Research Article

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Synthesis, Structure and Properties of Novel S-Substituted BIS-1,2,4-Triazoles

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ABSTRACT

The aim of the work was to describe the method of combining two 1,2,4-triazole systems in a molecule, the alkylation reaction of the thiol group to obtain the previously undiscovered S-derivatives of 1,2,4-triazole and the fragmentation pathway of the substances under hard ionization using gas chromatography-mass spectrometry. The structures of the synthesized compounds were confirmed by elemental analysis, 1H and 13C NMR spectroscopy and GC-MS analysis. The characteristic signals for S-alkyl residues were observed in the region typical for aliphatic compounds. The fragmentation of molecules was represented by the gradual cleavage of radicals and the opening of the second 1,2,4-triazole heterocycle.

Keywords: 1,2,4-triazole, heterocyclic compound, synthesis, fragmentation pathway.

ÖZET

Çalışmanın amacı, bir molekülde iki 1,2,4-triazol sistemini birleştirme yöntemini, daha önce keşfedilmemiş 1,2,4-triazolün *S*-türevlerini elde etmek için tiyol grubunun alkilasyon reaksiyonunu ve gaz kromatografisi-kütle spektrometrisi kullanılarak sert iyonizasyon altındaki maddelerin parçalanma yolu. Sentezlenen bileşiklerin yapıları element analizi, ^{1}H ve ^{13}C NMR spektroskopisi ve GC-MS analizi ile doğrulandı. S-alkil tortuları için karakteristik sinyaller, alifatik bileşikler için tipik olan bölgede gözlendi. Moleküllerin parçalanması, radikallerin kademeli olarak bölünmesi ve ikinci 1,2,4-triazol heterosiklinin açılması ile temsil edildi.

Anahtar Kelimeler: 1,2,4-triazol, heterosiklik bileşik, sentez, parçalanma yolu.

Introduction

Five-membered nitrogen-containing heterocyclic compounds are important structural fragments, many of which possess biological activities. 1,2,4-Triazole falls into the group of five-membered, aromatic nitrogen heterocycles, which is comprised of two carbon and three nitrogen atoms present at the 1-,2- and 4-positions of the ring.

The most classic synthesis method of 1,2,4-triazolethiones is the cyclization with water splitting of hydrazinecarbothioamides in alkaline medium [1]. 1,2,4-Triazoles can be synthesized in the laboratory *via* Pellizzari reaction, which takes place between an amide and a hydrazide giving an intermediate compound that undergoes further intramolecular cyclization [2]. It allows for reacting various hydrazines to yield different triazole derivatives, such as, for instance, 1,2-di(butan-2-ylidene) hydrazine to obtain the corresponding 1,2,4-triazole [3]. Other approaches of obtaining 1,2,4-triazoles have also been described, including catalysed oxidative coupling of organic nitrile with amidine [4], from amidine and trialkylamines [5] and from the reaction of carboxylic acid and amidines followed by treatment with monosubstituted hydrazines [6]. In addition, 3,4,5-trisubstituted 1,2,4-triazoles have been obtained by oxidative cyclization of amidrazones and aldehydes, catalysed by cerium and ammonium nitrate [7].

Considering current advancements in organic chemistry, it is safe to say that the interest for creating new effective drugs, including those based on heterocyclic compounds, is strong [8-10]. The heterocyclic 1,2,4-triazole system is also found in many natural products and pharmaceuticals [11]. The molecule of 1,2,4-triazole is among the most promising heterocyclic systems for the search of new biologically active substances [12]. Also merits attention a novel series of triazole-piperazine hybrids which used as potent anticancer agents and showed better anticancer activity than the standard drug [13]. Among the main advantages of 1,2,4-triazole-based systems is their low toxicity and broad range of pharmacological potentialities [14-16]. Some of 1,2,4-triazole derivatives exhibit hypotensive effect, while many of them possess anticonvulsant [17], anticancer [18, 19], antibacterial [20], anti-HIV [21], analgesic [22], antitumor [23], fungicidal [24], anti-inflammatory activity [25].

Based on the previous research [26] some of novel piperazine-containing 3-(furan-2-yl)-1,2,4-triazole *bis*-Mannich bases exhibited significant *in vitro* antifungal activities towards several plant fungi, [27] a series of new 1,4-bis(1,2,4-triazol-1-ylmethyl)naphthalene and 4,4'-bis(1,2,4-triazol-1-ylmethyl)biphenyl perform systematic structural variation by the employment of different metal ion or counter-anions, a new compound was derived, which contains two bridged 1,2,4-triazole moieties. This system is of great interest in terms of biological activity as the number of reactivity sites is doubled, which means broader range of chemical modifications possible in various directions [28].

Materials and Methods

Generalities

Melting point values were determined on OptiMelt MPA100 apparatus (USA) with platinum RTD sensor allowing temperature measurements up to 400°C with a resolution of 0.1°C using open capillary method.

Elemental analysis of the synthesized compounds was performed on Elementar Vario L cube multipurpose (CHNS) elemental analyser (Germany) with 4-aminobenzenesulfonamide used as the standard.

^{1}H and *^{13}C NMR* spectra of the compounds were acquired using Mercury 400 spectrometer (400, 100 MHz) with DMSO-d_6 used as the solvent and tetramethylsilane (TMS) as the internal standard. The coupling constants (J) are reported in Hertz (Hz). Spectra were decrypted using SpinWorks software.

The alkylated *S*-derivatives of *bis*-1,2,4-triazole was confirmed by gas chromatography–mass spectrometry (GC-MS) analysis on Agilent 7890B GC system connected with Agilent 5977B mass spectrometry detector (USA). DB-5ms column with dimensions of 30 m x 250 μm x 0.25 μm was used for the separation. Carrier gas (helium) velocity was 2.0 mL/min. Injection volume was 0.5 μL. Split flow ratio was set at 1:20. Type of ionization: electron impact (EI) with electron energy of 70 eV. The range of scanned mass to charge ratios was 50-500 m/z.

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General procedure for obtaining the starting material used in the synthesis of 4-(5-(((5-(alkylthio)-4-methyl-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine derivatives (7-16)

Compound 1 was resynthesized according to the reported method, namely the closure of thiosemicarbazide in an alkaline medium [29]. Compound **1** (0.01 mol) was treated with an equivalent amount of isopropyl 2-chloroacetate in methanol abs (30 mL) and with the addition of an equivalent amount of alkali and heating under reflux for 4 h. The resulting ester 2 (0.1 mol) was placed into a flask containing hydrazine hydrate (0.3 mol) and methanol (50 mL). Further, the mixture was heated under reflux for 8 h, cooled and filtered, yielding the solid 3, which was dried subsequently. Next, the obtained hydrazide 3 (0.1 mol) was transferred into a beaker, to which methanol (50 mL) and methylisothiocyanate were added. The mixture was stirred for 8 h, during which the temperature was monitored. The beaker containing the mixture was cooled with cold water precipitating the solid 4, which was further filtered and recrystallized from methanol. The resulting thiosemicarbazide 4 (0.1 mol) was taken into a flask and dissolved in methanol (30 mL), after which 30% NaOH solution was added to set pH at 10-11. After 4 h of boiling, the solution 5 was cooled and brought to pH 5-6 with acetic acid. The formed solid product was filtered and recrystallized from ethanol to afford the corresponding compound 6. The progress of the reaction was monitored by TLC with n-hexane/EtOAc (4:1).

