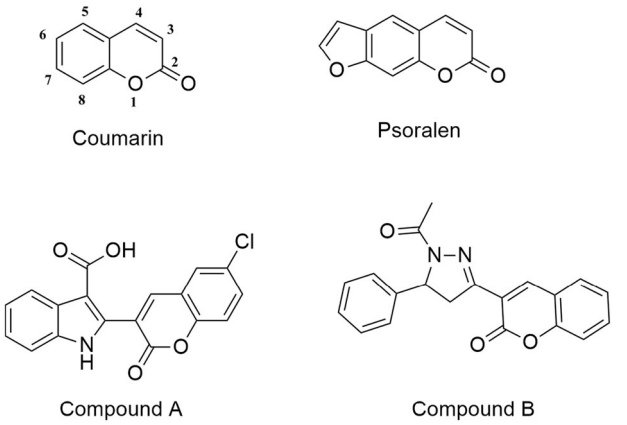


Fig. 1 Chemical structure of coumarin and coumarin related compound

(Wang et al. 2013). Therefore, coumarins were considered as a privileged structure for designing novel agents.

Nitric oxide is one of the most important signaling molecular, which plays important role in physiological activity of human body. In recent years, it has been found that high concentration of NO displayed cytotoxic effect and could induce apoptosis of tumor cells, hold back tumor cells proliferation, and assist macrophage to devour tumor cells (Wink et al. 1998; Hofseth et al. 2003; Huang et al. 2017). Furoxans is an important class of NO-donors, which can produce high concentration of NO and display strong antitumor activity (Cerecetto et al. 2005; Boiani et al. 2001). Compound C, a furoxans-based nitric oxide releasing coumarin derivative (Liu et al. 2014), displayed strong antiproliferation activities on A549 ($IC_{50} = 0.024\ \mu M$), HeLa ($IC_{50} = 0.11\ \mu M$), A2780 ($IC_{50} = 0.014\ \mu M$), and HUVEC ($IC_{50} = 0.034\ \mu M$) cell lines and three drug resistant tumor cell lines MDA-MB-231/Gem ($IC_{50} = 0.14\ \mu M$), A2780/CDDP ($IC_{50} = 0.062\ \mu M$), and SKOV3 ($IC_{50} = 0.083\ \mu M$) cell lines in vitro, superior than cisplatin. More importantly, compound C exhibited lower cytotoxicity on normal cell T29 ($IC_{50} > 2.0\ \mu M$), showing good selectivity against tumor cells (Fig. 2).

Our research group once synthesized a series of NO-donating N-alkyl matrinol and NO-donating N-alkyl matrinic acid derivatives (Wu et al. 2015; He et al. 2010, 2015). Their antitumor activity in vitro showed that some of compounds exhibited high anti-proliferative activity on human hepatocellular carcinoma cells Bel-7402 (IC_{50} values ranging from 2.55 to 3.26 μM) and SMMC-7721 (IC_{50} values ranging from 5.26 to 5.79 μM) (Fig. 3). With the aim to develop a new lead possessing efficient pharmacological activities, using the molecular hybridization approach, we designed new analogs of coumarin, containing the furoxan NO donor moiety. It was expected that the novel furoxan-based coumarin derivatives would improve the potency and spectrum. All chemical structures were

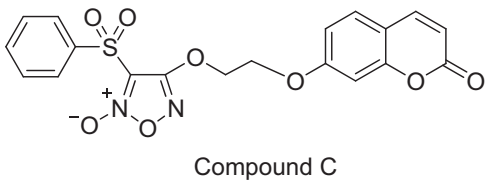


Fig. 2 Chemical structure of hybrid of 7-hydrocoumarin and furoxan

established by infrared (IR), high resolution mass spectrometry (HRMS), and ^{1}H and ^{13}C nuclear magnetic resonance (NMR), and these target compounds were evaluated for their inhibitory activity against some cancer cells proliferation. Herein, we report the synthesis and biological evaluation of these derivatives.

Materials and methods

General

Melting points were measured using a WRS-1B apparatus without any correction. ^{1}H and ^{13}C NMR spectra were recorded on 400 MHz Bruker Avance DPX spectrometers and referenced with tetramethylsilane as an internal standard. All NMR spectra were recorded in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 at room temperature. IR spectra were collected on Nicolet Avatar 6700 spectrometer using KBr film. ESI mass spectra were acquired using a Thermo Fisher LTQ Orbitrap XL Liquid chromatography-mass spectrometry instrument.

Ethyl 2-oxo-2*H*-chromene-3-carboxylate (2)

The mixture of Salicylaldehyde (4.2 mL, 40 mmol), diethyl malonate (8.5 mL, 56 mmol), anhydrous EtOH (20 mL), piperidine (0.5 mL, 5 mmol), and AcOH (0.1 mL) was heated under 80 °C. After 2 h, water (30 mL) was added to the reaction mixture, and the resulting suspension was placed in 0 °C. The crystalline was collected by filtration, washed with 50% of EtOH, and dried. Finally, the crude product was purified by recrystallized from 25% EtOH to give white solid 2 (7.5 g, 85.4%), m.p. 92.0–93.1 °C (Gao et al. 2014).

