Synthesis and Antibacterial Activity of Some Triazole, Thiadiazole and Oxadiazole Derivatives

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This study presents synthesis of new heterocyclic compounds from 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole class obtained by cyclization of corresponding acylthiosemicarbazide, in different media. The new intermediate acylthiosemicarbazide was obtained by treatment of 4-(4-chlorophenylsulfonyl)-benzoic acid hydrazide 1 with 4-fluorophenyl isothiocyanate. Structures of the new compounds were identified by spectroscopic technique (IR, UV-VIS, 1H-NMR, 13C-NMR, MS) and also confirmed by elemental analysis. The new compounds have been screened for their in vitro antibacterial activities against several type strains of oral streptococci.

Keywords: 1,2,4-triazole, 1,3,4-thia(oxa)diazole, acylthiosemicarbazide, 4-(4-chlorophenyl-sulfonyl)phenyl moiety, 4-fluorophenyl moiety, antibacterial activity

The resistance of bacteria against antimicrobial agents has become a widespread medical problem. Because of this development of resistance, many drugs which were very effective long ago before became now useless. Moreover, the toxic effects produced by many antibiotics must not be forgotten. So, the need for new antimicrobial agents is a priority in the medical world.

Several five-membered aromatic systems having three heteroatoms at symmetrical position have been studied because of their interesting biological properties. It is well established that various derivatives of 1,2,4-triazole, 1,3,4-thiadiazole and 1,3,4-oxadiazole exhibit broad spectrum of biological properties including antibacterial [1-11]. Because diarylsulfones are known in the literature for their antibacterial activity [12,13], the goal of the present study was to synthesize new derivatives from triazole, thiadiazole and oxadiazole class containing a diarylsulfone fragment in order to discover new antibacterial agents.

In previous studies [14,15] we reported the synthesis of some new heterocyclic compounds from these classes that contain both 4-(4-X-phenylsulfonyl)phenyl (X = H, Br) and 4-fluorophenyl radical moieties.

It is well known that the presence of halogenated groups in organic molecules often confers significant and useful changes in their chemical, physical and biological properties due to the elevated electronegativity and lipophilic character of halogen atoms [16].

Knowing this data from the literature, the aim of this study was to synthesize new derivatives of these classes that contain on the 4-(4-X-phenylsulfonyl)phenyl fragment, instead of bromine, the chlorine atom, more electronegative, because the presence of this atom could increase the biological activity of these compounds.

For this purpose, new derivatives from triazole, thiadiazole and oxadiazole class, containing in their molecule both 4-(4-chlorophenylsulfonyl)phenyl and 4-fluorophenyl fragments, were synthesized by cyclization of corresponding acylthiosemicarbazide, in different media, and were tested for their antibacterial activity against some oral streptococcal type strains. The oral streptococci belong to the normal flora of the oral cavity, but sometimes they can produce infective endocarditis and other kind of infections.

Experimental part

Melting points were determined on a Böetius apparatus and were not corrected. The IR spectra were recorded with a Vertex 70 Bruker spectrophotometer (in KBr pellet). The IR bands are given as w – weak, m – medium, s – intense, vs – very intense. The 1H-NMR and 13C-NMR spectra were recorded, in DMSO-d6, on a Vario Gemini 300BB instrument, at room temperature, operating at 300 MHz for 1H and 75 MHz for 13C-NMR. Chemical shifts were given as δ values in parts per million (ppm) relative to tetramethylsilane as internal standard and coupling constants J in Hz. Spin multiplets are given as: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet) and w (wide). 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 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. The UV spectra were recorded, in methanic solutions, on a SPECORD 40 Analytik Jena spectrophotometer.

Synthesis of new compounds

The title compounds were synthesized according to the sequence shown in the Scheme 1.

The new compounds (2-6) (X= Cl) were obtained by the same procedures as derivatives (X-C4H4SO-C6H4-; X= H, Br) described previously [14,15]. The 4-(4-chloro-

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(1) was synthesized following the method described in literature [17].

The new intermediate from acylthiosemicarbazide class (2) was obtained by reaction of hydrazide (1) with 4-fluorophenyl isothiocyanate.

