

DEVELOPMENT OF NEW 5-(CHROMENE-3-YL) METHYLENE-2,4-THIAZOLIDINEDIONES AS ANTIMICROBIAL AGENTS

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Abstract

Background and aims. In the context of the increasing phenomenon of microbial resistance to usual drugs, the development of new treatment strategies and new therapeutic protocols is a constant need. Thiazolidinedione and chromone represent two important scaffolds in medicinal chemistry due to their large pharmacological applicability.

Methods. We synthesized a new 5-(chromene-3-yl)methylene-2,4-thiazolidinedione starting from 6,8-dichloro-4-oxo-4H-chromene-3-carbaldehyde. Then, by treating with different α -bromoalkylarylketones, we obtained N-substituted derivatives. All new compounds were investigated for their antimicrobial potential, using the diffusion method, against *Listeria monocytogenes* ATCC 13932, *Staphylococcus aureus* ATCC 49444, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Candida albicans* ATCC 10231. Three concentrations, 10 mg/ml, 5 mg/ml and 1 mg/ml of compounds were used. The results were evaluated by the measurement of the inhibition zone diameters and compared to those of gentamicin and fluconazole respectively, as reference drugs.

Results. All new synthesized compounds were characterized using physico-chemical and spectrometric methods. They displayed modest to good antimicrobial activity. New molecules **8**, **9** and **10** may represent promising candidates, showing zone inhibition diameters superior to those of reference drugs.

Conclusions. This work presents chemical synthesis, characterization and investigation of the antibacterial and antifungal potential of 5-(chromene-3-yl)methylene-2,4-thiazolidinedione derivatives, which may be worthy of future research for designing new chemical entities.

Keywords: chromone, 2,4-thiazolidinedione, antibacterial, antifungal

Background and aims

Bacterial infections can cause some of the most serious diseases and widespread epidemics in the world. With the increase in resistance of bacteria to antibiotic treatment, it is essential to develop novel approaches and new antibacterial agents, as alternatives to various existing antimicrobial therapies [1-4].

Several studies have been reported that thiazolidinediones have acquired much importance in medicinal chemistry due to their diverse pharmaceutical effects, such as antihyperglycemic [5], antitumor [6], anti-inflammatory, antioxidant [7], bactericidal, fungicidal [8-11], etc. Different possibilities of heterocyclic modifications with a wide spectrum of pharmacological properties are the most important grounds for investigation of this class of compounds.

There have been many reports in literature depicting that the presence of heterocyclic moieties such as chromone, at fifth position of thiazolidinedione, proves to be more potent and efficacious than a simple aryl group [12]. Chromone and related compounds are widespread in nature and exhibit a wide range of pharmacological activity such as antibacterial, antifungal [13,14], antitumor, antioxidant, anti-HIV, antiulcerant, anti-inflammatory and immune-stimulatory. Therefore, the vast range of biological effects associated with this scaffold has resulted in the chromone ring system being considered as a privileged structure in medicinal chemistry.

Synthetic organic chemistry has always been a vital part of highly integrated and multidisciplinary process of various drug developments. Motivated by these findings and in continuation of our ongoing efforts endowed with the discovery of nitrogen and sulfur-containing heterocycles with potential chemotherapeutic activities, we present here the synthesis and screening of the antimicrobial activities of new 5-(chromene-3-yl)methylene-2,4-thiazolidinedione derivatives.

Methods

Chemistry

General

Solvents and reagents were obtained from commercial sources. Analytical thin layer chromatography was carried out on precoated Silica Gel 60F₂₅₄ sheets using UV absorption for visualization. The melting points were taken with MPM-H1 Schorpp melting point meter and are uncorrected. The ¹H NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 500 MHz and were in accordance 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) as solvent. Mass spectra were recorded by Agilent 1100, type SL spectrometer (positive ionization) and with a Varian

MAT CH-5 spectrometer (70 eV). Elemental analysis was registered with a Vario El CHNS instrument.

Synthesis

General procedure

Synthesis of 5-(chromene-3-yl)methylene-2,4-thiazolidinedione (3)

1 mmol of 6,8-dichloro-4-oxo-4*H*-chromene-3-carbaldehyde **1** was refluxed for 3 h with 1 mmol (0.117 g) of 2,4-thiazolidinedione **2** and 4 mmol (0.328 g) of anhydrous sodium acetate in 5 ml of acetic acid, according to the literature data [12]. The reaction mixture was cooled, and the crude product was filtered under reduced pressure, washed with water on the filter and purified by recrystallization from ethanol.

