Investigation of the cytotoxicity of bioinspired coumarin analogues towards human breast cancer cells

Leonidas Gkionis1 · Eleni Kavetsou2 · Alexandros Kalospyros2 · Dimitris Manousakis2 · Miguel Garzon Sanz1 · Sam Butterworth1,3 · Anastasia Detsi2 · Annalisa Tirella1,3

Received: 7 February 2020 / Accepted: 1 April 2020 / Published online: 23 April 2020 © The Author(s) 2020

Abstract
Coumarins possess a wide array of therapeutic capabilities, but often with unclear mechanism of action. We tested a small library of 18 coumarin derivatives against human invasive breast ductal carcinoma cells with the capacity of each compound to inhibit cell proliferation scored, and the most potent coumarin analogues selected for further studies. Interestingly, the presence of two prenyloxy groups (5,7-diprenyloxy-4-methyl-coumarin, 4g) or the presence of octyloxy substituent (coumarin 4d) was found to increase the potency of compounds in breast cancer cells, but not against healthy human fibroblasts. The activity of potent compounds on breast cancer cells cultured more similarly to the conditions of the tumour microenvironment was also investigated, and increased toxicity was observed. Results suggest that tested coumarin derivatives could potentially reduce the growth of tumour mass. Moreover, their use as (combination) therapy in cancer treatment might have the potential of causing limited side effects.

Leonidas Gkionis and Eleni Kavetsou: Co-first authors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11030-020-10082-6) contains supplementary material, which is available to authorized users.

Anastasia Detsi adetsi@chemeng.ntua.gr
Annalisa Tirella annalisa.tirella@manchester.ac.uk

1 Division of Pharmacy and Optometry, Faculty of Biology, Medicine and Health, Manchester Academic Health Science Centre, University of Manchester, Oxford Road, Manchester M13 9PL, UK
2 Laboratory of Organic Chemistry, School of Chemical Engineering, National Technical University of Athens, Herouon Polytechniou 9, Zografou Campus, 15780 Athens, Greece
3 NorthWest Centre for Advanced Drug Delivery (NoWCADD), Faculty of Biology, Medicine and Health, University of Manchester, Oxford Road, Manchester M13 9PT, UK
Keywords Alkoxy-coumarins · Auraptene · Umbelliprenin · Breast cancer

Introduction

Breast carcinoma is considered the predominant and more common malignancy in women worldwide, with one in eight women potentially developing breast cancer during their lifetime [1] and predictions of 3.2 million newly diagnosed cases per year by 2050 [2]. Early detection and intervention are essential to increase patients’ survival rate, yet the treatment of advanced cancer remains an issue. While many biological and physicochemical factors have been identified in cancer development, there is an increasing interest in the role of inflammation and involvement of stromal component of the tumour microenvironment [3]. The challenge of treating breast cancer resides not only in the identification of active compounds capable of targeting the cancer, but mainly in identifying potent therapies with low side effects [4]. In this perspective, natural compounds like coumarins have gained significant interest in the recent years for their numerous pharmacological activities including chemopreventive and antiproliferative properties against various cancer types [5–8].

Coumarins are heterocyclic organic compounds that are widely distributed in nature [4, 9]. Coumarin derivatives have gained high scientific interest as promising drug candidates since they possess multiple pharmacological properties [10–13], such as antioxidant [14–16], antibacterial [17, 18], antimicrobial [18], antiviral [13], hepatoprotective [19] and anti-inflammatory effects [20–22]. Natural and synthetic coumarins have been also reported as effective chemopreventive and anticancer agents in vitro [23–26] and in vivo [27].

Chemical modification such as alkylation (the addition of unsaturated or saturated chain to the coumarin scaffold) has been shown to enhance the pharmacological profile of several coumarins, especially their anticancer activity [25]. In particular, the insertion of an unsaturated chain (prenyl, geranyl or farnesyl side chain) is known as prenylation and constitutes a metabolic pathway in nature (including plant kingdom and microorganism such as fungi and bacteria [28]). The process of prenylation is considered to further enhance the pharmacological activity of these metabolites mostly because it strengthens the lipophilicity of the molecules [29]. Recently, natural oxyprenylated coumarins (iso-pentenyloxy (C5), geranyloxy (C10) and farnesyloxy (C15) compounds and their biosynthetic derivatives) have been studied for their pharmacological properties [28], mainly as potential anticancer agents [30]. Auraptene (7-geranyloxy coumarin) and umbelliprenin are the most common plant-derived oxyprenylated coumarins, first isolated from citrus fruits and Ferula plant species, respectively, and present a wide range of bioactivities [25, 31–35]. The addition of an
aliphatic chain to the coumarin scaffold is another modification shown to have anticancer effects as reported by Farley et al. [25], who reported that octyloxy-coumarins possess cytotoxicity against pancreatic cancer cells with concentrations in the order of tens of nM. As a continuation to our previous work concerning the biological evaluation of structurally modified coumarin analogues [14, 15], a series of bioinspired synthetic alkoxy coumarin derivatives (bearing saturated and unsaturated chains) were synthesized, structurally characterized and evaluated for their cytotoxicity against breast cancer cell lines (MCF-7 and MDA-MB-231) and fibroblasts. Interestingly we found that the more potent coumarin compounds have no effect on fibroblast (off-target control) and increase their potency on breast cancer cells cultured under nutrient-deprived conditions similar to the tumour microenvironment.

Materials and methods

Coumarins analogues

Synthesis

The chemicals used for synthesis and analysis were purchased from Sigma-Aldrich or Alfa Aesar (7-hydroxycoumarin, 98%) and used without further purification. NMR spectra were recorded on a Varian 300 MHz and 600 MHz spectrometer at the Institute of Chemical Biology of the National Hellenic Research Foundation. The HRMS spectra were obtained using a UHPLC-MSn Orbitrap Velos-Thermo mass spectrometer. Melting points were determined on a Gallenkamp MFB-595 melting point apparatus and are uncorrected.

General procedure for the synthesis of hydroxy or dihydroxy-4-substituted coumarin analogues The desired compounds 3a and 3b were synthesized according to the method of Prousis et al. [36].

7-Hydroxy-4-propyl-2H-chromen-2-one (3a) Beige solid; yield 80% (735.4 mg, 3.60 mmol); mp 130 °C (lit. m.p. 127–128 °C) [36]. 1H NMR (300 MHz, CDCl3): δ(ppm) 7.48 (d, J = 8.7 Hz, 1H, H-5), 6.79 (d, J = 8.7 Hz, 1H, H-6), 6.77 (s, 1H, H-8), 6.08 (s, 1H, H-3), 2.70 (t, J = 7.5 Hz, 2H, 4-CH2CH2CH3), 1.62 (m, 2H, 4-CH2CH2CH3), 0.96 (t, J = 7.2 Hz, 3H, 4-CH2CH2CH3).