2-Oxo-2*H*-chromene-3-carboxylic acid (3)

The mixture of compound 2 (8.0 g, 28 mmol), NaOH (6.0 g, 150 mmol), 95% EtOH (30 mL) and water (20 mL) was refluxed and stirred for 20 min. After cooling, the mixture was added to a solution of 20 mL concentrated hydrochloric acid in 100 mL water under stirring. Then the crude product was reduced pressure suction filter and washed with ice water. Finally, the crude product was purified by

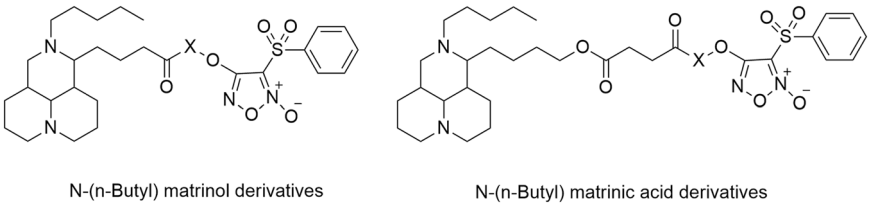


Fig. 3 Chemical structure of NO-donation N-(*n*-Butyl) matrinic acid and N-(*n*-Butyl) matrinol derivatives

recrystallized from 50 % EtOH to give white crystal 3 (5.0 g, 95.6%), m.p. 193.2–194.7 °C (Gao et al. 2014).

2-Oxo-2*H*-chromene-3-carbonyl chloride (4)

Compound 3 (4 g, 19 mmol) and pyridine (0.3 mL) was dissolved in sulfoxide chloride (15 mL) and refluxed 6 h. After cooling, the mixture was removed redundant sulfoxide chloride by reduced pressure to give crude product. Finally, the crude product was purified by recrystallized from petroleum ether to give faint yellow solid 4 (3.3 g, 81.2%), m.p. 143.6–145.8 °C (Mo et al. 2006).

General procedure for the synthesis of compound 10a–k

Compound **9a–k** (1.0 mmol) and triethylamine (TEA) (0.83 mL, 6.0 mmol) was dissolved in dichloromethane (DCM) (10 mL), then added drop wise the solution of **4** (312 mg, 1.5 mmol) in CH_2Cl_2 at room temperature. The mixture was stirred at room temperature for 2 h, then the reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography to give white solid **10a–k**.

4-(2-((2-oxo-2*H*-chromene-3-carbonyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10a)

White solid; yield: 81.5%; m.p. 165.4–166.6 °C. APCI-HRMS (*m*/*z*): calcd for $C_{20}H_{14}N_2O_9S$ (neutral M + H) 459.0454; found 459.0494; IR (ν_{max}/cm^{-1}): 1764, 1628, 1349, 1167, 1565; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H, =CH), 8.06 (s, 2H, Ph-H), 7.76 (s, 1H, Ph-H), 7.65 (s, 3H, Ph-H), 7.37 (d, J = 4.0 Hz, 2H, Ph-H), 4.63 (s, 2H, O–CH_2), 4.52 (s, 2H, O=CCH_2); ^{13}C NMR (400 MHz, DMSO-d_6) δ 162.68, 159.30, 156.19, 155.07, 149.89, 137.63, 136.40, 135.23, 130.71, 130.31 (2C), 128.75 (2C), 125.39, 118.14, 117.43, 116.69, 111.00, 69.56, 63.01.

4-(3-((2-oxo-2*H*-chromene-3-carbonyl)oxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10b)

White solid; yield: 82.3%; m.p. 119.9–120.2 °C. APCI-HRMS (*m*/*z*): calcd for $C_{21}H_{16}N_2O_9S$ (neutral M + H) 473.0577; found 473.0695; IR (ν_{max}/cm^{-1}) 2917, 2850, 1751, 1709, 1626, 1556, 1447, 1255, 1165; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H, =CH), 8.07 (s, 2H, Ph-H), 7.76 (s, 1H, Ph-H), 7.65 (s, 3H, Ph-H), 7.37 (d, J = 4.5 Hz, 2H, Ph-H), 4.65 (s, 2H, O–CH_2), 4.57 (s, 2H, O=CCH_2), 2.39 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.11, 158.85, 156.57, 155.21, 149.06, 138.00, 135.69, 134.57, 129.73 (3C), 128.48 (2C), 124.95, 117.87, 117.81, 116.74, 110.49, 68.13, 61.80, 27.90.

