SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 2-HYDRAZONE-THIAZOLINE-4-ONES

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Abstract

A new series of 2-hydrazone-thiazoline-4-ones 3a-d and 2-hydrazone-5-arylidene-thiazoline-4-ones 4a-h, 5a-f and 6a-b were synthesized starting from various thiosemicarbazones by the Hantzsch condensation with chloroacetic acid. The newly synthesized compounds were screened for their antimicrobial activity against 4 strains of bacteria: Staphylococcus aureus (ATCC 29213), Bacillus subtilis (ATCC 60511), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 10145) and one fungal strain: Candida albicans (ATCC 10231). The compounds 3a-c demonstrated a good inhibitory activity against E. coli. The results of the antifungal screening showed that the 2-hydrazone-thiazolin-4-ones 3c, 3d and 4b presented an excellent activity against Candida albicans.

Keywords: thiazolin-4-ones, thiosemicarbazones, antibacterial, antifungal

Rezumat

Au fost sintetizate noi serii de 2-hidrazon-tiazolin-4-one 3a-d şi 2-hidrazon-5-ariliden-tiazolin-4-one 4a-h, 5a-f si 6a-b, pornind de la diverse thiosemicarbazone prin condensare Hantzsch cu acid cloroacetic. A fost investigată activitatea antimicrobiană a noilor compuşi sintetizaţi pe 4 tulpini bacteriene: Staphylococcus aureus (ATCC 29213), Bacillus subtilis (ATCC 60511), Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 10145) şi pe o tulpină fungică: Candida albicans (ATCC 10231). Compuşi 3a-c au demonstrat o activitate inhibitoare bună pe E. coli. Rezultatele screeningului antifungic au arătat faptul că 2-hidrazon-tiazolin-4-onele 3c, 3d şi 4b au prezentat o activitate foarte bună pe Candida albicans.

Keywords: thiazolin-4-ones, thiosemicarbazones, antibacterial, antifungal
Introduction

The treatment of bacterial and fungal infectious diseases remains a challenging problem because of the increasing number of multi-drug microbial pathogens [9, 11].

Nowadays, the design of new compounds able to deal with resistant bacteria, having new structures and new targets of action, has become one of the most important areas in the antibacterial research purpose [17].

It has been observed that thiazoles and their derivatives represent a prevalent scaffold in antimicrobial drug discovery because of their varied biological activity [2, 5, 6, 8]. Also, thiazolin-4-ones are an important group of heterocyclic compounds, having a wide range of pharmacological activities, including antibacterial and antifungal effects [1, 3, 10].

In addition, the chromone derivatives are gaining importance as medicinal agents, such as antibacterial and antifungal [7, 13].

It has been reported that the introduction of an arylidene moiety in position 5 of the thiazolin-4-one ring and of a hydrazone group in position 2 enhances the antimicrobial activity [4, 15, 18].

Prompted by these reports, we decided to synthesize some new compounds containing the thiazoline-4-one moiety linked to chromone or arylidene rings. Our aim was also to study their antimicrobial activity against 4 bacterial strains: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, and one fungal strain: Candida albicans.

Materials and Methods

The melting points were registered using an Electrothermal melting point meter and were uncorrected. FT-IR spectra were recorded on a Nicolet 210 FT-IR spectrometer using potassium bromide. The $^1$H NMR spectra were recorded at room temperature on a Bruker Avance NMR spectrometer operating at 500 MHz. Chemical shift values were reported relative to tetramethylsilane (TMS) as internal standard. The samples were prepared by dissolving the compounds in DMSO-d$_6$ (δH= 2.51 ppm) as solvent and the spectra were recorded using a single excitation pulse of 10.1 μs. GC-MS analyses were performed on an Agilent gas chromatograph 6890 equipped with an apolar Macherey Nagel Permabond SE 52 capillary column. Elemental analysis was performed using a Vario El CHNS instrument. All compounds gave satisfactory CHNS quantitative elemental analysis results. The purity of the synthesized compounds was verified by thin layer chromatography (TLC) and was carried out on precoated Silica Gel 60F254 sheets using heptan-ethylacetate 1:3 system and UV light for visualization.
The synthesis of 4-formyl-2-phenyl-thiazole 1d was previously reported [14]. The 3-formyl-chromones are Merck products.

