Digest Journal of Nanomaterials and Biostructures Vol. 8, No. 3, July - September 2013, p. 1079 - 1087

NEW *N*-SUBSTITUTED 5-CHROMENYL-THIAZOLIDINEDIONES AS ANTIMICROBIAL AND ANTIPROLIFERATIVE AGENTS

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The continuous need for discovering new antimicrobial agents, resulted from the alarming development process of bacterial resistance, and for new antiproliferative compounds, derived from the alarming increase of cancers all over the world, determined us to obtain new *N*-substituted 5-chromenyl thiazolidinediones and evaluate them as antimicrobial and antiproliferative agents. The synthetic route started with the condensation of 2,4-thiazolidinedione with 3-formyl-chromones. The new derivatives were treated after with various alkyl halides or 2-iodoacetamide, in order to obtain *N*-substituted compounds. The purity was confirmed by TLC, and all new molecules were characterized by elemental analysis and spectroscopic data (NMR, MS). The antimicrobial activity was assessed on Gram-positive and Gram-negative bacteria and one fungal strain. The compounds showed a promising potential. The antiproliferative effect was evaluated on both murine and human cancer lines and demonstrated a moderate active of the tested new derivatives.

(Received June 3, 2013; Accepted August 10, 2013)

Keywords: Chromenyl-thiazolidinediones; Antibacterial; Antifungal; Antiproliferative

1. Introduction

Infectious diseases are responsible for a great number of deaths in the world population. Given the evidence for the rapid global spread of resistant clinical isolates and the appearance of drug-resistant strains among community acquired infections, the need for discovery or optimization of efficient antimicrobial agents is substantially important. Despite the fact that the antiinfectious arsenal can be considered rich in number of molecules used in therapy, the appearance of the so-called "super bacteria" [1], characterized by a high level of resistance to antibiotics, raises big issues in the antiinfectious treatment area, the therapeutically methods being very restricted. Over the years, different strategies of overcoming the resistance problem were proposed. One is represented by the design of innovative agents having a different mechanism of action, so that it can't occur any cross-resistance with the therapeutic agents already in use. In addition, the treatment of fungal infections is confronting with resistant strains [2], also, involving consequences for the immunosuppressed.

Malignant tumors represent one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor [3]. It is expected to increase by more than 45% in the next 20 years [4]. More than 80% of the chemotherapeutics used today to combat cancers are pro-apoptotic agents, while numerous cancer types are naturally resistant to apoptosis such as gliomas, melanomas, pancreas cancers, and esophageal cancers, and for diverse reasons

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metastatic cancers. It is therefore mandatory to identify novel therapeutics aiming to kill cancer cells resistant to apoptosis.

In the family of heterocyclic compounds, nitrogen-containing heterocycles represent an important class in medicinal chemistry. Thiazolidinediones display a large range of biological activities, such as hypoglycemic, antibacterial, antifungal [5], antituberculous, anti-HIV and antitumor [6, 7].

Chromone represents a core of natural compounds, found in fruits, vegetables, seeds, flowers. Due to their widespread in plants and to their low toxicity, they are found also in the human diet. Molecules containing the chromone fragment (chromones, flavones) are important pharmacophores, manifesting many important biological activities, for example: antioxidant activity [8], anti-inflammatory, antibacterial [9], antifungal, antitumor [10,11].

Considering the large interest for this type of substances and consisting with our group's previous focus of research [12], we have developed a series of new *N*-substituted 5-chromenyl-thiazolidinediones and investigated them as antimicrobial and antiproliferative agents.

2. Experimental

Chemistry

General

Solvents were obtained from commercial sources; the reagents were synthesized in our laboratory. Analytical thin layer chromatography was carried out on precoated Silica Gel $60F_{254}$ sheets using UV absorption for visualization. The melting points were taken with two melting point meters, Electrothermal and MPM-H1 Schorpp and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 400 MHz and 100 MHz, respectively and were in accord with the assigned structures. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The samples were prepared by dissolving the synthesized powder of the compounds in DMSO-d_6 (δ_H= 2.51 ppm, δ_C= 40.1 ppm) as solvent. GC-MS analyses were realized with an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was registered with a Vario El CHNS instrument.