4-(4-((2-oxo-2H-chromene-3-carbonyl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10c)

White solid; yield: 80.6%; m.p. 134.6–135.9 °C; APCI-HRMS (*m*/*z*): calcd for $C_{22}H_{18}N_2O_9S$ (neutral M + H) 487.0733; found 48 7.0845; IR (ν_{max}/cm^{-1}): 2959, 2850, 1764, 1623, 1559, 1453, 1360, 1248, 1165; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H, =CH), 8.08 (d, J = 0.9 Hz, 2H, Ph-H), 7.77 (s, 1H, Ph-H), 7.66 (s, 4H, Ph-H), 7.37 (d, J = 3.3 Hz 2H, Ph-H), 4.51 (d, J = 24.6 Hz, 4H, O=CCH_2, O–CH_2), 2.18–1.96 (m, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.23, 158.94, 156.59, 155.21, 148.86, 138.05, 135.65, 134.47, 129.67 (3C), 128.51 (2C), 124.91, 118.14, 117.85, 116.75, 110.47, 70.99, 65.09, 25.30, 25.02.

4-((5-((2-oxo-2*H*-chromene-3-carbonyl)oxy)pentyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10d)

White solid, yield: 81.3%, m.p. 119.9–121.1 °C. ESI-HRMS (*m*/*z*): calcd for $C_{23}H_{20}N_2O_9S$ (neutral M + H) 501.0923; found 523.0775 (M + Na); IR (ν_{max}/cm^{-1}): 3061, 2961, 2926, 1754, 1619, 1562, 1449, 1392, 1248, 1212, 1158; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H, =CH), 8.05 (s, 2H, Ph-H), 7.77 (s, 1H, Ph-H), 7.64 (s, 4H, Ph-H), 7.34 (s, 2H, Ph-H), 4.45 (d, J = 16.8 Hz, 4H, O=CCH_2, O–CH_2), 1.96 (d, J = 29.5 Hz, 4H, CH_2), 1.69 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.19, 159.35, 156.46, 154.99, 149.16, 137.66, 136.55, 134.98, 130.73, 130.45 (2C), 128.74 (2C), 125.28, 118.24, 118.16, 116.62, 110.92, 71.77, 66.42, 27.98, 27.89, 22.06.

4-((6-((2-oxo-2*H*-chromene-3-carbonyl)oxy)hexyl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10e)

White solid; 79.5%, m.p. 113.7–114.2 °C. ESI-HRMS (*m*/*z*): calcd for $C_{24}H_{22}N_2O_9S$ (neutral M + H) 515.1080; found

537.0928 (M + Na); IR (ν_{max}/cm^{-1}): 2959, 2933, 2853, 1745, 1697, 1617, 1559, 1450, 1309, 1245, 1162; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H, =CH), 8.06 (d, J = 3.7 Hz, 2H, Ph-H), 7.77 (s, 1H, Ph-H), 7.71–7.60 (m, 4H, Ph-H), 7.36 (d, J = 5.7 Hz, 2H, Ph-H), 4.45 (s, 2H, O–CH_2), 4.40 (s, 2H, O=CCH_2), 1.93 (d, J = 1.3 Hz, 2H, CH_2), 1.86 (d, J = 9.9 Hz 2H, CH_2), 1.57 (s, 4H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.25, 159.04, 156.63, 155.16, 148.61, 138.11, 135.62, 134.37, 129.65, 129.57 (2C), 128.47 (2C), 124.86, 118.34, 117.87, 116.74, 110.48, 71.42, 65.69, 28.44, 28.28, 25.43, 25.25.

4-((4-((2-oxo-2*H*-chromene-3-carbonyl)oxy)but-2-yn-1-yl) oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10f)

White solid; yield: 78.7%; m.p. 135.1–138.1 °C; APCI-HRMS (*m/z*): calcd for $C_{22}H_{14}N_2O_9S$ (neutral M + H) 483.0420; found 483.0532; IR (ν_{max}/cm^{-1}): 2914, 1762, 1609, 1556, 1490, 1372, 1208, 1168, 998 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (s, 1H, =CH), 8.11 (d, J = 1.8 Hz, 2H, Ph-H), 7.79 (s, 1H, Ph-H), 7.68 (dd, J = 3.4, 0.8 Hz, 4H, Ph-H), 7.40 (d, J = 4.3 Hz, 2H, Ph-H), 5.16 (d, J = 1.0 Hz, 2H, O–CH_2), 5.04 (d, J = 1.4 Hz, 2H, O=CCH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.85, 137.81, 137.79, 135.80, 135.76, 134.93, 129.83, 129.76, 129.73, 128.61 (2C), 125.08, 117.73, 116.85, 110.62, 88.28, 83.51, 58.90, 53.05, 50.90, 50.18, 29.68.

