

THE REACTIONS OF CYCLIZATION OF SEMICARBAZIDE DERIVATIVES
OF 1,3-DIPHENYL-1,2,4-TRIAZOLIN-5-THIONE-4-ACETIC ACIDMARIA DOBOSZ¹, MONIKA PITUCHA¹ and ALINA CHUDNICKA²¹ Department of Organic Chemistry, Faculty of Pharmacy, Medical University,
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Abstract: In the reaction of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid with isocyanates, semicarbazide derivatives of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid [**I–X**] were obtained. Cyclization of these compounds in the presence of 2% NaOH led to the formation of derivatives of 1,2,4-triazolin-5-one [**XI–XX**].

Keywords: 1,2,4-triazolin-5-one, semicarbazide derivatives, reaction of cyclization.

Depending on the nature of substituents, the derivatives of 1,2,4-triazolin-5-one can show different pharmacological action, e.g. antidepressant (1–4), anticonvulsant (5), analgesic (6), antiinflammatory (7,8), antitumor (9), antibacterial (10,11) or can be used as herbicides (12–16) or fungicides (17–21).

One of the methods of synthesis of these compounds is the cyclization of acyl derivatives of semicarbazide in alkaline media (22–25). The present paper is a continuation of the research work connected with chemical properties of ethyl ester of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid (26), which led to obtaining many new interesting compounds by transformation in the ester group. These compounds can show varied pharmacological activity. Here, the ethyl ester was used for the preparation of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid (27). In the reaction of hydrazide with isocyanates the respective semicarbazide derivatives [**I–X**] were obtained. The cyclization of semicarbazide derivatives led to new derivatives composed of 1,2,4-triazolin-5-thione and 1,2,4-triazolin-5-one linked through the methylene group.

The reactions were performed according to the Scheme 1.

EXPERIMENTAL

Chemistry

Melting points were determined in a Fisher-Johns block and are presented without any corrections. IR spectra were recorded in KBr using a Specord IR-75 spectrophotometer. The ¹H NMR spectra were recorded on a Tesla BS-567 A spect-

rometer (100 MHz) in DMSO-d₆ with TMS as internal standard. The mass spectra were taken with an AMD-604 mass spectrometer using a 70 eV electron beam. ¹³C NMR spectra were recorded on a Bruker AC 200F instrument. Chemicals were purchased from Merck Co. or Fluka Ltd. and used without further purification. The purity of obtained compounds was checked by TLC on Merck Co. plates Aluminium oxide 60 F₂₅₄ in a CHCl₃/C₂H₅OH (10:1 and 10:2) solvent system with UV or in iodide steam visualization. Hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid was obtained in the reaction of ethyl ester of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid with anhydrous hydrazine (27).