Compounds **2b** and **5** were synthesized according to the literature data [13, 14].

Synthesis of 5-chromenyl-2,4-thiazolidinediones (2b-d)

For synthesis, 1 mmol of the 3-formyl-chromones **1b-d** was refluxed for 3 h with 1 mmol (0.117 g) of 2,4-thiazolidinedione and 4 mmol (0.328 g) of anhydrous sodium acetate in 5 ml of acetic acid. The reaction mixture was cooled, and the crude product was filtered under reduced pressure, washed with water on the filter and purified by recrystallisation from ethanol.

5-((6-Chloro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione (**2b**)

Yield 74%. White powder, mp: 286-8 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.51 (s, 1H, C=CH); 7.71 (d, 1H, C_8-chromone-H); 7.92 (dd, 1H, C_7-chromone-H); 8.09 (d, 1H, C_5-chromone-H); 8.90 (s, 1H, C_2-chromone-H); 12.45 (br, s, *N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 118.4 (chromone-C_8); 121.6 (chromone-C_{4a}); 124.2 (CH); 124.6 (chromone-C_5); 124.9 (chromone-C_3); 126 (chromone-C_6); 131.3 (thiazolidinedione-C_5); 135.3 (chromone-C_7); 154.4 (chromone-C_{8a}); 161.4 (chromone-C_2); 167.9 (thiazolidinedione-C_4); 169.4 (thiazolidinedione-C_2); 174.2 (chromone-C_4). Anal. Calcd. (%) for $C_{13}H_6ClNO_4S$ (307.71): C, 50.74; H, 1.97; N, 4.55; S, 10.42. Found: C, 50, 77; H, 1.96; N, 4.56; S, 10.38. MS (EI, 70 eV): *m/z* 308 [M^+].

5-((6-Fluoro-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione (**2c**)

Yield 85%. Light yellow powder, mp: 288 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.63 (s, 1H, C=CH); 7.83 (d, 1H, C_8-chromone-H); 7.88 (dd, 1H, C_7-chromone-H); 7.91 (d, 1H,

C_5-chromone-H); 8.91 (s, 1H, C_2-chromone-H); 12.52 (br, s, *N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 114.8 (chromone-C_8); 119.2 (chromone-C_{4a}); 122.7 (CH); 123.8 (chromone-C_5); 125.2 (chromone-C_3); 126.2 (chromone-C_6); 132.4 (thiazolidinedione-C_5); 137.1 (chromone-C_7); 156.1 (chromone-C_{8a}); 160.4 (chromone-C_2); 167.6 (thiazolidinedione-C_4); 168.8 (thiazolidinedione-C_2); 175.1 (chromone-C_4). Anal. Calcd. (%) for $C_{13}H_6FNO_4S$ (291.25): C, 53.61; H, 2.08; N, 4.81; S, 11.01. Found: C, 53.55; H 2.09; N 4.79; S, 11.03. MS (EI, 70 eV): *m/z* 292 [M^+].

5-((6,8-Dibromo-4-oxo-4H-chromen-3-yl)methylene)thiazolidine-2,4-dione (**2d**)

Yield 90%. Light yellow powder, mp: 229-300 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.68 (s, 1H, C=CH); 8.16 (d, 1H, C_7-chromone-H); 8.38 (d, 1H, C_5-chromone-H); 9.02 (s, 1H, C_2-chromone-H); 12.47 (br, s, *N*H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 117.4 (chromone-C_6); 122.6 (chromone-C_8); 124.0 (chromone-C_3); 124.3 (CH); 125.8 (chromone-C_{4a}); 126.2 (thiazolidinedione-C_5); 129.9 (chromone-C_5); 136.8 (chromone-C_7); 153.1 (chromone-C_{8a}); 159.9 (chromone-C_2); 166.9 (thiazolidinedione-C_4); 168.9 (thiazolidinedione-C_2); 177.1 (chromone-C_4). Anal. Calcd. (%) for $C_{13}H_5Br_2NO_4S$ (431.06): C, 36.22; H, 1.17; N, 3.25; S, 7.44. Found: C, 36.19; H, 1.18; N, 3.23; S, 7.51. MS (EI, 70 eV): *m/z* 432 [M^+].