The starting compound 6 appears as white crystalline substance, insoluble in water and soluble in organic solvents. For analytical purposes, the compound was purified by recrystallization from water-methanol mixture.

In the next step, a mixture potassium carbonate (0.01 mol, 1.38 g) and 4-methyl-5-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol (6) (0.01 mol, 3.05 g) in methanol (50 mL) was stirred at room temperature until the solid was dissolved and after this, one of bromo derivatives (0.01 mol) was added. Further the residue was filtered, dried and recrystallized from methanol-DMF (1:1).

4-Methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol (6).White powder in 86% yield, m.p. 220-222°C. ^{1}H NMR δ ppm 2.46 (br.s, 3H); 4.00 (s, 2H); 7.83 (d, *J*= 5.0 Hz, 2H); 8.66 (d, *J*= 5.0 Hz, 2H). MS (m/z): 305.0 (M^+). Calc. for $C_{11}H_{11}N_7S_2$ (305.38): C, 43.26; H, 3.63; N, 32.11, S, 21.00 %. Found: C, 43.08; H, 3.96; N, 31.86, S, 21.10%.

4-(5-(((4-Methyl-5-(methylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (7). Yellow powder in 80% yield, 176-178 m.p. 120-122°C. ^{1}H NMR δ ppm 3.57 (s, 3H); 3.69 (s, 3H); 4.20 (s, 2H); 7.82 (d, *J*= 4.5 Hz, 1H); 8.30 (d, *J*= 6.0 Hz, 1H); 8.60 (d, *J*= 4.5 Hz, 1H); 8.74 (d, *J*= 6.0 Hz, 1H). ^{13}C NMR δ 154.90, 148.90, 147.01, 37.96, 19.12. MS (m/z): 319 (M^+). Calc. for $C_{12}H_{13}N_7S_2$ (319.41): C, 45.12; H, 4.10; N, 30.70, S 20.08 %. Found: C, 45.27; H, 4.02; N, 30.76, S 19.86%.

4-(5-(((4-Methyl-5-(ethylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (8). Yellow powder in 75% yield, m.p. 190-192°C. ^{1}H NMR δ ppm 1.79 (s, 3H); 3.11 (t, *J*= 5.0 Hz, 2H); 3.45 (s, 3H); 4.37 (s, 2H); 7.76 (d, *J*=5.0 Hz, 1H); 7.82 (d, *J*=5.0 Hz, 1H); 8.42 (d, *J*= 5.0 Hz, 1H); 8.57 (d, *J*= 5.0 Hz, 1H). MS (m/z): 333 (M^+). Calc. for $C_{13}H_{15}N_7S_2$ (333.43): C, 46.83; H, 4.53; N, 29.41, S 19.23 %. Found: C, 46.65; H, 4.67; N, 29.26, S 19.08%.

4-(5-(((4-Methyl-5-(propylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (9). Yellow powder in 77% yield, m.p. 181-183°C. ^{1}H NMR δ ppm 0.84 (t, *J*= 7.0 Hz , 3H); 1.73-1.88 (m, 2 H); 3.46-3.57 (m, 2H); 3.65 (br.s, 3H); 4.03 (s, 2H); 7.82 (d, *J*= 4.5 Hz, 2H); 8.58 (d, *J*= 5.5 Hz, 2H). MS (m/z): 347 (M^+). Calc. for $C_{14}H_{17}N_7S_2$ (347.46): C, 48.39; H, 4.93; N, 28.22, S 18.32%. Found: C, 48.64; H, 4.83; N, 28.36, S 18.33%.

4-(5-(((4-Methyl-5-(butylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (10). Yellow powder in 69% yield, m.p. 168-170°C. ^{1}H NMR δ ppm 0.87 (t, *J*= 7.2 Hz, 3H); 1.22-1.31 (m, 2H); 1.80-1.88 (m, 2H); 3.77 (s, 3H); 4.07 (s, 2H); 4.47 (t, *J*= 7.2 Hz, 2H); 7.83 (d, *J*= 5.0 Hz, 2H); 8.63 (d, *J*= 5.0 Hz, 2H). ^{13}C NMR δ 153.4, 148.4, 117.9, 38.1, 37.9, 37.7, 23.5. MS (m/z): 347 (M^+). Calc. for $C_{15}H_{19}N_7S_2$ (361.49): C, 49.84; H, 5.30; N, 27.12, S 17.04 %. Found: C, 49.70; H, 5.11; N, 27.01, S 17.21%.

4-(5-(((4-Methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (11). Yellow powder in 85% yield, m.p. 118-120°C.

^{1}H NMR δ ppm 0.67-0.85 (m, 3H); 1.10-1.32 (m, 4H); 1.79-1.95 (m, 2H); 2.63 (br.s, 1 H); 3.82 (br.s, 3H); 4.07 (s, 2H); 4.49 (t, *J*= 7.0 Hz, 2H); 7.83 (d, *J*= 4.5 Hz, 2H); 8.65 (d, *J*= 4.5 Hz, 2H). MS (m/z): 374 (M^+). Calc. for $C_{16}H_{21}N_7S_2$ (375.51): C, 51.18; H, 5.64; N, 26.11; S, 17.08 %. Found: C, 51.30; H, 5.60; N, 26.06, S 17.01%.

4-(5-(((4-Methyl-5-(hexylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (12). Yellow powder in 82% yield, m.p. 223-225°C. ^{1}H NMR δ ppm 0.71-0.84 (m, 3H); 1.01 (t, *J*= 6.7 Hz, 2H); 1.10-1.27 (m, 4H); 3.40 (q, *J*= 7.0 Hz, 1H); 3.75 (s, 3H); 4.07 (s, 2H); 4.45 (t, *J*= 7.0 Hz, 2H); 7.83 (d, *J*= 5.5 Hz, 2H); 8.62 (d, *J*= 5.5 Hz, 2H). MS (m/z): 389 (M^+). Calc. for $C_{17}H_{23}N_7S_2$ (389.54): C, 52.42; H, 5.89; N, 25.17, S 16.46 %. Found: C, 52.34; H, 5.95; N, 25.15, S 16.43%.

4-(5-(((4-Methyl-5-(heptylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (13). Yellow powder in 78% yield, m.p. 180-182°C. ^{1}H NMR δ ppm 0.72-0.84 (m, 3H); 0.98-1.27 (m, 4H); 1.44-1.55 (m, 2H); 1.86 (d, *J*= 7.0 Hz, 2H); 3.72 (s, 3H); 3.98-4.09 (m, 2H); 4.38-4.48 (m, 2H); 4.56 (s, 2H); 7.83 (d, *J*= 5.0 Hz, 2H); 8.60 (d, *J*= 5.0 Hz, 2H), MS (m/z): 403 (M^+). Calc. for $C_{18}H_{25}N_7S_2$ (403.57): C, 53.57; H, 6.24; N, 24.30, S 15.89 %. Found: C, 53.44; H, 6.30; N, 24.40, S 15.73%.

*4-(5-(((4-Methyl-5-(octylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (14).*Yellow powder in 73% yield, m.p. 235-237°C, ^{1}H NMR δ ppm 0.75-0.84 (m, 3H); 1.21 (d, *J*= 7.5 Hz, 2H); 1.80-1.89 (m, 2H); 3.00 (t, *J*= 6.5 Hz, 2H); 3.50-3.56 (m, 2H); 3.70 (s, 3H); 3.98-4.07 (m, 4H); 4.43 (t, *J*= 7.0 Hz, 2H); 4.53 (s, 2H); 7.82 (d, *J*= 5.5 Hz, 2H); 8.59 (d, *J*= 5.5 Hz, 2H). MS (m/z): 417 (M^+). Calc. for $C_{19}H_{27}N_7S_2$ (417.59): C, 54.65; H, 6.52; N, 23.48 , S 15.36 %. Found: C, 54.53; H, 6.54; N, 23.40, S 15.58 %.