4-((1-((2-oxo-2*H*-chrom3ene-3-carbonyl)oxy)propan-2-yl) oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10g)

White solid, yield: 80.4%; m.p. 152.9–154.7 °C. ESI-MS (*m/z*): calcd for $C_{21}H_{16}N_2O_9S$ (neutral M + H) 473.0; IR (ν_{max}/cm^{-1}): 2917, 2850, 1761, 1613, 1553, 1460, 1392, 1293, 1248, 1165, 1085, 1005; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H, =CH), 8.06 (d, J = 2.0 Hz, 2H, Ph-H), 7.76 (s, 1H, Ph-H), 7.65 (dd, J = 2.8, 1.0 Hz, 4H, Ph-H), 7.36 (s, J = 0.5 Hz, 2H, Ph-H), 4.61 (d, J = 32.7 Hz, 4H, O=CCH_2, O–CH), 2.38 (d, J = 5.2 Hz, 2H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.14, 158.85, 156.51, 155.24, 149.05, 138.02, 135.66, 134.56, 129.72 (2C), 129.67, 128.50 (2C), 124.93, 117.93, 117.82, 116.78, 110.49, 68.11, 61.80, 27.91.

4-(2-(2-((2-oxo-2*H*-chromene-3-carbonyl)oxy)ethoxy) ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10h)

White solid, yield: 77.5%; m.p. 98.9–101.0℃; ESI-HRMS (*m/z*): calcd for $C_{22}H_{18}N_2O_{10}S$ (neutral M + H) 503.0682; found 503.0797; IR (ν_{max}/cm^{-1}): 2834, 2895, 1723, 1564, 1449, 1345, 1169, 1020; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (s, 1H, =CH), 8.08 (s, 2H, Ph-H), 7.77 (s, 1H, Ph-H), 7.66 (s, 4H, Ph-H), 7.38 (s, 2H, Ph-H), 4.54–4.43 (m, 4H, O=CCH_2, O–CH_2), 2.10 (d, J = 4.7 Hz, 2H, O–CH_2), 2.02 (d, J = 2.6 Hz, 2H, O–CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.01, 159.36, 156.36, 155.00, 149.29, 137.65, 136.52, 135.04, 130.71 (2C), 130.41, 128.71 (2C), 125.27, 118.16, 117.90, 116.61, 110.92, 71.33, 68.77, 68.29, 64.96.

4-(2-(2-oxo-2*H*-chromene-3-carboxamido)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10i)

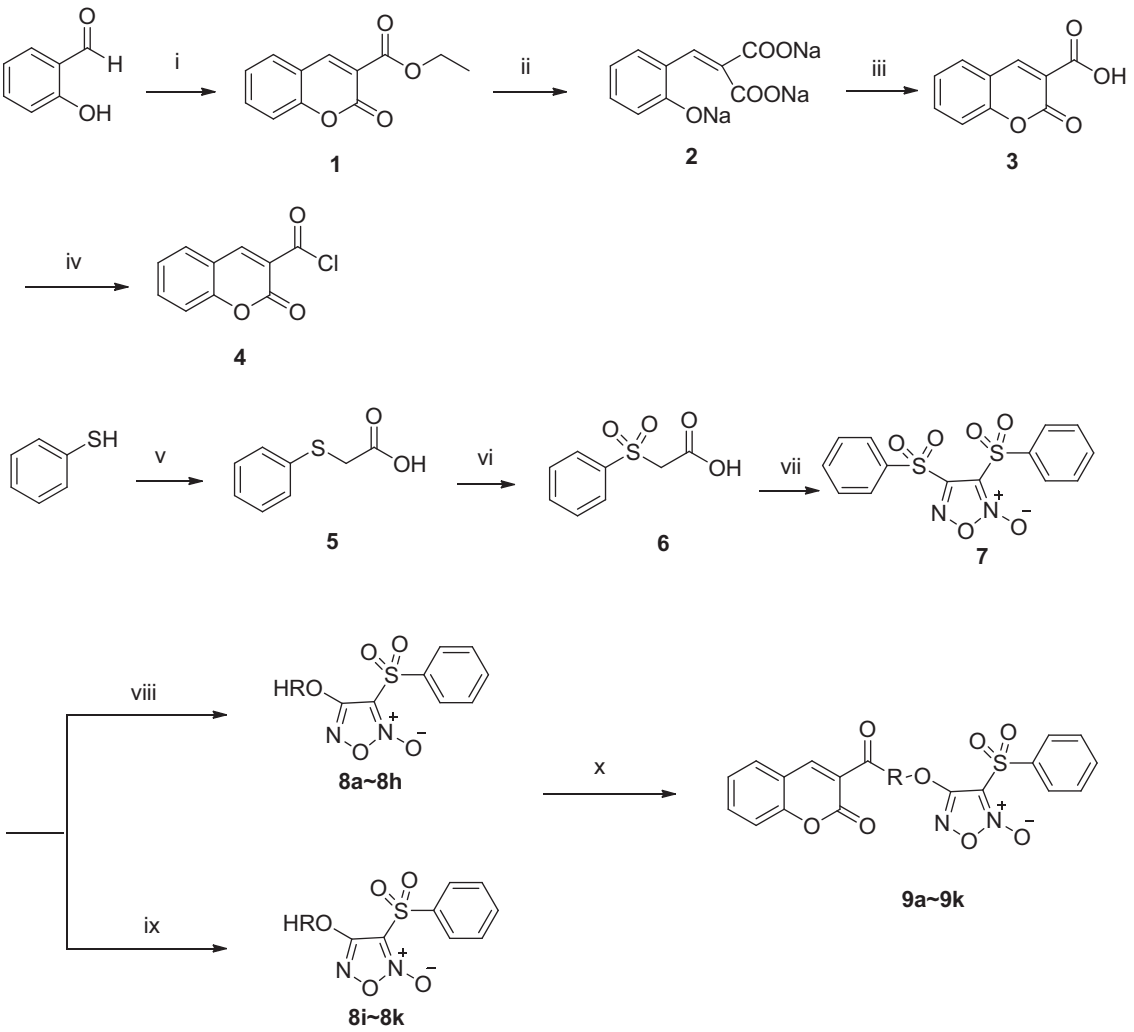
White solid, yield: 64.2%; m.p. 142.3–145.7℃; APCI-HRMS (*m/z*): calcd for $C_{20}H_{15}N_3S$ (neutral M + H) 458.0580; found 458.0658; IR (ν_{max}/cm^{-1}): 3273, 3022, 2924, 1710, 1650, 1632, 1531, 1450, 1362, 1254, 1154 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (s, 1H, =CH), 8.93 (s, 1H, O=C–NH), 8.14 (s, 2H, Ph-H), 7.76–7.70 (m, 3H, Ph-H), 7.65 (d, J = 5.0 Hz, 2H, Ph-H), 7.43 (d, J = 8.7 Hz, 2H, Ph-H), 4.61 (s, 2H, O–CH_2), 3.98 (d, J = 2.9 Hz, 2H, O=C–NH–CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.13, 160.78, 159.26, 154.43, 148.44, 134.76, 132.95, 130.82, 130.43, 129.37, 128.86, 126.95, 126.69, 125.31, 119.01, 118.89, 116.67, 111.08, 70.31, 38.28.

