This paper presents synthesis of new compounds from 1,3,4-thiadiazole and 1,3,4-oxadiazole class obtained by cyclization of some hydrazinecarbothioamides containing arylsulfonfylphenyl and 2,4-difluorophenyl moieties that were described in a previous paper [22]. The structures of these compounds were confirmed by elemental analysis and spectral techniques (IR, $^1$H-NMR, $^{13}$C-NMR, UV). The 1,3,4-thiadiazoles and 1,3,4-oxadiazoles were tested for their antioxidant activity by DPPH method.

Keywords: 1,3,4-thiadiazole, 1,3,4-oxadiazole, antioxidant activity

1,3,4-Thiadiazoles are a class of five-membered heterocyclic compounds which have significant interest in synthesis and in medicinal chemistry due to their wide range of biological properties. Among the biological properties of these compounds are distinguished: antitumoral [1-3], antibacterial [4-7], antifungal [4-7], antioxidant [8,9], analgesic [10], anti-inflammatory [11], antidepressant, anxiolytic and anticonvulsant [12] activities.

Similarly, a survey of literature revealed that 1,3,4-oxadiazoles are five-membered heterocyclic compounds that have attracted much attention during the years because this nucleus plays an important role in biological fields having various properties such as: antitumoral [13-15], antibacterial [4,6,7], antifungal [4,6,7,16], anti-inflammatory [6,11], muscle relaxants [17], antioxidant [18,19], anticonvulsant [20], spasmolytic, hipotensive [21].

We have recently reported synthesis and antioxidant activity of some hydrazinecarbothioamides and their cyclization products from 1,2,4-triazole-3-thione class that contain arylsulfonfylphenyl and 2,4-difluorophenyl moieties in their molecule [22]. The promising results obtained regarding to antioxidant activity of 1,2,4-triazole-3-thiones and especially of hydrazinecarbothioamides prompted us to synthesize other heterocyclic compounds from 1,3,4-thiadiazole and 1,3,4-oxadiazole class starting from these acyclic derivatives.

Thus, there were synthesized and characterized some new compounds from 1,3,4-thiadiazole and 1,3,4-oxadiazole class in order to investigate their antioxidant activity.

This work is a continuation of our research in the domain of heterocyclic compounds [23-26].

Experimental part

All reagents and solvents were purchased from Sigma-Aldrich, Merck and Fluka Companies. Melting points were determined on a Böetius apparatus and are uncorrected. The IR spectra were recorded in KBr pellets on a Vertex 70 Bruker spectrometer. The UV spectra were determined, using methanolic solutions (~ 2.5 10$^{-4}$ M), on a SPECORD 40 Analytik Jena 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 at 75 MHz for $^{13}$C-NMR) in DMSO-$d_6$ as the solvent and tetramethylsilane (TMS) as the internal standard. The content of C, H and N was determined with a ECS-40-10-Costech microdosimeter.

Synthesis of compounds

The synthetic reactions leading to the 5-substituted N-(2,4-difluorophenyl)-1,3,4-thiadiazol-2-aminos and N-(2,4-difluorophenyl)-1,3,4-oxadiazol-2-aminos are outlined in Scheme 1. The 2-(4-(4-X-phenylsulfonyl)benzoyl)-N-(2,4-difluorophenyl)hydrazinecarbothioamides 2a-c (X = H, Cl or Br) [22] are the key intermediates for the synthesis of the title compounds 3a-c and 4a-c. These acyclic compounds obtained by reaction of hydrazides 1a-c with 2,4-difluorophenyl isothiocyanate were reported previously [22]. The starting materials, 4-(4-X-phenylsulfonyl)benzoic acid hydrazides 1a-c were prepared in several steps according to the method described in literature [27,28]. 5-(4-(4-X-Phenylnsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-thiadiazol-2-aminos 3a-c were obtained by refluxing of hydrazinecarbothioamides 2a-c with phosphorus oxychloride. 5-(4-(4-X-Phenylnsulfonyl)phenyl)-(N-(2,4-difluorophenyl))1,3,4-oxadiazol-2-aminos 4a-c were obtained by refluxing of the same hydrazine-carbothioamides 2a-c with mercury oxide in ethanol.