**Synthesis of thiosemicarbazones (2a-d) (General procedure) [12]**

In a flask equipped with a reflux condenser, a mixture of carbonyl compound 1a-d (30 mmol) and thiosemicarbazide (30 mmol) reacted in 40mL ethanol in the presence of a catalytic amount of acetic acid. The reaction mixture was heated under reflux 3h, whereupon the solid product partially crystallized out. The solution was left to cool and the separated solid product was filtered off, washed with water, dried, and recrystallized from ethanol to obtain compounds 2a-d.

**Synthesis of thiazolin-4-ones 3a-d (General procedure)**

A mixture of thiosemicarbazone 2a-d (20mmol), chloroacetic acid (20mmol), anhydrous sodium acetate (40mmol) and absolute ethanol (50mL) was refluxed for 8h. The products obtained upon cooling were collected by filtration, washed with water, dried, and recrystallized from ethanol.

**Synthesis of 5-arylidene-substituted thiazolin-4-ones 4a-j, 5a-f and 6a-b (General procedure)**

There were added equimolar amounts of the appropriate aldehyde (10mmol) and anhydrous sodium acetate (40mmol) to a solution of 3a-d (10mmol) in glacial acetic acid (5mL). The reaction mixture was heated under reflux for 8 h. After cooling, the precipitated solid was filtered off, washed with water and recrystallized from ethanol-DMF.

**In vitro antibacterial and antifungal activity**

The newly synthesized compounds were screened for their antimicrobial activity against 4 bacterial strains: *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 60511), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145) and one fungal strain: *Candida albicans* ATCC 10231, by the agar diffusion technique and MIC (minimal inhibitory concentration) determination. The organisms were obtained from the Microbiological Laboratory of University of Medicine and Pharmacy Cluj-Napoca, Romania.

**Experimental procedures for the antimicrobial activity**

**Disk diffusion method**

The antimicrobial activity of the newly synthesized compounds was evaluated according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997) using the agar disk diffusion method [16]. Ciprofloxacin and Fluconazole were purchased from the Romanian market and used as reference for the antibacterial and antifungal activity, respectively. Petri plates containing 20 mL of Mueller Hinton Agar were
used for all the bacteria tested. Candida albicans strain was cultivated in Sabouraud’s dextrose agar. The inoculum was spread on the surface of the solidified media. Solutions of the tested compounds were prepared in DMSO at a concentration of 5 mg/0.5mL. Sterile Whatman no. 1 filter paper disks (6mm in diameter) impregnated with the solution in DMSO of the test compounds (20μL solution corresponding to 200μg compound/disk) were placed on the Petri plates. Ciprofloxacin (200μg/disc) was used as positive control for bacteria. Fluconazole (200μg/disc) was used as positive control for Candida albicans. A paper disk impregnated with dimethylsulfoxide (DMSO) was used as negative control. Plates inoculated with the bacteria were incubated for 24h at 37⁰C and the fungal culture was incubated for 72h at 25⁰C. The inhibition zone diameters were measured in millimeters. All the tests were performed in duplicate and the average was taken as final reading.

**Determination of MIC**

The MIC (µg/mL) were determined by the binary microdilution method in 96 multi-well microtitre plates. Solutions of the test compounds, Ciprofloxacin and Fluconazole were prepared in DMSO at a concentration of 100 μg/mL. From this stock solutions, serial dilutions of the compounds (50, 25, 12.5, 6.25, 3.12 and 1.56 µg/mL) were prepared under aseptic conditions in a final volume of 200μL of nutrient medium. 50μL of microbial inoculums were added to all tubes, which were incubated at 37⁰C for 24h. The MIC were recorded in each case as the minimum concentration of the compound which inhibited the visible growth of the tested microorganism. All determinations were performed in duplicate and the average was taken as final reading. 50μL of DMSO were used as a negative control.

**Results and Discussion**

**Chemistry**

The synthetic strategies adopted to obtain the targeted compounds are outlined in figures 1 and 2. In order to synthesize the thiosemicarbazones 2a-d, we used as start compounds various aromatic aldehydes: 2,6-dichlorobenzaldehyde, salicylaldehyde, 2,4-dichlorobenzaldehyde and 4-formyl-2-phenyl-thiazole. By the reaction of benzaldehydes 1a-d with thiosemicarbazide in refluxing ethanol, the correspondent thiosemicarbazones 2a-d were obtained in very good yields. The condensation of 2a-d with chloroacetic acid in boiling ethanol and in the presence of anhydrous sodium acetate yielded the 2-hydrazone-thiazolin-4-ones 3a-d.
The presence of active methylene group in position 5 of the thiazoline-4-one nucleus allows the possibility of condensation with various aromatic aldehydes, in order to obtain a series of 5-arylidene-derivatives. Thus, refluxing 3a-c with various aryl-aldehydes in the presence of anhydrous sodium acetate, in boiling acetic acid, we obtained 5-arylidene-thiazolino-4-ones 4a-h and 5a-f (Figure 1). In the same conditions were obtained the 5-arylidene-thiazolin-4-ones 6a-b, starting from 3d (Figure 2).