4-(3-(2-oxo-2*H*-chromene-3-carboxamido)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10j)

White solid, yield: 67.1%; m.p. 148.5–156.3 °C; APCI-HRMS (*m/z*): calcd for $C_{21}H_{17}N_3O_8S$ (neutral M + H) 472.0770; found 472.0805; IR (ν_{max}/cm^{-1}): 3355, 3045, 2913, 1699, 1648, 1533, 1449, 1369, 1248, 1161; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (s, 1H, =CH), 8.92 (s, 1H, O=C–NH), 8.10 (d, J = 6.0 Hz, 2H, Ph-H), 7.74–7.63 (m, 5H, Ph-H), 7.42 (d, J = 7.0 Hz, 2H, Ph-H), 4.55 (s, 2H, O–CH_2), 3.70 (s, 2H, O=C–NH–CH_2), 2.25 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_{63}) δ 161.94, 160.68, 159.38, 154.32, 147.75, 137.62, 136.61, 134.50, 130.69 (2C), 130.50, 128.90 (2C), 125.58, 119.69, 118.92, 116.59, 110.99, 69.89, 36.27, 28.67.

4-((1-(2-oxo-2*H*-chromene-3-carboxamido)propan-2-yl)oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (10k)

White solid, yield: 65.2%; m.p. 152.9–154.7 °C; APCI-HRMS (*m/z*): calcd for $C_{21}H_{17}N_3O_8S$ (neutral M + H) 472.0770; found 472.0807; IR (ν_{max}/cm^{-1}) 3356, 2917, 2850, 1703, 1623, 1655, 1527, 1453, 1354, 1162; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H, =CH), 8.90 (s, 1H, O=C–NH), 8.10 (d, J = 4.8 Hz, 2H, Ph-H), 7.74–7.63 (m, 5H, Ph-H), 7.42 (d, J = 6.0 Hz, 2H, Ph-H), 4.50 (s, 1H, O–CH), 3.52–3.37 (s, 2H, O=C–NH–CH_2), 1.52 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.20, 160.81, 158.79, 154.39, 136.49, 134.73, 130.38 (3C), 128.93, 128.07, 125.95, 125.69, 125.55, 119.17, 118.88, 116.66, 111.10, 78.41, 43.17, 17.27.



9a,10a	RO=O$(CH_2)_2$O	**9b,10b**	RO=O$(CH_2)_3$O
9c,10c	RO=O$(CH_2)_4$O	**9d,10d**	RO=O$(CH_2)_5$O
9e,10e	RO=O$(CH_2)_6$O	**9f,10f**	RO=O$CH_2C{\equiv}CCH_2$O
9g,10g	RO=OCH$(CH_3)CH_2$O	**9h,10h**	RO=O$(CH_2)_2$O$(CH_2)_2$O
9i,10i	RO=NH$(CH_2)_2$O	**9j,10j**	RO=NH$(CH_2)_3$O
9k,10k	RO=NHCH$(CH_3)CH_2$O		

