PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2019, **53**(3), p. 147–155

Chemistry

SYNTHESIS OF NEW 1,2,4-TRIAZOLE DERIVATIVES AND STUDY OF THEIR ANTINEMATODE ACTIVITY

A. S. GALSTYAN [1*], T. V. GHOCHIKYAN [1**], V. R. FRANGYAN [1***], M. SARFRAZ [2****], C. JACOB [2*****]

[1] *Chair of Organic Chemistry, YSU, Armenia*
[2] *Chair of Pharmacy, Saarland University, Germany*

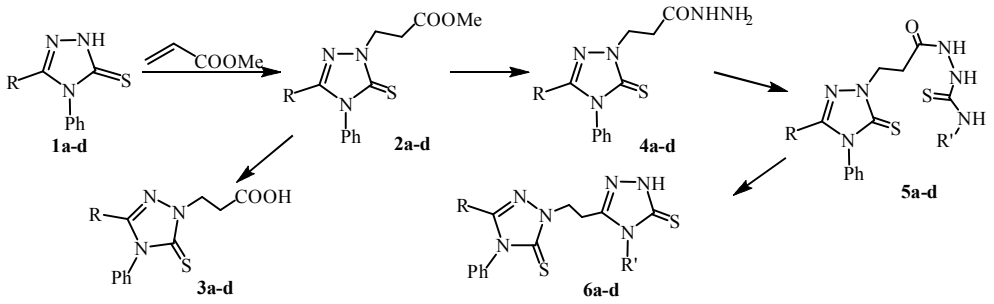
A method for obtaining methyl esters of β-1,2,4-triazole-substituted alanines by condensation of methyl acrylate with 3,4-disubstituted 5-mercapto-1,2,4-triazoles has been developed. Based on the products obtained, a number of new biheterocyclic systems, previously not described in the literature, have been synthesized. By testing the obtained compounds it was established that individual representatives of the latter exhibited antinematode activity for agricultural nematodes *Steinernema feltiae*, increasing the viability of the latter by 3–5%.

Keywords: 1,2,4-triazoles, β-heterocyclic alanines, triazolo-triazoles, antinematode activity.

Introduction. It is known that most of the drugs contain derivatives of heterocyclic compounds as active ingredients. Among the latter, a special place is occupied by nitrogen-containing five-membered heterocyclic compounds, in particular, 1,2,4-triazoles. The interest in this class of compounds is explained by a wide range of biological activities; a large number of them are already used in practical medicine as drugs, for example, voriconazole, triazolam, fluconazole, itraconazole, furacilin, alprazolam, estazolam, etc. On the other hand, intensive research on elaborating synthesis of new representatives of the afore mentioned series of compounds and studying their biological properties is in progress. In recent years, numerous methods have been developed for the synthesis of new 1,2,4-triazole derivatives combined with various heterocycles [1–9], condensed systems [10–12], and in some cases with application of microwave radiation [13, 14]. Biological studies have shown that the new 1,2,4-triazole derivatives exhibit antioxidant [15], insecticidal [16, 17], antimicrobial [18, 19], antibacterial [20, 21], anticonvulsant [22, 23], antineoplastic [24, 25], anti fungal [26, 27] properties, they are inhibitors of p38 mitogen-activated protein (MAP) kinases [28], etc. The data presented unequivocally show that studies of 1,2,4-triazoles are relevant and appropriate.

* E-mail: a_galstyan@ysu.am
** E-mail: ghochikyan@ysu.am
*** E-mail: frangyan@bk.ru
**** E-mail: s8musarf@stud.uni-saarland.de
***** E-mail: c.jacob@mx.uni-saarland.de

Results and Discussion. In order to expand the assortment of 1,2,4-triazole derivatives and to search for new useful features in their series, new representatives of methyl esters of β-traizole-substituted alanines were synthesized according to the method developed by us earlier [29]. Based on compounds **2**, **a**–**d**, according to the Scheme below, a number of transformations leading to the new derivatives of functionally substituted 1,2,4-triazoles were carried out.



1a. R = 3-Me-C_6H_4; **1b.** R = 4-Me-C_6H_4; **1c.** R = CH_2OPh; **1d.** R = Bn
2a. R = 3-Me-C_6H_4; **2b.** R = 4-Me-C_6H_4; **2c.** R = CH_2OPh; **2d.** R = Bn
3a. R = 3-Me-C_6H_4; **3b.** R = 4-Me-C_6H_4; **3c.** R = CH_2OPh; **3d.** R = Bn
4a. R = 3-Me-C_6H_4; **4b.** R = 4-Me-C_6H_4; **4c.** R = CH_2OPh; **4d.** R = Bn
5a. R = 3-Me-C_6H_4, R' = All; **5b.** R = 4-Me-C_6H_4, R' = Ph; **5c.** R = CH_2OPh, R' = Ph; **5d.** R = Bn; R' = Ph
6a. R = 3-Me-C_6H_4, R' = All; **6b.** R = 4-Me-C_6H_4, R' = Ph; **6c.** R = CH_2OPh, R' = Ph; **6d.** R = Bn; R' = Ph

Hydrolysis and hydrazinolysis of compounds **2**, **a**–**d** have been studied and it has been established that alkaline hydrolysis with a 40% aqueous alkali solution resulted in the appropriate acids that are analogs of β-alanine (**3**, **a**–**d**), and the hydrazinolysis products are hydrazides of acids **4**, **a**–**d** that have a unique structure and could be employed in fine organic synthesis for the construction of various biheterocyclic systems. In particular, based on **4**, **a**–**d** a method for the synthesis of triazolo-triazoles of a new structure (**6**, **a**–**d**) has been developed. It should be noted that the substituents in position 3 of the triazole ring do not influence the course of the reactions, all processes proceed chemoselectively, under mild conditions and provide high yields of the target products.

