Synthesis and Antibacterial Activity Investigation of New Heterocyclic Compounds from Triazole, Thiadiazole and Oxadiazole Class

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*Novel derivatives of 1,2,4,-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole were synthesized from cyclization of new 1-[4-(phenylsulfonyl)benzoyl]-4-(4-fluorophenyl)-thiosemicarbazide **2** which was obtained by reaction of the 4-(phenylsulfonyl)-benzoic acid hydrazide **1** with 4-fluorophenyl isothiocyanate. The compound **2**, in basic medium, gave 1,2,4-triazole-3(4H)-thione **3**, whereas in acidic medium 1,3,4-thiadiazol-2-amine **5** was obtained. The synthesis of 1,3,4-oxadiazol-2-amine **6** was carried out by reaction of the same acylthiosemicarbazide **2** with yellow mercuric oxide. Treatment of 1,2,4-triazole **3** with ethyl bromoacetate led to the S-alkylated 1,2,4-triazole derivative **4**. The newly synthesized compounds were characterized by elemental analysis and spectral studies (IR, UV-VIS, ^{1}H-NMR, ^{13}C-NMR, MS). All the synthesized compounds have been evaluated in vitro for their antibacterial activity against several strains of pneumococci and different type/reference strains of oral streptococci.*

Keywords: 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole, acylthiosemicarbazide, cyclization, antibacterial activity, S. pneumoniae, oral streptococci

Streptococcus pneumoniae plays a special role in human pathology, and sometimes, different species of oral streptococci may be involved in serious infections which need antimicrobial therapy too. Due to the increasing incidence of antibiotic resistant isolates [1,2], the investigation of the antimicrobial activity of some new synthesis organic compounds against such bacteria, as potential antimicrobial agents, remains of great interest.

The chemistry of heterocyclic compounds continues to be an explore field in the organic chemistry. 1,2,4-Triazole derivatives have occupied an unique position in penta-atomic heterocyclic chemistry, mainly due to their antimicrobial activity [3-9]. It is known that many derivatives from 1,3,4-thiadiazole and 1,3,4-oxadiazole class show various biological properties including the antimicrobial effect [4,9-13]. Also, many reports in the literature have indicated that diarylsulfones have antimicrobial activity [14].

As part of our program aimed to develop new antimicrobial compounds, this paper is a continuation of research in the field of heterocyclic compounds containing a diarylsulfone moiety with potential biological action [15-18] and reports the syntheses of some new derivatives from 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole class derived from 1-[4-(phenylsulfonyl)-benzoyl]-4-(4-fluorophenyl)-thiosemicarbazide **2** and their antibacterial activity against *S. pneumoniae* (reference strain and isolates from paediatric patients with respiratory infections) and different type or reference strains of oral streptococci.

The structure of the newly compounds was determined by spectral methods and elemental analysis.

Experimental part

All melting points were determined on a Böetius instrument and were not corrected. The IR spectra were recorded in KBr pellet for the solid compounds on a Vertex 70 Bruker spectrophotometer. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian Gemini 300BB spectrometer (at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR), in DMSO-d_6, using tetramethylsilane (TMS) as reference, the chemical shifts being reported in ppm and the coupling constants in Hz. The UV spectra were recorded in methanolic solutions on a SPECORD 40 Analytik Jena spectrophotometer. The mass spectra ESI-MS were recorded with a triple quadrupole mass spectrometer Varian 1200 L/MS/MS, coupled with a high performance liquid chromatograph with Varian ProStar 240 SDM ternar pump. The sample solution (2 μg/mL in cloroform/metanol 2/1, v/v) was introduced in the ESI interface by direct infusion, after a tenth dilution with methanol (at a flow rate of 20 μL/min.).