4-(5-(((4-Methyl-5-(nonylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (15). Yellow powder in 79% yield, m.p. 201-203°C. ^{1}H NMR δ ppm 0.76-0.84 (m, 3H); 1.14-1.27 (m, 8H); 1.74-1.86 (m, 4H); 3.01 (t, *J*= 7.0 Hz, 2H); 3.54-3.59 (m, 1H); 3.62 (s, 3H); 4.36 (t, *J*= 6.5 Hz, 1H); 4.61 (s, 2H); 7.82 (d, *J*= 5.0 Hz, 2H); 8.57 (d, *J*= 5.0 Hz, 2H). MS (m/z): 431 (M^+). Calc. for $C_{20}H_{29}N_7S_2$ (431.62): C, 55.65; H, 6.77; N, 22.72, S 14.86 %. Found: C, 55.52; H, 6.84; N, 22.75, S 14.79%.

4-(5-(((4-Methyl-5-(decylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (16). Yellow powder in 82% yield, m.p. 186-188°C. ^{1}H NMR δ ppm 0.77-0.83 (m, 3H); 1.13-1.27 (m, 14 H); 1.76-1.79 (m, 2H); 3.62 (s, 3H) 3.76 (s, 2H); 4.36 (t, *J*= 7.0 Hz, 2H); 7.81 (d, *J*= 4.0 Hz, 2H); 8.56 (d, *J*= 4.0 Hz, 2H), MS (m/z): 445 (M^+). Calc. for $C_{21}H_{31}N_7S_2$ (445.65): C, 56.60; H, 7.01; N, 22.00, S 14.39 %. Found: C 56.48, 7.02; N, 22.02, S 14.47%.

Results and Discussion

Briefly, in all cases, an equimolar ratio of reagents is sufficient for a successful reaction. All synthesized compounds were crystalline substances of yellow or white colour, with narrow melting point intervals (118-225°C), water solubility decreases with lengthening of the carbon chain, sparingly soluble in alcohols and practically insoluble in non-polar solvents. The experimental data shows that the introduction of functional groups in *bis*-1,2,4-triazole-3-thione at the sulphur atom leads to a shift in solubility towards more polar substances. The alkyl S-substituted derivatives with one 1,2,4-triazole ring are almost insoluble in water and poorly soluble in highly polar solvents. By contrast, most of *bis*-1,2,4-triazole derivatives are soluble in water, acetic acid, DMF and alcohols..

The desired *S*-derivatives of 1,2,4-triazole were afforded using widely known alkylation methods [30]. Cyclization of the second 1,2,4-triazole ring was the first step and is demonstrated in Scheme 1.

Structures of the novel 4-methyl-5-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5-yl)thio)methyl)-4*H*-1,2,4-triazole-3-thiol (**6**) and 4-(5-(((5-(alkylthio)-4-methyl-4*H*-1,2,4-triazole-3-yl)methyl)thio)-1*H*-1,2,4-triazole-3-s)pyridines (7-16) were characterized by elemental analysis, ^{1}H and ^{13}C NMR, GC-MS. The syntheses are presented in Schemes 1, 2.

The selectivity of the reaction was demonstrated by ^{13}C NMR analysis for the compounds. In the ^{13}C NMR spectra of the synthesized compound 10 the intense signals of the carbon atoms of the triazole ring [155.69 ppm (C-3, C-5, C-14, C-16)], pyridine ring [147.59 ppm (C-6, C-8, C-10), 117.34 ppm (C-7, C-11)] and methylene groups [37.56-38.12 ppm (C-13, C-21, C-22, C-23)] are observed (Figure 1).

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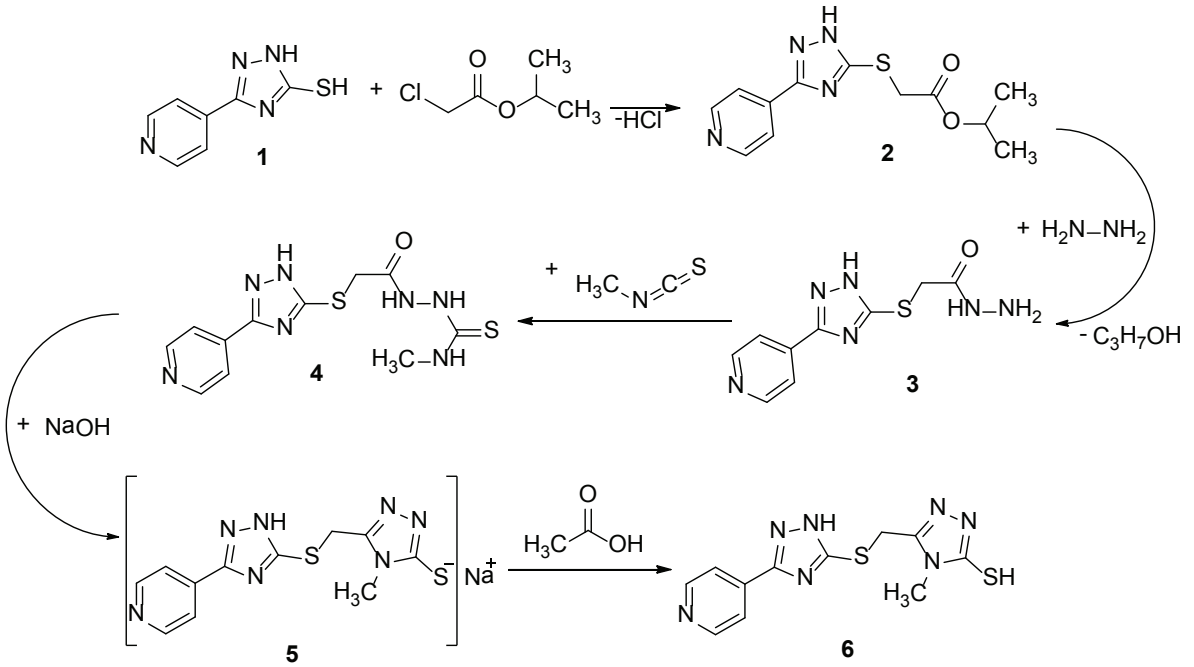
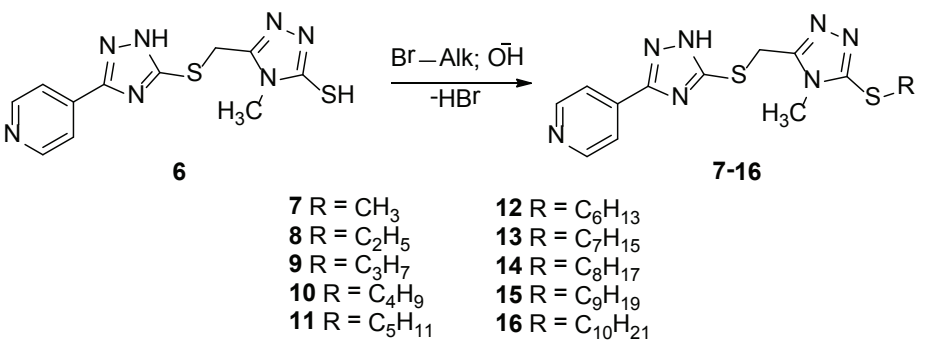


Scheme 1. Synthesis of 4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)methyl)-4H-1,2,4-triazole-3-thiol (6).