![Diagram](image_url)

**Figure 1**
Synthesis of thiazolin-4-ones 3a-c, 5-arylidene-thiazolin-4-ones 4a-h and 5a-f
Figure 2

Synthesis of 5-arylidene-thiazolin-4-ones 6a-b

The 2-hydrazone-thiazolin-4-ones 3a-d were characterized by the presence of a strong band at 1710-1735 cm⁻¹ in the IR spectra attributed to C=O group from thiazolin-4-one. This is considered to be an important confirmation data for the thiazolin-4-one nucleus formation. Other spectral data important for the cyclisation was the appearance of a singlet signal for the 2 protons in position 5 of the thiazolin-4-one ring, in the 1H NMR spectra, in the 3.90-4.05 ppm area. Mass spectra showed molecular ion peaks in agreement with the molecular formula. The most important fragmentation is that of the N-N bond.

The disappearance of the singlet signal of the 2 protons in position 5 of the thiazolyl-4-one ring and the appearance of a singlet in the 6.55-6.99 ppm area in the 1H NMR spectra, attributed to CH=C thiiazoline, confirmed the condensation in 5 and the formation of 5-arylidene-derivatives 4a-h, 5a-f and 6a-b.

2-(2,6-dichlorobenzylidene)-hydrazinecarbothioamide (2a). White powder; Yield 90%; mp: 245-246°C. 1H NMR (DMSO-d6): δ ppm = 7.51-7.88 (m, 3H, Ar-H), 7.92 (br, s, 1H, -NH), 8.09 (s, 1H, CH=N), 8.12 (br, s, 1H, -NH-), 11.32 (s, 1H, NH). Anal. Calcd. (%) for C₈H₇Cl₂N₃S (248.13): C 38.72; H 2.84; N 16.93; S 12.92. Found: C 38.56; H 2.83; N 16.85; S 12.86.

2-(2-methoxybenzylidene)-hydrazinecarbothioamide (2b). White powder; Yield 88%; mp: 214-215°C. 1H NMR (DMSO-d6): δ ppm = 3.78 (s, 3H, OCH₃), 7.14-7.59 (m, 4H, Ar-H), 7.88 (br, s, 1H, -NH-), 8.13 (s, 1H, CH=N), 8.16 (br, s, 1H, -NH-), 11.22 (s, 1H, NH). Anal. Calcd. (%) for C₉H₁₁N₃OS (209.27): C 51.65; H 5.30; N 20.08; S 15.32. Found: C 51.63; H 5.32; N 20.12; S 15.4.
2-(2,4-dichlorobenzylidene)-hydrazinecarbothioamide (2c). White powder; Yield 83%; mp: 244-246°C. 1H NMR (DMSO-d6): δ_{ppm} = 7.45-7.77 (m, 3H, Ar-H), 7.78 (br, s, 1H, -NH), 8.09 (br, s, 1H, -NH-), 8.18 (s, 1H, CH=N), 11.15 (s, 1H, NH). Anal. Calcd. (%) for C₈H₇Cl₂N₃S (248.13): C 38.72; H 2.84; N 16.93; S 12.92. Found: C 38.53; H 2.83; N 16.99; S 12.91.

2-((2-phenylthiazol-4-yl)-methylene)-hydrazinecarbothioamide (2d). Yellow powder; Yield 80%; mp: 245-246°C. 1H NMR (DMSO-d6): δ_{ppm} = 7.65 (s, 1H, C₅-H thiazol), 7.51-8.57 (m, 5H, Ar-H), 7.70 (br, s, 1H, CH=N), 8.02 (br, s, 1H, CH=N), 8.09 (s, 1H, CH=N), 11.25 (s, 1H, NH). Anal. Calcd. (%) for C₁₁H₁₀N₄S₂ (262.35): C 50.36; H 3.84; N 21.36; S 24.44. Found: C 50.46; H 3.83; N 21.4; S 24.42.