Synthesis of *N*-substituted 5-(chromene-3-yl)methylene-2,4-thiazolidinedione (4-10)

For synthesis, 1 mmol of 5-chromenyl-2,4-thiazolidinedione **3** was stirred for 30 minutes, at room temperature, with 1.1 mmol (0.062 g) of anhydrous potassium hydroxide, in 6 ml of dimethylformamide (DMF). After the potassium salt was formed, 1.1 mmol of α-bromoalkylarylketones were added. The crude product was filtered under reduced pressure, washed with water on the filter and purified by recrystallization from ethanol.

Microbiology

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 (10⁶ 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°C.

The effects of the new compounds were assessed by measuring the diameter of the growth inhibition zone. Zone diameters were measured to the nearest whole millimeter at a point in which there will be no visible growth after 24–48 h.

All the tests were performed in duplicate and the average was taken as final reading.

Results

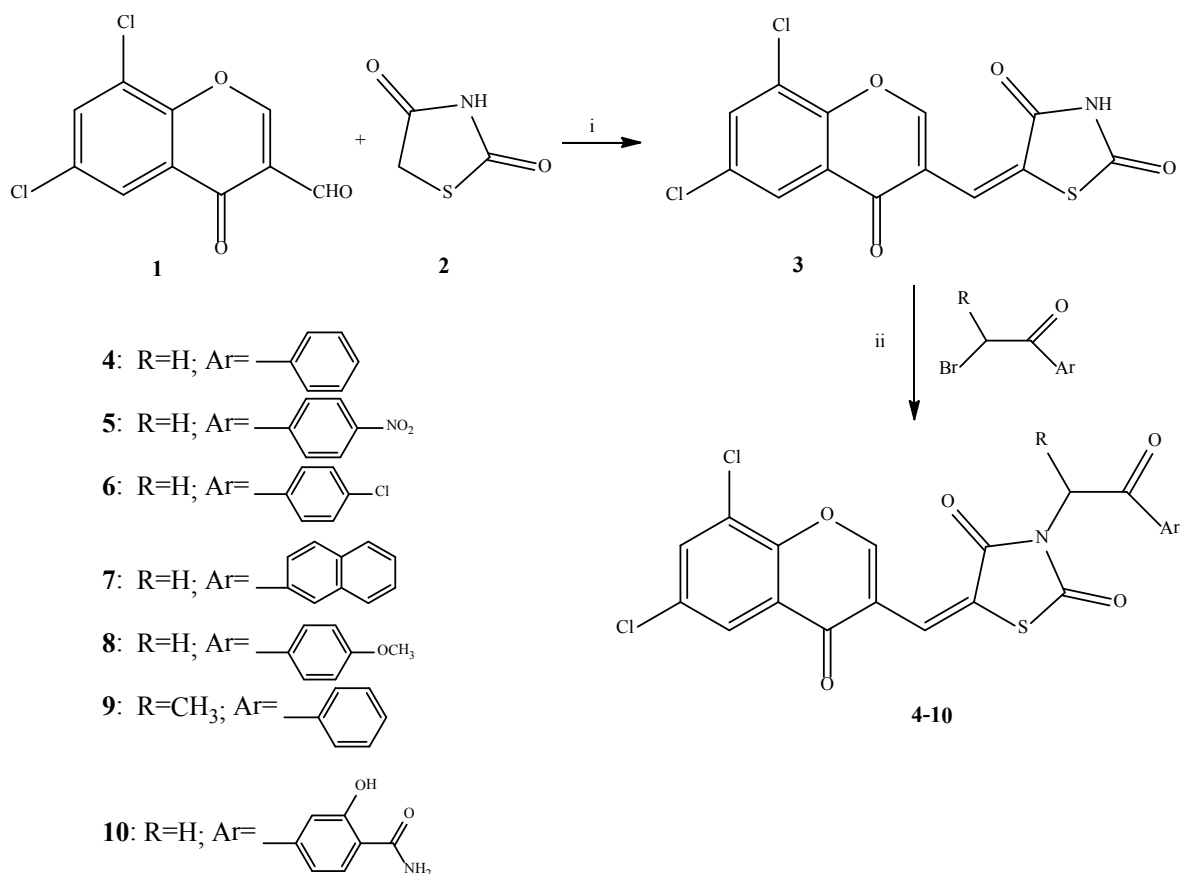
Chemistry

5-(chromene-3-yl)methylene-2,4-thiazolidinedione **3** was obtained by the condensation of 6,8-dichloro-4-oxo-4*H*-chromene-3-carbaldehyde **1** with 2,4-thiazolidinedione **2**, according to the literature data (**Scheme 1**) [12]. The new derivatives were treated with various α -bromoalkylarylketones, in order to obtain the new *N*-substituted molecules **4–10**.