The structures of all synthesized compounds were established by modern physicochemical methods. The purity and individuality were checked by TLC.

Biological Part. Some of the synthesized compounds were subjected to biological studies and positive nematodes *Steinernema feltiae*, which are of great importance both in plant growth (as regulators) and in animal husbandry, were chosen as an object [30].

The use of nematodes in biological studies is due to the fact that they are easily obtained, cultivated, processed and can be used as a reliable organism for preliminary toxicological research. Nematodes are an ideal model of a multicellular living organism to be studied under a microscope as well as in studying the absorption and distribution of a biologically active compound in a small and primitive multicellular organism [31].

As a result of processing the experimental data, the following graphs were obtained that clearly illustrate the nematode activity of some compounds. In particular, Fig. 1 shows that compounds **3a** and **3c** increase the viability of nematodes by 5 and 4%, respectively, and compound **3b** does not display significant activity.

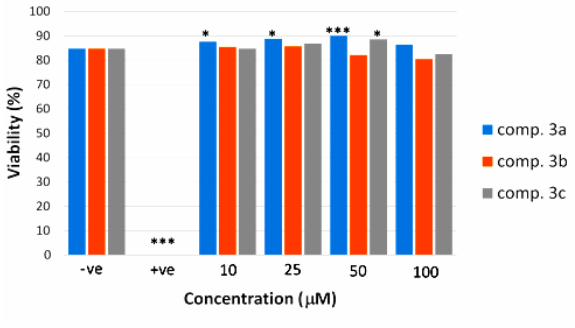


Fig. 1. Impact of compounds **3a**, **3b, 3c** on survival of *S. feltiae* after 24 *h*. Data expressed as mean ±SD; $n = 9$;

* – significant difference against negative control ($p < 0.05$);
*** – significant difference against negative control ($p < 0.001$);
–ve – negative control (DMSO);
+ve – positive control (EtOH).

A similar picture is observed in studying compounds **4, a–c**. While compounds **4b** and **4c** increase the viability of nematodes by 3 and 4%, respectively, compound **4a** is inactive.

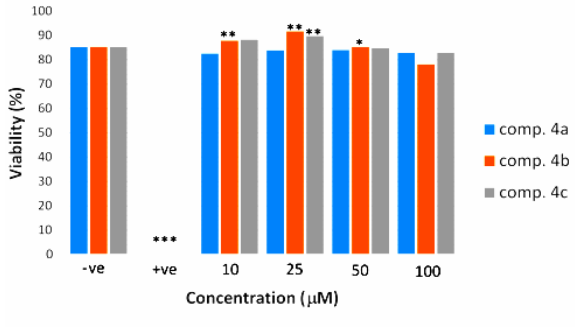


Fig. 2. Impact of compounds **4a**, **4b, 4c** on survival of *S. feltiae* after 24 *h*. Data expressed as mean ±SD; $n = 9$;

* – significant difference against negative control ($p < 0.05$);
** – very significant difference against negative control ($p < 0.05$);
*** – significant difference against negative control ($p < 0.001$);
–ve – negative control (DMSO);
+ve – positive control (EtOH).

Studies of compounds **6a** and **6b** have shown that these compounds do not exhibit significant activity.

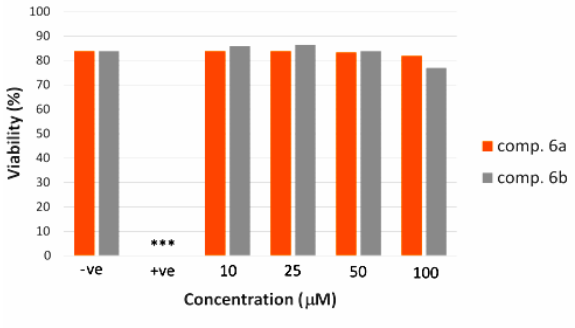


Fig. 3. Impact of compounds **6a** and **6b** on survival of *S. feltiae* after 24 *h*. Data expressed as mean ± SD; $n = 9$;

* – significant difference against negative control ($p < 0.05$);
** – very significant difference against negative control ($p < 0.05$);
*** – significant difference against negative control ($p < 0.001$);
–ve – negative control (DMSO);
+ve – positive control (EtOH).

As follows from the formulas given, the sulfur atom can be in different states – thiol or thionic. Thiol or unblocked sulfur is easily oxidized and the produced forms rapidly affect and inhibit thiol-proteins and enzymes. In consequence of this, they are considered to be an independent class of oxidative stressors [32]. The thionic form has an antioxidant property, which is clearly established by studies on ergothioneine [33].

The thionic form is a fixed state of sulfur, does not have a higher oxidative state, and consequently acts as an antioxidant providing the growth of nematodes. All that has been said is clearly observed in the case of compounds **3** and **4**. As for

compounds **6, a–d**, the molecules of these compounds contain both closed and unblocked sulfur, hence the inactivity of **6, a–d** can be interpreted as the antagonistic interaction between the two forms. The thiol form acts as an oxidative stressor, and the thionic form acts as an antioxidant.