Synthesis of 5-(4-(4-X-Phenylnsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-thiadiazol-2-aminos 3a-c

To 0.001 mol of hydrazinecarbothioamide 2, 5 mL of phosphorous oxychloride was added and the mixture was refluxed for 4h. After removing of phosphorus oxychloride by vacuum distillation the residue obtained was added in a glass with ice and a precipitate was obtained. Then, a diluted aqueous solution of NaHCO$_3$ (pH ~ 8) was added and the precipitate product was filtered off, washed with water, dried and recrystallized from chloroform/petroleum ether (~ 1:2, v/v).
**Scheme 1. Synthesis of 1,3,4-thiadiazoles 3a-c and 1,3,4-oxadiazoles 4a-c**

![Scheme 1](image)

**N-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-1,3,4-thiadiazole-2-amine 3a**

m.p. 230-232 °C; yield: 77.3 %; IR (KBr, ν, cm⁻¹): 3313 m, 3088 w, 3050 w, 1610 m, 1596 w, 1555 s, 1502 s, 1308 m, 1289 m, 1156 s, 1105 m; UV (methanol, λ_max, nm): 227.3, 252.3, 335.7; Elemental analysis: anal. calcd for C_{20}H_{13}F_{2}N_{3}O_{2}S_{2} (429.46): C, 55.93; H, 3.05; N, 9.78. Found: C, 55.88; H, 2.99; N, 9.82%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

**5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-thiadiazole-2-amine 3b**

m.p. 235-238 °C; yield: 73.4 %; IR (KBr, ν, cm⁻¹): 3344 m, 3094 w, 3050 w, 1608 m, 1556 s, 1501 s, 1321 m, 1283 m, 1159 s, 1103 m, 766 m; UV (methanol, λ_max, nm): 252.9, 335.7; Elemental analysis: anal. calcd for C_{20}H_{12}ClF_{2}N_{3}O_{2}S_{2} (463.91): C, 51.78; H, 2.61; N, 9.06. Found: C, 51.86; H, 2.54; N, 9.03%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

**5-(4-(4-bromophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-thiadiazole-2-amine 3c**

m.p. 237-239 °C; yield: 69.6 %; IR (KBr, ν, cm⁻¹): 3345 m, 3089 w, 3060 w, 1609 m, 1573 m, 1556 s, 1501 s, 1321 m, 1289 m, 1159 s, 1102 m, 608 m; UV (methanol, λ_max, nm): 254.6, 334.8; Elemental analysis: anal. calcd for C_{20}H_{12}BrF_{2}N_{3}O_{2}S_{2} (508.36): C, 47.25; H, 2.38; N, 8.27. Found: C, 47.32; H, 2.34; N, 8.21%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

**Synthesis of 5-(4-(4-X-phenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-oxadiazole-2-amines 4a-c**

To a solution of hydrazinecarbothioamide 2a-c (0.001 mol) in ethanol was added yellow mercury oxide (0.002 mol) and the mixture was refluxed for 8 h. The reaction mixture was filtered off for removing the mercury sulfide and after cooling of the filtrate, the precipitate of oxadiazole was obtained.

**N-(2,4-difluorophenyl)-5-(4-(phenylsulfonyl)phenyl)-1,3,4-oxadiazole-2-amine 4a**

m.p. 227-228 °C; yield: 37.8 %; IR (KBr, ν, cm⁻¹): 3304 m, 3092 m, 3048 m, 1658 s, 1579 m, 1509 s, 1323 m, 1218 s, 1164 s, 1102 s; UV (methanol, λ_max, nm): 245.8, 321.6; Elemental analysis: anal. calcd for C_{20}H_{13}F_{2}N_{3}O_{3}S (413.40): C, 58.11; H, 3.17; N, 10.16. Found: C, 58.09; H, 3.20; N, 10.15%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

