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Synthesis and cellular characterization of novel isoxazolo- and thiazolohydrazinylidene-chroman-2,4-diones on cancer and non-cancer cell growth and death

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ABSTRACT

Coumarins are extensively studied anticoagulants that exert additional effects such as anticancerogenic and even anti-inflammatory. In order to find new drugs with anticancer activities, we report here the synthesis and the structural analysis of new coumarin derivatives which combine the coumarin core and five member heterocycles in hydrazinylidene-chroman-2,4-diones. The derivatives were prepared by derivatization of the appropriate heterocyclic amines which were used as electrophiles to attack the coumarin ring. The structures were characterized by spectroscopic techniques including IR, NMR, 2D-NMR and MS. These derivatives were further characterized especially in terms of a potential cytotoxic and apoptogenic effect in several cancer cell lines including the breast and prostate cancer cell lines MCF-7, MDA-MB-231, PC-3, LNCaP, and the monocytic leukemia cell line U937. Cell viability was determined after 48 h and 72 h of treatment with the novel compounds by MTT assay and the 50% inhibitory concentrations (EC_{50} values) were determined. Out of the 8 novel compounds screened for reduced cell viability, **4c**, **4d** and **4e** were found to be the most promising and effective ones having EC_{50} values that were several fold reduced when compared to the reference substance 4-hydroxycoumarin. However, the effects were cancer cell line dependent. The breast cancer MDA-MB-231 cells, the prostate cancer LNCaP cells, and U937 cells were most sensitive, MCF-7 cells were less sensitive, and PC-3 cells were more resistant. Reduced cell viability was accompanied by increased apoptosis as shown by PARP-1 cleavage and reduced activity of the survival protein kinase Akt.

In summary, this study has identified three novel coumarin derivatives that in comparison to 4-hydroxycoumarin have a higher efficiency to reduce cancer cell viability and trigger apoptosis and therefore may represent interesting novel drug candidates.

1. Introduction

Coumarins are a group of heterocyclic compounds synthesized by numerous plant species as well as by some bacteria and fungi.[1,2] According to their chemical structure, they belong to the family of benzopyrones. So far, more than 1300 different coumarins have been identified. The most representative molecule, that is coumarin, has been extensively studied both in biochemical and pharmaceutical fields.[3–5]

Over the past decades, many studies have reported that coumarins and derivatives exert a plethora of biological activities including anti-microbial, anti-viral, anti-coagulant, anti-inflammatory, and anti-cancer effects.[6–13] Best known is the anti-coagulant effect of the 4-hydroxycoumarin derivative warfarin ((*RS*)-4-hydroxy-3-(3-oxo-1-phenyl-butyl)-coumarin) that reached market approval early on.[14–16] A beneficial effect of warfarin in cancer patients leading to prolonged survival was shown by Zacharski and colleagues.[17] Meanwhile, many studies have reported a beneficial effect of coumarins on other cancer types including malignant

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melanoma, leukemia, renal cell carcinoma, prostate and breast cancer cells progression.[18–20] Also, certain platinum(II) complexes of aminocoumarins showed very good in vitro cytotoxicity.[21] A variety of mechanisms have been proposed such as interfering with estrogen synthesis, interfering with cell cycle progression or even acting as inhibitors of cytochrome P450 1.[22]

Also, a number of coumarins with substituents in position 7 (usually some electron-releasing group) and position 3, especially imines,[23,24] were reported and, in general their photosensitivity was tested. Finally, a number of nitrogen-rich compounds were found to be good chemotherapeutic agents[25] especially if a thiazole ring was introduced as shown by Gouda et al.[26] Recent studies have suggested that several coumarin derivatives showed antiproliferative activity in various tumor cells.[13,20,27] Considering those inputs, coumarin as a very versatile biological agent, and nitrogen-rich heterocyclic compounds as good chemotherapeutic agents, it was tempting to combine these moieties and evaluate their activity. Therefore, in this study, we have synthesized several novel 3-substituted thiazolo and isoxazolo hydrazinylidene-chroman-2,4-dione compounds. These compounds were tested for cell viability in different cancer and non-cancer cell lines. We found that three of the novel compounds effectively reduced cell viability in a concentration-dependent manner. A decrease in the level of phospho-Akt and an increase in the level of PARP-1 cleavage strongly argues for the induction of the intrinsic pathway of apoptosis.

