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

STEFANIA FELICIA BARBUCEANU[1], OCTAVIAN TUDOREL OLARU[2], GEORGE MIHAI NITULESCU[3], CONSTANTIN DRAGHICI[4], LAURA ILEANA SOCEA[1]*, FLORICA BARBUCEANU[5], CRISTIAN ENACHE[6], GABRIEL SARAMET[7]

[1] Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Organic Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

[2] Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Botany Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

[3] Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

[4] Military Medical Scientific Research Center, 24-28 Gr. Cobalcescu Str., 010195, Bucharest, Romania

[5] Institute for Diagnosis and Animal Health, 63 Dr. Staicovici Str., 050557, Bucharest, Romania

[6] Central Laboratory for Fito-Sanitary Quarantine, 11 Soseaua Afumati Str., 077190, Bucharest, Romania

[7] Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Technology and Biopharmacy Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

*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)benzoyl)-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 natrium hidroxide and the 5-(4-(4-X-phenylsulfonyl)phenyl)-N-(3-fluorophenyl)-1,3,4-thiadiazol-2-amines **4a-c** were synthesized by refluxing of hydrazinecarbothioamides **2a-c** with phosphorus oxychloride. The newly synthesized compounds were characterized by IR, ^{1}H-NMR, ^{13}C-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 a 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 ^{1}H-NMR and at 75 MHz for ^{13}C-NMR, using DMSO-d_6 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 ECS 4010 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).

* email: laurasocea@gmail.com

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) hydrazine-carbo-thioamides **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 $POCl_3$ (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;

^{1}H-NMR (DMSO-d_6, δ ppm, *J* Hz): 10.84 (s; 1H; NH-4); 9.97 (s; 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 (dd; 8.4; 7.9; 1H; H-22); 7.27 (bd; 8.0; 1H; H-19); 6.99 (bt; 8.4; 1H; H-21);

^{13}C-NMR (DMSO-d_6, δppm, *J* Hz): 180.91 (C-3); 164.73 (C-5); 161.50 (d; 241.3; C-20); 143.86 (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); 129.84 (C-8;C-10); 129.30 (C-13;C-17); 127.57 (C-7;C-11); 121.51 (C-23); 111.65 (m, C-19;C-21);

Elemental analysis: found: C:55.99; H:3.71; N:9.87%; calcd. for $C_{20}H_{16}FN_3O_3S_2$ (429.49 g/mol): C:55.93; H:3.76; N:9.79%;

ESI-MS, *m/z* (%): 430 $[M+H]^+$; 319 (38) $[M+H-FC_6H_4NH_2]^+$, 277 (100, BP) $[C_6H_5SO_2C_6H_4CONHNH_2+H]^+$, 245 (27.2) $[C_6H_5SO_2C_6H_4CO]^+$, 154 (6.8) $[FC_6H_4NHCS]^+$, 112 (10.8) $[FC_6H_4NH_2+H]^+$.

***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, 1295s, 1233m, 1156s, 1090m, 859m, 753m;

^{1}H-NMR (DMSO-d_6, δppm, *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);

^{13}C-NMR (DMSO-d_6, δppm, *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 $C_{20}H_{15}ClFN_3O_3S_2$ (463.93 g/mol): C:51.78; H:3.26; N:9.06 %;