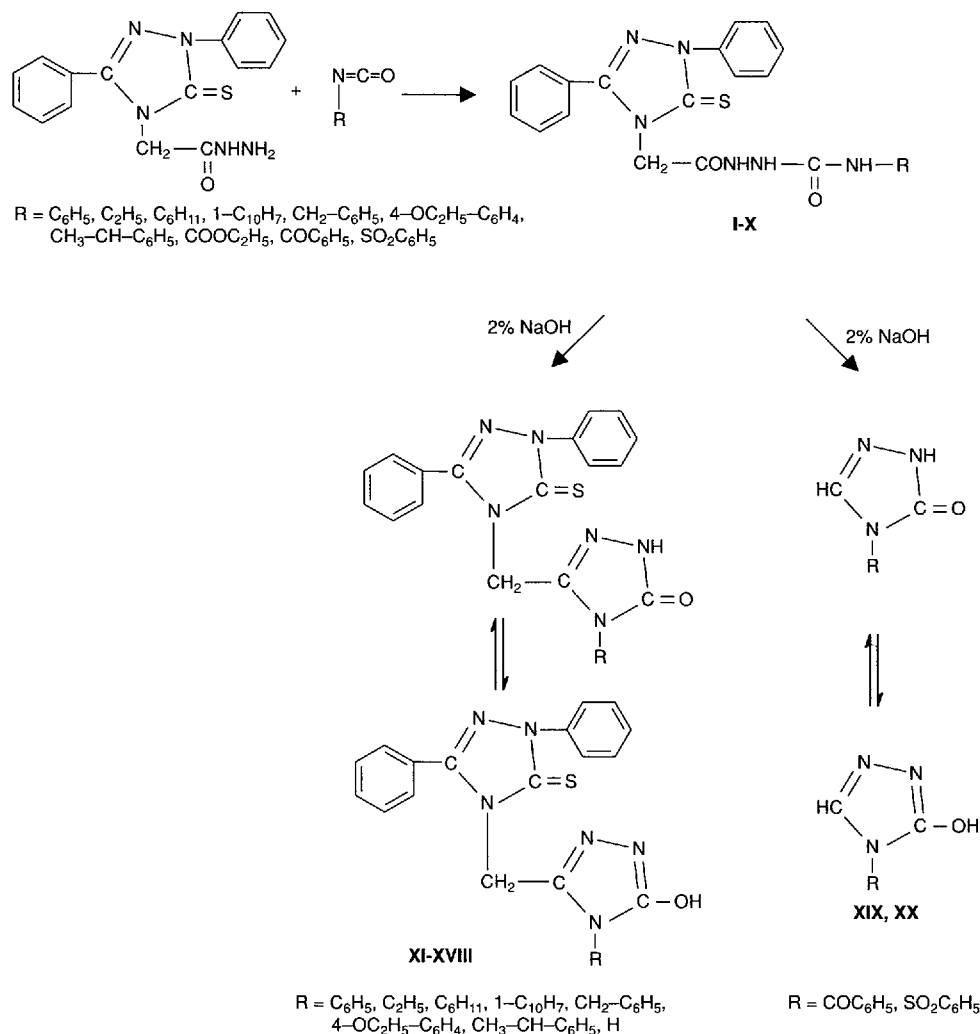
1. 1,3-Diphenyl-1,2,4-triazolin-5-thione-4-acetic acid semicarbazide derivatives [**I–X**]

Method A

The 0.01 mole of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid and 0.01 mole of isocyanate were mixed carefully and then placed in a round-bottomed flask equipped with a reflux condenser. Then, the mixture was heated in an oil bath at a temperature of 70–90°C for 20 h. Then, the product of the reaction was washed with diethyl ether in order to remove the unreacted isocyanate. The washed product was dried and crystallized from ethanol. The data relating to compounds [**I–X**] are listed in Table 1.

Method B [**V**, **VI**, **VIII**, **IX**, **X**]

The 0.01 mole of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid was dissolved in 10 cm³ of N,N-dimethylacetamide and 0.01



Scheme 1.

mole of isocyanate was added. This mixture was kept for 24 h at room temperature. Then, 40 cm³ of water was added. Precipitated compound was filtered and crystallized from ethanol. In the case of compound [IX] N,N-dimethylacetamide was distilled under reduced pressure and the sediment was crystallized from anhydrous ethanol.

Method C [I-III, VII, IX]

The 0.01 mole of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid and 0.01 mole of isocyanate in 10 cm³ of anhydrous diethyl ether was kept for 24 h at room temperature. Then, the formed compound was filtered, washed with diethyl ether and crystallized from ethanol.

Mixed melting points have not shown any variations. IR and ¹H NMR spectra of the studied

compounds taken by methods A, B, C were identical. Yields of semicarbazide derivatives of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid obtained by these three methods were similar.

2. 3,4-Disubstituted-1,2,4-triazolin-5-one derivatives [XI-XVII]

Method A

0.01 mole of semicarbazide derivatives [I-VII] was placed in a round-bottomed flask equipped with reflux and 40–50 cm³ of 2% solution of sodium hydroxide was added. The flask was heated at the boiling state for 5 h for semicarbazide derivative [IV], 15 h for semicarbazide [I, II], 20 h for semicarbazide [V-VII] and 40 h for derivative [III]. Afterwards the solution was cooled and