2. Materials and methods

2.1. General synthetic procedure

The heterocyclic amines (**1a–h**, 10 mmol) were dissolved in 10 mL water followed by addition of 40 mL of 6 M HCl and the systems were cooled in the ice-salt bath down to −10 °C. Afterwards, an aqueous solution of $NaNO_2$ (10 mmol, 0.7 g/5 mL H_2O) was added slowly drop by drop and stirred vigorously on a magnetic stirrer. After 15 min, fresh solution of 4-hydroxycoumarin (**3**, 10 mmol, 1.62 g) in 10 mL NaOH (10 wt.) was added. Intensively colored and voluminous precipitates (**4a–h**) were obtained immediately which were stirred 15 min. in the bath and 30 min. on room temperature. Finally, they were filtrated by vacuum, washed 3 times with distilled water and dried on air. The purification was carried out by the technique of recrystallization using ethanol as solvent.

2.1.1. 3-[2-(4*H*-1,2,4-Triazol-3-yl)hydrazinylidene]chroman-2,4-dione (4a)

Yellow powder (93%), mp 225–227 °C. FTIR (KBr, ν/cm^{-1}): 3616-3175 (NH, stretching, broad), 3120 (CH, aromatic stretching), 1736 (C=O, stretching), 1617, 1544 (aromatic deformations). ^{1}H NMR (DMSO-d_6, δ/ppm, *J*/Hz): 8.01 dd (7.5, 1.5, H5-coum.), 7.30–7.45 m (H6&H8-coum.), 7.80 dd (7.5, 1.5, H7-coum.), 8.63 br s (H5-triazole), 10.77 s (NH-triazole). ^{13}C NMR (DMSO-d_6, δ/ppm): 157.9 (C2-coum.), 124.4 (C3-coum.), 178.6 (C4-coum.), 126.7 (C5-coum.), 124.8 (C6-coum.), 136.2 (C7-coum.), 117.3 (C8-coum.), 120.3 (C4a-coum.), 154.0 (C8a-coum.), 164.0 (C2-triazole), 145.3 (C5-triazole). TOF-MS-ES+ (*m*/*z*): 280.0582 [M+Na]$^+$, $C_{11}H_7N_5O_3$.

2.1.2. 3-[2-(5-Methylisoxazol-3-yl)hydrazinylidene]chroman-2,4-dione (4b)

Yellow small needles (92%), mp 203–205 °C. FTIR (KBr, ν/cm^{-1}): 3620–3053 (NH, stretching, broad), 1740 (C=O, stretching), 1602, 1525 (aromatic deformations). ^{1}H NMR (DMSO-d_6, δ/ppm, *J*/Hz): 8.00 dd (7.8, 1.7, H5-coum.), 7.30–7.45 m (H6&H8-coum.), 7.80 ddd (8.0, 8.0, 1.5, H7-coum.), 6.63 (H4-isoxazole), 2.46 s (CH_3-isoxazole). ^{13}C NMR (DMSO-d_6, δ/ppm): 157.6 (C2-coum.), 125.7 (C3-coum.), 178.4 (C4-coum.), 126.8 (C5-coum.), 124.8 (C6-coum.), 136.9 (C7-coum.), 117.4 (C8-coum.), 120.4 (C4a-coum.), 154.1 (C8a-coum.), 162.7 (C3-isoxazole), 94.1(C4-isoxazole), 172.0 (C5-isoxazole), 12.3 (CH_3-isoxazole). TOF-MS-ES+ (*m*/*z*): 272.0677 [M+H]$^+$, 294.0440 [M+Na]$^+$, $C_{13}H_{10}N_3O_4$.

2.1.3. 3-[2-(Thiazol-2-yl)hydrazinylidene]chroman-2,4-dione (4c)

Orange-red crystals (82%), mp 209–211 °C. FTIR (KBr, ν/cm^{-1}): 3625-3250 (NH, stretching, broad), 3125, 3080 (CH, aromatic stretching), 1765 (C=O, stretching), 1623, 1606, 1521 (aromatic deformations). ^{1}H NMR (DMSO-d_6, δ/ppm, *J*/Hz): 8.00 dd (8.2, 1.6, H5-coum.), 7.30–7.45 m (H6&H8-coum.), 7.81 ddd (8.0, 8.0, 1.8, H7-coum.), 7.70 d (2.5, H4-thiazole), 7.57 d (2.5, H5-thiazole). ^{13}C NMR (DMSO-d_6, δ/ppm): 157.3 (C2-coum.), 125.0 (C3-coum.), 177.9 (C4-coum.), 126.7 (C5-coum.), 124.8 (C6-coum.), 136.8 (C7-coum.), 117.4 (C8-coum.), 120.4 (C4a-coum.), 154.0 (C8a-coum.), 166.3 (C2-thiazole), 140.4 (C4-thiazole), 117.6 (C5-thiazole). TOF-MS-ES+ (*m*/*z*): 274. 0345 [M+H]$^+$, 296.0160 [M+Na]$^+$, $C_{12}H_7N_3O_3S$.