5,7-Dihydroxy-4-methyl-2H-chromen-2-one (3b) Beige solid; yield 93% (707.2 mg, 3.68 mmol); mp 288–289 °C (lit. m.p. 289–290 °C) [36]. 1H NMR (600 MHz, DMSO-d6): δ(ppm) 10.51 (s, 1H, 7-OH), 10.29 (s, 1H, 5-OH), 6.25 (s, 1H, H-8), 6.16 (s, 1H, H-6), 5.84 (s, 1H, H-3), 2.48 (d, J = 9.6 Hz, 3H, 4-CH3).

Synthesis of geranylgeranyl iodide The following method was adapted from Alvarez-Manzaneda et al. [37]; briefly, 1170.0 mg (1.72 mmol, 1 eq.) of imidazole and 450.0 mg (1.72 mmol, 1 eq.) of triphenylphosphine were dissolved in 10 mL of anhydrous dichloromethane (DCM) in a round-bottom flask. 435.0 mg (1.72 mol, 1 eq.) of iodine was added slowly, and the mixture was stirred for 30 min. Then, the flask was covered with aluminium foil and placed in an ice bath, followed by slow addition of 0.57 mL (1.72 mol, 1 eq.) of geranylgeranyl. The mixture was stirred for approximately 2 h. After the reaction was complete (monitored by TLC in pure hexane), the mixture was filtered through a plug of silica, which was then washed with pure hexane. The solvent was evaporated in vacuo, resulting in a dark oily film. Yield 52% (51.9 mg).

General procedure for the synthesis of alkoxy-coumarins 4a–4m One eq. of the hydroxy- or dihydroxy-4-substituted coumarins, 3a–3c, and 1 eq. of potassium carbonate (K2CO3) were dissolved in dry acetone. Then, 1.2 eq. of the appropriate alkoxy-bromide or geranylgeranyl iodide was added dropwise at room temperature, and the mixture was refluxed for 6 h. After the completion of the reaction, K2CO3 was filtrated, the precipitate was washed with acetone and the solvent was removed in vacuo. The desired products were purified via silica gel column chromatography in a solvent system of petroleum ether/ethyl acetate (9:1). Diprenyloxy coumarins were obtained in high purity after two steps of silica gel chromatography.

7-Prenyloxy-4-methyl-2H-chromen-2-one (4a) White solid; yield 70% (61.1 mg, 0.25 mmol); mp 84 °C (lit. m.p. 84–85 °C) [38]. 1H NMR (300 MHz, CDCl3): δ(ppm) 7.48 (d, J = 8.7 Hz, 1H, H-5), 6.86 (dd, J = 8.7 Hz & J = 2.4 Hz, 1H, H-6), 6.82 (d, J = 2.4 Hz, 1H, H-8), 5.48 (t, J = 6.9 Hz, 1H, H-2′), 4.59 (d, J = 6.9 Hz, 2H, H-1′), 2.42 (s, 3H, 4-CH3), 1.83 (s, 3H, 3′-CH3), 1.79 (s, 3H, 4′-CH3).

7-Geranyloxy-4-methyl-2H-chromen-2-one (4b) Brown gummy solid; yield 68% (296.8 mg, 0.95 mmol). 1H NMR (300 MHz, DMSO-d6): δ(ppm) 7.64 (d, J = 9.0 Hz, 1H, H-5), 6.82 (d, J = 2.4 Hz, 1H, H-6), 6.82 (d, J = 2.4 Hz, 1H, H-8), 5.48 (t, J = 6.9 Hz, 1H, H-2′), 4.59 (d, J = 6.9 Hz, 2H, H-1′), 2.42 (s, 3H, 4-CH3), 1.83 (s, 3H, 3′-CH3), 1.79 (s, 3H, 4′-CH3).
7-Farnesoyloxy-4-methyl-2H-chromen-2-one (4c) Yellow gummy solid; yield 60% (319.6 mg, 0.84 mmol). 1H NMR (300 MHz, CDCl3): δ (ppm) 7.48 (d, J = 8.7 Hz, 1H, H-5), 6.86 (dd, J = 8.7 Hz & 1.8 Hz, 1H, H-1), 6.62 (d, J = 1.8 Hz, 1H, H-8), 6.13 (s, 1H, H-3), 5.47 (t, J = 6.9 Hz, 1H, H-2'), 5.10–5.07 (m, 1H, H-6'), 4.60 (d, J = 6.6 Hz, 1H, H-1'), 2.39 (s, 3H, 4-CH3), 2.12–1.95 (m, 8H, H-4' & H-5' & H-8' & H-9'), 1.76 (s, 3H, 3'-CH3), 1.67 (s, 3H, 7'-CH3), 1.59 (s, 6H, 11''-CH3 & 12''-CH3). 13C NMR (75 MHz, DMSO-d6): δ (ppm) 162.0, 160.6, 155.1, 145.1, 145.1, 135.1, 131.1, 126.8, 124.5, 123.9, 119.5, 113.5, 113.1, 111.5, 101.8, 65.6, 39.7, 39.3, 26.6, 26.0, 25.9, 18.6, 17.9, 16.9, 16.3. HRMS (ESI) calcd for C25H32O3Na: m/z: 403.2244, found: 403.2245.

4-Methyl-7-octyloxy-2H-chromen-2-one (4d) Pale yellow solid; yield 55% (180 mg, 0.62 mmol); mp 51 °C. 1H NMR (600 MHz, CDCl3): δ (ppm) 8.58 (d, J = 8.4 Hz, 1H, H-5), 6.78 (dd, J = 9 Hz & J = 2.4 Hz, 1H, H-6), 6.68 (d, J = 2.4 Hz, 1H, H-8), 6.15 (s, 1H, H-3), 4.06 (t, J = 6.6 Hz, 2H, H-1'), 2.39 (s, 3H, 4-CH3), 1.72 (m, 2H, H-2'), 1.40 (m, 2H, H-7'), 1.29 (m, 8H, H-3' & H-5' & H-4' & H-6'), 0.86 (t, J = 6.9 Hz, 3H, 7'-CH3).