Cyclodehydration of this compound (2), in presence of sodium hydroxide, occurred with obtaining of 5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-2\textsuperscript{H}-1,2,4-triazole-3(4\textsuperscript{H})-thione (3), while in presence of sulfuric acid led to 5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (5). Alkylation of 1,2,4-triazole (3) with ethyl bromoacetate, in presence of sodium ethoxide, afforded ethyl 2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-4\textsuperscript{H}-1,2,4-triazol-3-ylthio)acetate (4).

Treat the same acylthiosemicarbazide (2) with yellow mercury oxide, 5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (6) was obtained due to the cyclodesulfurization.

**Preparation of 1-(4-(4-chlorophenylsulfonyl)benzoyl)-4-(4-fluorophenyl)-thiosemicarbazide (2)**

A mixture of (1) (1 mmol) and 4-fluorophenyl isothiocyanate (1 mmol) in absolute ethanol (3 mL) was refluxed for 10 h. After cooling, the formed solid was filtered off and crystallized from ethanol to give white crystals of acylthiosemicarbazide (2).

- m.p.: 175-176°C; yield 92.0%;
- Elemental analysis (%) - Found C: 51.86; H: 3.33; N: 8.98; Calcd. for C\textsubscript{20}H\textsubscript{15}ClFN\textsubscript{3}O\textsubscript{3}S\textsubscript{2} (463.93 g/mol): C: 51.78; H: 3.26; N: 9.06
- IR (KBr; cm\textsuperscript{-1}): 3316s, 3170s, 3088w, 3045w, 1681s, 1530s, 1509s, 1478m, 1319s, 1297s, 1260m, 1220s, 1158s, 836m, 756s; UV-Vis (CH\textsubscript{3}OH) (λ\textsubscript{max}/nm, (log ε)): 203.5 (4.58); 225 (4.34); 252 (4.48); 359.5 (2.71); ESI-MS, m/z (%): [M+H]\textsuperscript{+} 464 (56\textsuperscript{Cl}); [M+H]\textsuperscript{+} 466 (58\textsuperscript{Cl})

The spectral data \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR are presented in tables 1 and 2.

**Preparation of 5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-2\textsuperscript{H}-1,2,4-triazole-3(4\textsuperscript{H})-thione (3)**

A mixture of (2) (1 mmol) and NaOH 8% solution (8 mL) was heated under reflux for 4 h. The obtained solution was cooled, acidified with a diluted solution of HCl 1% (pH = ~ 5.5). The obtained precipitated was separated by filtration, washed with water, dried and recrystallized from CHCl\textsubscript{3}/petroleum ether (1:1, v/v).

- m.p.: 284-285°C; yield 97.2%;
- Elemental analysis (%) - Found C: 53.98; H: 2.88; N: 9.33; Calcd. for C\textsubscript{20}H\textsubscript{13}ClFN\textsubscript{3}O\textsubscript{2} (445.92 g/mol): C: 53.87; H: 2.94; N: 9.42
- IR (KBr; cm\textsuperscript{-1}): 3418w, 3086m, 3022w, 1601s, 1580m, 1536m, 1512s, 1475m, 1327s, 1283m, 1250m, 1160s, 840m, 768s; UV-Vis (CH\textsubscript{3}OH) (λ\textsubscript{max}/nm, (log ε)): 205 (4.63); 255 (4.47); 322.5 (3.92); ESI-MS, m/z (%): [M+H]\textsuperscript{+} 446 (56\textsuperscript{Cl}); [M+H]\textsuperscript{+} 448 (58\textsuperscript{Cl})

The spectral data \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR are presented in tables 1 and 2.

**Preparation of ethyl 2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-4\textsuperscript{H}-1,2,4-triazol-3-ylthio)acetate (4)**

To a solution of compound (3) (1 mmol) in sodium ethoxide (prepared from 2.5 mg metallic sodium in 10 mL ethanol), ethyl bromoacetate (1 mmol) was added with stirring at room temperature. The reaction mixture was stirred at room temperature for 12 h, and then was poured into ice water. The obtained precipitate was filtered off, washed with water, dried and recrystallized from ethanol.