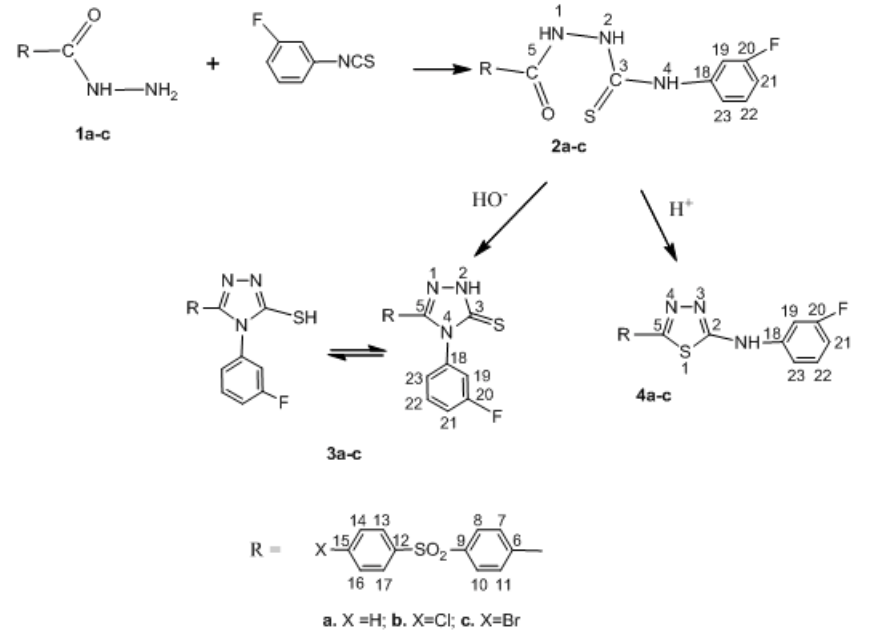
ESI-MS, *m/z* (%): 464 $[M+H]^+$ (^{35}Cl), 466 $[M+H]^+$ (^{37}Cl); 353/355(50.7/57.7)$[M+H-FC_6H_4NH_2]^+$, 311/313 (100, BP) $[ClC_6H_4SO_2C_6H_4CONHNH_2+H]^+$, 279/281 (14.2/7.0) $[ClC_6H_4SO_2C_6H_4CO]^+$, 112 (19.6/11) $[FC_6H_4NH_2+H]^+$.

***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, 1102m, 1069m, 859m, 615s, 578m;

^{1}H-NMR (DMSO-d_6, 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

7.35 (dt; 8.2; 7.1; 1H; H-22); 7.25 (bd; 8.6; 1H; H-19); 6.99 (bt; 8.2; 1H; H-21);

^{13}C-NMR (DMSO-d$_6$, δppm, *J* Hz): 180.94 (C-3); 164.90 (C-5); 161.52 (d; 241.5; C-20); 143.33 (C-9); 140.87 (d; 10.5; C-18); 139.85 (C-12); 137.24 (C-6); 130.15 (C-22); 129.60 (C-14;C-16); 129.37 (C-13;C-17); 128.40 (C-8;C-10); 128.30 (C-15); 127.62 (C-7;C-11); 121.54 (C-23); 111.66 (m, C-19;C-21);

Elemental analysis: found: C:47.32; H:2.88; N:8.24 %; calcd. for $C_{20}H_{15}BrFN_3O_3S_2$ (508.38 g/mol): C:47.25; H:2.97; N:8.27 %;

ESI-MS, *m/z* (%): 508 $[M+H]^+$ (^{79}Br), 510 $[M+H]^+$ (^{81}Br); 397/399 (29.,6/23.4) $[M+H-FC_6H_4NH_2]^+$, 355/357 (100, BP) $[BrC_6H_4SO_2C_6H_4CONHNH_2+H]^+$, 112 (64.5/30.7) $[FC_6H_4NH_2+H]^+$.

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

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 *p*H ~ 5. The solid product obtained was filtered off, washed with water, dried and recrystallized from $CHCl_3$/petroleum ether (1:2,v/v).

