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-hydrazon-thiazolin-4-ones **3c, 3d** and **4b** presented an excellent activity against *Candida albicans*.

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 tiosemicarbazone prin condensare Hantzsch cu acid cloroacetic. A fost investigată activitatea antimicrobiană a noilor compuşi sintetizati 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).* Compuşii **3a-c** au demonstrat o activitate inhibitorie 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-*d6* (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, where upon 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 37^0C and the fungal culture was incubated for 72h at 25^0C. 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

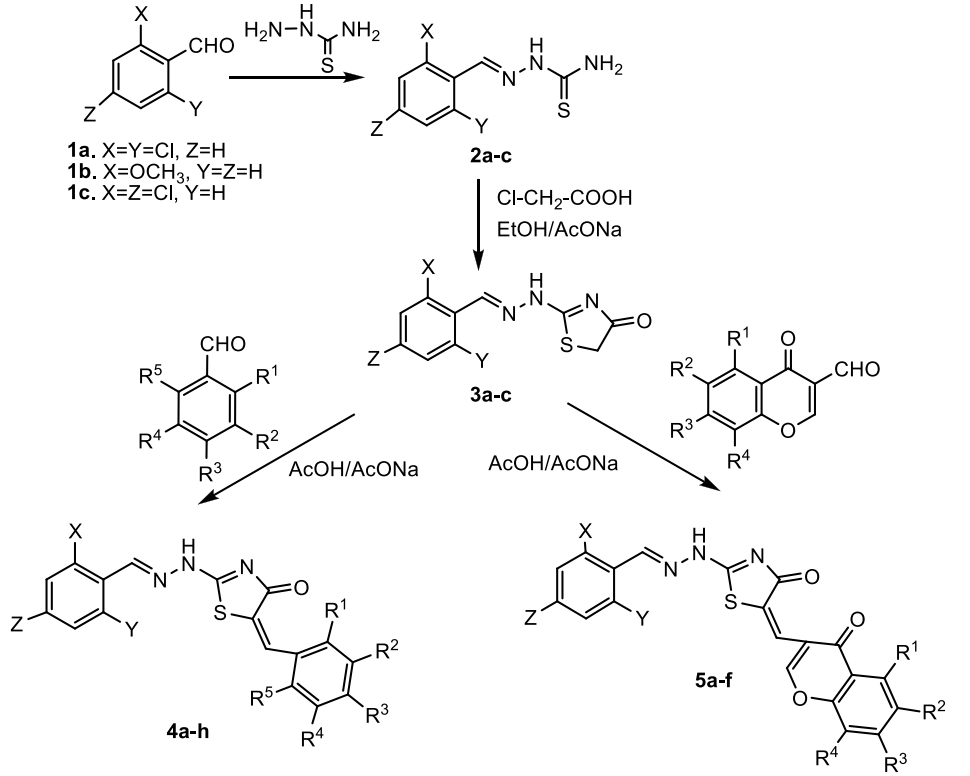
The MIC (µg/mL) were determined by the binary microdilution method in 96 multi-well microtitre plates. Solutions of the test compounds, Ciprofloxacin and Fluconazole were prepared in DMSO at a concentration of 100 µg/mL. From this stock solutions, serial dilutions of the compounds (50, 25, 12.5, 6.25, 3.12 and 1.56µg/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 37^0C 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-dichloro-benzaldehyde 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-thiazolin-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**).



4a: X=Y=Cl, Z=H, R^1=R^5=Cl, R^2=R^3=R^4=H
4b: X=Y=Cl, Z=H, R^1=OCH_3, R^2= R^3=R^4=R^5=H
4c: X=Y=Cl, Z=H, R^1=R^2=R^4=R^5=H, R^3=NO_2
4d: X=OCH_3, Y=Z=H, R^1=OCH_3, R^2=R^3=R^4=R^5= H
4e: X=OCH_3, Y=Z=H, R^1=R^5=Cl, R^2=R^3=R^4=H
4f: X=Y=Cl, Z=H, R^3=Br, R^1=R^2=R^4=R^5=H
4g: X=Y=Cl, Z=H, R^1=R^3=Cl, R^2=R^4=R^5=H
4h: X=Z=Cl, Y=H, R^3=Br, R^1=R^2=R^4=R^5=H

5a: X=Y=Cl, Z=H, R^1=R^3=R^4=H, R^2=CH_3
5b: X=Y=Cl, Z=H, R^1=R^3=R^4=H, R^2=Cl
5c: X=Y=Cl, Z=H, R^1=R^3=R^4=H, R^2=F
5d: X=Y=Cl, Z=H, R^1=R^3=H, R^2=R^4=Br
5e: X=OCH_3, Y=Z=H, R^1=R^3=R^4=H, R^2=CH_3
5f: X=OCH_3, Y=Z=H, R^1=R^3=R^4=H, R^2=Cl

