SYNTHESIS AND ANTIMICROBIAL ACTIVITY EVALUATION OF SOME N[1]-ARYLIDENE-THIOSEMICARBAZONE AND 1,3,4-THIADIAZOLINE DERIVATIVES

IOANA IONUȚ[1], CRISTINA NASTASĂ[1], BRÎNDUŞA TIPERCIUC[1], SMARANDA ONIGA[1], ADRIAN PÎRNĂU[2], LAURIAN VLASE[3], IOANA COLOSI[4], MIHAELA DUMA[5], OVIDIU ONIGA[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[2]National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania
[3]Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[4]Department of Microbiology, Faculty of Pharmacy, Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[5]State Veterinary Laboratory for Animal Health and Food Safety, Cluj-Napoca, Romania

Abstract

Aims. *Synthesis of novel and potential antimicrobial agents, with new targets of action.*

Materials and Methods. *A total of 20 derivatives of N[1]-arylidene-thiosemicarbazones (series **A**) and 4,5-dihydro-1,3,4-thiadiazoles (series **B**) were synthesized and their antimicrobial activities were assessed in vitro against bacteria (Salmonella typhimurium ATCC 14028, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 49444, Enterococcus faecalis ATCC 29212, Bacillus cereus ATCC 11778, Listeria monocytogenes ATCC 13932) and fungal strains (Candida albicans ATCC 10231, Candida glabrata, Candida krusei and Candida tropicalis) using gentamicin and ketoconazole as reference drugs. The structures of the synthesized compounds were confirmed by ^{1}H NMR, MS and elemental (carbon, hydrogen, nitrogen and sulphur) analysis.*

Results. *11 compounds showed potent bactericidal effects against bacterial strains, with the size of the zone of inhibition ranging between 10 and 20 mm (100 µg compound/well); and 12 compounds exhibited antifungal activities, with the size of the zone of inhibition ranging between 12 and 28 mm (100 µg compound/well).*

Conclusions. *We have synthesized 20 compounds and evaluated them for their in vitro antimicrobial activities.Most of them showed good antimicrobial activities.*

Keywords: thiosemicarbazones, 1,3,4-thiadiazolines, antibacterial, antifungal, size of the zone of inhibition.

Introduction

The emergence of bacterial resistance to different classes of antibacterial agents, such as β-lactams, quinolones, and macrolides is an alarming problem that seriously affects human health. To combat this situation, numerous efforts have been made in the development of new approaches to treat bacterial infections, particularly for therapeutics with novel mechanism of action and little or no cross-resistance. As a result, new antibacterial agents against multi-drug resistant strains have become the center of attention in this highlighted research field [1].

In recent years, there has been a growing interest pertaining to the synthesis of bioactive compounds in the field of organic chemistry. Among the family of heterocyclic compounds, nitrogen containing heterocycles, especially azoles compounds, gain considerable importance owing to their varied biological properties such

Adress for correspondence: ionut.ioana@umfcluj.ro

as antibacterial, antifungal, anti-inflamatory [2-4], antihypertensive, anti-HIV, antitumor, anticonvulsivant, herbicidal, insecticidal [5-8], antiprotozoal [9]. Furthermore, synthesis of novel chemical entities, which are still in resemblance with bioactive molecules by virtue of the presence of some critical structural features, is an essential direction of the research for new leads in drug designing programs. Hence, our continued interest in the development of simple and convenient synthetic routes has induced us to synthesize a class of new molecules having thiadiazoline scaffolds. Five membered aromatic systems having three heteroatoms at symmetrical positions have been studied because of their interesting physiological properties. In the recent decades, the synthesis of substituted thiadiazolines and related compounds has attracted considerable attention because these compounds constitute the structural frameworks of several naturally occurring alkanoids that show a wide range of pharmaceutical and industrial importance. Thiadiazolines possess a wide range of biological properties and they act as antibacterial, anti-inflamatory, anticancer, antihypertensive, analgesic [10] and fungicidal [11]. Thus the thiadiazoline nucleus has attracted much interest in the development of pharmacologically active compounds. Since the thiadiazoline moiety seems to be a possible pharmacophore in various pharmacologically active agents, we decided to synthesize compounds with this functionality as possible antimicrobial agents which could furnish better therapeutic results.

Materials and methods

Chemistry

General synthetic route towards the derivatives of N^1-arylidene-thiosemicarbazones **(A1-10)** is illustrated in Figure 1: a simple condensation between substituted aromatic/hetaromatic aldehydes or substituted acetophenones and N^4-substituted thiosemicarbazides, in absolute ethanol, using concentrated sulphuric acid as catalyst [12-14]. The synthesis and the structure analysis of all the thiosemicarbazone derivatives (**A1-4** and **A7-10**), with the exception of **A5** and **A6**, have been reported earlier [14-20]. N^4-substituted thiosemicarbazides (phenyl and methyl) were obtained, with good yields, through the addition reaction of hydrazine hydrate to phenylisothiocyanate and methylisothiocyanate respectively, by stirring in absolute ethanol, at room temperature, for 3 hours - Figure 2 [13].