2-(2,6-Dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (3a). White powder; Yield 75%; mp: 227-228°C. IR(KBr): ν/cm⁻¹ = 3246 (NH), 1729 (C=O), 1605 (C=N). 1H NMR (DMSO-d6): δ_{ppm} = 3.95 (s, 2H, S-CH₂), 7.55-7.90 (m, 3H, Ar-H), 8.10 (s, 1H, CH=N), 11.34 (s, 1H, NH). MS: m/z = 288 (M⁺). Anal. Calcd. (%) for C₁₀H₇Cl₂N₃O (288.15): C 41.68; H 2.45; N 14.58; S 11.13. Found: C 41.47; H 2.44; N 14.51; S 11.16.

2-(2-Methoxybenzylidene)hydrazinyl)-thiazol-4(5H)-one (3b). White-yellow powder; Yield 78%; mp: 236-237°C. IR(KBr): ν/cm⁻¹ = 3248 (NH), 1733 (C=O), 1606 (C=N). 1H NMR (DMSO-d6): δ_{ppm} = 3.92 (s, 2H, S-CH₂), 7.55-7.90 (m, 3H, Ar-H), 8.10 (s, 1H, CH=N), 11.44 (s, 1H, NH). MS: m/z = 249 (M⁺). Anal. Calcd. (%) for C₁₁H₁₀N₃O₂S (249.29): C 53.00; H 4.45; N 16.86; S 12.86. Found: C 52.88; H 4.44; N 16.77; S 12.73.

2-(2,4-Dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (3c). White powder; Yield 69%; mp: 301-302°C. IR(KBr): ν/cm⁻¹ = 3230 (NH), 1730 (C=O), 1609 (C=N). 1H NMR (DMSO-d6): δ_{ppm} = 3.95 (s, 2H, S-CH₂), 7.55-7.90 (m, 3H, Ar-H), 8.10 (s, 1H, CH=N), 11.44 (s, 1H, NH). MS: m/z = 288 (M⁺). Anal. Calcd. (%) for C₁₀H₇Cl₂N₃O (288.15): C 41.68; H 2.45; N 14.58; S 11.13. Found: C 41.47; H 2.44; N 14.51; S 11.16.

2-(2-Phenylthiazol-4-yl)methylene)hydrazinyl)-thiazol-4(5H)-one (3d). White-yellow powder; Yield 76%; mp: 230-232°C. IR(KBr): ν/cm⁻¹ = 3241 (NH), 1720 (C=O), 1602 (C=N). 1H NMR (DMSO-d6): δ_{ppm} = 3.92 (s, 2H, S-CH₂), 7.55 (s, 1H, C₅-H thiazol), 7.65-8.02 (m, 5H, Ar-H), 8.12 (s, 1H, CH=N), 11.37 (s, 1H, NH). MS: m/z = 302 (M⁺). Anal. Calcd. (%) for C₁₃H₁₀N₃O₂S (302.37): C 51.64; H 3.33; N 18.53; S 21.21. Found: C 51.77; H 3.32; N 18.61; S 21.31.
5-(2,6-Dichlorobenzylidene)-2-(2-(2,6-dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (4a). Yellow powder, Yield 55%; mp: 290-291 °C. IR(KBr): ν/cm\(^{-1}\)=3243 (NH), 1727 (C=O), 1605 (C=N). \(^1\) H NMR (DMSO-d\(_6\)): \(\delta_{ppm}\) = 6.70 (s, 1H, CH=C\(_{5\text{thiazoline}}\)), 7.25-7.95 (m, 6H, Ar-H), 8.03 (s, 1H, CH=N), 11.12 (s, 1H, NH). MS: m/z = 445 (M\(^+\)). Anal. Calcd. (%) for C\(_{17}\)H\(_9\)Cl\(_2\)N\(_2\)O\(_2\): C 53.18; H 3.23; N 10.37; S 7.91. Found: C 53.32; H 3.22; N 10.33; S 7.86.

2-(2-(2,6-Dichlorobenzylidene)hydrazinyl)-5-(2-methoxybenzylidene)-thiazol-4(5H)-one (4b). Yellow powder, Yield 58%; mp: 258-260 °C. IR(KBr): ν/cm\(^{-1}\)=3235 (NH), 1710 (C=O), 1609 (C=N). \(^1\) H NMR (DMSO-d\(_6\)): \(\delta_{ppm}\) = 6.79 (s, 1H, CH=C\(_{5\text{thiazoline}}\)), 7.12-7.65 (m, 7H, Ar-H), 8.83 (s, 1H, CH=N), 11.32 (s, 1H, NH). MS: m/z = 406 (M\(^+\)). Anal. Calcd. (%) for C\(_{18}\)H\(_{12}\)Cl\(_2\)N\(_2\)O\(_2\): C 53.21; H 3.22; N 10.34; S 7.89. Found: C 53.32; H 3.22; N 10.33; S 7.86.