7-Prenyloxy-4-propyl-2H-chromen-2-one (4e) Yellow solid; yield 60% (135.0 mg, 0.49 mmol); mp 89 °C. 1H NMR (600 MHz, CDCl3): δ (ppm) 7.51 (d, J = 8.4 Hz, 1H, H-5), 6.85 (dd, J = 9.0 Hz & J = 2.4 Hz, 1H, H-6), 6.82 (d, J = 2.4 Hz, 1H, H-8), 6.11 (s, 1H, H-3), 5.47 (t, J = 6.9 Hz, 1H, H-2'), 4.57 (d, J = 7.2 Hz, 2H, H-1'), 2.69 (t, J = 7.5 Hz, 2H, 4-CH2CH2CH3), 1.80 (s, 3H, 3'-CH3). 1.76 (s, 3H, 4'-CH3). 1.72–1.69 (m, 2H, 4-CH2CH2CH3), 1.04 (t, J = 7.5 Hz, 3H, 4-CH3). 13C NMR (150 MHz, CDCl3): δ (ppm) 161.9, 161.7, 156.5, 155.6, 139.3, 125.4, 118.8, 113.0, 112.9, 110.9, 101.8, 77.6, 77.2, 76.7, 67.6, 65.5, 33.9, 25.9, 21.6, 18.4, 14.1. HRMS (ESI) calcd for C17H17O3 (M + H)+: m/z: 273.1485, found: 273.1485.

7-Octyloxy-4-propyl-2H-chromen-2-one (4f) Beige solid; yield 60% (350.0 mg, 1.11 mmol); mp 47 °C. 1H NMR (600 MHz, CDCl3): δ (ppm) 7.51 (d, J = 9.0 Hz, 1H, H-5), 6.84 (dd, J = 8.4 Hz & J = 1.8 Hz, 1H, H-6), 6.81 (d, J = 2.4 Hz, 1H, H-8), 6.12 (s, 1H, H-3), 4.01 (t, J = 6.6 Hz, 2H, H-1'), 2.71 (t, J = 7.5 Hz, 2H, 4-CH2CH2CH3), 1.80 (m, 2H, H-2'), 1.73 (m, 2H, 4-CH2CH2CH3), 1.45 (m, 2H, H-7'), 1.31 (m, 8H, H-3' & H-5' & H-4' & H-6'), 1.04 (t, J = 7.2 Hz, 3H, 4-CH2CH2CH3), 0.88 (t, J = 6.9 Hz, 3H, 7'-CH3). 13C NMR (150 MHz, CDCl3): δ (ppm) 162.2, 161.8, 156.6, 155.7, 125.4, 112.9, 110.8, 101.6, 68.8, 33.9, 31.9, 29.4, 29.3, 29.1, 26.1, 22.8, 21.7, 14.2, 14.1. HRMS (APCI) calcd for C32H35O3 (M + H)+: m/z: 493.3312, found: 493.3302.
7-Prenyloxy-2H-chromen-2-one (4j) White solid; yield 65% (59.9 mg, 0.26 mmol); mp 77 °C (lit. m.p. 77–78 °C) [39]. 1H NMR (300 MHz, CDCl3): δ (ppm) 7.63 (d, J=9.6 Hz, 1H, H-4), 7.36 (d, J=8.4 Hz, 1H, H-5), 6.86–6.82 (m, 2H, H-6 & H-8), 6.25 (d, J=9.3 Hz, 1H, H-6), 5.48 (t, J=8.1 Hz, 1H, H-2'), 4.59 (d, J=6.9 Hz, 2H, H-1'), 1.84 (s, 3H, 3'-CH3), 1.79 (s, 3H, 4'-CH3).

7-Geranyloxy-2H-chromen-2-one (aurapten) (4k) White solid; yield 60% (268.5 mg, 0.90 mmol); mp 63 °C (lit. m.p. 62–63 °C) [40]. 1H NMR (300 MHz, CDCl3): δ (ppm) 7.63 (d, J=9.3 Hz, 1H, H-4), 7.36 (d, J=8.4 Hz, 1H, H-5), 6.86–6.82 (m, 2H, H-6 & H-8), 6.24 (d, J=9.3 Hz, 1H, H-3), 5.47 (t, J=6 Hz, 1H, H-2'), 5.09 (t, J=5.7 Hz, 1H, H-6'), 4.62 (d, J=6.6 Hz, 2H, H-1'), 2.15–2.11 (m, 4H, H-4' & H-5'), 1.79 (s, 3H, 3'-CH3), 1.69 (s, 3H, 8'-CH3), 1.63 (s, 3H, 7'-CH3).

Farnesyl-coumarin (umbelliprenin) (4l) Yellowish solid; yield 80% (439.8 mg, 1.20 mmol); mp 61 °C (lit. m.p. 58–60 °C) [40]. 1H NMR (300 MHz, CDCl3): δ (ppm) 7.62 (d, J=9.3 Hz, 1H, H-4), 7.35 (d, J=8.1 Hz, 1H, H-5), 6.86–6.82 (m, 2H, H-6 & H-8), 6.24 (d, J=9.6 Hz, 1H, H-3), 5.48 (t, J=6.6 Hz, 1H, H-2'), 4.62 (d, J=6.6 Hz, 1H, H-1'), 2.18–2.01 (m, 8H, H-4' & H-5' & H-8' & H-9'), 1.79 (s, 3H, 3'-CH3), 1.70 (s, 3H, 8'-CH3), 1.63 (s, 6H, 11'-CH3 & 12'-CH3).

Geranylgeranyloxy-coumarin (4m) Yellow solid; yield 64% (365.0 mg, 0.94 mmol); mp 72–73 °C (lit. m.p. 77–78 °C). 1H NMR (300 MHz, CDCl3): δ (ppm) 7.63 (d, 1H), 7.36 (d, 1H), 6.86–6.82 (m, 2H, 6H & 8H), 2.15–2.03 (m, 8H, 8H), 1.99–1.95 (m, 4H), 1.76 (s, 3H), 1.67 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H), 1.58 (s, 3H).

5,7-Diacetyloxy-4-methyl-2H-chromen-2-one (5) 1 eq of the coumarin 3b and 2 eq of acetic anhydride were added to the appropriate amount of pyridine, and the mixture was heated at 80 °C. After the completion of the reaction, pyridine was removed in vacuo and a solid product was obtained. The final pure coumarin was selected after recrystallization. Green solid; yield 72% (273.5 mg, 0.99 mmol); mp 143–144 °C (lit. m.p. 150–151 °C) [42]. 1H NMR (600 MHz, CDCl3): δ (ppm) 7.06 (s, 1H, H-8), 6.87 (s, 1H, H-6), 6.20 (s, 1H, H-5), 2.50 (s, 3H, 4-CH3), 2.37 (s, 3H, 7-CH3CO), 2.32 (s, 3H, 5-CH3CO).