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

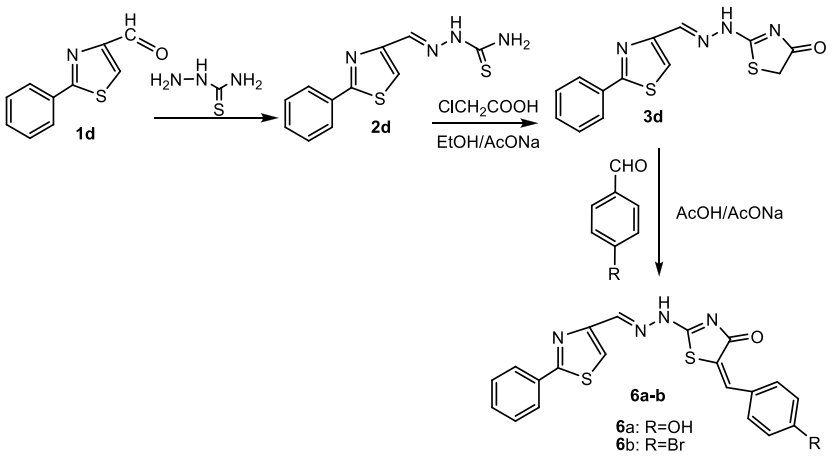


Figure 2

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 $CH=C_{5thiazoline}$, 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 ^0C. ^1H NMR (DMSO-d_6): 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 $C_8H_7Cl_2N_3S$ (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: 214-215^0 C. ^1H NMR (DMSO-d_6): δ_{ppm} = 3.78 (s, 3H, OCH_3), 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 $C_9H_{11}N_3OS$ (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: 244-246^0 C. ^{1}H NMR (DMSO-d_6): 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 $C_8H_7Cl_2N_3S$ (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: 245-246^0 C. ^{1}H NMR (DMSO-d_6): δ_{ppm} = 7.65 (s, 1H, C_5-H thiazol), 7.51-8.57 (m, 5H, Ar-H), 7.70 (br, s, 1H, -NH-), 8.02 (br, s, 1H, -NH-), 8.24 (s, 1H, CH=N), 11.25 (s, 1H, NH). Anal. Calcd. (%) for $C_{11}H_{10}N_4S_2$ (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-(2,6-Dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (3a). White powder; Yield 75%; mp: 227-228^0 C. IR(KBr): ν/cm^{-1}=3246 (NH), 1729 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 4.02 (s, 2H, S-CH_2), 7.55-7.98 (m, 3H, Ar-H), 8.04 (s, 1H, CH=N), 11.32 (s, 1H, NH). MS: m/z = 288 (M^+). Anal. Calcd. (%) for $C_{10}H_7Cl_2N_3OS$ (288.15): C 41.68; H 2.45; N 14.58; S 11.13. Found: C 41.48; H 2.44; N 14.56; S 11.17.

2-(2-(2-Methoxybenzylidene)hydrazinyl)-thiazol-4(5H)-one (3b). White-yellow powder; Yield 78%; mp: 236-237^0 C. IR(KBr): ν/cm^{-1}=3248 (NH), 1733 (C=O), 1606 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 3.73 (s, 3H, OCH_3), 3.98 (s, 2H, S-CH_2), 7.18-7.55 (m, 4H, Ar-H), 8.09 (s, 1H, CH=N), 11.34 (s, 1H, NH). MS: m/z = 249 (M^+). Anal. Calcd. (%) for $C_{11}H_{11}N_3O_2S$ (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-(2,4-Dichlorobenzylidene)hydrazinyl)-thiazol-4(5H)-one (3c). White powder; Yield 69%; mp: 301-302^0 C. IR(KBr): ν/cm^{-1}=3230 (NH), 1730 (C=O), 1609 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 3.95 (s, 2H, S-CH_2), 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 $C_{10}H_7Cl_2N_3OS$ (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-((2-Phenylthiazol-4-yl)methylene)hydrazinyl))-thiazol-4(5H)-one (3d). White-yellow powder; Yield 76%; mp: 230-232^0 C. IR(KBr): ν/cm^{-1}=3241 (NH), 1720 (C=O), 1602 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 3.92 (s, 2H, S-CH_2), 7.55 (s, 1H, C_5-H thiazol), 7.56-8.02 (m, 5H, Ar-H), 8.12 (s, 1H, CH=N), 11.37 (s, 1H, NH). MS: m/z = 302 (M^+). Anal. Calcd. (%) for $C_{13}H_{10}N_4OS_2$ (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 ^{0}C. IR(KBr): ν/cm^{-1}=3243 (NH), 1727 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-*d*$_6$): δ_{ppm}= 6.70 (s, 1H, CH=C$_{5thiazoline}$), 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 $C_{17}H_9Cl_4N_3OS$ (445.15): C 45.87; H 2.04; N 9.44; S 7.20. Found: C 45.75; H 2.03; N 9.41; S 7.17.