5-(2-Methoxybenzylidene)-2-(2-(2-methoxybenzylidene)hydrazinyl)-thiazol-4(5H)-one (4c). Yellow powder, Yield 61%; mp: 295-297 °C. IR(KBr): ν/cm\(^{-1}\)=3237 (NH), 1715 (C=O), 1603 (C=N). \(^1\) H NMR (DMSO-d\(_6\)): \(\delta_{ppm}\) = 3.71 (s, 3H, OCH\(_3\)), 6.55 (s, 1H, CH=C\(_{5\text{thiazoline}}\)), 7.13-7.70 (m, 8H, Ar-H), 8.04 (s, 1H, CH=N), 11.26 (s, 1H, NH). MS: m/z = 367(M\(^+\)). Anal. Calcd. (%) for C\(_{19}\)H\(_{13}\)Cl\(_2\)N\(_2\)O\(_2\): C 48.47; H 2.39; N 13.30; S 7.61. Found: C 48.55; H 2.39; N 13.33; S 7.64.

5-(2-Dichlorobenzylidene)-2-(2-(2-dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (4d). Yellow powder, Yield 69%; mp: 246-247\(^\circ\) C. IR(KBr): ν/cm\(^{-1}\)=3237 (NH), 1723 (C=O), 1611 (C=N). \(^1\) H NMR (DMSO-d\(_6\)): \(\delta_{ppm}\) = 3.31 (s, 3H, OCH\(_3\)), 3.74 (s, 3H, OCH\(_3\)), 6.55 (s, 1H, CH=C\(_{5\text{thiazoline}}\)), 7.13-7.70 (m, 8H, Ar-H), 8.04 (s, 1H, CH=N), 11.26 (s, 1H, NH). MS: m/z = 367(M\(^+\)). Anal. Calcd. (%) for C\(_{19}\)H\(_{13}\)Cl\(_2\)N\(_2\)O\(_2\): C 62.11; H 4.66; N 11.44; S 8.73. Found: C 62.21; H 4.65; N 11.39; S 8.75.

5-(2,6-Dichlorobenzylidene)-2-(2-(2,6-dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (4e). Yellow powder, Yield 63%; mp: 249-250 °C. IR(KBr): ν/cm\(^{-1}\)=3240 (NH), 1722 (C=O), 1604 (C=N). \(^1\) H NMR (DMSO-d\(_6\)): \(\delta_{ppm}\) = 3.79 (s, 3H, OCH\(_3\)), 6.79 (s, 1H, CH=C\(_{5\text{thiazoline}}\)), 7.15-7.66 (m, 7H, Ar-H), 8.08 (s, 1H, CH=N), 11.30 (s, 1H, NH). MS: m/z = 406 (M\(^+\)). Anal. Calcd. (%) for C\(_{19}\)H\(_{13}\)Cl\(_2\)N\(_2\)O\(_2\): C 53.21; H 3.23; N 10.34; S 7.89. Found: C 53.18; H 3.23; N 10.37; S 7.91.

5-(4-Bromobenzylidene)-2-(2-(2,6-dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (4f). Yellow powder, Yield 66%; mp: 305 °C. IR(KBr): ν/cm\(^{-1}\)=3229 (NH), 1718 (C=O), 1605 (C=N). \(^1\) H NMR (DMSO-d\(_6\)): \(\delta_{ppm}\)=
6.60 (s, 1H, CH=C₃thiazole), 7.16-7.59 (m, 7H, Ar-H), 8.11 (s, 1H, CH=N), 11.35 (s, 1H, NH). MS: m/z = 455 (M⁺). Anal. Calcd. (%) for C₁₇H₂₀BrCl₂N₃OS (455.16): C 55.03; H 2.86; N 9.17; S 7.00. Found: C 55.12; H 2.85; N 9.2; S 6.97.

5-(2,4-Dichlorobenzylidene)-2-(2-(2,4-dichlorobenzylidene)hyrazinyl)thiazol-4(5H)-one (4g). Yellow powder, Yield 57%; mp: 267-268°C. IR(KBr): ν/cm⁻¹=3236 (NH), 1717 (C=O), 1604 (C=N). 1H NMR (DMSO-d₆): δppm= 6.66 (s, 1H, CH=C₃thiazole), 7.15-7.55 (m, 6H, Ar-H), 8.09 (s, 1H, CH=N), 11.12 (s, 1H, NH). MS: m/z = 445 (M⁺). Anal. Calcd. (%) for C₁₇H₁₂BrCl₂N₃OS (445.15): C 45.87; H 2.04; N 9.44; S 7.20. Found: C 45.69; H 2.03; N 9.41; S 7.19.