Cell culture

Dulbecco’s modified Eagle’s medium (DMEM, D6429), foetal bovine serum (FBS, F9665), trypsin (T3924), l-glutamine (G7513), antibiotics (penicillin – streptomycin, P0781) and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT, M2128) were purchased from Sigma-Aldrich (Gillingham, UK). Dulbecco’s modified Eagle’s medium with no glucose (DMEM, A1443001) was purchased from Gibco Thermo Fisher Scientific, UK. Human breast adenocarcinoma cell lines MCF-7 (HTB-22™) and MDA-MB-231 (HTB-26™) were kindly donated from Manchester Cancer Research Labs (University of Manchester, UK). Human colon fibroblasts 18-Co (CRL-1459™) were purchased from ATCC.

General cell culture

Unless otherwise specified, all cell culture experiments were performed in a humidified 5% (v/v) CO2 air atmosphere at 37 °C in complete medium, and cell culture growth media were supplemented with 10% (v/v) foetal bovine serum and 2 mM l-glutamine. Human breast adenocarcinoma cell lines were cultured, maintained at densities lower than 1 × 10⁶ cells/cm² and discarded upon reaching passage number 60. Stromal healthy cells (human colorectal fibroblasts, 18Co) were cultured using complete DMEM medium supplemented also with 1% (v/v) penicillin–streptomycin. Cells were maintained at densities less than 1 × 10⁶ cells/cm² and discarded upon reaching passage number 12.

Nutrient-deprived conditions

Cells were culture using nutrient-deprived cell culture conditions (i.e. cell culture media with no glucose, l-glutamine, HEPES and sodium pyruvate) to mimic conditions similar to the tumour microenvironment. Note that these experiments were performed only using breast cancer cells. MCF-7 and MDA-MB-231 cells were seeded in 96-well plates (Corning Inc., NY, USA) at a density of 1 × 10⁴ and 6.7 × 10³ cells/cm², respectively. Cells were incubated with coumarin derivatives at concentrations of 0.1, 1, 10, 50, 100 and 250 μM up to 48 h. Untreated cells (negative) and cells incubated with 0.5% (v/v) DMSO in complete media (positive) were used as controls.

Coumarins cytotoxicity

The full library of coumarins was tested in both breast cancer cell lines, i.e. MCF-7 and MDA-MB-231. Fibroblast were used as “healthy” control. Stocks of compounds 3a–3c, 4a–4m and 5 were dissolved in pure DMSO and then diluted in complete media. (Note that DMSO concentration was kept lower than 0.5% (v/v).) Briefly, MCF-7 and MDA-MB-231 cells were seeded in 96-well plates (3799, Corning Inc., NY, USA) at a density of 1 × 10⁴ and 6.7 × 10³ cells/cm², respectively, whereas 18Co fibroblasts were seeded at a density of 1 × 10⁴ cells/cm². Cells were incubated with coumarin.
derivatives at concentrations 0.1, 1, 10, 50, 100 and 250 μM for 48 h. Untreated cells (negative) and cells incubated with 0.5% (v/v) DMSO in complete media (positive) were used as controls.

**Cell metabolism assay**

For each treatment, cell viability was measured via MTT assay after 48-h incubation as following described. Cell culture medium was replaced with 150 μL of fresh medium and 30 μL of MTT solution, and cells were incubated for 4 h (37 °C, 5% CO₂). After the formazan crystal formation, cell culture medium was removed from each well and replaced with 200 μL of DMSO. The absorbance was measured at 540-nm wavelength using a plate reader (Synergy 2 Biotek plate reader, Gen5 software).

**Toxicity (IC₅₀) and identification of potent coumarins**

IC₅₀ values were calculated (nonlinear regression, normalized response–variable slope) with GraphPad Prism (version 7.04). Values were ranked and classified as: high toxicity, moderate toxicity, poor toxicity and no toxicity. Threshold were set as 60, 80 and > 100 μM, respectively. The selection was used in order to test only the most toxic coumarins under cell culture condition more relevant to the tumour microenvironment, i.e. deprived cell culture media (Sect. 3.3).

**Results and discussion**

**Design and synthesis of coumarin analogues**

Our previous results concerning the cytotoxic activity evaluation of natural oxyprenylated coumarins [14] in combination with the latest literature data led us to design, a new series of diprenyloxy as well as dialkyloxy coumarins. The new series were designed in order to evaluate the influence of the disubstitution as well as the length of the lipophilic chain in the cytotoxicity against breast cancer cell lines.

In order to efficiently synthesize the desired coumarin analogues and the naturally occurring oxyprenylated coumarins, the appropriate hydroxy-4-substituted coumarins 3a and 3b were firstly synthesized via Pechmann reaction using iron (III) chloride (FeCl₃) as the catalyst [36]. Compounds 3a and 3b as well as the commercially available 7-hydroxycoumarin (umbelliferone, 3c) were subsequently alkylated with the appropriate commercially available alkyl bromide using potassium carbonate (K₂CO₃) in acetone (Scheme 1). For the preparation of 4m, the required geranylgeranyl iodide was prepared according to the method of Alvarez-Manzaneda et al. [37].

All the coumarin derivatives were purified using flash column chromatography and were structurally identified using ¹H and ¹³C NMR spectroscopy and HRMS spectrometry.

![Scheme 1](image_url)
Cytotoxicity towards breast cancer cells

The systematic variations on the alkyl chain as well as the position of substitution at the coumarin scaffold were examined as potential factors which could affect anticancer activity. The first set of experiments identified the most potent candidates among the coumarin derivatives herein synthesized. Cytotoxicity was firstly evaluated on two breast cancer cell lines: MCF-7 and MDA-MB-231. MCF-7 cells were selected as they retain several characteristics of differentiated mammary epithelium proliferation, as well as expressing oestrogen receptor, whereas MDA-MB-231 was selected as expressing a more aggressive and metastatic cells that do not express high levels of the oestrogen, progesterone or HER2 receptors (i.e. triple negative).