Synthesis of new compounds

The starting compound, 4-(4-phenylsulfonyl)-benzoic acid hydrazide **1**, was synthesized according to the previously reported procedure [19]. Acylthiosemicarbazide **2** was obtained by nucleophilic addition of hydrazide **1** to 4-fluorophenyl isothiocyanate. The 5-(4-(phenylsulfonyl) phenyl)-4-(4-fluorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione **3** was obtained by cyclization of acylthiosemicarbazide **2** in presence of sodium hydroxide. The ethyl 2-{[5-(4-(phenylsulfonyl)phenyl)-4-(4-fluorophenyl)-4*H*-1,2,4-triazol-3yl]-thio}acetate **4** was synthesized by reaction of

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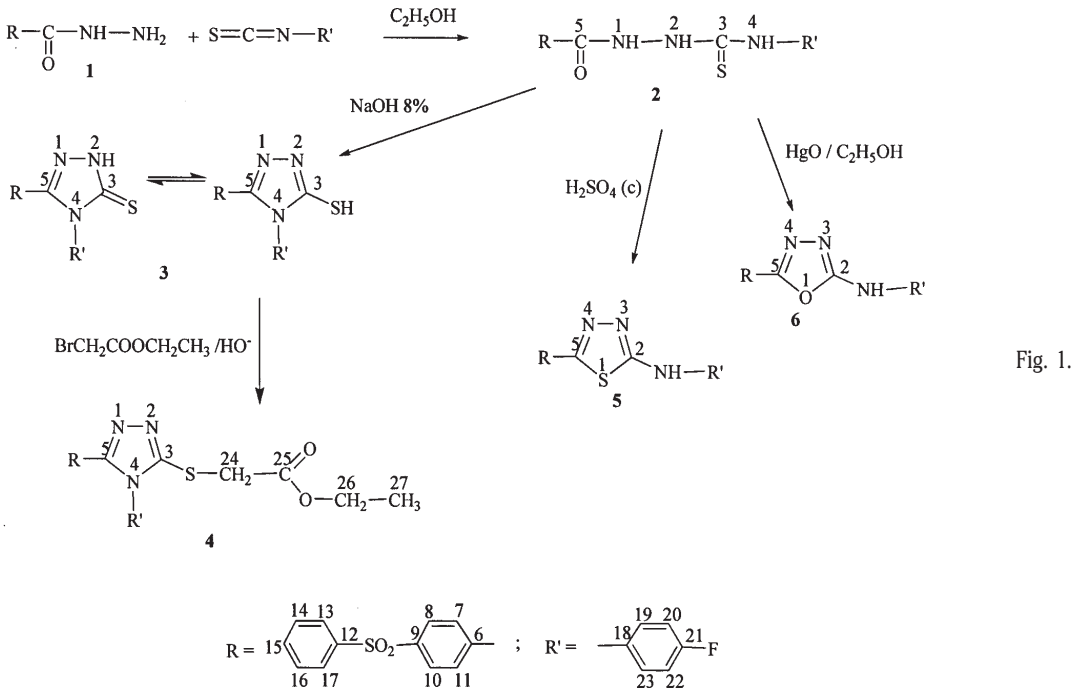

Fig. 1.

1,2,4-triazole **3** with ethyl bromoacetate, in basic media. The 5-(4-(phenylsulfonyl)-phenyl)-N-(4-fluoro-phenyl)-1,3,4-thiadiazol-2-amine **5** and 5-(4-(phenylsulfonyl)phenyl) -N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine **6** have been prepared by the cyclization of the same acylthiosemicarbazide **2** with sulfuric acid and yellow mercury oxide respectively.

The synthetic route for these new compounds is presented in figure 1.

4-(4-Fluorophenyl)-1-(4-(phenylsulfonyl)benzoyl)-thiosemicarbazide 2

An equimolar mixture of hydrazide **1** (2 mmol) and 4-fluorophenyl isothiocyanate in ethanol (5 mL) was heated to reflux for 10 h. The reaction mixture was cooled and the separated product was filtered off, dried and recrystallized from ethanol.

m.p.: 184-185°C; yield 97.5%; Elemental analysis (%) - Found C:56.01; H:3.70; S:14.88; N:9.85; Calcd. for $C_{20}H_{16}FN_3O_3S_2$ (429.49g/mol): C:55.93; H:3.77; S:14.93; N:9.78