Synthesis of *N*-substituted 5-chromenyl-2,4-thiazolidinedione (3, 4, 6-9)

For synthesis, 1 mmol of 5-chromenyl-2,4-thiazolidinedione **2b-d** was stirred for 3 h at room temperature with 1.1 mmol of alkyl halides or iodoacetamide in 6 ml of DMF in the presence of 1.1 mmol (0.062 g) of anhydrous potassium hydroxide. The crude product was filtered under reduced pressure, washed with water on the filter and purified by recrystallisation from ethanol.

5-(6-Chloro-4-oxo-4H-chromen-3-yl-methylene)-3-methyl-thiazolidine-2,4-dione (**3**)

Yield 42%. Light brown powder, mp: 237-238 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 3.25 (s, 3H, -CH_3); 7.50 (d, 1H, C_8-chromone-H); 7.65 (dd, 1H, C_7-chromone-H); 7.82 (s, 1H, C=CH); 8.21 (s, 1H, C_2-chromone-H); 8.38 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 26.3 (CH_3); 119.2 (chromone-C_8); 121.5 (chromone-C_{4a}); 124.3 (CH); 124.7 (chromone-C_5); 124.9 (chromone-C_3); 125.6 (chromone-C_6); 132.1 (thiazolidinedione-C_5); 135.3 (chromone-C_7); 154.3 (chromone-C_{8a}); 162.1 (chromone-C_2); 167.8 (thiazolidinedione-C_4); 168.7 (thiazolidinedione-C_2); 174.3 (chromone-C_4). Anal. Calcd. (%) for $C_{14}H_8ClNO_4S$ (321.74): C, 52.26; H, 2.51; N, 4.35; S, 9.97. Found: C, 52.00; H, 2.50; N, 4.33; S, 10.01. MS (EI, 70 eV): *m/z* 322 [M^+].

5-(6-Chloro-4-oxo-4H-chromen-3-yl-methylene)-3-ethyl-thiazolidine-2,4-dione (**4**)

Yield 40%. Yellow powder, mp: 210-211 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 1.30 (s, 3H, -CH_3); 3.80 (s, 2H, -CH_2-); 7.56 (d, 1H, C_8-chromone-H); 7.77 (dd, 1H, C_7-chromone-H); 7.79 (s, 1H, C=CH); 8.15 (s, 1H, C_2-chromone-H); 8.22 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 13.1 (CH_3); 39.9 (CH_2); 118.8 (chromone-C_8); 121.5 (chromone-C_{4a}); 123.1 (CH); 124.8 (chromone-C_5); 124.9 (chromone-C_3); 125.6 (chromone-C_6); 132.2 (thiazolidinedione-C_5); 135.3 (chromone-C_7); 153.9 (chromone-C_{8a}); 162.1 (chromone-C_2); 167.2 (thiazolidinedione-C_4); 168.9 (thiazolidinedione-C_2); 173.8 (chromone-C_4). Anal. Calcd. (%) for $C_{15}H_{10}ClNO_4S$ (335.76): C, 53.66; H, 3.00; N, 4.17; S, 9.55. Found: C, 53.40; H, 2.99; N, 4.15; S, 9.59. MS (EI, 70 eV): *m/z* 336 [M^+].