Coumarins were classified as possessing high (IC$_{50}$ < 80 µM), moderate (80 µM < IC$_{50}$ < 100 µM) and poor toxicity (100 µM < IC$_{50}$ < 250 µM); compounds with IC$_{50}$ values > 250 µM were classified as non-toxic. Coumarin (IC$_{50}$ < 80 µM), moderate (80 µM < IC$_{50}$ < 100 µM) and HER2 receptors (i.e. triple negative).

One of the main drawbacks of cytotoxic compounds is the poor selectivity towards cancer cells, with undesired effects on healthy cells, e.g. fibroblasts and epithelia. In an effort to understand whether coumarins have any effect on ‘healthy’ cells, human fibroblasts (18-Co) were treated with the most potent coumarin derivatives. As control, fibroblasts were also treated with a non-toxic coumarin (i.e. umbelliferone, 3c). Interestingly, cytotoxicity data evidenced no effect of the selected coumarins on fibroblasts (shown in Table 2) with the exception of coumarin 4k that showed some toxicity towards ‘healthy’ cells. The tested compounds were not as toxic as typical chemotherapeutics with IC$_{50}$s at the scale of few hundred nM such as doxorubicin [43–46] or gemcitabine [47–49], but they did appear to have no significant effects on ‘healthy’ fibroblasts as compared to the aforementioned chemo-agents. This is a very positive result in view of development of (nano)formulations and further translation of such compounds.

Auraptene (4j) was found to be the most potent compound among the library of coumarins tested in this study, confirming what has been already observed in other in vitro studies and in various in vivo animal models [50]. Its effect on cancer cells is still not clear and could be associated with induction of carcinogen-detoxifying enzymes, inhibition of free radical generation or metalloproteinase production [51]. The length of the prenyl chain seems to affect the activity of the compounds: auraptene (4k, 10 carbons) is more potent compared to its prenyloxy analogue (4j, 5 carbons) against the tested breast cancer cells. However, umbelliprenin (4l, 15 carbons) and coumarin 4m (20 carbons) exhibited lower antitumour potency, with umbelliprenin being more toxic than coumarin 4m in both cancer cell lines (Fig. 1).

The number of substituents on the aromatic ring could also play a role in the activity: 5,7-diprenyloxy-4-methyl-coumarin (4g) is approximately 2.5 times more cytotoxic compared to its monosubstituted analogue (compound 4a) against MCF-7 cells. Increasing the chain length of the substituents, as in 7-geranylgeranyloxy-coumarin (4n), results in complete loss of activity against both cell lines (Fig. 1, Table 1).

In an effort to better investigate the role of unsaturation on cytotoxicity, the coumarin analogues 4d and 4f, which possess a saturated alkyloxy substituent, were synthesized. Only coumarin 4d exhibited a moderate potency against MCF7 cells, whereas only a slight toxic effect was observed on MDA-MB-231 (Table 1); moreover, no toxicity was observed on fibroblasts (Table 2). These results suggest that this specific modification can participate in different biochemical pathways compared to unsaturated substituents; however, further research is necessary to confirm this and identify specific pathways.

Finally, we investigated the activity of derivatives as function of lipophilicity through the introduction of a methyl group at position 4 of the coumarin scaffold. The methylated coumarin derivatives 4b, 4a and 4c exhibited a rather moderate cytotoxic effect compared to their corresponded non-methylated analogues 4k, 4j and 4l. This observation suggests that substitution at position 4 might not directly link to increased toxicity. Furthermore, comparing the compounds with different substitutions at position4 (e.g. coumarins 4d vs 4f, coumarins 4g vs 4h) increased toxicity was observed in both cancer cell lines for compounds with methyl substitution (Fig. 1).

Cytotoxicity in tumour relevant in vitro models

In accordance with the work of Jun et al. [52] and Devji et al. [41], we were motivated to assess the activity of some selected derivatives under nutrient-deprived conditions (NDCs). Cancer cells are programmed in a non-ordinary way to exhibit high glycolytic activity even under sufficient aerobic conditions [53]. In hypoxic tumour conditions, when oxygen depletion and low vascularization take place, cancer cells often find the way to proliferate rapidly by foregoing oxidative phosphorylation and instead ferment large
Table 1 IC₅₀ values (µM) of coumarin derivatives (18 compounds) on MCF-7 and MDA-MB-231 breast cancer cells obtained after 48-h incubation

| Coumarin derivative | Chemical structure | MCF-7 IC₅₀ values (µM) | MDA-MB-231 IC₅₀ values (µM) |
|---------------------|-------------------|------------------------|----------------------------|
| 3a                  | ![Chemical structure 3a](image) | 225 ± 19               | 206 ± 9                    |
| 3b                  | ![Chemical structure 3b](image) | > 250                  | > 250                      |
| 3c                  | ![Chemical structure 3c](image) | > 250                  | > 250                      |
| 4a                  | ![Chemical structure 4a](image) | 179 ± 35               | 169 ± 23                   |
| 4b                  | ![Chemical structure 4b](image) | 129 ± 13               | 137 ± 9                    |
| 4c                  | ![Chemical structure 4c](image) | 105 ± 3                | 110 ± 29                   |
| 4d                  | ![Chemical structure 4d](image) | 82 ± 19                | 113 ± 7                    |
Table 1 (continued)

| Coumarin derivative | Chemical structure | MCF-7 IC₅₀ values (µM) | MDA-MB-231 IC₅₀ values (µM) |
|---------------------|--------------------|------------------------|----------------------------|
| 4e                  | ![Chemical structure](#) | > 250                  | > 250                      |
| 4f                  | ![Chemical structure](#) | 124 ± 39               | 143 ± 34                   |
| 4g                  | ![Chemical structure](#) | 69 ± 14                | 144 ± 16                   |
| 4h                  | ![Chemical structure](#) | 234 ± 76               | 159 ± 61                   |
| 4i                  | ![Chemical structure](#) | > 250                  | 133 ± 34                   |
| 4j                  | ![Chemical structure](#) | 143 ± 11               | 175 ± 33                   |
| 4k                  | ![Chemical structure](#) | 70 ± 5                 | 60 ± 4                     |
amounts of glucose into lactate under aerobic glycolysis, known as the Warburg effect [54, 55]. Moreover, hypoxia tends to boost this phenomenon by up-regulating the HIF-1α factor that “switches on” glycolytic and glucose transporter gene expression [56]. Breast carcinoma cell lines behave in a glucose-dependent manner and derive the majority of energy needed from high-throughput glycolysis [56, 57]. Hyperglycaemic systemic conditions, i.e. diabetes, have been proved to further promote the migratory invasiveness of breast malignancies in patients [55].