**5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-oxadiazole-2-amine 4b**

m.p. 229-232 °C; yield: 37.4 %; IR (KBr, ν, cm⁻¹): 3398 m, 3087 w, 3065 w, 1623 s, 1581 s, 1548 s, 1504 s, 1325 m, 1291 m, 1157 s, 1101 s, 771 m; UV (methanol, λ_max, nm): 229.1, 248.5, 322.5; Elemental analysis: anal. calcd for C_{20}H_{12}ClF_{2}N_{3}O_{3}S (447.84): C, 53.64; H, 2.70; N, 9.38. Found: C, 53.69; H, 2.77; N, 9.34%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

**5-(4-(4-chlorophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-oxadiazole-2-amine 4c**

m.p. 229-232 °C; yield: 37.4 %; IR (KBr, ν, cm⁻¹): 3398 m, 3087 w, 3065 w, 1623 s, 1581 s, 1548 s, 1504 s, 1325 m, 1291 m, 1157 s, 1101 s, 771 m; UV (methanol, λ_max, nm): 229.1, 248.5, 322.5; Elemental analysis: anal. calcd for C_{20}H_{12}ClF_{2}N_{3}O_{3}S (447.84): C, 53.64; H, 2.70; N, 9.38. Found: C, 53.69; H, 2.77; N, 9.34%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

**Table 1**

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The 1H-NMR spectra of 1,3,4-thiadiazoles 3a-c and 1,3,4-oxadiazoles 4a-c (DMSO-d_6, δ ppm, J Hz).
5-(4-(4-bromophenylsulfonyl)phenyl)-N-(2,4-difluorophenyl)-1,3,4-oxadiazol-2-amine 4c

m.p. 231-233 °C; yield: 34.6 %; IR (KBr, ν, cm⁻¹): 3244 m, 3087 w, 3064 w, 1622 s, 1579 s, 1550 m, 1506 m, 1315 m, 1291 m, 1157 s, 1102 m, 568 m; UV (methanol, λ max, nm): 248.5, 320.7; Elemental analysis: anal. calcd for C₂₀H₁₂BrF₂N₃O₃S (492.29): C, 48.79; H, 2.46; N, 8.54. Found: C, 48.69; H, 2.40; N, 8.49%.

1H-NMR spectral data are shown in table 1 and 13C-NMR spectral data are shown in table 2.

Antioxidant activity testing

Protocol used for testing of the antioxidant activity of compounds 3a-c and 4a-c is similarly with that described in the previous work [22]. The antioxidant activity of the thiadiazoles 3a-c and oxadiazoles 4a-c was evaluated by DPPH method [8,29] with some modifications and compared with standards, ascorbic acid (AA), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT).

The 400 μM solution of DPPH (2 mL) in ethanol was added to tested sample solutions (2 mL) at a concentration of 500 μM in acetone - ethanol 4:96 v/v. The samples were kept in the dark at room temperature. After 30 min the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity (%), % = {1–[(Asample–Asampleblank)/Acontrol] 100}, where Acontrol was the absorbance of DPPH solution without sample, Asample was the absorbance of sample solution with DPPH, A sampleblank was the absorbance of the sample solutions without the DPPH. All analyses were undertaken on three replicates and the results averaged. Because of their low antioxidant activity the determination of IC₅₀ of the compounds was not undertaken.

Results and discussions

Chemistry

Obtaining of 1,3,4-thiadiazoles 3a-c by cyclization of hydrazinecarbothioamides with phosphorus oxychloride took place in good yield. Instead, obtaining of 1,3,4-oxadiazoles 4a-c by cyclodesulfurization of same hydrazinecarbothioamides with mercury oxide took place with low yields.