4-(3-fluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-2H-1,2,4-triazole-3(4H)-thione **3a**

m.p.=241-243°C; yield = 84.8%

IR (KBr; cm^{-1}): 3327m, 3080m, 3073m, 1630m, 1599s, 1537m, 1491s, 1329s, 1316s, 1290s, 1247m, 1158vs, 1105s, 1073m, 867m;

^{1}H-NMR (DMSO-d$_6$, δ ppm, *J* Hz): 14.36 (s; 1H; NH); 7.69 (bt; 7.0; 1H; H-15); 7.51 (bt; 7.5; 1H; H-22); 7.40-8.00 (m; 8H; H-7;H-8;H-10;H-11;H-13;H-17;H-14;H-16); 7.32-7.48 (m; 2H; H-19;H-21); 7.22 (bd; 1H; H-23);

^{13}C-NMR (DMSO-d$_6$, δppm, *J* Hz): 169.02 (C-3); 161.91 (d; 245.6; C-20); 149.01 (C-5); 142.53 (C-9); 140.36 (C-12); 135.59 (d; 10.6; C-18); 134.16 (C-15); 131.22 (d; 9.2; C-22); 130.60 (C-14;C-16); 130.38 (C-6); 129.93 (C-7;C-11); 127.70 (C-13;C-17); 127.62 (C-8;C-10); 125.15 (C-23); 116.95 (d; 20.6; C-21); 116.54 (d; 24.3; C-19);

Elemental analysis: found: C:58.47; H:3.51; N:10.17 %; calcd. for $C_{20}H_{14}FN_3O_2S_2$ (411.47 g/mol): C:58.38; H:3.43; N:10.21 %;

ESI-MS, *m/z* (%): 412 $[M+H]^+$; 271 $[M+H-C_6H_5SO_2]^+$.

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

m.p.=260-261°C; yield = 76.3%

IR (KBr; cm^{-1}): 3256m, 3090m, 1599s, 1547m, 1491s, 1326s, 1288m, 1261m, 1245m, 1160vs, 1106m, 1089s, 868m, 768s;

^{1}H-NMR (DMSO-d$_6$, δ ppm, *J* Hz): 14.37 (s; 1H; NH); 7.97 (d; 8.2; 2H; H-13;H-17); 7.96 (d; 8.5; 2H; H-8;H-10); 7.68 (d; 8.5; 2H; H-7;H-11); 7.55 (d; 8.2; 2H; H-14;H-16); 7.52 (bt; 7.4; 1H; H-22); 7.33-7.50 (m; 2H; H-19;H-21); 7.20 (bd; 7.4; 1H; H-23);

^{13}C-NMR (DMSO-d$_6$, δppm, *J* Hz): 169.06 (C-3); 161.92 (d; 245.3; C-20); 148.99 (C-5); 142.08 (C-9); 139.33 (C-12); 135.58 (d; 10.6; C-18); 131.25 (d; 9.1; C-22); 130.59 (C-6); 130.12 (C-14;C-16); 129.63 (C-13;C-17); 129.55 (C-7;C-11;C-15); 127.77 (C-8;C-10); 125.16 (C-23); 116.98 (d; 20.9; C-21); 116.55 (d; 24.3; C-19);

Elemental analysis: found: C:54.00; H:3.11; N:9.36 %; calcd. for $C_{20}H_{13}ClFN_3O_2S_2$ (445.92 g/mol): C:53.87; H:2.94; N:9.42%;

ESI-MS, *m/z* (%): 446 $[M+H]^+$ (^{35}Cl); 448 $[M+H]^+$ (^{37}Cl); 271 (100, BP) $[M+H-ClC_6H_4SO_2]^+$.

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}): 3260m, 3082m, 1599s, 1573s, 1492s, 1325s, 1287s, 1239m, 1158vs, 1105s, 1067s, 869m, 614s, 580s;

^{1}H-NMR (DMSO-d$_6$, δppm, *J* Hz): 14.37 (s; 1H; NH); 7.96 (d; 8.4; 2H; H-13;H-17); 7.87 (d; 8.7; 2H; H-8;H-10); 7.83 (d; 8.7; 2H; H-7;H-11); 7.54 (d; 8.4; 2H; H-14;H-16); 7.51 (bt; 7.5; 1H; H-22); 7.30-7.50 (m; 2H; H-19;H-21); 7.20 (bd; 7.3; 1H; H-23);