The derivatives of 4,5-dihydro-1,3,4-thiadiazoles **(B1-10)** were prepared via the route shown in Figure 1. These compounds were obtained by reacting the thiosemicarbazones (**A1-10)** with acetic anhydride, as cyclization reagent, in presence of small quantities of pyridine as catalyst [10,11,21]. The synthesis and the structure analysis of the compounds **B1** and **B3** have been reported earlier [15,22].

An objective of this research was to evaluate the influence that different substituents on the phenyl ring and the presence of a thiazole heterocycle inserted between a phenyl ring and the thiadiazoline moiety may have on the antimicrobial activity.

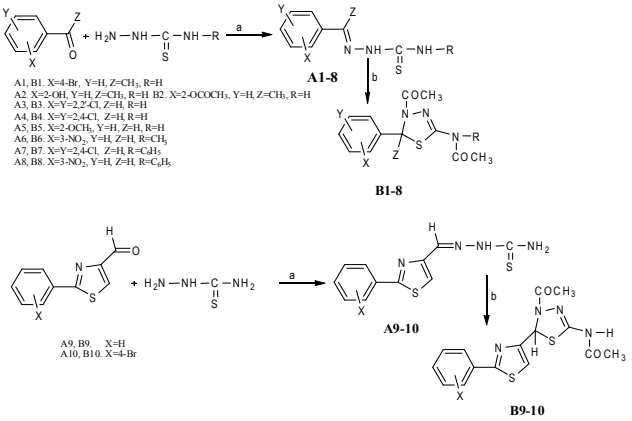

Figure 1. Synthesis of N^1-arylidene-thiosemicarbazones **A1-10** and substituted thiadiazolines **B1-10**. Reagents and conditions: (a) $EtOH_{abs}/_{conc}H_2SO_4$, reflux; (b) Ac_2O/pyridine, reflux.

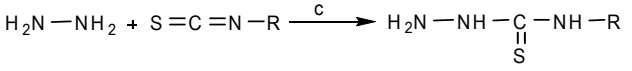

Figure 2. Synthesis of N^4-substituted-thiosemicarbazides. Reagents and conditions: (c) EtOH abs, room temperature, 3h stirring.

Most of the reagents were purchased from Acros, Aldrich Sigma and Merck, and used without further purification. To synthesize compounds **A9-10** and **B9-10**, previously reported 2-aryl-4-formylthiazoles were used [23].

The melting points were taken with an Electrothermal melting point meter and were uncorrected. The ^{1}HNMR 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 12 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 registered with a Vario El CHNS instrument. The purity of the synthesized compounds was verified by thin layer chromatography (TLC) and was carried out on precoated Silica Gel 60F254 sheets using heptane–ethyl-acetate 1:3 system and UV light for visualization.

Synthesis of thiosemicarbazone derivatives (Series A, General procedure)

In a flask equipped with a reflux condenser, a mixture of aldehyde or cetone (50 mmol) and various

thiosemicarbazides (50 mmol) was reacted in 50 ml absolute ethanol in the presence of a catalytic amount of concentrated sulphuric acid (98%). 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.

2-(1-(4-bromophenyl)ethylidene)hydrazinecarbothioamide (A1)

The title compound was obtained in 83% yield. Melting point: 197ºC [15]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.39 (s, 1H, NH), 7.88 and 8.10 (2 br s, 1H each, NH_2), 7.29-7.58 (m, 4H, phenyl-H), 2.38 (s, 3H, CH_3). Anal. Calcd. (%) for $C_9H_{10}BrN_3S$ (272.16): C, 39.72; H, 3.70; N, 15.44; S, 11.78. Found: C, 39.91; H, 3.44; N, 15.68; S, 11.68. MS (EI, 70 eV): *m/z* 273(M^+).

2-(1-(2-hydroxiphenyl)ethylidene)hydrazinecarbothioamide (A2)

The title compound was obtained in 87% yield. Melting point: 198ºC [16]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.07 (s, 1H, NH), 9.61 (s, 1H, OH), 7.66 and 7.87 (2 br s, 1H each, NH_2), 7.32-7.56 (m, 4H, phenyl-H), 2.37 (s, 3H, CH_3). Anal. Calcd. (%) for $C_9H_{11}N_3OS$ (209.27): C, 51.65; H, 5.30; N, 20.08; S, 15.32. Found: C, 51.80; H, 5.57; N, 20.47; S, 15.68. MS (EI, 70 eV): *m/z* 210 (M^+).

