New Heterocyclic Compounds from 1,3,4-Thiadiazole, 1,3,4-Oxadiazole and 1,2,4-Triazole Class with Potential Antibacterial Activity

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*In this paper new heterocyclic compounds from 2-amino-1,3,4-thiadiazole, 2-amino-1,3,4-oxadiazole and 1,2,4-triazol-3(4H)-thione class were obtained from acylthiosemicarbazide **2** by intramolecular cyclization in different conditions. In the reaction of 4-(4-bromophenylsulfonyl)-benzoic acid hydrazide **1** with 4-fluorophenyl isothiocyanate, the new 1-[4-(4-bromophenylsulfonyl)benzoyl]-4-(4-fluorophenyl)-thiosemicarbazide **2** was obtained. 5-(4-(4-Bromophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine **3** was prepared by the intramolecular cyclization of acylthiosemicarbazide **2** in concentrated sulphuric acid media. 5-(4-(4-Bromophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine **4** was obtained from reaction of acylthiosemicarbazide **2** with HgO, in alcoholic media, or with I_2/KI, in basic media. The synthesis of 5-(4-(4-bromophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione **5** was carried out by cyclodehydration of acylthiosemicarbazide **2** in sodium hydroxide media. Alkylation of 1,2,4-triazole **5** with ethyl bromoacetate afforded S-substituted 1,2,4-triazole **6**. Acylation of same 1,2,4-triazole **5** with acetyl chloride led to the N-substituted 1,2,4-triazole **7**. The structures of these new compounds were elucidated by FTIR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR, MS spectra and elemental analysis. All the new compounds were screened for their antibacterial activity against 11 type strains of different species of oral streptococci.*

Keywords: acylthiosemicarbazide, 2-amino-1,3,4-thiadiazole, 2-amino-1,3,4-oxadiazole, 1,2,4-triazole-3(4H)-thione, oral streptococci.

In the last few decades, the chemistry of pentaatomic heterocyclic compounds from 1,3,4-thiadiazole, 1,3,4-oxadiazole and 1,2,4-triazole class has received considerable attention owing to their biological importance.

Many 2-amino-1,3,4-oxadiazole derivatives are reported in the literature to have a wide range of therapeutic activities like antimicrobial [1-3], antifungal, [2,3], antitubercular [3], anti-inflammatory [4,5], analgesic [5], etc. Also, it is known that many 2-amino-1,3,4-thiadiazole and 1,2,4-triazol-3-thione derivatives have biological activity: antibacterial [6-11], antifungal [11-16], tuberculostatic [11,17], anti-inflammatory, analgesic [4,5,10,18], etc.

Meanwhile, diphenylsulfone derivatives were found to possess antibacterial activity [19,20].

Taking these data into account, and continuing our research on the synthesis of heterocyclic compounds with potential biological activity [21-28], in the present study, some new 1,3,4-thiadiazole, 1,3,4-oxadiazole and 1,2,4-triazole derivatives having a diphenylsulfone moiety have been synthesized and tested from their antibacterial activity. The structure of these new compounds was confirmed by elemental and spectral (FT-IR, UV-Vis, ^{1}H-NMR, ^{13}C-NMR, MS) analyses.

Experimental part

The melting points of the new compounds were determined with a Böetius apparatus and are not corrected. The IR spectra were recorded using KBr pallets on a Vertex 70 Bruker spectrophotometer, at 4000-400 cm^{-1}, and the UV spectra on a SPECORD 40 Analytik Jena spectrophotometer, within the range 200-600 nm. The NMR spectra were recorded in DMSO-d_6 on a Varian Gemini 300BB apparatus, at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR using tetramethylsilane (TMS) as internal standard. The mass spectra were registered with a triple quadrupole mass spectrometer Varian 1200 L/MS/MS, with electrospray interface (ESI), coupled with a high performance liquid chromatograph with Varian ProStar 240 SDM ternar pump. The sample solution (2 µg/mL in chloroform/methanol 2/1, v/v) was introduced in the ESI interface by direct infusion, after a tenth dilution with methanol, at a flow rate of 20µL/min.