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

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

Yield 80%. Light yellow powder, mp: 300°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 7.56 (s, 1H, C=CH); 8.00 (d, 1H, C₇-chromone-H); 8.24 (d, 1H, C₅-chromone-H); 8.93 (s, 1H, C₂-chromone-H); 12.51 (s, 1H, -NH). Anal. Calcd. (%) for C₁₃H₅Cl₂NO₄S (342.15): C, 45.63; H, 1.47; N, 4.09; S, 9.37. Found: C, 45.74; H, 1.47; N, 4.08; S, 9.39. MS (EI, 70 eV): *m/z* (%) 343 [M+1] (100%), 313 (30%), 268 (40%), 183 (30%).



Scheme 1. Synthesis of new *N*-substituted 5-(chromene-3-yl)methylene-2,4-thiazolidinediones.

i: anhydrous sodium acetate/acetic acid, 3h reflux; ii: α -bromoalkylarylketones, anhydrous potassium hydroxide, DMF, 30 minutes, stirring, room temperature

5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-3-(2-oxo-2-phenylethyl)thiazolidine-2,4-dione (4)

Yield 75%. Yellow powder, mp: 224°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 5.31 (s, 2H, -CH₂-); 7.64 (t, 2H, phenyl); 7.74 (d, 1H, C₇-chromone-H); 7.75 (t, 1H, phenyl); 7.78 (s, 1H, C=CH); 7.92 (d, 1H, C₅-chromone-H); 8.10 (d, 2H, phenyl); 8.90 (s, 1H, C₂-chromone-H). Anal. Calcd. (%) for C₂₁H₁₁Cl₂NO₅S (460.29): C, 54.80; H, 2.41; N, 3.04; S, 6.97. Found: C, 54.59; H, 2.41; N, 3.03; S, 6.99. MS (EI, 70 eV): *m/z* (%) 461 [M+1], 301 (100%), 271 (70%).

5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-3-(2-(4-nitrophenyl)-2-oxoethyl) thiazolidine-2,4-dione (5)

Yield 65%. Light brown powder, mp: 234°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 5.20 (s, 2H, -CH₂-); 7.14 (d, 2H, phenyl); 7.68 (dd, 1H, C₇-chromone-H); 7.78 (s, 1H, C=CH); 7.95 (s, 1H, C₅-chromone-H); 8.04 (d, 2H, phenyl); 8.98 (s, 1H, C₂-chromone-H). Anal. Calcd. (%) for C₂₁H₁₀Cl₂N₂O₇S (505.28): C, 49.92; H, 1.99; N, 5.54; S, 6.35. Found: C, 49.81; H, 1.98; N, 5.53; S, 6.36. MS (EI, 70 eV): *m/z* (%) 505 [M+1] (100%), 279 (55%), 205 (35%), 149 (10%).

3-(2-(4-chlorophenyl)-2-oxoethyl)-5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-thiazolidine-2,4-dione (6)

Yield 65%. Light yellow powder, mp: 222°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 5.24 (s, 2H, -CH₂-); 7.14 (d, 2H, phenyl); 7.70 (dd, 1H, C₇-chromone-H); 7.78 (s, 1H, C=CH); 7.96 (s, 1H, C₅-chromone-H); 8.04 (d, 2H, phenyl); 8.98 (s, 1H, C₂-chromone-H). Anal. Calcd. (%) for C₂₁H₁₀Cl₃NO₅S (494.73): C, 50.98; H, 2.04; N, 2.83; S, 6.48. Found: C, 50.78; H, 2.04; N, 2.83; S, 6.50. MS (EI, 70 eV): *m/z* (%) 495 [M+1], 299 (100%), 271 (90%).

5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-3-(2-(naphthalen-2-yl)-2-oxoethyl) thiazolidine-2,4-dione (7)

Yield 72%. Light yellow powder, mp: 220°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 5.31 (s, 2H, -CH₂-); 7.68 (d, 1H, C₇-chromone-H); 7.68-7.74 (m, 1H, naphthyl); 7.70 (dd, 1H, naphthyl); 7.75 (d, 1H, naphthyl); 7.79 (s, 1H, C=CH); 7.95 (s, 1H, C₅-chromone-H); 8.05 (d, 1H, naphthyl); 8.09 (d, 1H, naphthyl); 8.17 (d, 1H, naphthyl); 8.90 (s, 1H, naphthyl); 8.97 (s, 1H, C₂-chromone-H). Anal. Calcd. (%) for C₂₅H₁₃Cl₂NO₅S (510.35): C, 58.84; H, 2.57; N, 2.74; S, 6.28. Found: C, 58.63; H, 2.56; N, 2.75; S, 6.27. MS (EI, 70 eV): *m/z* (%) 511 [M+1], 372 (90%), 354 (40%), 328 (100%), 300 (30%).