- m.p.: 174-175°C; yield 72.0%;
- Elemental analysis (%) - Found C: 54.27; H: 3.54; N: 7.81; Calcd. for C\textsubscript{24}H\textsubscript{19}ClFN\textsubscript{3}O\textsubscript{4}S\textsubscript{2} (532.01 g/mol): C: 54.18; H: 3.60; N: 7.90

The spectral data \textsuperscript{1}H-NMR and \textsuperscript{13}C-NMR are presented in tables 1 and 2.
IR (KBr; cm⁻¹): 3090m, 3074m, 2982m, 2936w, 2902w, 2840w, 1724s, 1601m, 1582m, 1511s, 1473m, 1439m, 1322s, 1286s, 1229m, 1160vs, 845m, 768s; UV-Vis (CH₃OH) (λmax/nm, (log ε)): 204 (4.63); 244 (4.21); 281 (4.25); ESI-MS, m/z (%): [M+H]+ 532 (35Cl), [M+H]+ 534 (37Cl)

The spectral data ¹H-NMR and ¹³C-NMR are presented in tables 1 and 2.

**Preparation of 5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (5)**

A solution of (2) (1 mmol) in conc. sulfuric acid (40 mL) was stirred at 0°C for 3 h and then at room temperature for 3 h. The reaction mixture was neutralized with a diluted solution of ammonium hydroxide and then, the obtained solid was collected by filtration, washed with water, dried and recrystallized from CHCl₃/petroleum ether (1:1, v/v).

m.p.: 245-247°C; yield 89.2%; Elemental analysis (%) - Found C:53.94; H:2.83; N:9.50; Calcd. for C₂₀H₁₃ClFN₃O₂S₂ (445.92 g/mol): C:53.87; H:2.94; N:9.42

IR (KBr; cm⁻¹): 3307m, 3089m, 3060m, 1617m, 1558s, 1510s, 1494s, 1327s, 1280m, 1230m, 1157s, 834m, 765s; UV-Vis (CH₃OH) (λmax/nm, (log ε)): 205 (4.40); 262 (4.25); 346 (4.18); ESI-MS, m/z (%): [M+H]+ 446 (35Cl); [M+H]+ 448 (37Cl)

The spectral data ¹H-NMR and ¹³C-NMR are presented in tables 1 and 2.

**Preparation of 5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(4-fluorophenyl)-1,3,4-oxadiazol-2-amine (6)**

To a solution of thiosemicarbazide (2) (5 mmol) in ethanol, the yellow mercuric oxide (10 mmol) was added and the reaction mixture was refluxed for 8 h. The residue obtained after removal of solvent by distillation under reduced pressure was dissolved in DMF. At this solution was added ethanol (ethanol:DMF 1:1,v/v) and allowed to stand overnight. The obtained precipitated was filtered off, dried and recrystallized from ethanol.

m.p.: 293-295°C; yield 56%; Elemental analysis (%) - Found C:55.77; H:2.94; N:9.69; Calcd. for C₂₀H₁₃ClFN₃O₃S (429.85 g/mol): C:55.88; H:3.05; N:9.78

IR (KBr; cm⁻¹): 3304m, 3073w, 3015w, 1622s, 1590s, 1516s, 1582s, 1510s, 1494s, 1327s, 1280m, 1230m, 1157s, 834m, 765s; UV-Vis (CH₃OH) (λmax/nm, (log ε)): 203 (4.38); 251 (4.29); 327 (4.21); ESI-MS, m/z (%): [M+H]+ 430 (35Cl); [M+H]+ 432 (37Cl)

The spectral data ¹H-NMR and ¹³C-NMR are presented in tables 1 and 2.

**Antibacterial activity testing**

The in vitro testing of the antibacterial activity of compounds was performed using the broth microdilution method, in order to detect the minimum inhibitory concentrations (MIC). The compounds were dissolved in dimethyl sulfoxide (2048 μg/mL). This solvent showed no antibacterial activity against the tested bacterial strains. Series of binary dilutions of the new compounds (from 1:2 to 1:256) were performed in Mueller-Hinton cation-adjusted broth supplied with 3% lysed horse blood, in 96-well plates, in a broth volume of 50μL/well.