2-(2,6-dichlorobenzylidene)hydrazinecarbothioamide (A3)

The title compound was obtained in 79% yield. Melting point: 245-246ºC [6]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.38 (s, 1H, NH), 9.05 (s, 1H, CH=N), 7.81 and 8.20 (2 br s, 1H each, NH_2), 7.23-7.52 (m, 3H, phenyl-H). Anal. Calcd. (%) for $C_8H_7ClN_3S$ (248.13): C, 38.72; H, 2.84; N, 16.93; S, 12.92. Found: C, 39.04; H, 2.55; N, 17.14; S, 13.08. MS (EI, 70 eV): *m/z* 249 (M^+).

2-(2,4-dichlorobenzylidene)hydrazinecarbothioamide (A4)

The title compound was obtained in 83% yield. Melting point: 245ºC [6]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.39 (s, 1H, NH), 9.12 (s, 1H, CH=N), 7.94 and 8.12 (2 br s, 1H each, NH_2), 7.46-7.91 (m, 3H, phenyl-H). Anal. Calcd. (%) for $C_8H_7ClN_3S$ (248.13): C, 38.72; H, 2.84; N, 16.93; S, 12.92. Found: C, 38.94; H, 2.68; N, 17.21; S, 13.14. MS (EI, 70 eV): *m/z* 249 (M^+).

2-(2-methoxibenzylidene)hydrazinecarbothioamide (A5)

The title compound was obtained in 92% yield. Melting point: 213-214ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.49 (s, 1H, NH), 8.33 (s, 1H, CH=N), 7.72 and 8.07 (2 br s, 1H each, NH_2), 7.36-7.63 (m, 4H, phenyl-H), 3.86 (s, 1H, OCH_3). Anal. Calcd. (%) for $C_9H_{11}N_3OS$ (209.27): C, 51.65; H, 5.30; N, 20.08; S, 15.32. Found: C, 51.42; H, 5.57; N, 20.27; S, 15.68. MS (EI, 70 eV): *m/z* 210 (M^+).

***N*-methyl-2-(3-nitrobenzylidene)hydrazinecarbothioamide (A6)**

The title compound was obtained in 80% yield. Melting point: 243-244ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.67 (s, 1H, $CSNHCH_3$), 11.26 (s, 1H, NHCS), 8.40 (s, 1H, CH=N), 7.52-7.77 (m, 4H, phenyl-H), 3.42 (s, 3H, CH_3). Anal. Calcd. (%) for $C_9H_{10}N_4O_2S$ (238.27): C, 45.37; H, 4.23; N, 23.51; S, 13.46. Found: C, 45.62; H, 4.57; N, 23.77; S, 13.68. MS (EI, 70 eV): *m/z* 239 (M^+).

***N*-phenyl-2-(2,4-dichlorobenzylidene)hydrazinecarbothioamide (A7)**

The title compound was obtained in 84% yield. Melting point: 251ºC [14]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.59 (s, 1H, $CSNHC_6H_5$), 11.06 (s, 1H, NHCS), 8.36 (s, 1H, CH=N), 7.72-7.87 (m, 3H, phenyl-H), 7.29-7.56 (m, 5H, phenyl-H). Anal. Calcd. (%) for $C_{14}H_{11}Cl_2N_3S$ (324.23): C, 51.86; H, 3.42; N, 12.96; S, 9.89. Found: C, 51.62; H, 3.67; N, 13.17; S, 9.68. MS (EI, 70 eV): *m/z* 325 (M^+).

***N*-phenyl-2-(3-nitrobenzylidene)hydrazinecarbothioamide (A8)**

The title compound was obtained in 88% yield. Melting point: 202-203ºC [18]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.54 (s, 1H, $CSNHC_6H_5$), 11.11 (s, 1H, NHCS), 8.42 (s, 1H, CH=N), 7.37 - 7.61 (m, 4H, phenyl-H), 7.46-7.59 (m, 5H, phenyl-H). Anal. Calcd. (%) for $C_{14}H_{12}N_4O_2S$ (300.34): C, 55.99; H, 4.03; N, 18.65; S, 10.68. Found: C, 55.62; H, 4.37; N, 18.75; S, 10.43. MS (EI, 70 eV): *m/z* 301 (M^+).

2-((2-phenylthiazol-4-yl)methylene)hydrazinecarbothioamide (A9)

The title compound was obtained in 81% yield. Melting point: 233-235ºC [19]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.47 (s, 1H, NH), 8.35 (s, 1H, CH=N), 7.84 and 8.06 (2 br s, 1H each, NH_2), 7.46-7.63 (m, 5H, phenyl-H), 7.58 (s, 1H, thiazole-H), 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, 50.52; H, 3.51; N, 21.72; S, 24.52. MS (EI, 70 eV): *m/z* 263 (M^+).