Scheme 2. Synthesis of 4-(5-(((5-(alkylthio)-4-methyl-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-s)pyridines .

The structure of the starting compound was confirmed using a set of appropriate instrumental methods of analysis. Samples of all substances were prepared at the same concentrations and analysed by GC-MS under the same conditions. Electron ionization parameters remained the same for all compounds. Elemental analysis data, along with other physical properties of these compounds, are reported in Table 1. The difference between the found content of CHNS elements was no more than ±0.25%. The yield for all substances was about 69-86%. Melting points showed the individuality of the synthesized compounds.

^{1}H NMR spectra of the compound **7** are characterized by the presence of a singlet in high field in the region of 4.00-4.91 ppm (Figure 2), which corresponds to the methylene linker. The elongation of the alkyl fragment (C1-C6) is indicated by a decrease in the values of the singlet signals of the S-CH_3 group from 4.20 to 4.07 ppm. Particularly, the spin-spin interaction constant for carbon atoms no. 10-11 of the pyri-

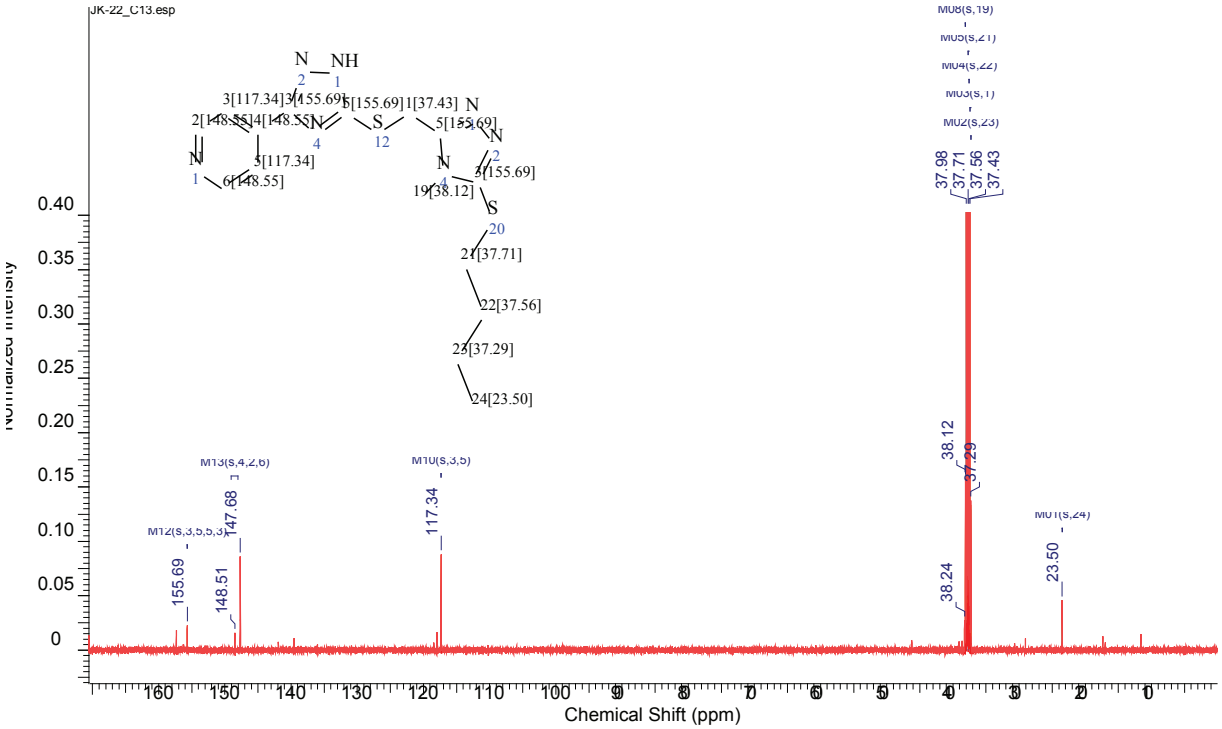


Figure 1. ^{13}C NMR spectrum of 4-(5-(((4-methyl-5-(butylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl) pyridine (10).

dine ring must be noted. The significant reduction in frequency is indicative for the acceptor effect of the 1,2,4-triazole ring. There are characteristic signals of *S*-alkyl residues observed in the region typical for aliphatic compounds, which appear in varied forms in the region of 0.78-3.57 ppm. Methyl substituent at the second 1,2,4-triazole ring is registered at 3.69 ppm, in a lower field than the *S*-methyl moiety due to the manifestation of the acceptor effect of 1,2,4-triazole.

Hard ionization conditions produced a large number of MS peaks. Molecular peak in the spectrum appeared at 305.2 m/z, while the retention time (RT) for the thiol was 11.061 min (Figure 3).

Mass spectrometric fragmentation of the molecule allowed for drawing conclusions about the presence of certain functional groups and fragments (Scheme 3, 4). Specifically, the model of sequential fragmentation using 4-methyl-5-(((3-pyridin-4-yl)thio) methyl)-4H-1,2,4-triazole-3-thiol as an example was proposed. Under the electron energy of 70 eV, the molecule begins to lose its structural bonds one by one. Usually, such a breakup occurs selectively and encompasses a number of bonds. In this instance, possible fragment formation pathways are demonstrated based on the most intensive peaks in the MS spectrum. Structure identification was started from searching for the molecular peak at 305 m/z with normalized abundance 5.42%, which corresponds to the molecular mass of the compound. In many cases, the excess energy of the molecular peak is too high, making it disappears from the mass spectrum. However, in this case, the stability of the formed radical cation was sufficiently high. The initial bis-1,2,4-triazole was subjected to a heterolytic cleavage at C-N and C=N bonds of the Nitrogen atom in the second 1,2,4-triazole ring. In addition, the first ionization threshold led to the dissociation of –SH group yielding N,N-dimethyl-2-((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio)acetimidamide (m/z 262.10; ab. 5.33%), a similar fragmentation pathway with ring opening of 1,2,4-triazole was reflected in manuscript [31]. Furthermore, most probably the detachment of two methylene groups from the nitrogen took place leading to the formation of 2-((3-(pyridine-4-yl)-1H-1,2,4-triazole-5-yl)thio)acetimidamide (m/z 234.07; ab. 1.34%). The next functional group that was sub-

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Table 1. Physicochemical properties of the synthesized compounds and elemental analysis results.