Table 1. Physical and analytical data for compounds I–X

No	R	Formula Molecular Weight	Yield % M.p. °C	Analysis Calculated/Found			IR (cm ⁻¹) KBr	¹ H NMR – δ (ppm) DMSO – d ₆
				%C	%H	%N		
I	C ₆ H ₅	C ₂₃ H ₂₀ N ₆ O ₂ S 444.5	70 229–230	62.14 61.9	4.53 4.6	18.91 19.1	3291 NH 3060 CH arom. 2961, 1427 CH aliph. 1702 C=O 1544 C=N	5.05 (s, 2H, CH ₂) 6.98–8.11 (m, 15H, arom.) 8.99; 9.55; 10.81 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
II	C ₂ H ₅	C ₁₉ H ₂₀ N ₆ O ₂ S 396.5	65 214–216	57.56 57.1	5.08 5.0	21.20 21.1	3229 NH 3076 CH arom. 2971, 1428 CH aliph. 1704 C=O 1547 C=N	1.00 (t, 3H, CH ₃), 2.85 (q, 2H, CH ₂), 4.98 (s, 2H, CH ₂) 7.44–8.11 (m, 10H, arom.) 8.29; 9.42; 10.12 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
III	C ₆ H ₁₁	C ₂₃ H ₂₆ N ₆ O ₂ S 450.5	63 138–140	61.31 61.5	5.82 6.0	18.65 18.7	3268 NH 3020 CH arom. 2937, 1428 CH aliph. 1718 C=O 1557 C=N	0.98–1.78 (m, 10H, 5CH ₂) 4.95 (s, 2H, CH ₂) 6.23 (t, 1H, CH) 7.37–8.08 (m, 10H, arom.) 8.10; 9.37; 10.11 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
IV	l-C ₁₀ H ₂₂	C ₂₇ H ₂₀ N ₆ O ₂ S 494.6	72 258–260	65.56 65.5	4.48 4.8	16.99 16.8	3220 NH 3049 CH arom. 2923, 1427 CH aliph. 1710 C=O 1556 C=N	4.93 (s, 2H, CH ₂) 7.39–8.80 (m, 17H, arom.) 8.94; 9.20; 10.43 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
V	CH ₂ –C ₆ H ₅	C ₂₄ H ₂₂ N ₆ O ₂ S 458.5	68 228–231	62.86 63.0	4.84 4.6	18.34 18.0	3222 NH 3062 CH arom. 2923, 1426 CH aliph. 1710 C=O 1561 C=N	4.24 (d, 2H, CH ₂), 4.85 (s, 2H, CH ₂), 6.97–8.16 (m, 15H, arom.) 8.44; 9.48; 10.19 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
VI	4–OC ₂ H ₅ C ₆ H ₄	C ₂₅ H ₂₄ N ₆ O ₃ S 488.4	71 205–208	61.47 61.5	4.95 5.0	17.19 17.3	3316 NH 3065 CH arom. 2979, 1419 CH aliph. 1669 C=O 1545 C=N 1234 C–O–C	1.29 (t, 3H, CH ₃), 3.75 (q, 2H, CH ₂), 4.89 (s, 2H, CH ₂) 6.79–8.59 (m, 14H, arom.) 8.79; 9.53; 10.29 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
VII	CH ₃ CH–C ₆ H ₅ 	C ₂₅ H ₂₄ N ₆ O ₂ S 472.6	68 242–243	63.52 63.1	5.11 5.2	17.78 17.8	3322 NH 3059 CH arom. 2969, 1428 CH aliph. 1670 C=O 1560 C=N	1.34 (d, 3H, CH ₃), 4.96 (s, 2H, CH ₂), 6.66 (d, 1H, CH) 7.17–8.08 (m, 15H, arom.) 8.25; 9.41; 10.17 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
VIII	COOC ₂ H ₅	C ₂₀ H ₂₀ N ₆ O ₄ S 440.5	72 186–188	54.53 54.1	4.57 4.8	19.08 19.0	3316 NH 3068 CH arom. 2978, 1426 CH aliph. 1731 C=O estr. 1695 C=O 1558 C=N	1.26 (t, 3H, CH ₃), 4.18 (q, 2H, CH ₂), 4.92 (s, 2H, CH ₂) 7.49–8.16 (m, 10H, arom.) 9.22; 9.35; 10.41 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
IX	COC ₆ H ₅	C ₂₄ H ₂₀ N ₆ O ₃ S 472.5	70 276–278	61.00 61.0	4.26 4.3	17.78 17.7	3255 NH 3070 CH arom. 2060, 1425 CH aliph. 1750 COC ₆ H ₅ 1713 C=O 1524 C=N	4.92 (s, 2H, CH ₂) 7.45–8.12 (m, 15H, arom.) 9.81; 10.18; 10.76 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]
X	SO ₂ C ₆ H ₅	C ₂₃ H ₂₀ N ₆ O ₄ S ₂ 508.4	68 215–217	54.33 54.1	3.96 4.0	16.52 16.5	3207 NH 3063 CH arom. 2960, 1425 CH aliph. 1713 C=O 1558 C=N 1356, 1165 S=O	4.93 (s, 2H, CH ₂) 7.44–8.10 (m, 15H, arom.) 8.87; 9.25; 10.99 (3s, 3H, 3NH) [–NH–NH–C(=O)–NH–]