2-((2-(4-bromophenyl)thiazol-4-l)methylene)hydrazinecarbothioamide (A10)

The title compound was obtained in 79% yield. Melting point: 248-249ºC [20]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.76 (s, 1H, NH), 8.29 (s, 1H, CH=N), 7.74 and 7.96 (2 br s, 1H each, NH_2), 7.55 (s, 1H, thiazole-H), 7.68-7.84 (m, 4H, phenyl-H). Anal. Calcd. (%) for $C_{11}H_9BrN_4S_2$ (341.25): C, 38.72; H, 2.66; N, 16.42; S, 18.79. Found: C, 38.94; H, 2.41; N, 16.79; S, 18.58. MS (EI, 70 eV): *m/z* 342 (M^+).

Synthesis of 1,3,4-thiadiazolidine derivatives (Series B, General procedure)

0.005 mol of correspondent thiosemicarbazone was refluxed with 10 ml acetic anhydride and 0,5 ml pyridine for 3-4 hours. After cooling, the mixture was poured into water, stirred 30 minutes hours at room temperature. The resulted precipitate was filtered, washed with water and recrystallized from absolute ethanol.

***N*-(4-acetyl-5-(4-bromophenyl)-5-methyl-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (B1)**

The title compound was obtained in 60% yield. Melting point: 202-204ºC [15]. ^{1}H NMR(DMSO-d_6, 500 MHz, ppm): δ 11.67 (s, 1H, NH), 7.54-7.56 (dd, 2H, phenyl-H, J_{1-2} = 8,65), 7.32-7.34 (dd, 2H, phenyl-H, J_{2-1} = 8.7), 2.41 (s, 3H, N^3-thiadiazoline-$COCH_3$), 2.26 (s, 3H, $NHCOCH_3$), 2.03 (s, 3H, C_2-thiadiazoline-CH_3). Anal. Calcd. (%) for $C_{13}H_{14}BrN_3O_2S$ (356.24): C, 43.83; H, 3.96; N, 11.80; S, 9.00. Found: C, 43.61; H, 3.81; N, 11.55; S, 8.74. MS (EI, 70 eV): *m/z* 357 (M^+).

2-(5-acetamido-3-acetyl-2-methyl-2,3-dihydro-1,3,4-thiadiazol-2-yl)phenylacetate (B2)

The title compound was obtained in 63% yield. Melting point: 205-208ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.63 (s, 1H, NH), 7.30-7.55 (m, 4H, phenyl-H), 2.9 (s, 3H, C_2-thiadiazoline-CH_3), 2.45 (s, 3H, N^3-thiadiazoline-$COCH_3$), 2,31 (s, 3H, $OCOCH_3$), 2.26 (s, 3H, $NHCOCH_3$). Anal. Calcd. (%) for $C_{15}H_{17}N_3O_4S$ (335.38): C, 53.72; H, 5.11; N, 12.53; S, 9.56. Found: C, 53.36; H, 4.95; N, 12.24; S, 9.62. MS (EI, 70 eV): *m/z* 336 (M^+).

***N*-(4-acetyl-5-(2,6-dichorophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (B3)**

The title compound was obtained in a 72% yield. Melting point: 212ºC [15]. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.82 (s, 1H, NH), 7.48 (dd, 1H, C_3-phenyl-H, J_{1-2} = 8.5, J_{2-1} = 8.1), 7.48 (dd, 1H, C_5-phenyl-H), 7.35 (t, 1H, C_4-phenyl-H, J_{2-3} = 8.1, J_{3-2} = 8.5), 7.31 (s, 1H, C_2-thiadiazoline-H), 2.13 (s, 3H, N-thiadiazoline-$COCH_3$), 2.07 (s, 3H, $NHCOCH_3$). Anal. Calcd. (%) for $C_{12}H_{11}Cl_2N_3O_2S$ (332.21): C, 43.39; H, 3.34; N, 12.65; S, 9.65. Found: C, 43.64; H, 3.59; N, 13.04; S, 9.59. MS (EI, 70 eV): *m/z* 333 (M^+).

***N*-(4-acetyl-5-(2,4-dichorophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (B4)**

The title compound was obtained in a 64% yield. Melting point: 172ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.85 (s, 1H, NH), 7.72 (s, 1H, C_3-phenyl-H, J_{1-2}=2.2, J_{2-1}=2.1), 7.45 (dd, 1H, C_5-phenyl-H, J = 8.4), 7.11 (s, 1H, C_2-thiadiazoline-H), 7.1 (d, 1H, C_6-phenyl-H, J = 8.45), 2.27 (s, 3H, *N*-thiadiazoline-$COCH_3$), 2.04 (s, 3H, $NHCOCH_3$). Anal. Calcd. (%) for $C_{12}H_{11}Cl_2N_3O_2S$ (332.21): C, 43.39; H, 3.34; N, 12.65; S, 9.65. Found: C, 43.71; H, 3.51; N, 12.45; S, 9.31. MS (EI, 70 eV): *m/z* 333 (M^+).