2-(5-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)-2,4-dioxo-thiazolidin-3-yl)acetamide (**6**)

Yield 72%. White powder, mp: 258-264 °C. ^1H NMR (DMSO-d_6, 500 MHz, ppm): δ 4.22 (s, 2H, -CH_2-); 7.31 and 7.73 (2 br s, 1H each, *N*H_2); 7.64 (d, 1H, C_8-chromone-H); 7.69 (dd, 1H, C_7-chromone-H); 7.72 (s, 1H, C=CH); 7.93 (s, 1H, C_5-chromone-H); 8.91 (s, 1H, C_2-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 40.5 (CH_2); 118.1 (chromone-C_8); 118.8 (chromone-C_{4a}); 123.1 (CH); 124.0 (chromone-C_5); 125.1 (chromone-C_3); 126.8 (chromone-C_6); 136.6 (thiazolidinedione-C_5); 136.7 (chromone-C_7); 154.1 (chromone-C_{8a}); 162.2 (chromone-C_2); 166.2

(thiazolidinedione-C_4); 167.4 (thiazolidinedione-C_2); 169.1 (H_2N-C=O); 175.3 (chromone-C_4). Anal. Calcd. (%) for $C_{15}H_9ClN_2O_5S$ (364.76): C, 49.39; H, 2.49; N, 7.68; S, 8.79. Found: C, 49.37; H, 2.48; N, 7.69; S, 8.80. MS (EI, 70 eV): *m/z* 366 [M^+].

2-(5-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)-2,4-dioxo-thiazolidin-3-yl)acetamide (**7**)

Yield 70%. White powder, mp: 324-326 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 2.45 (s, 2H, -CH_3-); 4.21 (s, 2H, -CH_2-); 7.33 and 7.74 (2 br s, 1H each, *N*H_2); 7.65 (d, 1H, C_8-chromone-H); 7.70 (dd, 1H, C_7-chromone-H); 7.72 (s, 1H, C=CH); 7.94 (s, 1H, C_5-chromone-H); 8.93 (s, 1H, C_2-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 20.9 (CH_3); 40.5 (CH_2); 118.1 (chromone-C_8); 118.9 (chromone-C_{4a}); 123.1 (CH); 123.2 (chromone-C_5); 125.3 (chromone-C_3); 126.8 (chromone-C_6); 136.7 (thiazolidinedione-C_5); 136.7 (chromone-C_7); 154.1 (chromone-C_{8a}); 162.3 (chromone-C_2); 166.2 (thiazolidinedione-C_4); 167.4 (thiazolidinedione-C_2); 169.1 (H_2N-C=O); 175.3 (chromone-C_4). Anal. Calcd. (%) for $C_{16}H_{12}N_2O_5S$ (344.34): C, 55.81; H, 3.51; N, 8.14; S, 9.31. Found: C, 55.80; H, 3.51; N, 8.13; S, 9.32. MS (EI, 70 eV): *m/z* 345 [M^+].

2-[5-(6-Fluoro-4-oxo-4H-chromen-3-yl-methylene)-2,4-dioxo-thiazolidin-3-yl]-acetamide (**8**)

Yield 40%. Light yellow powder, mp: 270-271 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 4.24 (s, 2H, -CH_2-); 7.28 and 7.72 (2 br s, 1H each, *N*H_2); 7.69 (d, 1H, C_8-chromone-H); 7.75 (dd, 1H, C_7-chromone-H); 7.77 (s, 1H, C=CH); 7.84 (s, 1H, C_5-chromone-H); 8.98 (s, 1H, C_2-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 40.5 (CH_2); 112.3 (chromone-C_8); 117.8 (chromone-C_{4a}); 122.7 (CH); 124.2 (chromone-C_5); 125.1 (chromone-C_3); 126.3 (chromone-C_6); 150.3 (thiazolidinedione-C_5); 158.7 (chromone-C_7); 160.2 (chromone-C_{8a}); 163.1 (chromone-C_2); 166.2 (thiazolidinedione-C_4); 166.4 (thiazolidinedione-C_2); 168.1 (H_2N-C=O); 174.9 (chromone-C_4). Anal. Calcd. (%) for $C_{15}H_9FN_2O_5S$ (348.31): C, 51.72; H, 2.60; N, 8.04; S, 9.21. Found: C, 51.48; H, 2.59; N, 8.00; S, 9.18. MS (EI, 70 eV): *m/z* 349 [M^+].