^{13}C-NMR (DMSO-d$_6$, δppm, *J* Hz): 169.04 (C-3); 161.92 (d; 245.6; C-20); 148.96 (C-5); 142.03 (C-9); 139.58 (C-12); 135.58 (d; 10.6; C-18); 133.05 (C-14;C-16); 131.25 (d; 8.9; C-22); 130.59 (C-6); 129.63 (C-7;C-11); 129.52 (C-13;C-17); 128.47 (C-15); 127.77 (C-8;C-10); 125.15 (C-23); 116.98 (d; 20.9; C-21); 116.55 (d; 24.3; C-19);

Elemental analysis: found: C:48.75; H:2.81; N:8.48 %; calcd. for $C_{20}H_{13}BrFN_3O_2S_2$ (490.37 g/mol): C:48.99; H:2.67; N:8.57 %;

ESI-MS, *m/z* (%): 490 $[M+H]^+$ (^{79}Br); 492 $[M+H]^+$ (^{81}Br); 271 (100, BP) $[M+H-BrC_6H_4SO_2]^+$.

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

A mixture of the hydrazinecarbothioamide **2a,b** (3 mmol) and phosphorous oxychloride (15 mL) was refluxed for 5 h. The solvent was removed under reduced pressure. The residue obtained was poured onto to crushed ice with the obtaining of a precipitate and then a diluted solution of $NaHCO_3$ was added till *p*H ~8. The solid was filtered off, washed with water, dried and recrystallized from $CHCl_3$/petroleum ether (1:2,v/v).

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

m.p.= 244-246 °C; yield = 55%

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

^{1}H-NMR (DMSO-d$_6$ δppm, *J* Hz): 10.93 (s; 1H; NH); 8.07 (s; 4H; H-7;H-8;H-10;H-11); 7.98 (bd; 8.1; 2H; H-13;H-17); 7.60-7.75 (m; 4H; H-14;H-15;H-16;H-19); 7.20-7.43 (m; 2H; H-22;H-23); 6.82 (bt; 8.1; 1H; H-21);

^{13}C-NMR (DMSO-d$_6$, δppm, *J* Hz): 164.78 (C-2); 162.58 (d; 240.5; C-20); 156.45 (C-5); 141.98 (C-12); 141.87 (d; 11.3; C-18); 140.75 (C-9); 134.71 (C-6); 134.05 (C-15); 130.82 (d; 9.4; C-22); 129.96 (C-14;C-16); 128.43 (C-8;C-10); 127.96 (C-13;C-17); 127.52 (C-7;C-11); 113.61 (C-23); 108.71 (d; 20.9; C-21); 104.65 (d; 26.5; C-19);

Elemental analysis: found: C:58.57; H:3.27; N:10.11 %; calcd. for $C_{20}H_{14}FN_3O_2S_2$ (411.47 g/mol): C:58.38; H:3.43; N:10.21 %;

ESI-MS, *m/z* (%): 412 $[M+H]^+$; 271 (35) $[M+H-C_6H_5SO_2]^+$, 169 (18.5) $[FC_6H_4NHNCS+H]^+$.

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

m.p.= 246-248°C; yield = 80.7%

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

^{1}H-NMR (DMSO-d$_6$ δppm, *J* Hz): 10.99 (s; 1H; NH); 8.09 (s; 4H; H-7;H-8;H-10;H-11); 8.01 (d; 8.5; 2H; H-13;H-17); 7.71 (d; 8.5; 2H; H-14;H-16); 7.70 (bd; 1H; 10.2; H-19); 7.39 (q; 7.6; 1H; H-22); 7.32 (bd; 7.6; 1H; H-23); 6.85 (bt; 8.0; 1H; H-21);