IR (KBr; cm^{-1}): 3331s, 3306s, 3171s, 3091m, 3071m, 3045m, 1685s, 1547s, 1510s, 1483m, 1311s, 1296s, 1262s, 1222s, 1159s, 837m; ^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 8.13 (d, 9.2; 2H; H-7; H-11); 8.09 (d, 9.2; 2H; H-8, H-10); 8.00 (dd, 7.7;1.8; 2H; H-13, H-17); 7.72 (dd, 7.7;1.5; 2H; H-14, H-16); 7.70 (tt, 7.1;1.5; 1H, H-15); 7.40 (ws, 1H; H-19); 7.17 (t, 8.8; 1H, H-20); 7.17 (t, 8.8; 1H; H-22); 7.40 (ws; 1H; H-23); 9.85 (ws, 1H, NH); 9.95 (ws, 1H, NH); 10.81 (ws, 1H; NH); ^{13}C-NMR (DMSO-d_6, δ, ppm): 181.90 (C-3); 164.63 (C-5); 137.07 (C-6); 127.52 (C-7, C-11); 127.52 (C-8, C-10); 143.77 (C-9); 140.62 (C-12); 129.84 (C-13, C-17); 129.26 (C-14, C-16); 134.05 (C-15); 135.42 (C-18); 128.35 (d, 16.0; C-19); 114.70 (d, 22.6; C-20); 159.20 (d, 242.3; C-21); 114.70 (d, 22.6; C-22); 128.30 (d, 16.0; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 203 (4.60); 247 (3.46); 353 (3.33); ESI-MS, *m/z* (%): $[M+H]^+$ 430

4-(4-Fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-2H-1,2,4-triazole-3(4H)-thione 3

Thiosemicarbazide **2** (0.5 mmol) was added to 4 mL of NaOH 8% solution and the reaction mixture was heated under reflux for 4 h . After cooling, the solution was acidified with a diluted solution of HCl. Crude product was precipitated, filtered off and washed with water. The solid thus separated was dried and recrystallized from $CHCl_3$/ petroleum ether (1:1, v/v).

m.p.: 267-268°C; yield 70.3%; Elemental analysis (%) - Found C:58.49; H:3.31; S:15.53; N:10.09; Calcd. for $C_{20}H_{14}FN_3O_2S_2$ (411.47g/mol): C:58.38; H:3.43; S:15.59, N:10.21;

IR (KBr; cm^{-1}): 3429m, 3086s, 3065w, 2923m, 1601m, 1579w, 1535m, 1512s, 1470m, 1324s, 1290s, 1249s, 1228s, 1160s, 842s; ^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 7.52 (d, 8.8; 2H; H-7; H-11); 7.92 (d, 8.8; 2H; H-8, H-10); 7.92 (dd, 7.4;1.4; 2H; H-13, H-17); 7.43 (wt, 7.4; 2H; H-14, H-16); 7.52 (tt, 7.4;1.4; 1H, H-15); 7.55 (dd, 8.8;5.0; 1H; H-19); 7.43 (t, 8.8; H-20); 7.43 (t, 8.8; 1H; H-22); 7.55 (dd; 8.8; 5.0; 1H; H-23); ^{13}C-NMR (DMSO-d_6, δ, ppm): 169.16 (C-3); 149.08 (C-5); 130.46 (C-6); 129.44 (C-7, C-11); 127.70 (C-8, C-10); 142.46 (C-9); 140.36 (C-12); 127.60 (C-13, C-17); 129.92 (C-14, C-16); 134.13 (C-15); 131.05 (C-18); 131.5 (d, 9.2; C-19); 116.50 (d, 22.9; C-20); 162.80 (d, 247.1; C-21); 116.50 (d, 22.9; C-22); 131.5 (d, 9.2; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 203.5 (4.62); 253 (4.44); 317 (3.96); ESI-MS, *m/z* (%): $[M+H]^+$ 412

Ethyl 2-(4-(4-fluorophenyl)-5-(4-(phenylsulfonyl) phenyl)-4H-1,2,4-triazol-3-ylthio)acetate 4

The 1,2,4-triazole **3** (1 mmol) was added to a solution of sodium ethoxide (1 mmol of sodium dissolved in 10 mL ethanol and the mixture was shaken for a few minutes at the room temperature until a solution was obtained. The ethyl bromoacetate (1 mmol) was added at this solution and the reaction mixture was stirred at room temperature for 12 h. The mixture was poured into ice water and the obtained compound was filtered off, washed with water and recrystallized from ethanol.