Scheme 1 Synthesis of NO-donating coumarin derivatives **10a–k**. reagents and conditions: (i) diethyl malonate, piperidine (cat.), EtOH, reflux, 2 h; (ii) NaOH, H_2O, EtOH, reflux, 20 min; (iii) HCl, rt; (iv) $SOCl_2$, pyridine, reflux, 6 h; (v) Chloroacetic acid, NaOH, Na_2CO_3, rt, 4 h and reflux 1 h; (vi) HOAc, H_2O_2, rt. 4 h; (vii) 98% HNO_3, reflux, 30 min; (viii) HO-R-OH, THF, 25%NaOH, rt. 40 min; (ix) HO-R-NH_2, dry THF, NaH, −5 °C, 30 min; (x) Compound **4**, DCM, TEA, 2 h

Anticancer activity study (MTT assay)

Human hepatocellular carcinoma HepG2, human colorectal cancer SW620, HCT116, human cervical cancer HeLa and human breast cancer MCF-7, human intestinal epithelial CCD841 cells per well at 10^4 cell were cultured in 10% FBS DMEM high glucose solution and a 37 ℃ in 5% CO_2 in 96-well flat-bottom microplates overnight. The cells were incubated in triplicate with, or without, different concentrations of each test compound for 72 h. Then 20 μL of 5 mg/mL MTT solution was added to each well and incubated for 4 h. Moved 150 μL DMSO in each well and detection absorbent value in 470 nm wavelength. The inhibition rate

of each compound in different concentrations was expressed as a percentage.

Results and discussion

Chemistry

The synthetic route of these target compounds **10a–k** is outlined in Scheme 1. Salicylaldehyde 1 which stirred with diethyl malonate in the presence of piperidine in ethanol afforded ethyl coumarin-3-carboxylate 2. The ethyl ester was hydrolyzed in the presence of NaOH to give coumarin-3-carboxylic acid 3, which was then converted to related acid chloride 4 by using thionyl chloride. Subsequently, the obtained acid chloride was reacted with various mono (phenylsulfonyl) furoxans, synthesized in a four-step sequence as previously described, in the presence of TEA in DCM to afford the final compounds. All target compounds were purified by column chromatography, and their structures were confirmed by IR, ^{1}H and ^{13}C NMR, MS, and HRMS.

The in vitro activity of target compounds **10a**−**k** was examined against human cervical cancer HeLa cell by MTT assay, using coumarin-3-carboxylic acid and doxorubicin as control. The activity was expressed by half maximal inhibitory concentration (IC_{50}) and was provided in Table 1. The results revealed that all the coumarin–furoxan hybrids possessed higher activity than coumarin-3-carboxylic acid, and five out of eleven compounds, **10b–c**, **10f**, and **10h–i**, displayed significantly inhibitory effects, which were superior to one classical anticancer drug, Doxorubicin.

Table 1 The structure and cytotoxic activity of the target compounds against HeLa human cervical cancer cells

Compounds	–R–	IC_{50} (μM)
10a	$-(CH_2)_2O-$	32.32
10b	$-(CH_2)_3O-$	4.85
10c	$-(CH_2)_4O-$	5.16
10d	$-(CH_2)_5O-$	38.61
10e	$-(CH_2)_6O-$	34.96
10f	$-CH_2C{\equiv}CCH_2O-$	5.95
10g	$-CH_2CH(CH_3)O-$	14.82
10h	$-(CH_2)_2O(CH_2)_2O-$	4.72
10i	$-(CH_2)_2NH-$	0.88
10j	$-(CH_2)_3NH-$	17.75
10k	$-CH_2CH(CH_3)NH-$	44.12
3	/	460
Doxorubicin	/	10.21

IC_{50}: a concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean IC_{50} from the does-response curves of at least three independent experiments

Especially, compound **10i** ($IC_{50} = 0.88$ μM) showed extraordinary antitumor activity, with IC_{50} values below 1 μM, being much more active than coumarin-3-carboxylic acid and more effective than doxorubicin ($IC_{50} = 10.21$ μM). These data suggest that these compounds possess significant antiproliferative activity suggesting that the furoxan pharmacophores play crucial roles in manifesting anticancer activity. This finding is coherent with previous conclusions made by Liu MM group (Liu et al. 2014).

In vitro antitumor activity evaluation

To examine how the target compounds affected other cancer cells, we selected the most promising compounds **10b–c**, **10f**, and **10h–i** and evaluated their respective cytotoxicity against on the other four different human cancer cells, including human hepatocellular carcinoma (HepG2), human colorectal cancer (HCT116 and SW620) and human breast cancer (MCF-7) by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay. As shown in Table 2, that compounds **10c** and **10h** displayed much less inhibition on the growth of HepG2, HCT116 and SW620 cells, likewise, **10b** was weaker antiproliferative activity on HCT116 and SW620 cells, with IC_{50} values exceed 25 μM. On the contrary, compound **10b** showed selective cytotoxic effect against HepG2 cancer cell line with an IC_{50} of 3.86 μM. Moreover, all five compounds displayed potent cytotoxic activity against breast cancer cell lines, with IC_{50} values ranging from 0.61 to 8.93 μM, and showed selective cytotoxic effect against MCF-7 cancer cell line. Notably, compound **10i** was found to be the most potent compound with an IC_{50} value of 0.61 μM against MCF-7 cells and 0.88 μM against HeLa cells. Especially, among all the compounds, compound **10f** had higher anti-proliferative activity on MCF-7 ($IC_{50} = 7.90$ μM), SW620 ($IC_{50} = 1.86$ μM), HCT-116 ($IC_{50} = 3.46$ μM), and HepG2 ($IC_{50} = 9.85$ μM), displayed the prominent and broader spectrum of cytotoxic activities against all the five human tumor cell lines.