Synthesis of new compounds

N^1-[4-(4-Bromophenylsulfonyl)benzoyl]-N^4-(4-fluorophenyl)-thiosemicarbazide **2** was synthesized through the refluxing of an equimolecular mixture formed by the 4-(4-bromophenylsulfonyl)-benzoic acid hydrazide **1**, known in the literature [19], and the 4-fluorophenyl izothiocyanate, in anhydrous ethanol. Acylthiosemicarbazide **2** underwent three different cyclization reactions. Thus, when thiosemicarbazide **2** was treated with cold concentrated H_2SO_4 resulted in dehydrative cyclization giving the 5-(4-(4-bromophenylsulfonyl) phenyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine **3**. By treatment of **2** with a NaOH 8% solution, under reflux, the

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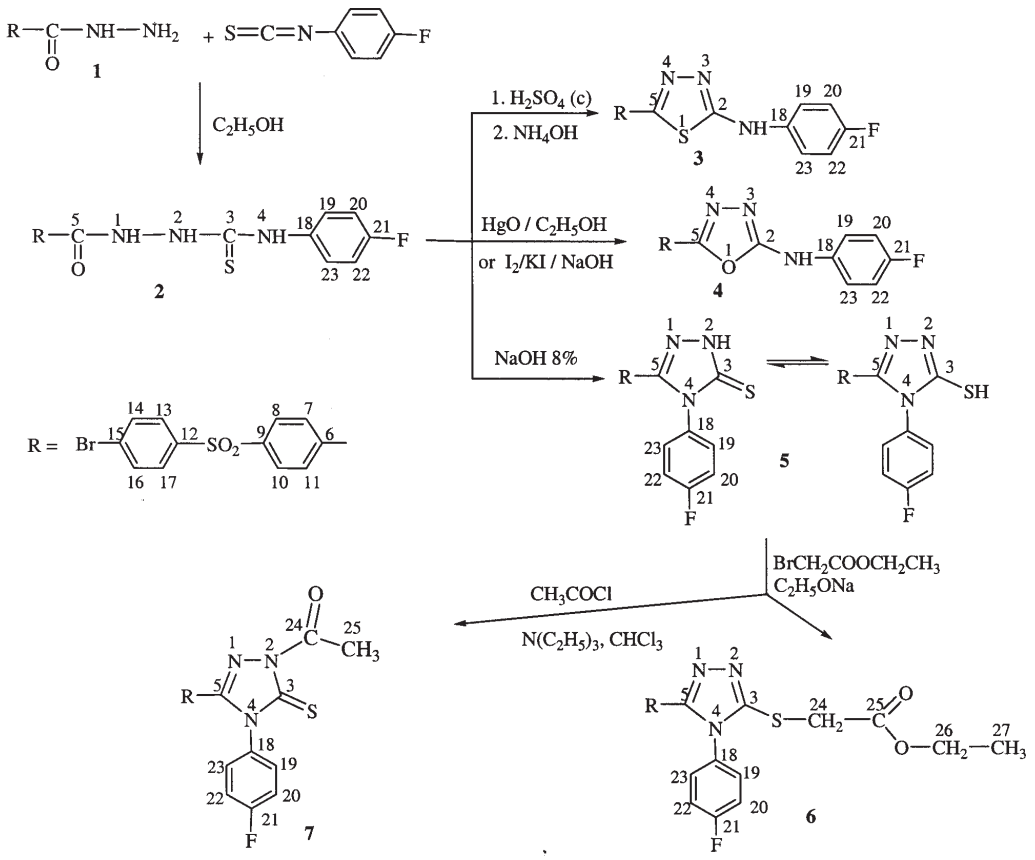

Fig. 1.

5-(4-(4-bromophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-2*H*-1,2,4-triazole-3(4*H*)-thione **5** was obtained. 5-(4-(4-Bromophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine **4** was synthesized from thiosemicarbazide **2** by two methods: by reaction with HgO in ethanol media or by reaction with I_2/KI in NaOH solution media. Alkylation of 1,2,4-triazole **5** with ethyl bromoacetate, in the presence of sodium ethoxide gave the corresponding ethyl 2-{[5-(4-((4-bromophenyl) sulfonyl) phenyl)-4-(4-fluorophenyl)-4*H*-1,2,4-triazol-3yl]-thio}acetate **6**. When the same triazole **5** was treated with acethyl chloride, in basic media, the acylated derivatives, ethyl 2-(5-(4-(4-bromophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazol-2-yl)ethanone **7**, was obtained (fig. 1).