5-(4-Bromobenzylidene)-2-(2-(2,4-dichlorobenzylidene)hyrazinyl)thiazol-4(5H)-one (4h). Yellow powder, Yield 60%; mp: 277-278°C. IR(KBr): ν/cm⁻¹=3235 (NH), 1723 (C=O), 1610 (C=N). 1H NMR (DMSO-d₆): δppm= 6.59 (s, 1H, CH=C₃thiazole), 7.18-7.49 (m, 7H, Ar-H), 8.04 (s, 1H, CH=N), 11.29 (s, 1H, NH). MS: m/z = 455 (M⁺). Anal. Calcd. (%) for C₁₇H₁₂BrCl₂N₃OS (455.16): C 45.87; H 2.04; N 9.44; S 7.20. Found: C 45.76; H 2.21; N 9.41; S 7.05.

2-(2-(2,6-Dichlorobenzylidene)hyrazinyl)-5-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)thiazol-4(5H)-one (5a). White-yellow powder, Yield 44%; mp: 308°C. IR(KBr): ν/cm⁻¹=3244 (NH), 1750 (C=O chromone), 1740 (C=O thiazolin-4-one), 1607 (C=N). 1H NMR (DMSO-d₆): δppm= 2.59, (s, 3H, CH₃), 6.99 (s, 1H, CH=C₃thiazole), 7.15-7.65 (m, 3H, Ar-H), 7.41 (s, 1H, C₂-chromone-H), 7.47 (s, 1H, C₅-chromone-H), 7.55 (d, 1H, C₈-chromone-H), 7.66 (d, 1H, C⁷-chromone-H), 8.66 (s, 1H, CH=N), 12.62 (s, 1H, NH). MS: m/z = 458 (M⁺). Anal. Calcd. (%) for C₂₁H₁₃Cl₂N₃O₃S (458.32): C 55.03; H 2.86; N 9.17; S 7.00. Found: C 55.12; H 2.85; N 9.2; S 6.97.

2-(2-(2,6-Dichlorobenzylidene)hyrazinyl)-5-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)thiazol-4(5H)-one (5b). White-yellow powder, Yield 49%; mp: 309°C. IR(KBr): ν/cm⁻¹=3239 (NH), 1753 (C=O chromone), 1720 (C=O thiazolin-4-one), 1602 (C=N). 1H NMR (DMSO-d₆): δppm= 6.99 (s, 1H, CH=C₃thiazole), 7.20-7.55 (m, 3H, Ar-H), 7.47 (s, 1H, C₅-chromone-H), 7.48 (s, 1H, C₂-chromone-H), 7.54 (d, 1H, C₈-chromone-H), 7.62 (d, 1H, C⁷-chromone-H), 8.23 (s, 1H, CH=N), 12.69 (s, 1H, NH). MS: m/z = 479 (M⁺). Anal. Calcd. (%) for C₂₀H₁₀Cl₃N₃O₃S (478.74): C 50.18; H 2.11; N 8.78; S 6.70. Found: C 50.26; H 2.11; N 8.75; S 6.73.

2-(2-(2,6-Dichlorobenzylidene)hyrazinyl)-5-((6-fluoro-4-oxo-4H-chromen-3-yl)methylene)thiazol-4(5H)-one (5c). White-yellow powder,
Yield 56%; mp: 305-307 °C. IR(KBr): ν/cm⁻¹=3239 (NH), 1755 (C=O chromone), 1722 (C=O thiazolin-4-one), 1606 (C=N). ¹H NMR (DMSO-d₆): δ ppm= 6.84 (s, 1H, CH=C₅-thiazoline), 7.25-7.41 (m, 3H, Ar-H), 7.47 (s, 1H, C₆-chromone-H), 7.55 (d, 1H, C₈-chromone-H), 7.58 (s, 1H, C₅-chromone-H), 7.74 (d, 1H, C₇-chromone-H), 8.72 (s, 1H, CH=N), 12.54 (s, 1H, NH). MS: m/z = 462 (M⁺). Anal. Calcd. (%) for C₂₀H₁₅Cl₂N₃O₅S (462.28): C 51.96; H 2.18; N 9.09; S 6.94. Found: C 51.76; H 2.17; N 9.05; S 6.91.