2-(2-(2,6-Dichlorobenzylidene)hydrazinyl)-5-(2-methoxybenzylidene)-thiazol-4(5H)-one (4b). Yellow powder, Yield 58%; mp: 258-260 ^{0}C. IR(KBr): ν/cm^{-1}=3235 (NH), 1710 (C=O), 1609 (C=N). ^{1}H NMR (DMSO-*d*$_6$): δ_{ppm}= 3.71 (s, 3H, OCH_3), 6.74 (s, 1H, CH=C$_{5thiazoline}$), 7.25-7.97 (m, 7H, Ar-H), 8.89 (s, 1H, CH=N), 11.32 (s, 1H, NH). MS: m/z = 406 (M^+). Anal. Calcd. (%) for $C_{18}H_{13}Cl_2N_3O_2S$ (406.29): C 53.21; H 3.23; N 10.34; S 7.89. Found: C 53.32; H 3.22; N 10.33; S 7.86.

2-(2-(2,6-Dichlorobenzylidene)hydrazinyl)-5-(4-nitrobenzylidene)-thiazol-4(5H)-one (4c). Yellow powder, Yield 61%; mp: 295-297 ^{0}C. IR(KBr): ν/cm^{-1}=3234 (NH), 1715 (C=O), 1603 (C=N). ^{1}H NMR (DMSO-*d*$_6$): δ_{ppm}= 6.79 (s, 1H, CH=C$_{5thiazoline}$), 7.12-7.65 (m, 7H, Ar-H), 8.83 (s, 1H, CH=N), 11.36 (s, 1H, NH). MS: m/z = 421 (M^+). Anal. Calcd. (%) for $C_{17}H_{10}Cl_2N_4O_3S$ (421.26): 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-Methoxybenzylidene)-2-(2-(2-methoxybenzylidene)hydrazinyl)-thiazol-4(5H)-one (4d). Yellow powder, Yield 69%; mp: 246-247^0 C. IR(KBr): ν/cm^{-1}=3237 (NH), 1723 (C=O), 1611 (C=N). ^{1}H NMR (DMSO-*d*$_6$): δ_{ppm}= 3.31 (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 6.55 (s, 1H, CH=C$_{5thiazoline}$), 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 $C_{19}H_{17}N_3O_3S$ (367.43): 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-methoxybenzylidene)hydrazinyl)-thiazol-4(5H)-one (4e). Yellow powder, Yield 63%; mp: 249-250 ^{0}C. IR(KBr): ν/cm^{-1}=3240 (NH), 1722 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-*d*$_6$): δ_{ppm}= 3.79 (s, 3H, OCH_3), 6.79 (s, 1H, CH=C$_{5thiazoline}$), 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 $C_{18}H_{13}Cl_2N_3O_2S$ (406.29): 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 ^{0}C. IR(KBr): ν/cm^{-1}=3229 (NH), 1718 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-*d*$_6$): δ_{ppm}=

6.60 (s, 1H, CH=$C_{5thiazoline}$), 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 $C_{17}H_{10}BrCl_2N_3OS$ (455.16): C 44.86; H 2.21; N 9.23; S 7.04. Found C 44.69; H 2.22; N 9.27; S 7.07.

5-(2,4-Dichlorobenzylidene)-2-(2-(2,6-dichlorobenzylidene)hydrazinyl)-thiazol-4(5H) one (4g). Yellow powder, Yield 57%; mp: 267-268^0 C. IR(KBr): ν/cm^{-1}=3236 (NH), 1717 (C=O), 1604 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 6.66 (s, 1H, CH=$C_{5thiazoline}$), 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 $C_{17}H_9Cl_4N_3OS$ (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)hydrazinyl)-thiazol-4(5H)-one (4h). Yellow powder, Yield 60%; mp: 277-278^0 C. IR(KBr): ν/cm^{-1}=3235 (NH), 1723 (C=O), 1610 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 6.59 (s, 1H, CH=$C_{5thiazoline}$), 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 $C_{17}H_{10}BrCl_2N_3OS$ (455.16): C 44.86; H 2.21; N 9.23; S 7.04. Found: C 44.76; H 2.21; N 9.28; S 7.05.