Table 2. Physical and analytical data for compounds **XI–XVIII**

No	R	Formula Molecular Weight	Yield % M.p. °C	Analysis Calculated/Found			IR (cm ⁻¹) KBr	¹ H NMR – δ (ppm) DMSO – d ₆
				%C	%H	%N		
XI	C ₆ H ₅	C ₂₃ H ₁₈ N ₆ OS 426.5	70 110–112	64.77 64.6	4.25 4.3	19.78 20.0	3065 CH arom. 2966, 1425 CH aliph. 1707 C=O 1596 C=N 1547 C–N	4.90 (s, 2H, CH ₂) 7.44–8.11 (m, 15H, arom.) 11.94 (s, 1H, –NH) –C=O
XII ^x	C ₂ H ₅	C ₁₉ H ₁₈ N ₆ OS 378.4	67 205–207	60.30 60.6	4.79 4.8	22.21 22.1	3063 CH arom. 2981, 1423 CH aliph. 1719 C=O 1592 C=N 1557 C–N	1.15 (t, 3H, CH ₃) 3.66 (q, 2H, CH ₂) 5.36 (s, 2H, CH ₂) 7.44–8.08 (m, 10H, arom.) 11.62 (s, 1H, –NH) –C=O
XIII [*]	C ₆ H ₁₁	C ₂₃ H ₂₄ N ₆ OS 432.5	71 164–166	63.87 63.85	5.59 5.5	19.43 19.4	3028 CH arom. 2930, 1424 CH aliph. 1703 C=O 1574 C=N 1507 C–N	1.14–1.65 (m, 10H, 5CH ₂) 4.75 (s, 2H, CH ₂) 5.53 (t, 1H, CH) 7.50–8.26 (m, 10H, arom.) 11.54 (s, 1H, –NH) –C=O
XIV	l-C ₁₀ H ₇	C ₂₇ H ₂₀ N ₆ OS 476.5	68 263–265	68.05 68.0	4.23 4.1	17.64 17.9	3049 CH arom. 2923, 1451 CH aliph. 1706 C=O 1556 C=N 1495 C–N	5.26 (s, 2H, CH ₂) 7.55–8.14 (m, 17H, arom.) 11.48 (s, 1H, –NH) –C=O
XV ^{xx}	CH ₂ –C ₆ H ₅	C ₂₄ H ₂₀ N ₆ OS 440.5	73 228–230	65.47 65.7	4.57 4.6	19.07 19.1	3030 CH arom. 2923, 1427 CH aliph. 1707 C=O 1571 C=N 1520 C–N	4.95 (d, 2H, CH ₂) 5.34 (s, 2H, CH ₂) 7.12–7.97 (m, 15H, arom.) 11.76 (s, 1H, –NH) –C=O
XVI	4–OC ₂ H ₅ C ₆ H ₄	C ₂₅ H ₂₂ N ₆ O ₂ S 470.4	68 118–120	63.82 63.4	4.71 4.8	17.85 17.6	3062 CH arom. 2981, 1421 CH aliph. 1713 C=O 1549 C=N 1507 C–N	1.32 (t, 3H, CH ₃) 4.41 (q, 2H, CH ₂) 4.92 (s, 2H, CH ₂) 7.06–8.09 (m, 14H, arom.) 11.79 (s, 1H, –NH) –C=O
XVII	CH ₃ CH–C ₆ H ₅ 	C ₂₅ H ₂₂ N ₆ OS 454.5	60 140–142	60.05 60.2	4.87 4.6	18.49 18.3	3060 CH arom. 2928, 1423 CH aliph. 1671 C=O 1596 C=N 1530 C–N	1.29 (d, 3H, CH ₃) 4.96 (s, 2H, CH ₂) 6.44 (q, 1H, CH) 7.24–8.09 (m, 15H, arom.) 10.69 (s, 1H, –NH) –C=O
XVIII ^{**}	H	C ₁₇ H ₁₄ N ₆ OS 350.3	64 200–202	58.28 58.1	4.03 4.0	23.98 24.0	3060 CH arom. 2944, 1426 CH aliph. 1714 C=O 1557 C=N 1493 C–N	4.90 (s, 2H, CH ₂) 7.51–8.11 (m, 10H, arom.) 9.79; 10.77 (2s, 2H, 2NH)