Thus, based on 1,2,4-triazoles, methods for the preparation of new functional derivatives and biheterocyclic compounds have been developed, a nematode property in the series of 1,2,4-triazoles has been revealed. An attempt has been made to establish a relationship between the structure and biological activity of the compounds proposed.

Experimental Part.

Biologycal Part: Analysis of Antinematode Activity. The viability of fresh nematode suspensions was observed with a microscope (TR 200, VWR Inter.) at a fourfold magnification. For the experiment, nematodes with a viability greater than 90% were used. For the analysis of antinematode activity, 200 *mg* of nematode powder was suspended in 50 *mL* of phosphate buffered saline solution (PBS) (pH 7) or distilled water. Under moderate illumination, the mixture was stirred for 10 *min* at room temperature. 10 μL of the suspension was added to each well of a 96-well plate, followed by the addition of the test substance at different concentrations. The final volume was adjusted to 100 μL with buffer. The number of live (N_0) and total number of nematodes (N) were immediately determined. The plate was then incubated in the dark for 24 *h* at room temperature, 50 μL of warm water (50°*C*) was added and the number of live nematodes (N_{24}) was counted. Viability is determined by the following formula:

$$Viability_0 = \frac{N_0}{N} \cdot 100\%, \qquad Viability_{24} = \frac{N_{24}}{N} \cdot 100\%.$$

Based on experimental data, graphs of the dependence of the viability of nematodes on the concentration of the substances under study are constructed.

Preparation of PBS buffer

Reagent	Quantity added for 10 × solution, *g*	Final concentration (×10), *mM*
NaCl	80	1.37
KCl	2	27
Na_2HPO_4	14.4	100
KH_2PO_4	2.4	18
If necessary, PBS was supplemented with the following:		
$CaCl_2 \cdot 2H_2O$	1.33	10
$MgCl_2 \cdot 6H_2O$	1.0	5

Chemical Part. ^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury-300 *MHz* in DMSO–CCl_4 mixture (1 : 3) or on Bruker AVANCE 400 *MHz* spectrometer in $CDCl_3$, DMSO-d_6. Chemical shifts (δ, *ppm*) are reported as quoted relative to the residual signals of chloroform-*d* (7.25 for ^{1}H NMR and 77.0 for ^{13}C NMR) or DMSO-d_6 (2.5 for ^{1}H NMR and 39.5 for ^{13}C NMR) as internal references. ESI–MS spectra were measured with a MicroTof Bruker Daltonics instrument. TLC analysis was performed on Silufol UV-254 plates. All reagents were of reagent grade and were used as such or distilled prior to use. Starting 1,2,4-triazoles **1**, **a–d** were prepared as previously reported [34]. Melting points were determined on Boetius micro-heating stage.

General Procedure for the Preparation of Esters (2, a–d). To a mixture of 8 *mmol* of the corresponding triazole in 40 *mL* of acetonitrile was added 0.8 *mL* of 1 *M* solution of sodium methylate in methanol, 9.7 *mmol* of methyl acrylate and the whole was stirred for 4 *h* at room temperature and 5 *h* at 50–60ºC. The mixture was cooled, and the solvent was removed in vacuum. The crystals formed were recrystallized.

*Methyl 3-[3-(3-methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4--triazol-1-yl]propanoate (**2a**)*. Yield 86%, m.p. 125–126ºC (H_2O : MeOH = 1: 10). ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 7.53–7.42 m ($3H_{arom.}$), 7.35–7.26 m ($2H_{arom.}$, 7.21–7.15 m ($2H_{arom.}$), 7.15–7.07 m ($1H_{arom.}$), 7.02–6.91 m ($1H_{arom.}$), 4.64 t (*J* = 7.4 *Hz*, 2H, N$\underline{CH_2}CH_2$), 3.74 s (3H, OCH_3), 3.03 t (*J* = 7.4 *Hz*, 2H, $NCH_2\underline{CH_2}$), 2.25 s (3H, $\underline{CH_3}$Ar). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 171.2, 168.4, 149.8, 138.7, 135.2, 131.5, 129.8, 129.7, 129.0, 128.5, 128.4, 125.3, 125.2, 52.1, 45.2, 32.5, 21.4. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{19}H_{19}N_3O_2S^+$: 354.1276. Found: 354.1284.

*Methyl 3-[3-(4-methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4--triazol-1-yl]propanoate (**2b**)*. Yield 88%, m.p. 137–139ºC (H_2O : EtOH = 1: 10). ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 7.54–7.42 m ($3H_{arom.}$), 7.33–7.24 m ($2H_{arom.}$), 7.20–7.10 m ($2H_{arom.}$), 7.10–7.02 m ($2H_{arom.}$), 4.63 t (*J* = 7.4 *Hz*, 2H, N$\underline{CH_2}CH_2$), 3.73 s (3H, OCH_3), 3.02 t (*J* = 7.4 *Hz*, 2H, $NCH_2\underline{CH_2}$), 2.30 s (3H, $\underline{CH_3}$Ar). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 171.2, 168.3, 149.8, 141.1, 135.2, 129.8, 129.7, 129.4, 128.4, 128.1, 122.4, 52.1, 45.2, 32.5, 21.5. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{19}H_{19}N_3O_2S^+$: 354.1276. Found: 354.1284.

*Methyl 3-[3-(phenoxymethyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4--triazol-1-yl]propanoate (**2c**)*. Yield 78%, colorless oil [35, 36].