On that basis, we exposed the breast cancer cells to nutrient-deprived conditions where culture media were supplemented only with 2.5% v/v FBS, but not additional glucose, l-glutamine, sodium pyruvate. The coumarins tested were auraptene (4k), umbelliprenin (4l) and analogues 4d, 4c and 4g. As shown in Table 3, the tested compounds showed selective preferential cytotoxicity under nutrient-deprived conditions with umbelliprenin (4l) to be the most potent candidate as its pharmacological activity was remarkably enhanced by 15 times (IC₅₀ = 9.0 and 7.0 for MCF7 and MDA-MB231 cells, respectively). In similar studies, Zhang et al. and Jun et al. reported the high preferential cytotoxicity of umbelliprenin (4l) and its C6 analogue under NDC against pancreatic cancer cells [52, 58]. It should be therefore noted that these derivatives could represent a potential new tool for treating aggressively metastatic hypoxic tumours. The exact mechanism of action, though, should be further investigated.
Table 2  IC₅₀ values (µM) of the most potent coumarin derivatives (five compounds: 4c, 4d, 4g, 4k and 4l) on 18Co colon fibroblasts (healthy control)

| Coumarin derivative | Chemical structure | 18-Co IC₅₀ values (µM) |
|---------------------|--------------------|-----------------------|
| 3c                  | ![Image]           | > 250                 |
| 4c                  | ![Image]           | > 250                 |
| 4d                  | ![Image]           | 206 ± 17              |
| 4g                  | ![Image]           | 216 ± 23              |
| 4k                  | ![Image]           | 176 ± 62              |
| 4l                  | ![Image]           | > 250                 |

Data are expressed as mean ± SD and are obtained from n = 2 independent experiments
Fig. 1 Effect of substituents on coumarins on MCF-7 cells: cell viability was measured after 48-h incubation with different concentrations of coumarins and IC_{50} values determined using nonlinear regression (GraphPad Prism, v7). Coumarins were compared on the basis of the following structural features: a length of lipophilic chain, coumarin 4k versus 4j (10 vs 5 carbons) and coumarin 4l versus 4m (15 vs 20 carbons); b position of the substituent on the coumarin scaffold, coumarin 4g vs 4a; c effect of the saturation degree (substitution at position C7), coumarin 4d vs 4f; d the presence of methyl group (substitution at position C4), coumarin 4b versus 4k and coumarin 4a versus 4j (data not shown), coumarin 4c versus 4l (data not shown); e the presence of propyl group (substitution at position C4), coumarins 4g versus 4h and coumarins 4d versus 4f (data not shown)

A | Length of lipophilic chain

4j vs 4k

4m vs 4l

B | Group substitution

4a vs 4g

4d vs 4f

C | Saturation degree in 7’ carbon

D | Substitution in 3’ carbon

4b vs 4k

4g vs 4h

E | Alkylation in 3’ carbon
Conclusions

A series of novel alkoxy-coumarin derivatives were synthesized and tested for their cytotoxicity against the MCF7 and MDA-MB-231 breast cancer cells. The results of this study indicate that alkylation modification induces noticeable differentiation in pharmacological activity of coumarins. Auraptene (4k) was found to possess the most potent cytotoxic activity among the tested derivatives followed by compounds 4c, 4d, 4g and 4l. The tested compounds seemed not to affect the cell viability of the healthy 18Co fibroblasts but for the highest dose only. The amplification of the cytotoxic effect of the above pharmacophores under nutrient-deprived conditions, with umbel-liprenin (4l) being the lead compound, indicates that these compounds could lead to potential new therapeutics for highly metastatic hypoxic tumours once their mechanisms are fully understood.

| Coumarin derivative | Chemical structure | MCF-7 IC50 values (µM) | MDA-MB-231 IC50 values (µM) |
|---------------------|--------------------|------------------------|-----------------------------|
| 4c                  | ![Chemical structure](image) | 19 ± 3                 | 51 ± 9                      |
| 4d                  | ![Chemical structure](image) | 29 ± 3                 | 63 ± 5                      |
| 4g                  | ![Chemical structure](image) | 35 ± 9                 | 11 ± 2                      |
| 4k                  | ![Chemical structure](image) | 14 ± 5                 | 12 ± 8                      |
| 4l                  | ![Chemical structure](image) | 9 ± 1                  | 7 ± 2                       |

Data are expressed as average ± SD of n = 3 independent experiments

Acknowledgements  Eleni Kavetsou gratefully acknowledges financial support from State Scholarships Foundation (IKY): The realization of the doctoral dissertation was co-funded by the “Grant Scheme for Postgraduate Studies in Secondary Education” of the Operational Program “Human Resource Development, Education and Lifelong Learning” of the NSRF 2014-2020 with the co-financing of the European Social Fund. Leonidas Gkionis is indebted to EPSRC for a Ph.D. studentship as part of the Graphene NOWNANO Doctoral Training Centre.

Funding  Not applicable.

Availability of data and material (data transparency)  The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Compliance with ethical standards