2-(2-(2,6-Dichlorobenzylidene)hydrazinyl)-5-((6-methyl-4-oxo-4H-chromen-3 yl)methylene)-thiazol-4(5H)-one (5a). White-yellow powder, Yield 44%; mp: 308 ^{0}C. IR(KBr): ν/cm^{-1}=3244 (NH), 1750 (C=O chromone), 1740 (C=O thiazolin-4-one), 1607 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 2.59, (s, 3H, CH_3), 6.99 (s, 1H, CH=$C_{5\text{-}thiazoline}$), 7.15-7.65 (m, 3H, Ar-H), 7.41 (s, 1H, $C_{2\text{-}chromone}$-H), 7.47 (s, 1H, $C_{5\text{-}chromone}$-H), 7.55 (d, 1H, $C_{8\text{-}chromone}$-H), 7.66 (d, 1H, $C_{7\text{-}chromone}$-H), 8.66 (s, 1H, CH=N), 12.62 (s, 1H, NH). MS: m/z = 458 (M^+). Anal. Calcd. (%) for $C_{21}H_{13}Cl_2N_3O_3S$ (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)hydrazinyl)-5-((6-chloro-4-oxo-4H-chromen-3-yl)-methylene)-thiazol-4(5H)-one (5b). White-yellow powder, Yield 49%; mp: 309 ^{0}C. IR(KBr): ν/cm^{-1}=3239 (NH), 1753 (C=O chromone), 1720 (C=O thiazolin-4-one), 1602 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 6.99 (s, 1H, CH=$C_{5\text{-}thiazoline}$), 7.20-7.55 (m, 3H, Ar-H), 7.47 (s, 1H, $C_{5\text{-}chromone}$-H), 7.48 (s, 1H, $C_{2\text{-}chromone}$-H), 7.54 (d, 1H, $C_{8\text{-}chromone}$-H), 7.62 (d, 1H, $C_{7\text{-}chromone}$-H), 8.23 (s, 1H, CH=N), 12.69 (s, 1H, NH). MS: m/z = 479 (M^+). Anal. Calcd. (%) for $C_{20}H_{10}Cl_3N_3O_3S$ (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)hydrazinyl)-5-((6-fluoro-4-oxo-4H-chromen-3-yl)methylene)thiazol-4(5H)-one (5c). White-yellow powder,