Compd.	Alk	Molecular formula	M. wt.	m.p. (°C)	Yield (%)	Elemental analysis: calc. (found) (%)			
						C	H	N	S
6	-	$C_{11}H_{11}N_7S_2$	305	220-222	86	43.26 (43.08)	3.63 (3.96)	32.11 (31.86)	21.00 (21.10)
7	CH_3	$C_{12}H_{13}N_7S_2$	319	176-178	80	45.12 (45.27)	4.10 (4.02)	30.70 (30.76)	20.08 (19.86)
8	C_2H_5	$C_{13}H_{15}N_7S_2$	333	190-192	75	46.83 (46.65)	4.53 (4.67)	29.41 (29.26)	19.23 (19.08)
9	C_3H_7	$C_{14}H_{17}N_7S_2$	347	181-183	77	48.39 (48.24)	4.93 (4.83)	28.22 (28.36)	18.22 (18.33)
10	C_4H_9	$C_{15}H_{19}N_7S_2$	361	168-170	69	49.84 (49.70)	5.30 (5.11)	27.12 (27.01)	17.04 (17.21)
11	C_5H_{11}	$C_{16}H_{21}N_7S_2$	375	118-120	85	51.18 (51.30)	5.64 (5.60)	26.11 (26.06)	17.08 (17.01)
12	C_6H_{13}	$C_{17}H_{23}N_7S_2$	389	223-225	82	52.42 (52.34)	5.89 (5.95)	25.17 (25.15)	16.46 (16.43)
13	C_7H_{15}	$C_{18}H_{25}N_7S_2$	403	180-182	78	53.57 (53.44)	6.24 (6.30)	24.30 (24.40)	15.89 (15.73)
14	C_8H_{17}	$C_{19}H_{27}N_7S_2$	417	235-237	73	54.65 (54.53)	6.52 (6.54)	23.48 (23.40)	15.36 (15.58)
15	C_9H_{19}	$C_{20}H_{29}N_7S_2$	431	201-203	79	55.65 (55.52)	6.77 (6.84)	22.72 (22.75)	14.86 (14.79)
16	$C_{10}H_{21}$	$C_{21}H_{31}N_7S_2$	445	186-188	82	56.60 (56.46)	7.01 (7.02)	22.00 (22.02)	14.39 (14.47)

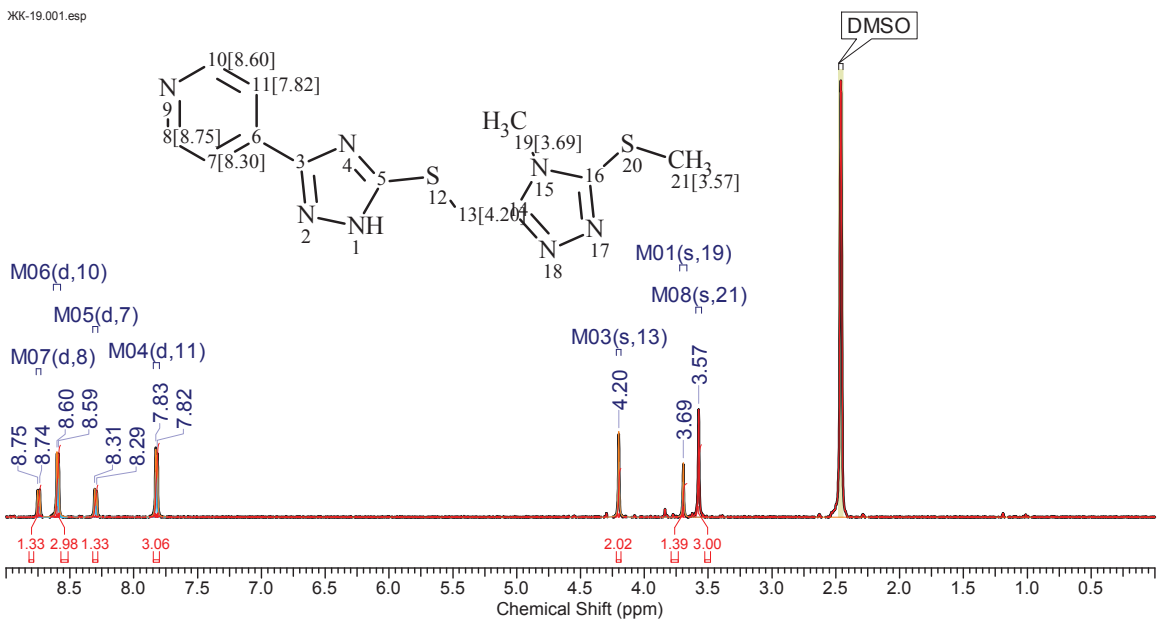


Figure 2. ^{1}H NMR spectrum of 4-(5-(((4-methyl-5-(methylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl) pyridine (7).

jected to the dissociative ionization was –NH2 and the Nitrogen in the pyridine cycle (m/z 223.09; ab. 3.56% and m/z 208.08; ab. 1.45%). The emergence of [C8H8N4S]+ with m/z 192 is possible only by the saturation of -CH2- bond, relocation of the protons from the part of hydrocarbon bonding, shortening of

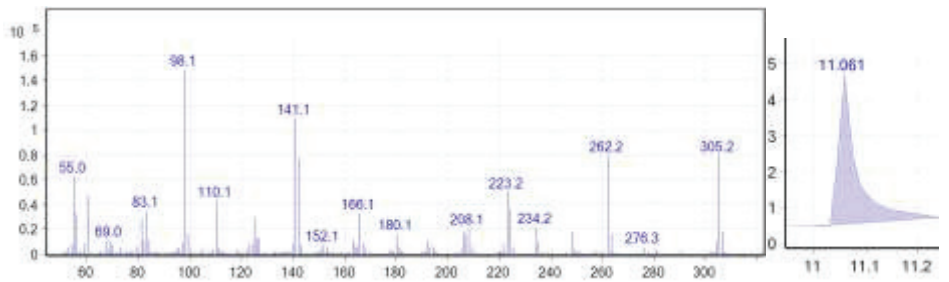
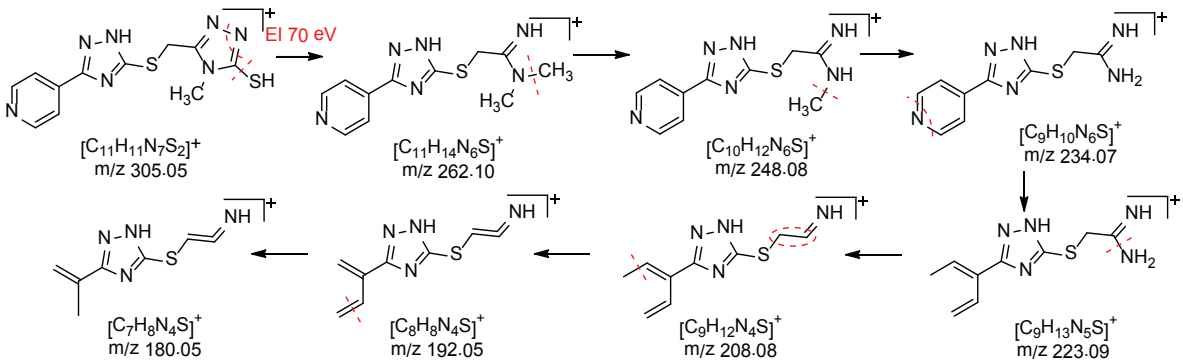


Figure 3. Chromatographic and mass spectrometric parameters of 4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl)thio) methyl)-4H-1,2,4-triazole-3-thiol (6).

the structural framework of the molecule due to the detachment of =CH2 and unsaturation of –CH3 radicals (m/z 192.05 – 180.05 – 166,03; ab. 0.45 – 1.14 – 2.28 %).