Synthesis of 1-(4-(4-bromophenylsulfonyl)benzoyl) - 4-(4-fluorophenyl)-thiosemicarbazide 2

At 5 mmol of hydrazide **1** was added 5 mmol of 4-fluorophenyl isothiocyanate and 13 mL ethanol. The mixture was refluxed for 10 h. The solid product was filtered, washed with few cold ethanol and recrystalized from ethanol.

m.p.: 189-190°C; yield 92.0%

Elemental analysis (%) - Found C:47.19; H:2.88; S:12.57; N:8.19; Calcd. for $C_{20}H_{15}BrFN_3O_3S_2$ (508.38g/mol): C:47.25; H:2.97; S:12.61; N:8.27;

IR (KBr; cm^{-1}): 3320s, 3290s, 3086w, 3060w, 1678s, 1533s, 1509s, 1473m, 1318s, 1293m, 1260m, 1220s, 1158s, 837m, 577m

^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 8.16 (d, 8.8; 2H; H-7; H-11); 8.10 (d, 2H; H-8, H-10); 7.93 (d, 8.8; 2H; H-13, H-17); 7.86 (d, 8.8, 2H; H-14, H-16); 7.40 (ws, 1H; H-19); 7.15 (t, 8.8; 1H; H-20); 7.15 (t, 8.8; 1H; H-22); 7.40 (ws; 1H; H-23); 9.80-9.85 (ws, 2H, NH); 10.80 (ws, 1H, NH)

^{13}C-NMR (DMSO-d_6, δ, ppm): 181.62 (C-3); 164.58 (C-5); 137.26 (C-6); 129.33 (C-7, C-11); 127.52 (C-8, C-10); 143.25 (C-9); 139.84 (C-12); 128.54 (C-13, C-17); 133.00 (C-14, C-16); 128.33 (C-15); 135.27 (C-18); 129.60 (d, 16.0; C-19); 114.73 (d, 22.3; C-20); 159.23 (d, 232.0; C-21); 114.73 (d, 22.3; C-22); 129.6 (d, 16.0; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 203.5 (4.62); 230 (4.34); 255 (4.49); 363 (3.25); ESI-MS, *m/z* (%): $[M+H]^+$ 508 (^{79}Br); $[M+H]^+$ 510 (^{81}Br)

Synthesis of 5-(4-(4-bromophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine 3

The thiosemicarbazide **2** (1 mmol) was added in portions to 40 mL of concentrated sulphuric acid at 0°C with continuous stirring. The reaction mixture was stirred for 3 h at 0°C and still 3 h at room temperature. The solution obtained was neutralized, at 0°C, with a diluted solution of ammonium hydroxide. The solid precipitated was filtered, washed with water, dried and recrystallized from $CHCl_3$/petroleum ether (1:1, v/v).

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

m.p.: 251-252°C; yield 90%

Elemental analysis (%) - Found C:49.05; H:2.59; S:13.03; N:8.51; Calcd. for $C_{20}H_{13}BrFN_3O_2S_2$ (490.37g/mol): C:48.99; H:2.67; S:13.08, N:8.57;

IR (KBr; cm^{-1}): 3340w, 3058w, 3013w, 1629m, 1573s, 1558m, 1509s, 1495s, 1325s, 1290m, 1231m, 1157s, 836m, 571m

^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 8.07 (ws; 2H; H-7; H-11); 8.07 (ws, 2H; H-8, H-10); 7.92 (d, 8.8; 2H; H-13, H-17); 7.85 (d, 8.8, 2H; H-14, H-16); 7.68 (dd, 8.8;4.9; 1H; H-19); 7.23 (t, 8.8; 1H; H-20); 7.23 (t, 8.8; 1H; H-22); 7.68 (dd; 8.8;4.9; 1H; H-23); 10.78 (ws, 1H, NH)

^{13}C-NMR (DMSO-d_6, δ, ppm): 165.31 (C-2); 155.62 (C-5); 135.01 (C-6); 127.81 (C-7, C-11); 128.42 (C-8, C-10); 139.99 (C-9); 136.73 (C-12); 129.42 (C-13, C-17); 132.95 (C-14, C-16); 128.15 (C-15); 141.23 (C-18); 119.55 (d, 7.7; C-19); 115.72 (d, 22.6; C-20); 157.55 (d, 239.6; C-21); 115.72 (d, 22.6; C-22); 119.55 (d, 7.7; C-23); UV-Vis (CH_3OH) λ_{max}/nm, (log ε)): 204 (4.40); 262 (4.30); 348