Table 2 In vitro cytotoxicity (IC_{50}, μM) for compounds **10b**, **10c**, **10f**, **10h**, and **10i** against four human cancer cell lines

Compounds	In vitro cytotoxicity (IC_{50}, μM)			
	SW620	HepG2	HCT116	MCF-7
10b	>25	3.86	>25	7.72
10c	>25	>25	>25	8.93
10f	1.86	9.85	3.46	7.92
10h	>25	>25	>25	7.68
10i	12.61	17.32	14.43	0.61
Doxorubicin	2.32	3.54	0.48	3.25

IC_{50}: a concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean IC_{50} from the does-response curves of at least three independent experiments

As seen in Table 1, the antiproliferative activity of the novel coumarin–furoxan hybrids was influenced by the linker. For example, variation in the length of alkyl chain affected significantly the in vitro anticancer activity of these derivatives. Compounds **10b–c** with a 2–4 methylenes linker displayed stronger anticancer activity in comparison to those possessing with a 5–6 methylenes linker. For example, compound **10i** containing the aminoethanol linkage showed better selectivity and enhanced activity. Particularly, it was also observed that compound **10f** with the butynelene linker enhanced broader spectrum antitumor potency.

Cancer cell selectivity

Given the significant antiproliferative activity of **10f** in vitro, the selectivity profile was investigated by examining the inhibitory effects of **10f** on the growth of human colorectal cancer SW620 cells and normal intestinal epithelial CCD841 cells. It was found that treatment with increased dose of **10f** had no significant effect on the survival of non-tumor CCD841 cells, whereas the same treatment induced death of the majority of SW620 cells (Fig. 4). These results suggest that **10f** possesses selectivity in antiproliferation activity against colorectal tumor cell over normal cell.

Conclusion

In summary, a series of hybrids of furoxan–coumarin were synthesized and evaluated. Some hybrids possessed potent antiproliferative activities against five human cancer cell lines in vitro. In particular, compound **10i** was identified as the most antiproliferative activities for human breast and cervical cancer cell lines. More importantly, compound **10f** selectively inhibited colorectal tumor cell proliferation but not normal cells. Together, given their potent antitumor activities, these furoxan/coumarin hybrids warrant further investigation as candidates for the potential treatment of human cancer.

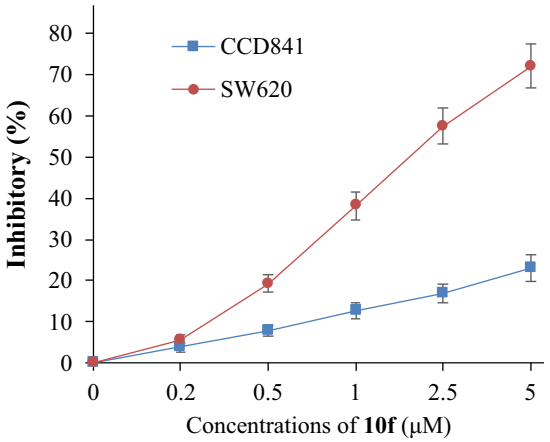


Fig. 4 Inhibitory effects of **10f** on the proliferation of SW620 and CCD841 cells. Cells were incubated with the indicated concentrations of **10f** for 72 h. Cell proliferation was assessed using the MTT assay. Data are means ± SD of the inhibition (%) from three independent experiments

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adams M, Ettl S, Kunert O, Wube AA, Haslinger E, Bucar F, Bauer R (2006) Antimycobacterial activity of geranylated furocoumarins from tetradium daniellii. Planta Med 72:1132–1135

Appendino G, Bianchi F, Bader A, Campagnuolo C, Fattorusso E, Scafati O, Molina M, Macho A, Fiebich B, Bremner P, Heinrich M, Ballero M, Muñoz E (2004) Coumarins from opopanax chironium. New dihydrofurancoumarins and differential induction of apoptosis by imperatorin and heraclenin. J Nat Prod 67:532–536

Boiani M, Cerecetto H, Gonzalez M, Risso M, Olea-Azar C, Piro OE, Castella EE, de Cerain AL, Ezpeleta O, Monge -Vega A (2001) 1,2,5-Oxadiazole *N*-oxide derivatives as potential anti-cancer agents: synthesis and biological evaluation. Part IV. Eur J Med Chem 36:771–782