2.1.4. 3-[2-(5-Methylthiazol-2-yl)hydrazinylidene]chroman-2,4-dione (4d)

Red crystals (63%), mp 218–220 °C. FTIR (KBr, ν/cm^{-1}): 3616-3175 (NH, stretching, broad), 3120 (CH, aromatic stretching), 1736 (C=O, stretching), 1617, 1544 (aromatic deformations). ^{1}H NMR (DMSO-d_6, δ/ppm, *J*/Hz): 8.00 dd (8.0, 1.7, H5-coum.), 7.30–7.45 m (H6&H8-coum. &H4-thiazole), 7.78 ddd (8.0, 8.0, 1.5, H7-coum.), 2.44 d (1.2, CH_3-thiazole). ^{13}C NMR (DMSO-d_6, δ/ppm): 157.3 (C2-coum.), 124.6 (C3-coum.), 177.6 (C4-coum.), 126.6 (C5-coum.), 124.7 (C6-coum.), 136.7 (C7-coum.), 117.3 (C8-coum.), 120.4 (C4a-coum.), 153.9 (C8a-coum.), 164.5 (C2-thiazole), 137.5 (C4-thiazole), 131.7 (C5-thiazole), 11.9 (CH_3-thiazole). TOF-MS-ES+ (*m*/*z*): 288.0403 [M+H]$^+$, 310.0294 [M+Na]$^+$, $C_{13}H_9N_3O_3S$.

2.1.5. 3-[2-(4,5-Dimethylthiazol-2-yl)hydrazinylidene]chroman-2,4-dione (4e)

Carmine-red crystals (65%), mp 206–208 °C. FTIR (KBr, ν/cm^{-1}): 3629-3075 (NH, stretching, broad), 1752 (C=O, stretching), 1616, 1558 (aromatic deformations). ^{1}H NMR (DMSO-d_6, δ/ppm, *J*/Hz): 8.01 dd (7.8, 1.4, H5-coum.), 7.25-7.45 m (H6&H8-coum.), 7.77 ddd (8.0, 8.0, 1.8, H7-coum.), 2.34 s (5-CH_3-thiazole), 2.23 s (4-CH_3-thiazole). ^{13}C NMR (DMSO-d_6, δ/ppm): 157.3 (C2-coum.), 124.0 (C3-coum.), 177.2 (C4-coum.), 126.7 (C5-coum.), 124.7 (C6-coum.), 136.5 (C7-coum.), 117.3 (C8-coum.), 120.6 (C4a-coum.), 153.9 (C8a-coum.), 163.9 (C2-thiazole), 145.0 (C5-thiazole), 144.5 (C4-thiazole), 11.2 (5-CH_3-thiazole), 14.0 (4-CH_3-thiazole). TOF-MS-ES+ (*m*/*z*): 302.0834 [M+H]$^+$, 324.0802 [M+Na]$^+$, $C_{14}H_{11}N_3O_3S$.

2.1.6. 3-[2-(5-*tert*-Butylisoxazol-3-yl)hydrazinylidene]chroman-2,4-dione (4f)

Yellow crystals (86%), mp 225–227 °C. FTIR (KBr, ν/cm^{-1}): 3616–3175 (NH, stretching, broad), 3120 (CH, aromatic stretching), 1736 (C=O, stretching), 1617, 1544 (aromatic deformations). ^{1}H NMR (DMSO-d_6, δ/ppm, *J*/Hz): 8.00 dd (8.0, 1.5, H5-coum.), 7.30–7.45 m (H6&H8-coum.), 7.80 ddd (8.0, 8.0, 1.5, H7-coum.), 6.58 s (H4-isoxazole), 1.35 s (*tert*-CH_3-isoxazole). ^{13}C NMR (DMSO-d_6, δ/ppm): 157.6 (C2-coum.), 125.7 (C3-coum.), 178.3 (C4-coum.), 126.8 (C5-coum.), 124.9 (C6-coum.), 137.0 (C7-coum.), 117.4 (C8-coum.), 120.4 (C4a-coum.), 154.1 (C8a-coum.), 162.4 (C3-isoxazole), 91.2 (C4-isoxazole), 182.8 (C5-isoxazole), 32.1 (*tert*-$C(CH_3)_3$-isoxazole), 28.2 (*tert*-$C(CH_3)_3$-isoxazole). TOF-MS-ES+ (*m*/*z*): 314.1189 [M+H]$^+$, 336.0926 [M+Na]$^+$, 649.2048 [2M+Na]$^+$ $C_{16}H_{15}N_3O_4$.