m.p.: 147°C; yield 77.2%; Elemental analysis (%) - Found C:58.04; H:3.89; S:12.80; N:8.36; Calcd. for $C_{24}H_{20}FN_3O_4S_2$ (497.56g/mol): C:57.93; H:4.05; S:12.89, N:8.45;

IR (KBr; cm^{-1}): 3072m, 3045w, 2984w, 2930w, 1732s, 1601w, 1541w, 1509s, 1444m, 1307s, 1290m, 1222m, 1159s, 842m; ^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 7.58 (d, 8.5; 2H; H-7; H-11); 7.96 (d, 8.5; 2H; H-8, H-10); 7.95 (dd, 7.5;1.4; 2H; H-13, H-17); 7.62 (t, 7.5, 2H; H-14, H-16); 7.70 (tt, 7.5;1.4; 1H; H-15); 7.56 (dd, 8.5;6.9; 1H; H-19); 7.43 (t, 8.5; 1H; H-20); 7.43 (t, 8.5; 1H; H-22); 7.56 (dd; 8.5;6.9;

1H; H-23); 4.10 (s, 2H, H-24); 4.21 (q, 7.1; 2H; H-26); 1.18 (t, 7.1; 3H, H-27); ^{13}C-NMR (DMSO-d_6, δ, ppm): 153.05 (C-3); 152.39 (C-5); 131.14 (C-6); 127.50 (C-7, C-11); 127.78 (C-8, C-10); 141.95 (C-9); 140.47 (C-12); 129.88 (C-13, C-17); 130.01 (C-14, C-16); 134.05 (C-15); 130.13 (C-18); 130.10 (d, 9.4; C-19); 117.28 (d, 23.2; C-20); 162.63 (d, 248.2; C-21); 117.28 (d, 23.2; C-22); 130.10 (d, 9.4; C-23); 33.98 (C-24); 167.99 (C-25); 61.36 (C-26); 13.98 (C-27); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 204 (4.63); 240 (4.16); 280 (4.26); ESI-MS, *m/z* (%): $[M+H]^+$ 498

N-(4-fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-1,3,4-thiadiazol-2-amine 5

A mixture of thiosemicarbazide **2** (0.1 mmol) and concentrated H_2SO_4 (4 mL) was stirred at 0°C for 3 h and then, at room temperature, for another 3 h. The reaction mixture was neutralized with a diluted solution of ammonium hydroxide, at 0°C. The obtained precipitated was filtered off, washed with water, dried and recrystallized from $CHCl_3$/petroleum ether (1:1, v/v).

m.p.: 270-271°C; yield 76.7%; Elemental analysis (%) - Found C:58.43; H:3.39; S:15.53; N:10.30; Calcd. for $C_{20}H_{14}FN_3O_2S_2$ (411.47g/mol): C:58.38; H:3.43; S:15.59, N:10.21;

IR (KBr; cm^{-1}): 3340m, 3057m, 3008m, 1628m, 1583s, 1558s, 1510s, 1495s, 1317s, 1291s, 1228s, 1156s, 836m; ^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 8.06 (s; 2H; H-7; H-11); 8.06 (s; 2H; H-8, H-10); 8.00 (wd, 8.0; 2H; H-13, H-17); 7.68 (m; 4H; H-14, H-15, H-16, H-19, H-23); 7.20 (t, 8.5; H-20); 7.20 (t, 8.5; 1H; H-22); 10.70 (ws, 1H; NH); ^{13}C-NMR (DMSO-d_6, δ, ppm): 165.26 (C-2); 155.68 (C-5); 134.75 (C-6); 127.75 (C-7, C-11); 128.32 (C-8, C-10); 140.75 (C-9); 136.75 (C-12); 127.41 (C-13, C-17); 129.84 (C-14, C-16); 133.91 (C-15); 141.77 (C-18); 119.52 (d, 8.0; C-19); 115.71 (d, 22.7; C-20); 157.55 (d, 239.6; C-21); 115.71 (d, 22.7; C-22); 119.52 (d, 8.0; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 203 (4.38); 260 (4.23); 345 (4.19); ESI-MS, *m/z* (%): $[M+H]^+$ 412

N-(4-fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-1,3,4-oxadiazol-2-amine 6