2-[5-(6,8-Dibromo-4-oxo-4H-chromen-3-yl-methylene)-2,4-dioxo-thiazolidin-3-yl]-acetamide (**9**)

Yield 34%. Light brown powder, mp: 309-313 °C. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 4.21 (s, 2H, -CH_2-); 7.34 and 7.74 (2 br s, 1H each, NH_2); 7.62 (d, 1H, C_7-chromone-H); 7.68 (s, 1H, C=CH); 8.17 (s, 1H, C_5-chromone-H); 8.88 (s, 1H, C_5-chromone-H). ^{13}C NMR (DMSO-d_6, 500 MHz, ppm): δ 43.6 (CH_2); 113.6 (chromone-C_8); 118.4 (chromone-C_{4a}); 118.9 (CH); 124.3 (chromone-C_5); 125.4 (chromone-C_3); 126.1 (chromone-C_6); 127.8 (thiazolidinedione-C_5); 136.6 (chromone-C_7); 140.2 (chromone-C_{8a}); 153.0 (chromone-C_2); 162.1 (thiazolidinedione-C_4); 166.7 (thiazolidinedione-C_2); 169.0 (H_2N-C=O); 174.6 (chromone-C_4). Anal. Calcd. (%) for $C_{15}H_8Br_2N_2O_5S$ (488.11): C, 36.91; H, 1.65; N, 5.74; S, 6.57. Found: C, 36.74; H, 1.64; N, 5.72; S, 6.60. MS (EI, 70 eV): *m/z* 488 [M^+].

Microbiology

Zone diameters were measured to the nearest whole millimeter at a point in which there will be no visible growth after 24 – 48 h.

The antimicrobial activity of the newly synthesized compounds was evaluated according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 1997) using the agar diffusion method [15]. Gentamicin and Fluconazole were purchased from the drug market and used as reference for antibacterial and antifungal activity, respectively. Petri plates containing 20 mL of Mueller Hinton Agar were used for all the bacteria tested and Mueller-Hinton medium supplemented with 2% glucose (providing adequate growth of yeasts) and 0.5 g/L methylene blue (providing a better definition of the inhibition zone diameter) was used for antifungal testing.

After 18h, the bacterial strains were put on a saline solution of NaCl (0.9%), so that the turbidity would be that of MacFarland (10^6 UFC/mL). The inoculum was spread on the surface of

the solidified media. Solutions of the tested compounds were prepared in DMSO. There were three concentrations tested: 10 mg/mL, 5 mg/mL and 1 mg/mL.

Six-millimeter diameter wells were cut from the agar using a sterile cork-borer. A sterile swab was soaked in suspension and then the Mueller-Hinton agar plates were inoculated by streaking the entire surface. After drying for 10-15 minutes, the six millimeter diameter wells were inoculated with 50 µL from each solution. Gentamicin (10 µg/well) and Fluconazole (25 µg/well) were used as antibacterial and antifungal reference, respectively. Plates inoculated with bacteria were incubated for 24 h and those with fungus 48 h, at 37 ^{o}C.

The inhibition zone diameters were measured in millimeters. All the tests were performed in duplicate and the average was taken as final reading.

Antiproliferative

Determination of In Vitro Growth Inhibition Activity in Murine Cancer Cells. Two murine cancer cell lines were used: B16 (mouse melanoma) and CT26 (colorectal carcinoma) [16]. They were grown in Dulbecco's modified essential medium (DMEM) containing 2mM L-glutamine, 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (37°C, 5% CO_2). Exponentially growing 2 cancer cells were plated onto 96-well plates at 5000 cells per well in 200 µl DMEM, and 24 h later the cells were exposed for 48 h to the solvent alone or to the compounds at the indicated concentrations. A stock solution of each compound with the concentration 10 mM and several dilutions (100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM and 3.125 µM) were prepared. Viability was assessed using the MTT (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium) test. The assessment of cell growth using this colorimetric assay is based on the capability of living cells to reduce the yellow product MTT (3-(4,5)-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) to a blue product, formazan, by a reduction reaction that occurs in the mitochondria. The number of living cells after 72 h of culture in the presence (or absence for the negative control) of the various compounds was directly proportional to the intensity of the blue color, which was measured quantitatively at 562 nm by spectrophotometry using a microplate reader (BioKinetics Reader EL340, Fisher Bioblock Scientific, Illkirch, France). Control cells were exposed to 1% DMSO.