(4.21); ESI-MS, *m/z* (%): $[M+H]^+$ 490 (^{79}Br); $[M+H]^+$ 492 (^{81}Br)

Synthesis of 5-(4-(4-bromophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine 4

a. To a solution of thiosemicarbazide **2** (1 mmol) in ethanol was added yellow mercuric oxide (2 mmol). The mixture was refluxed for 8 h. The solvent was distilled off in vacuo and the residue was dissolved in DMF. At solution was added ethanol (ethanol:DMF 1:1,v/v) and allowed to stand overnight. The solide obtained was filtered off.

b. To a suspension of the thiosemicarbazide **2** (1 mmol) in ethanol (25 mL), sodium hydroxide solution (5N, 2 mL) was added with shaking. A solution of iodine in potassium iodide was added dropwise with stirring till the colour of iodine persisted. The reaction mixture was then refluxed for 5 h on a water bath. The solid mass was filtered off, washed with water and recrystallized from $CHCl_3$/ petroleum ether (1:1, v/v).

*5-(4-(4-bromophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine **4***

m.p.: 300-302°C; yield 69%

Elemental analysis (%) - Found C:50.57; H:2.82; S:6.71; N:8.97; Calcd. for $C_{20}H_{13}BrFN_3O_3S$ (474.3g/mol): C:50.65; H:2.76; S:6.76, N:8.86;

IR (KBr; cm^{-1}): 3306m, 3074w, 3015w, 1621s, 1591s, 1580s, 1516m, 1323m, 1292m, 1236m, 1157s, 840m, 564m

^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 8.17 (d, 8.8; 2H; H-7; H-11); 8.04 (d, 8.8; 2H; H-8, H-10); 7.92 (d, 8.8; 2H; H-13, H-17); 7.85 (d, 8.8; 2H; H-14, H-16); 7.62 (dd, 8.8;4.8; 1H; H-19); 7.20 (t, 8.8; 1H; H-20); 7.20 (t, 8.8; 1H; H-22); 7.62 (dd; 8.8;4.8 1H; H-23); 10.80 (s, 1H, NH)

^{13}C-NMR (DMSO-d_6, δ, ppm): 160.44 (C-2); 156.60 (C-5); 128.42 (C-6); 128.53 (C-7, C-11); 126.65 (C-8, C-10); 141.99 (C-9); 139.75 (C-12); 129.50 (C-13, C-17); 133.00 (C-14, C-16); 128.31 (C-15); 156.58 (C-18); 118.88 (d, 7.8; C-19); 115.70 (d, 22.4; C-20); 157.47 (d, 239.0; C-21); 115.70 (d, 22.4; C-22); 118.88 (d, 7.8; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 203 (4.35); 252 (4.24); 326 (4.12); ESI-MS, *m/z* (%): $[M+H]^+$ 474 (^{79}Br); $[M+H]^+$ 476 (^{81}Br)

Synthesis of 5-(4-(4-bromophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione 5

To corresponding thiosemicarbazide **2** (1 mmol), a solution of NaOH 8% (8 mL) was added. The reaction mixture was heated under reflux for 4 h and then a diluted solution of HCl 1N was added until it reached pH 7 when a solid product was formed. The product was filtered, washed with water and recrystallized from ethanol.

m.p.: 292-294°C; yield 83.2 %

Elemental analysis (%) - Found C:49.10; H:2.60; S:13.01; N:8.49; Calcd. for $C_{20}H_{13}BrFN_3O_2S_2$ (490.37g/mol): C:48.99; H:2.67; S:13.08, N:8.57;

IR (KBr; cm^{-1}): 3429w, 3084m, 3022w, 1601w, 1572m, 1535w, 1512s, 1470m, 1327s, 1283m, 1248m, 1225m, 1160s, 840m, 573m

^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 7.86 (d, 9.0; 2H; H-7; H-11); 7.85 (d, 9.0; 2H; H-8, H-10); 7.98 (t, 8.5; 2H; H-13, H-17); 7.52 (d, 8.5; 2H; H-14, H-16); 7.45 (dd, 8.8;5.0; 1H, H-19); 7.36 (t, 8.8; 1H; H-20); 7.36 (t, 8.8; H-22); 7.45 (dd, 8.8;5.0; 1H; H-23)

