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Biscoumarin derivatives: Synthesis, crystal structure, theoretical studies and induced apoptosis activity on bladder urothelial cancer cell

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HIGHLIGHTS

- Five new biscoumarin derivatives (1–5) were synthesized.
- Their anti-cancer activities on two human bladder urothelial cell lines were evaluated.
- The cell cycle analysis and apoptosis change of the compounds were also observed.
- The HB energies of compounds 1–5 were calculated.

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ABSTRACT

In this study, five new biscoumarin derivatives (1–5) were synthesized and compound 4 inhibited the proliferation of the bladder urothelial cells (J82 cell line) obviously after 48 h treatment at different concentration (1, 5 and 10 μmol/L), and J82 cells were predominantly induced to apoptotic cell death after compound 4 treatment. Morphologic changes of bladder urothelial cancer cells were also observed under transmission electron microscopy (TEM) after compound 4 treatment. In addition, compound 4 had much less toxicity to human umbilical vein endothelial cells. To explore the possible anti-cancer mechanism of compound 4, two classical intramolecular O—H···O hydrogen bonds (HBs) in their structures and the corresponding HB energies were performed with the density functional theory (DFT) [B3LYP/6-31G*] method. Anti-bladder cancer activity of compound 4 is possible due to the intramolecular weakest HB energies.

Introduction

Bladder urothelial cancer is diagnosed at an increasing rate in the world, which is the second most common urologic malignancy, while the clinical outcomes remain highly controversial [1–3]. Treatment for the bladder urothelial cancer takes different approaches depending on the conditions, and chemotherapy is one of the important treatments, which may be used before surgery, after surgery, or instead of surgery for those cases in which surgery is considered unsuitable [4–6]. However, chemotherapeutic agents against bladder urothelial cancer are still limited, more novel anti-cancer agents with great selectivity and specificity need to be developed for bladder urothelial cancer treatment.

Biscoumarins have received considerable attention in the past few years for their versatile biological and medical properties, such as antioxidant, anti-inflammatory, antibacterial, and anticancer activities [7–9]. It was reported that biscoumarin derivatives can strongly inhibit tubulin aggregation and played an efficient role against cancer, so the cancer cells were able to prevent progression through the cell cycle. Other mechanism of anti-cancer activity was due to the antiangiogenesis and promotion of apoptosis [10–12]. These results indicated that coumarin derivatives might represent interesting novel drug candidates.

In this work, we synthesized five new biscoumarin derivatives, namely, 3,3′-benzylidene-bis-(4-hydroxycoumarin) (1), 3,3′-(2-nitrobenzylidene)-bis-(4-hydroxycoumarin) (2), 3,3′-(3-nitrobenzylidene)-bis-(4-hydroxycoumarin) (3), 3,3′-(2-chloro-5-nitrobenzylidene)-bis-(4-hydroxycoumarin) (4) and 3,3′-(3-nitro-4-hydroxybenzylidene)-bis-(4-hydroxycoumarin) (5) (Fig. 1), and then evaluated their anti-cancer activities on two human bladder urothelial cell lines. In addition, the cell cycle analysis

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and apoptosis change induced by compound treatment were also observed.

Experimental

Apparatus and materials

IR spectra (400–4000 cm^{-1}) were obtained using a Bruker Equinox-55 spectrophotometer. ^1H NMR spectra were obtained using a Varian Inova-400 spectrometer (at 400 MHz). Mass spectra were obtained using a micrOTOF-Q II mass spectrometer. The melting points were taken on a XT-4 micro melting apparatus, and the thermometer was uncorrected.

The bladder urothelial carcinoma cell line J82 and human umbilical vein endothelial cells were purchased from ATCC (Manassas, VA, USA). Cells were initially transferred into uncoated plastic tissue plates and were grown in Eagle's minimal essential medium (EMEM) with Earle's balanced salt solution (BSS) and 2 mM L-glutamine, modified to contain 1.0 mM sodium pyruvate, 0.1 mM nonessential amino acids, 1.5 g/L sodium bicarbonate, and 10% FBS. Cells were incubated at 37 °C in 95% air/5% CO_2. Medium was refreshed 3 times per week, and the cancer cells were harvested when they formed a confluent monolayer on the tissue plate.

Synthesis and characterization of compounds 1–5

Compounds 1–5 were synthesized according to a reported procedure [13]. A mixture of benzaldehyde (2-nitrobenzaldehyde, 3-nitrobenzaldehyde, 2-chloro-5-nitrobenzaldehyde and 3-nitro-4-hydroxybenzaldehyde) (10 mmol) and 4-hydroxycoumarin (20 mmol) was dissolved in 100 mL of EtOH. A few drops of piperidine were added, and the mixture was stirred for 3 h at room temperature. After reaction completion as determined by TLC, water was added until precipitation occurred. After filtering the precipitates, they were sequentially washed with ice-cooled water and ethanol and then dried in a vacuum.

3,3′-Benzylidene-bis-(4-hydroxycoumarin) (1): m.p. 208–209 °C. IR (KBr pellet cm^{-1}): 3446 (OH), 1652 (C=O), 1615 (C—O), 1572 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.528 (s, 1H, OH), 11.299 (s, 1H, OH), 7.994–8.080 (q, 2H), 7.606–7.649 (m, 2H), 7.215–7.421 (m, 9H), 6.104 (s, 1H, CH). HRMS (ESI^+): *m*/*z*: calcd for $C_{25}H_{16}O_6$: 435.0839 [M+Na^+]; found: 435.0899.