2-(2-(2,6-Dichloro-benzylidene)hydrazinyl)-5-((6-dibromo-4-oxo-4H-chromen-3-yl)methylene)-thiazol-4(5H)-one (5d). White-yellow powder, Yield 55%; mp: 342-343 °C. IR(KBr): ν/cm⁻¹=3242 (NH), 1751 (C=O chromone), 1733 (C=O thiazolin-4-one), 1600 (C=N). ¹H NMR (DMSO-d₆): δ ppm= 6.88 (s, 1H, CH=C₅-thiazoline), 7.15-7.46 (m, 3H, Ar-H), 7.43 (s, 1H, C₅-chromone-H), 7.44 (s, 1H, C₂-chromone-H), 7.51 (s, 1H, C₇-chromone-H), 8.59 (s, 1H, CH=N), 12.57 (s, 1H, NH). MS: m/z = 602 (M⁺). Anal. Calcd. (%) for C₂₀H₁₉Br₂Cl₂N₃O₅S (602.08): C 39.90; H 1.51; N 6.98; S 5.33. Found: C 39.78; H 1.51; N 6.96; S 5.34.

2-(2-(2-Methoxybenzylidene)hydrazinyl)-5-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)-thiazol-4(5H)-one (5e). White-yellow powder, Yield 61%; mp: 296-298 °C. IR(KBr): ν/cm⁻¹=3239 (NH), 1740 (C=O chromone), 1730 (C=O thiazolin-4-one), 1604 (C=N). ¹H NMR (DMSO-d₆): δ ppm= 2.51 (s, 3H, CH₃), 3.93 (s, 3H, OCH₃), 6.88 (s, 1H, CH=C₅-thiazoline), 7.25-7.55 (m, 4H, Ar-H), 7.48 (s, 1H, C₅-chromone-H), 7.54 (d, 1H, C₈-chromone-H), 7.57 (s, 1H, C₂-chromone-H), 7.62 (d, 1H, C₇-chromone-H), 8.53 (s, 1H, CH=N), 12.54 (s, 1H, NH). MS: m/z = 420 (M⁺). Anal. Calcd. (%) for C₂₂H₁₇FN₃O₅S (419.45): C 63.00; H 4.09; N 10.02; S 7.64. Found: C 63.05; H 4.08; N 10.08; S 7.63.

2-(2-(2-Methoxybenzylidene)hydrazinyl)-5-((6-chloro-4-oxo-4H-chromen-3-yl)methylene)-thiazol-4(5H)-one (5f). White-yellow powder, Yield 67%; mp: 296-298 °C. IR(KBr): ν/cm⁻¹=3239 (NH), 1739 (C=O chromone), 1728 (C=O thiazolin-4-one), 1600 (C=N). ¹H NMR (DMSO-d₆): δ ppm= 3.87 (s, 3H, OCH₃), 6.99 (s, 1H, CH=C₅-thiazoline), 7.15-7.44 (m, 4H, Ar-H), 7.45 (s, 1H, C₂-chromone-H), 7.49 (s, 1H, C₅-chromone-H), 7.51 (d, 1H, C₈-chromone-H), 7.66 (d, 1H, C₇-chromone-H), 8.62 (s, 1H, CH=N), 12.59 (s, 1H, NH). MS: m/z = 440 (M⁺). Anal. Calcd. (%) for C₂₃H₁₄ClN₃O₅S (439.87): C 57.34; H 3.21; N 9.55; S 7.29. Found: C 57.42; H 3.22; N 9.57; S 7.32.

2-(2-((2-Phenylthiazol-4-yl)methylene)-5-(4-hydroxybenzylidene)-hydrazinyl)-thiazol-4(5H)-one (6a). Yellow-orange powder, Yield 46%;
mp: 284-285 °C. IR(KBr): ν/cm⁻¹=3307 (OH), 3255 (NH), 1727 (C=O), 1605 (C=N). ¹H NMR (DMSO-d₆): δppm= 5.88 (s, 1H, OH), 6.59 (s, 1H, CH=C₅-thiazoline), 7.11-7.67 (m, 9H, Ar-H), 7.88 (s, 1H, C₅-H thiazol), 8.30 (s, 1H, CH=N), 12.40 (s, 1H, NH). MS: m/z = 407 (M⁺). Anal. Calcd. (%) for C₂₀H₁₄N₄O₂S₂ (406.48): C 59.10; H 3.47; N 13.78; S 15.78. Found: C 59.25; H 3.46; N 13.74; S 15.76.