^{13}C-NMR (DMSO-d_6, δ, ppm): 169.17 (C-3); 149.05 (C-5); 130.67 (C-6); 133.03 (C-7, C-11); 129.63 (C-8, C-10); 141.94 (C-9); 139.59 (C-12); 127.60 (C-13, C-17); 129.48 (C-14, C-16); 128.42 (C-15); 139.58 (C-18); 131.10 (d, 8.9; C-19); 116.42 (d, 23.2; C-20); 162.19 (d, 247.3; C-21); 116.42 (d, 23.2; C-22); 131.10 (d, 8.9; C-23); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 204 (4.62); 258 (4.46); 320 (3.86); ESI-MS, *m/z* (%): $[M+H]^+$ 490 (^{79}Br), $[M+H]^+$ 492 (^{81}Br)

Synthesis of ethyl 2-{[5-(4-((4-bromophenyl) sulfonyl)phenyl)-4-(4-fluorophenyl)-4H-1,2,4-triazol-3yl]-thio}acetate 6

To a solution of 1,2,4-triazole **5** (1 mmol) in sodium ethoxide (1 mmol of sodium dissolved in 10 mL ethanol) was added ethyl bromoacetate (1 mmol). The reaction mixture was stirred at room temperature for 12 h and then poured into ice water. The solid obtained was filtered off, washed with water and recristallized from ethanol.

m.p.: 159-160°C; yield 65.0%

Elemental analysis (%) - Found C:50.12; H:3.23; S:11.05; N:7.36; Calcd. for $C_{24}H_{19}BrFN_3O_4S_2$ (576.46g/mol): C:50.00; H:3.32; S:11.12, N:7.29;

IR (KBr; cm^{-1}): 3082m, 2982m, 2935w, 1733s, 1600m, 1573m, 1511s, 1468s, 1438s, 1322s, 1287m, 1228m, 1160s, 845m, 577m

^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 7.80 (d, 8.5; 2H; H-7; H-11); 7.95 (d, 8.5; 2H; H-8, H-10); 7.98 (d, 8.5; 2H; H-13, H-17); 7.61 (d, 8.5; 2H; H-14, H-16); 7.59 (dd, 8.8;6.9; 1H; H-19); 7.46 (t, 8.8; 1H; H-20); 7.46 (t, 8.8; 1H; H-22); 7.59 (dd; 8.8;6.9; 1H; H-23); 4.10 (s, 2H, H-24); 4.11 (q, 7.1; 2H; H-26); 1.19 (t, 7.1; 3H, H-27)

^{13}C-NMR (DMSO-d_6, δ, ppm): 153.05 (C-3); 152.47 (C-5); 131.36 (C-6); 133.02 (C-7, C-11); 129.61 (C-8, C-10); 141.46 (C-9); 139.73 (C-12); 127.89 (C-13, C-17); 128.94 (C-14, C-16); 128.35 (C-15); 131.37 (C-18); 130.11 (d, 9.4; C-19); 117.35 (d, 23.2; C-20); 163.64 (d, 248.5; C-21); 117.35 (d, 23.2; C-22); 130.11 (d, 9.4; C-23); 34.01 (C-24); 168.14 (C-25); 61.39 (C-26); 14.01 (C-27); UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 204 (4.55); 249 (4.15); 280 (4.14); ESI-MS, *m/z* (%): $[M+H]^+$ 576 (^{79}Br), $[M+H]^+$ 578 (^{81}Br)

Synthesis of ethyl 2-(5-(4-(4-bromophenylsulfonyl) phenyl)-4-(4-fluorophenyl)-3-thioxo-3,4-dihydro-1,2,4-triazol-2-yl)ethanone 7

A mixture of triazole **5** (0,1 mol), acethyl chloride (0,1 mol), triethylamine (0,2 mol) and 3 mL chloroform were refluxed for 6 h. The solvent was distilled off in vacuo and the obtained precipitate was filtered off, washed with water and recrystallized from ethanol.

m.p.: 245°C; yield 70.0%

Elemental analysis (%) - Found C:49.50; H:2.95; S:12.14; N:7.97; Calcd. for $C_{22}H_{15}BrFN_3O_3S_2$ (532.41g/mol): C:49.63; H:2.84; S:12.05, N:7.89;

IR (KBr; cm^{-1}): 3088w, 3056w, 3034w, 2966w, 2875w, 1755s, 1601w, 1574m, 1510s, 1325s, 1269s, 1245m, 1212m, 1155s, 840m, 569m