¹³C NMR (DMSO – d₆) δ (ppm):

* 24.27 (C_{3'} + C_{5'}; C₆H₁₁), 25.08 (C_{4'}; C₆H₁₁), 32.08 (C_{2'} + C_{6'}; C₆H₁₁), 47.50 (C_{1'}; C₆H₁₁), 47.83 (CH₂–C), 123.63 (C₂ + C₆; C₆H₅), 125.41 (C₄; C₆H₅), 127.91 (C₂ + C₆; C₆H₅), 128.64 (C₂ + C₅; C₆H₅), 128.76 (C_{3'} + C_{5'}; C₆H₅), 128.94 (C₄; C₆H₅), 131.05 (C₁; C₆H₅), 137.96 (C_{1'}; C₆H₅), 151.61 (2x C=N), 164.11 (C=O), 166.80 (C=S),

** 47.18 (CH₂–C), 123.71 (C₂ + C₆; C₆H₅), 124.8 (C₄; C₆H₅), 125.31 (C₂ + C₆; C₆H₅), 127.10 (C_{3'} + C_{5'}; C₆H₅), 128.52 (C_{3'} + C_{5'}; C₆H₅), 128.58 (C₄; C₆H₅), 132.9 (C₁; C₆H₅), 145.6 (C₁; C₆H₅), 151.31 (C=N), 156.31 (CH₂–C), 166.77 (C=O), 167.30 (C=S)

MS m/e (%):

^x 3799(15, M⁺+1), 378(5), 307(30), 289(14), 252(5), 154(100), 135(68)

^{xx} 441(48, M⁺+1), 440(11, M+), 307(35), 252(10), 174(33), 145(100), 135(62), 89(25)

neutralized with dilute hydrochloric acid. The precipitated compound was filtered and then crystallized from ethanol. More detailed data relating to compounds [XI–XVII] are listed in Table 2.

Method B [XIV, XVII]

The 0.01 mole of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid and 0.01 mole of isocyanate were mixed carefully and then placed in a round-bottomed flask equipped with a reflux condenser. Then, the flask was heated in an oil bath at a temperature of 160–180°C for 20 h. Then, the reaction product was washed with diethyl ether in order to remove the unreacted isocyanate. The washed product was dried and crystallized from ethanol.

The same products as in method A were obtained. Mixed melting points have not shown any variations. IR and ¹H NMR spectra of the compound obtained by method A are identical. The highest yield was observed for method A.

3. 3-Substituted-1,2,4-triazolin-5-one derivatives [XVIII]

Method A

The procedure was the same as in the case of method A for compounds [XI–XVII]. The reaction was carried out by heating substrates for 2 h.

The data relating to compound [XVIII] are listed in Table 2.