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

Ethics approval (include appropriate approvals or waivers)  Not applicable.
Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Marcom PK (2017) Breast cancer. In: Ginsburg G, Willard H. (eds) Genomic and precision medicine: primary care, 3rd edn. Elsevier Ltd., pp 181–194
2. Tao ZQ, Shi A, Lu C et al (2015) Breast cancer: epidemiology and etiology. Cell Biochem Biophys 72:333–338. https://doi.org/10.1007/s12013-014-0459-6
3. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674
4. Guerra AR, Duarte MF, Duarte IF (2018) Targeting tumor metabolism with plant-derived natural products: emerging trends in cancer therapy. J Agric Food Chem 66:10663–10685. https://doi.org/10.1021/acs.jafc.8b04104
5. Lee KW, Bode AM, Dong Z (2011) Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer 11:211–218
6. Zhang L, Xu Z (2019) Coumarin-containing hybrids and their anticancer activities. Eur J Med Chem 181:111587
7. Fayed EA, Sabour R, Harras MF, Mehany ABM (2019) Design, synthesis, biological evaluation and molecular modeling of new coumarin derivatives as potent anticancer agents. Med Chem Res 28:1284–1297. https://doi.org/10.1007/s00044-019-02373-x
8. Kaur M, Kohli S, Sandhu S et al (2015) Coumarin: a promising scaffold for anticancer agents. Anticancer Agents Med Chem 15:1032–1048. https://doi.org/10.2174/1871520615666151011250503
9. O’Kennedy R, Thones DR (1997) Coumarins: biology, applications and mode of action. Wiley, Chichester
10. Detsi A, Kontogiorgis C, Hadjipavlou-Litina D (2017) Coumarin derivatives: an updated patent review (2015–2016). Expert Opin Ther Pat 27:1201–1226
11. Stefanachi A, Leonetti F, Pisani L et al (2018) Coumarin: a natural, privileged and versatile scaffold for bioactive compounds. Molecules 23:1–34
12. Barot KP, Jain SV, Kremer L et al (2015) Recent advances and therapeutic journey of coumarins: current status and perspectives. Med Chem Rev 24:2771–2798
13. Venugopala KN, Rashmi V, Odhav B (2015) Review on natural coumarin lead compounds for their pharmacological activity. Biomed Res Int 2013:1–14
14. Kavetsou E, Gkionis L, Galani G et al (2017) Synthesis of prenyloxy coumarin analogues and evaluation of their antioxidant, lipoxygenase (LOX) inhibitory and cytotoxic activity. Med Chem Res 26:856–866. https://doi.org/10.1007/s00044-017-1800-6
15. Roussaki M, Zelianakos K, Kavetsou E et al (2014) Structural modifications of coumarin derivatives: determination of antioxidant and lipoxygenase (LOX) inhibitory activity. Bioorganic Med Chem 22:6586–6594. https://doi.org/10.1016/j.bmc.2014.10.008
16. Al-Majedy YK, Al-Duhaidaahawi DL, Al-Azawi KF et al (2016) Coumarins as potential antioxidant agents complemented with suggested mechanisms and approved by molecular modeling studies. Molecules 21:2–11. https://doi.org/10.3390/molecules211020135
17. Tamene D, Endale M (2019) Antibacterial activity of coumarins and carbazole alkaloid from roots of clausena anisata. Adv Pharmacol Sci 2019:1–8. https://doi.org/10.1155/2019/5419854
18. Yang L, Wu L, Yao X et al (2018) Hydroxycoumarins: new, effective plant-derived compounds reduce Ralstonia pseudosolanae-carum populations and control tobacco bacterial wilt. Microbiol Res 215:15–21. https://doi.org/10.1016/j.micres.2018.05.011
19. Tian D, Wang F, Duan M et al (2019) Coumarin analogues from the Citrus grandis (L.) osbeck and their hepatoprotective activity. J Agric Food Chem 67:1937–1947. https://doi.org/10.1021/acs.jafc.8b06489
20. Berrino E, Milazzo L, Micheli L et al (2019) Synthesis and evaluation of carbonic anhydrate inhibitors with carbon monoxide releasing properties for the management of rheumatoid arthritis. J Med Chem 62:7233–7249. https://doi.org/10.1021/acs.jmedchem.9b00845
21. Zhao YL, Yang XW, Wu BF et al (2019) Anti-inflammatory effect of Pomelo peel and its bioactive coumarins. J Agric Food Chem 67:8810–8818. https://doi.org/10.1021/acs.jafc.9b02511
22. Peperidou A, Bua S, Bozdağ M et al (2018) Novel 6- and 7-substituted coumarins with inhibitory action against lipoxygenase and tumor-associated carbonic anhydrase IX. Molecules 23:13–17. https://doi.org/10.3390/molecules23010153
23. Zhao H, Donnelly AC, Kusuma BR et al (2011) Engineering an antibiotic to fight cancer: optimization of the novobiocin scaffold to produce anti-proliferative agents. J Med Chem 54:3839–3853. https://doi.org/10.1021/jm200148p
24. Herrera-R A, Castrillón W, Otero E et al (2018) Synthesis and antiproliferative activity of 3- and 7-styrylcoumarins. Med Chem Res 27:1893–1905. https://doi.org/10.1007/s00044-018-2202-0
25. Farley CM, Dibwe DF, Ueda JY et al (2016) Evaluation of synthetic coumarins for antiasthmatic cytotoxicity against pancreatic cancers. Bioorganic Med Chem Lett 26:1471–1474. https://doi.org/10.1016/j.bmcl.2016.01.054
26. Ricci F, Carrassa L, Christodoulou MS et al (2018) A high-throughput screening of a chemical compound library in ovarian cancer stem cells. Comb Chem High Throughput Screen 21:50–56. https://doi.org/10.2174/187620732166618012401340406
27. Cao D, Liu Y, Yan W et al (2016) Design, synthesis, and evaluation of in vitro and in vivo anticancer activity of 4-substituted coumarins: a novel class of potent tubulin polymerization inhibitors. J Med Chem 59:5721–5739. https://doi.org/10.1021/acs.jmedchem.6b00158
28. Fiorito S, Epifano F, Teddeo VA, Genovese S (2018) Recent acquisitions on oxyprenylated secondary metabolites as anti-inflammatory agents. Eur J Med Chem 153:116–122. https://doi.org/10.1016/j.ejmech.2017.08.038
29. Alhassan AM, Abdullahi ML, Uba A, Umar A (2014) Prenylation of aromatic secondary metabolites: a new frontier for development of novel drugs. Trop J Pharm Res 13:307–314
30. Hasan M, Genovese S, Fiorito S et al (2017) Oxyprenylated phenylpropanoids bind to MT1 melatonin receptors and inhibit breast cancer cell proliferation and migration. J Nat Prod 80:3324–3329. https://doi.org/10.1021/acs.jnatprod.7b00853
31. Okuyama S, Minami S, Shimada N et al (2013) Anti-inflammatory and neuroprotective effects of auraptene, a citrus coumarin, following cerebral global ischemia in mice. Eur J Pharmacol 699:118–123. https://doi.org/10.1016/j.ejphar.2012.11.043