^{1}H-NMR (DMSO-d_6, δ, ppm, *J*, Hz): 7.61 (d, 8.5; 2H; H-7; H-11); 8.01 (d, 8.5; 2H; H-8, H-10); 7.88 (d, 9.1; 2H; H-13, H-17); 7.84 (d, 9.1, 2H; H-14, H-16); 7.48 (dd, 8.8;5.2; 1H; H-19); 7.36 (t, 8.8; 1H; H-20); 7.48 (t, 8.8; 1H; H-22); 7.48 (dd; 8.8;5.2; 1H; H-23); 2.72 (s, 3H, H-25);

^{13}C-NMR (DMSO-d_6, δ, ppm): 169.55 (C-3); 148.69 (C-5); 130.05 (C-6); 133.02 (C-7, C-11); 127.77 (C-8, C-10); 142.72 (C-9); 139.44 (C-12); 127.77 (C-13, C-17); 129.60 (C-14, C-16); 128.47 (C-15); 130.05 (C-18); 131.37 (d, 9.2; C-19); 116.71 (d, 23.2; C-20); 162.35 (d, 247.6; C-21); 116.71 (d, 23.2; C-22); 131.37 (d, 9.2; C-23); 167.76 (C-24); 24.68 (C-25);

UV-Vis (CH_3OH) (λ_{max}/nm, (log ε)): 202 (4.46); 228.5 (4.27); 256 (4.33); 292 (4.11);

Antibacterial activity

The *in vitro* antimicrobial activity of the compounds was tested for determination of the minimum inhibitory concentration (MIC) by the broth microdilution method. All tested compounds were dissolved in dimethyl sulfoxide (DMSO) at the concentration of 2048 μg/mL. DMSO was previously determined to have no antimicrobial activity against any of the tested microorganisms. Binary serial dilutions from 1:2 to 1:256 in Mueller-Hinton II (cation-adjusted broth) with 3% lysed horse blood (BMHII) was performed in 96-well plates, in a 50 μL volume/well.

The 6 new heterocyclic compounds were tested for their *in vitro* growth inhibitory activity against the following oral streptococcal type strains: *S. anginosus* NCTC 10713, *S. mutans* ATCC 25175, *S. salivarius* ATCC 13419, *S. mitis* ATCC 6249, *S. sanguinis* ATCC 10556, *S. parasanguinis* ATCC 15909, *S. vestibularis* ATCC 49124, *S. constellatus* LMG 14507 (BCCM™/LMG, Ghent University), *S. intermedius* LMG 17840 (BCCM™/LMG, Ghent University), *S. oralis* LMG 14532 (BCCM™/LMG, Ghent University) and *S. gordonii* LMG 14516 (BCCM™/LMG, Ghent University). The bacterial inoculum was adjusted to the turbidity of 0.5Mc.Farland standard and was diluted 1:100 in Mueller-Hinton broth for achieving a density of 1 x 10^6 CFU/mL. Aliquots of 50 μL of the diluted inoculum were added to each well containing the tested compounds and in the positive growth control well, filled already with 50 μL broth without compound. The sterility control well contained only compound-free broth (100 μL). The final liquid volume in each well was of 100 μL.

The inoculum control was performed by removing 10 μL from the growth control well immediately after inoculation and diluting it in 10 mL Mueller-Hinton broth. After vortex-mixing, 100 μL of this dilution were spread onto the surface of a Columbia blood agar plate, which was incubated aerobically at 37^0C for 24h.

The 96-microwell plate was sealed with a sterile adhesive sheet, covered with a tight lid and incubated aerobically at 37°C for 24 h. The amount of growth in each well was compared to that of the positive control and the MIC value was determined by macroscopic observations of microbial growth and it corresponded to the well with the lowest concentration of the tested substance where microbial growth was clearly inhibited (last dilution with clear liquid, respectively).

For the minimum bactericidal concentration (MBC) determination, an amount of 10 μL from all wells without visible bacterial growth and from the positive and negative controls too, was applied in spot (using an electronic pipette) onto Columbia blood agar plates, with incubation in atmosphere of 5%CO_2 at 37^0C for 48h. The MBC was considered the lowest compound concentration at which ≥99.9% bacterial killing was achieved, based on the bacterial count in the inoculum control.