The antibacterial activity of the compounds was tested against the following strains of oral streptococci: S. anginosus NCTC 10713, S. mitis ATCC 6249, S. mutans ATCC 25175, S. parasanguis ATCC 15909, S. salicarius ATCC 13419, S. sanguinis ATCC 10556 and S. vestibularis ATCC 49124. An inoculum adjusted at 0.5 McFarland turbidity was made from each type strain and was afterwards diluted in Mueller-Hinton broth to 1/100, in order to obtain a bacterial density of 1 x 10⁶ CFU/mL. From the diluted inoculum, 50 μL were added in all the wells containing the tested compounds and in the wells with the positive growth control (which already contained 50 μL of compound-free broth). The wells with the negative growth (the sterility control) were filled in only with 100 μL compound-free broth.

The 96-microwell plates were sealed with sterile adhesive sheet and covered by their lid. An inoculum control for each bacterial strain was prepared by removing an aliquot of 10 μL just after inoculating the positive growth control wells, diluting it with 10 mL of Mueller-Hinton broth by vortexing, and afterwards, by spreading of 100 μL of the diluted broth.
inoculum on a Columbia blood agar plate with further aerobic incubation 5% CO₂ atmosphere, at 37°C for 24 h.

The MIC were determined the next day, after checking the macroscopic aspect of the positive and negative growth controls. The MIC values were considered the lowest concentrations of the tested compounds that inhibited the microbial growth, indicated by the last well without any turbidity or growth button.

For detecting the minimum bactericidal concentrations (MBC), aliquots of 10 μL were removed from all the wells without visible bacterial growth and from the positive and negative growth controls too, and inoculated in spots on Columbia blood agar plates by an electronic pipette. After a 48h period of incubation in 5% CO₂ atmosphere, at 37°C, the MBC values were considered to be the lowest concentrations of the compounds able to kill ≥ 99.9% of the final bacterial inoculum.

**Results and discussions Chemistry**

The structures of compounds (2-6) were established by their IR, 1H-NMR, 13C-NMR and MS spectra. The IR absorptions due to the C=O and C=S functions from new aclythiosemicarbazide (2) appeared at 1681 and 1260 cm⁻¹, respectively. In the 1H-NMR spectrum of this aclyclic compound (2), three singlets were observed at the 9.86, 9.96 and 10.86 ppm region representing the protons of the NH group. Also, the 13C-NMR spectrum exhibited two important signals characteristic to C=S and C=O carbons which appear at 181.80 ppm and 164.58 ppm, respectively [18].

Absence of carbonyl absorption band in IR spectrum of new heterocyclic compounds (3), (5) and (6) confirmed that cyclization, of aclythiosemicarbazide (2) took place, in different media.

Since 1,2,4-triazol-3-thione may exist in thiole-thione tautomeric forms, our investigation showed that in this case thione structure dominates both in solid state and in solution. Thus, the IR spectrum of (3) showed two characteristic absorption bands at 1250 cm⁻¹ and 3418 cm⁻¹ attributed to νC=S and νNH group respectively. The νSH band which appears in region 2500-2650 cm⁻¹ [19-21] is not presented in IR spectra of this compound. Also, the presence in 1H-NMR spectrum of the signal due to the NH proton, at 14.10 ppm [22,23] and in 13C-NMR spectrum of C=O carbons, at 161.98 ppm [18,21], supported that the thione form of the triazoline ring of compound (3) is predominant in the solution.

In case of alkylate 1,2,4-triazole (4), the carbonyl group of ester which appears in IR at 1724 cm⁻¹ and in 1H-NMR spectra of the signal due to the NH proton, at 14.10 ppm [22,23] and in 13C-NMR spectrum of C=O carbons, at 161.98 ppm [18,21], supported that the thione form of the triazoline ring of compound (3) is predominant in the solution.