^{13}C-NMR (DMSO-d_6, δ ppm, *J* Hz): 164.62 (C-2); 162.58 (d; 241.6; C-20); 156.41 (C-5); 141.50 (C-12); 141.86 (d; 11.0; C-18); 139.65 (C-9); 134.92 (C-6;C-15); 130.84 (d; 9.5; C-22); 130.11 (C-14;C-16); 129.65 (C-8;C-10); 129.52 (C-13;C-17); 128.03 (C-7;C-11); 113.65 (C-23); 108.75 (d; 20.9; C-21); 104.66 (d; 26.6; C-19);

Elemental analysis: found: C:54.05; H:2.79; N:9.33 %; calcd. for $C_{20}H_{13}ClFN_3O_2S_2$ (445.92 g/mol): C:53.87; H:2.94; N:9.42 %;

ESI-MS, *m/z* (%): 446 $[M+H]^+$ (^{35}Cl); 448 $[M+H]^+$ (^{37}Cl); 271 (100, BP) $[M+H\text{-}ClC_6H_4SO_2]^+$.

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

m.p.= 247-249°C; yield = 73.6%

IR (KBr; cm^{-1}): 3310m, 3089m, 3056m, 1614s, 1573s, 1553m, 1497s, 1323s, 1157s, 1103m,1069m, 832m, 602m,571m;

^{1}H-NMR (DMSO-d_6, δppm, *J* Hz): 10.98 (s; 1H; NH); 8.09 (s; 4H; H-7;H-8;H-10;H-11); 7.92 (d; 8.2; 2H; H-13;H-17); 7.85 (d; 8.2; 2H; H-14;H-16); 7.70 (bd; 1H; 11.8; H-19); 7.30-7.43 (m; 2H; H-22;H-23); 6.85 (bt; 8.2; 1H; H-21);

^{13}C-NMR (DMSO-d_6, δppm, *J* Hz): 164.83 (C-2); 162.58 (d; 234.8; C-20); 156.41 (C-5); 141.90 (d; 11.2; C-18); 141.46 (C-12); 139.99 (C-9); 134.92 (C-6); 133.56 (C-14;C-16); 130.85 (d; 9.7; C-22); 129.54 (C-8;C-10); 129.45 (C-13;C-17); 128.30 (C-15); 128.04 (C-7;C-11); 113.63 (C-23); 108.76 (d; 20.9; C-21); 104.65 (d; 26.6; C-19);

Elemental analysis: found: C:48.78; H:2.79; N:8.68 %; calcd. for $C_{20}H_{13}BrFN_3O_2S_2$ (490.37 g/mol): C:48.90; H:2.67; N:8.57 %;

ESI-MS, *m/z* (%): 490 $[M+H]^+$ (^{79}Br); 492 $[M+H]^+$ (^{81}Br); 271 (100, BP) $[M+H\text{-}BrC_6H_4SO_2]^+$.

Acute toxicity assessment using Daphnia magna

Daphnia magna test was performed in 12-wells TPP tissue culture plates, using 10 daphnids for each well. All experiments were conducted in the dark, in a plant growth chamber (Sanyo MLR-351 H, USA) at 25°C. Each compound was tested in duplicate. Lethality was recorded after 24, 48 and 72 h, considering dead the organisms that did not move their appendages for 30 s [27]. The bioassay was performed at concentrations ranging from 3.75 to 100 µM for each compound. The 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 were deduced on the basis of their IR spectra which revealed the disappearance of the carbonyl group band from hydrazinecarbothioamides (1676-1697 cm^{-1}) due to the intramolecular cyclization of these acyclic compounds. The IR spectra of these compounds indicated a single stretching vibration due to the NH group which appeared in region 3256-3327 cm^{-1}. Moreover, in case of triazoles, the thiocarbonyl stretching vibration at 1239-1247 cm^{-1} confirming the predominance of the thione tautomer.