5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-3-(2-(4-methoxyphenyl)-2-oxoethyl)

thiazolidine-2,4-dione (8)

Yield 70%. White powder, mp: 224°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 3.03 (s, 3H, -CH₃); 5.24 (s, 2H, -CH₂-); 7.12 (d, 2H, phenyl); 7.68 (d, 1H, C₇-chromone-H); 7.68-7.75 (s, 1H, C=CH); 7.94 (s, 1H, C₅-chromone-H); 8.06 (d, 2H, phenyl); 8.97 (s, 1H, C₂-chromone-H). Anal. Calcd. (%) for C₂₂H₁₃Cl₂NO₆S (490.31): C, 53.89; H, 2.67; N, 2.86; S, 6.54. Found: C, 53.68; H, 2.66; N, 2.85; S, 6.56. MS (EI, 70 eV): *m/z* (%) 491 [M+1], 372 (95%), 326 (100%).

5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-3-(1-oxo-1-phenylpropan-2-yl) thiazolidine-2,4-dione (9)

Yield 70%. Light brown powder, mp: 220°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 2.46 (s, 3H, -CH₃); 5.45 (s, 1H, -CH(CH₃)-); 7.64 (t, 2H, phenyl); 7.72 (d, 1H, C₇-chromone-H); 7.75 (t, 1H, phenyl); 7.79 (s, 1H, C=CH); 7.95 (d, 1H, C₅-chromone-H); 8.10 (d, 2H, phenyl); 8.92 (s, 1H, C₂-chromone-H). Anal. Calcd. (%) for C₂₂H₁₃Cl₂NO₅S (474.31): C, 55.71; H, 2.76; N, 2.95; S, 6.76. Found: C, 55.60; H, 2.76; N, 2.96; S, 6.78. MS (EI, 70 eV): *m/z* (%) 475 [M+1], 299 (100%), 273 (60%).

5-(2-(5-((6,8-dichloro-4-oxo-4H-chromen-3-yl)methylene)-2,4-dioxothiazolidin-3-yl)acetyl)-2-hydroxybenzamide (10)

Yield 70%. Light yellow powder, mp: 299°C. ¹H NMR (DMSO-*d*₆, 500 MHz, ppm): δ 5.30 (s, 2H, -CH₂-); 7.09 (d, 1H, phenyl); 7.44 (d, 1H, phenyl); 7.70 (d, 1H, C₇-chromone-H); 7.76 (s, 1H, C=CH); 7.96 (s, 1H, C₅-chromone-H); 7.98 (br, 1H, -CO-NH₂); 8.10 (dd, 1H, phenyl); 8.42 (br, 1H, -CO-NH₂); 8.94 (s, 1H, C₂-chromone-H); 13.90 (s, 1H, Ar-OH). Anal. Calcd. (%) for C₂₂H₁₂Cl₂N₂O₇S (519.31): C, 50.88; H, 2.33; N, 5.39; S, 6.17. Found: C, 50.77; H, 2.32; N, 5.40; S, 6.15. MS (EI, 70 eV): *m/z* (%) 520 [M+1] (100%), 371 (20%).

Microbiology

The new synthesized compounds were investigated for their antimicrobial potential, using the agar diffusion technique. Two Gram-positive bacteria (*Listeria monocytogenes* ATCC 13932, *Staphylococcus aureus* ATCC 49444), two Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028) and one fungus strain (*Candida albicans* ATCC 10231) were used in this screening. The compounds were tested in three concentrations, 10 mg/ml, 5 mg/ml and 1 mg/ml, respectively. The effects were quantified by the measurement of the growth inhibition zone diameter (in millimeters) and the comparison with those displayed by the drugs used as reference: gentamicin for the antibacterial activity and fluconazole for the antifungal, respectively. DMSO was tested as control. It did not present growth inhibitory effect. The registered diameters are presented in **Table I**.

Table I. Diameters of the growth inhibition zones (mm) for the new 5-(chromene-3-yl)methylene-2,4-thiazolidinedione derivatives.