To a solution of thiosemicarbazide **2** (1 mmol) in ethanol, the yellow mercuric oxide (2 mmol) was added and the reaction mixture was refluxed for 8 h. The solvent was removed under reduced pressure and the residue was treated with DMF and then was filtered off. At the obtained filtrate was added ethanol (ethanol:DMF 1:1,v/v) and allowed to stand overnight. The obtained precipitated was filtered off.

m.p.: 280-282°C; yield 77.5%; Elemental analysis (%) - Found C:60.85; H:3.48; S:8.05; N:10.71; Calcd. for $C_{20}H_{14}FN_3O_3S$ (395.41g/mol): C:60.75; H:3.57; S:8.11, N:10.63;

IR (KBr; cm^{-1}): 3269m, 3072m, 1617s, 1585s, 1576s, 1510s, 1321m, 1292s, 1222m, 1159s, 832m; ^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 8.15 (d, 8.8; 2H; H-7; H-11); 8.09 (d, 8.8; 2H; H-8, H-10); 7.65 (t, 6.9; 2H; H-13, H-17); 8.00 (dd, 6.9;1.4; 2H; H-14, H-16); 7.73 (tt, 7.4;1.4; 1H; H-15); 7.63 (dd, 9.0;5.0; 1H; H-19); 7.21 (t, 9.0; 1H; H-20); 7.21 (t, 9.0; 1H; H-22); 7.63 (dd; 9.0;5.0; 1H; H-23); 10.82 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6, δ, ppm): 160.41 (C-2); 158.55 (C-5); 128.22 (C-6); 128.46 (C-7, C-11); 126.60 (C-8, C-10); 142.53 (C-9); 140.52 (C-12); 127.49 (C-13, C-17); 129.90 (C-14, C-16); 134.04 (C-15); 156.62 (C-18); 118.87 (d, 8.02; C-19); 115.78 (d, 22.5; C-20); 157.46 (d, 239.0; C-21); 115.78 (d, 22.5; C-22); 118.87 (d, 8.0; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 202 (4.41); 249 (4.32); 323 (4.26); ESI-MS, *m/z* (%): $[M+H]^+$ 396

Investigation of the antimicrobial activity of the new compounds

The antimicrobial activity of the 5 new heterocyclic compounds was tested by the broth microdilution method in order to determine the minimum inhibitory concentrations (MIC) and afterwards, the minimum bactericidal concentrations (MBC) against the reference strain *S. pneumoniae* ATCC 49619 and 11 type/reference strains of different species of oral streptococci. In addition, the antimicrobial activity of these compounds was tested against 30 clinical isolates of *S. pneumoniae* recovered in 2009 from paediatric patients with various respiratory infections (mostly in the *otorhinolaryngeal sphere*) and stored at -70°C at the laboratory of the Microbiology Department of the Faculty of Dentistry, University of Medicine and Pharmacy "Carol Davila" – Bucharest, during an exploratory research project (contract no.1136/2009) granted by the National University Research Council (CNCSIS) and the Executive Agency for Higher Education and Research Funding (UEFISCSU) from Romania.

The dimethyl sulfoxide (DMSO) was used to perform the stock solution of each compound at a concentration of 2048μg/mL. The antimicrobial activity of DMSO has been determined previously against the pneumococcal strains and the following type/reference strains of oral streptococci used in this research: *S. mitis* ATCC 6249, *S. oralis* LMG 14532 (BCCM™/LMG, Ghent University), *S. sanguinis* ATCC 10556, *S. parasanguinis* ATCC 15909, *S. gordonii* LMG 14516 (BCCM™/LMG, Ghent University), *S. anginosus* NCTC 10713, *S. constellatus* LMG 14507 (BCCM™/LMG, Ghent University), *S. intermedius* LMG 17840 (BCCM™/LMG, Ghent University), *S. mutans* ATCC 25175, *S. salivarius* ATCC 13419 and *S. vestibularis* ATCC 49124, and showed no growth-inhibitory effect.