***N*-(4-acetyl-5-(2-methoxyphenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (B5)**

The title compound was obtained in a 76% yield. Melting point: 245ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.84 (s, 1H, NH), 7.2-7.76 (m, 4H, phenyl-H), 7.13 (s, 1H, C_2-thiadiazoline-H), 3.81 (s, 3H, OCH_3), 2.20 (s, 3H, *N*-thiadiazoline-$COCH_3$), 2.05 (s, 3H, $NHCOCH_3$). Anal. Calcd. (%) for $C_{13}H_{15}N_3O_3S$ (293.34): C, 53.23; H, 5.15; N, 14.32; S, 10.93. Found: C, 52.89; H, 4.86; N, 16.71; S, 11.22. MS (EI, 70 eV): *m/z* 294 (M^+).

***N*-(4-acetyl-5-(3-nitrophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-*N*-methylacetamide (B6)**

The title compound was obtained in a 70% yield. Melting point: 163-165ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.28-7.65 (m, 4H, phenyl-H), 7.23 (s, 1H, C_2-thiadiazoline-H), 2.44 (s, 3H, N-CH_3), 2.27 (s, 3H, *N*-thiadiazoline-$COCH_3$), 2.03 (s, 3H, N-$COCH_3$). Anal. Calcd. (%) for $C_{13}H_{14}N_4O_4S$ (322.34): C, 48.44; H, 4.38; N, 17.38; S, 9.95. Found: C, 48.71; H, 4.66; N, 17.59; S, 10.21. MS (EI, 70 eV): *m/z* 323 (M^+).

***N*-(4-acetyl-5-(2,4-dichorophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-*N*-phenylacetamide (B7)**

The title compound was obtained in a 62% yield. Melting point: 188ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.43-7.86 (m, 3H, phenyl-H), 7.21-7.54 (m, 5H, phenyl-H), 7.33 (s, 1H, C_2-thiadiazoline-H), 2.35 (s, 3H, N-thiadiazoline-$COCH_3$), 2.12 (s, 3H, N-$COCH_3$). Anal. Calcd. (%) for $C_{18}H_{15}Cl_2N_3O_2S$ (408.30): C, 52.95; H, 3.70; N, 10.29; S, 7.85. Found: C, 53.14; H, 4.11; N, 10.43; S, 8.12. MS (EI, 70 eV): *m/z* 409 (M^+).

***N*-(4-acetyl-5-(3-nitrophenyl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)-*N*-phenylacetamide (B8)**

The title compound was obtained in a 68% yield. Melting point: 160-163ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 7.11-7.56 (m, 4H, phenyl-H), 7.48-7.59 (m, 5H, phenyl-H), 7.29 (s, 1H, C_2-thiadiazoline-H), 2.26 (s, 3H, N-thiadiazoline-$COCH_3$), 2.09 (s, 3H, N-$COCH_3$). Anal. Calcd. (%) for $C_{18}H_{16}N_4O_4S$ (384.41): C, 56.24; H, 4.20; N, 14.57; S, 8.34. Found: C, 56.55; H, 4.36; N, 14.66; S, 8.21. MS (EI, 70 eV): *m/z* 385 (M^+).

***N*-(4-acetyl-5-(2-phenylthiazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (B9)**

The title compound was obtained in a 76% yield. Melting point: 218ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.62 (s, 1H, NH), 7.42-7.61 (m, 5H, phenyl-H), 7.57 (s, 1H, thiazole-H), 7.25 (s, 1H, C_2-thiadiazoline-H), 2.22 (s, 3H, N-thiadiazoline-$COCH_3$), 2.01 (s, 3H, NH-$COCH_3$). Anal. Calcd. (%) for $C_{15}H_{14}N_4O_2S_2$ (346.43): C, 52.01; H, 4.07; N, 16.17; S, 18.51. Found: C, 52.33; H, 4.16; N, 16.47; S, 18.39. MS (EI, 70 eV): *m/z* 347(M^+).

***N*-(4-acetyl-5-(2-(4-bromophenyl)thiazol-4-yl)-4,5-dihydro-1,3,4-thiadiazol-2-yl)acetamide (B10)**

The title compound was obtained in a 66% yield. Melting point: 208ºC. ^{1}H NMR (DMSO-d_6, 500 MHz, ppm): δ 11.78 (s, 1H, NH), 7.70-7.85 (m, 4H, phenyl-H), 7.71 (s, 1H, thiazole-H), 6.96 (s, 1H, C_2-thiadiazoline-H), 2.30 (s, 3H, N-thiadiazoline-$COCH_3$), 2.13 (s, 3H, NH-$COCH_3$). Anal. Calcd. (%) for $C_{15}H_{13}BrN_4O_2S_2$ (425.32): C, 42.36; H, 3.08; N, 13.17; S, 15.08. Found: C, 42.71; H, 2.89; N, 13.40; S, 15.22. MS (EI, 70 eV): *m/z* 426 (M^+).