Cp 10/5/1 (mg/ml)	Gram-positive bacteria		Gram-negative bacteria		Fungus
	<i>L. monocytogenes</i> ATCC 13932	<i>S. aureus</i> ATCC 49444	<i>E. coli</i> ATCC 25922	<i>S. typhi</i> ATCC 14028	<i>C. albicans</i> ATCC 10231
3	18/18/18	12/12/16	14/14/14	15/16/15	18/18/18
4	18/18/18	20/20/20	14/14/6	15/16/13	20/18/18
5	14/14/16	14/14/12	14/14/14	16/18/15	18/18/22
6	16/16/16	16/14/14	18/18/14	18/16/15	22/20/20
7	14/16/14	10/10/10	18/18/18	18/18/20	16/16/16
8	18/22/18	22/12/12	12/14/14	15/19/20	20/18/18
9	22/22/20	24/28/28	18/18/16	20/18/16	18/18/16
10	28/28/28	28/28/28	18/18/18	18/18/18	22/22/22
Gentamicin	18	19	22	18	NT
Fluconazole	NT	NT	NT	NT	28

NT: not tested

Discussion

The synthesis of the new *N*-substituted 5-(chromene-3-yl)methylene-2,4-thiazolidinediones had two steps. Firstly, 5-(6,8-dichloro-chromenyl)-2,4-thiazolidinedione **3** was obtained by Knoevenagel condensation between 6,8-dichloro-4-oxo-4*H*-chromene-3-carbaldehyde and 1,3-thiazolidine-2,4-dione, in acetic acid and in the presence of anhydrous sodium acetate. The substitution at the level of thiazolidinedione nitrogen was realized with different α -bromoalkylarylketones and took place in the presence of anhydrous potassium hydroxide and DMF.

The purity of all compounds was verified by TLC. The combined use of elemental analysis and spectral methods confirmed the structures proposed for the synthesized compounds. The ¹H NMR spectra showed characteristic singlets due to –CH= proton coming from formyl group, at δ 7.56–7.79 ppm, demonstrating the synthesis of 5-chromenyl-thiazolidinedione. The disappearance, in the spectra, of the signal due to the H from –NH– in thiazolidinedione heterocycle and the appearance of signals specific for the phenyl protons, evidenced the *N*-substitution and thus, the synthesis of the new derivatives.

The antimicrobial screening revealed modest to good growth inhibitory effect against the standardized strains used. Substances **3**, **4** and **8** showed the same activity as gentamicin, the reference drug, against the strain of *L. monocytogenes* ATCC 13932, while derivatives **9** and **10** had a better effect. The most powerful inhibition was demonstrated by compound **10**, which, for all the concentrations used (10 mg/ml, 5 mg/ml and 1 mg/ml) displayed a 28 mm diameter of the growth inhibition zone. The activity against the strain of *S. aureus* ATCC

49444 was promising for molecules **4** and **8** and

very good for **9** and **10**. These 4 substances had a better effect than the reference antibacterial drug.

The inhibition against Gram-negative strains was not remarkable. All new synthesized chromenyl-thiazolidinediones showed diameters lower than that of gentamicin, in the case of *E. coli* ATCC 25922, while against *S. typhi* ATCC 14028, compounds **5**, **6**, **7**, **9** and **10** had a similar effect to the reference. Molecules **7** and **8**, both at 1 mg/ml concentration, and **9**, at 10 mg/ml, displayed a better effect than of the antibiotic used.

The growth of the strain of *C. albicans* ATCC 10231 was inhibited by all the investigated derivatives, but the diameters were lower than of fluconazole. Compound **10**, in all three concentrations, displayed the most promising effect.

We used three concentrations of the new molecules in this screening, in order to see if there is a direct relationship between the concentration and the antimicrobial effect. Due to the fact that some substances showed a better activity at a lower concentration and vice-versa, we can say that we could not establish a direct influence of the concentration on the inhibitory activity.

Conclusions

The present work describes the synthesis of eight new 5-(chromene-3-yl)methylene-2,4-thiazolidinediones **3–10**. The structures of all synthesized molecules were fully confirmed by physical data, elemental analyses, MS and ¹H NMR spectroscopy in solution.

As far as biological activity is concerned, all new compounds showed moderate to good antimicrobial effect. Some of them exerted a growth inhibitory activity superior to the reference drugs used. The screening showed promising

potential for the new molecules **8**, **9** and **10**. There was no direct proportionality between the concentrations used and the inhibitory effect.

Our results suggest that selected 5-(chromene-3-yl)methylene-2,4-thiazolidinedione derivatives may be worthy of future research for designing new chemical entities with antibacterial or antifungal activity.

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