Cardellina JH, Bokesch HR, McKee TC, Boyd MR (1995) Resolution and comparative anti-HIV evaluation of the enantiomers of calanolides A and B. Bioorg Med Chem Lett 4:1011–1014

Cerecetto H, Porcal W (2005) Pharmacological properties of furoxans and benzofuroxans: recent developments. Mini-Rev Med Chem 5:57–71

Chen Y, Wang HD, Xia X, Kung HF, Pan Y, Kong LD (2007) Behavioral and biochemical studies of total furo-coumarins from seeds of Psoralea corylifolia in the chronic mild stress model of depression in mice. Phytomedicine 14:523–529

Cui HH, Gao YH, Liang SL, Cai HF, Wei ZX (2007) Chemical constituents of Citrus medica var. sarcodactylis from Sichuan Province (I). Chin Tradit Herb Drugs 9:1304–1306

Gao LL, Huang JK, He LQ (2014) Design, synthesis, and evaluation of coumarin-3-carboxylic acid nicotinic acid prodrugs as novel platelet aggregation inhibitors. Chem World 7:430

He LQ, Liu J, Yin DK, Zhang YH, Wang XS (2010) Synthesis and biological evaluation of nitric oxide-releasing matrine derivatives as anticancer agents. Chin Chem Lett 21:381–384

He LQ, Yang Q, Wu YX, Wang XS (2015) Novel hybrids of (phenylsulfonyl)furoxan and N-benzyl matrinol as anti-hepatocellular carcinoma agents. Acta Pharm Sin 50:574–578

Hofseth LJ, Hussain SP, Wogan GN, Harris CC (2003) Nitric oxide in cancer and chemoprevention. Free Radic Biol Med 34:955–968

Hsieh MJ, Chen MK, Yu YY (2014) Psoralen reverses docetaxel-induced multidrug resistance in A549/D16 human lung cancer cells lines. Phytomedicine 49:970–977

Huang Z, Fu J, Zhang Y (2017) Nitric Oxide Donor-Based Cancer Therapy: Advances and Prospects Journal of Medicinal Chemistry 60(18):7617–7635

Kamath PR, Sunil D, Ajees AA (2015) Some new indole–coumarin hybrids; synthesis, anticancer and Bcl-2 docking studies. Bioorg Chem 63:101–109

Liu MM, Chen XY, Huang YQ, Feng P, Guo YL, Yang G, Chen Y (2014) Hybrids of phenylsulfonylfuroxan and coumarin as potent antitumor agents. J Med Chem 57:9343–9356

Mo ZL, Liu YZ, Chen H, Sun WH, Li HJ (2006) Study on the fluorescence of poly(amidoamine) dendrimers decorated with coumarin-3-methyl acyl chloride on the periphery. Spectrosc Spectr 11:2080–2084

Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79:629–661

Piao XL, Yoo HH, Kim HY, Kang TL, Hwang GS, Park JH (2004) Antioxidative activity of furanocoumarins isolated from *angelicae dahuricae*. J Ethnopharmacol 93:243–246

Vilar S, Quezada E, Santana L, Uriarte E, Yánez M, Fraiz N, Alcaide C, Canob E, Orallob F (2006) Design, synthesis, and vasorelaxant and platelet anti-aggregatory activities of coumarin–resveratrol hybrids. Bioorg Med Chem Lett 16:257–261

Wang JX, Zhang BH, Xu DD (2013) Synthesis and antitumor activity of NO-donating derivatives of gambogic acid. Chin J Nat Med 11:87–96

Wang L, Wu HL, Yin XL, Hu Y, Gu HW, Yu RQ (2017) Simultaneous determination of umbelliferone and scopoletin in Tibetan medicine Saussurea laniceps and traditional Chinese medicine Radix angelicae pubescentis using excitation-emission matrix fluorescence coupled with second-order calibration method. Spectrochim Acta A 170:104–110

Wink DA, Vodovotz Y, Cook JA, Krishna MC, Kim S, Coffin D, DeGraff W, Deluca AM, Liebmann J, Mitchell JB (1998) The role of nitric oxide chemistry in cancer treatment. Biochemistry 63:802–803

Wu SH, Zhang ZH, Zhao JB (1998) An experimental study on antitumor activity of psoralen on mammary cancer cell line EMT6 in vitro and in vivo. Chin J Chin Mater Med 23:303–305

Wu YX, Huang JK, Gao LL, He LQ, Huang P, Wang XS (2015) Design, synthesis and evaluation of nitric oxide-releasing derivatives of N-(n-butyl)matrinic acid and N-(n-butyl) matrinol as anti-hepatocellular carcinoma agents. Heterocycles 91:301–312

Zhou HY, Hong JL, Shu P, Ni YJ, Qin MJ (2009) A new dicoumarin and anticoagulant activity from *Viola yedoensis* Makino. Fitoterapia 80:283–285