Synthesis and Structural Characterization of Some Potential Anti-Virulence 1,2,4-Triazoles and 1,3,4-Thiadiazoles

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This paper presents a continuation of our studies in the synthesis of some 1,2,4-triazole-3-thiones and 1,3,4-thiadiazol-2-amines containing in their molecules the fluorine atom. For the synthesis of these heterocycles, the hydrazinecarbothioamides, 2-(4-(4-X-phenylsulfonyl)phenyl)-N-(3-fluorophenyl) hydrazinecarbothioamides 2a-c, were obtained by treatment of some 4-(4-X-phenylsulfonyl)-benzoic acid hydrazides 1a-c with 3-fluorophenyl isothiocyanate. The new 5-(4-(4-X-phenylsulfonyl)phenyl)-4-(3-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thiones 3a-c were obtained by refluxing of hydrazinecarbothioamide 2a-c with a solution of natrium hydroxide and the 5-(4-(4-X-phenylsulfonyl)phenyl)-N-(3-fluorophenyl)-1,3,4-thiadiazol-2-aminos 4a-c were synthesized by refluxing of hydrazinecarbothioamides 2a-c with phosphorus oxychloride. The newly synthesized compounds were characterized by IR, ¹H-NMR, ¹³C-NMR, mass spectral data and by elemental analysis. The compounds have been tested on Daphnia magna for their toxicity assessment.

Keywords: 1,2,4-triazole-3-thione, 1,3,4-thiadiazol-2-amine, hydrazinecarbothioamides, heterocyclization, anti-virulence activity

The literature data indicate that the fluoro-organic compounds are very important in the medicinal chemistry due to their unique physical and biological properties [1]. The presence of the fluorine into a molecule may increase the lipophilicity of the molecule leading to compounds with superior biological properties as against their non-fluorinated analogues [2].

On the other hand, substituted 1,2,4-triazoles and 1,3,4-thiadiazoles are heterocycles with important biological activity. It is known that 1,2,4-triazole-3-thiones and 2-amino-substituted 1,3,4-thiadiazoles continue to be of great interest to a large number of researchers due to their pharmacological importance, the most important biological properties of these compounds being anticancer, antibacterial, antifungal, analgesic, anti-inflammatory, anticonvulsant, antiviral, antioxidant activities [3-14].

In our previous studies, we reported the heterocycles analogues from 1,2,4-triazole-3-thiones and 1,3,4-thiadiazoles 2-amino-substituted class carrying diphenylsulfone and different radicals containing fluorine atoms (ex. 2-fluoro-, 4-fluoro-, 2,4-difluorophenyl) as potent antimicrobial agents [15-20].

The Daphnia magna test is intensively used to assess potential adverse effects produced by chemical compounds on the environment, especially on non-target organisms, particularly under conditions of chronic exposure [21]. The tests are easy to carry out at low cost and their usefulness was extended in the drug development process as a first step for further experiments before pharmacological screening [22]. The freshwater micro-crustacean Daphnia magna was successfully employed as a prerequisite toxicity screening both for original synthesized compounds [23-24], and also for plant extracts [25]. The test on daphnids provided valuable results that were used as a starting point for further research [26].

Based on all above considerations and as an extension of our researches to development new compounds with biological potential, we have designed and synthesized new derivatives from triazole and thiadiazole class having in the molecule the fluorine atom, namely 3-fluorophenyl radical, in order to discover new anti-virulence agents.

Experimental part

The melting points were determined on a Böetius apparatus and are uncorrected. The NMR spectra were measured with a Varian Gemini 300BB spectrometer, at 300 MHz for ¹H-NMR and at 75 MHz for ¹³C-NMR, using DMSO-d₆ as solvent. The chemical shifts are expressed in δ values (ppm) relative to TMS as internal standard and the coupling constants are expressed in Hz. The IR spectra were measured in potassium bromide pellets with a Vertex 70 Bruker spectrophotometer. The elemental analyses were performed using a Costech ECS4010 microdosimeter.

For determination of the mass spectra, the compound solution with a concentration about 100 µg/mL in methanol (with 0.1% formic acid) was direct infused into electrospray interface of a Varian 1200 MS MS mass spectrometer. Due to ionization in positive mode, were obtained protonated molecular ions [M+H]⁺. Due to triple quadrupole configuration, these molecular ions could suffer fragmentations using CID (collision induced dissociation) with an inert gas (argon).

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Synthesis of the new compounds

The new heterocyclic compounds 3 and 4 have been synthesized according with our general procedures [15,19]. For the synthesis of the heterocycles from 1,2,4-triazoles and 1,3,4-thiadiazoles that have been presented in detail below, the key intermediates from the hydrazine-carbothioamides class have been obtained by treatment of some 4-(4-X-phenylsulfonyl)-benzoic acid hydrazides 1a-c [15,19] with 3-fluorophenyl isothiocyanate. The 1,2,4-triazole-3-thiones and 1,2,4-thiadiazoles have been synthesized by cyclization of hydrazine-carbothioamides.