Scheme 4 demonstrates the further fragmentation of the ionized bis-1,2,4-triazole-3-thiol molecule until the formation of the final ion, which corresponded to the structure of the five-membered heterocycle, namely 1,2,4-triazole with a mass to charge ratio of 69. The next step was the dissociation of imine group (=NH) and the formation of [C6H7N3S]+ ion. The fragmented ions with m/z of 141.04 (ab. 7.62%), 127.02 (ab. 0.97%), 83.05 (ab. 2.02%) are produced due to the dissociation of the alkyl chain present at both sides of 1,2,4-triazole ring at 3- and 5-positions and detachment of the thiol group. Along with that, fragment ion structures produced from pyridine and 1,2,4-triazole heterocycles were proposed, which also were visible on the mass spectrum. All of the structures agree with the nitrogen rule except for 3,4-dimethyl-1H-1,2,4-triazole-4-ium (m/z 98; ab. 10.14%), which is characterized by the most intensive peak on the mass spectrum. Some of the fragments have double peaks differing by ± 1 to 2 m/z, which is possible due to regrouping, bond saturation as well as migration of H+. It is important to remember that normal understandings of ion structure and mechanism of their formation are largely inapplicable to the characterization of fragmentation patterns. This is because under hard ionization and high temperature conditions fragment ions exist in various hardly predictable vibrationally and electronically induced states, and only the fragment composition may be determined with a high precision.

The spectra of *bis*-1,2,4-triazolethiones confirmed that the analysed compounds are *S*-substituted alkyls. As the hydrocarbon chain grows, the molecular weight increases by 14 mass units, corresponding to the methylene group (–CH_2–). Considering the high degree of structural similarities between the target compounds 7-16, for informative purposes, only GC-MS data for the 4-(5-(((4-methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine will be illustrated (Figure 4).



Scheme 3. Mass spectrometric fragmentation pattern of 4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl) thio)methyl)-4H-1,2,4-triazole-3-thiol (6) 1/2.

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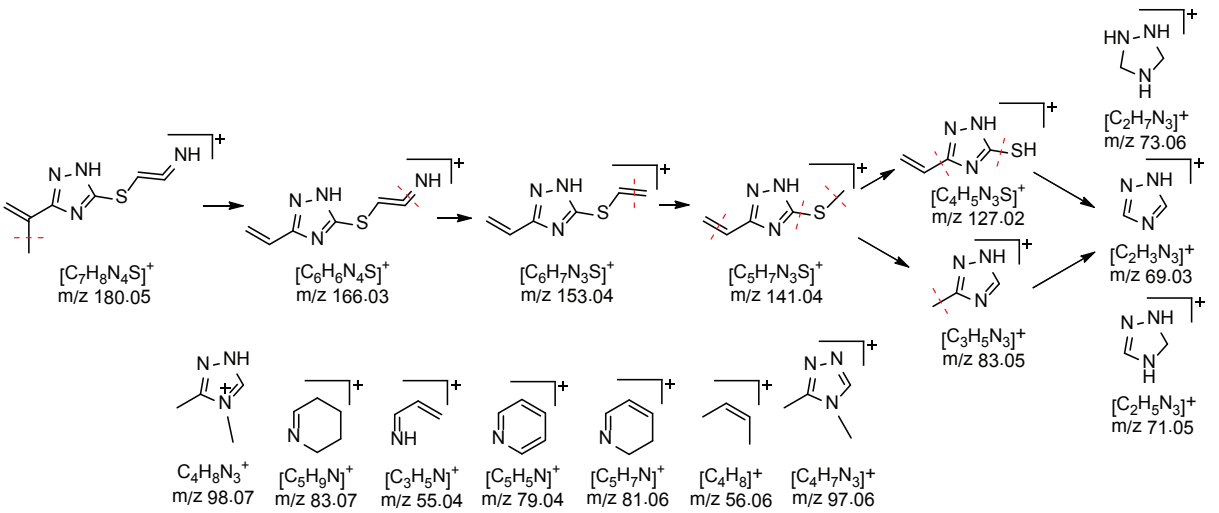
According to Figure 4, pseudo-molecular peak appeared in the mass spectrum at 374.2 m/z, while the peak of the compound on the chromatogram was at 11.266 min. Since stationary phase of the used GC column was nonpolar, components with aliphatic residues were expected to be strongly retained in the column. Given all compounds have aliphatic substituents, components with lower molecular weight eluted earlier, despite the slight decrease in polarity.

Fragmentation pattern of 4-(5-(((4-methyl-5-(pentylthio)-4*H*-1,2,4-triazole-3-yl)methyl) thio)-1*H*-1,2,4-triazole-3-yl)pyridine (11) is similar to the fragmentation pattern of 4-methyl-5-(((3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5-yl)thio) methyl)-4*H*-1,2,4-triazole-3-thiol (**6**), however there are also atypical fragmented ions that can appear on the mass spectrum (Scheme 5).

The continuation of this research will be directed at testing for the presence of various biological activities. In summary, *bis*-1,2,4-triazolethione moiety holds potential for becoming a favoured synthetic scaffold in pharmaceutical chemistry. The exceptional properties of this promising heterocycle facilitate its wide range of applications.

Conclusions

The purpose of this work was to synthesize new *S*-alkyl *bis*-1,2,4-triazoles in a number of 10 compounds and describe their physicochemical properties (^{1}H and ^{13}C NMR, GC-MS, elemental analysis, molecular fragmentation).



Scheme 4. Mass spectrometric fragmentation pattern of 4-methyl-5-(((3-(pyridin-4-yl)-1H-1,2,4-triazole-5-yl) thio)methyl)-4H-1,2,4-triazole-3-thiol (6) 2/2.

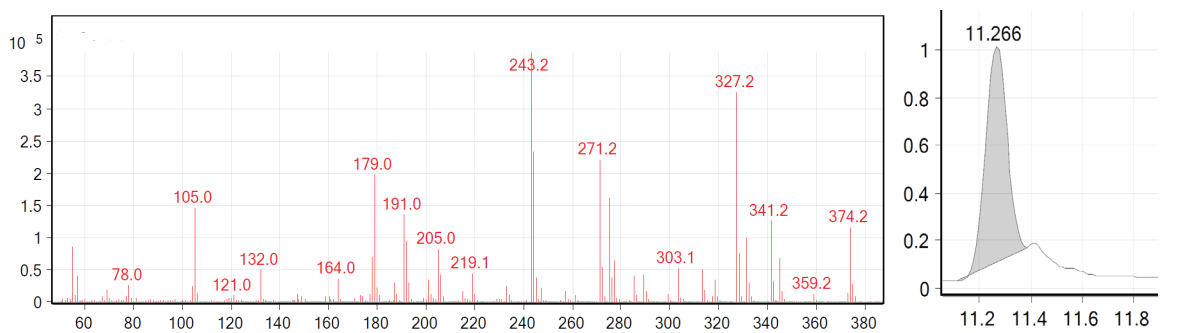
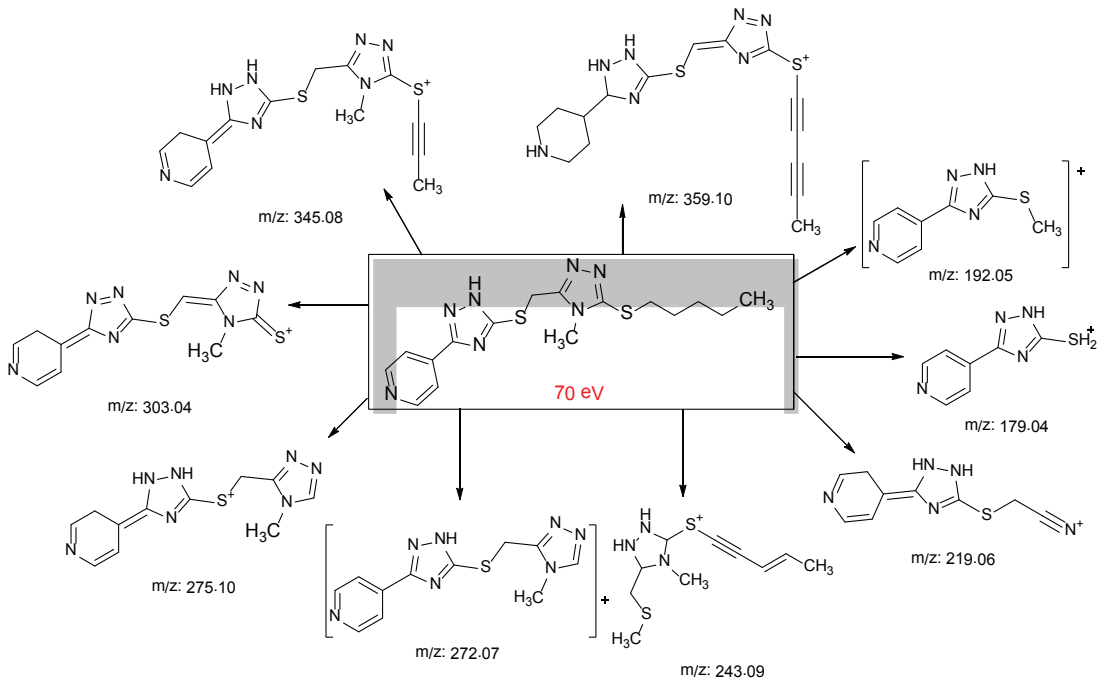