3,3′-(2-Nitrobenzylidene)-bis-(4-hydroxycoumarin) (2): m.p. 218–219 °C. IR (KBr pellet cm^{-1}): 3446 (OH), 1652 (C=O), 1615 (C—O), 1520 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.550 (s, 1H, OH), 11.219 (s, 1H, OH), 7.978–8.073 (m, 2H), 7.612–7.658 (m, 3H), 7.540–7.582 (m, 1H), 7.386–7.464 (m, 6H), 6.628 (s, 1H, CH). HRMS (ESI^+): *m*/*z*: calcd for $C_{25}H_{15}NO_8$: 480.0690 [M+Na^+]; found: 480.0611.

3,3′-(3-Nitrobenzylidene)-bis-(4-hydroxycoumarin) (NBH): m.p. 220–221 °C. IR (KBr pellet cm^{-1}): 3446 (OH), 1652 (C=O), 1615 (C—O), 1555 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.579 (s, 1H, OH), 11.384 (s, 1H, OH), 8.137–8.162 (m, 1H), 8.070–8.104 (t, 2H), 7.990–8.008 (d, 1H), 7.651–7.690 (t, 2H), 7.568–7.591 (m, 1H), 7.496–7.536 (t, 1H), 7.385–7.453 (m, 4H), 6.129 (s, 1H, CH). HRMS (ESI^+): *m*/*z*: calcd for $C_{25}H_{15}NO_8$: 480.0690 [M+Na^+]; found: 480.0689.

3,3′-(2-Chloro-5-nitrobenzylidene)-bis-(4-hydroxycoumarin) (CBH): m.p. 150–151 °C. IR (KBr pellet cm^{-1}): 3489 (OH), 1684 (C=O), 1623 (C—O), 1520 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.752 (s, 1H, OH), 11.006 (s, 1H, OH), 8.359–8.365 (t, 1H), 8.064–8.144 (m, 3H), 7.640–7.683 (m, 2H), 7.533–7.555 (d, 1H), 7.402–7.438 (t, 4H), 6.192 (s, 1H, CH). HRMS (ESI^+): *m*/*z*: calcd for $C_{25}H_{14}ClNO_8$: 514.0300 [M+Na^+]; found: 514.0321.

3,3′-(3-Nitro-4-hydroxybenzylidene)-bis-(4-hydroxycoumarin) (NHH): m.p. 238–239 °C. IR (KBr pellet cm^{-1}): 3251 (OH), 1655 (C=O), 1582 (C—O), 1536 (C=C) cm^{-1}. ^1H NMR ($CDCl_3$, δ, ppm): 11.593 (s, 1H, OH), 11.396 (s, 1H, OH), 10.545–10.558 (d, 1H), 7.944–8.108 (m, 3H), 7.686 (s, 2H), 7.402–7.466 (m, 4H), 7.287 (s, 1H), 7.142–7.176 (q, 1H), 6.046 (s, 1H, CH). HRMS (ESI^+): *m*/*z*: calcd for $C_{25}H_{15}NO_9$: 496.0639 [M+Na^+]; found: 496.0632.

Crystal structure determination

For X-ray diffraction experiments, single crystals of compound 4 were both grown from methanol. The X-ray diffraction data were collected on a Bruker SMART APEX II CCD diffractometer equipped

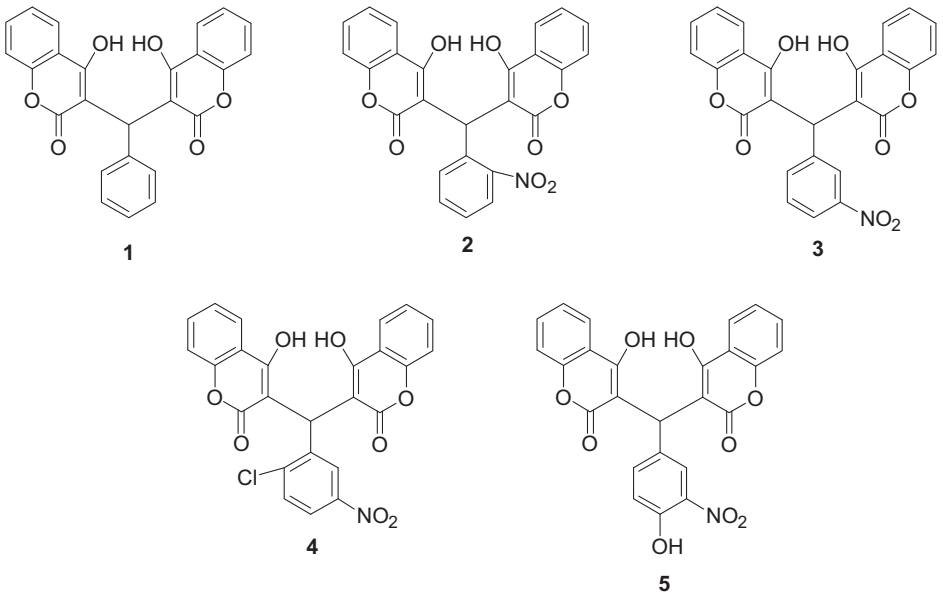

Fig. 1. Chemical structures of compounds 1–5.