Thus, for the synthesis of the 1,2,4-triazole-3-thiones 3a-c containing the 3-fluorophenyl radical linked to the triazole nitrogen atom from four position, the 2-(4-(4-X-phenylsulfonyl)benzoyl)-N-(3-fluorophenyl) hydrazinecarbothioamides 2a,b were refluxed into a solution of NaOH 8%. The 1,3,4-thiadiazoles 2-amino-substituted with 3-fluorophenyl radical have been obtained from the refluxing of the same hydrazinecarbothioamides 2a-c with POCI₃ (scheme 1).

General procedure for the synthesis of 2-(4-(4-X-phenylsulfonyl)benzoyl)-N-(3-fluorophenyl) hydrazinecarbothioamide 2a-c

A mixture of hydrazide 1 (10 mmol) and 3-fluorophenyl isothiocyanate (10 mmol) in absolute ethanol (50 mL) was refluxed for 16 h. The mixture was then cooled, filtered off, washed with cold alcohol, dried and the product obtained was recrystallized from ethanol.

N-(3-fluorophenyl)-2-(4-(phenylsulfonyl)benzoyl) hydrazinecarbothioamide 2a

m.p.=181-183 °C; yield = 84.1%
IR (KBr; cm⁻¹): 3368m, 3297m, 3149m, 3098m, 1697s, 1553m, 1519s, 1356s, 1317s, 1260s, 1226m, 1151vs, 1130m, 1105m, 855m;
1H-NMR (DMSO-d₆, δ ppm, J Hz): 10.84 (s; 1H; NH-4); 9.97 (bs; 1H; NH-1); 9.87 (s; 1H; NH-2); 8.13 (s; 4H; H-7;H-11;H-8;H-10); 8.00 (dd, 7.6;1.8; 2H; H-13;H-17); 7.64 (bt, 7.6; 2H; H-14; H-16); 7.60 (td, 7.6;1.8; 1H; H-15); 7.43 (bs; 1H; H-23, in exchange); 7.35 (m; 1H; H-22); 7.26 (bd; 7.4; 1H; H-19); 6.99 (bt; 8.3; 1H; H-21);
13C-NMR (DMSO-d₆, δ ppm, J Hz): 180.91 (C-3); 164.73 (C-5); 161.50 (d; 241.3; C-20); 143.82 (C-9); 140.94 (d; 10.9; C-18); 140.65 (C-12); 137.06 (C-6); 134.10 (C-15); 130.20 (C-22); 129.94 (C-14;C-16); 129.30 (C-13;C-17); 127.57 (C-7;C-11); 121.51 (C-23); 116.85 (m, C-19;C-21);
Elemental analysis: found: C:55.99; H:3.71; N:9.87%; calcd. for C₃₀H₂₄FN₃O₃S₂ (493.49 g/mol): C:55.93; H:3.76; N:9.79%;
ESI-MS, m/z (%): 494 [M+H]+; 383 (22) [M+H-C₂H₅NH₂]+, 341 (100, BP) [C₆H₄SO₂C₆H₄CONHNH₂+H]+, 309 (60.8) [C₆H₄SO₂C₆H₄CO]+, 158 (6.8) [C₆H₄NHCS]+, 116 (10.8) [C₆H₄NH₂]^+.

2-(4-(4-chlorophenylsulfonyl)benzoyl)-N-(3-fluorophenyl) hydrazinecarbothioamide 2b

m.p.=191-193 °C; yield = 80%
IR (KBr; cm⁻¹): 3319m, 3150w, 3087m, 1680s, 1599s, 1483s, 1360m, 1311m, 1293s, 1255s, 1216s, 1156s, 1095m, 753m;
1H-NMR (DMSO-d₆, δ ppm, J Hz): 10.85 (s; 1H; NH-4); 9.97 (bs; 1H; NH-1); 9.85 (s; 1H; NH-2); 8.13 (s; 4H; H-7;H-11;H-8;H-10); 8.02 (d, 8.8; 2H; H-13;H-17); 7.71 (d, 8.8; 2H; H-14; H-16); 7.45 (bs; 1H; H-23, in exchange); 7.35 (m; 1H; H-22); 7.26 (bd; 7.4; 1H; H-19); 6.99 (bt; 8.3; 1H; H-21);
13C-NMR (DMSO-d₆, δ ppm, J Hz): 180.91 (C-3); 164.74 (C-5); 161.54 (d; 245.6; C-20); 143.42 (C-9); 140.96 (d; 10.5; C-18); 140.89 (C-15); 139.48 (C-12); 137.28 (C-6); 130.70 (C-22); 130.14 (C-14;C-16); 129.41 (C-8;C-10); 129.63 (C-13;C-17); 127.66 (C-7;C-11); 121.55 (C-23); 111.80 (m, C-19;C-21);
Elemental analysis: found: C:51.85; H:3.19; N:9.13 %; calcd. for C₂₀H₁₅ClFN₃O₃S₂ (463.93 g/mol): C:51.78; H:3.26; N:9.06 %;
ESI-MS, m/z (%): 464 [M+H]+ (35Cl), 466 [M+H]+ (37Cl); 353/355(50.7/57.7)[M+H-FC₆H₄NH₂]+, 311/313 (100, BP) [ClC₆H₄SO₂C₆H₄CONHNH₂+H]+, 279/281 (14.2/7.0) [ClC₆H₄SO₂C₆H₄CO]+, 112 (19.6/11) [FC₆H₄NH₂+H]+.