*Methyl 3-[3-benzyl-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanoate (**2d**)*. Yield 82%, m.p. 54–55ºC [29].

General Procedure for the Preparation of Acids (3, a–d). A mixture of 0.015 *mol* of the corresponding **2**, **a**–**d**, 3 *mL* of ethanol and 1.2 *g* of sodium hydroxide as a 40% solution was heated at 80–90ºC for 5 *h*. After cooling, the mixture was diluted with water and acidified with hydrochloric acid to pH 2–3. The precipitated crystals were filtered off, washed with water, dried and recrystallized.

*3-[3-(3-Methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanoic acid (**3a**)*. Yield 92%, m.p.183–184ºC (EtOH : H_2O = 1: 1.125). ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 7.54–7.43 m ($3H_{arom.}$), 7.35–7.27 m ($2H_{arom.}$), 7.23–7.16 m ($2H_{arom.}$), 7.13 t (*J* = 7.6 *Hz*, $1H_{arom}$), 6.98 d (*J* = 7.6 *Hz*, $1H_{arom}$), 4.65 t (*J* = 7.3 *Hz*, 2H, CH_2N), 3.11 t (*J* = 7.3 *Hz*, 2H, $CH_2C{=}O$), 2.26 s (3H, $\underline{CH_3}$Ar). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 176.0, 167.9, 149.4, 138.2, 134.6, 131.0, 129.4, 129.2, 128.5, 128.0, 127.9, 124.8, 124.6, 44.3, 31.8, 20.9. Found, %: C 63.57; H 5.16; N 12.50; S 9.31. $C_{18}H_{17}N_3O_2S$. Calculated, %: C 63.70; H 5.05; N 12.38; S 9.45.

*3-[3-(4-Methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanoic acid (**3b**)*. Yield 94%, m.p. 185–187ºC. ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 7.55–7.39 m ($3H_{arom.}$), 7.36–7.22 m ($2H_{arom.}$), 7.17 d (*J* = 8.2 *Hz*, $2H_{arom}$), 7.08 d (*J* = 8.1 *Hz*, $2H_{arom.}$), 4.64 t (*J* = 7.3 *Hz*, 2H, CH_2N), 3.09 t (*J* = 7.3 *Hz*, 2H, $CH_2C{=}O$), 2.31 s (3H, $\underline{CH_3}$Ar). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 175.8, 167.9, 149.4, 140.6, 134.7, 129.3, 129.3, 129.0, 127.9, 127.7, 121.9, 44.3, 31.8, 21.0. Found, %: C 63.55; H 4.89; N 12.55; S 9.56. $C_{18}H_{17}N_3O_2S$. Calculated, %: C 63.70; H 5.05; N 12.38; S 9.45.

*3-[3-(Phenoxymethyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanoic acid (**3c**)*. Yield 95%, m.p. 109–110ºC [33].

*3-[3-Benzyl-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol-1-yl]--propanoic acid (**3d**)*. Yield 93%, m.p. 164–165ºC (H_2O). ^{1}H NMR (300 *MHz*, DMSO:CCl_4= 1:3), *δ, ppm*: 12.22 br.s (1H, OH), 7.52–7.37 m ($3H_{arom.}$), 7.28–7.01 m ($5H_{arom.}$), 6.96–6.81 m ($2H_{arom.}$), 4.49–4.30 m (2H, CH_2N), 3.86 s (2H, CH_2Ph), 2.87–2.79 m (2H, CH_2C=O). ^{13}C NMR (75 *MHz*, DMSO : CCl_4 = 1: 3), *δ, ppm*: 171.2, 167.0, 149.2, 133.9, 133.7, 129.0, 128.8, 128.1, 128.0, 126.5, 44.3, 31.9, 31.2. Found, %: C 63.56; H 5.18; N 12.49; S 9.30. $C_{18}H_{17}N_3O_2S$. Calculated, %: C 63.70; H 5.05; N 12.38; S 9.45.

General Procedure for the Synthesis of Hydrazides (4, a–d). To a mixture of 6 *mmol* of compounds ***2, a–d*** in 20 *mL* of ethanol was added 0.34 *mL* of 85% solution of hydrazine, stirred for 5 *h* at room temperature and 2 *h* at 50–60ºC. After removal of the solvent, the residue was recrystallized.

*3-[3-(3-Methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanehydrazide (**4a**)*. Yield 93%, m.p. 199–201ºC. ^{1}H NMR (400 *MHz*, DMSO), *δ, ppm*: 9.19 s (1H, NH), 7.58–7.41 m ($3H_{arom.}$), 7.41–7.28 m ($2H_{arom.}$), 7.28–7.08 m ($3H_{arom.}$), 7.06–6.88 m ($1H_{arom.}$), 4.43 t (*J* = 7.6 *Hz*, 2H, CH_2N), 4.25 br.s (2H, NH_2), 2.68 t (*J* = 7.6 *Hz*, 2H, CH_2C=O), 2.20 s (3H, $\underline{CH_3}$Ar). ^{13}C NMR (101 *MHz*, DMSO), *δ, ppm*: 168.6, 167.4, 149.3, 138.0, 135.0, 131.2, 129.6, 129.4, 128.9, 128.7, 128.4, 125.4, 125.3, 45.3, 31.8, 20.8. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{18}H_{20}N_5OS^+$: 354.1389. Found: 354.1394.