Antimicrobial activity assay

Twenty compounds were screened *in vitro* for their antimicrobial activities against six strains of bacteria, *Staphylococcus aureus* ATCC 49444, *Enterococcus*

faecalis ATCC 29212, *E.coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Bacillus cereus* ATCC 11778 *Listeria monocytogenes* ATCC 13932, and one strain of fungi *Candida albicans* ATCC 10231 by the agar diffusion technique. Some of the compounds that were active against *Candida albicans* were tested against other three strains of fungi *Candida krusei* ATCC 6285, *Candida glabrata* ATCC, *Candida tropicalis* ATCC. 2 mg/mL solutions in DMSO were used (100 µg compound/well). The obtained results were compared with those of gentamicin (10 µg/well) as antibacterial, and fluconazole (25 µg/well) as antifungal reference.

Culture media

For antibacterial testing, Mueller-Hinton agar was used.

For antifungal testing Mueller-Hinton medium supplemented with 2% glucose (providing adequate growth of yeasts) and 0.5 mg/mL methylene blue (providing a better definition of the inhibition zone diameter) was used.

Inoculum

Standardization of the inoculum is essential for accurate and reproducible antimicrobial susceptibility tests. The inoculum was prepared by suspending five representative colonies, obtained from an 18-24 h culture on non-selective nutritive agar medium, in sterile distilled water. The cell density was adjusted to the density of a 0.5 McFarland standard by measuring the absorbance in a spectrophotometer at a wavelength of 530 nm and adding sterile distilled water as required (corresponding to a population of 1-5 × 10^6 CFU/ml).

Six-millimeter diameter wells were cut from the agar using a sterile cork-borer, and a predetermined volume of each compound solution will be delivered into the wells.

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.

The plates were incubated at 35°C. Zone diameters were measured to the nearest whole millimeter at a point in which there will be no visible growth after 24-48 h.

Results and discussions

Twenty compounds with new structures were synthesized, in good yelds. All of them gave satisfactory CHNS quantitative elemental analysis, MS analysis and ^{1}HNMR analysis results. They were investigated for their *in vitro* antimicrobial activity. The results of antibacterial and antifungal activities of series **A** and series **B** compounds against a panel of selected Gram positive, Gram negative and fungi are presented in Table I in comparison with those of the reference drugs gentamicin and fluconazole, respectively.

Growth inhibitory activities against Gram-positive and negative bacteria: 11 compounds showed antibacterial activities - **A2, B1-10,** most of them being more active against Gram-positive than Gram-negative

Table I

Compound	Inhibition zone in mm						
	Bacteria						Fungi
	Gram positive bacteria				Gram negative bacteria		
	S.aureus	*E.faecalis*	*L.monocytogenes*	*B.cereus*	*E.coli*	*S.typhi*	*C.albicans*
A1	-	-	-	-	-	-	28
A2	16	-	-	-	-	10	14
A3	-	-	-	-	-	-	-
A4	-	-	-	-	-	-	22
A5	-	-	-	-	-	-	-
A6	-	-	-	-	-	-	-
A7	-	-	-	-	-	-	-
A8	-	-	-	-	-	-	-
A9	-	-	-	-	-	-	18
A10	-	-	-	-	-	-	-
B1	-	16	12	18	18	-	18
B2	16	12	-	18	18	-	18
B3	12	12	10	18	12	-	18
B4	16	16	12	18	18	-	22
B5	-	-	-	16	14	-	22
B6	-	12	-	18	14	-	22
B7	-	12	-	16	-	-	22
B8	16	18	-	16	16	-	22
B9	-	12	16	18	20	-	-
B10	-	12	16	18	12	-	-
Gentamicin	19	8	18	18	22	18	NT
Fluconazole	NT	NT	NT	NT	NT	NT	25

Legend:

Θ - No antimicrobial activity
NT - Not Tested

C. glabrata: **A1 (**23 mm); **A9** (12 mm); Fluconazole (17 mm)
C. krusei: **A1 (**14 mm); Fluconazole (no activity)
C. tropicalis: **A1 (**16 mm); Fluconazole (23 mm).

bacteria. Thus, 5 compounds (100 µg compound/well) were active against *Staphylococcus aureus* ATTC 49444 (**A2**, **B2-4** and **B8**), but with lower activities than reference gentamicin (10 µg/well); 9 compounds were more active against *Enterococcus faecalis* ATCC 29212 (**B1-4**, **B6-10**) than gentamicin; 5 of them were less active against *Listeria monocytogenes* ATCC 13932 (**B1,3,4,9,10)** than the reference drug; all of the compounds belonging to series **B** showed a comparable activity against *Bacillus cereus* ATCC 11778 with that of gentamicin. Gram-negative strains were less susceptible to the synthesized compounds: 9 compounds from series **B** showed an antibacterial activity against *Esherichia coli* ATCC 25922, compound **B9** being the most active, however less active than gentamicin; only 1 compound belonging to series **A-A2-**was active against *Salmonella typhimurium* ATCC 14028. The size of the zone of the inhibition ranged between 10 and 20 mm.