In the ^{1}H-NMR spectra of thiosemicarbazides, the three singlets at ~ 9.8 ppm, ~ 9.9 ppm and ~ 10.8 ppm were assigned to the NH protons. The ^{1}H-NMR spectra of triazoles and thiadiazoles compounds supported the formation of heterocycles via the appearance of a single band for NH group which appeared at ~ 14.3 ppm for triazole and ~ 10.9 ppm for thiadiazole. The ^{13}C-NMR spectra of hydrazinecarbothioamides showed characteristic signals at ~ 164.7 ppm and ~ 180.9 ppm due to the C=O and C=S.

In the ^{13}C-NMR spectra of heterocyclic compounds, the C-3 and C-5 carbons from 1,2,4-triazole ring resonated at chemical shift δ~ 169 ppm and ~ 149 ppm, while the C-2 and C-5 carbons from 1,3,4-thiadiazole ring resonated at 164.8 and 156.4 ppm. The C-3 carbon signal (169 ppm) from triazoles indicated the presence of thiocarbonyl group (C=S), confirming thus presence of this heterocycle 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 that belong from protonated molecular ions leaving phenylsulphone moiety (see experimental part).

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 compound **2b** induced a lethality between 40 and 60%. By comparing to 24 and 48 h results, at 72 h, no significant modifications were observed for compounds **2a**, **3c**, **4a**, whereas for compound **4c** a 30% lethality was observed at highest concentration. Compounds **2c**, **3a, 3b** and **4b** induced lethality over 50% at 100µM and therefore their LC50 could be calculated by using the lethality curves presented in figure 1. The LC50 values are presented in table1. The highest toxicity was exhibited by hydrazine-carbothioamide **2c**, followed by triazoles **3a**, **3b** and thiadiazole **4b**. The CI95% values indicate that triazole **3b** induced a similar lethality to thiadiazole **4b**.

Table 1

TOXICITY EVALUATION ON *DAPHNIA MAGNA*

Compound	LC50 (µM)	CI95%
2c	2.87	1.07 - 7.69
3a	39.88	28.19 - 56.43
3b	68.12	52.21 - 88.89
4b	74.56	55.63 - 99.95