*3-[3-(4-Methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanehydrazide (**4b**)*. Yield 89%, m.p. 194–197ºC (H_2O : EtOH = 1: 1). ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 9.20 s (1H, NH), 7.53–7.42 m ($3H_{arom.}$), 7.38–7.28 m ($2H_{arom.}$), 7.23–7.15 m ($2H_{arom.}$), 7.15–7.06 m ($2H_{arom.}$), 4.43 t (*J* = 7.5 *Hz*, 2H, CH_2N), 4.28 br.s (2H, NH_2), 2.68 t (*J* = 7.5 *Hz*, 2H, CH_2C=O), 2.25 s (3H, $\underline{CH_3}$Ar). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 168.6, 167.4, 149.3, 140.5, 135.0, 129.6, 129.4, 129.2, 128.7, 128.3, 122.5, 45.3, 31.9, 20.9. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{18}H_{20}N_5OS^+$: 354.1389. Found: 354.1376.

*3-[3-(Phenoxymethyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol--1-yl]propanehydrazide (**4c**)*. Yield 94%, m.p. 149ºC (H_2O : EtOH = 4 : 1) [34].

*3-(3-Benzyl-4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-1-yl)propane--hydrazide (**4d**)*. Yield 86%, m.p. 113ºC (H_2O : EtOH = 40 : 1) [29].

General Procedure for the Synthesis of Hydrazinecarbothioamides (5, a–d). 4.95 *mmol* of the appropriate isothiocyanate was added to a mixture of 4.5 *mmol* of the corresponding hydrazide ***4, a–d*** in 10 *mL* of ethanol, stirred for 1 *h* at room temperature and 4 *h* at 75–80ºC. The mixture was cooled the precipitate was filtered off, washed with ethanol, dried and recrystallized.

*2-{3-[3-(3-methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol-1-yl]propanoyl}-N-(prop-2-en-1-yl)hydrazine-1-carbothioamide (**5a**)*. Yield 92%, m.p. 166–168ºC. ^{1}H NMR (300 *MHz*, DMSO : CCl_4 = 1: 3), *δ, ppm*: 10.02–9.28 m (1H, NH), 9.26–9.00 m (1H, NH), 7.82 t (*J* = 5.7 *Hz*, 1H, NH), 7.58–7.39 m ($3H_{arom.}$), 7.39–7.24 m ($2H_{arom.}$), 7.22–7.07 m ($4H_{arom.}$), 5.81 ddt (*J* = 17.0, 10.3, 5.2 *Hz*, 1H, $\underline{CH}$=CH_2), 5.24–5.08 m ($1H^a$, CH=$\underline{CH_2}$), 5.08–4.96 m ($1H^b$, CH=$\underline{CH_2}$), 4.53 t (*J* = 7.4 *Hz*, 2H, N$\underline{CH_2}CH_2C$=O), 4.07 t (*J* = 5.4 *Hz*, 2H, $\underline{CH_2}CH$=CH_2), 2.84 t

(*J* = 7.4 *Hz*, 2H, CH_2C=O), 2.33 s (3H, $\underline{CH_3}$Ar). Found, %: C 58.21; H 5.51; N 18.77; S 14.02. $C_{22}H_{24}N_6OS_2$. Calculated, %: C 58.38; H 5.34; N 18.57; S 14.17.

N-Phenyl-2-{3-[3-(4-methylphenyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H--1,2,4-triazol-1-yl]propanoyl}hydrazine-1-carbothioamide ***(5b)***. Yield 97%, m.p. 194–196°*C*. ^{1}H NMR (300 *MHz*, DMSO : CCl_4 = 1: 3), *δ, ppm*: 10.20–9.75 m (1H, NH), 9.70–9.03 m (2H, NH), 7.61–7.40 m ($5H_{arom}$), 7.34–7.22 m ($4H_{arom.}$), 7.19–7.01 m ($5H_{arom.}$), 4.62–4.42 m (2H, $N\underline{CH_2}CH_2CO$), 3.03–2.83 m (2H, $NCH_2\underline{CH_2}CO$), 2.32 s (3H, $\underline{CH_3}$Ar). Found, %: C 61.63; H 4.78; N 17.38; S 13.21. $C_{25}H_{24}N_6OS_2$. Calculated, %: C 61.45; H 4.95; N 17.20; S 13.12.

N-Phenyl-2-{3-[3-(phenoxymethyl)-4-phenyl-5-sulfanylidene-4,5-dihydro-1H--1,2,4-triazol-1-yl]propanoyl}hydrazine-1-carbothioamide ***(5c)***. Yield 81%, m.p. 80–82°*C* (H_2O : EtOH= 1: 1.5). ^{1}H NMR (300 *MHz*, DMSO : CCl_4= 1: 3), *δ, ppm*: 10.24–9.75 m (1H, NH), 9.72–9.11 m (2H, NH), 7.57–7.44 m ($6H_{arom.}$), 7.32–7.14 m ($6H_{arom.}$), 6.94–6.84 m ($1H_{arom.}$), 6.81–6.67 m ($2H_{arom.}$), 4.85 s (2H, CH_2O), 4.51 t (*J* = 7.3 *Hz*, 2H, CH_2N), 2.85 t (*J* = 7.3 *Hz*, 2H, CH_2C=O). Found, %: C 59.32; H 4.60; N 16.86; S 12.90. $C_{25}H_{24}N_6O_2S_2$. Calculated, %: C 59.50; H 4.79; N 16.65; S 12.71.