Results and discussions

Chemistry

The structures of the new compounds were confirmed by spectral analyses.

The IR Spectra

The IR spectra of the acylthiosemicarbazide **2** have C=O stretching band at 1678 cm^{-1}. In 1,3,4-thiadiazole **3**, 1,3,4-oxadiazole **4** and 1,2,4-triazol-3(4*H*)-thione **5**, the disappearance of C=O streatching band of the acylthiosemicarbazides and detecting of C=N stretching band at 1601-1629 cm^{-1} is an evidence for ring closure. The 1,3,4-thiadiazole **3** and 1,3,4-oxadiazole **4** showed NH stretching band at 3340 cm^{-1} and 3306 cm^{-1}, respectively. 1,2,4-Triazole **5** may exist in thiole and thione forms. The IR spectra of this compound **5** presents an absorption band due to stretching vibration of NH group in the region of 3429 cm^{-1} and that of C=S group at 1248 cm^{-1} which shows that this compound is found, in solid, predominantly in thione form. In the IR spectra of S-substituted 1,2,4-triazole **6** presence of a new strong band at 1733 cm^{-1} characteristic for C=O from carboxyl group confirmed alkylation reaction of 1,2,4-triazole **5** with ethyl bromoacetate. The absence of the stretching band C=S demonstrated that the alkylation took place at sulphur atom. The IR spectra of 1,2,4-triazole **7** showed a peak at 1755 cm^{-1} due to carbonyl function derived from acetyl group. Presence of absorption band due to stretching vibration of the group thiocarbonyl (1245 cm^{-1}) demonstrates that the acylation took place at the nitrogen atom. Also, the absorption band due to ν_{NH} group which in triazole **5** appear at 3429 cm^{-1}, in compound **6** and **7** is not present.

The ^{1}H-NMR spectra

In the ^{1}H-NMR spectra of all new compounds **2-6**, all protons were seen accordingly to the expected chemical shift and integral values.

In the acylthiosemicarbazide **2** NH signals were observed as singlets at a chemical displacement between δ = 9.80-10.80 ppm. Also, NH proton of the compounds having thiadiazole **3** and oxadiazole **4** nucleus were seen at about 10.78 ppm and 10.80 ppm, respectively. The signal singlet attributed for two protons of S-CH_2 group from S-alkylated 1,2,4-triazole **6** appears at δ= 4.10 ppm. Besides, in this derivative **6** appear another two signals characteristic to $COOCH_2CH_3$ group: triplet for CH_3 at 1.19 ppm and quartet for CH_2 at 4.11 ppm. The singlet signal corresponding to the three protons of methyl group from compound **7** is present at 2.72 ppm.

The ^{13}C-NMR spectra

In the ^{13}C-NMR spectra of the acylthiosemicarbazide **2** are present two characteristic signals to the carbon from C=O (164.58 ppm) and C=S (181.62 ppm) groups, which disappear in the new heterocyclic compounds from 1,3,4-thiadiazole **3**, 1,3,4-oxadiazole **4** and 1,2,4-triazole **5** class.

Cyclization of this acylthiosemicarbazide **2** to 1,3,4-thiadiazole **3** and 1,3,4-oxadiazole **4** was proved in the ^{13}C-NMR spectra by presence of two new signals characteristic to C-2 heterocyclic carbon (165.31 ppm for thiadiazole and 160.44 ppm for oxadiazole) and to C-5 heterocyclic carbon (155.62 ppm for thiadiazole and 156.60 ppm for oxadiazole). In the 1,2,4-triazole **5**, C-3 (C=S) and C-5 heterocyclic carbons resonated at 169.17 ppm and 149.05 ppm, respectively. Presence of the C=S signal demonstrates that this compounds exists, in solution, predominantly in thione form. The ^{13}C-NMR of the S-alkylated derivative **6** showed signals at 61.39 and 14.01 ppm due to C-26 and C-27 carbons from $COOCH_2CH_3$ group. Also, the signals attributed to carbons from C=O and SCH_2 groups appeared at 168.14 ppm and 34.01 ppm, respectively. In acylated 1,2,4-triazole **7**, the carbon signals of C=O from acethyl and CH_3 groups are present at 167.76 ppm and 24.68 ppm, respectively.