2-(4-(4-bromophenylsulfonyl)benzoyl)-N-(3-fluorophenyl) hydrazinecarbothioamide 2c

m.p.=202-203 °C; yield = 90.9%
IR (KBr; cm⁻¹): 3319m, 3149w, 3085m, 3050m, 1676s, 1598m, 1573m, 1541s, 1487m, 1361m, 1293s, 1266m, 1235m, 1156s, 1026m, 859m, 615s, 578m;
1H-NMR (DMSO-d₆, δ ppm, J Hz): 10.84 (s; 1H; NH-4); 9.96 (bs; 1H; NH-1); 9.86 (s; 1H; NH-2); 8.13 (s; 4H; H-7;H-11;H-8;H-10); 7.93 (d, 8.6; 2H; H-13;H-17); 7.88 (d, 8.6; 2H; H-14; H-16); 7.44 (bs; 1H; H-23, in exchange);

Scheme 1
A mixture of hydrazinecarbothioamide 2a,b (3 mmol) and a solution of NaOH 8% (45 mL) was refluxed for 5 h. The obtained solution was filtered and the filtrate was cooled and acidified with a diluted solution of HCl till pH ~ 5. The solid product obtained was filtered off, washed with water, dried and recrystallized from CHCl3/petroleum ether (1:2,v/v).

IR (KBr; cm⁻¹): 3256m, 3090m, 1599s, 1547m, 1491s, 1398m, 1325s, 1276s, 1239m, 1158vs, 1075m, 1067s, 696m, 614s, 580s;

ESI-MS, m/z (%): 508 [M+H]+ (79Br), 510 [M+H]+ (81Br);

General procedure for the synthesis of 5-(4-(4-phenylsulfonyl)phenyl)-4-(3-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione 3a-c

A mixture of hydrazinecarbothioamide 2a,b (3 mmol) and phosphorous oxychloride (15 mL) was refluxed for 5 h. The solid product obtained was filtered off, washed with water, dried and recrystallized from CHCl3/petroleum ether (1:2,v/v).

IR (KBr; cm⁻¹): 3310m, 3080m, 3064m, 1616s, 1553m, 1503s, 1323s, 1292m, 1157vs, 1106m, 844m;

ESI-MS, m/z (%): 446 [M+H]+ (35Cl), 448 [M+H]+ (37Cl);

General procedure for the synthesis of 5-(4-(4-phenylsulfonyl)phenyl)-N-(3-fluorophenyl)-1,3,4-thiadiazol-2-amine 3a-c

A mixture of the hydrazinecarbothioamide 2a,b (3 mmol) and phosphorous oxychloride (15 mL) was refluxed for 5 h. The solid product obtained was filtered off, washed with water, dried and recrystallized from CHCl3/petroleum ether (1:2,v/v).

IR (KBr; cm⁻¹): 3405m, 3090m, 1656s, 1553m, 1503s, 1471s, 1323s, 1292m, 1157vs, 1106m, 844m;

ESI-MS, m/z (%): 402 [M+H]+ (35Cl); 404 [M+H]+ (37Cl);

5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(3-fluorophenyl)-2H-1,2,4-triazole-3(4H)-thione 3b

m.p.= 244-246°C; yield = 55%

IR (KBr; cm⁻¹): 3405m, 3090m, 1656s, 1553m, 1503s, 1471s, 1323s, 1292m, 1157vs, 1106m, 844m;

ESI-MS, m/z (%): 442 [M+H]+; 271 [M+H+C6H5SO2]+.