N-Phenyl-2-{3-[3-Benzyl-4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4-triazol-1-yl]propanoyl}hydrazine-1-carbothioamide ***(5d)***. Yield 89%, m.p. 137–139°*C* (H_2O: EtOH = 2 : 1) [29].

General Procedure for the Synthesis of bis-1,2,4-triazoles (6, a–d). To a solution of 4 *mL* of 10% KOH was added 3.5 *mmol* of the corresponding thiosemicarbazide, stirred for 2 *h* at room temperature and 4 *h* at 85–90°*C*. After cooling, the mixture was diluted with water and acidified with hydrochloric acid to pH 2–3. The precipitated crystals were filtered off, washed with water, dried and recrystallized.

4-Phenyl-5-(3-methylphenyl)-2-{2-[4-(prop-2-en-1-yl)-5-sulfanylidene-4,5--dihydro-1H-1,2,4-triazol-3-yl]ethyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione ***(6a)***. Yield 88%, m.p. 79–81°*C*. ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 12.01–11.38 m (1H, NH), 7.54–7.43 m ($3H_{arom.}$), 7.34–7.27 m ($2H_{arom}$), 7.22–7.16 m ($2H_{arom}$), 7.16–7.09 m ($1H_{arom}$), 7.00–6.93 m ($1H_{arom.}$), 5.92 ddt (*J* = 15.7, 10.5, 5.3 *Hz*, 1H, $\underline{CH}$=CH_2), 5.29 br.d (*J* = 10.3 *Hz*, $1H^a$, CH=$\underline{CH_2}$), 5.22 br.d (*J* = 17.2 *Hz*, $1H^b$, CH=$\underline{CH_2}$), 4.77 br.d (*J* = 5.3 *Hz*, 2H, $\underline{CH_2}$–CH=CH_2), 4.75–4.69 m (2H, $N\underline{CH_2}CH_2$), 3.47–3.20 m (2H, $NCH_2\underline{CH_2}$), 2.26 s (3H, Ar$\underline{CH_3}$). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 168.1, 149.1, 138.3, 134.5, 131.2, 129.9, 129.4, 129.3, 128.5, 128.1, 127.8, 124.9, 124.4, 118.5, 45.7, 45.6, 23.6, 20.9. HRMS (ESI) m/z: $[M + H]^+$ calculated for $C_{22}H_{23}N_6S_2^+$: 435.1426. Found: 435.1410.

4-Phenyl-5-(4-methylphenyl)-2-{2-[4-phenyl-5-sulfanylidene-4,5-dihydro-1H--1,2,4-triazol-3-yl]ethyl}-2,4-dihydro-3H-1,2,4-triazole-3-thione ***(6b)***. Yield 97%, m.p. 132–135°*C*. ^{1}H NMR (400 *MHz*, $CDCl_3$), *δ, ppm*: 12.47–11.93 m (1H, NH), 7.62–7.50 m ($3H_{arom.}$), 7.50–7.37 m ($5H_{arom.}$), 7.34–7.21 m ($2H_{arom.}$), 7.16–7.02 m ($4H_{arom.}$), 4.61 t (*J* = 7.1 *Hz*, 2H, CH_2N), 3.16 t (*J* = 7.1 *Hz*, 2H, $NCH_2\underline{CH_2}$), 2.30 s (3H, Ar$\underline{CH_3}$). ^{13}C NMR (101 *MHz*, $CDCl_3$), *δ, ppm*: 168.8, 168.6, 150.1, 149.6, 141.2, 135.0, 133.2, 130.3, 130.1, 129.8, 129.8, 129.5, 128.3, 128.2, 128.1, 122.2, 45.7, 24.6, 21.5. HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{25}H_{23}N_6S_2^+$: 471.1426. Found: 471.1433.

5-Phenoxymethyl-4-phenyl-2-[2-(4-phenyl-5-sulfanylidene-4,5-dihydro-1H--1,2,4-triazol-3-yl)ethyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione ***(6c)***. Yield 82%, m.p. 85–90°*C*. ^{1}H NMR (300 *MHz*, DMSO : CCl_4= 1: 3), *δ, ppm*: 13.67 br.s (1H, NH), 7.61–7.39 m ($10H_{arom.}$), 7.21–7.14 m ($2H_{arom.}$), 6.94–6.85 m ($1H_{arom.}$), 6.80–6.71 m

($2H_{arom.}$), 4.88 s (2H, CH_2O), 4.46 t (*J* = 7.2 *Hz*, 2H, $N\underline{CH_2}CH_2$), 3.03 t (*J* = 7.2 *Hz*, 2H, $NCH_2\underline{CH_2}$). Found, %: C 61.52; H 4.68; N 17.45; S 13.05. $C_{25}H_{22}N_6OS_2$. Calculated, %: C 61.71; H 4.56; N 17.27; S 13.18.

5-Benzyl-4-phenyl-2-[2-(4-phenyl-5-sulfanylidene-4,5-dihydro-1H-1,2,4--triazol-3-yl)–ethyl]-2,4-dihydro-3H-1,2,4-triazole-3-thione ***(6d)***. Yield 93%, m.p. 113–114ºC (H_2O : EtOH = 2 : 1) [29].