Yield 56%; mp: 305-307 ^{0}C. IR(KBr): ν/cm^{-1}=3239 (NH), 1755 (C=O chromone), 1722 (C=O thiazolin-4-one), 1606 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 6.84 (s, 1H, CH=$C_{5\text{-thiazoline}}$), 7.25-7.41 (m, 3H, Ar-H), 7.47 (s, 1H, $C_{2\text{-chromone}}$-H), 7.55 (d, 1H, $C_{8\text{-chromone}}$-H), 7.58 (s, 1H, $C_{5\text{-chromone}}$-H), 7.74 (d, 1H, $C_{7\text{-chromone}}$-H), 8.72 (s, 1H, CH=N), 12.54 (s, 1H, NH). MS: m/z = 462 (M^+). Anal. Calcd. (%) for $C_{20}H_{10}Cl_2FN_3O_3S$ (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-((6,8-dibromo-4-oxo-4H-chromen-3-yl)methylene)-thiazol-4(5H)-one (5d). White-yellow powder, Yield 55%; mp: 342-343 ^{0}C. IR(KBr): ν/cm^{-1}=3242 (NH), 1751 (C=O chromone), 1733 (C=O thiazolin-4-one), 1600 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 6.88 (s, 1H, CH=$C_{5\text{-thiazoline}}$), 7.15-7.46 (m, 3H, Ar-H), 7.43 (s, 1H, $C_{5\text{-chromone}}$-H), 7.44 (s, 1H, $C_{2\text{-chromone}}$-H), 7.51 (s, 1H, $C_{7\text{-chromone}}$-H), 8.59 (s, 1H, CH=N), 12.57 (s, 1H, NH). MS: m/z = 602 (M^+). Anal. Calcd. (%) for $C_{20}H_9Br_2Cl_2N_3O_3S$ (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: 296-298^0 C. IR(KBr): ν/cm^{-1}=3239 (NH), 1740 (C=O chromone), 1730 (C=O thiazolin-4-one), 1604 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 2.51 (s, 3H, CH_3), 3.93 (s, 3H, OCH_3), 6.88 (s, 1H, CH=$C_{5\text{-thiazoline}}$), 7.25-7.55 (m, 4H, Ar-H), 7.48 (s, 1H, $C_{5\text{-chromone}}$-H), 7.54 (d, 1H, $C_{8\text{-chromone}}$-H), 7.57 (s, 1H, $C_{2\text{-chromone}}$-H), 7.62 (d, 1H, $C_{7\text{-chromone}}$-H), 8.53 (s, 1H, CH=N), 12.54 (s, 1H, NH). MS: m/z = 420 (M^+). Anal. Calcd. (%) for $C_{22}H_{17}N_3O_4S$ (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 ^{0}C. IR(KBr): ν/cm^{-1}=3239 (NH), 1739 (C=O chromone), 1728 (C=O thiazolin-4-one), 1600 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 3.87 (s, 3H, OCH_3), 6.99 (s, 1H, CH=$C_{5\text{-thiazoline}}$), 7.15-7.44 (m, 4H, Ar-H), 7.45 (s, 1H, $C_{2\text{-chromone}}$-H), 7.49 (s, 1H, $C_{5\text{-chromone}}$-H), 7.51 (d, 1H, $C_{8\text{-chromone}}$-H), 7.66 (d, 1H, $C_{7\text{-chromone}}$-H), 8.62 (s, 1H, CH=N), 12.59 (s, 1H, NH). MS: m/z = 440 (M^+). Anal. Calcd. (%) for $C_{21}H_{14}ClN_3O_4S$ (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 ^{0}C. IR(KBr): ν/cm^{-1}=3307 (OH), 3255 (NH), 1727 (C=O), 1605 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 5.88 (s, 1H, OH), 6.59 (s, 1H, CH=$C_{5\text{-thiazoline}}$), 7.11-7.67 (m, 9H, Ar-H), 7.88 (s, 1H, C_5-H thiazol), 8.30 (s, 1H, CH=N), 12.40 (s, 1H, NH). MS: m/z = 407 (M^+). Anal. Calcd. (%) for $C_{20}H_{14}N_4O_2S_2$ (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 ^{0}C. IR(KBr): ν/cm^{-1}=3237 (NH), 1731 (C=O), 1598 (C=N). ^{1}H NMR (DMSO-d_6): δ_{ppm}= 6.55 (s, 1H, CH=$C_{5\text{-thiazoline}}$), 7.20-7.79 (m, 9H, Ar-H), 7.91 (s, 1H, C_5-H thiazol), 8.33 (s, 1H, CH=N), 12.32 (s, 1H, NH). MS: m/z = 469 (M^+). Anal. Calcd. (%) for $C_{20}H_{13}BrN_4OS_2$ (469.38): C 51.18; H 2.79; N 11.94; S 13.66. Found: C 51.41; H 2.78; N11.99; S 13.71.

In vitro antibacterial and antifungal activity

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

Table I

Antimicrobial activity of **3a-d**, **4a-h**, **5a-f** and **6a-b** compounds

Compounds	Zone of inhibition (mm) and MIC[a] values (μg.mL^{-1})				
	S.aureus	*E.coli*	*B.subtilis*	*P.aeruginosa*	*C.albicans*
3a	-	25(6.25)	-	-	6
3b	-	20(6.25)	-	-	8
3c	-	20(6.25)	-	-	35(6,25)
3d	-	-	-	-	42(6,25)
4a	11 (>50)	-	-	-	16(12.5)
4b	10(>50)	10(50)	-	-	24(6.25)
4c	7	10(50)	-	-	12(>50)
4d	5	-	-	-	9
4e	8	-	-	-	9
4f	6	-	-	-	9
4g	6	-	-	-	16(50)
4h	-	-	-	-	-
5a	10(>50)	12(50)	-	-	-
5b	-	-	-	-	-
5c	-	10(>50)	-	-	-
5d	-	-	-	-	-
5e	-	-	-	-	-
5f	-	-	-	-	-
6a	-	-	-	-	-
6b	-	-	-	-	-
Ciprofloxacin	23 (1.56)	25 (1.56)	22 (1.56)	20 (3.12)	-
Fluconazole	-	-	-		25 (1.56)
DMSO (dimethyl sulfoxide)	-	-	-		-

[a]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 arylidene 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-arylidene-thiazoline-4-ones **4a-h**, **5a-f** and **6a-b** obtained from various arylidene-thiosemicarbazones. Some of the compounds were found to be very active against *E. coli* and *Candida albicans*. The introduction of the arylidene 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.

Acknowledgements

This research was done with the financial support of PNII-ID1271/2008 project/CNCSIS, Romania.

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Manuscript received: December 11th 2010