Growth inhibitory activities against fungal strains: 12 compounds (100 µg compound/well) exhibited antifungal activity against *Candida albicans* ATCC 10231 (**A1,2,4,9** and **B1-8**), most of them presenting comparable activities with that of reference fluconazole (25 µg compound/well). The active compounds belonging to series **A** were also tested against other three strains of *Candida* species. Compound **A1** was the most active, presenting a better activity agains *C. albicans* and *C. glabrata* than fluconazole. The size of the zone of the inhibition ranged between 12 and 28 mm.

The growing incidence and frequency of bacterial resistance to current therapeutic agents remains a huge challenge for infectious disease specialist and pharmaceutical companies. In order to keep ahead of this growing issue, novel compounds working by new mechanisms of action are required [1].

In this study, we have described the synthesis of 20 novel compounds, derivatives of N^1-arylidene-thiosemicarbazones and 4,5-dyhidro-1,3,4-thiadiazoles, and have investigated for their in vitro antimicrobial activity.

The results presented in Table I demonstrated that series **B** compounds possessed much better antibacterial efficacy than series **A** compounds. Actually, only one compound (**A2**) presented antibacterial activity from the first synthesized series. So, we could presume that, by transforming the derivatives of thiosemicarbazones into thiadiazoline derivatives, through cyclisation in presence of acetic anhydride, antibacterial activity is gained.

It can be noted that most of the series **B** compounds were highly selective against Gram-positive microorganisms tested. The lack of efficacy may be attributed to their poor ability to penetrate the additional outer membrane barier of Gram-negative bacteria [1].

The above results allowed us to draw some observations. The introduction of halogen atoms on the phenyl ring in position 2 of the thiadiazoline scaffold, enlarges the antibacterial effect, whereas compounds with other substituents (-OH, $-OCH_3$, $-NO_2$) are less active. It is interesting to point out that double substitution of the phenyl ring in position 2 (**B3, B4**) also increases the antimicrobial effect, expecially 2,4-disubstituted compounds (**B4**). Concerning the **B** series, compounds with an acetylamino group in position 5 of the thiadiazoline (**B1, B2, B3, B4, B5**) are more active against bacteria strains than those with an *N*-R-substituted-acetylamino group (**B6**, **B7, B8**). On the other hand, triple substitution of the amino group increases the antifungal effect.

Introduction of a thiazole ring in position 2 of the thiadiazolines (**B9,10**) has no major influence on the antibacterial activity, but the antifungal effect disappears.

In summary, we have designed, synthesized, and evaluated the antimicrobial activities of some new N^1-arylidene-thiosemicarbazone and 1,3,4-thiadiazoline. Of all the compounds tested **A2, B1-10** showed antibacterial effects against a spectrum of Gram-positive microorganisms, but had lower activities against Gram-negative bacteria. Concerning the antifungal activity, **A1-2, A4, A9** and **B1-8** were active against tested *Candida* species, most of them against *Candida albicans*. **A1** exhibited the best antifungal activity.

Conclusions

This study presents the synthesis of novel thiosemicarbazone and thiadiazoline derivatives, most of them with good antimicrobial activities. It can be noticed that bacteria were in general more sensitive than fungal species to these compounds. These classes of compounds, especially those belonging to series **B**, may constitute potential antibacterial agents against certain drug-resistant strains of bacteria.

Acknowledgements

This research was financed from the European Social Fund, POS DRU/107/1.5/S/78702 project of the Iuliu Haţieganu University of Medicine and Pharmacy, Cluj-Napoca, and also by the PNII-ID1271/2008 project/CNCSIS, Romania.

References

1. Yu WY, Yang LX, Xie JS, et al. Derivatives of aryl-4-guanidinomethylbenzoate and *N*-aryl-4-guanidinomethylbenzamide as new antibacterial agents: synthesis and bioactivity. Acta Pharmacol Sin, 2008; 29:267-277.

2. Karegoudar P, Karthikeyan MS, Prasad DJ, Mahalinga M, Holla BS, Kumari NS. Synthesis of some novel 2,4-disubstituted thiazoles as possible antimicrobial agents. Eur J Med Chem, 2008; 43:261-267.