Method B

The 0.01 mole of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid and 0.01 mole of ethyl isocyanatoformate, benzoyl and benzenesulphonyl isocyanate were mixed carefully and then placed in a round-bottomed flask equipped with a reflux condenser. Then, the flask was heated in an oil bath at a temperature of 180°C for 10–15 h. Then, the reaction product was washed with diethyl ether, dried and crystallized from ethanol. Yield 50–55%.

Method C

The 0.01 mole of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid and 0.01 mole of ethyl isocyanatoformate, benzoyl or benzenesulphonyl isocyanate and 10 cm³ of N,N-dimethylacetamide was heated in a round-bottomed flask at a temperature of 100°C for 10 h. Then the reaction mixture was distilled under reduced pressure (water-solution pump) and the product was crystallized from anhydrous ethanol. Yield 49–51%.

The same product as in methods A and B was obtained. Mixed melting points have not shown any variations. IR and ¹H NMR spectra of the compound obtained by methods A, B, C were identical. The highest yield was observed for method A.

4. 4-Substituted-1,2,4-triazolin-5-one derivatives [XIX, XX]

General procedure:

0.01 mole of semicarbazide derivatives [IX, X] was placed in a round-bottomed flask equipped with reflux and then 30–40 cm³ of 2% solution of sodium hydroxide was added. The flask was heated for 3–5 h. After cooling the solution was neutralized with dilute hydrochloric acid. The precipitated compounds was filtered and then crystallized from ethanol.

Yield for [XIX] 1.05 g (56%) m.p. 115–117°C

Analysis for C₉H₇N₃O₂ (189.15)

Calculated: %C=75.14; %H=3.70; %N=22.22

Found: %C=75.0; %H=3.7; %N=22.3

IR (cm⁻¹): 3071 (CH arom.); 1688 (C=O); 1582 (C=N); 1547 (C–N).

¹H NMR (DMSO-d₆) δ: 7.41 (s, 1H, CH); 7.44–8.02 (m, 5H, arom.); 12.70 (s, 1H, –NH)



Yield for [XX] 1.33 g (53%) m.p. 150–151°C

Analysis for C₈H₇N₃O₃S (225.14)

Calculated: %C=42.67; %H=3.13; %N=18.65

Found: %C=42.8; %H=3.6; %N=18.7

IR (cm⁻¹): 3041 (CH arom.); 1708 (C=O); 1512 (C=N); 1454 (C–N)

¹H NMR (DMSO-d₆) δ: 7.44 (s, 1H, CH); 7.50–8.02 (m, 5H, arom.); 10.54 (s, 1H, –NH)



Microbiology

Determination of *in vitro* antimicrobial activity of the compound tested was performed using the macrodilution method, according to NCCLS (National Committee for Clinical Laboratory Standards) (28–30) and the disc-diffusion method by Kirby–Bauer.

In the macrodilution method, the following appropriate concentrations of the tested compound were prepared: 1) 512 µg/ml, 2) 256 µg/ml, 3) 128 µg/ml, 4) 64 µg/ml, 5) 32 µg/ml, 6) 16 µg/ml, 7) 8 µg/ml, 8) 4 µg/ml, 9) 2 µg/ml, 10) 1 µg/ml, in sterile plastic plates (Biokom System Firm) with wells adequate for 1 ml. For each dilution of the compound tested, the used strains were typical for the bacterial species, as follow: *Staphylococcus*

aureus ATCC25923, *Escherichia coli* ATCC35218, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus faecalis* ATCC29212, *Streptococcus pneumoniae* ATCC49619, *Candida albicans* ATCC14053.

Because of poor solubility in water, the compound tested was dissolved in dilute alcohol (1:10 alcohol/water) (31). The inocula of the microorganisms (5×10^5 cfu/ml per well of the plate) with adequate dilutions of the compound were incubated (bacteria – 35°C, *C. albicans* – 30°C) for 20 h. The same test was carried out with cultures of the microorganisms used as control.

In the disc-diffusion method by Kirby-Bauer, sterile paper disc (\varnothing 5 mm) were used impregnated with alcohol solution (1:10 ethanol/water) of the dissolved compound at concentrations of 500 µg/ml and 750 µg/ml. Discs containing dilute alcohol were used as control. The strains typical for the bacterial species were used as in the macrodilution method.