The C-3 heterocyclic carbon of this new S-alkylated 1,2,4-triazole **6** (δ= 153.05 ppm) is more shielded than the C-3 heterocyclic carbon from 1,2,4-triazole **5** (δ= 169.17 ppm). These results indicate that the alkylation took place at sulphur atom and not to N-2 nitrogen atom. Unlike the S-alkylated derivative **6**, the signal of carbon atom C-3

Table 1
THE MIC AND MBC VALUES OF THE 6 NEWLY SYNTHESIZED COMPOUNDS TESTED AGAINST 11 TYPE STRAINS OF ORAL STREPTOCOCCI

Nr. Comp	*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
	CMI/CMB	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*	*CMI/CMB*
2	128/256	64/128	64/128	128/512	64/256	64/256	128/256	64/256	64/256	64/512	64/128
3	256/512	128/256	128/512	128/512	128/256	64/256	128/256	128/512	128/512	128/512	128/128
4	256/512	128/128	128/256	256/512	256/512	128/512	128/256	128/512	128/256	128/512	128/512
5	64/256	32/64	32/128	64/256	64/256	32/256	64/256	64/256	64/256	64/256	32/128
6	128/512	128/128	64/256	128/512	128/256	64/256	128/256	128/256	128/256	128/256	64/256
7	128/256	64/256	128/256	64/256	128/256	64/256	256/512	64/256	64/256	128/256	64/128

from 1,2,4-triazole **7** occurs at 169.55 ppm which shows that the acylation took place at the nitrogen atom [29,30].

Antibacterial activity

The MICs and MBCs (μg/mL) of the newly prepared compounds tested for their antimicrobial activity on type strains belonging to different species of oral streptococci are presented in table 1. The MIC values ranged between: 64-128 μg/mL for compounds **2** and 6, 64-256 μg/mL for compounds **3** and **7**, 128-256 μg/mL for compound **4** and 32-64 μg/mL for compound **5**, whereas the MBC values ranged between: 128-512 μg/mL for all compounds, except for compound **5**, with MBC values ranging from 64 to 256 μg/mL. However, the MBC/MIC ratios were ≤ 4, indicating a bactericidal effect of all the compounds on the tested bacteria.

The newly synthesized substances showed not a very important antimicrobial activity against the tested strains, except for 1,2,4-triazol-3(4*H*)-thione **5**, which exhibited inhibitory activity on all 11 species of oral streptococci. Compared to the other newly prepared substances presented in this article, 2-amino-1,3,4-oxadiazole **4** seemed to be the less active one on growth inhibition of the oral streptococci. All compounds were able to inhibit *S. oralis* growth in concentration of 32-64 μg/mL, except for 1,3,4-oxadiazole **4**, while only 1,2,4-triazole **5** presented lower MIC values for *S. anginosus*, *S. sanguinis* and *S. oralis*. Compounds obtained by alkylation or acylation of 1,2,4-triazole **5** (S-alkylated 1,2,4-triazole **6** and N-acetylated 1,2,4-triazole **7**) are less active than this. With the exception of *S. mutans*, *S. mitis* and *S. oralis* strains where 1,3,4-thiadiazole **3** is more active compared to 1,3,4-oxadiazole **4**, in the other strains both compounds had the same values of MIC .

Conclusions

This study reports the synthesis and characterization of new pentaatomic heterocyclic compounds from 2-amino-1,3,4-thiadiazole **3**, 2-amino-1,3,4-oxadiazole **4** and 1,2,4-triazol-3(4*H*)-thione **5** class having a diphenylsulfone moiety which were synthesized by intramolecular cyclization of new acylthiosemicarbazide **2** under different conditions. 1,2,4-Triazole derivative **6** was obtained by alkylation of 1,2,4-triazol-3(4*H*)-thione **5** with ethyl bromoacetate in basic media. 1,2,4-Triazole **7** was synthesized by acylation of 1,2,4-triazol-3(4*H*)-thione **5** with acetyl chloride in basic media. The spectral data and the elemental analysis confirmed the structure of these new compounds.

The potential antibacterial activity of these new compounds was also studied and 1,2,4-triazol-3(4*H*)-thione **5** was found to be the most active one against the oral streptococcal type strains and deserves further investigation on clinical isolates of oral streptococci. Since these streptococci can produce sometimes serious infections which need antimicrobial treatment and due to the considerable concern regarding their increasing resistance to beta-lactam and other commonly used antibiotics, investigation of the antimicrobial activity of newly synthesized compounds on these bacteria should be of great interest.

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