3. Rostom SAF, El-Ashmawy IM, El Razik HA, Badr MH, Ashour HMA. Design and synthesis of some thiazolyl and thiadiazolyl derivatives of antipyrine as potential non-acidic anti-inflammatory, analgesic and antimicrobial agents. Bioorg Med Chem, 2009; 17:882-895.

4. Vicini P, Geronikaki A, Incerti M, Zani F, Dearden J,

Hewitt M. 2-Heteroarylimino-5-benzylidene-4-thiazolidinones analogues of 2-thiazolylimino-5-benzylidene-4-thiazolidinones with antimicrobial activity: Synthesis and structure-activity relationship. Bioorganic Med Chem, 2008; 16:3714-3724.
5. Bharti SK, Tilak R, Singh SK. Synthesis, antibacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubsituted thiazole ring. Eur J Med Chem, 2010; 45:651-660.
6. Gouda MA, Berghot MA, El-Ghani GEA, Khalil AM. Synthesis and antimicrobial activities of some new thiazole and pyrazole derivatives based on 4,5,6,7-tetrahydrobenzothiophene moiety. Eur J Med Chem, 2010; 45:1338-1345.
7, Khalil AM, Berghot MA, Gouda MA. Synthesis and antibacterial activity of some new thiazole and thiophene derivatives. Eur J Med Chem, 2009; 44: 4434-4440.
8, Güzeldemirci NU, Küçükbasmaci Ö. Synthesis and antimicrobial activity evaluation of new 1,2,4-triazoles and 1,3,4-thiadiazoles bearing imidazol[2,1-b]thiazole moiety. Eur J Med Chem, 2010; 45:63-68.
9, Branowska D, Farahat AA, Kumar A, et al. Synthesis and antiprotozoal activity of 2,5-bis[amidinoaryl]thiazoles. Bioorg Med Chem, 2010; 18:3551-3558.
10. Yusuf M, Jain P. Synthesis and biological significances of 1,3,4-thiadiazolines and related heterocyclic compounds. Arab J Chem, 2011: in press.
11. Jin SH, Liang XM, Yang X, Chen FH, Wang DQ. Synthesis, structural characterization and solubility of 2-(1-substituted-1,11-undecylidene)-5-arylimino-Δ^3-1,3,4-thiadiazolines. Chin Chem Lett, 2009; 20:1267-1270.
12. Bondock S, Khalifa W, Fadda AA. Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3,4-dihydronaphatelene-2-carboxaldehyde. Eur J Med Chem, 2007; 42: 948-954.
13. Suni V, Kurup MRP, Nethaji M. Structural and spectral perpectives of a novel thiosemicarbazone synthesized from di-2-pyridyl ketone and 4-phenyl-3-thiosemicarbazide. Spectrochim Acta Part A, 2006; 63:174-181.
14. M. de Aquino T, Liesen AP, A. da Silva RE, et al. Synthesis, anti-Toxoplasma gondii and antimicrobial activities of benzaldehyde 4-phenyl-3-thiosemicarbazones and 2-[(phenylme thylene)hydrazono]-4-oxo-3-phenyl-5-thiazolidineacetic acids. Bioorg Med Chem, 2008; 16: 446-456.
15. Somogyi L. Synthesis, Oxidation and Dehydrogenation of cyclic N,O- and N,S-Acetals. Part 1. Transformation of N,S-Acetals: 3-Acyl-1,3,4-thiadiiazolines. Heterocycles, 2004; 63:2243-2267.
16. Varma RHK, Prabhakaran CP. Synthesis and characterisation of silver(I) complexes of some dithiocarbamates and thiosemicarbazones. Indian J Chem A, 1989; 28:1119-1121.
17. El-Gendy Z, Abdel-Rahman RM, Fawzy MM, Mahmoud MB. Biologically Active Thiazolidinones. Part-II. Synthesis and Fungitoxicities of isolated and fused Thiazolidinones derived from Thiosemicarbazone. J Indian Chem Soc, 1990; 67:927-929.
18. Tenorio RP, Carvalho CS, Pessanha CS, et al. Synthesis of thiosemicarbazone and 4-thiazolidinone derivatives and their in vitro anti-Toxoplasma gondii activity. Bioorg Med Chem Lett, 2005; 15:2575-2578.
19. Simiti I, Demian H. Farmacia, 1966; 14:735-738.
20. Simiti I, Demian H. Farmacia, 1973; 21:713-716.
21. Shih MH, Wu CL. Efficient synthesis of thiadiazoline and thiadiazole derivatives by the cyclization of 3-aryl-4-formylsydnone thiosemicarbazones with acetic anhydride and ferric chloride. Tetrahedron, 2005; 61:10917-10925.
22. Kyowa HK. Patent EP1454903 A1, 2004.
23. Silberg A, Simiti I, Mantsch H. About the synthesis and properties of 2-aryl-4-halomethyl-thiazoles, Chem Ber, 1961; 11:2887-2894.