The overnight cultures of the microorganisms were adjusted to approximately 1.5×10^8 cfu/ml, and were spread over the following appropriate media: Mueller-Hinton agar for *S. aureus*, *E. coli*, *P. aeruginosa*, Mueller-Hinton agar enriched with blood for *S. pneumoniae*, *E. faecalis* and Sabouraud agar for the yeasts, in petri dishes. Then, the paper discs impregnated with the solutions of the compound tested were placed on the surface of the media inoculated with the microorganism. The plates were incubated at 35°C/20 h for bacteria and at 30°C/20 h for *C. albicans* at. After incubation, the zones of growth inhibition around the discs were observed indicating that the examined compound inhibits the growth of microorganism.

RESULTS AND DISCUSSION

Chemistry

New semicarbazide derivatives [I–X] were obtained in the reaction of hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid with isocyanates. The reactions were carried out by heating the molten substrates for 20 h. The same compounds were obtained in the reaction of hydrazide and isocyanates in anhydrous ether at room temperature and in N,N-dimethylacetamide medium. The conditions of the reactions were established experimentally. Semicarbazide derivatives [I–X] were cyclized in a 2% solution of sodium hydroxide. The direction of these reactions depended on the substituents in acyl derivatives of semicarbazide. In the case of semicarbazide derivatives containing aliphatic or aromatic group [I–VII] the cyclization led to obtaining 3,4-disubstituted-1,2,4-triazolin-5-one [XI–XVII]. These

compounds possess aliphatic or aromatic groups at position 4 of the 1,2,4-triazolin-5-one system. The same compounds were obtained by heating hydrazide of 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid with isocyanates at a temperature higher than the formation of semicarbazide derivatives. The course of these reaction includes formation of intermediate semicarbazide derivatives which cyclize spontaneously to the 1,2,4-triazole system. This method was unknown in the literature up to now. In the case of the semicarbazide derivative, which was obtained from hydrazide and ethyl isocyanatoformate [VIII] in the cyclization reaction in alkaline media hydrolysis and decarboxylation took place and a 3-substituted-1,2,4-triazolin-5-one derivative was obtained [XVIII]. These compounds were obtained by heating hydrazide 1,3-diphenyl-1,2,4-triazolin-5-thione-4-acetic acid with ethyl isocyanatoformate, benzoyl and also benzenesulphonyl isocyanate. The reactions were performed in molten state and with N,N-dimethylacetamide at a temperature higher than the formation of semicarbazide derivatives. The reactions of cyclization proceeded immediately without separation of semicarbazide derivatives and thermal degradation and decarboxylation to 3-substituted-1,2,4-triazolin-5-one was observed. The process of the cyclization reaction of semicarbazide derivatives [IX, X] was quite different. During the reaction, which was carried out in an alkaline medium, 1,2,4-triazole system connected with the methylene group was detached from the initial compound. The same compounds were obtained in the reaction of hydrazide of formic acid with benzoyl and benzenesulphonyl isocyanate.

The purity of the obtained compounds was checked by TLC. The structure of the new obtained products was confirmed by elemental analysis as well as by IR and ^1H NMR and MS (for [XII, XV]), ^{13}C NMR (for [XIII, XVIII]) spectra.

Taking into account the possibility of existence of enol-keto isomerism in the obtained products of cyclization reaction, we have assumed that all compounds [XI–XX] exist in the keto-form.

Microbiology

Using the macrodilution method, no inhibition growth of microorganisms was observed in the prepared dilutions (512 µg/ml – 1 µg/ml) of the compound tested. Determination of the activity by the disc-diffusion method, according to Kirby-Bauer, confirmed that the compound tested [XII] shows no activity towards the tested microorganisms. There were no affirmation of that activity at concentrations 500 µg/ml and 750 µg/ml.

The examinations of antimicrobial activity of the presented compound are just initial, rough tests performed only with the five most popular bacterial species and one yeast's species. The negative results do not exclude antimicrobial activity of the compound against other bacterial species as from genus *Mycrobacterium* or other fungal species as: *Aspergillus*, *Penicillium*, *Microrosporum*, *Trichophyton*. The investigations on that field will be conducted.

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