Received 05.06.2019
Reviewed 20.06.2019
Accepted 16.12.2019

REFERENCES

1. Zieliński W., Kudelkoand A., Czardybon W. The Synthesis of 4-Acylamino-1,2,4-triazole Derivatives in the Reaction of α-Hydroxyacid Hydrazidesand Orthoesters. *J. Het. Chem*., **42** : 7 (2005), 1393–1397.
2. Wille S., Hein M., Miethchen R. First Cross-Coupling Reactions on Halogenated 1H-1,2,4-Triazole Nucleosides. *Tetrahedron*, **62** : 14 (2006), 3301–3308.
3. Dolzhenko A.V., Dolzhenko A.V., Chui Wai-Keung. Practical Synthesis of Regioisomeric 5(7)-Amino-6,7(4,5)-dihydro[1,2,4]triazolo[1,5-a][1,3,5]triazines. *Tetrahedron*, **63** : 52 (2007), 12888–12895.
4. Bayrak H., Demirbas A., Demirbas N., Karaoglu S.A. Cyclization of Some Carbothioamide Derivatives Containing Antipyrine and Triazole Moieties and Investigation of Their Antimicrobial Activities. *Eur. J. Med. Chem*., **45** : 11 (2010), 4726–4732.
5. Bakavoli M., Rahimizadeh M., Shiri A., Akbarzadeh M., Mousavi S.-H., Tayarani-Najaran Z., Atapour-Mashhad H., Nikpour M. Synthesis of New Derivatives of 3-Aryl-1,5-dimethyl-1H--[1,2,4]triazolo[4′,3′:1,2]pyrimido[4,5-e][1,3,4]oxadiazines as Potential Antiproliferative Agents. *J. Het. Chem*., **48** : 1 (2011), 183–187.
6. Eliazyan K.A., Shahbazyan L.V., Pivazyan V.A., Yengoyan A.P. Synthesis and Growth Regulatory Activity of Pyrazolesulfanyl- and Isoxazolesulfanyl-1H-[1,2,4]-triazoles and Their Azinyl Derivatives. *J. Het. Chem*., **48** : 1 **(**2011), 188–191.
7. Li Yan-Hong, Zhang Bei, Yang Hai-Kui, Li Qiu, Diao Peng-Cheng, You Wen-Wei, Zhao Pei-Liang. Design, Synthesis, and Biological Evaluation of Novel Alkylsulfanyl-1,2,4-triazoles as cis-Restricted Combretastatin A-4 Analogues. *Eur. J. Med. Chem*., **125** (2017), 1098–1106.
8. Prieto A., Uzel A., Bouyssi D., Monteiro N. Thiocyanation of N,N-Dialkylhydrazonoyl Bromides: An Entry to Sulfur-Containing 1,2,4-Triazole Derivatives. *Eur. J. Org. Chem*., **2017** : 28 (2017) 4201–4204;
9. Szabó K.E., Páhi A., Somsák L. C-Glycosyl 1,2,4-triazoles: Synthesis of the 3-β-D-glucopyranosyl--1,5-disubstituted and 5-β-D-glucopyranosyl-1,3-disubstituted Variants. *Tetrahedron*, **73** : 27–28 (2017), 3810–3822.
10. Song Yang-Heon, Son Young Hoon. Synthesis of New Sulfur-linked 1,2,4-Triazolothieno-pyrimidine and 1,2,4-Triazolopyrazolopyrimidine Derivatives Containing Fused Heterocyclic pPyrimidines. *J. Het. Chem*., **47** : 5 (2010), 1183–1187.
11. Murlykina M.V., Sakhno Y.I., Desenko S.M., Shishkina S.V., Shishkin O.V., Sysoiev D.O., Kornet M.N., Schols D., Goeman J.L., Van der Eycken J., Van der Eycken E.V., Chebanov V.A. Study of the Chemoselectivity of Multicomponent Heterocyclizations Involving 3-Amino-1,2,4-triazole and Pyruvic Acids as Key Reagents, and Biological Activity of the Reaction Products. *Eur. J. Org.Chem*., **2015** : 20 (2015), 4481–4492.
12. Milen M., Földesi T., Dancsó A., Simig G., Volk B. Synthesis of Three New Tricyclic Ring Systems: Pyrrolotriazepines Condensed with an Imidazole, a Triazole, or a Tetrazole Ring. *Synlett*, **26** : 17 (2015), 2418–2424.
13. Jian Fangfang, Xiao Hailian, Zhu Chongyi, Xu Liangzhong. Synthesis, Structure and Biological Activities of [α-(Alkylcarbonyl)-2-(1,2,4-triazol-1-yl)]ethyl-N,N-dialkydithiocarbamate derivatives. *J. Het. Chem*., **43** : 4 (2006), 925–929.