Figure 4. Chromatographic and mass spectrometric parameters of 4-(5-(((4-methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl)methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (11).



Scheme 5. Mass spectrometric fragmentation pattern of 4-(5-(((4-methyl-5-(pentylthio)-4H-1,2,4-triazole-3-yl) methyl)thio)-1H-1,2,4-triazole-3-yl)pyridine (11).

The present paper describes a short and efficient synthesis of novel *S*-alkyl *bis*-1,2,4-triazoles. Starting from *bis*-1,2,4-triazole-3-thiol (**6**) *via* its intermediate isopropyl 2-((3-(pyridin-4-yl)-1*H*-1,2,4-triazole-5-yl)thio)acetate **2** and hydrazide **3**, the compounds were synthesized at overall yields of ~ 50-89%.

The structures of new *bis*-1,2,4-triazole compounds were identified by GC-MS and confirmed using elemental analysis as well as ^{1}H and ^{13}C NMR spectroscopy. The mass spectra of *bis*-1,2,4-triazolethiones at constant electron energy have been shown.

A fragmentation pattern was proposed for 4-methyl-5-(((3-pyridin-4-yl)-1*H*-1,2,4-triazole-5-yl)thio) methyl)-4*H*-1,2,4-triazole-3-thiol produced by electron impact and possible formation pathways of fragments possessing the highest peak intensities in the mass spectrum. Hard ionization of the starting thiol leads to a significant weakening of single bonds in the second 1,2,4-triazole heterocycle and in the pyridine ring. The proposed fragmentation scheme for *bis*-1,2,4-triazole-3-thiol is significant for further research of the behaviour of these group of derivatives in mass-spectrometric conditions.

References

1. Orlewska, C.; Pancechowska-Ksepko, D.; Foks, H.; Zwolska, Z.; Augustynowicz-Kopec, E. Reactivity of N 1-Dithioester Substituted Pyridinand Pyrazincarboxamidrazones. Phosphorus, Sulfur, and Silicon and the Related Elements, 2006, 181(4), pp. 737–744. https://doi.org/10.1080/10426500500270065
2. Pellizzari, G.; Gazz Chim Ital, 1911, no. 41, pp. 93.
3. Ji Ram, V.; Sethi, A.; Nath, M.; Pratap, R. Five-Membered Heterocycles. The Chemistry of Heterocycles, 2019, pp. 365. https://doi.org/10.1016/b978-0-08-101033-4.00005-x
4. Ueda, S.; Nagasawa, H. Facile synthesis of 1,2,4-triazoles *via* a copper-catalyzed tandem addition−oxidative cyclization. Journal of the American Chemical Society, 2009, 131(42), pp. 15080-15081. DOI: https://doi.org/10.1021/ja905056z
5. Huang, H.; Guo, W.; Wu, W.; Li, C.-J.; Jiang, H. Copper-catalyzed oxidative C(sp3)–H functionalization for facile synthesis of 1,2,4-triazoles and 1,3,5-triazines from amidines. Organic Letters, 2015, 17(12), pp. 2894-2897. DOI: https://doi.org/10.1021/acs.orglett.5b00995
6. Castanedo, G. M.; Seng, P. S.; Blaquiere, N.; Trapp, S.; Staben, S. T. Rapid synthesis of 1,3,5-substituted 1,2,4-triazoles from carboxylic acids, amidines, and hydrazines. The Journal of Organic Chemistry, 2011, 76(4), pp. 1177-1179. DOI: https://doi.org/10.1021/jo1023393

ISSN: 2458 - 8806

7. Vidavalur, S.; Nakka, M.; Tadikonda, R.; Rayavarapu, S.; Sarakula, P. A Simple and efficient synthesis of 3,4,5-trisubstituted/n-fused 1,2,4-triazoles via ceric ammonium nitrate catalyzed oxidative cyclization of amidrazones with aldehydes using polyethylene glycol as a recyclable reaction medium. Synthesis, 2014, 47(04), pp. 517-525. DOI: https://doi.org/10.1055/s-0034-1378909

8. Minozzi, C.; Caron, A.; Grenier-Petel, J.; Santandrea, J.; Collins, S. Heteroleptic copper(I)-based complexes for photocatalysis: combinatorial assembly, discovery, and optimization. Angewandte Chemie International Edition, 2018, 57(19), pp. 5477-5481. DOI: 10.1002/anie.201800144

9. Arnold, F. Directed evolution: bringing new chemistry to life. Angewandte Chemie International Edition, 2017, 57(16), pp. 4143-4148. DOI: 10.1002/anie.201708408

10. Klimešová, V.; Zahajská, L.; Waisser, K.; Kaustová, J.; Möllmann, U. Synthesis and antimycobacterial activity of 1,2,4-triazole 3-benzylsulfanyl derivatives. Il Farmaco, 2004, 59(4), pp. 279-288. DOI: 10.1016/j.farmac.2004.01.006

11. Kapro ´n, B.; Łuszczki, J.J.; Płazi ´nska, A.; Siwek, A.; Karcz, T.; Grybo´s, A.; Nowak, G.; Makuch-Kocka, A.;11. Takahashi, M., Dodo, K., Sugimoto, Y., Aoyagi, Y., Yamada, Y., Hashimoto, Y., & Shirai, R. Synthesis of the novel analogues of dysidiolide and their structure–activity relationship. Bioorganic & Medicinal Chemistry Letters, 2000, 10(22), pp. 2571–2574. https://doi.org/10.1016/s0960-894x(00)00527-8 Timur, ˙I.; Kocyigit, Ü.M.; Dastan, T.; Sandal, S.; Ceribası, A.O.; Taslimi, P.; Gulcin, ˙I.; Koparir, M.; Karatepe, M.; Çiftçi, M.J. In vitro cytotoxic and in vivo antitumoral activities of some aminomethyl derivatives of 2,4-dihydro-3H-1,2,4-triazole-3-thiones—Evaluation of their acetylcholinesterase and carbonic anhydrase enzymes inhibition profiles. Biochem. Mol. Toxicol, 2019, 33, pp. 22239–22250 https://doi.org/10.1002/jbt.22239