32. Okuyama S, Semba T, Toyoda N et al (2016) Auraptene and other prenyloxyprenylpropanoids suppress microglial activation and dopaminergic neuronal cell death in a...
lipo polysaccharide-induced model of Parkinson’s disease. Int J Mol Sci 17:1–11. https://doi.org/10.3390/ijms17101716
33. Iranshahi M, Askari M, Sahebkar A, Hadjipavlou-Litina D (2009) Evaluation of antioxidant, anti-inflammatory and lipoygenase inhibitory activities of the prenylated coumarin umbelliprenin. Daru 17:99–103
34. Yan H, Ma Z, Peng S, Deng X (2013) Anti-inflammatory effect of auraptene extracted from trifoliate orange (Poncirus trifoliata) on LPS-stimulated RAW 264.7 cells. Inflammation 36:1523–1532. https://doi.org/10.1016/j.inflammas 0.2013.09.004
35. La VD, Zhao L, Epifano F et al (2013) Anti-inflammatory and wound healing potential of citrus aurapten. J Med Food 16:961–964. https://doi.org/10.1089/jmf.2013.0029
36. Prousis KC, Avlonitis N, Heropoulos GA, Calogeropoulou T (2014) Prenylated coumarins: Design, synthesis and evaluation of isoprenylated coumarins as potential anti-pancreatic cancer agents. Bioorganic Med Chem Lett 24:4654–4658. https://doi.org/10.1016/j.bmcl.2014.08.038
37. Alvarez-Manzaneda EJ, Chahboun R, Cabrera Torres E et al (2005) Reaction of allylic and benzylic alcohols and esters with PPh 3:12: one-pot synthesis of β, γ-unsaturated compounds. Tetrahedron Lett 46:3755–3759. https://doi.org/10.1016/j.tetlett.2005.03.132
38. Takeuch N, Kasama T, Aida Y et al (1991) Pharmacological activities of the prenylcoumarins, developed from folk usage as a medicine of Peucedanum japonicum thumb. Chem Pharm Bull 39:1415–1421. https://doi.org/10.1248/cpb.39.1415
39. Row EC, Brown SA, Stachulski AV, Lennard MS (2006) Design, synthesis and evaluation of furanocoumarin monomers as inhibitors of CYP3A4. Org Biomol Chem 4:1604–1610. https://doi.org/10.1039/b601096b
40. Askari M, Sahebkar A, Iranshahi M (2009) Synthesis and purification of 7-prenyloxyxycoumarins and herniarin as bioactive natural coumarins. Iran J Basic Med Sci 12:63–69. https://doi. org/10.22038/ijbms.2009.5145
41. Devji T, Reddy C, Woo C et al (2011) Pancreatic anticancer activity of a novel geranylgeranylated coumarin derivative. Bioorganic Med Chem Lett 21:5770–5773. https://doi. org/10.1016/j.bmcl.2011.08.005
42. Canter FW, Curd FH, Robertson A (1931) Hydroxy-carbonyl compounds. Part III. The preparation of coumarins and 1,4-benzopyrones from phloroglucinol and resorcinol. J Chem Soc. https://doi.org/10.1039/p9310001255
43. Wen Sh, Su Sc, Liu B et al (2018) Sulbutactam-enhanced cytotoxicity of doxorubicin in breast cancer cells. Cancer Cell Int 18:1–18. https://doi.org/10.1186/s12935-018-0625-9
44. Arif IS, Hooper CL, Greco F et al (2013) Increasing doxorubicin activity against breast cancer cells using PPARγ-ligands and by exploiting circadian rhythms. Br J Pharmacol 169:1178–1186. https://doi.org/10.1111/bph.2012.11.007
45. Fang XJ, Jiang H, Zhu YQ et al (2014) Doxorubicin induces drug resistance and expression of the novel CD44st via NF-κB pathway in human breast cancer MCF-7 cells. Oncol Rep 31:2735–2742. https://doi.org/10.3892/or.2014.3131
46. You Y, Xu Z, Chen Y (2018) Doxorubicin conjugated with a trastuzumab epitope and an MMP-2 sensitive peptide linker for the treatment of HER2-positive breast cancer. Drug Deliv 25:448–460. https://doi.org/10.1080/10717544.2018.1435746
47. Tao XM, Wang JC, Wang JB et al (2012) Enhanced anticancer activity of gemcitabine coupling with conjugated linoleic acid against human breast cancer in vitro and in vivo. Eur J Pharm Biopharm 82:401–409. https://doi.org/10.1016/j.ejpb.2012.06.007
48. Li A, Fridley B, Kalari K et al (2008) Gemcitabine and cyto sine arabinoside cytotoxicity: association with lymphoblas tod cell expression. Cancer Res 68:7050–7058. https://doi. org/10.1158/0008-5472.CAN-08-0405
49. Hernández-Vargas H, Rodríguez-Pinilla SM, Julián-Tendero M et al (2007) Gene expression profiling of breast cancer cells in response to gemcitabine: NF-κB pathway activation as a potential mechanism of resistance. Breast Cancer Res Treat 102:157–172. https://doi.org/10.1007/s10549-006-9322-9
50. Krishnan P, Yan KJ, Windler D et al (2009) Citrus aurapten suppresses cyclin D1 and significantly delays N-methyl nitrosourea induced mammary carcinogenesis in female Sprague-Dawley rats. BMC Cancer 9:1–12. https://doi.org/10.1186/1471-2407-9-259
51. Krishnan P, Kleiner-Hancock H (2012) Effects of Auraptene on IGF-1 stimulated cell cycle progression in the human breast cancer cell line, MCF-7. Int J Breast Cancer 2012:1–8. https://doi.org/10.1155/2012/502092
52. Jun M, Bacy AF, Moyer J et al (2014) Synthesis and biological evaluation of isoprenylated coumarins as potential anti-pancreatic cancer agents. Bioorganic Med Chem Lett 24:4654–4658. https://doi.org/10.1016/j.bmcl.2014.08.038
53. Izuishi K, Kato K, Ogura T et al (2000) Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. Cancer Res 60:6201–6207
54. Zhuang Y, Chan DK, Haugrud AB, Miskimins WK (2014) Mechanisms by which low glucose enhances the cytotoxicity of metformin to cancer cells both in vitro and in vivo. PLoS ONE. https://doi.org/10.1371/journal.pone.0108444
55. Takatani-Nakase T, Matsui C, Maeda S et al (2014) High glucose level promotes migration behavior of breast cancer cells through zinc and its transporters. PLoS ONE 9:e90136. https:// doi.org/10.1371/journal.pone.0090136
56. O’Mahony F, Razandi M, Pedram A et al (2012) Estrogen modulates metabolic pathway adaptation to available glucose in breast cancer cells. Mol Endocrinol 26:2056–2070. https://doi. org/10.1210/me.2012-1191
57. Simões RV, Serganova IS, Kruchevsky N et al (2015) Metabolic plasticity of metastatic breast cancer cells: adaptation to changes in the microenvironment. Neoplasia 17:671–684. https://doi.org/10.1159/000451323
58. Zhang H, Zhou R, Jun M et al (2016) Identification of the factors responsible for the selective in vitro cytotoxic activity of isoprenylated coumarin derivatives under nutrient-deprived conditions. J Cancer 7:160–166. https://doi.org/10.7150/jca.13243

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.