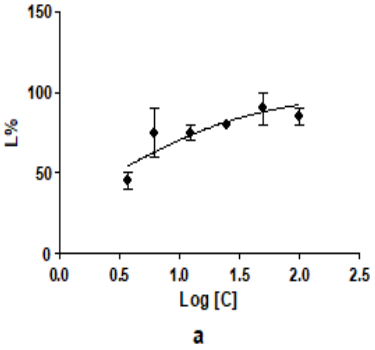


Fig.1a. Toxicity evaluation on *Daphnia magna*

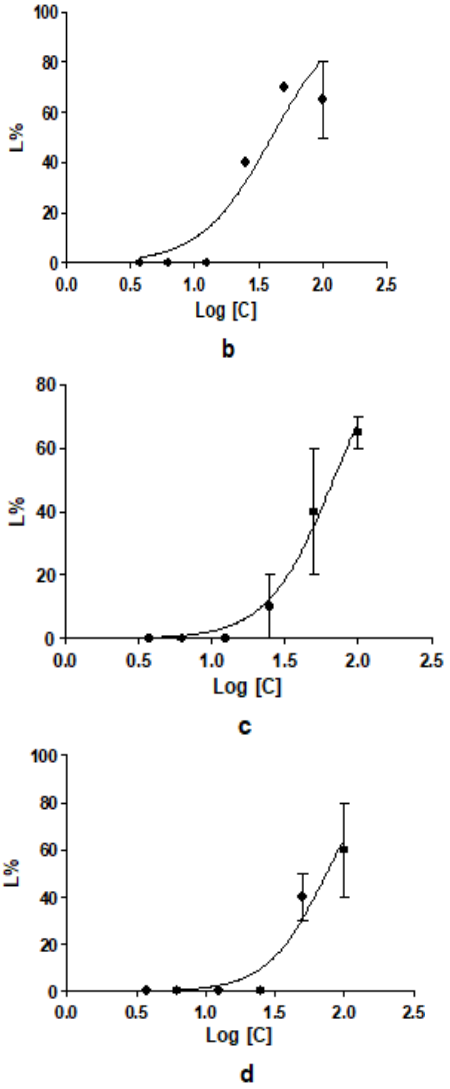


Fig.1. Toxicity evaluation on *Daphnia magna*

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-X-phenylsulfonyl)-benzoic acid hydrazides with 3-fluorophenyl isothiocyanate. The heterocyclic compounds from 1,2,4-triazole and 1,3,4-thiadiazole class have been obtained by cyclization of hydrazinecarbothioamides in the presence of some cyclization agent (a solution of NaOH for triazole and $POCl_3$ for thiadiazole). The structure of the compounds synthesized was deduced on the basis of their spectral data (IR, ^{1}H-NMR and ^{13}C-NMR, MS) and elemental analysis. All compounds were not toxic in the first 48 h of exposure, whereas at 72 h **2b**, **2c, 3a**, **3b** and **4b** exhibited medium lethality. Compounds **2a**, **3c**, **4a** and **4c** were practically non-toxic throughout the experiment. Based on these preliminary results we will perform future research on their antimicrobial activity.

Acknowledgements: The authors acknowledge the financial support offered by Romanian National Authority for Scientific Research, UEFISCDI, through grant PN-II-RU-TE-2014-4-1670, no. 342/2015.