12. Mishra, C.B.; Mongre, R.K.; Kumari, S.; Jeong, D.K.; Tiwari, M. Novel Triazole-Piperazine Hybrid Molecules Induce Apoptosis via Activation of the Mitochondrial Pathway and Exhibit Antitumor Efficacy in Osteosarcoma Xenograft Nude Mice Model. ACS Chem. Biol, 2017, 17, pp. 753–768. https://doi.org/10.1021/acschembio.6b01007

13. Mermer, A.; Demirbaş, N.; Şirin, Y.; Uslu, H.; Özdemir, Z.; Demirbaş, A. Conventional and microwave prompted synthesis, antioxidant, anticholinesterase activity screening and molecular docking studies of new quinolone-triazole hybrids. Bioorganic Chemistry, 2018, 78, pp. 236-248. DOI: 10.1016/j.bioorg.2018.03.017

14. Qiao, K.; Fu, W.; Jiang, Y.; Chen, L.; Li, S.; Ye, Q.; Gui, W. QSAR models for the acute toxicity of 1,2,4-triazole fungicides to zebrafish (Danio rerio) embryos. Environmental Pollution, 2020, 265, pp. 114837. https://doi.org/10.1016/j.envpol.2020.114837

15. Singh, R.; Pujar, G.V.; Purohit, M.N.; Chandrashekar, V.M. Synthesis, *in vitro* cytotoxicity, and antibacterial studies of new asymmetric bis-1,2,4-triazoles. Medicinal Chemistry Research, 2012, 22(5), pp. 2163-2173. DOI: https://doi.org/10.1007/s00044-012-0209-5

16. ŁUszczki, J. J., Lepiech, J., Zagaja, M., Wróblewska-Łuczka, P., Florek-Łuszczki, M., Bojar, H., Walczak, A., & Plech, T. Anticonvulsant and neurotoxic effects of a novel 1,2,4-triazole-3-thione derivative (TPF-34) and its isobolographic interaction profile with classical antiepileptic drugs in mice. Pharmacological Reports, 2019, 72(1), pp. 87–95. https://doi.org/10.1007/s43440-019-00044-7

17. El-Sherief, H. A., Youssif, B. G., Bukhari, S. N. A., Abdel-Aziz, M., & Abdel-Rahman, H. M. Novel 1,2,4-triazole derivatives as potential anticancer agents: Design, synthesis, molecular docking and mechanistic studies. Bioorganic Chemistry, 2018, 76, pp. 314–325. https://doi.org/10.1016/j.bioorg.2017.12.013

18. Turky, A., Bayoumi, A. H., Sherbiny, F. F., El-Adl, K., & Abulkhair, H. S. (2020). Unravelling the anticancer potency of 1,2,4-triazole-N-arylamide hybrids through inhibition of STAT3: synthesis and in silico mechanistic studies. Molecular Diversity, 25(1), 403–420. https://doi.org/10.1007/s11030-020-10131-0

19. Strzelecka, M., & ŚWiątek, P. (2021). 1,2,4-Triazoles as Important Antibacterial Agents. Pharmaceuticals, 14(3), 224. https://doi.org/10.3390/ph14030224

20. Küçükgüzel, ¸S.G.; Çıkla-Süzgün, P. Recent advances bioactive 1,2,4-triazole-3-thiones. Eur. J. Med. Chem, 2015, 97, pp. 830–870. https://doi.org/10.1016/j.ejmech.2014.11.033

21. Ahmadi, F.; Ghayahbashi, M.; Sharifzadeh, M.; Alipoiur, E.; Ostad, S.; Vosooghi, M.; khademi, H.; Amini, M. Synthesis and Evaluation of Anti-inflammatory and Analgesic Activities of New 1,2,4-triazole Derivatives. Medicinal Chemistry, 2014, 11(1), pp. 69–76. https://doi.org/10.2174/1573406410666140613154507

22. Jiang, G. W., Chang, Q., Liang, D. Y., Zhang, Y. T., Meng, Y. J., & Yi, Q. Q. Preparation and antitumor effects of 4-amino-1,2,4-triazole Schiff base derivative. Journal of International Medical Research, 2020, 48(2), 030006052090387. https://doi.org/10.1177/0300060520903874

23. Wu, W. N.; Jiang, Y. M.; Fei, Q.; Du, H. T. Synthesis and fungicidal activity of novel 1,2,4-triazole derivatives containing a pyrimidine moiety. Phosphorus, Sulfur, and Silicon and the Related Elements, 2019, 194(12), pp. 1171–1175. https://doi.org/10.1080/10426507.2019.1633321

24. Khan, B., Naiyer, A., Athar, F., Ali, S., & Thakur, S. C. Synthesis, characterization and anti-inflammatory activity evaluation of 1,2,4-triazole and its derivatives as a potential scaffold for the synthesis of drugs against prostaglandin-endoperoxide

synthase. Journal of Biomolecular Structure and Dynamics, 2020, 39(2), pp. 457–475. https://doi.org/10.1080/07391102.2019.1711193

25. Zhang, Y.; Zhan, Y. Z.; Ma, Y.; Hua, X. W.; Wei, W.; Zhang, X.; Song, H. B.; Li, Z. M.; Wang, B. L. Synthesis, crystal structure and 3D-QSAR studies of antifungal (bis-)1,2,4-triazole Mannich bases containing furyl and substituted piperazine moieties. Chinese Chemical Letters, 2018, 29(3), pp. 441–446. https://doi.org/10.1016/j.cclet.2017.08.035
26. Wang, D.-Z.; Hu, T.-L.; Zhao, J.-P.; Bu, X.-H. Metal–organic coordination architectures of bis(1,2,4-triazole) ligands bearing different spacers: syntheses, structures and luminescent properties. CrystEngComm, 2010, 12(11), pp. 3587-3592. DOI: https://doi.org/10.1039/b913147g
27. Singh, R.; Pujar, G. V.; Purohit, M. N.; Chandrashekar, V. M. Synthesis, in vitro cytotoxicity, and antibacterial studies of new asymmetric bis-1,2,4-triazoles. Medicinal Chemistry Research, 2012, 22(5), pp. 2163–2173. DOI: https://doi.org/10.1007/s00044-012-0209-5
28. Bharty, M. K., Kushawaha, S. K., Chaudhari, U. K., Dani, R. K., Maiti, B., & Butcher, R. J. (2017). Synthesis, structural characterization, electrochemical studies and DFT calculations on nickel(II) complexes of N-picolinoyl-N′-benzothioylhydrazide and 5-(pyridine-4-yl)-2H-1,2,4-triazole-3-thione. Transition Metal Chemistry, 2017, 42(3), pp. 243–252. https://doi.org/10.1007/s11243-017-0128-x
29. Kochikyan, T. V., Samvelyan, M. A., Arutyunyan, V. S., Avetisyan, A. A., Tamazyan, R. A., & Aivazyan, A. G. Synthesis of 1,2,4-triazole-3-thiols and their S-substituted derivatives. Russian Journal of Organic Chemistry, 2010, 46(4), pp. 551–555. doi:10.1134/s1070428010040184
30. Heitke, B. T., & McCarty, C. G. Mass Spectra of C-Amino- and C-Azido-l,2,4-triazoles. Canadian Journal of Chemistry, 1974, 52(16), pp. 2861–2865. https://doi.org/10.1139/v74-416

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