A two-fold dilution series from 1/2 to 1/1024 in Muller-Hinton broth (MHB) supplemented with 3% lysed horse blood was performed in 96 well microplates in a 50 mL volume per well. The bacterial inoculum was prepared for each strain from a 24 h culture on blood agar (BA) by transferring several colonies into MHB and adjusting the suspension at the turbidity of 0.5 McFarland standard, yielding approximately 1.5 x 10^8 colony forming units (CFU)/mL. The inoculum was diluted 1/100 by transferring an aliquot of 100 μL into a tube with 9900 μL MHB to obtain a density of 1 x 10^6 CFU/mL, of which aliquots of 50 μL were added into each well containing the newly synthesized substances and into the positive growth control well (already containing 50 μL compound-free MHB), within 30 min for achieving a final bacterial density of 5 x 10^5CFU/mL. All wells contained a final liquid volume of 100 μL, including the negative growth control well (the sterility control well, filled only with compound-free MHB). An inoculum control was performed by removing 10 μL from the growth control well immediately after inoculation, diluting them into 10 mL MHB and spreading of 100 μL of this dilution onto a BA plate.

The microplates were sealed with sterile adhesive sheets and covered with proper lids and were incubated at 37°C for 24 h, together with the inoculum control plates. The bacterial growth in the wells containing the tested compounds was examined macroscopically and compared to the aspect of the positive and negative growth controls. The *MIC* was recorded as the lowest concentration of the respective compound that inhibited the visible bacterial growth (absence of turbidity or growth button).

In order to determine the MBC, 10 µL were taken from the wells without microbial growth (as well as from the negative and positive growth controls) and applied in spot on BA plates, using an electronic pipette. After 48h of incubation in an atmosphere supplemented with 5% CO_2 at 37°C, the MBC value was considered to be achieved in the well containing the lowest concentration of the tested compound which was able to kill ≥ 99.9% of the bacterial amount determined previously in the inoculum control plate.

Results and discussions

Chemistry

The formation of the mentioned compounds was confirmed on the basis of their spectral data and elemental analysis.

Acylthiosemicarbazide **2** shows in the IR spectrum the following characteristic absorption bands: $\nu_{C=O}$ (1685 cm^{-1}), $\nu_{C=S}$ (1262 cm^{-1}) and ν_{NH} (3331, 3306, 3171 cm^{-1}).

In the IR spectra of 1,2,4-triazole **3**, 1,3,4-thiadiazole **5** and 1,3,4-oxadiazole **6** no absorption bands were detected about 1685 cm^{-1} indicating the absence of C=O group of acylthiosemicarbazide which is an evidence for the conversion of compound **2** to these heterocyclic compounds. Also, in the IR spectra of these new heterocyclic compounds **3,5,6** a stretching band characteristic of the C=N group from heterocyclic nucleus at 1601 cm^{-1} (from triazole), 1628 cm^{-1} (from thiadiazole) and 1617 cm^{-1} (from oxadiazole) appear too.

Although two types of tautomers, thione or thiole, could be expected from the cyclization of acylthiosemicarbazide **2**, under basic media, only the thione type compounds were observed in the IR spectra of the 1,2,4-triazole **3**. Existence of the thione form predominantly in solid state is demonstrated by the presence of two absorption bands at 1249 cm^{-1} and 3429 cm^{-1} belonging to the $\nu_{C=S}$ and ν_{NH} groups, respectively, and by absence of ν_{SH} [20].

The stretching band due to NH group from 1,3,4-thiadiazole **5** and 1,3,4-oxadiazole **6** was present at 3340 cm^{-1} and 3269 cm^{-1} respectively.

In 1,2,4-triazole derivative **4**, obtained by alkylation of compound **3** with ethyl bromoacetate, a new stretching band appears, at 1732 cm^{-1}, which corresponding to the C=O from ester group. Absence of stretching vibration of C = S group in compound **4** shows that alkylation occurred at the sulfur atom [21].

In ^{1}H-NMR spectrum of the 1,2,4-triazole S-alkylated **4**, the signals from -$COOCH_2CH_3$ group appear as triplet, at δ = 1.18 ppm, for methyl protons and as quartet, at 4.21 ppm, for methylene protons. Also, the methylene group linked to sulfur atom is present, as singlet, at 4.10 ppm.

The singlet signal characteristic to NH proton from 1,3,4-thiadiazole **5** and 1,3,4-oxadiazole **6** appears at 10.70 ppm and 10.82 ppm, respectively.