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

m.p.= 240-245°C; yield = 63%

IR (KBr; cm⁻¹): 3405m, 3090m, 1656s, 1553m, 1503s, 1471s, 1323s, 1292m, 1157vs, 1106m, 844m;

ESI-MS, m/z (%): 442 [M+H]+ (35Cl); 444 [M+H]+ (37Cl);

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

m.p.= 266-268°C; yield = 52.5%

IR (KBr; cm⁻¹): 3310m, 3091m, 3050m, 1614s, 1555m, 1505s, 1398m, 1325s, 1291m, 1157vs, 1089m, 832m, 764m;

ESI-MS, m/z (%): 490 [M+H]+ (79Br), 492 [M+H]+ (81Br);

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

m.p.= 246-268°C; yield = 52.5%

IR (KBr; cm⁻¹): 3310m, 3091m, 3050m, 1614s, 1555m, 1505s, 1398m, 1325s, 1291m, 1157vs, 1089m, 832m, 764m;
characteristic stretching vibration of C=S, C=O and NH deduced on the basis of their spectral data.

Results and discussions
value ranging from 0.6 to 2.1
validity criterion according to OECD guideline 202, a LC50 for each compound. The lethal concentration (LC50), presented in figure 1. The LC50 values are presented in table 1. The LC50 values indicate that triazole 3b induced a similar lethality to thiadiazole 4b.

Acute toxicity assessment using Daphnia magna
All new synthesized compounds induced lethality under 20% in the first 48 h of exposure. After 72 h of exposure, lethal concentration (LC50) which produces a 50% lethality, was determined by interpolation and the upper and lower limits of the 95% confidence interval (95% CI) were calculated [28]. The statistical analysis was performed using GraphPad Prism version 5.01 software (GraphPad Software, Inc., La Jolla, CA, USA).

The reference test with potassium dichromate was conducted to check the sensitivity of Daphnia to meet the validity criterion according to OECD guideline 202, a LC50 value ranging from 0.6 to 2.1 µg/mL [29].

Results and discussions
The formation of the compounds synthesized was deduced on the basis of their spectral data.

In the IR spectra of hydrazinecarbothioamides, the characteristic stretching vibration of C=S, C=O and NH groups appeared at 1226-1235 cm⁻¹, 1676-1697 cm⁻¹ and 3149-3368 cm⁻¹, respectively. The formation of the 1,2,4-triazoles and 1,3,4-thiadiazoles compounds supported the formation of heterocycles via the appearance of a single band for NH group which appeared at ~ 1433 cm⁻¹ confirming the predominance of the thione tautomer.

In the 1H-NMR spectra of thiocarbamides, the three singlets at ~ 9.8 ppm, ~ 9.9 ppm and ~ 10.8 ppm were assigned to the NH protons. The 1H-NMR spectra of triazoles and thiadiazoles compounds showed characteristic signals at ~ 16.47 ppm and ~ 18.09 ppm due to the C=O and C=S.

In the 13C-NMR spectra of heterocyclic compounds, the C-3 carbon signal (169 ppm) appeared predominantly in the thione form. The remaining protons and carbons from the molecule of these compounds appeared at their corresponding regions.

Other proofs that confirm the structures of these compounds are the mass spectrometry data. The fragmentations are very similar for all compounds, the main fragment from heterocyclic compounds was m/z=271 which belong from protonated ions leaving phenylsulphonyl moiety (see experimental part).

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>LC50 (µM)</th>
<th>CI95%</th>
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<tbody>
<tr>
<td>2c</td>
<td>2.87</td>
<td>1.07 - 7.69</td>
</tr>
<tr>
<td>3a</td>
<td>39.88</td>
<td>28.19 - 56.43</td>
</tr>
<tr>
<td>3b</td>
<td>68.12</td>
<td>52.21 - 88.89</td>
</tr>
<tr>
<td>4b</td>
<td>74.56</td>
<td>55.63 - 99.95</td>
</tr>
</tbody>
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Fig.1a. Toxicity evaluation on Daphnia magna

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spectral data (IR, 1H-NMR and 13C-NMR, MS) and elemental compounds synthesized was deduced on the basis of their presence of some cyclization agent (a solution of NaOH obtained by cyclization of hydrazinecarbothioamides in the from 1,2,4-triazole and 1,3,4-thiadiazole class have been fluorophenyl isothiocyanate. The heterocyclic compounds intermediates from hydrazinecarbothioamides class compounds from 1,2,4-triazole and 1,3,4-thiadiazole class. The intermediates from hydrazinecarbothioamides class experienced 96 h exposure, whereas at 72 h medium lethality. Compounds practically non-toxic throughout the experiment. Based on these preliminary results we will perform future research on their antimicrobial activity.

Conclusions
In conclusion, we have synthesized some heterocyclic compounds from 1,2,4-triazole and 1,3,4-thiadiazole class. The intermediates from hydrazinecarbothioamides class have been synthesized by treatment of some 4-((4-phenylsulfanyl)-benzoic acid hydrazides with 3-phenylsulfonyl)-benzoic acid hydrazides with 3.

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