References

1. SHELKE, S., SALUNKHE, N., SANGALE, S., BHALERAO, S., NAIK, N., MHASKE, G., JADHAV, R., KARALE B., J. Korean Chem. Soc., **54**, no. 1, 2010, p. 59
2. KARALE, B. K., NIRMAL, P. R., AKOLKAR, H.N., Indian J. Chem., 54B, 2015, p. 434
3. SHAKER, R.M., Arkivoc, **ix**, 2006, p. 59
4. MAVROVA, A. Ts., WESSELINOVA, D., TSENOV, J. A., LUBENOV, L. A., Eur. J. Med. Chem., **86**, 2014, p. 676
5. ZOUMPOULAKIS, P., CAMOUTSIS, Ch., PAIRAS, G., SOKOVIC, M., GLAMOCLIJA, J., POTAMITIS, C., PITSAS, A., Bioorg. Med. Chem., **20**, 2012, p. 1569
6. MOHAMMAD, A., American Journal of Current Organic Chemistry, **1**, no. 1, 2015, p. 17
7. KUCUKGUZEL, S. G., CIKLA-SUZGUN, P., Eur. J. Med. Chem., **97**, 2015, p. 830
8. BAROT, K. P., MANNA, K. S., GHATE, M. D., J. Saudi Chem. Soc., **21**, 2017, p. S35
9. ARIFFIN, A., RAHMAN, N. A., YEHYE, W. A., ALHADI, A. A., KADIR, F. A., Eur. J. Med. Chem., **87**, 2014, p. 564
10. JAIN, A. K., SHARMA, S., VAIDYA, A., RAVICHANDRAN, V., AGRAWAL, R. K., Chem. Biol. Drug. Des., **81**, 2013, p. 557
11. TATAR, E., KUCUKGUZEL, S.G., KARAKUS, S., De CLERCQ, E., ANDREI, G., SNOECK, R., PANNECOUQUE, C., OKTEM OKULLU, S., UNUBOL, N., KOCAGOZ, T., KALAYCI, S., SAHÝN, F., KUCUKGUZEL, Ý., Marmara Pharm. J., **19**, 2015, p. 88
12. MOISE, M., SUNEL, V., PROFIRE, L., POPA, M., DESBRIERES, J., PEPTU, C., Molecules, **14**, 2009, p. 2621
13. SAH, P., SEN, K., Int. J. Cur. Res. Rev., **7**, no. 14, 2015, p. 1
14. El-GAZZAR, Y. I., GEORGEY, H. H., El-MESSERY, S. M., EWIDA, H. A., HASSAN, G. S., RAAFAT, M. M., EWIDA, M. A., El-SUBBAGH H. I., Bioorganic Chemistry, **72**, 2017, p. 282
15. BARBUCEANU, S.-F., ALMAJAN, G. L., DRAGHICI, C., SARAMET, G., ENACHE, C., BANCESCU, G., Rev. Chim. (Bucharest), **62**, no. 3, 2011, p. 308
16. BARBUCEANU, S.-F., BANCESCU, G., CRETU, O. D., DRAGHICI, C., BANCESCU, A., RADU-POPESCU, M., Rev. Chim. (Bucharest), **61**, no. 2, 2010, p. 140
17. BANCESCU, G., BARBUCEANU, S.-F., BARBUCEANU, F., SARAMET, G., BANCESCU, A., HIRJAU, M., Farmacia, **61**, no. 1, 2013, p. 193
18. BARBUCEANU, S.-F., BANCESCU, G., DRAGHICI, C., BARBUCEANU, F., CRETU, O. D., APOSTOL, T. V., BANCESCU, A., Rev. Chim. (Bucharest), **63**, no. 4, 2012, p. 362
19. BARBUCEANU, S.-F., BANCESCU, G., SARAMET, G., BARBUCEANU, F., DRAGHICI, C., RADULESCU, F. S., IONESCU, A., NEGRES, S., Heteroat. Chem., **24**, no. 4, 2013, p. 309
20. BARBUCEANU, S.-F., BANCESCU, G., SARAMET, G., RADULESCU, F. S., BARBUCEANU, F., SOCEA, L.-I., BANCESCU, A., Farmacia, **64**, no. 2, 2016, p. 237
21. MADDEN, J. C., ENOCH, S. J., HEWITT, M., CRONIN, M. T. D., Toxicol. Lett., **185**, nr. 2, 2009, p. 85.
22. VESELA, S., ONDRUSKA, V., KUCA, K., PATOCKA, J., J. Enzyme Inhib. Med. Chem., **21**, nr. 4, 2006, p. 427.
23. SOCEA, L.-I., SOCEA, B., SARAMET, G., BARBUCEANU, S.-F., DRAGHICI, C., CONSTANTIN, V. D., OLARU, O. T., Rev. Chim. (Bucharest), **66**, no. 8, 2015, p. 1122.
24. PAHONTU, E., ILIES, D.-C., SHOVA, S., OPREAN, C., PAUNESCU, V., OLARU, O. T., RADULESCU, F. S., GULEA, A., ROSU, T., DRAGANESCU, D., Molecules, **22**, no. 4, 2017, p. 650.
25. OLARU, O. T., NITULESCU, G. M., ORTAN, A., BABEANU, N., POPA, O., IONESCU, D., DINU-PIRVU, C. E., Rom. Biotechnol. Lett., **22**, no. 6, 2016, p. 12054.
26. OLARU, O. T., VENABLES, L., VAN DE VENTER, M., NITULESCU, G. M., MARGINA, D., SPNDIDOS, D. A., TSATSAKIS, A. M., Oncol. Lett., **10**, no. 3, 2015, p. 1323.
27. NITULESCU, G. M., DRAGHICI, C., CHIFIRIUC, M.-C., MISSIR, A.-V., Farmacia, **57**, no. 5, 2009, p. 527.
28. NITULESCU, G. M., IANCU, G., NITULESCU, G., IANCU, R. C., BOGDANICI, C., VASILE, D. Rev. Chim. (Bucharest), **68**, no. 4, 2017, p. 754.
29. MARGINA, D., OLARU, O. T., ILIE, M., GRADINARU, D., GUTU, C., VOICU, S., DINISCHIOTU, A., SPANDIDOS, D. A., TSATSAKIS, A. M., Exp. Ther. Med., **10**, no. 5, 2015, p. 1681.

Manuscript received: 14.09.2017