In ^{13}C-NMR spectra of new heterocyclic compounds **3,5,6** the absence of the signals for carbonyl (164.63 ppm) and thiocarbonyl (181.90 ppm) carbon from compounds **2**, and appearance of two new signals in the range of 149.08-158.55 ppm and 160.81-169.16 pm corresponding to C-5 and C-2 (from oxa(thia)diazole)/C-3 (from triazole) heterocyclic carbon also confirmed that cyclization of acylthiosemicarbazide took place. In 1,2,4-triazole **3** the signal at 169.16 ppm indicated that this compound exists predominantly, in solution, in the thione tautomeric form [22]. The ^{13}C-NMR spectra of S-alkylated compound **4** showed four characteristic signals at 33.98 ppm, 167.99 ppm, 61. 36 ppm and 13.98 ppm for the S-CH_2, C=O, CH_2 and CH_3, respectively. In this compound **4**, the presence of the C-3 signal at 153.05 ppm, which is more shielded than the carbon atom C=S from 1,2,4-triazole **3**, shows that alkylation occurred at the sulfur and not at nitrogen atom. In the mass spectra of these compounds, the presence of the molecular ion confirmed the proposed structure.

Antimicrobial activity

The values of MIC and MBC of the 5 newly synthesized compounds tested against the 11 type/reference strains of different oral streptococcal species are presented in table 1. The values of MIC ranged between: 16-128 µg/mL for triazole **3**, 32-128 µg/mL for both thiosemicarbazide **2** and S-alkylated triazole **4**, and 128-256 µg/mL for thiadiazole **5** and oxadiazole **6**, while the values of MBC ranged between: 64-128 µg/mL for both triazole **3** and **4**, 128-256 µg/mL for thiosemicarbazide **2** and thiadiazole **5**, and 128-512 µg/mL for oxadiazole **6,** which exhibited the highest MBC value when tested against *S. anginosus* NCTC 10713 and *S. mitis* ATCC 6249 (table 1).

Table 1

THE MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BACTERICIDAL CONCENTRATIONS (MBC) OF THE NEW COMPOUNDS AGAINST THE 11 TYPE/REFERENCE STRAINS OF ORAL STREPTOCOCCI

Compound	Values of MIC / MBC (µg / mL) against:										
	S. anginosus NCTC 10713	*S. constellatus* LMG 14507	*S. intermedius* LMG 17840	*S. mutans* ATCC 25175	*S. mitis* ATCC 6249	*S. oralis* LMG 14532	*S. sanguinis* ATCC 10556	*S. parasanguinis* ATCC 15909	*S. gordonii* LMG 14516	*S. salivarius* ATCC 13419	*S. vestibularis* ATCC 49124
2	128/256	32/128	32/128	64/128	128/128	64/128	128/128	128/256	64/128	128/128	64/256
3	128/128	32/128	16/128	32/64	64/128	64/128	64/128	64/128	64/128	64/128	32/64
4	128/128	32/128	32/128	32/64	64/128	128/128	128/128	64/128	128/128	64/128	32/64
5	128/256	256/256	128/128	128/256	256/256	256/256	128/256	128/256	256/256	256/256	128/256
6	256/512	256/256	128/128	128/256	256/512	256/256	128/256	128/256	256/256	256/256	256/256

Table 2

THE MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF THE NEW COMPOUNDS AGAINST 30 ISOLATES OF *S. PNEUMONIAE*

Compound	Values of MIC (µg / mL)				Values of MBC (µg / mL)			
	MIC_{min} [a]	MIC_{max} [b]	MIC_{50} [c]	MIC_{90} [d]	MBC_{min} [e]	MBC_{max} [f]	MBC_{50} [g]	MBC_{90} [h]
2	16	128	64	128	32	256	128	256
3	8	128	32	64	32	256	128	128
4	16	128	64	128	32	256	128	256
5	64	256	128	256	64	256	256	256
6	64	512	128	256	128	512	256	256

[a]The lowest MIC value, [b]the highest MIC value, [c]the MIC value at which 50% of the isolates were inhibited, [d]the MIC value at which 90% of the isolates were inhibited, [e]the lowest MBC value, [f]the highest MBC value, [g]the MBC value at which 50% of the isolates were killed, [h]the MBC value at which 90% of the isolates were killed.