14. Casas-Solvas J.M., Martos-Maldonado M.C., Vargas-Berenguel A. Synthesis of β-Cyclodextrin Derivatives Functionalized with Azobenzene. *Tetrahedron*, **64** : 48 (2008), 10919–10923.
15. Ünver Y., Sancak K., Çelik F., Birinci E., Küçük M., Soylu S., Burnaz N.A. New Thiophene--1,2,4-triazole-5(3)-ones: Highly Bioactive Thiosemicarbazides, Structures of Schiff Bases and Triazole–thiols. *Eur. J. Med. Chem*., **84** (2014), 639–650.
16. Luo Yan-Ping, Lin Long, Yang Guang-Fu. Design and Synthesis of Diheterocyclic Compounds Containing Tetrazolinone and 1,2,4-Triazole. *J. Het. Chem*., **44** : 4 (2007), 937–943.
17. Maddila S., Pagadala R., Jonnalagadda S.B. Synthesis and Insecticidal Activity of Tetrazole-Linked Triazole Derivatives. *J. Het. Chem*., **52** : 2 (2015), 487–491.
18. Swamy S. N., Basappa Priya B.S., Prabhuswamy B., Doreswamy B.H., Prasad J. Sh., Rangappa K. S. Synthesis of Pharmaceutically important Condensed Heterocyclic 4,6-Disubstituted-1,2,4-triazolo--1,3,4-thiadiazole Derivatives as Antimicrobials. *Eur. J. Med. Chem*., **41** : 4 (2006), 531–538.
19. Kalhor M., Mobinikhaledi A., Dadras A., Tohidpour M. Synthesis and Antimicrobial Activity of Some Novel Substituted 1,2,4-Triazoles Bearing 1,3,4-Oxadiazoles or Pyrazoles. *J. Het. Chem*., **48** : 6 (2011), 1366–1370.
20. Parmar K., Suthar B., Prajapati S., Suthar A. Synthesis and Biological Activity of Novel 1,3,5--Trisubstituted 1,2,4-Triazole Derivatives. *J. Het. Chem*., **47** : 1 (2010), 156–161.
21. Arafa W.A.A. Preparation and Evaluation of Antibacterial Activity of Some New 1,2,4-Triazole Derivatives. *J. Het. Chem*., **47** : 5 (2010), 1109–1115.
22. Plech T., Kaproń B., Łuszczki J.J., Paneth A., Siwek A., Kołaczkowski M., Zołnierek M., Nowak G. Studies on the Anticonvulsant Activity of 4-Alkyl-1,2,4-triazole-3-thiones and Their Effect on GABAergic System. *Eur. J. Med. Chem*., **86** (2014), 690–699.
23. Ayati A., Emami S., Foroumadi A. The Importance of Triazole Scaffold in the Development of Anticonvulsant Agents. *Eur. J. Med. Chem*., **109** (2016), 380–392.
24. Zhang G., Hu Y. Synthesis and Antitumor Activities of 2-(Substituted)phenyl-1,2,4-triazolo -[1,5-a]pyridines. *J. Het. Chem*., **44** : 4 (2007), 919–922.
25. Zhang B., Li Y.-H., Liu Y., Chen Y.-R., Pan En-Sh., You W.-W., Zhao P.-L. Design, Synthesis and Biological Evaluation of Novel 1,2,4-Triazolo [3,4-b][1,3,4]thiadiazines Bearing Furan and Thiophene Nucleus. *Eur. J. Med. Chem*., **103** (2015), 335–342.
26. Gupta M. Synthesis and Antifungal Activity of Novel 3,6-Diaryl-5*H*-[1,2,4]triazolo -[4,3-b][1,2,4]triazepines. *J. Het. Chem*., **44** : 5 (2007), 1023–1027.
27. Borthakur S.K., Borthakur S., Goswami D., Boruah P., Kalita P.K. Synthesis and Antifungal Activities of Some New 5,7-Disubstituted[1,2,4]triazolo[1,5-a]pyrimidin-6-one Derivatives. *J. Het. Chem*., **53** : 6 (2016), 2079–2083.
28. Thiel O.R., Achmatowicz M., Milburn R.M. Process Research and Development for Heterocyclic p38 MAP Kinase Inhibitors. *Synlett*., **23** : 11 (2012), 1564–1574.
29. Ghochikyan T.V., Samvelyan M.A., Galstyan A.S., Frangyan V.R. Synthesis of New Bis-1,2,4-triazoles. *Khim. Zh. Arm*. (*Chemical Journal of Armenia*), **69** : 1–2 (2016), 171–176.
30. Blaxter M., Koutsovoulos G. The Evolution of Parasitism in Nematoda. *Parasitology*, **142(S1)** (2015), S26–S39.
31. Manikova D., Medvekova-Letavayova L., Vlasakova D., Kosik P., Estevam E.C., Nasim M.J., Gruhlke M., Slusarenko A., Burkholz T., Jakob C., Chovanec M. Intracellular Diagnostics: Hunting for the Mode of Action of Redox-Modulating Selenium Compounds in Selected Model Systems. *Molecules*, **19** : 8 (2014), 12258–12279.
32. Giles G.I., Jacob C. Reactive Sulfur Species: An Emerging Concept in Oxidative Stress. *Biol. Chem*., **383** : 3–4 (2002), 375–388.
33. Yamashita Y., Yabu T., Yamashita M. Discovery of the Strong Antioxidant Selenoneine in Tuna and Selenium Redox Metabolism. *World J. Biol. Chem*., **1** : 5 (2010), 144–150
34. Kochikyan T.V., Arutyunyan E.V., Arutyunyan V.S., Avetisyan A.A. New Syntheses on the Basis of Ethyl 2-Oxotetrahydrofuran-3-carboxylates. *Russ. J. Org. Chem*., **44** : 6 (2008), 849–854.
35. Ghochikyan T.V., Samvelyan M.A., Galstyan A.S., Harutyunyan V.S., Frangyan V.R., Ghochikyan A.T., Nikitina L.E. The Condensation of 4,5-Disubstituted-1H-1,2,4-triazole-3-thioles with Methylacrylate and Acrylonitrile. *Khim. Zh. Arm*. (*Chemical Journal of Armenia*), **68** : 1 (2015), 153–158.
36. Galstyan A.S., Ghochikyan T.V., Frangyan V.R. Synthesis and Characterization of New Biheterocyclic Systems. *Khim. Zh. Arm*. (*Chemical Journal of Armenia*), **70** : 1–2 (2017), 237–245.