Experiments were run in triplicate. Results are presented as the inhibitory concentrations for 50% of cells (IC_{50}) for a 48 h exposure time.

Determination of In Vitro Growth Inhibition Activity in Human Cancer Cells. Seven human cancer cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA), the European Collection of Cell Culture (ECACC, Salisbury, UK) and the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ, Braunschweig, Germany). These seven cell lines included the A549 non-small-cell lung cancer (NSCLC; DSMZ code ACC107), the SKMEL-28 melanoma (ATCC code HTB-72), the Hs683 oligodendroglioma (ATCC code HTB-138), the U373 (ECACC code 08061901), the T98G (ATCC; code CRL-1690) and U251 (ECACC code 09063001) glioblastoma, and the MCF-7 breast cancer (DSMZ; code ACC115) cell lines.

The cells were cultured in RPMI (Lonza, Verviers, Belgium) culture medium supplemented with 10% heat-inactivated fetal calf serum (Lonza), 4 mM glutamine, 100 µg/ml gentamicin, and penicillin-streptomycin (200 U/ml and 200 µg/ml; Lonza).

The overall growth level of each cell line was determined using the colorimetric MTT (3-[4,5-dimethylthiazol-2yl-diphenyl tetrazolium bromide, Sigma, Belgium) assay as detailed previously [17].

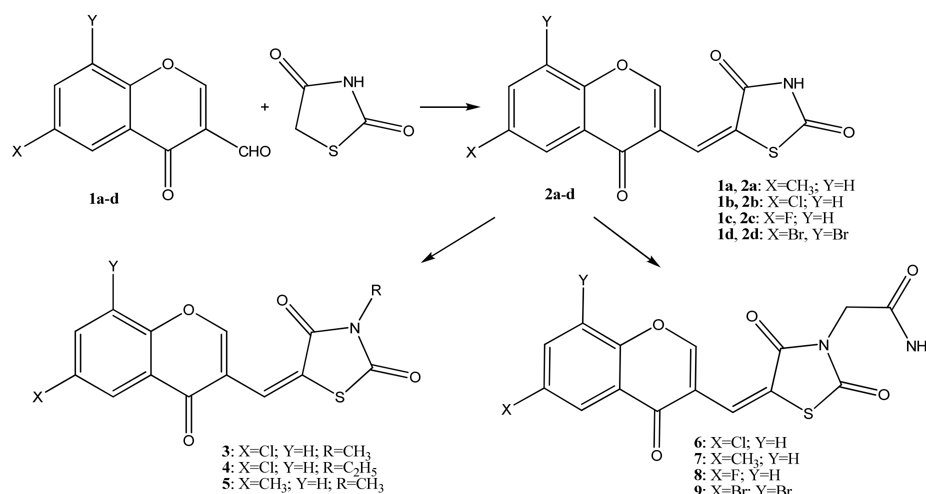
3. Results and discussion

Chemistry

5-chromenyl-methylene-thiazolidinediones **2a-d** were obtained by the condensation of 2,4-thiazolidinedione with 3-formyl-chromones **1a-d** (**Scheme 1**). The new derivatives were

treated with various alkyl halides or 2-iodoacetamide, in order to obtain the new *N*-substituted molecules **3-9**. Compounds 2b and 5 were synthesized according to the literature data [13, 14].

The purity of compounds was confirmed by TLC, and all new molecules were characterized by elemental analysis and spectroscopic data (NMR, MS).