The MIC values against *S. pneumoniae* ATCC 49619 were the following: 32 μg/mL for acylthiosemicarbazide **2** and S-alkylated triazole **4**, 16 μg/mL for triazole **3** and 128 μg/mL for both thiadiazole **5** and oxadiazole **6**, while the MBC values were: 32 μg/mL for compounds **2**, **3** and **4** and 128 μg/mL for compound **5** and **6**. The minimum and maximum values of the MIC (MIC_{min} and MIC_{max}, respectively) and MBC (MBC_{min} and MBC_{max}, respectively) of the new heterocyclic compounds against the 30 pneumococcal isolates are indicated in table 2. In addition, the compounds concentrations that inhibited or killed 50% of the isolates (MIC_{50} and MBC_{50}, which were equivalent to the median values of the MIC and MBC, respectively) are mentioned in the same table, together with the concentrations values that inhibited or killed 90% of the isolates (MIC_{90} and MBC_{90}, which corresponded to the 90th percentile of the MIC and MBC distributions, respectively) (table 2).

The MBC/MIC ratios obtained in the case of these new heterocyclic compounds tested against the strains mentioned above were ≤ 4, and only 1,2,4-triazole **3** has shown a MBC/MIC ratio of 8, when tested against *S. intermedius*. However, of the 5 new compounds investigated, 1,2,4-triazole-3(4*H*)-thione **3** exhibited the highest degree of inhibition against all strains used in this research work, except for *S. anginosus*. The alkylation of 1,2,4-triazole failed to confer compound **4** a better antibacterial effect. Nevertheless, S-alkylated 1,2,4-triazole derivative **4** was as active as 1,2,4-triazole-3(4*H*)-thione **3** against the type/reference strains belonging to the following oral streptococcal species: *S. anginosus*, *S. constellatus*, *S. mutans*, *S. mitis*, *S. parasanguinis*, *S. salivarius* and *S. vestibularis*, and against one half of the pneumococcal isolates. For the rest of the strains, S-alkylated 1,2,4-triazole **4** showed a lower antimicrobial effect compared to 1,2,4-triazole **3**, inhibiting the growth of *S. intermedius* and *S. sanguinis* at the same MIC as acylthiosemicarbazide **2**, but at a higher concentration than compound **2** in the case of *S. oralis* and *S. gordonii*. Among the new compounds described in this article, 1,3,4-oxadiazol-2-amine **6** has shown the lowest antimicrobial activity against both type/reference strains and clinical isolates, being closely followed by 1,3,4-thiadiazol-2-amine **5**.

Comparing the MIC values achieved against *S. pneumoniae* ATCC 49619 to those obtained against the pneumococcal clinical isolates, compounds **2**, **3** and **4** seemed to be less active against most of the clinical isolates than against the reference strain.

Conclusions

New heterocyclic compounds from 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole class were synthesized, characterized and tested *in vitro* for their antibacterial activities against some pneumococcal strains and several type/reference strains of oral streptococci. The new 1,2,4-triazole **3** has been resulted by reaction of acylthiosemicarbazide **2** with sodium hydroxide, and afterwards was treated with ethyl bromoacetate in order to obtain the S-alkylated derivative **4**. The new 1,3,4-thiadiazole **5** and 1,3,4-oxadiazole **6** have been obtained by treatment of acylthiosemicarbazide **2** with sulfuric acid and yellow mercury oxide, respectively. New intermediate from thiosemicarbazide class **2**, necessary for the synthesis of these heterocyclic compounds, was obtained by reaction of 4-(phenylsulfonyl)-benzoic acid hydrazide with 4-fluorophenyl isothiocyanate.

The structure of the new obtained compounds was confirmed by different spectral methods and elemental analysis.

The results of the present study have indicated the necessity for antimicrobial activity investigation of every newly synthesised compounds against both reference strains and clinical isolates. In this regard, acylthiosemicarbazide **2**, S-alkylated 1,2,4-triazole derivative **4**, and especially, 1,2,4-triazole-3(4H)-thione **3**, which exhibited a higher degree of growth inhibition compared to the other 2 new compounds (thiadiazole **5** and oxadiazole **6**), need further screening for their antibacterial activity against clinically important isolates belonging to different species oral streptococcal.

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