2-(2-((2-Phenylthiazol-4-yl)methylene)hydrazinyl)-5-(4-bromobenzylidene)-thiazol-4(5H)-one(6b). Yellow-orange powder, Yield 51%; mp: 310-312 °C. IR(KBr): ν/cm⁻¹=3237 (NH), 1731 (C=O), 1598 (C=N). ¹H NMR (DMSO-d₆): δppm= 6.55 (s, 1H, CH=C₅-thiazoline), 7.20-7.79 (m, 9H, Ar-H), 7.91 (s, 1H, C₅-H thiazol), 8.33 (s, 1H, CH=N), 12.32 (s, 1H, NH). MS: m/z = 469 (M⁺). Anal. Calcd. (%) for C₂₀H₁₃BrN₄OS₂ (469.38): C 51.18; H 2.79; N 11.94; S 13.66. Found: C 51.41; H 2.78; N 11.99; S 13.71.

In vitro antibacterial and antifungal activity

The results of the antimicrobial evaluation are summarised in table I.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Zone of inhibition (mm) and MIC* values (µg.mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.aureus</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
</tr>
<tr>
<td>3b</td>
<td>-</td>
</tr>
<tr>
<td>3c</td>
<td>-</td>
</tr>
<tr>
<td>3d</td>
<td>-</td>
</tr>
<tr>
<td>4a</td>
<td>11 (&gt;50)</td>
</tr>
<tr>
<td>4b</td>
<td>10(&gt;50)</td>
</tr>
<tr>
<td>4c</td>
<td>7</td>
</tr>
<tr>
<td>4d</td>
<td>5</td>
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<td>4e</td>
<td>8</td>
</tr>
<tr>
<td>4f</td>
<td>6</td>
</tr>
<tr>
<td>4g</td>
<td>6</td>
</tr>
<tr>
<td>4h</td>
<td>-</td>
</tr>
<tr>
<td>5a</td>
<td>10(&gt;50)</td>
</tr>
<tr>
<td>5b</td>
<td>-</td>
</tr>
<tr>
<td>5c</td>
<td>-</td>
</tr>
<tr>
<td>5d</td>
<td>-</td>
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<tr>
<td>5e</td>
<td>-</td>
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<tr>
<td>5f</td>
<td>-</td>
</tr>
<tr>
<td>6a</td>
<td>-</td>
</tr>
<tr>
<td>6b</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>23 (1.56)</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>-</td>
</tr>
<tr>
<td>DMSO (dimethyl sulfoxide)</td>
<td>-</td>
</tr>
</tbody>
</table>

*The MIC values were determined only for the active compounds with a zone of inhibition > 10mm. The MIC values were evaluated in the range 1.56-50µg/mL.
None of the compounds inhibited the growth of *Pseudomonas aeruginosa* and *Bacillus subtilis*. The activity against *S. aureus* was also moderate. The compounds 3a-c had an interesting activity against *E. coli*. The results of the anti-fungal screening showed that only the compounds 3c, 3d and 4b presented a good activity against *C. albicans*. However, for all the tested compounds, MIC values were lower than for Ciprofloxacin and Fluconazole, the two drugs used as reference.

Unexpected, the presence of the chromone ring in the structures of 5a-f did not enhance the antimicrobial activity. Despite the literature data [3, 15, 18], the introduction of the aryldiene moiety in position 5 of the thiazolin-4-ones diminished or even canceled the antibacterial and antifungal activities. In the case of 6a-b, the presence of p-bromo-benzylidene or 4-hydroxy-benzylidene fragments in the 5-position of thiazolin-4-one ring cancels the activity against *Candida albicans*.

In addition, the presence of thiazole ring in the structure of 3d and 6a-b didn't show to be favorable for the activity against the bacterial strains used in this study. On the other hand, the activity against *C. albicans* for 3d was increased by the presence of the thiazole ring.

**Conclusion**

We reported the synthesis and antimicrobial activity of a new series of 2-hydrazone-thiazoline-4-ones 3a-d and 2-hydrazone-5-aryldene-thiazoline-4-ones 4a-h, 5a-f and 6a-b obtained from various aryldene-thiosemicarbazones. Some of the compounds were found to be very active against *E. coli* and *Candida albicans*. The introduction of the aryldiene moiety in position 5 of the thiazolin-4-ones or the presence of the chromone nucleus diminished or even canceled the antibacterial and antifungal activities.

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**References**


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