Scheme 1. Synthesis of new chromenyl-thiazolidinedones N-substituted

Microbiology

The new synthesized thiazolidinediones were screened for their antimicrobial activity, using the agar diffusion technique. There were 6 bacterial strains used, 4 Gram-positive (*Listeria monocytogenes* ATCC 13932, *Staphylococcus aureus* ATCC 49444, *Bacillus cereus* ATCC 11778, *Enterococcus faecalis* ATCC 29212) and 2 Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028) and one fungal strain, *Candida albicans* ATCC 10231. The growth inhibitory effects displayed by the new derivatives are shown in **Table 1**.

Table 1. Antimicrobial activity of the new 5-chromenyl-thiazolidinediones

Cp	Gram-positive bacteria				Gram-negative bacteria		Fungus
	L. monocytogenes ATCC 13932	***S. aureus*** ATCC 49444	***B. cereus*** ATCC 11778	***E. faecalis*** ATCC 29212	***E. coli*** ATCC 25922	***S. typhi*** ATCC 14028	***C. albicans*** ATCC 10231
2a	10/10/14	9/14/18	10/11/11	10/11/12	8/12/12	11/20/22	18/18/18
2b	10/14/16	12/14/14	13/14/14	8/9/8	9/18/18	13/24/20	18/18/18
2c	8/14/10	10/16/14	11/12/11	11/13/13	10/20/14	10/20/18	18/16/16
2d	9/20/22	10/16/18	12/13/14	10/11/11	6/16/16	11/20/22	18/18/18
3	14/14/14	12/12/12	6/7/7	10/10/10	10/20/16	10/22/20	13/18/18
4	14/14/18	7/14/12	9/10/10	11/12/12	10/16/16	10/20/22	20/18/18
5	14/14/14	10/14/14	11/12/12	12/12/12	12/12/12	12/24/24	20/18/16
6	18/18/14	20/12/12	13/12/12	13/14/14	18/16/18	18/22/22	14/18/20
7	16/14/14	20/12/12	13/13/12	14/13/14	18/12/14	22/22/24	24/14/14
8	12/14/14	10/14/12	10/12/11	12/12/12	8/14/14	11/18/20	15/16/14

Cp	Gram-positive bacteria				Gram-negative bacteria		Fungus
	L. monocytogenes ATCC 13932	***S. aureus*** ATCC 49444	***B. cereus*** ATCC 11778	***E. faecalis*** ATCC 29212	***E. coli*** ATCC 25922	***S. typhi*** ATCC 14028	***C. albicans*** ATCC 10231
9	16/14/15	18/12/12	13/12/12	14/13/13	18/12/16	20/22/24	22/14/16
Gentamicin	18	19	18	8	22	18	NT
Fluconazole	NT	NT	NT	NT	NT	NT	28

The compounds investigated in this screening showed moderate to good antimicrobial activity on the selected strains. The weakest effect was registered for compound **3** on *Bacillus cereus*. In general, Gram-negative bacteria were more susceptible to the new molecules. For some of the compounds, the diameter of the zone inhibition was bigger than of the reference drugs, suggesting a more powerful activity. For example, the effect of **2d** on *Listeria monocytogenes* at a concentration of 1 mg/mL was higher than of Gentamicin, the diameters of **6** and **7** on *Staphylococcus aureus*, at a concentration of 10 mg/mL were bigger than that of the reference antibiotic. In addition, all derivatives (excepting **2b**) displayed a more powerful effect on *Enterococcus faecalis* than Gentamicin. A good inhibitory activity was displayed also against *Salmonella typhi* by all the compounds, some of them manifesting, at different concentrations, a superior effect to the reference drug. The best activity against this bacterial strain was noted for compounds **2b** (concentration of 5 mg/mL), **5** (concentrations of 5 mg/mL and 1 mg/mL), **7** and **9** (concentration of 1 mg/mL).

The antifungal potential was assessed against *Candida albicans*. All new derivatives displayed moderate to good activity against this strain, but were not superior to Fluconazole, used as reference. The most efficient was compound **7**, with a diameter of 24 mm, at a concentration of 10 mg/mL.