Obtaining the 1,3,4-thiadiazoles by cyclization of hydrazinecarbothioamides in acidic media is confirmed in the IR spectra of compounds 3a-c by the disappearance of absorption band of the carbonyl group from hydrazinecarbothioamides (1663-1682 cm⁻¹) [22] and by the appearance of a new absorption band due to the stretching vibrations of the C=N group at 1622-1658 cm⁻¹. Also, in the IR spectra of compounds 4a-c, the absence of the absorption band generated by the stretching vibration of carbonyl group and the appearance of a new band generated by the stretching vibration of a C=N group (1622-1658 cm⁻¹) are regarded as a proof of the cyclization of hydrazinecarbothioamides at 2-amino-1,3,4-oxadiazoles.

In 1H-NMR spectra of compounds 3a-c and 4a-c is distinguished the presence of a unique singlet signal characteristic to proton from NH group unlike the 1H-NMR spectra of hydrazinecarbothioamides where three singlet signals for the three NH groups appeared [22]. This singlet signal for NH group from thiadiazoles appeared at δ = 10.60-10.68 ppm and respectively at 10.60-10.61 ppm in case of oxadiazoles.

In 13C-NMR spectra of compounds 3a-c and 4a-c the absence of the carbonyl and thiocarbonyl groups signals from hydrazinecarbothioamides [22], the raw materials, is a proof of their cyclization. Also, the presence of two new signals at ~ 166 ppm and ~ 157 ppm characteristic to the carbon atoms C-2 and C-5 from the 1,3,4-thiadazole nucleus. In case of compounds 4a-c, the signals characteristic to the carbon atoms C-2 and C-5 from the 1,3,4-oxadiazole nucleus are presented at ~ 161 and ~ 157 ppm.

The signals of the protons and those of the carbon atoms from arylsulfonylphenyl and 2,4-difluorophenyl fragments are presented at the corresponding chemical shifts (tables 1 and 2).

Antioxidant activity

The experimental results obtained by antioxidant screening of compounds 3a-c and 4a-c indicated that the antioxidant activity of 1,3,4-thiadazole derivatives is weak and of 1,3,4-oxadiazole is negligible (table 3). Cyclization in acid media of hydrazinecarbothioamides which have
excellent antioxidant activity at 250μM concentration (96.90 - 97.18%) [22], led to a decrease in antioxidant activity of obtained compounds from 1,3,4-thiadiazole 2-amino-substituted class, at the same concentration (250 μM) as follows: 10.42 ± 0.96 % (for 3a), 19.51 ± 0.61 % (for 3b) and 17.52 ± 0.72 % (for 3c). Comparative with standards (AA, BHA and BHT), 1,3,4-thiadiazoles have lower antioxidant activity than BHT (23.05 ± 1.32 %) and very low than AA (91.26 ± 0.49 %) and BHA (89.30 ± 1.37 %).

Making a correlation between structure and biological activity of these compounds, it was found that the presence of halogens on the arylsulfonylphenyl moiety determine a better antioxidant activity. The highest activity was found in case of thiadiazole containing the chlorine atom, while the antioxidant activity of the derivative containing the bromine atom had a slight decrease.

Cyclodesulfurization of same hydrazinecarbothioamides in 1,3,4-oxadiazoles 2-aminosubstituted led to decrease in antioxidant activity at insignificant values: 2.34 ± 1.14% (for 4a), 3.16 ± 1.20 (for 4b) and 3.77 ± 1.44 (for 4c).

Conclusions

In this study, the synthesis of some new 1,3,4-thiadiazole and 1,3,4-oxadiazole compounds possessing arylsulfonylphenyl and 2,4-difluorophenyl moieties was reported. The chemical structure of these compounds was determined by elemental analysis and spectral methods. The compounds were tested for their antioxidant activity. The antioxidant screening revealed that 1,3,4-thiadiazoles showed low activity while 1,3,4-oxadiazoles have no antioxidant activity.

The presence of the oxygen atom in the 1,3,4-oxadiazole nucleus with electronegativity greater than the sulfur atom from 1,3,4-thiadiazole nucleus caused a very large decrease in antioxidant activity. These results do not encourage continuation of the antioxidant screening in this compounds class.

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