Antibacterial activity

The values of the MIC and MBC (μg/mL) of the new compounds against the 7 oral streptococcal type strains are presented in table 3. The values of MIC were ranging between: 32-64 μg/mL for triazole (3), 32-128 μg/mL for S-alkylated 1,2,3-triazole (4), 64-128 μg/mL for thiosemicarbazide (2) and thiadiazole (5), and 64-256 μg/mL for oxadiazole. The values of MIC were ranging between: 64-256 μg/mL for compounds (2), (4) and (5), and 128-512 μg/mL for (6). In all cases, the MIC/MIC ratio was less or equal to 4, suggesting a bactericidal effect of the compounds against the tested oral streptococcal type strains.

For all compounds, the lowest MIC values were obtained when their action was investigated against S. mutans, while in case of S. anginosus, all MIC values were higher than 64 μg/mL, except for triazole (3), when MIC was of 64 μg/L. The last one expressed the most important antibacterial activity among all compounds, but its alkylation, leading to derivative (4), has not improved the antibacterial effect. In contrast, the derivative belonging to the 1,3,4-oxadiazole classes showed the weakest antimicrobial action.

Comparing the results of this study with those previously reported [14,15] in describing the synthesis of some

**Table 3**

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<tr>
<th>No. Comp.</th>
<th>S. anginosus (NCTC 10713)</th>
<th>S. mutans (ATCC 25175)</th>
<th>S. mitis (ATCC 6249)</th>
<th>S. sanguinis (ATCC 10556)</th>
<th>S. parasanguinis (ATCC 15909)</th>
<th>S. salivarius (ATCC 13419)</th>
<th>S. vestibularis (ATCC 49124)</th>
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<tbody>
<tr>
<td>(2)</td>
<td>128/256</td>
<td>64/128</td>
<td>64/128</td>
<td>64/128</td>
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<td>64/256</td>
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<tr>
<td>(3)</td>
<td>64/128</td>
<td>32/64</td>
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heterocyclic compounds belonging to the same classes containing both 4-fluro-phenyl and 4-(X phenylsulfonyl) phenyl fragments, but X being H or Br, and their antimicrobial action against the same oral streptococcal strains, the following aspects were observed: a) the presence of chlorine atom in the molecule of the compounds has improved the antibacterial action of the respective: thiosemicarbazide against S. sanguinis, thiazole against S. mutans, and oxadiazole against S. mitis from S. mutans; b) the compounds with chlorine atom in their molecule and those without halogen atom exhibited a better antimicrobial activity than those containing the bromine atom, such as thiosemicarbazide and triazole-thione against S. mutans, thiadiazole against S. anginosus, and alkylate-triazole against: S. mutans, S. mitis, S. salivarius, S. vestibularis and S. parasanguinis; c) the compounds containing a chloride or a bromine atom in the molecule proved to be more active on some oral streptococcal strains than those in which the halogen atom was missing, as in case of triazole-thione against S. anginosus, oxadiazole against S. salivarius and S. vestibularis, thiazole against S. mitis and S. salivarius, and also in case of thiosemicarbazide against: S. mitis, S. salivarius and S. parasanguinis.

Conclusions

In this paper, we have prepared some new derivatives from 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole class which have 4-(4-chlorophenylsulfonyl)phenyl and 4-flurophenyl fragments. The structure of the obtained compounds was confirmed by different spectral methods and elemental analysis.

Testing in vitro these new compounds for their antimicrobial activity against several type strains of oral streptococci, it was noticed that 5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(4-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione (3) showed the best antibacterial activity, suggesting the need of further investigation on more bacterial strains.

Based on the MIC values presented by the compounds containing the 4-(X-phenylsulfonyl)phenyl (X = H, Cl, Br) and 4-fluorophenyl fragments, it might be concluded that, in general, those derivatives containing a chlorine atom had a better antibacterial activity against some oral streptococcal strains than those containing one atom of bromine or those without halogen atom.

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References

17. MAVRODIN, A., ZOTTA, V., STOENESCU, V.M., OTELEANU, D., Pharm. Zentralhalde Dschl. 95, 1956, p. 353
20. PINTILIE, O., PROFIRE, L., SUNEL, V., POPA, M., PUI, A., Molecules, 12, 2007, p. 103

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