In regard of the antimicrobial screening, it can be observed that for some compounds, the highest activity was registered for the highest concentration, 10 mg/mL, while for others, it was the 5 mg/mL or even 1 mg/mL concentration which led to a bigger inhibition zone diameter. Therefore, we could not establish a direct proportionality relationship between the concentrations of the tested compounds and their effects on the microbial strains used in this assay.

Antiproliferative

Determination of In Vitro Growth Inhibition Activity in Murine Cancer Cells

Some of the compounds were investigated for their antiproliferative effect against two murine cancer lines, B16 and CT26. The obtained results are presented in **Table 2**.

Table 2. Characterization of the in vitro growth inhibitory activity (using the MTT colorimetric assay) of compounds 2a-d, 3, 4, 5, 8.

Compound	IC50 (µM)	
	B 16	CT26
2a	85	67
2b	40	33
2c	72	73
2d	42	52
3	28	[a]
4	66	[a]
5	69	62
8	56	[a]

[a]: >100 µM

The investigated molecules showed good inhibitory activity against the two cell lines, excepting **3**, **4** and **8** which were active only on B16. The highest effect was displayed by compound **3** on B16 (28 μM) and by compound **2b** on CT26 (33 μM).

Determination of In Vitro Growth Inhibition Activity in Human Cancer Cells

Four of the new synthesized compounds were also investigated on seven human cell lines: A549 non-small-cell lung cancer (NSCLC; DSMZ code ACC107), the SKMEL-28 melanoma (ATCC code HTB-72), the Hs683 oligodendroglioma (ATCC code HTB-138), the U373 (ECACC code 08061901), the T98G (ATCC; code CRL-1690) and U251 (ECACC code 09063001) glioblastoma, and the MCF-7 breast cancer (DSMZ; code ACC115) cell lines.

The data (**Table 3**) obtained in this screening are represented as the mean values from one experiment with six replicates in each experimental condition.

Table 3. Characterization of the in vitro growth inhibitory activity (using the MTT colorimetric assay) of compounds 3, 4, 8, 9

Compounds	**IC_{50} concentrations (μM) after having cultured the cancer cells for 3 days with the compound of interest**							
	A549	**SKMEL-28**	**U373**	**U251**	**Hs683**	**MCF-7**	**Min-Max**	**Mean ± SEM**
3	85	*	*	56	*	*	56-100	**
4	80	*	*	34	*	69	34-100	**
8	62	71	82	70	50	56	50-82	65 ± 5
9	72	66	*	64	88	56	56-100	**

* *IC_{50}> 100 μM*

** *The mean value could not be calculated because at least one cell line displayed an IC_{50} > 100 μM*

The data in **Table 3** show that the most potent compound displayed an *in vitro* IC_{50} of 34 μM (**4**), while the remaining compounds in the study displayed weak or no (> 100 μM) *in vitro* growth inhibition activity. In fact, only one (**8**) of the four compounds in the study displayed an antiproliferative activity of < 100 μM against all six cancer cell lines that were analyzed. For this new derivative, the substitution of the chromenyl moiety with a fluorine atom led to a higher growth inhibition effect than substitution with a chlorine or bromine atom.

4. Conclusions

9 new molecules (3 new chromenyl-methylene-thiazolidinediones and 6 *N*-substituted derivatives) were synthesized, characterized and evaluated, together with two derivatives (**2a** and **5**) that we reproduced according to the literature data for their antimicrobial and antiproliferative potentials. Some of the compounds showed a very promising potential as antibacterial and antifungal agents, against the Gram-positive and Gram-negative bacterial strains and against the fungal strain, respectively. There was not a direct proportionality relationship between the different concentrations of the tested compounds and their inhibitory activities.

The antiproliferative effect was evaluated on two murine and seven human cancer cell lines and demonstrated a moderate antiproliferative effect of the tested compounds.

Acknowledgements

This work is published within the internal Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca (Romania) grant financed contract having the no. 27020/5/15.11.2011.

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